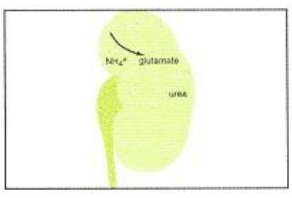
Chapter 11—
Amino Acid Metabolism

Marguerite W. Coomes



11.1— Overview

Amino acids and the relationship between their structure and the structure and function of proteins were presented in Chapter 2. This chapter describes the metabolism of amino acids, emphasizing the importance of dietary protein as the major source of amino acids for humans.

Molecular nitrogen, N_2, exists in the atmosphere in great abundance. Before it can be utilized by animals it must be "fixed," that is, reduced from N_2 to NH_3 by microorganisms, plants, and electrical discharge from lightning. Ammonia is then incorporated into amino acids and proteins, and these become part of the food chain (Figure 11.1). Humans can synthesize only 11 of the 20 amino acids needed for protein synthesis. Those that cannot be synthesized *de novo* are termed **"essential"** because they must be obtained from dietary foodstuffs that contain them (Table 11.1).

This chapter includes discussion of interconversions of amino acids, removal and excretion of ammonia, and synthesis of "nonessential" amino acids

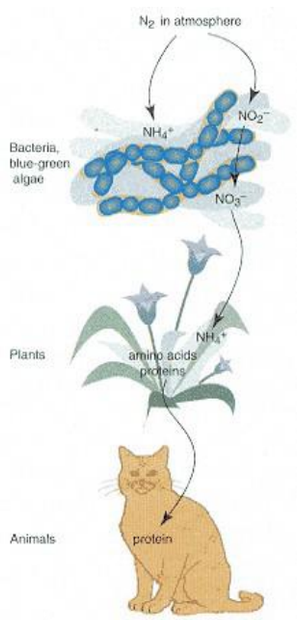


Figure 11.1
Outline of entry of atmospheric nitrogen into the human diet.
This occurs initially by reduction of nitrogen to ammonia by enzymes in microorganisms and plants.

TABLE 11.1 Dietary Requirements of Amino Acids

Essential	*Nonessential*
Arginine[a]	Alanine
Histidine	Aspartate
Isoleucine	Cysteine
Leucine	Glutamate
Lysine	Glycine
Methionine[b]	Proline
Phenylalanine[c]	Serine
Threonine	Tyrosine
Tryptophan	
Valine	

[a] Arginine is synthesized by mammalian tissues, but the rate is not sufficient to meet the need during growth.

[b] Methionine is required in large amounts to produce cysteine if the latter is not supplied adequately by the diet.

[c] Phenylalanine is needed in larger amounts to form tyrosine if the latter is not supplied adequately by the diet.

by the body. As part of ammonia metabolism, synthesis and degradation of glutamate, glutamine, aspartate, asparagine, alanine, and arginine are discussed. Synthesis and degradation of other nonessential amino acids are then described, as well as the degradation of the essential amino acids. Synthetic pathways of amino acid derivatives and some diseases of amino acid metabolism are also presented.

Carbons from amino acids enter intermediary metabolism at one of seven points. Glucogenic amino acids are metabolized to pyruvate, 3-phosphoglycerate, α-ketoglutarate, oxaloacetate, fumarate, or succinyl CoA. Ketogenic amino acids produce acetyl CoA or acetoacetate. Metabolism of some amino acids results in more than one of the above and they are therefore both glucogenic and ketogenic (Figure 11.2). Products of amino acid metabolism can be used to provide energy. Additional energy-generating compounds, usually NADH, are also produced during degradation of some of the amino acids.

11.2— Incorporation of Nitrogen into Amino Acids

Most Amino Acids Are Obtained from the Diet

A healthy adult eating a varied and plentiful diet is generally in **"nitrogen balance,"** a state where the amount of nitrogen ingested each day is balanced by the amount excreted, resulting in no net change in the amount of body nitrogen. In the well-fed condition, excreted nitrogen comes mostly from digestion of excess protein or from normal turnover. Protein turnover is defined as the synthesis and degradation of protein. Under some conditions the body is either in negative or positive nitrogen balance. In **negative nitrogen balance** more nitrogen is excreted than ingested. This occurs in starvation and certain diseases. During starvation carbon chains of amino acids from proteins are needed for gluconeogenesis; ammonia released from amino acids is excreted mostly as urea and is not reincorporated into protein. A diet deficient in an essential amino acid also leads to a negative nitrogen balance, since body proteins are degraded to provide the deficient essential amino acid, and the

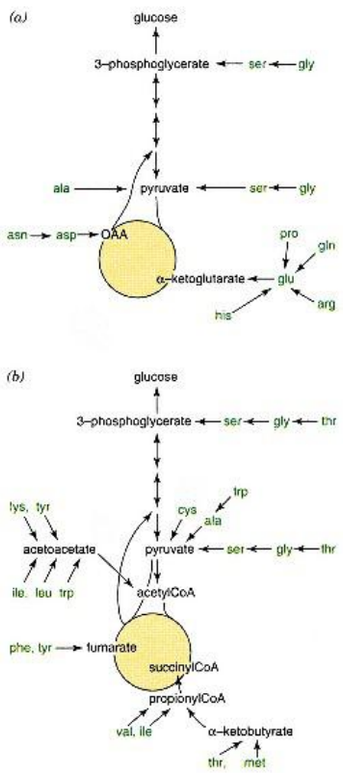


Figure 11.2
Metabolic fate of
(a) nonessential amino acids;
(b) essential amino acids plus cysteine and tyrosine.

other 19 amino acids liberated are metabolized. Negative nitrogen balance may also exist in senescence. **Positive nitrogen balance** occurs in growing children, who are increasing their body weight and incorporating more amino acids into proteins than they break down. Cysteine and arginine are not essential in adults but are essential in children because they are synthesized from methionine and ornithine. These amino acids are readily available in adults but limited in children because of their greater use of all amino acids. Positive nitrogen balance also occurs in pregnancy and during refeeding after starvation.

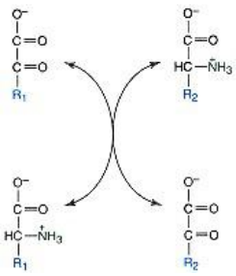


Figure 11.3
Aminotransferase reaction.

Amino Groups Are Transferred from One Amino Acid to Form Another

Most amino acids used by the body to synthesize protein or as precursors for amino acid derivatives are obtained from the diet or from protein turnover. When necessary, nonessential amino acids are synthesized from α-keto acid precursors via transfer of a preexisting amino group from another amino acid by **aminotransferases,** also called **transaminases** (Figure 11.3). Transfer of amino groups also occurs during degradation of amino acids. Figure 11.4 shows how the amino group of alanine is transferred to α-ketoglutarate to form glutamate. In this reaction the pyruvate produced provides carbons for gluconeogenesis or for energy production via the TCA cycle. This reaction is necessary since ammonia cannot enter the urea cycle directly from alanine but can be donated by glutamate. The opposite reaction would occur if there were a need for alanine for protein synthesis that was not being met by dietary intake or protein turnover. Transamination involving essential amino acids is normally unidirectional since the body cannot synthesize the equivalent α-keto acid. Figure 11.5 shows transamination of valine, an essential amino acid. The resulting α-ketoisovalerate is further metabolized to succinyl CoA as discussed on page 477. Transamination is the most common reaction involving free amino acids, and only threonine and lysine do not participate in an aminotransferase reaction. An obligate amino and α-keto acid pair in all of these reactions is glutamate and α-ketoglutarate. This means that amino group transfer between alanine and aspartate would have to occur via coupled reactions, with a glutamate intermediate (Figure 11.6). The equilibrium constant for aminotransferases is close to one so that the reactions are freely reversible. When nitrogen excretion is impaired and **hyperammonemia** occurs, as in liver failure, amino acids, including the essential amino acids, can be replaced in the diet by α-keto acid analogs, with the exception of threonine and lysine as mentioned above. The α-keto acids are transaminated by aminotransferases to produce the different amino acids. Figure 11.5 shows valine formation after administration of α-ketoisovalerate as therapy for hyperammonemia.

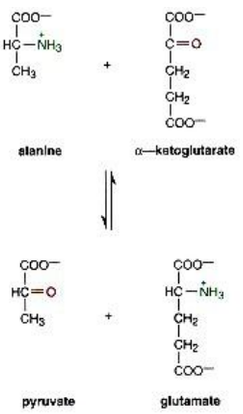


Figure 11.4
Glutamate–pyruvate aminotransferase reaction.

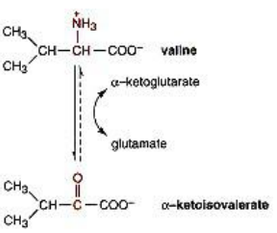


Figure 11.5
Transamination of valine.
Valine can be formed from -ketoisovalerate only when this compound is administered therapeutically.

Tissue distribution of some of the aminotransferase family is used diagnostically by measuring the release of a specific enzyme during tissue damage; for instance, the presence of glutamate oxaloacetate aminotransferase in plasma is a sign of liver damage (see p. 166).

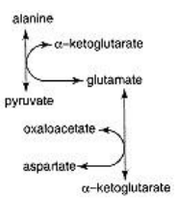


Figure 11.6
A coupled transamination reaction.

Pyridoxal Phosphate Is Cofactor for Aminotransferases

Transfer of amino groups occurs via enzyme-associated intermediates derived from **pyridoxal phosphate,** the functional form of vitamin B_6 (Figure 11.7). The active site of the "resting" aminotransferase contains pyridoxal phosphate covalently attached to a ε-amino group of a lysine residue that forms part of the amino acid chain of the transferase (Figure 11.8) The complex is further stabilized by ionic and hydrophobic interactions. The linkage, –CH=N–, is called a **Schiff base.** The carbon originates in the aldehyde group of pyridoxal phosphate, and the nitrogen is donated by the lysine residue. When a substrate amino acid, ready to be metabolized, approaches the active site, its amino group displaces the lysine ε-amino group and a Schiff base linkage is formed with the amino group of the amino acid substrate (Figure 11.9). At this point the pyridoxal phosphate-derived molecule is no longer covalently attached to the enzyme but is held in the active site only by ionic and hydrophobic interactions between it and the protein. The Schiff base linkage involving the amino acid substrate is in tautomeric equilibrium between an aldimine, –CH=N–CHR_2, and a ketimine, –CH_2–N=CR. Hydrolysis of the ketimine liberates an α-keto acid, leaving the amino group as part of the pyridoxamine structure. A reversal of the process is now possible; an α-keto acid reacts with the amine group, the double bond is shifted, and then hydrolysis liberates an amino acid. Pyridoxal phosphate now reforms its Schiff base with the "resting" enzyme (Figure 11.8). Most pyridoxal phosphate-requiring reactions involve transamination, but the ability of the Schiff base to transfer electrons between different atoms allows this cofactor to participate

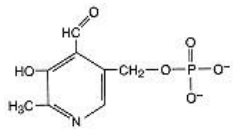


Figure 11.7
Pyridoxal phosphate.

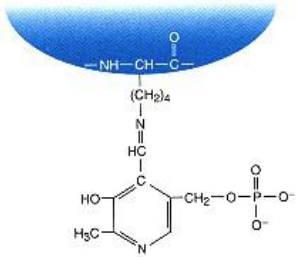


Figure 11.8
Pyridoxal phosphate in aldimine linkage to protein lysine residue.

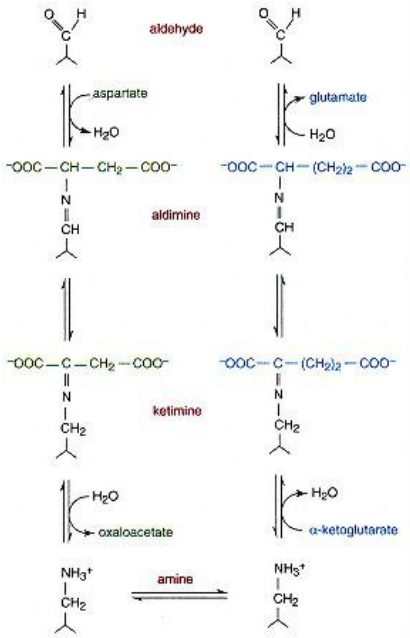


Figure 11.9
Different forms of pyridoxal phosphate during a transamination reaction.

when other groups, such as carboxyls, are to be eliminated. Figure 11.10 shows the reaction of a **pyridoxal-dependent decarboxylase** and an **α-, β-elimination.**

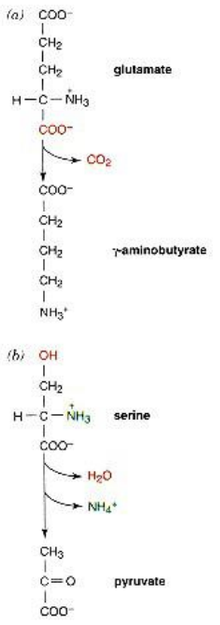


Figure 11.10
Glutamate decarboxylase and serine dehydratase are pyridoxal phosphate-dependent reactions.

The effective concentration of vitamin B_6 in the body may be decreased by administration of certain drugs, such as the antitubercular, isoniazid, which forms a Schiff base with pyridoxal making it unavailable for catalysis.

Glutamate Dehydrogenase Incorporates and Produces Ammonia

In the liver ammonia is incorporated as the amino group of nitrogen by **glutamate dehydrogenase** (Figure 11.11). This enzyme also catalyzes the reverse reaction. Glutamate always serves as one of the amino acids in transaminations and is thus the "gateway" between free ammonia and amino groups of most amino acids (Figure 11.12). NADPH is used in the synthetic reaction, whereas NAD^+ is used in liberation of ammonia, a degradative reaction. The enzyme is involved in the production of ammonia from amino acids when these are needed as glucose precursors or for energy. Formation of NADH during the oxidative deamination reaction is a welcome bonus, since it can be reoxidized by the respiratory chain with formation of ATP. The reaction as shown is readily reversible in the test tube but it is likely that *in vivo* it occurs more frequently in the direction of ammonia formation. The concentration of ammonia needed for the reaction to produce glutamate is toxic and under normal conditions would rarely be attained except in the perivenous region of the liver. A major source of ammonia is **bacterial metabolism** in the intestine, the released ammonia being absorbed and transported to the liver. Glutamate dehydrogenase incorporates this ammonia, as well as that produced locally, into glutamate. The enzyme's dominant role in ammonia removal is emphasized by its location inside liver mitochondria, where the initial reactions of the urea cycle occur.

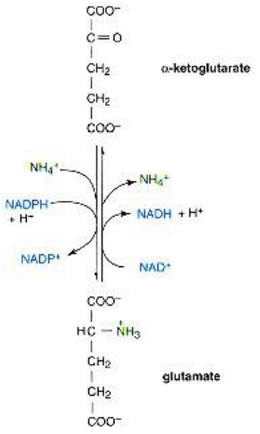


Figure 11.11
Glutamate dehydrogenase reaction.

Glutamate dehydrogenase is regulated allosterically by purine nucleotides. When there is need for oxidation of amino acids for energy, the activity is increased in the direction of glutamate degradation by ADP and GDP, which are indicative of a low cellular energy level. GTP and ATP, indicative of an ample energy level, are allosteric activators in the direction of glutamate synthesis (Figure 11.13).

Free Ammonia Is Incorporated into and Produced from Glutamine

Free ammonia is toxic and is preferentially transported in the blood in the form of amino or amide groups. Fifty percent of circulating amino acids are **glutamine,** an ammonia transporter. The amide group of glutamine is important as a nitrogen donor for several classes of molecules, including purine bases, and the amino group of cytosine. Glutamate and ammonia are substrates for **glutamine synthetase** (Figure 11.14). ATP is needed for activation of the α-carboxyl group to make the reaction energetically favorable.

Removal of the amide group is catalyzed by **glutaminase** (Figure 11.15). There are tissue-specific isozymes. Mitochondrial glutaminase I of kidney and

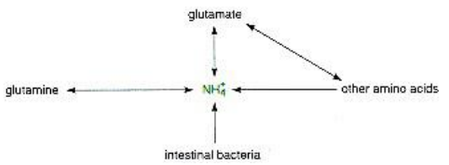


Figure 11.12
Role of glutamate in amino acid synthesis, degradation, and interconversion.

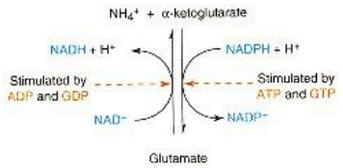


Figure 11.13
Allosteric regulation of glutamate dehydrogenase.

liver requires phosphate for activity. Liver contains glutamine synthetase and glutaminase but is neither a net consumer nor a net producer of glutamine. The two enzymes are confined to parenchymal cells in different segments of the liver. The **periportal region** is in contact with blood coming from skeletal muscle and contains glutaminase (and the urea cycle enzymes). The **perivenous area** represents 5% of parenchymal cells; blood from it flows to the kidney and cells in this area contain glutamine synthetase. This **"intercellular glutamine cycle"** (Figure 11.16) can be considered a mechanism for scavenging ammonia that has not been incorporated into urea. The enzymes of urea synthesis are found in the same periportal cells as glutaminase, whereas the uptake of glutamate and α-ketoglutarate for glutamine synthesis predominates in the perivenous region. The glutamine cycle makes it possible to control flux of ammonia either to urea or to glutamine and thence to excretion of ammonia by the kidney under different pH conditions (see p. 1045).

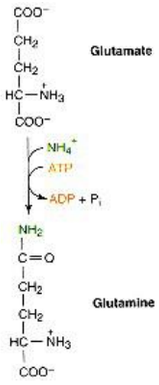


Figure 11.14
Reaction catalyzed by glutamine synthetase.

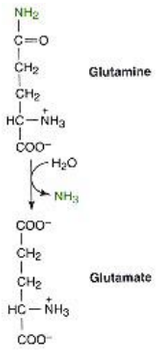


Figure 11.15
Reaction catalyzed by glutaminase.

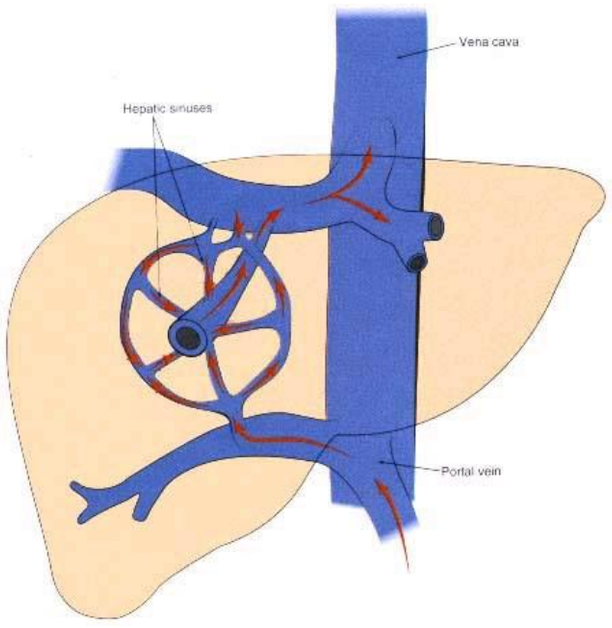


Figure 11.16
Intercellular glutamine cycle.
Periportal cells surround incoming blood vessels, and perivenous cells surround outgoing blood vessels.

Amide Group of Asparagine Is Derived from Glutamine

The amide group of **asparagine** comes from that of glutamine (Figure 11.17), and not from free ammonia, as in the synthesis of glutamine. ATP is needed to activate the receptor α-carboxyl group. Asparagine is readily synthesized in most cells, but some leukemic cells seem to have lost this ability. A therapeutic approach that has been tried for patients with **asparagine synthetase**-deficient tumors is treatment with exogenous **asparaginase** to hydrolyze the blood-borne asparagine on which these cells rely (Figure 11.18). Normal cells synthesize and degrade asparagine.

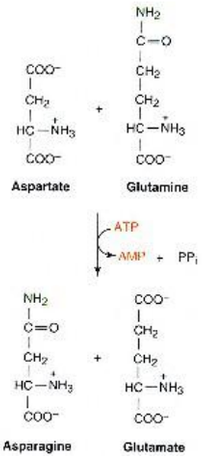


Figure 11.17
Synthesis of asparagine.

Amino Acid Oxidases Remove Amino Groups

Many amino acids are substrates for **L-amino acid oxidase** (Figure 11.19). The significance of this reaction in the metabolism of amino acids is uncertain, but appears to be small. The enzyme contains flavin mononucleotide (FMN) and produces hydrogen peroxide. After the hydrogen peroxide is reduced to water, the final products are an α-keto acid, ammonia, and water, the same products as those of the glutamate dehydrogenase reaction. In the amino acid oxidase reaction, unlike the reaction catalyzed by glutamate dehydrogenase, there is no concomitant production of NADH, and therefore no production of ATP.

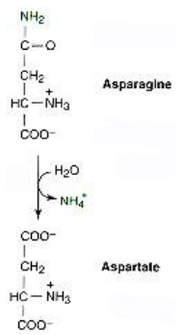


Figure 11.18
Reaction catalyzed by asparaginase.

A **D-amino acid oxidase** also occurs in human cells. Very little of the D-amino acid isomer is found in humans and the role of D-amino acid oxidase may be in degradation of D-amino acids derived from intestinal bacteria.

11.3— Transport of Nitrogen to Liver and Kidney

Protein Is Degraded on a Regular Basis

Whole cells die on a regular and planned basis, and their component molecules are metabolized. This **"planned cell death"** is called **apoptosis.** Individual proteins also undergo regular turnover under normal conditions. Even though the reactions involved in intracellular protein degradation have been identified, an understanding of the regulation of protein degradation is in its infancy. The half-life of a protein can be an hour or less, such as for ornithine decarboxylase, phosphokinase C, and insulin, several months for hemoglobin and histones,

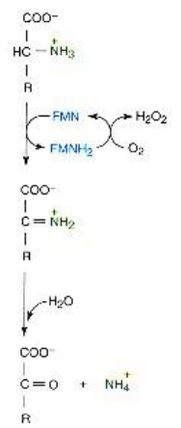


Figure 11.19
Reaction of L-amino acid oxidase, a flavoprotein.

or the life of the organism for the crystallins of the lens. The majority, however, turn over every few days. Selection of a particular protein molecule for degradation is not well understood but may, in many cases, occur by "marking" with covalently bound molecules of an oligopeptide, termed **ubiquitin.** Ubiquitin contains 76 amino acid residues and is attached via its C-terminal glycine residue to the terminal amino group and to lysine residues in the protein to be marked for degradation. This is a nonlysosomal, ATP-dependent process and requires a complex of three enzymes known as ubiquitin protein ligase. Recently, ubiquitination and protein degradation have been found to regulate the cell cycle by influencing the availability of proteins required in the S and G_1 phases. Other protein degradation occurs in the lysosomes, or extralysosomally by calcium-dependent enzymes.

Amino Acids Are Transported from Muscle after Proteolysis

The majority of protein, and consequently of amino acids, is in skeletal muscle. Under conditions of energy need, this protein is degraded and amino groups from the amino acids are transferred to glutamine and alanine and transported to liver or kidney. Urea is produced in liver and ammonia (from glutamine) in kidney (Figure 11.20). Carbon skeletons are either used for energy or transported to the liver for gluconeogenesis. Muscle protein responds to conditions such as starvation, trauma, burns, and septicemia, by undergoing massive degradation. Of the amino acids released, most important as a source of fuel are **branched-chain amino acids** (valine, leucine, and isoleucine). The first step in their degradation is transamination, which occurs almost exclusively in muscle. Protein is, of course, degraded throughout the body, but muscle is by far the greatest source of free amino acids for metabolism.

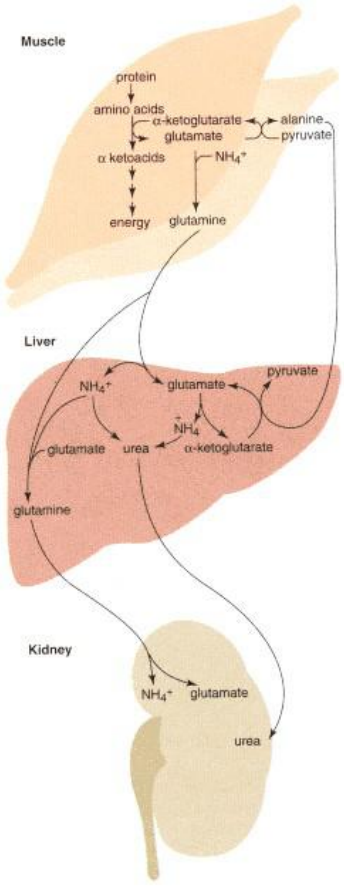


Figure 11.20
Major pathways of interorgan nitrogen transport following muscle proteolysis.

Ammonia Is Released in Liver and Kidney

The main destination of glutamine and alanine in the blood is the liver (see Figure 11.20). Here ammonia is released by alanine aminotransferase, glutaminase, and glutamate dehydrogenase. Glutamate dehydrogenase not only releases ammonia but also produces NADH and α-ketoglutarate, a glucogenic intermediate. Under conditions of energy need these products are very beneficial. Many tumors produce a condition called **cachexia,** characterized by wasting of muscle. This is caused not at the level of regulation of the rate of muscle protein breakdown, but rather by an increase in the rate at which liver removes amino acids from plasma, which, in turn, has a potentiating effect on muscle proteolysis. When circulating glucagon concentration is high (a signal that carbon is required by the liver for gluconeogenesis), it also potentiates amino acid metabolism by stimulating amino acid uptake by the liver.

Some glutamine and alanine is taken up by the kidney. Ammonia is released by the same enzymes that are active in liver, protonated to ammonium ion and excreted. When acidosis occurs the body shunts glutamine from liver to kidney to **conserve bicarbonate,** since formation of urea, the major mechanism for removal of NH_4^+, requires bicarbonate. To avoid use and excretion of this anion as urea during acidosis, uptake of glutamine by liver is suppressed, and more is transported to kidney for excretion as ammonium ion (see p. 1045).

11.4— Urea Cycle

Nitrogens of Urea Come from Ammonia and Aspartate

The **urea cycle** and the tricarboxylic acid (TCA) cycle were discovered by Sir Hans Krebs and co-workers. In fact, the urea cycle was described before the

TCA cycle. In land-dwelling mammals, the urea cycle is the mechanism of choice for nitrogen excretion. The two nitrogens in each urea molecule (Figure 11.21) are derived from two sources, free ammonia and the amino group of aspartate. The cycle starts and finishes with **ornithine.** Unlike the TCA cycle, where carbons of oxaloacetate at the start are different from those at the end, the carbons in the final ornithine are the same carbons with which the molecule started.

Ammonia (first nitrogen for urea) enters the cycle after condensation with bicarbonate to form **carbamoyl phosphate** (Figure 11.22), which reacts with ornithine to form **citrulline.** Aspartate (the donor of the second urea nitrogen) and citrulline react to form **argininosuccinate,** which is then cleaved to arginine and fumarate. Arginine is hydrolyzed to urea and ornithine is regenerated. Urea is then transported to the kidney and excreted in urine. The cycle requires 4 ATPs to excrete each two nitrogen atoms. It is therefore more energy efficient to incorporate ammonia into amino acids than to excrete it. The major regulatory step is the initial synthesis of carbamoyl phosphate, and the cycle is also regulated by induction of the enzymes involved.

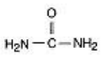


Figure 11.21
Urea

Synthesis of Urea Requires Five Enzymes

Carbamoyl phosphate synthetase I is technically not a part of the urea cycle, although it is essential for urea synthesis. Free ammonium ion and bicarbonate are condensed, at the expense of 2 ATPs, to form carbamoyl phosphate. One ATP activates bicarbonate, and the other donates the phosphate group of carbamoyl phosphate. Carbamoyl phosphate synthetase I occurs in the mitochondrial matrix, uses ammonia as nitrogen donor, and is absolutely dependent on ***N*-acetylglutamate** for activity (Figure 11.23). Another enzyme with similar activity, **carbamoyl phosphate synthase II,** is cytosolic, uses the amide group of glutamine, and is not affected by *N*-acetylglutamate. It participates in pyrimidine biosynthesis (see p. 505).

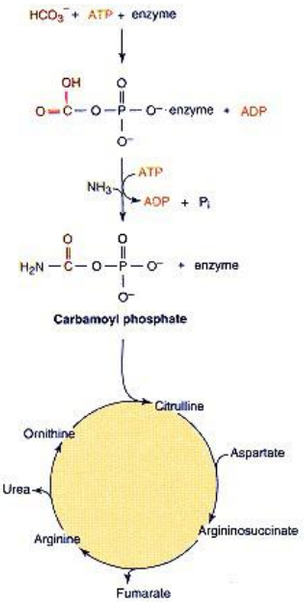


Figure 11.22
Synthesis of carbamoyl phosphate and entry into urea cycle.

Formation of citrulline is catalyzed by **ornithine transcarbamoylase** (11.24) in the mitochondrial matrix. Citrulline is transported from the mitochondria, and other reactions of the urea cycle occur in the cytosol. Argininosuccinate production by **argininosuccinate synthetase** requires hydrolysis of ATP to AMP and PP_i, the equivalent of hydrolysis of two molecules of ATP. Cleavage of argininosuccinate by **argininosuccinate lyase** produces fumarate and arginine. Arginine is cleaved by **arginase** to ornithine and urea. Ornithine reenters the mitochondrion for another turn of the cycle. The inner mitochondrial membrane contains a **citrulline/ornithine exchange transporter.**

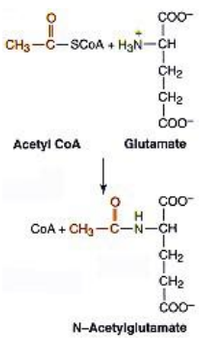


Figure 11.23
Reaction catalyzed by *N*-acetylglutamate synthetase.

Synthesis of additional ornithine from glutamate for the cycle will be described later. Since arginine is produced from carbons and nitrogens of ornithine, ammonia, and aspartate, it is a nonessential amino acid. In growing children, however, where there is net incorporation of nitrogen into the body, *de novo* synthesis of arginine is inadequate and the amino acid becomes essential.

Carbons from aspartate, released as fumarate, may enter the mitochondrion and be metabolized to oxaloacetate by the TCA enzymes fumarase and malate dehydrogenase, transaminated, and then theoretically enter another turn of the urea cycle as aspartate. Most oxaloacetate (about two-thirds) from fumarate is metabolized via phosphoenolpyruvate to glucose (Figure 11.25). The amount of fumarate used to form ATP is approximately equal to that required for the urea cycle and gluconeogenesis, meaning that the liver itself gains no net energy in the process of amino acid metabolism.

Since humans cannot metabolize urea it is transported to the kidney for filtration and excretion. Any urea that enters the intestinal tract is cleaved by the intestinal **urease**-containing bacteria, the resulting ammonia being absorbed and used by the liver.

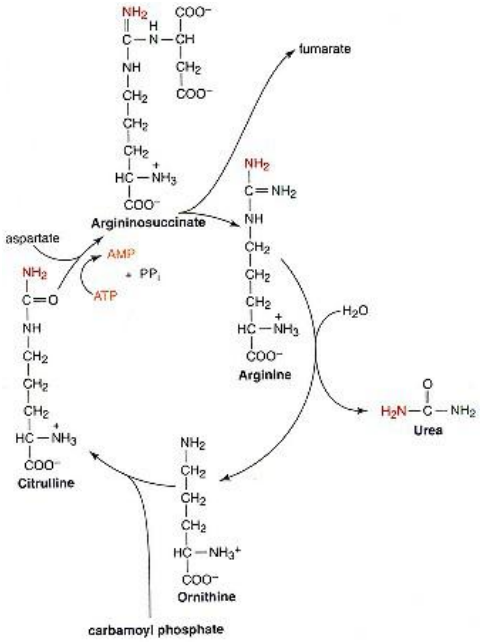


Figure 11.24
Urea cycle.

Urea Synthesis Is Regulated by an Allosteric Effector and Enzyme Induction

Carbamoyl phosphate synthetase has a mandatory requirement for the allosteric activator ***N*-acetylglutamate** (see Figure 11.23). This compound is synthesized from glutamate and acetyl CoA by ***N*-acetylglutamate synthetase,** which is activated by arginine. Acetyl CoA, glutamate, and arginine are needed to supply intermediates or energy for the urea cycle, and the presence of *N*-acetylglutamate indicates that they are all available. Tight regulation is desirable for a pathway that controls the plasma level of potentially toxic ammonia and that is also highly energy dependent.

Induction of urea cycle enzymes occurs (10- to 20-fold) when delivery of ammonia or amino acids to liver rises. Concentration of cycle intermediates also plays a role in its regulation through mass action. A high-protein diet (net excess amino acids) and starvation (need to metabolize excess nitrogen in order to provide carbons for energy production) result in induction of urea cycle enzymes.

Metabolic Disorders of Urea Synthesis Have Serious Results

The urea cycle is the major mechanism for the elimination of ammonia, a very toxic substance. Metabolic disorders that arise from abnormal function of enzymes of urea synthesis are potentially fatal and cause coma when ammonia concentrations become high. Loss of consciousness may be a consequence of ATP depletion. The major source of ATP is oxidative phosphorylation, which

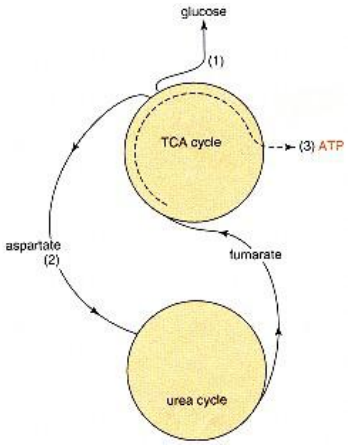


Figure 11.25
Fumarate from the urea cycle is a source of glucose (1), aspartate (2), or energy (3).

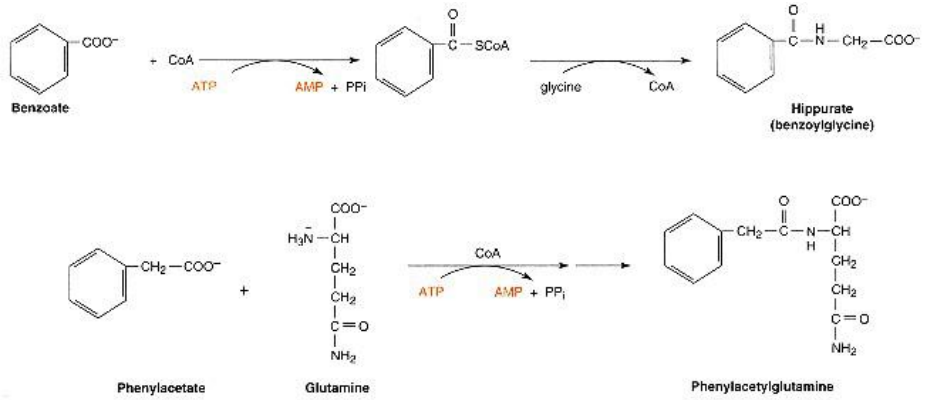


Figure 11.26
Detoxification reactions as alternatives to the urea cycle.

is linked to transfer of electrons from the TCA cycle down the electron transport chain. A high concentration of ammonia sequesters α-ketoglutarate to form glutamate, thus depleting the TCA cycle of important intermediates and reducing ATP production.

Patients with a deficiency in each of the urea cycle enzymes have been found. Therapy for these deficiencies has a threefold basis: (1) to limit protein intake and potential buildup of ammonia, (2) to remove excess ammonia, and (3) to replace any intermediates missing from the urea cycle. The first is accomplished by limiting ingestion of amino acids, replacing them if necessary with the equivalent α-keto acids to be transaminated *in vivo*. The bacterial source of ammonia in the intestines can be decreased by a compound that acidifies the colon, such as levulose, a poorly absorbed synthetic disaccharide that is metabolized by colonic bacteria to acidic products. This promotes the excretion of ammonia in feces as protonated ammonium ions. Antibiotics can also be administered to kill ammonia-producing bacteria. The second is achieved by compounds that bind covalently to amino acids and produce nitrogen-containing molecules that are excreted in urine. Figure 11.26 shows condensation of benzoate and glycine to form **hippurate,** and of phenylacetate and glutamine to form **phenylacetylglutamine.** Phenylacetate is extremely unpalatable and is given as the precursor sodium phenylbutyrate. Both reactions require energy for activation of the carboxyl groups by addition of CoA.

Clinical Correlations 11.1 and 11.2 give examples of therapy for specific enzyme deficiencies, which often includes administration of urea cycle intermediates.

CLINICAL CORRELATION 11.1

Carbamoyl Phosphate Synthetase and *N*-Acetylglutamate Synthetase Deficiencies

Hyperammonemia has been observed in infants with 0–50% of the normal level of carbamoyl synthetase activity in their livers. In addition to the treatments described in the text, these infants have been treated with arginine, on the hypothesis that activation of *N*-acetylglutamate synthetase by arginine would stimulate the residual carbamoyl phosphate synthetase. This enzyme deficiency generally leads to mental retardation. A case of *N*-acetylglutamate synthetase deficiency has been described and treated successfully by administering carbamoyl glutamate, an analog of *N*-acetylglutamate, that is also able to activate carbamoyl phosphate synthetase.

11.5— Synthesis and Degradation of Individual Amino Acids

Other aspects of metabolism of glutamate, glutamine, aspartate, asparagine, pyruvate, and arginine, the amino acids whose basic metabolism has already been covered, are now discussed. Synthesis of other nonessential amino acids and degradation of all the amino acids will be covered, as well as synthesis of physiologically important amino acid derivatives.

CLINICAL CORRELATION 11.2

Deficiencies of Urea Cycle Enzymes

Ornithine Transcarbamoylase Deficiency

The most common deficiency involving urea cycle enzymes is lack of ornithine transcarbamoylase. Mental retardation and death often result, but the occasional finding of normal development in treated patients suggests that the mental retardation usually associated is caused by the excess ammonia before adequate therapy. The gene for ornithine transcarbamoylase is on the X chromosome, and males generally are more seriously affected than heterozygotic females. In addition to ammonia and amino acids appearing in the blood in increased amounts, orotic acid also increases, presumably because carbamoyl phosphate that cannot be used to form citrulline diffuses into the cytosol, where it condenses with aspartate, ultimately forming orotate (Chapter 12).

Argininosuccinate Synthetase and Lyase Deficiency

The inability to condense citrulline with aspartate results in accumulation of citrulline in blood and excretion in urine (citrullinemia). Therapy for this normally benign disease requires specific supplementation with arginine for protein synthesis and for formation of creatine. Impaired ability to split argininosuccinate to form arginine resembles argininosuccinate synthetase deficiency in that the substrate, in this case argininosuccinate, is excreted in large amounts. The severity of symptoms in this disease varies greatly so that it is hard to evaluate the effect of therapy, which includes dietary supplementation with arginine.

Arginase Deficiency

Arginase deficiency is rare but causes many abnormalities in development and function of the central nervous system. Arginine accumulates and is excreted. Precursors of arginine and products of arginine metabolism may also be excreted. Unexpectedly, some urea is also excreted; this has been attributed to a second type of arginase found in the kidney. A diet including essential amino acids but excluding arginine has been used effectively.

Brusilow, S. W., Danney, M., Waber, L. J., Batshaw, M., et al. Treatment of episodic hyperammonemia in children with inborn errors of urea synthesis. *N. Engl. J. Med.* 310:1630, 1984.

Glutamate Is a Precursor of Glutathione and γ-Aminobutyrate

Glutamate is a component of **glutathione,** which is discussed at the end of this chapter (see p. 484). It is also a precursor for **γ-aminobutyric acid,** a neurotransmitter (Figure 11.27), which will be discussed in Chapter 21, and of proline and ornithine, described below.

Arginine Is Also Synthesized in Intestines

Production of arginine for protein synthesis, rather than as an intermediate in the urea cycle, occurs in kidney, which lacks arginase. The major site of synthesis of citrulline to be used as an arginine precursor is intestinal mucosa, which has all necessary enzymes to convert glutamate (via ornithine as described below) to citrulline, which is then transported to the kidney to produce arginine. Arginine is also a precursor for **nitric oxide** (Chapter 22); in brain, **agmatine,** a compound that may have antihypertensive properties, is an arginine derivative (Figure 11.28).

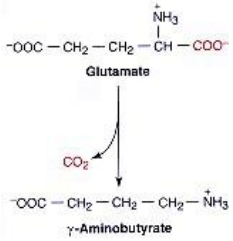


Figure 11.27
Synthesis of -aminobutyric acid.

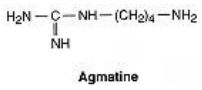


Figure 11.28
Agmatine.

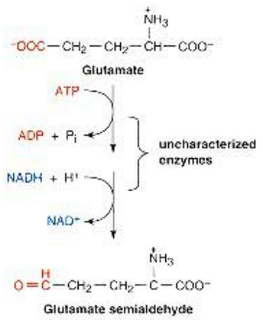


Figure 11.29
Synthesis of glutamic semialdehyde.

Ornithine and Proline

Ornithine, the precursor of citrulline and arginine, and **proline** are both synthesized from glutamate and degraded, by a slightly different pathway, to glutamate. Synthesis of these two nonessential amino acids starts from α-ketoglutarate with a shared reaction that uses ATP and NADH (Figure 11.29) and forms **glutamic semialdehyde.** This spontaneously will cyclize to form a Schiff base between the aldehyde and amino groups, which is then reduced by NADPH to proline. Glutamic semialdehyde can undergo transamination of the aldehyde group, preventing cyclization and producing ornithine (Figure 11.30).

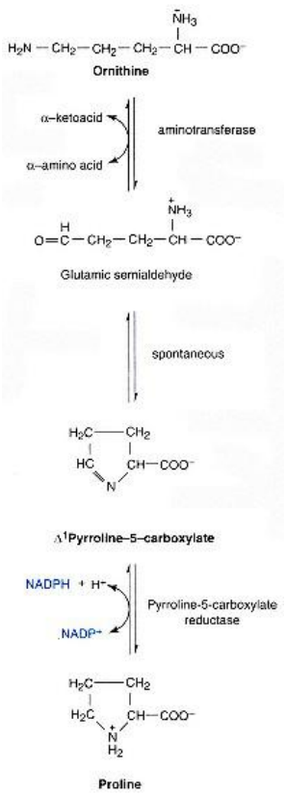


Figure 11.30
Synthesis of ornithine and proline from glutamic semialdehyde, a shared intermediate.

Proline is converted back to the Schiff base intermediate, Δ^1-pyrroline 5-carboxylate, which is in equilibrium with glutamic semialdehyde. The transaminase reaction in the ornithine synthetic pathway is freely reversible and forms glutamic semialdehyde from ornithine (Figure 11.30). Proline residues can be hydroxylated after incorporation into a protein. This posttranslational modification forms **3-** or **4-hydroxyproline** (Figure 11.31). When these are released by protein degradation and metabolized they produce glyoxalate and pyruvate, and 4-hydroxy-2-ketoglutarate, respectively.

Ornithine is a precursor of putrescine, the foundation molecule of polyamines, highly cationic molecules that interact with DNA. Ornithine decarboxylase catalyzes this reaction (Figure 11.32). It is regulated by phosphorylation at several sites, presumably in response to specific hormones, growth factors, or cell cycle regulatory signals. It can also be induced, and this is often the first easily measurable sign that cell division is imminent, since polyamines must be synthesized before mitosis can occur. Other common **polyamines** are **spermidine** and **spermine** (see Figure 11.59), which are synthesized from putrescine by addition of propylamine, a product of methionine metabolism (see p. 472).

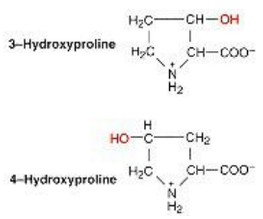


Figure 11.31
Hydroxyprolines.

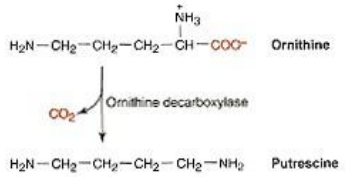


Figure 11.32
Decarboxylation of ornithine to putrescine.
Structures of spermidine and
spermine are shown in Figure 11.59.

Serine and Glycine

Serine is synthesized *de novo* starting with 3-phosphoglycerate from the glycolytic pathway. When serine provides gluconeogenic intermediates this is also the product of its degradation, although the enzymes and intermediates in the two pathways are different. Synthesis of serine uses phosphorylated intermediates between 3-phosphoglycerate and serine (Figure 11.33*a*), loss of the phosphate being the last step. From serine to 3-phosphoglycerate the intermediates are unphosphorylated, the addition of a phosphate being the last step. The enzymes that catalyze the reactions in the two pathways are not the same (Figure 11.33*b*). Another reaction for entry of serine into intermediary metabolism is via **serine dehydratase,** which forms pyruvate with loss of the amino group as NH_4^+ (Figure 11.34). The same enzyme catalyzes a similar reaction with threonine (see p. 463).

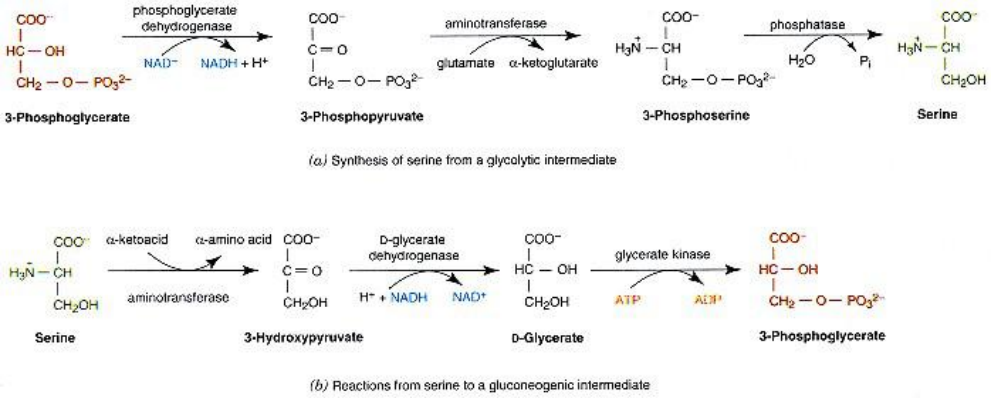


Figure 11.33
Pathways for
(a) synthesis of serine and
(b) metabolism of serine for gluconeogenesis.

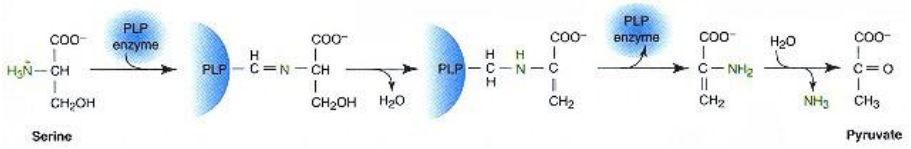


Figure 11.34
Reaction of serine dehydratase requires pyridoxal phosphate.

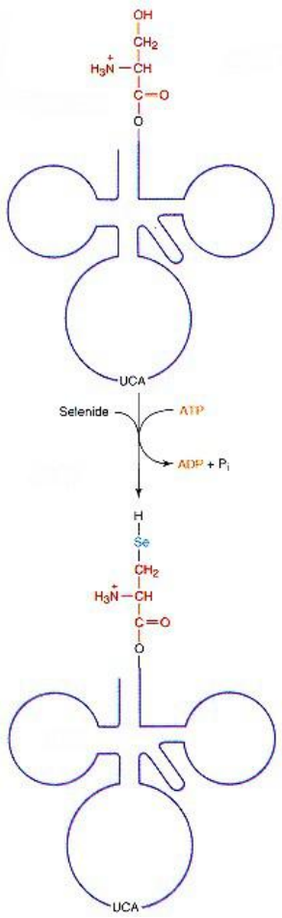


Figure 11.35
Formation of selenocysteinyl tRNA from seryl tRNA is via a phosphoseryl tRNA intermediate.

Serine is precursor of an unusual but important amino acid. Certain proteins, notably **glutathione peroxidase,** contain **selenocysteine** (Figure 11.35). In mRNA for selenoproteins the codon UGA, which generally serves as a termination codon, codes for selenocysteine. This amino acid is formed from serine after formation of the seryl–tRNA complex (serine bound to a specific $tRNA^{Ser}$ with the anticodon to UGA).

Ethanolamine, choline, and **betaine** (Figure 11.36) are derivatives of serine. Ethanolamine and choline are components of lipids, and betaine is a methyl donor in a minor pathway leading to methionine salvage (see p. 472). Serine is also a sulfhydryl group acceptor from homocysteine in cysteine synthesis (see p. 470).

In some enzymes a serine residue is modified to form a prosthetic group. In humans the only example described so far is ***S*-adenosylmethionine decarboxylase** (discussed below in relation to polyamine formation; (see p. 473). The prosthetic group formed is similar to pyruvate. *S*-Adenosylmethionine de-carboxylase is synthesized in precursor form that is then cleaved autocatalytically between a glutamate and a serine residue to form two polypeptides. During cleavage other reactions convert the new N-terminal serine of one of the resulting peptides into a pyruvate (Figure 11.37). The pyruvate functions in decarboxylation by forming a Schiff base with the amino group of *S*-adenosylmethionine.

Serine is converted reversibly to **glycine** in a reaction that requires pyridoxal phosphate and **tetrahydrofolate. N^5, N^{10}-methylenetetrahydrofolate** (N^5, N^{10}-THF) is produced (Figure 11.38). The demand for serine or glycine and the amount of N^5, N^{10}-THF available determine the direction of this reaction. Glycine is degraded to CO_2 and ammonia by a **glycine cleavage complex** (Figure 11.39; see Clin. Corr. 11.3). This reaction is reversible in the test tube, but not *in vivo*, as the K_m values for ammonia and N^5, N^{10}-THF are much higher than their respective physiological concentrations.

Glycine is the precursor of **glyoxalate,** which can be transaminated back to glycine or oxidized to **oxalate** (Figure 11.40). Excessive production of oxalate forms the insoluble calcium oxalate salt, which may lead to kidney stones. In Chapter 21 the role of glycine as a neurotransmitter is described.

Tetrahydrofolate Is a Cofactor in Many Reactions of Amino Acids

The **tetrahydrofolate** molecule is the reduced form of folic acid, one of the B vitamins, and often occurs as a polyglutamyl derivative (Figure 11.41). Tetrahydrofolate, involved in two reactions described earlier in the chapter, is a **one-carbon carrier** that facilitates interconversion of methenyl, formyl, formimino,

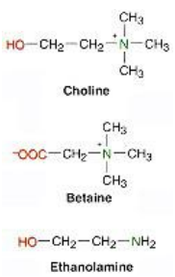


Figure 11.36
Choline and related compounds.

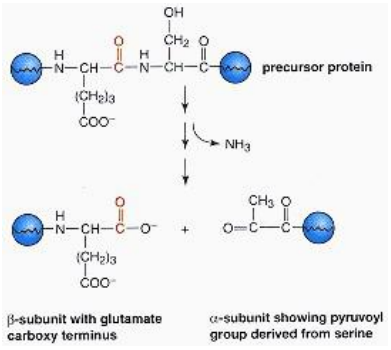


Figure 11.37
Formation of enzyme with covalently bound pyruvoyl prosthetic group.

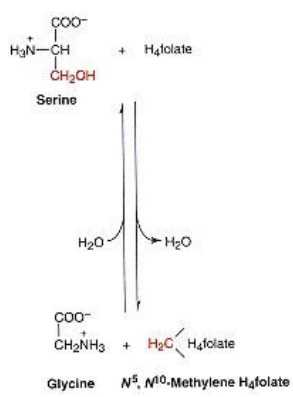


Figure 11.38
Serine hydroxymethyltransferase.

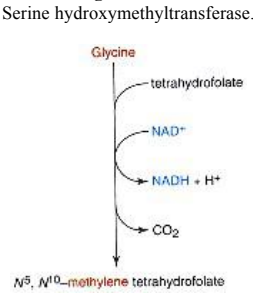


Figure 11.39
Glycine cleavage is pyridoxal phosphate dependent.

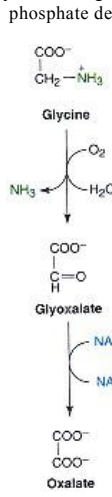


Figure 11.40
Oxidation of glycine.

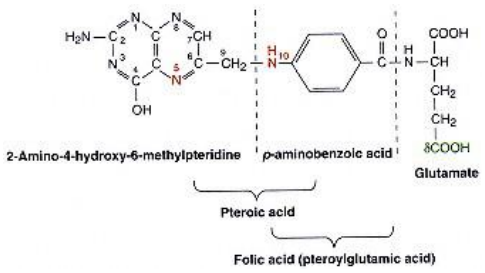


Figure 11.41
Components of folate.
Polyglutamate can be added to the -carboxyl group.

CLINICAL CORRELATION 11.3

Nonketotic Hyperglycinemia

Nonketotic hyperglycinemia is characterized by severe mental deficiency, and many patients do not survive infancy. The name of this very serious disease is meant to distinguish it from ketoacidosis in abnormalities of branched-chain amino acid metabolism in which, for unknown reasons, the glycine level in the blood is also elevated. Deficiency of glycine cleavage complex has been demonstrated in homogenates of liver from several patients, and isotopic studies *in vivo* have confirmed that this enzyme is not active in these patients. The glycine cleavage complex consists of four different protein subunits. Inherited abnormalities have been found in three of these. The severity of this disease suggests that glycine cleavage is of major importance in the catabolism of glycine. Glycine is a major inhibitory neurotransmitter, which probably explains some neurological complications of the disease. Vigorous measures to reduce the glycine levels fail to alter the course of the disease.

Nyhan, W. L. Metabolism of glycine in the normal individual and in patients with non-ketotic hyperglycinemia. *J. Inherit. Metab. Dis.* 5:105, 1982.

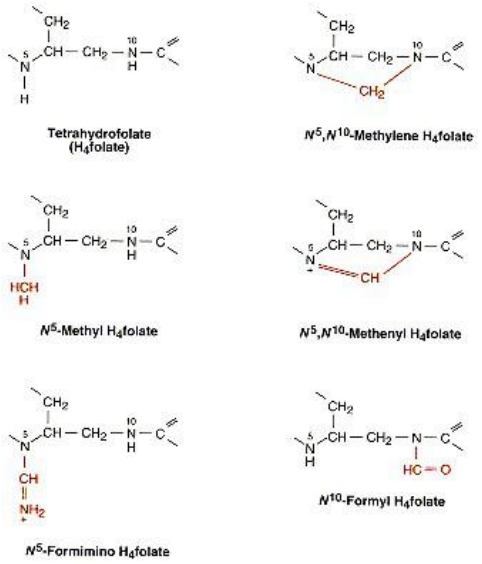


Figure 11.42
Active center of THF.
N^5 is the site of attachment of methyl groups; N^{10} is the site for formyl and formimino; methylene and methenyl groups form bridges between N^5 and N^{10}.

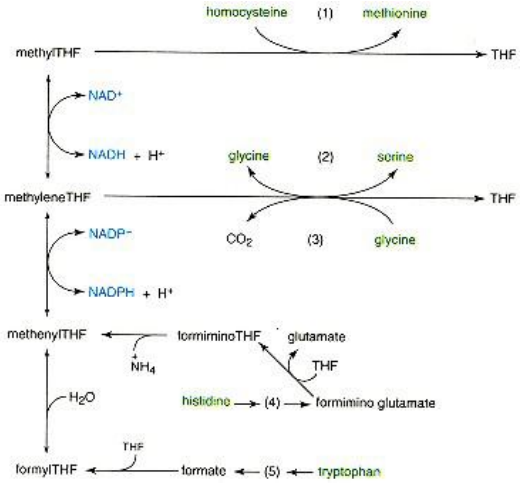


Figure 11.43
Inter-conversion of derivatized THF and roles in amino acid metabolism.
(1) Methionine salvage,
(2) serine hydroxymethyltransferase,
(3) glycine cleavage complex,
(4) histidine degradation, and
(5) tryptophan metabolism.

methylene, and methyl groups (Figure 11.42). This occurs at the expense of pyridine nucleotide reduction or oxidation and occurs while the carbon moiety is attached to THF (Figure 11.43). The most oxidized forms, formyl and methenyl, are bound to N^{10} of the pteridine ring, methylene forms a bridge between N^5 and N^{10}, and methyl is bound to N^5. The interconversions permit use of a carbon that is removed from a molecule in one oxidation state for addition in a different oxidation state to a different molecule (Fig. 11.42).

In reduction of the N^5,N^{10}-methylene bridge of tetrahydrofolate to a methyl group for transfer to the pyrimidine ring (Figure 11.44), a reaction found in **thymidylate synthesis** (Chapter 12), the reducing power comes not from pyridine nucleotide but from the pteridine ring itself. The resulting oxidized form of folate, dihydrofolate, has no physiological role and must be reduced back to tetrahydrofolate. The reaction is catalyzed by NADPH-dependent dihydrofolate reductase (see Clin. Corr. 11.4). The net result of the two reactions is oxidation of NADPH and reduction of the methylene bridge to a methyl group, analogous to the one-step reactions shown in Figure 11.43.

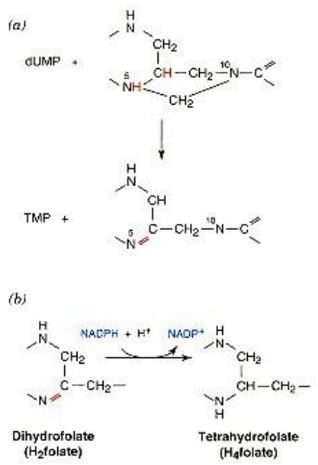


Figure 11.44
Reduction reactions involving THF.
(a) Reduction of methylene group on THF to a methyl group and transfer to dUMP to form TMP.
(b) Reduction of resulting dihydrofolate to tetrahydrofolate.

Threonine

Threonine is usually metabolized to lactate (Figure 11.45), but an intermediate in this pathway can undergo thiolysis with CoA to acetyl CoA and glycine. Thus the α-carbon atom of threonine can contribute to the one-carbon pool. In an alternative, but less common pathway, the enzyme described earlier in serine metabolism, **serine dehydratase** (see p. 459), converts threonine to α-ketobutyrate. A complex similar to pyruvate dehydrogenase metabolizes this to propionyl CoA.

Phenylalanine and Tyrosine

Tyrosine and **phenylalanine** are discussed together, since tyrosine results from hydroxylation of phenylalanine and is the first product in phenylalanine degradation. Because of this, tyrosine is not usually considered to be essential, whereas phenylalanine is. Three-quarters of ingested phenylalanine is metabo-

CLINICAL CORRELATION 11.4

Folic Acid Deficiency

The 100–200 mg of folic acid required daily by an average adult can theoretically be obtained easily from conventional Western diets. Deficiency of folic acid, however, is not uncommon. It may result from limited diets, especially when food is cooked at high temperatures for long periods, which destroys the vitamin. Intestinal diseases, notably celiac disease, are often characterized by folic acid deficiency caused by malabsorption. Inability to absorb folate is rare. Folate deficiency is usually seen only in newborns and produces symptoms of megaloblastic anemia. Of the few cases studied, some were responsive to large doses of oral folate but one required parenteral administration, suggesting a carrier-mediated process for absorption. Besides the anemia, mental and other central nervous system symptoms are seen in patients with folate deficiency, and all respond to continuous therapy although permanent damage appears to be caused by delayed or inadequate treatment. A classical experiment was carried out by a physician, apparently serving as his own experimental subject, to study the human requirements for folic acid. His diet consisted only of foods (boiled repeatedly to extract the water-soluble vitamins) to which vitamins (and minerals) were added, omitting folic acid. Symptoms attributable to folate deficiency did not appear for seven weeks, altered appearance of blood cells and formiminoglutamate excretion were seen only at 13 weeks, and serious symptoms (irritability, forgetfulness, and macrocytic anemia) appeared only after four months. Neurological symptoms were alleviated within two days after folic acid was added to the diet; the blood picture became normal more slowly. The occurrence of folic acid in essentially all natural foods makes deficiency difficult, and apparently a normal person accumulates more than adequate reserves of this vitamin. For pregnant women the situation is very different. Needs of the fetus for normal growth and development include constant, uninterrupted supplies of coenzymes (in addition to amino acids and other cell constituents). Recently, folate deficiency has been implicated in spina bifida.

Herbert, V. Experimental nutritional folate deficiency in man. *Trans. Assoc. Am. Physicians* 75:307, 1962.

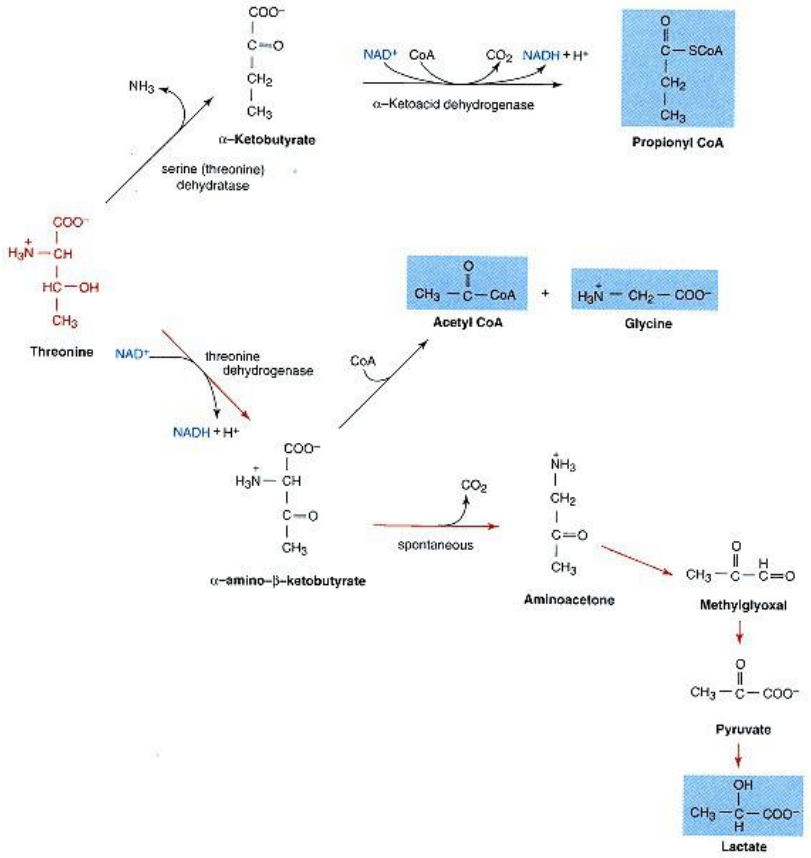


Figure 11.45
Outline of threonine metabolism.
Major pathway is in color.

lized to tyrosine. This is catalyzed by **phenylalanine hydroxylase** (Figures 11.46 and Clin. Corr. 11.5), which is **tetrahydrobiopterin dependent** (Figure 11.48). This reaction occurs only in the direction of tyrosine formation, and phenylalanine cannot be synthesized from tyrosine. **Biopterin,** unlike folic

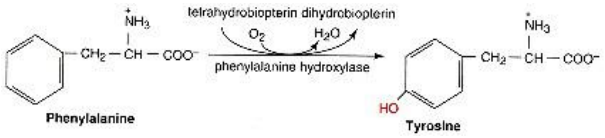


Figure 11.46
Phenylalanine hydroxylase.

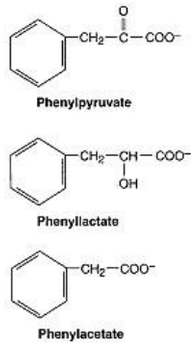


Figure 11.47
Minor products of phenylalanine metabolism.

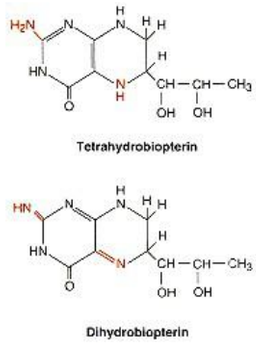


Figure 11.48
Biopterin.
The dihydro- (quinonoid) form is produced during oxidation of aromatic amino acids and is then reduced to the tetrahydro- form by a dehydrogenase using NADH and H^+.

acid, which it resembles in containing a pteridine ring, is not a vitamin. It is synthesized from GTP. (See Clin. Corr. 11.5.)

Tyrosine Is the First Intermediate in Phenylalanine Metabolism

The first step in metabolism of tyrosine is transamination by **tyrosine amino-transferase** to *p*-hydroxyphenylpyruvate (Figure 11.49). The enzyme is inducible, its synthesis being increased by glucocorticoids and dietary tyrosine. ***p*-Hydroxyphenylpyruvate oxidase** produces **homogentisic acid.** This complex reaction involves decarboxylation, oxidation, migration of the carbon side chain, and hydroxylation. Ascorbic acid is required for at least one of these activities, but all four are catalyzed by the one enzyme. The aromatic ring is next cleaved by an iron-containing enzyme, homogentisate oxidase, to maleyla-

CLINICAL CORRELATION 11.5

Phenylketonuria

Phenylketonuria (PKU) is the most common disease caused by a deficiency of an enzyme of amino acid metabolism. The name comes from the excretion of phenylpyruvic acid, a phenylketone, in the urine. Phenyllactate is also excreted (Figure 11.47), as is an oxidation product of phenylpyruvate, phenylacetate, which gives the urine a "mousey" odor. These three metabolites are found only in trace amounts in urine in the healthy person. The symptoms of mental retardation associated with this disease can be prevented by a phenylalanine-free diet. Routine screening is required by governments in many parts of the world. Classical PKU is an autosomal recessive deficiency of phenylalanine hydroxylase. Over 170 mutations in the gene have been reported. In some cases there are severe neurological symptoms and very low IQ. These are generally attributed to toxic effects of phenylalanine, possibly because of reduced transport and metabolism of other aromatic amino acids in the brain due to competition from the high phenylalanine concentration. Another characteristic is light color of skin and eyes, due to underpigmentation because of tyrosine deficiency. Conventional treatment is to feed affected infants a synthetic diet low in phenylalanine, but including tyrosine, for about four to five years, and impose dietary protein restriction for several more years or for life. About 3% of infants with high levels of phenylalanine have normal hydroxylase but are defective in either synthesis or reduction of biopterin. Biopterin deficiency can be treated by addition to the diet. Deficiency in dihydrobiopterin reductase is more serious. Since biopterin is also necessary for the synthesis of catecholamines and serotonin, which function as neurotransmitters, central nervous system functions are more seriously affected and treatment at this time includes administration of precursors of serotonin and catecholamines.

Brewster, T. G., Moskowitz, M. A., Kaufman, S., et al. Dihydrobiopterin reductase deficiency associated with severe neurologic disease and mild hyperphenylalanemia. *Pediatrics* 63:94, 1979; Kaufman, S. Regulation of the activity of hepatic phenylalanine hydroxylase. *Adv. Enzyme Regul.* 25:37, 1986; Scriver, C. R. and Clow, L. L. Phenylketonuria: epitome of human biochemical genetics. *N. Engl. J. Med.* 303:1336,1980; Woo, S. L. C. Molecular basis and population genetics of phenylketonuria. *Biochemistry* 28:1, 1989.

cetoacetate. This will isomerize from cis to trans to give fumarylacetoacetate, in a reaction catalyzed by maleylacetoacetate isomerase, an enzyme that seems to require glutathione for activity. Fumarylacetoacetate is then cleaved to fumarate and acetoacetate. Fumarate can be further utilized in the TCA cycle for energy or for gluconeogenesis. Acetoacetate can be used, as acetyl CoA, for lipid synthesis or energy. (See Clin. Corr. 11.6.)

Dopamine, Epinephrine, and Norepinephrine Are Derivatives of Tyrosine

Most tyrosine not incorporated into proteins is metabolized to acetoacetate and fumarate. Some is used as precursor of **catecholamines.** The eventual metabolic fate of tyrosine carbons is determined by the first step in each pathway. Catecholamine synthesis (Figure 11.50) starts with **tyrosine hydroxylase,** which, like phenylalanine and tryptophan hydroxylase, is dependent on tetrahydrobiopterin. All three are affected by biopterin deficiency or a defect in dihydrobiopterin reductase (see Figure 11.48). Tyrosine hydroxylase produces dihydroxyphenylalanine, also known as DOPA, dioxophenylalanine. **DOPA decarboxylase,** with pyridoxal phosphate as cofactor, forms **dopamine,** the active neurotransmitter, from DOPA. In the substantia nigra and some other parts of the brain, this is the last enzyme in this pathway (see Clin. Corr. 11.7). The adrenal medulla converts dopamine to **norepinephrine** and **epinephrine**

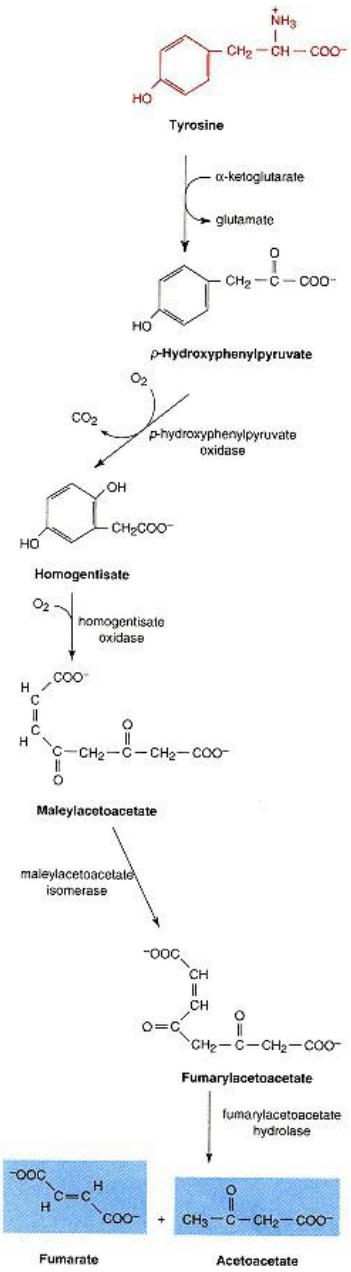


Figure 11.49
Degradation of tyrosine.

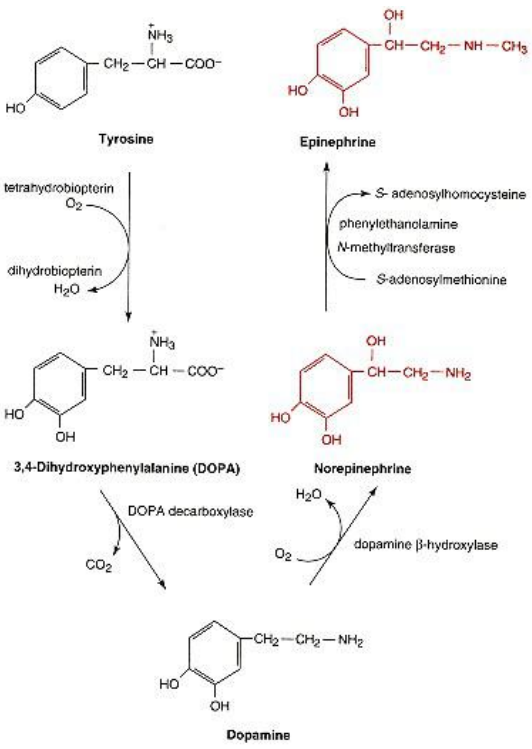


Figure 11.50
Synthesis of catecholamines.

CLINICAL CORRELATION 11.6

Disorders of Tyrosine Metabolism

Tyrosinemias

The absence or deficiency of tyrosine aminotransferase produces accumulation and excretion of tyrosine and metabolites. The disease, oculocutaneous or type II tyrosinemia, results in eye and skin lesions and mental retardation. Type I, hepatorenal tyrosinemia, is more serious, involving liver failure, renal tubular dysfunction, rickets, and polyneuropathy, caused by a deficiency of fumarylacetoacetate hydrolase. Accumulation of fumarylacetoacetate and maleylacetate, both of which are alkylating agents, can lead to DNA alkylation and tumorigenesis. Both diseases are autosomal recessive and rare.

Alcaptonuria

The first condition identified as an "inborn error of metabolism" was alcaptonuria. Individuals deficient in homogentisate oxidase excrete almost all ingested tyrosine as the colorless homogentisic acid in their urine. This auto-oxidizes to the corresponding quinone, which polymerizes to form an intensely dark color. Concern about the dark urine is the only consequence of this condition early in life. Homogentisate is slowly oxidized to pigments that are deposited in bones, connective tissue, and other organs, a condition called ochronosis because of the ochre color of the deposits. This is thought to be responsible for the associated arthritis, especially in males. The study of alcaptonuria by Archibald Garrod, who first indicated its autosomal recessive genetic basis, includes an unusual historical description of the iatrogenic suffering of the first patient treated for the condition, which is frequently benign.

Albinism

Skin and hair color are controlled by an unknown number of genetic loci in humans and exist in infinite variation; in mice, 147 genes have been identified in color determination. Many conditions have been described in which the skin has little or no pigment. The chemical basis is not established for any except classical albinism, which results from a lack of tyrosinase. Lack of pigment in the skin makes albinos sensitive to sunlight, increasing carcinoma of the skin in addition to burns; lack of pigment in the eyes may contribute to photophobia.

Fellman, J. H., Vanbellinghan, P. J., Jones, R. T., and Koler, R. D. Soluble and mitochondrial tyrosine aminotransferase. Relationship to human tyrosinemia. *Biochemistry* 8:615, 1969; Kvittingen, E. A. Hereditary tyrosinemia type I. An overview. *Scand. J. Clin. Lab. Invest.* 46:27, 1986

(also called **adrenaline).** The methyl group of epinephrine is derived from *S*-adenosylmethionine (see p. 469).

Brain plasma tyrosine regulates norepinephrine formation. Estrogens decrease tyrosine concentration and increase tyrosine aminotransferase activity, diverting tyrosine into the catabolic pathway. Furthermore, estrogen sulfate competes for the pyridoxal phosphate site on DOPA decarboxylase. These three effects combined may help explain mood variations during the menstrual cycle. Tyrosine is therapeutic in some cases of depression and stress. Its transport appears to be reduced in skin fibroblasts from schizophrenic patients, indicating other roles for tyrosine derivatives in mental disorders.

CLINICAL CORRELATION 11.7

Parkinson's Disease

Usually in people over the age of 60 years but occasionally earlier, tremors may develop that gradually interfere with motor function of various muscle groups. This condition is named for the physician who described "shaking palsy" in 1817. The primary cause is unknown, and there may be more than one etiological agent. The defect is caused by degeneration of cells in certain small nuclei of the brain called substantia nigra and locus caeruleus. Their cells normally produce dopamine as a neurotransmitter, the amount released being proportional to the number of surviving cells. A dramatic outbreak of parkinsonism occurred in young adult drug addicts using a derivative of pyridine (methylphenyl-tetrahydropyridine, MPTP). It (or a contaminant produced during its manufacture) appears to be directly toxic to dopamine-producing cells of substantia nigra. Symptomatic relief, often dramatic, is obtained by administering DOPA, the precursor of dopamine. Clinical problems developed when DOPA (L-DOPA, levo-DOPA) was used for treatment of many people who have Parkinson's disease. Side effects included nausea, vomiting, hypotension, cardiac arrhythmias, and various central nervous system symptoms. These were explained as effects of dopamine produced outside the central nervous system. Administration of DOPA analogs that inhibit DOPA decarboxylase but are unable to cross the blood–brain barrier has been effective in decreasing side effects and increasing effectiveness of the DOPA. The interactions of the many brain neurotransmitters are very complex, cell degeneration continues after treatment, and elucidation of the major biochemical abnormality has not yet led to complete control of the disease. Recently, attempts have been made at treatment by transplantation of fetal adrenal medullary tissue into the brain. The adrenal tissue synthesizes dopamine and improves the movement disorder.

Calne, D. B., and Langston, J. W. Aetiology of Parkinson's disease. *Lancet* 2:1457, 1983; and Cell and tissue transplantation into the adult brain. *Ann. N.Y. Acad. Sci.* 495, 1987.

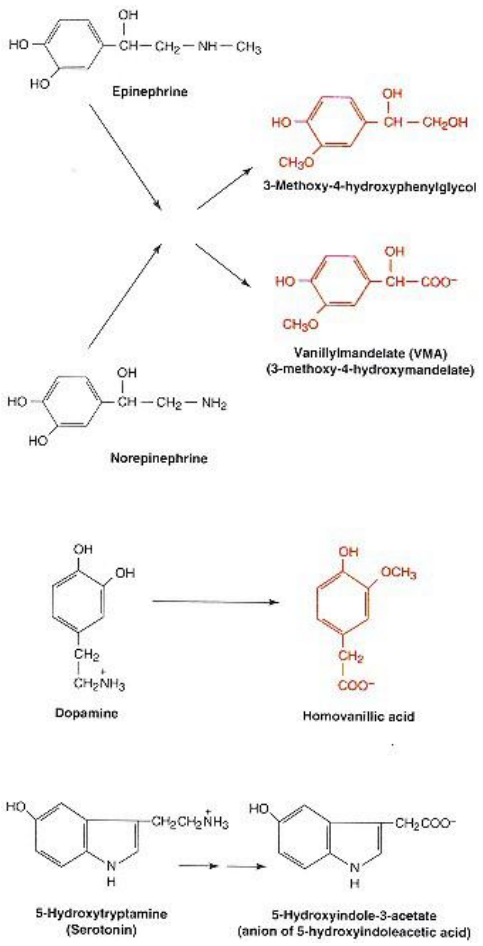


Figure 11.51
Major urinary excretion products of dopamine, epinephrine, norepinephrine, and serotonin.

Catecholamines are metabolized by **monoamine oxidase** and catecholamine ***O*-methyltransferase.** Major metabolites are shown in Figure 11.51. Absence of these metabolites in urine is diagnostic of a deficiency in synthesis of catecholamines. Lack of synthesis of serotonin (see p. 866) is indicated by lack of 5-hydroxyindole-3-acetic acid, shown in the same figure.

Tyrosine Is Involved in Synthesis of Melanin, Thyroid Hormone, and Quinoproteins

Conversion of tyrosine to melanin requires **tyrosinase,** a copper-containing protein (Figure 11.52*a*). The two-step reaction uses DOPA as a cofactor internal to the reaction and produces **dopaquinone.** During melanogenesis, following

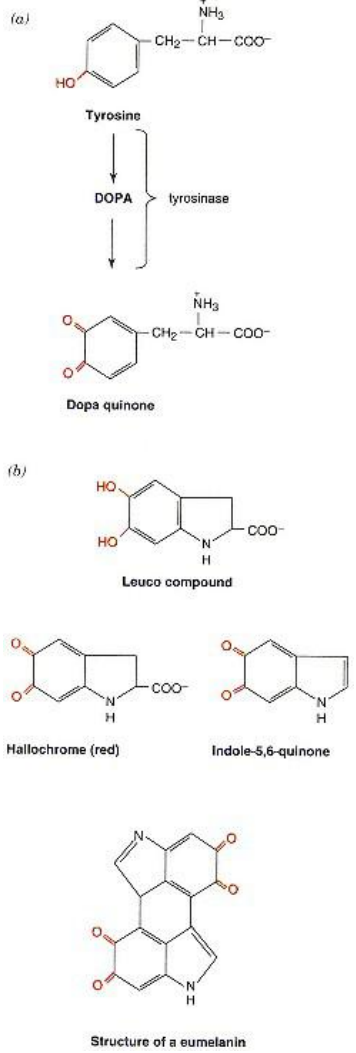


Figure 11.52
(a) Tyrosinase uses DOPA as a cofactor/intermediate;
(b) some intermediates in melanin synthesis and an example of the family of black eumelanins.

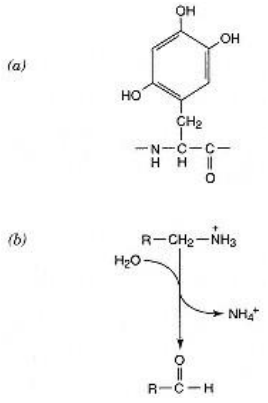


Figure 11.53
(a) Topaquinone and
(b) amine oxidase reaction.

exposure to UVB light, tyrosinase and a protein called tyrosinase-related protein, which may function in posttranslational modification of tyrosinase, are induced. A lack of tyrosinase activity produces **albinism.**

There are various types of **melanin** (Figure 11.52*b*). All are aromatic quinones and the conjugated bond system gives rise to color. The dark pigment that is usually associated with melanin is eumelanin, from the Greek for "good melanin." Other melanins are yellow or colorless. The role of tyrosine residues of thyroglobulin in thyroid hormone synthesis is presented in the chapter on hormones (Chapter 20).

Some proteins use a modified tyrosine residue as a prosthetic group in oxidation–reduction reactions. The only example reported in humans is **topaquinone** (trihydroxyphenylalanylquinone), which is present in some plasma amine oxidases (Figure 11.53).

Methionine and Cysteine

De novo synthesis of **methionine** does not occur and methionine is essential. **Cysteine,** however, is synthesized by transfer of the sulfur atom derived from methionine to the hydroxyl group of serine. As long as the supply of methionine is adequate, cysteine is nonessential. The disposition of individual atoms of methionine and cysteine is a prime example of how cells regulate pathways to fit their immediate needs for energy or for other purposes. Conditions under which various pathways are given preference will be emphasized.

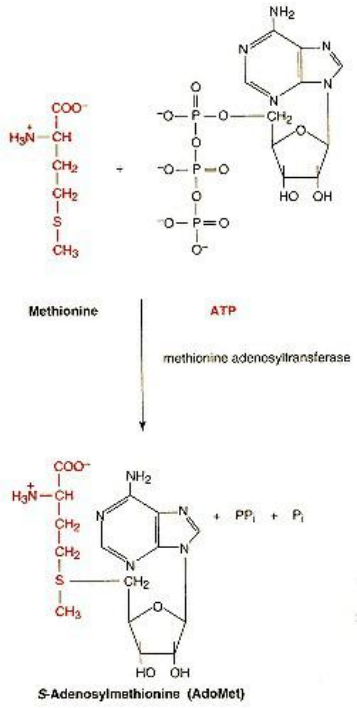


Figure 11.54
Synthesis of AdoMet.

Methionine Is First Reacted with Adenosine Triphosphate

When excess methionine is present its carbons can be used for energy or for gluconeogenesis, and the sulfur retained as the sulfhydryl of cysteine. Figure 11.54 shows the first step, catalyzed by **methionine adenosyltransferase.** All phosphates of ATP are lost, and the product is ***S*-adenosylmethionine** (abbreviated **AdoMet,** or **SAM** in older references). The sulfonium ion is highly reactive, and the methyl is a good leaving group. AdoMet as a methyl group donor will be described below. After a methyltransferase removes the methyl group, the resulting ***S*-adenosylhomocysteine** is cleaved by **adenosylhomocysteinase** (Figure 11.55). Note that homocysteine is one carbon longer than cysteine. Although the carbons are destined for intermediary metabolism, the

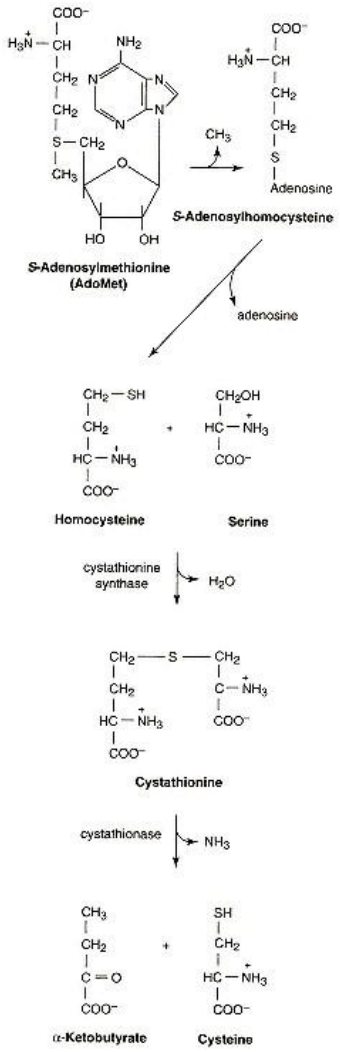


Figure 11.55
Synthesis of cysteine from *S*-adenosylmethionine.

sulfur, a more specialized atom, will be conserved through transfer to serine to form cysteine. This requires the pyridoxal phosphate-dependent **cystathionine synthase** and **cystathionase** (Figure 11.55; see Clin. Corr. 11.8). Since the bond to form cystathionine is made on one side of the sulfur, and that cleaved is on the other side, the result is a **transsulfuration** (see Clin. Corr. 11.9). Homocysteine produces α-ketobutyrate and ammonia. α-Ketobutyrate is decarboxylated by a multienzyme complex resembling pyruvate dehydrogenase to

CLINICAL CORRELATION 11.8

Hyperhomocysteinemia and Atherogenesis

Deficiency of cystathionine synthase causes homocysteine to accumulate, and remethylation leads to high levels of methionine. Many minor products of these amino acids are formed and excreted. No mechanism has been established to explain why accumulation of homocysteine should lead to some of the pathological changes. Homocysteine may react with and block lysyl aldehyde groups on collagen. The lens of the eye is frequently dislocated some time after the age of 3, and other ocular abnormalities often occur. Osteoporosis develops during childhood. Mental retardation is frequently the first indication of this deficiency. Attempts at treatment include restriction of methionine intake and feeding of betaine (or its precursor, choline). In some cases significant improvement has been obtained by feeding pyridoxine (vitamin B_6), suggesting that the deficiency may be caused by more than one type of gene mutation; one type may affect the K_m for pyridoxal phosphate and others may alter the K_m for other substrates, V_{max}, or the amount of enzyme. A theory relating hyperhomocysteinemia to atherogenesis has been proposed. Excess homocysteine can form homocysteine thiolactone, a highly reactive intermediate, which thiolates free amino groups in low density lipoproteins (LDLs) and causes them to aggregate and be endocytosed by macrophages. The lipid deposits form atheromas. Homocysteine can have other effects, including lipid oxidation and platelet aggregation, which in turn lead to fibrosis and calcification of atherosclerotic plaques. About one-quarter of patients with atherosclerosis who exhibit none of the other risk factors (such as smoking or oral contraceptive therapy) have been found to be deficient in cystathionine synthase activity.

Kaiser-Kupfer, M. I., Fujikawa, L., Kuwabara, T., et al. Removal of corneal crystals by topical cysteamine in nephrotic cystinosis. *N. Engl. J. Med.* 316:775, 1987; McCully, K. S. Chemical pathology of homocysteine I. Atherogenesis. *Ann. Clin. Lab. Sci.* 23:477, 1993.

yield propionyl CoA, which is then converted to succinyl CoA as described on page 479.

When the need is for energy, and not for cysteine, homocysteine produced in the above pathway is metabolized by **homocysteine desulfhydrase** to α-ketobutyrate, NH_3, and H_2S (Figure 11.56).

S-Adenosylmethionine Is a Methyl Group Donor

The role of tetrahydrofolate as a one-carbon group donor has been described (see p. 460). Although this cofactor could in theory serve as a source of methyl groups, the vast majority of methyltransferase reactions utilize ***S*-adenosylmethionine.** Methyl group transfer from AdoMet to a methyl acceptor is irre-

CLINICAL CORRELATION 11.9

Other Diseases of Sulfur Amino Acids

Congenital deficiency of any of the enzymes involved in transsulfuration results in accumulation of sulfur-containing amino acids. Hypermethioninemia has been attributed to deficiency of methionine adenosyltransferase, probably caused by a K_m mutant that requires higher than normal concentrations of methionine for saturation, but functions normally in methylation reactions. Lack of cystathionase does not seem to cause any clinical abnormalities other than cystathioninuria. The first reported case of this deficiency was about a mentally retarded patient and the retardation was attributed to the deficiency. Apparently the mental retardation was coincidental, the condition being benign. The amount of cysteine synthesized in these deficiencies is unknown, but treatment with a low-methionine diet for hypermethioninemia is unnecessary.

Diseases Involving Cystine

Cystinuria is a defect of membrane transport of cystine and basic amino acids (lysine, arginine, and ornithine) that results in their increased renal excretion. Extracellular sulfhydryl compounds are quickly oxidized to disulfides. Low solubility of cystine results in crystals and the formation of calculi, a serious feature of this disease. Treatment is limited to removal of stones, prevention of precipitation by drinking large amounts of water or alkalinizing the urine to solubilize cystine, or formation of soluble derivatives by conjugation with drugs. Much more serious is cystinosis in which cystine accumulates in lysosomes. The stored cystine forms crystals in many cells, with a serious loss of function of the kidneys, usually causing renal failure within ten years. The defect is believed to be in the cystine transporter of lysosomal membranes.

Seashore, M. R., Durant, J. L., and Rosenberg, L. E. Studies on the mechanisms of pyridoxine responsive homocystinuria. *Pediatr. Res.* 6:187, 1972; Mudd, S. H. The natural history of homocystinuria due to cystathione β-synthase deficiency. *Am. J. Hum. Genet.* 37:1, 1985; and Frimpter, G. W. Cystathionuria: nature of the defect. *Science* 149:1095, 1965.

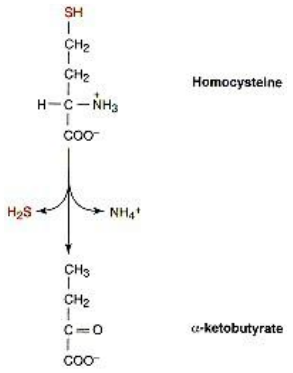


Figure 11.56
Homocysteine desulfhydrase.

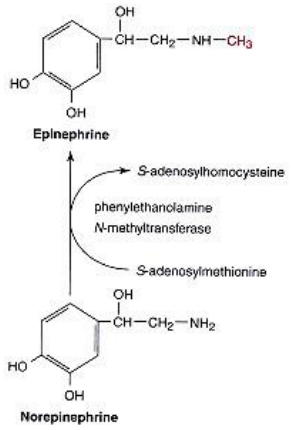


Figure 11.57
S-adenosylmethyltransferase reaction.

versible. An example is shown in Figure 11.57. *S*-Adenosylhomocysteine left after methyl group transfer can be metabolized to cysteine, α-ketobutyrate, and ammonia. When cells need to resynthesize methionine, since the methyltransferase reaction is irreversible, another enzyme is required (Figure 11.58). **Homocysteine methyltransferase** is one of two enzymes known to require a vitamin B_{12} cofactor (the other is described on p. 479). The methyl group comes from ***N*^{5}-methyltetrahydrofolate.** This is the only reaction known that uses this form of tetrahydrofolate as a methyl donor. The net result of reactions in Figures 11.57 and 11.58 is donation of a methyl group and regeneration of methionine under methionine-sparing conditions. A minor salvage pathway uses a methyl group from betaine instead of *N*^{5}-methyltetrahydrofolate.

AdoMet Is the Precursor of Spermidine and Spermine

Propylamine added to putrescine (see p. 459) to form spermidine and spermine is also derived from AdoMet, leaving methylthioadenosine. Putrescine is formed by decarboxylation of ornithine (see p. 459), and with propylamine forms spermidine. Addition of another propylamine gives spermine (Figure 11.59). The methylthioadenosine that remains can be used to resynthesize methionine. Much of the polyamine needed by the body is provided by microflora in the gut or from the diet and is carried by the enterohepatic circulation. Meat has

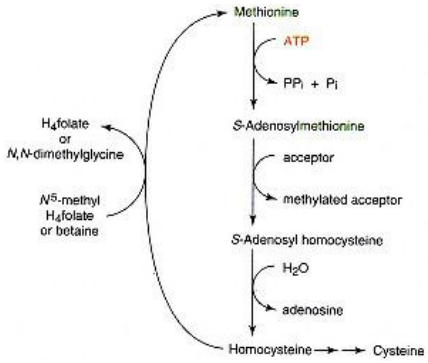


Figure 11.58
Resynthesis of methionine, a methylcobalamin-dependent reaction.

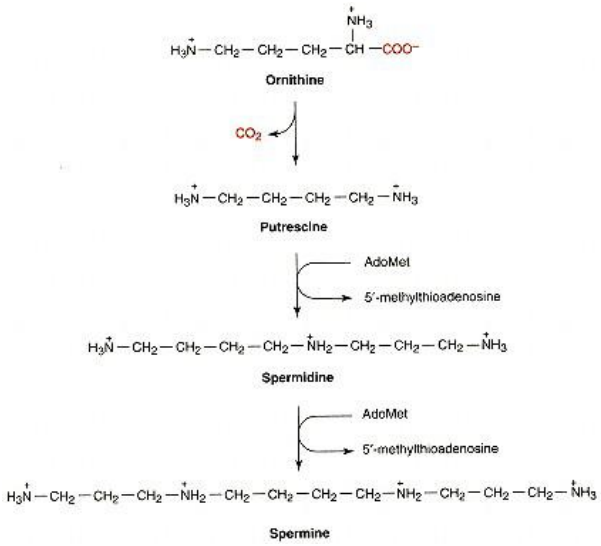


Figure 11.59
Polyamine synthesis.

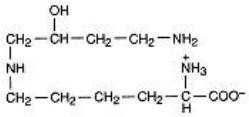


Figure 11.60
Hypusine.

a high content of putrescine, but other foods contain more spermidine and spermine.

The butylamino group of spermidine is used for posttranslational modification of a specific lysine residue in eIF-4D, an initiation factor for eukaryotic protein synthesis. The group is then hydroxylated, and the modified residue that results is called **hypusine** (Figure 11.60).

Metabolism of Cysteine Produces Sulfur-Containing Compounds

Cysteine, derived from the sulfur of homocysteine and a molecule of serine, is metabolized in several ways. The pathway chosen is determined by the needs of the cell. The major metabolite is **cysteinesulfinate** (Figure 11.61). This is further metabolized to sulfite and pyruvate, or to hypotaurine and taurine.

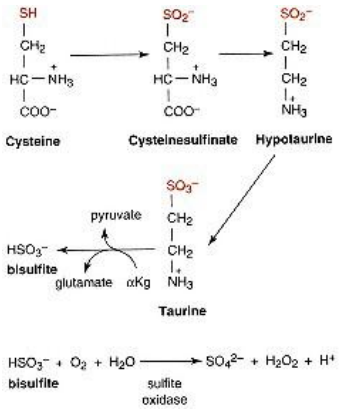


Figure 11.61
Formation of taurine and sulfate from cysteine.

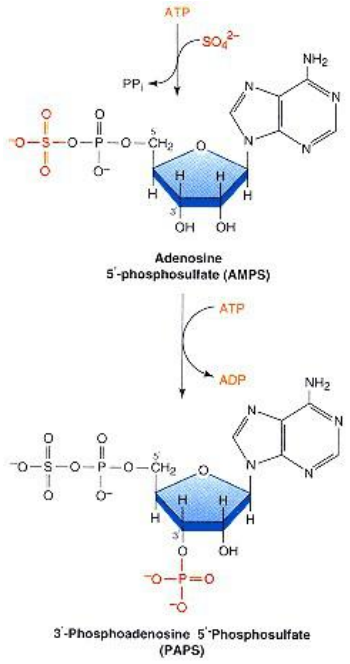


Figure 11.62
Synthesis of PAPS.

Taurine is an abundant intracellular free amino acid, but its exact role is unknown. It appears to play a necessary role in brain development. It forms conjugates with bile acids (see p. 418) and may enhance bile flow and increase cholesterol clearance by the liver. Taurine may also play a role in salvaging toxic intermediates, in regulating intracellular calcium, and, because of its abundance, in osmoregulation.

Sulfite produced from cysteine metabolism can be oxidized to **sulfate** (Figure 11.61), and this can be used in formation of **3′-phosphoadenosine-5′-phosphosulfate (PAPS),** the source of sulfate groups for addition to biological molecules (Figure 11.62).

Another reaction of cysteine metabolism catalyzed by cystathionase moves the sulfur from one cysteine to another cysteine (Figure 11.63) to form **thio-cysteine.** Thiosulfate is formed from cysteine as shown in Figure 11.64. An enzyme called **rhodanese** can incorporate a sulfur from thiosulfate or thiocysteine into other molecules such as cyanide ion (Figure 11.65).

Tryptophan

Metabolism of **tryptophan** has many branch points. The dominant or oxidative pathway of tryptophan in the human (Figure 11.66, in color) starts with oxidation of tryptophan to *N*-formylkynurenine by a heme-containing enzyme, **tryptophan dioxygenase,** also called **tryptophan pyrrolase** or **tryptophan oxygenase,** because the pyrrole ring is cleaved in the reaction. Tryptophan dioxygenase is induced by glucocorticoids and glucagon. It is found in liver; other tissues contain a similar enzyme called indolamine dioxygenase, which is less substrate specific. Formamidase then hydrolyzes formylkynurenine to formate and kynurenine. At this point the pathway begins to branch. In the dominant pathway, reactions lead to 3-hydroxykynurenine, 3-hydroxyanthranilic acid and alanine, amino-carboxymuconic semialdehyde, and, by decarboxylation, to aminomuconic semialdehyde. This can be further metabolized in several steps to glutarate and eventually acetoacetyl CoA, or recyclized nonenzymatically to **picolinic acid,** which is excreted in the urine.

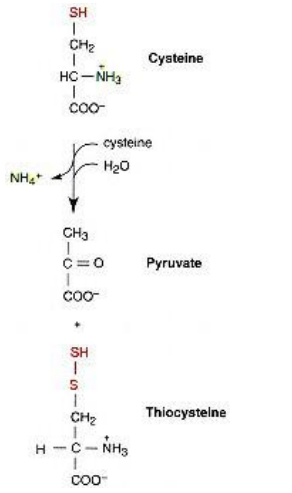


Figure 11.63
Synthesis of thiocysteine.

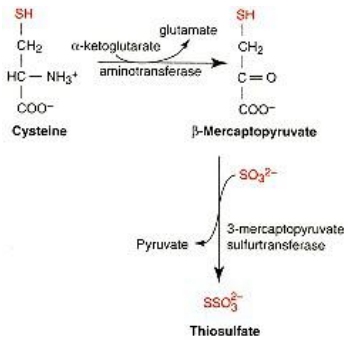


Figure 11.64
Formation of thiosulfate.

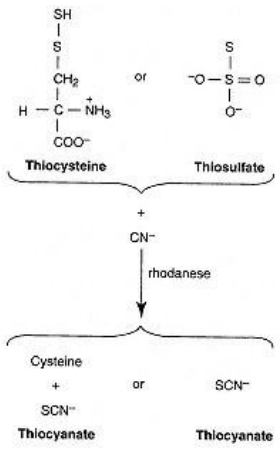


Figure 11.65
Detoxification of cyanide by products of cysteine metabolism.

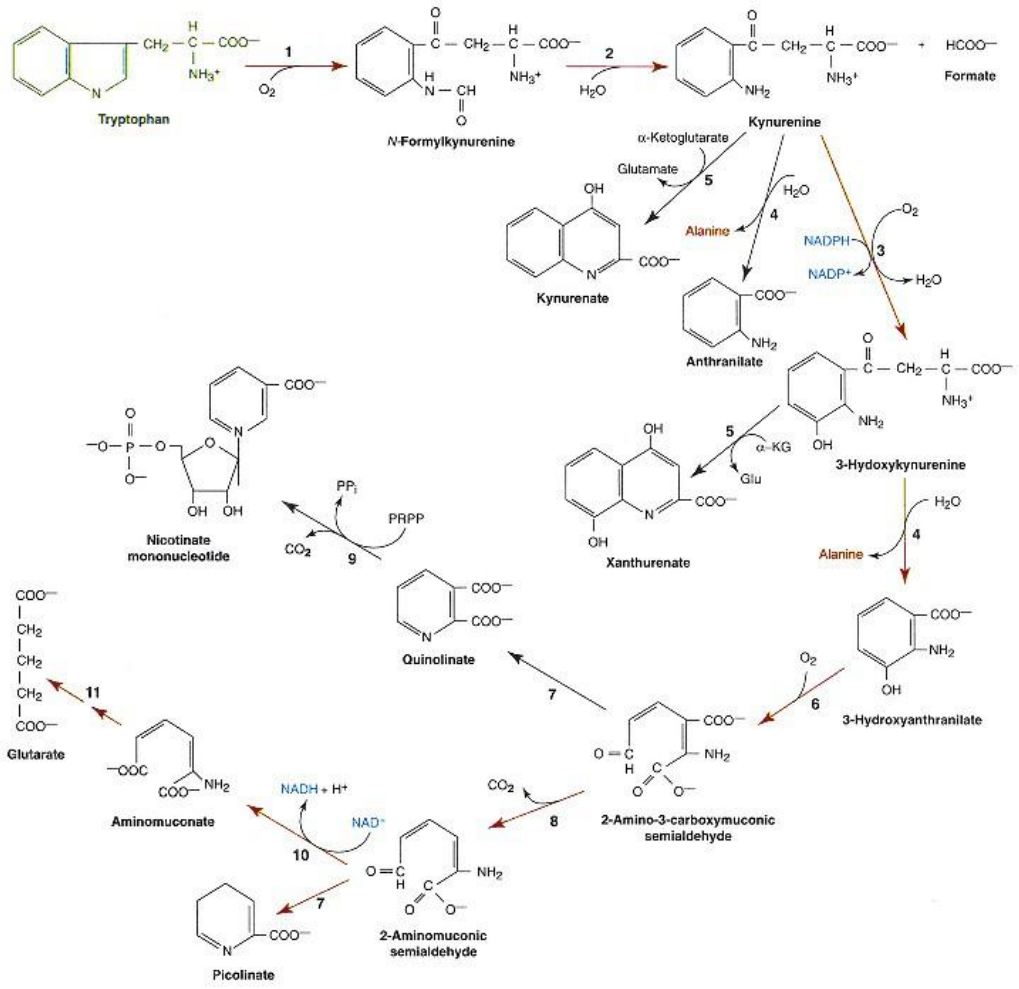


Figure 11.66
Metabolism of tryptophan.
Major pathway is shown in red. Enzymes indicated by number are
(1) tryptophan oxygenase,
(2) kynurenine formamidase,
(3) kynurenine hydroxylase,
(4) kynureninase,
(5) aminotransferase,
(6) 3-hydroxyanthranilate oxidase,
(7) spontaneous nonenzymatic reaction,
(8) picolinate carboxylase,
(9) quinolinate phosphoribosyltransferase,
(10) aldehyde dehydrogenase, and
(11) complex series of reactions.

Tryptophan Is a Precursor of NAD

Tryptophan is the precursor of approximately 50% of the body's pyridine nucleotides. The rest is obtained from the diet. The branch point leading to nicotinate mononucleotide can be seen in Figure 11.66 at the stage of amino-carboxymuconic semialdehyde. The enzyme that forms 2-aminomuconic semi-

aldehyde, **picolinate carboxylase,** from this compound has a low K_m and is easily saturated with substrate. Since picolinate carboxylase has low activity in liver, some amino-carboxymuconic semialdehyde is cyclized in a nonenzymatic reaction to quinolinic acid. Phosphoribosylpyrophosphate provides a ribonucleotide moiety and the final step is a decarboxylation leading to nicotinate mono-nucleotide. Note that the nicotinic acid ring is synthesized as a part of a nucleotide. Because **kynurenine hydroxylase** is inhibited by estrogen, women are more susceptible to **pellagra,** the disease produced by niacin deficiency (from the Italian *pelle,* skin, and *agra,* rough).

Pyridoxal Phosphate Has a Prominent Role in Tryptophan Metabolism

Many enzymes in this lengthy pathway are pyridoxal phosphate dependent. **Kynureninase** is one of them and is affected by a vitamin B_6 deficiency (Figure 11.66), resulting in excess kynurenine and xanthurenate excretion and giving urine a greenish-yellow color. This is a diagnostic symptom of vitamin B_6 deficiency.

Kynurenine Gives Rise to Neurotransmitters

Another pathway that kynurenine can follow is transamination and condensation of the side chain to form a two-ring compound, kynurenic acid. This reaction is also depicted in Figure 11.66. **Kynurenic acid,** its decarboxylated metabolite **kynuramine,** and **quinolinate** have all been shown to act as tryptophan-derived neurotransmitters, possibly as antiexcitotoxics and anticonvulsives.

Serotonin and Melatonin Are Tryptophan Derivatives

Serotonin (5-hydroxytryptamine) results from hydroxylation of tryptophan by a tetrahydrobiopterin-dependent enzyme and decarboxylation by a pyridoxal phosphate-containing enzyme (Figure 11.67*a*). It is a neurotransmitter in brain and causes contraction of smooth muscle of arterioles and bronchioles. It is found widely in the body and may have other physiological roles. **Melatonin,** a sleep-inducing molecule, is ***N*-acetyl-5-methoxytryptamine** (Figure 11.67*b*). The acetyltransferase needed for its synthesis is present in pineal gland and retina. Melatonin is involved in regulation of circadian rhythm, being synthesized mostly at night. It appears to function by inhibiting synthesis and secretion of other neurotransmitters such as dopamine and GABA (see p. 866).

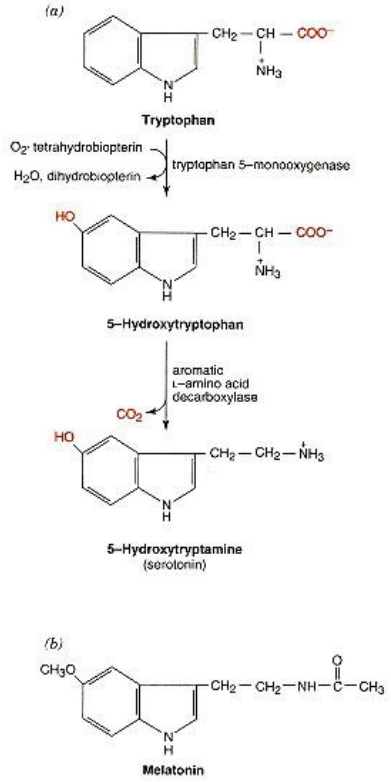


Figure 11.67
(a) Synthesis of serotonin (5-hydroxytryptamine) and
(b) structure of melatonin.

Tryptophan Induces Sleep

Ingestion of foods rich in tryptophan leads to sleepiness because serotonin is also sleep-inducing. Reducing availability of tryptophan in the brain can interfere with sleep. Tryptophan availability is reduced when other amino acids compete with it for transport through the blood-brain barrier. Elevated plasma concentrations of other amino acids, after a high-protein meal, diminish transport of tryptophan and induce wakefulness. The sleep-inducing effect of carbohydrates is due to decreased plasma amino acid levels, since carbohydrate stimulates release of insulin, and insulin causes removal of amino acids from plasma and uptake into muscle. This alleviates competition and increases the amount of tryptophan that can enter the brain. Strangely, extra serotonin appears to lead to sleepiness in females, but only calmness in males.

Branched-Chain Amino Acids

Metabolism of **branched-chain amino acids** (BCAAs)—**valine, isoleucine,** and **leucine**—is unusual, being initiated in muscle. NADH is formed during their metabolism, making them an excellent source of energy. BCAA aminotransferase is present at a much higher concentration in muscle than liver. Although

the three amino acids produce different products, the first steps in their metabolism are similar.

Initial Reactions of BCAA Metabolism Are Shared

BCAA aminotransferase exists in three isozymes distributed differently between tissues, sometimes found in cytosol and sometimes in mitochondria (Figure 11.68). Two handle all three BCAAs, and one is specific for leucine and methionine. Starvation induces the muscle aminotransferases but does not affect these enzymes in liver. The resulting α-keto branched-chain acids are oxidatively decarboxylated by an inner mitochondrial membrane enzyme complex similar to the pyruvate dehydrogenase complex, which produces NADH and CO_2. When phosphorylated the dehydrogenase component of the complex has some activity, but this is greatly increased by dephosphorylation. All three α-keto branched-chain acids appear to be metabolized by the same enzyme. The more active form is found in liver in the fed state, and in muscle during starvation, reflecting the metabolism of dietary BCAAs by liver, and of muscle BCAAs to provide energy during fasting. The resulting CoA compounds are one carbon shorter than the original amino acids and are next acted on by an enzyme that resembles the first dehydrogenase found in fatty acid β-oxidation.

Pathways of Valine and Isoleucine Metabolism Are Similar

Valine and isoleucine continue down a common pathway, with addition of water across the double bond to form a hydroxylated intermediate (Figure 11.69). The hydroxyl group on the isoleucine derivative is oxidized by NAD^+ followed by thiolysis to give acetyl CoA and propionyl CoA. The valine derivative loses CoA and is oxidized by NAD^+ to methylmalonate semialdehyde, which is then converted to propionyl CoA.

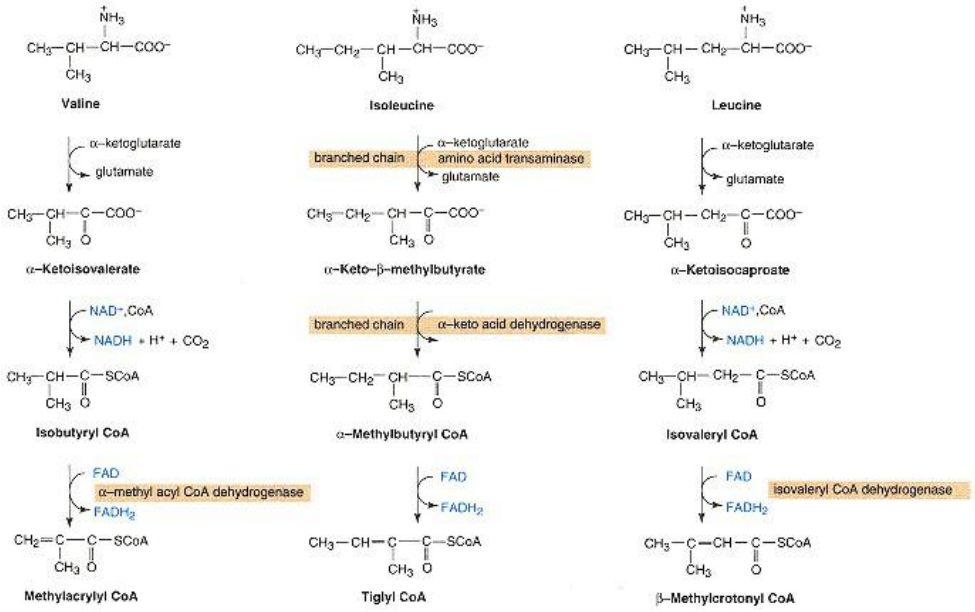


Figure 11.68
Common reactions in degradation of branched-chain amino acids.

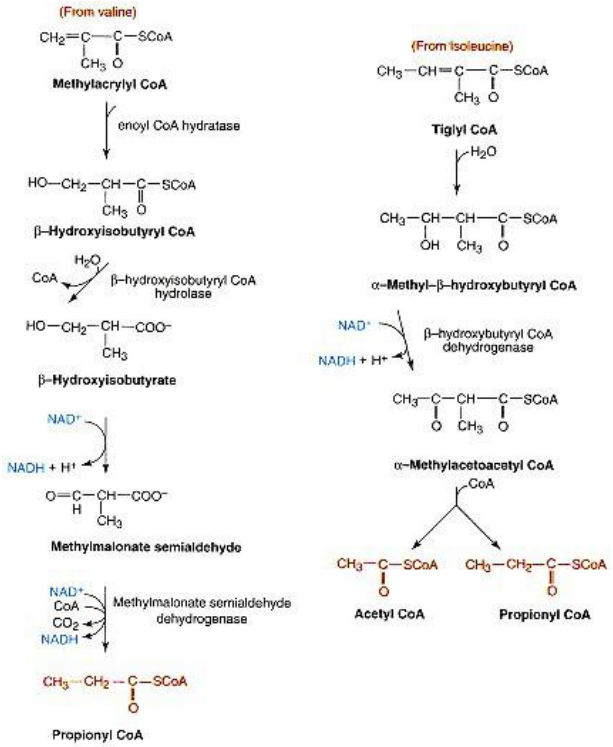


Figure 11.69
Terminal reactions in degradation of valine and isoleucine.

The Leucine Pathway Differs from Those of the Other Two Branched-Chain Amino Acids

The position of the methyl side chain in leucine prohibits the oxidation step found in the metabolism of the other BCAAs (Figure 11.70). The double bond-containing derivative is carboxylated, hydroxylated, and cleaved to acetoacetate and acetyl CoA. One intermediate is **β-hydroxy-β-methylglutaryl CoA,** an

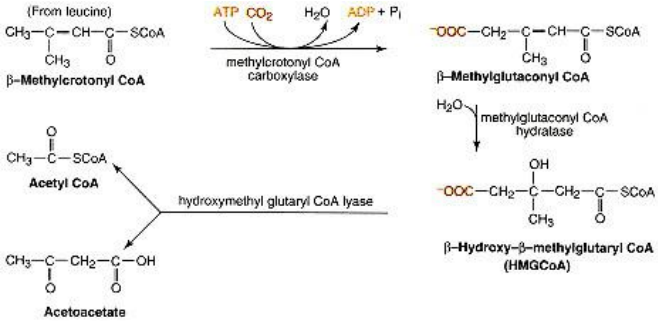


Figure 11.70
Terminal reactions of leucine degradation.

intermediate in cytosolic sterol synthesis (Chapter 10). Since BCAA degradation occurs in mitochondria the two pools do not mix. Leucine also has a minor alternative pathway (not shown), which results in excretion of 3-hydroxyvaleric acid, and can be utilized in the case of blockage in the leucine degradative pathway (Clin. Corr. 11.10).

CLINICAL CORRELATION 11.10

Diseases of Metabolism of Branched-Chain Amino Acids

Enzyme deficiencies in catabolism of branched-chain amino acids are not common. In general, they produce acidosis in newborns or young children. Very rare instances have been reported of hypervalinemia and hyperleucine–isoleucinemia. It has been suggested that the two conditions indicate existence of specific aminotransferases for valine and for leucine and isoleucine. Alternatively, mutation could alter the specificity of a single enzyme. The most common abnormality is deficiency of branched-chain keto acid dehydrogenase complex activity. There are several variations, but all patients excrete the branched-chain α-keto acids and corresponding hydroxy acids and other side products; an unidentified product imparts characteristic odor associated with the name maple syrup urine disease. Some cases respond to high doses of thiamine. A large percentage show serious mental retardation, ketoacidosis, and short life span. Dietary treatment to reduce the branched-chain ketoacidemia is effective in some cases. Some cases have been reported of deficiency of enzymes in later reactions of branched-chain amino acids. These include a blockage of oxidation of isovaleryl CoA with accumulation of isovalerate (which gives urine a sweaty feet smell), β-methylcrotonyl CoA carboxylase deficiency (in which urine smells like that of a cat), deficiency of β-hydroxy-β-methylglutaryl CoA lyase, and deficiency of β-ketothiolase that splits α-methylacetoacetyl CoA (with no defect in acetoacetate cleavage). In the latter condition, development is normal and symptoms appear to be related only to episodes of ketoacidosis.

Zhang, B., Edenberg, H. J., Crabb, D. W., and Harris, R. A. Evidence for both a regulatory and structural mutation in a family with maple syrup urine disease. *J. Clin. Invest*. 83:1425, 1989.

Propionyl CoA Is Metabolized to Succinyl CoA

Propionyl CoA is an end product of isoleucine, valine, and methionine metabolism, odd-chain fatty acid oxidation, and degradation of the side chain of cholesterol. The first step in the conversion of the 3-carbon propionyl CoA to the 4-carbon succinyl CoA is initiated by **propionyl-CoA carboxylase,** which is biotin dependent (Figure 11.71; see 11.11). This gives D-methylmalonyl CoA, an isomerase that converts to a mixture of D- and L-methymalonyl CoA. **Methylmalonyl mutase,** which requires 5 -deoxyadenosylcobalamin (a derivative of vitamin B_{12}) converts the L-isomer to succinyl CoA. This is the second enzyme known to be dependent on vitamin B_{12} (see p. 473). The reaction is very unusual, removing a methyl side chain and inserting it as a methylene group into the backbone of the compound.

Lysine

Lysine is the other entirely ketogenic amino acid. The carbons enter intermediary metabolism as acetoacetyl CoA. Lysine has an ε- and an α-amino group.

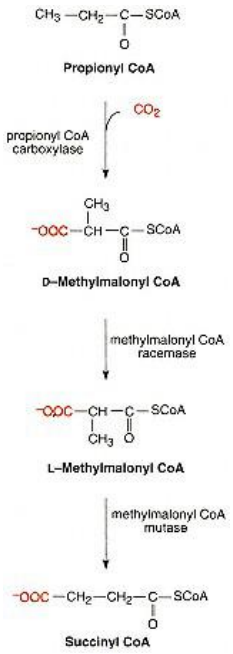


Figure 11.71
Interconversion of propionyl CoA, methylmalonyl CoA, and succinyl CoA.
The mutase requires 5 -deoxyadenosyl-cobalamin for activity

CLINICAL CORRELATION 11.11

Diseases of Propionate and Methylmalonate Metabolism

Deficiencies of the three enzymes shown in Figure 11.71 contribute to ketoacidosis. Propionate is formed in the degradation of valine, isoleucine, methionine, threonine, the side chain of cholesterol, and odd-chain fatty acids. The amino acids appear to be the main precursors since decreasing or eliminating dietary protein immediately minimizes acidosis. A defect in propionyl-CoA carboxylase results in accumulation of propionate, which is diverted to alternative pathways, including incorporation into fatty acids for an acetyl group forming odd-chain fatty acids. The extent of these reactions is very limited. In one case large amounts of biotin were reported to produce beneficial effects, suggesting that more than one defect decreases propionyl-CoA carboxylase activity. Possibilities are a lack of intestinal biotinidase that liberates biotin from ingested food for absorption or a lack of biotin holocarboxylase that incorporates biotin into biotin-dependent enzymes. Children have been found with acidosis caused by high levels of methylmalonate, which is normally undetectable in blood. Enzymes analyzed from liver taken at autopsy or from cultured fibroblasts have shown that some cases were due to deficiency of methylmalonyl-CoA mutase. One group was unable to convert methylmalonyl CoA to succinyl CoA under any conditions, but another group carried out the conversion when 5 -adenosylcobalamin was added. Clearly, those with an active site defect in the enzyme cannot metabolize methylmalonate, but those with defects in handling vitamin B_{12}, respond to massive doses of the vitamin. Other cases of methylmalonic aciduria suffer from a more fundamental inability to use vitamin B_{12} that leads to deficiency in methylcobalamin (coenzyme of methionine salvage) and in 5 -adenosylcobalamin deficiency (coenzyme of methylmalonyl CoA isomerization).

Mahoney, M. J., and Bick, D. Recent advances in the inherited methylmalonic acidemias. *Acta Paediatr. Scand*. 76:689, 1987.

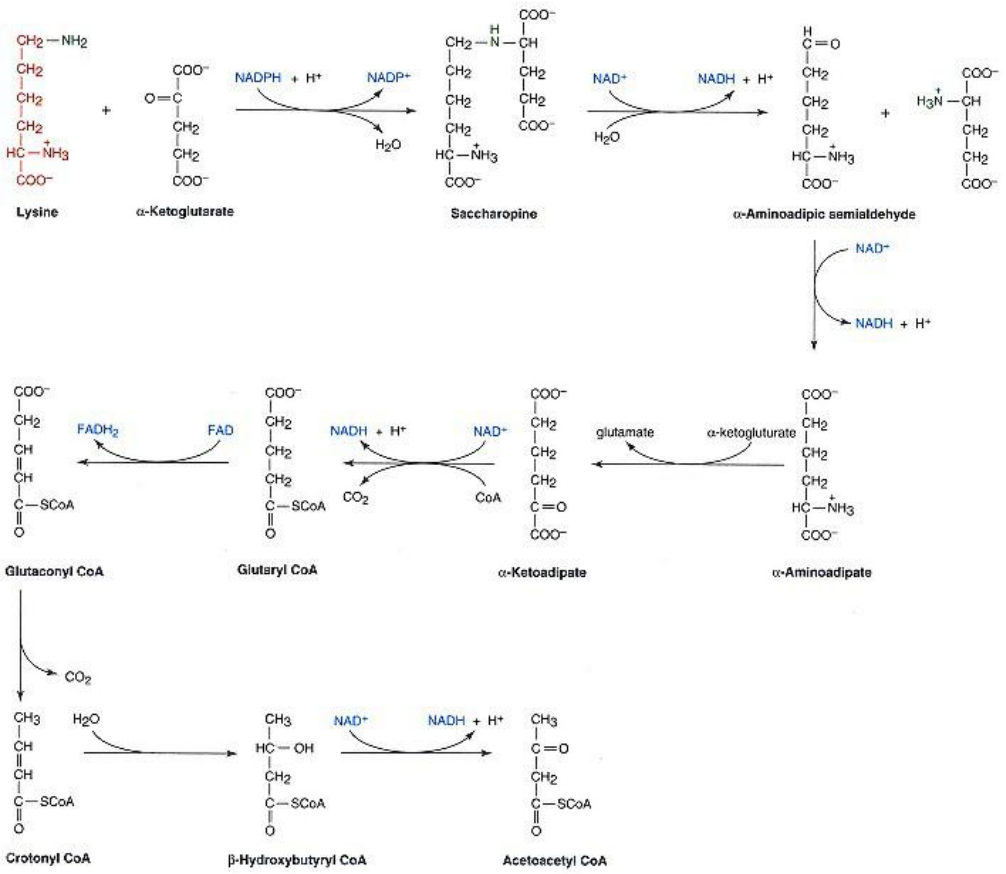
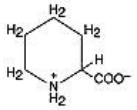


Figure 11.72
Principal pathway of lysine degradation.

The initial transamination of the ε-amino group requires α-ketoglutarate as acceptor and cosubstrate (Figure 11.72). Instead of the pyridoxal phosphate–Schiff base mechanism, an intermediate called **saccharopine** is formed, which is then cleaved to glutamate and a semialdehyde compound. The usual Schiff base electronic rearrangement mechanism is replaced by an oxidation and a reduction, but the products are effectively the same. The semialdehyde is then oxidized to a dicarboxylic amino acid, and a transamination of the α-amino group occurs in a pyridoxal-dependent manner. Further reactions lead to acetoacetyl CoA.



Pipecolate

Figure 11.73
Minor product of lysine metabolism.

A minor pathway starts with removal of the α-amino group and goes via the cyclic compound **pipecolate** (Figure 11.73) to join the major pathway at the level of the semialdehyde intermediate. This does not replace the major pathway even in a deficiency of enzymes in the early part of the pathway (see Clin. Corr. 11.12).

Carnitine Is Derived from Lysine

Medium- and long-chain fatty acids are transported into mitochondria for β-oxidation as **carnitine** conjugates (see p. 382). Carnitine is synthesized not from free lysine but rather from lysine residues in certain proteins. The first step is trimethylation of the ε-amino group of the lysine side chain, with AdoMet as the methyl donor (Figure 11.74). Free trimethyllysine is obtained from hydrolysis of the protein and is metabolized in four steps to carnitine.

Histidine

The first reaction catalyzed by **histidase** (Clin. Corr. 11.13) removes free ammonia and leaves a compound with a double bond called urocanate (Figure 11.75). Two other reactions lead to **formiminoglutamate (FIGLU).** The formimino group is then transferred to tetrahydrofolate.

Urinary Formiminoglutamate Is Diagnostic of Folate Deficiency

The formimino group of formiminoglutamate must be transferred to tetrahydrofolate before the final product, glutamate, can be produced. When there is

CLINICAL CORRELATION 11.12

Diseases Involving Lysine and Ornithine

Lysine

Two metabolic disorders of lysine are recognized. α-Amino adipic semialdehyde synthase is deficient in a small number of patients who excrete lysine and smaller amounts of saccharopine. This has led to the discovery that the enzyme has both lysine-α-ketoglutarate reductase and saccharopine dehydrogenase activities. Single proteins with multiple enzymatic activities are also found in pyrimidine synthesis and fatty acid synthesis. It is thought that hyperlysinemia is benign. More serious is familial lysinuric protein intolerance due to failure to transport dibasic amino acids across intestinal mucosa and renal tubular epithelium. Plasma lysine, arginine, and ornithine are decreased to one-third or one-half of normal. Patients develop marked hyperammonemia after a meal containing protein. This is thought to arise from deficiency of the urea cycle intermediates ornithine and arginine in liver, limiting the capacity of the cycle. Consistent with this view, oral supplementation with citrulline prevents hyperammonemia. Other features are thin hair, muscle wasting, and osteoporosis, which may reflect protein malnutrition due to lysine and arginine deficiency.

Ornithine

Elevated ornithine levels are generally due to deficiency of ornithine δ-aminotransferase. A well-defined clinical entity, gyrate atrophy of the choroid and retina, characterized by progressive loss of vision leading to blindness by the fourth decade, is caused by deficiency of this mitochondrial enzyme. The mechanism of changes in the eye is unknown. Progression of the disease may be slowed by dietary restriction in arginine and/or pyridoxine therapy, which reduces ornithine in body fluids.

O'Donnell, J.J., Sandman, R. P., and Martin, S. R. Gyrate atrophy of the retina: inborn error of L-ornithine: 2-oxoacid aminotransferase. *Science* 200:200, 1978; Rajantil, J., Simell, O., and Perheentupa, J. Lysinuric protein intolerance. Basolateral transport defect in renal tubuli. *J. Clin. Invest*. 67:1078, 1981.

CLINICAL CORRELATION 11.13

Histidinemia

Histidinemia is due to histidase deficiency. A convenient assay for this enzyme uses skin, which produces urocanate as a constituent of sweat; urocanase and other enzymes of histidine catabolism found in liver do not occur in skin. A finding that urocanate is absent in sweat can only be interpreted as a lack of synthesis, and not as accelerated disappearance by further metabolism. Histidase deficiency can be confirmed by enzyme assay in skin biopsies. Incidence of the disorder is high, about 1 in 10,000 newborns screened. Most reported cases of histidinemia have shown normal mental development. Restriction of dietary histidine normalizes the biochemical abnormalities but is not usually required.

Scriver, C. R., and Levy, H. L. Histidinemia: reconciling retrospective and prospective findings. *J. Inherit. Metab. Dis*. 6:51, 1983.

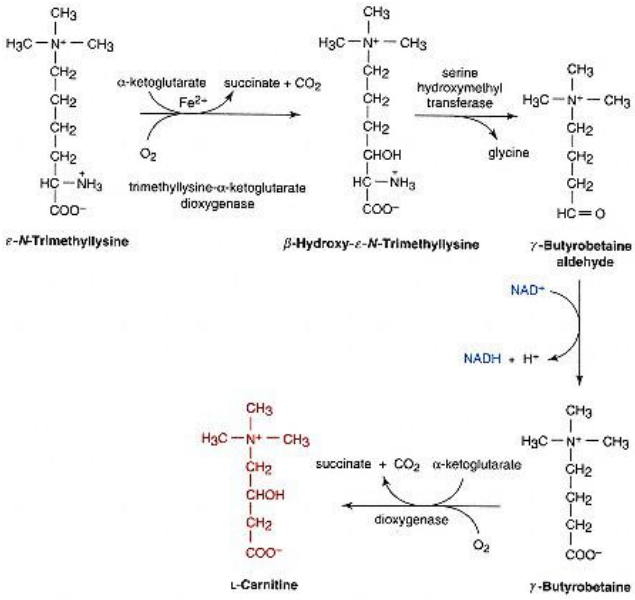


Figure 11.74
Biosynthesis of carnitine.

insufficient tetrahydrofolate available, this reaction decreases and FIGLU is excreted in urine. This is a diagnostic sign of folate deficiency if it happens after a test dose of histidine is ingested (see Clin. Corr. 11.14).

Histamine, Carnosine, and Anserine Are Produced from Histidine

Histamine (Figure 11.76), released from cells as part of an allergic response, is produced from histidine by **histidine decarboxylase.** Histamine has many

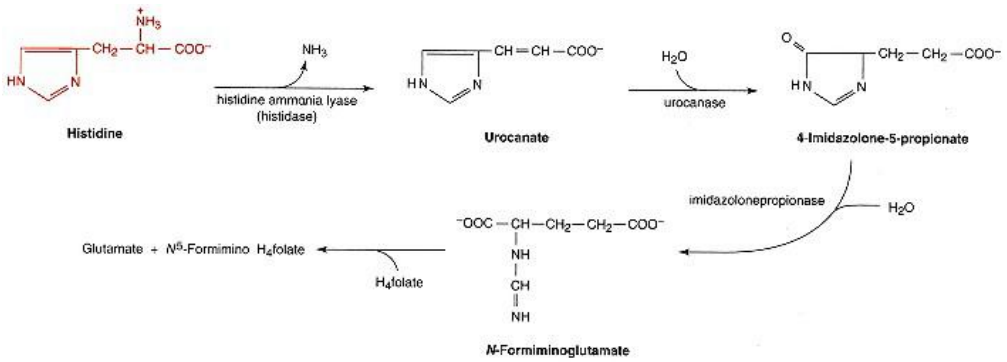


Figure 11.75
Degradation of histidine.

CLINICAL CORRELATION 11.14

Diseases of Folate Metabolism

A significant fraction of absorbed folic acid must be reduced to function as a coenzyme. Symptoms of folate deficiency may be due to deficiency of dihydrofolate reductase. Parenteral administration of N^5-formyltetrahydrofolate, the most stable of the reduced folates, is effective in these cases. In some cases of central nervous system abnormality attributed to deficiency of methylene folate reductase there is homocystinuria. Decreased enzyme activity lowers the N^5-methyltetrahydrofolate formed so that the source of methyl groups for the salvage of homocysteine is limiting. Large amounts of folic acid, betaine, and methionine reversed the biochemical abnormalities and, in at least one case, the neurological disorder. Patients with widely divergent presentations had shown deficiencies in transfer of the formimino group from formiminoglutamate to tetrahydrofolate. They excreted varying amounts of FIGLU; some responded to large doses of folate, but others did not. The mechanism whereby a deficiency of formiminotransferase produces pathological changes is unclear. It is not sure whether this deficiency causes a disease state. One patient showed symptoms of folate deficiency and had tetrahydrofolate methyltransferase deficiency. The associated anemia did not respond to vitamin B_{12} but showed some improvement with folate. It was suggested that the patient formed inadequate N^5-methyltetrahydrofolate to promote remethylation of homocysteine. This left the coenzyme "trapped" in the methylated form and unavailable for use in other reactions.

physiological roles, including dilation and constriction of certain blood vessels. An overreaction to histamine can lead to asthma and other allergic reactions. **Carnosine** (β-alanylhistidine) and **anserine** (β-alanylmethylhistidine) are dipeptides (Figure 11.77) found in muscle. Their function is unknown.

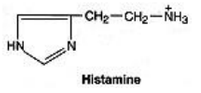
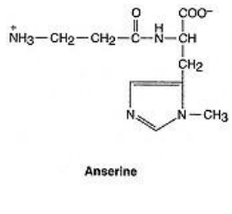


Figure 11.76
Histamine.

Creatine

Storage of "high-energy" phosphate, particularly in cardiac and skeletal muscle, occurs by transfer of the phosphate group from ATP to **creatine** (Figure 11.78). Creatine is synthesized by transfer of the guanidinium group of arginine to glycine, and subsequent addition of a methyl group from AdoMet. The amount of creatine in the body is related to muscle mass, and a certain percentage of this undergoes turnover each day. About 1–2% of preexisting creatine phosphate is cyclized nonenzymatically to **creatinine** (Figure 11.79) and excreted in urine, and new creatine is synthesized to replace it. The amount of creatinine excreted by an individual is therefore constant from day to day. When a 24-hour urine sample is requested, the amount of creatinine in the sample can be used to determine whether the sample truly represents a whole day's urinary output.



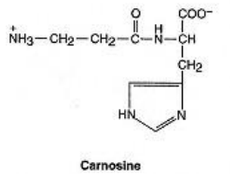


Figure 11.77
Anserine and carnosine.

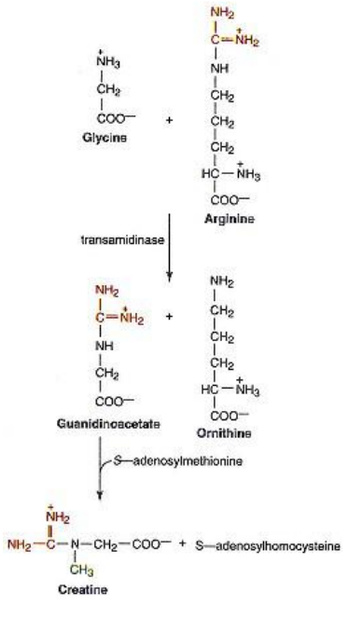


Figure 11.78
Synthesis of creatine.

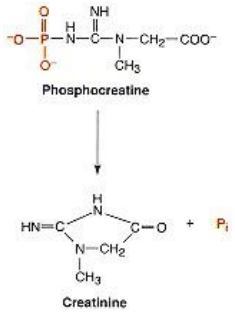


Figure 11.79
Spontaneous reaction forming creatinine.

Glutathione

Glutathione, the tripeptide γ-glutamylcysteinylglycine, has several important functions. It is a reductant, conjugated to drugs to make them more water soluble, involved in transport of amino acids across cell membranes, part of some leukotriene structures (see p. 438), a cofactor for some enzymatic reactions, and an aid in the rearrangement of protein disulfide bonds.

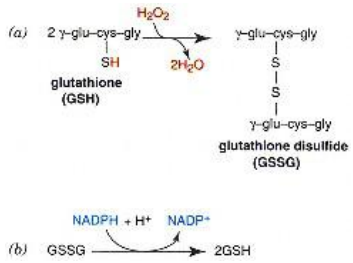


Figure 11.80
(a) Scavenging of peroxide by glutathione peroxidase and
(b) regeneration of reduced glutathione by glutathione reductase.

Glutathione as reductant is very important in maintaining stability of erythrocyte membranes. Its sulfhydryl group can be used to reduce peroxides formed during oxygen transport (see p. 1026). The resulting oxidized form of GSH consists of two molecules joined by a disulfide bond. This is reduced to two molecules of GSH at the expense of NADPH (Figure 11.80). The usual steady-state ratio of GSH to GSSG in erythrocytes is 100:1.

Conjugation of drugs by glutathione, often after a preliminary reaction catalyzed by cytochrome P450 (Chapter 23), renders them more polar for excretion (Figure 11.81).

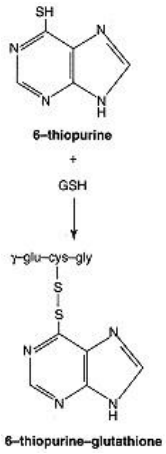


Figure 11.81
Conjugation of a drug by glutathione transferase.

Glutathione Is Synthesized from Three Amino Acids

Glutathione is synthesized by formation of the dipeptide γ-glutamylcysteine and the subsequent addition of glycine. Both reactions require activation of carboxyl groups by ATP (Figure 11.82). Synthesis of glutathione is largely regulated by cysteine availability.

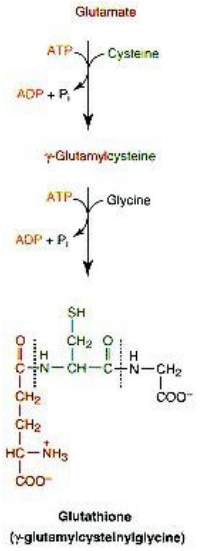


Figure 11.82
Synthesis of glutathione.

The γ-Glutamyl Cycle Transports Amino Acids

There are several mechanisms for transport of amino acids across cell membranes. Many are symport or antiport mechanisms (see p. 200) and are coupled to sodium transport. The ***γ*-glutamyl cycle** is an example of "group transfer" transport. It is more energy-requiring than other mechanisms, but is rapid and has high capacity, and functions in the kidney and some other tissues. It is particularly important in renal epithelial cells.

The enzyme ***γ*-glutamyl transpeptidase** is located in the cell membrane. It shuttles GSH to the cell surface to interact with an amino acid. γ-Glutamyl amino acid is transported into the cell, and the complex is hydrolyzed to liberate the amino acid (Figure 11.83). Glutamate is released as **5-oxoproline,** and cysteinylglycine is cleaved to its component amino acids. To regenerate GSH glutamate is reformed from oxoproline in an ATP-requiring reaction, and GSH is resynthesized from its three component parts. Three ATPs are used in the regeneration of glutathione, one in formation of glutamate from oxoproline and two in formation of the peptide bonds.

Glutathione Concentration Affects the Response to Toxins

When the body encounters toxic conditions such as peroxide formation, ionizing radiation, alkylating agents, or other reactive intermediates, it is beneficial to increase the level of GSH. Cysteine and methionine have been administered as GSH precursors, but they have the disadvantage of being precursors of an energy-expensive pathway to GSH. A more promising approach is administration of a soluble diester of GSH, such as γ-(α-ethyl)glutamylcysteinylethylglycinate.

Very premature infants have a very low concentration of cysteine because of low cystathionase activity in liver. This keeps the GSH concentration low and makes them more susceptible to oxidative damage, especially from hydro-peroxides formed in the eye after hyperbaric oxygen treatment. Under certain circumstances, such as rendering tumor cells more sensitive to radiation or parasites more sensitive to drugs, it is desirable to lower GSH levels. This can be achieved by the glutamate analog **buthionine** sulfoximine (Figure 11.84) as a competitive inhibitor of GSH synthesis.

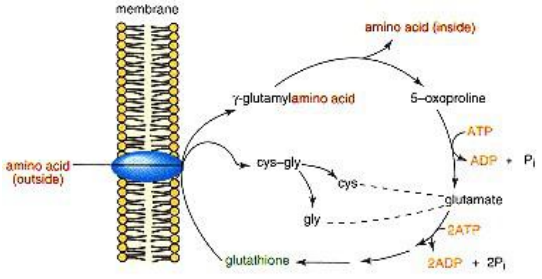


Figure 11.83
-Glutamyl cycle for transporting amino acids.

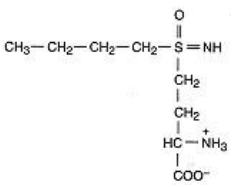


Figure 11.84
Buthionine sulfoximine.

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Questions

C. N. Angstadt and J. Baggott

1. Amino acids considered nonessential for humans are:

   A. those not incorporated into protein.

   B. not necessary in the diet if sufficient amounts of precursors are present.

   C. the same for adults as for children.

   D. the ones made in specific proteins by posttranslational modifications.

   E. generally not provided by the ordinary diet.

2. Aminotransferases:

   A. usually require α-ketoglutarate or glutamine as one of the reacting pair.

   B. catalyze reactions that result in a net use or production of amino acids.

   C. catalyze irreversible reactions.

   D. require pyridoxal phosphate as an essential cofactor for the reaction.

   E. are not able to catalyze transamination reactions with essential amino acids.

3. The production of ammonia in the reaction catalyzed by glutamate dehydrogenase:

   A. requires the participation of NADH or NADPH.

   B. proceeds through a Schiff base intermediate.

   C. may be reversed to consume ammonia if it is present in excess.

   D. is favored by high levels of ATP or GTP.

   E. would be inhibited when gluconeogenesis is active.

4. The amide nitrogen of glutamine:

   A. represents a nontoxic transport form of ammonia.

   B. is a major source of ammonia for urinary excretion.

   C. is used in the synthesis of asparagine, purines, and pyrimidines.

   D. can be recovered as ammonia by the action of glutaminase.

   E. all of the above are correct.

5. In the formation of urea from ammonia by the urea cycle, all of the following are correct EXCEPT:

A. aspartate supplies one of the nitrogens found in urea.

B. part of the large negative free-energy change of the process may be attributed to the hydrolysis of pyrophosphate.

C. the rate of the cycle is independent of diet.

D. fumarate is produced.

E. genetic deficiency of any one of the enzymes can lead to hyperammonemia.

6. Carbamoyl phosphate synthetase I:

A. is a flavoprotein.

B. is controlled primarily by feedback inhibition.

C. is unresponsive to changes in arginine.

D. requires acetylglutamate as an allosteric effector.

E. requires ATP as an allosteric effector.

7. All of the following are correct about ornithine EXCEPT it:

A. may be formed from or converted to glutamic semialdehyde.

B. can be converted to proline.

C. plays a major role in the urea cycle.

D. is a precursor of putrescine, a polyamine.

E. is in equilibrium with spermidine.

8. Serine:

A. is in equilibrium with threonine via a reaction catalyzed by a hydroxymethyltransferase.

B. may enter intermediary metabolism either as 3-phosphoglycerate or as pyruvate.

C. can be converted to selenoserine for incorporation into proteins.

D. is a prosthetic group for *S*-adenosylmethionine decarboxylase.

E. all of the above are correct.

9. In folic acid-dependent carriage of a one-carbon group:

A. the formation of the methyl group of thymine involves a direct transfer from methyl THF.

B. the first entry of carbons into the THF pool is via methylene THF.

C. carbons are fixed at the oxidation level at which they enter the pool.

D. the only acceptor for the methyl form is homocysteine.

E. carbons are always carried on nitrogen 10 of the pteridine ring.

10. An inability to generate tetrahydrobiopterin might be expected to:

A. inhibit the normal degradative pathway of phenylalanine.

B. lead to albinism.

C. directly prevent formation of melatonin.

D. reduce the body's ability to transfer one-carbon fragments.

E. have little or no effect on the production of catecholamines.

11. Both tyrosine aminotransferase and tryptophan oxygenase are enzymes that can be induced by adrenal glucocorticoids. This is reasonable because:

A. tyrosine and tryptophan are precursors of physiologic amines.

B. glucocorticoids work by inducing enzymes.

C. tryptophan is the precursor of nicotinic acid needed for NAD^+ synthesis.

D. tyrosine is the precursor of catecholamines in the adrenal gland.

E. these two enzymes initiate the major catabolic pathways in the liver of tyrosine and tryptophan.

12. *S*-Adenosylmethionine:

A. contains a positively charged sulfur (sulfonium) that facilitates the transfer of substituents to suitable acceptors.

B. yields α-ketobutyrate in the reaction in which the methyl is transferred.

C. donates a methyl group in a freely reversible reaction.

D. generates H_2S by transsulfuration.

E. provides the carbons for the formation of cysteine.

13. In humans, sulfur of cysteine may participate in all of the following EXCEPT:

A. the conversion of cyanide to less toxic thiocyanate.

B. the formation of thiosulfate.

C. the formation of taurine.

D. the donation of the sulfur for methionine formation.

E. the formation of PAPS.

14. All of the following are true about the branched-chain amino acids EXCEPT they:

A. are essential in the diet.

B. differ in that one is glucogenic, one is ketogenic, and one is classified as both.

C. are catabolized in a manner that bears a resemblance to β-oxidation of fatty acids.

D. are oxidized by a dehydrogenase complex to branched-chain acyl CoAs one carbon shorter than the parent compound.

E. are metabolized initially in the liver.

15. Lysine as a nutrient:

A. may be replaced by its α-keto acid analog.

B. produces pyruvate and acetoacetyl CoA in its catabolic pathway.

C. is methylated by *S*-adenosylmethionine.

D. is the only one of the common amino acids that is a precursor of carnitine.

E. all of the above are correct.

16. Histidine:

A. unlike most amino acids, is not converted to an α-keto acid when the amino group is removed.

B. is a contributor to the tetrahydrofolate one-carbon pool.

C. decarboxylation produces a physiologically active amine.

D. forms a peptide with β-alanine.

E. all of the above are correct.

17. Glutathione does all of the following EXCEPT:

A. participate in the transport of amino acids across some cell membranes.

B. scavenge peroxides and free radicals.

C. form sulfur conjugates for detoxification of compounds.

D. convert hemoglobin to methemoglobin.

E. act as a cofactor for some enzymes.

Answers

1. B A: All of the 20 common amino acids are incorporated into protein. B and E: Although most of our supply of nonessential amino acids comes from the diet, we can make them if necessary, given the precursors. C: Arginine is not believed to be required for adults (pp. 446–447).

2. D A: Most mammalian aminotransferases use glutamate or α-ketoglutarate. B: One amino acid is converted into another amino acid; there is neither net gain nor net loss. C: The reactions are freely reversible. E: Only lysine and threonine do not have aminotransferases (pp. 448–449).

3. C This is an important mechanism for reducing toxic ammonia concentrations. A: This would favor ammonia consumption. B: The cofactor is a pyridine nucleotide not pyridoxal phosphate. D: These are inhibitory. E: Since part of the role is to provide amino acid carbon chains for gluconeogenesis, this would be active (p. 450).

4. E It is in the form of the amide nitrogen of glutamine that much of amino acid nitrogen is made available in a nontoxic form (pp. 450–452).

5. C Rate must fluctuate to accommodate the amount of ammonia to be removed. A, B, and D: One of the nitrogen atoms is supplied as aspartate, with its carbon atoms being released as fumarate. This reaction is physiologically irreversible because of the hydrolysis of pyrophosphate. E: Since this is the main pathway for disposal of ammonia, any defect leads to hyperammonemia (pp. 454–456).

6. D B, C, and D: The primary control is by the allosteric effector, *N*-acetylglutamate. Synthesis of the effector, and therefore activity of CPSI, is increased in the presence of arginine. E: ATP is a substrate (p. 455).

7. E Spermidine is formed by adding propylamine to putrescine. A and B: Both amino acids give rise to glutamic semialdehyde and are formed from it. C: It is both a substrate and product of the cycle. D: This is a decarboxylation (p. 458).

8. B It is also synthesized from 3-phosphoglycerate. A: Threonine is an essential amino acid. C: It is converted to selenocysteine after forming a Ser-tRNA. D: It is *converted* to the prosthetic group (pyruvate) for that enzyme (p. 459).

9. D This forms methionine, which would become a general methyl donor. A: Methylene THF is reduced during the process of transfer to dUMP to form the thymine nucleotide. B: This is true for serine but not for other donors. C: An important aspect of the pool is interconvertibility of its forms. E: They can also be carried on nitrogen 5 or between nitrogens 5 and 10 (pp. 459–463).

10. A Tetrahydrobiopterin is a necessary cofactor for phenylalanine, tyrosine, and tryptophan hydroxylases. The first catalyzes the major pathway of phenylalanine catabolism. B: Albinism stems from a deficiency of tyrosinase, which, while giving the same product as tyrosine hydroxylase, is not a tetrahydrobiopterin-requiring enzyme. C: It would reduce the formation of the precursor, serotonin, but not melatonin itself. D: One-carbon fragments are transferred from either *S*-adenosylmethionine or the THF one-carbon pool. E: Catecholamine formation, catalyzed by the second enzyme, would also be deficient (pp. 464–467).

11. E Although all the statements are true, only E offers a suitable rationale. Tyrosine and tryptophan both yield a glucogenic fragment (fumarate and alanine) upon catabolism. Glucocorticoids are secreted in response to low blood glucose or stress (pp. 465 and 474).

12. A The reactive, positively charged sulfur reverts to a neutral thioether when the methyl group is transferred to an acceptor. B: The product, *S*-adenosylhomocysteine, is hydrolyzed to homocysteine. C: Transmethylations from AdoMet are irreversible. D: Transsulfuration refers to the combined action of cystathionine synthase and cystathionase transferring methionine's sulfur to serine to yield cysteine. E: Methionine provides only the sulfur; carbons are from serine (pp. 469–472).

13. D Methionine is the source of sulfur for cysteine (via homocysteine), but the reverse is not true in humans. A and B: Transamination to β-mercaptopyruvate with subsequent formation of thiosulfate and/or conversion of cystine to thiocysteine allows transfer of the sulfur to detoxify cyanide. C: Taurine is deaminated cysteine. E: SO_4^{2-}, the most oxidized form of sulfur found physiologically, is either excreted or activated as PAPS for use in detoxifying phenolic compounds or in biosynthesis (pp. 470 and 473).

14. E BCAA aminotransferase, the first enzyme, is much higher in muscle than in liver. B, C, and D: Although their catabolism is similar, the end products are different because of the differences in the branching. After transamination, the α-keto acids are oxidized by a dehydrogenase complex in a fashion similar to pyruvate dehydrogenase. The similarity to β-oxidation comes in steps like oxidation to an α,β-unsaturated CoA, hydration of the double bond, and oxidation of a hydroxyl to a carbonyl (pp. 476 and 479).

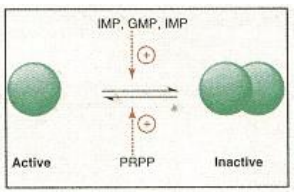
15. D A: Lysine does not participate in transamination probably in part because the α-keto acid exists as a cyclic Schiff base. B: This is one of two purely ketogenic amino acids. C and D: Free lysine is not methylated, but lysyl residues in a protein are methylated in a posttranslational modification. Intermediates of carnitine synthesis are derived from trimethyllysine liberated by proteolysis (pp. 479–481).

16. E A: Elimination of ammonia from histidine leaves a double bond (urocanate) unlike both transamination and oxidative deamination reactions. B: A portion of the ring is released as formimino THF. C and D: Histamine; carnosine (pp. 481–483).

17. D Most of the functions of glutathione listed are dependent on the sulfhydryl group (–SH). A major role of glutathione in red blood cells is reduction of methemoglobin. Glutathione reductase helps to maintain the ratio of GSH/GSSG at about 100 : 1 (p. 484).

Chapter 12—
Purine And Pyrimidine Nucleotide Metabolism

Joseph G. Cory



12.1— Overview

The material in this chapter is limited to mammalian cells and where possible to nucleotide metabolism in humans. There are major differences between nucleotide metabolism in bacteria and mammalian cells and even some differences between humans and other mammals. Purine and pyrimidine nucleotides participate in many critical cellular functions. The metabolic roles of the nucleotides range from serving as the monomeric precursors of RNA and DNA to serving as second messengers. The sources of the purine and pyrimidine nucleotides are via *de novo* synthetic pathways and salvage of exogenous and endogenous nucleobases and nucleosides. Amino acids, CO_2, and ribose 5-phosphate (from the hexose monophosphate shunt) serve as sources for carbon, nitrogen, and oxygen atoms of purines and pyrimidine nucleotides.

The intracellular concentrations of nucleotides are finely regulated by allosterically modulated enzymes in the pathways in which nucleotide end products control key steps in the pathways. 2 -Deoxyribonucleotides required for DNA replication are generated directly from ribonucleotides and these reactions are also carefully regulated by nucleotides acting as positive and negative effectors. In addition to the regulation of nucleotide metabolism via allosteric regulation, concentrations of key enzymes in the metabolic pathway are altered during the cell cycle with many of the increases in enzyme activity occurring during late G1/early S phase just preceding DNA replication.

Defects in the metabolic pathways for *de novo* synthesis or salvage of nucleotides result in clinical diseases or syndromes. Furthermore, defects in degradation of nucleotides also lead to clinical problems. These include gout (defect in *de novo* purine nucleotide synthesis), Lesch–Nyhan syndrome (defect in purine nucleobase salvage), orotic aciduria (defect in *de novo* pyrimidine nucleotide synthesis), and immunodeficiency diseases (defects in purine nucleoside degradation). Because nucleotide synthesis is required for DNA replication and RNA synthesis in dividing cells, drugs that block *de novo* pathways of nucleotide synthesis have been used successfully as antitumor and antiviral agents.

12.2— Metabolic Functions of Nucleotides

Nucleotides and their derivatives play critical and diverse roles in cellular metabolism. Many different nucleotides are present in mammalian cells. Some, such as ATP, are present in the millimolar range while others, such as cyclic AMP, are orders of magnitude lower in concentration. The functions of nucleotides include the following:

1. Role in **Energy Metabolism:** As seen in earlier chapters, ATP is the principal form of chemical energy available to cells. ATP is generated in cells via either oxidative or substrate-level phosphorylation. ATP drives reactions as a phosphorylating agent and is involved in muscle contraction, active transport, and maintenance of ion gradients. ATP also serves as phosphate donor for generation of other nucleoside 5 -triphosphates.

2. **Monomeric Units of Nucleic Acids:** RNA and DNA consist of sequences of nucleotides. Nucleoside 5 -triphosphates are substrates for reactions catalyzed by RNA and DNA polymerases.

3. **Physiological Mediators:** Nucleosides and nucleotides serve as physiological mediators of key metabolic processes. Adenosine is important in control of coronary blood flow; ADP is critical in platelet aggregation and hence blood coagulation; cAMP and cGMP act as second messengers; and GTP is required for capping of mRNA, signal transduction through GTP-binding proteins, and in microtubule formation.

4. **Precursor Function:** GTP is the precursor for formation of the cofactor, tetrahydrobiopterin, required for hydroxylation reactions and nitric oxide generation.

5. **Components of Coenzymes:** The coenzymes NAD^+, $NADP^+$, FAD and their reduced forms and coenzyme A all contain as part of their structures a 5 -AMP moiety.

6. **Activated Intermediates:** Nucleotides also serve as carriers of "activated" intermediates required for a variety of reactions. UDP-glucose is a key intermediate in synthesis of glycogen and glycoproteins. GDP-mannose, GDP-fucose, UDP-galactose, and CMP-sialic acid are all key intermediates in reactions in which sugar moieties are transferred for synthesis of glycoproteins. CTP is utilized to generate CDP-choline, CDP-ethanolamine, and CDP-diacylglycerols, which are involved in phospholipid metabolism. Other activated intermediates include *S*-adenosylmethionine (SAM) and 3 -phosphoadenosine 5 -phosphosulfate (PAPS). *S*-Adenosylmethionine is a methyl donor in reactions involving methylation of sugar and base moieties of RNA and DNA and in formation of compounds such as phosphatidylcholine from phosphatidylethanolamine and carnitine from lysine. *S*-Adenosylmethionine also provides aminopropyl groups for synthesis of spermine from ornithine. PAPS is used as the sulfate donor to generate sulfated biomolecules such as proteoglycans and sulfatides.

7. **Allosteric Effectors:** Many of the regulated steps of metabolic pathways are controlled by intracellular concentrations of nucleotides. Many examples have already been discussed in previous chapters, and the roles of nucleotides in regulation of mammalian nucleotide metabolism will be discussed in this chapter.

Distributions of Nucleotides Vary with Cell Type

The principal purine and pyrimidine compounds found in cells are the 5 -nucleotide derivatives. ATP is the nucleotide found in the highest concentration in cells. The distributions of nucleotides in cells vary with cell type. In red blood cells, adenine nucleotides far exceed the concentrations of guanine, cytosine, and uridine nucleotides; in other tissues, such as liver, there is a complete spectrum of nucleotides and their derivatives, which include NAD^+ NADH, UDP-glucose, and UDP-glucuronic acid. In normally functioning cells, nucleoside 5 -triphosphates predominate, whereas in hypoxic cells the concentrations of nucleoside 5 -monophosphates and nucleoside 5 -diphosphates are greatly increased. Free nucleobases, nucleosides, nucleoside 2 - and 3 -monophosphates, and "modified" bases represent degradation products of endogenous or exogenous nucleotides or nucleic acids.

The concentrations of **ribonucleotides** in cells are in great excess over the concentrations of **2′-deoxyribonucleotides**. For example, the concentration of ATP in Ehrlich tumor cells is 3600 pmol per 10^6 cells compared to dATP concentration of 4 pmol per 10^6 cells. However, at the time of DNA replication the concentrations of dATP and other deoxyribonucleoside 5 -triphosphates are markedly increased to meet the substrate requirements for DNA synthesis.

In normal cells, the total concentrations of nucleotides are essentially constant. Thus the total concentration of AMP plus ADP plus ATP remains constant, but there can be major changes in the individual concentration such that the ratio of ATP/(ATP + ADP + AMP) is altered depending on the energy state of the cell. The same is true for NAD^+ and NADH. The total concentration of NAD^+ plus NADH is normally fixed within rather narrow concentration limits. Consequently, when it is stated that the NADH level is increased, it follows that the concentration of NAD^+ is correspondingly decreased in that cell. The basis for this "fixed" concentration of nucleotides is that *de novo* synthesis and salvage pathways for nucleotides, nucleosides, and nucleobases are very rigidly controlled under normal conditions.

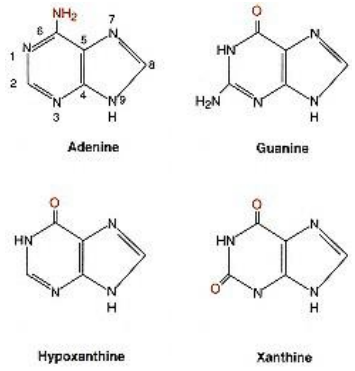


Figure 12.1
Purine bases.

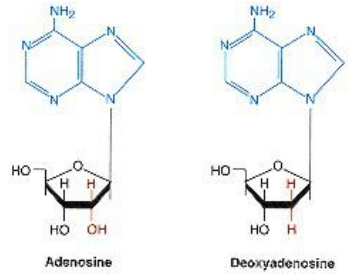


Figure 12.2
Adenosine and deoxyadenosine.

12.3— Chemistry of Nucleotides

The major purine derivatives in cells are those of **adenine** and **guanine**. Other purine bases encountered are hypoxanthine and xanthine (Figure 12.1). Nucleoside derivatives of these molecules will contain either ribose or 2-deoxyribose linked to the purine ring through a ***β*-*N*-glycosidic bond** at N-9. Ribonucleosides contain ribose, while deoxyribonucleosides contain deoxyribose as the sugar moiety (Figure 12.2). Nucleotides are **phosphate esters** of purine nucleosides (Figure 12.3). 3 -Nucleotides such as adenosine 3 -monophosphate (3 -AMP) may occur in cells as a result of nucleic acid degradation. In normally functioning cells, tri- and diphosphates are found to a greater extent than monophosphates, nucleosides, or free bases.

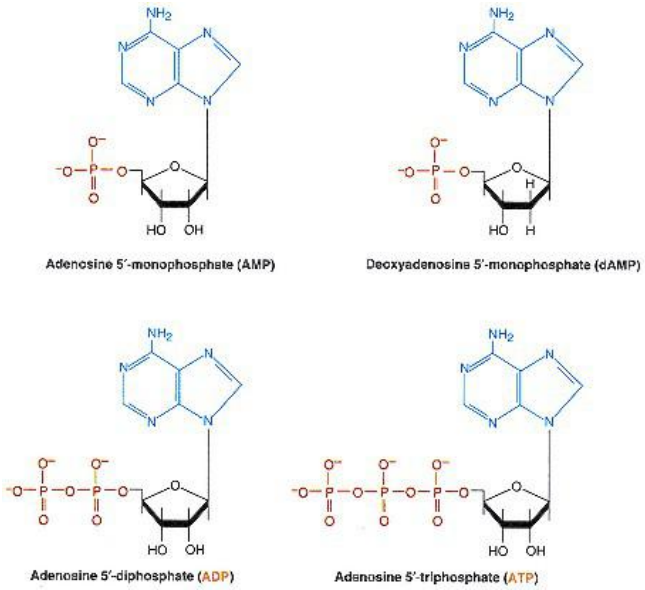


Figure 12.3
Adenine nucleotides.

The pyrimidine nucleotides found in highest concentrations in cells are those containing **uracil** and **cytosine**. The structures of the bases are shown in Figure 12.4. Uracil and cytosine nucleotides are the major pyrimidine components of RNA; cytosine and thymine are the major pyrimidine components of DNA. As with purine derivatives, the pyrimidine nucleosides or nucleotides contain either ribose or 2-deoxyribose. The sugar moiety is linked to the pyrimidine in a β-*N*-glycosidic bond at N-1. Nucleosides of pyrimidines are uridine, cytidine, and thymidine (Figure 12.5). **Phosphate esters** of pyrimidine nucleosides are UMP, CMP, and TMP. In cells the major pyrimidine derivatives are tri- and diphosphates (Figure 12.6).

See the Appendix for a summary of the nomenclature and chemistry of the purines and pyrimidines.

Figure 12.4
Pyrimidine bases.

Properties of Nucleotides

Cellular components containing either purine or pyrimidine bases can be easily detected because of their strong absorption of UV light. Purine bases, nucleosides, and nucleotides have stronger absorptions than pyrimidines and their derivatives. The wavelength of light at which maximum absorption occurs varies with the particular base component, but in most cases the UV maximum is close to 260 nm. The UV spectrum for each derivative responds differently to changes in pH. The UV absorptions provide the basis for sensitive methods in assaying these compounds. For example, deamination of adenine nucleosides or nucleotides to the corresponding hypoxanthine derivatives causes a marked shift in λ_{max} from 265 to 250 nm, which is easily determined. Because of the high molar extinction coefficients of the purine and pyrimidine bases and their high concentrations in nucleic acids, the absorbance at 260 nm can be used to quantitate the amount of nucleic acid in RNA and DNA preparations.

The *N*-glycosidic bond of nucleosides and nucleotides is stable to alkali. However, stability of this bond to acid hydrolysis differs markedly. The *N*-glycosidic bond of purine nucleosides and nucleotides is easily hydrolyzed by dilute acid at elevated temperatures (e.g., 60°C) to yield free purine base and sugar or sugar phosphate. On the other hand, the *N*-glycosidic bond of uracil, cytosine, and thymine nucleosides and nucleotides is very stable to acid treatment. Strong conditions, such as perchloric acid (60%) and 100°C, releases free pyrimidine but with complete destruction of the sugar. The *N*-glycosidic bond of dihydrouracil nucleoside and dihydrouracil nucleotide is labile in mild acid.

Because of the highly polar phosphate group, purine and pyrimidine nucleotides are much more soluble in aqueous solutions than are their nucleosides and free bases. In general, nucleosides are more soluble than free bases.

Purine and pyrimidine bases and their nucleoside and nucleotide derivatives can be easily separated by a variety of techniques. These methods include paper chromatography; thin-layer chromatography (TLC), utilizing plates with cellulose or ion-exchange resins, electrophoresis; and ion-exchange column chromatography. With high-performance liquid chromatography (HPLC) nanomole quantities of these components are easily and quickly separated and detected.

12.4— Metabolism of Purine Nucleotides

The purine ring is synthesized *de novo* in mammalian cells utilizing amino acids as carbon and nitrogen donors and also CO_2 as a carbon donor. The ***de novo* pathway** for purine nucleotide synthesis leading to **inosine 5′-monophosphate (IMP)** consists of ten metabolic steps. Hydrolysis of ATP is required to drive several reactions in this pathway. Overall, the *de novo* pathway for purine nucleotide synthesis is expensive in terms of moles of ATP utilized per mole of IMP synthesized.

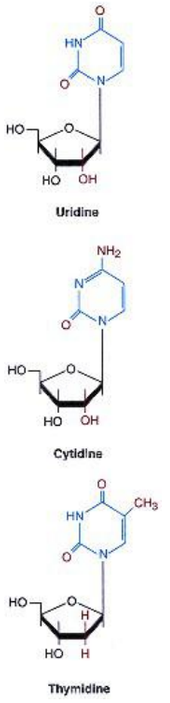


Figure 12.5
Pyrimidine nucleosides.

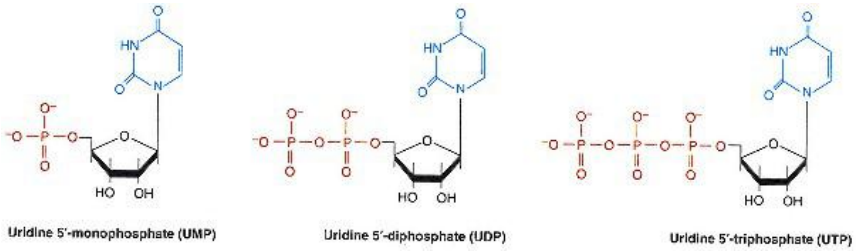


Figure 12.6
Uracil nucleotides.

Purine Nucleotides Are Synthesized by a Stepwise Buildup of the Ring to Form IMP

All enzymes involved in synthesis of purine nucleotides are found in the cytosol of the cell. However, not all cells (e.g., red cells) are capable of *de novo* purine nucleotide synthesis. In the *de novo* pathway, a stepwise series of reactions leads to synthesis of IMP, which in turn serves as the precursor for both **adenosine 5′-monophosphate** (AMP) and **guanosine 5′-monophosphate** (GMP). Since IMP serves as the common precursor for AMP and GMP and this pathway is highly regulated by AMP and GMP, IMP is not normally found to any extent in cells.

Formation of IMP is shown in Figure 12.7. Several points should be emphasized about this pathway: phosphoribosylpyrophosphate (PRPP) is synthesized from ribose 5-phosphate generated by the hexose monophosphate pathway; the equivalent of 6 moles of ATP are utilized per mole of IMP synthesized; formation of 5-phosphoribosylamine (the first step) is the committed step. In formation of **5-phosphoribosylamine**, the N–C bond is formed that will ultimately be the *N*-glycosidic bond of the purine nucleotide; there are no known regulated steps between 5-phosphoribosylamine and IMP. **Tetrahydrofolate** serves as a "C_1" carrier (***N*^{10}-formyl H_4folate**, Figure 12.8) in this pathway.

The enzyme activities catalyzing several steps in the pathway reside on separate domains of **multifunctional proteins**. The activities of 5 -phosphoribosylglycinamide synthetase, 5 -phosphoribosylglycinamide transformylase, and 5 -phosphoribosylaminoimidazole synthetase form part of a trifunctional protein. 5 -Phosphoribosylaminoimidazole carboxylase and 5 -phosphoribosyl-4-(*N*-succinocarboxamide)-5-aminoimidazole synthetase activities are on a bifunctional protein. 5 -Phosphoribosyl-4-carboxamide-5-aminoimidazole transformylase and IMP cyclohydrolase activities are present on another bifunctional protein.

To summarize, *de novo* synthesis of purine nucleotides requires amino acids as carbon and nitrogen donors, CO_2 as a carbon source, and "C_1 units" transferred via H_4folate. The contributions of these sources to the purine ring are shown in Figure 12.9. Several amino acids including serine, glycine, tryptophan, and histidine can yield "C_1 units" to H_4folate (Chapter 11) and therefore they can contribute to C-2 and C-8 of the ring. 5 -Phosphoribosyl-5-aminoimidazole carboxylase, which catalyzes the reaction in which CO_2 is used to introduce C-6 of the ring, is not a biotin-dependent carboxylase.

IMP Is the Common Precursor for AMP and GMP

IMP, the first ribonucleotide formed in the *de novo* pathway, serves as the common precursor for AMP and GMP synthesis (Figure 12.10). AMP and GMP are converted to ATP and GTP, respectively, utilizing nucleoside 5 -monophos-

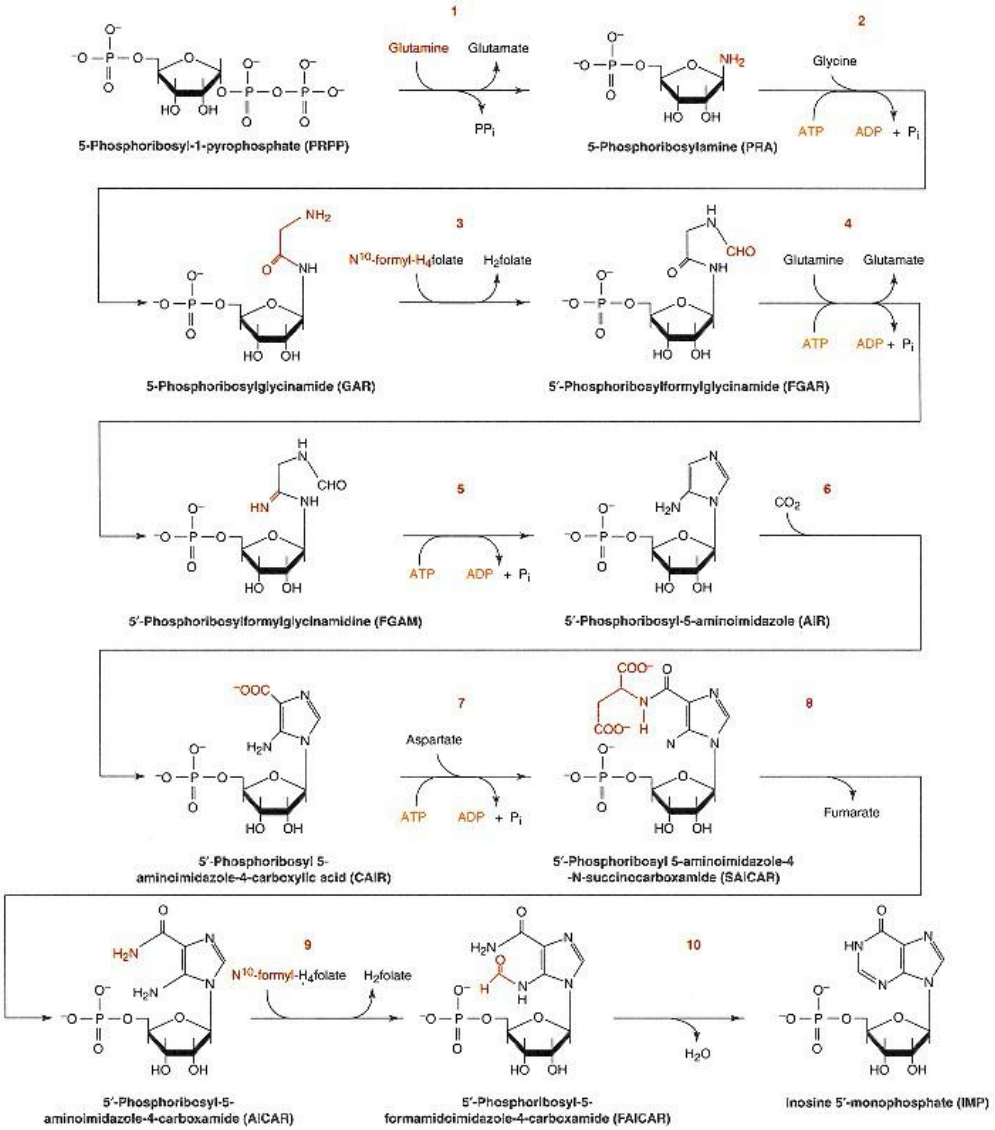


Figure 12.7
De *novo* synthesis of purine ribonucleotides.
The enzymes catalyzing the reactions are:
① glutamine PRPP amidotransferase;
② GAR synthetase;
③ GAR transformylase;
④ FGAM synthetase;
⑤ AIR synthetase;
⑥ AIR carboxylase;
⑦ SAICAR synthetase;
⑧ adenylosuccinate lyase;
⑨ AICAR transformylase; and
⑩ IMP cyclohydrolase.

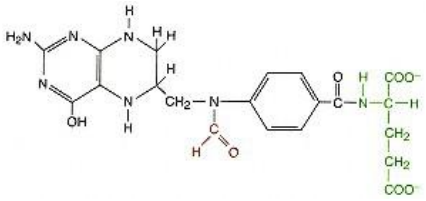


Figure 12.8
Structure of N^{10}-formyl H_4folate.

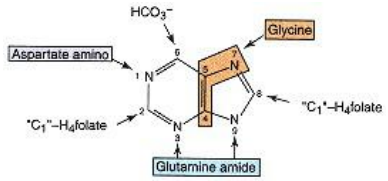


Figure 12.9
Sources of carbon and nitrogen atoms in the purine ring.
C-4, C-5, and N-7 are from glycine;
N-3 and N-9 from glutamine; C-2 and C-8 from
"C_1"-H_4folate; N-1 from aspartate; and C-6 from CO_2.

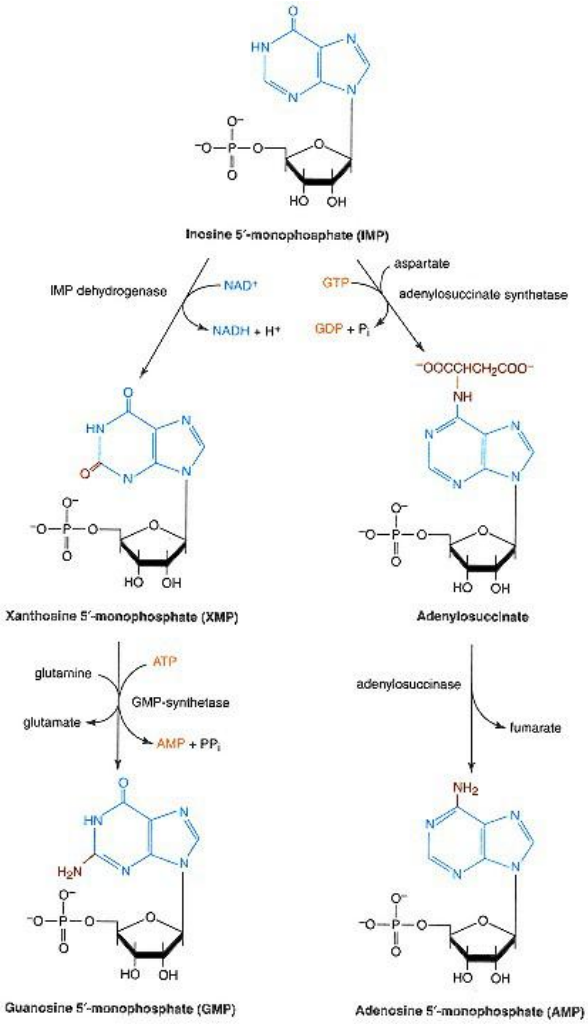


Figure 12.10
Formation of AMP and GMP from IMP branch point.

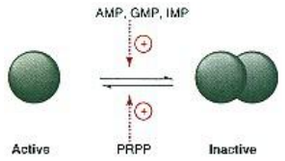


Figure 12.11
Effects of allosteric modulators on molecular forms of glutamine PRPP amido-transferase.

phate kinases and **nucleoside 5′-diphosphate kinases**. Conversion of IMP to AMP and GMP, from this branch point, does not occur randomly. Formation of GMP from IMP requires ATP as the energy source, whereas formation of AMP from IMP requires GTP as the energy source. This can be thought of as a reciprocal relationship. That is, when there is sufficient ATP in the cell, GMP will be synthesized and when there is sufficient GTP, AMP will be synthesized.

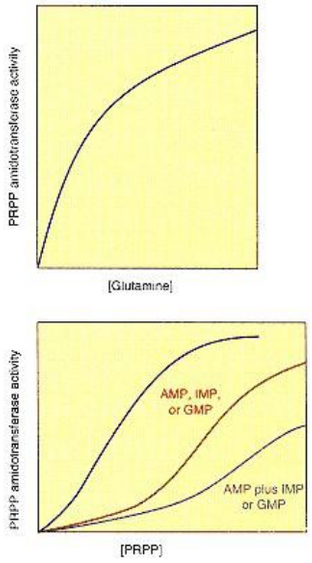


Figure 12.12
Glutamine PRPP amidotransferase activity as a function of glutamine or PRPP concentrations.

Purine Nucleotide Synthesis Is Highly Regulated

The committed step of a metabolic pathway is generally the site of metabolic regulation. In the *de novo* pathway of purine nucleotide synthesis, formation of 5-phosphoribosylamine from glutamine and **5-phosphoribosyl-1-pyrophosphate** is the committed step in IMP formation. The enzyme catalyzing this reaction, **glutamine PRPP amidotransferase**, is rate-limiting and is regulated allosterically by the end products of the pathway—IMP, GMP, and AMP. These nucleotides serve as negative effectors. On the other hand, PRPP is a positive effector. Glutamine PRPP amidotransferase is a monomer of 135 kDa that is enzymatically active. In the presence of IMP, AMP, or GMP, the enzyme forms a dimer that is much less active. The presence of PRPP favors the active monomeric form of the enzyme (Figure 12.11).

The enzyme from human tissues has distinct nucleotide-binding sites. One site specifically binds oxypurine nucleotides (IMP and GMP) while the other site specifically binds aminopurine nucleotides (AMP). When AMP and GMP or IMP are simultaneously present, the enzyme activity is synergistically inhibited. Glutamine PRPP amidotransferase displays hyperbolic kinetics with respect to glutamine as the substrate and sigmoidal kinetics with respect to PRPP (Figure 12.12). Since the intracellular concentration of glutamine is close to its K_m and the concentration varies relatively little, the glutamine concentration has little effect in regulating IMP synthesis. The intracellular concentration of PRPP, however, varies widely and can be 10 to 100 times less than the K_m for PRPP. As a result, the concentration of PRPP plays an important role in regulating synthesis of purine nucleotides.

Between the formation of 5-phosphoribosylamine and IMP, there are no known regulated steps. However, there is regulation at the branch point of IMP to AMP and IMP to GMP. From IMP to GMP, **IMP dehydrogenase** is the rate-limiting enzyme and it is regulated by GMP acting as a competitive inhibitor. **Adenylosuccinate synthetase** is the rate-limiting enzyme in conversion of IMP to AMP with AMP acting as a competitive inhibitor.

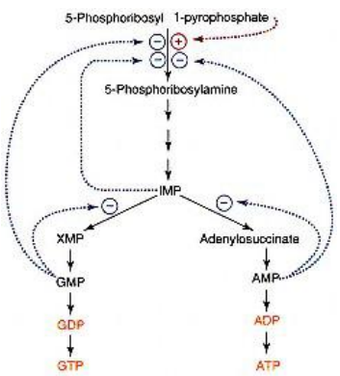


Figure 12.13
Regulation of purine nucleotide synthesis.
The dashed lines represent sites of active ⊕ or inhibition ⊖.

There must be other as yet unknown mechanisms that regulate the ATP/GTP ratio within relatively narrow limits. In most cells the total cellular concentration of adenine nucleotides (ATP plus ADP plus AMP) is four to six times that of guanine nucleotides (GTP plus GDP plus GMP). The overall regulation of purine nucleotide synthesis is summarized in Figure 12.13. Defects in the metabolic pathway that lead to loss of regulation of purine nucleotide synthesis result in overproduction of purine nucleotides and the end product, uric acid.

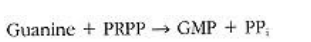
This results in a relatively common clinical condition known as gout (see Clin. Corr. 12.1).

Purine Bases and Nucleosides Can Be Salvaged to Reform Nucleotides

The efficiency of normal metabolism is shown by the presence of two distinct **"salvage pathways."** One pathway utilizes the bases, hypoxanthine, guanine, and adenine as substrates while the other pathway utilizes preformed nucleosides as the substrates. In each pathway there is specificity with respect to the base or nucleoside being "salvaged." The "salvage" of bases requires the activity of **phosphoribosyl transferases.** There are two distinct phosphoribosyl transferases. **Hypoxanthine–guanine phosphoribosyltransferase** (HGPRTase) catalyzes the reactions

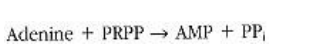
Hypoxanthine + PRPP → IMP + PP_i

and



Guanine + PRPP → GMP + PP_i

and **adenine phosphoribosyltransferase** (APRTase) catalyzes



Adenine + PRPP → AMP + PP_i

CLINICAL CORRELATION 12.1

Gout

Gout is characterized by elevated uric acid levels in blood and urine due to a variety of metabolic abnormalities that lead to overproduction of purine nucleotides via the *de novo* pathway. Many, if not all, of the clinical symptoms associated with elevated levels of uric acid arise because of the very poor solubility of uric acid in the aqueous environment. Sodium urate crystals deposit in joints of the extremities and in renal interstitial tissue, and these events tend to trigger the sequelae. Hyperuricemia from overproduction of uric acid via the *de novo* pathway can be distinguished from hyperuricemia that results from kidney disease or excessive cell death (e.g., increased degradation of nucleic acids from radiation therapy). Feeding of [^{15}N]glycine to a patient who is an overproducer will result in uric acid excreted in urine that is enriched in ^{15}N at the N-7 of uric acid while in a patient who is not an overproducer, there will be no enrichment of ^{15}N in uric acid from these patients.

Studies of "gouty" patients have shown that multiple and heterogeneous defects are the cause of overproduction of uric acid. In some cases, biochemical defects have not been defined. Examples of biochemical defects that result in increased purine nucleotide synthesis include the following:

1. Increased PRPP synthetase activity: Increased PRPP synthetase activity results in increased intracellular levels of PRPP. As discussed in the section on regulation of purine nucleotide synthesis, PRPP acts as a positive effector of glutamine–PRPP amidotransferase, leading to increased flux through the *de novo* pathway since activity of the rate-limiting step is markedly increased.

2. Partial HGPRTase activity: Partial decrease in HGPRTase activity has two fallouts with respect to the *de novo* pathway for purine nucleotide synthesis. First, since there is decreased salvage of hypoxanthine and guanine, PRPP is not consumed by the HGPRTase reaction and PRPP can activate glutamine–PRPP amidotransferase activity. Second, with decreased salvage of hypoxanthine and guanine, IMP and GMP are not formed via this pathway so that regulation of the PRPP amidotransferase step by IMP and GMP as negative effectors is compromised.

3. Glucose 6-phosphatase deficiency: In patients who have glucose 6-phosphatase deficiency (von Gierke's disease, type I glycogen storage disease) there is frequently hyperuricemia and gout as well. Loss of glucose 6-phosphatase activity results in more glucose 6-phosphate being shunted to the hexose monophosphate shunt. As a result of increased hexose monophosphate shunt activity, more ribose 5-phosphate is generated and the intracellular level of PRPP is increased. PRPP is a positive effector of PRPP amidotransferase.

These examples show that factors that increase the rate-limiting step in *de novo* synthesis of purine nucleotide synthesis lead to increased synthesis and degradation to uric acid.

There are different approaches to the treatment of gout that include colchicine, antihyperuricemic drugs, and allopurinol. Allopurinol and its metabolite, alloxanthine, are effective inhibitors of xanthine oxidase and will cause a decrease in uric acid levels. In "overproducers" who do not have a severe deficiency of HGPRTase activity, allopurinol treatment inhibits xanthine oxidase, thereby increasing the concentrations of hypoxanthine and xanthine. These purine bases are then salvaged to form IMP and XMP. These reactions consume PRPP and generate inhibitors of PRPP amidotransferase. The overall effect is that allopurinol treatment decreases both uric acid formation and *de novo* synthesis of purine nucleotides.

Becker, M. A., and Roessler, B. J. Hyperuricemia and gout. In: C. R. Scriver, A. L. Beaudet, W. S. Sly, and D. Valle (Eds.), *The Metabolic and Molecular Bases of Inherited Disease*, 7th ed., Vol. II, Chap. 49. New York: McGraw-Hill, 1995, pp. 1655–1677.

These two enzymes do not overlap in substrate specificity. The phosphoribosyl-transferase reactions are regulated by the end products of the reactions. IMP and GMP are competitive inhibitors, with respect to PRPP, of HGPRTase while AMP is a competitive inhibitor, with respect to PRPP, of APRTase. In this way, salvage of purine bases is regulated.

The hypoxanthine and guanine for salvage arise from degradation of endogenous or exogenous purine nucleotides. On the other hand, the source of adenine utilized in the APRTase reaction appears to be mainly from synthesis of polyamines (see p. 473). For each molecule of **spermine** synthesized, two molecules of **5′-methylthioadenosine** are generated that are degraded to **5-methylthioribose-1-phosphate** and adenine via the **5′-methylthioadenosine phosphorylase**-catalyzed reaction. The adenine base is salvaged through the APRTase reaction.

Generation of AMP and GMP through these phosphoribosyltransferase reactions effectively inhibits the *de novo* pathway at the PRPP amidotransferase step. First, PRPP is consumed, decreasing the rate of formation of 5-phosphoribosylamine; and second, AMP and GMP serve as feedback inhibitors at this step.

HGPRTase activity is markedly depressed in the **Lesch–Nyhan syndrome** (see Clin. Corr. 12.2), which is characterized clinically by hyperuricemia, mental retardation, and self-mutilation.

CLINICAL CORRELATION 12.2

Lesch–Nyhan Syndrome

The Lesch–Nyhan syndrome is characterized clinically by hyperuricemia, excessive uric acid production, and neurological problems, which may include spasticity, mental retardation, and self-mutilation. This disorder is associated with a very severe or complete deficiency of HGPRTase activity. The gene for HGPRTase is on the X chromosome, hence the deficiency is virtually limited to males. In a study of the available patients, it was observed that if HGPRTase activity was less than 2% of normal, mental retardation was present, and if the activity was less than 0.2% of normal, the self-mutilation aspect was expressed. This defect also leads to excretion of hypoxanthine and xanthine.

There are more than a hundred disease-related mutations defined in the HGPRTase gene from Lesch–Nyhan patients. These have led to the loss of HGPRTase protein, loss of HGPRTase activity, "K_m mutants," HGPRTase protein with a short half-life, and so on.

The role of HGPRTase is to catalyze reactions in which hypoxanthine and guanine are converted to nucleotides. The hyperuricemia and excessive uric acid production that occur in patients with the Lesch–Nyhan syndrome are easily explained by the lack of HGPRTase activity. Hypoxanthine and guanine are not salvaged, leading to increased intracellular pools of PRPP and decreased levels of IMP or GMP. Both of these factors promote *de novo* synthesis of purine nucleotides without regard for proper regulation of this pathway.

It is not understood why a severe defect in this salvage pathway leads to neurological problems. Adenine phosphoribosyltransferase activity in these patients is normal or elevated. With this salvage enzyme, presumably the cellular needs for purine nucleotides could be met by conversion of AMP to GMP via IMP if the cell's *de novo* pathway were not functioning. The normal tissue distribution of HGPRTase activity perhaps could explain the neurological symptoms. The brain (frontal lob, basal ganglia, and cerebellum) has 10–20 times the enzyme activity found in liver, spleen, or kidney and from 4 to 8 times that found in erythrocytes. Individuals who have primary gout with excessive uric acid formation and hyperuricemia do not display neurological problems. It is argued that products of purine degradation (hypoxanthine, xanthine, and uric acid) cannot be toxic to the central nervous system (CNS). However, it is possible that these metabolites are toxic to the developing CNS or that lack of this enzyme leads to an imbalance in the concentrations of purine nucleotides at critical times during development.

If IMP dehydrogenase activity in brain were extremely low, lack of HGPRTase could lead to decreased levels of intracellular GTP due to decreased salvage of guanine. Since GTP is a precursor of tetrahydrobiopterin, a required cofactor in the biosynthesis of neurotransmitters, and is required in other functions such as signal transduction via G-proteins and protein synthesis, low levels of GTP during development could be the triggering factor in the observed neurological manifestations.

Treatment of Lesch–Nyhan patients with allopurinol will decrease the amount of uric acid formed, relieving some of the problems caused by sodium urate deposits. However, since the Lesch–Nyhan patient has a marked reduction in HGPRTase activity, hypoxanthine and guanine are not salvaged, PRPP is not consumed, and consequently *de novo* synthesis of purine nucleotides is not shut down. There is no treatment for the neurological problems. These patients usually die from kidney failure, resulting from high sodium urate deposits.

Rossiter, B. J. F., and Caskey, C. T. Hypoxanthine-guanine phosphoribosyl-transferase deficiency: Lesch–Nyhan syndrome and gout. In: C. R. Scriver, A. L. Beaudet, W. S. Sly, and D. Valle (Eds.), *The Metabolic and Molecular Bases of Inherited Disease*, 7th ed., Vol II, Chap. 50. New York: McGraw Hill, 1995, pp. 1679–1706.

Overall, these salvage reactions not only conserve energy but also permit cells to form nucleotides from the bases. The erythrocyte, for example, does not have glutamine PRPP amidotransferase and hence cannot synthesize 5-phosphoribosylamine, the first unique metabolite in the pathway of purine nucleotide synthesis. As a consequence, red cells must depend on purine phosphoribosyltransferases and 5 -phosphotransferase to replenish their nucleotide pools.

Purine Nucleotides Can Be Interconverted to Maintain the Appropriate Balance of Adenine and Guanine Nucleotides

De novo synthesis of purine nucleotides is under very fine control, executed at the committed step catalyzed by **glutamine PRPP amidotransferase** and at the branch point of IMP to AMP and IMP to GMP. Additional enzymes present in mammalian cells allow for interconversions of adenine and guanine nucleotides to maintain the appropriate balance of cellular concentrations of these purine nucleotides. These interconversions occur by indirect steps. There is no direct one-step pathway for conversion of GMP to AMP or AMP to GMP. In each case, AMP or GMP is metabolized to IMP (Figure 12.14). These reactions are catalyzed by separate enzymes, each of which is under separate controls. Reductive deamination of GMP to IMP is catalyzed by **GMP reductase**. GTP activates this step while XMP is a strong competitive inhibitor of the reaction. GTP, while not required by the enzyme, increases enzyme activity by lowering the K_m with respect to GMP and by increasing V_{max}.

AMP deaminase (5 -**AMP aminohydrolase**) catalyzes deamination of AMP to IMP and is activated by K^+ and ATP and inhibited by P_i, GDP, and GTP. In the absence of K^+, the υ versus [AMP] curve is sigmoidal. The presence of K^+ is not required for maximum activity; rather K^+ is a positive allosteric effector reducing the apparent K_m for AMP.

The net effect of these reactions is that cells can interconvert adenine and guanine nucleotides to meet cellular needs, while maintaining control over these reactions.

GTP Is Precursor of Tetrahydrobiopterin

GTP is the direct precursor for **tetrahydrobiopterin** synthesis (Figure 12.15). Reactions from GTP to tetrahydrobiopterin are catalyzed by **GTP cyclohydrolase I, 6-pyruvoyl-tetrahydropterin synthase,** and **sepiapterin reductase,** with GTP cyclohydrolase I being rate-limiting. Many cell types can synthesize tetrahydrobiopterin. Tetrahydrobiopterin is a required cofactor in hydroxylation reactions involving phenylalanine, tyrosine, and tryptophan (see p. 476) and is involved in the generation of nitric oxide. Inhibitors of IMP dehydrogenase cause a marked reduction in cellular levels of tetrahydrobiopterin, demonstrating the importance of GTP as the precursor of tetrahydrobiopterin and of IMP dehydrogenase as the rate-limiting enzyme in GTP formation.

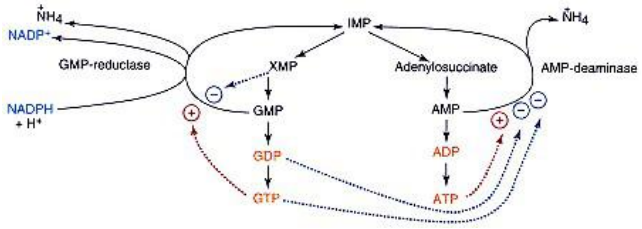


Figure 12.14
Interconversions of purine nucleotides.
The dashed lines represent sites of regulation; ⊖ inhibition.

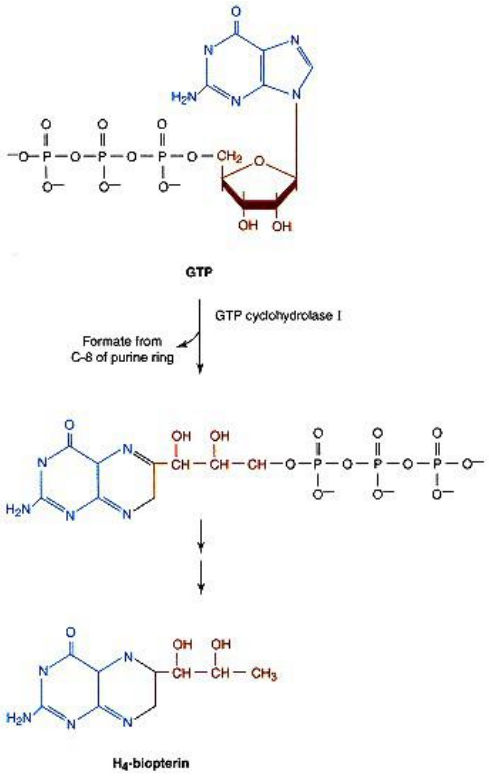


Figure 12.15
Synthesis of tetrahydrobiopterin from GTP.

End Product of Purine Degradation in Humans Is Uric Acid

The degradation of purine nucleotides, nucleosides, and bases funnel through a common pathway leading to formation of uric acid (Figure 12.16). The enzymes involved in degradation of nucleic acids and nucleotides and nucleosides vary in specificity. **Nucleases** show specificity toward either RNA or DNA and also toward the bases and position of cleavage site at the 3 ,5 -phosphodiester bonds. **Nucleotidases** range from those with relatively high specificity, such as 5 -AMP nucleotidase, to those with broad specificity, such as the acid and alkaline phosphatases, which will hydrolyze any of the 3 - or 5 -nucleotides. **AMP deaminase** is specific for AMP. **Adenosine deaminase** is less specific, since not only adenosine but also 2 -deoxyadenosine and many other 6-amino-purine nucleosides are deaminated by this enzyme.

Purine nucleoside phosphorylase catalyzes the reversible reactions

$$\text{Inosine} + P_i \rightleftharpoons \text{hypoxanthine} + \text{ribose 1-P}$$

or

$$\text{Guanosine} + P_i \rightleftharpoons \text{guanine} + \text{ribose 1-P}$$

or

$$\text{Xanthosine} + P_i \rightleftharpoons \text{xanthine} + \text{ribose 1-P}$$

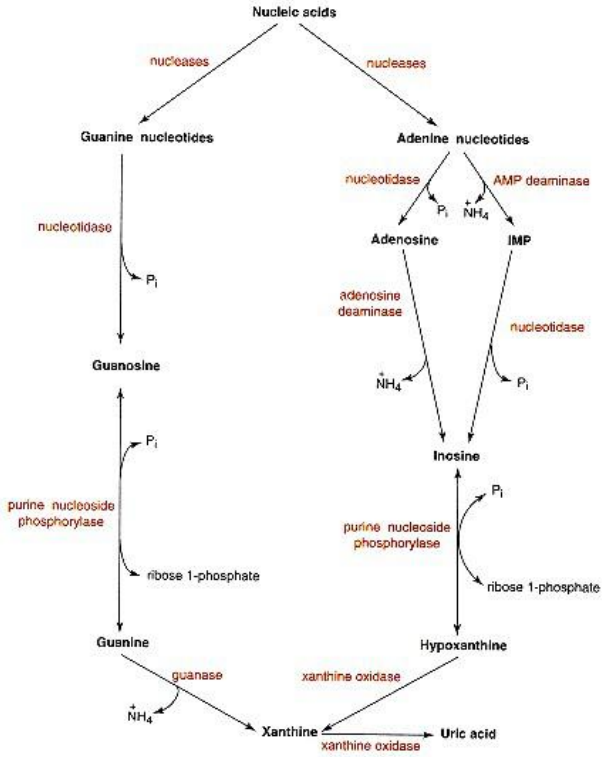
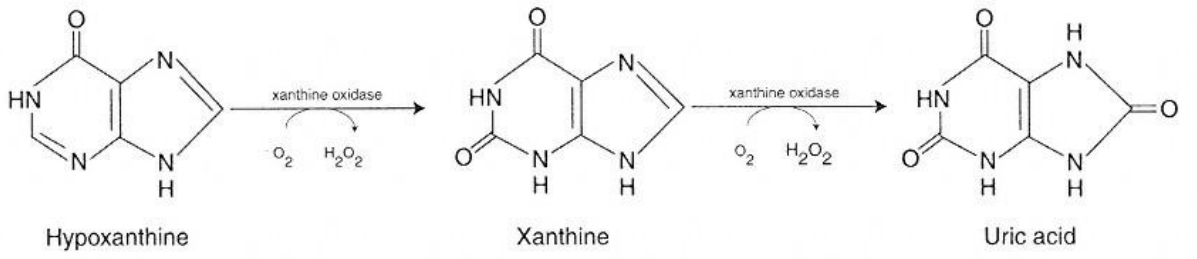


Figure 12.16
Degradation of purine nucleotides.

Deoxyinosine and deoxyguanosine are also excellent substrates for purine nucleoside phosphorylase. This is important for removal of deoxyguanosine to prevent uncontrolled accumulation of dGTP, which is toxic to cells at high concentrations. While the equilibrium constants for reactions catalyzed by purine nucleoside phosphorylase favor the direction of nucleoside synthesis, cellular concentrations of free purine base and ribose 1-phosphate are too low to support nucleoside synthesis under normal conditions. The main function of the enzyme is the degradative rather than synthetic pathway. Deficiencies in adenosine deaminase and purine nucleoside phosphorylase have been correlated with disease states in humans. Adenosine deaminase deficiency is associated with **severe combined immunodeficiency,** and purine nucleoside phosphorylase deficiency with a defective **T-cell immunity** but normal B-cell immunity (see Clin. Corr. 12.3).

Formation of Uric Acid

As seen in Figure 12.16, adenine nucleotides end up as hypoxanthine while guanine nucleotides are metabolized to xanthine. These purines are metabolized by **xanthine oxidase** to form **uric acid,** a unique end product of purine nucleotide degradation in humans. The reactions are as follows:



Xanthine oxidase is an enzyme that contains FAD, Fe, and Mo and requires molecular oxygen as a substrate. Since uric acid is not very soluble in aqueous medium, there are clinical conditions in which elevated levels of uric acid result in deposition of sodium urate crystals primarily in joints (see Clin. Corr. 12.1).

12.5— Metabolism of Pyrimidine Nucleotides

The pyrimidine ring is synthesized *de novo* in mammalian cells utilizing amino acids as carbon and nitrogen donors and CO_2 as a carbon donor. *De novo* synthesis of pyrimidine nucleotide leads to **uridine 5′-monophosphate** (UMP) in six metabolic steps. ATP hydrolysis (or equivalent) is required to drive several steps in the pathway.

CLINICAL CORRELATION 12.3

Immunodeficiency Diseases Associated with Defects in Purine Nucleoside Degradation

Two distinct immunodeficiency diseases are associated with defects in adenosine deaminase (ADA) and purine nucleoside phosphorylase (PNP), respectively. These enzymes are involved in the degradative pathways leading to formation of uric acid. Substrates for adenosine deaminase are adenosine and deoxyadenosine, while substrates for purine nucleoside phosphorylase are inosine, guanosine, deoxyinosine, and deoxyguanosine. A deficiency in ADA is associated with a severe combined immunodeficiency involving both T-cell and B-cell functions. PNP deficiency is associated with an immunodeficiency involving T-cell functions with the sparing of effects on B-cell function. In neither case is the mechanism(s) by which the lack of these enzymes leads to immune dysfunction known. However, in ADA-deficient patients, intracellular concentrations of dATP and S-adenosylhomocysteine are greatly increased. Several hypotheses have been put forth to explain the biochemical consequences of a lack of ADA: (1) high levels of dATP inhibit ribonucleotide reductase activity and as a consequence inhibit DNA synthesis; (2) deoxyadenosine inactivates S-adenosyl homocysteine hydrolase, leading to decreased *S*-adenosylmethionine required for methylation of bases in RNA and DNA; and (3) increased levels of adenosine result in increased cAMP levels. It is possible that each of these mechanisms contributes to the overall effect of immune dysfunction. There is not, however, a suitable explanation for the specificity of the effects on only T cells and B cells.

Treatment of children with ADA deficiency have included blood transfusions, bone marrow transplantation, enzyme replacement therapy with ADA–polyethylene glycol (ADA–PEG), and, most recently, gene therapy. Each of these treatments has disadvantages. Blood transfusions produce problems of "iron overload" and safety of the source. Bone marrow transplantation, while curative, requires a suitably matched donor. Enzyme replacement therapy with ADA–PEG has been the most successful to date, but the treatment requires constant monitoring of ADA levels and frequent injections of ADA–PEG, and there is considerable cost involved for the ADA–PEG. Gene therapy presents the hope for the future. While it has not been unequivocally established that gene therapy is curative, there are strong indications in early gene therapy trials that the ADA gene has been successfully transfected into stem cells of ADA-deficient children.

Cournoyer, D., and Caskey, C. T. Gene therapy of the immune system. *Annu. Rev. Immunol.* 11:297, 1993; Hershfield, M. S. PEG-ADA: an alternative to haploidentical bone marrow transplantation and an adjunct to gene therapy for adenosine deaminase deficiency. *Hum. Mutat.* 5:107, 1995; Hershfield, M. S., and Mitchell, B. S. Immunodeficiency diseases caused by adenosine deaminase deficiency and purine nucleoside phosphorylase deficiency. In: C. R. Scriver, A. L. Beaudet, W. S. Sly, and D. Valle (Eds.), *The Metabolic and Molecular Bases of Inherited Disease*, 7th ed., Vol. II, Chap. 52. New York: McGraw-Hill, 1995, pp. 1725–1768; Hoogerbrugge, P M., von Beusechem, V. W., Kaptein, L. C., Einerhard, M. P., and Valerio, D. Gene therapy for adenosine deaminase deficiency. *Br. Med. Bull.* 51:72, 1995; Markert, M. L. Molecular basis for adenosine deaminase deficiency. *Immunodeficiency* 5:141, 1994; and Markert, M. L. Purine nucleoside phosphorylase deficiency. *Immunodeficiency Rev.* 3:45, 1991.

Pyrimidine Nucleotides Are Synthesized by a Stepwise Series of Reactions to Form UMP

In contrast to *de novo* purine nucleotide synthesis, all enzymes for *de novo* synthesis of pyrimidine nucleotides are not found in the cytosol of the cell. Reactions leading to formation of UMP are shown in Figure 12.17. The following

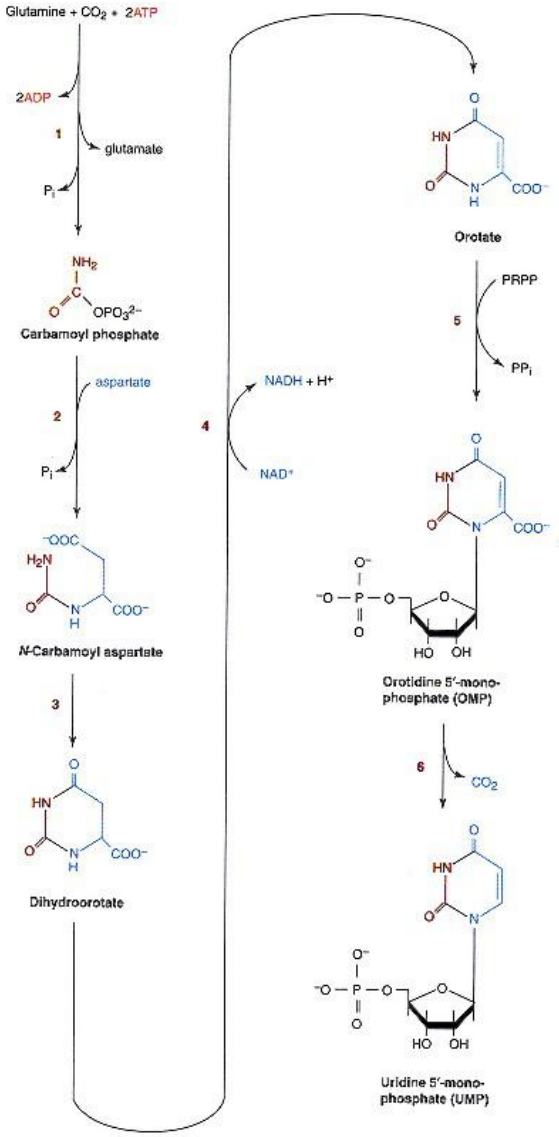


Figure 12.17
De *novo* synthesis of pyrimidine nucleotides.
Enzyme activities catalyzing the reactions are ① carbamoyl phosphate synthetase II, ② aspartate carbamoyl-transferase, ③ dihydroorotase, ④ dihydroorotate dehydrogenase, ⑤ orotate phosphoribosyltransferase, and ⑥ OMP decarboxylase. The activities of ③ are on a trifunctional protein (CAD); the activities of ⑥ are on a bifunctional protein (UMP synthase).

CLINICAL CORRELATION 12.4

Hereditary Orotic Aciduria

Hereditary orotic aciduria results from a defect in *de novo* synthesis of pyrimidine nucleotides. This genetic disease is characterized by severe anemia, growth retardation, and high levels of orotic acid excretion. The biochemical basis for this orotic aciduria is a defect in one or both of the activities (orotate phosphoribosyltransferase or orotidine decarboxylase) associated with UMP synthase, the bifunctional protein. It is a very rare disease (only 15 patients are known) but the understanding of the metabolic basis for this disease has led to successful treatment of the disorder. Patients are fed uridine, which leads not only to reversal of the hematologic problem but also to decreased formation of orotic acid. Uridine is taken up by cells and converted by uridine phosphotransferase to UMP that is converted to UDP and then to UTP. UTP formed from exogenous uridine, in turn, inhibits carbamoyl phosphate synthetase II, the major regulated step in the *de novo* pathway. As a result, orotic acid via the *de novo* pathway is markedly decreased to essentially normal levels. UTP is also a substrate for CTP synthesis. In effect, then, exogenous uridine bypasses the defective UMP synthase and supplies cells with UTP and CTP required for nucleic acid synthesis and other cellular functions. The success of treatment of hereditary orotic aciduria with uridine provides *in vivo* data regarding the importance of the carbamoyl phosphate synthase step as the site of regulation of pyrimidine nucleotide synthesis in humans.

Webster, D. R., Becroft, D. M. O., and Suttle, D. P. Hereditary orotic aciduria and other disorders of pyrimidine metabolism. In: C. R. Scriver, A. L. Beaudet, W. S. Sly, and D. Valle (Eds.), *The Metabolic and Molecular Bases of Inherited Disease*, 7th ed., Vol. II, Chap. 55. New York: McGraw-Hill, 1995, pp. 1799–1837.

important aspects of the pathway should be noted. The pyrimidine ring is formed first and then ribose 5-phosphate is added via PRPP. The enzyme catalyzing formation of carbamoyl phosphate, **carbamoyl phosphate synthetase II,** is cytosolic and is distinctly different from **carbamoyl phosphate synthetase I** found in the mitochondria as part of the urea cycle. Synthesis of *N*-carbamoylaspartate is the committed step in pyrimidine nucleotide synthesis but formation of cytosolic carbamoyl phosphate is the regulated step. Formation of **orotate** from dihydroorotate is catalyzed by a mitochondrial enzyme. Other enzymes of the pathway are found in the cytosol on multifunctional proteins. The enzyme activities of carbamoyl phosphate synthetase II, aspartate carbamoyl-transferase, and dihydroorotase are found on a trifunctional protein (CAD), and orotate phosphoribosyltransferase and OMP decarboxylase activities are found on a bifunctional protein, defined as UMP synthase. A defect in this bifunctional protein that affects either phosphoribosyltransferase activity or decarboxylase activity leads to a rare clinical condition known as hereditary orotic aciduria (see Clin. Corr. 12.4).

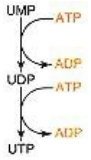


Figure 12.18
Formation of UTP from UMP.

This series of reactions produces UMP. Other major pyrimidine nucleotides found in cells are cytidine nucleotides, which are formed from UTP; UMP is converted to UTP by **nucleotide diphosphokinase** (Figure 12.18). **CTP synthetase** catalyzes formation of CTP from UTP with glutamine being the amino group donor (Figure 12.19). CTP synthetase displays homotropic sigmoi-

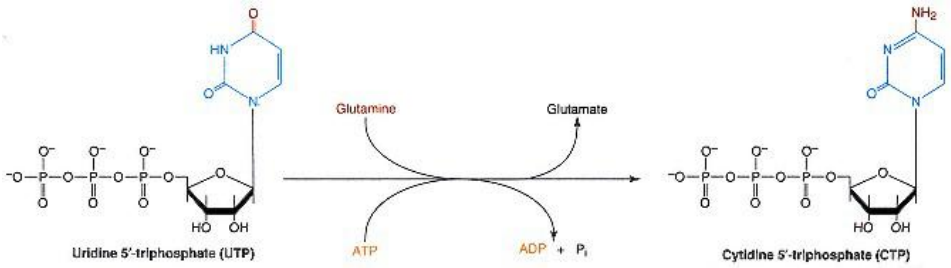


Figure
12.19 Formation of CTP from UTP catalyzed by CTP synthetase.

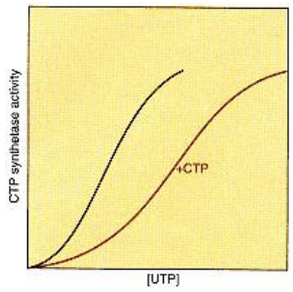


Figure 12.20
Regulation of CTP synthetase.

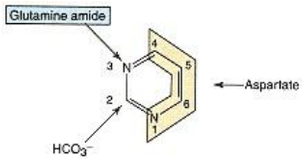


Figure 12.21
Sources of carbon and nitrogen atoms in pyrimidines.
C-4, C-5, and C-6, and N-1 are from aspartate; N-3 is from glutamine; and C-2 from CO_2.

dal kinetics; CTP, the product, is a negative effector of the reaction as shown in Figure 12.20.

To summarize, *de novo* synthesis of pyrimidine nucleotides requires aspartate and glutamine as carbon and nitrogen donors and CO_2 as a carbon donor (Figure 12.21). Five of the six reactions in the pathway take place in the cytosol of the cell, while the other reaction occurs in the mitochondria. The enzyme activities involved with the cytosolic reactions reside on multifunctional proteins. UTP is the direct precursor of CTP.

Pyrimidine Nucleotide Synthesis in Humans Is Regulated at the Level of Carbamoyl Phosphate Synthetase II

Regulation of pyrimidine nucleotide synthesis in mammalian cells occurs at the carbamoyl phosphate synthetase II step. As mentioned earlier, carbamoyl phosphate synthetase II is a cytosolic enzyme and distinct from carbamoyl phosphate synthetase I, which is mitochondrial, utilizes ammonia as the amino donor, and is activated by *N*-acetylglutamate. Carbamoyl phosphate synthetase II is inhibited by UTP, an end product of the pathway, and is activated by PRPP. K_i for UTP and K_a for PRPP are in the range of values that would allow intracellular levels of UTP and PRPP to have an effect on the control of pyrimidine nucleotide synthesis. Carbamoyl phosphate synthetase II is the only source of carbamoyl phosphate in extrahepatic tissues. However, in liver, under stressed conditions in which there is excess ammonia, carbamoyl phosphate synthetase I can generate carbamoyl phosphate in mitochondria, which ends up in the cytosol and serves as a substrate for pyrimidine nucleotide synthesis. This pathway serves to detoxify excess ammonia. Elevated levels of orotic acid are excreted as a result of ammonia toxicity in humans. This points to carbamoyl phosphate synthetase II as being the major regulated activity in pyrimidine nucleotide metabolism.

UMP does not inhibit carbamoyl phosphate synthetase II activity but does compete with OMP to inhibit the **OMP decarboxylase** (Figure 12.22). As discussed earlier, conversion of UTP to CTP is also regulated so that cells can maintain a balance between uridine and cytidine nucleotides.

Pyrimidine Bases Are Salvaged to Reform Nucleotides

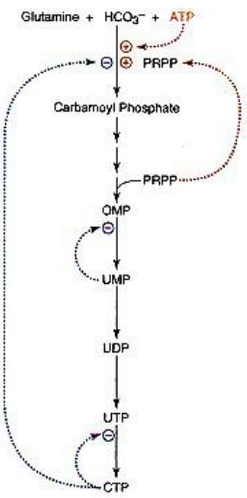


Figure 12.22
Regulation of pyrimidine nucleotide synthesis.
Solid arrows represent enzyme catalyzed reactions and dashed arrows represent activation ⊖

Pyrimidines are "salvaged" by conversion to the nucleotide level by reactions involving **pyrimidine phosphoribosyltransferase.** The general reaction is

Pyrimidine + PRPP → pyrimidine nucleoside 5′-monophosphate + PP_i

The enzyme from human erythrocytes can utilize orotate, uracil, and thymine as substrates but not cytosine. These salvage pathways divert the pyrimidine base from the degradative pathway to the nucleotide level for cellular utilization. As a pyrimidine base becomes available to cells, there are competing

reactions that will either result in degradation and excretion or reutilization of the bases. For example, when normal liver is presented with uracil, it is rapidly degraded to β-alanine, whereas proliferating tumor cells would convert uracil to UMP. This is the result of the availability of PRPP, enzyme levels, and metabolic state of the animal.

12.6— Deoxyribonucleotide Formation

As indicated previously, the concentrations of **deoxyribonucleotides** are extremely low in nonproliferating cells. Only at the time of DNA replication (S phase) does the deoxyribonucleotide pool increase to support the required DNA synthesis.

Deoxyribonucleotides Are Formed by Reduction of Ribonucleoside Diphosphates

Nucleoside 5′-diphosphate reductase (ribonucleotide reductase) catalyzes the reaction in which 2 -deoxyribonucleotides are synthesized from the corresponding ribonucleoside 5 -diphosphate. The reaction is controlled not only by the amount of enzyme present in cells but also by a very finely regulated allosteric control mechanism. The reaction can be summarized as shown in Figure 12.23. Reduction of a particular substrate requires the presence of a specific nucleoside 5 -triphosphate as a positive effector. For example, reduction of CDP or UDP requires ATP as the positive effector, while reduction of ADP and GDP require the presence of dGTP and dTTP, respectively. A small molecular weight protein, **thioredoxin** or **glutaredoxin,** is involved in reduction at the 2 position through oxidation of its sulfhydryl groups. To complete the catalytic cycle, NADPH is used to regenerate free sulfhydryl groups on the protein. **Thioredoxin reductase,** a flavoprotein, is required if thioredoxin is involved; glutathione and **glutathione reductase** are involved if glutaredoxin is the protein.

Mammalian ribonucleotide reductase consists of two nonidentical protein subunits (heterodimer), neither of which alone has enzymatic activity. The larger subunit has at least two different effector-binding sites. The smaller subunit contains a nonheme iron and a stable tyrosyl free radical. The two subunits make up the active site of the enzyme. The two subunits are encoded by different genes on separate chromosomes. The mRNAs for these subunits, and consequently the proteins, are differentially expressed as cells transit the cell cycle.

As mentioned earlier, the activity of ribonucleotide reductase is under allosteric control. While reduction of each substrate requires the presence of a specific positive effector, the products serve as potent negative effectors of the enzyme. DeoxyATP is a potent inhibitor of the reduction of all four substrates: CDP, UDP, GDP, and ADP; dGTP inhibits reduction of CDP, UDP, and GDP; dTTP inhibits reduction of CDP, UDP, and ADP. From this it is seen that dGTP

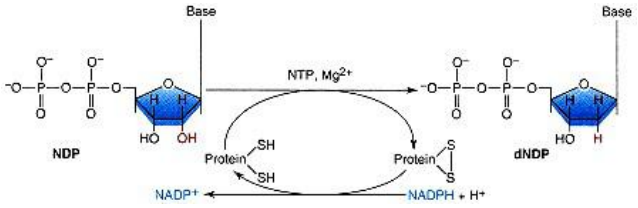


Figure 12.23
De novo synthesis of 2 -deoxyribonucleotides from ribonucleotides. This reaction is catalyzed by ribonucleotide reductase.

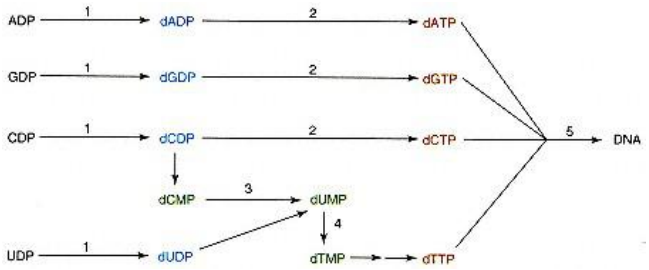


Figure 12.24
Role of ribonucleotide reductase in DNA synthesis.
The enzymes catalyzing the reactions are
(1) ribonucleotide reductase,
(2) nucleoside 5 -diphosphate kinase,
(3) deoxycytidylate deaminase,
(4) thymidylate synthase, and
(5) DNA polymerase.

and dTTP can serve as either positive or negative effectors of ribonucleotide reductase. Effective inhibition of ribonucleotide reductase by dATP, dGTP, or dTTP explains the toxicity of deoxyadenosine, deoxyguanosine, and thymidine to a variety of mammalian cells.

Ribonucleotide reductase is uniquely responsible for catalyzing the rate-limiting reactions by which 2 -deoxyribonucleoside 5 -triphosphates are synthesized *de novo* for DNA replication as summarized in Figure 12.24. Effective inhibitors of ribonucleotide reductase are potent inhibitors of DNA synthesis and hence of cell replication.

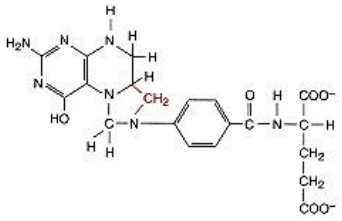
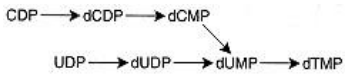


Figure 12.25
Structure of N^5,N^{10}-methylene H_4folate.

Deoxythymidylate Synthesis Requires N^5,N^{10}-Methylene H_4Folate

Deoxythymidylate (dTMP) is formed from 2 -deoxyuridine 5 -monophosphate (dUMP) in a reaction that is unique. **Thymidylate synthase** catalyzes the reaction in which a one-carbon unit from N^5,N^{10}-methylene H_4folate (Figure 12.25) is transferred to dUMP and simultaneously reduced to a methyl group. The reaction is presented in Figure 12.26. In this reaction, N^5,N^{10}-methylene H_4folate serves as the one-carbon donor and as a reducing agent. This is the only reaction in which H_4folate, acting as a one-carbon carrier, is oxidized to H_2folate. There are no known regulatory mechanisms for this reaction.

The substrate for this reaction can come from two different pathways as shown below:



In both pathways deoxyribonucleotides, dCDP or dUDP, are generated by ribonucleotide reductase. In one pathway, dUMP is generated from dUDP while in the other pathway, dCMP is deaminated to dUMP. From labeling studies it

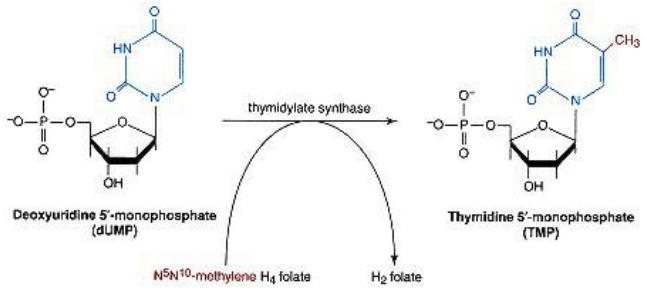


Figure 12.26
Synthesis of deoxythymidine nucleotide.

appears that the major pathway for formation of dUMP involves deamination of dCMP by **dCMP deaminase,** an enzyme that is subject to allosteric regulation by dCTP (positive) and dTTP (negative) (Figure 12.27).

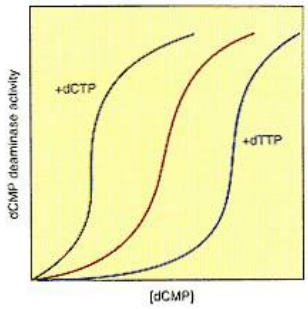


Figure 12.27
Regulation of dCMP deaminase.

Pyrimidine Interconversions with Emphasis on Deoxyribopyrimidine Nucleosides and Nucleotides

As shown in Section 12.4, there are metabolic pathways for interconversions of purine nucleotides and these pathways are regulated to maintain an appropriate balance of adenine and guanine nucleotides. Pathways also exist for interconversion of pyrimidine nucleotides and these pathways are of particular importance for pyrimidine deoxyribonucleosides and deoxyribonucleotide as summarized in Figure 12.28. Note that dCTP and dTTP are major positive and negative effectors of the interconversions and salvage of deoxyribonucleosides.

Pyrimidine Nucleotides Are Degraded to β-Amino Acids

Turnover of nucleic acids results in release of pyrimidine nucleotides and purine nucleotides (discussed previously). Degradation of pyrimidine nucleotides follows the pathways shown in Figure 12.29. In these degradative pathways the pyrimidine nucleotides are converted to nucleosides by nonspecific phosphatases. Cytidine and deoxycytidine are deaminated to uridine and deoxyuridine by pyrimidine **nucleoside deaminase. Uridine phosphorylase** catalyzes phosphorolysis of uridine, deoxyuridine, and thymidine resulting in formation of uracil and thymine as pyrimidine base products.

Uracil and thymine are then further degraded by analogous reactions, although the final products are different as shown in Figure 12.30. Uracil is degraded to β-alanine, NH_4^+, and CO_2. None of these products is unique to uracil degradation, and consequently the turnover of cytosine or uracil nucleotides cannot be estimated from the end products of this pathway. Thymine degradation proceeds to **β-aminoisobutyric acid,** NH_4^+, and CO_2. β-Aminoisobutyric acid is excreted in urine of humans and originates exclusively from degradation of thymine. Thus it is possible to estimate the turnover of DNA or thymidine nucleotides by measurement of β-aminoisobutyric acid excretion. Increased levels of β-aminoisobutyric acid are excreted in cancer patients undergoing chemotherapy or radiation therapy in which large numbers of cells are killed and DNA is degraded.

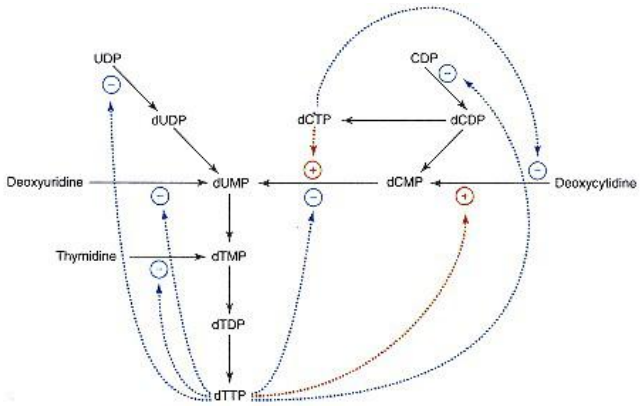


Figure 12.28
Interconversions of pyrimidine nucleotides with emphasis on deoxyribonucleotide metabolism.
The solid arrows indicate enzyme-catalyzed reactions; the dashed lines represent sites of activation ⊖.

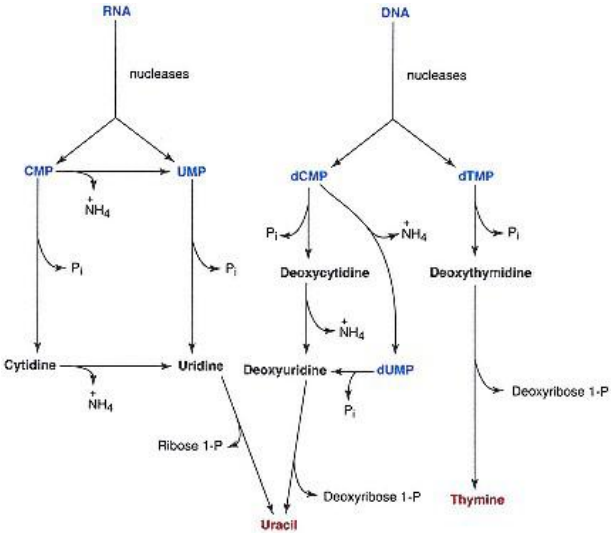


Figure 12.29
Pathways for degradation of pyrimidine nucleotides.

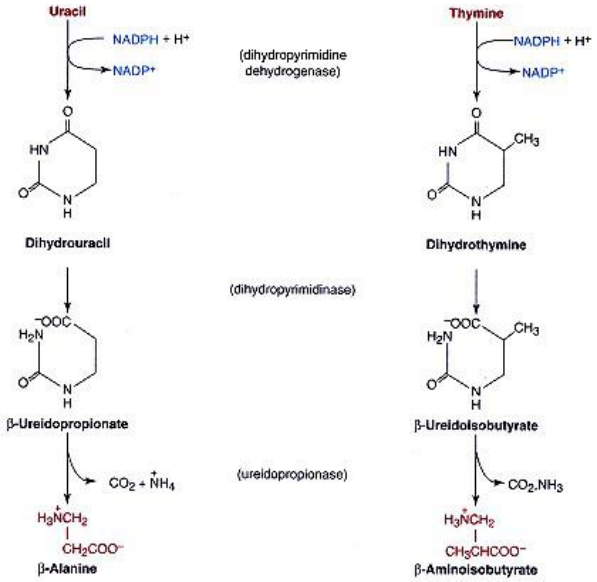


Figure 12.30
Degradation of uracil and thymine to end products.

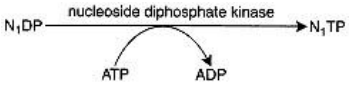
Enzymes catalyzing degradation of uracil and thymine (**dihydropyrimidine dehydrogenase, dihydropyrimidinase,** and **uriedopropionase**) do not show a preference for either uracil or thymine or their degradative intermediates.

12.7—
Nucleoside and Nucleotide Kinases

As shown in Figures 12.7 and 12.17, *de novo* synthesis of both purines and pyrimidine nucleotides yields nucleoside 5 -monophosphates. Cells contain specific nucleoside kinases that utilize nucleosides from endogenous or exogenous sources to form nucleoside 5 -monophosphates. This is particularly important in a cell such as the red cell that cannot form nucleotides *de novo*.

In addition to nucleoside kinases, there are nucleotide kinases that convert a nucleoside 5 -monophosphate to nucleoside 5 -diphosphate and nucleoside 5 -diphosphates to nucleoside 5 -triphosphates. These are important reactions since most reactions in which nucleotides function require nucleoside 5 -triphosphate (primarily) or nucleoside 5 -diphosphate.

Nucleoside kinases show a high level of specificity with respect to the base and sugar moieties. There is also substrate specificity in nucleotide kinases. On the other hand, mammalian cells contain, in high concentration, nucleoside diphosphate kinase, which is relatively nonspecific for either phosphate donor or phosphate acceptor in terms of purine or pyrimidine base or the sugar. This reaction is as follows:



Since ATP is present in the highest concentration and most readily regenerated on a net basis via glycolysis or oxidative phosphorylation, ATP is probably the major donor for these reactions.

12.8—
Nucleotide-Metabolizing Enzymes As a Function of the Cell Cycle and Rate of Cell Division

For cell division to occur, essentially all of the components of cells must double. The term **cell cycle** describes the events that lead from formation of a daughter cell, as a result of mitosis, to completion of processes needed for its own division into two daughter cells. The cell cycle is represented in Figure 12.31. The phases of the cell cycle have been defined as mitosis (M), gap 1 (G1), synthesis (S) and gap 2 (G2). Some cells will enter G0, a state in which cells are viable and functional but are in a nonproliferative or quiescent phase. The total period of the cell cycle will vary with the particular cell type. In most mammalian cell types, times for the cell cycle phases of M, S, and G2 are relatively constant, while time periods for the G1 phase vary widely, causing cells to have long or short doubling times. There are many "factors" that will cause cells to leave the G0 state and reenter the cell cycle. In preparation for **DNA replication** (S phase), there are considerable increases in synthesis of enzymes involved in nucleotide metabolism, especially during late G1/early S. While protein and RNA synthesis occur throughout G1, S, and G2 phases of the cell cycle, DNA replication occurs only during S phase.

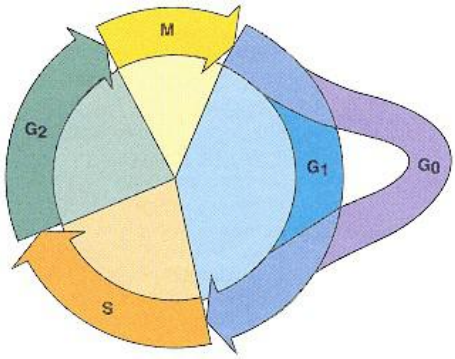


Figure 12.31
Diagrammatic representation of the cell cycle.
For a mammalian cell with a doubling time of 24 h, G1 would last ~12 h; S, 7 h; G2, 4 h; and M, 1 h. Cells would enter the G0 state if they became quiescent or nonproliferative.

Enzymes of Purine and Pyrimidine Nucleotide Synthesis Are Elevated during S Phase

Strict regulation of nucleotide synthesis requires that certain mechanisms must be available to the cell to meet the requirements for ribonucleotide and deoxyribonucleotide precursors at the time of increased RNA synthesis and DNA replication. To meet these needs, cells respond by increasing levels of specific enzymes involved with nucleotide formation during very specific periods of the cell cycle.

Enzymes involved in purine nucleotide synthesis and interconversions that are elevated during the S phase of the cell cycle are PRPP amidotransferase and IMP dehydrogenase. Adenylosuccinate synthetase and adenylosuccinase do not appear to increase. Enzymes involved in pyrimidine nucleotide synthesis that are elevated during the S phase of the cell cycle include aspartate carbamoyl-transferase, dihydroorotase, dihydroorotate dehydrogenase, orotate phosphoribosyltransferase, and CTP synthetase. Many enzymes involved in synthesis and interconversions of deoxyribonucleotides are also elevated during the S phase of the cell cycle. Included in these enzymes are ribonucleotide reductase, thymidine kinase, dCMP deaminase, thymidylate synthase, and TMP kinase. The importance of increased levels of enzyme activities during late G1/early S phase to DNA replication is worthy of further discussion with a specific example.

As discussed previously, the deoxyribonucleotide pool is extremely small in "resting" cells (less than 1 μM). As a result of the increase in ribonucleotide reductase, deoxyribonucleotides reach levels of 10–20 μM during DNA synthesis. However, this concentration would sustain DNA synthesis for only minutes, while complete DNA replication would require hours. Consequently, levels of ribonucleotide reductase activity not only must increase but must be sustained during S phase in order to provide the necessary substrates for DNA synthesis.

If we look at a population of cells (i.e., tissue) rather than individual cells going through the cell cycle, we observe that rapidly growing tissues such as regenerating liver, embryonic tissues, intestinal mucosal cells, and erythropoietic cells are geared toward DNA replication and RNA synthesis. These tissues will show elevated levels of those key enzymes involved with purine and pyrimidine nucleotide synthesis and interconversions and complementary decreases in levels of enzymes that catalyze reactions in which these precursors are degraded. These changes reflect the proportion of cells in that tissue that are in S phase.

There is an ordered pattern of biochemical changes that occur in tumor cells. Utilizing a series of liver, colon, and kidney tumors of varying growth rates, it has been possible to define these biochemical changes (1) **transformation-linked** (meaning that all tumors regardless of growth rate show certain increased and certain decreased enzyme levels), (2) **progression-linked** (alterations that correlate with growth rate of tumors), and (3) **coincidental alterations** (not connected to the malignant state). As very limited examples, levels of ribonucleotide reductase, thymidylate synthase, and IMP dehydrogenase increase as a function of tumor growth rate. PRPP amidotransferase, UDP kinase, and uridine kinase are examples of enzymes whose activity is increased in all tumors, whether they are slow-growing or the most rapidly growing tumors.

Alterations in gene expression in tumor cells are not only quantitative changes in enzyme levels but also qualitative changes (isozyme shifts). While some enzymes are increased in both fast-growing normal tissue (e.g., embryonic and regenerating liver) and tumors, the total quantitative and qualitative patterns for normal and tumor tissue can be distinguished.

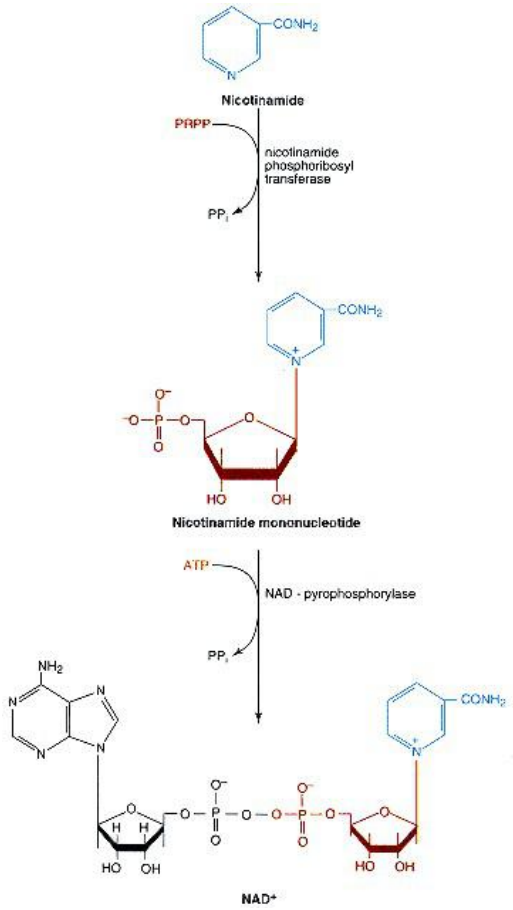


Figure 12.32
Pathway for NAD^+ synthesis.

12.9— Nucleotide Coenzyme Synthesis

Nicotinamide adenine dinucleotide (NAD^+), flavin adenine nucleotide (FAD), and coenzyme A (CoA) serve as important coenzymes in intermediary metabolism. These coenzymes are synthesized by a variety of mammalian cell types. Figures 12.32 (p. 513), 12.33, and 12.34 present the biosynthetic pathways for each. NAD^+ synthesis requires niacin, FAD synthesis requires riboflavin, and CoA requires pantothenic acid. NAD can be synthesized by three different pathways starting from tryptophan (see p. 475), nicotinate, or nicotinamide. When tryptophan is in excess of the amount needed for protein synthesis and serotonin synthesis, it is used for NAD^+ synthesis. This situation is not likely in most normal diets and, consequently, niacin is required in the diet.

Each of these coenzymes has an AMP moiety as part of the molecule.

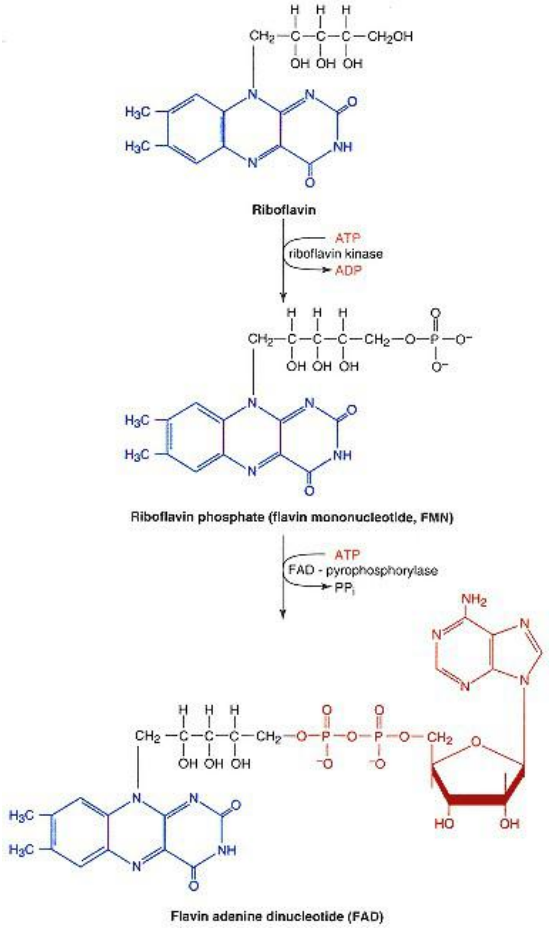


Figure 12.33
Synthesis of FAD.

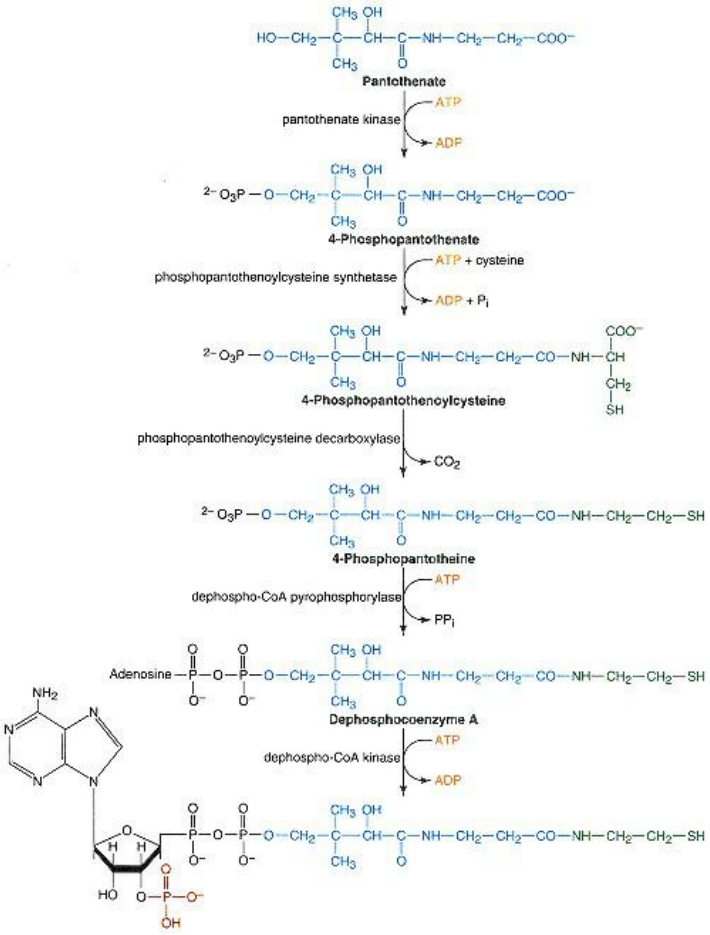


Figure 12.34
Synthesis of CoA.

However, the AMP is not directly involved in the functional part of the molecule since electron transfer in NAD^+ or FAD occurs via the niacin or riboflavin rings, respectively, and activation of acyl groups occurs through the –SH group of CoA. Synthesis of NAD^+ by any of the three pathways requires utilization of PRPP as the ribose 5-phosphate donor. Nicotinamide adenine dinucleotide phosphate ($NADP^+$) is synthesized from NAD^+. NAD^+ is used not only as a cofactor in oxidation–reduction reactions but also as a substrate in ADP-ribosylation reactions (e.g., DNA repair and pertussis toxin poisoning). These reactions lead to the turnover of NAD^+. The end product of NAD^+ degradation is 2-pyridone-5-carboxamide, which is excreted in urine. Synthesis of nucleotide coenzymes is regulated such that there are essentially "fixed" concentrations of these coenzymes in the cell. When the statement is made that a certain

metabolic condition is favored when the concentration of NAD^+ is low, it means that the concentration of NADH is correspondingly high.

12.10— Synthesis and Utilization of 5-Phosphoribosyl-1-Pyrophosphate

5-Phosphoribosyl-1-pyrophosphate (PRPP) is a key molecule in *de novo* synthesis of purine and pyrimidine nucleotides, salvage of purine and pyrimidine bases, and synthesis of NAD^+. PRPP synthetase catalyzes the reaction presented in Figure 12.35. Ribose 5-phosphate used in this reaction is generated from glucose 6-phosphate metabolism via the **hexose monophosphate shunt** or from ribose 1-phosphate (generated by phosphorolysis of nucleotides) via a phosphoribomutase reaction.

The enzyme has an absolute requirement for inorganic phosphate and is strongly regulated. The *v* versus [P_i] curve for PRPP synthetase activity is sigmoidal rather than hyperbolic, meaning that at the normal cellular concentration of P_i, the enzyme activity is depressed. The enzyme activity is further regulated by ADP, 2,3-bisphosphoglycerate, and other nucleotides. ADP serves as a competitive inhibitor of PRPP synthetase with respect to ATP; 2,3-bisphosphoglycerate is a competitive inhibitor with respect to ribose 5-phosphate; and nucleotides serve as noncompetitive inhibitors with respect to both substrates. 2,3-Bisphosphoglycerate may be important in regulating PRPP synthetase activity in red cells.

Levels of PRPP are low in "resting" or confluent cells but increase rapidly at the time of rapid cell division. Increased flux of glucose 6-phosphate through the hexose monophosphate shunt can result in increased cellular levels of PRPP and increased production of purine and pyrimidine nucleotides. PRPP is important not only because it serves as a substrate in the glutamine PRPP amidotransferase reaction and the phosphoribosyltransferase reactions, but also because it serves as a positive effector of the major regulated steps in purine and pyrimidine nucleotide synthesis, namely, PRPP amidotransferase and carbamoyl phosphate synthetase II.

Reactions and pathways in which PRPP is required are as follows:

1. *De novo* purine nucleotide synthesis

a. PRPP + glutamine → 5-phosphoribosylamine + glutamate + PP_i

2. Salvage of purine bases

a. PRPP + hypoxanthine (guanine) → IMP (GMP) + PP_i

b. PRPP + adenine → AMP + PP_i

3. *De novo* pyrimidine nucleotide synthesis

a. PRPP + orotate → OMP + PP_i

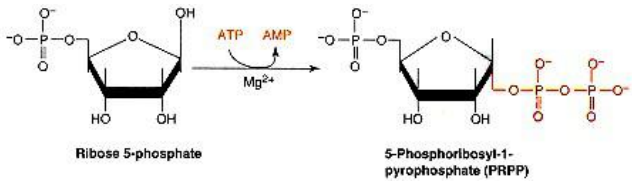


Figure 12.35
Synthesis of PRPP.

4. Salvage of pyrimidine bases

a. PRPP + uracil → UMP + PP_i

5. NAD^+ synthesis

a. PRPP + nicotinate → nicotinate mononucleotide + PP_i

b. PRPP + nicotinamide → nicotinamide mononucleotide + PP_i

c. PRPP + quinolinate → nicotinate mononucleotide + PP_i

12.11— Compounds That Interfere with Cellular Purine and Pyrimidine Nucleotide Metabolism: Chemotherapeutic Agents

De novo synthesis of purine and pyrimidine nucleotides is critical to normal cell replication, maintenance, and function. Regulation of these pathways is important since disease states arise from defects in the regulatory enzymes. Many compounds have been synthesized or isolated as natural products from plants, bacteria, or fungi that are structural analogs of the bases or nucleosides used in metabolic reactions. These compounds are relatively specific inhibitors of enzymes involved in nucleotide synthesis or interconversions and have proved to be useful in therapy of diverse clinical problems. They are generally classified as antimetabolites, antifolates, glutamine antagonists, and other compounds.

Antimetabolites Are Structural Analogs of Bases or Nucleosides

Antimetabolites, generally, are structural analogs of purine and pyrimidine bases or nucleosides that interfere with very specific metabolic sites. They include **6-mercaptopurine** and **6-thioguanine** for treatment of acute leukemia, **azathioprine** for immunosuppression in patients with organ transplants, **allopurinol** for treatment of gout and hyperuricemia, and **acyclovir** for treatment of herpesvirus infection. The detailed understanding of purine nucleotide metabolism aided in the development of these drugs. Conversely, study of the mechanism of action of these drugs has led to a better understanding of normal nucleotide metabolism in humans.

Only a few of these will be discussed to show (1) the importance of *de novo* pathways in normal cell metabolism, (2) that regulation of these pathways occurs *in vivo*, (3) the concept of the requirement for metabolic activation of the drugs, and (4) that inactivation of these compounds can greatly influence their usefulness.

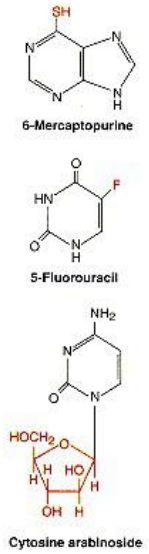


Figure 12.36
Structures of 6-mercaptopurine, 5-fluorouracil, and cytosine arabinoside.

6-Mercaptopurine (6-MP) (Figure 12.36) is a useful antitumor drug in humans. The cytotoxic activity of this agent is related to formation of 6-mercaptopurine ribonucleotide by the tumor cell. Utilizing PRPP and HGPRTase, 6-mercaptopurine ribonucleoside 5 -monophosphate accumulates in cells and is a negative effector of PRPP amidotransferas, the committed step in the *de novo* pathway. This nucleotide also acts as an inhibitor of the conversion of IMP to GMP at the IMP dehydrogenase step and IMP to AMP at the adenylosuccinate synthetase step. Since 6-mercaptopurine is a substrate for xanthine oxidase and is oxidized to 6-thiouric acid, allopurinol is generally administered to inhibit degradation of 6-MP and to potentiate the antitumor properties of 6-MP.

5-Fluorouracil (Fura) (Figure 12.36) is a pyrimidine analog of uracil. 5-Fluorouracil is, of itself, not the active species. It must be converted by cellular enzymes to the active metabolites 5-fluorouridine 5 -triphosphate (FUTP) and

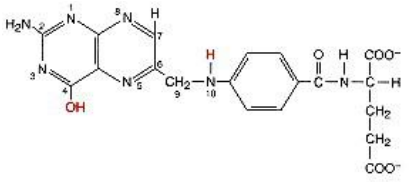
5-fluoro-2′-deoxyuridine 5′-monophosphate (FdUMP). FUTP is efficiently incorporated into RNA and once incorporated into RNA inhibits maturation of 45S precursor rRNA into the 28S and 18S species and alters splicing of pre-mRNA into functional mRNA. FdUMP is a potent and specific inhibitor of thymidylate synthase. In the presence of H_4folate, FdUMP, and thymidylate synthase, a ternary complex is formed that results in covalent bonding of FdUMP to thymidylate synthase. This results in inhibition of dTMP synthesis and leads, in effect, to what is called a "thymineless death" for cells.

Cytosine arabinoside (araC) (Figure 12.36) is used in treatment of several forms of human cancer. AraC must be metabolized by cellular enzymes to cytosine arabinoside 5 -triphosphate (araCTP) to exert its cytotoxic effects. AraCTP competes with dCTP in the DNA polymerase reaction and araCMP is incorporated into DNA. This results in inhibition of synthesis of the growing DNA strand. Clinically, the efficacy of araC as an antileukemic drug correlates with the concentration of araCTP that is achieved in the tumor cell, which in turn determines the level of araCMP incorporated into DNA. Formation of araCMP via deoxycytidine kinase appears to be the rate-limiting step in activation to araCTP.

Antifolates Inhibit Formation of Tetrahydrofolate

Antifolates interfere with formation of H_4folate from H_2folate or folate by inhibition of H_2folate reductase. **Methotrexate** (MTX), a close structural analog of folic acid, is used as an antitumor agent in treatment of human cancers. The comparison of the two structures is seen in Figure 12.37. Differences are at C-4 where an amino group replaces a hydroxyl group and at N-10 where a methyl group replaces a hydrogen atom. The mode of action of MTX is specific; it inhibits H_2folate reductase with a K_i in the range of 0.1 nM. The reactions inhibited are shown in Figure 12.38.

MTX at very low concentrations is cytotoxic to mammalian cells in culture. The effects can be prevented by addition of thymidine and hypoxanthine to the culture medium. Reversal of the MTX effects by thymidine and hypoxanthine indicates that MTX causes depletion of thymidine and purine nucleotides in cells. Figure 12.39 shows the relationship between H_4folate, *de novo* purine



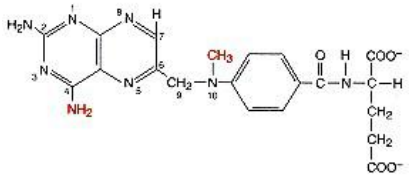


Figure 12.37
Comparison of the structures of folic acid and methotrexate.

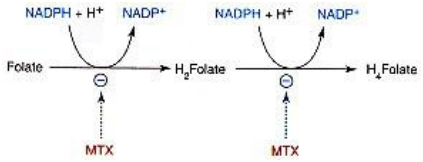


Figure 12.38
Sites of inhibition of methotrexate.

nucleotide synthesis, and dTMP formation. It is important to note that in the thymidylate synthase reaction, H_2folate is generated and unless it can readily be reduced back to H_4folate via dihydrofolate reductase, cells would not be capable of *de novo* synthesis of purine nucleotides or thymidylate synthesis due to depletion of H_4folate pools.

In treatment of human leukemias, normal cells can be rescued from the toxic effects of "high-dose MTX" by N^5-formyl-H_4folate **(leucovorin).** This increases the clinical efficacy of MTX treatment.

Glutamine Antagonists Inhibit Enzymes that Utilize Glutamine as Nitrogen Donors

Many reactions in mammalian cells utilize glutamine as the amino group donor. This is different from bacterial cells that primarily utilize ammonia as the amino donor in a similar reaction. These amidation reactions are critical in *de novo* synthesis of purine nucleotide (N-3 and N-9), synthesis of GMP from IMP, formation of cytosolic carbamoyl phosphate, synthesis of CTP from UTP, and synthesis of NAD^+.

Compounds that inhibit these reactions are referred to as glutamine antagonists. **Azaserine** (*O*-diazoacetyl-L-serine) and **6-diazo-5-oxo-L-norleucine** (DON) (Figure 12.40), which were first isolated from cultures of *Streptomyces*, are very effective inhibitors of enzymes that utilize glutamine as the amino donor. Since azaserine and DON inactivate the enzymes involved, addition of glutamine alone will not reverse the effects of either of these two drugs. It would necessitate that many metabolites such as guanine, cytosine, hypoxanthine (or adenine), and nicotinamide be provided to bypass the many sites blocked by these glutamine antagonists. As expected from the fact that so many key steps

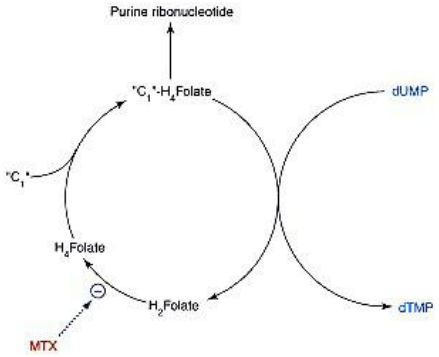


Figure 12.39
Relationships between
H_4folate, *de novo* purine nucleotide
synthesis, and dTMP synthesis.

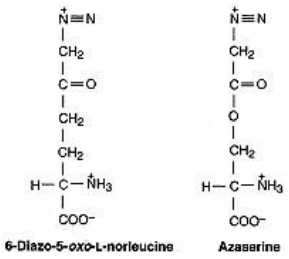


Figure 12.40
Structure of glutamine antagonists.

are inhibited by DON and azaserine, these agents are extremely toxic and not of clinical use.

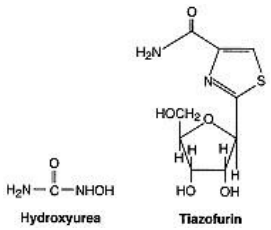


Figure 12.41
Structure of hydroxyurea and tiazofurin.

Other Agents Inhibit Cell Growth by Interfering with Nucleotide Metabolism

Tumor cells treated with **hydroxyurea** (Figure 12.41) show a specific inhibition of DNA synthesis with little or no inhibition of RNA or protein synthesis. Hydroxyurea is an inhibitor of ribonucleotide reductase, blocking reduction of CDP, UDP, GDP, and ADP to the corresponding 2 -deoxyribonucleoside 5 -diphosphates. Toxicity of this drug results from depletion of 2 -deoxyribonucleoside 5 -triphosphates required for DNA replication. Although hydroxyurea is specific for inhibition of ribonucleotide reductase, its clinical use is limited because of its rapid rate of clearance and the high drug concentration required for effective inhibition.

Tiazofurin (Figure 12.41) is converted by cellular enzymes to the NAD^+ analog, **tiazofurin adenine dinucleotide** (TAD). TAD inhibits IMP dehydrogenase, the rate-limiting enzyme in GTP synthesis, with a K_i of 0.1 μM. As a result of IMP dehydrogenase inhibition, the concentration of GTP is markedly depressed.

These clinically useful drugs serve as examples in which knowledge of basic biochemical pathways and mechanisms leads to generation of effective drugs. An important point regarding many of the antimetabolites used as drugs is that they must be activated to the nucleotide level by cellular enzymes to exert their cytotoxic effects.

Purine and Pyrimidine Analogs As Antiviral Agents

Herpesvirus (HSV) and **human immunodeficiency virus** (HIV) infections (AIDS) present major clinical problems. Two antimetabolites have been identified that can be used in the control/treatment (but not cure) of HSV and HIV infections. These drugs—**acyclovir** (acycloguanosine), a purine analog, and **3′-azido-3′-deoxythymidine** (AZT), a pyrimidine analog (Figure 12.42)—require metabolism to phosphorylated compounds to yield the active drug. Acycloguanosine is activated to the monophosphate by a specific HSV–thymidine kinase, encoded by the HSV genome, which can catalyze phosphorylation of acycloguanosine. The host cellular thymidine kinase cannot utilize acyclovir as a substrate. Acycloguanosine monophosphate is then phosphorylated by the cellular enzymes to the di- and triphosphate forms. Acycloguanosine triphosphate serves as a substrate for the HSV-specific DNA polymerase and is incorporated into the growing viral DNA chain causing chain termination. The specificity of acycloguanosine and its high therapeutic index therefore reside in the fact that only HSV-infected cells can form the acycloguanosine monophosphate.

AZT is phosphorylated by cellular kinases to AZT triphosphate, which blocks HIV replication by inhibiting HIV–DNA polymerase (an RNA-dependent polymerase). The selectivity of AZT for HIV-infected versus uninfected cells occurs because DNA polymerase from HIV is at least 100-fold more sensitive to AZT triphosphate than is host cell DNA-dependent DNA polymerase.

Figure 12.42
Structure of the antiviral agents, acyclovir and AZT.

These two antiviral agents demonstrate the diversity of responses required for selectivity. In one case, enzyme activity encoded by the viral genome is mandatory for activation of the drug (acycloguanosine); in the second example, although cellular enzymes activate the drug (AZT), the viral gene product (HIV–DNA polymerase) is the selective target.

Biochemical Basis for Development of Drug Resistance

Failure of chemotherapy in treatment of human cancer is often related to development of tumor cell populations that are resistant to the cytotoxic effects of

the particular drug. Tumors represent a very heterogenous population of cells and in many instances drug-resistant cells are present. Upon therapy, drug-sensitive cells are killed off and a resistant cell population becomes enriched. In some cases, drug treatment causes genetic alterations that result in the drug-resistant phenotype. Resistance to drugs can be categorized as "specific drug resistance" or "multidrug resistance."

Biochemical and molecular mechanisms that account for drug resistance have been determined for many drugs. For example, resistance to methotrexate can develop as a result of several different alterations. These include a defect or loss of the transporter for N^5-formyl-H_4folate and N^5-methyl-H_4folate, which results in decreased cellular uptake of MTX; amplification of the dihydrofolate reductase gene, which results in a marked increase in cellular dihydrofolate reductase, the target enzyme; alterations in the dihydrofolate reductase gene that result in a "mutant" dihydrofolate reductase that is less sensitive to inhibition by MTX; and decreased levels of folylpolyglutamate synthetase, which results in lower levels of polyglutamylated MTX, the "trapped" form of MTX. A MTX-resistant population could have any one or a combination of these alterations. The net result of any of these resistance mechanisms is to decrease the ability of MTX to inhibit dihydrofolate reductase at clinically achievable MTX concentrations. Other specific drug resistance mechanisms could be described for compounds such as cytosine arabinoside, 5-fluorouracil, and hydroxyurea.

In multiple drug resistance, the drug-resistant population is cross-resistant to a series of seemingly unrelated antitumor agents. These compounds include drugs such as the vinca alkaloids, adriamycin, actinomycin D, and etoposide. All of these drugs are natural products or derived from natural products and they are not chemically related in structure. They have different mechanisms of action as antitumor agents but appear to act on some nuclear event.

Multidrug-resistant tumor cells express high levels (compared to the drug-sensitive tumor cell phenotype) of a protein called MDR1 (P-glycoprotein) or another protein called MRP (multidrug resistance protein). These proteins are membrane bound, have a mass around 170 kDa, but are distinctly separate proteins. These proteins function as "pumps" to efflux drugs from cells. As a result of increased efflux of drugs, caused by the ATP-dependent efflux proteins, the cellular concentration of drug is decreased below its cytotoxic concentration.

Development of drug-resistant tumor cells presents major clinical problems. Study of the mechanisms of drug resistance has greatly aided in our understanding of cancer cells.

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Questions

C. N. Angstadt and J. Baggott

1. Nucleotides serve all of the following roles EXCEPT:

   A. monomeric units of nucleic acids.

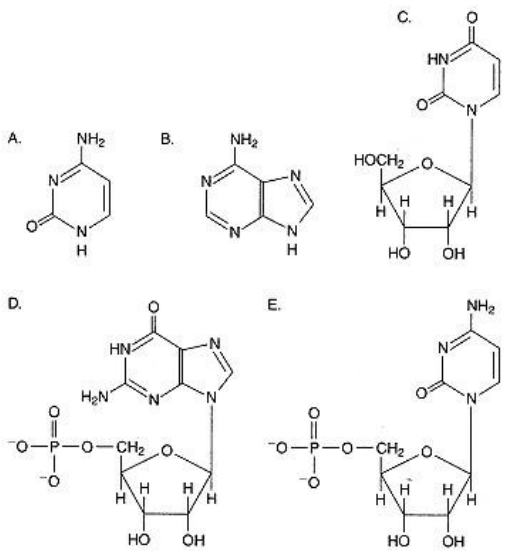
   B. physiological mediators.

   C. sources of chemical energy.

   D. structural components of membranes.

   E. structural components of coenzymes.

Refer to the following figure for Questions 2–4.



2. Identify adenine.

3. Identify a pyrimidine nucleoside.

4. Identify CMP.

5. The amide nitrogen of glutamine is a source of nitrogen for the:

A. *de novo* synthesis of purine nucleotides.

B. *de novo* synthesis of pyrimidine nucleotides.

C. synthesis of GMP from IMP.

D. all of the above.

E. none of the above.

6. The enzyme catalyzing the rate-limiting step of the *de novo* synthesis of purine nucleotides:

A. is a multifunctional protein.

B. uses PRPP as a substrate.

C. requires AMP for activity.

D. is controlled primarily by substrate availability.

E. shows sigmoidal kinetics with respect to both of its substrates.

7. The two purine nucleotides found in RNA:

A. are formed in a branched pathway from a common intermediate.

B. are formed in a sequential pathway.

C. must come from exogenous sources.

D. are formed by oxidation of the deoxy forms.

E. are synthesized from nonpurine precursors by totally separate pathways.

8. Which of the following is/are aspects of the overall regulation of *de novo* purine nucleotide synthesis?

A. AMP, GMP, and IMP cause a shift of PRPP amidotransferase from a small form to a large form.

B. PRPP levels in the cell can be severalfold less than the K_m of PRPP amidotransferase for PRPP.

C. GMP is a competitive inhibitor of IMP dehydrogenase.

D. All of the above are correct.

E. None of the above is correct.

9. The type of enzyme known as a phosphoribosyltransferase is involved in all of the following EXCEPT:

A. salvage of pyrimidine bases.

B. the *de novo* synthesis of pyrimidine nucleotides.

C. the *de novo* synthesis of purine nucleotides.

D. salvage of purine bases.

10. Uric acid is:

A. formed from xanthine in the presence of O_2.

B. a degradation product of cytidine.

C. deficient in the condition known as gout.

D. a competitive inhibitor of xanthine oxidase.

E. oxidized, in humans, before it is excreted in urine.

11. In nucleic acid degradation, all of the following are correct EXCEPT:

A. there are nucleases that are specific for either DNA or RNA.

B. nucleotidases convert nucleotides to nucleosides.

C. the conversion of a nucleoside to a free base is an example of hydrolysis.

D. because of the presence of deaminases, hypoxanthine rather than adenine is formed.

E. a deficiency of adenosine deaminase leads to an immunodeficiency.

12. In the *de novo* synthesis of pyrimidine nucleotides:

A. reactions take place exclusively in the cytosol.

B. a free base is formed as an intermediate.

C. PRPP is required in the rate-limiting step.

D. UMP and CMP are formed from a common intermediate.

E. UMP inhibition of OMP decarboxylase is the major control of the process.

13. Deoxyribonucleotides:

A. cannot be synthesized so they must be supplied preformed in the diet.

B. are synthesized *de novo* using dPRPP.

C. are synthesized from ribonucleotides by an enzyme system involving thioredoxin.

D. are synthesized from ribonucleotides by nucleotide kinases.

E. can be formed only by salvaging free bases.

14. β-Aminoisobutyrate:

A. is an intermediate in the degradation of both uracil and thymine.

B. in the urine can be used to estimate the turnover of DNA.

C. arises from uracil by cleavage of the pyrimidine ring.

D. is in equilibrium with β-alanine.

E. is the end product common to the degradation of both uracil and thymine.

15. The conversion of nucleoside 5 -monophosohates to nucleoside 5 -triphosphates:

A. is catalyzed by nucleoside kinases.

B. is a direct equilibrium reaction.

C. utilizes a relatively specific nucleotide kinase and a relatively nonspecific nucleoside diphosphate kinase.

D. generally uses GTP as a phosphate donor.

E. occurs only during the S phase of the cell cycle.

16. If a cell were unable to synthesize PRPP, which of the following processes would likely be directly impaired?

A. FAD synthesis

B. NAD synthesis

C. coenzyme A synthesis

D. ribose 5-phosphate synthesis

E. dTMP synthesis

17. Which of the following antitumor agents works by impairing *de novo* purine synthesis?

A. acyclovir (acycloguanosine)

B. 5-fluorouracil (antimetabolite)

C. methotrexate (antifolate)

D. hydroxyurea

E. allopurinol

Answers

1. D Both cAMP and cGMP are physiological mediators. NAD, FAD, and CoA all contain AMP as part of their structures (pp. 490–491).

2. B Adenine is the free purine (A is a pyrimidine.)

3. C A nucleoside contains a base plus sugar but no phosphate.

4. E CMP is a pyrimidine nucleotide. (D is a purine nucleotide; pp. 492–493).

5. D Nitrogen atoms 3 and 9 of purine nucleotides (p. 495, Fig. 12.7) and N-3 of pyrimidine nucleotides (p. 504, Fig. 12.17) are supplied by glutamine in *de novo* synthesis. The 2-amino group of GMP also comes from this source.

6. B The rate-limiting step of purine nucleotide synthesis is the amido transfer between glutamine and PRPP (p. 494). A: There are several multifunctional proteins in the pathway but this is not one. C: It is inhibited by AMP. D: This is a typical allosteric enzyme. Know the things that control it. E: As expected for an allosteric enzyme, it does show sigmoidal kinetics but only with respect to PRPP. The kinetics for glutamine are hyperbolic.

7. A GMP and AMP are both formed from the first purine nucleotide, IMP, in a branched pathway (p. 494). B: The pyrimidine nucleotides UMP and CTP are formed in a sequential pathway from orotic acid (p. 505). D: Deoxy forms are formed by reduction of the ribose forms.

8. D A is the mechanism of inhibition since the large form of the enzyme is inactive (p. 497). B: PRPP amidotransferase shows sigmoidal kinetics with respect to PRPP so large shifts in concentration of PRPP have the potential for altering velocity (p. 497). C plays a major role in controlling the branched pathway of IMP to GMP or AMP (p. 497).

9. C In purine nucleotide synthesis, the purine ring is built up stepwise on ribose-5-phosphate and not transferred to it (p. 497). A, B, and D: Phosphoribosyltransferases are important salvage enzymes for both purines and pyrimidines (pp. 506–507) and are also part of the synthesis of pyrimidines since OPRT catalyzes the conversion of orotate to OMP (pp. 503–504).

10. A The xanthine oxidase reaction produces uric acid. B and E: Uric acid is an end product of purines, not pyrimidines. C: Gout is characterized by excess uric acid (p. 502, Fig. 12.16).

11. C The product is ribose 1-phosphate rather than the free sugar, a phosphorolysis. A: They can also show specificity toward the bases and positions of cleavage. B: A straight hydrolysis. D: AMP deaminase and adenosine deaminase remove the 6-NH_2 as NH_3. The IMP or inosine formed is eventually converted to hypoxanthine (p. 502, Fig. 12.16). E: This is called severe combined immunodeficiency (pp. 501–502).

12. B This is in contrast to purine *de novo* synthesis. A: One enzyme is mitochondrial. C: PRPP is required to convert orotate to OMP but this is not rate-limiting. D: OMP to UMP to CTP is a sequential process. E: This does occur but the rate-limiting step is that catalyzed by CPS II (pp. 502–506).

13. C Deoxyribonucleotides are synthesized from the ribonucleoside diphosophates by nucleoside diphosphate reductase that uses thioredoxin as the direct hydrogen-electron donor (p. 507). A, B, and E: There is a synthetic mechanism as just described but it is not a *de novo* pathway. D: Nucleotide kinases are enzymes that add phosphate to a base or nucleotide.

14. B This compound originates exclusively from thymine, which is found primarily in DNA. A and E: It is an end product of degradation but only of thymine. C and D: β-Alanine arises from cleavage of the uracil ring (p. 509).

15. C These two enzymes are important in interconverting the nucleotide forms. A: These convert nucleosides to nucleoside monophosphates. B: Two steps are required. D: ATP is present in highest concentration and is the phosphate donor. E: Occurs during the S phase but this is a general reaction for the cell (pp. 509–510).

16. D PRPP is formed from ribose 5-phosphate in an irreversible reaction. E: dTMP is formed directly from dUMP, which doesn't have to be made *de novo* (p. 507).

17. C Antifolates reduce the concentration of THF compounds that are necessary for two steps of purine synthesis. A: This is an antiviral agent that inhibits DNA synthesis. B: 5-Fluorouracil is a pyrimidine analog not a purine analog. D: Hydroxyurea inhibits the reduction of ribonucleotides to deoxyribonucleotides so it is not involved in *de novo* purine synthesis. E: Allopurinol potentiates the effect of 6-mercaptopurine but is not an inhibitor of purine synthesis (pp. 517–520).