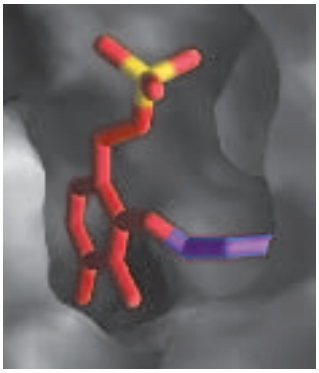


chapter 18

AMINO ACID OXIDATION AND THE PRODUCTION OF UREA

I chose the study of the synthesis of urea in the liver because it appeared to be a relatively simple problem.

—*Hans Krebs, article in* Perspectives in Biology and Medicine, *1970*

We now turn our attention to the amino acids, the final class of biomolecules that, through their oxidative degradation, make a significant contribution to the generation of metabolic energy. The fraction of metabolic energy obtained from amino acids, whether they are derived from dietary protein or from tissue protein, varies greatly with the type of organism and with metabolic conditions. Carnivores can obtain (immediately following a meal) up to 90% of their energy requirements from amino acid oxidation, whereas herbivores may fill only a small fraction of their energy needs by this route. Most microorganisms can scavenge amino acids from their environment and use them as fuel when required by metabolic conditions. Plants, however, rarely if ever oxidize amino acids to provide energy; the carbohydrate produced from CO_2 and H_2O in photosynthesis is generally their sole energy source. Amino acid concentrations in plant tissues are carefully regulated to just meet the requirements for biosynthesis of proteins, nucleic acids, and other molecules needed to support growth. Amino acid catabolism does occur in plants, but its purpose is to produce metabolites for other biosynthetic pathways.

In animals, amino acids undergo oxidative degradation in three different metabolic circumstances:

1. During the normal synthesis and degradation of cellular proteins (protein turnover; Chapter 27), some amino acids that are released from protein breakdown and are not needed for new protein synthesis undergo oxidative degradation.
2. When a diet is rich in protein and the ingested amino acids exceed the body's needs for protein synthesis, the surplus is catabolized; amino acids cannot be stored.
3. During starvation or in uncontrolled diabetes mellitus, when carbohydrates are either unavailable or not properly utilized, cellular proteins are used as fuel.

Under all these metabolic conditions, amino acids lose their amino groups to form α-keto acids, the "carbon skeletons" of amino acids. The α-keto acids undergo oxidation to CO_2 and H_2O or, often more importantly, provide three- and four-carbon units that can be converted by gluconeogenesis into glucose, the fuel for brain, skeletal muscle, and other tissues.

The pathways of amino acid catabolism are quite similar in most organisms. The focus of this chapter is on the pathways in vertebrates, because these have received the most research attention. As in carbohydrate and fatty acid catabolism, the processes of amino acid degradation converge on the central catabolic pathways, with the carbon skeletons of most amino acids finding their way to the citric acid cycle. In some cases the reaction pathways of amino acid breakdown closely parallel steps in the catabolism of fatty acids (Chapter 17).

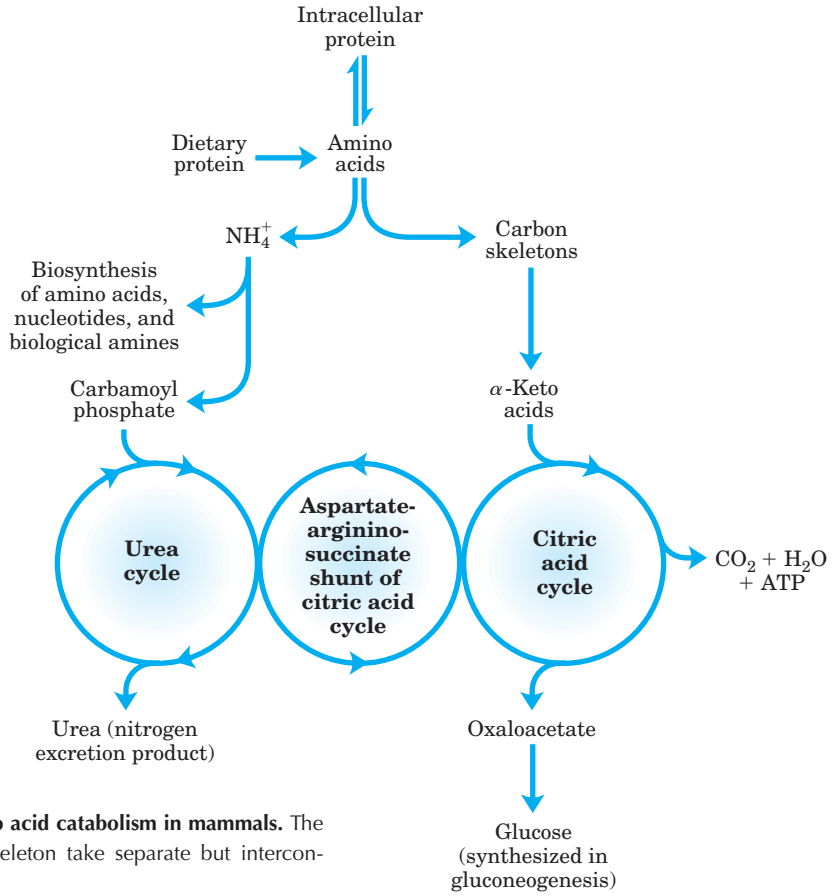


FIGURE 18–1 Overview of amino acid catabolism in mammals. The amino groups and the carbon skeleton take separate but interconnected pathways.

One important feature distinguishes amino acid degradation from other catabolic processes described to this point: every amino acid contains an amino group, and the pathways for amino acid degradation therefore include a key step in which the α-amino group is separated from the carbon skeleton and shunted into the pathways of amino group metabolism (Fig. 18–1). We deal first with amino group metabolism and nitrogen excretion, then with the fate of the carbon skeletons derived from the amino acids; along the way we see how the pathways are interconnected.

18.1 Metabolic Fates of Amino Groups

Nitrogen, N_2, is abundant in the atmosphere but is too inert for use in most biochemical processes. Because only a few microorganisms can convert N_2 to biologically useful forms such as NH_3 (Chapter 22), amino groups are carefully husbanded in biological systems.

Figure 18–2a provides an overview of the catabolic pathways of ammonia and amino groups in vertebrates. Amino acids derived from dietary protein are the source of most amino groups. Most amino acids are metabolized in the liver. Some of the ammonia generated in this process is recycled and used in a variety of biosynthetic pathways; the excess is either excreted directly or converted to urea or uric acid for excretion, depending on the organism (Fig. 18–2b). Excess ammonia generated in other (extrahepatic) tissues travels to the liver (in the form of amino groups, as described below) for conversion to the excretory form.

Glutamate and glutamine play especially critical roles in nitrogen metabolism, acting as a kind of general collection point for amino groups. In the cytosol of hepatocytes, amino groups from most amino acids are transferred to α-ketoglutarate to form glutamate, which enters mitochondria and gives up its amino group to form NH_4^+. Excess ammonia generated in most other tissues is converted to the amide nitrogen of glutamine, which passes to the liver, then into liver mitochondria. Glutamine or glutamate or both are present in higher concentrations than other amino acids in most tissues.

In skeletal muscle, excess amino groups are generally transferred to pyruvate to form alanine, another important molecule in the transport of amino groups to the liver.

We begin with a discussion of the breakdown of dietary proteins, then give a general description of the metabolic fates of amino groups.

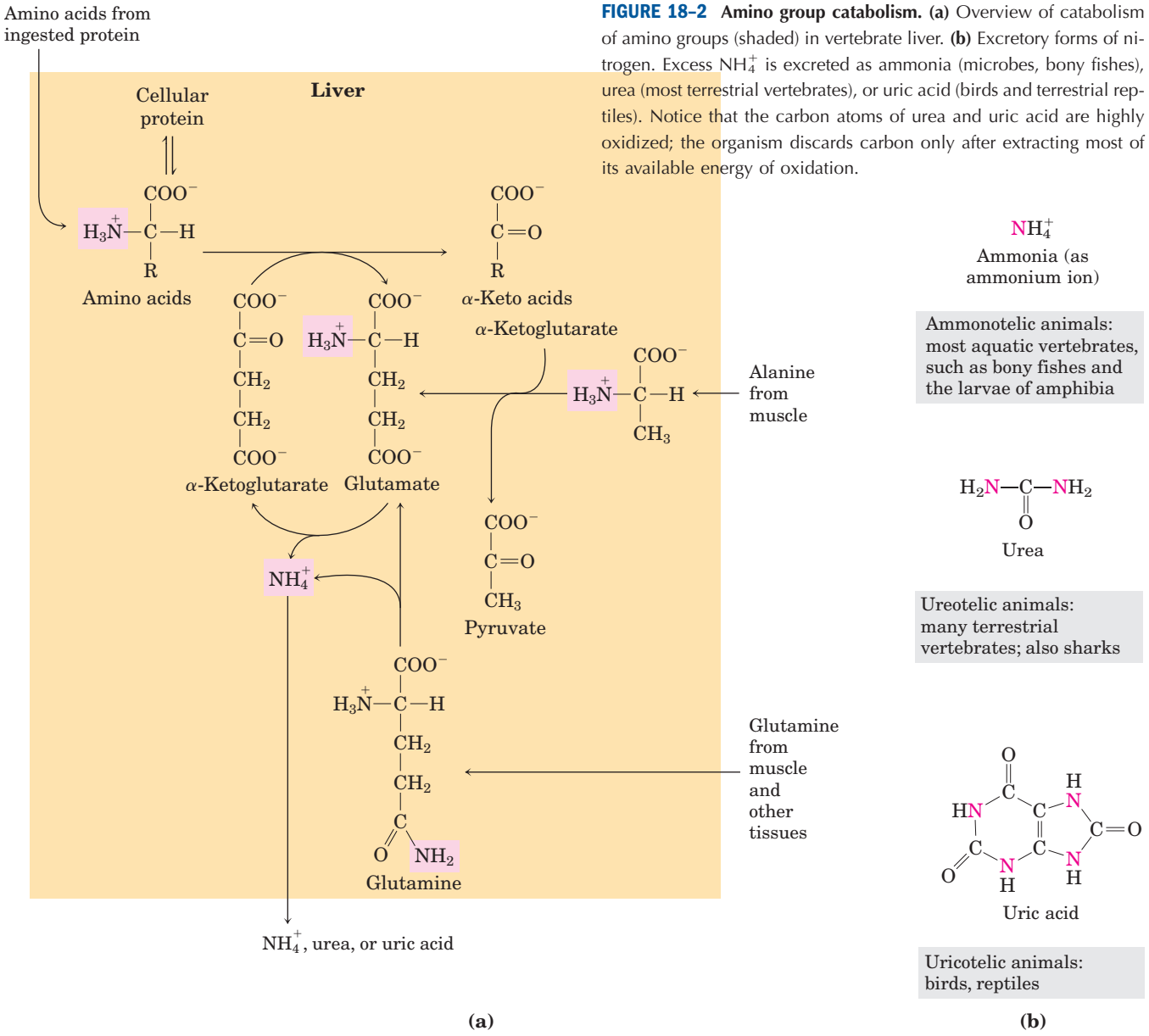


FIGURE 18–2 **Amino group catabolism. (a)** Overview of catabolism of amino groups (shaded) in vertebrate liver. **(b)** Excretory forms of nitrogen. Excess NH_4^+ is excreted as ammonia (microbes, bony fishes), urea (most terrestrial vertebrates), or uric acid (birds and terrestrial reptiles). Notice that the carbon atoms of urea and uric acid are highly oxidized; the organism discards carbon only after extracting most of its available energy of oxidation.

Dietary Protein Is Enzymatically Degraded to Amino Acids

In humans, the degradation of ingested proteins to their constituent amino acids occurs in the gastrointestinal tract. Entry of dietary protein into the stomach stimulates the gastric mucosa to secrete the hormone **gastrin,** which in turn stimulates the secretion of hydrochloric acid by the parietal cells and pepsinogen by the chief cells of the gastric glands (Fig. 18–3a). The acidic gastric juice (pH 1.0 to 2.5) is both an antiseptic, killing most bacteria and other foreign cells, and a denaturing agent, unfolding globular proteins and rendering their internal peptide bonds more accessible to enzymatic hydrolysis. **Pepsinogen** (M_r 40,554), an inactive precursor, or zymogen (p. 231), is converted to active pepsin (M_r 34,614) by the enzymatic action of pepsin itself. In the stomach, pepsin hydrolyzes ingested proteins at peptide bonds on the amino-terminal side of the aromatic amino acid residues Phe, Trp, and Tyr (see Table 3–7), cleaving long polypeptide chains into a mixture of smaller peptides.

As the acidic stomach contents pass into the small intestine, the low pH triggers secretion of the hormone **secretin** into the blood. Secretin stimulates the pancreas to secrete bicarbonate into the small intestine to neutralize the gastric HCl, abruptly increasing the pH to about 7. (All pancreatic secretions pass into the small intestine through the pancreatic duct.) The digestion of proteins now continues in the small intestine. Arrival of amino acids in the upper part of the intestine (duodenum) causes release into the blood of the hormone

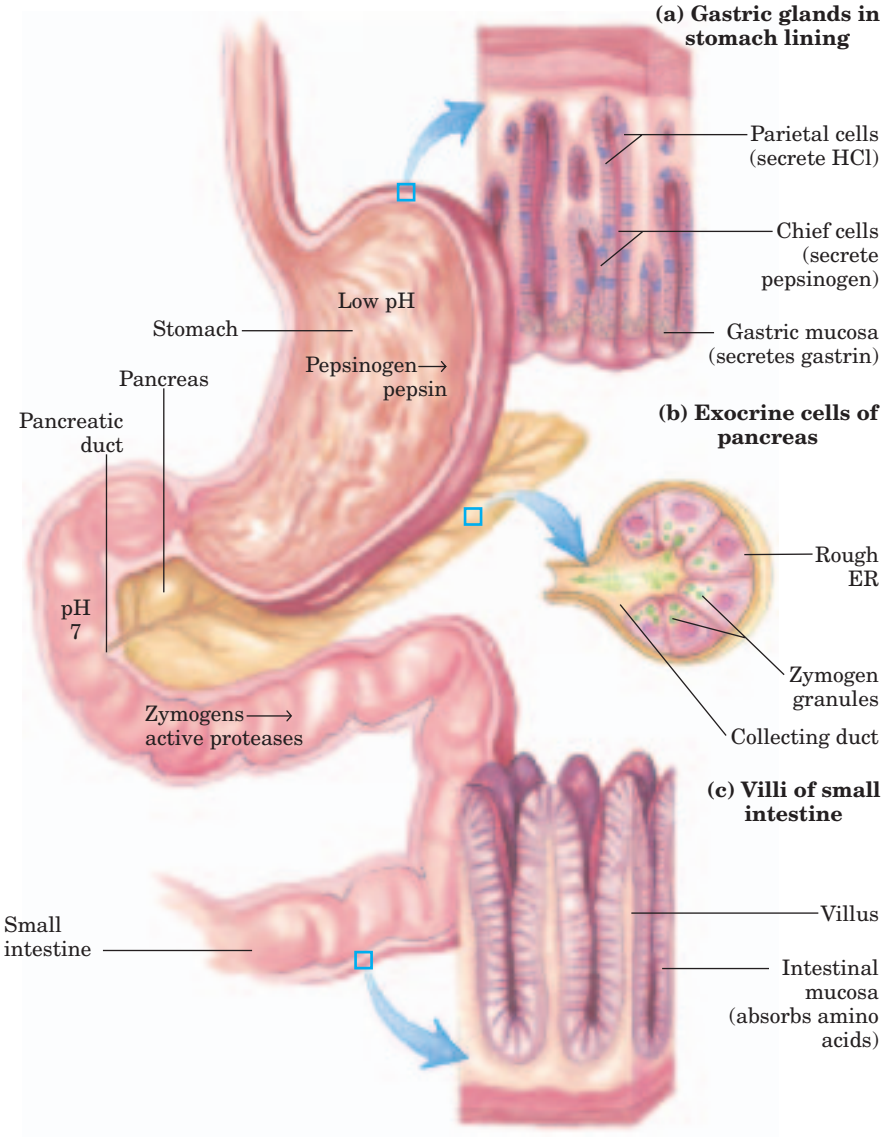


FIGURE 18–3 Part of the human digestive (gastrointestinal) tract. (a) The parietal cells and chief cells of the gastric glands secrete their products in response to the hormone gastrin. Pepsin begins the process of protein degradation in the stomach. **(b)** The cytoplasm of exocrine cells is completely filled with rough endoplasmic reticulum, the site of synthesis of the zymogens of many digestive enzymes. The zymogens are concentrated in membrane-enclosed transport particles called zymogen granules. When an exocrine cell is stimulated, its plasma membrane fuses with the zymogen granule membrane and zymogens are released into the lumen of the collecting duct by exocytosis. The collecting ducts ultimately lead to the pancreatic duct and thence to the small intestine. **(c)** Amino acids are absorbed through the epithelial cell layer (intestinal mucosa) of the villi and enter the capillaries. Recall that the products of lipid hydrolysis in the small intestine enter the lymphatic system after their absorption by the intestinal mucosa (see Fig. 17–1).

cholecystokinin, which stimulates secretion of several pancreatic enzymes with activity optima at pH 7 to 8. **Trypsinogen, chymotrypsinogen,** and **procarboxypeptidases A** and **B,** the zymogens of **trypsin, chymotrypsin,** and **carboxypeptidases A** and **B,** are synthesized and secreted by the exocrine cells of the pancreas (Fig. 18–3b). Trypsinogen is converted to its active form, trypsin, by **enteropeptidase,** a proteolytic enzyme secreted by intestinal cells. Free trypsin then catalyzes the conversion of additional trypsinogen to trypsin (see Fig. 6–33). Trypsin also activates chymotrypsinogen, the procarboxypeptidases, and proelastase.

Why this elaborate mechanism for getting active digestive enzymes into the gastrointestinal tract? Synthesis of the enzymes as inactive precursors protects the exocrine cells from destructive proteolytic attack. The pancreas further protects itself against self-digestion by making a specific inhibitor, a protein called **pancreatic trypsin inhibitor** (p. 231), that effectively prevents premature production of active proteolytic enzymes within the pancreatic cells.

Trypsin and chymotrypsin further hydrolyze the peptides that were produced by pepsin in the stomach. This stage of protein digestion is accomplished very efficiently, because pepsin, trypsin, and chymotrypsin have different amino acid specificities (see Table 3–7). Degradation of the short peptides in the small intestine is then completed by other intestinal peptidases. These include carboxypeptidases A and B (both of which are zinc-containing enzymes), which remove successive carboxyl-terminal residues from peptides, and an **aminopeptidase** that hydrolyzes successive amino-terminal residues from short peptides. The resulting mixture of free amino acids is transported into the epithelial cells lining the small intestine (Fig. 18–3c), through which the amino acids enter the blood capillaries in the villi and travel to the liver. In humans, most globular proteins from animal

sources are almost completely hydrolyzed to amino acids in the gastrointestinal tract, but some fibrous proteins, such as keratin, are only partly digested. In addition, the protein content of some plant foods is protected against breakdown by indigestible cellulose husks.

Acute pancreatitis is a disease caused by obstruction of the normal pathway by which pancreatic secretions enter the intestine. The zymogens of the proteolytic enzymes are converted to their catalytically active forms prematurely, *inside* the pancreatic cells, and attack the pancreatic tissue itself. This causes excruciating pain and damage to the organ that can prove fatal. ■

Pyridoxal Phosphate Participates in the Transfer of α-Amino Groups to α-Ketoglutarate

The first step in the catabolism of most L-amino acids, once they have reached the liver, is removal of the α-amino groups, promoted by enzymes called **aminotransferases** or **transaminases.** In these **transamination** reactions, the α-amino group is transferred to the α-carbon atom of α-ketoglutarate, leaving behind the corresponding α-keto acid analog of the amino acid (Fig. 18–4). There is no net deamination (loss of amino groups) in these reactions, because the α-ketoglutarate becomes aminated as the α-amino acid is deaminated. The effect of transamination reactions is to collect the amino groups from many different amino acids in the form of L-glutamate. The glutamate then functions as an amino group donor for biosynthetic pathways or for excretion pathways that lead to the elimination of nitrogenous waste products.

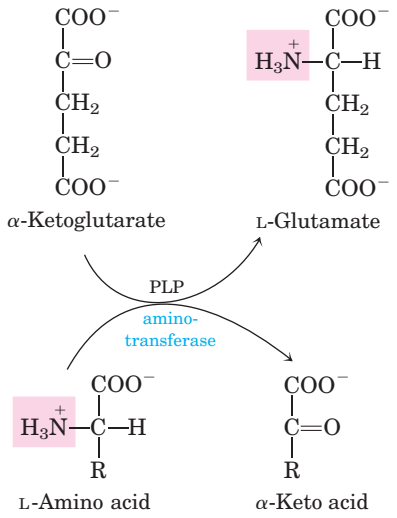


FIGURE 18–4 **Enzyme-catalyzed transaminations.** In many aminotransferase reactions, α-ketoglutarate is the amino group acceptor. All aminotransferases have pyridoxal phosphate (PLP) as cofactor. Although the reaction is shown here in the direction of transfer of the amino group to α-ketoglutarate, it is readily reversible.

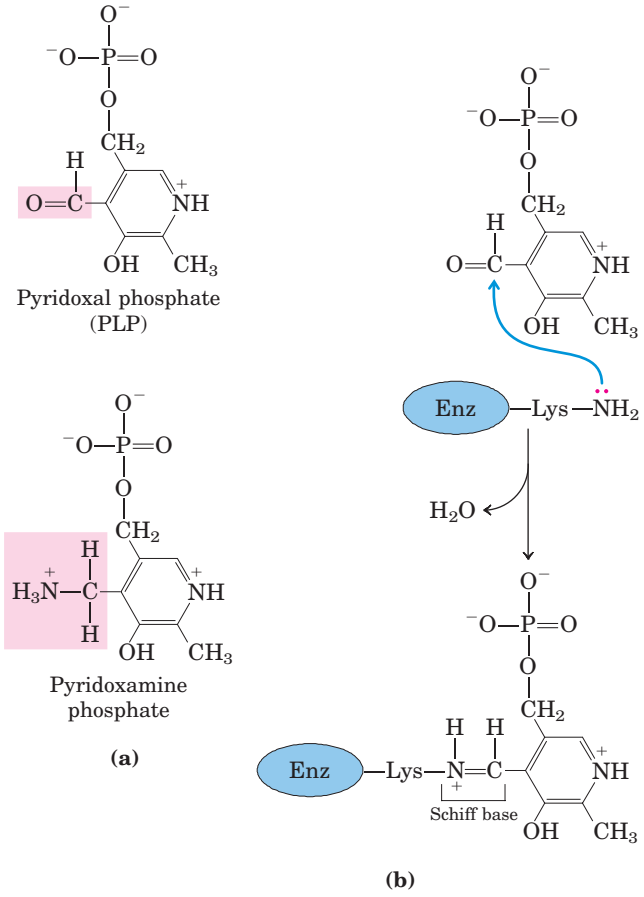
Cells contain different types of aminotransferases. Many are specific for α-ketoglutarate as the amino group acceptor but differ in their specificity for the L-amino acid. The enzymes are named for the amino group donor (alanine aminotransferase, aspartate aminotransferase, for example). The reactions catalyzed by aminotransferases are freely reversible, having an equilibrium constant of about 1.0 ($\Delta G'^{\circ} \approx 0$ kJ/mol).

All aminotransferases have the same prosthetic group and the same reaction mechanism. The prosthetic group is **pyridoxal phosphate (PLP),** the coenzyme form of pyridoxine, or vitamin B_6. We encountered pyridoxal phosphate in Chapter 15, as a coenzyme in the glycogen phosphorylase reaction, but its role in that reaction is not representative of its usual coenzyme function. Its primary role in cells is in the metabolism of molecules with amino groups.

Pyridoxal phosphate functions as an intermediate carrier of amino groups at the active site of aminotransferases. It undergoes reversible transformations between its aldehyde form, pyridoxal phosphate, which can accept an amino group, and its aminated form, pyridoxamine phosphate, which can donate its amino group to an α-keto acid (Fig. 18–5a). Pyridoxal phosphate is generally covalently bound to the enzyme's active site through an aldimine (Schiff base) linkage to the ε-amino group of a Lys residue (Fig. 18–5b, d).

Pyridoxal phosphate participates in a variety of reactions at the α, β, and γ carbons (C-2 to C-4) of amino acids. Reactions at the α carbon (Fig. 18–6) include racemizations (interconverting L- and D-amino acids) and decarboxylations, as well as transaminations. Pyridoxal phosphate plays the same chemical role in each of these reactions. A bond to the α carbon of the substrate is broken, removing either a proton or a carboxyl group. The electron pair left behind on the α carbon would form a highly unstable carbanion, but pyridoxal phosphate provides resonance stabilization of this intermediate (Fig. 18–6 inset). The highly conjugated structure of PLP (an electron sink) permits delocalization of the negative charge.

Aminotransferases (Fig. 18–5) are classic examples of enzymes catalyzing bimolecular Ping-Pong reactions (see Fig. 6–13b), in which the first substrate reacts and the product must leave the active site before the second substrate can bind. Thus the incoming amino acid binds to the active site, donates its amino group to pyridoxal phosphate, and departs in the form of an α-keto acid. The incoming α-keto acid then binds, accepts the amino group from pyridoxamine phosphate, and departs in the form of an amino acid. As described in Box 18–1 on page 664, measurement of the alanine aminotransferase and aspartate aminotransferase levels in blood serum is important in some medical diagnoses.



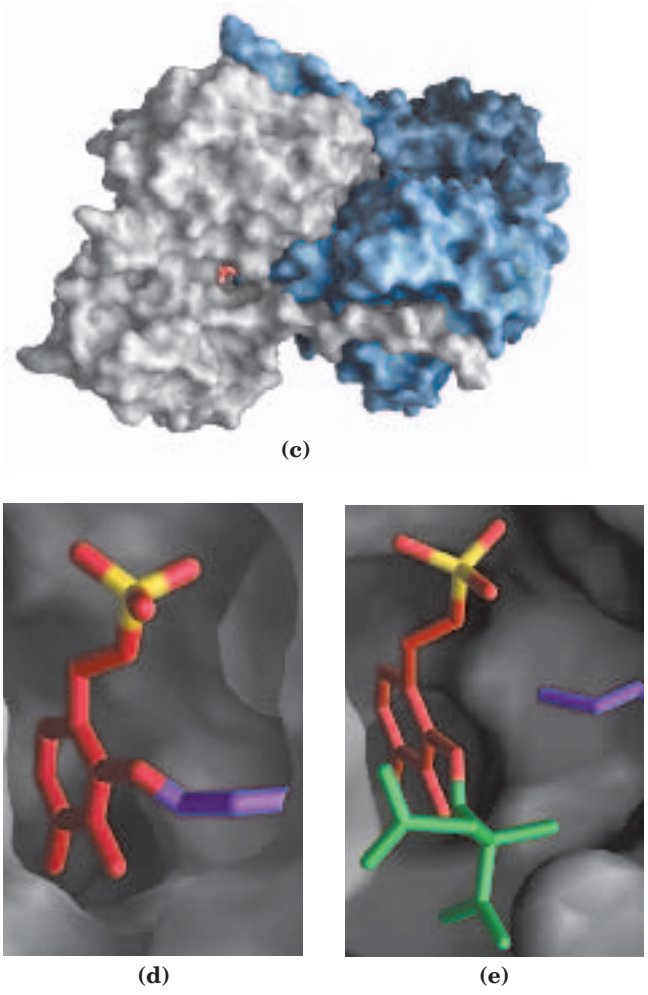


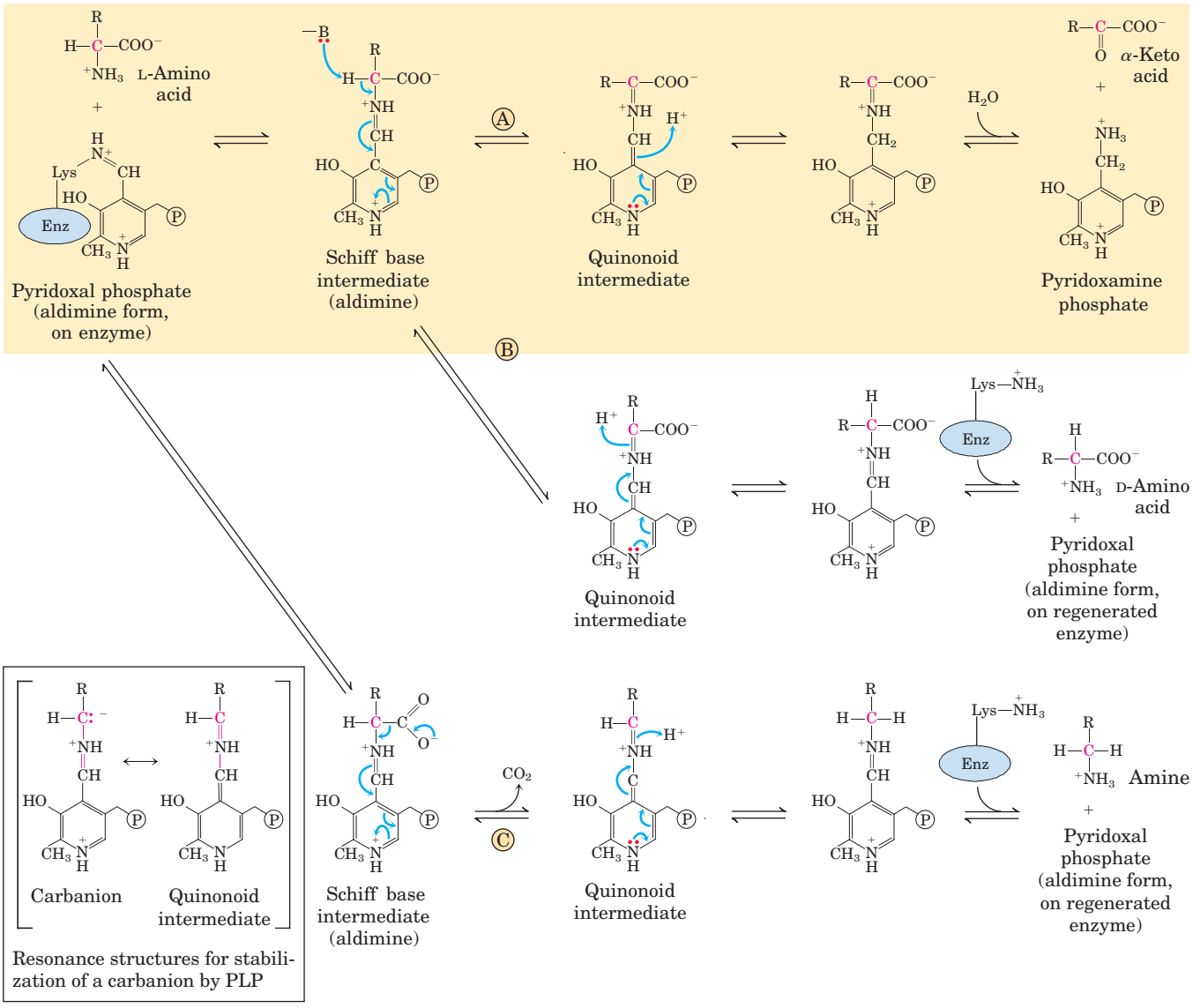
FIGURE 18–5 Pyridoxal phosphate, the prosthetic group of aminotransferases. (a) Pyridoxal phosphate (PLP) and its aminated form, pyridoxamine phosphate, are the tightly bound coenzymes of aminotransferases. The functional groups are shaded. **(b)** Pyridoxal phosphate is bound to the enzyme through noncovalent interactions and a Schiff-base linkage to a Lys residue at the active site. The steps in the formation of a Schiff base from a primary amine and a carbonyl group are detailed in Figure 14–5. **(c)** PLP (red) bound to one of the two active sites of the dimeric enzyme aspartate aminotransferase, a typical aminotransferase; **(d)** close-up view of the active site, with PLP (red, with yellow phosphorus) in aldimine linkage with the side chain of Lys^{258} (purple); **(e)** another close-up view of the active site, with PLP linked to the substrate analog 2-methylaspartate (green) via a Schiff base (PDB ID 1AJS).

Glutamate Releases Its Amino Group as Ammonia in the Liver

As we have seen, the amino groups from many of the α-amino acids are collected in the liver in the form of the amino group of L-glutamate molecules. These amino groups must next be removed from glutamate to prepare them for excretion. In hepatocytes, glutamate is transported from the cytosol into mitochondria, where it undergoes **oxidative deamination** catalyzed by **L-glutamate dehydrogenase** (M_r 330,000). In mammals, this enzyme is present in the mitochondrial matrix. It is the only enzyme that can use either NAD^+ or $NADP^+$ as the acceptor of reducing equivalents (Fig. 18–7).

The combined action of an aminotransferase and glutamate dehydrogenase is referred to as **transdeamination.** A few amino acids bypass the transdeamination pathway and undergo direct oxidative deamination. The fate of the NH_4^+ produced by any of these deamination processes is discussed in detail in Section 18.2. The α-ketoglutarate formed from glutamate deamination can be used in the citric acid cycle and for glucose synthesis.

Glutamate dehydrogenase operates at an important intersection of carbon and nitrogen metabolism. An allosteric enzyme with six identical subunits, its activity is influenced by a complicated array of allosteric modulators. The best-studied of these are the positive modulator ADP and the negative modulator GTP. The metabolic rationale for this regulatory pattern has not been elucidated in detail. Mutations that alter the allosteric binding site for GTP or otherwise cause permanent activation of glutamate dehydrogenase lead to a human genetic disorder called hyperinsulinism-hyperammonemia



MECHANISM FIGURE 18–6 **Some amino acid transformations at the *α* carbon that are facilitated by pyridoxal phosphate.** Pyridoxal phosphate is generally bonded to the enzyme through a Schiff base (see Fig. 18–5b, d). Reactions begin (top left) with formation of a new Schiff base (aldimine) between the α-amino group of the amino acid and PLP, which substitutes for the enzyme-PLP linkage. Three alternative fates for this Schiff base are shown: (A) transamination, (B) racemization, and (C) decarboxylation. The Schiff base formed between PLP and the amino acid is in conjugation with the pyridine ring, an electron sink that permits delocalization of an electron pair to avoid formation of an unstable carbanion on the α carbon (inset). A quinonoid intermediate is involved in all three types of reactions. The transamination route (A) is especially important in the pathways described in this chapter. The pathway highlighted here (shown left to right) represents only part of the overall reaction catalyzed by aminotransferases. To complete the process, a second α-keto acid replaces the one that is released, and this is converted to an amino acid in a reversal of the reaction steps (right to left). Pyridoxal phosphate is also involved in certain reactions at the β and γ carbons of some amino acids (not shown). Pyridoxal Phosphate Reaction Mechanisms

syndrome, characterized by elevated levels of ammonia in the bloodstream and hypoglycemia.

Glutamine Transports Ammonia in the Bloodstream

Ammonia is quite toxic to animal tissues (we examine some possible reasons for this toxicity later), and the levels present in blood are regulated. In many tissues, including the brain, some processes such as nucleotide degradation generate free ammonia. In most animals much of the free ammonia is converted to a nontoxic compound before export from the extrahepatic tissues into the blood and transport to the liver or kidneys. For this transport function, glutamate, critical to *intracellular* amino group metabolism, is supplanted by L-glutamine. The free ammonia produced in tissues is combined with glutamate to yield glutamine by the action of **glutamine synthetase.** This reaction requires

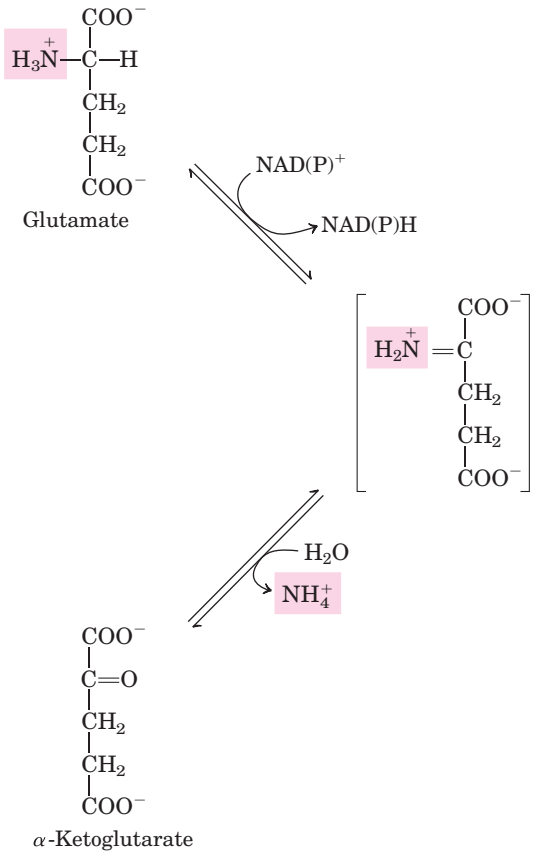


FIGURE 18–7 Reaction catalyzed by glutamate dehydrogenase. The glutamate dehydrogenase of mammalian liver has the unusual capacity to use either NAD^+ or $NADP^+$ as cofactor. The glutamate dehydrogenases of plants and microorganisms are generally specific for one or the other. The mammalian enzyme is allosterically regulated by GTP and ADP.

ATP and occurs in two steps (Fig. 18–8). First, glutamate and ATP react to form ADP and a γ-glutamyl phosphate intermediate, which then reacts with ammonia to produce glutamine and inorganic phosphate. Glutamine is a nontoxic transport form of ammonia; it is normally present in blood in much higher concentrations than other amino acids. Glutamine also serves as a source of amino groups in a variety of biosynthetic reactions. Glutamine synthetase is found in all organisms, always playing a central metabolic role. In microorganisms, the enzyme serves as an essential portal for the entry of fixed nitrogen into biological systems. (The roles of glutamine and glutamine synthetase in metabolism are further discussed in Chapter 22.)

In most terrestrial animals, glutamine in excess of that required for biosynthesis is transported in the blood to the intestine, liver, and kidneys for processing. In these tissues, the amide nitrogen is released as ammonium ion in the mitochondria, where the enzyme **glutaminase** converts glutamine to glutamate and NH_4^+ (Fig. 18–8). The NH_4^+ from intestine and kidney is transported in the blood to the liver. In the liver, the ammonia from all sources is disposed of by urea synthesis. Some of the glutamate produced in the glutaminase reaction may be further processed in the liver by glutamate dehydrogenase, releasing more ammonia and producing carbon skeletons for metabolic fuel. However, most glutamate enters the transamination reactions required for amino acid biosynthesis and other processes (Chapter 22).

In metabolic acidosis (p. 652) there is an increase in glutamine processing by the kidneys. Not all the excess NH_4^+ thus produced is released into the bloodstream or converted to urea; some is excreted directly into the urine. In the kidney, the NH_4^+ forms salts with metabolic acids, facilitating their removal in the urine. Bicarbonate produced by the decarboxylation of α-ketoglutarate in the citric acid cycle can also serve as a buffer in blood plasma. Taken together, these effects of glutamine metabolism in the kidney tend to counteract acidosis. ■

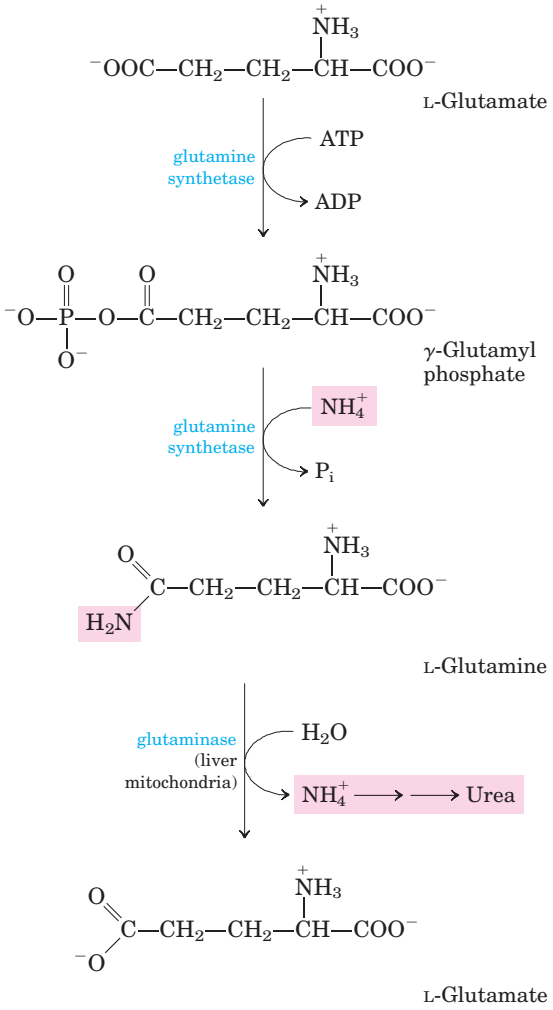


FIGURE 18–8 Ammonia transport in the form of glutamine. Excess ammonia in tissues is added to glutamate to form glutamine, a process catalyzed by glutamine synthetase. After transport in the bloodstream, the glutamine enters the liver and NH_4^+ is liberated in mitochondria by the enzyme glutaminase.

BOX 18–1 BIOCHEMISTRY IN MEDICINE

Assays for Tissue Damage

Analyses of certain enzyme activities in blood serum give valuable diagnostic information for a number of disease conditions.

Alanine aminotransferase (ALT; also called glutamate-pyruvate transaminase, GPT) and aspartate aminotransferase (AST; also called glutamate-oxaloacetate transaminase, GOT) are important in the diagnosis of heart and liver damage caused by heart attack, drug toxicity, or infection. After a heart attack, a variety of enzymes, including these aminotransferases, leak from the injured heart cells into the bloodstream. Measurements of the blood serum concentrations of the two aminotransferases by the SGPT and SGOT tests (S for serum)—and of another enzyme, **creatine kinase,** by the SCK test—can provide information about the severity of the damage. Creatine kinase is the first heart enzyme to appear in the blood after a heart attack; it also disappears quickly from the blood. GOT is the next to appear, and GPT follows later. Lactate dehydrogenase also leaks from injured or anaerobic heart muscle.

The SGOT and SGPT tests are also important in occupational medicine, to determine whether people exposed to carbon tetrachloride, chloroform, or other industrial solvents have suffered liver damage. Liver degeneration caused by these solvents is accompanied by leakage of various enzymes from injured hepatocytes into the blood. Aminotransferases are most useful in the monitoring of people exposed to these chemicals, because these enzyme activities are high in liver and can be detected in very small amounts.

Alanine Transports Ammonia from Skeletal Muscles to the Liver

Alanine also plays a special role in transporting amino groups to the liver in a nontoxic form, via a pathway called the **glucose-alanine cycle** (Fig. 18–9). In muscle and certain other tissues that degrade amino acids for fuel, amino groups are collected in the form of glutamate by transamination (Fig. 18–2a). Glutamate can be converted to glutamine for transport to the liver, as described above, or it can transfer its α-amino group to pyruvate, a readily available product of muscle glycolysis, by the action of **alanine aminotransferase** (Fig. 18–9). The alanine so formed passes into the blood and travels to the liver. In the cytosol of hepatocytes, alanine aminotransferase transfers the amino group from alanine to α-ketoglutarate, forming pyruvate and glutamate. Glutamate can then enter mitochondria, where the glutamate dehydrogenase reaction releases NH_4^+ (Fig. 18–7), or can undergo transamination with oxaloacetate to form aspartate, another nitrogen donor in urea synthesis, as we shall see.

The use of alanine to transport ammonia from skeletal muscles to the liver is another example of the intrinsic economy of living organisms. Vigorously contracting skeletal muscles operate anaerobically, producing pyruvate and lactate from glycolysis as well as

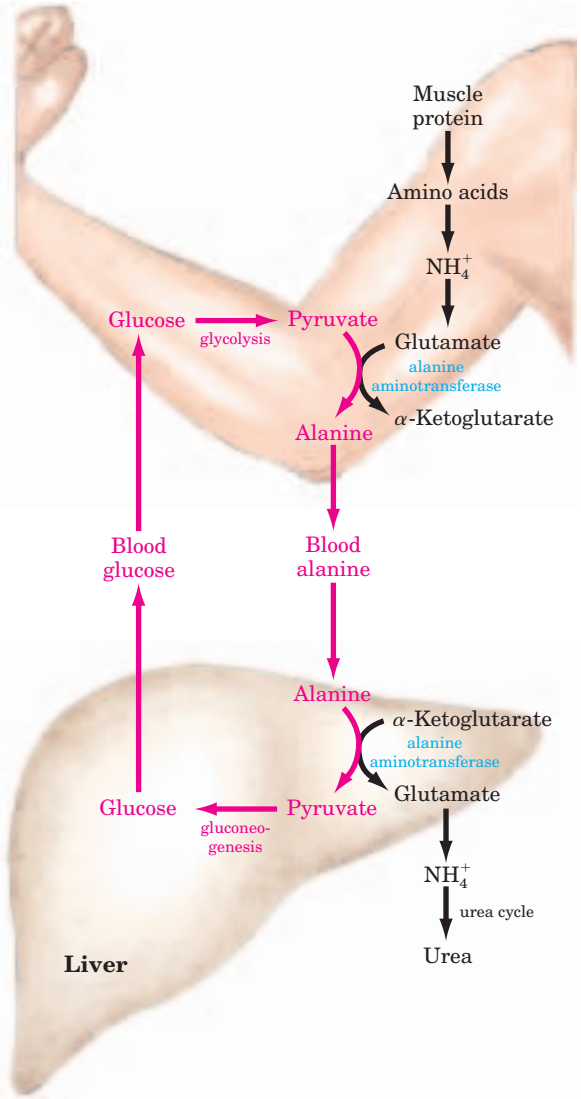


FIGURE 18–9 Glucose-alanine cycle. Alanine serves as a carrier of ammonia and of the carbon skeleton of pyruvate from skeletal muscle to liver. The ammonia is excreted and the pyruvate is used to produce glucose, which is returned to the muscle.

ammonia from protein breakdown. These products must find their way to the liver, where pyruvate and lactate are incorporated into glucose, which is returned to the muscles, and ammonia is converted to urea for excretion. The glucose-alanine cycle, in concert with the Cori cycle (see Box 14–1 and Fig. 23–18), accomplishes this transaction. The energetic burden of gluconeogenesis is thus imposed on the liver rather than the muscle, and all available ATP in muscle is devoted to muscle contraction.

Ammonia Is Toxic to Animals

The catabolic production of ammonia poses a serious biochemical problem, because ammonia is very toxic. The molecular basis for this toxicity is not entirely understood. The terminal stages of ammonia intoxication in humans are characterized by onset of a comatose state accompanied by cerebral edema (an increase in the brain's water content) and increased cranial pressure, so research and speculation on ammonia toxicity have focused on this tissue. Speculation centers on a potential depletion of ATP in brain cells.

Ridding the cytosol of excess ammonia requires reductive amination of α-ketoglutarate to glutamate by glutamate dehydrogenase (the reverse of the reaction described earlier; Fig. 18–7) and conversion of glutamate to glutamine by glutamine synthetase. Both enzymes are present at high levels in the brain, although the glutamine synthetase reaction is almost certainly the more important pathway for removal of ammonia. High levels of NH_4^+ lead to increased levels of glutamine, which acts as an osmotically active solute (osmolyte) in brain astrocytes, star-shaped cells of the nervous system that provide nutrients, support, and insulation for neurons. This triggers an uptake of water into the astrocytes to maintain osmotic balance, leading to swelling and the symptoms noted above.

Depletion of glutamate in the glutamine synthetase reaction may have additional effects on the brain. Glutamate and its derivative γ-aminobutyrate (GABA; see Fig. 22–29) are important neurotransmitters; the sensitivity of the brain to ammonia may reflect a depletion of neurotransmitters as well as changes in cellular osmotic balance. ■

As we close this discussion of amino group metabolism, note that we have described several processes that deposit excess ammonia in the mitochondria of hepatocytes (Fig. 18–2). We now look at the fate of that ammonia.

SUMMARY 18.1 Metabolic Fates of Amino Groups

- Humans derive a small fraction of their oxidative energy from the catabolism of amino acids. Amino acids are derived from the normal breakdown (recycling) of cellular proteins, degradation of ingested proteins, and breakdown of body proteins in lieu of other fuel sources during starvation or in uncontrolled diabetes mellitus.
- Proteases degrade ingested proteins in the stomach and small intestine. Most proteases are initially synthesized as inactive zymogens.
- An early step in the catabolism of amino acids is the separation of the amino group from the carbon skeleton. In most cases, the amino group is transferred to α-ketoglutarate to form glutamate. This transamination reaction requires the coenzyme pyridoxal phosphate.
- Glutamate is transported to liver mitochondria, where glutamate dehydrogenase liberates the amino group as ammonium ion (NH_4^+). Ammonia formed in other tissues is transported to the liver as the amide nitrogen of glutamine or, in transport from skeletal muscle, as the amino group of alanine.
- The pyruvate produced by deamination of alanine in the liver is converted to glucose, which is transported back to muscle as part of the glucose-alanine cycle.

18.2 Nitrogen Excretion and the Urea Cycle

If not reused for the synthesis of new amino acids or other nitrogenous products, amino groups are channeled into a single excretory end product (Fig. 18–10). Most aquatic species, such as the bony fishes, are **ammonotelic,** excreting amino nitrogen as ammonia. The toxic ammonia is simply diluted in the surrounding water. Terrestrial animals require pathways for nitrogen excretion that minimize toxicity and water loss. Most terrestrial animals are **ureotelic,** excreting amino nitrogen in the form of urea; birds and reptiles are **uricotelic,** excreting amino nitrogen as uric acid. (The pathway of uric acid synthesis is described in Fig. 22–45.) Plants recycle virtually all amino groups, and nitrogen excretion occurs only under very unusual circumstances.

In ureotelic organisms, the ammonia deposited in the mitochondria of hepatocytes is converted to urea in the **urea cycle.** This pathway was discovered in 1932 by Hans Krebs (who later also discovered the citric acid cycle) and a medical student associate, Kurt Henseleit. Urea production occurs almost exclusively in the liver and is the fate of most of the ammonia channeled there. The urea passes into the bloodstream and thus to the kidneys and is excreted into the urine. The production of urea now becomes the focus of our discussion.

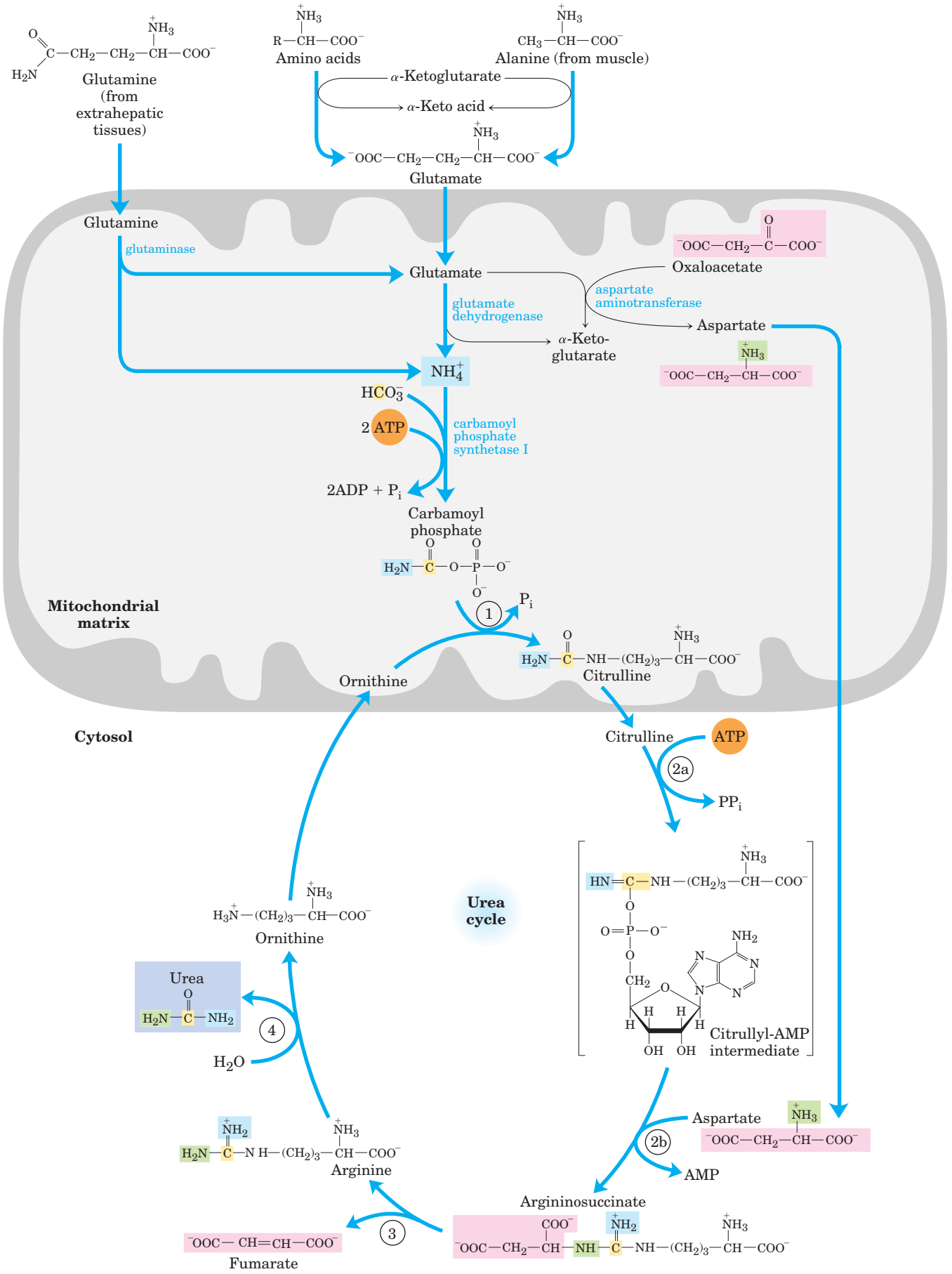

Glutamine
(from
extrahepatic
tissues)
Amino acids
Alanine (from muscle)
α-Ketoglutarate
α-Keto acid
Glutamate
Glutamine
glutaminase
Glutamate
Oxaloacetate
aspartate
aminotransferase
Aspartate
glutamate
dehydrogenase
α-Keto-
glutarate
NH4+
HCO3−
2 ATP
carbamoyl
phosphate
synthetase I
2ADP + Pi
Carbamoyl
phosphate
Mitochondrial
matrix
Pi
1
Ornithine
Citrulline
Cytosol
Citrulline
ATP
2a
PPi
Urea
cycle
Ornithine
Citrullyl-AMP
intermediate
Urea
4
H2O
Aspartate
2b
AMP
Arginine
Argininosuccinate
3
Fumarate

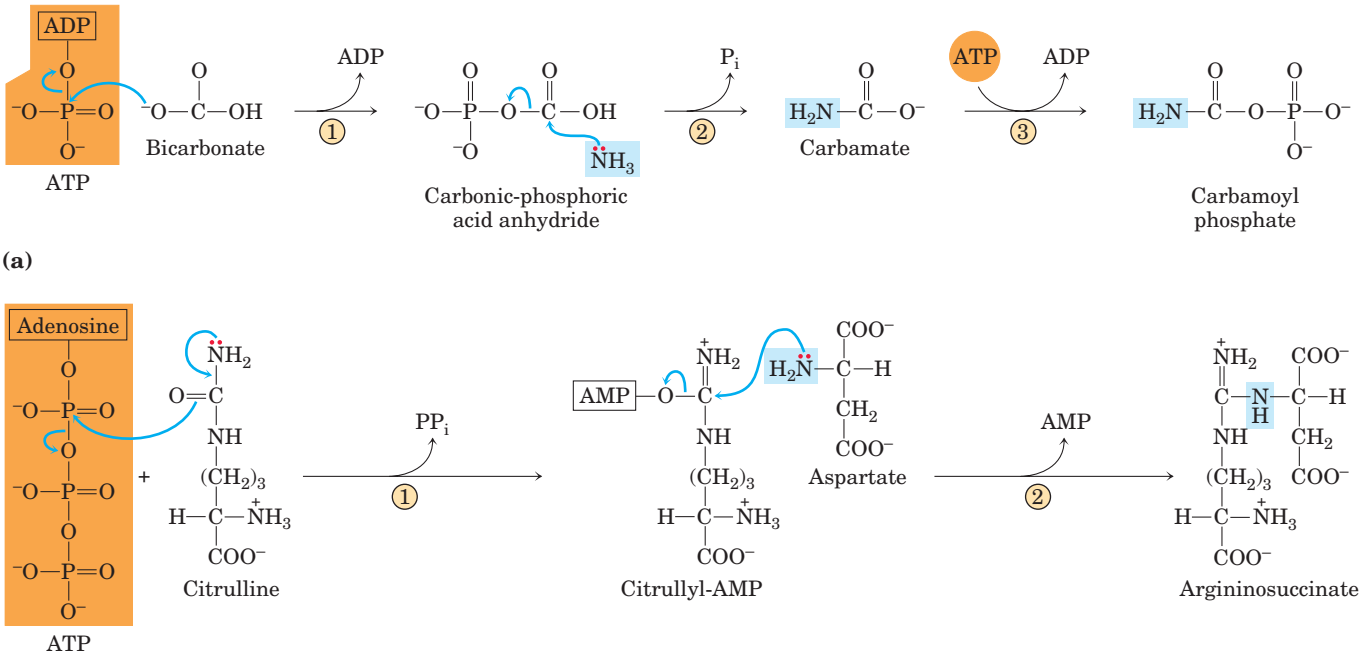
FIGURE 18–10 (facing page) **Urea cycle and reactions that feed amino groups into the cycle.** The enzymes catalyzing these reactions (named in the text) are distributed between the mitochondrial matrix and the cytosol. One amino group enters the urea cycle as carbamoyl phosphate, formed in the matrix; the other enters as aspartate, formed in the matrix by transamination of oxaloacetate and glutamate, catalyzed by aspartate aminotransferase. The urea cycle consists of four steps. ① Formation of citrulline from ornithine and carbamoyl phosphate (entry of the first amino group); the citrulline passes into the cytosol. ② Formation of argininosuccinate through a citrullyl-AMP intermediate (entry of the second amino group). ③ Formation of arginine from argininosuccinate; this reaction releases fumarate, which enters the citric acid cycle. ④ Formation of urea; this reaction also regenerates, ornithine. The pathways by which NH_4^+ arrives in the mitochondrial matrix of hepatocytes were discussed in Section 18.1.

Urea Is Produced from Ammonia in Five Enzymatic Steps

The urea cycle begins inside liver mitochondria, but three of the subsequent steps take place in the cytosol; the cycle thus spans two cellular compartments (Fig. 18–10). The first amino group to enter the urea cycle is derived from ammonia in the mitochondrial matrix—NH_4^+ arising by the pathways described above. The liver also receives some ammonia via the portal vein from the intestine, from the bacterial oxidation of amino acids. Whatever its source, the NH_4^+ generated in liver mitochondria is immediately used, together with CO_2 (as HCO_3^-) produced by mitochondrial respiration, to form carbamoyl phosphate in the matrix (Fig. 18–11a; see also Fig. 18–10). This ATP-dependent reaction is catalyzed by **carbamoyl phosphate synthetase I,** a regulatory enzyme (see below). The mitochondrial form of the enzyme is distinct from the cytosolic (II) form, which has a separate function in pyrimidine biosynthesis (Chapter 22).

The carbamoyl phosphate, which functions as an activated carbamoyl group donor, now enters the urea cycle. The cycle has four enzymatic steps. First, carbamoyl phosphate donates its carbamoyl group to ornithine to form citrulline, with the release of P_i (Fig. 18–10, step ①). Ornithine plays a role resembling that of oxaloacetate in the citric acid cycle, accepting material at each turn of the cycle. The reaction is catalyzed by **ornithine transcarbamoylase,** and the citrulline passes from the mitochondrion to the cytosol.

The second amino group now enters from aspartate (generated in mitochondria by transamination and transported into the cytosol) by a condensation reaction between the amino group of aspartate and the ureido



MECHANISM FIGURE 18–11 **Nitrogen-acquiring reactions in the synthesis of urea.** The urea nitrogens are acquired in two reactions, each requiring ATP. **(a)** In the reaction catalyzed by carbamoyl phosphate synthetase I, the first nitrogen enters from ammonia. The terminal phosphate groups of two molecules of ATP are used to form one molecule of carbamoyl phosphate. In other words, this reaction has two activation steps (① and ③). Carbamoyl Phosphate Synthetase I Mechanism **(b)** In the reaction catalyzed by argininosuccinate synthetase, the second nitrogen enters from aspartate. The ureido oxygen of citrulline is activated by the addition of AMP in step ①; this sets up the addition of aspartate in step ②, with AMP (including the ureido oxygen) as the leaving group. Argininosuccinate Synthetase Mechanism

(carbonyl) group of citrulline, forming argininosuccinate (step ② in Fig. 18–10). This cytosolic reaction, catalyzed by **argininosuccinate synthetase,** requires ATP and proceeds through a citrullyl-AMP intermediate (Fig. 18–11b). The argininosuccinate is then cleaved by **argininosuccinase** (step ③ in Fig. 18–10) to form free arginine and fumarate, the latter entering mitochondria to join the pool of citric acid cycle intermediates. This is the only reversible step in the urea cycle. In the last reaction of the urea cycle (step ④), the cytosolic enzyme **arginase** cleaves arginine to yield **urea** and ornithine. Ornithine is transported into the mitochondrion to initiate another round of the urea cycle.

As we noted in Chapter 16, the enzymes of many metabolic pathways are clustered (p. 605), with the product of one enzyme reaction being channeled directly to the next enzyme in the pathway. In the urea cycle, the mitochondrial and cytosolic enzymes appear to be clustered in this way. The citrulline transported out of the mitochondrion is not diluted into the general pool of metabolites in the cytosol but is passed directly to the active site of argininosuccinate synthetase. This channeling between enzymes continues for argininosuccinate, arginine, and ornithine. Only urea is released into the general cytosolic pool of metabolites.

The Citric Acid and Urea Cycles Can Be Linked

Because the fumarate produced in the argininosuccinase reaction is also an intermediate of the citric acid cycle, the cycles are, in principle, interconnected—in a process dubbed the "Krebs bicycle" (Fig. 18–12). However, each cycle can operate independently and communication between them depends on the transport of key intermediates between the mitochondrion and cytosol. Several enzymes of the citric acid cycle, including fumarase (fumarate hydratase) and malate dehydrogenase (p. 612), are also present as isozymes in the cytosol. The fumarate generated in cytosolic arginine synthesis can therefore be converted to malate in the cytosol, and these intermediates can be further metabolized in the cytosol or transported into mitochondria for use in the citric acid cycle. Aspartate formed in mitochondria by transamination between oxaloacetate and glutamate can be transported to the cytosol, where it serves as nitrogen donor in the urea cycle reaction catalyzed by argininosuccinate synthetase. These reactions, making up the **aspartate-argininosuccinate shunt,** provide metabolic links between the separate pathways by which the amino groups and carbon skeletons of amino acids are processed.

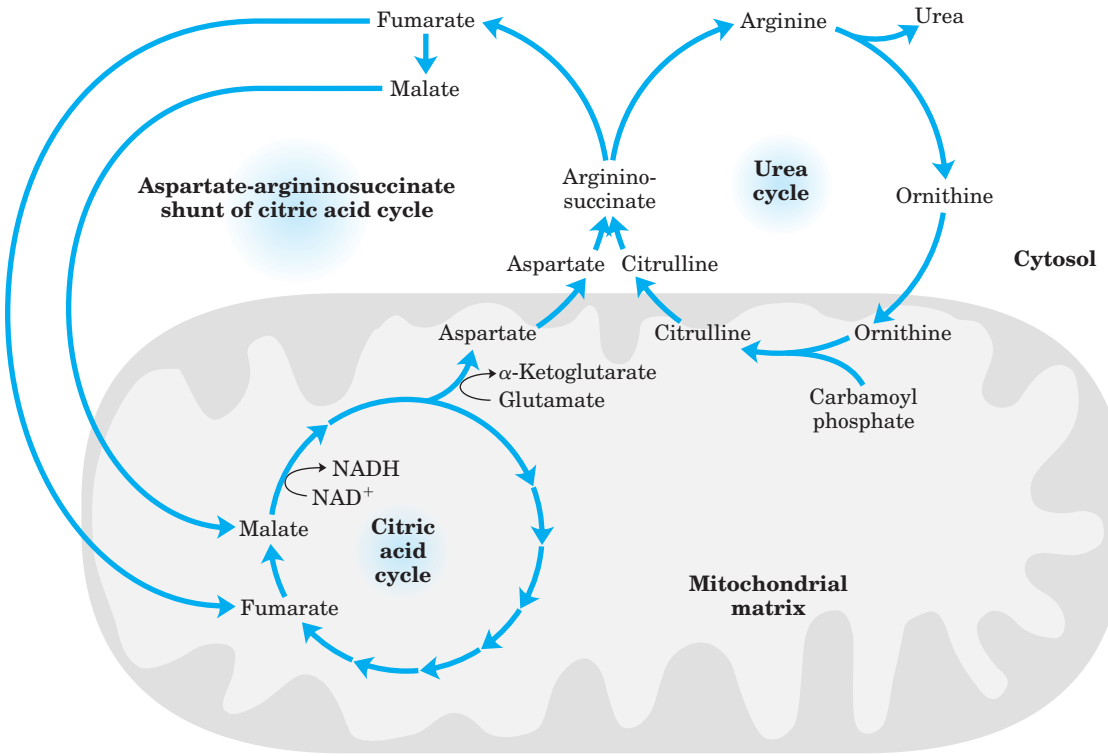


FIGURE 18–12 Links between the urea cycle and citric acid cycle. The interconnected cycles have been called the "Krebs bicycle." The pathways linking the citric acid and urea cycles are called the aspartate-argininosuccinate shunt; these effectively link the fates of the amino groups and the carbon skeletons of amino acids. The interconnections are even more elaborate than the arrows suggest. For example, some citric acid cycle enzymes, such as fumarase and malate dehydrogenase, have both cytosolic and mitochondrial isozymes. Fumarate produced in the cytosol—whether by the urea cycle, purine biosynthesis, or other processes—can be converted to cytosolic malate, which is used in the cytosol or transported into mitochondria (via the malate-aspartate shuttle; see Fig. 19–27) to enter the citric acid cycle.

The Activity of the Urea Cycle Is Regulated at Two Levels

The flux of nitrogen through the urea cycle in an individual animal varies with diet. When the dietary intake is primarily protein, the carbon skeletons of amino acids are used for fuel, producing much urea from the excess amino groups. During prolonged starvation, when breakdown of muscle protein begins to supply much of the organism's metabolic energy, urea production also increases substantially.

These changes in demand for urea cycle activity are met over the long term by regulation of the rates of synthesis of the four urea cycle enzymes and carbamoyl phosphate synthetase I in the liver. All five enzymes are synthesized at higher rates in starving animals and in animals on very-high-protein diets than in well-fed animals eating primarily carbohydrates and fats. Animals on protein-free diets produce lower levels of urea cycle enzymes.

On a shorter time scale, allosteric regulation of at least one key enzyme adjusts the flux through the urea cycle. The first enzyme in the pathway, carbamoyl phosphate synthetase I, is allosterically activated by ***N*-acetylglutamate,** which is synthesized from acetyl-CoA and glutamate by ***N*-acetylglutamate synthase** (Fig. 18–13). In plants and microorganisms this enzyme catalyzes the first step in the de novo synthesis of arginine from glutamate (see Fig. 22–10), but in mammals *N*-acetylglutamate synthase activity in the liver has a purely regulatory function (mammals lack the other enzymes needed to convert glutamate to arginine). The steady-state levels of *N*-acetylglutamate are determined by the concentrations of glutamate and acetyl-CoA (the substrates for *N*-acetylglutamate synthase) and arginine (an activator of *N*-acetylglutamate synthase, and thus an activator of the urea cycle).

Pathway Interconnections Reduce the Energetic Cost of Urea Synthesis

If we consider the urea cycle in isolation, we see that the synthesis of one molecule of urea requires four high-energy phosphate groups (Fig. 18–10). Two ATP molecules are required to make carbamoyl phosphate, and one ATP to make argininosuccinate—the latter ATP undergoing a pyrophosphate cleavage to AMP and PP_i, which is hydrolyzed to two P_i. The overall equation of the urea cycle is

$$2NH_4^+ + HCO_3^- + 3ATP^{4-} + H_2O \longrightarrow \text{urea} + 2ADP^{3-} + 4P_i^{2-} + AMP^{2-} + 2H^+$$

However, the urea cycle also causes a net conversion of oxaloacetate to fumarate (via aspartate), and the regeneration of oxaloacetate (Fig. 18–12) produces NADH in the malate dehydrogenase reaction. Each NADH molecule can generate up to 2.5 ATP during mitochondrial respiration (Chapter 19), greatly reducing the overall energetic cost of urea synthesis.

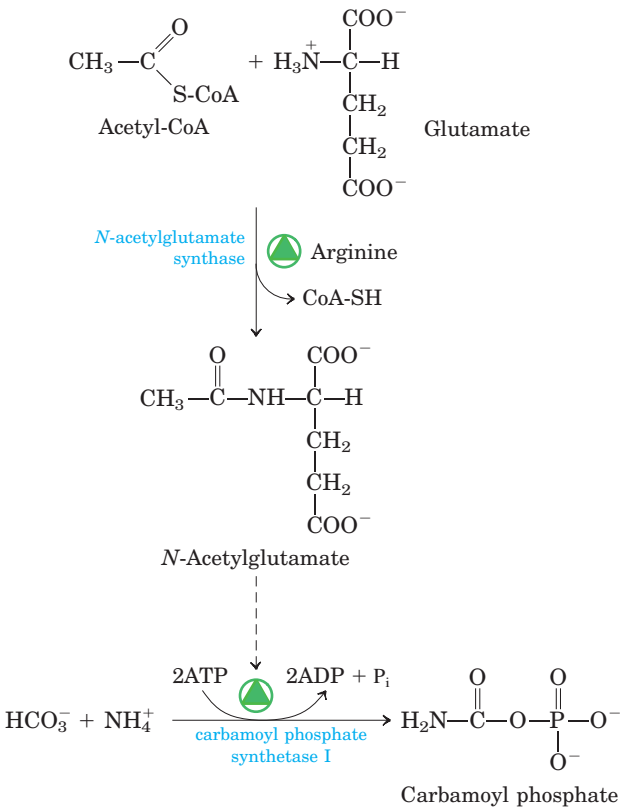


FIGURE 18–13 Synthesis of *N*-acetylglutamate and its activation of carbamoyl phosphate synthetase I.

Genetic Defects in the Urea Cycle Can Be Life-Threatening

People with genetic defects in any enzyme involved in urea formation cannot tolerate protein-rich diets. Amino acids ingested in excess of the minimum daily requirements for protein synthesis are deaminated in the liver, producing free ammonia that cannot be converted to urea and exported into the bloodstream, and, as we have seen, ammonia is highly toxic. The absence of a urea cycle enzyme can result in hyperammonemia or in the build-up of one or more urea cycle intermediates, depending on the enzyme that is missing. Given that most urea cycle steps are irreversible, the absent enzyme activity can often be identified by determining which cycle intermediate is present in especially elevated concentration in the blood and/or urine. Although the breakdown of amino acids can have serious health consequences in individuals with urea cycle deficiencies, a protein-free diet is not a treatment option. Humans are incapable of synthesizing half of the 20 common amino acids, and these **essential amino acids** (Table 18–1) must be provided in the diet.

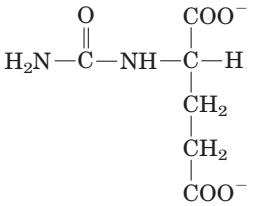
TABLE 18–1 Nonessential and Essential Amino Acids for Humans and the Albino Rat

Nonessential	*Conditionally essential**	*Essential*
Alanine	Arginine	Histidine
Asparagine	Cysteine	Isoleucine
Aspartate	Glutamine	Leucine
Glutamate	Glycine	Lysine
Serine	Proline	Methionine
	Tyrosine	Phenylalanine
		Threonine
		Tryptophan
		Valine

*Required to some degree in young, growing animals, and/or sometimes during illness.

A variety of treatments are available for individuals with urea cycle defects. Careful administration of the aromatic acids benzoate or phenylbutyrate in the diet can help lower the level of ammonia in the blood. Benzoate is converted to benzoyl-CoA, which combines with glycine to form hippurate (Fig. 18–14, left). The glycine used up in this reaction must be regenerated, and ammonia is thus taken up in the glycine synthase reaction. Phenylbutyrate is converted to phenylacetate by β oxidation. The phenylacetate is then converted to phenylacetyl-CoA, which combines with glutamine to form phenylacetylglutamine (Fig. 18–14, right). The resulting removal of glutamine triggers its further synthesis by glutamine synthetase (see Eqn 22–1) in a reaction that takes up ammonia. Both hippurate and phenylacetylglutamine are nontoxic compounds that are excreted in the urine. The pathways shown in Figure 18–14 make only minor contributions to normal metabolism, but they become prominent when aromatic acids are ingested.

Other therapies are more specific to a particular enzyme deficiency. Deficiency of *N*-acetylglutamate synthase results in the absence of the normal activator of carbamoyl phosphate synthetase I (Fig. 18–13). This condition can be treated by administering carbamoyl glutamate, an analog of *N*-acetylglutamate that is effective in activating carbamoyl phosphate synthetase I.



Carbamoyl glutamate

Supplementing the diet with arginine is useful in treating deficiencies of ornithine transcarbamoylase, argininosuccinate synthetase, and argininosuccinase. Many of these treatments must be accompanied by strict dietary control and supplements of essential amino acids. In the rare cases of arginase deficiency, arginine, the substrate of the defective enzyme, must be excluded from the diet. ■

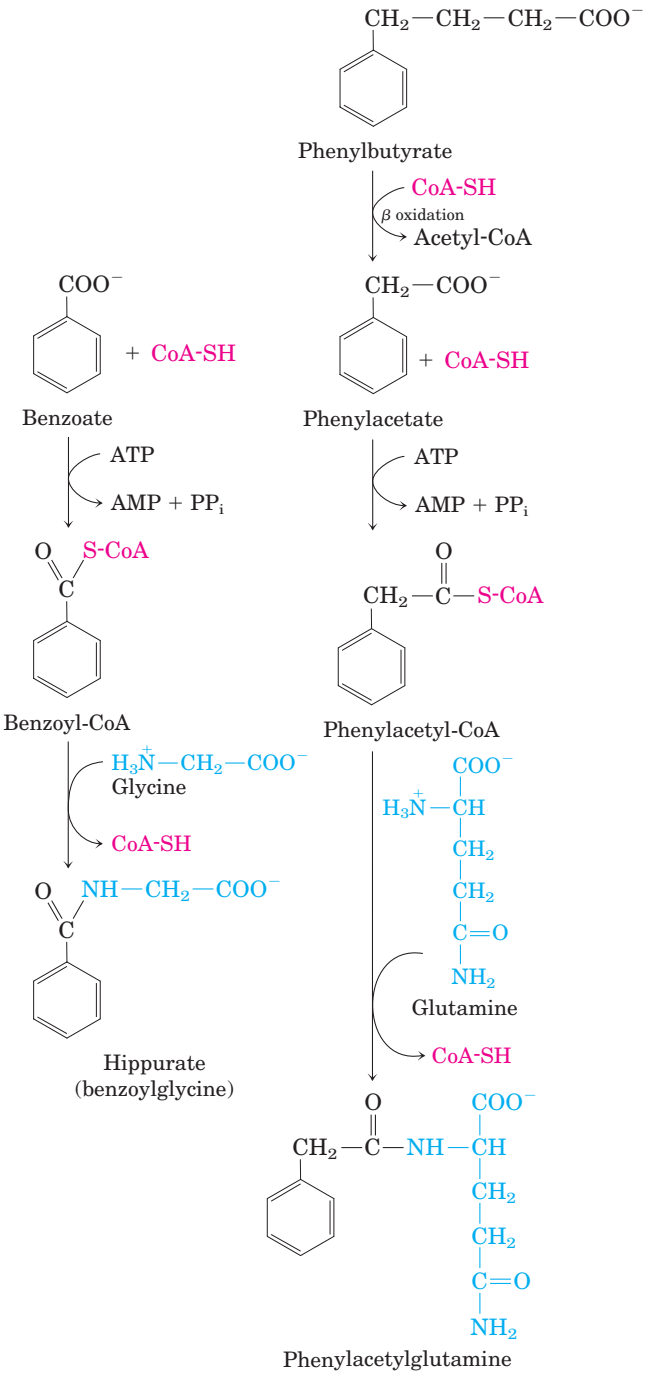


FIGURE 18–14 Treatment for deficiencies in urea cycle enzymes. The aromatic acids benzoate and phenylbutyrate, administered in the diet, are metabolized and combine with glycine and glutamine, respectively. The products are excreted in the urine. Subsequent synthesis of glycine and glutamine to replenish the pool of these intermediates removes ammonia from the bloodstream.

SUMMARY 18.2 Nitrogen Excretion and the Urea Cycle

- Ammonia is highly toxic to animal tissues. In the urea cycle, ornithine combines with ammonia, in the form of carbamoyl phosphate, to form citrulline. A second amino group is transferred to citrulline from aspartate to form arginine—the immediate precursor of urea. Arginase catalyzes hydrolysis of arginine to urea and ornithine; thus ornithine is regenerated in each turn of the cycle.
- The urea cycle results in a net conversion of oxaloacetate to fumarate, both of which are intermediates in the citric acid cycle. The two cycles are thus interconnected.
- The activity of the urea cycle is regulated at the level of enzyme synthesis and by allosteric regulation of the enzyme that catalyzes the formation of carbamoyl phosphate.

18.3 Pathways of Amino Acid Degradation

The pathways of amino acid catabolism, taken together, normally account for only 10% to 15% of the human body's energy production; these pathways are not nearly as active as glycolysis and fatty acid oxidation. Flux through these catabolic routes also varies greatly, depending on the balance between requirements for biosynthetic processes and the availability of a particular amino acid. The 20 catabolic pathways converge to form only six major products, all of which enter the citric acid cycle (Fig. 18–15). From here the carbon skeletons are diverted to gluconeogenesis or ketogenesis or are completely oxidized to CO_2 and H_2O.

All or part of the carbon skeletons of seven amino acids are ultimately broken down to acetyl-CoA. Five amino acids are converted to α-ketoglutarate, four to succinyl-CoA, two to fumarate, and two to oxaloacetate. Parts or all of six amino acids are converted to pyruvate, which can be converted to either acetyl-CoA or oxaloacetate. We later summarize the individual pathways for the 20 amino acids in flow diagrams, each leading to a specific point of entry into the citric acid cycle. In these diagrams the carbon atoms that enter the citric acid cycle are shown in color. Note that some amino acids appear more than once, reflecting different fates for different parts of their carbon skeletons. Rather than examining every step of every pathway in amino acid catabolism, we single out for special discussion some enzymatic reactions that are particularly noteworthy for their mechanisms or their medical significance.

Some Amino Acids Are Converted to Glucose, Others to Ketone Bodies

The seven amino acids that are degraded entirely or in part to acetoacetyl-CoA and/or acetyl-CoA—phenylalanine, tyrosine, isoleucine, leucine, tryptophan, threonine, and lysine—can yield ketone bodies in the liver,

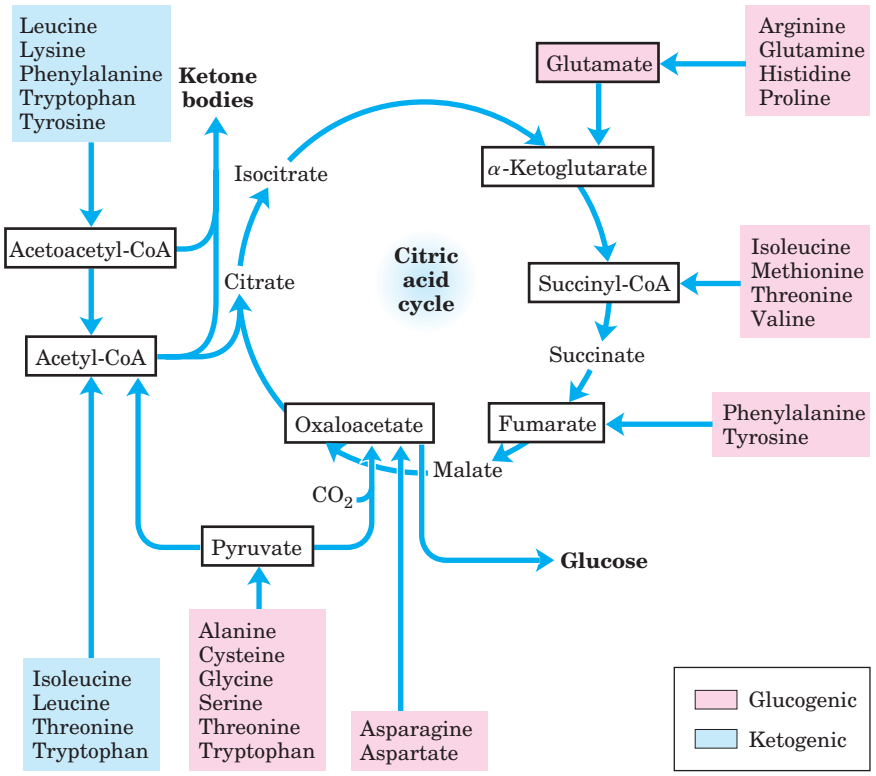


FIGURE 18–15 Summary of amino acid catabolism. Amino acids are grouped according to their major degradative end product. Some amino acids are listed more than once because different parts of their carbon skeletons are degraded to different end products. The figure shows the most important catabolic pathways in vertebrates, but there are minor variations among vertebrate species. Threonine, for instance, is degraded via at least two different pathways (see Figs 18–19, 18–27), and the importance of a given pathway can vary with the organism and its metabolic conditions. The glucogenic and ketogenic amino acids are also delineated in the figure, by color shading. Notice that five of the amino acids are both glucogenic and ketogenic. The amino acids degraded to pyruvate are also potentially ketogenic. Only two amino acids, leucine and lysine, are exclusively ketogenic.

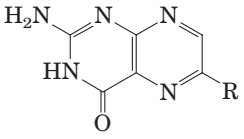
where acetoacetyl-CoA is converted to acetoacetate and then to acetone and β-hydroxybutyrate (see Fig. 17–18). These are the **ketogenic** amino acids (Fig. 18–15). Their ability to form ketone bodies is particularly evident in uncontrolled diabetes mellitus, in which the liver produces large amounts of ketone bodies from both fatty acids and the ketogenic amino acids.

The amino acids that are degraded to pyruvate, α-ketoglutarate, succinyl-CoA, fumarate, and/or oxaloacetate can be converted to glucose and glycogen by pathways described in Chapters 14 and 15. They are the **glucogenic** amino acids. The division between ketogenic and glucogenic amino acids is not sharp; five amino acids—tryptophan, phenylalanine, tyrosine, threonine, and isoleucine—are both ketogenic and glucogenic. Catabolism of amino acids is particularly critical to the survival of animals with high-protein diets or during starvation. Leucine is an exclusively ketogenic amino acid that is very common in proteins. Its degradation makes a substantial contribution to ketosis under starvation conditions.

Several Enzyme Cofactors Play Important Roles in Amino Acid Catabolism

A variety of interesting chemical rearrangements occur in the catabolic pathways of amino acids. It is useful to begin our study of these pathways by noting the classes of reactions that recur and introducing their enzyme cofactors. We have already considered one important class: transamination reactions requiring pyridoxal phosphate. Another common type of reaction in amino acid catabolism is one-carbon transfers, which usually involve one of three cofactors: biotin, tetrahydrofolate, or *S*-adenosylmethionine (Fig. 18–16). These cofactors transfer one-carbon groups in different oxidation states: biotin transfers carbon in its most oxidized state, CO_2 (see Fig. 14–18); tetrahydrofolate transfers one-carbon groups in intermediate oxidation states and sometimes as methyl groups; and *S*-adenosylmethionine transfers methyl groups, the most reduced state of carbon. The latter two cofactors are especially important in amino acid and nucleotide metabolism.

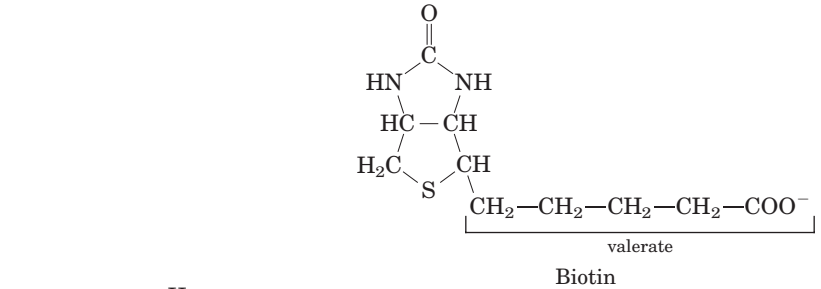
Tetrahydrofolate (H_4 folate), synthesized in bacteria, consists of substituted pterin (6-methylpterin),



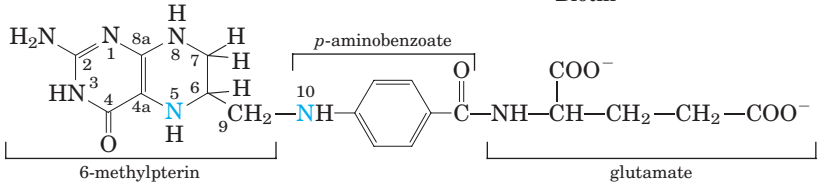
Pterin

p-aminobenzoate, and glutamate moieties (Fig. 18–16). The oxidized form, folate, is a vitamin for mammals; it is converted in two steps to tetrahydrofolate by the enzyme dihydrofolate reductase. The one-carbon group undergoing transfer, in any of three oxidation states, is bonded to N-5 or N-10 or both. The most reduced form of the cofactor carries a methyl group, a more oxidized form carries a methylene group, and the most oxidized forms carry a methenyl, formyl, or formimino group (Fig. 18–17). Most forms of tetrahydrofolate are interconvertible and serve as donors of one-carbon units in a variety of metabolic reactions. The primary source of one-carbon units for tetrahydrofolate is the carbon removed in the conversion of serine to glycine, producing N^5,N^{10}-methylenetetrahydrofolate.

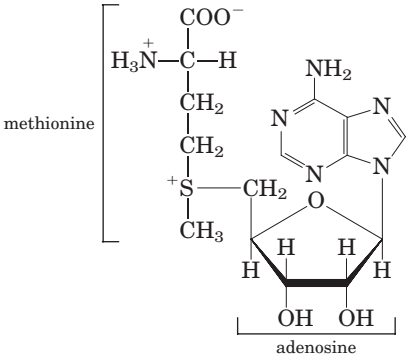
Although tetrahydrofolate can carry a methyl group at N-5, the transfer potential of this methyl group is insufficient for most biosynthetic reactions. ***S*-Adenosylmethionine (adoMet)** is the preferred cofactor for biological methyl group transfers. It is synthesized from ATP and methionine by the action of **methionine**



Biotin



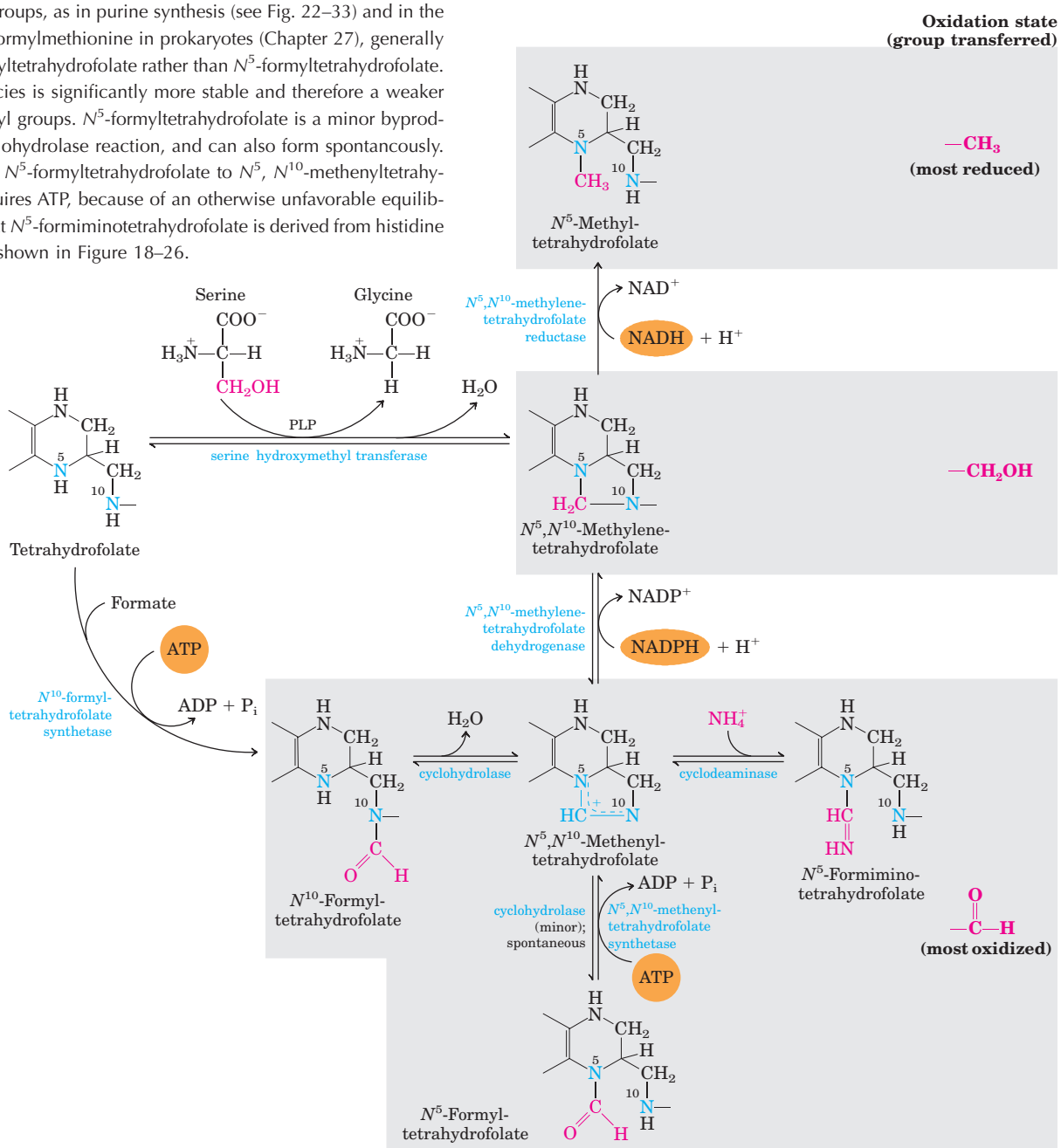
Tetrahydrofolate (H_4 folate)



S-Adenosylmethionine (adoMet)

FIGURE 18–16 Some enzyme cofactors important in one-carbon transfer reactions. The nitrogen atoms to which one-carbon groups are attached in tetrahydrofolate are shown in blue.

FIGURE 18–17 Conversions of one-carbon units on tetrahydrofolate. The different molecular species are grouped according to oxidation state, with the most reduced at the top and most oxidized at the bottom. All species within a single shaded box are at the same oxidation state. The conversion of N^5,N^{10}-methylenetetrahydrofolate to N^5-methyltetrahydrofolate is effectively irreversible. The enzymatic transfer of formyl groups, as in purine synthesis (see Fig. 22–33) and in the formation of formylmethionine in prokaryotes (Chapter 27), generally uses N^{10}-formyltetrahydrofolate rather than N^5-formyltetrahydrofolate. The latter species is significantly more stable and therefore a weaker donor of formyl groups. N^5-formyltetrahydrofolate is a minor byproduct of the cyclohydrolase reaction, and can also form spontaneously. Conversion of N^5-formyltetrahydrofolate to N^5, N^{10}-methenyltetrahydrofolate, requires ATP, because of an otherwise unfavorable equilibrium. Note that N^5-formiminotetrahydrofolate is derived from histidine in a pathway shown in Figure 18–26.



adenosyl transferase (Fig. 18–18, step ①). This reaction is unusual in that the nucleophilic sulfur atom of methionine attacks the 5′ carbon of the ribose moiety of ATP rather than one of the phosphorus atoms. Triphosphate is released and is cleaved to P_i and PP_i on the enzyme, and the PP_i is cleaved by inorganic pyrophosphatase; thus three bonds, including two bonds of high-energy phosphate groups, are broken in this reaction. The only other known reaction in which triphosphate is displaced from ATP occurs in the synthesis of coenzyme B_{12} (see Box 17–2, Fig. 3).

S-Adenosylmethionine is a potent alkylating agent by virtue of its destabilizing sulfonium ion. The methyl group is subject to attack by nucleophiles and is about

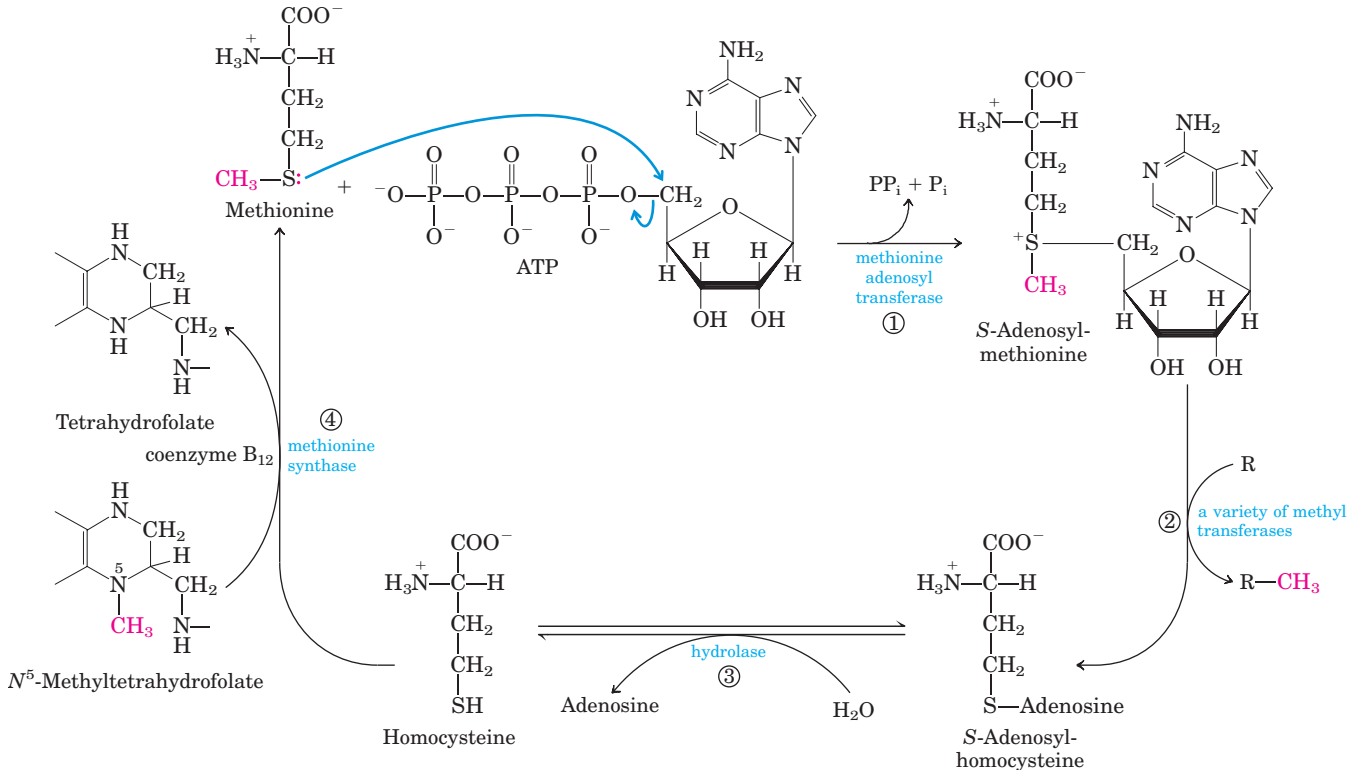


FIGURE 18–18 Synthesis of methionine and *S*-adenosylmethionine in an activated-methyl cycle. The steps are described in the text. In the methionine synthase reaction (step ④), the methyl group is transferred to cobalamin to form methylcobalamin, which in turn is the methyl donor in the formation of methionine. *S*-Adenosylmethionine, which has a positively charged sulfur (and is thus a sulfonium ion), is a powerful methylating agent in a number of biosynthetic reactions. The methyl group acceptor (step ②) is designated R.

1,000 times more reactive than the methyl group of N^5-methyltetrahydrofolate.

Transfer of the methyl group from *S*-adenosylmethionine to an acceptor yields ***S*-adenosylhomocysteine** (Fig. 18–18, step ②), which is subsequently broken down to homocysteine and adenosine (step ③). Methionine is regenerated by transfer of a methyl group to homocysteine in a reaction catalyzed by methionine synthase (step ④), and methionine is reconverted to *S*-adenosylmethionine to complete an activated-methyl cycle.

One form of methionine synthase common in bacteria uses N^5-methyltetrahydrofolate as a methyl donor. Another form of the enzyme present in some bacteria and mammals uses N^5-methyltetrahydrofolate, but the methyl group is first transferred to cobalamin, derived from coenzyme B_{12}, to form methylcobalamin as the methyl donor in methionine formation. This reaction and the rearrangement of L-methylmalonyl-CoA to succinyl-CoA (see Box 17–2, Fig. 1a) are the only known coenzyme B_{12}–dependent reactions in mammals. In cases of vitamin B_{12} deficiency, some symptoms can be alleviated by administering not only vitamin B_{12} but folate. As noted above, the methyl group of methylcobalamin is derived from N^5-methyltetrahydrofolate. Because the reaction converting the N^5,N^{10}-methylene form to the N^5-methyl form of tetrahydrofolate is irreversible (Fig. 18–17), if coenzyme B_{12} is not available for the synthesis of methylcobalamin, then no acceptor is available for the methyl group of N^5-methyltetrahydrofolate and metabolic folates become trapped in the N^5-methyl form. This sequestering of folates in one form may be the cause of some symptoms of the vitamin B_{12} deficiency disease pernicious anemia. However, we do not know whether this is the only effect of insufficient vitamin B_{12}. ■

Tetrahydrobiopterin, another cofactor of amino acid catabolism, is similar to the pterin moiety of tetrahydrofolate, but it is not involved in one-carbon transfers; instead it participates in oxidation reactions. We consider its mode of action when we discuss phenylalanine degradation (see Fig. 18–24).

Six Amino Acids Are Degraded to Pyruvate

The carbon skeletons of six amino acids are converted in whole or in part to pyruvate. The pyruvate can then be converted to either acetyl-CoA (a ketone body precursor) or oxaloacetate (a precursor for gluconeogenesis). Thus amino acids catabolized to pyruvate are both ketogenic and glucogenic. The six are alanine, tryptophan, cysteine, serine, glycine, and threonine (Fig. 18–19). **Alanine** yields pyruvate directly on transamination with

α-ketoglutarate, and the side chain of **tryptophan** is cleaved to yield alanine and thus pyruvate. **Cysteine** is converted to pyruvate in two steps; one removes the sulfur atom, the other is a transamination. **Serine** is converted to pyruvate by serine dehydratase. Both the β-hydroxyl and the α-amino groups of serine are removed in this single pyridoxal phosphate–dependent reaction (Fig. 18–20a).

Glycine is degraded via three pathways, only one of which leads to pyruvate. Glycine is converted to serine by enzymatic addition of a hydroxymethyl group (Figs 18–19 and 18–20b). This reaction, catalyzed by **serine hydroxymethyl transferase,** requires the coenzymes tetrahydrofolate and pyridoxal phosphate. The serine is converted to pyruvate as described above. In the second pathway, which predominates in animals, glycine undergoes oxidative cleavage to CO_2, NH_4^+, and a methylene group (—CH_2—) (Fig. 18–19). This readily reversible reaction, catalyzed by **glycine cleavage enzyme** (also called glycine synthase), also requires tetrahydrofolate, which accepts the methylene group. In this oxidative cleavage pathway the two carbon atoms of glycine do not enter the citric acid cycle. One carbon is lost as CO_2 and the other becomes the methylene group of N^5,N^{10}-methylenetetrahydrofolate (Fig. 18–17), a one-carbon group donor in certain biosynthetic pathways.

This second pathway for glycine degradation appears to be critical in mammals. Humans with serious defects in glycine cleavage enzyme activity suffer from a condition known as nonketotic hyperglycinemia. The condition is characterized by elevated serum levels of glycine, leading to severe mental deficiencies and death in very early childhood. At high levels, glycine is an inhibitory neurotransmitter, perhaps explaining the neurological effects of the disease. Many genetic defects of amino acid metabolism have been identified in humans (Table 18–2). We will encounter several more in this chapter. ■

In the third and final pathway of glycine degradation, the achiral glycine molecule is a substrate for the enzyme D-amino acid oxidase. The glycine is converted to glyoxylate, an alternative substrate for hepatic lactate

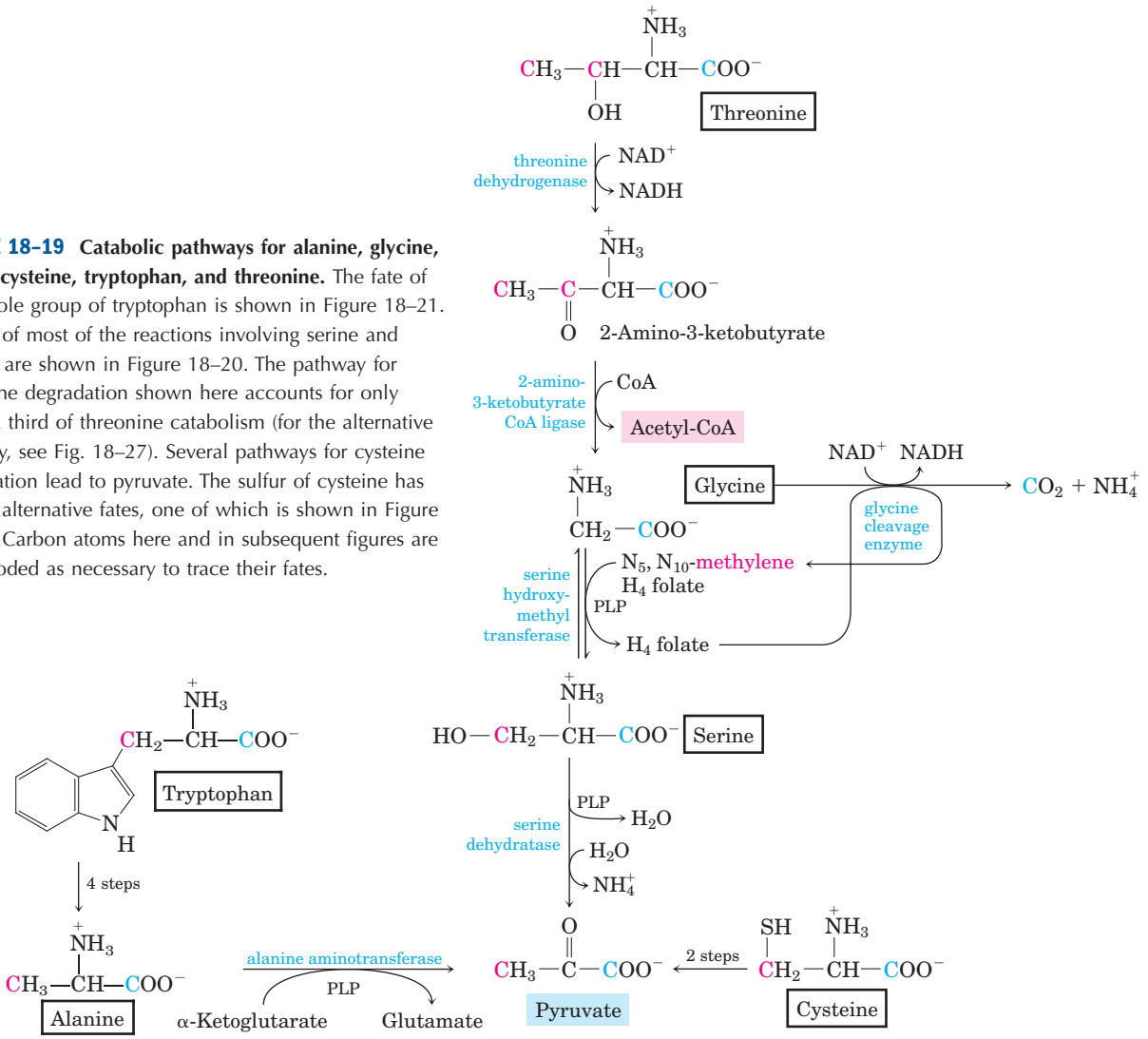
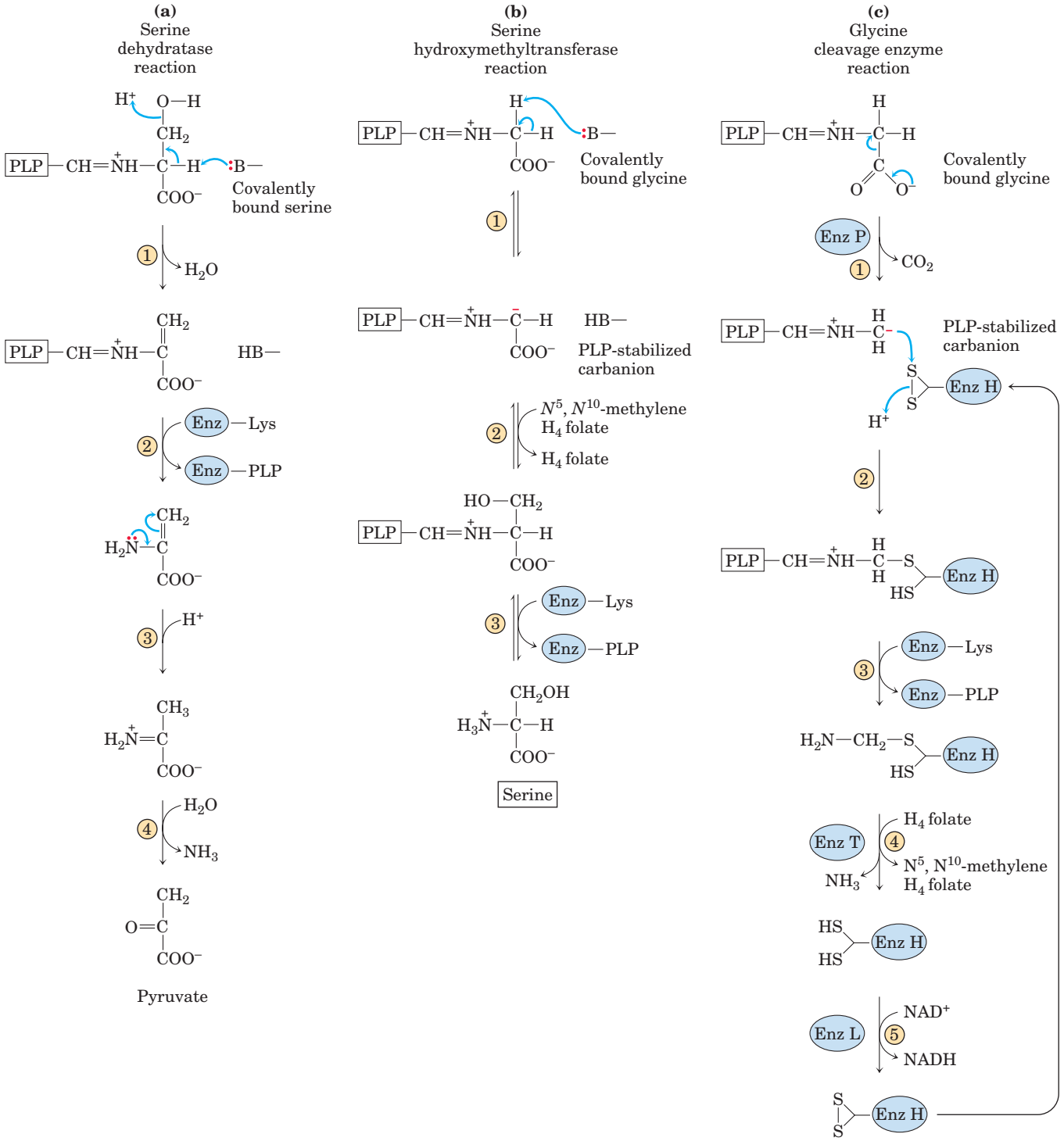


FIGURE 18–19 Catabolic pathways for alanine, glycine, serine, cysteine, tryptophan, and threonine. The fate of the indole group of tryptophan is shown in Figure 18–21. Details of most of the reactions involving serine and glycine are shown in Figure 18–20. The pathway for threonine degradation shown here accounts for only about a third of threonine catabolism (for the alternative pathway, see Fig. 18–27). Several pathways for cysteine degradation lead to pyruvate. The sulfur of cysteine has several alternative fates, one of which is shown in Figure 22–15. Carbon atoms here and in subsequent figures are color-coded as necessary to trace their fates.

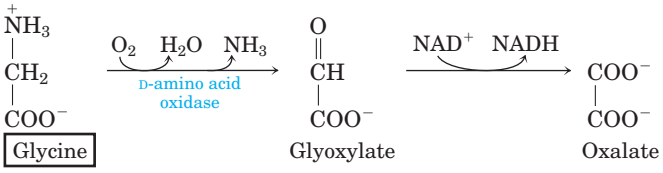


MECHANISM FIGURE 18–20 Interplay of the pyridoxal phosphate and tetrahydrofolate cofactors in serine and glycine metabolism. The first step in each of these reactions (not shown) involves the formation of a covalent imine linkage between enzyme-bound PLP and the substrate amino acid—serine in **(a),** glycine in **(b)** and **(c). (a)** The serine dehydratase reaction entails a PLP-catalyzed elimination of water across the bond between the α and β carbons (step 1), leading eventually to the production of pyruvate (steps 2 through 4). **(b)** In the serine hydroxymethyltransferase reaction, a PLP-stabilized carbanion on the α carbon of glycine (product of step 1) is a key intermediate in the transfer of the methylene group (as $—CH_2—OH$) from N^5,N^{10}-methylenetetrahydrofolate to form serine. This reaction is reversible. **(c)** The glycine cleavage enzyme is a multienzyme complex, with components P, H, T, and L. The overall reaction, which is reversible, converts glycine to CO_2 and NH_4^+, with the second glycine carbon taken up by tetrahydrofolate to form N^5,N^{10}-methylenetetrahydrofolate. Pyridoxal phosphate activates the α carbon of amino acids at critical stages in all these reactions, and tetrahydrofolate carries one-carbon units in two of them (see Figs 18–6, 18–17).

TABLE 18–2 Some Human Genetic Disorders Affecting Amino Acid Catabolism

Medical condition	*Approximate incidence (per 100,000 births)*	*Defective process*	*Defective enzyme*	*Symptoms and effects*
Albinism	<3	Melanin synthesis from tyrosine	Tyrosine 3-monooxygenase (tyrosinase)	Lack of pigmentation: white hair, pink skin
Alkaptonuria	<0.4	Tyrosine degradation	Homogentisate 1,2-dioxygenase	Dark pigment in urine; late-developing arthritis
Argininemia	<0.5	Urea synthesis	Arginase	Mental retardation
Argininosuccinic acidemia	<1.5	Urea synthesis	Argininosuccinase	Vomiting; convulsions
Carbamoyl phosphate synthetase I deficiency	<0.5	Urea synthesis	Carbamoyl phosphate synthetase I	Lethargy; convulsions; early death
Homocystinuria	<0.5	Methionine degradation	Cystathionine β-synthase	Faulty bone development; mental retardation
Maple syrup urine disease (branched-chain ketoaciduria)	<0.4	Isoleucine, leucine, and valine degradation	Branched-chain α-keto acid dehydrogenase complex	Vomiting; convulsions; mental retardation; early death
Methylmalonic acidemia	<0.5	Conversion of propionyl-CoA to succinyl-CoA	Methylmalonyl-CoA mutase	Vomiting; convulsions; mental retardation; early death
Phenylketonuria	<8	Conversion of phenylalanine to tyrosine	Phenylalanine hydroxylase	Neonatal vomiting; mental retardation

dehydrogenase (p. 538). Glyoxylate is oxidized in an NAD^+-dependent reaction to oxalate:



The primary function of D-amino acid oxidase, present at high levels in the kidney, is thought to be the detoxification of ingested D-amino acids derived from bacterial cell walls and from cooked foodstuffs (heat causes some spontaneous racemization of the L-amino acids in proteins). Oxalate, whether obtained in foods or produced enzymatically in the kidneys, has medical significance. Crystals of calcium oxalate account for up to 75% of all kidney stones. ■

There are two significant pathways for **threonine** degradation. One pathway leads to pyruvate via glycine (Fig. 18–19). The conversion to glycine occurs in two steps, with threonine first converted to 2-amino-3-ketobutyrate by the action of threonine dehydrogenase. This is a relatively minor pathway in humans, accounting for 10% to 30% of threonine catabolism, but is more important in some other mammals. The major pathway in humans leads to succinyl-CoA and is described later.

In the laboratory, serine hydroxymethyltransferase will catalyze the conversion of threonine to glycine and acetaldehyde in one step, but this is not a significant pathway for threonine degradation in mammals.

Seven Amino Acids Are Degraded to Acetyl-CoA

Portions of the carbon skeletons of seven amino acids—**tryptophan, lysine, phenylalanine, tyrosine, leucine, isoleucine,** and **threonine**—yield acetyl-CoA and/or acetoacetyl-CoA, the latter being converted to acetyl-CoA (Fig. 18–21). Some of the final steps in the degradative pathways for leucine, lysine, and tryptophan resemble steps in the oxidation of fatty acids. Threonine (not shown in Fig. 18–21) yields some acetyl-CoA via the minor pathway illustrated in Figure 18–19.

The degradative pathways of two of these seven amino acids deserve special mention. Tryptophan breakdown is the most complex of all the pathways of amino

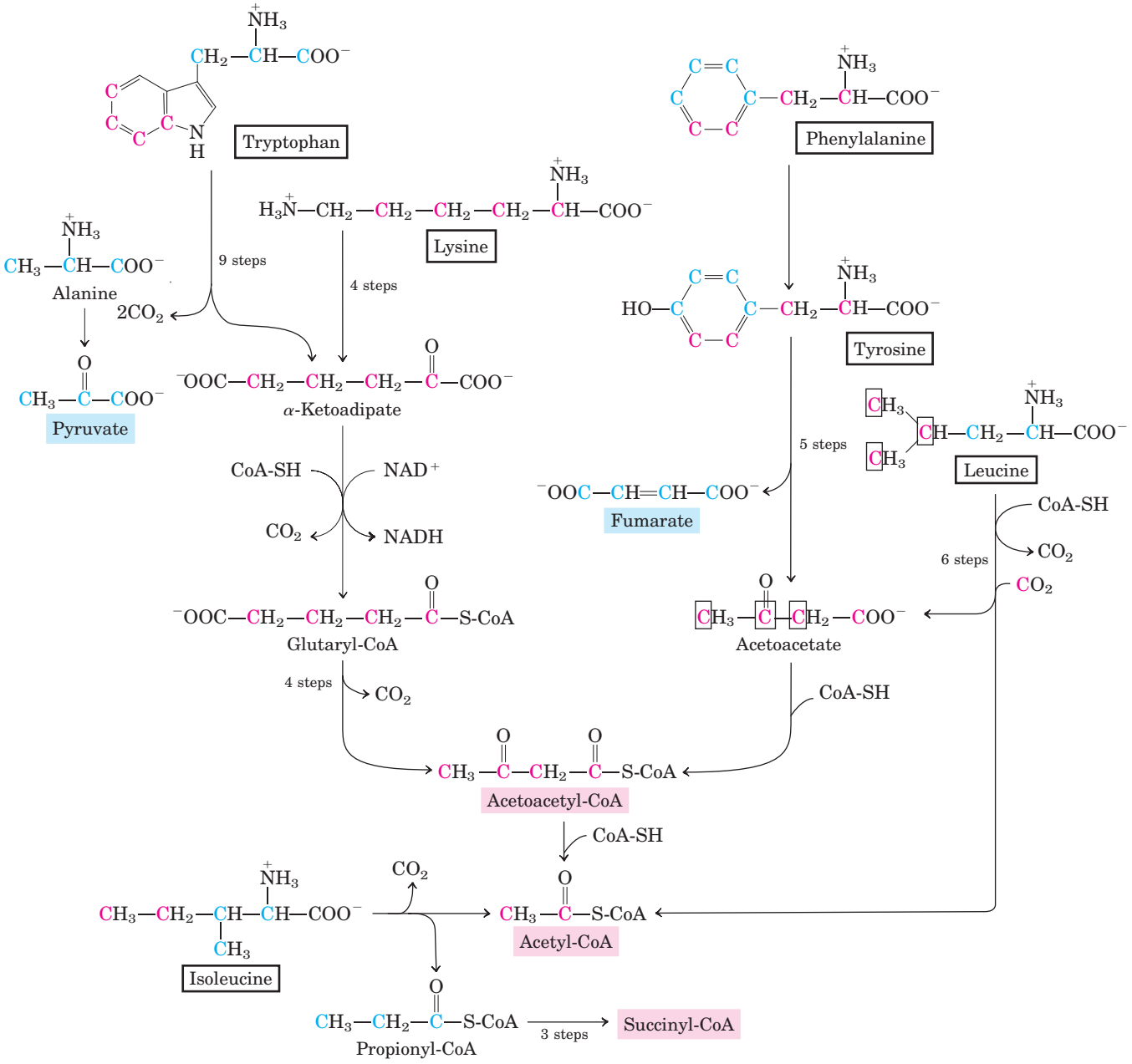


FIGURE 18–21 Catabolic pathways for tryptophan, lysine, phenylalanine, tyrosine, leucine, and isoleucine. These amino acids donate some of their carbons (red) to acetyl-CoA. Tryptophan, phenylalanine, tyrosine, and isoleucine also contribute carbons (blue) to pyruvate or citric acid cycle intermediates. The phenylalanine pathway is described in more detail in Figure 18–23. The fate of nitrogen atoms is not traced in this scheme; in most cases they are transferred to α-ketoglutarate to form glutamate.

acid catabolism in animal tissues; portions of tryptophan (four of its carbons) yield acetyl-CoA via acetoacetyl-CoA. Some of the intermediates in tryptophan catabolism are precursors for the synthesis of other biomolecules (Fig. 18–22), including nicotinate, a precursor of NAD and NADP in animals; serotonin, a neurotransmitter in vertebrates; and indoleacetate, a growth factor in plants. Some of these biosynthetic pathways are described in more detail in Chapter 22 (see Figs 22–28, 22–29).

The breakdown of phenylalanine is noteworthy because genetic defects in the enzymes of this pathway lead to several inheritable human diseases (Fig. 18–23), as discussed below. Phenylalanine and its oxidation product tyrosine (both with nine carbons) are degraded into two fragments, both of which can enter the citric acid cycle: four of the nine carbon atoms yield free acetoacetate, which is converted to acetoacetyl-CoA and thus acetyl-CoA, and a second four-carbon fragment is recovered as fumarate. Eight of the nine carbons of these two amino acids thus enter the citric acid cycle; the remaining carbon is lost as CO_2. Phenylalanine, after its hydroxylation to tyrosine, is also the precursor of dopamine, a neurotransmitter, and of norepinephrine and epinephrine, hormones secreted by the adrenal medulla (see Fig. 22–29). Melanin, the black pigment of skin and hair, is also derived from tyrosine.

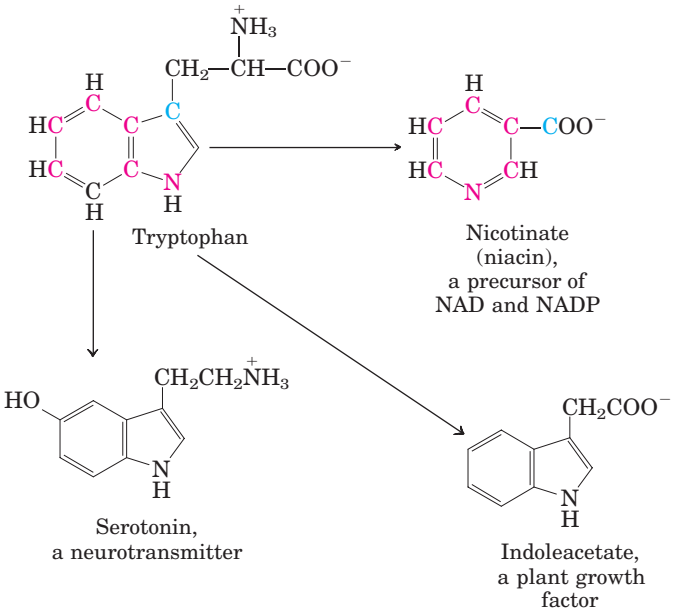


FIGURE 18–22 Tryptophan as precursor. The aromatic rings of tryptophan give rise to nicotinate, indoleacetate, and serotonin. Colored atoms trace the source of the ring atoms in nicotinate.

Phenylalanine Catabolism Is Genetically Defective in Some People

Given that many amino acids are either neurotransmitters or precursors or antagonists of neutrotransmitters, genetic defects of amino acid metabolism can cause defective neural development and mental retardation. In most such diseases specific intermediates accumulate. For example, a genetic defect in **phenylalanine hydroxylase,** the first enzyme in the catabolic pathway for phenylalanine (Fig. 18–23), is responsible for the disease **phenylketonuria (PKU),** the most common cause of elevated levels of phenylalanine (hyperphenylalaninemia).

Phenylalanine hydroxylase (also called phenylalanine-4-monooxygenase) is one of a general class of enzymes called **mixed-function oxidases** (see Box 21–1), all of which catalyze simultaneous hydroxylation of a substrate by an oxygen atom of O_2 and reduction of the other oxygen atom to H_2O. Phenylalanine hydroxylase

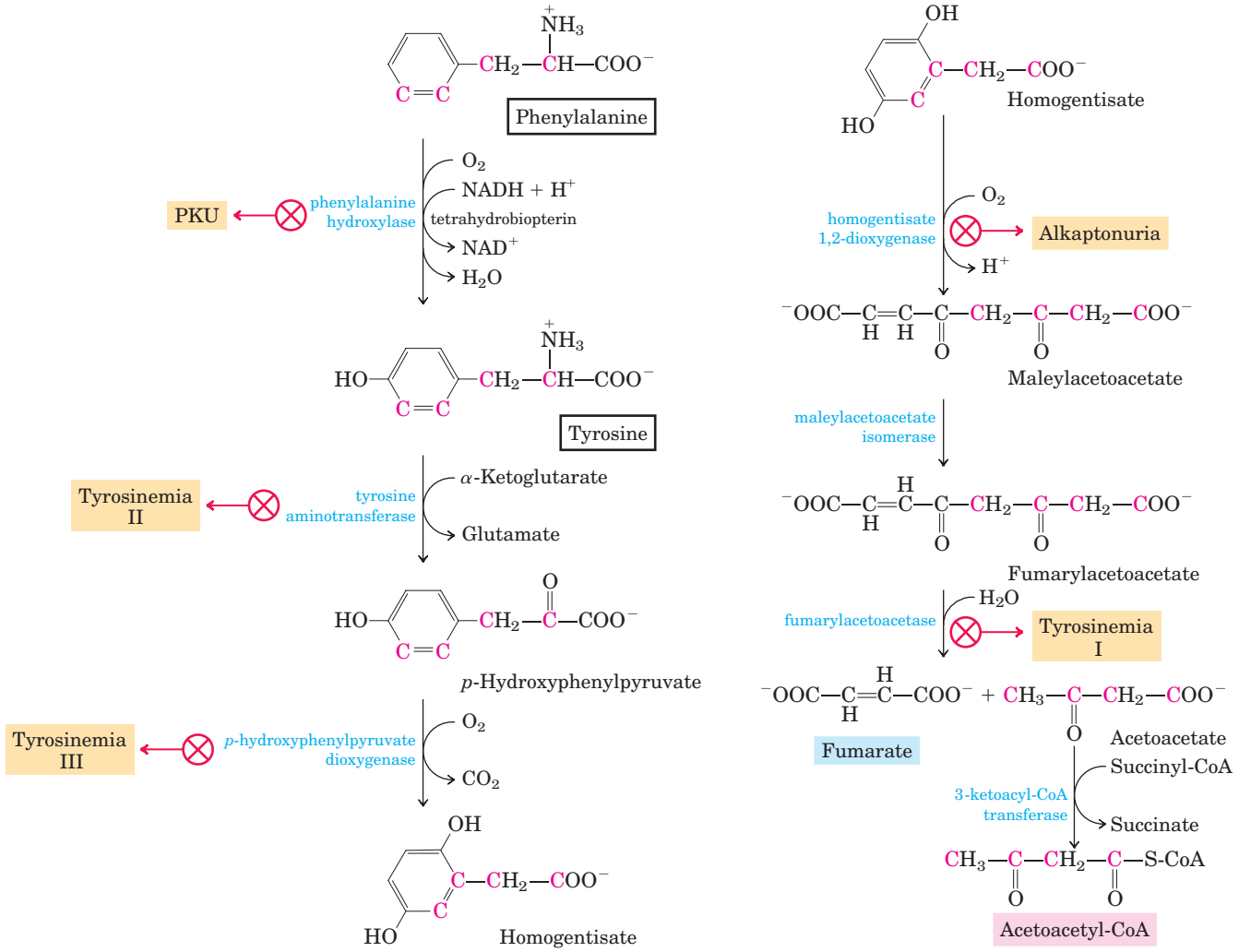


FIGURE 18–23 Catabolic pathways for phenylalanine and tyrosine. In humans these amino acids are normally converted to acetoacetyl-CoA and fumarate. Genetic defects in many of these enzymes cause inheritable human diseases (shaded yellow).

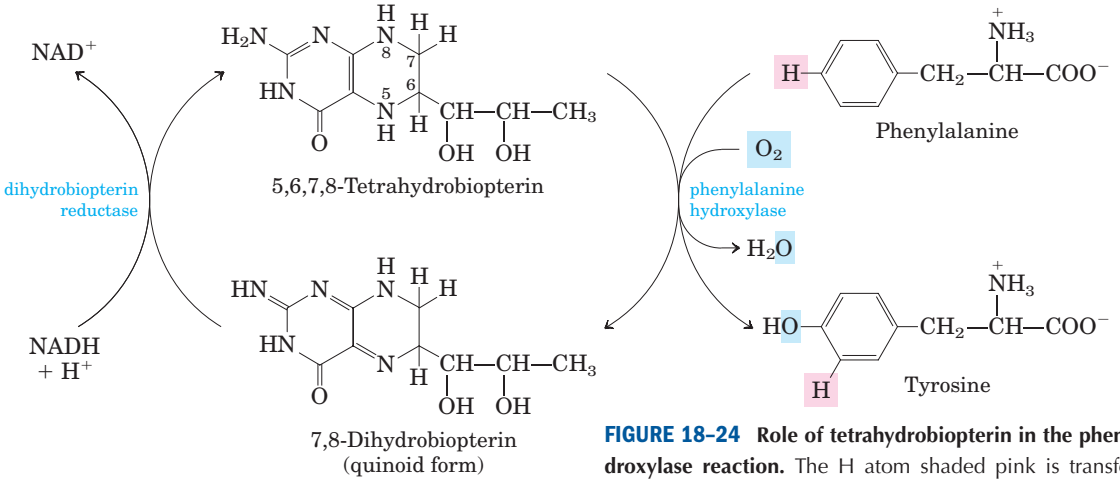


FIGURE 18–24 Role of tetrahydrobiopterin in the phenylalanine hydroxylase reaction. The H atom shaded pink is transferred directly from C-4 to C-3 in the reaction. This feature, discovered at the NIH, is called the NIH Shift.

requires the cofactor tetrahydrobiopterin, which carries electrons from NADH to O_2 and becomes oxidized to dihydrobiopterin in the process (Fig. 18–24). It is subsequently reduced by the enzyme **dihydrobiopterin reductase** in a reaction that requires NADH.

In individuals with PKU, a secondary, normally little-used pathway of phenylalanine metabolism comes into play. In this pathway phenylalanine undergoes transamination with pyruvate to yield **phenylpyruvate** (Fig. 18–25). Phenylalanine and phenylpyruvate accumulate in the blood and tissues and are excreted in the urine—hence the name "phenylketonuria." Much of the phenylpyruvate, rather than being excreted as such, is either decarboxylated to phenylacetate or reduced to phenyllactate. Phenylacetate imparts a characteristic odor to the urine, which nurses have traditionally used to detect PKU in infants. The accumulation of phenylalanine or its metabolites in early life impairs normal development of the brain, causing severe mental retardation. This may be caused by excess phenylalanine competing with other amino acids for transport across the blood-brain barrier, resulting in a deficit of required metabolites.

Phenylketonuria was among the first inheritable metabolic defects discovered in humans. When this condition is recognized early in infancy, mental retardation can largely be prevented by rigid dietary control. The diet must supply only enough phenylalanine and tyrosine to meet the needs for protein synthesis. Consumption of protein-rich foods must be curtailed. Natural proteins, such as casein of milk, must first be hydrolyzed and much of the phenylalanine removed to provide an appropriate diet, at least through childhood. Because the artificial sweetener aspartame is a dipeptide of aspartate and the methyl ester of phenylalanine (see Fig. 1–23b), foods sweetened with aspartame bear warnings addressed to individuals on phenylalanine-controlled diets.

Phenylketonuria can also be caused by a defect in the enzyme that catalyzes the regeneration of tetrahydrobiopterin (Fig. 18–24). The treatment in this case is more complex than restricting the intake of phenylalanine and tyrosine. Tetrahydrobiopterin is also required for the formation of L-3,4-dihydroxyphenylalanine (L-dopa) and 5-hydroxytryptophan—precursors of the neurotransmitters norepinephrine and serotonin, respectively—and in phenylketonuria of this type, these precursors must be supplied in the diet. Supplementing the diet with tetrahydrobiopterin itself is ineffective because it is unstable and does not cross the blood-brain barrier.

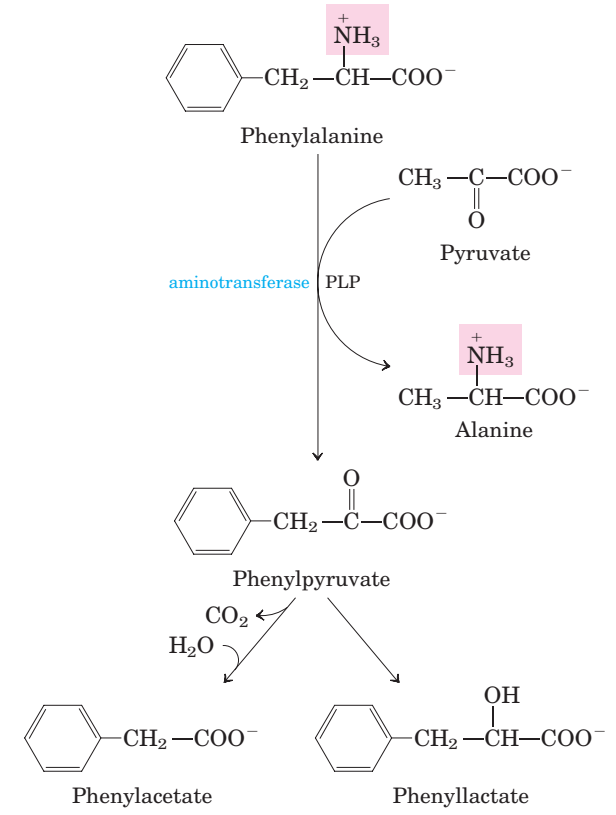


FIGURE 18–25 Alternative pathways for catabolism of phenylalanine in phenylketonuria. In PKU, phenylpyruvate accumulates in the tissues, blood, and urine. The urine may also contain phenylacetate and phenyllactate.

Screening newborns for genetic diseases can be highly cost-effective, especially in the case of PKU. The tests (no longer relying on urine odor) are relatively inexpensive, and the detection and early treatment of PKU in infants (eight to ten cases per 100,000 newborns) saves millions of dollars in later health care costs each year. More importantly, the emotional trauma avoided by early detection with these simple tests is inestimable.

Another inheritable disease of phenylalanine catabolism is **alkaptonuria,** in which the defective enzyme is **homogentisate dioxygenase** (Fig. 18–23). Less serious than PKU, this condition produces few ill effects, although large amounts of homogentisate are excreted and its oxidation turns the urine black. Individuals with alkaptonuria are also prone to develop a form of arthritis. Alkaptonuria is of considerable historical interest. Archibald Garrod discovered in the early 1900s that this condition is inherited, and he traced the cause to the absence of a single enzyme. Garrod was the first to make a connection between an inheritable trait and an enzyme, a great advance on the path that ultimately led to our current understanding of genes and the information pathways described in Part III. ■

Five Amino Acids Are Converted to α-Ketoglutarate

The carbon skeletons of five amino acids (proline, glutamate, glutamine, arginine, and histidine) enter the citric acid cycle as α-ketoglutarate (Fig. 18–26). **Proline, glutamate,** and **glutamine** have five-carbon skeletons. The cyclic structure of proline is opened by oxidation

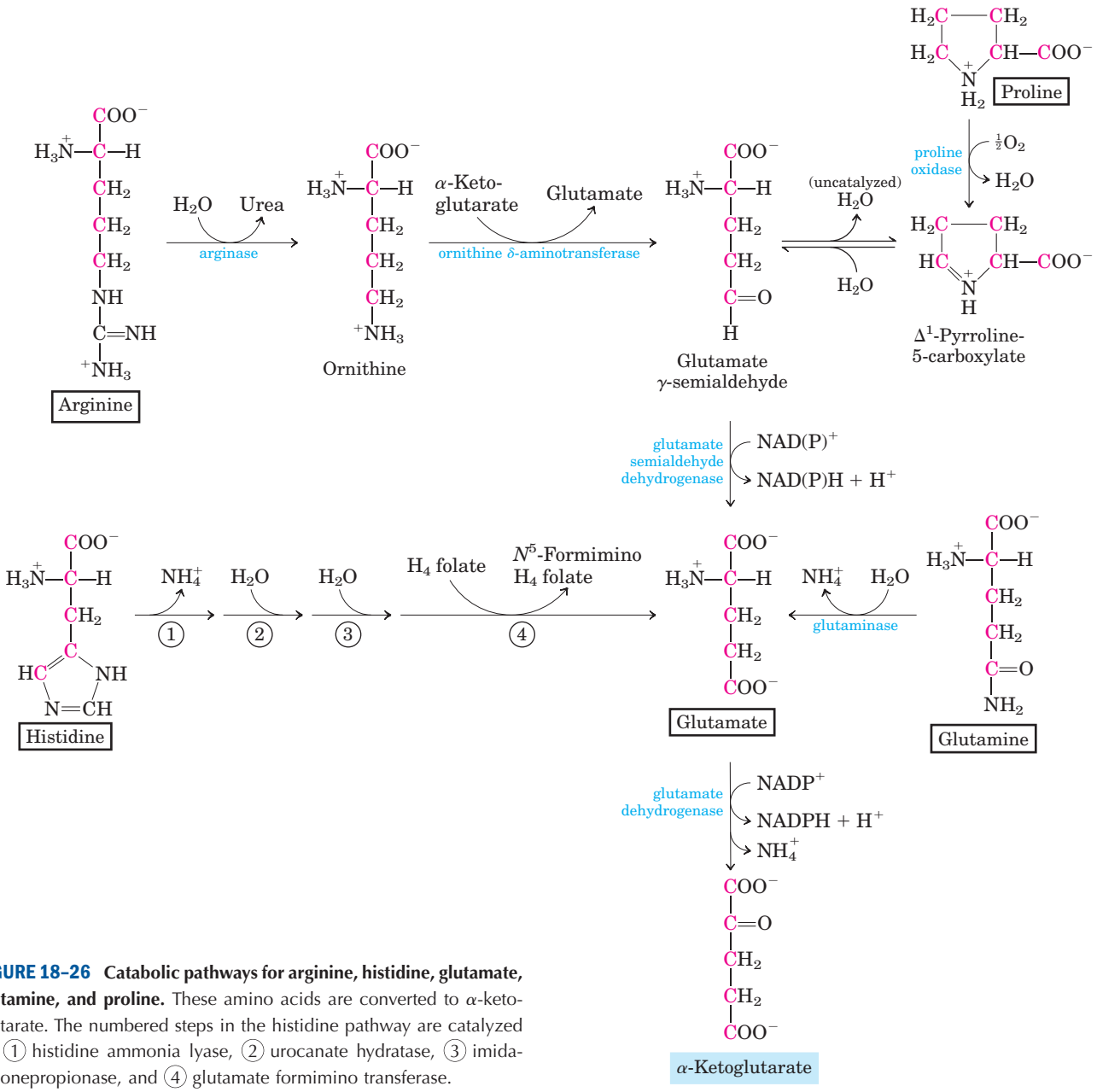


FIGURE 18–26 Catabolic pathways for arginine, histidine, glutamate, glutamine, and proline. These amino acids are converted to α-ketoglutarate. The numbered steps in the histidine pathway are catalyzed by (1) histidine ammonia lyase, (2) urocanate hydratase, (3) imidazolonepropionase, and (4) glutamate formimino transferase.

of the carbon most distant from the carboxyl group to create a Schiff base, then hydrolysis of the Schiff base to a linear semialdehyde, glutamate γ-semialdehyde. This intermediate is further oxidized at the same carbon to produce glutamate. The action of glutaminase, or any of several enzyme reactions in which glutamine donates its amide nitrogen to an acceptor, converts glutamine to glutamate. Transamination or deamination of glutamate produces α-ketoglutarate.

Arginine and **histidine** contain five adjacent carbons and a sixth carbon attached through a nitrogen atom. The catabolic conversion of these amino acids to glutamate is therefore slightly more complex than the path from proline or glutamine (Fig. 18–26). Arginine is converted to the five-carbon skeleton of ornithine in the urea cycle (Fig. 18–10), and the ornithine is transaminated to glutamate γ-semialdehyde. Conversion of histidine to the five-carbon glutamate occurs in a multistep pathway; the extra carbon is removed in a step that uses tetrahydrofolate as cofactor.

Four Amino Acids Are Converted to Succinyl-CoA

The carbon skeletons of methionine, isoleucine, threonine, and valine are degraded by pathways that yield succinyl-CoA (Fig. 18–27), an intermediate of the citric acid cycle. **Methionine** donates its methyl group to one of several possible acceptors through *S*-adenosylmethionine,

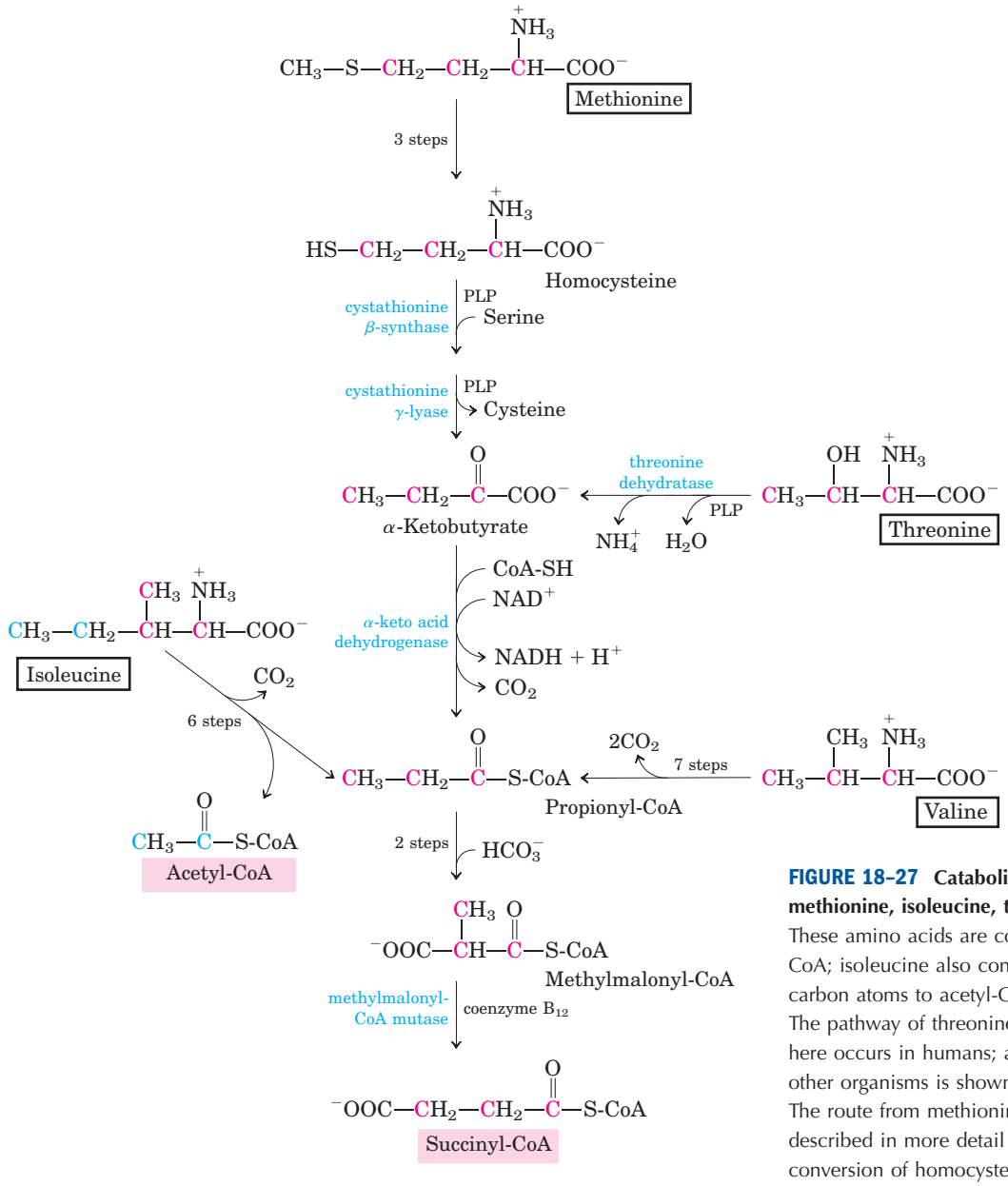


FIGURE 18–27 Catabolic pathways for methionine, isoleucine, threonine, and valine. These amino acids are converted to succinyl-CoA; isoleucine also contributes two of its carbon atoms to acetyl-CoA (see Fig. 18–21). The pathway of threonine degradation shown here occurs in humans; a pathway found in other organisms is shown in Figure 18–19. The route from methionine to homocysteine is described in more detail in Figure 18–18; the conversion of homocysteine to α-ketobutyrate in Figure 22–14; the conversion of propionyl-CoA to succinyl-CoA in Figure 17–11.

and three of its four remaining carbon atoms are converted to the propionate of propionyl-CoA, a precursor of succinyl-CoA. **Isoleucine** undergoes transamination, followed by oxidative decarboxylation of the resulting α-keto acid. The remaining five-carbon skeleton is further oxidized to acetyl-CoA and propionyl-CoA. **Valine** undergoes transamination and decarboxylation, then a series of oxidation reactions that convert the remaining four carbons to propionyl-CoA. Some parts of the valine and isoleucine degradative pathways closely parallel steps in fatty acid degradation (see Fig. 17–8a). In human tissues, **threonine** is also converted in two steps to propionyl-CoA. This is the primary pathway for threonine degradation in humans (see Fig. 18–19 for the alternative pathway). The mechanism of the first step is analogous to that catalyzed by serine dehydratase, and the serine and threonine dehydratases may actually be the same enzyme.

The propionyl-CoA derived from these three amino acids is converted to succinyl-CoA by a pathway described in Chapter 17: carboxylation to methylmalonyl-CoA, epimerization of the methylmalonyl-CoA, and conversion to succinyl-CoA by the coenzyme B_{12}–dependent methylmalonyl-CoA mutase (see Fig. 17–11). In the rare genetic disease known as methylmalonic acidemia, methylmalonyl-CoA mutase is lacking—with serious metabolic consequences (Table 18–2; Box 18–2).

Branched-Chain Amino Acids Are Not Degraded in the Liver

Although much of the catabolism of amino acids takes place in the liver, the three amino acids with branched side chains (leucine, isoleucine, and valine) are oxidized as fuels primarily in muscle, adipose, kidney, and brain tissue. These extrahepatic tissues contain an aminotransferase, absent in liver, that acts on all three branched-chain amino acids to produce the corresponding α-keto acids (Fig. 18–28). The **branched-chain α-keto acid dehydrogenase complex** then catalyzes oxidative decarboxylation of all three α-keto acids, in each case releasing the carboxyl group as CO_2 and producing the acyl-CoA derivative. This reaction is formally analogous to two other oxidative decarboxylations encountered in Chapter 16: oxidation of pyruvate to acetyl-CoA by the pyruvate dehydrogenase complex (see Fig. 16–6) and oxidation of α-ketoglutarate to succinyl-CoA by the α-ketoglutarate dehydrogenase complex (p. 610). In fact, all three enzyme complexes are similar in structure and share essentially the same reaction mechanism. Five cofactors (thiamine pyrophosphate, FAD, NAD, lipoate, and coenzyme A) participate, and the three proteins in each complex catalyze homologous reactions. This is clearly a case in which enzymatic machinery that evolved to catalyze

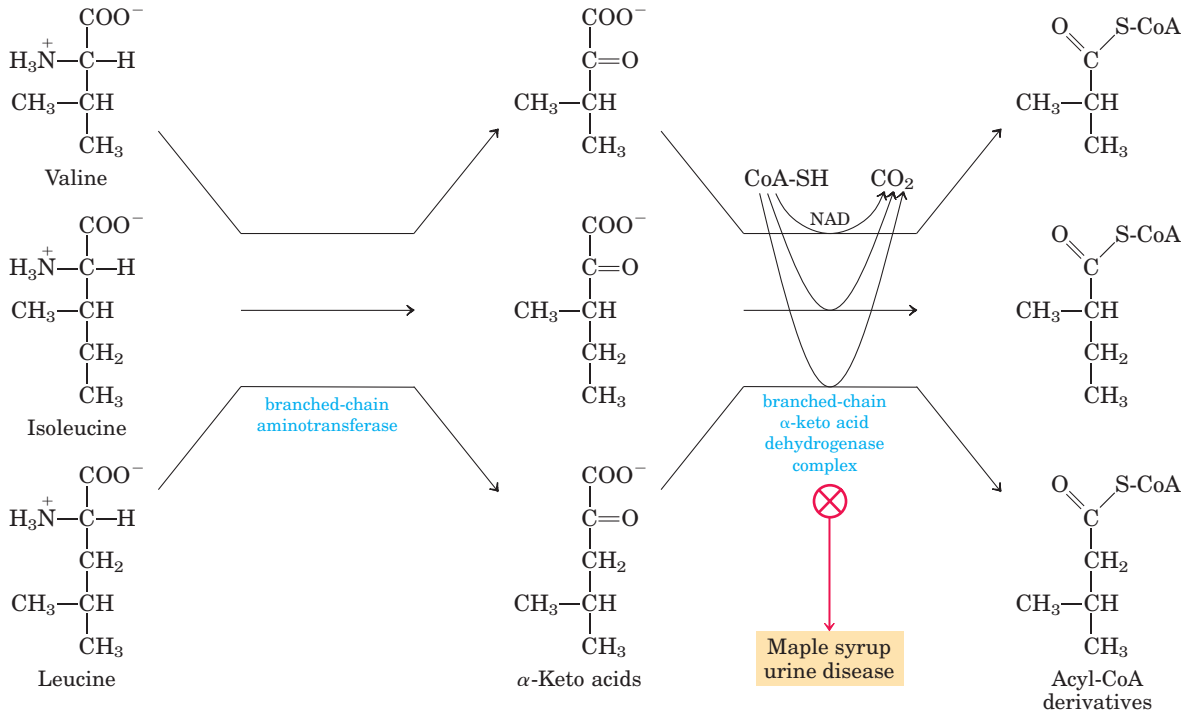


FIGURE 18–28 Catabolic pathways for the three branched-chain amino acids: valine, isoleucine, and leucine. The three pathways, which occur in extrahepatic tissues, share the first two enzymes, as shown here. The branched-chain α-keto acid dehydrogenase complex is analogous to the pyruvate and α-ketoglutarate dehydrogenase complexes and requires the same five cofactors (some not shown here). This enzyme is defective in people with maple syrup urine disease.

BOX 18–2 BIOCHEMISTRY IN MEDICINE

Scientific Sleuths Solve a Murder Mystery

Truth can sometimes be stranger than fiction—or at least as strange as a made-for-TV movie. Take, for example, the case of Patricia Stallings. Convicted of the murder of her infant son, she was sentenced to life in prison—but was later found innocent, thanks to the medical sleuthing of three persistent researchers.

The story began in the summer of 1989 when Stallings brought her three-month-old son, Ryan, to the emergency room of Cardinal Glennon Children's Hospital in St. Louis. The child had labored breathing, uncontrollable vomiting, and gastric distress. According to the attending physician, a toxicologist, the child's symptoms indicated that he had been poisoned with ethylene glycol, an ingredient of antifreeze, a conclusion apparently confirmed by analysis at a commercial lab.

After he recovered, the child was placed in a foster home, and Stallings and her husband, David, were allowed to see him in supervised visits. But when the infant became ill, and subsequently died, after a visit in which Stallings had been briefly left alone with him, she was charged with first-degree murder and held without bail. At the time, the evidence seemed compelling as both the commercial lab and the hospital lab found large amounts of ethylene glycol in the boy's blood and traces of it in a bottle of milk Stallings had fed her son during the visit.

But without knowing it, Stallings had performed a brilliant experiment. While in custody, she learned she was pregnant; she subsequently gave birth to another son, David Stallings Jr., in February 1990. He was placed immediately in a foster home, but within two weeks he started having symptoms similar to Ryan's. David was eventually diagnosed with a rare metabolic disorder called methylmalonic acidemia (MMA). A recessive genetic disorder of amino acid metabolism, MMA affects about 1 in 48,000 newborns and presents symptoms almost identical with those caused by ethylene glycol poisoning.

Stallings couldn't possibly have poisoned her second son, but the Missouri state prosecutor's office was not impressed by the new developments and pressed forward with her trial anyway. The court wouldn't allow the MMA diagnosis of the second child to be introduced as evidence, and in January 1991 Patricia Stallings was convicted of assault with a deadly weapon and sentenced to life in prison.

Fortunately for Stallings, however, William Sly, chairman of the Department of Biochemistry and Molecular Biology at St. Louis University, and James Shoemaker, head of a metabolic screening lab at the university, got interested in her case when they heard about it from a television broadcast. Shoemaker performed his own analysis of Ryan's blood and didn't detect ethylene glycol. He and Sly then contacted Piero Rinaldo, a metabolic disease expert at Yale University School of Medicine whose lab is equipped to diagnose MMA from blood samples.

When Rinaldo analyzed Ryan's blood serum, he found high concentrations of methylmalonic acid, a breakdown product of the branched-chain amino acids isoleucine and valine, which accumulates in MMA patients because the enzyme that should convert it to the next product in the metabolic pathway is defective. And particularly telling, he says, the child's blood and urine contained massive amounts of ketones, another metabolic consequence of the disease. Like Shoemaker, he did not find any ethylene glycol in a sample of the baby's bodily fluids. The bottle couldn't be tested, since it had mysteriously disappeared. Rinaldo's analyses convinced him that Ryan had died from MMA, but how to account for the results from two labs, indicating that the boy had ethylene glycol in his blood? Could they both be wrong?

When Rinaldo obtained the lab reports, what he saw was, he says, "scary." One lab said that Ryan Stallings' blood contained ethylene glycol, even though the blood sample analysis did not match the lab's own profile for a known sample containing ethylene glycol. "This was not just a matter of questionable interpretation. The quality of their analysis was unacceptable," Rinaldo says. And the second laboratory? According to Rinaldo, that lab detected an abnormal component in Ryan's blood and just "assumed it was ethylene glycol." Samples from the bottle had produced nothing unusual, says Rinaldo, yet the lab claimed evidence of ethylene glycol in that, too.

Rinaldo presented his findings to the case's prosecutor, George McElroy, who called a press conference the very next day. "I no longer believe the laboratory data," he told reporters. Having concluded that Ryan Stallings had died of MMA after all, McElroy dismissed all charges against Patricia Stallings on September 20, 1991.

By Michelle Hoffman (1991). *Science* **253,** 931.

one reaction was "borrowed" by gene duplication and further evolved to catalyze similar reactions in other pathways.

Experiments with rats have shown that the branched-chain α-keto acid dehydrogenase complex is regulated by covalent modification in response to the content of branched-chain amino acids in the diet. With little or no excess dietary intake of branched-chain amino acids, the enzyme complex is phosphorylated and thereby inactivated by a protein kinase. Addition of excess branched-chain amino acids to the diet results in dephosphorylation and consequent activation of the enzyme. Recall that the pyruvate dehydrogenase complex is subject to similar regulation by phosphorylation and dephosphorylation (p. 621).

There is a relatively rare genetic disease in which the three branched-chain α-keto acids (as well as their precursor amino acids, especially leucine) accumulate in the blood and "spill over" into the urine. This condition, called **maple syrup urine disease** because of the characteristic odor imparted to the urine by the α-keto acids, results from a defective branched-chain α-keto acid dehydrogenase complex. Untreated, the disease results in abnormal development of the brain, mental retardation, and death in early infancy. Treatment entails rigid control of the diet, limiting the intake of valine, isoleucine, and leucine to the minimum required to permit normal growth. ■

Asparagine and Aspartate Are Degraded to Oxaloacetate

The carbon skeletons of **asparagine** and **aspartate** ultimately enter the citric acid cycle as oxaloacetate. The enzyme **asparaginase** catalyzes the hydrolysis of asparagine to aspartate, which undergoes transamination with α-ketoglutarate to yield glutamate and oxaloacetate (Fig. 18–29).

We have now seen how the 20 common amino acids, after losing their nitrogen atoms, are degraded by dehydrogenation, decarboxylation, and other reactions to yield portions of their carbon backbones in the form of six central metabolites that can enter the citric acid cycle. Those portions degraded to acetyl-CoA are completely oxidized to carbon dioxide and water, with generation of ATP by oxidative phosphorylation.

As was the case for carbohydrates and lipids, the degradation of amino acids results ultimately in the generation of reducing equivalents (NADH and $FADH_2$) through the action of the citric acid cycle. Our survey of catabolic processes concludes in the next chapter with a discussion of respiration, in which these reducing equivalents fuel the ultimate oxidative and energy-generating process in aerobic organisms.

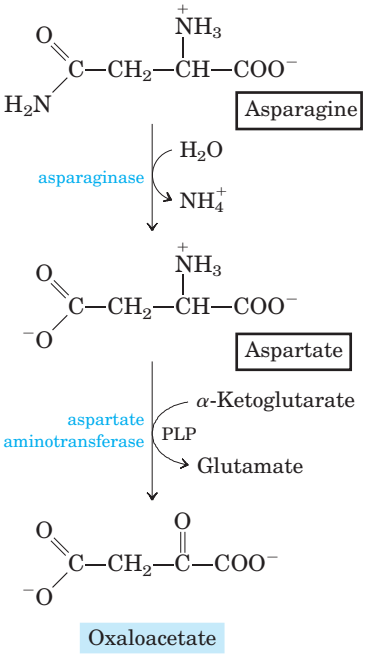


FIGURE 18–29 Catabolic pathway for asparagine and aspartate. Both amino acids are converted to oxaloacetate.

SUMMARY 18.3 Pathways of Amino Acid Degradation

- After removal of their amino groups, the carbon skeletons of amino acids undergo oxidation to compounds that can enter the citric acid cycle for oxidation to CO_2 and H_2O. The reactions of these pathways require a number of cofactors, including tetrahydrofolate and *S*-adenosylmethionine in one-carbon transfer reactions and tetrahydrobiopterin in the oxidation of phenylalanine by phenylalanine hydroxylase.
- Depending on their degradative end product, some amino acids can be converted to ketone bodies, some to glucose, and some to both. Thus amino acid degradation is integrated into intermediary metabolism and can be critical to survival under conditions in which amino acids are a significant source of metabolic energy.
- The carbon skeletons of amino acids enter the citric acid cycle through five intermediates: acetyl-CoA, α-ketoglutarate, succinyl-CoA, fumarate, and oxaloacetate. Some are also degraded to pyruvate, which can be converted to either acetyl-CoA or oxaloacetate.

- The amino acids producing pyruvate are alanine, cysteine, glycine, serine, threonine, and tryptophan. Leucine, lysine, phenylalanine, and tryptophan yield acetyl-CoA via acetoacetyl-CoA. Isoleucine, leucine, threonine, and tryptophan also form acetyl-CoA directly.
- Arginine, glutamate, glutamine, histidine, and proline produce α-ketoglutarate; isoleucine, methionine, threonine, and valine produce succinyl-CoA; four carbon atoms of phenylalanine and tyrosine give rise to fumarate; and asparagine and aspartate produce oxaloacetate.
- The branched-chain amino acids (isoleucine, leucine, and valine), unlike the other amino acids, are degraded only in extrahepatic tissues.
- A number of serious human diseases can be traced to genetic defects in the enzymes of amino acid catabolism.

Key Terms

Terms in bold are defined in the glossary.

aminotransferases 660
transaminases 660
transamination 660
pyridoxal phosphate (PLP) 660
oxidative deamination 661
L-glutamate dehydrogenase 661
glutamine synthetase 662
glutaminase 663
creatine kinase 664
glucose-alanine cycle 664
ammonotelic 665
ureotelic 665
uricotelic 665
urea cycle 665
urea 668
essential amino acids 669
ketogenic 672
glucogenic 672
tetrahydrofolate 672
***S*-adenosylmethionine (adoMet)** 672
tetrahydrobiopterin 674
phenylketonuria (PKU) 679
mixed-function oxidases 679
alkaptonuria 681
maple syrup urine disease 685

Further Reading

General

Arias, I.M., Boyer, J.L., Chisari, F.V., Fausto, N., Schachter, D., & Shafritz, D.A. (2001) *The Liver: Biology and Pathobiology,* 4th edn, Lippincott Williams & Wilkins, Philadelphia.

Bender, D.A. (1985) *Amino Acid Metabolism,* 2nd edn, Wiley-Interscience, Inc., New York.

Brosnan, J.T. (2001) Amino acids, then and now—a reflection on Sir Hans Krebs' contribution to nitrogen metabolism. *IUBMB Life* **52,** 265–270.
An interesting tour through the life of this important biochemist.

Campbell, J.W. (1991) Excretory nitrogen metabolism. In *Environmental and Metabolic Animal Physiology,* 4th edn (Prosser, C.L., ed.), pp. 277–324, John Wiley & Sons, Inc., New York.

Coomes, M.W. (1997) Amino acid metabolism. In *Textbook of Biochemistry with Clinical Correlations,* 5th edn (Devlin, T.M., ed.), pp. 779–823, Wiley-Liss, New York.

Hayashi, H. (1995) Pyridoxal enzymes: mechanistic diversity and uniformity. *J. Biochem.* **118,** 463–473.

Mazelis, M. (1980) Amino acid catabolism. In *The Biochemistry of Plants: A Comprehensive Treatise* (Stumpf, P.K. & Conn, E.E., eds), Vol. 5: *Amino Acids and Derivatives* (Miflin, B.J., ed.), pp. 541–567, Academic Press, Inc., New York.
A discussion of the various fates of amino acids in plants.

Walsh, C. (1979) *Enzymatic Reaction Mechanisms,* W. H. Freeman and Company, San Francisco.
A good source for in-depth discussion of the classes of enzymatic reaction mechanisms described in the chapter.

Amino Acid Metabolism

Christen, P. & Metzler, D.E. (1985) *Transaminases,* Wiley-Interscience, Inc., New York.

Curthoys, N.P. & Watford, M. (1995) Regulation of glutaminase activity and glutamine metabolism. *Annu. Rev. Nutr.* **15,** 133–159.

Fitzpatrick, P.F. (1999) Tetrahydropterin-dependent amino acid hydroxylases. *Annu. Rev. Biochem.* **68,** 355–382.

Kirsch, J.F. & Eliot, A.C. (2004) Pyridoxal phosphate enzymes: mechanistic, structural and evolutionary considerations. *Annu. Rev. Biochem.* **73** [in press].

Pencharz, P.B. & Ball, R.O. (2003) Different approaches to define individual amino acid requirements. *Annu. Rev. Nutr.* **23,** 101–116.
Determination of which amino acids are essential in the human diet is not a trivial problem, as this review relates.

The Urea Cycle

Brusilow, S.W. & Horwich, A.L. (2001) Urea cycle enzymes. In *The Metabolic Bases of Inherited Disease,* 8th edn (Scriver, C.R., Beaudet, A.C., Sly, W.S., Valle, D., Childs, B., Kinzler, K., & Vogelstein, B., eds), pp. 1909–1963, McGraw-Hill Companies Inc., New York.
An authoritative source on this pathway.

Holmes, F.L. (1980) Hans Krebs and the discovery of the ornithine cycle. *Fed. Proc.* **39,** 216–225.
A medical historian reconstructs the events leading to the discovery of the urea cycle.

Kirsch, J.F., Eichele, G., Ford, G.C., Vincent, M.G., Jansonius, J.N., Gehring, H., & Christen, P. (1984) Mechanism of action of aspartate aminotransferase proposed on the basis of its spatial structure. *J. Mol. Biol.* **174,** 497–525.

Morris, S.M. (2002) Regulation of enzymes of the urea cycle and arginine metabolism. *Annu. Rev. Nutr.* **22,** 87–105.
This review details what is known about some levels of regulation not covered in the chapter, such as hormonal and nutritional regulation.

Disorders of Amino Acid Degradation

Ledley, F.D., Levy, H.L., & Woo, S.L.C. (1986) Molecular analysis of the inheritance of phenylketonuria and mild hyperphenylalaninemia in families with both disorders. *N. Engl. J. Med.* **314,** 1276–1280.

Nyhan, W.L. (1984) *Abnormalities in Amino Acid Metabolism in Clinical Medicine,* Appleton-Century-Crofts, Norwalk, CT.

Scriver, C.R., Beaudet, A.L., Sly, W.S., Valle, D., Childs, B., Kinzler, A.W., & Vogelstein, B. (eds) (2001) *The Metabolic and Molecular Bases of Inherited Disease,* 8th edn, Part 5: *Amino Acids,* McGraw-Hill, Inc., New York.

Scriver, C.R., Kaufman, S., & Woo, S.L.C. (1988) Mendelian hyperphenylalaninemia. *Annu. Rev. Genet.* **22,** 301–321.

Problems

1. Products of Amino Acid Transamination Name and draw the structure of the α-keto acid resulting when each of the following amino acids undergoes transamination with α-ketoglutarate: (a) aspartate, (b) glutamate, (c) alanine, (d) phenylalanine.

2. Measurement of Alanine Aminotransferase Activity The activity (reaction rate) of alanine aminotransferase is usually measured by including an excess of pure lactate dehydrogenase and NADH in the reaction system. The rate of alanine disappearance is equal to the rate of NADH disappearance measured spectrophotometrically. Explain how this assay works.

3. Distribution of Amino Nitrogen If your diet is rich in alanine but deficient in aspartate, will you show signs of aspartate deficiency? Explain.

4. A Genetic Defect in Amino Acid Metabolism: A Case History A two-year-old child was taken to the hospital. His mother said that he vomited frequently, especially after feedings. The child's weight and physical development were below normal. His hair, although dark, contained patches of white. A urine sample treated with ferric chloride ($FeCl_3$) gave a green color characteristic of the presence of phenylpyruvate. Quantitative analysis of urine samples gave the results shown in the table.

	Concentration (mM)	
Substance	*Patient's urine*	*Normal urine*
Phenylalanine	7.0	0.01
Phenylpyruvate	4.8	0
Phenyllactate	10.3	0

(a) Suggest which enzyme might be deficient in this child. Propose a treatment.

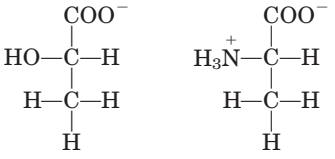
(b) Why does phenylalanine appear in the urine in large amounts?

(c) What is the source of phenylpyruvate and phenyllactate? Why does this pathway (normally not functional) come into play when the concentration of phenylalanine rises?

(d) Why does the boy's hair contain patches of white?

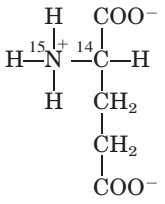
5. Role of Cobalamin in Amino Acid Catabolism Pernicious anemia is caused by impaired absorption of vitamin B_{12}. What is the effect of this impairment on the catabolism of amino acids? Are all amino acids equally affected? (Hint: See Box 17–2.)

6. Lactate versus Alanine as Metabolic Fuel: The Cost of Nitrogen Removal The three carbons in lactate and alanine have identical oxidation states, and animals can use either carbon source as a metabolic fuel. Compare the net ATP yield (moles of ATP per mole of substrate) for the complete oxidation (to CO_2 and H_2O) of lactate versus alanine when the cost of nitrogen excretion as urea is included.



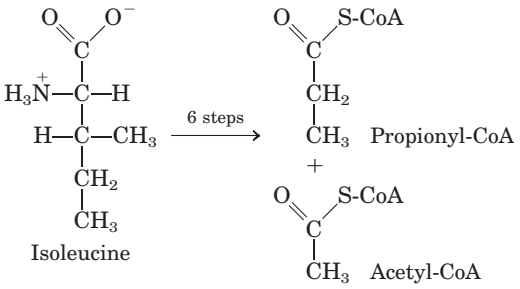
Lactate Alanine

7. Pathway of Carbon and Nitrogen in Glutamate Metabolism When [2-^{14}C,^{15}N] glutamate undergoes oxidative degradation in the liver of a rat, in which atoms of the following metabolites will each isotope be found: (a) urea, (b) succinate, (c) arginine, (d) citrulline, (e) ornithine, (f) aspartate?

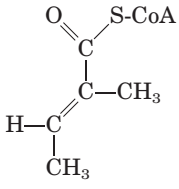


Labeled glutamate

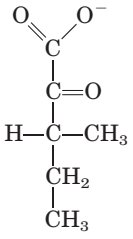
8. Chemical Strategy of Isoleucine Catabolism Isoleucine is degraded in six steps to propionyl-CoA and acetyl-CoA:



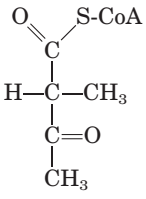
(a) The chemical process of isoleucine degradation includes strategies analogous to those used in the citric acid cycle and the β oxidation of fatty acids. The intermediates of isoleucine degradation (I to V) shown below are not in the proper order. Use your knowledge and understanding of the citric acid cycle and β-oxidation pathway to arrange the intermediates in the proper metabolic sequence for isoleucine degradation.



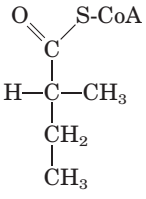
I



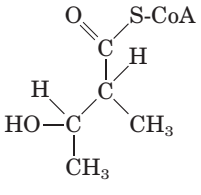
II



III



IV

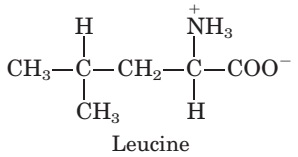


V

(b) For each step you propose, describe the chemical process, provide an analogous example from the citric acid cycle or β-oxidation pathway (where possible), and indicate any necessary cofactors.

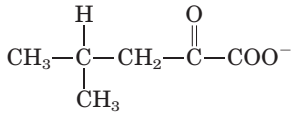
9. Role of Pyridoxal Phosphate in Glycine Metabolism The enzyme serine hydroxymethyltransferase requires pyridoxal phosphate as cofactor. Propose a mechanism for the reaction catalyzed by this enzyme, in the direction of serine degradation (glycine production). (Hint: See Figs 18–19 and 18–20b.)

10. Parallel Pathways for Amino Acid and Fatty Acid Degradation The carbon skeleton of leucine is degraded by a series of reactions closely analogous to those of the citric acid cycle and β oxidation. For each reaction, (a) through (f), indicate its type, provide an analogous example from the citric acid cycle or β-oxidation pathway (where possible), and note any necessary cofactors.



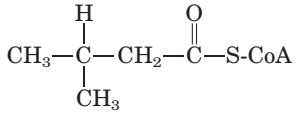
Leucine

(a)



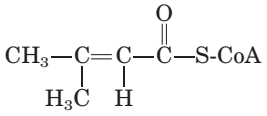
α-Ketoisocaproate

(b) $CoA\text{-}SH$, CO_2



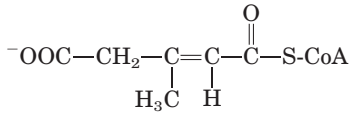
Isovaleryl-CoA

(c)



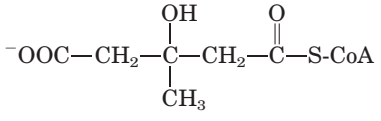
β-Methylcrotonyl-CoA

(d) HCO_3^-



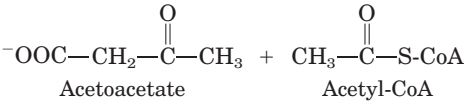
β-Methylglutaconyl-CoA

(e) H_2O



β-Hydroxy-β-methylglutaryl-CoA

(f)



Acetoacetate + Acetyl-CoA

11. Ammonia Toxicity Resulting from an Arginine-Deficient Diet In a study conducted some years ago, cats were fasted overnight then given a single meal complete in all amino acids except arginine. Within 2 hours, blood ammonia levels increased from a normal level of 18 μg/L to 140 μg/L, and the cats showed the clinical symptoms of ammonia toxicity. A control group fed a complete amino acid diet or an amino acid diet in which arginine was replaced by ornithine showed no unusual clinical symptoms.

(a) What was the role of fasting in the experiment?

(b) What caused the ammonia levels to rise in the experimental group? Why did the absence of arginine lead to ammonia toxicity? Is arginine an essential amino acid in cats? Why or why not?

(c) Why can ornithine be substituted for arginine?

12. Oxidation of Glutamate Write a series of balanced equations, and an overall equation for the net reaction, describing the oxidation of 2 mol of glutamate to 2 mol of α-ketoglutarate and 1 mol of urea.

13. Transamination and the Urea Cycle Aspartate aminotransferase has the highest activity of all the mammalian liver aminotransferases. Why?

14. The Case against the Liquid Protein Diet A weight-reducing diet heavily promoted some years ago required the daily intake of "liquid protein" (soup of hydrolyzed gelatin), water, and an assortment of vitamins. All other food and drink were to be avoided. People on this diet typically lost 10 to 14 lb in the first week.

(a) Opponents argued that the weight loss was almost entirely due to water loss and would be regained very soon after a normal diet was resumed. What is the biochemical basis for this argument?

(b) A number of people on this diet died. What are some of the dangers inherent in the diet, and how can they lead to death?

15. Alanine and Glutamine in the Blood Normal human blood plasma contains all the amino acids required for the synthesis of body proteins, but not in equal concentrations. Alanine and glutamine are present in much higher concentrations than any other amino acids. Suggest why.