CHAPTER 18

Amino Acid Oxidation and the Production of Urea

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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 consume primarily protein and thus must obtain most of their energy from amino acids, 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 (see Fig. 17-9).

One important feature distinguishes amino acid degradation from other catabolic processes described to

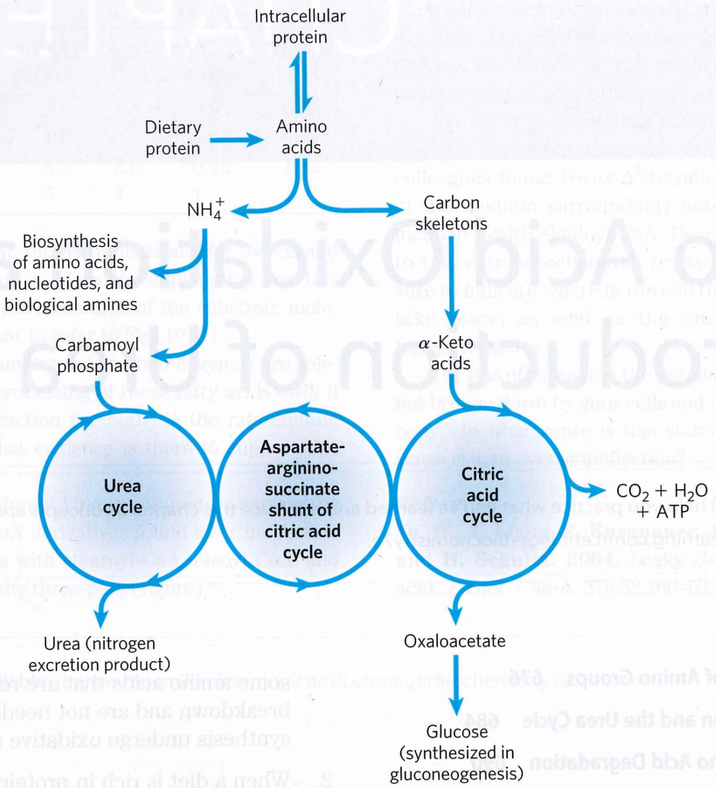


FIGURE 18-1 Overview of amino acid catabolism in mammals. The amino groups and the carbon skeleton take separate but interconnected pathways.

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.

Four amino acids play central roles in nitrogen metabolism: glutamate, glutamine, alanine, and aspartate. The special place of these four amino acids in nitrogen metabolism is not an evolutionary accident. These particular amino acids are the ones most easily converted into citric acid cycle intermediates: glutamate and glutamine to α-ketoglutarate, alanine to pyruvate, and aspartate to oxaloacetate. Glutamate and glutamine are especially important, acting as a kind of general collection point for amino groups. In the cytosol of liver cells (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,

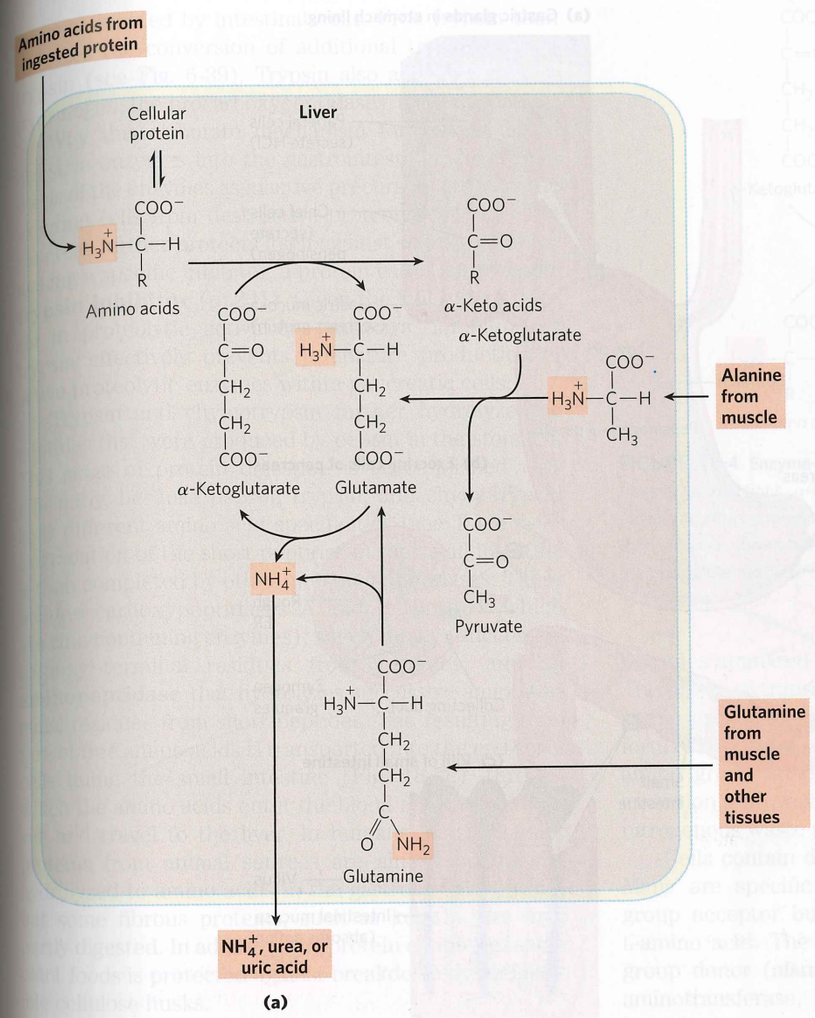


FIGURE 18-2 Amino group catabolism. **(a)** Overview of catabolism of amino groups (shaded) in vertebrate liver. **(b)** Excretory forms of nitrogen. Excess NH_4^+ is excreted as ammonia (microbes, bony fishes), urea (most terrestrial vertebrates), or uric acid (birds and terrestrial reptiles). Notice 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.

another important molecule in the transport of amino groups to the liver. We will see in Section 18.2 that aspartate comes into play in the metabolic processes that occur once the amino groups are delivered to the liver.

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

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. 230), is converted to active pepsin (M_r 34,614) by an autocatalytic cleavage (a cleavage mediated by the pepsinogen itself) that occurs only at low pH. In the stomach, pepsin hydrolyzes ingested proteins at peptide bonds on the amino-terminal side of Leu and the aromatic amino acid residues Phe, Trp, and Tyr (see Table 3-6), cleaving long polypeptide chains into a mixture of smaller peptides.

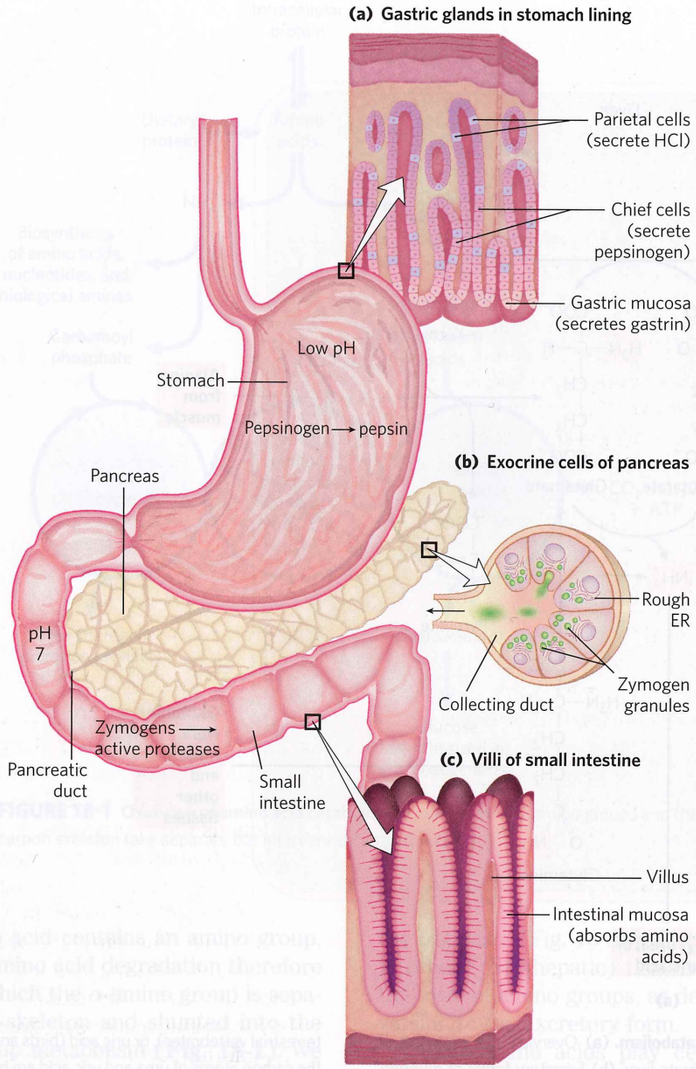


FIGURE 18-3 Part of the human digestive (gastrointestinal) tract. **(a)** The parietal cells and chief cells of the gastric glands secrete their products in response to the hormone gastrin. Pepsin begins the process of protein degradation in the stomach. **(b)** The cytoplasm of exocrine cells of the pancreas 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)** In the small intestine, 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).

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 **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. 6-39). 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). Given the key role of trypsin in proteolytic activation pathways, inhibition of trypsin effectively prevents premature production of active proteolytic enzymes within 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 3-6). 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

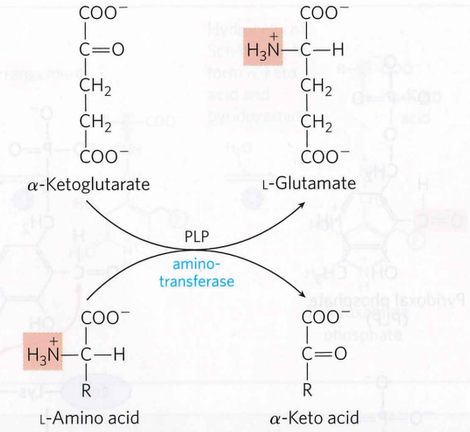


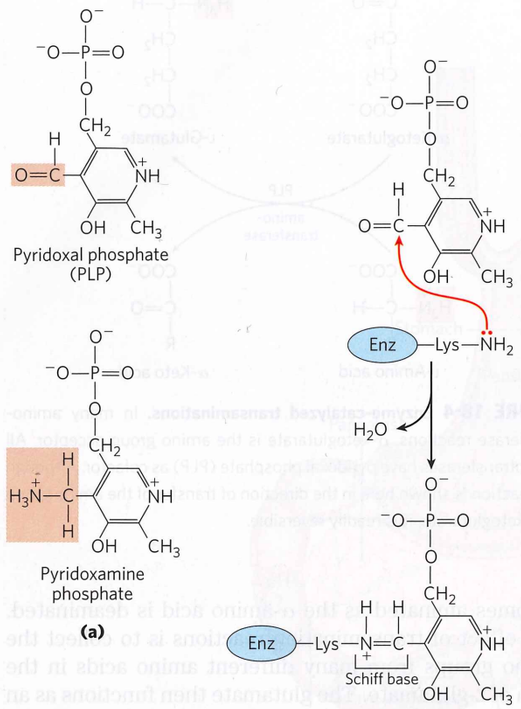
FIGURE 18-4 Enzyme-catalyzed transaminations. In many aminotransferase reactions, α-ketoglutarate is the amino group acceptor. All aminotransferases have pyridoxal phosphate (PLP) as cofactor. Although the reaction is shown here in the direction of transfer of the amino group to α-ketoglutarate, it is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

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.

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 and aspartate aminotransferase, for example). The reactions catalyzed by aminotransferases are freely reversible, having an equilibrium constant of about 1.0 ($\Delta G' \approx 0$ 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).



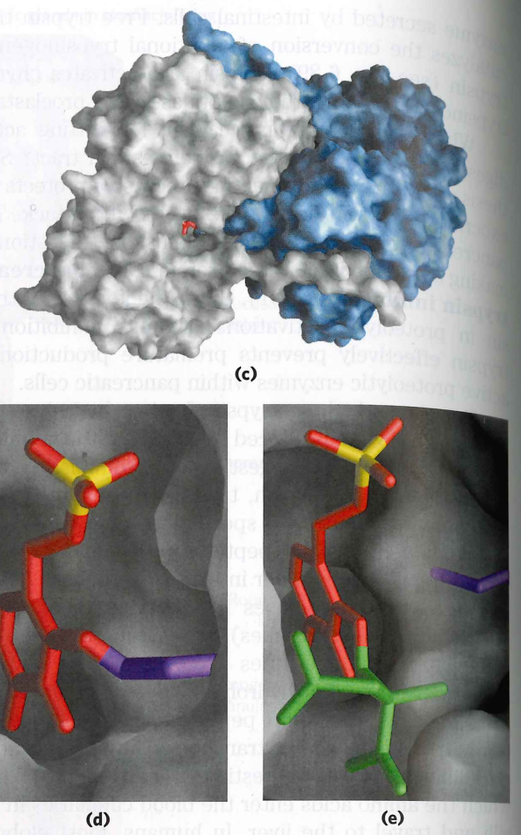


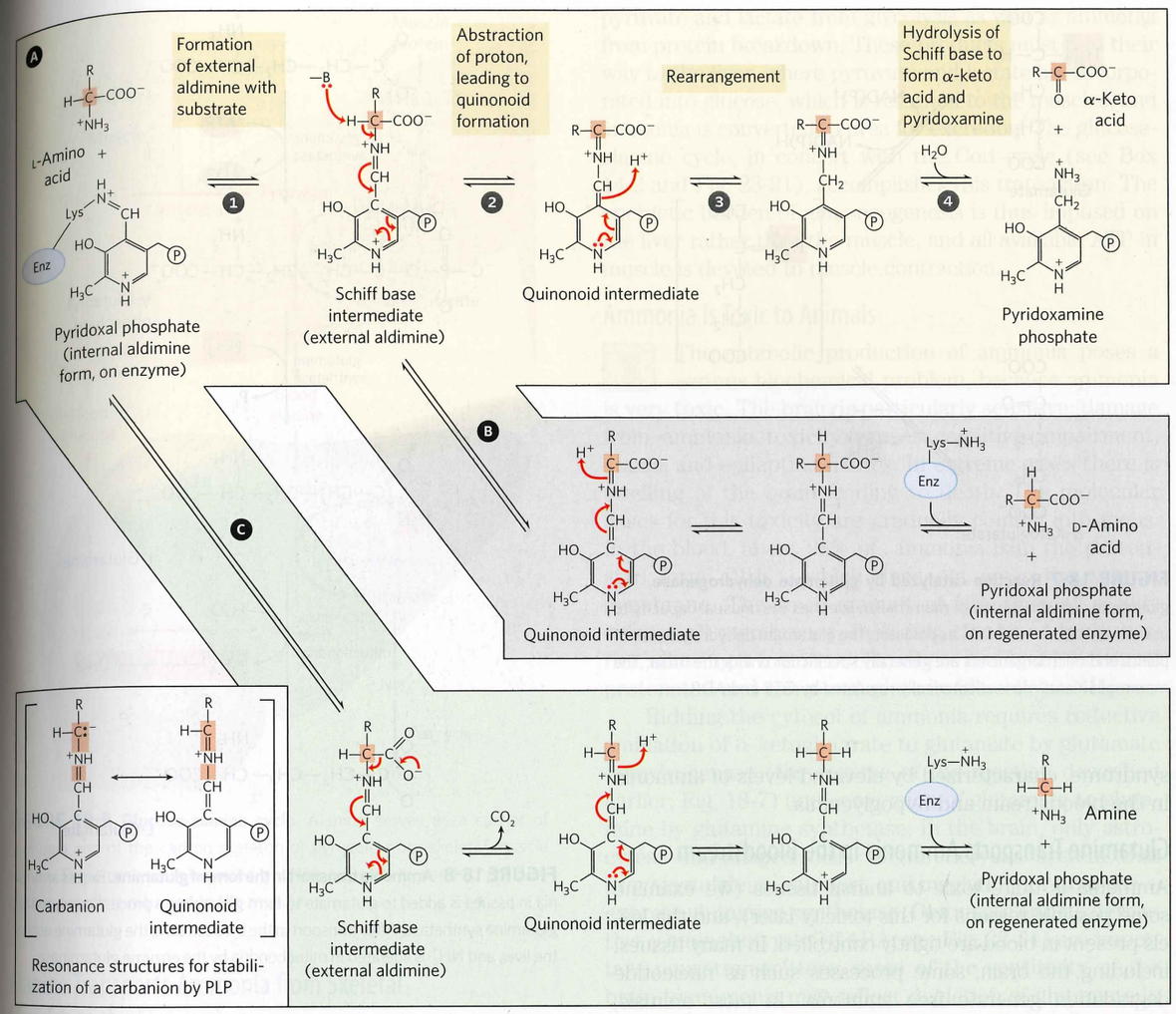
FIGURE 18-5 Pyridoxal phosphate, the prosthetic group of aminotransferases. (a) Pyridoxal phosphate (PLP) and its aminated form, pyridoxamine phosphate, are the tightly bound coenzymes of aminotransferases. The functional groups are shaded. **(b)** Pyridoxal phosphate is bound to the enzyme through noncovalent interactions and a Schiff-base (aldimine) 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 14-6. **(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. [Source: (c, d, e) PDB ID 1AJS, S. Rhee et al., *J. Biol. Chem.* 272:17,293, 1997.]

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, d), 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.

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.



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, also called an internal aldimine. This activated form of PLP readily undergoes transimination to form a new Schiff base (external aldimine) with the α-amino group of the substrate amino acid (see Fig. 18-5b, d). Three alternative fates for the external aldimine are shown: A transamination, B racemization, and C decarboxylation. The PLP–amino acid Schiff base 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 in yellow (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).

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

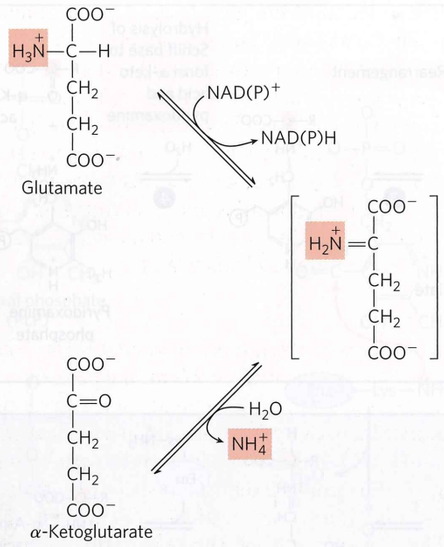


FIGURE 18-7 Reaction catalyzed by glutamate dehydrogenase. The glutamate dehydrogenase of mammalian liver has the unusual capacity to use either NAD^+ or $NADP^+$ as cofactor. The glutamate dehydrogenases of plants and microorganisms are generally specific for one or the other. The mammalian enzyme is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

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 tightly controlled. 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 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

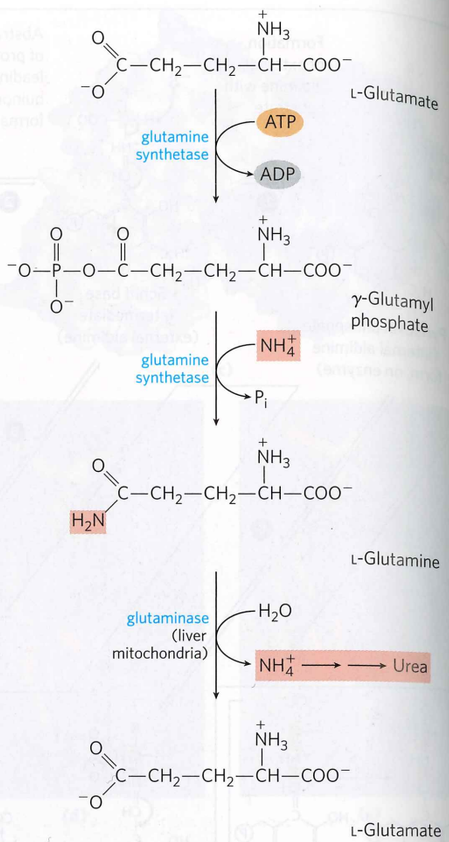


FIGURE 18-8 Ammonia transport in the form of glutamine. Excess ammonia in tissues is added to glutamate to form glutamine, a process catalyzed by glutamine synthetase. After transport in the bloodstream, the glutamine enters the liver, and NH_4^+ is liberated in mitochondria by the enzyme glutaminase.

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. 670) 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. ■

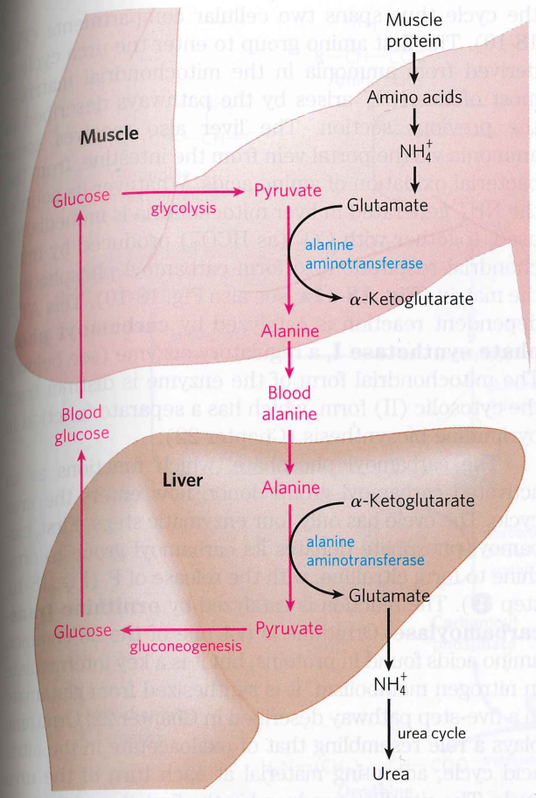


FIGURE 18-9 Glucose-alanine cycle. Alanine serves as a carrier of ammonia and of the carbon skeleton of pyruvate from skeletal muscle to liver. The ammonia is excreted and the pyruvate is used to produce glucose, which is returned to the muscle.

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 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 14-2 and Fig. 23-21), 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 brain is particularly sensitive; damage from ammonia toxicity causes cognitive impairment, ataxia, and epileptic seizures. In extreme cases there is swelling of the brain leading to death. The molecular bases for this toxicity are gradually coming into focus. In the blood, about 98% of ammonia is in the protonated form (NH_4^+), which does not cross the plasma membrane. The small amount of NH_3 present readily crosses all membranes, including the blood-brain barrier, allowing it to enter cells, where much of it becomes protonated and can accumulate inside cells as NH_4^+.

Ridding the cytosol of 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. In the brain, only astrocytes—star-shaped cells of the nervous system that provide nutrients, support, and insulation for neurons—express glutamine synthetase. Glutamate and its derivative γ-aminobutyrate (GABA; see Fig. 22-31) are important neurotransmitters; some of the sensitivity of the brain to ammonia may reflect depletion of glutamate in the glutamine synthetase reaction. However, glutamine synthetase activity is insufficient to deal with excess ammonia, or to fully explain its toxicity.

Increased $[NH_4^+]$ also alters the capacity of astrocytes to maintain potassium homeostasis across the membrane. NH_4^+ competes with K^+ for transport into the cell through the Na^+K^+ ATPase, resulting in elevated extracellular $[K^+]$. The excess extracellular K^+ enters neurons through a symporter, **Na^+-K^+-2Cl^- cotransporter 1 (NKCC1)**, bringing Na^+ and $2Cl^-$ with it. Excess Cl^- in these neurons alters their response when the neurotransmitter GABA interacts with their $GABA_A$ receptors, producing abnormal depolarization and increased neuronal activity that likely account for the neuromuscular incoordination and seizures that often result from ammonia poisoning. If extracellular $[NH_4^+]$ remains elevated, the perturbation of ion and aquaporin channels in astrocytes causes the cells to swell, resulting in fatal brain edema. ■

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. 22-48.) Plants recycle virtually all amino groups—they excrete nitrogen only under rare 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.

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—most of this NH_4^+ arises by the pathways described in the previous section. 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 only 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 ❶). The reaction is catalyzed by **ornithine transcarbamoylase**. Ornithine is not one of the 20 common amino acids found in proteins, but it is a key intermediate in nitrogen metabolism. It is synthesized from glutamate in a five-step pathway described in Chapter 22. Ornithine plays a role resembling that of oxaloacetate in the citric acid cycle, accepting material at each turn of the urea cycle. The citrulline produced in the first step of the urea cycle passes from the mitochondrion to the cytosol.

The next two steps bring in the second amino group. The source is aspartate generated in mitochondria by transamination and transported into the cytosol. A condensation reaction between the amino group of aspartate and the ureido (carbonyl) group of citrulline forms argininosuccinate (step ❷ 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 ❸ in Fig. 18-10) to form free arginine and fumarate, the latter being converted to malate before entering

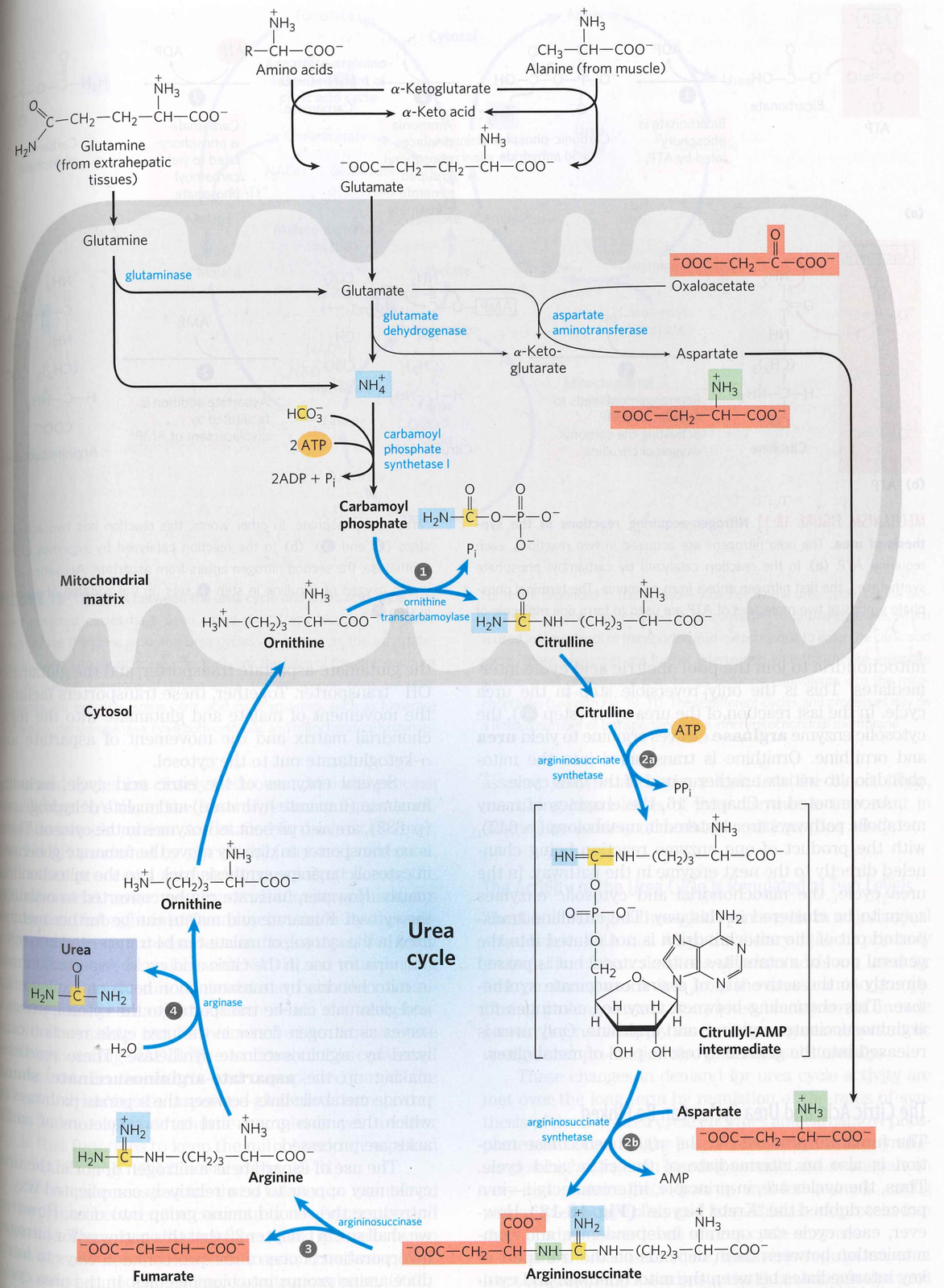
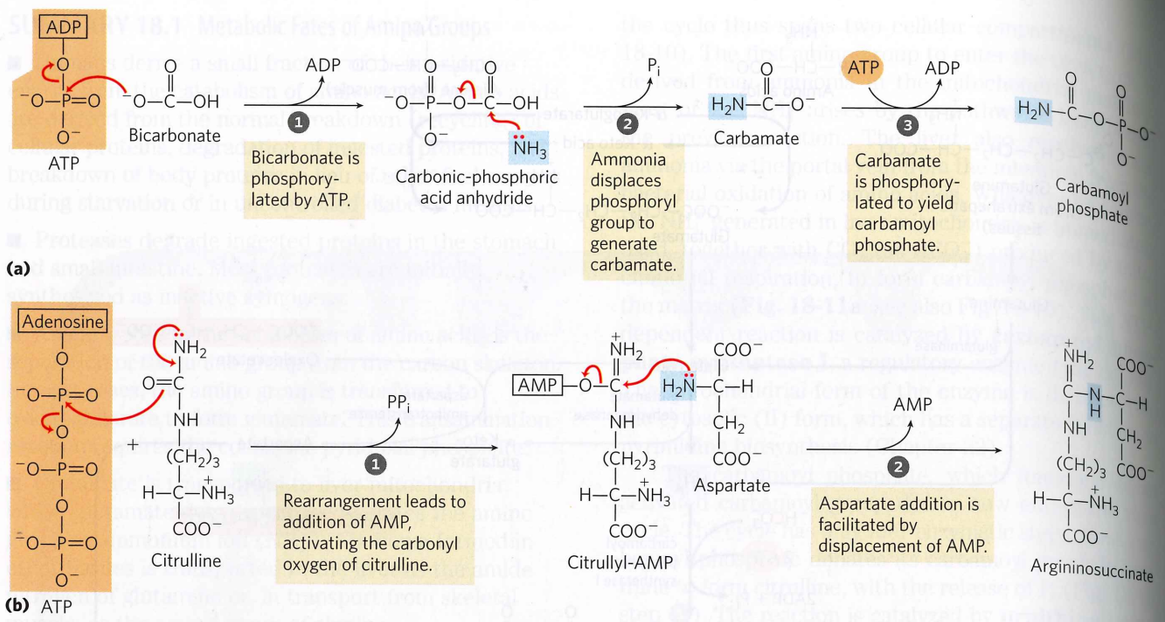


FIGURE 18-10 The urea cycle and reactions that feed amino groups into the cycle. The enzymes catalyzing these reactions (named in the text) are distributed between the mitochondrial matrix and the cytosol. One amino group enters the urea cycle as carbamoyl phosphate, formed in the matrix; the other enters as aspartate, formed in the matrix by transamination of oxaloacetate and glutamate, catalyzed by aspartate aminotransferase. The urea cycle consists of four steps. ❶ Formation of citrulline from ornithine and carbamoyl phosphate (entry of the first amino group); the citrulline passes into the cytosol. ❷ Formation of argininosuccinate through a citrullyl-AMP intermediate (entry of the second amino group). ❸ Formation of arginine from argininosuccinate; this reaction releases fumarate, which enters the citric acid cycle. ❹ 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.



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 (❶ and ❸). **(b)** In the reaction catalyzed by argininosuccinate synthetase, the second nitrogen enters from aspartate. Activation of the ureido oxygen of citrulline in step ❶ sets up the addition of aspartate in step ❷.

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 ❹), 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 in metabolons (p. 642), 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 seem 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

The fumarate produced in the argininosuccinase reaction is also an intermediate of the citric acid cycle. Thus, 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. Major transporters in the inner mitochondrial membrane include the malate-α-ketoglutarate transporter, the glutamate-aspartate transporter, and the glutamate-OH^- transporter. Together, these transporters facilitate the movement of malate and glutamate into the mitochondrial matrix and the movement of aspartate and α-ketoglutarate out to the cytosol.

Several enzymes of the citric acid cycle, including fumarase (fumarate hydratase) and malate dehydrogenase (p. 633), are also present as isozymes in the cytosol. There is no transporter to directly move the fumarate generated in cytosolic arginine synthesis back into the mitochondrial matrix. However, fumarate can be converted to malate in the cytosol. Fumarate and malate can be further metabolized in the cytosol, or malate can be 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.

The use of aspartate as a nitrogen donor in the urea cycle may appear to be a relatively complicated way to introduce the second amino group into urea. However, we shall see in Chapter 22 that this pathway for nitrogen incorporation is one of the two common ways to introduce amino groups into biomolecules. In the urea cycle, additional pathway interconnections can help explain why aspartate is used as a nitrogen donor. The urea and

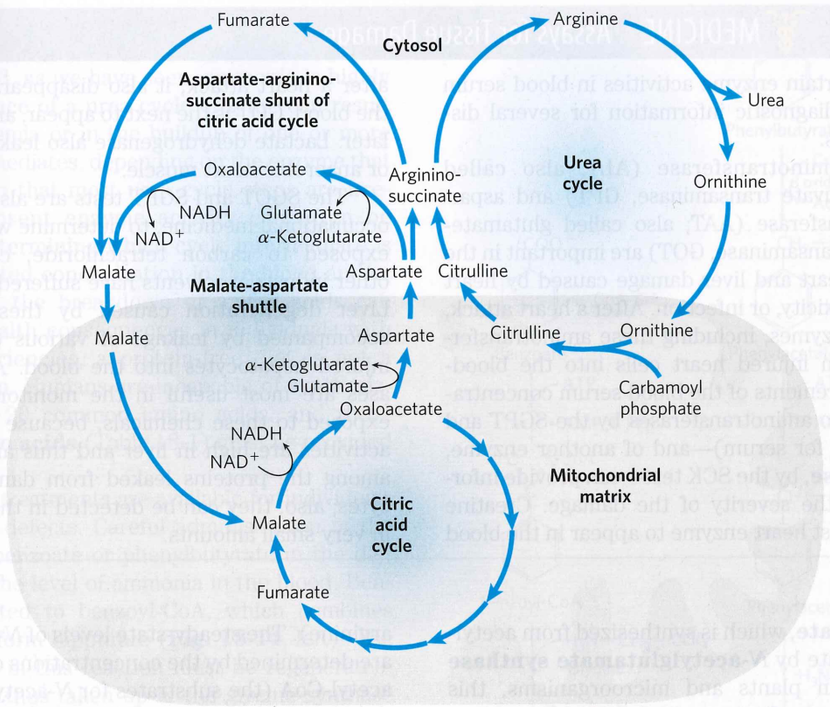


FIGURE 18-12 Links between the urea cycle and citric acid cycle. The interconnected cycles have been called the "Krebs bicycle." The pathways linking the citric acid and urea cycles are known as the aspartate-argininosuccinate shunt; these effectively link the fates of the amino groups and the carbon skeletons of amino acids. The interconnections are quite elaborate. For example, some citric acid cycle enzymes, such as fumarase and malate dehydrogenase, have both cytosolic and mitochondrial isozymes.

citric acid cycles are closely tied to an additional process that brings NADH, in the form of reducing equivalents, into the mitochondrion. As detailed in the next chapter, the NADH produced by glycolysis, fatty acid oxidation, and other processes cannot be transported across the mitochondrial inner membrane. Reducing equivalents are instead brought into the mitochondrion by converting aspartate to oxaloacetate in the cytosol, reducing the oxaloacetate to malate with NADH, and transporting the malate into the mitochondrial matrix via the malate–α-ketoglutarate transporter. Once inside the mitochondrion, the malate can be reconverted to oxaloacetate while generating NADH. The oxaloacetate is converted to aspartate in the matrix and transported out of the mitochondrion by the aspartate-glutamate transporter. This malate-aspartate shuttle completes yet another cycle that functions to keep the mitochondrion supplied with NADH (Fig. 18-12; see also Fig. 19-31).

These processes require that a balance be maintained in the cytosol between the concentrations of glutamate and aspartate. The enzyme that transfers amino groups between these key amino acids is aspartate aminotransferase, AAT (also called glutamate-oxaloacetate transaminase, GOT). This is one of the most active enzymes in hepatocytes and other tissues. When tissue damage occurs, this easily assayed enzyme and others leak into the blood. Thus, measuring blood levels of liver enzymes is important in diagnosing a variety of medical conditions (Box 18-1).

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 to enter the citric acid cycle. These processes are further intertwined with the malate-aspartate shuttle, a set of reactions that brings reducing equivalents into the mitochondrion (see also Fig. 19-31). These different cycles and processes rely on a limited number of transporters in the inner mitochondrial membrane.

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

BOX 18-1 MEDICINE Assays for Tissue Damage

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

Alanine aminotransferase (ALT; also called glutamate-pyruvate transaminase, GPT) and aspartate aminotransferase (AAT; 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 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 thus are likely to be among the proteins leaked from damaged hepatocytes; also, they can be detected in the bloodstream in very small amounts.

***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. 22-12), 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).

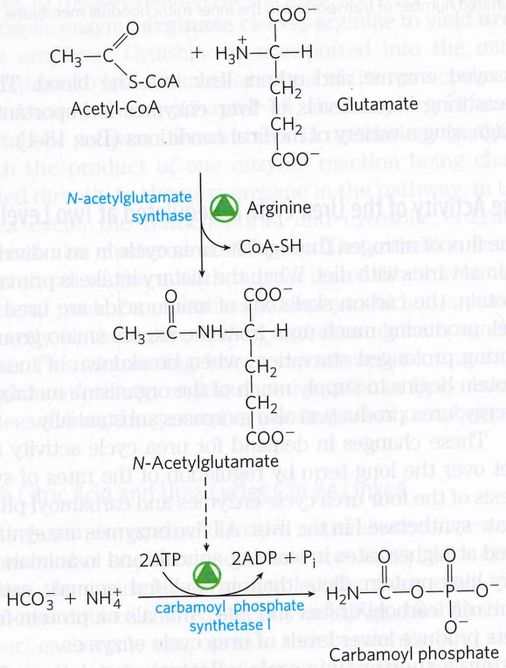


FIGURE 18-13 Synthesis of *N*-acetylglutamate and its activation of carbamoyl phosphate synthetase I.

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, this apparent cost is compensated for by the pathway interconnections detailed above. The fumarate generated by the urea cycle is converted to malate, and the malate is transported into the mitochondrion (Fig. 18-12). Inside the mitochondrial matrix, NADH is generated 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.

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 buildup 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 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.

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.

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

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

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

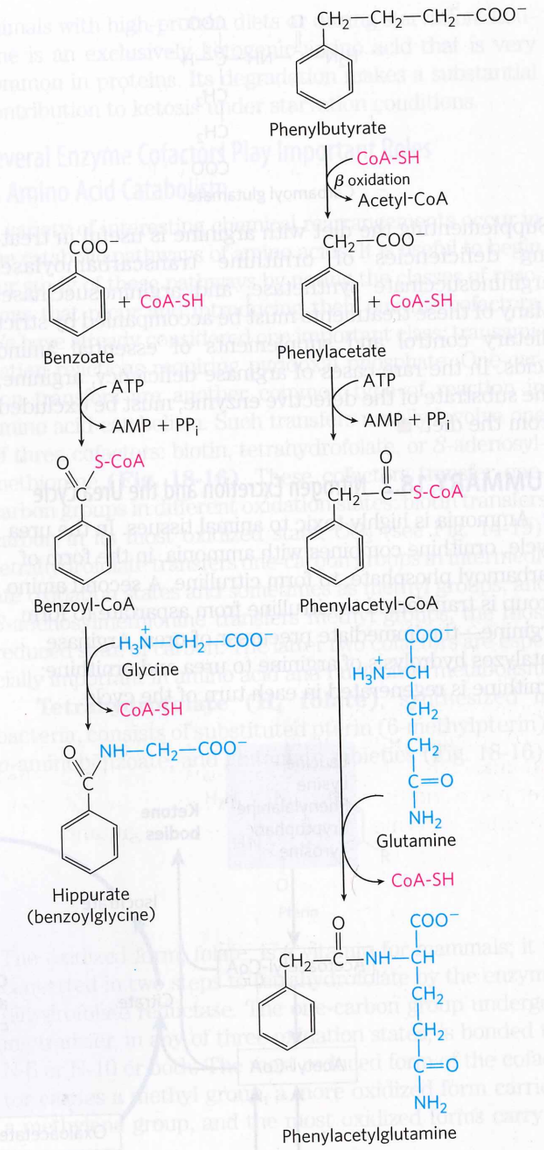
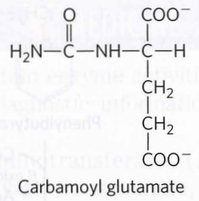


FIGURE 18-14 Treatment for deficiencies in urea cycle enzymes. The aromatic acids benzoate and phenylbutyrate, administered in the diet, are metabolized and combine with glycine and glutamine, respectively. The products are excreted in the urine. Subsequent synthesis of glycine and glutamine to replenish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

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. ■

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; 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

Amino acid catabolism normally accounts 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.

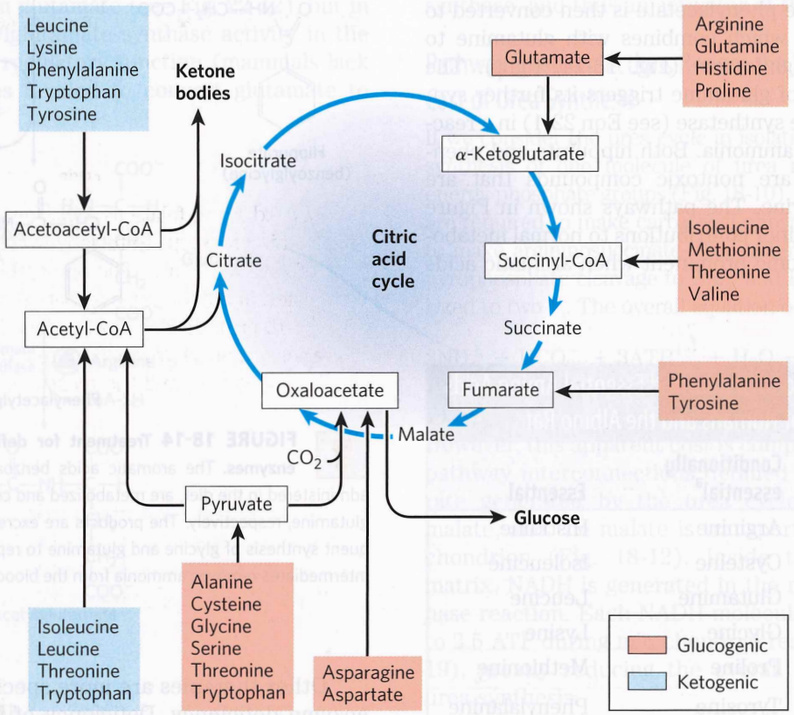


FIGURE 18-15 Summary of amino acid catabolism. Amino acids are grouped according to their major degradative end product. Some amino acids are listed more than once because different parts of their carbon skeletons are degraded to different end products. The figure shows the most important catabolic pathways in vertebrates, but there are minor variations among vertebrate species. Threonine, for instance, is degraded by 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.

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

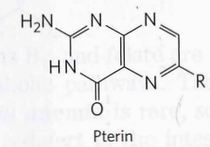
The seven amino acids that are degraded entirely or in part to acetoacetyl-CoA and/or acetyl-CoA—phenylalanine, tyrosine, isoleucine, leucine, tryptophan, threonine, and lysine—can yield ketone bodies in the liver, where acetoacetyl-CoA is converted to acetoacetate and then to acetone and β-hydroxybutyrate (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. One-carbon transfers are another common type of reaction in amino acid catabolism. Such transfers 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. 14-19); 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), *p*-aminobenzoate, and glutamate moieties (Fig. 18-16).



Pterin

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

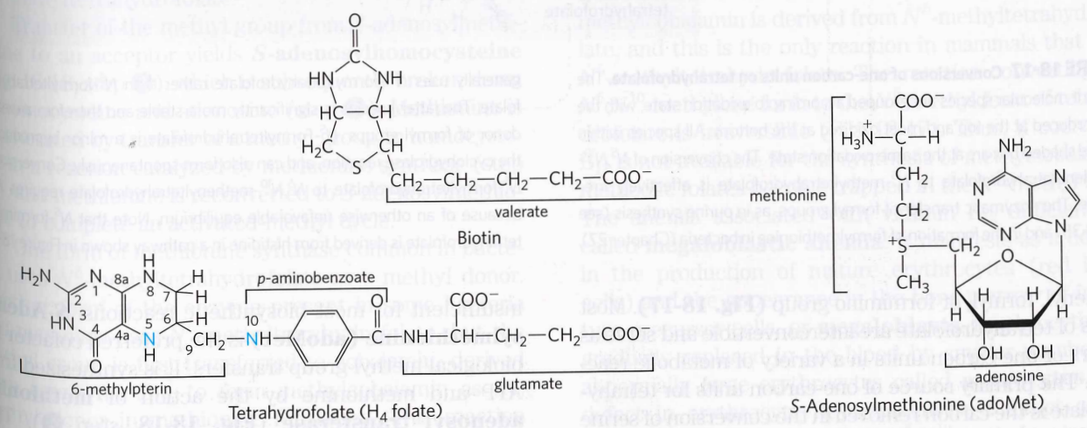


FIGURE 18-16 Some enzyme cofactors important in one-carbon transfer reactions. The nitrogen atoms to which one-carbon groups are attached in tetrahydrofolate are shown in blue.