

38 Fate of Amino Acid Nitrogen: Urea Cycle

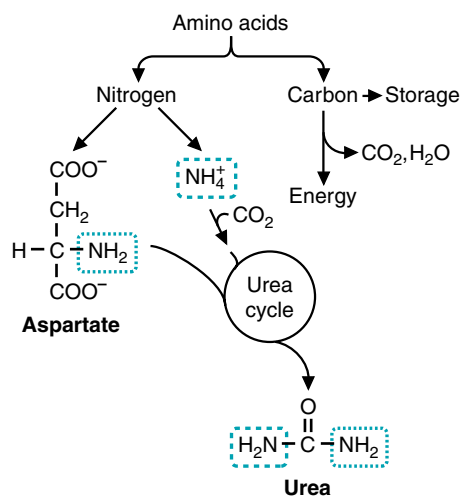


Fig 38.1. Fate of amino acid carbons and nitrogen. Amino acid carbon can be used either for energy storage (glycogen, fatty acids) or for energy. Amino acid nitrogen is used for urea synthesis. One nitrogen of urea comes from NH_4^+ , the other from aspartate.

In comparison with carbohydrate and lipid metabolism, the metabolism of amino acids is complex. We must be concerned not only with the fate of the carbon atoms of the amino acids but also with the fate of the **nitrogen**. During their metabolism, amino acids travel in the blood from one tissue to another. Ultimately, most of the nitrogen is converted to **urea** in the **liver** and the carbons are oxidized to CO_2 and H_2O by a number of tissues (Fig. 38.1).

After a meal that contains protein, amino acids released by digestion (see Chapter 37) pass from the gut through the hepatic portal vein to the liver (see Fig. 38.2A). In a normal diet containing 60 to 100 g protein, most of the amino acids are used for the synthesis of proteins in the liver and in other tissues. Excess amino acids may be converted to glucose or triacylglycerol.

During fasting, muscle protein is cleaved to amino acids. Some of the amino acids are partially oxidized to produce energy (see Fig. 38.2B). Portions of these amino acids are converted to **alanine** and **glutamine**, which, along with other amino acids, are released into the blood. Glutamine is oxidized by various tissues, including the **lymphocytes**, **gut**, and **kidney**, which convert some of the carbons and nitrogen to alanine. Alanine and other amino acids travel to the liver, where the carbons are converted to glucose and ketone bodies and the nitrogen is converted to urea, which is excreted by the kidneys. Glucose, produced by gluconeogenesis, is subsequently oxidized to CO_2 and H_2O by many tissues, and ketone bodies are oxidized by tissues such as muscle and kidney.

Several enzymes are important in the process of interconverting amino acids and in removing nitrogen so that the carbon skeletons can be oxidized. These include **dehydratases**, **transaminases**, **glutamate dehydrogenase**, **glutaminase**, and **deaminases**.

The conversion of amino acid nitrogen to urea occurs mainly in the liver. Urea is formed in the urea cycle from NH_4^+ , bicarbonate, and the nitrogen of **aspartate** (see Fig. 38.1). Initially, NH_4^+ , bicarbonate, and ATP react to produce **carbamoyl phosphate**, which reacts with **ornithine** to form **citrulline**. Aspartate then reacts with citrulline to form **argininosuccinate**, which releases **fumarate**, forming **arginine**. Finally, **arginase** cleaves arginine to release urea and regenerate ornithine. The cycle is regulated in a **feed-forward** manner, such that when amino acid degradation is occurring, the rate of the cycle is increased.



THE WAITING ROOM



Percy Veere and his high school friend decided to take a Caribbean cruise, during which they sampled the cuisine of many of the islands on their itinerary. One month after their return to the United States, Percy complained of

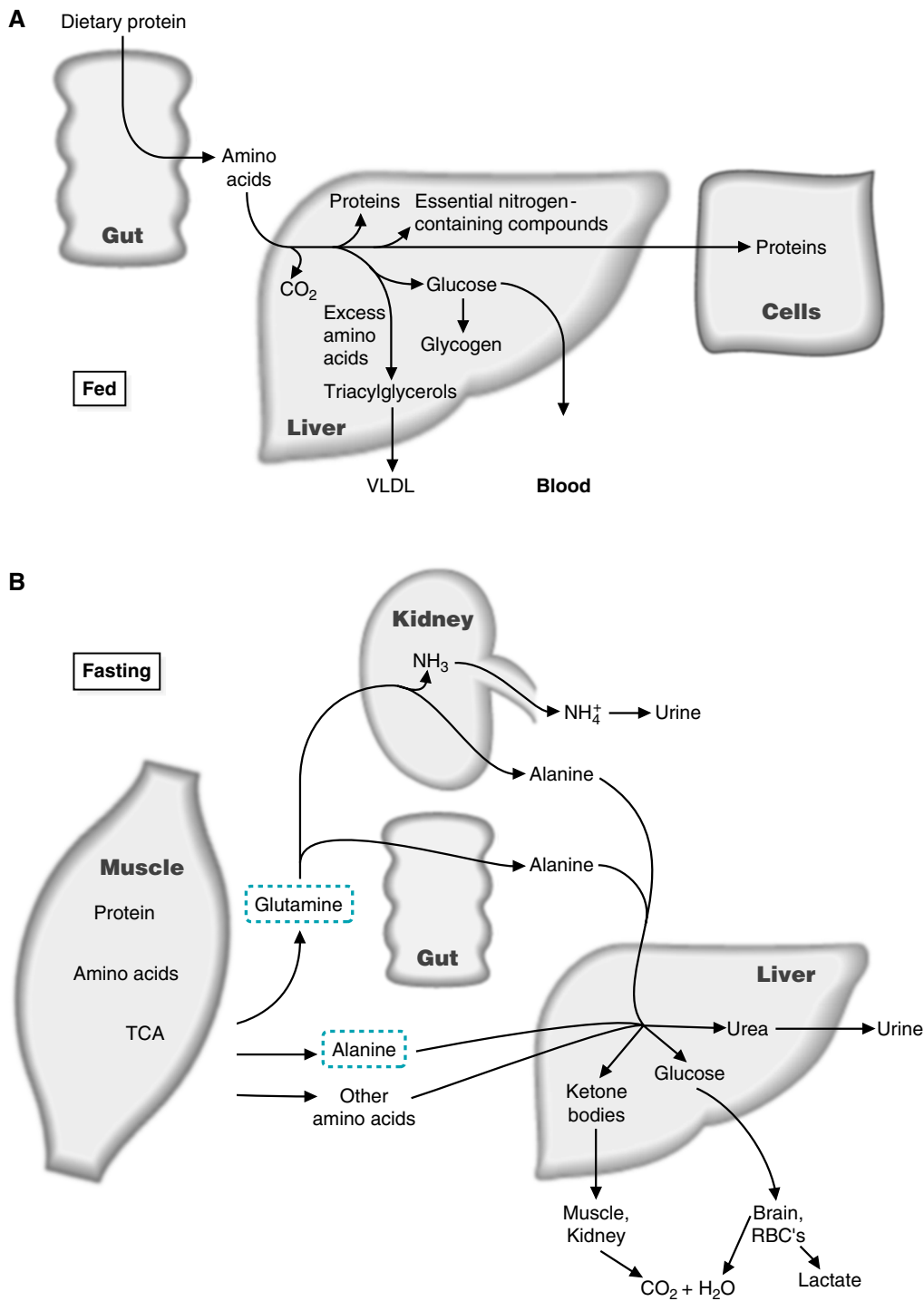


Fig 38.2. Roles of various tissues in amino acid metabolism. **A.** In the fed state, amino acids released by digestion of dietary proteins travel through the hepatic portal vein to the liver, where they are used for the synthesis of proteins, particularly the blood proteins, such as serum albumin. Excess amino acids are converted to glucose or to triacylglycerols. The latter are then packaged and secreted in VLDL. The glucose produced from amino acids in the fed state is stored as glycogen or released into the blood if blood glucose levels are low. Amino acids that pass through the liver are converted to proteins in cells of other tissues. **B.** During fasting, amino acids are released from muscle protein. Some enter the blood directly. Others are partially oxidized and the nitrogen stored in the form of alanine and glutamine, which enter the blood. In the kidney, glutamine releases ammonia into the urine and is converted to alanine and serine. In the cells of the gut, glutamine is converted to alanine. Alanine (the major gluconeogenic amino acid) and other amino acids enter the liver, where their nitrogen is converted to urea, which is excreted in the urine, and their carbons to glucose and ketone bodies, which are oxidized by various tissues for energy.

severe malaise, loss of appetite, nausea, vomiting, and arthralgias (joint pains). He had a low-grade fever and noted a persistent and increasing pain in the area of his liver. His friend noted a yellow discoloration of the whites of Percy's eyes and skin. Percy's urine turned the color of iced tea, and his stool became a light-clay color. His doctor found his liver to be enlarged and tender. Liver function tests were ordered.

Serologic testing for viral hepatitis type B, C, and D were nonreactive, but fecal studies showed "shedding" of hepatitis virus type A. Tests for antibodies to antigens of the hepatitis A virus (anti-HAV) in the serum were positive for the immunoglobulin M type.

A diagnosis of acute viral hepatitis type A was made, probably contracted from virus-contaminated food Percy had eaten while on his cruise. His physician explained that there was no specific treatment for type A viral hepatitis but recommended symptomatic and supportive care and prevention of transmission to others by the fecal-oral route. Percy took acetaminophen 3 to 4 times a day for fever and arthralgias throughout his illness.

I. FATE OF AMINO ACID NITROGEN

A. Transamination Reactions

Transamination is the major process for removing nitrogen from amino acids. In most instances, the nitrogen is transferred as an amino group from the original amino acid to α -ketoglutarate, forming glutamate, whereas the original amino acid is converted to its corresponding α -keto acid (Fig. 38.3). For example, the amino acid aspartate can be transaminated to form its corresponding α -keto acid, oxaloacetate. In the process, the amino group is transferred to α -ketoglutarate, which is converted to its corresponding amino acid, glutamate.

All amino acids except lysine and threonine undergo transamination reactions. The enzymes catalyzing these reactions are known as transaminases or aminotransferases. For most of these reactions, α -ketoglutarate and glutamate serve as one of the α -keto acid-amino acid pairs. Pyridoxal phosphate is the cofactor, and the mechanism of the reaction is indicated in Figure 38.4.

Overall, in a transamination reaction, an amino group from one amino acid becomes the amino group of a second amino acid. Because these reactions are readily reversible, they can be used to remove nitrogen from amino acids or to transfer nitrogen to α -keto acids to form amino acids. Thus, they are involved both in amino acid degradation and in amino acid synthesis.

B. Removal of Amino Acid Nitrogen as Ammonia

Cells in the body and bacteria in the gut release the nitrogen of certain amino acids as ammonia or ammonium ion (NH_4^+) (Fig. 38.5). Because these two forms of nitrogen can be interconverted, the terms are sometimes used interchangeably. Ammonium ion releases a proton to form ammonia by a reaction with a pK of 9.3 (Fig. 38.6). Therefore, at physiologic pH, the equilibrium favors NH_4^+ by a factor of approximately 100/1 (see Chapter 4, the Henderson-Hasselbalch equation). However, it is important to note that NH_3 is also present in the body, because this is the form that can cross cell membranes. For example, NH_3 passes into the urine from kidney tubule cells and decreases the acidity of the urine by binding protons, forming NH_4^+ . Once the NH_4^+ is formed, the compound can no longer freely diffuse across membranes.

Glutamate can be oxidatively deaminated by a reaction catalyzed by glutamate dehydrogenase that produces ammonium ion and α -ketoglutarate (Fig. 38.7). Either NAD^+ or NADP^+ can serve as the cofactor. This reaction, which occurs in the mitochondria of most cells, is readily reversible; it can incorporate ammonia

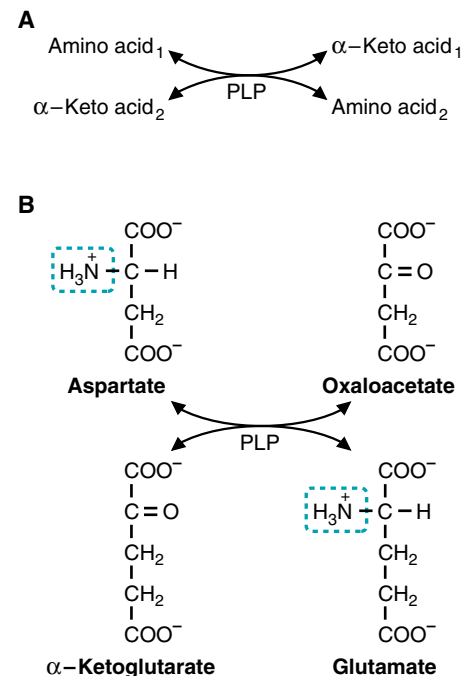


Fig. 38.3. Transamination. The amino group from one amino acid is transferred to another. Pairs of amino acids and their corresponding α -keto acids are involved in these reactions. α -ketoglutarate and glutamate are usually one of the pairs. The reactions, which are readily reversible, use pyridoxal phosphate (PLP) as a cofactor. The enzymes are called transaminases or aminotransferases. A. A generalized reaction. B. The aspartate transaminase reaction.



Whereas pyridoxal phosphate is used primarily for reactions involving amino acids, it is also required for the glycogen phosphorylase reaction, in which it acts as a general acid/base catalyst.

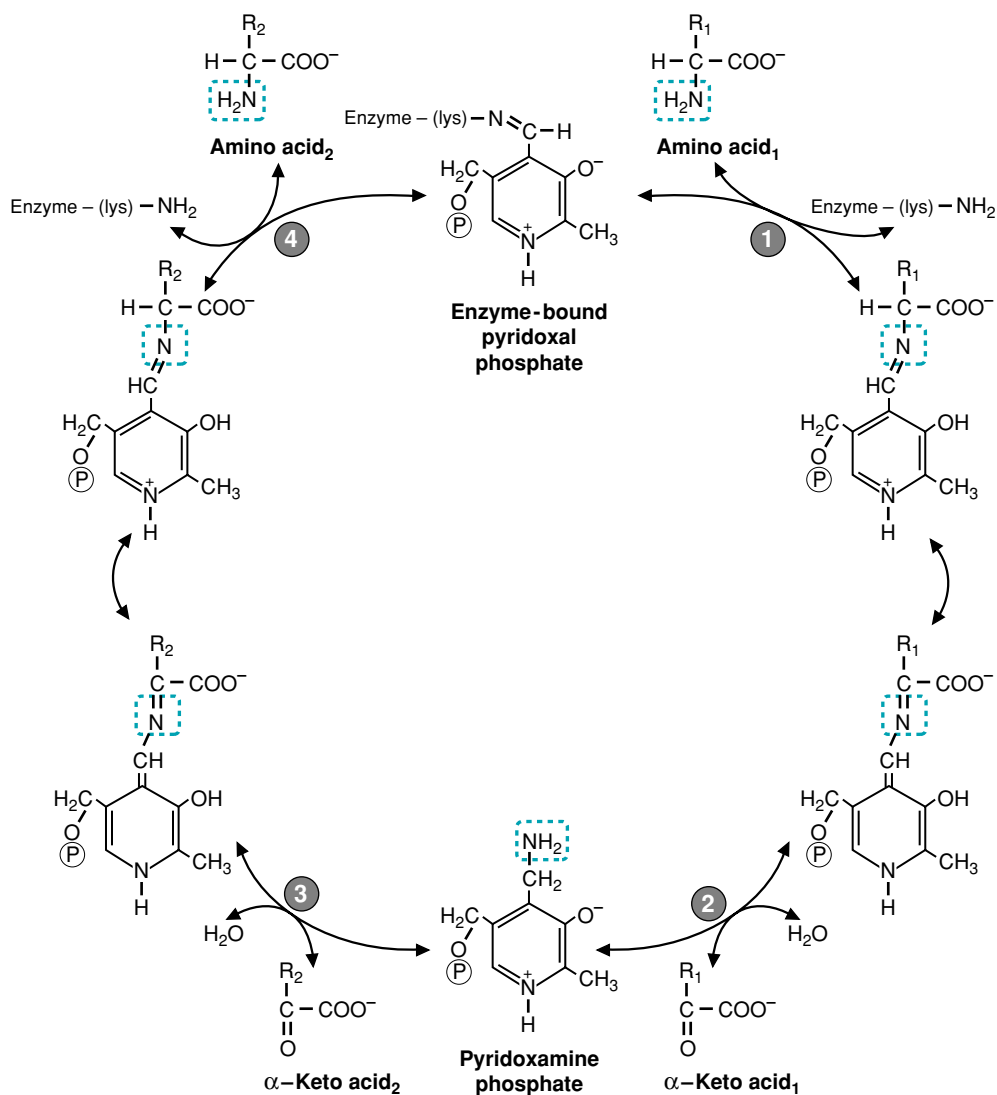


Fig. 38.4. Function of pyridoxal phosphate (PLP) in transamination reactions. The order in which the reactions occur is 1 to 4. Pyridoxal phosphate (enzyme-bound) reacts with amino acid₁, forming a Schiff base (a carbon–nitrogen double bond). After a shift of the double bond, α-keto acid₁ is released through hydrolysis of the Schiff base, and pyridoxamine phosphate is produced. Pyridoxamine phosphate then forms a Schiff base with α-keto acid₂. After the double bond shifts, amino acid₂ is released through hydrolysis of the Schiff base and enzyme-bound pyridoxal phosphate is regenerated. The net result is that the amino group from amino acid₁ is transferred to amino acid₂.

into glutamate or release ammonia from glutamate. Glutamate can collect nitrogen from other amino acids as a consequence of transamination reactions and then release ammonia through the glutamate dehydrogenase reaction. This process provides one source of the ammonia that enters the urea cycle.

In addition to glutamate, a number of amino acids release their nitrogen as NH_4^+ (see Fig. 38.5). Histidine may be directly deaminated to form NH_4^+ and urocanate. The deaminations of serine and threonine are dehydration reactions that require pyridoxal phosphate and are catalyzed by serine dehydratase. Serine forms pyruvate, and threonine forms α-ketobutyrate. In both cases, NH_4^+ is released.

Glutamine and asparagine contain R group amides that may be released as NH_4^+ by deamidation. Asparagine is deamidated by asparaginase, yielding aspartate and NH_4^+ . Glutaminase acts on glutamine, forming glutamate and NH_4^+ . The glutaminase



Glutamate dehydrogenase is one of three mammalian enzymes that can “fix” ammonia into organic molecules. The other two are glutamine synthetase and carbamoyl phosphate synthetase I.

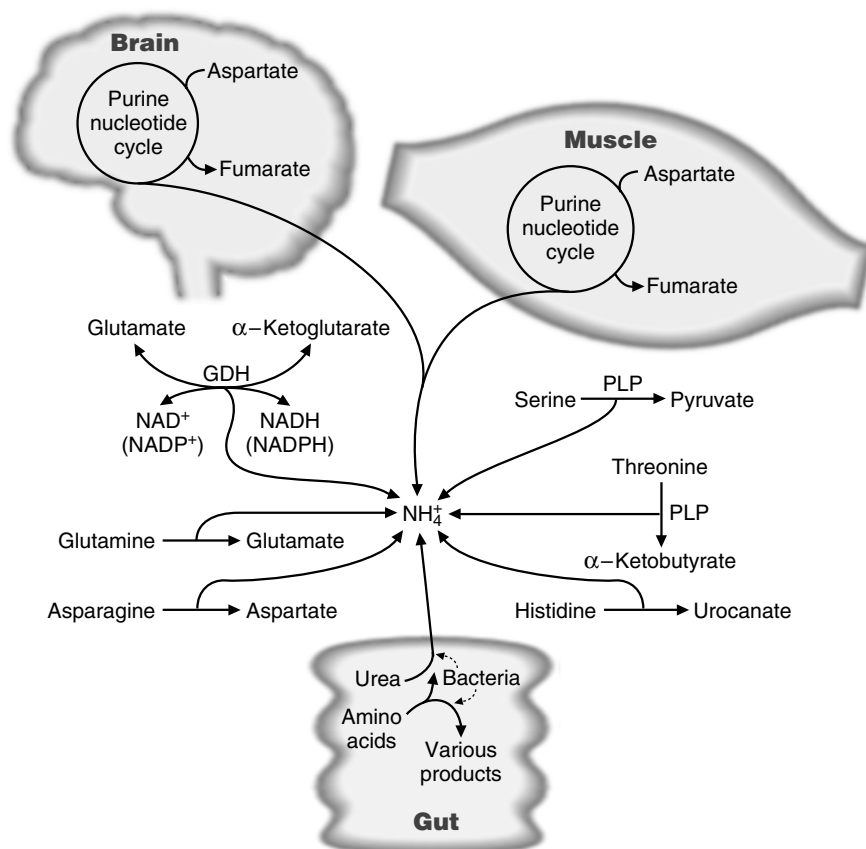


Fig. 38.5. Summary of the sources of NH_4^+ for the urea cycle. All of the reactions are irreversible except glutamate dehydrogenase (GDH). Only the dehydratase reactions, which produce NH_4^+ from serine and threonine, require pyridoxal phosphate as a cofactor. The reactions that are not shown occurring in the muscle or the gut can all occur in the liver, where the NH_4^+ generated can be converted to urea. The purine nucleotide cycle of the brain and muscle is further described in Chapter 41.

reaction is particularly important in the kidney, where the ammonium ion produced is excreted directly into the urine, where it forms salts with metabolic acids, facilitating their removal in the urine.

In muscle and brain, but not in liver, the purine nucleotide cycle allows NH_4^+ to be released from amino acids (see Fig. 38.5). Nitrogen is collected by glutamate from other amino acids by means of transamination reactions. Glutamate then transfers its amino group to oxaloacetate to form aspartate, which supplies nitrogen to the purine nucleotide cycle (see Chapter 41). The reactions of the cycle release fumarate and NH_4^+ . The ammonium ion formed can leave the muscle in the form of glutamine.

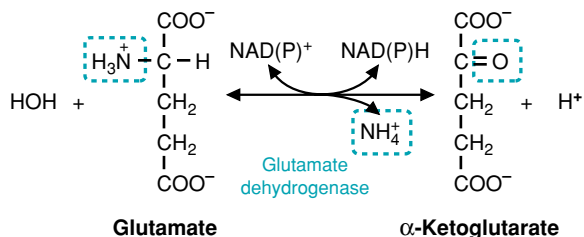


Fig. 38.7. Reaction catalyzed by glutamate dehydrogenase. This reaction is readily reversible and can use either NAD^+ or NADP^+ as a cofactor. The oxygen on α -ketoglutarate is derived from H_2O .



Percy Veere's laboratory studies showed that his serum alanine transaminase (ALT) level was 294 units/L (reference range = 5–30), and his serum aspartate transaminase (AST) level was 268 units/L (reference range = 10–30). His serum alkaline phosphatase level was 284 units/L (reference range for an adult male = 40–125), and his serum total bilirubin was 9.6 mg/dL (reference range = 0.2–1.0). Bilirubin is a degradation product of heme, as described in Chapter 44.

Cellular enzymes such as AST, ALT, and alkaline phosphatase leak into the blood through the membranes of hepatic cells that have been damaged as a result of the inflammatory process. In acute viral hepatitis, the serum ALT level is often elevated to a greater extent than the serum AST level. Alkaline phosphatase, which is present on membranes between liver cells and the bile duct, is also elevated in the blood in acute viral hepatitis.

The rise in serum total bilirubin occurs as a result of the inability of the infected liver to conjugate bilirubin and of a partial or complete occlusion of the hepatic biliary drainage ducts caused by inflammatory swelling within the liver. In fulminant hepatic failure, the serum bilirubin level may exceed 20 mg/dL, a poor prognostic sign.

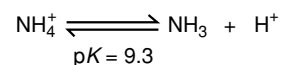


Fig. 38.6. Formation of ammonia from ammonium ion. Because the pK is 9.3, at physiologic pH, the concentration of NH_4^+ is almost 100 times that of NH_3 .



Vitamin B_6 deficiency symptoms include dermatitis, a microcytic, hypochromic anemia, weakness, irritability, and, in some cases, convulsions. Xanthurenic acid (a degradation product of tryptophan) and other compounds appear in the urine because of an inability to completely metabolize amino acids. A decreased ability to synthesize heme from glycine may cause the microcytic anemia (see Chapter 44), and decreased decarboxylation of amino acids to form neurotransmitters (see Chapter 48) may explain the convulsions.

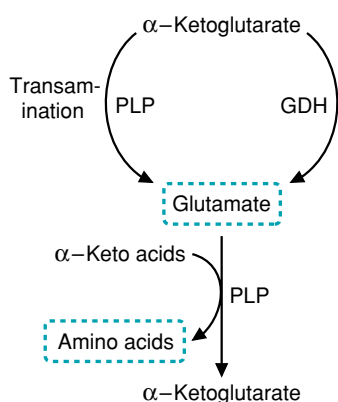
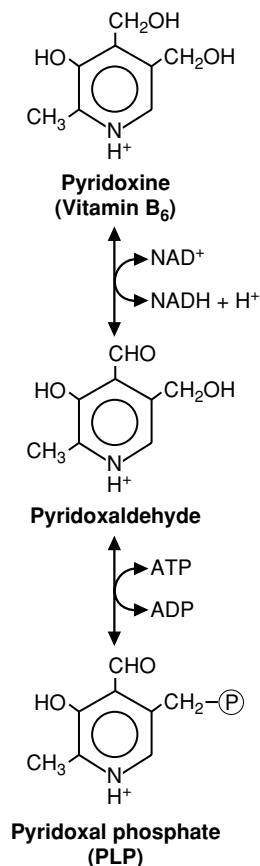


Fig. 38.8. Role of glutamate in amino acid synthesis. Glutamate transfers nitrogen by means of transamination reactions to α -keto acids to form amino acids. This nitrogen is either obtained by glutamate from transamination of other amino acids or from NH_4^+ by means of the glutamate dehydrogenase (GDH) reaction. PLP = pyridoxal phosphate.



Pyridoxal phosphate is derived from pyridoxine (vitamin B_6). Pyridoxal phosphate is the cofactor not only for transamination reactions but also for decarboxylations and a number of other reactions involving amino acids.



In summary, NH_4^+ that enters the urea cycle is produced in the body by deamination or deamidation of amino acids (see Fig. 38.5). A significant amount of NH_4^+ is also produced by bacteria that live in the lumen of the intestinal tract. This ammonium ion enters the hepatic portal vein and travels to the liver.

C. Role of Glutamate in the Metabolism of Amino Acid Nitrogen

Glutamate plays a pivotal role in the metabolism of amino acids. It is involved in both synthesis and degradation.

Glutamate provides nitrogen for amino acid synthesis (Fig. 38.8). In this process, glutamate obtains its nitrogen either from other amino acids by transamination reactions or from NH_4^+ by the glutamate dehydrogenase reaction. Transamination reactions then serve to transfer amino groups from glutamate to α -keto acids to produce their corresponding amino acids.

When amino acids are degraded and urea is formed, glutamate collects nitrogen from other amino acids by transamination reactions. Some of this nitrogen is released as ammonia by the glutamate dehydrogenase reaction, but much larger amounts of ammonia are produced from the other sources shown in Figure 38.5. NH_4^+ is one of the two forms in which nitrogen enters the urea cycle (Fig. 38.9).

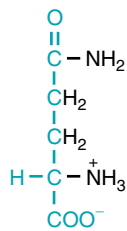
The second form of nitrogen for urea synthesis is provided by aspartate (see Fig. 38.9). Glutamate can be the source of the nitrogen. Glutamate transfers its amino group to oxaloacetate, and aspartate and α -ketoglutarate are formed.

D. Role of Alanine and Glutamine in Transporting Amino Acid Nitrogen to the Liver

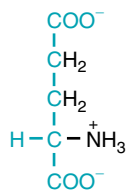
Protein turnover and amino acid degradation occur in all tissues; however, the urea cycle enzymes are primarily active in the liver (the intestine expresses low levels of activity of these enzymes; see Chapter 42). Thus, a mechanism needs to be in place to transport amino acid nitrogen to the liver. Alanine and glutamine are the major carriers of nitrogen in the blood. Alanine is primarily exported by the muscle. Because the muscle is metabolizing glucose through glycolysis, pyruvate is available in the muscle. The pyruvate is transaminated by glutamate to form alanine, which travels to the liver (Fig. 38.10). The glutamate is formed by transamination of an amino acid that is being degraded. On arriving at the liver, alanine is transaminated to pyruvate, and the nitrogen will be used for urea synthesis. The pyruvate formed is used for gluconeogenesis and the glucose exported to the muscle for use as energy. This cycle of moving carbons and nitrogen between the muscle and liver is known as the glucose/alanine cycle.



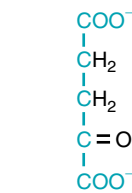
Compounds that contain "glut" in their name have five carbons in a straight chain. At each end of the chain, the carbon is part of a carboxyl group. In glutamine, the carboxyl group has formed an amide, and in hydroxymethylglutaryl CoA (HMG-CoA), it has formed a thioester with coenzyme A.



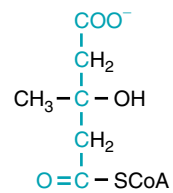
Glutamine



Glutamate



α -Ketoglutarate



Hydroxymethylglutaryl CoA (HMG CoA)

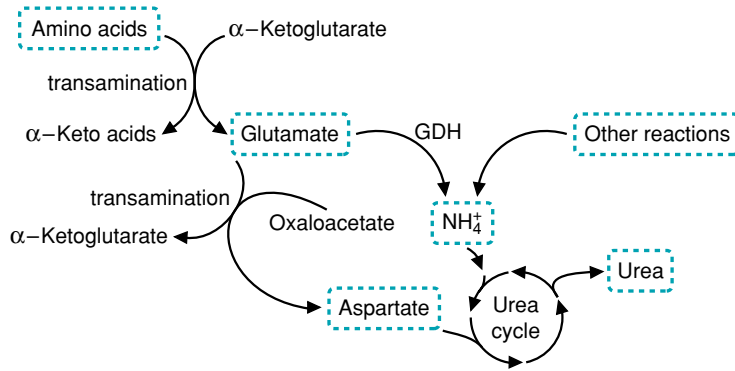


Fig. 38.9. Role of glutamate in urea production. Glutamate collects nitrogen from other amino acids by transamination reactions. This nitrogen can be released as NH_4^+ by glutamate dehydrogenase (GDH). NH_4^+ is also produced by other reactions (see Fig. 38.5). NH_4^+ provides one of the nitrogens for urea synthesis. The other nitrogen comes from aspartate and is obtained from glutamate by transamination of oxaloacetate.

Glutamine is synthesized from glutamate by the fixation of ammonia, requiring energy (adenosine triphosphate [ATP]) and the enzyme glutamine synthetase (Fig. 38.11), which is a cytoplasmic enzyme found in all cells. Under conditions of rapid amino acid degradation within a tissue, such that ammonia levels increase, the glutamate that has been formed from transamination reactions will accept another nitrogen molecule to form glutamine. The glutamine travels to the liver, kidney, or intestines, where glutaminase (see Fig. 38.11) will remove the amide nitrogen to form glutamate plus ammonia. In the kidney, the release of ammonia, and the formation of ammonium ion, serves to form salts with metabolic acids in the urine. In the intestine, the glutamine is used as a fuel (see Chapter 42). In the liver, the ammonia is used for urea biosynthesis.



Glutamine synthetase in liver is located in cells surrounding the portal vein. Its major role is to convert any ammonia that has escaped from urea production into glutamine, such that free ammonia does not leave the liver and enter the circulation.

II. UREA CYCLE

The normal human adult is in nitrogen balance; that is, the amount of nitrogen ingested each day, mainly in the form of dietary protein, is equal to the amount of nitrogen excreted. The major nitrogenous excretory product is urea, which exits from the body in the urine. This innocuous compound, produced mainly in the liver by the urea cycle, serves as the disposal form of ammonia, which is toxic, particularly to the brain and central nervous system. Normally, little ammonia (or NH_4^+) is present in the blood. The concentration ranges between

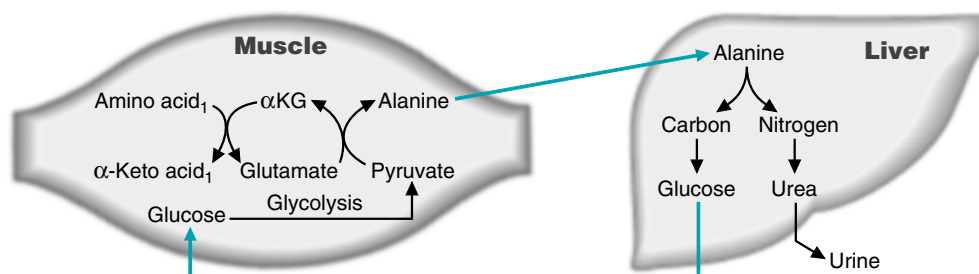


Fig. 38.10. The glucose/alanine cycle. Within the muscle, amino acid degradation leads to the transfer of nitrogens to α -ketoglutarate and pyruvate. The alanine formed travels to the liver, where the carbons of alanine are used for gluconeogenesis and the alanine nitrogen is used for urea biosynthesis. This could occur during exercise, when the muscle uses blood-borne glucose (see Chapter 47).

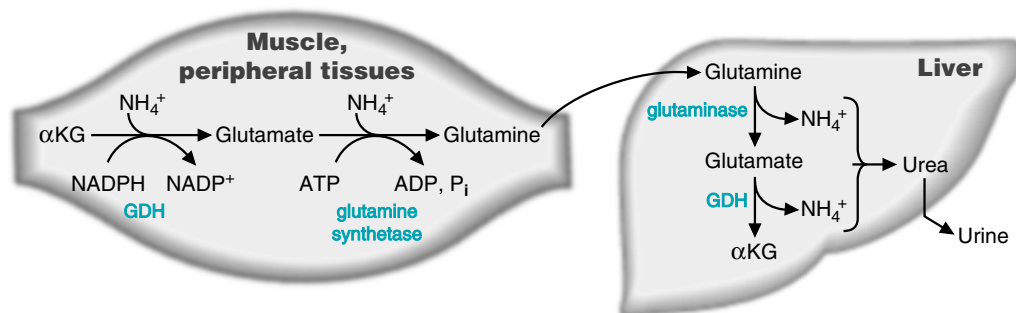
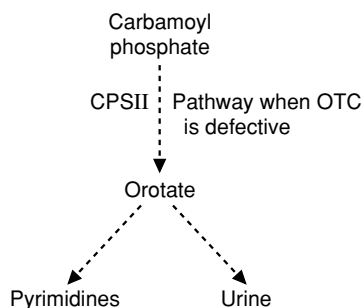


Fig. 38.11. Synthesis of glutamine in peripheral tissues and its transport to the liver. Within the liver, glutaminase converts glutamine to glutamate. Note how α -ketoglutarate can accept two molecules of ammonia to form glutamine. GDH = glutamate dehydrogenase.

Percy Veere's symptoms and laboratory abnormalities did not slowly subside over the next 6 weeks as they usually do in uncomplicated viral hepatitis A infections. Instead, his serum total bilirubin, ALT, AST, and alkaline phosphatase levels increased further. His vomiting became intractable, and his friend noted jerking motions of his arms (asterixis), facial grimacing, restlessness, slowed mentation, and slight disorientation. He was admitted to the hospital with a diagnosis of hepatic failure with incipient hepatic encephalopathy (brain dysfunction caused by accumulation of various toxins in the blood), a rare complication of acute type A viral hepatitis alone. The possibility of a superimposed acute hepatic toxicity caused by the use of acetaminophen was considered.

When ornithine transcarbamoylase (OTC) is deficient, the carbamoyl phosphate that normally would enter the urea cycle accumulates and floods the pathway for pyrimidine biosynthesis. Under these conditions, excess orotic acid (orotate), an intermediate in pyrimidine biosynthesis, is excreted in the urine. It produces no ill effects but is indicative of a problem in the urea cycle.



30 and 60 μM . Ammonia is rapidly removed from the blood and converted to urea by the liver. Nitrogen travels in the blood mainly in amino acids, particularly alanine and glutamine.

A. Reactions of the Urea Cycle

Nitrogen enters the urea cycle as NH_4^+ and aspartate (Fig. 38.12). NH_4^+ forms carbamoyl phosphate, which reacts with ornithine to form citrulline. Ornithine is the compound that both initiates and is regenerated by the cycle (similar to oxaloacetate in the TCA cycle). Aspartate reacts with citrulline, eventually donating its nitrogen for urea formation. Arginine is formed in two successive steps. Cleavage of arginine by arginase releases urea and regenerates ornithine.

1. SYNTHESIS OF CARBAMOYL PHOSPHATE

In the first step of the urea cycle, NH_4^+ , bicarbonate, and ATP react to form carbamoyl phosphate (see Fig. 38.12). The cleavage of 2 ATPs is required to form the high-energy phosphate bond of carbamoyl phosphate. Carbamoyl phosphate synthetase I (CPSI), the enzyme that catalyzes this first step of the urea cycle, is found mainly in mitochondria of the liver and intestine. The Roman numeral suggests that another carbamoyl phosphate synthetase exists, and indeed, CPSII, located in the cytosol, produces carbamoyl phosphate for pyrimidine biosynthesis, using nitrogen from glutamine (see Chapter 41).

2. PRODUCTION OF ARGININE BY THE UREA CYCLE

Carbamoyl phosphate reacts with ornithine to form citrulline (see Fig. 38.12). The high-energy phosphate bond of carbamoyl phosphate provides the energy required for this reaction, which occurs in mitochondria and is catalyzed by ornithine transcarbamoylase. The product citrulline is transported across the mitochondrial membranes in exchange for cytoplasmic ornithine and enters the cytosol. The carrier for this transport reaction catalyzes an electroneutral exchange of the two compounds.

In the cytosol, citrulline reacts with aspartate, the second source of nitrogen for urea synthesis, to produce argininosuccinate (see Fig. 38.12). This reaction, catalyzed by argininosuccinate synthetase, is driven by the hydrolysis of ATP to adenosine monophosphate (AMP) and pyrophosphate. Aspartate is produced by transamination of oxaloacetate.

The urea cycle was proposed in 1932 by Hans Krebs and a medical student, Kurt Henseleit, based on their laboratory observations. It was originally called the Krebs-Henseleit cycle. Subsequently, Krebs used this concept of metabolic cycling to explain a second process that also bears his name, the Krebs (or TCA) cycle.

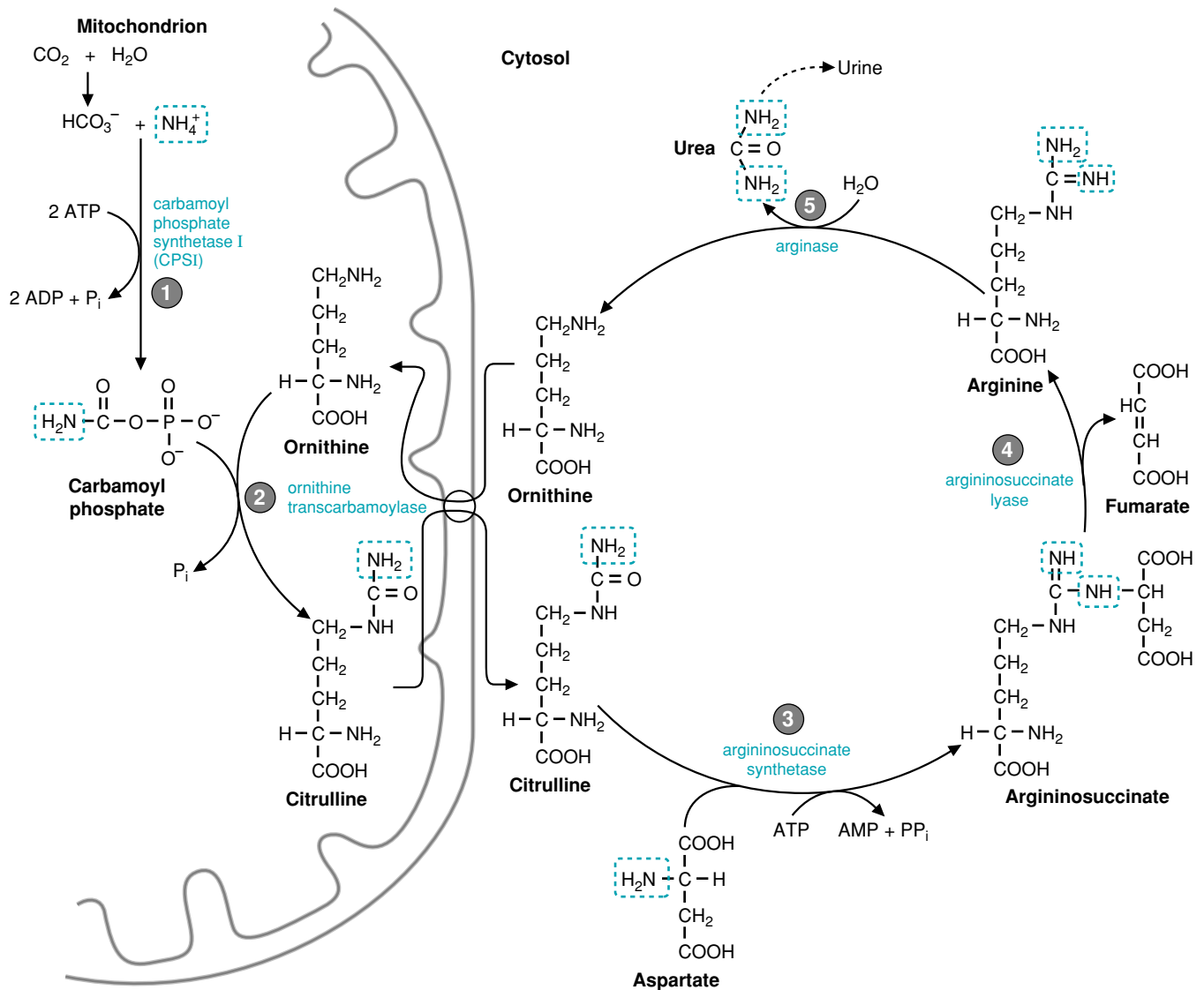


Fig. 38.12. Urea cycle. The steps of the cycle are numbered 1 to 5.

Argininosuccinate is cleaved by argininosuccinate lyase to form fumarate and arginine (see Fig. 38.12). Fumarate is produced from the carbons of argininosuccinate provided by aspartate. Fumarate is converted to malate (using cytoplasmic fumarase), which is used either for the synthesis of glucose by the gluconeogenic pathway or for the regeneration of oxaloacetate by cytoplasmic reactions similar to those observed in the TCA cycle (Fig. 38.13). The oxaloacetate that is formed is transaminated to generate the aspartate that carries nitrogen into the urea cycle. Thus, the carbons of fumarate can be recycled to aspartate.

3. CLEAVAGE OF ARGININE TO PRODUCE UREA

Arginine, which contains nitrogens derived from NH_4^+ and aspartate, is cleaved by arginase, producing urea and regenerating ornithine (see Fig. 38.12). Urea is produced from the guanidinium group on the side chain of arginine. The portion of arginine originally derived from ornithine is reconverted to ornithine.

The reactions by which citrulline is converted to arginine and arginine is cleaved to produce urea occur in the cytosol. Ornithine, the other product of the arginase

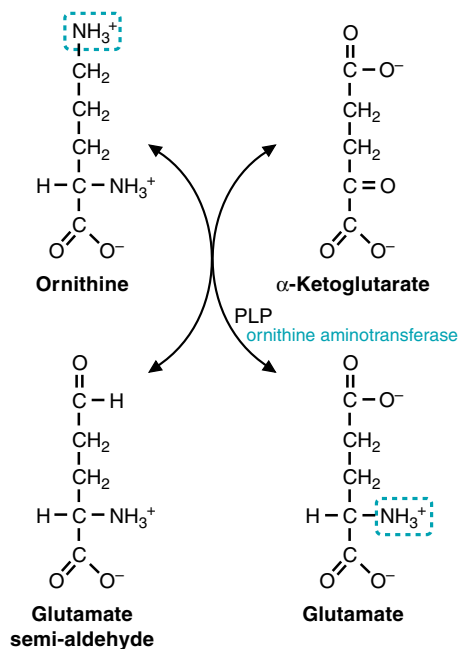


Fig. 38.14. The ornithine aminotransferase reaction. This is a reversible reaction dependent on pyridoxal phosphate, which normally favors ornithine degradation.



The precise pathogenesis of the central nervous system (CNS) signs and symptoms that accompany liver failure (hepatic encephalopathy) in patients such as **Percy Veere** is not completely understood. These changes are, however, attributable in part to toxic materials that are derived from the metabolism of nitrogenous substrates by bacteria in the gut that circulate to the liver in the portal vein. These materials “bypass” their normal metabolism by the liver cells, however, because the acute inflammatory process of viral hepatitis severely limits the ability of liver cells to degrade these compounds to harmless metabolites. As a result, these toxins are “shunted” into the hepatic veins unaltered and eventually reach the brain through the systemic circulation (“portal-systemic encephalopathy”).

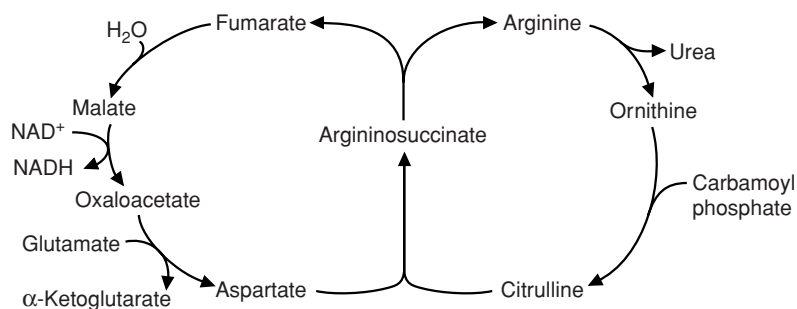


Fig. 38.13. The Krebs bicycle, indicating the common steps between the TCA and urea cycles. All reactions shown occur in the cytoplasm except for the synthesis of citrulline, which occurs within the mitochondria.

reaction, is transported into the mitochondrion in exchange for citrulline, where it can react with carbamoyl phosphate, initiating another round of the cycle.

B. Origin of Ornithine

Ornithine is an amino acid. However, it is not incorporated into proteins during the process of protein synthesis because no genetic codon exists for this amino acid. Although ornithine is normally regenerated by the urea cycle (one of the products of the arginase reaction), ornithine also can be synthesized *de novo* if needed. The reaction is an unusual transamination reaction catalyzed by ornithine aminotransferase under specific conditions in the intestine (Fig. 38.14). The usual direction of this reaction is the formation of glutamate semialdehyde, which is the first step of the degradation pathway for ornithine.

C. Regulation of the Urea Cycle

The human liver has a vast capacity to convert amino acid nitrogen to urea, thereby preventing toxic effects from ammonia, which would otherwise accumulate. In general, the urea cycle is regulated by substrate availability; the higher the rate of ammonia production, the higher the rate of urea formation. Regulation by substrate availability is a general characteristic of disposal pathways, such as the urea cycle, which remove toxic compounds from the body. This is a type of “feed-forward” regulation, in contrast to the “feedback” regulation characteristic of pathways that produce functional endproducts.

Two other types of regulation control the urea cycle: allosteric activation of CPSII by *N*-acetylglutamate (NAG) and induction/repression of the synthesis of urea cycle enzymes. NAG is formed specifically to activate CPSII; it has no other known function in mammals. The synthesis of NAG from acetyl CoA and glutamate is stimulated by arginine (Fig. 38.15). Thus, as arginine levels increase within the liver, two important reactions are stimulated. The first is the synthesis of NAG, which will increase the rate at which carbamoyl phosphate is produced. The second is to produce more ornithine (via the arginase reaction), such that the cycle can operate more rapidly.

The induction of urea cycle enzymes occurs in response to conditions that require increased protein metabolism, such as a high-protein diet or prolonged fasting. In both of these physiologic states, as amino acid carbon is converted to glucose, amino acid nitrogen is converted to urea. The induction of the synthesis of urea cycle enzymes under these conditions occurs even though the uninduced enzyme levels are far in excess of the capacity required. The ability of a high-protein diet to increase urea cycle enzyme levels is another type of “feed-forward” regulation.

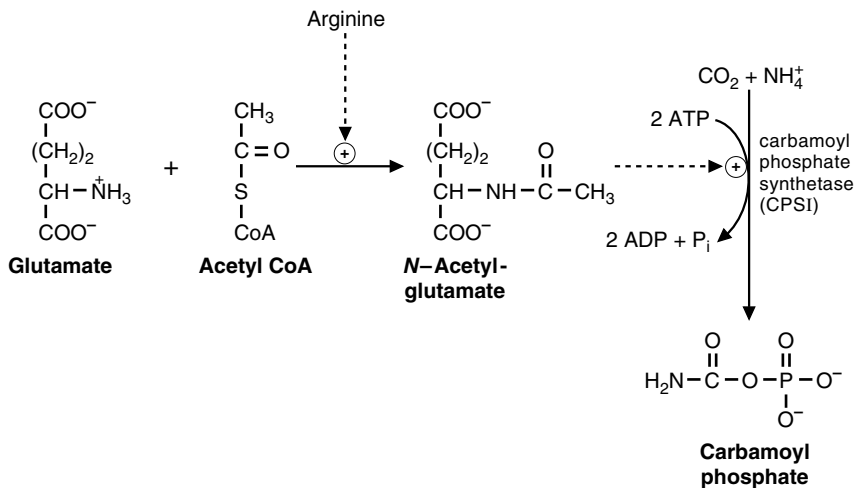


Fig. 38.15. Activation of carbamoyl phosphate synthetase I (CPSI). Arginine stimulates the synthesis of *N*-acetylglutamate, which activates CPSI.

D. Function of the Urea Cycle during Fasting

During fasting, the liver maintains blood glucose levels. Amino acids from muscle protein are a major carbon source for the production of glucose by the pathway of gluconeogenesis. As amino acid carbons are converted to glucose, the nitrogens are converted to urea. Thus, the urinary excretion of urea is high during fasting (Fig. 38.16). As fasting progresses, however, the brain begins to use ketone bodies, sparing blood glucose. Less muscle protein is cleaved to provide amino acids for gluconeogenesis, and decreased production of glucose from amino acids is accompanied by decreased production of urea (see Chapter 31).

The major amino acid substrate for gluconeogenesis is alanine, which is synthesized in peripheral tissues to act as a nitrogen carrier (see Fig. 38.10). Glucagon release, which is expected during fasting, stimulates alanine transport

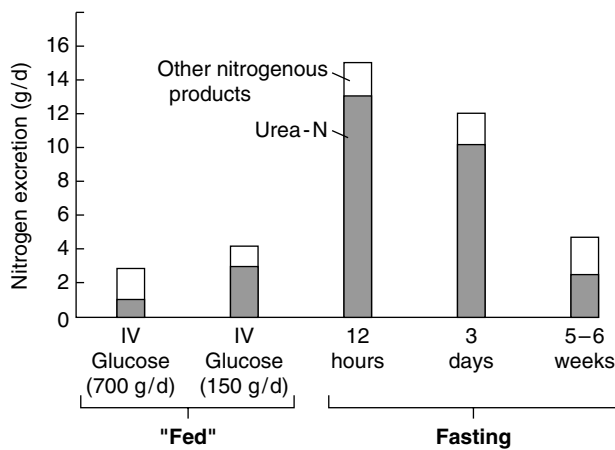


Fig. 38.16. Nitrogen excretion during fasting. Human subjects were initially given intravenous (IV) glucose as indicated, then fasted. Total nitrogen excretion was measured as well as the nitrogen in urea (dark area). Based on Ruderman NB, et al. Gluconeogenesis and its disorders in man. In: Hanson RW, Mehler MA, eds. Gluconeogenesis: Its Regulation in Mammalian Species. New York: John Wiley, 1976:518.



NH_4^+ is one of the toxins that results from the degradation of urea or proteins by intestinal bacteria and is not metabolized by the infected liver. The subsequent elevation of ammonia concentrations in the fluid bathing the brain causes depletion of tricarboxylic acid (TCA) cycle intermediates and ATP in the central nervous system. α -Ketoglutarate, a TCA cycle intermediate, combines with ammonia to form glutamate in a reaction catalyzed by glutamate dehydrogenase. Glutamate subsequently reacts with ammonia to form glutamine.

The absolute level of ammonia and its metabolites, such as glutamine, in the blood or cerebrospinal fluid in patients with hepatic encephalopathy correlates only roughly with the presence or severity of the neurologic signs and symptoms. γ -Aminobutyric acid (GABA), an important inhibitory neurotransmitter in the brain, is also produced in the gut lumen and is shunted into the systemic circulation in increased amounts in patients with hepatic failure. In addition, other compounds (such as aromatic amino acids, false neurotransmitters, and certain short-chain fatty acids) bypass liver metabolism and accumulate in the systemic circulation, adversely affecting central nervous system function. Their relative importance in the pathogenesis of hepatic encephalopathy remains to be determined.



In addition to producing urea, the reactions of the urea cycle also serve as the pathway for the biosynthesis of arginine. Therefore, this amino acid is not required in the diet of the adult; however, it is required in the diet for growth.



Urea is not cleaved by human enzymes. However, bacteria, including those in the human digestive tract, can cleave urea to ammonia and CO_2 , using the enzyme urease. Urease is not produced by humans.

To some extent, humans excrete urea into the gut and saliva. Intestinal bacteria convert urea to ammonia. This ammonia, as well as ammonia produced by other bacterial reactions in the gut, is absorbed into the hepatic portal vein. It is normally extracted by the liver and converted to urea. Approximately one fourth of the total urea released by the liver each day is recycled by bacteria.

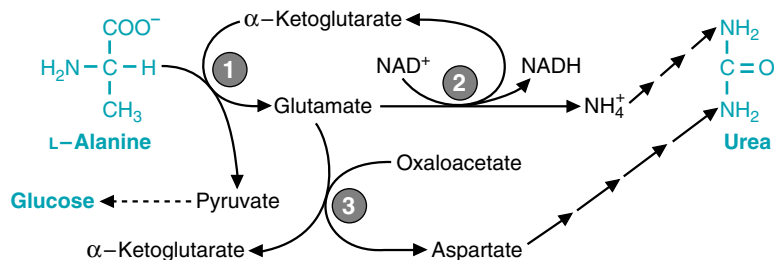


Fig. 38.17. Conversion of alanine to glucose and urea. 1. Alanine, the key gluconeogenic amino acid, is transaminated to form pyruvate, which is converted to glucose. The nitrogen, now in glutamate, can be released as NH_4^+ (2) or transferred to oxaloacetate to form aspartate (3). NH_4^+ and aspartate enter the urea cycle, which produces urea. In summary, the carbons of alanine form glucose and the nitrogens form urea. Two molecules of alanine are required to produce one molecule of glucose and one molecule of urea.

into the liver by activating the transcription of transport systems for alanine. Two molecules of alanine are required to generate one molecule of glucose. The nitrogen from the two molecules of alanine is converted to one molecule of urea (Fig. 38.17).

CLINICAL COMMENTS



The two most serious complications of acute viral hepatitis found in patients such as **Percy Veere** are massive hepatic necrosis leading to fulminant liver failure and the eventual development of chronic hepatitis. Both complications are rare in acute viral hepatitis type A, however, suggesting that acetaminophen toxicity may have contributed to Percy's otherwise unexpectedly severe hepatocellular dysfunction and early hepatic encephalopathy.

Fortunately, bed rest, rehydration, parenteral nutrition, and therapy directed at decreasing the production of toxins that result from bacterial degradation of nitrogenous substrates in the gut lumen (e.g., administration of lactulose, which reduces gut ammonia levels by a variety of mechanisms, the use of enemas and antibiotics to decrease the intestinal flora, a low-protein diet) prevented **Percy Veere** from progressing to the later stages of hepatic encephalopathy. As with most patients who survive an episode of fulminant hepatic failure, recovery to his previous state of health occurred over the next 3 months. Percy's liver function studies returned to normal, and a follow-up liver biopsy showed no histologic abnormalities.

BIOCHEMICAL COMMENTS



Disorders of the urea cycle are dangerous because of the accumulation of ammonia in the circulation. Ammonia is toxic to the nervous system, and its concentration in the body must be carefully controlled. Under normal conditions, free ammonia is rapidly fixed into either α -ketoglutarate (by glutamate dehydrogenase, to form glutamate) or glutamate (by glutamine synthase, to form glutamine). The glutamine can be used by many tissues, including the liver; the glutamate donates nitrogens to pyruvate to form alanine, which travels to the liver. Within the liver, as the nitrogens are removed from their carriers, carbamoyl phosphate synthetase I fixes the ammonia into carbamoyl phosphate to initiate the urea

cycle. However, when a urea cycle enzyme is defective, the cycle is interrupted, which leads to an accumulation of urea cycle intermediates before the block. Because of the block in the urea cycle, glutamine levels increase in the circulation, and because α -ketoglutarate is no longer being regenerated by removal of nitrogen from glutamine, the α -ketoglutarate levels are too low to fix more free ammonia, leading to elevated ammonia levels in the blood. So how does one reduce ammonia and glutamine levels in such patients?

The key to treating patients with urea cycle defects is to diagnose the disease early and then aggressively treat with compounds that can aid in nitrogen removal

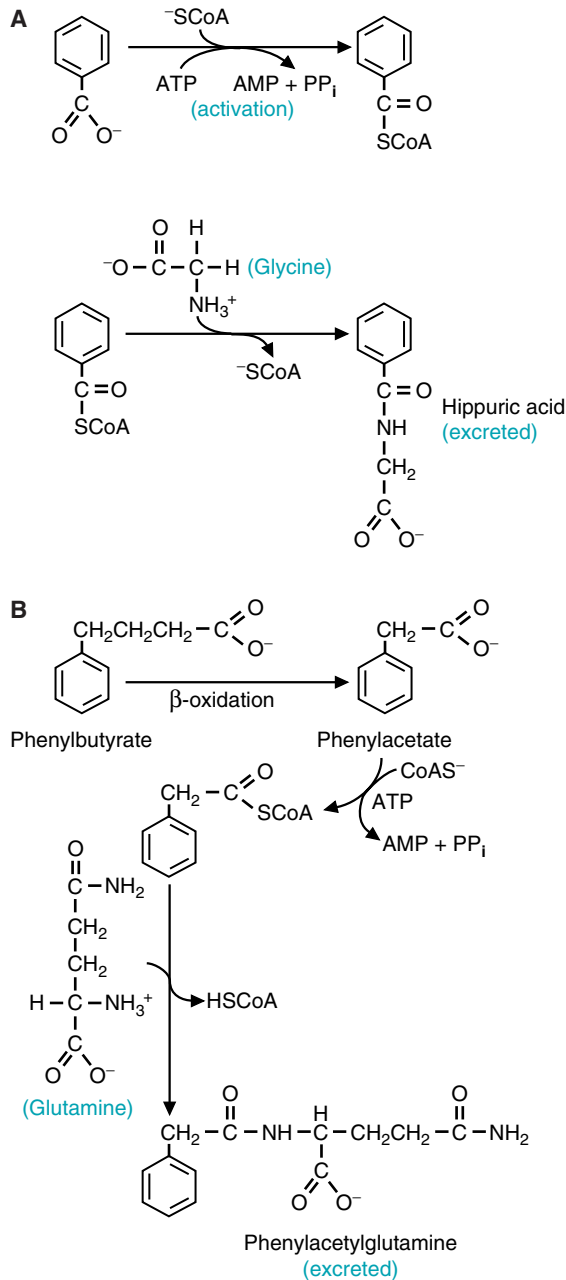


Fig. 38.18. The metabolism of benzoic acid (A) and phenylbutyrate (B), two agents used to reduce nitrogen levels in patients with urea cycle defects.

from the patient. Low-protein diets are essential to reduce the potential for excessive amino acid degradation. If the enzyme defect in the urea cycle comes after the synthesis of argininosuccinate, massive arginine supplementation has proved beneficial. Once argininosuccinate has been synthesized, the two nitrogen molecules destined for excretion have been incorporated into the substrate; the problem is that ornithine cannot be regenerated. If ornithine could be replenished to allow the cycle to continue, argininosuccinate could be used as the carrier for nitrogen excretion from the body. Thus, ingesting large levels of arginine leads to ornithine production by the arginase reaction, and nitrogen excretion via argininosuccinate in the urine can be enhanced.

Arginine therapy will not work for enzyme defects that exist in steps before the synthesis of argininosuccinate. For these disorders, drugs are used that form conjugates with amino acids. The conjugated amino acids are excreted, and the body then has to use its nitrogen to resynthesize the excreted amino acid. The two compounds most frequently used are benzoic acid and phenylbutyrate (the active component of phenylbutyrate is phenylacetate, its oxidation product. Phenylacetate has a bad odor, which makes it difficult to take orally). As indicated in Figure 38.18A, benzoic acid, after activation, reacts with glycine to form hippuric acid, which is excreted. As glycine is synthesized from serine, the body now uses nitrogens to synthesize serine, so more glycine can be produced. Phenylacetate (see Fig. 38.18B) forms a conjugate with glutamine, which is excreted. This conjugate removes two nitrogens per molecule and requires the body to resynthesize glutamine from glucose, thereby using another two nitrogen molecules.

Urea cycle defects are excellent candidates for treatment by gene therapy. This is because the defect only has to be repaired in one cell type (in this case, the hepatocyte), which makes it easier to target the vector carrying the replacement gene. Preliminary gene therapy experiments had been carried out on individuals with ornithine transcarbamoylase deficiency (the most common inherited defect in the urea cycle), but the experiments came to a halt when one of the patients died of a severe immunologic reaction to the vector used to deliver the gene. This incident has placed gene replacement therapy in the United States “on hold” for the foreseeable future.

Suggested Readings

- Brusilow S, Horwich A. Urea cycle enzymes. In: Scriver CR, Beaudet AL, Valle D, Sly WS, et al., eds. *The Metabolic and Molecular Bases of Inherited Disease*, vol II, 8th Ed. New York: McGraw-Hill, 2001:1909–1963.
- Marshall E. Gene therapy death prompts review of adenovirus vector. *Science* 1999;286:2244–2245.
- Morris SM Jr. Regulation of enzymes of the urea cycle and arginine metabolism. *Annu Rev Nutr* 2002;22:87–105.



REVIEW QUESTIONS—CHAPTER 38

1. Deficiency diseases have been described that involve each of the five enzymes of the urea cycle. Clinical manifestations may appear in the neonatal period. Infants with defects in the first four enzymes usually appear normal at birth, but after 24 hours progressively develop lethargy, hypothermia, and apnea. They have high blood ammonia levels, and the brain becomes swollen. One possible explanation for the swelling is the osmotic effect of the accumulation of glutamine in the brain produced by the reactions of ammonia with α -ketoglutarate and glutamate. Arginase deficiency is not as severe as deficiencies of the other urea cycle enzymes.

Given the following information about five newborn infants (identified as I to V) who appeared normal at birth but developed hyperammonemia after 24 hours, determine which urea cycle enzyme might be defective in each case (for each infant, choose from the same five answers, lettered A through E). All infants had low levels of blood urea nitrogen (BUN). (Normal citrulline levels are 10-20 μM .)

Infant	Urine Orotate	Blood Citrulline	Blood Arginine	Blood Ammonia
I	Low	Low	Low	High
II	-*	High (> 1,000 μM)	Low	High
III	-	-	High	Moderately high
IV	High	Low	Low	High
V	-	High (200 μM)	Low	High

* = Value not determined; low = below normal; high = above normal.

- (A) Carbamoylphosphate synthetase I
- (B) Ornithine transcarbamoylase
- (C) Argininosuccinate synthetase
- (D) Argininosuccinate lyase
- (E) Arginase

2. The nitrogens in urea are directly derived from which of the following compounds?

- (A) Ornithine and carbamoyl phosphate
- (B) Ornithine and aspartate
- (C) Ornithine and glutamate
- (D) Carbamoyl phosphate and aspartate
- (E) Carbamoyl phosphate and glutamine
- (F) Aspartate and glutamine

3. Which one of the following enzymes can fix ammonia into an organic molecule?

- (A) Alanine-pyruvate aminotransferase
- (B) Glutaminase
- (C) Glutamate dehydrogenase
- (D) Arginase
- (E) Argininosuccinate synthetase

4. Pyridoxal phosphate, which is required for transaminations, is also required for which of the following pathways?

- (A) Glycolysis
- (B) Gluconeogenesis
- (C) Glycogenolysis
- (D) TCA cycle
- (E) Fatty acid oxidation

5. The major regulated step of the urea cycle is which of the following?

- (A) Carbamoyl phosphate synthetase I
- (B) Ornithine transcarbamoylase
- (C) Argininosuccinate synthetase
- (D) Argininosuccinate lyase
- (E) Arginase