19: Amino Acids: Nitrogen Disposal

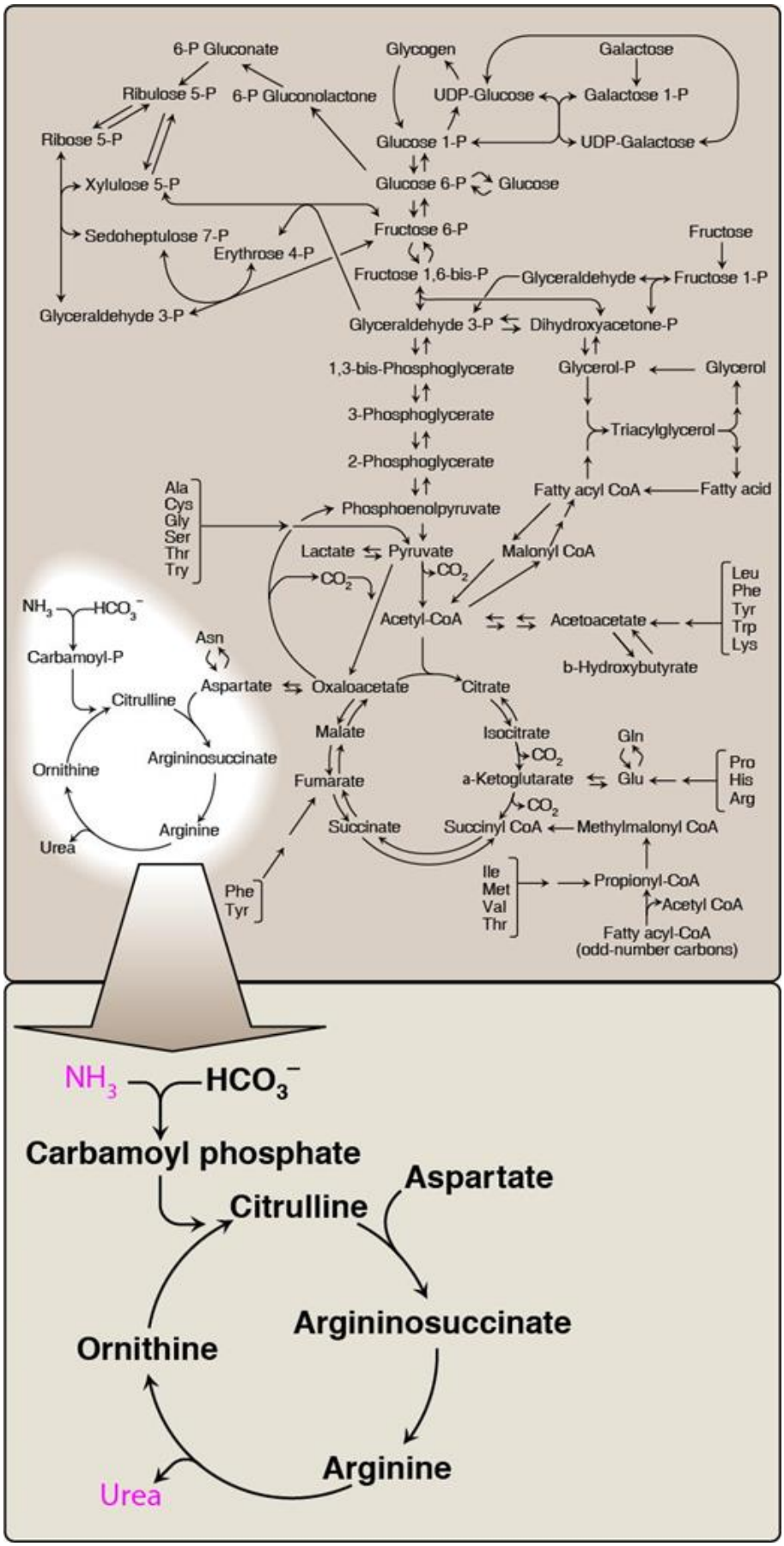
Overview

Unlike fats and carbohydrates, amino acids are not stored by the body. That is, no protein exists whose sole function is to maintain a supply of amino acids for future use. Therefore, amino acids must be obtained from the diet, synthesized *de novo*, or produced from the degradation of body protein. Any amino acids in excess of the biosynthetic needs of the cell are rapidly degraded. The first phase of catabolism involves the removal of the α-amino groups (usually by transamination and subsequent oxidative deamination), forming ammonia and the corresponding α-keto acids, the carbon skeletons of amino acids. A portion of the free ammonia is excreted in the urine, but most is used in the synthesis of urea (Fig. 19.1), which is quantitatively the most important route for disposing of nitrogen from the body. In the second phase of amino acid catabolism, described in Chapter 20, the carbon skeletons of the α-keto acids are converted to common intermediates of energy-producing metabolic pathways. These compounds can be metabolized to carbon dioxide (CO_2) and water (H_2O), glucose, fatty acids, or ketone bodies by the central pathways of metabolism described in Chapters 8, Chapters 8, Chapters 9, Chapters 10, Chapters 11, Chapters 12, Chapters 13, and Chapters 16.

FIGURE 19.1

Urea cycle shown as part of the essential pathways of energy metabolism.

(Note: Fig. 8.2, p. 101, for a more detailed map of metabolism.) NH_3 = ammonia; CO_2 = carbon dioxide.



Overall Nitrogen Metabolism

Amino acid catabolism is part of the larger process of the metabolism of nitrogen-containing molecules. Nitrogen enters the body in a variety of compounds present in food, the most important being amino acids contained in dietary protein. Nitrogen leaves the body as urea, ammonia, and other products derived from amino acid metabolism (such as creatinine, see p. 320). The role of body proteins in these transformations involves two important concepts: the amino acid pool and protein turnover.

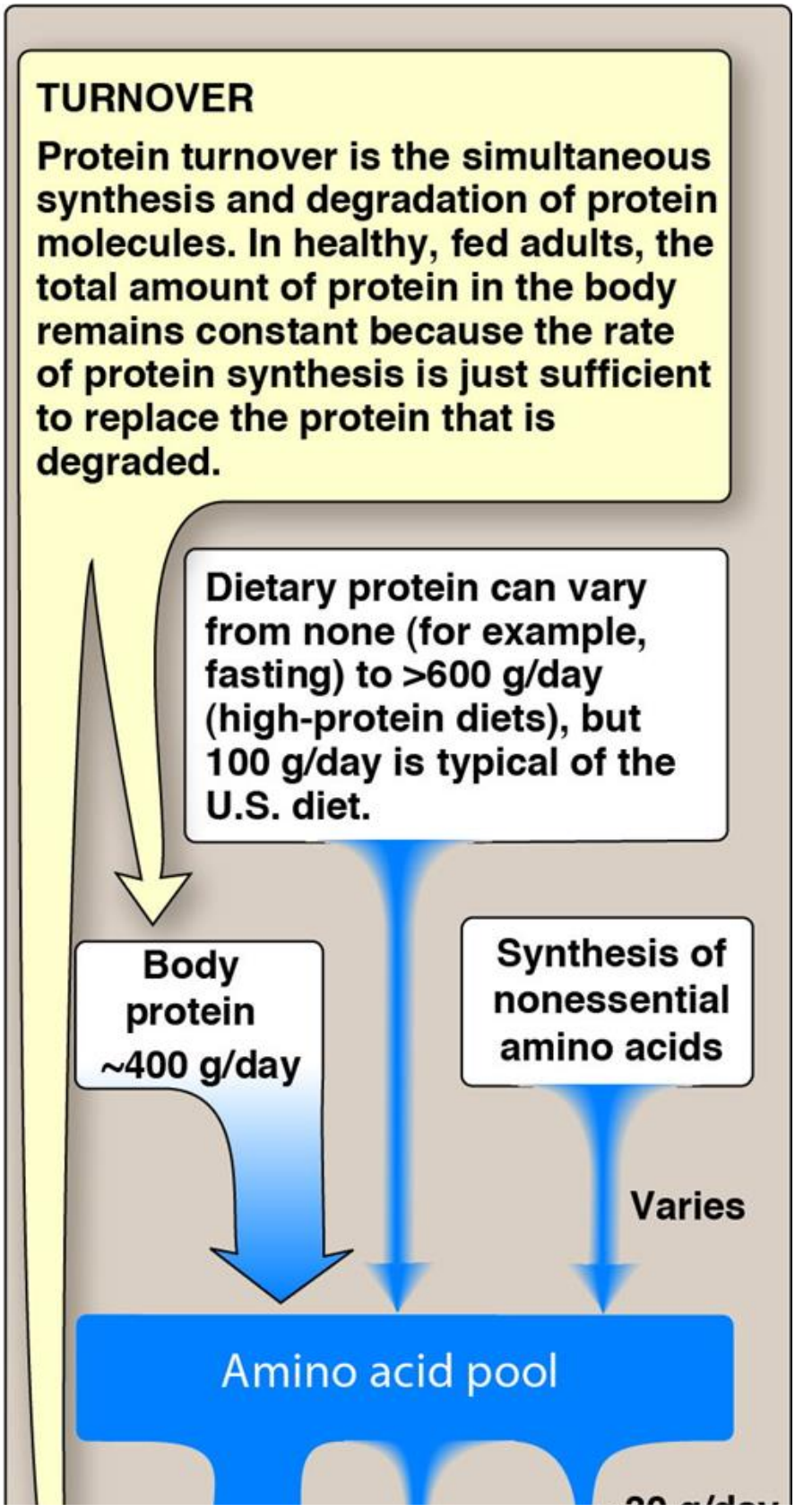
Amino acid pool

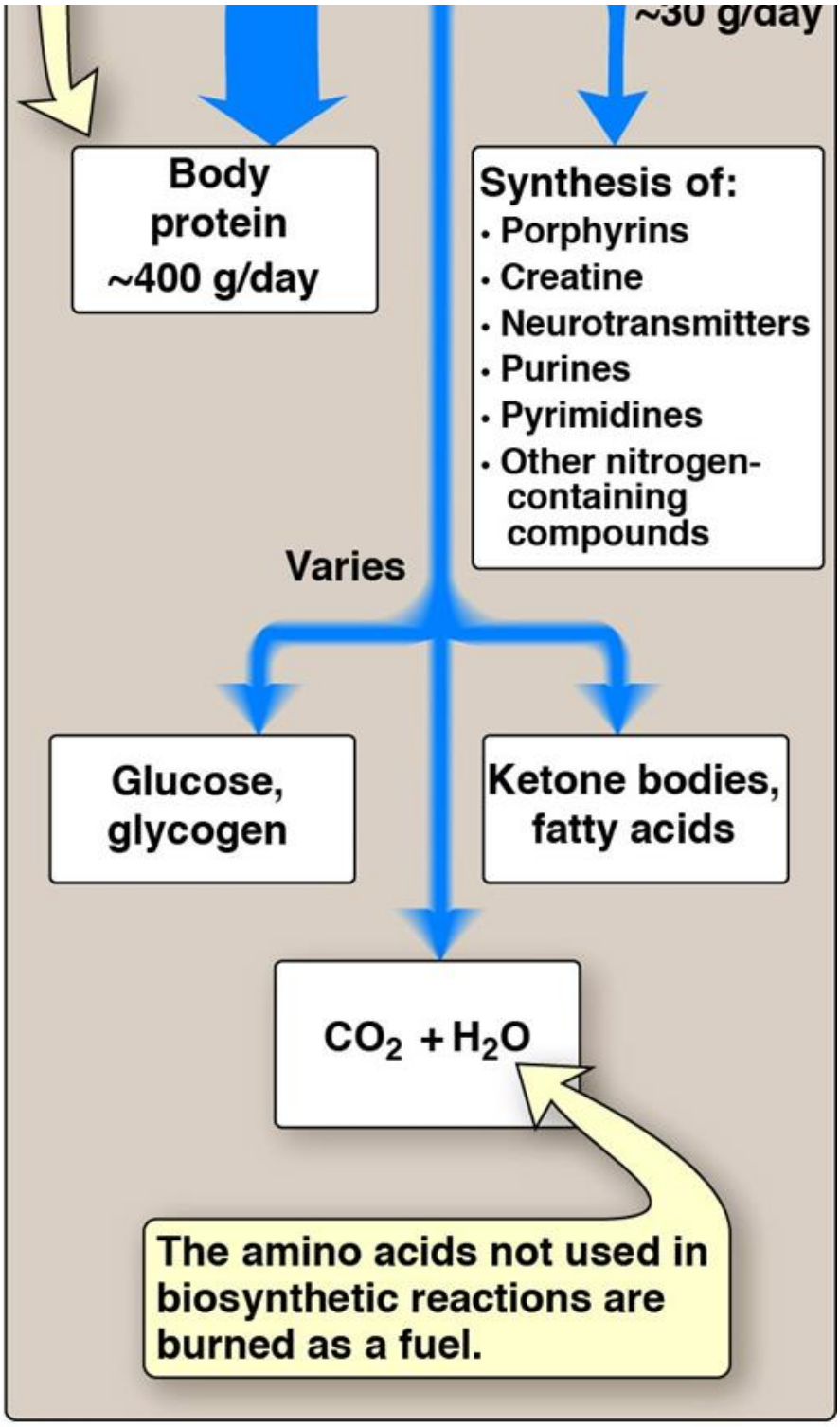
Free amino acids are present throughout the body, such as in cells, blood, and the extracellular fluids. For the purpose of this discussion, envision all of these amino acids as if they belonged to a single entity, called the amino acid pool. This pool is supplied by three sources: (1) amino acids provided by the degradation of endogenous (body) proteins, most of which are reutilized; (2) amino acids derived from exogenous (dietary) protein; and (3) nonessential amino acids synthesized from simple intermediates of metabolism (Fig. 19.2). Conversely, the amino acid pool is depleted by three routes: (1) synthesis of body protein, (2) consumption of amino acids as precursors of essential nitrogen-containing small molecules, and (3) conversion of amino acids to glucose, glycogen, fatty acids, and ketone bodies or oxidation to CO_2 + H_2O (Fig. 19.2). Although the amino acid pool is small (comprising ~90 to 100 g of amino acids) in comparison with the amount of protein in the body (~12 kg in a 70-kg man), it is conceptually at the center of whole-body nitrogen metabolism.

FIGURE 19.2

Sources and fates of amino acids.

(Note: Nitrogen from amino acid degradation is released as ammonia, which is converted to urea and excreted.) CO_2 = carbon dioxide.





In healthy, well-fed individuals, the input to the amino acid pool is balanced by the output. That is, the amount of amino acids contained in the pool is constant. The amino acid pool is said to be in a steady state, and the individual is said to be in nitrogen balance (see p. 412).

Protein turnover

Most proteins in the body are constantly being synthesized and then degraded (turned over), permitting the removal of abnormal or unneeded proteins. For many proteins, regulation of synthesis determines the concentration of protein in the cell, with protein degradation assuming a minor role. For other proteins, the rate of synthesis is constitutive (i.e., essentially constant), and cellular levels of the protein are controlled by selective degradation.

Rate

In healthy adults, the total amount of protein in the body remains constant because the rate of protein synthesis is just sufficient to replace the protein that is degraded. This process, called protein turnover, leads to the hydrolysis and resynthesis of 300 to 400 g of body protein each day. The rate of protein turnover varies widely for individual proteins. Short-lived proteins (e.g., many regulatory proteins and misfolded proteins) are rapidly degraded, having half-lives measured in minutes or hours. Long-lived proteins, with half-lives of days to weeks, constitute the majority of proteins in the cell. Structural proteins, such as collagen, are metabolically stable and have half-lives measured in months or years.

Protein degradation

There are two major enzyme systems responsible for degrading proteins: the ATP-dependent ubiquitin (Ub)–proteasome system of the cytosol and the ATP-independent degradative enzyme system of the lysosomes. Proteasomes selectively degrade damaged or short-lived proteins. Lysosomes use acid hydrolases (see p. 178) to nonselectively degrade intracellular proteins (autophagy) and extracellular proteins (heterophagy), such as plasma proteins that are taken into the cell by endocytosis.

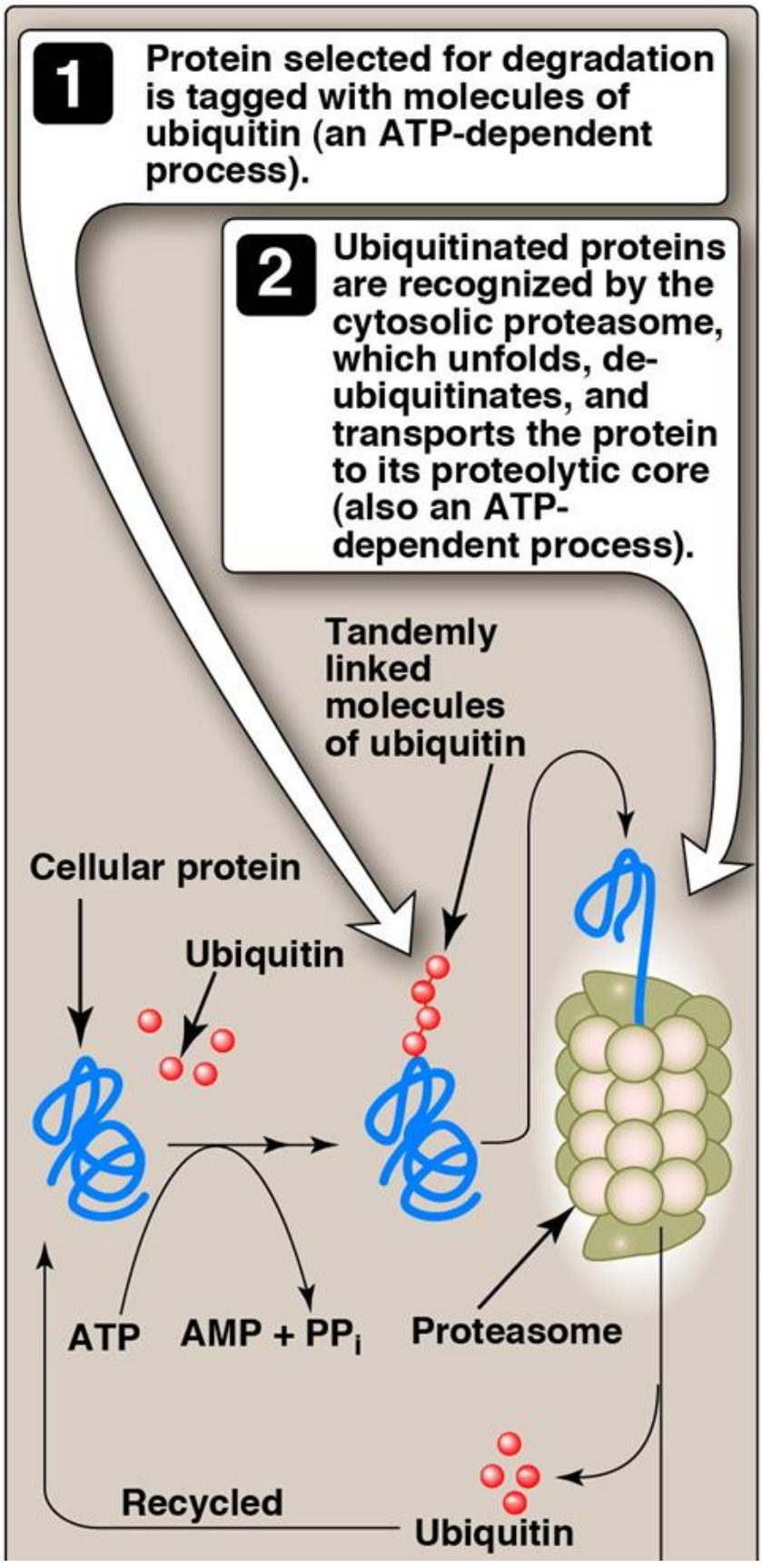
Ubiquitin–proteasome system

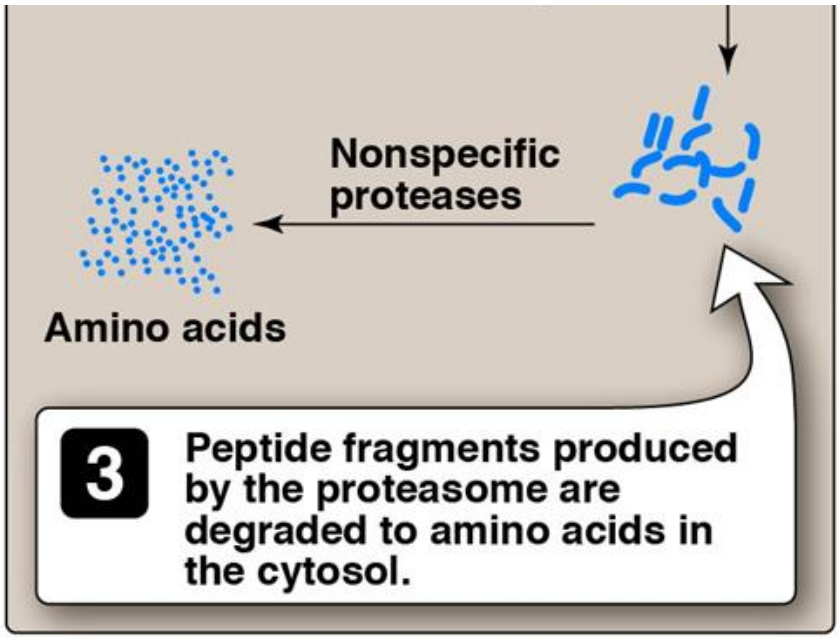
Proteins selected for degradation by the cytosolic Ub–proteasome system are first modified by the covalent attachment of Ub, a small, globular, nonenzymic protein that is highly conserved across eukaryotic species. Ubiquitination of the target substrate occurs through isopeptide linkage of the α-carboxyl group of the C-terminal glycine of Ub to the ε-amino group of a lysine in the protein substrate by a three-step, enzyme-catalyzed, ATP-dependent process. (Note: Enzyme 1 [E1, an activating enzyme] activates Ub, which is then transferred to E2 [a conjugating enzyme]. E3 [a ligase] identifies the protein to be degraded and interacts with E2-Ub. There are many more E3 proteins than there are E1 or E2.) The consecutive addition of four or more Ub molecules to the target protein generates a polyubiquitin chain. Proteins tagged with Ub chains are recognized by a large, barrel-shaped, macromolecular, proteolytic complex called a proteasome (Fig. 19.3). The proteasome unfolds, deubiquitinates, and cuts the target protein into fragments that are then further degraded by cytosolic proteases to amino acids, which enter the amino acid pool. The Ub is recycled. It is noteworthy that the selective degradation of proteins by the Ub–proteosome complex (unlike simple hydrolysis by proteolytic enzymes) requires ATP hydrolysis.

FIGURE 19.3

The ubiquitin–proteasome degradation pathway of proteins.

AMP = adenosine monophosphate; PP_i = pyrophosphate.





Degradation signals

Because proteins have different half-lives, it is clear that protein degradation cannot be random but, rather, is influenced by some structural aspect of the protein that serves as a degradation signal, which is recognized and bound by an E3. The half-life of a protein is also influenced by the amino (N)-terminal residue, the so-called N-end rule, and ranges from minutes to hours. Destabilizing N-terminal amino acids include arginine and posttranslationally modified amino acids such as acetylated alanine. In contrast, serine is a stabilizing amino acid. Additionally, proteins rich in sequences containing proline, glutamate, serine, and threonine (called PEST sequences after the one-letter designations for these amino acids) are rapidly ubiquitinated and degraded and, therefore, have short half-lives.

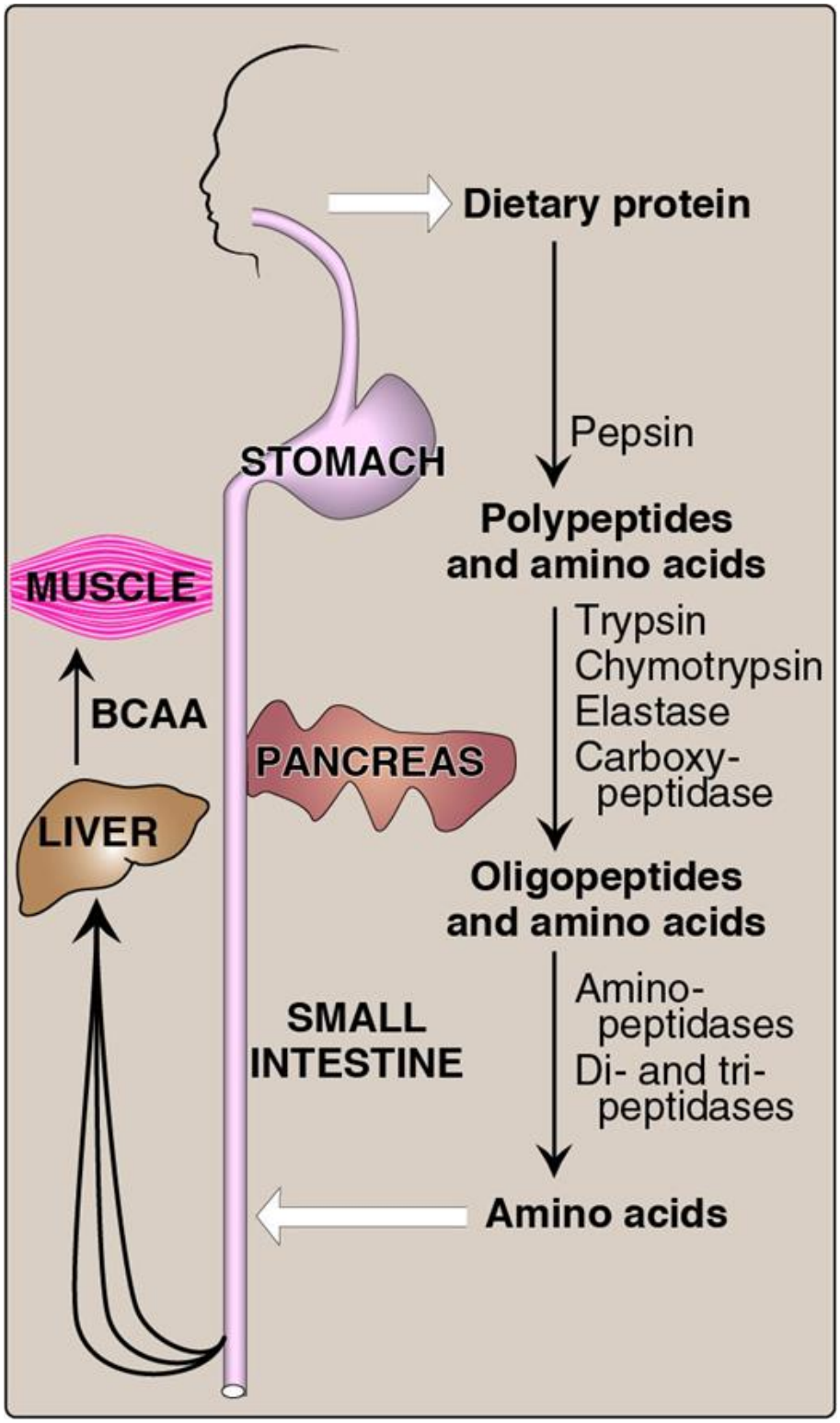
Dietary Protein Digestion

Most of the nitrogen in the diet is consumed in the form of protein, typically amounting to 70 to 100 g/day in the American diet (Fig. 19.2). Proteins are generally too large to be absorbed by the intestine. (Note: An example of an exception to this rule is that newborns can take up maternal antibodies in breast milk.) Therefore, proteins must be hydrolyzed to yield di- and tripeptides as well as individual amino acids, which can be absorbed. Proteolytic enzymes responsible for degrading proteins are produced by three different organs: the stomach, the pancreas, and the small intestine (Fig. 19.4).

FIGURE 19.4

Digestion of dietary proteins by the proteolytic enzymes of the gastrointestinal tract.

BCAA = branched chain amino acids.



Digestion by gastric secretion

The digestion of proteins begins in the stomach, which secretes gastric juice, a unique solution containing hydrochloric acid (HCl) and the proenzyme pepsinogen.

Hydrochloric acid

Stomach HCl is too dilute (pH 2 to 3) to hydrolyze proteins. The acid, secreted by the parietal cells of the stomach, functions instead to kill some bacteria and to denature proteins, thereby making them more susceptible to subsequent hydrolysis by proteases.

Pepsin

This acid-stable endopeptidase is secreted by the chief cells of the stomach as an inactive zymogen (or proenzyme), pepsinogen. (Note: In general, zymogens contain extra amino acids in their sequences that prevent them from being catalytically active. Removal of these amino acids permits the proper folding required for an active enzyme.) In the presence of HCl, pepsinogen undergoes a conformational change that allows it to cleave itself (autocatalysis) to the active form, pepsin, which releases polypeptides and a few free amino acids from dietary proteins.

Digestion by pancreatic enzymes

On entering the small intestine, the polypeptides produced in the stomach by the action of pepsin are further cleaved to oligopeptides and amino acids by a group of pancreatic proteases that include both endopeptidases (that cleave within) and exopeptidases (that cut at an end). (Note: Bicarbonate [HCO_3^-], secreted by the pancreas in response to the intestinal hormone secretin, raises the intestinal pH.)

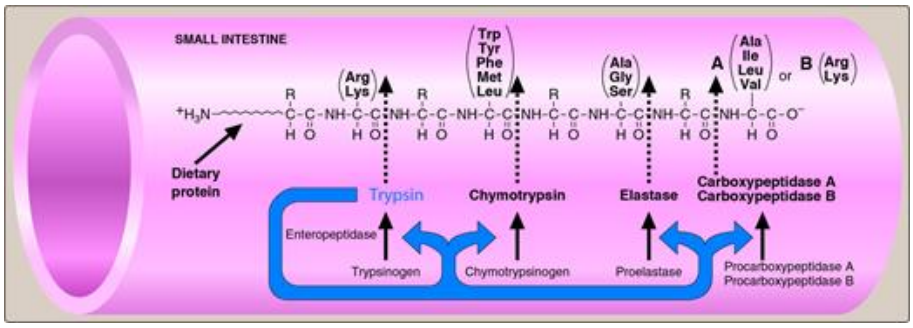
Specificity

Each of these enzymes has a different specificity for the amino acid R-groups adjacent to the susceptible peptide bond (Fig. 19.5). For example, trypsin cleaves only when the carbonyl group of the peptide bond is contributed by arginine or lysine. These enzymes, like pepsin described above, are synthesized and secreted as inactive zymogens.

FIGURE 19.5

Cleavage of dietary protein in the small intestine by pancreatic proteases.

The peptide bonds susceptible to hydrolysis are shown for each of the five major pancreatic proteases. (Note: The first three are serine endopeptidases, whereas the last two are exopeptidases. Each is produced from an inactive zymogen.)



Zymogen release

The release and activation of the pancreatic zymogens are mediated by the secretion of cholecystokinin, a polypeptide hormone of the small intestine (see p. 194).

Zymogen activation

Enteropeptidase (also called enterokinase), a serine protease synthesized by and present on the luminal (apical) surface of intestinal mucosal cells (enterocytes) of the brush border, converts the pancreatic zymogen trypsinogen to trypsin by removal of a hexapeptide from the N-terminus of trypsinogen. Trypsin subsequently converts other trypsinogen molecules to trypsin by cleaving a limited number of specific peptide bonds in the zymogen. Thus, enteropeptidase unleashes a cascade of proteolytic activity because trypsin is the common activator of all the pancreatic zymogens (Fig. 19.5).

Digestion abnormalities

In individuals with a deficiency in pancreatic secretion (e.g., because of chronic pancreatitis, cystic fibrosis, or surgical removal of the pancreas), the digestion and absorption of fat and protein are incomplete. This results in the abnormal appearance of lipids in the feces (a condition called steatorrhea; see p. 196) as well as undigested protein.

Celiac disease (celiac sprue) is a disease of malabsorption resulting from immune-mediated damage to the small intestine in response to ingestion of gluten (or gliadin produced from gluten), a protein found in wheat, barley, and rye.

Digestion of oligopeptides by small intestine enzymes

The luminal surface of the enterocytes contains aminopeptidase, an exopeptidase that repeatedly cleaves the N-terminal residue from oligopeptides to produce even smaller peptides and free amino acids.

Amino acid and small peptide intestinal absorption

Most free amino acids are taken into enterocytes via sodium-dependent secondary active transport by solute carrier (SLC) proteins of the apical membrane. At least seven different transport systems with overlapping amino acid specificities are known. Di- and tripeptides, however, are taken up by a proton-linked peptide transporter (PepT1). The peptides are then hydrolyzed to free amino acids. Regardless of their source, free amino acids are released from enterocytes into the portal system by sodium-independent transporters of the basolateral membrane. Therefore, only free amino acids are found in the portal vein after a meal containing protein. These amino acids are either metabolized by the liver or released into the general circulation. (Note: Branched-chain amino acids [BCAAs] are not metabolized by the liver but, instead, are sent from the liver to muscle via the blood [Fig. 19.4].)

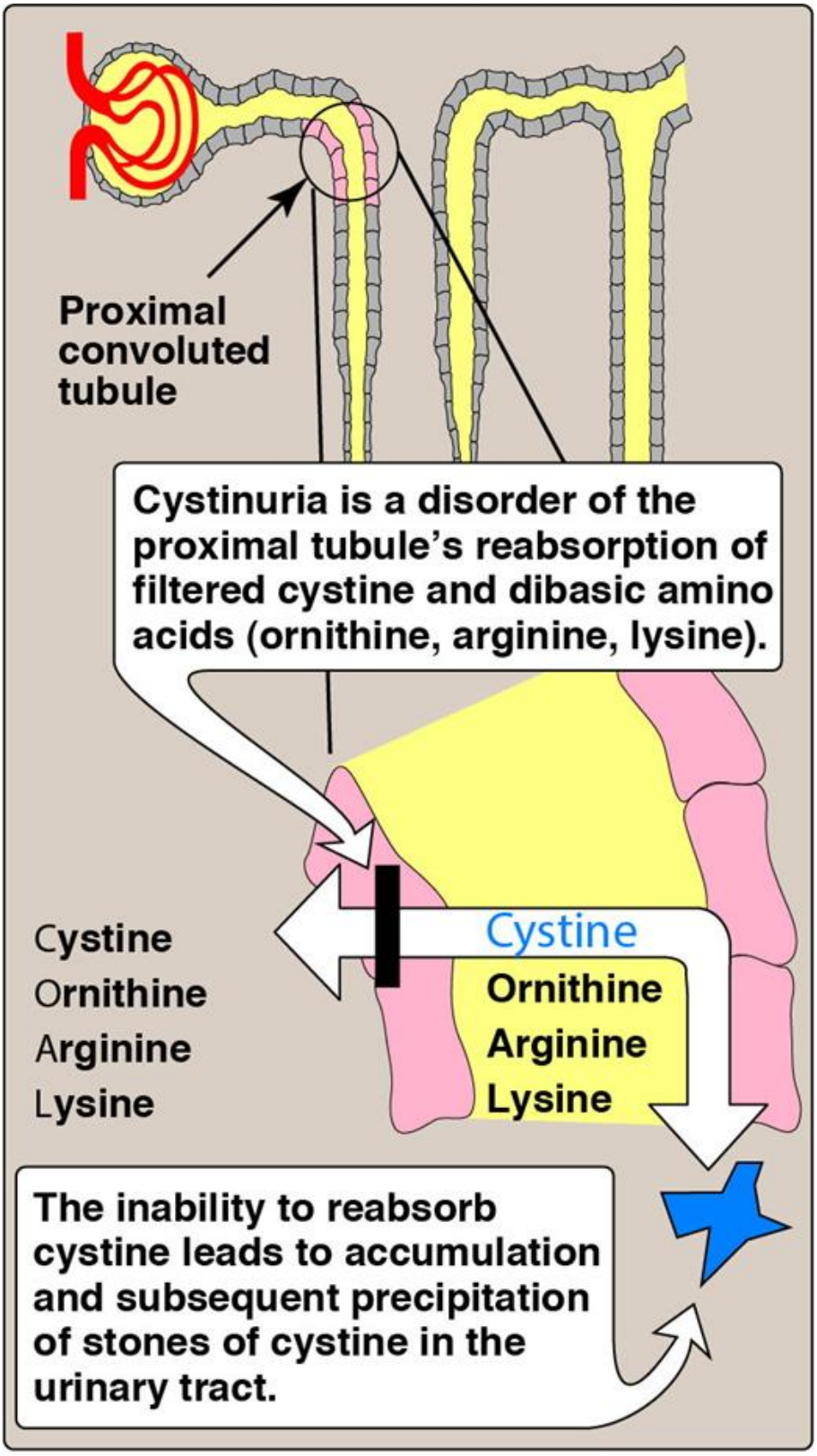
Absorption abnormalities

The small intestine and the proximal tubules of the kidneys have common transport systems for amino acid uptake. Consequently, a defect in any one of these systems results in an inability to absorb particular amino acids into the intestine and into the kidney tubules. For example, one system is responsible for the uptake of cystine and the dibasic amino acids ornithine, arginine, and lysine (represented as COAL). In the inherited disorder cystinuria, this carrier system is defective, and all four amino acids appear in the urine (Fig. 19.6). Cystinuria occurs at a frequency of 1 in 7,000 individuals, making it one of the most common inherited diseases and the most common genetic error of amino acid transport. The disease expresses itself clinically by the precipitation of cystine to form kidney stones (calculi), which can block the urinary tract. Oral hydration is an important part of treatment for this disorder. (Note: Defects in the uptake of tryptophan by a neutral amino acid transporter can result in Hartnup disorder and pellagra-like [see p. 430] dermatologic and neurologic symptoms.)

FIGURE 19.6

Genetic defect seen in cystinuria.

(Note: Cystinuria is distinct from cystinosis, a rare defect in the transport of cystine out of lysosomes that results in the formation of cystine crystals within the lysosome and widespread tissue damage.)



Nitrogen Removal from Amino Acids

The presence of the α-amino group keeps amino acids safely locked away from oxidative breakdown. Removing the α-amino group is essential for producing energy from any amino acid and is an obligatory step in the catabolism of all amino acids. Once removed, this nitrogen can be incorporated into other compounds or excreted as urea, with the carbon skeletons being metabolized. This section describes transamination and oxidative deamination, reactions that ultimately provide ammonia and aspartate, the two sources of urea nitrogen (see p. 279).

Transamination: funneling amino groups to form glutamate

The first step in the catabolism of most amino acids is the transfer of their α-amino group to α-ketoglutarate (Fig. 19.7), producing an α-keto acid (derived from the original amino acid) and glutamate (derived from α-ketoglutarate). The citric acid cycle ketoacid intermediate α-ketoglutarate plays a pivotal role in amino acid metabolism by accepting the amino groups from most amino acids, thereby becoming its structurally related amino acid, glutamate. Glutamate produced by transamination can be oxidatively deaminated (see B. below) or used as an amino group donor in the synthesis of nonessential amino acids. This transfer of amino groups from one carbon skeleton to another is catalyzed by a family of readily reversible enzymes called aminotransferases (also called transaminases). These enzymes are found in the cytosol and mitochondria of cells throughout the body. All amino acids, with the exception of lysine and threonine, participate in transamination at some point in their catabolism. (Note: These two amino acids lose their α-amino groups by deamination [see pp. 294 and 295].)

Substrate specificity

Each aminotransferase is specific for one or, at most, a few amino group donors. Aminotransferases are named after the specific amino group donor, because the acceptor of the amino group is almost always α-ketoglutarate. Two important aminotransferase reactions are catalyzed by alanine aminotransferase (ALT) and aspartate aminotransferase (AST), as shown in Figure 19.8. All aminotransferases require the coenzyme pyridoxal phosphate (a derivative of vitamin B_6; see p. 428), which is covalently linked to the ε-amino group of a specific lysine residue at the active site of the enzyme.

Alanine aminotransferase

ALT is present in many tissues. The enzyme catalyzes the transfer of the amino group of alanine to α-ketoglutarate, resulting in the formation of pyruvate and glutamate. The reaction is readily reversible. However, during amino acid catabolism, this enzyme (like most aminotransferases) functions in the direction of glutamate synthesis. (Note: In effect, glutamate acts as a collector of nitrogen from most amino acids.)

FIGURE 19.7

***Aminotransferase* reaction using α-ketoglutarate as the amino group acceptor.**

PLP = pyridoxal phosphate.

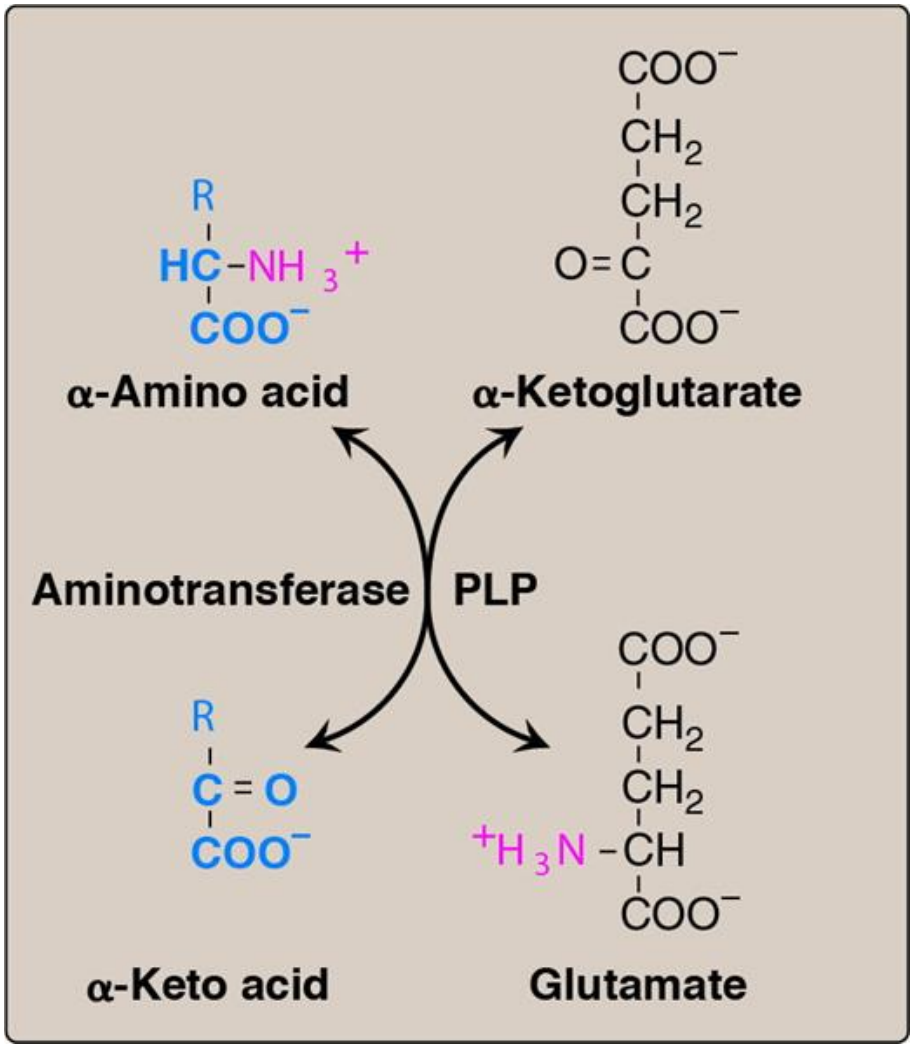
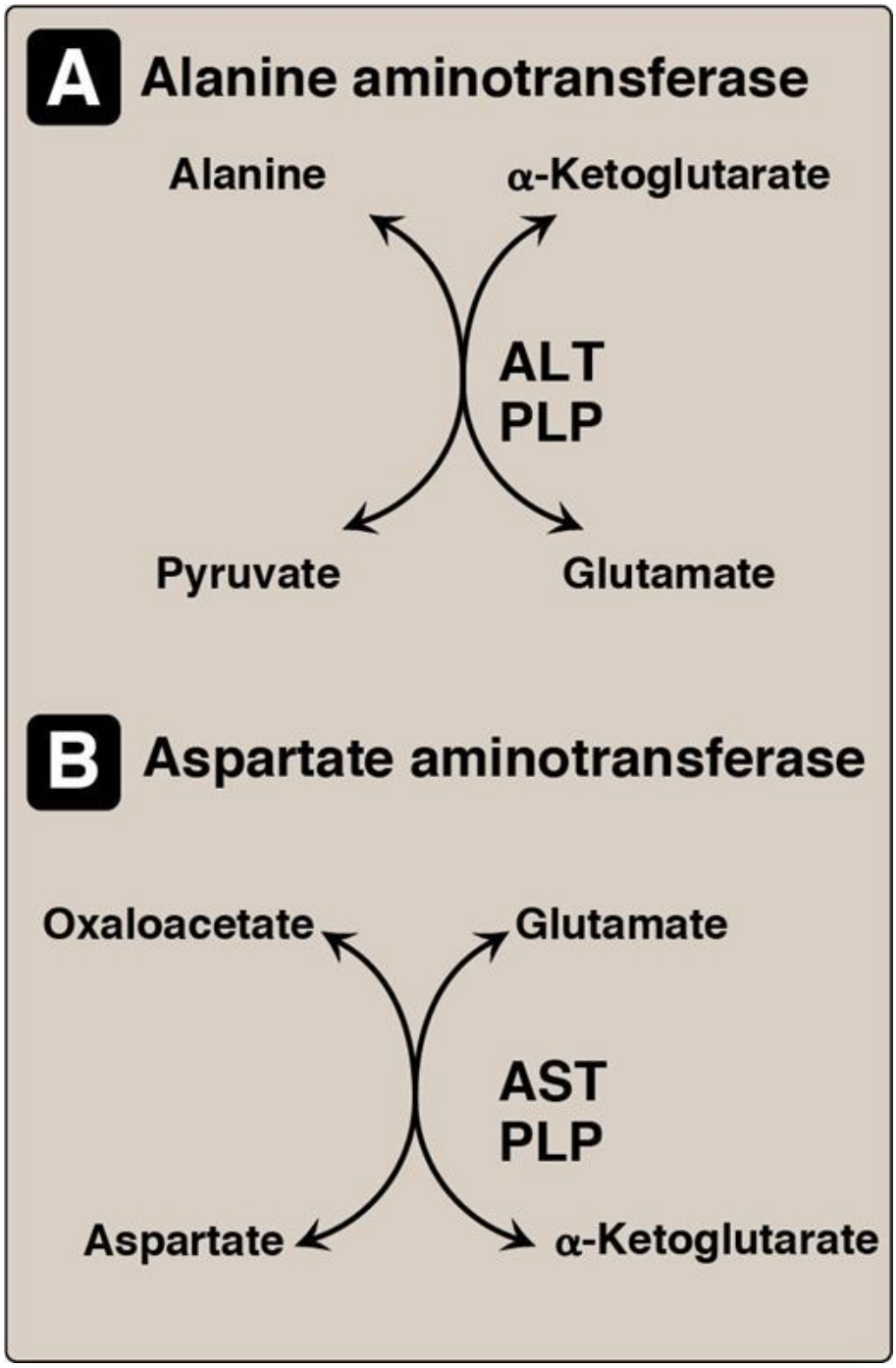


FIGURE 19.8

Reactions catalyzed during amino acid catabolism.

A: *Alanine aminotransferase* (*ALT*). **B:** *Aspartate aminotransferase* (*AST*). PLP = pyridoxal phosphate.



Aspartate aminotransferase

AST is an exception to the rule that aminotransferases funnel amino groups to form glutamate. During amino acid catabolism, AST primarily transfers amino groups from glutamate to oxaloacetate, forming α-ketoglutarate and aspartate, respectively. Aspartate is used as a source of nitrogen in the urea cycle (see p. 281). Like other transaminations, the AST reaction is reversible.

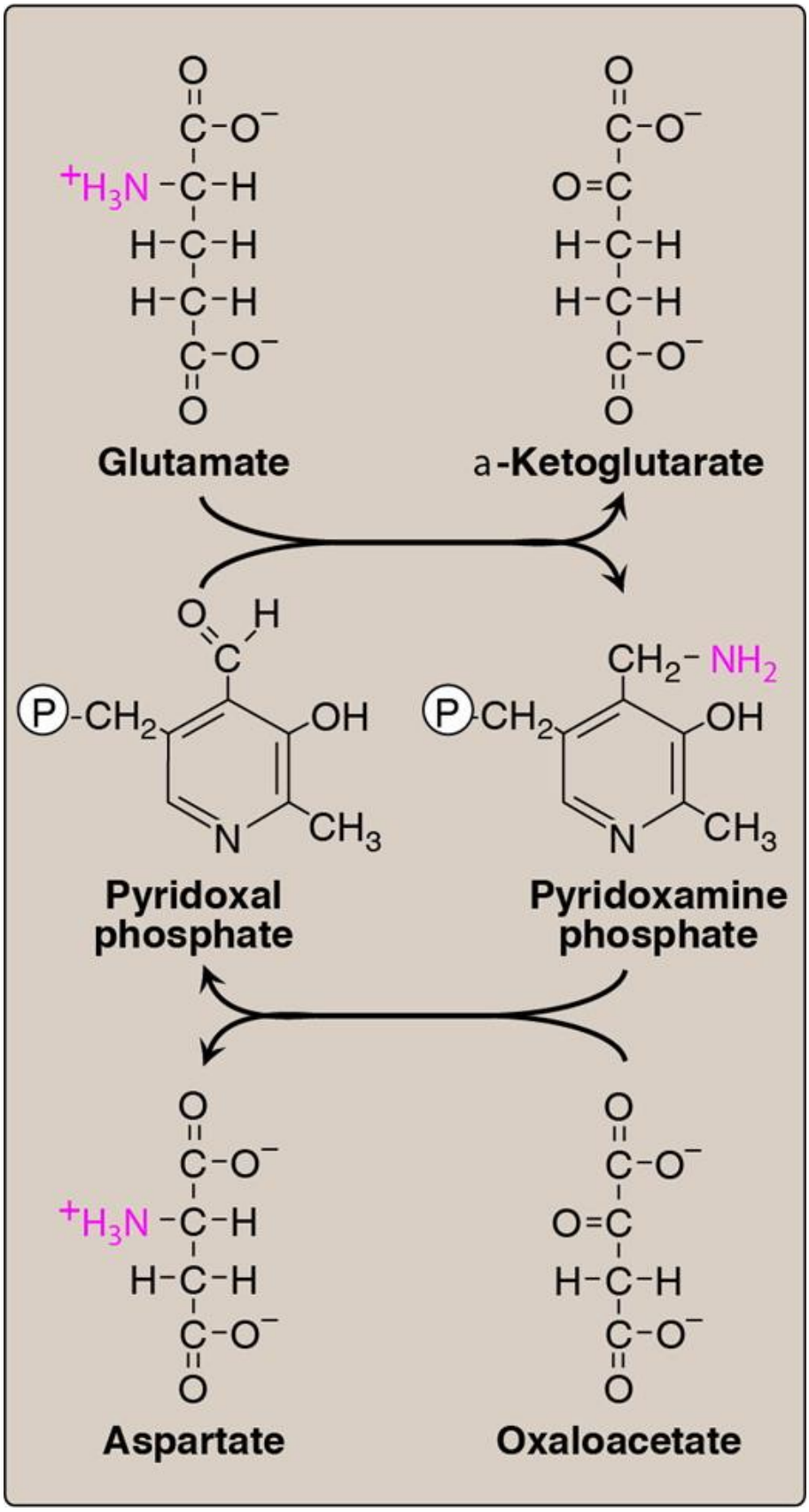
Mechanism

Figure 19.9 shows the mechanistic reactions for the transamination catalyzed by AST. Aminotransferases act by transferring the amino group of an amino acid substrate (glutamate) to the pyridoxal part of the coenzyme to generate pyridoxamine phosphate. The amino acid substrate glutamate is thus converted to an α-keto acid product (α-ketoglutarate). The pyridoxamine form of the coenzyme then reacts with an α-keto acid substrate (oxaloacetate) to form an amino acid product (aspartate), at the same time regenerating the original aldehyde form of the coenzyme.

FIGURE 19.9

Cyclic interconversion of pyridoxal phosphate and pyridoxamine phosphate during the *aspartate aminotransferase* reaction.

Ⓟ = phosphate group.



Equilibrium

For most transamination reactions, the equilibrium constant is near 1. This allows the reaction to function in both amino acid degradation through removal of α-amino groups (e.g., after consumption of a protein-rich meal) and biosynthesis of nonessential amino acids through addition of amino groups to the carbon skeletons of α-keto acids (e.g., when the supply of amino acids from the diet is not adequate to meet the synthetic needs of cells).

Diagnostic value

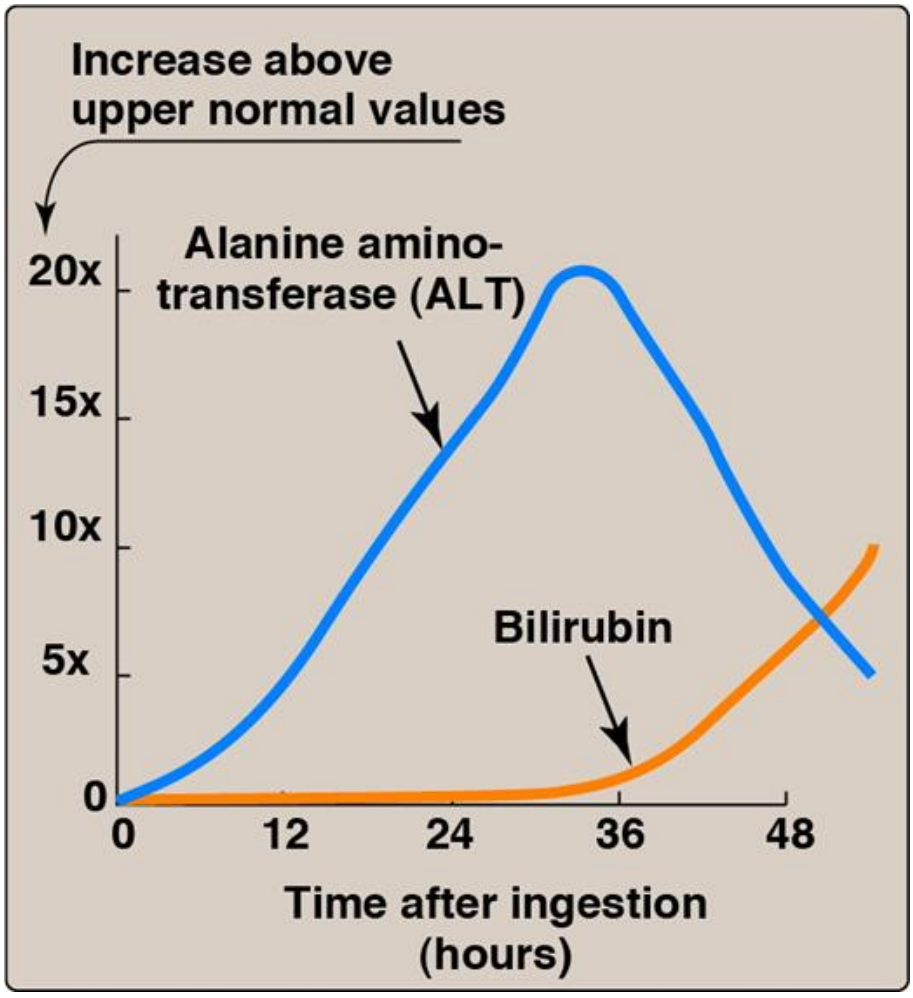
Aminotransferases are normally intracellular enzymes, with the low levels found in the plasma representing the release of cellular contents during normal cell turnover. Elevated plasma levels of aminotransferases indicate damage to cells rich in these enzymes. For example, physical trauma or a disease process can cause cell lysis, resulting in release of intracellular enzymes into the blood. Two aminotransferases, AST and ALT, are of particular diagnostic value when they are found in the plasma.

Hepatic disease

Plasma AST and ALT are elevated in nearly all hepatic diseases but are particularly high in conditions that cause extensive cell necrosis, such as severe viral hepatitis, toxic injury, and prolonged circulatory collapse. ALT is more specific than AST for liver disease, but the latter is more sensitive because the liver contains larger amounts of AST. Serial measurements of AST and ALT (liver function tests) are often useful in determining the course of liver damage. Figure 19.10 shows the early release of ALT into the blood, following ingestion of a liver toxin. (Note: The elevation in bilirubin results from hepatocellular damage that decreases the hepatic conjugation and excretion of bilirubin [see p. 316].)

FIGURE 19.10

Pattern of *ALT* and bilirubin in the plasma, following poisoning by ingestion of the toxic mushroom *Amanita phalloides*.



Nonhepatic disease

Aminotransferases may be elevated in nonhepatic diseases such as those that cause damage to cardiac or skeletal muscle. However, these disorders can usually be distinguished clinically from liver disease using additional lab tests. When muscle damage is suspected, creatine kinase, lactate dehydrogenase, and myoglobin plasma levels, in addition to AST and ALT levels, may also be increased. Blood urea nitrogen, bilirubin, γ-glutamyl transferase (GGT) and alkaline phosphatase (ALP) levels would be in the normal range. If bone disease is suspected, ALP levels will be disproportionately higher than the AST, ALT, and GGT levels.

Oxidative deamination: amino group removal

In contrast to transamination reactions that transfer amino groups, oxidative deamination reactions result in the liberation of the amino group as free ammonia (Fig. 19.11). These reactions occur primarily in the liver and kidney. They provide α-keto acids that can enter the central pathways of energy metabolism and ammonia, which is a source of nitrogen in hepatic urea synthesis. (Note: Ammonia exists primarily as ammonium [NH_4^+] in aqueous solution, but it is the unionized form [NH_3] that crosses membranes.)

Glutamate dehydrogenase

As described above, the amino groups of most amino acids are ultimately funneled to glutamate by means of transamination with α-ketoglutarate. Glutamate is unique in that it is the only amino acid that undergoes rapid oxidative deamination, a reaction catalyzed by glutamate dehydrogenase ([GDH], Fig. 19.11). Therefore, the sequential action of transamination (resulting in the transfer of amino groups from most amino acids to α-ketoglutarate to produce glutamate) and the oxidative deamination of that glutamate (regenerating α-ketoglutarate) provide a pathway whereby the amino groups of most amino acids can be released as ammonia.

Coenzymes

GDH, a mitochondrial enzyme, is unusual in that it can use either nicotinamide adenine dinucleotide (NAD^+) or its phosphorylated reduced form (NADPH) as a coenzyme (Fig. 19.11). NAD^+ is used primarily in oxidative deamination (the simultaneous loss of ammonia coupled with the oxidation of the carbon skeleton, as shown in Fig. 19.12A), whereas NADPH is used in reductive amination (the simultaneous gain of ammonia coupled with the reduction of the carbon skeleton, as shown in Fig. 19.12B).

FIGURE 19.11

Oxidative deamination by *glutamate dehydrogenase*.

(Note: The enzyme is unusual in that it uses both nicotinamide adenine dinucleotide [NAD^+] and nicotinamide adenine dinucleotide phosphate [NADPH].) NH_3 = ammonia.

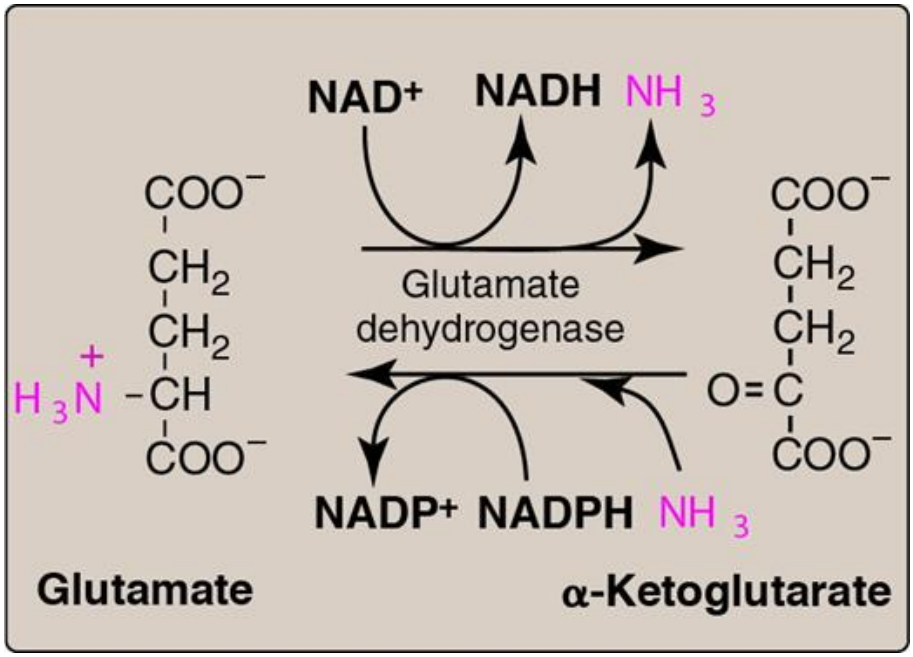
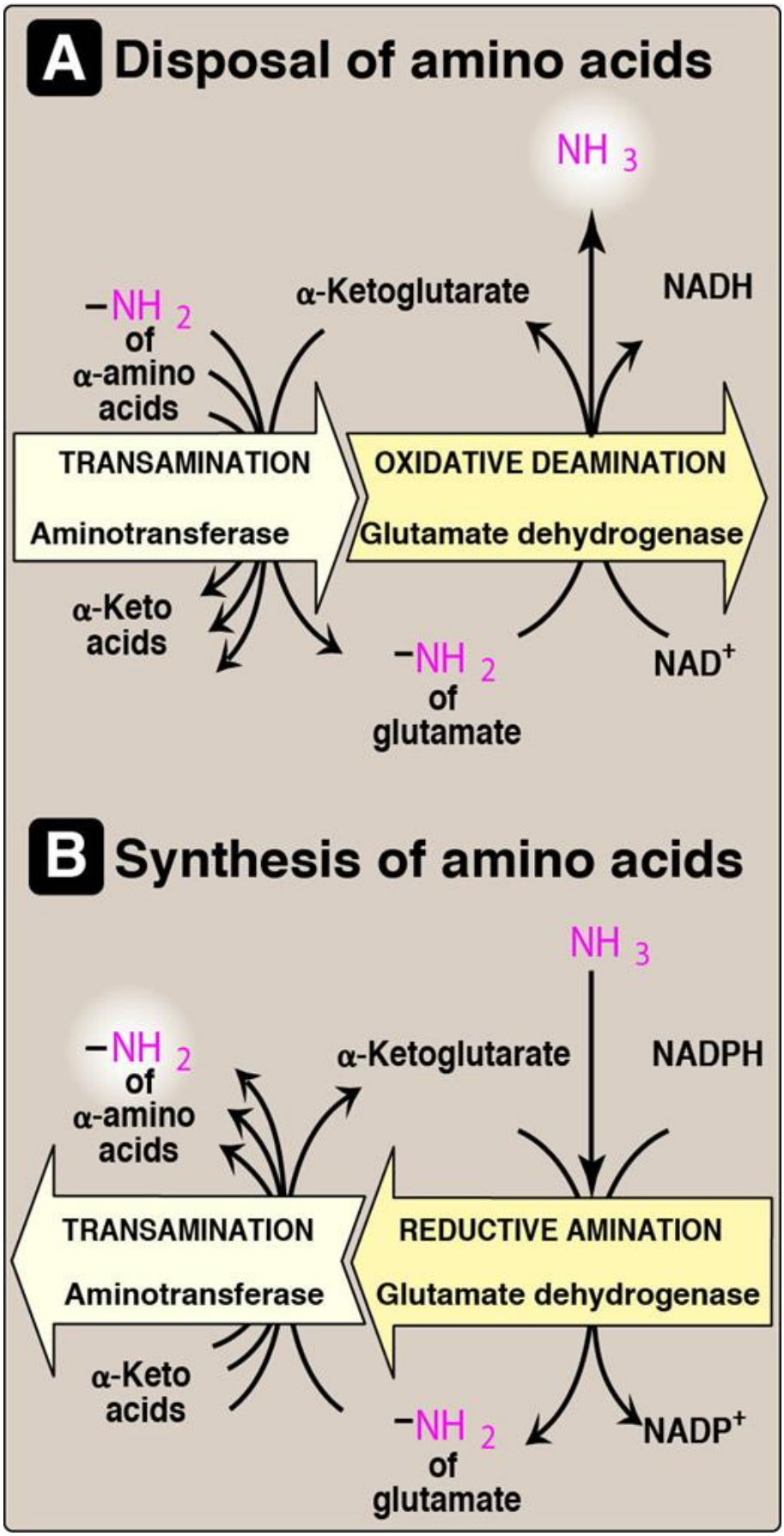


FIGURE 19.12

A, B: Combined actions of *aminotransferase* and *glutamate dehydrogenase* reactions. (Note: Reductive amination occurs only when ammonia [NH_3] level is high.) NAD(H) = nicotinamide adenine dinucleotide; NADP(H) = nicotinamide adenine dinucleotide phosphate.



Reaction direction

The direction of the reaction depends on the relative concentrations of glutamate, α-ketoglutarate, and ammonia and the ratio of oxidized to reduced coenzymes. For example, after ingestion of a meal containing protein, glutamate levels in the liver are elevated, and the reaction proceeds in the direction of amino acid degradation and the formation of ammonia (Fig. 19.12A). High ammonia levels are required to drive the reaction to glutamate synthesis.

Allosteric regulators

Guanosine triphosphate is an allosteric inhibitor of GDH, whereas adenosine diphosphate is an activator. Therefore, when energy levels are low in the cell, amino acid degradation by GDH is high, facilitating energy production from the carbon skeletons derived from amino acids.

D-Amino acid oxidase

D-Amino acids (see p. 5) are supplied by the diet but are not used in the synthesis of mammalian proteins. They are, however, efficiently metabolized to α-keto acids, ammonia, and hydrogen peroxide in the peroxisomes of liver and kidney cells by flavin adenine dinucleotide–dependent D-amino acid oxidase (DAO). The α-keto acids can enter the general pathways of amino acid metabolism and be reaminated to L-isomers or catabolized for energy. (Note: DAO degrades D-serine, the isomeric form of serine that modulates N-methyl-D-aspartate [NMDA]-type glutamate receptors. Increased DAO activity has been linked to increased susceptibility to schizophrenia. DAO also converts glycine to glyoxylate [see p. 292].) L-Amino acid oxidases are found in snake venom.

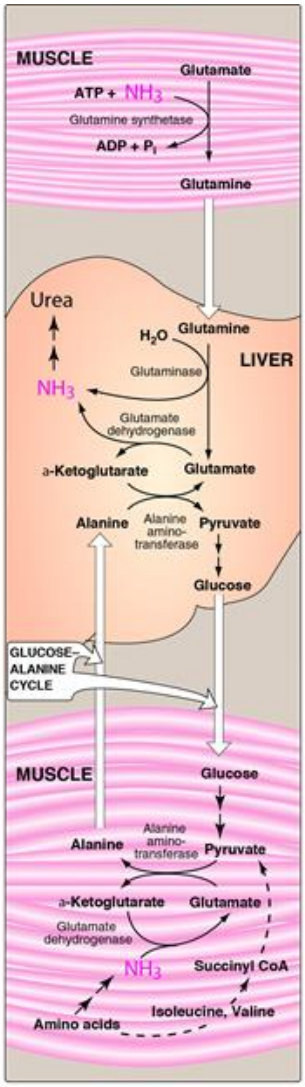
Ammonia transport to the liver

Two mechanisms are available in humans for the transport of ammonia from peripheral tissues to the liver for conversion to urea. Both are important in, but not exclusive to, skeletal muscle. The first uses glutamine synthetase to combine ammonia with glutamate to form glutamine, a nontoxic transport form of ammonia (Fig. 19.13). The glutamine is transported in the blood to the liver where it is cleaved by glutaminase to glutamate and ammonia (see p. 283). The glutamate is oxidatively deaminated to ammonia and α-ketoglutarate by GDH. The ammonia is converted to urea. The second transport mechanism involves the formation of alanine by the transamination of pyruvate produced from both aerobic glycolysis and metabolism of the succinyl coenzyme A (CoA) generated by the catabolism of the BCAA isoleucine and valine. Alanine is transported in the blood to the liver, where it is transaminated by ALT to pyruvate. The pyruvate is used to synthesize glucose, which can enter the blood and be used by muscle, a pathway called the glucose–alanine cycle. The glutamate product of ALT can be deaminated by GDH, generating ammonia. Thus, both alanine and glutamine carry ammonia to the liver.

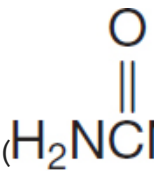
FIGURE 19.13

Transport of ammonia (NH_3) from muscle to the liver.

ADP = adenosine diphosphate; P_i = inorganic phosphate; CoA = coenzyme A.



Urea Cycle



Urea (H_2NCNH_2) is the major disposal form of amino groups derived from amino acids and accounts for ~90% of the nitrogen-containing components of urine. One nitrogen of the urea molecule is supplied by free ammonia and the other nitrogen by aspartate. (Note: Glutamate is the immediate precursor of both ammonia [through oxidative deamination by GDH] and aspartate nitrogen [through transamination of oxaloacetate by AST].) The carbon and oxygen of urea are derived from CO_2 (as HCO_3^-). Urea is produced by the liver and then is transported in the blood (blood urea nitrogen) to the kidneys for excretion in the urine.

Reactions

The first two reactions leading to the synthesis of urea occur in the mitochondrial matrix, whereas the remaining cycle enzymes are located in the cytosol (Fig. 19.14). (Note: Gluconeogenesis [see p. 128] and heme synthesis [see p. 309] also involve both the mitochondrial matrix and the cytosol.)

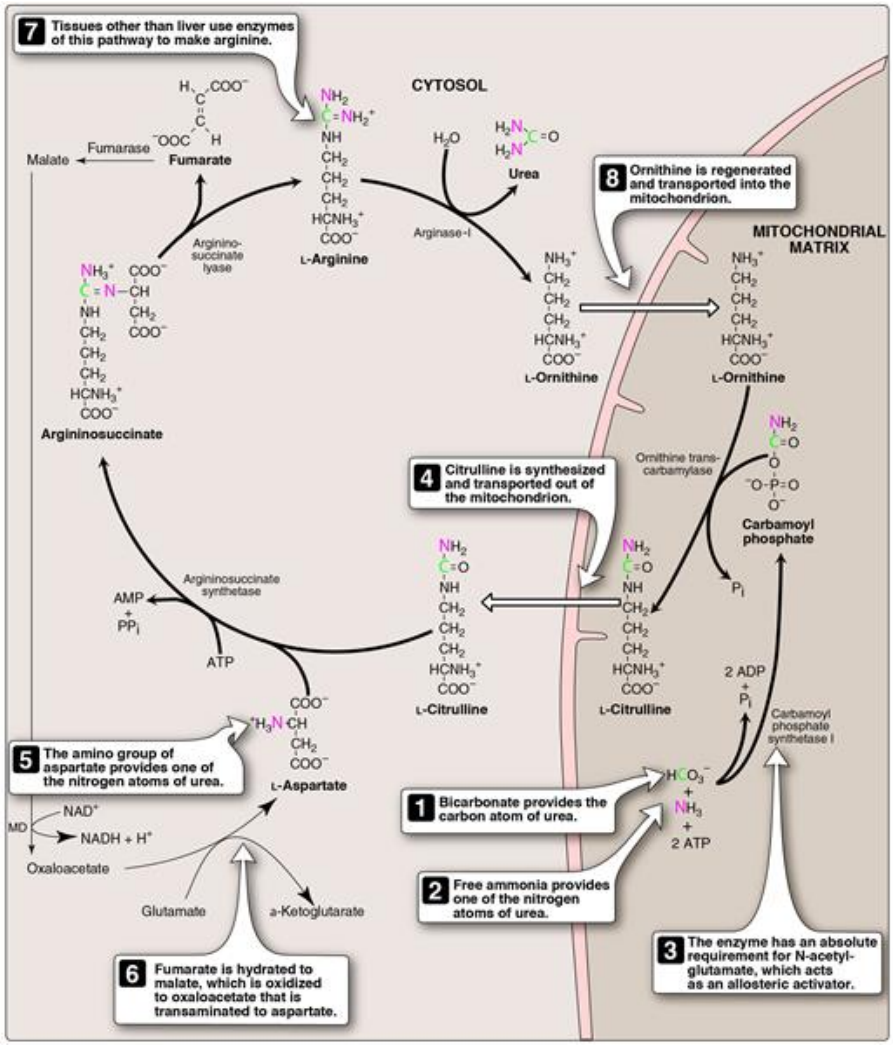
Carbamoyl phosphate formation

Formation of carbamoyl phosphate by carbamoyl phosphate synthetase I (CPS I) is driven by cleavage of two molecules of ATP. Ammonia incorporated into carbamoyl phosphate is provided primarily by the oxidative deamination of glutamate by mitochondrial GDH (Fig. 19.11). Ultimately, the nitrogen atom derived from this ammonia becomes one of the nitrogens of urea. CPS I requires N-acetylglutamate (NAG) as a positive allosteric activator (Fig. 19.14). (Note: Carbamoyl phosphate synthetase II participates in the biosynthesis of pyrimidines [see p. 335]. It does not require NAG, uses glutamine as the nitrogen source, and occurs in the cytosol.)

FIGURE 19.14

Reactions of the urea cycle.

(Note: An antiporter transports citrulline and ornithine across the inner mitochondrial membrane.) ADP = adenosine diphosphate; AMP = adenosine monophosphate; PP_i = pyrophosphate; P_i = inorganic phosphate; NAD(H) = nicotinamide adenine dinucleotide; *MD = malate dehydrogenase*.



Citrulline formation

The carbamoyl portion of carbamoyl phosphate is transferred to ornithine by ornithine transcarbamylase (OTC) as the phosphate is released as inorganic phosphate. The reaction product, citrulline, is transported to the cytosol. (Note: Ornithine and citrulline move across the inner mitochondrial membrane via an antiporter. These basic amino acids are not incorporated into cellular proteins because there are no codons for them [see p. 496].) Ornithine is regenerated with each turn of the urea cycle, much in the same way that oxaloacetate is regenerated by the reactions of the tricarboxylic acid (TCA) cycle (see p. 120).

Argininosuccinate formation

Argininosuccinate synthetase combines citrulline with aspartate to form argininosuccinate. The α-amino group of aspartate provides the second nitrogen that is ultimately incorporated into urea. The formation of argininosuccinate is driven by the cleavage of ATP to adenosine monophosphate and pyrophosphate. This is the third and final molecule of ATP consumed in the formation of urea.

Argininosuccinate cleavage

Argininosuccinate is cleaved by argininosuccinate lyase to yield arginine and fumarate. The arginine serves as the immediate precursor of urea. The fumarate is hydrated to malate, providing a link with several metabolic pathways. Malate can be oxidized by malate dehydrogenase to oxaloacetate, which can be transaminated to aspartate (Fig. 19.8) and enter the urea cycle (Fig. 19.14). Alternatively, malate can be transported into mitochondria via the malate–aspartate shuttle (see p. 87), reenter the TCA cycle, and get oxidized to oxaloacetate, which can be used for gluconeogenesis (see p. 131). (Note: Malate oxidation generates NADH for oxidative phosphorylation [see p. 84], thereby reducing the energy cost of the urea cycle.)

Arginine cleavage to ornithine and urea

Arginase-I hydrolyzes arginine to ornithine and urea and is virtually exclusive to the liver. Therefore, only the liver can cleave arginine, thereby synthesizing urea, whereas other tissues, such as the kidney, can synthesize arginine from citrulline. (Note: Arginase-II in kidneys controls arginine availability for nitric oxide synthesis [see p. 165].)

Fate of urea

Urea diffuses from the liver and is transported in the blood to the kidneys, where it is filtered and excreted in the urine (Fig. 19.19). A portion of the urea diffuses from the blood into the intestine and is cleaved to CO_2 and ammonia by bacterial urease. The ammonia is partly lost in the feces and is partly reabsorbed into the blood. In patients with kidney failure, plasma urea levels are elevated, promoting a greater transfer of urea from blood into the gut. The intestinal action of urease on this urea becomes a clinically important source of ammonia, contributing to the hyperammonemia often seen in these patients. Oral administration of antibiotics reduces the number of intestinal bacteria responsible for this ammonia production.

Overall stoichiometry

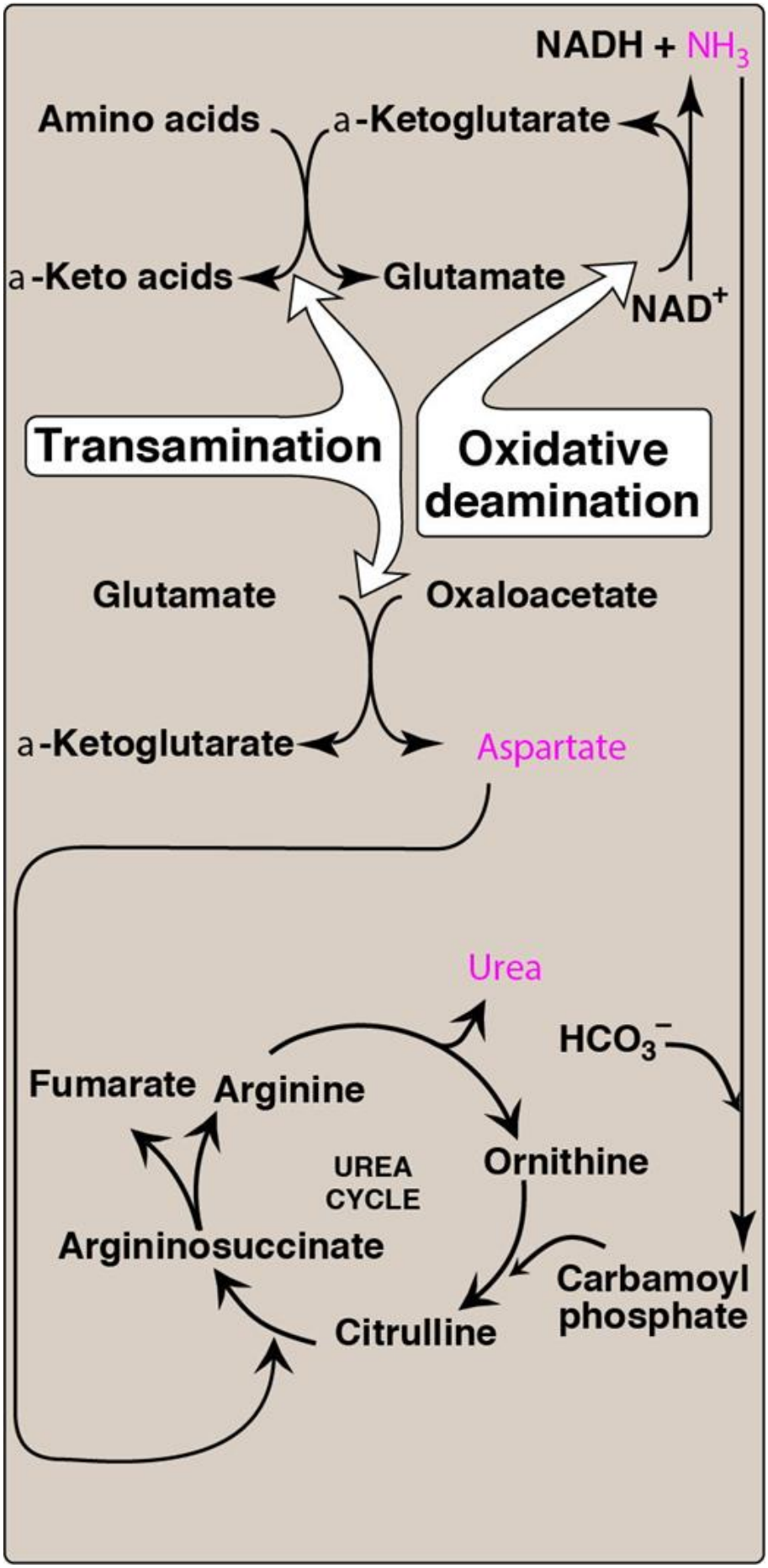
$$\text{Aspartate} + NH_3 + HCO_3^- + 3ATP + H_2O \rightarrow \text{urea} + \text{fumarate} + 2ADP + AMP + 2P_i + PP_i$$

Because four high-energy phosphate bonds are consumed in the synthesis of each molecule of urea, the synthesis of urea is irreversible, with a large, negative ΔG (see p. 78). One nitrogen of the urea molecule is supplied by free ammonia and the other nitrogen by aspartate. Glutamate is the immediate precursor of both ammonia (through oxidative deamination by GDH) and aspartate nitrogen (through transamination of oxaloacetate by AST). In effect, both nitrogen atoms of urea arise from glutamate, which, in turn, gathers nitrogen from other amino acids (Fig. 19.15).

FIGURE 19.15

Flow of nitrogen from amino acids to urea.

Amino groups for urea synthesis are collected in the form of ammonia (NH_3) and aspartate. NAD(H) = nicotinamide adenine dinucleotide; HCO_3^- = bicarbonate.


NADH + NH_3
Amino acids
a-Ketoglutarate
a-Keto acids
Glutamate
NAD^+
Transamination
Oxidative deamination
Glutamate
Oxaloacetate
a-Ketoglutarate
Aspartate
Urea
HCO_3^-
Fumarate
Arginine
Ornithine
UREA CYCLE
Argininosuccinate
Carbamoyl phosphate
Citrulline

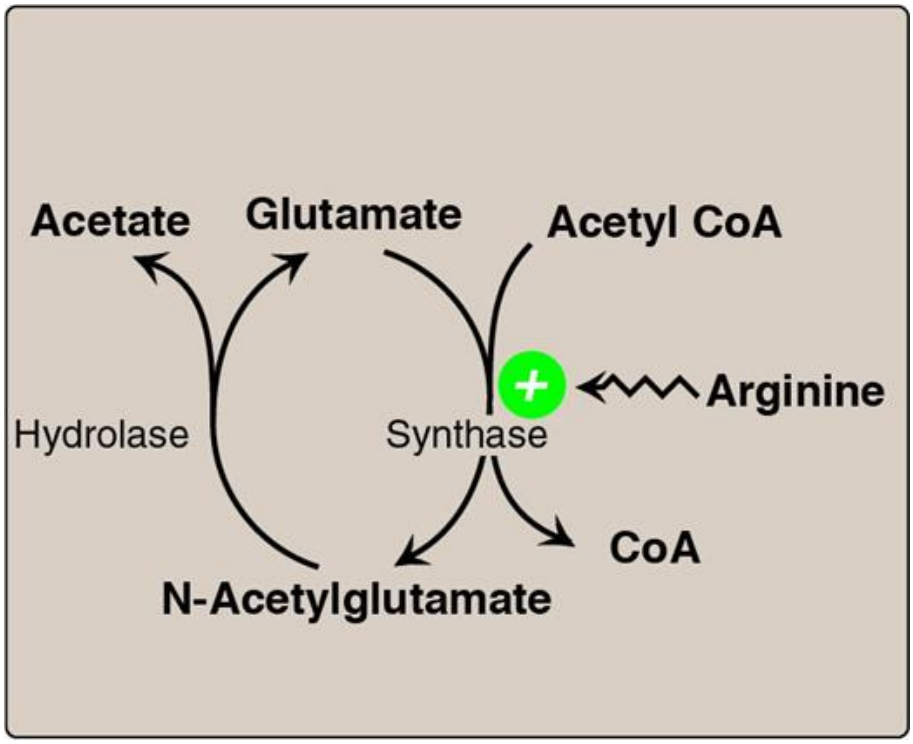
Regulation

NAG is an essential activator for CPS I, the rate-limiting step in the urea cycle. It increases the affinity of CPS I for ATP. NAG is synthesized from acetyl CoA and glutamate by N-acetylglutamate synthase (NAGS), as shown in Figure 19.16, in a reaction for which arginine is an activator. The cycle is also regulated by substrate availability (short-term regulation) and enzyme induction (long term).

FIGURE 19.16

Formation and degradation of N-acetylglutamate, an allosteric activator of *carbamoyl phosphate synthetase I*.

CoA = coenzyme A.



Ammonia Metabolism

Ammonia is produced by all tissues during the metabolism of a variety of compounds, and it is disposed of primarily by formation of urea in the liver. However, the blood ammonia level must be kept very low, because even slightly elevated concentrations (hyperammonemia) are toxic to the central nervous system (CNS). Therefore, a mechanism is required for the transport of nitrogen from the peripheral tissues to the liver for ultimate disposal as urea while keeping circulating levels of free ammonia low.

Sources

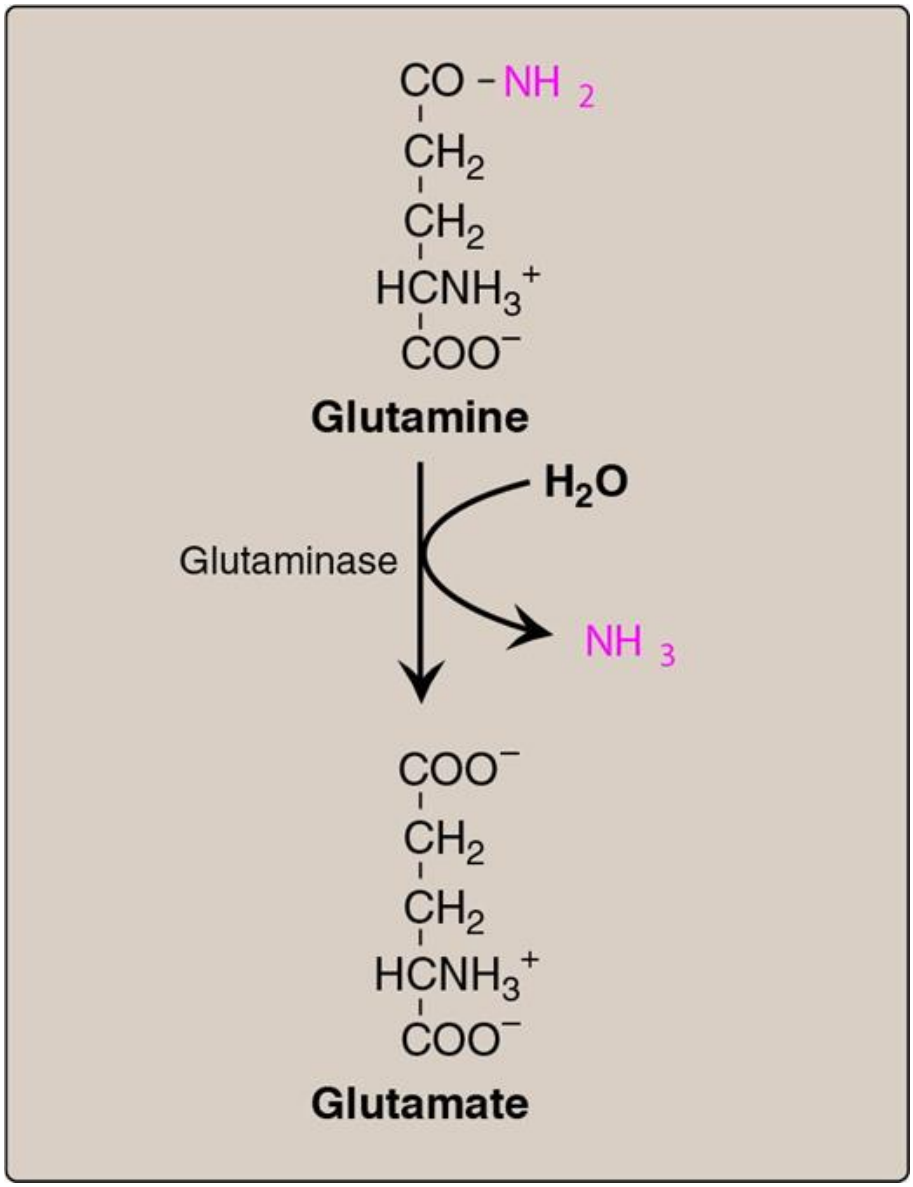
Amino acids are quantitatively the most important source of ammonia because most Western diets are high in protein and provide excess amino acids, which travel to the liver and undergo transdeamination (i.e., the linking of the aminotransferase and GDH reactions), producing ammonia. (Note: The liver catabolizes straight-chain amino acids, primarily.) However, substantial amounts of ammonia can be obtained from other sources.

Glutamine

An important source of plasma glutamine is from the catabolism of BCAA in skeletal muscle. This glutamine is taken up by cells of the intestine, the liver, and the kidneys. The liver and kidneys generate ammonia from glutamine by the actions of glutaminase (Fig. 19.17) and GDH. In the kidneys, most of this ammonia is excreted into the urine as NH_4^+, which provides an important mechanism for maintaining the body's acid–base balance through the excretion of protons. In the liver, the ammonia is detoxified to urea and excreted. (Note: α-Ketoglutarate, the second product of GDH, is a glucogenic precursor in the liver and kidneys.) Ammonia is also generated by intestinal glutaminase. Enterocytes obtain glutamine either from the blood or from digestion of dietary protein. (Note: Intestinal glutamine metabolism also produces alanine, which is used by the liver for gluconeogenesis, and citrulline, which is used by the kidneys to synthesize arginine.)

FIGURE 19.17

Hydrolysis of glutamine to form ammonia (NH_3).



Intestinal bacteria

Ammonia is formed from urea by the action of bacterial urease in the lumen of the intestine. This ammonia is absorbed from the intestine by way of the portal vein, and virtually all is removed by the liver via conversion to urea.

Amines

Amines obtained from the diet and monoamines that serve as hormones or neurotransmitters give rise to ammonia by the action of monoamine oxidase (see p. 318).

Purines and pyrimidines

In the catabolism of purines and pyrimidines, amino groups attached to the ring atoms are released as ammonia (see Fig. 22.15, p. 333).

Transport in the circulation

Although ammonia is constantly produced in the tissues, it is present at very low levels in blood. This is due both to the rapid removal of blood ammonia by the liver and to the fact that several tissues, particularly muscle, release amino acid nitrogen in the form of glutamine and alanine, rather than as free ammonia (Fig. 19.13).

Urea

Formation of urea in the liver is quantitatively the most important disposal route for ammonia. Urea travels in the blood from the liver to the kidneys, where it passes into the glomerular filtrate.

Glutamine

This amide of glutamate provides a nontoxic storage and transport form of ammonia (Fig. 19.18). The ATP-requiring formation of glutamine from glutamate and ammonia by glutamine synthetase occurs primarily in skeletal muscle and the liver but is also important in the CNS, where it is the major mechanism for the removal of ammonia in the brain. Glutamine is found in plasma at concentrations higher than other amino acids, a finding consistent with its transport function. (Note: The liver keeps blood ammonia levels low through glutaminase, GDH, and the urea cycle in periportal [close to inflow of blood] hepatocytes and through glutamine synthetase as an ammonia scavenger in the perivenous hepatocytes.) Ammonia metabolism is summarized in Figure 19.19.

FIGURE 19.18

Synthesis of glutamine.

ADP = adenosine diphosphate; P_i = inorganic phosphate; NH_3 = ammonia.

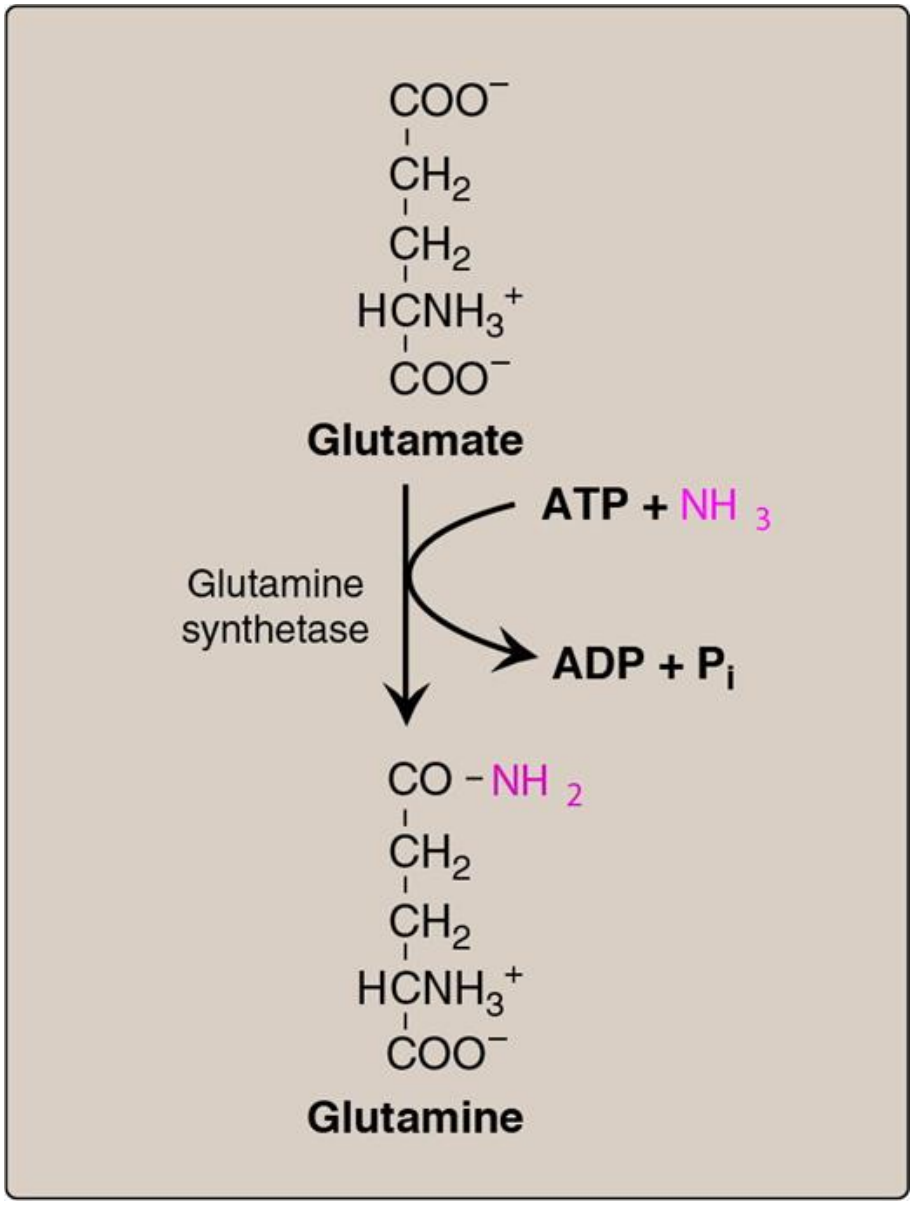
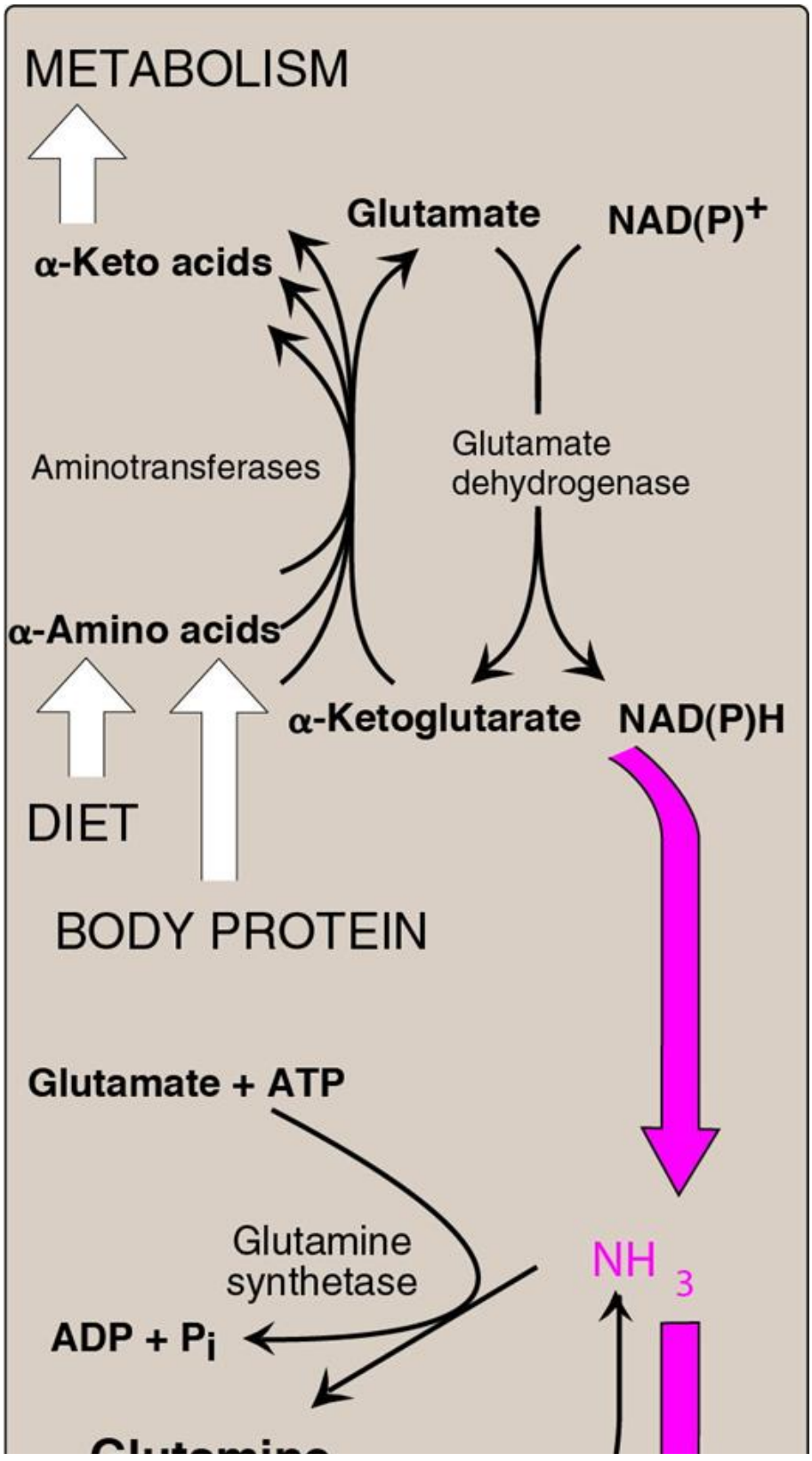
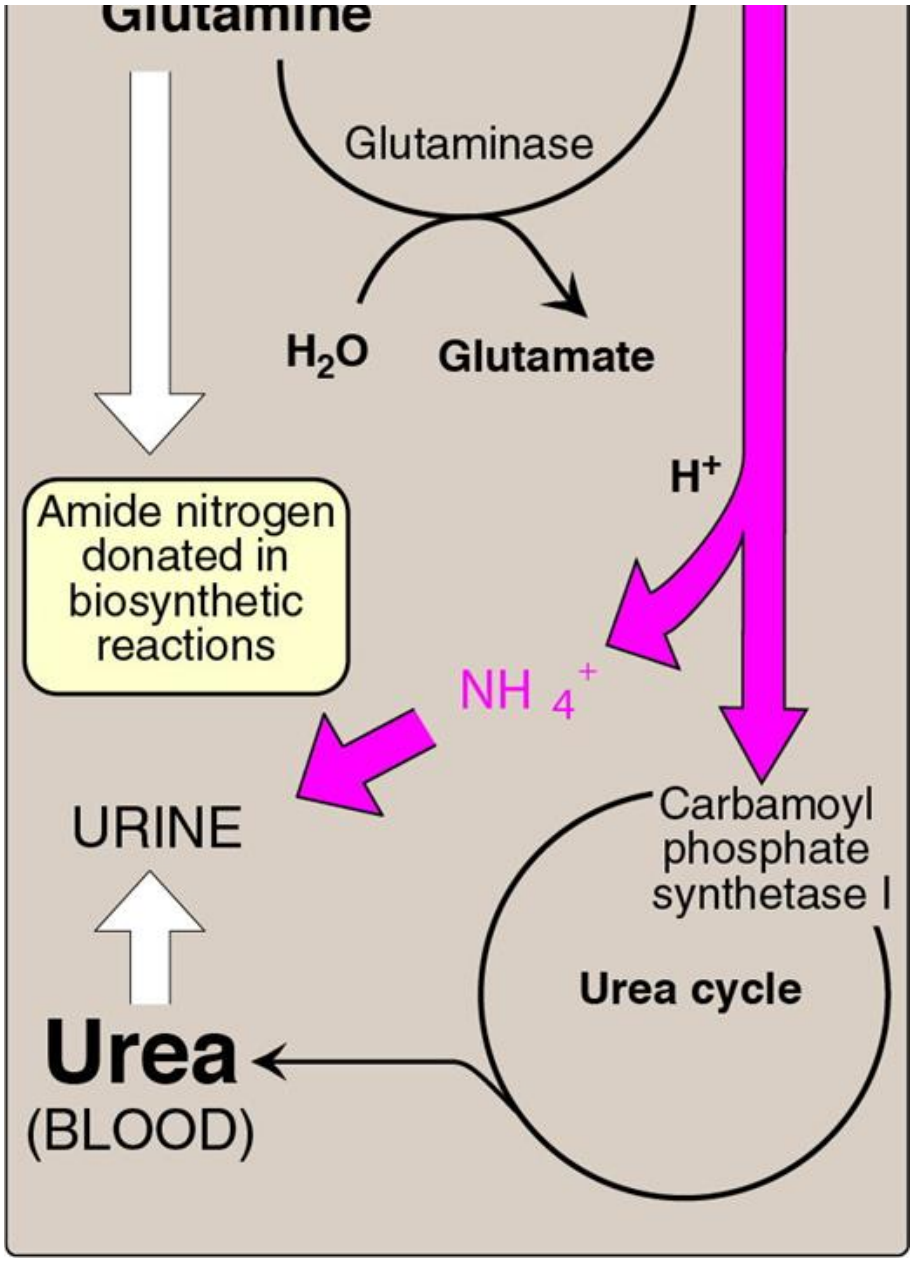


FIGURE 19.19

Ammonia (NH_3) metabolism.

Urea content in the urine is reported as urinary urea nitrogen, or UUN. Urea in blood is reported as BUN (blood urea nitrogen). (Note: The enzymes *glutamate dehydrogenase*, *glutamine synthetase*, and *carbamoyl phosphate synthetase I* fix NH_3 into organic molecules.)





Hyperammonemia

The capacity of the hepatic urea cycle exceeds the normal rates of ammonia generation, and the levels of blood ammonia are normally low (5 to 35 µmol/l). However, when liver function is compromised, due to either genetic defects of the urea cycle or liver disease, blood levels can be >1,000 µmol/l. Such hyperammonemia is a medical emergency, because ammonia has a direct neurotoxic effect on the CNS. For example, elevated concentrations of ammonia in the blood cause the symptoms of ammonia intoxication, which include tremors, slurring of speech, somnolence (drowsiness), vomiting, cerebral edema, and blurring of vision. At high concentrations, ammonia can cause coma and death. There are two major types of hyperammonemia.

Acquired

Liver disease is a common cause of acquired hyperammonemia in adults and may be due, for example, to viral hepatitis or to hepatotoxins such as alcohol. Cirrhosis of the liver may result in formation of collateral circulation around the liver. As a result, portal blood is shunted directly into the systemic circulation and does not have access to the liver. Therefore, the conversion of ammonia to urea is severely impaired, leading to elevated levels of ammonia.

Congenital

Genetic deficiencies of each of the five enzymes of the urea cycle (and of NAGS) have been described, with an overall incidence of ~1:25,000 live births. OTC deficiency is X linked, predominantly affecting males, although female carriers may become symptomatic. All of the other urea cycle disorders follow an autosomal-recessive inheritance pattern. In each case, the failure to synthesize urea leads to hyperammonemia during the first weeks following birth. Combinations of other symptoms common to hyperammonemia (tremors, slurred speech, drowsiness, vomiting cerebral edema, blurred vision, intellectual and developmental disability, and in severe hyperammonemia, even coma and death) can also be seen in different urea cycle deficiencies. Diagnosis is based upon symptoms, laboratory testing, and genetic testing. Historically, congenital urea cycle defects have a high morbidity (neurologic manifestations) and mortality. Additional information for specific urea cycle deficiencies are summarized in the following sections.

Ornithine transcarbamylase deficiency

OTC deficiency is the most common urea cycle disorder. Specific laboratory test results include a decrease in reaction and the downstream products citrulline and arginine. Interestingly, there is also an increase in detectable serum and urinary orotic acid levels. Carbamoyl phosphate, one of the OTC substrates, instead becomes a substrate for pyrimidine biosynthesis, entering into the pathway downstream of the regulatory reaction (see Fig. 22.21, p. 336). As a result, orotic acid is an overproduced pyrimidine biosynthesis pathway intermediate. (Note: Elevated orotic acid is also seen in hereditary orotic aciduria, due to a pyrimidine biosynthesis enzyme deficiency in UMP synthase [UMPS]. Along with genetic testing, OTC deficiency can be differentially diagnosed from UMPS deficiency based on other symptoms. Hyperammonemia is a symptom of OTC deficiency, but not of UMPS deficiency; instead, megaloblastic anemia may be a symptom of UMPS deficiency.)

Argininosuccinate synthetase deficiency

This deficiency is also referred to as citrullinemia type 1, as there is an accumulation of the substrate for the reaction, citrulline, in blood and urine. There may be a neonatal acute (classic) form, a milder late-onset form, a form that begins during or after pregnancy, and an asymptomatic form. In the neonatal acute form, citrulline can be detected as part of newborn screening. This detection is critical to prevent hyperammonemia and brain damage.

Argininosuccinate lyase deficiency

In argininosuccinate lyase deficiency, there is an accumulation of the substrate for the reaction, argininosuccinate, in the urine, resulting in argininosuccinic aciduria. This is diagnostic and part of the newborn screening. In more severe- and late-onset forms of the deficiency, the aciduria may be associated with neurologic abnormalities, developmental delays, and cognitive impairment.

Arginase-I deficiency

In arginase-I deficiency, there is an accumulation of the substrate for the reaction, arginine, in the blood and urine, and is often referred to as argininemia or hyperargininemia. The hyperammonemia seen with arginase deficiency is often less severe because arginine contains two waste nitrogens, and can be excreted in the urine. As such, patients with this deficiency may appear to be healthy at birth, and have normal development during the first 1 to 3 years. After this, the first symptoms of arginase deficiency may appear with apparent developmental delays, loss of developmental milestones, and intellectual disability. Hyperammonemia may be episodic, associated with high-protein meals or periods of stress, such as illness or fasting.

N-acetylglutamate synthase deficiency

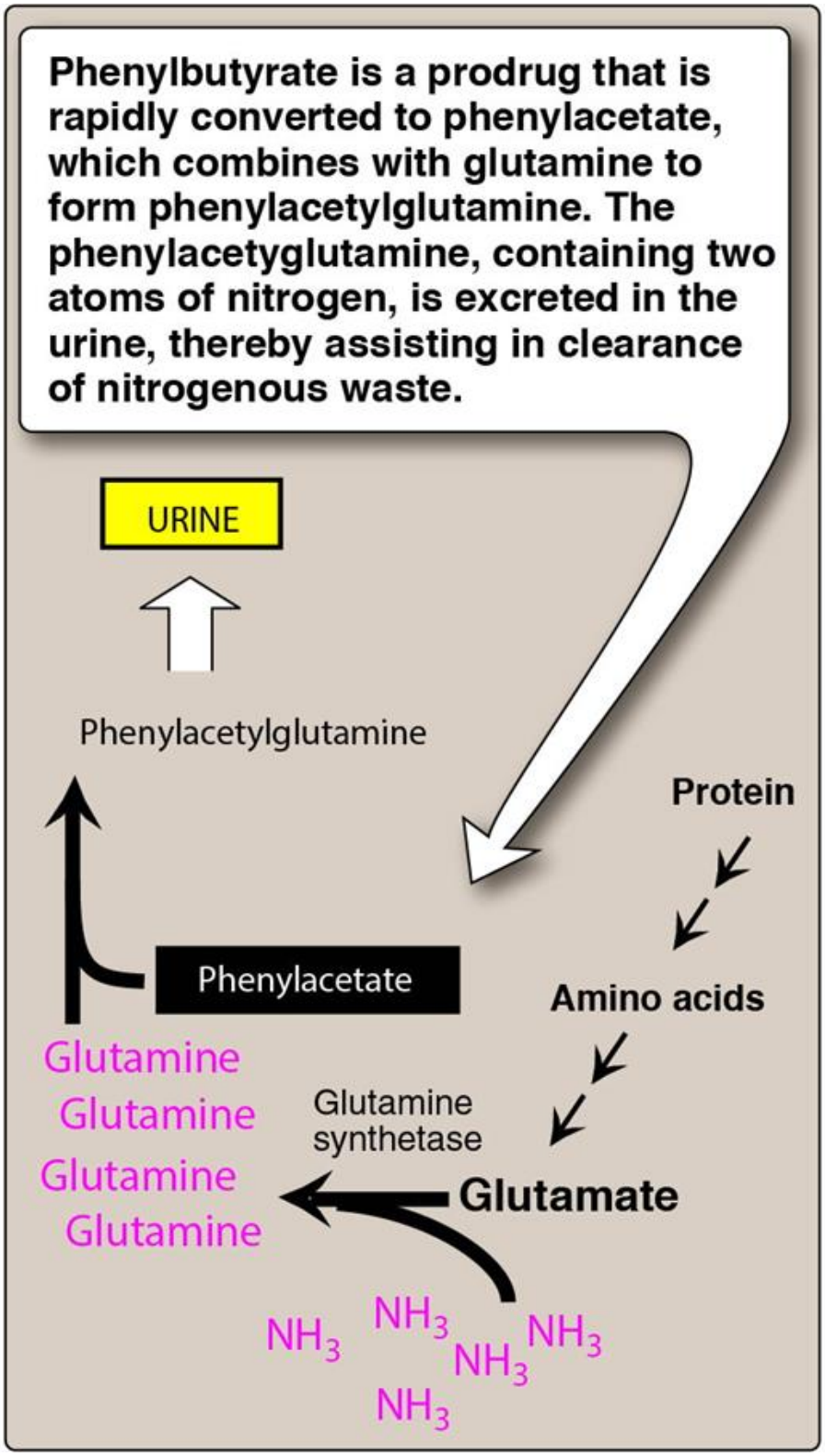
Like the aginase-I deficiency, a deficiency in NAGS can result in developmental delays and intellectual disability. Less severe forms may episodic later in life, associated with periods of high-protein meals, stress, or fasting. Carglumic acid is an FDA-approved therapy for NAGS deficiency. It is a synthetic form of NAG, the positive allosteric activator of carbamoyl phosphate synthetase I.

Treatment for hyperammonemia

Treatment for urea cycle enzyme deficiencies involves both limiting protein intake in the diet in the presence of sufficient calories to prevent protein catabolism, and the removal of excess ammonia in the blood. This can vary depending on the enzyme deficiency and the defect severity. Patients adhere to a low-protein diet, with minimal protein levels needed to maintain good health. This can vary depending on the age and weight of the patient. Drinks with special formulas and/or medical foods can be purchased in which protein levels are tailored to the patient's needs. Nitrogen-scavenging medications, including the aromatic acids benzoate and phenylbutyrate, can reduce ammonia levels in the blood. Benzoate combines with glycine to form hippurate. Phenylbutyrate is converted to phenylacetate, and combines with glutamine to form phenylacetylglutamine (Fig. 19.20). Both end products, hippurate and phenylacetylglutamine, are readily excreted in the urine. The combined excretion of glycine and glutamine, and their subsequent biosynthesis, effectively lowers ammonia levels and the potential for hyperammonemia. During severe hyperammonemia, patients may also require dialysis, intravenous fluids, or other treatments to quickly reduce blood ammonia levels and prevent permanent brain damage.

FIGURE 19.20

Treatment of patients with urea cycle defects by administration of phenylbutyrate to aid in excretion of ammonia (NH_3).



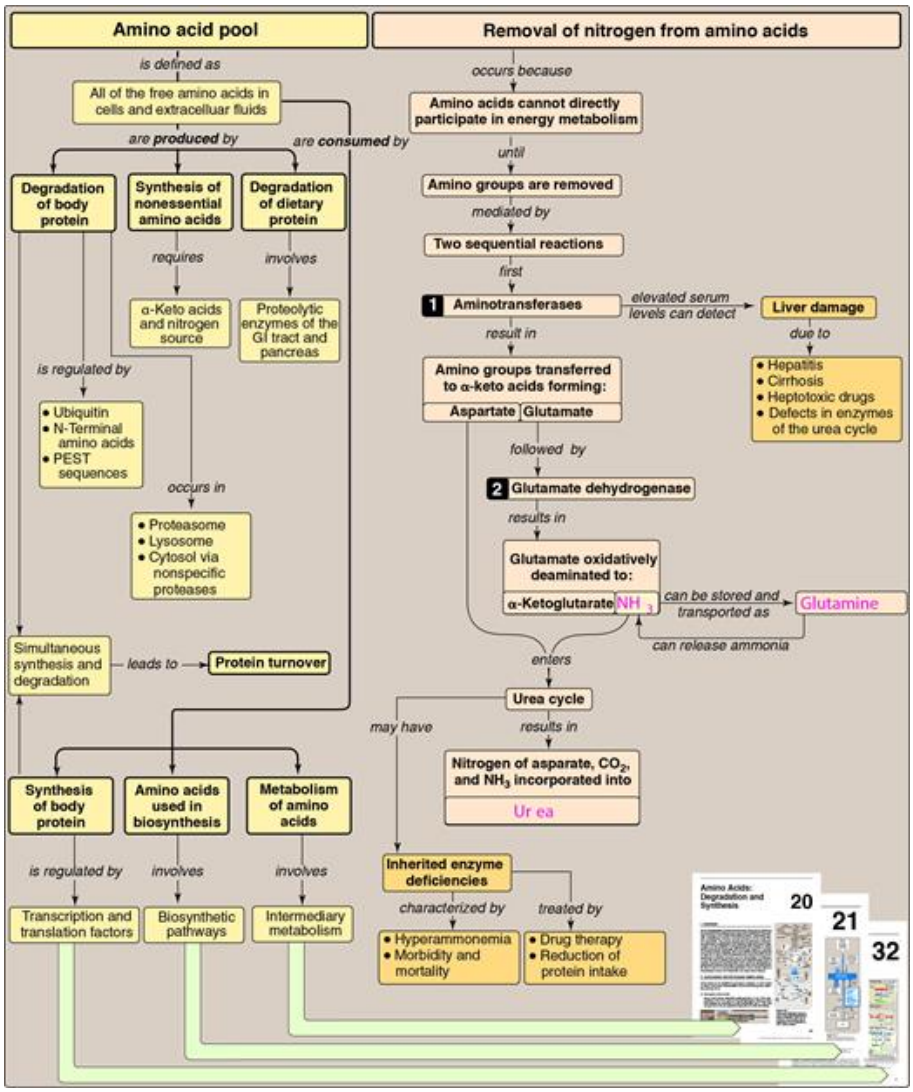
Chapter Summary

- **Nitrogen enters** the body in a variety of compounds present in food, the most important being **amino acids** contained in **dietary protein**.
- **Nitrogen leaves** the body as **urea**, **ammonia**, and other products derived from amino acid metabolism (Fig. 19.21).

FIGURE 19.21

Key concept map for nitrogen metabolism.

GI = gastrointestinal; PEST = proline, glutamate, serine, threonine; NH_3 = ammonia; CO_2 = carbon dioxide.



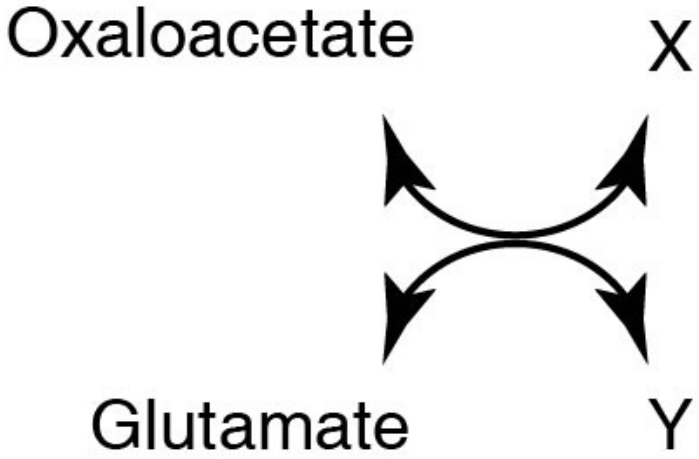
- Free amino acids in the body are produced by hydrolysis of dietary protein by **proteases** activated from their **zymogen** form in the stomach and intestine, degradation of tissue proteins, and *de novo* synthesis. This **amino acid pool** is consumed in the synthesis of body protein, metabolized for energy, or its members used as precursors for other nitrogen-containing compounds.
- Free amino acids from digestion are taken up by intestinal **enterocytes** via **sodium-dependent secondary active transport**. Small peptides are taken up via **proton-linked transport**.

- Body protein is simultaneously degraded and resynthesized, a process known as **protein turnover**. The concentration of a cellular protein may be determined by regulation of its synthesis or degradation. The ATP-dependent, cytosolic, selective **Ub–proteasome** and ATP-independent, relatively nonselective **lysosomal acid hydrolases** are the two major enzyme systems that are responsible for **degrading proteins**.
- Nitrogen cannot be stored, and amino acids in excess of the biosynthetic needs of the cell are quickly degraded. The first phase of **catabolism** involves the transfer of the α-amino groups through **transamination** by **pyridoxal phosphate**–dependent **aminotransferases** (**transaminases**), followed by **oxidative deamination of glutamate** by **GDH**, forming **ammonia** and the corresponding **α-keto acids**.
- A portion of the **free ammonia** is excreted in the **urine**. Some ammonia is used in converting glutamate to **glutamine** for safe transport, but most is used in the hepatic synthesis of **urea**, which is quantitatively the most important route for disposing of nitrogen from the body. **Alanine** also carries nitrogen to the liver for disposal as urea.
- The two major causes of **hyperammonemia** (with its neurologic effects) are acquired liver disease and congenital deficiencies of urea cycle enzymes such as X-linked **OTC**.

Study Questions

Choose the ONE best answer.

19.1. In this transamination reaction (right), which of the following are the products X and Y?



A. Alanine, α-ketoglutarate

B. Aspartate, α-ketoglutarate

C. Glutamate, alanine

D. Pyruvate, aspartate

E. Alanine, pyruvate

Correct answer = B. Transamination reactions always have an amino acid and an α-keto acid as substrates. The products of the reaction are also an amino acid (corresponding to the α-keto substrate) and an α-keto acid (corresponding to the amino acid substrate). Three amino acid/α-keto acid pairs commonly encountered in metabolism are alanine/pyruvate, aspartate/oxaloacetate, and glutamate/α-ketoglutarate. In this question, glutamate is deaminated to form α-ketoglutarate, and oxaloacetate is aminated to form aspartate.

19.2. Which one of the following statements about amino acids and their metabolism is correct?

A. Free amino acids are taken into the enterocytes by a single proton-linked transport system.

B. In healthy, well-fed individuals, the input to the amino acid pool exceeds the output.

C. The liver uses ammonia to buffer protons.

D. Muscle-derived glutamine is deaminated in liver and kidney tissue to ammonia + a gluconeogenic precursor.

E. The first step in the catabolism of most amino acids is their oxidative deamination.

F. The toxic ammonia generated from the amide nitrogen of amino acids is transported through blood as arginine.

Correct answer = D. Glutamine, produced by the catabolism of branched-chain amino acids in muscle, is deaminated by glutaminase to ammonia + glutamate. The glutamate is deaminated by glutamate dehydrogenase to ammonia + α-ketoglutarate, which can be used for gluconeogenesis. Free amino acids are taken into enterocytes by several different sodium-linked transport systems. Healthy, well-fed individuals are in nitrogen balance, in which nitrogen input equals output. The liver converts ammonia to urea, and the kidneys use ammonia to buffer protons. Amino acid catabolism begins with transamination that generates glutamate. The glutamate undergoes oxidative deamination. Toxic ammonia is transported as glutamine and alanine. Arginine is synthesized and hydrolyzed in the hepatic urea cycle.

For Questions 19.3 to 19.5, use the following scenario.

A female neonate appeared healthy until age ~24 hours, when she became lethargic. A sepsis workup proved negative. At 56 hours, she started showing focal seizure activity. The plasma ammonia level was found to be 887 μmol/l (normal 5 to 35 μmol/l). Quantitative plasma amino acid levels revealed a marked elevation of citrulline but not argininosuccinate.

19.3. Which one of the following enzymic activities is most likely to be deficient in this patient?

A. Arginase

B. Argininosuccinate lyase

C. Argininosuccinate synthetase

D. Carbamoyl phosphate synthetase I

E. Ornithine transcarbamylase

Correct answer = C. Genetic deficiencies of each of the five enzymes of the urea cycle, as well as deficiencies in N-acetylglutamate synthase, have been described. The accumulation of citrulline (but not argininosuccinate) in the plasma of this patient means that the enzyme required for the conversion of citrulline to argininosuccinate (argininosuccinate synthetase) is defective, whereas the enzyme that cleaves argininosuccinate (argininosuccinate lyase) is functional.

19.4. Which one of the following would also be elevated in the blood of this patient?

A. Asparagine

B. Glutamine

C. Lysine

D. Urea

E. Arginine

Correct answer = B. Deficiencies of the enzymes of the urea cycle result in the failure to synthesize urea and lead to hyperammonemia in the first few weeks after birth. Glutamine will also be elevated because it acts as a nontoxic storage and transport form of ammonia. Therefore, elevated glutamine accompanies hyperammonemia. Asparagine, lysine, and arginine do not serve this sequestering role. Urea would be decreased because of impaired activity of the urea cycle. (Note: Alanine would also be elevated in this patient.)

19.5. Why might supplementation with arginine be of benefit to this patient?

The arginine will be cleaved by arginase to urea and ornithine. Ornithine will be combined with carbamoyl phosphate by ornithine transcarbamylase to form citrulline. Citrulline, containing one waste nitrogen, will be excreted.