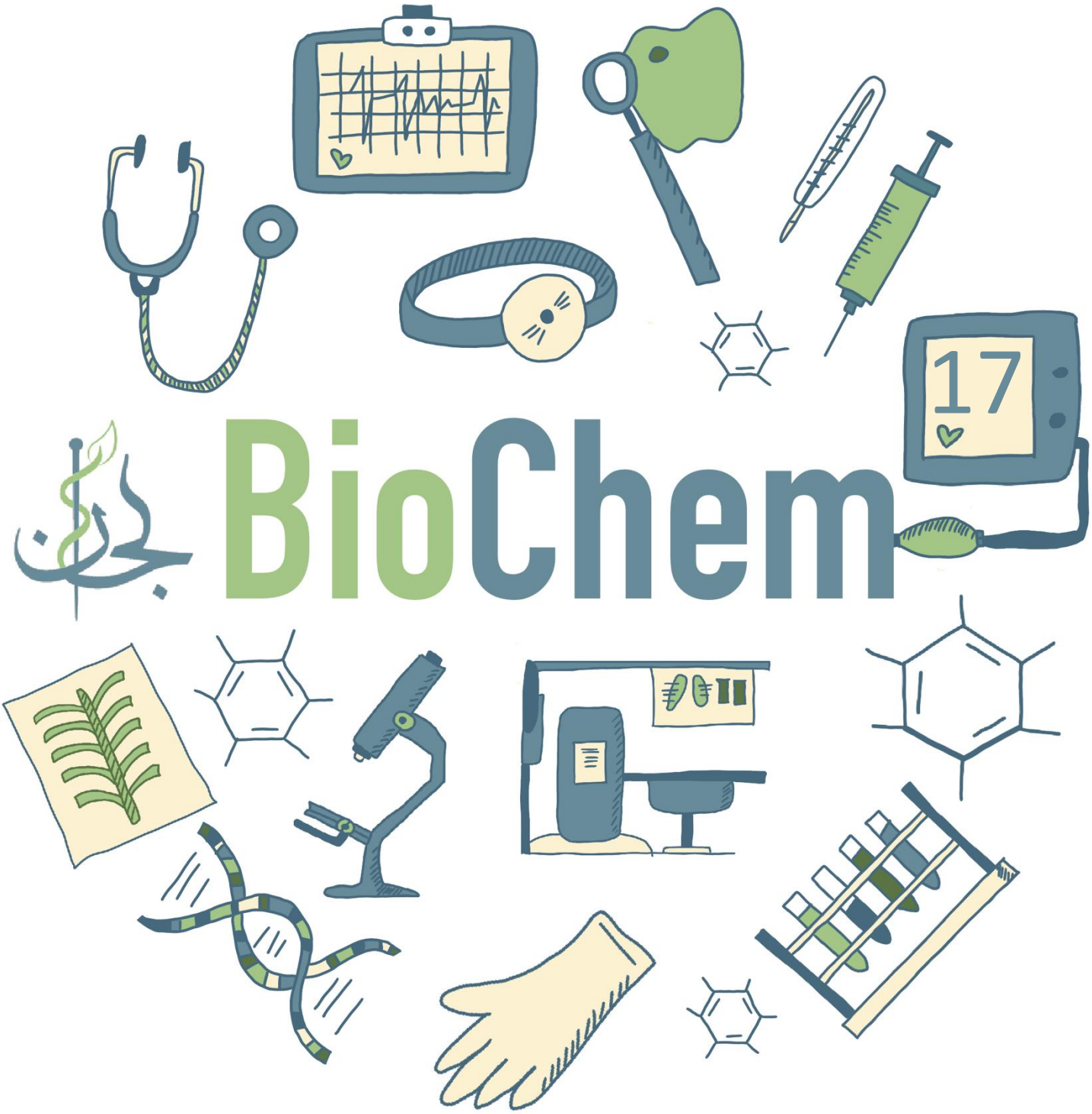

BioChem

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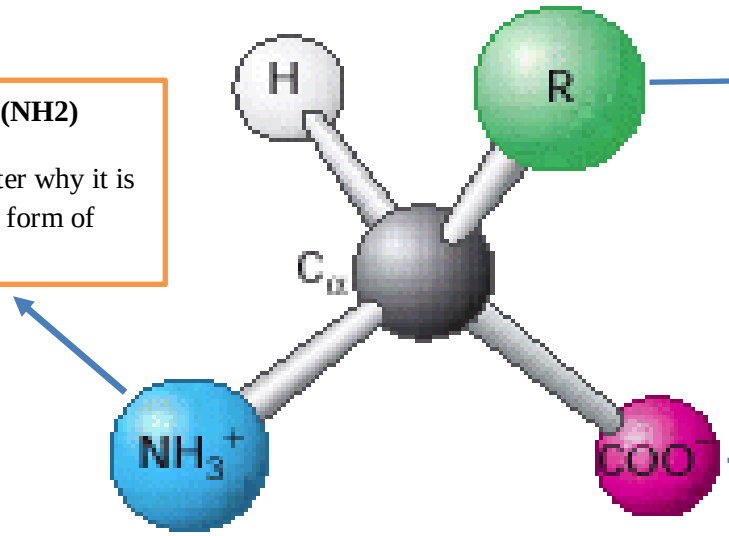
Amino Acids

- Amino acids as the name suggests are molecules that contain an amino group as well as an acidic group (carboxylic).
- They contain a carbon that is called an alpha carbon (α-carbon), which is a chiral carbon attached to four different groups; an **Amino group** (NH2), a **Carboxyl group** (COOH**),** a **Hydrogen**, and an **R group** (the variable part of an amino acid that specifies the type of the amino acid)

Amino group (NH2)

We will explain later why it is presented in the form of (NH3+)

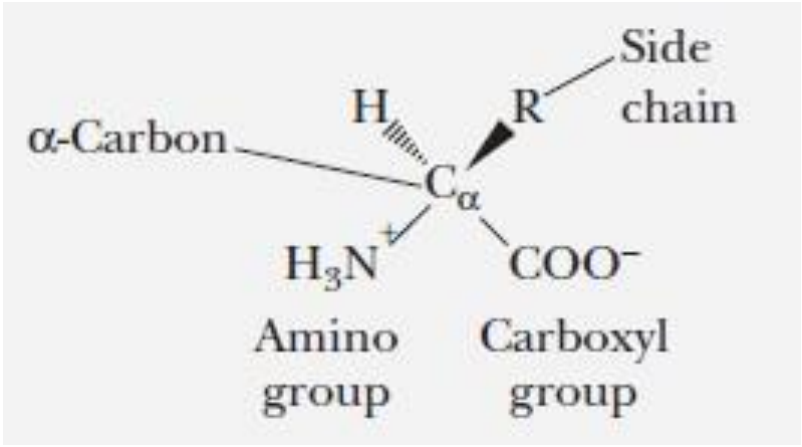


R group

Variable among different amino acids, each one contains a special R group.
R groups are the part of an amino acid that specifies its type

Carboxyl group (COOH)

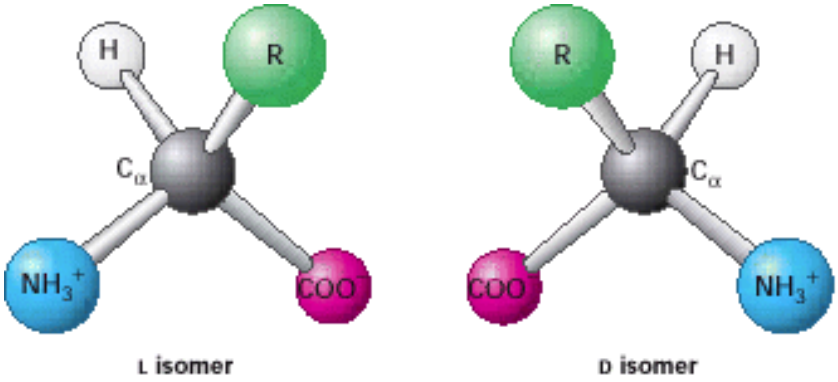
We will explain later why it is attached in the form of (COO-)



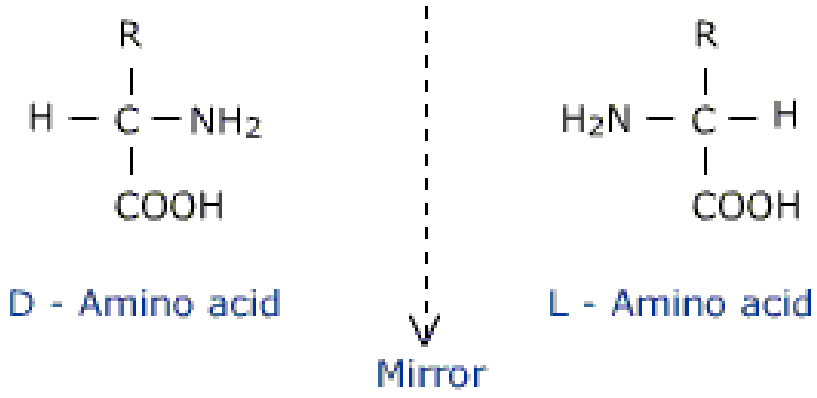
- Three of the four bonds around the (α-carbon) are similar, while the last one differs according to the type of R group, hence resulting in a large array of 20 different types of amino acids that constitute our proteins.

- There are other amino acids (other than the 20 that can be presented as just single molecules), performing different functions.

- Amino acids with their attached groups may be oriented in space in different ways around the (α-carbon).

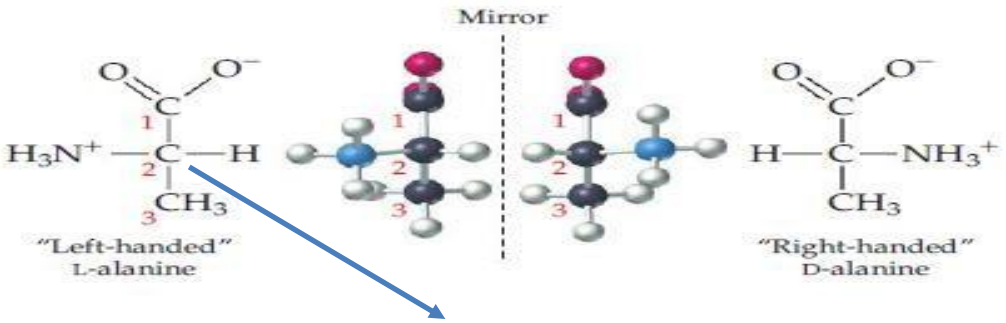


  - Here each molecule of these 2 is the mirror image of the other, and they are isomers (D-isomer and L-isomer)
  - To determine which isomer is the D-isomer and which is the L-isomer, we look at the amino group:
    - If the amino group is on the right, then this amino acid is a **D-amino acid**
    - If the amino group is on the left, then this amino acid is an **L-amino acid**



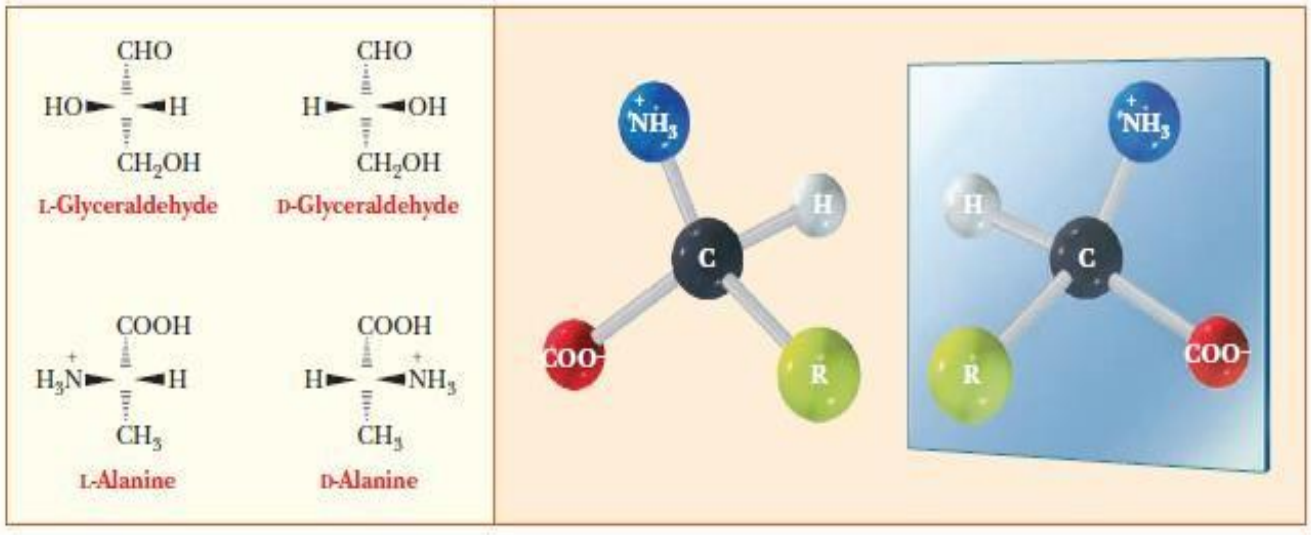
  - ✓ In proteins that are presented naturally in our cells, the type of amino acids that are presented are **L-amino acids**, although this doesn't necessarily mean that we don't have D-amino acids (we have D-amino acids but they're not presented in the proteins that are synthesized In our cells).

Alanine, a chiral molecule



- Having four different groups attached to the central carbon (α-carbon), gives us a chiral center, with D and L isomers. This can be achieved in all amino acids (meaning that they all have isomers) **except for glycine** because its R group is a hydrogen, therefore there would be 2 hydrogens, thus the (α-carbon) of glycine is achiral
- The amino acids in proteins are not superimposable on their mirror images (except for glycine).
- The Latin terms *laevus* and *dexter*, meaning "left" and "right", respectively, (the ability to rotate polarized light to the left or the right).

Amino acids stereoisomers

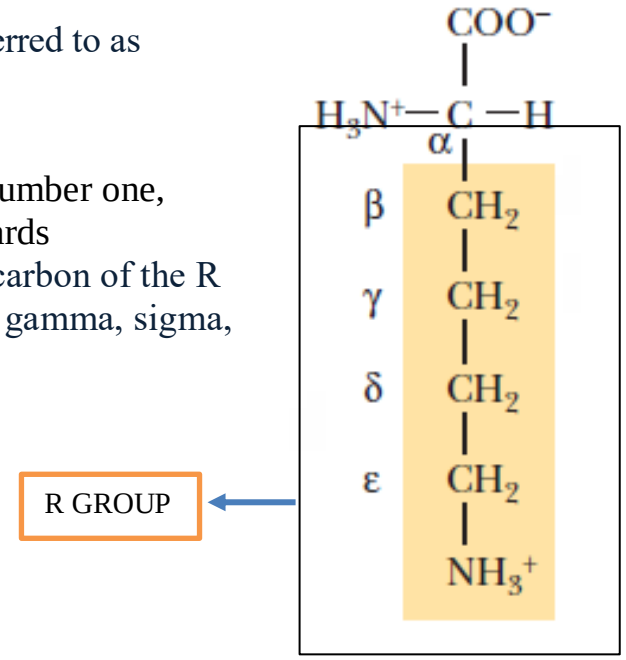


- It's the same idea here, all amino acids have stereoisomers; D and L (except for glycine), just like carbohydrates (they also have the concept of D and L isomers). But in sugars the D-isomer is mainly presented in our cell and body, while in amino acids the L-isomer is mainly the type presented in our proteins.

- **D-amino acids** occur in nature, in bacterial cell walls and in some antibiotics, but not in proteins.

Designation of carbons

- Side-chain carbon atoms are designated with letters of the Greek alphabet, starting from the α-carbon. These carbon atoms are, in turn, the β-, γ-, d-, and ε-carbons.
- If a carbon atom is terminal, it is referred to as the ω-carbon.
  - The α-carbon is like carbon number one, we start counting from it towards the R group, so then the first carbon of the R group is the beta carbon, then gamma, sigma, epsilon…etc.



Types of amino acids

- There are 20 kinds of amino acids, depending on their side chains varying in size, shape, charge, hydrogen-bonding capacity, hydrophobic character and chemical reactivity.

- The most effective classification of amino acids; is according to the polarity **of their R group** (because if you look at amino acids, they generally have polar groups, the amino group, the hydroxyl group whether the R group is polar or not):

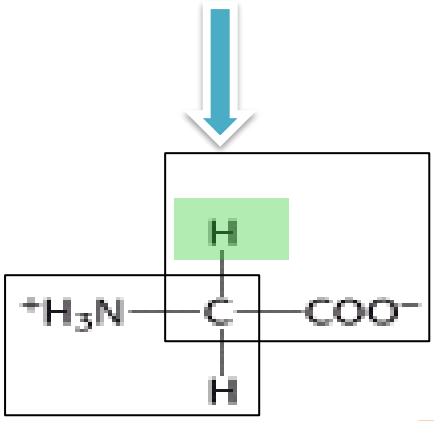
Non-polar	Polar (uncharged)	Charged (positive)	Charged (negative)
Alanine	Serine	Lysine	Glutamate

Valine	Threonine	Arginine	Aspartate
Leucine	Glutamine	Histidine	
Isoleucine	Asparagine		
Methionine	Cysteine		
Tryptophan	Tyrosine		
Phenylalanine			
Proline			
Glycine			

Non-polar amino acids

❖ Glycine

- The simplest amino acid
- Its R group is Hydrogen
- Is a derivative of acetic acid
- Could be considered as a derivative of ethylamine.



Acetic acid

Glycine (Gly, G)

You are required to know the three-letter abbreviation only **(GLY)**

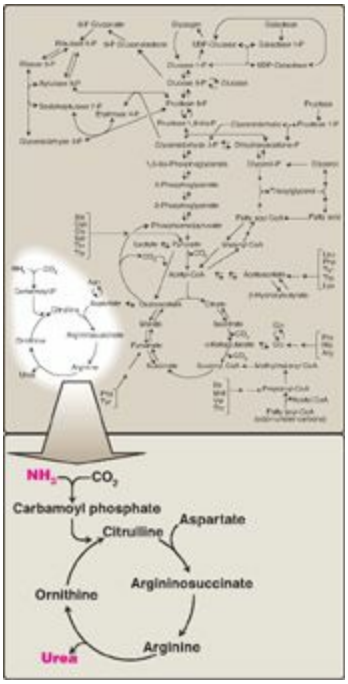
Ethylamine (ALMOST)

Amino Acids: Disposal of Nitrogen 19

I. 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 normal protein degradation. 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 (Figure 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 CO_2 and water, glucose, fatty acids, or ketone bodies by the central pathways of metabolism described in Chapters 8–13 and 16.

Figure 19.1 Urea cycle shown as part of the essential pathways of energy metabolism. (See Figure 8.2, p. 92, for a more detailed view of intermediary metabolism.)



II. OVERALL NITROGEN METABOLISM

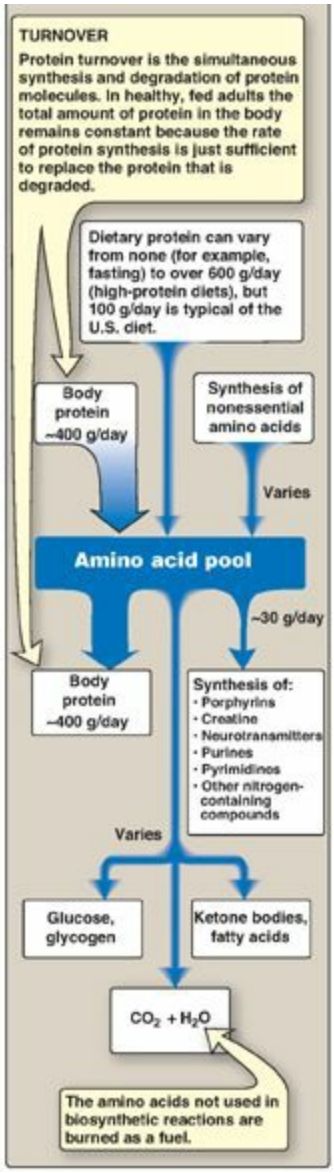
Amino acid catabolism is part of the larger process of the metabolism of nitrogen-containing molecules. Nitrogen enters the body in a variety of compounds present in food, the most important being amino acids contained in dietary protein. Nitrogen leaves the body as urea, ammonia, and other products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

A. 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 (Figure 19.2). Conversely, the amino 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 (see Figure 19.2). Although the amino acid pool is small (comprising about 90–100 g of amino acids) in comparison with the amount of protein in the body (about 12 kg in a 70-kg man), it is conceptually at the center of whole-body nitrogen metabolism.

> 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.

Figure 19.2 Sources and fates of amino acids.



B. Protein turnover

Most proteins in the body are constantly being synthesized and then degraded, 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 (that is, essentially constant), and cellular levels of the protein are controlled by selective degradation.

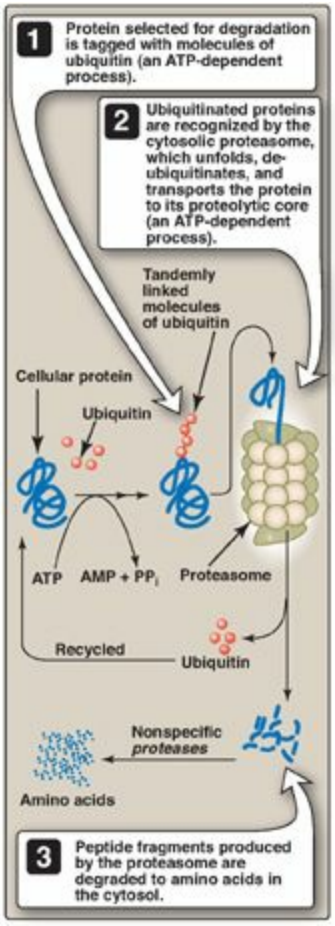
1. **Rate of turnover:** In healthy adults, the total amount of protein in the body remains constant because the rate of protein synthesis is just sufficient to replace the protein that is degraded. This process, called protein turnover, leads to the hydrolysis and resynthesis of 300–400 g of body protein each day. The rate of protein turnover varies widely for individual proteins. Short-lived proteins (for example, 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.

2. **Protein degradation:** There are two major enzyme systems responsible for degrading proteins: the adenosine triphosphate (ATP)-dependent ubiquitin-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. 162) to nonselectively degrade intracellular proteins ("autophagy") and extracellular proteins ("heterophagy"), such as plasma proteins, that are taken into the cell by endocytosis.

   a. **Ubiquitin–proteasome proteolytic pathway:** Proteins selected for degradation by the cytosolic ubiquitin-proteasome system are first modified by the covalent attachment of ubiquitin (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 on the protein substrate by a three-step, enzyme-catalyzed, ATP-dependent process. [Note: Enzyme 1 (E1, or activating enzyme) activates Ub, which is then transferred to E2 (conjugating enzyme). E3 (a ligase) identifies the protein to be degraded and interacts with E2-Ub.] The consecutive addition of four or more Ub molecules to the target protein generates a polyubiquitin chain. Proteins tagged with Ub are recognized by a large, barrel-shaped, macromolecular, proteolytic complex called a proteasome (Figure 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. Ub is recycled. It is noteworthy that the selective degradation of proteins by the ubiquitin-proteosome complex (unlike simple hydrolysis by proteolytic enzymes) requires energy in the form of ATP.

   b. **Chemical signals for protein degradation:** 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. For example, some proteins that have been chemically altered by oxidation or tagged with ubiquitin are preferentially degraded. The half-life of a protein is also influenced by the amino (N)-terminal residue. For example, proteins that have serine as the N-terminal amino acid are long-lived, with a half-life of more than 20 hours, whereas those with aspartate at their N-terminus have a half-life of only 3 minutes. 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 degraded and, therefore, have short half-lives.

Figure 19.3 The ubiquitin-proteasome degradation pathway of proteins. AMP = adenosine monophosphate; PP_i = pyrophosphate.


1
Protein selected for degradation is tagged with molecules of ubiquitin (an ATP-dependent process).
2
Ubiquitinated proteins are recognized by the cytosolic proteasome, which unfolds, de-ubiquitinates, and transports the protein to its proteolytic core (an ATP-dependent process).
Tandemly linked molecules of ubiquitin
Cellular protein
Ubiquitin
ATP
AMP + PPi
Proteasome
Recycled
Ubiquitin
Nonspecific proteases
Amino acids
3
Peptide fragments produced by the proteasome are degraded to amino acids in the cytosol.

III. DIGESTION OF DIETARY PROTEINS

Most of the nitrogen in the diet is consumed in the form of protein, typically amounting to 70–100 g/day in the American diet (see Figure 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.] They must, therefore, 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 (Figure 19.4).

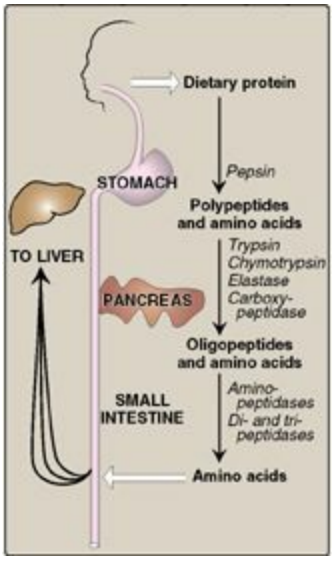
A. Digestion by gastric secretion

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

1. **Hydrochloric acid:** Stomach acid is too dilute (pH 2–3) 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.

2. **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.] Pepsinogen is activated to pepsin, either by hydrochloric acid or autocatalytically by pepsin molecules that have already been activated. Pepsin releases peptides and a few free amino acids from dietary proteins.

Figure 19.4 Digestion of dietary proteins by the proteolytic enzymes of the gastrointestinal tract.



B. Digestion by pancreatic enzymes

On entering the small intestine, large 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 (cleave within) and exopeptidases (cut at an end). [Note: Bicarbonate (HCO_3^-), also secreted by the pancreas, raises the pH.]

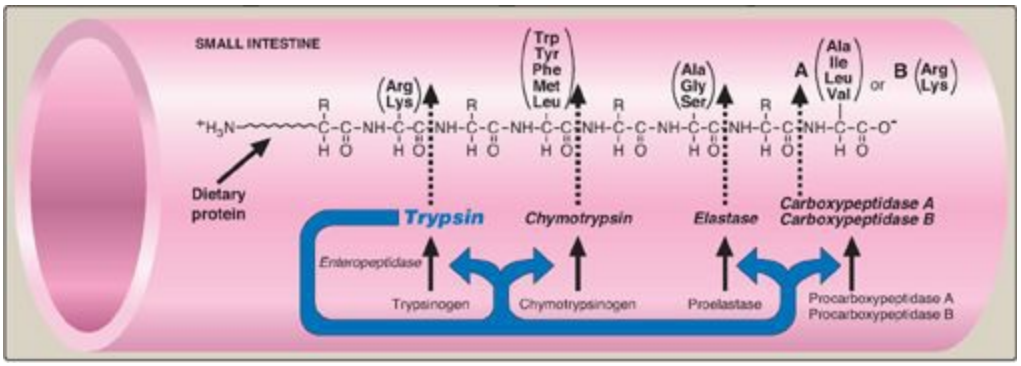
1. **Specificity:** Each of these enzymes has a different specificity for the amino acid R-groups adjacent to the susceptible peptide bond (Figure 19.5). For example, trypsin cleaves only when the carbonyl group of the peptide bond is contributed by arginine or lysine. These enzymes, like pepsin described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

2. **Release of zymogens:** The release and activation of the pancreatic zymogens is mediated by the secretion of cholecystokinin and secretin, two polypeptide hormones of the digestive tract (see p. 176).

3. **Activation of zymogens:** Enteropeptidase (formerly called enterokinase), an enzyme synthesized by and present on the luminal surface of intestinal mucosal cells of the brush border membrane, 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. Enteropeptidase, thus, unleashes a cascade of proteolytic activity because trypsin is the common activator of all the pancreatic zymogens (see Figure 19.5).

4. **Abnormalities in protein digestion:** In individuals with a deficiency in pancreatic secretion (for example, due to 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. 177) as well as undigested protein.

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.]



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.

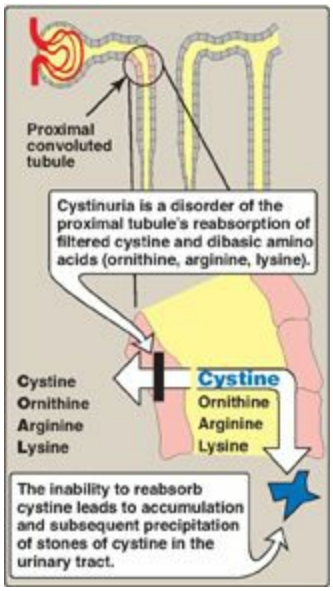
C. Digestion of oligopeptides by enzymes of the small intestine

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

D. Absorption of amino acids and small peptides

Free amino acids are taken into the enterocytes by a sodium-linked secondary transport system of the apical membrane. Di- and tripeptides, however, are taken up by a proton-linked transport system. The peptides are hydrolyzed in the cytosol to amino acids that are released into the portal system by facilitated diffusion. 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 are important examples of amino acids that are not metabolized by the liver but, instead, are sent from the liver primarily to muscle via the blood.]

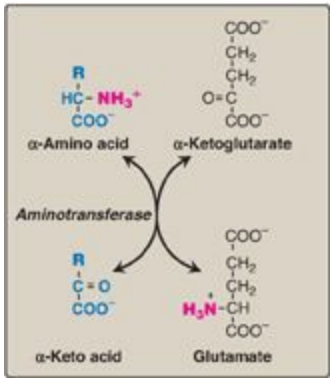
Figure 19.6 Genetic defect seen in cystinuria. [Note: Cystinuria is distinct from cystinosis, a rare defect in the transport of cystine out of lysosomes that results in the formation of cystine crystals within the lysosome and tissue damage.]



IV. TRANSPORT OF AMINO ACIDS INTO CELLS

The concentration of free amino acids in the extracellular fluids is significantly lower than that within the cells of the body. This concentration gradient is maintained because active transport systems, driven by the hydrolysis of ATP, are required for movement of amino acids from the extracellular space into cells. At least seven different transport systems are known that have overlapping specificities for different amino acids. Because the small intestine and the proximal tubule of the kidney have common transport systems for amino acid uptake, a defect in any one of these systems results in an inability to absorb particular amino acids into the gut 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 (Figure 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 transport of tryptophan can result in Hartnup disorder and pellagra-like (see p. 380) dermatologic and neurologic symptoms.]

Figure 19.7 Aminotransferase reaction using α-ketoglutarate as the amino group acceptor.



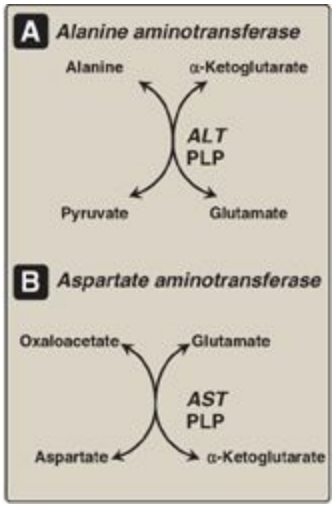
V. REMOVAL OF NITROGEN FROM AMINO ACIDS

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. 253).

A. Transamination: the funneling of amino groups to glutamate

The first step in the catabolism of most amino acids is the transfer of their α-amino group to α-ketoglutarate (Figure 19.7), producing an α-keto acid (derived from the original amino acid) and glutamate. α-Ketoglutarate plays a pivotal role in amino acid metabolism by accepting the amino groups from most amino acids, thereby becoming glutamate. Glutamate produced by transamination can be oxidatively deaminated (see 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 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. 265–266).]

Figure 19.8 Reactions catalyzed during amino acid catabolism. A. Alanine aminotransferase (ALT). B. Aspartate aminotransferase (AST). PLP = pyridoxal phosphate (see p. 251).



1. **Substrate specificity of aminotransferases:** 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

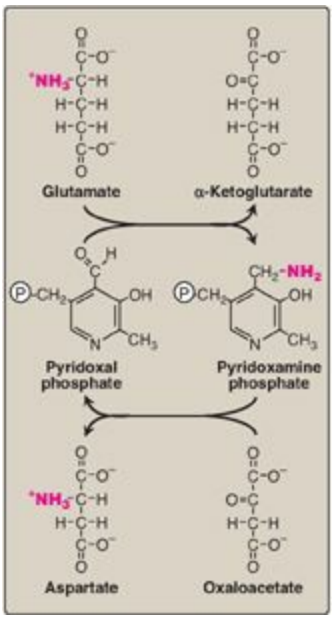
alanine aminotransferase (ALT) and aspartate aminotransferase (AST), as shown in Figure 19.8).

a. 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: Glutamate, in effect, acts as a "collector" of nitrogen from most amino acids.]

b. Aspartate aminotransferase: AST is an exception to the rule that aminotransferases funnel amino groups to form glutamate. During amino acid catabolism, AST transfers amino groups from glutamate to oxaloacetate, forming aspartate, which is used as a source of nitrogen in the urea cycle (see p. 253). Like other transaminations, the AST reaction is reversible.

2. Mechanism of action of aminotransferases: All aminotransferases require the coenzyme pyridoxal phosphate (a derivative of vitamin B_6; see p. 378), which is covalently linked to the ε-amino group of a specific lysine residue at the active site of the enzyme. Aminotransferases act by transferring the amino group of an amino acid to the pyridoxal part of the coenzyme to generate pyridoxamine phosphate. The pyridoxamine form of the coenzyme then reacts with an α-keto acid to form an amino acid, at the same time regenerating the original aldehyde form of the coenzyme. Figure 19.9 shows these two component reactions for the reaction catalyzed by AST.

Figure 19.9 Cyclic interconversion of pyridoxal phosphate and pyridoxamine phosphate during the aspartate aminotransferase reaction. Ⓟ = phosphate group.



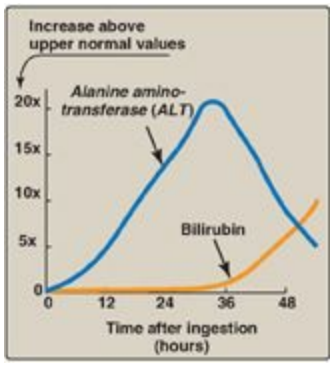
3. Equilibrium of transamination reactions: For most transamination reactions,

the equilibrium constant is near 1. This allows the reaction to function in both amino acid degradation through removal of α-amino groups (for example, 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 (for example, when the supply of amino acids from the diet is not adequate to meet the synthetic needs of cells).

4. **Diagnostic value of plasma aminotransferases:** Aminotrans-ferases 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.

   a. **Liver disease:** Plasma AST and ALT are elevated in nearly all liver 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 (so-called "liver function tests") are often useful in determining the course of liver damage. Figure 19.10 shows the early release of ALT into the serum, following ingestion of a liver toxin. [Note: Elevated serum bilirubin results from hepatocellular damage that decreases the hepatic conjugation and excretion of bilirubin (see p. 284).]

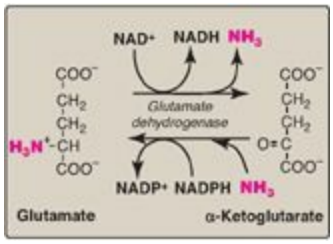
Figure 19.10 Pattern of serum ALT and bilirubin in the plasma, following poisoning with the toxic mushroom Amanita phalloides.



   b. **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.

Figure 19.11 Oxidative deamination by glutamate dehydrogenase. [Note: The enzyme

is unusual in that it uses both NAD^+ (nicotinamide adenine dinucleotide) and NADPH (nicotinamide adenine dinucleotide phosphate).]



B. Oxidative deamination of amino acids

In contrast to transamination reactions that transfer amino groups, oxidative deamination reactions result in the liberation of the amino group as free ammonia (Figure 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 un-ionized form (NH_3) that crosses membranes.]

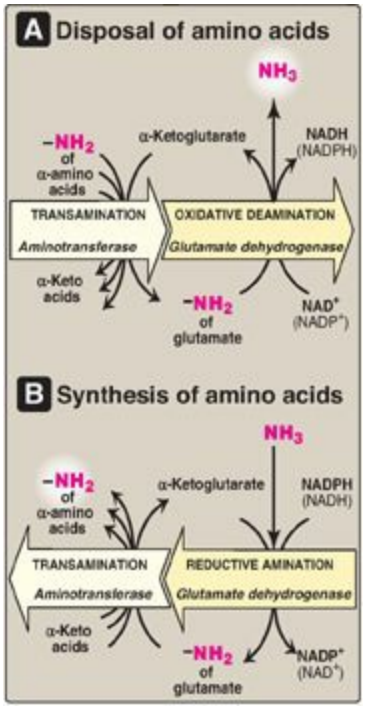
1. **Glutamate dehydrogenase:** As described above, the amino groups of most amino acids are ultimately funneled to glutamate by means of transamination with α-ketoglutarate. Glutamate is unique in that it is the only amino acid that undergoes rapid oxidative deamination, a reaction catalyzed by glutamate dehydrogenase (see Figure 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.

    a. **Coenzymes:** Glutamate dehydrogenase, a mitochondrial enzyme, is unusual in that it can use either nicotinamide adenine dinucleotide (NAD^+) or its phosphorylated reduced form (NADPH) as a coenzyme (see Figure 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 Figure 19.12A), and NADPH is used in reductive amination (the simultaneous gain of ammonia coupled with the reduction of the carbon skeleton, as shown in Figure 19.12B).

    b. **Direction of reactions:** 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 (see Figure 19.12A). High ammonia levels are required to drive the reaction to glutamate synthesis.

c. Allosteric regulators: Guanosine triphosphate is an allosteric inhibitor of glutamate dehydrogenase, whereas adenosine diphosphate (ADP) is an activator. Therefore, when energy levels are low in the cell, amino acid degradation by glutamate dehydrogenase is high, facilitating energy production from the carbon skeletons derived from amino acids.

Figure 19.12 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.



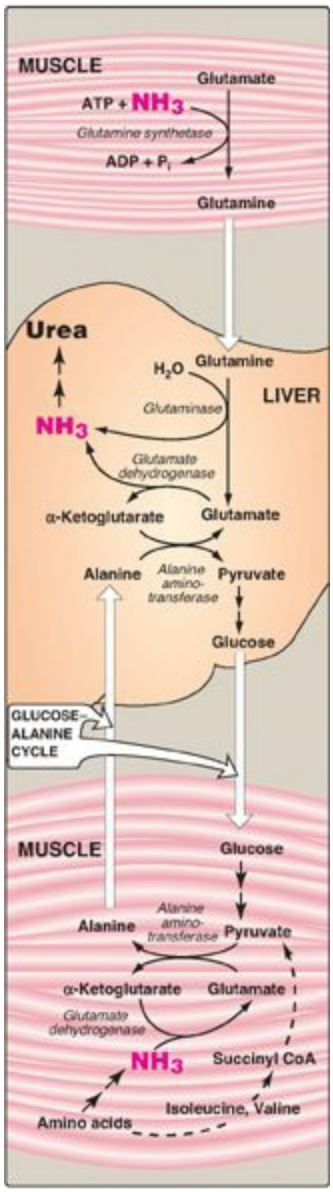
2. D-Amino acid oxidase: D-Amino acids (see p. 5) are found in plants and in the cell walls of microorganisms but are not used in the synthesis of mammalian proteins. D-Amino acids are, however, present in the diet and are efficiently metabolized by the kidney and liver. D-Amino acid oxidase (DAO) is a flavin adenine dinucleotide–dependent peroxisomal enzyme that catalyzes the oxidative deamination of these amino acid isomers, thereby producing α-keto acids, ammonia, and hydrogen peroxide. 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.] L-amino acid oxidases are known, but their physiologic significance is unclear.

C. Transport of ammonia to the liver

Two mechanisms are available in humans for the transport of ammonia from the peripheral tissues to the liver for its ultimate 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 (Figure 19.13). The glutamine is transported in the blood to the liver where it is cleaved by glutaminase to produce glutamate and free ammonia (see p. 256). 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 branched-chain amino acids isoleucine and valine. Alanine is transported by the blood to the liver, where it is converted to pyruvate, again by transamination. The pyruvate is used to synthesize glucose, which can enter the blood and be used by muscle, a pathway called the glucose–alanine cycle.

Figure 19.13 Transport of ammonia (NH_3) from muscle to the liver. ADP = adenosine diphosphate; Pi = inorganic phosphate; CoA = coenzyme A.



VI. UREA CYCLE

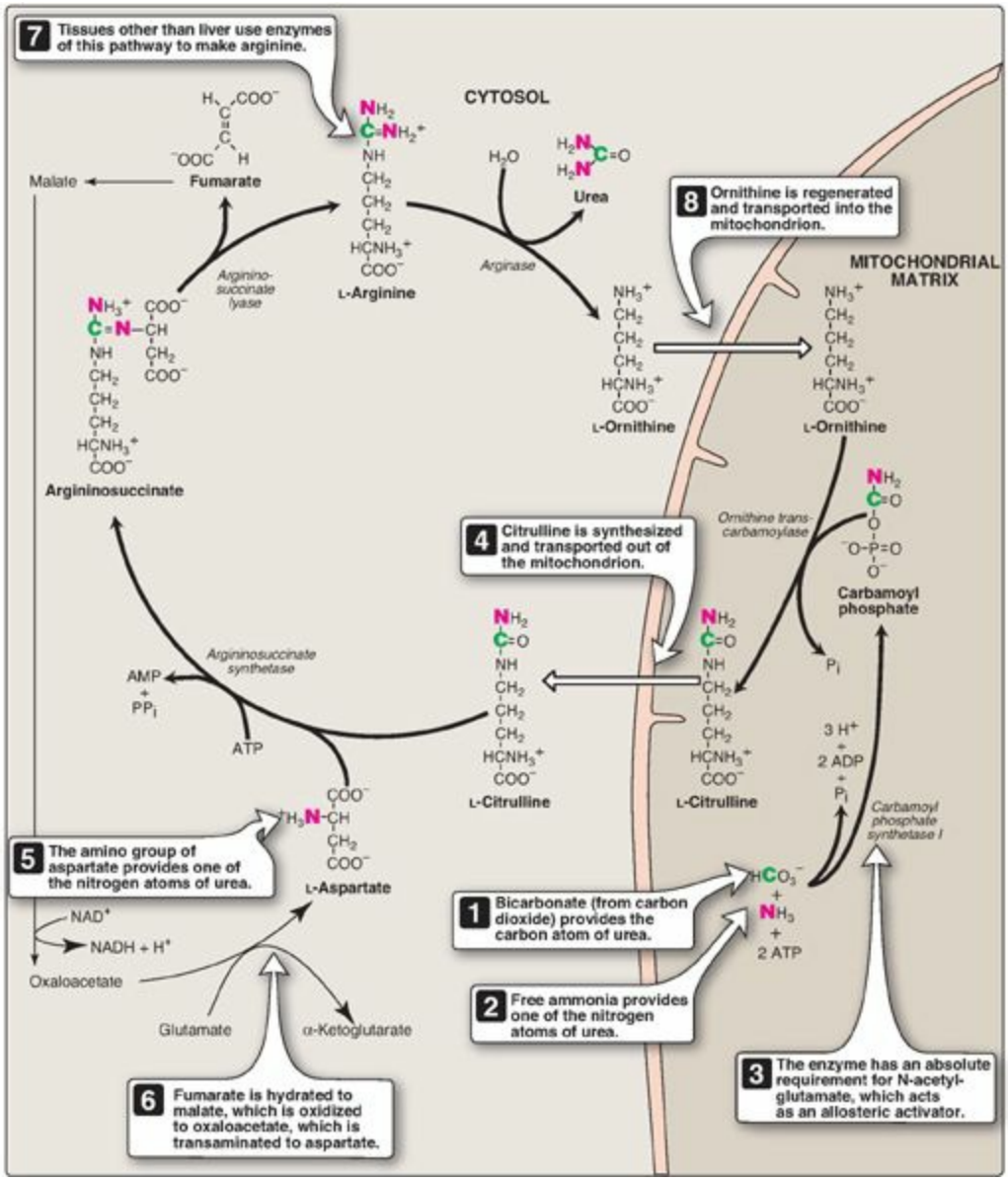
Urea (H_2NCONH_2) is the major disposal form of amino groups derived from amino acids and accounts for about 90% of the nitrogen-containing components of urine. One nitrogen of the urea molecule is supplied by free ammonia and the other nitrogen by aspartate. [Note: Glutamate is the immediate precursor of both ammonia (through oxidative deamination by glutamate dehydrogenase) 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 to the kidneys for excretion in the urine.

A. Reactions of the cycle

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 (Figure 19.14). [Note: Gluconeogenesis (see p. 117) and heme synthesis (see p. 278) also involve both the mitochondrial matrix and the cytosol.]

1. **Formation of carbamoyl phosphate:** 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 glutamate dehydrogenase (see Figure 19.11). Ultimately, the nitrogen atom derived from this ammonia becomes one of the nitrogens of urea. CPS I requires N-acetylglutamate as a positive allosteric activator (see Figure 19.14). [Note: Carbamoyl phosphate synthetase II participates in the biosynthesis of pyrimidines (see p. 302). It does not require N-acetylglutamate, 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

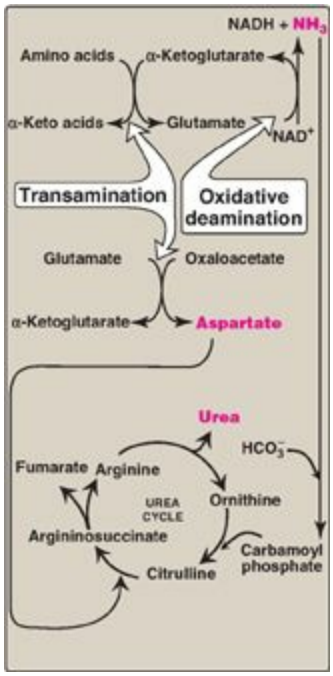


2. **Formation of citrulline:** The carbamoyl portion of carbamoyl phosphate is transferred to ornithine by ornithine transcarbam-oylase (OTC) as the high-energy phosphate is released as inorganic phosphate. The reaction product, citrulline, is transported to the cytosol. [Note: Ornithine and citrulline are basic amino acids that participate in the urea cycle, moving across the inner mitochondrial membrane via a cotransporter. They are not incorporated into cellular proteins because there are no codons for these amino acids (see p. 432).] Ornithine is regenerated with each turn of the urea cycle, much in the same way that oxaloacetate is regenerated by the reactions of the citric acid cycle (see p. 109).

3. **Synthesis of argininosuccinate:** 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.

Figure 19.15 Flow of nitrogen from amino acids to urea. Amino groups for urea

synthesis are collected in the form of ammonia and aspartate. NAD(H) = nicotinamide adenine dinucleotide.

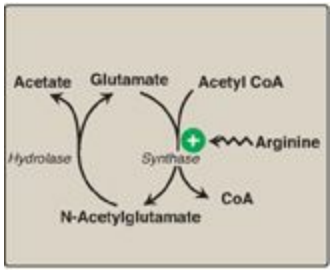


4. **Cleavage of argininosuccinate:** Argininosuccinate is cleaved by argininosuccinate lyase to yield arginine and fumarate. The arginine formed by this reaction serves as the immediate precursor of urea. Fumarate produced in the urea cycle is hydrated to malate, providing a link with several metabolic pathways. For example, the malate can be transported into the mitochondria via the malate–aspartate shuttle, reenter the tricarboxylic acid cycle, and get oxidized to oxaloacetate, which can be used for gluconeogenesis (see p. 120). [Note: Malate oxidation generates NADH and, subsequently, ATP.] Alternatively, the oxaloacetate can be converted to aspartate via transamination (see Figure 19.8) and can enter the urea cycle (see Figure 19.14).

5. **Cleavage of arginine to ornithine and urea:** Arginase 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 by these reactions.

6. **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 (see Figure 19.19). A portion of the urea diffuses from the blood into the intestine and is cleaved to CO_2 and NH_3 by bacterial urease. This 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 NH_3 production.

Figure 19.16 Formation and degradation of Nacetylglutamate, an allosteric activator of carbamoyl phosphate synthetase I. CoA = coenzyme A.



B. Overall stoichiometry of the urea cycle

$$\text{Aspartate} + NH_3 + HCO_3^- + 3\text{ATP} + H_2O \rightarrow$$

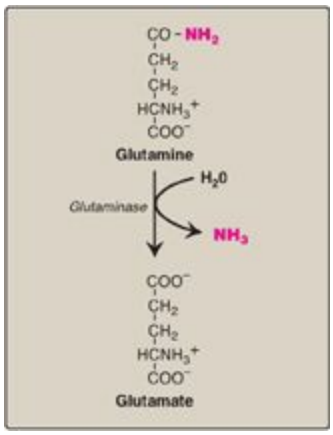
$$\text{urea} + \text{fumarate} + 2\text{ADP} + AMP + 2\ P_i + PP_i$$

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. 70). One nitrogen of the urea molecule is supplied by free NH_3, and the other nitrogen by aspartate. Glutamate is the immediate precursor of both ammonia (through oxidative deamination by glutamate dehydrogenase) 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 (Figure 19.15).

C. Regulation of the urea cycle

N-Acetylglutamate (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 (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 19.17 Hydrolysis of glutamine to form ammonia (NH_3).



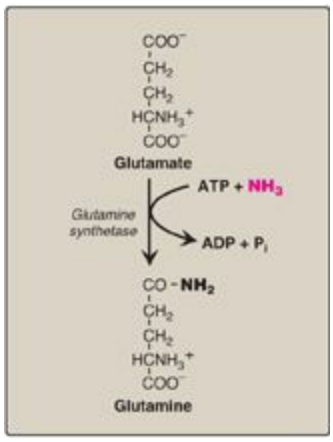
VII. METABOLISM OF AMMONIA

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 level of ammonia in the blood must be kept very low, because even slightly elevated concentrations (hyperammonemia) are toxic to the central nervous system (CNS). Therefore, there must be a metabolic mechanism by which nitrogen is moved from peripheral tissues to the liver for ultimate disposal as urea, at the same time maintaining low levels of circulating ammonia.

A. Sources of ammonia

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 (that is, the linking of aminotransferase and glutamate dehydrogenase reactions), producing ammonia. [Note: Liver catabolizes straight-chain amino acids, primarily.] However, substantial amounts of ammonia can be obtained from other sources.

Figure 19.18 Synthesis of glutamine. ADP = adenosine diphosphate; P_i = inorganic phosphate.



1. **From glutamine:** An important source of plasma glutamine is from the catabolism of branched-chain amino acids in skeletal muscle. This glutamine is taken up by cells of the intestine, the liver, and the kidney. The liver and kidneys generate ammonia from glutamine by the actions of glutaminase (Figure 19.17) and glutamate dehydrogenase. 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 glutamate dehydrogenase, is a glucogenic precursor in liver and kidney.] Ammonia is also generated by intestinal glutaminase. The intestinal mucosal cells 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.]

2. **From bacterial action in the intestine:** 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.

3. **From 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. 286).

4. **From purines and pyrimidines:** In the catabolism of purines and pyrimidines, amino groups attached to the ring atoms are released as ammonia (see Figure 22.15 and p. 304).

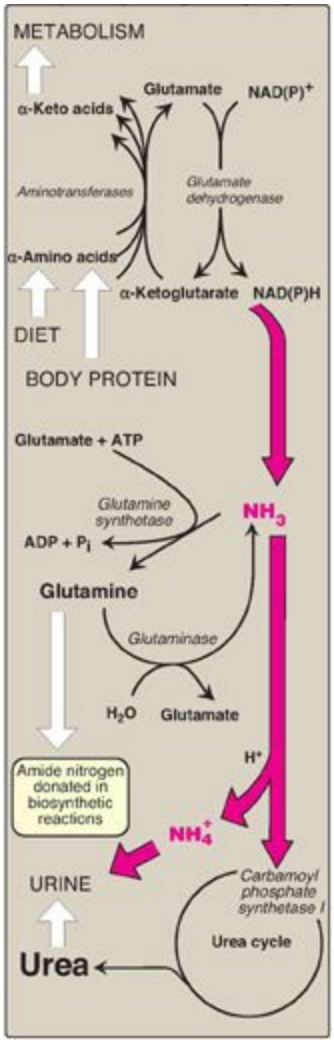
B. Transport of ammonia in the circulation

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 or alanine, rather than as free ammonia (see Figure 19.13).

1. **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.

2. **Glutamine:** This amide of glutamate provides a nontoxic storage and transport form of ammonia (Figure 19.18). The ATP-requiring formation of glutamine from glutamate and ammonia by glutamine synthetase occurs primarily in skeletal muscle and 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 and the urea cycle in periportal (close to inflow of blood) hepatocytes and via glutamine synthetase as an ammonia "scavenger" in the perivenous hepatocytes.] The metabolism of ammonia is summarized in Figure 19.19.

Figure 19.19 Metabolism of ammonia (NH_3). [Note: Glutamate dehydrogenase is one of several sources of NH_3.] Urea content in the urine is reported as urinary urea nitrogen, or UUN. Urea in blood is reported as BUN (blood urea nitrogen). The enzymes glutamate dehydrogenase, glutamine synthetase, and carbamoyl phosphate synthetase I fix NH_3 into organic molecules.



C. Hyperammonemia

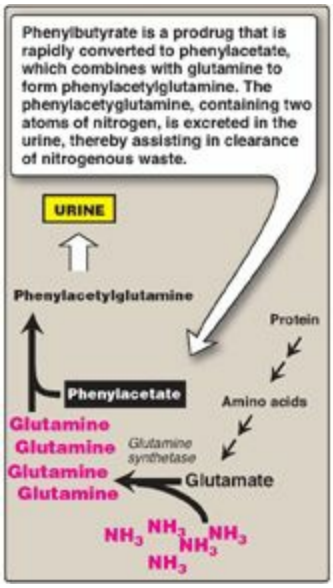
The capacity of the hepatic urea cycle exceeds the normal rates of ammonia generation, and the levels of serum ammonia are normally low (5–35 μmol/l). However, when liver function is compromised, due either to genetic defects of the urea cycle or liver disease, blood levels can rise above 1,000 μmol/l. 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.

1. **Acquired hyperammonemia:** Liver disease is a common cause of 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.

2. **Congenital hyperammonemia:** Genetic deficiencies of each of the five enzymes

of the urea cycle have been described, with an overall incidence estimated to be 1:25,000 live births. X-linked ornithine transcarbamoylase deficiency is the most common of these disorders, 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. [Note: The hyperammonemia seen with arginase deficiency is less severe because arginine contains two waste nitrogens and can be excreted in the urine.] Historically, urea cycle defects had high morbidity (neurologic manifestations) and mortality. Treatment included restriction of dietary protein in the presence of sufficient calories to prevent catabolism. Administration of compounds that bind covalently to amino acids, producing nitrogen-containing molecules that are excreted in the urine, has improved survival. For example, phenylbutyrate given orally is converted to phenylacetate. This condenses with glutamine to form phenylacetylglutamine, which is excreted (Figure 19.20).

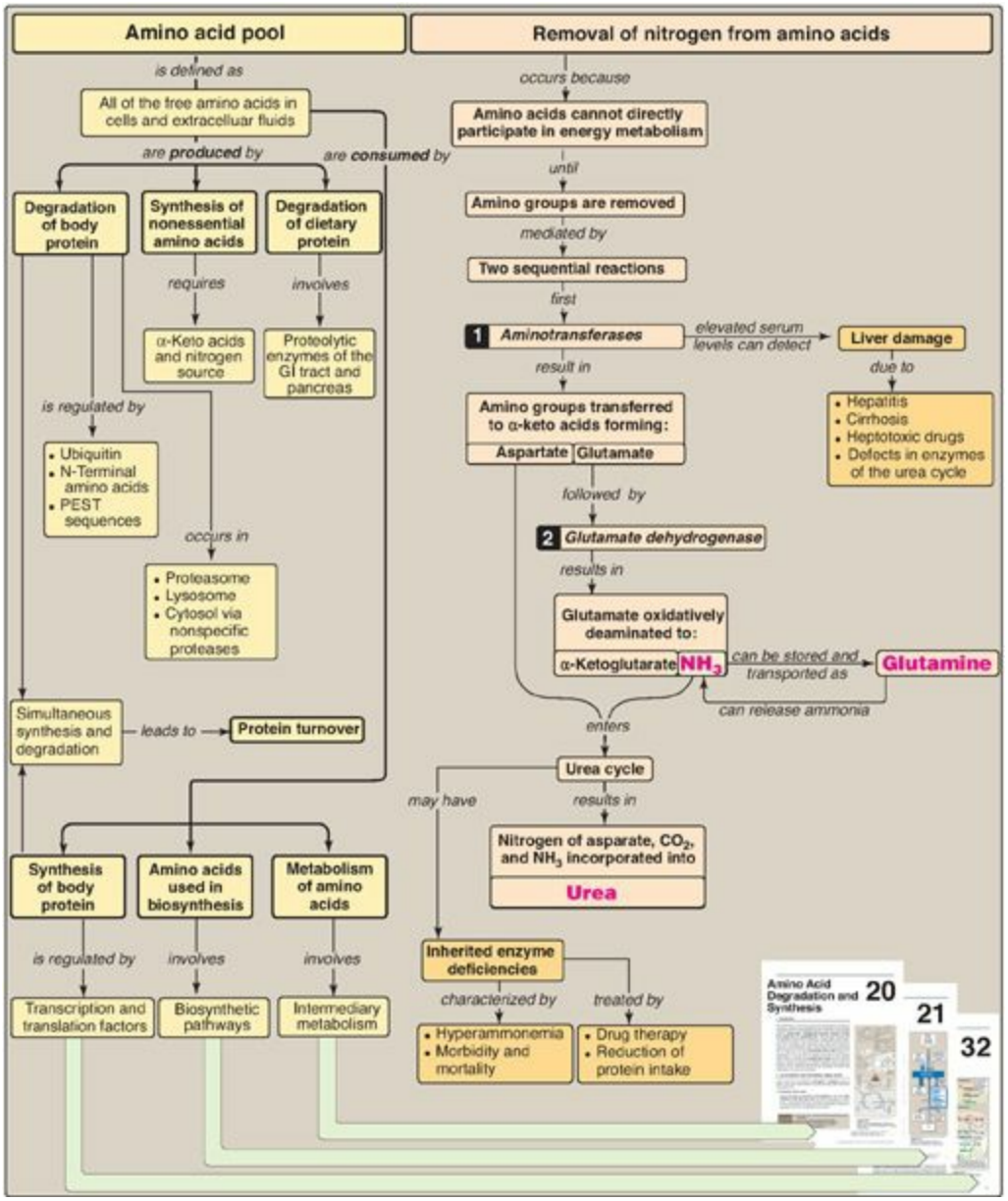
Figure 19.20 Treatment of patients with urea cycle defects by administration of phenylbutyrate to aid in excretion of ammonia (NH_3).



VIII. 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 (Figure 19.21). 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 cells via sodium-linked secondary active transport. Note that 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 adenosine triphosphate (ATP)-dependent, cytosolic, selective **ubiquitin–proteasome** and ATP-independent, 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 **glutamate dehydrogenase**, forming **ammonia** and the corresponding **α-keto acids**. A portion of the **free ammonia** is excreted in the **urine**, some of which 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. The two major causes of **hyperammonemia** (with its neurologic effects) are liver disease and inherited deficiencies of urea cycle enzymes such as X-linked **ornithine transcarbamolyase**.

Figure 19.21 Key concept map for nitrogen metabolism. GI = gastrointestinal; PEST = proline, glutamate, serine, threonine; NH_3 = ammonia.

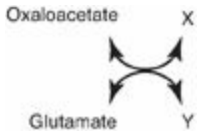

Amino acid pool
Removal of nitrogen from amino acids
is defined as
All of the free amino acids in cells and extracellular fluids
are produced by
are consumed by
Degradation of body protein
Synthesis of nonessential amino acids
Degradation of dietary protein
requires
involves
α-Keto acids and nitrogen source
Proteolytic enzymes of the GI tract and pancreas
is regulated by
Ubiquitin
N-Terminal amino acids
PEST sequences
occurs in
Proteasome
Lysosome
Cytosol via nonspecific proteases
Simultaneous synthesis and degradation
leads to
Protein turnover
Synthesis of body protein
Amino acids used in biosynthesis
Metabolism of amino acids
is regulated by
involves
involves
Transcription and translation factors
Biosynthetic pathways
Intermediary metabolism
occurs because
Amino acids cannot directly participate in energy metabolism
until
Amino groups are removed
mediated by
Two sequential reactions
first
1 Aminotransferases
elevated serum levels can detect
Liver damage
due to
Hepatitis
Cirrhosis
Heptotoxic drugs
Defects in enzymes of the urea cycle
result in
Amino groups transferred to α-keto acids forming:
Aspartate
Glutamate
followed by
2 Glutamate dehydrogenase
results in
Glutamate oxidatively deaminated to:
α-Ketoglutarate
NH3
can be stored and transported as
Glutamine
can release ammonia
enters
Urea cycle
may have
results in
Nitrogen of asparate, CO2, and NH3 incorporated into
Urea
Inherited enzyme deficiencies
characterized by
treated by
Hyperammonemia
Morbidity and mortality
Drug therapy
Reduction of protein intake
Amino Acid Degradation and Synthesis
20
21
32

Study Questions:

Choose the ONE best answer.

19.1 In the transamination reaction shown to the right, which of the following are the products X and Y?

A. Alanine, α-ketoglutarate
B. Asparate, α-ketoglutarate
C. Glutamate, alanine
D. Pyruvate, aspartate



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 proton-linked transport system.
B. In healthy, fed individuals, the input to the amino acid pool exceeds the output.
C. Liver uses ammonia to buffer protons.
D. Muscle-derived glutamine is metabolized in liver and kidney tissue to ammonia plus 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 deamidated to ammonia plus glutamate. The glutamate is deaminated to ammonia plus α-

ketoglutarate, which can be used for gluconeogenesis. Free amino acids are taken into enterocytes by a sodium-linked transport system. Healthy, fed individuals are in nitrogen balance, in which nitrogen input equals output. Liver converts ammonia to urea, and kidney uses 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– 19.5:

A female neonate did well until approximately 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 5–35 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 transcarbamoylase

Correct answer = C. Genetic deficiencies of each of the five enzymes of the urea cycle, as well as deficiencies in N-acetyglutamate 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 (argininosucinate 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

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 always accompanies hyperammonemia. Asparagine and lysine do not serve this sequestering role. Urea would be decreased due to 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 transcarbamoylase to form citrulline. Citrulline, containing one waste nitrogen, will be excreted.