

BMLT-2nd SEM

Biochemistry & Biophysics: Paper-V, Unit-9

Topic: PROTEIN METABOLISM

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PROTEIN 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, for example, in cells, blood, and the extracellular fluids. For the purpose of this discussion, envision all 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 body proteins,
- 2) amino acids derived from dietary protein, and
- 3) synthesis of nonessential amino acids from simple intermediates of metabolism.

Conversely, the amino pool is depleted by three routes:

- 1) synthesis of body protein, 2) amino acids consumed as precursors of essential nitrogen-containing small molecules, and 3) conversion of amino acids to glucose, glycogen, fatty acids, ketone bodies, or $\text{CO}_2 + \text{H}_2\text{O}$.

Although the amino acid pool is small (comprised of 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.

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, relatively constant, and cellular levels of the protein are controlled by selective degradation.

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.

Proteins are generally too large to be absorbed by the intestine. 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.

A. Digestion of proteins 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, functions instead to kill some bacteria and to denature proteins, thus 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. In general, zymogens contain extra amino acids in their sequences that prevent them from being catalytically active. [Note: Removal of these amino acids permits the proper folding required for an active enzyme.] Pepsinogen is activated to pepsin , either by HCl, or autocatalytically by other pepsin molecules that have already been activated. Pepsin releases peptides and a few free amino acids from dietary proteins.

B. Digestion of proteins 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.

1. Specificity: Each of these enzymes has a different specificity for the amino acid R-groups adjacent to the susceptible peptide bond. 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.

3. Activation of zymogens:

Enteropeptidase (formerly called enterokinase) —an enzyme synthesized by and present on the l uminal 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.

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 and undigested protein in the feces.

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 Na⁺-linked secondary transport system of the apical membrane. Di- and tri- peptides, however, are taken up by a H⁺-linked transport system. The peptides are hydrolyzed in the cytosol to amino acids that are released into the portal system by facilitated diffusion. Thus, 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.

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.
- The small intestine and the proximal tubule of the kidney have common transport systems for amino acid uptake; therefore, 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

Amino acid deamination

The first reaction in the breakdown of an amino acid is almost always removal of its alpha-amino group with the object of excreting excess nitrogen and degrading the remaining carbon skeleton or converting it to glucose. Urea, the predominant nitrogen excretion product in terrestrial mammals, is synthesized from ammonia and aspartate. Both of these latter substances are derived mainly from glutamate, a product of most deamination reactions.

Most amino acids are deaminated by transamination, the transfer of their amino group to an alpha-keto acid to yield the alpha-keto acid of the original amino acid and a new amino acid, in reactions catalyzed by aminotransferases.

REMOVAL OF NITROGEN FROM AMINO ACIDS

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).

The products are 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, thus becoming glutamate.

Glutamate produced by transamination can be oxidatively deaminated 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 (formerly called transaminases). These enzymes are found in the cytosol and mitochondria of cells throughout the body—especially those of the liver, kidney, intestine, and muscle. All amino acids, with the exception of lysine and threonine, participate in transamination at some point in their catabolism.

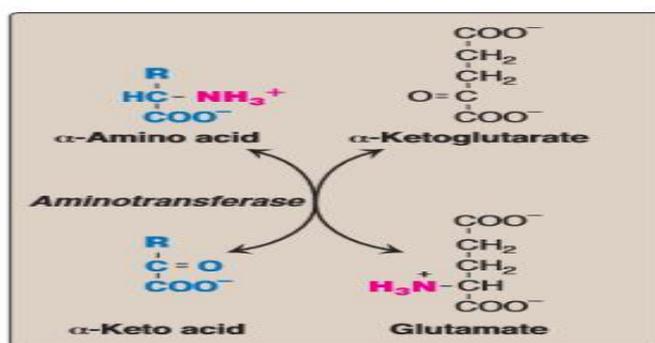


Figure 19.7
Aminotransferase reaction using α -ketoglutarate as the amino-group acceptor.

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. The two most important aminotransferase reactions are catalyzed by alanine aminotransferase (ALT) and aspartate aminotransferase (AST),

a. Alanine aminotransferase (ALT): 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. Thus, glutamate, in effect, acts as a “collector” of nitrogen from alanine.

b. Aspartate aminotransferase (AST): 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

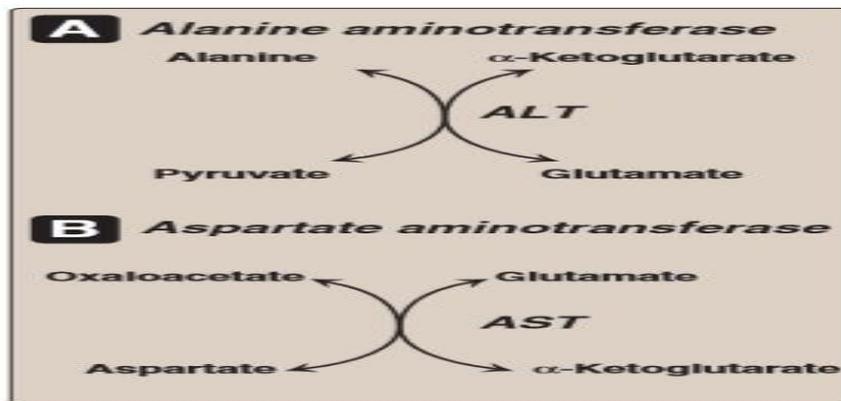


Figure 19.8
 Reactions catalyzed during amino acid catabolism. A. Alanine aminotransferase (ALT). B. Aspartate aminotransferase (AST).

2. Mechanism of action of aminotransferases:

All aminotransferases require the coenzyme pyridoxal phosphate (a derivative of vitamin B6), 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

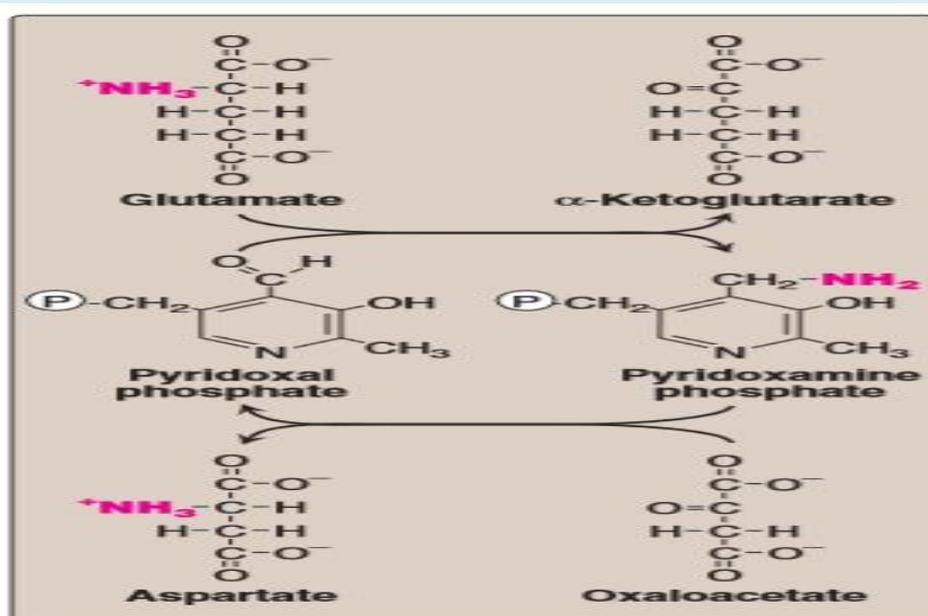


Figure 19.9
 Cyclic interconversion of pyridoxal phosphate and pyridoxamine phosphate during the aspartate aminotransferase reaction. [Note: (P) = phosphate group.]

UREA CYCLE

Urea 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 CO₂. ***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 mitochondria***, whereas the remaining cycle ***enzymes are located in the cytosol*** (Figure 19.14).

1. Formation of carbamoyl phosphate: Formation of carbamoyl phosphate by carbamoyl phosphate synthetase 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. Ultimately, the nitrogen atom derived from this ammonia becomes one of the nitrogens of urea. Carbamoyl phosphate synthetase I requires N-acetylglutamate as a positive allosteric activator (see Figure 19.14).

2. Formation of citrulline: The carbamoyl portion of carbamoyl phosphate is transferred to ornithine by ornithine transcarbamoylase (OTC) as the high-energy phosphate is released as Pi. 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. 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.

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 (AMP) and pyrophosphate. This is the third and final molecule of ATP consumed in the formation of urea.

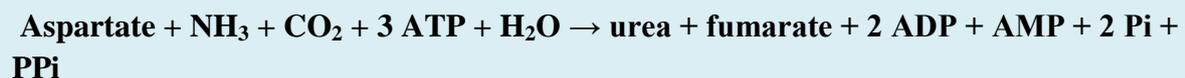
4. Cleavage of argininosuccinate: Argininosuccinate is cleaved by argininosuccinate lyase to yield arginine and fumarate. The arginine formed by this reaction serves as the immediate precursor of urea. Fumarate produced in the urea cycle is hydrated to malate, providing a link with several metabolic pathways. For example, the malate can be transported into the mitochondria via the malate shuttle, reenter the tricarboxylic acid cycle, and get oxidized to oxaloacetate (OAA), which can be used for gluconeogenesis. Alternatively, the OAA can be converted to aspartate via transamination, and can enter the urea cycle

5. Cleavage of arginine to ornithine and urea: Arginase cleaves arginine to ornithine and urea, and occurs almost exclusively in the liver. Thus, whereas other tissues, such as the

kidney, can synthesize arginine by these reactions, only the liver can cleave arginine and, thereby, synthesize urea.

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. 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 neomycin reduces the number of intestinal bacteria responsible for this NH_3 production.

Overall stoichiometry of the urea cycle



Four high-energy phosphate bonds are consumed in the synthesis of each molecule of urea; therefore, the synthesis of urea is irreversible, with a large, negative ΔG .

[Note: The ATP is replenished by oxidative phosphorylation.] 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.

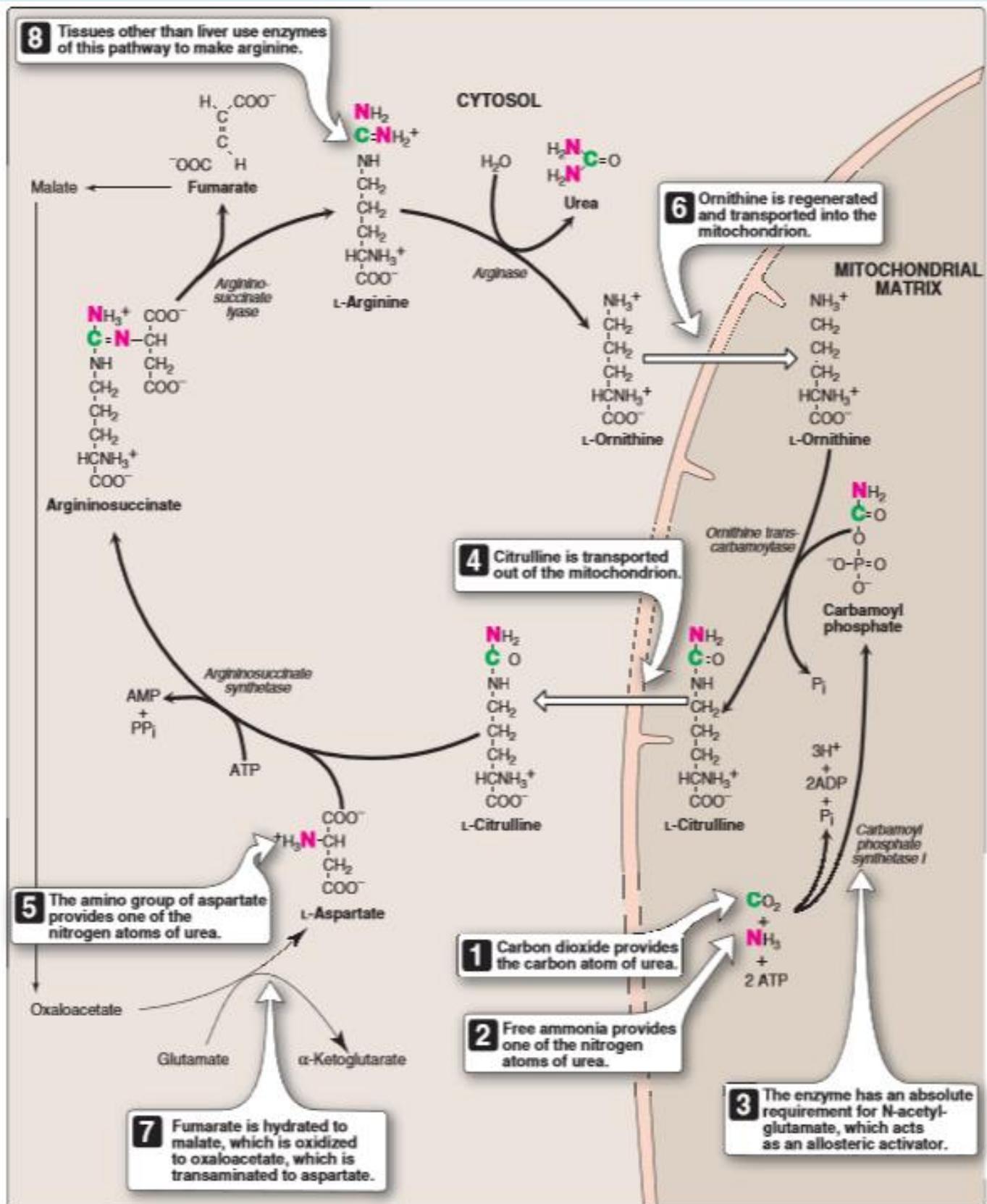


Figure 19.14
Reactions of the urea cycle.

METABOLIC DEFECTS IN AMINO ACID METABOLISM

Inborn errors of metabolism are commonly caused by mutant genes that generally result in abnormal proteins, most often enzymes.

The inherited defects may be expressed as a total loss of enzyme activity or, more frequently, as a partial deficiency in catalytic activity.

Without treatment, the inherited defects of amino acid metabolism almost invariably result in mental retardation or other developmental abnormalities as a consequence of harmful accumulation of metabolites.

Although more than 50 of these disorders have been described, many are rare, occurring in less than 1 per 250,000 in most populations. Collectively, however, they constitute a very significant portion of pediatric genetic diseases. Phenylketonuria is the most important disease of amino acid metabolism because it is relatively common and responds to dietary treatment.

A. Phenylketonuria

Phenylketonuria (PKU), caused by a deficiency of phenylalanine hydroxylase (Figure 20.15), is the most common clinically encountered inborn error of amino acid metabolism (prevalence 1:15,000). Biochemically, it is characterized by accumulation of phenylalanine (and a deficiency of tyrosine). Hyper phenyl alaninemia may also be caused by deficiencies in any of the several enzymes required to synthesize BH₄, or in dihydropteridine reductase, which regenerates BH₄ from BH₂ (Figure 20.16). Such deficiencies indirectly raise phenylalanine concentrations, because phenyl alanine

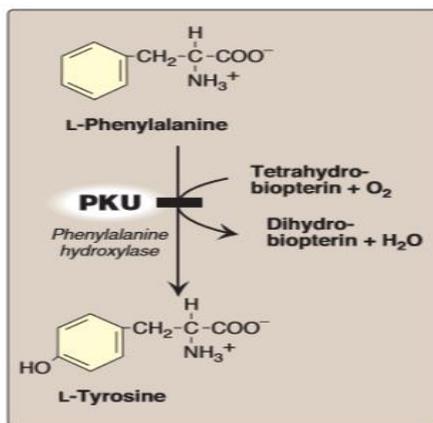


Figure 20.15
A deficiency in *phenylalanine hydroxylase* results in the disease phenylketonuria (PKU).

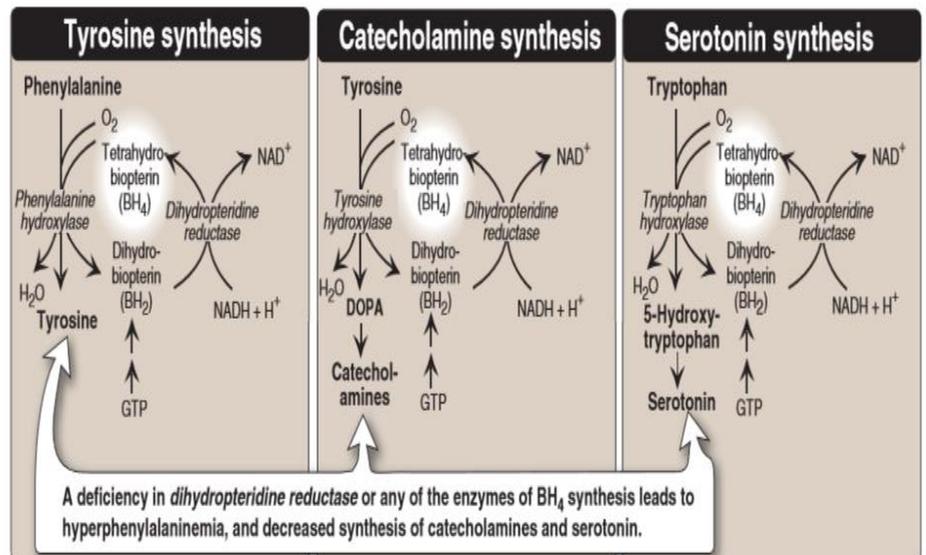


Figure 20.16
Biosynthetic reactions involving amino acids and tetrahydrobiopterin.

hydroxylase requires BH₄ as a coenzyme. BH₄ is also required for tyrosine hydroxylase and tryptophan hydroxylase, which catalyze reactions leading to the synthesis of neurotransmitters, such as serotonin and the catecholamines. Simply restricting dietary phenylalanine does not reverse the central nervous system (CNS) effects due to deficiencies in neurotransmitters. Replacement therapy with BH₄ or L-DOPA and 5-hydroxytryptophan (products of the affected tyrosine hydroxylase- and tryptophan hydroxylase -catalyzed reactions) improves the clinical outcome in these variant forms of hyper phenyl alaninemia, although the response is unpredictable.

1. Characteristics of classic PKU:

a. *Elevated phenylalanine*: Phenylalanine is present in elevated concentrations in tissues, plasma, and urine. Phenyllactate, phenyl acetate, and phenylpyruvate, which are not normally produced in significant amounts in the presence of functional phenylalanine hydroxylase, are also elevated in PKU. These metabolites give urine a characteristic musty (“mousey”) odor. [Note: The disease acquired its name from the presence of a phenylketone (now known to be phenyl- pyruvate) in the urine.]

b. *CNS symptoms*: Mental retardation, failure to walk or talk, seizures, hyperactivity, tremor, microcephaly, and failure to grow are characteristic findings in PKU. The patient with untreated PKU typically shows symptoms of mental retardation by the age of 1 year, and rarely achieves an IQ greater than 50

c. *Hypopigmentation*: Patients with phenylketonuria often show a deficiency of pigmentation (fair hair, light skin color, and blue eyes). The hydroxylation of tyrosine by tyrosinase, which is the first step in the formation of the pigment melanin, is competitively inhibited by the high levels of phenylalanine present in PKU.

2. Neonatal screening and diagnosis of PKU:

Early diagnosis of phenylketonuria is important because the disease is treatable by dietary means. Because of the lack of neonatal symptoms, laboratory testing for elevated blood levels of phenylalanine is mandatory for detection.

However, the infant with PKU frequently has normal blood levels of phenylalanine at birth because the mother clears increased blood phenylalanine in her affected fetus through the placenta. Normal levels of phenylalanine may persist until the new born is exposed to 24–48 hours of protein feeding. Thus, screening tests are typically done after this time to avoid false negatives. For newborns with a positive screening test, diagnosis is confirmed through quantitative determination of phenylalanine levels.

4. Treatment of PKU:

Most natural protein contains phenylalanine, and it is impossible to satisfy the body’s protein requirement when ingesting a normal diet without exceeding the phenylalanine limit. Therefore, in PKU, blood phenylalanine is maintained close to the normal range by feeding synthetic amino acid preparations low in phenylalanine, supplemented with some natural

foods (such as fruits, vegetables, and certain cereals) selected for their low phenylalanine content. The amount is adjusted according to the tolerance of the individual as measured by blood phenylalanine levels. The earlier treatment is started, the more completely neurologic damage can be prevented. [Note: Treatment must begin during the first 7–10 days of life to prevent mental retardation.] Because phenylalanine is an essential amino acid, overzealous treatment that results in blood phenylalanine levels below normal is avoided because this can lead to poor growth and neurologic symptoms. In patients with PKU, tyrosine cannot be synthesized from phenylalanine and, therefore, it becomes an essential amino acid and so must be supplied in the diet.

B. Maple syrup urine disease:

Maple syrup urine disease (MSUD) is a rare (1:185,000), autosomal recessive disorder in which there is a partial or complete deficiency in branched-chain α -keto acid dehydrogenase, an enzyme complex that decarboxylates leucine, isoleucine, and valine. These amino acids and their corresponding α -keto acids accumulate in the blood, causing a toxic effect that interferes with brain functions. The disease is characterized by feeding problems, vomiting, dehydration, severe metabolic acidosis, and a characteristic maple syrup odor to the urine. If untreated, the disease leads to mental retardation, physical disabilities, and even death.

1. Classification:

The term “maple syrup urine disease” includes a classic type and several variant forms of the disorder. The classic form is the most common type of MSUD. Leukocytes or cultured skin fibroblasts from these patients show little or no branched-chain α -keto acid dehydrogenase activity. Infants with classic MSUD show symptoms within the first several days of life. If not diagnosed and treated, classic MSUD is lethal in the first weeks of life. Patients with intermediate forms have a higher level of enzyme activity (approximately 3–15% of normal).

The symptoms are milder and show an onset from infancy to adulthood. Patients with the rare thiamine-dependent variant of MSUD achieve increased activity of branched-chain α -keto acid dehydrogenase if given large doses of this vitamin.

2. Screening and diagnosis:

As with PKU, prenatal diagnosis and neonatal screening are available, and most affected individuals are compound heterozygotes.

3. Treatment:

The disease is treated with a synthetic formula that contains limited amounts of leucine, isoleucine, and valine—sufficient to provide the branched-chain amino acids necessary for normal growth and development without producing toxic levels. Early diagnosis and lifelong dietary treatment is essential if the child with MSUD is to develop normally. [Note: Branched-chain amino acids are an important energy source in times of metabolic need, and individuals with MSUD are at risk of decompensation during periods of increased protein catabolism.]

C. Albinism

Albinism refers to a group of conditions in which a defect in tyrosine metabolism results in a deficiency in the production of melanin. These defects result in the partial or full absence of pigment from the skin, hair, and eyes. Albinism appears in different forms, and it may be inherited by one of several modes: autosomal recessive (primary mode), autosomal dominant, or X-linked. Complete albinism (also called tyrosinase -negative oculocutaneous albinism) results from a deficiency of copper-requiring tyrosinase, causing a total absence of pigment from the hair, eyes, and skin. It is the most severe form of the condition. In addition to hypopigmentation, affected individuals have vision defects and photophobia (sunlight hurts their eyes). They are at increased risk for skin cancer.

D. Homocystinuria

The homocystinurias are a group of disorders involving defects in the metabolism of homocysteine. The diseases are inherited as autosomal recessive illnesses, characterized by high plasma and urinary levels of homocysteine and methionine and low levels of cysteine. The most common cause of homocystinuria is a defect in the enzyme cystathionine β -synthase, which converts homocysteine to cystathionine (Figure 20.21). Individuals who are homozygous for cystathionine β -synthase deficiency exhibit ectopia lentis (displacement of the lens of the eye), skeletal abnormalities, a tendency to form thrombi (blood clots), osteoporosis, and neurological deficits. Patients can be responsive or nonresponsive to oral administration of pyridoxine (vitamin B₆)—a coenzyme of cystathionine β -synthase. Vitamin B₆-responsive patients usually have a milder and later onset of clinical symptoms compared with B₆-nonresponsive patients. Treatment includes restriction of methionine intake and supplementation with vitamins B₆, B₁₂, and folate.

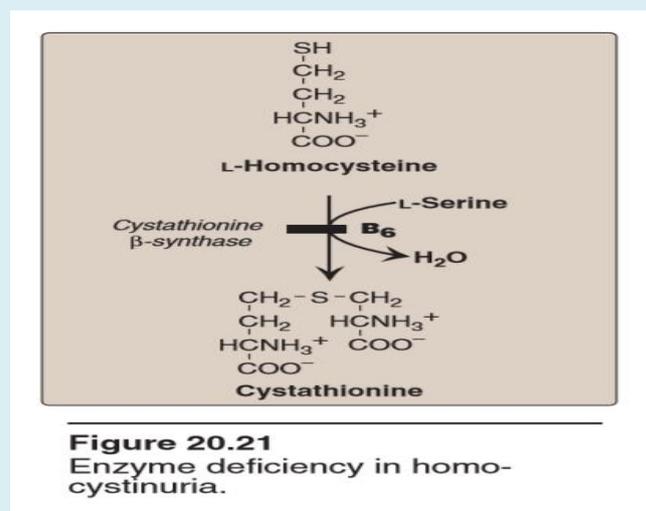


Figure 20.21
Enzyme deficiency in homocystinuria.

E. Alkaptonuria (Alcaptonuria)

Alkaptonuria is a rare metabolic condition involving a deficiency in homogentisic acid oxidase, resulting in the accumulation of homogentisic acid. The condition has three characteristic symptoms: homogentisic aciduria (the patient's urine contains elevated levels of homogentisic acid, which is oxidized to a dark pigment on standing), large joint arthritis, and black ochronotic pigmentation of cartilage and collagenous tissue. Patients with alkaptonuria are usually asymptomatic until about age 40. Dark staining of the diapers sometimes can indicate the disease in infants, but usually no symptoms are present until later in life. Diets low in protein—especially in phenylalanine and tyrosine—help reduce the levels of homogentisic acid, and decrease the amount of pigment deposited in body tissues. Although alkaptonuria is not life-threatening, the associated arthritis may be severely crippling.

Questions

1. Define amino acid pool.
2. What do you mean by protein turnover.
3. How protein can be digested by pancreatic enzymes.
4. What are the abnormalities of protein digestion.
5. Explain transamination of amino acids.
6. Explain in detail about the urea cycle along with its stoichiometric equation.
7. Short notes on:
 - a. Phenylketonuria
 - b. Alkaptonuria
 - c. Albinism
8. Explain the term “maple syrup urine disease”.