Nitrogen Metabolism

ietary proteins are the primary source of the nitrogen that is metabolized by the body (Fig. 1). Amino acids, produced by digestion of dietary proteins, are absorbed through intestinal epithelial cells and enter the blood. Various cells take up these amino acids, which enter the cellular pools. They are used for the synthesis of proteins and other nitrogen-containing compounds or they are oxidized for energy.

Protein synthesis, the translation of mRNA on ribosomes (see Chapter 15), is a dynamic process. Within the body, proteins are constantly being synthesized and degraded, partially draining and then refilling the cellular amino acid pools.

Compounds derived from amino acids include cellular proteins, hormones, neurotransmitters, creatine phosphate, the heme of hemoglobin and the cytochromes, the skin pigment melanin, and the purine and pyrimidine bases of nucleotides and nucleic acids. In fact, all of the nitrogen-containing compounds of the body are synthesized from amino acids. Many of these pathways are outlined in the following chapters of the book.

In addition to serving as the precursors for the nitrogen-containing compounds of the body and as the building blocks for protein synthesis, amino acids are also a source of energy. Amino acids are directly oxidized or they are converted to glucose and then oxidized or stored as glycogen. They also may be converted to fatty acids and stored as adipose triacylglycerols. Glycogen and triacylglycerols are oxidized during periods of fasting. The liver is the major site of amino acid oxidation. However, most tissues can oxidize the branched-chain amino acids (leucine, isoleucine, and valine).

Before the carbon skeletons of amino acids are oxidized, the nitrogen must be removed. Amino acid nitrogen forms ammonia, which is toxic to the body. In the liver, ammonia and the amino groups from amino acids are converted to urea, which is nontoxic, water-soluble, and readily excreted in the urine. The process by which urea is produced is known as the urea cycle. The liver is the organ responsible for producing urea. Branched-chain amino acids can be oxidized in many tissues, but the nitrogen must always travel to the liver for disposal.

Although urea is the major nitrogenous excretory product of the body, nitrogen is also excreted in other compounds (Table 1). Uric acid is the degradation product

Table 1. Major Nitrogenous	Urinary Excretory I	Products
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Amount excreted in urine/day ^a		
12–20 g urea nitrog	gen (12,000–20,000 mg)	
140–1,500 mg ammonia nitrogen		
Males:	14–26 mg/kg	
Females:	11–20 mg/kg	
250–750 mg		
	12–20 g urea nitrog 140–1,500 mg amr Males: Females:	

^aThe amounts are expressed in units generally reported by clinical laboratories. Note that the amounts for creatinine and uric acid are for the whole compound, whereas those for urea and ammonia are for the nitrogen content.

^bUnder normal circumstances, approximately 90% of the nitrogen excreted in the urine is in the form of urea. The exact amounts of each component vary, however, depending on dietary protein intake and physiologic state. For instance, NH₄⁺ excretion increases during an acidosis because the kidney secretes ammonia to bind protons in the urine.



Fig. 1. Summary of amino acid metabolism. Dietary proteins are digested to amino acids in the stomach and intestine, which are absorbed by the intestinal epithelium, transferred to the circulation, and taken up by cells. Amino acids are used to synthesize proteins and other nitrogen-containing compounds. The carbon skeletons of amino acids are also oxidized for energy, and the nitrogen is converted to urea and other nitrogenous excretory products.



The healthy human adult is in nitrogen balance; in other words, the

amount of nitrogen excreted each day (mainly in the urine) equals the amount consumed (mainly as dietary protein). Negative nitrogen balance occurs when the amount of nitrogen excreted is greater than the amount consumed, and positive nitrogen balance occurs when the amount excreted is less than that consumed (see Chapter 1).

Table 2. Amino Acids Synthesized in the Body^a

From Glucose	From an Essential Amino Acid
Serine Glycine Cysteine ^b Alanine Aspartate Aspartagine Glutamate Glutamine	Tyrosine (from phenylalanine)
Proline Arginine	

^aThese amino acids are called "nonessential" or "dispensable," terms that refer to dietary requirements. Of course, within the body, they are necessary. We cannot survive without them. ^bAlthough the carbons of cysteine can be derived from glucose, its sulfur is obtained from the essential amino acid methionine.

of the purine bases, creatinine is produced from creatine phosphate, and ammonia is released from glutamine, particularly by the kidney, where it helps to buffer the urine by reacting with protons to form ammonium ions (NH_4^+) . These compounds are excreted mainly in the urine, but substantial amounts are also lost in the feces and through the skin. Small amounts of nitrogen-containing metabolites are formed from the degradation of neurotransmitters, hormones, and other specialized amino acid products and excreted in the urine. Some of these degradation products, such as bilirubin (formed from the degradation of heme), are excreted mainly in the feces.

Eleven of the 20 amino acids used to form proteins are synthesized in the body if an adequate amount is not present in the diet (Table 2). Ten of these amino acids can be produced from glucose; the 11th, tyrosine, is synthesized from the essential amino acid phenylalanine. It should be noted that cysteine, one of the 10 amino acids produced from glucose, obtains its sulfur atom from the essential amino acid methionine.



Fig. 2. Overview of nitrogen metabolism. The metabolism of nitrogen-containing compounds is shown on the right and that of glucose and fatty acids is shown on the left. This figure shows a hypothetical, composite cell. No single cell type has all of these pathways. Many of the pathways shown are described in the next few chapters. $\alpha KG = \alpha$ -ketoglutarate; OAA = oxaloacetate; G-6-P = glucose 6-phosphate; G-1-P = glucose 1-phosphate.

Nine amino acids are essential in the human. "Essential" means that the carbon skeleton cannot be synthesized and, therefore, these amino acids are required in the diet (Table 3). The essential amino acids are also called the indispensable amino acids. Arginine is essential during periods of growth; in adults it is no longer considered essential.

After nitrogen is removed from amino acids, the carbon skeletons are oxidized (Fig. 2). Most of the carbons are converted to pyruvate, intermediates of the tricarboxylic acid (TCA) cycle, or to acetyl CoA. In the liver, particularly during fasting, these carbons may be converted to glucose or to ketone bodies and released into the blood. Other tissues then oxidize the glucose and ketone bodies. Ultimately, the carbons of the amino acids are converted to CO_2 and H_2O .

Table 3. Amino Acids Essential in theDiet

Lysine Isoleucine Leucine Threonine Valine Tryptophan Phenylalanine Methionine Histidine Arginine (not required by the adult, but required for growth)

37 Protein Digestion and Amino Acid Absorption

Proteolytic enzymes (also called **proteases**) break down dietary proteins into their constituent amino acids in the **stomach** and the **intestine**. Many of these digestive proteases are synthesized as larger, inactive forms known as **zymogens**. After zymogens are secreted into the digestive tract, they are cleaved to produce the active proteases.

In the stomach, **pepsin** begins the digestion of proteins by hydrolyzing them to smaller polypeptides. The contents of the stomach pass into the small intestine, where enzymes produced by the exocrine pancreas act. The pancreatic proteases (**trypsin**, **chymotrypsin**, **elastase**, and the **carboxypeptidases**) cleave the polypeptides into oligopeptides and amino acids.

Further cleavage of the oligopeptides to amino acids is accomplished by enzymes produced by the intestinal epithelial cells. These enzymes include **aminopeptidases** located on the brush border and other peptidases located within the cells. Ultimately, the amino acids produced by protein digestion are absorbed through the **intestinal epithelial cells** and enter the **blood**.

A large number of overlapping transport systems exist for amino acids in cells. Some systems contain *facilitative transporters*, whereas others express *sodium-linked tranporters*, which allow the *active transport* of amino acids into cells. Defects in amino acid transport can lead to disease.

Proteins are also continually synthesized and degraded (turnover) in cells. A wide variety of proteases exist in cells to carry out this activity. Lysosomal proteases (cathepsins) degrade proteins that enter lysosomes. Cytoplasmic proteins targeted for turnover are covalently linked to the small protein ubiquitin, which then interacts with a large protein complex, the proteasome, to degrade the protein in an adenosine triphosphate (ATP)-dependent process. The amino acids released from proteins during turnover can then be used for the synthesis of new proteins or for energy generation.



THE WAITING ROOM

Sissy Fibrosa, a young child with cystic fibrosis, has had repeated bouts of bronchitis caused by *Pseudomonas aeruginosa*. With each of these infections, her response to aerosolized antibiotics has been good. However,

her malabsorption of food continues, resulting in foul-smelling, glistening, bulky stools. Her growth records show a slow decline. She is now in the 24th percentile for height and the 20th percentile for weight. She is often listless and irritable, and she tires easily. When her pediatrician discovered that her levels of the serum proteins



Fig. 37.1. Digestion of proteins. The proteolytic enzymes, pepsin, trypsin, chymotrypsin, elastase, and the carboxypeptidases, are produced as zymogens (the [pro] and [ogen] accompanying the enzyme name) that are activated by cleavage after they enter the gastrointestinal lumen (see Fig. 37.2).



Kwashiorkor, a common problem of children in Third World countries, is caused by a deficiency of protein in a diet that is adequate in calories. Children with kwashiorkor suffer from muscle wasting and a decreased concentration of plasma proteins, particularly albumin. The result is an increase in interstitial fluid that causes edema and a distended abdomen that make the children appear "plump" (see Chapter 44). The muscle wasting is caused by the lack of essential amino acids in the diet; existing proteins must be broken down to produce these amino acids for new protein synthesis.

These problems may be compounded by a decreased ability to produce digestive enzymes and new intestinal epithelial cells because of a decreased availability of amino acids for the synthesis of new proteins.

albumin, transferrin, and thyroid hormone binding prealbumin (transthyretin) were low to low-normal (indicating protein malnutrition), Sissy was given enteric-coated microspheres of pancreatic enzymes. Almost immediately, the character of Sissy's stools became more normal and she began gaining weight. In the next 6 months, her growth curves showed improvement, and she seemed brighter, more active, and less irritable.

For the first few months after a painful episode of renal colic, during which he passed a kidney stone (see Chapter 6), Cal Kulis had faithfully maintained a high daily fluid intake and had taken the medication required to increase the pH of his urine. Because he has cystinuria, these measures were necessary to increase the solubility of the large amounts of cystine present in his urine and, thereby, to prevent further formation of kidney stones (calculi). With time, however, he became increasingly complacent about his preventive program. After failing to take his medication for a month, he experienced another severe episode of renal colic with grossly bloody urine. Fortunately, he passed the stone spontaneously, after which he vowed to faithfully comply with therapy.

His mother heard that some dietary amino acids were not absorbed in patients with cystinuria and asked whether any dietary changes would reduce Cal's chances of developing additional renal stones.

I. PROTEIN DIGESTION

The digestion of proteins begins in the stomach and is completed in the intestine (Fig. 37.1). The enzymes that digest proteins are produced as inactive precursors (zymogens) that are larger than the active enzymes. The inactive zymogens are secreted from the cells in which they are synthesized and enter the lumen of the digestive tract, where they are cleaved to smaller forms that have proteolytic activity (Fig. 37.2). These active enzymes have different specificities; no single enzyme can completely digest a protein. However, by acting in concert, they can digest dietary proteins to amino acids and small peptides, which are cleaved by peptidases associated with intestinal epithelial cells



Fig. 37.2. Activation of the gastric and pancreatic zymogens. Pepsinogen catalyzes its own cleavage as the pH of the stomach drops. Trypsinogen is cleaved by enteropeptidase in the intestine to form the active protease trypsin. Trypsin then plays a key role by catalyzing the cleavage and activation of the other pancreatic zymogens.

A. Digestion of Proteins in the Stomach

Pepsinogen is secreted by the chief cells of the stomach. The gastric parietal cells secrete HCl. The acid in the stomach lumen alters the conformation of pepsinogen so that it can cleave itself, producing the active protease pepsin. Thus, the activation of pepsinogen is autocatalytic.

Dietary proteins are denatured by the acid in the stomach. This serves to inactivate the proteins and partially unfolds them such that they are better substrates for proteases. However, at the low pH of the stomach, pepsin is not denatured and acts as an endopeptidase, cleaving peptide bonds at various points within the protein chain. Although pepsin has a fairly broad specificity, it tends to cleave peptide bonds in which the carboxyl group is provided by an aromatic or acidic amino acid (Fig. 37.3). Smaller peptides and some free amino acids are produced.

B. Digestion of Proteins by Enzymes from the Pancreas

As the gastric contents empty into the intestine, they encounter the secretions from the exocrine pancreas. One of these secretions is bicarbonate, which, in addition to neutralizing the stomach acid, raises the pH such that the pancreatic proteases, which are also present in pancreatic secretions, can be active. As secreted, these pancreatic proteases are in the inactive proenzyme form (zymogens). Because the active forms of these enzymes can digest each other, it is important for their zymogen forms all to be activated within a short span of time. This feat is accomplished by the cleavage of trypsinogen to the active enzyme trypsin, which then cleaves the other pancreatic zymogens, producing their active forms (see Fig. 37.2).

The zymogen trypsinogen is cleaved to form trypsin by enteropeptidase (a protease, formerly called enterokinase) secreted by the brush-border cells of the small intestine. Trypsin catalyzes the cleavages that convert chymotrypsinogen to the active enzyme chymotrypsin, proelastase to elastase, and the procarboxypeptidases to the carboxypeptidases. Thus, trypsin plays a central role in digestion because it both cleaves dietary proteins and activates other digestive proteases produced by the pancreas.

Trypsin, chymotrypsin, and elastase are serine proteases (see Chapter 9) that act as endopeptidases. Trypsin is the most specific of these enzymes, cleaving peptide bonds in which the carboxyl (carbonyl) group is provided by lysine or arginine (see Fig. 37.3). Chymotrypsin is less specific but favors residues that contain hydrophobic or acidic amino acids. Elastase cleaves not only elastin (for which it was named) but also other proteins at bonds in which the carboxyl group is contributed by amino acid residues with small side chains (alanine, glycine, or serine). The actions of these pancreatic endopeptidases continue the digestion of dietary proteins begun by pepsin in the stomach.



Elastase is also found in neutrophils, white blood cells that have the job of engulfing and destroying invading bacteria. Neutrophils frequently act in the lung, and elastase is sometimes released into the lung as the neutrophils work. In normal individuals, the released elastase is blocked from destroying lung cells by the action of circulating α -1-antitrypsin, a protease inhibitor synthesized and secreted by the liver. Certain individuals have a genetic mutation that leads to the production of an inactive α -1-antitrypsin protein (α -1-antitrypsin deficiency). The lack of this enzyme activity leads to the development of emphysema caused by proteolytic destruction of lung cells, which results in a reduction in the expansion/contraction capability of the lungs.



Patients with cystic fibrosis, such as Sissy Fibrosa, have a genetically determined defect in the function of the chloride channels. In the pancreatic secretory ducts, which carry pancreatic enzymes into the lumen of the small intestines, this defect causes inspissation (drying and thickening) of pancreatic exocrine secretions, eventually leading to obstruction of these ducts. One result of this problem is the inability of pancreatic enzymes to enter the intestinal lumen to digest dietary proteins.



Fig. 37.3. Action of the digestive proteases. Pepsin, trypsin, chymotrypsin, and elastase are endopeptidases; they hydrolyze peptide bonds within chains. The others are exopeptidases; aminopeptidases remove the amino acid at the N-terminus and the carboxypeptidases remove the amino acid at the C terminus. For each proteolytic enzyme, the amino acid residues involved in the peptide bond that is cleaved are listed beside the R group to the right of the enzyme name.

Recall that the exocrine pancreas, in addition to secreting proteolytic zymogens, also secretes amylase for starch digestion and lipase and co-lipase for dietary triacylglycerol digestion.



The pancreas synthesizes and stores the zymogens in secretory granules. The pancreas also syn-

thesizes a secretory trypsin inhibitor. The need for the inhibitor is to block any trypsin activity that may occur from accidental trypsinogen activation. If the inhibitor were not present, trypsinogen activation would lead to the activation of all of the zymogens in the pancreas, which would lead to the digestion of intracellular pancreatic proteins. Such episodes can lead to pancreatitis.





Hartnup disease is another genetically determined and relatively rare autosomal recessive disorder. It is caused by a defect in the transport of neutral amino acids across both intestinal and renal epithelial cells. The signs and symptoms are, in part, caused by a deficiency of essential amino acids (see Clinical Comments). Cystinuria and Hartnup disease involve defects in two different transport proteins. In each case, the defect is present both in intestinal cells, causing malabsorption of the amino acids from the digestive products in the intestinal lumen and in kidney tubular cells, causing a decreased resorption of these amino acids from the glomerular filtrate.



Trace amounts of polypeptides pass into the blood. They may be transported through intestinal epithelial

cells, probably by pinocytosis, or they may slip between the cells that line the gut wall. This process is particularly troublesome for premature infants, because it can lead to allergies caused by proteins in their food.

The smaller peptides formed by the action of trypsin, chymotrypsin, and elastase are attacked by exopeptidases, which are proteases that cleave one amino acid at a time from the end of the chain. Procarboxypeptidases, zymogens produced by the pancreas, are converted by trypsin to the active carboxypeptidases. These exopeptidases remove amino acids from the carboxyl ends of peptide chains. Carboxypeptidase A preferentially releases hydrophobic amino acids, whereas carboxypeptidase B releases basic amino acids (arginine and lysine).

C. Digestion of Proteins by Enzymes from Intestinal Cells

Exopeptidases produced by intestinal epithelial cells act within the brush border and also within the cell. Aminopeptidases, located on the brush border, cleave one amino acid at a time from the amino end of peptides. Intracellular peptidases act on small peptides that are absorbed by the cells.

The concerted action of the proteolytic enzymes produced by cells of the stomach, pancreas, and intestine cleaves dietary proteins to amino acids. The digestive enzymes digest themselves as well as dietary protein. They also digest the intestinal cells that are regularly sloughed off into the lumen. These cells are replaced by cells that mature from precursor cells in the duodenal crypts. The amount of protein that is digested and absorbed each day from digestive juices and cells released into the intestinal lumen may be equal to, or greater than, the amount of protein consumed in the diet (50–100 g).

II. ABSORPTION OF AMINO ACIDS

Amino acids are absorbed from the intestinal lumen through secondary active Na⁺dependent transport systems and through facilitated diffusion.

A. Cotransport of Na⁺ and Amino Acids

Amino acids are absorbed from the lumen of the small intestine principally by semispecific Na⁺-dependent transport proteins in the luminal membrane of the intestinal cell brush border, similar to that already seen for carbohydrate transport (Fig 37.4). The cotransport of Na^+ and the amino acid from the outside of the apical membrane to the inside of the cell is driven by the low intracellular Na⁺ concentration. Low intracellular Na⁺ results from the pumping of Na⁺ out of the cell by a Na⁺,K⁺-ATPase on the serosal membrane. Thus, the primary transport mechanism is the creation of a sodium gradient; the secondary transport process is the coupling of amino acids to the influx of sodium. This mechanism allows the cells to concentrate amino acids from the intestinal lumen. The amino acids are then transported out of the cell into the interstitial fluid principally by facilitated transporters in the serosal membrane (see Fig. 37.4).

At least six different Na⁺-dependent amino acid carriers are located in the apical brush border membrane of the epithelial cells. These carriers have an overlapping specificity for different amino acids. One carrier preferentially transports neutral amino acids, another transports proline and hydroxyproline, a third preferentially transports acidic amino acids, and a fourth transports basic amino acids (lysine, arginine, the urea cycle intermediate ornithine) and cystine (two cysteine residues linked by a disulfide bond). In addition to these Na⁺-dependent carriers, some amino acids are transported across the luminal membrane by facilitated transport carriers. Most amino acids are transported by more than one transport system.

As with glucose transport, the Na⁺-dependent carriers of the apical membrane of the intestinal epithelial cells are also present in the renal epithelium. However, different isozymes are present in the cell membranes of other tissues. Conversely, the facilitated transport carriers in the serosal membrane of the intestinal epithelia are similar to those found in other cell types in the body. During starvation, the intestinal epithelia, like these other cells, take up amino acids from the blood to use as an energy source. Thus, amino acid transport across the serosal membrane is bidirectional.

System Name	Sodium-dependent?	Specificity	Tissues Expressed
А	Yes	Small amino acids (ala, ser, gln)	Many
ASC	Yes	Small amino acids (ala, ser, cys)	Many
Ν	Yes	Gln, asn, his	Liver
L	No	Branched and aromatic amino acids	Many
B ^{0,+}	Yes	Basic amino acids	Intestine (brush border) ^b
ATB°	Yes	Zwitterionic amino acids (monoamino, monocarboxylic acid amino acids)	Intestine and kidney ^c
X _{AG}	Yes	Anionic amino acids	Intestine (brush border)
Imino	Yes	Pro, hypro, gly	Intestine (brush border)

Table 37.1 A Partial Listing of Amino Acid Transport Systems

^a Not all transport systems are listed.

^b This system is most likely defective in cystinuria.

^c This system is most likely defective in Hartnup disease.

B. Transport of Amino Acids into Cells

Amino acids that enter the blood are transported across cell membranes of the various tissues principally by Na⁺-dependent cotransporters and, to a lesser extent, by facilitated transporters (Table 37.1). In this respect, amino acid transport differs from glucose transport, which is Na⁺-dependent transport in the intestinal and renal epithelium but facilitated transport in other cell types. The Na⁺ dependence of amino acid transport in liver, muscle, and other tissues allows these cells to concentrate amino acids from the blood. These transport proteins have a different genetic basis, amino acid composition, and somewhat different specificity than those in the luminal membrane of intestinal epithelia. They also differ somewhat between tissues. For instance, the N system for glutamine uptake is present in the liver but either not present in other tissues or present as an isoform with different properties. There is also some overlap in specificity of the transport proteins, with most amino acids being transported by more than one carrier.

III. PROTEIN TURNOVER AND REPLENISHMENT OF THE INTRACELLULAR AMINO ACID POOL

The amino acid pool within cells is generated both from dietary amino acids and from the degradation of existing proteins within the cell. All proteins within cells have a half-life $(t_{1/2})$, a time at which 50% of the protein that was synthesized at a particular time will have been degraded. Some proteins are inherently short-lived, with half-lives of 5 to 20 minutes. Other proteins are present for extended periods, with half-lives of many hours, or even days. Thus, proteins are continuously being

Cal Kulis and other patients with cystinuria have a genetically determined defect in the transport of cystine and the basic amino acids, lysine, arginine, and ornithine, across the brush-border membranes of cells in both their small intestine and renal tubules. However, they do not appear to have any symptoms of amino acid deficiency, in part because the amino acids cysteine (which is oxidized in blood and urine to form the disulfide cystine) and arginine can be synthesized in the body (i.e., they are "nonessential" amino acids). Ornithine (an amino acid that is not found in proteins but serves as an intermediate of the urea cycle) can also be synthesized. The most serious problem for these patients is the insolubility of cystine, which can form kidney stones that may lodge in the ureter, causing bleeding and severe pain.

Patients with cystinuria and Hartnup disease have defective transport proteins in both the intestine and the kidney. These patients do not absorb the affected amino acids at a normal rate from the digestive products in the intestinal lumen. They also do not readily resorb these amino acids from the glomerular filtrate into the blood. Therefore, they do not have a hyperaminoacidemia (a high concentration in the blood). Normally, only a few percent of the amino acids that enter the glomerular filtrate are excreted in the urine; most are resorbed. In these diseases, much larger amounts of the affected amino acids are excreted in the urine, resulting in a hyperaminoaciduria.



Fig. 37.4. Transepithelial amino acid transport. Na⁺-dependent carriers transport both Na⁺ and an amino acid into the intestinal epithelial cell from the intestinal lumen. Na⁺ is pumped out on the serosal side (across the basolateral membrane) in exchange for K⁺ by the Na⁺,K⁺-ATPase. On the serosal side, the amino acid is carried by a facilitated transporter down its concentration gradient into the blood. This process is an example of secondary active transport.



Protein turnover is guite extensive. For example, red blood cells have a lifespan of 120 days. Every day 3 imes10¹¹ (300,000 million) red blood

cells die and are phagocytosed. The hemoglobin in these cells is degraded to amino acids by lysosomal proteases, and these amino acids are reutilized. Approximately 6 lb hemoglobin is recycled in this way every year. As the aged cells are dying, newly generated reticulocytes are synthesizing hemoglobin in preparation for their conversion into new red blood cells, which replace the dying cells.

Adults cannot increase the amount of muscle or other body proteins by eating an excess amount of protein. If dietary protein is consumed in excess

of our needs, it is converted to glycogen and triacylglycerols, which are then stored.

synthesized and degraded in the body, using a variety of enzyme systems to do so (Table 37.2). Examples of proteins that undergo extensive synthesis and degradation are hemoglobin, muscle proteins, digestive enzymes, and the proteins of cells sloughed off from the gastrointestinal tract. Hemoglobin is produced in reticulocytes and reconverted to amino acids by the phagocytic cells that remove mature red blood cells from the circulation on a daily basis. Muscle protein is degraded during periods of fasting, and the amino acids are used for gluconeogenesis. After ingestion of protein in the diet, muscle protein is resynthesized.

A large amount of protein is recycled daily in the form of digestive enzymes, which are themselves degraded by digestive proteases. In addition, approximately one fourth of the cells lining the walls of the gastrointestinal tract are lost each day and replaced by newly synthesized cells. As cells leave the gastrointestinal wall, their proteins and other components are digested by enzymes in the lumen of the gut, and the products are absorbed. Only approximately 6% (roughly 10 g) of the protein that enters the digestive tract (including dietary proteins, digestive enzymes, and the proteins in sloughed-off cells) is excreted in the feces each day. The remainder is recycled.

Proteins are also recycled within cells. The differences in amino acid composition of the various proteins of the body, the vast range in turnover times $(t_{1/2})$, and the recycling of amino acids are all important factors that help to determine the requirements for specific amino acids and total protein in the diet. The synthesis of many enzymes is induced in response to physiologic demand (such as fasting or feeding). These enzymes are continuously being degraded. Intracellular proteins are also damaged by oxidation and other modifications that limit their function. Mechanisms for intracellular degradation of unnecessary or damaged proteins involve lysosomes and the ubiquitin/proteasome system.

A. Lysosomal Protein Turnover

Lysosomes participate in the process of autophagy, in which intracellular components are surrounded by membranes that fuse with lysosomes, and endocytosis (see Chapter 10). Autophagy is a complex regulated process in which cytoplasm is sequestered into vesicles and delivered to the lysosomes. Within the lysosomes, the cathepsin family of proteases degrades the ingested proteins to individual amino acids. The recycled amino acids can then leave the lysosome and rejoin the intracellular amino acid pool. Although the details of how autophagy is induced are still not known, starvation of a cell is a trigger to induce this process. This will allow old proteins to be recycled and the newly released amino acids used for new protein synthesis, to enable the cell to survive starvation conditions.

Classification	Mechanism	Role
Cathepsins Caspases	Cysteine proteases Cysteine proteases, which cleave after aspartate	Lysosomal enzymes Apoptosis; activated from pro-caspases (see Chapter 18)
Matrix metalloproteinases	Require zinc for catalysis	Model extracellular matrix components; regulated by TIMPs (tissue inhibitors of matrix metalloproteinases)
Proteasome	Large complex that degrades ubiquitin-tagged proteins	Protein turnover
Serine proteases	Active site serine in a catalytic triad with histidine and aspartic acid	Digestion and blood clotting; activated usually from zymogens (see Chapter 45)
Calpains	Calcium-dependent cysteine proteases	Many different cellular roles

Table 37.2. Proteases Involved in Protein Turnover/Degradation

B. The Ubiquitin-Proteasome Pathway

Ubiquitin is a small protein (76 amino acids) that is highly conserved. Its amino acid sequence in yeast and humans differs by only three residues. Ubiquitin targets intracellular proteins for degradation by covalently binding to the ϵ -amino group of lysine residues. This is accomplished by a three-enzyme system that adds ubiquitin to proteins targeted for degradation. Oftentimes, the target protein is polyubiquitinylated, in which additional ubiquitin molecules are added to previous ubiquitin molecules, forming a long ubiquitin tail on the target protein. After polyubiquitinylation is complete, the targeted protein is released from the threeenzyme complex.

A protease complex, known as the proteasome, then degrades the targeted protein, releasing intact ubiquitin that can again mark other proteins for degradation (Figure 37.5). The basic proteasome is a cylindrical 20S protein complex with multiple internal proteolytic sites. ATP hydrolysis is used both to unfold the tagged protein and to push the protein into the core of the cylinder. The complex is regulated by cap protein complexes, which bind the ubiquinylated protein (a step that requires ATP) and deliver them to the complex. After the target protein is degraded, the ubiquitin is released intact and recycled. The resultant amino acids join the intracellular pool of free amino acids. Different cap complexes alter the specificity of the proteasome. For example, the PA700 cap is required for ubiquinylated proteins, whereas the PA28 cap targets only short peptides to the complex.

CLINICAL COMMENTS

Sissy Fibrosa's growth and weight curves were both subnormal until her pediatrician added pancreatic enzyme supplements to her treatment plan. These supplements digest dietary protein, releasing essential and other amino acids from the dietary protein that are then absorbed by the endothelial cells of Sissy's small intestine, through which they are transported into the blood. A discernable improvement in Sissy's body weight and growth curves was noted within months of the start of this therapy.



Fig 37.5. The proteasome and cap proteins. The cap proteins (PA700 and PA28) regulate the activity of this proteolytic complex by recruiting to the complex the substrates for proteolysis. The ATP requirement is to unfold and denature the proteins targeted for destruction, although the PA28 protease complex does not require ATP.



Another protein modification, which occurs through a three-enzyme complex similar to that required for ubiquitin addition, is SUMOylation. SUMO stands for small ubiqutin-like modifier, and when proteins are tagged with SUMO their activites are altered (either positively or negatively, depending on the protein). SUMOylation presents yet another means of fine-tuning existing regulatory systems.

Many proteins that contain regions rich in the amino acids proline (P), glutamate (E), serine (S), and threonine (T) have short half-lives. These regions are known as PEST sequences, based on the one-letter abbreviations used for these amino acids. Most of the amino acids that contain PEST sequences are hydrolyzed by the ubiquitin-proteasome system.

Apart from the proportions of essential amino acids present in various foods, the quality of a dietary protein is also determined by the rate at which it is digested and, in a more general way, by its capacity to contribute to the growth of the infant. In this regard, the proteins in foods of animal origin are more digestible than are those derived from plants. For example, the digestibility of proteins in eggs is approximately 97%; that for meats, poultry, and fish is 85 to 100%, and that from wheat, soybeans, and other legumes ranges from 75 to 90%.

The official daily dietary "protein requirement" accepted by the U.S. and Canadian governments is 0.8 g of protein per kilogram of desirable body weight for adults (approximately 56 g for an adult male and 44 g for an adult female). On an average weight basis, the requirement per kilogram is much greater for infants and children. This fact underscores the importance of improving **Sissy Fibrosa's** protein digestion to optimize her potential for normal growth and development.

In patients with cystinuria, such as **Cal Kulis**, the inability to normally absorb cystine and basic amino acids from the gut and the increased loss of these amino acids in the urine would be expected to cause a deficiency of these compounds in the blood. However, because three of these amino acids can be synthesized in the body (i.e., they are nonessential amino acids), their concentrations in the plasma remain normal, and clinical manifestations of a deficiency state do not develop. It is not clear why symptoms related to a lysine deficiency have not been observed.

In the disorder that was first observed in the Hartnup family and bears their name, the intestinal and renal transport defect involves the neutral amino acids (monoamine, monocarboxylic acids), including a number of the essential amino acids (isoleucine, leucine, phenylalanine, threonine, tryptophan, and valine) as well as certain nonessential amino acids (alanine, serine, and tyrosine). A reduction in the availability of these essential amino acids would be expected to cause a variety of clinical disorders. Yet children with the Hartnup disorder identified by routine newborn urine screening almost always remain clinically normal.

However, some patients with the Hartnup biochemical phenotype eventually develop pellagra-like manifestations, which usually include a photosensitivity rash, ataxia, and neuropsychiatric symptoms. Pellagra results from a dietary deficiency of the vitamin niacin or the essential amino acid tryptophan, which are both precursors for the nicotinamide moiety of NAD and NADP. In asymptomatic patients, the transport abnormality may be incomplete and so subtle as to allow no phenotypic expression of Hartnup disease. These patients also may be capable of absorbing some small peptides that contain the neutral amino acids.

The only rational treatment of patients having pellagra-like symptoms is the administration of niacin (nicotinic acid) in oral doses up to 300 mg/day. Although the rash, ataxia, and neuropsychiatric manifestations of niacin deficiency may disappear, the hyperaminoaciduria and intestinal transport defect do not respond to this therapy. In addition to niacin, a high-protein diet may benefit some patients.

BIOCHEMICAL COMMENTS

The γ -glutamyl cycle is necessary for the synthesis of glutathione, a compound that protects cells from oxidative damage (see Chapter 24). When originally discovered, the cycle was thought to be important in amino acid transport, but its involvement in such transport is now thought to be limited to salvage of cysteine. The enzymes of the cycle are present in many tissues, although certain tissues lack one or more of the enzymes of the cycle.



Fig. 37.6. γ -Glutamyl cycle. In cells of the intestine and kidney, amino acids can be transported across the cell membrane by reacting with glutathione (γ -glutamyl-cysteinyl-glycine) to form a γ -glutamyl amino acid. The amino acid is released into the cell, and glutathione is resynthesized. However, the major role of this cycle is glutathione synthesis, and many tissues lack the transpeptidase and 5-oxo-prolinase activities.

The entire cycle is presented in Figure 37.6. In this case, the extracellular amino acid reacts with glutathione (γ -glutamyl-cysteinyl-glycine) in a reaction catalyzed by a transpeptidase present in the cell membrane. A γ -glutamyl amino acid is formed, which travels across the cell membrane and releases the amino acid into the cell. The other products of these two reactions are reconverted to glutathione.

The reactions converting glutamate to glutathione in the γ -glutamyl cycle are the same reactions required for the synthesis of glutathione. The enzymes for glutathione synthesis, but not the transpeptidase, are found in most tissues. The oxoprolinase is also missing from many tissues, such that the major role of this pathway is one of glutathione synthesis from glutamate, cysteine, and glycine. The transpeptidase is the only protease in the cell that can break the γ -glutamyl linkage in glutathione. Glutathione is also involved in reducing compounds such as hydrogen peroxide (see Chapter 21). It also protects cells, in particular erythrocytes, from oxidative damage, through formation of oxidized glutathione, two glutathione residues connected by a disulfide bond.

Suggested References

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REVIEW QUESTIONS-CHAPTER 37

- 1. An individual with a deficiency in the conversion of trypsinogen to trypsin would be expected to experience a more detrimental effect on protein digestion than an individual who was defective in any of the other digestive proteases. This is due to which of the following?
 - (A) Trypsin has a greater and wider range of substrates to act on.
 - (B) Trypsin activates pepsinogen, so digestion can begin in the stomach.
 - (C) Trypsin activates the other zymogens that are secreted by the pancreas.
 - (D) Trypsin activates enteropeptidase, which is needed to activate the other pancreatic zymogens.
 - (E) Trypsin inhibits intestinal motility, so the substrates can be hydrolyzed for longer periods.
- 2. An individual has been shown to have a deficiency in an intestinal epithelial cell amino acid transport system for leucine. However, the individual shows no symptoms of amino acid deficiency. This could be due to which of the following?
 - (A) The body synthesizes leucine to compensate for the transport defect.
 - (B) The kidney reabsorbs leucine and sends it to other tissues.
 - (C) There are multiple transport systems for leucine.
 - (D) Isoleucine takes the place of leucine in proteins.
 - (E) Leucine is not necessary for bulk protein synthesis.
- 3. Kwashiorkor can result from which of the following?
 - (A) Consuming a calorie-deficient diet that is also deficient in protein
 - (B) Consuming a calorie-adequate diet that is deficient in carbohydrates
 - (C) Consuming a calorie-adequate diet that is deficient in fatty acids
 - (D) Consuming a calorie-adequate diet that is deficient in proteins
 - (E) Consuming a calorie-deficient diet that is primarily proteins
- 4. Which of the following enzymes is activated through an autocatalytic process?
 - (A) Enteropeptidase
 - (B) Trypsinogen
 - (C) Pepsinogen
 - (D) Aminopeptidase
 - (E) Proelastase

5. Children with kwashiorkor usually have a fatty liver. This is due to which of the following?

- (A) The high fat content of their diet
- (B) The high carbohydrate content of their diet
- (C) The high protein content of their diet
- (D) The lack of substrates for gluconeogenesis in the liver
- (E) The lack of substrates for protein synthesis in the liver
- (F) The lack of substrates for glycogen synthesis in the liver