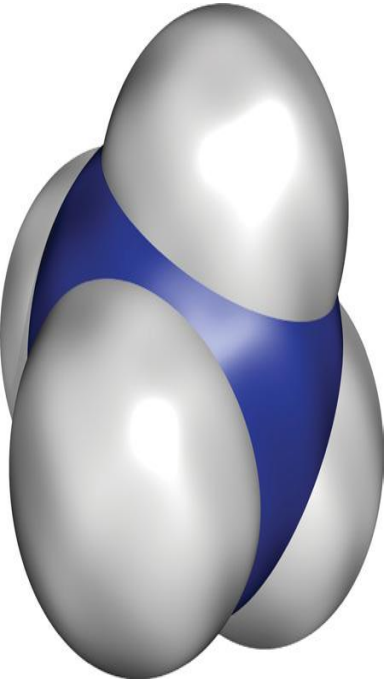
NITROGEN METABOLISM

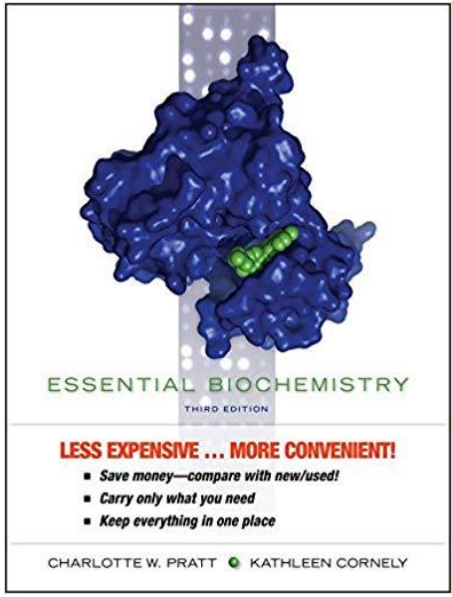


For

Forth stage – Chemistry dept.

Assistant Professor

Dr. ABDULKADIR MOHAMMED NOORI



Syllabus

Protein Matabolism:

- Protein degradation
- Chemical signals for protein degradation
- Nitrogen Balance
- Digestion of Dietary Proteins
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- Jaundice
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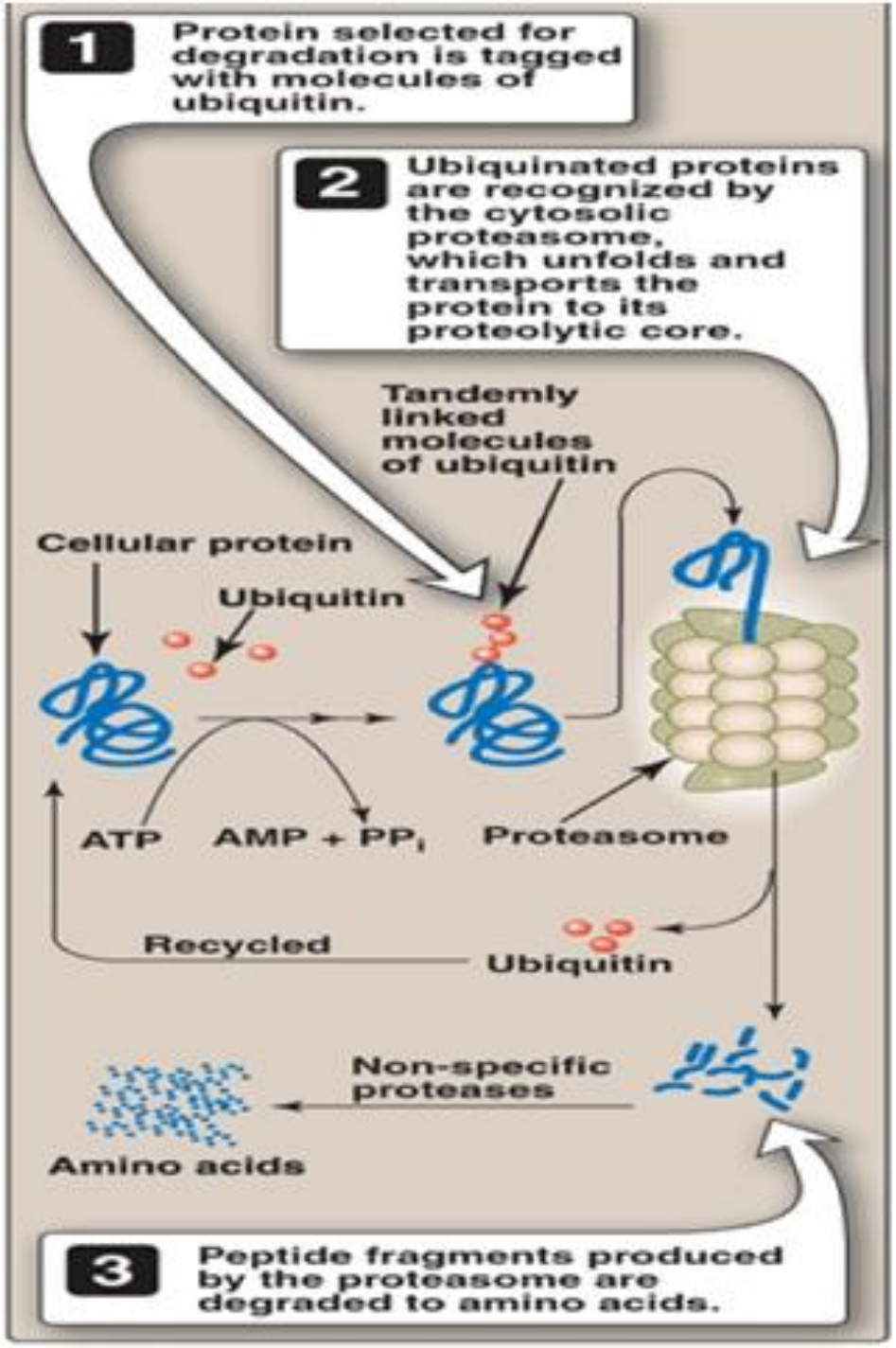
Protein degradation: There are two major enzyme systems responsible for degrading damaged or unneeded proteins:

1-**The energy-dependent ubiquitin-proteasome mechanism**: Proteasomes mainly degrade **endogenous proteins,** that is, proteins that were synthesized within the cell.

2- The nonenergy-dependent degradative enzymes (acid hydrolases) of the lysosomes: Lysosomal enzymes degrade **primarily extracellular proteins**, such as plasma proteins that are taken into the cell by endocytosis, and cell-surface membrane proteins that are used in receptor-mediated endocytosis.

Ubiquitin-proteasome proteolytic pathway: Proteins selected for degradation by the ubiquitin-proteasome mechanism are first covalently attached to ubiquitin, a small, globular protein. Ubiquitination of the target substrate occurs through linkage of the α-carboxyl glycine of ubiquitin to a lysine ε-amino group on the protein substrate by a three-step, enzyme-catalyzed process.

Proteins tagged with ubiquitin are then recognized by a large, barrel-shaped اسطواني الشكل , macromolecular, proteolytic complex called a **proteasome**, (Figure 2). The proteasome cuts the target protein into fragments that are then further degraded to amino acids, which enter the amino acid pool. The degradation of proteins by the ubiquitin-proteasome complex (unlike simple hydrolysis by proteolytic enzymes) requires adenosine triphosphate **(ATP)—**that is, it is energy-dependent.


1
Protein selected for degradation is tagged with molecules of ubiquitin.
2
Ubiquinated proteins are recognized by the cytosolic proteasome, which unfolds and transports the protein to its proteolytic core.
Tandemly linked molecules of ubiquitin
Cellular protein
Ubiquitin
ATP
AMP + PPi
Proteasome
Recycled
Ubiquitin
Non-specific proteases
Amino acids
3
Peptide fragments produced by the proteasome are degraded to amino acids.

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. **The half-life of a protein is influenced by the nature of the 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**. In contrast, proteins with **aspartate** as the N-terminal amino acid have a half-life of only **three minutes**. Furthermore, 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, exhibit short intracellular half-lives.

Nitrogen Balance

Catabolism of amino acids leads to a net loss of nitrogen from the body .This loss must be compensated by the diet in order to maintain a constant amount of body protein ,Nitrogen balance studies evaluate the relationship between the nitrogen intake (in the form of protein) and nitrogen excretion.

Three situations are possible as follows:

1- Nitrogen equilibrium:

In normal adults nitrogen intake =nitrogen excretion. The subject is said to be in nitrogen equilibrium or balance. In this situation, the rate of protein synthesis is equal to the rate of the rate of degradation.

2- Positive nitrogenbalance:

In whish nitrogen intake > nitrogen excretion. It shows that nitrogen is retained in the body, which means that protein is laid down. This occurs in growing infants and pregnant women.

3- Negative nitrogen balance:

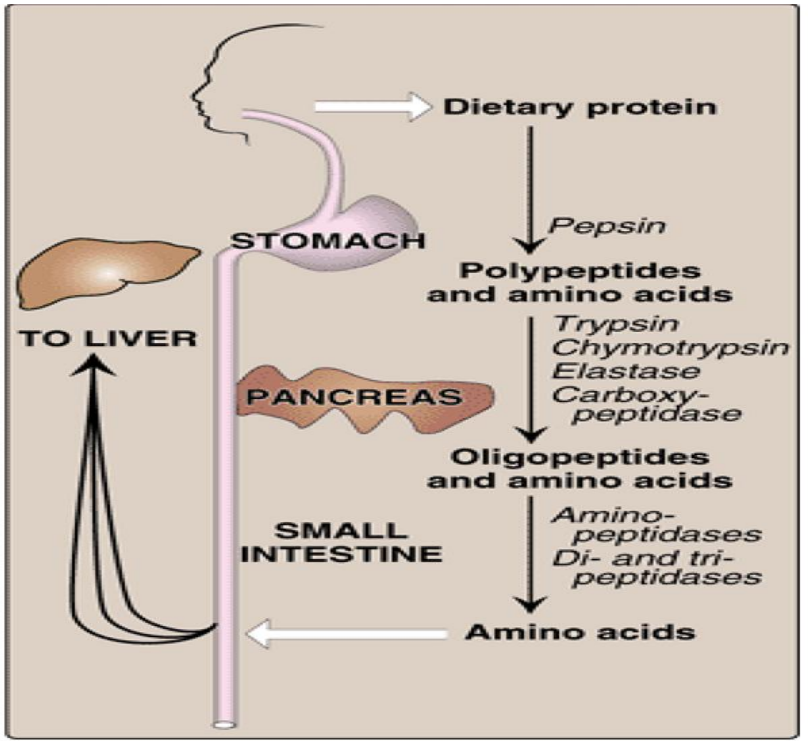
In which nitrogen intake <nitrogen excretion. this occurs during serious illness and major injury and trauma, in advanced cancer and following failure to ingest adequate or sufficient high-quality protein (malnutrition). If the situation is prolonged, it will ultimately lead to death.

Digestion of Dietary Proteins

Proteins are generally too large to be absorbed by the intestine. Proteins must, therefore, be hydrolyzed to yield their constituent 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).

[Note: An example of an exception to this rule is that newborns can take up maternal antibodies in breast milk, e.g. immunoglobin IgA from colostrum of maternal milk are absorbed intact without loss of biologic activity , so that they provide passive immunity to the infant. In contrast to the situation encountered in the newborn infants, in some adult individuals, small amount of intact proteins may be absorbed through the intestinal mucosa. These proteins often cause undesirable immunological responses (formation of antibodies against the foreign protein)and are responsible for the symptoms of food allergies.

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



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 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 serous cells of the stomach as an inactive zymogen (or proenzyme), pepsinogen. In general, zymogens contain extra amino acids in their sequences, which 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) 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 NH2-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.

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 is incomplete. This results in the abnormal appearance of lipids (called steatorrhea,)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 free amino acids and smaller peptides.

D. Absorption of amino acids and dipeptides

Free amino acids are taken into the enterocytes up by a Na+-linked secondary transport system. Di- and tripeptides, however, are taken up by a H+-linked transport system. There, the peptides are hydrolyzed in the cytosol to amino acids before being released into the portal system. 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.

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 systemsare known that have overlapping specificities for different amino acids.

V. Removal of Nitrogen from Amino Acids

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, with the carbon skeletons being metabolized.

A. Transamination: the funneling of amino groups to glutamate

1-The first step in the catabolism of most amino acids is the transfer of their α-amino group to α-ketoglutarate (Figure 19.7).

2- The products are an α-keto acid (derived from the original amino acid) and glutamate.

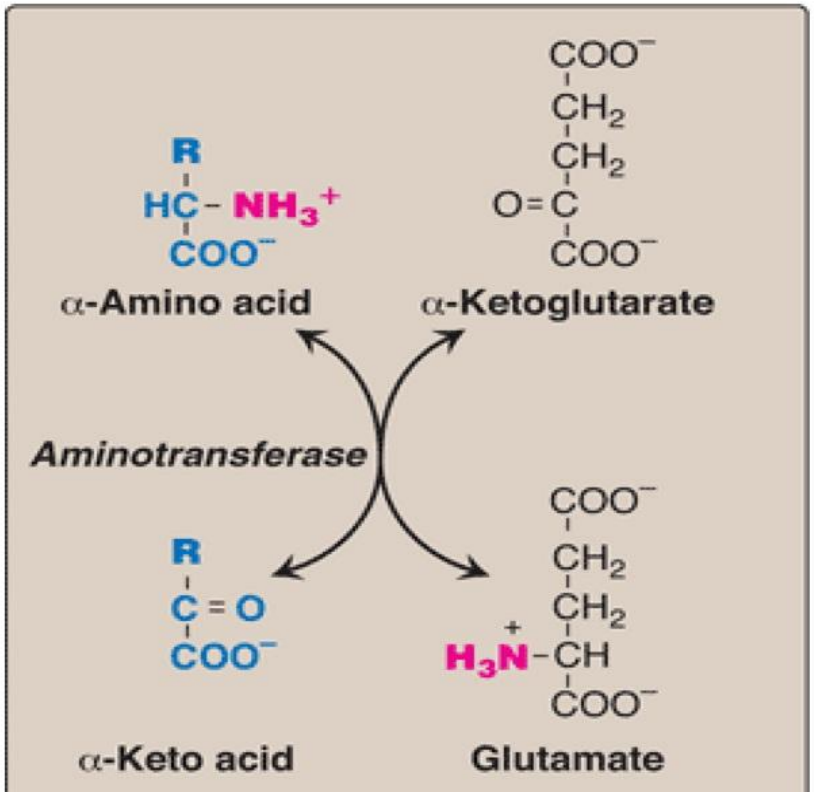


Figure 19.7 Aminotransferase reaction using α-ketoglutarate as the amino-group acceptor

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), Figure 19.8.

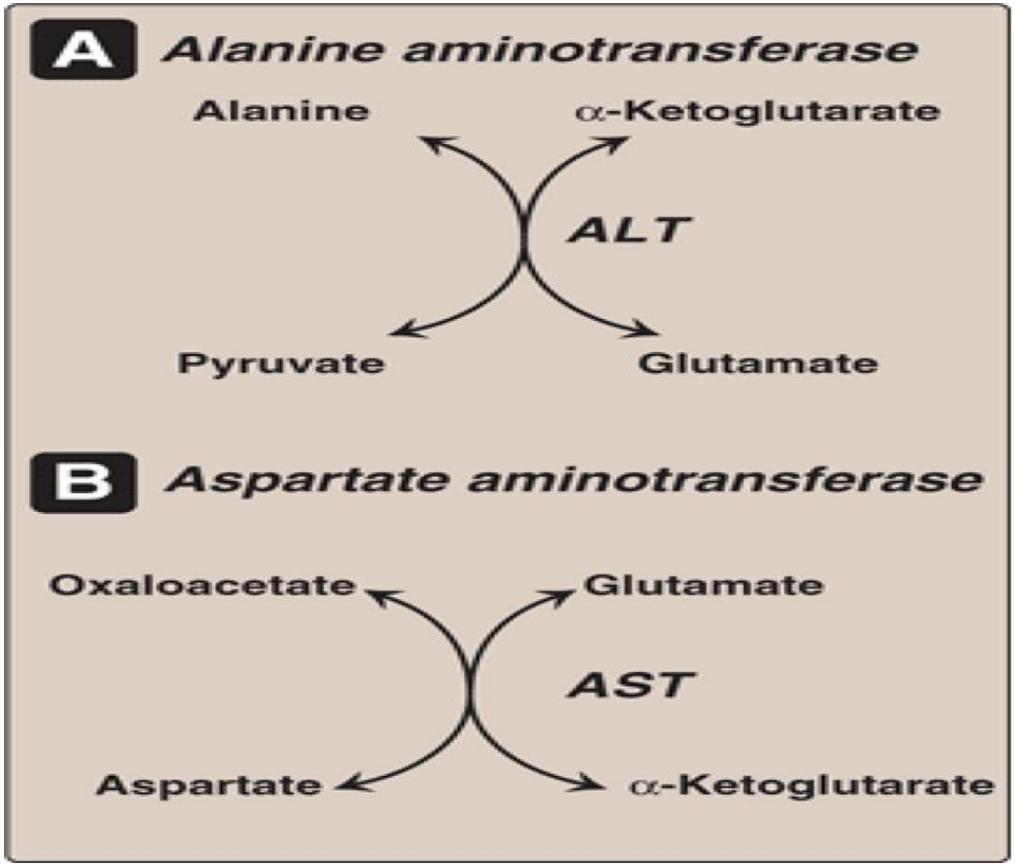


Figure 19.8 Reactions catalyzed during amino acid catabolism. A. Alanine aminotransferase (ALT). B. Aspartate aminotransferase (AST).

a-Alanine aminotransferase (ALT): Formerly called glutamate-pyruvate transaminase, 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.

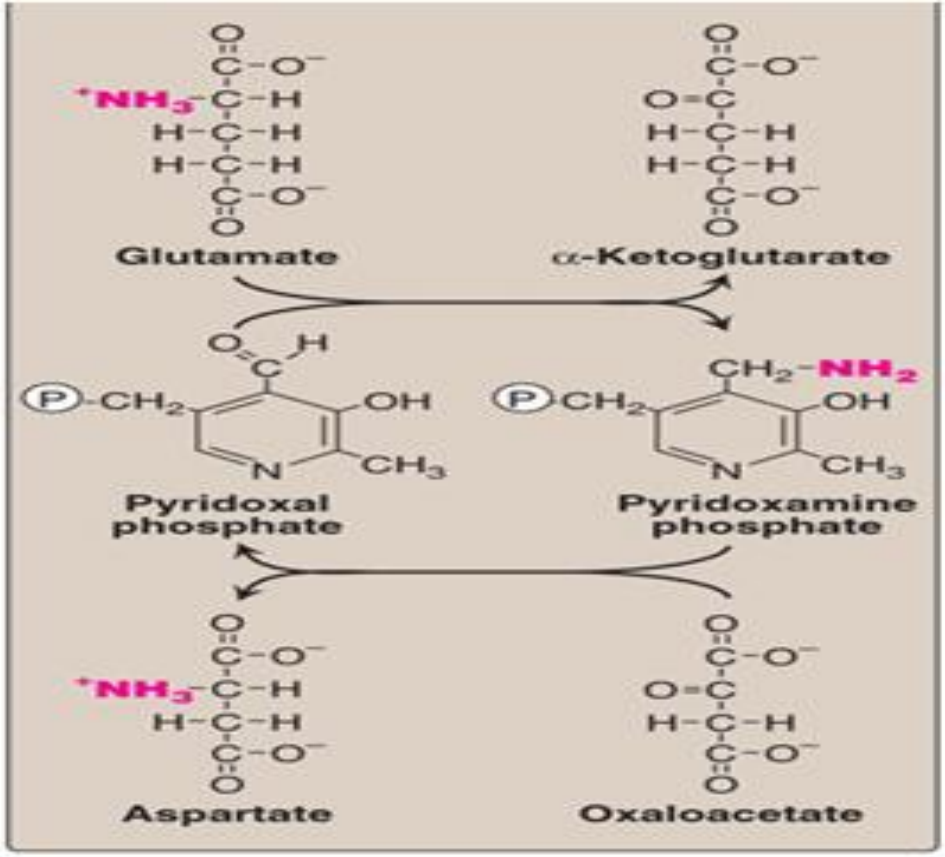
b-Aspartate aminotransferase (AST): AST formerly called glutamate-oxaloacetate transaminase, AST is an exception to the rule that aminotransferases funnel amino groups to form glutamate. During amino acid catabolism, AST transfers amino groups from glutamate to oxaloacetate, forming aspartate, which is used as a source of nitrogen in the urea cycle.

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 .Figure 19.9 shows the reaction catalyzed by AST.

3-Diagnostic value of plasma aminotransferases:

Aminotransferases are normally intracellular enzymes, with the low levels found in the plasma representing the release of cellular contents during normal cell turnover. The presence of elevated plasma levels of aminotransferases indicates 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.

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



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 enzyme measurements are often useful in determiningthe course of liver damage.

b-Nonhepatic disease: Aminotransferases may be elevated in nonhepatic disease, such as myocardial infarction and muscle disorders. However, these disorders can usually be distinguished clinically from liver disease.

B. Glutamate dehydrogenase: the oxidative deamination of amino acids

In contrast to transamination reactions that transfer amino groups, oxidative deamination by glutamate dehydrogenase results 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 pathway of energy metabolism, and ammonia, which is a source of nitrogen in urea synthesis.

c-Allosteric regulators: guanosine triphosphate is an allosteric inhibitor of glutamate dehydrogenase, whereas adenosine diphosphate (ADP) is an activator. Thus, 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.

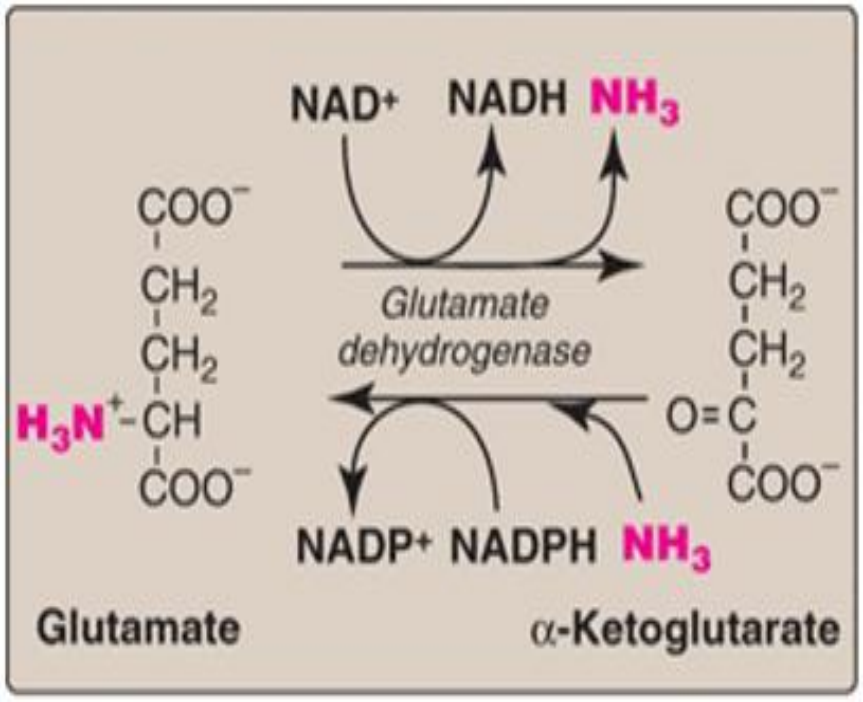


Figure 19.11 Oxidative deamination by glutamate dehydrogenase

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.

The first, found in most tissues, 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.

The second transport mechanism, used primarily by muscle, involves transamination of pyruvate (the end product of aerobic glycolysis) to form alanine (see Figure 19.8). Alanine is transported by the blood to the liver, where it is converted to pyruvate, again by transamination. In the liver, the pathway of gluconeogenesis can use the pyruvate 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 from peripheral tissues to the liver

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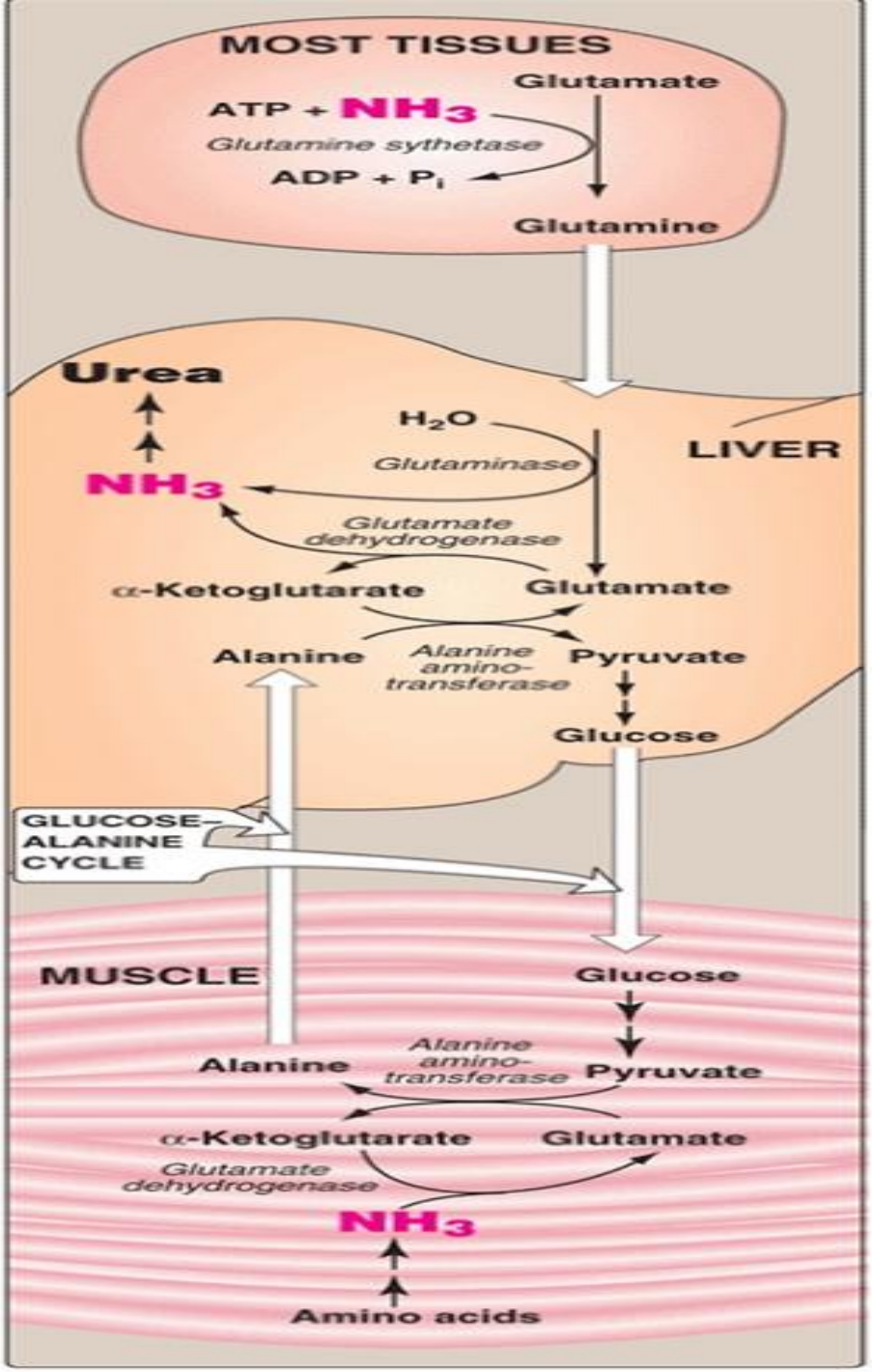
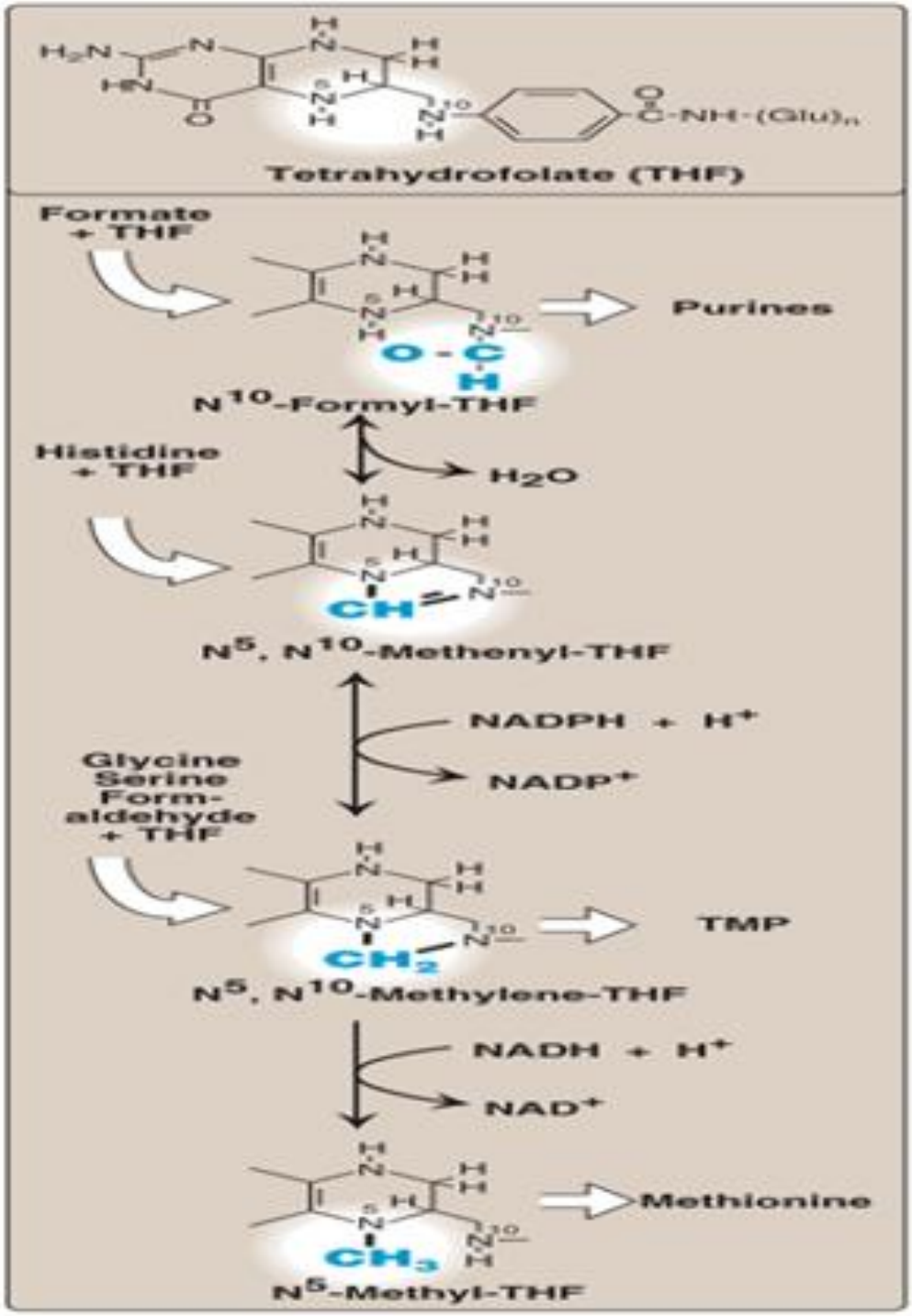


Role of Folic Acid in Amino Acid Metabolism

Some synthetic pathways require the addition of single carbon groups. These “one-carbon units” can exist in a variety of oxidation states. These include methane, methanol, formaldehyde, formic acid, and carbonic acid. It is possible to incorporate carbon units at each of these oxidation states, except methane, into other organic compounds. These single carbon units can be transferred from carrier compounds such as tetrahydrofolic acid and S-adenosylmethionine to specific structures that are being synthesized or modified.

A. Folic acid: a carrier of one-carbon units The active form of folic acid, tetrahydrofolic acid (THF), is produced from folate by dihydrofolatereductase in a two-step reaction requiring two moles of NADPH. The carbon unit carried by THF is bound to nitrogen N^5 or N^{10}, or to both N^5 and N^{10}. THF allows one-carbon compounds to be recognized and manipulated by biosynthetic enzymes. Figure(20.11) shows the structures of the various members of the THF family and their interconversions, and indicates the sources of the one-carbon units and the synthetic reactions in which the specific members participate.

Figure 20.11 Summary of the interconversions and uses of the carrier, tetra-hydrofolate



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 result 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. Phenylketonuria is the most important disease of amino acid metabolism because it is relatively common and responds to dietary treatment.

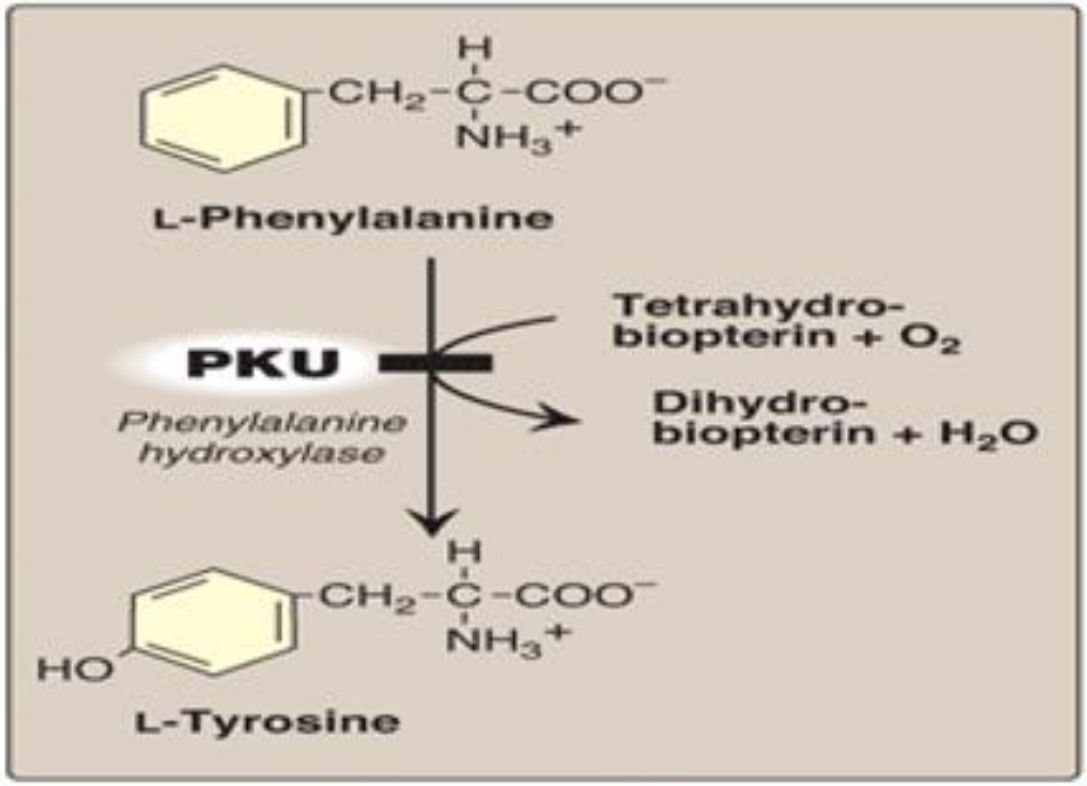


Figure 20.15 A deficiency in phenylalanine hydroxylase results in the disease phenylketonuria (PKU).

A. Phenylketonuria

Phenylketonuria (PKU), caused by a deficiency of phenylalanine hydroxylase (Figure 20.15), PKU 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). **Hyperphenylalaninemia** may also be caused by deficiencies in any of the several enzymes required to synthesize BH4, or in dihydropteridine (BH2) reductase, which regenerates BH4 from BH2 .

Such deficiencies indirectly raise phenylalanine concentrations, because phenylalanine hydroxylaserequires 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 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 hyperphenylalaninemia, although the response is unpredictable.

Characteristics of classic PKU

Elevated phenylalanine: Phenylalanine is present in elevated concentrations in tissues, plasma, and urine. Since patients can not convert phenylalanine to tyrosine by normal pathway ,some minor pathway of phenylalanine becomes prominent in phenylketonurics and accumulation of toxic derivatives of phenylalanine such as Phenyllactate, phenylacetate, and phenylpyruvate, which are not normally produced in significant amounts in the presence of functional phenylalanine hydroxylase (Figure 20.17). The disease acquired its name (PKU) from the high levels of the ketoacid , phenyl pyruvate in urine .

CNS symptoms: Mental retardation, failure to walk or talk, seizures نوبة مرضية, 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 one year.

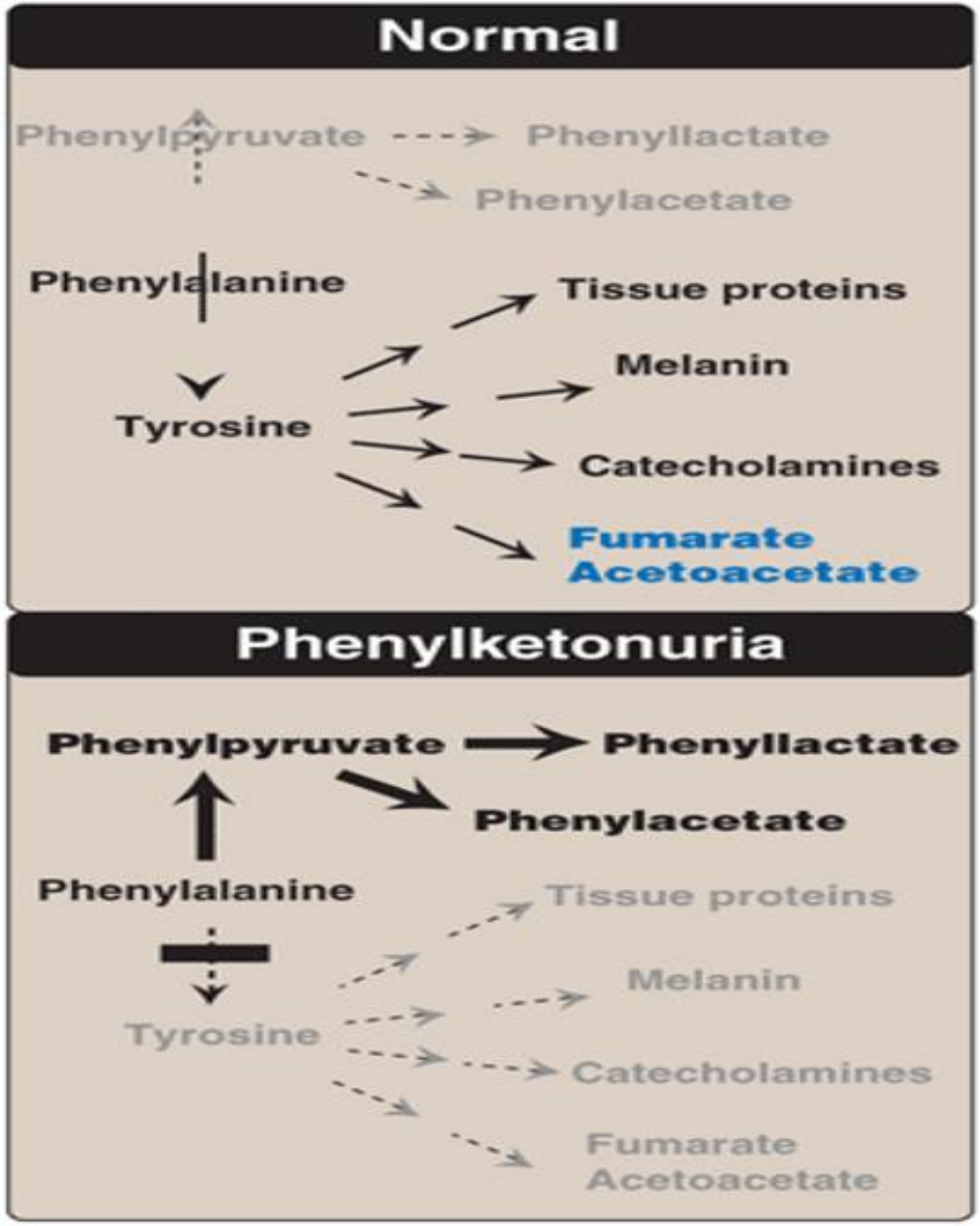


Figure 20.17 Pathways of phenylalanine metabolism in normal individuals and in patients with phenylketonuria.

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 decarboxylatesleucine, isoleucine, and valine (see Figure 20.10). 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.

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. In addition to hypopigmentation, affected individuals have vision defects and photophobia (sunlight hurts their eyes). They are at increased risk for skin cancer.

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.

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).

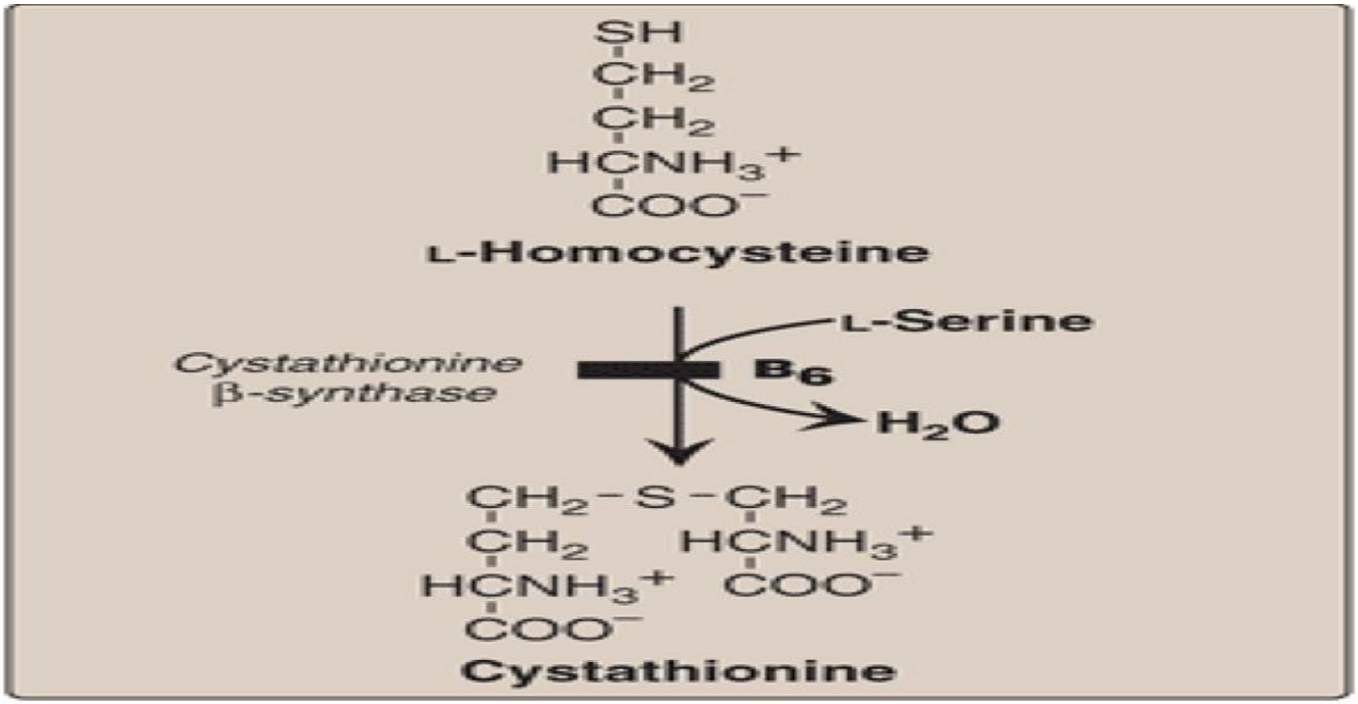


Figure 20.21 Enzyme deficiency in homo-cystinuria

E. Alkaptonuria

Alkaptonuria is a rare metabolic disease involving a deficiency in homogentisic acid oxidase, resulting in the accumulation of **homogentisic acid**. [Note: This reaction occurs in the degradative pathway of tyrosine] , The illness has three characteristic symptoms: homogentisicaciduria (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. 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.

يكونون عادة بدون أعراض حتى سن الأربعين تقريبًا. تساعد الأنظمة الغذائية alkaptonuriaالمرضى الذين يعانون من التي تحتوي على نسبة منخفضة من البروتين - خاصة في الفينيل ألانين والتيروزين - على تقليل مستويات حمض الهوموجنتيسيك ، وتقليل كمية الصبغة المترسبة في أنسجة الجسم.

Biologically important compounds derived from Tryptophan

1- **Vitamin niacin (vitamin B_3)**

2- **Neurotransmitter serotonin**

3- **Hormone melatonin**: is a hormone produced by the pineal gland which has effects on the hypothalamic pituitary system .Synthesis of melatonin is regulated by light –dark cycle and blood levels of melatonin rise at night.

Parkinson, s disease :

in Parkinson, s disease , dopamine levels in the CNS are decreased because of a deficiency of cells that produce dopamine and depression is associated with low levels of serotonin.

Conversion of Amino Acids to Specialized Products

In addition to serving as building blocks for proteins, amino acids are precursors of many nitrogen-containing compounds that have important physiologic functions. These molecules include porphyrins, neurotransmitters, hormones, purines, and pyrimidines.

I. Porphyrin Metabolism: Porphyrins are cyclic compounds that readily bind metal ions—usually Fe^{2+} or Fe^{3+}. The most prevalent metalloporphyrin in humans is **heme**, which consists of one ferrous (Fe^{2+}) iron ion coordinated in the center of the tetrapyrrole ring of protoporphyrin IX. **Heme** is the **prosthetic group** for hemoglobin, myoglobin, the cytochromes, catalase, and tryptophan pyrrolase. These hemeproteins are rapidly synthesized and degraded. For example, 6 to 7 g of hemoglobin is synthesized each day to replace heme lost through the normal turnover of erythrocytes.

Biosynthesis of heme:

The major sites of heme biosynthesis are the liver, which synthesizes a number of heme proteins, and the erythrocyte-producing cells of the bone marrow, which are active in hemoglobin synthesis. The initial reaction and the last three steps in the formation of porphyrins occur in mitochondria, whereas the intermediate steps of the biosynthetic pathway occur in the cytosol. [Note: Mature red blood cells lack mitochondria and are unable to synthesize heme] .

1-Formation of δ-aminolevulinic acid (ALA): All the carbon and nitrogen atoms of the porphyrin molecule are provided by two simple building blocks: **glycine (a nonessential amino acid) and succinyl CoA (an intermediate in the citric acid cycle)**. Glycine and succinyl CoA condense to form ALA in a reaction catalyzed by ALA synthase (Figure 21.3) this reaction requires pyridoxal phosphate as a coenzyme, and is the committed and rate-controlling step in hepatic porphyrin biosynthesis.

2-Formation of porphobilinogen: The condensation of two molecules of ALA to form porphobilinogen by **ALA dehydratase** is extremely sensitive to inhibition by **heavy metal ions** (see Figure 21.3). This inhibition is, in part, responsible for the elevation in ALA and the anemia seen in lead poisoning.

3-Formation of uroporphyrinogen: The condensation of four porphobilinogens produces the linear tetrapyrrole, hydroxymethylbilane, which is isomerized and cyclized by uroporphyrinogen III synthase to produce the asymmetric uroporphyrinogen III. This cyclic tetrapyrrole undergoes decarboxylation of its acetate groups, generating coproporphyrinogen III (Figure 21.4). These reactions occur in the cytosol.

4-Formation of heme: Coproporphyrinogen III enters the mitochondrion, and two propionate side chains are decarboxylated to vinyl groups generating protoporphyrinogen IX, which is oxidized to protoporphyrin IX. The introduction of iron (as Fe+2) into protoporphyrin IXoccurs spontaneously, but the rate is enhanced by ferrochelatase, an enzyme that, like ALA dehydratase, is inhibited by lead (Figure 21.5).

Figure 21.3 Pathway of porphyrin synthesis: Formation of porphobilinogen. (Continued in Figures 21.4 and 21.5).

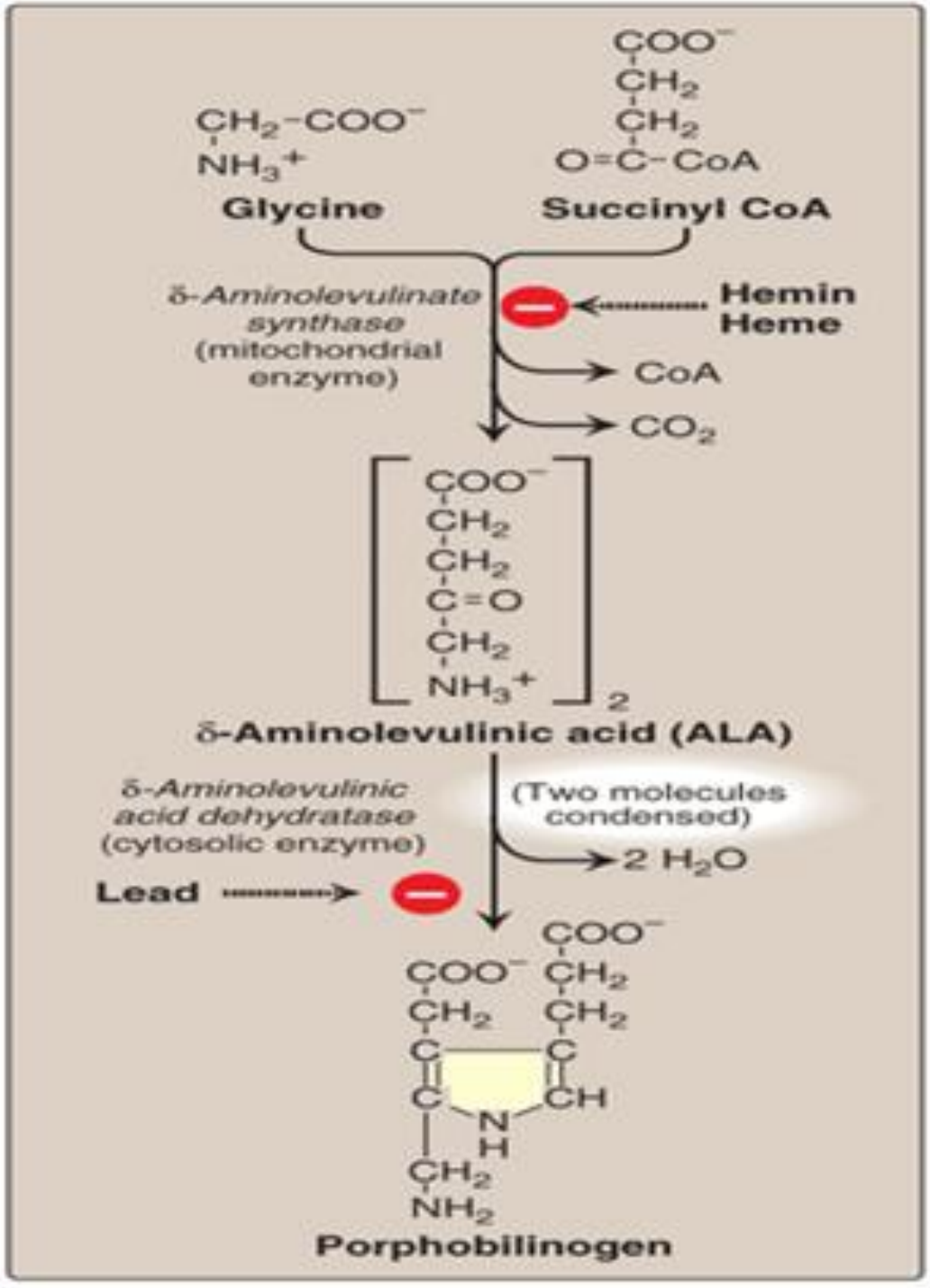
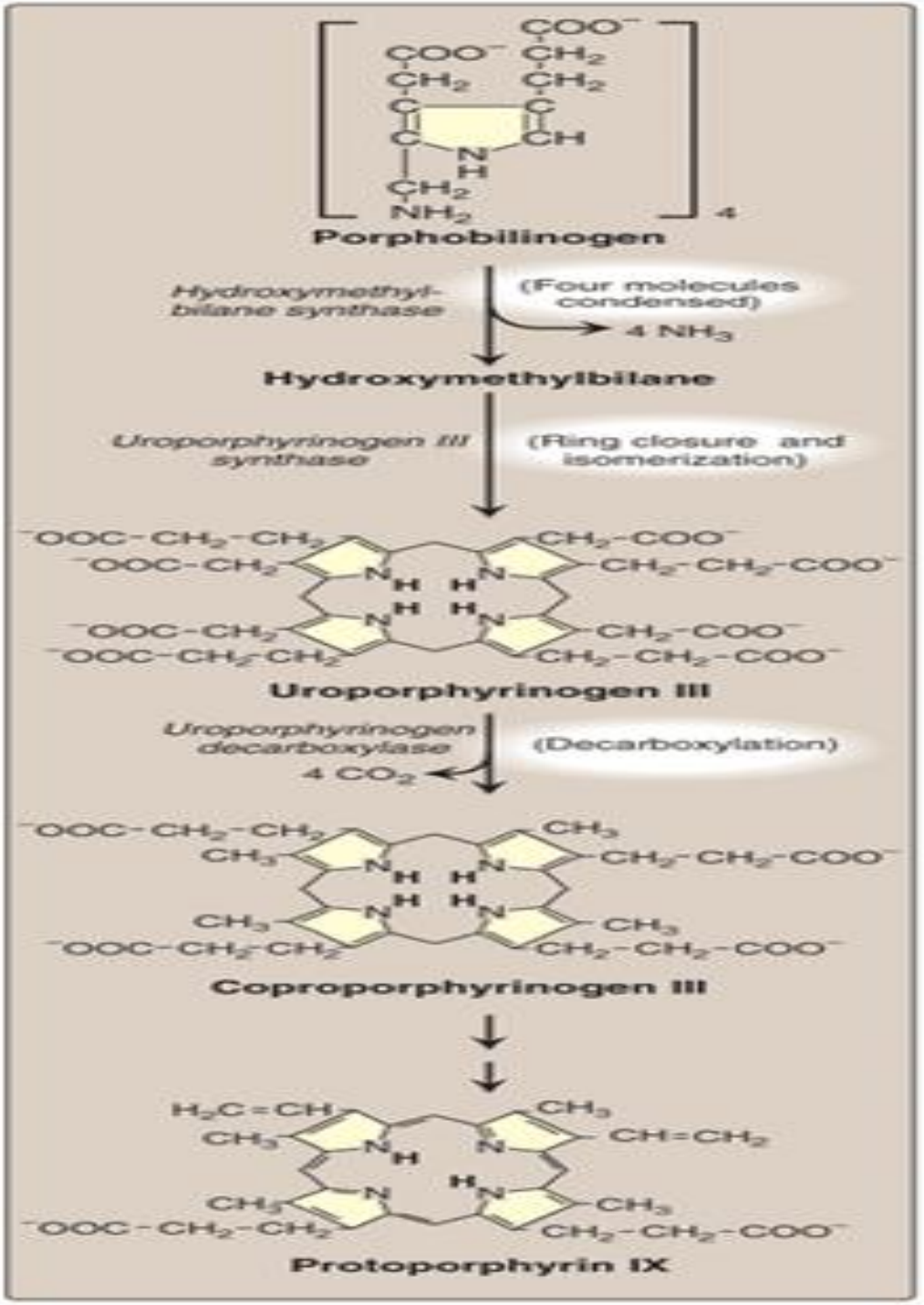


Figure 21.4 Pathway of porphyrin synthesis: Formation of protoporphyrin IX. Continued from Figure 21.3



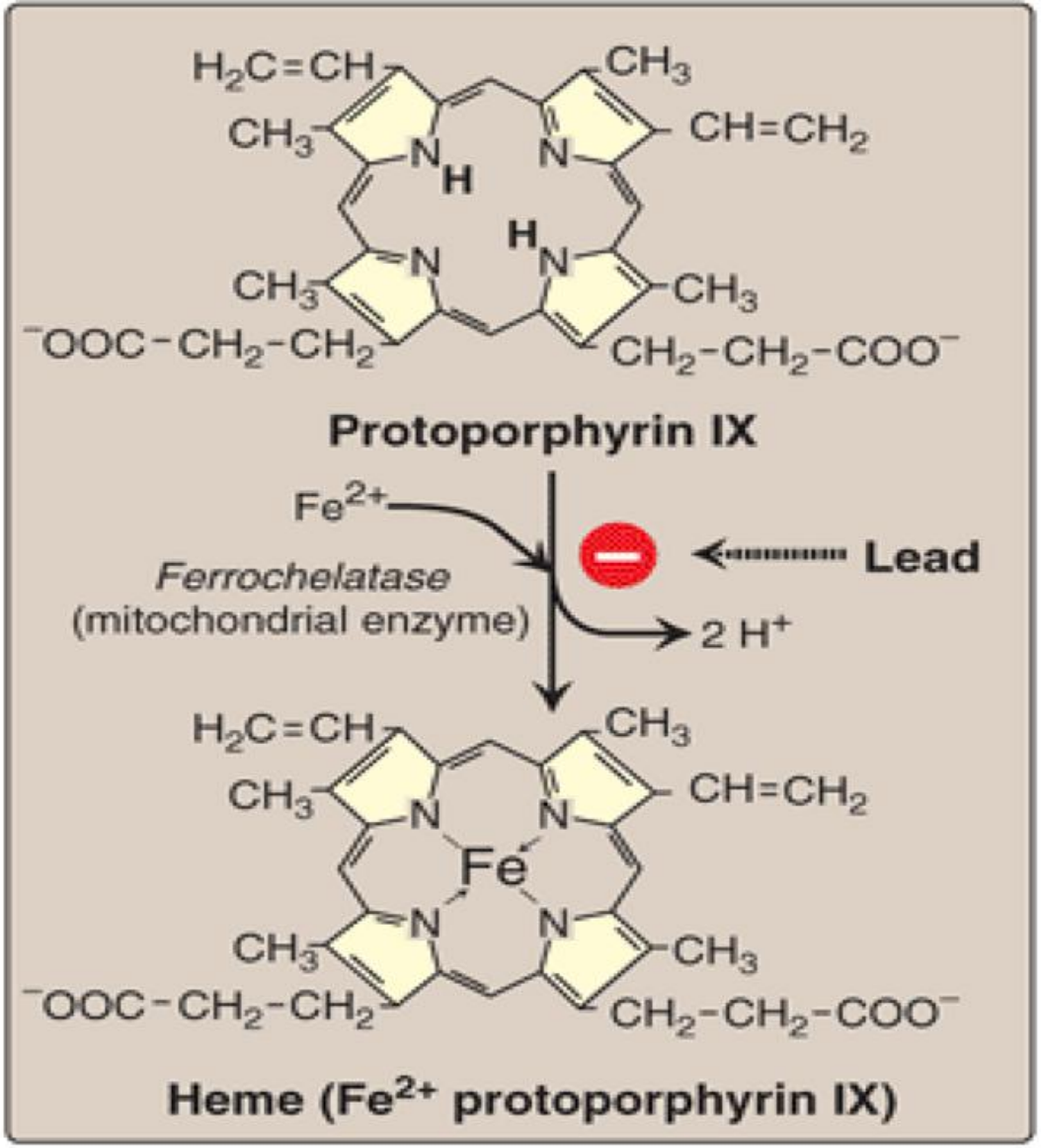


Figure 21.5 Pathway of porphyrin synthesis: Formation of heme. (Continued from Figures 21.3 and 21.4)

Increased ALA synthase activity:

One common feature of the **porphyrias** is a decreased synthesis of heme. In the liver, heme normally functions as a repressor of ALA synthase. Therefore, the absence of this end product results in an increase in the synthesis of ALA synthase (derepression). This causes an increased synthesis of intermediates that occur prior to the genetic block. The accumulation of these toxic intermediates is the major pathophysiology of the porphyrias.

Degradation of heme

After approximately 120 days in the circulation, red blood cells are taken up and degraded by the reticuloendothelial system, particularly in the liver and spleen (Figure 21.9). Approximately 85% of heme destined for degradation comes from red blood cells, and 15% is from turnover of immature red blood cells and cytochromes from extraerythroid tissues.

Bilirubin, unique to mammals, appears to function as an antioxidant. In this role, it is oxidized to biliverdin, which is then reduced by biliverdinreductase, regenerating bilirubin.

1-Formation of bilirubin: The first step in the degradation of heme is catalyzed by the microsomal hemeoxygenase system of the reticuloendothelial cells. In the presence of NADPH and O2, the enzyme adds a hydroxyl group to the methenyl bridge between two pyrrole rings, with a concomitant oxidation of ferrous iron to Fe3+. A second oxidation by the same enzyme system results in cleavage of the porphyrin ring. The green pigment biliverdin is produced as ferric iron and CO are released Biliverdin is reduced, forming the red-orange bilirubin. Bilirubin and its derivatives are collectively termed bile pigments.

2-Uptake of bilirubin by the liver: Bilirubin is only slightly soluble in plasma and, therefore, is transported to the liver by binding non-covalently to albumin. [Note: Certain anionic drugs, such as salicylates and sulfonamides, can displace bilirubin from albumin, permitting bilirubin to enter the central nervous system. This causes the potential for neural damage in infants.] Bilirubin dissociates from the carrier albumin molecule and enters a hepatocyte, where it binds to intracellular proteins, particularly the protein ligandin.

3-Formation of bilirubin diglucuronide: In the hepatocyte, the solubility of bilirubin is increased by the addition of two molecules of glucuronic acid. [Note: This process is referred to as **conjugation**.] The reaction is catalyzed by microsomal bilirubin **glucuronyltransferase** using **uridinediphosphate-glucuronic acid** as the glucuronate donor.

4-Secretion of bilirubin into bile: Bilirubin diglucuronide **(conjugated bilirubin)** is actively transported against a concentration gradient into the bile canaliculi and then into the bile. This energy-dependent, rate-limiting step is susceptible to impairment in liver disease. Unconjugated bilirubin is normally not secreted.

5-Formation of urobilins in the intestine: Bilirubin diglucuronide is hydrolyzed and reduced by bacteria in the gut to yield **urobilinogen**, a colorless compound. Most of the urobilinogen is oxidized by intestinal bacteria to **stercobilin**, which gives feces the characteristic brown color. However, some of the urobilinogen is reabsorbed from the gut and enters the portal blood. A portion of this urobilinogen participates in the enterohepaticurobilinogen cycle in which it is taken up by the liver, and then resecreted into the bile. The remainder of the urobilinogen is transported by the blood to the kidney, where it is converted to **yellow urobilin** and excreted, giving urine its characteristic color. The metabolism of bilirubin is summarized in Figure 21.9

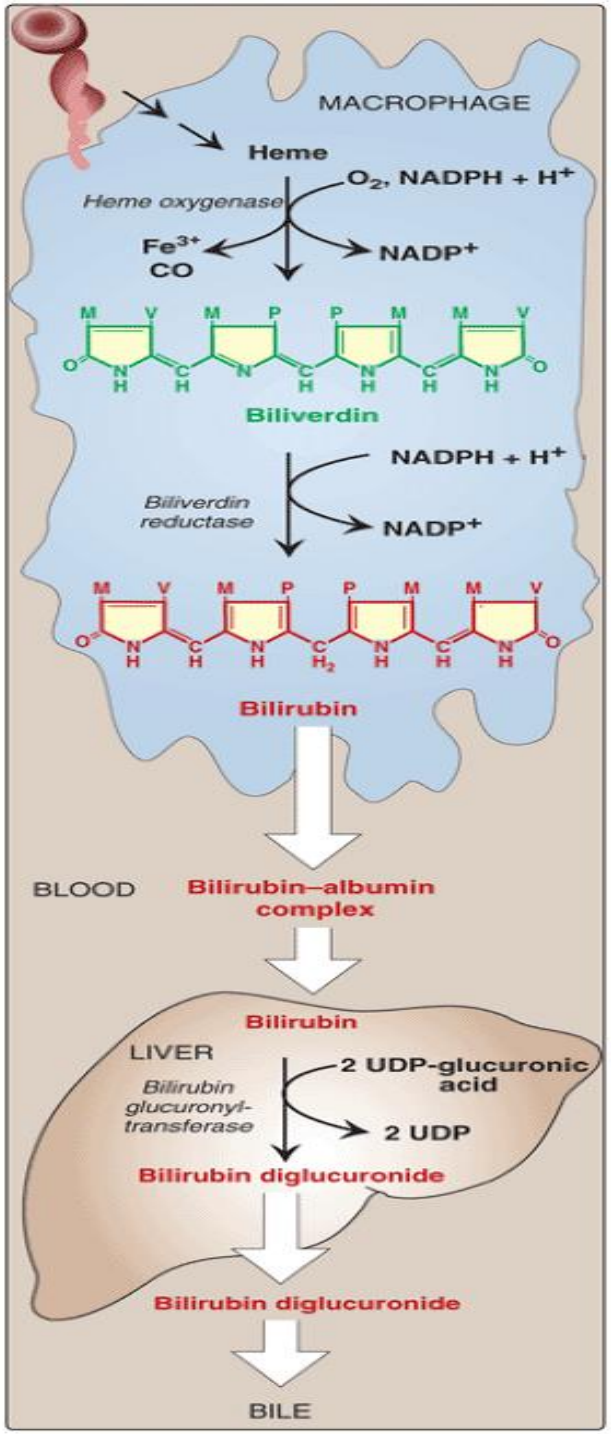


Figure 21.9 Formation of bilirubin from heme. UDP = uridinediphosphate

Jaundice

Jaundice refers to the yellow color of skin, nail beds, and sclerae (whites of the eyes) caused by deposition of bilirubin, secondary to increased bilirubin levels in the blood (hyper-bilirubinemia).

1-Types of jaundice:

a-Hemolytic jaundice: The liver has the capacity to conjugate and excrete over 3,000 mg of bilirubin per day, whereas the normal production of bilirubin is only 300 mg/day. This excess capacity allows the liver to respond to increased heme degradation with a corresponding increase in conjugation and secretion of bilirubin diglucuronide. However, massive lysis of red blood cells (for example, in patients with sickle cell anemia, glucose 6-phosphate dehydrogenase deficiency) may produce bilirubin faster than it can be conjugated. More bilirubin is excreted into the bile, the amount of urobilinogen entering the enterohepatic circulation is increased, and urinary urobilinogen is increased. Unconjugated bilirubin levels become elevated in the blood, causing jaundice.

b-Hepatocellular jaundice: Damage to liver cells (for example, in patients with cirrhosis or hepatitis) can cause unconjugated bilirubin levels to increase in the blood as a result of decreased conjugation. Plasma levels of AST (SGOT) and ALT (SGPT) are elevated, and the patient experiences nausea and anorexia.

C-Obstructive jaundice: In this instance, jaundice is not caused by overproduction of bilirubin or decreased conjugation, but instead results from obstruction of the bile duct. For example, the presence of a hepatic tumor or bile stones may block the bile ducts, preventing passage of bilirubin into the intestine. Patients with obstructive jaundice experience gastrointestinal pain and nausea, and produce stools that are a pale, clay color. The liver "regurgitates" conjugated bilirubin into the blood (hyperbilirubinemia).
The compound is eventually excreted in the urine. [Note: Prolonged obstruction of the bile duct can lead to liver damage and a subsequent rise in unconjugated bilirubin].

2-Jaundice in newborns: Newborn infants, particularly if premature, often accumulate bilirubin, because the activity of hepatic bilirubin **glucuronyltransferase** is low at birth—it reaches adult levels in about four weeks. Elevated bilirubin, in excess of the binding capacity of albumin, can diffuse into the basal ganglia and cause toxic encephalopathy (kernicterus). Thus, newborns with significantly elevated bilirubin levels are treated with blue fluorescent light, which converts bilirubin to more polar and, hence, water-soluble isomers. These photoisomers can be excreted into the bile without conjugation to glucuronic acid.

Other Nitrogen-Containing Compounds

A. Histamine

Histamine is a chemical messenger that mediates a wide range of cellular responses, including allergic and inflammatory reactions, gastric acid secretion, and possibly neurotransmission in parts of the brain. A powerful vasodilator, histamine is formed by decarboxylation of histidine in a reaction requiring **pyridoxal phosphate.** It is secreted by mast cells as a result of allergic reactions or trauma. Histamine has no clinical applications, but agents that interfere with the action of histamine have important therapeutic applications.

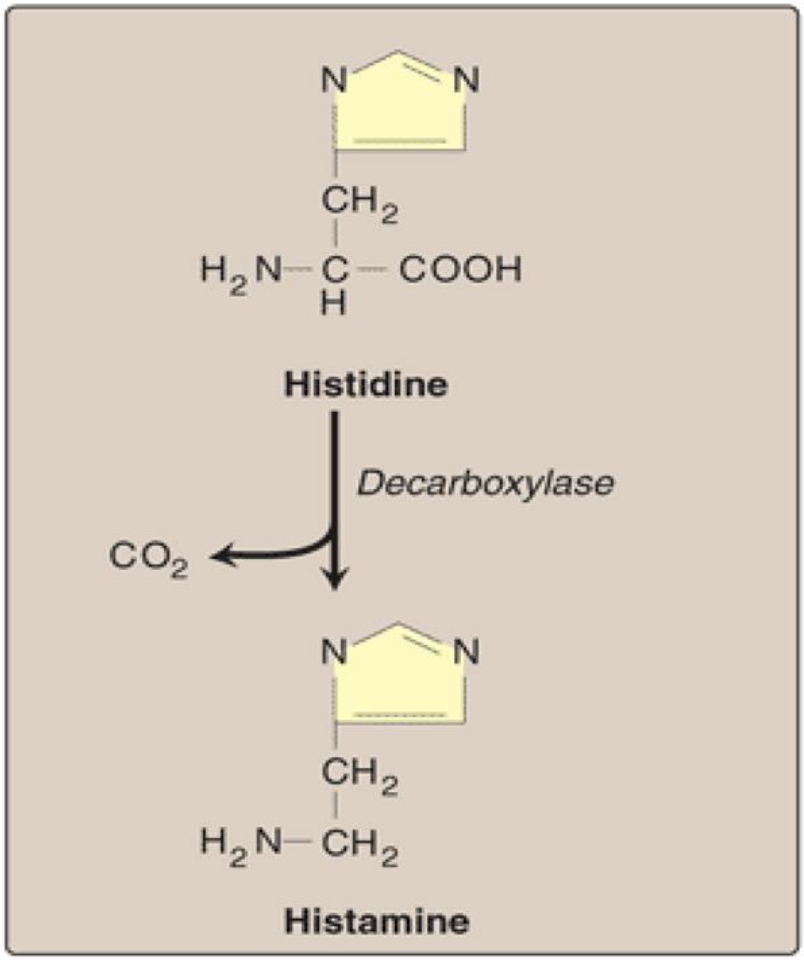
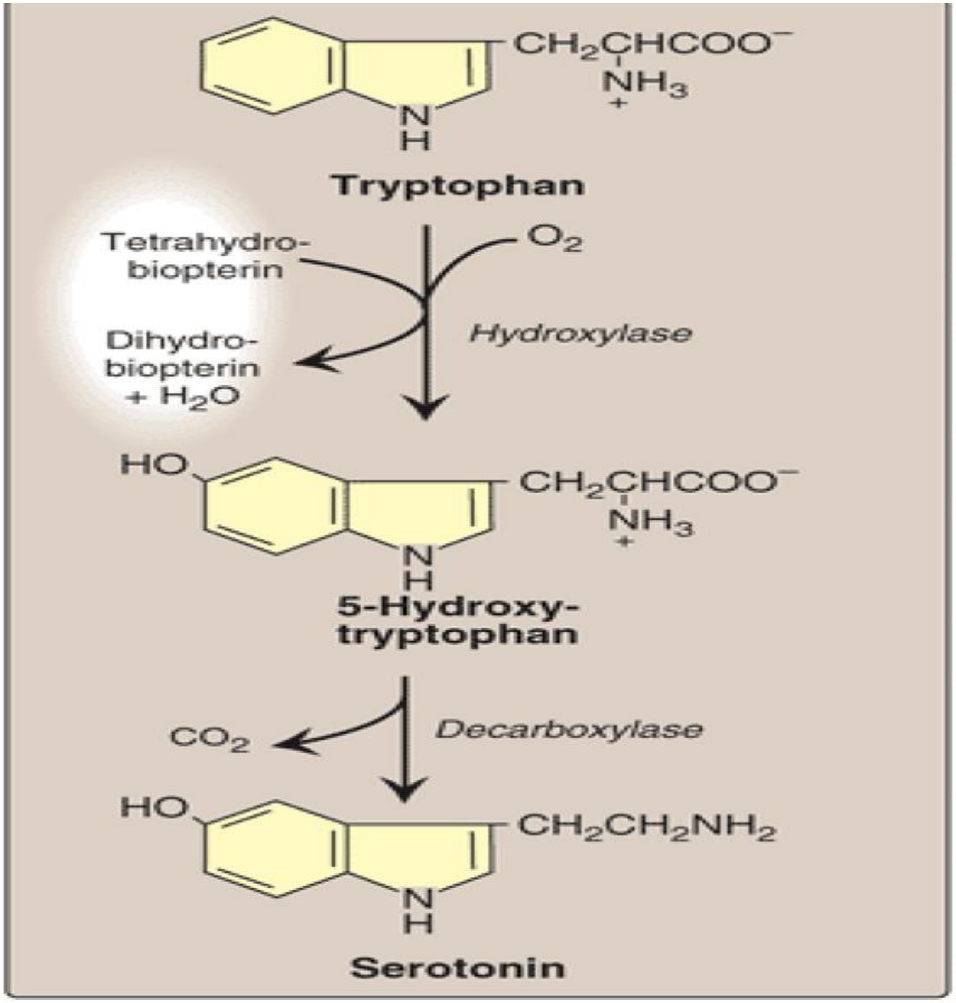


Figure 21.17 Biosynthesis of histamine

B-Serotonin

Serotonin, also called 5-hydroxytryptamine, is synthesized and stored at several sites in the body (Figure 21.18). By far the largest amount of serotonin is found in cells of the intestinal mucosa. Smaller amounts occur in the central nervous system, where it functions as a neurotransmitter, and in platelets. Serotonin is synthesized from tryptophan, which is hydroxylated in a reaction analogous to that catalyzed by phenylalanine hydroxylase. The product, 5-hydroxytryptophan, is decarboxylated to serotonin. Serotonin has multiple physiologic roles, including pain perception, affective disorders, and regulation of sleep, temperature, and blood pressure.

Figure 21.18 Synthesis of serotonin.



C-Creatine

Creatine phosphate (also called phosphocreatine), the phosphorylated derivative of creatine found in muscle, is a high-energy compound that can reversibly donate a phosphate group to adenosine diphosphate to form ATP (Figure 21.19). Creatine phosphate provides a small but rapidly mobilized reserve of high-energy phosphates that can be used to maintain the intracellular level of adenosine triphosphate (ATP) during the first few minutes of intense muscular contraction. [Note: The amount of creatine phosphate in the body is proportional to the muscle mass.

1-Synthesis: Creatine is synthesized from **glycine** and the **guanidino group of arginine,** plus **a methyl group from S-adenosylmethionine** (see Figure 21.19). Creatine is reversibly phosphorylated to creatine phosphate by creatine kinase, using ATP as the phosphate donor. [Note: The presence of **creatine kinase** in the plasma is indicative of tissue damage, and is used in the diagnosis of myocardial infarction.

2-Degradation: Creatine and creatine phosphate spontaneously cyclize at a slow but constant rate to form creatinine, which is excreted in the urine. The amount of creatinine excreted is proportionalto the total creatine phosphate content of the body, and thus can be used to estimate muscle mass. When muscle mass decreases for any reason (for example, from paralysis or muscular dystrophy), the creatinine content of the urine falls. In addition, any rise in blood creatinine is a sensitive indicator of kidney malfunction, because creatinine normally is rapidly removed from the blood and excreted. A typical adult male excretes about 15 mmol of creatinine per day.

Figure 21.19 Synthesis of creatine