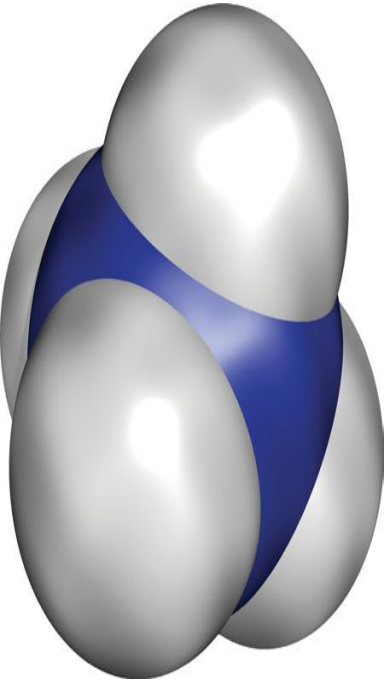
Protein Matabolism



For

Forth stage – Chemistry dept.

Professor

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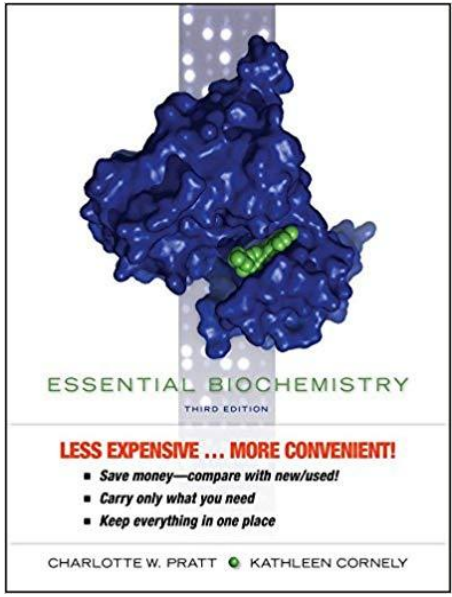


Syllabus

Protein Matabolism:

- Protein degradation
- Chemical signals for protein degradation
- Nitrogen Balance
- Digestion of Dietary Proteins
- Transport of ammonia to the liver
- Role of Folic Acid in Amino Acid Metabolism
- Metabolic Defects in Amino Acid Metabolism
- Biologically important compounds derived from Tryptophan
- Conversion of Amino Acids to Specialized Products
- Biosynthesis of heme:
- Jaundice
- Other Nitrogen-Containing Compounds

Protein degradation: There are two major enzyme systems responsible for degrading damaged or unneeded proteins:

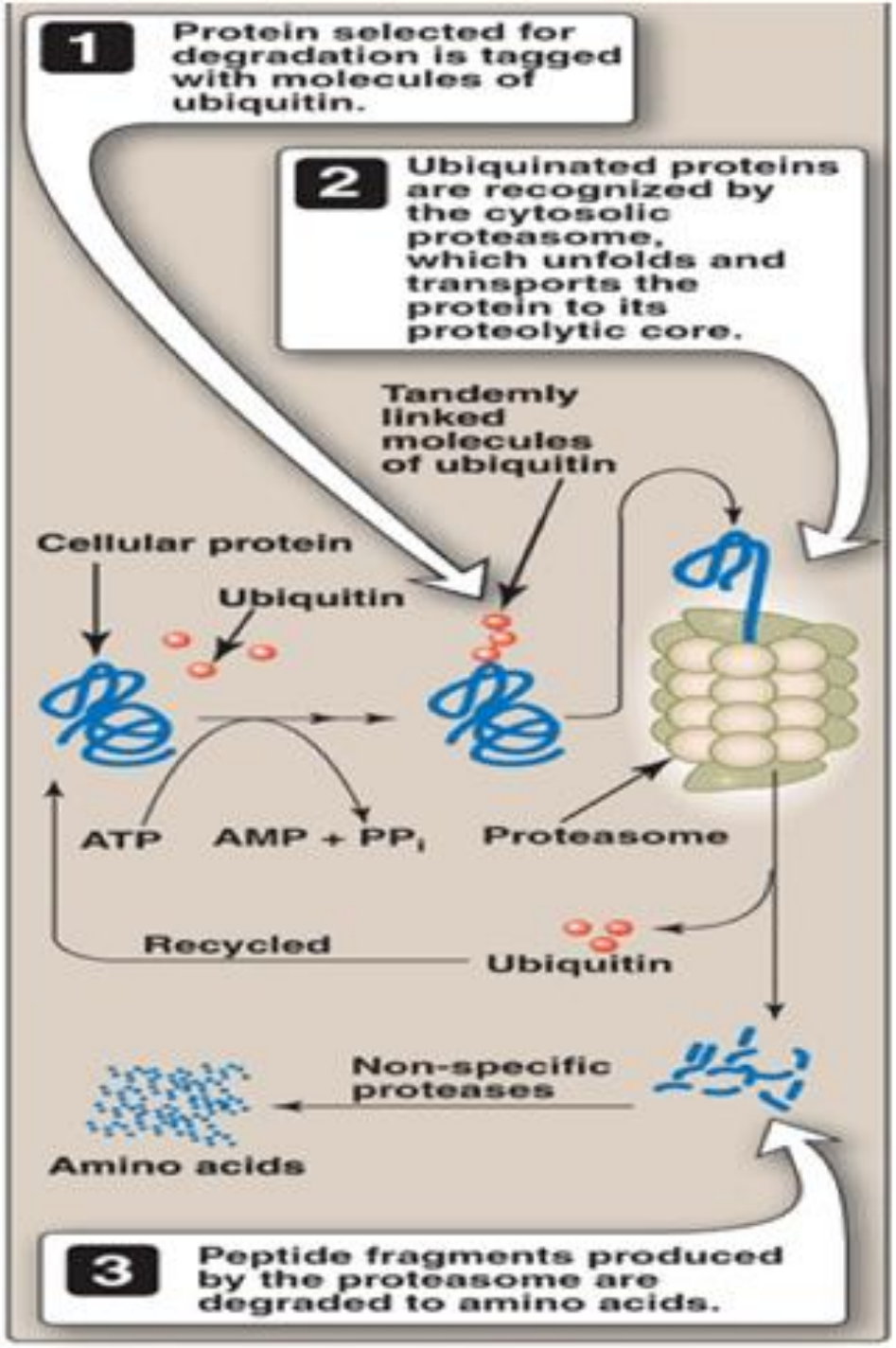
1-**The energy-dependent ubiquitin-proteasome mechanism**: Proteasomes mainly degrade **endogenous proteins,** that is, proteins that were synthesized within the cell.

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.

Endocytosis هي العملية التي من خلالها تقوم الخلايا بامتصاص الجزيئات (مثل البروتينات) التي تحتاج إليها. يتم استخدامه من قبل جميع خلايا الجسم، لأن معظم المواد ذات الأهمية بالنسبة لهم هي عبارة عن جزيئات بقطبية كبيرة التي لا يمكن ان تمر من خلال البلازما الكارهة للماء أو غشاء الخلية. هذه العملية هي نقيض للعملية التى تسمى الإخراج الخلوي أو الإيماس[٤] (الاسم العلمي: exocytosis.)

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.

Nitrogen Balance

Catabolism of amino acids leads to a net loss of nitrogen from the body .This loss must be compensated تعوض 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 nitrogen balance:

In whish nitrogen intake > nitrogen excretion. It shows that nitrogen is retained in the body, which means that protein is laid down يوضع او يستقر في الجسم. 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 الصدمة او الاذى الكبير, 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 milkاللبأ من الام are absorbed intact سليم 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 الحساسية.

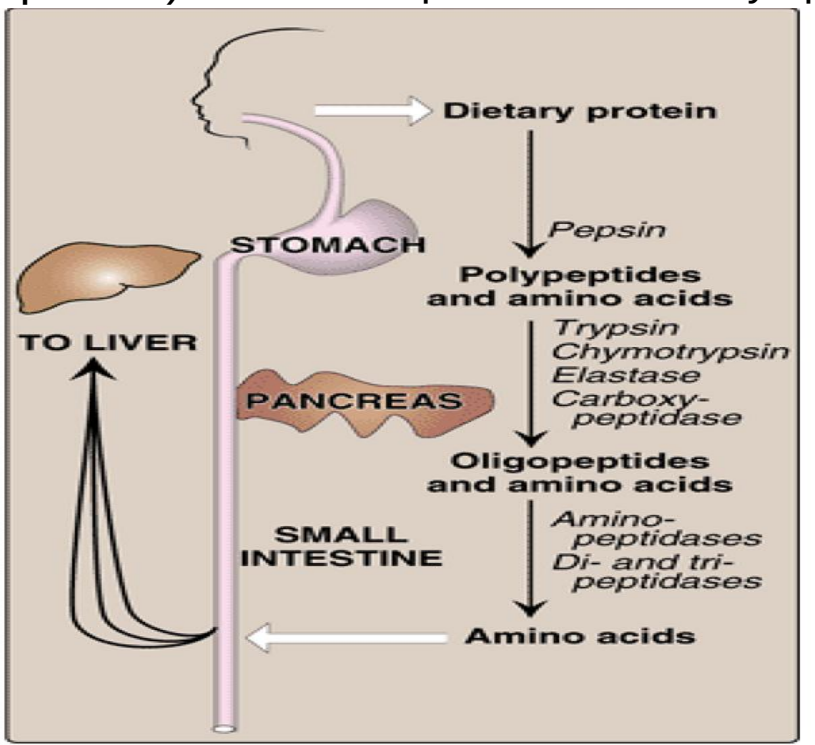


Figure 19.4 Digestion of dietary proteins by the proteolytic enzymes of the gastrointestinal tract.

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 الغدد المصلية 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 surfaceاللمعي السطحof intestinal mucosal cells of the brush border membrane-converts the pancreatic zymogen trypsinogen to trypsin by removal of a hexapeptide from the N-terminus of trypsinogen. Trypsin subsequently converts other trypsinogen molecules to trypsin by cleaving a limited number of specific peptide bonds in the zymogen. Enteropeptidase thus unleashes a cascade of proteolytic activity تطلق سلسلة من النشاط البروتيني,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 التليف الكيسي, 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,إسهال دهني) 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 المعوية 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 النظام البوابي. Thus, only free amino acids are found in the portal vein after a meal containing protein.These amino acids are either metabolized by 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.

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 خطوة واجبة 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.

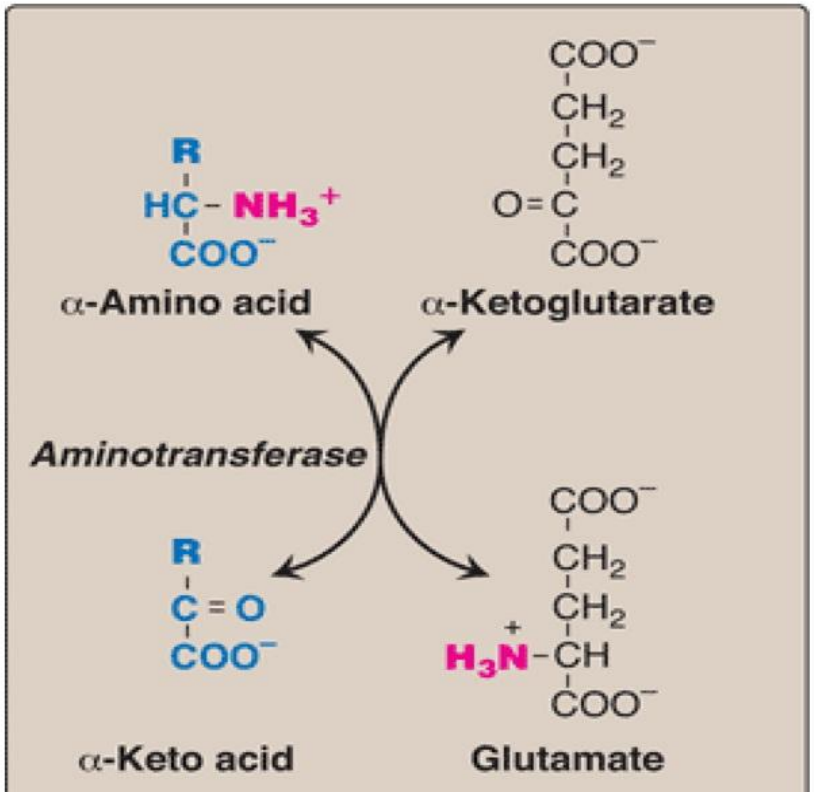


Figure 19.7 Aminotransferase reaction using α-ketoglutarate as the amino-group acceptor

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)or glutamate-pyruvate transaminase (GPT) and aspartate aminotransferase (AST) or glutamic oxaloacetic transaminase (GOT), Figure 19.8.

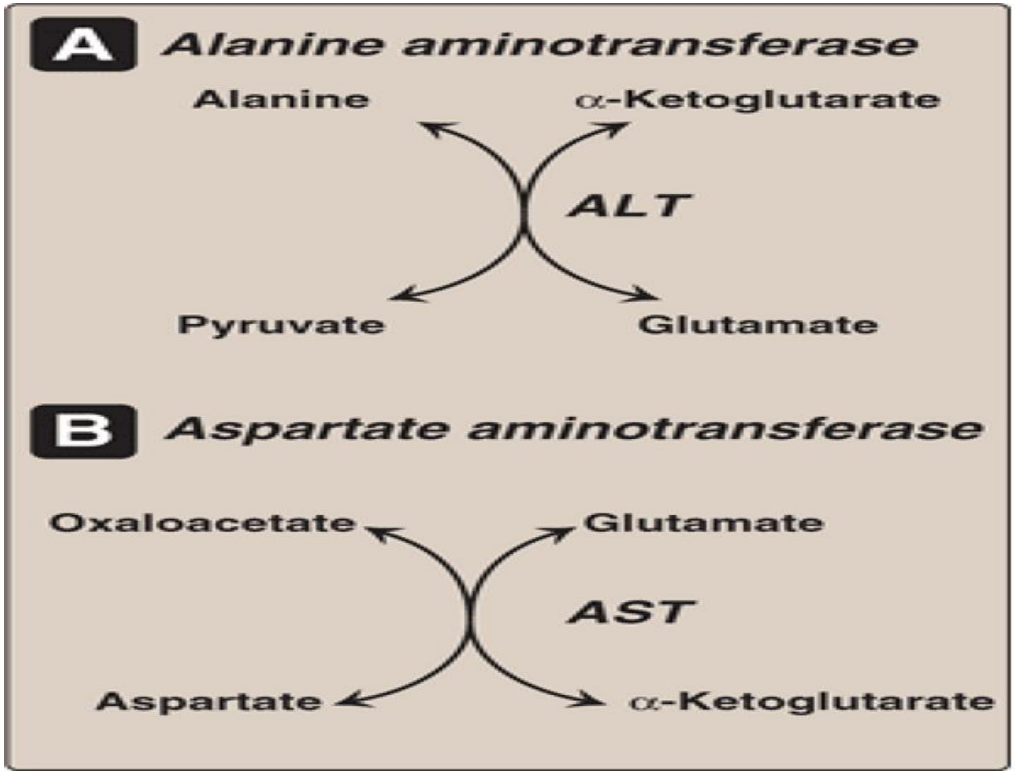


Figure 19.8 Reactions catalyzed during amino acid catabolism. A. Alanine aminotransferase (ALT). B. Aspartate aminotransferase (AST).

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

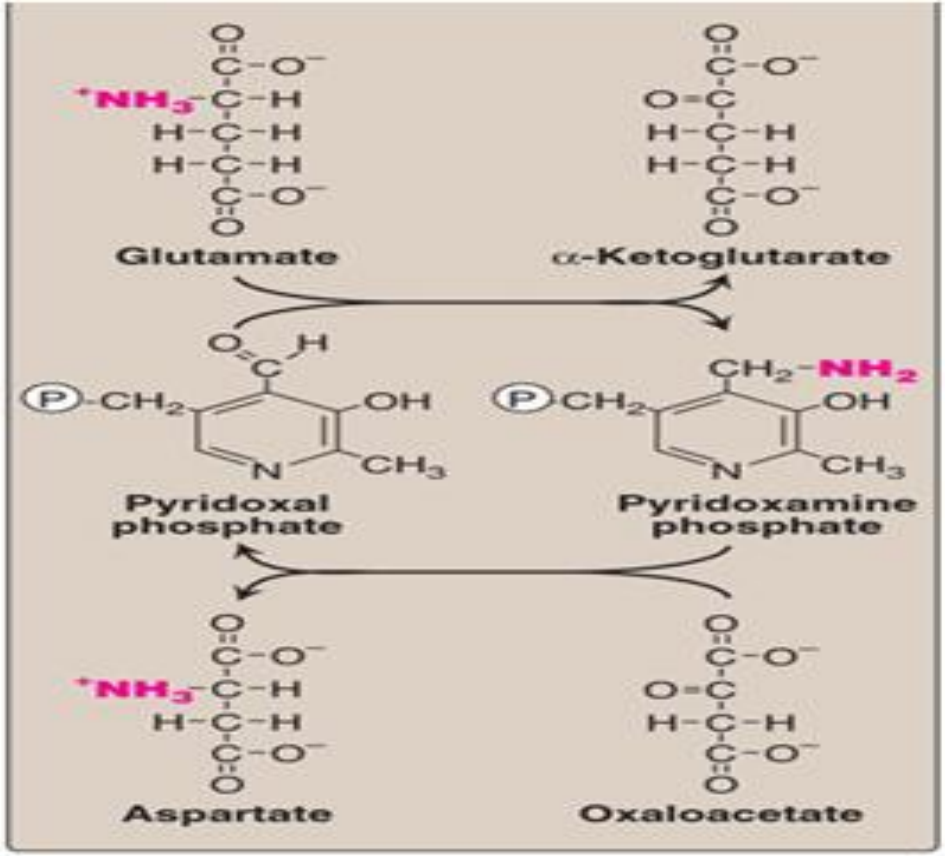
b-Aspartate aminotransferase (AST): AST formerly called glutamate-oxaloacetate transaminase, **AST (or GOT) is an exception to the rule that aminotransferases funnel amino groups to form glutamate**. During amino acid catabolism, **AST transfers amino groups from glutamate to oxaloacetate**, **forming 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 انهيار الدورة الدموية لفترات طويلة . **ALT (or GPT) is more specific than AST for liver disease اكثر تخصصية لان كمية اقل, but the latter is more sensitive because the liver contains larger amounts of AST اكثر تحسس لان كمية اكثر** . Serial enzyme measurements are often useful in determining the 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 (من خلال قياس مثلا troponin, CK).

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 (GTP) 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.

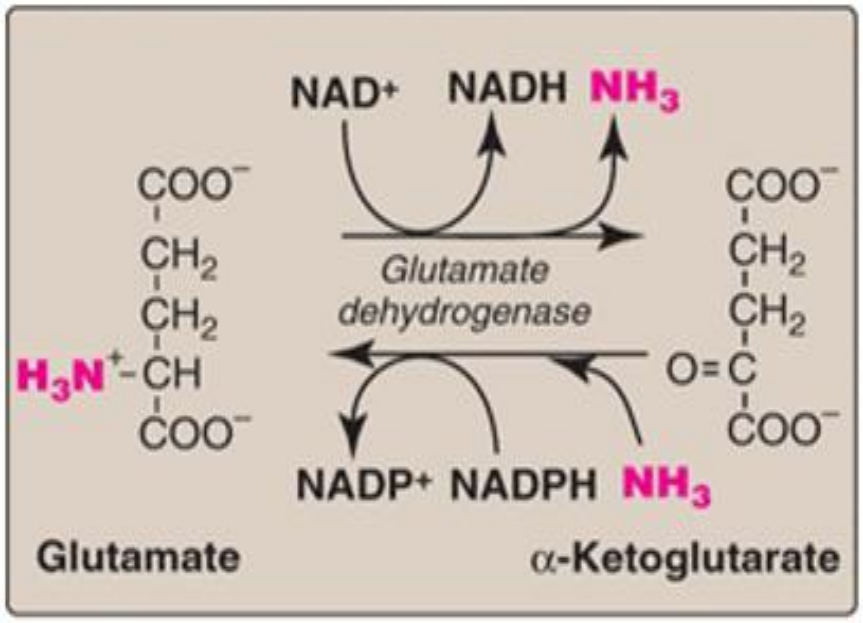


Figure 19.11 Oxidative deamination by glutamate dehydrogenase

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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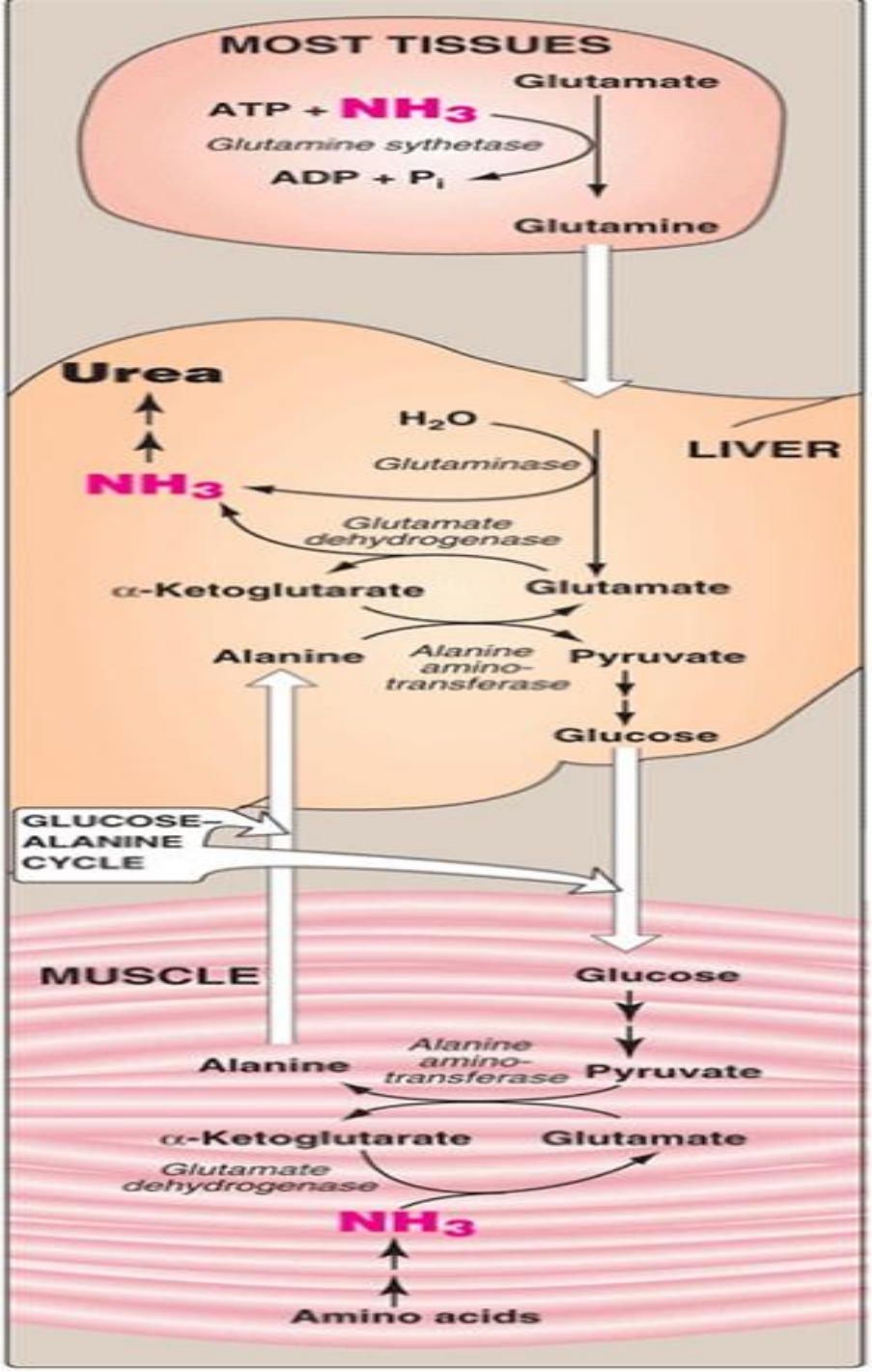


Metabolic Defects in Amino Acid Metabolism

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 خلل الموروثة 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.

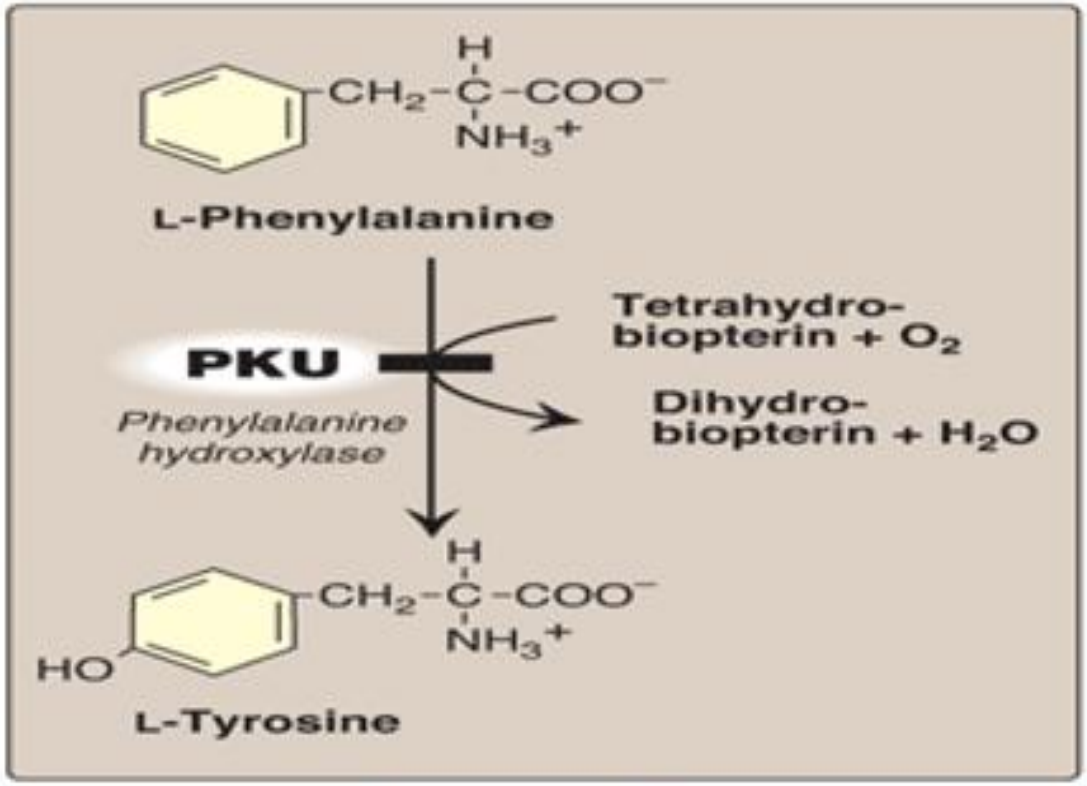


Figure 20.15 A deficiency in phenylalanine hydroxylase results in the disease phenylketonuria (PKU).

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 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 catecholamines**. Simply restricting الحد من 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 لا يمكن التنبؤ به .

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 فرط النشاط , tremor ارتعاش , microcephaly ضمور الراس, 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.

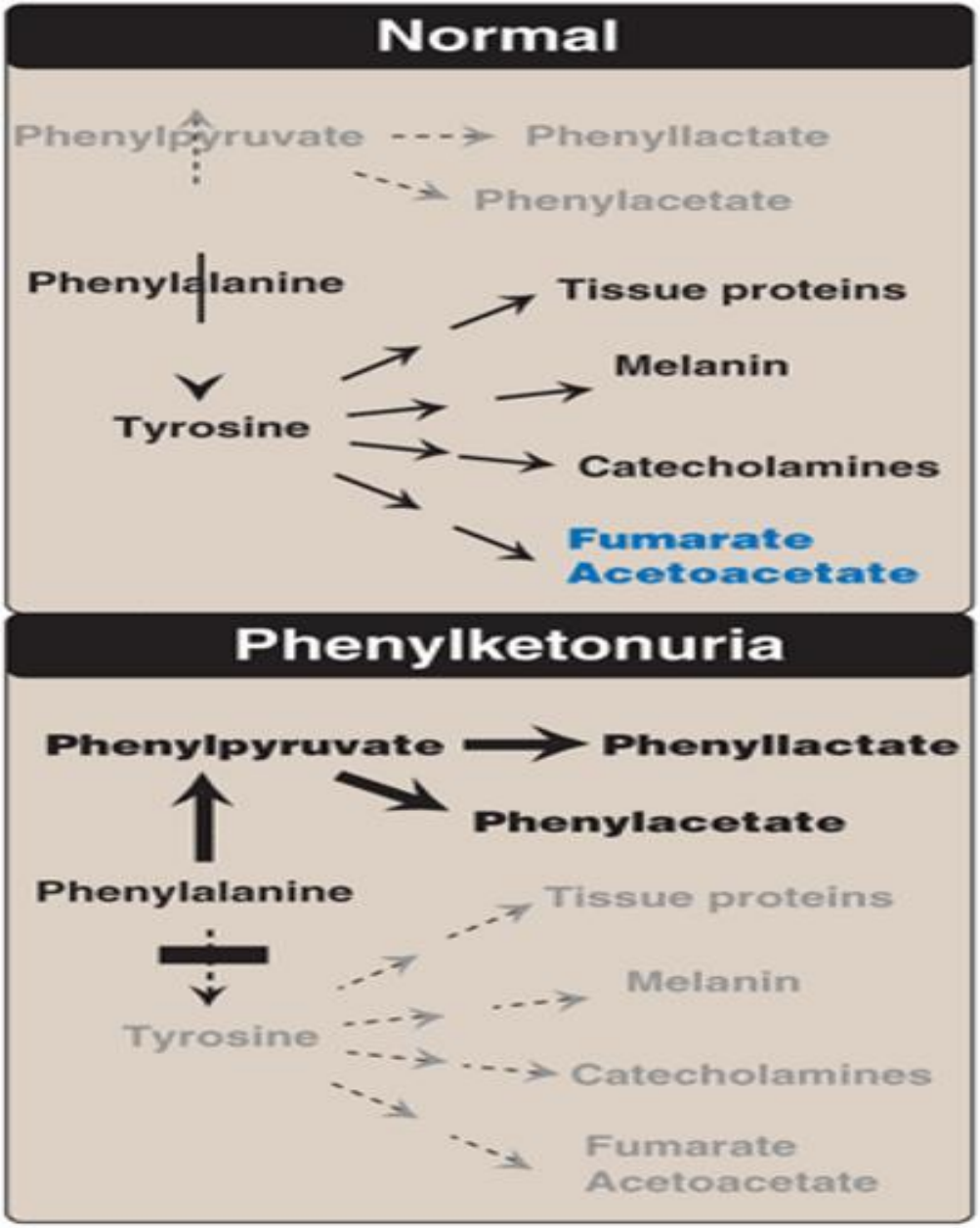


Figure 20.17 Pathways of phenylalanine metabolism in normal individuals and in patients with phenylketonuria.

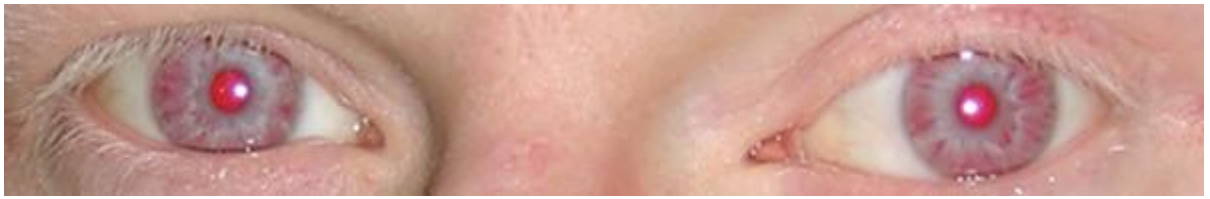
B. Maple syrup urine disease

Maple syrup urine disease داء البول القيقبي(MSUD) is a rare (1:185,000), autosomal recessive disorder مرض وراثي متنحي 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.

هو خلل وراثي في الجينات فالجسم لا يستطيع تكسير أجزاء عديدة من البروتين أثناء عملية الأيض. إن الشخص المصاب بهذا المرض لا يستطيع استخدام مكونات من البروتين (أحماض امينية) وهي الليوسين والايزوليوسين والفالين وهذه الأحماض هامة للنمو والتطور ولكن تناوله بكثرة يمكن أن يحدث تراكم هذه المواد الكيميائية في الدم

C. Albinism

Albinism البرصrefers 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 hairاشقر, 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 مرض وراثي متنحي, 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).

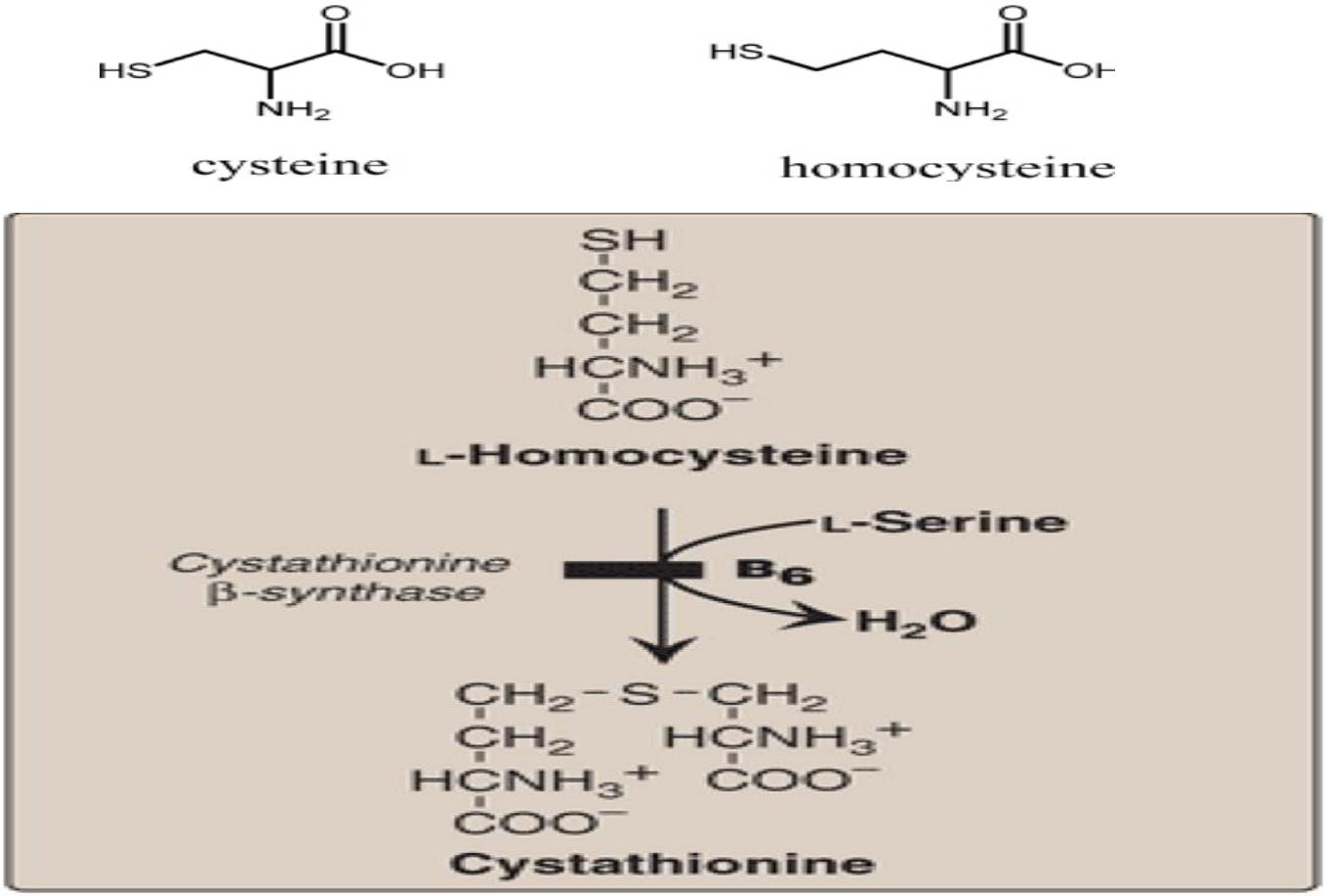
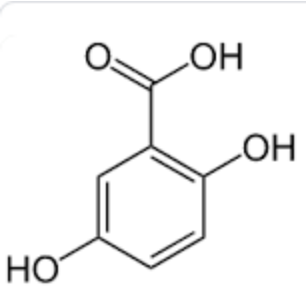


Figure 20.21 Enzyme deficiency in homo-cystinuria

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 يعطل عمل المفاصل.

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



homogentisic acid