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Enzymes

Enzymes are a proteins that synthesized by living cells and act as biological catalysts that speed up biochemical reactions without appearing in the net final equation.

Substrates: - a reactants in biochemical reaction upon which enzymes act to give product.

Characteristics of Enzymes

1. They enter the biochemical reaction in small quantity without changing in its chemical structure.

2. Catalysis occurs in a region within the enzyme known as the active site.

3. Enzymes convert the substrate to product in high efficient with high reaction rates where the rates of enzymatically catalyzed reactions are typically 10^6 to 10^{12} greater than those uncatalyzed reactions.

4. Enzymes have a greater degree of specificity as compared to the chemical catalysts; that is, enzymatic reactions rarely have side products.

5. The catalytic activity of many enzymes depends on the presence of small non-protein molecules inside them termed ***cofactors***. Cofactors can be subdivided into two groups: *metals ions* (Cu^{+2}, Fe^{+2}, Ni^{+2}…etc.) *and small organic molecules* called **coenzyme**. For example the enzyme carbonic anhydrase requires Zn^{+2} for its activity. Enzyme without its cofactor is called an *apoenzyme* (inactive enzyme); the complete active enzyme is called a *holoenzyme*.

Apoenzyme + cofactor = holoenzyme

Enzyme unit (or activity):- amount of enzyme which convert one Micro-mole (10^{-6} mole) of substrate to product in one minute under determined measurement condition.

Turnover number: - The number of moles of the substrate that converted to the product per one mole of the enzyme in one minute.

Specific activity: - enzyme units per milligram of protein. It consider a measure of the enzyme purity, thus increases with purification.

Factors affecting the rate of enzyme-catalysed reactions

1. Enzyme concentration
2. Substrate concentration
3. product concentration
4. Temperature
5. pH
6. presence of inhibitors or activators
7. Time
8. Light and radiation

[**Note**] The optimum temperature for most human enzymes is between 35 and 40°C. Human enzymes start to denature at temperatures above 40°C, but thermophilic bacteria found in the hot springs have optimum temperatures of 70°C.

Enzyme Classification

Enzymes can be classified into six distinct classes. These are:

1. Hydrolases: Enzymes that hydrolyze substrates.
2. Isomerases: Enzymes involved in isomerization reactions.
3. Ligases: Enzymes involved in condensation reactions.
4. Lyases: Enzymes that promote addition to double bonds.
5. Oxidoreductases: Enzymes involved in redox reactions.
6. Transferases: Enzymes that catalyze group transfer reactions

How substrate binds to the enzyme's active side

Active side: - a region in the enzyme at which the substrate binds to the enzyme, it consist of amino acids residues which participate in the catalysis.

There are two theories to explain how the enzyme binds to substrate

1. "Lock and key" theory.

In this model, the substrate has a specific shape match the active site of enzyme, like a lock and key [figure (1)]. The limitation of this theory is the rigidity of active side related to the substrate. However, this theory is applied on the number of simple kinetics enzymes.

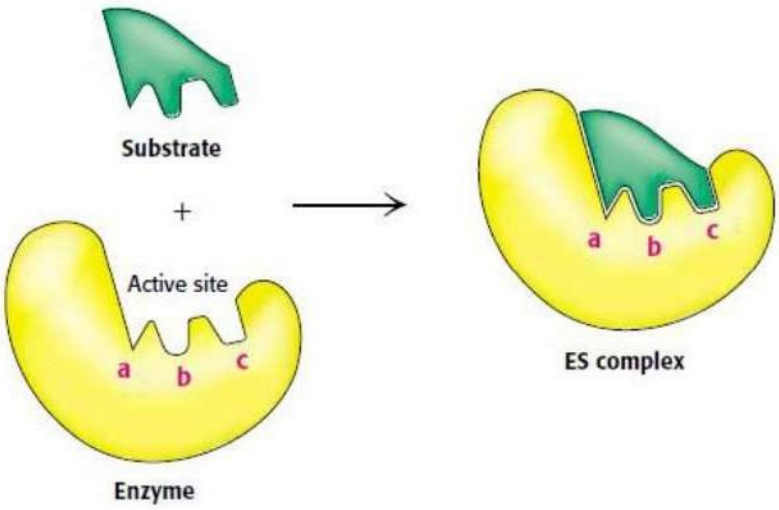


Figure (1) Lock-and-key model of enzyme–substrate binding. In this model, the active site of the unbound enzyme is complementary in shape to the substrate.

2- induced-fit model.

In this model, the active site changes shape as it interacts with the substrate [figure (2)]. Once the substrate is fully locked in and in the exact position, the catalysis can begin. In essence, substrate binding alters the conformation of the protein, so that the protein and the substrate "fit" each other more precisely.

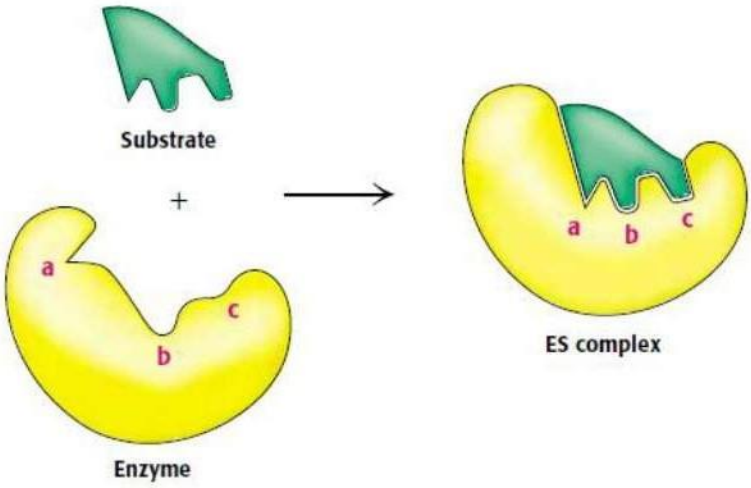


Figure (2) Induced-fit model of enzyme–substrate binding. In this model, the enzyme changes shape on substrate binding. The active site forms a shape complementary to the substrate only after the substrate has been bound

Michaelis-Menten equation

Michaelis and Menten suggests that the enzyme reversibly combines with its substrate to form an ES complex that subsequently yields product, regenerating the free enzyme.

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_2} E + P$$

where

S is the substrate

E is the enzyme

ES is the enzyme–substrate complex

P is the product

k1, k-1, and K2 are rate constants

The Michaelis-Menten equation describes how reaction velocity varies with substrate concentration:

$$v_o = \frac{V_{max}\,[s]}{k_m + [s]} \ldots\ldots (1)$$

where v_o = initial reaction velocity

V_{max} = maximal velocity

Km = Michaelis constant = (k-1 + k2)/k1

[S] = substrate concentration

Important conclusions about Michaelis-Menten kinetics

I- Relationship of velocity to enzyme concentration:

The rate of the reaction is directly proportional to the enzyme concentration at all substrate concentrations. For example, if the enzyme concentration is halved, the initial rate of the reaction (v_o), as well as V_{max}, are reduced to half.

II- Order of reaction:

When [S] is much less than Km, the velocity of the reaction is approximately proportional to the substrate concentration (Figure 3).
The rate of reaction is then said to be first order with respect to substrate. When [S] is much greater than Km, the velocity is constant and equal to V_{max}.
The rate of reaction is then independent of substrate concentration, and is said to be zero order with respect to substrate concentration.

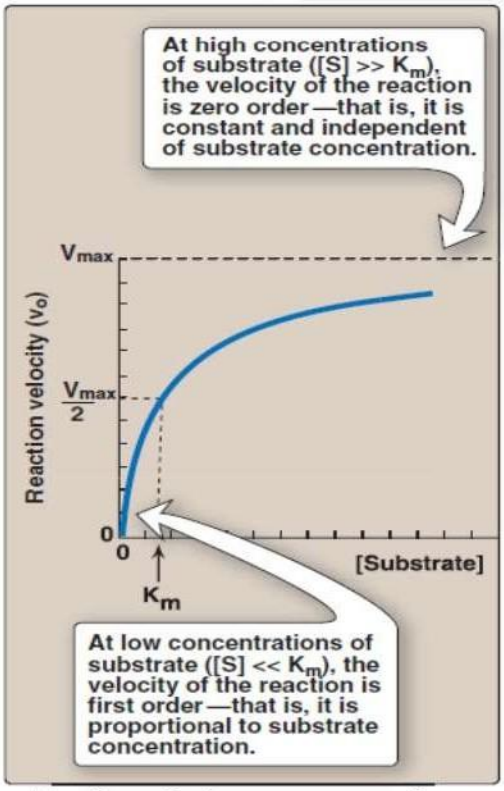


Figure (3) Effect of substrate concentration on reaction velocity for an enzyme catalyzed reaction.

III- Characteristics of K_m: -

K_m is the substrate concentration at which the reaction velocity equal to (1/2 V_{max}), it indicates how efficiently an enzyme selects its substrate and converts it to product. *K_m is often used as a measure of an enzyme's affinity for a substrate*. Let us assume that the measured velocity (v_o) is equal to 1/2 V_{max}. Then the equation (1) may be substituted as follows:

$$\frac{1}{2}V_{max} = \frac{V_{max}[S]}{K_m + [S]}$$

$$K_m + [S] = \frac{2V_{max}[S]}{V_{max}}$$

$$K_m + [S] = 2[S]$$

$$K_m = [S]$$

A low Km value indicates a strong affinity between enzyme and substrate figure (4), whereas **a high Km value** indicates a weak affinity between them. For majority of enzymes, the Km values are in the range of 10^{-5} to 10^{-2} moles. It may however, be noted that Km is **not dependent on the concentration of enzyme**.

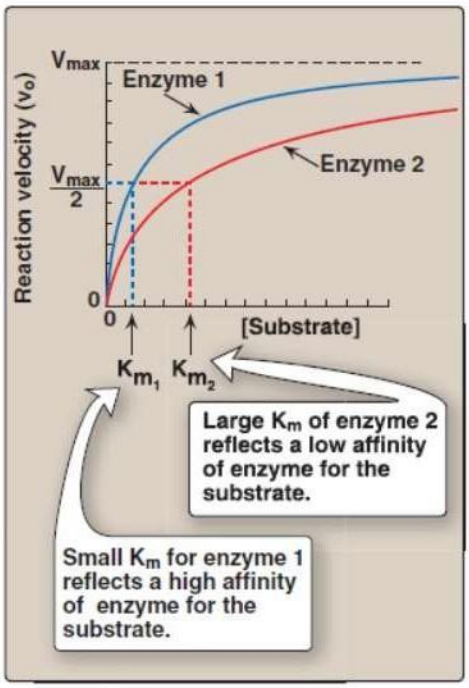


Figure (4) Effect of substrate concentration on reaction velocities for two enzymes:
enzyme 1 with a small Km, and
enzyme 2 with a large Km.

Isoenzymes

Isoenzymes (also called isozymes) are enzymes that catalyze the same reaction (act on the same substrate), but they differ in their physical and chemical properties which include (the structure, immunological properties, pH optimum, K_m and V_{max} values), also they possess different amino acid composition. For this reason, isoenzymes may contain different numbers of charged amino acids and may, therefore, be separated from each other by electrophoresis.

Regulation of enzymes by covalent modification

Many enzymes may be regulated by covalent modification, by the addition or removal of some groups from amino acid of enzyme such as (serine, threonine, or tyrosine). Common modifying groups include **phosphoryl, acetyl, adenylyl, methyl, amide, Carboxyl, hydroxyl, and sulfate**. In this case, a donor molecule is required to provide a functional group that modifies the properties of the enzyme. Most modifications are reversible. Thus, a fully active enzyme can be converted into an inactive form by the covalent attachment of a functional group. For example, the addition of a hydrophobic group can trigger association of enzyme with a cell membrane.

Phosphorylation and dephosphorylation are the most common but not the only way of covalent modification.

1. Phosphorylation and dephosphorylation:

Phosphorylation reactions of enzyme are catalyzed by a family of enzymes called protein kinases that use adenosine triphosphate (ATP) as a phosphate donor figure (5). Removal of the phosphate group by a protein phosphatase returns the enzyme to its original state.

Addition of phosphate group may convert enzyme from **inactive state** form into the **active form** or from **active** form into **inactive** form.

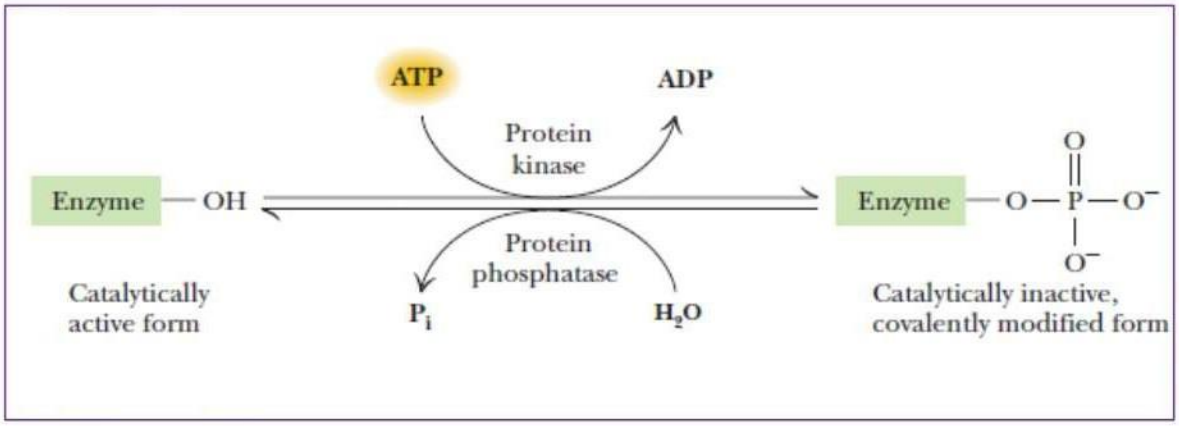


Figure (5) covalent modification by the addition and removal of phosphate groups.

2- Adenylation, acetylation, and methylation

In the adenylation process, an adenylyl group is added using ATP as adenylyl group donor. In the same way the acetylation and methylation, an acetyl and methyl group is added respectively figure (6).

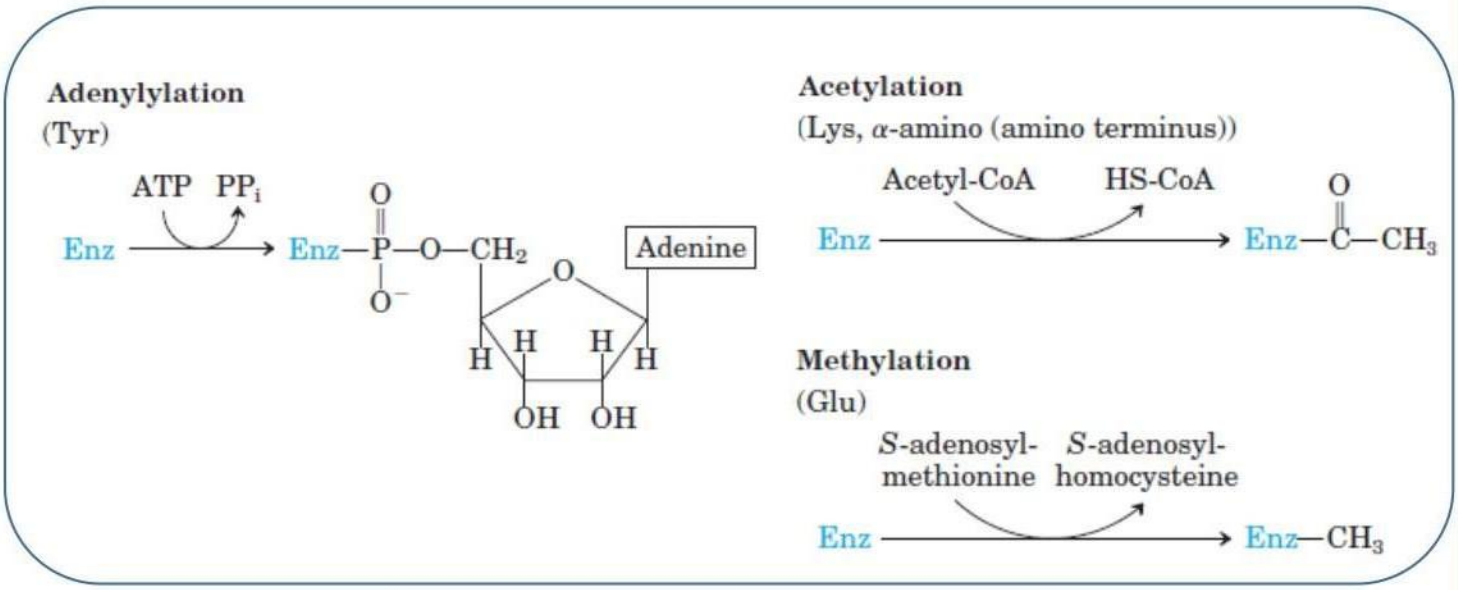


Figure (6) some enzyme modification reactions.

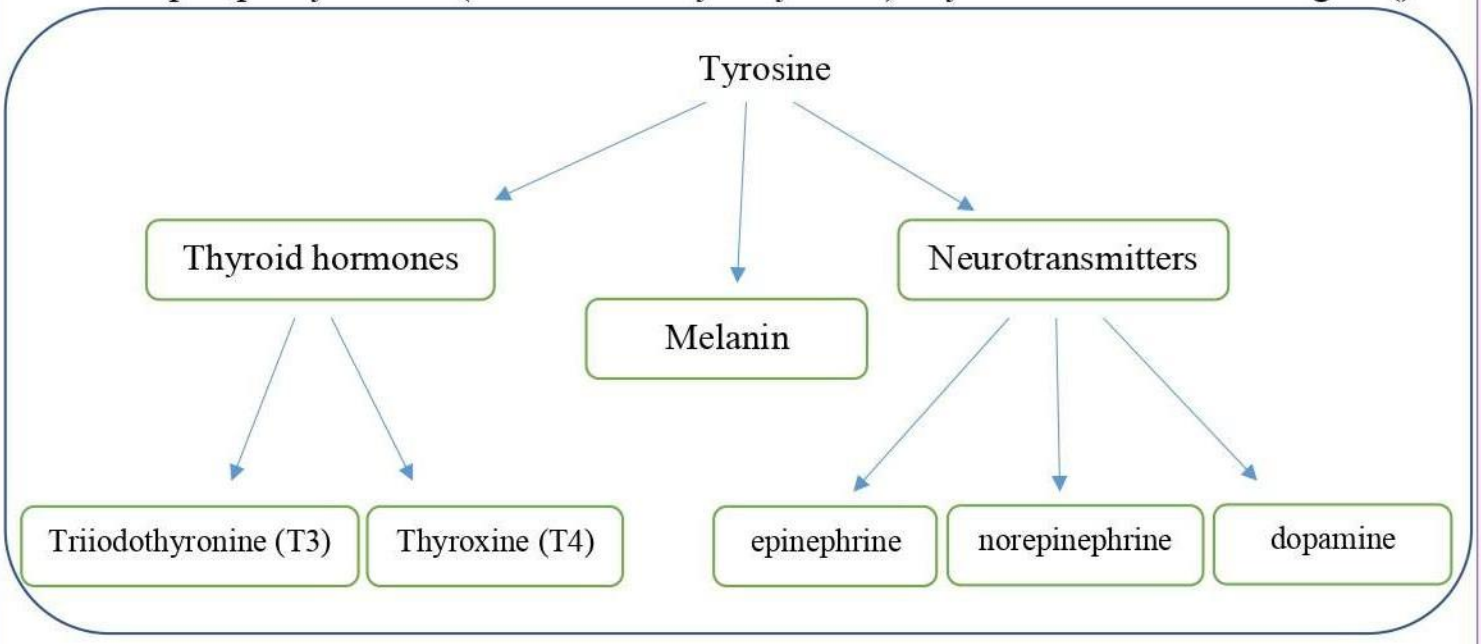
Enzyme deficiency diseases

1. Phenylketonuria

Phenylketonuria (PKU) the commonest inborn error of metabolism, is so named because of accumulation of amino acid called phenylalanine in the body, a type of phenylketone. Amino acids are the building blocks of protein. Phenylalanine is found in all proteins and some artificial sweeteners.

Phenylalanine hydroxylase is an enzyme your body uses to convert phenylalanine into tyrosine, which your body needs to create thyroid hormones (Triiodothyronine (T3), Thyroxine (T4)) and neurotransmitters such as (epinephrine, norepinephrine, and dopamine) and also to synthesis of melanin figure ().

PKU is caused by a defect in the gene that helps to create phenylalanine hydroxylase. When this enzyme is missing, your body can't break down phenylalanine. This causes a buildup of phenylalanine (and a deficiency of tyrosine) in your tissues and blood figure ().



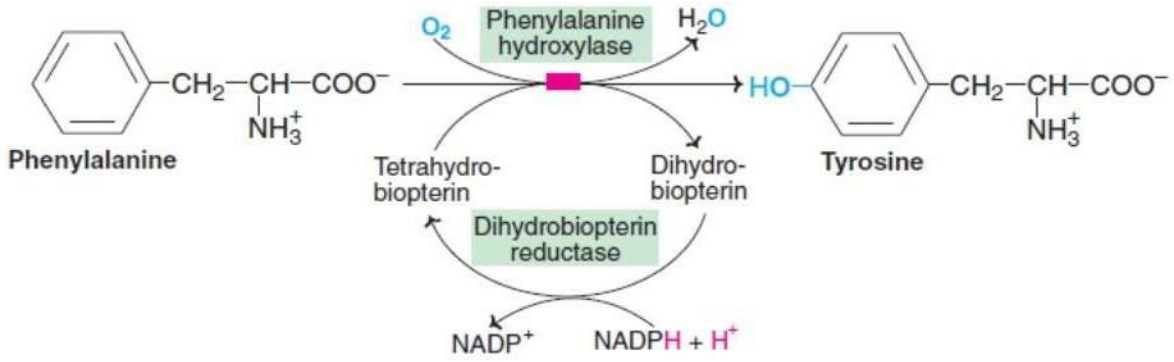
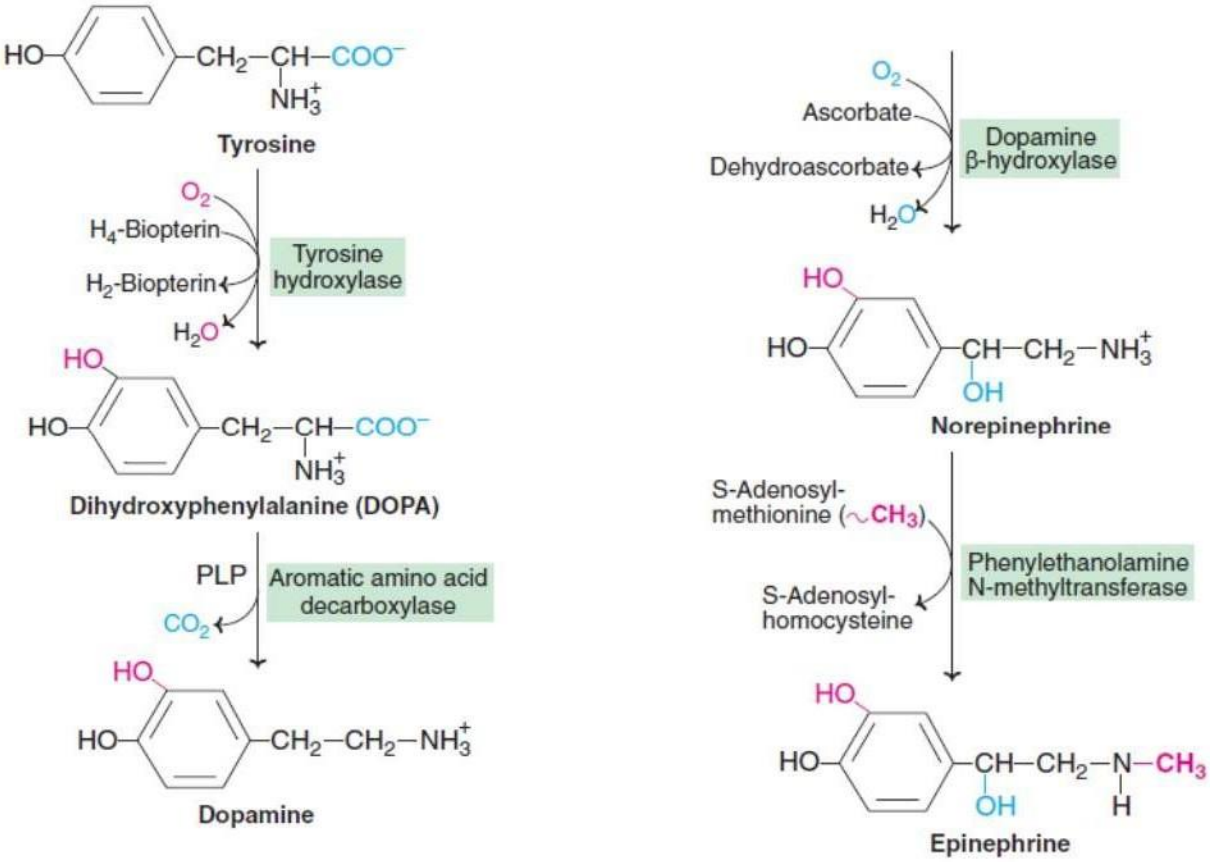


Fig. 15.18 : Synthesis of tyrosine from phenylalanine (—Block in phenylketonuria).



Metabolism of tyrosine-synthesis (dopamine, norepinephrine, epinephrine)

Due to disturbances in the routine metabolism, phenylalanine is diverted to **alternate pathways** (**Fig.15.23**), resulting in the excessive production of phenylpyruvate, phenylacetate, phenyllactate and phenylglutamine. All these metabolites are excreted in urine in high concentration in PKU. Phenylacetate gives the urine a mousey odour.

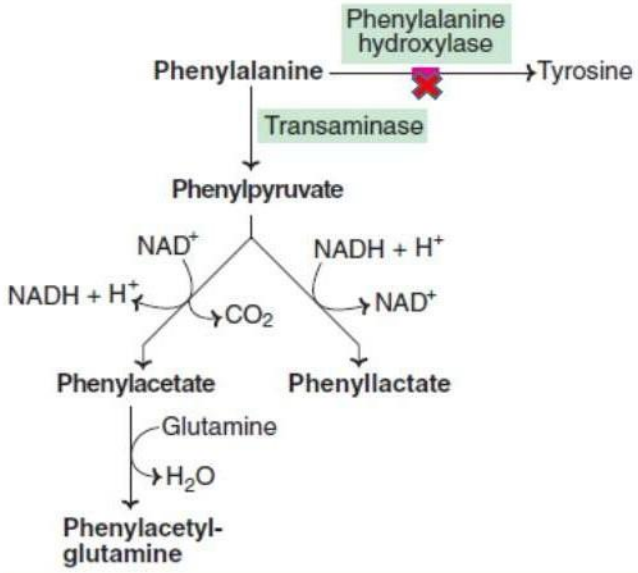
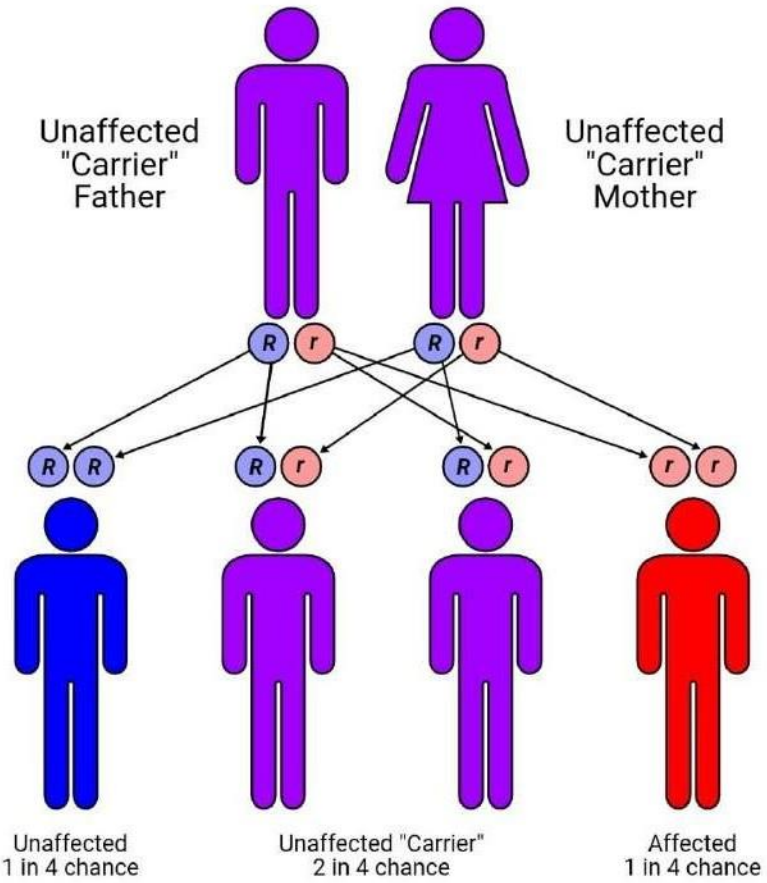


Fig. 15.23 : Metabolites that accumulate in phenylketonuria (▬–Block in phenylketonuria).

Genetic Cause of phenylketonuria

PKU is an inherited condition caused by a mutation in the PAH gene. The PAH gene helps to create phenylalanine hydroxylase, the enzyme responsible for breaking down phenylalanine. A dangerous buildup of phenylalanine can occur when someone eats high-protein foods, such as eggs and meat.

It is autosomal recessive, meaning that both copies of the gene must be mutated for the condition to develop. If both parents are carriers for PKU, there is a 25% chance any child they have will be born with the disorder, a 50% chance the child will be a carrier, and a 25% chance the child will neither develop nor be a carrier for the disease.



Inheritance of PKU: Phenylketonuria is inherited in an autosomal recessive fashion.

Symptoms and effects of phenylketonuria

1. Effects on central nervous system :

Mental retardation, hyperactivity, failure to walk or talk, failure of growth, seizures and tremor are the characteristic findings in PKU. If untreated, the patients show very low IQ (below 50).

2. Accumulation of phenylalanine in brain forbid the transport and metabolism of other aromatic amino acids (tryptophan and tyrosine).

3. The synthesis of **serotonin** (an excitatory neurotransmitter) from tryptophan is insufficient. This is due to the competition of phenylalanine and its metabolites with tryptophan that impairs the synthesis of serotonin.

4. **Effect on pigmentation:**

Melanin is the pigment synthesized from tyrosine by tyrosinase. Accumulation of phenylalanine competitively inhibits tyrosinase and forbid melanin formation. The result is **hypopigmentation** that causes light skin colour, fair hair, blue eyes etc.

Treatment

PKU is not curable. However, if PKU is diagnosed early enough, an affected newborn can grow up with normal brain development by managing and controlling phenylalanine ("Phe") levels through diet, or a combination of diet and medication.

2. Lactose intolerance

Also called (hypolactasia) is an inability to digest a type of natural sugar called lactose (milk sugar) because of the absence of the enzyme **lactase** in the intestines of adults.

When the small intestine of patient with lactose intolerant stops making enough of the enzyme lactase to digest and break down the lactose sugar to glucose and galactose. When this happens, the undigested lactose moves into the large intestine. The bacteria that are normally present in large intestine interact with the undigested lactose and cause symptoms such as bloating, gas, abdominal cramps and diarrhea figure (4).

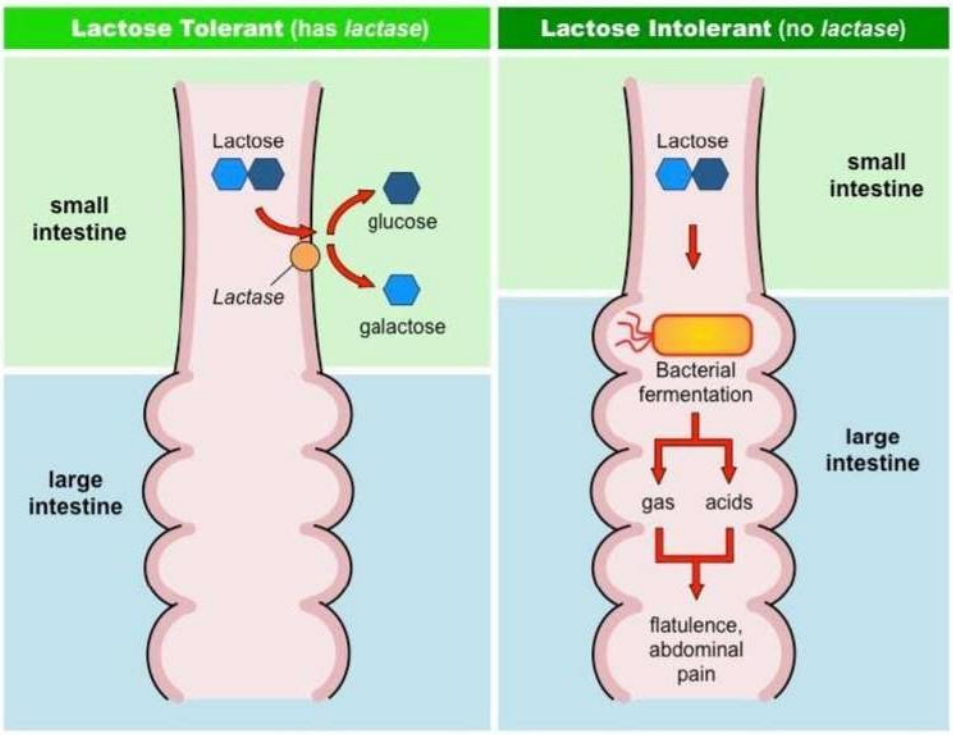


Figure (4)

There are four types lactose intolerance include:

1. Primary lactose intolerance:- occurs as the amount of lactase declines as people age.

2. Secondary lactose intolerance:- is due to injury to the small intestine such as from infection, celiac disease, inflammatory bowel disease, or other diseases.

3. Developmental lactose intolerance:- may occur in premature babies and usually improves over a short period of time.

4. Congenital lactose intolerance:- is an extremely rare genetic disorder in which little or no lactase is made from birth.

Diagnosis

1. Hydrogen breath test

2. Blood test

3. Stool acidity test

4. Intestinal biopsy

5. Stool sugar chromatography

6. Genetic diagnostic

Treatment

The simplest treatment of lactose intolerance is to avoid the consumption of products containing much lactose. Alternatively, the enzyme lactase can be ingested with milk products or by taking lactase in pill form prior to eating.