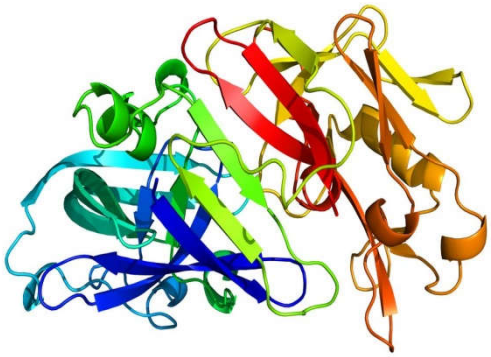
Enzymes, Classification, Mechanism and Diagnostic Importance

1. ENZYMES:

- Enzymes are biocatalysts which are also known as the catalysts of life. They are synthesized in living cells and speed up the biochemical reactions. Almost all metabolic processes in the cell need enzyme catalysis in order to occur. Like all catalysts, enzymes increase the reaction rate by lowering the energy of activation. The enzymes convert substrate to product.Chemically, enzymes are like catalyst and but they are not consumed in chemical reactions. Enzymes differ from most other catalysts and are fast enough to sustain life.



2. Catalyst:

- A catalyst is defined as a substance that increases the rate of a chemical reaction without itself undergoing any change in the overall process.

➢ **Example:**

- The student-teacher relationship may be a good example to understand how a catalyst works. The students often find it difficult to learn from a text-book on their own. The teacher explains the subject to the students and increases their understanding capability. In this example, the teacher acts like a catalyst in enhancing the understanding ability of students.A good teacher is always a good catalyst in student's life.

➢ Definition of Enzyme:

- Enzymes may be defined as biocatalysts synthesized by living cells. They are protein in nature, colloidal and thermolabile in character and specific in their action.

- **Colloidal:**

- The colloidal means that they do not form solution and remain in suspended form in solution.

- **Thermolabile:**

- Thermolabile means that they are very sensitive from high temperature. The enzymes structurally denature and do not work efficiently.
- The enzymes speed up the biochemical reactions and a small quantity of enzyme can bring about the decomposition of large amount of the substrate.
- The enzymes itself do not particicipate in the reaction.
- They bring about many complex reactions like oxidation,reduction and hydrolyses.
- The enzymes are characterized by three distinctive features.

- **Catalytic power:**

- The enzymes has ability to catalyze the biochemical reaction. They accelerate the reaction at higher rates.

- **Specificity:**

- The enzymes are very selective in their nature. They are very specific in the enzyme catalyzing reactions.

- **Regulation:**

- The enzymes are metabolic activators.Sometimes they may inhibit the reactions due to inhibitors.

- **Example:**

- The enzymes control our body reactions. Without enzymes the reactions would takes place slowly **e.g** In the laboratory, hydrolysis of proteins by a strong acid at 100°C takes place at least in two days.The same protein is fully digested by the enzymes in gastro intestinal tract at body temperature (37°C) within two hours.
- This remarkable difference in the chemical reactions taking place in the living system is exclusively due to enzymes.The very existence of life is unimaginable without the presence of enzymes.

3. Structure of enzyme:

- Enzymes are globular complex protein structures made up of long linear chains of amino acids ranging from just 62 amino acids rsidues in size to over 25,000 amino acids residues.
- Most enzym are much larger than the subtrate on which they act.

- Unlike most catalysts enzymes are not consumed by the reactions that they catalyze.
- During reactions only a small area is directly involved. This area is called active site.

➢ **Active site:**

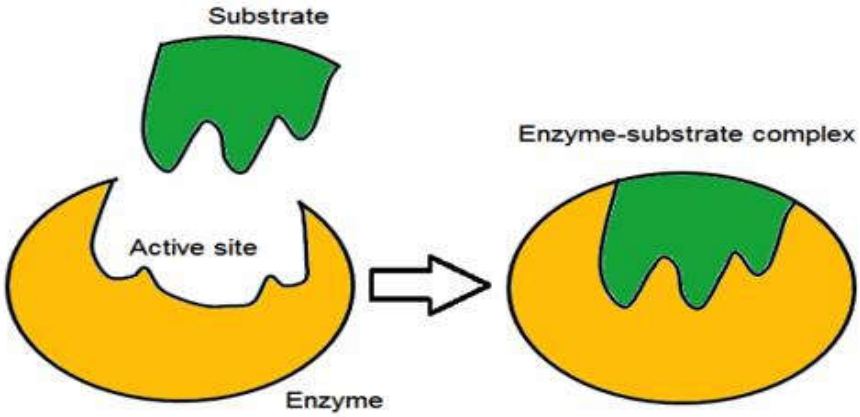
- It is region where substrate is recognized and then bound for reaction. This regionis called active site. The active site is not rigid it may change according to substrate.

➢ **Substrate:**

- An enzyme substrate is the material on which an enzyme acts. The enzymec specifically reacts on a specific substrate .

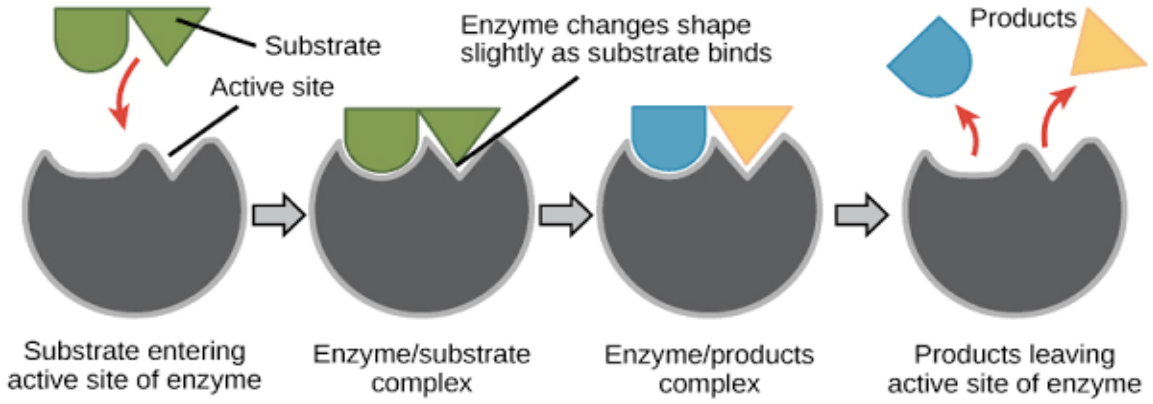
➢ **Product:**

- A product is something manufactured by an enzyme from its substrate.



4. Mechanism of Enzyme Action:

- The enzymes are very specific in their action. They reacts with a specific substrate.
- The substrate approaches the active site.
- The substrate binds to the active site and it forms enzyme substrate complex.
- The substrate is then transformed into product.
- The product is released.
- The enzymes are recycled at the end of reaction. They are not used in the reaction. The enzymes can be used again and again.



- **Enzymes are sometimes considered under two broad categories:**

➢ **Intracellular enzymes** :

- They are synthesized and retained in the cell to perform functions of cell itself. These are known as intracellular enzymes.
- They are found in nucleus ,mitochondria and choloroplasts.

➢ **Example:**

- Oxireductase catalyses the biological oxidation.

➢ **Extracellular enzymes:**

- These enzymes are synthesized in the cell but secreted from cell to work externally and are called extracellular enzymes.

➢ **Examples:**

- Digestive enzymes are secreted in the pancrease but are not used by pancrease. They are transported to duodenum.

5. Classification of enzymes:

➢ **Oxidoreductases :**

- These enzymes are involved in oxidation-reduction reactions.

➢ **Transferases :**

- Enzymes that catalyse the transfer of functional groups are called transferases.

➢ **Hydrolases :**

- These are enzymes that bring about hydrolysis of various compounds.

- **Lyases :**

- These enzymes specialised in the addition or removal of water, ammonia, CO2 etc.
- **Isomerases :**
- Enzymes involved in all the isomerization reactions are called isomerases.
- **Ligases:** Enzymes catalysing the synthetic reactions. It originated from Greek word "ligate" which means to bind. The two molecules are join together and ATP is used.

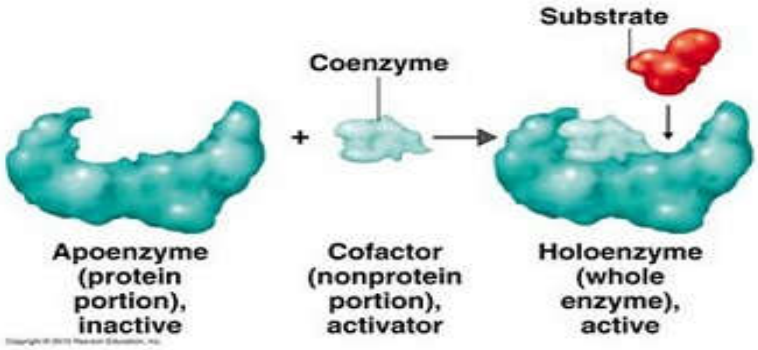
6. Characteristics of enzymes:

- Enzymes are generally globular proteins which are made up of amino acids. The sequence of the amino acids specifies the structure which in turn determines the catalytic activity of the enzyme.Although structure determines function but sometimes it cannot be predicted from structure alone because some other factors also effects its reactivity.
- Enzyme structures unfold when heated or exposed to chemical denaturants and this disruption to the structure typically causes a loss of activity and enzymes do not work properly Enzyme denaturation is normally linked to temperatures above a species normal survival level.
- As a result enzymes from bacteria living in volcanic environments such as hot springs are used for industrial purpose due to their ability to function at high temperatures.

Holoenzyme:

- The basic functional unit of the enzyme is known as holoenzyme.It is complete and catalytically active.
- The holoenzyme is often made up of :

- **Apoenzyme:**It is the protein part.
- **Coenzyme:**It is non-protein part.

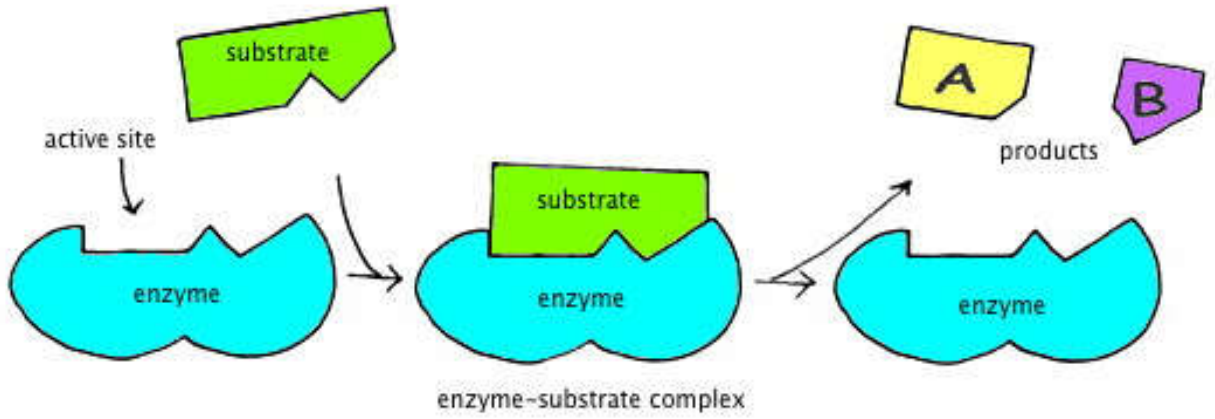


➢ Active site:

- Enzymes are big in size as compared to substrates which are relatively smaller. Evidently, a small portion of the huge enzyme molecule is directly involved in the substrate binding and catalysis.
- The active site of an enzyme represents as the small region at which the substrate(s) binds and participates in the catalysis of enzyme.

➢ **Salient features of active site:**

- The existence of active site is due to the tertiary structure of protein.
- Active sites are regarded as small pockets occupying a small region in a big enzyme molecule.
- The active site is not rigid in structure and shape. It is rather flexible to promote the specific substrate binding.
- Generally, the active site possesses a substrate binding site and a catalytic site .The catalytic site is for the catalysis of the specific reaction.
- The coenzymes or cofactors on which some enzymes depend are present as a part of the catalytic site.
- The substrate(s) binds at the active site by weak non-covalent bonds.
- Enzymes are specific in their function due to the existence of active sites.
- The commonly found amino acids at the active sites are serine, aspartate, histidine, cysteine, lysine, arginine, glutamate, tyrosine etc. Among these amino acids, serine is the most frequently found.
- The substrate[S] binds the enzyme (E) at the active site to form enzyme-substrate complex (ES). The product (P) is released after the catalysis and the enzyme is available again to perform another reaction.



➢ Coenzymes:

- The non-protein part of the enzyme is called coenzyme.This part is not always adequate to bring about the catalytic activity.Many enzymes require certain non-protein small additional factors, collectively referred to as cofactors for catalysis.
- The cofactors may be organic or inorganic in nature.

➢ Definition:

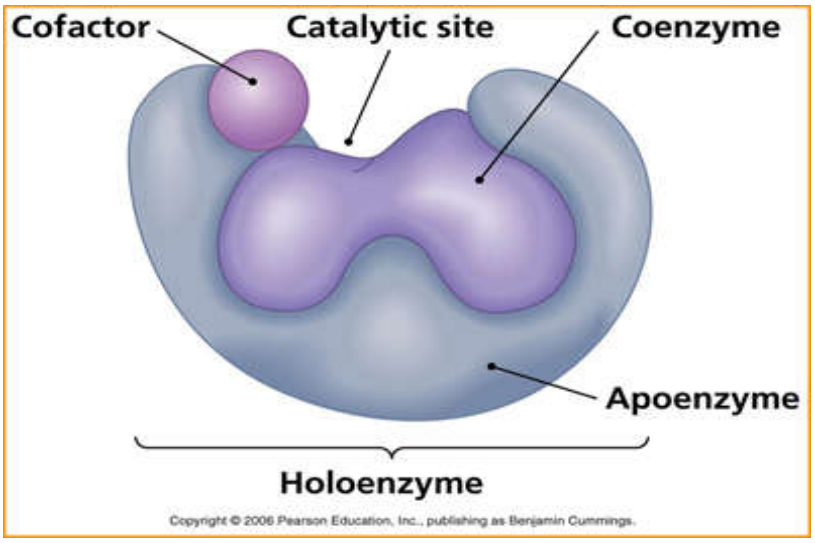
- The non-protein, organic, low molecular weight and dialysable substance associated with enzyme function is known as coenzyme .
- The functional enzyme is referred to as holoenzyme.
- It is made up of a protein part which is known as apoenzyme and a non-protein part called coenzyme.
- The term activator is referred to the inorganic cofactor like Ca2+, Mg2+, Mn2+ etc, which are necessary to enhance enzyme activity.

➢ Properties of coenzymes:

- Coenzymes act as second substrate.
- Coenzymes are often regarded as the second substrates or co-substrates.
- Coenzymes undergo alterations during the enzymatic reactions, which are later regenerated .
- Coenzymes do not decide enzyme specificity.
- A particular coenzyme may participate in catalytic reactions along with different enzymes.

➢ **Example:**

- NAD+ acts as a coenzyme for lactate dehydrogenase and alcohol dehydrogenase. In both the enzymatic reactions, NAD+ is involved in hydrogen transfer.
- The specificity of the enzyme is mostly dependent on the apoenzyme and not on the coenzyme .



➢ Enzymes Specific Nature:

- Enzymes are highly specific in their actions interacting with one or few substrates and catalyze only one type of chemical reaction. They are very sensitive in nature. The contact between the enzyme and substrate is the most essential pre-requisite for enzyme activity.

➢ **The enzymes activity is affected by following factors:**

- Temperature
- pH
- Concentration of substrate
- Concentration of enzyme
- Concentration of product
- Effect of time

➢ Temperature:

- The velocity of an enzyme reaction increases with increase in temperature up to a maximum level and then declines at higher temperature.Like most chemical reactions,the rate of an enzyme-catalyzed reaction increases as the temperature is raised.

- **Optimum temperature:**

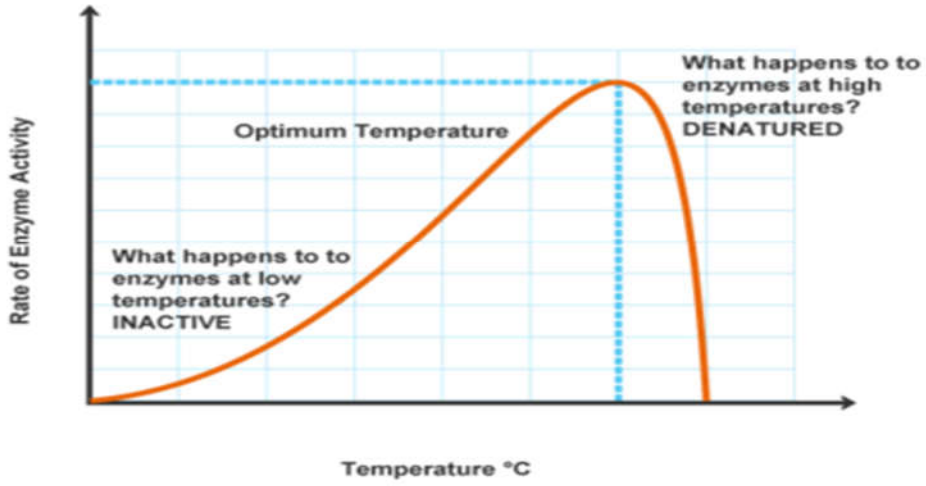
- The temperature at which the rate of reaction is maximum is called optimum temperature.
- The enzymes work at the maximum rate at optimum temperature. A ten degree Centigrade rise in temperature will increase the activity of most enzymes by 50 to 100%.

- **Temperature coefficient or Q 10:**

- It is defined as increase in enzyme velocity when the temperature is increased by 10°C. For a majority of enzymes, Q10 is 2 between 0°C and 40°C. Increase in temperature results in higher activation energy of the molecules and more molecular collision and interaction for the reaction to proceed faster.

- **Examples:**

- The optimum temperature for most of the enzymes is between 35°C–40°C.
- Some plant enzymes like urease have optimum activity around 60°C.
- Majority of the enzymes become inactive at higher temperature (above 70°C)
- and their structure destroy and they are denatured.



- **pH:**

- The enzyme catalyzing reactions are mostly pH dependent. Increase in the hydrogen ion concentration which is known as pH considerably influences the enzymes activity. Each enzyme has an optimum pH at which the rate of reaction is maximum.

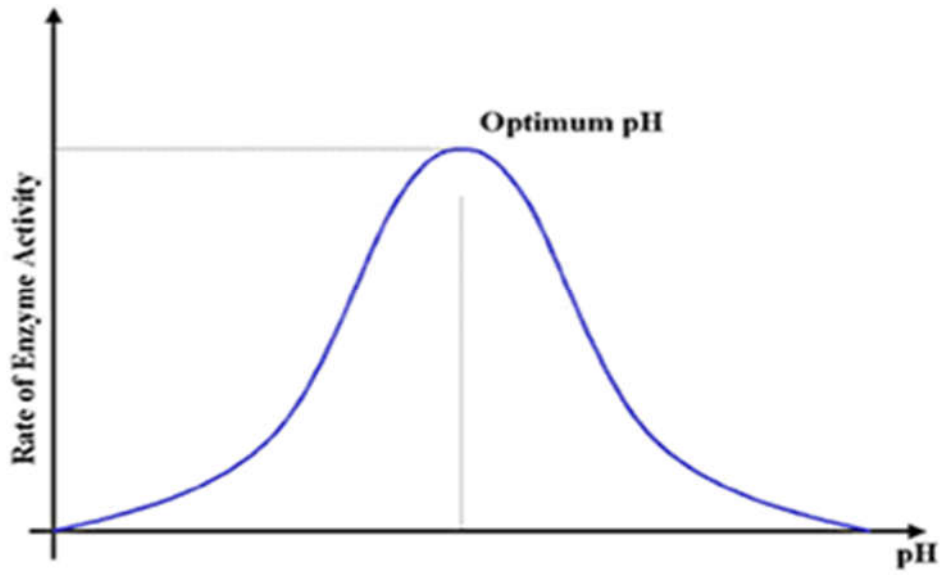
- **Optimum pH:**

- The pH at which rate of enzyme action is maximum and enzyme works efficiently is called optimum pH.
- Below and above this pH, the enzyme activity is much lower and at extreme pH, the enzyme becomes totally inactive.

➢ **Examples:**

- Most of the enzymes of higher organisms show optimum activity around neutral pH
  - (6-8).
- There are, however, many exceptions like pepsin (1-2), acid phosphatase (4-5) and alkaline phosphatase (10-11).

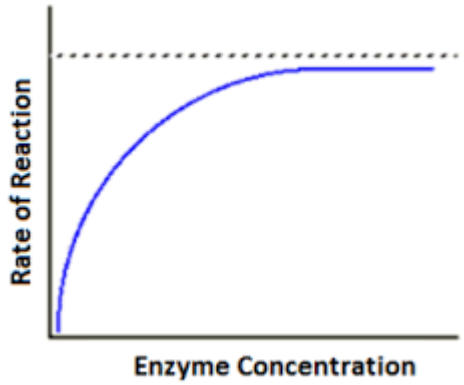
➢ The enzymes of fungi and plants are mostly active between pH 4-6.



➢ Concentration of enzymes:

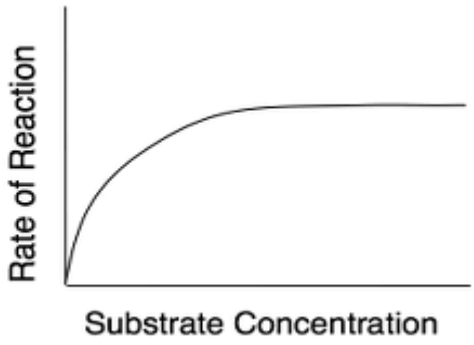
- As the concentration of the enzyme is increased, the velocity of the reaction proportionately increases. This is because more active sites are available for enzyme activity.

- In fact, this property of enzyme is made use in determining the serum enzymes for the diagnosis of diseases. By using a known volume of serum, and keeping all the other factors at optimum level, we can diagnose diseases.



➢ Concentration of substrate:

- Increase in the substrate concentration gradually increases the velocity of enzyme reaction within the limited range of substrate. As the substrate concentration increases the more products are formed within limited range but the reaction rate may increase when no active site is available for substrate reaction. Then by increasing enzyme concentration the reaction rate may not be affected.



➢ Concentration of products:

- The accumulation of reaction products generally decreases the enzyme velocity.The products combine with the active site of enzyme and form a loose complex and inhibit the enzyme activity and enzymes active sites may not be available for reaction and rate of reaction slows down.

➢ Effect of time:

- The time itself had no role. Time is dependent on other conditions. If the conditions are optimum then less time is required for reaction to proceed.But under unsuitable condition reactions do not proceed properly and require time to proceed and complete.

➢ Enzymes are not damaged:

- The enzymes which are used in reaction, they are not damaged. The enzymes are available unchanged at the end of reaction. The same enzyme can be used for further reactions. They only catalyze the eaction by lowering the energy of activation but are not used in the reaction.

➢ Enzymes speed up chemical reactions:

- The enzymes work by lowering the energy of activation.

➢ **Activation energy:**

- The energy which is used to start the reaction is called activation energy.
- The reaction proceeds much faster as compared to other reactions taking place in absence of enzymes. So enzymes play a vital role in proceeding the reactions.

7. DIAGNOSTIC IMPORTANCE OF ENZYMES:

- Enzyme activities in biological fluids is of great clinical importance. Enzymes play critical role in the metabolic activities of all living organisms whether humans, animals, plants or microorganisms and are widely applied in microbialtechnology and their diagnosis processes.
- Enzymes act as preferred bio-markers in various disease conditions, such as myocardial infarction, renal disease, liver disease, rheumatoid arthritis, cancer, and so for. They provide insight into the diseased condition by diagnosis, or by assessment of response therapy.

➢ Definition:

- The enzymes that are used for the detection or diagnosis of disease conditions are called "diagnostic enzymes".
- Enzymes in the circulation are divided into two groups:
- Plasma functional
- Plasma non-functional

➢ Plasma specific or plasma functional enzymes:

- Certain enzymes are normally present in the plasma and they have specific functions to perform. Generally, these enzyme activities are higher in plasma than in the tissues. They are mostly synthesized in the liver and enter the circulation.

➢ **Examples:**

- Lipoprotein lipase, plasmin, thrombin, choline esterase, ceruloplasmin etc.

➢ Non-plasma specific or plasma non-functional enzymes:

- These enzymes are either totally absent or present at a low concentration in plasma compared to their levels found in the tissues.

➢ **Example:**

- The digestive enzymes of the gastrointestinal tract (e.g. amylase, pepsin, trypsin, lipase etc.) present in the plasma are known as secretory enzymes .

➢ Constitutive enzymes:

- All the other plasma enzymes associated with metabolism of the cell are collectively referred to as constitutive enzymes.

➢ **Example:**

- lactate dehydrogenase, transaminases, acid and alkaline phosphatases, creatine phosphokinase.
- The non-plasma specific enzymes is very important for the diagnosis of several diseases. The normal serum level of an enzyme indicates the balance between its synthesis and release in the routine cell turnover. The raised enzyme levels could be due to cellular damage, increased rate of cell turnover, proliferation of cells, increased synthesis of enzymes etc. Serum enzymes are conveniently used as markers to detect the cellular damage which ultimately helps in the diagnosis of diseases .

➢ Amylase :

- The activity of serum amylase is increased in acute pancreatitis. The peak value is observed within 8-12 hours after the onset of disease which returns to normal by 3rd or 4th day. Elevated activity of amylase is also found in urine of the patients of acute pancreatitis. Serum amylase is also important for the diagnosis of chronic pancreatitis, acute parotitis (mumps) and obstruction of pancreatic duct.

➢ Alanine transaminase (ALT):

- It is elevated in acute hepatitis of viral or toxic origin, jaundice. ALT is increased with liver damage and is used to screen for or monitor liver disease.
- In normal healthy human adult, the ALT concentration range is from 5 to 35 U/L and its concentration above this range indicates a damaged liver, heart, and muscle.

➢ Aspartate transaminase (AST) :

- The activity in serum is increased in myocardial infarction and also in liver diseases. This blood test is used to diagnose liver damage. Aspartate transaminase (AST) is an enzyme that is released when your liver or muscles are damaged. Although AST is found mainly in your liver and heart. AST can also be found in small amounts in other muscles. This test can also be used to monitor liver disease.The serum level of AST helps people to diagnose damaged body organs, especially the heart and liver. In a healthy human adult,AST has a concentration of around 5–40 U/L. However, after severe damage, the AST level rises 10–20-times higher than the normal range. AST is also found in the red blood cells, muscle tissue, and other organs, including the kidney and pancreas. It can be used in combination with other enzymes to monitor myocardial, hepatic parenchymal, and muscle diseases in humans and animals. Moreover, to screen the liver fibrosis in chronic hepatitis B.

➢ Alkaline phosphatase (ALP) :

- Alkaline phosphatase (ALP) is an enzyme found in several tissues throughout the body. It is elevated in certain bone and liver disease. ALP is useful for the diagnosis of rickets , hyperparathyroidism, carcinoma of bone, and obstructive jaundice.In the liver, ALP is found on the edges of cells that join to form bile ducts, tiny tubes that drain bile.

➢ Acid phosphatase (ACP):

- It is increased in the cancer of prostate glands.The tartarate labile ACP is useful for the diagnosis and of prostate cancers i.e. ACP is a good tumor marker. It is an enzyme that acts to liberate

phosphate under acidic conditions and is made in the liver, spleen, bone marrow, and prostate gland. Abnormally high serum levels of acid phosphatase may indicate infection, injury, or cancer of the prostate.

- **Lactate dehydrogenase (LDH):**

- LDH is useful for the diagnosis of myocardial infarction, infective hepatitis, leukemia and muscular destruction. The lactate dehydrogenase (LDH) test looks for signs of damage to the body's tissues. LDH is an enzyme found in almost every cell of your body, including your blood, muscles, brain, kidneys, and pancreas. The enzyme turns sugar into energy. The LDH test measures the amount of LDH in your blood or other body fluid and by measuring the amount we can diagnose diseases.

- **Creatine kinase (CK):**

- It is elevated in myocardial infarction (early detection) and muscular dystrophy. Clinically, creatine kinase is used in blood tests as a marker of damage of CK-rich tissue such as in myocardial infarction (heart attack), rhabdomyolysis (severe muscle breakdown), muscular dystrophy, and acute kidney injury.