Unit Four

Chemistry of Enzymes

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Unit 4. Chemistry of Enzymes

Definition of Enzymes

An enzyme is a protein that catalyzes one or more specific biochemical reactions. However, measurement of the enzyme protein concentration is more specific and less prone to analytical variation. Generally, enzymes are present in cells at much higher concentrations than in plasma. Some occur predominantly in cells of certain tissues, where they may be located in different cellular compartments such as the cytoplasm or the mitochondria. 'Normal' plasma enzyme concentrations reflect the balance between the rate of synthesis and release into plasma during cell turnover, and the rate of clearance from the circulation.

Life is possible due to the coordination of numerous metabolic reactions inside the cells. Proteins can be hydrolyzed with hydrochloric acid by boiling for a very long time; but inside the body, with the help of enzymes, proteolysis takes place within a short time at body temperature. Enzyme catalysis is very rapid; usually 1 molecule of an enzyme can act upon about 1000 molecules of the substrate per minute. Lack of enzymes will lead to block in metabolic pathways causing inborn errors of metabolism. The substance upon which an enzyme acts, is called the substrate. The enzyme will convert the substrate into the product or products.

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Classification of Enzymes

When early workers isolated certain enzymes, whimsical names were given. Some of these, such as Pepsin, Trypsin, Chymotrypsin, etc. are still used. Later, it was agreed to call the enzymes by adding the suffix "-ase" to the substrate. Thus, enzyme Lactase acts on the substrate lactose, and the products glucose and galactose are formed. Enzymes that hydrolyze starch (amylose) are termed as amylases; those that dehydrogenate the substrates are called dehydrogenases. These are known as the trivial names of the enzymes.

The enzymes are grouped into following **six** major classes:

1- Oxidoreductases: They catalyze oxidation of one substrate with reduction of another substrate or co-enzyme.

2- Transferases: They transfers one group (other than hydrogen) from the substrate to another substrate.

3- Hydrolases: They hydrolyze ester, ether, peptide or glycosidic bonds by adding water and then breaking the bond.

4- Lyases: They remove groups from substrates or break bonds by mechanisms other than hydrolysis.

5- Isomerases: They produce optical, geometric or positional isomers of substrates.

6- Ligases: They link two substrates together (usually with the simultaneous hydrolysis of ATP).

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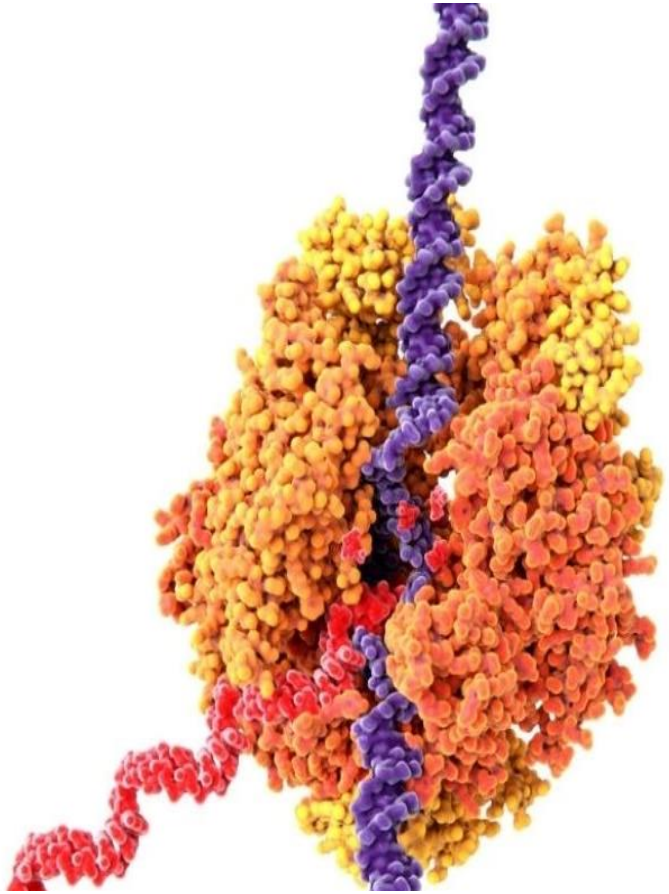
Characteristics of Enzymes

1. Almost all enzymes are proteins. Enzymes follow the physical and chemical reactions of proteins.
2. They are heat labile.
3. They are water-soluble.
4. They can be precipitated by protein precipitating reagents.
5. They contain 16% weight as nitrogen.



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Properties of Enzymes

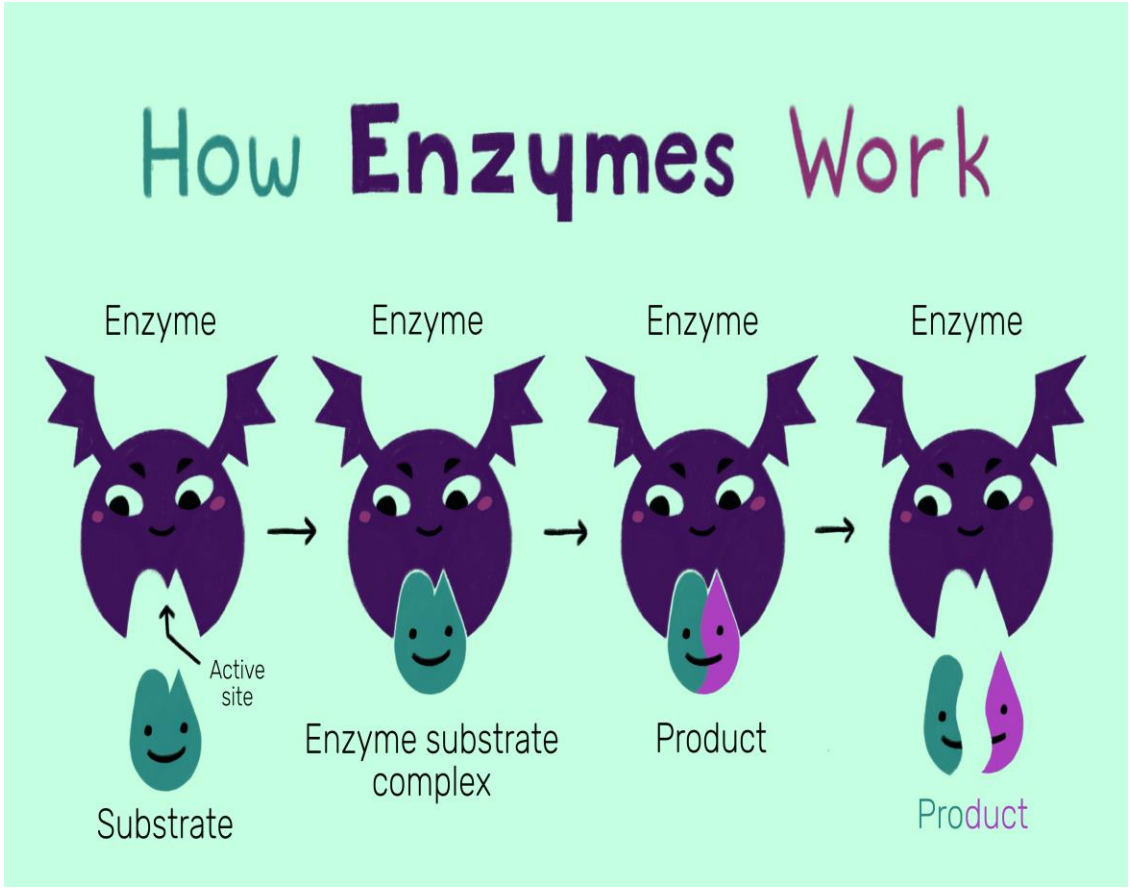
1- Active Site: Catalysis occurs at the active center or active site. The region of the enzyme where substrate binding and catalysis occurs is referred to as active site or active center.

2- Catalytic Efficiency: Reactions in presence of enzymes are very rapid compared with reactions without enzymes.

3- Specificity: Some enzymes are specific for certain reactions or substrate group.

4- Co-Factor: Some enzymes need to combine to some metals (like: iron or zinc) to be active for reaction.

5- Regulation: Enzymes can activate or inhibit the reaction inside the cell and the reaction will not occur unless the cell need it.



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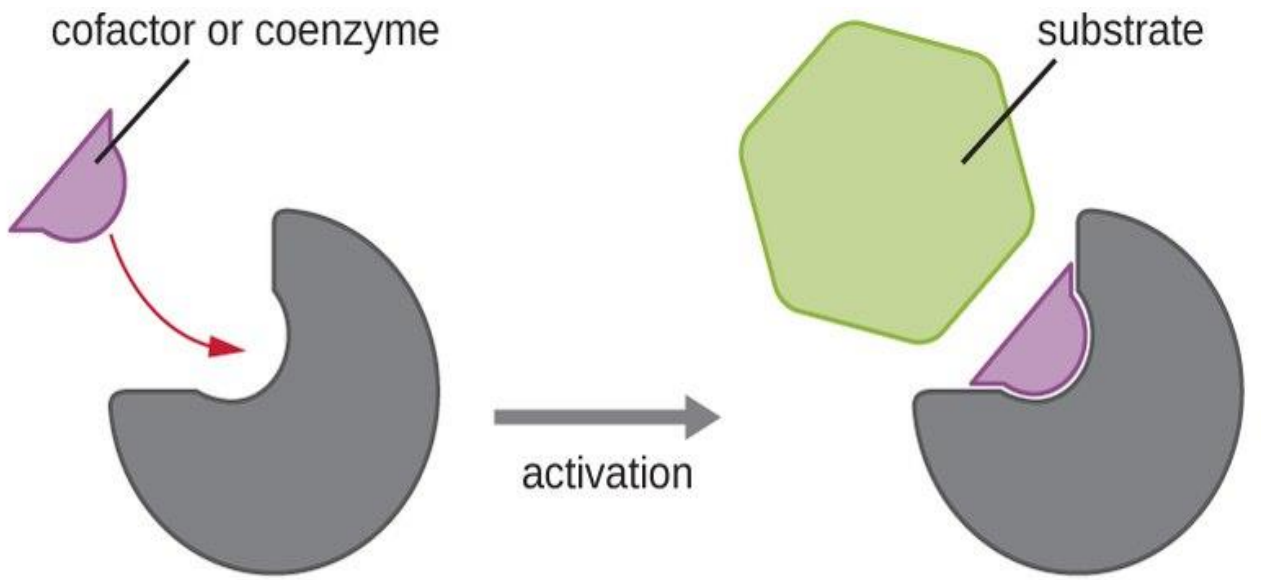
Co-Enzymes

Enzymes may be simple proteins and called simple enzymes. Some enzymes are complexed and called complex enzymes because they are containing a non-protein part called the prosthetic group, this prosthetic group is called the co-enzyme. The protein part of the complexed enzyme is then named the Apo-enzyme (it is heat labile). These two portions (Co-enzyme & Apo-enzyme) combined together are called the Holo-enzyme.



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Salient Features of Co-Enzymes

1. The co-enzyme is essential for the biological activity of the enzyme.

2. Co-enzyme is a low molecular weight organic substance. It is heat stable.

3. Generally, the co-enzymes combine loosely with the enzyme molecules. The enzyme and co-enzyme can be separated easily by dialysis.

4. Inside the body, when the reaction is completed, the coenzyme is released from the apo-enzyme, and can bind to another enzyme molecule.

5. One molecule of the co-enzyme is able to convert a large number of substrate molecules with the help of enzyme.

6. Most of the co-enzymes are derivatives of vitamin B complex substances.

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Factors Influencing Enzyme Activity

1- Enzyme Concentration: Velocity of reaction is increased proportionately with the concentration of enzyme, provided substrate concentration is unlimited. This property is made use of determining the level of particular enzyme in plasma, serum or tissues.

2- Substrate Concentration: As substrate concentration is increased, the velocity is also correspondingly increased in the initial phases; but later it will be constant.

3- Concentration of Products: In a reversible reaction, when equilibrium is reached, the reaction rate is slowed down. So when product concentration is increased, the reaction is slowed, stopped or even reversed.

4- Temperature: The velocity of enzyme reaction increases when temperature of the medium is increased; reaches a maximum and then falls. When temperature is more than 50°C, heat denaturation and consequent loss of tertiary structure of protein occurs. So activity of the enzyme is decreased. Most human enzymes have the optimum temperature around 37°C.

5- pH: Each enzyme has an optimum pH. The pH decides the charge on the amino acid residues at the active site. The net charge on the enzyme protein would influence substrate binding and catalytic activity. Optimum pH may vary depending on the temperature, concentration of substrate, presence of ions, etc.

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Inhibition of Enzymes

An enzyme inhibitor is a molecule that binds to an enzyme and decreases its activity. By binding to enzymes' active sites, inhibitors reduce the compatibility of substrate and enzyme and this leads to the inhibition of Enzyme-Substrate complexes' formation, preventing the catalysis of reactions and decreasing the amount of product produced by a reaction. It can be said that as the concentration of enzyme inhibitors increases, the rate of enzyme activity decreases, and thus, the amount of product produced is inversely proportional to the concentration of inhibitor molecules. Since blocking an enzyme's activity can kill a pathogen or correct a metabolic imbalance, many drugs are enzyme inhibitors. Not all molecules that bind to enzymes are inhibitors; enzyme activators bind to enzymes and increase their enzymatic activity, while enzyme substrates bind and are converted to products in the normal catalytic cycle of the enzyme. The binding of an inhibitor can stop a substrate from entering the enzyme's active site and/or hinder the enzyme from catalyzing its reaction. Inhibitor binding is either reversible or irreversible. Irreversible inhibitors usually react with the enzyme and change it chemically (e.g. via covalent bond formation) like Non-Competitive Inhibition. In contrast, reversible inhibitors bind non-covalently and different types of inhibition are produced depending on whether these inhibitors bind to the enzyme, the enzyme-substrate complex, or both such as Competitive Inhibition .

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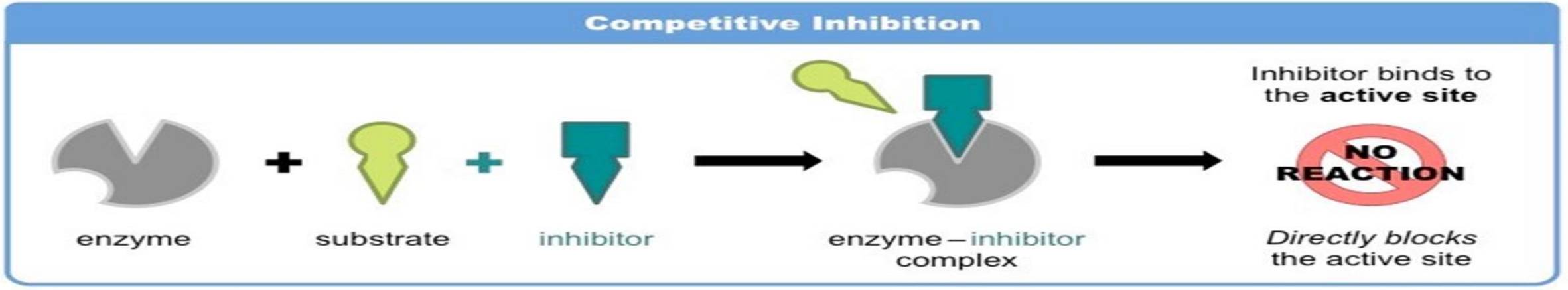
Competitive Inhibition

Here inhibitor molecules are competing with the normal substrate molecules for attaching with the active site of the enzyme. Since (enzyme-inhibitor complex) can react only to reform the enzyme and inhibitor, the number of enzyme molecules available for Enzyme-Substrate complex formation is reduced. Suppose 100 molecules of substrate and 100 molecules of inhibitor are competing for 100 molecules of the enzyme. So, half the enzyme molecules are trapped by the inhibitor and only half the molecules are available for catalysis to form the product. Since effective concentration of enzyme is reduced, the reaction velocity is decreased. In competitive inhibition, the inhibitor will be a structural analog of the substrate. There will be similarity in three-dimensional structure between substrate (S) and inhibitor (I).



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Clinical Importance of Competitive Inhibition

Pharmacological action of many drugs may be explained by the principle of competitive inhibition. A few important examples are given below:

1. Sulfonamides: They are commonly employed antibacterial agents. Bacteria synthesize folic acid by combining paraaminobenzoicacid (PABA) with pteroylglutamic acid. Bacterial wall is impermeable to folic acid. Sulfa drugs, being structural analogs of PABA, will inhibit the folic acid synthesis in bacteria, and they die. The drug is nontoxic to human cells, because human beings cannot synthesized folic acid.

2. Methotrexate: It is a structural analog of folic acid, and so can competitively inhibit folate reductase enzyme. This is essential for DNA synthesis and cell division. Therefore, methotrexate is used as an anticancer drug.

3. Dicoumarol: It is structurally similar to vitamin K and can act as an anticoagulant by competitively inhibiting the vitamin K activity.

4. Isonicotinic acid hydrazide (INH): It is a commonly used antituberculous drug. It is structurally similar to pyridoxal, and prolonged use of INH may cause pyridoxal deficiency and peripheral neuropathy.

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Non-Competitive Inhibition

A variety of poisons, such as iodoacetate, heavy metal ions (lead, mercury) and oxidizing agents act as irreversible non-competitive inhibitors. There is no competition between substrate and inhibitor. The inhibitor usually binds to a different domain on the enzyme, other than the substrate binding site. Since these inhibitors have no structural resemblance to the substrate, an increase in the substrate concentration generally does not relieve this inhibition. For example, fluoride will remove magnesium and manganese ions and so will inhibit the enzyme "enolase" and consequently the glycolysis. In addition, Enzymes present in human being act on the drugs, Penicillin blocks cell wall synthesis in bacteria by irreversible binding of the enzyme transpeptidase and leads to bacteria dies. The inhibitor combines with the enzymes by forming a covalent bond and then the reaction becomes irreversible. The velocity of reaction is reduced because the remaining enzyme molecules have the same affinity for the substrate. Increasing the substrate concentration will abolish the competitive inhibition, but will not abolish non-competitive inhibition.

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Plasma Enzymes

Enzymes are present in cells at much higher concentrations than in plasma. Normal plasma enzyme concentrations reflect the balance between the rate of synthesis and release into plasma during cell turnover, and the rate of clearance from the circulation. Plasma enzyme levels depend on the extent of cell damage and the rate of release from damaged cells, which in turn, depends on the rate at which damage is occurring. In the absence of cell damage, the rate of release depends on the degree of induction of enzyme synthesis and the rate of cell proliferation. These factors are balanced by the rate of enzyme clearance from the circulation. Acute cell damage, for example in viral hepatitis, may cause very high plasma aminotransferase activities that reduce as the condition resolves. By contrast, the liver may be much more extensively involved in advanced cirrhosis but the rate of cell damage is often low, and consequently plasma enzyme activities may be only slightly raised or within the reference range. In very severe liver disease, plasma enzyme activities may even fall terminally when the number of hepatocytes is grossly reduced. Relatively small enzymes, such as amylase, can be cleared by the kidneys. Thus, plasma amylase activity may be high as a result of renal glomerular impairment rather than pancreatic damage. However, most enzymes are large proteins and may be catabolized by plasma proteases before being taken up by the reticulo-endothelial system. In healthy individuals, each enzyme has a fairly constant and characteristic biological half-life, a fact that may be used to assess the time since the onset of an acute illness.

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Normal Plasma Enzyme Activities

1- Aspartate aminotransferase (AST): it's also called glutamate oxaloacetate aminotransferase (GOT). It is present in high concentrations in cells of cardiac and skeletal muscle, liver, kidney and erythrocytes. Damage to any of these tissues may increase plasma AST levels.

2- Alanine aminotransferase (ALT): It's also called glutamate pyruvate aminotransferase (GPT). It is present in high concentrations in liver and, to a lesser extent, in skeletal muscle, kidney and heart. Damage to any of these tissues may increase plasma ALT levels. ALT is more specifi c for hepatic disease than AST.

3- Lactate dehydrogenase (LDH): It catalyzes the reversible interconversion of lactate and pyruvate. The enzyme is widely distributed in the body, with high concentrations in cells of cardiac and skeletal muscle, liver, kidney, brain and erythrocytes; measurement of plasma total LDH activity is therefore a non-specific marker of cell damage. Predominant elevation of LDH occurs after myocardial infarction in megaloblastic anaemia, after renal infarction, acute leukaemia, malignancy of many tissues and after damage to the liver or skeletal muscle.

4- Creatine kinase (CK): It is most abundant in cells of cardiac and skeletal muscle and in brain, but also occurs in other tissues such as smooth muscle. Its plasma activity is always high after myocardial infarction, hepatic disease, critically ill patients, rheumatoid arthritis, during and for a few days after parturition, after brain damage (trauma or cerebrovascular accident) and in association with malignant tumors of the bronchus, prostate and breast.

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5- Amylase: It breaks down starch and glycogen to maltose. It is present at a high concentration in pancreatic juice and in saliva and may be extracted from other tissues such as the gonads, skeletal muscle and adipose tissue. Being of relatively low molecular weight, it is excreted in the urine. Estimation of plasma amylase activity is mainly requested to help in the diagnosis of acute pancreatitis, in which the plasma activity may be very high. However, it may also be raised in association with other intraabdominal and extra-abdominal conditions that cause similar acute abdominal pain; thus a high result is not a specific diagnostic marker for acute pancreatitis. If the plasma amylase activity fails to fall after an attack of acute pancreatitis, there may be leakage of pancreatic fluid into the lesser sac (a pancreatic pseudocyst).

6- Lipase: Sometimes, when it is difficult to interpret plasma amylase results, it may be more useful to measure plasma lipase This enzyme is also derived from the pancreas but is more specific for pancreatic pathology. In addition, lipase has a longer half-life than amylase and therefore may be more useful in the diagnosis of late-presenting acute pancreatitis.

7- Alkaline phosphatase (ALP): It hydrolyse organic phosphates at high pH. It is present in most tissues but in particularly high concentration in the osteoblasts of bone and the cells of the hepatobiliary tract, intestinal wall, renal tubules and placenta. In adults, plasma ALP is derived mainly from bone and liver in approximately equal proportions; the proportion due to the bone fraction is increased when there is increased osteoblastic activity.