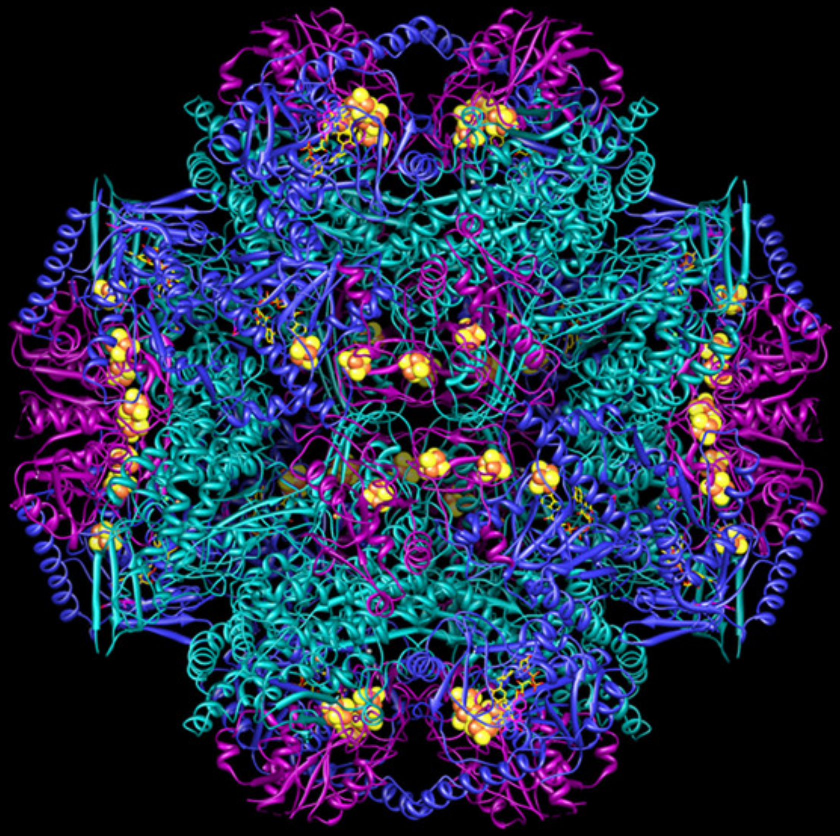
ENZYMES



ENZYME

"Enzymes can be defined as biological polymers that catalyze biochemical reactions."

The vast majority of enzymes are proteins with catalytic capabilities that are essential for maintaining various life processes. Metabolic processes and other chemical reactions in the cell are carried out by a set of enzymes that are necessary to sustain life.

The initial stage of metabolic process depends upon the enzymes, which react with a molecule and is called the **substrate.** Enzymes convert the substrates into other distinct molecules and are called the **products**. The regulation of enzymes has been a key element in clinical diagnosis because of their role in maintaining life processes.

The macromolecular component of all enzymes consists of protein, except in the class of RNA catalysts called ribozymes. The word ribozyme is derived from the ribonucleic acid enzyme. Many ribozymes are molecules of ribonucleic acid which catalyze reactions in one of their own bonds or among other RNAs.

Enzymes exist in all fluids and tissues of the body. Intracellular enzymes catalyze all the reactions that occur in metabolic pathways. The enzymes in plasma membrane regulate catalysis in the cells in response to cellular signals and enzymes in the circulatory system regulate clotting of blood. Almost all the significant life processes are based on the enzyme functions.

ENZYME STRUCTURE

Enzyme Structure: Enzymes are a linear chain of amino acids that generate the three-dimensional structure. The sequence of amino acids enumerates the structure which in turn identifies the catalytic activity of the enzyme. The structure of the enzyme denatures when heated, leading to loss of enzyme activity, which is typically connected to the temperature. Enzymes are larger than their substrates and their size vary, which range from sixty-two amino acid residues to an average of two thousand five hundred residues present within fatty acid synthase. Only a small section of the structure is involved in catalysis and are situated next to binding sites. The catalytic site and binding site together constitute the enzyme’s active site. A small number of ribozymes exists which serves as an RNA-based biological catalyst. It reacts in complex with proteins.

Classification of Enzyme

According to the International Union of Biochemists (I U B), enzymes are divided into six functional classes and are classified based on the type of reaction in which they are used to catalyze. The 6 types of enzymes are

Following are the enzymes classifications in detail:

Types	Biochemical Property
1. Oxidoreductases	The enzyme Oxidoreductase catalyzes the oxidation reaction where the electrons tend to travel from one form of a molecule to the other.
1. Transferases	The Transferases enzymes help in the transportation of the functional group among acceptors and donors molecules.
1. Hydrolases	Hydrolases are hydrolytic enzymes, which catalyze the hydrolysis reaction by adding water to cleave the bond and hydrolyze it.
1. Lyases	Adds water, carbon dioxide or ammonia across double bonds or eliminate these to create double bonds.
1. Isomerases	The Isomerases enzymes catalyze the structural shifts present in a molecule, thus causing the change in the shape of the molecule.
1. Ligases	The Ligases enzymes are known to charge the catalysis of a ligation process.

CLASSIFICATION OF ENZYME

Oxidoreductases: These catalyze oxidation and reduction reactions, e.g. pyruvate dehydrogenase, which catalyzes the oxidation of pyruvate to acetyl coenzyme A.

Transferases: These catalyze the transfer of a chemical group from one compound to another. An example is a transaminase, which transfers an amino group from one molecule to another.

Hydrolases: They catalyze the hydrolysis of a bond. For example, the enzyme pepsin hydrolyzes peptide bonds in proteins.

Lyases: These catalyze the breakage of bonds without catalysis, e.g. aldolase (an enzyme in glycolysis) catalyzes the splitting of fructose-1, 6-bisphosphate to glyceraldehyde-3-phosphate and dihydroxyacetone phosphate.

Isomerases: They catalyze the formation of an isomer of a compound, example, phosphoglucomutase catalyzes the conversion of glucose-1-phosphate to glucose-6-phosphate (transfer of a phosphate group from one position to another in the same compound) in glycogenolysis (conversion of glycogen to glucose for quick release of energy.

Ligases: Ligases catalyze the joining of two molecules. For example, DNA ligase catalyzes the joining of two fragments of DNA by forming a phosphodiester bond.

Cofactors

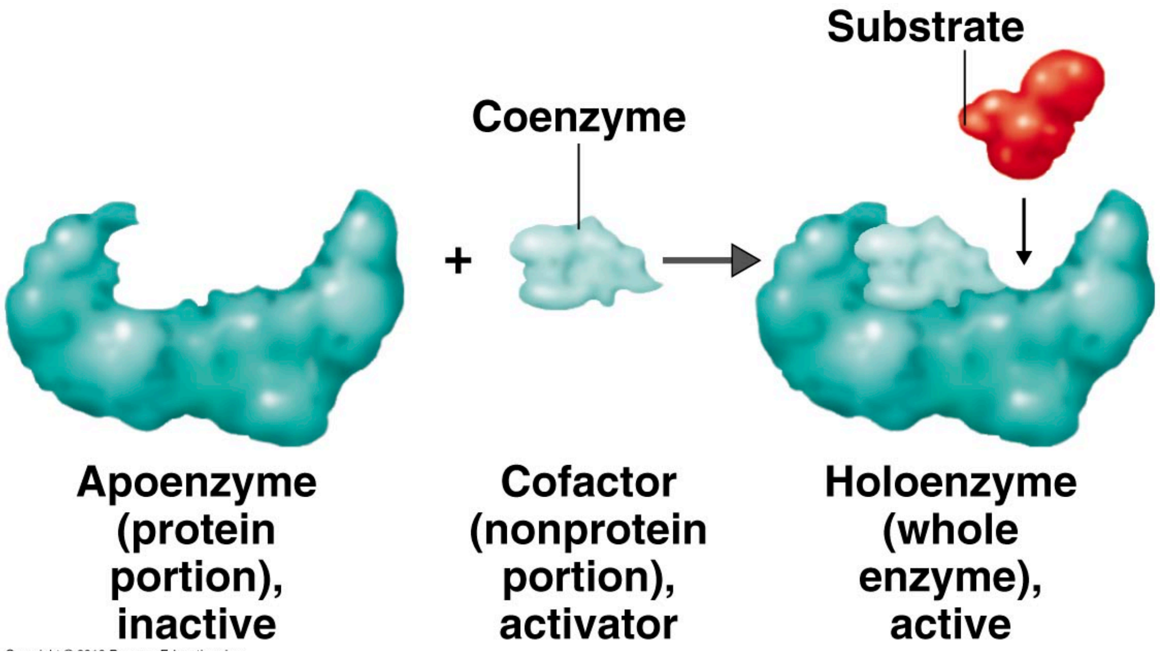
Co-factors are non-proteinous substances that associate with enzymes. A cofactor is essential for the functioning of an enzyme. **An enzyme without a cofactor is called an apoenzyme. An apoenzyme and its cofactor together constitute the holoenzyme.**

There are three kinds of cofactors present in enzymes:

Prosthetic groups: These are cofactors tightly bound to an enzyme at all times. A **fad** is a prosthetic group present in many enzymes.

Coenzyme: A coenzyme is bound to an enzyme only during catalysis. At all other times, it is detached from the enzyme. NAD^+ is a common coenzyme.

Metal ions: For the catalysis of certain enzymes, a metal ion is required at the active site to form coordinate bonds. Zn^{2+} is a metal ion cofactor used by a number of enzymes.



ISOENZYMES

Isoenzymes (or *isozymes*) are a group of enzymes that catalyze the same reaction but have different enzyme forms and catalytic efficiencies. Isozymes are usually distinguished by their electrophoretic mobilities. All living systems apparently require multiple molecular forms of certain enzymes in order to maximize biological capacity.

Isozymes arise from gene duplications and/or different epigenetic modifications of a gene product(s). In this sense, most of the recombinant enzymes with deletion, insertion, and/or other mutations at the genetic level fall into the category of isozymes (89). In a restricted definition, "isozymes" are different in genetic origins. An example is the human alkaline phosphatases which have at least three different genetic origins, i.e., for placental, intestinal, and liver/bone/kidney enzymes (see Section I,B, Chapter 5). The enzymes that have epigenetic differences due to differential precursor processings, covalent modifications, and/or tissue distributions are then called *isoforms.* Examples of isoforms are the liver/bone/kidney alkaline phosphatases which are encoded by the same gene but differentially modified in a tissue-specific manner.time

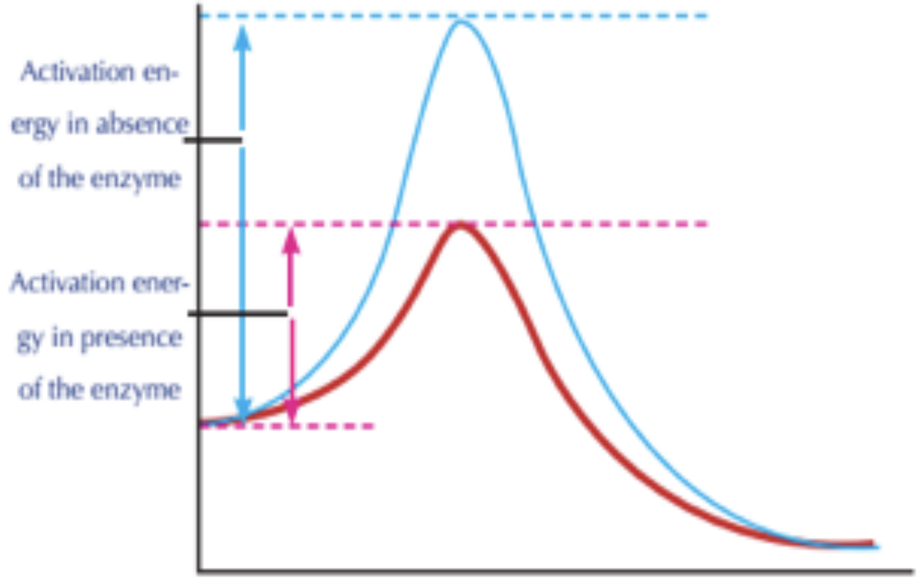
Mode of Action

https://www.britannica.com/science/protein/The-mechanism-of-enzymatic-action

CLICK THIK LINK

Factors Affecting Enzyme Activity

Introduction: Biochemical reactions are necessary for growth, repairing damaged tissues, and obtaining energy and they take place in all living organisms' bodies. These reactions are called 'metabolism' and they happen all the time in living organisms. If they stop working, this leads to the death of the organism. All the reactions that occur in living organisms require high activation energy to take place. To reduce the cell's consumption of energy, there is a catalyst to ensure that the chemical reactions occur rapidly and reduce the activation of energy. **This catalyst is the enzymes**.

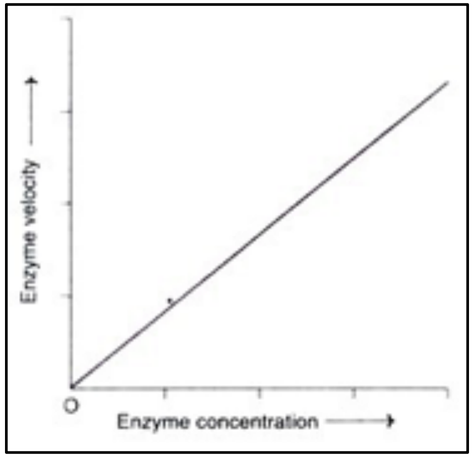


Enzymes are biological catalysts made up of large protein molecules. They speed up the chemical reactions inside the cell. The enzyme is made up of a combination of amino acids which for a chain of polypeptides between each other.
Enzymes are similar to other chemical catalysts. They participate in the reaction without getting affected. In other words, they speed up the chemical reactions inside the cells without getting consumed. Enzymes are affected by the hydrogen ion concentration (pH) and the temperature. Enzymes are highly specific compared to other catalysts, and each enzyme is specialized for one reactant substance. This reactant substance is called substrate, and it is specialized for one type of reaction or a few reactions. Enzymes lower the activation energy required to get the reaction started. Collectively, these are the most important properties of the enzyme.

There are several factors that affect the speed of an enzyme's action, such as the concentration of the enzyme, the concentration of the substrate, temperature, hydrogen ion concentration (pH), and the presence of inhibitors.

Factor 1. Concentration of Enzyme:

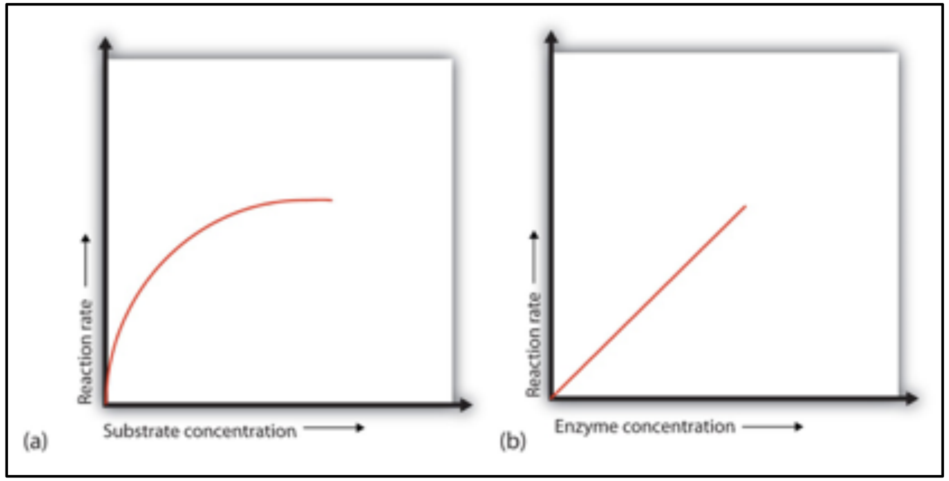
As the concentration of the enzyme is increased, the velocity of the reaction proportionately increases. This property is used for determining the activities of serum enzymes during the diagnosis of diseases.



Factor 2: Concentration of Substrate:

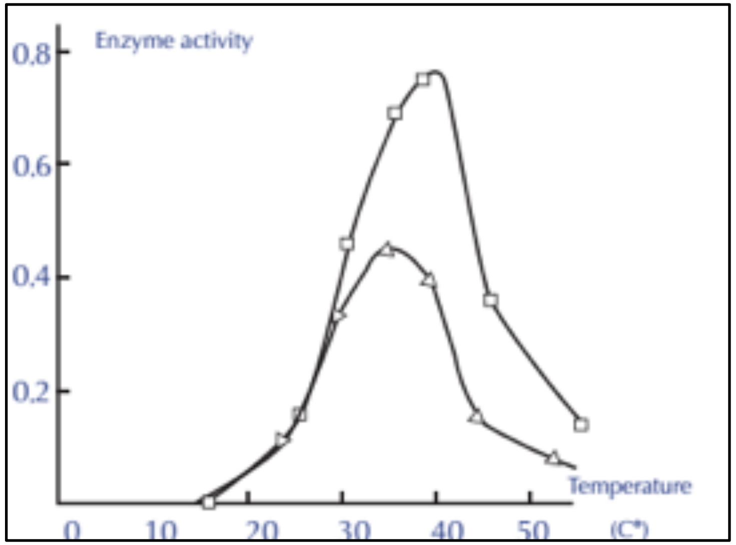
n the presence of a given amount of enzyme, the rate of enzymatic reaction increases as the substrate concentration increases until a limiting rate is reached, after which further increase in the substrate concentration produces no significant change in the reaction rate. At this point, so much substrate is present that essentially all of the enzyme active sites have substrate bound to them.

In other words, the enzyme molecules are saturated with substrate. The excess substrate molecules cannot react until the substrate already bound to the enzymes has reacted and been released (or been released without reacting).



Factor 3: Effect of Temperature

•The protein nature of the enzymes makes them extremely sensitive to thermal changes. Enzyme activity occurs within a narrow range of temperatures compared to ordinary chemical reactions. As you have seen, each enzyme has a certain temperature at which it is more active. This point is called the optimal temperature, which ranges between 37 to 40C°.



•The enzyme activity gradually lowers as the temperature rises more than the optimal temperature until it reaches a certain temperature at which the enzyme activity stops completely due to the change of its natural composition.

•On the other hand, if the temperature lowers below the optimal temperature, the enzyme activity lowers until the enzyme reaches a minimum temperature at which the enzyme activity is the least. The enzyme activity stops completely at 0C°, but if the temperature rises again, then the enzyme gets reactivated once more.

Factor 4: Effect of pH

•The potential of hydrogen (pH) is the best measurement for determining the concentration of hydrogen ion (H^+)in a solution. It also determines whether the liquid is acidic, basic or neutral. Generally, all liquids with a pH below 7 are called acids, whereas liquids with a pH above 7 are called bases or alkalines. Liquids with pH 7 are neutral and equal the acidity of pure water at 25 C°. You can determine pH of any solution using the pH indicators.

Enzymes are protein substances that contain acidic carboxylic groups ($COOH^-$) and basic amino groups (NH_2). So, the enzymes are affected by changing the pH value.

Each enzyme has a pH value that it works at with maximum efficiency called the optimal pH. If the pH is lower or higher than the optimal pH, the enzyme activity decreases until it stops working. For example, pepsin works at a low pH, i.e, it is highly acidic, while trypsin works at a high pH, i.e, it is basic. Most enzymes work at neutral pH 7.4.

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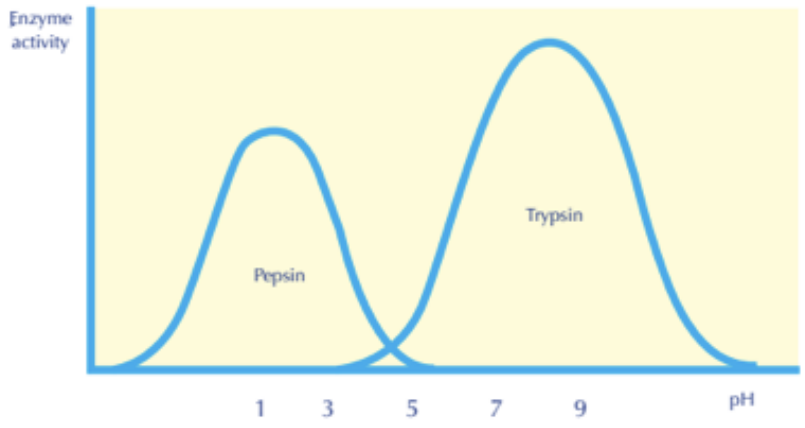

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Factor 5: Effect of Activators

- Some of the enzymes require certain inorganic metallic cations, like Mg^{2+}, Mn^{2+}, Zn^{2+}, Ca^{2+}, Co^{2+}, Cu^{2+}, Na^+, K^+ etc., for their optimum activity. Rarely, anions are also needed for enzyme activity, e.g. a chloride ion (Cl^-) for amylase.

Enzyme Marker

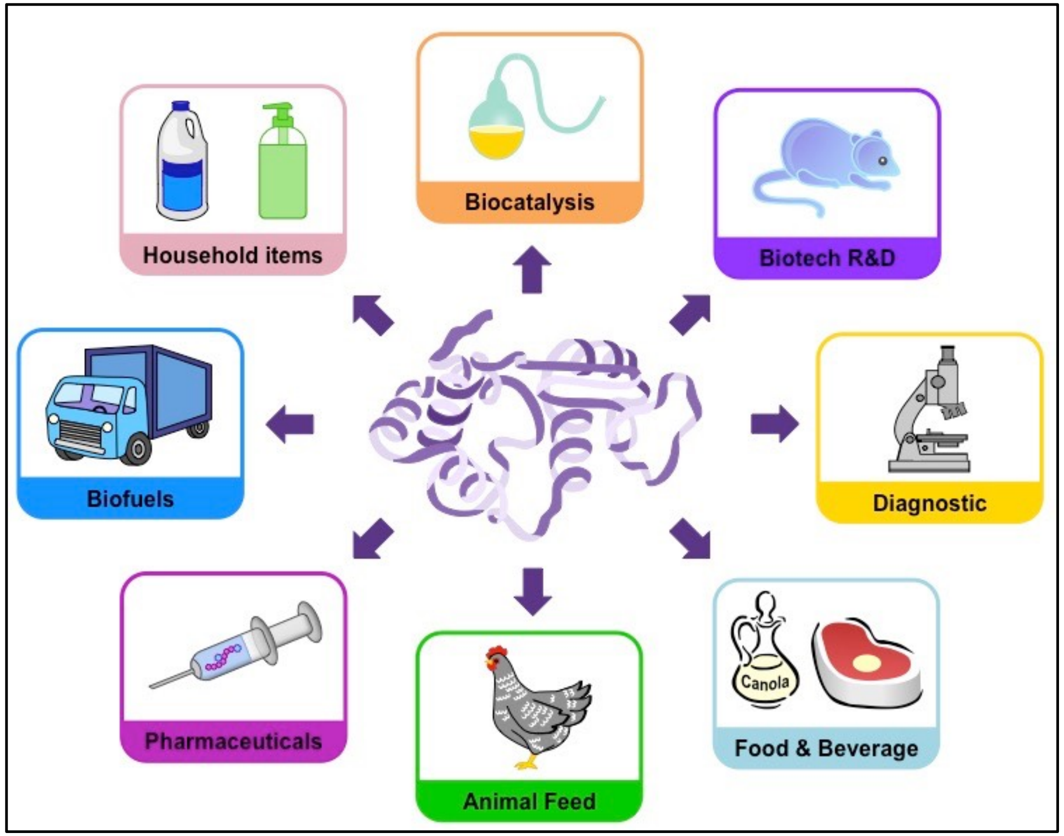
Enzymes are highly specialized complex proteins that assist chemical changes in every part of the body. For example, they help break down food so your body can use it effectively. They also help your blood clot. And they’re present in every organ and cell in your body. Enzymes are necessary for your body to function properly.

Enzyme markers are blood tests that analyze specific enzyme activity in the body. Some inherited diseases or conditions can cause these enzymes to stop working or be less efficient. Monitoring the rise or fall of enzyme levels can aid in the diagnosis of a variety of conditions.

You can test blood for using enzyme markers, or a routine blood test to help uncover abnormalities. In some cases, you may need to take a test multiple times over the course of several days to measure changes over time.

Uses of Enzyme

1. Diagnostics uses of enzyme
2. Therapeutic uses of enzyme
3. Lab uses of enzyme
4. Industrial uses of enzyme



1. Diagnostics Uses of Enzyme

Liver diseases

1. AST**(SGOT)**: **5 to 40** units per liter of serum (the liquid part of the blood).
2. ALT**(SGPT): 7 to 56** units per liter of serum
3. ALP(**Alkaline phosphatase**): **20 to 140 IU/LT**, but this can vary from laboratory to laboratory.
4. 5'NT **(5'nucleotidase)**: **2-17 Units per liter.**
5. GGT(**Gamma- Glutamyl Transpeptidase**): **9–48 Units per liter (U/L).** (Normal values can vary due to age and sex.)
6. LDH 4,5 (**Lactate Dehydrogenase**): **100–190 U/L(Older than 12 years**)

ALT specific for liver disease

AST**(SGOT)**/ALT**(SGPT) ratio is known as De Ritis**

Normal<1 liver disease
Normal >2 Alcoholic liver disease
Normal >3 more alcoholic liver disease

AST/ALT: Slight ↑
ALP: ↑↑↑↑

Obstructive jaundice

AST/ALT: ↑↑↑↑↑
ALP: ↑

Hepatitis

ALP
5'NT
GGT

It are marker of obstructive liver disease

ALP

Increases level 2-3time to the Normal: **Viral hepatitis**
Increases level 10-12 to the Normal: Obstructive liver disease
Increases level more than 25 to Normal: **Malignancy, tumor, rickets, bone involvement metastasis,** Paget's disease

What does the test result mean?

High ALP usually means that either the liver has been damaged or a condition causing increased bone cell activity is present. If other liver tests such as bilirubin, aspartate aminotransferase (AST), or alanine aminotransferase (ALT) are also high, usually the increased ALP is coming from the liver. If GGT **or 5'-nucleotidase** is also increased, then the high ALP is likely due to liver disease. If either of these two tests is normal, then the high ALP is likely due to a bone condition. Likewise, if calcium and/or phosphorus measurements are abnormal, usually the ALP is coming from bone.

If it is not clear from signs and symptoms or from other routine tests whether the high ALP is from liver or bone, then a test for ALP isoenzymes may be necessary to distinguish between bone and liver ALP.

ALP in liver disease

ALP results are usually evaluated along with other tests for liver disease. In some forms of liver disease, such as hepatitis, ALP is usually much less elevated than AST and ALT. When the bile ducts are blocked (usually by gallstones, scars from previous gallstones or surgery, or by cancers), ALP and bilirubin may be increased much more than AST or ALT. ALP may also be increased in liver cancer.

ALP in bone disease

In some bone diseases, such as Paget's disease, where bones become enlarged and deformed, or in certain cancers that spread to bone, ALP may be increased.

If a person is being successfully treated for Paget's disease, then ALP levels will decrease or return to normal over time. If someone with bone or liver cancer responds to treatment, ALP levels should decrease.

Moderately elevated ALP may result from other conditions, such as Hodgkin's lymphoma, congestive heart failure, ulcerative colitis, and certain bacterial infections.

Low levels of ALP may be seen temporarily after blood transfusions or heart bypass surgery. A deficiency in zinc may cause decreased levels. A rare genetic disorder of bone metabolism called hypophosphatasia can cause severe, protracted low levels of ALP. Malnutrition or protein deficiency as well as Wilson disease could also be possible causes for lowered ALP.

Cardiac Marker

CPK-MB (Creatine Phosophokinase): 5-25 international units per liter (UI/L).

LDH-I (Lactate Dehydrogenase): Age Older than 12 years 100–190 U/L

MYGLOBIN: Normal range 0 to 85 nanogram per milliliter (ng/mL)

TROPONIN T: Normal Range Below 0.04 ng/ml

TROPONIN R: The **normal range** for **troponin** is between 0 and 0.4 ng/mL

IMA(Ischemia- modified albumin): IMA is produce when albumin comes into contact with the ischemic tissue.Altering it and making it more resistant to binding metals, IMA is produced continually during ischemia and rises within 2-3 hours of an ischemic event

Glycogen Phosphorylase BB: More sensitive than the other markers during the first 3-4hours after onset of chest pain

Pancreatic diseases

AMYLASE

LIPASE

Normal values: In a **healthy** individual, a **normal blood amylase level** is around 23-85 units per liter (U/L), although some lab **ranges** for **normal amylase** go up to 140 U/L. A **normal** lipase **level** can **range** from 0-160 U/L depending on the lab.

BONE DISEASE

ALP Bone isoenzyme pre β: Biochemical Markers of Bone Turnover in Osteoporosis

6 TYPES OF ALP

α_1: Biliary pathology(antitrypsin deficiency)

α_2HL: lLiver

A_2 HS: Placenta

PRE β: Bone

ʒ: Intestine

LAP (Leukocyte alkaline phosphatase)**:** leukemoid reaction (20 and 100 being considered normal)

CALCITONIN: normal value is less than 10 pg/mL.

Nagao(Germ cell variant)(alkaline phosphatase gene): These results indicate that the tumor

Kasahara (fetus intestinal variant): renal cell carcinoma

Regan (Placental varient): Tumour marker for renal cell carcinoma.

1. Alanine amino transferase (ALT)
   Marked increase in parenchymal liver diseases
2. Aspartate amino transferase (AST)
   Elevated in parenchymal liver disease
3. Alkaline phosphatase (ALP)
   Marked increase in obstructive liver disease
4. Gamma glutamyl transferase (GGT)
   Increase in obstructive and alcoholic liver

II. Myocardial infarction

1. Creatine kinase (CK-MB)
   First enzyme to rise following infarction
   CK-MB isoenzyme is specific
2. Aspartate amino transferase (AST)
   Rises after the rise in CK and returns to normal in 4-5 days
3. Lactate dehydrogenase (LDH)
   LDH-1 becomes more than 2 (flipped pattern)

III. Muscle diseases

1. Creatine kinase (CK-MM)
   Marked increase in muscle diseases.
   CK-MM fraction is elevated
2. Aspartate amino transferase (AST)
   Increase in muscle disease; not specific
3. Aldolase (ALD)
   Earliest enzyme to rise, but not specific

IV.Bone diseases

1. Alkaline phosphatase (ALP)
   Marked elevation in rickets and Paget's disease
   Heat labile bone isoenzyme is elevated (BAP).

V. Prostate cancer

1. Prostate specific antigen (PSA)
   Marker for prostate cancer.
   Mild increase in benign prostate enlargement
2. Acid phosphatase (ACP)
   Marker for prostate cancer. Metastatic bone disease especially from a primary from prostate.
   Inhibited by L tartrate.

2. THERAPEUTIC USES OF ENZYME

A S P A R Ginase

Asparginase
Acute lymphoblastic leukamia

Rtpa (Recombinant tissue plasminogen): A form of **tissue plasminogen activator** that is made in the laboratory. It helps dissolve blood clots and is **used** to treat heart attacks, strokes, and clots in the lungs

Alpha1 Antistripsin: used for diagnosis Emphysema

Streptokinase/ Urokinase: break down clots in some cases of myocardial infarction
Streptodornase: It is used topically on surface lesions and by instillation in closed body cavities to remove clotted blood or fibrinous or purulent accumulations.

Papin: Anti Inflammatory agent
Pancreatin: Used for diagnosis digestive disorders

3.LAB USES OF ENZYME

Glucose: Hexokinase- GOD,POD
Urea: Urease
Cholesterol: CHOD-POD
TG:LIPASE
UA: Urease

ESTIMATION

Horseradish peroxidase:
ALP:
Recom-DNA:
Rt- Endoneuclease
DNA polymerase:
Nucleosidase:

It is used extensively in biochemistry applications.

4. INDUSTRIAL USES OF ENZYME

www.mdpi.com/2073-4344/8/6/238/pdf

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