Beni-suef University
Faculty of science
Chemistry department
Biochemistry division

LECTURE NOTES IN CLINICAL ENZYMOLOGY

FOR

BIO-CHEMISTRY DIPLOMA STUDENTS

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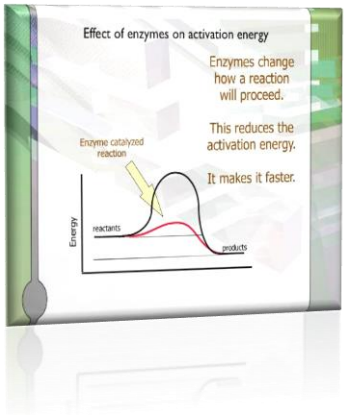


Contents

Clinical Enzymology

- Clinical enzymology can be described as the branch of science which deals with the application of enzyme analysis to the diagnosis and treatment of disease.
- Enzymes are ‘protein catalysts’ and can be best described as substances that increase the rate of a particular chemical reaction without being permanently consumed or altered in any reaction.
- Enzymes are required for all biological reactions occurring in the body. As a catalyst they are required in amounts much less than the substrate they act on. And it is this catalytic property which makes enzymes a very sensitive indicator of any biochemical or pathological change occurring in the body.
- The measurement of the serum levels of numerous enzymes has been shown to be of diagnostic significance. This is because the presence of these enzymes in the serum indicates that tissue or cellular damage has occurred dresulting in the release of intracellular components into the blood. Hence, when a physician indicates that he/she is going to assay for liver enzymes, the purpose is to ascertain thepotential for liver cell damage. An increase in the enzymatic levels in the blood compared to their normal levels is a good indicator of altered patterns.

- ***Historical relevance of clinical enzymology***

This diagnostic relevance of enzymes was put into practice as early as the ١٩٠٠s. One of the earliest reported enzyme measurement in body fluids was that of amylase in urine by Wohlgemoth in ١٩٠٨. The use of serum as the diagnostic fluid for measuring enzyme activity started in ١٩٢٠s and ١٩٣٠s with studies on alkaline phosphatase (ALP) in bone and liver disease conducted by Kay, King, Bodansky and Roberts. The relevance of alterations in acid phosphatases (ACP) activity was first appreciated by Kutscher and Wolbergs; and Gutmanand Gutman in prostate cancer. The modern diagnostic

enzymology with better sensitivity of detection and analytical methods took shape in the ١٩٤٠s and ١٩٥٠s. Warburg and Christian observed an increased activity of glycolytic enzymes in sera of tumor-bearing mice and later ,Wroblewski and Karmen reported a transitory rise in the levels of glutamic-oxaloacetic transaminase activity (also known as aspartate aminotransferase) in serum after acutemyocardial infarction.

- ***Enzymes nomenclature*:**

١- By adding the suffix"ase"after the name of substrate

e.g. Lactase, Arginase

٢- By adding the enzymeaction to the name of the substrate

e.g. Malate dehydrogenase

٣- Some enzymes still retain their old name

e.g. Pepsine, Trypsine

- ***Enzyme Structure:***

Enzymes are proteins. Their folded conformation creates an area known as the activesite. Amino acids arranged in the active sitelinked by peptide bonds {CO-NH}.

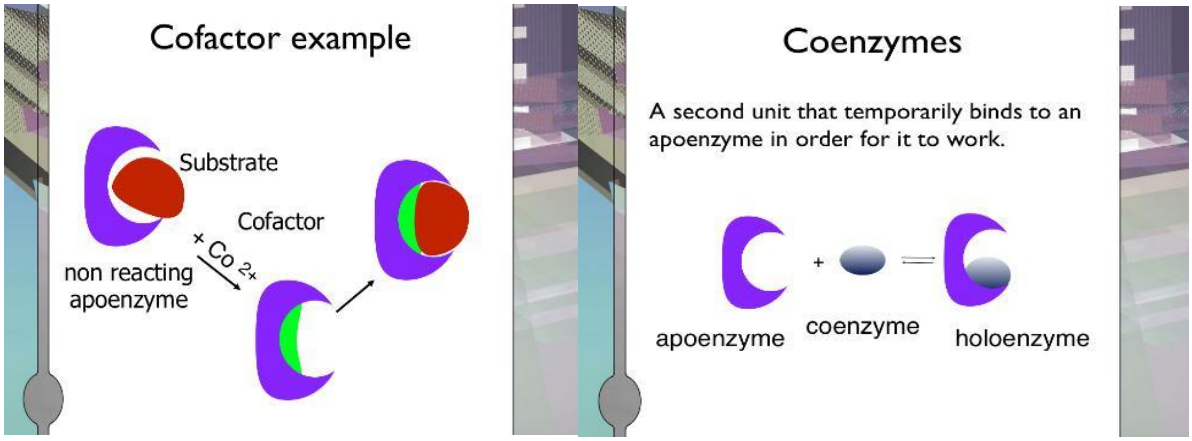
Many enzymes contain small non-protein molecules and metal ions (called the prosthetic groups, cofactors and coenzymes) that participate directly in substrate binding or catalysis along with the protein part of the enzymes.

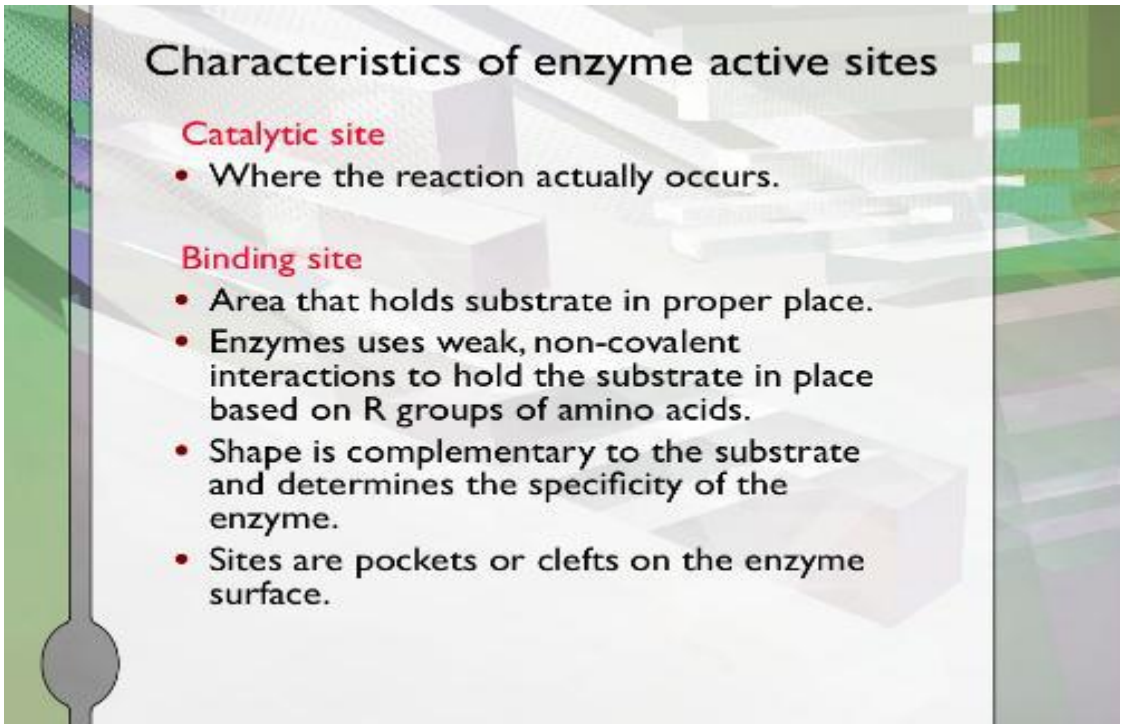
- Prosthetic groups the non-protein part of holoenzymes , it is firmly bounded to Apo enzymes with covalent forces and may be organic or inorganic.
- Cofactors the non-protein part of holoenzyme, it is loosely bounded to Apo enzymes with non-covalent bond, it has low Mwt and it is organic molecule.

Prosthetic groups and Cofactors facilitate the binding of substrates to the enzymes by making them more electrophilic and neucleophilic. Coenzymes serves as a group transfer

reagents, they help in transfer of the substrate from their point of generation to the point of utilization.

Cofactor or Prosthetic group must bind to apoenzyme forming what known by holoenzyme





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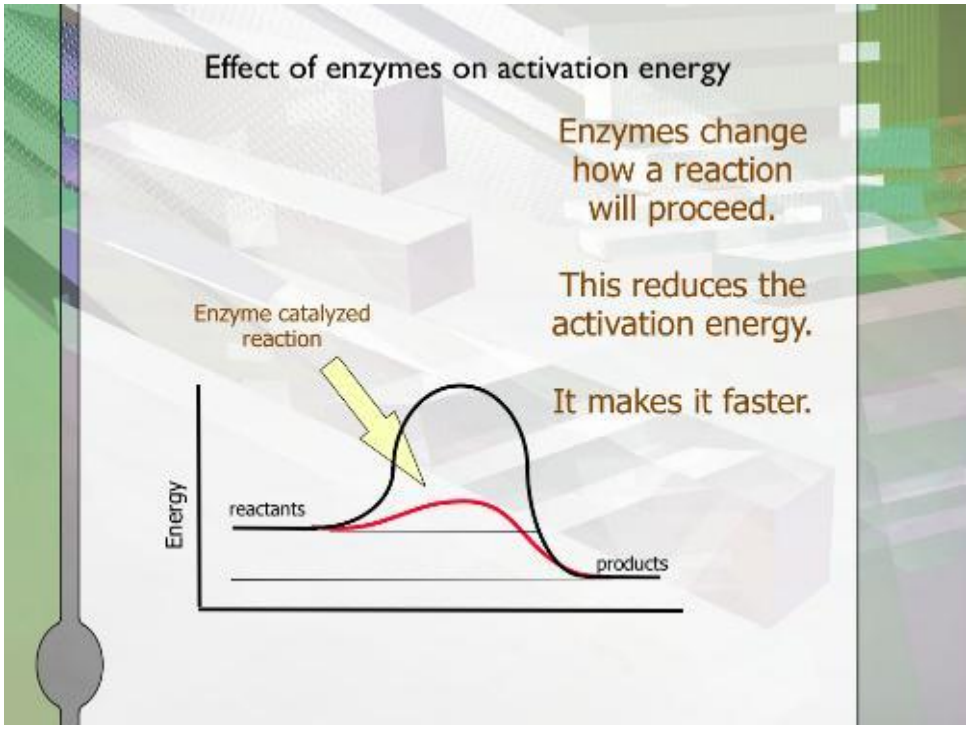


Enzyme can increase reaction rate of reaction at the order of x ١٠

- ***Specificity of enzymes***

The nature and arrangement of amino acids in the active site of the enzyme make it specific for only one type of substrate

Enzymes classified according to specificity into ٤ categories:

١- Absolutely specific

Only reacte with a single substrate.

٢- Group specific

Works with similar molecules with the same functional group

٣- Linkage specific

Catalyzes specific combination of bonds

٤- Sterio chemically specific

Only will work with the proper D- or L- form

- ***Classification of enzymes***

Enzymes can be classified on the basis of the type of reaction it catalyzes (International Union of Biochemists [IUB] classes). They are:

١. *Oxidoreductases:* Involved in oxidation and reduction of substrates.

٢. *Transferase:* Help in transfer of a particular group such as methyl or glycosyl groups from one substrate to another.

٣. *Hydrolases:* Bring about hydrolytic cleavage of bonds like C-C, C-O, C-N, etc.

٤. *Lyases:* Facilitate removal of small molecule from a large substrate leaving double bonds; also add groups to double bonds.

٥. *Isomerases:* Isomerisation of substrate.

٦. *Ligases:* Involved in joining together of two substrates, coupled to the hydrolysis of an ATP.

- ***How enzyme works***

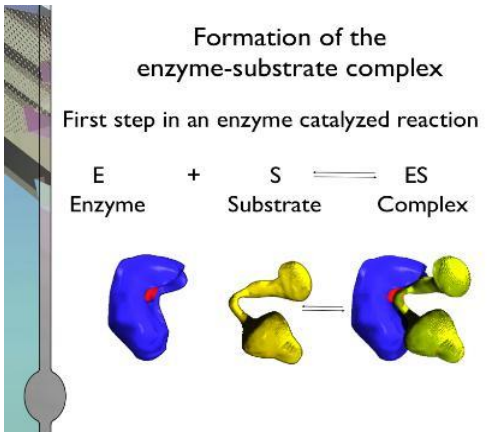
I- Enzyme and substrate combine to form a complex

II- Complex goes through transition state not quite substrate or product

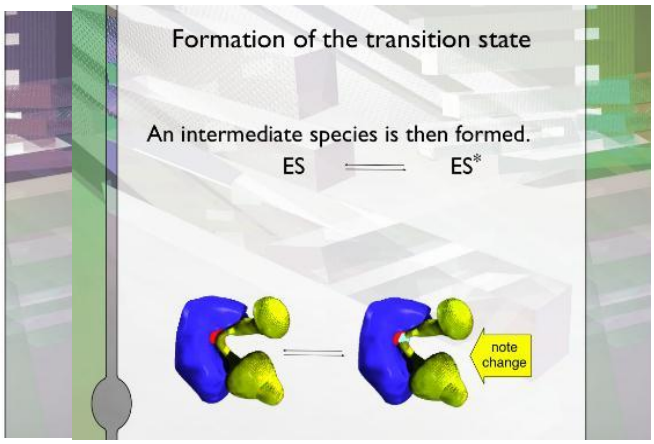
III- Complex of enzyme and product is formed

IV- Finally enzyme and product separate

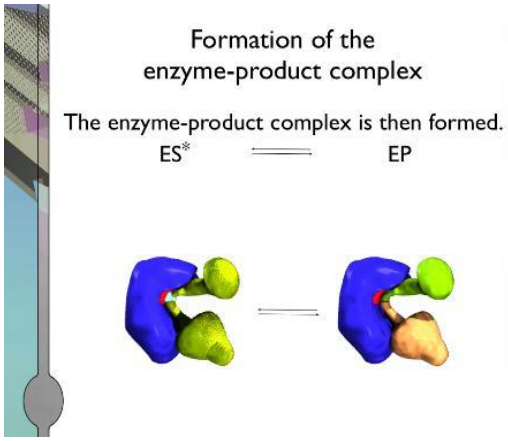
I-

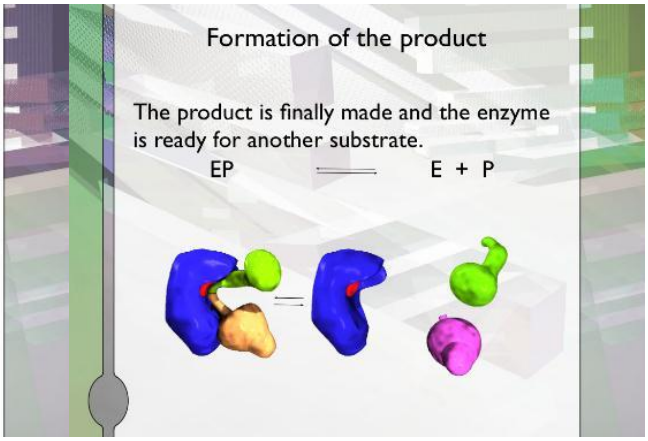


II-



III- IV-





Principle behind diagnosing diseases with enzymes

The basic principle of using the enzyme levels for diagnosing disease is based on comparing the changes in activity in serum or plasma of enzymes which are predominantly present intracellularly and are secreted in the serum in very low active amounts. A sensitive analysis would give insight into the pathological changes and nature of the disease.

- ***Factors affecting enzyme activity***

Enzymes are very efficient catalysts for biochemical reactions. They speed up reactions by providing an alternative reaction pathway of lower activation energy.

Like all catalysts, enzymes take part in the reaction - that is how they provide an alternative reaction pathway. But they do not undergo permanent changes and so remain unchanged at the end of the reaction. They can only alter the rate of reaction, not the position of the equilibrium.

The proteins in enzymes are usually globular. The intra- and inter-molecular bonds that hold proteins in their secondary and tertiary structures are disrupted by changes in temperature and pH . This affects shapes and so the catalytic activity of an enzyme is pH and temperature sensitive.

١) Temperature

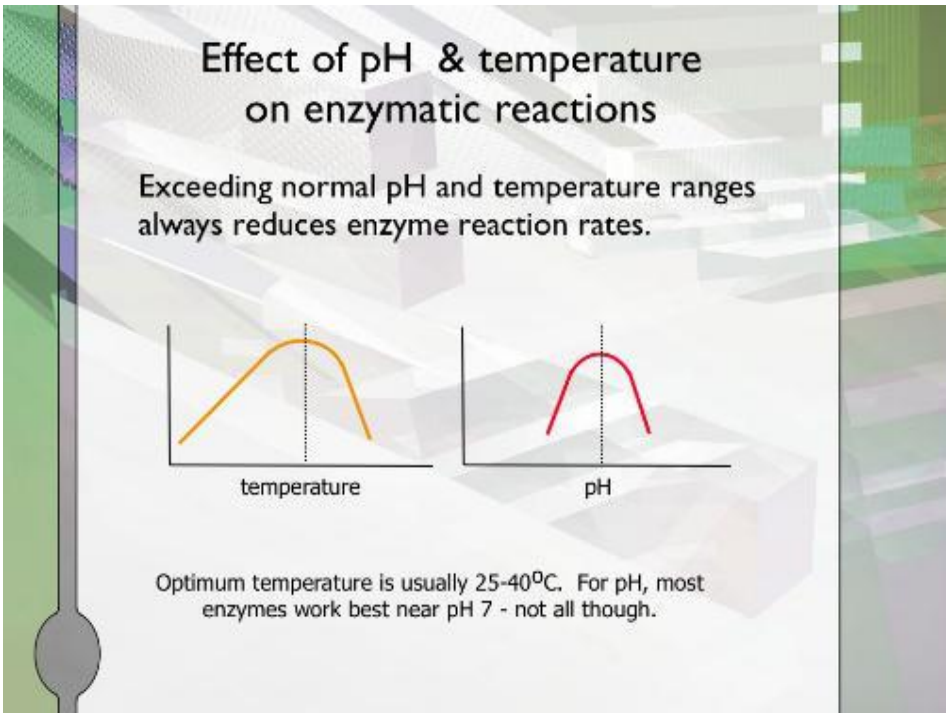
As the temperature rises, reacting molecules have more and more kinetic energy. This increases the chances of a successful collision and so the rate increases. There is a certain temperature at which an enzyme's catalytic activity is at its greatest (see graph). This optimal temperature is usually around human body temperature (٣٧,٥ oC) for the enzymes in human

cells.

Above this temperature the enzyme structure begins to break down (**denature**) since athigher temperatures intra- and intermolecular bonds are broken as the enzyme molecules gain even more kinetic energy.

٢) pH

Each enzyme works within quite a small pH range. There is a pH at which its activity is greatest (the optimal pH). This is because changes in pH can make and break intra- and intermolecular bonds, changing the shape of the enzyme and, therefore, its effectiveness.

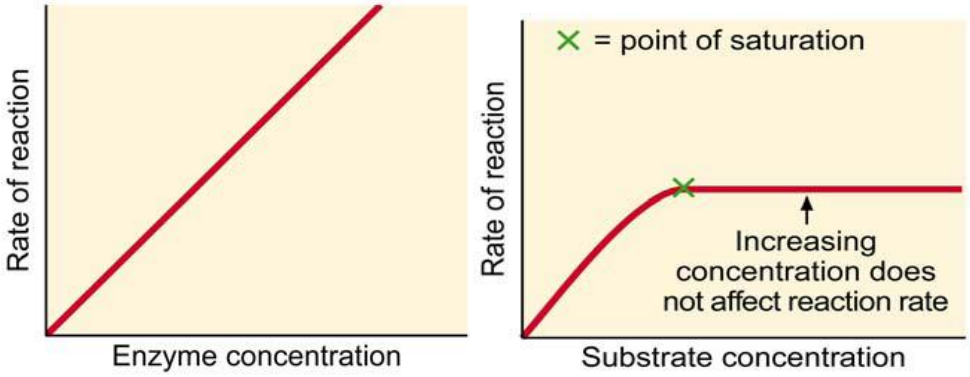


٣) Concentration of enzyme and substrate

The rate of an enzyme-catalysed reaction depends on the concentrations of enzyme and substrate. As the concentration of either is increased the rate of reaction increases (curve ١).

For a given enzyme concentration, the rate of reaction increases with increasing substrate concentration up to a point, above which any further increase in substrate concentration produces no significant change in reaction rate. This is because the active sites of the enzyme molecules at any given moment are virtually saturated with substrate. The enzyme/substrate complex has to dissociate before the active sites are free to accommodate more substrate.

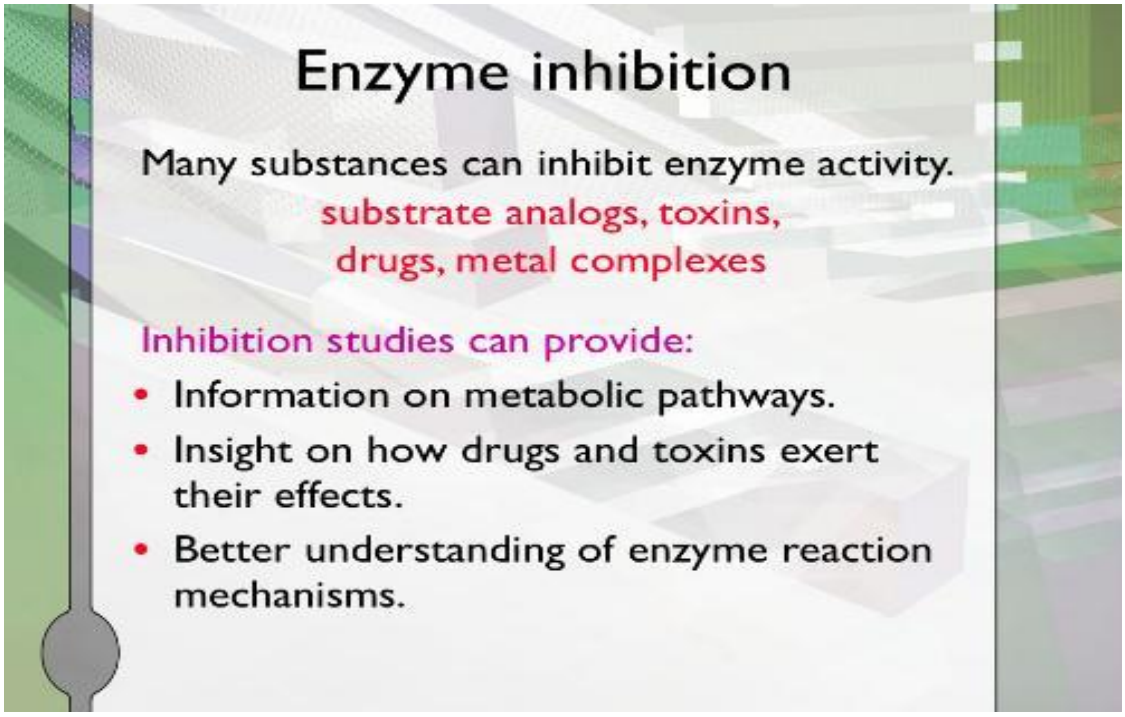
Provided that the substrate concentration is high and that temperature and pH are kept constant, the rate of reaction is proportional to the enzyme concentration.



curve ١: Effect of the concentrations of enzyme and substrate on the rate of an enzymecatalyzed reaction

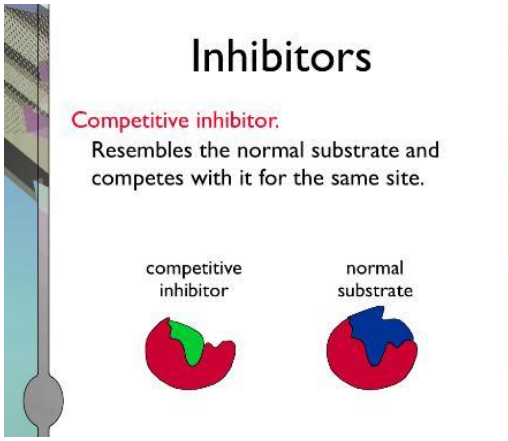
٤) Inhibitors of enzyme activity

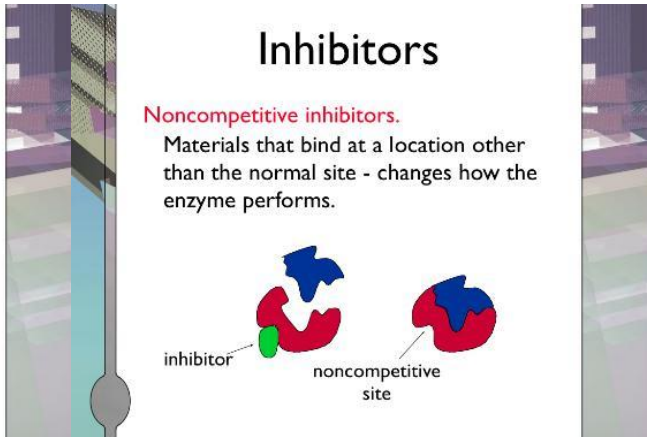
Some substances reduce or even stop the catalytic activity of enzymes in biochemical reactions. They block or distort the active site. These chemicals are called inhibitors, because they inhibit reaction.



Inhibitors that occupy the active site and prevent a substrate molecule from binding to the enzyme are said to be **a**ctive site-directed (or competitive, as they 'compete' with the substrate for the active site).

Inhibitors that attach to other parts of the enzyme molecule, perhaps distorting its shape, are said to be non-active site-directed (or non competitive).





- ***Isozymes and their relevance in diagnosis***

Enzymes exist in multiple forms in the human body. These multiple molecular forms of the enzymes are referred to as the isoenzymes or isozymes. The various forms of an enzyme can be distinguished from each other on the basis of differences in various physical properties, such as electrophoretic mobility or resistance to chemical or thermal inactivation. They often also show quantitative differences in their catalytic properties. Such enzyme variants are found sometimes within a single organ or even within a single cell-type. However, all iso enzymes retain the ability to catalyze the basic reaction unique to that particular enzyme.

Isoenzymes

- catalyse same reactions but are formed from structurally different polypeptides.
- They perform the same catalytic function.
- Different isoenzymes may arise from different tissues and their specific detection may give clues to the site of pathology.
- Various isoenzymes of an enzyme can differ in three major ways:

- enzymatic properties

- physical properties (e.g heat stability)

- biochemical properties such as amino acid composition and immunological reactivities.

Unit of enzyme activity:

The amount of enzyme in a sample is measured by the rate of reaction catalyzed by the enzyme. This rate is directly proportioned to the amount of enzyme and is expressed in enzyme unit, IU/L. Isoenzyme Physically distinct and separable form of the given enzyme present in different cell types. It is of diagnostic value. Can be separated by electrophoresis.
e.g. isoenzymes of lactate dehydrogenase are LD١, LD٢, LD٣, LD٤ and LD٥

Regulation of enzyme levels in serum and plasma

The balance between the rate of influx of active enzyme into the circulation and its eventual clearance from the blood determines the level of activity of the enzyme. There are two crucial factors which determine the rate of entry of enzymes into the circulation from the cells of origin. The first being those that affect the rate of leak from the cells and the second are those that actually reflect altered rates of enzyme production, due to either increased synthesis of the enzyme in response to metabolic alterations in the cell or due to increased proliferation of the cell itself.

How enzymes leak from cells

Enzymes are essentially harbored inside cells of their origin and restrained within the plasma membrane. As long as the integrity of the plasma membrane is maintained the enzymes do not leak out of the cell. This integrity is maintained by the cell's ATP production. ATP production of the cell can be hampered in many ways, such as the loss of oxygen carrying capacity and blood supply; treatment with chemicals and drugs and other environmental pollutants, extreme physical stress such as heat, radiation; exposure to microbial agents and subsequent infection; disruption or malfunction of the immune system; genetic defects leading to metabolic disorders and nutritional disorders. One or many of such distress cause

the plasma membrane to deteriorate. During the early stages of this loss of integrity there is an efflux of potassium ions and an increased influx of sodium ions. This leads to increased water retention in the cell leading to swelling. In later stages, calcium influx occurs which acts as a stimulus to the intracellular enzymes leading to their hyperactivity and an increase in cell damage and disruption of the cell membrane. Finally, all these processes lead to an increased production of free radical and oxidative damage and the membrane become leaky and molecules of all sizes eventually leak out depending on the

extent of damage. The rate at which these enzymes leak out of the cell also depends on the sub-cellular location of the enzymes. The mitochondrial enzymes and others which are bound to the membranes of subcellular structures are not readily released into the circulations. Sensitive detection of such enzymes gives information to distinguish between damage only to the cell membrane from that of a necrotic damage. There is also a distinct variation of enzyme release with respect to the type of tissue damaged. For example, in case of myocardial infarction, the short episode of an attack leads to a rapid release of myocardial enzymes and in about ٢٤ hours the enzyme levels resemble those present in the myocardial tissue. However, in chronic diseases such as those of liver, the enzyme release may continue over a prolonged period of time and may show a varied pattern depending on the variation in clearance of the enzyme and its leakage at various stages of the disease.

Information from enzymes measurements in serum

- Presence of disease
- Organs involved
- Aetiology /nature of disease: differential diagnosis
- Extent of disease-more damaged cells-more leaked enzymes in blood
- Time course of disease

Some important enzymes in clinical practice

Estimation of enzymes activities in the serum has many applications in the diagnosis, differential diagnosis (e.g. in myocardial infarction both AST and LDH are increased in the serum but in case of pulmonary embolism AST is normal but LDH is increased), assess ingprognosis of diseases, and early detection of disease (e.g. increase level of ALT in serum in viral hepatitis before the occurrence of jaundice). Some important enzymes of clinical significances are discussed below:

NAME OF THE ENZYME	PRESENT IN
Aspartate Amino transferase (AST)Serum glutamate-oxaloacetate transaminase(SGOT)	Heart and Liver
Alanine Amino transferase (ALT)Serum glutamate-pyruvate transaminase(SGPT)	Heart and Liver
Alkaline Phosphatase (ALP)	Bone, intestine and other tissues
Acid Phosphatase (ACP)	Prostate
γ glutamyl Transferase (γ GT)	Liver
Creatine kinase (CK)	Muscle Including cardiac muscle
Lactate Dehydrogenase (LDH)	Heart, liver, muscle, RBC
α Amylase	Pancreas

I. Aspartate transaminase (AST) and Alanine transaminase (ALT)

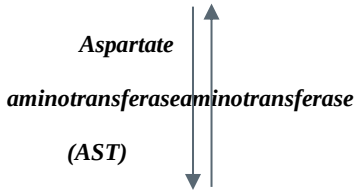
Transaminases are present in most of the tissues of the body. They catalyze the inter conversions of the amino acids and ٢-oxacids by transfer of amino groups.

Transaminases are specific for the amino acid from which the amino group has to be transferred to a keto acid. ٢-oxoglutarate and glutamate couple serves as one amino group acceptor and donor pair in all amino transfer reactions.

AST catalyzes the inter conversion of oxaloacetate to aspartate coupled with glutamate tooxoglutarate.

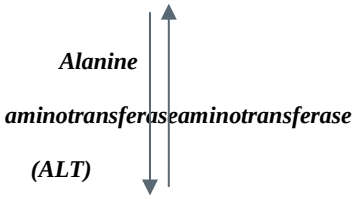
ALT catalyzes the inter conversion of pyruvate to alanine coupled with glutamate to oxo glutarate.

α- Oxoglutarate + L-aspartate



L- glutamate + oxaloacetate

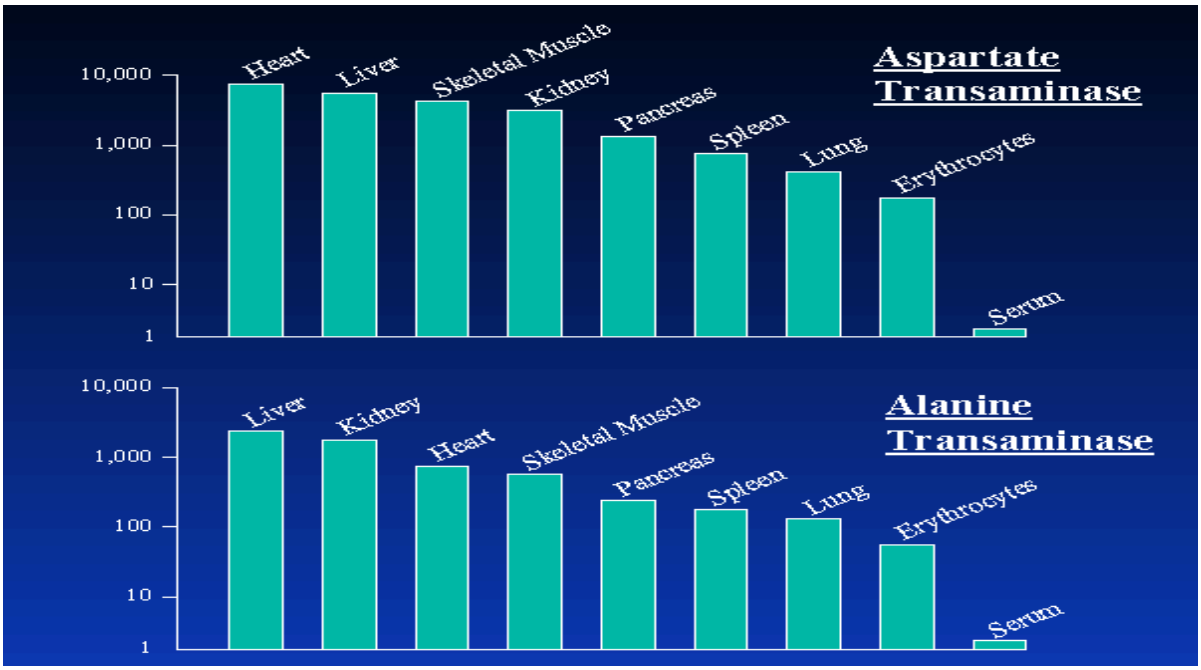
α- Oxo glutarate + L-alanine



L- glutamate + pyruvate

- Alanine transaminase (ALT) and Aspartate transaminase (AST) enzymes are the most abundantly present in the liver and is elevated in blood as a result of leakage from damaged cells
- Measurement of these transaminases is useful for the diagnosis of liver diseases
- In viral hepatitis the enzyme levels are increased ٢٠-٥٠ times above the upper limit of the normal range

➲ **Differences in bodily distribution between AST and ALT**



Aspartate transaminase (AST)

➲ This enzyme is widely distributed in the body.

➲ Main sources: Heart, liver, skeletal muscle, and kidney.

➲ Useful in the diagnosis of MI, liver disorders and muscle damage.

➲ Causes of ↑serum AST levels:

➲ Physiological : Neonates.

➲ Liver diseases: Hepatitis, hepatic necrosis , cholestasis

➲ Cardiac disease: Myocardial Infarction.

➲ Diseases of skeletal muscle: Crush injury, trauma, myopathy

➲ From Erythrocytes: Hemolysis

➲ increase is specific for liver damage involving hepatocellular damage

➲ is moderately increased in Muscular dystrophy and acute myocardial infarction.

Alanine transaminase (ALT)

- Widely distributed, although the largest amounts found in the liver.
- Congestive cardiac failure →release from the liver
- More specific for liver disease than AST.

Clinical significance

The activities of both AST and ALT are high in tissues especially liver, heart, and muscles.

Any damage or injury to the cells of these tissues may cause release of these enzymes along with other intracellular proteins/enzymes into the circulation leading to increase activities of these enzymes in the blood. Some increases in the activities of both the enzymes are seen after alcohol intake.

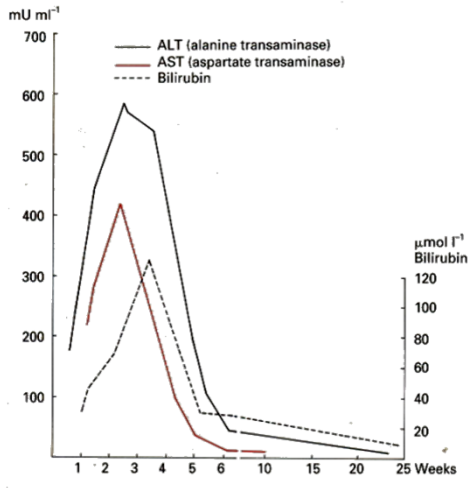
Liver diseases:

Determinations of activities of AST and ALT in serum in patients with liver diseases like viral hepatitis and other forms of liver diseases with necrosis, give high values even before the appearance of clinical signs and symptoms like jaundice. Activity levelsof٢٠ to ٥٠ fold higher than normal are frequently seen in liver cells damage but it may reach as high as ١٠٠ times in severe damage to cells. Highest serum activities are seen between ٧th and١٢th days and return to normal levels by the ٣rd to ٥th week. In sever tissue damage ALT activity is higher than AST and the ALT:AST ratio becomes ≥١(normally<١). In cirrhosis the level of activities vary with the severity of the disease. It may increase only up to ٥ fold of the norm al activities. Up to ١٠ fold increase is seen in carcinoma of the liver. Even though the activities of both AST and ALT are elevated in the serum of the patients with liver diseases, ALT is more liver specific enzyme as increased ALT activity in serum is hardly seen in tissues other than
liver cell damage.

Heart diseases:
In myocardial infarction high activity of AST is seen in serum. ALT activity is within normal range or slightly increased in uncomplicated myocardial infarction. Rise in AST is seen within ٦ to ٨ hours of the onset of chest pain, highest level at ١٨ to ٢٤ hours and returns to pre infarction levels by ٤th to ٥th day. There are other superior markers available for myocardial infarction as AST lacks the tissue specific characteristics, as its activity may also increased in diseases of other tissues like liver and skeletal muscles.

Skeletal muscle diseases:
AST and occasionally ALT activity levels are increased in progressive muscular dystrophy and dermatomyositis. Level of AST may go as high as ٨times of the normal. There is no increase in the enzyme activity in the muscle diseases of neurogenic origin. Increased AST activity, ٢ to ٥ times of normal, is also seen after crushed muscle injuries. In other conditions like pulmonary emboli, acute pancreatitis ,hemolytic disease and gangrene the activity of AST is found to be ٢ to ٥ times higher than the normal activity.

LEVELS OF ENZYMES IN DISEASES INVOLVING LIVER DAMAGE



In viral hepatitis rapid rise in transaminases (AST & ALT) in serum occurs even before bilirubin rise is seen

II. γ-Glutamyltransferase (GGT)

A microsomal enzyme, its synthesis induced by ethanol and anticonvulsant drugs

Amino acid + Glutathione $\rightleftharpoons$ ***γ-glutamyl amino acid + Cysteinylglycine***

• Found mainly in the kidney and significant amounts in liver, brain, prostate, and pancreas.

• Used primarily for diagnosis of hepatobiliary problems .

• ALT, AST and GGT are the main liver function tests .

• Marked elevation of serum GGT level is seen in alcoholic liver disease.

• Normal serum value – ٨٠-١٨٠ units /١٠٠ ml

Clinical significances

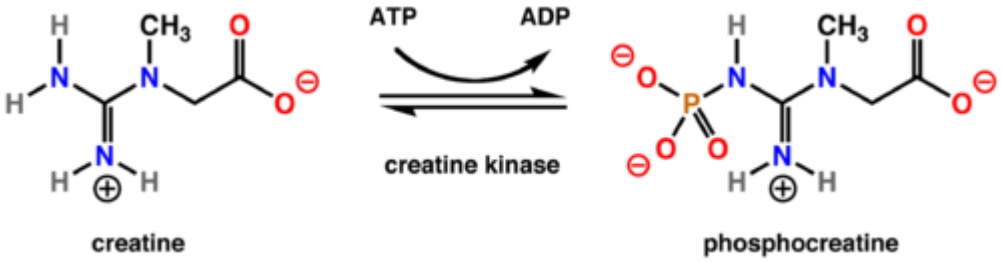
Liver diseases: The activity of GGT in serum is elevated in all forms of liver disease. Highest activity is seen in cases of intra- and post-hepatic biliary obstruction. GGT is more sensitive than ALP in detecting obstructive jaundice, cholangitis and cholecystitis. In infectious hepatitis there is only moderate elevation of GGT activity in the serum. In cases of primary and secondary liver cancer the level of GGT increase earlier and more pronounced than other enzymes of the liver. Increased levels of GGT are seen in heavy drinkers and patients with alcoholic liver cirrhosis. High levels are also found in patients receiving anticonvulsant drugs. Increased levels of GGT are found in almost all the diseases involve in gliver but are of little value in discriminating different kinds of liver disease. Normal levels of GGT are seen in patients with muscle diseases, children older than ١ year or in healthy pregnant women – conditions in which ALP is elevated. Thus measurement of GGT levels in serum can be used to ascertain whether observed elevations of ALP are due to skeletal disease or reflect the presence of hepatobiliary disease.

Pancreatic diseases: GGT activity may increase in acute as well as chronic pancreatitis and in some cases of pancreatic malignancies especially if associated with hepatobiliary obstruction.

Tumors: Prostate malignancy may at time show increased level of GGT in serum. The irradiation of tumors in cancer patients may be accompanied by a rise in GGT activity. In general if there is increased GGT level in serum of cancer patients there is a chance that the tumor might have metastasize to the liver.

III. Creatine Kinase (CK)

Creatine kinase catalyzes the reversible phosphorylation of creatine by ATP.



The equilibrium position for the reaction is pH dependent. At neutral pH, phosphocreatine has a much higher phosphorylating potential than does ATP, thus favors the reverse reaction.

The reverse reaction proceeds ٢-٦ times faster than the forward reaction under optimum reaction conditions. During muscle contraction ATP is consumed to form ADP, this ADP is again rephosphorylated to ATP by enzyme creatine kinase (CK) using phosphocreatine as a phosphate donor. Phosphocreatine is the major phosphorylated compound present in muscles, eight times more than that of ATP. Enzyme activity is inhibited by excess ADP, urate, cysteine and metal ions like Mn^{2+}, Ca^{2+}, Zn^{2+} & Cu^{2+}. Mg^{2+} is required for the activity of CK but excess of it inhibit the CK activity.

The enzyme is dimeric, composed of ٢subunits namely B (Brain) and M (muscle). Names indicate major tissue of origin. B and Mare the product of two different genes.

These subunits form three different dimers and form three different isoenzymes namely BB (CK-١), MB (CK-٢) and MM (CK-٣). Isoenzymes BB predominates in brain, prostate, gut, l ungs, bladder, uterus, placenta and thyroid, MM predominates in skeletal muscle and heart muscle and MB isoenzymes is present in varying degree in heart muscle (٢٥٪-٤٦٪ of CK activity) and some in skeletal muscle.

Isoenzyme name	**Composition**	**The most predominates**	**Elevated in**
CK-١	BB	Brain	CNS diseases
CK-٢	MB	Myocardium/ Heart	Acute myocardial infarction
CK-٣	MM	Skeletal muscle, Myocardium	

Isoenzyme	**Distribution**	**% serum normal**	**Causes of increased activity in serum**
CK-MM	-sk. muscles - miocardum	٩٥-١٠٠٪	- muscles diseases (dystrofy, myositis),rhabdomyolysis, trauma - AMI - CNS tumors, enkefalites - hypothyroidism, acromegaly (endocrine miopathy)
CK-BB	- tub digestiv, pancreas, uter, prostată,	٠٪	- brain infarct, CNS surgery -intestinal infarct, cancer of prostate, testis, ovaries, postpartum, renalfailure, anoxia, digestive cancer, prostatectomy
CK-MB	- myocardial - musculature striată,	٠-٥٪	- AMI - cardiac surgery, miocardită, cardioversion(>٤٠٠J),

	Prostate , uter		prolongedsupraventricular tachycardia,percutaneous coronary angioplasty) - NONCARDIAC CAUSES: sk muscle diseases or trauma,carcinomas (eg. prostate, breast), drugs (e.g. tranquilizers), postpartum,

Clinical significance

Serum CK activity is elevated in tissue damages involving skeletal muscle, heart muscle ,brain injury etc. Elevation of particular CK isoenzymes activity in serum is of dia gnostic value.

Heart diseases:

CK activity in serum invariably increases after myocardial infarction (MI).CK-٢ levels rise ٣ to ٦ hours after a heart attack. If there is no further damage to the heart muscle, the level peaks at ١٢ to ٢٤ hours and returns to preinfarction level in ١٢ to ٤٨ hours .The use of total CK and CK-٢ in the diagnosis of myocardial infarction is the most important single application of CK measurements in clinical chemistry. Percentage of CK-٢ over total CK activity is valuable in diagnosis of MI. Preinfarction values of CK-٢ are usually less than٦٪ of the total CK activity, but following an infarction values can increase up to ٣٠٪depending on the extent of myocardial damage, location of the infarct or the methods used for analysis.

The diagnostic sensitivity of total CK in myocardial infarction is ٩٣-٩٨٪ and for CK-٢ it is nearly ١٠٠٪. And the diagnostic specificity is ٧٥-٨٥٪ for total CK and nearly ١٠٠٪ for CK-٢ if the level of CK-٢ is >٦٪ of total CK.

Elevation of total CK and CK-٢ is seen in cardiac trauma following heart surgery which may mask elevation due to intraoperative myocardial infarction. Other cardiac conditions like angina pectoris, cardiogenic shock, electrical counter shock, myocarditis, congestive

heart failure, cardiac intra arterial procedures have reportedly shown elevations of total serum CK or CK-٢ or both. CK-٢ level of <٥٪ or ٦٪ of total CK usually exclude the myocardial damage.

Skeletal muscle diseases:

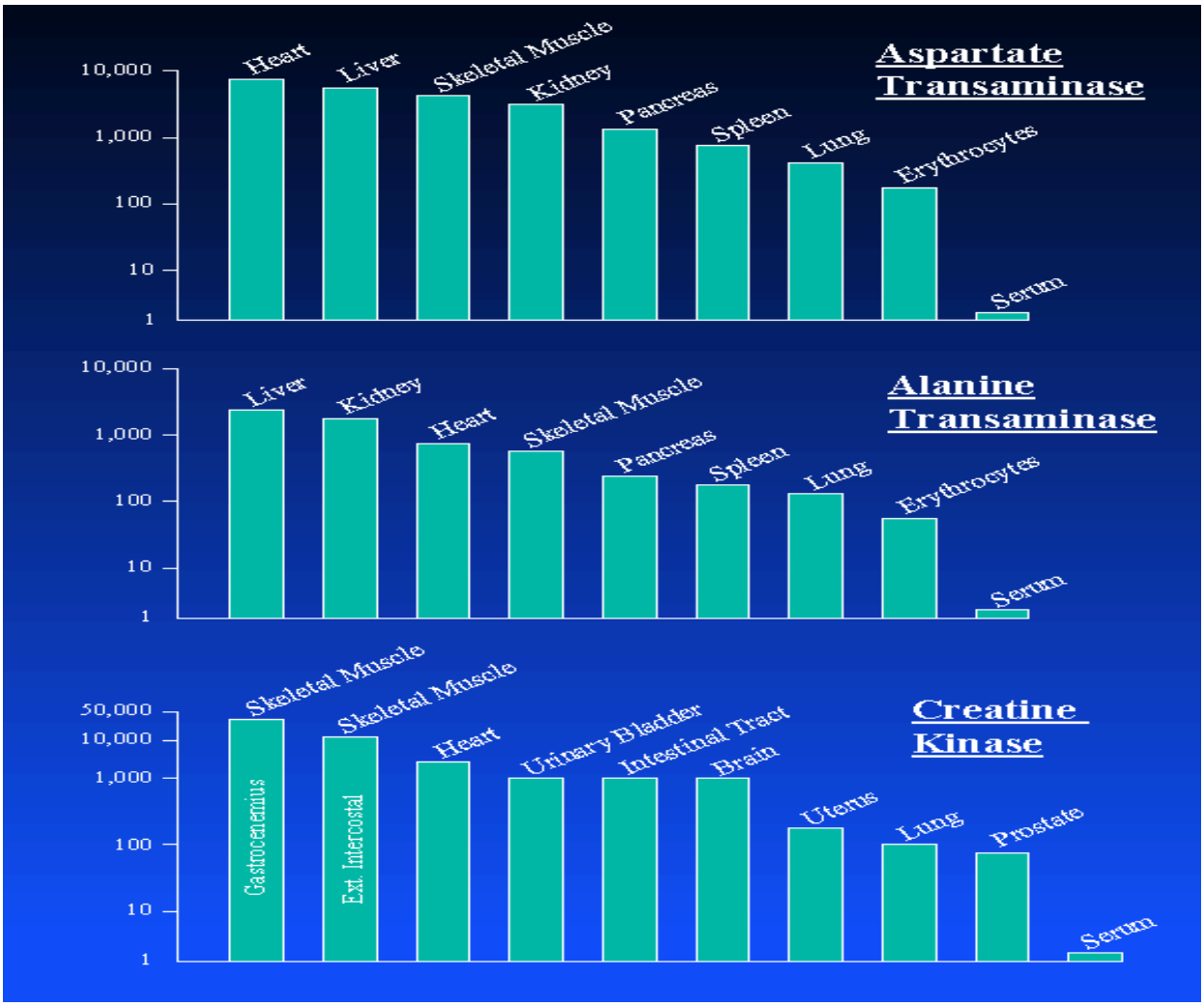
High CK activity is found in all types of muscular dystrophy. Incase of Duchenne muscular dystrophy the level may go as high as ٥٠ times that of the normal level. In progressive muscular dystrophy, enzyme activity in serum is highest in child hood and may be elevated long before the disease is clinically apparent, About ٥٠ to ٨٠٪ of asymptomatic female carriers of Duchenne dystrophy show ٣-٦ fold elevations of CK activity. Elevation of CK activity is observed in other muscular diseases including malignant hyperthermia. Muscle disease of neurogenic origin may have normal CK activity in the serum.

CNS diseases:

In cerebral ischemia , acute cerebro vascular disease, head injury and neurological interventions the level of CK in the serum may increase. In Reye's syndrome, which is characterized by acute brain swelling with fatty infiltration, the CK activity may riseto upto ٧٠ fold of normal level.

Thyroid diseases:

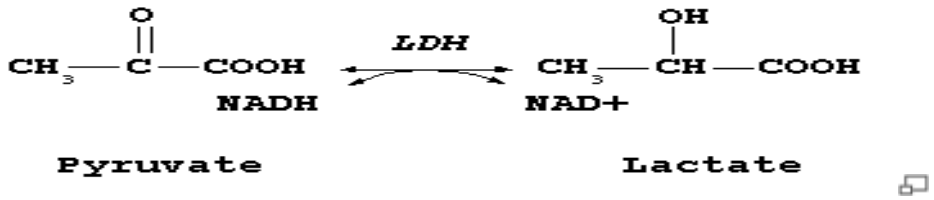
There is an inverse relationship with thyroid activity. Elevation of CK activity up to ٥ times the normal level is seen in about ٦٠٪ of the hypothyroid subjects. Them ajor enzyme present is CK-٣, CK-٢ may go up to ١٣٪ of the total CK activity if myocardial involment is there in the hypothyroid subjects.



WHAT MAJOR DIFFERENCES DO YOU NOTICE BETWEEN TRANSAMINASES AND CREATIN KINASE? "There is no CK in the liver"

IV. Lactate dehydrogenase (LDH)

Lactate dehydrogenase catalyzes the oxidation of L-lactate to pyruvate. In the reaction hydrogen is transferred from lactate with the mediation of NAD+ as hydrogen acceptor.



LDH is elevated in myocardial infarction, blood disorders LDH is composed of four subunits of two types namely H = Heart, M = skeletal muscleIt exists as ٥ different isoenzymes with various combinations of H and

Isoenzyme name	**Composition**	**Composition**	**Present in**	**Elevated in**
LDH١	($H_٤$)	HHHH	Myocardium, RBC	myocardial infarction
LDH٢	($H_٣M_١$)	HHHM	Myocardium, RBC	
LDH٣	($H_٢M_٢$)	HHMM	Kidney,Skeletal muscle	
LDH٤	($H_١M_٣$)	HMMM	Kidney, Skeletal muscle	
LDH٥	($M_٤$)	MMMM	Skeletal muscle, Liver	Skeletal muscle and liverdiseases

Clinical significance

Heart diseases: In myocardial infarction the level of total LDH activity in serum is ٣-٤ times that of normal but it may go up to ١٠ times the normal value. In myocarditis and cardiac failure with hepatic congestion the serum enzyme activity may be elevated. Moderate elevation of the enzyme activity may be seen in severe shock and anoxia. But no change in serum enzyme activity in angina and in pericarditis. Hemolysis due to any cause may elevate level of LDH١ & ٢ in the serum.

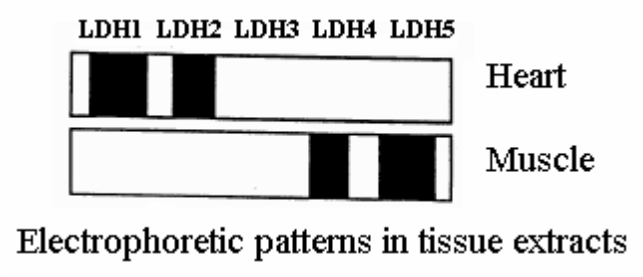
Liver diseases: In toxic hepatitis with jaundice serum activity of LDH may be elevated upto١٠ times the normal value. Increased activity in serum is also seen in viral hepatitis. SerumLDH٥ activity is often elevated in patients with primary liver disease and liver anoxia secondary to decreased O٢ perfusion.

Muscle diseases: The patients with progressive muscular dystrophy often show elevated LDH activity especially LDH٥ isoenzyme in the serum. In the later stages of the disease when the muscle mass is lost the LDH level may come down to normal level.

Kidney diseases: In chronic glomerulonephritis, systemic lupus erythematosus, diabetic nephrosclerosis and bladder & kidney malignancies the LDH activity in the urine is found to be elevated ٣ to ٦ times the normal.

Tumors: Patients with malignant disease show increased LDH activity in serum especiallyLDH٤ & ٥. In germ cell tumors like teratomas, seminoma of the testis high level of LDH١ isseen.

Note :The efficiency of the conversion of pyruvate tolactate increases with the number of M chains.



Electrophoretic patterns in tissue extracts

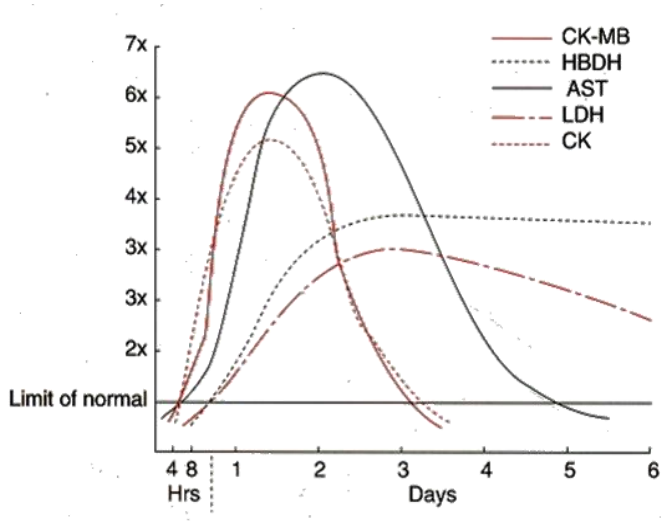
What are the metabolic consequences for heart and muscles?

The high concentration of LDH ٥ (٤ M subunits) in skeletal muscle rapidly converts pyruvate to lactate, while the high concentration of LDH ١ (٤ H subunits) in heart tissue favorsconversion of pyruvate to acetyl CoA which enters the citric acid cycle.

In skeletal muscle, where oxygen deprivation is common during exercise, the reaction is efficient and large amounts of lactate can be formed.

In tissues that preferentially oxidize glucose aerobically to $CO_2$and water, such as cardiac muscle, the reaction is not efficient and pyruvate is preferentially converted to acetyl CoA which enters the citric acid cycle.

- ***LEVELS OF ENZYMES IN MYOCARDIAL INFARCTION***



AST and CK rise in 6 hours following acute myocardial infarction
HBDH and LDH are elevated much later and remains high for a longer period of days

V. Alkaline phosphatase(ALP)

Alkaline phosphatases are present in almost all tissues of the body. They are membrane bound and are zinc containing metaloenzymes. Alkaline phosphatases are a family of isoenzymes. They hydrolyze a variety of organic phosphate esters transferring phosphate groups from a donor substrate to an acceptor containing a hydroxyl group. The active center of the enzyme contains a serine residue. High levels of enzyme are present in intestinal epithelium (I), Kidney tubules (K), osteoblasts in the bone (B), bile canalicular and sinusoidal membrane of the liver (L), placenta and the lactating breast (P).

Alkaline phosphatases from different sources exhibit three types of activity

١. Hydrolytic R-P + H-OH $\longrightarrow$ R-OH + H-P

٢. Phosphotransferase R-P + R'-OH $\longrightarrow$ R-OH + R'P

٣. Pyrophosphatase R-P-P-R' + H-OH $\longrightarrow$ R-P + R'-P

Alkaline phosphatase act on large variety of physiologic and non-physiologic substances. Though the precise natural substrate of alkaline phosphatase in the body is not known, it is associated with calcification and mineralization process in bone and probably in lipid transport in the intestine.

Alkaline phosphatases are a group of true isoenzymes, encoded by at least four different genes: tissue non-specific, intestinal, placental and germ-line ALP. The isoforms derived from the tissue non-specific isoenzyme by post translational modification include the variants of the enzyme found in the liver, bone, kidney and the placenta. Some malignant tumors can produce a placental form of the enzyme called the Regan's isoenzymes.

In serum of normal adults most of the enzyme activity is contributed by liver and nearly half by bone. The respective contributions of these two forms to the total activity are markedly age dependent. Intestine contributes very little amount. The contribution from

the kidney is negligible. Placenta contributes a considerable amount during pregnancy. In urine ALP is from renal tissue. The kidneys do not clear the serum ALP.
Normal serum value:٣-١٣ KA units/١٠٠ml.

Clinical significance

Physiological bone growth elevates ALP in serum and hence in the sera of growing children enzyme activity is ١,٥-٢,٥ times that in normal adult serum. The level of ALP in the serum of women in the third trimester of pregnancy is ٢-٣ times more than that of normal level.

Liver diseases: Biliary obstruction due to any cause may elevate ALP level by increasing its synthesis from the hepatocytes adjacent to the biliary canaliculi. This newly synthesized ALP enters the circulation and elevates the enzyme level in the serum. Elevation of ALP in the serum is more with extra hepatic obstruction by stones or by carcinoma head of pancreas than in intrahepatic obstruction. The enzyme level may return to normal on removal of the obstruction. Liver diseases affecting parenchymal cells like infectious hepatitis show only moderate elevation or normal serum ALP levels.

Bone diseases: Bone diseases with increased osteoblastic activity shows increased ALP level in the serum. High ALP levels sometimes up to ٢٥ times the normal value are seen in osteitisdeformans (Paget's disease). In Paget's disease there is resorption of bones due to uncontrolled osteoclastic activity and body tries to rebuild bone by increasing osteoblastic activity leading to high ALP level. Moderate increase in ALP level in the serum is seen in osteomalacia, rickets (comes down to normal on treatment with vitamin D), Fanconi's syndrome, primary and secondary hyperparathyroidism. Secondaries in bone from prostate

Cancer: show high serum ALP. Very high ALP levels are present in patients with osteogenic bone cancer.

VI. Prostaticacid phosphatase (PAP):

Acid phosphatase include all the phosphatases catalyzing the following reaction at an optimal pH below

Ortho phosphoric monoester + H_2O ⟶ alcohol + H_3PO_4

Acid phosphatase is an enzyme found throughout the body, but primarily in the prostate gland. The male prostate gland has ١٠٠ times more acid phosphatase than any other body tissue. Tissues other than prostate have small amounts of acid phosphatase, including bone, liver, spleen, kidney, and red blood cells and platelets. Acid phosphatases are present in lysosomes, some extra lysosomal acid phosphatases are also found in many cells. Damage to these tissues causes a moderate increase in acid phosphatase levels. Different forms of acid phosphatase are found in different organs, and their serum levels are used as a diagnostic for disease in the corresponding organs. Acid phosphatase from prostate contributes to ١/٣rd to ½ of the enzyme activity present in the serum of a healthy male. The source of the remainder of the acid phosphatase in the serum from healthy males and females is not clear. Normal serum value - ١-٥ KA units/ ١٠٠ml.

Clinical significance

The highest levels of acid phosphatase are found in metastasized prostate cancer. It is of clinical importance to differentiate prostatic and non prostatic form of acid phosphatase. The prostatic enzyme is strongly inhibited by dextrorotatory tartrate ions, whereas the erythrocyte isoenzyme is not. Formaldehyde and cupric ions inhibit erythrocyte acid phosphatase but not the prostate acid phosphatase. Diseases of the bone, such as Paget's disease or hyperparathyroidism; diseases of blood cells, such as sickle cell disease or multiple myeloma; or lysosomal disorders, such as Gaucher's disease, also show moderately increased levels of acid phosphatase.

VII. Amylase

Amylases are a group of hydrolases that hydrolyze complex carbohydrates containing α-Dglucose units linked through carbon ١and ٤ located on adjacent glucose residues. Both straight chain and branched polyglucans are hydrolyzed at α-١,٤-linkages. The branch points (α-١,٦-linkages) are not attacked by the enzyme. There are two types of amylases – α- amylase and β-amylase.

β-amylase are found in plants and bacteria, they are also called exo amylases as they hydrolyze α-١,٤-linkages only at the terminal reducing end of a polyglycan chain.

α-amylase are found in humans, they are also called endo amylases as they can hydrolyze α- ١,٤-linkages anywhere along the polyglycan chain randomly, so the large polysaccharides are hydrolyzed rapidly into smaller molecules like dextrins, maltose, glucose etc.

Amylase require calcium ions and are activated by chloride and bromide, pH optimum is ٧,٠.

The molecular weight of serum amylase range from ٥٥ to ٦٠ kDa.

Amylase is present in many organs and tissues. The highest concentration is found in pancreas. It is synthesized by the aciner cells of the pancreas and then through pancreatic duct it is secreted into the intestinal tract. Salivary gland also secretes amylase in the mouth where the hydrolysis of starch takes place while the food is still in the mouth. The salivary enzymes become inactive when it reaches stomach due to the presence of acid. When food reaches duodenum, pancreatic and intestinal amylase act on the polyglycans present in the bolus and hydrolyze them to produce maltose. Maltose is then acted upon by intestinal maltase and hydrolyzed to glucose. In the lower part of the intestine most of the amylase is destroyed by trypsin, but some amylase activity is present in the feces.

Other then pancreatic and salivary amylase, Amylase activity is present in striated muscle, adipose tissue, lung, ovaries, fallopian tubes, semen and testes. The enzyme is also secreted in milk, colostrums and tears. Tumors of lung and ovary, ascetic fluid due to pancreatic tumor may contain amylase. The serum amylase is mainly contributed by pancreatice (P-type) and salivary gland (S-type) and amylase in urine is derived from the plasma. Even after pancreatectomy the level of serum amylase activity is not reduced much as the salivary glands and other sources contribute to the serum amylase activity.

Macroamylasemia: In the serum of macroamylasemia patients, macroamylase is present.

They are probably a complex of ordinary amylase and other high molecular weight plasma proteins like IgA, IgG and other molecules. They are not filtered in the kidneys and increases serum amylase activity by ٦ to ٨ folds. Amylase activity in the urine is found to be lower than normal. No clinical symptoms are however associated with this disorder. The differentiation of macroamylases from the increased serum amylase in e.g. acute pancreatitis is most simply made by determination of the urinary amylase which will be increased if the amylase is of the usual molecular weight.

Clinical significance

Pancreas: Amylase activity assay in serum and urine are mainly done in the investigation of the pancreatic function and the diagnosis of diseases of the pancreas. In acute pancreatitis, serum amylase activity increased within ٢ to ١٢ hours of the onset of the disease with maximal levels in ١٢-٧٢ hours and the level returns to normal by the third or fourth day.

There is ٤ to ٦ fold increased in amylase activity above the reference limit. However, up to ٢٠٪ of the cases may have normal amylase activity in the serum. A significant amount of serum amylase is excreted in the urine, so the rise in serum amylase is reflected in the rise of urine amylase activity.

In quiescent chronic pancreatitis both the serum and urine amylase activity is found to be subnormal.

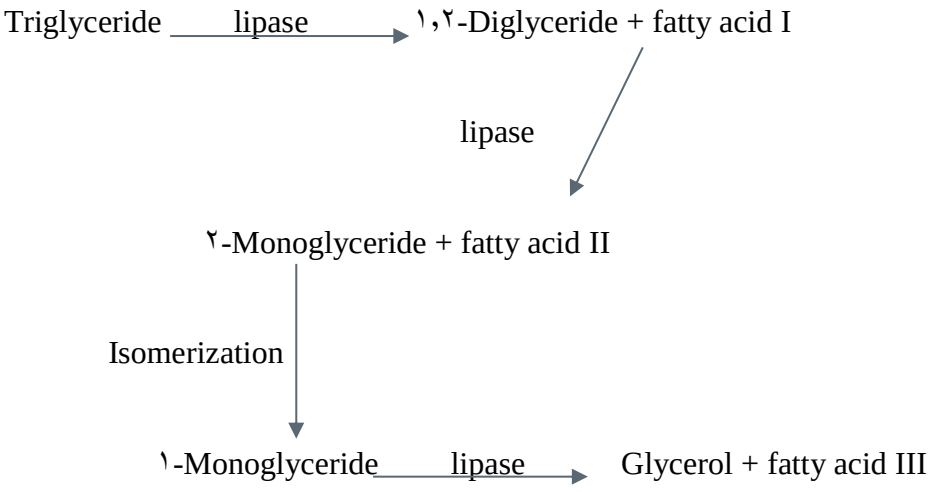
Amylase activity assay is also used in detecting the development of complications following acute pancreatitis like, pseudocyst, ascites, pleural effusion etc. Pacreatic abscess and traumatic lesion to the pancreas, e.g surgical trauma, may cause transcient rise in serum amylase activity. Pancreatic cancer leading to the obstruction of the duct may elevate the serum amylase activity

Salivary gland: Any lesion to the salivary gland due to infection (mumps), surgical traumaor any other type of trauma, calculus and tumors may lead to high amylase activity in the serum which is mainly of S-type.

Other causes of hyper amylasemia include renal insufficiency, tumor of the lung and ovary, cholecystitis, ruptured ectopic pregnancy, cerebral trauma, diabetic ketoacidosis.

VIII. Lipase

Lipases are enzymes that hydrolyze glycerol esters of long chain fatty acids. Ester bonds at carbon ١ and ٣ only are attacked by the enzyme and from each substrate it produce two moles of fatty acids and one mole of ٢-acylglycerol. The enzyme cannot act on ٢-acylglycerol due to the steric hindrance but ٢-acylglycerol spontaneously isomerizes to ٣-acylglycerol which is then attacked by the enzyme and split off the third fatty acid molecule from the glycerol.



Lipase act on the substrate only when it is present in emulsified form. The sequence of lipase acting on the substrate is as follows: first colipase binds to a micelle of bile salt forming colipase-bile salt complex that attaches to the surface of the substrate. To colipase-bile salt complex the enzyme lipase binds with high affinity. The enzyme lipase is activated by colipase and the lipase action starts efficiently. Normal serum value : <١٥٠ units /L.

The main source of lipase is pancreas and the serum lipase is mainly contributed by pancreas, but some lipase is also produced by gastric, pulmonary and intestinal mucosa and also by the tongue.

Clinical significance

Pancreas: Lipase activity in the serum and other body fluid is measured exclusively for pancreatic disorders. In acute pancreatitis, increased lipase activity in the serum is seen after ٤ to ٨ hours of an attack, peaks at about ٢٤ hours, and come to the normal level by ٨ to ١٤ days. Increased lipase activity parallels that of amylase, but lipase activity may increase sooner and remain longer than that of amylase activity in the serum and the extant of rise is higher with lipase activity. As ٢٠٪ cases of acute pancreatitis show normal amylase activity, it is necessary to estimate both the amylase and lipase activity in the serum of a patient suspected of acute pancreatitis. Many patient with severe acute pancreatitis develop a pseudocyst in which there is delayed improvement in the clinical condition of the patient.

Estimation of lipase show persistent increase in the activity. Serum lipase assay is a more specific diagnostic test in case of patient presenting with acute abdomen to differentiate pancreatic disorder with other acute abdominal disorders like ulcers with perforation, intestinal obstruction etc.

Other conditions in which high serum lipase activity is seen are obstruction of the pancreatic duct by calculus or by carcinoma of the pancrease, acute and chronic renal diseases.

X. ***Cholinesterases***

Cholinesterases are defined as enzymes that promptly hydrolyze acetylcholine released at the nerve endings. There are two types of enzymes i) acetyl cholinesterase and ii) acyl choline acyl hydrolase (SChE)

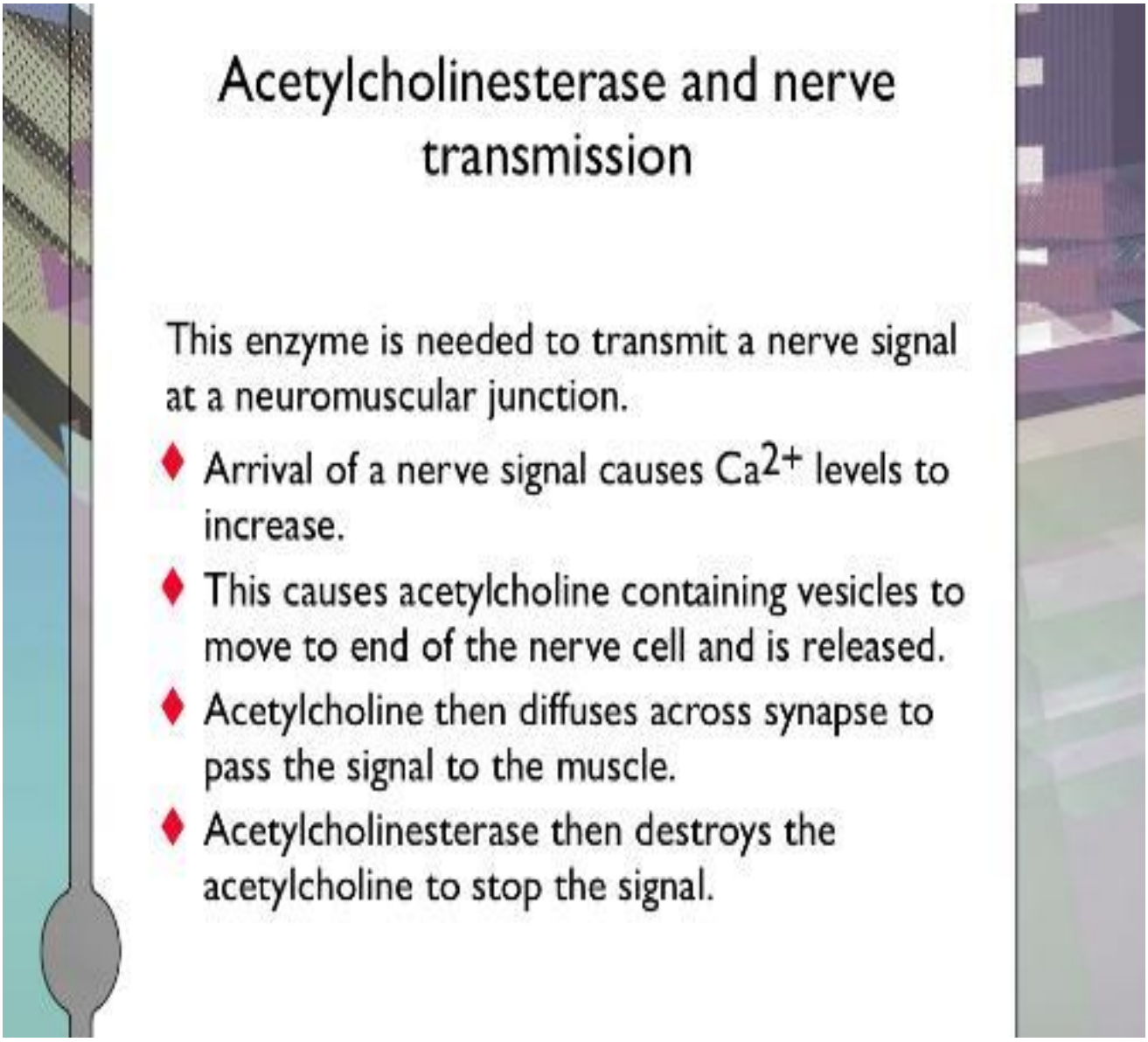
i) Acetyl cholinesterase : It is also known as true cholinesterase or cholinesterase I. It is responsible for the prompt hydrolysis of acetylcholine released at the nerve endings. Acetylcholine degradation is required for depolarization of the nerve so that it can be repolarized in the next conduction event. Acyl cholinesterase is found in nerve endings, the gray matter of the brain, spleen, lungs and erythrocytes.

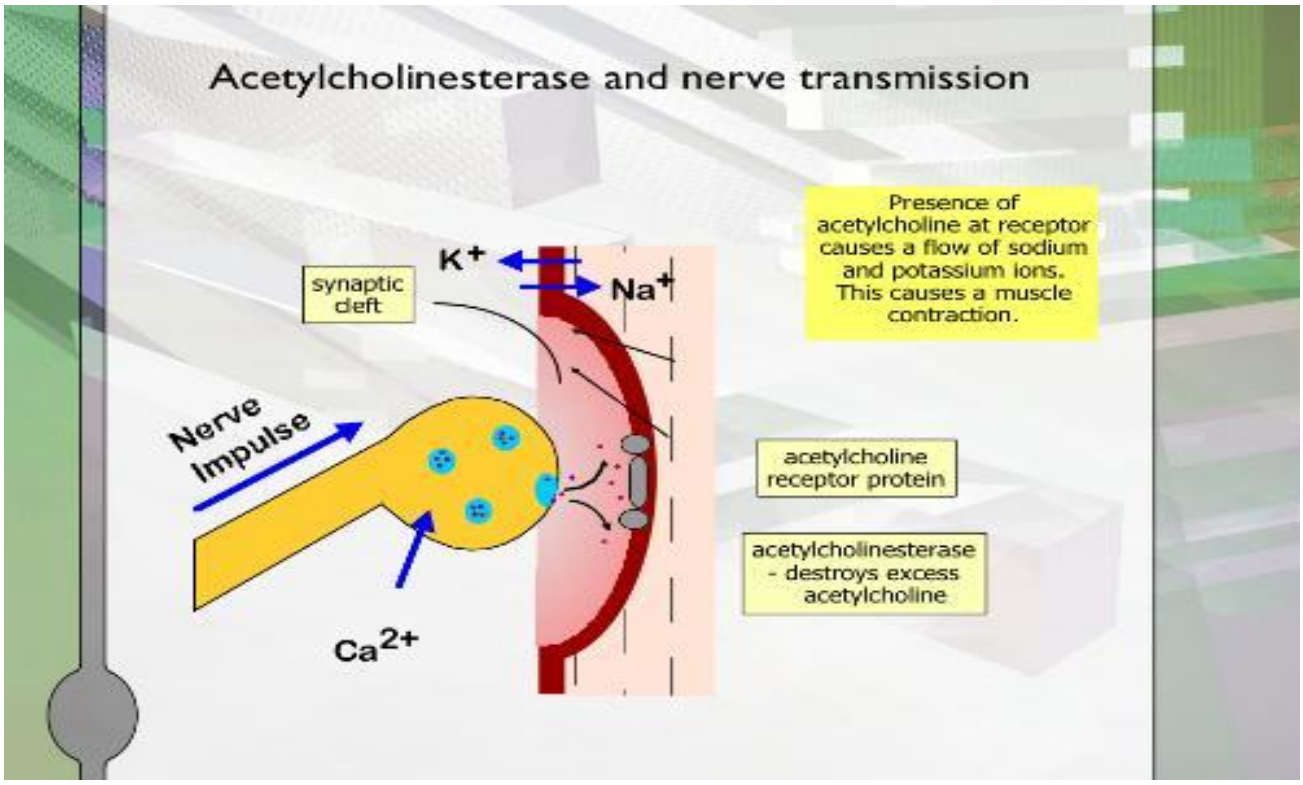
ii) Acyl choline acyl hydrolase: It is also known as pseudo cholinesterase or cholinesterase II or benzoyl cholinesterase. Its biological role is unknown but is found in the white matter of the brain, liver, heart, pancreas and serum. The serum enzyme assay of the pseudo cholinesterase is clinically useful.
Both the enzymes catalyze the same type of reaction but the specificity towards some of the substrate differs.

Acetylcholine bromide Cholinesterase $\longrightarrow$ Choline bromide + Acetate ion + H^+

Both the enzymes are inhibited by the prostigmine and physostigmine in a competitive manner and are irreversibly inhibited by some organic phosphorous compounds such as

Cholinesterase diisopropylfluoro phosphate. Other inhibitors are morphine, quinine, pheno lthiazines, pyrophosphate, bile salts etc.





Clinical Significance

Cholinesterase activity in serum is useful as an indicator of possible insecticide poisoning.

Organic phosphorus compound like parathion, sarin and tetraethyl pyrophosphate which are used as insecticides are inhibitor of cholinesterase activity. Acute organophosphate (OP) poisoning is a significant cause of morbidity and mortality in developing countries including India. Workers in agriculture and in organic chemical industries are exposed to these chemicals and are subject to poisoning by inhalation or by contact.

In India they are freely available in shops and are widely used as insecticides in agriculture and in homes. The anticholinesterase organophosphate compounds - OPCs cause toxicity after their absorption from skin, mucous membranes and respiratory tract

following accidental exposure, or from gastrointestinal tract following suicidal ingestion. They are metabolically subjected to hydrolysis by esterases. Although they bind to and interact with a number of enzymes in the human body, yet, it is their action on the enzyme acetyl cholinesterase (AChE) that is of clinical importance. These compounds bind to the esteratic site on the acetyl cholinesterase molecule phosphorylating the enzyme, leading to inhibition of its normal action. The net result is the accumulation of excess acetylcholine (ACh) at the cholinergic nerve endings all over the body resulting in the characteristic clinical manifestations. Following classical OP poisoning, three well defined clinical phases are seen:

initial acute cholinergic crisis, the intermediate syndrome and delayed polyneuropathy (OPIDN). In addition, OPs on chronic exposure affect several of the physiological systems which include central nervous system, neuromuscular junctions, cardiovascular system, metabolic and endocrine systems including reproduction. These effects have been reported both in humans and animals.

Enzyme has importance to the anaesthetist primarily for its rôle in the metabolism of suxamethonium, although other anaesthetic related drugs that this enzyme metabolises are also increasingly important. Clinical applications are primarily centered on subnormal levels of enzyme activity. The decreased activity levels can be caused by inhibitors, reduced biosynthesis, or dysfunctional genetic variants. Changes in enzyme activity should be related to baseline levels because there is wide individual variation as well as methodological variation. Once baseline levels have been established, cholinesterase activity becomes a sensitive indicator of pesticide intoxication and hepatic biosynthetic capacity. A more sophisticated assay, performed in the presence of an inhibitor, is required to detect the atypical genetic variants of serum or plasma

Plasma cholinesterase decreased in:

- Liver cell damage (hepatitis, cirrhosis, +/-metastatic carcinoma, obstructive jaundice).
- Also decreased in organophosphate (pesticide) poisoning due to inhibition by pesticide.
- Degrades succinylcholine, a muscle relaxant given during general anesthesia in surgery.
- Some people are deficient in plasma cholinesterase (congenital inherited recessive disease), so the normal dose of succinylcholine would kill them
- Therefore, a determination of plasma cholinesterase is made prior to major surgery.

Low Plasma Cholinesterase

Category of cause	**Examples**
Physiological reasons	Infancy, ٣rd Trimester of Physiological reasons pregnancy
Inherited abnormality	Succynilcoline sensitivity (ChE variants)
Acquired abnormality:	
A) Liver disease	Impaired protein synthesis
B) Industrial poisoning	Organophosphorus insecticides
C) Drug effects	Oral contraceptives, MAO inhibitors, Cytotoxic drugs
D) Condition that may have decreased albumin	Malnutrition, infections, etc.

Case study

- A ٣٦-year old man was admitted to a hospital following episodes of nausea, vomiting, and general malaise.
- His urine was darker than usual.
- Upon examination it was discovered that his liver was enlarged and tender to palpation.
- Liver function tests were; plasma ALT was ١٥٠٠ IU/L; AST was ٤٠٠ IU/L.
- During the next ٢٤ hours the man developed jaundice, and his plasma total bilirubin was ٩,٠ mg/dL (٠,٢ – ١ mg/dL).

Biochemical Questions:

١. Discuss this case

٢. What reactions are catalyzed by AST and ALT?

٣. What conditions are important to maintain in performing the enzyme assays?

٤. Which other enzymes might have been elevated in the plasma?

Practice Examination

١. **An enzyme is known to move an amine group from one material to another. It would be referred to as a(n):**

- lyase
- oxioreductase
- transferase
- hydrolase
- movase

٢. **A catalytically inactive protein formed by removal of the cofactor from an active enzyme is called a(n):**

- activator
- apoenzyme
- proenzyme
- preenzyme

٣. **The inactive precursor of an enzyme is called a(n):**

- activator
- apoenzyme
- proenzyme
- preenzyme

٤. **A measure of how many subtrate molecules can be acted on by an enzyme molecule is called:**

- heat of reaction
- turnover rate
- catalytic speed
- activation energy

٥. **Which would you expect to have an effect on activity of an enzyme.**

- substrate concentration
- pH
- temperature
- enzyme concentration
- all of the above

٦. **A zymogen is classified as an inactive form of an enzyme.**

- True
- False

٧. **Which enzyme is useful for detecting pancreatic disorders?**

- amylase
- alkaline phosphatase
- creatine kinase
- lysozyme

٨.The enzymatic model that assumes that enzymes have flexable conformations is called

- lock and key
- induced fit
- active site modification
- noncompetitive inhibition

٩.For a biological process to occur a free energy of activation must be overcome. Enzymes work in this process to:

a. Lower the free energy of activation
b. Raise the free energy of activation
c. Enzymes have no effect on free energy of activation
d. The effect on free energy of activation is dependent on the enzyme in question
e. None of the above

١٠.CK-M and CK-B are examples of what type of enzyme?

a. Homogeneous enzymes
b. Isoenzymes
c. Heterogeneous enzymes
d. Co-factors
e. None of the above

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