ENZYMES

→ Catalysts for biological reactions
→ Most of the enzymes are proteins
→ Activity lost if denatured.
→ Heat ~~labile~~ Labile, water-soluble
→ ~~precipitated~~ Precipitated by protein – precipitating reagents

Naming Enzymes

→ The name of an enzyme identifies the reacting substance. usually ends in -ase.
→ for example sucrase catalyses the hydrolysis of sucrose.
→ The name also describes the function of the enzyme.
→ Some names describe both the substrate and the function
eg: oxidases catalyses oxidation reactions.
eg: alcohol dehydrogenase oxidise ethanol.

IUBMB System of Classification

IUBMB System – 4 digits
- First digit – class
- Second digit – sub-class.
- Third digit – sub-sub class.
- Fourth digit – No. of particular enzyme in the list.

Enzyme Classification – 4 Major Classes

1. Oxidoreductase
- Catalyse oxidation of one substrate with simultaneous reduction of another substrate also co-enzyme.

$AH_2 + B \longrightarrow A + BH_2$

eg: Alcohol dehydrogenase (IUB name = Alcohol – NAD Oxidoreductase)

Alcohol + NAD^+ $\xrightarrow{\text{alcohol dehydrogenase}}$ Aldehyde + NADH + H^+.

- Subclasses includes oxidases, oxygenases, dehydrogenases and reductases.

2. Transferase
- Transfers one group (other than hydrogen) from one substrate to another substrate

$AR + B \longrightarrow A + B-R$.

eg: 1) Hexokinase (ATP-Hexose-6-transferase)

Hexose + ATP $\xrightarrow{HE}$ Hexose-6-phosphate + ADP

2) Transaminases – transfer amino group

3) Hydrolases
- Hydrolysis of the bond (cleave bond and add water).
- Cleave ester, ether, peptide or glycosidic bonds by adding water.

eg: 1) Acetyl choline esterase (Acetyl choline hydrolase)

Acetyl choline + H_2O $\xrightarrow{ACE}$ choline + Acetate

2) Proteases - hydrolyse peptide bonds.

4. Lyases
- Cleave bonds by mechanisms other than hydrolysis.

eg: Aldolase, HMG CoA lyase, ATP Citrate lyase.

Fructose 1,6-BP $\xrightarrow{aldolase}$ glyceraldehyde-3-P + DHAP

5 Isomerase
- Produce optical, geometric or positional isomers of substrate (racemase & epimerase).

eg: Triose phosphate isomerase.

Glyceraldehyde-3-P $\xrightarrow{isomerase}$ DHAP.

6 Ligases (question)
- These enzymes link 2 substrates together, with the simultaneous hydrolysis of ATP

eg: Carboxylase and synthetase (Fatty acyl CoA synthetase)

eg: Acetyl CoA carboxylase

Acetyl CoA + CO_2 + ATP $\xrightarrow{\text{Acetyl CoA carboxylase}}$ Malonyl CoA + ADP + Pi

7. Translocases
- Transfer of molecules across membranes.
- Catalyse the translocation of ions and other molecules across membranes.

eg: There are specific translocases for transferring hydrogen ions, inorganic anions, cations, amino acids.
- Carnitine shuttle system (Carnitine-acyl-translocase)

Enzymes
- Non-protein part → Prosthetic group (coenzyme)
- protein part → Apo-enzyme

Holoenzyme

Co-enzyme
→ Non protein part of the enzyme.

(2)

→ Low mol. wt. organic substance.

→ Most of them are derivatives of vit B complex, heat stable.

→ Able to convert a large no. of substrate molecules with the help of enzyme.

→ Enzyme and coenzyme can be separated by dialysis.

divided into two groups –

1) In reactions catalyzed by oxido reductases.

2) In reactions where groups are transferred other than hydrogen.

1) Reactions catalyzed by oxidoreductases.

eg: NAD - NADH.
NADP - NADPH.
FAD - $FADH_2$.
FMN - $FMNH_2$.

Glyceraldehyde-3-P $\xrightarrow[NAD]{\text{dehydrogenase}}$ 1,3, bisphosphoglycerate + NADH+H+.

Lactate $\xleftarrow[LDH]{NAD}$ Pyruvate + NADH +H+

2) In reactions where groups are transferred other than hydrogen

Examples of co-enzyme.

Co enzyme	Vit.	Rx catalyzed
TPP	Thiamine	oxidative decarboxylation
PLP	Vit. B6	Transfer of amino group
BIOTIN	Biotin	carboxylation
Coenzyme A	Pantothenic acid	Acyl group transfer
Tetrahydro folate	Folic acid	one carbon metabolism.

Iso-enzymes.

→ Multiple molecular forms of the same enzyme synthesized from different tissues are called iso-enzymes.

→ Iso enzymes have different physical properties, but catalyse same chemical reactions.

→ Isoenzymes exhibit subunit structure.

The subunits are all the same type (produced by a single gene) - Homomultimer

The subunits are different (produced by diff gene) - Heteromultimer.

→ Isoenzymes have different electrophoretic mobility

→ Different cofactor requirement

→ Different heat stability.

→ Km value is different for isoenzymes.

→ One of the isoenzyme may be sensitive to one inhibitor eg: tartarate labile ACP (acid phosphatase)

→ different tissue localisation. So, it is useful to understand diseases of different organs.

→ ~~Allozyme~~ Allozymes: The same locus of the gene have diff. alleles. Only one form will be present in one individual.

→ Examples of enzymes which exhibit isoenzyme pattern are:

1. Lactate Dehydrogenase - LDH
2. Creatine Kinase - CK
3. Alkaline Phosphatase - ALP

Lactate dehydrogenase.

→ catalyse

Lactate ⟷ pyruvate

NAD+ ⟵ NAD+H+

Normal serum level of LDH in serum is 100-200 IU/L

- LDH level is more inside the RBC than in plasma, so minor hemolysis will also increase total LDH level.

Isoenzymes of LDH.

5 isoenzymes

LDH-1, LDH-2, LDH-3, LDH-4, LDH-5.

→ LDH is present in different tissues like heart, RBC, brain, liver and skeletal muscle.

→ LDH is a tetramer with 4 subunits (2 types of polypeptide chains).

- H (Heart) polypeptide chain and M (Muscle) polypeptide chain so 5 combinations of H and M chains are possible.

H_4, H_3M_1, H_2M_2, H_1M_3 and M_4

→ when subjected to electrophoresis, LDH-1 moves fastest to anode.

- Followed by LDH-2, LDH-3

LDH-4 and LDH-5.

→ LDH-5 has least mobility

→ The acidic and basic aa in the isoenzyme determines its electrophoretic mobility.

* Separated by cellulose acetate electrophoresis at pH 8.6.

LDH-1 — H_4

LDH-2 — H_3M

LDH-3 — H_2M_2

LDH-4 — HM_3

LDH-5 — M_4.

→ Normally LDH-2 (H_3M_1) conc. in blood is greater than LDH-1 (H_4).

→ But in myocardial infarction LDH 1 conc. is greater than LDH-2.

→ This reversed pattern is called flipped pattern (1/3 mark)

Characteristic features of LDH isoenzyme.

Isoenzyme	Subunit structure	Tissue localisation	Electrophoretic mobility	Activity at 60°C for 20 min
LDH-1	H_4	Heart	fastest	stable
2	H_3M_1	RBC	faster	Stable
3	H_2M_2	Brain	fast	Partially stable
4	H_1M_3	Liver	slow	unstable
5	M_4	skeletal muscle	slowest	Unstable

Isoenzymes of creatine kinase (CK)

- Catalyze the following reaction

Creatine → Creatine phosphate
(ATP, ADP)

→ CK is a dimer with B (Brain) chain and M (muscle) chain

→ 2 chains can combine in 3 different ways.

BB - CK1 Brain isoenzyme
MB - CK-2 Heart
MM - CK-3 Skeletal muscle

→ Normal level in serum
Male = 15 - 100 IU/L
Female = 10 - 80 IU/L

→ Total CK level in serum is increased in muscular dystrophies

→ CK-MB isoenzyme level in serum is elevated in myocardial infarction.

→ It is the earliest enzyme marker in MI

Alkaline phosphatase

- Catalyze the hydrolysis of phosphate esters at alkaline pH (9-10).

→ Zn containing enzyme

→ It is an ectoenzyme

→ Isoenzymes of ALP arise from,
Biliary canaliculi
Liver
Bone
Intestine
Placenta.

→ Normal level

VIVA
Normal level of ALP is
40 - 125 IU/L.

Isoenzymes of ALP

→ Alpha-1 ALP :- synthesized by epithelial cells of biliary canaliculi.

→ Increased in obstructive jaundice

→ Alpha-2-heat labile ALP: produced by hepatic cells

→ This isoenzyme is stable at 56°C but loses its activity when kept at 65°C for 30 minutes

→ Alpha-2 heat labile:
placental origin
→ It will not be destroyed at 66°C
→ found in blood in normal pregnancy
→ Inhibited by phenylalanine
→ Regan isoenzyme (named after the first patient in whom it was detected)
→ An isoenzyme closely resembling the placental form of ALP
→ Seen in circulation in almost 15% cases of carcinoma of lung, liver and gut

1 mark
→ Pre-beta ALP: Bone origin
Elevated levels are seen in liver diseases
→ Gamma ALP: produced from intestinal cells.
→ Level increased in ulcerative colitis
→ Inhibited by phenylalanine
→ Leukocyte ALP: level increased in lymphomas.

Clinical Enzymology

Functional Enzymes:
→ Enzymes which are actively secreted and seen in higher conc in plasma. Level decreases during some disorders
eg: enzymes of blood coagulation

Non functional Enzymes
→ Enzymes which are coming out from cells of various tissues
→ Their normal levels in blood are very low.
→ This level is drastically increased during cell death or diseases.

Cardiac Biomarkers
Markers of MI or Enzyme profile in MI or cardiac diseases.
→ A biomarker helps in detecting any dysfunction of an organ.
→ Cardiac biomarkers are used to detect cardiac diseases
→ Cardiac biomarkers are
• Creatine kinase (CK-MB

- Aspartate amino transferase (AST)
- Lactate dehydrogenase (LDH)
- Cardiac troponins (TnI and TNT)
- Brain Natriuretic peptide (BNP)
- Myoglobin

→ 4 biomarkers for early detection of acute MI are,
- Cardiac Troponins
- CK-MB
- Myoglobin (sensitive, but not specific)

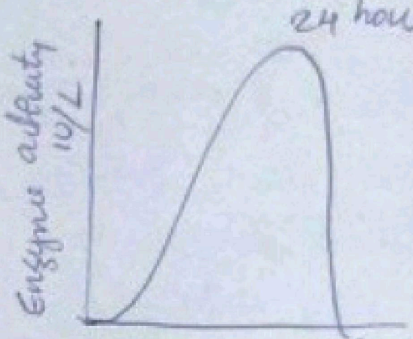
→ Predictors of risk in cardiac diseases include
- The atherogenic lipoproteins
- hsCRP (Inflammatory marker)

Creatine kinase (CK)

→ Large amount of CK is present in heart, skeletal muscle and moderate amount in brain.

(Write Normal level.)

→ CK level increased in muscular dystrophies and myocardial infarction (MI).
→ CK-MB is high in MI
→ CK level starts to rise within 3-6 hours of infarction (first enzyme to rise).
→ Peak level reaches by 24 hour
→ Value returns to normal by 2-4 days.



→ CK has an advantage over CDH. (1 mark)
The CK level is not increased in hemolysis or in congestive cardiac failure

Aspartate amino transferase (AST)

→ Also called SGOT (Serum glutamate Oxaloacetate transaminase)

Aspartic acid + alpha keto glutarate $\xleftrightarrow[AST]{PLP}$ Oxaloacetate + Glutamic acid.

→ Requires Pyridoxal phosphate (PLP) as coenzyme
Normal level in serum = 20-40 IU/L.

2 Isoenzyme forms.

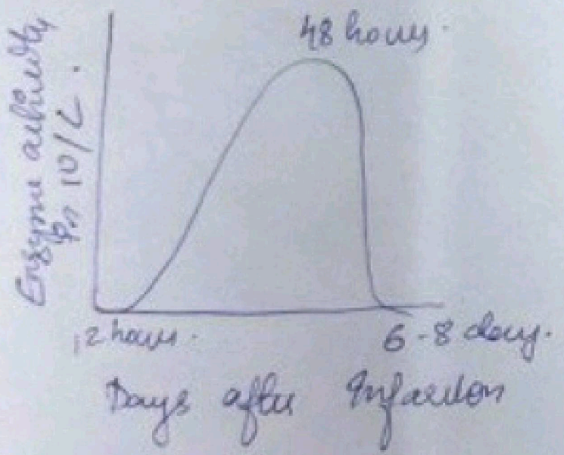
Mitochondrial (elevated in severe cell injury).

Cytoplasmic (elevated in mild cell injury).

→ The level of AST is significantly elevated in MI

→ Rise start at 12 hours after MI.
Peak value reaches by 48 hours.
Value returns to normal by 6 - 8 days.

→ AST level moderately elevated in liver diseases.



Lactate Dehydrogenase (LDH)

Normal level - 100 - 200 IU/L

→ LDH conc. is more inside the RBC than in plasma, so minor amount of hemolysis will give false positive test.

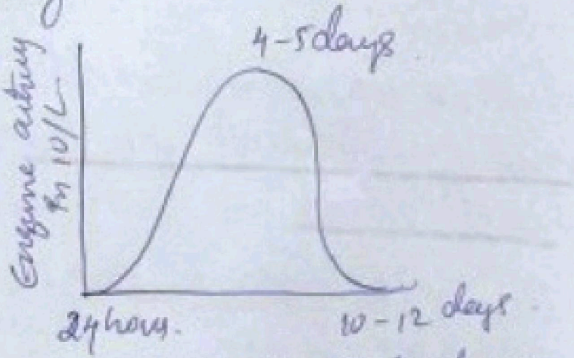
→ Increase in total LDH level is seen in hemolytic anemias hepatocellular damage, muscular dystrophy, carcinomas etc

→ LDH-1 (H_4 isoenzyme) level is increased 5 - 10 times in MI.

→ Rise starts at 24 hours after the onset of coronary artery block.

→ Peak values are observed by 4 - 5 days

→ Value returns to normal by 10 - 12 days



— End of marker ENZYMES —

Troponins

→ Troponins are NOT enzyme.

→ Troponin is a complex of three regulatory proteins that is integral to muscle contraction in skeletal and cardiac muscle but not smooth muscle.

→ Troponin has 3 subunits

TnC, TnT and InI

→ Troponin C binds to calcium ions.

→ Troponin T binds to tropomyosin.

→ Troponin I binds to actomyosin.

→ Trop I is released into blood within 4 hours after the onset of cardiac symptoms.

→ Peak value observed at 14 - 24 hrs and remains elevated for 7-10 days post infarction.

→ It is a useful marker at any time interval after the latest attack.

→ Trop T increases within 6 hours of MI.
Peak value 48 hours and then remains elevated upto 7 - 14 days.

→ Degree of elevation of troponin value can give prognostic information.

Marker	Onset	Peak	Duration	Remarks
Troponin I	4-6 hr.	24-48 hr.	8-14 days.	Preferred marker.
CK MB.	3-6 hr.	48-24 hr.	36-48 hrs.	Useful marker.
Myoglobin.	1-4 hr.	6-7 hr.	24 hrs.	Non specific.
AST.	6-12 hr.	24-48 hr.	6-8 days.	Not used now.
LDH.	24 hrs.	4-5 days.	10-12 days.	

HW draw graphs.

BNP (Brain Natriuretic peptide)

→ Natriuretic peptide family
- ANP, BNP, CNP.

→ ANP - produced in atria.

→ BNP - produced in ventricles, brain.

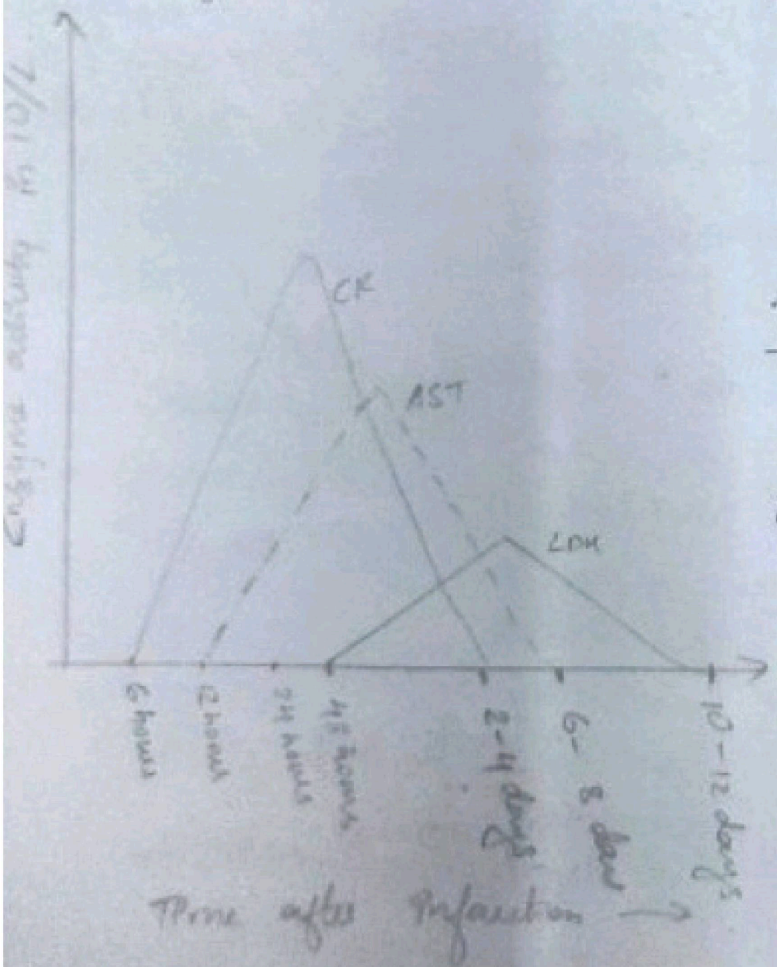
→ high conc. of ANP and BNP seen in congestive cardiac failure.

Other markers of MI

→ Myoglobin

→ IMA (Ischaemia modified albumin)

CK	
rise	3 - 6 hours.
peak	24 hours.
normal	2 - 4 days
AST	
rise	12 hours.
peak	48 hours.
normal	6 - 8 days.
LDH.	
rise	24 hours
peak	4 - 5 days.
Normal	10 - 12 days.
Trop I	
rise	4 hours.
peak.	14 - 24 hours.
Normal	7 - 10 days.
Trop T.	
rise	6 hours.
peak	48 hours.
Normal	7 - 14 days.



Markers of Liver Dysfunction

→ The enzymes used in the assessment of liver disease may be divided into 2 groups.

(a) Those indicating hepato cellular damage and

(b) Those indicating cholestasis (obstruction).

- Enzymes indicating Hepatic cell damage.

  ALT, AST.

- Enzymes indicating cholestasis (obstructive liver disease)

  ALP

  GGT

  NTP.

  (5' Nucleotidase)

Enzymes indicating cholestasis

1. Alkaline phosphatase.

→ catalyse the hydrolysis of phosphate esters at alkaline pH (9–10).

→ Zn containing enzyme.

→ It is an ectoenzyme – associated with membranes of liver, kidney, intestine, bone osteoblasts.

→ Normal serum value.
40 – 125 I.U/L.

Activated by Mg & Mn.

→ Increase in ALP

- Moderately high (hepatic d/s)
  2 – 3 times.
  → infective
  → alcoholic
  → carcinoma

- Very high ALP noted in obstructive jaundice or hepatic carcinoma.

  - Extra hepatic obstn:
    → gallstones
    → Carcinoma head of pancreas
  - Intrahepatic obstn:
    → Viral hepatitis
    → drugs.

- Drastically high ALP. (10 – 25 times of upper limit) noted in bone diseases.
  → paget's
  → rickets
  → Osteoblastoma
  → Metastatic Carcinoma of bone
  → Hyperparathyroidism.

2. Gamma glutamyl transferase (GGT)

→ Normal serum value.
10 – 30 IU/L.

→ clinically important as it is sensitive to detect alcohol abuse.

→ Elevated levels seen in chronic alcoholism.

→ In liver diseases, GGT level increases like that of ALP

→ Very sensitive of biliary tract diseases.

3. Nucleotide Phosphatase (NTP)

→ ~~5' nucleotide ph~~
5' nucleotidase.

→ Marker enzyme of plasma membrane.

→ Nickel inhibits NTP.

→ Ectoenzyme.

→ Normal level – 2–10 IU/L

Enzymes indicating Hepatic Cell Damage

1. Alanine aminotransferase (ALT).

→ Serum glutamate pyruvate transaminase SGPT

→ Normal serum level.
Male 13 – 35 IU/L
Female 10 – 30 IU/L

In liver diseases, ALT > AST

→ Very high ALT (300 – 1000 U/L)
- Acute hepatitis – toxic or viral.

→ Moderately high ALT (50 – 100 U/L)
- Chronic hepatitis, cirrhosis, hepatitis C, NASH (non alcoholic steatohepatitis)

2. AST.
Normal level 20 – 40 IU/L.

→ AST is significantly elevated in liver disease.
primary hepatomas.
Alcoholic hepatitis.

Markers of Prostate Diseases

PSA (1 mark)

ACP (Acid phosphatase)

① Prostate specific Antigen (PSA)

→ Specific for prostate tissue.
Normal value 1 – 5 Mg/L

→ Value above 10 ng/L indicative of prostate cancer.
→ Bound and free forms present.
→ 4 - 10 Ng/L - Benign prostatic hypertophy.
→ >10 Ng/L - prostate cancer.

② Acid phosphatase (ACP).
→ hydrolyzes phosphoric acid ester.
pH 4 - 6 (acidic.
→ Site of secretion: prostate, RBC, WBC, platelets
→ Normal serum value - 2.5 - 12 U/L.
→ Elevated in bone metastasis of prostate cancer.
→ Tumour marker.

→ Blood for ACP should be collected before rectal examination.
As prostate massage may increase the value.

Markers of Pancreatic Disease
→ Amylase
→ Lipase

Amylase
→ Serum amylase 50 - 120 U/L
produced by pancreas, salivary gland
→ Amylase highly increased (1000 times) in acute pancreatitis.
Rises in 5 - 12 hours, lasts for 2 - 4 days.
→ moderately increased in chronic pancreatitis.
Mumps (parotitis)
pancreatic duct obstruction

② Lipase
50 - 175 U/L.
advantage over amylase:
→ elevated for 7 - 14 days.
→ Not increased in mumps (more specific).
→ Highly ↑ lipase (upto 5 times) acute pancreatitis
→ Moderately ↑ lipase.
carcinoma of pancreas.
biliary disease.
peptic ulcer.

Enzyme profile in muscle disease

→ CK-MM.
→ ALD (aldolase) - earliest (specific) enzyme to rise.
→ LDH.
→ AST - not specific.

Enzyme profile in bone dis.

→ Marker of osteoblast activity - ALP
Increased in pagets, rickets, osteoblastoma
→ Marker of osteoclast activity
TRAP
(tartarate resistant acid phosphatase).

Other enzyme patterns in diseases

→ cholinesterase - OP poisoning (organic phosphate)
→ G6PD - drug induced hemolytic anaemia.
→ Ceruloplasmin - Wilsons disease.
→ Neuron specific enolase - neuroendocrine tumours.
→ Enolase / neurospecific enolase
- Glycolytic enzyme.
- NSE is a tumour marker for tumours of neuroendocrine origin (small cell lung cancer, neuroblastoma)

Use of Enzyme

- Therapeutic agents
- Diagnostic agents

Therapeutic use of Enzymes

1) Streptokinase (streptococci) } lyse intravascular clots in MI
2) Urokinase (from urine) }
3) Asparaginase - anti cancer drug.
4) Pepsin } - given to patients with defective digestion.
5) Pancreatin (trypsin, lipase) }
6) Streptodornase (DNAse) - applied locally.
7) Papain - anti inflammatory
8) Alpha-1- antitrypsin - AAT deficiency / emphysema

Enzymes as Diagnostic agents

→ Enzymes are useful in clinical lab to estimate blood analytes
1) Glucose oxidase - glucose.
2) Urease - urea
3) Uricase - uric acid
4) Hexokinase - glucose.

(15)

5) Cholesterol oxidase - cholesterol.
6) Lipase - Triglyceride
7) Peroxidase - glucose, cholesterol
8) Horse radish peroxidase } ELISA
Alkaline Phosphatase
9) Restriction endonuclease - RFLP, Southern blotting.
10) Reverse transcriptase - PCR (RT-PCR)

- A 52 year old male is a heavy smoker and is brought to the casuality with history of chest pain of 3 hours associated with profuse sweating. Investigations showed the following results.

LDH - normal
SGOT - normal
SGPT - normal
CKMB - Elevated
What is the probable diagnosis?

Ans: Myocardial infarction.

- A 60 year old female patient is brought to the casuality with history of profuse sweating and chest discomfort. Investigations showed the following result.

LDL - 240mg/dl
FBS - 210mg/dl
SGPT - 200 IU/L
Trop I - Elevated
CK-MB - elevated.

→ what is the probable diagnosis?
→ what advice would you give to this patient on discharge?

Ans: MI.
Diet control, Exercise.
(diabetic, cholesterol ↑).

SUBSTRATE
→ Substance upon which an enzyme acts is called substrate

Enzyme Specificity (4 or 8 mark)

→ Enzymes have varying degrees of specificity for substrate
Enzymes may recognize and catalyse
- a single substrate
- a group of similar substrate
- a particular type of bond.

1. Absolute Specificity

→ Catalyse one type of reaction for single substrate
→ Urea is the only substrate for urease
→ Glucose oxidase will oxidize only beta-D-glucose.

2. Bond Specificity

→ Catalyse one type of reaction for a specific type of bond.
→ Most of the proteolytic enzyme are showing bond specificity.
→ Trypsin can hydrolyse peptide bonds formed by carboxyl groups of arginine or lysine residues in any proteins.

3. Group Specificity

One enzyme can catalyse the same reaction on a group of structurally similar compounds.
→ Hexokinase can catalyse phosphorylation of glucose, galactose and mannose.

4. Stereospecificity

→ Human enzymes are specific for L amino acids and D carbohydrates.

→ Lactate dehydrogenase acts on pyruvate will form only L lactate, but no the D variety.

Absolute → catalyse one type of rxn.
eg: urease

Enzyme units:

→ One standard unit or international unit of enzyme activity is the amount of enzyme that will convert one micromole of substrate per minute per litre of sample
10/L.

Active site / Active centre

→ The region of the enzyme where substrate binding and catalysis occurs is referred to as active site or active centre of enzyme.
→ Active site occupies only small portion of the whole enzyme
→ Substrate binds to enzyme at active site by non-covalent bonds.
→ The binding of substrate to active site depends on the alignment of specific groups

(17)

a atoms at the active site.

→ Amino acids or groups that directly participate in making or breaking bonds are called catalytic residue or catalytic groups.

→ Active site contains substrate binding site and catalytic site sometimes these two may be different.

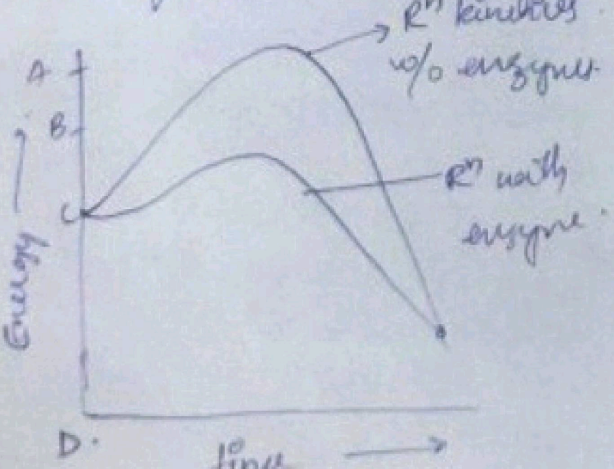
Mode of Enzymes.

Theories of Enzyme Actions (Qn)

→ Lowering of Activation Energy

Activation energy – the energy required to convert all molecules of a reacting substance from the ground state to the transition state.

→ Enzyme reduce the magnitude of activation energy and thereare the rate of reaction.



Michaelis - Menten theory

Enzyme - substrate complex theory.

$E + S \rightleftharpoons E\text{-}S \text{ complex} \rightarrow E + P$

eg: Alkaline phosphatase hydrolyses phosphate esters

E-serine-OH + Glucose-6-P ← E-S

E-serine-O-P + Glucose ← E-S complex

E-serine-OH + Pi ← E + P.

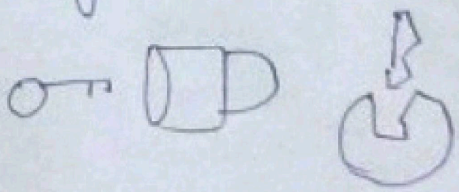
Fischer's Template theory

(Lock and key model)

→ three dimensional structure of active site of enzyme is complementary to substrate.

→ Enzyme and substrate fit each other similar to lock and key.

(a)



Koshland's Induced fit theory

→ The enzyme is flexible.

→ Substrate binds to specific part of the enzyme.

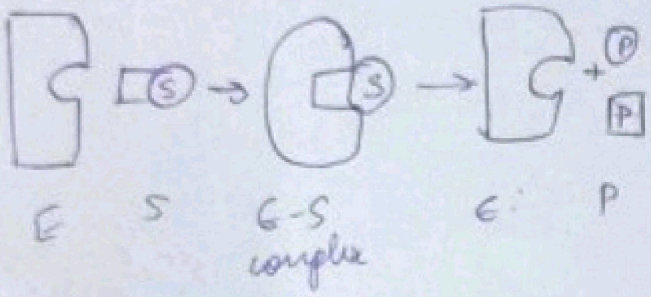
→ This leads to more secondary bonding and conformational change of active site of enzyme

18

OR

→ The substrate induces conformational change in the enzyme

→ It is the basis of allosteric regulation.



Other mechanisms

→ Substrate strain theory

→ Acid Base catalysis

→ covalent catalysis

→ Entropy effects

Substrate Strain Theory

→ Binding of substrate to the active site of the enzyme can induce a strain in the 'S'

→ The strain induction increases the energy level of the substrate - attains transition state and easily converted to products.

Acid Base Catalysis.

→ Here the protonated form an amino acid at the active site act as an acid, which interact with its conjugated base (non-protonated form) - product is released.

→ At physiological pH, the protonated form of the AA histidine function as an acid and its corresponding conjugate as base.
eg: action of Ribonuclease.

Covalent Catalysis

→ Here, the negatively charge (nucleophile) or +vely charged (electrophile) group present in the active site attack the substrate and result in covalent binding of 'S' to 'E'.

→ The action of Chymotrypsin - a ~~obs~~ Serine protease is a combination of acid base and covalent catalysis

Entropy Effects

→ Entropy is defined as the extent of disorder in a system

→ Enzyme enhances reaction rates by decreasing entropy

Factors Affecting Enzyme Activity

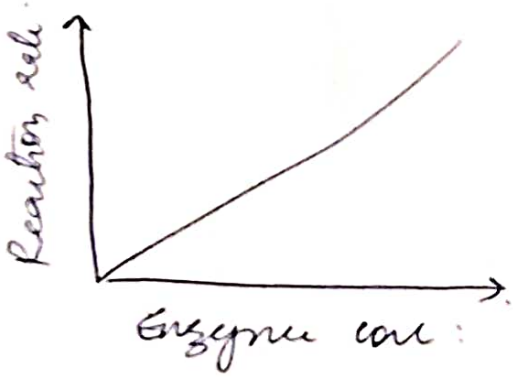
- → Enzyme concentration
- → Substrate concentration
- → Temperature
- → pH (H^+ concentration)
- → product accumulation
- → Time
- → presence of activators
- → presence of inhibitors

→ The rate or velocity of a reaction (V) is the no of substrate molecules converted to product per unit time

① Enzyme concentration.

→ Rate of a reaction or velocity (V) is directly proportional to the enzyme concentration, when sufficient substrate is present.

→ This property is made use in determining the level of particular enzyme in plasma, serum or tissues.

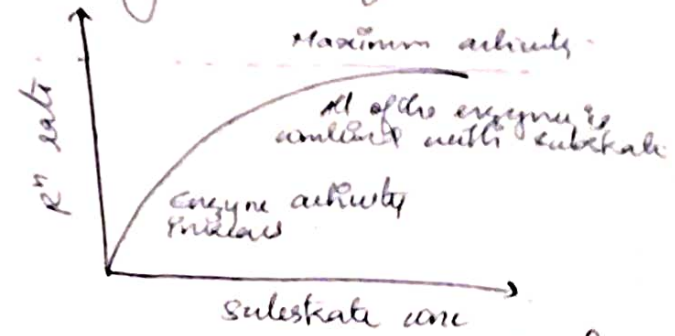


Effect of substrate concentration

As substrate conc. is increased, velocity increases until it reaches a maximum value, Vmax.

- Vmax is the maximum velocity obtained in the presence of excess substrate.

→ The leveling of reaction rate at high (S) conc reflects the saturation of all binding sites of 'E' with 'S'



* The plot of initial velocity (Vo) against substrate conc. is hyperbolic.

→ Michaelis Menton equation describes how reaction velocity varies with 'S' concentration

$$V = \frac{V_{max}(S)}{K_m + (S)}$$

→ when conc. of [S] is made equal to Km (michaelis constant)

$$V = \frac{1}{2} V_{max}$$

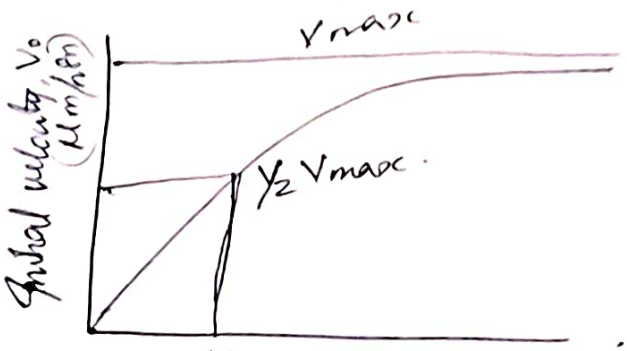
Velocity at which half the no. of enzyme is saturated with the substrate – ½ Vmax

Q/ What is Km?

- **Km is the substrate conc. at half maximal velocity**

→ It denotes that half of the enzyme molecule (ES) are bound with the substrate at that particular substrate concentration.

→ Km is expressed in moles/L



→ Km value of most of the enzymes are within the range 10^{-5} to 10^{-2} moles/L.

→ Km is the signature of the enzyme.

OR.

It is constant for an enzyme and is the characteristic feature of a particular enzyme for a specific substrate

Q/ Significance of Km.

(i) Km denotes the affinity of enzyme for substrate.
- lower the Km, higher the affinity and vice versa.

(ii) Km help to identify the natural substrate of an enzyme.

(iii) Km is the evaluation of enzyme inhibition.

(iv) For enzyme estimation in clinical laboratories Km of the enzyme gives an idea of the amount of S to be added to the reaction medium.

(v) Km is independent of the enzyme concentration.
If enzyme concentration is doubled, the Vmax will be double. But the Km will remain exactly the same.