INTRODUCTION

➢ **ENZYME :-**

Enzymes are biological catalytic increases the velocity of reaction , inactive at o°c and destroyed by moist heat at 100°c.

➢ Enzymes are present in virtually all organs but with slightly different forms in different locations.

➢ Enzymes can also acts as reagents for various Bio-chemical estimations and detections.

ISO-ENZYMES

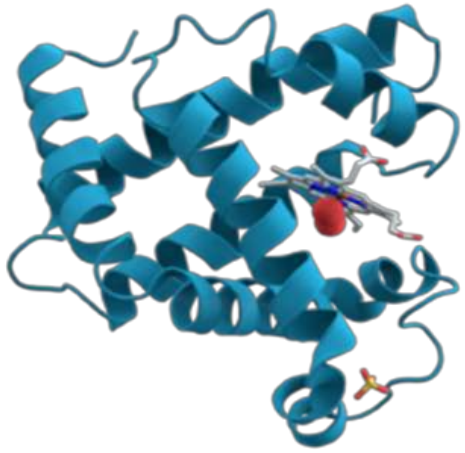
- Iso-enzymes (or) isozymes are multiple forms(isomers) of the same enzyme that catalyze the same biochemical reaction.
- Iso-enzymes show different chemical and physical properties like:

  Electrophoretic mobility.

  kinetic properties.

  Amino acid sequence.

  Amino acid compositin.



- All iso-enzymes are enzymes but all enzymes are not iso-enzymes.

  E.g.:- LDH, Creatine kinase.

CLASSIFICATION:-

enzymes are divided into two types.

they are :

* **Functional plasma enzymes.**

* **Non-functional plasma enzymes.**

1)Functional plasma enzymes (or) Plasma derived enzymes

- Certain enzymes, proenzymes, and their substrates are present at all times in the circulation of normal individuals and perform a physiologic function in the blood

Examples of these **functional plasma enzymes**

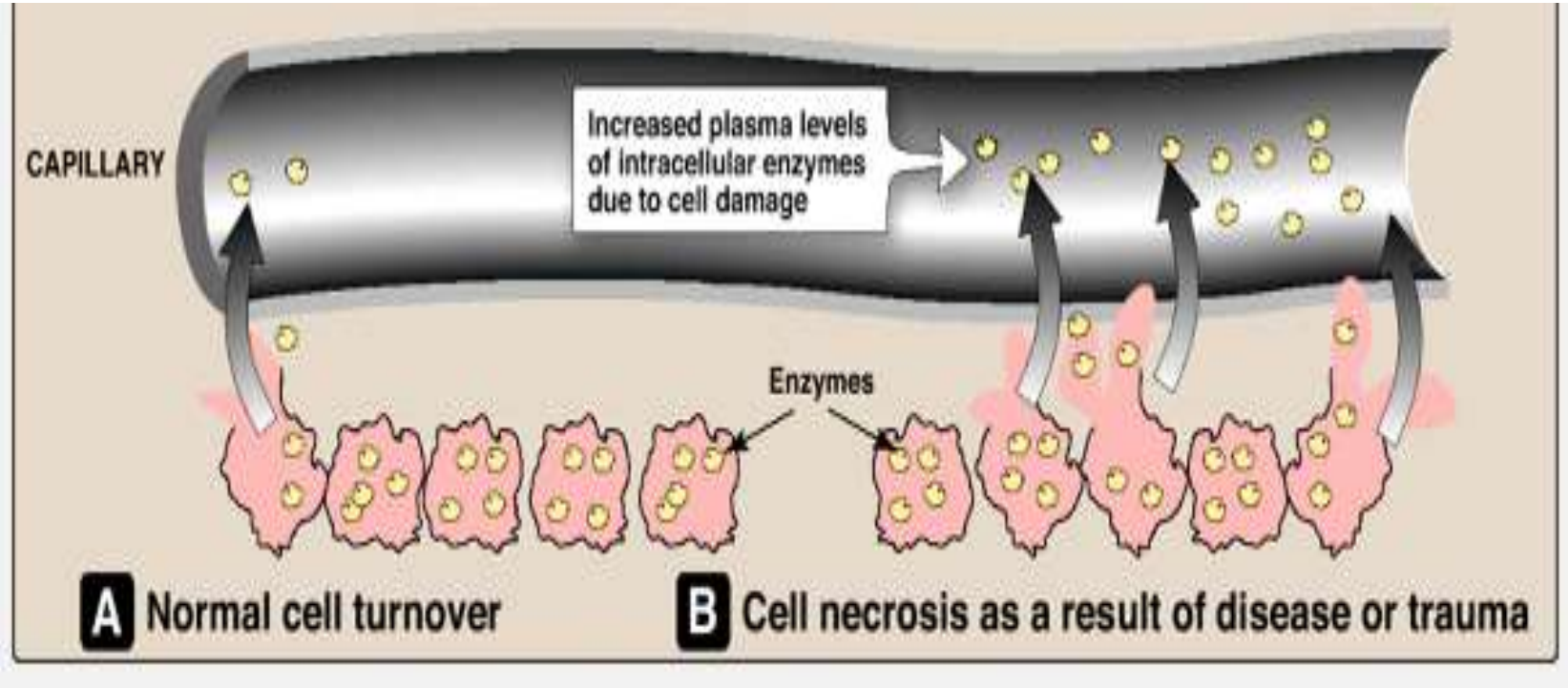
- Lipoprotein lipase
- Pseudo cholinesterase
- Widely secreted from liver .

Nonfunctional plasma enzymes (Cell derived enzymes) :-

- Plasma also contains numerous other enzymes that perform no known physiologic function in blood.
- These apparently **nonfunctional plasma enzymes** arise from the routine normal destruction of erythrocytes, leukocytes, and other cells.

Diagnostic Enzymes :-

Tissue damage or necrosis resulting from injury or disease is generally accompanied by increases in the levels of several nonfunctional plasma enzymes.



Units of serum enzyme activity:-

International unit:-

One IU is defined as the activity of the enzyme which transforms one micro mole of substrate in to products per minute per liter of sample under optimal conditions and at defined temperature .

- It is expressed as IU/L.

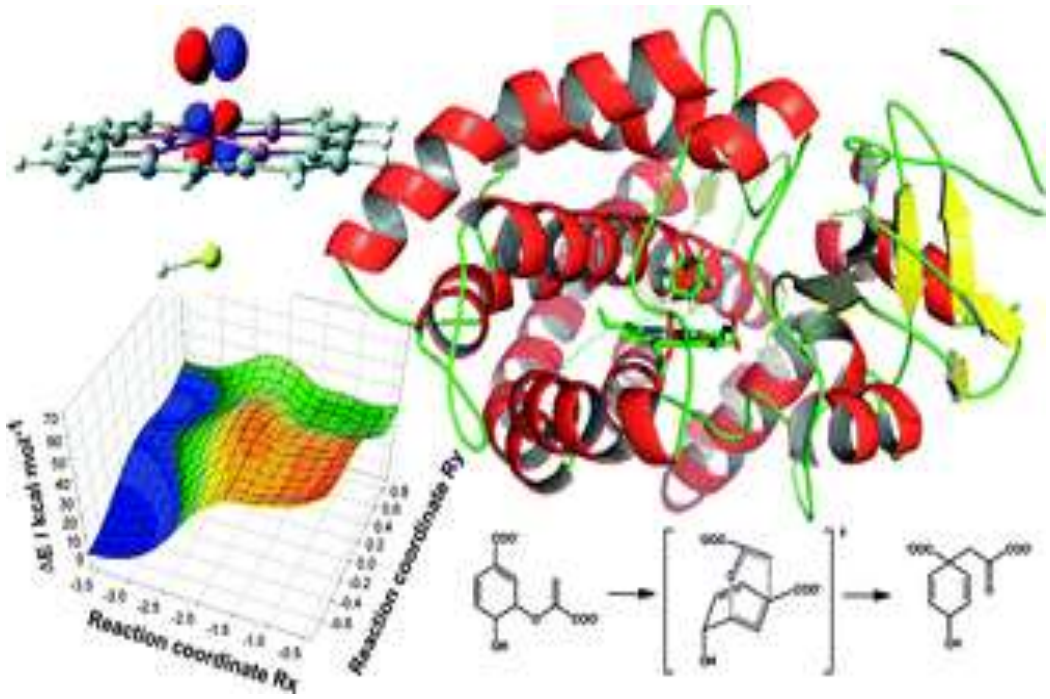
Units of serum enzyme activity:-

2-Katal – catalytic unit – One Katal is defined as thenumber of mole of substrate transformed per secondper litre of sample. It is abbreviated as kat or k.60 U=mkat and 1nkat=0.06U

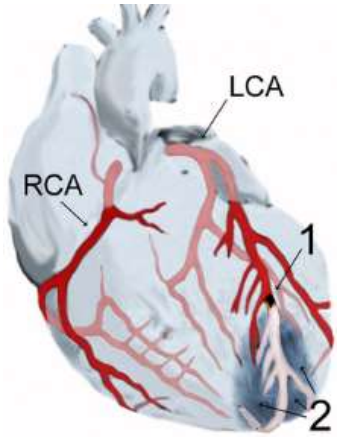
Diagnostic Enzymes in different diseases:-

Enzyme estimations are helpful in the diagnosis of –

1) Myocardial Infarction
2) Liver diseases
3) Bone disease
4) Cancers
5) GI Tract diseases



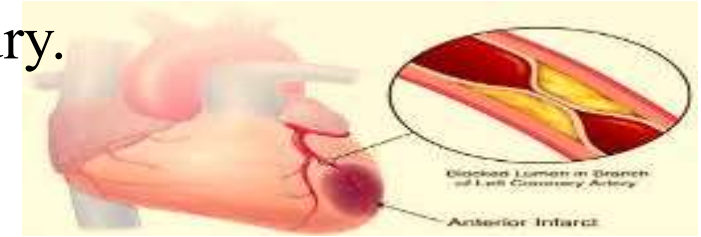
Myocardial infarction :



- commonly known as a heart attack,
- occurs when blood flow stops to a part of the heart causing damage to the heart muscle
- . The most common symptom is chest pain or discomfort which may travel into the shoulder, arm, back, neck, or jaw.
- Often it is in the center or left side of the chest and lasts for more than a few minute

Diagnosis of Acute Myocardial Infarction (AMI):-

- The diagnosis of AMI is usually predicated on the WHO criteria of chest pain, ECG changes, and increases in biochemical markers of myocardial injury.



- Half of the patients with "typical" symptoms do not have AMI.

- In contrast, biochemical markers have excellent sensitivity diagnosing AMI.

Serum enzymes in Acute Myocardial Infarction:-

Enzyme assays routinely carried out for the diagnosis of Acute Myocardial Infarction are**:-**

- **Creatine Phosphokinase**
- **Aspartate transaminase**
- **Lactate dehydrogenase**
- **Troponins**
- **Myoglobin**

1) Creatine Kinase (CK/ CPK) :-

- Creatine + ATP $\rightleftharpoons$ phosphocreatine + ADP

  (Phosphocreatine – serves as energy reserve during muscle contraction)

- It is an enzyme found primarily in the heart and skeletal muscles, and to a lesser extent in the brain but not found at all in liver and kidney
- Catalyzes the transfer of phosphate between creatine and ATP/ADP
- Provides rapid regeneration of ATP when ATP is low

Creatine Kinase (CK/ CPK) :-

- Normal range for total CK: Male : 46-171 U/L= 0.78-2.90 μkat/L
- Female: 34-145 U/L= 0.58-2.47 μkat/L

Creatine Kinase (CK/ CPK) Isoenzymes:-

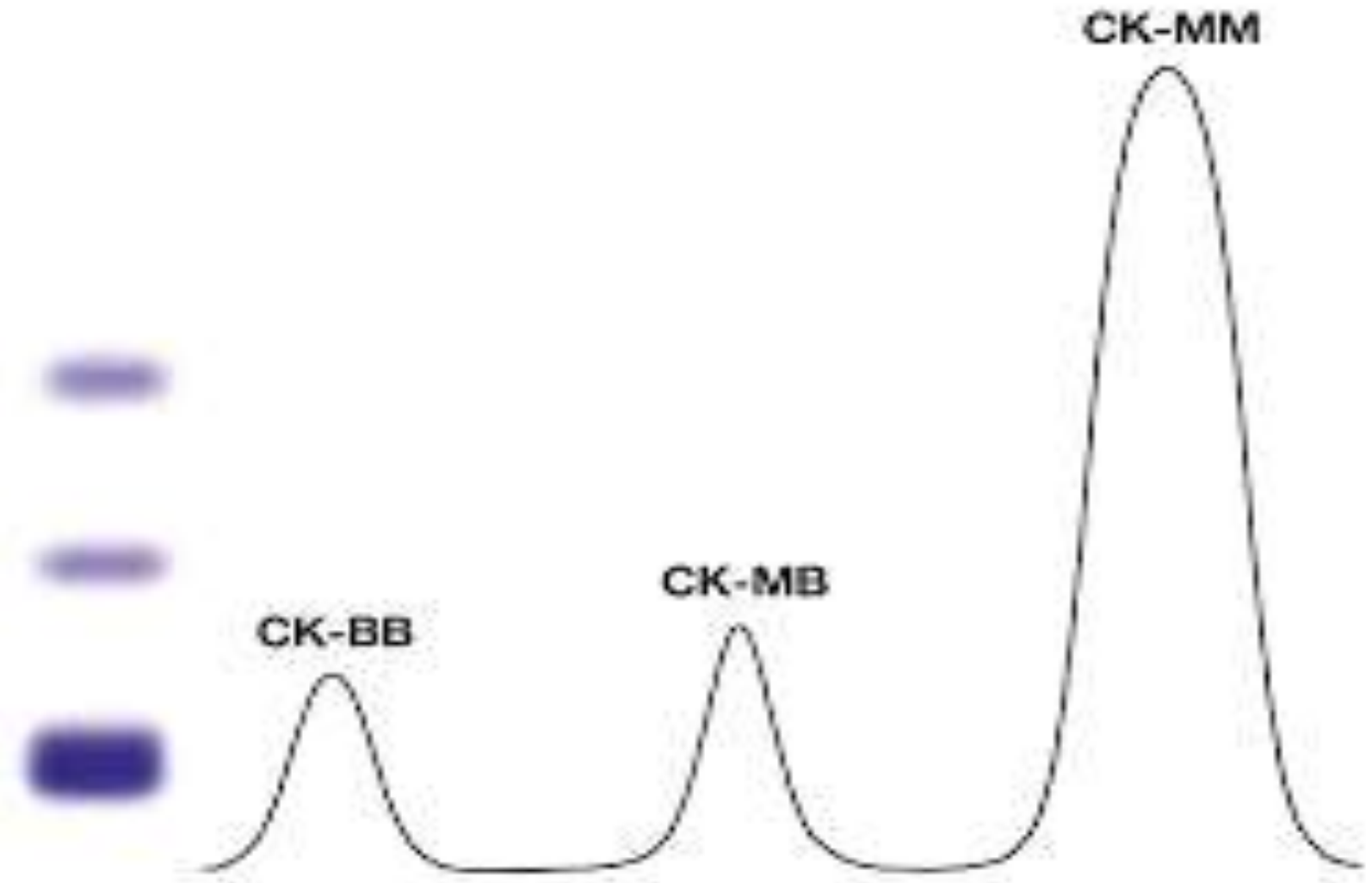
There are three Isoenzymes.

- Measuring them is of value in the presence of elevated levels of CK or CPK to determine the source of the elevation.
- Each iso enzyme is a dimer composed of two promoters ‘M’ (for muscles) and ‘B’(for Brain).
- These isoenzymes can be separated by Electrophoresis or by Ion exchange Chromatography.

Creatine kinase (CK)

- Three main CK isoenzymes with two polypeptide chains B or M

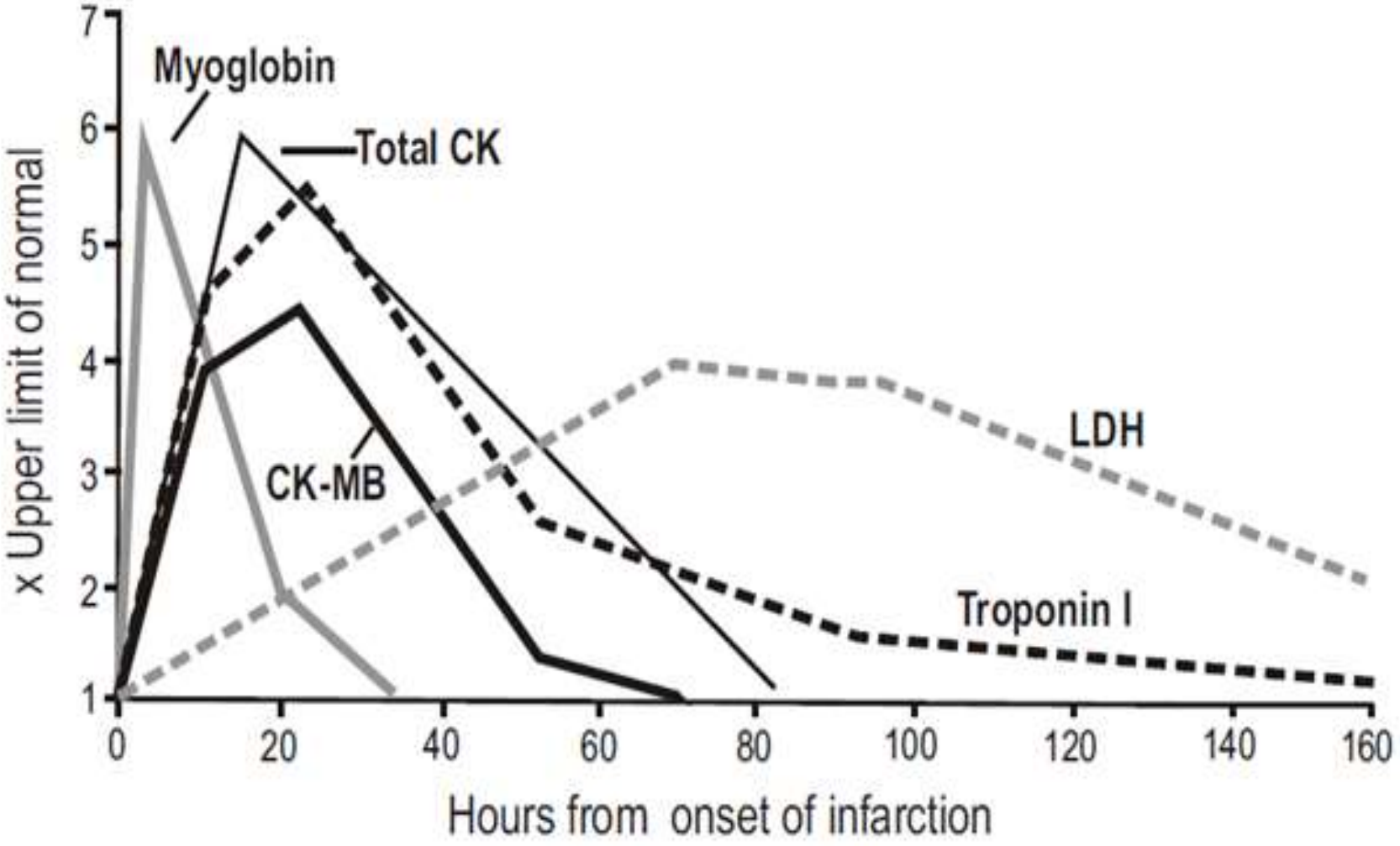
Type	Composition	Comment
Skeletal Muscle	98% CK-MM 2% CK-MB	Elevated in muscle disease
Cardiac muscle	70-80% CK-MM 20-30% CK-MB	Cardiac muscle has highest amount of CK-MB
Brain	CK-BB	
Plasma	Mainly CK-MM	

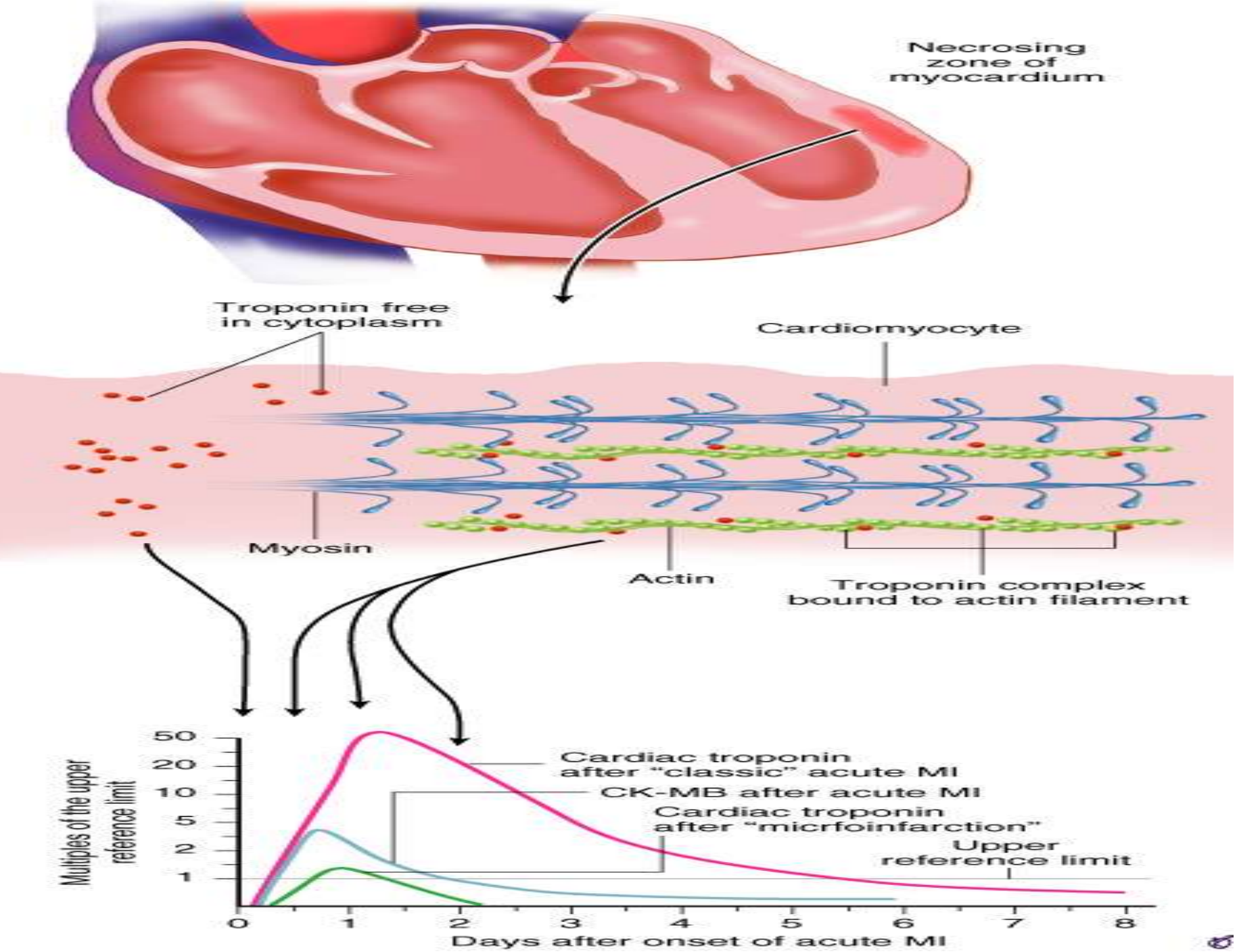

CK-MM
CK-MB
CK-BB

Creatine Kinase diagnosis

- After myocardial infarction- serum value is found to increase within 3-6 hours, reaches a peak level in 24- 30 hours and returns to normal level in 2-4days (usually in 72 hours). Normal Value- serum activity varies from 10-50IU/L at 30°C. CK is a sensitive indicator in the early stages of myocardial ischemia. No increase in activity is found in heart failure and coronary insufficiency. In acute MI, CPK usually rises faster than SGOT and returns to normal faster than the SGOT

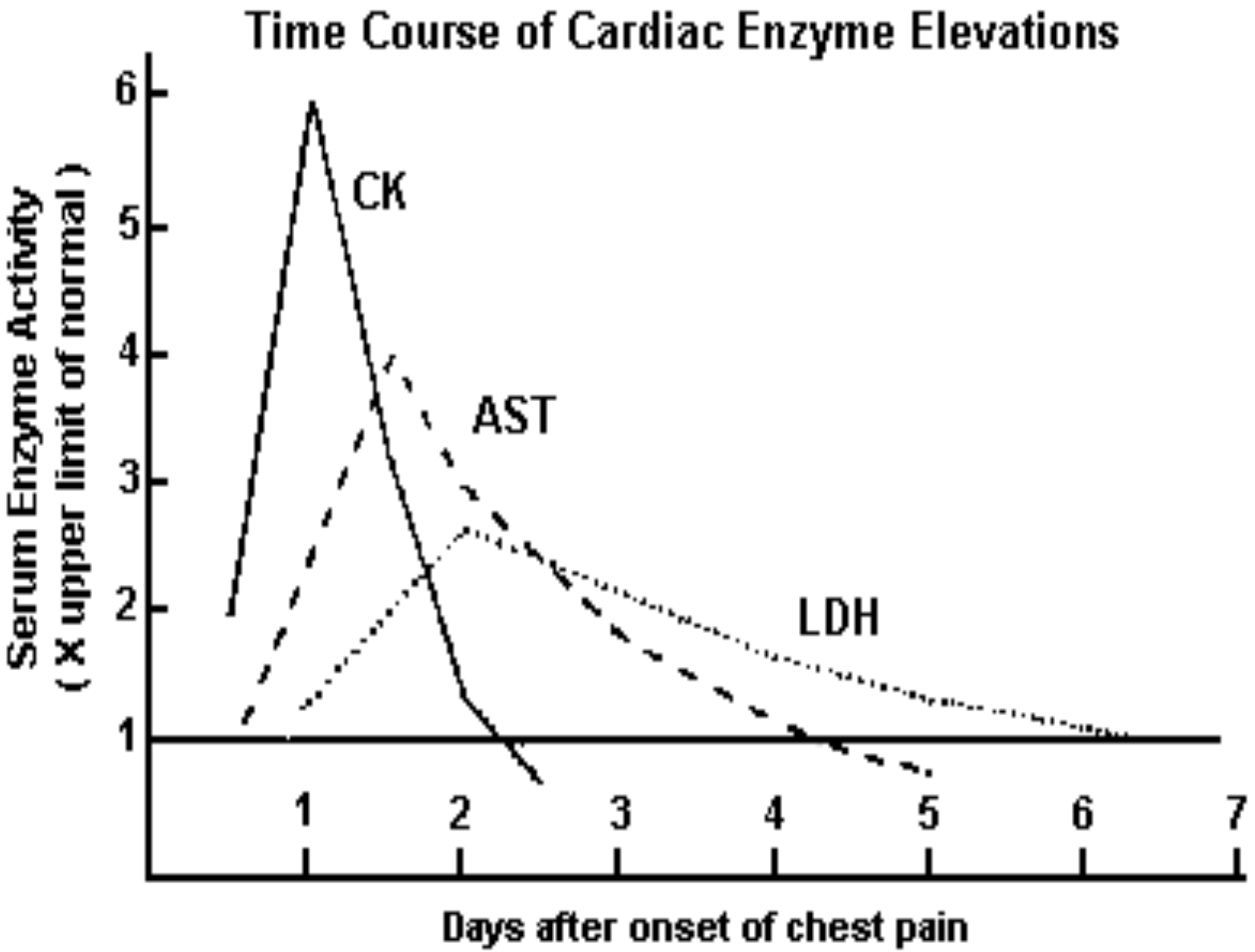
Figure 1: Cardiac Marker Pattern Associated with Myocardial Infarction (5)





2) Aspartate amino Transferase (AST):-

- It is also called as Serum Glutamate Oxalo acetate Transaminase (SGOT).
- The level is significantly elevated in Acute MI.
- Normal Value:- 0-41 IU/L at 37°C
- In acute MI- Serum activity rises sharply within the first 12 hours, with a peak level at 24 hours or over and returns to normal within 3-5 days.
- The rise depends on the extent of infarction.


Time Course of Cardiac Enzyme Elevations
Serum Enzyme Activity
(X upper limit of normal)
6
5
4
3
2
1
CK
AST
LDH
1
2
3
4
5
6
7
Days after onset of chest pain

Prognostic significance-

- Levels> 350 IU/L are due to massive infarction (Fatal),
- > 150 IU/L are associated with high mortality and levels,
- < 50 IU/L are associated with low mortality.

Other diseases-

- The rise in activity is also observed in muscle and hepatic diseases.

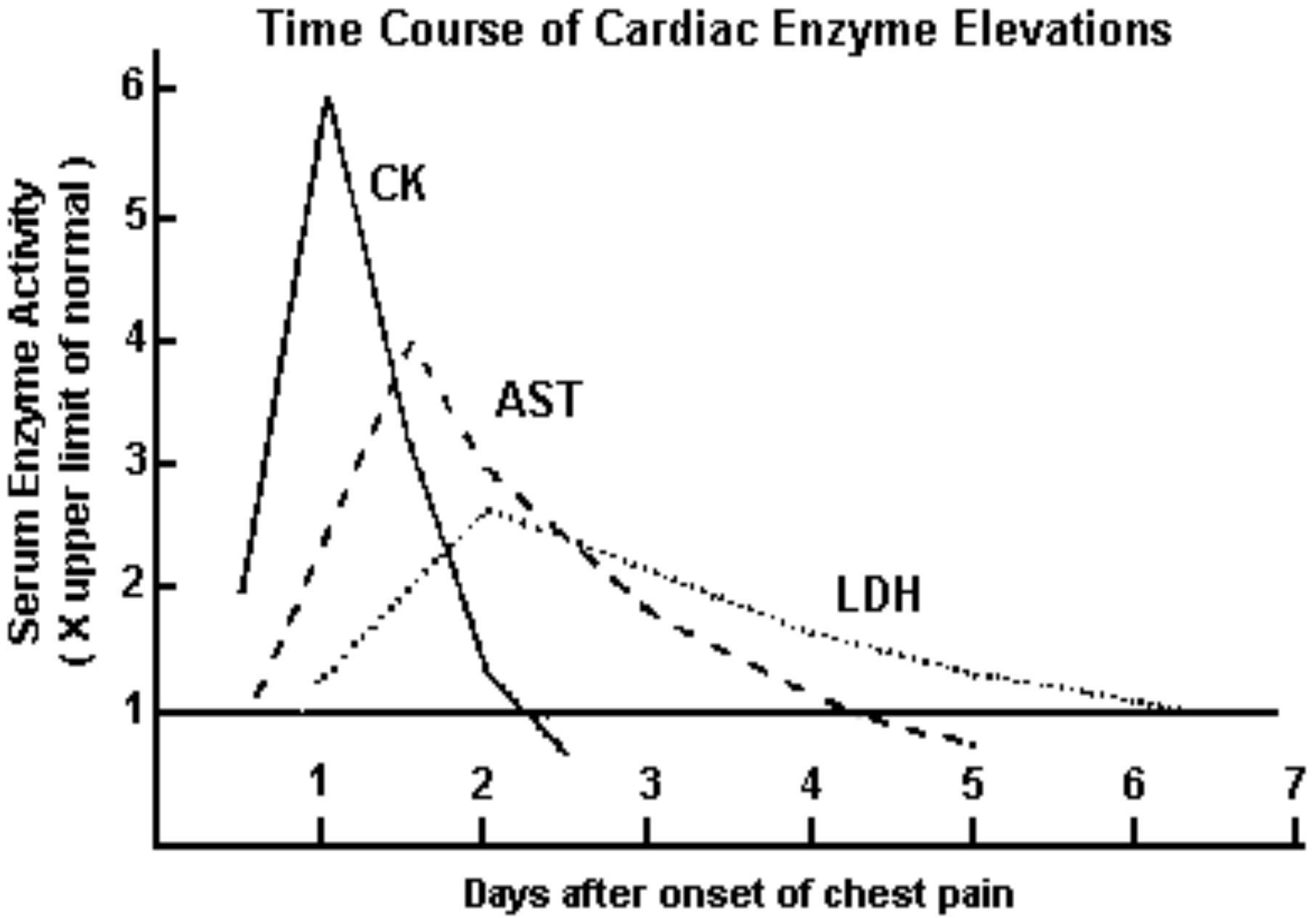
These can be well differentiated from simultaneous estimations of other enzyme activities like SGPT etc, which do not show and rise in activity in Acute MI.

3) Lactate dehydrogenase (LDH):-

- Lactate dehydrogenase catalyzes the reversible conversion of pyruvate and lactate.
- **Normal level :-** 55-140 IU/L at 30°C.
- The levels in the upper range are generally seen in children.
- LDH level is 100 times more inside the RBCs than in plasma, and therefore minor amount of hemolysis results in false positive result.

In Acute MI:-

- The serum activity rises within 12 to 24 hours, attains a peak at 48 hours (2 to 4 days) reaching about 1000 IU/L and then returns gradually to normal from 8th to 14th day.
- The magnitude of rise is proportional to the extent of myocardial infarction.
- Serum LDH elevation may persist for more than a week after CPK and SGOT levels have returned to normal levels.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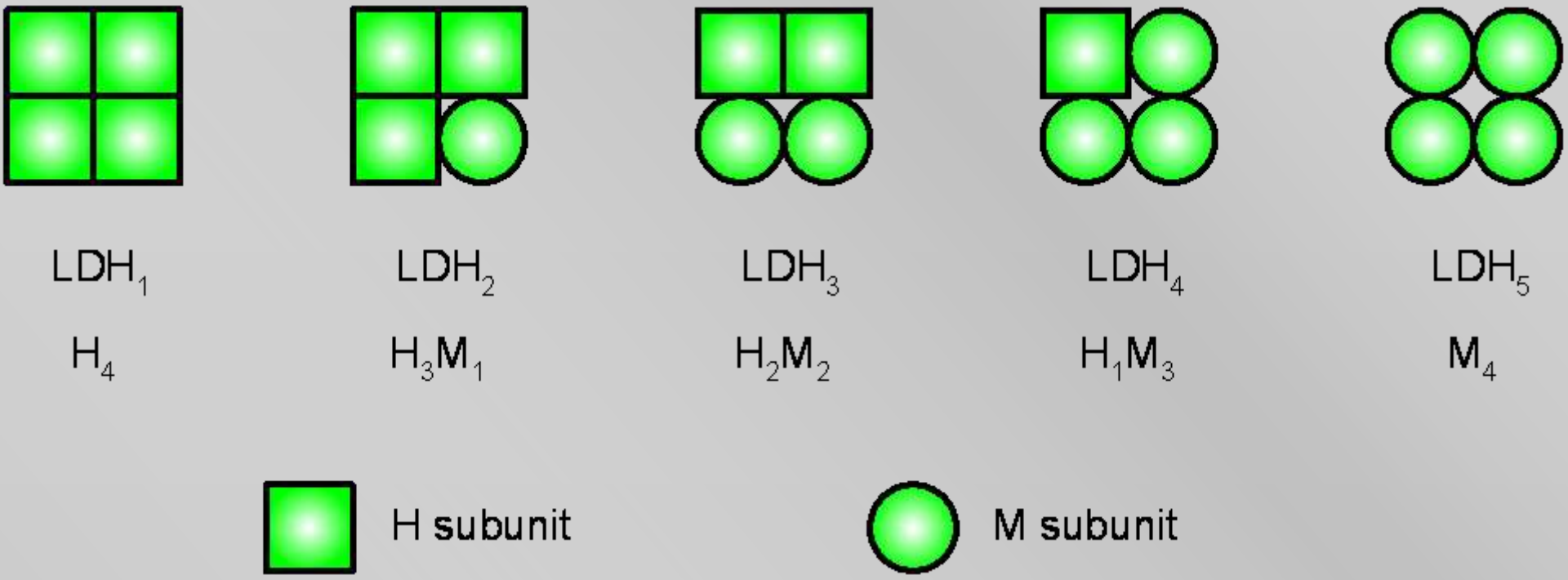
Days after onset of chest pain

Other diseases :-

- Hemolytic anemia's,
- Hepatocellular damage,
- Carcinoma,
- Leukemia's.

Isoenzymes of LDH:-

- LDH enzyme is tetramer with 4 subunits.
- The subunit may be either H(Heart) or M(Muscle) polypeptide chains.
- These two chains are the product of 2 different genes.
- Although both of them have the same molecular weight, there are minor amino acid variations.
- There can be 5 possible combinations; H4, H3M1, H2M2, H1M3. M4, these are 5 different types of isoenzymes seen in all individuals.


LDH1
H4
LDH2
H3M1
LDH3
H2M2
LDH4
H1M3
LDH5
M4
H subunit
M subunit

Liver diseases:-

Serum enzyme tests can be grouped into two categories:

- Enzymes whose elevation in serum reflects damage to hepatocytes

- Enzymes whose elevation in serum reflects cholestasis.

Enzymes that Reflect Damage to Hepatocytes:-

The **Aminotransferases** (transaminases) are sensitive indicators of liver cell injury and are most helpful in recognizing acute hepatocellular diseases such as hepatitis.

These include-

1) Aspartate aminotransferase (AST),
2) Alanine aminotransferase (ALT).

Amino Transferases :-

- **AST** is found in the liver, cardiac muscle, skeletal muscle, kidneys, brain, pancreas, lungs, leukocytes, and erythrocytes in decreasing order of concentration.
- **Normal level :- 0-41 IU/L.**
- The Aminotransferases are normally present in the serum in low concentrations. These enzymes are released into the blood in greater amounts when there is damage to the liver cell membrane resulting in increased permeability.

Diagnostic significance of Aminotransferases:-

- Levels of up to 300 U/L are nonspecific and may be found in any type of liver disorder.
- Striking elevations i.e., aminotransferases > 1000 IU/L occur almost exclusively in disorders associated with extensive hepatocellular injury such as

  viral hepatitis,

  Ischemic liver injury (prolonged hypotension),
- In most acute hepatocellular disorders, the ALT is higher than or equal to the AST.

Enzymes that reflect Cholestasis:-

The activities of three enzymes—

1)Alkaline phosphatase,

2) 5'-nucleotidase,

3) γ-Glutamyl transpeptidase (GGT).

- Alkaline phosphatase and 5'-nucleotidase are found in or near the bile canalicular membrane of hepatocytes, while GGT is located in the endoplasmic reticulum and in bile duct epithelial cells.

2) γ- Glutamyl transferase (γ GT):-

- It is involved in **amino acid transport** across the membranes.
- Found mainly in **biliary ducts of the liver**, kidney and pancreas.
- Enzyme activity is induced by a **number of drugs** and in particular **alcohol.**
- γ-GT increased in liver diseases especially in obstructive jaundice.
- γ-GT levels are used as a marker of alcohol induced liver disease and in liver cirrhosis.

Bone diseases:-

1) **Alkaline Phosphatase:-**Rises in Rickets, osteomalacia, hyperparathyroidism and in Paget's disease. Also rises in primary and secondary malignancies of bones.

2) **Acid Phosphatase:-**Highly increased in bony metastasis of carcinoma prostate

GI tract diseases:-

Amylase:-

- Serum activity > 1000 units is seen within 24 hours in acute Pancreatitis, values are diagnostic.
- A raised serum activity is also seen in perforated peptic ulcer and intestinal obstruction.

Lipase:-

Levels as high as 2800 IU/L are seen in acute pancreatitis. Also reported high in perforated duodenal and peptic ulcers and intestinal obstruction.

Enzymes in diagnostic use

Serum Enzymes	Location of serum enzymes	Concentration increased in	Concentration decreased in
Lipase	Pancreas	Acute pancreatitis, Pancreatic carcinoma	Liver disease, vit-A deficiency, diabetes mellitus
Amylase	Saliva	High intestinal obstruction, Acute pancreatitis, Parotitis, Diabetes	Liver disease
Trypsin	Stomach	Acute disease of pancreas	-
Cholinesterase		Nephrotic syndrome	Liver disease, Malnutrition
Alkaline phosphatase	Bone, liver	Rickets, Jaundice, Metastatic carcinoma, kidney disease	-
Acid phosphatase	Prostrate	Metastatic prostatic carcinoma	-

Serum Enzymes	Location of serum enzymes	Concentration increased in	Concentration decreased in
Lactate dehydrogenase	Heart, Kidney, RBC, Liver, Muscle	MI, acute hepatitis, anaemia	-
Isocitrate dehydrogenase	Liver	Cirrhosis	-
Creatine kinase	Brain, Bowel, Heart, Skeletal muscle	MI, Muscular dystrophy	-
Glucose-6- phosphate dehydrogenase	Heart	Myocardial Infraction	Congenital deficiency causes haemolytic anaemia
ϒ-glutamyl-transferase	Liver, Kidney, Pancreas	Hepatitis, Cholestatic liver diseases	-

Serum Enzymes	Location of serum enzymes	Concentration increased in	Concentration decreased in
Ceruloplasmin (Ferroxidase activity)	Liver	Cirrhosis, Bacterial infection, Pregnancy	Wilsons disease (hepatolenticular degenaration)
Aldolase	Muscle, Liver, RBC	Muscular dystrophy, Hepatitis, Haemolytic anemia, Leukemia	-
Oxytocinase	Uterus	Normal pregnancy from fourth month. Increasing level shows good foetal prognosis.	Intrauterine foetal birth.

ENZYMES AS DIAGNOSTIC AGENTS

Enzyme	Used for testing
Urease	Urea
Uricase	Uric acid
Glucose oxidase	Glucose
Cholesterol oxidase	Cholesterol
Lipase	Triglyceride
Alkaline phosphatase	ELISA
Horse radish peroxidase	ELISA
Restriction endonuclease	Recombinant DNA technology
Reverse transcriptase	Polymerase chain reaction