Clinical Enzymology

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Specific Learning Objectives

At the end of session, I MBBS students shall be able to

- Define plasma **functional** and plasma **non-functional** enzymes with examples
- Explain mechanism of presence of plasma non functional enzymes in serum / plasma with examples
- Explain possible mechanisms of abnormal levels of serum enzymes in pathological conditions.
- Describe **isoenzymes** with examples and clinical significance.
- Describe the medical applications of various enzymes like **diagnostic, therapeutic and analytical**
- Interpret the **laboratory results** of various serum enzymes of liver, cardiac, skeletal muscle, the biliary tract, and the pancreas in pathological conditions

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.

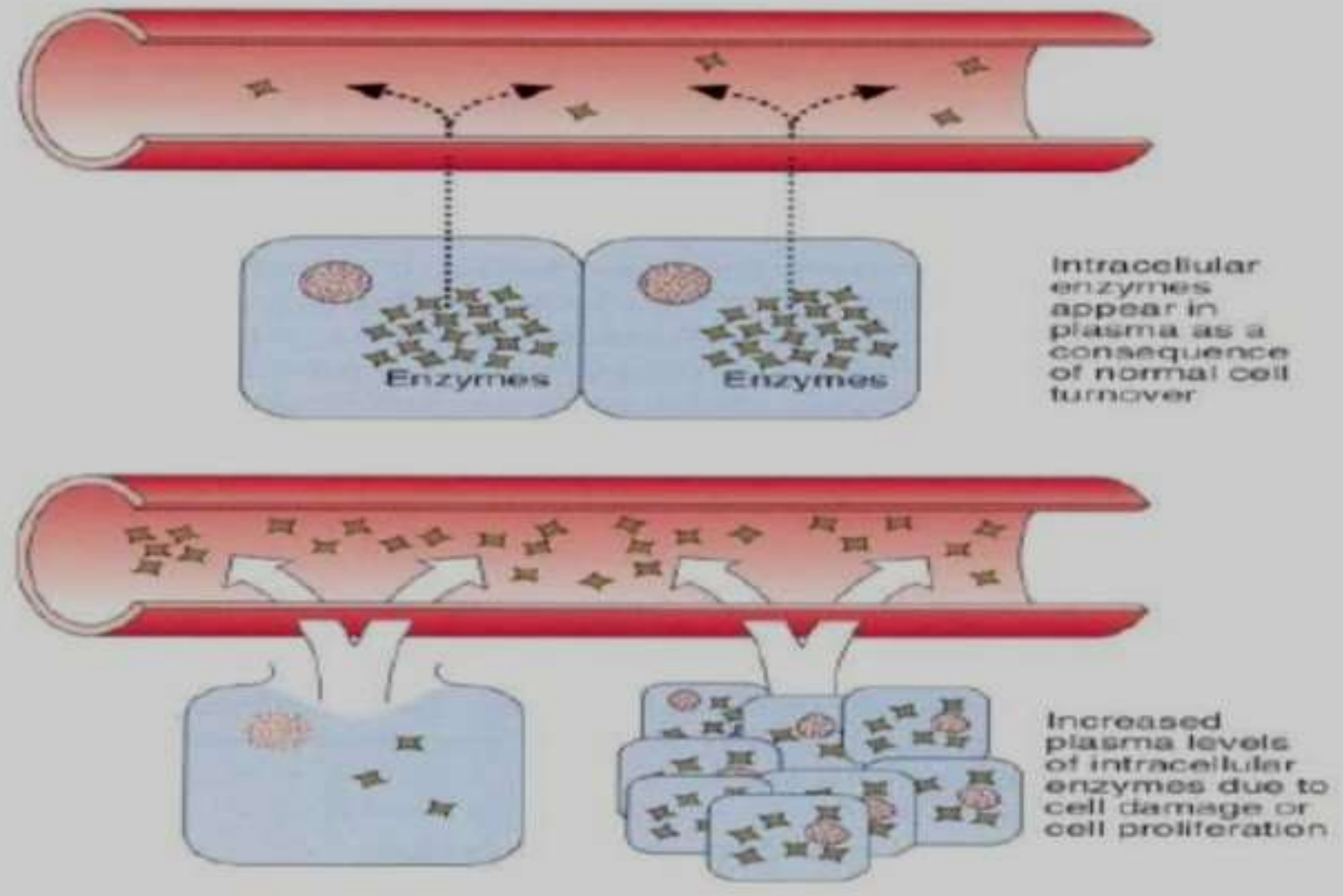
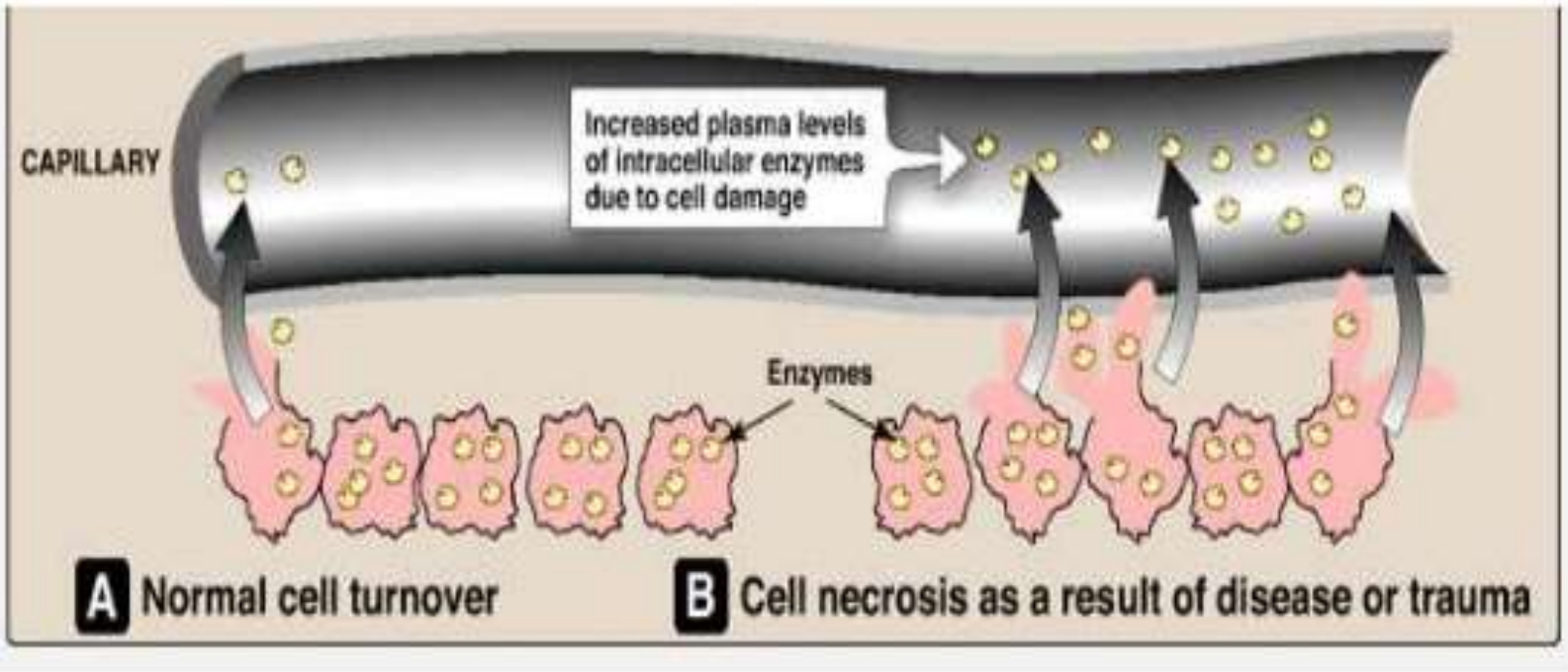


Fig. 2 **Plasma levels of intracellular enzymes.**

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.

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.

CLINICAL APPLICATION/USES OF ENZYMES

- **Diagnostic**
- **Therapeutic**
- **Analytical**

Diagnostic Enzymes in different diseases:-

Enzyme estimations are helpful in the diagnosis of –

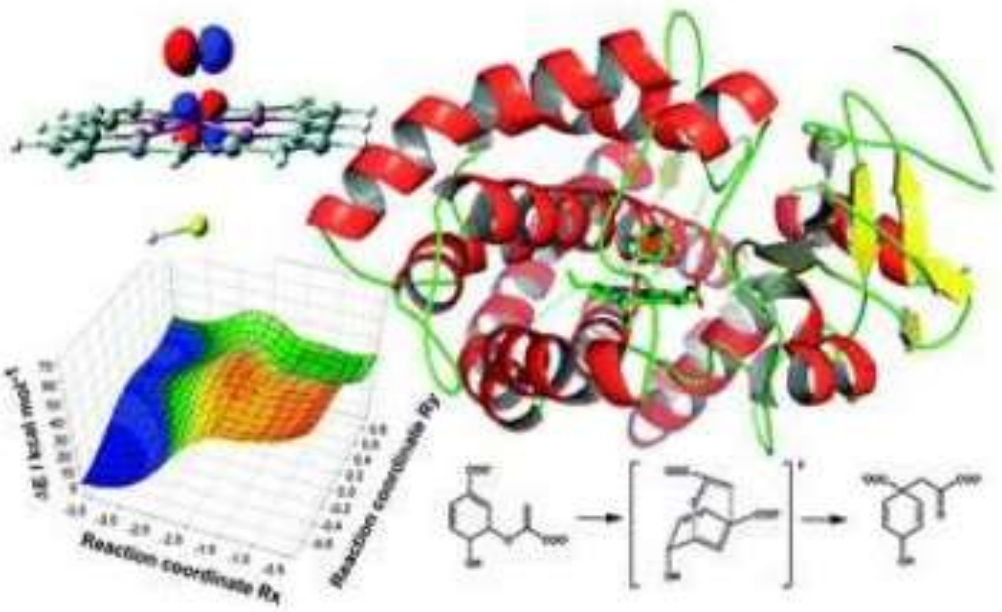
1) Myocardial Infarction

2) Liver diseases

3) Bone disease

4) Cancers

5) GI Tract diseases



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**

Other biochemical markers include

- Troponins T and I
- Myoglobin

Creatine Phosphokinase (CPK) or Creatine Kinase (CK)

Creatine + ATP ⟷ Creatine phosphate + ADP

Creatine kinase is a dimer that are made up of two types of polypeptide chains, which may be either *M (muscle)* type or B *(brain)* type, generating three isoenzymes.

- CK1 (BB) : Brain
- CK2 (MB) : Heart (N.R. 5-25 IU/L)
- CK3 (MM) : Skeletal muscle

Clinical Application

1. CK BB may be elevated in neonates particularly in damaged brain or very low birth weight new-born

2. Increased level of CK- MB occurs in myocardial infarction

Cardiac tissue is the only tissue which has mixed MB isoenzyme.

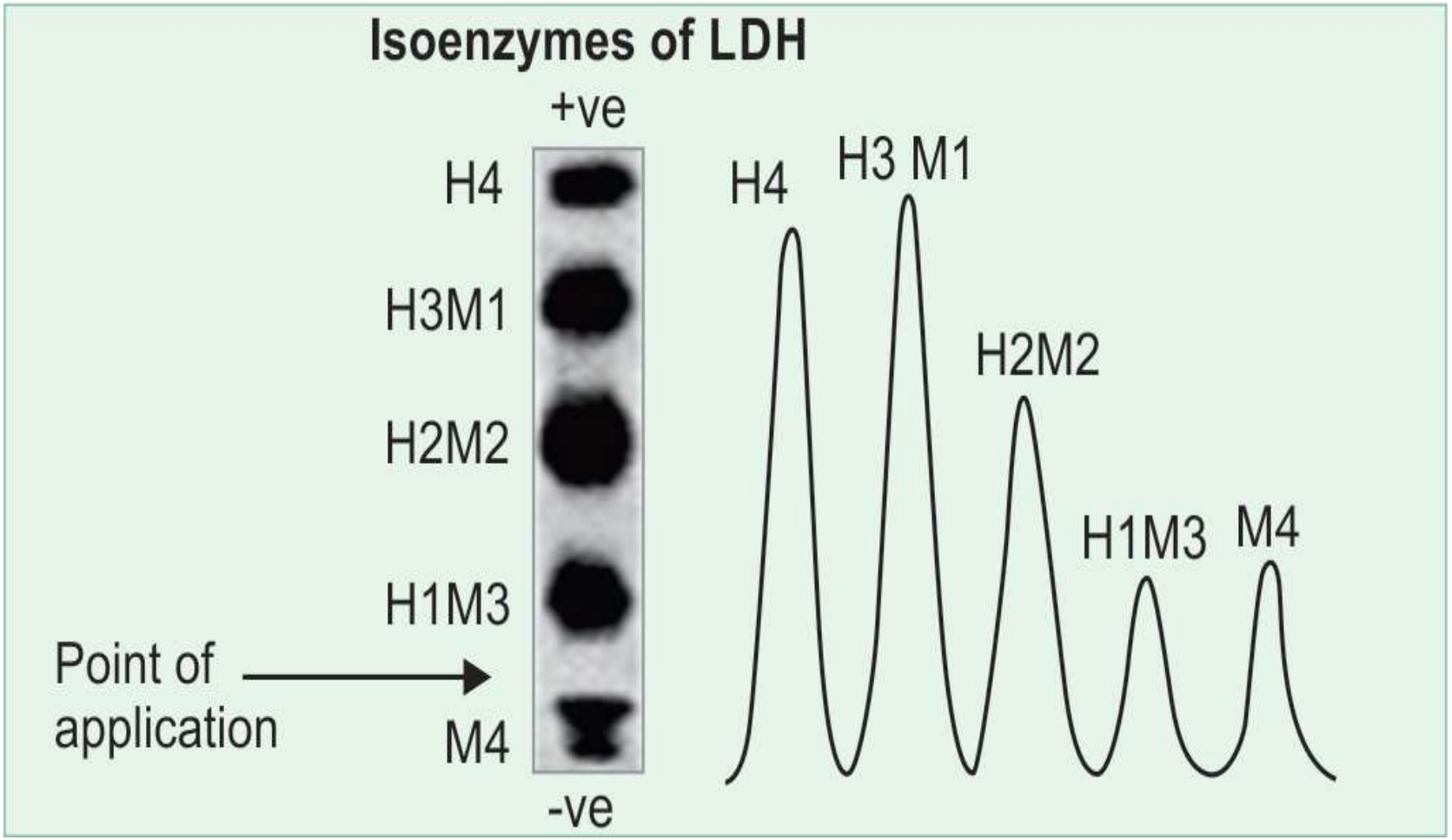
3. CK-MB isoenzyme starts to increase within 4 hours after an acute myocardial infarction (AMI) and reaches a maximum within 24 hrs.

4. Elevated levels of CK-MM in serum occur in muscle dystrophies and myopathies.

LACTATE DEHYDROGENASE (LDH)

Pyruvate  Lactate (anaerobic glycolysis)

- LDH is elevated in myocardial infarction, blood disorders
- It is a tetrameric protein and made of two types of subunits namely H = Heart, M = skeletal muscle
- It exists as 5 different isoenzymes with various combinations of H and M subunits



Electrophoresis pattern of LDH isoenzymes.

ISOENZYME NAME	COMPOSITION	PRESENT IN	ELEVATED IN
LDH1	(H_4) HHHH	Heart	myocardial infarction
LDH2	(H_3M_1) HHHM	RBC	Hemolytic disorders
LDH3	(H_2M_2) HHMM	Brain	Leukemia, malignancy
LDH4	(H_1M_3) HMMM	Liver	Pulmonary diseases
LDH5	(M_4) MMMM	Skeletal muscle	Skeletal muscle and liver diseases

- LDH5(M4) form is seen in skeletal muscles while **LDH1(H4) form is seen in heart**.

- Normally LDH2(H3M1) concentration in blood is greater than LDH1(H4); but this pattern is reversed in myocardial infarction; this is called **flipped pattern**.

- LDH level is 100 times more inside the RBC than in plasma, and therefore minor amount of **hemolysis** will result in a false positive test. Since total LDH is increased in many conditions, LDH is non specific cardiac marker .

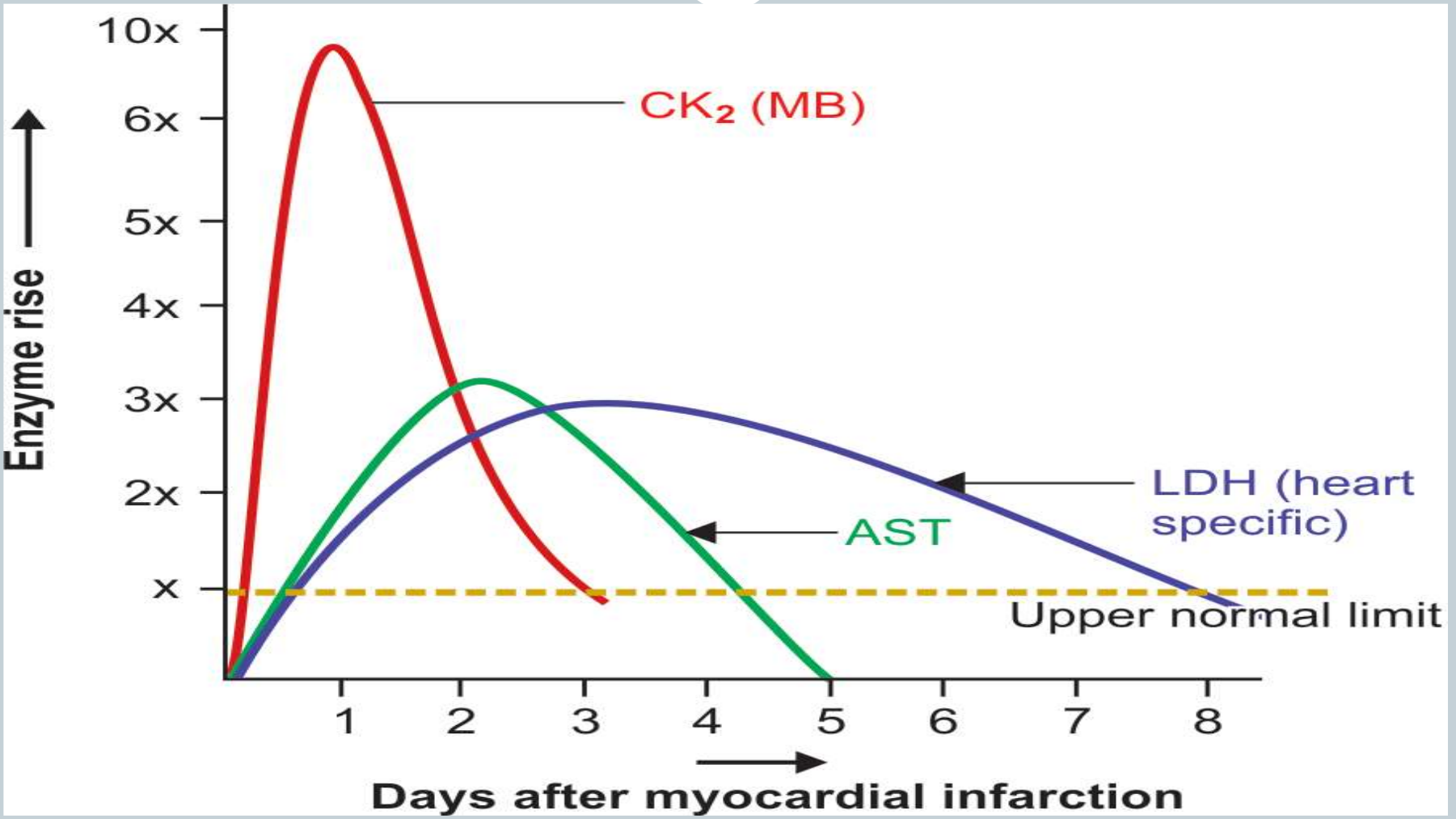
Aspartate transaminase (AST)

- It was known formerly as serum glutamate oxaloacetate transminase (SGOT).
- The plasma AST normal value for adults is 2 to 20 IU/L.
- Increased AST level occurs after myocardial infarction.
- It is moderately elevated in liver disease.
- The plasma AST level starts increasing after 6 to 12 hours after the onset of chest pain with peak values 24 to 48 hours and the values fall to normal level by the 4^{th} to 6^{th} day.

: Cardiac markers with time course after onset of acute myocardial infarction.

Markers	*Abnormal activity detectable (hours)*	*Time for maximum rise (hours)*	*Time for return to normal (days)*
CK_2 (MB)	3–10	10–24	2–3
AST/SGOT	6–12	24–48	4–6
LDH (heart specific)	8–16	48–72	7–12
Myoglobin (Mb)	1–3	6–9	1
Troponin-I (cTnI)	3–8	24–48	3–5
Troponin-T (cTnT)	3–8	72–100	5–10

Various enzyme assays and their time course after onset of acute myocardial infarction.



Enzyme Assays in Liver Diseases

1. **Enzymes in hepatocyte damage**:

- Aspartate aminotransferase (ALT)
- Alanine aminotransferase (AST)

ALT is the more liver-specific enzyme.

2. **Enzymes in cholestasis**:

- Alkaline phosphatase
- 5’-nucleotidase
- γ-glutamyl transferase

Alanine transaminase (ALT)

- Alanine transaminase was known formerly as serum glutamate pyruvate transaminase (SGPT).
- The plasma ALT normal value for adult is 2 to 15 IU/L.
- ALT level is elevated in liver diseases (viral or toxic hepatitis jaundice) and cirrhosis of liver.

Alkaline phosphatase (ALP)

- ALP hydrolyzes organic phosphate at alkaline pH.
- Normal serum level for adults is 3-13 KA units/dl.
- It is elevated in certain **bone and liver disease.**
- Very high levels may be noticed in obstructive jaundice, bone diseases such as Paget's disease, rickets, osteomalacia, carcinoma of bone and hyperparathyroidism

Isoenzymes of Alkaline Phosphatase

- **Alpha-1 ALP -** synthesized by epithelial cells of biliary canaliculi. Its activity is increased in obstructive jaundice.
- **Alpha-2 heat labile ALP -** It is produced by hepatic cells. Increased in hepatitis.
- **Alpha-2 heat stable** ALP - It is of placental origin, which is found in blood in normal pregnancy.

  Regan isoenzyme is similar to placental which is seen in carcinoma of lung, liver and gut
- **Pre-beta ALP -** is of bone origin and elevated levels are seen in bone diseases.
- **Gamma ALP –** is produced by intestines and level increased in ulcerative colitis

Gamma Glutamyl Transferase (GGT)

- It can transfer gamma glutamyl residues to substrate. It is used for the synthesis of glutathione. It is seen in liver, kidney, pancreas, intestinal cells, and prostate gland.

- **Reference serum** value of GGT is 10–30 U/L.

- GGT is clinically important because of its sensitivity to detect **alcohol abuse**.
- GGT is increased in alcoholics even when other liver function tests are within normal limits.
- GGT level is rapidly decreased within a few days when the person stops to take alcohol.
- Increase in GGT level is generally proportional to the amount of alcohol intake.

Acid phosphatase (ACP)

- It hydrolyzes phosphoric acid ester at pH 5 to 6.
- Normal serum value for ACP is 0.5 to 4 KA units/dL.
- Acid phosphatase enzyme is useful for the diagnosis and prognosis of prostate cancer. ACP is therefore an important tumor marker.

Enzyme Assays in Pancreatitis

- Serum Amylase
- Urine amylase
- Lipase

Amylase

- It catalyzes hydrolysis of starch and glycogen.
- Normal serum value is 50-120 U/L.
- The activity of serum amylase is increased in acute pancreatitis, chronic pancreatitis, mumps and obstruction of pancreatic duct.

Lipase

It will hydrolyze triglyceride to beta monoglyceride and fatty acid. The enzyme is present in pancreatic secretion.

The level in blood is highly elevated in **acute pancreatitis** and this persists for 7–14 days. Thus, lipase remains elevated longer than amylase. Moreover, lipase is not increased in mumps. Therefore, lipase estimation has advantage over amylase.

It is moderately increased in carcinoma of pancreas, biliary diseases and perforating peptic ulcers.

Enolase

It is a glycolytic enzyme. Neuron-specific enolase (NSE) is an isoenzyme seen in neural tissues and Apudomas.

NSE is a **tumor marker** for cancers associated with neuroendocrine origin, small cell lung cancer, neuroblastoma, pheochromocytoma, medullary carcinoma of thyroid, etc.

Therapeutic use of Enzymes

- **Streptokinase** (from Streptococcus) or **Urokinase** (from urine) can lyse intravascular clots and are therefore used in myocardial infarction.
- **Pepsin** and **trypsin** are given to patients with defective digestion.
- **Asparaginase** is used as an anticancer drug.
- **Alpha 1- Antitrypsin** used in treatment of emphysema

Analytical Uses of Enzymes

Enzyme	*Used for testing*
Urease	Urea
Uricase	Uric acid
Glucose oxidase	Glucose
Peroxidase	Glucose; Cholesterol
Hexokinase	Glucose
Cholesterol oxidase	Cholesterol
Lipase	Triglycerides
Horse radish peroxidase (HRP)	ELISA
Alkaline phosphatase	ELISA
Restriction endonuclease	Southern blot; RFLP
Reverse transcriptase Taq polymerase	Polymerase chain reaction (PCR)

Summary

I. Hepatic diseases

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

II. Myocardial infarction

1. Cardiac troponins (CTnT and CTnI). (These are not enzymes, but are specific and sensitive and elevated very early in MI).
2. Creatine kinase (CK-MB): CK-MB isoenzyme is specific

III. Muscle diseases

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

IV. Bone diseases

1. Alkaline phosphatase (ALP) Marked elevation in rickets and Paget's disease

V. Prostate cancer

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

VI. Pancreatic disease

1. Amylase: Marker for acute pancreatitis and inflammation of salivary glands
2. Lipase: Marker of pancreatitis, more specific than amylase