

Isoenzymes

Multiple forms of an enzyme which differ in physical and chemical properties and catalyze the same reaction as an enzyme.

Isoenzymes are produced by a single gene and some may result from more than one gene.

Isoenzymes can be separated by:

1-Heat inactivation

2-Chemical inhibition

3-Electrophoretic techniques (specific)

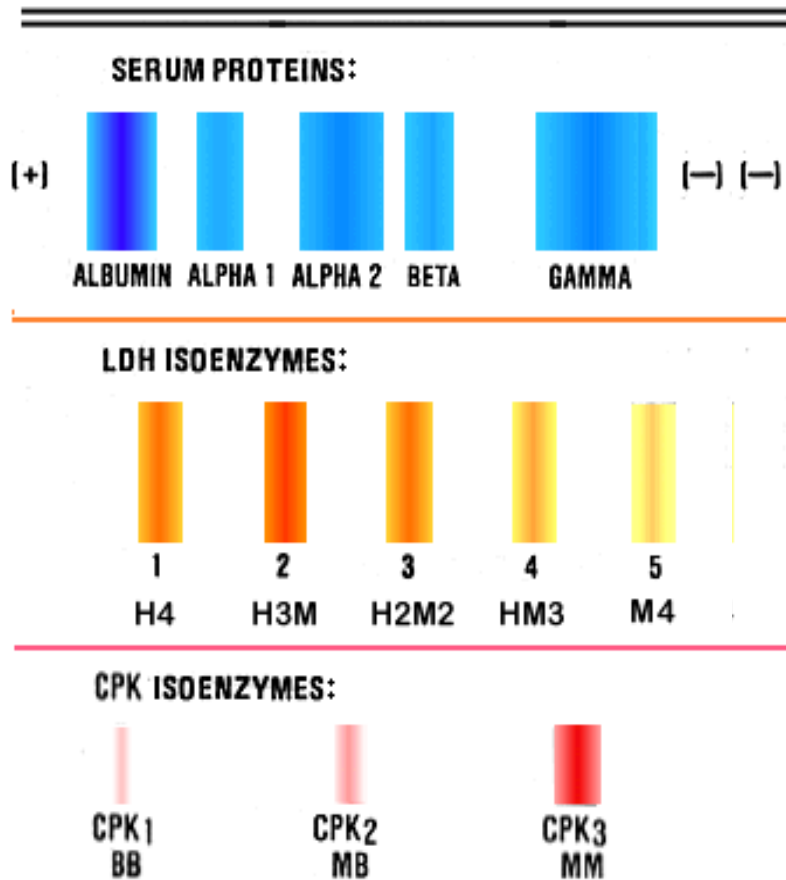
I SOENZYMES

- **Catalyze the same reaction.**
- **Differ in AA sequence and physical properties.**
- **Separable on the basis of charge.**
- **Are tissue specific.**

Different Isoenzymes may arise from different tissues and their specific detection may give clues to the site of pathology.

Electrophoresis

Is a technique by which • separation of Movement of charged particles through an electrolyte when subjected to electrical field.



Advantages of Isoenzyme measurement

1. Isoenzyme variants are **derived** from **different tissue** sources.
2. So separation renders **increased specificity** to enzyme analysis.
3. **Tissue** or **organ** effected can be **detected** (where isoenzyme elevation occurs)

Types

1. CPK (creatinine Phospho Kinase)
2. Troponin
3. LDH (Lactate Dehydrogenase)
4. ALP (Alkaline phosphatase)
5. Aldolase
6. Amylase

CPK-Creatinine Phospho kinase

- CPK **Isoenzymes** are performed when the **total CPK** level is **elevated**.
- Isoenzyme testing can help **differentiate** the **source** of the **damaged tissue**.
- **CPK** is an enzyme found predominantly in the **heart, brain, and skeletal muscle**.
- CPK is composed of **3 Isoenzymes** that differ slightly in structure:
- CPK is a **dimer** made up of **2 subunits** called B for brain and M for muscle.

CREATINE KINASE (CK)

- Creatine kinase is a dimer made of 2 monomers

occurs in the tissues
- Skeletal muscle contains M subunit, Brain contains

B subunits
- Three different isoenzymes are formed

CPK- Isoenzymes

- CPK-1 (also called **CPK-BB**) is concentrated in the **brain and lungs**
- CPK-2 (also called **CPK-MB**) is found mostly in the **heart**
- CPK-3 (also called **CPK-MM**) is found mostly in **skeletal muscle**
- Because the CPK-1 isoenzyme is predominately found in the brain and lungs, injury to either of these organs (for example, **stroke** or lung injury due to a **pulmonary embolism**) are associated with elevated levels of this isoenzyme.

Isoenzyme name	Composition	Present in	Elevated in
CK-1	BB	Brain	CNS diseases
CK-2	MB	Myocardium Heart	Acute myocardial infarction
CK-3	MM	Skeletal muscle, Myocardium	

CPK (CK)

- Creatinine Phospho kinase or Creatinine Kinase catalyses the conversion of creatine to creatine Phosphate.
- $\text{Creatine} + \text{ATP} \xrightarrow{\text{Creatinine kinase}} \text{creatine phosphate} + \text{ADP}$ Creatinine Phospho kinase
- Normal level: 15-100U/L (males)
: 10-80 U/L (females)
- Sample: in serum it is estimated and not increased in hemolysis.

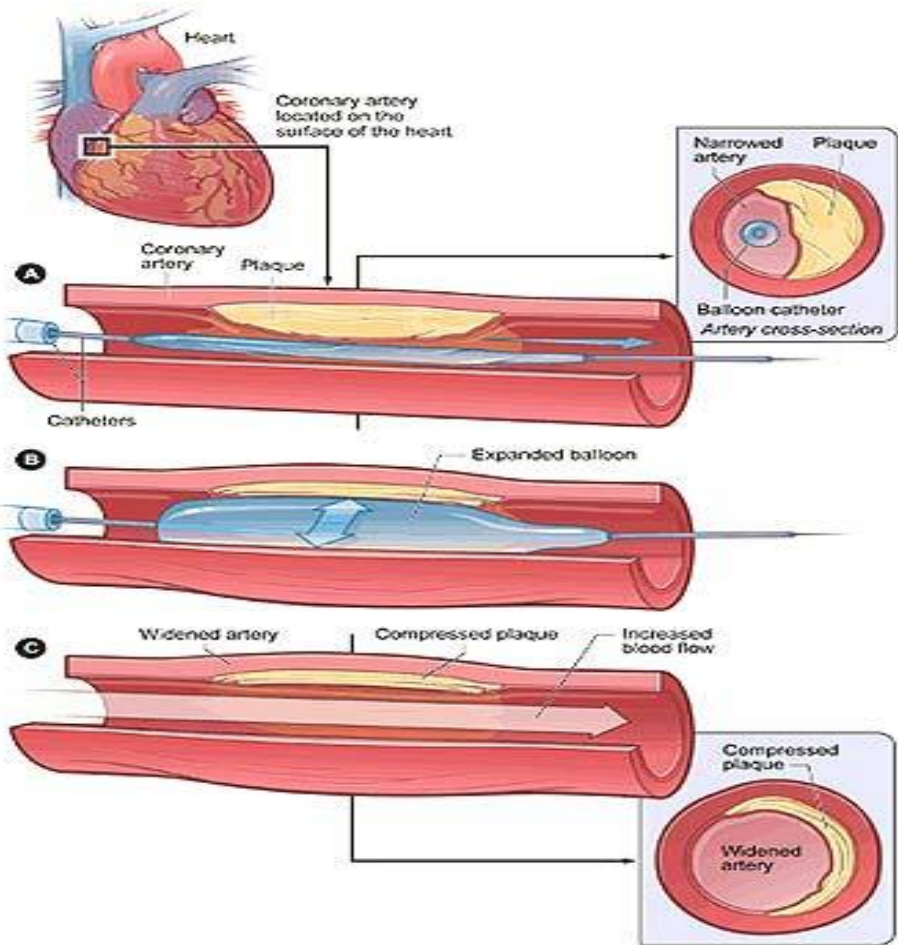
Clinical significance of CK

- ▶ CPK & heart attack:
- ▶ CPK2 isoenzymes is very small, (2% of total CPK activity) & undetectable in plasma.
- ▶ In myocardial infarction (MI), CPK2 levels are increased within 4 hrs, then falls rapidly.
- ▶ Total CPK level is elevated upto 20-folds in MI.

Clinical significance

- ▶ It is present in serum when there is extensive tissue damage causing breakdown of mitochondrial & cell wall.
- ▶ Its presence in serum indicates cellular damage, seen in malignancies.

Atherosclerosis



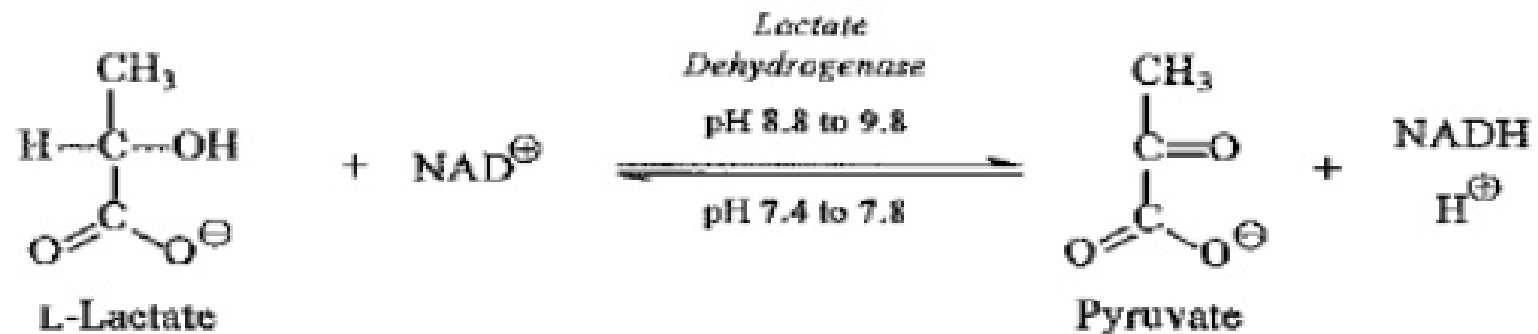
- Is a condition in which arteries are blocked to a greater or lesser extent by deposition of cholesterol plaques , leading most commonly to coronary heart disease by blocking of coronary arteries i.e
- (myocardial infarction MI).

LACTATE DEHYDROGENASE (LDH)

Pyruvate \rightleftharpoons 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**

Lactate dehydrogenase (LDH)



Normal values

Serum -100 -200 U/L

CSF - 7 -30 U/L

Urine - 40 -100 U/L

LDH-Isoenzymes

- The LDH has **five** Isoenzymes which are:
- LDH-1 (H₄) is found mainly in the **heart**.
- LDH-2 (H₃M₁) **Reticuloendothelial system**.
- LDH-3 (H₂M₂) is found in the **lungs**.
- LDH-4 (H₁M₃) in the **kidney, placenta, and pancreas**, and
- LDH-5 (M₄) in **liver** and **striated (skeletal) muscle**.
- **Normally**, levels of **LDH-2** are higher than those of the other Isoenzymes

Isoenzyme name	Composition	Composition	Present in	Elevated in
LDH1	(H ₄)	HHHH	Myocardium, RBC	myocardial infarction
LDH2	(H ₃ M ₁)	HHHM	Myocardium, RBC	
LDH3	(H ₂ M ₂)	HHMM	Kidney, Skeletal muscle	
LDH4	(H ₁ M ₃)	HMMM	Kidney, Skeletal muscle	
LDH5	(M ₄)	MMMM	Skeletal muscle, Liver	Skeletal muscle and liver diseases

Description

LDH

- LDH is found in the cells of almost all body tissues.
- Because this enzyme is actually composed of **five different Isoenzymes**, however, analysis of the different LDH isoenzyme levels in the blood can help in the diagnosis of some diseases.

LDH

- LDH is an **oxidoreductase** enzyme whose activity is necessary for the reversible reaction in which **Pyruvate and lactate are inter converted**. It is important in glycolysis.
- LDH Isoenzyme is a **tetramer** with **4 subunits**. The subunit may be either H (heart) or M (muscle) .

Clinical significance of LDH

- ▶ In normal serum, LDH2 (H3M) predominant isoenzyme & LDH5 is rarely seen.
- ▶ In myocardial infarction, LDH1(H4) levels are greater than LDH2.
- ▶ Megaloblastic anemia (50 times upper limit of LDH 1 and LDH 2)
- ▶ Muscular dystrophy, LDH5 (M4) is increased.
- ▶ Toxic hepatitis with jaundice (10 times more LDH5)

- ▶ Renal disease- tubular necrosis or pyelonephritis, pulmonary embolism
LDH 3 (massive destruction of platelets)
- ▶ Total LDH is increased in neoplastic diseases.
- ▶ LDH5 is increased in breast cancer, malignancies of CNS, prostatic carcinoma.
- ▶ In leukaemias, LDH2 & LDH3 levels are increased.
- ▶ In malignant tumors of testis & ovaries, LDH2, LDH3 & LDH 5 levels are increased.

LDH and Heart Attack

- One of the most important **diagnostic uses** for the LDH Isoenzymes test is in the **differential diagnosis** of **myocardial infarction** or heart attack.
- The total LDH level rises within **24-48** hours after a heart attack, **peaks** in **two to three days**, and returns to normal in approximately **five to ten days**.
- The LDH-1 isoenzyme level, however, is more sensitive and specific than the total LDH.

LDH and Heart Attack

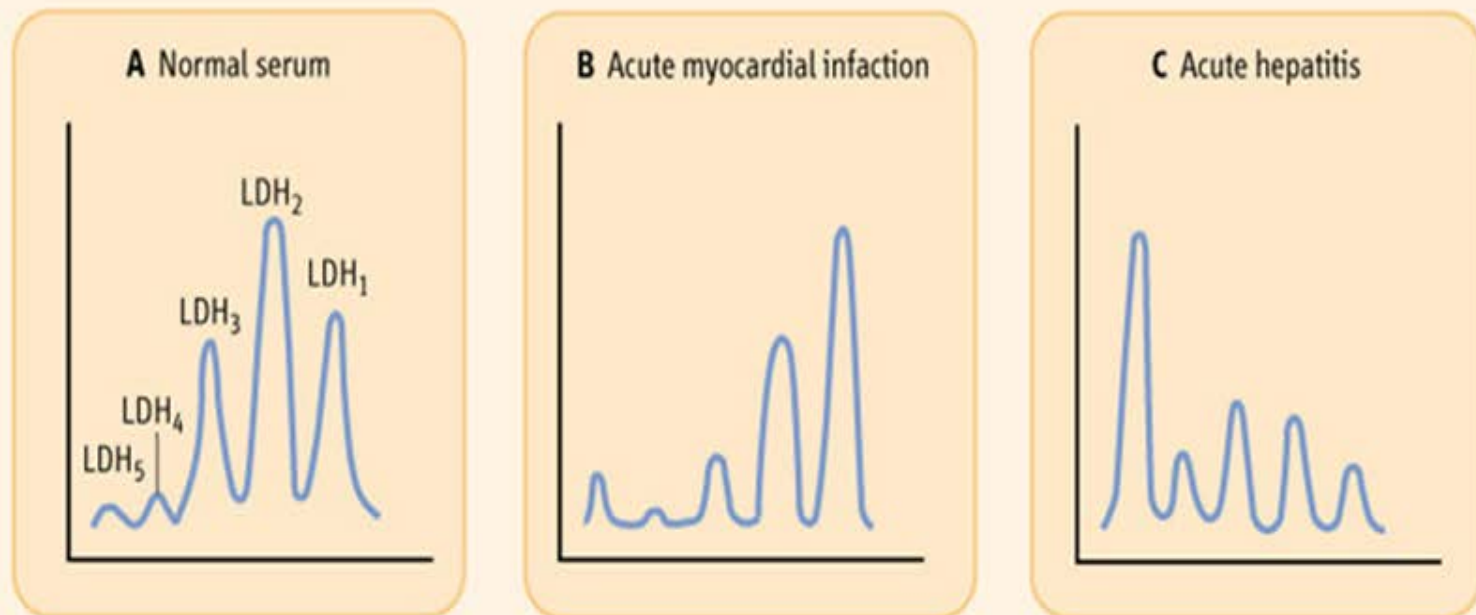
- Normally, the level of LDH-2 is higher than the level of LDH-1.
- An LDH-1 level higher than that of LDH-2, a phenomenon known as "flipped LDH," is strongly indicative of a myocardial infarction.
- The flipped LDH usually appears within 12-24 hours after a heart attack.
- A normal LDH-1/LDH-2 ratio is considered reliable evidence that a heart attack has not occurred.

LACTATE DEHYDROGENASE IN MI

- LDH** is a tetramer of two non-identical subunits (**LDH5**(M4), **LDH4**(M3H), **LDH3**(M2H2), **LDH2**(MH3), **LDH1**(H4))



Densitometric patterns of LDH isozymes in normal and patient serum



Alkaline Phosphatase (ALP)

- Isoenzymes are **five**:
 1. **ALP-1** present in **liver** increased in obstructive jaundice, biliary cirrhosis.
 2. **ALP-2** in **bone** increased in rickets and Pagets
 3. **ALP-3** in **placenta** increase in 2nd and 3rd trimester of pregnancy and decrease indicates placental insufficiency and foetal death.

Alkaline Phosphatase (ALP)

4. ALP-4 in intestine increased in intestinal disease and after gastrectomy surgery
5. ALP-5 in kidney increases in kidney disorders.
 - In normal serum liver and bone fractions are present.
 - Abnormal ALP Isoenzymes Regan and nagao are present in carcinomas and metastasis.
 - Normal level: 40-125 U/L

Amylase

- **Two** Isoenzymes:
 1. **Salivary** in normal serum 60% increased in Parotitis/ mumps
 2. **Pancreatic** 40% increased in pancreatitis
- The peak levels are seen between 5-12hours after onset of disease and returns to normal level with in 2-4 days.
- Normal level: 50-120 IU/L

Aldolase

- Three Isoenzymes:
 1. Aldolase-A present in muscle increased in muscular dystrophies.
 2. Aldolase-B in liver increased in hepatitis.
 3. Aldolase-C in brain
- Normal level: 1.5-7 U/L

Acid phosphatase

- Present in Prostate, RBC and platelets.
- Increased in Prostate cancer, Gaucher's disease and thromboembolic disorders
- Normal level: 2.5-12 U/L

Enzymes in Liver diseases

The following enzymes when elevated are useful in the diagnosis of liver diseases and disfunction due to viral hepatitis, toxic hepatitis, cirrhosis and hepatic necrosis

1. Alanin transaminas(ALT).
2. Aspartate transaminase (AST).
3. Lactate dehydrogenase (LDH).

The enzymes that markedly increase in intrahepatic and extra hepatic cholestasis are

- 4- Alkaline Phsphatase.
- 5- Nucleotidase.

Coenzymes: the protein part of the enzyme on its own is not always adequate to bring about the catalytic activity and many enzymes require certain non protein small additional factor regarded as cofactor these cofactors are called (Coenzymes)

Coenzymes are non protein organic low molecular wt associated with enzyme function. It differs from enzyme by physical, chemical and immunological properties.

Coenzymes for B complex vitamins:

Most of the coenzymes are the derivatives of water soluble vit B, the biochemical function of B-complex are exerted through their respective coenzymes.

Non Vit Coenzymes:

Organic substances which has no relation with vit but function as coenzymes Ex: ATP, CDP (cytidine monophosphate) UDP (uridine diphosphate)

NAD, NADP FMN, FAD.

Enzymes as therapeutic agents;

Enzymes are used as drug for treatment of medical problem

Streptokinase; is a useful for clearing the blood clot .

Streptokinase activates plasma plasminogen to plasmin which in turn attacks fibrin to convert into soluble products