Class 14:

Plasma lipoproteins and enzymes

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Objectives for this lecture

- Discuss the different type of Plasma lipoproteins and enzymes.
- understand the effect of liver and kidney disease on the level of plasma lipoproteins and enzymes.

Plasma Enzymes

Plasma derived

These enzymes act on substrates in plasma & their activity is higher in plasma than cells. E.g. coagulation enzymes.

<u>Cell Derived:</u> These enzymes have a high activity in cells & overflow into the plasma.

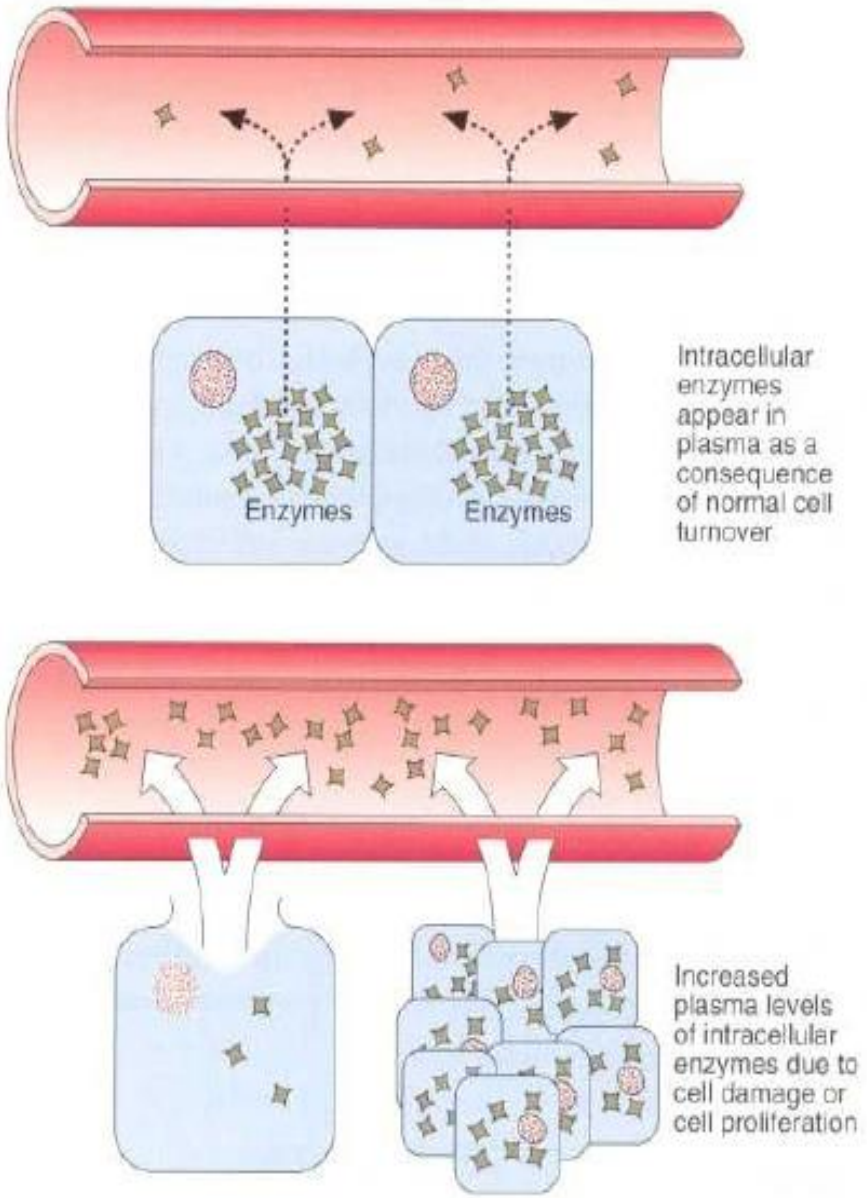
- Secretary enzymes
- Metabolic enzymes

- Blood plasma contains many enzymes which are classified into:

1. Functional plasma enzymes
2. Non functional plasma enzyme

Differences between functional and non functional enzymes

	Functional plasma enzymes	Non functional plasma enzymes
Concentration in plasma	Present in plasma in higher concentrations in comparison to tissue	Normally, Present in plasma in very low concentrations in comparison to tissue
Function	Have known functions	No known functions
Substrate	Their substrates are always present in plasma	Their substrates are absent from plasma
Site of synthesis	liver	Different organs .g. liver heart, skeletal muscles and brain
Effect of disease	Decrease in liver disease	Increase in different organ diseases
Examples	Clotting factors e.g. Prothrombin Lipoprotein lipase, Pseudocholinesterase	ALT, AST, CK, LDH, alkaline phosphatase, acid phosphatase and lipase



- Small amounts of intracellular enzymes are present in the blood as a result of normal cell turnover.
- 'Normal' plasma enzyme levels reflect the balance between the rate of synthesis and release into plasma during cell turnover, and the rate of clearance from the circulation.
- The presence of elevated enzyme activity in the plasma may indicate tissue damage that is accompanied by increased release of intracellular enzymes

Source of non functional enzymes

- **Cell damage** with the release of its content of enzymes into blood e.g. Myocardial infarction and viral hepatitis
- **Obstruction of normal pathways** e.g. Obstruction of bile duct increases alkaline phosphatase
- **Increase of the enzyme synthesis** e.g. bilirubin increases the rate of synthesis of alkaline phosphatase in obstructive liver disease
- **Increased permeability** of cell membrane as in hypoxia

Medical importance of non functional enzymes

- Measurement of non functional enzymes is important for:

1. ***Diagnosis of diseases*** as disease of different organs cause elevation of different plasma enzymes
2. ***Prognosis of the disease*** we can follow up of the treatment by measuring plasma enzymes before and after treatment

Disadvantages of enzyme assays

- A major disadvantage for the diagnosis of tissue damage is their lack of specificity to a particular tissue or cell type. Many enzymes are common to more than one tissue.
- This problem may be obviated in 2 ways:
  - **First**, different tissues may contain (and thus release when they are damaged) two or more enzymes in different proportions
  - **Second**, some enzymes exist in different forms (isoforms)

Isoenzymes

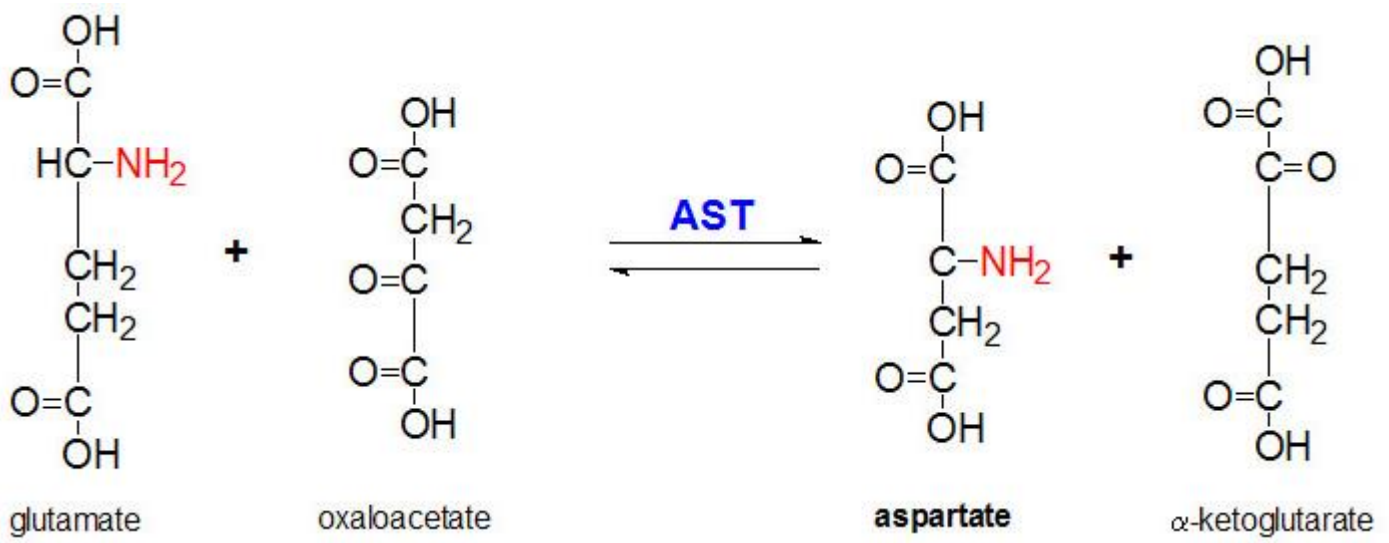
- Isoenzymes (or isozymes) are a group of enzymes that catalyze the same reaction but they differ in amino acid sequence
- Isoenzymes can be:
  - produced by different genes (= true isozymes)
  - produced by different posttranslational modification (= isoforms)
- found in different compartments of a cell
- found in different tissues of an organism
- can be oligomers of various subunits (monomers)

Isoenzymes

- They differ in:
  - electrophoretic mobility
  - enzymatic properties
  - physical properties (e.g heat stability)
  - biochemical properties such as amino acid composition, immunological reactivities
- Because isoenzymes are originated from different tissues, their determination give more information than measurement of total enzyme activity in plasma

Abnormal plasma enzyme activities

- **Aspartate Transaminase (AST)/ Serum Glutamate oxaloacetate (SGOT)**



Diagnostic Significance

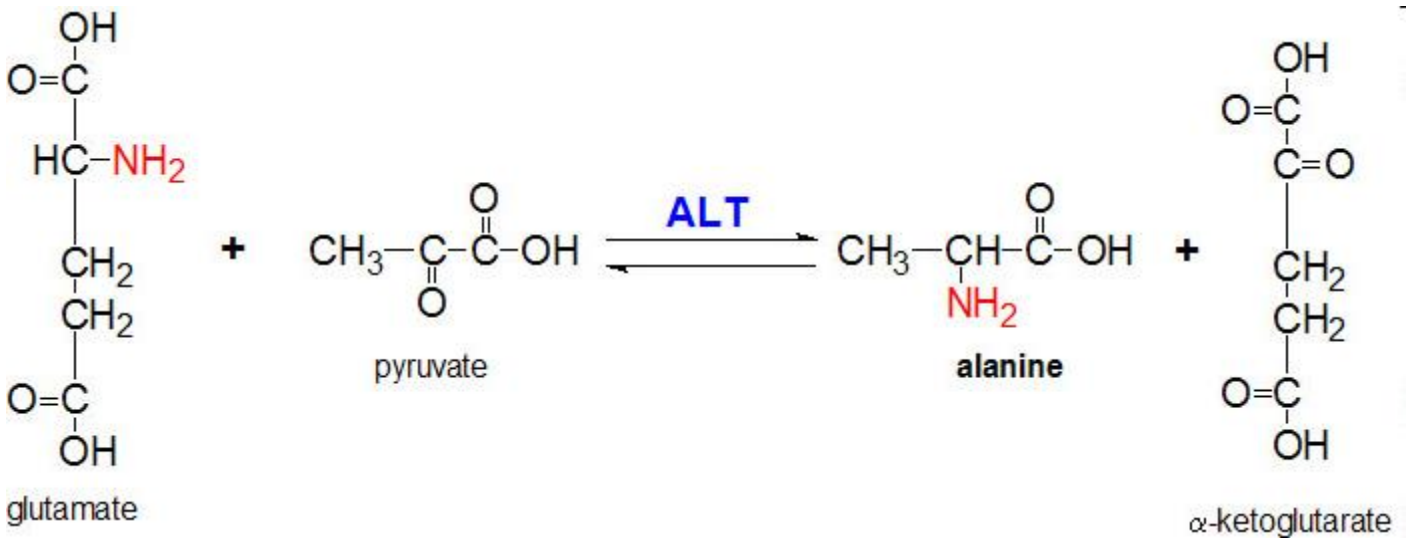
- The clinical use of AST is limited mainly to the evaluation of hepatocellular disorders and skeletal muscle involvement.
- Post AMI (Acute Myocardial Infarction)
  - Rises 6 – 8 hours
  - Peaks at 24 hours
  - Returns to normal by day 5
- AST levels are highest in acute hepatocellular disorders, viral hepatitis, cirrhosis.
  - Viral hepatitis may reach 100 x ULN (Upper limit of Normal)

Diagnostic Significance

- There are two isoenzyme fractions located in the cell cytoplasm and mitochondria,
  - the cytoplasmic isoenzyme is predominant in serum
  - while the mitochondrial one may be increased following cell necrosis.
- Isoenzyme analysis of AST is not routinely performed in the clinical laboratory.

Abnormal plasma enzyme activities

- **Alanine Transaminase (ALT)/ glutamate pyruvate transaminase (GPT)**

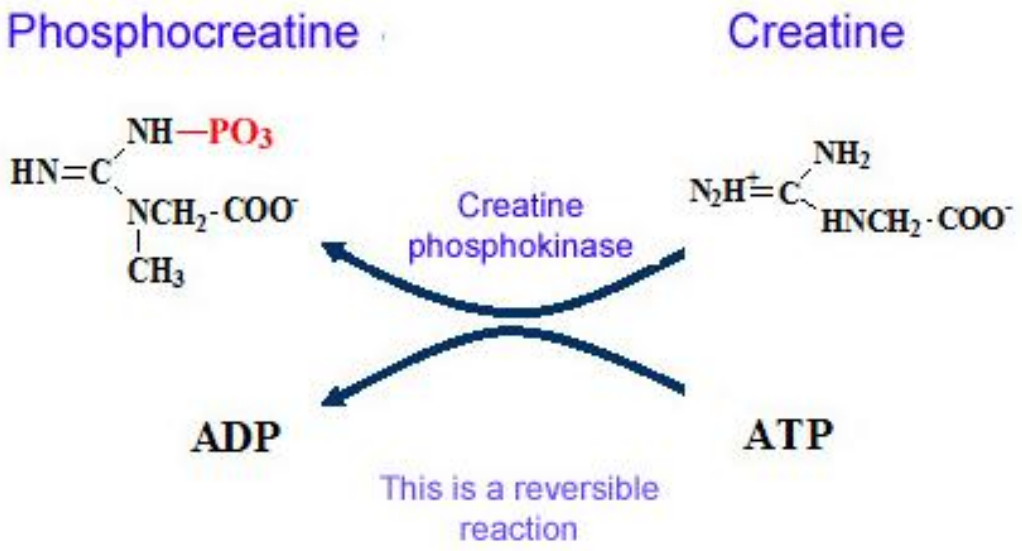


Alanine aminotransferase (ALT)

- Very high values are seen in acute hepatitis, either toxic or viral in origin.
- Both ALT and AST are increased in liver diseases, but ALT >AST.
- Moderate increase may be seen in chronic liver disease such as cirrhosis, and malignancy in liver.
- (AST/ALT) in normal conditions is 1.33 ± 0,42.

Abnormal plasma enzyme activities

- Creatine Kinase (CK, CPK)



Creatine Kinase (CK)

- High concentrations of CK in:
  - **skeletal muscle**
  - **cardiac muscle**
  - **brain tissue**
- Increased plasma CK activity is associated with damage to these tissues

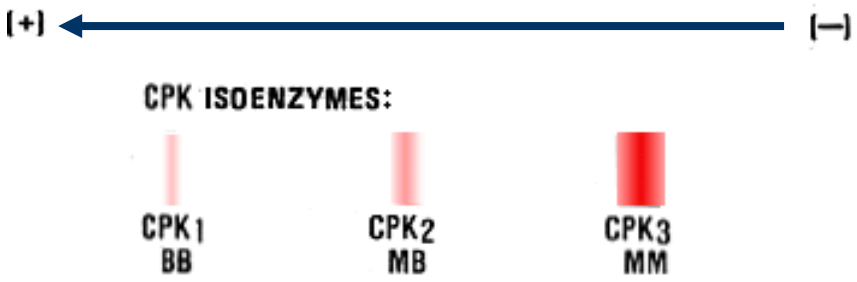
- ↑ CK is especially useful to diagnose:
  - AMI
  - Skeletal muscle diseases (Muscular Dystrophy)

Creatine Kinase isoenzymes

- CK occurs in 3 isoenzymes, each is a dimer composed of 2 subunits (B & M): **CK1** = **BB**, **CK2** = **MB** and **CK3** = **MM**
- Normal serum consists of:
  - Approximately 94% to 100% CK-MM
  - Values for the MB isoenzyme range from undetectable to trace (<6% of total CK).
  - CK-BB is also present in small quantities
- Cardiac muscle CK is 80% CK-MM and 20% CK-MB

Creatine Kinase isoenzymes

- Each CK isozyme shows a characteristic electrophoretic mobility.



Diagnostic Significance

- The value of CK isoenzyme separation can be used principally in detection of myocardial damage.
  - increased CK – MB (> 6% of the total CK activity) is a strong indication of AMI
- Post AMI
  - CK-MB increases 4 – 8 hours
  - Peaks at 12 - 24 hours
  - Returns to normal 48 - 72 hours

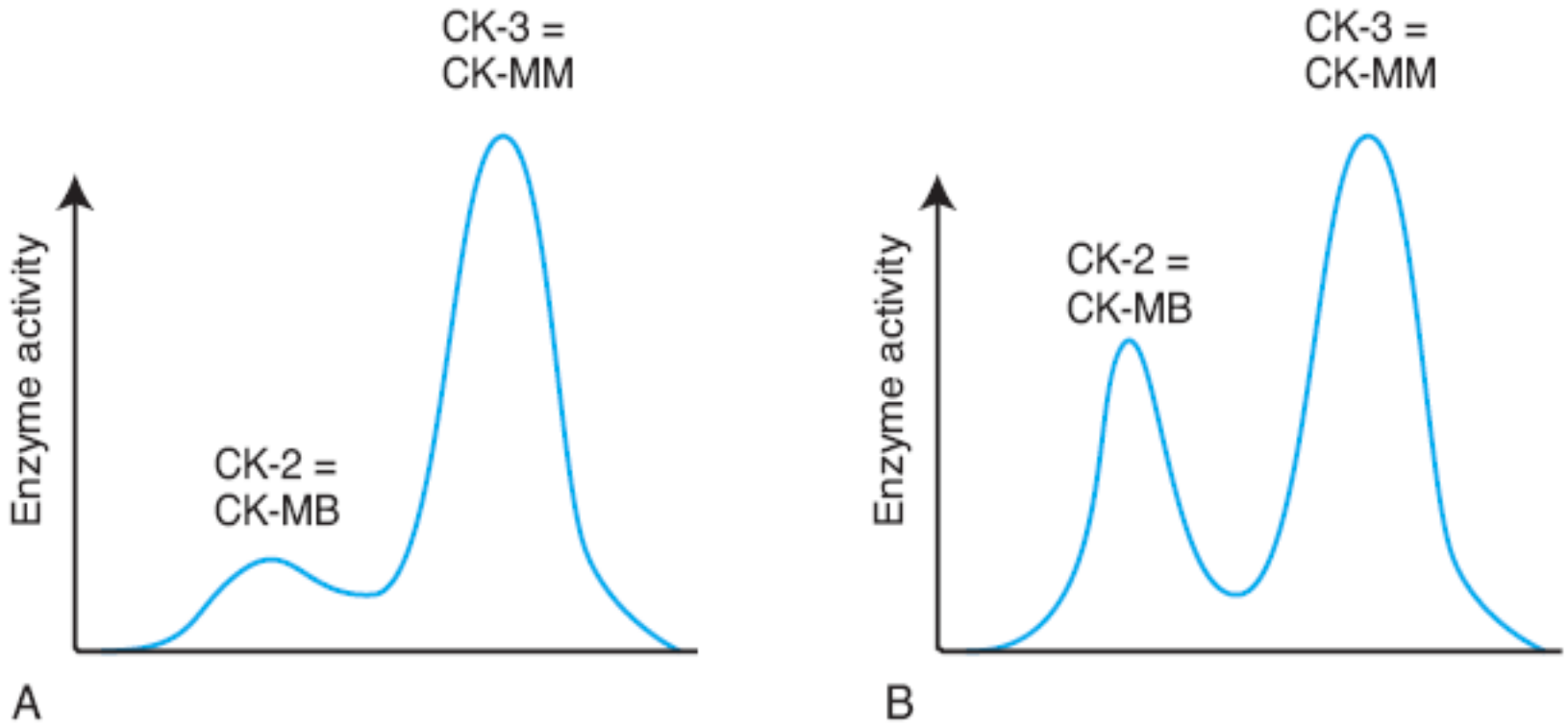
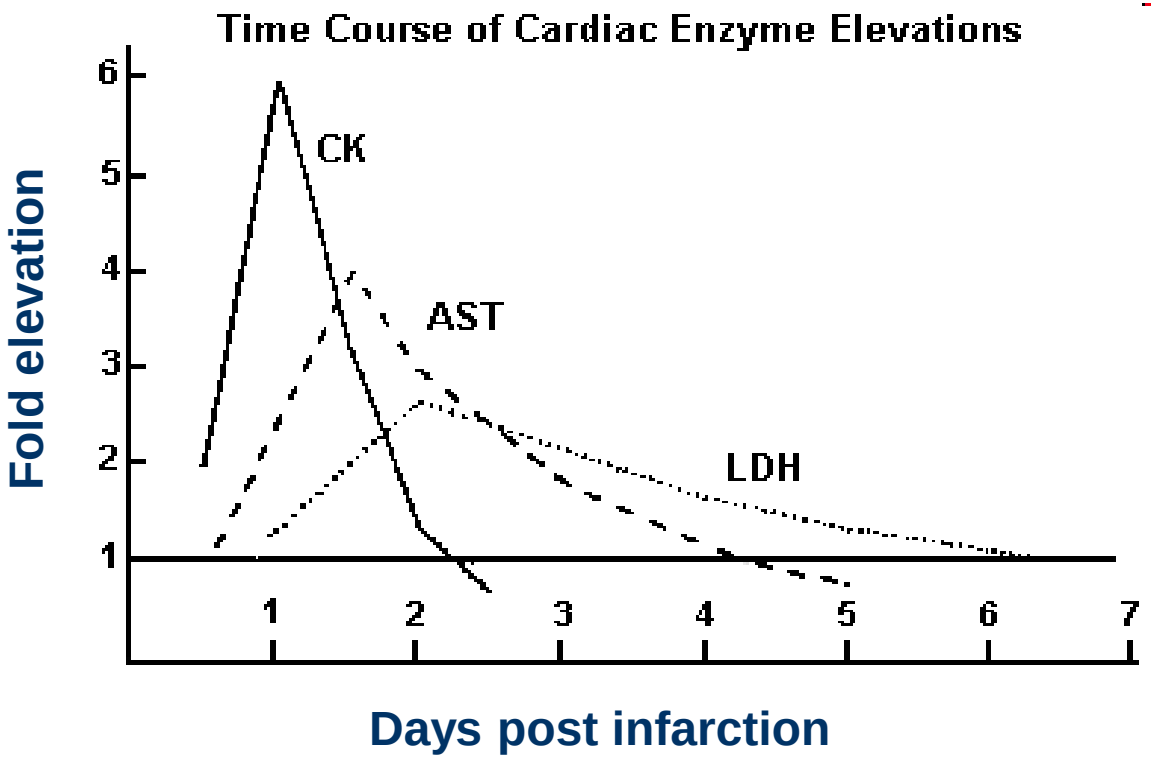


Figure 8–1. Electrophoretic separation of the CK isoenzymes in the serum of (*A*) a healthy individual and (*B*) a patient with acute myocardial infarction. Isoenzymes are numbered on the basis of their electrophoretic mobility, with the most anodal form receiving the lowest number.

Cardiac Disorders

- The **CK** rise the earliest, the **LDH** rise is latest
- The **LDH** elevatithose of **CK** and **AST** ons are present longer than



Abnormal plasma enzyme activities

- *α-Amylase*
  - hydrolyses alpha-bonds of large alpha-linked polysaccharides such as starch and glycogen, yielding glucose and maltose
  - It is used as a marker to detect acute pancreatitis and appendicitis

Abnormal plasma enzyme activities

- *Gamma-glutamyl-transferase (GGT)*

  carboxypeptidase which cleaves C-terminal glutamyl groups and transfers them to peptides and other suitable acceptors

Abnormal plasma enzyme activities

- *Alkaline Phosphatase (ALP)*
  - Widely distributed throughout the body
  - High levels are seen is liver, bone, placenta and intestine
  - Physiological increases are been in pregnancy, due to the placental isoenzyme, and in childhood (when bones are growing), due to the bone isoenzyme.

Diagnostic Significance

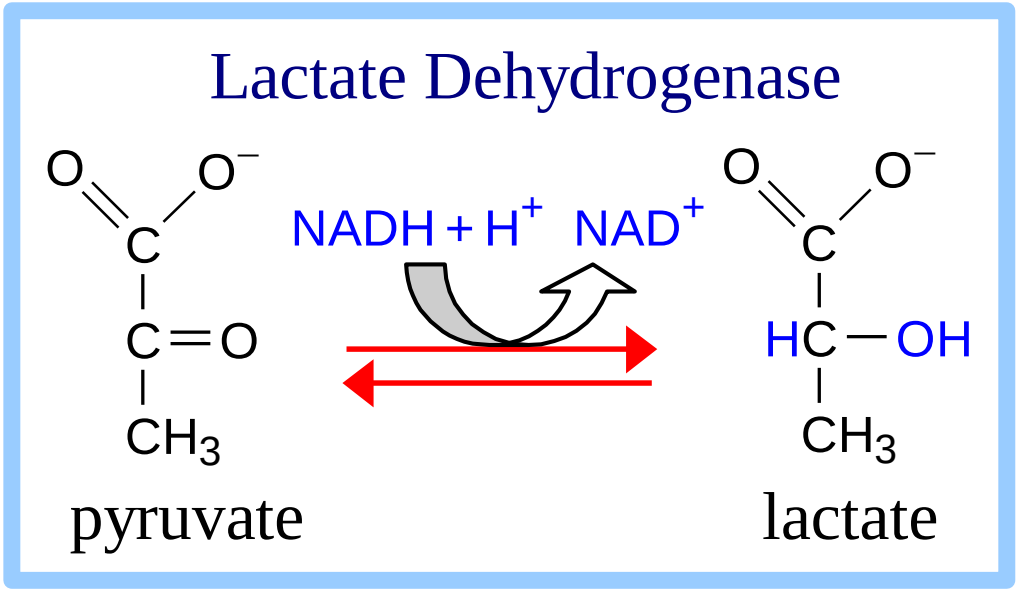
- *Alkaline Phosphatase (ALP)*
  - In hepatobiliary obstruction, hepatocytes lining the biliary ducts induces the ALP synthesis.
  - High levels of ALP is indicative of extrahepatic obstruction rather than intrahepatic obstruction
  - In bones, the enzyme is derived from osteoblasts. Hence increased in bone diseases like rickets, osteomalacia, neoplastic diseases with bone metastates and healing fractures

Abnormal plasma enzyme activities

- *Acid Phosphatase (ACP)*
  - ACP is secreted by prostate cells, RBC, platelets and WBC.
  - The main source of ACP is prostate gland and so can be used as a marker for prostate disease.
  - 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.

Abnormal plasma enzyme activities

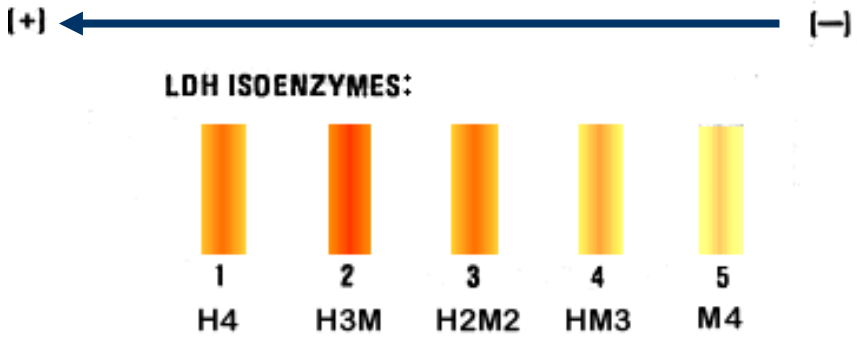
- **LDH**



  - High activities in heart, liver, muscle, kidney, and RBC
  - Lesser amounts: Lung, smooth muscle and brain

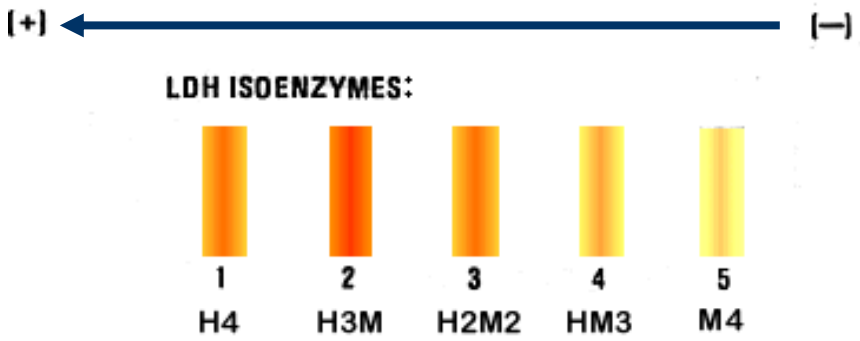
Abnormal plasma enzyme activities

- *LDH*
  - LDH occurs in 5 isoenzymes: LDH1 (H4), LDH2 (H3M), LDH3 (H2M2), LDH4 (HM3) and LDH5 (M4)



Abnormal plasma enzyme activities

- *LDH*
  - LDH occurs in 5 isoenzymes:
    - LDH1 (H4): Cardiac , RBCs
    - LDH2 (H3M): Cardiac , RBCs
    - LDH3 (H2M2): Lung, spleen, pancreas
    - LDH4 (HM3): Hepatic
    - LDH5 (M4): Skeletal muscle



Diagnostic Significance

- *LDH is elevated in a variety of disorders:*
  - in cardiac,
  - hepatic,
  - skeletal muscle,
  - and renal diseases,
  - as well as in several hematologic and neoplastic disorders
- The highest levels of LD-1 are seen in pernicious anemia and hemolytic disorders
- LD-3 with pulmonary involvement
- LD-5 predominates with liver & muscle damage

Diagnostic Significance

- In healthy individuals
  - LD-2 is in highest quantity then LD-1, LD-3, LD-4 and LD-5
- Heart problems:
  - If problem is not MI, both LD1 and LD2 rise, with LD2 being greater than LD1
  - If problem is MI, LD1 is greater than LD2.

Diagnostic Significance

- LDH-6 has been present in patients with arteriosclerotic cardiovascular failure
- Its appearance signifies a grave prognosis and impending death
- It is suggested, that LDH-6 may reflect liver injury secondary to severe circulatory insufficiency

Abnormal plasma enzyme activities

- *Lipase*

  It is highly elevated in acute pancreatitis and this persists for 7-14 days. Thus, lipase remains elevated longer than amylase.

Enzymes as Tumor Markers

Enzyme	Disease
Serum acid phosphatase	Prostrate cancer
Serum Alkaline phosphatase	Metastasis in liver, jaundice due to carcinoma head of pancreas, osteoblastic metastasis in bones
Serum LDH	Advanced malignancies and Leukemias
β- Glucuronidase	Cancer of urinary bladder
Leucine Amino Peptidase (LAP)	Liver cell carcinoma
Neuron specific Enolase	Malignancies of nervous tissue and brain

Major Enzymes of Clinical Significance

Disease	Enzyme
Cardiac Disorders	**AST-LDH1-CK**
Hepatocellular Disorders Viral hepatitis: Hepatitis B & Hepatitis C. Toxic hepatitis: caused by chemicals & Toxins	**ALT-AST-LDH5**
Skeletal Muscle Disorders	**CK-AST**
Biliary tract disorders	**ALP- GGT**
Bone Disorders	**ALP**
Acute Pancreatitis	**Lipase-AMS**
Salivary Gland Inflammation	**AMS**

References

- Victor A Hoffbrand, Paul Moss, J Pettit; ***Essential Haematology***. Essentials Series Blackwell Science, New York; 2008.
- Victor W. Rodwell, David A. Bender, Kathleen M. Botham, Peter J. Kennelly, P. Anthony Weil. ***Harper's Illustrated Biochemistry.*** McGraw-Hill Ed, 31 ed, 2018.