# Clinical Enzymology

# General Properties of Enzymes

**Proteins having catalytic properties.**

**High specificity for substrates.**

**Some enzymes have (isoenzymes)**

**They can be differentiated by their physical or chemical properties e.g., electrophoretic mobility, heat stability, antigenicity.**

**Require coenzymes for activity, e.g., vit. B1 for PDH(**pyruvate dehydrogenase ) **& vit B6 for transaminase activity.**

**Enzymes are also antigens e.g., the M and B subunits of CK can be recognised antigenically.**

**High tissue : plasma activity ratio,**

**e.g., activity of transaminases in liver cells, or**

**CK in muscle cells, is more than 10 000 times the normal plasma levels.**

# Serum enzymes increases may be due to

**Cell death** - this results in a small short-lived increase (e.g., following myocardial infarction).

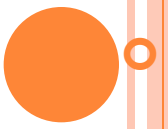
**↑cell membrane permeability** in living cells (due to hypoxia, inflammation, drugs/poisons, cellular swelling) gives rise to a large protracted increase in serum enzymes ( acute viral hepatitis).

**↑ synthesis in a specific cell type**

GGT in liver cells is induced by alcohol or anticonvulsant

ALP in liver cells is induced by obstruction,

LDH is induced in neoplastic tissues).



# Classification of enzymes

**Plasma specific enzymes**:.

**Secreted enzymes**: pancreatic digestive enzymes (amylase, lipase), prostatic acid phosphatase.

**Cellular enzymes**: normally intracellular - leak out when tissue damaged

# FUNCTIONAL PLASMA ENZYMES

- **Present in plasma in higher concentrations than in tissues**
- **Have known functions**
- **Their substrates are present in blood**
- **Mostly synthesized in liver**
- **Usually decreased in case of diseased condition**
- **Examples: clotting factors, lipoprotein lipase, etc.**

# Non-Functional Plasma Enzymes

- **Present in plasma in lower concentrations than in tissues**
- **No known functions in plasma**
- **Their substrates are absent from blood**
- **Synthesized in liver, heart, skeletal muscles, brain, etc**
- **Usually elevated in diseased conditions**
- **Examples: AST, ALT, CPK, LDH**

# Lactate Dehydrogenase

**Conversion of pyruvate to lactate in a reversible manner**

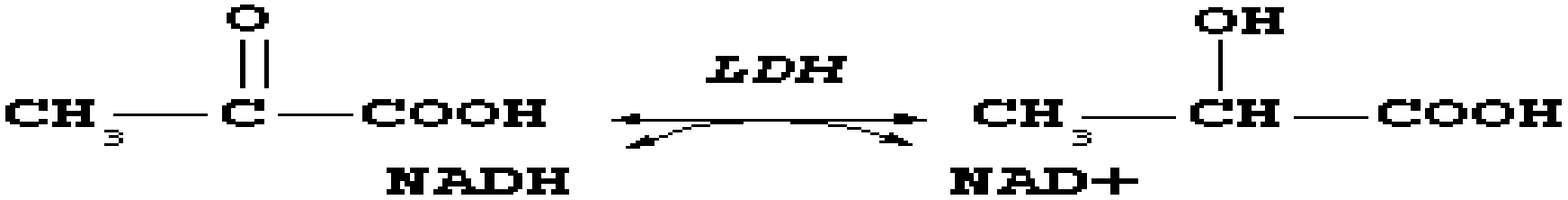
**Isoenzyme, exist in 5 forms.**

**Normal values: 60–250 IU/L**

**Isoenzymic variations in different disease conditions**

**Five forms: LDH1, LDH2, LDH3,LDH4, LDH5**

**LDH is commonly addressed based on their location as hepatic LDH, muscle LDH, cardiac LDH, and so on**



# LDH is a tetramer consisting of sub-units H or M coded by two different genes

| | | | | | |
|---|---|---|---|---|---|
| Thus 5 possible isoenzymes | H H H H ($H_4$) | H H H M ($H_3M$) | H H M M ($H_2M_2$) | H M M M ($HM_3$) | M M M M ($M_4$) |
| | $LDH_1$ Heart, RBCs | $LDH_2$ Reticuloendothelial system | $LDH_3$ lung | $LDH_4$ Kidney, placenta,pancreas | $LDH_5$ liver |

# LDH

**Important biological markers**

**Diseases of live, heart, muscle, and malignancies**

**Elevated in myocardial infarction within 12 h and peaks around 48 h. Returns to normal in 8–14 days. late and long-lasting increase in total LDH**.

**(**The predominant isoenzyme is $LDH_1 > LDH_2$**)**

**Hepatic cell damage** (Increase in total LDH, exclusively due to LD5)

**Also elevated in leukemia, carcinomas, renal and muscular dystrophy**

**Haematological disorders :** elevation in total LDH due to breakdown circulating red cells or red cell precursors in bone marrow.

**Intra-vascular haemolysis** e.g., due to an **auto-immune** disorder, inherited **enzyme deficiency (G-6-PDH, PK)**

Both **LDH1 & LDH2 increased**.

Associated features: ↑ serum unconjugated bilirubin, ↑ urobilinogen, & ↓ haptoglobin.

**Megaloblastic anemia** due to **folate** or vitamin $B_{12}$ deficiency

**Failure of cell division** leads to **cell lysis** and ***enzyme release from the bone marrow*** - predominant increase in $LDH_1$

**Malignant tumors** may manifest an isolated increase in serum LDH due to ↑**glycolytic enzymes by** a wide variety of **neoplasms**.

Typically isoenzyme pattern (**LDH2, LDH3 and LDH4**) due to **expression** of both subunits (**H and M**)

# Creatine Phosphokinase

**Conversion of creatine to phosphocreatine in an energy-dependent reaction**

**Exists in 3 isoenzyme forms**

**Can be differentiated on an electrophoretic gel**

**Exist as dimers (B and M forms) CPK-1, CPK-2,CPK-3**

CK-BB is expressed in all tissues at low levels and has little clinical relevance. Skeletal muscle expresses CK-MM (98%) and low levels of CK-MB (1%).

The myocardium (heart muscle), in contrast, expresses CK-MM at 70% and CK-MB at 25–30%.

**Differ in electrophoretic mobilities (BB is fast moving and MM is slow moving)**

**Normal values: 4–60 IU/L**

# CPK

**CPK-1:**

**Injury to lungs or brain (e.g., brain injury such as trauma, stroke, or bleeding in the brain, lung injury due to a pulmonary embolism, brain cancer, electroconvulsive therapy, pulmonary infarction**

**CPK-2:**

**Levels rise 3–6 h after a heart attack (myocardial infarction)**

**If there is no further damage to the heart muscle, the level peaks at 12–24 h and returns to normal 12–48 h after tissue death.**

**Elevation is observed in myocarditis (inflammation of the heart electrical injuries, trauma to the heart, heart defibrillation, open heart surgery**

early increase of total CK, specifically the MB isoenzyme

# CPK

## CPK-3:

**Elevation is observed in crush injuries of skeletal muscle, muscular dystrophy, myositis (skeletal muscle inflammation), post-electromyography, recent surgery, and strenuous exercise**

# Alkaline Phosphatase

**Hydrolases:** is a hydrolase enzyme responsible for removing phosphate groups from many types of molecules, including nucleotides, proteins, and alkaloids.

**Exists in several isoenzymic forms**

**Six forms identified; 4 of them are key forms: hepatic, bone, placental, and intestinal isoenzymes**

**Differentiated by electrophoresis, chemical inhibition, and heat inactivation assays.**

# ALP

**Non-specific marker enzyme**

**Observed to be elevated under conditions of:**

**Hepatic damage (e.g., liver cirrhosis, hepatocarcinoma, hepatobiliary diseases like obstructive jaundice)**

**Osteoblastic activity in children–Rickets, osteomalacia**

**Hyperparathyroidism**

**Last 6 weeks of pregnancy**

**Oncogenic markers**

**Observed to be decreased during**

**–Defective calcification**

**–Anaemia**

**–Scurvy**

# Acid Phosphatase

**Prostatic ACP is found in the prostate and also in other tissues like the spleen, kidneys, liver, and the pancreas**

**Non-prostatic ACP is observed in the erythrocytes and the leukocytes**

**important marker enzyme for prostate cancer**

**Moderate elevations observed in**

**–Hyperparathyroidism**

**–Breast cancer**

**–Gaucher's disease**

**–Hemolytic anemia**

Recently, ACP assays have been largely replaced by measurement of prostate-specific antigen

# Plasma Amylase

**Breakdown of complex carbohydrates.**

**This enzyme is found in the salivary glands and pancreas, also present in Fallopian tubes and small intestine.**

**Activity at pH 6.9–7.0**

**Maximum in pancreas**

**Useful for the determination of pancreatic disorders**

**Elevation (3–6 times) at 2–12 h after attack and returns to normal in 2–3 days**

# Amylase

**Causes of increase**

in **acute pancreatitis**.

in **renal failure** (Amylase is a small molecule, and is rapidly excreted by the kidneys)

in conditions **with acute abdominal pain** (perforated duodenal **ulcer** - intestinal **obstruction** – ruptured **Fallopian** tube)

Salivary glands disorder (**Mumps**)

**Morphine** adminstration

# CHOLINESTERASE

Hydrolysis of Ach

– True cholinesterase (Cholinesterase I)

- RBCs, lungs, spleen, nerve endings

–Pseudocholinesterase (Cholinesterase II)

- Liver, pancreas, heart, white matter of brain, serum

Important marker for cardiac and liver function

–Decreased in hepatitis, cirrhosis, carcinoma, chronic renal disease, pregnancy, poisoning (organophosphorous)

–Elevated in myocardial infarction (within 3–12h)–Slightly elevated in thyrotoxicosis,

# Adenosine Deaminase (ADA)

Adenosine + H2O -----> inosine + NH3

Measured as a marker of underlying TB infection.

**synthesis** of ADA is **enhanced** in **T and B lymphocytes responding to tuberculous** infection

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ADA is useful in diagnosis of pulmonary TB and Tuberculous ascites