

CLINICAL ENZYMOLOGY

Veterinary Analytical Biochemistry



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- Clinical enzymology is the application of Enzymes in the diagnosis, Prognosis and treatment of diseases.
- Measurements of the activity of digestive enzymes in the body fluids as an aid to diagnosis date back to the early 1900s (Amylase in urine was first studied by Wohlgemuth in 1908)
- Measurements of enzymes activity in serum began in the 1920s and 1930s.
- The scientists who began these studies were Kay, King, Bodansky and Roberts in their work on Alkaline phosphatase.
- In 1955 La Due, Wroblewski and Karmen reported the rise of Aspartate (SGOT) after acute myocardial infarction → from this period a great stimulus was received in the measurement of cellular enzyme released into the plasma as a consequence of tissue damage.

Plasma/ Serum Enzymes

Enzymes in circulation are divided into two groups

- PLASMA SPECIFIC OR PLASMA FUNCTIONAL ENZYMES
- NON- PLASMA SPECIFIC OR PLASMA NON-FUNCTIONAL ENZYMES

Plasma/ Serum Enzymes

(A) PLASMA SPECIFIC OR PLASMA FUNCTIONAL ENZYMES

- Few enzymes in plasma are functionally important & they are involved in blood clotting, lipoprotein metabolism & drug metabolism.
- Synthesized in liver & released into plasma. E.g. Pseudocholinesterase, Lipoprotein lipase, Ceruloplasmin, Blood coagulation enzymes

Clinical significance:

- These are present in **higher** concentrations in plasma than cells.
- These are clinically significant when the serum level is **decreased** below the reference range.

Plasma/ Serum Enzymes

B) NON- PLASMA SPECIFIC OR PLASMA NON-FUNCTIONAL ENZYMES

- Most of the enzymes present in plasma serve **no function** in the plasma.
- Non-functional enzymes are derived cells of tissues & organs (from routine normal destruction of erythrocytes, leukocytes, and other cells).
- Present in high concentrations within cells E.g. AST, ALT, LDH,CK,ALP, amylase

Clinical significance:

- Non-functional enzymes in plasma are present in **low** concentrations.
- These are clinically important when the serum level is **increased** above the reference range.
- **Secretory Enzymes** (function in extracellular space) eg. Digestive Enzymes (amylase, pepsin, trypsin, lipase)
- **Constitutive enzymes** i.e. Enzymes associated with metabolism of cell (LDH, Transaminases , Acid and Alkaline phosphatases, Creatine phosphokinase etc.)

	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 e.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,	ALT, AST, CK, LDH, alkaline phosphatase, acid phosphatase

Localization of enzymes

- **Intracellular**

Lysosome

Nucleus

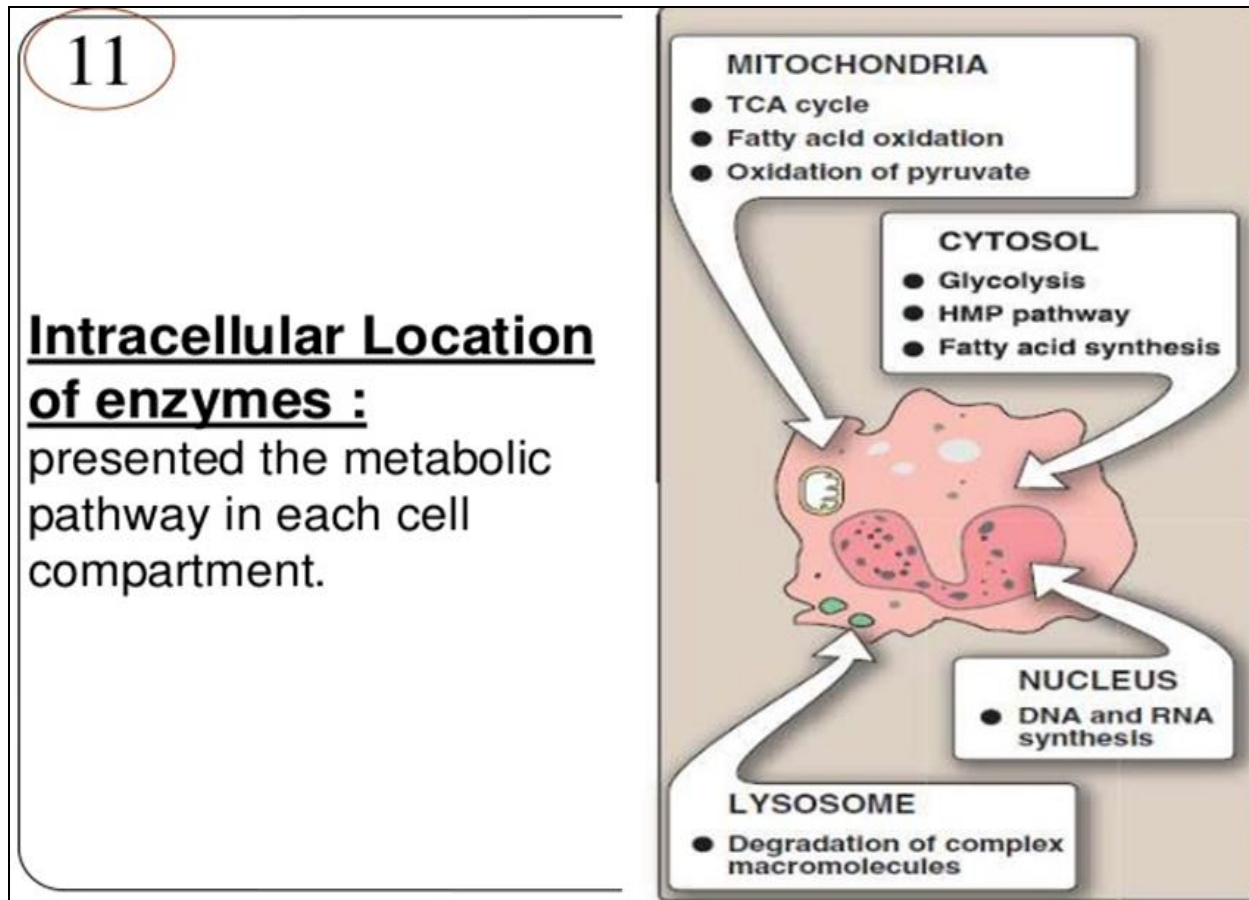
Cytosol

Mitochondria

- **Extracellular**

Secreted and function out from the Cell

Mainly digestive enzymes (alpha amylase)



Clinical Use of Enzymes

- Diagnosis and Prognosis of Diseases -
Diagnostic Markers/ Biomarkers/
Laboratory Analytes
- Analytic Reagents
- Therapeutic Agents

Enzymes in Diagnosis and Prognosis

- Estimation of the activity of **Non-Plasma Specific or Plasma Non-functional Enzymes** is very important for diagnosis and prognosis of the disease.
- Normal serum levels indicate a balance between its synthesis and release in routine cell turnover.
- Serum enzymes are used as markers to detect cellular damage, which helps in diagnosis.

Clinical Significance of Enzyme Estimation

Single or serial assay of serum activity of a selected enzyme

- 1) Helps in making the diagnosis/differential diagnosis/ early detection of a disease.
- 2) Helps in ascertaining prognosis of a disease.
- 3) Helps in ascertaining the response to drugs in a disease.
- 4) Also help in ascertaining the time course of disease.

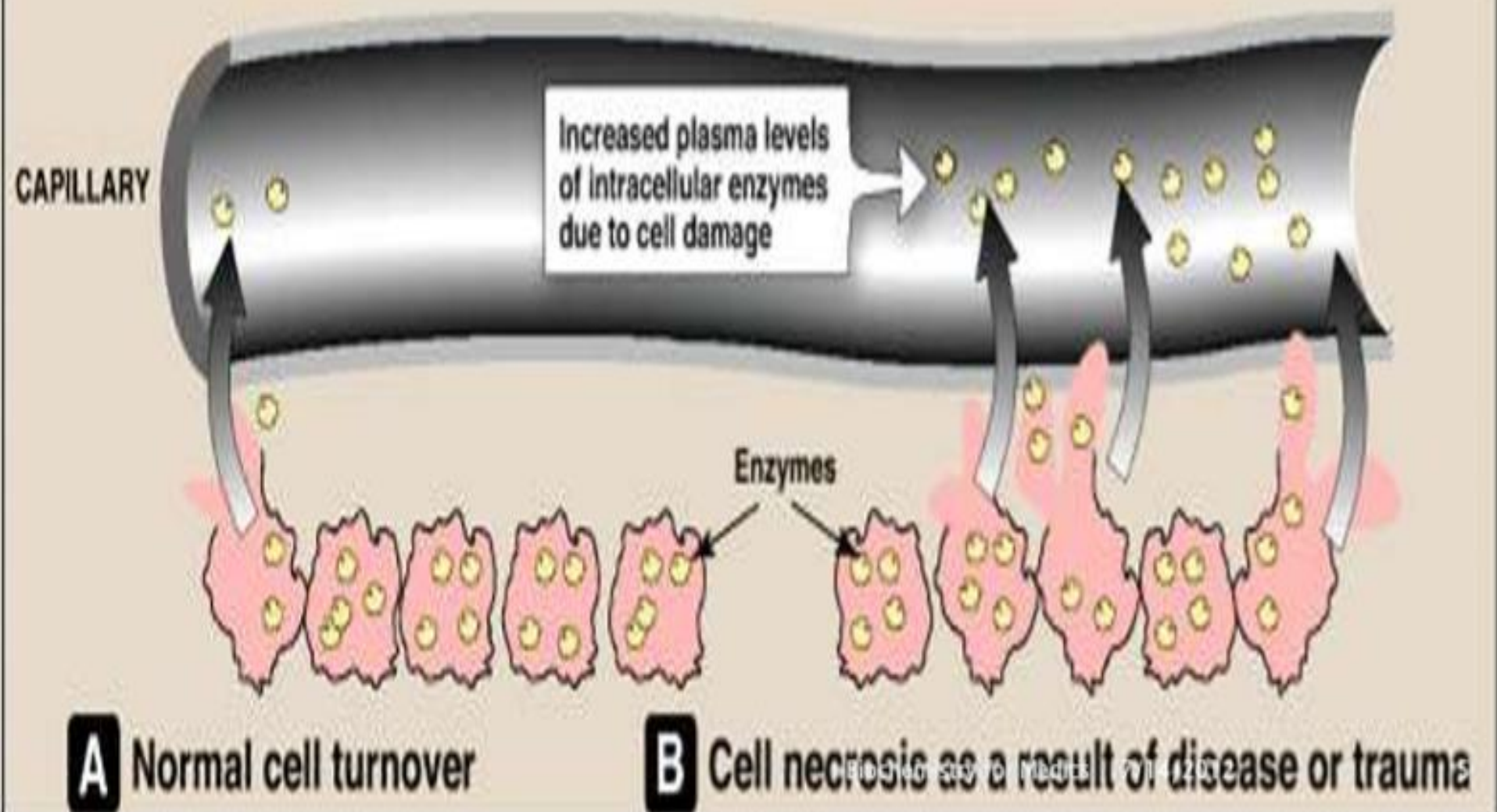
Enzymes as Diagnostic Markers

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

- A) Increased release i. Necrosis of cell ii. Increased permeability of cell without gross cellular damage iii. Increased production of enzyme within the cell resulting in increase in serum by overflow iv. Increase in tissue source of enzyme as in malignancy
- B) Impaired disposition i. Increased levels in obstructive jaundice ii. Increased levels in renal failure

Decreased Serum Levels

- A. Decreased formation which may be i. Genetic ii. Acquired
- B. Enzyme inhibition
- C. Lack of cofactors



Possible mechanisms responsible for increased serum levels

Enzymes as Diagnostic Markers

<i>Serum enzyme (elevated)</i>	<i>Disease (most important)</i>
Amylase	Acute pancreatitis
Serum glutamate pyruvate transaminase (SGPT)	Liver diseases (hepatitis)
Serum glutamate oxaloacetate transaminase (SGOT)	Heart attacks (myocardial infarction)
Alkaline phosphatase	Rickets, obstructive jaundice
Acid phosphatase	Cancer of prostate gland
Lactate dehydrogenase (LDH)	Heart attacks, liver diseases
Creatine phosphokinase (CPK)	Myocardial infarction (early marker)
Aldolase	Muscular dystrophy
5'-Nucleotidase	Hepatitis
γ -Glutamyl transpeptidase (GGT)	Alcoholism

Enzyme	Reference values	Disease(s) in which decreased
Amylase	80–180 Somogyi units/dl	Liver diseases
Pseudocholinesterase (ChE II)	10–20 IU/dl	Viral hepatitis, malnutrition, liver cancer, cirrhosis of liver
Ceruloplasmin	20–50 mg/dl	Wilson's disease (hepatolenticular degeneration)
Glucose 6-phosphate dehydrogenase (G6PD) in RBC	120–280 IU/ 10^{12} RBC	Congenital deficiency with hemolytic anemia

<i>Disease/enzyme(s)</i>	<i>Significance</i>
I Myocardial infarction (Refer Table 6.12 and Fig. 6.16)	
II Hepatic disease Alanine transaminase (ALT) Aspartate transaminase (AST) γ -Glutamyl transpeptidase (GGT) 5'-Nucleotidase	Markedly elevated in viral hepatitis Increased in liver diseases. Significantly elevated in obstructive jaundice (gall stones). Markedly increased in alcoholic liver diseases. Elevated in hepatic cholestasis.
III Muscle disease Creatine kinase (CK) Aldolase (ALD) Aspartate transaminase (AST)	Markedly increased in muscle disease (CK-MM more sensitive). Early marker (not specific) Significantly increased, although not specific.
IV Bone disease Alkaline phosphatase (ALP)	Increased in rickets and Paget's disease.
V Pancreatic disease Amylase Lipase	Significantly elevated in acute pancreatitis. Markedly increased in acute pancreatitis.
VI Prostate cancer Acid phosphatase (ACP) Prostate specific antigen (PSA)	Marker enzyme for prostate cancer. Significantly elevated in prostate cancer (not an enzyme).

Isoenzymes

- The multiple forms of an enzyme catalysing the same reaction are isoenzymes or isozymes.
- They, however, differ in their physical and chemical properties which include the structure, electrophoretic and immunological properties, K_m and V_{max} values, pH optimum, relative susceptibility to inhibitors, degree of denaturation, Amino acid sequence and their composition.

Enzymes as Diagnostic Markers

Enzyme estimations are helpful in the diagnosis of –

- 1) Myocardial Infarction
- 2) Liver diseases
- 3) Muscle diseases
- 4) Bone diseases
- 5) Cancers
- 6) GI Tract diseases

Diagnosis of Myocardial Infarction (MI/AMI)

- The diagnosis of Acute Myocardial Infarction (AMI) is usually predicated on the WHO criteria of chest pain, ECG changes, and increases in biochemical markers of myocardial injury.
- The ECG is specific for AMI, but lacks sensitivity. In contrast, **biochemical markers** have excellent sensitivity for diagnosing AMI.

Serum enzymes in Myocardial Infarction

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

- 1) Creatine Phosphokinase/ Creatine kinase (CPK2- MB)- earliest marker
- 2) Aspartate transaminase (AST/ SGOT) and
- 3) Lactate dehydrogenase (LDH1)

Non-enzymatic biomarkers in MI:

Cardiac troponins (CT) Although not enzymes, the proteins cardiac troponins are highly useful for the early diagnosis of MI. Among these, **troponin I** (inhibitory element of actomyosin ATPase) and **troponin T** (tropomyosin binding element) are important. The protein **myoglobin** is also an early marker for the diagnosis of MI. However, it is not specific to cardiac diseases. High serum concentration of brain natriuretic peptide is a marker for congestive cardiac failure.

TABLE 6.12 Summary of diagnostic markers used for the evaluation of acute myocardial infarction

<i>Diagnostic marker</i>	<i>Time of peak elevation</i>	<i>Time of return to normal level</i>	<i>Diagnostic importance</i>
Myoglobin	4-6 hrs	20-25 hrs	Earliest marker, however not cardiac specific.
Cardiac troponin I	12-24 hrs	5-9 days	Early marker and cardiac specific.
Cardiac troponin T	18-36 hrs	5-14 days	Relatively early marker and cardiac specific. However, elevated in other degenerative diseases.
Creatine phosphokinase (MB)	20-30 hrs	24-48 hrs	Cardiac specific and early marker.
Lactate dehydrogenase (LDH I)	48-72 hrs	10-15 days	Relatively late marker and cardiac specific.
Aspartate transaminase	30-48 hrs	4-6 days	Not cardiac specific.

Isoenzymes of CK/CPK

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 isoenzyme is a **dimer** composed of two protomers '**M**' (for muscles) and '**B**' (for Brain).
- These isoenzymes can be separated by Electrophoresis or by Ion exchange Chromatography.

Isoenzyme name	Composition	Present in	Elevated in	Electrophoretic mobility	Mean percentage in blood
CK-1	BB	Brain	CNS diseases	Maximum	0%
CK-2	MB	Myocardium / Heart	Acute myocardial infarction	Intermediate	0-3%
CK-3	MM	Skeletal muscle, Myocardium	-	Least	97-100%

Serum Enzymes in Liver Diseases

Serum enzyme tests can be grouped into two categories:

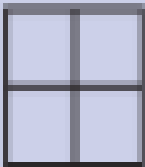
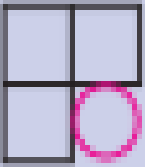
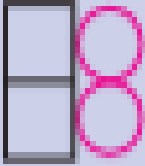
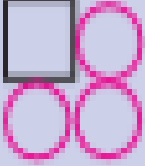
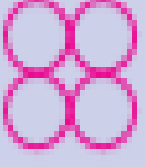
- enzymes whose elevation in serum reflects **damage to hepatocytes** i.e. ALT, AST & LDH5
- enzymes whose elevation in **serum reflects cholestasis** i.e. Alkaline phosphatase, 5'-nucleotidase, and γ -Glutamyl transpeptidase (GGT)
- In viral hepatitis Rapid rise in transaminases (AST & ALT) in serum occurs even before bilirubin rise is seen

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- Biomarker of MI
- LDH₅- Biomarker of Liver Diseases

TABLE 6.11 Lactate dehydrogenase (LDH) isoenzymes and their characteristics

<i>Isoenzyme</i>	<i>Subunit constitution</i>	<i>Principal tissue of origin</i>	<i>Electrophoretic mobility</i>	<i>Whether destroyed by heat (at 60°C)</i>	<i>Percentage of normal serum in humans</i>
LDH ₁	H ₄ 	Heart and RBC	Fastest	No	25%
LDH ₂	H ₃ M 	Heart and RBC	Faster	No	35%
LDH ₃	H ₂ M ₂ 	Brain and kidney	Fast	Partially	27%
LDH ₄	HM ₃ 	Liver and skeletal muscle	Slow	Yes	8%
LDH ₅	M ₄ 	Skeletal muscle and liver	Slowest	Yes	5%

LACTATE DEHYDROGENASE (LDH) ISOENZYMES—TISSUE LOCALIZATION AND SOURCES OF ELEVATION

ISOENZYME	TISSUE	DISORDER
LDH-1 (HHHH)	Heart	Myocardial infarction
	Red blood cells	Hemolytic anemia
LDH-2 (HHHM)	Heart	Megaloblastic anemia
	Red blood cells	Acute renal infarct
		Hemolyzed specimen
LDH-3 (HHMM)	Lung	Pulmonary embolism
	Lymphocytes	Extensive
	Spleen	Pulmonary pneumonia
	Pancreas	Lymphocytosis
		Acute pancreatitis
		Carcinoma
LDH-4 (HMMM)	Liver	Hepatic injury or inflammation
LDH-5 (MMMM)	Skeletal muscle	Skeletal muscle injury

Serum enzymes in Bone Diseases

- **Alkaline Phosphatase (ALP)** - Rises in Rickets, osteomalacia, hyperparathyroidism and in Paget's disease. Also rises in primary and secondary malignancies of bones.
- **Acid Phosphatase (ACP)** - Highly increased in bony metastasis of carcinoma prostate

Isoenzymes of Alkaline phosphatase (ALP)

- **Six** isoenzymes of alkaline phosphatase (ALP) have been identified.
- ALP is a monomer, the isoenzymes are due to the difference in the carbohydrate content (sialic acid residues).
- The most important ALP isoenzymes are D1-ALP, D2-heat labile ALP, D2-heat stable ALP, pre-E ALP, J-ALP etc.
- Increase in D2-heat labile ALP suggests hepatitis whereas pre E-ALP indicates bone diseases.

Isoenzymes of Alcohol dehydrogenase

- Alcohol dehydrogenase (ADH) has **two heterodimer isoenzymes**.
- Among the white Americans and Europeans, DE1 isoenzyme is predominant whereas in Japanese and Chinese (Orientals) DE2 is mostly present.
- The isomer DE2 more rapidly converts alcohol to acetaldehyde. Accumulation of acetaldehyde is associated with tachycardia (increase in heart rate) and facial flushing among Orientals which is not commonly seen in whites.
- It is believed that Japanese and Chinese have increased sensitivity to alcohol due to the presence of DE2–isoenzyme of ADH.

Serum enzymes in Muscle diseases

- **Aldolase** - Moderate increase in Dermatomyositis, muscular dystrophies, highest values are seen in Deuchenne type of muscular dystrophies
- **CPK** - Elevated in neurogenic muscular dystrophies, highest values are seen in Deuchenne type of muscular dystrophies
- **AST/ SGOT**

Serum enzymes in 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 U/l are seen in acute pancreatitis. Also reported high in perforated duodenal and peptic ulcers and intestinal obstruction

6) Enzymes as tumor markers

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

Enzymes as Analytical Reagents

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

Enzymes as therapeutic agents

Enzyme	Therapeutic Application
Streptokinase/Urokinase	Acute MI, Pulmonary embolism, DVT(Deep vein thrombosis)
Trypsin, lipase and amylase	Pancreatic insufficiency
Asparaginase/Glutaminase	Acute lymphoblastic leukemias
Hyaluronidase	Enhanced local anesthesia and for easy diffusion of fluids
Papain	Anti inflammatory
Chymotrypsin	Pain killer and Anti inflammatory
Alpha- 1 Antitrypsin	Deficiency and Emphysema
Serrapeptidase	Pain killer and Anti inflammatory

Industrial Applications of Enzymes

- Rennin - Cheese preparation
- Glucose isomerase - Production of high fructose syrup
- Alpha -Amylase - In food industry
- Proteases - Washing powder

THANKS