Therapeutic and diagnostic applications of enzymes and isoenzymes

Clinical significance of enzymes:

Enzyme units

International units (one micromole of substrate conversion / per minute/l of serum sample (IU /l)

Standard international : /SYSTEM INTERNATIONAL /KATAL (catalytic activity) number of moles of substrate transformed /second /l of sample KATOr K(IU= 60 MICROKATAL)

Techniques for estimation of enzymes

- ✓ Colorimetry /spectrophotometry
- ✓ Fluorometry
- ✓ RIA
- ✓ ELISA
- ✓ Chemiluminescence

Factors affecting enzyme estimations

- ✓ Age
- ✓ Sex
- ✓ Pregnancy
- ✓ Time of sampling
- ✓ Temperature
- ✓ p H
- ✓ Substrate concentration
- ✓ Product concentration
- ✓ Presence of drugs in plasma

Therefore strict control on estimation of enzyme is needed.

Enzyme appear in plasma by 3 ways

- ➢ Functional plasma enzymes
- ➢ Non Functional plasma enzymes
- ➢ Obstruction to secretory pathway

Functional plasma enzymes	**Non Functional plasma enzymes**
High concentration in plasma in physiological conditions	low concentration in plasma in physiological conditions
Low concentration in tissue in physiological conditions	High concentration in tissue in physiological conditions
low concentration in plasma in pathological conditions decreased synthesis by damaged liver cells	High concentration in plasma in pathological conditions(tissue damage)
eg Psuedocholine esterase, ipase	SGPT , SGOT, LDH, CPK

Obstruction to Secretory Pathway

Physiological conditions balance between synthesis & release

Pathological conditions loss of balance between synthesis & release

CONDITIONS RELATED TO INCREASED SERUM ENZYME LEVELS

Significant elevation in serum levels of enzymes is observed under following conditions :

1. Cellular damaged
2. Increase rate of cell turnover
3. Proliferation of cells
4. Increased synthesis

PRINCIPLE OF ESTIMATION OF ENZYMES BY COLORIMETRY /SPECTROMETRY

A:Buffered Substrate + Serum (Enzyme) → Product

Product + Chemical Reagent → Colored Complex

Measurement of optical density of colored complex

B :NADH dependent estimations using UV light as a source : increase or decrease of Absorbance

Clinical significance of Enzymes:

Enzyme	Function	Normal range	Occurrence	Clinical significance
Aldolase	F1,6 Phosphate to Triose Phosphate	1.5-7.2micromoles /l	Myocardium,Skeletal muscles and liver	Sensitive index in muscle wasting Muscular dystrophy Poliomyelitis Myasthenia Gravis
α-Amylase	Starch to Maltose	Serum – 50-120 IU/L URINE < 375 IU /L	Salivary gland Pancreas placenta	MUMPS > 1000IU/L Ectopic pregnancy Acute pancreatitis
Acid phosphatase (optimum p H)	Hydrolysis of esters of phosphoric acid	2.5 – 12 IU /L	Prostrate, RBC, WBC Platelet semen	Prostrate Cancer Forensic rape case
PSA-PROSTRATE SENTSITIVE ANTIGEN	(Serine protease)	1 -5 Microgram /L	Prostrate semen (LIQUIFICATION OF COAGULUM)	Prostrate Cancer (>10Microgram/L)before rectal examination beningn Prostrate Enlargement (5- 10

				Microgram/L)

ASPARTATE TRANSAMINASE (AST/SGOT)

PRINCIPLE OF ESTIMATION OF SGOT

α KGA +Aspartate ↔ Glutamate + Oxaloacetate

↓

Pyruvate

Pyruvate +DNPH □ BROWN COLOR COMPLEX (Alkaline pH)

Clinical Significance of SGOT

- ✓ Normal range of Serum SGOT = 2-20 IU/L
- ✓ Significant increase observed in Myocardial Infarction
- ✓ Moderate increase observed in liver disease including Hepatoma
- ✓ Isoenzymes –Cytosolic (Mild Injury)/Mitochondrial (Severe Injury)

ALANINE TRANSAMINASE (ALT/SGPT)

PRINCIPLE OF ESTIMATION OF SGPT

α KGA +Alanine ↔ Glutamate +Pyruvate

Pyruvate +DNPH □ BROWN COLOR COMPLEX (alkaline medium)

Clinical Significance of SGPT

- ✓ Normal range of SGPT =(13-40 IU/L)
- ✓ Significant increase observed in ACUTE HEPITITIS (100-1000 IU/L)
- ✓ Moderate increase observed in liver disease including Hepatoma
- ✓ Increase in Serum ALT>>>Serum AST is observed before clinical manifestation
- ✓ Chronic Liver Diseases (25-100 Iu/L) /Cirrhosis /Malignancy
- ✓ Bad prognosis is indicated by SUDDEN FALL in serum levels of SGPT

Enzymes indicated Liver Diseases

Hepatic Disease	**Enzyme of choice for diagnosis**
Parenchymal diseases	SGPT
Liver dysfunction, cholestasis	Nucleotide Phosphatase
Obstructive Jaundice	Alkaline Phosphatase

Alcoholic liver	Gamma Glutamyl Trans peptidase (γGT)
Hepatitis	LDH 5
Alcoholic liver	Alcohol Dehydrogenase

ENZYMES INDICATED IN HEART DISEASES

Enzymes	Pattern in heart disease (AMI)
CPK -MB	**First enzyme to increase in AMI**
Aspartate Amino Transferase	increase after CPK, half life 4-5days
Lactate Dehydrogenase (LDH1)	Last enzyme to get elevated in AMI ,significant half life

ENZYMES INDICATED IN MUSCLE

Enzyme which show significant increase in muscle disease

- **Creatinine Phosphokinase (CPK -MM)**
- SGPT
- **Aldolase (non specific)**

ENZYME INDICATED IN BONE DISEASE

Serum Alkaline Phosphatase increases significantly in paget Disease, Rickets ,Hyperthyroidism.

ENZYME INDICATED IN PROSTRATE DISEASE

- ✓ Acid phosphatase (Tartaric acid labile) - Prostrate Cancer (Malignant / Benign)
- ✓ Diagnosis conformed by estimation of PROSTRATE SPECIFIC ANTIGEN (PSA)–Prostrate Cancer (Malignant / Benign)

ENZYME INDICATED IN KIDNEY DISEASE

- ✧ Beta Glucuronidase –for diagnosis of urinary bladder diseases

Therapeutic uses of Enzymes:

Enzyme	**Therapeutic use**
Asparginase	Acute Lymphatic Leukemia (cells need Asparagine for its growth)
Streptokinase	Lyse intracellular clot
Uro kinase	Lyse Intracellular Clot
Plasminogen	Plasmin /Clot lysis
Streptokinase	DNA ase applied locally
Hyaluronidase	Enhance local anesthesia
Pancreatic (Lipase & Trypsin)	Pancreatic insufficiency – oral administration
Papain	Anti-inflammatory
Alpha Anti Trypsin	Emphysema

Diagnostic uses of enzymes:

PRINCIPLE :

SUBSTRATE (SERUM)+ENZYME to PRODUCT -CHEMICAL REAGENT –OD OF COLOR COMPLEX (α CONC OF SUBSTRATE)

ENZYME FOR DIAGNOSTIC PURPOSE	ESTIMATION OF
UREASE	UREA
URICASE	URIC ACID
GLUCOSE OXIDASE	GLUCOSE
PERIOXIDASE	GLUCOSE /CHOLESTEROL
HEXOKINASE	GLUCOSE
CHOLESTEROL OXIDASE	CHOLESTEROL
LIPASE	TRIGLYCERIDE
HORSE RADDISH PERROXIDASE	ELISA
ALKALINE PHOSPHATASE	ELISA
RESTRICTION ENDONUCLEASE	SOURTHEN BLOT
REVERSE TRANSCRIPTASE	POLYMERASE CHAIN REACTION

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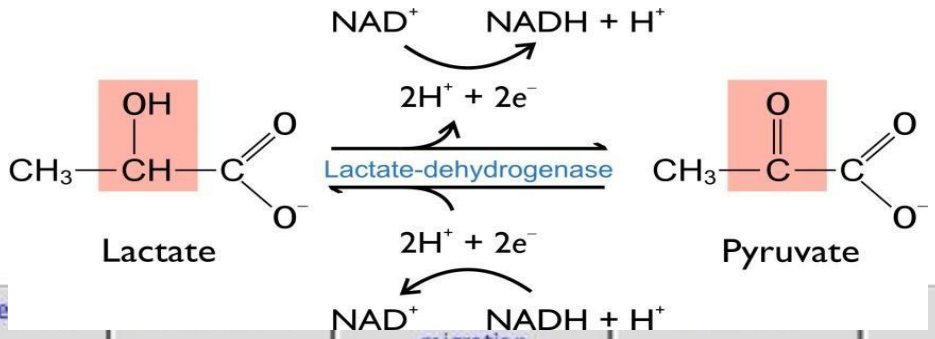
ISOENZYMES

Definition : Enzymes occurring in different molecular forms which differ in their physiochemical properties but catalyze the same reaction

Physio-chemical properties of Isoenzymes
1. differential mobility on electrophoresis
2. differential mobility in column chromatography
3. differential kinetic properties

Km, V max, Optimum temperature, Optimum pH, Relative sensitivity to inhibitors and degree of denaturation.

Iso enzymes of Lactate dehydrogenase :



Isoenzyme		migration		Elevated in
LDH 1 Heat resistant	(H_4)	Fastest moving	Myocardium, RBC, kidney	myocardial infarction
LDH2 Heat resistant	(H_3M_1)		Myocardium, RBC, kidney	Kidney disease, megaloblastic anemia
LDH3	(H_2M_2)		brain	Leukemia, malignancy
LDH4 Heat labile	(H_1M_3)		Lung, spleen	Pulmonary infarction
LDH5 Heat labile Inhibited by urea	(M_4)	Slowest moving	Skeletal muscle, Liver	Skeletal muscle and liver diseases