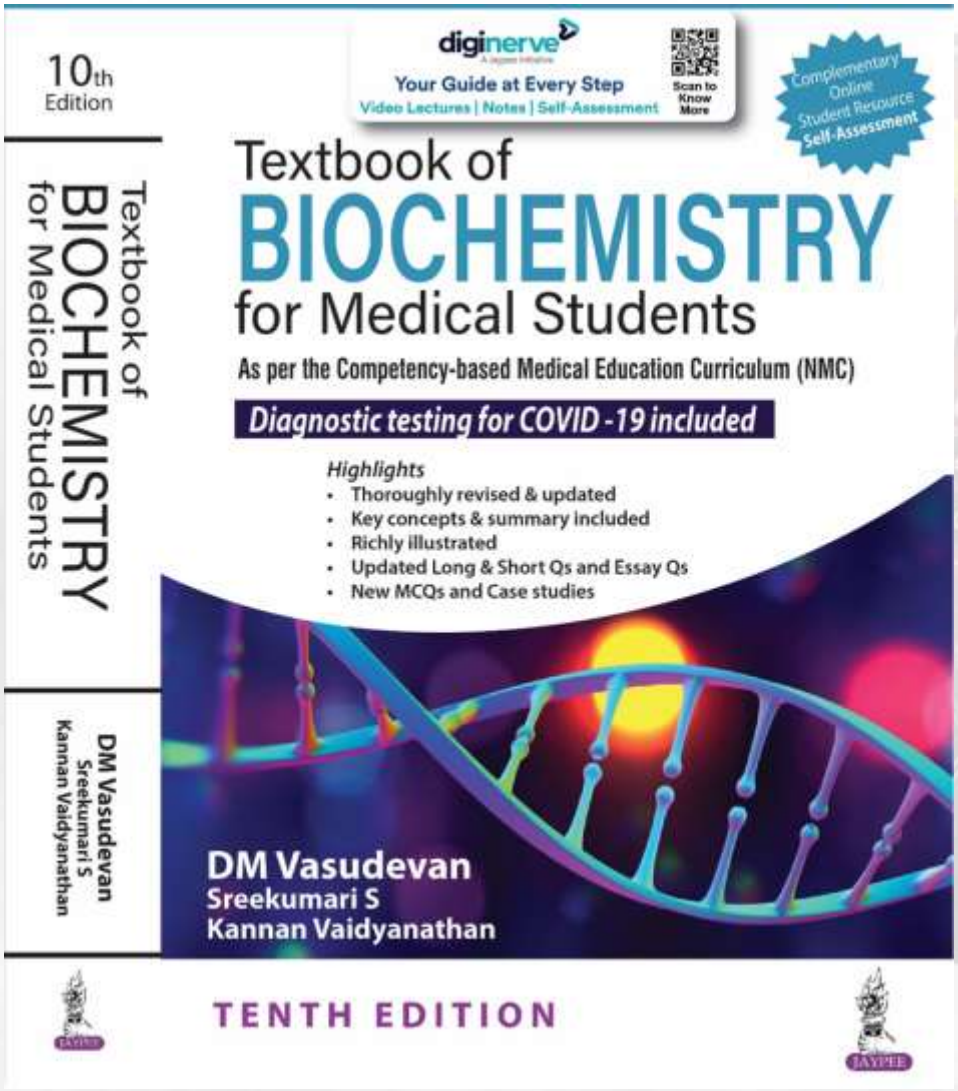


Chapter 4B: Clinical Enzymology

Textbook of
BIOCHEMISTRY
for Medical Students

By DM Vasudevan, *et al.*

TENTH EDITION

Isoenzymes

They are **physically distinct forms of the same enzyme activity**. Different molecular forms of the same enzyme synthesized from various tissues are called isoenzymes.

Hence study of isoenzymes is very useful to understand diseases of different organs.

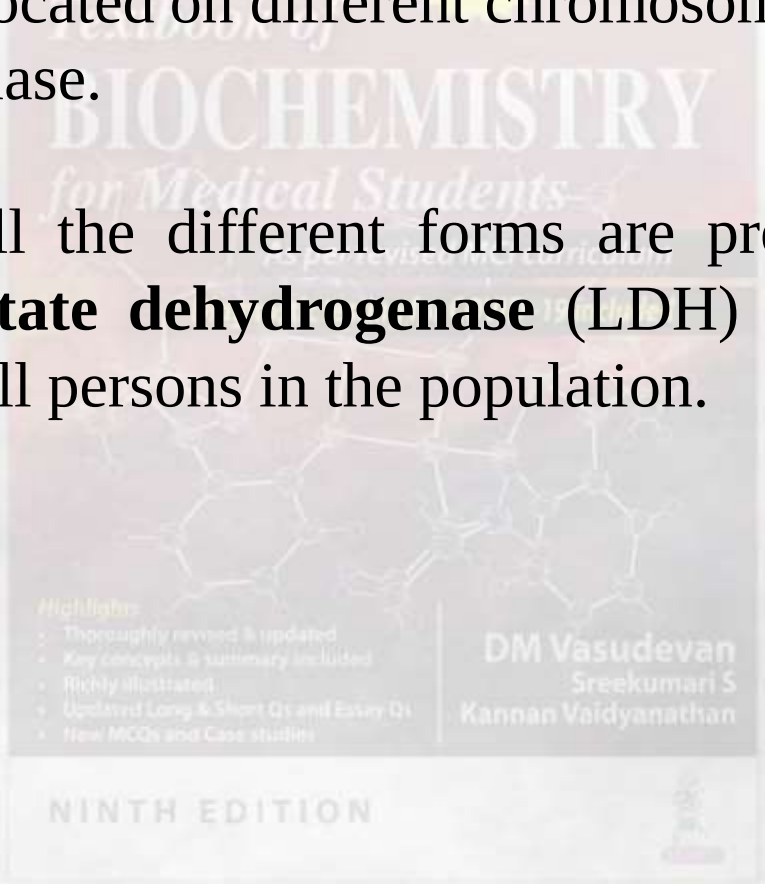
If the subunits are all the same, the protein is a **homomultimer** represented by a single gene.

If the subunits are different, the protein is said to be a **heteromultimer**, produced by different genes.

Isoenzymes may be Formed in Different Ways

They may be products of different genes (more than one locus) in which case they are known as **true isoenzymes**.
The genes may be located on different chromosomes, e.g. salivary and pancreatic amylase.

In certain cases, all the different forms are present in the same individual, e.g. **lactate dehydrogenase** (LDH) has 5 isoenzymes and all are seen in all persons in the population.



Isoenzymes may be Formed in Different Ways

The same locus of the gene may have different alleles (alternate forms). Such allelic isoenzymes are called **allozymes**. In this case, only one form will be present in one individual; but all the different forms will be seen in total population. For example, more than 400 distinct forms of **glucose-6-phosphate dehydrogenase** (GPD) have been identified; all of them are produced by the same locus on the X-chromosome.

When isoenzymes due to variation at a single locus occur with appreciable frequency (more than 1% in population), it is said to be **polymorphism.**

Molecular heterogeneity of enzymes may also be produced after the protein is synthesized (post-translational modification). These are called **isoforms**, e.g. sialic acid content in alkaline phosphatase (ALP) isoenzymes. Different types of isoforms may be seen in the same individual.

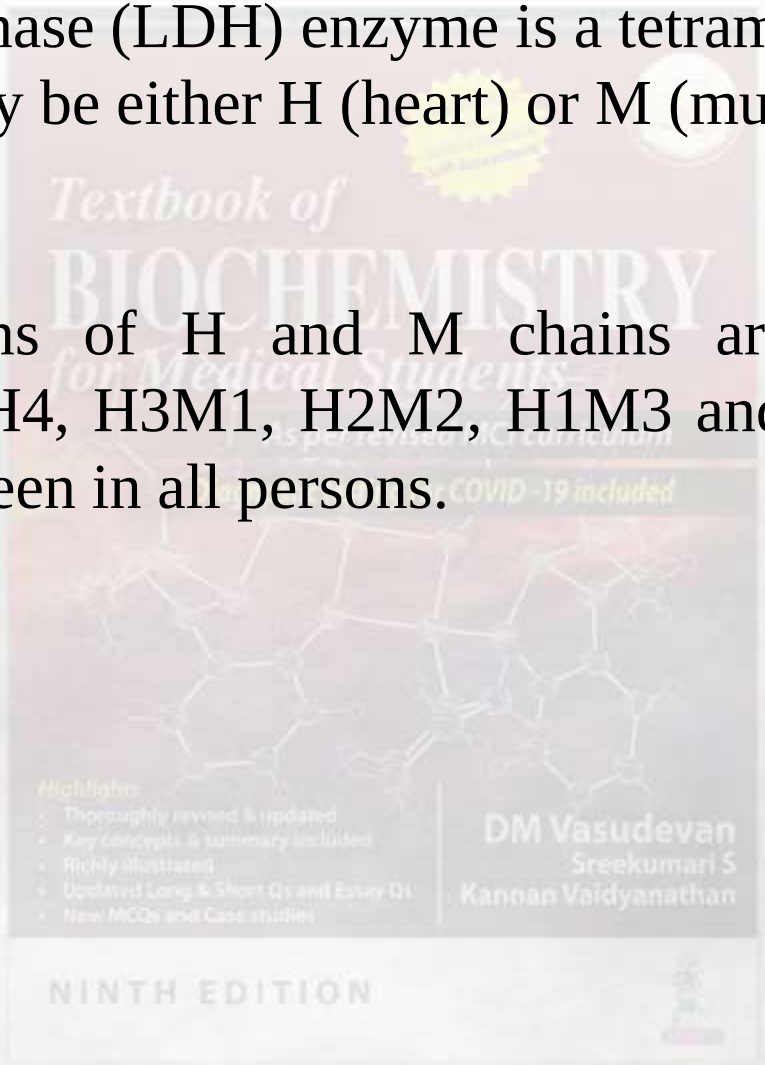
Identification of Isoenzymes

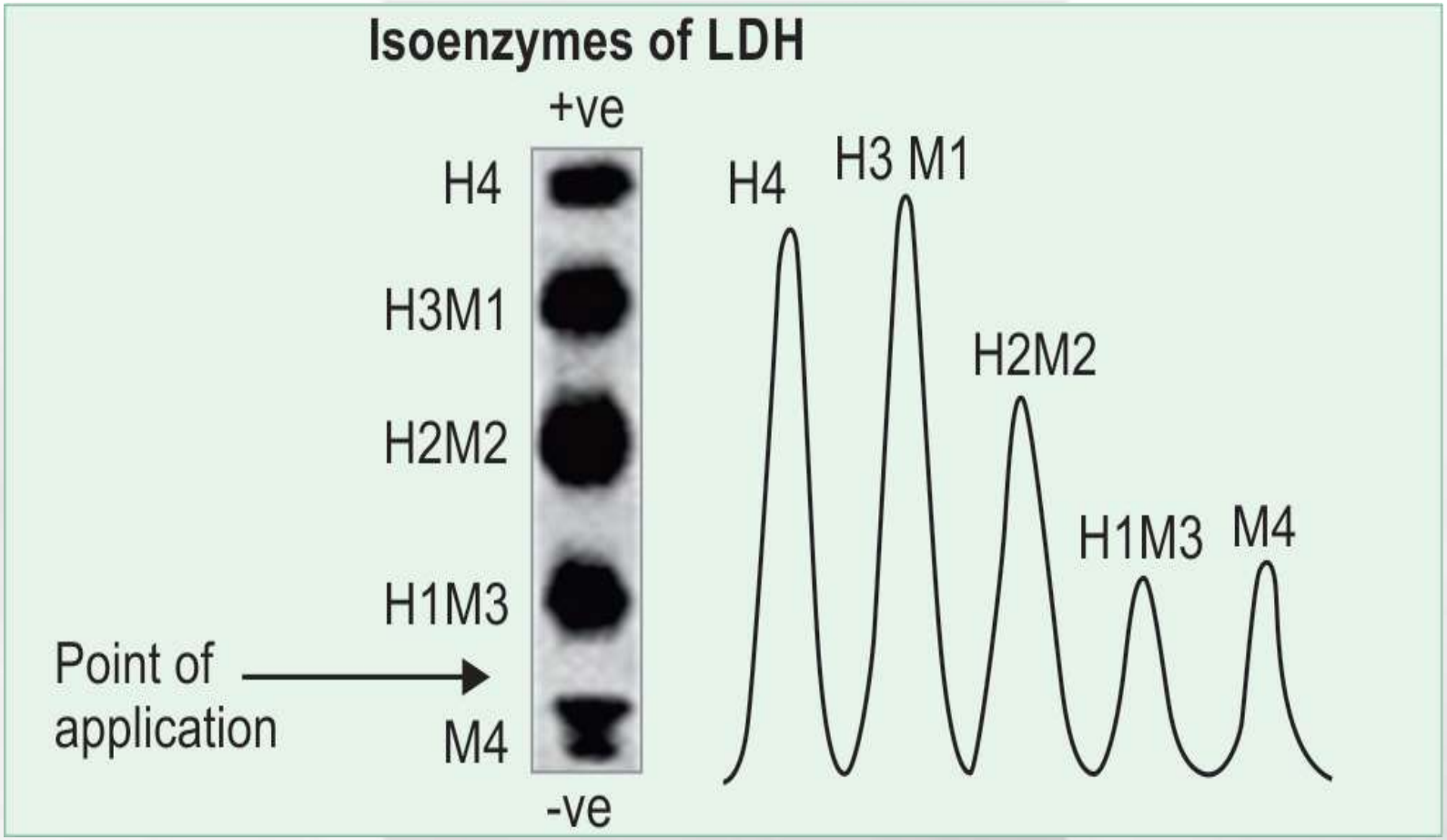
- **In electrophoresis**, the isoenzymes have different mobility. LDH and CK isoenzymes can be separated by electrophoresis
- **Heat stability**: One of the isoenzymes may be easily denatured by heat, e.g. bone isoenzyme of ALP (BALP).
- **Inhibitors**: One of the isoenzymes may be sensitive to one inhibitor, e.g. tartrate labile ACP.
- **The Km value** or substrate specificity may be different for isoenzymes, e.g. glucokinase has high Km and hexokinase has low Km for glucose.
- **Cofactor** requirements may be different for isoenzymes. Mitochondrial isocitrate dehydrogenase is NAD+ dependent and the cytoplasmic isoenzyme is NADP+ dependent.
- **Tissue** localization may be different for isoenzymes. H4 form of LDH is present in heart, while M4 variety is seen in skeletal muscle.
- **Specific antibodies** may identify different types of isoenzymes. For example, CK isoenzymes are separated by antibodies

Isoenzymes of LDH

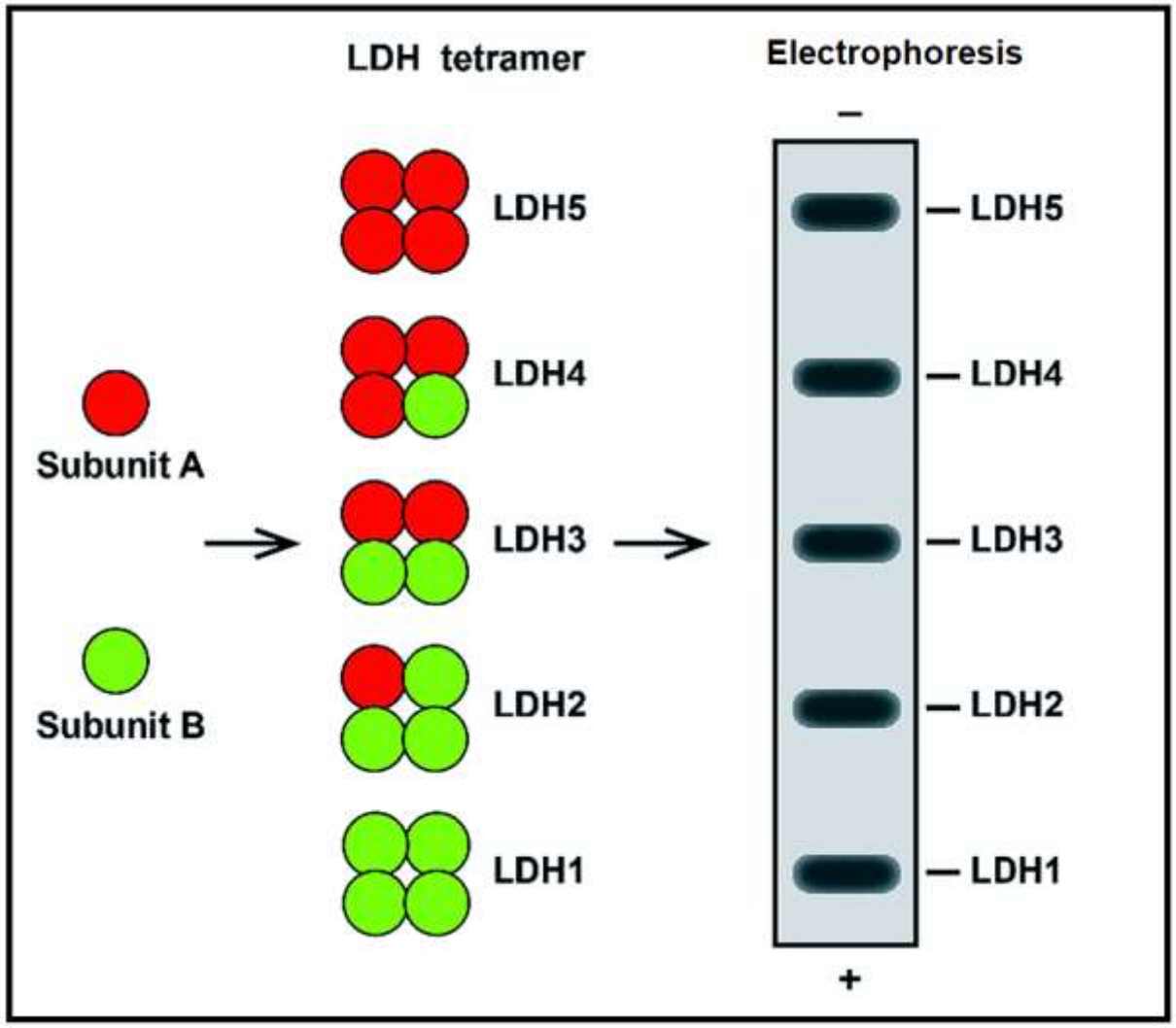
Lactate dehydrogenase (LDH) enzyme is a tetramer with 4 subunits. But the subunit may be either H (heart) or M (muscle) polypeptide chains.

So 5 combinations of H and M chains are possible. These combinations are H4, H3M1, H2M2, H1M3 and M4 varieties. All these 5 forms are seen in all persons.





Electrophoresis pattern of LDH isoenzymes.



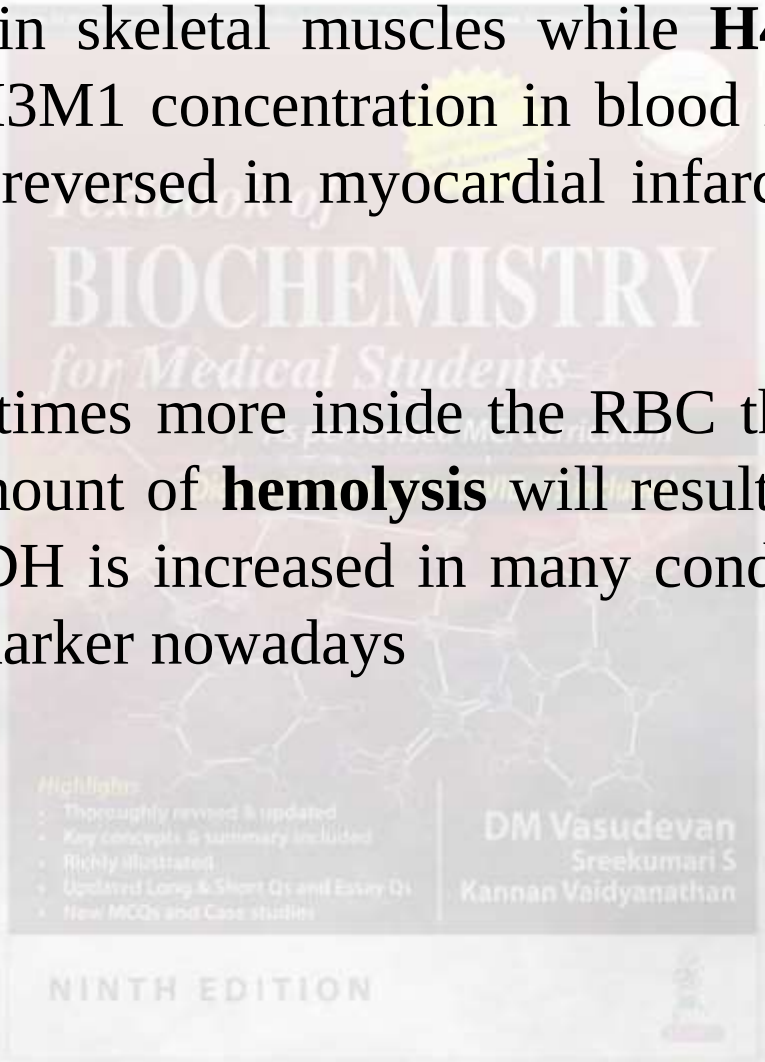
Formation of isoenzymes of lactate dehydrogenase.

Characteristic Features of LDH Isoenzymes.

Subunit make up of isoenzyme	*Electrophoretic mobility at pH 8.6*	*Activity at 60°C for 30 min*	*Tissue of origin*	*Percentage in human serum (Mean)*
H4	Fastest	Not destroyed	Heart muscle	30%
H3M1	Faster	Not destroyed	RBC, Brain	35%
H2M2	Fast	Partially destroyed	Brain, Leukocytes	20%
H1M3	Slow	Destroyed	Liver, Leukocytes	10%
M4	Slowest	Destroyed	Skeletal muscle	5%

M4 form is seen in skeletal muscles while **H4 form is seen in heart**. Normally H3M1 concentration in blood is greater than H4; but this pattern is reversed in myocardial infarction; this is called **flipped pattern**.

LDH level is 100 times more inside the RBC than in plasma, and therefore minor amount of **hemolysis** will result in a false positive test. Since total LDH is increased in many conditions, LDH is not used as a cardiac marker nowadays



Clinical Enzymology

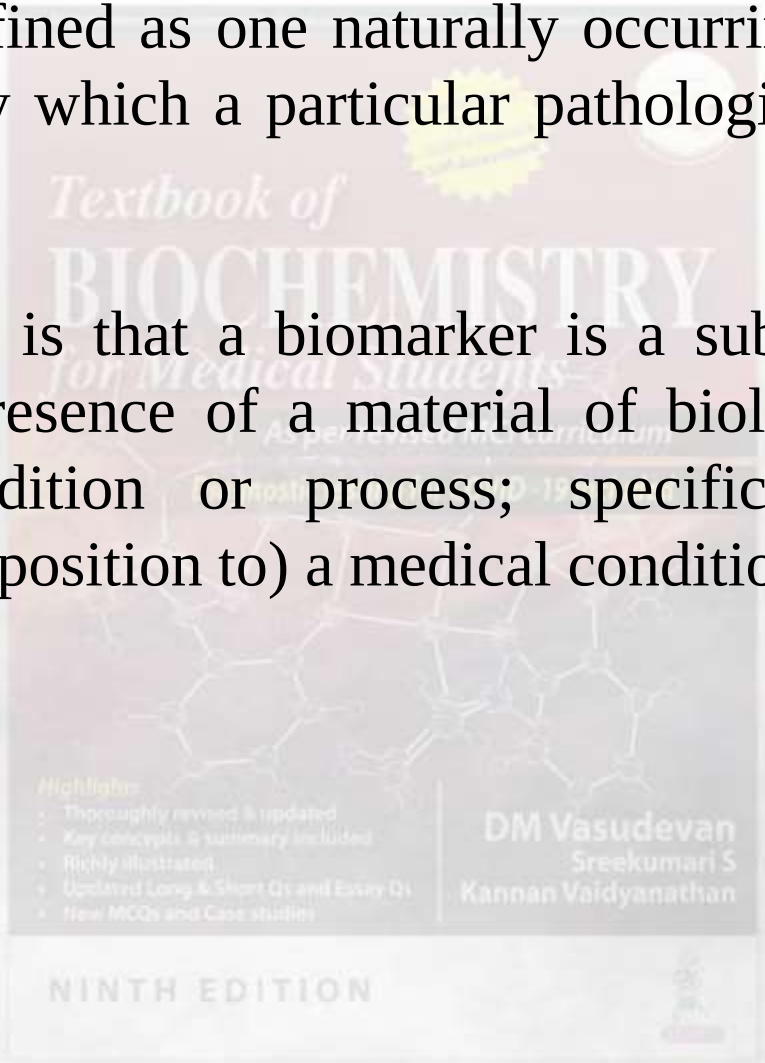
Plasma contains many **functional enzymes,** which are actively secreted into plasma. For example, enzymes of blood coagulation.

On the other hand, there are a few **nonfunctional enzymes** in plasma, which are coming out from cells of various tissues due to normal wear and tear. Their normal levels in blood are very low; but are drastically increased during cell death (necrosis) or disease. Therefore, assays of these enzymes are very useful in **diagnosis of Diseases.**

Biomarker

A biomarker is defined as one naturally occurring molecule, gene, or characteristic by which a particular pathological process can be identified.

Another definition is that a biomarker is a substance used as an indicator of the presence of a material of biological origin, or a physiological condition or process; specifically a diagnostic indicator of (predisposition to) a medical condition



Cardiac Biomarkers

They are used to detect cardiac diseases. These are discussed in Chapter 13. The cardiac markers may be classified as:

1. Cardiac markers of myocardial infarction (MI)
2. Markers for risk prediction
3. Markers of myocardial stress or congestive cardiac failure

Commonly used biomarkers for early detection of acute myocardial infarction are: Cardiac troponins, (TnI and TnT), and creatine kinase isoenzyme (CK-MB).

The brain natriuretic peptide (BNP) is a useful indicator of congestive cardiac failure.

Enzyme Profile in Liver Diseases

Enzymes commonly studied for diagnosis of liver diseases are:

1. Alanine aminotransferase (ALT)
2. Aspartate aminotransferase (AST)
3. Alkaline phosphatase (ALP)
4. Nucleotide phosphatase (NTP)
5. Gamma glutamyl transferase (GGT)

Alanine Aminotransferase (ALT)

Normal serum level of ALT for male is 13–35 U/L and for female is 10–30 U/L.

Very high values (300–1000 U/L) are seen in **acute hepatitis,** either toxic or viral in origin. Both ALT and AST levels are increased in liver disease, but ALT > AST.
Rise in ALT levels may be noticed several days before clinical signs such as jaundice are manifested.

Moderate increase (50–100 U/L) of ALT may be seen in chronic liver diseases such as cirrhosis, hepatitis C and non-alcoholic steatohepatitis (NASH).

Aspartate Aminotransferase (AST)

Reference serum level of AST ranges from 8 to 20 U/L.

It is a marker of liver injury and shows moderate to drastic increase in parenchymal liver diseases like hepatitis and malignancies of liver.

AST was used as a marker of myocardial ischemia in olden days.
The level is significantly elevated in myocardial infarction.
As AST is raised in various other conditions, the troponins have replaced AST as a diagnostic marker in ischemic heart disease. AST is now used as a marker for hepatic injury.

Alkaline Phosphatase (ALP)

ALP hydrolyzes aliphatic, aromatic or heterocyclic compounds. The pH optimum for the enzyme reaction is between 9 and 10. It is activated by magnesium and manganese. Zinc is a constituent ion of ALP.

It is produced by **osteoblasts** of bone and is associated with the calcification process.

Moderate (2–3 times) increase in ALP level is seen in **hepatic diseases** such as infective hepatitis, alcoholic hepatitis or hepatocellular carcinoma.

Alkaline Phosphatase (ALP)

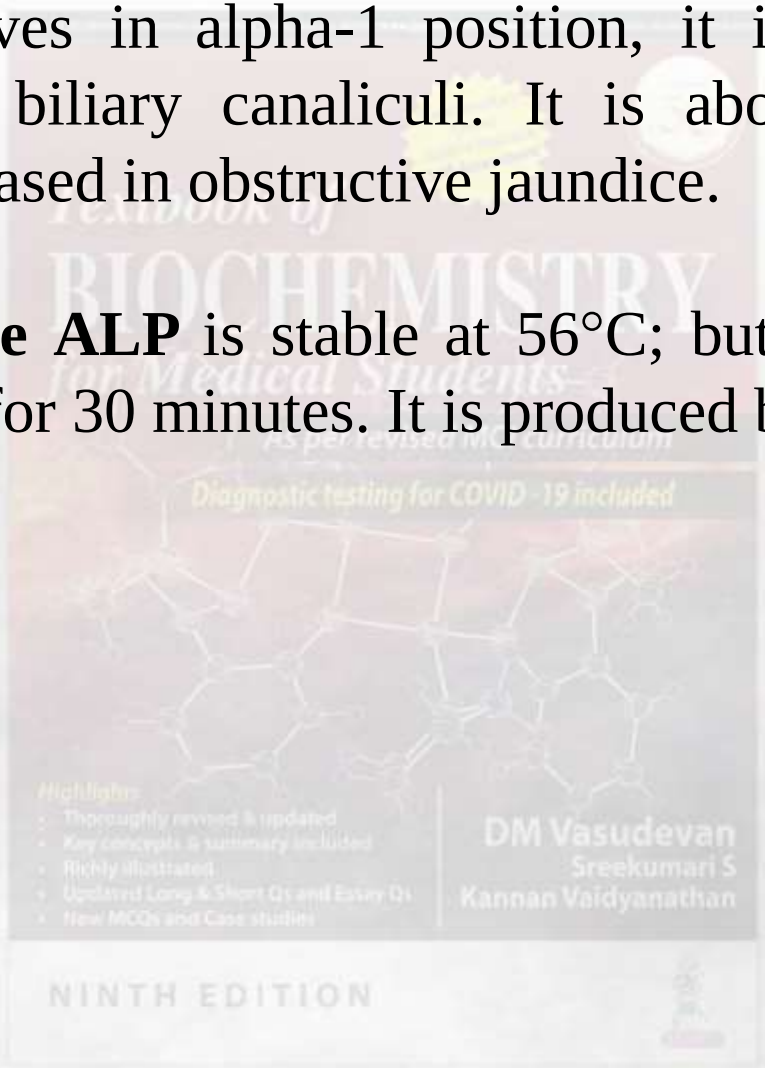
Very high levels of ALP (10–12 times of upper limit) may be noticed in **extrahepatic obstruction** (obstructive jaundice) caused by gallstones or by pressure on bile duct by carcinoma of head of pancreas. **Intrahepatic cholestasis** may be due to virus (infective hepatitis) or by drugs (chlorpromazine). ALP is produced by epithelial cells of biliary canaliculi and obstruction of bile with consequent irritation of epithelial cells leads to secretion of ALP into serum.

Drastically high levels of ALP (10–25 times of upper limit) are seen in **bone diseases** where osteoblastic activity is enhanced such as Paget's disease (osteitis deformans), rickets, osteomalacia, osteoblastoma, metastatic carcinoma of bone.

Isoenzymes of Alkaline Phosphatase

Alpha-1 ALP moves in alpha-1 position, it is synthesized by epithelial cells of biliary canaliculi. It is about 10% of total activity and is increased in obstructive jaundice.

Alpha-2 heat labile ALP is stable at 56°C; but loses its activity when kept at 65°C for 30 minutes. It is produced by hepatic cells.



Isoenzymes of Alkaline Phosphatase

Alpha-2 heat stable ALP will not be destroyed at 65°C but is **inhibited by phenylalanine**. It is of placental origin, which is found in blood in normal pregnancy. An isoenzyme closely resembling the placental form is characteristically seen in circulation in about 15% cases of carcinoma of lung, liver and gut and named as **Regan isoenzyme** or carcino placental isoenzyme.

Pre-beta ALP is of bone origin and elevated levels are seen in **bone diseases**. This is heat labile (destroyed at 56°C, 10 min). Heat labile bone isoenzyme of alkaline phosphatase (BAP) is a marker of bone disease.

Gamma Glutamyl Transferase (GGT)

It can transfer gamma glutamyl residues to substrate. It is used for the synthesis of glutathione. It is seen in liver, kidney, pancreas, intestinal cells, and prostate gland.

Reference serum value of GGT is 10–30 U/L. It is moderately increased in infective hepatitis and prostate cancers.

GGT is clinically important because of its sensitivity to detect **alcohol abuse**. GGT is increased in alcoholics even when other liver function tests are within normal limits. GGT level is rapidly decreased within a few days when the person stops to take alcohol. Increase in GGT level is generally proportional to the amount of alcohol intake.

Prostate Specific Antigen

Prostate specific antigen (PSA) is produced from the secretory epithelium of prostate gland. It is normally secreted into seminal fluid, where it is necessary for the liquefaction of seminal coagulum. It is a serine protease and is a 32 kD glycoprotein.

In blood it is bound to alpha-2 macroglobulin and alpha-1-antitrypsin; a very small fraction is in the free form also. Reference serum value is 1–5 units/L.

It is very specific for prostate activity. Values above 10 units/L is indicative of **prostate cancer**.

Acid Phosphatase

Acid phosphatase (ACP) hydrolyses phosphoric acid ester at pH between 4 and 6. **Reference serum** value for ACP is 2.5–12 U/L. ACP is secreted by prostate cells, RBC, platelets and WBC.

The prostate isoenzyme is inactivated by **tartaric acid**. Normal level of tartrate labile fraction of ACP is 1 U/L. ACP total value is increased in **prostate cancer** and highly elevated in bone metastasis of prostate cancer. In these conditions, the tartrate labile isoenzyme is elevated. This assay is very helpful in follow-up of treatment of prostate cancers. So, ACP is an important **tumor marker**.

Since blood cells contain excess quantity of ACP, care must be taken to prevent hemolysis while taking blood from the patient.

Cholinesterase (ChE)

Acetylcholinesterase or true ChE or Type 1 ChE can act mainly on acetylcholine. It is present in **nerve endings and in RBCs.** Newly formed RBC will have high levels of ChE which is slowly reduced according to the age of the cell. Therefore, ChE level in RBCs will be proportional to the reticulocyte count.

Organophosphorus insecticides (Parathion) irreversibly inhibit ChE in RBCs. Measurement of ChE level in RBCs is useful to determine the amount of exposure in persons working with these insecticides.

Glucose-6-phosphate Dehydrogenase (GPD)

It is an important enzyme in the hexose monophosphate shunt pathway of glucose. It is mainly used for production of NADPH. **Hydrogen peroxide** is continuously formed inside the RBC. Peroxide will destroy RBC cell membrane. Glutathione and NADPH will prevent this process. Therefore, NADPH is very essential for preserving the RBC integrity.

Drug-induced hemolytic anemia: In the GPD deficient individuals, RBC lifespan may be reduced, but there will be no disease manifestations. But when certain drugs (**aspirin**, mepacrine, primaquine, **sulpha**) are taken by such individuals, there will be sudden damage to RBCs. Primaquine stimulates peroxide formation. In GPD deficient cells the level of NADPH is low, leading to unchecked buildup of peroxides resulting in premature cell lysis. This drug-induced hemolytic anemia is characteristic of GPD deficiency. Fava beans (star beans, corner beans) may also induce hemolytic anemia which is called **favism.**

Carrier State has Biological Advantage

The gene for GPD is located in **X-chromosome**. Therefore hemizygous males and homozygous females will manifest the disease, while heterozygous females are carriers.

In heterozygous condition, where one gene is abnormal and the allelic form is normal, the GPD level in RBC is half the normal value. GPD deficiency seems to protect the person from falciparum **malaria**. The malarial parasites require NADPH for optimal growth. Thus, GPD deficiency has a selective advantage in malaria infested regions.

Met-hemoglobinemia: NADPH is also necessary for reduction of met-hemoglobin (oxidized form) to hemoglobin. Hence in GPD deficient individuals, met-hemoglobinemia may also be manifested.

Amylase

This enzyme splits starch to maltose. It is activated by calcium and chloride ions. It is produced by pancreas and salivary glands.

Reference serum value is 50–120 IU/L. The value is increased about 1000 times in **acute pancreatitis** which is a life-threatening condition. The peak values are seen between 5–12 hours after the onset of disease and returns to normal levels within 2–4 days after the acute phase has subsided. Moderate increase in serum levels are seen in chronic pancreatitis, mumps (parotitis) and obstruction of pancreatic duct.

Reference value of **amylase in urine** is less than 375 U/L. It is increased in acute pancreatitis. It is increased on the 1st day and remains to be elevated for 7–10 days.

Lipase

It will hydrolyze triglyceride to beta monoglyceride and fatty acid. The enzyme is present in pancreatic secretion.

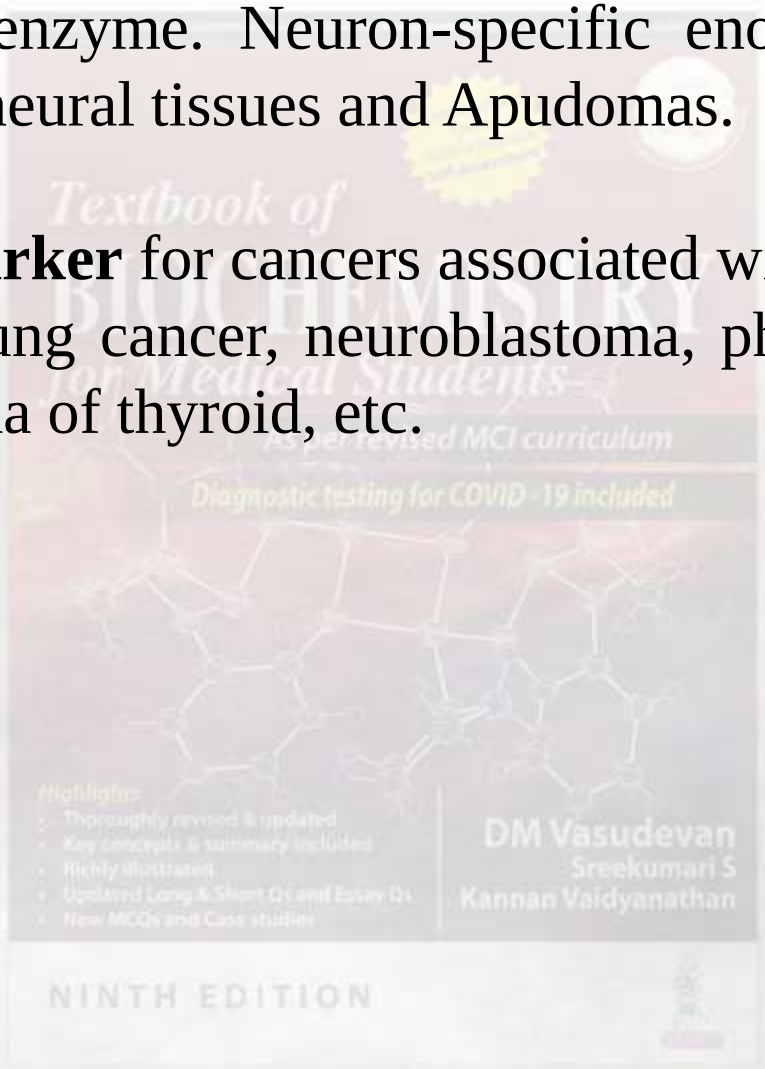
The level in blood is highly elevated in **acute pancreatitis** and this persists for 7–14 days. Thus, lipase remains elevated longer than amylase. Moreover, lipase is not increased in mumps. Therefore, lipase estimation has advantage over amylase.

It is moderately increased in carcinoma of pancreas, biliary diseases and perforating peptic ulcers.

Enolase

It is a glycolytic enzyme. Neuron-specific enolase (NSE) is an isoenzyme seen in neural tissues and Apudomas.

NSE is a **tumor marker** for cancers associated with neuroendocrine origin, small cell lung cancer, neuroblastoma, pheochromocytoma, medullary carcinoma of thyroid, etc.



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Pseudo cholinesterase or type II ChE is nonspecific and can hydrolyze acyl esters. It is produced mainly by **liver cells**. Succinyl choline is a widely used muscle relaxant. It is a structural analogue of ACh, and so competitively fixes on post-synaptic receptors of ACh. **Succinylcholine** is hydrolyzed by the liver ChE within 2–4 minutes. But in certain persons the ChE activity may be absent; this is a genetically transmitted condition. In such individuals when succinylcholine is given during surgery, it may take hours to get the drug metabolized. Very prolonged '**scoline apnea**' may result in 'nightmare of anesthetist

Ceruloplasmin

It is otherwise called ferroxidase. Reference serum level is 25–50 mg/dL. Modern techniques based on radial immunodiffusion or immunoturbidimetric methods are also available.

Since it is an acute phase protein, it is increased in all inflammatory conditions, collagen diseases, malignancies and pregnancy.

A value less than 20 mg/dL is pathognomonic of Wilson's hepatolenticular degeneration, in which copper toxicity is manifested.

Enzyme Patterns (Enzyme Profiles) in Diseases

I. Hepatic diseases

1. Alanine aminotransferase (ALT): Marked increase in parenchymal liverdiseases
2. Aspartate aminotransferase (AST): Elevated in parenchymal liver disease
3. Alkaline phosphatase (ALP): Marked increase in obstructive liver disease
4. Gamma glutamyl transferase (GGT): Increase in obstructive and alcoholic liver

II. Myocardial infarction

1. Cardiac troponins (CTnT and CTnI). (These are not enzymes, but are specific and sensitive and elevated very early in MI).
2. Creatine kinase (CK-MB): CK-MB isoenzyme is specific

III. Muscle diseases

1. Creatine kinase (CK-MM): Marked increase in muscle diseases.
2. Aspartate aminotransferase (AST): Increase in muscle disease; not specific
3. Aldolase (ALD): Earliest enzyme to rise, but not specific

IV. Bone diseases

1. Alkaline phosphatase (ALP) Marked elevation in rickets and Paget's disease

V. Prostate cancer

1. Prostate specific antigen (PSA): Marker for prostate cancer. Mild increase in benign prostate enlargement
2. Acid phosphatase (ACP): Marker for prostate cancer. Metastatic bone disease especially from a primary form prostate. Inhibited by L tartrate.

VI. Pancreatic disease

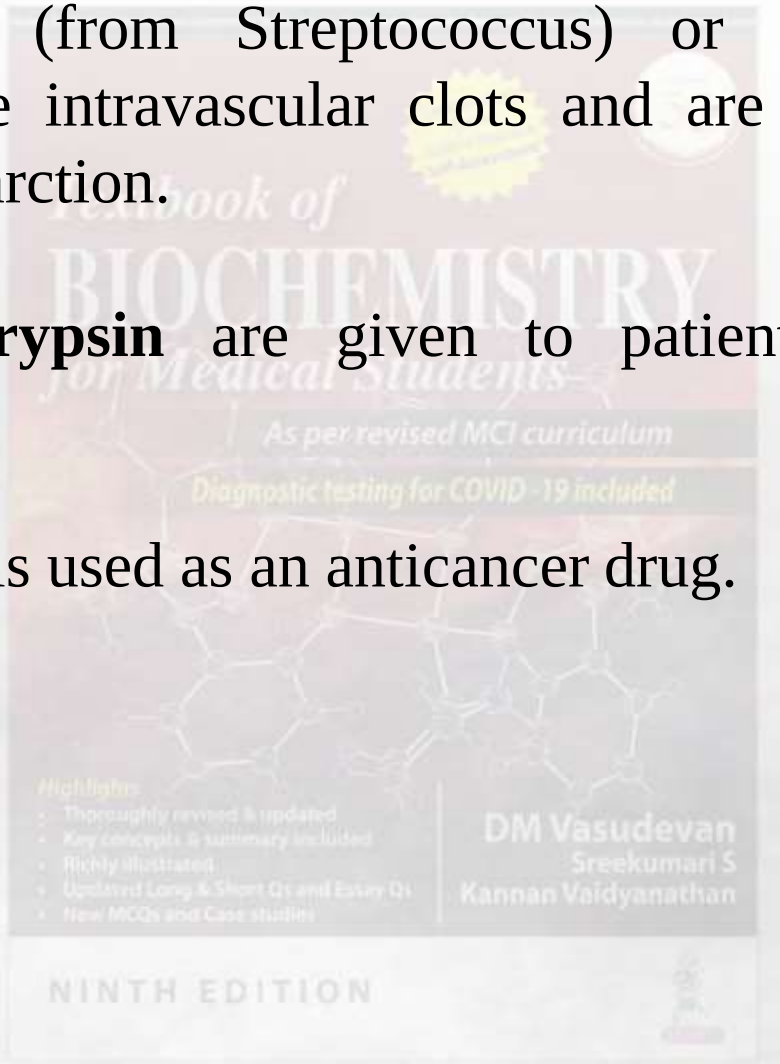
1. Amylase: Marker for acute pancreatitis and inflammation of salivary glands
2. Lipase: Marker of pancreatitis, more specific than amylase

Enzymes as Therapeutic Agents

- **Streptokinase** (from Streptococcus) or **Urokinase** (from urine) can lyse intravascular clots and are therefore used in myocardial infarction.

- **Pepsin** and **trypsin** are given to patients with defective digestion.

- **Asparaginase** is used as an anticancer drug.



Therapeutic use of Enzymes

Enzyme	*Therapeutic application*
Asparaginase	Acute lymphoblastic leukemia
Streptokinase	To lyse intravascular clot
Urokinase	To lyse intravascular clot
Recombinant tissue prothrombin activator (rtPA)	Lysis of clot, especially for cerebrovascular thrombolysis
Streptodornase DNAse	applied locally
Pancreatin (trypsin and lipase)	Pancreatic insufficiency; oral administration
Papain	Anti-inflammatory
Alpha-1-antitrypsin	AAT deficiency; emphysema

Enzymes used for Diagnostic Purpose

Enzyme	*Used for testing*
Urease	Urea
Uricase	Uric acid
Glucose oxidase	Glucose
Peroxidase	Glucose; Cholesterol
Hexokinase	Glucose
Cholesterol oxidase	Cholesterol
Lipase	Triglycerides
Horse radish peroxidase (HRP)	ELISA
Alkaline phosphatase	ELISA
Restriction endonuclease	Southern blot; RFLP
Reverse transcriptase Taq polymerase	Polymerase chain reaction (PCR)