2. The enzyme **asparaginase** is used in the treatment of leukemias. Tumor cells are dependent on asparagine of the host plasma for their multiplication. By administering asparaginase the host's plasma levels of asparagine are drastically reduced. This leads to impairment in the viability of tumor cells.

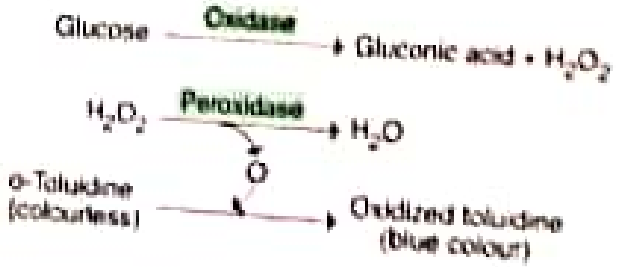
Enzymes as analytical reagents

Some enzymes are useful in the clinical laboratory for the measurement of substrates, drugs, and even the activities of other enzymes. The biochemical components (e.g. glucose, urea, uric acid, cholesterol) can be more accurately and specifically estimated by enzymatic procedures compared to the conventional chemical methods. A good example is the estimation of plasma glucose by glucose oxidase and peroxidase method.

Immobilized enzymes

Enzymes can be used as catalytic agents in industrial and medical applications. Some of these enzymes are immobilized by binding them to a solid insoluble matrix which will not affect the enzyme stability or its catalytic activity. Beaded gels and cyanogen bromide activated sepharose are commonly used for immobilization of enzymes. The bound enzymes can be preserved for long periods without loss of activity.

Glucose oxidase and peroxidase, immobilized and coated on a strip of paper, are used in the clinical laboratory for the detection of glucose in urine.



The intensity of the blue colour depends on the concentration of glucose. Hence, the strip method is useful for semi-quantitative estimation of glucose in urine.

DIAGNOSTIC IMPORTANCE OF ENZYMES

Estimation of enzyme activities in biological fluids, particularly plasma/serum, is of great clinical importance. Enzymes in the circulation are classified into two groups: plasma functional and plasma non functional.

1. Plasma specific or plasma functional enzymes

Certain enzymes are normally present in the plasma and they have specific functions to perform. Generally these enzyme activities are higher in plasma than in the tissues. They are mainly synthesized in the liver and enter the circulation e.g. lipoprotein lipase, plasmin, prothrombin, cholinesterase, ceruloplasmin etc.

Impairment in liver function or genetic disorders often leads to a fall in the activities of plasma functional enzymes e.g. deficiency of ceruloplasmin in Wilson's disease.

2. Non-plasma specific or plasma non- functional enzymes

These enzymes are either totally absent or present at a low concentration in plasma compared to their levels found in the tissues. The digestive enzymes of the gastrointestinal tract (e.g. amylase, pepsin, trypsin, lipase etc.) present in the plasma are known as **secretory enzymes.** All the other plasma enzymes associated with metabolism of the cell are collectively referred to as **constitutive enzymes** (e.g. lactate dehydrogenase, transaminases, acid and alkaline phosphatases, creatine phosphokinase).

Estimation of the activities of non-plasma specific enzymes is very important for the diagnosis and prognosis of several diseases.

The normal serum level of an enzyme indicates the balance between its synthesis and release in the routine cell turnover. The raised enzyme levels could be due to cellular damage, increased rate of cell turnover, proliferation of cells, increased synthesis of enzymes etc. Serum

Table 6.8 Important enzymes in the diagnosis of diseases

Serum enzyme (elevated)	Disease (most important)
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

enzymes are conveniently used as ***markers*** to detect the cellular damage which ultimately helps ***in the diagnosis of diseases***.

A summary of the important enzymes useful for the diagnosis of specific diseases is given in ***Table 6.8***. Detailed information on the diagnostic enzymes including reference values is provided in ***Table 6.9***. A brief account of selected diagnostic enzymes is discussed.

Amylase : The activity of serum amylase is increased in ***acute pancreatitis*** (normal 80-180 Somogyi units/dl). The peak value is observed within 8-12 hours after the onset of disease which returns to normal by 3rd or 4th day. Elevated activity of amylase is also found in urine of the patients of acute pancreatitis. Serum amylase is also important for the diagnosis of chronic pancreatitis, acute parotitis (mumps) and obstruction of pancreatic duct.

Alanine transaminase (ALT/SGPT) : SGPT is elevated in ***acute hepatitis*** of viral or toxic origin, jaundice and cirrhosis of liver (normal 3-40 IU/l).

Aspartate transaminase (AST/SGOT) : SGOT activity in serum is increased in ***myocardial infarction*** and also in liver diseases (normal

It may be noted that SGPT is more specific for the diagnosis of liver diseases while SGOT is for heart diseases. This is mainly because of their cellular distribution. SGPT is a cytosomal enzyme while SGOT is found in cytosol and mitochondria.

Alkaline phosphatase (ALP) : It is elevated in certain bone and liver diseases (normal 3-13 KA units/dl). ALP is useful for the diagnosis of ***rickets***, hyperparathyroidism, carcinoma of bone, and ***obstructive jaundice***.

Acid phosphatase (ACP) : It is increased in the ***cancer of prostate gland*** (normal 0.5-4 KA units/dl). The tartarate labile ACP (normal <1 KA units/dl) is useful for the diagnosis and prognosis of prostate cancers i.e. ACP is a good tumor marker.

Lactate dehydrogenase (LDH) : LDH is useful for the diagnosis of ***myocardial infarction, infective hepatitis***, leukemia and muscular dystrophy (serum LDH normal 50-200 IU/l). LDH has five isoenzymes, the details of which are described later.

Creatine kinase (CK) : It is elevated in ***myocardial infarction*** (early detection) and muscular dystrophy (normal 10-50 IU/l). CK has three isoenzymes (described later).

Table 6.9 Increase in plasma (serum) enzymes in the diagnosis of diseases

Enzymes	Reference value	Disease(s) in which increased
I. Digestive enzymes		
Amylase	80–180 Somogyi units/dl or 2.5–5.5 μKat	Acute pancreatitis, mumps (acute parotitis), obstruction in pancreatic duct, severe diabetic ketoacidosis
Lipase	0.2–1.5 IU/l	Acute pancreatitis, moderate elevation in carcinoma of pancreas
II. Transaminases		
Alanine transaminase (ALT) or serum glutamate pyruvate transaminase (SGPT)	3–40 IU/l or 40–250 nKat	Acute hepatitis (viral or toxic), jaundice, cirrhosis of liver
Aspartate transaminase (AST) or serum glutamate oxaloacetate transaminase (SGOT)	4–45 IU/l or 50–320 nKat	Myocardial infarction, liver diseases, liver cancer, cirrhosis of liver
III. Phosphatases		
Alkaline phosphatase (ALP) (pH optimum 9–10)	In adults-3–13 King Armstrong (KA) units/dl or 25–90 IU/l or 500–1400 nKat. In children–15–30 KA/dl	Bone diseases (related to higher osteoblastic activity)-rickets, Paget's disease, hyperparathyroidism, carcinoma of bone. Liver diseases-obstructive jaundice (cholestasis), infective hepatitis, cirrhosis of liver
Acid phosphatase (ACP) (pH optimum 4–6)	0.5–4 KA units/dl or 2.5–12 IU/l or 10–100 nKat. Tartarate labile ACP-0–0.9 KA units/dl	Prostatic carcinoma i.e. cancer of prostate gland (tartarate labile ACP serves as a marker for diagnosis and follow up), Paget's disease
IV. Enzymes of carbohydrate metabolism		
Aldolase	2–8 IU/l	Muscular dystrophy, liver diseases, myocardial infarction, myasthenia gravis, leukemias
Isocitrate dehydrogenase (ICD)	1–4 IU/l	Liver diseases (inflammatory toxic or malignant)
Lactate dehydrogenase (LDH)	50–200 IU/l or 1–5 μKat	Myocardial infarction, acute infective hepatitis, muscular dystrophy, leukemia, pernicious anaemia
V. Miscellaneous enzymes		
Creatine kinase (CK) or creatine phosphokinase (CPK)	10–50 IU/l	Myocardial infarction (CK useful for early detection), muscular dystrophy, hypothyroidism, alcoholism
Choline esterase (CHE)	2–10 IU/l	Nephrotic syndrome, myocardial infarction
5' Nucleotidase or nucleotide phosphatase (NTP)	2–15 IU/l	Hepatitis, obstructive jaundice, tumors
γ-Glutamyl transpeptidase (GGT)	5–40 IU/l	Alcoholism, infective hepatitis, obstructive jaundice
Ceruloplasmin (ferroxidase)	25–50 mg/dl	Bacterial infections, collagen diseases, cirrhosis, pregnancy

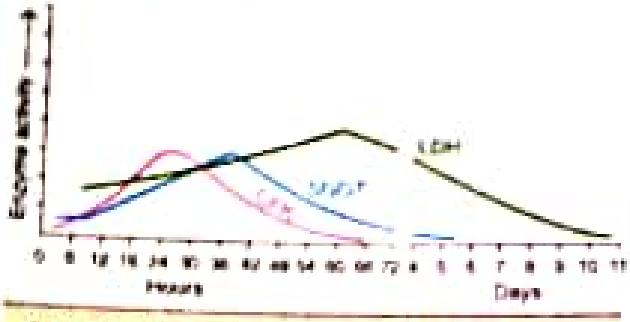


Fig. 6.16 : *Enzyme pattern in myocardial infarction (CPK-Creatine phosphokinase, SGOT-Serum glutamate oxaloacetate transaminase; LDH-Lactate dehydrogenase)*

Isoenzymes of alcohol dehydrogenase

Alcohol dehydrogenase (ADH) has two heterodimer isoenzymes. Among the white Americans and Europeans, $\alpha\beta_1$ isoenzyme is predominant whereas in Japanese and Chinese (Orientals) $\alpha\beta_2$ is mostly present. The isomer $\alpha\beta_2$ 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 $\alpha\beta_2$-isoenzyme of ADH.

ENZYME PATTERN IN DISEASES

For the right diagnosis of a particular disease, it is always better to estimate a few (three or more) serum enzymes, instead of a single enzyme. Examples of enzyme patterns in important diseases are given here.

Enzymes in myocardial infarction

The enzymes, namely creatine phosphokinase (CPK), aspartate transaminase (AST) and lactate dehydrogenase (LDH) –are important for the diagnosis of myocardial infarction (MI). The elevation of these enzymes in serum in relation to hours/days of MI is given in the ***Fig.6.16***.

Creatine phosphokinase (precisely isoenzyme MB) is the first enzyme to be released into circulation within 6–18 hours after the infarction. Therefore, CPK estimation is highly useful for the early diagnosis of MI. This enzyme reaches a peak value within 24–30 hours, and returns to normal level by the 2nd or 3rd day.

Aspartate transaminase (AST or SGOT) rises sharply after CPK, and reaches a peak within 48 hours of the myocardial infarction. AST takes 4–5 days to return to normal level.

Lactate dehydrogenase (LDH) generally rises from the second day after infarction, attains a peak by the 3rd or 4th day and takes about 10–15 days to reach normal level. Thus, LDH is the last enzyme to rise and also the last enzyme to return to normal level 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. Cardiac troponin I (CTI) is released into circulation within four hours after the onset of MI, reaches a peak value by 12–24 hours, and remains elevated for about a week.

The protein ***myoglobin*** is also an early marker for the diagnosis of MI. Myoglobin is however, not commonly used as it is not specific to cardiac diseases. In the ***Table 6.12***, a summary of the diagnostic markers used in MI is given.

Enzymes in liver diseases

The following enzymes—when elevated in serum – are useful for the diagnosis of liver dysfunction due to viral hepatitis (jaundice), toxic hepatitis, cirrhosis and hepatic necrosis.

1. Alanine transaminase;
2. Aspartate transaminase;
3. Lactate dehydrogenase;

The enzymes that markedly increase in intrahepatic and extrahepatic cholestasis are :

1. Alkaline phosphatase. 2. 5'-Nucleotidase

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

Diagnostic marker	Time of peak elevation	Time of return to normal level	Diagnostic importance
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.

Serum-γ-glutamyl transpeptidase is useful in the diagnosis of alcoholic liver diseases.

Enzymes in muscle diseases

In the muscular dystrophies, probably due to the increased leakage of enzymes from the damaged cells, serum levels of certain muscle enzymes are increased. These include creatine phosphokinase, aldolase and aspartate transaminase. Of these, CPK is the most reliable indicator of muscular diseases, followed by aldolase.

Enzymes in cancers

Increase in the serum acid phosphatase (tartarate labile) is specific for the detection of prostatic carcinoma.

[Note : *Prostate specific antigen* (PSA; mol wt. 32 KD), though not an enzyme, is a more reliable marker for the detection of prostate cancer. Normal serum concentration of PSA is 1-4 ng/ml].

A non-specific increase in certain enzymes like LDH, alkaline phosphatase and transaminase may be associated with malignancy in any part of the body.

β-Glucuronidase estimation in urine is useful for detecting the cancers of urinary bladder, pancreas etc.