Lectures Notes

On

CLINICAL ENZYMEOLOGY
(In Diagnosis and Medical Application)

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CLINICAL ENZYMEOLOGY

OBJECTIVES

After reading this lesson, you will be able to:
- Describe plasma enzymes.
- Explain about the assessment of cell damage and proliferation.
- Describe the role of enzymes in health and diseases.
- Explain enzyme application in diagnosis, prognosis, treatment, and biotechnology.

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1. INTRODUCTION

Enzymes are catalysts that increase the rate of physiologic reactions. Each and every reaction in our body catalyzed by enzyme.

In general, most enzymes are present in cells at much higher concentrations than in plasma. Measurement of their levels in plasma indicates whether their tissue of origin is damaged leading to the release of intracellular components into the blood.

This forms the basis of clinical enzymology.

Thus clinical enzymology refers to measurement of enzyme activity for the diagnosis and treatment of diseases.

Since the tight control of enzyme activity is essential for homeostasis, any malfunction of a single critical enzyme (mutation, overproduction, underproduction or deletion) can lead to a genetic disease - commonly called inborn errors of metabolism.

- One example is the most common type of phenylketonuria caused by a mutation of a single amino acid in the enzyme phenylalanine hydroxylase, which catalyzes the first step in the degradation of phenylalanine.
- The deficiency results in build-up of phenylalanine and related un-physiological by-products. This can lead to mental retardation if the disease is untreated early.

1.1. DIAGNOSTIC CLINICAL ENZYMEOLOGY:

One major item of the diagnostic clinical enzymology is the investigation of changes in the level of enzymes and their correlation to the differential diagnosis of diseases and to establish cut-off levels for normal, benign and malignant diseases.

These enzymes changes could be followed up in plasma, serum, urine, urine, blood cells or tissue biopsies.

Cross-sectional single or longitudinal serial assays of the serum activity of a selected enzyme(s) may support the diagnosis of a specific disease location and/or extent, disease prognosis, recurrence and or monitoring the response to treatment.

Thus, detection of the plasma level of an enzyme immunologically (for its protein amount) or colorimetrically (for its activity, preferred) have the following applications:

- Diagnosis: As example, high serum creatine phosphokinase (CPK2) on the day of a suspected case of myocardial infarction strengthen the diagnosis if ECG changes are doubtful.
- Differential diagnosis: e.g., chest pain associates myocardial infarction and pulmonary embolism. Elevated serum glutamate-oxaloacetate transaminase (GOT) and lactate dehydrogenase (LDH) characterizes myocardial infarction, whereas, elevated serum LDH only characterizes pulmonary embolism.
- Therapeutic follow up and/or early detection of a disease:
  - Chronic administration of several therapeutics - e.g., anti-depressant and anticancer chemotherapies - elevates serum isocitrate dehydrogenase or ornithine carbamoyl-transferase level when they induce minimal hepatotoxicity.
  - Serum glutamate-pyruvate transaminase (GPT) level elevates in sub-clinical early viral hepatitis.

1.2. PLASMA ENZYMES:

Enzymes present in plasma can be classified into 2 types, they are:

- Functional Plasma enzymes and Non-functional plasma enzymes
1.2.1. Plasma-derived enzymes (functional plasma enzymes):
- They are normally occurring functional plasma enzymes.
- Mostly synthesized by the liver.
- Usually decreased in disease conditions.
- Their field of activity is plasma components and their activity is higher in plasma than in cells, e.g., coagulation and lipoprotein-metabolizing enzymes.
- Their clinical importance is limited to diseases related to their own synthesis and function; i.e., abnormalities of metabolism of plasma lipoproteins and blood clotting, and the organ function of their synthesizing tissues, e.g., thromboplastin as a liver function test.

1.2.2. Cell-Derived enzymes (Non-functional plasma enzymes):
- Normally they locate to intracellular compartments; i.e., they are non-functional plasma enzymes.
- A very low plasma level normally exists due to normal wear and tear and diffusion through undamaged cell membranes.
- Gross damage to the cells or abnormal membrane permeability, overproduction of the enzymes or abnormal high cellular proliferation may allow their leakage in abnormally high amount into plasma and other body fluids.
- The amount and nature of the plasma enzyme(s) reflects the extent and nature of the damaged tissue.
- Measurement of these enzymes in plasma can be used to assess cell damage and proliferation i.e. diagnosis of disease.
- They are further subdivided into: secretory and metabolic non-functional plasma enzymes:
  - Secretory: They are synthesized and secreted by specialized glands into body lumens mainly for digestion.
    - Their retrograde escape into blood reflects damage in the tissue of their origin, e.g., pancreatic amylase and lipase in pancreatitis.
  - Metabolic: They are intracellular metabolic enzymes and their appearance in the plasma is mainly due to cellular damage among other factors.

INCREASED non-functional plasma enzymes could be due to increased release and/or impaired clearance.

A- Abnormally increased release from cells may be due to:
1. Pathological apoptosis and/or necrosis of cells, e.g., elevated levels of aldolase (EC 4.1.2.13), CK, LDH and GOT in progressive muscular dystrophy.
2. Increased membrane permeability without gross cellular damage, e.g., elevated levels of GPT in early stage of viral hepatitis.
3. Increased intracellular enzyme concentration due to:
   ☼ Protein anabolic drugs, e.g., increased synthesis of liver transaminases.
   ☼ Higher cellular proliferation and increased cell mass as in malignancies, e.g., elevation of acid phosphatase in cancer prostate.

B- Impaired clearance:
   ☼ As the case for other plasma proteins, enzymes have specific plasma half-life after which they are disposed by cellular reuptake, degradation and/or excretion in bile or urine.
   ☼ Examples include:
     - Elevation of serum Leucine Amino-Peptidase (LAP) and ALP in obstructive jaundice and,
Elevation of several enzymes in nephrotic syndrome and renal failure. **DECREASED** activity of non-functional plasma enzymes could be due to decreased enzyme synthesis, increased enzyme inhibition and/or deficiency of its activating factors.

- Decreased synthesis of an enzyme could be;
  - Genetically **inherited** as most metabolic inborn errors, e.g., hypophosphatasia with low serum ALP level and Wilson’s disease with low serum ceruloplasmin level.
  - It could be **acquired** as low serum pseudocholinesterase level in hepatitis, and, low serum amylase level in chronic hepatic and pancreatic diseases and severe malnutrition.
- Increased enzyme inhibition, e.g.,
  - Insecticide poisoning that leads to low serum pseudocholinesterase activity, but assaying the protein with immunoassays will show normal enzyme level.
- Lack of cofactors, e.g., pregnancy and liver cirrhosis displays low serum GOT level.

### 1.3. ISOENZYMES:

- Isoenzymes (also known as isozymes) are enzymes that differ in amino acid sequence but catalyze the same chemical reaction
- Believed to be originating from closely linked genes or from multiple gene loci.
- Evolution from a single form possibly due to long-term mutations
- They vary with respect to their kinetic parameters, electrophoretic mobility, and localization.
- They all have independent action E.g. Lactate dehydrogenase have 5 isoenzymes (LDH1, LDH2, LDH3, LDH4 & LDH5)
- They can be used to identify the specific affected tissues.
- They can be differentiated from each other and can be clinically quantified in the lab.

### 1.4. ENZYMES IN DIAGNOSIS AND PROGNOSIS

- Plasma enzyme activities can be used in the diagnosis of disease and prognosis of treatment.
- Plasma enzyme levels depend on balance between the rate of influx of active enzyme into the circulation and its eventual clearance from the blood.
- The rate of influx is determined by the rate of release from damaged cells and altered rate of enzyme synthesis.

#### 1.4.1. Localization of Damage:

- Enzymes used to measure tissue damage are present in nearly all cells with varying concentration.
- So the measurement may indicate an abnormality, but the specific diagnosis cannot be made.
- For example if there is circulatory failure after a cardiac arrest very high plasma levels of enzymes originating from many tissues may occur because of hypoxic damage to cells and reduced rates of clearance:
  - Then the raised plasma levels of ‘cardiac’ enzymes do not necessarily mean that a myocardial infarct caused the arrest.

#### 1.4.2. The diagnostic precision of plasma enzyme analysis may be improved by;

1- Estimation of more than one enzyme.
- Many enzymes are widely distributed, but their relative concentrations may vary in different tissues.
- E.g. Alanine and aspartate transaminases are abundant in the liver, the concentration of AST is much greater than that of alanine transaminase in heart muscle.
2. Isoenzyme determination.
- Some enzymes exist in more than one form: these isoenzymes may be separated by their different physical or chemical properties.
- If they originate in different tissues such identification will give more information than the measurement of plasma total enzyme activity:
- For example, creatine kinase may be derived from skeletal or cardiac muscle, but one of its isoenzymes is found predominantly in the myocardium (CK2).

3- Serial enzyme estimations.
- The rate of change of plasma enzyme activity is related to a balance between the rate of entry and the rate of removal from the circulation.
- A persistently raised plasma enzyme activity is suggestive of a chronic disorder or occasionally of impaired clearance.
- The distribution of enzymes within cells may differ. ALT and LDH are predominantly located in cytoplasm and glutamate dehydrogenase in mitochondria, whereas AST occurs in both these cellular compartments.

2. ENZYMES OF CLINICAL SIGNIFICANCE

Table -1 lists the commonly analyzed enzymes, including their systematic names and clinical significance.

Each enzyme is discussed in this chapter with respect to tissue source, diagnostic significance, and reference range.  
Table 1: Major enzymes of clinical significance
2.1. CREATINE KINASE

(EC 2.7.3.2; adenosine triphosphate: creatine N phosphotransferase CK)

口 CK is an enzyme that is generally associated with ATP regeneration in contractile or transport systems.
口 Its predominant physiologic function occurs in muscle cells, where it is involved in the storage of high-energy creatine phosphate.
口 Every contraction cycle of muscle results in creatine phosphate use, with the production of ATP.
口 This results in relatively constant levels of muscle ATP. The reversible reaction catalyzed by CK is shown in the following Equation.

\[
\text{Creatine} + \text{ATP} \rightleftharpoons \text{Creatine phosphate} + \text{ADP}
\]

2.1.1. Tissue Source

口 CK is widely distributed in tissue, with highest activities found in skeletal muscle, heart muscle, and brain tissue.
口 CK is present in much smaller quantities in other tissue sources, including the bladder, placenta, gastrointestinal tract, thyroid, uterus, kidney, lung, prostate, spleen, liver, and pancreas.

2.1.2. Diagnostic Significance

口 Because of the high concentrations of CK in muscle tissue, CK levels are frequently elevated in disorders of cardiac and skeletal muscle.
口 The CK level is considered a sensitive indicator of acute myocardial infarction (AMI) and muscular dystrophy.
口 Total CK levels are not entirely specific indicators in as much as CK elevation is found in various other abnormalities of cardiac and skeletal muscle.
口 Levels of CK also vary with muscle mass and, therefore, may depend on:
  - Gender, race, degree of physical conditioning, and age.
口 Elevated CK levels are also occasionally seen in central nervous system disorders such as cerebrovascular accident, seizures, nerve degeneration, and central nervous system shock.
  - Damage to the blood–brain barrier must occur to allow enzyme release to the peripheral circulation.
口 Serum CK levels and CK/progesterone ratio have been useful in the diagnosis of ectopic pregnancies.

CK Isoenzyme:

口 Because enzyme elevation is found in numerous disorders, the separation of total CK into its various ISOENZYME fractions is considered a more specific indicator of various disorders than total levels.
  - Typically, the clinical relevance of CK activity depends more on isoenzyme fractionation than on total levels.
  - CK occurs as a dimer consisting of two subunits that can be separated readily into three distinct molecular forms.
  - The three isoenzymes have been designated as CK-BB (brain type), CK-MB (hybrid type), and CK-MM (muscle type).
  - Table 2 indicates the tissue localization of the isoenzymes and the major conditions associated with elevated levels.
  - Hypothyroidism results in CK-MM elevations because of the involvement of muscle tissue (increased membrane permeability), the effect of thyroid hormone on enzyme activity, and, possibly, the slower clearance of CK as a result of slower metabolism.
However, the degree of exercise in relation to the exercise capacity of the individual is the most important factor in determining the degree of elevation.

Levels may be elevated for as long as 48 hours following exercise.

It has been observed that CK-BB may be significantly elevated in patients with carcinoma of various organs.

These findings indicate that CK-BB may be a useful tumor-associated marker.

The most common causes of CK-BB elevations are central nervous system damage, tumors, childbirth, and the presence of macro-CK, an enzyme–immunoglobulin complex.

The value of CK isoenzyme separation can be found principally in detection of myocardial damage.

Cardiac tissue contains significant quantities of CK-MB, approximately 20% of all CK-MB.

Whereas CK-MB is found in small quantities in other tissue, myocardium is essentially the only tissue from which CK-MB enters the serum in significant quantities.

Demonstration of elevated levels of CK-MB, greater than or equal to 6% of the total CK, is considered a good indicator of myocardial damage, particularly AMI.

The typical time course of CK-MB elevation following AMI is not found in other conditions.

Following myocardial infarction, the CK-MB levels begin to rise within 4 to 8 hours, peak at 12 to 24 hours, and return to normal levels within 48 to 72 hours.

This time frame must be considered when interpreting CK-MB levels.

The specificity of CK-MB levels in the diagnosis of AMI can be increased if
interpreted in conjunction with LDH isoenzymes and/or troponins and if measured sequentially over a 48-hour period to detect the typical rise and fall of enzyme activity seen in AMI.

Other non-enzyme proteins, called troponins ((troponin I and troponin T), have been found to be even more sensitive and more specific marker of myocardial damage and may elevate in the absence of CK-MB elevations.

2.1.3. Reference Range

- Male, 15–160 U/L (37°C).
- Female, 15–130 U/L (37°C).
- The higher values in males are attributed to increased muscle mass.
- Note that enzyme reference ranges are subject to variation, depending on the method used and the assay conditions.

2.2. LACTATE DEHYDROGENASE (EC 1.1.1.27; L-lactate: NAD+ oxidoreductase; LD)

LDH is an enzyme that catalyzes the interconversion of lactic and pyruvic acids. It is a hydrogen-transfer enzyme that uses the coenzyme NAD+ according to the following equation:

\[
\begin{align*}
\text{CH}_3\text{CHOH} + \text{NAD}^+ & \rightarrow \text{CH}_3\text{COOH} + \text{NADH} + \text{H}^+ \\
\text{Lactate} & \rightarrow \text{Pyruvate}
\end{align*}
\]

2.2.1. Tissue Source

LDH is widely distributed in the body. High activities are found in the heart, liver, skeletal muscle, kidney, and erythrocytes; lesser amounts are found in the lung, smooth muscle, and brain.

2.2.2. Diagnostic Significance

- Because of its widespread activity in numerous body tissues, LDH is elevated in a variety of disorders.
- Increased levels are found in cardiac, hepatic, skeletal muscle, and renal diseases, as well as in several hematologic and neoplastic disorders.
- Liver disorders, such as viral hepatitis and cirrhosis, show slight elevations of two to three times.
- AMI and pulmonary infarct also show slight elevations of approximately the same degree.
- In AMI, LDH levels begin to rise within 12 to 24 hours, reach peak levels within 48 to 72 hours, and may remain elevated for 10 days.
- Skeletal muscle disorders and some leukemias contribute to increased LDH levels. Marked elevations can be observed in most patients with acute lymphoblastic leukemia in particular.
- Because of the many conditions that contribute to increased activity, an elevated total LDH value is a rather nonspecific finding.

LDH Isoenzymes

- LDH assays, therefore, assume more clinical significance when separated into isoenzyme fractions.
- The enzyme can be separated into five major fractions, each comprising four subunits. It has a molecular weight of 128,000 daltons.
- Each isoenzyme comprises four polypeptide chains with a molecular weight of 32,000 daltons each. Two different polypeptide chains, designated H (heart) and M (muscle), combine in five arrangements to yield the five major isoenzyme fractions.
Table 3 indicates the tissue localization of the LDH isoenzymes and the major disorders associated with elevated levels.

- In the sera of healthy individuals, the major isoenzyme fraction is LDH-2, followed by LDH-1, LDH-3, LDH-4, and LDH-5.
- However, cardiac tissue and red blood cells contain a higher concentration of LDH-1. Therefore, in conditions involving cardiac necrosis (AMI) and intravascular hemolysis.

**Table 3: LDH Isoenzymes – Tissue Localization and Tissue Sources**

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

This flipped pattern is suggestive of AMI. However, LDH is not specific to cardiac tissue and is not a preferred marker of diagnosis of AMI.

- LDH-1/LDH-2 ratios greater than 1 also may be observed in hemolyzed serum samples.
- Elevations of LDH-3 occur most frequently with pulmonary involvement and are also observed in patients with various carcinomas.
- The LDH-4 and LDH-5 isoenzymes are found primarily in liver and skeletal muscle tissue, with LDH-5 being the predominant fraction in these tissues.
- LDH-5 levels have greatest clinical significance in the detection of hepatic disorders, particularly intrahepatic disorders.
  - Disorders of skeletal muscle will reveal elevated LDH-5 levels, as depicted in the muscular dystrophies.

### 2.2.3. Reference Range

LDH, 100–225 U/L (37°C).

### 2.3. ASPARTATE AMINOTRANSFERASE

(EC 2.6.1.1; L-aspartate: 2-oxoglutarate aminotransferase; AST)

- Aspartate aminotransferase (AST) is an enzyme belonging to the class of transferases. It is commonly referred to as a transaminase and is involved in the transfer of an amino group between aspartate and α-keto acids.
- The older terminology, serum glutamic-oxaloacetic transaminase (SGOT, or GOT), may also be used.
- Pyridoxal phosphate functions as a coenzyme.
- The reaction proceeds according to the following equation:
- The transamination reaction is important in intermediary metabolism because of its function in the synthesis and degradation of amino acids.
The keto-acids formed by the reaction are ultimately oxidized by the tricarboxylic acid cycle to provide a source of energy.

### 2.3.1. Tissue Source
- AST is widely distributed in human tissue. The highest concentrations are found in cardiac tissue, liver, and skeletal muscle, with smaller amounts found in the kidney, pancreas, and erythrocytes.

### 2.3.2. Diagnostic Significance
- The clinical use of AST is limited mainly to the evaluation of hepatocellular disorders and skeletal muscle involvement.
- AST elevations are frequently seen in pulmonary embolism.
- In AMI, AST levels begin to rise within 6 to 8 hours, peak at 24 hours, and generally return to normal within 5 days.
  - However, because of the wide tissue distribution, AST levels are not useful in the diagnosis of AMI.
  - Following congestive heart failure.
- AST levels also may be increased, probably reflecting liver involvement as a result of inadequate blood supply to that organ.
  - AST levels are highest in acute hepatocellular disorders.
  - In viral hepatitis, levels may reach 100 times.
  - In cirrhosis, only moderate levels—approximately four times—are detected.
- Skeletal muscle disorders, such as the muscular dystrophies, and inflammatory conditions also cause increases in AST levels.
- The intracellular concentration of AST may be 7,000 times higher than the extracellular concentration.
  - AST exists as two isoenzyme fractions located in the cell cytoplasm and mitochondria.
  - The cytoplasmic isoenzyme is the predominant form occurring in serum.
  - In disorders producing cellular necrosis, the mitochondrial form may be significantly increased.

### 2.3.3. Reference Range
- AST, 5 to 30 U/L (37°C).

### 2.4. ALANINE AMINOTRANSFERASE (EC 2.6.1.2; L-alanine: 2-oxoglutarate aminotransferase; ALT)
- Alanine aminotransferase (ALT) is a transferase with enzymatic activity similar to that of AST. Specifically, it catalyzes the transfer of an amino group from alanine to α-ketoglutarate with the formation of glutamate and pyruvate.
- The older terminology was serum glutamic-pyruvic transaminase (SGPT, or GPT).
- The following equation indicates the transferase reaction. Pyridoxal phosphate acts as the coenzyme.

### 2.4.1. Tissue Source
- ALT is distributed in many tissues, with comparatively high concentrations in the liver. It is considered the more liver-specific enzyme of the transferases.
2.4.2. Diagnostic Significance

- Clinical applications of ALT assays are confined mainly to evaluation of hepatic disorders. Higher elevations are found in hepatocellular disorders than in extrahepatic or intrahepatic obstructive disorders.
- In acute inflammatory conditions of the liver, ALT elevations are frequently higher than those of AST and tend to remain elevated longer as a result of the longer half-life of ALT in serum (16 and 24 hours, respectfully).
- Cardiac tissue contains a small amount of ALT activity, but the serum level usually remains normal in AMI unless subsequent liver damage has occurred.
- ALT levels have historically been compared with levels of AST to help determine the source of an elevated AST level and to detect liver involvement concurrent with myocardial injury.

2.4.3. Reference Range

- ALT, 6–37 U/L (37°C).

2.5. ALKALINE PHOSPHATASE
(EC 3.1.3.1; orthophosphoric-monoester phosphohydrolase [alkaline optimum]; ALP)

- Alkaline phosphatase (ALP) belongs to a group of enzymes that catalyze the hydrolysis of various phosphormonoesters at an alkaline pH.
- Consequently, ALP is a non-specific enzyme capable of reacting with many different substrates.
- Specifically, ALP functions to liberate inorganic phosphate from an organic phosphate ester with the concomitant production of an alcohol.
- The reaction proceeds according to the following equation:

\[
\text{Phosphomonoester} \rightarrow \text{Alcohol} + \text{Phosphate ion}
\]

- The optimal pH for the reaction is 9.0 to 10.0, but optimal pH varies with the substrate used. The enzyme requires \(\text{Mg}^{2+}\) as an activator.

2.5.1. Tissue Source

- ALP activity is present on cell surfaces in most human tissue.
- The highest concentrations are found in the intestine, liver, bone, spleen, placenta, and kidney.
  - In the liver, the enzyme is located on both sinusoidal and bile canalicular membranes;
  - Activity in bone is confined to the osteoblasts, those cells involved in the production of bone matrix.

2.5.2. Diagnostic Significance

- Elevations of ALP are of most diagnostic significance in the evaluation of hepatobiliary and bone disorders.
- In hepatobiliary disorders, elevations are more predominant in obstructive conditions than...
in hepatocellular disorders;

- Increases are primarily a result of increased synthesis of the enzyme induced by cholestasis.

In bone disorders, elevations are observed when there is involvement of osteoblasts.

- The highest elevations of ALP activity occur in Paget’s disease (osteitis deformans).
- Other bone disorders include osteomalacia, rickets, hyperparathyroidism, osteogenic sarcoma, during healing bone fractures and during periods of physiologic bone growth.

In normal pregnancy, increased ALP activity can be detected between weeks 16 and 20.

- ALP activity increases and persists until the onset of labor. Activity then returns to normal within 3 to 6 days.
- Elevations also may be seen in complications of pregnancy such as hypertension, preeclampsia, and eclampsia, as well as in threatened abortion.

ALP levels are significantly decreased in the inherited condition of hypo-phosphatasia.

- Subnormal activity is a result of the absence of the bone isoenzyme and results in inadequate bone calcification.

### 2.5.3. Reference Range

- ALP, 30 to 90 U/L (30°C).

### 2.6. ACID PHOSPHATASE

**EC 3.1.3.2; orthophosphoric acid-monoester phosphohydrolase [acid optimum]; ACP**

- Acid phosphatase (ACP) belongs to the same group of phosphatase enzymes as ALP and is a hydrolase that catalyzes the same type of reactions.
- The major difference between ACP and ALP is the pH of the reaction. ACP functions at an optimal pH of approximately 5.0.
- The following equation outlines the reaction sequence:

![Reaction Equation]

**2.6.1. Tissue Source**

- Acid phosphatase is present in lysosomes, which are organelles present in all cells with the possible exception of erythrocytes.
  - The lysosomal and prostatic enzymes are strongly inhibited by d-tartrate ions (tartrate-labile ACP), whereas the erythrocyte and bone isoenzymes are not (TR-ACP).

- Extra-lysosomal ACP activity are also present in many cells; in the prostate, bone, liver, spleen, kidney, erythrocytes, and platelets. The prostate is the richest source, with many times the activity found in other tissue.

**2.6.2. Diagnostic Significance**

- Historically, ACP measurement has been used as an aid in the detection of prostatic carcinoma, particularly metastatic carcinoma of the prostate.
- Total ACP determinations are relatively insensitive techniques, detecting elevated ACP levels resulting from prostatic carcinoma in the majority of cases only when the tumor has metastasized.
- Newer markers, such as prostate-specific antigen (PSA), are more useful screening and diagnostic tools.
• PSA is more likely than ACP to be elevated at each stage of prostatic carcinoma, even though a normal PSA level may be found in stage D tumors.
• PSA is more specific and sensitive for diagnosis of prostatic cancer.
• However, PSA is elevated in conditions other than prostatic carcinoma, such as benign prostatic hypertrophy and prostatitis.
• ACP is more useful for monitoring the treatment of a known case of disseminated prostatic carcinoma than for making the diagnosis.

Other prostatic conditions in which ACP elevations have been reported include hyperplasia of the prostate and prostatic surgery.

Serum ACP activity may frequently be elevated in bone disease. Activity has been shown to be associated with the osteoclasts.

• Elevations of ACP have been noted in Paget’s disease, and in breast cancer with bone metastases.
• Because of ACP activity in platelets, elevations are observed when platelet damage occurs, as in the thrombocytopenia resulting from excessive platelet destruction from idiopathic thrombocytopenic purpura.

2.6.3. Reference Range

• Prostatic ACP, 0 to 3.5 ng/mL.

2.7. γ-Glutamyltransferase (EC 2.3.2.21; γ-glutamyl-peptide: amino acid γ-glutamyletransferase; GGT)

γ-Glutamyltransferase (GGT) is an enzyme involved in the transfer of the γ-glutamyl residue from γ-glutamyl peptides to amino acids, H₂O, and other small peptides.

In most biologic systems, glutathione serves as the γ-glutamyl donor. The following equation outlines the reaction sequence:

\[ \text{Glutathione} + \text{amino acid} \rightarrow \text{glutamyl - peptide} + \text{l-cysteinylglycine} \]

The specific physiologic function of GGT has not been clearly established, but it is suggested that GGT is involved in:

• Peptide and protein synthesis,
• Regulation of tissue glutathione levels, and
• The transport of amino acids across cell membranes.

2.7.1. Tissue Source

• GGT activity is found primarily in tissue of the kidney, brain, prostate, pancreas, and liver.
• Clinical applications of assay, however, are confined mainly to evaluation of liver and biliary system disorders.

2.7.2. Diagnostic Significance

• In the liver, GGT is located in the canaliculi of the hepatic cells and particularly in the epithelial cells lining the biliary ductules.
• Because of these locations, GGT is elevated in virtually all hepatobiliary disorders, making it one of the most sensitive of enzyme assays in these conditions.
• Higher elevations are generally observed in biliary tract obstruction.
• Within the hepatic parenchyma, GGT exists to a large extent in the smooth endoplasmic reticulum and is, therefore, subject to hepatic microsomal induction.

• Because of the susceptibility to enzyme induction, any interpretation of GGT levels must be done with consideration of the consequent effects of drugs and alcohol.
• Therefore, GGT levels will be increased in patients receiving enzyme-inducing drugs such as warfarin, phenobarbital, and phenytoin.
Because of the effects of alcohol on GGT activity, elevated GGT levels may indicate alcoholism, particularly chronic alcoholism.

GGT assays are useful in monitoring the effects of abstention from alcohol and are used as such by alcohol treatment centers. GGT levels are also elevated in other conditions, such as acute pancreatitis, diabetes mellitus, and myocardial infarction.

2.7.3. Reference Range

- GGT: male, 6–45 U/L (37°C); female, 5–30 U/L (37°C).
- Values are lower in females, presumably because of suppression of enzyme activity resulting from estrogenic or progestational hormones.

2.8. Amylase (EC3.2.1.1; 1, 4-\(\alpha\)-D-glucan glucanohydrolase; AML)

- Amylase (AMS) is an enzyme belonging to the class of hydrolases that catalyze the breakdown of starch and glycogen.
- Starch consists of both amylose and amylopectin.
- Amylose is a long, unbranched chain of glucose molecules, linked by \(\alpha 1–4\) glycosidic bonds; amylopectin is a branched-chain polysaccharide with \(\alpha 1–6\) linkages at the branch points.
- The structure of glycogen is similar to that of amylopectin but is more highly branched.
- \(\alpha\)AMS attacks only the \(\alpha 1–4\) glycosidic bonds to produce degradation products consisting of glucose; maltose; and intermediate chains, called dextrins, which contain \(\alpha 1–6\) branching linkages.
- Cellulose and other structural polysaccharides consisting of linkages are not attacked by \(\alpha\)AMS. AMS is therefore an important enzyme in the physiologic digestion of starches. The reaction proceeds according to the following equation:

\[
\text{AMS \ requires calcium and chloride ions for its activation.}
\]

2.8.1. Tissue Source

- The acinar cells of the pancreas and the salivary glands are the major tissue sources of serum AMS.
- Lesser concentrations are found in skeletal muscle and the small intestine and fallopian tubes.
- AMS is the smallest enzyme, with a molecular weight of 50,000 to 55,000.
  - Because of its small size, it is readily filtered by the renal glomerulus and also appears in the urine.
- Digestion of starches begins in the mouth with the hydrolytic action of salivary AMS.
- Salivary AMS activity, however, is of short duration because, on swallowing, it is inactivated by the acidity of the gastric contents.
- Pancreatic AMS then performs the major digestive action of starches once the polysaccharides reach the intestine.

2.8.2. Diagnostic Significance

- The diagnostic significance of serum and urine AMS measurements is in the diagnosis of acute pancreatitis.
- Disorders of tissue other than the pancreas can also produce elevations in AMS levels.
- Therefore, an elevated AMS level is a non-specific finding.
However, the degree of elevation of AMS is helpful, to some extent, in the differential diagnosis of acute pancreatitis.

Other disorders causing an elevated serum AMS level include

- Salivary gland lesions, such as mumps and parotitis, and
- Other intra-abdominal diseases, such as perforated peptic ulcer, intestinal obstruction, cholecystitis, ruptured ectopic pregnancy, mesenteric infarction, and acute appendicitis.

In addition, elevations have been reported in renal insufficiency and diabetic ketoacidosis.

2.8.3. Reference Range

- Because of the various AMS procedures currently in use, activity is expressed according to each procedure.

2.9. LIPASE (EC 3.1.1.3; triacylglycerol acylhydrolase; LPS)

- Lipase (LPS) is an enzyme that hydrolyzes the ester linkages of fats to produce alcohols and fatty acids.
- Specifically, LPS catalyzes the partial hydrolysis of dietary triglycerides in the intestine to the 2-monoglyceride intermediate, with the production of long-chain fatty acids.
- The reaction proceeds according to the following equation:

\[
\begin{align*}
\text{Triacylglycerol} & \rightarrow \text{2-Monoglyceride} + \text{Fatty acids} \\
\text{LPS} & : \text{CH} & : \text{OH} \\
\text{CH} & : \text{OH} & : \text{R} _ 1 & + 2 \text{H}_2\text{O} \\
\text{CH} & : \text{OH} & : \text{R} _ 2 & + 2 \text{fatty acids}
\end{align*}
\]

- The enzymatic activity of pancreatic LPS is specific for the fatty acid residues at positions 1 and 3 of the triglyceride molecule, but substrate must be an emulsion for activity to occur.
- The reaction rate is accelerated by the presence of colipase and a bile salt.

2.9.1. Tissue Source

- LPS concentration is found primarily in the pancreas, although it is also present in the stomach and small intestine.

2.9.2. Diagnostic Significance

- Clinical assays of serum LPS measurements are confined almost exclusively to the diagnosis of acute pancreatitis.
- It is similar in this respect to AMS measurements but is considered more specific for pancreatic disorders than AMS measurement.
- Both AMS and LPS levels raise quickly, but LPS elevations persist for approximately 5 days in acute pancreatitis, whereas AMS elevations persist for only 2 to 3 days.
- Elevations have been reported in cases of penetrating duodenal ulcers and perforated peptic ulcers, intestinal obstruction, and acute cholecystitis.
- In contrast to AMS levels, LPS levels are normal in conditions of salivary gland involvement.
  - Therefore, LPS levels are useful in differentiating serum AMS elevation as a result of pancreatic versus salivary involvement.

2.9.3. Reference Range

- LPS, 0–1.0 U/mL.
2.10. GLUCOSE-6-PHOSPHATE DEHYDROGENASE (EC 1.1.1.49); D-Glucose -6-phosphate: NADP+ oxidoreductase; G6PD):

 marketed oxidoreductase that catalyzes the oxidation of glucose-6-phosphate to 6-phosphogluconate or the corresponding lactone.

 The reaction is important as the first step in the pentose-phosphate shunt of glucose metabolism with the ultimate production of NADPH. The reaction is outlined in the following equation:

\[
\text{G6PD} + \text{NADP} + \text{G-6-PD} \rightarrow \text{6-Phosphogluconate} + \text{NADPH} + \text{H}^+.
\]

2.10.1. Tissue Source

Sources of G-6-PD include the adrenal cortex, spleen, thymus, lymph nodes, lactating mammary gland, and erythrocytes.

Little activity is found in normal serum.

2.10.2. Diagnostic Significance

Most of the interest of G-6-PD focuses on its role in the erythrocyte. Here, it functions to maintain NADPH in reduced form.

An adequate concentration of NADPH is required to regenerate sulphydryl-containing proteins, such as glutathione, from the oxidized to the reduced state.

Glutathione in the reduced form, in turn, protects hemoglobin from oxidation by agents that may be present in the cell.

A deficiency of G-6-PD results in an inadequate supply of NADPH and, ultimately, in the inability to maintain reduced glutathione levels.

When erythrocytes are exposed to oxidizing agents, hemolysis occurs because of oxidation of hemoglobin and damage of the cell membrane.

G-6-PD deficiency is an inherited sex-linked trait. The disorder can result in several different clinical manifestations, one of which is drug-induced hemolytic anemia.

When exposed to an oxidant drug such as primaquine, an antimalarial drug, affected individuals experience a hemolytic episode.

The severity of the hemolysis is related to the drug concentration.

Increased levels of G-6-PD in the serum have been reported in myocardial infarction and megaloblastic anemias.

2.10.3. Reference Range

G-6-PD, 10–15 U/g Hb.

2.11. CHOLINESTERASE (EC 3.1.1.7, acetycholine acetylhydrolase).

 marketed cholinesterase or choline esterase I.

2.11.1. Tissue source

It is found in:

- Erythrocytes, Lung and spleen.
- Nerve endings.
2.11.2. Diagnostic Significance
- Measurements of CHE activity in serum are used:
  1. as a test of liver function.
  2. as an indicator of possible insecticide poisoning.
- Causes of decreased plasma cholinesterase activity:
  1. Hepatic parenchymal disease (reduced synthesis)
  2. Ingestion or absorption through the skin, of such anticholinesterases as organophosphates.
- Causes of increased plasma cholinesterase activity:
  1. Recovery from liver damage (actively growing hepatocytes).
  2. Nephrotic syndrome.

2.11.3. Reference range
- Normal values for CHE: 4.9-11.9 U/mL.

2.12. GLUTAMATE DEHYDROGENASE
(EC 1.4.1.3; L-glutamate: NAD (P)+ oxidoreductase, deamination; GLD)
- It is a mitochondrial enzyme.

2.12.1. Tissue source
- It is found mainly in the:
  - Liver, Heart muscle
  - Kidneys but small amounts occur in other tissue, including
  - Brain, Skeletal muscle tissue, and Leukocytes.

2.12.2. Diagnostic Significance
- GLD is increased in serum of patients with hepatocellular damage offering differential diagnostic potential in the investigation of liver disease, particularly when interpreted in conjunction with other enzyme test results.
- The key to this differential diagnostic potential is to be found in the intra-organ and intracellular distribution of the enzyme.
- As an exclusively mitochondrial enzyme, GLD is released from necrotic cells and is of value in estimation of the severity of liver cell damage.

2.12.3. Reference range
- The GLD upper reference limits are 6U/L (women) and 8U/L (men), when a method optimized at 37ºC is used.

3- ENZYMES & DISEASE DIGAGNOSIS

3.1. PANCREATIC ENZYMES:

3.1.1. Acute pancreatitis
- It is an inflammatory process in which pancreatic enzymes are activated and cause auto-digestion of the gland.
- It is a result of anatomical changes that arise from two events:
  1. The first is the autodigestion of the acinar cells by inappropriate activation of the pancreatic enzymes (especially trypsinogen) within the cell.
  2. The second is the cellular injury response that is mediated by pro-inflammatory cytokines.
- There are some enzymes that are synthesized and stored as the active enzymes in the zymogen granules.
These include α-amylase and lipase.

3.1.2. α-Amylase: (EC 3.2.1.1; 1, 4-α-D-glucan glucohydrolase; AML)

- See above.
- Raised plasma amylase activity caused by:
  1. Marked increase (five to 10 times the upper reference limit):
     - Acute pancreatitis.
     - Severe glomerular impairment.
  2. Moderate to higher increase (up to five times the upper reference limit):
     - Perforated peptic ulcer.
     - Acute cholecystitis.
     - Intestinal obstruction.
     - Salivary gland disorders like mumps, salivary calculi.

3.1.3. Lipase: (EC 3.1.1.3; triacylglycerol acylhydrolase; LPS)

- See above.
- Plasma lipase levels are elevated in:
  - Acute pancreatitis and
  - Carcinoma of the pancreas.
- Serum amylase is increased in mumps, pancreatic disease or due to some other cause, whereas lipase is increased only in pancreatitis.
- Therefore, the determination of both amylase and lipase together helps in the diagnosis of acute pancreatitis.

3.2. LIVER ENZYMES:

- There are three types of enzymes:
  1. Enzymes which are normally present inside the hepatocytes released into the blood when there is a hepatocellular damage = markers of hepatocellular damage.
  2. Enzymes which are primary membrane bound (plasma membrane or side of hepatocytes) = markers of cholestasis.
  3. Enzymes which are synthesized in the hepatocyte = indicates disturbances in the hepatocellular synthesis.

3.2.1. MARKERS OF HEPATOCYTOPLASMIC DAMAGE:

1. Amino-transaminases:

- See above.
- In the liver, the concentration of ALT per unit weight of the tissue is more than AST.
- These enzymes are more important in assessing and monitoring the degree of liver cell inflammation and necrosis.
- Hepatocytes have very high activity of ALT, therefore elevations in serum ALT are considered to be relatively specific for liver disease.
- AST may be elevated in other forms of tissue damage, such as myocardial infarction, muscle necrosis and renal disorders.
- In acute viral hepatitis there is a 100-1000 times increase in both ALT and AST but ALT level is increased more than that of AST.

2. Aspartate Transaminase (EC 2.6.1.1; L-aspartate: 2-oxoglutarate aminotransferase; AST)

- See above.
- Causes of Raised Plasma AST Activities:
  - Artefactual:
    - Due to in vitro release from erythrocytes if there is haemolysis or if separation of plasma from cells is delayed.
3. ALT (glutamate pyruvate transaminase. GPT)

- It is present in high concentrations in liver and to a lesser extent, in skeletal muscle, kidney and heart. Half-life = 47 hours
- In liver damage, both enzymes are increased but ALT increases more. In myocardial infarction AST is increased with little or no increase in ALT.

**Causes of Raised Plasma ALT Activities**
- **Marked increase** (10 to 100 times the upper limit of the adult reference range)
  - Circulatory failure with 'shock' and hypoxia:
  - Acute viral or toxic hepatitis.
- **Moderate to higher increase**:
  - Cirrhosis (may be normal or up to twice the upper adult reference limit): infectious mononucleosis (due to liver involvement):
  - Liver congestion secondary to congestive cardiac failure:
  - Cholestatic jaundice (up to 10 times the upper reference limit in adults); surgery or extensive trauma and skeletal muscle disease (much less affected than AST)

3.2.2. MARKERS OF CHOLESTASIS:

1. Alkaline phosphatase (EC 3.1.3.1; orthophosphoric-monoester phosphohydrolase [alkaline optimum]; ALP). Half-life= 10 days.

- In adults plasma ALP is derived mainly from bone and liver in approximately equal proportions.
- They are present in most tissues but are in particularly high concentration in the osteoblasts of bone and the cells of the hepatobiliary tract, intestinal wall, renal tubules and placenta.
- The exact metabolic function of ALP is unknown but it is probably important for calcification of bone.

**Causes of raised Plasma ALP activity**
- **Physiological**:
  - There is a gradual increase in the proportion of liver ALP with age: in the elderly the plasma bone isoenzyme activity may increase slightly.
- **Pathologically**:
  - Bone disease: Bone diseases with increased osteoblastic activity, or liver disease with involvement of the biliary tracts, are the commonest causes of an increased total alkaline phosphatase activity.
    - Rickets and osteomalacia.
    - Secondary hyperparathyroidism.
  - Liver disease.
  - Malignancy.
  - Bone or liver involvement or direct tumor production.
Possible Causes of Low Plasma ALP Activity:

- Arrested bone growth.
- Hypophosphatasia: an autosomal recessive disorder, associated with rickets or osteomalacia.

2. Gamma-glutamyl-transferase (EC 2.3.2.21; γ-glutamyl-peptide: amino acid γ-glutamyltransferase; GGT):

- See above.
- Causes of raised plasma GGT activity
  - Induction of enzyme synthesis, without cell damage, by drugs or alcohol.
  - Hepatocellular damage, such as that due to infectious hepatitis:
  - A patient should never be labeled an alcoholic because of a high plasma GGT activity alone.

### 3.3. MUSCLE ENZYMES

3.3. 1. Creatine Kinase (EC 2.7.3.2; adenosine triphosphate: creatine Nphosphotransferase CK)

- See above.
- CK is most abundant in cells of cardiac and skeletal muscle and in brain, but also occurs in other tissues such as smooth muscle (GIT & UT).
- Serum CK activity is greatly elevated in all types of muscular dystrophy. In progressive muscular dystrophy.
- Serum CK activity characteristically falls as patients get older and as the mass functioning muscle diminishes with the progression of the disease.
- Quite high values of CK are noted in viral myositis, polymyositis and similar muscle disease.

3.3. 2. Lactate Dehydrogenase (EC 1.1.1.27; L-lactate: NAD+ oxidoreductase; LD)

- It catalyzes the reversible interconversion of lactate and pyruvate.
- The enzyme is widely distributed in the body, with high concentrations in cells of cardiac and skeletal muscle, liver, kidney, brain and erythrocytes: measurement of plasma total LD activity is therefore a nonspecific marker of cell damage.
- It is increased in plasma in M.I., acute leukemias, generalized carcinomatosis and in acute hepatitis. Estimation of its isoenzymes in more useful in clinching diagnosis between hepatic disease and M.I.
- Isoenzymes of LD
  - See above.

3.3.3. Muscle Disease

- In the muscular dystrophies plasma levels of the muscle enzymes, CK and the transaminases are increased, probably because of leakage from the diseased cells.
- Results of plasma CK estimation are the more specific.
- Although plasma enzyme activities are usually normal in neurogenic muscular atrophy; the number of false positives make such tests unreliable in differentiating these conditions from primary muscle disease.

### 3.4. CARDIAC ENZYMES

Myocardial Infarction

- All plasma enzyme activities (including that of CK-MB) may be normal until at least four hours after the onset of chest pain due to a myocardial infarction; blood should not be taken for enzyme assay until this time has elapsed.
- The simultaneous measurement of plasma CK-MB activity, which is shown to exceed six
6% of the total CK activity, may occasionally help in the early diagnosis: a raised plasma CK-MB activity or concentration alone is not diagnostic of an infarction.

- In most cases of suspected myocardial infarction, measurement of plasma total CK-MB and LD1 activities, together with the clinical and ECG findings, are adequate to make a diagnosis. Plasma total CK activity alone can be very misleading.

- Newer markers for myocardial infarctions: troponin T and troponin I are regulatory proteins involved in myocardial contractility. Both being evaluated as an early and specific marker of acute myocardial infarction.

- Elevated serum troponins are more predictive of adverse outcomes in unstable angina or myocardial infarction than the conventional assay of CK2 (CK-MB).

### 3.5. ENZYMES IN MALIGNANCY

- Plasma total enzyme activities may be raised or an abnormal isoenzyme detected, in several neoplastic disorders.

- Serum prostatic (tartrate-labile) acid phosphatase activity rises in some cases of malignancy of the prostate gland.

- Any malignancy may be associated with a non-specific increase in plasma LD1 and occasionally, transaminase activity.

- Plasma transaminase and alkaline phosphatase estimations may be of value to monitor treatment of malignant disease.

- Raised levels may indicate secondary deposits in liver or of alkaline phosphatase, in bone. Liver deposits may also cause an increase in plasma LD or GGT.

- Tumors occasionally produce a number of enzymes, such as the ALP isoenzyme, LD (HBD) or CK-BB, assays of which may be used as an aid to diagnosis or for monitoring treatment.

### 3.6. OTHER CLINICAL CORRELATIONS

- Every catabolic or anabolic reaction in the body is catalysed by an enzyme that is synthesized from a specific gene(s).

- Mutation in this gene(s), defective transcription, posttranscriptional or translational processing of the enzyme leads to deficiency of the enzyme and hence deficiency or absence of that metabolic reaction product and accumulation of its substrate.

- This is the base of the metabolic inborn errors of metabolism as hereditary diseases.

1. Niemann-Pick disease: Acid Sphingomyelinase Deficiency

- Sphingomyelin, a ubiquitous component of cell membranes, especially neuronal membranes, is normally degraded within lysosomes by the enzyme sphingomyelinase.

- In patients with Niemann-Pick disease, inherited deficiency of this enzyme causes sphingomyelin to accumulate in lysosomes of the brain, bone marrow, and other organs.

- Enlargement of the lysosomes interferes with their normal function, leading to cell death and consequent neuropathy.

- Symptoms include failure to thrive and death in early childhood as well as learning disorders in those who survive the postnatal period.

2. Homocysteinuria: Cystathionine β-synthase Deficiency

1. Cystathionine β-synthase catalyzes conversion of homocysteine to cystathionine, a critical precursor of cysteine.

2. Deficiency of this enzyme leads to the most common form of homocystinuria; a pediatric disorder characterized by accumulation of homocysteine and reduced activity of several sulfotransferase reactions that require this compound or its derivatives as substrate.

3. Accumulation of homocysteine and reduced transsulfation of various compounds leads to abnormalities in connective tissue structures that cause altered blood vessel wall structure,
loss of skeletal bone density (osteoporosis), dislocated optic lens (ectopia lentis), and increased risk of blood clots.

4. ENZYME APPLICATION

4.1. APPLIED EXAMPLES OF PLASMA ENZYMES DISEASE PATTERN (ENZYMOMGRAM):

- In heart diseases within the first day of infarction, elevation of serum CK is noticed followed by GOT and GPT (AST, and, ALT) that peaks at 3rd days and LDH that peaks at 5th days.
- Other enzymes are also used and include; γ-glutamyl-transpeptidase (γGTP), histaminase, pseudocholinesterase and aldolase.
- However, the serum level of these enzymes also increases in non-cardiac diseases, e.g.,
  - CK in hypothyroidism, muscular dystrophy, and dermatomyositis;
  - GOT in muscular and hepatic diseases;
  - LDH in cancer, pulmonary embolism, renal diseases, pernicious anemia, and muscle and liver diseases; aldolase in dermatomyositis, muscular dystrophy and viral hepatitis, and,
  - γGTP in hepatobiliary disorders, alcoholism and pancreatic diseases.
- The detection of tissue-specific isoenzyme would resolve confusion about tissue origin of some of these enzymes, e.g., cardiac CK-MB or CPK2 and LDH-1 and -2.

- In liver disease abnormally elevated levels of the following enzymes are detected;
  - GPT, GOT, ALP (particularly in post-hepatic jaundice, cancer liver and metastatic carcinoma),
  - 5'-nucleotidase (particularly in biliary tract diseases),
  - LDH, isocitrate dehydrogenase (particularly in infective hepatitis, malignancy and drug toxicity),
  - Ornithine carbamoyl transferase (particularly in viral hepatitis, obstructive jaundice, cirrhosis and metastatic carcinoma) and
  - Sorbitol dehydrogenase (particularly in viral hepatitis and chemical poisoning).
- However, ALP is also elevated in rickets, osteomalacia, hyperparathyroidism, Paget’s disease and bone malignancy.
- In gastrointestinal diseases, elevated serum pancreatic amylase and lipase levels are detected in acute pancreatitis, mumps, perforated peptic ulcer and intestinal obstruction.

- In malignancy elevated serum levels of
  - LDH, aldolase, phosphohexose isomerase are detected in widespread malignancies and leukemia;
  - Cathepsins, plasmin (serine endopeptidase; EC 3.4.21.7) and other proteases in metastatic tumors;
  - LAP in liver carcinoma;
  - Acid phosphatase in prostate carcinoma, osteolytic metastasis from breast, leukemia and myeloproliferative disorders (but also in Gaucher’s disease, hemolytic anemia, thrombocytosis, Paget’s disease and pulmonary embolism);
  - β-glucuronidase in cancer of urinary bladder, cancer head of pancreas, breast and cervix cancer; and
  - Alkaline phosphatase in liver and bone metastasis and carcinoma of pancreas.
4.2. ENZYME ENGINEERING & INDUSTRIAL APPLICATIONS OF ENZYMES

- Modern enzyme biotechnology began in 1874 when Christian Hansen extracting dried calves' stomachs with saline solution to prepare rennet for cheese manufacturing.
- However, enzymes were used for long time either in the form of vegetables rich in enzymes, or in the form of microorganisms, e.g., for brewing processes, in baking, and in the production of alcohol.
- Therefore, enzymes are the engineers of biotechnology and are engineered by biotechnology, e.g., amplifying their genes for larger production, and, by mutating their genes to be constitutively active, not to be inhibited by feedback effectors, to have high stability against temperature and pH changes, to withstand the organic environment, and, change substrate specificity.

- Enzyme engineering (or enzyme biotechnology) is the usage of the catalytic activity of isolated enzymes, to produce new metabolites or to convert some compounds into another's (biotransformation) useful as chemicals, pharmaceuticals, fuel, food or agricultural additives.
- Natural source of these enzymes are animal tissues, plants, fungi and bacteria, and, cloned genes for specific recombinant enzymes production in foreign host organisms.
- Enzyme reactor consists of a vessel containing a reaction medium, used to perform a desired conversion by natural or recombinant enzymes.
- Enzymes used in this process are free in the solution or immobilized in particulate, membranous or fibrous support.

1- Amylase - instead of the conventional acid hydrolysis;
   - Breaks starch into simpler sugars useful, e.g., for baking and high-fructose corn syrup preparation after isomerizing glucose into fructose using glucose isomerase. These syrups have enhanced sweetening properties and lower calorific values than sucrose for the same level of sweetness.
   - Liquefaction of starch was also improved by using a heat-stable α-amylase.
   - Textile desizing (starch removal) by amylase was used instead of the long difficult and textile damaging methods of treatment with acid, alkali or oxidizing agents, or soaked in water for several days so that naturally occurring microorganisms could break down the starch.

2- Likewise, proteases are used to lower flour protein content for a smoother biscuit manufacturing and to predigest baby food.
   - In photographic industry, a protease is used to dissolve gelatin off scrap film to recover its silver content.

3- Natural (from calf stomach) or recombinant renin is used to hydrolyze proteins during cheese manufacturing.

4- Lipases are used during the production of Roquefort cheese to enhance the ripening of the blue-mould cheese, and,

5- Lactase hydrolyses milk lactose for the usage of lactase-deficient people.

6- Papain (a di and tri-peptidase from the papaya fruit; EC 3.4.22.2) is used to soften meat for cooking.

7- Cellulase is used to break down cellulose into sugars that can be fermented into ethanol in biofuel production. As biological detergents and contact lens cleaners, proteases (e.g., the peptidase subtilisin; EC 3.4.21.62),

8- Amylase, lipase and cellulase are used. In rubber industry,

9- Catalase is used to generate O₂ from peroxide to convert latex into foam rubber.

10- In molecular biology reagents industry, restriction enzymes, nucleotide transferases, DNA
ligase and polymerases are used to manipulate DNA in genetic engineering and polymerase chain reactions,

11- Important in pharmacology, agriculture, medicine e.g.:
- Urokinase to activate intravascular blood clot dissolution through activating plasminogen into plasmin, and,
- Encapsulated digestive pancreatic proteolytic enzymes in cases of pancreatic insufficiency, e.g., cystic fibrosis.
- Several enzymes are applied as reagents in laboratory diagnostic techniques, e.g., glucose, glycerol and cholesterol oxidases in determination of glucose, triglycerides or cholesterol levels in clinical samples.

12- However, the important application is the investment of enzyme kinetics and mechanisms in developing enzyme inhibitors as drugs that targets specific metabolic pathways as:
- Antibacterial, antiviral, anticancer, and, anti-metabolic drugs.
- Although designed to be specific for these conditions, the close similarity between metabolic enzymes in viruses, bacteria and cancer cells to the normal cells made it inevitable that the patient would succumb some side-effects.

13- Biosensors are composed of immobilized enzyme(s) that react with substrate to generate a product which is used by a transducer to generate an electrical signal.
- They express the rate of substrate consumption and/or product generation. An example is the glucose electrode that is composed of a layer of glucose oxidase immobilized on polyacrylamide gel around a platinum oxygen electrode.
- Contact with a solution containing glucose activates the reaction with O₂ to generate H₂O₂ and gluconolactone.
- The electrode detects the corresponding reduction in O₂.
- Different types of biosensors are used as detecting devices in diagnostic clinical biochemistry, food hygiene and detection of environmental pollution.

4.3. THE ENZYME AS DRUGS: (Primary and Replacement Therapies)

4.3.1. Introduction:
- Enzymes as therapy are either used to replenish a missing enzyme due to an inherited gene defect or as a primary therapy, i.e., unrelated to such diseases.
- Enzymes as drugs are specific to substrate and catalytically highly active on such substrate.
- They are administrated through injection, topical application, inhalation and orally.
- The first recombinant enzyme as a drug, was the clot dissolving Activase 1 (alteplase) - the recombinant human tissue plasminogen activator, was approved for human heart attacks in 1987.
- Polyethylene glycol-conjugated bovine adenosine deaminase (Adagen1) was approved in 1990, to treat patients afflicted with inherited adenosine deaminase deficiency type of severe combined immunodeficiency disease (SCID).
- Because of their specificity and potency, therapeutic enzymes now cover a wide range of diseases and conditions that include:
  - Inherited diseases (e.g., Gaucher's, Fabry's, mucopolysaccharidoses I, II and VI, Pompe's glycogen storage, cystic fibrosis, phenylketonuria, and adenosine deaminase deficiency).
  - Pro- and anticoagulants, antineoplastic enzymes and prodrug activator enzymes, antifungal, antiprotozoal and antibacterial enzymes, burn debridement and others.
  - Pompe’s disease was the first muscle disorder to be treated by enzyme replacement therapy.
4. 3.2. Examples:

1- The replaced adenosine deaminase conjugated to polyethylene glycol has enhanced half-life (originally less than 30 min) and reduced possibility of immunological reactions due to the bovine origin of the drug. The enzyme cleaves the excess circulating adenosine of the patients to reduce its toxicity to the immune system.

2- Ceredase1 (alglucerase injection; glucocerebrosidase) for the treatment of Gaucher's disease, a lysosomal storage disease.
   - It is the first enzyme replacement therapy in which an exogenous enzyme was targeted to its correct compartment within the body.
   - First the source was a modified placental glucocerebrosidase (Ceredase1) and subsequently recombinant human enzyme (imiglucerase) was used.

3- The second lysosomal storage disease to follow was Fabry’s disease; a fat (glycolipid) storage disorder caused by a deficiency in α-galactosidase.
   - It primarily affects the vasculature and results in renal failure, pain, and corneal clouding.
   - Recombinant human α-galactosidase was used.

4- Chondroitinases promote regeneration of injured spinal cord by removing, in the glial scar, the accumulated chondroitin sulfate that inhibits axon growth.
   - Hyaluronidase has a similar hydrolytic activity on chondroitin sulfate and may also help in the regeneration of damaged nerve tissue.

5- Phenylketonuria (PKU) is another genetic disorder requiring strict compliance with a specialized diet.
   - PKU is caused by low or non-existent phenylalanine hydroxylase activity, which catalyzes the conversion of phenylalanine to tyrosine.
   - An oral treatment, Phenylase™, is a recombinant yeast phenylalanine ammonia lyase that is able to degrade phenylalanine in the gastrointestinal tract.

6- Fat malabsorption; A mixture of pancreatic enzymes, including lipases, proteases and amylases, has been shown to be useful in the treatment of fat malabsorption in patients with human immunodeficiency virus and pancreatic insufficiency in cystic fibrosis patients.

7- Lysozyme has been used as a naturally occurring antibacterial agent in many foods and consumer products, because of its ability to break carbohydrate chains in the cell wall of bacteria.
   - Lysozyme has also been shown to possess activity against HIV, as has RNase A and urinary RNase U, which selectively degrade viral RNA.

8- Eletik: One of the side-effects of cancer chemotherapy is hyperuricemia, a build-up of uric acid that results in gouty arthritis and chronic renal disease. Urate oxidase is able to degrade the poorly soluble uric acid. Interestingly, the gene for this enzyme is present in humans, but possesses a nonsense codon. Recombinant Rasburicase (Eletik) is safe and effective as uricolytic agent particularly in its polyethylene glycol conjugated.

4.3.3. Enzyme Replacement Therapy for Inborn Errors of Metabolism

- Lysosomal enzyme deficiencies, which frequently result in disease due to accumulation of the substrate for the missing enzyme, are suitable targets for enzyme replacement therapy (ERT).
- In ERT, intravenously administered enzymes are taken up directly by the affected cells through a receptor-mediated mechanism.
- ERT provides temporary relief of symptoms but must be given repeatedly and is not a permanent cure.
4.3.4. Drugs acts as enzyme inhibitors (Application):

- At least half of the ten most commonly dispensed drugs in the United States act as inhibitors of enzymes.
- For example, the widely prescribed lactam antibiotics, such as penicillin and amoxicillin, act by inhibiting one or more of the enzymes of bacterial cell wall synthesis.
- Drugs may also act by inhibiting extracellular reactions. This is illustrated by angiotensin-converting enzyme (ACE) inhibitors that lower blood pressure by blocking the enzyme that cleaves angiotensin I to form the potent vasoconstrictor, angiotensin II.
- These drugs are including captopril, enalapril, and lisinopril, cause vasodilatation and a resultant reduction in blood pressure.

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<th>Table 4: Pharmaceutical Uses of Enzyme Inhibitors</th>
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<td>The Inhibitor</td>
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<td>5-Flourouracil</td>
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<td>Mercaptopurine</td>
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4.4. ANTIENZYME:

- Intestinal parasites, e.g., Ascaris express on its surface substances that are protein in nature which inhibits pepsin and trypsin.
- These substances are called antienzyme. For this reason the parasite worm is not digested in the intestine, where the inhibition occurs only around the parasite.
- Trypsin inhibitors are also found in raw egg white, potatoes, and soya bean.
- Antibodies against several non-functional plasma enzymes have a clinical diagnostic importance, since they are longer living than the enzyme itself and hence reflect the disease history better. Also autoimmune antibodies are clinically important.

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Best Regards

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