SEMIIOLOGY OF ENZYMES USED IN CLINICAL DIAGNOSIS
BONE DISEASES: ALP

- The phosphatases, which are analysed in the clinical laboratory, are generally phosphomonoesterases, catalyzing the hydrolysis reaction of the phosphoric esters (α-glycerophosphate, p-nitrophenylphosphate) and production of orthophosphoric acid.

\[
R - O - PO_3H_2 + H_2O \rightarrow R - OH + H_3PO_4
\]

- The phosphatases are not very specific but their optimum pH for the activity is varying with the organ of origin. From this point of view, they can be classified in:

1) Phosphomonoesterases type I (alkaline phosphatases):
- the optimum pH is 9.0-10.4;
- they are activated by the presence of Mg\(^{2+}\);
- they are widely distributed in the body, being present in high concentration in bones (osteoblasts-cells of growing bone), intestinal mucosa, renal tubule cells, and in lower concentration in the liver (cells lining the sinusoids, bile canaliculi), leukocytes, placenta, mammary gland; normal serum contains a mixture of isoenzymes derivated primarily from liver, intestines, bones; the one from the liver is predominant excepting the cases of rapid skeletal growth.

2) Phosphomonoesterases type II (acidic phosphatases):
- the optimum pH is 5.0-5.5;
- they are not activated by Mg\(^{2+}\), and are inhibited by fluorides and oxalates;
- they exist in the prostate gland, kidney, spleen, platelets and erythrocytes; they have a low concentration in plasma.
Diagnostic significance:
The values that are obtained represent the activity of more isoenzymes. The alkaline phosphatases are eliminated by bile, except the bone fraction (it has a high molecular mass and is catabolized by the reticulo-endothelial system cells).

Reference values:
- adults: 20 - 48 IU/L
- children: 38 - 138 IU/L
- third trimester of pregnancy: 28 - 115 IU/L

The children high values reflect the increased osteoblastic activity that occurs during periods of rapid skeletal growth.

The physiological increased value in the third trimester of pregnancy is due to the elaboration of a placental isoenzyme that is absorbed into the maternal bloodstream.

After a meal rich in carbohydrates and lipids the intestinal isoenzyme is increased, especially in the persons with B or O blood groups.
Pathological significance:

Increased activity can be noticed after treatment with drugs for epilepsy, anticoagulant, antidiabetic, especially in women.

In order to determine the organ origin of the increased values the isoenzymes can be separated by electrophoresis.

High values are present in:

1) bone isoenzyme:
   - bone disorders with increased osteoblastic activity - Paget’s disease (osteitis deformans), osteoblastic tumours with metastases;
   - hyperparathyroidism (mobilization of calcium and phosphorus from bone);
   - deficiency of vitamin D $3$: rickets, osteomalacia.

Decreased values exist in:

- hypophosphatasemia, rare congenital defect;
- depressed osteoblastic activity - dwarfs;
- hypothyroidism - deficiency of thyroid hormone;
- pernicious anemia (deficiency of vitamin B$_{12}$).
MUSCLE DISEASES: CPK-MM, LDH, ALD, AST,

- CREATINPHOSPHOKINASE (CPK, CK)

Creatine phosphokinase catalyzes the phosphorylation of creatine by ATP in muscle cells and brain tissue.

Because of its role closely linked to energy production, CPK reflects normal tissue catabolism. A rise above normal in serum indicates an alteration of cells containing a high amount of enzyme. CPK isoenzymes can be separated into three distinct molecular structures:

- CPK-BB (CPK1) located mainly in brain tissue
- CPK-MB (CPK2) located mainly in cardiac muscle and less in skeletal muscle
- CPK-MM (CPK3) located in skeletal muscle.

Elevated CPK due to skeletal muscle cell damage prevents detection of myocardial infarction by detection of total CPK. Fractionation and measurement of CPK isoenzymes replace the use of total CPK for a more precise location of tissue damage.
Reference values may vary depending on the method of determination used:

- Total CPK: men 40-175 U/l, women 25-140 U/l
- CPK-BB: indetectable
- CPK-MB: indetectable - 7 U/l
- CPK-MM: 5-70 U/l

Clinical implications:
- Elevated CPK-MM follows the skeletal muscle damage after an injury or muscle disease. Moderate increase is observed in hypothyroidism, a marked increase in muscle activity caused by intense agitation.
- Total CPK elevations may be due alcoholic cardiomyopathy, carbon monoxide poisoning, severe hyperthermia, post seizure, severe hypokalemia.
LACTATE DEHYDROGENASE (LD, LDH)

LDH catalyzes the reversible conversion of pyruvic acid into lactic acid during anaerobic glycolysis.

Because LDH exists in almost all tissues (in high concentrations in the liver, myocardium, kidney, skeletal muscle, erythrocytes) specific cell damage cause a general increase in total serum LDH. This limits the diagnostic usefulness of LDH. However, inactivation by heat or electrophoresis can identify and measure five isoforms in particular tissues.

- LDH1 and LDH2 occur mainly in the heart, kidney and erythrocytes
- LDH3 occurs mainly in the lungs
- LDH4 and LDH5 exist in liver and skeletal muscle.
Reference values:

Total LDH  48-115 U/L with the following distribution of the isoenzymes:

- \( \text{LDH}_1 \)  17,5-28,3 %
- \( \text{LDH}_2 \)  30,4-36,4 %
- \( \text{LDH}_3 \)  19,2-24,8 %
- \( \text{LDH}_4 \)  9,6-15,6 %
- \( \text{LDH}_5 \)  5,5-12,7 %

Clinical implications:

Many common diseases may increase total LDH level. Clinical use of total LDH activity are in particular:
CARDIOVASCULAR DISEASES: CPK, AST, ALT, LDH

- CPK-BB detection may indicate cerebral tissue damage, certain malignant brain tumors, severe shock, and kidney failure.
- CPK-MB > 5% of total CPK (or more than 10 U / l) suggest an acute myocardial infarction (AMI). In IMA and after cardiac surgery CPK-MB begins to rise after 2-4 hours, reaches maximum values in 12-24 hours and usually returns to normal within 24-48 hours. The persistence of high values indicates the evolution of myocardial damage.
- Total CPK following much the same pattern as CPK-MB but grows a little later. Total CPK elevations may be due alcoholic cardiomyopathy, carbon monoxide poisoning, severe hyperthermia, post seizure, severe hypokalemia.
LDH1 and LDH2 occur mainly in the heart, kidney and erythrocytes.
LDH3 occurs mainly in the lungs.
LDH4 and LDH5 exist in liver and skeletal muscle.

The wide clinical application (in combination with other cardiac enzymes) is in the diagnosis of acute myocardial infarction (AMI). It is useful when creatine phosphokinase (CK) was not measured in the first 24 hours of evolution of IMA. Myocardial LDH level increases later than CK (12-48 hours after infarction) peaking after 2-5 days and returns to normal in 7-10 days, if not persistent tissue necrosis. The test is useful in the diagnosis of liver, lung, erythrocyte diseases.

Increase in myocardial infarction occurs later than the one of CK and SGOT, and is less intense; the clinical value lies in the longer period of time (7-10 days) of persistent growth, long after the CK and SGOT returned to normal.

Isoenzyme electrophoresis is required for the diagnosis; some conditions can maintain normal levels of total LDH but isoenzyme distribution may change. This indicates a specific organ damage. For example, in AMI LDH1 concentration becomes higher than LDH2 in 12-48 hours of onset. This reversal of the normal distribution is typical for AMI.
CARDIOVASCULAR DISEASES: CPK, AST, ALT, LDH

- THE SERUM TRANSAMINASES

- The serum transaminases act at intracellular level, catalyzing the transfer reaction of the amino group (-NH$_2$) from one $\alpha$-amino acid to an $\alpha$-keto acid. Their activity does not manifest in the serum, so they may be considered as nonfunctional plasmatic enzymes.

- The transamination reaction is important in the intermediate metabolism for the synthesis of the own amino acids using the $\alpha$-amino acids and $\alpha$-keto acids in excess in the metabolic “pool”. Through this reaction, the connection between the protein and carbohydrate metabolisms is established, using the $\alpha$-keto acids produced in Krebs Cycle as intermediates.
Glutamate oxalylacetate aminotransferase (GOT) or aspartate aminotransferase (AST) catalyzes the transfer of amino group from L-aspartic acid to \( \alpha \)-ketoglutaric acid (2-oxoglutaric acid) with the production of oxalylacetic acid and glutamic acid:

GOT (AST) exists in high concentration in the myocardium and liver and in low concentration in the skeletal muscles.
- Glutamate pyruvate aminotransferase (GPT) or alanine aminotransferase (ALT) catalyzes the transfer of amino group from L-alanine to $\alpha$-ketoglutaric acid with the production of pyruvic acid and glutamic acid.

- GPT (ALT) is predominant in the liver; its concentration is low in the myocardium and skeletal muscles.
The coenzyme of aminotransferases is pyridoxal-5-phosphate (PALP) a derivative of pyridoxine (vitamin B₆) that acts as intermediate acceptor of amino group, transforming into pyridoxamine-5-phosphate (PAMP).
Diagnostic significance

Reference values:

GOT (AST):
- children younger than 3 months maximum: 40 IU
- children 3 months-5 years old: 2 - 28 IU
- adults: 2 - 20 IU

GPT (ALT):
- children younger than 5 years: 0.2 - 13 IU
- adults: 2 - 16.5 IU

GOT/GPT (AST/ALT) ratio (De Ritis) 1.3
Pathological significance:

- The aminotransferases are cellular enzymes; their activity in the serum is normally decreased.
- When the tissue cells containing large amounts of these enzymes are injured or killed, the enzymes diffuse into the bloodstream, where a temporary high degree of enzyme activity occurs. The degree of the activity depends on the extent of the tissue damage, the prior concentration of the enzyme in the tissue and the time course following the tissue injury.
AST(GOT) serum activity is increased in:

1) heart affections:
- myocardial infarction - the activity begins to rise about 6 to 12 hours after the infarction and usually reaches its maximum value in about 24-48 hours; it usually returns to normal 4-6 days after the infarction; it is a much less specific indication of the myocardial infarction than the rise in creatinephosphokinase (CK) because many other conditions can cause a rise of GOT (liver, muscle, hemolytic diseases);
- prolonged myocardial ischemia;
- congestive heart failure (hepatic ischemia and anoxia are produced).

2) hepatic affections:
- activity may rise 100 times the normal value in severe acute hepatitis, acute toxicity of a drug that severely damages the liver tissue (chloroform ingestion, CCl₄, phosphorus compounds); the rise in the acute hepatitis begins early in the disease, frequently before jaundice is visible; it may be the only sign of a hepatitis without jaundice and an early sign for a new active period of the disease;
- moderate elevation (1-10 times the normal value) usually occurs in cholestasis, chronic hepatitis, cirrhosis, hepatic tumours, mononucleosis.

3) muscular disorders:
- all types of progressive muscular dystrophy (10 times the normal value, then, the values become progressively lower because of the decreased muscle mass),
- neurogenic muscle atrophies, muscle trauma, surgery, intramuscular injections when long lasting preparation are used.
ALT (GPT) serum activity increased values occur in:

1) hepatic affections - hepatitis (viral, toxic), cholestasis, tumors;
2) pancreatic diseases - acute pancreatitis, tumors;
3) GPT is used to resolve some ambiguous increases in serum GOT in cases of suspected myocardial infarction, when CK, CK-MB or LD isoenzymes are not available or electrocardiogram signs are not characteristic:

when both GOT and GPT are elevated in serum, the liver is the primary source of the enzymes (liver ischemia because of congestive heart failure or other sources of liver cells injury);

if the serum GOT (AST) is elevated while the GPT (ALT) remains within normal limits in a case of suspected myocardial infarction, the results are compatible with myocardial infarction.

GOT/GPT ratio (de Ritis):

1) In hepatic affections: GOT/GPT <1.3
2) In myocardial infarction: GOT is increased and GPT normal. Thus, GOT/GPT > 1.3
HEMATOLOGIC DISEASES: LDH
PANCREATIC DISEASES: AMY, LIP

THE SERUM $\alpha$-AMYLASE

The amylase catalyzes the specific hydrolysis of the $\alpha$ 1-4 glycosidic bonds in the starch and glycogen, with the formation of a series of intermediates - olygoglucides with reducing properties.

Depending on the position of the broken bond in the glycosidic chain, the amylases are:

- $\alpha$-amylase (endoamylase) - $\alpha$ 1-4 bonds that are splitted are in the middle of the chain;
- $\beta$-amylase (exoamylase) - splits off the maltose units from the free nonreducing end of the chain.
Amylases that act in the human digestive tract are $\alpha$-amylases. They are secreted by the salivary glands and pancreas and reach the gastrointestinal tract. They are important for the digestion of the ingested starch (especially the pancreatic isoenzyme, because the salivary one is inactivated in the acidic medium of the stomach). The amylose chains are broken down into smaller fragments until the maltose is obtained. The intermediates are called dextrines and give specific coloured products in reaction with iodine, depending on their molecular weight:

- blue-violet colour: amylodextrines;
- red colour: erythrodextrines;
- yellow colour: flavodextrines;
- colourless: acrodextrines.
The serum $\alpha$-amylase has its origin in the salivary glands, pancreas, liver (3/4 of normal serum amylase), intestine, fat tissue. To specify the origin of the amylases, it is necessary to determine the different isoenzymes.

The catalytic activity of the enzymes depends on a series of factors: pH, temperature ($30-40^\circ C$), the presence or absence of specific ions.

The optimal conditions for $\alpha$-amylase are: pH 7.1; $38^\circ C$; the presence of Cl$^-$ (NaCl).

To determine the serum amylase activity, the serum is reacted with the starch in standard conditions of pH, temperature, ionic force, time. One can determine either the amount of starch that is transformed (observing the decrease of the intensity of the blue colour which appears normally in reaction with iodine) or the amount of the reducing oses that appear by starch hydrolysis (slower method, influenced by the hyperglycemia).

Diagnostic significance:

Reference values depend on the method used for the determination:

- 60 - 200 Amylase Units/100 ml serum (Smith & Roe method);
- 10 - 32 Wohlgemuth Units/100 ml serum (amyloclastic);
- 60 - 80 Somogyi Units/100 ml serum (saccharogenic).
Pathological significance:

Increased activity:

- considerably raised (6 - 10 times the normal) values in acute pancreatitis, obstruction of the pancreatic ducts (stones, inflammation, cancer of the head of pancreas) are rapid and temporary, reaching a maximum value in about 24 hours with a return to normal in 2-3 days; in hemorrhagic pancreatitis the values may be higher;
- mildly increased values can be noticed in the obstruction of the parotide glands (stones) or mumps (parotiditis);
- less than 4 times the normal values:
  - acute abdominal affections; peptic ulcer that has perforated the duodenum wall; high intestinal occlusion (reabsorption); acute hepatitis and cholecystitis; tubal pregnancy (tubal isoenzyme); after the administration of morphine, analgetic narcotics, the values can be high for 24 hours; the ingestion of alcohol, Azathioprin, Chlorthalidone, Chlorothiazide, oral contraceptives can lead to falsely increased values; renal failure may decrease the excretion; after medication with heparin and hemodialysis the concentration is increased if the method is with iodine-starch reaction (Pasternack-Stenman method).

Decreased values: pancreatic atrophy; acute or chronic hepatocellular damage (but is not a sensitive liver function test).
In urine only those enzymes whose molecular weight is smaller than 60,000 Daltons are normally found. They are filtered through the glomerular membrane and are not reabsorbed in the tubules.

Examples: lysosome (MW 11,450-14,500), amylase (MW 45,000).

Amylase, with its two isoenzymes, salivary and pancreatic is the most analysed in the clinical laboratory.

The increase of amylasemia determines the increase of amylasuria.

The last one persists longer than the elevation in serum amylase activity and can help to establish the diagnosis of acute pancreatitis.

The urinary amylase may be elevated for 7 to 10 days, whereas the serum amylase returns to normal in 2 or 3 days after attack.
Reference values:
- 8 - 64 WU.

Pathological significance:
- Increased values appear in:
  - Acute pancreatitis and persist for as long as a week after the serum amylase has returned to normal;
  - Obstruction of the pancreatic ducts (stone, inflammation, compression of the common bile duct by a cancer of the head of the pancreas);
  - Obstruction by a stone in the parotid duct, mumps (parotiditis).
- Decreased excretion is present in:
  - Chronic renal disease with decreased glomerulus’s filtration;
  - Severe damage of hepatic cells.
LIPASE

- The lipases or carboxyester hydrolases are enzymes that catalyse the hydrolysis of carboxylic esters with the production of one molecule of fatty acid and one of alcohol, as in the following reaction:

  \[ R - \text{CO} - \text{O} - R' + \text{HOH} \rightleftharpoons R - \text{COOH} + R' - \text{OH} \]

- The lipases that act on the simple lipids exist in the pancreatic juice, blood, lungs, kidneys, stomach.

- The pancreatic lipases breakdown the triacylglycerides of the unsaturated fatty acids with a high rate. The hydrolysis takes place in steps with the production of diacylglycerides and monoacylglycerides and finally simple compounds such as glycerol and fatty acids.

- The activity of lipases is stimulated by the presence of activators: \( \text{Ca}^{2+} \) salts, bile acids, albumins.

- They are inhibited by heavy metals.

- Pancreatic lipase has optimum pH 8.0 and is inhibited by quinine.
Diagnostic importance:

Reference values:

0 - 0.05 IU

Pathological significance:

The concentration of serum lipase is related with the activity of the pancreas being more specific than the amylase. In all the pancreatitis that have been verified by surgical or necroptical diagnosis the lipasemia have been increased even if the amylasemia could have shown normal values.

In acute pancreatitis the increase of lipase is shown but after few hours the serum level is high for longer time, while its excretion is delayed. Its determination is useful especially after the first 3 days when amylasemia becomes normal. That is the reason why for the monitoring the evolution of an acute pancreatitis is indicated to dose both enzymes.

In pancreatic cancer the hyperlipasemia is more frequently noticed than hyperamylasemia.

Lipase and amylase may be tested in the pleural or peritoneal liquid. In acute pancreatitis their concentration is higher than that in the serum, helping the diagnosis.
PROSTATE CANCER: ACP

- Reference values:
  - Total ACP
    - serum
      - male: 4.7 - 13.5 IU/L
      - female: 5.0 - 11.0 IU/L
    - plasma
      - male: 1.5 - 8.6 IU/L
      - female: 3.0 - 16.0 IU/L
  - Prostatic ACP
    - maximum: 3.6 IU/L
    - maximum: 1.0 IU/L
- Diagnostic significance
- Increased concentration:
  - Total ACP and Prostatic ACP are increased in metastasizing carcinoma of the prostate (more than 3-15 times the upper level of the normal); the carcinoma has to invade blood capillaries, lymph channels, other tissues before the elevation in the serum of ACP occurs; a discrete prostatic cancer that has not penetrated beyond the capsule does not cause the rise in serum ACP; the decrease of values after therapy indicates its efficiency. Massage of the prostate increases ACP activity for 1 or 2 days.
  - Nonprostatic ACP is increased in sphingolipidoses (Gaucher’s disease).
  - Occasionally, the tartrate-inhibitable (prostatic) ACP may be elevated in some bone diseases: Paget’s disease, female breast cancer that has metastasized to bone.
- Decreased concentration has no pathologic significance.
Various immunotechniques (RIA, counterimmunoelectrophoresis, enzyme immunoassay) based on a monoclonal antibody against a prostate-specific ACP isoenzyme (PAP) are used in attempt to detect a prostatic carcinoma before it is detectable by touch (palpable) in a rectal examination (early prostatic carcinoma). These tests are about 1,000-fold more sensitive than conventional activity measurements.