**Regulation of enzyme activity in the living system (allosteric regulation, feedback regulation, covalent modification of enzymes, activation of latent enzymes by limited proteolysis, cyclic nucleotides in regulation of enzymatic processes).**

Allosteric regulation. Certain substances referred to as allosteric modulators (effectors or modifiers) bind at the allosteric site and regulate the enzyme activity. The enzyme activity is increased when a positive (+) allosteric effector binds at the allosteric site known as activator site. On the other hand, a negative (-) allosteric effector binds at the allosteric site called inhibitor site and inhibits the enzyme activity. Most of the allosteric enzymes are oligomeric in nature. Binding of effector changes the shape of enzyme including its active site.

The process of inhibiting the first step (sometimes the second step) by the final product, in a series of enzyme catalysed reactions of a metabolic pathway is referred to as feedback regulation. An end product of a pathway binds to the allosteric site of a regulatory enzyme and inhibiting it stops the pathway:



Feedback inhibition is necessary to control metabolic pathways for efficient cellular function. E.g. Feedback inhibition also controls nucleotide production. The pyrimidines (Thymine, Cytosine, and Uracil) have different pathways and feedback mechanisms than the Purines (Adenine and Guanine). Aspartate transcarbamoylase regulates pyrimidine synthesis in bacteria. The regulation for purine production begins as PRPP or 5-phosphoribosyl-1-pyrophosphate which is converted into Phosphoribosylamine. This pathway is inhibited by IMP, AMP, and GMP. Then Phosphribosylamine is converted into IMP. IMP is a common precursor to both Adenosine and Guanine. The pathways from IMP to the Adenosine and Guanine precursors of AMP and GMP, respectively, are separated. IMP to AMP is inhibited by AMP(adenosine precursor) and IMP to GMP(guanine precursor) are inhibited by GMP, thus the products are inhibiting the precursors.

Covalent modification of enzymes is a process in which enzyme activity is altered by covalently modifying structure of the enzyme. Involves adding or removing a group from an enzyme. Covalent modification of enzymes includes reactions of phosphorylation, methylation, acetylation, UDP-rybosylation, adenylation, ets. The most common covalent modification ia addition or removal of phosphate group:

* phosphate group is often derived from an ATP molecule;
* addition of the phosphate group (phosphorylation) is catalysed by a kinase enzyme;
* removal of the phosphate group (dephosphorylation) is catalysed by a phosphatase enzyme;
* phosphate group is added to (or removed from) the R group of a serine tyrosine or threonine amino acid residue in the enzyme regulated.

Some enzymes are synthesized as inactive latent forms called zymogens or proenzymes e.g. trypsinogen and pepsinogen. Zymogens are inactive because their catalytic sites are masked by a polypeptide chain. To activate zymogens, the polypeptide chain is cleaved to open the catalytic site for its substrate. This mechanism is called limited proteolysis.

Action of second messengers. Nerve impulses and the binding of many hormones to cell surface receptors elicit changes in the rate of enzyme-catalyzed reactions within target cells by inducing the release or synthesis of specialized allosteric effectors called second messengers. The primary, or “first,” messenger is the hormone molecule or nerve impulse. Second messengers include 3′, 5′-cAMP, synthesized from ATP by the enzyme adenylyl cyclase in response to the hormone epinephrine, and Ca2+, which is stored inside the endoplasmic reticulum of most cells. Membrane depolarization resulting from a nerve impulse opens a membrane channel that releases calcium ions into the cytoplasm, where they bind to and activate enzymes involved in the regulation of muscle contraction and the mobilization of stored glucose from glycogen to supply the increased energy demands of muscle contraction. Other second messengers include 3′,5′-cGMP and the polyphosphoinositols produced by the hydrolysis of inositol phospholipids by hormone-regulated phospholipases.

1. **Metabolism of ketone bodies.(enzymatic reactions of ketone bodies biosynthesis; reactions of utilization of ketone bodies, energetic significance; metabolism of ketone bodies in pathology.**

Ketone bodies (acetoacetate, β-hydroxybulyrate, and acetone) are made in liver when β-oxidation of fatty acids is in excess of that required by the liver. These water-soluble, energy-rich compounds are transported to other tissues for generation energy. Excess production of ketone bodies, that occurs during starvation or untreated diabetes can be harmful.

Once fatty acids are degraded in liver mitochondria the resulting acetyl-CoA can undergo a number of metabolic fates. The utilization of acetyl-CoA is of central importance in the tricarboxylic acid cycle. Alternatively acetyl-CoA is involved in the synthesis of ketone bodies, which takes place only in the mitochondria. In the first reaction, catalyzed by aceloacelyl-CoA thiolase, two acetyl-CoAs condense to form acetoacetyl-CoA. This reaction is a reversal of the last reaction in β-oxidation and is thermodynamically unfavorable in that the equilibrium favors thiolytic cleavage of acelyl-CoA. Hence, this compound is formed the levels of acetyl-CoA rise, which pushes the reaction towards acetoacetyl-CoA synthesis.



*Ketone bodies are converted back to acyl-CoA in the mitichondria or nonhepatic tissues and used as a source of energy in these tissues*

A third molecule of acelyl-CoA reacts with aceloacetyl-CoA to yield β-hydroxy- β-methylglularyl-CoA (HMG-CoA) in a reaction catalyzed by HMG-CoA synthase. In the formation of ketone bodies, the next reaction is calalyzed by HMG-CoA lyase and yields acetoacetate and acetyl-CoA. The acetoacetate can be reduced to β-hydroxybutyrate by an enzyme on the inner membrane of the mitochondrion, β-hydroxybutyrate dehydrogenase. Although acetoacetate can also be decarboxylated to form acetone, this is normally of minor importance. However, patients with uncontrolled type I diabetes (a disease caused by a lack of production of insulin in the islet cells of the pancreas) have high levels of ketone bodies in their plasma, and their breath has characteristic odor of acetone. The rise in ketone bodies occurs because cells and tissues are glucose starved. As a result fatty acids are mobilized to provide energy, which results in an excess production of acetyl-CoA in the liver. Hence, the liver converts this excess acetyl-CoA into keione bodies which can be used in extmhepalic tissues as a source of energy. An additional complication is that a large rise in the serum levels of ketone bodies lowers the pH of blood because of the increased concentration of these acids. Amongother reasons, this condition can be hazardous because of the effect of lower pH on oxygen binding to hemoglobin.

Ketone body synthesis is primarily a liver function, since mitochondrial HMG-CoA synthase is present in large quantities only in this tissue. Acetoacetate and β- hydroxybutyrate are secreted into the blood and carried to other tissues where they are converted into acetyl-CoA as described. The reactions catalyzed by β-hydroxybutyrate dehydrogenase and thiolase are common to both the synthesis and degradation of the ketone bodies. However, the second enzyme in the sequence for degradation, β-oxyacid-CoA transferase, is present in all tissues but liver. Hence, ketone bodies are made in the liver and metabolized to CO2 and energy in nonhepatic (nonliver) tissues. Ketone bodies can also be used to supply these tissues with acetyl-CoA for fatty acid and cholesterol biosynthesis. Ketone bodies are important sources of energy for the brain during starvation. Normally, glucose is the major source of energy in the brain, and the brain doesn't use fatty acids as a major source of energy.

1. **Hormones of thyroid gland. Structure and function of thyroid hormones. Pathology of thyroid gland, metabolic disorders in hypo- and hyper- thyreosis. Endemic goiter and its prevention.**

The thyroid gland produces and secretes in blood thyroid hormones – thyroxine, triiodothyronine, which are important in regulating general metabolism, development and tissue differentiation. The peculiarity of the thyroid hormonal synthesis is the presence of iodine in their structure. Thyroxine (T4) and the more potent triiodothyronine are leaved from a large precursor protein – thyroglobulin. It is a storage protein for iodine and can be considered a prohormone of the ci rculating thyroid hormones. Thyroglobulin is secreted into the lumen of the thyroid gland, where specific residues are iodinated in one or two positions by a special peroxidase.

Thyroxine action happens through:

1) the activation of the genetic apparatus of the cell with the stimulation of the biosynthesis of specific proteins in cells that is shown as growth and differentiation reactions;

2) the stimulation action on the biosynthesis of the protein and DNA in mitochondrias that leads to the increase of metabolism in cells.

There are the following effects of the thyroid action:

1. The metabolic action – the increased of the common metabolism of the cell, the absorption of the oxygen and warmth producing in the tissues; after leading of thyroxine the absorption of the oxygen is increased by neurons of CNS, muscles, kidneys, liver and is decreased after thyroidectomia.
2. Increase the lipid metabolism (by the way of the intensification of the processes of fatty utilization, the breaches of the producing of β-lipoproteins, the decrease of the hydrolysis of triglycerides) and carbohydrate metabolism (intensify the absorption of glucose in the gastro-intestinal tract, take part in the sugar regulation in blood, activate the processes of the splitting of glucose, decrease the synthesis of glycogen in the liver);
3. Provide the morphogenetic and differentiation action. The deficiency of the processes of tissue’s differentiation, particularly of CNS, causes the difficult breaches in mind. The deficiency of thyroxine in adult person is connected with back breach of the functions of CNS, sleepy and brake state, lifelessness, apathy, lowering of capacity for work.
4. Influence water and electronic metabolism through the mineral corticoids of the adrenal cortex. Hyperthyroidism causes polyuria but hypothyroidism develops oligouria.

Disorders of thyroid function:

* + - Myxedema - Hypothyroidism occurring in adulthood. Clinical features include lethargy, cold intolerance, decreased sweating, bradycardia, tongue enlargement, and non-pitting edema of the skin due to infiltration of the subcutaneous tissues by metachromatic proteoglycans.
		- Cretinism: A type of mental retardation and bodily malformation caused by severe, uncorrected thyroid deficiency in infancy and early childhood.
		- Grave’s disease is an autoimmune disease in which the immune system produces antibodies which stimulate the TSH receptors of the thyroid gland, resulting in overproduction of thyroid hormones. Symptoms: increased pulse rate, increased sweating, heat intolerance, hair loss, inflammation of the eyes, swelling of the tissues around the eyes, and protrusion of the eyes.
		- Goiter: an enlargement of the thyroid gland, often resulting from the deficiency of iodine in the diet (simple goiter) or other causes of hyperthytoidism.
1. **Definition of total and residual nitrogen in blood. Nonprotein nitrogen containing compounds of blood, their diagnostic significance. Nitrogenemia, its kinds and causes of development, differentiation in clinical conditions.**

The main component of blood plasma nitrogen are proteins (albumins, globulins and fibrinogen). The non-protein nitrogen of the blood is occasionally referred to as the residual nitrogen, that is, nitrogen that remains in the filtrate after protein precipitation. In healthy persons, the variation in non-protein, or residual, nitrogen of the blood is insignificant and is mainly due to the dietary supply of proteins.

The concentration of non-protein nitrogen in the whole blood and in plasma is nearly constant and amounts to 15-25 mmol/L (in blood). The non-protein nitrogen of the blood includes:

* urea nitrogen (50% of the total non-protein nitrogen),
* amino acid nitrogen (25%),
* ergothioneine nitrogen (8%),
* uric acid nitrogen (4%),
* creatine nitrogen (5%),
* creatinine nitrogen (2.5%),
* ammonia and indican nitrogen (0.5%),
* and the nitrogen of other non-protein species (polypeptides, nucleotides, nucleosides, glutathione, bilirubin, choline, histamine, etc).

Thus, compositionally, the non-protein nitrogen of the blood is mainly represented by the nitrogen of end metabolites of simple and conjugated proteins.

The major product of protein metabolism in the organism is **urea.** Commonly, urea is 18 times less toxic than other nitrogenous compounds. In acute renal insufficiency, the urea concentration in the blood can reach 50-83 mmol/L (the normal is 3.3-6.6 mmol/L).

The increased level of urea in the blood (as high as 16-20 mmol/L, based on the urea nitrogen) is a symptom of a moderate disturbance of the renal function; the level of 35 mmol/L is a grave state, and above 50 mmol/L, is a very grave state with a poor prognosis.

Occasionally, the blood nitrogen urea is defined as a percentage of the residual nitrogen of the blood:

Urea nitrogenx 100

 Residual nitrogen

Normally, this percentage is less than 48%. In renal insufficiency, this ratio may reach 90%; in an impaired ureapoietic function of the liver, its value is lowered (below 45%).

**Uric acid** is also regarded as an essential non-protein component of the blood. In humans, uric acid is the end product of purine base metabolism. Normally, the uric acid concentration in the whole blood is 0.18-0.24 mmol/L (in blood serum, about 0.29 mmol/L). An increased level of uric acid in the blood (the state referred to as hyperuricemia) is a major symptom of gout - the uric acid level in the blood serum may be as high as 0.5-0.9 mmol/L, or even 1.1 mmol/L.

The nitrogen of **amino acids** and **polypeptides** is also a constituent of residual nitrogen. Free amino acids are permanently present in small amounts in the blood. A part of them are of exogenous origin, that is, supplied to the blood from the gastrointestinal tract; the other part of amino acids are formed by degradation of tissue proteins. Glutamic acid and glutamine account for one fifth of the amino acids contained in the blood plasma. The concentration of free amino acids in blood plasma and blood serum is practically the same, but it is quite different from the amino acid level in the erythrocytes.

The blood analysis for total peptide content is carried out, as a rule, on special occasions. It should be kept in mind, however, that many of the blood peptides are biologically active agents and their quantitation is of great diagnostic interest in the clinic.

In certain pathologies, the non-protein nitrogen level in the blood increases. Such a state is referred to nitrogenemia. Depending on the causative factors, there are two major types:

* productive nitrogenemia
* retention nitrogenemia

The productive nitrogenemia is observed in an excessive delivery of nitrogenous products to blood as sequent to accelerated degradation of tissue proteins in diffuse inflammation, wounds, extensive burns, cachexia, and other states.

The retention nitrogenemia sets in because of an incomplete urinary discharge of nitrogen-containing products on their normal delivery in the bloodstream. This type is subdivided into:

* renal nitrogenemia, the residual nitrogen concentration in the blood increases because of the reduced excretory function of the kidney (reduced renal clearance). Urea is mainly nitrogenemia, the urea nitrogen accounting for 90% of non-protein nitrogenemia may arise from an acute circulatory insufficiency, low arterial pressure, or reduced renal blood flow.
* extrarenal nitrogenemia - also, the common cause of extrarenal retention nitrogenemia is obstruction of urine outflow from the kidney.
1. **Determination of alanine aminotransferase and aspartate aminotransferase activities. The principle of the method. Clinical and diagnostic value of these enzymes.**

*Alanine aminotransferase* (ALT) catalyses the following reaction: ****

Pyruvate forms a colored hydrazone after reaction with dinitrophenylhydrazine. In alkaline medium pyruvate hydrazone gives a product of red-brown color, which intensity is proportional to concentration of pyruvate. According to the quantity of formed pyruvate the activity of subsequent amino transferase can be evaluated quantitatively.

**Clinical diagnostic significance**. In human body the process of transamination occurs in the liver, hear, skeletal muscles, kidneys and other organs. In blood plasma transaminase activity is very low during nirmal conditions. When a cell membrane is damaged and the integrity of cell is breacked aminotranferase enzymes migrate into the blood. Thus the estimation of aminotranferase activity in blood serum is important in diagnoses, especially in myocardial infarction, viral hepatitis and liver cirrosis.

Considerable increase in ALT activity (10 – 100 times normal values) is observed in cases of viral and toxic hepatitis, blood circulation insufficiency during shock and hypoxia.

Moderate increase of ALT activity occurs during liver cirrhosis, chronic hepatitis during the acute phase, obstructive jaundice, liver swelling during cardiac insufficiency and degenerative processes of the kidneys, lungs, muscles and pancreatic gland.

Considerable increase in AST activity is observed during myocardial infarction, viral hepatitis, toxic liver destruction and blood circulation insufficiency during shock and hypoxia.

Moderate increase of AST activity is detected during liver cirrhosis, obstructive jaundice, liver malignancies, skeletal muscules destruction, pancreatitis, pneumonia and hemolytic anemia.

Normal values in blood serum: ALT - 0.1 - 0.7 moles/hr´ml,

 AST - 0.1 - 0.45 moles/hr´ml.