Министерство здравоохранения Республики Беларусь

УЧРЕЖДЕНИЕ ОБРАЗОВАНИЯ
«ГРОДНЕНСКИЙ ГОСУДАРСТВЕННЫЙ МЕДИЦИНСКИЙ УНИВЕРСИТЕТ»

Кафедра биологической химии

Н. Э. Петушок
А. Г. Виницкая

БИОЛОГИЧЕСКАЯ ХИМИЯ

Практикум
для студентов, обучающихся
по специальности 1-79 01 01 «Лечебное дело»
(английский язык обучения)

Под общей редакцией профессора В. В. Лелевича

N. E. Petushok
A. G. Vinitskaya

BIOCHEMISTRY

Workbook
for the medical faculty for international students
(in English)

Edited by professor V. V. Lelevich

Гродно
ГрГМУ
2021

УДК 577.1(07)=11
ББК 28.072я73
П 29

Рекомендовано Центральным научно-методическим советом ГрГМУ (протокол № 5 от 26.06.2020).

Авторы: доц. каф. биологической химии, канд. биол. наук
Н. Э. Петушок;
доц. каф. биологической химии, канд. биол. наук
А. Г. Виницкая.

Рецензент: доц. каф. общей и биоорганической химии,
канд. хим. наук А. Л. Бубен.

Петушок, Н. Э.

П 29 Биологическая химия : практикум для студентов, обучающихся по специальности 1-79 01 01 «Лечебное дело» (англ. яз. Обучения) = Biochemistry : workbook for the faculty of foreign students (in English) / Н. Э. Петушок, А. Г. Виницкая; под общ. ред. проф. В. В. Лелевича. – Гродно : ГрГМУ, 2021. – 136 с.
ISBN 978-985-595-280-1.

The workbook contains theoretical questions and laboratory works to perform by students in accordance with the curriculum on Biochemistry. The reference ranges of some diagnostically important substances and enzymes for memorizing are also presented.

УДК 577.1(07)=11
ББК 28.072я73

ISBN 978-985-595-280-1

BIOCHEMISTRY LABORATORY SAFETY RULES

1. When starting work in a biochemical laboratory, students should be acquainted with the main safety regulations. Some rules are NOT made to be broken.
2. A student has no right to cause situations that threaten personal life and health and these of other people.
3. A student must:
   - Follow the Rules of internal regulations of labour of the University, the Department of Biochemistry, Chemical Laboratory Safety Rules, and Fire Safety Rules.
   - Avoid injuries, cuts, and burns in the laboratory; know first aid skills in electrical hazards, poisonings, and exposure to chemicals.
   - Follow the rules of behavior in the departmental rooms, and laboratories.
   - Be dressed appropriately; wear medical attire in the laboratory.
   - Notify the teacher at once about each accident occurred at classes, extra-curricular events.
   - Follow the instructions given by the teacher or laboratory manual. Don't start a lab until you know all of the steps, from start to finish. If you have questions about any part of a procedure, get the answer before starting.

Following rules for handling must be observed:

1. With biological material

1.1. The use of biological material (blood, urine, saliva, gastric juice, spinal fluid, tissue homogenates, and others) shall be carried out with the maximal care. This is necessary for preventing the transmission of various infectious diseases (HIV, syphilis, hepatitis, etc.).

1.2. Wash your hands thoroughly after the work.

2. With reagents

2.1. Pay attention to the order in which chemicals are to be added to each other and do not deviate from the instructions. All vials containing reagents are labelled with the name of the reagent and its concentration. The vials must be sealed tight.

2.2. Special care shall be taken in the handling of poisonous, flammable reagents, as well as concentrated acids and alkalis.

Work with such reagents should be carried under the switched-on fume cupboard.

2.3. Reagents should be protected against contamination and consumed in a more economical way.

2.4. Do not sniff, or taste reagents. Never pipette reagents by mouth. Take reagents by automated pipettes with disposal tips, or use the pipette bulb with glass graduated pipettes.

3. With electric equipment

3.1. Measuring devices (photocolorimeters, spectrophotometers, and others) shall be grounded and be in proper working order.

3.2. Students are not allowed to damage electric equipment, switches, power sockets, lighters, etc.

3.3. Avoid touching electric equipment with wet hands.

3.4. Water thermostats, centrifuges shall be in working order and electrically grounded. The covers of these appliances must be closed during the work.

3.5. Make sure that water is always available in the water thermostat.

4. With gas and other heating equipment

4.1. Dishes made of plain glass must not be heated on an open flame.

4.2. Flammable substances shall not be heated in open flames but only in a thermostat.

4.3. Make sure that water is always available in the water thermostat.

5. Chemical glassware and other laboratory tools

5.1. Laboratory glassware (test tubes, pipettes, flasks, burettes, measuring cylinders, etc.) require careful handling. Otherwise, it may break down and cut by a sharp edge of a piece of chipped glassware.

5.2. Test tubes should be stored in the tube racks.

5.3. Automatic pipettes also require careful handling. The plastic they’re made of is fragile enough. If used inappropriately, the pipettes may get broken.

6. At the end of class students are obliged to:

6.1. Switch off all measuring devices, fume cupboard, and thermostat.

6.2. Turn off the gas burner and shut off the gas.

6.3. Clean their workplace and wash glassware.

6.4. Make sure to check whether cold and hot water taps are switched off.

*Date*_____

CLASS № 1

THEME: INTRODUCTION INTO BIOCHEMISTRY

THEORETICAL PART

1. History of biochemistry.
2. Major objectives, branches and research trends of biochemistry.
3. Objects and methods of biochemistry.
4. Role of biochemistry in medical education.

LABORATORY WORK № 1

WORK WITH PIPETTES

There are two kinds of pipettes typically used in biochemistry laboratories: glass graduated pipettes and automatic pipettes.

1. Rules of using graduated glass pipettes:

- Glass pipettes are calibrated to deliver accurately the fixed volumes of liquids. Their typical volumes are 1, 2, 5, and 10 ml, and they are graduated from 0 to the largest volume.
- Examine glass pipettes. Note graduating marks (numbers and lines) on their sides. The numbers and lines indicate the volume of a liquid in milliliters which the pipette holds for transportation.
- Place the rubber bulb at the top of the pipette and squeeze it to empty it of air. Then, with the tip of the pipette submerged in the water, gently relax the bulb to draw water up into the pipette.
- Allow the level of the fluid in the pipette to rise a couple centimeters above the line or mark on the side. While you are drawing up fluid make sure the tip of the pipette always remains beneath the surface of the fluid. Do not allow the fluid to rise up into the bulb itself.
- Remove the bulb and quickly cap the open top of the pipette with your finger. By tilting your finger to one side, allow a little air into the pipette so the fluid drains out until the bottom of the meniscus (the curve-shaped depression in the top of the fluid) reaches the fill mark or line.

- Remove the pipette from the solution and transfer it to the receiving flask or test tube. Allow the pipette to drain into the flask or test tube.

2. Rules of using air displacement micropipettes (automatic pipettes):

- Piston-driven air displacement pipettes are a type of micropipettes, which are tools to handle volumes of liquid in the microliter scale. They are of two types: Uni and Vary. The Uni pipettes provide the transport of the fixed volume of liquids from 0,01 to 1 ml (100 to 1000 microliters). The Vary pipettes are graduated to deliver liquids within the certain range.
- **All automatic pipettes must be used only with disposal plastic tips!**
- Examine the given micropipette and define its type. At the top there is a plunger that you can push in to empty the micropipette; next to the plunger is an ejector you can use to eject the plastic tip from the end of the micropipette. Along the side, it has a volume adjustment wheel you can use to adjust the volume the pipette will take up or contain.
- Look at the volume dial along the side of the micropipette. Working with a Vary pipettes, determine what the volume is set to at present and adjust that volume with the volume adjustment wheel to reach the appropriate volume.
- Insert the end of the micropipette shaft into a plastic tip.
- Depress the plunger with your thumb until you reach the first stop.
- Insert the plastic tip of the pipette just below the surface of the fluid in a vial.
- Release your thumb pressure on the plunger, slowly and gently, drawing fluid into the plastic tip of the micropipette. Once the plunger has traveled all the way out, remove the pipette tip from the solution.
- Transfer the pipette to a receiving vessel or test tube and place the tip just below the surface of the fluid in the receiving vessel. Do not submerge it completely.
- Depress the plunger slowly and gently to expel all the fluid in the micropipette tip. This time, continue to apply pressure past the first stop until you reach the second stop.

- Remove the pipette tip from the solution. Then release your thumb pressure on the plunger of the pipette.

LABORATORY WORK № 2
COLORIMETRY. WORK WITH PHOTOELECTROCOLORIMETER

Colorimetry is a technique that is used to determine the concentration of coloured compounds in solutions. The method is widely used in medical laboratories to determine the concentration of substances in the patient's blood (urine).

PRINCIPLE OF THE METHOD: the method is based on the **Beer-Lamber-Bouger law**, which states that the absorbance of solution is directly proportional to the concentration of absorbing solute and depend on the thickness of the absorbing layer (*d*, path length). Absorbance of light is called **EXTINCTION**, expressed by the letter "E":

$$E = k \cdot [c] \cdot d$$

Where: k – a light absorbance coefficient, c –concentration of solute, d - width of a cuvette (usually 0.5 or 1 sm)

In colorimetry monochromatic light (of a single wavelength, λ) is used. The method requires a **"blank" solution** for calibration (**zero**) and reports results in absorbance units (extinction), transmittance or if it is calibrated, as a concentration.

Construction of photoelectrocolorimeter (PEC)

1. ON/OFF switch.
2. LIGHT SOURCE (often an ordinary low-voltage lamp).
3. MONOCHROMATOR (*a set of colored filters that choose particular wavelength of light).*
4. READOUT (scale that display a visual information on absorbance.
5. SAMPLE COMPARTMENT with a holder for 2 cuvettes, (In a manual colorimeter the cuvettes are inserted and removed by hand).
6. LEVER for cuvette movement (turn the lever).

7. BUTTONS for adjusting of the device.
8. TWO CUVETTE to hold the working and “blank” solution; (they enables comparison between the working solution and a "blank", consisting of pure solvent, to improve accuracy).
9. DETECTOR (photoregister) to measure the transmitted light.

A **cuvette** is a small tube with square cross section, sealed at one end, made of glass or plactic, is designed to hold samples for spectroscopic measurements. 2 sides are clear and used to passing a single beam of light/ Another 2 sides are unclear and used to handle the cuvette.

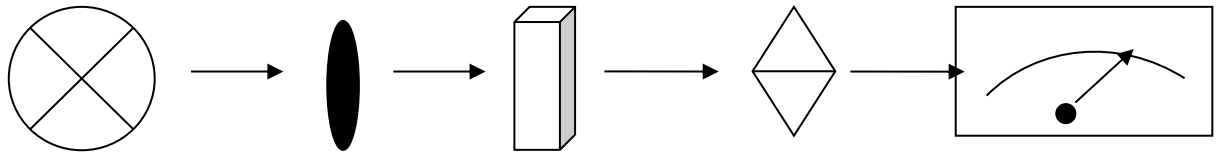


Figure 1. Principal components of photoelectrocolorimeter

A light source emits light along a broad spectrum, then the monochromator selects and transmits light of a particular wavelength. The monochromatic light passes through the sample in a cuvette of path length *d* and is absorbed by the sample in proportion to the concentration of the absorbing species. The transmitted light is measured by a detector and demonstrated on the scale.

How to operate with PEC?

- Plug the unit into a power outlet.
- Switch on PEC.
- Select necessary wavelength (λ).
- Pour distilled water or “control” solution to the “blank” cuvette and “sample” solution to the sample cuvette.
- Place the cuvettes to the sample compartment, as the ‘blank’ cuvette appears on the way of the light beam.
- Close the cover of the sample compartment and light beam immediately passes through the blank.
- Press button ‘Γ’ than ‘E’.

- Open the cover of the sample compartment. Press ‘НУЛЬ’ button.
- Close the cover of the sample compartment. Press button ‘E’. Zero appears on the scale. Your device is adjusted to zero.
- Turn the lever for cuvette movement. Sample appears on the way of light and light passes through it.
- Extinction of the sample is displayed on the READOUT.
- Open the cover of sample compartment and remove the sample cuvette, insert the next one and close the cover. The next values appear on the READOUT
- After finishing of work remove cuvettes, wash them with distilled water.
- Switch off PEC.

Calculation of substance concentration:

1) according to the formula (using the standard solution with known concentration):

$C_{standart} \rightarrow E_{st}$

$C_{sample} = ? \rightarrow E_x$

$$C_{sample} = \frac{E_{sample}}{E_{standard}} \times C_{standard}$$

where

C_{sample} – concentration of the sample;

E_{sample} – extinction of the sample;

$C_{standard}$ – concentration of the standard solution;

$E_{standard}$ – extinction of the standard solution.

2) according to calibration graph (drawing the concentration-to-extinction graph).

STEPS OF WORK:

1. Using a PEC measure and write down extinctions of 2% and 5% $CuSO_4$ solutions:

$E_{2\%}=$, $E_{5\%}=$

2. Based on the data obtained and concentrations of two solutions of $CuSO_4$, draw a calibration graph (graph of concentration versus extinction):

Questions to laboratory work

1. Biochemistry laboratory safety rules.
2. Pipettes, types, rules for working with them.
3. Colorimetry, principle of the method. Construction of photoelectrocolorimeter, device operation rules.
4. Ways of calculating of substance concentration in the colorimetry.

_____Teacher's signature

*Date*_____

CLASS № 2

THEME: PROPERTIES AND FUNCTIONS OF PROTEINS

THEORETICAL PART

1. History of protein studies.
2. Proteins as the major components of the body. Functions of proteins. Protein content in the tissues.
3. Structure of amino acids. Classification. Shape of proteins. Molecular mass of protein
4. Physicochemical properties of proteins. Precipitation reactions of proteins.
5. Methods for separation and purification of protein: ultracentrifugation, electrophoresis, chromatography, dialysis.
6. Colour reactions of amino acids and proteins, practical use.
7. Methods for the quantitative measurement of proteins in a solution. Total serum protein.

LABORATORY WORK № 1

COLOUR REACTIONS OF AMINO ACIDS AND PROTEINS

Colour reactions of amino acids and proteins detect the presence of protein in biological fluids or identify their amino acid composition.

These reactions are used for qualitative and quantitative determination of proteins and amino acids.

1. Biuret test

is used for detecting the presence of peptide bonds in polypeptides and proteins.

PRINCIPLE OF THE METHOD: when a solution of polypeptides or proteins is treated with cupper ions in a moderately alkaline medium ($NaOH/CuSO_4$), a blue-violet colored Cu^{2+}-peptide complex is formed. The blue-violet complex is formed only in case when the tested substance has 2 and more peptide bonds. The colour turns from blue to violet in the presence of proteins, blue to pink in the presence of short-chain polypeptides.

The biuret reaction can be used for both qualitative and quantitative analysis of protein.

STEPS OF WORK:

1) take 1 test tube and place it in a test tube rack,
2) add to the test tube the solutions:

COMPONENTS	Amount of each solution
Protein solution NaOH, 10% $CuSO_4$, 1%	5 drops 5 drops 2 drops
Mix carefully	

RESULT:

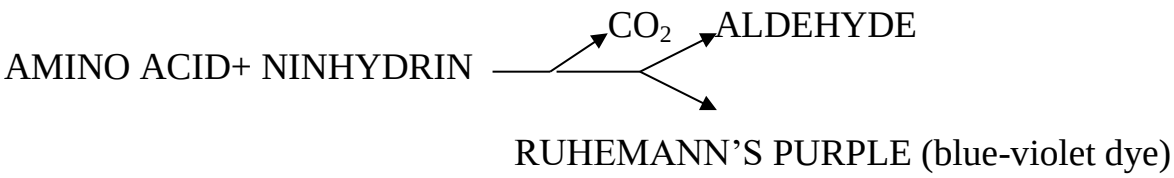
CONCLUSION:

2. Ninhydrin reaction

is used to detect the presence of free α-amino acids as well as amino acid residues in proteins and peptides.

PRINCIPLE OF THE METHOD: amino acids, peptides and proteins, when heated to boiling in the presence of ninhydrin, undergo deamination. After that ammonia interacts with ninhydrin with formation of a purple colored product (Ruhemann's pyrple). Reaction is specific for alpha-amino acids. All primary amines and ammonia react similarly but without the liberation of carbon dioxide. The imino acids proline and hydroxyproline also react with ninhydrin, but they give a yellow colored complex instead of a purple one. Besides amino acids, other complex structures such as peptides and proteins also react positively when subjected to the ninhydrin reaction.

A ninhydrin solution in ethanol or other volatile solvents is often used as a developer for amino acids in paper chromatography or thin layer chromatography.



STEPS OF WORK:

1) take 1 test tube and place it in a test tube rack.

2) add to the test tube the solutions:

COMPONENTS	Amount of each solution
Protein solution Ninhydrin 0,5%	5 drops 5 drops
Mix the tube and heat to boiling fot 1-2 minutes until developing of blue color	

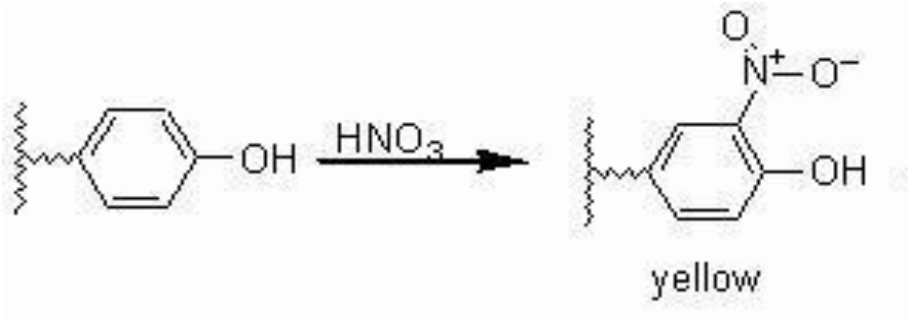
RESULT:

CONCLUSION:

3. Xanthoproteic reaction (for aromatic amino acids)

a method that can be used to detect the presence of proteins in a solution, using concentrated nitric acid. The test gives a positive result in those proteins with aromatic amino acids (phenylalanine, tyrosine, tryptophan). This chemical reaction is a qualitative test, determining the presence or absence of proteins.

PRINCIPLE OF THE METHOD: aromatic amino acids after heating with nitric acid undergo nitration. This process leads to formation yellow colored nitro-derivatives:



STEPS OF WORK:

1) take 1 test tube and place it in a test tube rack,

2) add to the test tube the solutions:

COMPONENTS	Amount of each solution
Protein solution HNO_3concentrated	5 drops 5 drops
Mix the tube and heat to boiling until appearance of yellow color	

RESULT:

CONCLUSION:

4. Fohl reaction (Lead sulfide test)

reaction is specific for cysteine and cystine (qualitative determination).

PRINCIPLE OF THE METHOD: sulphur containing amino acids, such as cysteine and cystine upon boiling with sodium hydroxide yield sodium sulphide. This reaction is due to partial conversion of the organic sulphur to inorganic sulphide, which can be detected by precipitating it to lead sulphide (black coloured sediment), using lead acetate solution.

Protein/Cystein + 2 NaOH $\rightarrow$ Na_2S

$Na_2S + (CH_3COO)_2Pb \rightarrow \underline{PbS}\downarrow + 2\ CH_3COONa$

STEPS OF WORK:

1) take 1 test tube and place it in a test tube rack.
2) add to the test tube the solutions:

COMPONENTS	Amount of each solution
Protein solution NaOH, 30% $(CH_3COO)_2Pb$, 5%	5 drops 5 drops 1 drop
Mix the tube and heat to boiling until developing of black precipitate	

RESULT:

CONCLUSION:

LABORATORY WORK № 2

QUANTITATIVE DETERMINATION OF TOTAL PROTEIN IN BLOOD SERUM

This is a biochemical test for measuring the total amount of protein in blood plasma or serum, based on the Biuret reaction. Protein in the plasma is made up of albumin and globulin. **Albumin** is made mainly in the liver. It helps keep the blood from leaking out of blood vessels. Albumin also helps carry some medicines and other substances through the blood and is important for tissue growth and healing.

The **globulin** is made up of α1, α2, β, and γ globulins. Some globulins are made by the liver, while others are made by the immune system. These fractions can be quantitated using protein electrophoresis, but the total protein test is a faster and cheaper test that estimates the total of all fractions together. The traditional method for measuring total protein uses the Biuret reagent. The Biuret reaction can be used to assess the concentration of proteins because peptide bonds occur with the same frequency per amino acid in the peptide.

PRINCIPLE OF THE METHOD: protein in serum forms a violet colored complex when reacted with cupric ions in an alkaline solution:

$$\text{PROTEIN} + \text{NaOH/CuSO}_4 = \text{violet colour}$$

The intensity of the violet color is proportional to the amount of protein present and detected photometrically.

STEPS OF WORK:

1) take 2 test tubes, label them and place in a test tube rack.

2) add to the each test tube the solutions:

COMPONENTS / Amount of each solution, ml	CONTROL	SAMPLE
Gornal's reagent	4,0	4,0
serum	-	0,1
NaCl 0,9%	0,1	-

Stir and allow standing for 20 min. Read the extinction for sample versus the control at 540 nm Cuvette 1 cm

RESULT: E_{sample}=

Determine concentration of protein in g/l in the serum using calibration graph:

C_{sample} = g/L

DIAGNOSTIC IMPORTANCE

The reference range for total protein in serum (total protein = albumins + globulins) is **65-85 g/L** for adult persons.

Concentrations above the normal range of serum total protein (**hyperproteinemia)** are found in paraproteinemia, collagenases, myeloma disease, Hodgkin's lymphoma, leukaemia hyperimmunoglobulinemia, dehydration (vomiting, diarrhea).

A decreased concentration of serum total protein (**hypoproteinemia)** is found in: alimentary distrophy, pregnancy, chronic nephritis, cirrhosis, hepatitis, gastroenteropathies, carcinoma, immunodeficiency

CONCLUSION:

_____Teacher's signature

*Date*_____

CLASS № 3

THEME: *STRUCTURES OF PROTEINS*

THEORETICAL PART

1. Primary structure of proteins. Determination of primary structure.
   Properties of the peptide bond.
2. Secondary structure of proteins. Supersecondary structure.
3. Tertiary structure of proteins. Types of stabilizing bonds.
4. Relation between tertiary structure and function of proteins. Denaturation of proteins, factors, practical use.
5. Quaternary structure of proteins.

The structure of proteins can be classified into four levels: primary, secondary, tertiary, quaternary. The basic structure, primary structure, is made up with covalent bond which is the strongest binding in the chemical bonds, so the primary structure is hardly affected even if the protein changes its form. When the conformation of proteins is altered, this process is called **denaturation**, whereas the intact protein is referred to the **native protein**. Secondary, tertiary, quaternary structures were made up of weaker bindings than covalent bonds. Therefore, the high plane structures above primary structure is disrupted easily. This alternation results in denaturation and loses of the biological activity. The factors of denaturation are heat, strong acid, organic compounds, and heavy metal ions. Each sources of denaturation reacts on different bonds in the protein and makes the protein visible as a precipitate or coagulation. Through this experiment, we observed the alternation by means of applying heat and several reagents to the protein solution.

LABORATORY WORK № 1.
DENATURATION OF PROTEIN BY NITRIC ACID

Work demonstrates the influence of chemical factors on stability of protein in solutions. The acid disrupts the bonds between the amino acids that make up the tertiary structure of the protein. The disruption

causes the protein to denature which causes a change in shape of the protein and leads to its precipitation.

Precipitation of proteins by concentrated nitric acid is used in urinalysis for detecting proteinuria.

PRINCIPLE OF THE METHOD: nitric acid causes denaturation of proteins with the formation of a white precipitate (this differs from the nitration reaction in “xanthoproteic acid test”).

STEPS OF WORK:

1) take 1 test tube and place it in a test tube rack.
2) add to the test tube the solutions:

COMPONENTS	Amount of each solution
HNO_3 concentrated	10 drops
Protein solution	10 drops

Take the test tube with concentrated HNO_3 under the angle 45^o and carefully, slowly add protein solution along side of a test tube.

RESULT:

CONCLUSION:

LABORATORY WORK № 2

SEPARATION OF ALBUMINS AND GLOBULINS OF AN EGG WHITE BY SALTING-OUT

The properties of the protein such as ionic strength, hydrophobicity, hydrophilicity etc., determine their solubility. At a certain salt concentration, water molecules surrounding hydrophobic patches on the surface of a protein are removed by the added salt ions. Exposed hydrophobic areas in a polar solvent are not energetically favorable so these patches will interact with each other, resulting in aggregation and precipitation.

The solubility of proteins varies according to the ionic strength of the solution, and hence according to the salt concentration. Two distinct effects are observed: at low salt concentrations, the solubility of the protein increases with increasing salt concentration, an effect termed **salting in**. As the salt concentration is increased further, the solubility of the protein begins to decrease. At sufficiently high concentration, the protein will be almost completely precipitated from the solution (**salting out**). Salting out is usually **reversible.**

Since proteins differ markedly in their solubilities at different salt concentrations, salting-out is a very useful procedure to assist in the purification of a given protein. The commonly used salt is ammonium sulfate, as it is very water soluble and has no adverse effects upon enzyme activity. It is generally used as a saturated aqueous solution which is diluted to the required concentration, expressed as a percentage concentration of the saturated solution (a 100% solution).

The two main fractions in blood serum are globulins and albumins. Globulins are insoluble in distilled water and slightly soluble in dilute salt solutions and can be precipitated using 50%-saturated ammonium sulfate. Albumins are readily soluble in water and in dilute salt solutions. Albumins precipitate first when using saturated ammonium sulfate concentrations. The difference in solubility of proteins is in this lab utilized to fractionate egg white solution into its two main components.

PRINCIPLE OF THE METHOD: due to the fact that protein contains multiple charged groups, its solubility depends on the concentrations of dissolved salts. The additional ions shield the

protein's multiple ionic charges, thereby weakening the attractive forces between individual protein molecules (such forces can lead to aggregation and precipitation). So, the solubility of protein decreases. This "salting out" effect is primarily a result of the competition between the added salt ions and the protein molecules for molecules of water.

STEPS OF WORK:

1) take 2 test tubes, label them as the Sample 1 and Sample 2 and place in a test tube rack.

2) add to each test tube following solutions:

COMPONENTS / Amount of each component	SAMPLE 1	SAMPLE 2
Protein solution NaCl (crystalline) $(NH_4)_2SO_4$-saturated solution (100%)	1,0 ml till full saturation (100%) - Incubation 10 min	1,0 ml - 1,0 ml (till 50% saturation) Without incubation
RESULT:		
	Filtration	Filtration
$(NH_4)_2 SO_4$ (powder)	Boil	Add salt to saturation
RESULT:		

Attention! *You will indicate saturation of solution when the crystals of NaCl remain insoluble on the bottom of the tube.*

CONCLUSION:

_____Teacher’s signature

*Date*_____

CLASS № 4

THEME: DIVERSITY AND CLASSIFICATION OF PROTEINS

THEORETICAL PART

1. Biologically important peptides; classification, representatives, biological functions. Glutathione.
2. Dynamic state of native proteins. Complementarity. Ligands and function of proteins.
3. A variety of proteins and their functions. Quantitative determination of protein for functional properties. Protein medicines (hormones, enzymes, etc.).
4. Changes of proteins in ontogenesis and disease.
5. Simple proteins; representatives, characteristics, biological functions.
6. Conjugated proteins: representatives, characteristics, biological functions.

LABORATORY WORK
ACIDIC HYDROLYSIS OF PROTEINS

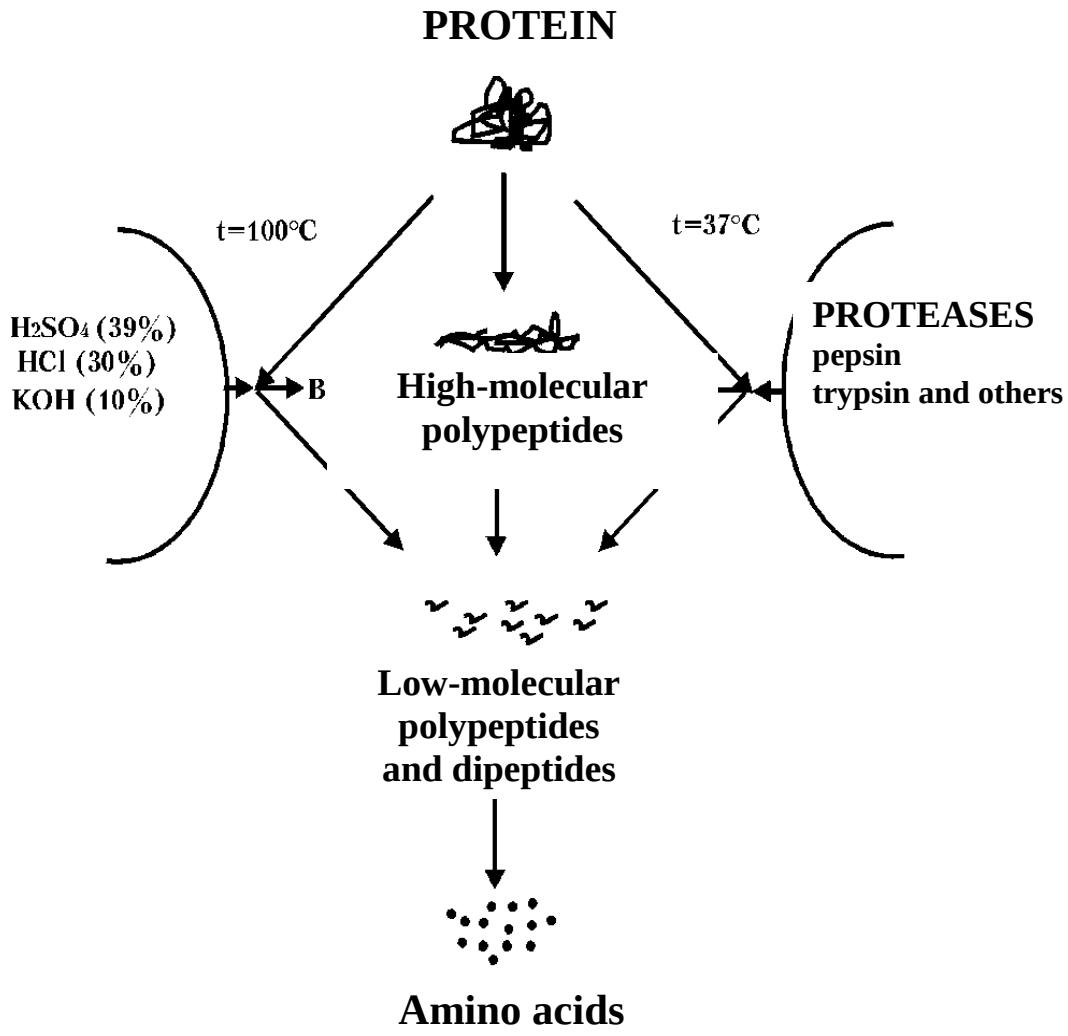
Hydrolysis of protein is a process of biopolymer's degradation with cleavage of peptide bonds through the assistance of water molecules under the action of acids, alkalis or proteases. A solution containing hydrolyzed protein is called hydrolysate.

Application of protein hydrolysis:

- in laboratory conditions hydrolysis of protein is used for determination of primary structure and amino acid composition;
- protein hydrolysates are used in clinical practice for parenteral nutrition (feeding a person intravenously bypassing the usual process of eating and digestion);
- protein hydrolysis can be used to modify the allergenic properties of infant formula (manufactured food designed and marketed for feeding to babies and infants);
- hydrolysis of proteins constantly occurs in gastrointestinal tract and in cells of humans and animals under the action of proteolytic enzymes.

PRINCIPLE OF THE METHOD: biopolymer (protein) in reaction with water degrades to monomers (amino acids). Hydrolysis is catalyzed by protons, hydroxyl ions and enzymes on mechanisms of nucleophilic substitution. Total acidic hydrolysis of proteins proceeds in 12-96 hours at 100 °C.

Sequence of events in hydrolysis of protein is presented in the scheme:



STEPS OF WORK:

1. Add 20 ml of the egg white solution to the flask.

↓

2. Add 5 ml of concentrated HCl.

↓

3. Close the flask by rubber stopper with long glass tube (reverse condenser).

↓

4. Boil the mixture for 45 min.

Hydrolysate for this lab is made by departmental assistants

↓

5. Take 0,5 – 1 ml (5 drops) of the ready-made hydrolysate to the tube № 1 and 5 drops of the egg white solution to the tube (№ 2).

↓

6. Place a piece of **litmus paper** into the hydrolysate sample (№ 1). Adjust the pH of the solution to neutral by addition of 20 % NaOH (to blue colour of litmus paper).

↓

7. Perform a biuret reaction in both test tubes with the hydrolysate and the egg white solution, using instruction from the Class 2.

RESULT:

CONCLUSION:

_____Teacher's signature

*Date*_____

CLASS № 5

THEME: ENZYMES: PROPERTIES AND MECHANISM OF ACTION

THEORETICAL PART

1. History of enzymes study.
2. Properties of enzymes. Active and allosteric centers in enzymes.
3. Simple and conjugated enzymes. Cofactors of enzymes. Co-enzymatic functions of vitamins.
4. Mechanism of enzyme catalysis.
5. Specificity of enzymes.
6. Classification and nomenclature of enzymes.
7. Isoenzymes.
8. Units of enzyme activity.

LABORATORY WORK № 1

EFFECT OF TEMPERATURE ON AMYLASE ACTIVITY

Enzymes are proteins which can catalyze a reaction. They cause an increase in the rate of the reaction, while not being consumed in the reaction. Enzyme activity is affected by several factors such as temperature, pH, and presence of inhibors and activators.

The factor which we are testing in this experiment is temperature. Enzyme activity is changed by variation in temperature. As temperature raises the rate of chemical reactions increases because temperature increases the rate of motion of molecules. This leads to more interactions between an enzyme and its substrate. However, if the temperature is too high, enzymes can be denatured and they can no longer bind to a substrate and catalyze reactions.

PRINCIPLE OF THE METHOD: amylase is an enzyme found in saliva and pancreas gland. It catalyzes hydrolysis of alpha-glycoside bonds in the starch, which is a polysaccharide of glucose:

The activity of enzyme might be determined by estimation either loss in the substrate, or the product income. In this work we determine the presence of a substrate or intermediate product. Reaction of starch with iodine gives dark blue dye. Reaction of dextrins with iodine gives violet, red-brown dye, depending on the dextrin types (Violet or red-brown color might be resulted from the mixture of long- or medium chained polysaccharides).

If in the sample present only maltose (final product), after addition of iodine can see yellow colour of solution (color of iodine).

STEPS OF WORK:

1. For enzymatic studies the amylase from saliva is used. Students must collect (spit in a tube) about 1 ml of saliva in the graduated tube and dilute it in proportion 1 to 10. (Pour distilled water in the tube with saliva till the upper graduation – 10 ml). The total amount of diluted saliva is 10 ml.
2. Take 3 test tubes, label them and place in a test tube rack,
3. Add to each test tube the solutions, according to table:

COMPONENTS / Amount of each solution	SAMPLE 1	SAMPLE 2	SAMPLE 3
Starch (1 %), ml Amylase of saliva (1:10), ml	0,5 0,5	0,5 0,5	0,5 0,5
Allow test tubes to standing for 10 min at	Room temperature (20^oC)	Thermostat (40^oC)	Boiling water bath (100^oC)
After 10 min add 1 drop of KJ (1%)to all test tubes. Mark color of every sample.			

RESULT:

CONCLUSION:

LABORATORY WORK № 2

EFFECT OF ACTIVATOR AND INHIBITOR ON AMYLASE ACTIVITY

Enzyme inhibitors are substances which alter the catalytic action of the enzyme and consequently slow down, or in some cases, stop catalysis. On the other hand, activators are able to increase the rate of reaction and shorten the time of the substrate transformation.

The aim of the work is comparing the action of different salt solutions on the duration of the completed amylase reaction (time of the total hydrolysis of starch to maltose).

STEPS OF WORK:

1) take 3 test tubes, label them and place in a test tube rack,
2) add to the each test tube the solutions:

COMPONENTS / Amount of each solution	CONTROL	SAMPLE 1	SAMPLE 2
H_20	10 drops	8 drops	8 drops
NaCl, 1%	-	2 drops	-
$CuSO_4$, 1%	-	-	2 drops
Amylase of saliva (1:10)	20 drops	20 drops	20 drops
Starch, 1%	5 drops	5 drops	5 drops

Allow test tubes to stand for 5 min (10, 15 min) at a room temperature.

↓

Add 1-2 drops of KJ (1%) to all test tubes. Mark color of every sample and control.

RESULT:

CONCLUSION:

LABORATORY WORK № 3

DETERMINATION OF AMYLASE ACTIVITY IN BLOOD SERUM

The pancreas and salivary gland produce α-amylase to hydrolyze dietary starch into disaccharide maltose which is then converted by other enzymes to glucose to supply the body with energy. When the pancreas is diseased or inflamed, amylase releases into the blood. A test can be done to measure the level of this enzyme in blood. Amylase may also be measured with a urine test. Determination of amylase activity in the urine and blood serum is used for the diagnosis of diseases of pancreatic gland or salivary gland.

PRINCIPLE OF THE METHOD. α-Amylase catalyzes the hydrolysis of para-nitrophenyl-2-D-maltoheptazide to intermediate metabolites, which, under the action of α-glucosidase, decompose to para-nitrophenol and glucose. The rate of formation of para-nitrophenol, measured photometrically at a wavelength of 405 nm, is proportional to the catalytic activity of α-amylase in a sample of blood serum or urine.

STEPS OF WORK:

1) take a test tube and place it in a test tube rack,
2) add to the test tube the solutions:

COMPONENTS	SAMPLE
Working reagent, ml	2
Heat 2 min in thermostat at 37°C	
Blood serum, ml	0,04
Mix well and incubate 2 min at 37 ^{0}C	
Read the extinctions (E_1) for sample versus the distilled water at λ = 405 nm, cuvette 1 cm	
Incubation 3 min at 37 ^{0}C	
Read the extinctions (E_2) for sample versus the distilled water at λ = 405 nm, cuvette 1 cm	

RESULT: E_1 =
E_2 =

α-Amylase activity = ($E_2 - E_1$) x 843,3 = U/L.

DIAGNOSTIC IMPORTANCE

Activity of α-amylase in blood serum is normal **up to 90 U/L**.

Increased blood amylase levels may occur due to pancreatitis, infection of the salivary glands (such as mumps), and malignancy of the pancreas, pancreatic or bile ducts blockage, perforated ulcer, intestinal blockage, tubal pregnancy, diabetic ketoacidosis.

Decreased of α-amylase activity is observed in atrophy of the pancreas, cystic fibrosis, hypothyroidism cahexia.

CONCLUSION:

_____Teacher's signature

*Date*_____

CLASS № 6

THEME: KINETICS OF ENZYMATIC REACTIONS

THEORETICAL PART

1. Enzyme kinetics (Michaelis-Menten and Lineweaver-Burk equations).
2. Factors affecting enzymatic reaction rate (temperature, pH, substrate and enzyme concentration).
3. Regulation of enzyme activity:
   3.1. Activation and inhibition of enzymes
   3.2. Allosteric regulation. Covalent modification of the structure of enzymes (phosphorylation - dephosphorylation, limited proteolysis).
4. Drugs as the inhibitors of enzymes.

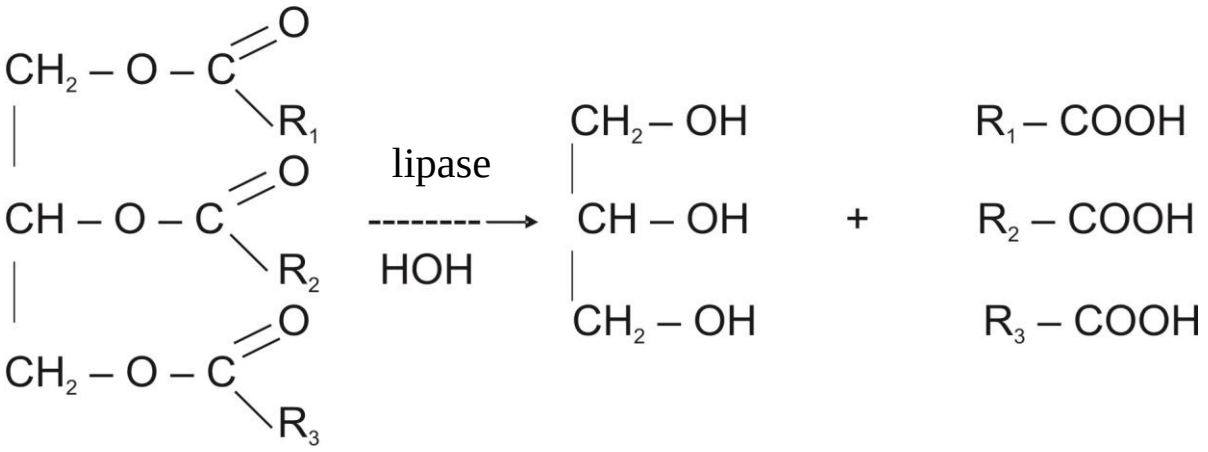
LABORATORY WORK
KINETICS OF LIPASE-CATALYZED HYDROLYSIS OF TRIACYLGLYCEROLS

Lipolytic enzymes of pancreatic gland hydrolyze dietary lipids in the small intestine. Bile acids emulsify lipids, activate lipase and participate in absorption of products of lipid digestion. Studying the kinetics of lipase action, you can watch the dynamics of enzyme activity and factors which affect this process (temperature, concentrations of substrate and products of reaction, presence of bile).

The aim of the present laboratory work is the study the kinetics of lipase, or the rate of lipase reaction affected by some factors (concentrations of substrate and products of reaction, presence of bile).

PRINCIPLE OF THE METHOD. Lipase catalyzes the reaction:

$$\text{Triacylglycerols} \xrightarrow[+H_2O]{\textbf{\textit{lipase}}} \text{glycerol + fatty acids}$$



The rate of lipase-catalyzed reaction can be estimated by the quantity of fatty acids, formed within a defined time interval. Quantity of fatty acids is determined by alkaline titration with phenolphthalein as indicator and is expressed in ml of 0,01n NaOH solution.

STEPS OF WORK:

1) take 2 test tubes, label them and place in a test tube rack,
2) add to each test tube the solutions:

Components	SAMPLE 1 (without bile)	SAMPLE 2 (with bile)
Milk, ml	10,0	10,0
H_2O, ml	1,0	-
Bile, ml	-	1,0
Lipase, ml (homogenate of pancreatic gland)	1,0	1,0

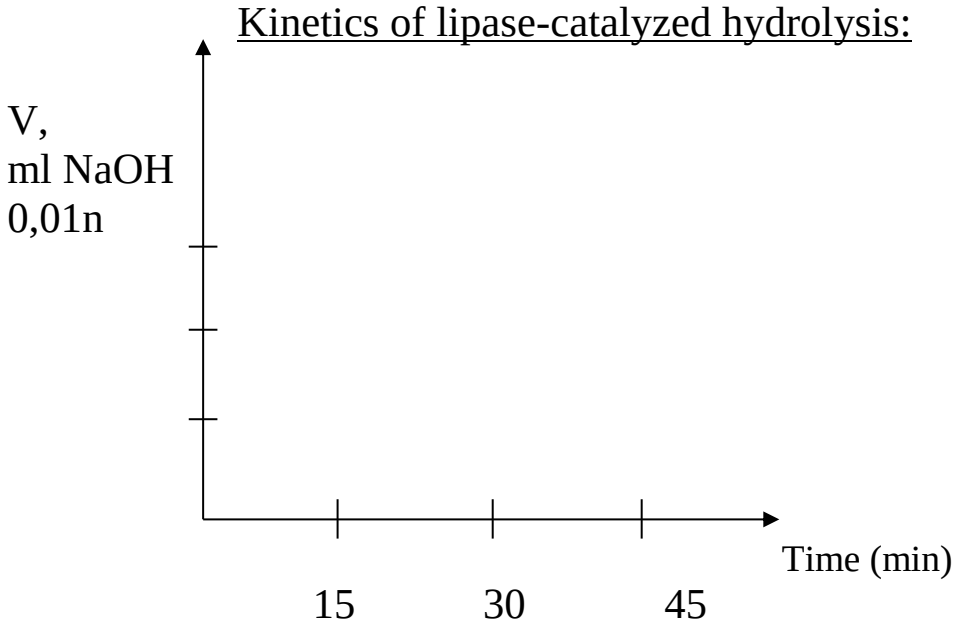
1) Stir and take 2 ml from each test tube to 2 small flasks.
2) Put the test tubes into the thermostat at 37^{o}C. **The time of the beginning of the reaction is point 0 min!**
3) Add 1-2 drops of phenolphthalein to the flasks with the samples 1 and 2.
4) Perform titration of medium in the flasks by adding of 0,01n NaOH to pink color. **Then measure the volume of sodium hydroxide used for titration.** The amount of alkali used for titration is equal to the amount of acids in the medium.
5) Take 2 ml of mixture from each test tube after 15, 30 and 45 min of incubation and perform appropriate titrations.

During the first titration you neutralized all acids, which present in the milk before the lipase action. The results of the first titration (before the lipase action) are subtracted from the results of the following titrations.

RESULT: write down your result into the table:

Time of incubation, min	Volume of 0,01 n NaOH solution, used for titration		
		- bile	+ bile
0	**X**		
15	Y – X = Δ Y		
30	Z – X = Δ Z		
45	E – X = Δ E		

Draw appropriate curves for the samples without and with a bile on the graph using the results of subtraction



CONCLUSION:

_____Teacher's signature

*Date*_____

CLASS № 7

THEME: APPLIED ASPECTS OF ENZYMOLOGY

THEORETICAL PART

1. Tissue-specific enzymes.
2. Serum enzymes used in clinical diagnosis. Origin of serum enzymes.
3. Enzymes in genetic diseases.
4. Practical applications of enzymes in medicine. Immobilized enzymes.

MCQ «Proteins. Enzymes»

STUDENTS' INDIVIDUAL WORK «PROTEINS, ENZYMES»

Exercises for the individual work

1. Write a complete structural formula and name peptide formed from LYSINE, GLUTAMATE, PROLINE, TYROSINE, CYSTEINE, and in which C-terminal residue is CYSTEINE and N-terminal residue is LYSINE.
   Which classes do these amino acids belong to?
2. Draw a diagram of the structure of the molecule of the complex enzyme.
3. Write down the diagram of the amino acid analyzer, indicate the area of application of amino acid analysis.
4. What types of interactions may exist between the following amino acids: Tyr and Glu; Cys and Cys; His and Asp; Ala and Val.
5. Explain how food preservation by freezing utilizes one of the factors that influence enzyme activity?

_____Teacher’s signature

CLASS № 8

MINI-EXAM «PROTEINS & ENZYMES»

1. History of protein study.
2. Proteins as the major components of the body. A variety of proteins and their functions.
3. Structure of amino acids. Classification. Shape of proteins. Molecular mass of proteins, methods of determination.
4. Physicochemical properties of proteins. Precipitation reactions of proteins.
5. Methods for separation and purification of protein: ultracentrifugation, electrophoresis, chromatography, dialysis.
6. Colour reactions of amino acids and proteins.
7. Methods for the quantitative measurement of proteins in a solution. Total serum protein.
8. Primary structure of proteins. Determination of primary structure. Peptide bond formation.
9. Secondary structure of proteins. Supersecondary structure.
10. Tertiary structure of proteins. Types of stabilizing bonds.
11. Relation between tertiary structure and function of proteins. Denaturation of proteins, factors, practical use.
12. Quaternary structure of proteins.
13. Factors responsible for the stability of proteins in solution. Salting out.
14. Biologically important peptides; classification, representatives, biological functions. Glutathione.
15. Dynamic state of native proteins. Complementarity. Ligands and function of proteins. Quantitative determination of protein for functional properties.
16. Protein medicines (hormones, enzymes, etc.).
17. Changes of proteins in ontogenesis and disease.
18. Simple proteins; representatives, characteristics, biological functions.
19. Conjugated proteins; representatives, characteristics, biological functions.
20. History of enzymes study.
21. Properties of enzymes. Active and allosteric centers in enzymes.

22. Simple and conjugated enzymes. Cofactors of enzymes. Co-enzymatic functions of vitamins.
23. Mechanism of enzyme catalysis.
24. Specificity of enzymes.
25. Classification and nomenclature of enzymes.
26. Isoenzymes.
27. Definition of enzyme activity. Units of enzyme activity.
28. Enzyme kinetics (Michaelis-Menten and Lineweaver-Burk equations).
29. Factors affecting enzymatic reaction rate (temperature, pH, substrate and enzyme concentration).
30. Regulation of enzyme activity.
31. Activation and inhibition of enzymes.
32. Drugs as the inhibitors of enzymes.
33. Tissue-specific enzymes.
34. Serum enzymes used in clinical diagnosis. Origin of serum enzymes.
35. Enzymes in genetic diseases.
36. Practical applications of enzymes in medicine. Immobilized enzymes.

Date: _____

CLASS № 9

THEME: GENERAL PATHWAYS OF AMINO ACID METABOLISM

THEORETICAL PART

1. Dynamic state of body proteins. Nitrogen balance.
2. Sources of amino acids in the body and ways of their use.
3. Digestion of proteins in the gastrointestinal tract. Absorption of amino acids.
4. Intestinal putrefaction of proteins (conversion of amino acids by intestinal bacteria).
5. General pathways of amino acid metabolism.
6. Transamination of amino acids, enzymes, biological role. Coenzyme function of vitamin B_6. Mechanism of transamination. Aminotransferases, their tissue specificity and diagnostic significance.
7. Types of deamination of amino acids. Oxidative deamination and reductive amination. Biological role.
8. Transdeamination. Biological role.

LABORATORY WORK

DETERMINATION OF ALANINE AMINOTRANSFERASE ACTIVITY IN THE BLOOD SERUM

Aminotransferases are enzymes catalyzing the transfer of amino group from amino acids to keto acids with the formation of a new amino acid and a new keto acid without intermediary release of ammonia. Aminotransferase contains derivative of vitamin B_6 as a coenzyme. The activity of aminotransferases is used to assess the amino acid metabolism in different tissues.

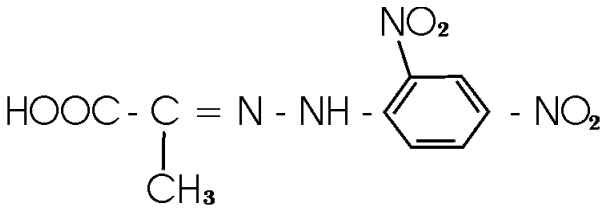
Alanine aminotransferase (ALT) is also called serum glutamic-pyruvic transaminase (SGPT). ALT is found in plasma and in various bodily tissues, but is most commonly associated with the liver. It is commonly measured clinically as a part of a diagnostic evaluation of hepatocellular injury, to determine liver health. When used in

diagnostics, it is almost always measured in international units/liter (IU/L or, mmol/l/h).

PRINCIPLE OF THE METHOD: alanine aminotransferase catalyzes the reaction in which amino group from alanine is transferred to alpha-ketoglurate to form pyruvate and glutamate:

$$\text{Alanine} + \alpha\text{-Ketoglutarate} \xrightleftharpoons[B_6]{ALT} \text{Glutamate} + \text{Pyruvate}$$

Pyruvate reacts with 2,4-dinitrophenylhydrazine, and in the presence of KOH, forms 2,4-dinitrophenylhydrazone pyruvate of brown-and-red colour:



The intensity of the colour is measured colorimetrically. The amount of pyruvate produced in the transamination reaction is proportional to the activity of ALT in the blood serum.

STEPS OF WORK:

1) take 2 test tubes, label them and place in a test tube rack,
2) add to the each test tube the solutions:

COMPONENTS	CONTROL	SAMPLE
Substrate mixture, ml	0,25	0,25
NaCl 1%, ml	0,05	-
Blood serum, ml	-	0,05
Incubate 30 min at 37 ^{0}C		
2,4-Dinitrophenylhydrazine, ml	0,25	0,25

Mix well, leave for 20 min at room temperature

NaOH, ml	2,5	2,5

Mix well, leave for 10 min at room temperature

Read the extinction for sample versus the control, λ= 500-530 nm, cuvette 1 cm.

RESULT: E = ;

According to the calibration graph the activity of alanine aminotransferase is …….. mmol/ L / h·.

DIAGNOSTIC VALUE

Normally, ALT activity is low in the blood serum. In certain diseases accompanied by cell destruction (necrosis) or the increased cell membrane permeability (inflammation) ALT releases from cells into the blood plasma.

Reference range of alanine aminotransferase activity is **5-42 IU**/L.

Significantly elevated levels of ALT (SGPT) often suggest the existence of medical problems such as viral hepatitis, liver damage, bile duct problems (obstructive jaundice), congestive heart failure, infectious mononucleosis, miopathy. Elevated ALT may also be caused by dietary choline deficiency or strenuous physical exercise.

CONCLUSION:

_____Teacher's signature

Date: _____

CLASS № 10

THEME: DETOXIFICATION OF AMMONIA. METABOLISM OF CERTAIN AMINO ACIDS

THEORETICAL PART

1. Decarboxylation of amino acids. Types of decarboxylation, biological role. Biogenic amines: synthesis, their functions. Oxidation of biogenic amines.
2. Ways for the formation and detoxification of ammonia.
3. Intracellular detoxification of ammonia: reductive amination, synthesis of glutamine and asparagine. Role of glutaminase in the maintenance of acid-base balance in the body.
4. Biosynthesis of urea. Disorders of the urea synthesis and excretion.
5. Catabolism of amino acids in the organism. Glucogenic and ketogenic amino acids.
6. Metabolism of methionine: formation of S-adenosylmethionine, its role in transmethylation reactions. Synthesis of creatine.
7. Metabolism of phenylalanine and tyrosine. Disorders of phenylalanine and tyrosine metabolism (phenylketonuria, alkaptonuria, albinism).

LABORATORY WORK
DETERMINATION OF UREA IN THE BLOOD SERUM (enzymatic kinetic method)

Urea is the major product of ammonia detoxification in the body. Urea is the end product of the amino acid and protein metabolism. The liver is the only site of urea synthesis. The liver produces urea in the urea cycle. Urea is released from hepatocytes into the blood and then is filtered by glomeruli into the urine. Urea concentration in the blood and urine may serve as an indicator of the liver and renal function.

In healthy persons urea nitrogen in the blood constitutes approximately 50% of all nitrogen-containing compounds (proteins,

amino acids, creatinine, uric acid, indican, etc). Urea nitrogen in urine constitutes approximately 80% of all nitrogen-containing compounds.

PRINCIPLE OF THE METHOD: urea is hydrolyzed in the presence of urease to form ammonia and CO_2. Then ammonia in the reaction catalyzed by glutamate dehydrogenase (GDH) interacts with α-ketoglutarate to form glutamate.

In the course of the reaction, the reduced NAD is oxidized. The decrease in the concentration of NADH is proportional to the urea content.

$$\text{Urea} + 2H_2O \xrightarrow{\textit{urease}} 2NH_4^+ + CO_3^{2-}$$

$$NH_4^+ + \alpha\text{-ketoglurate} + NADH \xrightarrow{\textit{GDH}} \text{L-glutamate} + NAD^+ + 2H_2O$$

STEPS OF WORK:

1) take 3 test tubes, label them and place in a test tube rack,
2) add to each test tube the solutions:

COMPONENTS	CONTROL	STANDARD	SAMPLE
Working reagent, ml	–	2,0	2,0
H_2O, ml	2,0	–	–
Warm up tubes in thermostat at 37^0C for 1 min.			
Blood serum, ml	–	–	0,02
Standard solution, ml (C_{st}= 13,33 mmol/L)	–	0,02	–
Mix well and read the extinction for sample and standard versus the control (water) after 1min ($E_{1sample}$ и $E_{1standart}$), than after 2 min ($E_{2sample}$ и $E_{2standart}$), λ= 340-365 nm, cuvette 0,5 cm.			

RESULT: $E_{1sample}$= , $E_{1standard}$= , $E_{2sample}$= , $E_{2standard}$=

ΔE_{sample}= ($E_{1sample} - E_{2sample}$) =
$\Delta E_{standard}$= ($E_{1standard} - E_{2standard}$) =

CALCULATION:

$$C_{urea} = \frac{\Delta E_{test}}{\Delta E_{stand}} \cdot C_{st} = \quad \text{mmol/L.}$$

DIAGNOSTIC VALUE

The normal urea level in the blood serum **is 2,5-8,3 mmol/L.** Urinary excretion of urea is **333-583 mmol/day.**

Urea level is decreased in the blood and urine in hepatitis, cirrhosis and genetic disorders of the urea cycle (as a result of impaired urea synthesis).

In nephritis and renal failure, when renal filtration is impaired, urea is retained, and hence its concentration is increased in the blood (synthesis of urea in the liver is normal), but is decreased in the urine (renal filtration is impaired; therefore urea accumulates in the blood but does not excrete in the urine).

The high protein diet, hypovolemia, congestive heart failure, gastrointestinal hemorrhage, fever and increased catabolism ccan also lead to an increase in blood urea.

CONCLUSION:

STUDENTS' INDIVIDUAL WORK
«METABOLISM OF AMINO ACIDS»
Assignments for the individual work

1. Compose metabolic scheme of amino acid metabolism.
2. Accentuate:
   2.1. Sources of amino acids in tissues.
   2.2. General pathways of amino acid metabolism.
   2.3. Intracellular detoxification of ammonia.
   2.4. Biosynthesis of urea.
   2.5. Ketogenic amino acids which breakdown leads to formation of acetyl-CoA.
   2.6. Substrates of the citric acid cycle which are intermediates of amino acid metabolism.
   2.7. End products of amino acid and nucleotides metabolism, and their levels in the blood.

_____Teacher signature

Date: _____

CLASS № 11

THEME: STRUCTURE OF NUCLEOTIDES AND NUCLEIC ACIDS

THEORETICAL PART

1. History of nucleic acids study.
2. Chemical composition of nucleic acids. Differences between DNA and RNA.
3. DNA: composition, structure, cell localization, biological role.
4. RNA: types, composition, structures, cell localization, biological role.
5. Nucleoproteins: structure of ribosomes of eucaryotes and chromatin.
6. Biosynthesis of purine nucleotides: synthesis of phosphoribosylamine, origin of atoms in the purine ring.
7. Inosinic acid as a precursor for synthesis of adenylic and guanylic acids. Regulation of biosynthesis of purine nucleotides.

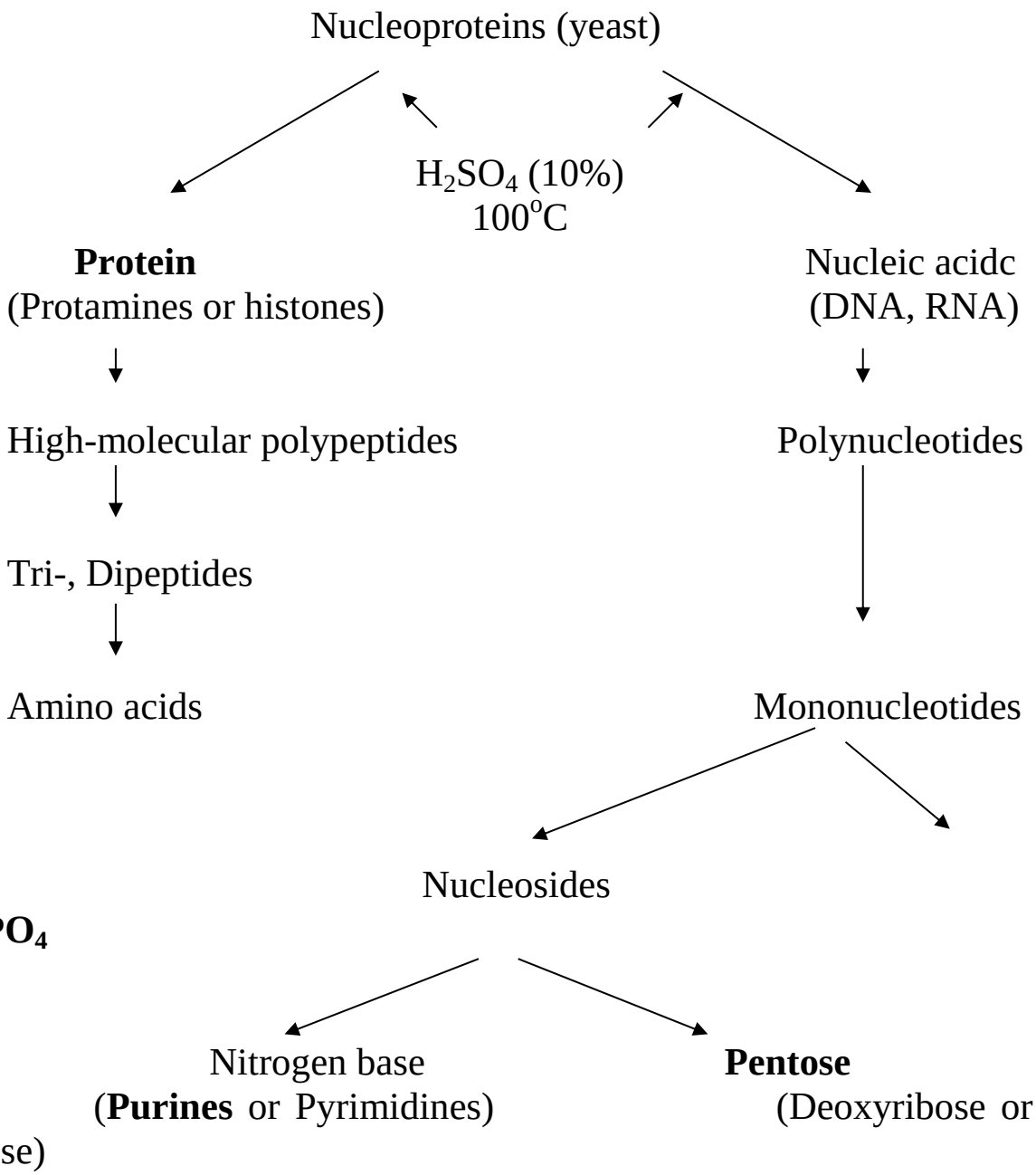
LABORATORY WORK
HYDROLYSIS OF NUCLEOPROTEINS

Nucleoproteins belong to the group of conjugated proteins, consisting of protein linked to a nucleic acid (DNA or RNA). The proteins combined with DNA are found in chromosomes. Ribosomes are made up of proteins and RNA.

Nucleoproteins play an important role in heredity, protein synthesis, mutation, viral infections, cancer and engineering. Nucleoproteins contain a nucleic acid molecule attached to one or more molecules of simple proteins, usually a basic protein, such as a protamine or histone, because nucleic acid molecules are acidic.

Like carbohydrates and proteins, the complex molecules of nucleic acid are also made up of simple molecules. In order to understand nucleoproteins architecture, the molecule is hydrolyzed and the hydrolytic products are studied.

Acidic hydrolysis of nucleoproteins yield following simple substances:



Components of nucleoproteins can be detected using qualitative reactions.

Color reactions for components of nucleoproteins:

Components	Reaction	Observation
Protein	Biuret reaction	Blue-violet color
Purine	Silver test	Light brown precipitation
Pentose	Trommer test	Red color and precipitation (Cu_2O)
Phosphoric acid	Molybdenum test	Lemon-yellow color

STEPS OF WORK:

1) take 4 test tubes, label them and place in a test tube rack,
2) carry out the following reactions in each:

Biuret reaction	
Hydrolyzate of nucleoproteins NaOH 10% $CuSO_4$ 1%	5 drops 10 drops 1-2 drops
Result:	
Silver test	
Hydrolyzate of nucleoproteins $(NH_4)OH$ (conc). $AgNO_3$ 1%	10 drops 1 drop 5 drops Leave for 5 min
Result:	
Trommer test	
Hydrolyzate of nucleoproteins NaOH 30 % $CuSO_4$ 7 %	5 drops 10 drops 3 drops Boil 10 sec
Result:	
Molybdenum test	
Hydrolyzate of nucleoproteins Molybdenum reagent	5 drops 20 drops Boil 1-2 min
Result:	

CONCLUSION:

_____Teacher’s signature

Date: _____

CLASS № 12

THEME: METABOLISM OF NUCLEOTIDES AND NUCLEIC ACIDS

THEOREICAL PART

1. Biosynthesis of pyrimidine nucleotides. Regulation of biosynthesis of pyrimidine nucleotides.
2. Synthesis of deoxyribonucleotides. Synthesis of thymidylic acid.
3. Digestion of nucleic acids in the gastrointestinal tract. Degradation of nucleic acids in tissues. Re-utilization of nucleosides and nitrogenous bases for synthesis of nucleotides.
4. Degradation of purine and pyrimidine nucleotides.
5. Disorders of metabolism of nucleotides: xanthinuria, orotaciduria, gout.
6. Biosynthesis of DNA in eukaryotic cells: substrates, enzymes, scheme.

LABORATORY WORK
DETERMINATION OF URIC ACID CONCENTRATION IN THE BLOOD SERUM

Uric acid is a waste product of purine metabolism in man. Two purines, adenine and guanine are important constituents of nucleic acid, and of free nucleotides such as ATP, cAMP and GTP. Xanthine and hypoxanthine are other body purines. Purines can be synthesized in the body or can be ingested. Food stuffs which contain abnormal amounts of nuclear proteins such as liver and pancreas, contains large quantities of purines. The stimulants in coffee and tea (caffeine) and in cocoa (theobromine) are xanthines.

The serious consequences of abnormal uric acid metabolism depend in part upon the insolubility of uric acid and its sodium salt. The former crystallizes in the kidney and urinary tract while the latter in cartilage and other tissues around the joints in gout.

Plasma uric acid is filtered by the glomerulus's and is subsequently reabsorbed to about 90% by the tubules. Uric acid

concentration in serum is greatly affected by extra renal as well as renal factors.

PRINCIPLE OF THE METHOD: uric acid is enzymatically (enzyme – uricase) oxidized by oxygen to produce hydrogen peroxide, allantoin, and carbon dioxide. The H_2O_2 reacts with chromogen (reduced - colorless) in the presence of peroxidase to form a chromogen (oxidized) dye. The intensity of color (pink) formed is proportional to the uric acid concentration and can be measured photometrically.

STEPS OF WORK:

1) take 2 test tubes, label them and place in a test tube rack,
2) add to each test tube the solutions:

COMPONENTS	SAMPLE	STANDARD
Blood serum, ml	0,025	-
Standard of uric acid, ml (357 μmol/L)	-	0,025
Working solution, ml	1,0	1,0
	Stir and incubate 10 min at 37 ºC	
	On completion of incubation read the extinction for sample and standard versus water, λ= 500 nm, cuvette 0,5 cm.	

Attention! The colour of the solution is stable for 15 minutes

RESULT: E_{st} = ; E_{sample}= .

CALCULATION:

$$C_{sample} = \frac{C_{st} \cdot E_{sample}}{E_{st}} = \quad \mu mol/L.$$

DIAGNOSTIC VALUE

Normal ranges of uric acid in the blood - **140-340 μmol/L (female**) and **200-415 μmol/L (male).** Normal values of uric acid excretion in urine are **1,5-4,5 mmol/day.**

There are 3 major causes for elevated level of uric acid in blood: gout, increased nuclear breakdown and renal diseases.

Hyperuricemia is also a common feature of toxemia of pregnancy and lactic acidosis. The cause of increased plasma levels have is apparently competition for binding sites in the renal tubules.

Elevated levels may also be found after ingestion of a diet rich in purines, or a marked decrease in total dietary intake, resulting in increased tissue breakdown.

Hypouricemia is associated with the following clinical disorders: Fanconi syndrome, Wilson's disease, syndrome of inappropriate antidiuretic hormone secretion, low purine diet.

Uric acid excretion depends on the purines content in food and intensity of nucleoproteins metabolism. **Hypouricuria** (decrease of uric excretion with urine) is noted in gout, nephritis, renal insufficiency; **hyperuricuria** (increase of uric excretion with urine) – in leukemia, accelerated breakdown of nucleoproteins. In gout uric acid salts (urates) precipitate in cartilages, muscles and joints. The content of uric acid in the blood can be increased while in the urine – decreased.

CONCLUSION:

_____Teacher signature

Date: _____

CLASS № 13

THEME: BIOSYNTHESIS OF NUCLEIC ACIDS AND PROTEIN

THEORETICAL PART

1. Biosynthesis of RNA in eukaryotic cells: substrates, enzymes, steps, scheme.
2. RNA processing.
3. Reverse transcription: scheme, biological role.
4. The genetic code: its characteristic features.
5. Stages of protein synthesis. Activation of amino acids.
6. Eukaryotic translation: initiation, elongation, termination.
7. Posttranslational processing of proteins.
8. Regulation of gene expression.

MCQ «Metabolism of nucleotides and nucleic acids»

STUDENTS' INDIVIDUAL WORK

«Metabolism of nucleic acids and proteins»

1. List the main enzymes involved in DNA replication in eukaryotes, name their functions:

	Enzyme	Its function
1		
2		
3		
4		
5		

2. Write structure and reaction of synthesis of valyl-tRNA.

3. Depictp the scheme of biosynthesis of dipeptide methionyl-glutamate (initiation and elongation stages).

4. What are the main mechanisms of gene expression control

- at the genome level:

- at the level of transcription:

- at the level of translation:

_____Teacher’s signature

*Date*_____

CLASS № 14

THEME: PRINCIPLES OF MOLECULAR BIOLOGY

THEORETICAL PART

1. Antibiotics as inhibitors of protein synthesis.
2. Enzymes and techniques used in molecular biology.
3. The blot-analysis of DNA and RNA. Methods for protein identifying: Western blot analysis.
4. Polymerase chain reaction: stages and practical applications.
5. Restriction fragment length polymorphism. DNA fingerprint.
6. Sequencing of nucleic acids.
7. Genetic engineering, recombinant DNA technology.

Watching training videos.

STUDENTS' INDIVIDUAL WORK
"PRINCIPLES OF MOLECULAR BIOLOGY
Assignments for the individual work

1. Fill in the table:

Characteristic	**Southern** blot	**Northern** blot	**Western** blot
Tested molecule			
Using of restriction enzymes (yes/no)			
Using of electrophoresis (yes/no)			
Probe nature			

2. Draw the scheme of polymerase chain reaction (PCR).

3. Explain the role of genetic engineering in the production of protein. Draw the scheme of process. What are the examples of proteins obtained in this way?

-

_____Teacher’s signature

CLASS № 15

MINI-EXAM «METABOLISM OF NUCLEIC ACIDS AND NUCLEOTIDES. PRINCIPLES OF MOLECULAR BIOLOGY»

1. History of nucleic acids study.
2. Chemical composition of nucleic acids. Differences between DNA and RNA.
3. DNA: composition, structure, cell localization, biological role.
4. RNA: types, composition, structures, cell localization, biological role.
5. Nucleoproteins: structure of ribosomes of eucaryotes and chromatin.
6. Biosynthesis of purine nucleotides: synthesis of phosphoribosyl amine, origin of atoms in the purine ring.
7. Inosinic acid as a precursor for synthesis of adenylic and guanylic acids. Regulation of biosynthesis of purine nucleotides.
8. Biosynthesis of pyrimidine nucleotides. Regulation of biosynthesis of pyrimidine nucleotides.
9. Synthesis of deoxyribonucleotides. Synthesis of thymidylic acid.
10. Digestion of nucleic acids in the gastrointestinal tract. Degradation of nucleic acids in tissues. Re-utilization of nucleosides and nitrogenous bases for synthesis of nucleotides.
11. Degradation of purine and pyrimidine nucleotides.
12. Disorders of metabolism of nucleotides: xanthinuria, orotaciduria, gout.
13. Biosynthesis of DNA in eukaryotic cells: substrates, enzymes, scheme.
14. Biosynthesis of RNA in eukaryotic cells: substrates, enzymes, steps, scheme.
15. RNA processing.
16. Reverse transcription: scheme, biological role.
17. The genetic code: its characteristic features.
18. Stages of protein synthesis. Activation of amino acids.
19. Eukaryotic translation: initiation, elongation, termination.
20. Posttranslational processing of proteins.
21. Regulation of gene expression.
22. Antibiotics as inhibitors of protein synthesis.
23. Enzymes and techniques used in molecular biology.

24. The blot-analysis of DNA and RNA. Methods for protein identifying: Western blot analysis.
25. Polymerase chain reaction: stages and practical applications.
26. Restriction fragment length polymorphism. DNA fingerprint.
27. Sequencing of nucleic acids.
28. Genetic engineering, recombinant DNA technology.

Date: _____

CLASS № 16

THEME: BASICS OF BIOENERGETICS

THEORETICAL PART

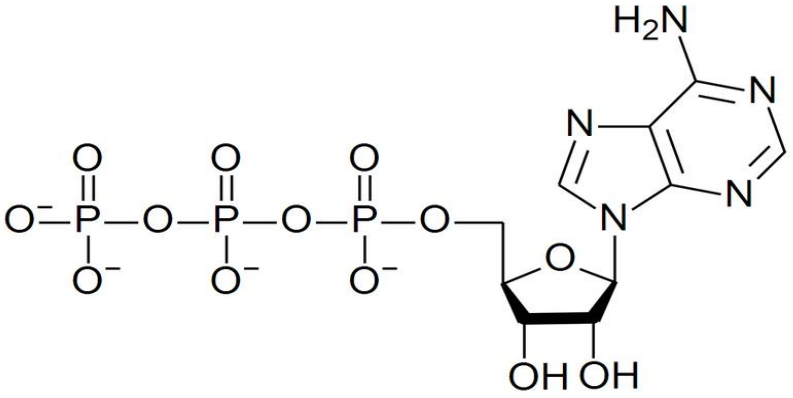
1. Bioenergetics of the cell.
2. High-energy compounds: structure, biological role (ATP and other nucleoside triphosphates, 1,3-bisphosphoglycerate, phosphoenolpyruvate, creatine phosphate, acetyl CoA, succinyl CoA).
3. Electron transport chain (ETC), its structural organization and functioning. Electron transport chain complexes.
4. NAD^+($NADP^+$)-dependent dehydrogenases, structure of coenzyme, biological role.
5. FAD(FMN)-dependent dehydrogenases, structure of coenzyme, biological role.
6. Coenzyme Q, structure, biological role.
7. Cytochromes, structure, biological role.

LABORATORY WORK

QUANTITATIVE DETERMINATION OF HIGH-ENERGY COMPOUNDS IN THE MUSCULAR TISSUE

Macroergic (nigh-enegy) compounds are the substances containing so-called “macroergic bonds” marked by “tilda”. Dissociation of these bonds is followed by a huge energy release.

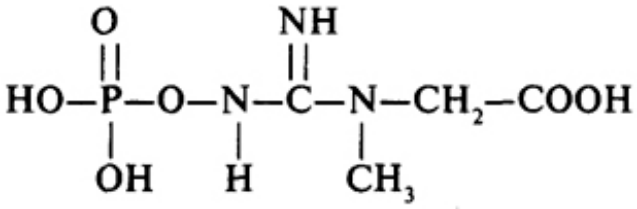
Adenosine-5'-triphosphate (ATP) is a multifunctional nucleotide that plays an important role in cell biology as the "molecular unit of currency" of intracellular energy transfer.



ATP contains two macroergic bonds and transports chemical energy within cells for metabolism. ATP is made from adenosine diphosphate (ADP) or adenosine monophosphate (AMP) and its use in metabolism converts it back into these precursors.

In animals it is an energy source produced during cellular respiration and consumed by many enzymes and a multitude of cellular processes, including biosynthetic reactions, and cell division. ATP is also is required for the shortening of actin and myosin filament crossbridge required for muscle contraction. This latter process is one of the main energy requirements of animals and is essential for locomotion and respiration.

Creatine phosphate is a phosphorylated creatine molecule containing an one macroergic bond. It serves as a rapidly mobilizable reserve of high-energy phosphates in skeletal muscle and brain: Creatine phosphate can anaerobically donate a phosphate group to ADP to form ATP during the first 2 to 7 seconds following an intense muscular or neuronal effort.



The muscle cell is able to generate creatine phosphate from excess ATP during rest, as well as its use of creatine phosphate for quick regeneration of ATP during intense activity, provides a spatial and temporal buffer of ATP concentration. Phosphocreatine plays a particularly important role in tissues that have high, fluctuating energy demands such as muscle and brain.

PRINCIPLE OF THE METHOD: in acidic medium ATP and creatine phosphate hydrolyzed to ADP (or AMP), creatine and phosphate. Phosphate will readily react with ammonium molybdate in the presence of ascorbic acid to form a blue colored complex, the intensity of which is directly proportional to the concentration of phosphate in the solution. .

STEPS OF WORK:

A. Preparation of protein-free muscle filtrate:

0.5 g of a rat muscle is homogenized in 5 ml of 2.5% trichloro-acetic acid. The muscle homogenate is filtered, and protein-free filtrate is used for the phosphate quantitative determination.

The protein-free filtrate is preparing by departmental assistants!

B. Determination of labile phosphates

1) take 2 test tubes, label them and place in a test tube rack,
2) add to the each test tube the solutions:

COMPONENTS, ml	CONTROL	SAMPLE
Protein-free muscle filtrate	0,5	0,5
HCl, 1M	1,0	1,0
	-	Boil for 10 min then cool
NaOH, 1M	1,0	1,0
H_2O	2,5	2,5
Ammonium molybdate, 1%	0,5	0,5
Ascorbic acid	0,5	0,5
	Stir and incubate at room temperature for 10 min	
	On completion of incubation read extinction of versus control, λ = 640 nm, cuvette 1 cm	

RESULT:

Ex =

Calculation is performed according to calibration graph.

Received result of concentration of phosphate in the sample (A) put into the formula:

C = A · 3,3 · 40 = mg ATP/g tissue

Find amount of ATP in 1g of tissue:

Amount of ATΦ = A ˙ 260 = μmol /g of tissue

CONCLUSION:

Note: concentration of ATP ≈ 5μmol per 1 g of muscle (at rest condition)

_____Teacher's signature

*Date*_____

CLASS № 17

THEME: THE CENTRAL PATHWAY OF METABOLISM. BIOCHEMISTRY OF MEMBRANES

THEORETICAL PART

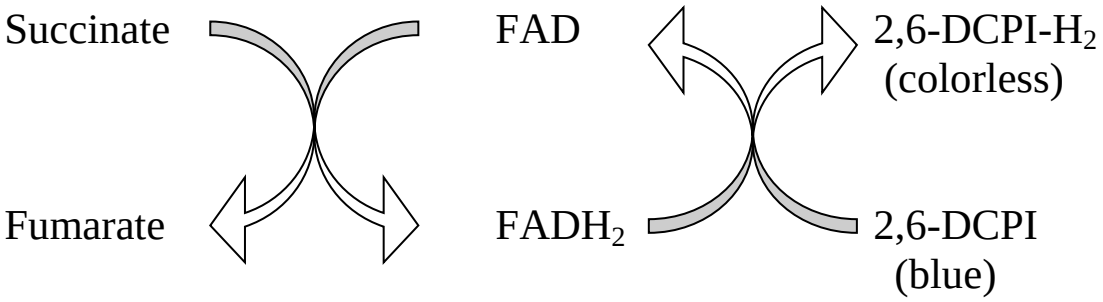
1. ATP: structure, biological role; the ways of its formation (oxidative and substrate-level phosphorylation) and use.
2. Oxidative phosphorylation, mechanisms. The chemiosmotic theory of oxidative phosphorylation. The P/O ratio.
3. Regulation of ETC. Activators and inhibitors of the electron transport chain. Uncoupling agents.
4. The citric acid cycle: reactions, regulation and biological role.
5. Relation of the citric acid cycle with the electron transport chain, energy yield of the citric acid cycle.
6. Chemical composition and structure of biological membranes. Lipids and proteins of biological membranes.
7. General properties and functions of biological membranes.
8. Types of transport mechanisms across membranes.

LABORATORY WORK № 1
DETECTION OF THE SUCCINATE DEHYDROGENASE ACTIVITY

Succinate dehydrogenase or respiratory **Complex II** is an enzyme complex bound to the inner mitochondrial membrane. It is the only enzyme that participates in both the citric acid cycle and the electron transport chain. The enzyme catalyzes the oxidation (removal of succinate to fumarate. Coenzyme is FAD joined with the enzyme by covalent bond. FAD is intermediary acceptor of hydrogen:

Assignment: write the formulas of the substrate and product of the reaction

PRINCIPLE OF THE METHOD. Substrate is succinate. Final acceptor of hydrogen is 2,6-dichlorophenolindophenol (dark-blue colour) which, due to reduction, is converted to its colorless (reduced) form. Conversion of dark-blue oxidized chromogen into its colorless form allows detecting the catalytic activity of the enzyme.



Source of the enzyme is homogenate of muscles.
Malonate is competitive inhibitor of succinate dehydrogenase.

STEPS OF WORK:

1) take 4 test tubes, label them and place in a test tube rack,
2) add to each test tube the solutions:

Components, ml	1	2	3	4
Homogenate of muscles	1,0 (Boil 2 min)	1,0	1,0	1,0
H_2O	0,5	0,5	1,5	-
Malonate	-	-	-	0,5
Succinate	1,0	1,0	-	1,0
2,6-dichlorphenol-indophenol	2 drops	2 drops	2 drops	2 drops
	Stir and incubate 15 min at 37 ºC			
RESULT (color)				

CONCLUSION:

LABORATORY WORK № 2.

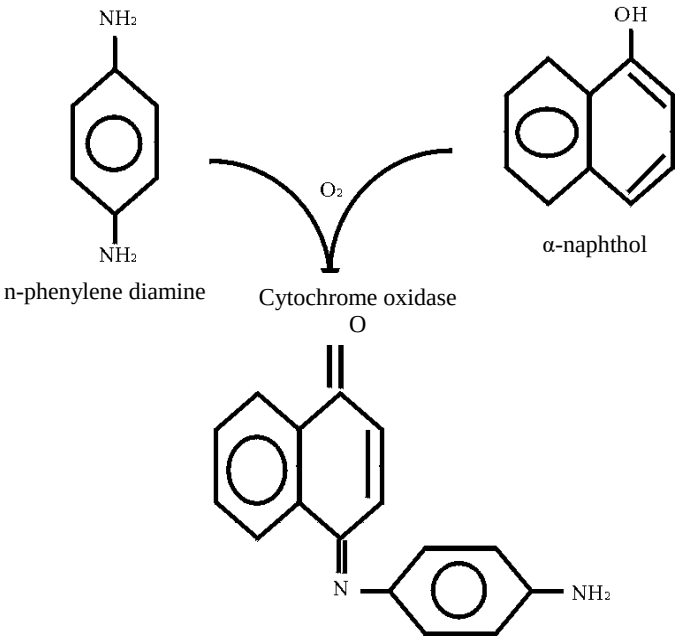
DETECTION OF THE CYTOCHROME OXIDASE ACTIVITY

The enzyme cytochrome oxidase is the last enzyme in the respiratory electron transport chain of mitochondria located in the mitochondrial membrane. It receives electrons from *cytochrome c* molecules and transfers them to one oxygen molecule, converting molecular oxygen to molecule of water, and in addition translocates four protons across the membrane, helping to establish a transmembrane difference of proton electrochemical potential that the ATP synthase then uses to synthesize ATP.

PRINCIPLE OF THE METHOD. Mixture of α-naphthol and *p*-phenylene diamine (reagent NADI) is oxidized by cytochrome oxidase and in the presence of oxygen forms product of blue colour.

Qualitative detection of the cytochrome oxidase activity in muscles is performed by a "oxidase test'. http://en.wikipedia.org/wiki/Oxidase_test - cite_note-urlOxidase_Test_and_Modified_Oxidase_Test-1It is based on the application of the reagent called NADI (mixture of *α-naphthol* and *p-phenylene diamine*). The reagent is a dark-blue to color when oxidized, and colorless when reduced.

In a viable tissue the samples possess cytochrome oxidase activity. This enzyme catalyzes the transport of electrons from donor compounds to electron acceptors (usually oxygen). The test reagent NADI acts as an artificial electron donor for the enzyme oxidase. The oxidized reagent forms the colored compound indophenol blue:



STEPS OF WORK:

Rat muscle is divided into two parts. One part is placed on the filter paper, the other part is put into the test-tube with 1 ml of water and boiled for 1 min. After cooling, the boiled muscle tissue is taken from the test-tube by a glass stick and is put on the filter paper.

Both portions of the muscle are applied by 2 drops of reagent NADI. Incubation is performed for 5-10 min at room temperature.

RESULT:

CONCLUSION:

STUDENTS' INDIVIDUAL WORK
"ENERGY METABOLISM"

Assignments for the individual work

1. Draw a common scheme of energy metabolism, including the Electron Transport Chain (ETC) and Tricarboxylic Acid Cycle (TCA cycle).
   1.1. Illustrate the relationship between TCA cycle and ETC.
   1.2. Mark vitamin-dependent enzymes reactions.
   1.3. Illustrate anabolic function of TCA cycle.
   1.4. Highlight the regulatory enzymes of TCA cycle.

.

_____Teacher's signature

Date: _____

CLASS № 18

THEME: OXIDATIVE PROCESSES IN THE CELL. INTRODUCTION INTO METABOLISM.
CREDIT SESSION

THEORETICAL PART

1. General characteristics of oxidation processes. Oxidase and peroxidase types of oxidation: schemes, enzymes, biological role.
2. Dioxygenase and monooxygenase types of oxidation: schemes, enzymes, biological role. Microsomal oxidation: scheme, cytochrome P_{450}, biological role.
3. Reactive oxygen species: their tissue-damaging effects.
4. Antioxidant systems, role of enzymes and non-enzymatic antioxidants.
5. Metabolism and metabolic pathways. Interrelations between anabolism and catabolism.
6. Experimental study of metabolism, the use of radioisotope tracers.
7. The specific and common pathways of catabolism.

Date: _____

CLASS № 19

THEME: CARBOHYDRATES OF PHYSIOLOGICAL SIGNIFICANCE

THEORETICAL PART

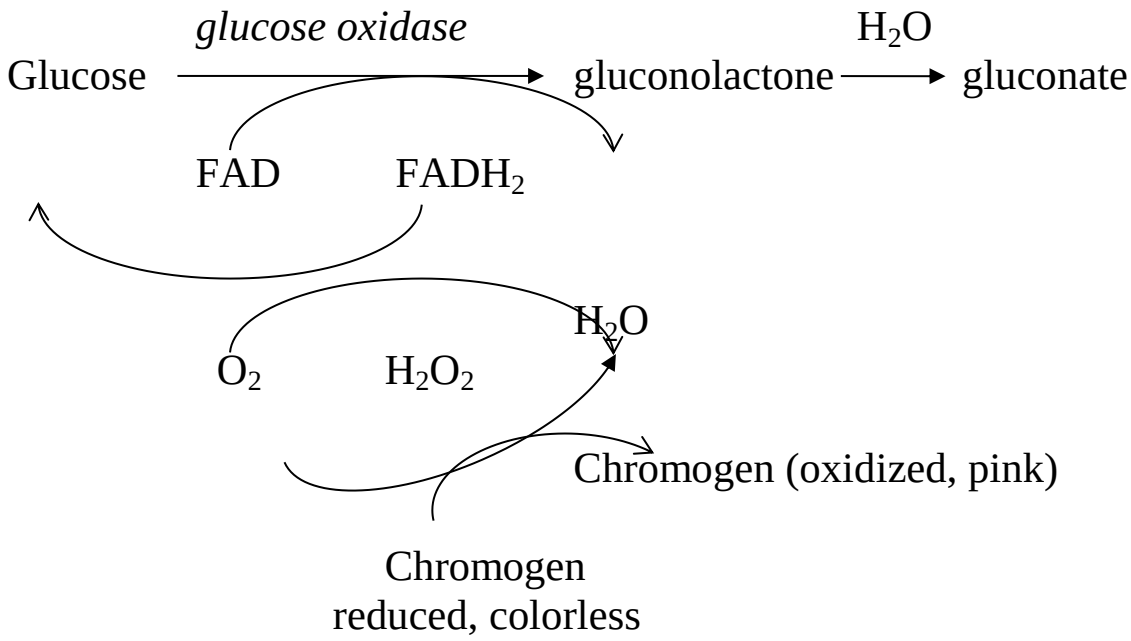
1. Carbohydrates: general characteristics and classification, biological functions, contents in human tissues.
2. Dietary carbohydrates, their characteristics.
3. Digestion and absorption of carbohydrates in the gastrointestinal tract. Lactose intolerance.
4. The general scheme of pathways of glucose metabolism and their estimation.
5. Reactions of glucose phosphorylation and dephosphorylation of glucose 6-phosphate. Regulation.
6. Galactose metabolism. Galactosemia.
7. Fructose metabolism. Essential fructosuria, hereditary fructose intolerance.
8. Lactose metabolism, regulation of synthesis.

LABORATORY WORK

QUANTITATIVE DETERMINATION OF GLUCOSE IN SERUM BY GLUCOSE OXIDASE KIT

Determination of glucose in the blood is used in diagnosis and treatment of carbohydrates metabolism disorders, including hypoglycemic and hyperglycemic conditions. The enzymatic method for determination of glucose is based on application of two enzymes: glucose oxidase and peroxidase.

PRINCIPAL OF THE METHOD. The diagnostic kit contains glucose oxidase and peroxidase. Glucose oxidase is complex FAD-containing enzyme, that catalyses the oxidation of glucose to hydrogen peroxide (H_2O_2) and gluconolactone. The H_2O_2 reacts with chromogen (reduced - colorless) in the presence of peroxidase to form a chromogen (oxidized) - pink. Staining intensity is proportional to glucose concentration and measured photometrically.



STEPS OF WORK:

1) take 3 test tubes, label them and place in a test tube rack,
2) add to each test tube the solutions:

COMPONENTS, ml	CONTROL	STANDARD	SAMPLE
Blood serum	-	-	0,02
Standard of glucose (5,55 mmol/L)	-	0,02	-
H_2O (dist)	0,02	-	-
Working solution of enzymes	2,0	2,0	2,0
Stir and incubate for 20 min at 37ºC			
On completion of incubation read extinction of sample and standard versus control, λ = 500 nm, cuvettes 0,5 cm			

RESULT: E_{st} = ; E_{sample} = ;

Cst = 5,55 mmol/L

Calculation is performed according to the formula:

$$C_{sample} \frac{C_{st} \cdot E_{sample}}{E_{st}} = \quad \text{mmol/L.}$$

DIAGNOSTIC VALUE

Normal values of glucose concentration in serum in adults is **3,33-6,4 mmol/L.**

Increase of glucose content in the blood (**hyperglycemia**) is observed in physiological condition (stress, carbohydrates intake) and pathology: diabetes mellitus, acute pancreatitis, myocardial infarction, hyperfunction of same endocrine glands (thyrotoxicosis, glucagonoma, Icenko-Cushing's syndrome, pheochromocytoma).

Decrease of blood glucose level (**hypoglycemia**) occurs in physiological condition (starvation, insufficiency of diet carbohydrates, hard physical work) and same diseases: insulinoma, Addison's disease, arsenic, phosphorus, benzol, chlorophorm poisoning, disturbances of carbohydrates absorption in GIT, glycogen storage diseases, and overdosage of insulin while treating diabetes mellitus.

CONCLUSION:

_____Teacher’s signature

Date: _____

CLASS № 20

THEME: WAYS FOR GLUCOSE UTILIZATION – I

THEORETICAL PART

1. Aerobic glycolysis: reactions.
   1.1. Pyruvate dehydrogenase complex: components, mechanism of the reaction, regulation, biological role.
   1.2. Energy yield and biological role of aerobic glycolysis.
2. Anaerobic glycolysis: reactions.
   2.1. Reactions of substrate-level phosphorylation and oxidation-reduction reactions in anaerobic glycolysis.
   2.2. Energy yield, biological role and regulation of anaerobic glycolysis.
3. Formation of ethanol during fermentation.
4. Glucuronic acid pathway, scheme, biological role.

LABORATORY WORK
GLUCOSE TOLERANCE TEST

A glucose tolerance test (GTT) is a medical test in which glucose is administered and blood samples taken afterward to determine how the body metabolizes blood sugar. The test is usually used to test for diabetes, Cushing syndrome, insulin resistance, and sometimes reactive hypoglycemia and rarer disorders of carbohydrate metabolism.

How the Test is Performed

1. A patient is instructed not to eat or drink anything after midnight before the test. In addiction he shouldn't eat during the test, and warn his health care provider if he is using medications that can interfere with the test results.
2. For the test, he is asked to drink a liquid containing 1.5g/kg of sucrose, or 1 g/kg of glucose. In practice that will be about 100 g sugar, dissolved in a glass of water.

3. The patient's blood is taken before he/she do this (0 minutes), and again 30 minutes, 1 and 2 hours after he had drunk the solution. As a rule, the test takes up to about 2-3 hours.
4. The glucose concentration in serum is determined in fasting (0 min), 1 and 2 hours after sucrose consumption.

According to the results obtained following graph is drawn. This graph is called glycemic curve:

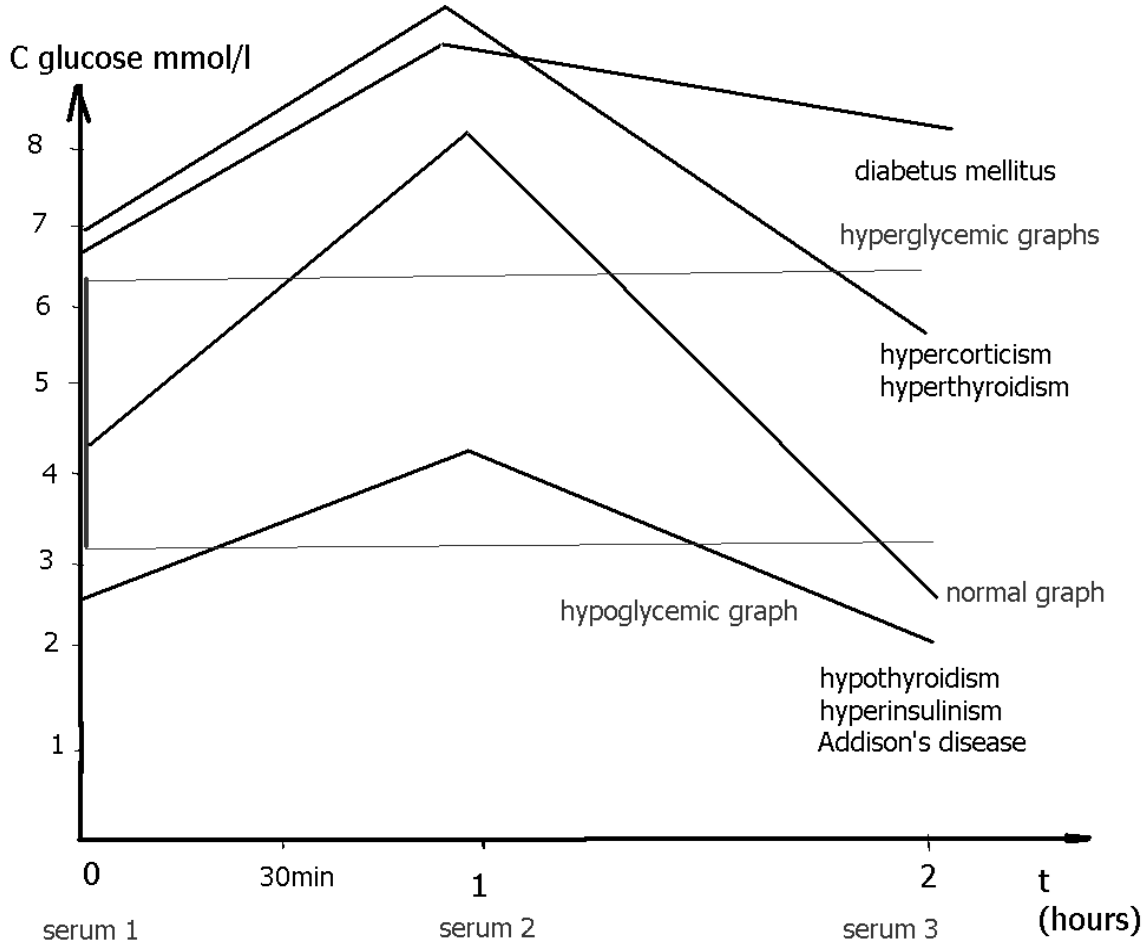


Figure – Examples of glycemic curves

Interpretation of the Glucose Tolerance Test's results

Fasting plasma glucose (measured before the GTT begins) in healthy person should be between 3,3-6,4 mmol/L (110 mg/dL).

Fasting levels between 6.4 and 8.0 mmol/l are borderline ("impaired fasting glycaemia"), and fasting levels repeatedly at or above 8.0 mmol/l are diagnostic of diabetes.

Between 0-1 hour a sucrose breaks up to glucose and fructose in gut, absorbs, and go to blood, causing higher level of glucose in serum. The glucose concentration reaches its maximum for 60 min (Note! No more than 9,9 mmol/L – renal threshold of glucose) and returns to a start point for 120 min (often it becomes lower than initial). The glucose level after 2 hours should not exceed 7.8 mmol/L. Levels between this and 11.1 mmol/L (200 mg/dL) indicate "impaired glucose tolerance." Glucose levels above 11.1 mmol/L (200 mg/dL) at 2 hours confirms a diagnosis of diabetes.

In case of disturbances of carbohydrates metabolism glycemic curves are distinguished from normal (hyper- and hipoglycemic curve).

STEPS OF WORK:

using glucose oxidase method (see previous class) determine concentration of glucose in 3 samples: 1^{st} – fasting levels (0 min), 2^{nd} – after 60 min and 3^{d} - after 120 min of sugar consumption. Draw the glycemic curve.

RESULT:

E_{st} = ; E_{sample} 1= ; E_{sample} 2= ; E_{sample} 3=

Cst = 5,55 mmol/L

C_1= mmol/L; C_2= mmol/L ; C_3= mmol/L

According to the results obtained draw glycemic curve:

C
mmol/L

t (hours)

CONCLUSION:

_____Teacher’s signature

Date: _____

CLASS № 21

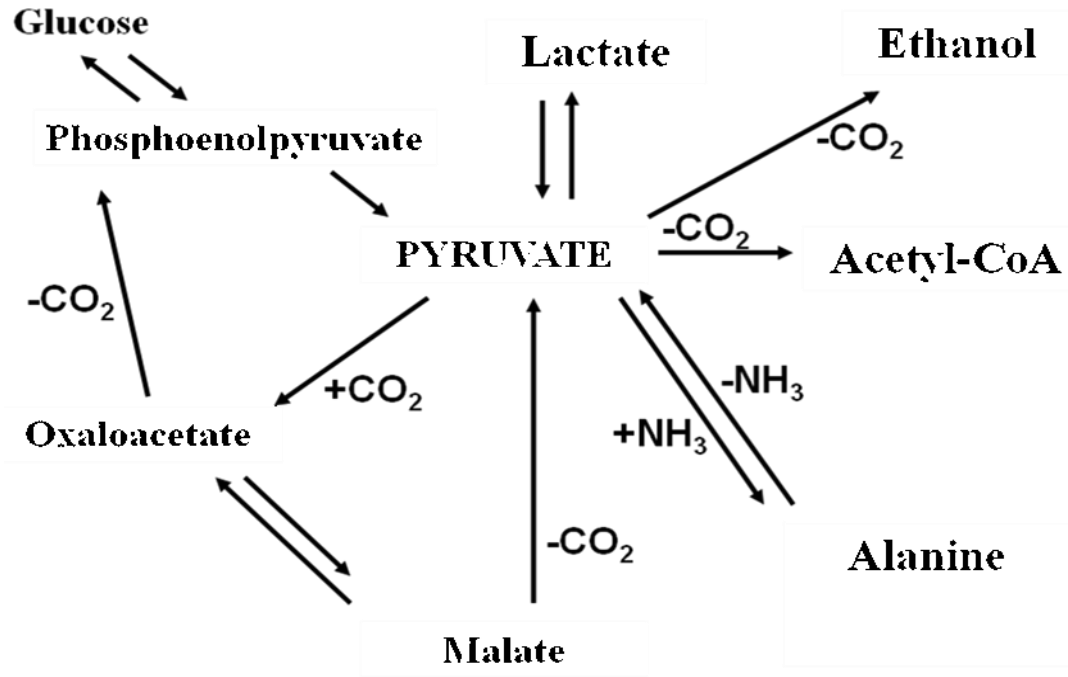
THEME: WAYS FOR GLUCOSE UTILIZATION – II

THEORETICAL PART

1. Scheme of pyruvate metabolism.
2. Metabolism of lactate. Cori's cycle.
3. Gluconeogenesis: metabolic precursors of glucose, scheme, biological role, regulation.
4. Key reactions of gluconeogenesis. Role of biotin.
5. Pentose phosphate pathway: oxidative and non-oxidative reactions, biological role.

LABORATORY WORK
DETERMINATION OF PYRUVATE IN THE URINE

Pyruvate is one of central intermediate products of carbohydrate metabolism and produced in all tissues in great amount. Metabolic pathways of pyruvate illustrate in the scheme:



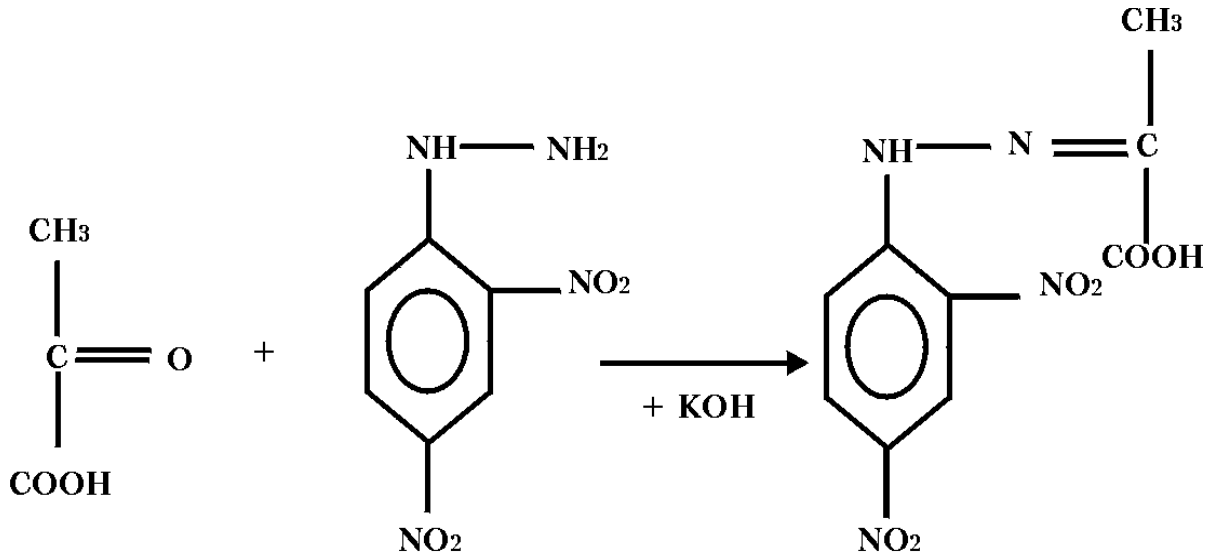
Pyruvate is primarily derived from glucose via glycolysis. Provided that sufficient oxygen is available, pyruvate is converted by pyruvate dehydrogenase into acetyl CoA which enters the citric acid

cycle where is metabolized to produce ATP. Pyruvate can also be converted into carbohydrates via gluconeogenesis or to fatty acid through acetyl CoA.

Recently pyruvate has been used as a dietary supplement to aid in weight loss. Pyruvate can improve exercise endurance capacity, effectively reduce cholesterol, and serves as a potent antioxidant. Therefore, the measurement of its concentration can give valuable information to the progress of specific biochemical reactions.

The lactate/pyruvate ratio reflects state of the cell and describes the balance between NAD^+ and NADH, which is dependent on the interconversion of lactate and pyruvate via lactate dehydrogenase.

PRINCIPLE OF THE METHOD. Pyruvate interacts with 2,4-dinitrophenylhydrasine in alkaline medium to form 2,4-dinitrophenylhydrasone derivatives of yellow-orange color, the staining intensity of which is proportional to concentration of pyruvate.



2,4-dinitrophenylhydrasine 2,4-dinitrophenylhydrasone

STEPS OF WORK:

1) take 3 test tubes, label them and place in a test tube rack,
2) add to each test tube the solutions:

COMPONENTS, ml	SAMPLE	CONTROL

Urine	1,0	-
H_2O	-	1,0
KOH (25 %, ethanol solution)	1,0	1,0
	Shake 1 min	
2,4-Dinitrophenylhydrasine (0,1%)	0,5	0,5
	Shake and leave for 15 min at room temperature	
	Read extinction of sample versus control, λ = 470-480 nm, cuvettes 0,5 sm	

RESULT:

E = .

Calculation is performed according to calibration graph. The received result (in μg) should be multiplied by empiric coefficient 11,366.

C = ……. x 11,366 = μmol/day.

DIAGNOSTIC VALUE

Normal value of pyruvate excretion in urine is **114-284 μmol/day (10-25 μg/day).** In the blood – **56,8-113,6 μmol/L.**

Abnormal blood pyruvate levels are reported in a number of disorders including shock, liver disease, congestive heart failure, diabetes mellitus, thiamine deficiency, metabolic disorders, obesity.

The pyruvate excretion increases in the states followed by hypovitaminosis of B_1 (pyruvate dehydrogenase deficiency**)**, in diabetes mellitus, cardiac insufficiency, hyperfunction of the hypophysis-adrenal system.

The lower pyruvate excretion may be determined in anesthetized patients.

CONCLUSION:

_____Teacher’s signature

Date _____

CLASS № 22

THEME: METABOLISM OF GLYCOGEN. REGULATUION OF CARBOHYDRATE METABOLISM

THEORETICAL PART

1. Physiological role of glycogen. Synthesis of glycogen, regulation.
2. Degradation of glycogen, regulation.
3. Disorders of glycogen metabolism: glycogenoses, aglycogenoses.
4. Glycemia, regulation of glycemia.
5. Hyperglycemia and hypoglycemia, their causes.
6. Disorders of carbohydrate metabolism in diabetes mellitus.

MCQ "METABOLISM OF CARBOHYDRATES"

STUDENTS' INDIVIDUAL WORK
"METABOLISM OF CARBOHYDRATES"

Assignments for the individual work

1. Compose metabolic scheme of carbohydrates metabolism.
   1.1. Mark diagnostically important substrates, that are measured in blood or urine (glucose, pyruvate, lactate, glycogen, galactose, fructose).
   1.2. Mark diagnostically important enzymes, that are measured in blood (glucose-6-phoshatase, fructose-1,6-diphosphate aldolase, lactate dehydrogenase (LDH), glycogen phosphorylase, glucose-6-phoshate dehydrogenase).
   1.3. Highlight the regulatory enzymes of:
   - glycolysis - hexokinase, 6-phosphofructokinase, pyruvate kinase, PDH complex
   - gluconeogenesis – pyruvate carboxylase, PEP-carboxykinase, fructose-1,6-diphosphatase, glucose-6-phosphatase.
   - synthesis of glycogen – glycogen synthase
   - degradation of glycogen – glycogen phosphorylase
   - pentose phosphate pathway – glucose-6-phosphate dehydrogenase.

   1.4. Mark vitamin-dependent reactions.

CLASS № 23

MINI-EXAM «METABOLISM OF CARBOHYDRATES»

1. Carbohydrates: general characteristics and classification, biological functions, contents in human tissues.
2. Dietary carbohydrates, their characteristics.
3. Digestion and absorption of carbohydrates in the gastrointestinal tract. Lactose intolerance.
4. The general scheme of pathways of glucose metabolism and their estimation.
5. Reactions of glucose phosphorylation and dephosphorylation of glucose 6-phosphate. Regulation.
6. Galactose metabolism. Galactosemia.
7. Fructose metabolism. Essential fructosuria, hereditary fructose intolerance.
8. Lactose metabolism, regulation of synthesis.
9. Aerobic glycolysis: reactions.
10. Pyruvate dehydrogenase complex: components, mechanism of the reaction, regulation, biological role.
11. Energy yield and biological role of aerobic glycolysis.
12. Anaerobic glycolysis: reactions.
    - 12.1. Reactions of substrate-level phosphorylation and oxidation-reduction reactions in anaerobic glycolysis.
    - 12.2. Energy yield, biological role and regulation of anaerobic glycolysis.
13. Formation of ethanol during fermentation: reactions.
14. Scheme of pyruvate metabolism. Diagnostic value of pyruvate determination in the blood and urine.
15. Metabolism of lactate, Cori’s cycle.
16. Gluconeogenesis: metabolic precursors of glucose, scheme, biological role, regulation.
17. Key reactions of gluconeogenesis. Role of biotin.
18. Pentose phosphate pathway: oxidative and non-oxidative reactions, biological role.
19. Glucuronic acid pathway, major reactions, biological role.
20. Physiological role of glycogen, synthesis of glycogen, regulation.
21. Degradation of glycogen, regulation.
22. Disorders of glycogen metabolism: glycogenoses, aglycogenoses.
23. Glycemia, regulation of glycemia.

24. Hyperglycemia and hypoglycemia, their causes.
25. Disorders of carbohydrate metabolism in diabetes mellitus.
26. Methods for determination of glucose in the blood serum. Diagnostic value.
27. Glucose tolerance test.

CLASS № 24

THEME: LIPIDS OF PHYSIOLOGICAL SIGNIFICANCE

THEORETICAL PART

1. Classification of lipids. Lipids of human tissues. Biological functions of lipids.
2. Dietary lipids, polyunsaturated fatty acids – essential nutrients. Digestion and absorption of lipids in the gastrointestinal tract (emulsification, enzymatic hydrolysis, formation of micelles). Role of bile acids.
3. Re-synthesis of triacylglycerols in the intestinal wall. Formation of chylomicrons. Composition and metabolism of chylomicrons.
4. Intracellular lipolysis. Hormonal regulation of triacylglycerols mobilization (lipolysis) in adipose tissue.
5. Fatty acids of human tissues: classification, representatives. Activation of fatty acids, transport of acyl CoA into mitochondrion. Role of carnitine.
6. β-Oxidation of fatty acids: reactions, energy production of β-oxidation, biological role.
7. Oxidation of odd-chain fatty acids.
8. Lipid peroxidation.

LABORATORY WORK

DETERMINATION OF TRIACYLGLYCEROLS IN THE BLOOD SERUM

Triacylglycerols (TAG) are fatty acid esters of glycerol and represent the main lipid component of dietary fat and fat depots of animals. TAG, being nonpolar lipid substances (insoluble in water), need to be transported in the blood associated with various lipoprotein particles.

PRINCIPLE OF THE METHOD. TAG are hydrolyzed by lipoprotein lipase to produce glycerol and free fatty acids. The glycerol participates in a series of coupled enzymatic reactions, in which glycerol kinase and glycerol phosphate oxidase are involved and H_2O_2 is generated. The H_2O_2 reacts with chromogen (reduced – colorless) in the presence of peroxidase to form a chromogen dye

(oxidized). The intensity of color (pink) formed is proportional to the TAG concentration and can be measured photometrically.

STEPS OF WORK:

1) take 3 test tubes, label them and place in a test tube rack,
2) add to each test tube the solutions:

COMPONENTS, ml	CONTROL	STANDARD	SAMPLE
Blood serum	-	-	0,02
Standard of TAG (2,5 mmol/l)	-	0,02	-
H_2O (dist)	0,02	-	
Working solution	2,0	2,0	2,0
Stir and incubate for 10 min at 37ºC			
On completion of incubation read extinction of sample and standard versus control, λ = 500 nm, cuvettes 0,5 cm			

RESULT:

E st = ; Esample = ; Cst = 2,5 mmol/L

Calculation is performed according to the formula:

$$Csample = \frac{Cst \cdot Esample}{Est} = \text{mmol/L.}$$

From the received result 0,11 mmol/L should be subtracted (concentration of free glycerol in the blood).

DIAGNOSTIC VALUE

Normal values of TAG in the blood are **0,40-1,54 mmol/L (female), 0,45-1,82 mmol/L (male)**.

Reasons of **hypertriacylglycerolemia:** high-fat food intake, obesity, starvation, diabetes mellitus, cirrhosis, glicogenosis type I, III and IV, excessive alcohol intake.

Hypotriacylglycerolemia is observed in the absence of synthesis of apoprotein B (apoB) in the liver.

CONCLUSION:

_____Teacher’s signature

Date: _____

CLASS № 25

THEME: METABOLISM OF FATTY ACIDS. KETONE BODIES

THEORETICAL PART

1. Reactions of synthesis and utilization of ketone bodies. Their biological role.
2. Mechanism of ketosis in diabetes mellitus and starvation. Ketoacidosis.
3. Biosynthesis of fatty acids:
   - 3.1. Sources of acetyl CoA and NADPH in the cytoplasm;
   - 3.2. Synthesis of malonyl CoA;
   - 3.3. Fatty acid synthase: structure;
   - 3.4. Biosynthesis of palmitic acid: reactions.
4. Biosynthesis of triacylglycerols.
5. Biosynthesis of phospholipids.
6. Fatty infiltration of the liver. Lipotropic agents.

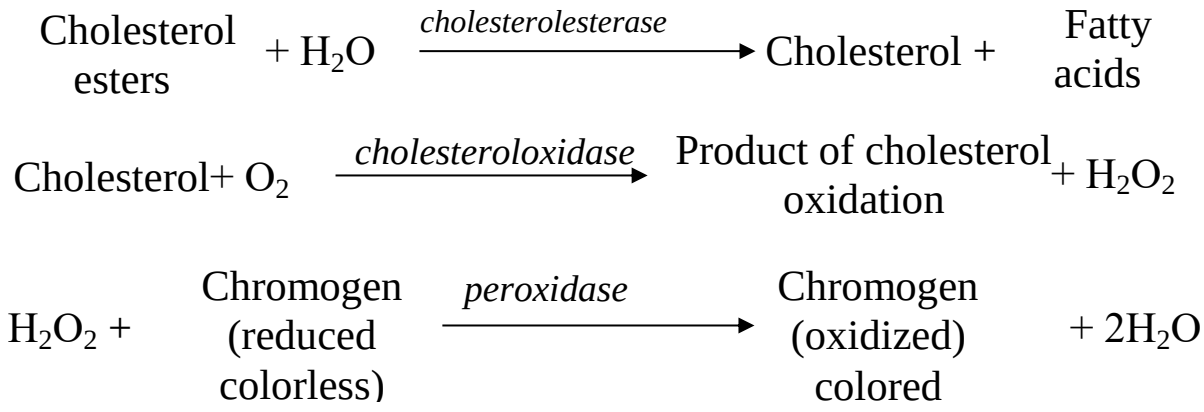
LABORATORY WORK

DETERMINATION OF TOTAL CHOLESTEROL IN BLOOD SERUM BY ENZYMATIC METHOD

Cholesterol is an unsaturated alcohol of the steroid family of compounds; it is essential for the normal function of all animal cells and is a fundamental element of their cell membranes. It is also a precursor of various critical substances such as adrenal and gonadal steroid hormones, bile acids and vitamin D. Since cholesterol is insoluble in water, it is transported in the blood plasma within lipoproteins. Total cholesterol is defined as the sum of HDL, LDL, and VLDL.

PRINCIPLE OF THE METHOD.

Cholesterol esters are hydrolyzed to free cholesterol by cholesterol ester hydrolase. The free cholesterol produced is oxidized by cholesterol oxidase to cholest-4-en-3-one with the simultaneous production of hydrogen peroxide. The H_2O_2 reacts with chromogen (redused – colorless) in the presence of peroxidase to form an oxidized chromogen dye:



The intensity of color (pink) formed is proportional to the cholesterol concentration and can be measured photometrically:

STEPS OF WORK:

1) take 3 test tubes, label them and place in a test tube rack,
2) add to each test tube the solutions:

COMPONENTS, ml	CONTROL	STANDARD	SAMPLE
Blood serum	-	-	0,02
Standard of cholesterol (5,17 mmol/L)	-	0,02	-
Water (dist)	0,02	-	
Working solution of enzymes and chromogen	2,0	2,0	2,0
Stir and incubate for 10 min at 37ºC			
On completion of incubation read extinction of sample and standard versus control, λ = 500 nm, cuvettes 0,5 cm			

RESULT: E st = ; E sample = ; Cst = 5,17 mmol/L

Calculation is performed according to the formula:

$$C_{sample} = \frac{C_{st} \cdot E_{sample}}{E_{st}} = \text{mmol/L.}$$

DIAGNOSTIC VALUE

Normal value of total cholesterol in blood is **3,6-5,2 mmol/L.** The increased plasma cholesterol level (**hypercholesterolemia**) is observed in atherosclerosis, diabetes mellitus, mechanic and parenchymatous jaundice, nephritis, hypothyroidism. Decrease of plasma cholesterol level (**hypocholesterolemia**) is observed in anemias, fasting, tuberculosis, hyperthyroidism, cancerous cachexia, feverish states

CONCLUSION:

_____Teacher's signature

Date: _____

CLASS № 26

METABOLISM OF CHOLESTEROL AND COMPLEX LIPIDS

THEORETICAL PART

1. Metabolism of cholesterol in the body.
2. Biosynthesis of cholesterol: main steps, scheme. Regulation of cholesterol synthesis.
3. Initial reactions of cholesterol biosynthesis.
4. Bile acids: representatives, structure, metabolism, biological functions.
5. Metabolism of sphingolipids. Disorders of sphingolipid metabolism.
6. Transport of lipids and fatty acids in the blood. Role of albumins. General characteristics of lipoproteins.
7. Metabolism of lipoproteins: formation and utilization. Lipoprotein lipase. Role of apoproteins.

LABORATORY WORK
DETERMINATION OF LOW DENSITY LIPOPROTEINS (LDL) IN THE BLOOD SERUM

LDL is one of the five major groups of lipoproteins. Lipoprotein molecules enable the transportation of lipids, within the water around cells including the bloodstream

PRINCIPLE OF THE METHOD. The method is based on the ability of LDL to sediment in the presence of calcium chloride and heparin; the solution becomes turbid. Concentration of LDL in plasma is determined by the degree of solution turbidity.

STEPS OF WORK: take a test tube, add the solutions:

	Experimental sample
Blood serum	0,2 ml
$CaCl_2$ (0,27%)	2 ml
	Stir and measure read extinction of sample versus water (E_1), λ = 630 nm, cuvettes 0,5 cm
	Put back mixture in to test tube
Heparin	0,04 ml
	Stir, incubate 4 min (exactly!) and measure extinction (E_2) under the same condition

RESULT:

E_1 = ;
E_2 = .

Concentration of LDL = (E_2 – E_1) x 10 = g/L

DIAGNOSTIC VALUE

Normal value of LDL in blood serum is **2-4 g/L**.

The increased level of plasma LDL is observed in atherosclerosis, diabetes mellitus, obesity, hepatitis, hypothyroidism hyperlipoproteinemias (type II).

CONCLUSION:

_____Teacher's signature

*Date*_____

CLASS № 27

THEME: DISTURBANCES OF LIPID METABOLISM

THEORETICAL PART

1. Disorders in digestion and absorption of lipids in the gastrointestinal tract.
2. Dislipoproteinemias: hyper- and hypolipoproteinemias.
3. Hypercholesterolemia and atherosclerosis. Biochemical principles of treatment.
4. Cholelithiasis. Formation of cholesterol gall stones.
5. Obesity: disturbances of lipid metabolism.

MCQ "METABOLISM OF LIPIS"

STUDENTS' INDIVIDUAL WORK
"METABOLISM OF LIPIS"

Assignments for the individual work

1. Compose metabolic scheme of lipids metabolism.
   1.1. Mark diagnostically important substrates, that are measured in blood or urine: triacylglycerols, cholesterol, lipoproteins, ketone bodies.
   1.2. Mark diagnostically important enzymes, that are measured in blood: lipase, lipoproteinlipase.
   1.3. Highlight the regulatory enzymes of: β-oxidation of FA (carnitine-acyl transferase-I), biosynthesis of fatty acids (acetyl-CoA carboxylase), biosynthesis of cholesterol (HMG-CoA-reductase), lipolysis (TAG-lipase).
   1.4. Mark vitamin-dependent reactions

CLASS № 28

MINI-EXAM «METABOLISM OF LIPIDS»

1. Classification of lipids. Lipids of human tissues. Biological functions of lipids.
2. Dietary lipids, polyunsaturated fatty acids – essential nutrients. Digestion and absorption of lipids in the gastrointestinal tract (emulsification, enzymatic hydrolysis, formation of micelles). Role of bile acids.
3. Resynthesis of triacylglycerols in the intestinal wall. Formation of chylomicrons. Composition and metabolism of chylomicrons.
4. Intracellular lipolysis. Hormonal regulation of triacylglycerols mobilization (lipolysis) in adipose tissue.
5. Fatty acids of human tissues: classification, representatives. Activation of fatty acids, transport of acyl CoA into mitochondrion. Role of carnitine.
6. β-Oxidation of fatty acids: reactions, energy production of β-oxidation, biological role.
7. Oxidation of odd-chain fatty acids.
8. Reactions of synthesis and utilization of ketone bodies. Their biological role.
9. Mechanism of ketosis in diabetes mellitus and starvation. Ketoacidosis.
10. Sources of acetyl CoA and NADPH for fatty acids biosynthesis. Synthesis of malonyl CoA.
11. Biosynthesis of palmitic acid: reactions. Fatty acid synthase.
12. Biosynthesis of triacylglycerols.
13. Biosynthesis of phospholipids.
14. Fatty infiltration of the liver. Lipotropic agents.
15. Metabolism of cholesterol in the body.
16. Biosynthesis of cholesterol: main steps, scheme. Regulation of cholesterol synthesis.
17. Initial reactions of cholesterol biosynthesis.
18. Bile acids: representatives, structure, metabolism, biological functions.
19. Metabolism of sphingolipids. Disorders of sphingolipid metabolism.

20. Transport of lipids and fatty acids in the blood. Role of albumins. General characteristics of lipoproteins.
21. Metabolism of lipoproteins: formation and utilization. Lipoprotein lipase. Role of apoproteins.
22. Disorders in digestion and absorption of lipids in the gastrointestinal tract.
23. Dislipoproteinemias: hyper- and hypolipoproteinemias.
24. Hypercholesterolemia and atherosclerosis. Biochemical principles of treatment.
25. Cholelithiasis. Formation of cholesterol gall stones.
26. Obesity: disturbances of lipid metabolism.
27. Major lipid components of the blood serum. Diagnostic value.

Date: _____

CLASS № 29

THEME*: *HORMONE ACTION MECHANISM

THEORETICAL PART

1. General characteristics of hormones: properties, types of biological action. Classification of hormones on the chemical structure, site of formation, mechanism of action. Target tissues and the cell receptors of hormones.
2. Mechanisms of action of hormones binding with the membrane receptors. Second messengers: cyclic purine nucleotides, calcium ions, products of hydrolysis of phosphatidylinositol. Diversity of protein kinases and their role in transmission of hormonal signal.
3. Mechanism of action of hormones binding with the intracellular receptors.
4. Thyroid hormones: structure, target tissues, biological effects. Hyper- and hypoproduction of the hormones.
5. Parathyroid hormone, calcitonin: target tissues, biological effects. Hyper- and hypofunction of parathyroid hormone.
6. Pancreatic hormones: insulin, glucagon. Target tissues, biological effects. Hyper- and hypoproduction of the hormones.
7. Adrenaline and noradrenaline: structure, target tissues, biological effects. Hyperproduction of adrenaline.

LABORATORY WORK

DETECTION OF ADRENALIN IN THE URINE (QUALITATIVE REACTION ON ADRENALIN)

In the adrenal medulla, catecholamines are synthesized from amino acid tyrosine. In the liver and muscles, adrenaline and noradrenaline activate phosphorylase which degrades glycogen. In the adipose tissue, the hormones activate degradation of triacylglycerols. Release of adrenalin and noradrenalin from the adrenal medulla is part of the fight-or-flight response.

PRINCIPLE OF THE METHOD. The molecule of both adrenaline and noradrenaline contains pyrocatechin ring which forms product of green colour reacting with $FeCl_3$. In subsequent adding NaOH the solution obtains red-brown colour.

STEPS OF WORK:

COMPONENTS	CONTROL	TEST
H_2O	10 drops	-
Solution of adrenaline	-	10 drops
$FeCl_3$	1 drop	1 drop
	Light-yellow colour	Green colour
NaOH , 10 %	3 drops	3 drops
	Colour is not changed	Red-brown colour

RESULT:

DIAGNOSTIC VALUE

Adrenaline may be quantified in blood, plasma, or serum as a diagnostic aid, to monitor therapeutic administration, or to identify the causative agent in a potential poisoning victim.

Endogenous plasma adrenaline concentrations in resting adults are normally less than 10 ng/L, but may increase by 10-fold during exercise and by 50-fold or more during times of stress.

Normal concentration of adrenaline in the blood serum is not **more than 6.28 nmol/L**, the excretion in the urine is **27,3 – 81,9 nmol/day.**

Increased excretion of adrenaline is observed in pheochromocytoma, hypertension stroke, acute period of myocardial infarction, hepatitis and liver cirrhosis, physical exertion and emotional stress reaction.

Pheochromocytoma patients often have plasma adrenaline levels of 1000-10,000 ng/L. Parenteral administration of adrenaline to acute-care cardiac patients can produce plasma concentrations of 10,000 to 100,000 ng/L.

CONCLUSION:

_____Teacher’s signature

Date: _____

CLASS № 30

THEME: BIOLOGICAL ACTION OF HORMONES

THEORETICAL PART

1. Glucocorticoids: structure of cortisol, target tissues, effects on metabolism and functions. Hyper- and hypoproduction of the hormones.
2. Mineralocorticoids: structure of aldosterone, target tissues, biological effects. Hyper- and hypoproduction of the hormones.
3. Female sex hormones: structure of estradiol and progesterone, target tissues, effects on metabolism and functions. Hyper- and hypoproduction of the hormones.
4. Male sex hormones: structure of testosterone, target tissues, effects on metabolism and functions**.** Hyper- and hypoproduction of the hormones.
5. Hormones of hypothalamus and hypophysis, their biological action. Growth hormone, adrenocorticotropic hormone: target tissues, effects on metabolism. Hyper- and hypoproduction of growth hormone.
6. Eicosanoids (prostaglandins, thromboxanes, leukotrienes) and their role in the regulation of metabolism and functions.
7. Disorders of functions of endocrine glands: hyper- and hypoproduction of hormones.

STUDENTS' INDIVIDUAL WORK
"CHARACTERISTICS OF THE MAJOR HORMONES"

Assignments for the individual work

Fill in the table for following hormones: **thyroxin, insulin, glucagon, glucocorticoids, mineralocorticoids, parathyroid hormone, calcitonin, male and female sex hormones, Growth hormone, adrenocorticotropic hormone.**

Hormone	Chemical structure	Site of synthesis	Mechanism of action	Target-tissue

Biological effect	**Hypoproduction of the hormone**		**Hyperproduction of the hornmone**	
	Name	Signs	Name	Signs

Hormone	Chemical structure	Site of synthesis	Mechanism of action	Target-tissue

Biological effect	**Hypoproduction of the hormone**		**Hyperproduction of the hornmone**	
	Name	Signs	Name	Signs

Date: _____

CLASS № 31

THEME: BIOCHEMISTRY OF NUTRITION. VITAMINS

THEORETICAL PART

1. Components of human's food. The significance of nutrition for the vital activity. Essential food components. Causes and biochemical characteristics of malnutrition syndrome
2. Pathological states related to nutrition disorders: protein-energy malnutrition – kwashiorkor, marasmus; causes and disturbance of metabolism.
3. Vitamins, general characteristics, classification, biological functions. Vitamin-like substances.
4. Sources of vitamins for a human. Causes of hypo- and hypervitaminoses. Role of microflora of large intestine in synthesis of some vitamins.
5. Fat-soluble vitamins: A, D, E, and K, biological role, daily requirements, dietary sources, symptoms of deficiency. Hypervitaminosis of some vitamins.
6. Water-soluble vitamins: B_1, B_2, B_6, B_{12}, PP, C, pantothenic acid, biotin, folic acid, biological role, daily requirements, dietary sources, symptoms of deficiency.
7. The use of vitamins in clinical practice. Polyvitamin medications.
8. Anti-vitamins, mechanism of action, representatives, their application in medical practice and scientific investigations.

Formulas for memorizing: structure of vitamins A, D, B_1, B_2, B_6, PP, C, and pantothenic acid.

LABORATORY WORK

QUANTITATIVE DETERMINATION OF THE VITAMIN C IN THE URINE

Vitamin C (ascorbic acid) is an essential nutrient for humans. Vitamin C is a cofactor in at least eight enzymatic reactions, including several collagen synthesis reactions that, when dysfunctional, cause the most severe symptoms of scury. Ascorbate may also act as an antioxidant.

Vitamin C participates:

- in the oxidative-reduction processes,
- in the synthesis of steroid hormones and catecholamines in adrenals,
- appears to be coenzyme of hydroxylases catalyzing conversion of proline into hydroxyproline, which are sued in the synthesis of collagen
- furthers absorption of iron from the intestine,
- activates pepsinogen in the stomach.
- Interacts with the immune system through modulation of the activities of phagocytes, lymphocytes.

Vitamin C deficiency in the organism leads to the impairment of these processes.

PRINCIPLE OF THE METHOD. Vitamin C is capable of reducing 2,6-dichlorophenolindophenol (2,6-DCPIP). This chromogen when oxidized is colored (dark-blue colour in alkaline medium and red colour in acidic medium). When reduced, the chromogen becomes colorless. Reaction mixture is titrated by 2,6-dichlorophenolindophenol in the acidic medium until the light-red colour appears.

STEPS OF WORK:

COMPONENTS	TEST SAMPLE
U r i n e	10.0 ml
H_2O	10.0 ml
HCl 10%	20 drops
2,6-DCPIP 0,001N	Titrate until the light-red colour is appeared

RESULT: A =

CALCULATION:

$$X = \frac{0{,}088 \cdot A \cdot 1500}{10} = \quad \text{mg/day},$$

where 0,088 – amount of vitamin C (mg) corresponds to 1ml 0,001N 2,6-DCPIP;

A – result of titration, ml;

1500 – average daily diuresis, ml;

10 – the volume of the urine taken for titration, ml.

DIAGNOSTIC VALUE

Normal content of vitamin C in the blood serum is **34-114 µmol/L**. Normal excretion in the urine is **20-30 mg/day.**

Determination of vitamin C in the urine allows assessing the content of vitamin C in the organism, for there is normally proportion between concentration of the vitamin in the blood serum and amount of the vitamin excreted in the urine. However, in hypovitaminosis C, the content of ascorbic acid in the urine is not always decreased. Often it is normal despite the appreciable deficiency of the vitamin in tissues.

In healthy people administration of 100 mg of vitamin C *per os* results in the increase of its concentration in the both blood serum and urine. In hypovitaminosis C, tissues being experienced need for the vitamin retain administered load of ascorbic acid and its concentration in the urine is not increased.

Concentration of vitamin C in the urine is decreased in acute and chronic infectious diseases, anemia, steatorrhea, malabsorption, alcoholism.

CONCLUSION:

_____Teacher's signature

*Date*_____

CLASS № 32

THEME*: *BIOCHEMISTRY OF THE BLOOD

THEORETICAL PART

1. Blood, general characteristics and functions.
2. Specific features of chemical composition, structure and metabolism of blood cells.
3. Hemoglobin, structure, derivatives. Variants of hemoglobin in ontogenesis. Hemoglobinopathies.
4. Transport of oxygen and carbon dioxide in blood.
5. Metabolism of iron. Transferrin and ferritin. Iron deficiency anemia.
6. Plasma proteins: their classification and characteristics.
7. Hemostasis. Blood coagulation factors. Intrinsic and extrinsic pathways of blood coagulation. Scheme of blood coagulation. Role of vitamin K in blood coagulation.
8. Anticoagulant and fibrinolytic system. Disorders of coagulation and anticoagulation systems. Thrombosis and hemophilia.
9. Biochemical analysis of blood, major laboratory results, use for diagnosis.

LABORATORY WORK № 1.
DETERMINATION OF HEMOGLOBIN CONCENTRATION IN THE BLOOD

Hemoglobin concentration measurement is among the most commonly performed blood tests, usually as part of a complete blood count. Hemoglobin determination, or hemoglobinometry, is the measurement of the concentration of hemoglobin in the blood. Hemoglobin's main function in the body is to carry oxygen from the lungs to the tissues and to assist in transporting carbon dioxide from the tissues to the lungs. The formation of hemoglobin takes place in the developing red cells located in bone marrow.

Hemoglobin values are affected by age, sex, pregnancy, disease, and altitude. During pregnancy, gains in body fluids cause the red cells to become less concentrated, causing the red cell count to fall. Since hemoglobin is contained in red cells, the hemoglobin concentration also falls. Disease may also affect the values of

hemoglobin. For example, iron deficiency anemia may drop hemoglobin values from a normal value Above-normal hemoglobin values may occur when dehydration develops. Changes in altitude affect the oxygen content of the air and, therefore, also affect hemoglobin values. At higher altitudes there is less oxygen in the air, resulting in an increase in red cell counts and hemoglobin values. At lower altitudes there is more oxygen, resulting in a decrease in red cell counts and hemoglobin values.

PRINCIPLE OF THE METHOD: hemoglobin oxidized by $K_3[Fe(CN)_6]$ to hemiglobin. Hemiglobin react with acetoanhydrine with formation of coloured hemiglobincyanide. Concentration of hemiglobincyanide is detected photometrycally.

STEPS OF WORK: take test tube

COMPONENTS	Sample
Blood	0,02 ml
Reagent	5,0 ml

Stir and incubate10 min,

read the extinction of sample versus the control (reagent) at 540 nm, cuvette 1 cm

RESULT:

E_s =

Calculation is performed according to the formula:

C = E_s x 392 = g/L

DIAGNOSTIC IMPORTANCE

Normal concentration of hemoglobin in the blood is **115-145 g/L (female), 130-160 g/L (male).**

Increased concentration of hemoglobin is observed hypohydration, tissue hypoxia, ulcer disease, in neonates within first hours of life.

Decreased concentration of hemoglobin is observed in anemias, hemoglobinopathies, deficiency of vitamins B_{12}, E and folic acid.

CONCLUSION:

LABORATORY WORK № 2

DETERMINATION OF CALCIUM CONCENTRATION IN THE BLOOD SERUM

Calcium is a mineral that is important in the regulation and processes of many body functions including bone formation, hormone release, muscle contraction, and nerve and brain function. Calcium levels are tightly regulated in the body. Calcium regulation is primarily controlled by parathyroid hormone (PTH), vitamin D, and calcitonin.

- Parathyroid hormone is a hormone produced by the parathyroid glands, which are four small glands that surround the thyroid and are found in the anterior part of the lower neck.
- Vitamin D is obtained through a process that begins with sun exposure to the skin, the process then continues in the liver and kidneys. Vitamin D can also be found in foods such as eggs and dairy products.
- Calcitonin is produced in specialized cells in the thyroid gland.

Together, these three hormones act on the bones, the kidneys, and the GI tract to regulate calcium. One of the most common causes of high

calcium levels (hypercalcemia), is an overproduction of parathyroid hormone, or hyperparathyroidism.

PRINCIPLE OF THE METHOD: calcium in alkaline medium reacts with glioxalbis (2-hydroxyanil) (GBHA) to produce dye. Intensity of colour is directly depends on concentration of calcium.

STEPS OF WORK:

STEPS OF WORK:

1) take 3 test tubes, label them and place in a test tube rack,
2) add to each test tube the solutions:

COMPONENTS, ml	CONTROL	STANDARI	SAMPLE
Reagent	1,0	1,0	1,0
H_2O	0,01	-	-
Ca, standard solution	-	0,01	-
Blood serum	-	-	0,01

Stir and incubate 5 min at 18°C.

Read the extinction of samples versus the control at λ = 574 nm, 0,5 cm cuvettes (colour is stable 5-12 min).

RESULT:

E_s =

E_{st} =

CALCULATIONS: $C_s = \frac{E_s}{E_{st}} \cdot 2,5 =$ mmol/L

DIAGNOSTIC IMPORTANCE

Normal concentration of calcium in the blood is **2,25-2,75 mmol/L.**

Physiologic **hypercalcemia** is observed in neonates, in some persons after mails. Pathologic **hypercalcemia** is observed in hyperparathyrosis (calcium is washed out of the bones into the blood plasma), acromegaly, myeloma disease, thyrotoxicosis, cancer with bones destruction, cancer of lung, kidney, pancreas, liver.

Hypocalcemia is observed in deficiency of vitamin D, hypoparathyrosis, nephrosis, chronic renal insufficiency, glomerulonephritis, cirrhosis, acute pancreatitis.

CONCLUSION:

_____Teacher's signature

*Date*_____

CLASS № 33

THEME: BIOCHEMISTRY OF THE LIVER

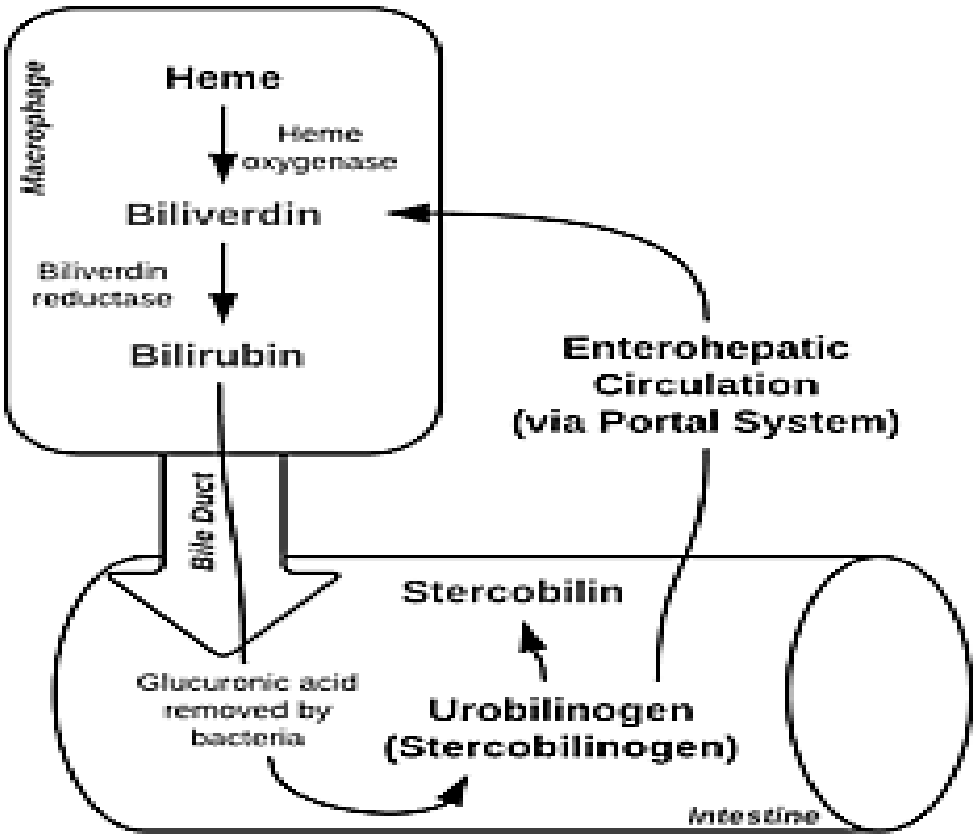
THEORETICAL PART

1. Role of the liver in carbohydrate, lipid, amino acid and protein metabolism.
2. Detoxification functions of the liver.
3. Heme synthesis, reactions.
4. Degradation of heme. Bilirubin metabolism, scheme.
5. Disorders in bilirubin metabolism: jaundice, its types.
6. Biochemical mechanisms of hepatic failure and hepatic coma. Biochemical tests for diagnosis of liver disorders.

LABORATORY WORK
QUANTITATIVE DETERMINATION OF THE BILIRUBIN IN THE BLOOD SERUM

When hemoglobin is destroyed in the body, heme is also degraded, mainly in the reticuloendothelial cells of the liver, spleen, and bone marrow. Indirect bilirubin (the end product of heme degradation) formed in peripheral tissues is transported to the liver by plasma albumin. In the liver indirect bilirubin is detoxified by conjugating with glucuronic acid.

The blood serum naturally contains two types of bilirubin: indirect and direct. Indirect bilirubin is also called unconjugated, or “free”, and is easily adsorbed by plasma proteins. These proteins required to be precipitated with ethanol in order to allow the indirect bilirubin to react with diazo reagent (indirect reaction). The direct (conjugated) bilirubin does not require addition of ethanol (direct reaction). Both indirect and direct bilirubin form “total” bilirubin.



Disturbances of bilirubin metabolism lead to development of jaundice, or yellow discoloration of the skin, sclerae, and mucous membrane. Determination of total, direct and indirect bilirubin in the blood serum is used in differential diagnosis for jaundice of different types.

PRINCIPLE OF THE METHOD: diazo reagent interacts with direct bilirubin with formation of pink dye. Indirect bilirubin is insoluble in water and gives indirect reaction with diazo reagent, that is, the reaction produces a specific colour after preliminary treatment.

STEPS OF WORK:

1) take 3 test tubes, label them and place in a test tube rack,
2) add to each test tube the solutions:

	Volume, ml		
	CONTROL	TOTAL BILIRUBIN	DIRECT BILIRUBIN
Serum	0,5	0,5	0,5
Caffein reagent	1,75	1,75	–
NaCl, 0,9%	0,25	–	1,75
Diazo reagent	–	0,25	0,25

Stir and incubate at room temperature:

DIRECT BILIRUBIN – 10 min after diazo reagent adding.
Read the extinction of samples versus the control at λ = 500-600 nm, 0,5 cm cuvettes

E_{direct} =

TOTAL BILIRUBIN – 20 min after diazo reagent adding.
Read the extinction of samples versus the control at λ = 500-600 nm, 0,5 cm cuvettes

E_{total} =

RESULTS:

$C_{direct\ bilirubin} = E_{direct} \cdot 222,3$ μmol/L =

$C_{total\ bilirubin} = E_{total} \cdot 222,3$ μmol/L =

$C_{undirect\ bilirubin} = C_{total\ bilirubin} - C_{direct\ bilirubin}$ = μmol/L

DIAGNOSTIC IMPORTANCE

Normal level of total bilirubin in blood serum **5,0-20,5 μmol/L**; conjugated bilirubin (direct) – **1,0-7,5 μmol/L**.

The increased level of total bilirubin is observed in neonatal jaundice, liver dysfunction (toxin- or inflammation-induced), blockage of the bile ducts, hemolytic anemias.

In **parenchymatous (hepatic)** jaundice:
- in the blood increased content of direct and indirect bilirubin;
- in the urine present direct bilirubin and urobilinogen.

In **obstructive (mechanic)** jaundice:
- in the blood increased content of direct bilirubin;
- in the urine present direct bilirubin (chyluria);
- the fecal level of stercobilin is decreased (acholic stools);

In **hemolytic** jaundice:
- in the blood increased content of indirect bilirubin;
- in the urine increased the level stercobilinogen;
- the fecal level of stercobilin is increased.

CONCLUSION:

_____Teacher's signature

*Date*_____

CLASS № 34

THEME*: *BIOCHEMISTRY OF KIDNEY AND URINE

THEORETICAL PART

1. The kidney, biochemical functions, metabolism of the kidney.
2. Role of the kidney in regulation of pH balance.
3. Synthesis of biologically active compounds in kidney. Role of renin, erythropoietin, calcitriol.
4. Electrolytes composition of body fluids.
5. Regulation of sodium, water balance and pH in body fluids.
6. Water and electrolyte imbalance, acid-base imbalance. Dehydration, oedema, acidosis, alkalosis.
7. Mineral components of tissues: classification, representatives, biological role.
8. Sodium, potassium; their biological role, metabolism, regulation of balance.
9. Calcium, phosphorus; their biological role, metabolism, regulation of balance.
10. Trace elements, biological role (Fe, Cu, Co, I, Zn, Mn, Se).

LABORATORY WORK
BIOCHEMICAL ANALYSIS OF THE URINE

The compounds that occur in the normal urine at very low concentration escaping their determination by routine analytical techniques are called “pathologic components of urine”. These compounds are proteins, glucose, ketone bodies, bile and blood pigments. Determination of these compounds is used for diagnosis and treatment watch.

1. Qualitative reaction on protein

PRINCIPLE OF THE METHOD: denaturation of protein by nitric acid (or sulphsalicilic acid)

STEPS OF WORK:

1) take 2 test tubes, label them and place in a test tube rack,

2) add to each test tube the solutions:

COMPONENTS	Control	Sample
URINE	-	1,0 ml
H_2O	1,0 ml	-
Sulphosalicilic acid 20% (or HNO_3 concentrated)	3 drops	3 drops

RESULT:

DIAGNOSTIC VALUE

In the urine protein may occur in nephritis, cystitis, hypertension, sometimes in pregnancy, nephrosis.

CONCLUSION:

2. Quantitative determination of protein in the urine

PRINCIPLE OF THE METHOD: the method based on the Heller's test with concentrated nitric acid.

STEPS OF WORK:

1) take 1 ml of HNO_3 to 4 test tubes;
2) in other 2 test tubes prepare urine solutions as described in the table

COMPONENTS	sample 1	sample 2	sample 3	sample 4
Dissolution	-	1 : 10	1 : 20	1 :30
Urine	1,0 ml	0,1 ml	0,1 ml	0,1 ml
H_2O	-	0,9 ml	1,9 ml	2,9 ml
Add with pipette dissolved urine (1 ml) on nitric acid				
HNO_3 concentrated	1,0 ml	1,0 ml	1,0 ml	1,0 ml

RESULT:

CONCLUSION:

3. Quantitative determination of glucose in the urine

PRINCIPLE OF THE METHOD: method is based on ability of glucose to reduce CuOH in alkaline medium to Cu_2O (red-colored sediment).

STEPS OF WORK:

COMPONENTS	Sample
Hainesse reagent Urine	9 drops 2 drops
Stir and heat	

RESULT:

DIAGNOSTIC VALUE

The presence of glucose in the urine (glucosuria) is observed in diabetes mellitus, kidney damage, chlorophorm poisoning.

CONCLUSION:

4. Qualitative reaction on blood pigments

PRINCIPLE OF THE METHOD: test is based on the oxidation of benzidine by oxygen which formed in reaction of hydrogen peroxide degradation catalyzed by hemoglobin.

STEPS OF TEST:

- take 20 drops of urine to the test tube, boil it then cool;
- to the urine add 20 drops of benzidine solution and 2 drops of hydrogen peroxide.

The solution obtains blue or green colour in presence of blood pigments.

RESULT:

CONCLUSION:

5. Urine dipstick express tests

A urine dipstick consists of a white plastic strip with absorbent microfiber cellulose pads attached to it. Each pad contains dried reagents needed for a specific test.

1. Insert the reactive portion of the dipstick into urine sample, completely, but briefly.
2. Remove the dipstick from the urine samples by sliding the back of the dipstick along the rim to remove excess urine.
3. Adhere to the reaction time stated on the package insert; and note that not all the tests are to be read at the same time.

4. Compare the color of the test areas on the dipstick with the color chart on the bottle label by holding the strip close to the color blocks.
5. Record the results for each test using the concentration given by the closest color match.

RESULT:

CONCLUSION:

_____Teacher’s signature

*Date*_____

CLASS № 35

THEME*: *BIOCHEMISTRY OF NERVOUS, MUSCULAR AND CONNECTIVE TISSUE

THEORETICAL PART

1. Morphochemical composition of nervous tissue. Myelin membrane: composition and structure.
2. Specific features of carbohydrate, lipid and amino acid metabolism in nervous tissue. Energy metabolism in the brain.
3. Molecular mechanisms of synaptic transmission.
4. Mediators, biogenic amines and neuropeptides
5. Structure and composition of muscle tissue. Muscle proteins, their functions.
6. Biochemical mechanisms of muscle contraction and relaxation. Role of ions in regulation of muscle contraction.
7. Muscle energy metabolism. Sources of ATP for muscle contraction, role of creatine phosphate, creatine kinase.
8. Chemical composition and metabolism of extracellular matrix. Collagen, elastin; specific features of their structure and metabolism.
9. Proteoglycans, glycosaminoglycans and glycoproteins of the connective tissue; specific features of their synthesis and degradation, biological role.

LABORATORY WORK

QUANTITATIVE MEASUREMENT OF PROTEIN IN THE LIQUOR

PRINCIPLE OF THE METHOD. Proteins in solution react with $CuSO_4$ and NaOH to give violet colour, which intensity is proportional to the concentration of proteins, that may be detected photometrically.

STEPS OF WORK:

1) take 2 test tubes, label them and place in a test tube rack.

2) add to each test tube the solutions:

COMPONENTS	Control	Sample
Gornal's reagent	4,0 ml	4,0 ml
Liquor	-	0,1 ml
NaCl 0,9%	0,1 ml	-
Stir and allow incubate 20 min. Read the extinction for sample versus the control at 540 nm, cuvette 10 mm		

RESULT:

E_{sample} =

Find out the protein concentration in the sample from the calibration graph

C_{sample} = g/L

DIAGNOSTIC IMPORTANCE

Normal level of total protein in liquor is **0,22-0,33 g/L.**

An increased content of protein in the liquor (**hyperproteinrhachia**) is observed in meningitis, brain tumors.

Hypoproteinrhachia is observed in hydrocephaly, liquor hypersecretion, intracranial hypertension.

CONCLUSION:

_____Teacher's signature

*Date*_____

CLASS № 36

THEME: INTEGRATION AND REGULATION OF METABOLISM. PRINCINPLES OF CLINICAL BIOCHEMISTRY. FINAL COMPUTER TESTING

THEORETICAL PART

1. The role of regulation of metabolism in functioning of organs and systems. Intracellular location of major metabolic pathways.
2. Regulation of metabolism: major mechanisms.
3. The levels of metabolism integration. The substrate-level interrelationships in metabolism. The role of TCA substrates in integration of metabolism.
4. Energy interrelations among catabolic and anabolic pathways.
5. Substrate-level relations among metabolism of carbohydrates and amino acids. Biosynthesis of lipids from carbohydrates and amino acids. Integration of metabolism by coenzymes.
6. The role of clinical biochemistry in diagnostics and treatment of metabolic pathology.
7. Basic and special biochemical tests.
8. Laboratory values of clinical importance.

FINAL COMPUTER TESTING

NORMAL CONCENTRATIONS OF SOME LABORATORY TESTED SUBSTRATES AND ENZYMES

BLOOD	
Alanine aminotransferase (AlAT)	5-42 IU/L
Albumins	33-53 g/L
Amylase	up to90 U/L
Aspartate aminotransferase (AsAT)	5-37 IU/L
Bilirubin total	5,0-20,5 μmol/L
Bilirubin conjugated (direct)	1,0-7,5 μmol/L
Calcium	2,25-2,75 mmol/L
Ceruloplasmin	150-600 mg/L
Chlorides	95-110 mmol/L
Copper	4,4-12,6 mmol/L (female); 11-24 mmol/L (male)
C-reactive protein	0-10 mg/L
Creatinine	53-115 μmol/L
Creatine kinase	25-200 U/L
Fibrinogen	2-4 g/L
Gamma glutamyl transpeptidase	11-50 U/L
Globulins	20-30 g/L
Glucose (serum)	3,3-6,4 mmol/L
Glucose (capillary blood)	3,3-5,55 mmol/L
Hemoglobin	115-145 g/L (female); 130-160 g/L (male)
Hemoglobin glycosylated	up to 6.5%
Iron	8,8-31,0 μmol/L
Lactate dehydrogenase	174-516 U/L
LDL	2-4 g/L
Magnesium	0,8-1,0 mmol/L
Phosphatase alkaline	18/306 U/L
Phosphorus	0,8-1,6 mmol//L
Potassium	3,2-5,6 mmol/L
Sodium	130-155 mmol/L
Transferrin	1,74-3,82 g/L

Total protein	65-85 g/L
Triacylglycerols	0,4-1,54 mmol/L (female); 0,45-1,82 mmol/L (male)
Urea	2,50-8,3 mmol/L
Uric acid	140-340 μmol/L (female); 200-415 μmol/L (male)
Cholesterol	3,6-5,2 mmol/L
URINE	
Amylase	28-160 g/h·L
Urea	333-583 mmol/day
Uric acid	1,6-6,4 mmol/day
CEREBROSPINAL FLUID	
Chlorides	120-130 mmol/L
Glucose	2,5-3,89 mmol/L
Protein	0,22-0,33 g/L

LIST OF EXAMINATION QUESTIONS
for the medical faculty for international students

I. INTRODUCTION

1. Major objectives of biochemistry. Role of biochemistry in medical education. Objects and methods of biochemistry.
2. History of biochemistry. Branches and research trends of biochemistry.

II. STRUCTURE AND FUNCTION OF PROTEINS

3. History of protein study. Proteins as the major components of the body. Functions of proteins. Shape of proteins. Protein content in the tissues
4. Hydrolysis of proteins. Amino acids: structure and classification.
5. Colour reactions of amino acids and proteins. Methods for the quantitative determination of proteins in a solution.
6. Physico-chemical properties of proteins and protein solutions.
7. Methods for separation and purification of protein: ultracentrifugation, chromatography, electrophoresis.
8. Peptides: classification, representatives, biological functions.
9. Protein functioning. Complementarity. Interactions between ligands and proteins.
10. Primary structure of proteins. Determination of primary structure, bonds which stabilize primary structure.
11. Secondary structure of proteins: types, bonds which stabilize secondary structure. Determination of secondary structure. Supersecondary structure.
12. Tertiary structure of proteins. Factors which stabilize tertiary structure. Determination of three-dimensional structure.
13. Denaturation of proteins, factors, practical use.
14. Quaternary structure of proteins. Factors which stabilize quaternary structure.
15. Proteins as the major components of the body. Functions of proteins.
16. Proteins of organs and tissues. Changes of proteins in ontogenesis and disease.
17. Simple proteins; representatives, characteristics, biological functions.
18. Conjugated proteins; representatives, characteristics, biological functions.

III. ENZYMES

19. History of enzymes study. Active and allosteric centers in enzymes.
20. Mechanism of enzyme catalysis. Properties of enzymes.
21. Classification and nomenclature of enzymes. Isoenzymes.
22. The kinetics of enzymatic reactions. The Michaelis-Menten equation and Lineweaver-Burk plot.
23. Factors affecting enzymatic reaction rate (temperature, pH, substrate and enzyme concentration).
24. Simple and conjugated enzymes. Cofactors of enzymes. Co-enzymatic functions of water-soluble vitamins.
25. Regulation of enzyme activity. Allosteric activators and inhibitors, covalent modifications, selective proteolysis.
26. Inhibition of enzymes. Application of inhibitors in medical practice (drugs as the inhibitors of enzymes).
27. Tissue-specific enzymes. Changes of enzymes in ontogenesis.
28. Changes of enzymes in disease. Enzymes in genetic diseases.
29. Blood plasma enzymes. Serum enzymes used in clinical diagnosis.
30. Use of enzymes as therapeutic agents.
31. Methods for enzyme activity determination. Units of enzyme activity.

IV. STRUCTURE AND FUNCTION OF NUCLEIC ACIDS. BIOSYNTHESIS OF NUCLEIC ACIDS AND PROTEIN. MOLECULAR BIOLOGY TECHNICS

32. History of nucleic acids study
33. DNA: composition, structure, cell localization, biological role. Denaturation and hybridization of nucleic acids.
34. RNA: types, composition, structures, cell localization, biological role.
35. Nucleoproteins: role of protein in higher structural organization of nucleic acids. Structure of chromatin.
36. Biosynthesis of DNA in eukaryotic cells: scheme, enzymes, regulation.
37. Reverse transcription, biological role.
38. Biosynthesis of RNA in eukaryotic cells: steps, enzymes. Regulation of transcription. Processing of RNA.
39. The genetic code: its characteristic features.

40. Activation of amino acids. Adaptor function of tRNA. Formation and structure of aminoacyl-tRNA.
41. Structure of eukaryotic ribosomes, their function in protein synthesis.
42. Biosynthesis of protein in eukaryotic cells: steps, scheme. Posttranslational processing of proteins.
43. Regulation of protein synthesis. Antibiotics as inhibitors of protein synthesis.
44. DNA fingerprint.
45. Polymerase chain reaction: stages and practical applications.
46. The blot-analysis of DNA and RNA. Western blot analysis.
47. Sequencing of DNA by the Sanger's method.
48. Genetic engineering, cloning of DNA.

V. HORMONES

49. General characteristics of hormones: classification, properties, types of biological action.
50. Classification of hormones on the chemical structure, on the place of their synthesis and on the mechanism of action. Target tissues and the cell receptors of hormones.
51. Mechanisms of action of hormones binding with the membrane receptors. Second messengers: cyclic purine nucleotides, calcium ions, products of hydrolysis of phosphatidylinositol. Diversity of protein kinases and their role in transmission of hormonal signal
52. Mechanism of action of hormones binding with the intracellular receptors.
53. Thyroid hormones: structure, synthesis; target tissues, biological effects. Hyper- and hypofunction.
54. Parathyroid hormone and calcitonin: structure, target tissues, biological effects. Hyper- and hypofunction of parathyroid hormone.
55. Pancreatic hormones: insulin, glucagon. Structure, target tissues, biological effects. Hyper- and hypofunction.
56. Epinephrine (adrenaline) and norepinephrine (noradrenaline): structure, synthesis and inactivation, target tissues, biological effects. Hyperproduction of adrenaline.
57. Glucocorticoids: structure, target tissues, biological effects. Hyper- and hypofunction.

58. Mineralocorticoids: structure, target tissues, biological effects. Disorders of mineralocorticoid excess.
59. Female sex hormones: structure, target tissues, biological effects. Hyper- and hypofunction.
60. Male sex hormones: structure, target tissues, biological effects. Hyper- and hypofunction.
61. Hormones of hypothalamus and hypophysis, their biological action. Growth hormone, adrenocorticotropic hormone: target tissues, effects on metabolism. Hyper- and hypoproduction of growth hormone.
62. Eicosanoids (prostaglandins, thromboxanes, leukotrienes) and their role in the regulation of metabolism and functions.

VI. BIOCHEMISTRY OF NUTRITION AND DIGESTION. VITAMINS

63. Components of human's food. The significance of nutrition for the vital activity. Pathological states related to nutrition disorders.
64. Dietary carbohydrates, lipids and proteins: daily requirements, characteristics, nutritional importance.
65. Essential food components: amino acids, fatty acids, their characteristics and biological importance.
66. Vitamins, general characteristics, classification, biological functions. Vitamin-like substances. Causes of hypo- and hypervitaminoses.
67. Vitamin A: biological role, symptoms of deficiency, daily requirements, dietary sources. Hypervitaminosis A.
68. Vitamin E: biological role, symptoms of deficiency, daily requirements, dietary sources.
69. Vitamin D: biological role, symptoms of deficiency, daily requirements, dietary sources. Hypervitaminosis D.
70. Vitamin K: biological role, symptoms of deficiency, daily requirements, dietary sources.
71. Vitamin B_1: coenzyme forms, biological role, symptoms of deficiency, daily requirements, dietary sources.
72. Vitamin B_2: coenzyme forms, biological role, symptoms of deficiency, daily requirements, dietary sources.
73. Vitamin PP: coenzyme forms, biological role, symptoms of deficiency, daily requirements, dietary sources.

74. Vitamin B_6: coenzyme forms, biological role, symptoms of deficiency, daily requirements, dietary sources.
75. Pantothenic acid: coenzyme forms, biological role, symptoms of deficiency, daily requirements, dietary sources.
76. Folic acid): coenzyme forms, biological role, symptoms of deficiency, daily requirements, dietary sources.
77. Vitamin H: coenzyme forms, biological role, symptoms of deficiency, daily requirements, dietary sources.
78. Vitamin C: coenzyme forms, biological role, symptoms of deficiency, daily requirements, dietary sources.
79. Vitamin B_{12}: coenzyme forms, biological role, symptoms of deficiency, daily requirements, dietary sources.
80. Anti-vitamins, mechanism of action, representatives, their application in medical practice and scientific investigations

VII.STRUCTURE AND FUNCTIONS OF MEMBRANES

81. Structure and functions of membranes. Lipids and proteins of membranes.
82. Properties of membranes. Transport mechanisms. Types of transport processes across membrane.

VIII. INTRODUCTION INTO METABOLISM

83. Metabolism and metabolic pathways. Experimental study of metabolism, use of radioisotope tracers.
84. The pathways for the catabolism of carbohydrates, proteins and lipids. The specific and common pathways of catabolism. Interrelations between anabolism and catabolism.

IX. ENERGY METABOLISM. TCA.

85. Bioenergetics of the cell. Free energy. High-energy compounds: structure, biological role.
86. ATP: structure, biological role; the ways of its formation and use.
87. Biological oxidation and tissue respiration.
88. NAD^+($NADP^+$)-dependent dehydrogenases, structure of coenzyme, biological role.
89. FAD(FMN)-dependent dehydrogenases, structure of coenzyme, biological role.
90. Coenzyme Q, structure, biological role.
91. Cytochromes of ETC, structure, biological role.
92. Electron transport chain (ETC), its structural organization and functioning. Electron transport chain complexes.

93. Oxidative phosphorylation. The chemiosmotic theory of oxidative phosphorylation. The P/O ratio. Substrate-level phosphorylation.
94. Regulation of ETC. Activators and inhibitors of the electron transport chain. Uncoupling of oxidation and phosphorylation.
95. General characteristics of oxidation processes. The role of oxygen in oxidative processes in the cell. Types of oxidation: enzymes, biological role.
96. Microsomal oxidation: scheme, biological role.
97. Oxygen free radicals: their tissue-damaging effects. Lipid peroxidation.
98. Antioxidant systems, role of enzymes.
99. The citric acid cycle: reactions.
100. The scheme of the cytric acid cycle, its regulation and biological role.
101. Energy yield of the cytric acid cycle. Relation of the citric acid cycle with the respiratory chain.

X. METABOLISM OF CARBOHYDRATES

102. General characteristics and classification of carbohydrates, biological functions. Carbohydrates of human tissues.
103. Dietary carbohydrates. Digestion and absorption of carbohydrates in the gastrointestinal tract.
104. The general scheme of pathways of glucose metabolism and their estimation. Reactions of glucose phosphorylation and dephosphorylation of glucose 6-phosphate, biological role. Regulation.
105. Galactose and lactose metabolism. Hereditary disorders of galactose and lactose metabolism.
106. Fructose metabolism. Hereditary disorders of fructose metabolism.
107. Anaerobic glycolysis: reactions and biological significance.
108. Oxidation-reduction reactions in anaerobic glycolysis. Reactions of substrate-level phosphorylation in glycolysis.
109. Energy-producing reactions and biological role of anaerobic glycolysis. Regulation of anaerobic glycolysis.
110. Catabolism of glucose under aerobic conditions: reactions.
111. Pyruvate dehydrogenase complex: components, mechanism of the reaction, regulation, biological role.

112. Energy yield and biological role of aerobic degradation of glucose. Scheme of pyruvate metabolism.
113. Metabolism of lactate. Gluconeogenesis: scheme, metabolic precursors of glucose.
114. Key reactions of gluconeogenesis. Role of biotin. Biological role and regulation of gluconeogenesis.
115. Pentose phosphate pathway: oxidative and non-oxidative reactions. biological role.
116. Glucuronic acid pathway of glucose: major reactions, biological role.
117. Synthesis of glycogen. Regulation of glycogenesis.
118. Glycogen degradation, regulation. Physiological role of glycogen.
119. Disorders of glycogen metabolism: glycogenoses, aglycogenoses.
120. Hormonal regulation of glycemia. The role of insulin, adrenaline, glucagon and corticosteroids. Hyperglycemia and hypoglycemia, their causes. Methods for determination of glucose in the blood serum.
121. Disorders of carbohydrate metabolism in diabetes mellitus.
122. Glucose tolerance test. Diagnostic value.

XI. METABOLISM OF LIPIDS

123. Classification of lipids. Lipids of human tissues. Biological functions of lipids.
124. Dietary lipids. Digestion of lipids: emulsification, enzymatic hydrolysis, formation of micelles. The role of bile acids. Disorders in digestion and absorption of lipids in the gastrointestinal tract.
125. Resynthesis of fats in the intestinal wall. Formation of chylomicrons. Composition and metabolism of chylomicrons.
126. Fatty acids of human tissues: classification, representatives. Activation of fatty acids, transport of acyl CoA into mitochondrion.
127. β-Oxidation of fatty acids: reactions, energy production of β-oxidation, relation with citric acid cycle and electron transport chain.
128. Oxidation of odd-chain fatty acids.
129. Reactions of synthesis and utilization of ketone bodies. Mechanism of ketosis in diabetes mellitus and starvation. Ketoacidosis.

130. Biosynthesis of fatty acids: sources of acetyl CoA and NADPH in the cytoplasm, synthesis of malonyl CoA, fatty acid synthase.
131. Biosynthesis of palmitic acid: reactions. The fatty acid synthase complex.
132. Metabolism of triacylglycerols. Biosynthesis and catabolism of triacylglycerols, regulation.
133. Biosynthesis of phospholipids. Fatty infiltration of the liver.
134. Metabolism of cholesterol in the body. Transport of cholesterol in the blood.
135. Biosynthesis of cholesterol: main steps, scheme. Regulation of cholesterol synthesis. Initial reactions of cholesterol biosynthesis.
136. Bile acids: representatives, structure, metabolism, biological functions. Cholelithiasis. Formation of cholesterol gall stones.
137. Metabolism of sphingolipids. Disorders of sphingolipid metabolism.
138. Hypercholesterolemia and atherosclerosis. Biochemical principles of treatment.
139. Transport of lipids in the blood, role of albumins. General characteristics of lipoproteins.
140. Metabolism of lipoproteins: formation and utilization. Lipoprotein lipase. Role of apoproteins.
141. Hyperlipoproteinemias.

XII. METABOLISM OF AMINO ACIDS

142. Dynamic state of body proteins. Nitrogen balance. Sources of amino acids in the body and ways of their use.
143. Dietary proteins. Digestion of proteins in the gastrointestinal tract. Absorption of amino acids.
144. Intestinal putrefaction of proteins (conversion of amino acids by intestinal bacteria).
145. Types of deamination of amino acids. Oxidative deamination and reductive amination. Biological role.
146. Transamination of amino acids, biological role. Coenzyme functions of vitamin B_6. Mechanism of transamination. Clinical significance of transaminase activity in the blood serum.
147. Transdeamination. Biological role.
148. Decarboxylation of amino acids. Types of decarboxylation, biological role. Biogenic amines: synthesis, functions, oxidation of biogenic amines.

149. Ways for formation and detoxification of ammonia. Intracellular detoxification of ammonia. Role of glutaminase in the maintenance of acid-base balance in the body.
150. Biosynthesis of urea (urea cycle). Disorders of the urea synthesis and excretion.
151. Catabolism of amino acids in the organism. Glucogenic and ketogenic amino acids.
152. Metabolism of methionine, formation of S-adenosylmethionine, its role in transmethylation reactions. Synthesis of creatine.
153. Metabolism of phenylalanine and tyrosine. Disorders of phenylalanine and tyrosine metabolism (phenylketonuria, alkaptonuria, albinism).

XIII. METABOLISM OF NUCLEOTIDES

154. Biosynthesis of purine nucleotides: synthesis of phosphoribosylamine, origin of atoms in the purine ring. Inosinic acid as a precursor for synthesis of adenylic and guanylic acids. Regulation of synthesis of purine nucleotides.
155. Biosynthesis of pyrimidine nucleotides. Regulation of biosynthesis of pyrimidine nucleotides.
156. Degradation of nucleic acids in the gastrointestinal tract and tissues. Degradation of purine and pyrimidine nucleotides.
157. Disorders of metabolism of nucleotides: xanthinuria, orotaciduria, gout.

XIV. WATER AND ELECTROLYTE METABOLISM. BIOCHEMISTRY OF THE KIDNEY AND URINE

158. Body water compartments. Composition, volume and osmolality of body fluids. Functions of water in the organism. The water balance.
159. Regulation of electrolyte and water balance and pH in body fluids.
160. Disturbances in water-mineral and acid-base balance. Dehydration, edema, acidosis and alkalosis.
161. Mineral components of tissues: representatives, biological role.
162. Sodium, potassium; their biological role, metabolism, regulation of balance.
163. Calcium, phosphate; their biological role, metabolism, regulation of balance.

164. Trace elements (Fe, Cu, Co, I, Mg, Zn, Mn, Se), their biological role.
165. Kidney, biochemical functions, metabolism of the kidney. Role of kidney in regulation of pH balance.
166. General characteristics and composition of urine.
167. Pathologic components of urine. Role of urine analysis in diagnosis.

XV. INTEGRATION OF METABOLISM

168. The levels of metabolism integration. The substrate-level interrelationships in metabolism. The role of TCA substrates in integration of metabolism.
169. Energy interrelations among catabolic and anabolic pathways.
170. Substrate-level relations among metabolism of carbohydrates and amino acids. Biosynthesis of lipids from carbohydrates and amino acids. Integration of metabolism by coenzymes.

XVI. REGULATION OF METABOLISM

171. The role of regulation of metabolism in functioning of organs and systems. Intracellular location of major metabolic pathways.
172. Regulation of metabolism: major mechanisms.

XVII. BIOCHEMISTRY OF THE LIVER

173. Role of the liver in carbohydrate, lipid, amino acid and protein metabolism.
174. Detoxifying function of liver.
175. Heme synthesis, reactions.
176. Function of liver in pigment metabolism. Bilirubin metabolism, scheme.
177. Disorders in bilirubin metabolism: jaundice, its types. Differential diagnosis for jaundices of different types.
178. Biochemical mechanisms of hepatic failure and hepatic coma. Biochemical tests for diagnosis of liver disorders.

XVIII. BIOCHEMISTRY OF THE BLOOD

179. Blood, general characteristics and functions. Specific features of metabolism in blood cells.
180. Hemoglobin, structure, derivatives. Transport of oxygen and carbon dioxide. Variants of hemoglobin in ontogenesis. Hypoxias and hemoglobinopathies.
181. Metabolism of iron. Iron deficiency anemia.

182. Plasma proteins, their characteristics. Classification of plasma proteins on the functions: transport proteins, complement proteins, kinins, blood clotting proteins, proteins of fibrinolytic system, immunoglobulins, inhibitors of proteolysis.
183. Blood serum enzymes, its diagnostic value. Acute phase proteins.
184. Hemostasis. Blood coagulation factors. Role of platelets.
185. Intrinsic and extrinsic pathways of blood coagulation. Cascade mechanism for activation of coagulation factors. Role of vitamin K in blood coagulation.
186. Anticoagulant and fibrinolytic system.
187. Abnormalities in coagulation: disorders of coagulation and anticoagulation systems. Thrombosis and hemophilias.
188. Biochemical analysis of blood serum, major components, use for diagnosis in health and pathology.

XIX. BIOCHEMISTRY OF NERVOUS SYSTEM

189. Morphochemical composition of nervous tissue. Transport of substrates into the brain, role of the blood/brain barrier. Axonal transport.
190. Specific features of carbohydrate, lipid and amino acid metabolism in nervous tissue. Energy metabolism in the brain.
191. Biochemical mechanisms of generation and transmission of nervous impulses. Molecular mechanisms of synaptic transmission.
192. Neurotransmitters: acetylcholine, catecholamines, serotonin, GABA. Synthesis and metabolism in nervous tissue, functions.

XX. BIOCHEMISTRY OF MUSCLES

193. Structure and composition of muscle tissue. Muscle proteins, their functions.
194. Biochemical mechanisms of muscle contraction and relaxation. Role of ions in regulation of muscle contraction.
195. Muscle energy metabolism. Sources of ATP for muscle contraction, role of creatine phosphate, creatine kinase.

XXI. BIOCHEMISTRY OF CONNECTIVE TISSUE

196. Chemical composition and structure of extracellular matrix (ground substance). Collagen, elastin; specific features of their structure and metabolism.
197. Proteoglycans and glycoproteins of connective tissue; specific features of their synthesis and degradation, biological role.

XXII. INTRODUCTION TO CLINICAL BIOCHEMISTRY

198. The role of clinical biochemistry in diagnoctic and treatment of metabolic pathology.
199. Basic and special biochemical tests.
200. Laboratory values of clinical importance.

Учебное издание

Петушок Наталья Эдуардовна
Виницкая Анна Георгиевна

БИОЛОГИЧЕСКАЯ ХИМИЯ

Практикум
для студентов, обучающихся
по специальности 1-79 01 01 «Лечебное дело»
(английский язык обучения)

Ответственный за выпуск В. В. Воробьев

Компьютерная верстка С. В. Петрушиной

Подписано в печать 06.08.2020.
Формат 60x84/16. Бумага офсетная.
Гарнитура Times New Roman. Ризография.
Усл. печ. л. 7,90. Уч.-изд. л. 3,52. Тираж 300 экз. Заказ 97.

Издатель и полиграфическое исполнение
учреждение образования
«Гродненский государственный медицинский университет».
ЛП № 02330/445 от 18.12.2013.
Ул. Горького, 80, 230009, Гродно.