Ministry of Education and Science of Ukraine
Petro Mohyla Black Sea National University

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WORKING BOOK FOR SELF-PREPARATORY WORK ON BIOLOGICAL AND BIOORGANIC CHEMISTRY

(MODULE 2)

in the field of knowledge 22 «Health care»
in the specialty 222 «Medicine»

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Larycheva O. M. Workivg book for self-preparatory work on biological and bioorganic chemistry (Module 2) : in the field of knowledge 22 «Health care» in the specialty 222 «Medicine». – Mykolaiv : PMBSNU Publishing House, 2020. – 404 p. (Methodical series ; issue 300).

The workbook covers all stages of students' preparation for practical classes in Biological and Bioorganic Chemistry in 3^{d} semester including independent and classroom work. It is structured with topics that contain a list of theoretical questions and tasks for self-preparation, as well as methods of experiments for practical classes and examples of Krock-1 tests.

For students of medical specialties of higher educational institutions.

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CONTENTS

LABORATORY HAZARDS AND FIRST AID

HAZARDS

The student is surrounded by many dangers such as:

- ✓ Broken glassware.
- ✓ Corrosive reagents.
- ✓ Mechanical hazards.
- ✓ Poisonous fumes that could be inhaled.
- ✓ Inflammable chemicals.
- ✓ Gas leakages.
- ✓ Electrical hazards.

SAFETY MEASURES

✓ Lab coat has to be worn in order to protect oneself from corrosive splashes.

✓ One has to be careful while handling gases.

✓ Chemical work involving irritating chemicals and dangerous infectious materials should always be conducted under hoods with good exhaust and adequate ventilation.

✓ Safety cabinets and hoods should be used while handling corrosive reagents.

✓ Bunsen burner should never be used around inflammable material like ether, and acetone.

✓ Hands should be washed with soap water followed by washing with disinfectant material.

✓ Eating and drinking in the laboratory must be avoided.

PRECAUTIONS REGARDING FIRE SAFETY

- ✓ Open flames should not be left unattended.
- ✓ Smoking should be strictly prohibited.
- ✓ Burning match sticks should not be thrown in waste baskets.

IN CASE OF ACCIDENTAL FIRE

- ✓ Sand or blanket should be used to put off the small fire.
- ✓ For longer blazes, «Fire Extinguishers» have to be used.
- ✓ Water should NOT be used on electrical fire.

✓ Water should NOT be used on a fire caused by organi solvents such as ether, alcohol, petrol, etc.

✓ While trying to escape from fire, in case if it cannot be extinguished quickly, it is safe to stay close to the floor and crawl by covering mouth with damp cloth.

ACCIDENTAL SWALLOW OF CORROSIVE SOLUTIONS

1. If the corrosive solution swallowed is an acid:

✓ Spit the corrosive solution.

✓ Promptly rinse the mouth.

✓ Antidotes such as 8% magnesium hydroxide (milk of magnesia) or egg white mixed in water can be used orally to neutralize the acid.

✓ Seek medical help immediately.

2. If the corrosive solution swallowed is an alkali:

✓ Promptly rinse the mouth.

✓ **Antidotes such as lemon juice or 5% acetic acid can be taken orally to neutralize the alkali.**

✓ Seek medical help immediately.

INHALATION OF CORROSIVE GASES

Take the student to fresh air and seek medical help immediately.

BURNS

From strong acids:

✓ First wash with LOTS of water and then wash with 5% sodium carbonate or 5% ammonium hydroxide.

✓ Seek medical help immediately.

From strong alkalies:

✓ Wash immediately with LOTS of water and later with 5% boric acid or dilute acetic acid solution.

✓ Seek medical help immediately.

IN CASE OF ACCIDENTS IN THE LABORATORY

✓ One should not be panic.

✓ Alarm should be raised as soon as possible.

✓ The laboratory should be evacuated, to minimize further damage to property.

✓ Gas and electricity connections have to be turned off immediately.

✓ In case of fire attacks, fire extinguishers should be used to tackle them.

✓ In case of large fires, the fire brigade has to be called.

WASTE DISPOSAL

✓ Neutralization of acids and alkalies should be done prior to their washing in the sink.

✓ Organic solvents should be stored in metal drums and later it must be washed off.

✓ Some chemicals can be cleared or disposed by INCINERATION.

LABORATORY SAFETY RULES

GENERAL GUIDELINES

1. Conduct yourself in a responsible manner at all times in the laboratory.
2. Follow all written and verbal instructions carefully. If you do not understand a direction or part of a procedure, ASK YOUR TEACHER BEFORE PROCEEDING WITH THE ACTIVITY.
3. Do not touch any equipment, chemicals or other materials in the laboratory area until you are instructed to do so.
4. Be prepared for your work in the laboratory. Read all procedures thoroughly before entering the laboratory. Never fool around in the laboratory.
5. Always work in a well-ventilated area.
6. Observe good housekeeping practices. Work areas should be kept clean and tidy at all times.
7. Be alert and proceed with caution at all times in the laboratory. Notify the teacher immediately, if you observe any unsafe conditions.
8. Dispose all chemical wastes properly. Never mix chemicals in sink drains. Sinks are to be used only for water. Check with your teacher for disposal of chemicals and solutions.
9. Keep hands away from face, eyes, mouth and body while using chemicals or lab equipment. Wash your hands with soap after performing all experiments.
10. Dress properly during a laboratory activity. Long hair, dangling jewelry, and loose or baggy clothing are hazardous in the laboratory. Long hair must be tied back, and dangling jewelry and baggy clothing must be secured. Shoes must completely cover the foot. No sandals allowed in the laboratory.
11. A lab coat should be worn during laboratory experiments.

ACCIDENTS AND INJURIES

1. Report any accident (spill, breakage, etc.) or injury (cut, burn, etc.) to the teacher immediately, no matter how trivial it is. Do not panic.
2. If a chemical splashed into your eye(s) or on your skin, immediately flush with running tab water for at least 20 minutes.

HANDLING CHEMICALS

1. All chemicals in the laboratory are to be considered dangerous. Avoid handling chemicals with fingers. Do not taste or smell any chemicals.

2. Check the label on all chemical bottles twice before removing any of the contents. Take only as much chemical as you need.
3. Never return unused chemicals to their original container.
4. Never remove chemicals or other materials from the laboratory area.
5. Never pipette by mouth.

HANDLING GLASSWARE AND EQUIPMENT

1. Never handle broken glass with your bare hands. Use a brush and dustpan to clean up broken glass. Place broken glass in the designated glass disposal container.
2. Examine glassware before each use. Never use chipped, cracked or dirty glassware.
3. If you do not understand how to use an equipment, ASK THE TEACHER FOR HELP!
4. Do not immerse hot glassware in cold water. The glassware may shatter.
5. Heated glassware remains very hot for a long time. They should be set aside in a designated place to cool, and picked up with caution, using tongs.
6. Never look into a container that is being heated as there are chances of it getting splashed to the face or eyes.

AFTER EXPERIMENTS

1. Clean all the glassware you have and put them on the shelf in a proper order.
2. Wipe and clean the table.
3. Put all chemicals back to their respective places.
4. Put off the gas burner.

PIPETTING TECHNIQUES

1. Use a pipette bulb to draw liquid above the calibration mark (Fig. 1).
2. Remove the bulb and cover the pipette with your forefinger.
3. Dry the pipette tip with a tissue.
4. Rotate the pipette using the thumb and the other fingers to let in air so that the liquid drains slowly until the meniscus reaches the calibration mark.
5. To deliver the liquid, hold the pipette vertically and let the pipette tip touch the wall of the receiving container.
6. When the delivery is completed, touch the tip of the pipette to the wall of the container.

Caution: Always keep the pipette tip under liquid surface when you draw up liquid. Never use the bulb to blow air inside the pipette, this will introduce dust and make the pipette dirty.

Fig. 1. Pipetting techniques

TOPIC №1.
An object and assignments of biochemistry, its principal trends and parts. Objectives and methods of biochemical investigation; their clinical and diagnostic significance

1. Objective: Introduction to the assignments and methods of biochemical investigation; to learn specific methods of investigation of biologically active substances as well to make an acquaintance with instruments and devices used in biochemistry.

2. Actuality of the theme: Biochemistry is a science which investigates chemical composition of living organisms, chemical structure of constituents, their properties, localization, the pathways of their appearance and transformations, as well as chemical processes, which take place in living cell and provide turnover of matter and energy in the cell.

Biochemistry is on the way of solution of important problems and questions of natural history and medicine, e.g. problem of protein synthesis, life span prolongation, etc.

Modern physical, chemical and mathematical methods are used in biochemical investigations. Biochemical data are used in medical diagnostics, treatment and prevention of diseases

3. Specific aims:

✓ To know principal stages and regularities in origin and development of biochemistry as fundamental medical and biological science and educational discipline.

✓ To recognize priciples of methods of investigation of functional status in human body in health and disease.

✓ To interprete data of biochemical investigations and evaluate the status of selected metabolic pathways

✓ To determine optical density of coloured solutions at distinct light wavelength using a photocolourimeter, to interpret obtained results properly.

4. Reference card for the separate study of educational literature for the lesson preparation

	Questions:	References:
1.	**Biochemistry as fundamental biomedical science**	Gubsky Yu. Biological chemistry: textbook / edited by Yu. Gubsky. – Vinnytsia : Nova Knyha, 2018. – 2nd edition. – P. 13–14.
2.	**A short history of biochemistry and its main periods.**	Laboratory manual

3.	**Chemical constitution of living organisms, the characteristic features of living systems:** ✓ The major biomolecules of cells (proteins, carbohydrates, DNA, RNA, lipids).	1. Satyanarayana U., Chakrapani U. «Bio-chemistry», Fours Edition. – 2013. – P. 3–4. 2. Gubsky Yu. Biological chemistry: textbook / edited by Yu. Gubsky. – Vinnytsia: Nova Knyha, 2018. – 2nd edition. – P. 14–18.
4.	**The structural components of the prokaryotic and eukaryotic cells**: ✓ Differences between prokaryotic and eukaryotic cells; ✓ Role of different organelles (nucleus, mitochondria, endoplasmic reticulum, Golgi apparatus, lysosomes and peroxisomemes) in metabolic processes in cells.	1. Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 4–8. 2. Gubsky Yu. Biological chemistry: textbook / edited by Yu. Gubsky. – Vinnytsia: Nova Knyha, 2018. – 2nd edition. – P. 72–74.
5.	**Biological material used in biochemical investigations:** ✓ General rules of blood collection; ✓ Types of laboratory tests; ✓ Collection of urine; ✓ Cerebrospinal fluid; ✓ Quality of control.	Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 769–771.
6.	**Methods in biochemical investigations**	Laboratory manual.

5. Tasks for independent work and self-control

5.1. Fill in the chart «Composition, structure and functions of biomolecules»:

Biomolecules	Monomers (components)	Type of bonds	Functions
Carbohydrates: – monosaccharides			
– disaccharides			
– oligosaccharides			
– polysaccharides			

Lipids: – triacylglycerols			
– phospholipids			
– sphingomyelins			
– glycolipids			
– steroids			
Peptides			
Proteins			
Nucleotides			
Nucleic acids			

5.2. Choose corect answer.

1. Ukrainian biochemist is:

A. Watson
B. Sumner
C. Knoop
D. Palladin
E. Krebs

2. Functional biochemistry essentially close to:

A. Physiology
B. Pathology
C. Histology
D. Microbiology
E. Physics

3. Qualitative reaction for peptide bond are:

A. Fohl reaction
B. Xanthoproteic reaction
C. Nitroprusside reaction
D. Ninhydrin reaction
E. Biuret reaction

4. How many kinds of amino acids is part of the protein molecules?

A. 10
B. 20
C. 30
D. 40
E. 50

5. Amino acids amphotery caused by the presence of functional groups:

A. COOH and $–NH_2$
B. COOH and –OH
C. COOH and –SH
D. NH_2 and –OH
E. NH_2 and –SH

6. Primary structure of the protein molecule is:

A. Disulfide bond
B. Peptide bond
C. Hydrogen bond
D. Hydrophobic bond
E. Ion bond

7. Purely biochemical methods are:

A. Colourimetric
B. Nephelometric
C. Potentiometric
D. Enzymatic
E. Refractometric

8. Select sulfur-containing amino acids:

A. histidine
B. serine
C. methionine
D. arginine
E. asparagine

9. Structural monomers for proteins is:

A. monosaccharides
B. nucleotides
C. glycerin
D. amino acids
E. nucleosides

10. The peptide bonds in proteins are formed between groups:

A. COOH and $–NH_2$
B. COOH and –OH
C. COOH and –SH

D. NH_2 and –OH
E. NH_2 and –SH

11. Denaturation – is the destruction of the protein molecule structures:

A. quaternary and primary
B. tertiary and primary
C. secondary and primary
D. only primary
E. secondary, tertiary, quaternary

12. The simple proteins include all named, except:

A. Albumin;
B. Protamine
C. Protamine
D. Proteoglycans
E. Globulins

6. Individual independent students work

1. History of biochemistry and its main periods. The significance of biochemistry in the development of medical sciences and practical health care.
2. The fundamental discoveries in a branch of structural and functional significances in proteins and nucleic acids.

7. Theoretical materials

The objectives and assignments of biochemistr and its principal trends and parts

Biochemistry is the science of the chemical constituents of living cells and of the reactions and processes they undergo. Although the term «biochemistry» seems to have been first used in 1882, it is generally accepted that the word "biochemistry" was first proposed in 1903 by Carl Neuberg, a German chemist. Life depends on various biochemical reactions, that is why biochemistry has become the basic language of all biological sciences. Controlling information flow through biochemical signaling and the flow of chemical energy through metabolism, biochemical processes give rise to the incredible complexity of life. Biochemistry mostly deals with the structures and functions of cellular components such as proteins, carbohydrates, lipids, nucleic acids and other biomolecules as well as with their conversion. Over the last 40 years biochemistry has become so successful at explaining living processes that now almost all areas of the life sciences from botany to medicine are engaged in biochemical research. Today the main focus of pure biochemistry lies in understanding how

biological molecules give rise to the processes that occur within living cells which in turn relates greatly to the study and understanding of whole organisms. Biochemistry is a field of science concerned with the study of:

- ✓ chemical properties of the compounds constitutive of the living organism (static biochemistry);
- ✓ their conversions (dynamic biochemistry);
- ✓ relation of these conversions to the activity of organs and tissues (functional biochemistry).

Biochemistry is concerned with the entire spectrum of life forms, from relatively simple viruses and bacteria to complex human beings. Depending on the **object of study** biochemistry is divided into:

- ✓ biochemistry of humans and animals
- ✓ biochemistry of plants
- ✓ biochemistry of microorganisms

Major objective of biochemistry is complete understanding of all the chemical processes associated with living cells at the molecular level.

Methods used in biochemistry

- ✓ *for separating and purifying biomolecules*
  salt fractionation
  chromatography
  electrophoresis
  ultracentrifugation
- ✓ *for determining biomolecular structure*
  elemental analysis
  spectroscopy
  mass spectrometry
  X-ray crystallography
  use hydrolysis and enzymes to degrade the biomolecules
- ✓ *for determining substances concentrations*
  spectrophotometry
  colourimetry

History of biochemistry

I period: ancient time – 15th century. In this period people used biochemical processes to make bread, cheese, wine, though the essence of these processes was unknown to them.

II period: 15th century – first half of the 19th century. In this period German physician Paracelsus (1493–1541) put forward the concept of a close relationship between chemistry and medicine: chemical reactions formed the basis of vital activity and the cause of any disease is a disturbance of the natural course of chemical processes within the organism.

The first controlled experiments in human metabolism were published by Santorio Santorio in 1614 in his book *«Ars de statica medecina»*. This book describes how he weighed himself before and after eating, sleeping, working, sex, fasting, drinking, and excreting. He found that most of the food he took in was lost through what he called "insensible perspiration". Russian scientist Lomonosov (1711–1765) formulated the law of conservation of mass. French chemist Lavoisier (1743–1794) proposed that in respiration of living organism oxygen consumed and carbon dioxide evolved. Russian chemist Kirchhoff (1764–1833) described in 1814 enzy-matic process of starch saccharization by the action of an extract from the germinated barleycorn. By the 1850s, other enzymes were discovered:salivary amylase, pepsin in gastric juice, trypsin of pancreatic juice.

III period: second half of the 19th century – first half of the 20th century. In this period French scientist Pasteur (1821–1895) performed studies of fermentation with participation of living yeast cells. German chemist Buchner in 1897 provided evidence for the ability of a cellfree yeast juice to produce alcoholic fermentation. For his works on cell-free fermentation Buchner was awarded a Nobel Prize in chemistry in 1907. In the second half of the 19th century special chairs of medical, or physiological, chemistry were instituted at the medical departments of many European universities.

IV period: 1950s – present time. Advent of biochemistry, development of new methodologic principles and techniques such as chromatography, X-ray diffraction, nuclear magnetic resonance spectroscopy, radioisotopic labeling, electron microscopy and molecular dynamics simulations. These techniques allowed for the discovery and detailed analysis of many molecules and metabolic pathways of the cell, such as glycolysis and the Krebs cycle (citric acid cycle). In 1960, the biochemist Robert K. Crane revealed his discovery of the sodium-glucose cotransport as the mechanism for intestinal glucose absorption. This was the very first proposal of a coupling between the fluxes of ions and a substrate that has been seen as sparking a revolution in biology.

Today, the findings of biochemistry are used in many areas from genetics to molecular biology and from agriculture to medicine.

The study of human biochemistry will open your eyes to how the body works as a chemical system. From a physician's point of view, biochemistry not only describes how the system works, but also provides a foundation for understanding how to improve its operation, how to diagnose problems and, where possible, how to remedy them. To understand links between nutrients, metabolism, health and disease, is one of the most important reasons to study biochemistry. Knowing biochemistry helps to understand

current therapies, which include recombinant proteins, such as human insulin or erythropoietin synthesized by bacteria. It helps to understand the action of new drugs. In the future, therapies will possibly involve gene rather than organ transplants. Pharmacogenomics and nutritional genomics will create a basis for designer treatments, customized to an individual's genetic makeup.

Practice protocol №1 «____» _____ 20___

Rules of work in biochemical laboratory

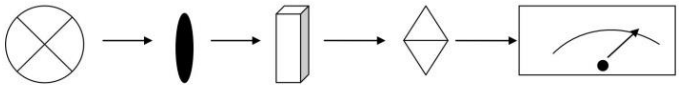
1. To begin laboratory researches after instructing by the teacher, strictly adhering to a methodology (algorithm) of researches.
2. To be attentive, accurate and exact at carrying out of laboratory researches.
3. To carry out the work with dangerous reagents under the hood.
4. To adhere to instructions to the work on devices and the equipment.

Colourimetry

Experiment 1. Work with photoelectrocolourimeter

The photoelectrocolourimetric method of analysis serves for determination of substances concentrations in coloured solutions, biological liquids or tissue extracts. It may be also used for determination of concentrations of colourless substances if they can be transformed into coloured state with the specific reagents. The method is one of the most widespread in biochemistry and clinical medicine.

This method is based on Lambert-Beer law, which postulates that the absorbance of solution is directly proportional to the concentration of the absorbing solute and depends on the thickness of the absorbing layer (path length, d). In colourimetry monochromatic light (of a single wavelength, λ) is used.



The operating procedure on FEC

1. To switch on the colourimetre for 15 minutes prior to the beginning of measurements. During warming up cuvette branch should be open.
2. To expose a colour optical filter necessary for measurement.
3. To set the minimal sensitivity of a colourimetre.

4. Before measurement to check up the setting of the pointer of a colourimetre on «0» at closed cuvette branch.
5. To place a cuvette with a solution at a light beam.
6. To close a cover of a cuvette branch.
7. To write down parameters from the scale of colourimetre.
8. To switch off the device from a network.

Principle. 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.

Calculation of substances concentrations:

1) according to the formula (the standard solution with known concentration must be used);

$$C\ sample = \frac{E\ sample}{E\ standard} \times C\ standard,$$

where

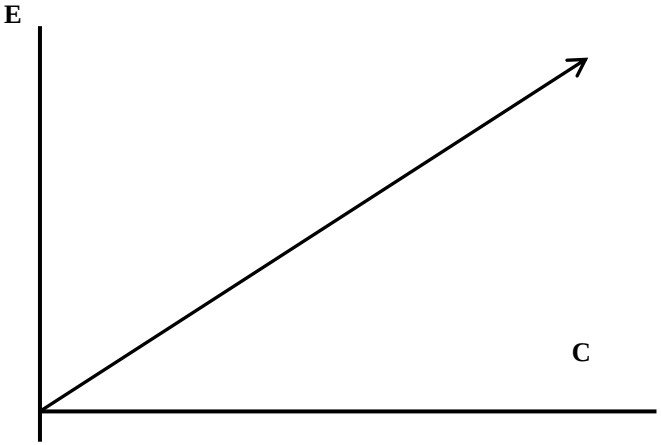
C_{sample} – concentration of the sample;

E_{sample} – extinction (absorbance) of the sample;

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

$E_{standard}$ – extinction (absorbance) of the standard solution.

2) according to calibration graph (graph of dependence of extinction onconcentration).



Methods of quantitative determination of proteins

Protein quantity in the specimen can be estimated by physical and chemical methods. Physical methods include refractometry, spectrophotometry, etc.

Chemical methods employ colour reactions of proteins and the intensity of colour is measured by colourimetry (e.g. biuret test, Lowry method). Chemical methods include also determination of nitrogen by Quieldahl method.

Experiment 2. Biuret method of protein quantitation.

PrincipleE. Proteins in alkaline medium interact with copper sulfate forming a compound with a violet colour. Optical density of this compound solution is directly proportional to the concentration of protein.

Reagents. Blood serum, standard protein solution (50 g/l), biuret reagent (0.15 g copper sulfate, 0.6 g sodium, potassium tartrate are dissolved in 60 ml of water, 30 ml of 10% NaOH solution are added, volume adjusted to 100 ml, then 0.1 g KJ is added).

Method. Take five clean dry tubes and do the following procedures:

1. Add to the first tube (control) 0.1 ml of saline.
2. Add to the second tube 0.1 ml of standard protein solution.
3. Add to the third, fourth and fifth tubes 0.1 ml of tested blood serum A, B and C respectively.
4. Add 5 ml of biuret reagent to all tubes, mix and incubate for 10–15 min.
5. Measure the extinction (optical density) on a photocolourimeter using a green light filter against control solution (λ = 540 – 560 nm).

Calculation. The concentration of protein in the test solution is calculated according to the formula:

$$X = (A \times B)/C,$$

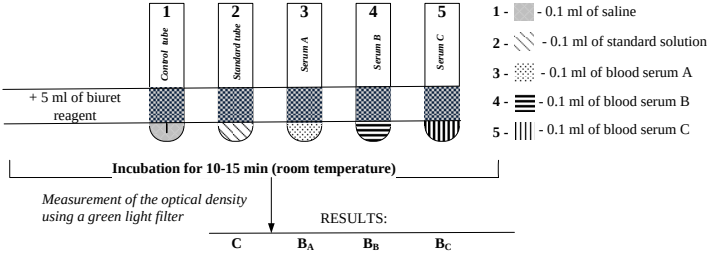
where

X – protein concentration in test solution, (g/l),

A – protein concentration in standard solution (50 g/l),

B – extinction of tested protein solution,

C – extinction of standard protein solution.



Results:

Conclusion:

Clinical and diagnostic significance. In a healthy person the content of protein consists from 60 g/l to 80 g/1. Below 60 g/1 is hypoproteinemia and above 80 g/1 is hyperproteinemia. A decrease in blood protein concentration occurs as a result of a decrease in protein biosynthesis, of water balance disorders, or as a result of an increase in protein breakdown and protein loss. Hypoproteinemia is observed in nephritic syndrome, malabsorption syndrome (enteritis, chronic pancreatitis), exudative enteropathias, skin diseases (combustion, exematous lesions), during massive blood loss, in retention of water and mineral salts (chronic kidney diseases), agammaglobulinemias, hypogamma-globulinemias, and during starvation or improper nutrition.

Decrease in blood protein concentration is observed during cardiac failure as a result of water retention causing a swelling of tissues or during kidney diseases when proteins are excreted in the urine. Protein biosynthesis disturbances take place during cancer cachecsia and during chronic inflammatory diseases, when accompanied by degenerative processes.

Hyperproteinemia is observed in plasmocytoma, Waldenstrom macroglobulinemia, rheumatoid arthritis, collagenoses, liver cirrhosis as well as in diseases accompanied with dehydration, that is during diarrhea, vomiting, and diabetes insipidus. As a result of heavy mechanic lesions (traumas) the increase in blood protein concentration may be due to the loss of a substantial part of the intravascular fluid. In acute infections the increase in blood protein concentration may due to the increased production of acute phase proteins, in chronic infectious diseases it may be due to enhanced synthesis of immunoglobulins.

The report is checked up _____

(The signature of the teacher, date)

Examples of Krock-1 tests

1. Fatty acids are used for the synthesis of many biologically important compounds in human body. Some of them are not synthesized because mammals do not possess the enzymes for their biosynthesis. Which of the following fatty acids is an essential fatty acid?

A. α-Linoleic acid
B. Palmitic acid
C. Oleic acid
D. Stearic
E. Butyric

2. In hydrolysis of proteins and peptides 20 different α-L-amino acids are released that are called proteinogenic amino acids. Furthermore, in humans there are also some other amino acids which are not part of the proteins, select them:

A. Ornithine
B. Alanine
C. Cysteine
D. Methionine
E. Serine

3. Protein molecules are biopolymers that enforce a number of important functions. The enzymatic function of proteins is:

A. Catalysis of biochemical reactions
B. Release of free chemical energy
C. Protection against infection
D. Formation of biological membranes
E. Transport of oxygen

4. Proteins have several levels of structural organization. Hemoglobin is a complex protein which serves as an oxygentransporting protein and has:

A. Quaternary structure
B. α-structure
C. Fibrillar structure
D. Structure similar to collagen
E. Primary structure

5. Which class of proteins by the chemical structure hemoglobin belongs to?

A. Chromoproteins
B. Metalloproteins
C. Phosphoproteins

D. Glycoproteins
E. Lipoproteins

6. Bioorganic high-molecular compounds that consist of residues of α-L-amino acids and connected by amide (peptide) bonds are called:

A. Proteins
B. Carbohydrates
C. Lipids
D. Nucleic acids
E. Heterocyclic compounds

7. What bioorganic compounds are aldehyde and ketone derivatives of polyhydric alcohols?

A. Carbohydrates
B. Proteins
C. Heterocyclic compounds
D. Lipids
E. Nucleic acids

8. What class of bioorganic compounds has a distinctive feature of being soluble in the nonpolar solvents but insoluble in water and other polar solvents?

A. Lipids
B. Carbohydrates
C. Proteins
D. Nucleic acids
E. Heterocyclic compounds

9. Transfer RNAs play an important role in the biosynthesis of protein molecules on ribosomes. The tertiary structure of tRNA is:

A. Cloverleaf
B. Letter S
C. Globular
D. Fibrillar
E. Helix

10. The cyclic compounds which in addition to carbon contain in the ring at least one atom of another element (heteroatom) are called:

A. Heterocyclic compounds
B. Proteins
C. Carbohydrates
D. Lipids
E Nucleic acids

11. What are the products of hydrolysis of trioleylglycerol?

A. Glycerol and three oleic acids
B. Glycerol and three palmitic acids
C. Water and carboxylic acid

D. Glycerol and water
E. Glycerol and base

12. What are polymers of mononucleotides?
A. Nucleic acids
B. Proteins
C. Carbohydrates
D. Lipids
E. Heterocyclic compounds

13. Which homopolysaccharide is the molecular form of glucose storage in the human body?
A. Glycogen
B. Cellulose
C. Amylose
D. Starch
E. Dextran

14. A nucleotide is composed of:
A. Nitrogenous base, pentose and phosphate
B. Hexose, nitrogenous base and phosphate
C. Nitrogenous base and pentose
D. Hexose and phosphate
E. Nitrogenous base and phosphate

15. What qualitative reaction is used to determine the residues of α-amino acids in the protein structure and the free α-amino acids?
A. Ninhydrin test
B. Xanthoproteic test
C. Lead-sulfide test
D. Sakaguchi test
E. Ehrlich test

16. Lipoproteins are particles that consist of noncovalently associated lipids and proteins. What is the main function of lipoproteins in the blood plasma?
A. Transport
B. Plastic
C. Energy
D. Regulatory
E. Catalytic

17. What is the biological function of messenger RNA (mRNA)?
A. mRNA is a matrix for biosynthesis of proteins
B. mRNA transports amino acids
C. mRNA hydrolyzes proteins
D. mRNA catalyzes peptide bonds formation
E. mRNA activates rRNA

18. A nucleoside is composed of:

A. Nitrogenous base and pentose
B. Hexose, nitrogenous base and phosphate
C. Nitrogenous base, pentose and phosphate
D. Hexose and phosphate
E. Nitrogenous base and phosphate

19. What branch of biochemistry studies a chemical composition of living organisms and a structure of bioorganic molecules, which are a part of living matter?

A. Static biochemistry
B. Dynamic biochemistry
C. Functional biochemistry
D. Medical biochemistry
E. Enzymology

20. What branch of biochemistry studies transformation of substances that form in its entirety metabolism of living organisms?

A. Dynamic biochemistry
B. Static biochemistry
C. Functional biochemistry
D. Medical biochemistry
E. Enzymology

21. What branch of biochemistry studies the biochemical reactions in various organs and tissues underlying the physiological functions?

A. Functional biochemistry
B. Static biochemistry
C. Dynamic biochemistry
D. Medical biochemistry
E. Enzymology

22. What branch of biochemistry studies metabolic patterns in normal and pathological conditions (pathochemistry) in the human body?

A. Clinical biochemistry
B. Static biochemistry
C. Dynamic biochemistry
D. Functional biochemistry
E. Enzymology

23. Choose amino acids that are in a fraction of basic amino acids and have positively charged radicals according to the results of chromatography:

A. Lysine, arginine, histidine
B. Aspartate, Glutamate

C. Leucine, isoleucine, methionine
D. Alanine, proline, tyrosine
E. Tryptophan, cysteine, glycine

24. Choose amino acids that are in a fraction of acidic amino acids and have negatively charged radicals according to the results of chromatography:

A. Aspartate, Glutamate
B. Lysine, arginine, histidine
C. Leucine, isoleucine, methionine
D. Alanine, proline, tyrosine
E. Tryptophan, cysteine, glycine

References:

1. Gubsky Yu. Biological chemistry : textbook / edited by Yu. Gubsky. – Vinnytsia : Nova Knyha, 2018. – 2nd edition. – 488 p.
2. Lecture materials.
3. Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – 799 p.
4. Textbook «Biological chemistry. Collection of test tasks» for English-speaking students of higher medical educational institutions of the Ministry of Public Health of Ukraine. – Poltava, 2018. – 148 p.

TOPIC №2.
Structure and physico-chemical properties of enzymes. The employment of special methods for investigation of enzymes in biological material

1. Objective: Life in all its diverse manifestations is an extremely complex system of chemical reactions which are catalyzed by enzymes. The last play vital role in nearly all life processes. They are involved in living organisms in a vast multitude of interrelated chemical reactions such as synthesis, degradation and interconversion of a large number of chemical compounds. An understanding of their implications provides a deep insight into the sense and innermost enigmas of the fascinating phenomenon that we call life.

2. Actuality of the theme: Enzymes are biological catalysts of reactions of a metabolism. Protein nature of enzymes causes their high lability depending on many factors. So, depending on a body temperature of the person, and also on pH of the internal environment of an organism, the activity of enzymes can change that results in the development of pathological processes.

3. Specific aims:

– To interpret biochemical principles of structure and functioning of different classes of enzymes.

– On the basis of physical and chemical properties of enzymes as proteins to explain the dependence of enzymatic activity from pH of medium, temperature and other factors.

4. Reference card for the separate study of educational literature for the lesson preparation

	Questions:	References:
1.	**Enzymes: definition, properties of enzymes as biological catalysts, difference between enzymes and inorganic catalysts.**	1. Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 85. 2. Gubsky Yu. Biological chemistry: textbook / edited by Yu. Gubsky. – Vinnytsia: Nova Knyha, 2018. – 2nd edition. – P. 80–81.
2.	**Nomenclature and classification of enzymes:** ✓ Trival and systematic names of enzymes; ✓ Classification of enzymes according IUB (six classes of enzymes with short characteristics and examples to each one)	1. Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 86–87 2. Gubsky Yu. Biological chemistry: textbook / edited by Yu. Gubsky. – Vinnytsia: Nova Knyha, 2018. – 2nd edition. – P. 84–90.

3.	**Simple and conjugated enzymes. Role of non-protein part of conjugated enzymes** ✓ Prosthetic groups; ✓ Coenzymes; ✓ Cofactors.	1. Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 87, 96–98. 2. Gubsky Yu. Biological chemistry: textbook / edited by Yu. Gubsky. – Vinnytsia: Nova Knyha, 2018. – 2nd edition. – P. 94–99.
4.	**Structure of enzymes: active centres and allosteric sites:** ✓ Structure of active centre, its binding and catalytic sites; ✓ Role of allosteric site	1. Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 87, 91–92. 2. Gubsky Yu. Biological chemistry: textbook / edited by Yu. Gubsky. – Vinnytsia: Nova Knyha, 2018. – 2nd edition. – P. 92.
5.	**Levels of structural organization of enzymes. Multi-enzyme complexes their advantages.**	Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 87, 96–98.
6.	**Specificity of enzymes:** ✓ Stereo-specificity; ✓ Reaction specificity; ✓ Substrate specificity (relative, absolute and broad).	Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 95–96.

5. Tasks for independent work and self-control

5.1. Define the following terms:

a) enzyme

b) catalyst

c) coenzyme

d) prosthetic group

e) cofactor

f) holoenzyme

g) apoenzyme

h) active site

5.2. Complete the table.

Classification of enzymes

№	Class	Type of reactions with examples	Subclasses
1			
2			
3			
4			
5			

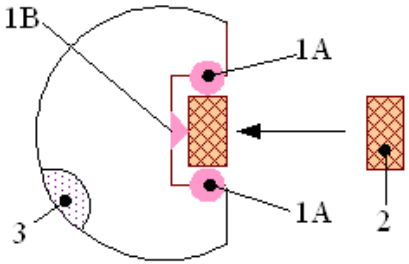
5.3. Select appropriate to enzyme's class type of catalysed reaction and example:

	Enzyme Class		Reaction catalyzed		Example
I	Oxidoreductases	A.	$A + B + ATP \rightarrow A\text{-}B + ADP$	1.	Phosphohexose isomerase
II	Transferases	B.	$A\text{-}B + H_2O \rightarrow AH + BOH$	2.	Glutamine synthetase
III	Hydrolases	C.	$AH_2 + B \rightarrow A + BH_2$	3.	Aldolase
IV	Lyases	D.	$A \rightarrow A'$	4.	Pepsin
V	Isomerases	E.	$A\text{-}B + X\text{-}Y \rightarrow AX\text{-}BY$	5.	Alcohol dehydrogenase
VI	Ligases	F.	$A\text{-}X + B \rightarrow A + B\text{-}X$	6.	Hexokinase

5.4. What components of active sites into simple and conjugated enzymes?

How are active sites formed?

5.5. Find on the picture bellow the structural components of enzyme, its active centre with binding and catalytic sites, allosteric centre and the substrate.



5.6. Describe the following types of enzyme specificity. Give the examples.

A. Substrate specificity:

1) absolute

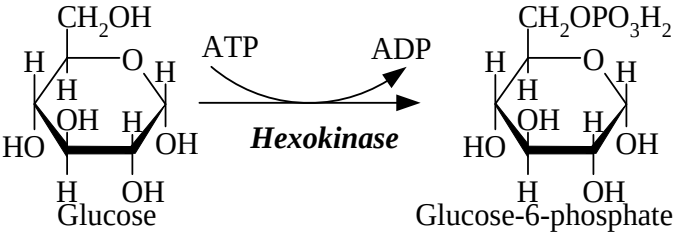
2) relative (group)

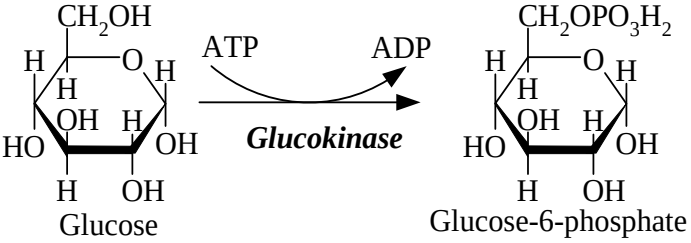
3) broad

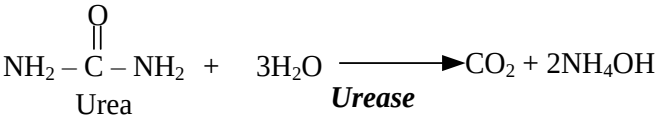
B. Stereochemical specificity.

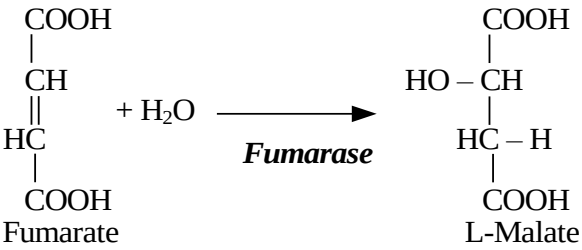
C. Reaction specificity.

5.7. Choose the appropriate type of enzyme specificity (stereo-, relative, absolute, for each enzyme catalyzing the following reactions:

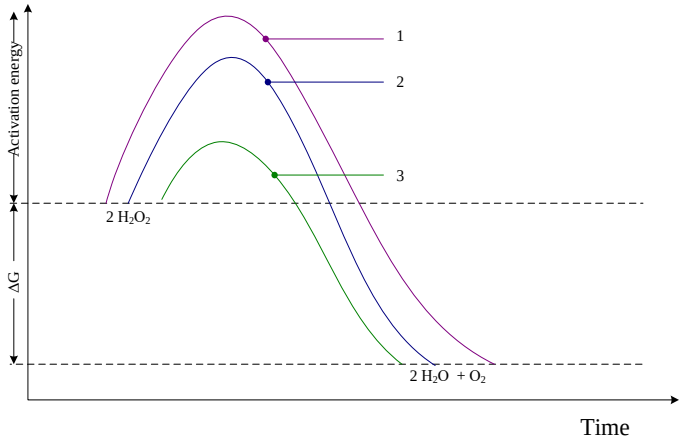








5.8. The picture bellow represents the activation energy for the hydrolysis of hydrogen peroxide. Which of them (1, 2, 3) correspond to spontaneous reaction, the reaction catalyzed by inorganic catalyst and the reaction catalyzed by an enzyme (catalase). Explain your answer.



5.9. Situational tasks:

a) In patients with hypoacidity gastritis found reduce pepsin activity in gastric juice.

To what class of enzymes belongs pepsin?

What kind of reactions catalyzed by this enzyme?

The structure of this enzyme is a simple or complex?

b) In a patient with myocardial infarction found increased activity of lactate dehydrogenase in blood serum

To what class of enzymes belongs lactate dehydrogenase?

What kind of reactions catalyzed by this enzyme?

The structure of this enzyme is a simple or complex?

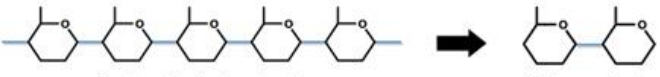
6. Individual independent students work

1. Multi-enzyme complexes and their advantages.

Practice protocol №2 «____» _____ 20___

Salivary amylase

Amylase – is an enzyme that catalyses the hydrolysis of starch into sugars. Amylase is present in the saliva of humans and some other mammals, where it begins the chemical process of carbohydrates digestion. Salivary gland makes amylase to hydrolyse α-1,4-glycosidic bonds of starch into disaccharides (maltose) which are converted by other enzymes to glucose to supply the body with energy. As *diastase*, amylase was the first enzyme to be discovered and isolated (by a. Payen in 1833).



Experiment. Investigation of substrate specificity of salivary amylase and yeast sucrase.

Principle. Enzymes exhibit selectivity to substrates, which is called substrate specificity. In many cases this property is the essential characteristic that distinctly differs enzymes from inorganic catalysts. The high specificity of enzymes depends from the conformational complementarity between the molecules of enzyme and substrate due to the unique structure of active centre of the enzyme.

Method:

I. Specificity of amylase:

1. Add 1 ml of two fold diluted saliva in each of two tubes.
2. Add 2 ml of 1% solution of starch into the first tube.
3. Add 2 ml of 1% solution of sucrose into the second tube.
4. Incubate both tubes at 37^{o}C in thermostat for 15 min.
5. Conduct the Trommer's reaction with the content of both tubes.

Trommer's reaction. Add 5 ml of 5% NaOH and several drops of $CuSO_4$ solution, mix the content of these tubes and heat it on a flame to boiling. The appearance of yellow-red sediment indicates on the presence of reducing substances, namely, reducing disaccharide maltose.

II. Specificty of yeasts sucrase:

1. Add 1 ml of sucrase from yeasts solution in each of two tubes.
2. Add 2 ml of 1% solution of sucrose to the first tube

3. Add 2 ml of 1% starch solution to the second tube.
4. Incubate both tubes at 37°C in thermostat for 15 min.
5. Conduct the Trommer's reaction with the content of both tubes.

III. Note the results of the experiment in the table.

Tube №	*Enzyme*	Substrate	Result of Trommer reaction
1	*Amylase*	Starch	
2	*Amylase*	Sucrose	
3	*Sucrase*	Sucrose	
4	*Sucrase*	Starch	

Conclusion:

Clinical and diagnostic significance. Amylase (E. C. 3.2.1.1) hydrolyzes starch and dextrins. It is produced predominantly in salivary and pancreatic glands, although amylase activity can be detected in tissues of liver, kidneys, intestines and lungs. During normal conditions amylase activity in blood serum corresponds to 0.42–0.96 g of starch, hydrolyzed by 1m1 of enzyme in 1 minute.

An increase of amylase activity in serum is observed in pancreatitis, peritonitis, mesenteric vessels thrombosis, rupture of oviduct in case of extrauterine pregnancy and after the injection of some drugs, e.g. morphine, caffeine, ACTH and cortisol. The increase of the activity of amylase in saliva is observed in renal insufficiency, stomatitis, neuralgia. A decrease in amylase activity is noted in some cases of psychoses, accompanied by depression or excitation and in gastric secretion disorders (anaciditas).

The report is checked up _____
(The signature of the teacher, date)

Examples of Krock-1 tests

1. Which enzymes catalyze the conversion of proline to hydroxyproline and lysine to hydroxylysine in the collagen molecule?

A. Hydroxylases
B. Hydrolases
C. Dehydrogenases
D. Oxidases
E. Dehydratases

2. The enzyme oxidase of D-amino acids only catalyzes a deamination of D-amino acids. Which property of enzymes is shown in this case?

A. Stereochemical specificity
B. Thermolability
C. Relative specificity
D. Dependence on pH
E. Absolute specificity

3. What is a region of an enzyme where substrate molecules bind and undergo a chemical reaction called?

A. Active site
B. Allosteric Site
C. Inhibitor
D. Holoenzyme
E. Activator

4. The absolute specificity of enzymes is only an ability to catalyze a conversion of:

A. One substrate
B. One of stereoisomers
C. One specific type of a chemical bond
D. One specific group of substrates
E. Many substrates

5. The relative specificity of enzymes is only an ability to catalyze a conversion of:

A. A group of substrates that have the same chemical structure
B. Specific stereoisomers
C. One metabolic process
D. One substrate
E. Two substrates

6. A lot of enzymes consist of several subunits (protomers) that are joined by noncovalent bonds. What are they called?

A. Oligomeric enzymes
B. Coenzymes

C. Apoenzymes
D. Multienzyme complexes
E. Isoenzymes

7. What is a non-protein part of enzyme called?

A. Coenzyme
B. Apoenzyme
C. Activator
D. Holoenzyme
E. Inhibitor

8. What is a protein part of the enzyme called?

A. Apoenzyme
B. Coenzyme
C. Activator
D. Inhibitor
E. Holoenzyme

9. What is a non-protein part of enzyme covalently binding with the protein part called?

A. Prosthetic group
B. Coenzyme
C. Activator
D. Inhibitor
E. Holoenzyme

10. Enzymes speed up chemical reactions due to:

A. Reduction of activation energy
B. Increasing of activation energy
C. Transition of molecules in an active state
D. The formation of additional bonds
E. The formation of covalent bonds

11. What is a chemical nature of enzymes?

A. Protein
B. Lipid
C. Polysaccharide
D. Nucleic acid
E. Vitamin

12. What are substrates of proteolytic enzymes?

A. Proteins
B. Carbohydrates
C. Vitamins
D. Lipids
E. Nucleic acids

13. What are common properties between enzymes and inorganic catalysts?

A. They catalyse thermodynamically possible reactions only
B. Their activity is dependent upon pH
C. Specificity of action
D. Dependence on an amount of substrate
E. Dependence on an effector's action

14. What are functions of enzymes connected with?
A. Increasing of chemical reactions
B. Decreasing of chemical reactions
C. Decrease in the pH optimum of the chemical reactions
D. Decrease in the temperature optimum of chemical reactions
E. Neutralization of products of chemical reactions

15. What properties do enzymes have?
A. Thermolability
B. The small molecular weight
C. Luminescence
D. Resistance to the effects of heavy metal salts
E. Thermostability

16. What is a role of an active site of an enzyme?
A. Binding and conversion of substrate
B. Attaching of enzymes to the membrane
C. Regulation of enzyme's activity
D. Interaction between enzymes
E. Binding of allosteric effectors

17. What determines electrochemical properties of enzymes?
A. Qualitative and quantitative composition of amino acids
B. Presence of a peptide bond
C. Denaturation
D. Renaturation
E. Presence of protomers of protein molecules

18. What determines a solubility of an enzyme?
A. The ratio of hydrophobic and hydrophilic amino acid radicals in the structure of an enzyme
B. Molar mass of the solvent
C. Concentration of a solvent
D. Atomic composition of a solvent
E. The presence of a proper catalyst

19. The enzyme catalyzes the transfer of a functional group from one substrate to another one. Specify a class of the enzyme:
A. Transferases
B. Hydrolases
C. Isomerases
D. Oxidoreductases
E. Ligases

20. Biogenic amines are produced by decarboxylases. What a class of enzymes decarboxylases belong to?

A. Lyases
B. Isomerases
C. Oxidoreductases
D. Hydrolases
E. Transferases

21. Glucokinase catalyzes a reaction of a transfer of a phosphate group from ATP to glucose. What a class of enzymes glucokinase belongs to?

A. Transferases
B. Oxidoreductases
C. Isomerases
D. Hydrolases
E. Lyases

22. Biological oxidation is a main energy molecular process. Which class of enzymes catalyzes this process?

A. Oxidoreductases
B. Hydrolases
C. Lyases
D. Ligases
E. Transferases

23. How many numbers are in the enzyme code by the systematic nomenclature?

A. Four
B. Six
C. Three
B. Five
E. Seven

24. What class of the enzymes aerobic dehydrogenases belongs to?

A. Oxidoreductases
B. Lyases
C. Transferases
D. Hydrolases
E. Ligases

25. What class of the enzymes protein kinases belongs to?

A. Transferases
B. Lyases
C. Oxidoreductases
D. Hydrolases
E. Ligases

26. What class of the enzymes pepsin belongs to?

A. Hydrolases
B. Lyases
C. Oxidoreductases
D. Transferases
E. Ligases

27. What are enzymes catalyzing reactions of an intramolecular transfer of groups or atoms called?

A. Isomerases
B. Ligases
C. Hydrolases
D. Transferases
E. Oxidoreductases

28. According to the type of a chemical reaction all enzymes are divided into:

A. Six classes
B. Nine classes
C. Five classes
D. Seven subclasses
E. Seven classes

29. What are enzymes catalyzing the splitting of the intramolecular bonds of organic substances by the use of water called?

A. Hydrolases
B. Ligases
C. Lyases
D. Oxidoreductases
E. Transferases

30. What are enzymes?

A. Biocatalysts of protein nature
B. Structural components of biological membranes
C. Inorganic catalysts
D. Microelements that increase a rate of chemical reactions
E. Organic molecules of a non-protein nature

31. Monooxygenases belong to the class of:

A. Oxidoreductases
B. Lyases
C. Transferases
D. Hydrolases
E. Ligases

32. What principle is used to classify all enzymes into six classes?

A. The type of the chemical reaction
B. The type of bonds
C. The type of the substrate
D. Mechanism of an enzyme action
E. The type of the product

33. What nomenclature is used to give a name to the enzymes in our days?

A. Systematic
B. Usual
C. Common
D. United
E. The main

34. The enzymes of the cytochrome system participate in:

A. Transfer of electrons
B. Transfer of hydrogen atoms
C. Transfer of oxygen atoms
D. Transfer of proteins
E. Transfer of molecules

35. Reactive oxygen species, including superoxide radical, are produced in the human body. Which enzyme is necessary for the inactivation of superoxide radical?

A. Superoxide dismutase
B. Catalase
C. Glutathione
D. Peroxidase
E. Glutathione peroxidase

36. Enzyme sucrase breaks down sucrose to glucose and fructose with water molecule participation. Which class is this enzyme concerned to?

A. Hydrolases
B. Isomerases
C. Lyases
D. Ligases
E. Oxidoreductases

37. Peptidases catalyze the splitting of:

A. Polypeptides
B. Nucleic acids
C. Polysaccharides
D. Lipids
E. Oligosaccharides

38. What enzyme belongs to peptidases?

A. Trypsin
B. ATP-ase
C. RNA-polymerase
D. Amylase
E. Urease

39. Specify the class of enzymes that uses ATP energy for the synthesis of new bonds:

A. Ligases
B. Oxidoreductases
C. Hydrolases
D. Isomerases
E. Transferases

40. The first position in classification of enzymes is occupied by:

A. Oxidoreductases
B. Transferases
C. Isomerases
D. Hydrolases
E. Ligase

41. Indicate a class of enzymes, which performs the process of phosphorylation of substrates:

A. Transferases
B. Oxidoreductases
C. Isomerases
D. Lyases
E. Ligases

42. In a patient was detected disorder in digestion of protein in stomach and small intestines. What group of enzymes may cause this disorder?

A. Proteinases
B. Amylase
C. Lipase
D. Lyases
E. Aminotransferases

43. Which of the below mentioned properties is characteristic only for biologic catalysts?

A. Ability to regulation
B. Increase a velocity of reaction, decreasing energy of activation
C. Don’t change the state of equilibrium of chemical reaction
D. Increase a velocity of reaction, but are not consumed and aren’t irreversibly changed
E. Increase a velocity of reaction, inreasing energy of activation

44. After applying the extract from pancreas into a tube with a solution of starch, it was observed a decline of blue staining in the sample with a solution of iodine, which indicates to hydrolysis of starch. Under the influence of what pancreatic enzyme it happens?

A. α-Amylase
B. Chymotrypsin
C. Lipases
D. Aldolases
E. Trypsin

45. Indicate the substrate of salivary amylase:

A. Starch
B. Protein
C. Sucrose
D. Glucose
E. Amino acid

46. Enzymes of the class of lyases are able to catalyze the type of reactions:

A. Decarboxylation
B. Hydrolysis
C. Oxidation
D. Reduction
E. Transamination

47. Give the full name of conjugated enzyme, polypeptide chains of which are combined with nonprotein part:

A. Holoenzyme
B. Prosthetic group
C. Cofactor
D. Coenzyme
E. Apoenzyme

48. A reaction mixture gives yellow colour with iodine and positive Fellinge's reaction after 10 minutes of incubation of starch with saliva. The mixture contains:

A. Maltose and dextrins
B. Fructose and glucose
C. Sucrose
D. Lactose
E. Galactose

49. D-oxidase of alanine is able to deaminize of D-alanine only, but it doesn't break down the structure of L-alanine. Give the type of specificity of this enzyme:

A. Stereochemical
B. Absolute
C. Absolute group
D. Relative group
E. All answers

50. Yeast extract was added to the test-tube with unknown substrate. Mixture in the test-tube gave the positive reaction of Fellinge after 15 minutes of incubation. Which substrate was in the test-tube?

A. Sucrose
B. Starch
C. Lactose
D. Glycogen
E. Cellulose

51. Call the enzyme, which belongs to oxidoreductases:

A. Catalase
B. Peptidase
C. Amylase
D. Lipase
E. Lactase

52. Oxidoreductases do not catalyze the reactions of:

A. Dehydration
B. Electron transfer
C. Dehydrogenation
D. Oxygen incorporation
E. Hydrogen peroxide destruction

53. Call the reaction, which is catalyzed by enzymes from transferases class:

A. Amino group carrying from alanine to α-ketoglutarate
B. Taking of carboxyl group from pyruvate
C. Electron transfer from cytochrome oxydase to oxygen
D. Water combining with fumarate
E. Glucose conversion to galactose

54. The enzymes, which belong to III class are:

A. Amylase, pepsin, lipase
B. Trypsin, catalase, arginase
C. Elastase, lactate dehydrogenase, thiolase
D. Glycogen phosphorylase, gastrixin, maltase
E. Citrate synthase, pyruvate carboxylase, sucrase

55. Enzymes are not characteristic by:

A. Photolability
B. Specificity
C. Thermolability

D. Ability to regulation
E. pH-lability

56. Enzymes, consisting of some polypeptide chains, are called:
A. Olygomeric
B. Thermolabile
C. Allosteric
D. Isomeric
E. Monomeric

57. The structure of the active site of a simple enzyme contains only:
A. Amino acid radicals
B. Co-substrates
C. Radicals of the allosteric site
D. Coenzymes
E. Radicals of inhibitors in complex with ions of metals

58. Enzymes of V class catalyze reactions of:
A. Isomerization
B. Oxydation
C. Intermolecular carrying of chemical groups and radicals
D. Reduction
E. Hydrolysis

59. What is a membrane-bound multienzyme complex?
A. Specific enzymes are bound with the lipid bilayer of subcellular organelles
B. Specific enzymes are bound together by hydrogen bonds
C. Specific enzymes are not themselves bound
D. An enzyme is consisted of several protomers
E. Certain enzymes are bound together by covalent bonds

References:

1. Gubsky Yu. Biological chemistry: textbook / edited by Yu. Gubsky. – Vinnytsia : Nova Knyha, 2018. – 2nd edition. – 488 p.
2. Lecture materials.
3. Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – 799 p.
4. Textbook «Biological chemistry. Collection of test tasks» for English-speaking students of higher medical educational institutions of the Ministry of Public Health of Ukraine. – Poltava, 2018. – 148 p.

TOPIC №3.
Research of the mechanism of enzymes action and the kinetics of ferment catalysis. Principles of determination and units of the enzymatic activity

1. Objective: To learn the basic properties of enzymes, kinetics of enzymatic reactions to apply this knowledge to explain the role of enzymes in providing functioning of the body and biomedical practice.

2. Actuality of the theme: The complex structure and the functional organization of enzymes in part is a key to understanding of characteristic properties of enzymes – high specificity and velocity of catalysis. Classical works of Michaelis and Menten who have developed regulations about enzyme – substrate complexes have played the great role in development of representations about the mechanism of enzymes action.

3. Specific aims:

– To analyze values of the activity of enzymes in blood plasma in dependence from their localization in the cell, tissue or organ.

– To explain the features of kinetics and energetic of enzymatic reactions.

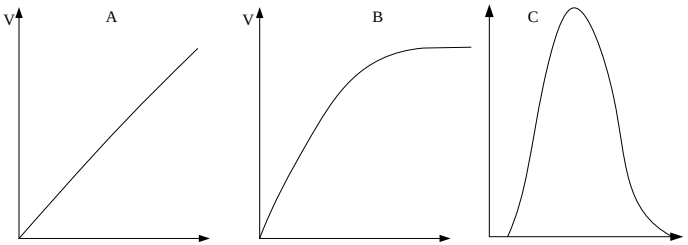
4. Reference card for the separate study of educational literature for the lesson preparation

	Questions:	References:
1.	**Enzyme kinetics. Factors affecting enzymatic activity:** ✓ definition of enzyme kinetics; ✓ plot of dependence of enzymatic reaction velocity on concentration of enzyme (explain it); ✓ plot of dependence of enzymatic reaction velocity on concentration of substrate (explain it); ✓ plot of dependence of enzymatic reaction velocity on temperature (explain it); ✓ plots of dependence of enzymatic reaction velocity on effect of pH for different enzymes (explain them).	1. Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 88–90. 2. Gubsky Yu. Biological chemistry: textbook / edited by Yu. Gubsky. – Vinnytsia: Nova Knyha, 2018. – 2nd edition. – P. 83–84, 100.

2.	**Michaelis-Menten kinetics:** ✓ Michaelis-Menten model of enzymatic reaction ✓ Michaelis-Menten equation; ✓ Biological meaning of Michaelis constant.	1. Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 88–89 2. Gubsky Yu. Biological chemistry: textbook / edited by Yu. Gubsky. – Vinnytsia: Nova Knyha, 2018. – 2nd edition. – P. 101–102.
3.	**Mechanisms of enzyme catalysis.**	1. Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 99–100. 2. Gubsky Yu. Biological chemistry: textbook / edited by Yu. Gubsky. – Vinnytsia: Nova Knyha, 2018. – 2nd edition. – P. 91–92.
4.	**Units of enzymatic activity:** ✓ katal; ✓ International unit	1. Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 104–106. 2. Gubsky Yu. Biological chemistry: textbook / edited by Yu. Gubsky. – Vinnytsia: Nova Knyha, 2018. – 2nd edition. – P.82.

5. Tasks for independent work and self-control

5.1. Which of the following curves describes Michaelis-Menten equation: line – A, a hyperbola – B or a parabola – C?



5.2. Describe the stages of enzyme-catalyzed reaction according the common equation:

$E + S \leftrightarrow ES^* \rightarrow EP \rightarrow E + P$

a) Which stage is reversible?

b) Which ones are irreversible?

c) Which stage is slow?

d) Which ones are quick?

e) What bonds are formed between enzyme and substrates?

5.3. Situational tasks:

a) In patients with chronic gastritis observed decrease in activity of pepsin, gastric juice pH is 5.0. Explain the reason for decreased activity of pepsin.

Reason for such patients previously administered to take weak solution of hydrochloric acid before meals?

What type specificity typical for this enzyme?

b) At acute pancreatitis activation of proteolytic enzymes (trypsin, chymotrypsin) in pancreas taking place. To prevent autolysis of the pancreas at the preclinical stage recommended starvation and cooling of the abdominal wall in the pancreas region.

What can explain necessity of use these measures?

What kind of specificity typical for pancreatic enzymes?

In what units activity of trypsin measured in the serum in SI?

6. Individual independent students work

1. The employment of enzymes in biochemical investigations.

Practice protocol №3 «____» _____ 20___

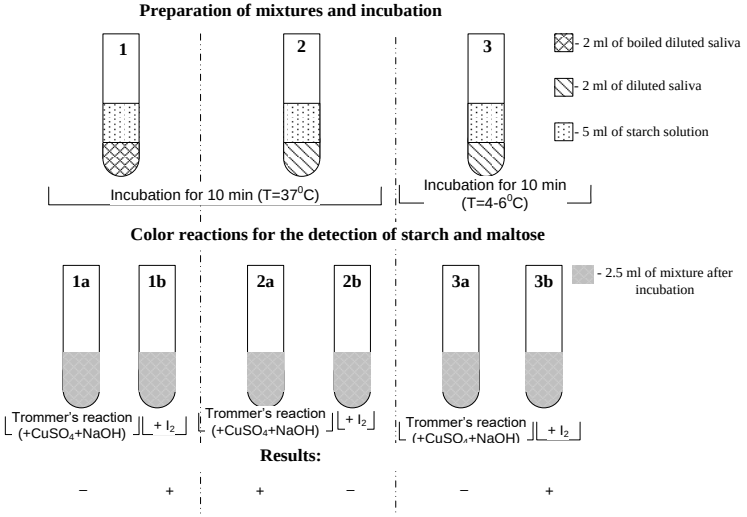
Experiment 1. Investigation of the thermolability of salivary amylase.

Principle. Salivary amylase is a protein and consequently is thermosensitive. Boiling inactivates enzymes due to denaturation, low temperature (near 0°C) substantially lowers the rate of reaction. The hydrolysis of starch is monitored by probes with iodine and the appearance of the of maltose, which is the final product of starch hydrolysis by salivary amylase. Starch with iodine gives a blue coloured complex. Dextrins (intermediate products of starch hydrolysis) in presence of iodine form red-brown colour or no change in colour at all. Maltose also does not form a coloured complex with iodine, but it can be detected with the Trommer's reaction.

Method.

I. Dilution of saliva. To obtain diluted saliva (the source of amylase), rinse one's mouth with distilled water during 1–2 min and collect that portion of fluid (saliva + water) into separate tube and use it for the analysis.

II. Preparation of experimental mixtures and incubation. Take 3 clean tubes and do the following procedures:



1. Add 2 ml of diluted saliva to the first tube and boil it for two min on a flame.
2. Add 2 ml of diluted (nonboiled) saliva to the second and the third tubes.

3. Add 5 ml of starch solution into each tube.
4. Place the first and the second tubes into the termostate at 37°C for 10 min.
5. Place the third tube in a cold water (near 4-6°C) for 10 min.
6. Divide the content of each tube into two equal portions.

III. Colour reactions for the detection of starch and maltose.

1. Add 3 drops of iodine to the first portion of each test solution. Register changes in colour.

2. Use the second portion of the probes for the performance of the Trommer's reaction.

Conclusion:

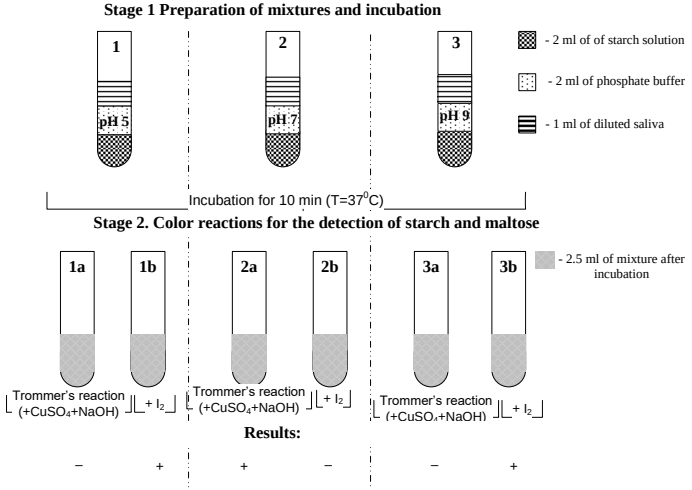
Experiment 2. The investigation of the influence of pH of medium on amylase activity.

Principle. Each enzyme has an optimum pH at which the velocity is maximum. Below and above this pH, the enzyme activity is much lower and at extreme pH, the enzyme becomes totally inactive. Most of the enzymes of higher organisms show optimum activity around neutral pH (6–8).

Method.

I. Dilute saliva as described above (Experiment 1)

II. Preparation of experimental mixtures and incubation. Take 3 clean tubes and do the following procedures:



1. Add 2 ml of starch solution to each of the three tubes.
2. Add to the first tube 2 ml of phosphate buffer, pH 5.0.
3. Add to the second 2 ml of phosphate buffer, pH 7.0.
4. Add to the third 2 ml of phosphate buffer, pH 9.0.
5. Add into each tube 1 ml of diluted saliva.
6. Incubate tubes at 37^oC for 10 minutes.
7. Divide the content of each tube into two equal portions.

III. Colour reactions for the detection of starch and maltose.

1. Add 3 drops of iodine to the first portion of each test solution. Register changes in colour.

2. Use the second portion of the probes for the performance of the Trommer's reaction (see Experiment 1).

Conclusion:

The report is checked up _____
(The signature of the teacher, date)

Examples of Krock-1 tests

1. Metabolic acidosis was developed in the patient. An activity of enzymes was decreased, because of:

A. A charge of enzymes was changed;
B. Activity of mitochondrial enzymes was inhibited and an increased activity of lysosomal enzymes led to the activation of metabolic processes;
C. An activity of intracellular enzymes didn't change significantly;
D. There was a total inhibition of tissue enzymes;
E. There was a total activation of tissue enzymes.

2. In accordance with the Kosland theory, the substrate is able to induce changes in the configuration of an enzyme molecule in accordance with its structure and as a result, binding with the substrate molecule occurs in the active site of an enzyme. What is not a function of the active site?

A. Interaction with effectors;
B. Formation of an enzyme-substrate complex;
C. Catalytic transformation of the substrate;
D. Specific binding to the substrate;
E. –.

3. Specify an enzyme with optimal pH = 11.0:

A. Arginase;
B. Catalase;
C. Lipase;
D. Pyruvate dehydrogenase;
E. Collagenase.

4. Specify an enzyme with optimal pH = 2.0:

A. Pepsin;
B. Trypsin;
C. Chymotrypsin;
D. α-Amylase;
E. Collagenase.

5. The absence of a change in the rate of the enzymatic reaction with increasing substrate concentration is associated with:

A. Saturation of an active site;
B. Denaturation of the enzyme;
C. Saturation of allosteric site;
D. Blocking of the allosteric site;
E. Increase in the temperature of the medium.

6. How does the increasing of enzyme concentration effect enzyme reaction rate?

A. It proportionally increases;
B. It proportionally reduces;
C. It doesn't change;
D. It increases and then remains constant;
E. It decreases and then remains constant.

7. What happens with enzymes by the action of a high temperature?

A. Denaturation;
B. Hydrolysis;
C. Formation of an enzyme-substrate complex;
D. Blocking of an active site;
E. Violation of a primary structure.

8. At what temperature does an inactivation of enzymes begin as a result of their denaturation?

A. 50-60°C;
B. 39°C;
C. 80°C;
D. 100°C;
E. 40°C.

9. Saliva contains an enzyme that catalyses the hydrolysis of α-1,4-glycosidic bonds of starch. What is the enzyme called?

A. Amylase;
B. Lactase;
C. Nuclease;
D. Lysozyme;
E. Peptidase.

10. The Michaelis constant is the substrate concentration when the velocity of the reaction is:

A. A half of the maximum;
B. Minimum;
C. Maximum;
D. A half of the minimum;
E. One-third of the maximum.

11. A patient has a reduced gastric acidity and the violated digestion of proteins in the stomach. What is the enzymatic property observed in this case?

A. The pH dependence of an enzymaticactivity;
B. Specificity of the enzymes;
C. Thermolability of enzymes;
D. Denaturation of enzymes;
E. Effect of inhibitors on an enzymatic activity.

12. Enzymes are placed in the cell in such way to ensure the performance of the functions of certain organelles. Which of the following enzymes are the lysosomal ones?

A. Hydrolytic enzymes;
B. Enzymes of protein synthesis;
C. Enzymes for the synthesis of urea;
D. Fatty acid synthesis enzymes;
E. Glycogen synthesis enzymes.

13. The enzyme inactivation under its heating till 100^{o}C is caused by:

A. Denaturation;
B. Decarboxylation;
C. Renaturation;
D. Phosphorylation;
E. Desamination.

14. An international unit for an enzyme activity is katal, defined as an amount of enzyme that transforms:

A. 1 mol of the substrate per 1 second;
B. 1 gram of the substrate per 1 second;
C. 1 gram of the substrate per 1 hour;
D. 1 mol of the product per 1 hour;
E. 1 μmol of the substrate per 1 second.

15. What phenomenon is the basis of the mechanism of enzyme's action?

A. Formation of an enzyme-substrate complex;
B. Approximation of functional groups that enter the active site of the enzyme;
C. Changing of the spatial configuration;
D. Changing of the enzyme charge;
E. Hydrolysis of the enzyme.

References:

1. Gubsky Yu. Biological chemistry: textbook / edited by Yu. Gubsky. – Vinnytsia : Nova Knyha, 2018. – 2nd edition. – 488 p.
2. Lecture materials.
3. Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – 799 p.
4. Textbook «Biological chemistry. Collection of test tasks» for English-speaking students of higher medical educational institutions of the Ministry of Public Health of Ukraine. – Poltava, 2018. – 148 p.

TOPIC №4.
Regulation of enzymatic activity and mechanisms of enzymopathias

1. Objective: To learn the main principles of regulation of metabolic pathways, the consequences of alteration of enzymatic activity in the cell.

2. Actuality of the theme: Enzymes are biocatalysts with changeable activity, submitted to regulatory influences. Estimation of enzymatic activity is routinely used in laboratory investigations with diagnostic purposes.

3. Specific aims:

✓ To analyze pathways and mechanisms of regulation of enzymatic reactions as a background of metabolism in health and disease.

✓ To explain the application of activators and inhibitors of enzymes as medicines and pharmaceuticals for correction of metabolic disorders in pathology.

4. Reference card for the separate study of educational literature for the lesson preparation

	Questions:	References:
1.	**Enzyme inhibition:** ✓ reversible (competitive, non-competitive); ✓ irreversible. Each type of inhibition should be defined, explained, given examples, shown on plots.	1. Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 92–95. 2. Gubsky Yu. Biological chemistry: textbook / edited by Yu. Gubsky. – Vinnytsia: Nova Knyha, 2018. – 2nd edition. – P. 103–107.
2.	**Regulation of enzyme activity in the living system:** ✓ allosteric regulation; ✓ feedback regulation; ✓ covalent modification; ✓ activation of latent enzymes by limited proteolysis.	1. Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 100–103. 2. Gubsky Yu. Biological chemistry: textbook / edited by Yu. Gubsky. – Vinnytsia: Nova Knyha, 2018. – 2nd edition. – P. 107–112.
3.	**Control of enzymes synthesis:** ✓ constitutive enzymes; ✓ adaptive enzymes; ✓ induction and repression.	1. Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 104. 2. Gubsky Yu. Biological chemistry: textbook / edited by Yu. Gubsky. – Vinnytsia: Nova Knyha, 2018. – 2nd edition. – P. 112–113.

5. Tasks for independent work and self-control

5.1. Complete the table

Activation of proenzyme

Active enzyme	Proenzyme (zymogen)	Mechanism of activation
Pepsin		
Chymotrypsin		
Trypsin		
Thrombin		
Plasmin		

5.2. Write the reactions of enzyme molecule phosphorylation and dephosphorylation.

a) What enzymes catalyze these reactions?

b) What amino acids residues of enzymes are phosphorylated?

c) How do phosphorylation or dephosphorylation influence on enzyme activity?

d) Is this way of enzyme activity regulation reversible or irreversible? Explain the answer.

e) Give examples of enzymes which activity is regulated by phosphorylation and dephosphorylation.

5.3. Allosteric regulation of enzyme activity.

a) Where is placed allosteric site of enzyme?

b) Effect of allosteric modulator binding on enzyme activity?

c) Mechanism of allosteric regulation.

d) What substances can serve as allosteric modulators (effectors)?

5.4. Regulation of enzyme quantity (genetic control).

Define the following terms:

constitutive enzymes

adaptive enzymes

enzyme induction

enzyme repression

5.5. Describe different inhibitors:

Inhibitor(s)	Enzyme (group of enzymes)	Substrate(s)	Type of inhibition	Significance/ usage of inhibitor(s)
Malonic acid				
Sulfa drugs	Dihydropteroate synthase	PABA		
Methotrexate, aminopterin	Dihydrofolate reductase	Dihydrofolic acid		
Allopurinol	Xanthine oxidase	Xanthine		
Lovastatin	HMG-CoA Reductase	HMG-CoA		
Captopril, enalapril	Angiotensin-converting enzyme	Angiotensin I		
Succinylcholine Organophosphates DFP etc.)	Acetylcholine esterase	Acetylcholine		
Iodoacetate, heavy metal ions	Enzymes with HS-groups in active site	Different		
Cyanides	Cytochrome oxidase	O_2		
Disulfiram (antabuse)	Aldehyde dehydrogenase	Acetaldehyde		
Penicillin	Transpeptidase	Glycopeptide		
Aspirin	Cyclooxygenase	Arachidonic acid		
Allosteric inhibitors	Allosteric enzymes	Different		

5.6. Complete the table

Enzyme inhibitors

	Competitive inhibitors	**Noncompetitive inhibitors**	**Irreversible inhibitors**
Examples of inhibitors			
Structural similarity of substrate and inhibitor (yes/no)			
Competition with substrate (yes/no)			
Binding site on the enzyme molecule (where?)			
Type of bonds between enzyme and inhibitor			
Reversibility of action (yes/no)			

5.7. The inhibitor X decreases the activity of enzyme Y by 70 %. The increase of the concentration of the substrate S returns up to 60 % of Y activity. Which type of inhibition represents inhibitor X?

5.8. Situational tasks

a) The patient after stroke appointed proserin among other drugs to restore muscle mobility.

Which inhibits enzyme activity proserin?

What type inhibitors it belongs?

Concentration of which metabolite (neurotransmitters) in the muscles will grow under the action proserin?

b) After receiving sulfanilamides patient appeared abdominal distension and diarrhea due to violation of intestnal microflora (dysbiosis).

What is the mechanism underlying the bactericidal action of sulphanilamides?

What type inhibitors belong sulfanilamide drugs?

What vitamin expedient to assign patient?

6. Individual independent students work

1. Regulators of enzymatic activity and their employment in clinical practice.

Practice protocol №4 «____» _____ 20___

Experiment 1. Study of the influence of activators and inhibitors on activity of salivary amylase.

Principle. Compounds, which enhance the activity of enzymes – called activators – are a number of metal ions, e.g. Na^+, Mg^{2+}, Mn^{2+}, Co^{2+}, as well as organic substances, especially metabolic intermediates. Amylase is activated by sodium chloride (NaCl), inhibited – by copper sulphate ($CuSO_4$). As indicator of the influence of mentioned compounds on the activity of amylase is a degree of starch cleavage under the action of amylase in the presence of NaCl or $CuSO_4$.

Method.

I. Dilution of saliva. Dilute saliva two fold.

II. Preparation of experimental mixtures and incubation. Take 3 clean dry tubes and do the following procedures:

1. Add 1 ml of distilled water into the first tube.
2. Add 0.8 ml of distilled water and 0.2 ml of 1% NaCl into the second tube.
3. Add 0.8 ml of distilled water and 0.2 ml of 1% solution of $CuSO_4$ into the third tube.
4. Add 1 ml of diluted saliva into each tube.
5. Add 2 ml of 1% starch solution into each tube. Mix them well and place tubes to thermostat at 37°C during 15 min.

III. Colour reaction. Add 0.1% solution of iodine in 0.2% sodium iodide. Changes in colour development are observed and registered in a table (see below).

Tube content	№ of tube		
	1	2	3
Water (ml)	1	0.8	0.8
NaCl, 1%-solution (ml)	–	0.2	–
$CuSO_4$, 1%-solution (ml)	–	–	0.2
Saliva 2 fold diluted (ml)	1	1	1
Starch, 1%-solution (ml)	2	2	2
Final colour after addition of iodine			

Conclusion:

Clinical and diagnostic significance. Inhibitors of enzymes are widely used in medicine as drugs and medicinals, e.g. acetylosalicylic acid (aspirin) is an inhibitor of cyclooxygenase (prostaglandin syntase) and employed as anti-inflammatory drug. Trasylol (inhibitor of trypsin) and contrical – inhibitor of different proteinases – (one of them are kallikreins) are used in treatment of pancreatitis, allopurinol – inhibitor of xanthine oxidase – is used for treatement of gout, etc.

Experiment 2. The influence of phosphacol and calcium ions on activity of cholinesterase.

Principle. Organic phosphate compounds are irreversible inhibitors of cholinesterase and acetyl-cholinesterase due to irreversible binding with an active site of enzyme and suppress its activity. Preparations of phosphorganics are strong poisons for insects (pesticides) as well as for higher animals. Mechanism of inhibitory action consists in covalent binding with OH group of serine in active center of enzyme.

In conduction of nerve excitation an increase of calcium ions in nerve ending occurs and this is a signal for release of acetylcholine into the synaptic cleft and subsequent interaction with acetylcholine receptors of postsynaptic membrane and hydrolytic cleavage by cholinesterase. Besides, calcium ions are strong acticvators of cholinesterase.

Method of quantitative determination of cholinesterase is based on the titration with alkali the acetic acid, which is liberated after acetylcholine

hydrolysis. The quantity of alkaline solution, expended for titration, is a measure of enzyme activity.

Method. Take three clean dry tubes and add reagents according to the table:

Reagents in tubes	№ of tube		
	1	2	3
Blood serum (ml)	0.5	0.5	0.5
0.5% solution of $CaCl_2$,(drops)	–	5	–
0.5% solution of phosphacol (drops)	–	–	5
Incubation at room temperature 5 min			
2% solution of acetylcholine (ml)	1.5	1.5	1.5
Incubation for 10 min			
Phenolphthaleine (drops)	2	2	2
Quantity of 0.1 M NaOH expended for titration, ml			

Conclusion:

Clinical and diagnostic significance. Cholinesterase catalyses hydrolysis of acetylcholine, a known neuromediator with formation of choline and acetic acid. In human blood there are two forms of cholinesterase. In blood plasma is present nonspecific cholinesterase (EC 3.1.1.8), which cleaves not only acetylcholine, but also other esters of choline. In red blood cells there is a specific, or true, cholinesterase, which cleaves acetylcholine only (EC 3.1.1.7).

In normal conditions the activity of cholinesterase is about 44.4–94.4 ucat/l (colourimetric method according to hydrolysis of acetylcholine chloride).

Physiologically active substances, which are inhibitors of cholinesterase, have an important pharmacological and toxicological significance, as they cause a significant increase in concentration of neuromediator in some parts of CNS, as well as in body in general. Reversible inhibitors of acetylcholine esterase are employed in medicine for enhancement of cholinergic impulsation, which is alterede in some neurological diseases, e.g. atonia of intestines or bladder. In these cases are used preparations proserine, physostigmine, galantamine.

Irreversible inhibitors of acetylcholinesterase are potent neurotoxins, which causes a marked excitation of nerve system manifested as convultions, disorders of cardiovascular, digestive and other systems of the organism.

The most wide spreded irreversible inhibitors are organic phosphates. They are employed for killing of harmfull insects (dichlophos, chlorophos, metaphos, etc.). Neuroparalytic toxins which are used as warfare, are zarine, soman, tabun and others.

High sensitivity of cholinesterase to this phosphorganic compounds permits to use this enzyme as a marker for detection of traces of phosphorganic compounds in human body.

The report is checked up _____
(The signature of the teacher, date)

Examples of Krock-1 tests

1. Azaserine is a structural analogue of glutamine. It is inhibitor of purine nucleotide biosynthesis. Which type of inhibition is characteristic of this drug?

A. Competitive
B. Irreversible
C. Noncompetitive
D. Incompetitive
E. Allosteric

2. The activators of the enzymes are substances that:

A. Increase the rate of a reaction
B. Decrease the rate of a reaction
C. Stimulate a denaturation of the enzymes
D. Increase a reversibility of a reaction
E. Cause enzymes' destruction

3. The buffer solution and an enzyme were incubated at 80°C for 30 minutes under modeling the biochemical process. The qualitative reaction is negative to the reaction product. What property of enzymes has led to the stop of the chemical reaction?

A. Denaturation of enzymes
B. Absolute specificity
C. Stereospecificity
D. Dependence on pH
E. Relative specificity

4. Pharmacological preparations which contain mercury, arsenic and other heavy metals inhibit enzymes which have a sulfhydryl group. What amino acids are used for the reactivation of these enzymes?

A. Cysteine
B. Isoleucine

C. Histidine
D. Aspartic acid
E. Glycine

5. An unknown substance was added to the enzyme-substrate system during studying the properties of an enzyme. As a result, the Michaelis constant was increased by 2 times. What phenomenon was observed?

A. Competitive inhibition
B. Allosteric activation
C. Uncompetitive inhibition
D. Noncompetitive inhibition
E. Irreversible inhibition

6. Proteolytic enzymes of stomach and pancreas are synthesized in an inactive form as zymogens, and then activated in the digestive tract. What is a proteolytic enzyme of stomach synthesized in an inactive form called?

A. Pepsin
B. Trypsin
C. Chymotrypsin
D. Elastase
E. Collagenase

7. Sulfonamide preparations inhibit the growth of bacteria. They are used for the treatment of the infectious diseases. What is the mechanism of their action?

A. Competitively inhibit the synthesis of folic acid
B. Allosteric inhibition of bacterial enzymes
C. Participate in oxidation-reduction processes
D. Inhibit the absorption of folic acid
E. Irreversibly inhibit the synthesis of folic acid necessary for the normal functioning of bacteria

8. Sulfanilamide preparations were prescribed to the patients with angina. The antimicrobial effect of sulfanilamides is caused by the violation of the synthesis of folic acid. With what substance do sulfanilamides compete for the active site of the enzyme?

A. Para-aminobenzoic acid
B. Glutamic acid
C. Citrate
D. Succinate
E. Malate

9. The composition of saliva includes enzymes that break down carbohydrates. Specify saliva enzyme involved in hydrolysis of starch:

A. Amylase
B. Lactase

C. Nuclease
D. Maltose
E. Peptidase

10. The enzyme inhibitors are substances that:

A. Decrease the rate of the reaction
B. Increase the rate of the reaction
C. Stimulate enzymes' denaturation
D. Increase the reversibility of the reaction
E. Cause enzymes' destruction

11. Digestion of different food components occurs in the duodenum under the influence of pancreatic enzymes. Which of the following enzymes hydrolyses O-glycoside bonds of carbohydrates?

A. Alpha-Amylase
B. Carboxypeptidase
C. Lipase
D. Trypsin
E. Urease

12. Methotrexate is a competitive inhibitor of dehydrofolate reductase. It is used for the bladder cancer treatment. On the interaction with which component is the mechanism of action of this drug based on?

A. The active site of the enzyme
B. Apoenzyme
C. Allosteric site of the enzyme
D. Prosthetic group
E. Substrate

13. Salicylates are reversible non-competitive inhibitors of glutamate dehydrogenase. Choose a method that can be used to reduce a degree of an enzyme inhibition?

A. To reduce a concentration of an inhibitor
B. Increasing substrate concentration
C. To reduce the pH value of the medium
D. Enter a structural analog of the substrate
E. Reduce the substrate concentration

14. Acetylcholinesterase is an enzyme that catalyses a breakdown of acetylcholine. Insecticides, pesticides and poisons with nervously-paralytic action based on fluorophosphates irreversibly inhibit acetylcholineste-rase. Specify a mechanism of the inhibition:

A. Inhibitors bind to serine residue in the active site of the enzyme
B. Inhibitors bind to histidine residue in the allosteric site
C. Inhibitors are the structural analogs of the substrate

D. Inhibitors form complex with acetylcholine
E. Inhibitors cause denaturation of the enzyme

15. What is a competitive inhibitor of succinate dehydrogenase?

A. Malonate
B. Alanine
C. Succinate
D. Fumarate
E. Alfa-ketoglutarate

16. Saliva contains an enzyme that has bactericidal effect due to its ability to destroy the peptidoglycan of the bacterial cell wall. Name this enzyme:

A. Lysozyme (muramidase)
B. α-amylase
C. Trypsin
D. Phosphatase
E. Ribonuclease

17. A structural feature of regulatory enzymes is presence of an allosteric site. Specify its role:

A. It binds a regulatory effector
B. It binds a substrate
C. It changes a structure of a substrate
D. It promotes a dissociation of a coenzyme
E. It binds a coenzyme

18. Name the type of enzyme inhibition where the chemical structure of an inhibitor resembles the structure of the substrate:

A. Competitive
B. Non-competitive
C. Uncompetitive
D. Substrate
E. The irreversible

19. Organophosphorus compounds are highly toxic poisons with nervously-paralytic action based on an inhibition of an activity of acetylcholinesterase by forming covalent bonds with the OH groups of serine in the active site of an enzyme. What type of the inhibition is characteristic for this class of compounds?

A. Irreversible
B. Reversible
C. Competitive
D. Non-competitive
E. Retroinhibition

20. The interaction of carbomoyl phosphate and aspartate is the first step in the synthesis of pyrimidine nucleotides in the E. coli cell,

which is catalyzed by the enzyme aspartate carbamoyltransferase. The synthesis of pyrimidine nucleotides is stopped under the increasing of the concentration of UTP in the cell. Specify the type of regulation of aspartate carbamoyltransferase:

A. Allosteric regulation
B. Partial proteolysis
C. Phosphorylation of an enzyme
D. Effect of inhibitory proteins
E. Cleavage of inhibitory proteins

21. Acetyl-Coa carboxylase is the key enzyme in the synthesis of fatty acids. One way to regulate the activity of Acetyl-CoA carboxylase is feedback inhibition by the final product palmitoyl-Coa. What kind of inhibition feedback inhibition belongs to?

A. Allosteric inhibition
B. Competitive inhibition
C. Irreversible inhibition
D. Covalent modification of the enzyme
E. Non-competitive inhibition

22. Tabun, Sarin and diisopropylfluorophosphate are organophosphorus compounds. They are highly toxic poisons with nervously-paralytic action. Which of the following enzymes is inhibited by organophosphorus compounds?

A. Acetylcholinesterase
B. Phospholipase A_2
C. Angiotensin converting enzyme
D. Tyrosine aminotransferase
E. Cytochrome P450

23. Name the type of enzyme inhibition where the inhibitor binds at a site other than the enzyme's active site:

A. Allosteric
B. Non-competitive
C. Uncompetitive
D. Substrate
E. Competitive

24. The anti-inflammatory drug that blocks the action of cyclooxygenase was used for treatment of a patient. What is an antiinflammatory drug called?

A. Aspirin
B. Analgin
C. Allopurinol

D. Thiamine
E. Creatine

25. The transformation of inactive proinsulin into an active one occurs by:
A. Partial proteolysis
B. Attachment of the regulatory subunit
C. Changes in the tertiary structure
D. Phosphorylation-dephosphorylation
E. Attachment of C-peptide

26. A patient has an acute pancreatitis. What medications should a doctor prescribe to prevent a pancreatic autolysis?
A. Inhibitors of proteases
B. Activators of proteases
C. Trypsin
D. Chymotrypsin
E. Amylase

27. A doctor prescribed trasylol (contrykal, gordox) to prevent attacks of acute pancreatitis, which inhibits the activity of:
A. Trypsin
B. Chymotrypsin
C. Gastricin
D. Carboxypeptidases
E. Elastases

28. Which part of an enzyme the regulators interact with changing enzyme's activity?
A. Allosteric site
B. Anchor part of the active site
C. Catalytic part of the active site
D. Proenzyme (zymogen)
E. Parallosteric site

29. A patient suffering from tuberculosis was prescribed isoniazid. It is a structural analogue of nicotinamide and pyridoxine. What type of an inhibition by the mechanism of action isoniazid causes?
A. Competitive
B. Irreversible
C. Non-competitive
D. Allosteric
E. Uncompetitive

30. ATP is an effector for the hexokinase that is a regulatory enzyme of glycolysis. As a result of their interaction with the enzyme,

structural changes occur and hexokinase loses its catalytic activity. Which structural unit of the enzyme does ATP bind to?

A. Allosteric site
B. Apoenzyme
C. Anchor part of the active site
D. Catalytic part of the active site
E. All of the above

31. It was revealed a significant decrease in pepsin activity in the analysis of the gastric juice of a patient with hypoacid gastritis. Indicate the possible biochemical mechanism of this phenomenon:

A. Disruption of an enzyme formation from a proenzyme
B. Denaturation of the enzyme molecule
C. Competitive inhibition of the enzyme
D. Decrease in the activation energy of the enzymatic reaction
E. The absence of the intrinsic factor in the gastric juice

32. One of the methods of treatment for methanol poisoning is that the patient is prescribed ethanol inside or intravenously in an amount that causes intoxication in a healthy person. Why is this treatment effective?

A. Ethanol competes with methanol for the active site of alcohol dehydrogenase
B. Ethanol binds the allosteric site of alcohol dehydrogenase, which is inactivated
C. Ethanol competes with methanol for the allosteric site of alcohol dehydrogenase
D. Ethanol blocks the enzyme alcohol dehydrogenase
E. Ethanol is split faster than methanol, resulting in less toxic products

33. Which of the following substancesactivates conversion of pepsinogen into pepsin by a partial proteolysis?

A. HCl
B. Enterokinase
C. NaCl
D. ATP
E. Bile acids

34. What type of inhibition is observed under the use of proserin that is an acetylcholinesterase inhibitor?

A. Reversible
B. Competitive
C. Non-competitive
D. Uncompetitive
E. Allosteric

35. The competitive inhibition proceeds by:

A. Inhibitor binding at the active site of enzyme
B. Enzyme dephosphorylation
C. Inhibitor binding at the allosteric site of enzyme
D. Enzyme denaturation
E. Enzyme hydroxylation

36. In the human body chymotrypsin is secreted by pancreas and converted to an active chymotrypsin by a partial proteolysis in the lumen of the small intestine under the action of:

A. Trypsin
B. Enterokinases
C. Pepsin
D. Aminopeptidases
E. Carboxypeptidases

37. When studying the composition of pancreatic juice, it was found that it contains a large number of enzymes. Some of them are secreted in an inactive form. What are these enzymes?

A. Trypsinogen, chymotrypsinogen
B. Nuclease, pepsin
C. Sucrase, amylase
D. Catalase, lipase
E. Nuclease, peptidase

38. In medical practice teturam is widely used for the prevention of alcoholism. Teturam is an inhibitor of acetaldehyde dehydrogenase. The increase of what metabolite in the blood, forming during the dehydrogenation of ethanol, causes an aversion to alcohol?

A. Acetaldehyde
B. Acetoacetate
C. Malondialdehyde
D. Propionaldehyde
E. Methanol

39. An allosteric modulator influences enzyme activity by:

A. Binding to a site on the enzyme molecule distinct from catalytic site
B. Competing for the catalytic site with the substrate
C. Changing the nature of the product formed
D. Changing the specificity of the enzyme for its substrate
E. Covalent modifying the enzyme

40. What is the mechanism of inhibition of folic acid synthesis by sulfanylamides?

A. Competitive
B. Irreversible
C. Denaturation of enzyme
D. Noncompetitive
E. Binding with allosteric site of enzymes

41. Call the type of inhibition, under which enzyme is not reactivated after inhibitor removal:

A. Irreversible
B. Substrate
C. Noncompetitive
D. Reversible
E. Competitive

42. Choose the activator of salivary amylase:

A. Sodium chloride
B. Ammonium sulfate
C. Copper sulfate
D. Magnesium chloride
E. Calcium gluconate

43. Many enzymes are formed from proenzymes in the result of cleavage of part of their polypeptide chain. Name the process

A. Limited proteolysis
B. Transamination
C. Phosphorylation
D. Dephosphorylation
E. Deamination

44. Succinate dehydrogenase catalyses the dehydrogenation of succinate. Malonic acid HOOC-CH_2-COOH is used to interrupt the action of this enzyme. Choose the inhibition type:

A. Competitive
B. Non-competitive
C. Limited proteolysis
D. Allosteric
E. Dephosphorylation

45. One of the means of regulating enzyme activity in a human body is the covalent modification. Glycogen phosphorylase and glycogen synthetase activities are regulated by the following type of covalent modification:

A. Phosphorylation-dephosphorylation
B. ADP-ribosylation
C. Methylation

D. Hydrolysis
E. Sulfonation

46. Certain infections caused by bacteria are treated with sulphanilamides that block the synthesis of bacterial growth factor. What is the mechanism of these drugs action?

A. They are antivitamins of para-aminobenzoic acid
B. They inhibit the folic acid absorption
C. They are allosteric enzyme inhibitors
D. They are involved in redox processes
E. They are allosteric enzymes

47. Indicate the type of inhibition, in which the product of reaction is the inhibitor of enzyme:

A. Retroinhibition
B. Noncompetitive
C. Stereochemical
D. Competitive
E. Absolut

References:

1. Gubsky Yu. Biological chemistry : textbook / edited by Yu. Gubsky. – Vinnytsia : Nova Knyha, 2018. – 2nd edition. – 488 p.
2. Lecture materials.
3. Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – 799 p.
4. Textbook «Biological chemistry. Collection of test tasks» for English-speaking students of higher medical educational institutions of the Ministry of Public Health of Ukraine. – Poltava, 2018. – 148 p.

TOPIC №5.
Intracellular organization of enzyme activity. Isoenzymes, multienzymes complexes. Basics of medical enzymology

1. Objective: to use information about the enzymes for diagnostics diseases, enzyme therapy and disclosure mechanisms of enzymopathies development.

2. Actuality of the theme: the main feature of living organisms is a constant exchange of substances occurs with the participation of enzymes. Hereditary metabolic defects is a result of defects in the genes responsible for the synthesis of certain enzymes. Metabolic disorders manifested in some cases of severe enzymopathies. Determination of enzyme activity in the body bioliquids allows diagnosing various diseases. Enzymes are widely used as medicines. This underscores the need for knowledge of enzymology by medical doctor.

3. Specific aims:

- ✓ Intracellular organization of enzyme activity
- ✓ Structure of isoenzymes, multienzymes complexes , and their role in metabolism
- ✓ Diagnostic value in determining the spectrum of isoenzymes in differentiation of diseases
- ✓ Causes of molecular (genetic) diseases – enzymopathies.
- ✓ Normal levels of activity of certain enzymes and their changes in diseases (enzymodiagnostics)
- ✓ Principles of the use of enzymes, coenzymes and inhibitors as drugs (enzymotherapy)

4. Reference card for the separate study of educational literature for the lesson preparation

Questions:	References:
1. **The localization of enzymes in cells (compartmentation) and organs.**	Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 103.
2. **Applications of enzymes:** ✓ enzymes as therapeutic agents; ✓ immobilized enzymes.	Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 105–106.
3. **Diagnostic importance of enzymes:** ✓ plasma specific or plasma functional enzymes; ✓ non-plasma specific or plasma non-functional enzymes; ✓ decreased plasma enzyme activities.	Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 106–109.

4.	**Changes in enzymatic activity of blood plasma and serum as diagnostic indexes (markers) of pathological processes in distinct organs:** ✓ myocardial infarction; ✓ pancreatic disease; ✓ liver diseases; ✓ pathology of muscle tissue.	Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 113.
5.	**Isoinzymes:** ✓ explanation for the existence of isoenzymes; ✓ isoenzymes of lactate dehydrogenase (LDH); ✓ isoenzymes of creatine phosphokinase; ✓ isoenzymes of alkaline phosphatase; ✓ isoenzymes of alcohol dehydrogenase.	Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 109–112.
6.	**Enzyme pattern in diseases:** ✓ enzymes in myocardial infarction; ✓ enzymes in liver diseases; ✓ enzymes in muscle diseases; ✓ enzymes in cancers.	Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 112–113.
7.	**Diagnostic importance of enzymes in other body fluids and tissues.**	Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 113–114.

5. Tasks for independent work and self-control

5.1. Select proper enzymes to their compartmentation:

	Organelle		Enzyme/metabolic pathway
1	Cytoplasm	**A**	Catalase, urate oxidase, D-amino acid oxidase.
2	Mitochondria	**B**	Protein biosynthesis; triacylglycerol pnospholipids and steroid synthesis, cytochrome P450, esterase.
3	Nucleus	**C**	Biosynthesis of DNA and RNA.
4	Endoplasmic reitciculum	**D**	Aminotransferases, glycolysis, hexose monophosphate shunt, fatty acids synthesis, purine and pyrimidine catabolism.
5	Lysosomes	**E**	Fatty acid oxidation, amino acid oxidation, Krebs cycle, urea synthesis, electron transport chain.
6	Peroxisomes	**F**	Lysozyme, phosphatases, phospholiases, hydrolases, proteseas, lipases, nucleases.

5.2. Describe the therapeutic applications of the following enzymes:

a) streptokinase

b) asparaginase

c) papain

d) antitrypsin

e) pancreatic enzymes (trypsin, lipase)

5.3. Enzymodiagnostics.

a) List specific (functional) plasma enzymes. What possible reasons can lead to a fall in the activities of these enzymes?

1.

2.

3.

4.

5.

b) Nonfunctional (nonspecific) plasma enzymes are present at a low concentration in blood plasma. What their sources in plasma at normal condition? Why are their activities in plasma elevated during different diseases?

c) Complete the table

Enzyme and isozyme assays

Diseases	What enzymes and isozymes have elevated activity in the plasma?
Heart (myocardial infarction)	
Liver (hepatitis, cirrhosis)	
Skeletal muscle (dystrophy)	

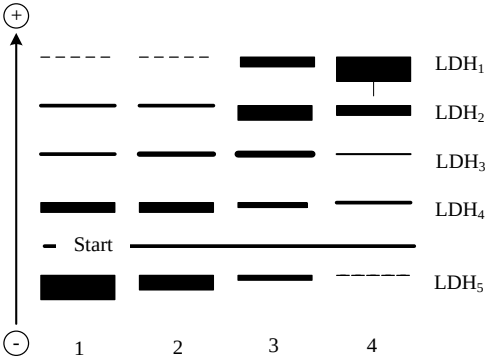
Pancreas (acute pancreatitis)	
Prostate gland (cancer)	
Bone diseases	
Kidney diseases	

5.4. Complete the table.

Isozyme

Isozyme	Combination of subunits	Tissue specificity	% of normal blood serum in humans
LDH_1			
LDH_2			
LDH_3			
LDH_4			
LDH_5			
CPK_1			
CPK_2			
CPK_3			

5.5. Which bands (1, 2, 3, 4) at the electrophoregram of lactate dehydrogenase (LDH) below correspond to organs (liver, muscle, heart, kidneys)?



5.6. Select which enzymes activities increase under conditions of following diseases:

	Enzymes:		**Disease:**
1	Lactate dehydrogenase-1	A	Acute pancreatitis
2	Alkaline phosphatase	B	Cancer of prostate gland
3	Acidic phosphatase	C	Muscular dystrophy
4	Amylase	D	Myocardial infarction
5	Aldolase	E	Rickets

5.7. Situational tasks:

a) In urgent clinic brought a patient suspected of having acute pancreatitis.

Growing activity of which enzymes in the blood and urine will confirm the diagnosis?

What pancreatic enzyme determined by the Volgemut method in urine?

Specify the normal values of this enzyme in Volgemut units.

b) Patient 58 yars old was hospitalized with complaints on pain in the retrosternal area, sudden weakness, sweating, fear, dizziness. The preliminary diagnosis – myocardial infarction.

Activity of which three enzymes is necessary to determine in patient's blood?

Which ones have isoenzyme form?

Which isoenzyme is most informative in the early hours of myocardial infarction?

6. Individual independent students work

1. Enzymopathology.
2. Enzymodiagnostics of a myocardium, a liver diseases.
3. Enzymotherapy.

Practice protocol №5 «____» _____ 20___

Experiment. Determination of the amylase activity in urine by Volgemut's method

Principle. The Volgemut's method is based on the minimal quantity of the enzyme determination, which is capable to split completely 1 ml of 1% starch solution. This quantity of enzyme is accepted for a unit of the amylase activity. Normal values of the amylase activity in the urine (by Volgemut) are 16-64 units. At acute pancreatitis the activity of amylase in the urine and blood serum arises 10-30 times. At kidney insufficiency amylase in urine is absent.

Method. Pour 1 ml of 0.85% sodium chloride solution into each test tube (7 test tubes). Add 1 ml of patient's urine into the 1-st test tube and mix thoroughly. Then transfer 1 ml of the mixture into the 2^{nd} test tube and repeat all the operations with the rest tubes: from the 2^{nd} one into the 3^{rd} one, etc. Pour 1 ml of liquid out of the 7^{th} test tube. Add 2 ml of 0.1% starch solution into each test tube, mix and put them into the thermostat at 38°C for 30 minutes. At the end of the incubation take the test tubes out, cool them and add 2 drops of the iodine solution into each one. Mix the content of the tubes and mark the latest test tube with no coloured solution (where there was full starch splitting).

№ of tube	1	2	3	4	5	6	7
Urine dilution	1:2	1:4	1:8	1:16	1:32	1:64	1:128
Colouring after the addition of iodine							

The calculation is made according to the formula:
X (units) = 1 × 2 × dilution;
1 – urine volume in ml;
2 – volume of 0.1% starch solution in ml;
X – amylase activity.

Results:

Conclusion:

The report is checked up _____
(The signature of the teacher, date)

Examples of Krock-1 tests

1. It was observed an increased hemolysis of erythrocytes in a 3-year-old child with an increased body temperature after aspirin taking. Congenital insufficiency of what enzyme causes hemolytic anemia?

A. Glucose-6-phosphate dehydrogenase
B. Glucose-6-phosphatase
C. Glycogen phosphorylase
D. Glycerolphosphate dehydrogenase
E. γ-glutamyltransferase

2. Dyspepsia is appeared in a newborn baby after the breast-feeding. The symptoms of dyspepsia disappear after the replacing of milk with glucose solution. The decreased activity of which enzyme is the cause of the indicated disorders?

A. Lactase
B. Sucrase
C. Maltase
D. Amylase
E. Isomaltase

3. A sick child is diagnosed with the galactose-1 -phosphate uridyltransferase deficiency. What is the most likely pathology?

A. Galactosemia
B. Fructosemia
C. Hyperglycemia
D. Hypoglycemia
E. Hyperlactatacidemia

4. A patient is diagnosed with steatorrhea under a laboratory investigation. The deficiency of what enzyme is the cause of the indicated disorder?

A. Lipase
B. Amylase
C. Pepsin
D. Lactase
E. Chymotrypsin

5. A 1-year-old child has lag of mental development. Vomiting, convulsions and loss of consciousness are observed in the morning. Hypoglycemia is revealed on an empty stomach. The deficiency of what enzyme is the cause of the indicated disorders?

A. Glycogen synthetase
B. Phosphorylase
C. Arginase
D. Sucrase
E. Lactase

6. Laboratory testing of a sick child's blood revealed a high amount of galactose and decreased concentration of glucose. Lag of mental development and cataract are observed in a child. What is the most likely disease?

A. Galactosemia
B. Lactosemia
C. Diabetes mellitus
D. Steroid diabetes
E. Fructosemia

7. A patient was brought to the surgical department with a diagnosis of acute pancreatitis. Conservative therapy was started. The prescription of what drug is pathogenetically grounded?

A. Contrykal
B. Trypsin
C. Chymotrypsin
D. Pancreatin
E. Fibrinolysin

8. Hydrogen cyanide and cyanides are strong poisons. Depending on the dose, death occurs within a few seconds or minutes. The inhibition of what enzyme causes the death?

A. Cytochromoxydase
B. Acetylcholinesterase
C. ATP synthase
D. Catalase
E. Methemoglobinreductase

9. A 47-year-old man was removed a salivary gland under medical indications. The content of amylase in saliva sharply decreased after the operation. Which gland was removed?

A. Parotid
B. Submandibular
C. The palatine
D. Gingival
E. Sublingual

10. An 8-month-old child was observed vomiting and diarrhea after taking fruit juices. The hereditary deficiency of what enzyme causes the disorders?

A. Fructose-1-phosphate aldolase
B. Fructokinase
C. Hexokinase
D. Phosphofructokinase
E. Fructose-1,6-diphosphatase

11. Five isoforms of lactate dehydrogenase were identified in human serum. Which property proves that marked isoenzyme forms are of the same enzyme?

A. Catalyze the same reaction
B. The same molecular weight
C. Tissue localization
D. The same physical and chemical properties
E. The same electrophoretic mobility

12. A patient complains of chronic fatigue and dizziness. He works at a chemical plant for the production of hydrocyanic acid. The disorder of what enzyme's function is the cause of the indicated symptoms?

A. Cytochromeoxidase
B. Lactate dehydrogenase
C. Succinate Dehydrogenase
D. Catalase
E. Pyruvate dehydrogenase

13. A 38-year-old patient is observed an enhanced hemolysis after taking aspirin and sulfanilamides caused by the deficiency of glucose-6-phosphate dehydrogenase. The impaired formation of what coenzyme causes the pathology?

A. $NADPH_{2;}$
B. $FADH_2$
C. Pyridoxal phosphate
D. $FMNH_{2;}$
E. Ubiquinone

14. Under the treatment of a patient's wound surface of an oral mucosa with hydrogen peroxide, the blood was coloured brown instead of foaming. The decrease of the concentration of what enzyme causes such disorders?

A. Catalase
B. Pseudocholinesterase
C. Glucose-6-phosphate dehydrogenase
D. Acetyltransferase
E. Methemoglobin reductase

15. Secretory activity of parotid salivary glands is reduced with age. The activity of what salivary enzyme will be decreased sharply?

A. Amylase
B. Lysozyme
C. Phosphatase
D. Hexokinase
E. Maltase

16. What protease inhibitor used to treat pancreatitis was first isolated from the salivary glands of cattle?

A. Trasylol
B. Allopurinol
C. Kallikrein
D. Amylase
E. Lysozyme

17. A 42 year-old man suffering from gout has increased concentration of uric acid in the blood. He was prescribed allopurinol to reduce the level of uric acid. The competitive inhibitor of what enzyme is allopurinol?

A. Xanthine oxidase
B. Adenosine deaminase
C. Adenine phosphoribosyltransferase
D. Hypoxanthine phosphoribosyltransferase
E. Guanine deaminase

18. Organisms that in the process of evolution have not created protection against H_2O_2, can live only under anaerobic conditions. Which of the listed enzymes can destroy hydrogen peroxide?

A. Peroxidase and catalase
B. Oxygenases and hydroxylases
C. Cytochrome oxidase, cytochrome B_5
D. Oxygenase and catalase
E. Flavine-dependent oxidases

19. Digestion of proteins in the stomach is the initial stage of protein digestion in the human gastrointestinal tract. Name the enzymes involved in the digestion of proteins in the stomach:

A. Pepsin and gastricin
B. Trypsin and catheptins
C. Chymotrypsin and lysozyme
D. Enteropeptidase and elastase
E. Carboxypeptidase and aminopeptidase

20. A 50-year-old woman was brought to a clinic with diagnosis of myocardial infarction. The activity of what enzyme will be increased during the first two days of the disease?

A. Aspartate aminotransferase
B. Alanine aminotransferase
C. Alanine aminopeptidase
D. LDH_4
E. LDH_5

21. The activity of lactate dehydrogenase in the patient's blood is increased in 6 hours after an acute myocardial injury. Which isoform of this enzyme is increased?

A. 1
B. 2
C. 3
D. 4
E. 5

22. A study of a secretory function of the stomach revealed a decrease in the concentration of hydrochloric acid in the gastric juice. The activity of which enzyme is reduced in this case?

A. Pepsin
B. Amylase
C. Lipase
D. Dipeptidase
E. Hexokinase

23. A child suffering from Lesch-Nyhan syndrome has a severe form of hyperuricemia, accompanied by the appearance of tophi, urate stones in the urinary tract and severe neuropsychiatric disorders. A decrease in the activity of which enzyme is the cause of this disease?

A. Hypoxanthine-guanine phosphoribosyltransferase
B. Xanthine oxidase
C. Hydrofolate reductase
D. Thymidylate synthase
E. Carbomoylphosphate synthetase

24. A 49-year-old patient suffering from acute pancreatitis has a threat of pancreonecrosis development, which was accompanied by the leaking of active pancreatic proteinases into the blood and tissues and the cleavage of tissue proteins. What protective factors of the body can inhibit these processes?

A. A_2-macroglobulin, α_1-antitrypsin
B. Immunoglobulins
C. Cryoglobulin, interferon
D. Ceruloplasmin, transferrin
E. Hemoplexin, haptoglobin

25. After taking milk diarrhea, bloating of the intestine were observed in a one-year-old child. What enzyme deficiency caused the symptoms in the baby?

A. Lactase
B. Maltase
C. Aldolase
D. Hexokinase
E. Glycosidase

26. A patient has an acute pancreatitis. What drugs prevent the autolysis of the pancreas?

A. Inhibitors of proteases
B. Protease activators
C. Trypsin
D. Chymotrypsin
E. Amylase

27. The increased activities of $LDH_{1,2}$, AST, MB-isoform of creatine phosphokinase were found in a patient's blood. In which of the following organs is a pathological process probably developed?

A. Cardiac muscle
B. Pancreas
C. The liver
D. Kidneys
E. Skeletal muscles

28. In the human body chymotrypsinogen is secreted by the pancreas and converted to an active chymotrypsin by the partial proteolysis in the lumen of the small intestine. What class of enzymes it belongs to?

A. Hydrolases
B. Lyases
C. Isomerases
D. Oxidoreductases
E. Synthetases

29. A man of 50 years who abused alcohol for a long time arose pain in the abdomen. The doctor suspected acute pancreatitis. The increased activity of which enzyme in the blood confirms this diagnosis?

A. Amylase
B. Transaminase
C. Lipase
D. Lactate dehydrogenase
E. Creatine phosphokinase

30. Isoenzymes widely used in the diagnosis of diseases. Thus, in case of myocardial infarction analyze isozyme composition:

A. Lactate dehydrogenase
B. Aspartate aminotransferase
C. Alanine aminotransferase
D. Malate dehydrogenase
E. Protein kinase

31. In tissue homogenates allocated enzymes that catalyze the mutual conversion of lactate and pyruvate. The proteins differ in electrophoretic mobility and molecular weight. These enzymes are called:

A. Isoenzymes
B. Holoenzymes
C. Coenzymes
D. Cofactors
E. Proenzyme

32. Elevation or depression of activity of specific enzymes may indicate either the presence of a disease or damage to a specific tissue. The increase of alkaline phosphatase indicates:

A. Liver disease, bone disorders
B. Acute pancreatitis
C. Muscle disorders
D. Prostate cancer
E. Heart attack

33. How are enzymes called, which catalyze the same reaction, but differ one from another by their primary structure and physico-chemical properties?

A. Isoenzymes
B. Holoenzymes
C. Zymogens
D. Cofactors
E. Apoenzymes

34. Choose isoforms of LDH, concentration of which increase in blood plasma of patients with myocardial infarction:

A. LDH_1 and LDH_2
B. LDH_3 and LDH_4
C. LDH_3
D. LDH_4 and LDH_5
E. LDH_5

35. Indicate the pathology, in which the activity of blood amylase increases 10 fold and more:

A. Acute pancreatitis
B. Virus hepatitis
C. Chronic cholecystitis
D. Miocardial infarction
E. Diabetes mellitus

36. Name the enzyme, the activity of which is determined in blood plasma of patients with pathology of bone tissue:

A. Alkaline phosphatase
B. Pepsin
C. Trypsin
D. Amylase
E. Acid phosphatase

37. The degree of the liver parenchyma lesion is estimated by the determination of:

A. Concentration of isoforms LDH_4 (HM3) and LDH_5 (M4) of blood plasma
B. Concentration of isoforms LDH_1 (H4) and LDH_2 (H3M) of blood plasma
C. Activity of amylase of urine
D. Activity of acid phosphatase
E. Concentration of isoform LDH_3 (H2M2) of blood plasma

38. A 47-year-old patient with diagnosis of myocardium infarction was admitted at the reanimation department. What fraction of lactate dehydrogenase (LDH) will prevail in blood serum for the first two days?

A. LDH_1
B. LDH_4
C. LDH_2
D. LDH_3
E. LDH_5

39. Choose the enzyme of blood plasma, which is used in therapeutic practice for the decrease of blood pressure:

A. Kallikrein
B. LDH_1
C. Trypsin
D. Chymotrypsin
E. Cholinesterase

40. Name the enzyme, which activity should be determined in patient's urine in acute pancreatitis:

A. Amylase
B. Protein kinase
C. Cholinesterase
D. Leucine aminopeptidase
E. Alkaline phosphatase

41. Which of the below-mentioned changes of biochemical parameters are characteristic for myocardial infarction?

A. Increased MB fraction of serum creatine phosphokinase
B. Increased α-amylase in blood
C. Increased MM fraction of serum creatine phosphokinase
D. Decreased creatinine level in the urine
E. Increased LPH5 fraction in blood serum

42. Activity of blood serum aspartate aminotransferase is significantly increased in the patient organism in 12 hours after acute attack of poststernum pain. Indicate the pathology, which is characterized by these changes

A. Miocardial infarction
B. Collagenosis
C. Viral hepatitis
D. Diabetes mellitus
E. Diabetes insipidus

43. The reasons causing liberation of intracellular enzymes in blood are:

A. Breakdown of cellular membranes
B. Dehydration
C. Enzyme secretion
D. Increase in body temperature
E. Increase in osmotic pressure

44. What of the following enzyme, referring to multyenzyme complexes:

A. Pyruvate dehydrogenase
B. Pyruvate decarboxylase
C. Lactate dehydrogenase
D. Malate dehydrogenase
E. Alcohol dehydrogenase

45. The activities of LDH_4, LDH_5, alanine aminotransferase, ornithine carbamoyltransferase are increased in a patient's blood. What organ is involved in pathological process?

A. Liver (hepatitis)
B. Skeletal muscle
C. Heart (myocardial infarction)

D. Kidney
E. Connective tissue

46. In clinical practice thromboses are prevented or cured by administration a certain medical preparation in the human organism. Name this preparation

A. Streptokinase
B. Lydase
C. Hyaluronidase
D. Collagenase
E. Pepsin

47. A 60-years-old man was admitted to the hospital with complains of sever chest pain and deficiency breathing. Biochemical analysis of blood revealed high levels of MB isoenzyme of creatine kinase and H4 of lactate dehydrogenase (LDH_1). Which disease may be diagnosed in that patient?

A. Myocardial infarction
B. Acute hepatitis
C. Prostate cancer
D. Acute pancreatitis
E. Muscle disorders

48. The increase of activities of LDH_1, LDH_2, aspartate amino-transferase, creatine phosphokynase was revealed at the examination of a patient's blood. Which organ (organs) are possibly involved in development of pathological process?

A. Heart (initial stage of myocardial infarction)
B. Skeletal muscle (dystrophy, atrophy)
C. Kidneys and adrenal glands
D. Connective tissue
E. Liver and kidneys

49. One of diagnostic test for acute pancreatitis is determination of the following enzyme activity in urine:

A. Amylase
B. Lactate dehydrohenase
C. Creatine kinase
D. Aldolase
E. Alanine aminopeptidase

50. In the process of metabolism human body produces active oxygen forms, including superoxide anion radical O_2. This anion is inactivated by the following enzyme:

A. Superoxide dismutase
B. Catalase
C. Peroxidase

D. Glutathione reductase
E. Glutathione peroxidase

51. A patient with acute pancreatitis has sharply increased blood and urine activity of one of the enzymes mentioned below in blood and urine that confirms the diagnosis. Give this enzyme

A. α-Amylase
B. Pepsin
C. Dipeptidase
D. Sucrase
E. Lactase

52. A patient has myocardial infarction. The first several hours of such medical condition will be characterized by significant increase of activity of the following enzyme in his blood serum:

A. Creatine phosphokinase
B. Lactate dehydrogenase 5
C. Aspartate aminotransferase
D. Alanine aminotransferase
E. Lactate dehydrogenase 4

53. Oral mucosa of a patient was treated with hydrogen peroxide. Instead of foaming, the blood turned brown. That is possible in case of reduced concentration of the following enzyme:

A. Catalase
B. Pseudocholinesterase
C. Glucose-6-phosphate dehydrogenase
D. Acetyltransferase
E. Methemoglobin reductase

54. A 60-year-old female patient presents with hypoactivity of the principal digestive enzyme of saliva. This is usually accompanied by disturbed primary hydrolysis of:

A. Carbohydrates
B. Fats
C. Proteins
D. Cellulose
E. Lactose

55. A 46-year-old female patient has a continuous history of progressive muscular (Duchenne's) dystrophy. Which blood enzyme changes will be of diagnostic value in this case?

A. Creatine phosphokinase
B. Lactate dehydrogenase
C. Pyruvate dehydrogenase
D. Glutamate dehydrogenase
E. Adenylate cyclase

56. Researchers isolated 5 isoenzymic forms of lactate dehydrogenase from the human blood serum and studied their properties. What property indicates that the isoenzymic forms were isolated from the same enzyme?

A. Catalyzation of the same reaction
B. The same molecular weight
C. The same physicochemical properties
D. Tissue localization
E. The same electrophoretic mobility

57. 12 hours after an acute attack of retrosternal pain a patient presented a jump of aspartate aminotransferase activity in blood serum. What pathology is this deviation typical for?

A. Myocardium infarction
B. Viral hepatitis
C. Collagenosis
D. Diabetes mellitus
E. Diabetes insipidus

58. 6 hours after the myocardial infarction a patient was found to have elevated level of lactate dehydrogenase in blood. What isoenzyme should be expected in this case?

A. LDH_1
B. LDH_2
C. LDH_3
D. LDH_4
E. LDH_5

59. A patient is diagnosed with cardiac infarction. Blood test for cardiospecific enzymes activity was performed. Which of the enzymes has three isoforms?

A. Creatine kinase
B. Pyruvate kinase
C. Alanine transaminase
D. Aspartate transaminase
E. Lactate dehydrogenase

60. Blood test of the patient revealed albumine content of 20 g/l and increased activity of lactate dehydrogenase isoenzyme 5 (LDH_5). These results indicate disorder of the following organ:

A. Liver
B. Kidneys
C. Heart
D. Lungs
E. Spleen

61. There is increased activity of AST, LDH_1, LDH_2, and CPK in the patient's blood. Pathological process most likely occurs in the:

A. Heart
B. Skeletal muscles
C. Kidneys
D. Liver
E. Adrenal glands

62. A patient is diagnosed with pancreatitis. Starch decomposition disturbance occurs in the patient's intestine due to deficiency of the following pancreatic enzyme:

A. Amylase
B. Tripsin
C. Chymotrypsin
D. Lipase
E. Carboxypeptidase

63. Activity of a number of enzymes and their isoforms is determined in the blood for the biochemical diagnosis of myocardial infarction. Which enzyme test is considered the best to confirm or exclude the diagnosis of the myocardial infarction in the early period after the onset of chest pain?

A. MB isoform of creatine phosphokinase
B. MM isoform of creatine phosphokinase
C. LDH_1 isoform of lactate dehydrogenase
D. LDH_5 isoform of lactate dehydrogenase
E. Cytoplasmic isoenzyme of aspartate aminotransferase

64. For biochemical diagnostics of myocardial infarction it is necessary to measure activity of a number of enzymes and their isoenzymes. What enzymatic test is considered to be the best to prove or disprove the diagnosis of infarction in the early period after the chest pain is detected?

A. Creatine kinase isoenzyme CK-MB
B. Creatine kinase isoenzyme CK-MM
C. LDH_1 lactate dehydrogenase isoenzyme
D. LDH_2 lactate dehydrogenase isoenzyme
E. Aspartate aminotransferase cytoplasmic isoenzyme

65. Vomiting, flatulence and diarrhea were appeared in a newborn baby during the breastfeeding. The hereditary insufficiency of what enzyme is a cause of this state?

A. Lactase
B. Maltase
C. Isomerase
D. Oligo-1,6-glucosidase
E. Pepsin

References:

1. Gubsky Yu. Biological chemistry : textbook / edited by Yu. Gubsky. – Vinnytsia : Nova Knyha, 2018. – 2nd edition. – 488 p.
2. Lecture materials.
3. Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – 799 p.
4. Textbook «Biological chemistry. Collection of test tasks» for English-speaking students of higher medical educational institutions of the Ministry of Public Health of Ukraine. – Poltava, 2018. – 148 p.

TOPIC №6.
The role of cofactors, vitamins and their coenzyme forms in enzyme catalysis

1. Objectives: to know the structure of complex enzymes and coenzymes role in their functioning; learn the structure of coenzymes and their participation in redox processes in the body and directions of biomedical applications.

2. Actuality of theme*:* by chemical nature and type of catalytic reaction coenzymes divided into 3 groups: I) carriers of hydrogen atoms and electrons; II) carriers of chemical groups III) coenzymes of isomerisation, synthesis and cleavage of C-C bonds. Group I coenzymes provide oxidoreductases activity and are widely used in medical practice as drugs to improve respiration and other redox processes.

Water soluble vitamin take part in metabolism as coenzymes and activators for many enzymatic reactions. Deficiency in vitamin supply of the body or disorders of their metabolism which is caused by alteration of their absorption or transformation into coenzyme forms, substantially decrease the intensity of energetic and plastic metabolism. This is accompanied with functional disorders of brain, heart, liver and other organs, suppression of immune response to infection, loss of ability to accommodate effectively to unfavorable environmental conditions.

3. Specific aims:

- ✓ To interprete the structure of complex enzymes, role of apoenzyme and cofactor in their functioning
- ✓ To classify cofactors by the chemical nature and mechanism of action
- ✓ To explain the role of metals in mechanisms of enzymatic catalysis.
- ✓ To explain the structure and mechanism of actions of the group I coenzymes – carriers of electrons, protons and hydrogen atoms
- ✓ To explain the structure and metabolic role of the group II coenzymes
- ✓ To explain the structure and metabolic role of the group III coenzymes

4. Reference card for the separate study of educational literature for the lesson preparation

Questions:	References:
1. **Cofactors, coenzymes and prosthetic groups of enzymes** (to explain difference).	1. Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 96–97. 2. Gubsky Yu. Biological chemistry: textbook / edited by Yu. Gubsky. – Vinnytsia: Nova Knyha, 2018. – 2nd edition. – P. 94.

2.	**Role of metal ions in function of enzymes.**	Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 91.
3.	**Classification of coenzymes due to their chemical nature and type of catalytic reaction.**	Gubsky Yu. Biological chemistry: textbook / edited by Yu. Gubsky. – Vinnytsia: Nova Knyha, 2018. – 2nd edition. – P. 94.
4.	**Coenzymes as transporters of hydrogen atoms and electrons** (chemical structure and short information about role): ✓ NAD^+, $NADP^+$ coenzymes – derivatives of vitamin PP; ✓ FAD, FMN coenzymes – derivatives of vitamin B_2 – riboflavin; ✓ Role of vitamin C in oxidative-reductive reactions	1. Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 132, 137–141. 2. Gubsky Yu. Biological chemistry: textbook / edited by Yu. Gubsky. – Vinnytsia: Nova Knyha, 2018. – 2nd edition. – P. 95–97, 354–357, 367–368.
5.	**Coenzymes as transporters of chemical groups** (chemical structure and short information about role): ✓ pyridoxal phosphate; ✓ HS-CoA – coenzyme of acylation; ✓ lipoic acid; ✓ THF – derivatives of folic acid	1. Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 143–144, 148–151, 157–158. 2. Gubsky Yu. Biological chemistry: textbook / edited by Yu. Gubsky. – Vinnytsia: Nova Knyha, 2018. – 2nd edition. – P. 358–359, 363–366.
6.	**Coenzymes of isomerisation, synthesis and cleavage of C-C bonds** (chemical structure and short information about role): ✓ thiamine pyrophosphate – coenzyme form of vitamin B_1; ✓ biocytin – coenzyme form of vitamin H – biotin; ✓ methylcobalamin and deoxyadenosylcobalamin – coenzyme forms of vitamin B_{12}	1. Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 135, 146–147, 152–155. 2. Gubsky Yu. Biological chemistry: textbook / edited by Yu. Gubsky. – Vinnytsia : Nova Knyha, 2018. – 2nd edition. – P. 353–354, 360–363.

5. Tasks for independent work and self-control

5.1. Complete the tables:

Redox coenzymes

Coenzyme	Oxidized form / Reduced form	Derived from vitamin	Trans-ferred	Dependent enzyme (example)
Nicotinamide adenine dinucleotide (NAD^+)				
Nicotinamide adenine dinucleotide phosphate ($NADP^+$)				
Flavin adenine dinucleotide (FAD)				
Flavin mononucleotide (FMN)				
Ascorbic acid				

Group-transferring coenzymes

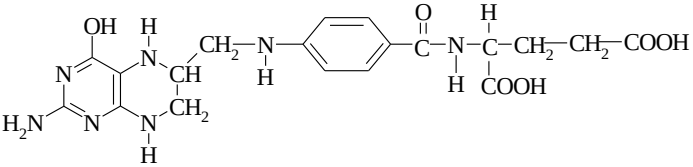
Coenzyme	Free form/ Charged form	Derived from vitamin	Trans-ferred	Important enzyme
Nucleoside phosphates (ADP, ATP)				
Coenzyme A				
Pyridoxal phosphate				
Lipoic acid				
Tetrahydrofolate				

Coenzymes of isomerisation, synthesis and cleavage of C-C bonds

Coenzyme	Free form/ Charged form	Derived from vitamin	Trans-ferred	Important enzyme
Thiamine diphosphate				
Biotin				
Cobalamins				

5.2. Find on the picture below following coenzymes:

- ✓ thiamine pyrophosphate
- ✓ FAD
- ✓ Folic acid
- ✓ methylcobalamin
- ✓ ascorbic acid
- ✓ biocytin
- ✓ pyridoxal phosphate
- ✓ lipoic acid
- ✓ NAD^+



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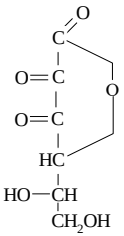

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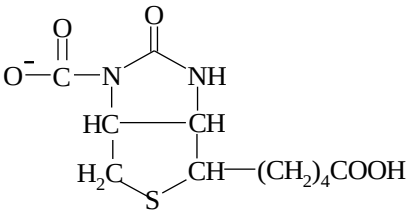

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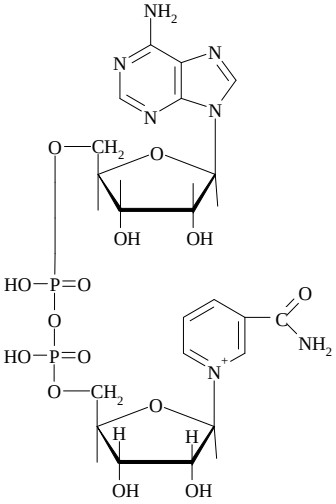

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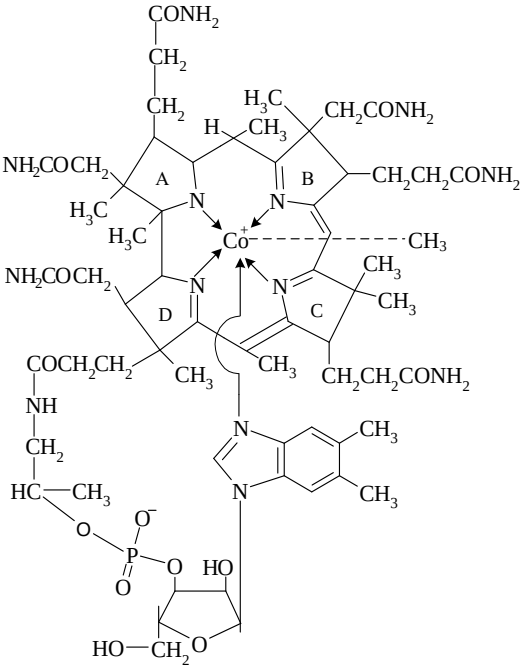

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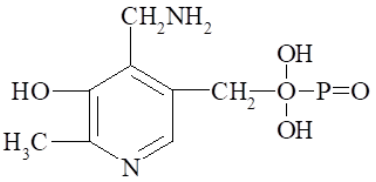

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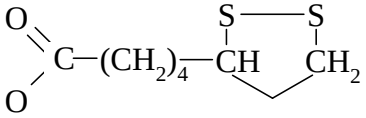

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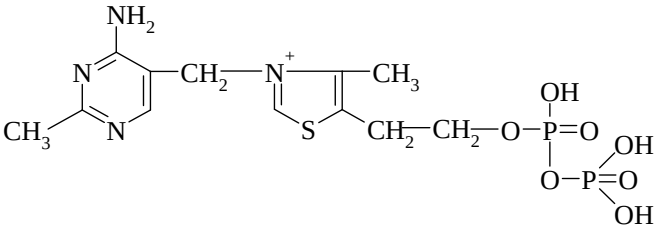

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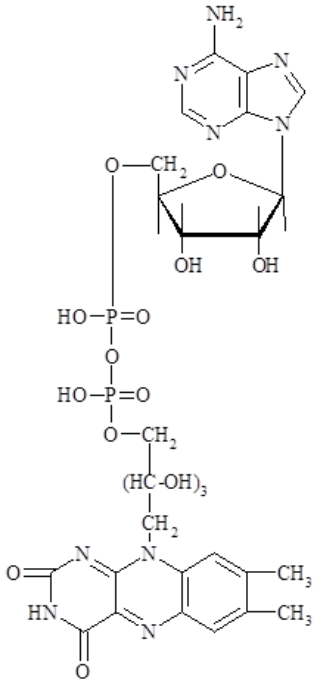

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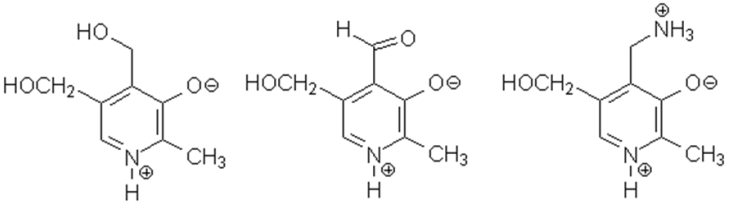
5.3. Select appropriate coenzyme forms for vitamins:

	Vitamin:		**Coenzyme:**
1	Thiamine (B_1)	A	Biocytin
2	Pyrodoxine (B_6)	B	Tetrahydrifolic acid
3	Nicotinic acid	C	Pyridoxalphosphate
4	Pantothenic acid	D	Thiamine pyrophosphate
5	Biotin	E	NAD and NADP
6	Folic Acid	F	Coenzyme A

5.4. Name types of vitamin B_6 shown on the picture bellow:



1 **2** **3**

5.5. Situational tasks:

a) In hypoxia cases toxic product – hydrogen peroxide (H_2O_2) accumulate in the tissues, which causes oxidative damage of cell membranes.

Which enzymes neutralize H_2O_2 in the cells?

Which cofactors involved in H_2O_2 neutralization?

Write down the mechanism of action of one of these cofactors

b) To improve the redox processes in clinical practice, patients prescribed vitamin E and B_2.

Which coenzyme form of these vitamins do you know?

Give examples of redox processes in which they participate.

Write down the mechanism of action of one of them.

6. Individual independent students work

1. Application of coenzymes in medical practice.

Practice protocol №6 «____» _____ 20___

In complex enzymes a protein component (apoenzyme) can be found out with the biuretic reaction. An unprotein component, containing a derivative of any vitamin, can be opened with the appropriate qualitative reactions for each vitamin.

Experiment 1. A method for the detecton of nicotinic acid.

Principle. Heating of a mixture of nicotinic acid and copper acetate gives a blue sediment of copper nicotinate.

Method. Dissolve 10 mg of nicotinic acid in 10–20 drops of 10% acetic acid. Heat the tube to boiling and then add an equal volume of 5% solution of copper acetate. The liquid turns blue and after standing blue sediment appears.

Results:

Conclusion:

Experiment 2. Reduction of $K_3Fe(CN)_6$ by ascorbic acid.

Principle. Ascorbic acid reduces $K_3Fe(CN)_6$ to $K_4Fe(CN)_6$. The latter reacts with $FeCl_3$ to produce a bright blue pigment – Berliner blue.

$$\mathbf{4FeCl_3 + 3K_4[Fe(CN)_6] \rightarrow Fe_4[Fe(CN)_6]_3 + 12KCl}$$

Method.

1. Add into two tubes one drop of 5% solution of $K_3Fe(CN)_6$ and one drop 1% solution of $FeCl_3$.
2. Add to the first tube 5–10 drops of an extract from canine rose fruits. Colour changes to blue (sometimes a blue sediment of Berliner blue pigment appears).
3. Add into the second 5–10 drops of distilled water. No changes occur in the second tube.

Results:

Conclusion:

Experiment 3. Qualitative reaction to vitamin B_1.

Principle. In alkaline environment thiamin is oxidated in tiochrom by ferrycianid of potassium. Tiochrom is coloured to yellow when heated.

Method.

№		Reactants, the sequence of addition	Tube
1.		5 % solution of vitamin B_1	2–3 drops
2.		10 % solution of NaOH and mix	0.5 ml
3.		5 % solution of $K_3Fe(CN)_6$	2–3 drops
4.		Heat, watching the colour change	

Conclusion:

Experiment 4. Reaction to vitamin B_6.

Principle. Vitamin B_6 with iron (III) chloride forms a type of iron phenolate red colour.

Method.

№	Reactants, the sequence of addition	Tube
1.	1 % Vitamin B_6 solution	5 drops
2.	1 % $FeCl_3$ solution	1 drop
3.	Mix, watching the colour change	

Conclusion:

The report is checked up _____

(The signature of the teacher, date)

Examples of Krock-1 tests

1. The iron deficiency was established by a laboratory investigation. What enzyme activity is decreased in this case?

A. Catalase
B. Carbonic anhydrase
C. Ceruloplasmin
D. Carboxypeptidase
E. Glutathione peroxidase

2. Biological oxidation and neutralization of xenobiotics is due to the heme-containing enzymes. Which metal is an obligatory component of these enzymes?

A. Fe
B. Zn
C. Co
D. Mg
E. Mn

3. The doctor prescribed pyridoxal phosphate for a patient according to the clinical indication. For the correction of what pathological processes was this medicine recommended?

A. Transamination and decarboxylation of amino acids
B. Oxidative decarboxylation of keto acids
C. Deamination of purine nucleotides
D. Synthesis of purine and pyrimidine bases
E. Synthesis of protein

4. Some amino acids, vitamin derivatives and phosphoric esters of ribose are involved in the biosynthesis of purine nucleotides. The coenzyme form of which vitamin is a carrier of one-carbon fragments in the synthesis of purine nucleotides?

A. Folate
B. Pantothenic acid
C. Niacin
D. Riboflavin
E. Pyridoxine

5. A 32-year-old patient is suffering from vitamin B_2 hypovitaminosis. The cause of an occurrence of specific symptoms (defeat of the epithelium, mucous membranes, skin, cornea of the eye) is most likely a deficit of:

A. Flavin coenzymes
B. Cytochrome a1
C. Cytochrome oxidase
D. Cytochrome b
E. Cytochrome c

6. A doctor prescribed isoniazid for a 39-yearold patient suffering from tuberculosis of the lungs. The deficiency of what vitamin could be developed due to prolonged use of this drug?

A. Pyridoxine
B. Thiamine
C. Cobalamin
D. Biotin
E. Folic acid

7. The activity of blood transaminases is determined for the diagnosis of certain diseases. Which vitamin is a cofactor of these enzymes?

A. B_6
B. B_2
C. B_1
D. B_{12}
E. B_c

8. These coenzymes are prosthetic groups of oxidoreductases, part of succinate dehydrogenase and xanthine oxidase. What are they?

A. FAD, FMN
B. NAD, NADP
C. THF
D. TPP
E. CoA

9. The structural analogs of vitamin B_2 (riboflavin) are prescribed for the patients suffering from malaria. Violations of the synthesis of what enzymes in plasmodium are caused by using of these drugs?

A. FAD-dependent dehydrogenases
B. Cytochrome
C. Peptidase
D. NAD-dependent dehydrogenases
E. Aminotransferase

10. Oxidative decarboxylation of α-ketoglutaric acid is decreased as a result of vitamin B_1 deficiency. The synthesis of which of the following coenzymes is disordered in this case?

A. Thiamine pyrophosphate
B. Nicotinamide adenine dinucleotide
C. Flavin adenine dinucleotide
D. Lipoic acid
E. Coenzyme A

11. Which vitamin is the precursor of NAD^+ and $NADP^+$ coenzymes?

A. PP
B. B_2
C. B_{12}
D. B_3
E. B_1

12. Oxidoreductases are the first class of enzymes. What are their coenzymes?

A. NAD, NADP, FAD, FMN
B. Methylcobalamin, THF
C. TGF, CoA, 4-phosphopantetein
D. TPP, carboxibiotin
E. TPP, pyridoxal-5-phosphate

13. Cocarboxylase (thiamine pyrophosphate) is used for the treatment of many diseases, normalizing energy metabolism. Which process is activated?

A. Oxidative decarboxylation of pyruvate
B. Deamination of glutamate
C. Decarboxylation of amino acids
D. Deamination of biogenic amines
E. Detoxification of xenobiotics in the liver

14. This is the most important coenzyme in the metabolism of amino acids. It is a part of transaminases and decarboxylases of amino acids. What is the coenzyme called?

A. Pyridoxal-5-phosphate
B. NAD, NADP

C. THF
D. TPP
E. CoA

15. A patient was diagnosed with seborrheic dermatitis associated with the deficiency of vitamin H (biotin). The reduced activity of which enzyme is observed in the patient?

A. Acetyl-CoA carboxylase
B. Pyruvate decarboxylase
C. Alcohol dehydrogenase
D. Aminotransferase
E. Carbamoyl phosphate synthetase

16. In case of enterobiasis acrihine – the structural analogue of vitamin B_2 – is administered. The synthesis disorder of which enzymes does this medicine cause in microorganisms?

A. FAD-dependent dehydrogenases
B. Cytochromeoxidases
C. Peptidases
D. NAD-dependet dehydrogenases
E. Aminotransferases

17. Which of the following vitamins provides a cofactor for transfer of one-carbon units?

A. Folate
B. Pyridoxine
C. Niacin
D. Riboflavin
E. Thiamin

18. What vitamin-like substens participates in the process of oxidative decarboxylation of pyruvate:

A. Lipoic acid
B. Ascorbic acid
C. Folate
D. Ubiquinone
E. Para-aminobenzoic acid

19. Hypovitaminosis C leads to a decrease in the formation of organic matrix, delay remineralization processes, impaired collagen synthesis, because this vitamin as a cofactor in the process of:

A. Hydroxylation of proline and lysine
B. Carboxylation of proline and lysine
C. Deamination of glutamate and aspartate
D. Transamination of alanine and aspartate
E. Amination of lysine and proline

20. Some amino acids, vitamin derivatives and phosphoric esters of ribose are involved in the biosynthesis of purine nucleotides. The coenzyme form of which vitamin is a carrier of one-carbon fragments in the synthesis of purine nucleotides?

A. Folate
B. Pantothenic acid
C. Niacin
D. Riboflavin
E. Pyridoxine

21. In experimental animals lipoic acid was excluded from the food, while they observed inhibition pyruvate dehydrogenase multyenzyme complex. Lipoic acid for this enzyme are:

A. The coenzyme
B. The substrate
C. The inhibitor
D. The allosteric regulator
E. The product

22. Structural analogs of vitamin B_2 (riboflavin) prescribed for patients with malaria. Violation of the synthesis of which plasmodium enzymes cause these drug?

A. FAD-dependent dehydrogenases
B. Cytochromeoxydase
C. Peptidase
D. NAD-dependent dehydrogenase
E. Aminotransferase

23. Pyridoxal phosphate assigned to the patient to correction of processes:

A. Transamination and amino acid decarboxylation
B. Oxidative decarboxylation of ketoacids
C. Deamination purine nucleotides
D. Synthesis of purine and pyrimidine bases
E. Protein synthesis

24. In the treatment of many diseases cocarboxylase (thiamine pyrophosphate) is used to provide cells with energy. This will start the process:

A. Oxidative decarboxylation of pyruvate
B. Deamination glutamate
C. Decarboxylation of amino acids
D. Deamination biogenes amines
E. Oxidative phosphorylation

25. Coenzyme, which is included in the composition of subclass «dehydrogenases» enzymes, was prescribed to the child with a medical purpose. Which of these vitamins may be involved in its formation?

A. PP and B_2
B. P and B_1
C. B_2 and B_6
D. B_1 and B_2
E. B_1 and B_5

26. A woman has hypovitaminosis B_2. The cause of specific symptoms (damage of epithelium, mucous membranes, skin, cornea) is probably the deficit of:

A. Flavin coenzyme
B. Cytochrome a_1
C. Cytochrome b
D. Cytochrome oxidase
E. Cytochrome c

27. Nicotinamide coenzyme form:

A. NAD^+, $NADP^+$
B. TDP, TPP
C. FAD, FMN
D. ATP, ADP
E. CoA-SH, acetyl CoA

28. Coenzyme form vit B_2:

A. FAD, FMN
B. TDP, TPP
C. NAD^+, $NADP^+$
D. ATP, ADP
E. CoA-SH, acetyl CoA

29. Heme (cofactor of the mitochondrial cytochrome chain) transports:

A. Electrons
B. The hydrogen atoms
C. Protons
D. Methyl groups
E. Hydroxyl groups

30. Vitamin PP is part of the respiratory chain in the form of coenzyme:

A. NAD^+
B. FAD
C. FMN
D. PALP
E. CoA-SH

31. In experimental animals lipoic acid was excluded from the food, while they observed inhibition pyruvate dehydrogenase multyenzyme complex. Lipoic acid for this enzyme are:

A. The coenzyme
B. The product
C. The substrate
D. The inhibitor
E. The allosteric regulator

32. It is known that the carbon dioxide is used in the body in the biosynthesis of fatty acids, urea, gluconeogenesis etc. Which vitamin participates in carboxylation reactions?

A. Biotin
B. Thiamin
C. Riboflavin
D. Nicotinamide
E. Retinol

33. Coenzymes that contain vitamin B_6:

A. Pyridoxal
B. Cobalamine
C. Flavin
D. Nicotinamide
E. Folate

34. Biotin carries next group:

A. Carboxyl
B. Acetyl
C. Methyl
D. Hydroxyl
E. Phosphate

35. Vitamin B_1 is a component of coenzyme:

A. TDP
B. FH_4
C. CoA
D. PALP (Pyridoxal phosphate)
E. NAD

36. Folic acid performs a cofactor function in the reactions:

A. Transfer of one-carbon groups
B. Phosphorylation
C. Deamination
D. Transamination
E. Hydrolysis

37. Which vitamin is the component of coenzyme A?

A. Pantothenic acid
B. Paraaminobenzoic acid

C. Pyridoxin
D. Carnitine
E. Orotic acid

38. Coenzyme for amino acid decarboxylation is:

A. PALP (Pyridoxal phosphate)
B. FH_4
C. CoA
D. TDP
E. NAD

39. The activity of blood transaminases is determined for the diagnosis of certain diseases. Which vitamin is a cofactor of these enzymes?

A. B_6
B. B_2
C. B_1
D. B_{12}
E. B_c

40. Coenzyme A participates in numerous important metabolic reactions. It is a derivative of the following vitamin:

A. Pantothenic acid
B. Calciferol
C. Thiamine
D. Niacin
E. Ubiquinone

41. Which of the following vitamins provides a cofactor for transfer of one-carbon units?

A. Folate
B. Riboflavin
C. Pyridoxine
D. Niacin
E. Thiamin

42. Coenzyme forms of pantothenic acid (vitamin B_3) are:

A. CoA-SH
B. FAD, FMN
C. TDF, TTF
D. NAD^+, $NADP^+$
E. PALP

43. Vitamin B_1 deficiency results in disturbance of oxidative decarboxylation of pyruvate. The synthesis of which of the following coenzymes is disturbed?

A. Thiamine pyrophosphate
B. Nicotinamide adenine dinucleotide
C. Lipoic acid

D. Flavine adenine dinucleotide
E. Coenzyme A

44. This is the most important coenzyme in the metabolism of amino acids. It is a part of transaminases and decarboxylases of amino acids. What is the coenzyme called?

A. Pyridoxal-5-phosphate
B. NAD, NADP
C. THF
D. TPP
E. CoA

45. Vitamin B_6 deficiency can potentiate vitamin PP insufficiency, as the coenzyme form of vitamin B_6 is involved in the synthesis of NAD from tryptophan. Specify the coenzyme form of vitamin B_6:

A. Pyridoxal phosphate
B. Calcitriol
C. Thiamine pyrophosphate
D. Methylcobalamin
E. Carboxybiotin

46. Reactions of intermolecular transfer of one-carbon radicals are essential for the synthesis of proteins and nucleic acids. Which vitamin is necessary for the formation of coenzyme for the above mentioned reactions?

A. Folic acid
B. Thiamine
C. Pantothenic acid
D. Riboflavin
E. Ascorbic acid

47. Biotin is involved in which of the following types of reactions?

A. Carboxylation
B. Deaminations
C. Hydroxylation
D. Oxidation
E. Reduction

48. Pyridoxal-P is a cofactor for which of the following enzymatic reactions:

A. Decarboxylation reaction
B. Fixation of CO_2
C. Phosphate group transfer
D. Transmethylation reaction
E. Dehydrogenase reaction

49. A number of diseases can be diagnosed by evaluating activity of blood transaminases. What vitamin is cofactor of these enzymes?

A. B_6
B. B_2;
C. B_1
D. B_8
E. B_5

50. Pyridoxal phosphate assigned to the patient to correction of processes:

A. Transamination and amino acid decarboxylation
B. Oxidative decarboxylation of ketoacids
C. Deamination purine nucleotides
D. Synthesis of purine and pyrimidine bases
E. Protein synthesis

References:

1. Gubsky Yu. Biological chemistry: textbook / edited by Yu. Gubsky. – Vinnytsia : Nova Knyha, 2018. – 2nd edition. – 488 p.
2. Lecture materials.
3. Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – 799 p.
4. Textbook «Biological chemistry. Collection of test tasks» for English-speaking students of higher medical educational institutions of the Ministry of Public Health of Ukraine. – Poltava, 2018. – 148 p.

TOPIC №7.
Metabolic pathways and bioenergetics

1. Objectives: To acquire close interrelation of different kinds of a metabolism and the general mechanisms of its regulation in norm and at a pathology.

2. Actuality of the theme: Metabolism – is one of the main attributes of alive organisms. All kinds of a metabolism closely cooperate among themselves. Three major systems: nervous, endocrine and vascular systems carry out the coordination of activity of all organs and tissues the base of which is the metabolism and exchange of energy. They carry out thin regulation of a metabolism in norm and pathologies.

It is important to take into account that in many processes of biosynthesis the using of the general sources of energetic maintenance, existence of the general predecessors (for example, acetyl-CoA – is the binding link of all metabolic ways) and the general final way of a metabolism (TCA, tissue respiration).

The knowledge of the general laws of a metabolism is necessary for deep understanding of mechanisms of development of metabolic changes at many pathological processes (for example, ketosis at a diabetes mellitus).

3. Specific aims:

✓ To interprete biochemical principles of metabolic pathways: catabolic, anabolic, amphibolic pathways.

✓ To explain biochemical mechanisms of regulation of catabolic and anabolic reactions.

4. Reference card for the separate study of educational literature for the lesson preparation

Questions:	References:
1. **Conception of turnover of material and energy (metabolism). Characterization of catabolic, anabolic and amphibolic reactions and their significance.**	1. Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 241–243. 2. Gubsky Yu. Biological chemistry: textbook / edited by Yu. Gubsky. – Vinnytsia: Nova Knyha, 2018. – 2nd edition. – P. 77–79.
2. **Exergonic and endergonic biochemical reactions, role of ATP and other macroergic phosphate containing compounds in their coupling.**	1. Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 222–224. 2. Gubsky Yu. Biological chemistry: textbook / edited by Yu. Gubsky. – Vinnytsia: Nova Knyha, 2018. – 2nd edition. – P. 114–115, 124–125.

3. **Intracellular location of metabolic pathways, compartmentalization of metabolic reactions in the cell. Methods of investigation of metabolism.**	Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 243.
4. **Catabolic transformation of biomolecules: proteins, carbohydrates, lipids, its characterization.**	1. Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 241–243. 2. Gubsky Yu. Biological chemistry: textbook / edited by Yu. Gubsky. – Vinnytsia: Nova Knyha, 2018. – 2nd edition. – P. 116–117.

5. Tasks for independent work and self-control

5.1. Define the following terms:

a) bioenergetics

b) free energy

c) enthalpy

d) entropy

e) exergonic reaction

f) endergonic reaction

g) standard free energy change (ΔG^0)

5.2. Compare endergonic and exergonic reactions (processes):

	Endergonic	Exergonic
Change of free energy (ΔG)		
How do they proceed (spontaneously or no)?		
Do they proceed in anabolic or catabolic pathways?		

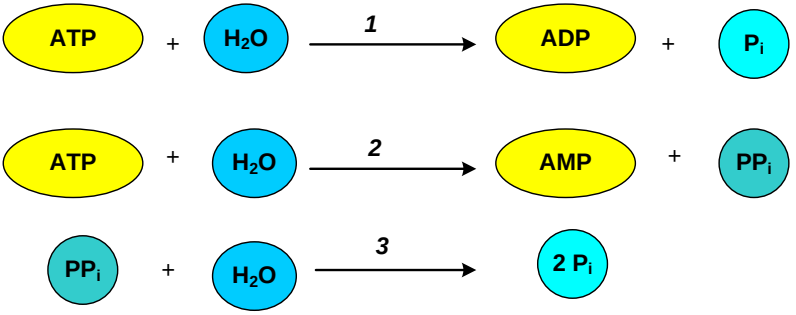
5.3. High-energy bonds and compounds.

a) What is meant by the term high-energy bond and compound?

b) Write the structure of ATP and indicate the high-energy bonds.

c) Explaine why ATP is the most important high-energy biomolecule (universal energy currency for living systems).

5.4. ATP may be hydrolysed to form ADP and Pi (orthophosphate) or AMP and PPi (pyrophosphate). Pyrophosphate may be subsequently hydrolyzed to orthophosphate releasing additional free energy. Note the ΔGo values (1, 2 and 3), which are released in each reaction.



5.5. Define the following terms:

a) metabolism and metabolic pathway

b) catabolism and catabolic pathway

c) anabolism and anabolic pathway

d) amphibolic pathway

e) metabolite.

6. Individual independent students work

1. Stages of biomolecule's catabolism

Practice protocol №7 «____» _____ 20___

Experiment. Isolation of mitochondria from liver by method of differential centrifugation (a demonstration).

Principle. Liver cells contain a great amount of specific subcellular organelles – mitochondria, in which reactions of tricarboxylic acid (TCA) cycle take place as well as processes of tissue respiration and oxidative phosphorylation.

Enzymes of TCA cycle are localized in mitochondrial matrix, while components of respiratory chain – on inner mitochondrial membrane.

Mitochondria are isolated from the tissue after its breakdown (homogenization) in special devices, called homogenizer. There exists several types of homogenizers, the most frequently is used glass homogenizer with a teflon pestle. Tissue is homogenized in 0.25 M sucrose solution. Thereafter homogenate is submitted to centrifugation at different speeds (revolutions per minute – rpm). At low speeds are sedimented nuclei and cell debris, which are discarded, mitochondria are sedimented at higher speeds (about 7-10 thousand rpm).

Method. Liver of experimental animal (albino rat) is washed from the blood, minced with a scissors and homogenized in 10 fold volume of cold solution N1 (0.25 M sucrose in 0.01 M solution of EDTA, pH 7.6). All

subsequent procedures are conducted at 1-2° C. Homogenate is centrifuged at 700 rpm during 6 min in order to eliminate nuclei and large cell debris. Supernatant is collected, transferred to another tube and submitted to centrifugation 10 min at 7000 rpm. The sediment of mitochondria is washed with solution N1 by suspending it in original volume of the solution and recentrifugation 10 min at 7000 prm. The obtained sediment is suspended in a small volume of solution N2 (0.25 M sucrose in 0.02 M tris buffer, pH 7.5), protein concentration is determined and obtained suspension is used for investigation of TCA cycle reactions, tissue respiration, oxidative phosphorylation etc.

Conclusion:

The report is checked up _____

(The signature of the teacher, date)

Examples of Krock-1 tests

1. Bioenergetics of the brain is significantly dependent upon oxygen supply. What substrate of oxidation is the main energy source for neurons?

A. Glucose
B. Fatty acids
C. Ketone bodies
D. Glycerol-3-phosphate
E. Phosphoenolpyruvate

2. Indicate the category of metabolism, which is characterized by processes of synthesis of complex substances from simpler ones with energy absorption:

A. Anabolism
B. Catabolism
C. Amphibolic pathway
D. Dualism
E. Anaplerotic reactions

3. Exergonic metabolic reactions take place with:

A. Energy release
B. Energy absorption
C. Heat generation
D. Absorption of heat
E. ATP absorption

4. Endergonic metabolic reactions take place with:

A. Energy absorption
B. Energy release
C. Heat generation
D. Absorption of heat
E. ATP absorption

5. Indicate the category of metabolism, which is characterized by processes of breakdown of complex substances to simpler ones with energy release:

A. Catabolism
B. Anabolism
C. Amphibolic pathway
D. Dualism
E. Anaplerotic reactions

6. Macroergic compounds are necessary for normal metabolism in the cells. Which of these components belongs to macroergic compounds?

A. Phosphocreatine
B. Creatine
C. Creatinine
D. Glucose-6-phosphate
E. Adenosine monophosphate

7. Amphibolic process serves as a source of metabolites for:

A. Both anabolic and catabolic processes
B. Catabolic processes
C. Anabolic processes
D. Protein synthesis
E. Anaplerotic reactions

8. How many percents of the chemical energy of the substance are transformed into ATP during its oxidation?

A. 70
B. 30
C. 20
D. 65
E. 42

9. In which cellular compartment does the third stage of catabolism occur?

A. Mitochondria
B. Nucleous
C. Cytosol
D. Lysosomes
E. Golgi apparatus

10. What type of membrane transport requires ATP?

A. Active transport
B. Simple diffusion
C. Osmosis
D. Filtration
E. Facilitated diffusion

11. What biochemical process of general pathway of catabolism of proteins, lipids and carbohydrates produces carbon dioxide as an end product of metabolism?

A. Krebs circle
B. Glycolysis
C. Tissue respiration
D. Gluconeogenesis
E. Lipolysis

12. What biochemical process of general pathway of catabolism of proteins, lipids and carbohydrates produces water as an end product of metabolism?

A. Tissue respiration
B. Krebs circle
C. Glycolysis
D. Gluconeogenesis
E. Lipolysis

13. Which of the following substances maximally promotes the utilization of inorganic phosphate during the oxidation?

A. Palmitoyl-CoA
B. Acetyl-CoA
C. Glucose
D. Succinate
E. Glycerol

14. Central intermediate of protein, lipid, carbohydrate metabolisms is:

A. Acetyl-CoA
B. Succinyl-CoA
C. Oxaloacetate
D. Lactate
E. Citrate

15. What stage of catabolism releases maximum of energy?

A. Third stage of catabolism
B. Glycolysis
C. First stage of catabolism
D. Oxidation of pyruvate
E. β-oxidation of fatty acids

16. What class of enzymes is involved in anabolism?

A. Ligases
B. Lyases
C. Hydrolases
D. Isomerases
E. Transferases

17. What class of enzymes is involved in the first stage of catabolisn?

A. Hydrolases
B. Lyases
C. Ligases
D. Isomerases
E. Transferases

18. Which of these metabolites are universal?

A. Acetyl-CoA and pyruvate
B. Acyl-CoA and malate
C. Stearoyl-CoA and citrate
D. Palmitoyl CoA and isocitrate
E. Acetoacetyl-CoA and fumarate

19. What is a central intermediate metabolite of protein, lipid and carbohydrate metabolism?

A. Acetyl-CoA
B. Succinyl-CoA
C. Oxaloacetate
D. Lactate
E. Citrate

20. In which cellular compartment does the third stage of catabolism occur?

A. Mitochondria
B. Nucleous
C. Cytosol
D. Lysosomes
E. Golgi apparatus

References:

1. Gubsky Yu. Biological chemistry: textbook / edited by Yu. Gubsky. – Vinnytsia : Nova Knyha, 2018. – 2nd edition. – 488 p.
2. Lecture materials.
3. Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – 799 p.
4. Textbook «Biological chemistry. Collection of test tasks» for English-speaking students of higher medical educational institutions of the Ministry of Public Health of Ukraine. – Poltava, 2018. – 148 p.

TOPIC №8.
Tricarboxylic acid cycle and its regulation

1. Objectives: To learn the sequence of reactions in tricarboxylic acids (TCA) cycle and biological significance of TCA cycle as the final stage of catabolic pathway in the cell. To make an aquaintance with methods of TCA cycle investigation in mitochondria and to examine the effect of malonic acid upon this process.

2. Actuality of the theme: The peculiarities of TCA cycle functioning have an important significance in evaluation of its role for providement of the cell with energy as well as for understanding of its amphibolic significance. The analysis of TCA cycle function is necessary for estimation of its role in turnover of matter and energy in the cell.

3. Specific aims:

✓ To interprete biochemical principles of TCA cycle functioning and its anaplerotic reactions and their amphibolic sense.

✓ To explain biochemical regulatory mechanisms in TCA cycle and its principal position in turnover of matter and energy.

4. Reference card for the separate study of educational literature for the lesson preparation

Questions:	References:
1. **Tricarboxylic acid (TCA) cycle:** ✓ Cellular location of TCA cycle enzymes; ✓ Sequence of TCA cycle reactions; ✓ Characterization of enzymes and coenzymes participating TCA cycle; ✓ Reactions of substrate phosphorylation in TCA cycle; ✓ The effect of allosteric modulators upon TCA cycle reactions; ✓ Energetic effect of TCA cycle.	1. Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 254–257. 2. Gubsky Yu. Biological chemistry: textbook / edited by Yu. Gubsky. – Vinnytsia : Nova Knyha, 2018. – 2nd edition. – P. 117–123.
2. **Anaplerotic and amphibolic reactions of TCA cycle.**	Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 257–258.

5. Tasks for independent work and self-control

5.1. The tricarboxylic acid (TCA) cycle or the Krebs cycle or citric acid cycle (CAC)

№	Substrates (name and the chemical formulae)	Products reactions	Enzyme, coenzymes	Type of reaction, its significance

5.2. Write the net equation of the CAC.

5.3. Molecular oxygen does not used in CAC reactions. Why than does the CAC function only in aerobic conditions?

5.4. Which of the reactions in CAC involves a substrate-level phosphorylation? What is the significance of this process?

5.5. Regulation of CAC.

Regulated enzymes	Activators and mechanism of activation	Inhibitors and mechanism of inhibition
Citrate synthase		
Isocitrate dehydrogenase		
α-Ketoglutarate dehydrogenase		

5.6. CAC is an amphibolic pathway. What is meant?

a) Explain the function of CAC in anabolic processes.

b) What anaplerotic reactions replenish the intermediates of CAC?

5.7. Situational task:

The patient entered to hospital with a diagnosis of diabetes mellitus. Among the metabolic disorders is a reduction of oxaloacetate, citrate and α-ketoglutarate.

Activity metabolic process which decreases in these conditions?

What are the consequences for the organism have decreased activity of this metabolic process?

Write the anaplerotic reaction which replenishes reserves of oxaloacetate.

6. Individual independent students work

1. Anaplerotic and amphibolic role of tricarboxylic acid cycle.
2. Role of the most important metabolites (pyruvate, α-ketoglutarate, acetyl-CoA, succinyl-CoA) in the integration of metabolism.

Practice protocol №8 «____» _____ 20___

Experiment 1. Investigation of TCA cycle functioning in mitochondria and the effect of malonate upon this process.

Principle. The transformations of acetyl-CoA in presence of mitochondrial enzymes is accompanied with production of CO_2. As a source of acetyl-CoA, which is further incorporated into TCA cycle, is used pyruvate. The last under the action of multimeric pyruvate dehydrogenase complex is submitted to oxidative decarboxylation and acetyl-CoA and CO_2

are produced. If TCA cycle is inhibited with malonate bubbles of gaze does not occur. Malonate is a classic competitive inhibitor of succinate dehydrogenase – enzyme of TCA cycle. It binds with active center of this enzyme and hinder binding of true substrate – succinic acid (or succinate).

For binding of released CO_2 into incubation medium is added $Ca(OH)_2$. At the end of incubation a bound CO_2 is detected due to a production of gaze bubbles after addition of sulphuric acid solution into incubation medium.

Method. Fill three tubes – a control one, experimental 1 and 2 with reagents as indicated in the table:

Content of tubes	Tubes		
	Control Tube 1	Exp. 1 Tube 2	Exp. 2 Tube 3
Phosphate buffer, pH 7.4, ml	2.0	2.0	2.0
Sodium pyruvate solution, ml	0.5	0.5	0.5
Malonic acid, ml			0.5
Saline, ml	0.5	0.5	
$Ca(OH)_2$ solution, ml	0.5	0.5	0.5
Suspension of mitochondria		0.5	0.5
Boiled suspension of mitochondria	0.5		
Incubation in a thermostate for 15 min at 37°C			
0,1 M solution of sulphuric acid	1.0	1.0	1.0
Result: production of CO_2 bubbles			

Place tubes in a thermostat for 15 min at 37 °C. Thereafter add 1.0 ml of 0.1M solution of sulphuric acid into each tube and observe the appearance of CO_2 bubbles.

Conclusion:

Experiment 2. Investigation of TCA cycle activity in mitochondria according to the rate of reductive equivalents production and the influence of malonate upon this process.

Principle. During the oxidation of acetyl-S-CoA in TCA cycle NAD^+ and FAD are reduced by hydrogen taken from the substrates. Reduced NAD and FAD in turn reduce methylene blue and make it colourless. Time of decolouration of reaction medium, containing methylene blue, corresponds to the intensity of reactions in TCA cycle of mitochondria.

Method. Fill three tubes – a control one, experimental 1 and 2 with reagents as indicated in the table:

Reagents added	Tubes		
	Control Tube 1	Exp. 1 Tube 2	Exp. 2 Tube 3
Phosphate buffer, pH 7.4, ml	2.0	2.0	2.0
Solution of sodium pyruvate, ml	0.5	0.5	0.5
Solution of malonate, ml	–	–	0.5
Saline	0.5	0.5	–
Suspension of mitochondria, ml	–	0.5	0.5
Suspension of boiled mitochondria, ml	0.5	–	–
Solution of methylene blue, ml	0.5	0.5	0.5
Incubation at 37 ^{o}C in a thermostate			
Results: time of medium decolouration			

Conclusion:

Clinical and diagnostic significance. Many substances, including medicinals and drugs, may influence the bioenergetics of the cell by changing the effectiveness of oxidative phosphorylation and ATP production. They can be devided into activators and inhibitors of energetic metabolism. Activators are represented by acid participants of TCA cycle (citric, succinic, malic acids) as well as by other compounds (glucose, amino acids, etc.). They are used in medical practice.

Citric acid as sodium salt is additionally used as anticoagulant, as well as a component of some other drugs.

The report is checked up _____

(The signature of the teacher, date)

Examples of Krock-1 tests

1. What vitamins are necessary for the reactions of the Krebs cycle?

A. B_1, B_2, B_3, PP
B. B_c, B_{12}, B_{15}, A
C. D, K, C, B_{15}
D. D, E, P, B_{15}
E. C, B_{12}, D, K

2. In which cellular compartment does the Krebs cycle occur?

A. Matrix of mitochondria
B. Nucleous
C. Cytosol
D. Lysosomes
E. Golgi apparatus

3. How many ATP may be synthesized during full oxidation of acetyl-CoA in the Krebs cycle?

A. 12
B. 1
C. 5
D. 8
E. 3

4. Indicate an enzyme of the Krebs cycle which catalyzes the reaction of substrate-level phosphorylation:

A. Succinate thiokinase
B. Citrate synthase
C. Pyruvate dehydrogenase
D. Malate dehydrogenase
E. Succinate dehydrogenase

5. Oxidation of α-ketoglutarate in the Krebs cycle is catalyzed by multienzyme complex. Choose the coenzymes of the complex from the listed below:

A. NAD, FAD, HS-CoA, TPP, lipoic acid
B. NADP, FMN, THF, TPP
C. THF, FAD, lipoic acid, TPP
D. FMN, NAD, TPP, HS-CoA, THF
E. HS-CoA, THF, lipoic acid, FAD, NAD

6. What metabolites of the Krebs cycle are substrates of tissue respiration?

A. Isocitrate, α-ketoglutarate, succinate, malate
B. Succinate, citrate, malate, oxaloacetate
C. Citrate, α-ketoglutarate, succinate, malate
D. Succinyl-CoA, α-ketoglutarate, succinate, malate
E. Acetyl-CoA, citrate, malate, oxaloacetate

7. What metabolites of the Krebs cycle are sources of protons and electrons for the tissue respiration?

A. Isocitrate, α-ketoglutarate, succinate, malate
B. Succinate, citrate, malate, oxaloacetate
C. Citrate, α-ketoglutarate, succinate, malate
D. Succinyl-CoA, α-ketoglutarate, succinate, malate
E. Acetyl-CoA, citrate, malate, oxaloacetate

8. A patient was admitted into hospital with a diagnosis diabetes mellitus type I. In metabolic changes the decrease of oxaloacetate synthesis rate is detected. What metabolic pathway is damaged as a result?

A. Tricarboxylic acid cycle
B. Glycolysis
C. Cholesterol biosynthesis
D. Glycogen mobilization
E. Urea synthesis

9. What enzyme of the Krebs cycle refers to flavin-depending dehydrogenases?

A. Succinate dehydrogenase
B. Isocitrate dehydrogenase
C. Alpha-ketoglutarate dehydrogenase
D. Malate dehydrogenase
E. Succinate thiokinase

10. Which metabolite integrates small (urea synthesis) and large (citric acid cycle) Krebs cycles?

A. Fumarate
B. Oxaloacetate
C. Isocitrate
D. Malate
E. Succinate

11. What are the allosteric inhibitors of the Krebs cycle?

A. ATP, $NADH_2$
B. ADP, NAD^+
C. AMP, FAD
D. ATP, NAD^+
E. ADP, FMN

12. What metabolite of the Krebs cycle provides its closed system?

A. Oxaloacetate
B. Isocitrate
C. Fumarate
D. Malate
E. Succinate

13. Which tricarboxylic acid cycle enzyme's activity is dramatically increased due to high concentration of ADP?

A. Isocitrate dehydrogenase
B. Succinate dehydrogenase
C. Fumarase
D. Malate dehydrogenase
E. Succinate thiokinase

14. Which tricarboxylic acid cycle enzyme's activity is dramatically decreased due to high concentration of acyl-CoA?

A. Citrate synthase
B. Isocitrate dehydrogenase
C. Fumarase
D. Malate dehydrogenase
E. Succinate thiokinase

15. Anaplerotic reactions replenish the intermediates in the citric acid cycle. Which amino acid is converted to oxaloacetate in the transamination reaction?

A. Aspartate
B. Isoleucine
C. Glycine
D. Leucine
E. Glutamate

16. Oxidation of α-ketoglutarate in the Krebs cycle is catalyzed by multienzyme complex. Choose the vitamins that are included in the complex:

A. B_1, B_2, B_3, PP, lipoic acid
B. B_{12}, B_2, B_3, PP, biotin
C. B_1, B_2, H, PP, lipoic acid
D. H, B_2, B_3, PP, lipoic acid
E. B_1, B_2, B_3, PP, biotin

17. What are anaplerotic reactions?

A. They replenish the intermediates in the citric acid cycle
B. They can function both in catabolic mode and as a source of precursors for anabolic pathways
C. Chemical reactions that involve a transfer of electrons between two species
D. Chemical reactions that result in the breakdown of more complex organic molecules into simpler substances
E. Chemical reactions in which simpler substances are combined to form more complex molecules

18. Substrate phosphorylation is a process of phosphate residue transfer from macroergic donor substance to ADP or some other nucleoside diphosphate. What enzyme of tricarboxylic acid cycle participates in reaction of substrate phosphorylation?

A. Succinyl thiokinase
B. Succinate dehydrogenase
C. Fumarase
D. Alpha-ketoglutarate dehydrogenase complex
E. Citrate synthase

19. The main function of the Krebs cycle is energy (generation of hydrogen). Which coenzymes are acceptors of hydrogen as a result of one turn of the Krebs cycle?

A. 3NADH+H and $FADH_2$
B. 2NADH+H and $2FADH_2$
C. NADH+H and $3FADH_2$
D. 2NADH+H and $2FMNH_2$
E. 2NADPH+H and $2FADH_2$

20. How many carbon dioxide molecules are generated in the Krebs cycle?

A. 2
B. 3
C. 10
D. 1
E. 12

21. In a patient are manifested symptoms of intoxication with arsenic compounds. What metabolic process is damaged taking into account that arsen containing substances inactivate lipoic acid?

A. Oxidative decarboxylation of α-ketoglutarate
B. Fatty acids biosynthesis
C. Neutralization of superoxide anions
D. Coupling of oxidation and phopsphorylation
E. Microsomal oxidation

22. Mitochondria are subcellular organelles and are present in a cytoplasm of every cell except mature red blood cells, bacteria, blue-green algae. What method is used principally for their isolation?

A. Differential centrifugation
B. Electrophoresis
C. Spectrophotometry
D. Chromatography
E. Gel-filtration

23. Examination of a patient revealed II grade obesity. It is known that he consumes a lot of sweets and rich food, has sedentary way of lifE. That's why anabolic metabolism has the priority in his organism. Which of the following pathways is amphibolic?

A. Cycle of tricarboxylic acids
B. Glyconeogenesis
C. Lipolysis
D. Glycolysis
E. Fatty acids oxidation

24. What coenzyme of flavin-dependent dehydrogenases participates in the reactions of tricarboxylic acid cycle?

A. Flavin adenine dinucleotide (FAD)
B. Flavin mononucleotide (FMN)
C. Nicotinamide-adenine dinucleotide (NAD^+)
D. Thymidine diphosphate (TDP)
E. Heme

References:

1. Gubsky Yu. Biological chemistry: textbook / edited by Yu. Gubsky. – Vinnytsia : Nova Knyha, 2018. – 2nd edition. – 488 p.
2. Lecture materials.
3. Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – 799 p.
4. Textbook «Biological chemistry. Collection of test tasks» for English-speaking students of higher medical educational institutions of the Ministry of Public Health of Ukraine. – Poltava, 2018. – 148 p.

TOPIC №9.
Biological oxidation

1. Objective: To learn general principles of enzymatic respiratory chain organization in mitochondria, distinct oxidoreductases and their functional significance in tissue respiration. To master the methods of investigation of the next oxido-reductases: phenol oxidase, aldehyde dehydrogenase and peroxidase.

2. Actuality of the theme: Oxidoreductases catalyse reactions connected with transfer of electrons and protons and are in the background of macroergic compounds production. Investigation of their activity is necessary for detailed understanding of the mechanisms of tissue respiration and its changes in different functional status of the body.

3. Specific aims:

✓ To explain processes of biological oxidation of different substrates in the cell and reservation of released energy in a form of macroergic bonds of ATP.

✓ To analyze reactions of biological oxidation and their role in providement of fundamental biochemical processes in tissues.

✓ Malate-aspartate and glycerophosphate shuttle systems of transmembrane transfer of reduced $NADH_2$ into mitochondria and their significance.

✓ To explain the structural organization of electron transport chain and its macromolecular.

✓ To interpret role of biological oxidation, tissue respiration and oxidative phosphorylation in generation of ATP in aerobic conditions.

4. Reference card for the separate study of educational literature for the lesson preparation

	Questions:	**References:**
1.	**Biological oxidation of substrates in cells. Redox potential (E_0)**	Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 224–225.
2.	**Enzymes involved in biological oxidation and their functional significance:** ✓ oxidases; ✓ dehydrogenases; ✓ peroxidases; ✓ oxygenases (mono- and dioxygenases)	1. Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 235–236. 2. Gubsky Yu. Biological chemistry: textbook / edited by Yu. Gubsky. – Vinnytsia : Nova Knyha, 2018. – 2nd edition. – P. 95–97.
3.	**Electron transport chain**	1. Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 225. 2. Gubsky Yu. Biological chemistry:

		textbook / edited by Yu. Gubsky. – Vinnytsia : Nova Knyha, 2018. – 2nd edition. – P. 126–128.
4.	**Mitochondria – the power houses of cell**	Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 225–226.
5.	**Pyridine dependent dehydrogenases:** ✓ chemical structure of NAD^+ and $NADP^+$; ✓ mechanism of oxidation and reduction of NAD^+; ✓ role of pyridine dependent dehydrogenases in reactions of oxidation and reduction.	Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 140–141; 226–227.
6.	**Flavine dependent dehydrogenases**: ✓ structure of FAD and FMN; ✓ mechanism of oxidation and reduction of FAD; ✓ role of flavine dependent dehydrogenases in reactions of oxidation and reduction.	Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 137–139, 227.
7.	**Iron-sulfur proteins**	Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 227.
8.	**Coenzyme Q**	Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 228.
9.	**Cytochromes and their role in tissue respiration.** Structure of their prosthetic group Cytochromes composition of respiratory chain	Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 228.
10.	**Molecular organization of electron transport chain of mitochondria** ✓ constituents of respiratory chain in mitochondria ✓ the sequence of electron transporters in respiratory chain ✓ the significance of redox potentials in transport of electrons and protons ✓ five complexes, their names and consttuents	1. Satyanarayana U., Chakrapani U. «Biochemistry», Third Edition. – 2006. – P. 226–228. 2. Gubsky Yu. Biological chemistry: textbook / edited by Yu. Gubsky. – Vinnytsia : Nova Knyha, 2018. – 2nd edition. – P. 129–131.

5. Tasks for independent work and self-control

5.1. Define

a) oxidation

b) redox potential

5.2. What indicates a more positive redox potential?

5.3. A more negative redox potential represents a greater tendency to lose _____.

5.4. Choose the standard reduction potential for each redox pair of electron-transport chain in mitochondria

	Redox pair		**$E^{o'}$ (Volts)**
1.	$NAD^+/NADH$	**A**	+0.82
2.	$FMN/FMNH_2$	**B**	+0.10
3.	Succinate/α-ketoglutarate	**C**	+0.23
4.	$FAD/ FADH_2$	**D**	– 0.22
5.	$2H^+/H_2$	**E**	+ 0.07
6.	Coenzyme Q (ox/red)	**F**	– 0. 30
7.	Cytochrome b ($Fe^{3+/}Fe^{2+}$)	**G**	+0.29
8.	Cytochrome c_1 ($Fe^{3+/}Fe^{2+}$)	**H**	– 0.67
9.	Cytochrome c ($Fe^{3+/}Fe^{2+}$)	**I**	– 0.42
10.	Cytochrome a ($Fe^{3+/}Fe^{2+}$)	**J**	+ 0.25
11.	½ O_2/H_2O	**K**	– 0.32

5.5. Describe these enzymes:

a) dehydrogenases

b) oxidases

c) peroxidases

d) dioxygenases

e) monooxygenases (hydroxylases)

5.6. Select appropriate type of biological oxidation:

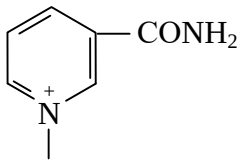
	Reaction		**Type**
1.	$SH_2 + R \rightarrow S + RH_2$	A	Dioxygenase
2.	$SH_2 + 1/2O_2 \rightarrow S - OH$	B	Oxidase
3.	$SH_2 + O_2 \rightarrow S + H_2O_2$	C	Dehydrogenase
4.	$S + O_2 \rightarrow SO_2$	D	Monooxygenase

5.7. Indicate biochemical processes that occur in the parts of a mitochondria:

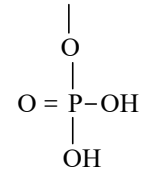
a) the matrix space

b) the inner mitochondrial membrane.

5.8. Which component of the NAD^+ structure takes part in the binding of hydrogen?



A



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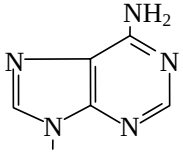
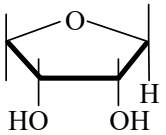


C



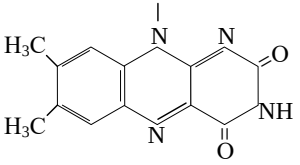
D

5.9. Description of components of mitochondrial ETC:

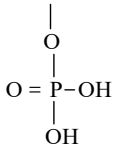
Prosthetic group (redoxcentre)	Flavoproteins	Iron-sulfur proteins	Ubiquinone (coenzyme Q)	Cytochromes
oxidized form				
reduced form				
how many electrons and protons are transferred?				

a) Write the equation of reduction of FMN (FAD) to $FMNH_2$ ($FADH_2$) (to simplify the equation, only the flavin ring write).

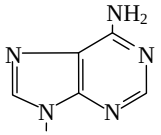
b) Which component of the FAD structure takes part in the binding of hydrogen?



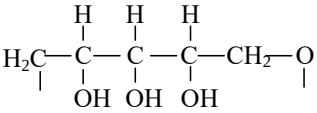
A



B



C



D

c) Write the equation of reduction of CoQ to $CoQH_2$.

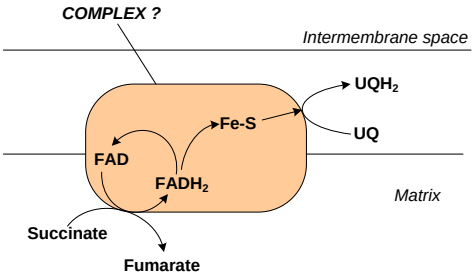
What component of ETC is not associated with protein?

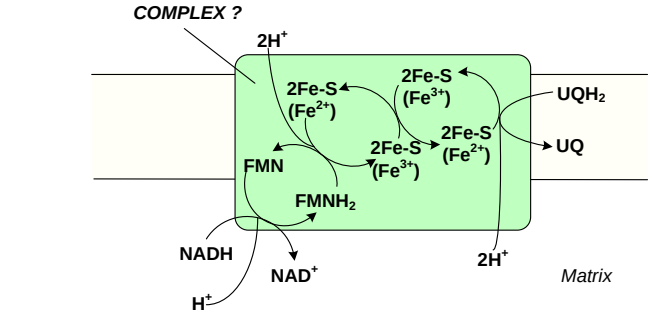
5.10. Description of complexes of mitochondrial ETC:

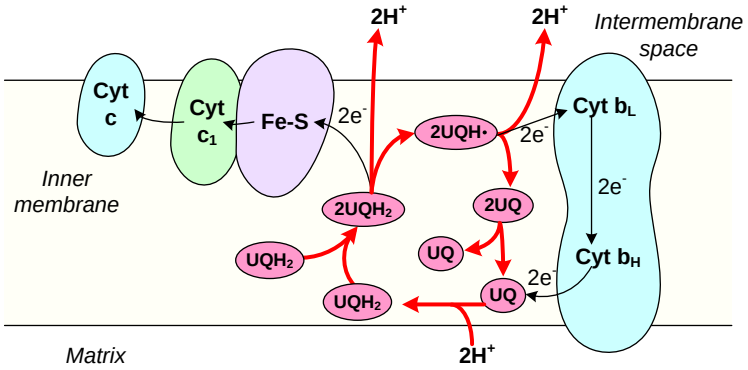
	I	II	III	IV
Complex name				
Composition of prosthetic groups (redox-centres)				
Reducing agent (electron donor)				
Oxidizing agent (electron acceptor)				
Ability to the translocation of protons (has or no)				

5.11. Give example of short ETC.

5.12. Name complexes shown at the pictures below:







6. Individual independent students work

1. Development of knowledge on biological oxidation, works of A. Bakh, V. Palladin, O.Warburg in this field.
2. Modern data about the organization and functioning of respiratory chain as a single polyenzyme complex.

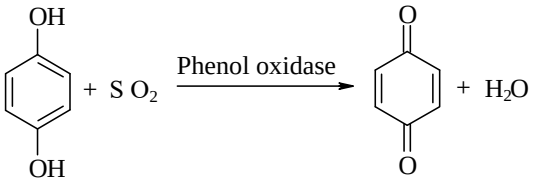
Practice protocol №9 «____» _____ 20___

Oxidoreductases

Oxidoreductases are enzymes that catalyse the transfer of electrons from one molecule, the reductant, also called the electron donor, to another the oxidant, also called the electron acceptor. Phenol oxidase, aldehyde dehydrogenase, peroxidase and many others belong to oxidoreductases.

Polyphenol oxidase is an enzyme that catalyses the hydroxylation of monophenols to *o*-diphenols. They can also further catalyse the oxidation of *o*-diphenols to produce *o*-quinones. It is the rapid polymerisation of *o*-

quinones to produce black, brown or red pigments (polyphenols) that is the cause of fruit browning.



Hydroquinon (o-diphenol) Quinon

Aldehyde dehydrogenases are a group of enzymes that catalyze the oxidation (dehydrogenation) of aldehydes. To date, nineteen ALDH genes have been identified within the human genome. These genes participate in a wide variety of biological processes including the detoxification of exogenously and endogenously generated aldehydes.

Peroxidases are a large family of enzymes that typically catalyse a reaction of the form:

$$ROOR' + \text{electron donor } (2\ e^-) + 2H^+ \rightarrow ROH + R'OH$$

For many of these enzymes the optimal substrate is hydrogen peroxide, but others are more active with organic hydroperoxides such as lipid peroxides

Experiment 1. Study of phenol oxidase activity.

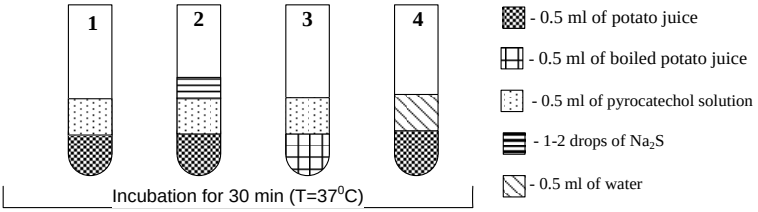
Principle. Method is based on the phenomenon of pyrocatechol oxidation by oxygen in the presence of phenol oxidase.

Method.

I. The source of enzyme is fresh potato juice. Mince in a mortar 1–2 g of potato add 25 ml of distilled water, mix it and press the juice into tube. (Iron instruments must not be used because ions of iron induce the darkening of juice).

II. Add 0.5 ml of potato juice into 4 tubes. Add 0.5 ml of pyrocatechol solution to the first tube; 1–2 drops of Na_2S (an inhibitor of phenol oxidase) and 0,5 ml of pyrocatechol – to the second; boiled juice and 0.5 ml of pyrocatechol – to the third and 0.5 ml of water to fourth tube. Place tubes into the thermostat at 37 °C for 30 min. Periodically shake the tube's content for better aeration.

Result. In the first tube brown colour appears due to oxidation of pyrocatechol. The colours of other tubes don't change: due to the presence of inhibitor (2 tube), denaturation of enzyme (3 tube) and absence of substrate (4 tube).



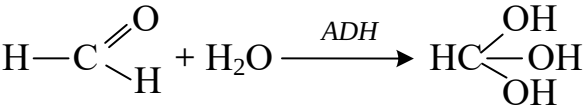
Result:

1. brown 2. colorless 3. colorless 4. colorless

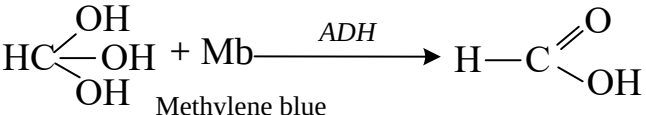
Conclusion:

Experiment 2. A study of aldehydedehydrogenase activity.

Principle. Method is based on the decolouration of methylene blue during its reduction by aldehyde in presence of an enzyme aldehyde dehydrogenase (ADH).



Formaldehyde Hydrated form of formaldehyde



Methylene blue Formiatic acid

Method. Add the following solutions into three ennumerated tubes:

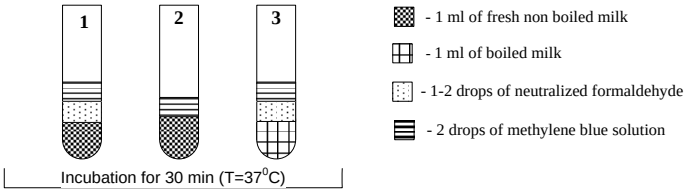
(1) – 1 ml of fresh non boiled milk, 1–2 drops of neutralized formaldehyde, 2 drops of methylene blue solution;

(2) – 1 ml of fresh milk and 2 drops of methylene blue;

(3) – 1 ml of boiled milk, 1–2 drops of formaldehyde and 2 drops of methylene blue.

Place tubes to the termostate for 30 min to at 37 °C.

Result. Content of the first tube is decolourized due to enzyme activity. Colour in the rest of the tubes does not change, as in the second tube – substrate is absent, in the third – enzyme is heat denaturated.



Result:

1. colorless 2. blue 3. blue

Conclusion:

Clinical and diagnostical importance. Aldehyde dehydrogenase plays a crucial role in maintaining low blood levels of acetaldehyde during alcohol oxidation. In this pathway, the intermediate structures can be toxic, and health problems arise when those intermediates cannot be cleared. When high levels of acetaldehyde occur in the blood, facial flushing, light headedness, palpitations, nausea, and general «hangover» symptoms occur.

Experiment 3. A study of peroxidase activity.

Principle. Method is based on the oxidation of benzidine by hydrogen peroxide in the presence of peroxidase. Blue product is formed during the oxidation of benzidine.

Method. Add the following reagents into 3 tubes:

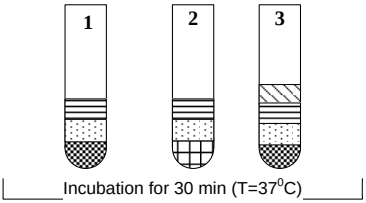
(1) – 3–4 drops of benzidine solution in acetic acid, 3–4 drops of 3 % solution of H_2O_2, 3–4 drops of horseradish extract.

(2) – 3–4 drops of benzidine solution, 3–4 drops of H_2O_2 solution, 3–4 drops of a boiled horseradish extract.

(3) – 3–4 drops of benzidine solution, peroxidase inhibitor, 3–4 drops of horse radish peroxidase and 3–4 drops of H_2O_2 solution.

Place tubes to the termostate for 30 min to at 37 °C.

Result. In the first tube a blue colour is developed due to oxidation of benzidine by H_2O_2 under catalysis of peroxidase. In the 2 and 3 tubes colour is not developed.



- 3-4 drops of horseradish extract
- 3 - 4 drops of a boiled horseradish extract
- 3-4 drops of 3 % solution of H_2O_2
- 3-4 drops of benzidine solution in acetic acid
- 3-4 drops of peroxidase inhibitor

Result:

1. blue 2. colorless 3. colorless

Conclusion:

Clinical and diagnostical importance. Horseradish peroxidase can be conjugated to a labeled molecule. It produces a coloured, fluorimetric, or luminescent derivative of the labeled molecule when incubated with a proper substrate, allowing it to be detected and quantified. Horseradish peroxidase is often used in conjugates (molecules that have been joined genetically or chemically) to determine the presence of a molecular target. For example, an antibody conjugated to horseradish peroxidase may be used to detect a small amount of a specific protein in a western blot. Here, the antibody provides the specificity to locate the protein of interest, and the horseradish peroxidase enzyme, in the presence of a substrate, produces a detectable signal. Horseradish peroxidase is also commonly used in techniques such as ELISA and Immunohistochemistry due to its monomeric nature and the ease with which it produces coloured products. Peroxidase, a heme-containing oxidoreductase, is a commercially important enzyme which catalyses the reductive cleavage of hydrogen peroxide by an electron donor.

The report is checked up _____

(The signature of the teacher, date)

Examples of Krock-1 tests

1. What enzymes catalyze transfer of hydrogen from some metabolite to oxygen?

A. Aerobic dehydrogenases
B. Oxygenases
C. Anaerobic dehydrogenases
D. Dioxygenases
E. Cytochromes

2. Transfer of electrons in the respiratory chain leads to the releasing of energy that is used for:

A. Oxidative phosphorylation
B. Substrate phosphorylation
C. Microsomal oxidation
D. Peroxidation
E. –

3. What enzymes oxidize a substrate by transferring the oxygen from molecular oxygen to it?

A. Oxygenases
B. Aerobic dehydrogenases
C. Anaerobic dehydrogenases
D. Dioxygenases
E. Cytochromes

4. What are metal-containing enzymes of the respiratory chain of mitochondrial inner membrane called?

A. Cytochromes
B. Aerobic dehydrogenases
C. Anaerobic dehydrogenases
D. Dioxygenases
E. Oxygenases

5. Flavin-containing coenzymes play an important role in catalysis being a part of the aerobic and anaerobic dehydrogenases. What are they?

A. FAD, FMN
B. NAD^+
C. Carboxybiotin
D. THF
E. TPP

6. Which of the following components is a part of a complex I of the respiratory chain (NADH-coenzyme Q reductase)?

A. FMN
B. NAD

C. NADP
D. FAD
E. Heme

7. What is a coenzyme of pyridine-dependent enzymes?

A. NAD^+, NADP
B. FAD, FMN
C. Carboxybiotin
D. THF
E. TPP

8. What is the order of cytochromes in the respiratory chain?

A. b-c_1-c-a-a_3
B. b-c-c_1-a-a_3
C. b-c-c_1-a_3-a
D. b-c-a-c_1-a_3
E. b-c_1-c-a_3-a

9. What are coenzymes of anaerobic dehydrogenases that involved in the biological oxidation of substrates?

A. FAD, NAD
B. NAD, TPP
C. NADP, FMN
D. TPP, FAD
E. THF, NAD

10. Which of the following components is a part of complex I, II and III of the respiratory chain?

A. Ubiquinone
B. NAD
C. NADP
D. FMN
E. Heme

11. What is a coenzyme of NADH+H-dehydrogenase of the respiratory chain?

A. FMN
B. NAD
C. NADP
D. TPP
E. THF

12. Choose a coenzyme of pyridine-dependent enzymes:

A. NAD^+
B. FAD
C. Carboxybiotin
D. THF
E. TPP

13. Which of the following components is a part of a complex II of the respiratory chain (Succinate-coenzyme Q reductase)?

A. FAD
B. NAD
C. NADP
D. FMN
E. Heme

14. Cytochromes are metal-containing enzymes. Which metal atom is in the structure of cytochrome b and cytochrome c?

A. Fe
B. Mo
C. Cu
D. Mg
E. Zn

15. What components of the respiratory chain are mobile and not fixed rigidly in the inner mitochondrial membrane?

A. Ubiquinone, cytochrome c
B. Cytochromes a and a_3
C. Cytochrome b, cytochrome c
D. Cytochrome a, ubiquinone
E. Cytochrome a, cytochrome b

16. Which complex of the respiratory chain carries out oxidative phosphorylation?

A. V
B. I
C. II
D. III
E. IV

17. What enzymes catalyze the dehydrogenation reactions where oxygen is the acceptor?

A. Aerobic dehydrogenases
B. Cytochromes
C. Anaerobic dehydrogenases
D. Dioxygenases
E. Oxygenases

18. What are flavin-containing coenzymes of anaerobic and aerobic dehydrogenases?

A. FAD, FMN
B. NAD^+
C. Carboxybiotin
D. THF
E. TPP

19. Researches of the latest decades established that immediate «executors» of cell apoptosis are special enzymes called caspases. Generation of one of them proceeds with participation of cytochrome C. What is its function in a normal cell?

A. Enzyme of respiratory chain of electron transport
B. Enzyme of tricarboxylic acid cycle
C. Enzyme of beta-oxidation of fatty acids
D. Component of H^+ATP system
E. Component of pyruvate-dehydrogenase system

20. Cytochrome oxidase is a hemeprotein that is an end component of the mitochondrial respiratory chain. What reaction is catalyzed with this enzyme?

A. Transfer of reduced equivalents to molecular oxygen
B. Cytochrome synthesis
C. Transfer of reduced equivalents to ubiquinone
D. Cytochrome splicing
E. Adenosine triphosphate synthesis

21. Redox potential (E_O volts) of ubiquinone, OX/RED system is:

A. +0.10
B. +0.08
C. +0.04
D. +0.29
E. + 0.35

22. The correct sequence of cytochrome carriers in respiratory chain is:

A. Cyt b→cyt c_1→cyt c→cyt aa_3
B. Cyt aa_3→ cyt b→cyt c→cyt c_1
C. Cyt b→cyt c→cyt c_1→cyt aa_3
D. Cyt b→cyt aa_3→cyt c→ cyt c
E. Cyt aa_3→ cyt c_1→cyt c→ cyt b

23. Enzymes of the respiratory chain perform oxidation of substrates and transfer of reductive equivalents to oxygen with production of water molecules. Where are they located?

A. On inner mitochondrial membrane
B. On cytoplasmic membrane
C. In cytoplasm
D. On outer mitochondrial membrane
E. In nucleus

24. Cytochromes are components of respiratory chain in mitochondrias, which transfer electrons from ubiquinon to molecular

oxygen. What part of cytochrome molecule take part in oxydative-reductive reactions?

A. Iron atom
B. Vinyl residue
C. Pyrrole cycle
D. Proteinous part
E. Methen bridge

References:

1. Gubsky Yu. Biological chemistry: textbook / edited by Yu. Gubsky. – Vinnytsia : Nova Knyha, 2018. – 2nd edition. – 488 p.
2. Lecture materials.
3. Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – 799 p.
4. Textbook «Biological chemistry. Collection of test tasks» for English-speaking students of higher medical educational institutions of the Ministry of Public Health of Ukraine. – Poltava, 2018. – 148 p.

TOPIC №10.
Chemiosmotic theory of oxidative phosphorylation. Inhibitors and uncouplers of oxidative phosphorylation

1. Objective: to study the chemiosmotic theory of oxidative phosphorylation and conditions of its effective course.

2. Actuality of the theme: mitochondrial system of interface of oxidative processes with generation high-energy intermediate ATP, name the oxidative phosphorylation. Oxidative phosphorylation allows to organism to absorb a significant share of potentially free energy of oxidation of substrata. Chemiosmotic theory allows to make a substantiation of the mechanism of oxidative phosphorylation. Oxidative phosphorylation is very important process, infringement of its course is incompatible with a life.

3. Specific aims:

✓ To treat a role of biochemical oxidation, tissue breathing and oxidative phosphorylation in generation of ATP at aerobic conditions.

✓ To analyze infringement of synthesis of ATP under conditions of action on an organism of the person of pathogenic factors of a chemical, physical and biological origin.

4. Reference card for the separate study of educational literature for the lesson preparation

Questions:	References:
1. **Oxidative phosphorylation:** ✓ P/O ratio. ✓ Sites of oxidative phosphorylation. ✓ Energetics of oxidative phosphorylation ✓ Chemical coupling hypothesis, ✓ Chemiosmotic theory; ✓ Inherited disorders of oxidative phosphorylation.	1. Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 228–232. 2. Gubsky Yu. Biological chemistry: textbook / edited by Yu. Gubsky. – Vinnytsia: Nova Knyha, 2018. – 2nd edition. – P. 131–134.
2. **Molecular structure and principles of functioning of ATP-synthase.**	1. Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 231. 2. Gubsky Yu. Biological chemistry: textbook / edited by Yu. Gubsky. – Vinnytsia: Nova Knyha, 2018. – 2nd edition. – P. 134–135.
3. **Inhibitors of electron transport in a respiratory chain of mitochondria.** ✓ To draw the scheme of respiratory chain and to show three possible sites of action of inhibitors; ✓ Characteristics of inhibitors.	Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 232–233.

4. **Uncouplers of electron transport and oxidative phosphorylation in a respiratory chain of mitochondria:** ✓ Physiological and pathological uncouplers; ✓ Significance of uncoupling	1. Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 233. 2. Gubsky Yu. Biological chemistry: textbook / edited by Yu. Gubsky. – Vinnytsia: Nova Knyha, 2018. – 2nd edition. – P. 135.
5. **Modes of ATP biosynthesis in cells:** ✓ Oxidative phosphorilation; ✓ Substrate level phosphorylation (examples of reactions).	Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 224.
6. **Transport of reducing equivalents:** ✓ Glycerol-phosphate shuttle; ✓ Malate-aspartate shuttle.	Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 234–235.
7. **Free radicals and mechanisms of their production and inactivation:** ✓ Types of free radicals; ✓ Sources and generation of free radicals; ✓ Lipid peroxidation; ✓ The antioxidant enzyme system.	Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 655–661.

5. Tasks for independent work and self-control

5.1. The degree of coupling of electron transfer with OP:

a) What is meant by the P/O ratio?

b) How many is P/O ratio when the pair of electrons are transferred to O_2 from 1) NADH, 2) $FADH_2$? Explain the reason of different values.

5.2. Chemiosmotic coupling theory.

a) Who is the author?

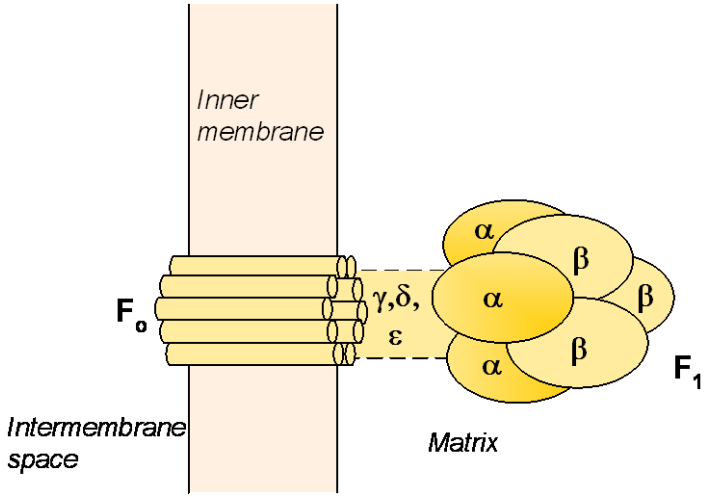
b) What intermediate couples electron transport through ETC and ATP synthesis?

c) What are the two components of proton-motive force Δp (electrochemical potential difference or gradient) and how are they formed?

5.3. Inner mitochondrial membrane is impermeable to: (a) H^+; (b) K^+; (c) OH^-; (d) All of them.

5.4. Synthesis of ATP.

a) Explain the role of ATP-synthase components, shown below:



b) Write the reaction equation of ATP synthesis.

c) Explain the mechanism of ATP-synthase catalytic action (binding-change or rotational mechanism).

5.5. Complete the table

Inhibitors

Inhibitors of ETC			Inhibitors of oxidative phosphorylation	
NADH and CoQ (complex I)	**Between cytochrome b & c_1 (complex III)**	**Cytochrome oxidase (cyt aa_3) (complex IV)**	**Physiological uncouplers**	**Pathological uncouplers**
Mechanism of action				

5.6. Define uncouplers.

5.7. Uncoupling of electron transport and OP.

a) Explain mechanism of uncoupling effect of 2,4-dinitrophenol, pentachlorophenol and similar uncouplers.

b) What possible function of uncoupling proteins (UCP) from brown adipose tissue and another tissues? (Inhumans, brown adipose tissue is abundant in infants, but it gradually diminishes and is barely detectable in adults). What mechanism of UCP effect?

c) What possible mechanisms of thermogenic effects of thyroid hormones?

5.8. Give the definitions of the terms:

a) Substrate-level phosphorylation (SP)

b) Oxidative phosphorylation (OP)

5.9. Describe two pathways of transport of reducing equivalents from cytosol to mitochondria:

a) Glycerol-phosphate shuttle

b) Malate-aspartate shuttle

5.10. Complete the table

Free radicals	Reactive oxygen species (ROS)

5.11. Describe lipid peroxidation:

Initiation phase:

Propagation phase:

Termination phase:

5.12. Define a biological antioxidant.
Give the examples of:
a) Enzymatic antioxidant

b) Non-enzymatic antioxidants

5.13. Situational tasks:
a) X gas poisoning that has the smell of rotten eggs, is accompanied by tissue respiration disorders, consciousness and can cause instant death.
Name the substance X.

Explain the mechanism of toxic action of a substance X in tissue respiration.

What substances have similar mechanism of action to the X on the respiratory chain?

b) During poisoning antimycin A patient observed signs of tissue hypoxia due to violation of the mitochondrial respiratory chain.
Explain the mechanism of toxic action antymycin A in tissue respiration?

Should this patient designate ubiquinone for therapeutic purposes?

Introduction of which vitamin can improve tissue respiration under these conditions?

c) High resistance of «winter-swimmers» (so-called «walruses») to low temperatures is explained by the increased production of certain hormones that stimulate the processes of biological oxidation and heat formation in the cells through the uncoupling of mitochondrial electron transfer and the oxidative phosphorylation.

Name the hormon?

How the electrochemical potential does change as a whole and its parts ($\Delta\varphi$ and ΔpH) in the mitochondria at high concentrations of thyroxine?

What is the name of substances with such effects on the electrochemical potential?

d) One of the side effects of prolonged use of antibiotic gramicidin is fever.

Explain the mechanism of pyrogenic action of this antibiotic?

How the electrochemical potential does change as a whole and its parts ($\Delta\varphi$ and ΔpH) in the mitochondria at gramicidin presens?

How gramicidin influence the activity of tissue respiration.

6. Individual independent students work

1. Uncouplers of oxidative phosphorylation and regulation of thermogenesis.
2. Universality of chemiosmotic theory for alive systems.

Practice protocol №10 «____» _____ 20___

Experiment. Assay of ATP in biological fluids

Principle. ATP content in the filtrate of erythrocytes is determined after acid hydrolysis by growth of inorganic phosphate (phosphate level measured at a colour reaction with ammonium molybdate in the presence of a reducing agent ascorbic acid).

Method.

Reactants, the sequence of addition	**Test tubes**	
	№1	**№2**
Filtrate of erythrocytes, ml	0.5	0.5
Distilled water, ml	1.0	1.0
Boiling in a water bath (7 min)	-	+
Ammonium molybdate (2.5% sol.), ml	0.25	0.25
Ascorbic acid (1% fresh sol.), ml	0.25	0.25
Incubation during 5 min.		
Samples eximane by photocolourimeter at a wavelength of 590 nm in 0.3 cm cuvette against distilled water		
Extinction	E_1=	E_2=
Number of ATP in mmol (available by calibration graph)	C_1=	C_2=

Calculation.

$$X = \frac{(C_2 \_ C_1) \times 0.5 \times 2000}{2 \times 10 \times 1000}, mmol/L$$

C_2 – ATP amount (in mmol) in the filtrate of red blood cells after hydrolysis;

C_1 – ATP amount (in mmol) in the filtrate of red blood cells befor hydrolysis;

2 – conversion factor of inorganic phosphate in ATP;

10, 1000, 2000 – conversion factors in mmol/L;

0.5 – filtrate volume of red blood cells, ml.

In norm in the erythrocyte filtrate ATP content is 0.9–1.5 mmol/L.

Result:

Conclusion:

The report is checked up _____

(The signature of the teacher, date)

Examples of Krock-1 tests

1. Incomplete reduction of oxygen molecule in respiratory chain and accumulation of hydrogen peroxide occur in pathology processes, which are accompanied by hypoxia. What enzyme provides hydrogen peroxide degradation?

A. Catalase
B. Cytochrome oxidase
C. Succinate dehydrogenase
D. α-Ketoglutarate dehydrogenase
E. Aconitase

2. A forensic medicine expert performing postmortem of a 20-year-old girl's body has found that the cause of death was poisoning by cyanides. What process disturbance is the most possible cause of the girl's death?

A. Tissue respiration
B. Hemoglobin synthesis
C. Transport of oxygen by hemoglobin
D. Synthesis of urea
E. Hydrogen transport by means of malate-aspartate mechanism

3. Mechanisms of many pathologic processes are realized through hypoxia state. The result of the latter is formation of hydrogen peroxide. Name the enzyme which evokes its breakdown.

A. Catalase
B. Cytochrome oxidase
C. Lactate dehydrogenase
D. Creatine phosphokinase
E. Elastase

4. What is the driving force for ATP synthesis in the electron transport chain?

A. Formation of chemiosmotic gradient
B. Transport of protons of hydrogen
C. Influence of electron transport inhibitors
D. Disorders of mitochondrial membrane integrity
E. Changes in redox potential of respiratory chain enzymes

5. The organisms, which have not created a system of protection from H_2O_2 in process of evolution, are able to exist only under anaerobic conditions. What of the enzymes mentioned below are able to destroy hydrogen peroxide?

A. Peroxidase and catalase
B. Oxygenases and hydroxylases
C. Cytochrome oxidase
D. Oxygenase and catalase
E. Flavin-dependent oxidases

6. A forensic medicine expert performing postmortem of a 20-year-old girl's body has found that the cause of death was poisoning by cyanides. What enzyme activity was inhibited by cyanides in the greatest extent?

A. Cytochrome oxidase
B. Malate dehydrogenase
C. Heme syntase
D. Aspartate aminotransferase
E. Carbamoyl phosphate synthase

7. How many ATP molecules are produced during oxidation of $NADH_2$ in the respiratory chain?

A. 3 ATP
B. 1 ATP
C. 10 ATP
D. 2 ATP
E. 12 ATP

8. How many ATP molecules are produced during oxidation of $FADH_2$ in the respiratory chain?

A. 2 ATP
B. 1 ATP
C. 10 ATP
D. 3 ATP
E. 12 ATP

9. For which of the following substrates of the tissue respiration P/O ratio is equal to 2?

A. Succinate
B. Isocitrate

C. Citrate
D. Succinyl-CoA
E. Acetyl-CoA

10. Which of the following substances uncouples electron transport from oxidative phosphorylation?

A. 2,4-Dinitrophenol
B. Carbone Monoxide
C. Rotenone
D. Antimycin
E. Oligomycin

11. Choose the complexes of the electron transport chain where the coupling of biological oxidation and oxidative phosphorylation occurs:

A. All of the above
B. I
C. III
D. IV
E. –

12. An instant death occurs with cyanides poisoning. What is the mechanism of cyanide action?

A. They inhibit cytochrome oxidase
B. They combine substances of Krebs cycle
C. They block succinate dehydrogenase
D. They inactivate oxygen
E. They inhibit cytochrome b

13. The coupling between electron transport and oxidative phosphorylation for ATP synthesis is carried out by:

A. Formation of chemiosmotic gradient
B. Spontaneous processes
C. Influence of electron transport inhibitors
D. Disorders of mitochondrial membrane integrity
E. The redox potentials of the enzymes of the respiratory chain

14. The sequence of electron transfer in the respiratory chain is due to the difference in the redox potential of its components. Which of the following components has the highest redox potential in the electron transport chain?

A. Cytochrome a_3
B. Cytochrome c_1
C. NADH+H
D. $FADH_2$
E. Cytochrome b

15. Increased production of thyroid hormones T3 and T4, weight loss, tachycardia and physic excitement are present at thyrotoxicosis.

How do thyroid hormones effect the energy metabolism in the mitochondria of cells?

A. Uncoupling of oxidation and oxidative phosphorylation
B. Activation of substrate phosphorylation
C. Inhibition of substrate phosphorylation
D. Inhibition of respiratory chain
E. Activate oxidative phosphorylation

16. The increased tolerance of winter swimmers to cold water is explained by the synthesis of large amounts of hormones that induce the process of oxidation and heat production in mitochondrion by uncoupling the biological oxidation and oxidative phosphorylation in their organisms. What are these hormones (hormone)?

A. Thyroid hormones
B. Adrenaline and noradrenaline
C. Glucagon
D. Insulin
E. Corticosteroids

17. What inhibitor blocks electron transport in complex I of the respiratory chain?

A. Rotenone
B. Carbone Monoxide
C. Cyanides
D. Antimycin
E. Oligomycin

18. What inhibitor blocks electron transport in complex III of the respiratory chain?

A. Antimycin
B. Carbone Monoxide
C. Cyanides
D. Oligomycin
E. Rotenone

19. The uncouplers of oxidative phosphorylation cause uncontrolled mitochondrial respiration. What is the mechanism of their action?

A. They collapse a chemiosmotic gradient
B. They inhibit transport of hydrogen protons c. They inhibit electron transport
D. They violate the mitochondrial membrane integrity
E. They change redox potential of the respiratory chain enzymes

20. The chemiosmotic hypothesis involves all of the following except:

A. Only proton transport is strictly regulated and other positively charged ions can diffuse freely across the mitochondrial membrane

B. Membrane is impermeable to protons

C. Electron transport by the respiratory chain pumps protons out of the mitochondria

D. ATP synthase activity is reversible

E. Proton flow into the mitochondria depends on the presence of ADP and Pi

21. What components of the respiratory chain only transport electrons?

A. Cytochrome b

B. Succinate dehydrogenase

C. Ubiquinone

D. NADH+H-dehydrogenase

E. ATP synthase

22. What component of the respiratory chain is included in complexes I, II and III?

A. Ubiquinone

B. NAD

C. NADP

D. FMN

E. Heme

23. What is a specific acceptor of inorganic phosphate in the process of oxidative phosphorylation?

A. ADP

B. AMP

C. ATP

D. FAD

E. NAD

24. What are metal-containing enzymes of the respiratory chain of mitochondrial inner membrane called?

A. Cytochromes

B. Aerobic dehydrogenases

C. Anaerobic dehydrogenases

D. Dioxygenases

E. Oxygenases

25. The sequence of electron transfer in the respiratory chain is due to the difference in the redox potential of its components. Which of the following components has the lowest redox potential in the electron transport chain?

A. $NADH+H^+$

B. Cytochrome c_1

C. Cytochrome a_3
D. $FADH_2$
E. Cytochrome b

26. A patient complains about attacks of laboured breathing, dizziness. He works at a chemical plant producing hydrocyanic acid. The described symptoms might be associated with dysfunction of the following enzyme:

A. Cytochrome oxidase
B. Lactate dehydrogenase
C. Succinate dehydrogenase
D. Catalase
E. Pyruvate dehydrogenase

27. ATP synthase is an enzyme with a quaternary structure, which consists of several protomers combined into the regions. Select them:

A. F_0 and F_1
B. F and F_1
C. F_2 and F_1
D. F_3 and F_1
E. F and F_2

28. Potassium cyanide is a poison, which leads to momentary death of the organism. What mitochondrial enzymes are influenced by potassium cyanide?

A. Cytochrome oxidase (aa_3)
B. Flavin-dependent enzymes
C. Cytochrome b
D. NAD-dependent dehydrogenases
E. Cytochrome P450

29. What inhibitors block electron transport in complex IV of the respiratory chain?

A. Cyanides
B. Amytal
C. Rotenone
D. Antimycin
E. Oligomycin

30. Show the point of coupling oxidation and phosphorylation in respiratory chain, which is blocked by barbiturate:

A. $FMNH_2DH \rightarrow CoQ$
B. $CoQH_2 \rightarrow 2b(Fe^{3+})$
C. $2b(Fe^{2+}) \rightarrow 2c_1\ (Fe^{3+})$
D. Cytochrome oxidase$\rightarrow 1/2O_2$
E. NADH$\rightarrow$FMNDH

31. Experimental animals were treated by preparation, which removes the pH gradient on the inner mitochondrial membrane, to uncouple the tissue respiration and oxidative phosphorylation. Which substance has been injected?

A. Dinitrophenol
B. Cholesterol
C. Ketone bodies
D. Urea
E. Somatotropin

32. Stimulation of lipid and biopolymer peroxidation is one of basic mechanisms of damage of structure and functions of cellular membranes and cell destruction. The cause of that is:

A. Increased production of oxygen free radicals and inhibition of antioxidant system
B. Hypovitaminosis of B_1
C. Hypervitaminosis of B_1
D. Hypovitaminosis of B_{12}
E. Hypervitaminosis of B_{12}

33. Increased production of thyroid hormones T_3 and T_4, weight loss, tachycardia, psychic excitement and so on are present at thyrotoxicosis. How do thyroid hormones affect the energy metabolism in the mitochondria of cells?

A. Disconnect oxidation and oxidative phosphorylation
B. Stop respiratory chain
C. Activate substrate level phosphorylation
D. Stop substrate level phosphorylation
E. Activate oxidative phosphorylation

34. A patient with abscess of the cut wound applied to the traumatological department. The wound was washed with 3% hydrogen peroxide to be cleaned from the pus. Foam was not observed. What caused inefficiency of the drug?

A. Inherited insufficiency of catalase
B. Pus in the wound
C. Shallow wound
D. Low concentration H_2O_2
E. Inherited insufficiency erythrocyte's phosphatdehydrogenase

35. Poisoning with cyanides results in momentary death. What is the mechanism of cyanide action on molecular level?

A. They inhibit cytochrome oxidase
B. They combine substances of Krebs cycle

C. They block succinate dehydrogenase
D. They inactivate oxygen
E. They inhibit cytochrome b

36. In course of metabolic process active forms of oxygen including superoxide anion radical are formed in the human body. By means of what enzyme is this anion inactivated?

A. Superoxide dismutase
B. Peroxidase
C. Catalase
D. Glutathioneperoxidase
E. Glutathionereductase

37. Cyanide is a poison that causes instant death of the organism. What enzymes found in mitochondria are affected by cyanide?

A. Cytochrome oxidase (aa_3)
B. Flavin enzymes
C. Cytochrome b_5
D. NAD^+-dependent dehydrogenase
E. Cytochrome P-450

38. Rotenone is known to inhibit respiratory chain. What complex of mitochondrial respiratory chain is inhibited by this substance?

A. NADH-coenzyme Q reductase
B. Succinate-coenzyme Q reductase
C. Cytochrome oxidase
D. Coenzyme Q-cytochrome c reductase
E. Adenosine triphosphate synthetase

39. The process of metabolism in the human body produces active forms of oxygen, including superoxide anion radical O^-_2. This anion is inactivated by the following enzyme:

A. Superoxide dismutase
B. Glutathione reductase
C. Catalase
D. Peroxidase
E. Glutathione peroxidase

40. The patient, who suffers from insomnia, appointed sleeping pills (class of barbiturates). Name the mitochondrial enzyme for which this drug is an inhibitor.

A. NADH-dehydrogenase
B. Cytochrome oxidase
C. Succinate dehydrogenase
D. Isocitrate dehydrogenase
E. α-ketoglutarate dehydrogenase

41. A woman 40-year-old suffering from diffuse toxic goiter presents with constant increase of her body temperature. What mechanism results in such clinical presentation?

A. Separation of oxidation and phosphorylation in cell mitochondria
B. Increased breakdown of glycogen in hepatic cells
C. Increased catabolism of protein in cells
D. Increased excitability of nerve cells
E. Increased cell sensitivity to catecholamines

42. Studies in recent decades have shown that direct «performers» in cell apoptosis are special enzymes – caspase. In the formation of one of them participates cytochrome c. Specify its function in normal cells.

A. The enzyme of the respiratory chain
B. Enzyme of CAC
C. The enzyme of β-oxidation of fatty acids
D. Component of ATP synthetase system
E. Component pyruvate dehydrogenase system

43. Periodontitis induces development of lipid peroxidation in the periodontal tissues, as well as increase in malondialdehyde and hydrogen peroxide concentration in the oral cavity. Which of the following enzymes provides antioxidant protection?

A. Catalase
B. Amylase
C. Maltase
D. Lactase
E. Invertase

44. In pathological processes that are accompanied by hypoxia, taking place the full restoration of oxygen molecules in the respiratory chain and the accumulation of hydrogen peroxide. Specify enzyme that ensures its destruction.

A. Catalase
B. Cytochrome oxidase
C. Succinate dehydrogenase
D. α-ketoglutarate dehydrogenase
E. Akonitase

45. When carbon monoxide poisoning in human tissue respiration is inhibited. Name the respiratory chain enzyme which activity sharply reduced under these conditions.

A. Cytochrome aa_3
B. Succinate dehydrogenase
C. NADH-dehydrogenase
D. Cytochrome b_1
E. Cytochrome c

46. Patient taken to the hospital with poisoning insecticide – rotenone. Which section of the mitochondrial electron transport chain is blocked by this substance?

A. NADH-coenzyme Q reductase
B. Succinate-coenzyme Q reductase
C. Coenzyme Q-cytochrome c reductase
D. Cytochrome c oxidase.
E. ATP- synthetase

47. Potassium cyanide, which is the poison got into the patient and caused death in a few minutes. The most likely reason for its toxic effect is:

A. Cytochrome oxidase
B. Catalase
C. ATP synthase
D. NADPH dehydrogenase
E. Violation of the synthesis of hemoglobin

48. The patient has high body temperature after administration to him high doses of thyroxine. Hyperthermia in this case caused by separation of processes of tissue respiration and:

A. Oxidative phosphorylation
B. Oxidative deamination of amino acids
C. Peroxidation of lipids
D. Oxidative decarboxylation of pyruvate
E. β-oxidation of fatty acids

49. A patient is followed up in an endocrinological dispensary on account of hyperthyreosis. Weight loss, tachycardia, finger tremor are accompanied by hypoxia symptoms - headache, fatigue, eye flicker. What mechanism of thyroid hormones action underlies the development of hypoxia?

A. Disjunction of oxidation and phosphorylation
B. Intensification of respiratory ferment synthesis
C. Inhibition of respiratory ferment synthesis
D. Specific binding of active centres of respiratory ferments
E. Competitive inhibition of respiratory ferments

50. What is an inhibitor of ATP synthase?

A. Carbone Monoxide
B. Antimycin
C. Cyanides
D. Oligomycin
E. Rotenone

References:

1. Gubsky Yu. Biological chemistry: textbook / edited by Yu. Gubsky. – Vinnytsia : Nova Knyha, 2018. – 2nd edition. – 488 p.
2. Lecture materials.
3. Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – 799 p.
4. Textbook «Biological chemistry. Collection of test tasks» for English-speaking students of higher medical educational institutions of the Ministry of Public Health of Ukraine. – Poltava, 2018. – 148 p.

TOPIC №11.
Investigation of glycolysis – anaerobic oxidation of carbohydrates

1. Objective: To learn fundamental principles of intracellular oxidation of glucose in anaerobic conditions and pathways of its regulation. To interpret the role of coenzymes and enzymes in glycolytic pathway.

2. Actuality of the theme: The occurrence of uninterrupted glycolysis is very essential in skeletal muscle during strenuous exercise where oxygen supply is very limited. Glycolysis in the erythrocytes leads to lactate production, since mitochondria – the centres for aerobic oxidation – are absent. Brain, retina, skin, renal medulla and gastrointestinal tract derive most of their energy from glycolysis. Concentration of lactate in blood increases after hard muscle exercises and in some diseases. Under anaerobic conditions, 2 ATP are synthesized while, under aerobic conditions, 8 or 6 ATP are synthesized-depending on the shuttle pathway that operates.

3. Specific aims:

✓ To interpret biochemical pathways of intracellular oxidation of glucose in anaerobic conditions

✓ To analyze peculiarities of glycolytic reactions, which occur with involvement of ATP

✓ To analyze peculiarities of substrate phosphorylation and production of ATP in this way

✓ To interpret role of coenzymes and enzymes in glycolytic reactions

✓ To analyze regulatory mechanisms of glucose oxidation in anaerobic conditions.

4. Reference card for the separate study of educational literature for the lesson preparation

Questions:	References:
1. **Glucose as an important metabolite in carbohydrate metabolism**: ✓ Major pathways of carbohydrate metabolism; ✓ Sources of glucose in the organism; ✓ Entry of glucose into cells	1. Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 244–245. 2. Gubsky Yu. Biological chemistry: textbook / edited by Yu. Gubsky. – Vinnytsia: Nova Knyha, 2018. – 2nd edition. – P. 137–139.
2. **Anaerobic oxidation of glucose:** ✓ The sequence of reactions in glycolysis; ✓ Enzymatic reactions of anaerobic and aerobic glycolysis; ✓ Characterization of glycolytic reactions, which occur with utilization of energy;	1. Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 245–248. 2. Gubsky Yu. Biological chemistry: textbook / edited by Yu. Gubsky. – Vinnytsia: Nova Knyha, 2018. – 2nd edition. – P. 139–148.

✓ Characterization of enzymatic reactions of substrate phosphorylation in glycolysis; ✓ Mechanism of glycolytic oxido-reduction and reactions, which provide this process.	
3. **The role of lactate dehydrogenase (LDH) in glycolysis, mechanism of reaction and its peculiarities.** ✓ Isoenzymes of LDH and their clinical diagnostic significance; ✓ Conversion of pyruvate to lactate-signilicance.	1. Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 248. 2. Gubsky Yu. Biological chemistry: textbook / edited by Yu. Gubsky. – Vinnytsia: Nova Knyha, 2018. – 2nd edition. – P. 147.
4. **Mechanisms of regulation of the rate of reactions in anaerobic glycolysis** ✓ Allosteric regulation of glycolysis; ✓ Role of fructose 2,6-bisphosphate in glycolysis; ✓ Irreversible steps in glycolysis.	1. Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 250–251. 2. Gubsky Yu. Biological chemistry: textbook / edited by Yu. Gubsky. – Vinnytsia: Nova Knyha, 2018. – 2nd edition. – P. 148–149.
5. **Energetic effect of anaerobic oxidation of glucose:** ✓ Production of ATP in glycolysis	1. Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 249. 2. Gubsky Yu. Biological chemistry: textbook / edited by Yu. Gubsky. – Vinnytsia: Nova Knyha, 2018. – 2nd edition. – P. 146–147.

5. Tasks for independent work and self-control

5.1. Major pathways of carbohydrate metabolism

Choose the feature for each major pathways of carbohydrate metabolism

1. Glycolysis	**A.** The oxidation of acetyl CoA to CO_2
2. Citric acid cycle	**B.** The formation of glycogen from glucose
4. Gluconeogenesis	**C.** This pathway is an alternative to glycolysis and TCA cycle for the oxidation of glucose (directly to carbon dioxide and water)
5. Glycogenesis	**D.** The oxidation of glucose to pyruvate or lactate
6. Glycogenolysis	**E.** Glucose is converted to glucuronic acid, pentoses and, in some animals, to ascorbic acid (not in man).
7. Hexose monophosphate shunt (pentose phosphate pathway or direct oxidative pathway)	**F.** The synthesis of amino sugars and other sugars for the formation of mucopolysaccharides and glycoproteins

8. Uronic acid pathway	**G.** The breakdown of glycogen to glucose
9. Galactose metabolism	**H.** The synthesis of glucose from non-carbohydrate precursors
10. Fructose metabolism	**I.** The pathways concerned with the conversion of galactose to glucose and the synthesis of lactose.
11. Amino sugar and mucopolysaccharide metabolism	**J.** The oxidation of fructose to pyruvate and the relation betweenfructose and glucose metabolism

5.2. Entry of glucose into cells

a) Glucose is transported into the cells by specific transport systems. What difference between insulin-independent and insulin-dependent transport system?

b) After the entry into the cells glucose is transformed to glucose-6-phosphate by ATP. This process is called _____. The reaction is catalysed by the specific enzyme _____ in liver cells and by non-specific _____ in liver and extrahepatic tissues.

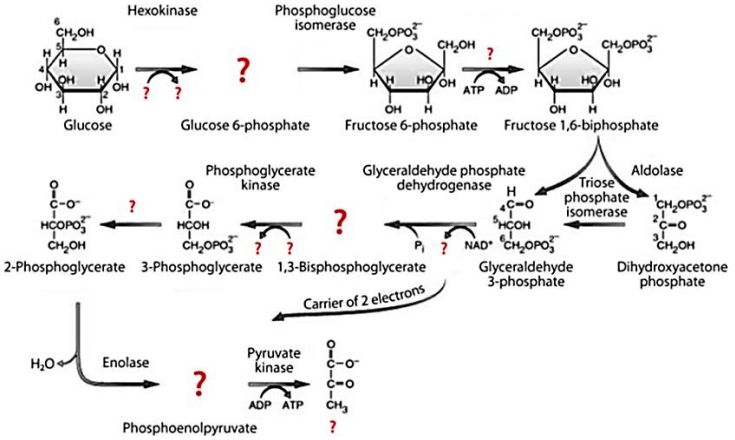
c) What principle difference between hexokinase and glucokinase?

5.3. Reactions of glycolysis.

a) What stages (phases) of glycolysis? Describe the differences between them.

b) Define substrate-level phosphorylation. What reactions in glycolysis are in this category?

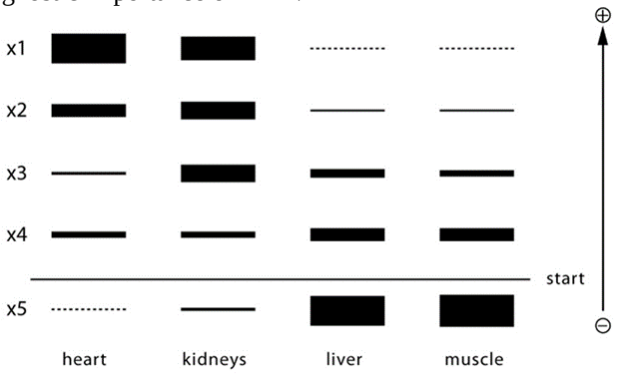
c) Fill in the blanks.



d) Write irreversible reactions of glycolysis. Which enzymes catalyse these ones?

5.4. Isoenzymes of LDH and their clinical diagnostic significance.

a) LDH has five distinct isoenzymes LDH_1, LDH_2, LDH_3, LDH_4 and LDH_5. They can be separated by electrophoresis (cellulose or starch gel or agarose gel). LDH_1 has more positive charge and fastest in electrophoretic mobility while LDH_5 is the slowest. Put the correct izoforms of LDHs and explain diagnostic importance of LDH.



b) Examination of a patient revealed increased activity of LDH_1, LDH_2 and creatine. What human organ might be damaged?

5.5. Mechanisms of regulation of the rate of reactions in anaerobic glycolysis.

a) Hexokinase is inhibited by _____.

b) Glucokinase is induced by _____.

c) Which enzyme is the most important regulatory enzyme in glycolysis?

d) Describe allosteric regulation of phosphofructokinase activity.

e) Describe regulation of pyruvate kinase activity:

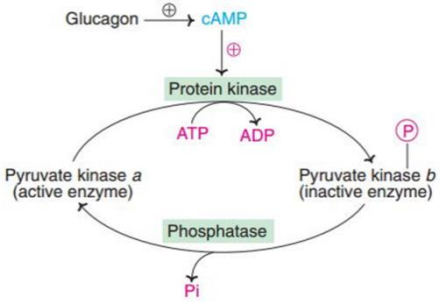
✓ by allosteric effectors

✓ by covalent modification (phosphorylation / dephosphorylation)

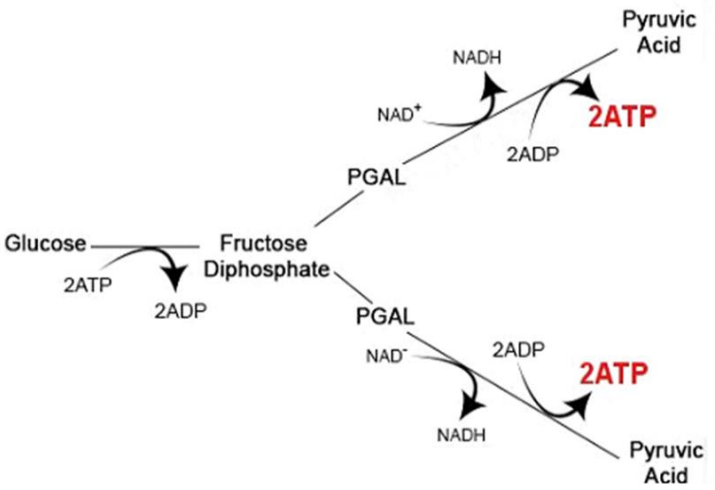
f) Choose the correct word to complete the sentence.

The hormone glucagon *inhibits / activates* hepatic glycolysis by covalent modification.

g) Fill in the scheme.



5.6. Indicate points of ATP generation in glycolysis and net production of ATP in anaerobic conditions; indicate enzymes of energy generation phase.



5.7. Situational tasks:

Clinical examination of the patient M. revealed presumptive diagnosis: stomach cancer. Lactic acid was found in gastric juice.

What type of glucose catabolism occurs in cancer cells?

Which enzyme involved in the formation of lactate?

Write the the reaction that catalyses by this enzyme.

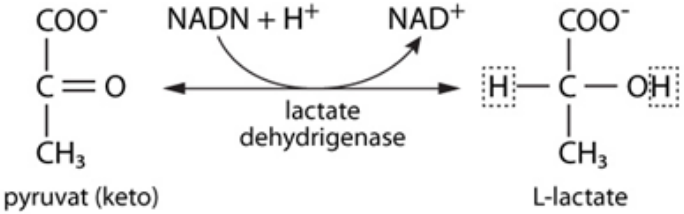
6. Individual independent students work

1. Disorders of carbohydrate metabolism and its pharmacological correction.
2. Principles of regulation of glucose metabolism. Characterization of regulatory enzymes in glycolysis.

Practice protocol №11 «____» _____ 20___

Experiment. Determination of the end-product of anaerobic glycolysis – lactic acid by the method of Uffelman.

The occurrence of uninterrupted glycolysis is very essential in skeletal muscle during strenuous exercise where oxygen supply is very limited. Glycolysis in the erythrocytes leads to ***lactate production***, since mitochondria – the centres for aerobic oxidation – are absent. Brain, retina, skin, renal medulla and gastrointestinal tract derive most of their energy from glycolysis.



Principle. At interaction of complex compound of iron phenolate of violet colour with lactic acid lactate of iron of yellow-green colour is formed .

Method. Place in a test tube 1 ml of blood serum, add 5 drops of 1 % solution of $FeCl_3$ and 1.0 ml of phenol solution. We observe occurrence of yellow-green colour.

Result:

Conclusion:

Clinical diagnostic significance. Venous blood of healthy person contains 0.5–2.2 mmol/1 of lactic acid. An increase of lactic acid content is associated with strenuous muscular effort during short time term intervals when there is a deficiency of oxygen. In this case the oxidative decarboxylation of pyruvate to acetyl-CoA does not occur and the production of the lactate takes place. Lactate can be utilized during a restoration period with a sufficient supply of oxygen. The increase

production of lactic acid is also observed during epilepsy, tetanies, convulsions and hypoxia, and it is associated with cardiac and pulmonary failure, malignant tumors, liver diseases and other pathologies.

The report is checked up _____

(The signature of the teacher, date)

Examples of Krock-1 tests

1. An untrained person who has not been practicing physical exercises for a long time complains of a muscle pain as a result of intensive manual work. What is the probable reason of the pain syndrome?

A. Accumulation of lactate in muscles
B. Decreasing of lipids level in muscles
C. Increased disintegration of muscle proteins
D. Accumulation of creatinine in muscles
E. Increase of ATP level in muscles.

2. Under relative deficiency of oxygen lactate is accumulated during intense muscular work. What is its further fate?

A. It is included in the gluconeogenesis in the liver
B. It is used by tissues for the synthesis of ketone bodies
C. It is removed with the urine
D. It is used in the muscles for synthesis of amino acids
E. It is used in the tissues for the synthesis of fatty acids

3. The high speed sprint causes a feeling of pain in skeletal muscles of untrained people that occurs due to lactate accumulation. The activation of what biochemical process is it resulting from?

A. Glycolysis
B. Pentose phosphate pathway
C. Gluconeogenesis
D. Lipogenesis
E. Glycogenesis

4. A person who was at hypodynamia state for a long time develops intensive ache in the muscles after physical work. What is the cause of this ache?

A. Lactic acid accumulation in muscles
B. Enhanced breakdown of muscle lipids
C. Creatine accumulation in muscles
D. Decrease in lipid contents of muscles
E. Increase in ADP contents of muscles

5. A 7-year-old girl manifests obvious signs of anemia. Laboratory tests showed the deficiency of pyruvate kinase activity in erythrocytes. The disorder of what biochemical process is a major factor in the development of anemia?

A. Anaerobic glycolysis
B. Deamination of amino acids
C. Tissue respiration
D. Oxidative phosphorylation
E. Breaking up of peroxides

6. What is the biological role of anaerobic glycolysis?

A. It is the only source of energy in the conditions of hypoxia
B. It supplies glucogenic amino acids for protein synthesis
C. It is the only way for rapid resynthesis of ATP in the muscle
D. It is the main way of energy production in uremia
E. It is the main way of energy formation in the nervous tissue

7. During consumption of cakes or sweets in mixed saliva a transient increase in lactate level takes place. Activation of what biochemical process causes this effect?

A. Anaerobic glycolysis
B. Tissue respiration
C. Aerobic glycilysis
D. Gluconeogenesis
E. Microsomal oxidation

8. What reactions of the anaerobic glycolysis require energy in the form of ATP?

A. Hexokinase and phosphofructokinase
B. Glucokinase and phosphoglycerate kinase
C. Glucokinase and succinylthiokinase
D. Hexokinase and pyruvate kinase
E. Glucokinase and creatine kinase

9. Which of the following compounds is a substrate of glycolytic oxidoreduction?

A. Glyceraldehyde-3-phosphate
B. Lactate
C. 1,3-Bisphosphoglycerate
D. Fructose 1,6-bisphosphate
E. Pyruvate

10. Anaerobic oxidation of glucose to lactic acid is regulated by certain enzymes. Which enzyme is the main regulator of this process?

A. Phosphofructokinase
B. Glucose 6-phosphate isomerase
C. Aldolase
D. Enolase
E. Lactate dehydrogenase

11. What of the following compounds inhibits phosphofructokinase?

A. ATP, citrate
B. ADP, lactate
C. AMP, citrate
D. Citrate, pyruvate
E. Fructose 1,6-bisphosphate, ATP

12. What of the following compounds are activators of phosphofructokinase?

A. Fructose-6-phosphate, AMP
B. ADP, lactate
C. ATP, citrate
D. Citrate, pyruvate
E. Fructose 1,6-bisphosphate, ATP

13. It is known that erythrocytes don't have mitochondria. Which compound is produced from pyruvate in the red blood cells?

A. Lactate
B. Carbon dioxide and water
C. Citrate
D. Acetyl-CoA
E. Isocitrate

14. At the second stage of glycolysis formation of substances containing macroenergy bonds occurs as a result of:

A. Kinase reactions
B. Endergonic reactions
C. Exergonic reactions
D. Transferase reactions
E. Dehydrogenase reactions

15. What is the rate-limiting enzyme of glycolysis?

A. Phosphofructokinase
B. Hexokinase
C. Aldolase
D. Enolase
E. Lactate dehydrogenase

16. What is the final metabolite of anaerobic glycolysis?

A. Lactate
B. Acetyl-CoA

C. Pyruvate
D. Water
E. Acyl-CoA

17. There are many metabolites of glucose oxidation in the cytoplasm of myocytes. Choose one of them that is directly converted to lactate:

A. Pyruvate
B. Oxaloacetate
C. Glycerol-3-phosphate
D. Glucose-6-phosphate
E. Fructose-6-phosphate

18. After a sprint an untrained person develops muscle hypoxia. What metabolite accumulates in muscles under this state?

A. Lactate
B. Ketone bodies
C. Acetyl CoA
D. Oxaloacetate
E. Glucose-6-phosphate

19. Human red blood cells don't have mitochondria. What is the main pathway for ATP production in these cells?

A. Anaerobic glycolysis
B. Aerobic glycolysis
C. Oxidative phosphorylation
D. Creatine kinase reaction
E. Cyclase reaction

20. Lactate and energy are produced in anaerobic glycolysis. How many ATP molecules are made using anaerobic glycolysis?

A. 2
B. 4
C. 36
D. 8
E. 12

21. Which one of the following statements about glycolysis in muscles is true?

A. It is activated at the first minute of muscle contraction
B. It is the only source of energy during prolonged muscle contraction
C. It is activated only during a long-term rhythmic contraction of the myofibrils
D. It is the last pathway included in the ATP resynthesis
E. This is the most advantageous energy pathway in comparison with other ones

22. Which organs, tissues and cells arecharacterized by high intensity of glycolysis?

A. Red blood cells, muscles, atypical cells (tumor)
B. Lymphocytes, liver, connective tissue
C. Bone and connective tissue, eye tissue
D. The liver, bone marrow, lymphoid tissue
E. The adipose and nervous tissues, leukocytes

23. What are the key reactions of glycolysis?

A. Hexokinase, phosphofructokinase and pyruvate kinase
B. Hexokinase, succinylthiokinase and pyruvate kinase
C. Pyruvate kinase, phosphoglycerate kinase, and creatine kinase
D. Glucokinase, pyruvate kinase, creatine kinase
E. Phosphofructokinase, phosphoglycerate kinase and pyruvate kinase

24. Diseases of the respiratory system and circulatory disorders impair the transport of oxygen, thus leading to hypoxia. Under these conditions the energy metabolism is carried out by anaerobic glycolysis. As a result, the following substance is generated and accumulated in blood:

A. Lactic acid
B. Pyruvic acid
C. Glutamic acid
D. Citric acid
E. Fumaric acid

25. Choose reactions of the anaerobic glycolysis where ATP is produced:

A. Phosphoglycerate kinase and pyruvate kinase
B. Glucokinase and succinylthiokinase
C. Hexokinase and pyruvate kinase
D. Glucokinase and creatine kinase
E. Glucokinase and phosphoglycerate kinase

26. What is the mechanism of ATP synthesis under anaerobic conditions?

A. Substrate level phosphorylation
B. Posttranslational phosphorylation
C. Oxidative phosphorylation
D. Covalent modification
E. Reductive phosphorylation

27. What are the consequences of hyperlactatemia?

A. Metabolic acidosis
B. Respiratory acidosis
C. Metabolic alkalosis
D. Respiratory alkalosis
E. –

28. Where does anaerobic glycolysis occur in the cell?

A. Cytosol
B. Nucleus

C. Mitochondrion
D. Membrane
E. Ribosome

29. Bioenergetics of brain considerably depends on oxygen supply. What substrate of oxidation has the greatest significance for providing brain with energy?

A. Glucose
B. Fatty acids
C. Ketone bodies
D. Glycerol-3-phosphate
E. Phosphoenolpyruvate

30. Energy in form of ATP is necessary for vital functions of erythrocytes. Which process provides these cells by sufficient amount of ATP?

A. Anaerobic glycolysis
B. Aerobic oxidation of glucose
C. Pentose phosphate pathway
D. Tricarboxylic acid cycle
E. β-Oxidaton of fatty acids

31. After running a sprint distance muscular rigility is observed in untrained people as a result of lactate accumulation. What biochemical process intensification is it linked with?

A. Glycolysis
B. Gluconeogenesis
C. Pentose phosphate pathway
D. Lipogenesis
E. Glycogenesis

32. A 32-year-old female patient suffers from gingivitis accompanied by gum hypoxia. What metabolite of carbohydrate metabolism is produced in the periodontium tissues more actively in this case?

A. Lactate
B. Ribose 5-phosphate
C. Glycogen
D. Glucose 6-phosphate
E. NADPH-H

33. Sarcoplasm contains a wide spectrum of glucose metabolites oxidation. Name one of them that is directly converted to lactate:

A. Pyruvate
B. Glycerophosphate

C. Glucose 6-phosphate
D. Oxaloacetate
E. Fructose 6-phosphate

34. After a sprint an untrained person develops muscle hypoxia. This leads to the accumulation of the following metabolite in muscles:

A. Lactate
B. Acetyl CoA
C. Ketone bodies
D. Oxaloacetate
E. Glucose 6-phosphate

35. A 60 year old patient was found to have a dysfunction of main digestive enzyme of saliva. This causes the disturbance of primary hydrolysis of:

A. Carbohydrates
B. Fats
C. Proteins
D. Cellulose
E. Lactose

36. When investigating human saliva it is necessary to assess its hydrolytic properties. What substance should be used as a substance in the process?

A. Starch
B. Amino acids
C. Proteins
D. Fiber
E. Fats

37. Pneumonia caused the development of respiratory hypoxia in the patient. What process of carbohydrate metabolism is activated in this case?

A. Anaerobic oxidation of glucose to lactate
B. Aerobic oxidation of glucose with the formation of CO_2 and H_2O
C. Gluconeogenesis
D. Synthesis of glycogen
E. Breakdown of glycogen

38. Some students developed myodynia after continuous physical activity during physical education. The reason for such condition was accumulation of lactic acid in the skeletal muscles. It was generated in the students' bodies after activation of the following process:

A. Glycolysis

B. Gluconeogenesis
C. Pentose-phosphate cycle
D. Lipolysis
E. Glycogenesis

39. Name sequentially enzymes that convert glucose into two trioses:

A. Hexokinase, phosphoglucomutase, phosphofructokinase, fructose-1,6-bisphosphate aldolase
B. Hexokinase, glucose-6-phosphatase, fructose-6-phosphate dehydrogenase
C. Glucokinase, glucose-6-phosphate dehydrogenase, phosphofructokinase, aldolase
D. Glucokinase, phosphohexoisomerase, fructose-1-phosphate-aldolase, triose phosphate isomerase
E. Hexokinase, phosphofructokinase, fructose-1,6-bisphosphate aldolase, dihydroxyacetone phosphate dehydrogenase

40. Which reaction is catalysed by lactate dehydrogenase?

A. The conversion of pyruvate into lactic acid
B. The conversion of glucose into glucose 6-phosphate
C. The conversion of succinate into fumarate
D. The conversion of isocitrate into alphaketoglutarate
E. The conversion of UDP-galactose into galactose

41. Concentration of glucose in blood plasma of healthy human varies:

A. 3.3–5.5 mmol/L
B. 2–4 mmol/L
C. 10–25 mmol/L
D. 6–9.5 mmol/L
E. 1–2 mmol/L

References:

1. Gubsky Yu. Biological chemistry: textbook / edited by Yu. Gubsky. – Vinnytsia : Nova Knyha, 2018. – 2nd edition. – 488 p.
2. Lecture materials.
3. Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – 799 p.
4. Textbook «Biological chemistry. Collection of test tasks» for English-speaking students of higher medical educational institutions of the Ministry of Public Health of Ukraine. – Poltava, 2018. – 148 p.

TOPIC №12.
Glucose oxidation under aerobic conditions

1. Objective: To learn the role of pyruvate dehydrogenase multienzyme complex in aerobic metabolism of glucose.

2. Actuality of the theme: Metabolism of carbohydrates includes all complex process of carbohydrates transformation starting from digestion, absorption, transport and utilization in cells up to formation of end products – CO_2 and H_2O. In aerobic conditions pyruvate, as a product of glycolysis, releases CO_2 and is transformed to acetyl-CoA, which is further oxidized in tricarboxylic acid cycle (Crebs cycle) to CO_2 and H_2O. The rate of reactions in TCA cycle depends from requirements of the cell in ATP. Regulatory reactions in TCA cycle are synthesis of citrate and oxidative decarboxylation of alpha-oxoglutarate, which are regulated by amount of ADP, succinyl-CoA and $NADH_2$.

3. Specific aims:

✓ To interpret mechanisms of monosaccharides transformation to final metabolic products and energetic effect in aerobic conditions.

✓ To analyze structural and functional peculiarities of pyruvate dehydrogenase complex.

4. Reference card for the separate study of educational literature for the lesson preparation

Questions:	References:
1. **Stages of aerobic oxidation of glucose.**	1. Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 249, 252, 254. 2. Gubsky Yu. Biological chemistry: textbook / edited by Yu. Gubsky. – Vinnytsia: Nova Knyha, 2018. – 2nd edition. – P. 149-150.
2. **Pasteur effect – switching over of anaerobic to aerobic oxidation of glucose, peculiarities of regulation:** ✓ Regulation of Pasteur effect; ✓ Enzymatic reactions of Pasteur effect.	Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 251.
3. **Crabtree effect**	Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 251.

4. **Oxidative decarboxylation of pyruvic acid.** ✓ structure of multienzyme pyruvate dehydrogenase complex. ✓ peculiarities of function of pyruvate tdehydrogenase complex. ✓ mechanism of oxidative decarboxylation of pyruvate. ✓ role of vitamins and coenzymes in transformation of pyruvate to acetyl-CoA.	1. Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 252-253. 2. Gubsky Yu. Biological chemistry: textbook / edited by Yu. Gubsky. – Vinnytsia: Nova Knyha, 2018. – 2nd edition. – P. 149–150.
5. **Energetic effect of aerobic oxidation of glucose.** ✓ Total ATP per mole of glucose under aerobic condition ✓ Total ATP per mole of glucose under anaerobic condition	1. Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 249. 2. Gubsky Yu. Biological chemistry: textbook / edited by Yu. Gubsky. – Vinnytsia: Nova Knyha, 2018. – 2nd edition. – P. 150.

5. Tasks for independent work and self-control

5.1. Write the stages of aerobic oxidation of glucose.

1) _____
2) _____
3) _____

5.2. Describe Pasteur effect.

5.3. Describe Crabtree effect.

5.4. Write the overall reaction of oxidative decarboxylation of pyruvic acid.

5.5. Structure of multienzyme pyruvate dehydrogenase complex (or α-ketoglutarate dehydrogenase complex).

Enzymes:

Coenzymes:

5.6. Describe regulation of PDH

✓ by allosteric effectors (feedback inhibition)

✓ by covalent modification (phosphorylation / dephosphorylation)

5.7. Biochemical importance of PDH

5.8. Medical importance of pyruvate

5.9. Energetic effect of aerobic oxidation of glucose

Stage	Substrate-level phosphorylation	Oxidative phosphorylation	Total number
Glycolysis (in the cytosol)			
Conversion of pyruvate to acetyl-CoA (inside mitochondria)			
TAC (inside mitochondria)			
Total ATP number (net yield) per molecule of glucose –			

5.10. Situational tasks:

a) In patients with chronic alcoholism observed increase of pyruvate content in blood serum and increase its excretion in the urine due to thiamine deficiency.

The activity of what metabolic process is reduced in these patients?

Write the scheme of the process, indicate enzymes and coenzymes.

Using of wich coenzyme enhance the metabolic activity of this pathway?

b) After the restoration of blood flow in damaged tissue the accumulation of lactate stops and glucose consumption rate decreases.

Activation of which processes are caused by these metabolic shifts?

What are the biochemical mechanisms underlying the Pasteur effect?

What allosteric enzymes (in glycolysis) are inhibited by ATP.

6. Individual independent students work

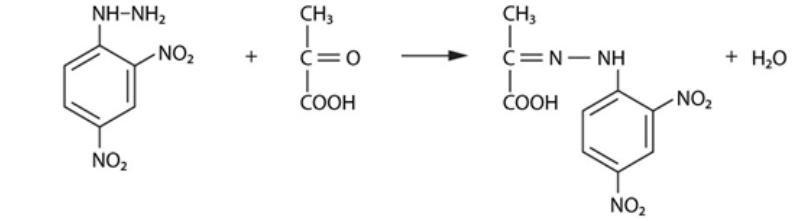
1. Energetics of glucose oxidation.

Practice protocol №12 «____» _____ 20___

Experiment. Quantitative determination of pyruvic acid in urine by colourimetric method.

Pyruvate is converted to acetyl CoA by oxidative decarboxylation. This is an irreversible reaction, catalysed by a multienzyme complex, known as ***pyruvate dehydrogenase complex (PDH)***, which is found only in the mitochondria. High activities of PDH are found in cardiac muscle and kidney. The enzyme PDH requires five cofactors (coenzymes), namely- TPP, lipoamide, FAD, coenzyme A and NAD^+ (lipoamide contains lipoic acid linked to amino group of lysine).

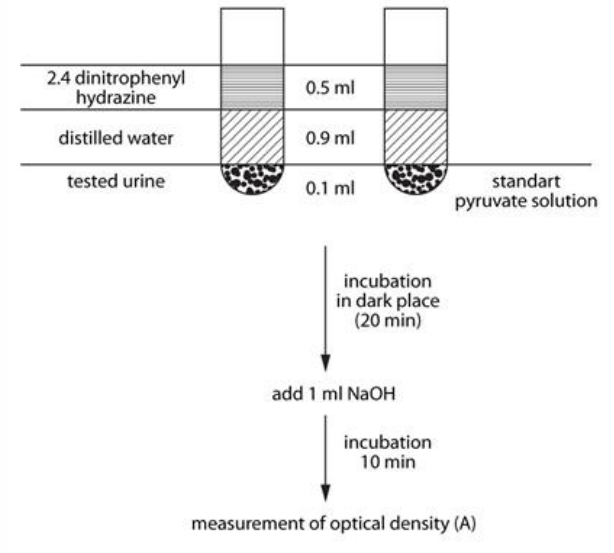
Principle. Pyruvic acid and 2,4-dinitrophenyl hydrazine form in alkaline medium a 2,4-dinitrophenylhydrazone pyruvate (brown-red colour compound). The intensity of the colour is proportional to the pyruvic acid concentration and is evaluated calorimetrically.



2.4-dinitrophenylhydrazone pyruvate 2.4-dinitrophenylhydrazone pyruvate (brown-red color compound)

Materials and reagents. Sample of urine, standard solution of pyruvic acid (625 mg in 100 ml of water), 0.1% solution of 2,4-dinitrophenylhydrazine (in 2 N hydrochloric acid), 12% solution of sodium hydroxide, distilled water, tubes, pipettes, colourimeter.

Method. To one tube is added 0.1 ml of tested urine, to another tube – 0.1 ml of standard pyruvate solution. To each tube is added 0.9 ml of distilled water. Thereafter is added 0.5 ml of 2,4-dinitrophenylhydrazine and tubes are leaved for 20 min in a dark place. Then to each tube is added 1 ml of 12% solution of sodium hydroxide and after 10 min the intensity of colour is measured in colourimeter with a blue light filter.



Calculation:

Pyruvic acid concentration (C_{exp}) is calculated according to the formula:

$$C_{exp} = \frac{C_{stand} \times A_{exp} \times V}{A_{stand} \times a}$$

where:

$C_{stand.}$ – concentration of standard pyruvate
$C_{exper.}$ – concentration of pyruvate in a sample of urine
$A_{exper.}$ – optical density of tested urine
$A_{stand.}$ – optical density of standard pyruvate
V – daily volume of excreted urine (1500 ml)
a – 0.1 ml of urine, taken for analysis.
Compare the obtained result with normal value. Draw the conclusion.

Result:

Conclusion:

The report is checked up _____

(The signature of the teacher, date)

Examples of Krock-1 tests

1. In a patient are manifested symptoms of intoxication with arsenic compounds. What metabolic process is damaged taking into account that arsenic containing substances inactivate lipoic acid?

A. Oxidative decarboxylation of pyruvate
B. Fatty acids biosynthesis
C. Neutralization of superoxide anions
D. Coupling of oxidation and phopsphorylation
E. Microsomal oxidation

2. Nicotinamide is widely used in the treatment of atherosclerosis. What biochemical process is improved under the influence of this metabolite?

A. Aerobic oxidation of substrates of metabolism
B. Synthesis of antiatherogenic lipoproteins
C. Activation of glucokinase reaction

D. Inhibition of cholesterol synthesis
E. Anaerobic oxidation of glucose

3. Patient has been prescribed energy correctors due to the disorders of energy metabolism in the tissues. Specify the metabolites that improve energy metabolism:

A. Succinate, $NADH_2$, $FADH_2$
B. Pyruvate, fumarate, $NADH_2$, $FADH_2$
C. α-ketoglutarate, succinyl-CoA, lipoic acid
D. Succinate, malate, $NADH_2$
E. Lactate, oxaloacetate, vitamin e

4. Treatment of many diseases involves using of cocarboxylase (thiamine pyrophosphate) for supplying cells with energy. What metabolic process is activated in this case?

A. Oxidative decarboxylation of pyruvate
B. Glutamate deamination
C. Amino acid decarboxylation
D. Decarboxylation of biogenic amines
E. Detoxication of harmful substances in liver

5. What is the inhibition of glycolysis reactions under conditions of active cellular respiration called?

A. Pasteur effect
B. Crabtree effect
C. Koch effect
D. Bohr effect
E. Mechnikov effect

6. Which metabolite is produced at the second stage of carbohydrate, lipid and amino acid catabolism?

A. Acetal-CoA
B. Ketone bodies
C. Pyruvate
D. Lactate
E. Citrate

7. Which enzymes catalyze oxidative decarboxylation of pyruvic acid?

A. Pyruvate dehydrogenase, dihydrolipoyl dehydrogenase, dihydrolipoyl transacetylase
B. Pyruvate dehydrogenase, isocitrate dehydrogenase, succinate dehydrogenase
C. Pyruvate dehydrogenase, phosphofructokinase, aldolase
D. Pyruvate kinase, citrate synthase, fumarase
E. Pyruvate dehydrogenase, dihydrolipoyl transacetylase, cytochrome oxidase

8. Four pairs of hydrogen atoms are produced in the Krebs cyclE. What are their further transformations?

A. $NADH_2$ and $FADH_2$ are oxidized by transfer of electrons and protons in the respiratory chain to molecular oxygen to form water

B. They included in the oxidative phosphorylation

C. They included in substrate level phosphorylation

D. They form water by reacting with oxygen

E. They provide accumulation of energy in macroenergy compounds

9. What are coenzymes of pyruvate dehydrogenase complex?

A. TPP, HS-CoA, lipoic acid, NAD^+ and FAD

B. NAD, FAD, pyridoxal phosphate, lipoic acid, HS-CoA

C. NAD, FAD, HS-CoA, lipoic acid, pyruvate kinase

D. Lipoic acid, NAD, FAD, HS-CoA, ions of Mg^{2+}

E. NAD, FAD, cAMP, lipoic acid, pyruvate dehydrogenase

10. What is the role of acetyl-CoA and NADH in the oxidative decarboxylation of pyruvate?

A. They are allosteric inhibitors of pyruvate dehydrogenase complex

B. They are activators of the pyruvate dehydrogenase complex

C. They are end products of oxidative decarboxylation of pyruvate

D. They are intermediate products of oxidative decarboxylation of pyruvate

E. They activate pyruvate dehydrogenase complex

11. Complete oxidation of glucose molecule and its coupling with phosphorylation is equivalent to the following total amount of ATP molecules formation:

A. 38

B. 8

C. 12

D. 2

E. 58

12. The formation of oxaloacetate from pyruvate is an important anaplerotic reaction. What enzyme catalyzes this reaction?

A. Pyruvate carboxylase

B. Citrate synthase

C. Pyruvate dehydrogenase

D. Malate dehydrogenase

E. Pyruvate decarboxylase

13. What reaction allows to convert pyruvate into the component of TCA using pyruvate carboxylase enzyme?

A. The conversion of pyruvate into oxaloacetate

B. The conversion of pyruvate into citrate

C. The conversion of pyruvate into malate

D. The conversion pyruvate into fumarate
E. The conversion pyruvate into succinate

14. At what stage of glycolysis are trioses formed such as dyhydroxyacetone phosphate and glyceraldehyde-3-phosphate?

A. At the first stage
B. At the second stage
C. In the process of oxidative decarboxylation of pyruvate
D. In the process of substrate phosphorylation
E. In the process of the formation of phosphoenolpyruvate

15. What substrates can be synthesized from the components of the citric acid cycle?

A. Amino acids, glucose, heme
B. Acetyl-CoA, pyruvate, fatty acids
C. Acetyl-CoA, fatty acids, glycogen
D. Amino acids, citric acid, heme
E. Amino acids, fatty acids, glycerol

16. Choose the regulators of Krebs cycle:

A. ATP/ADP ratio, $NADH_2/NAD^+$, $FAD/FADH_2$
B. The concentration of ATP in the cell, CO_2
C. ADP/AMP ratio, $FADH_2/FAD$
D. The concentration of pyruvate, lactate
E. ADP/AMP ratio, $NADH_2/NAD^+$

17. Formation of oxaloacetate from pyruvate is an important anaplerotic reaction that is catalyzed by pyruvate carboxylase. What is a coenzyme of pyruvate carboxylase?

A. Carboxybiotin
B. NAD^+
C. FAD
D. THF
E. TPP

18. It has been found out that one of pesticide's components is sodium arsenate that blocks lipoic acid. Which enzyme activity is impaired by this pesticide?

A. Pyruvate dehydrogenase complex
B. Microsomal oxidation
C. Methemoglobin reductase
D. Glutathione peroxidase
E. Glutathione reductase

19. Oxidative decarboxylation of pyruvate is the second stage of aerobic oxidation of glucose. What is the main product of this process?

A. Acetyl-CoA
B. Citrate
C. Glutamate

D. Oxaloacetate
E. Succinyl-CoA

20. The second stage of aerobic oxidation of glucose in cell is oxidative decarboxylation of pyruvate. Indicate the main product of this process:

A. Acetyl-CoA
B. Succinyl-CoA
C. Pyruvate
D. Citrate
E. Oxaloacetate

21. The thiamine deficiency results in beriberi disease which is characterised by carbohydrate metabolism disturbances. What metabolite is accumulated in blood at this state?

A. Pyruvate
B. Lactate
C. Succinate
D. Citrate
E. Malate

22. The thiamine deficiency results in beri-beri disease onset (polyneuritis) which is characterized by carbohydrate metabolism disturbances. What metabolite is accumulated in blood at this state?

A. Pyruvate
B. Lactate
C. Succinate
D. Citrate
E. Malate

23. After restoration of blood circulation in damaged tissue accumulation of lactate comes to a stop and speed of glucose consumption slows down. These metabolic changes are caused by activation of the following process:

A. Aerobic glycolysis
B. Anaerobic glycolysis
C. Lipolysis
D. Gluconeogenesis
E. Glycogen biosynthesis

24. When blood circulation in the damaged tissue is restored, then lactate accumulation comes to a stop and glucose consumption decelerates. These metabolic changes are caused by activation of the following process:

A. Aerobic glycolysis
B. Anaerobic glycolysis
C. Lipolysis

D. Gluconeogenesis
E. Glycogen biosynthesis

25. A woman-worker of chemical industry is delivered to clinics. She possesses the symptoms of poisoning. Her hair contains the high level of arsenate. The latter blocks lipoic acid activity. What biochemical process is most likely to suffer from this?

A. Oxidative decarboxylation of pyruvic acid
B. Microsomal oxidation
C. Methemoglobin renovation
D. Organic oxides renovation
E. Superoxide ions detoxification

26. What are the shuttle systems of glycolytic NADH2 oxidation?

A. Malate-aspartate, glycerol phosphate
B. Glycerol phosphate, glucose-lactate
C. Glycerol phosphate, aspartate-lactate
D. Malate-aspartate, glycine phosphate
E. Citrate-malate, malate-aspartate

References:

1. Gubsky Yu. Biological chemistry: textbook / edited by Yu. Gubsky. – Vinnytsia : Nova Knyha, 2018. – 2nd edition. – 488 p.
2. Lecture materials.
3. Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – 799 p.
4. Textbook «Biological chemistry. Collection of test tasks» for English-speaking students of higher medical educational institutions of the Ministry of Public Health of Ukraine. – Poltava, 2018. – 148 p.

TOPIC №13.
Alternative ways of exchange of monosaccharides. Metabolism of fructose and galactose

1. Objective: To interpret metabolic pathways of fructose and galactose in human body. To learn the sequence of enzymatic reactions in Pentose Phosphate Pathway (PPP).

2. Actuality of the theme: Pentose phosphate and glucuronate ways of an exchange of glucose, an exchange of fructose and galactose belong to alternative ways of an exchange of monosaccharides. The pentose-phosphate pathway of an exchange of glucose – the source of pentoses for synthesis of nucleotides, nucleic acids, coenzymes, except for it is a source of restored NADPH which are used at synthesis of fatty acids, cholesterol, steroid hormones and other compounds. Glucuronate pathway of an exchange of glucose – the source of glucoronate acid which is used for synthesis of glucosaminoglycans (carbohydrate derivatives of proteoglycans – proteins of a connective tissue) and takes part in neutralization of toxins in a liver. Fructose and galactose are included in a way of an exchange of glucose. At infringement of transformation of fructose and galactose fructosemia and galactosemia are developed.

3. Specific aims:

✓ To interpret mechanisms of monosaccharides transformation to final metabolic products and energetic effect in aerobic conditions

✓ To analyze structural and functional peculiarities of pyruvate dehydrogenase complex

✓ To explain the sequence of reactions in PPP and significance of this process

✓ To analyze metabolic pathways of fructose and galactose transformations in human body.

4. Reference card for the separate study of educational literature for the lesson preparation

Questions:	References:
1. **PPP and glucose utilization:** ✓ scheme of reactions in oxidative and nonoxidative stages of PPP; ✓ enzymes and coenzymes of PPP reactions; ✓ biological significance of PPP; ✓ disorders of PPP in red blood cells; ✓ enzymopathias of glucose-6-phosphate dehydrogenase.	1. Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 270–275. 2. Gubsky Yu. Biological chemistry: textbook / edited by Yu. Gubsky. – Vinnytsia : Nova Knyha, 2018. – 2nd edition. – P. 150–155.

2. **Enzymatic reactions of fructose turnover in human body. Hereditary enzymopathias of fructose metabolism.** ✓ Reactions of fructose turnover; ✓ Enzymopathias of fructose metabolism	Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 87, 278–281.
3. **Enzymatic reactions of galactose metabolism in human body. Hereditary enzymopathias of galactose metabolism.** ✓ Reactions of galactose metabolism. ✓ Enzymopathias of galactose metabolism	Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 276–278.

5. Tasks for independent work and self-control

5.1. PPP and glucose utilization.

a) Describe hexose monophosphate pathway (HMP).

b) Location of the pathway.

c) Characteristic of reactions of HMP (PPP) oxidative phase

Enzymes	Substrates	Products	Type of reaction
Glucose-6-phosphate dehydrogenase			
Gluconolactone hydrolase (lactonase)			
Phosphogluconate dehydrogenase			

d) Describe non-oxidative stage of HMP (PPP).

e) Write the overall reaction of HMP (PPP).

f) Biological significance of HMP (PPP). Importance of pentoses

g) Explain the differences between NAD and NADP:

	NAD/NADH	NADP/NADPH
Metabolic pathways that produced NADH or NADPH		
Main cell localization		
Functions of reduced forms		

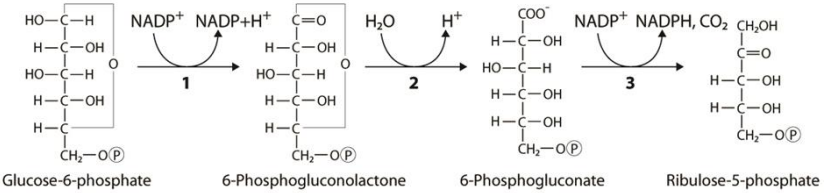
h) The direction of the HMP (PPP) reactions:

Cellular need	Direction of pathway, predominant phase
NADPH only	
NADPH and ribose-5-P	
Ribose-5-P only	
NADPH and pyruvate	

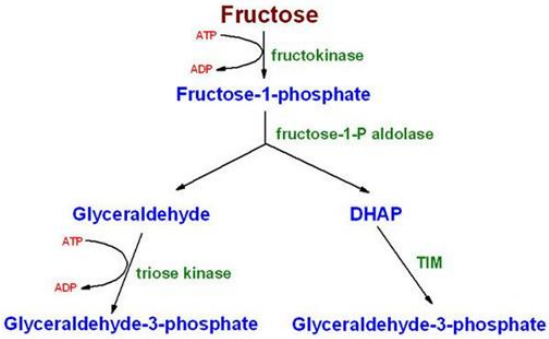
j) Disorders of PPP in red blood cells. Enzymopathias of glucose-6-phosphate dehydrogenase.

k) Wernicke-Korsakoff syndrome.

5.2. Indicate enzymes catalyzing the following reactions of HMP:



5.3. Name enzymes of fructose metabolism.



5.4. Genetic disorders of fructose and galactose metabolism.

a) Fill in the chart:

	Essential fructosuria	Hereditary fructose intolerance (HFI)	Classical galactosemia	Nonclassical galactosemia
Defective enzyme				
What substances are accumulated in blood and urine?				
What substances are accumulated in cells?				
Clinical symptoms				

b) Describe the influence of accumulation of fructose-1-phosphate or galactose-1-phosphate in the liver cells on:

1) glycogenolysis and gluconeogenesis

2) level of inorganic phosphate

3) level of ATP

c) Describe the influence of accumulation of sugars and sugar alcohols (sorbitol, galactotitol) in the lens of patients.

5.5. Situational tasks:

a) Scientist and anthropologist who was going in an expedition to South Africa to prevent malaria appointed quinacrine. On the background quinacrine intake hemolytic jaundice appeared of the patient.

What is the reason haemolysis of red blood cells while taking the drug against malaria?

The formation of which reducing agent violated in a red blood cells?

Which way in carbohydrate metabolism provides it?

b) The 10-month-old child was found mental retardation, liver enlargement, blurred vision. The physician connects these symptoms with congenital enzymopathies and recommends to exclude all dairy products from the diet.

Which enzyme deficiency occurs in a child?

Write the reaction that it catalyzes.

High concentration of what substance in the blood can confirm the diagnosis?

6. Individual independent students work

1. Pathobiochemistry of disorders in pentosophosphate pathway.
2. Pathobiochemistry of enzymopathias in connection with genetic defects in synthesis of enzymes of fructose and galactose metabolism.

Practice protocol №13 «____» _____ 20___

Experiment 1. Quantitative determination of pentoses in blood.

Principle. The method is based on colour reaction between pentose (ribose or deoxiribose) and orcine in the sour environment at presence Fe^{3+}. At their interaction the complex of green colour is formed. Intensity of painting depends on quantity of pentoses.

Method. To add in a test tube to 0.5 ml of researched solutions 0.5 ml of 1 % orcine (prepared on 0.1 % solution of $FeCl_3$ in concentrated HCl). Simultaneously to put the standard: to take 0.5 ml of pentose solution

known concentration and to add 0.5 ml of orcine reagent. To place test tubes in a boiling water bath for 40 minutes. Then to extend, add on 5 ml of water; to mix the contents of test tubes and to determine optical density of solutions on FEC in a ditch on 5 mm at a red optical filter (λ = 665 nanometers). On the bases of values of optical density of the standard and a researched solution to make a proportion and to calculate the contents of pentoses.

In norm concentration of pentoses: in blood – it is less 133 μmol/l (2 mg %), in urine – on the average about 250 mg / day.

Result:

Conclusion:

Experiment 2. Revealing fructose by Selivanov's reaction.

Principle. At heating of fructose solution with the concentrated hydrochloric acid oximethilphurphurol is formed that gives with resorcine the product of condensation painted in intensive red colour.

Method. In a test tube place 2–3 drops of a solution of fructose of 1 %, 4-5 drops of Selivanov's reagent (0.05 g of resorcine dissolve in 100 ml of the dissolved hydrochloric acid 1:1). Heat up and observe the change of painting of a solution.

Result:

Conclusion:

The report is checked up _____

(The signature of the teacher, date)

Examples of Krock-1 tests

1. What inherited disorder of galactose metabolism leads to mental retardation in a child?

A. Galactosemia
B. Glycogenosis
C. Fructosemia
D. Aglycogenosis
E. Fructose intolerance

2. Which of the following biochemical processes metabolism of fructose interacts with?

A. Glycolysis
B. Gluconeogenesis
C. Glycogenolysis
D. Glycogen synthesis
E. Protein synthesis

3. Fatty liver, galactosuria and aminoaciduria are observed in a newborn. Which substance must be excluded from the diet?

A. Milk sugar
B. Fatty acids
C. Phenylalanine
D. Cholesterol
E. Sucrose

4. What stage of the pentose phosphate pathway forms a large set of monosaccharides with different numbers of carbon atoms?

A. Nonoxidative reversible stage
B. Oxidative reversible stage
C. Anabolic stage
D. Catabolic stage
E. Transketolase stage

5. What mechanism controls the intensity of the pentose phosphate pathway?

A. The ratio of NADP/NADPH2 in the cell
B. ATP/ADP ratio
C. The ratio of creatine and creatinine
D. The activity of the pyruvate dehydrogenase multienzyme complex
E. Partial proteolysis

6. What is the mechanism of hemolytic anemia development under inherited deficiency of glucose-6-phosphate dehydrogenase?

A. Decreased concentration of reduced glutathione in red blood cells
B. Decreased content of hemoglobin

C. Lack of fructose-6-phosphate
D. Lack of ribose-5-phosphate
E. Activation of glycolysis

7. What is the key regulatory enzyme of the pentose phosphate pathway called?

A. Glucose-6-phosphate dehydrogenase
B. Phosphorylase
C. Glucokinase
D. Pyruvate dehydrogenase
E. Succinate dehydrogenase

8. What are the functions of reduced glutathione in red blood cells?

A. It reduces oxidizing agents such as H_2O_2
B. It produces NADPH
C. It reduces methemoglobin to hemoglobin
D. It reduces pyruvate to lactate
E. It produces NADH

9. Which one of the following biochemical processes supplies the cells by ribose-5-phosphate for the synthesis of nucleotides, nucleic acids and other pentose containing compounds?

A. Pentose phosphate pathway
B. Aerobic glycolysis
C. Anaerobic glycolysis
D. Microsomal oxidation
E. Glucuronate pathway

10. The child had vomiting and cramps after eating honey. It has been suspected a congenital intolerance to fructose. Which of the following enzyme's activity determination confirms the diagnosis?

A. Fructose-1-phosphate aldolase
B. Glycogen phosphorylase
C. Fructokinase
D. Hexokinase
E. 6-Phosphofructokinase

11. $NADPH_2$ is produced in the pentose phosphate pathway of glucose oxidation and used as a donor of reduced equivalents in anabolic reactions. What other processes actively occurring especially in the liver, require $NADPH_2$?

A. Phosphorylation
B. Decarboxylation
C. Transamination
D. Detoxification
E. Esterification

12. It was revealed in the experiment that intolerance to fructose is associated with a hereditary deficiency of the enzyme fructose-1-phosphate aldolase. Which of the following products of fructose metabolism accumulates in this case?

A. Fructose-1-phosphate
B. Fructose-1,6-bisphosphate
C. Fructose-6-phosphate
D. Glucose-6-phosphate
E. Glucose-1-phosphate

13. A newborn has good feeling after breastfeeding. Vomiting, abdominal ache, diarrhea and hypoglycemia appear after adding fruits and juices to the diet. What is the cause of such state?

A. Hereditary fructose intolerance
B. Ketosis
C. Gierke's disease
D. Glucosuria
E. Hyperglycemia

14. Reduced $NADPH_2$ is generated in the oxidative phase of the pentose phosphate pathway. Which of the following vitamins is the precursor of NADP coenzyme?

A. PP
B. B_c
C. B_2
D. B_1
E. B_{12}

15. Hexuronic acids play an important role in many biochemical processes. In which of the following pathways of glucose metabolism are they produced?

A. Glucuronate pathway
B. Glucuroniltransferase reaction
C. Pentose phosphate pathway
D. Gluconeogenesis
E. Glycogenolysis

16. It is known that the nonoxidative phase of the pentose phosphate pathway of glucose oxidation has several transketolase reactions. What is the coenzyme of transketolase?

A. TPP
B. $NADH_2$
C. $FADH_2$
D. $NADPH_2$
E. Pyridoxal phosphate

17. Hemolytic anemia has been developed in a patient in result of using of antibiotics for treatment of pneumonia. Laboratory tests established deficiency of glucose-6-phosphate dehydrogenase. Which metabolic pathway is affected in erythrocytes of the patient?

A. Pentose phosphate pathway
B. Glycolysis
C. Gluconeogenesis
D. Lipolysis
E. Phosphorylation of glucose

18. Vomiting, diarrhea, general dystrophy, hepato- and splenomegaly were observed in a newborn. These symptoms decrease after exclusion of milk from the diet. Which main hereditary defect results in the pathology?

A. Disturbance of galactose metabolism
B. Disturbance of phenylalanine metabolism
C. Hypersecretion of endocrine glands
D. Disturbance of glucose metabolism
E. Deficiency of glucose-6-phosphate dehydrogenase

19. Glucose-6-phosphate dehydrogenase reaction is a key regulatory reaction of the pentose phosphate pathway. Indicate the products of the reaction:

A. 6-Phosphogluconolactone, $NADPH_2$
B. Fructose-1,6-bisphosphate, $NADPH_2$
C. 6- Phosphogluconolactone, $HADH_2$
D. Fructose-6-phosphate, $NADPH_2$
E. Ribose-5-phosphate, $NADPH_2$

20. Fatty liver, galactosuria and aminoaciduria are observed in a newborn. Which substance must be excluded from diet?

A. Milk sugar
B. Fatty acids
C. Phenylalanine
D. Cholesterol
E. Sucrose

21. A 2-year–old boy is observed with cataract and increased liver and spleen size. The sugar blood level is increased too, although glucose tolerance test results are normal. The cause of the state is hereditary impaired metabolism of a substance. What is the substance?

A. Galactose
B. Glucose
C. Fructose
D. Maltose
E. Sucrose

22. Dyspepsia phenomenon (diarrhea, vomiting) was observed in a newborn fed with milk. These symptoms disappeared after feeding with glucose solution. Give the enzyme, which takes part in carbohydrate digestion and deficiency of which leads to these disturbances.

A. Lactase
B. Sucrase
C. Amylase
D. Isomerase
E. Maltase

23. A baby has been delivered to the clinics emergency department. It has the symptoms of vomitting, diarhea, growth impairment, cataract, mental retardation. The diagnosis is galactosemia. What enzyme deficiency is present at this case?

A. Hexose-1-phophate uridyl transferase
B. Glukokinase
C. UDP- glucose-4-epimerase
D. UDP- glucose pyrophosphorylase
E. Glucose-6-phosphate dehydrogenase

24. Fructosuria is known to be connected with inherited deficiency of fructose 1-phosphate aldolase. Which product of fructose metabolism will accumulate in the organism resulting in toxic action?

A. Fructose1-phosphate
B. Fructose 1,6-biphosphate
C. Glucose 1-phosphate
D. Fructose 6-phosphate
E. Glucose 6-phosphate

25. Galactosemia has been revealed in a child. Concentration of glucose in the blood has not considerably changed. What enzyme deficiency caused this illness?

A. Galactose-1-phosphate uridyltransferase
B. Galactokinase
C. Hexokinase
D. Amylo-1,6-glucosidase
E. Phosphoglucomutase

26. The biosynthesis of purine ring proceeds with the participation of ribose-5-phosphate by gradual upbuilding nitrogen and carbon atoms and uplocking the rings. What is the source of ribose-5-phosphate?

A. Penthose phosphate pathway
B. Glycolysis
C. Glycogenesis
D. Gluconeogenesis
E. Glycogenolysis

27. A newborn develops dyspepsia after the milk feeding. When the milk is substituted by the glucose solution the dyspepsia symptoms disappear. The newborn has the subnormal activity of the following enzyme:

A. Lactase
B. Invertase
C. Maltase
D. Amylase
E. Isomaltase

28. A child's blood presents high content of galactose, glucose concentration is low. There are such presentations as cataract, mental deficiency, adipose degeneration of liver. What disease is it?

A. Galactosemia
B. Steroid diabetes
C. Lactosemia
D. Diabetes mellitus
E. Fructosemia

29. It is known that the pentose phosphate pathway occuring in the adipocytes of adipose tissue acts as a cycle. What is the main function of this cycle in the adipose tissue?

A. $NADPH_2$ generation
B. Ribose-phosphate production
C. Xenobiotic detoxification
D. Energy generation
E. $NADH_2$ generation

30. A 40-year-old patient suffers from intolerance of dairy food products. This condition has likely developed due to insuficiency of the following digestive enzyme:

A. Lactase
B. Lipase
C. Maltase
D. Invertase
E. Amylase

31. It is known that pentose-phosphate pathway actively functions in the erythrocytes. What is the main function of this metabolic pathway in the erythrocytes?

A. Counteraction to lipid peroxidation
B. Activation of microsomal oxidation
C. Neutralization of xenobiotics
D. Oxidation of glucose into lactate
E. Increase of lipid peroxidation

32. A cataract and fatty degeneration of the liver develop in the conditions of high galactose and low glucose level in blood. What disease do these symptoms testify to?

A. Galactosemia
B. Diabetes mellitus
C. Lactosemia
D. Steroid diabetes
E. Fructosemia

33. A 7-year-old child presents with marked signs of hemolytic anemia. Biochemical analysis of erythrocytes determined low concentration of NADPH and reduced glutathione. What enzyme is deficient in this case leading to the biochemical changes and their clinical manifestations?

A. Glucose-6-phosphate dehydrogenase
B. Pyruvate kinase
C. Fructokinase
D. Lactate dehydrogenase
E. Hexokinase

34. A newborn child with the signs of cataract, growth and mental retardation, who manifested vomiting and diarrhea, was brought to an emergency clinic. A presumptive diagnosis of galactosemia was made. The deficiency of what enzyme occurs in case of this disease?

A. Galactose-1-phosphate uridyl transferase
B. Glucokinase
C. UDP-galactose-4-epimerase
D. Hexokinase
E. Glucose-6-phosphate dehydrogenase

35. Which of the following enzymes cleaves the disaccharide in the milk?

A. Lactase
B. Hexokinase
C. Maltase
D. Pyruvate kinase
E. Glucose-6-phosphatase

36. A 40 year-old patient suffers from whole milk intolerance. Which digestion enzyme deficiency explains the phenomenon?

A. Lactase
B. Amylase
C. Lactate dehydrogenase
D. Maltase
E. Lipase

37. It is known that the rate of the pentose phosphate pathway is limited by NADP/$NADPH_2$ ratio in the cell. What key enzymes of oxidative phase are regulated by NADP and $NADPH_2$?

A. Glucose-6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase
B. Glucose-6-phosphatase, hexokinase
C. Fructose-1,6-diphosphatase, phosphofructokinase
D. Lactonase, 6-phosphogluconate dehydrogenase
E. Galactose-1-phosphate-uridyltransferase, fructose-1-phosphate aldolase

38. Essential fructosuria is a hereditary disease, connected with disorders of fructose metabolism. The symptoms of lesions of liver and kidneys are manifested. This disease is caused by insufficiency of enzyme, which catalyze transformation of fructose to the next compound:

A. Fructoso-1-phosphate
B. Fructoso-6-phosphate
C. Fructoso-1,6-bisphosphate
D. Glucoso-6-phosphate
E. Glyceraldehyde phosphate

References:

1. Gubsky Yu. Biological chemistry: textbook / edited by Yu. Gubsky. – Vinnytsia : Nova Knyha, 2018. – 2nd edition. – 488 p.
2. Lecture materials.
3. Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – 799 p.
4. Textbook «Biological chemistry. Collection of test tasks» for English-speaking students of higher medical educational institutions of the Ministry of Public Health of Ukraine. – Poltava, 2018. – 148 p.

TOPIC №14.
Breakdown and biosynthesis of glycogen. Regulation of glycogen metabolism, biosynthesis of glucose – gluconeogenesis

1. Objectives: To learn reactions of synthesis and breakdown of glycogen and mechanisms of humoral regulation of glycogen metabolism in liver and muscles. To interpret reactions of gluconeogenesis, their peculiarities and principles of their regulation.

2. Actuality of the theme: Glycogen is the storage form of glucose in animals, as is starch in plants. It is stored mostly in liver (6-8 %) and muscle (1–2 %). Due to more muscle mass, the quantity of glycogen in muscle (250 g) is about three times higher than that in the liver (75 g). Glycogen is stored as granules in the cytosol, where most of the enzymes of glycogen synthesis and breakdown are present.

3. Specific aims:

✓ To explain characteristic features of glycogen breakdown and biosynthesis.

✓ To analyze mechanisms of humoral regulation of glycogen metabolism in liver and muscles.

✓ To explain hereditary disorders of glycogen metabolism.

✓ To analyze specific features of gluconeogenesis reactions and substrates of this process.

✓ To explain and interpret regulatory mechanisms of gluconeogenesis.

4. Reference card for the separate study of educational literature for the lesson preparation

Questions:	References:
1. **Mechanism and peculiarities of enzymatic reactions of glycogenesis.**	1. Satyanarayana U., Chakrapani U. «Biochemistry», Fourth Edition. – 20013. – P. 263–265. 2. Gubsky Yu. Biological chemistry: textbook / edited by Yu. Gubsky. – Vinnytsia: Nova Knyha, 2018. – 2nd edition. – P. 156-157, 160–162.
2. **Glycogenolysis.**	1. Satyanarayana U., Chakrapani U. «Biochemistry», Fourth Edition. – 20013. – P. 265–266. 2. Gubsky Yu. Biological chemistry: textbook / edited by Yu. Gubsky. – Vinnytsia: Nova Knyha, 2018. – 2nd edition. – P. 157–160.
3. **Cascade mechanisms of ATP-dependent regulation of glycogen phosphorylase and glycogen synthase activities.**	1. Satyanarayana U., Chakrapani U. «Biochemistry», Fourth Edition. – 20013. – P. 266–268. 2. Gubsky Yu. Biological chemistry: textbook / edited by Yu. Gubsky. – Vinnytsia: Nova Knyha, 2018. – 2nd edition. – P. 163–166.

4. **Peculiarities of hormonal regulation of glycogen metabolism in liver and muscles.**	1. Satyanarayana U., Chakrapani U. «Biochemistry», Fourth Edition. – 20013. – P. 266–268. 2. Gubsky Yu. Biological chemistry: textbook / edited by Yu. Gubsky. – Vinnytsia: Nova Knyha, 2018. – 2nd edition. – P. 163–166.
5. **Hereditary disorders in enzymes of glycogen synthesis and breakdown. Glycogenoses, aglycogenoses.**	1. Satyanarayana U., Chakrapani U. «Biochemistry», Fourth Edition. – 20013. – P. 269–270. 2. Gubsky Yu. Biological chemistry: textbook / edited by Yu. Gubsky. – Vinnytsia: Nova Knyha, 2018. – 2nd edition. – P. 166–167.
6. **Metabolic pathways and substrates of gluconeogenesis.** ✓ compartmentalization of enzymes, ✓ biological significance of the process.	1. Satyanarayana U., Chakrapani U. «Biochemistry», Fourth Edition. – 20013. – P. 258–259. 2. Gubsky Yu. Biological chemistry: textbook / edited by Yu. Gubsky. – Vinnytsia: Nova Knyha, 2018. – 2nd edition. – P. 167–171.
7. **Relations between glycolisis and gluconeogenesis (Cori cycle). Irreversible reactions of glycolysis and their shunt pathways. Glucose-lactate and glucose-alanine cycles.**	1. Satyanarayana U., Chakrapani U. «Biochemistry», Fourth Edition. – 20013. – P. 259–263.
8. **Regulation of gluconeogenesis in human organism.**	Satyanarayana U., Chakrapani U. «Biochemistry», Fourth Edition. – 20013. – P. 262–263.

5. Tasks for independent work and self-control

5.1. Write structural formula of glycogen fragment with branch point. Indicate the types of bonds.

5.2. What functions of glycogen in different tissues?

Liver	Skeletal muscle

5.3. Fill in the chart «Characteristic of glycogenesis reactions»

	Enzymes	Substrates	Products	Type of reaction
1	Glucokinase/ Hexokinase			
2	Phosphoglucomutase			
3	UDP-glucose pyrophosphorylase			
4	Glycogen Synthase			
5	Branching enzyme			

5.4. Fill in the chart «Characteristic of glycogenolysis reactions»

	Enzymes	Substrates	Products	Type of reaction
	Glycogen phosphorylase			
	Debranching enzyme (α-1,6-glucosidase)			
	Phosphoglucomutase			
	Glucose-6-phosphatase			

5.5. Compare glycogenolysis and functions of glycogen in: 1) liver, 2) muscles. Explain the difference.

5.6. Explain why glycogen stored in muscle is not available for maintenance of blood glucose level.

5.7. Fill in the chart:

Regulated Enzymes	Activators and mechanism of activation	Inhibitors and mechanism of inhibition
Glycogen synthase		
Glycogen phosphorylase		
Phosphorylase kinase		

5.8. Glycogen synthesis is activated when glycogen degradation is inhibited, and vice versa. Explain how and why.

Hormone	Endocrine gland	Initiating factor	Effect on glycogenolysis	Effect on glycogenesis
Glucagon				
Epinephrin				
Insulin				

5.9. Glycogen storage diseases: causes and clinical symptoms.

Type	Name of disease	Defective enzyme	Organ affected	Clinical features
I				
II				
III				
IV				
V				
VI				

5.10. Write the equations of unique reactions of gluconeogenesis (write the chemical formulae of metabolites). Name the enzymes.

1) pyruvate to posphoenolpyruvate

2) fructose-1,6-diphosphate to fructose-6-phosphate

3) glucose-6-phosphate to glucose

5.11. What noncarbohydrate precursors of glucose (gluconeogenesis substrates) are used: 1) during starvation, 2) during strenuous exercixe?

5.12. Compose the scheme of cyclic process between the liver and skeletal muscles (the Cori cycle). Describe the significance of Cori cycle.

5.13. Fill in the chart «Regulation of gluconeogenesis in human organism»

Regulated enzymes	Activators and mechanism of activation	Inhibitors and mechanism of inhibition
Pyruvate carboxylase		
Phosphoenolpyruvate carboxykinase		
Fructose-1,6- bisphosphatase		

5.14. Situational tasks:

a) To determine the cause of hypoglycemia newborn held glucagon test, that caused no increase in blood glucose.

Which way glucagon raises blood glucose level?

Name the possible reasons for the lack hyperglycemic effect of glucagon.

What other biochemical tests allow establish the diagnosis of a child?

b) The child has been delayed physical and mental development, deep disorders of the connective tissue of internal organs, keratan sulfate found in the urine.

Exchange of which substances violated a child?

Enter the name of these diseases and the cause of development.

What is this class of compounds excreted in urine?

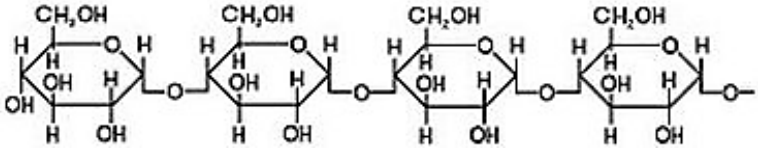
6. Individual independent students work

1. Principles of regulation of glycogen biosynthesis and breakdown.
2. Hereditary disorders of synthesis and breakdown of glycogen and glycoconjugates.

Practice protocol №14 «____» _____ 20___

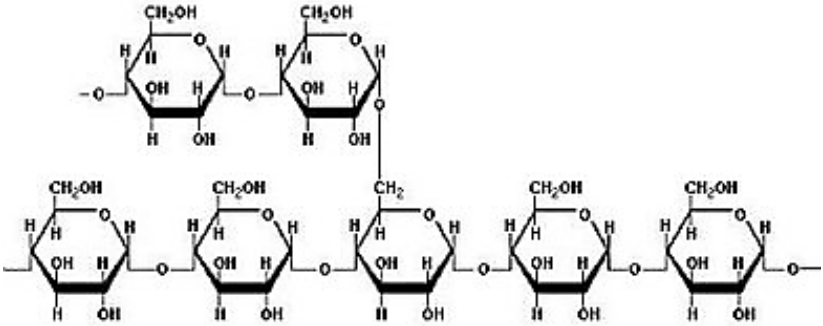
Experiment 1. Iodine test for starch.

Principle. Starch is a polysaccharide consisting of glucose units joined together by glycosidic bonds. The chains formed during the condensation reaction are either linear or highly branched molecules.



Linear – both straight and helical – molecules of starch are referred to as *Amylose*.

Whereas branched molecules of starch are called ***Amylopectin***.



The Iodine test for starch is used to determine the presence of starch in biological materials. The chains formed during the condensation reaction are either linear or highly branched molecules. Iodine on its own (small non-polar molecule) is insoluble in water. Therefore Potassium triiodide solution – Iodine dissolved in potassium iodide solution – is used as a reagent in the test. To be more specific, potassium iodide dissociates, and then the Iodide ion reacts reversibly with the Iodine to yield the triiodide ion. A further reaction between a triiodide ion and an iodine molecule yields the pentaiodide ion.

$(C_6H_{10}O_5)_n + I_2 \xrightarrow{\text{heating}} (C_6H_{10}O_5)_n\, I_2$

starch — complex of starch with Iodine

Since molecular iodine is always present in solution, the bench iodine solution appears brown; the iodide and triiodide pentaiodide ions are colourless.

The triiodide and pentaiodide ions formed are linear and slip inside the helix of the amylose (form of starch).

Reagents: 1 % solution of starch, Lugol solution (iodine dissolved in potassium iodide), tubes.

Method.

1. Add 1ml of the starch solution to a clean, dry test tube.
2. Add about 5 drops of Lugol solution to the test tube.
3. Note any colour changes. Write a conclusion.

Result:

Conclusion:

Experiment 2. Detection of glycogen in the liver.

Principle. A positive test for glycogen is a brown-blue colour. A negative test is the brown-yellow colour of the test reagent. Glycogen, as well as starch, forms a coloured compound with iodine (starch forms blue, glycogen – a red-brown compound). It is thought that starch and glycogen form helical coils. Iodine atoms can then fit into the helices to form a starch-iodine or glycogen-iodine complex. Starch in the form of amylose and amylopectin has less branches than glycogen. This means that the helices of starch are longer than glycogen, therefore binding more iodine atoms. The result is that the colour produced by a starch-iodine complex is more intense than that obtained with a glycogen-iodine complex.

Reagents. Fresh or frozen liver tissue, Lugol solution (iodine dissolved in potassium iodide), 1% solution of acetic acid, porcelain mortar, water bath, paper filters.

Generation of filtrate

1. Put 0.5 g of liver tissue into a tube, add 4 ml of distilled water and boil it (2–3 min in order to inactivate enzymes).
2. Transfer liver into the mortar and grind it.
3. Transfer the obtained homogenate into the tube, add 1 ml of distilled water and boil the solution on a water bath (20 min). Add 5–10 droplets of acetic acid solution for protein precipitation.
4. Eliminate precipitated proteins by filtration using paper filters.

Method.

1. Take a clean, dry tube. Put 1 ml of filtrate.
2. Add 2–3 droplets of Lugol solution.
3. Note any colour changes. (In presence of glycogen a red-violet colour is observed). Compare the colour with a colour obtained in the previous experiment. Explain the results.

Result:

Conclusion:

Clinical diagnostic significance. Glycogen is a polysaccharide, which serves as a main reserve of carbohydrates in the body. It is stored mainly in liver and muscles. Normal blood level – 16.2–38.7 mg/l. The prime function of liver glycogen is to maintain the blood glucose levels, particularly between meals. Liver glycogen stores increase in a well-fed state which are depleted during fasting. Muscle glycogen serves as a fuel reserve for the supply of ATP during muscle contraction.

The metabolic defects concerned with the glycogen synthesis and degradation are collectively referred to as glycogen storage diseases. These disorders are due to defects in the enzymes which may be either generalized (affecting all tissues) or tissue-specific. The inherited disorders are characterized by deposition of normal or abnormal type of glycogen in one or more tissues. Increase in blood glycogen concentration is observed in some infection diseases, which are accompanied with leukocytosis, diabetes mellitus, and malignancies.

The report is checked up _____

(The signature of the teacher, date)

Examples of Krock-1 tests

1. Gluconeogenesis was activated in an athlete in some time after intense exercises. What is its main substrate?

A. Lactate
B. Alpha-ketoglutarate
C. Glutamate
D. Aspartate
E. Phenylpyruvate

2. What are the major organs where glycogen is accumulated under glycogenoses?

A. Liver, muscles, kidneys
B. Liver, kidneys, heart
C. Liver, kidney, bone
D. Liver, kidneys, brain
E. Liver, lungs, heart

3. The conversion of glucose-6-phosphate into glucose is impaired under Von Gierke's disease which leads to the accumulation of glycogen in the liver. Which enzyme's deficiency causes the disease?

A. Glucose-6-phosphatase
B. Glycogen synthase
C. Phosphorylase
D. Hexokinase
E. Aldolase

4. Cori disease is the type III of glycogenosis where breakdown of glycogen only occurs by linear bonds. Glycogen is stored in the liver and muscles. Which enzyme's deficiency causes the disease?

A. Amylo-1,6-glucosidase
B. Glycogen phosphorylase
C. Phosphorylase kinase
D. Phosphoglucomutase
E. Glucokinase

5. The degradation of glycogen in the liver is activated by glucagon. What second messenger is formed for the transmission of the hormonal signal in the hepatocytes?

A. cAMP
B. cGMP
C. IP_3
D. NO
E. DAG

6. Pompe disease is the second type of glycogenosis that accompanied by the accumulation of glycogen in muscles and liver. Which enzyme's deficiency causes the disease?

A. Acid alfa-glucosidase
B. Glycogen phosphorylase
C. Glucose-6-phosphatase
D. Phosphoglucomutase
E. Glucokinase

7. Glycogen is synthesized from active form of glucose. What is an immediate donor of glucose residues in the glycogenesis?

A. UDP-glucose
B. Glucose-1-phosphate
C. ADP-glucose
D. Glucose-6-phosphate
E. Glucose-3-phosphate

8. What are enzymes required for the glycogenolysis?

A. Glycogen phosphorylase, glucose-6-phosphatase
B. Glycogen synthase
C. Phosphorylase kinase

D. Phosphoglucomutase
E. Phosphofructokinase, pyruvate carboxylase

9. What hormones activate glycogenolysis?

A. Adrenaline, thyroxine, glucagon, growth hormone
B. Gonadotropin, glucagon, estradiol, thyroxine
C. Corticosteroids, calcitonin, parathyrin, growth hormone
D. Follicle stimulating hormone, growth hormone, ACTH
E. Glucagon, cortisol, testosterone, progesterone

10. Postsynthetic covalent modification of enzymes plays an important role in regulation of their activity. What of mechanisms mentioned below is the regulation of glycogen phosphorylase and glycogen synthetase activities performed with?

A. Phosphorylation-dephosphorylation
B. ADP-rybosylation
C. Methylation
D. Adenylation
E. Limited proteolysis

11. Andersen disease is the type IV of glycogenosis that accompanied by the appearance of abnormal glycogen with linear structure type. Which enzyme's deficiency causes the disease?

A. Glycogen branching enzyme
B. Glycogen phosphorylase
C. Phosphorylase kinase
D. Phosphoglucomutase
E. Amylo-1,6-glucosidase

12. Gluconeogenesis is activated in an athlete after long intense physical activity and exhaustion of carbohydrates' reserves. What is the main substrate for gluconeogenesis in the athlete?

A. Lactate
B. Glutamic acid
C. Acetoacetate
D. Serine
E. Aspartic acid

13. Excessive accumulation of glycogen in the muscles, cramps and myoglobinuria are observed under McArdle's disease. Which enzyme's deficiency causes the disease?

A. Muscle phosphorylase
B. Liver glycogen phosphorylase
C. Amylo-1,6-glucosidase
D. Phosphoglucomutase
E. Glycogen branching enzyme

14. What are enzymes required for the synthesis of glycogen?

A. Glycogen synthase, amylo-1,4–1,6-transglycosylase
B. Phosphorylase, α-glucosidase, amylase
C. Pyruvate carboxylase
D. Glucose-6-phosphatase
E. Phosphofructokinase, pyruvate carboxylase

15. Growth retardation and hepatomegaly are observed under Hers' disease. Increased cholesterol concentration and increased activity of aminotransferases are revealed during laboratory examination. Which enzyme's deficiency causes the disease?

A. Liver phosphorylase
B. Alfa-1,4-glucosidase
C. Glucose-6-phosphatase
D. Amylo-1,6-glucosidase
E. Glycogen branching enzyme

16. A patient with rheumatoid arthritis has been given hydrocortisone for a long time. He has developed hyperglycemia, polyuria, glycosuria, thirst. These complications of treatment result from the activation of the following process:

A. Gluconeogenesis
B. Glycogenolysis
C. Glycogenesis
D. Glycolysis
E. Lipolysis

17. Which hormone activates glycogenesis?

A. Insulin
B. Estradiol
C. Calcitonin
D. Cortisol
E. Glucagon

18. Where is the most of glycogen stored in the human body?

A. Liver, muscles
B. Liver, bones
C. Bones, kidney
D. The kidneys, muscles
E. Kidneys, heart

19. What is a group of relatively rare genetic disorders associated with defects of the enzymes of glycogenolysis called?

A. Glycogenosis
B. Addison's disease
C. Cushing's disease

D. Steroid diabetes
E. Graves' disease

20. The increase in glucose concentration in blood under the action of glucagon is connected with activation of the next enzyme:

A. Glycogen phosphorylase
B. Hexokinase
C. Glucokinase
D. Aldolase
E. Glycogen synthase

21. What types of bonds are present in the structure of glycogen?

A. α-1,4 and α-1,6 glycosidic bonds
B. α-1,4 glycosidic bonds
C. α-1,6 glycosidic bonds
D. N- glycoside bonds
E. Phosphodiester bonds

22. What are the most common types of glycogenoses?

A. Liver and muscle glycogenoses
B. Muscle and bone glycogenoses
C. Liver and bone glycogenoses
D. Liver and heart glycogenoses
E. Liver and kidney glycogenoses

23. What is the main function of glycogen in the human body?

A. Energy function
B. Structural function
C. The support function
D. The receptor function
E. Regulatory function

24. What are amino acids used as the substrates for gluconeogenesis called?

A. Glucogenic aminoacids
B. Ketogenic amino acids
C. Amines
D. Sulfur-containing amino acids
E. Negatively charged amino acids

25. Which hormone activates the breakdown of glycogen in the liver only?

A. Glucagon
B. Estradiol
C. Calcitonin
D. Cortisol
E. Insulin

26. Which organ is the most sensitive to a decrease in the intracellular glucose concentration?

A. Brain
B. Liver
C. Muscles
D. Bones
E. Kidneys

27. Gluconeogenesis is essentially reversal of glycolysis pathway with the exception of three irreversible reactions, requiring bypass reactions. What are these reactions?

A. Hexokinase, phosphofructokinase and pyruvate kinase reactions
B. Pyruvate carboxylase, pyruvate kinase, and hexokinase reactions
C. Phosphorylase, hexokinase and phosphofructokinase reactions
D. Carboxylase, phosphofructokinase and glucokinase reactions
E. Hexokinase, pyruvate kinase and reaction of decarboxylation

28. A baby is week and apathic. Its liver is increased and liver biopsy test reveals excessive contents of glycogen. The glucose blood level is reduced. What causes the reduction?

A. Decreased (minimal) activity of liver glycogen phosphorylase
B. Decreased (minimal) activity of glycogen synthetase
C. Increased activity of glycogen synthetase
D. Decreased (minimal) activity of glucose-6-phosphatase
E. The deficiency of gene which is responsible for the synthesis of glucose-1-phosphate uridyltransferase

29. What are the substrates for gluconeogenesis?

A. Substrates of non-carbohydrate origin
B. Lipids
C. Nucleotides
D. Glucose
E. Metabolites of glucuronate pathway

30. What process prevents excessive accumulation of blood lactate during intensive muscle work?

A. Aerobic glycolysis
B. The glucose-lactate cycle
C. Glycogenesis
D. Glycogenolysis
E. Anaerobic glycolysis

31. Specify the process that supports normoglycemia in post-adsorption period after the exhaustion of reserves of carbohydrates:

A. Gluconeogenesis
B. Glucuronate pathway
C. Pentose phosphate pathway
D. Aerobic glycolysis
E. Anaerobic glycolysis

32. Indicate the mechanism of hormone's action providing the biosynthesis of the key enzymes of gluconeogenesis:

A. Intracellular
B. Membrane-intracellular
C. Mediated by c-AMP
D. Hormone-sensitive
E. Membrane

33. What process plays an important role in maintaining of normoglycemia for the energy supply of neurons under conditions of complete starvation?

A. Krebs Cycle
B. Glycolysis
C. Gluconeogenesis
D. Glycogenolysis
E. Lipolysis

34. Phosphoenolpyruvate kinase is a key enzyme of gluconeogenesis. Which group of enzymes it belongs to?

A. Inducible enzyme
B. The enzyme of adenylate cyclase cascade
C. Cobalamin-dependent enzyme
D. Constitutive enzyme
E. Krebs cycle enzyme

35. Indicate hormone that activates and induces biosynthesis of the key enzymes of gluconeogenesis:

A. Cortisol
B. Thyroxine
C. Parathyroid hormone
D. Insulin
E. Dopamine

36. Choose the hormone that activates gluconeogenesis and inhibits glycolysis:

A. Glucagon
B. Insulin
C. Parathyroid hormone
D. Aldosterone
E. Thyrotropin

37. Indicate hormone that inhibits gluconeogenesis:

A. Dopamine
B. Glucagon

C. Parathyroid hormone
D. Cortisol
E. Insulin

38. Indicate the metabolite that is the substrate for gluconeogenesis:
A. Glycerol
B. Alanine
C. Oxalacetate
D. Lactate
E. All of the above

39. Which process Cori cycle refers to?
A. Gluconeogenesis
B. Aerobic glycolysis
C. Glycogenesis
D. Glycogenolysis
E. Anaerobic glycolysis

40. Indicate the metabolite that is the substrate for gluconeogenesis:
A. Glycerol-3-phosphate
B. Acetone
C. Arachidonic acid
D. Sedoheptulose-7-phosphate
E. $NADPH_2$

41. Metabolic map of gluconeogenesis includes three bypass steps of glycolysis. Specify the total number of the reactions of these bypasses:
A. 4
B. 3
C. 2
D. 5
E. 6

42. Glucose content of blood stays at sufficient level after one week of starvation. Is it caused by activation of the following process:
A. Gluconeogenesis
B. Glycogenolysis
C. Glycogen phosphorolysis
D. Glycolysis
E. Tricarboxylic acid cycle

43. Indicate an enzyme of the second bypass of gluconeogenesis:
A. Fructose-1,6-bisphosphatase
B. Phosphoenolpyruvate carboxykinase
C. Pyruvate carboxylase
D. Phosphofructokinase
E. Glucose-6-phosphatase

44. After 2 days of fasting the major process by which blood glucose is produced is:

A. Gluconeogenesis
B. Glycolysis
C. Glycogenolysis
D. The pentose phosphate pathw
E. All the mentioned

45. There are two stages in the transformation of pyruvate into phosphoenolpyruvate in the process of gluconeogenesis. Each stage has a different cellular compartmentalization. What shuttle system is used for the transport of oxaloacetate from mitochondria into the cytosol?

A. All of them
B. Aspartate
C. Malate
D. Citrate
E. –

46. Glucose may be synthesized in humans from non carbohydrate compounds. Each of the following metabolites provides carbons for glucose synthesis by the process of gluconeogenesis, except:

A. Even-chain fatty acids from adipose triacylglycerols
B. Amino acids from muscle proteins
C. Lactate from red blood cells and exercising muscle
D. Glycerol from adipose triacylglycerols
E. All the mentioned

47. Tolerance of a 34-year-old patient to physical load is decreased, but glycogen content in skeletal muscles of the patient is increased. Which enzyme activity decrease can explain the phenomenon?

A. Glycogen phosphorylase
B. Phosphofructokinase
C. Glucose-6-phosphate dehydrogenase
D. Glycogensynthase
E. Glucose-6-phosphatase

48. Indicate the enzymes of the first bypass of the gluconeogenesis metabolic map:

A. Phosphoenolpyruvate carboxykinase, pyruvate carboxylase
B. Alanine aminotransferase, aspartate aminotransferase
C. Succinate dehydrogenase, malate dehydrogenase
D. Phosphofructokinase, hexokinase
E. All of them

49. Glucose-6-phosphatase absence, hypoglycemia and hepatomegaly were found in a child with point mutation of genes. What pathology is characterized by these signs?

A. Gierke's disease
B. Cori's disease
C. Addison's disease
D. Parkinson's disease
E. Mc-Ardle's disease

50. Gierke's disease is an illness which develops the excessive accumulation glycogen in liver and kidney tissues. Which enzyme deficiency is the cause of the disease.

A. Glucose-6-phosphatase
B. Glycogenphosphorylase
C. Phosphorylase kinase
D. Phosphoglucomutase
E. Glucokinase

51. One of characteristic features of glycogenosis is muscle ache during the performance of physical work. Which enzyme hereditary deficiency causes this pathology?

A. Glycogen phosphorylase
B. Glucose-6-phosphatase
C. Glycogen synthase
D. Amilo-1-6-glucosidase
E. Lysosomal glucosidase

52. An individual accidently ingests a compound that inhibits glucose-6-phosphatase. After an overnight fast, this individual, compared with a healthy person, would have a higher

A. Level of liver glycogen
B. Level of blood glucose
C. Rate of gluconeogenesis
D. Rate of glycogenolysis
E. All the mentioned

53. Characteristic sign of glycogenosis is muscle pain during physical work. Blood examination usually reveals hypoglycemia. This pathology is caused by congenital deficiency of the following enzyme:

A. Glycogen phosphorylase
B. Glucose-6-phosphate dehydrogenase
C. α-Amylase
D. Lysosomal glycosidase
E. γ-Amylase

54. 34-year-old patient has low endurance of physical loads. At the same time skeletal muscles have increased concentration of glycogen. This is caused by the reduced activity of the enzyme:

A. Glycogen phosphorylase
B. Glucose-6-phosphate dehydrogenase
C. Glucose-6-phosphatase
D. Phosphofructokinase
E. Glycogen synthase

55. Carbohydrates are nonessential components of the human diet. They are synthesized in organism by means of gluconeogenesis from:

A. Alanine, glycerol, lactate
B. Glycerol, fatty acids, leucine
C. Lactate, cholesterol, carnitine
D. Choline, pyruvate, acetyl-CoA
E. Glutamate, leucine, butyrate

56. The consequence of long-term starvation is the quick carbohydrate reserves outrun. What process renovates the glucose blood level?

A. Gluconeogenesis
B. Aerobic glycolysis
C. Anaerobic glycolysis
D. Glycogenolysis
E. Penthose-phosphate pathway

57. During starvation muscle proteins are degraded into free amino acids. These compounds will be the most probably involved into the following process:

A. Gluconeogenesis in liver
B. Glycogenolysis
C. Decarboxylation
D. Gluconeogenesis in muscles
E. Synthesis of higher fatty acids

58. Chronic overdosage of glucocorticoids leads to the development of hyperglycemia. What process of carbohydrate metabolism is responsible for this effect?

A. Gluconeogenesis
B. Glycogenolysis
C. Aerobic glycolisis
D. Pentose-phosphate cycle
E. Glycogenesis

59. It has been revealed that intense physical exercise causes activation of gluconeogenesis in liver of experimental rats. Which substance is glucose precursor in this case?

A. Pyruvate
B. Glycogen
C. Palmitate
D. Urea
E. Stearate

60. During intensive physical exertion, one of the energy sources for the working muscles is glucose produced as the result of gluconeogenesis. This process is the most intensive in the following organ:

A. Liver
B. Muscles
C. Lungs
D. Brain
E. Stomach

61. During starvation normal rate of glucose is maintained by means of gluconeogenesis activation. What substance can be used as a substrate for this process?

A. Alanine
B. Ammonia
C. Adenine
D. Urea
E. Guanine

62. Gluconeogenesis in liver is activated in the organism of sportsman after intensive training. Indicate the main substrate of this process:

A. Lactate
B. Serine
C. α-Ketoglutarate
D. Aspartate
E. Glutamate

63. Characteristic sign of glycogenosis is muscle pain during physical work. Blood examination reveals usually hypoglycemia. This pathology is caused by congenital deficiency of the following enzyme:

A. Glycogen phosphorylase
B. Glucose 6-phosphate dehydrogenase
C. Alpha amylase
D. Gamma amylase
E. Lysosomal glycosidase

64. It is known that many hormones act through the adenylate cyclase system causing the enzyme activation by phosphorylation. What enzyme is activated by hormonal signals and catalyzes glycogen breakdown?

A. Phosphorylase
B. Phosphotransferase
C. Glucomutase
D. Phosphatase
E. Tyrosinase

65. Prolonged fasting causes hypoglycemia which is amplified by alcohol consumption, as the following process is inhibited:

A. Gluconeogenesis
B. Glycolysis
C. Glycogenolysis
D. Lipolysis
E. Proteolysis

66. Corticosteroid analogues induce breakdown of muscle proteins into free amino acids. Under such conditions these amino acids become involved with the following processes:

A. Gluconeogenesis in liver
B. Glycolysis in muscles
C. Synthesis of higher fatty acids
D. Glycogenolysis
E. Decarboxylation

67. A child has a history of hepatomegaly, hypoglycemia, seizures, especially on an empty stomach and in stressful situations. The child is diagnosed with Gierke disease. This disease is caused by the genetic defect of the following enzyme:

A. Glucose-6-phosphatase
B. Amyloid-1,6-glycosidase
C. Phosphoglucomutase
D. Glycogen phosphorylase
E. Glucokinase

68. Pancreas is known as a mixed gland. Endocrine functions include production of insulin by beta cells. This hormone affects the metabolism of carbohydrates. What is its effect upon the activity of glycogen phosphorylase (GP) and glycogen synthase (GS)?

A. It inhibits GP and activates GS
B. It activates both GP and GS
C. It inhibits both GP and GS
D. It activates GP and inhibits GS
E. It does not affect the activity of GP and GS

69. The patient exhausted by starvation presents with intensification of the following process in the liver and kidneys:

A. Gluconeogenesis
B. Uric acid synthesis

C. Urea synthesis
D. Hippuric acid synthesis
E. Bilirubin synthesis

70. What biochemical process is stimulated in the liver and kidneys of a patient exhausted by starvation?

A. Gluconeogenesis.
B. Synthesis of urea.
C. Synthesis of bilirubin.
D.Formation of hippuric acid.
E. Synthesis of uric acid

71. During biochemical investigation of blood in a patient was detected hypoglycemia in fasting condition. Investigation of liver bioptates revealed the failure of glycogen synthesis. What enzyme deficiency may cause such status?

A. Glycogen synthase
B. Phosphorylase
C. Aldolase
D. Fructose bis-phosphatase
E. Pyruvate carboxylase

72. In an infant with point mutations in genes the absence of glucose 6-phosphatase, hypoglycemia and hepatomegalia were revealed. What disease is characterized by these symptoms?

A. Gierke disease
B. Adison disease
C. Parkinson disease
D. Cori disease
E. Mac Ardle disease

73. A type I of glycogenosis, Gierke's disease, causes the disturbance of glucose-6-phosphate to glucose conversion. This results in accumulation of glycogen in the liver and kidney. Which enzyme deficiency is observed at this state?

A. Glucose-6-phosphatase
B. Phosphorylase
C. Glycogensynthetase
D. Hexokinase
E. Aldolase

74. In patients with glycogenolysis, that is von Gierke's disease, the conversion of glucose-6-phosphate into glucose is inhibited, which is accompanied by the improper breakdown of glycogen in the liver. The cause of this condition is the following enzyme deficiency:

A. Glucose-6-phosphatase
B. Glycogen phosphorylase
C. Glucose-6-phosphate dehydrogenase

D. Phosphofructokinase
E. Phosphoglucomutase

75. What is the Cori cycle?

A. Glucose-lactate cycle
B. Glucose-alanine cycle
C. Glycine-lactate cycle
D. Ornithine cycle
E. Citrate cycle

76. Which of the following processes is enhanced in the liver and kidney of patient exhausted by starvation?

A. Gluconeogenesis
B. Bilirubin synthesis
C. Urea synthesis
D. Hippuric acid formation
E. Uric acid synthesis

77. In a weak apathic infant an enlarged liver was detected, which in investigation of biopcia pieces showed an excess of glycogen. Blood glucose concentration is under the normal value. What may be the cause of this disease?

A. Lowered activity of glycogen phosphorylase in a liver
B. Lowered activity of glycogen synthase
C. Lowered activity of glucose 6-phosphate isomerase
D. Lowered activity of glucokinase
E. Deficiency of gene responsible for synthesis of glucose 1-phosphate uridyl transferase

78. The genetic defect of pyruvate carboxylase deficiency is the cause of delayed physical and mental development and early death in children. This defect is characterized by lacticemia, lactaciduria, disorder of a number of metabolic pathways. In particular, the following process is inhibited:

A. Citric acid cycle and gluconeogenesis
B. Glycolysis and glycogenolysis
C. Glycogenesis and glycogenolysis
D. Lipolysis and lipogenesis
E. Pentose phosphate pathway and glycolysis

79. What are the bypass reactions of gluconeogenesis providing conversion of pyruvate into glucose?

A. Conversion of pyruvate to phosphoenolpyruvate, the conversion of fructose-1,6-bisphosphate to fructose-6-phosphate, conversion of glucose-6-phosphate into glucose
B. Conversion of pyruvate to acetyl-CoA, conversion of acetyl-CoA to pyruvate, conversion of fructose-1,6-diphosphate to glucose

C. Conversion of acetyl-CoA into pyruvate; conversion of glucose into glucose-6-phosphate

D. Conversion of oxaloacetate to phosphoenolpyruvate; conversion of glucose into fructose-1,6-bisphosphate; conversion of fructose-6-phosphate into glucose

E. Convertion of glucose into fructose; convertion of citrate into isocitrate; conversion of oxaloacetate into pyruvate

80. In a patient a lowering in ability to physical load was revealed, while in skeletal muscles the glycogen content was increased. The decrease in activity of what enzyme may cause this condition?

A. Glycogen phosphorylase
B. Phosphofructokinase
C. Glucose 6-phosphate dehydrogenase
D. Glycogen synthase
E. Glucose 6-phosphatase

References:

1. Gubsky Yu. Biological chemistry: textbook / edited by Yu. Gubsky. – Vinnytsia : Nova Knyha, 2018. – 2nd edition. – 488 p.
2. Lecture materials.
3. Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – 799 p.
4. Textbook «Biological chemistry. Collection of test tasks» for English-speaking students of higher medical educational institutions of the Ministry of Public Health of Ukraine. – Poltava, 2018. – 148 p.

TOPIC №15.
Studies of mechanisms of metabolic and hormonal regulation of carbohydrate metabolism. Diabetes mellitus

1. Objectives: To interpret the role of hormones in regulation and maintenance of constant blood glucose level. To learn the peculiarities of changes in metabolism of carbohydrates, lipids and proteins in diabetes mellitus.

2. Actuality of the theme: Diabetes mellitus is a clinical condition characterized by increased blood glucose level (hyperglycemia) due to insufficient or inefficient insulin. In other words, insulin is either not produced in sufficient quantity or inefficient in its action on the target tissues. As a consequence, the blood glucose level is elevated which spills over into urine in diabetes mellitus. Determination of blood glucose level in clinical laboratory investigations is of great importance in diagnostics of diabetes mellitus and many other diseases and disorders.

3. Specific aims:

✓ To analyze the principal sources and metabolic pathways of utilization of blood glucose

✓ To explain the role of hormones in maintenance of constant glucose level in blood

✓ To explain disorders in metabolism of carbohydrates in diabetes mellitus.

4. Reference card for the separate study of educational literature for the lesson preparation

Questions:	References:
1. **Principal carbohydrates of diet.** ✓ Mechanisms of carbohydrates digestion in digestive tube. ✓ Mechanisms of carbohydrate absorption.	Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 165–169.
2. **Role of liver in carbohydrate metabolism.**	1. Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 261–262. 2. Gubsky Yu. Biological chemistry: textbook / edited by Yu. Gubsky. – Vinnytsia: Nova Knyha, 2018. – 2nd edition. – P. 400–401.

3.	**Hormonal regulation of carbohydrate metabolism:** ✓ Insulin, its structure, mechanism of action, role in carbohydrate metabolism. ✓ Adrenalin and glucagone, mechanism of their regulatory effects on carbohydrate metabolism. ✓ Glucocorticoids, their effect on carbohydrate metabolism.	1. Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 669–678. 2. Gubsky Yu. Biological chemistry: textbook / edited by Yu. Gubsky. – Vinnytsia: Nova Knyha, 2018. – 2nd edition. – P. 172–173.
4.	**Characterization of hypo- and hyperglycemia, glucosuria.** ✓ Characterization of hypoglycemia ✓ Characterization of hyperglycemia ✓ Characterization of glucosuria.	1. Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 678 - 679, 681. 2. Gubsky Yu. Biological chemistry: textbook / edited by Yu. Gubsky. – Vinnytsia: Nova Knyha, 2018. – 2nd edition. – P. 174–175.
5.	**Insulin dependent and noninsulin dependent forms of diabetes mellitus.** ✓ Insulin dependent forms of diabetes mellitus. ✓ Noninsulin dependent forms of diabetes mellitus.	1. Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 679–680. 2. Gubsky Yu. Biological chemistry: textbook / edited by Yu. Gubsky. – Vinnytsia: Nova Knyha, 2018. – 2nd edition. – P. 175–176.
6.	**Characterization of metabolic disorders in diabetes mellitus**	Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 681–682.
7.	**Biochemical tests for evaluation of conditions of patients with diabetes mellitus. Glucose tolerance test and its alteration in diabetes mellitus. Biochemical criteria of diabetes mellitus.** ✓ Glucose tolerance test ✓ Double sugar loading ✓ Glycosilative HbA_{C1}.	Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 679–684.

5. Tasks for independent work and self-control

5.1. Digestion of carbohydrates

Part of GIT	Enzymes and place of their production	Substrates → Products
Mouth	1)	
Small intestine	1)	

	2)	
	3)	
	4)	
	5)	

5.2. Absorption of monosaccharide's.

a) Glucose is transported into the absorptive epithelial cells of the small intestine by secondary active transport. What difference between primary and secondary active transport?

b) Explain the mechanism of glucose absorption by sodium-dependent glucose transporters.

c) What function of Na+,K+-ATPase for glucose absorption in small intestine?

d) Glucose is transported from epithelial cells of the small intestine into the blood by facilitative diffusion (passive transport). What difference between passive and active transport?

5.3. Write reactions common for the Cory cycle and glucose-alanine cycle.

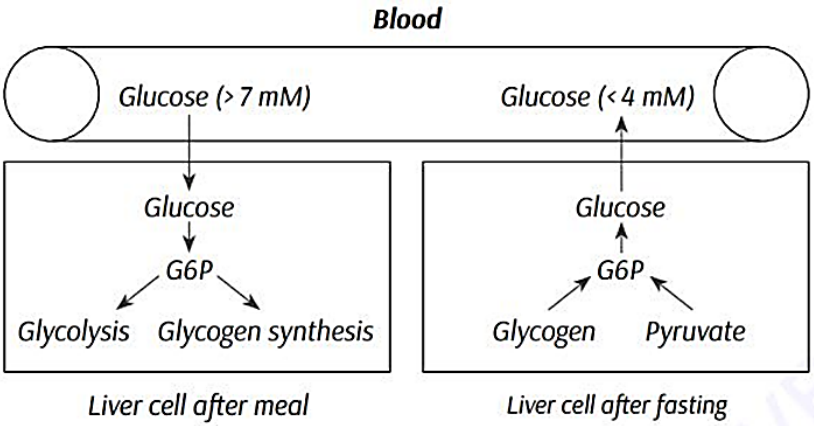
1)

2)

3)

4)

5.4. Complete the scheme.



5.5. Insulin plays a key role in the regulation of carbohydrate, lipid and protein metabolisms. Explain the net effect of insulin on the metabolic pathways represented above and effect on key enzymes.

№	Metaholism	Net effect	Effect on important enzyme(s)
1	Glycolysis	Increased	Glucokinase (↑) Phosphofructokinase (↑) Pyruvate kinaste (↑)
2	Gluconeogenesis		
3	Glycogenesis		
4	Glycogenolys		
5	HMPshunt		
6	Lipogenesis		

7	Lipolysis		
8	Ketogenesis		
9	Protein synthesis		
10	Protein degradation		

5.6. Regulation of glucose level in blood

Pathway	Insulin	Glucagon	Epinephrin	Glucocorticoids
Glucose transport from blood into cells				
Gluconeogenesis				
Glycogenesis				
Glycogenolysis				
Glycolysis				
Glucose level in blood				

5.7. Characterization of hypo- and hyperglycemia, glucosuria.

a) The symptoms of hypoglycemia can be divided into two categories. Adrenergic symptoms (anxiety, palpitation, tremor, and sweating) are mediated by epinephrine release regulated by the hypothalamus in response to hypoglycemia.Usually adrenergic symptoms (that is, symptoms mediated by elevated epinephrine) occur when blood glucose levels fall abruptly. The second category of hypoglycemic symptoms is neuroglycopenic Neuroglycopenia (the impaired delivery of glucose to the brain) results in impairment of brain function, causing headache, confusion, slurred speech, seizures, coma, and death. Neuroglycopenic symptoms often result from a

gradual decline in blood glucose, often to levels below 40 mg/dl. Describe the different types of hypoglycemia:

✓ in healthy individuals that may occur during exercise after a period of fasting;

✓ postprandial (sometimes called reactive hypoglycemia);

✓ due to alcohol drinking;

✓ due to excess of exogenous or endogenous insulin;

✓ a feature of endocrine disorders (what glands and hormons?);

b) Hyperglycemia.

✓ Hyperglycemia may cause a constellation of symptoms such as polyuria, dehydration and subsequent polydipsia (increased thirst). Explain why the kidney produces more urine in this case.

✓ If dehydration becomes severe, further cerebral dysfunction occurs and the patient may become comatose. Explain why.

✓ Chronic hyperglycemia also produces pathologic effects through the nonenzymatic glycosylation of a variety of proteins. This process distorts protein structure and slows protein degradation, which leads to an accumulation of these products in various organs, thereby adversely affecting organ function. These events contribute to the long-term microvascular and macrovascular complications of diabetes mellitus, which include diabetic retinopathy, nephropathy, and neuropathy (microvascular), in addition to coronary artery, cerebral artery, and peripheral artery insufficiency (macrovascular). Which assay can be used to control chronic hyperglycemia and protein glycosylation?

5.8. Comparison of two types of diabetes mellitus

Character	Insulin-dependent diabetes mellitus (IDDM)	Non-insulin diabetes mellitus (NIDDM)
General		
Prevalence	10-20% of diabetic population	80–90% of diabetic population
Age at onset		
Body weight		
Genetic predisposition		
Biochemical		
Defect		
Plasma insulin		
Auto antibodies		
Ketosis		
Acute complications		
Clinical		
Duration of symptoms		
Diabetic complications at diagnosis		
Oral hypoglycemic drugs		
Administration of insulin		

5.9. Glucosuria.

✓ At normal plasma concentration, all the glucose filtered through the renal glomeruli is reabsorbed in the proximal tubule, and none appears in the urine.At higher glucose concentrations, the capacity of the renal tubular transport system is exceeded, and glucose filters into the urine (glucosuria). Indicate the renal threshold for glucose.

✓ Rarely glucosuria can be detected at normal blood glucose levels. Explain why.

✓ Patient's urine was negative for glucose when measured with the glucose oxidase assay. However, glucose measured by a colourimetric test that determined total reducing sugar indicated that the concentration of sugar was quite high in both blood and urine.

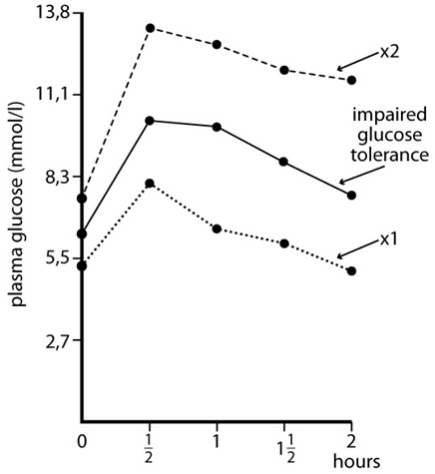
What reducing sugars could be identified?

What possible diagnoses?

5.10. – Explain the results of glucose tolerance test for patient X1 and patient X2.

X1 –

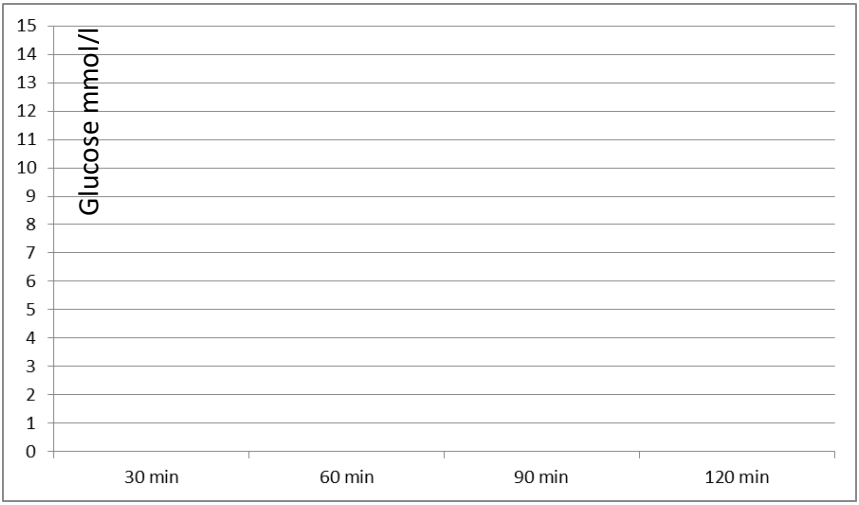
X2 –



5.11. Situational tasks:

1. In the patient's blood content of glucose in fasting – 5.65 mmol/l, 1 hour after sugar – 8.55 mmol/l, 2 hours – 4.95 mmol/ l. These indicators correspond to:
2. In the patient's blood content of glucose in fasting 5.6 mmol/l, 1 hour after sugar load – 11.0 mmol/l, 2 hours - 9.2 mmol/l. These indicators correspond to:
3. In the patient's blood content of glucose fasting 6.4 mmol/l, 1 hour after breakfast carbohydrate – 14.2 mmol/l, and after 2 hours – 12.6 mmol/l. These indicators correspond to:

Draw described sugar curves.



6. Individual independent students work

1. Features of a carbohydrate exchange under conditions of Cushing illness.
2. Metabolic changes and complications at diabetes mellitus.

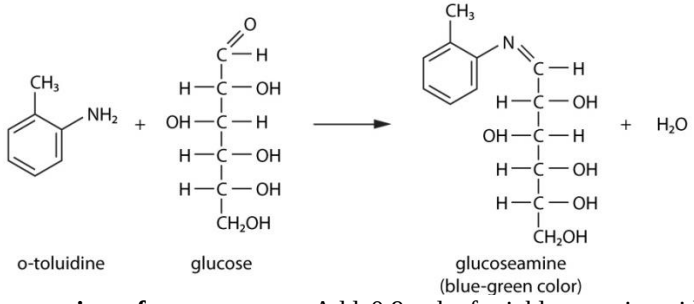
Practice protocol №15 «____» _____ 20___

Experiment. Estimation of blood glucose by o-toluidine method.

Principle. In this method of glucose determination, a primary aromatic amine, o-toluidine, reacts in hot glacial acetic acid with the terminal aldehyde group of glucose to produce a blue-green colour. The absorbance of this product is measured photometrically and glucose concentration can

be calculated. The absorbance in 600-700 nm region is directly proportional to the glucose concentration.

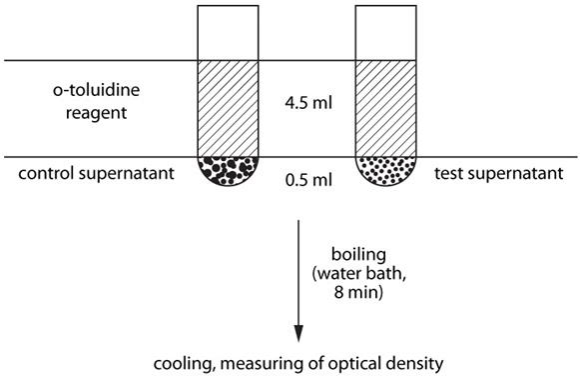
Reagents and materials. Blood sample, 3 % solution of trichloroacetic acid (TCA), o-toluidine reagent, glucose standard (4 mM/L, i.e. 720 mg of glucose dissolved in 1 L of water), distilled water, tubes, pipettes, micropipette 0.1 ml, centrifuge, centrifuge tubes, electrocolourimeter, water bath.



Preparation of supernatant. Add 0.9 ml of trichloroacetic acid into two centrifuge tubes. Into test tube add 0.1 ml of blood specimen, into control tube – 0.1 ml of standard glucose solution. Centrifuge the tubes at 3000 rpm for 10 min. Separate test and control supernatants for further investigations.

Method.

1. Take two clean, dry tubes. Into the first tube add 0.5 ml of control supernatant, into second – 0.5 ml of tested supernatant.
2. Add 4.5 ml of o-toluidine reagent to each tube.
3. Place the tubes on a boiling water bath for 8 min.
4. Cool tubes and measure optical density (A) in a colourimeter at wavelength 630 nm (red filter).



Calculation. The concentration of glucose is calculated using the formula:

$$C_{test} = \frac{C_{stand} \times A_{test}}{A_{stand}}$$

where

C_{test} – concentration of glucose in blood, mmoles/L;

C_{stand} – concentration of glucose in standard solution

A_{test} – optical density of test probe

A_{test} – optical density of standard glucose probe.

Compare the obtained result with normal value, draw the conclusion.

Result:

Conclusion:

Clinical diagnostic significance. The fasting blood glucose level in normal individuals is ***3.3–5.5 mmol/l*** and it is very efficiently maintained at this level. When the blood glucose concentration falls, the symptoms of ***hypoglycemia*** appear. The manifestations include headache, anxiety, confusion, sweating, slurred speech, seizures and coma, and, if not corrected, death. All these symptoms are directly and indirectly related to the deprivation of glucose supply to the central nervous system (particularly the brain) due to a fall in blood glucose level.

Elevation of blood glucose concentration is the hallmark of uncontrolled diabetes. ***Hyperglycemia*** is primarily due to reduced glucose uptake by tissues and its increased production via gluconeogenesis and glycogenolysis. When the blood glucose level goes beyond the renal threshold, glucose is excreted into urine (***glycosuria***).

Oral glucose-tolerant test. Principle of method: in the morning on an empty stomach determine concentration of glucose in the blood taken from a finger. After that give to the patient give to drink a solution of glucose at the rate of 1 g glucose on 1 kg of weight of a body. And then, during 3 hours, in each hour take blood and determine in it the concentration of glucose by ortotoluidine method (see the previous lesson).

On the found sizes build a sugar curve: across postpone time in hours, and on a vertical – concentration of glucose in blood.

In norm in blood of the healthy person: 1) The maximal increase of a level of glucose is observed in 1 hour after sugar loading; but does not exceed «kidney's threshold»; 2) by the second o'clock the level of glucose in blood is reduced below initial level; 3) by the third o'clock concentration of glucose in blood comes back to norm.

The report is checked up _____

(The signature of the teacher, date)

Examples of Krock-1 tests

1. Utilization of glucose occurs by means of sugar transport from the extracellular matrix through the plasma membrane into the cell. What hormone stimulates this process?

A. Insulin
B. Glucagon
C. Thyroxine
D. Aldosterone
E. Adrenaline

2. A patient with diabetes mellitus experienced loss of consciousness and convulsions after an injection of insulin. What might be the result of biochemical blood analysis for concentration of sugar?

A. 1.5 mmol / L
B. 8.0 mmol / L
C. 10.0 mmol / L
D. 3.3 mmol / L
E. 5.5 mmol / L

3. A patient with diabetes mellitus has been delivered to the hospital in the state of unconsciousness. Arterial pressure is low. The patient has acidosis. Indicate the substances, which accumulation in the blood results in these manifestations:

A. Acetone
B. Phenylalanine
C. Palmitic acid
D. Cholesterol esters
E. Glucose

4. Which diagnostic test is used to confirm the diagnosis of diabetes mellitus in patients with blood glucose level from 5.6 to 6.1 mmol/l?

A. Oral glucose tolerance test
B. Non-glycosylated hemoglobin
C. Determination of C-peptide in the blood

D. Determination of glucagon in the blood
E. Staub-Traugott test

5. Specify the biochemical effect of adrenalin that affects blood glucose concentration:

A. Activation of glycogen breakdown
B. Activation of glycogen synthesis in the liver
C. Activation of the pentose phosphate pathway
D. Activation of gluconeogenesis
E. The hypoglycemic effect

6. What diagnostic criterion of diabetes mellitus allows evaluating changes in the blood glucose concentration retrospectively?

A. Glycosylated hemoglobin
B. Glucagon
C. Insulin
D. Glucose tolerance test
E. Kidney «threshold» for glucose

7. What is the effect of insulin on carbohydrate metabolism in the liver?

A. It activates glycogenesis
B. It activates glycogenolysis
C. It activates gluconeogenesis
D. Hyperglycemic effect
E. It inhibits glycogenesis

8. Choose the reason for the development of hypoglycemia:

A. Hyperfunction of the pancreatic β-cells
B. Insulin insufficiency
C. Hyperfunction of the adrenal cortex
D. Hyperfunction of the pituitary gland
E. Hyperthyroidism

9. What mechanisms determine the hypoglycemic effect of insulin?

A. Stimulation of glycogen synthesis in the liver and muscles, increase of glucose permeability for membranes of target cells
B. Inhibition of glycogen synthesis and glucose uptake into the cytosol of the cell
C. Activation of gluconeogenesis
D. Inhibition of glycolysis and glycogenesis
E. Inhibition of glucose transformation into neutral fats

10. A patient complains of the dryness in the oral cavity, thirst, general weakness. Hyperglycemia, hyperketonemia were found during biochemical investigation. Ketone bodies and glucose were determined in the urine. What is a possible disease in the patient?

A. Diabetes mellitus
B. Acute pancreatitis

C. Diabetes insipidus
D. Ischemic heart disease
E. Alimentary hypoglycemia

11. Choose the reason for the development of hyperglycemia:

A. Hypothyroidism
B. Hyperinsulinism
C. Hypofunction of the pituitary gland
D. Hypofunction of the adrenal cortex
E. Diabetes mellitus

12. What is a blood sugar level in patients with hypoglycemic coma?

A. Below 1.0 mmol / L
B. Equal 3.5-4.0 mmol / L
C. Above 5.0 mmol / L
D. Above 6.0 mmol / L
E. Above the renal threshold for glucose

13. Specify one of the leading mechanisms of the development of hyperglycemia in diabetes mellitus:

A. Violation of glucose permeability into a target cell through a system of GLUT transporter
B. Glucosuria
C. Dehydration of the body
D. Activation of intracellular pathways of glucose metabolism
E. The increase of insulin concentration in the blood

14. A patient has diabetes mellitus that is accompanied by hyperglycemia of over 7.2 mmol/L on an empty stomach. What blood plasma protein level allows estimate the glycemia rate retrospectively (4–8 weeks before examination)?

A. Glycosylated hemoglobin
B. Ceruloplasmin
C. Fibrinogen
D. Albumin
E. C-reactive protein

15. What of the following hormones are antagonists of insulin?

A. Glucagon, thyroxine
B. Adrenaline, testosterone
C. Estradiol, cortisol
D. Corticotrophin, thyrotropin
E. All of the above

16. What is the normal range of glycosylated hemoglobin in the blood of healthy person?

A. 4–6 %
B. 1–2 %
C. 13–17 %
D. 7–9 %
E. 10–12 %

17. A patient has symptoms of the dryness of skin and mucous membranes, lowering the tone of the eyeballs. There is a smell of acetone in the air of the chamber. What can cause such condition?

A. Ketosis
B. Diabetes insipidus
C. Glycogenosis
D. Dehydration
E. –

18. What is the leading mechanism of the development of the diabetic hyperglycemic hyperosmolar coma?

A. Hyperglycemia and high blood osmotic pressure
B. Ketoacidosis, intoxication by ketone bodies
C. Ketosis, high blood osmotic pressure
D. Hyperpyruvatemia, high blood osmotic pressure
E. Hypoglycemia, cellular energy deficit

19. What is the diagnostic value of the C-peptide measurement (determination) in the blood of the patient?

A. The most accurate indicator of the functional activity of pancreatic β-cells
B. The most accurate indicator of the functional activity of pancreatic α-cells
C. The most accurate measure of the functional activity of the chromaffin cells of the adrenal medulla
D. It is used for the diagnostics of the diseases of the adenohypophysis
E. It is used for the diagnostics of the diabetes insipidus

20. The daily diuresis of a healthy person ranges from 1 to 2.5 liters. How will the excretion of urine be changed in patients with diabetes mellitus?

A. Diuresis increases according to the degree of glycosuria
B. Diuresis decreases, regardless of the degree of glycosuria
C. Diuresis does not change and does not depend on the degree of glycosuria

D. Diuresis reflects the degree of increase in the renal threshold figure with a maximum capacity at night

E. Diuresis decreases due to the decrease of glucose filtration through glomerulus of nephrons

21. A patient in comatose state was admitted to the medical institution. According to explanation of accompanying people the patient lost consciousness in training at the final stage of the Marathon distance. What coma is diagnosed?

A. Hypoglycemic
B. Hyperglycemic
C. Acidic
D. Hypothyreiod
E. Hepatic

22. An unconscious woman-patient was delivered to the clinics emergency department. The clinical analysis of b1ood reveals the following indexes: glucose – 1.98mmol/l, Hb – 82g/l, EPR – 18mm/hour. What is the most plausible diagnosis?

A. Hypoglycemia
B. Diabetes mellitus
C. Galactosemia
D. Somatotropin deficiency
E. Kidney diabetes

23. A 38 year-old man suffering from schizophrenia has been treated at clinics. The glucose, ketone bodies and urea blood levels of the patient were original. The shock therapy by regular insulin coma was provided and after that the state of the patient was getting better. What was the most probable cause of the insulin coma?

A. Hypoglycemia
B. Glucosuria
C. Dehydration of tissues
D. Metabolic acidosis
E. Ketonemia

24. A 45 year- old woman does not have any symptoms of sugar diabetes, although, she has increased glucose blood level (7,5 mmol/l) in the morning before the first meal. Which additional test be performed this case?

A. Determination of glucose tolerance before the first meal
B. Determination of glucose tolerance
C. Determination of ketone bodies blood level

D. Determination of residual nitrogen blood level
E. Determination of glycosilized hemoglobin level

25. A 62-year-old female patient has developed a cataract (lenticular opacity) secondary to the diabetes mellitus. What type of protein modification is observed in case of diabetic cataract?

A. Glycosylation
B. Methylation
C. Phosphorylation
D. ADP-ribosylation
E. Limited proteolysis

26. Which of the following liver enzymes becomes less active when a diabetic person is treated with insulin?

A. Fructose 1,6-bisphosphatase
B. Pyruvate kinase
C. Pyruvate dehydrogenase
D. Phosphofructokinase 1
D. All the mentioned

27. A 23-year-old patient with diabetes has hyperglycemia at the rate of 19 mmol/l which is clinically manifested by glucosuria, polyuria, polydipsia. Which of the listed below mechanisms is responsible for the development of glycosuria?

A. Exceedence of glucose renal threshold
B. Non-enzymatic glycosylation of proteins
C. Polyuria
D. Polydipsia
E. Tissue dehydration

28. A patient with insulin-dependent diabetes mellitus has been administered insulin. After a certain period of time the patient developed fatigue, irritability, excessive sweating. What is the main mechanism of such presentations developing?

A. Carbohydrate starvation of the brain
B. Increased lipogenesis
C. Increased ketogenesis
D. Increased glycogenolysis
E. Decreased glyconeogenesis

29. A 15-year-old patient has fasting plasma glucose level 4.8 mmol/1, one hour after glucose challenge it becomes 9.0 mmol/1, in 2 hours it is 7.0 mmol/1, in 3 hours it is 4.8 mmol/1. Such parameters are characteristic of:

A. Healthy person
B. Cushing’s disease
C. Subclinical diabetes mellitus

D. Diabetes mellitus type 1
E. Diabetes mellitus type 2

30. A diabetus mellitus patient developed unconsciousness and convulsions after administration of insulin. What result of blood glucose analysis is the most likely in this case?

A. 1.5 mmol/L
B. 3.3 mmol/L
C. 8 mmol/L
D. 5.5 mmol/L
E. 10 mmol/L

References:

1. Gubsky Yu. Biological chemistry: textbook / edited by Yu. Gubsky. – Vinnytsia : Nova Knyha, 2018. – 2nd edition. – 488 p.
2. Lecture materials.
3. Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – 799 p.
4. Textbook «Biological chemistry. Collection of test tasks» for English-speaking students of higher medical educational institutions of the Ministry of Public Health of Ukraine. – Poltava, 2018. – 148 p.

TOPIC №16.
Catabolism and biosynthesis of triacylglycerols. Intracellular lipolysis and molecular mechanisms of its regulation

1. Objective: To learn the processes of biosynthesis of triacylglycerols and the main pathways of intracellular metabolism of lipids.

2. Actuality of the theme: The knowledge of main pathways of intracellular metabolism of lipids under normal conditions and in pathology are necessary for medical students in further studies of general pathology, pharmacology and related clinical disciplines for correct interpretation of results of laboratory investigations and recognition of metabolic disorders in distinct cases.

3. Specific aims:

✓ To interpret biochemical function of simple and complex lipids in organism: their involvement in formation of structure and function of biological membranes, reserve and energetic significance, the role as precursors in biosynthesis of biologically active compounds of lipid nature.

✓ To explain the principal pathways of intracellular lipid metabolism.

✓ To explain enzymatic reactions of catabolism and biosynthesis of triacylglycerols.

✓ To analyze the main pathways of lipid metabolism in human body in normal conditions and in pathology.

✓ To explain hormonal regulation of lipid metabolism.

4. Reference card for the separate study of educational literature for the lesson preparation

Questions:	References:
1. **Classification of lipids. Biological functions of simple and complex lipids in human body:** ✓ reserve; ✓ energetic; ✓ thermoregulatory; ✓ production of biologically active substances.	1. Satyanarayana U., Chakrapani U. «Biochemistry», Fourth Edition. – 2013. – P. 28–29. 2. Gubsky Yu. Biological chemistry: textbook / edited by Yu. Gubsky. – Vinnytsia: Nova Knyha, 2018. – 2nd edition. – P. 57–58, 177–178.
2. **Structure and role of fatty acids.** To write formulas of main saturated, unsaturated and polyunsaturated fatty acids.	1. Satyanarayana U., Chakrapani U. «Biochemistry», Fourth Edition. – 2013. – P. 29–31. 2. Gubsky Yu. Biological chemistry : textbook / edited by Yu. Gubsky. – Vinnytsia : Nova Knyha, 2018. – 2nd edition. – P. 58–60.
3. **Triacylglycerols (TAG) their structure and properties.**	1. Satyanarayana U., Chakrapani U. «Biochemistry», Fourth Edition. – 2013. – P. 32–33.

		2. Gubsky Yu. Biological chemistry : textbook / edited by Yu. Gubsky. – Vinnytsia : Nova Knyha, 2018. – 2nd edition. – P. 61–62.
4.	**Structure and occurrence of main steroids**	1. Satyanarayana U., Chakrapani U. «Biochemistry», Fourth Edition. – 2013. – P. 37–38. 2. Gubsky Yu. Biological chemistry: textbook / edited by Yu. Gubsky. – Vinnytsia: Nova Knyha, 2018. – 2nd edition. – P. 67–70.
5.	**Structure and occurrence of complex lipids.**	1. Satyanarayana U., Chakrapani U. «Biochemistry», Fourth Edition. – 2013. – P. 28-29, 34–37. 2. Gubsky Yu. Biological chemistry: textbook / edited by Yu. Gubsky. – Vinnytsia: Nova Knyha, 2018. – 2nd edition. – P. 63–66.
6.	**Amphipathic lipids. Involvement of lipids in formation of structure and function of biological membranes. Liposomes.**	Satyanarayana U., Chakrapani U. «Biochemistry», Fourth Edition. – 2013. – P. 39–40.
7.	**Digestion of lipids:** ✓ digestion (action of lipases); ✓ absorption (micelles); ✓ re-synthesis and transport.	Satyanarayana U., Chakrapani U. «Biochemistry», Fourth Edition. – 2013. – P. 173–178, 306.
8.	**Catabolism of triacylglycerols:** ✓ characterization of intracellular lipolysis, its biological significance; ✓ enzymatic reactions; ✓ neurohumoral regulation of lipolysis: role of epinephrine, norepinephrine, glucagone, insulin; ✓ energetic balance of triacylglycerol oxidation.	1. Satyanarayana U., Chakrapani U. «Biochemistry», Fourth Edition. – 2013. – P. 285–287. 2. Gubsky Yu. Biological chemistry: textbook / edited by Yu. Gubsky. – Vinnytsia: Nova Knyha, 2018. – 2nd edition. – P. 179-181.
9.	**Biosynthesis of triacylglycerols.**	1. Satyanarayana U., Chakrapani U. «Biochemistry», Fourth Edition. – 2013. – P. 302–306. 2. Gubsky Yu. Biological chemistry: textbook / edited by Yu. Gubsky. – Vinnytsia: Nova Knyha, 2018. – 2nd edition. – P. 197–201.

5. Tasks for independent work and self-control

5.1. Complete the table (write the missed names or formulas of fatty acids):

	Common name	Systematic name	Structure
1	Butyric acid	n-Butanoic acid	
2		n-Hexa-decanaocicid	$CH_3(CH_2)_{14}COOH$
3	Steariac cid		$CH_3(CH_2)_{16}COOH$
4	Palmitoleic acid	Cis-9-Hexadeceancoid	
5		cis-9-Octadecenaocicid	$CH_3(CH_2)_7CH=CH(CH_2)_7COOH$
6	Linoleic acid	cis, cis-9, 12-octadecadienoic acid	
7	Arachidonic acid	All cis-5,8,11 Eicosatetranoic acid	

5.2. Write the structural formula of triacylglycerol (neutral fat): 1-stearyl-2-oleyl-3-linoleylglycerol

5.3. Write the structural formula of cholesterol and cholesteryl ester.

Cholesterol **Cholesteryl ester**

5.4. Digestion of lipids

Part of GIT	Enzymes	Substrates → Products
Mouth		
Stomach		
Small intestine	1) Degradation of triacylglycerol (fat)	
	2) Degradation of cholesteryl esters	
	3) Degradation of phospholipids	

5.5. Bile salts, free fatty acids and monoacylglycerols have detergent properties and emulsify dietary fat and oil. Describe the mechanism of this action.

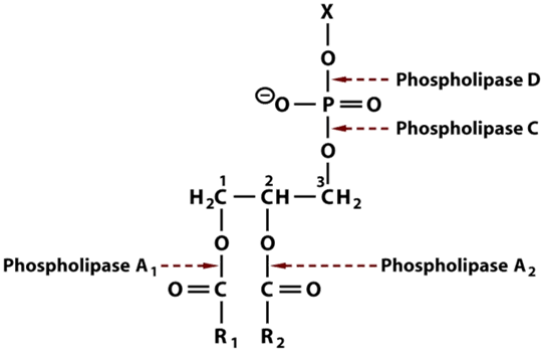
5.6. Fate of chylomicrons.

a) The enzyme lipoprotein lipase (LPL), which is located on the inner surface of the capillary endothelial cells of muscle and adipose tissue, digests TAG in the chylomicrons. What products are formed?

b) What major fate of the fatty acids in muscles?

c) What major fate of the fatty acids in adipose tissue?

5.7. Show the places for phospholipases A_1, A_2, C and D action. Which of them gives a lysophospholipid?



5.8. Define lipolysis. Describe biological significance of lipolysis.

5.9. Write the scheme of hydrolysis of triacylglycerol. Write the key regulating enzyme.

5.10. Control of lipolysis in adipose tissue through cyclic AMP.

Hormone	Effect	Lipolysis (increase or decrease)
Epinephrin		
Norepinephrine		
Glucagon		
Insulin		

5.11. Situational task:

Excessive intake of carbohydrates (600 g/day) in excess of energy needs, a woman in '28 has led to obesity.

Which process activation occurs in these conditions?

What products of carbohydrate catabolism is the metabolic precursors of the biosynthesis of fat?

Write them formula.

Which hormone activates lipogenesis?

6. Individual independent students work

1. To prepare the abstract on the theme «Obesity».
2. To create the scheme of hormonal regulation of lipolysis.

Practice protocol №16 «____» _____ 20___

Experiment 1. Quantitative determination of phospholipids in blood serum.

Principle. Phospholipids are precipitated with trichloroacetic acid together with plasma proteins. After mineralization of sediment the quantity of phosphorus is determined colourimetrically and content of phospholipids is calculated.

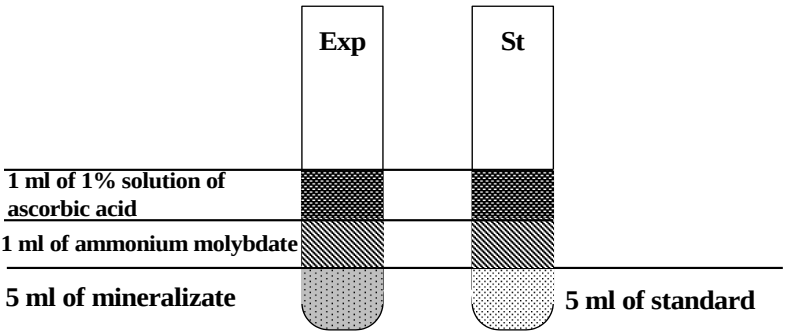
Method.

I. Precipitation of phospholipids with trichloroacetic acid (*Attention!!!! This part of experiment was previously done by technitions***):**

1. Take a clean dry centrifuge tubes.
2. Add 0.2 ml of blood serum, 2 ml of distilled water and 3 ml of 10 % solution of trichloroacetic acid.
3. Mix the content of the tube and after 2-3 min. centrifuge it during 5 min at 3000 rpm.
4. Carefully remove the supernatant. Sediment contains lipoproteins. Add to the sediment 1 ml of 56 % $HClO_4$ put the tube into a bath with boiling water for 30 min. The final mineralizate must be colourless.

II. Colour reaction:

1. Take 2 clean dry tubes.
2. To the 1^{st} (experimental) tube add 5 ml of mineralizate, 1 ml of ammonium molybdate and 1 ml of 1% solution of ascorbic acid.
3. To the 2^{nd} tube (standard) add 5 ml of standard phosphorus solution (0,05 g/l), 1 ml of ammonium molybdate and 1 ml of 1% solution of ascorbic acid.
4. Mix the content of both tubes and incubate 15-20 min at room temperature.



III. Measurement of optical desity:

After 5 min measure the optical density on a photoelectrocolourimeter with a red light filter.

IV. Calculation: Use the following formula:

$$\textbf{[Total phospholipids in serum]} = \frac{A_{exp} \times 0{,}05}{Ast \times 0{,}2} \times 25 \text{ g/l}$$

where A_{exp} – optical density of the experimental probe;

A_{st} – optical density of the standard probe;

0.05 – concentration of phosphorus in standard (mg/ml)

0.2 – volume of analyzed serum

25 – coefficient for calculation of total phospholipids content.

Result:

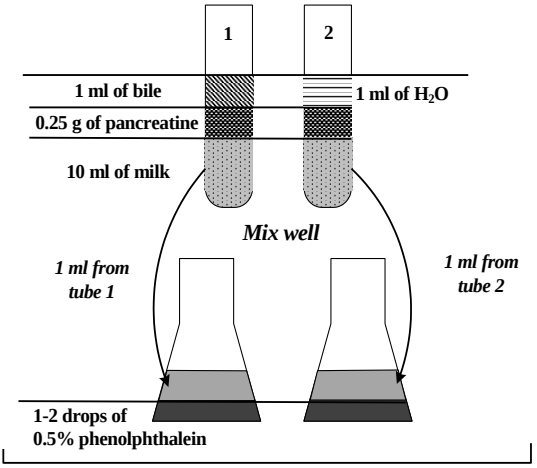
Conclusion:

Clinical and diagnostic significance. The determination of phospholipid content in blood has an important diagnostic significance. The concentration of total phospholipids in blood serum of healthy adult is 1.5–3.6 g/l. The increase of phospholipids level in blood serum (hyperphospholipidemia) is observed in heavy form of diabetes mellitus,

nephrosis, obturative jaundice. Decrease of phospholipid level (hypophospholipidemia) may be observed in atherosclerosis, anemias, feaver, alimentary distrophias, liver diseases.

Experiment 2. The influence of bile on the lipase activity.

Principle. The lipase activity is estimated according to the production of fatty acids during hydrolysis of fat. The quantity of fatty acids is determined by titration with alkali in the presence of phenolphthalein. Bile activates lipase thus accelerating the cleavage of lipids.



Titrate with 0.05 N NaOH to appearance the pink color, which doesn't disappear during 30 seconds.

Method.

I. Preparation of mixtures:

1. Take 2 clean dry tubes.
2. Add 10 ml of milk and 0.25 g of pancreatine into both tubes.
3. Add 1 ml of bile into 1st tube and 1 ml of water into 2nd. Mix them well.

II. Titration:

1. Take 1 ml of the mixture from tube 1 (with bile) and transfer it to the flask 1.
2. Take 1 ml of the mixture from tube 2 (without bile) and transfer it to the flask 2.
3. Add 1-2 drops of 0.5 % phenolphthalein solution into both flasks.
4. Titrate mixtures of both flasks with 0.05 N NaOH up to the pink colour, which doesn't disappear during 30 seconds. **Register the volume (in ml) of used alkali.**

Time, min	0 min	15 min	30 min	45 min	60 min
Quantity of NaOH expended for titration, ml					
Lipase activity in presence of bile					
Lipase activity in absence of bile					

III. Incubation. The first two tubes with digestive mixture (see I) place to the termostate at 38°C. Every 15 min take 1 ml of the mixture from each tube into flasks and repeat the titration (see II). Conduct 4-5 subsequent determinations. The results are registered as ml of alkali solution, expended for titration. Note the obtained result into the table bellow.

Explain the results, draw a conclusion.

Result:

Conclusion:

Clinical and diagnostic significance. Digestion of lipids occurs predominantly in intestines under the action of active pancreatic lipase, which acts only on emulsified lipids, e.g. milk fat. In gastric juice the activity of lipase is negligible. Lipids must be emulsified, which is achieved due to the action of bile acids. As bile emulsify lipids and activates lipase, hydrolysis of lipids proceeds more quickly. As lipase is the main enzyme in lipid digestion, changes in its activity may indicate on disorders in some processes of lipid metabolism. Deficiency of pancreatic lipase causes pancreatogenic steatorrhea, which is observed in chronic pancreatitis, hereditary or acquired deficiency of pancreatic gland, in mucoviscidosis. The amount of bile pigments in feces in such conditions is accompanying with the lowering of non esterified fatty acids content and significant increase in unhydrolized lipids. The alteration in emulcification, digestion and absorption of lipids and fat soluble vitamins as well may take place in diseases of liver, gall bladder or biliary ducts (cholelythiasis).

The report is checked up _____

(The signature of the teacher, date)

Examples of Krock-1 tests

1. Which of the following hormones reduces the rate of lipolysis in the adipose tissue?

A. Insulin
B. Adrenaline
C. Hydrocortisone
D. Growth hormone
E. Norepinephrine

2. Which hormone activates triglyceride lipase by adenylate cyclase cascade mechanism?

A. Adrenaline
B. Thyroxine
C. Melatonin
D. Vasopressin
E. Testosterone

3. A 35-year-old man has been delivered to a hospital in the state of a stressful situation. Laboratory investigation revealed increased free fatty acids' level in the blood above normal value. Which of the following processes has led to this state?

A. Breakdown of triacylglycerols in the adipose tissue
B. Synthesis of fatty acids
C. Breakdown of triacylglycerols in the gastrointestinal tract
D. Activation of lipoprotein lipase
E. Carnitine deficiency

4. Contrainsulin hormones stimulate lipolysis by the activation of the cAMPdependent protein kinases. What enzymes are activated by insulin causing the inhibition of lipolysis?

A. Phosphodiesterase, protein phosphatase
B. Phosphorylase, monoacylglycerol lipase
C. Adenylate cyclase, protein kinase
D. Diacylglycerol lipase, neuraminidase
E. Transpeptidase, glutamate dehydrogenase

5. Lipolysis is an enzymatic process of the hydrolysis of triacylglycerols to fatty acids and glycerol. How fatty acids are transported by the blood stream?

A. Albumins
B. Globulins
C. HDL
D. LDL
E. Chylomicrons

6. Glycerol and fatty acids were obtained in result of hydrolysis reaction. What compound was hydrolysed?

A. Triacilglycerol
B. Cholesterol
C. Phosphatidylcholine
D. Cholic acid
E. Sphingosine

7. A 36-year-old man was diagnosed with pheochromocytoma. Levels of epinephrine and the concentration of free fatty acids were increased in the blood. Which enzyme of lipolysis is activated under epinephrine influence?

A. Triglyceride lipase
B. Lipoprotein lipase
C. Phospholipase A_2
D. Phospholipase C
E. Cholesterol esterase

8. The function of what endocrine glands is examined in patients with overweight?

A. Anterior pituitary, thyroid, adrenal cortex, gonads
B. Thymus, hypothalamus, the posterior pituitary
C. Posterior pituitary, pancreatic alphacells, adrenal gland
D. Epiphysis, pancreas, parathyroid gland
E. Gonads and adrenal medulla

9. Which of the following hormones stimulates lipogenesis?

A. Insulin
B. Glucagon
C. Adrenaline
D. Thyroxine
E. Testosterone

10. Specify hormones that activate lipolysis in the adipose tissue:

A. Adrenalin, glucagon
B. Sex hormones
C. Growth hormone
D. Thyroxine
E. All of the above

11. Lipids are class of bioorganic compounds with very important functions. Specify them:

A. Energy, regulatory, structural
B. Protective, receptor, haemostatic
C. Structural, antioxidant, receptor
D. Digestive, excretory, regulatory
E. Regulatory, digestive, haemostatic

12. What hormones stimulate lipolysis by the activation of c-AMP-dependent protein kinase?

A. Adrenalin, glucagon
B. Estradiol, growth hormone
C. Vasopressin, insulin
D. Insulin, oxytocin
E. Insulin, corticotropin

13. Indicate the mechanism of tissue triglyceride lipase activation, which leads to breakdown of triacylglycerols:

A. Covalent modification
B. Protein cleavage
C. Feed-back inhibition
D. Methylation
E. All of the above

14. It is known that insulin inhibits lipolysis. Which type of the covalent modification of the tissue triglyceride lipase that is the key enzyme of the lipolysis used in this case?

A. Dephosphorylation
B. Acetylation
C. Methylation
D. Demethylation
E. Phosphorylation

15. Under lipolysis the activation of adenylate cyclase cascade mechanism leads to an increase in the intracellular concentration of the secondary messenger during the transmission of the hormonal signal. Specify it:

A. cAMP
B. cGMP
C. Ca^{2+}
D. Diacylglycerol
E. Inositol-3-phosphate

16. Glycerol is produced in the process of triacylglycerol mobilization. Specify the mechanism of glycerol activation:

A. Phosphorylation to form α-glycerol phosphate
B. Acetylation to form a glycerol-acetate
C. Amination to form glycerol-amine
D. Methylation to form methyl-glycerol
E. Formylation to form formyl-glycerol

17. It is known that the activity of the key enzyme of lipolysis of tissue triglyceride lypase is regulated by the covalent modification. What is the specific mechanism of this modification?

A. Phosphorylation-dephosphorylation
B. Protein cleavage
C. Amination-deamination
D. Methylation-demethylation
E. Acetylation-deacetylation

18. What is a frequent reason of alimentary obesity?

A. Increasing supply of energy substrates with food and reducing of their using for energy purposes
B. Hypodynamia
C. Decrease in the supply of energy substrates with food and increase of their use for energy purposes
D. Relevant gender
E. Disturbance of digestive processes

19. A 30-year-old man has been delivered to the hospital in the state of the stress. Laboratory investigation revealed increased free fatty acids' level in the blood. Which of the following enzymes was activated in this case?

A. Tissue triacylglycerol lipase
B. Pancreatic triacylglycerol lipase
C. Lipoprotein lipase
D. Acetyl-CoA carboxylase
E. Phospholipase A_2

20. A patient feels discomfort in their stomach after having eaten fatty food. The feces contain indigested drops of fat. The urinal bile acids test is positive. What substance deficit causes this state?

A. Bile acids
B. Phospholipids
C. Fatty acids
D. Chylomicrones
E. Triacylglycerols

21. Specify metabolite that is a precursor in the biosynthesis of triacylglycerols in adipocytes promoting development of obesity:

A. Dihydroxyacetone phosphate
B. Acetoacetate
C. Succinate
D. Oxalacetate
E. Malate

22. A patient complains of bad feeling after a meal of fatty food. They have frequent diarrhea, loss of weight. Causes of those events may be all the below mentioned except:

A. Gastritis
B. Pancreatitis

C. Enterocolitis
D. Cholelithiasis
E. Hepatitis

23. A patient has vomiting and steatorrhea after a meal of fatty fooD. The cause of that state may be:

A. Deficiency of bile acids
B. Deficiency of amylase
C. Increased secretion of lipase
D. Disturbance of phospholipase synthesis
E. Disturbance of trypsin synthesis

24. A patient has a disturbed absorption of fat hydrolysates. It might have been caused by a deficit in the small intestine cavity of:

A. Bile acids
B. Bile pigments
C. Sodium ions
D. Lipolytic enzymes
E. Liposoluble vitamins

25. A man, age 35, develops phechromocytosis. The epinephrine and norepinephrine as well as blood concentration of free fatty acids is increased. Which enzyme activation by epinephrine accelerates lipolysis?

A. TAG- lipase
B. Lipoprotein lipase
C. Phospholipase A_2
D. Phospholipase
E. Cholesterol esterase

26. Stool test detects in the patient's feces a large amount of undigested fats. This patient is the most likely to have disturbed secretion of the following enzymes:

A. Pancreatic lipases
B. Bile lipase
C. Pancreatic proteases
D. Gastric protease
E. Pancreatic amylase

27. A patient has normally coloured stool including a large amount of free fatty acids. The reason for this is a disturbance of the following process:

A. Fat absorption
B. Fat hydrolysis
C. Biliary excretion
D. Choleresis
E. Lipase secretion

28. What enzyme insufficient secretion causes the impaired digestion of lipids in gastro-intestinal tract and the appearance of the large quantities of neutral fats in feces.

A. Pancreatic lipase
B. Pepsin
C. Phospholipase
D. Enterokinase
E. Amylase

29. Obesity is a common disease. The aim of its treatment is to lower content of neutral fats in the body. What hormon-sensitive enzyme is the most important for intracellular lipolysis?

A. Triacylglycerol lipase
B. Diacylglycerol lipase
C. Monoacylglycerol lipase
D. Adenylate kinase
E. Protein kinase

30. Roentgenologically confirmed obstruction of common bile duct resulted in preventing bile from inflowing to the duodenum. What process is likely to be disturbed?

A. Fat emulgation
B. Protein absorption
C. Carbohydrate hydrolysis
D. Hydrochloric acid secretion in stomach
E. Salivation inhibition

31. In patients suffering from diabetes mellitus an increase in a content of non esterified fatty acids (NEFA) in blood is observed. It may be caused by:

A. Increase in activity of triacylglycerol lipase
B. Stimulation of ketone bodies utilization
C. Activation of synthesis of apolipoproteins A1, A2, A3
D. Decrease in activity of phosphatidylcholine-cholesterol-acyltransferase in blood plasma
E. Accumulation in cytosol of palmitoyl-CoA

32. Blood of the patients with diabetes mellitus shows increased content of free fatty acids. Name the most likely cause of this:

A. Increased activity of adipose triglyceride lipase
B. Accumulation of palmitoyl-CoA in cytosol
C. Activation of ketone bodies utilization
D. Activation of $apoA_1$, $apoA_2$, and apoA4 apolipoprotein synthesis
E. Decreased activity of plasma phosphatidylcholine-cholesterola-cyltransferase

33. Which one of the following statements about the absorption of lipids from the intestine is correct?

A. Dietary triacylglycerol is partially hydrolyzed and absorbed as free fatty acids and monoacyl glycerol

B. Release of fatty acids from triacylglycerol in the intestine is inhibited by bile salts

C. Dietary triacylglycerol must be completely hydrotyzed to tree fatty acids and glycerol before absorption

D. Fatty acids that contain ten carbons or less are absorbed and enter the circulation primarily via the lymphatic system

E. Formation of chylomicrons does not require protein synthesis in the intestinal mucosa

References:

1. Gubsky Yu. Biological chemistry: textbook / edited by Yu. Gubsky. – Vinnytsia : Nova Knyha, 2018. – 2nd edition. – 488 p.
2. Lecture materials.
3. Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – 799 p.
4. Textbook «Biological chemistry. Collection of test tasks» for English-speaking students of higher medical educational institutions of the Ministry of Public Health of Ukraine. – Poltava, 2018. – 148 p.

TOPIC №17.
Transport forms of lipids: lipoproteins of blood plasma

1. Objective: To learn the transport forms of lipids and their role in pathogenesis of some diseases.

2. Actuality of theme: Quantitative determination of β- and preβ-lipoproteins have great importance for diagnostics of an atherosclerosis, ischemic illness of heart (IHD), obesity, chronic diseases of a liver as it allows to reveal damage of liver parenchime.

3. Specific aims:

✓ To treat quantitative changes of lipoproteins for diagnostics of an atherosclerosis, IHD, chronic diseases of a liver.

✓ To be able to determine quantitatively lipoproteins of blood after Burstain.

4. Reference card for the separate study of educational literature for the lesson preparation

Questions:	References:
1. **Lipoproteins: structure, classification, characteristics of apolipoproteins.**	Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 317–318.
2. **Metabolism of lipoproteins – a general view.**	Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 318–320.
3. **Disorders of plasma lipoproteins** (classification of hyperlipoproteinemias, characteristics of hypolipoproteinemias.	Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 320–322.

5. Tasks for independent work and self-control

5.1. Complete the table:

	Chylomicrons	VLDL	LDL	HDL
Place of formation				
Density				
Apoprotein and its function				
Free cholesterol				

Phospholipid				
Cholesterol ester				
Triacylglycerols				

5.2. Describe the lipoprotein lipase.

Location	
Role	
Activator of lipoprotein lipase	

5.3. As the chylomicron loses TAG, its density increases and it becomes a chylomicron remnant, which is taken up by the liver by receptors that recognize apolipoprotein e. In the liver, the chylomicron remnant is degraded by lysosomal enzymes.

What products of lysosomal digestion of chylomicron remnants?

How are they used in the liver?

5.4. Describe the lecithin-cholesterol acyltransferase

Location	
Role	

5.5. Fill in the chart:

Name and type of hyperlipoprotei nemia	Defect	Changes of plasma lipoproteins	Changes of plasma lipid (most)	Risk of atheroscle-rosis and coronary disease
Familial lipoprotein lipase deficiency (type I)	Deficiency of LPL Abnormal LPL Deficiency of apoEII			
Familial hypercholesterol-emia (type IIa)	Defect of LDL receptor			
Familial combined hyperlipidemia (type IIb)	Over production of apoB100			
Familiar dysbetalipoprotein-emia (type III)	Abnormal Apolipoprotein E			
Familial hypertriacylglyce-rolemia (type IV)	Over production of VLDL			

5.6. Situational task:
The patient detected hereditary lipoprotein lipase deficiency.
What role in lipid metabolism lipoprotein lipase play?

Content which lipoproteins in these conditions will be elevated in the blood?

Representatives of which classes of lipids are the main components of lipoproteins?

6. Individual independent students work

1. Infringement of an exchange of lipids at different types of hyperlipoproteinemias.

Practice protocol №17 «____» _____ 20___

Experiment. Quantitative determination of β-lipoproteins in whey of blood after Burstain.

Principle. At interaction of lipoproteins with chloride of calcium and heparin colloidal stability of proteins of blood whey is broken, therefore the degree of its turbidity increases.

Method. In a test tube bring 2 ml of 0.02 M solution $CaCl_2$ and 0.2 ml of the researched whey. Mix and determine optical density of the mix (E1) on FEC in 5 mm a ditch at a red optical filter (λ = 630 nanometers). Pour a mix again in a test tube, add by micropipette of 0.04 ml heparin solution, mix. In 4 minutes exactly again determine optical density of the mix (E2)under the same conditions.

Calculation:

$$(E2\text{-}E1) \times 1000.$$

Result:

Conclusion:

Clinical and diagnostic significance. Ultracentrifugation can separate blood lipoproteins according to their different densities: high (HDL), low (LDL), very low (VLDL), etc. Lipoprotein fractions differ in their protein content and a percentage of certain lipid components. HDLs have large amounts of proteins (50–60%) and a higher density (1.063–1.210), while LDLs and VLDLs contain less protein, a significant amount of lipids (up to 95% by weight). In addition, they have low relative density (1.010–1.063). In norm the contents of β-liporpoteins makes 350-550 standard units of optical density that corresponds 3.0–4.5 g/L (300-450 mg of %). An enhanced blood serum concentration is most commonly observed for β-lipoproteins. Elevated lipoprotein levels are closely related to cholesterol excess in blood, since β-lipoproteins are especially rich in cholesterol. Elevation of β-and pre-β-lipoproteins is observed in atherosclerosis, diabetes mellitus, and other diseases.

The report is checked up _____

(The signature of the teacher, date)

Examples of Krock-1 tests

1. A 59-year-old man suffers from cerebral atherosclerosis. Hyperlipidemia is revealed under laboratory investigation. Which class of lipoproteins is most likely to be increased in the patient's serum?

A. Low density lipoproteins
B. High density lipoproteins
C. Complex of fatty acids with albumin
D. Chylomicrons
E. Free cholesterol

2. An 11-year-old boy has the serum level of cholesterol up to 25 mmol/L. There is a hereditary familial hypercholesterolemia in the anamnesis caused by the disorder of receptor synthesis for:

A. Low density lipoproteins
B. High density lipoproteins
C. Chylomicrons
D. Very low density lipoproteins
E. Intermediate density lipoproteins

3. A 68-year-old patient eats mainly eggs, bacon, butter, milk and meat. In the blood: cholesterol – 12.3 mmol / L, total lipids – 8.2 g/L, increased low-density lipoprotein fraction. What type of hyperlipoproteinemia is observed in the patient?

A. Hyperlipoproteinemia type IIa
B. Hyperlipoproteinemia type I
C. Hyperlipoproteinemia Type IIb
D. Hyperlipoproteinemia type IV
E. Hyperlipoproteinemia Type V

4. The level of anti-atherogenic lipoprotein fraction is increased in the patient's plasma after a course of the atherosclerosis treatment. The increased level of what class of lipoproteins confirms the effectiveness of the therapy of the disease?

A. HDL
B. VLDL
C. IDL
D. LDL
E. Chylomicrons

5. The increased level of high density lipoproteins leads to the decreased risk of atherosclerosis. What is the mechanism of the anti-atherogenic action of high density lipoproteins?

A. Eliminate cholesterol from the tissues
B. Activate conversion of cholesterol to steroid hormones
C. Participate in the cholesterol breakdown
D. Activate conversion of cholesterol to the bile acids
E. Contribute to the absorption of cholesterol in the intestine

6. A 60-year-old patient has been admitted to the cardiology clinic with the diagnosis of ischemic heart disease. Laboratory examination of patient's blood revealed hyperlipoproteinemia, the content of LDL is 6.5 g/l, the triacylglycerols' level is 3 g/l. What is the type of hyperlipoproteinemia?

A. Type II
B. Type I
C. Type III
D. Type IV
E. Type V

7. The level of cholesterol esters is low in the patient's blood, the serum is turbid. The deficiency of which enzyme causes such disorders?

A. Lecithin-cholesterol acyltransferase
B. Choline acetyltransferase
C. Cholesterol esterase
D. Lipoprotein lipase
E. 7-Cholesterol hydroxylase

8. What types of hyperlipoproteinemia are specific for patients with atherosclerosis?

A. II, IV
B. I, IV
C. I, III
D. IV
E. IV, V

9. It was revealed an increased level of cholesterol of β-lipoprotein fraction in the blood serum. What are possible consequences of such disorders for the human body?

A. Atherosclerosis
B. Gallstone disease
C. Obesity
D. Hypertension
E. Chronic renal failure

10. Concentration of low density lipoproteins is increased in the serum of a 45-year-old patient. The development of what disease could be expected in the patient?

A. Atherosclerosis
B. Pyelonephritis
C. Acute pancreatitis
D. Gastritis
E. Pneumonia

11. The examination of the patient suffering from atherosclerosis revealed a reduced high density lipoprotein concentration and increased low density lipoprotein concentration in the blood. Cholesterol concentration is 11 mmol/L. The activity of which enzyme is reduced?

A. Lecithin-cholesterol acyltransferase
B. Choline acetyltransferase
C. Cholesterol esterase
D. Lipoprotein lipase
E. 7-Cholesterol hydroxylase

12. Atherosclerosis is a disease manifested by the deposition of lipid structures (plaques) in the vascular wall. Cholesterol and its esters are the main components of plaques. Different classes of lipoproteins have different diagnostic value. What are atherogenic lipoproteins?

A. Low density lipoproteins, very low density lipoproteins
B. Chylomicrones
C. Free fatty acids
D. Intermediate density lipoproteins
E. High density lipoproteins

13. Examination of a 16-year-old patient revealed xanthomas, symptoms of pancreatitis, increased concentration of chylomicrons in the blood serum, reduced activity of lipoprotein lipase, slightly elevated very low density lipoproteins. Triacylglycerols' concentration is below 4.5 mmol/L. What is the type of hyperlipoproteinemia?

A. Type I
B. Type II
C. Type III
D. Type IV
E. Type V

14. Abnormal VLDL were found in the patient's blood plasma, which contain particularly large amounts of triacylglycerols. Xanthomas are observed, manifestations of atherosclerosis, cholesterol and triacylglycerols ratio in the VLDL is more than 1/5, increased apoE in VLDL. What is the type of hyperlipoproteinemia?

A. Type III
B. Type II
C. Type I
D. Type IV
E. Type V

15. A patient has elevated cholesterol – 7.76 mmol/L and concentration of triacylglycerols is 1.5 mmol/L. There is also a high level of low density lipoproteins – 7.1 g/L. What is the type of hyperlipoproteinemia?

A. Type II
B. Type I
C. Type III
D. Type IV
E. Type V

16. Triacylglycerols and cholesterol concentrations are increased in the patient's blood plasma, apoC_2 is decreased and apoC_3 is increased. What is the type of hyperlipoproteinemia?

A. Type IV
B. Type II
C. Type III
D. Type I
E. Type V

17. What are antiatherogenic lipoproteins called?

A. High density lipoproteins
B. Low density lipoproteins

C. Chylomicrons
D. Very low density lipoproteins
E. Intermediate density lipoproteins

18. What are atherogenic lipoproteins called?

A. Low density lipoproteins
B. High density lipoproteins
C. Chylomicrons
D. Albumins
E. Globulins

19. Which of the following lipoproteins will be elevated in the bloodstream about 2 hours after eating of a high fat meal?

A. Chylomicrons
B. High density lipoproteins
C. Intermediate lipoproteins
D. Low density lipoproteins
E. Very low density lipoproteins

20. The patient organism diagnosis displayed increased blood serum level of LDL. Which disease onset may be predicted?

A. Atherosclerosis
B. Gastritis
C. Kidney inflammation
D. Acute pancreatitis
E. Pneumonia

21. The patient's blood plasma was cloudy while being investigated in 4 hours after the patient had eaten fatty food. Which substance level is increased in the blood plasma which causes the state:

A. Chylomicrones
B. HDL
C. LDL
D. Cholesterol
E. Phospholipids

22. The adipose tissue of organism is the main TAG depot. Simultaneously their synthesis proceeds in the liver. What particles transport TAG from liver to adipose tissue?

A. VLDL
B. Chylomicrones
C. VDL
D. HDL
E. Albumin complex

23. A patient has symptoms of atherosclerosis. What plasma lipid transport forms should have an increased concentration?

A. LDL
B. HDL
C. IDL
D. VLDL
E. Chylomicrons

24. Cholesterol content in blood serum of 12-year-old is 25 mmol/l. Anamnesis states hereditary familial hypercholesterolemia caused by synthesis disruption of receptor-related proteins for:

A. Low-density lipoproteins
B. High-density lipoproteins
C. Middle-density lipoproteins
D. Very low-density lipoproteins
E. Chylomicrons

25. Blood serum of the patient has milky appearance. Biochemical analysis revealed high content of triacylglycerols and chylomicrons. This condition is caused by hereditary defect of the following enzyme:

A. Lipoprotein lipase
B. Phosphodiesterase
C. Adipose tissue hormone-sensitive lipase
D. Phospholipase
E. Pancreatic lipase

26. Increased HDL levels decrease the risk of atherosclerosis. What is the mechanism of HDL anti-atherogenic action?

A. They remove cholesterol from tissues
B. They supply tissues with cholesterol
C. They are involved in the breakdown of cholesterol
D. They activate the conversion of cholesterol to bile acids
E. They promote absorption of cholesterol in the intestine

27. A 67-year-old patient has atherosclerosis of cardiac and cerebral vessels. Examination revealed hyperlipidemia. What class of blood plasma lipoproteids is most important in atherosclerosis pathogenesis?

A. Low-density lipoproteids
B. α-lipoproteids
C. High-density lipoproteids
D. –
E. Chylomicrons

28. Examination of a child suffering from obesity revealed increased concentrations of low density lipoproteins, chylomicrons and

triacylglycerols in the blood serum. The serum is milky turbid on standing, the creamy layer is formed on the surface. What is the type of hyperlipoproteinemia?

A. Type V
B. Type II
C. Type III
D. Type IV
E. Type I

References:

1. Lecture materials.
2. Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – 799 p.
3. Textbook «Biological chemistry. Collection of test tasks» for English-speaking students of higher medical educational institutions of the Ministry of Public Health of Ukraine. – Poltava, 2018. – 148 p.

TOPIC №18.
β-Oxidation of fatty acids. Studies on metabolism of fatty acids and ketone bodies

1. Objective: To learn reactions of biosynthesis of fatty acids. To know metabolic pathways of ketone bodies under normal conditions and in pathology and to determine their amount in urine.

2. Actuality of the theme: Oxidation of lipids, respectively fatty acids, as well as ketone bodies metabolism are important constituents of energetic metabolism in sense of providing tissues and cells with ATP. Determination of ketone bodies concentration in blood and in urine has important significance in diagnostics of several pathological processes.

3. Specific aims:

- ✓ To study reactions β -oxidation of long chain fatty acids.
- ✓ To analyze the metabolism of ketone bodies.
- ✓ To explain the mechanism of excessive accumulation of ketone bodies in diabetes mellitus and in starvation.

4. Reference card for the separate study of educational literature for the lesson preparation

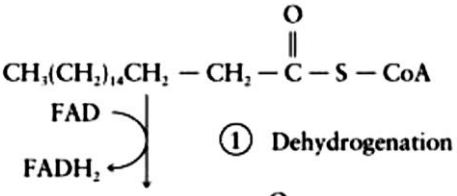
Questions:	References:
1. **β –Oxidation of long chain fatty acids:** ✓ localization of the process of β-oxidation of fatty acids; ✓ activation of fatty acids, the role of carnitin in transport of fatty acids into mitochondria; ✓ the sequence of enzymatic reactions in β –oxidation of fatty acids; ✓ energetic balance of β –oxidation of fatty acids.	1. Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 287-291. 2. Gubsky Yu. Biological chemistry: textbook / edited by Yu. Gubsky. – Vinnytsia: Nova Knyha, 2018. – 2nd edition. – P. 182–188.
2. **Mechanism of glycerol oxidation:** ✓ reactions; ✓ bioenergetics.	1. Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 287. 2. Gubsky Yu. Biological chemistry: textbook / edited by Yu. Gubsky. – Vinnytsia: Nova Knyha, 2018. – 2nd edition. – P. 179, 181.
3. **Metabolism of ketone bodies:** ✓ enzymatic reactions of ketone bodies biosynthesis (ketogenesis); ✓ reactions of ketone bodies utilization (ketolysis), energetic effect;	1. Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 294–297. 2. Gubsky Yu. Biological chemistry: textbook / edited by

✓ metabolism of ketone bodies in pathology. ✓ mechanism of excessive accumulation of ketone bodies in diabetes mellitus and in starvation.	Yu. Gubsky. – Vinnytsia: Nova Knyha, 2018. – 2nd edition. – P. 188–192.

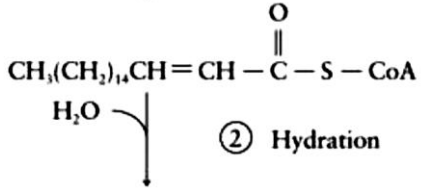
5. Tasks for independent work and self-control

5.1. Name enzymes of β-oxidation and explain net energetic effect of palmitoil-CoA oxidation:

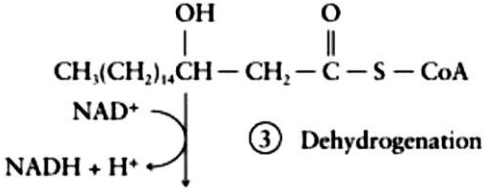
1)



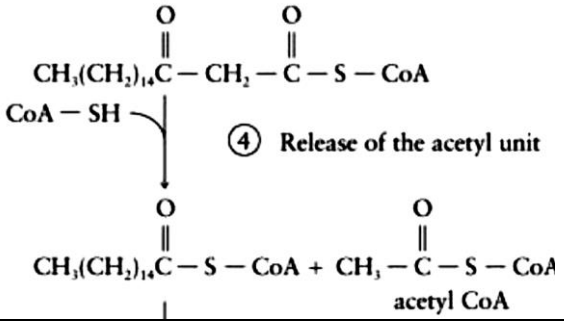
2)



3)



4)



5.2. How many cycles of β-oxidation are required for the complete oxidation of activated oleic acid, 18:1(Δ^9)?

5.3. Determine the number of ATP that can be generated from the fatty acids in 1 mol of tristearin. (Tristearin is a triacylglycerol composed of glycerol esterified to three stearic acid moleculs).

5.4. A number of inherited diseases in the metabolism of carnitine or acylcarnitines have been described. These include defects in the following enzymes or systems: the transporter for carnitine uptake into muscle; carnitine acyltransferase I (CATI or CPTI); carnitine-acylcarnitine translocase; and carnitine acyltransferase II (CATII or CPTII). Classical CATII deficiency, the most common of these diseases, is characterized byadolescent to adult onset of recurrent episodes of acute myoglobinuria precipitated by prolonged exercise or fasting. During these episodes, the patient is weak, and may be somewhat hypoglycemic with diminished ketosis (hypoketosis). Lipid deposits are found in skeletal muscles. CPK levels, and long-chain acylcarnitines are elevated in the blood. In contrast, when CATII deficiency has presented in infants, CAT II levels are below 10% of normal, the hypoglycemia and hypoketosis are severe, hepatomegaly occurs from the triacylglycerol deposits, and cardiomyopathy is also present.

a) What is the function of carnitine?

b) What is the structure of carnitine?

c) What metabolic pathway is impaired in patients with these defects?

d) Explain why these disorders lead to hypoglycemia.

e) Explain why these disorders lead to hypoketosis (low production of ketone bodies).

5.5. Very long-chain fatty acids (C_{22}, C_{24}, C_{26}) are shortened in peroxisomes. Peroxisomal β-oxidation generates hydrogen peroxide (H_2O_2), acetyl-CoA and a short- to medium-chain-length acyl-Coa. The acyl-CoA products are transferred to mitochondria to complete their oxidation.

a) Explaine why peroxisomal oxidation of fatty acids result in formation of H_2O_2.

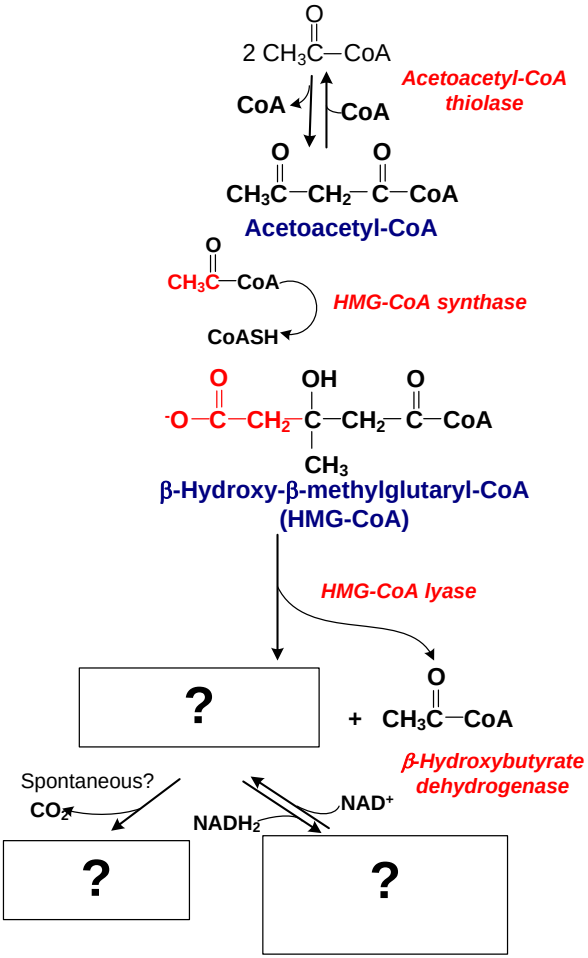
b) What enzyme catalyzes conversion of H_2O_2 to H_2O in peroxisomes?

c) A number of inherited deficiencies of peroxisomal enzymes have been described. Zellweger syndrome, resulting from the absence of peroxisomes, is characterized by accumulation of long-chain fatty acids in blood and leads to complex developmental and metabolic phenotypes affecting principally the liver and the brain. What groups of lipids contain very-long-chain fatty acid, especially in cells of the brain and nervous system?

d) Refsum's disease is caused by a deficiency in a single peroxisomal enzyme, the phytanoyl CoA hydroxylase that carries out α-oxidation of phytanic acid (branched fatty acid). Symptoms include retinitis pigmentosa, cerebellar ataxia, and chronic polyneuropathy. What dietary source of phytanic acid and what treatment of this disease?

5.6. Calculate ATP yield of glycerol oxidation.

5.7. Fill in the blanks:



5.8. Indicate the tissues where occurs: 1) ketogenesis, 2) ketolysis.

5.9. Overproduction of ketone bodies.

a) Define ketonemia.

b) Define ketonuria.

c) Define ketosis.

5.10. Situational tasks:

a) The unripe fruit of the ackee tree produces a toxin, hypoglycin, which causes a condition known as Jamaican vomiting sickness. The victims of the toxin are usually unwary children who eat this unripe fruit and develop a severe hypoglycemia, which is often fatal. Although hypoglycin causes hypoglycemia, it acts by inhibiting an acyl-CoA dehydrogenase involved in β-oxidation that has specificity for short- and medium-chain fatty acids. Explain why this disorder leads to severe hypoglycemia.

b) In patients with diabetes glucose revenues in adipocytes reduced. In accordance glucose-dependent inhibition of fatty acid mobilization decreases. Last come into the bloodstream and other tissues used them as an energy source.

Deficiency of which hormone causes this condition?

Explain the mechanism of action of this hormone on intracellular lipolysis.

How this hormone dependent enzyme of lipolysis goes from inactive to active form?

6. Individual independent students work

1. Genetic defects in fatty acid-CoA dehydrogenases.
2. Reasons and consequences of ketosis.

Practice protocol №18 «____» _____ 20___

Experiment 1. Qualitative reaction on acetone and acetoacetic acid (Lange reaction).

Principle. It is based on a property of acetone and acetoacetic acid to produce compounds with sodium nitroprussid of red colour in the basic pH of medium.

$$CH_3–CO–CH_3 + Na_2[Fe(CN)_5NO] + 2\ NaOH \rightarrow$$
$$\rightarrow Na_4[Fe(CN)_5NO = CHCOCH_3] + 2\ H_2O$$

Under the action of acetic acid it turns to a violet product:

$$Na_4[Fe(CN)_5NO{=}CHCOCH_3] + CH_3COOH \rightarrow$$
$$Na_3[Fe(CN)_5NOCH_3COCH_3] + CH_3COONa$$

compound after an overlay of conc. ammonia solution over the mixture of test sample, containing sodium nitroprussid and acetic acid. The rate of colour ring formation between two layers of liquids depends from acetone concentration. It is assumed, that appearance of the ring after 3-4 min corresponds to the acetone concentration 0.0085 g/l (8.5 mg/ l).

Method.

1. Take two clean dry tubes.
2. Add 0.5 ml of urine of a healthy person to the first tube (blank).
3. Add 0.5 ml of urine of a patient with diabetes mellitus to the second one (test sample).

4. Add 0.5 ml of 10% NaOH and 5-7 drops of fresh 10% sodium nitroprussid solution into both tubes. Colour in the 2nd tube turns red.

5. Add 5–7 drops of acetic acid into both tubes. Colour in the 2nd tube turns violet.

Explain the results, draw a conclusion.

Result:

Conclusion:

Experiment 2. Quantitative determination of ketone bodies in urine.

Principle. (see the previous experiment).

MethoD.

1. Take 5 clean dry tubes.
2. Add 1 ml of distilled water into each tube (except the first one).
3. Add 1 ml of urine of the patient with diabetes mellitus into the first and the second tubes. Thus, in the first tube urine is undiluted, in the second one the urine is two fold diluted.
4. Mix the content of the second tube thereafter transfer 1 ml of this mixture to the third tube and mix it. Now the third tube contains 4 fold diluted urine.
5. Remove 1 ml from the third tube – into the forth and mix it – 8 fold dilution.
6. Remove 1 ml from the fourth tube - into the fifth one and mix it – 16 fold dilution.
7. Add 8 drops of 50% ammonium sulfate solution into each tube for increasing the density of the solutions.
8. Add 8 drops of concentrated acetic acid into each tube.
9. Add 8 drops of sodium nitroprussid each tube. Mix well the content of the tubes.

10. Overlay **carefully** 1 ml of concentrated solution of ammonia into each tube starting from the last tube (**be careful with concentrated ammonia!**).

11. Register an appearance of red or violet ring in the tubes. Notice the last tube in which a coloured ring appears between the 3-d and the 4-th min.

Calculation:

$$X = 0.85 \times A \times 15,$$

were

X – concentration of acetone in urine (mg/day);

0.85 – empirical coefficient, corresponding to 0,85 mg of acetone in 100 ml;

A – the dilution of the urine sample;

15 – coefficient for calculation on the daily volume of urine (1500 ml).

Draw the conclusions.

Result:

Conclusion:

Clinical and diagnostic significance. Ketone bodies include the following substances – acetoacetic acid, β-hydroxybutyric acid, acetone. Biosynthesis of ketone bodies (ketogenesis) takes place in liver from intermediates of fatty acid oxidation, namely, from acetyl-CoA.

Acetoacetic acid, produced in liver, is transported to body tissues (brain, muscles, kidneys, heart etc.), where it serves as an energetic material. In healthy person the content of ketone bodies in blood is in ranges of 13–185 μmoles/l (1.5–20 mg/l). With urine is excreted 20–40 mg of ketone bodies daily, which are preferentially acetoacetic and β-hydroxybutyric acids. Acetone appears under pathological conditions.

The **increase** of ketone bodies concentration in blood (ketonemia) and in urine (ketonuria) is observed in diabetes mellitus, deficiency of sugar in nutrition, overproduction of hormones, antagonistic to insulin (corticosteroids, thyroxine, hormones of adenohypophysis). The **decrease** of ketone bodies content has no clinical value. In early childhood prolong

disorders of digestion (toxicosis, dysenteria) may lead to ketonemia due to the permanent starvation and exaggeration.

The report is checked up _____

(The signature of the teacher, date)

Examples of Krock-1 tests

1. The concentration of ketone bodies increases in the blood under conditions of diabetes mellitus type I. It leads to metabolic acidosis development. What is the source of ketone bodies biosynthesis?

A. Acetyl-CoA
B. Succinyl-CoA
C. Propionyl-CoA
D. Malonyl-CoA
E. Methylmalonyl-CoA

2. A 57 year-old man, suffering from diabetes mellitus develops ketoacidosis. The biochemical cause of this state is the decreased level of acetyl-CoA utilization due to the deficiency of:

A. Oxaloacetate
B. Alfa-ketoglutarate
C. Glutamate
D. Aspartate
E. Succinate

3. A process of fatty acid oxidation is associated with the process of ketone bodies biosynthesis. What pathology ketoacidosis is most often observed in?

A. Diabetes mellitus type I
B. Diabetes insipidus
C. Non-steroidal diabetes
D. Latent diabetes
E. Diabetes mellitus type II

4. Carnitine was recommended to the athlete for the improving of his results. What process is activated by carnitine?

A. Transport of fatty acids into the mitochondria
B. Synthesis of ketone bodies
C. Synthesis of lipids
D. Tissue respiration
E. Synthesis of steroid hormones

5. What is a complication of an excessive accumulation of ketone bodies in the body?

A. It is accompanied by metabolic acidosis
B. It inhibits transport of substances across the cell membrane
C. It directly leads to the destruction of phospholipids of biomembranes
D. It increases biosynthesis of cholesterol and atherogenic lipoproteins
E. It causes decreasing of osmotic pressure in the blood

6. Regarding diabetic ketoacidosis, select the one false statement:

A. Ketone bodies are hydroxyacetic and acetoacetic acid
B. Overproduction of ketone bodies is caused by the breakdown of fats due to the lack of insulin
C. Ketoacidosis is a result of ketone bodies overproduction
D. Plasma level of K^+ is often elevated
E. Administration of insulin and glucose is used for treatment of diabetic ketoacidosis

7. Explain why using of insulin in the combination with glucose reduces the concentration of ketone bodies in the patients with diabetes mellitus:

A. It stimulates glucose utilization in the tissues by activating the citric acid cycle and preventing the accumulation of acetyl-CoA
B. It stops the activity of key enzymes in carbohydrate metabolism
C. It increases the mobilization and catabolism of triglycerides
D. It speeds up the process of gluconeogenesis
E. It increases cholesterol and low density lipoprotein biosynthesis

8. Increased amount of free fatty acids is observed in the blood of patients with diabetes mellitus. It can be caused by:

A. Increased activity of triglyceride lipase in adipocytes
B. Storage of palmitoyl-CoA
C. Activation of a ketone bodies utilization
D. Activation of the biosynthesis of apolipoproteins
E. Decreased activity of phosphatidylcholine cholesterolacyltransferase in the blood plasma

9. Ketone bodies are found in the patient's urine. What is the most likely pathology?

A. Diabetes mellitus
B. Acute glomerulonephritis
C. Urolithiasis
D. Tuberculosis of the kidney
E. Renal infarction

10. What vitamins provide the dehydrogenation reactions in the β-oxidation of fatty acids?

A. PP, B_2
B. B_3, PP
C. B_2, C, B_{12}

D. Lipoic acid, B_1
E. B_2, B_6

11. Lipolysis is the enzymatic process of the hydrolysis of neutral fats into fatty acids and glycerol. Fatty acids go into the bloodstream and are transported by:

A. Albumins
B. Globulins
C. High density lipoproteins
D. Low density lipoproteins
E. Chylomicrons

12. Human body receives energy in the formof ATP during the oxidation of various substrates. Oxidation of which compound provides maximal utilization of an inorganic phosphate?

A. Palmitoyl-CoA
B. Acetyl-CoA
C. Glucose
D. Glycerol
E. Succinate

13. A 10-month-old child has been admitted to the hospital with the signs of damage of the limbs and trunk. Carnitine deficiency in muscles was detected after investigation. The biochemical basis for this disease is a disorder of the process:

A. Transport of fatty acids into the mitochondria
B. Regulation of Ca^{2+} levels in the mitochondria
C. Substrate phosphorylation
D. Utilization of lactic acid
E. Synthesis of actin and myosin

14. A patient with high rate of obesity was advised to use carnitine as a food additive in order to enhance «fat burning». What is the role of carnitine in the process of fat oxidation?

A. Transport of free fatty acids from the cytosol to the mitochondria
B. Transport of free fatty acids from fat depots to the tissues
C. It takes part in one of the reactions of beta-oxidation of free fatty acids
D. Activation of free fatty acids
E. Activation of intracellular lipolysis

15. Specify the energy value of the palmitic acid oxidation:

A. 130 ATP
B. 148 ATP
C. 38 ATP

D. 121 ATP
E. 12 ATP

16. Select the cells that synthesize ketone bodies:
A. Hepatocytes
B. Myocytes
C. Enterocytes
D. Pancreocytes
E. Fibroblasts

17. Specify metabolites that belong to ketone bodies:
A. Acetoacetate, β-hydroxybutyric acid, acetone
B. Acetone, malonyl-CoA, succinyl-CoA
C. β-hydroxybutyric acid, palmitoylCoA, acetone
D. Acetone, β-hydroxybutyric acid, acetyl-CoA
E. β-hydroxybutyric acid, 3-aminopentanic acid, acetoacetate

18. The woman, age 40, complained of thirst and enhanced appetite. She was delivered to the endocrine clinic with diagnose of diabetes mellitus. Which pathologic components can be identified during the laboratory analysis?
A. Glucose, ketone bodies
B. Protein, ketone bodies
C. Protein, creatine
D. Bilirubin, urobilin
E. Blood

19. Which ketone body is a precursor in the biosynthesis of two other ketone bodies?
A. Acetoacetate
B. Acetone
C. β-hydroxybutyrate
D. Acetyl-CoA
E. Aminopentanedioic acid

20. One of the factors that cause obesity is the inhibition of fatty acids oxidation due to:
A. Low level of carnitine
B. Impaired phospholipid synthesis
C. Excessive consumption of fatty foods
D. Choline deficiency
E. Lack of carbohydrates in the diet

21. A baby, age 1, was admitted to the clinics with the features of muscle impairment. The observation revealed the deficiency of carnitine in muscles. Which process is impaired?
A. Fatty acid transport to mitochondria
B. Ca level regulation in mitochondria
C. Substate phosphorylation

D. Uric acid utilization
E. Actin and myosin synthesis

22. A 39-year-old female patient with a history of diabetes was hospitalized in a precomatose state for diabetic ketoacidosis. This condition had been caused by an increase in the following metabolite level:

A. Acetoacetate
B. Citrate
C. Alpha-ketoglutarate
D. Malonate
E. Aspartate

23. Choose the correct sequence of ketone bodies biosynthesis:

A. Acetyl-CoA + acetyl-CoA → acetoacetylCoA → beta-hydroxy-beta-methyl-glutarylCoA → acetoacetate → beta-hydroxybutyrate and acetone
B. Acetyl-CoA + acetyl-CoA → acetoacetylCoA → methylmalonyl-CoA → betahydroxybutyrate and acetone
C. Acetyl-CoA + acetyl-CoA → succinylCoA → acetoacetate → beta-hydroxybutyrate and acetone
D. Acetyl-CoA + acetyl-CoA → propionylCoA → succinyl-CoA → acetoacetate → beta-hydroxybutyrate and acetone
E. Acetyl-CoA + malonyl-CoA → acetoacetyl-CoA → beta-hydroxy-betamethyl-glutaryl-CoA → acetoacetate → betahydroxybutyrate and acetone

24. Specify the sequence of reactions of β- oxidation of fatty acids:

A. Activation under the participation of ATP, carboxylation to form malonyl-CoA, $NADH_2$-hydrogenation, dehydration, FADdehydrogenation, condensation
B. Hydration, $FADH_2$ hydrogenation, condensation, carboxylation, hydrolysis to release acetyl-CoA
C. Enabling the participation of ATP and the HS-CoA, FAD-dehydrogenation, thiolysis to form acetyl-CoA
D. Condensation, ATP phosphorylation, isomerization, hydration, decarboxylation
E. Dehydrogenation, dehydration, FADdehydrogenation, esterification

25. An aerobic oxidation of substrates is specific for the cardiac muscle. What is the main source of energy in the myocardium?

A. Fatty acids
B. Triacylglycerols
C. Glycerol
D. Glucose
E. Amino acids

26. One of the diabetes mellitus symptoms is the increase in ketone bodies blood level. This results in metabolic acidosis. What substance are ketone bodies synthesized from?

A. Acetyl-CoA
B. Methylmalonyl-CoA
C. Succimyl-CoA
D. Propionyl-CoA
E. Malonyl-CoA

27. The patient diagnosis displayed the following indices: sugar blood level – 16 mmol/l, ketone bodies blood level – 0.52 mmol/l, daily diuresis – 10 l/day, sugar urinal contents – 2%, urinal ketone bodies – +++. What is the disease?

A. Diabetes mellitus
B. Kidney diabetes
C. Diabetes incipidum
D. Steroid diabetes
E. Kushing's disease

References:

1. Gubsky Yu. Biological chemistry: textbook / edited by Yu. Gubsky. – Vinnytsia : Nova Knyha, 2018. – 2nd edition. – 488 p.
2. Lecture materials.
3. Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – 799 p.
4. Textbook «Biological chemistry. Collection of test tasks» for English-speaking students of higher medical educational institutions of the Ministry of Public Health of Ukraine. – Poltava, 2018. – 148 p.

TOPIC №19.
Biosynthesis of fatty acids

1. Objective: To learn reactions of biosynthesis of fatty acids.

2. Actuality of the theme: Biosynthesis of fatty acids from glucose and their subsequent use for the synthesis of triacylglycerols – is the main way of accumulation of energy because of most cells the ability to form glycogen is limited. Food glucose, the amount of which exceeds the energy needs of the body, easily converted into fatty acids in adipocytes of adipose tissue, hepatocytes, epithelial cells of the mammary gland during lactation.

3. Specific aims:

✓ To know metabolic sources of fatty acid synthesis, acetyl-CoA shuttle transport mechanism from mitochondria into the cytosol, the formation of malonyl-CoA and biotinrole in this process.

✓ To know enzymes, coenzymes, mechanism and regulation of biosynthesis of saturated fatty acids and unsaturated fatty acids.

✓ To interpret biosynthesis of long chain fatty acids and regulation of biosynthetic process on the level of acetyl-CoA-carboxylase and fatty acid synthetase.

4. Reference card for the separate study of educational literature for the lesson preparation

Questions:	References:
1. **Biosynthesis of long chain fatty acids:** ✓ localization of biosynthesis of long chain fatty acids; ✓ metabolic sources for biosynthesis of fatty acids; ✓ stages in synthesis of saturated fatty acids; ✓ characteristic of the synthetase of long chain fatty acids, the significance of acyl transporting protein and biotin; ✓ sources of $NADPH_2$ for biosynthesis of long chain fatty acids; ✓ the sequence of enzymatic reactions in biosynthesis of long chain fatty acids ✓ regulation of biosynthetic process on level of acetyl-CoA-carboxylase and fatty acid synthetase; ✓ elongation of carbon chain of fatty acids.	1. Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 297–302. 2. Gubsky Yu. Biological chemistry: textbook / edited by Yu. Gubsky. – Vinnytsia: Nova Knyha, 2018. – 2nd edition. – P. 193–196.

5. Tasks for independent work and self-control

5.1. How does acetyl-CoA transfer from mitochondria to cytoplasm for fatty acid synthesis? Name the enzymes that are required.

5.2. Write the reaction, name enzyme and coenzyme of malonyl-CoA synthesis.

5.3. Describe fatty acid synthase multienzyme complex.

5.4. Write the overall reaction of the synthesis of palmitic acid (palmitate).

5.5. What sources of NADPH for fatty acid synthesis?

5.6. Fill in the chart:

Pathway	Regulatory enzyme	Activators	Inhibitors
Fatty acid synthesis	Acetyl-CoA carboxylase		
β-Oxidation	Carnitine acyltransferase I		

5.7. Describe dietary regulation of fatty acids metabolism. Indicate the influence of:

1) high-carbohydrate diet,

2) high-fat or low-fat diet,

3) starvation.

5.8. What unsaturated fatty acids can synthesize in mammals?

5.9. What fatty acids are essential? Why?

6. Individual independent students work

1. The biological significance of polyunsaturated fatty acids and their synthesis in the body.

Practice protocol №19 «____» _____ 20___

Experiment. Determination of iodine number.

Principle. Determination of iodine number is based on the ability of unsaturated fatty acids attach iodine on the location cleavage of double bonds. Iodine number – is the number of grams of iodine in the acceding 100 grams of fat. By iodine number is possible determine the type of fat.

Method.

№	Reactants, the sequence of addition	Flask (for 500ml)	
		№ 1 (test)	№ 2 (control)
1	A sample of fat, grams	0.1	-
2	Ethanol, ml	5	5
3	Iodine alcohol sol. (CN=0.1 mol), ml	10	10
4	H_2O, ml	till 200 ml	till 200 ml
Intensively mix. Incubate 5 minutes at room temperature			
Titrate the excess of 0,1N iodine by sodium thiosulphate in the presence of starch			
5	Starch, drops	1–2	1–2
6	0.1 N $Na_2S_2O_3$ sol.	Titrate to a complete disappearance of blue colour in mixtures	
Result (volume thiosulfate), ml			

Calculation iodine number (x) by the formula:

$$X = \frac{(A - B) \times 12.692 \times 100}{0.1 \times 1000}$$

where:

A – the volume of thiosulphate, which went on titration of control;

V – volume thiosulfate, which went on titration of test tube;

12.692 – iodine (mg), which used for titration 1 ml of 0.1 N $Na_2S_2O_3$ sol.;

100 – recalculation for 100 grams of fat;

0.1 – a sample of fat in grams;

1000 – conversion factor mg of iodine in grams

Physical and chemical constants of some lipids

Fat	The refractive index	Iodine number
Fat of human	1.452–1.457	62.5–73.3
Butter	1.475–1.476	26-38
Sunflower oil	1.475–1.476	118-120
Fish Oil	1.475–1.485	150-175
Castor oil	1.447–1.478	31-91

Explain the results, draw a conclusion.

Result:

Conclusion:

The report is checked up _____

(The signature of the teacher, date)

Examples of Krock-1 tests

1. Choose the main stages of the biosynthesis of saturated fatty acids:

A. Carboxylation of acetyl-CoA, condensation of malonyl-CoA with acetyl-CoA, followed by alternation of reduction of keto acid with $NADPH_2$ and condensation of fatty acid with malonyl-CoA

B. Condensation of acetyl-CoA, carboxylation, hydration, phosphorylation, condensation with acetyl-CoA

C. Condensation of acetyl-CoA molecules, decarboxylation, hydration, phosphorylation, condensation with malonyl-CoA

D. Condensation of mevalonic acid and acetyl-CoA, dehydration, reduction of $NADH_2$, further condensation with acetyl-CoA

E. Condensation of acetoacetyl-CoA and malonyl-CoA, decarboxylation, hydration, dehydrogenation involving NADP, condensation with oxaloacetate

2. Acetyl-CoA carboxylase reaction is a rate limiting in the control of the biosynthesis of fatty acids. Select the molecular mechanisms controlling the activity of acetyl-CoA carboxylase:

A. Allosteric regulation, covalent modification, changing of the synthesis of acetyl-CoA carboxylase

B. Reconstruction of operon, proteolysis, modification of the gene encoding the information about the structure of acetyl-CoA carboxylase

C. Changing of the penetration of gene, reversible inhibition, changes in the activity of the promoter region of the operon

D. Uncompetitive inhibition, partial proteolysis, decarboxylation of histidine residues in the structure of acetyl-CoA carboxylase

E. Irreversible inhibition, modification of the primary structure of the amino acid sequence of acetyl-CoA carboxylase

3. Specify the conditions required for the biosynthesis of fatty acids:

A. Acetyl-CoA, vitamins H, B_3, PP, ATP, $NADPH_2$, enzymatic Linen's complex

B. Vitamins B_2, C, B_1, glycerol, GTP, decarboxylase, dehydrogenases

C. Vitamins A, B, UTP, mevalonylpyrophosphate, alpha-ketoglutarate

D. Ethanolamine, methionine, vitamins P, E, UTP

E. Lipoic acid, squalene, choline, sphingosine

4. The increasing of the hydrocarbon chain in the palmitate synthase complex is a cyclical process and requires a sufficient amount of reducing equivalents in the form $NADPH_2$. Specify the number of $NADPH_2$ consumed in one cycle of the complex:

A. 2 molecule

B. 1 molecule

C. 3 molecules

D. 4 molecule

E. 6 molecules

5. Select the product of the key reaction of the fatty acid biosynthesis, vitamins and hormones that regulate the process:

A. Malonyl-CoA, insulin, H, PP, B_3

B. Beta-HMG-CoA, somatotropin, B_6

C. Mevalonic acid, norepinephrine, B_1, B_2

D. Mevalonylpyrophosphate, adrenaline, B_{12}, C, P

E. Phosphoric acid, cortisol, B_2, A, E

6. Specify a substrate that is transported from the mitochondria to the cytosol by means of the shuttle system and it is a direct donor of acetyl-CoA in the biosynthesis of fatty acids:

A. Citrate

B. Malonyl-CoA

C. Acetoacetyl-CoA

D. Methylmalonyl-CoA

E. Palmitoyl-CoA

7. It is found that the active form of dicarboxylic acid is the donor of two-carbon fragments in the biosynthesis of fatty acids. Name it:

A. Malonyl-CoA
B. Methylmalonyl-CoA
C. Acetyl-CoA
D. Stearoyl-CoA
E. Palmitoyl-CoA

8. Formation of malonyl-CoA from acetyl-CoA is a key reaction in the biosynthesis of fatty acids. Specify the enzyme that catalyzes this reaction:

A. Acetyl-CoA carboxylase
B. Malonyl-CoA decarboxylase
C. Malonyl-CoA hydratase
D. Acetyl-CoA dehydrogenase
E. Acetyl-CoA decarboxylase

9. Which of the following substances is a donor of reduction equivalents in the biosynthesis of palmitate?

A. $NADPH_2$
B. $FADH_2$
C. $NADH_2$
D. FMN
E. THF

10. It is known that fatty acid synthase is a multienzyme complex. What is called?

A. Linen
B. Knoop
C. Watson-Crick
D. Krebs
E. Krebs-Henseleit

11. Acyl carrier protein has two SHcontaining binding sites. Indicate their origin:

A. Cysteine residue, 4-phosphopantetheine
B. HS-CoA, methionine
C. Cysteine residue, dephosphopantetheine
D. Carboxybiotin, cysteine residue
E. Methionine, 4-phosphopantetheine

12. Citrate shuttle system is used for the transport of acetyl-CoA from the mitochondria into the cytosol. Select an enzyme which directly participates in the release of cytosolic acetyl-CoA:

A. Citrate lyase
B. Citrate dehydrogenese
C. Isocitrate dehydrogenese
D. Triacylglycerol lipase
E. Malate dehydrogenese

13. Oleic acid pool which is more than 50% of the total fatty acid composition of the human body formed by the system of:

A. Fatty acid desaturase
B. Dehydrogenation of fatty acids
C. Carboxylation of fatty acids
D. Elongation of fatty acids
E. Deamination of fatty acids

14. Specify the stage of the pentose phosphate pathway which produces the greatest number of $NADPH_2$, used for the fatty acids synthesis:

A. Oxidation stage
B. Substrate phosphorylation
C. Formation of phosphotriose
D. Glycogenolysis
E. Formation of glucuronic acid

15. Palmitic acid is a precursor in the formation of long-chain fatty acids (C_{18}-C_{24}). Two-carbon fragments connected to the palmitic acid. Specify the donors of twocarbon fragments:

A. Malonyl-CoA, acetyl-CoA
B. Methylmalonyl-CoA, acetyl-CoA
C. HS-CoA, acetyl-CoA
D. Stearoyl-CoA, carboxybiotin
E. Palmitoyl-CoA, acetyl-CoA

16. It is known that the activity of the key enzyme of fatty acid synthesis – acetyl-CoA carboxylase is regulated at a molecular level by a covalent modification. Specify the mechanism of this modification:

A. Phosphorylation-dephosphorylation
B. Proteolysis
C. Deamination-amination
D. Methylation-demethylation
E. Acetylation-deacetylation

17. A sufficient amount of reducing equivalents in the form $NADPH_2$ is required for the synthesis of the fatty acids. What process plays a key role in the replenishing of $NADPH_2$ pool?

A. Pentose phosphate pathway
B. Gluconeogenesis
C. Glucuronate pathway
D. Glycogenolysis
E. Lipogenesis

18. Specify a key metabolite that inhibits fatty acid biosynthesis reactions by the mechanism of allosteric feedback inhibition (negative feedback):

A. Palmitoyl-CoA
B. Malonyl-CoA
C. HS-CoA
D. Acetyl-CoA
E. Methylmalonyl-CoA

19. Specify hormone that activates the biosynthesis of fatty acids:

A. Insulin
B. Glucagon
C. Adrenaline
D. Growth hormone
E. Cortisol

20. The key reaction of fatty acid synthesis is production of malonyl-Coa. What metabolite is the source of malonyl-CoA synthesis?

A. Acetyl-CoA
B. Citrate
C. Succinyl-CoA
D. Malonate
E. Acyl-CoA

21. The biological role of polyunsaturated fatty acids is as follows:

A. Structure of biological membranes; they are a source of synthesis of prostaglandins and they inhibit the deposition of cholesterol in the vascular wall
B. They regulate blood pressure, activate lipid peroxidation
C. They stabilize unsaturated bonds in the structure of vitamin A and activate lipolysis
D. They involved in the synthesis of phospholipids to inhibit cholesterol esterification
E. They contribute to the absorption of fat-soluble vitamins, inhibit lipolysis and stimulate the synthesis of cholesterol

22. In addition to the fact that arachidonic acid performs an important structural function, it is still a precursor in the synthesis of biologically active substances. Indicate them:

A. All of them
B. Leukotrienes
C. Thromboxanes
D. Prostacyclin
E. Prostaglandin

References:

1. Gubsky Yu. Biological chemistry: textbook / edited by Yu. Gubsky. – Vinnytsia : Nova Knyha, 2018. – 2nd edition. – 488 p.
2. Lecture materials.
3. Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – 799 p.
4. Textbook «Biological chemistry. Collection of test tasks» for English-speaking students of higher medical educational institutions of the Ministry of Public Health of Ukraine. – Poltava, 2018. – 148 p.

TOPIC №20.
Metabolism of complex lipids

1. Objective: to learn the processes of metabolism of phospholipids and sphingolipids.

2. Actuality of the theme: Unlike simple fats and fatty acids which are used as an energetic material, compound lipids carry out plastic functions and are the main components of biological membranes of cells. Infringement of an exchange of compound lipids is a basis of development of such diseases as steatosis of liver, an atherosclerosis (reduction of antiatherogenic α-lipoproteins of blood plasma). Genetically caused infringements of lysosomal enzymes synthesis of disintegration of complex lipids result in development of sphingolipidoses.

3. Specific aims:

✓ To interpret biochemical function of complex lipids in organism: their involvement in formation of structure and function of biological membranes, the role as precursors in biosynthesis of biologically active compounds of lipid nature.

✓ To interpret enzymatic reactions of synthesis of phospholipids and sphingolipids.

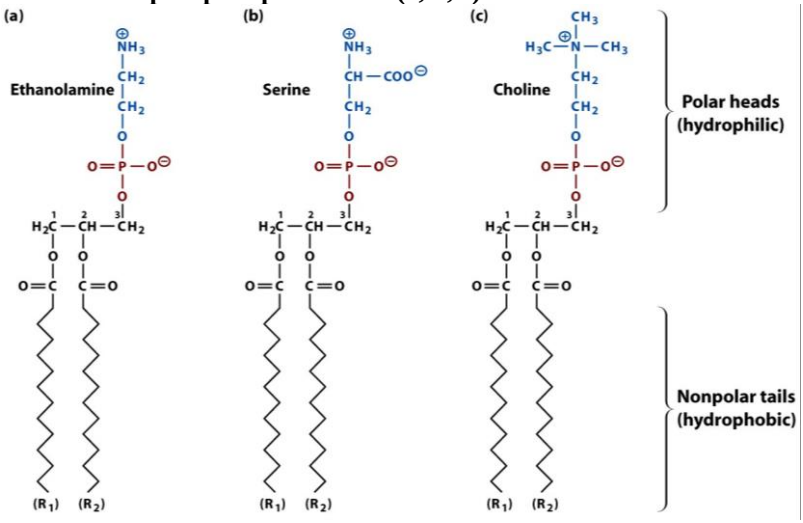
4. Reference card for the separate study of educational literature for the lesson preparation

Questions:	References:
1. **Metabolism of phospholipids:** ✓ biosynthesis of phospholipids; ✓ degradation of phospholipids; ✓ the role of LCAT in lecithin metabolism.	1. Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 303–307. 2. Gubsky Yu. Biological chemistry: textbook / edited by Yu. Gubsky. – Vinnytsia: Nova Knyha, 2018. – 2nd edition. – P. 200–201.
2. **Metabolism of sphingolipids.** Genetic anomalies of sphingolipid metabolism – sphingolipidoses. Lysosomal diseases	1. Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 307–309. 2. Gubsky Yu. Biological chemistry: textbook / edited by Yu. Gubsky. – Vinnytsia: Nova Knyha, 2018. – 2nd edition. – P. 201–206.

5. Tasks for independent work and self-control

5.1. Phospholipids are major components of cellular membranes. Glycerophospholipids are also components of blood lipoproteins, bile, and lung surfactant. Explain why they can fulfill these functions.

5.2. Name phospholipids bellow (a, b, c):



5.3. Glycerophospholipids are the source of the polyunsaturated fatty acids, particularly arachidonic acid.

What enzyme removes arachidonic acid from phospholipid molecule?

What significance of this fatty acid?

5.4. Complete the table (write the missed names, defective enzymes or symptoms of diseases):

Disease	Missing / defective enzyme	Major storage compound	Symptoms
	Sphingomyelinase	Sphingomyelins	Liver and spleen enlargement, mental retardation
Farber's disease		Ceramide	Painful and progressively deformed joints, skin nodules, death within a few years
Gaucher's disease	β-Glucosidase		Liver and spleen enlargement, erosion of long bones, mental retardation in infantile form only
Krabbe's disease	β-Galactosidase	Galactocerebrosides	
	Hexosaminidase A	Galactoside GM2	Mental degradation, blidness, death by age 3
Fabry's disease		Ceramide trihexoside	Skin rash, kidney failure, pain in lower extremities

5.5. Situational tasks:

a) A Jewish couple of Eastern European descent presents to the clinic for prenatal counseling after their only child died early in childhood. The family could not remember the name of the disorder but said it was common in their ancestry. Their first child was normal at birth, a slightly larger than normal head circumference, an abnormal «eye finding,» and a severe progressive neurologic disease with decreased motor skills and eventually death. The autopsy is consistent with Tay-Sachs disease.

What type of inheritance is this disorder?

What is the biochemical cause of the disorder?

b) Respiratory distress syndrome (RDS) accounts for 15–20% of neonatal mortality in Western countries. The disease affects only premature infants and its incidence is directly related to the degree of prematurity. RDS, in part, related to a deficiency in the synthesis of a substance known as lung surfactant. The major constituents of surfactant are dipalmitoylphosphatidyl choline, phosphatidyl glycerol, apoproteins (surfactant proteins: Sp-A, B, C), and cholesterol. These components of lung surfactant normally contribute to a reduction in the surface tension within the air spaces (alveoli) of the lung, preventing their collapse. The premature infant has not yet begun to produce adequate amounts of lung surfactant

Write the formula of dipalmitoylphosphatidyl choline, the major component of lung surfactant.

What clinical symptoms of RDS.

Practice protocol №20 «____» _____ 20___

Experiment. Determine the content of blood serum total lipids.

Principle. The method is based on the ability of the breakdown products of unsaturated lipids to form coloured compounds with phosphovanillyl reagents. The intensity of colour is proportional to the content of total lipids in blood serum.

Method. Add 0.1 ml of serum to a dry experimental test tube and gently add 2.9 ml of concentrated sulfuric acid. Then add 0.2 ml of water and 5.8 ml of concentrated sulfuric acid to the control test tube. Solutions in both test tubes should be thoroughly mixed with a glass rod and placed upon a boiling water bath for 10 min (caution!). Then both test tubes should be rapidly cooled to room temperature under running cold water. Add 0.2 ml from the experimental tubes and 0.4 ml of chilled mixture from the control tubes to other test tubes where the phosphovanillyl reagent is present: 3 ml should be in the experimental test tubes and 6 ml of the reagent should be in the control ones. After mixing with a glass rod, samples are placed in a dark place at room temperature for 45 minutes to provide conditions for changing their colour.

Photometry the experimental sample against the control one at PEC at 500-560 nm (green filter) in 0.5 cm thick cells. The content of serum total lipids X in g/L is calculated in accordance with the following formula:

$$X = (m \times 10{,}000 \times 3)/(0.2 \times 1{,}000)$$

where m is the amount of total lipid in a sample calculated according to the analytic curve (mg); 10,000 is the scaling factor for volume conversion per 1 liter of blood serum; 1,000 is the scaling factor for milligram-to-gram conversion; 3 is the total volume of the original mixture (0.1 ml serum plus 2.9 ml of concentrated sulfuric acid): 0.2 ml is a volume of mixture taken for the coloured reaction (ml).

Result:

Conclusion:

Clinical and diagnostic significance. The content of blood lipids (including triglycerides, phospholipids, cholesterol, fatty acids) attached to proteins (albumins) and as lipoproteins is an important diagnostic parameter. The normal content of serum total lipids is 4–8 g/L. High levels of lipids in the blood (hyperlipemia) as a physiological phenomenon are observed within 1–4 hours after consumption of lipid-rich food. Fasting levels of total lipids are usually lowered (hypolipemia). Elevated concentrations of lipids in the blood can be revealed in diabetes mellitus (up to 10–20 g/L), liver cirrhosis, atherosclerosis, obesity, coronary heart disease, lipoid nephrosis (kidney disease), acute hepatitis, pancreatitis; due to alcohol abuse.

The report is checked up _____

(The signature of the teacher, date)

Examples of Krock-1 tests

1. The methylation process is one of the stages is the endogenous synthesis of choline. Indicate vitamins, coenzyme forms of which are involved in this reaction:

A. Folic acid, B_{12}

B. B_2, PP

C. B_6, B_3
D. H, C
E. B_1, lipoic acid

2. It is possible to obtain phosphatidylcholine from phosphatidylethanolamine during the interaction with the following compound:

A. S-adenosyl methionine
B. Phosphatidic acid
C. Choline
D. CTP
E. Carboxybiotin

3. It is known that lipotropic factors are necessary for the synthesis of complex lipids in the body. Specify them:

A. All of them
B. Methionine
C. Polyunsaturated fatty acids
D. Vitamines B_6, B_{12}
E. Choline

4. A group of polyunsaturated fatty acids are used in the biosynthesis of the complex lipids. Which of them has four double bonds in its structure?

A. Arachidonic
B. Linoleic
C. Linolenic
D. Oleic
E. Palmitoleic

5. Specify a substance which is a donor of methyl groups in the synthesis of phospholipids:

A. Methionine
B. Ascorbic acid
C. Glucose
D. Glycerol
E. Citrate

6. Specify a substance which is a donor of methyl groups in the synthesis of phospholipids:

A. Methionine
B. DOPA
C. Cholesterol
D. Acetoacetate
E. Linoleic acid

7. The structure of phosphatidylcholine molecules includes:

A. Glycerol, fatty acids, choline, phosphoric acid
B. Choline, serine, mevalonic acid, methionine

C. Choline, cerebroside, sialic acid, galactose, phosphoric acid
D. Cholesterol, malonic acid, choline, mannose
E. Choline, sphingosine, lipoic acid, hexosamine and serine

8. A 2-year-old child suffers from neurological disorders since the birth. Objectively: the signs of blindness, macrocephaly. The child's condition progressively worsens. The doctor suspected Tay-Sachs disease. The activity of which enzyme should be investigated?

A. Hexosaminidase A
B. β-Galactosidase
C. Sphingomyelinase
D. Gglycogen phosphorylase
E. Glucocerebrosidase

9. A 5-year-old child was diagnosed with Niemann-Pick disease. Medical examination revealed neurological disorders. Which lipids are accumulated in the central nervous system?

A. Sphingomyelin
B. Leukotriene
C. Gangliosides
D. Cerebrosides
E. Cholesterol

10. Niemann-Pick disease is a sphingolipidosis characterized by an accumulation of sphingomyelin in the brain, liver and spleen. Indicate the defect of which of the listed enzymes leads to this pathology:

A. Sphingomyelinase
B. Glucocerebrosidase
C. β-galactosidase
D. Arylsulfatase
E. Iduronidase

11. Sphingolipidoses are enzyme pathologies developed due to the defects in the synthesis of certain hydrolytic enzymes that are involved in the catabolism of sphingolipids. What type of diseases includes this pathology?

A. Lysosomal
B. Mitochondrial
C. Secondary-induced
D. Heteronuclear
E. X-linked recessive

12. A 2-year-old child suffering from a congenital defect of the enzyme glucocerebrosidase was revealed hepato- and splenomegaly and the symptoms of the central nervous system disorders. What is the pathology?

A. Gaucher disease
B. Gierke disease

C. Tay-Sachs disease
D. Gout
E. Maroto-Lamy syndrome

13. What additional sources of energy are necessary for the synthesis of complex lipids in the human body?

A. CTP
B. GTP
C. TPP
D. UTP
E. ATP

14. Indicate the coenzymes of the methylation process in the endogenous synthesis of choline:

A. NADP, $NADPH_2$
B. FAD, FMN
C. PLP, PMP
D. Tetrahydrofolate, methylcobalamin
E. HS-CoA, 4-phosphopantetheine

15. One of the stages of the endogenous synthesis of choline is the process of decarboxylation of serine with the formation of ethanolamine, which is controlled by vitamin B_6. What are coenzyme forms of vitamin B_6?

A. PLP, PMP
B. FAD, FMN
C. NADP, $NADPH_2$
D. Cobalamin, THF
E. HS-CoA, 4-phosphopantetheine **16. One of the stages of the endogenous synthesis of choline is the process of the decarboxylation of serine with the formation of ethanolamine. The coenzyme forms of which vitamin are involved in this reaction?**

A. B_6
B. B_2
C. B_{12}
D. C
E. B_1

17. What substance is a common intermediate metabolite in the biosynthesis of phospholipids and triacylglycerols?

A. Phosphatidic acid
B. Diacylglycerol
C. 1,3-biphosphoglycerate

D. Mevalonate
E. Glycerophosphate

18. Arachidonic acid is an essential component of the diet. It serves as a precursor of biologically active substances. Which substance is synthesized from arachidonic acid?

A. Prostaglandine E_1
B. Ethanolamine
C. Choline
D. Norepinephrine
E. Triiodothyronine

19. Hepatic steatosis is developed in experimental rats being on protein-free diet due to the deficiency of methylating agents. The biosynthesis of which metabolite is disturbed in rats?

A. Choline
B. DOPA
C. Cholesterol
D. Acetoacetate
E. Linoleic acid

20. Examination of cell culture got from a patient with lysosomal pathology revealed accumulation of great quantity of lipids in the lysosomes. What of the following diseases is this disturbance typical for?

A. Tay-Sachs disease
B. Phenylketonuria
C. Galactosemia
D. Gout
E. Wilson disease

21. Synthesis of phospholipids is disturbed in fatty liver. Which of the below mentioned substances can stimulate the processes of methylation in synthesis of phospholipids?

A. Methionine
B. Vitamin C
C. Glucose
D. Glycerol
E. Citrate

22. Fat infiltration of liver is caused by impaired synthesis of phospholipids. What substance can enhance methylation processes in phospholipid synthesis.

A. Methionine
B. Ascorbic acid
C. Glucose
D. Glycerol
E. Citrate

23. Deficiency of linoleic and linolenic acids in an organism induces skin damages, hair loss, slow wound healing, thrombocytopenia, low resistance to infectious diseases. These symptoms are most likely to be caused by the disturbed synthesis of the following substances:

A. Eicosanoids
B. Interleukins
C. Interferons
D. Catecholamines
E. Corticosteroids

24. A 2-year-old child presents with acute psychomotor retardation, vision and hearing impairment, sharp enlargement of the liver and spleen. The child is diagnosed with hereditary Niemann-Pick disease. What genetic defect is the cause of this disease?

A. Sphingomyelinase deficiency
B. Glucose 6-phosphatase deficiency
C. Amylo-1,6-glucosidase deficiency
D. Acid lipase deficiency
E. Xanthine oxidase deficiency

25. Disruption of nerve fiber myelinogenesis causes neurological disorders and mental retardation. These symptoms are typical for hereditary and acquired alterations in the metabolism of:

A. Sphingolipids
B. Neutral fats
C. Higher fatty acids
D. Cholesterol
E. Phosphatidic acid

26. A 3-year-old girl with mental retardation has been diagnosed with sphingomyelin lipidosis (Niemann-Pick disease). In this condition synthesis of the following substance is disrupted:

A. Sphingomyelinase
B. Gangliosides
C. Ceramides
D. Glycosyltransferase
E. Sphingosine

27. Under a clinical picture a pediatrician has suspected the development of Tay-Sachs disease in a 2-year-old child. It is known that this sphingolipidosis is a similar in clinical manifestations with

gangliosidosis G_{M1}. The activity of what enzymes should be investigated for the differential diagnostics of the disease?

A. β-galactosidase and hexosaminidase A
B. Glucocerebrosidase and iduronsulfate sulfatase
C. Sphingomyelinase and heparan sulfate sulfatase
D. Arylsulfatase and glucocerebrosidase
E. Iduronidase and sphingomyelinase

28. A patient is diagnosed with glucocerebroside lipidosis (Gaucher's disease) that manifests as splenomegaly, liver enlargement, affected bone tissue, and neuropathies. What enzyme of complex lipid catabolism is deficient, thus causing this disease?

A. Glucocerebrosidase
B. Hyaluronidase
C. Sphingomyelinase
D. β-galactosidase
E. Hexosaminidase

References:

1. Gubsky Yu. Biological chemistry: textbook / edited by Yu. Gubsky. – Vinnytsia : Nova Knyha, 2018. – 2nd edition. – 488 p.
2. Lecture materials.
3. Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – 799 p.
4. Textbook «Biological chemistry. Collection of test tasks» for English-speaking students of higher medical educational institutions of the Ministry of Public Health of Ukraine. – Poltava, 2018. – 148 p.

TOPIC №21.
Biosynthesis and biotransformation of cholesterol. Pathology of lipid metabolism: steatorrhea, atherosclerosis, obesity

1. Objective: To learn the pathways of cholesterol metabolism and principal disorders of lipid metabolism. To interpret free radical reactions, mechanisms of lipid peroxidation and its significance in biological processes in normal conditions and in pathology.

2. Actuality of the theme: Disorders in cholesterol biotransformation processes cause several diseases, such as atherosclerosis, obesity et al. In this connection the investigation of lipid metabolism indexes is obvious for diagnostics and treatment of different diseases.

Production of free radicals and products of peroxide oxidation of lipids are normal metabolic processes. In normal conditions the level of products of peroxide oxidation of lipids is regulated by antioxidative enzymatic system. Disorders in relations between activity of pro- and anti- oxidative systems is manifested in form of different pathological features. This makes necessary the investigation an evaluation of oxidative stress indexes in pathology.

3. Specific aims:

- ✓ To interpret stages of cholesterol biosynthesis.
- ✓ To explain regulation of cholesterol production in human body.
- ✓ To analyze pathways of cholesterol biotransformation: esterification, synthesis of bile acids, steroid hormones, vitamin D_3, excretion of cholesterol from the body.
- ✓ To interpret pathology of lipid metabolism: atherosclerosis, diabetes mellitus, obesity, steatorrhea.
- ✓ To explain processes of lipid peroxidation under normal conditions and in pathology.
- ✓ To interpret regulation of free radical reactions in human body.

4. Reference card for the separate study of educational literature for the lesson preparation

Questions:	References:
1. **Biosynthesis of cholesterol in human body:** ✓ localization of the process and its significance; ✓ stages of cholesterol biosynthesis; ✓ enzymatic reactions of biosynthesis of mevalonic acid; ✓ regulation of cholesterol synthesis.	1. Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 308–312. 2. Gubsky Yu. Biological chemistry: textbook / edited by Yu. Gubsky. – Vinnytsia : Nova Knyha, 2018. – 2nd edition. – P. 207–209.

2.	**Pathways of cholesterol biotransformation** (esterification, production of bile acids and steroid hormones, synthesis of vitamin D_3, excretion from the body).	1. Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 313–315. 2. Gubsky Yu. Biological chemistry: textbook / edited by Yu. Gubsky. – Vinnytsia : Nova Knyha, 2018. – 2nd edition. – P. 210–211.
3.	**Atherosclerosis, mechanism of its development, role of genetic factors, hypercholesterolemia. Hypercolesterolemia in diabetes mellitus, myxoedema, obstructive jaundice, nephritic syndrome. Control of hypercholesterolemia**	1. Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P.315-317, 326–327. 2. Gubsky Yu. Biological chemistry: textbook / edited by Yu. Gubsky. – Vinnytsia : Nova Knyha, 2018. – 2nd edition. – P. 211–212.
4.	**Fatty liver (steatosis), lipotropic factors.**	Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 322–325.
5.	**Pathological processes which leads to the development of obesity.**	Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 325–326.
6.	**Lipid peroxidation and mechanisms of antioxidant enzymatic system action:** ✓ lipid peroxidation under normal conditions and in pathology. ✓ regulation of free radical reactions in human body. ✓ characterization of prooxidants and antioxidants, their significance in peroxide oxidation of lipid.	Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 656–657.

5. Tasks for independent work and self-control

5.1. Fill in the chart «Reactions of initial stage of cholesterol synthesis»:

Enzymes	Substrates	Products	Type of reaction
Thiolase			
HMG-CoA synthase			
HMG-CoA reductase			

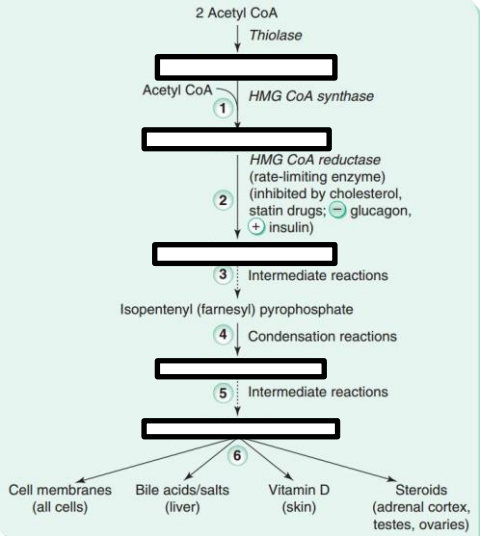
5.2. What tissues synthesize cholesterol?

5.3. What amount of cholesterol is synthesized per day in adults?

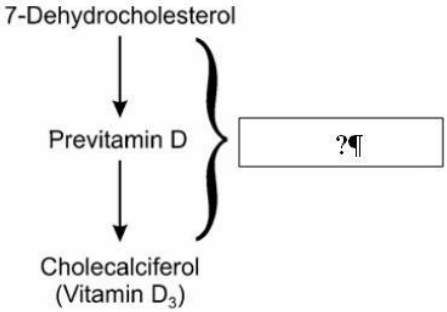
5.4. Cholesterol synthesis is controlled by feedback inhibition of the enzyme _____.

5.5. Describe the mechanism of cholesterol esterification and role of LCAT.

5.6. Rite the missed names of cholesterol synthesis metabolites on the scheme bellow:



5.7. Indicate the location of stage of vitamin D3 synthesis from cholesterol.



5.8. Fill in the chart «Lipid peroxidation»

Phase	Characteristic	Scheme of reactions

5.9. Situational tasks:

a) Cholesterol gallstone disease (cholelithiasis). Cholesterol cannot be broken down by mammalian cells into carbon dioxide and water. Removal from the body is thus dependent on transfer into the gut prior to excretion via the feces. There is a considerable flux of cholesterol from the liver into bile and then into the duodenum via the common bile duct. Bile supersaturated with cholesterol facilitates formation of cholesterol gallstones within the gall bladder. Gall stones occur in up to 20 % of the population of Western countries.

Cholesterol is present in high concentrations in bile, being solubilized in micelles. What components of bile keep cholesterol in micelles and prevent formation of cholesterol stones? Why can be their deficiency in the bile?

Describe the ways of cholelithiasis treatment. Administration of what drugs results in a gradual dissolution of cholesterol gallstones and excretion via the gut?

The elevated activity of blood alkaline phosphatase is a marker of partial blockage of the bile duct or cholestasis. Explain why?

b) Atherosclerosis is a complex process. Its main components are endothelial dysfunction, lipid deposition, and inflammatory reaction in the vascular wall. The key early event in atherosclerosis is the damage to the endothelium. This may be caused by excess of lipoproteins, hypertension, diabetes, or the components of cigarette smoke. The endothelium becomes more permeable to lipoproteins which move beneath the endothelial layer, in the underlying intima. Atherogenesis results in a gross disruption of the structure of the arterial wall and the formation of atherosclerotic plaque, which narrows the lumen of the affected artery. Clinically this may cause myocardial infarction (resulting from a complete blockage of coronary artery supplying the heart), stroke (a blockage of an artery supplying the brain), or peripheral vascular disease (a condition where narrowing of leg arteries leads to a characteristic pain on walking, known as intermittent claudication). Cardiovascular disease is presently the most frequent cause of death in the industrialized world.

What blood lipoproteins are the most atherogenic? Why?

List non-lipid cardiovascular risk factors. Explain their effects.

Excessive smoking elevates the risk of cardiovascular disease. Explain why.

Since arteriosclerosis is a multifactorial process, the effective cardiovascular prevention involves a comprehensive approach which combines lifestyle modification (smoking cessation, diet and exercise) with appropriate treatment of dyslipidemia, hypertension and diabetes. Indicate the dietary factors that can lower the plasma cholesterol and atherogenic lipoprotein levels. Explain their influence.

c) In a cleaner found fatty liver. The synthesis of what class of lipids in the liver is suppressed and which increases under these conditions?

How does vitamin like substanes choline acts in lipid metabolism in the liver? Write the structure.

Give examples of substances that prevent fatty liver.

Individual independent students work

1. Lipid peroxidation, its role under normal conditions and in pathology.

Practice protocol №21 «____» _____ 20___

Experiment 1. Determine the blood serum cholesterol concentration

A. by Ilc

Principle. Cholesterol at interaction with acetic anhydride at the presence of the concentrated sulfuric and acetic acids forms products of reaction of blue-green colour. Intensity of painting is directly proportional to quantity of cholesterol.

Ethero-bonded cholesterol of whey is 2/3 of general cholesterol.

Method.

№	Reactants, the sequence of addition	Standard tube	Test tube
1	The reagent (1 part of ice acetic acid, 5 parts of acetic anhydride and 1 part of concentrated sulfuric acid)	2 ml	2 ml
2	Standart solution of cholesterol (180 mg/100 ml)	0.1 ml	-
3	Serum	–	0.1 ml
Mix, incubate 20 minutes at room temperature.			
Samples eximane by photocolourimeter at a at a red optical filter (wavelength of 630–690 nm) against water			

Calculation:

$$C = E_{ex} / E_{st} \times C_{st},$$

where C – a concentration of cholesterol in the sample (mg/100 ml);

E_{ex} – an extinction of the experimental solution;

E_{st} – an extinction of the standart solution;

C_{st} – a concentration of cholesterol in the standart solution (180 mg/100 ml).

0.0258 – conversion rate per unit SI (mmol/l).

Result:

Conclusion:

B. by enzyme method

Principle. Free cholesterol is formed as a result of hydrolysis of cholesterol esters by cholesterol esterase. Cholesterol formed by hydrolysis from cholesterol esters and blood non-esterified cholesterol are oxidized by

atmospheric oxygen by cholesterol oxidase to form equimolar amounts of hydrogen peroxide. Peroxidase oxidizes the chromogenic substrates with the help of hydrogen peroxide to form a coloured compound whose colouration intensity is directly proportional to the blood cholesterol concentration. It is determined photometrically at the wavelength of 540 nm.

Method. Prepare three chemical test tubes (experimental, calibration, blank). Add 0.01 ml of serum to the experimental test tube, 0.01 ml of the calibration solution to the standard test tube, 0.01 ml of distilled water to the blank test tube. Take 1 ml of reagent and add it to the experimental, calibration and blank test tubes, respectively. Mix the content of test tubes and incubate them for 10 min at 37 °C or for 20 min at room temperature. Measure the extinction of the experimental and calibration test tubes against the blank one at a wavelength of 540 nm. Calculate the blood serum cholesterol concentration in accordance with the following formula:

$$C = E_{ex} / E_c \times 5.2$$

where

C – a concentration of cholesterol in the sample (mmol/L);

E_{ex} – an extinction of the experimental solution;

E_c – an extinction of the calibration solution;

5.2 – the cholesterol content in the calibration solution (mmol/L).

If the concentration of cholesterol exceeds 19.4 mmol/L, try to dilute the sample with isotonic sodium chloride solution and multiply the result by the dilution factor.

Clinical and diagnostic value: Exogenous cholesterol (0.3–0.5 g per day) comes from food and endogenous (0.8–2 g per day) is synthesized in the body. A lot of cholesterol is synthesized in the liver, intestine, and skin. Large amounts of cholesterol are present in the nervous tissue (20–30 g/kg). The concentration of cholesterol is determined in conjunction with other tests for the determination of hyperlipoproteinemia. Its serum concentration varies from 3.0 to 5.7 mmol/L in healthy individuals. Prolonged increased blood cholesterol concentrations contribute to atherosclerosis. Lipoproteins play a key role in pathogenesis of atherosclerosis. It has been established that atherosclerosis and related diseases develop when a significant increase in a plasma LDL fraction and, in many cases, VLDL is observed. It has been shown that chylomicrons cannot penetrate the vascular wall because of their large size, whereas HDL, LDL, and VLDL are partially able to do this. However, HDLs are the smallest lipoproteins, so that they can be easier removed from the vascular walls via the lymphatic system. In addition, HDLs have the highest protein and phospholipid content, so that they can be easier metabolized in the vascular wall and quickly removed from it

compared to cholesterol- and triglycerides-rich LDL and VLDL. The latter is referred to as atherogenic lipoproteins and can penetrate into the vascular wall from blood plasma and serve as the primary substrate causing atherosclerotic arterial damage. **Hypercholesterolemia** is observed in patients with hypertension, coronary heart disease, diabetes mellitus, obesity, jaundice, nephritis, nephrosis, syphilis, and hypothyroidism. The highest levels of cholesterol observed in genetic disorders of lipoprotein metabolism – family cholesterolemia. Secondary hypercholesterolemia occurs in liver disease, kidney disease, malignant pancreatic tumors, gout, hypertensive disease, obesity, diabetes. Reduced cholesterol concentrations (**hypocholesterolemia**) occur in tuberculosis, typhus, parenchymatous jaundice, hyperthyroidism, anemia, cancer cachexia, fever, some CNS disorders, and starvation.

Experiment 2. Qualitative reaction on bile acids (Petenkoffer reaction)

Principle. The reaction is based on formation of coloured products after condensation of bile acids with hydroxymethylfurfurol. The last is produced under the action of sulfuric acid upon fructose, which in turn is formed from sucrose due to its hydrolysis with sulfuric acid.

Method.

1. Take a clean dry tube.
2. Add 10–15 droplets of bile.
3. Add ~5 droplets of a fresh 20% sucrose solution and mix it.
4. **Carefully** overlay 1 ml of conc. sulfuric acid without mixing of two fluids.
5. Observe the precipitation of bile acids in the borderline between two fluids (appearance of violet colour ring).
6. Mix it well and observe the appearance of red-violet colour.

Result:

Conclusion:

Clinical and diagnostic significance. The bile acids and their salts are detergents that emulsify fats in the gut during digestion. They are synthesized from cholesterol in the liver by a series of reactions. The bile acids are produced in liver, about 10–15 g daily. Bile acids include cholic, deoxycholic, chenodeoxycholic, lithocholic et al., which are excreted in bile in free state or conjugated with glycine or taurine. Bile acids emulsify lipids, activate lipase, are involved in absorption of fatty acids, form choleinic complexes, stabilize cholesterol. Deficiency of bile acids in intestines may be caused with liver diseases (occlusive jaundice, hepatitis, cirrhosis), disorders in gallbladder or bile ducts (cholelithiasis, tumors of bile ducts). In coprologic investigations decrease or complete absence of bile pigments in feces are observed, as well as high content of soaps, espetially calcium soaps.

Experiment 3. Investigation of antioxidative enzymatic system. Quantitative determination of catalase activity.

Principle. Catalase belongs to a class of oxido-reductases and catalyzes the transformation of hydrogen peroxide to water and oxygen:

$$2H_2O_2 \rightarrow 2H_2O + O_2$$

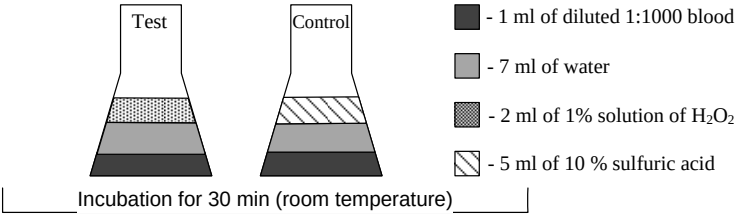
Biological significance of this enzyme consists in the defense of the body from harmful effect of hydrogen peroxide, which forms in the course of intracellular oxidation of different substances. According to chemical structure, catalase is a chromoprotein, containing heme as a prosthetic group. Molecular weight of catalase is 225–240 kda. It is widely distributed and extremely active enzyme.

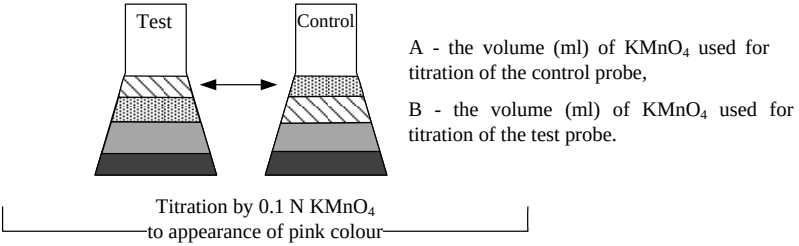
The determination of catalase is based on the estimation of hydrogen peroxide, which is decomposed by the enzyme in a distinct period of time. Hydrogen peroxide is titrated with permanganate, the reaction occurs according to the equation:

$$5H_2O_2 + 2KMnO_4 + 3H_2SO_4 \rightarrow K_2SO_4 + 2MnSO_4 + 8H_2O + 5O_2$$

Method. Take two flasks and do the following procedures:

1. Add 1 ml of diluted 1:1000 blood into both flasks then add 7 ml of water.
2. Add to the first flask (the test) 2 ml of 1% solution of hydrogen peroxide and 5 ml of 10% sulfuric acid to the other flask (the control).
3. Leave both flasks at room temperature for 30 min.
4. Then, add to the test flask 5 ml of 10% sulfuric acid, and to the control flask 2 ml of 1% solution of H_2O_2. Titrate the content of flasks by 0.1 N $KMnO_4$ to appearance of pink colour.





Calculate catalase activity according to formula:

$$C_f = (A - B) \times 1.7,$$

where

A – the volume of $KMnO_4$ solution, used for titration ofthe control probe,

B – the volume of $KMnO_4$ solution used for titration of the test probe.

Explain the results and draw a conclusion.

Result:

Conclusion:

Clinical and diagnostic significance. Catalase (E.C. 1.11.1.6.) is one of the most active and the most wide spread enzymes. It accelerates the breakdown of hydrogen peroxide and its derivatives. Normal value of catalase activity in blood is in ranges of 10–15 units/L. The estimation of catalase activity data on red blood cells count in blood is of low diagnostic value as catalase activity is tightly connected with red blood cells. In

clinical laboratory the catalase index determination is used, which is equal to catalase activity in units over number of: red blood cells (in ml/mm^3). In normal conditions this index is in $2 - 3 \times 10^{-6}$ ranges.

The report is checked up _____

(The signature of the teacher, date)

Examples of Krock-1 tests

1. A 65-year-old patient with signs of obesity and a risk of steatosis of the liver development is recommended a diet saturated with lipotropic factors. Specify it:

A. Methionine
B. Ascorbic acid
C. Glucose
D. Glycerin
E. Citrate

2. Specify the reaction of cholesterol biotransformation underlying the formation of its esters:

A. Esterification of cholesterol
B. Cholesterol hydroxylation
C. Oxidation of cholesterol
D. Amination of cholesterol
E. Reduction of the side chain

3. What is bile acid formed as a result of cholesterol hydroxylation at 3th, 7th and 12th position of carbon atoms called?

A. Cholic acid
B. Lithocholic acid
C. Chenodeoxycholic acid
D. Deoxycholic acid
E. Glycocholic acid

4. Which organs take part in the hydroxylation of cholesterol and formation of the active form of vitamin D_3?

A. Liver, kidneys
B. Kidneys, intestine
C. Liver, pancreas
D. Gonads, kidneys
E. Liver, intestine

5. Name a precursor in the biosynthesis of corticosteroids:

A. Pregnenolone
B. Estradiol

C. Corticosterone
D. Aldosterone
E. Androgens

6. What is normal concentration of cholesterol in the human blood serum?

A. 3.5–5.2 mmol / L
B. 5.2–6.5 mmol / L
C. 7.0–8.5 mmol / L
D. 2.0–3.5 mmol / L
E. 8–10 mmol / L

7. Specify the atherogenic lipoproteins:

A. LDL, VLDL
B. LDL, HDL
C. VLDL, chylomicrons
D. HDL, triacylglycerols
E. LDL, cholesterol, free fatty acids

8. What are the main substrates of lipid metabolism accumulated in the foam cells?

A. Cholesterol and its esters
B. Phospholipids
C. Triglycerides
D. Unsaturated fatty acids
E. Saturated fatty acids

9. What is a biological role of cholesterol?

A. Component of cell membranes, the source for the biosynthesis of physiologically active steroids
B. Component of biological membranes, involved in the synthesis of catecholamines
C. Synthesis of vitamin D_3, triacylglycerols
D. Synthesis of lipoproteins, vitamin D_3
E. Synthesis of phospholipids, eicosanoids

10. What are the most informative diagnostic criteria of atherosclerosis?

A. Concentration of the atherogenic lipoproteins, cholesterol and its esters
B. Concentration of HDL cholesterol, free fatty acids
C. Concentration of antiatherogenic lipoproteins, free fatty acids
D. Mevalonic acid concentration
E. Atherogenic coefficient

11. What are the antiatherogenic lipoproteins?

A. HDL
B. VLDL

C. LDL
D. Chylomicrons
E. Albumins

12. What is the key enzyme in the biosynthesis of cholesterol?
A. Beta-hydroxy-beta-methylglutarylCoA reductase
B. Monoamine oxidase
C. Citrate synthase
D. Acetyl-CoA carboxylase
E. Malonyl-CoA transferase

13. Mevalonic acid is a precursor of:
A. Cholesterol
B. Phospholipids
C. Saturated fatty acids
D. Sphingosine
E. Beta-hydroxy-methylglutaryl-CoA

14. Which class of lipoproteins containing apo-B-100 belongs to atherogenic ones?
A. LDL
B. HDL
C. IDL
D. Chylomicrons
E. Albumins

15. What is the main mechanism of cholesterol biosynthesis regulation?
A. Receptor-mediated
B. Competitive inhibition
C. Partial proteolysis
D. Lanosterol activation
E. Condensation of isoprenoid units

16. What is the most accepted theory to explain the mechanism of atherosclerosis development?
A. Autoimmune
B. Immune
C. Cholesterol
D. Systemic inflammatory response of the body
E. Damage of the endothelium

17. Which organ makes the greatest contribution to the endogenous biosynthesis of cholesterol?
A. Liver
B. Skin

C. Intestine
D. Kidneys
E. Connective tissue

18. What is the main pathway of cholesterol excretion from the body?

A. In the composition of bile through intestine
B. Sebaceous glands
C. Urinary excretion
D. Salivary glands
E. Conversion of 7-dehydrocholesterol

19. What is the most hydrophobic compound in the human body?

A. Triacylglycerols
B. Phospholipids
C. Cholesterol
D. Sphingosine
E. Mevalonic acid

20. What type of reaction is used for formation of squalene from isoprenoid units?

A. Condensation reaction
B. Isomerization reaction
C. Cyclization
D. Phosphorylation reaction
E. Reduction reaction

21. What key enzyme of cholesterol biosynthesis is inhibited under the using of antiatherogenic drugs (statins)?

A. Beta-hydroxy-methylglutaryl-CoA reductase
B. Monoamine oxidase
C. Citrate lyase
D. Acetyl-CoA carboxylase
E. Malonyl-CoA transferase

22. Which of the following compounds directly inhibits an expression of the HMGCoA reductase gene?

A. Cholesterol
B. HMG-CoA
C. Lanosterol
D. Isopentenyl pyrophosphate
E. Squalene

23. Fatty liver is prevented by lipotropic substances. Which of the below mentioned substances belongs to those?

A. Methionine
B. Cholesterol

C. Bilirubin
D. Glycine
E. Glucose

24. After having eaten fatty food, a patient feels nausea, flabbiness; with time passing steatorrhea appears. Cholesterol blood level – 9,2 mmol/l. What substance deficit causes the state:

A. Bile acids
B. Fatty acids
C. TAG
D. Phospholipids
E. Chylomicrones

25. A patient suffers from arterial hypertension, atherosclerotic impairment of vessels. Which lipid must they be recommended to reduce in their diet?

A. Cholesterol
B. Lecitine
C. Oleic acid
D. Monooleateglyceride
E. Phosphatidylserine

26. Colloidal properties of bile are disturbed in inflammatory processes of gallbladder. This can result in the formation of bile stones. Which substance crystallization is the basic cause of their formation?

A. Cholesterol
B. Urates
C. Chlorides
D. Oxalates
E. Phosphates

27. The data of subjective and objective diagnosis allow to suggest the occurrence of inflamation process in the patient gall-bladder, the disturbance of bile colloidal properties and the possibility of bile stones formation. What may cause the bile stones formation?

A. Cholesterol
B. Urates
C. Oxalates
D. Chlorides
E. Phosphates

28. The preventive radioprotector was given to a worker of a nuclear power station. What mechanism from the below mentioned is considered to be the main mechanism of radioprotection?

A. Inhibition of free radicals formation
B. Activation of oxidation reactions

C. Prevention of tissue's hypoxia
D. Increasing of tissue blood supply
E. Increasing of respiration

29. Steatosis is caused by accumulation of triacylglycerols in hepatocytes. One of the mechanisms of this disease is to reduce the utilization of neutral fat VLDL. What lipotropic substances prevent the steatosis development?

A. Methionine, B_6, B_{12}
B. Arginine, B_2, B_3
C. Alanine, B_1, PP
D. Valine, B_3, B_2
E. Isoleucine, B_1, B_2

30. Due to the blockage of the common bile duct (which was radiographically confirmed), the biliary flow to the duodenum was stopped. We should expect the impairment of:

A. Fat emulsification
B. Protein absorption
C. Carbohydrate hydrolysis
D. Secretion of hydrochloric acid
E. Salivation inhibition

31. A patient presents with steatorrhea. This disorder can be linked to disturbed supply of the intestine with the following substances:

A. Bile acids
B. Carbohydrates
C. Tripsin
D. Chymotrypsin
E. Amylase

References:

1. Gubsky Yu. Biological chemistry: textbook / edited by Yu. Gubsky. – Vinnytsia : Nova Knyha, 2018. – 2nd edition. – 488 p.
2. Lecture materials.
3. Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – 799 p.
4. Textbook «Biological chemistry. Collection of test tasks» for English-speaking students of higher medical educational institutions of the Ministry of Public Health of Ukraine. – Poltava, 2018. – 148 p.

TOPIC №22.
Studies on metabolism of amino acids (deamination, transamination, decarboxylation)

1. Objective: To learn the general pathways of amino acids metabolism, to make an acquaintance with methods of identification of amino acid metabolites, to interpret obtained results.

2. Actuality of the theme: Protein metabolism plays the most important role in general whole body metabolism. The competence and understanding of general pathways of amino acids transformations, their metabolic intermediates, determination of activity of enzymes, participating in these processes are criteria for evaluation of protein metabolism.

3. Specific aims:

✓ To determine the aminotransferases activity in blood serum.

✓ To evaluate the obtained results and draw the conclusions on possibilities of their application in clinical medicine.

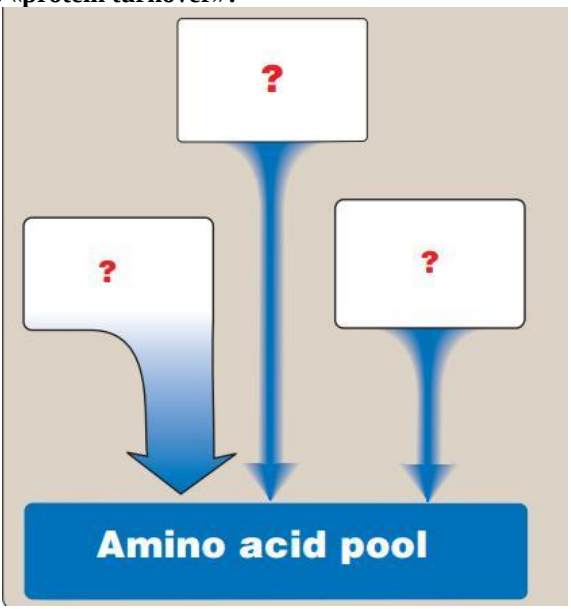
4. Reference card for the separate study of educational literature for the lesson preparation

Questions:	References:
1. **Pathways of formation and maintainance of free amino acid pool in human body. General pathways of free amino acid turnover.**	1. Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 330–332. 2. Gubsky Yu. Biological chemistry: textbook / edited by Yu. Gubsky. – Vinnytsia: Nova Knyha, 2018. – 2nd edition. – P. 213–216.
2. **Transamination of amino acids:** ✓ mechanism of transamination (role of pyridoxalphospate); ✓ examples of reactions of transamination; ✓ aminotransferases, their localization in tissues and organs; ✓ clinical diagnostic significance of determination of aminotransferases activity.	1. Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 332–333. 2. Gubsky Yu. Biological chemistry: textbook / edited by Yu. Gubsky. – Vinnytsia: Nova Knyha, 2018. – 2nd edition. – P. 216–219.
3. **Deamination of amino acids:** ✓ mechanism of oxidative deamination; ✓ oxidases of D- and L- amino acids, their enzymatic activity and specificity;	1. Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P.334–335. 2. Gubsky Yu. Biological chemistry: textbook / edited by Yu. Gubsky. – Vinnytsia: Nova Knyha, 2018. – 2nd edition. – P. 220–221.

	✓ glutamate dehydrogenase (equation of reaction, structure of GDH, regulation of its activity).	
4.	**Decarboxylation of amino acids:** ✓ production of biogenic amines (GABA, histamine, setrotonin, dopamine); ✓ decarboxylation of amino acids in putrifaction of proteins in intestines; ✓ oxidation of biogenic amines.	1. Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 375-377. 2. Gubsky Yu. Biological chemistry: textbook / edited by Yu. Gubsky. – Vinnytsia: Nova Knyha, 2018. – 2nd edition. – P. 221-222.

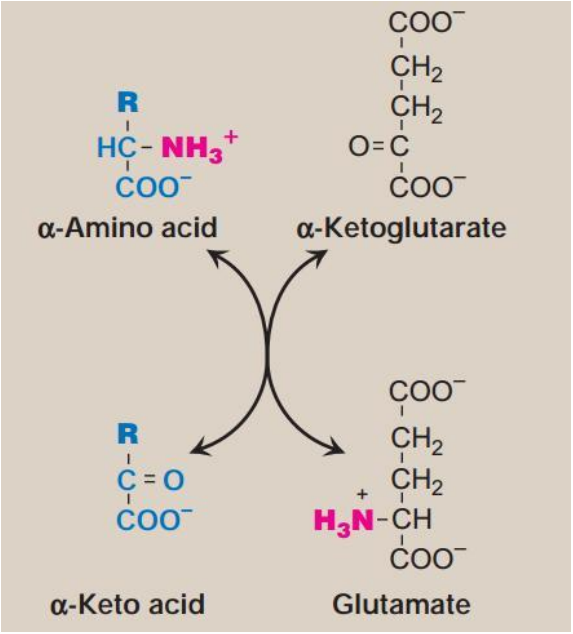
5. Tasks for independent work and self-control

5.1. What are the three inputs to the amino acid pool shown? What is «protein turnover»?

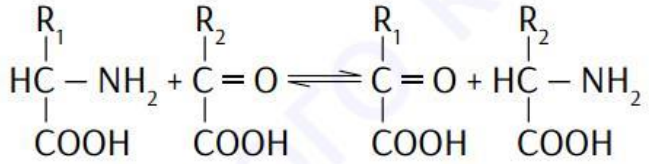


5.2. What does it mean for an individual to be in N balance? Positive N balance?

5.3. What is the general name of the enzymes that catalyze the reversible transfer of amino groups from one carbon skeleton to another, as shown? What vitamin is the source of the coenzyme used in the reaction?



5.4. Transamination involves the transfer of an α-amino group from an amino acid to an α-keto acid to form a new amino acid and a new α-keto acid:



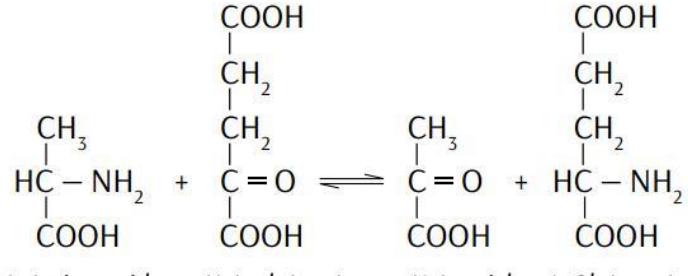
The enzymes that catalyze these reactions are called aminotransferases, or transaminases.

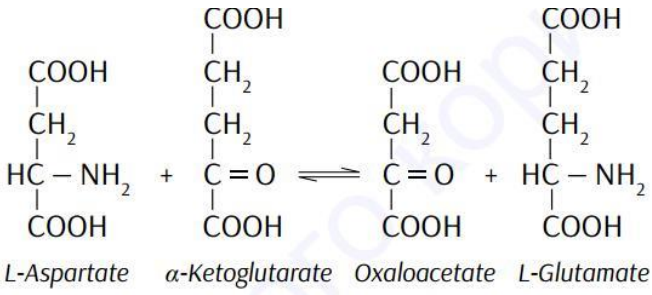
Some physiologically essential aminotransferases are:

Alanine aminotransferase (ALT), also known as glutamic-pyruvic transaminase (GPT);

Aspartate aminotransferase (AST), also known as glutamic-oxaloacetic transaminase (GOT).

Complete the reactions (write names of enzymes):





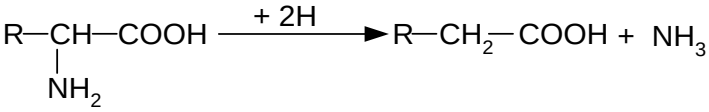
5.5. What is the primary fate of Glu during periods of amino acid catabolism?

5.6. Which set of clinical findings in blood is more suggestive of liver disease?

A. ↑AST, ↑ALT, ↑bilirubin

B. ↑AST, ↔ALT, ↔bilirubin

5.7. Name types of deamination of amino acids (choose correct name):



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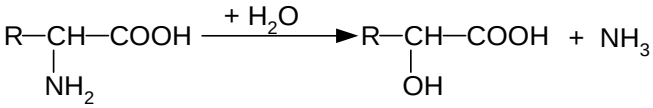

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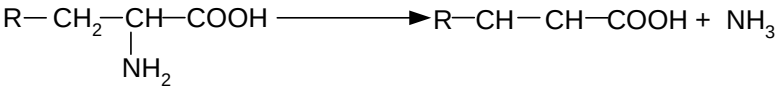

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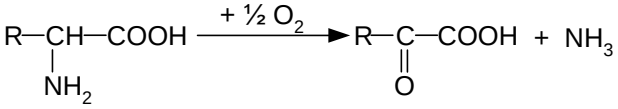


4

A. Oxidative deamination
B. Reductive deamination
C. Hydrolytic deamination
D. Intramolecular deamination

5.8. Complete the table «The biogenic amines and their functions»

Amino acid	Amine	Function(s)
Serine		
Glutamate		
Histidine		

Phenylalanine		
Tyrosine		
Tryptophan	1) 2) 3)	1) 2) 3)
Cysteine		

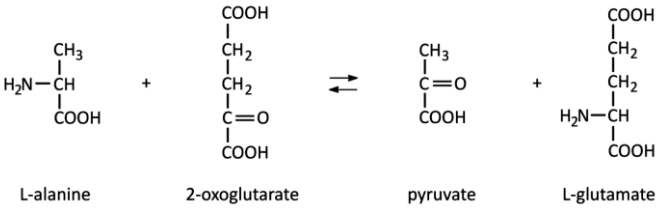
6. Individual independent students work

1. Clinical diagnostic significance of determination of amino-transferases activity.
2. The synthesis and breakdown of biogenic amines.

Practice protocol №22 «____» _____ 20___

Experiment. Determination of activity of alanine aminotransferase.

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



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

Method.

Pipette the solutions into the labelled test tubes according to the table:

	SAMPLE tube 1	**BLANK** tube 2
Substrate mixture	0.5 ml	0.5 ml
Saline	–	0.1 ml
Mix and preincubate at 37 °C for 5 minutes, then introduce:		
Sample (serum)	0.1 ml	–
Mix and incubate at 37 °C for exactly 30 minutes, then introduce:		
0.1% solution of dinitrophenylhydrazine	0.5 ml	0.5 ml
Mix and let stand at the laboratory temperature for 20 minutes, then introduce:		
sodium hydroxide (0.4 M)	5 ml	5 ml
Mix and incubate at the laboratory temperature for 10 minutes. Read the optical density of the sample at 510 nm against the blank.		

Calculate the quantity of pyruvate (in μg) according to the formula:

$$X = D / 0.09,$$

where

X – quantity of pyruvate in μg,
D – optical density.

The calculation of transaminase activity. 1 unit of alanine aminotransferase is such quantity of enzyme, which produce 1 μg of pyruvate under described conditions. The calculation of enzyme activity to micromoles of pyruvate, formed by 1 ml of serum in one hour is provided according to formula:

$$A = (X \times 2 \times 10) / 88,$$

where

X – quantity of pyruvate in μg,
2 – Coefficient for calculation for an hour of incubation,
10 – Coefficient for calculation for 1 ml of serum,
88 – Molecular weight of pyruvate.

Result:

Conclusion:

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

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

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

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

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

Normal values in blood serum: ALT – 0.1–0.7 μmoles/hr×ml,

AST – 0.1-0.45 μmoles/hr×ml.

The report is checked up _____

(The signature of the teacher, date)

Examples of Krock-1 tests

1. A patient with a cranial trauma manifests repeated epileptoid seizures. The biosynthesis of what biogenic amine is disturbed in this clinical situation?

A. GABA
B. Histamine
C. Adrenaline
D. Serotonin
E. Dopamine

2. In psychiatric practice biogenic amines and their derivatives are used for the treatment of certain diseases of the central nervous system. Name the substance which acts as an inhibitory mediator:

A. GABA
B. Histamine
C. Serotonin

D. Dopamine
E. Taurine

3. What would be the products for the transamination reaction in which glutamate and pyruvate are substrates?

A. Alfa-ketoglutarate and alanine
B. Glutamine and lactate
C. Alfa-ketoglutarate and acetyl-CoA
D. Alfa-ketoglutarate and lactate
E. Glutamine and acetyl-CoA

4. In result of decarboxylation of histidine biogenic amine is formed that has a powerful vasodilatation effect. Name it:

A. Histamine
B. Serotonin
C. Dioxyphenylalanine
D. Noradrenalin
E. Dopamine

5. Examination of a patient suffering from cancer of urinary bladder revealed a high rate of serotonin and 3-hydroxyanthranilic acid. It is caused by an excess of the following amino acid in the organism:

A. Tryptophan
B. Alanine
C. Histidine
D. Methionine
E. Tyrosine

6. A 7-year-old child was admitted to an emergency clinic in the state of allergic shock provoked by a wasp sting. High concentration of histamine was determined in the patient's blood. Which biochemical reaction leads to the production of this amine?

A. Decarboxylation
B. Hydroxylation
C. Dehydration
D. Deamination
E. Reduction

7. A patient diagnosed with carcinoid of bowels was admitted to the hospital. Analysis revealed high production of serotonin. It is known that this substance is formed from amino acid tryptophan. What biochemical mechanism underlies this process?

A. Decarboxylation
B. Deamination
C. Microsomal oxidation

D. Transamination
E. Formation of paired compounds

8. A patient complained about dizziness, memory impairment and periodical convulsions. It was revealed that these changes were caused by a product of decarboxylation of glutamic acid. Name this product:

A. GABA
B. Pyridoxal phosphate
C. Serotonin
D. ATP
E. Histamine

9. Pharmacological effects of antidepressants are connected with an inhibition of an enzyme catalyzing the oxidation of such biogenic amines as noradrenaline and serotonin in the mitochondrion of cerebral neurons. Which enzyme participates in this process?

A. Monoamine oxidase
B. Transaminase
C. Decarboxylase
D. Peptidase
E. Lyase

10. Biogenic amines, namely histamine, serotonin and dopamine are very active substances that affect markedly various physiological functions of the organism. What biochemical process is the principal pathway for biogenic amines production?

A. Decarboxylation of amino acids
B. Deamination of amino acids
C. Transamination of amino acids
D. Oxidation of amino acids
E. Reductive amination

11. Which of the following substances is an acceptor of amino groups in the reactions of amino acids transamination?

A. Alfa-ketoglutarate
B. Argininosuccinate
C. Lactate
D. Citrulline
E. Ornithine

12. An unusually active amine, a mediator of inflammation and allergy, appears via decarboxylation of histidine. Specify it:

A. Histamine
B. Serotonin
C. Dopamine

D. Gamma-Aminobutyrate
E. Tryptamine

13. The signs of skin depigmentation of a 19-year-old patient are caused by the disorder of melanin biosynthesis. The disturbance of the metabolism of what amino acid is it caused by?

A. Tyrosine
B. Tryptophan
C. Histamine
D. Proline
E. Lysine

14. Decarboxylation of glutamate results in the formation of the inhibitory transmitter in the central nervous system. Name it:

A. GABA
B. Glutathione
C. Histamine
D. Serotonin
E. Asparagine

15. A child manifests epileptic seizures caused by vitamin B6 deficiency. This is conditioned by the decrease of the gammaa-minobutyrate level in the nervous tissue which acts as an inhibiting neurotransmitter. The activity of which enzyme is decreased in this case?

A. Glutamate decarboxylase
B. Alanine aminotransferase
C. Glutamate dehydrogenase
D. Pyridoxal kinase
E. Glutamate synthase

16. Pyridoxal phosphate was prescribed to the patient according to the clinical indices. Which processes are corrected using this preparation?

A. Transamination and decarboxylation of amino acids
B. Oxidative decarboxylation of alfa-keto acids
C. Deamination of amino acids
D. Synthesis of purine and pyrimidine nucleotides
E. Protein synthesis

17. Psychopharmacological drugs with antidepressant effect inhibit the oxidative deamination of noradrenaline and serotonin in brain by means of inhibition of enzyme:

A. Monoaminoxidase
B. Cytochrome oxidase
C. Oxidase of L-amino acids
D. Glutamate dehydrogenase
E. Oxidase of D-amino acid

18. In diagnostics of an acute viral hepatitis estimation of the next enzymatic activity in the blood serum is the most valuable:

A. Alanine aminotransferase
B. Glutathion peroxidase
C. Creatine kinase
D. Amylase
E. Lactase

19. The transaminase activity sharply increases in the blood plasma of patients with hepatitis and myocardial infarction. Point the possible cause:

A. Damage of cellular membrane and entering enzymes to the blood
B. The increase of enzymes activity by hormones
C. Deficiency of pyridoxine
D. The increase of amino acids synthesis velocity in tissues
E. The increase of amino acids degradation velocity in tissues

20. In a child, consuming meal of plant origin for a prolong time, growth retardation, anemia, liver and kidney impairment were observed. The cause of such state is the deficiency in diet of the next nutrients:

A. Essential amino acids
B. Lipids
C. Carbohydrates
D. Mineral macroelements
E. Carotene

21. As a result of tryptophan hydroxylation in the presence of tryptophan-5-monooxygenase and next decarboxylation is produced:

A. Serotonin
B. Histamine
C. Dopamine
D. Melanin
E. Adrenalin

22. An important reaction for the biosynthesis of amino acid from carbohydrate intermediates is transamination which requires the cofactor:

A. Pyridoxal phosphate
B. Riboflavin
C. Niacin
D. Thiamin
E. Folic acid

23. What is a coenzyme of aminotransferases?

A. Pyridoxal phosphate
B. Thiamine pyrophosphate
C. Biotin
D. Riboflavin
E. Pantothenic acid

24. What is a coenzyme of the decarboxylases of amino acids?

A. Pyridoxal phosphate
B. Thiamine pyrophosphate
C. Biotin
D. Riboflavin
E. Pantothenic acid

25. Production of some toxic substances in large intestine occurs due to the decarboxylation of some amino acids. Indicate which substance is produced from ornithine:

A. Putrescin
B. Scatole
C. Indole
D. Cadaverine
E. Phenol

26. Negative nitrogen balance was found in 45-year-old man after prolonged vegetable diet. Which feature of diet is the cause of that state?

A. Insufficient amount of proteins
B. Excessive amount of water
C. Excessive amount of carbohydrates
D. Insufficient amount of fats
E. Insufficient amount of fats and proteins

27. In hepatitis, myocardial infarction the transaminase activity sharply increases in blood plasma of patients. Point the possible cause:

A. Damage of cellular membrane and entering enzymes to the blood
B. The increase of enzymes activity by hormones
C. Deficiency of pyridoxine
D. The increase of amino acids synthesis velocity in tissues
E. The increase of amino acids degradation velocity in tissues

28. Depressions and emotional insanities result from the deficit of noradrenaline, serotonin and other biogenic amines in the brain. Their concentration in the synapses may be increased by means of the antidepressants that inhibit the following enzyme:

A. Monoamine oxidase
B. Phenylalanine-4-monooxygenase
C. Diamine oxidase
D. D-amino-acid oxidase
E. L-amino-acid oxidase

29. Biogenic amines are used in clinical psychiatry to cure some CNS disease. Name the medicine of this group which is an inhibitory neurotransmitter.

A. γ-Aminobutyric acid
B. Dopamine
C. Histamine

D. Serotonin
E. Taurine

30. Biogenic amines – serotonin, dopamine etc. are very active substances, which influence on different physiological functions of the organism. Which process forms biogenic amines in organism tissues?

A. Amino acid decarboxylation
B. Amino acid deamination
C. Amino acid transamination
D. Amino acid oxidation
E. Reductive reamination

31. A 9-month-old infant is fed with artificial formulas with unbalanced vitamin B_6 concentration. The infant presents with pellagra-like dermatitis, convulsions, anemia. Convulsion development might be caused by the disturbed formation of:

A. GABA
B. Dopamine
C. Serotonin
D. Histamine
E. DOPA

32. A patient presents with dysfunction of cerebral cortex accompanied by epileptic seizures. He has been administered a biogenic amine synthetized from glutamate and responsible for central inhibition. What substance is it?

A. Gamma-amino butyric acid
B. Serotonin
C. Dopamine
D. Acetylcholine
E. Histamine

33. Glutamate decarboxylation results in formation of inhibitory transmitter in CNS. Name it:

A. GABA
B. Serotonin
C. Histamine
D. Glutathione
E. Asparagine

34. A female patient has scalded her hand with boiling water. The affected skin area became red, swollen and painful. This effect is caused by accumulation of the following substance:

A. Histamine
B. Lysine
C. Thiamine
D. Glutamine
E. Asparagine

35. It is known that the monoamine oxidase (MAO) enzyme plays an important part in the metabolism of catecholamine neurotransmitters. In what way does the enzyme inactivate these neurotransmitters (norepinephrine, epinephrine, dopamine)?

A. Oxidative deamination
B. Addition of an amino group
C. Removal of a methyl group
D. Carboxylation
E. Hydrolysis

36. Monoamine oxidase inhibitors are widely used as psychopharmacological drugs. They change the level of nearly all neurotransmitters in synapses, with the following neurotransmitter being the exception:

A. Acetylcholine
B. Serotonin
C. Dopamine
D. Adrenaline
E. Noradrenaline

37. It is knows that in catecholamine metabolism a special role belongs to monoamine oxidase (MAO). This enzyme inactivates mediators (noradrenalin, adrenalin, dopamine) by:

A. Oxidative deamination
B. Removing methyl groups
C. Adjoining amino groups
D. Hydrolysis
E. Carboxylation

38. A 24-year-old patient has been administered glutamic acid to treat epilepsy. Medicinal effect in this case occurs not due to glutamate itself, but due to the product of its decarboxylation:

A. γ-aminobutyric acid
B. Histamine 4-monooxygenase
C. Serotonin
D. Dopamine
E. Taurine

39. Depression and emotional disturbances result from the lack of noradrenaline, serotonin, and other biogenic amines in the brain. Their content in the synapses can be increased through administration of antidepressants that inhibit the following enzyme:

A. Monoamine oxidase
B. Diamine oxidase
C. L-amino acids oxidase
D. D-amino acid oxidase
E. Phenylalanine 4-monooxygenase

40. During hypersensitivity skin test a patient received an allergen subcutaneously, after which the patient developed skin redness, edema, and pain due to histamine action. This biogenic amine is produced as the result of the following transformation of histidine amino acid:

A. Decarboxylation
B. Methylation
C. Phosphorilation
D. Isomerization
E. Deaminization

41. 30 minutes after dental treatment the patient developed red itching spots on the face and oral mucosa. The patient I was diagnosed with urticaria. What bioactive substance with vasodilating and pruriginous effect is produced during this type of allergic reaction?

A. Histamine
B. Leukotriene B_4
C. Interleukin-1
D. Bradykinin
E. Prostaglandin E_2

42. A patient presents with dysfunction of the cerebral cortex accompanied by epileptic seizures. He has been administered a biogenic amine synthetized from glutamate and responsible for central inhibition. What substance is it?

A. γ-aminobutyric acid
B. Histamine
C. Acetylcholine
D. Serotonin
E. Dopamine

References:

1. Gubsky Yu. Biological chemistry : textbook / edited by Yu. Gubsky. – Vinnytsia : Nova Knyha, 2018. – 2nd edition. – 488 p.
2. Lecture materials.
3. Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – 799 p.
4. Textbook «Biological chemistry. Collection of test tasks» for English-speaking students of higher medical educational institutions of the Ministry of Public Health of Ukraine. – Poltava, 2018. – 148 p.

TOPIC №23.
Biosynthesis of glutathione and creatine

1. Objective: To acquire a method of quantitative determination of creatinine in urine, to be able to estimate results of the analysis. To know colour reaction on creatine with pycrine acid, to be able to calculate the result of the analysis. To know colour reaction on creatine with pycrine acid, to be able to calculate the result of the analysis.

2. Actuality of the theme: Creatinine is an end-product of an exchange of tissue's creatine phosphate. Increased excretion of creatinine in urine is observed at patients with a fever sharp infections, at a diabetes mellitus. Creatinine – nitrogenous slag, but due to nitrogen excretion function of kidneys clears blood At a pathology of kidneys that is accompanied by infringement of nitrogen excretion function, and also at sharp heart insufficiency, creatinine collects in blood, and its allocation with urine is reduced. Therefore quantitative determination of creatinine in blood and urine is one of obligatory analyses at diseases of kidneys. Creatine phosphate is applied in clinic for the treatment of patients with cardiovascular insufficiency.

3. Specific aims:

✓ To explain biochemical mechanisms of formation of creatine and creatinine.

✓ To analyze clinical value of infringements of an creatine and creatinine exchange.

✓ To treat a role of glutathione in an exchange of organic peroxides.

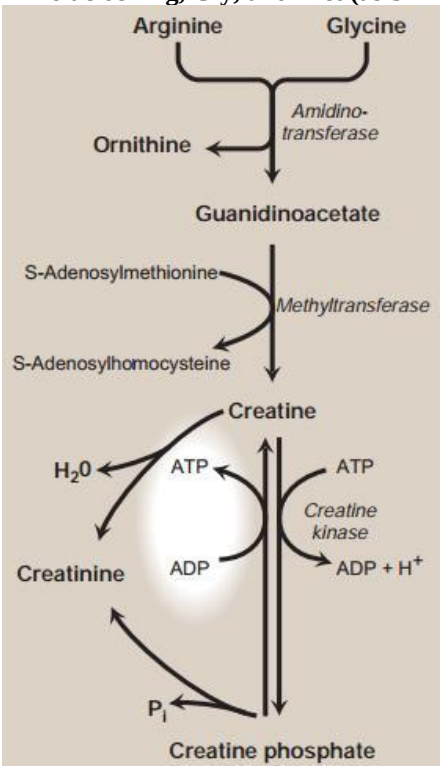
4. Reference card for the separate study of educational literature for the lesson preparation

Questions:	References:
1. **Glutathion, structure and role in metabolism of organic peroxides.**	Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 65-66, 172, 343.
2. **Production of creatine and creatinine, clinical and diagnostic significance of disorders in their metabolism.**	Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 342-344.

5. Tasks for independent work and self-control

5.1. Describe the structure and role of glutathion.

5.2. What is the function of creatine phosphate, the synthesis of which from the amino acids Arg, Gly, and Met (as SAM) is shown?



5.3. Explain clinical and diagnostic significance of creatine and creatinine.

6. Individual independent students work

1. Biological role of glutathione system in antioxidant protection.

Practice protocol №23 «____» _____ 20___

Experiment. Estimation of creatinine according to Jaffe colour reaction (Popper et al.).

Principle. After interaction of creatinine and picric acid in alkaline medium a coloured compound is formed, the intensity of colour is proportional to the concentration of creatinine.

$$\textbf{Creatinine + Picric acid} \xrightarrow{\text{NaOH}} \textbf{Creatinine picrate}$$

(Yellow) (Orange)

Method.

Pipette the solutions into the labelled test tubes according to the table:

	SAMPLE tube 1	**BLANK** tube 2	**STANDARD** tube 3
Picric acid	6 ml	6 ml	6 ml
Blood serum	2 ml	-	-
standard solution of creatinine	–	-	2 ml
Water	–	2 ml	-
Mix and boil in water bath for 20-30 sec. Eliminate the sediment by filtration, then introduce:			
Supernatant fluid (correspondent to tube)	4 ml	4 ml	4 ml
10% NaOH	0.2 ml	0.2 ml	0.2 ml
Mix and adjast volume to 10 ml and let stand at the laboratory temperature for 10 minutes:			
Read the optical density of the sample at 530 nm against the blank			

Concentration of creatinine is calculated according the formula:

$$X = (D_{exp} / D_{st}) \times 0{,}088 \text{ mMoles/l}$$, where:

D_{exp} – optical descity of tested sample

D_{st} – optical descity of standard solution

0.088 mMoles/l – concentration of creatinine in standard solution.

Result:

Conclusion:

Clinical diagnostic significance. Creatinine is an end product of creatine metabolism. Concentration of creatinine in blood plasma is relatively constant and reflects the amount of muscle mass and does not depend from diet or other factors. Creatinine is not reabsorbed in renal tubules and its clearance value is used for estimation of glomerular filtration in kidneys.

Under normal conditions concentration of creatinine in women – is 0,044–0,097 mMoles/l, in men – 0,044–0,115 mMoles/l. Increase in creatinine concentration is observed in acute and chronic kidney diseases, a decrease – in the 1–2 trimesters of pregnancy, in lowering of muscle mass due to age or myodystrophic changes.

Ecscretion of creatinine:

♂ – 1,0-2,0 g/day

♀ – 0,8-1,8 g/day

The report is checked up _____

(The signature of the teacher, date)

Examples of Krock-1 tests

1. Macroergic compounds are required for the normal metabolism of cells. Which of the compounds listed below refers to macroergic ones?

A. Creatine phosphate
B. Creatine
C. Creatinine
D. Glucose-6-phosphate
E. AMP

2. The considerable increase of MB-form of creatine kinase activity was found in a patient's blood. Point a possible pathology:

A. Myocardial infarction
B. Hepatitis
C. Rheumatism
D. Pancreatitis
E. Cholecystitis

3. The increase of urea and creatinine levels and the decrease of these parameters in the urine were revealed in a patient. Point possible causes of that state:

A. Renal disease
B. Liver disease
C. Muscle disease
D. Disturbances of binding, transport and excretion of ammonia with urine
E. Disturbance of acid-base balance

4. A 46-year-old female has been suffering from progressive myodystrophy (Duchenne's disease) for a long time. The change of catalytic activity of what blood enzyme proves to be a diagnostic test for the disease?

A. Creatine kinase
B. Lactate dehydrogenase
C. Pyruvate dehydrogenase
D. Glutamate dehydrogenase
E. Adenylate kinase

5. Methyl groups are used in organism for the synthesis of the important substances such as creatine, choline and adrenaline. The source of these groups is an essential amino acid:

A. Methionine
B. Glycine
C. Valine
D. Cysteine
E. Arginine

6. A patient with serious damage of muscular tissue was admitted to the traumatology department. What biochemical urine index will be increased in this case?

A. Creatinine
B. Lipids
C. Glucose
D. Mineral salts
E. Uric acid

7. The muscle dystrophy was diagnosed in an 18-year-old young malE. The increased level of what substance in the blood serum is the most probable in this pathology?

A. Creatine
B. Myosin
C. Myoglobin
D. Lactate
E. Alanine

8. Determination of which enzyme in the blood is the most informative in the first hours after the onset of myocardial infarction?

A. Creatine phosphokinase
B. Aspartate aminotransferase
C. Alanine aminotransferase
D. Lactate dehydrogenase
E. Glutamate dehydrogenase

9. What is a daily urinary excretion of creatinine?

A. 1–2 g
B. 2–3 g
C. 3–4 g
D. 0.5– 1 g
E. More than 4.0 g

10. The amino acids involved in the biosynthesis of creatine are:

A. Arginine, glycine, methionine
B. Arginine, alanine, glycine
C. Glycine, lysine, methionine
D. Arginine, lysine, methionine
E. Glycine, lysine, alanine

11. In preparation for a trip to an area of India where malaria is endemic, a young man is given primaquine prophylactically. Soon thereafter, he develops a hemolytic condition. The most likely cause of the hemolysis is a less than normal level of which of the following compounds?

A. Reduced form of glutathione
B. Oxidized form of NAD
C. Glucose 6-phosphate
D. Ribose-5-phosphate
E. Ribulose-5-phosphate

12. Long-term myocardial ischemia leads to the necrosis and hyperenzymemia. The determination of what enzyme activity in the blood is used in clinics to diagnose a myocardial infarction?

A. Creatine phosphokinase, AST, LDH1,2
B. Succinate dehydrogenase, amylase, lipase
C. Arginase, urease, maltase
D. Nucleases, trypsine, chymotripsin
E. Glycogen phosphorylase, glucogen synthase, malate dehydrogenase

13. The lack of selenium in the body is manifested by the development of cardiomyopathy. The decreased activity of what selenium-containing enzyme is observed in this case?

A. Glutathione peroxidase
B. Catalase
C. Cytochrome oxidase
D. Succinate dehydrogenase
E. Lactate dehydrogenase

14. Lipid peroxidation reactions are enhanced in a result of the decreased activity of antioxidant enzymes. The deficiency of what trace element leads to the decreased activity of glutathione peroxidase?

A. Selenium
B. Molybdenum
C. Cobalt
D. Magnesium
E. Copper

15. Detoxication of xenobiotics (drugs, epoxides, arene oxides, aldehydes, etc.) and endogenous metabolites (estradiol, prostaglandins, leukotrienes) occurs in the liver by conjugation with:

A. Glutathione
B. Aspartic acid
C. Glycine
D. S-adenosylmethionine
E. Phosphoadenosine

16. There is a peptide in a human body in which the formation of the gamma-carboxylic group of glutamate takes part. What is this peptide called?

A. Glutathione
B. Carnosine

C. Anserine
D. Oxytocin
E. Vasopressin

17. What biochemical indicator is used to evaluate the glomerular ultrafiltration in the kidneys?

A. Clearance of creatinine
B. Daily creatinine excretion in the urine
C. Proteinuria
D. The daily excretion of urea in the urine
E. Hematuria

18. The synthesis of creatine proceeds in two steps. What organs are directly involved in this process?

A. Kidney, liver
B. Spleen, kidneys
C. Liver, muscles
D. Kidney, myocardium
E. Kidney, muscles

19. The destruction of the membranes of the myocytes is caused by the deficiency of the vitamin E. The urinary excretion of what metabolite of muscle cells indicates to their damage?

A. Creatine
B. Glucose
C. Pyruvate
D. Ammonia
E. Lactate

20. Creatine phosphokinase (CK) is an enzyme that has an exceptional diagnostic value in many pathologies, specify its isoforms:

A. CK-MM, CK-MB, CK-BB
B. CK1, CK2, CK3, CK4, CK5
C. CK1, CK2, CK3
D. Cardiac-specific, liver-specific, bonespecific
E. All of the above

21. Amino acids are used for the synthesis of many biologically important compounds in the body. Which of the following amino acids is necessary for the synthesis of purine nucleotides, creatine, glutathione, paired bile acids?

A. Glycine
B. Arginine
C. Lysine

D. Methionine
E. Cysteine

22. Human organism has a peptide, which formation is performed with participation of γ-carboxylic group of glutamic acid. What is the name of this peptide?

A. Glutathione
B. Vasopressin
C. Carnosine
D. Anserine
E. Oxytocine

23. The considerable increase of MB-form creatine kinase activity was found in patient's blood. Point a possible pathology:

A. Myocardial infarction
B. Hepatitis
C. Rheumatism
D. Pancreatitis
E. Cholecystitis

24. What is the chemical structure of glutathione?

A. Tripeptide
B. Tetrapeptide
C. Nonapeptide
D. Derivative of 7-dehydrocholesterol
E. Dipeptide

25. The diagnosis of the young man, 18 years, is muscle dystrophy. Which substance the increased level in blood serum is the most possible in this pathology?

A. Creatine
B. Myoglobin
C. Myosin
D. Lactate
E. Alanine

References:

1. Gubsky Yu. Biological chemistry : textbook / edited by Yu. Gubsky. – Vinnytsia : Nova Knyha, 2018. – 2nd edition. – 488 p.
2. Lecture materials.
3. Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – 799 p.
4. Textbook «Biological chemistry. Collection of test tasks» for English-speaking students of higher medical educational institutions of the Ministry of Public Health of Ukraine. – Poltava, 2018. – 148 p.

TOPIC №24.
Detoxification of ammonia and urea biosynthesis

1. Objective: To interpret the pathways of production, transport and neutralization of ammonia in human body. To learn the methods of determination of urea in biological fluids and interpretation of obtained results.

2. Actuality of the theme: In course of amino acid metabolism are produced metabolites, which can be detected and quantified in blood and urine and may be used in diagnostics and treatment monitoring.

3. Specific aims:

✓ Quantitative determination of urea in biological fluids. Interpretation of obtained results and conclusions;

✓ To interpret metabolic pathways of production and neutralization of ammonia, circulatory transport of ammonia, urea biosynthesis;

✓ To analyze changes in processes of transport and neutralization of ammonia in hereditary anomalies of enzymes of ammonia turnover;

✓ To explain general metabolic pathways of nitrogen free residues of amino acids and peculiarities in transformation of aromatic and heterocyclic amino acids.

✓ To explain biochemical basis in development and manifestation of genetic anomalies in metabolism of aromatic and heterocyclic amino acids, accumulation of distinct metabolic intermediates in phenylketonuria, alkaptonuria, albinism.

4. Reference card for the separate study of educational literature for the lesson preparation

Questions:	References:
1. **General metabolic pathways of nitrogen free residues of amino acids in human body. Glycogenic and ketogenic amino acids.**	1. Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 372–374. 2. Gubsky Yu. Biological chemistry: textbook / edited by Yu. Gubsky. – Vinnytsia: Nova Knyha, 2018. – 2nd edition. – P. 228–231.
2. **Pathways of ammonia production. Toxicity of ammonia and mechanisms of its detoxification. Circulatory transport of ammonia (glutamine, alanine).**	1. Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 335–337. 2. Gubsky Yu. Biological chemistry: textbook / edited by Yu. Gubsky. – Vinnytsia: Nova Knyha, 2018. – 2nd edition. – P. 223.

3.	**Biosynthesis of urea:** ✓ enzymatic reactions; ✓ hereditary defects of enzymes involved in urea synthesis (enzymopathias of urea synthesis).	1. Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 337–341. 2. Gubsky Yu. Biological chemistry: textbook / edited by Yu. Gubsky. – Vinnytsia: Nova Knyha, 2018. – 2nd edition. – P. 224–227.
4.	**Special pathways of noncyclic amino acids metabolism.** ✓ Metabolism of glycine and serine; ✓ Role of tetrahydrofolate in transfer of one carbone fragments; ✓ Inhibitors of dihydrofolate reductase as antitumor agents.	1. Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 341–344, 363, 371–372. 2. Gubsky Yu. Biological chemistry: textbook / edited by Yu. Gubsky. – Vinnytsia: Nova Knyha, 2018. – 2nd edition. – P. 231–237.
5.	**Specific pathways of metabolism of aromatic amino acids phenylalanine and tyrosine, sequence of enzymatic reactions.**	1. Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 345–350. 2. Gubsky Yu. Biological chemistry: textbook / edited by Yu. Gubsky. – Vinnytsia: Nova Knyha, 2018. – 2nd edition. – P. 240–243.
6.	**Hereditary enzymopathias of phenylalanine and tyrosine metabolism – phenylketonuria, alkaptonuria, albinism.**	1. Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 351–353. 2. Gubsky Yu. Biological chemistry: textbook / edited by Yu. Gubsky. – Vinnytsia: Nova Knyha, 2018. – 2nd edition. – P. 244.
7.	**Peculiarities of metabolism of branched chain amino acids:** ✓ scheme of branched chain amino acids metabolism; ✓ role of vitamin B_{12} in metabolism of amino acid; ✓ metabolic defects of branched chain amino acids.	1. Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 363–366. 2. Gubsky Yu. Biological chemistry: textbook / edited by Yu. Gubsky. – Vinnytsia: Nova Knyha, 2018. – 2nd edition. – P. 237–240.

5. Tasks for independent work and self-control

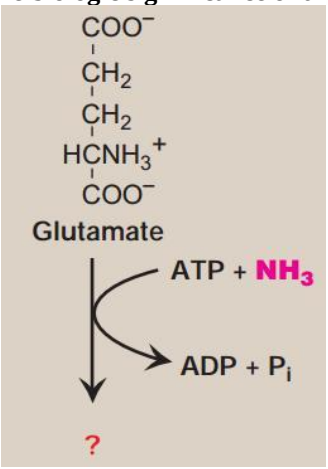
5.1. Which vertical column (A, B, or C) shown would most appropriately be labeled «(Solely) Ketogenic Amino Acids»?

	A	B	C
1	Alanine Arginine Asparagine Aspartate Cysteine Glutamate Glutamine Glycine Proline Serine	Tyrosine	
2	Histidine Methionine Threonine Valine	Isoleucine Phenylalanine Tryptophan	Leucine Lysine

Which horizontal row (1 or 2) would most appropriately be labeled «Essential Amino Acids»? What does it mean for an amino acid to be essential?

5.2. The pathways for catabolism of the C-skeletons of amino acids converge to form what seven intermediate products?

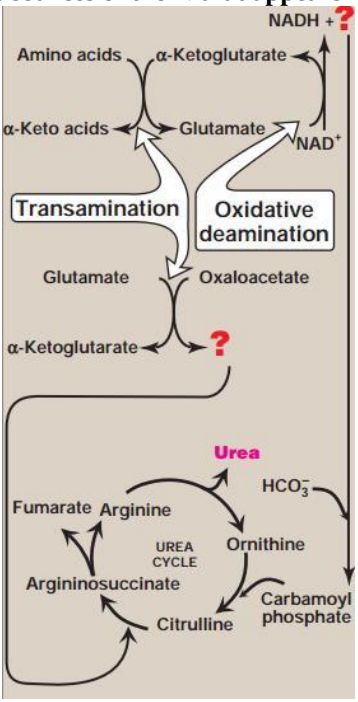
5.3. What is the amino acid product of the reaction shown? Would you expect the enzyme that catalyzes the reaction to be asynthase or a synthetase? What is the biologic signifi cance of the reaction?



5.4. What is the function of the urea cycle, and where does it occur? What is the regulated enzyme? What is the fate of the urea product?

5.5. How do the liver and the kidneys metabolize Arg differently? How does this relate to Arg being nonessential?

5.6. What are the sources of the N that appears in urea?



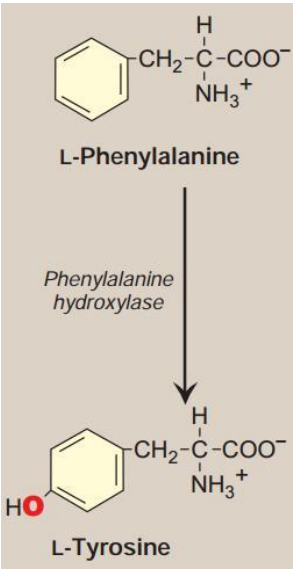
5.7. What happens to the fumarate produced by argininosuccinate lyase?

5.8. What is the significance of NH_4^+ production by the kidney?

5.9. What is blood blood urea nitrogen (BUN?) urine urea nitrogen (UUN)?

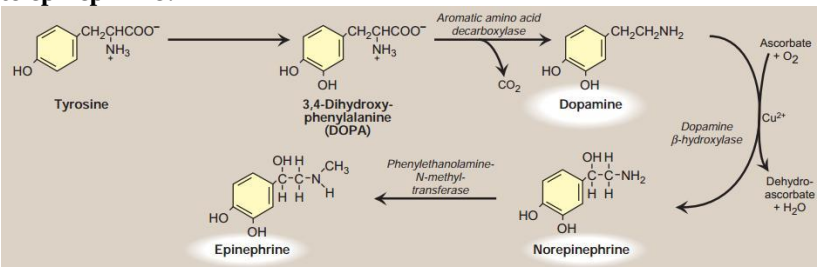
5.10. What is the function of tetrahydrofolate (THF) for metabolism of noncyclic amino acids?

5.11. What coenzyme is required by phenylalanine hydroxylase (PAH) reaction shown?



5.12. What is the cause of phenylketonuria (PKU), and how is it treated? Why are the CNS effects of PKU now rarely seen?

5.13. What enzyme catalyzes the rate-limiting conversion of Tyr to DOPA, as shown? What coenzyme does it require? What coenzyme is required in the conversion of DOPA to dopamine? Of norepinephrine to epinephrine?

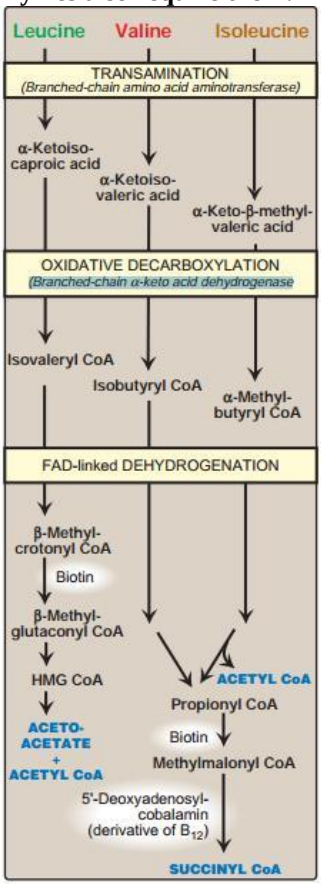


5.14. What is the function of the norepinephrine and epinephrine (catecholamines) released from the adrenals in response to physiologic stress?

5.15. What is the clinical consequence of tyrosinase deficiency?

5.16. What are the causes and clinical consequences of alkaptonuria?

5.17. What coenzymes are required by branched-chain α-keto acid dehydrogenase (BCKD), the enzyme that oxidatively decarboxylates the α-keto acid derivatives of the branched chain amino acids (BCAAs), as shown? What other enzymes also require them?



5.19. Situational task:

A 9-month-old boy was admitted to the hospital for evaluation of chronic vomiting and developmental delay. Lab studies revealed elevated levels of NH_3, Gln, Ala, and ornithine. Citrulline was low. Which urea cycle enzyme is deficient in the patient?

Why might antibiotics be used to treat UC disorders?

6. Individual independent students work

1. To estimate biochemical parameters of infringement of processes of ammonia neutralization at the congenital and got defects of a metabolism.

Practice protocol №24 **«____» _____ 20___**

Experiment 1. Quantitative determination of the contents of urea in blood with diacetylmonooxime.

Principle: urea forms with diacetylmonooxime the complex of rose-red colour. Intensity of painting is proportional to the concentration of urea in blood.

Method.

1 test tube – experiment	2 test tube – standard	3 test tube – control
0.1 ml of experiment 2 ml of working solution	0.1 ml of the standard 2 ml of working solution	0.1 ml of water 2 ml of working solution

To boil all test tubes on a water bath for 10 minutes, after cooling to colourimetrate contrary to the control at λ = 540 nanometers (a green optical filter), a ditch of 5 mm.

Calculation:

$$\text{Urea} = E_{ex}/E_{st} \times 16.65 = \text{mmol/L},$$

where

E_{ex} – extinction of skilled test;

E_{st} – extinction of standard test (in 1 ml is 16.65 mmol/L of urea).

Result:

Conclusion:

Experiment 2. Determine the content of urea in the urine.

Principle: the same.

Method.

0.1 ml of the urine collected during the day, filtered and diluted to 25 times and 0.1 ml of standard solution of urea are used for analysis. The samples are treated in the same way as blood serum. The content of urea (Y) in mmol/day in urine is calculated by the formula:

$$Y = (E_{ex} \times V \times 25 \times 1.665) / (E_{st} \times 0.1 \times 1000),$$

where

E_{ex} is the extinction of test sample;

V is the volume of daily urine;

1.665 is the concentration of urea in 0.1 ml of a standard sample (μmol);

0.1 is the volume of urine taken for the study (ml);

E_{st} is the extinction of the standard sample;

1000 is the scaling factor for micromol-to-millimol conversion;

25 is the dilution.

Excretion with urine is 20–30 g/day

Result:

Conclusion:

Experiment 3. Determine the content of ammonia in the urine.

Principle: the method is based on the interaction of ammonium salts with formaldehyde to form hexamethylene tetraamine and to release an acid, amount of which is equivalent to ammonia amount. An acid is titrated with a solution of the base.

Method.

Pour 10 ml of urine into the flask, add 1–2 drops of phenolphthalein and neutralize with 0.1 M solution of sodium hydroxide. Add an equal volume of fresh formaldehyde solution. Due to the formation of acid a pink colour

disappears. Titrate the mixture with 0.1 M sodium hydroxide to the appearance of the pink colour.

Calculate the concentration of ammonia (Z) in grams per daily urine by the volume of base consumed in the titration according to the formula:

$$Z=(a\times 0.0017 \times D)/10,$$

where

a is the volume of the base, which is used for titration, ml;

0.0017 is the amount of ammonia corresponding to 1 ml of 0.1 M solution of sodium hydroxide (titer of ammonia) (g);

D is the daily quantity of urine (ml);

10 is the amount of urine using for analysis (ml).

Result:

Conclusion:

Clinical and diagnostic significance. The normal content of urea in the blood varies between **3.3 and 8.3 mmol/L**. About 75 % of urea is excreted in the urine. The concentration of urea in the blood depends on the intensity of its synthesis and excretion. Determination of urea is an important diagnostic test that characterizes not only the state of protein metabolism but also functional status of kidney and liver. Increased concentration of urea in the blood (uremia) is observed in kidney diseases (disorder of their excretory function), enhanced protein breakdown, excessive protein diet, in the case of dehydration (relative azotemia), in poisoning by phosphorus. The reduction of urea in the blood and excretion of it in the urine are observed in diseases of the liver (hepatodystrophy, cirrhosis and hepatitis), pregnancy, as well as genetic defects of enzymes of urea synthesis. Under such conditions, there is an increase in blood ammonia called ammoniemia. The symptoms of hyperammonemia (nausea, vomiting, convulsions, syncope and oedema of the brain in severe cases) are manifestations of its

effects on the CNS. The increase of ammonia in the urine is also observed in a variety of processes accompanied by acidosis, fever, and diabetes mellitus. In diabetic acidosis the amount of ammonia in the urine exceeds the norm by more than 50 times. Excretion of ammonia in the urine decreases in some diseases that are accompanied by alkalosis (parathyroidotropic and children tetany, epylepsy, significant phosphaturia), as well as alkaline admission into the body.

The report is checked up _____

(The signature of the teacher, date)

Examples of Krock-1 tests

1. Ammonia is a very toxic substance, especially for nervous system. What substance takes the most active part in the ammonia detoxication in the brain tissue?

A. Glutamic acid
B. Lysine
C. Proline
D. Histidine
E. Alanine

2. A citrulline and a high level of ammonia are determined in the urine of a newborn child. The formation of what substance is the most credible to be disturbed?

A. Urea
B. Uric acid
C. Ammonia
D. Creatinine
E. Creatine

3. The greater amount of nitrogen is excreted from the organism in the form of urea. Inhibition of urea synthesis and accumulation of ammonia in blood and tissues are induced by the decreased activity of the following liver enzyme:

A. Carbamoyl phosphate synthetase
B. Aspartate aminotransferase
C. Urease
D. Amylase
E. Pepsin

4. A newborn child was found to have a reduced intensity of sucking, frequent vomiting and hypotonia. It was revealed an increased concentration of citrulline in urine and blood. What metabolic process is disturbed?

A. Ornithine cycle
B. Tricarboxylic acid cycle
C. Glycolysis
D. Glyconeogenesis
E. Cori cycle

5. Hyperargininemia and argininuria are observed in a 25-year-old patient. The urea level is decreased in blood and urine. Which enzyme deficiency is observed?

A. Arginase
B. Glutamate dehydrogenase
C. Ornithine carbamoyl transferase
D. Argininosuccinate synthetase
E. Tryptophan-5-monooxigenase

6. Toxicity of ammonia (especially for brain) is due to its capacity to disturb the functioning of the Krebs cycle as a result of the removal from the cycle of:

A. Alfa-ketoglutarate
B. Citrate
C. Malate
D. Succinate
E. Fumarate

7. A patient has all signs of the hepatic coma such as loss of consciousness, absence of reflexes, cramps, convulsion, disorder of heart activity, recurrent (periodical) respiration. What cerebrotoxic substances are accumulated in blood under hepatic insufficiency?

A. Ammonia
B. Urea
C. Necrosogenic substances
D. Autoantibodies
E. Ketone bodies

8. Secondary orotic aciduria is revealed in a patient with hereditary hyperammonemia due to disorders of the ornithine cycle. The increase of the concentration of what metabolite of the ornithine cycle causes enhanced synthesis of orotic acid?

A. Carbamoyl phosphate
B. Ornithine
C. Citrulline
D. Urea
E. Argininosuccinate

9. Glutamic acid plays the central role in the metabolism of amino acids in the nervous tissue. This is due to the fact that this amino acid:

A. It binds ammonia to form glutamine
B. It is used for the synthesis of glucose

C. It is used for the synthesis of lipids
D. It is used in the synthesis of neurospecific proteins
E. It is used for the synthesis of ketone bodies

10. Disorders of the central nervous system are observed in a child. Hyperammonemia is revealed in the blood serum. The deficiency of what enzyme can cause such pathological condition?

A. Ornithine transcarbamoylase
B. Glutathione transferase
C. Glycine transferase
D. Alanine aminotransferase
E. Glucuronyl transferase

11. Under metabolic acidosis acid products are neutralized by ammonia in the kidney and excreted in the form of salts of:

A. Ammonium
B. Potassium
C. Calcium
D. Sodium
E. Magnesium

12. Which amino acid is an intermediate in the biosynthesis of urea in the liver and is cleaved to form ornithine and urea?

A. Arginine
B. Citrulline
C. Valine
D. Leucine
E. Tryptophan

13. Under hyperammonemia convulsions, vomiting and loss of consciousness are observed. Ammonia concentration is increased in the biochemical analysis of blood. What is the pathogenetic mechanism of seizures under this condition?

A. Decreased biosynthesis of GABA
B. Inhibition of β-oxidation of fatty acids
C. Inhibition of glycolysis
D. Inhibition of the pentose phosphate pathway
E. Uncoupling of oxidation and oxidative phosphorylation

14. Neurons are very sensitive to the energy deficiency that caused by the high concentration of ammonia stimulating reducing amination of α-ketoglutarate and removing it from the following metabolic pathway:

A. The citric acid cycle
B. The ornithine cycle

C. Glycolysis
D. Glycogenolysis
E. Penthose phosphate pathway

15. A female neonate did well until approximately 24 hours of age when she became lethargic. A sepsis workup proved negative. At 56 hours, she started showing focal seizure activity. Hyperammonemia was found. Quantitative plasma amino acid levels revealed a marked elevation of argininosuccinate. Which one of the following enzymic activities is most likely to be deficient in this patient?

A. Argininosuccinate lyase
B. Arginase
C. Argininosuccinate synthase
D. Carbamoyl phosphate synthetase I
E. Ornithine transcarbamoylase

16. After a serious viral infection a 3-year-old child has repeated vomiting, loss of consciousness, convulsions. Examination revealed hyperammonemia. What caused changes of biochemical blood indices of this child?

A. Disorder of ammonia neutralization in the ornithine cycle
B. Activated processes of amino acid decarboxylation
C. Disorder of biogenic amines neutralization
D. Increased degradation of proteins in intestine
E. Inhibited activity of transamination enzymes

17. Which enzyme deficiency of the urea cycle causes hyperammonemia type I?

A. Carmamoyl phosphate synthetase I
B. Arginase
C. Argininosuccinate synthase
D. Ornithine transcarbamoylase
E. Argininosuccinate lyase

18. Ammonium cation excretion with urine is increased in the next condition:

A. Metabolic acidosis
B. Respiratory alkalosis
C. Hyperlipidemia
D. Hypoproteinemia
E. Obesity

19. Investigation of the patient's blood and urine showed that the concentration of urea in the daily urine is 180 mmol / L and in the blood is 1.5 mmol/L. The disorder of what metabolic pathway causes such state?

A. The ornithine cycle
B. Glycolysis

C. The Krebs cycle
D. Gluconeogenesis
E. Pentose phosphate pathway

20. According to blood analysis of a patient the residual nitrogen content is 48 mmol/L, urea – 15.3 mmol/L. The disease of which organ may cause such results of the laboratory investigation?

A. Kidneys
B. Liver
C. Stomach
D. Spleen
E. Intestine

21. The urea content in the blood serum of a healthy person is:

A. 3.3–8.3 mmol / L
B. 3.825.8 mmol /L
C. 10–20 mmol /L
D. 33–83 mmol / L
E. 333–585 mmol / L

22. There are several mechanisms of ammonia neutralization in humans. Which one takes place in the kidneys?

A. Ammoniogenesis
B. Ureogenesis
C. Reductive amination of 2-oxoglutarate
D. Synthesis of asparagine
E. Synthesis of glutamine

23. Pyridoxal phosphate was prescribed to patient according to clinical indices. Which processes correction is used this preparation for:

A. Transamination and decarboxylation of amino acids
B. Synthesis of purine and pyrimidine nucleotides
C. Oxidative decarboxylation of α-keto acids
D. Deamination of amino acids
E. Protein synthesis

24. As an effect of ultra-violet rays the human skin darkens, which is defensive reaction of the organism. What defensive substance – derivative of amino acids – is synthesized under influence of ultra-violet rays?

A. Melanin
B. Tyrosine
C. Phenylalanine
D. Tryptophan
E. Vitamin D

25. Olive green colour appears after treatment of newborn child's urine by $FeCl_3$ solution. Which amino acid metabolism disturbance is observed?

A. Phenylalanine
B. Histidine
C. Cysteine
D. Glutamine
E. Lysine

26. Albinos can't stand sun impact – they don't acquire sun-tan but get sunburns. Point the metabolism disturbance which is the base of this event:

A. Lack of tyrosinase
B. Degradation of melanin
C. Disturbance of cholesterol transport
D. Disturbance of serine hydroxylation
E. Degradation of vitamin D_3

27. A 6-month-old infant has physical and mental retardation, a lighter hair, skin and iris, positive Fellinge's reaction. Which disease was revealed in infant?

A. Phenylketonuria
B. Albinism
C. Down's disease
D. Galactosemia
E. Alkaptonuria

28. Alkaptonuria was diagnosed in patient. Which enzyme deficiency causes this pathology?

A. Homogentisate oxidase
B. Phenylalanine hydroxylase
C. Tyrosinase
D. Tyrosine hydroxylase
E. Monoaminoxidase

29. In urine of child the increased level of homogentisinic acid was found. The urine secreted darkens in the air. Which pathology is this characteristic for?

A. Alkaptonuria
B. Cystinuria
C. Phenylketonuria
D. Albinism
E. Amino aciduria

30. The suppression of conversion of phenylalanine to tyrosine is observed in one of the inherited pathologies. Biochemical indicator of disease is the accumulation in organism of some organic acids, including:

A. Phenylpyruvate

B. Aspartate
C. Pyruvate
D. Lactate
E. Glutamate

31. At repeated impact of ultra-violet rays the skin darkens owing to synthesizing melanin in it, which protects cells from damage. The main mechanism to start the defensive activity is:

A. Activation of a tyrosinase
B. Depression of tyrosinase
C. Activation of oxidase of homogentisic acid
D. Depression of oxidase of homogentisic acid
E. Depression of phenylalanine hydroxylase

32. The Kreb's citric acid cycle plays an important role in realization of glucoplastic effect of amino acids. This is provided by obligatory transformation of their carbon skeletons into:

A. Oxaloacetate
B. Citrate
C. Succinate
D. Malate
E. Fumarate

33. The increase of urea and creatinine levels and the decrease of these parameters in the urine were revealed in patient. Point possible causes of that state:

A. Renal disease
B. Liver disease
C. Muscle disease
D. Disturbances of binding, transport and excretion of ammonia with urine
E. Disturbance of acid-base balance

34. The known fact is that albinos get burns while sun tanning. Which amino acid metabolism disturbance lies in the base of this phenomenon?

A. Phenylalanine
B. Glutamate
C. Methionine
D. Tryptophan
E. Hystidine

35. A man, age 50, has been under severe stress. The blood level of epinephrine is high. Which enzyme deactivates the hormone?

A. Monoamine oxidase
B. Glucosidase

C. Peptidase
D. Carboxylase
E. Tyrosinase

36. A 6 day's newborn possesses the excess of phenylpyruvate and phenylacetate in the urine. Which amino acid metabolism is impaired?

A. Phenylalanine
B. Methionine
C. Tryptophan
D. Hystidine
E. Arginine

37. The urine of patient with alkaptonuria processes a great amount of homogentisinic acid. Which enzyme hereditary defect is observed?

A. Homogentisinic acid oxidase
B. Alanine aminotransferase
C. Tyrosinase
D. Phenylalanine-4-monooxigenase
E. Tyrosine aminotransferase

38. A breast-fed baby develops the darkening of sclera, mycoses membranes, ears. Its urine gets dark at the open air. The blood and urine tests reveal the presence of homogentisinic acid. What is the diagnosis?

A. Alkaptonuria
B. Albinism
C. Cysteinurea
D. Porphyria
E. Hemolytic anemia

39. A boy, age 9, was delivered to the clinics with the mental and physic retardation. The biochemical blood test revealed the increased quantity of phenylalanine. Which enzyme insufficient activity can lead to the state?

A. Phenylalanine-4-monooxygenase
B. Glutamate decarboxylase
C. Homogentisinic acid oxidase
D. Glutamine transaminase
E. Aspartate aminotransferase

40. A mother found out that her child's urine has a very dark colour. The child does not complain of anything. The urine does not contain bile pigments. The diagnosis is alkaptonuria. Which enzyme deficiency is observed?

A. Homogentisinic acid oxydase
B. Hydroxyphenylpyruvate oxydase
C. Phenylalanine hydroxylase
D. Tyrosinase
E. Phenylpyruvate decarboxylase

41. Ammonia is a very toxic substance, especially for central nervous system. Which compound takes exclusively active part in ammonia detoxification in brain tissue?

A. Glutamate
B. Lysine
C. Proline
D. Hystidine
E. Alanine

42. The urine of a newborn reveals citrulline and high level of ammonia. Which substance formation is most probably impaired?

A. Urea
B. Ammonia
C. Uric acid
D. Creatinine
E. Creatine

43. A child 10 months old, that parents are brunettes, has fair hair, very light skin and blue eyes. His outlook at birth was normal, but within the last 3 months failure of blood circulation in the brain, lagging in mental development were observed. The cause for such condition is:

A. Phenylketonuria
B. Galactosemia
C. Glycogenosis
D. Acute porphyria
E. Histidinemia

44. Mr S presents all signs of the hepatic coma: loss of consciousness, absence of reflexes, cramps, convulsion, disorder of heart activity, recurrent (periodical) respiration. What are cerebrotoxical substances which accumulate in blood under hepatic insufficiency?

A. Ammonia
B. Autoantibody
C. IL-l
D. Necrosogenic substances
E. Ketone body

45. A baby has dark colour of sclera, mucous membranes. The urine secreted darkens in the air. In blood and urine the homogentisinic acid is detected. What can cause this state?

A. Alkaptonuria

B. Cystinuria
C. Albinism
D. Galactosemia
E. Histidinemia

46. One of the forms of an inborn pathology is accompanied by retarded transformation of phenylalanine in tyrosine. Biochemical sign of the disease is accumulation of some organic acids in the organism, including:

A. Phenylpyruvate
B. Citrate
C. Pyruvate
D. Lactate
E. Glutamate

47. In a young man, 19 years old, signs of a depigmentation of the skin, conditioned by disturbance of melanin synthesis, are present. Which amino acid metabolism disturbance causes this condition?

A. Tyrosine
B. Histidine
C. Tryptophan
D. Proline
E. Glycine

48. At repeated impact of ultra-violet rays the skin darkens owing to synthesizing melanin in it, which protects cells from damage. The main mechanism to start the defensive activity is:

A. Activation of tyrosinase
B. Depression of tyrosinase
C. Activation of oxidase of homogentisic acid
D. Depression of oxidase of homogentisic acid
E. Depression of phenylalanine hydroxylase

49. A cerebral trauma caused increased ammonia generation. Which amino acid participates in the detoxification of ammonia from the brain?

A. Glutamic
B. Lysine
C. Tyrosine
D. Tryptophan
E. Valine

50. Nappies of a newborn have dark spots that witness of formation of homogentisic acid. Metabolic imbalance of which substance is it connected with?

A. Methionine
B. Tyrosine

C. Galactose
D. Tryptophan
E. Cholesterol

51. Ammonia is a very toxic substance, especially for nervous system. What substance takes the most active part in ammonia detoxification in brain tissues?

A. Glutamic acid
B. Alanine
C. Proline
D. Histidine
E. Lysine

52. A newborn child was found to have reduced intensity of sucking, frequent vomiting, hypotonia. Urine and blood exhibit increased concentration of citrulline. What metabolic process is disturbed?

A. Ornithine cycle
B. Cori cycle
C. Tricarboxylic acid cycle
D. Glyconeogenesis
E. Glycolysis

53. After a serious viral infection a 3-year-old child has, repeated vomiting, loss of consciousness, convulsions. Examination revealed hyperammoniemia. What may have caused changes of biochemical blood indices of this child?

A. Disorder of ammonia neutralization in ornithine cycle
B. Inhibited activity of transamination enzymes
C. Disorder of biogenic amines neutralization
D. Activated processes of amino acids decarboxylation
E. Increased putrefaction of proteins in intestines

54. An unconscious patient was taken by ambulance to the hospital. On objective examination the patient was found to have no reflexes, periodical convulsions, irregular breathing. After laboratory examination the patient was diagnosed with hepatic coma. Disorders of the central nervous system develop due to the accumulation of the following metabolite:

A. Ammonia
B. Urea
C. Glutamine
D. Bilirubin
E. Histamine

55. A 1,5-year-old child presents with both mental and physical lag, decolourizing of skin and hair, decrease in catecholamine

concentration in blood. When a few drops of 5% $FeCl_3$ solution have been added to the child's urine it turned olive green. Such alterations are typical for the following pathology of the amino acid metabolism:

A. Phenylketonuria
B. Albinism
C. Tyrosinosis
D. Alkaptonuria
E. Xanthinuria

56. A 4 year old boy has had recently serious viral hepatitis. Now there are such clinical presentations as vomiting, loss of consciousness, convulsions. Blood analysis revealed hyperammoniemia. Disturbance of which biochemical process caused such pathological condition of the patient?

A. Disturbed neutralization of ammonia in liver
B. Inhibition of transamination enzymes
C. Increased putrefaction of proteins in bowels
D. Activation of amino acid decarboxylation
E. Disturbed neutralization of biogenic amines

57. The greatest amount of nitrogen is excreted from the organism in form of urea. Inhibition of urea synthesis and accumulation of ammonia in blood and tissues are induced by the decreased activity of the following liver enzyme:

A. Carbamoyl phosphate synthetase
B. Urease
C. Aspartate aminotransferase
D. Amylase
E. Pepsin

58. Mother had noticed her 5-year-old child's urine to become dark in colour. Bile pigments in urine were not detected. The diagnosis of alkaptonuria was made. What pigment is deficient?

A. Homogentisic acid oxidase
B. Tyrosinase
C. Oxyphenylpyruvate oxidase
D. Phenylalanine hydroxylase
E. Phenylpyruvate decarboxylase

59. After severe viral hepatitis a 4-yea rold boy presents with vomiting, occasional loss of consciousness, convulsions. Blood test revealed hyperammoniemia. Such condition is caused by a disorder of the following biochemical hepatic process:

A. Disorder of ammonia neutralization
B. Disorder of biogenic amines neutralization

C. Protein synthesis inhibition
D. Activation of amino acid decarboxylation
E. Inhibition of transamination enzymes

60. A month after a serious operation a 38-year-old patient has recovered and has now positive nitrogen balance. Urine of this patient may be found to have low concentration of the following nitrogen containing substance:

A. Urea
B. Lactate
C. Stercobilinogen
D. Galactose
E. 17-ketosteroids

61. Analysis of a newborn's urine revealed phenylpyruvic acid. Its presence in urine is associated with the following pathology:

A. Phenylketonuria
B. Alkaptonuria
C. Albinism
D. Tyrosinosis
E. Gout

62. A patient has been diagnosed with alkaptonuria. Choose an enzyme whose deficiency can be the reason for this pathology:

A. Homogentisic acid oxidase
B. Phenylalanine hydroxylase
C. Glutamate dehydrogenase
D. Pyruvate dehydrogenase
E. Dioxyphenylalanine decarboxylase

63. Examination of urine in a newborn revealed presence of citrulline and high ammonia concentration. This baby is most likely to have the disorder of the following substance production:

A. Urea
B. Uric acid
C. Ammonia
D. Creatinine
E. Creatine

64. In case of alkaptonuria, homogentisic acid is excreted in urine in large amounts. The development of this disease is associated with a disorder of metabolism of the following amino acid:

A. Tyrosine
B. Phenylalanine
C. Alanine
D. Methionine
E. Asparagine

65. The greater amount of nitrogen is excreted from the organism in form of urea. Inhibition of urea synthesis and accumulation of ammonia in blood and tissues are induced by the decreased activity of the following liver enzyme:

A. Carbamoyl phosphate synthetase
B. Aspartate aminotransferase
C. Urease
D. Amylase
E. Pepsin

66. A 2-year-old child with mental and physical retardation has been delivered to a hospital. He presents with frequent vomiting after having meals. There is phenylpyruvic acid in urine. Which metabolism abnormality is the reason for this pathology?

A. Amino-acid metabolism
B. Lipid metabolism
C. Carbohydrate metabolism
D. Water-salt metabolism
E. Phosphoric calcium metabolism

67. A patient with hereditary hyperammonemia due to a disorder of ornithine cycle has developed secondary orotaciduria. The increased synthesis of orotic acid is caused by an increase in the following metabolite of ornithine cycle:

A. Carbamoyl phosphate
B. Citrulline
C. Ornithine
D. Urea
E. Argininosuccinate

68. A 2-year-old child presents with mental development retardation, intolerance of proteins, severe hyperammonemia against the background of low blood urea content. This condition is caused by the congenital deficiency of the following mitochondrial enzyme:

A. Carbamoyl phosphate synthetase
B. Citrate synthase
C. Succinate dehydrogenase
D. Malate dehydrogenase
E. Monoamine oxidase

69. Nitrogen is being excreted from the body mainly as urea. When activity of a certain enzyme in the liver is low, it results in inhibition of urea synthesis and nitrogen accumulation in blood and tissues. Name this enzyme:

A. Carbamoyl phosphate synthetase
B. Aspartate aminotransferase
C. Urease

D. Amylase
E. Pepsin

70. A sick child presents with high content of phenylpyruvate in urine (normally it is practically absent). Blood phenylalanine level is 350 mg/L (norm – 15 mg/L). What disease are these symptoms characteristic of?

A. Phenylketonuria
B. Alkaptonuria
C. Albinism
D. Tyrosinosis
E. Gout

71. During intensive muscle work there is a large amount of ammonia produced in the muscles. What amino acid plays the main role in the transportation of ammonia to the liver and participates in gluconeogenesis reactions?

A. Alanine
B. Aspartate
C. Ornithine
D. Lysine
E. Arginine

72. Nitrogen is being excreted from the body mainly as urea. When activity of a certain enzyme in the liver is low, it results in inhibition of urea synthesis and nitrogen accumulation in blood and tissues. Name this enzyme:

A. Carbamoyl phosphate synthetase
B. Amylase
C. Pepsin
D. Urease
E. Aspartate aminotransferase

73. A newborn presents with weak suckling, frequent vomiting, and hypotonia. Blood and urine citrulline are very high. What metabolic process is disturbed?

A. Ornithine cycle
B. Cori cycle
C. Glycolysis
D. Gluconeogenesis
E. Tricarboxylic acid cycle

74. Dopamine precursor – dioxyphenylalanine (DOPA) – is used in treatment of Parkinson's disease. This active substance is produced from the following amino acid:

A. Tyrosine
B. Cysteine
C. Alanine
D. Histidine
E. Tryptophan

75. An 84-year-old patient suffers from parkinsonism. One of the pathogenetic development elements of this disease is deficiency of a certain mediator in some of the brain structures. Name this mediator:

A. Dopamine
B. Noradrenaline
C. Histamine
D. Adrenaline
E. Acetylcholine

References:

1. Gubsky Yu. Biological chemistry : textbook / edited by Yu. Gubsky. – Vinnytsia : Nova Knyha, 2018. – 2nd edition. – 488 p.
2. Lecture materials.
3. Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – 799 p.
4. Textbook «Biological chemistry. Collection of test tasks» for English-speaking students of higher medical educational institutions of the Ministry of Public Health of Ukraine. – Poltava, 2018. – 148 p.

TOPIC № 25.
Metabolism of individual amino acids.
Biosynthesis of porphyrins. Porphyrias

1. Objective: To study pathways of sufur-containing amino acids, arginine and tryptophanE. To learn methods of hemoglobin determination, to perform benzidine test for detection of blood traces in biological material.

2. Actuality of the theme: Methionine and cysteine, besides being present in proteins, are involved in many important metabolic reactions. Methionine is also required for the initiation of protein biosynthesis. The sulfurcontaining amino acids are almost an exclusive dietary source of sulfur to the body. Arginine is a precursor of ornithine, which is used for synthesis of polyamines – spermidine and spermine (components of chromatin; they participate in DNA replication, transcription and translation) and it is a source of nitric oxide (NO). Tryptophan was the first to be identified as an essential amino acid. Tryptophan is both glucogenic and ketogenic in nature. It is a precursor for the synthesis of important compounds, namely NAD^+ and $NADP^+$ (coenzymes of niacin), serotonin and melatonin.

Porphyrins are organic compounds, which participate in principal processes of vital activity: tissue i.e. respiration and photosynthesis. In a free state porphyrins are not existing, their specific compounds are represented by complexes with proteins and metals. Hemoproteins, complexes of iron with porphyrins and proteins, represent different substances, connected with oxygen turnover. One group of hemoproteins includes respiratory enzymes. These are cytochromes, catalase, peroxidases. The second group of hemoproteins, which deals with transfer and retention of oxygen, includes hemoglobin and myoglobin. Accumulation of porphyrins in tissue over the normal limits cause the development of increased photosensitivity (skin or other exposed surfaces).

3. Specific aims:

✓ To explain biochemical basis of sufur-containing amino acids, arginine and tryptophane.

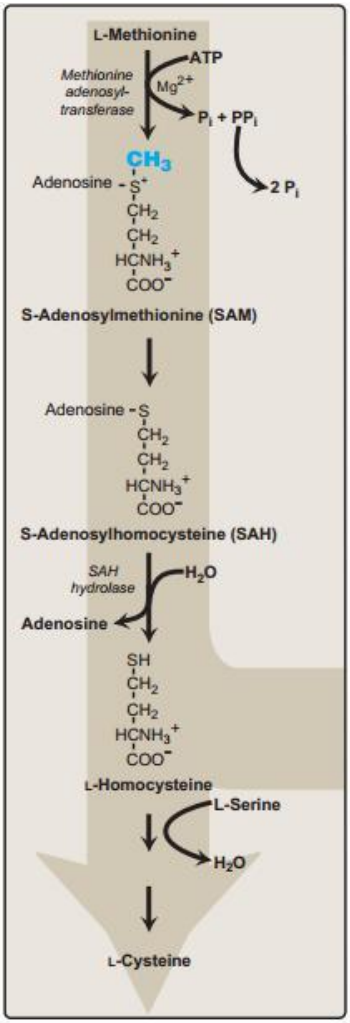
✓ To explain biochemical principles of regulation of an porphyrins exchange, occurrence and developments of hereditary infringements of porphyrins synthesis – porphyrias.

4. Reference card for the separate study of educational literature for the lesson preparation

Questions:	References:
1. **Metabolism of sulfur containing amino acids, reactions of methylation.**	1. Satyanarayana U., Chakrapani U. «Biochemistry», Fourth Edition. – 2013. – P. 358-362. 2. Gubsky Yu. Biological chemistry: textbook / edited by Yu. Gubsky. – Vinnytsia : Nova Knyha, 2018. – 2nd edition. – P. 235–237.
2. **Metabolism of arginine. Biological significance of nitric oxide, NO-synthase.**	Satyanarayana U., Chakrapani U. «Biochemistry», Fourth Edition. – 2013. – P. 366–368.
3. **Metabolism of tryptophane: kinurenic and serotonin pathways.**	1. Satyanarayana U., Chakrapani U. «Biochemistry», Fourth Edition. – 2013. – P. 354–358. 2. Gubsky Yu. Biological chemistry: textbook / edited by Yu. Gubsky. – Vinnytsia : Nova Knyha, 2018. – 2nd edition. – P. 243.
4. **Porphyrins. Structure of porphyrins.**	1. Satyanarayana U., Chakrapani U. «Biochemistry», Fourth Edition. – 2013. – P. 196–197. 2. Gubsky Yu. Biological chemistry: textbook / edited by Yu. Gubsky. – Vinnytsia: Nova Knyha, 2018. – 2nd edition. – P. 245–246.
5. **Biosynthesis of porphyrins**: ✓ scheme of enzymatic reactions of heme biosynthesis; ✓ regulation of porphyrin synthesis; ✓ hereditary disorders of porphyrin metabolism (enzymopathias).	1. Satyanarayana U., Chakrapani U. «Biochemistry», Fourth Edition. – 2013. – P.210–212. 2. Gubsky Yu. Biological chemistry: textbook / edited by Yu. Gubsky. – Vinnytsia: Nova Knyha, 2018. – 2nd edition. – P. 247–250.
6. **Classification of porphyries:** ✓ erythripoietic (Gunter's disease); ✓ liver dependent porphyrias; ✓ photodermatitis; ✓ neurologic infringements.	1. Satyanarayana U., Chakrapani U. «Biochemistry», Fourth Edition. – 2013. – P. 212–214. 2. Gubsky Yu. Biological chemistry: textbook / edited by Yu. Gubsky. – Vinnytsia: Nova Knyha, 2018. – 2nd edition. – P. 250–252.

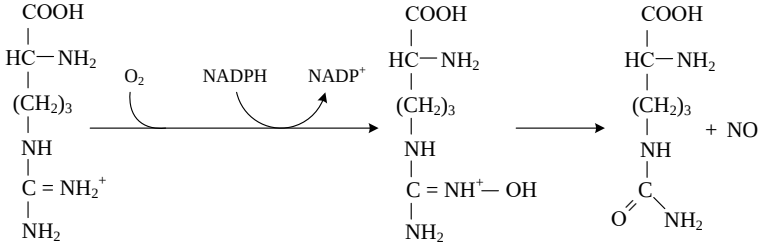
5. Tasks for independent work and self-control

5.1. What is the function of SAM, produced by the metabolism of Met, as shown?



5.2. Why is homocystinuria a concern? What role do vitamins B_6, B_{12}, and folate play in maintaining low homocysteine's levels?

5.3. Name the enzyme responsible for the catalysis of reaction bellow. What is the biological role NO.

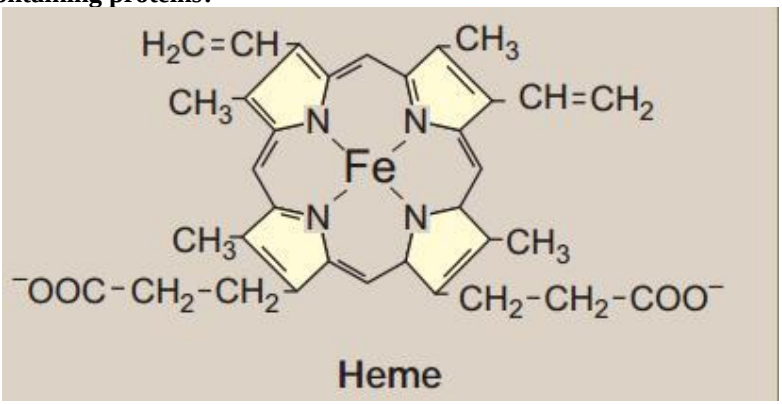


5.4. Where is the largest amount of serotonin synthesized in mammals?

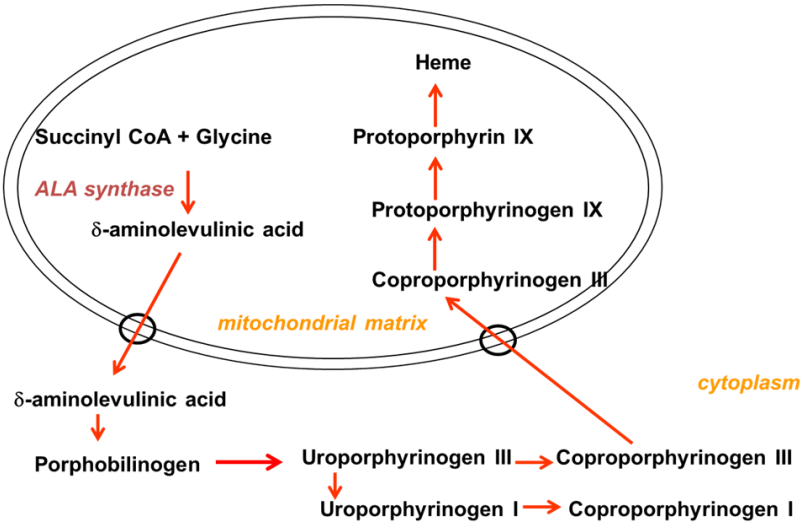
5.5. Write the name of enzyme which degrades serotonin to 5-hydroxyindoleacetate?

5.6. Write the functions of serotonin.

5.7. Based on the figure, to what series of the porphyrins (cyclic tetrapyrroles) does heme belong? What are some examples of heme-containing proteins?



5.8. Complete the scheme bellow:



5.9. Complete the table «Hereditary disorders of porphyrin metabolism (enzymopathias)»

Type of porphyria	Enzyme defect	Characteristics
Hepatic		
Erythropoietic (Gunter's disease)		

5.10. Situational task:

Daily excretion of porphyrins with urine at patient P. is 500 μg, and porphobilinogene – 800 μg, the contents of erythrocytes in blood is considerably reduced. From the anamnesis appeared, that patient P. used for preparation of food peep pottery in which as the analysis has shown, the contents of lead has been increased.

Infringement of what substance synthesis causes the given pathology?

What inhibition of enzyme has taken place?

6. Individual independent students work

1. Biological significance of nitric oxide, NO-synthase.

Practice protocol №25 «____» _____ 20___

Experiment. The determination of hemin group of hemoglobin.

Principle. The determination of hemin group of hemoglobin is based on its property to oxidize benzidine with hydrogen peroxide and the formation of a compound of blue colour.

Method.

1. Take a clean dry tube. Place into a tube 5 drops of 1% blood solution.
2. Add 5 drops of 1% benzidine in acetic acid.
3. Add 2–3 drops of 3% hydrogen peroxide.
4. The solution turns blue.

Result:

Conclusion:

The report is checked up _____

(The signature of the teacher, date)

Examples of Krock-1 tests

1. Hyperargininemia and argininuria are observed in a 25-year-old patient. The urea level is decreased in blood and urine. Which enzyme deficiency is observed?

A. Arginase
B. Glutamate dehydrogenase

C. Ornithine carbamoyl transferase
D. Argininosuccinate synthetase
E. Tryptophan-5-monooxigenase

2. In intestinal carcinoma about 60% of tryptophan is oxidized by means of serotonin pathway. Which vitamin requirement increases in that state?

A. Nicotinic acid
B. Folic acid
C. Pantothenic acid
D. Pyridoxine
E. Riboflavin

3. The urinary bladder cancer patient blood possesses high level of serotonin and hydroxyantranilic acid. Which amino acid metabolism disturbance is this connected?

A. Tryptophan
B. Phenylalanine
C. Tyrosine
D. Histidine
E. Alanine

4. Hormones and mediators are formed in metabolism of some amino acids. Which amino acid metabolism leads to the formation of gas mediator NO?

A. Arginine
B. Methionine
C. Leucine
D. Glycine
E. Serine

5. Obesity, necrotic changes in liver, adrenal gland insufficiency, low levels of phospholipids, choline and adrenaline in blood are revealed in 32-year-old patient. The most possible cause of that state development is the deficiency of:

A. Methionine
B. Alanine
C. Valine
D. Arginine
E. Glycine

6. Methyl groups are used in organism for the synthesis of the important substances such as creatine, choline, adrenaline. The source of these groups is the essential amino acid:

A. Methionine
B. Valine
C. Glycine

D. Cysteine
E. Arginine

7. A 57-year-old patient has ischemic disease of heart, arterial and venous thromboses, neuronal and psychiatric disorders, renal insufficiency. The level of homocysteine in blood is 3.5 times more than normal one (normal value is less than 10 mmol/l). The patient probably has:

A. Hyperhomocysteinemia
B. Hartnup's disease
C. Albinism
D. Alkaptonuria
E. Phenylketonuria

8. Vitamin-like substance choline is contained in phospholipids which are the main components of biological membranes. What sulphur-containing amino acid serves as the donor of methyl groups for the synthesis of choline?

A. Methionine
B. Serine
C. Glycine
D. Alanine
E. Threonine

9. Synthesis of phospholipids is disordered under the liver fat infiltration. Indicate which of the following substances can enhance the process of methylation during phospholipids synthesis?

A. Methionine
B. Citrate
C. Ascorbic acid
D. Glycerin
E. Glucose

10. The urinary bladder cancer patient blood possesses high level of serotonin and hydroxyantranilic acid. This is connected with the excessive delivery of an amino acid to the organism. Name the amino acid.

A. Tryptophan
B. Alanine
C. Hystidine
D. Methionine
E. Tyrosine

11. Pellagra may be caused by maize domination and low quantity of animal foodstuffs in the dietary intake. This pathology results from lack of the following amino acid:

A. Tryptophan
B. Isoleucine
C. Phenylalanine

D. Methionine
E. Histidine

12. An experimental animal that was kept on protein-free diet developed fatty liver infiltration, in particular as a result of deficiency of methylating agents. This is caused by disturbed generation of the following metabolite:

A. Choline
B. DOPA
C. Cholesterol
D. Acetoacetate
E. Linoleic acid

13. Vascular endothelium is characterized by high metabolic activity and synthesizes vasoactive substances. Among these substances there is a potent vasodilator synthesized from L-arginine. Name this vasodilator:

A. Nitrogen oxide
B. Acetylcholine
C. Adrenaline
D. Histamine
E. Bradykinin

14. The patient, who for a long time has been keeping to an unbalanced low-protein diet, developed fatty liver infiltration. Name the substance, absence of which in the diet can lead to this condition:

A. Methionine
B. Cholesterol
C. Biotin
D. Arachidonic acid
E. Alanine

15. Teeth fluoresce in the ultraviolet with a bright red colour and red colour of urine is observed in patients with erythropoietic porphyria (Gunther's disease). The deficiency of what enzyme causes the disease?

A. Uroporphyrinogen cosynthase III
B. Uroporphyrinogen synthase I
C. The delta-aminolevulinate synthase
D. Uroporphyrinogen decarboxylase
E. Ferrochelatase

16. Under the action of sunbeams blisters and increased skin pigmentation are revealed in a patient. The patient's urine becomes red in the opened air. Which of the following urine constituent's determination allows confirming the Gunther's disease?

A. Uroporphyrinogen I
B. Hemoglobin
C. Bilirubin
D. Creatinine
E. Acetone

17. A 33-year-old patient has been suffering from the disease for 10 years. Periodically he complains of acute abdominal pain, cramps, disorder of vision. His relatives are observed similar symptoms. The urine has red colour. The patient was hospitalized with the diagnosis of acute intermittent porphyria. The cause of the disease can be in the disorder of the synthesis of:

A. Heme
B. Insulin
C. Bile acids
D. Prostaglandins
E. Collagen

18. A 43-year-old workwoman of a chemical plant complains of general weakness, weight loss, apathy and somnolence. Chronic leadpoisoning is confirmed by laboratory tests – hypochromic anemia is revealed. In the blood the level of protoporphyrin is increased and the level of δ-aminolevulinic acid is reduced, which testifies to the disorder of the synthesis of:

A. Heme
B. DNA
C. RNA
D. Protein
E. Mevalonic acid

19. Increased skin sensitivity to sunlight is observed in a patient. The urine acquires a dark red colour after settling. Indicate the reason for such state:

A. Porphyria
B. Hemolytic jaundice
C. Albinism
D. Pellagra
E. Homogentisuria

20. A 12-year-old boy has an acute intermittent porphyria. The increase of concentration of what substance in the urine confirms the diagnosis?

A. Delta-aminolevulinic acid
B. Total bilirubin

C. Biliverdin
D. Heme
E. Indican

21. Under the action of sunbeams skin erythema, vesicular rash and itching are observed in a 5-year-old child. Laboratory investigation revealed the decrease of blood serum iron and increased urine excretion of uroporphyrinogen I. What is the most credible inhereditary pathology of the child?

A. Erythropoietic porphyria
B. Methemoglobinemia
C. Hepatic porphyria
D. Coproporphyria
E. Acute intermittent porphyria

22. A patient has the following symptoms: enlarged spleen, hemolytic anemia, ulcers, scars, erythema of the skin, increased sensitivity to ultraviolet, leukocytosis. Urine is of red-orange colour due to the presence of uroporphyrin I. What is the disease?

A. Gunther's disease
B. Hepatic porphyria
C. Hurler syndrome
D. Coproporphyria
E. Acute intermittent porphyria

23. What is the key enzyme of the heme biosynthesis?

A. Delta aminolevulinic acid synthase
B. Ala dehydrase
C. Uroporphyrinogen I synthase
D. Uroporphyrinogen III synthase
E. Uroporphyrinogen decarboxylase

24. Which of the following proteins contains porphyrins?

A. Myoglobin
B. Collagen
C. Globulins
D. Actin
E. Haptoglobin

25. The decreased activity of the enzyme delta aminolevulinic acid synthase causes the disorder of the heme biosynthesis and development of anemia. What is a cofactor of delta aminolevulinic acid synthase?

A. Pyridoxal phosphate
B. NAD^+

C. FAD
D. FMN
E. TPP

26. The ferrohelatase activity is drastically decreased in the liver cells of a patient as a result of poisoning by lead salts. The decrease of the synthesis of what substance is observed in the liver?

A. Heme
B. Porphobilinogen
C. Uroporphyrinogen III
D. Protoporphyrin IX
E. δ -aminolevulinic acid

27. A patient has hereditary erythropoietic porphyria as a result of the deficiency of uroporphyrinogen-III-cosyntase. Violation of the synthesis of what substance is observed in the patient?

A. Heme
B. Protein
C. AMP
D. GMP
E. Bile acids

28. Increased photosensitivity is one of the clinical symptoms of porphyria. Activation of which processes is the basis for the development of photodermatitis in porphyria?

A. Free radical oxidation
B. Microsomal oxidation
C. Mitochondrial oxidation
D. β-Oxidation of fatty acids
E. Oxidation of heme

29. Indicate a place of the porphyrin biosynthesis and their isomers in the human body:

A. Spleen
B. Kidneys
C. Lymph nodes
D. Muscular tissue
E. Heart

30. An increase in the concentration of what porphyrins in the urine is characteristic for the hepatic porphyria?

A. Uroporphyrin III
B. Coppropоrphyrin III
C. Uroporphyrin I

D. Coproporphyrin II
E. Protoporphyrin IX

31. Which precursors of the porphyrins have a diagnostic value?
A. δ-aminolevulinic acid
B. Succinic acid
C. Glutamate
D. β-keto-adipic acid
E. Pyruvate

32. What diseases can be attributed to the porphyrinuria group?
A. Chronic hepatitis
B. Hypovitaminosis
C. Hypervitaminosis
D. Gastritis
E. Pancreatitis

33. Succinyl-CoA is one of the substrates for the heme biosynthesis. Which metabolic pathway is a supplier of this substance?
A. Krebs cycle
B. Tissue respiration
C. Glycolysis
D. Glucuronate pathway
E. Pentose phosphate pathway

34. What substance is a direct precursor of carbon atoms in the heme part of hemoglobin?
A. Succinyl-CoA
B. Histidine
C. Alanine
D. Carbon dioxide
E. Aspartate

35. Which amino acid takes part in the biosynthesis of heme?
A. Glycine
B. Serine
C. Leucine
D. Tryptophan
E. Alanine

36. Which enzyme deficiency is involved in the development of hereditary coproporphyria?
A. Coproporphyrinogen oxidase
B. Uroporphyrinogen I synthase
C. Uroporphyrinogen III synthase
D. Delta aminolevulinic acid synthase
E. Uroporphyrinogen decarboxylase

37. A 64-year-old patient was a pilot in the past. In recent years, he worked with leaded petrol. Since that time, he has begun to notice a skin pigmentation of the hands. Periodically there are bubbles on the exposed parts of the body and limbs. Hepatomegaly and a disorder of the protein function of liver are observed too. The total protein in the blood is 100 g/l, albumin – 40 g/l globulin – 60 g/l. The iron content in the blood was reduced by 50%. Urine is of pink colour with a high content of coproporphyrins. Specify a possible pathology:

A. Porphyria
B. Hemolytic jaundice
C. Albinism
D. Pellagra
E. Homogentisuria

38. What is the main feature for the differential diagnostics is used to distinguish porphyrinuria from porphyria?

A. Decreased uroporphyrin III content in the urine
B. Anamnesis
C. The presence of porphobilinogen in the urine
D. Decreased coproporphyrin III content in the urine
E. Age

References:

1. Gubsky Yu. Biological chemistry : textbook / edited by Yu. Gubsky. – Vinnytsia : Nova Knyha, 2018. – 2nd edition. – 488 p.
2. Lecture materials.
3. Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – 799 p.
4. Textbook «Biological chemistry. Collection of test tasks» for English-speaking students of higher medical educational institutions of the Ministry of Public Health of Ukraine. – Poltava, 2018. – 148 p.

TOPIC №26.
Total module control

List of theoretical questions

1. Biological chemistry as a science. The objectives and assignments of biochemistry and its principal trends and parts. The significance of biochemistry in the development of medical science and practical health care.
2. Enzymes: definition, properties of enzymes as biological catalysts, difference between enzymes and inorganic catalysts. Specificity of enzymes.
3. Nomenclature and classification of enzymes.
4. Simple and conjugated enzymes. Role of non-protein part of conjugated enzymes. Structure of enzymes: active centres and allosteric sites. Levels of structural organization of enzymes.
5. Enzyme kinetics. Factors affecting enzymatic activity (concentration of enzyme, concentration of substrate, effect of temperature, effect of pH). Michaelis-Menten constant and equation.
6. Enzyme inhibition (reversible, irreversible, competitive, non-competitive).
7. Regulation of enzyme activity in the living system (allosteric regulation, feedback regulation, covalent modification of enzymes, activation of latent enzymes by limited proteolysis, cyclic nucleotides in regulation of enzymatic processes).
8. Diagnostical importance of enzymes (plasma specific and non-plasma specific enzymes. Changes in enzymatic activity of blood plasma and serum as diagnostic indexes (markers) of pathological processes in distinct organs – myocardial infarction, acute pancreatitis, liver disease, pathology of muscle tissue. Isoenzymes, their role in enzymodiagnostics.
9. Conception of turnover of material and energy (metabolism). Characterization of catabolic, anabolic and amphibolic reactions and their significance. Catabolic transformation of biomolecules: proteins, carbohydrates, lipids, its characterization.
10. Tricarboxylic acid (TCA) cycle (sequence of TCA cycle reactions, characterization of enzymes and coenzymes participating TCA cycle, energetic effect of TCA cycle).
11. Biological oxidation of substrates in cells. Reactions of biological oxidation and their functional significance.
12. Pyridine and dependent flavine dehydrogenases, structure of NAD, NADP, FAD and FMN, their role in reactions of oxidation and reduction.
13. Molecular organization of electron transport chain of mitochondria. Supramolecular complexes of respiratory chain in inner membrane of mitochondria.

14. Oxidative phosphorylation. Sites of oxidative phosphorylation. P/O ratio. Mechanisms of oxidative phosphorylation: chemical coupling hypothesis, chemiosmotic thery.

15. The scheme of chemiosmotic mechanism of coupling of electron transport in respiratory chain with ATP synthesis. Molecular structure and principles of functioning of ATP-synthetase. Inhibitors of electron transport in a respiratory chain of mitochondria. Uncouplers of electron transport and oxidative phosphorylation in a respiratory chain of mitochondria.

16. Glucose as an important metabolite in carbohydrate metabolism: general scheme of sources and turnover of glucose in the organism

17. Glucose oxidation under anaerobic conditions – glycolysis. Enzymatic reactions of glycolysis, energetic effect, regulation. Reactions of substrate level phosphorylation in glycolysis.

18. Metabolic pathways and substrates of gluconeogenesis, mechanisms of regulation, compartmentalization of enzymes, biological significance of the process.

19. Relations between glycolysis and gluconeogenesis (Cori cycle). Irreversible reactions of glycolysis and their shunt pathways. Glucose-lactate and glucose-alanine cycles.

20. Pentosophosphate pathway (PPP) of glucose utilization (scheme of reactions in oxidative and nonoxidative stages of PPP, enzymes and coenzymes of PPP reactions, biological significance of PPP, disorders of PPP in red blood cells, enzymopathias of glucose-6-phosphate dehydrogenase).

21. Oxidative decarboxylation of pyruvic acid: structure of multienzyme pyruvate dehydrogenase complex, peculiarities of function of pyruvate tdehydrogenase complex, mechanism of oxidative decarboxylation of pyruvate, role of vitamins and coenzymes in transformation of pyruvate to acetyl-CoA.

22. Enzymatic reactions of fructose turnover in human body. Hereditary enzymopathias of fructose metabolism. Enzymatic reactions of galactose metabolismr in human body. Hereditary enzymopathias of galactose metabolism.

23. Mechanism and peculiarities of enzymetic reactions of glycogenesis and glycogenolysis. Peculiarities of hormonal regulation of glycogen metabolism in liver and muscles.Hereditary disorders in enzymes of glycogen synthesis and breakdown. Glycogenoses, aglycogenoses, their characterization and causes.

24. Insulin dependent and noninsulin dependent forms of diabetes mellitus. Characterization of metabolic disorders in diabetes mellitus.

25. Catabolism of triacylglycerols: characterization of intracellular lipolysis, its biological significance; enzymatic reactions; neurohumoral

regulation of lipolysis: adrenalin, noradrenalin, glucagone, insulin; energetic balance of triacylglycerol oxidation.

26. Biosynthesis of triacylglycerols and phospholipids, the significance of phosphatidic acid.

27. β -Oxidation of long chain fatty acids: (location of the process of . β -oxidation of fatty acids; activation of fatty acids, the role of carnitin in transport of fatty acids into mitochondria;the sequence of enzymatic reactions in β -oxidation of fatty acids; energetic balance of β -oxidation of fatty acids)

28. Metabolism of ketone bodies.(enzymatic reactions of ketone bodies biosynthesis; reactions of utilization of ketone bodies, energetic significance; metabolism of ketone bodies in pathology. Mechanism of excessive accumulation of ketone bodies in diabetes mellitus and in starvation; the notions of ketoacidosis, ketonemia, ketonuria).

29. Biosynthesis of cholesterol in human body: (localization of the process and its signioficance stages of cholesterol biosynthesis, enzymatic reactions of biosynthesis of mevalonic acid regulation of cholesterol synthesis)

30. Pathways of cholesterol biotransformation (esterification, production of bile acids and steroid hormones, synthesis of vitamin D3, excretion from the body.

31. Atherosclerosis, mechanism of its development, role of genetic factors, hypercholesterolemia. Hypercolesterolemia in diabetes mellitus, myxoedema, obstructive jaundice, nephritic syndrome. Control of hyper-cholesterolemia

32. Pathways of formation and maintenance of free amino acid pool in human body. General pathways of free amino acid turnover.

33. Transamination of amino acids, substrates for transami nation reaction. Mechanism of transamination. Reaction. Aminotransferases, their localization in tissues and organs. Clinical diagnostic significance of determination of aminotransferases activity.

34. Types of reactions of amino acid deamination their final products. Mechanism of oxidative deamination, oxidases of D- and L- aminoacids, their enzymatic activity and specificity.

35. Decarboxylation of amino acids, decarboxylases. Production of biogenic amines (GABA, histamine. serotonin, dopamine). Decarboxylation of amino acids in putrifaction of proteins in intestines. Oxidation of biogenic amines.

36. Pathways of ammonia production. Toxicity of ammonia and mechanisms of its detoxification. Circulatory transport of ammonia (glutamine, alanine).

37. Biosynthesis of urea: enzymatic reactions, hereditary defects of enzymes involved in urea synthesis (enzymopathias of urea synthesis).

38. Specific pathways of metabolism of aromatic amino acids phenylalanine and tyrosine, sequence of enzymatic reactions. Hereditary enzymopathias of phenylalanine and tyrosine metabolism – phenylketonuria, alkaptonuria, albinism.

39. Metabolism of sulfur containing amino acids, reactions of methylation.

40. Biosynthesis of porphyrins, scheme of enzymatic reactions of heme biosynthesis. Regulation of porphyrin synthesis. Classification of porphyries – erythripoietic (Gunter's disease), liver.

APPENDIX 1

BIOCHEMICAL INDICES OF BLOOD SERUM

Component of blood serum	Concentration in molar units
1. Proteins of blood:	
total protein	65,0–85,0 g/L
globulins	23,0–35,0 g/L
albumins	35,0–50,0 g/L
protein coefficient	1,5–2,3
2. Non protein nitrogenous components:	
residual nitrogen	14,3–28,5 mmol/L
urea	3,3–8,3 mmol/L
uric acid	0,12–0,46 mmol/L
creatine	0,08–0,11 mmol/L
creatinine	0,06–0,076 mmol/L
ammonia nitrogen	29,4–47,0 mcmol/L
indican	1,19–3,13 mcmol/L
3. Indixes of carbohydrate metabolism:	
glucose	3,3–5,5 mmol/L
lactic acid (venous blood)	0,55–2,22 mmol/L
pyruvic acid	34–102 mcmol/L
sialic acids	2,0–2,33 mmol/L
4. Indixes of lipid metabolism:	
total lipids	4,0–8,0 g/L
triacylglycerols	0,59–1,77 mmol/L
cholesterol	3,0–6,5 mmol/L
phospholipids	2,0–4,6 mmol/L
lipoproteins:	
α-LP: for men	1,25–4,25 g/L
for women	2,5–6,5 g/L
β-LP	3,0–4,5 g/L
ketone bodies	0,034–0,43 mmol/L (no more than 2,5 mg %)
5. Indixes of pigment metabolism:	
total bilirubin	8,5–20,5 mcmol/L
direct bilirubin	1,0–5,0 mcmol/L
indirect bilirubin	1,7–17,0 mcmol/L
6. Indixes of mineral metabolism:	
sodium	137,0–144,0 mmol/L
potassium	3,8–5,3 mmol/L
total calcium	2,25–2,75 mmol/L
iron: for men	14,3–26,0 mcmol/L
for women	10,7–21,5 mcmol/L
chlorides	95,0–103,0 mmol/L
inorganic phosphate	1,0–2,0 mmol/L

APPENDIX 2

MAIN COMPONENTS OF THE ADULT PERSON URINE

Component	Concentration	
	in molar units	in units of mass (g/day)
Urea	333,0–583,0 mmol/day	20,0–35,0
Creatinine	8,0–16,0 mmol/day	0,8–2,0
Uric acid	1,5–4,4 mmol/day	0,3–0,8
Hippuric acid		0,4–0,8
Indican	46,0–56,0 mcmol/day	0,01
Sodium	100,0–200,0 mmol/day	2,0–4,0
Potassium	50,0–70,0 mmol/day	1,5–2,0
Calcium	1,2–3,7 mmol/day	0,1–0,3
Chlorides	100,0–250,0 mmol/day	
Phosphates	29,0–45,0 mmol/day	0,8–1,2
Total nitrogen		10,0–18,0
17-KS		5,0–25,0 mg/day
Ketone bodies	344,0–861,0 mcmol/day	20,0–50,0 mg/day
Creatinine clearance		80,0–120,0 mL/min
Relative density	1016–1022 g/L	

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