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THE WORKING BOOK FOR SELF-PREPARATORY WORK on biological and bioorganic chemistry (semester 4) in the field of knowledge 22 «Health care» in the specialty 222 «Medicine»

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L 31 **Larycheva O.** The Working Book for Self-Preparatory Work on Biological and Bioorganic Chemistry (Semester 4) in the Field of Knowledge 22 «Health Care»in the Specialty 222 «Medicine»: methodical instructions / O. Larycheva, O. Nevynskyi – Mykolaiv : Publishing edition of Petro Mohyla Black Sea National University, 2021. – 288 p. – (Methodical series ; issue 369).

The workbook covers all stages of students' preparation for practical classes in Biological and Bioorganic Chemistry 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.

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Topic 1.
Biochemical functions of nucleotides and nucleic acids

1. Objective: To study chemical structure of nucleotides, structure and functions of nucleic acids. To perform qualitative reactions for detection of nucleoproteins.

2. Actuality of the theme: DNA is the chemical basis of heredity and may be regarded as the reserve bank of genetic information. DNA is exclusively responsible for maintaining the identity of different species of organisms over millions of years.

Nucleic acids are the polymers of nucleotides (polynucleotides)held by 3' and 5' phosphate bridges. In other words, nucleic acids are built up by the monomeric units-nucleotides (it maybe recalled that protein is a polymer of amino acids). Nucleoproteins are proteins that are associated with nucleic acids. They can serve functional roles as enzymes, for example, telomerase in modifying the nucleic acid, or structural ones, as with histones in packaging chromatin.

3. Specific aims:

- ✓ To interpret chemical structure of nucleoprotein compounds, structure and function of nucleic acids, their role in protein biosynthesis.
- ✓ To know methods of isolation of nucleoproteins from tissues and qualitative reactions for detection of their components: a) biuret test for polypeptides, b) Trommer test for sugars (pentoses), c) silver probe for purine bases, d) molybdenum probe for phosphates.

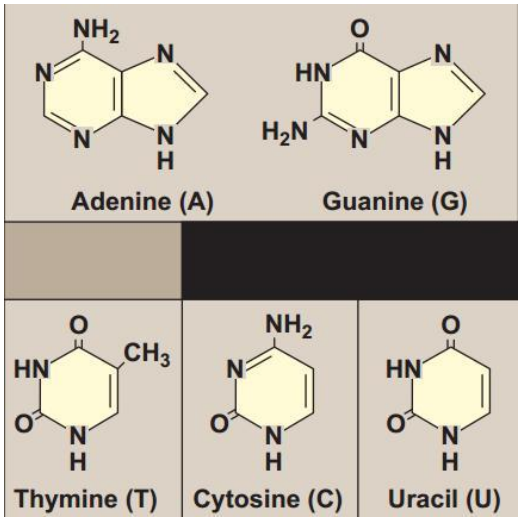
4. Reference card for the separate study of educational literature for the lesson preparation

Questions:	References:
1. **Biochemical functions of nucleic acids and nucleotides. Formation of nucleic acid chain from nucleotides.** ✓ Biochemical functions of nucleic acids; ✓ Biochemical functions of nucleotides; ✓ Formation of nucleic acid chain from nucleotides	1. Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 69–74. 2. Gubsky Yu. Biological chemistry: textbook / edited by Yu. Gubsky. – Vinnytsia: Nova Knyha, 2018. – 2nd edition. – P. 36. 3. Lecture notes.
2. **Constituents of nucleotides and nucleosides. Minor nitrogenous bases and nucleotides:** ✓ Constituents of nucleotides; ✓ Constituents of nucleosides; ✓ Minor nitrogenous bases and nucleotides	1. Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 69–73. 2. Gubsky Yu. Biological chemistry: textbook / edited by Yu. Gubsky. – Vinnytsia: Nova Knyha, 2018. – 2nd edition. – P. 37–41, 255–257. 3. Lecture notes.
3. **Free biologically active nucleotides and their biochemical functions: participation in metabolic reactions (ATP, NAD, NADP, FAD, FMN, CTP, UTP) and in their regulation (cyclic nucleotides – cAMP, cGMP):** ✓ biochemical functions of free biologically active nucleotides	1. Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 137–141, 223–224. 2. Gubsky Yu. Biological chemistry: textbook / edited by Yu. Gubsky. – Vinnytsia: Nova Knyha, 2018. – 2nd edition. – P. 270–272. 3. Lecture notes.
4. **Nucleic acids: structure, properties, stages of investigation. Primary structure of nucleic acids, polarity of polynucleotides, specific features of DNA and RNA structure**: ✓ Structure of nucleic acids; ✓ Properties of nucleic acids; ✓ Stages of investigation of nucleic acids; ✓ Primary structure of nucleic acids; ✓ Specific features of DNA and RNA structure	1. Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 73–82. 2. Gubsky Yu. Biological chemistry: textbook / edited by Yu. Gubsky. – Vinnytsia: Nova Knyha, 2018. – 2nd edition. – P. 42–43. 3. Lecture notes.

5.	**Structure, properties and biological significance of DNA. Experimental proves of DNA significance in heredity (phenomenon of transformation):** ✓ Structure of DNA; ✓ Properties of DNA; ✓ Biological significance of DNA; ✓ Experimental proves of DNA significance in heredity	1. Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 73–79. 2. Gubsky Yu. Biological chemistry: textbook / edited by Yu. Gubsky. – Vinnytsia: Nova Knyha, 2018. – 2nd edition. – P. 44–45, 272. 3. Lecture notes.
6.	**Secondary structure of DNA, role of hydrogen bonds in stabilization of secondary structure (Chargaff rule, Watson-Crick model), antiparallelism of chains:** ✓ Secondary structure of DNA; ✓ Role of hydrogen bonds in stabilization of secondary structure*;* ✓ Antiparallelism of chains.	1. Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 73–75. 2. Gubsky Yu. Biological chemistry: textbook / edited by Yu. Gubsky. – Vinnytsia: Nova Knyha, 2018. – 2nd edition. – P. 44–45, 272–273. 3. Lecture notes.
7.	**Tertiary structure of DNA. Physical-chemical properties of DNA: denaturation and renaturation of DNA:** ✓ Tertiary structure of DNA; ✓ Physical-chemical properties of DNA	1. Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 74–77. 2. Lecture notes.
8.	**Structure, properties and biological functions of RNA. Types of RNA: mRNA, tRNA, rRNA, snRNA; specific features of structure (secondary and tertiary) of different RNA types:** ✓ Structure, properties and biological functions of RNA; ✓ Types of RNA; ✓ Specific features of structure of different RNA types.	1. Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 79–82. 2. Gubsky Yu. Biological chemistry: textbook / edited by Yu. Gubsky. – Vinnytsia: Nova Knyha, 2018. – 2nd edition. – P. 44–45, 272–273. 3. Lecture notes.

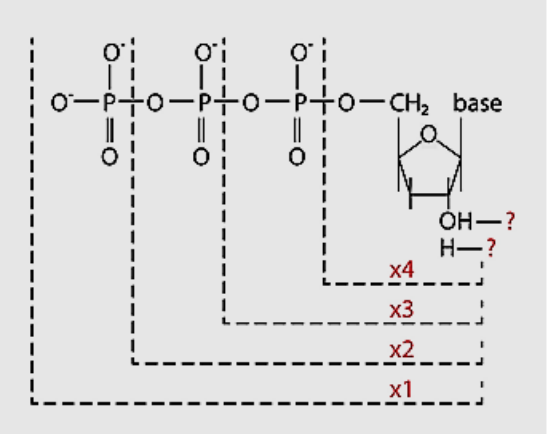
5. Tasks for independent work and self-control

5.1. Which nitrogenous base shown is used (as a component of a nucleotide) in DNA but not RNA synthesis? In addition to a purine or pyrimidine base, what are the other two components of a nucleotide?



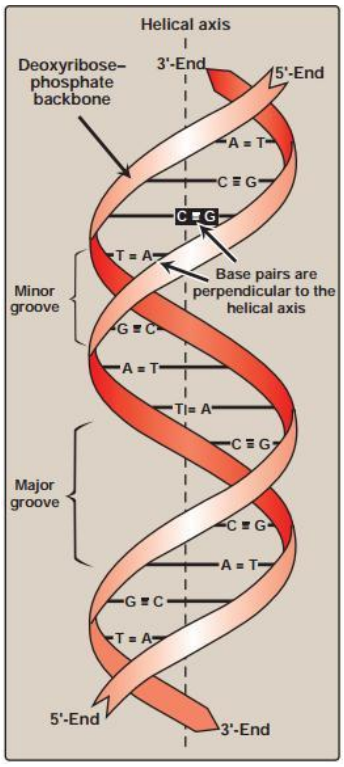
5.2. Why are nucleotides and nucleosides referred to as N-glycosides?

5.3. Name the structures represented below.



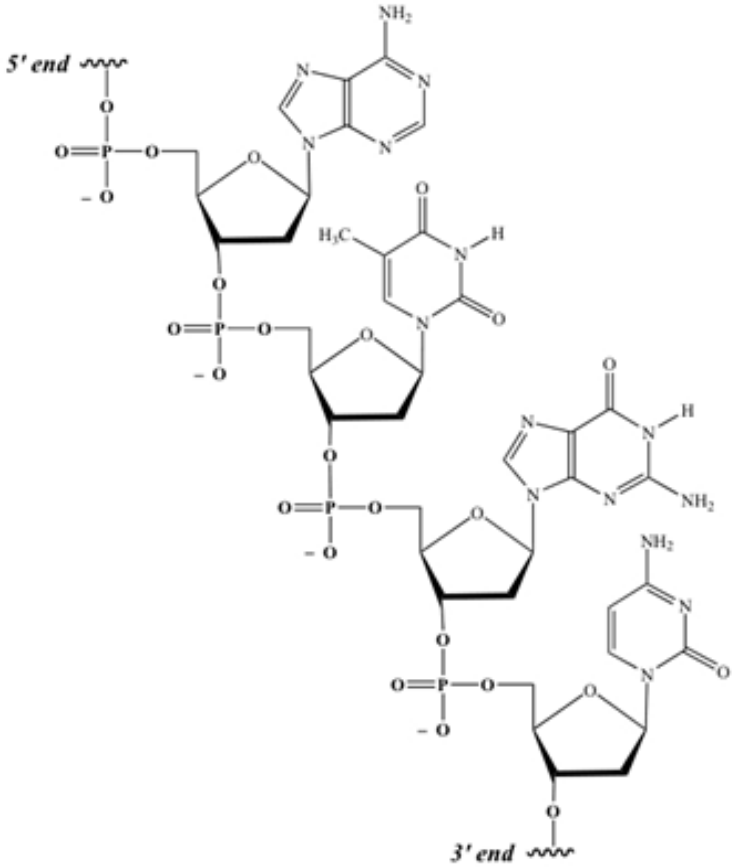
5.4. What type of bond links the dNMP monomers in each polymeric strand of the dsDNA molecule shown?

What holds the strands together?



5.5. What is the functional significance of A- and T-rich DNA compared to G- and C-rich DNA?

5.6. Make a daughters DNA strand using the template represented below. What nucleic acids do not obey Chargaff's rule?



5.7. Complete the chart «Classes of eukaryotic RNA»

Class	Types	Abundance	Functions
Ribosomal (rRNA)	28S, 18S, 5.8S, 5S	80 % of total	
Messenger (mRNA)	~10^5 different species	2–5 % of total	
Transfer (tRNA)	~60 different species	~15 % of total	
Small nuclear (snRNA)	~30 different species	≤ 1 % of total	
Heterogeneous nuclear (hnRNA)	~10^4 different species		

5.8. Which of the RNAs shown:

1. Accounts for the largest percentage of cellular RNA?

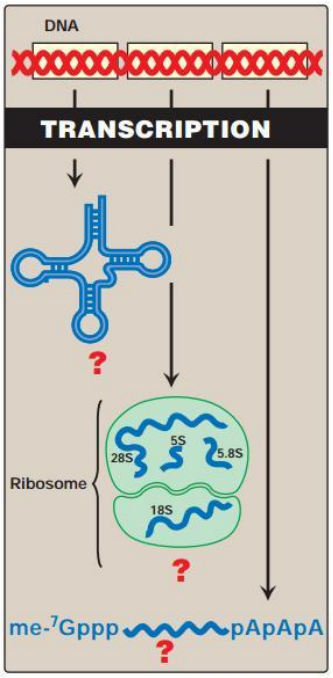
2. Carries amino acids to ribosomes?

3. Is extensively modified only in eukaryotes?

4. Contains a high percentage of modified bases?

5. Is a ribozyme in translation?

6. Contains extensive intrachain base-pairing?



5.9. How does RNA differ from DNA?

5.10. Situational tasks

a) DNA denaturation – is a violation of its native conformation to form single-stranded disordered molecules.

What structure of DNA stored after denaturation?

What bonds involved in the formation of DNA single chain?

Which proteins provide stability for DNA molecules?

b) Presents fragments of nucleic acids, such as:a) AUAUCG b) ATTCTCGA. Identify them as belonging to the RNA or DNA, and build a complementary chain.

6. Individual independent students work

1. Structure, properties and biological significance of nucleoproteins, phosphoproteins, lipoproteins, glycoproteins

2. Peculiarities in synthesis and in degradation of nucleoproteins, glycoproteins and proteoglycans.

Practice protocol №1 «____» _____ 20___

Qualitative reactions on structural components of nucleoproteins

Principle of the method. For investigation of chemical composition of nucleoproteins yeast cells are a convenient object. After hydrolysis the components of nucleoproteins can be detected in hydrolysate with reactions, which are specific to each constituent of nucleoproteins.

Reagents and materials. 500 mg of fresh yeasts or 100 mg of dried yeasts, 10 % solution of sulfuric acid, pipettes, filter paper, water bath.

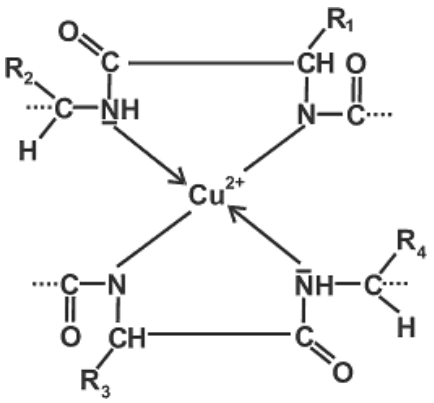
Preparation of the filtrate.

1. Place 100 mg of dried yeast into a clean, dry tube.
2. Add 4 ml of H_2SO_4 (10 %).
3. Place the tube into water bath (100°C) and incubate it for 60 min.
4. Cool and filtrate the liquid.

In the filtrate products of nucleoproteins are detected, i.e: polypeptides, purine and pyrimidine nitrogenous bases, ribose, deoxyribose, phosphoric acid.

Experiment 1. Biuret test for protein.

Principle of the method. The biuret test is a chemical test used for detecting the presence of peptide bonds. In the presence of peptides, a copper (II) ion forms violet-colored coordination complexes in an alkaline solution. The Biuret reagent is made of sodium hydroxide (NaOH) and hydrated copper (II) sulfate, together with potassium sodium tartrate. Potassium sodium tartrate is added to complex to stabilize the cupric ions. Proteins in the alkaline environment reduce Cu^{2+} to Cu^{+}, which forms a coordination complex with proteins, leading to a blue to pink color change.

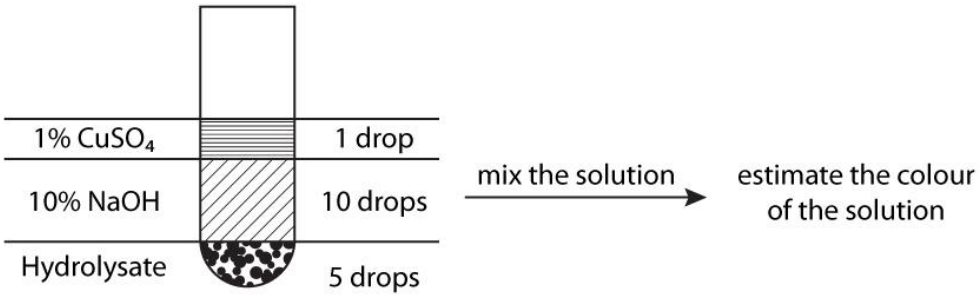


Procedure.

Take a clean and dry tube.

1. Introduce 5 drops of hydrolysate (obtained as described previously).
2. Add10 drops of NaOH (10 %). Mix the solution carefully.
3. Add 1 drop of $CuSO_4$ (1 %). Mix the solution carefully.

Estimate the color of the solution. Explain the results. Write conclusions and draw the peptide bond.



The biuret reaction can be used to assess the concentration of proteins because peptide bonds occur with the same frequency per amino acid in the peptide. The intensity of the color, and hence the absorption at 540 nm, is directly proportional to the protein concentration, according to the Beer-Lambert law.

Results:

Conclusion:

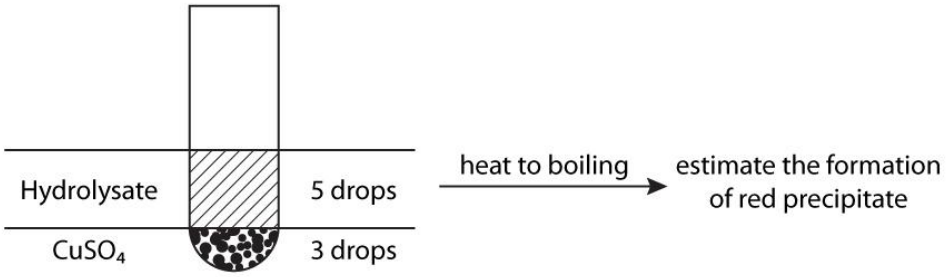
Experiment 2. Trommer's test for ribose and deoxyribose.

Principle of the method. Sugars with free hemiacetal group possess reductive properties and reduce some metal ions with changes in color or other effects. In Trommer's test copper ion (blue) is reduced to cuprous ion (orange red) in presence of reductive sugars (ribose, deoxyribose).

Procedure.

1. Add 3 drops of $CuSO_4$ solution to about 5 drops of hydrolysate (detected solution of sugar). Mix and then introduce drop by drop $CuSO_4$ solution. Stir incessantly until obtained $Cu(OH)_2$ dissolve.

2. Heat the blue solution to boiling. Red precipitate appears in the presence of reducing sugar. (Trommer's reagent contains copper sulphate with sodium hydroxide).



Results:

Conclusion:

Clinical diagnostic significance. Analysis of DNA is a routine investigation in diagnostics of hereditary diseases. It may be used for genotyping of fetal tissue in prenatal diagnostics, as well as in paternity determination.

Derivatives of nitrogenous bases are widely used in modern medicine. Mercaptopurine posses an antileukemic activity, as it serves as purines structural analogue and antimetabolite. Fluorouracil and fluorofur also has antitumor activity, as they are transformed into 5-fluoro-2-deoxyuridine 5'-monophosphate, which is a strong inhibitor of thymidylate synthase.

The report is checked up _____

(The signature of the teacher, date)

Examples of Krock-1 tests

1. The samples of the blood of a child and of a supposed father were directed for affiliation to the medical forensic examination. Which chemical components need to be identified in the explored samples of blood?
A. DNA
B. tRNA
C. rRNA
D. mRNA
E. mnRNA

2. There are about 200 clusters of genes synthesizing RNA in the nucleolar organizers of 13-15, 21 and 22 chromosomes of humans. The information of what type of RNA is encoded in these regions of chromosomes?
A. rRNA
B. tRNA
C. mRNA
D. snRNA
E. tRNA + rRNA

3. The body stores and transmits genetic information with the help of nucleic acids. Specify which type of RNA contains information about the order of amino acids in the protein:
A. mRNA
B. tRNA
C. 30s RNA
D. 70s RNA
E. 40s RNA

4. Nucleic acids include purine and pyrimidine nitrogen bases. Some nucleic acids contain minor nitrogenous bases. Which of the listed nitrogen bases belongs to minor ones?
A. Methyladenine
B. Thymine
C. Adenine
D. Guanine
E. Uracil

5. The nucleoli of the nuclei are damaged by an ionizing radiation in the tissue culture. The restoration of which organelles in the cytoplasm of cells becomes problematic?
A. Ribosomes
B. Lysosomes
C. Endoplasmic reticulum
D. Microtubules
E. The Golgi complex

6. Marked radioactive thymine was introduced into the culture medium with the cells. In what organelles of the cells will be a marked thymine detected?
A. Nucleus
B. Ribosomes
C. The Golgi apparatus
D. Lysosomes
E. Endoplasmic reticulum

7. What is necessary for the formation of the transport form of amino acids and the realization of the adaptive function?
A. tRNA
B. Revertase
C. GTP
D. mRNA
E. Ribosomes

8. DNA and RNA contain specific nitrogen bases. Which of the listed acids includes the thymidylate nucleotide?
A. DNA
B. tRNA
C. rRNA
D. mRNA
E. hnRNA

9. Nitrogenous bases of the purine and pyrimidine series, phosphoric acid residues and pentoses were found in the investigated hydrolyzate. Which class of organic substances the compound containing all these components belongs to?
A. Nucleotides
B. Chromoproteins
C. Phosphoproteins
D. Glycoproteins
E. Lipoproteins

10. A researcher discovered on an electronic microphotograph a structure formed by an octamer of histone proteins and a region of a DNA molecule that produces about 1.75 revolutions around them. What structure did the researcher discover?
A. Nucleosome
B. Elementary fibril
C. Semichromatide
D. Chromatide
E. Chromosome

11. Write the nucleotide sequence of one DNA strand, if the second chain has a sequence ATG-CCG-TAT-GCA-TT-:
A. – TAC-GGC-ATA-CGT-AA-
B. – GAC-GGC-ATA-CGT-AA-
C. – CAC-GGC-ATA-CGT-AA-
D. – ATG-TCG-TAT-GCA-TT-
E. – TCA-GCT-ATA-CGT-AA-

12. Under the administration of 5-bromouracil the biosynthesis of one of the listed substances is blocked. Specify it:
A. DNA
B. Protein
C. mRNA
D. rRNA
E. tRNA

13. In modern biochemical studies so-called «DNA diagnostics» is used to diagnose hereditary diseases, to detect the presence of certain viruses (including HIV) in the body, to identify an individual (gene fingerprinting in forensic medicine). Which method is used for this purpose?
A. Polymerase chain reaction
B. Chromatography
C. Electrophoresis
D. X-ray diffraction analysis
E. Immune-enzyme analysis

14. The transformation phenomenon discovered by F. Griffith in the study of the transformation of the non-pathogenic R-form of pneumococci into the pathogenic S-form in the interaction of the heat-killed S-form with the living R-form is determined by the transfer:
A. DNA
B. hnRNA
C. mRNA
D. tRNA
E. rRNA

15. Nucleotides containing minor nitrogenous bases are most often found in the molecules of:
A. tRNA
B. hnRNA
C. mRNA
D. DNA
E. rRNA

16. A peculiarity of the primary structure of all eukaryotic mRNA molecules and some viruses is the presence at the 5'-end:
A. 7-methylguanosine
B. 1-methylguanosine
C. 3-methylcytidine
D. 1-methyladenosine
E. 5-methylcytosine

17. A spatial model of the structure of a DNA molecule in the form of a double helix was proposed on the basis of studies of the nucleotide composition and structural analysis of this molecule. Who discovered the DNA structure?
A. D. Watson, F. Crick
B. O. Every, K. Mac McLeod, M. McCarthy
C. Chargaff
D. M. Wilkins
E. F. Griffith

18. Histones are positively charged proteins, which are concentrated in the nuclei of cells in the deoxyribonucleoproteins structure and they play an important role in the regulation of gene expression, containing a large number of:
A. Lysine and arginine
B. Asparagine and glutamine
C. Cysteine and methionine
D. Arginine and histidine
E. Lysine and histidine

19. Nucleic acids are characterized by a spatial configuration. The secondary structure of what of the nucleic acids looks like a leaf clover?
A. Transport RNA
B. Mitochondrial DNA
C. Messenger RNA
D. Nuclear DNA
E. Ribosomal RNA

20. The central dogma of a molecular biology is a realization of a hereditary information in the following direction:
A. DNA → RNA → protein
B. DNA → protein → RNA
C. Protein → RNA → DNA
D. RNA → DNA → protein
E. RNA → protein → DNA

21. Eukaryotic ribosomes consist of two subunits:
A. 60S and 40S
B. 50S and 30S
C. 70S and 40S
D. 60S and 30S
E. 50S and 40S

22. What is the specific nitrogenous base of ribonucleoproteins?
A. Uracil
B. Guanine
C. Adenine
D. Thymine
E. Cytosine

23. The spatial configuration of DNA is stabilized by hydrogen bonds. How many hydrogen bonds in the DNA molecule are formed between adenine and thymine?
A. 2
B. 3
C. 5
D. 8
E. 10

24. The spatial configuration of DNA is stabilized by hydrogen bonds. How many hydrogen bonds in the DNA molecule are formed between guanine and cytosine?
A. 3
B. 2
C. 5
D. 8
E. 10

25. The double helix of DNA is stabilized by hydrogen bonds. Between which nitrogenous bases of the DNA are hydrogen bonds formed?

A. A-T
B. G-A
C. C-T
D. U-A
E. C-A

Topic 2.
Research of the biosynthesis and catabolism of purine and pyrimidine nucleotides. Metabolic disorders

1. Objective: To learn reactions of synthesis and degradation of purine and pyrimidine nucleotides in normal conditions and in hereditary enzymopathias of this metabolic pathways. To learn the method of determination of uric acid in biological fluids and to interpret the obtained results.

2. Actuality of the theme: Uric acid is the end product of purinemetabolism in humans. The normalconcentrationof uric acid in the serumof adultsis in the range of 3-7 mg/dl. In women, it isslightly lower (by about 1 mg) than in men. Thedaily excretion of uric acid is about 500-700 mg.Hyperuricemia refers to an elevation in theserum uric acid concentration. This is sometimesassociated with increased uric acid excretion(uricosuria).Gout is a metabolic disease associated withoverproduction of uric acid. At the physiologicalpH, uric acid is found in a more soluble form assodium urate. In severe hyperuricemia, crystalsof sodium urate get deposited in the soft tissues,particularly in the joints. Such deposits arecommonly known as tophi. This causesinflammationin the joints result ina painfulgouty arthritis. Sodium urate and/or uric acidmay also precipitate in kidneys and ureters thatresults in renal damage and stone formation.

3. Specific objectives:

- ✓ To analyze the sequence of reactions of metabolism of purine nucleotides, disorders in uric acid metabolism and biochemical principles of gout development.
- ✓ To analyze the sequence of reactions of biosynthesis and catabolism of pyrimidine nucleotides.
- ✓ To conduct quantitative determination of uric acid in biological fluids and to interpret the obtained results.

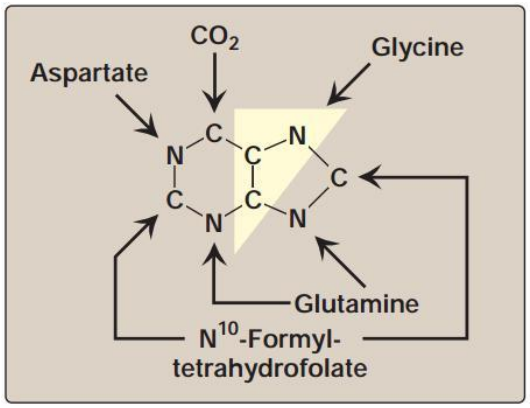
4. Reference card for the separate study of educational literature for the lesson preparation

	Questions:	References:
1.	**Biosynthesis of purine nucleotides; scheme of reactions of IMP synthesis; synthesis of AMP, GMP, ATP, GTP. Regulation of purine nucleotides synthesis on a principle of feedback inhibition:** ✓ Biosynthesis of purine nucleotides; ✓ Scheme of reactions of IMP synthesis; ✓ Synthesis of AMP, GMP, ATP, GTP; ✓ Regulation of purine nucleotides synthesis	1. Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 387–392. 2. Gubsky Yu. Biological chemistry: textbook / edited by Yu. Gubsky. – Vinnytsia: Nova Knyha, 2018. – 2nd edition. – P. 257–262. 3. Lecture notes.
2.	**Biosynthesis of pyrimidine nucleotides: reactions, regulation:** ✓ Reactions of biosynthesis of pyrimidine nucleotides; ✓ Regulation of biosynthesis of pyrimidine nucleotides.	1. Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 398–399. 2. Gubsky Yu. Biological chemistry: textbook / edited by Yu. Gubsky. – Vinnytsia: Nova Knyha, 2018. – 2nd edition. – P. 263–265. 3. Lecture notes.
3.	**Biosynthesis of deoxyribonucleotides. Formation of thymidyl nucleotides; inhibitors of dTMP biosynthesis as antitumor drugs:** ✓ Biosynthesis of deoxyribonucleotides; ✓ Formation of thymidyl nucleotides; ✓ Inhibitors of dTMP biosynthesis as antitumor drugs	1. Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 392; 399. 2. Lecture notes.

4.	**Catabolism of purine nucleotides; hereditary disorders of uric acid metabolism. Biochemical background of hyperuricemia, gout, Lesch-Nyhan syndrome:** ✓ Catabolism of purine nucleotides; ✓ Hereditary disorders of uric acid metabolism; ✓ Biochemical background of hyperuricemia; ✓ Biochemical background of gout; ✓ Biochemical background of Lesch-Nyhan syndrome	1. Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 392–397. 2. Gubsky Yu. Biological chemistry: textbook / edited by Yu. Gubsky. – Vinnytsia: Nova Knyha, 2018. – 2nd edition. – P. 265–268. 3. Lecture notes.
5.	**Catabolism of pyrimidine nucleotides:** ✓ Regulation of catabolism of pyrimidine nucleotides; ✓ Reactions of catabolism of pyrimidine nucleotides	1. Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 400. 2. Gubsky Yu. Biological chemistry: textbook / edited by Yu. Gubsky. – Vinnytsia: Nova Knyha, 2018. – 2nd edition. – P. 268. 3. Lecture notes.

5. Tasks for independent work and self-control

5.1. The origins of the atoms in a purine base during nucleotide de novo synthesis are shown. What is the order of addition of these atoms?

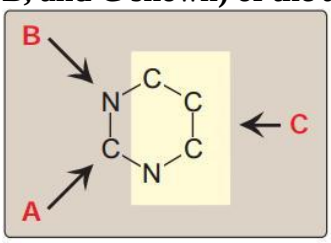


5.2. What enzyme catalyzes the committed step of purine nucleotide *de novo* synthesis. How is it regulated? What is the fate of IMP, the first purine nucleotide made?

5.3. Regulation of purine nucleotides biosynthesis.
Complete the chart:

Regulatory enzymes	Reaction	Inhibitors
PRPP synthetase	Ribose-5-phosphate + ATP → PRPP	
Glutamine PRPP amidotransferase	Glutamine + PRPP → 5-phosphoribosylamine	
Adenylosuccinate synthetase	IMP + GTP → Adenylosuccinate	
IMP dehydrogenase	IMP → Xanthosine monophosphate	

5.4. What are the sources (A, B, and C shown) of the atoms in a pyrimidine base?

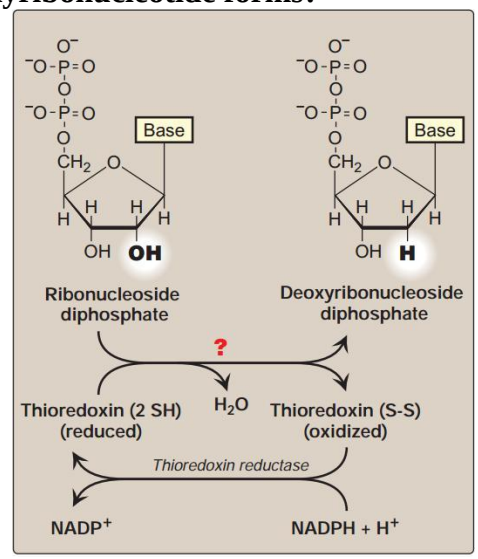


5.5. What is the regulated step in de novo pyrimidine synthesis? What is the first pyrimidine base formed, and how is it converted to the first pyrimidine nucleotide?

5.6. What enzyme (shown as a red question mark) catalyzes the reduction of the pyrimidine (and purine) ribonucleotides to their 2'-deoxyribonucleotide forms?

In what phase of the cell cycle is the enzyme active?

How is the enzyme regulated?



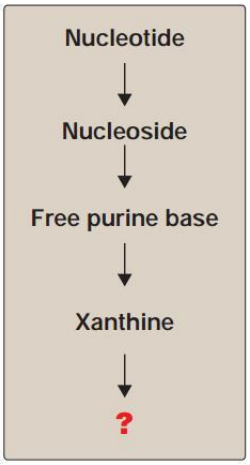
5.7. How is dTMP synthesis inhibited pharmacologically?

5.8. The molecular mechanisms of action of some antitumor drugs.

Drug	Affected enzyme and reaction	Explain why the inhibion of corresponding enzyme prevents thegrowth and division of cancer cells
5-Fluorouracil		
Methotrexate		
Hydroxyurea		

5.9. What highly oxidized purine (indicated by a red question mark) is the end product of purine nucleotide degradation?

How is it excreted from the body?



What enzyme catalyzes its production from xanthine?

Where is this enzyme found primarily?

5.10. Fill in the chart «Genetic defects associated with primary gout»

Genetic defect of enzyme	**Metabolic pathway**	**Explain why defect of corresponding enzyme leads to hyperuricemia and gout**
PRPP synthetase (lack of feedback control)	*De novo* synthesis of purine nucleotides	
HGPRT deficiency (Lesch-Nyhan syndrome)	Purine salvage pathway	
Glucose-6-phosphatase deficiency (Gierke's disease)	Glycogen degradation to glucose	

5.11. Oroticaciduria.

a) Indicate the defective enzymes.

b) Explain why this disorder lead to severe anemia and retarded growth.

c) Explain why feeding a diet rich in uridine results in improvement of the anemia, decreased excretion of orotic acid and is used to treat this disorder.

d) Deficiency of ornithine transcarbamoylase, enzyme of urea cycle, leads to the increased synthesis and excretion of orotic acid. This genetic disorder is called Reye's syndrome and considered as secondary orotic aciduria. Explain why urea cycle disorder leads to orotic aciduria.

5.12. Situational tasks

a) The 19-month-old baby detected retardation, spasticity, impulsive behaviour, auto aggression (with causing damage to itself). The content of uric acid in the blood – 1.96 mmol/L.

What is this disease?

Which enzyme deficiency occurs in a child?

What is the normal level of uric acid in the blood?

b) A 63-year-old woman was brought to the emergency department because of severe pain in her right big toe. The patient denied any trauma to the area. Gross examination revealed an overweight woman in distress. Examination of the toe revealed that it was swollen, red, and warm and tender to the touch. No other joints appeared to be affected. However, tophi were seen on the external part of her left ear. History revealed that this was the first episode of joint pain. It also revealed that the patient had begun taking «water pills» for hypertension about 4 months ago (her daughter later identified the medication as hydrochlorothiazide). The patient is a nondrinker. Fluid aspirated from the affected joint was negative for organisms but positive for needle-shaped crystals. The patient was started on ibuprofen (an NSAID) to reduce the inflammation and associated pain and was given a prescription for allopurinol.

Why might individuals with certain disorders of carbohydrate metabolism develop tophi?

6. Individual independent students work

Clinical and biochemical characteristics of uric acid metabolism disorders.

Practice protocol №2 «____» _____ 20___

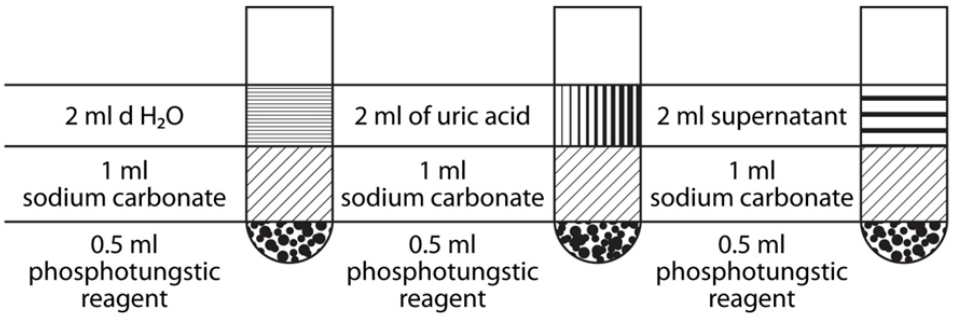
Experiment 1. Quantitative determination of uric acid in blood serum.

Principle of the method. Uric acid reduces phosphotungsten reagent with formation of blue color compound. The absorbance of light in 640 nm wavelength (optical density) is proportional to the concentration of uric acid in the tested specimen.

Reagents. Blood serum, 10 % solution of sodium carbonate, 0.35 M sulfuric acid, phosphotungstic reagent (Folin reagent), 30 μM solution of uric acid.

Procedure.

1. Transfer 0.5 ml of blood serum into centrifuge tube.
2. Add 4 ml of water. Mix the solution
3. Add 0.25 ml of 0.35 Mol sulfuric acid and 0.25 ml of 10 % solution of sodium dihydrogentungstate.
4. Incubate tubes for 5 min at room temperature
5. Centrifuge samples 10 min at 3000 rpm.
6. Collect a supernatant and determine the concentration of uric acid according to the scheme:



After 30 min the optical density of probes is measured in colorimeter on wavelength 640 nm (red filter, 590-700 nm).

Calculation of uric acid concentration is conducted according to the following formula:

$$C = \frac{A_{exp}}{A_{stand}} \times 30 \times 10$$

where:

C – concentration of uric acid in a blood serum sample, in mmoles/L

A_{exp} – optical density of the test sample

A_{stand} – optical density of uric acid standard

30 – concentration of uric acid in standard, μmoles/L

10 – dilution of serum sample.

Normal values of uric acid content in blood serum are 240–500 μmoles/L in males and 160–400 μmoles/L in females.

Results:

Conclusion:

Experiment 2. Quantitative determination of uric acid in urine.

Principle of the method is based on the property of uric acid to reduce phosphotungstic complex anion to a blue color compound. The quantity of phosphotungstic compound is determined by titration with potassium ferricyanide, which oxidizes phosphotungstic complex and decolorize it.

Reagents. Urine, phosphotungstig reagent (Folin reagent), 20 % solution of sodium carbonate, 0.01 n solution of potassium ferricyanide ($K_3[Fe(CN)_6]$), standard solution of uric acid (0.5 mg/ml).

Procedure.

1. Take two clean, dry flasks. To the first add 1.5 ml of urine (control) and to the second – 1.5 ml of standard solution of uric acid (control).

2. To both flasks add 1 ml of sodium carbonate solution (Na_2CO_3, 20 %) and 1 ml of Folin's reagent.

3. Titrate the content of flasks with $K_3[Fe(CN)_6]$ solution (0.01 N) up to disappearance of blue color.

The content of uric acid (in mg) in daily urine is calculated according to formula:

$$x = \frac{0.75 \times B \times D}{1.5 \times C}$$

where:

0.75 – amount of uric acid in standard, mg;

B – volume of $K_3[Fe(CN)_6]$ used for titration of urine sample, ml;

C – volume of $K_3[Fe(CN)_6]$ used for titration of standard sample, ml;

D – daily diuresis, ml.

Results:

Conclusion:

Clinical and diagnostic significance. Normal value of uric acid excretion with urine is 1.60–3.54 mmoles/day (270–700 mg/day). The normal values of uric acid concentration in blood serum is 0.05–0.06 mg/ml in males and 0.04-0.05 mg/ml in females. The increase of excretion of uric acid is observed in cases of diseases with an enhanced breakdown of nucleoproteins (leukoses, treatment with cytostatic drugs, action of ionizing radiation, combustion, rheumatism, hemolytic anemia, lead intoxication, toxicosis etc). A decrease of uric acid excretion is observed in kidney diseases (nephritis, renal insufficiency), progressive myodystrophie.

An increase in uric acid concentration in blood is called hyperuricemia. It is observed in gout, a disease, caused by hyperproduction of urates and their deposition in tissues, first of all in synovial sheets of joints. Deposition of uric acid is due to its poor solubility in water media. The drug of choice for the treatment ofgout is allopurinol. This is a structural analogue of hypoxanthine that competitively inhibits the enzyme xanthine oxidase. Further, allopurinol is oxidized to alloxanthine byxanthine oxidase. Alloxanthine, inturn, is a more effective inhibitor of xanthineoxidase. This type of inhibition is referred to as suicide inhibition. Inhibition of xanthine oxidase by allopurinol leads to the accumulation of hypoxanthine andxanthine. These two compounds are more soluble than uric acid, hence easily excreted. Besides the drug therapy, restriction in dietary intake of purines and alcohol is advised. Consumption of plenty of water will also beuseful. The anti-inflammatory drug colchicine is used for the treatment of gouty arthritis. Other anti-inflammatory drugs-such as phenylbutazone, indomethacin, oxyphenbutazone, corticosteroids are also useful.

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

Examples of Krock-1 tests

1. An oncologist prescribed methotrexate for a patient. With the lapse of time the target cells of the tumor lost sensitivity to this preparation. Gene expression of which enzyme is observed in this case?
A. Dihydrofolate reductase
B. Folate decarboxylase
C. Thiaminase
D. Folate oxidase
E. Desaminase

2. A 63-year-old man, suffering from gout, complains of pain in the region of kidneys. The presence of renal stone was discovered on ultrasonic examination. What process causes the renal stone to be formed?
A. Degradation of purine nucleotides
B. Catabolism of protein
C. Ornithine cycle
D. Degradation of a heme
E. Reduction of cysteine

3. A 60-year-old man had been operated on for prostate cancer. After 3 months he was given a course of radiation therapy and chemotherapy. The complex of drugs included 5-fluorodeoxyuridine that is the inhibitor of thymidylate synthase. The synthesis of what substance is primarily blocked by the action of this drug?
A. DNA
B. mRNA
C. rRNA
D. tRNA
E. Protein

4. A 6-year-old boy has signs of child cerebral paralysis. Laboratory investigation revealed an increased concentration of uric acid in the blood serum. Lesch-Nyhan syndrome is diagnosed. The genetic defect of which enzyme is the cause of such pathology?
A. Hypoxantine guanine phosphoribosyl transferase
B. UDP-glycosyl transferase
C. Hyaluronidase
D. Lactate dehydrogenase
E. UDP-glucuronyl transferase

5. The patient suffering from gout has been admitted to the clinic. What biochemical analysis should be performed to clarify the diagnosis?
A. Determination of the level of uric acid in the blood and urine
B. Determination of urea concentration in the blood and urine
C. Determination of the level of creatine in the blood
D. Determination of uricase activity in the blood
E. Determination of the concentration of amino acids in the blood

6. A protein thioredoxine participates in the reaction of ribose transformation to deoxyribose in course of deoxyribonucleotide production for DNA biosynthesis. It contains two SH groups, which in course of reaction are oxidized. What coenzyme is used in the restoration of a reduced form of thioredoxine?
A. $NADPH_2$
B. Glutathione
C. Coenzyme Q
D. $NADH_2$
E. AMP

7. A 50-year-old patient complains of joint pain, which is impaired by the weather change. The increase in the concentration of uric acid was revealed in the blood. The increased breakdown of what substance is the most likely cause of these changes?
A. AMP
B. CMP
C. UTP
D. UMP
E. TMP

8. A 69-year-old woman complains of pain in joints, restriction of movement in joints. The joints are swollen, looking as enlarged knots. An increased concentration of uric acid is detected in the blood and urine. What disease is characterized by these symptoms?
A. Gout
B. Pellagra
C. Alkaptonuria
D. Thyrosinosis
E. Liver cirrhosis

9. Allopurinol was administered to a patient with gout. What pharmacological property of allopurinol makes therapeutic effect in this case?
A. Competitive inhibition of xanthine oxidase
B. Increase of velocity of nitrogen substances excretion
C. Acceleration of catabolism of pyrimidine nucleotides
D. Retardation of reutilization of pyrimidine nucleotides
E. Acceleration of nucleic acid synthesis

10. Pterine derivatives – aminopterine and methotrexate are competitive inhibitors of dihydrofolate reductase, as result of which they depress tetrahydrofolic acid formation from dihydrofolate. These drugs lead to inhibition of single-carbon group transport. Which nucleotide biosynthesis is depressed?
A. dTMP
B. UMP
C. AMP
D. Carbamoyl phosphate
E. IMP

11. Patient complains of the pain in small joints. Joints are enlarged. It is an increased level of urates in the blood serum. Which substances metabolism is disordered?
A. Purines
B. Disaccharides
C. Amino acids
D. Pyrimidines
E. Glycerol

12. The decrease of uric acid concentration and the accumulation of xanthine and hypoxanthine were found in the blood of a 12-year-old boy. The genetic defect of the synthesis of what enzyme does it testifies to?
A. Xanthine oxydase
B. Arginase
C. Urease
D. Ornithine carbamoyltransferase
E. Glycerol kinase

13. Child shows delay of growth and psychic development, high amount of orotic acid is excreted with urine. This disease is observed as a result of the disorder of:
A. Pyrimidine nucleotide synthesis
B. Pyrimidine nucleotide breakdown
C. Purine nucleotide synthesis
D. Purine nucleotide breakdown
E. Convertion of ribonucleotides into deoxyribonucleotides

14. Physical and mental underdevelopment is observed in a child. Large quantity of orotic acid is excreted in the urine. This hereditary disease is a result of the next metabolic disorder:
A. Pyrimidine nucleotides synthesis
B. Pyrimidine nucleotides breakdown
C. Purine nucleotides synthesis
D. Purine nucleotides breakdown
E. Ornithine cycle of urea production

15. In a 1-month-old newborn baby an enhanced content of orotic acid in urine is detected. The child has a diminished weight gain. What treatment must be undertaken in order to correct metabolic disorders?
A. Injections of uridine
B. Injections of adenosine
C. Injections of guanosine
D. Injections of thymidine
E. Injections of histidine

16. Methotrexate is prescribed for the treatment of malignant tumors. It is a structural analogueue of folic acid, which is a competitive inhibitor of dihydrofolate reductase. The synthesis of what compounds is suppressed by the using of this drug?
A. Nucleotides of DNA
B. Fatty acids
C. Monosaccharides
D. Glycerophosphatides
E. Glycogen

17. For the treatment of gout a patient was administered allopurinol that is a structural analogueue of hypoxanthine. It has led to an increase in the excretion of the hypoxanthine with urine. What process is blocked with this treatment?
A. Formation of uric acid
B. The main way of synthesis of purine nucleotides
C. Synthesis of urea
D. Catabolism of pyrimidine nucleotides
E. Salvage pathway for the synthesis of purine nucleotides

18. Your 56-year-old male patient presents with intense redness, heat, and pain over his right great toe at the metatarsophalangeal joint. Fluid from this joint shows bifringent crystals. This disease is caused by the degradation of an excessive amount of which of the following?
A. Adenine
B. Thymine
C. Uracil
D. Cytosine
E. Ribose-5-phosphate

19. The biosynthesis of the purine ring occurs with the participation of ribose-5-phosphate by gradually building up of atoms of nitrogen, carbon, and ring closure. The source of ribose-5-phosphate is:
A. Pentose phosphate pathway
B. Glycolysis
C. Gluconeogenesis
D. Glycogenolysis
E. Lipolysis

20. A 50-year-old man is diagnosed with gout. Hyperuricemia is noted in biochemical blood analysis. Which of the following enzymes is involved in the formation of uric acid?
A. Xanthine oxidase
B. Pyruvate dehydrogenase
C. Succinate dehydrogenase
D. Epimerase
E. Transaminase

21. Gout is a disease developed due to the impaired exchange of nucleotides and the accumulation in the body of:
A. Uric acid
B. Urea
C. β-alanine
D. Homogentisic acid
E. Phenylpyruvic acid

22. Formation of deoxyribonucleotides of the purine series is derived from the corresponding ribonucleotides by:
A. Reduction of ribose into deoxyribose
B. Decarboxylation
C. Transamination
D. Hydroxylation
E. Phosphorylation

23. Thymidylate nucleotides are required for the normal replication process. Their synthesis occurs with the participation of thymidylate synthase. What is a coenzyme of thymidylate synthase?
A. Methylenetetrahydrofolate
B. Carboxybiotin
C. Thiamine diphosphate
D. Pyridoxal phosphate
E. Nicotinamide adenine dinucleotide

24. Some amino acids, derivatives of vitamins and phosphoric esters of ribose take part in the synthesis of purine nucleotides. The coenzyme form of which vitamin is the carrier of one-carbon fragments in the synthesis of purine nucleotides?
A. Folic acid
B. Pyridoxine
C. Riboflavin
D. Pantothenic acid
E. Nicotinic acid

25. Orotic aciduria is a hereditary disease characterized by a severe metabolic anemia and the deposition of orotic acid crystals in the organs and tissues. What drug should be prescribed in this case?

A. Uridine
B. Adenosine
C. Guanosine
D. Asparagine
E. Glutamine

27. Your 60-year-old female patient has psoriasis and has been treated with methotrexate for several years. She has no other medical problems and her preventive screenings, including fecal occult blood tests and colonoscopy, have all been normal. She has developed an anemia. Which of the following would you expect to find when working up her anemia?[2]

A. A macrocytic anemia
B. A microcytic anemia
C. Thalassemia
D. Spherocytes
E. A low vitamin B_{12} level

29. A patient has been recently diagnosed with colorectal cancer. The physician treats the patient with a combination of chemotherapeutic drugs, one of which is 5-fluorouracil (5-FU). 5-FU is effective as an anticancer drug because it inhibits which one of the following enzymes?[4]

A. Thymidylate synthase
B. Dihydrofolate reductase
C. Amidophosphoribosyl transferase
D. 5′-phosphoribosyl-1′-pyrophosphate (PRPP) synthetase
E. UMP synthase

31. A 6-month-old infant is seen by the pediatrician for developmental delay. Blood work shows megaloblastic anemia, although measurements of B_{12} and folate are in the high normal range. Urinalysis demonstrates, upon standing, the formation of a crystalline substance. Supplementation of the child's diet with uridine reversed virtually all of the clinical problems. The crystalline substance was most likely composed of which of the following?[6]

A. Orotate
B. Thymine
C. Uracil
D. Aspartate
E. Cytosine

26. Individuals with gout are given allopurinol for long-term management of the disease. In such individuals, which of the following bases would accumulate in the urine? [1]

A. Hypoxanthine and xanthine
B. Guanine and adenine
C. Hypoxanthine and guanine
D. Xanthine and guanine
E. Urate and xanthine

28. A researcher wants to develop a method of labeling purines with 15N for use in future spectroscopic studies. Purine synthesis will be done in a test tube using only the enzymes necessary to synthesize purines via the de novo pathway. Which starting materials should be labeled with the heavy nitrogen in order to maximize ^{15}N incorporation into purines?[3]

A. Aspartate, glycine, and glutamine
B. Aspartate, glycine, and N5-formimino tetrahydrofolate
C. Asparagine, glycine, and glutamine
D. Asparagine, glutamate, and glutamine
E. Aspartate, glycine, and glutamate

30. A patient exhibits fasting hypoglycemia and lactic acidosis under fasting conditions. Hepatomegaly is also evident. A glucagon challenge only releases about 10 % of the expected level of glucose from the liver. The patient has also developed gout due to an increase in the levels of which of the following metabolites?[5]

A. PRPP
B. Glutamine
C. ATP
D. NADH
E. dTTP

32. Your patient has sickle cell disease and is being treated with hydroxyurea. After 2 weeks on the drug, you find greatly reduced levels of most blood cell types, and the patient is removed from the drug to allow his blood cell counts to stabilize. One potential reason for this side effect of hydroxyurea treatment is its ability to alter the synthesis of which of the following metabolites?[7]

A. dUMP
B. 5′-phosphoribosyl-1′-amine
C. PRPP
D. Adenosylcobalamin
E. N5-methyltetrahydrofolate

33. An 18-month-old infant has had a history of recurrent bacterial and viral infections. The child has failure to thrive, developmental delay, and tremors. Physical exam shows a lack of peripheral lymphoid tissue. Blood work shows lymphopenia, but normal levels of B-cells and circulating immunoglobulins. This child most likely has a defect in which of the following enzymes?[8]

A. Purine nucleoside phosphorylase
B. Adenine phosphoribosyltransferase
C. Adenosine deaminase (ADA)
D. Adenosine kinase
E. Hypoxanthine guanine phosphoribosyltransferase

34. A 1-year-old boy was brought to the pediatrician due to a developmental delay, biting of his lips and fingers, and the presence of orange crystals in his diapers. Enzymatic analysis shows loss of 99 % of the activity of a particular enzyme. The defective enzyme in this disorder would normally utilize which of the following as a substrate?[9]

A. Guanine
B. Adenine
C. Adenosine
D. Guanosine
E. GMP

35. A 6-month-old boy was brought to the pediatrician due to frequent bacterial and viral infections. Blood work shows the complete absence of B and T cells. Radiographic analysis shows a greatly reduced thymic shadow. Treatment of the child with a stabilized protein reverses the deficiencies. This protein has which of the following activities?[10]

A. Converts adenosine to inosine
B. Converts adenine to AMP
C. Converts guanine to GMP
D. Converts IMP to XMP
E. Converts guanosine to inosine

36. A penicillin-allergic child was given a sulfonamide for otitis media. Human cells are resistant to sulfonamides due to which of the following?[11]

A. Sulfonamides inhibit a metabolic pathway not present in eukaryotic cells
B. Sulfonamides are specific for prokaryotic RNA polymerases
C. Sulfonamides are specific for prokaryotic DNA polymerases
D. Sulfonamides inhibit bacterial ribonucleotide reductase, but not eukaryotic ribonucleotide reductase
E. Sulfonamides inhibit prokaryotic mismatch repair, but not eukaryotic mismatch repair

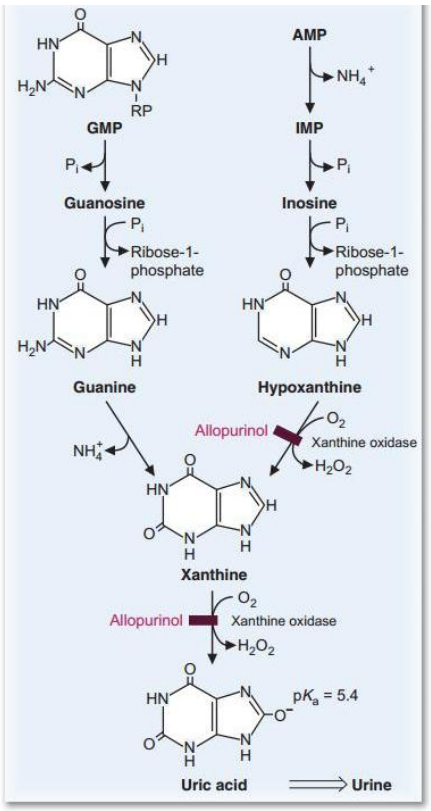
37. The primary route of carbon entry into the tetrahydrofolate (THF) pool is via the serine hydroxymethyl transferase reaction. Which of the following is required to convert that initial form of the THF into the form that can donate carbons to de novo purine synthesis?[12]

A. Water
B. FAD
C. Glycine
D. B_{12}
E. B_6

38. Many anticancer drugs are given to patients in their nucleoside form, rather than the nucleotide form. Which enzyme below will be required in the conversion of deoxyguanosine to dGTP?[13]

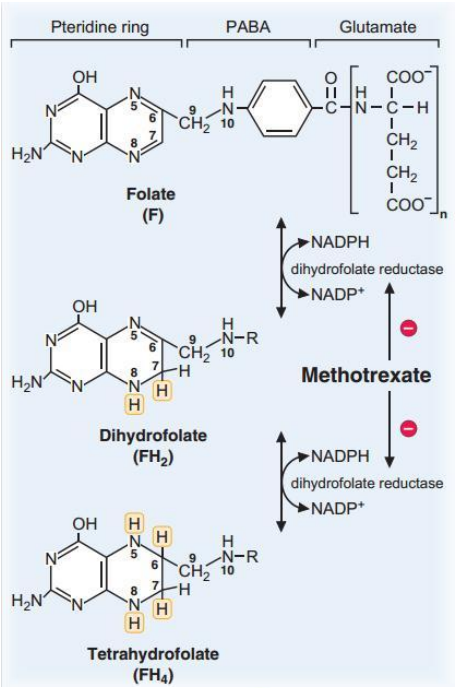
A. Ribonucleotide reductase
B. Deoxyguanosine kinase
C. Pyrimidine nucleoside phosphorylase
D. Adenine phosphoribosyltransferase
E. 5′-nucleotidase

1 The answer is: Hypoxanthine and xanthine. The target of allopurinol, the enzyme xanthine oxidase, catalyzes two reactions. The fi rst is the conversion of hypoxanthine (which is produced during the degradation of adenine) to xanthine and the second is the conversion of xanthine (which is produced during the degradation of guanine) to uric acid. Thus, in the presence of allopurinol, hypoxanthine accumulates from the degradation of adenine and xanthine accumulates via the guanine degradative pathway. Both of these compounds are more soluble than urate, thus alleviating the major problem in gout.



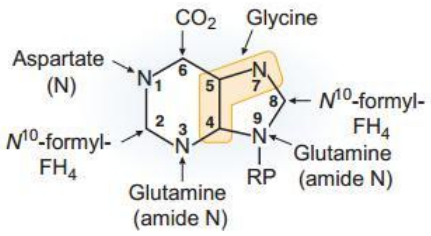
2 The answer is: A macrocytic anemia. Methotrexate acts by inhibiting dihydrofolate reductase such that THF cannot be formed (either from folate or dihydrofolate), and a functional folate deficiency results. The folate deficiency then results in a macrocytic anemia due to the lack of DNA synthesis. Red cell precursors increase in mass but cannot divide due to the lack of precursors for DNA replication. As a result, larger than normal cells are released into the circulation, although the overall red cell number decreases, resulting in an anemia. Both thalassemia and spherocytosis lead to microcytic anemia. Vitamin B12 levels would not be affected, and the normal occult blood tests and colonoscopy indicate that there is no bleeding leading to the anemia.

3 The answer is: Aspartate, glycine, and glutamine. The nitrogen in a purine ring is directly derived from glycine, glutamine, and aspartic acid. Glutamate, N5-formimino tetrahydrofolate, and asparagine do not directly donate nitrogen to the ring.



4 The answer is: Thymidylate synthase. 5-fluorouracil is a thymine analogue (thymine is 5-methyl uracil), which, after activa-tion in the cells to F-dUMP, binds tightly to thymidylate synthase and blocks the enzyme from converting dUMP to dTMP. By blocking thymidine synthesis, cells can no longer synthesize DNA and will not replicate. 5-FU has no direct effect on dihydrofolate reductase, amidopho-sphoribosyl transferase, PRPP synthase, or UMP synthase.

5 The answer is: PRPP. The patient has von Gierke disease, a lack of glucose-6-phosphatase activity. When this individual tries to produce glucose for export in the liver, glucose-6-phosphate accumulates, which then goes through either glycolysis (generating lactate) or the HMP shunt pathway, producing excess ribose-5-phosphate. The excess ribose-5-phosphate is converted to PRPP, which then stimulates the amidophosphoribosyl transferase reaction (the rate-limiting step of purine production) to produce 5′-phosphoribosy l1′-amine. This last reaction occurs because under normal cellular conditions, the concentrations of PRPP and glutamine aresignifi cantly below the Km values for

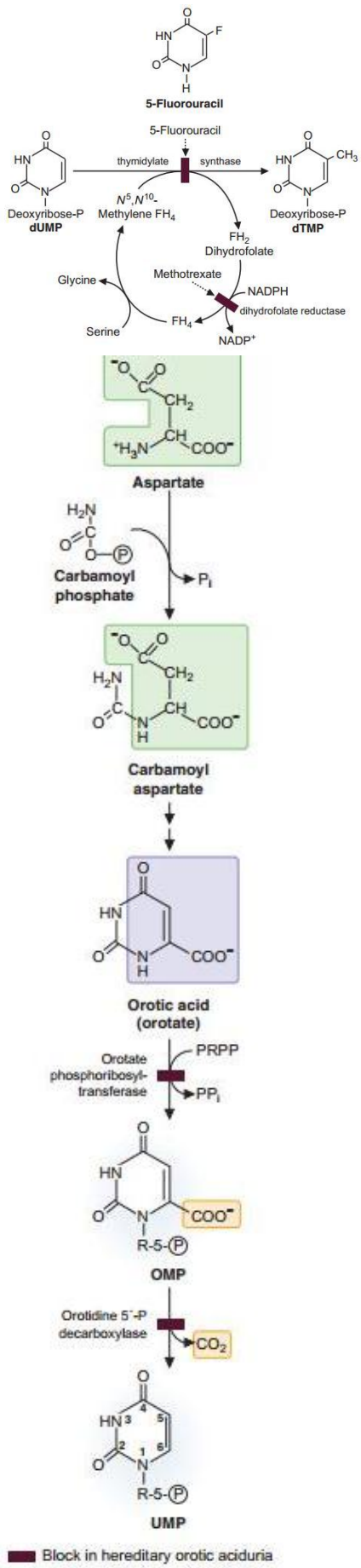


amidopho-sphoribosyl transferase. Any cellular perturbation that increases PRPP levels, then, will increase the rate of the reaction, producing purines that are not required by the cell. This leads to degradation of the excess purines, producing urate and leading to gout. The lactic acidosis associated with von Gierke disease also blocks the transport of urate from the blood into the urine, which contributes to the elevated uric acid levels seen in these patients. Von Gierke disease does not lead to elevated glutamine, ATP, NADH, or dTTP levels.

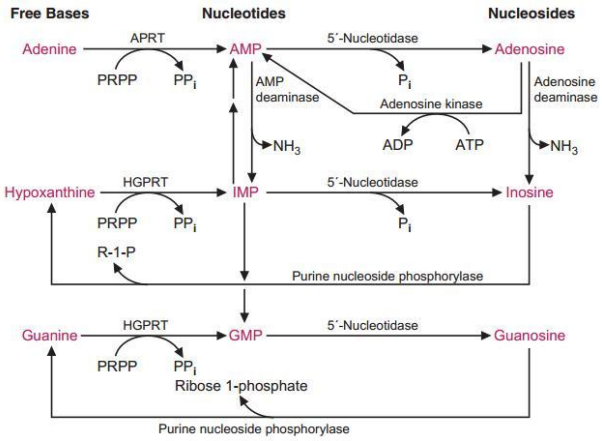
6 The answer is: Orotate. The child has hereditary orotic aciduria, a mutation in the UMP synthase that leads to orotic acid accumulation in the urine. Treatment with uridine bypasses the block and allows UTP, CTP, and dTTP synthesis. Uridine treatment also has the benefi cial effect of blocking further orotate production, as UTP inhibits carbamoyl phosphate synthetase II, the rate-determining step of pyrimidine production. As CPS-II is inhibited, less orotate is produced. The megaloblastic anemia is the result of inadequate DNA synthesis in the red cell precursors due to the lack of dTTP and dCTP. The crystals are made of orotate, as that is the compound that is accumulating. Uracil, thymine, and cytosine would not be synthesized in a patient with this disorder. Aspartate is very soluble and would not form crystals if it were to accumulate.

7 The answer is: dUMP. Hydroxyurea, in addition to inducing γ-chain synthesis of hemoglobin, is also an inhibitor of ribonucleotide reductase. If ribonucleotide reductase is inhibited, the cells' ability to generate deoxyribonucleotides will be impaired, and DNA synthesis will be hindered. Since blood cells are regenerated at a rapid rate, they are one of the first cells affected by an inhibition of DNA synthesis, and the result is a decrease of blood cells in the patient. Of the answers listed, the synthesis of only dUMP requires the activity of ribonucleotide reductase. Hydroxyurea does not interfere with the synthesis of N5-methyltetrahydrofolate, 5′-phosphoribosyl 1′-amine, PRPP, or adenosylcobalamin.

8 The answer is: Purine nucleoside phosphorylase. The child has purine nucleoside phosphorylase deficiency, which, for reasons not yet fully elucidated, specifi cally reduces T-cell counts but not B-cells. Purine nucleoside phosphorylase is one of the salvage enzymes that converts guanosine or inosine to the free base plus ribose-1-phosphate (adenosine is not a substrate for this enzyme). HGPRT defi ciency leads to Lesch-Nyhan syndrome, whose symptoms are quite different (there is no immune deficiency with an HGPRT defect). APRT deficiency leads to a buildup of an insoluble metabolite (2,8-dihydroxyadenine) that precipitates in the kidney and will lead to renal failure. ADA deficiency will lead to an immune disorder, but in ADA deficiency, both B and T cells are deficient. An adenosine kinase deficiency has not been reported in humans.



Salvage of bases. The purine bases hypoxanthine and guanine react with PRPP to form the nucleotides inosine and guanosine monophosphate, respectively. HGPRT catalyzes this reaction. Adenine forms AMP in a similar type of reaction catalyzed by APRT. Nucleotides are converted to nucleosides by 5′-nucleo-tidase. Free bases are generated from nucleosides by purine nucleoside phosphorylase (although note that adenosine is not a substrate of this enzyme). Deamination of the base adenine occurs with AMP and ADA. Of the purines, only adenosine can be phosphorylated by adenosine kinase directly back to a nucleotide.

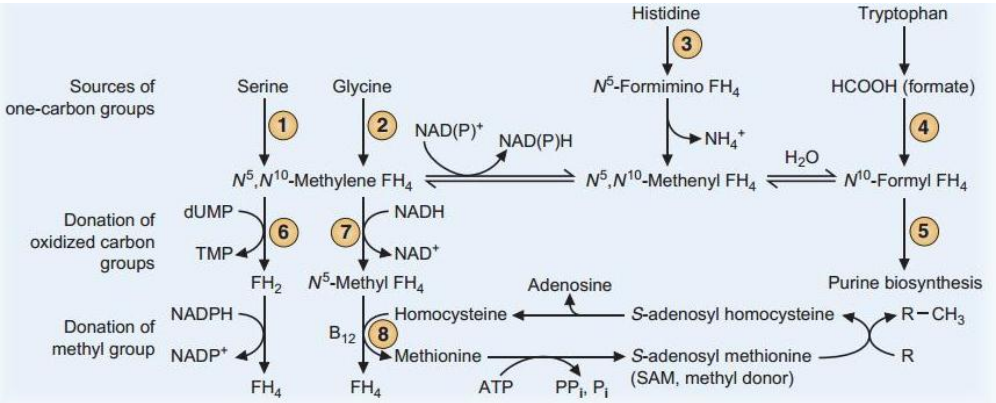


9 The answer is: Guanine. The patient has Lesch-Nyhan syndrome, a deficiency in HGPRT activity. HGPRT utilizes as substrates hypoxanthine, guanine, and PRPP, converting the free base to a nucleoside monophosphate (IMP and GMP). The enzyme does not utilize adenine, adenosine, guanosine, or GMP as a substrate. The reason for the aberrant behavior and developmental delay observed in this disorder has not yet been elucidated.

10 The answer is: Converts adenosine to inosine. The patient has the symptoms of ADA deficiency, which leads to severe combined immunodeficiency syndrome. ADA catalyzes the conversion of adenosine to inosine. IMP dehydrogenase converts IMP to XMP. APRT converts adenine to AMP. HGPRT converts guanine to GMP, and there is no enzyme that can convert guanosine to inosine in one step (guanase can convert guanine to xanthine in one step, but does not work on nucleoside substrates).

11 The answer is: Sulfonamides inhibit a metabolic pathway not present in eukaryotic cells. Sulfonamides inhibit the synthesis of THF, a compound that eukaryotic cells cannot synthesize (which is why folic acid is a required vitamin in the human diet). Via inhibition of THF synthesis, the target prokaryotic cells can no longer synthesize dTMP and purines and are unable to grow and replicate. Sulfonamides do not affect DNA polymerases directly, nor do they alter mismatch repair. Sulfonamides also have no effect on ribonucleotide reductase.

12 The answer is: Water. Serine donates a carbon to THF to form N5, N10-methylene THF. This is oxidized to form N5, N10-methenyl THF, which is then hydrolyzed with water to form N10-formyl THF. As such, glycine, FAD, B12, and B6 are not required for these conversions to take place.



13 The answer is: Ribonucleotide reductase. Deoxyguanosine would be first acted on by purine nucleoside phosphorylase, which would produce guanine and deoxyribose-1-phosphate. The guanine would be converted to GMP by HGPRT, and the GMP phosphorylated to GDP. The GDP would be reduced byribonucleotide reductase to dGDP, which is then phosphorylated again to produce dGTP. Since guanine is a purine, pyrimidine nucleoside phosphorylase is not required in this pathway. There is no deoxyguanosine kinase (the only purine nucleosides that can be phosphorylated by adenosine kinase are adenosine and deoxyadenosine). APRT only works for the adenine base, not guanine. The 5′-nucleotidase is not required as there are no dephosphorylation events in the pathway outlined.

Topic 3.
Research of DNA replication and transcription of RNA

1. Objective: To learn general principles of nucleic acids synthesis; stages of these processes; mechanisms of mutations and reparations of damaged DNA; development of hereditary diseases. To interpret mechanisms of action of antibiotics and other inhibitors of nucleic acids biosynthesis. To conduct quantitative determination of DNA in biological material.

2. Actuality of the theme: The biological information flows from DNA to RNA and from there to proteins. This is the central dogma of life. It is ultimately the DNA that controls every function of the cell through protein synthesis. In the process of nucleic acids biosynthesis may occurs various disorders in nucleotide sequence under the action of physical (ionizing and corpuscular irradiations), chemical (mutagens) and biological (viruses) agents. Pharmaceutical preparations are widely used in medical practice that can inhibit biosynthesis of nucleic acids in eukaryotes and retard tumor cells proliferation in oncologic patients.

3. Specific objectives:

- ✓ To conduct quantitative determination of DNA in biological material.
- ✓ To interpret molecular mechanisms of storage and transfer of genetic information, role of enzymatic systems, which provide semiconservative mechanism of DNA replication in prokaryotes and eukaryotes.
- ✓ To explain mechanism of action of enzymatic system of RNA transcription.
- ✓ To interpret biochemical mechanisms of gene recombination, gene amplification, specific features of regulation of gene expression in eukaryotes.
- ✓ To analyze consequences of genomic, chromosomal and gene mutations, mechanisms of action of the most known mutagens, biological significance and mechanisms of DNA reparations (reparation of UV-induced gene mutations).

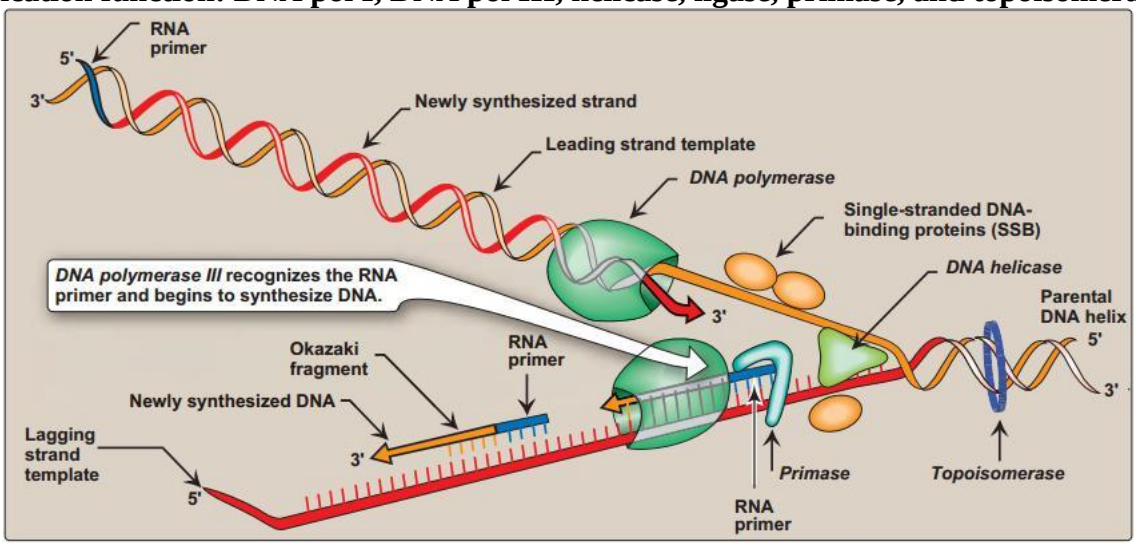
4. Reference card for the separate study of educational literature for the lesson preparation

	Questions:	References:
1.	**Biological significance of DNA replication. The sense of J.Watson and F.Crick discovery (1953). Semiconservative mechanism of replication, the scheme of Meselsohn's and Stahl's experiment:** ✓ Significance of DNA replication; ✓ Semiconservative mechanism of replication; ✓ The scheme of Meselsohn's and Stahl's experiment.	1. Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 523–524. 2. Gubsky Yu. Biological chemistry: textbook / edited by Yu. Gubsky. – Vinnytsia: Nova Knyha, 2018. – 2nd edition. – P. 274–278. 3. Lecture notes.
2.	**General scheme of DNA synthesis. Mechanisms of DNA replication in prokaryotes and eukaryotes:** ✓ Scheme of DNA synthesis; ✓ DNA replication in prokaryotes and eukaryotes.	1. Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 524–528. 2. Gubsky Yu. Biological chemistry: textbook / edited by Yu. Gubsky. – Vinnytsia: Nova Knyha, 2018. – 2nd edition. – P. 279–281. 3. Lecture notes.
3.	**General scheme of transcription. Stages and enzymes of RNA synthesis in prokaryotes and eukaryotes:** ✓ Scheme of transcription; ✓ Stages of RNA synthesis; ✓ Enzymes of RNA synthesis.	1. Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 543–547. 2. Gubsky Yu. Biological chemistry: textbook / edited by Yu. Gubsky. – Vinnytsia: Nova Knyha, 2018. – 2nd edition. – P. 288–290. 3. Lecture notes.
4.	**Posttranscriptional modifications of RNA.**	1. Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 547–549. 2. Gubsky Yu. Biological chemistry: textbook / edited by Yu. Gubsky. – Vinnytsia: Nova Knyha, 2018. – 2nd edition. – P. 290–291. 3. Lecture notes.

5.	**Inhibitors of replication and transcription as medical drugs; their mechanisms of action:** ✓ Inhibitors of replication as medical drugs; ✓ Inhibitors of transcription as medical drugs.	1. Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 528. 2. Lecture notes.
6.	**Regulation of gene expression in prokaryotes. Structure of Lac-operon of *Escherichia coli*: structural and regulatory genes, promotor, operator, regulator; repression and induction of Lac-operon function:** ✓ Regulation of gene expression in prokaryotes; ✓ Structure of Lac-operon of *Escherichia coli;* ✓ Repression and induction of Lac-operon function.	1. Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 567–569. 2. Lecture notes.
7.	**Peculiarities of structure and functioning of eukaryotic genome. Molecular organization of DNA in eukaryotes (exones, introns, repeated sequences). Nuclear chromatin and chromosomes of eukaryotes:** ✓ Structure and functioning of eukaryotic genome; ✓ Molecular organization of DNA in eukaryotes; ✓ Nuclear chromatin and chromosomes of eukaryotes.	1. Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 392–397. 2. Lecture notes.

5. Tasks for independent work and self-control

5.1. Use the figure to list the order in which the following enzymes of prokaryotic replication function: DNA pol I, DNA pol III, helicase, ligase, primase, and topoisomerase.

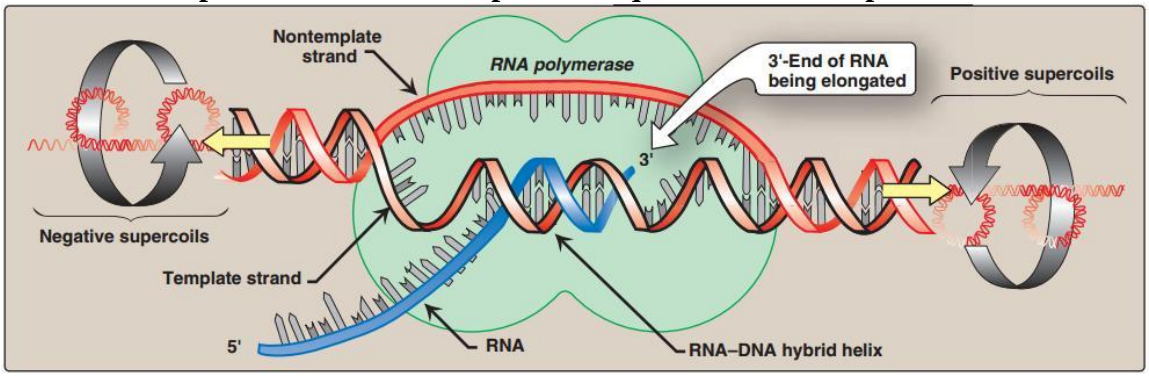


Describe the function of each of the listed enzymes.

What is the clinical significance of DNA gyrase inhibitors?

5.2. In what phase of the eukaryotic cell cycle does scheduled replication occur?

5.3. How does prokaryotic RNA pol recognize and bind the appropriate region of DNA to initiate transcription, as shown? Is a primer required in transcription?



5.4. Describe the properties of eukaryotic RNA polymerases:

Form of RNA polymerase	Products (classes of RNA)	Cell location	Sensitivity to α-amanitin
I			
II			
III			

5.5. What is an enhancer?

5.6 What three posttranscriptional processing events convert eukaryotic pre-mRNA to its functional form?

5.7. Individuals with tuberculosis are typically treated with a multidrug regimen that includes rifampin. How does rifampin work?

5.8. Why is α-amanitin (a cyclic peptide produced by some mushrooms) toxic to eukaryotic cells?

5.9. Complete the chart:

Inhibitors	Affected molecule	Affected process	Use for the treatment of
Doxorubicin (adriamycin)	DNA template (intercalates between the stacked base pairs)		
Dactinomycin (actinomycin D)	DNA template (interferes with the movement of RNA polymerase)		
Quinolones, novobiocin	Bacterial DNA gyrase (a topoisomerase)		
Analogueues of nucleosides: zidovudine (ZDV, AZT), dideoxyinosine, adenine arabinoside, cytosine arabinoside	Reverse transcriptase		
Efavirenz (nonnucleoside inhibitor)	Reverse transcriptase		
Rifampin	Prokaryotic RNA polymerase		

5.10. What is the basic structural unit of eukaryotic chromatin?

5.11. Situational task

A 23-year-old man, recently diagnosed with testicular cancer, is started on a drug regimen that includes dactinomycin (actinomycin D). Why is this drug cytotoxic?

6. Individual independent students work

Influence of physiologically active compounds on translation processes.

Practice protocol №3 «____» _____ 20___

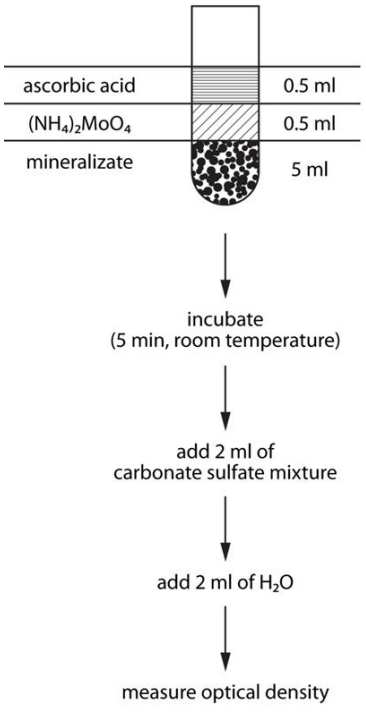
Experiment. Determination of DNA on basis of phosphorus content in a sample.

Principle of the method. The method consists on determination of phosphorus content as phosphate anion produced after mineralization of DNA specimen with concentrated sulfuric acid. The quantity of phosphorus is determined by ammonium molybdate in the presence of reducer (ascorbic acid). The product of reaction has a blue color; its intensity is proportional to quantity of phosphorus in a sample.

Procedure.

Isolation of DNA.

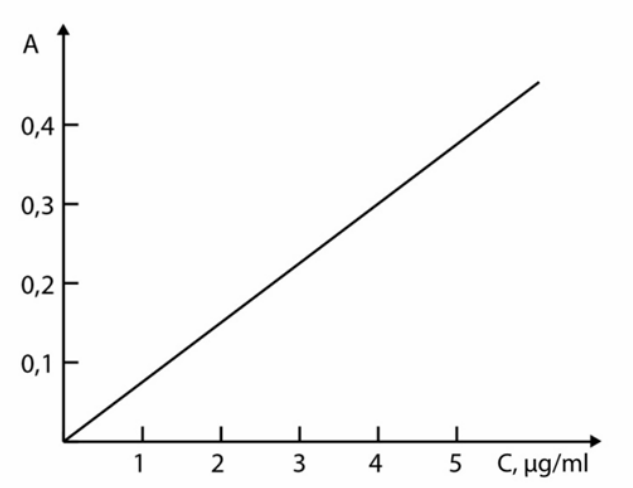
1. The equivalent of no more than 100 μL of liver tissue is placed in a microcentrifuge tube.
2. Add 200 μL of grinding buffer and dounce the tissue using a commercial or homemade dounce.
3. Add 200 μL of mini lysis solution.
4. Place tubes at 65°C for 30 min.
5. Add 60 μL of 5M potassium acetate and mix the solution by inverting.
6. Place tubes on ice for 30 min.
7. Spin tubes at max speed in a microcentrifuge and transfer supernatant to a new tube.
8. Add 1 mL of ice-cold 95-100 % ethanol and mix solution by inverting. Let tubes sit for 30 min at room temperature
9. Pellet the DNA by spinning at maximum speed in a microcentrifuge and remove ethanol supernatant
10. Resuspend pellet in 100 μL of 0.5 M ammonium acetate (NH_4OAc).
11. Add 400 μ1 of 70 % ethanol and mix the tubes by inverting. Let tubes sit at room temperature for 10 min.
12. Pellet the DNA by spinning at maximum speed in a microcentrifuge for 10 min.



13. Pour off ethanol and allow pellet at bottom of tube to air dry for 30 min to one hour.
14. Resuspend the pellet in 50 to 100 μL of dH_2O or TE buffer solution.

Determination of phosphorus.

1. Transfer 5 ml of obtained sample (mineralizate) into clean and dry tube.
2. Add 0.5 ml of $(NH_4)_2MoO_4$(5 %) and mix the tubes.
3. Add 0.5 ml of 1 % sol of ascorbic acid. Resuspend solution carefully.
4. Incubate tube for five min in room temperature.
5. Add 2 ml of carbonate-sulfite mixture and adjust the volume to 10 ml with dH_2O.
6. Incubate tube (10 min at room temperature)
7. Measured the optical density (extinction) on a photocolorimeter at a red filter.
8. Determine the content of phosphorus according to calibration curve.



Concentration of DNA in mg % is calculated according to formula:

$$C_{DNA} = a \times 10\ mg\%$$

where a –is a concentration of P in mg/ml.

The normal content of DNA in rat liver is 25–35 mg %

Results:

Conclusion:

Diagnostic and practical application. Determination of DNA content in tumor tissues has some prognostic significance in treatment of patients. More important is investigation of DNA in samples of biological origin (tissue bioptates, blood cells, spermatozoa, sediment obtained from urine, human hair, etc.) by the method of polymerase chain reaction (PCR) in diagnostics of viral, hereditary diseases and in identification of a person (DNA diagnostics). The content of DNA is used also for control the process of purification of subcellular fractions (microsomes, lysosomes, Golgi vesicles etc.).

The report is checked up _____

(The signature of the teacher, date)

Examples of Krock-1 tests

1. Name the nucleotides of DNA:
A. dAMP, dTMP, dGMP, dCMP
B. AMP, TMP, GMP, CMP
C. dUMP, dGMP, dCMP, dAMP
D. Adenine, guanine, cytosine, thymine
E. Deoxyadenosine, deoxyguanosine, deoxycytidine, deoxythymidine

2. The DNA molecule does not contain a nitrogenous base:
A. Uracil
B. Thymine
C. Deoxyribose
D. Guanine
E. Adenine

3. The primary transcript is:
A. Heteronuclear RNA that has not undergone posttranscriptional modification
B. DNA synthesized by a semiconservative method
C. RNA, obtained as a result of modification of the ends of the molecule
D. Connection of RNA with a protein in the cytoplasm with the formation of ribonucleoprotein
E. Matrix of DNA

4. Posttranscriptional processing includes:
A. Modification of 5'- and 3'-ends of mRNA, splicing
B. Modification of 5'- and 3'-ends of DNA, excision of introns, joining of introns
C. Modification of nitrogenous bases, modification of rRNA
D. DNA repair, exon splicing
E. DNA repair, splicing, and cross-linking of RNA introns

5. What is a process of synthesis of RNA on a DNA matrix called?
A. Transcription
B. Replication
C. Translation
D. Reparation
E. Recognition

6. What enzyme is involved in the process of repair in prokaryotes?
A. DNA-polymerase II
B. DNA-polymerase III
C. DNA-ligase
D. DNA-polymerase I
E. DNA-gyrase

7. The spatial correspondence of nitrogenous bases to each other in nucleic acid molecules is carried out according to the principle:
A. Complementarity
B. Cooperativity
C. Coplanarity
D. Collinearity
E. Induced correspondence

8. In case of poisoning by amanitine, a deathcap mushroom toxin, RNA-polymerase II is blocked. This leads to the blockage of:
A. Synthesis of mRNA
B. Synthesis of tRNA
C. Reverse transcription
D. Synthesis of primers
E. Processing of mRNA

9. mRNA is delivered in the granular endoplasmic reticulum to the ribosomes, containing both exons and introns. What process did not happen in the cell?
A. Processing
B. Translation
C. Prolongation
D. Transcription
E. Replication

10. For the treatment of urogenital infections, fluoroquinolones, inhibitors of the DNAgyrase enzyme, are used. Indicate the process that is disrupted by quinolones:
A. DNA replication
B. DNA repair
C. Amplification of genes
D. Recombination of genes
E. Reverse transcription

11. It has been established that toxins of fungi and some antibiotics can suppress the activity of RNA polymerase. What process is disrupted in the cell in case of inhibition of this enzyme?
A. Transcription
B. Processing
C. Replication
D. Translation
E. Reparation

12. During replication DNA-polymerase forms Okazaki fragments on the lagging polynucleotide chain of the «replicative fork». Name the enzyme, which stitches these fragments into a single chain:
A. DNA-ligase
B. Primase
C. Exonuclease
D. RNA-polymerase
E. DNA-polymerase

13. What is the process of synthesis of a new DNA molecule on a matrix of maternal DNA called?
A. Replication
B. Reparation
C. Transcription
D. Processing
E. Splicing

14. A patient is recommended to make DNA investigation to precise the diagnosis. Synthetic primers are known to be used, which contain:
A. Ribonucleotides
B. Desoxypibonucleotides
C. Amino acids
D. Pyrimidine nucleotides
E. Purine nucleotides

15. Antibiotic rifampicin, which is used for tuberculosis treatment, influences to the several biochemical processes. Point them:
A. Inhibits RNA-polymerase at the initiation stage
B. Inhibits DNA-polymerase at the initiation stage
C. Inhibits DNA-ligase
D. Inhibits amino-acyl-tRNA-synthetase
E. Inhibits the protein factors action at the protein synthesis

16. RNA of the human immunodeficiency virus (HIV) has penetrated into the leukocyte and with the help of the enzyme revertase (reverse transcriptase) it performs synthesis of the viral DNA in the cell. This process is based on:
A. Reverse transcription
B. Depression of the operon
C. Reverse translation
D. Inverse replication
E. Repression of the operon

17. The complementary RNA transcript is synthesized on the DNA template in the process of transcription in the nucleus of the cell. Which enzyme catalyzes this process?
A. DNA-dependent RNA polymerase
B. Primase
C. DNA-ligase
D. DNA-polymerase
E. DNA-ase

18. In case of a random use of death cap mushrooms (Amanita phalloides), containing the toxin α-amanitin, the inhibition of the following enzyme occurs:
A. RNA polymerase II
B. DNA-polymerase
C. DNA-synthetase
D. Peptidyltransferase
E. Translocase

19. Oncogenic viruses use the reverse transcription process to transfer their information from RNA to DNA. Indicate by which enzyme the hybrid RNA-DNA is formed:
A. Revertase
B. RNA-polymerase
C. Transcriptase
D. DNA-synthetase
E. Ribonuclease

20. 5-Fluorouracil is a medication used to treat cancer. It acts as a competitive inhibitor of enzymes that participate in the synthesis of DNA. Specify which enzyme is blocked in this process:
A. Thymidylate synthase
B. Uracil synthetase
C. Adenine synthetase
D. Guanosine synthase
E. Cytosine synthase

21. The medicinal preparation specifically inhibits the activity of reverse transcriptase. What is pharmacological action of the substance?
A. Antiviral
B. Antineoplastic
C. Antimicrobial
D. Immunosuppressive
E. Radioprotective

22. A patient suffering from tuberculosis was prescribed an antibiotic rifampicin. Inhibition of what biochemical process underlies the therapeutic effect of the drug?
A. Initiation of transcription
B. Termination of transcription
C. Elongation of transcription
D. Replication
E. Initiation of translation

23. An antibiotic rifampicin using to treat tuberculosis has the following pharmacological properties:
A. It inhibits RNA-polymerase at the initiation stage
B. It inhibits DNA-polymerase at the initiation stage
C. It inhibits DNA-ligase
D. It inhibits aminoacyl-tRNA synthetase
E. It inhibits the effect of protein translation factors

24. A number of hereditary diseases are caused by mutations in the areas of genes that determine the beginning or the end of introns. Which process removes introns, and then binds exons?
A. Splicing
B. Transcription
C. Recombination
D. Replication
E. Translation

25. According to the model of a double DNA helix that was suggested by Watson and Creek, it was established that one of chains would not be lost during replication and the second chain would be synthesized complementary to the first one. What way of replication is it?
A. Semiconservative
B. Analogueous
C. Identical
D. Dispersed
E. Conservative

26. Pterine derivatives – aminopterine and methotrexate are competitive inhibitors of dihydrofolate reductase, as result of which they depress tetrahydrofolic acid formation from dihydrofolate. These drugs lead to inhibition of single-carbon group transport. The biosynthesis of which polymer is inhibited?
A. DNA
B. Protein
C. Homopolysaccharide
D. Ganglioside
E. Glycosaminoglycan

27. The doubling of the DNA molecule, preceding mitosis, occurs according to the mechanism:
A. Semiconservative replication
B. Conservative replication
C. Polymerization
D. Disperse replication
E. Esterification

28. The high mutation rate of the human immunodeficiency virus (HIV) is due in part to a property of which of the following host cell enzymes?[1]
A. RNA polymerase
B. DNA polymerase
C. DNA primase
D. Telomerase
E. DNA ligase

29. The sequence of part of a DNA strand is the following: –ATTCGATTGCCCACGT–. When this strand is used as a template for DNA synthesis, the product will be which one of the following?[2]
A. ACGTGGGCAATCGAAT
B. UAAGCUAACGGGUGCA
C. ACGUGGGCAAUCGAAU
D. TAAGCTAACGGGTGCA
E. TGCACCCGTTAGCTTA

30. The isolation of nascent Okazaki fragments during DNA replication led to the surprising discovery of uracil in the fragment. The uracil is present due to which of the following?[3]
A. The need for a primer
B. Chemical modification of thymine
C. An error in DNA polymerase
D. Failure of mismatch repair
E. Deamination of cytosine

31. An IV drug user is tested, and found positive, for infection by HIV. If the patient is only placed on one antiviral medication, viral loads will initially be reduced, but will then rapidly increase. The resistance to the drug occurs due to which of the following?[4]
A. Lack of error checking in RNA polymerase
B. Lack of error checking in DNA polymerase
C. Lack of DNA repair enzyme systems in HIV-infected cells
D. Incorporation of uracil in the RNA genome of HIV
E. Incorporation of thymine in the RNA genome of HIV

32. A woman visits her physician due to fever and pain upon urination. Urinary analysis shows bacteria, leukocytes, and leukocyte esterase in the urine, and the physician places the woman on a quinolone antibiotic (ciprofloxacin). The mammalian counterpart to the bacterial enzyme inhibited by this drug is which of the following?[5]
A. Topoisomerase
B. DNA polymerase α
C. Ligase
D. Primase
E. Helicase

33. You see a 2-year-old child of Ashkenazi Jewish descent who is very small for her age. The patient exhibits a long, narrow face, small lower jaw, and prominent eyes and ears. The child is very sensitive to being outdoors in the sun, often burning easily, with butterfly-shaped patches of redness on her skin. Upon testing, the child is also slightly developmentally delayed. The defective protein in this child is which of the following?[6]
A. DNA helicase
B. DNA ligase
C. RNA polymerase
D. DNA polymerase
E. Reverse transcriptase

34. A scientist is replicating human DNA in a test tube and has added intact DNA, the replisome complex, and the four deoxyribonucleoside triphosphates. To the surprise of the scientist, there was no DNA synthesized, as determined by the incorporation of radio-labeled precursors into acid-precipitable material. The scientist's failure to synthesize DNA is most likely due to a lack of which of the following in his reaction mixture?[7]
A. Ribonucleoside triphosphates
B. Reverse transcriptase
C. Templates
D. Dideoxynucleoside triphosphates
E. Sigma factor

35. A careful analysis of cellular components discovers short-lived RNA species in which an adenine nucleotide is found with three phosphodiester bonds (linked to the 2′, 3′, and 5′ carbons). This transient structure is formed during which of the following processes?[8]
A. Splicing of hnRNA
B. mRNA polyadenylation
C. mRNA cap formation
D. Transcription of microRNAs
E. Transcription of rRNA

36. A patient suffering from chills, vomiting, and cramping was rushed to the emergency department. He had eaten wild mushrooms for dinner that he had picked earlier in the day. His symptoms are due to an inhibition of which of the following enzymes?[9]
A. RNA polymerase II
B. RNA polymerase I
C. RNA polymerase III
D. Telomerase
E. DNA primase

37. A 22-year-old woman sees her physician for a variety of complaints over the past year. These include fevers that come and go, fatigue, joint pain and stiffness, a butterfly rash on the face, sores in her mouth, easy bruising, and increased feelings of anxiety and depression. A diagnostic blood test is likely to show autoimmune antibodies directed against which class of molecules?[10]
A. Ribonuclear protein complexes
B. DNA polymerases
C. Carbohydrates
D. tRNA complexes
E. Peroxisomal proteins

38. A young child of Mediterranean parents was brought to the pediatrician due to lethargy, tiredness, and pallor. Blood analysis revealed a microcytic anemia, although iron levels were normal. What test hould be run to determine that the child has a variant of thalassemia?[11]
A. Western blotting of the peptide chains in hemoglobin
B. PCR of the gene for RNA polymerase
C. Western blot of snurps in the child
D. Western blot of TFIID
E. PCR of the gene for γ-globin in the child

39. When first discovered, introns were not thought to code for a functional product. Recently, however, introns have been found to code for products that can regulate the expression of a large number of genes. This regulation occurs at which stage of gene expression?[12]
A. Degradation of the mRNA
B. Posttranscriptional processing
C. Export of mRNA into the cytoplasm
D. Ribosome biogenesis
E. Transcription of mRNA

40. A man with a bacterial infection was prescribed rifampin to resolve the infection. Rifampin does not affect eukaryotic cells due to which of the following?[13]
A. Structural differences in RNA polymerase between eukaryotes and prokaryotes
B. Differences in ribosome structure between eukaryotes and prokaryotes
C. Differences in transcription factors between eukaryotes and prokaryotes
D. Inability of the drug to bind to DNA containing nucleosomes
E. Differences in snurp structure between eukaryotes and prokaryotes

41. A researcher, while studying a liver cell line, found the following anomalous result. He was studying protein X production within the liver cell. Western blot analysis using a polyclonal antibody showed a normal size, and amount, for protein X. Enzyme assays demonstrated normal levels of activity for protein X. Northern blot analysis, however, yielded two bands of equal intensity: one the expected size and the other 237 nucleotides longer. One possible explanation for this finding is which of the following?[14]
A. Loss of a transcription termination site
B. A loss of an intron/exon junction
C. Inefficient transcription initiation
D. A nonsense mutation in the DNA
E. Gain of an alternative splice site

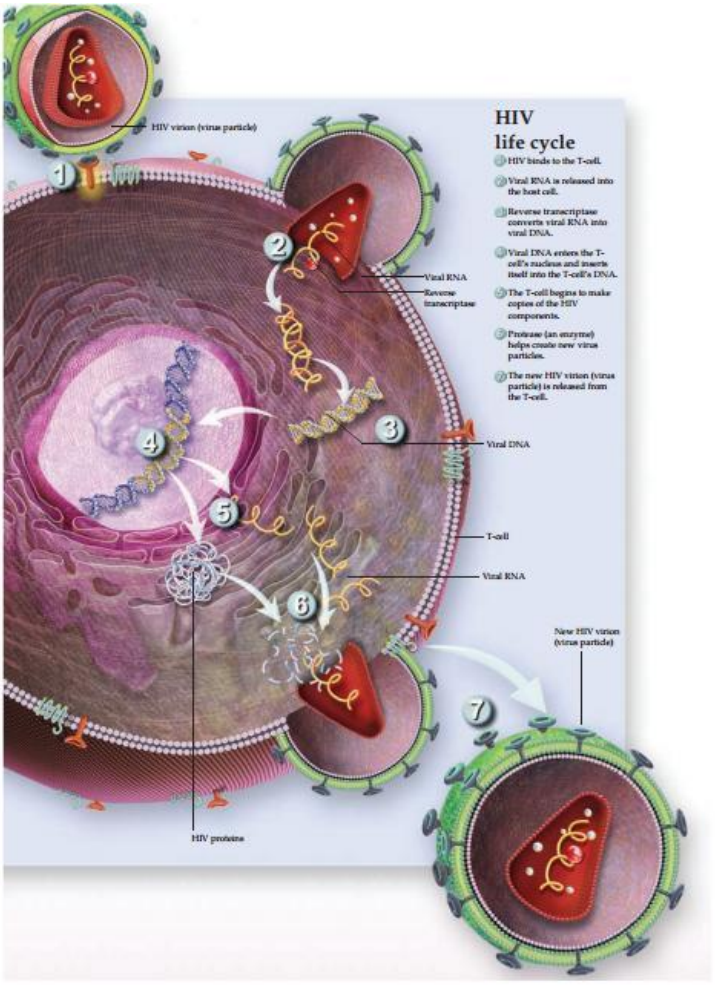
42. A patient displays tiredness and lethargy, and blood work demonstrates an anemia. Western blot analysis indicates significantly greater levels of α-globin than β-globin. Molecular analysis indicates a single nucleotide change in an intron of the β-globin gene. How does such a mutation lead to this clinical finding?[15]
A. Creation of an alternative splice site, such that β-globin levels are decreased
B. A microRNA is produced, which is targeted against the β-globin mRNA, thereby reducing β-globin production
C. Creation of a new transcription initiation site, such that the mRNA for β-globin is now out of frame
D. Creation of a stop codon in the β-globin mRNA
E. Elimination of the polyadenylation signal, thereby reducing β-globin production

43. Dideoxynucleotides are effective agents against DNA synthesis, but appear to have little, or no, effect on RNA synthesis. This is most likely due to which of the following?[16]
A. Lack of a 2′-OH group
B. Lack of a 3′-OH group
C. Presence of a 5′-phosphodiester bond
D. Presence of an N-glycosidic bond at carbon 1
E. Factor TFIID does not recognize deoxyribonucleotides

44. A cell line was derived, which was temperature sensitive for splicing hnRNA. At the nonpermissive temperature, splicing was unable to occur. A potential activity that is mutated in the splicesome is which of the following?[17]
A. Loss of endonuclease activity
B. Ability to carry out DNA synthesis
C. Loss of 3′–5′ exonuclease activity
D. Ability to carry out RNA synthesis
E. Loss of ability for transcription-coupled DNA repair

45. A 2-year-old has been diagnosed with a rhabdomyosarcoma and is placed on chemotherapy, including the drug dactinomycin. Dactinomycin exerts its effects by which of the following mechanisms?[18]
A. Binding of the drug to DNA, thereby blocking RNA synthesis
B. Binding of the drug to ribosomes, thereby blocking translation
C. Binding of the drug to transcription factors, thereby blocking RNA synthesis
D. Binding of the drug to RNA polymerase II, thereby blocking RNA synthesis
E. Binding of the drug to DNA, thereby blocking DNA synthesis

1 The answer is: RNA polymerase. During the life cycle of the HIV, the double-stranded DNA which was produced from the genomic RNA integrates randomly intothe host chromosome. Cellular RNA polymerase then transcribes the viral DNA to produce viral RNA, which is used in the translation of viral proteins, and as the genomic material for new virions. RNA polymerase lacks 3′-5′ exonuclease activity, thus the enzyme cannot correct any errors it may make while transcribing the viral DNA. The RNA produced, which carries errors in transcription, is then packaged into a new virus particle, and this mutation may lead to a change that confers a growth advantage to this strain of virus. The lack of proofreading by RNA polymerase is not usually a problem in eukaryotic cells, as many messages are produced from a single gene, and if 1 % of those messages produce a mutated protein it will be compensated by the 99 % of the messages which produce a normal protein. In the viral case, however, the mRNA turns into the genomic material, which will lead to mutations in all future descendants of that virus. This is why HIV is treated with multiple, different antivirals simultaneously, to destroy any virus which mutates to be resistant to the antiviral agents. DNA polymerase has error-checking capabilities, and will not signifi cantly increase the mutation rate of the integrated viral DNA. DNA primase may make errors, but they are corrected when the RNA primer is removed from the DNA. Telomerase only works on the ends of chromosomes, and the viral DNA does not usually integrate at those positions. DNA ligase activity is not required for viral RNA production.



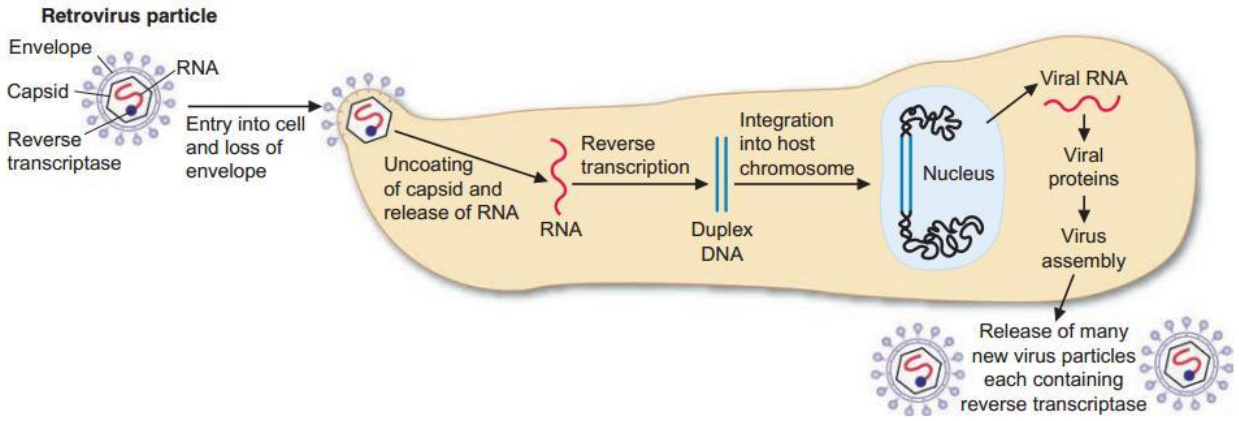
The HIV life cycle.

2 The answer is: ACGTGGGCAATCGAAT. The product of DNA replication will be complementary to the template, and antiparallel. Reading from the 5′ end of the template, the product will be 3′-TAAGCTAACGGGTGCA-. When written 5′-3′ (standard notation) one has -ACGTGGGCAATCGAAT-. Recall that uracil (U) is not placed into DNA by DNA polymerase.

3 The answer is: The need for a primer. DNA polymerase requires a primer in order to synthesize DNA. The primer is provided by a small piece of RNA, synthesized by DNA primase (an RNA-polymerase). RNA synthesis does not require a primer. Once a small piece of RNA is synthesized, DNA polymerase will begin to add deoxyribonucleotides to the end of the RNA. Later, during DNA replication, another DNA polymerase will come along and remove the RNA, replacing the RNA bases with deoxyribonucleotides. However, as initially synthesized, Okazaki fragments will contain uracil. While the deamination of cytosine can produce uracil, this is much more frequent in the more stable DNA than RNA. Thymine cannot be easily converted to uracil in DNA (it would require a demethylation), and does not contribute to uracil content in Okazaki fragments. Mismatch repair does not operate on DNA:RNA hybrids (which form when the primer is synthesized), and DNA polymerase does not recognize uracil, so it would not make the type of mistake in which uracil were placed into DNA.

4 The answer is: Lack of error checking in RNA polymerase. As part of the life cycle of the virus, the RNA genome of the virus is converted to DNA, which integrates randomly into the host chromosome. Host cell RNA polymerase II then transcribes the viral DNA, producing viral RNA, which is translated to produce viral proteins, and which is also utilized as the genome for new viral particles. RNA polymerase does not contain 3′-5′ exonuclease activity (which DNA

polymerase does), so RNA polymerase cannot check its work and cannot fix errors when a mismatch is made. The accumulated effect of these errors increases the mutation rate of the virus much more than organisms containing DNA genomes. Since the enzyme that creates the viral DNA is reverse transcriptase, which also has no error-checking capability, the risk for mutations is greatly enhanced. DNA polymerase does check its work but is not used in the viral life cycle. The DNA repair enzymes are not altered by HIV infection. Uracil is a normal component of the viral RNA genome, whereas thymine is not, but neither of these facts results in an increase in mutation rate.



An overview of the retroviral life cycle.

5 The answer is: Topoisomerase. The quinolone family of antibiotics is targeted toward the bacterial enzyme DNA gyrase, which is the bacterial counterpart of the mammalian topoisomerases. These are the enzymes which break the phosphodiester backbone to allow relief of torsional strain as the DNA helix is unwinding to allow replication to proceed. Through an inhibition of gyrase, DNA replication in the bacteria is inhibited, which leads to bacterial cell death. Since the topoisomerases are not affected by these drugs, there is no effect on eukaryotic DNA synthesis. DNA polymerase α is unique to eukaryotic cells (the bacteria have DNA polymerases I, II, or III). DNA ligase, primase, and DNA helicase are not targets of this class of drugs. The helicase is the enzyme which allows the DNA strands to unwind; however, it needs to work with gyrase (or topoisomerase) such that the tension created by unwinding can be relieved.

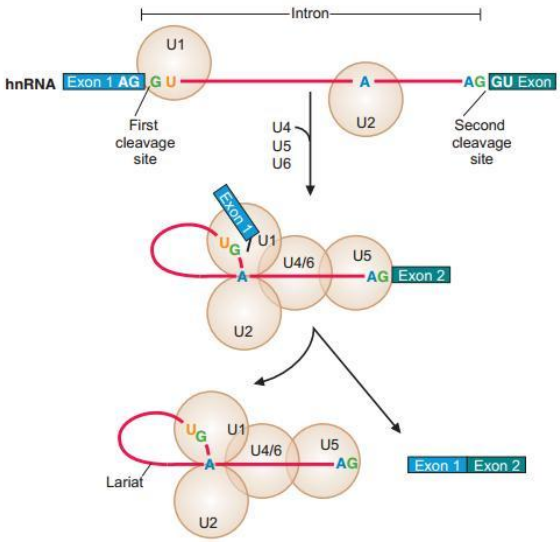
6 The answer is: DNA helicase. The child has the symptoms of Bloom syndrome, a disease in which DNA helicase is defective, and DNA replication is compromised. The DNA helicase is necessary to help stabilize the unwinding of the DNA as the replication fork passes through a stretch of DNA. With reduced helicase activity, genomic instability occurs, with increased risk of mutagenic effects and chromosome damage, including chromosome breaks and translocations. These secondary effects lead to the symptoms observed in the patients. The patients also have a higher than normal risk for various malignancies, due to the increased genomic instability. The mutation is in the BLM gene, which is on chromosome 15. This mutation does not alter, in a direct fashion, DNA polymerase or ligase activity nor RNA polymerase activity. Reverse transcriptase is not a normal component of eukaryotic cells (it is introduced to cells when they are infected by a retrovirus).

7 The answer is: Ribonucleoside triphosphates. DNA synthesis requires the synthesis of primers upon which deoxyribonucleotides will be added. The primers are composed of ribonucleotides (DNA primase is a DNAdependant RNA polymerase), and the scientist forgot to add NTPs to the reaction mixture (deoxyribonucleotides are not recognized by DNA primase, and cannot be used to synthesize a primer). Reverse transcriptase is not required for DNA

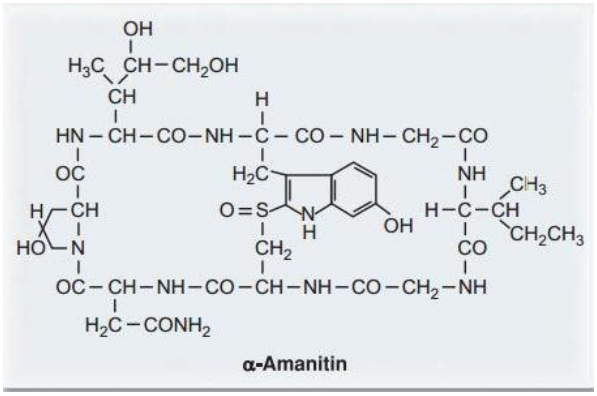
synthesis from a DNA template (as in this situation). Sigma factor is a required factor for bacterial RNA synthesis; it is not a required factor for eukaryotic DNA synthesis.

8 The answer is: Splicing of hnRNA. An adenine nucleotide in the middle of the intron is a required component for splicing to occur, and the sugar residue attached to this adenine is involved in three phosphodiester linkages; the normal 3′ and 5′ and also 2′ to the splice site. The resulting structure resembles a lariat. Such a structure does not form during capping, polyadenylation, or the normal transcription of genes. It is unique to the splicing mechanism.

9 The answer is: RNA polymerase II. The patient has ingested α-amanitin, a toxin that, at very low concentrations, inhibits RNA polymerase II and blocks the transcription of single-copy genes. RNA polymerase I and III are more resistant to the effects of amanitin, and this toxin has no effect on telomerase or any type of DNA polymerase. The inability to synthesize new proteins in all cells leads to the symptoms observed. Amanitin poisoning initially causes gastrointestinal disturbances, then electrolyte imbalance and fever, followed by liver and kidney dysfunction. Death can follow 2 to 3 days after ingestion.



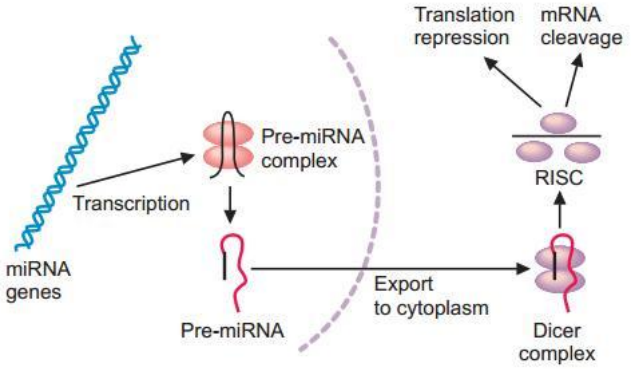
Lariat formation during splicing, showing the required intronic adenine nucleotide with three phosphodiester bonds.



10 The answer is: Ribonuclear protein complexes. The woman has lupus, an autoimmune disorder. One class of antibodies developed is against the snurps, small ribonuclear protein complexes, which are involved in mRNA splicing. Autoantibodies are not developed against DNA polymerase (although antibodies against DNA are often found), carbohydrates, tRNA complexes, or peroxisomal proteins.

11 The answer is: Western blotting of the peptide chains in hemoglobin. Thalassemias are the result of an imbalance in the synthesis of α- and β-globin genes. If this were to occur, a Western blot analysis of the α and β chains would show a difference in the amount of each in the red blood cells, suggesting either an α- or β-thalassemia (in an α-thalassemia, one would see less α chains or variants of α chains being produced, as compared to just one, normal β chain. The opposite would be true for a β-thalassemia). As many α-thalassemias are caused by gene deletions, FISH might be another way to determine this condition, using a probe specific for the α-globin gene. Most β-thalassemias are not due to gene deletions. PCR for γ-globin (fetal Hb) or RNA polymerase will not address an imbalance in α- and/or β-globin chain synthesis. Similarly, Western blots of snurps or TFIID will not address an imbalance in synthesis (if there was a problem with snurps, all RNA splicing would be affected, not just the α or β-globin gene; similarly, if TFIID were altered, all mRNA synthesis would be altered). Clinical labs will also

use hemoglobin electrophoresis to quantitate the levels of globin chains in a patient. The illustration used in the question was obtained from a patient with β-thalassemia.



12 The answer is: Degradation of the mRNA. Introns have been shown to contain genes for microRNAs, which are processed to small, interfering RNAs, which can regulate gene expression either by binding to and initiating degradation of a particular mRNA or by binding to a particular mRNA and blocking translation of the mRNA. These small RNA molecules do not affect the transcription of the target mRNA, nor posttranscriptional processing (capping and polyadenylation). They also do not affect the export of mRNA into the cytoplasm, nor do they alter ribosome biogenesis. As an example, the miR-17-92 cluster encodes seven microRNAs and resides within an intron of the C13 or F25 gene on chromosome 13. These miRNAs are upregulated in lung cancer, and may contribute to the progression of the disease by downregulating their target genes. An overview of microRNA transcription, processing, and mode of action. The miRNA genes are transcribed in the nucleus, processed to a pre-miRNA, and exported to the cytoplasm. In the cytoplasm, the pre-miRNA is further processed by an RNase (Dicer), and the resultant double-stranded miRNA forms part of the RISC (RNA-induced silencing complex). A strand selection separation process occurs that allows recognition of the appropriate mRNA to ablate (either by nuclease degradation or inhibition of translation).

13 The answer is: Structural dif erences in RNA polymerase between eukaryotes and prokaryotes. Rifampin binds to prokaryotic RNA polymerase but cannot bind (due to the different structures of the RNA polymerase between prokaryotic and eukaryotic cells) to eukaryotic RNA polymerase. The drug does not bind to ribosomes, DNA, snurps, or transcription factors. Snurps are not present in prokaryotic cells.

14 The answer is: Loss of a transcription termination site. One of the genes that encoded protein X had a mutation at the transcription termination site, which enabled the mRNA to be transcribed into a longer form (237 nucleotides longer). The reading frame of the mRNA was intact, as was the start and stop codons, so the protein produced from this lengthened mRNA was normal. If a nonsense mutation had been created in gene X, a truncated, likely inactive, protein would have been produced. The loss of an intron/exon junction would alter the splicing pattern of the mRNA, and would most likely alter the reading frame of the protein and create a nonfunctional protein. Another possibility is that the loss of an intron/exon junction would produce an elongated protein (due to intronic DNA being transcribed as part of the mRNA), with a concomitant loss of activity. Gaining an alternative splice site would potentially lead to two forms of the fi nal protein being produced, yet only one is seen by Western blot. Inefficient transcription initiation would not produce two distinct mRNAs.

15 The answer is: Creation of an alternative splice site, such that β-globin levels are decreased. If an intronic mutation created an alternative splice site, the splicesome would utilize this site for splicing a certain percentage of the time, forming an mRNA that would not code for functional β-globin protein. This would lead to a reduction in β-globin synthesis relative to α-globin synthesis, thereby creating a β-thalassemia. If a microRNA were created which targeted the β-globin mRNA, then there would be a drastic reduction in β-globin synthesis as all β-globin mRNA would be targeted for destruction, which is not observed. Since the introns would be normally spliced from the mature mRNA, the creation of a transcription initiation site would have no effect on the mature mRNA. Similarly, the creation of a stop codon in an intron would

have no effect on the mature mRNA. The polyadenylation signal is not found in introns, so the mutation could not be at this location within the gene.

16 The answer is: Lack of a 2′-OH group. RNA polymerase is looking for substrates that contain a 2′-hydroxyl group (recall, DNA polymerase utilized dNTPs, which normally lack a hydroxyl group at the 2′ position). As this substrate lacks a 2′-hydroxyl group, therefore the binding affi nity of this drug for RNA polymerase is very low, such that the likelihood that this chain terminator will be incorporated into a growing RNA chain is minimal. DNA polymerase, however, utilizes substrates lacking a 2′-hydroxyl group, and can bind and utilize this substrate.

17 The answer is: Loss of endonuclease activity. The act of splicing requires the breakage of internal phosphodiester bonds, which is the job of an endonuclease. Splicing does not require new RNA synthesis, DNA synthesis, error-checking (the 3′-5′ exonuclease activity), or DNA repair. The process of splicing is shown above (explanation 8 for question 35).

18 The answer is: Binding of the drug to DNA, thereby blocking RNA synthesis. Dactinomycin binds to DNA and blocks the ability of RNA polymerase to transcribe genes, thereby blocking transcription. The drug does not bind specifi cally to ribosomes, transcription factors, or RNA polymerase II. It also does not interfere with DNA synthesis.

Topic 4.
Research of the biosynthesis of protein on ribosomes. Antibiotics are inhibitors of matrix synthesis. Action of viruses and toxins

1. Objective: To learn general principles of protein synthesis, stages of this process, possible mechanisms of appearance and development of hereditary diseases. To interpret the mechanisms of antibiotics action as well as other inhibitors of protein synthesis.

2. Actuality of the theme: There are wide variations in the cells with respect to the quality and quantity of proteins synthesized. This largely depends on the need and ability of the cells. Erythrocytes lack the machinery for translation, and therefore cannot synthesize proteins. In general, the growing and dividing cells produce larger quantities of proteins. Some of the cells continuously synthesize proteins for export. For instance, liver cells produce albumin and blood clotting factors for export into the blood for circulation. The normal liver cells are very rich in the protein biosynthetic machinery, and thus the liver may be regarded as the protein factory in the human body.

Understanding of mechanisms of protein synthesis and the nature of hereditary diseases is based on knowledge of the genetic code which forms a set of codons. Biosynthesis of abnormal proteins underlies hereditary diseases caused by mutations – changes in nucleotide sequence of a gene. Antibacterial action of antibiotics is based on their ability to cooperate with proteins of prokaryote ribosomes and inhibit synthesis of bacterial proteins. Knowledge of molecular basis of protein biosynthesis is necessary to explain processes of regeneration, wound healing and ways of their correction. It is also important for analysis of pathological processes, that are based on infringement of anabolism and catabolism (protein deficiency, children hypotrophy, exhaustion, disfunction of endocrine glands, starvation, etc.).

3. Specific aims:

- ✓ To interpret the conception of protein synthesis on the ribosomes.
- ✓ To explain biochemical processes of posttranslational modification of proteins.
- ✓ To explain the influence of physiologically active substances and antibiotics on translation.

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

	Questions:	References:
1.	**Features of genetic code; triplet structure, its properties:** ✓ Features of genetic code; ✓ Triplet structure of genetic code; ✓ Properties of genetic code.	1. Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 551–552. 2. Gubsky Yu. Biological chemistry: textbook / edited by Yu. Gubsky. – Vinnytsia: Nova Knyha, 2018. – 2nd edition. – P. 291–292. 3. Lecture notes. 4. Lehninger A. Principles of Biochemistry. – Seventh Edition. David L. Nelson and Michael M. Cox. – 2017. – P. 2822–2847.
2.	**Protein synthesis on the ribosomes. Components of protein synthesis system:** ✓ Protein synthesis on the ribosomes; ✓ Components of protein synthesis system.	1. Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 554–560. 2. Gubsky Yu. Biological chemistry: textbook / edited by Yu. Gubsky. – Vinnytsia: Nova Knyha, 2018. – 2nd edition. – P. 293. 3. Lecture notes. 4. Lehninger A. Principles of Biochemistry. – Seventh Edition. David L. Nelson and Michael M. Cox. – 2017. – P. 2848–2859.

3.	**Transfer RNA, aminoacyl-tRNA synthetase:** ✓ Transfer RNA and amino acid activation; ✓ Aminoacyl-tRNA synthetase.	1. Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 554–555. 2. Gubsky Yu. Biological chemistry: textbook / edited by Yu. Gubsky. – Vinnytsia: Nova Knyha, 2018. – 2nd edition. – P. 294–295. 3. Lecture notes. 4. Lehninger A. Principles of Biochemistry. – Seventh Edition. David L. Nelson and Michael M. Cox. – 2017. – P. 2860–2867.
4.	**Stages and mechanisms of translation: initiation, elongation, termination. Initiating and terminating codons of mRNA:** ✓ Stages of translation; ✓ Mechanisms of translation; ✓ Initiating and terminating codons of mRNA.	1. Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 554–560. 2. Gubsky Yu. Biological chemistry: textbook / edited by Yu. Gubsky. – Vinnytsia: Nova Knyha, 2018. – 2nd edition. – P. 296–299. 3. Lecture notes. 4. Lehninger A. Principles of Biochemistry. – Seventh Edition. David L. Nelson and Michael M. Cox. – 2017. – P. 2867–2900.
5.	**Post-translational modification of polypeptide chains. Regulation of translation. Molecular mechanisms of translation control on example of globin synthesis:** ✓ Post-translational modification of polypeptide chains; ✓ Regulation of translation. ✓ Molecular mechanisms of translation control.	1. Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 561–562. 2. Lecture notes. 3. Lehninger A. Principles of Biochemistry. – Seventh Edition. David L. Nelson and Michael M. Cox. – 2017. – P. 2900–2904.
6.	**The influence of biologically active compounds on translation. Antibiotics as inhibitors of translation in prokaryotes and eukaryotes, their biomedical application:** ✓ The influence of biologically active compounds on translation; ✓ Inhibitors of translation in prokaryotes and eukaryotes.	1. Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 558–560. 2. Lecture notes. 3. Lehninger A. Principles of Biochemistry. – Seventh Edition. David L. Nelson and Michael M. Cox. – 2017. – P. 2906–2910.

5. Tasks for independent work and self-control

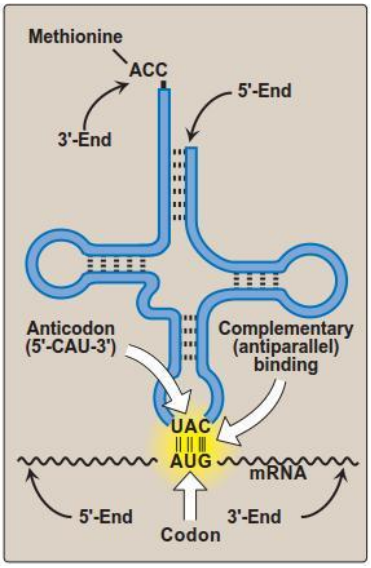
5.1. List the components that are required for protein synthesis.

5.2. The genetic code is a triplet code of four nucleotide bases (U, C, A, and G, as shown).

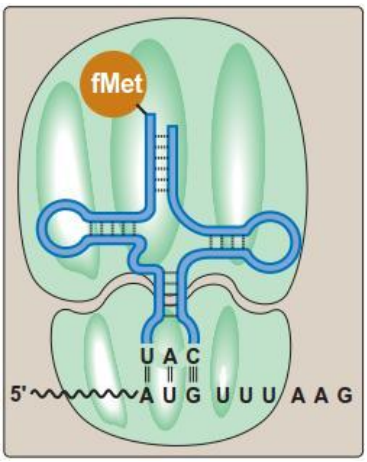
5'-BASE	MIDDLE BASE				3'-BASE
	U	C	A	G	
	Phe	Ser	Tyr	Cys	U
U	Phe	Ser	Tyr	Cys	C

Why do only 61 of the 64 triplets code for amino acids in the translation of mRNA?

5.3. What enzymes generate a charged tRNA (shown)? What two activities do these enzymes possess?



5.4. Does the figure show the product of translation initiation in a prokaryote or eukaryote? Which ribosome is depicted, a 70S or an 80S? To which site on the ribosome is the charged $tRNA_i$ bound?



5.4. What NTP gets hydrolyzed in all three steps of translation?

5.5. What role does a ribozyme play in elongation?

5.6. What elongation factor is required for translocation?

5.7. What is a «polysome»?

5.8. Explain the roles of the large and small subunits of ribosome.

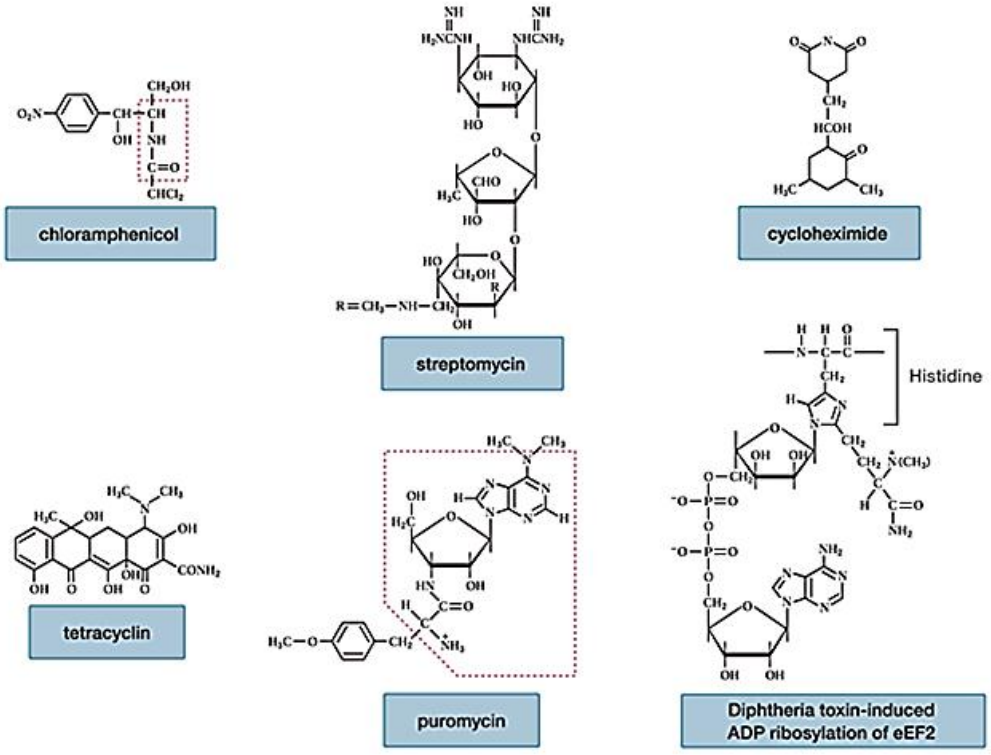
5.9. Complete the table «Post-translational modification of polypeptide chains»

Type	Explain, examples
1. Proteolytic degradation	
2. Intein splicing	
3. Covalent modifications	
i. Phosphorylation	
ii. Hydroxylation	
iii. Glycosylation	
iiii. Carboxylation	

5.10. Complete the table «Inhibitors of translation»

Inhibitor	Mechanism of inhibitory action	Prokaryotes or eukaryotes
Streptomycin		
Tetracycline		
Puromycin		
Chloramphenicol		
Diphtheria toxin		
Ricin (toxin from the castor bean)	Inactivates eukaryotic 28S ribosomal RNA by providing the N-glycolytic cleavage or removal of a single adenine.	
Erythromycin, clarithromycin	Bind to the 50S ribosomal subunit of bacteria and inhibit translocation.	

5.11. What stages of translation are inhibited by the antibiotics represented below?



5.12. Situational tasks

a) The patient was diagnosed with pneumonia doctor prescribed antibacterial drag (macrolides group) – azithromycin.

Inhibitor of which matrix process in bacterial cells is azithromycin?

Explain the mechanism of action of antibiotic azithromycin.?

Give examples of antibacterial drugs with similar mechanism of action of azithromycin.

b) In patients with diphtheria occurs typical lesions of the mucous membrane of the upper respiratory tract, due to the action of diphtheria toxin.

What stage of realization of genetic information in the epithelium of the upper respiratory tract diphtheria toxin inhibits?

What is the molecular mechanism underlying its cytotoxic action?

What epithelial cells coenzyme destroys the diphtheria toxin?

6. Individual independent students work

Influence of physiologically active compounds on translation processes.

Practical abilities.

1. Explain antitumor activity of antibiotics – inhibitors of initiation: streptomycin, riphamycin, rifampicin.
2. Explain mechanism of action of antibiotics – inhibitors of elongation: amycetin, chloramphenicol, erythromycin, cycloheximide, puromycin, tetracycline.
3. Explain mechanism of action of antibiotics – inhibitors of termination: anisomycin, chloramphenicol, erythromycin, lincomycin, streptomycin.
4. Explain mechanism of action of interferons.
5. Explain mechanism of action of diphtheria toxin.
6. Explain molecular mechanisms of mutation. What most abundant mutagens do you know?

Examples of Krock-1 tests

1. Examination of a patient revealed a reduced content of magnesium ions that are necessary for the attachment of ribosomes to the granular endoplasmatic reticulum. It is known that it causes disturbance of protein biosynthesis. What stage of protein biosynthesis will be disturbed?
A. Translation
B. Amino acid activation
C. Termination
D. Replication
E. Transcription

2. Indicate the sequence of stages of protein synthesis:
A. Initiation, elongation, termination, posttranslational modification
B. Termination, elongation, initiation, transcription
C. Translation, initiation, elongation, termination
D. Transcription, activation, elongation, posttranslational modification
E. Elongation, initiation, termination, posttranscriptional processing

3. Marked amino acids alanine and tryptophan were administered to a mouse in order to study localization of protein biosynthesis in its cells. Around what organelles will be the accumulation of marked amino acids observed?
A. Ribosomes
B. Golgi apparatus
C. Agranular endoplasmic reticulum
D. Nucleus
E. Lysosomes

4. A 5-year-old child with a sore throat and high fever was admitted to a clinic. A diagnosis of diphtheria was established. It is known that the causative agent of diphtheria releases an extremely virulent exotoxin. Specify the mechanism of its action:
A. It inhibits the elongation factor eEF-2
B. It causes translation errors
C. It modifies the translocation protein factor
D. It disrupts the binding of aminoacyl-tRNA to amino acids
E. It Inhibits termination

5. The inherited information is saved in DNA, though directly in the synthesis of protein in a cell it does not participate. What process provides the realization of the inherited information in a polypeptide chain?
A. Translation
B. Transcription
C. Translocation
D. Replication
E. Transformation

6. The translation of mRNA into the amino acid sequence of a polypeptide in prokaryotes is terminated at the end of the message by one of the three stop codons in the mRNA chain. The stop codon is recognized by:
A. A specific protein
B. A specific uncharged tRNA
C. A specific aminoacyl-tRNA
D. A specific ribosomal RNA
E. A specific ribosomal subunit

7. Degeneration of the genetic code is the ability of more than one triplet to encode a single amino acid. Which amino acid is encoded by only one triplet?
A. Methionine
B. Serine
C. Alanine
D. Leucine
E. Lysine

8. What is necessary for the formation of the transport form of amino acids during protein synthesis on ribosomes?
A. tRNA
B. Revertase
C. GTP
D. mRNA
E. Ribosome

9. Specify the function of aminoacyl-tRNA synthetases:
A. It binds aminoacyladenylate with tRNA
B. It activates the amino acid with GTP
C. It binds aminoacyl-t-RNA with ribosome
D. It forms peptide bonds between amino acids
E. It transfers aminoacyl-t-RNA into ribosomes

10. What amino acid is encoded by the triplet AUG?
A. Methionine
B. Serine
C. Tyrosine
D. Cysteine
E. Valine

11. Patient with inflammation is recommended to use erythromycin, which binds with 50S subunit of ribosome and blocks translocase. Inhibition of protein synthesis in prokaryotes occurs at the stage of:
A. Elongation
B. Amino acids activation
C. Termination
D. Posttranslational protein modification
E. Initiation

12. Eukaryotes have defined cells, which exhibit the next structural peculiarity:
A. Genetic information is stored in DNA, organized as nuclear chromatin
B. The cell possess a cell wall
C. The cell contains specific particles, responsible for cell respiration
D. Genetic information is stored in DNA, uniformly distributed throughout the cytoplasm
E. Genetic information is stored in a messenger RNA

13. A cell of granular endoplasmatic reticulum is at the stage of translation, when mRNA advances to the ribosomes. Amino acids get bound by peptide bonds in a certain sequence thus causing polypeptide biosynthesis. The sequence of amino acids in a polypeptide corresponds with the sequence of:
A. mRNA codons
B. tRNA nucleotides
C. tRNA anticodons
D. rRNA nucleotides
E. rRNA anticodons

14. A eukaryotic cell line contains an aberrant, temperature-sensitive ribonuclease that specifically cleaves the large rRNA molecule into many pieces, destroying its secondary structure and its ability to bind to ribosomal proteins. This cell line, at the nonpermissive temperature, has greatly reduced the rates of protein synthesis. This rate-limiting step is which of the following?[1]
A. Peptide bond formation
B. Termination
C. Elongation
D. Initiation
E. tRNA activation and charging

15. One of the protein synthesis stages is recognition. The first mRNA triplet starts with UAU triplet. What is complementary triplet found in tRNA?
A. AUA
B. AAA
C. GUG
D. UGU
E. CUC

16. Infectious diseases are treated with antibiotics (streptomycin, erythromycin, chloramphenicol). They inhibit the following stage of protein synthesis:
A. Translation
B. Transcription
C. Replication
D. Processing
E. Splicing

17. Some triplets of mRNA (UAA, UAG, UGA) do not encode any amino acids, but are terminators in the process of reading of information, so they can stop the translation. What are these triplets called?
A. Stop codons
B. Operators
C. Anticodons
D. Exons
E. Introns

18. Redundancy of the genetic code means that:
A. A given amino acid can be coded for by more then one base triplet
B. There is no punctuation in the code sequences
C. The third base in a codon is not important in coding
D. A given base triplet can code for more then one amino acid
E. Codons are not ambiguous

19. Genetic information of a nuclear DNA is transmitted to the site of protein synthesis by:
A. mRNA
B. rRNA
C. tRNA
D. Polysomes
E. DNA

20. In which of the following molecules would you find an anticodon?
A. Transfer RNA
B. Messenger RNA
C. Ribosomal RNA
D. Small nuclear RNA
E. Heterogenous RNA

21. Proteins are biopolymers of a principal significance in a cell building. They are composed from amino acids as monomers, which are connected into chain by the next main type of chemical bond:
A. Peptide bond
B. Phosphodiester bond
C. Ionic bond
D. Hydrogen bond
E. Glycosidic bond

22. Under the influence of mutagen the composition of some triplets in a gene was changed but the cell continued the synthesis of the same protein. What characteristics of the genetic code can it be connected with?
A. Degeneracy
B. Universality
C. Triplet nature
D. Specificity
E. Collinearity

23. Endemic goiter was diagnosed in a patient. What type of post-translational modification of thyroglobuline is damaged in a patient?
A. Iodination
B. Phosphorylation
C. Methylation
D. Acetylation
E. Glycosylation

24. It is known that the genetic code is degenerate and has a triplet nature. What nucleotide can be changed in the coding triplet without losing its sense?
A. Third
B. First
C. Second
D. Second and third
E. First or second

25. Out of 200 different amino acids form in nature the number of amino acids present in proteins:
A. 20
B. 25
C. 40
D. 35
E. 100

26. Polypeptide which has been synthesized on the ribosome includes 54 amino acids. How many codons does mRNA have in this case?
A. 54
B. 27
C. 108
D. 162
E. 44

27. The P-site in the translation is associated with:
A. Peptidyl-RNA
B. DNA
C. Aminoacyl-RNA
D. Ribosome
E. Protein

28. A-site in the translation is associated with:
A. Aminoacyl-RNA
B. DNA
C. Peptidyl-RNA
D. Ribosome
E. Protein

29. Under conditions of active exercise, protein synthesis is reduced in the muscle. Under these conditions, which aspect of translation is inhibited? [2]
A. Inability to initiate translation
B. Inability to elongate during translation
C. Inability to terminate translation
D. Inability to synthesize mRNA
E. Inability to produce rRNA

30. A youngster was prescribed erythromycin for a bacterial infection, but he developed hearing loss due to use of this drug. This occurred due to which of the following? [3]
A. Inhibition of mitochondrial protein synthesis
B. Inhibition of mitochondrial RNA synthesis
C. Inhibition of mitochondrial DNA replication
D. Weakening and tearing of the eardrum
E. Increased neuronal signalling in the inner ear

31. A 3-year-old boy, whose parents did not immunize him due to fears of postimmunization side effects, exhibited fever, chills, severe sore throat, lethargy, trouble breathing, and a husky voice. Physical exam indicated greatly enlarged lymph nodes, an increased heart rate, and swelling of the palate. The throat is dull red, and a gray exudate (pseudomembrane) is present on the uvula, pharynx, and tongue. A necessary cofactor for allowing these symptoms to appear in the child is which of the following?[4]

A. NAD^+
B. ATP
C. FAD
D. Acetyl-CoA
E. UDP-glucose

33. A 2-year-old girl exhibits a very high fever of sudden onset and complains of a stiff neck. Physical exam reveals a positive Brudzinski and Kernig sign and petechiae on the extremities. The pediatrician, in addition to rushing the child to the hospital, prescribes a drug that blocks prokaryotic peptide bond formation, even though it can have serious side effects. That drug is which of the following? [6]

A. Chloramphenicol
B. Rapamycin
C. Rifampin
D. Cycloheximide
E. Puromycin

35. A patient underwent a kidney transplant and among the many drugs she received posttransplant was rapamycin. Rapamycin aids in preventing an immune response to the transplant via which of the following mechanisms? [8]

A. The drug inhibits initiation of protein synthesis
B. The drug inhibits cap formation
C. The drug specifically inhibits RNA polymerase III
D. The drug inhibits ribosome subunit assembly
E. The drug inhibits antibody-specific transcription factors from binding to DNA

37. A 2-year-old boy with an ear infection was given amoxicillin, but it did not clear up the problem. Switching to azithromycin successfully eradicated the infection, and subsequent laboratory work indicated that the offending bacterium was resistant to amoxicillin. Bacterial resistance to antibiotics is often due to which of the following? [10]

A. Enzymatic destruction of the antibiotic
B. Altered cell wall
C. Altered ribosome structure
D. Inability to transport the drug into the bacteria
E. A mutation in RNA polymerase

32. A 3-year-old boy, whose parents did not immunize him due to fears of postimmunization side effects, exhibited fever, chills, severe sore throat, lethargy, trouble breathing, and a husky voice. Physical exam indicated greatly enlarged lymph nodes, an increased heart rate, and swelling of the palate. The throat is dull red, and a gray exudate (pseudomembrane) is present on the uvula, pharynx, and tongue. The molecular mechanism responsible for these physical observations in the boy is which of the following?[5]

A. Inhibition of an elongation factor for translation
B. Activation of an elongation factor for translation
C. Glycosylation of a G protein
D. Inhibition of protein kinase A
E. Activation of protein kinase A

34. An adult male is diagnosed with a typical pneumonia. His physician prescribes clarithromycin, which is specific for prokaryotic cells. Which of the following best explains the mechanism of prokaryotic specificity?[7]

A. The drug binds to the 50S ribosomal subunit of bacteria and blocks translocation
B. The drug binds to the 30S ribosomal subunit of bacteria and blocks initiation of protein synthesis
C. The drug binds to the 50S ribosomal subunit of bacteria and inhibits f-met-tRNAi binding
D. The drug binds to the 30S ribosomal subunit of bacteria and blocks peptide bond formation
E. The drug binds to both ribosomal subunits and prevents bacterial ribosome assembly

36. You see a very sick patient (vomiting and bloody diarrhoea, dehydration, and mental status changes) in the emergency department, who, you are told, was an amateur chef trying out a new creation in which he wanted to experiment with the extracts of castor beans. This person's symptoms are all due to which of the following? [9]

A. Ribosomal inactivation by covalent modification
B. Inhibition of RNA polymerase II
C. Inhibition of RNA polymerase I
D. Ribosomal disassembly due to covalent modification
E. Inhibition of amino-acyl tRNA synthetases

38. A patient with type 2 diabetes has been prescribed recombinant insulin to help control his disease. Three months after starting this regime, a blood test is done, which indicates that the patient is still producing endogenous insulin, in addition to the recombinant insulin the patient is taking. The blood test has, at its basis, which of the following? [11]

A. Posttranslational proteolytic processing
B. Posttranslational glycosylation
C. Posttranslational modification of amino acid side chains
D. Posttranslational acylation
E. Posttranslational quaternary structure formation

39. A young boy exhibits myopathy, encephalopathy, lactic acidosis, and strokelike episodes. All of his siblings have some aspects of the same symptoms. The boy most likely has which type of mutation?[12]

A. A defect in mitochondrial tRNA production
B. A defect in mRNA synthesis
C. A defect in mitochondrial rRNA production
D. A defect in cytoplasmic tRNA
E. A defect in cytoplasmic rRNA

41. A patient taking lovastatin and Zetia® for elevated cholesterol was found to produce lower levels of glycosylated proteins. This is most likely due to the unintended consequence of blocking the synthesis of which of the following compounds? [14]

A. Dolichol
B. Cholesterol
C. Coenzyme Q
D. HMG-CoA
E. Ketone bodies

40. A young boy has edema, a protruding abdomen, and very thin arms and legs. The edema has at its origins which of the following?[13]

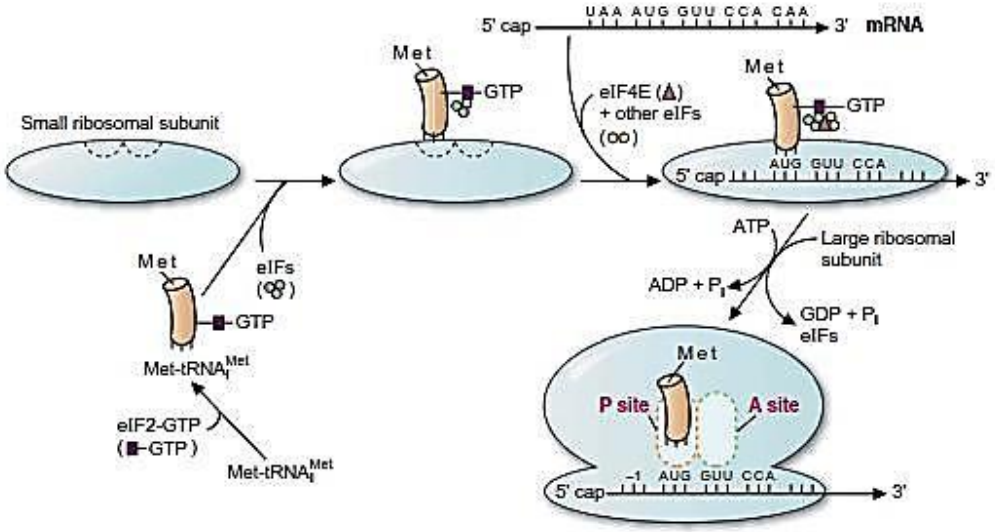
A. Lack of liver protein synthesis
B. Lack of muscle protein synthesis
C. Lack of intestinal protein synthesis
D. Excessive water production due to protein hydrolysis
E. Excessive water production due to triglyceride hydrolysis

42. A hospital laboratory made an error and mistyped a patient's blood as AB, instead of B. When given type A blood, the patient had an adverse reaction. The major difference between individuals with AB and B type blood is due to which of the following? [15]

A. The presence of a specific glycosyltransferase
B. The presence of a specific acyltransferase
C. The presence of a specific peptidase
D. The lack of dolichol pyrophosphate
E. Increased levels of dolichol pyrophosphate

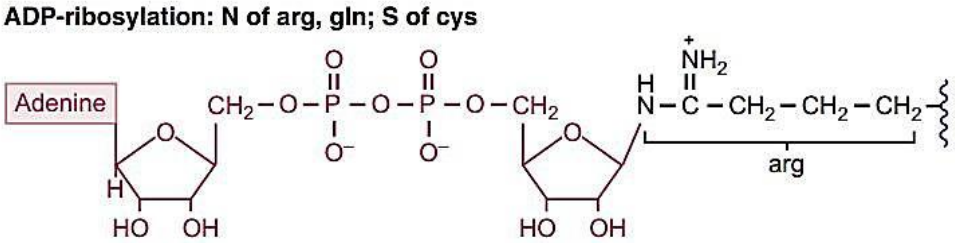
1 The answer is: Peptide bond formation. It is the large ribosomal RNA that catalyzes peptide bond formation, using peptides and amino acids in the «A» and «P» sites on the ribosome. Destroying the secondary structure of this rRNA via the aberrant ribonuclease will limit the ability of the ribosome to create peptide bonds. The large, ribosomal RNA molecule is not essential for the initiation, termination, elongation (moving the ribosome along the mRNA after peptide bond formation has occurred), or tRNA activation and charging.

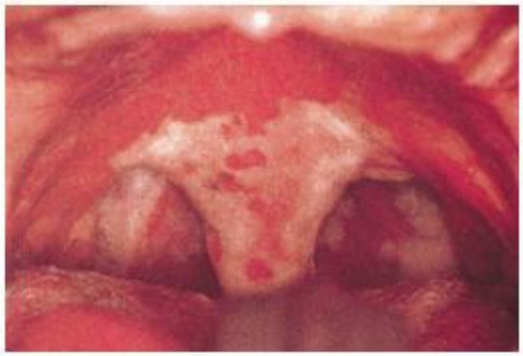
2 The answer is: Inability to initiate translation. As muscle works, and AMP levels rise, the muscle wants to preserve its ATP for muscle contraction, and not to use it for new protein synthesis. The increase in AMP levels leads to the activation of AMP-activated protein kinase, which will phosphorylate and inactivate eIF4E (eukaryotic initiation factor 4E), which is a necessary component in recognizing the 5′ cap structure of the mRNA to allow ribosome assembly on the mRNA. The activation of the AMP-activated protein kinase does not alter elongation or the termination of translation. It does not block overall transcription, either of mRNA or rRNA (although it may lead to an inhibition of ribosomal biogenesis as well as the transcription of certain specific genes).



3 The answer is: Inhibition of mitochondrial protein synthesis. Ototoxicity (hearing loss) occurs with a subset of antibiotics because, in addition to affecting prokaryotic ribosomes, the drugs also have an effect on mitochondrial ribosomes. Mitochondria contain their own DNA, RNA polymerase, and protein synthesizing apparatus (recall that it is thought that during evolution, bacteria invaded eukaryotic cells and formed a symbiotic relationship, with the bacteria eventually becoming the mitochondria), which is very similar to the prokaryotic apparatus. Thus, certain drugs will affect mitochondrial protein synthesis, and the effects seem to be greatest on those organs that have high energy needs (such as neuronal tissue). Erythromycin does not affect mitochondrial RNA synthesis or DNA replication. It does not affect the ear drum, nor does it increase neuronal signaling in the inner ear.

4 The answer is: NAD+. Diphtheria toxin, after entering cells, is cleaved by a protease to form an active enzyme, which, utilizing NAD+ as a substrate, ADP-ribosylates eEF2, thereby inhibiting protein translation. ATP, FAD, acetyl-CoA, and UDP-glucose are not required for the ADP-ribosylation reaction. The final modified product, an arginine with an ADP-ribose attached, is shown below.





5 The answer is: Inhibition of an elongation factor for translation. The child has diphtheria, which is caused by a bacterium (Corynebacterium diphtheriae), which produces a toxin that leads to the inhibition of eEF2 (eukaryotic elongation factor 2), which is required for the movement of tRNA from the «A» site to the «P» site. The toxin catalyzes the ADP-ribosylation (using NAD+ as a substrate) of eEF2 to bring about this inhibition. If one treats such a child with nicotinamide (the reaction product resulting from the loss of ADP-ribose from NAD+), one can reverse and block the ADP-ribosylation reaction catalyzed by the toxin. The toxin has no effect on protein kinase A, nor does it glycosylate a G protein. Diphtheria causes sore throat, fever, swollen nodes (bull neck),weakness, hoarseness, painful swallowing, and chills. The hallmark of the disease is a thick, gray membrane covering the pharynx.

6 The answer is: Chloramphenicol. Chloramphenicol blocks peptide bond formation in prokaryotic ribosomes (with no effect on eukaryotic ribosomes). This concept is the basis of certain antibiotic therapy; the differences in ribosome structure between eukaryotic and prokaryotic cells allow selective drug inhibition. The child has meningococcal meningitis, and chloramphenicol, despite its side effects of inhibiting mitochondrial protein synthesis, is a very effective agent for this disorder. Cycloheximide has the same effect as chloramphenicol in eukaryotic cells but has no effect on prokaryotes. Rapamycin leads to the blockage of translation initiation, not peptide bond formation. Puromycin is a chain terminator, stopping protein synthesis but not directly inhibiting peptide bond formation. Rifampin inhibits prokaryotic mRNA synthesis and has no direct effect on

translation. Rifampin might be used as prophylaxis for household contacts of meningococcal meningitis but is not as effective a treatment for the actual disease.

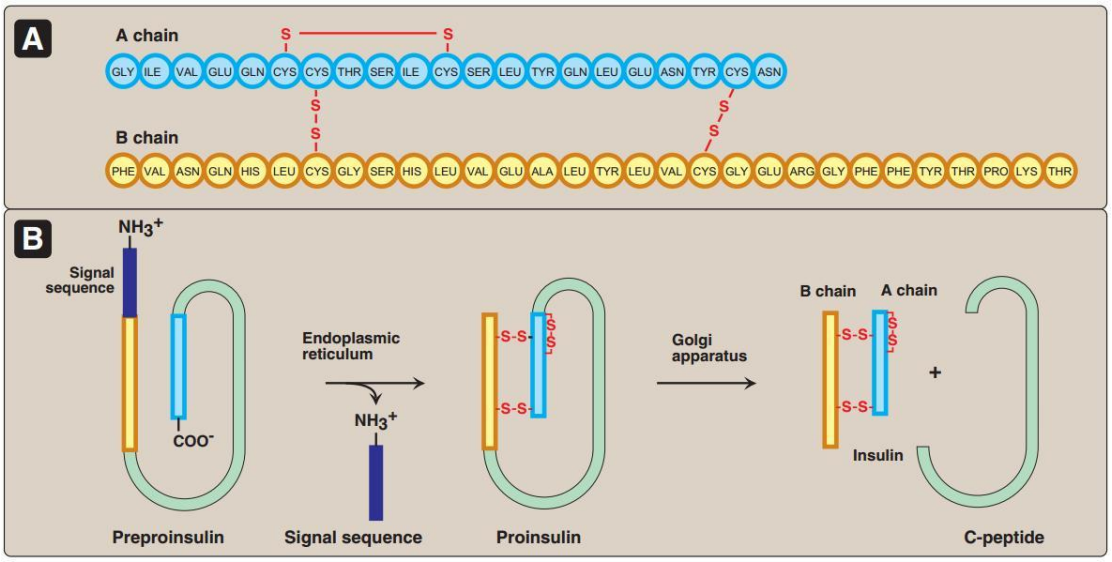
7 The answer is: The drug binds to the 50S ribosomal subunit of bacteria and blocks translocation. Clarithromycin (an antibiotic in the macrolide family with erythromycin and azithromycin) is specific for the large ribosomal subunit of prokaryotes (it will not bind to eukaryotic ribosomes). When this drug binds to the large ribosomal subunit, translocation of the ribosome (movement along the mRNA) is blocked, which blocks overall protein synthesis. tRNA binding is not affected by clarithromycin, nor is there a blockage of the formation of an initiation complex. It is the large subunit (50S) that contains the peptidyl transferase activity, which is also not blocked by this agent.

8 The answer is: The drug inhibits initiation of protein synthesis. Rapamycin inhibits the mammalian target of rapamycin (mTOR), which is a protein kinase. One of the many targets of mTOR is eIF4E binding protein (eIF4E is a required initiation factor for protein synthesis). When not phosphorylated, the binding protein binds tightly to eIF4E and prevents it from participating in the formation of the translational initiation complex, thereby blocking protein synthesis. When phosphorylated at multiple locations by mTOR, the binding protein falls off the initiation factor and allows translational initiation complexes to form. In the presence of rapamycin, mTOR has no kinase activity, and the binding protein remains bound to eIF4E, thereby inhibiting protein synthesis. The drug does not affect ribosome assembly. The drug also has no effect on transcription or RNA processing.

9 The answer is: Ribosomal inactivation by covalent modification. Ricin, a toxin found in castor oil beans, specifically cleaves an N-glycosidic bond in the 28S rRNA of the large ribosomal subunit (an adenine base is removed, but the phosphodiester backbone remains intact). The sequence of the rRNA that is altered is required for binding elongation factors during protein synthesis. As ribosomes become inactivated by ricin, protein synthesis in cells stops, leading to cell death. Ricin does not inhibit RNA polymerases or amino-acyl tRNA synthetases. Ricin does not initially affect ribosome assembly. The LD50 for ricin is 30 mg/kg body weight.

10 The answer is: Enzymatic destruction of the antibiotic. Bacteria that develop resistance to antibiotics usually do so by containing an enzymatic activity that destroys the structure of the antibiotic so that it cannot effectively inhibit its target within the cell. Amoxicillin works by destroying the bacterial cell wall, by being incorporated into the growing cell wall, which leads to a cessation of cell wall synthesis. It does not alter ribosome structure. Mutations in RNA polymerase will not lead to resistance to drugs. Azithromycin was effective because the bacteria did not produce an enzyme that destroyed the drug.

11 The answer is: Posttranslational proteolytic processing. Insulin is synthesized as a preproinsulin. The pre sequence is the signal sequence, which is cleaved when the protein enters the endoplasmic reticulum. Proinsulin contains the C-peptide, which is removed to form mature insulin, and disulfide bonds that hold the A and B chains together. The modification is thus proteolytic processing, and not glycosylation, modification of side chains, acylation, or altered quaternary structure. The test is a C-peptide level. If a person is producing insulin, a C-peptide level can show this. This is also a way to see if a person has type 1 diabetes mellitus (no C-peptide, since they cannot produce endogenous insulin) or type 2 (normal or high C-peptide levels, since this disease is a reduced response to normally produced insulin).

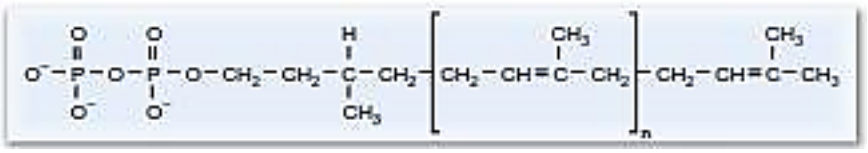


Panel A indicates the amino acid sequence of mature insulin while
panel B indicates the steps involved in converting preproinsulin to mature insulin.

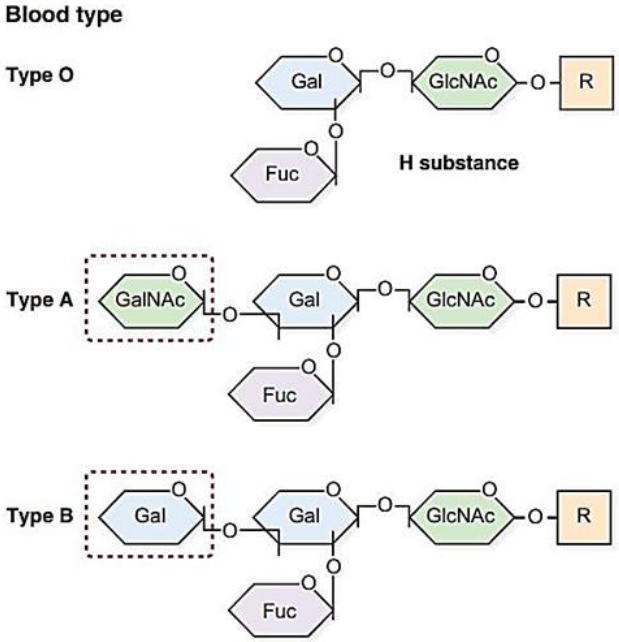
12 The answer is: A defect in mitochondrial tRNA production. The boy has MELAS (Mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke), a neurodegenerative disorder due to a mutation in mitochondrial tRNA, leading to defective protein synthesis within the mitochondria. The component most often affected is complex 1 of the respiratory chain. The severity of the disease will be dependent on the extent of heteroplasmy (what percentage of the mitochondria codes for the altered tRNA). The more mutant mitochondria present, the more severe the symptoms.

13 The answer is: Lack of liver protein synthesis. The boy is displaying kwashiorkor due to a calorie-deficient diet low in protein. Since the boy is taking in less protein than he needs, he is becoming deficient in the essential amino acids. Thus, to synthesize new proteins, existing proteins need to be degraded such that a pool of essential amino acids is available for the new protein synthesis. This protein degradation occurs in the muscles, leaving the boy with very thin arms and legs. The liver, despite the increased muscle protein turnover, is still deficient in essential amino acids and reduces its level of protein synthesis, including those proteins normally found in the blood, such as serum albumin. The reduced protein content in the blood reduces the osmotic strength of the blood such that when the blood flows through the body, the osmotic strength of the tissues is higher, and fluid leaves the blood and enters the interstitial space around the tissues. This leads to the protruding abdomen seen in these starving individuals. The problem is not related to reduced muscle protein synthesis (which does occur, but does not affect the osmotic strength of the blood), or reduced intestinal protein synthesis, for the same reason as the muscle. Both protein hydrolysis and triglyceride hydrolysis require water; water is not produced when these molecules are broken down.

14 The answer is: Dolichol. The biosynthesis of both coenzyme Q and dolichol is dependent on isoprene units, speci cally isopentenylpyrophosphate, which is derived from mevalonic acid in the de novo pathway of cholesterol biosynthesis. Lovastatin inhibits HMG-CoA reductase, which reduces mevalonate production. This can have, then, the unintended consequence of a reduction in dolichol production, thereby leading to underglycosylation of processed proteins. A reduction of coenzyme Q synthesis can lead to muscle weakness (a side effect of the statin class of drugs), but coenzyme Q is not involved in protein glycosylation. Cholesterol, HMG-CoA, and ketone bodies are not required for protein glycosylation as well. The structure of dolichol, in which the isoprene building block is highlighted, is shown below.



15 The answer is: The presence of a specifc glycosyltransferase. As shown below, type A and B blood differ by the presence of one sugar on glycosylated proteins. Individuals with type A blood add one type of sugar (N-acetylgalactosamine), while individuals with type B blood add a different sugar (galactose) due to differences in the specificity of a glycosyltransferase that recognizes the base carbohydrate structure. The differences in blood group antigens are not due to acylation or proteolytic processing on the cell surface. As these carbohydrates are O-linked, dolichol (which is required for N-linked glycosylation) is not required for their synthesis.



Structures of the blood group antigens.

Topic 5.
Analysis of mechanisms of mutations, DNA reparations. Mastering the principles of genetic engineering and cloning of genes, their application in modern medicine

1. Objective: To know principles of gene engineering and gene cloning, its application in modern medicine. To learn principle of polymerase chain reaction (PCR), its applications in diagnostics.

2. Actuality of the theme: Chemical mutagens (nitrogenous base analogueues, deaminating, alkylating agents) and physical (ultraviolet and ionizating radiation) cause damage of DNA, mutations that cause enzymopathies and hereditary diseases.

Restoration of damaged molecules of DNA performed by reparation systems.

Transplantation of the genes, production of hybrid DNA molecules, gene cloning are used to obtain biotechnology medicines and for diagnostics (hormones, enzymes, antibiotics, interferons, etc.).

3. Specific aims:

- ✓ To explain biochemical and molecular biology principles of methods used in recombinant DNA technologies, recombinant gene technology and formation of hybrid DNA molecules.
- ✓ To explain principles of gene cloning for production of drugs with biotechnologies methods.

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

Questions:	References:
1. **Mutations: genomic, chromosomal, gene (point mutations), their significance in appearance of enzymopatias and human hereditary diseases:** ✓ Genomic mutations; ✓ Chromosomal mutations; ✓ Gene mutations (point mutations).	1. Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 535–537. 2. Lecture notes.
2. **Biochemical mechanisms of action of chemical mutagens – analogues of nitrogenous bases, ultraviolet and ionizing radiation:** ✓ Biochemical mechanisms of action of chemical mutagens; ✓ Biochemical mechanisms of action of ultraviolet and ionizing radiation.	1. Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 534–535. 2. Lecture notes.
3. **Biological significance and mechanisms of DNA reparation. Reparation of UV-induced mutations. Xeroderma pigmentosum:** ✓ Mechanisms of DNA reparation; ✓ Reparation of UV-induced mutations; ✓ Xeroderma pigmentosum.	1. Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 537–539. 2. Lecture notes.
4. **Gene engineering: general principles, biomedical significance. The employment of enzymes. Gene cloning for obtaining of medicinals and diagnostic tools using methods of biotechnology (hormones, enzymes, antibiotics, antigens, interferons etc.):** ✓ Recombination; ✓ Recombinant DNA technology; ✓ Gene cloning.	1. Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 532–534, 578–597. 2. Gubsky Yu. Biological chemistry: textbook / edited by Yu. Gubsky. – Vinnytsia: Nova Knyha, 2018. – 2nd edition. – P. 284–287. 3. Lecture notes. 4. Lehninger A. Principles of Biochemistry. – Seventh Edition. David L. Nelson and Michael M. Cox. – 2017. – P. 2666–2701.

5.	**Polymerase chain reaction, its biomedical application in diagnostics of contagious and hereditary diseases, identification of a person (DNA-diagnostics).**	1. Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 535–537, 594–596. 2. Lehninger A. Principles of Biochemistry. – Seventh Edition. David L. Nelson and Michael M. Cox. – 2017. – P. 836–840. 3. https://www.youtube.com/watch?v=2KoLnIwoZKU.

5. Tasks for independent work and self-control

5.1. What unique DNA lesion is caused by UV radiation? What process repairs the lesion? Inability to perform this repair results in what rare genetic disease?

5.2. Compare two types of recombinations.

Homologous recombination	Non-homologous recombination

5.3. Give the definitions of terms:

Recombinant DNA technology

Restriction endonucleases

Gene clone

Polymerase chain reaction (PCR)

5.4. Write the essential requirements for PCR.

5.5. Complete the table «Applications of PCR»

Application	Explaining
1. PCR in clinical diagnosis Prenatal diagnosis of inherited diseases	
Diagnosis of retroviral infections	
Diagnosis of bacterial infections	
Diagnosis of cancers	
PCR in sex determination of embryos	
2. PCR in DNA sequencing	
3. PCR in comparative studies of genomes	
4. PCR in forensic medicine	

6. Individual independent students work

1. Consequences of genomic, chromosomal and gene mutations.
2. The mechanism of action of the most common mutagens.

Practice protocol №5 «____» _____ 20___

Experiment . Polymerase Chain Reaction

Principle of the method. The PCR procedure, relies on enzymes called DNA polymerases. These enzymes synthesize DNA strands from deoxyribonucleotides (dNTPs), using a DNA template. DNA polymerases do not synthesize DNA de novo, but instead must add nucleotides to preexisting strands, referred to as primers. In PCR, two synthetic oligonucleotides are prepared for use as replication primers that can be extended by a DNA polymerase. These oligonucleotide primers are complementary to sequences on opposite strands of the target DNA, positioned so that their 5′ ends define the ends of the segment to be amplified, and they become part of the amplified sequence. The 3′ ends of the annealed primers are oriented toward each other and positioned to prime DNA synthesis across the targeted DNA segment.

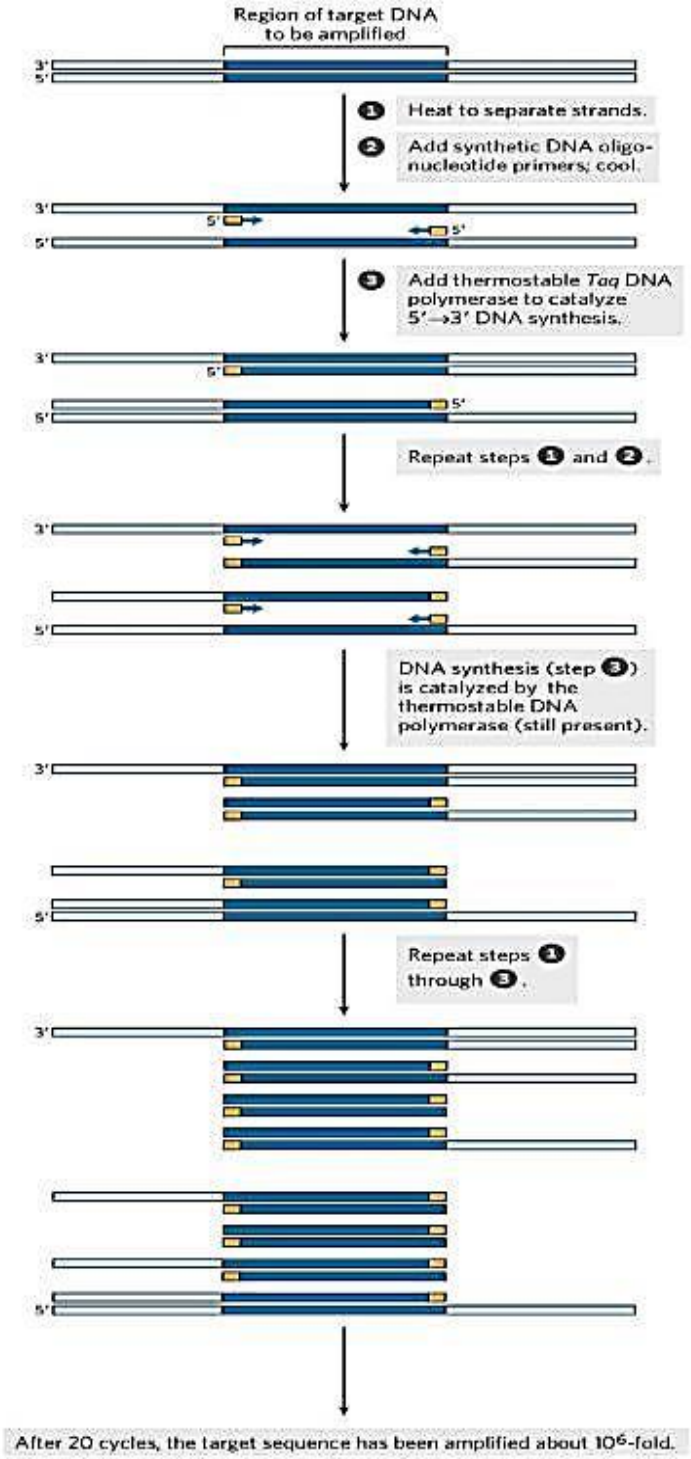


Figure. Amplification of a DNA segment by the polymerase chain reaction (PCR). The PCR procedure has three steps. DNA strands are **1** separated by heating, then **2** annealed to an excess of short synthetic DNA primers (orange) that flank the region to be amplified (dark blue); **3** new DNA is synthesized by polymerization catalyzed by DNA polymerase. The thermostable *Taq* DNA polymerase is not denatured by the heating steps. The three steps are repeated for 25 or 30 cycles in an automated process carried out in a small benchtop instrument called a thermocycler.

The PCR procedure has an elegant simplicity. Basic PCR requires four components: a DNA sample containing the segment to be amplified, the pair of synthetic oligonucleotide primers, a pool of deoxynucleoside triphosphates, and a DNA polymerase. There are three steps.

In step 1, the reaction mixture is heated briefly to denature the DNA, separating the two strands.

In step 2, the mixture is cooled so that the primers can anneal to the DNA. The high concentration of primers increases the likelihood that they will anneal to each strand of the denatured DNA before the two DNA strands (present at a much lower concentration) can reanneal to each other.

In step 3, the primed segment is replicated selectively by the DNA polymerase, using the pool of dNTPs. The cycle of heating, cooling, and replication is repeated 25 to 30 times over a few hours in an automated process, amplifying the DNA segment between the primers until the sample is large enough to be readily analyzed or cloned. In brief, the amplified DNA is joined to another DNA segment with sequences that allow it to be replicated in a host cell. Each replication cycle doubles the number of target DNA segment copies, so the concentration grows exponentially. The flanking DNA sequences increase in number linearly, but this effect is quickly rendered insignificant. After 20 cycles, the targeted DNA segment has been amplified more than a millionfold (2^{20}); after 30 cycles, more than a billionfold. Step 3 of PCR uses a heat-stable DNA polymerase such as the *Taq* polymerase, isolated from a thermophilic bacterium (*Thermus aquaticus*) that thrives in hot springs where temperatures approach the boiling point of water. The *Taq* polymerase remains active after every heating step (step 1) and does not have to be replenished.

This technology is highly sensitive: PCR can detect and amplify just one DNA molecule in almost any type of sample – including some ancient ones. The double-helical structure of DNA is highly stable, but DNA does degrade slowly over time through various nonenzymatic reactions. PCR has allowed the successful cloning of rare, undegraded DNA segments isolated from samples more than 40,000 years old. Investigators have used the technique to clone DNA fragments from the mummified remains of humans and extinct animals, such as the woolly mammoth, creating the research fields of molecular archaeology and molecular paleontology. DNA from burial sites has been amplified by PCR and used to trace ancient human migrations. Epidemiologists use PCR-enhanced DNA samples from human remains to trace the evolution of human pathogenic viruses. Due to its capacity to amplify just a few strands of DNA that might be present in a sample, PCR is a potent tool in forensic medicine. It is also being used to detect viral infections and certain types of cancers before they cause symptoms, as well as in the prenatal diagnosis of genetic diseases. Given the extreme sensitivity of PCR methods, contamination of samples is a serious issue. In many applications, including forensic and ancient DNA tests, controls must be run to make sure the amplified DNA is not derived from the researcher or from contaminating bacteria.

The report is checked up _____

(The signature of the teacher, date)

Examples of Krock-1 tests

1. The next technique is used for multiple amplification of distinct and selected segment of DNA:
A. Polymerase chain reaction
B. DNA fingerprint analysis
C. Southern blot analysis
D. Northern blot analysis
E. Restriction fragment length polymorphism analysis

2. A young man has been diagnosed with HIV-infection by the polymerase chain reaction. What is the main principle of this reaction?
A. Gene amplification
B. Genetic recombination
C. Transcription
D. Gene mutation
E. Chromosome mutation

3. Mercury ions were incorporated into human body. This led to the increase in the rate of transcription of the gene, responsible for detoxification of heavy metals. What protein gene amplification is in the background of this process?
A. Metallothioneine
B. Ceruloplasmin
C. Interferone
D. Transferrin
E. Ferritin

4. The samples of blood of a child and of a supposed father were directed for affiliation to medical forensic examination. Which chemical components need to be identified in the explored samples of blood?
A. DNA
B. tRNA
C. rRNA
D. mRNA
E. Protein

5. Restriction endonucleases are useful in the recombinant DNA technique because they:
A. Cut DNA at specific sites
B. Restrict the number of nucleotides that can be removed at one time
C. Restore the bonds in the DNA backbone
D. Synthesize cDNA from RNA
E. Can be used to locate genes for mapping

6. What analytical technique is used for the identification of a father of a child?
A. Polymerase chain reaction
B. Northern blot analysis
C. Restriction fragment length polymorphism analysis
D. DNA fingerprint analysis
E. Southern blot analysis

7. Restriction endonucleases have a bacterial origin, which are used in recombinant DNA technology. They belong to the next class of enzymes:
A. Hydrolases
B. Oxidoreductases
C. Transferases
D. Lyases
E. Isomerases

8. What enzyme is used for synthesis of genes from template RNA or DNA in gene engineering? (This enzyme was discovered in some RNA containing viruses)
A. Revertase
B. Exonuclease
C. Endonuclease
D. Topoisomerase I
E. Helicase

9. To precise the diagnosis a patient is recommended to make DNA investigation. Synthetic primers are known to be used containing:
A. Ribonucleotides
B. Deoxyribonucleotides
C. Amino acids
D. Pyrimidine nucleotides
E. Purine nucleotides

10. What are mutations caused by the changes in the amount of a complete set of chromosomes or individual chromosomes in a diploid set called?
A. Genomic
B. Haploid
C. Polyploid
D. Chromosomal
E. Gene

11. What are fragments of DNA containing one or several genes and a control element that can move from one part of the genome to another called?
A. Transposons
B. Exons
C. Introns
D. Plasmids
E. Promoters

12. In the tryptophan operon of E. coli the end product of biochemical pathway, tryptophan, binds to the repressor protein which then binds to the:
A. Operator to inhibit transcription
B. Promoter to accelerate transcription
C. Promoter to inhibit transcription
D. Operator to accelerate transcription
E. Repressor gene to accelerate transcription

13. In the lactose operon system in E. coli, the repressor is:
A. A protein
B. A product of a structural gene
C. Bound to the promoter sequence
D. Lactose
E. A short length of DNA

14. What is the function of the promoter region of a bacterial operon?
A. It is binding site for RNA polymerase
B. It codes for repressor proteins
C. It codes for inducer substances
D. It codes for corepressor substances
E. It is a binding site for inducers

15. What is the function of the regulatory gene of bacterial operon?
A. It codes for repressor proteins
B. It codes for β-galactosidase
C. It acts as an on-off switch for the structural genes
D. It is a binding site for RNA polymerase
E. It is a binding site for inducers

16. Which gene families encode information on the structure of light chains of immunoglobulins?
A. V, J, C
B. D, J, C
C. V, D, C
D. V, D, J
E. L, V, D

17. According to the Jacob-Monod model of gene regulation, inducer substances in bacterial cells probably:
A. Combine with repressor proteins, inactivating them
B. Combine with operator regions
C. Combine with structural genes, stimulating them to synthesize messenger RNA
D. Combine with promoter regions, activating RNA polymerase
E. Combine with nucleoli, triggering production of more ribosomes

18. What is an operon called?
A. Complex of genetic elements responsible for the coordinated synthesis of a group of functionally related proteins
B. Complex of factors of translation
C. A protein-synthesizing complex
D. Complex of genetic elements responsible for the regulation of gene modification
E. Combination of triplets of nucleotides, which are responsible for the operative recombination of genes

19. Which gene families encode information on the structure of heavy chains of immunoglobulins?
A. V, D, J, C
B. V, D, J, H
C. D, J, C, L
D. D, J, C, H
E. V, D, L, C

20. A repressor protein was found in the cell. Which gene encodes the amino acid sequence of this protein?
A. Regulator
B. Terminator
C. Promoter
D. Modifier
E. Operator

21. The structural genes of the lac operon are responsible for the synthesis of the following enzymes:
A. Galactosidase, galactoside permease, galactoside trans-acetylase
B. Lactoside transferase, galactosidase galactoside trans-acetylase
C. Lactase, galactosidase, galactoside permease
D. Galactokinase, lactase, galactosidase
E. Galactose-1-phosphate-uridyltransferase, galactosidase, lactase

22. Skin of the patients with xeroderma pigmentosum is very sensitive to sunrise, cancer of skin can develop. The reason is a hereditary deficiency of enzyme UVendonuclease. This defect leads to the disturbance of:
A. DNA repair
B. Transcription
C. DNA replication
D. Translation
E. Reverse transcription

23. An operon consists of:
A. Promoter, operator, structural genes
B. Terminator, operator, promoter
C. Regulator, structural genes, promoter
D. Modifier, structural genes, promoter
E. Operator, promoter, regulatory gene

24. The control sites of the operon are:
A. Operator, promoter
B. Terminator, promoter
C. Regulator, promoter
D. Modifier, operator
E. Promoter, regulatory gene

25. A promoter is a region of DNA that interacts with:
A. RNA polymerase
B. Repressor protein
C. Regulator
D. DNA polymerase
E. Ribosome

26. The operator is a region of DNA that specifically binds to:
A. Repressor
B. Terminator
C. The regulator
D. The modifier
E. Promoter

27. The CAP protein interacts with a specific site in the structure of:
A. Promoter
B. Terminator
C. Regulator
D. Modifier
E. Repressor

28. Structural genes are the region of the operon that contains information about:
A. The primary structure of polypeptides that are transcribed from this operon
B. The primary structure of protein-repressor
C. The primary structure of protein regulators
D. Primary structure of RNA polymerase II
E. Primary structure of DNA polymerase I

29. Worker of the chemical factory as result of infringement of prevention of accidents regulations is influenced by nitrite acid and nitrites, which causes the cytosine deamination in DNA molecule. Which enzyme starts the repair cascade?
A. Uracil-DNA-glycosidase
B. DNA-dependent-RNA-polymerase
C. CTP-synthetase
D. Amino-acyl-tRNA-synthetase
E. Glucose-6-phosphate dehydrogenase

30. From nitrates, nitrites and nitrosamines in the body is produced nitrous acid, which causes oxidative deamination of nitrogenous bases of nucleotides. This may lead to a point mutation by change of cytosine to one of the next base:
A. Uracil
B. Thymine
C. Adenine
D. Guanine
E. Inosine

31. Abnormal hemoglobin M appears as a result of change at the β-chain of globin amino acid valine to glutamate at the 67 position. Which type of mutation is in DNA?
A. Missense mutation
B. Deletion of one nucleotide
C. Insertion of one nucleotide
D. Deletion of three nucleotides
E. Insertion of three nucleotides

32. Under the influence of various physical, chemical and biological factors, damage of the DNA structure can occur. What is the ability of cells to repair such damage called?
A. Reparation
B. Transcription
C. Replication
D. Transduction
E. Transformation

33. In modern biochemical investigations for diagnostics of inherited diseases, detection of certain viruses (for example HIV), authentication of individual (gene dactylography in forensic medicine) so called «DNA-diagnostics» is employed. What method is used in these investigations?
A. Polymerase chain reaction
B. Chromatography
C. Electrophoresis
D. X-ray-structural analysis
E. Electron microscopy

34. An experiment proved that UV-radiated cells of patients with xeroderma pigmentosum restore the native DNA structure slower than the cells of healthy individuals as a result of the reparation enzyme defection. What enzyme helps in this process?
A. Endonuclease
B. DNA polymerase III
C. DNA hyrase
D. RNA ligase
E. Primase

35. What are enzymes involved in the DNA repair?
A. DNA-polymerase, DNA-ligase
B. RNA-ase and DNA-ase
C. DNA-dependent RNA-polymerase
D. Peptidyltransferase and peptidyltranslosase
E. Nucleosidase and nucleotidase

36. What are quantitative and qualitative changes in the genotype called?
A. Mutation
B. Reparation
C. Amplification
D. Induction
E. Reversion

37. Molecular analysis of the hemoglobin of a patient with anemia revealed a substitution of glutamic acid for valine in the 6th position of the β-chain of globin. What is the molecular mechanism of this pathology?
A. Gene mutation
B. Chromosomal mutation
C. Genomic mutation
D. Amplification of genes
E. Gene transduction

38. On the African continent, sickle-cell anemia is common, in which the erythrocytes have the shape of a sickle due to the replacement of the amino acid glutamate with valine in the hemoglobin molecule. What is the cause of this disease?
A. Gene mutation
B. Transduction defect
C. Violation of mechanisms for the implementation of genetic information
D. Crossover fault
E. Genomic mutation

39. Spontaneous mutations include those that are caused by:
A. Natural factors
B. Transduction
C. Short-range factors
D. Artificial factors
E. Reversion

40. Induced mutations are caused by:
A. Artificial factors
B. Transduction
C. Short-range factors
D. Natural factors
E. Reversion

41. At what kind of chromosomal mutation is there the transfer of a site of one chromosome to another, not homologous to it?
A. Translocation
B. Transposition
C. Inversion
D. Deletion
E. Duplication

42. At what kind of chromosome mutation is there a loss of certain areas of the chromosome?
A. Deletion
B. Translocation
C. Transposition
D. Inversion
E. Duplication

43. At what kind of chromosomal mutation is the sequence of genes reversed?
A. Inversion
B. Translocation
C. Transposition
D. Deletion
E. Duplication

44. At what kind of chromosomal mutation is a doubling of certain areas of chromosomes?
A. Duplication
B. Translocation
C. Transposition
D. Inversion
E. Deletion

45. At what kind of gene mutation is the purine nitrogen base replaced by purine?
A. Transition
B. Transversion
C. Inversion
D. Deletion
E. Insertion

46. At what kind of gene mutation is the purine nitrogen base replaced by pyrimidine?
A. Transversion
B. Transition
C. Inversion
D. Deletion
E. Insertion

47. Amplification of genes is:
A. Increase in the number of copies of genes
B. Correction of altered genes
C. Changing of the sequence of genes to the opposite
D. The loss of several consecutive genes
E. Gene insertion

48. Over 50 % of human tumours have developed an inactivating mutation in p53 activity. The lack of this activity contributes to tumour cell growth via which one of the following mechanisms?[1]
A. Increase in DNA mutation rates
B. Loss of Wnt signalling
C. Activation of MAP kinases
D. Increase in apoptotic events
E. Increase in transcription-coupled DNA repair

49. Analysis of a cell line that rapidly transforms into a tumour cell line demonstrated an increased mutation rate within the cell. Further analysis indicated that there was a mutation in the DNA polymerase enzyme that synthesizes the leading strand. This inactivating mutation is likely to be in which of the following activities of this DNA polymerase?[2]
A. 3′–5′ exonuclease activity
B. 5′–3′ exonuclease activity
C. Phosphodiester bond making capability
D. Uracil-DNA glycosylase activity
E. Ligase activity

50. An 8-month-old child is brought to the pediatrician's office due to excessive sensitivity to the sun. Skin areas exposed to the sun for only a brief period of time were reddened with scaling. Irregular dark spots have also appeared. The pediatrician suspects a genetic disorder in which of the following processes?[3]
A. Nucleotide excision repair
B. Transcription
C. Base excision repair
D. DNA replication
E. Translation

51. A 13-year-old exhibited developmental delay, learning disabilities, mood swings, and at times, autistic behaviour when he was younger. His current physical exam shows a long face, large ears, and large, fleshy hands. His fingers exhibit hyperextensible joints. Examination of fibroblasts cultured from the boy showed abnormal DNA damage, but only in the absence of folic acid. This disorder has, at the genetic level, which one of the following?[4]

A. An extended triplet nucleotide repeat
B. A large deletion
C. A single missense mutation
D. A nonsense mutation
E. Gene inactivation via methylation

52. You have been following a newborn who first presented with hypotonia and trouble sucking. Special feeding techniques were required for the child to gain nourishment. As the child aged, there appeared to be developmental delay, and the child then gained a great interest in eating, and rapidly became obese. Developmental delay was still evident, as was hypotonia. A karyotype analysis of this patient would indicate which of the following?[5]

A. A deletion
B. A trisomy
C. A duplication
D. A chromosomal inversion
E. A monosomy

53. Spontaneous deamination of certain bases in DNA occurs at a constant rate under all conditions. Such deamination can lead to mutations if not repaired. Which deamination indicated below would lead to a mutation in a resulting protein if not repaired?[6]

A. C to U
B. T to U
C. G to A
D. A to G
E. U to C

54. A couple sees an obstetrician due to difficulties of the woman keeping a pregnancy to term. She has had three miscarriages over the past 6 years, and the couple is searching for an answer. Karyotype analysis of the woman gave the result of 45, XX, der (14; 21). A likely potential cause of the miscarriages may be which of the following?[7]

A. Imbalance of DNA in euploid conceptions
B. Imbalance of DNA in polyploid conceptions
C. Triple X conceptions
D. Zero X conceptions
E. Trisomy 21 conceptions

55. Concerned parents are referred to a specialty clinic by their family physician due to abnormalities in their 18-month-old child's development. The child displays delayed psychomotor development, and is mentally retarded. The child is photosensitive, and also appears to be aging prematurely, with a stooped posture and sunken eyes. The altered process in this autosomal recessive disorder is which of the following?[8]

A. Transcription-coupled DNA repair
B. DNA replication
C. Base excision repair
D. Proofreading by DNA polymerase
E. Sealing nicks in DNA

56. A new patient visits your practice due to his concern of developing colon cancer. A large number of relatives have had premature (less than the age of 45) colon cancer, and all cases were right-sided, with the only visible polyps being found on that side. The molecular basis for this form of colon cancer is which of the following?[9]

A. A defect in DNA mismatch repair
B. A defect in base excision repair
C. A defect in the Wnt signaling pathway
D. A defect in repairing double-strand DNA breaks
E. A defect in telomerase

57. An individual having β-thalassemia minor exhibits two bands on a Northern blot using a probe against exon 1 of β-globin. The smaller band is of normal size and «heavier» than the other larger band, which consists of approximately 247 additional nucleotides. One explanation for this finding is which of the following?[10]

A. The presence of a nonsense mutation in the DNA
B. A mutation which creates an alternative splice site
C. A lack of capping of the mRNA
D. An extended poly-A tail
E. A loss of AUG codons

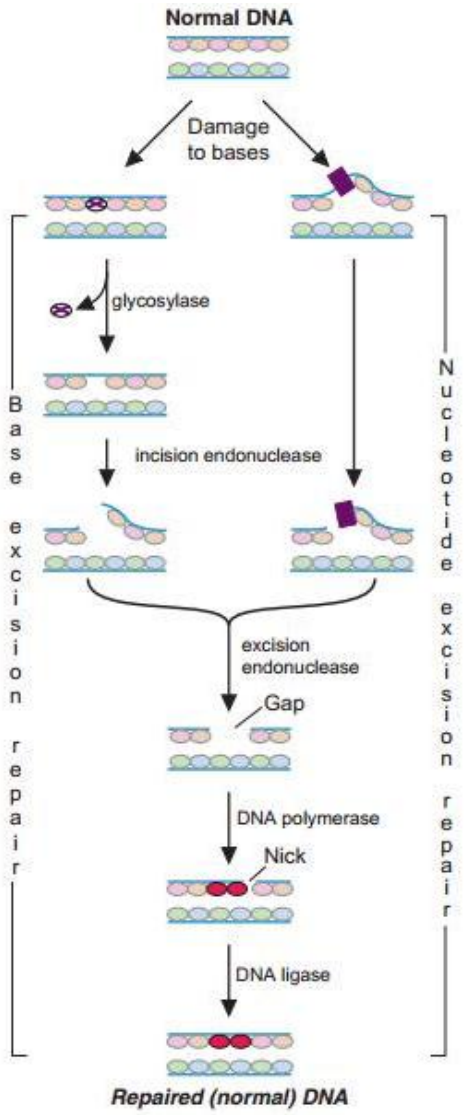
58. An intestinal cell line was being studied for its ability to produce lipid-containing particles. Surprisingly, a mutated variant of this cell line was unable to do so. Western blot analysis yielded a protein with the same size as apolipoprotein B100. A potential mutation in this cell line, which would lead to this result, is which of the following?[11]

A. RNA editing defect
B. Cap formation altered
C. Splicing defect
D. Inefficient poly-A tail addition
E. Promoter alteration

1 The answer is: Increase in DNA mutation rates. p53 is a protein which scans the genome for damage, and when damage is spotted, it induces the synthesis of genes which will stop the cell from continuing through the cell cycle. p53 will also lead to the synthesis of genes involved in repairing the DNA damage. Once the damage has been repaired, the cell will resume its passage through the cell cycle. If the damage cannot be repaired, apoptosis will be initiated in the cell. If p53 is missing, or mutated such that its functions are lost, damaged DNA will be replicated, and at times, the replisome will make errors repairing the damage. This will increase the overall mutation rate of the cell such that eventually mutations will appear in genes which regulate cell proliferation, and a cancer will develop. p53 is not involved in Wnt signaling or activation of MAP kinases. Functional p53 can increase apoptotic events, but the lack of p53 will actually decrease the frequency of apoptosis in cells. This protein is also not involved in transcriptioncoupled DNA repair.

2 The answer is: 3'-5' exonuclease activity. DNA polymerase rarely makes mistakes when inserting bases into a newly synthesized strand and base-pairing with the template strand. However, mistakes do occur at a frequency of about one in a million bases synthesized, but DNA polymerase has an error checking capability which enables it to remove the mispaired base before proceeding with the next base insertion. This is due to the 3′-5′ exonuclease activity of DNA polymerase by which, prior to adding the next nucleotide to the growing DNA chain, the base put into place in the previous step is examined for correct base-pairing properties. If it is incorrect, the enzyme goes «backwards» and removes the incorrect base, then replaces it with the correct base. The 5′-3′ exonuclease activity of DNA polymerase moves ahead, and is used to remove RNA primers from newly synthesized DNA. If the enzyme could no longer synthesize phosphodiester bonds (the primary responsibility of the enzyme), DNA synthesis would halt. A loss of uracil-DNA glycosylase activity is not a property of DNA polymerase, but that of a separate enzyme system which repairs spontaneous deamination of cytosine bases to uracil within DNA strands. If these were left intact, mutations would increase in DNA. A loss of ligase activity would lead to unstable DNA, as the Okazaki fragments would not be able to be sealed together to form one continuous piece of DNA, and this would most likely lead to cell death, not an increased mutation rate.

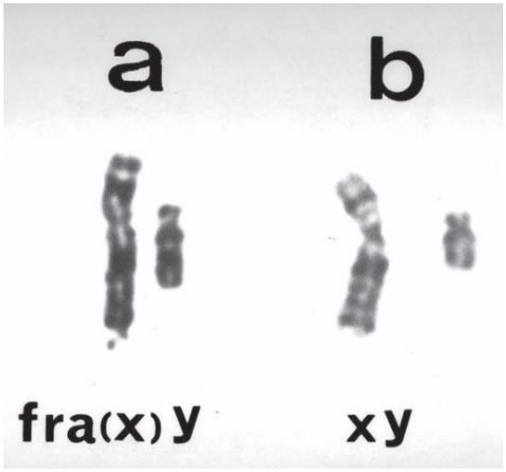
3 The answer is: Nucleotide excision repair. The child is suffering from a form of xeroderma pigmentosum, a disorder in which thymine dimers (created by exposure to UV light) cannot be appropriately repaired in DNA. Nucleotide excision repair enzymes recognize bulky distortions in the helix, whereas base excision repair recognizes only specifi c lesions of a small, single, damaged base. The mechanism whereby thymine dimers are removed from DNA is nucleotide excision repair in which entire nucleotides are removed from the damaged DNA. In base excision repair, only a single base is removed; the sugar phosphate backbone is initially left intact. This disorder is not due to alterations in transcription (synthesizing RNA from DNA), DNA replication, or translation (synthesizing proteins from mRNA). Another example of a disease resulting from a defect in nucleotide excision repair is Cockayne syndrome. Neurological diseases (such as Alzheimer's) may also have a deficiency in nucleotide excision repair.



A comparison of nucleotide excision repair and base excision repair.

4 The answer is: An extended triplet nucleotide repeat. The boy is displaying the symptoms of fragile X syndrome. Fragile X contains a triplet nucleotide repeat (CGG) on the X chromosome in the 5′ untranslated region of the FMR1 gene. The triplet repeat expansion leads to no expression of the FMR1 gene, which produces a protein required for brain development. Its function appears to be that of an mRNA shuttle, moving mRNA from the nucleus to appropriate sites in the cytoplasm for translation to occur. Depending on the level of expression of FMR1 (which is dependent on the number of repeats), the symptoms can vary from mild to severe. Less than 1 % of cases of fragile X are due to missense or nonsense mutations; the vast majority are due to the expansion of the triplet repeat at the 5’ end of the gene. Gene inactivation by methylation, or deletion, are not causes of fragile X syndrome. The syndrome was called fragile X because the X chromosome that carries the repeat expansion is subject to DNA strand break under certain conditions (such as lack of folic acid), which does not occur with normal X chromosomes. The area containing the repeat alters the staining pattern of the X chromosome, allowing this to be seen in a karyotype.

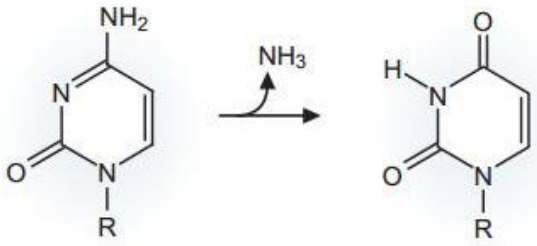
Fragile X is the most common inheritable cause of mental retardation. Males are more severely affected. In early childhood, developmental delay, speech and language problems, and autisticlike behavior are noticeable. After puberty, the classic physical signs develop (large testicles, long-thin face, mental retardation, large ears, and prominent jaw).



Picture of a fragile X chromosome and normal X and Y chromosomes. Note the end of the long arm (q), and the differences between the two chromosomes.

5 The answer is: A deletion. The child has PraderWilli syndrome, which is due to a deletion of a cluster of genes on chromosome 15, on the long arm. When this deletion is inherited from the father, Prader-Willi syndrome is observed. If the same deletion is inherited from the mother, an entirely different syndrome is observed, termed Angelmann syndrome. The diagnosis can be confirmed by FISH analysis using a probe specifi c for the 15q11–13 region.

6 The answer is: C to U. Cytosine spontaneously deaminates to form uracil while in DNA. This error is repaired by the uracil-DNA glycosylase system, which recognizes this abnormal base in DNA and initiates the process of base excision repair to correct the mistake. Neither thymine nor uracil contains an amino group to deaminate. When adenine deaminates, the base hypoxanthine is formed (inosine as part of a nucleoside), and guanine deamination will lead to xanthine production.



The deamination of cytidine to uridine (C to U within a DNA strand).

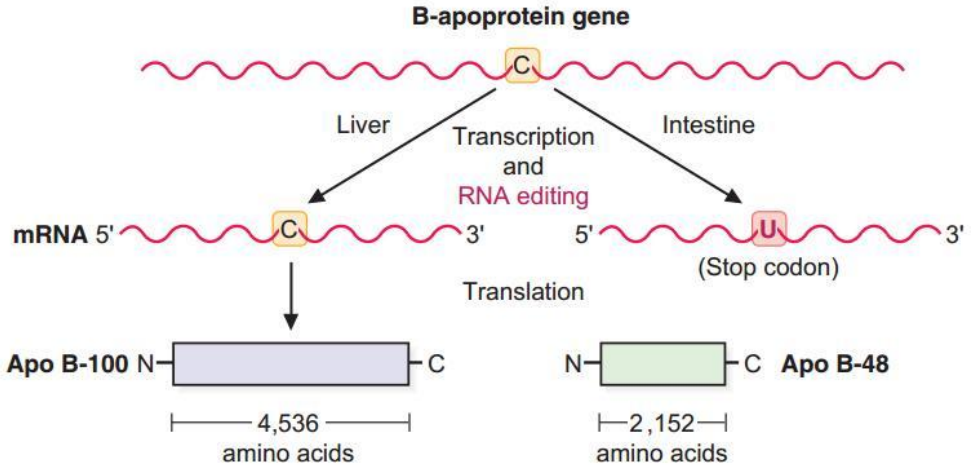
7 The answer is: Imbalance of DNA in euploid conceptions. The woman has a Robertsonian translocation between chromosomes 14 and 21 (the two chromosomes are fused together at their stalks. When she creates her eggs, there is an imbalance in the amount of DNA representing chromosomes 14 and 21 in the eggs, such that fertilization the eggs will lead to either monosomy or trisomy with these chromosomes, most of which are incompatible with life.. Polyploid outcomes would be three or more times the normal number of chromosomes, which does not occur here; and the Robertsonian translocation will not affect the distribution of the X chromosome. Trisomy 21 will lead to a live birth, Down syndrome, although there is still a risk of miscarriage with trisomy 21 conceptions. The risk is lower, however, than an imbalance of DNA brought about by the segregation of the chromosomes containing the Robertsonian translocation. Euploid cells have a number of chromosomes which are exact multiples of the haploids (in humans haploid is 23, diploid is 46, and polyploid is 69 or 92 chromosomes).

8 The answer is: Transcription-coupled DNA repair. The child is exhibiting the symptoms of Cockayne syndrome (CS), a defect in transcription-coupled DNA repair. Transcription-coupled DNA repair occurs only on actively transcribed genes; if RNA polymerase is halted due to damage to DNA on an actively transcribed gene, this repair system fi xes the DNA such that transcription can continue. Cells derived from patients with CS have a reduction in RNA synthesis in response to UV irradiation, as transcription-coupled DNA repair is reduced, thereby reducing the rate of RNA produced from genes which did contain thymine dimers. There are at least two forms of CS: CS 1 (or A), the form present at birth, and CS 2 (or B), one that occurs later in life, during early childhood. The two forms are due to mutations in two different genes (ERCC8, on chromosome 5, is responsible for CS-A, and ERCC6, on chromosome 10, is responsible for CS-B). The child's symptoms are not due to defects in base excision repair or in DNA ligase (sealing nicks in DNA). DNA replication is normal in these children, as is the proofreading capability of DNA polymerase.

9 The answer is: A defect in DNA mismatch repair. The patient is concerned about HNPCC, hereditary nonpolyposis colon cancer, which is due to mutations in genes which are involved in DNA mismatch repair. This colon tumor does not form large numbers of polyps within the intestine, as does the other form of inherited colon cancer, adenomatous polyposis coli (APC). HNPCC is also a right-sided colon cancer. Defects in base excision repair do not lead to HNPCC. A defect in the Wnt signaling pathway, which controls the action of β-catenin, an important transcription factor, may play a role in APC. Defects in repairing double-strand breaks in DNA are linked to breast cancer. Mutations in telomerase would lead to earlier cell senescence and death due to an inability to maintain the proper length of the chromosomes.

10 The answer is: A mutation which creates an alternative splice site. The patient has developed a mutation in an intron which acts, only a small percentage of the time, as a splice donor site instead of the normal site at the intron/exon boundary. Thus, when this site is utilized by the splicesome, a piece of the intron is incorporated into the mRNA product, producing a longer than normal mRNA. This is an infrequent event, however, as judged by the finding that the density of the normal sized mRNA band on the gel is darker than this abnormal band. A nonsense mutation in the DNA will not affect transcription (although it does affect the protein product made from the mRNA). The lack of a cap would result in an unstable mRNA that perhaps would not be translated but would not signifi cantly change the size of the mRNA. Poly-A polymerase adds the poly-A tail and would add the same size tail to both species of mRNA. If the polyadenylation signal were mutated, then the overall mRNA size would be larger, but there would not be two different proteins produced. Since the patient has a β-thalassemia, defective β-globin protein is being produced from the larger mRNA. Loss of methionine codons will affect translation, but not transcription.

11 The answer is: RNA editing defect. The intestine contains an RNA editing complex that alters one base in the apo B100 mRNA, which creates a stop codon, such that when the mRNA is translated, protein synthesis stops after 48 % of the codons have been translated. This is a unique type of posttranscriptional modification. The initial transcripts for both apo B48 and apo B100 are the same. Mutations that alter splicing, cap formation, or polyadenylation would not produce the full size protein in place of apo B48. Mutations in the promoter would alter initiation of transcription, but not the end product formed.



In intestinal cells, RNA editing converts a cytosine (C) to a uracil (U), producing a stop codon. Consequently, the B-apoprotein of intestinal cells (apo B48) contains only 2,152 amino acids. Apo B48 is 48 % the size of apo B100, the product synthesized in the liver from the same gene, and which was not edited at the RNA level.

Topic 6.
Investigation of the role of hormones of protein-peptide nature on metabolism

1. Objective: To learn biochemical and physiological function of hormones in a system of intercellular integration in the organism. To learn structure of hormones of protein nature, mechanisms of their action upon the target cells, role of secondary messengers in cell response to the hormones of protein or amino acid nature.

2. Actuality of the theme: Hormones are conventionally defined as organic substances, produced in small amounts by specific tissues (endocrine glands), secreted into the blood stream to control the metabolic and biological activities in the target cells. Hormones may be regarded as the chemical messengers involved in the transmission of information from one tissue to another and from cell to cell.

Understanding of biochemical mechanisms of realization of hormones effect on functioning of cell systems allows explaining mechanisms of development of pathological states, caused by disorders in functioning of endocrine glands and target cells as well as it also forms in students considerations about the correction of hypo- or hyperfunction of endocrine glands.

3. Specific aims:

- ✓ To interpret the biochemical and physiological functions of hormones and bioregulators in a system of intercellular integration of vital functions of human organism.
- ✓ To analyse and to explain correspondence between the structure of protein and peptide hormones to their function and mechanism of action on target cells.
- ✓ To interpret the molecular mechanisms of action of hormones of protein and peptide nature, up on target cells with involvement of signaling mediator molecules.

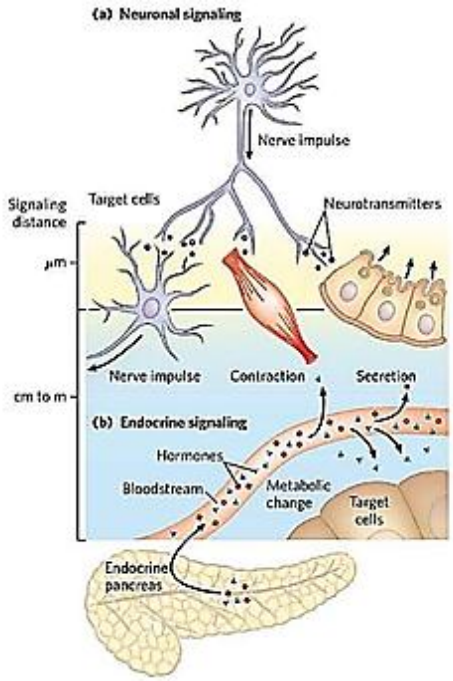
4. Reference card for the separate study of educational literature for the lesson preparation

	Questions:	References:
1.	**Hormones in a system of intercellular integration of physiological functions in human organism. Classification of hormones.**	1. Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 427–428. 2. Gubsky Yu. Biological chemistry: textbook / edited by Yu. Gubsky. – Vinnytsia: Nova Knyha, 2018. – 2nd edition. – P. 301–302. 3. Lecture notes.
2.	**Regulation of synthesis and secretion of hormones. Cyclic nature of hormone secretion, transport of hormones in blood. Factors, influencing the secretion and effect of hormone action:** ✓ Regulation of synthesis and secretion of hormones; ✓ Cyclic nature of hormone secretion. ✓ Transport of hormones in blood. ✓ Factors, influencing the secretion and effect of hormone action	1. Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 427–429. 2. Gubsky Yu. Biological chemistry: textbook / edited by Yu. Gubsky. – Vinnytsia: Nova Knyha, 2018. – 2nd edition. – P. 303. 3. Lecture notes.
3.	**Targets of hormonal action; types of cell response to the hormone. Membrane and cytosol receptors of hormones in the cell:** ✓ Targets of hormonal action; ✓ Types of cell response to the hormone; ✓ Membrane and cytosol receptors of hormones in the cell	1. Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 428–429. 2. Gubsky Yu. Biological chemistry: textbook / edited by Yu. Gubsky. – Vinnytsia: Nova Knyha, 2018. – 2nd edition. – P. 303. 3. Lecture notes.

4.	**Mechanisms of hormonal action – amino acid derivatives, peptide and protein hormones, steroid hormones. Regulatory sites in DNA, which interacts with hormone-receptor complexes. Messenger function of cyclic nucleotides, Ca/calmodulin system, phosphoinositides. Serine, threonine and tyrosine proteinkinases in effectory response of the cell:** ✓ Mechanisms of hormonal action; ✓ Messenger function of cyclic nucleotides; ✓ Ca/calmodulin system, phosphoinositides; ✓ Serine, threonine and tyrosine proteinkinases in effectory response of the cell	1. Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 428–431. 2. Gubsky Yu. Biological chemistry: textbook / edited by Yu. Gubsky. – Vinnytsia: Nova Knyha, 2018. – 2nd edition. – P. 303–307. 3. Lecture notes.
5.	**Hormones of hypothalamus. Mechanism of their action:** ✓ Hormones of hypothalamus; ✓ Mechanism of action of hypothalamus.	1. Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 431–437. 2. Gubsky Yu. Biological chemistry: textbook / edited by Yu. Gubsky. – Vinnytsia: Nova Knyha, 2018. – 2nd edition. – P. 308–310. 3. Lecture notes.
6.	**Tropic hormones of the anterior pituitary:** ✓ Group «growth hormone (somatropin) – prolactin – chorionic somatomamotropin»; pathological processes associated with impaired growth hormone, somatomedin, prolactin; ✓ A group of glycoproteins – pituitary trophic hormones (TSH, gonadotropins, FSH, LH), chorionic gonadotropin; ✓ POMK hormones – processing products of POMK (adrenokortykotropin, lipotropin, endorphins).	1. Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 432–437. 2. Gubsky Yu. Biological chemistry: textbook / edited by Yu. Gubsky. – Vinnytsia: Nova Knyha, 2018. – 2nd edition. – P. 311–317. 3. Lecture notes.
7.	**Hormones of the posterior pituitary: vasopressin (antidiuretic hormone) and oxytocin. Mechanism of action. The use of oxytocin in medical practice:** ✓ Vasopressin (antidiuretic hormone); ✓ Oxytocin$ ✓ Application of oxytocin in medical practice.	1. Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 437. 2. Gubsky Yu. Biological chemistry: textbook / edited by Yu. Gubsky. – Vinnytsia: Nova Knyha, 2018. – 2nd edition. – P. 318–321. 3. Lecture notes.
8.	**Characteristics of pancreatic hormones:** ✓ Endocrine function of the pancreas (insulin, glucagon, somatostatin, pancreatic polypeptide); ✓ Insulin – structure, biosynthesis and secretion; characterization of insulin receptors, molecular mechanisms of action (effect on metabolism of carbohydrates, lipids, amino acids and proteins); ✓ Gucagon – the chemical nature and the biological effect of the hormone.	1. Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 669–676. 2. Gubsky Yu. Biological chemistry: textbook / edited by Yu. Gubsky. – Vinnytsia: Nova Knyha, 2018. – 2nd edition. – P. 321–326. 3. Lecture notes.

5. Tasks for independent work and self-control

5.1. The coordination of metabolism in mammals is achieved by the _____ _____ system, which produces _____ _____ signaling, carried out by neurotransmitters (acetylcholine, norepinephrine etc.) and _____ _____ signaling.



5.2. Give the definition of term
Hormones

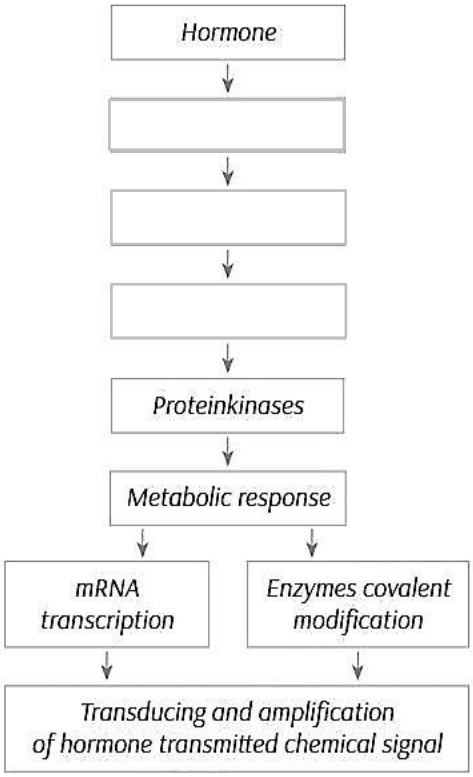
5.3. Complete the table «Classification of hormones based on the chemical nature»

Groups	Examples

5.4. Complete the table «Classification of hormones based on the mechanism of action»

Groups	Characteristic	Mechanism of action	Examples
Group I. Hormones that bind to intracellular receptors			
Group I. Hormones that bind to cell surface receptors		The second messenger is cAMP	
		The second messenger is phosphatidyl inositol/calcium	
		The second messenger is unknown/unsettled	

5.5. Complete the scheme «The consequence of biochemical events underlying the transformation of hormone induced regulatory signal to the target cells response»



5.6. It is known that the key event in the stimulation of the biochemical reactions cascade is the interaction of the hormone-bound receptor with a special transducing device which role is played by G proteins. Which types of G proteins are there?

5.7. Write the scheme of activation of protein kinase A.

5.8. Complete the table «Hormones of the hypothalamus»

Hormone	Chemical nature	Functions
GHRH		
Somatostatin		
TRH		
CRH		
GRH		
PRH		

In addition to the hypothalamus, somatostatin is also secreted from the δ-cells of the pancreatic islets, many areas of the central nervous system outside of the hypothalamus, and in gastric and duodenal mucosal cells. Indicate the effects of somatostatin in these tissues.

5.9. Complete the table «Anterior pituitary hormones»

Hormone	Chemical nature	Target tissues, functions
Growth hormone (GH)/somatotropin		
Prolactin		
Corticotrophin (ACTH)		
Thyrotropin, thyroid-stimulating hormone (TSH)		
Luteinizing hormone (LH)		
Follicle stimulating hormone (FSH)		

5.10. Complete the table «Hormones of the posterior pituitary»

Characteristic	Vasopressin	Oxytocin
Chemical nature		
Place of producrion		
Place of storage		
Stimulation of secretion		
The main target cells and effects	1) 2)	1) 2)
Mechanism of action		

5.11. Complete the table «Pancreatic hormones»

Characteristic		Insulin	Glucagon
Chemical structure, posttranslational modification			
Place of production			
Regulation of secretion 1) stimulators 2) inhibitors			
The main target tissues and cells		1) 2) 3)	1) 2)
Hormone-nondependent cells and tissues			
Effects on carbohydrate metabolism, key enzymes	Glucose uptake Glycogenesis Glycogenolysis Gluconeogenesis Glycolysis		
Effects on lipid metabolism	Lipogenesis Lipolysis Ketogenesis		
Effect on protein metabolism	AAs uptake Synthesis Degradation		
Receptor and signal transduction system			
Normal half-life in the plasma			

5.12. Situational task

1) Hormones exert two types of metabolic regulation. The first type of control occurs within minutes to hours of the hormone-receptor interaction and usually results from changes in the catalytic activity or kinetics of key preexisting enzymes, caused by phosphorylation or dephosphorylation of these enzymes. The second type of control involves regulation of the synthesis of key enzymes by mechanisms that stimulate or inhibit transcription and translation of mRNA. These processes are slow and require hours to days. List the hormones that belong to first and second types.

2) Diabetics appointed as the substitution therapy hormone insulin.
Specify the synthesis of insulin?

Specify the chemical nature of the hormone?

How regulated insulin secretion?

3) The patient 20 years (no pregnancy) was admitted to the hospital complaining of occurrence milk in the breast (galactorrhoea) and the absence of menstruation for 6 months, strong headaches.
Increased synthesis and secretion of which hormone is observed in this disease?

To which group of adenohypophysis hormones it belongs?

What is the name of this pathology.

6. Individual independent students work

1. Analysis of biochemical parameters that characterize the metabolism of carbohydrates, proteins and lipids in disorders of the endocrine glands.
2. Mechanism of action of protein-peptide, steroid, thyroid hormones and catecholamines on target cells.

Practice protocol №6 «____» _____ 20___

Experiment. Qualitative reactions of insulin.

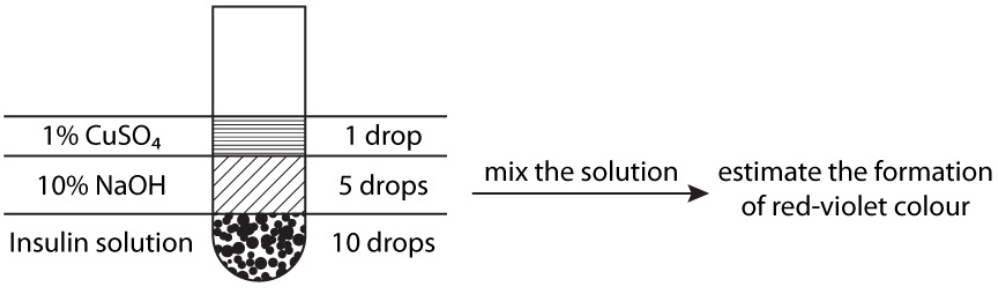
Insulin is a polypeptide hormone producedby the β-cells of islets of Langerhans of pancreas. It has profound influence on the metabolism of carbohydrate, fat and protein. Insulinis consideredas anabolic hormone, as it promotes the synthesis of glycogen, triacylglycerols and

proteins. This hormone has been implicated in the development of diabetes mellitus. Insulin gives all characteristic reactions of proteins.

Biuret probe.

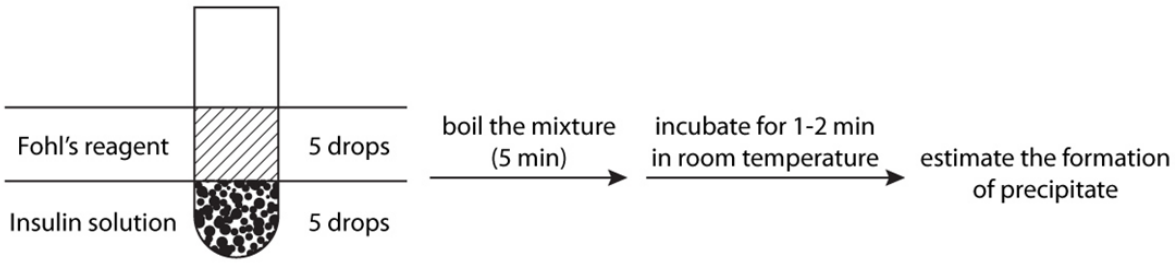
1. Into a clean, dry tube add 10 droplets of insulin solution.
2. Add 5 drops of 10 % solution of NaOH and one drop of $CuSO_4$ solution. Mix the tube carefully.

A red-violet color appears which is characteristic for proteins and peptides.



Fohl's reaction.

1. Into a clean, dry tube add 5 droplets of insulin solution.
2. Add 5 droplets of Fohl's reagent (alkaline solution of lead acetate).
3. Boil the mixture 5 min. Incubate the solution for 1-2 min at room temperature. Observe the formation of precipitate.



Millon's reaction.

1. Into a clean, dry tube add 5 droplets of insulin solution.
2. Add 1-2 ml of Millon's reagent.
3. Carefully heat the mixture on a flame. Observe the formation of red precipitate.

Results:

Conclusion:

The report is checked up _____

(The signature of the teacher, date)

Examples of Krock-1 tests

1. Indicate a substance which serves as a secondary messenger and increases Ca^{2+} ion concentration:
A. Inositol-1,4,5-triphosphate
B. Inositol-3,6-bisphosphate
C. Phosphatidyl-inositol-4,5-bisphosphate
D. Inositol-6-phosphate
E. Free inositol

2. Adrenalin is a water soluble hormone, its effect is mediated by second messengers, which are:
A. cAMP
B. NAD^+
C. ATP
D. Acetylcholine
E. Cytochrome c

3. All of the following are known to be a part of a signal transduction cascade except:
A. Phosphorylation of fibronectin
B. Dissociation of the components of a heterotrimeric G-protein
C. Enzymatic breakdown of phosphatidyl inositol bisphosphate
D. Elevation of intracellular Ca^{2+}
E. Activation of cGMP phosphodiesterase

4. Second messengers diacylglycerol and inositol triphosphate are produced from subsequent phospholipid of plasma membrane due to the activity of the following enzyme:
A. Phospholipase C
B. Phospholipase A_1
C. Phospholipase A_2
D. Phospholipase D
E. Phosphodiesterase

5. Ca^{2+} ions constitute one of the most ancient evolutionary second messengers. They are activators of glycogenolysis in case of reacting with:
A. Calmodulin
B. Calcitonin
C. Calciferol
D. Kinase of myosin light chains
E. Phosphorylase C

6. An emotional stress induces activation of hormone-sensitive triglyceride lipase in the adipocytes. What secondary mediator takes part in this process?
A. cAMP
B. cGMP
C. AMP
D. Diacylglycerol
E. Calcium ions

7. Which of the following enzymes is activated by cAMP?
A. Protein kinase A
B. Tyrosine kinase
C. Phospholipase C
D. Phosphodiesterase
E. Adenylyl cyclase

8. There are intracellular receptors for the following hormones:
A. Thyroxine
B. Follicle stimulating hormone (FSH)
C. Oxytocin
D. Insulin
E. ACTH

9. G-proteins act as:
A. Hormone carriers
B. Hormone receptors
C. Second messengers
D. Signal transducers
E. Enzyme carriers

10. ACTH induces rise in:
A. cAMP
B. cGMP
C. Calcium
D. Magnesium
E. Sodium

11. The formation of second messengers is obligatory in a membrane-intracellular mechanism of hormone action. Point out the substance that is unable to be a second messenger:
A. Glycerol
B. Diacylglycerol
C. Inositol-3,4,5-triphosphate
D. cAMP
E. Ca^{2+}

12. A patient is complaining of dryness of the mouth, blood plasma glucose is 8 mmol/L. An increased secretion of what hormone stimulates the development of hyperglycemia?
A. Glucagon
B. Insulin
C. Thyroxine
D. Triiodothyronine
E. Aldosterone

13. Ions of calcium may function as a second messenger. They are activators of many processes if they react with:
A. Calmodulin
B. Calcitonin
C. Calciferol
D. Myosin
E. Phosphorylase

14. Degeneration of glycogen in the liver is stimulated by glucagon. What second messenger is thus formed in the cell?
A. cAMP
B. cGMP
C. CO
D. NO
E. Triacylglycerol

15. What hormone is secreted from posterior pituitary gland?
A. Vasopressin
B. Thyrotropic hormone
C. Prolactin
D. Adrenocorticotropic hormone
E. Growth hormone

16. The number of amino acids in human growth hormone is:
A. 191
B. 19
C. 151
D. 291
E. 391

17. Acromegaly results due to an excessive release of:
A. Growth hormone
B. Insulin
C. Glucagon
D. Thyroxine
E. Oxytocin

18. The number of amino acids in the hormone oxytocin is:
A. 9
B. 7
C. 14
D. 18
E. 20

19. The receptors of which of the following hormones are not associated with G-protein?
A. Aldosterone
B. TSH
C. Epinephrine
D. Vasopressin
E. Glucagon

20. The patient is in a state of hypoglycaemic coma. An overdose of which of the following hormones can lead to this condition?
A. Insulin
B. Progesterone
C. Cortisol
D. Growth hormone
E. Corticotropin

21. TSH stimulates the synthesis of:
A. Thyroxine
B. Adrenocorticoids
C. Epinephrine
D. Insulin
E. Glucagon

22. Which of the following is least likely to contribute to the hyperglycaemia associated with an uncontrolled type I diabetes?
A. Increased skeletal muscle glycogenolysis
B. Decreased adipose lipogenesis
C. Increased adipose lipolysis
D. Increased hepatic gluconeogenesis
E. Decreased skeletal muscle glucose uptake

23. What hormone stimulates the reabsorption of water in kidney tubules?
A. Vasopressin
B. Parathyroid hormone
C. Calcitonin
D. Aldosterone
E. Atrial natriuretic peptide

24. Melanocyte stimulating hormone is secreted by:
A. Intermediate lobe of pituitary gland
B. Anterior lobe of pituitary gland
C. Posterior lobe of pituitary gland
D. Pineal gland
E. Thyroid gland

25. A 50-year-old patient complaints of thirst, polyuria, taking plenty of water. The blood glucose is 4.8 mmol/L. There is neither glucose nor acetone in the urine. The urine is colourless. Its relative density is 1002-1004. What caused the patient's polyuria?
A. Lack of vasopressin
B. Aldosteronism
C. Lack of insulin
D. Hypothyroidism
E. Thyrotoxicosis

26. Biologically active substances, especially hormones, are products of hydrolysis and modification of certain proteins. From which of the listed below proteins do lipotropin, cortcotropin, melanotropin and endorphins appear in hypophysis?
A. Proopiomelanocortin
B. Neuroalbumins
C. Neurostromin
D. Neuroglobulin
E. Thyreoglobulin

27. A 26-year-old woman complains of general weakness, loss of weight (18 kg), absence of menses. She has been ill for one year after a difficult delivery. Objectively: body height – 168 cm, weight – 50 kg, hypoglycaemia and atrophy of muscles. The failure of which gland is the loss of body weight connected with?
A. Adenohypophysis
B. Parathyroid
C. Thyroid
D. Sex glands
E. Adrenal cortex

28. A 23-year-old patient complains of a headache, change of appearance (increase in feet and wrists size, face features distortion). His voice grew harsh, the memory worsened. The disease set in three years ago without apparent causes. The analysis of the urine is without special changes. A possible cause of this status can be:
A. Overproduction of somatotropin
B. Deficiency of glucagon
C. Deficiency of thyroxine
D. Deficiency of aldosterone
E. Overproduction of corticosteroids

29. After a brain hemorrhage that led to the damage of hypothalamic nuclei, diabetes insipidus was developed in a 67-year-old patient. What was the reason of polyuria in this case?
A. Decrease of water reabsorption
B. Decrease of potassium ions reabsorption
C. Acceleration of glomerular filtration
D. Hyperglycemia
E. Hypoglycemia

30. Cushing's disease, which is characterized by obesity, hypertension and elevated blood glucose level, is caused by a disorder in the production and secretion of the next hormones:
A. ACTH and glucocorticoids overproduction
B. Insulin insufficiency
C. ACTH and glucocorticoids insufficiency
D. Thyroxine insufficiency
E. Estriol overproduction

31. A 47-year-old woman complains for persistent feeling of thirst, rapid fatigue, a loss of weight. Daily diuresis is 3-4 liters. Blood glucose level is 4.8 mmol/L, there is no glucose in urine. In this case it is reasonable to investigate blood content of:
A. Vasopressine
B. Estrogens
C. Aldosterone
D. Cortisole
E. Thyroxine

32. A 10-year-old boy was brought to a hospital for the inspection of the cause of growth retardation. He had grown only by three centimeters in the last two years. What hormone's deficiency is the cause of such state?
A. Somatotropin
B. Corticotropin
C. Gonadotropin
D. Thyrotropin
E. Parathormone

33. For analgesia a certain substance which imitates the physiological properties of morphine but is synthesized inside the human brain can be used. Name this substance.
A. Endorphine
B. Oxytocin
C. Vasopressin
D. Calcitonin
E. Somatoliberin

34. It is known that patients suffering from diabetes mellitus often have inflammatory processes, reduced regeneration and impaired wound healing. The reason for this is:
A. Inhibition of protein synthesis
B. Activation of lipolysis
C. Activation of gluconeogenesis
D. Activation of lipogenesis
E. Increased catabolism

35. A 13-year-old girl is observed a hypotension and polyuria. Preliminary diagnosis is diabetes insipidus. It is caused by the deficiency of:
A. Vasopressin
B. Aldosterone
C. Adrenalin
D. Cortisol
E. Oxytocin

36. After injection of insulin the glucose level is reduced within a few seconds. What process is activated in this case?
A. Transport of glucose into the cells from the extracellular space
B. Glycolysis
C. Synthesis of glycogen
D. Synthesis of lipids
E. Pentose phosphate pathway

37. Dysfunction of pancreatic islets of Langerhans leads to a decreased production of:
A. Glucagon and insulin
B. Thyroxine and calcitonin
C. Insulin and adrenalin
D. Kallikrein and angiotensin
E. Parathyroid hormone and cortisol

38. Which hormone stimulates glucose transport from the extracellular space through the plasma membrane into the cell?
A. Insulin
B. Glucagon
C. Thyroxine
D. Aldosterone
E. Adrenaline

39. A 15-year-old patient complains of constant thirsting, frequent urination, increased appetite, itching and weakness. In the blood: glucose is 16 mmol/L, ketone bodies – 100 mmol/L. What is the disease developed in the patient?
A. Insulin-dependent diabetes mellitus
B. Insulin-independent diabetes mellitus
C. Steroid diabetes
D. Diabetes insipidus
E. Thyrotoxicosis

40. A 40-year-old woman complaining of thirst and polyuria was delivered to the endocrinology department with a diagnosis of diabetes mellitus. What pathological components are identified in laboratory investigation of patient's urine?
A. Glucose, ketone bodies
B. Protein, amino acids
C. Protein, creatine
D. Bilirubin, urobilin
E. Blood

41. A patient suffering from diabetes mellitus after insulin injection lost consciousness, convulsions appeared. What was the result of the biochemical blood test for glucose?
A. 2.5 mmol/L
B. 3.3 mmol/L
C. 8.0 mmol/L
D. 10.0 mmol/L
E. 5.5 mmol/L

42. The patient's blood glucose level on an empty stomach is 4.65 mmol/L, in 1 hour after glucose loading is 6.55 mmol/L, and after 2 hours is 3.2 mol/L. These figures are typical for:
A. Healthy state
B. Latent diabetes mellitus
C. Noninsulin-dependent diabetes mellitus
D. Insulin-dependent diabetes mellitus
E. Thyrotoxicosis

43. A 46-year-old patient complains of dry mouth, frequent urination and a general weakness. Biochemical analysis revealed hyperglycemia and hyperketonemia. Glucose and ketone bodies are detected in urine. The diffuse changes in the myocardium are observed on the electrocardiogram. What is the diagnosis in the patient?
A. Diabetes mellitus
B. Alimentary hyperglycemia
C. Acute pancreatitis
D. Diabetes insipidus
E. Coronary heart disease

44. A 27-year-old man has been rushed to the emergency room following his sudden collapse and entry into a state of unconsciousness. Examination of personal belongings revealed the patient is an insulindependent diabetic. A rapid decline in which of the following humoral factors likely triggered the sudden collapse of the patient?
A. Glucose
B. Glucagon
C. Fatty acids
D. Insulin
E. Triglyceride

45. A patient has been delivered to a hospital in a coma. The patient has lost consciousness during the marathon. The development of what kind of coma can be assumed?
A. Hypoglycemic
B. Hyperglycemic
C. Renal
D. Hepatic
E. -

46. The patient's blood glucose level on an empty stomach is 5.6 mmol/L, one hour after sugar loading is 13.8 mmol/L, and in 3 hours is 9.2 mmol/L. These figures are typical for:
A. Latent diabetes mellitus
B. Cushing's Disease
C. Acromegaly
D. Thyrotoxicosis
E. Healthy state

47. An examination of a patient's blood revealed that glucose was 16 mmol/L, ketone bodies were 1.2 mmol/L. Polyuria, glycosuria, and ketonuria were observed. What disease can be assumed?
A. Diabetes mellitus
B. Diabetes insipidus
C. Steroid diabetes
D. Cushing's syndrome
E. Cushing's disease

48. 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 of adipocytes
B. Storage of palmitoyl-CoA
C. Activation of the ketone bodies utilization
D. Activation of the synthesis of the apolipoproteins
E. Decreased activity of phosphatidylcholine-cholesterolacyltransferase in blood plasma

49. After eating the food there is alimentary (food) hyperglycemia, which stimulates the secretion of:
A. Insulin
B. Adrenaline
C. Norepinephrine
D. Cortisol
E. Glucagon

50. Ketonuria is observed in a patient. What disease is the cause of ketone bodies appearance in the urine?
A. Diabetes mellitus
B. Acute glomeluronefritis
C. Urolithiasis
D. Tuberculosis of the kidney
E. Renal infarction

51. During the examination of a 58-year-old man the signs of the activation of lipogenesis were identified, which are caused by the increased secretion of:
A. Insulin
B. Sex hormones
C. STH
D. Adrenaline
E. Thyroxine

52. Which of the following changes in hepatic metabolism explains the increased incidence of ketoacidosis observed in type I diabetes?
A. Increased β-oxidation
B. Increased protein synthesis
C. Increased lipoprotein synthesis
D. Increased glucose uptake
E. Increased glycogen breakdown

53. What is the main stimulus for the release of glucagon?
A. Hypoglycemia
B. Hyperglycemia
C. Ketosis
D. Hypercholesterolemia
E. Hyperlipoproteinemia

54. What is the main stimulus for insulin release?
A. Hyperglycemia
B. Hyperpiruvatemia
C. Hypoglycemia
D. Ketoacidosis
E. Lactic acidosis

Topic 7.
Investigation of the role of hormones – derivatives of amino acids and biogenic amines. Study of hormonal regulation of calcium and phosphate homeostasis in the body

1. Objective: To learn structure of hormones of amino acid nature and derivatives of amino acids nature, mechanisms of their action upon the target cells, role of secondary messengers in cell response to the hormones of protein or amino acid nature. To learn mechanisms of hormonal regulation of calcium homeostasis: distribution of Ca^{+2} in organism, fractions of calcium in human blood plasma, the role of bone tissue, intestines and kidneys in support of calcium homeostasis.

2. Actuality of the theme: Endocrine system and nervous system are responsible for regulation of processes of life – metabolism, growth, development, propagation and adaptation. Understanding of ethyology and pathogenesis of endocrine diseases and illnesses of nonendocrine origins is only possible on the basis of sound knowledge of biochemistry of hormones, biosynthesis and secretion, mechanisms of their action and regulation, interaction with cells and metabolic changes in tissues and organs.

Calcium performs many important functions in organism:

A) Participates in mineralization of bones and solid tissues of teeth;

B) Cofactor of enzymes;

C) Second messenger in transduction of hormonal signals;

D) Participates in muscle contraction;

E) Participates in hemostasis.

The constancy of calcium in organism is adjusted by calcitropic hormones (calcitonin, parathyroid hormone and calcitriol). Infringement of hormonal regulation of calcium homeostasis of calcium can result in hypocalcemia (it is accompanied by tetanees, development of bony rarefication) or hypercalcemia (results in a hypotonia of muscles, nephrocalcinosis).

3. Specific aims:

- ✓ To interpret the molecular mechanisms of action of hormones of derivatives of amino acids (catecholamines) up on target cells with involvement of signaling mediator molecules.
- ✓ To interpret the mechanisms of hormonal regulation of calcium homeostasis: distribution of calcium in the body, fractions of calcium in human blood plasma, the role of bone tissue, small intestine and kidneys in support of calcium homeostasis.

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

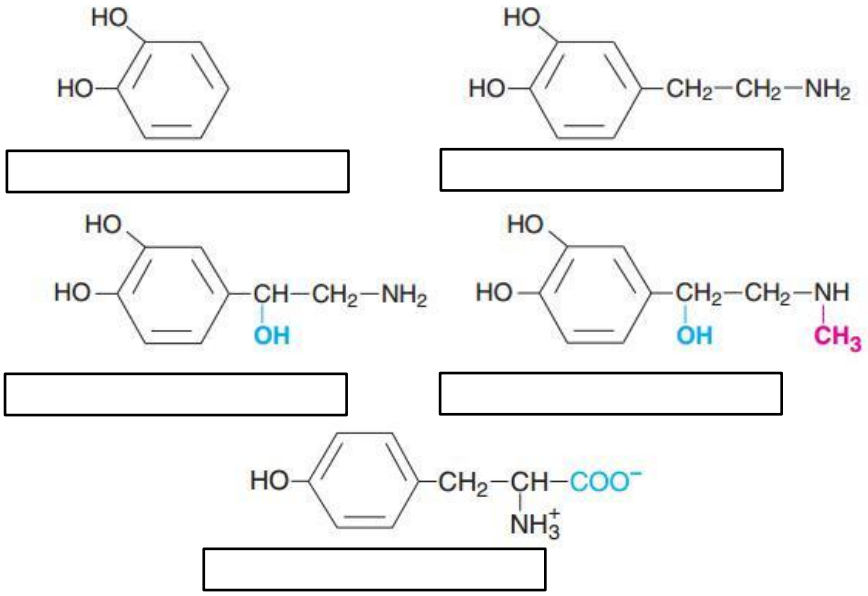
Questions:	References:
1. **Catecholamines: epinephrine, norepinephrine, dopamine. Chemical nature, biological effect, receptors. Their role in the stress response:** ✓ Epinephrine ✓ Norepinephrine ✓ Dopamine	1. Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 349–351, 444–445. 2. Gubsky Yu. Biological chemistry: textbook / edited by Yu. Gubsky. – Vinnytsia: Nova Knyha, 2018. – 2nd edition. – P. 327–330. 3. Lecture notes.
2. **The mechanism of action of parathyroid hormone and calcitonin. Parathyroid hormone – structure. Calcitriol: biosynthesis; effect on the absorption of Ca^{2+} and phosphate in the intestine. Calcitonin – structure, impact on calcium and phosphate:** ✓ The mechanism of action of parathyroid hormone and calcitonin; ✓ Structure of parathyroid hormone;	1. Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 407–408. 2. Gubsky Yu. Biological chemistry: textbook / edited by Yu. Gubsky. – Vinnytsia: Nova Knyha, 2018. – 2nd edition. – P. 342–346. 3. Lecture notes.

	✓ Biosynthesis; effect on the absorption of Ca^{2+} and phosphate in the intestine of calcitriol; ✓ Calcitonin – structure, impact on calcium and phosphate.	
3.	**Clinical and biochemical characteristics of disorders of calcium homeostasis (rickets, osteoporosis). Distribution of Ca^{2+} in the body; molecular forms of calcium in the blood plasma. The role of bone, small intestine and kidney in calcium homeostasis:** ✓ Clinical and biochemical characteristics of disorders of calcium homeostasis; ✓ Distribution of Ca^{2+} in the body; ✓ Molecular forms of calcium in the blood plasma; ✓ The role of bone, small intestine and kidney in calcium homeostasis.	1. Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 408–409. 2. Lecture notes.
4.	**General characteristics of hormone-like substances. Biochemical basis of hormonal regulation of digestion. Gastrin. Cholecystokinin. Secretin:** ✓ General characteristics of hormone-like substances; ✓ Biochemical basis of hormonal regulation of digestion.	1. Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 449–450. 2. Lecture notes.
5.	**Biogenic amines with hormonal and neurotransmitter properties: structure, biosynthesis, physiological effects, biochemical mechanisms of action (serotonin, melatonin, histamine). Receptors of biogenic amines; receptor action of drugs, histamine receptor antagonists:** ✓ Structure, biosynthesis, physiological effects, biochemical mechanisms of action (serotonin, melatonin, histamine) of the biogenic amines; ✓ Receptors of biogenic amines.	1. Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 356–358, 377. 2. Gubsky Yu. Biological chemistry: textbook / edited by Yu. Gubsky. – Vinnytsia: Nova Knyha, 2018. – 2nd edition. – P. 330. 2. Lecture notes.

5. Tasks for independent work and self-control

5.1. Describe the biosynthesis of catecholamines.

5.2. Name these catecholamines and their precursor:



5.3. Describe the biochemical functions of catecholamines

	Hormones	Effect on metabolic pathways/physiological functions	Overall effects
Carbohydrate metabolism	Epinephrine, norepinephrine	Glycogenolysis ↑ Gluconeogenesis ↑ Glycogenesis ↓	Blood glucose levels ↑
Lipid metabolism			
Physiological functions			

5.4. Enzymes which inactivate catecholamins: _____
_____.

The metabolic products of metabolism of catecholamines: _____
_____.

5.5. What is cause of pheochromocytoma? What substance is identified in urine to diagnose pheochromocytoma? Why?

5.6. Complete the table «Calcitonin and parathyroid hormone»

Characteristic	Parathyroid hormone	Calcitonin
Chemical nature		
Place of production		
Stimulation of secretion		
The main target cells and effects	1) 2) 3)	1) 2)
Mechanism of action		

5.7. Give clinical and biochemical characteristics of calcium homeostasis disorders

Disorder	Cause	Clinical and biochemical characteristics	Treatment
Hypercalcemia			
Hypocalcemia			
Rickets			
Renal rickets			
Osteoporosis			
Osteopetrosis (marble bone disease)			

5.8. A variety of peptides synthesized in the endocrine cells of the pancreatic islets, stomach, small bowel and large bowel or the cells of the enteric nervous system, influence digestion or absorption of nutrients, gastric and intestinal motility, gallbladder contraction, the synthesis or secretion of insulin or the counterregulatory hormones. Indicate the place of production and functions of the major gastrointestinal hormones:

Gastrointestinal hormones	Place of production	Mechanism of action	Functions	Stimulation	Inhibition
Gastrin					
Secretin					
Cholecystokinin (CCK)					
Gastric inhibitory peptide (GIP)					

5.9. Fill in the chart «Biogenic amines with hormonal and neurotransmitter properties»

Features	Serotonin	Histamine	Melatonin
The way of formation			
Derived from amino acid			
Functions			

5.10. Situational task

In 6 years old boy suffering from convulsions found hypocalcaemia, hyperphosphatemia, hipophosphaturia.

With the lack of what hormone synthesis it related?

What is the name of this disease?

Introduction of which substances reduce the manifestations of this disease?

6. Individual independent students work

1. Distribution of calcium ions in the body, forms of calcium in human blood plasma.
2. The contribution of bone tissue, small intestine and kidneys to calcium homeostasis.

Practice protocol №7 «____» _____ 20___

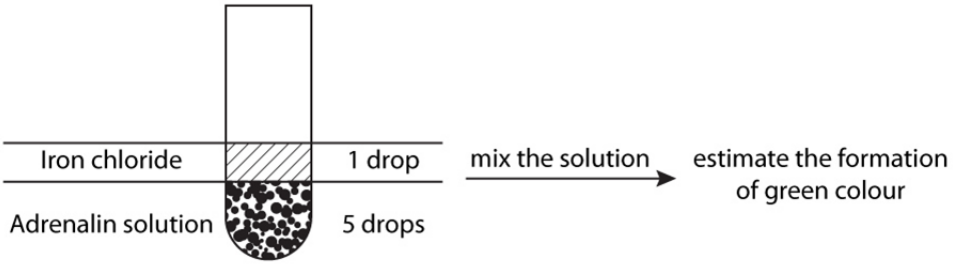
Experiment 1. Qualitative reactions of adrenalin.

Principle. Adrenalin is readily oxidized by air oxygen to adrenochrome which gives a characteristic green color with ferric chloride or red color with diazotisized sulfanilic acid (diazoreagent).

Reaction with iron chloride.

Procedure.

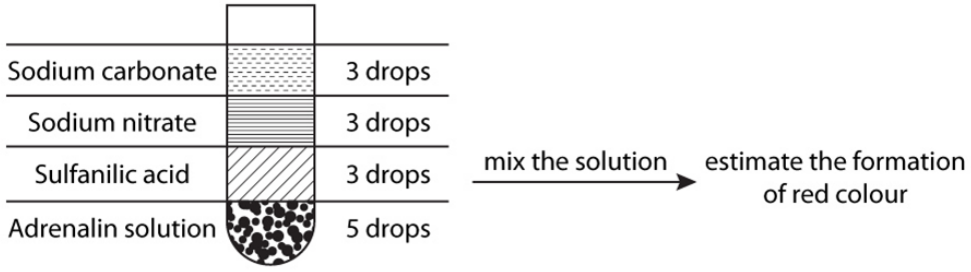
1. Into a clean, dry tube add 5 droplets of adrenalin solution.
2. Add 1 droplet of iron chloride solution. Green color appears.
3. After addition of 3 drops of 10 % NaOH a cherry-red compound is formed.



Diazoreaction on adrenalin.

Procedure.

1. Into a clean, dry tube add 5 droplets of adrenalin solution.
2. Add 3 drops of 1 % solution of sulfanilic acid and 3 droplets of sodium nitrite.
3. Add 3 droplets of 10 % solution of sodium carbonate. Mix the solution. Observe the formation of red color.



Results:

Conclusion:

Clinical and diagnostic significance. The investigation of hormones and mediators metabolism has an important significance in diagnostics of endocrine disorders, as well as for evaluation of body status in different pathological conditions, connected with alteration of function of central and vegetative nerve systems, heart, liver, kidneys and other parenchimatous organs.

Whatever disorders in a system hypothalamus-hypophysis-cortical adrenals lead to a change in corticosteroid secretion.

In interpretation of results it is useful to remember, that adrenalin secretion in females and males is almost equal, exception is in children in age of 12-15 years (in boys excretion is higher than in girls) and in 41-50 years old persons (in men higher then in women).

Secretion of noradrenalin is equal in boys and girls up to 8-11 years of life, but thereafter its secretion in women is higher, then in men. The secretion of adrenalin and noradrenalin is not constant during the day. In day time it is respectively 7.5 and 30.9 mg/min, in night it corresponds to 1.9 and 11.3 mg/min.

Smoking, physical load, emotional stress cause increase in excretion of catecholamines in urine. Enhanced catecholamines excretion is observed in liver cirrhosis, acute phase of ulcer disease of stomach and duodenum. Disorders in excretion have significance in pathogenesis of uremia.

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

Examples of Krock-1 tests

1. A patient consulted a doctor with complaints of dizziness, memory impairment, periodic convulsions. It was revealed that the cause of such changes is the decarboxylation product of glutamic acid. Name it:
A. GABA
B. Histamine
C. Serotonin
D. Tryptamine
E. Dopamine

2. The releasing of this biogenic amine in the human body regulates emotional stability and improve mood, so it is called «the hormone of good mood» and «hormone of happiness.» For which biogenic amine are the abovementioned effects?
A. Serotonin
B. Dopamine
C. Histamine
D. Epinephrine
E. GABA

3. The patient has an allergic reaction, accompanied by itching, swelling and reddening of the skin. The concentration of which biogenic amine is increased in the tissues?
A. Histamine
B. Serotonin
C. Tryptamine
D. Dopamine
E. Gamma-aminobutyric acid

4. Epileptiform convulsions are observed in an infant caused by the deficiency of vitamin B_6. It is a result of the deficiency of neurotransmitter – γ-aminobutyric acid. The activity of which enzyme is reduced?
A. Glutamate decarboxylase
B. Alanine aminotransferase
C. Glutamate dehydrogenase
D. Pyridoxalkinase
E. Glutamatsynthethase

5. The content of serotonin is sharply increased in the blood of the patient suffering from malignant carcinoid. What amino acid is used to produce this biogenic amine?
A. Tryptophan
B. Alanine
C. Leucine
D. Threonine
E. Methionine

6. This compound increases secretion of gastric acid, hydrochloric acid, tone of smooth muscle and decreases blood pressure. For which biogenic amine are the above-mentioned effects?
A. Histamine
B. Dopamine
C. GABA
D. Serotonin
E. Epinephrine

7. A 9-month-old child feeds on artificial mixtures that are not balanced by the content of vitamin B6. The child has pellagra-like dermatitis, convulsions and anemia. Violation of the synthesis of what substance led to the development of seizures?
A. GABA
B. Histamine
C. Serotonin
D. DOPA
E. Dopamine

8. Depression, emotional disorders are a consequence of a lack of norepinephrine, serotonin and other biogenic amines in the brain. The increase of their content in the synapses can be achieved due to antidepressants which inhibit the activity of the enzyme:
A. Monoamine oxidase
B. Diaminoooxidase
C. L-amino acid oxidase
D. D-amino acid oxidase
E. Phenylalanine-4-monooxygenase

9. What is the precursor of serotonin?
A. 5-Hydroxytryptophan
B. Tyrosine
C. Histidine
D. Phenylalanine
E. Serine

10. Which enzyme breaks down serotonin?
A. Monoamine oxidase
B. Creatine phosphokinase
C. Lactate dehydrogenase 1, 2
D. Gamma-glutamyltransferase
E. Lactate dehydrogenase 4, 5

11. During the decarboxylation of histidine, a biogenic amine is formed that has a potent vasodilating action. Specify it:
A. Histamine
B. Serotonin
C. DOPA
D. Norepinephrine
E. Dopamine

12. What are the main regulators of the calcium balance in the body?
A. Parathyroid hormone, calcitriol, calcitonin
B. Parathyroid hormone, calcitriol, calmodulin
C. Parathyroid hormone, thyroxine, Ca-binding proteins
D. Parathyroid hormone, calcitriol, aldosterone
E. Calcidiol, calcitonin, 7-dehydrocholesterol

13. What is the result of glutamate decarboxylation?
A. GABA
B. Glutathione
C. Histamine
D. Serotonin
E. Asparagine

14. Which reaction are biogenic amines produced?
A. Decarboxylation
B. Deamination
C. Amination
D. Transamination
E. Hydroxylation

15. Which reaction does neutralize biogenic amines?
A. Deamination
B. Decarboxylation
C. Amination
D. Transamination
E. Hydroxylation

16. Which of these biogenic amines is a catecholamine?
A. Epinephrine
B. Indolamine
C. Histamine
D. Serotonin
E. GABA

17. The deficiency of which biogenic amine is one of the factors in the depression development, obsessive-compulsive disorder and severe migraines?
A. Serotonin
B. Dopamine
C. Histamine
D. GABA
E. Epinephrine

18. The concentration of which biogenic amine is increased at such pathological processes as anaphylactic shock, burns, frostbite, fever, allergic reactions?
A. Histamine
B. Dopamine
C. GABA
D. Norepinephrine
E. Epinephrine

19. Which of biogenic amines is a mediator of inflammation and allergy?
A. Histamine
B. Dopamine
C. GABA
D. Norepinephrine
E. Epinephrine

20. Which of these biogenic amines is a catecholamine?
A. Dopamine
B. Indolamine
C. Histamine
D. Serotonin
E. GABA

21. What are the mechanisms determining the calcium homeostasis in the body?
A. Calcium deposition in the bones, calcium absorption in the small intestine, reabsorption of calcium ions in the tubules of nephrons
B. Inhibition of hepatocytes, intestinal absorption of calcium, calcium reabsorption in the renal tubules
C. Bone resorption by osteoclasts, calcium excretion in the faeces, calcium reabsorption in the renal tubules
D. Calcium binding to plasma proteins, calcium absorption in the small intestine, excessive excretion by the kidneys
E. Inhibition of phosphatase activity, synthesis activation calcitonin

22. What is the mechanism of calcitriol action?
A. Interaction with the receptor → translocation to the nucleus → transcription of the gene expression → synthesis of Ca-binding and transporting proteins
B. Interactions with the receptor protein → activation of synthesis of cholecalciferol from dehydrocholesterol in the skin → transport to the liver
C. Biotransformation of cholesterol into calcitriol in the kidney by enzymatic hydroxylation reactions
D. Biotransformation of cholesterol in the liver and kidneys under the influence of parathyroid hormone controls the hydroxylation reaction
E. Transformation of calcidiol in hepatocytes into calcitriol with α1-hydroxylase

23. What is the main biological function of calcitriol?
A. Stimulation of Ca^{2+} absorption in the intestine and phosphates, through the activation of Ca-binding proteins
B. Stimulation of transferrin synthesis of the blood and mineralization of bone
C. Regulation of sodium and potassium excretion by the kidneys
D. Reduction of calcium concentration in blood and bone resorption inhibition
E. Inhibition of calcium secretion in the nephron and bone resorption

24. What is the main effect of calcitonin on calcium homeostasis?
A. Hypocalcemic effect, inhibition of resorption of bone organic matrix
B. Hypocalcemic effect, increased resorption of the organic matrix of bone tissue
C. Hypercalcemic effect, increased bone resorption
D. Hypercalcemic effect, inhibition of bone resorption
E. Increasing of calcium and phosphate reabsorbtion in the kidney

25. What is the main characteristic of the biological effect of parathyroid hormone?
A. Hypercalcemic
B. Hypocalcemic
C. Hyperphosphatemic
D. Hypophosphatemic
E. Hyperglycemic

26. Specify a normal serum calcium concentration in adults:
A. 2.25-2.75 mmol/L
B. 2.5-3.5 mmol/L
C. 1.5-2.0 mmol/L
D. 1.0-2.25 mmol/l
E. 2.0-4.0 mmol/L

27. What is the cause of rickets in children?
A. Deficiency of vitamin D_3
B. Excess of vitamin D_3
C. Vitamin A deficiency
D. Hyperparathyroidism
E. Hypoparathyroidism

28. What is a precursor of active form of vitamin D_3 – 1,25-dihydroxycholecalciferol?
A. 7-dehydrocholesterol
B. PTH
C. Calcitonin
D. Alpha-ketoglutarate
E. Citrate

29. What changes in calcium-phosphorus metabolism are characteristic of hyperparathyroidism?
A. Increasing of serum calcium and phosphorus concentrations
B. Decreasing of serum calcium and phosphorus concentrations
C. Decreasing of calcium concentration and increase the phosphorus concentration in the blood
D. Increasing of potassium and sodium concentrations in the blood
E. Decreasing of potassium and sodium concentrations in the blood

30. Osteoporosis is the most common disorder of bone metabolism. What is the definition of this pathology?
A. It is systemic skeletal disease characterized by a reduction of bone mass per unit of volume, and its microarchitectural violation
B. It is systemic skeletal disease characterized by the violation of the organic matrix of bone mineralization
C. It is the softening of bone tissue due to lack of calcium and phosphorus
D. It is excessive bone mineralization
E. It is Loss of bone trabecular part

31. What component is produced during bone mineralization forming the basis of the inorganic structure of the skeleton?
A. Hydroxyapatite
B. Calcium citrate
C. Calcium phosphate
D. Carbonate apatite
E. Chlorapatite

32. In which organ is the most active form of vitamin D_3 – 1,25-dihydroxycholecalciferol synthesized?
A. In the kidneys
B. In the liver
C. In the intestine
D. In the skin
E. In the salivary glands

33. What is the universal messenger of intracellular effects of calcium ions with which its complexes are formed called?
A. Calmodulin
B. cAMP
C. cGMP
D. Diacylglycerol
E. Inositol-1,4,5-triphosphate

34. What is a biochemical indicator of vitamin D_3 deficiency?
A. 1,25-dihydroxycholecalciferol
B. Level of calcium in the saliva
C. Blood phosphorus
D. Content of mineral salts in the bone tissue
E. Binding of calcium complex of Ca^{2+} calmodulin

35. What is the main mechanism for hypercalcemic effects of PTH?
A. Mobilization of calcium and phosphorus from bone tissue by activation of osteoclast acid phosphatase
B. Inhibition of absorption of calcium and phosphorus in the intestine
C. Increases of urinary calcium
D. Deficiency of vitamin D_3
E. Increases of urinary phosphorus

36. What are the symptoms of hypoparathyroidism?
A. Hypocalcemia, hyperphosphatemia
B. Hypercalcemia, hyperphosphatemia
C. Hyperphosphatemia, osteomalacia
D. Hypercalcemia, decrease the synthesis of vitamin D_3
E. Deficiency of vitamin D_2, hypophosphatemia

37. What are the main symptoms of hyperparathyroidism?
A. Hypercalcemia, osteoporosis
B. Hypercalcemia, hyperphosphatemia
C. Hypocalcemia, hypophosphatemia
D. Hypocalcemia, osteodystrophy
E. Hypophosphatemia, skeletal deformity

38. What is the main clinical sign of hypoparathyroidism?
A. Tonic convulsions, up to the tetanus
B. Atony of muscles
C. Hypophosphaturia, nephrocalcinosis
D. Osteoporosis, osteodystrophy
E. Reduced neuromuscular excitability

39. Patient's blood calcium is 2.1 mmol/L, hyperphosphatemia is observed. What endocrine pathology is characterized by these changes?
A. Hypoparathyroidism
B. Hyperparathyroidism
C. Alkalosis
D. Hypervitaminosis of D_3
E. Hypervitaminosis of D_2

40. A one-year-old child has enlarged head and belly, retarded cutting of teeth, destruction of enamel structure. What hypovitaminosis causes these changes?
A. Hypovitaminosis D
B. Hypovitaminosis C
C. Hypovitaminosis A
D. Hypovitaminosis B_1
E. Hypovitaminosis B_2

41. A patient has the sudden decrease of Ca^{2+} content in blood. What hormone secretion will increase?
A. Thyrocalcitonin
B. Parathormone
C. Aldosterone
D. Vasopressin
E. Somatotropin

42. What bioregulators are regulators of calcium homeostasis?
A. Calcitonin, parathyroid hormone
B. Adrenalin, glucagon
C. Thyroxine, insulin
D. Cortisol, aldosterone
E. Testosterone, estrogen

Topic 8.
Investigation of the role of steroid and thyroid hormones

1. Objective: To analyze changes in carbohydrate, lipid and protein metabolism which occur in endocrine glands dysfunction and to interpret prognostic significance of distinct disorders. To know biochemical mechanisms of pathological processes development and typical manifestations in endocrine diseases, with special attention to thyroid gland dysfunction.

2. Actuality of the theme: Hormones play an important role in mechanisms of homeostasis. These substances regulate the activity of enzymes in cells, influence the expression of cell genome and change the intensity of metabolism in target cell and in the body as well. The knowledge of mechanisms of the neurohumoral regulation of metabolism gives basis for diagnostics and rational therapy at endocrinological disorders.

Diseases of the thyroid gland are encountered frequently enough among other forms of endocrine pathologies, they cause complications of various diseases. Severe dysfunction of the thyroid gland results in impairment of nervous, cardiovascular and other systems («thyrotoxic heart», «thyrotoxic liver»). Iodine deficiency diseases belong to the most widespread noninfectious diseases of mankind. Knowledge of biochemistry of thyroid hormones is necessary for understanding of molecular basis of its pathology, diagnostics, prognosis and preventive measures.

Steroid hormones (corticosteroids and sex hormones) play an important role in regulation of all kinds of metabolism and regulation of homeostasis. Corticosteroids have a wide spectrum of biological action and play an important role in realization of adaptation mechanisms. Sex hormones effect higher nervous activity, growth and development of the organism, determine sexual differentiation and reproductive function. Steroid hormones are widely applied clinically in therapy of endocrine diseases, and also for correction of metabolism at many nonendocrine diseases. That is why knowledge of biochemistry of steroid hormones and understanding of principles of their medical application is necessary in practical work of doctors.

3. Specific aims:

- ✓ To analyze metabolic changes and biochemical indexes, which characterize the carbohydrate metabolism, metabolism of proteins and lipids in disorders of endocrine glands and to summarize the prognostic estimation of these disorders.
- ✓ To explain the biochemical mechanisms of development and manifestation of pathological processes and typical disorders of endocrine system.

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

Questions:	References:
1. **The mechanism of action of thyroid hormones (cytosolic) of thyroid gland and steroid hormones (cytosolic and nuclear receptors).** ✓ The mechanism of action of thyroid hormones; ✓ The mechanism of action of steroid hormones.	1. Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 428. 2. Gubsky Yu. Biological chemistry: textbook / edited by Yu. Gubsky. – Vinnytsia: Nova Knyha, 2018. – 2nd edition. – P. 333. 3. Lecture notes.
2. **Thyroid hormones:** ✓ The structure and biosynthesis of thyroid hormones; ✓ Biological effects of T_4 and T_3; ✓ Pathology of the thyroid gland; features of metabolic disorders in conditions of hyper- and hypothyroidism; ✓ Mechanisms of endemic goiter formation and its prevention.	1. Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 437–441. 2. Gubsky Yu. Biological chemistry: textbook / edited by Yu. Gubsky. – Vinnytsia: Nova Knyha, 2018. – 2nd edition. – P. 331–335. 3. Lecture notes.

3.	**Steroid hormones: nomenclature, classification. Scheme of biosynthesis of the steroid hormones from cholesterol:** ✓ Nomenclature of steroid hormones; ✓ Classification of steroid hormones; ✓ Biosynthesis of the steroid hormones from cholesterol.	1. Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 441–449. 2. Gubsky Yu. Biological chemistry: textbook / edited by Yu. Gubsky. – Vinnytsia: Nova Knyha, 2018. – 2nd edition. – P. 335–336. 3. Lecture notes.
4.	**Steroid hormones of the adrenal cortex (C21-steroids):** ✓ The structure, physiological and biochemical effects of glucocorticoids (cortisol, corticosterone), the role of cortisol in the regulation of metabolism (carbohydrates, proteins, lipids); ✓ Biochemical basis of anti-inflammatory properties of glucocorticoids; ✓ The structure, physiological and biochemical effects of mineralocorticoids (e.g. aldosterone); role of aldosterone in the regulation of water metabolism; ✓ Cushing's disease, Adison's disease, aldosteronism, Cohn's disease.	1. Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 441–444. 2. Gubsky Yu. Biological chemistry: textbook / edited by Yu. Gubsky. – Vinnytsia: Nova Knyha, 2018. – 2nd edition. – P. 336–338. 3. Lecture notes.
5.	**Steroid hormones of the gonads:** ✓ Female hormones: estrogen – estradiol, estrone (C18-steroids), progesterone (C21-steroids); physiological and biochemical effects; connection with the phases of the menstrual cycle; regulation of synthesis and secretion; ✓ Male sex hormones (androgens) – testosterone, dihydrotestosterone (S19-steroids); physiological and biochemical effects, regulation of synthesis and secretion; ✓ Clinical application of hormone analogueues and antagonists gonads.	1. Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 445–449. 2. Gubsky Yu. Biological chemistry: textbook / edited by Yu. Gubsky. – Vinnytsia: Nova Knyha, 2018. – 2nd edition. – P. 338–341. 3. Lecture notes.

5. Tasks for independent work and self-control

5.1. Complete the scheme of mechanism of steroid and thyroid hormones action.

_____ → _____ → _____ _____ → Hormone-response element (HRE) of DNA → Change of transcription of specific genes → Change of protein synthesis → Cell response.

5.2. Write the formula of thyroxine (tetraiodothyronineT_4) and triiodothyronine (T_3). Which of them is a more active hormone?

5.3. Give the short characteristic of biosynthesis, storage, release and transport of thyroid hormones.

	Characteristic
Biosynthesis:	
1) Uptake of iodide	
2) Formation of active iodine	
3) Thyroglobulin and of T_3 and T_4	
Storage and release	
Transport	

5.4. Give the characteristic of biochemical functions of thyroid hormones
Influence on the metabolic rate:

Effect on protein synthesis:

Influence on carbohydrate metabolism:

Effect on lipid metabolism:

5.5. Which mechanism of regulation of T_3 and T_4 synthesis? Explain it.

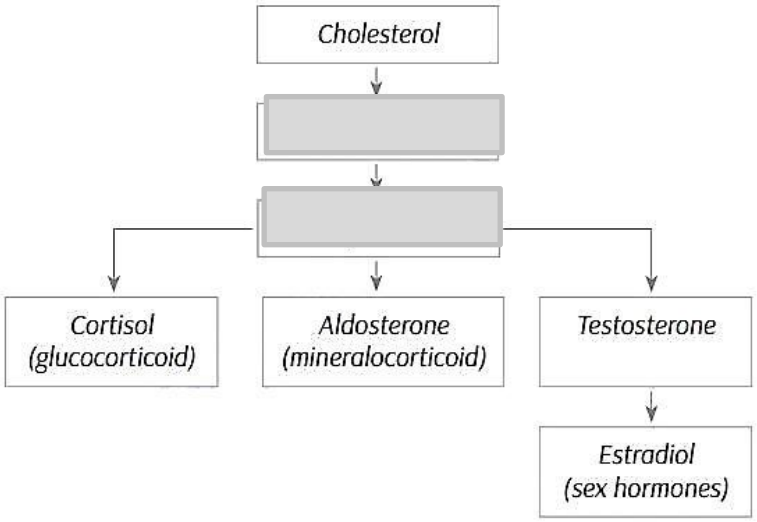
5.6. Abnormalities of thyroid function

Characteristic	Grave's disease	Diffuse toxic goiter	Endemic goiter
Causes			
Changes of metabolism			
Clinical symptoms			
Treatment			

5.6. List the ways which are used for diagnosis of thyroid function.

5.7. Write the formula of cholesterol. Number the carbon atoms.

5.8. Complete the scheme:



5.9. Give the general description of adrenocorticosteroids

Characteristic	Glucocorticoids	Mineralocorticoids	Androgens and estrogens
Number of carbon atoms			
Zone of production			
Functions			
Examples of hormones			

5.10. Complete the table «Biochemical functions of glucocorticoid hormones (cortisol, cortisone and corticosterone)»

Metabolism/physiological function	Effects
Carbohydrate metabolism	
Lipid metabolism	
Protein and nucleic acid metabolism	
Water and electrolyte metabolism	
Immune system	
Physiological functions	

5.11. Describe the biochemical functions of aldosterone.

5.12. Disturbances of the adrenal cortical functions (A – aldosterone, C- cortisol, ACTH).

Disorder	Changes of level ↑, ↓	Clinical symptoms
Cushing's disease (pituitary tumor)	A C ACTH	
Cushing's syndrome (tumor of adrenal cortex)	C ACTH	
Addison's disease	A C ACTH	
Primary aldosteronism (Conn's syndrome)	A Renin	
Secondary aldosteronism	A Renin	

5.13. Complete the table «Functions of sex hormones»

Hormones	Sex-related physiological functions	Biochemical functions
Androgens (dihydrotestosterone (DHT))		
Estrogens		
Progesterone	–	

5.14. Situational tasks

The patient was found hypernatremia, hypokalemia, increase in osmotic blood pressure, swelling.

With the increase secretion of which hormone this pathology is related?

Identify hormone of the adrenal cortex, its chemical nature and mechanism of action?

What is the name of this pathology?

6. Individual independent students work

1. Pathology of the thyroid gland; features of metabolic disorders in conditions of hyper- and hypothyroidism.
2. Mechanisms of endemic goiter and its prevention.

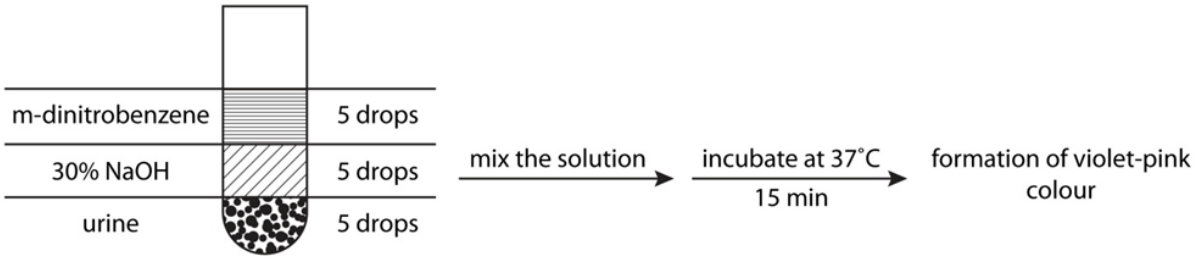
Practice protocol №8 «____» _____ 20___

Experiment 1. Determination of 17-ketosteroids in urine.

Principle. 17-ketosteroids after reaction with m-dinitrobenzene in strong alkaline medium give a condensation product with a violet-pink color (maximum of absorption at 530 nm). The intensity of color is proportional to a quantity of 17-ketosteroids in urine.

Procedure.

1. Into a clean, dry tube add 5 drops of urine and 5 drops of 30 % of NaOH solution (caution, hazard!).
2. Add 5 drops of m-dinitrobensene. Mix the solution.
3. Put the test tubes into the incubator (37˚C) for 15 minutes.
4.Observe the formation of violet-pink colored solution.



Results:

Conclusion:

Clinical significance. Maximal excretion of 17-ketosteroids is observed at 25 years age, thereafter the quantity of excreted steroids diminishes. During stress situation quantity of 17-ketosteroids in blood and urine is increased. In pathology the content of ketosteroids is changed depending from the function of suprarenal glands. At hypofunction of suprarenal glands (Addison's disease) excretion of 17-ketosteroids consists of 20-30 % of normal value. The excretion of ketosteroids is also decreased at hyperthyreosis, hepatic cyrrhosis, suprarenal gland tumors. 17-ketosteroids are excreted mainly in conjugated form (with sulfuric or glucuronic acid). Simultaneous determination of free and conjugated 17-ketosteroids in urine permits more precisely evaluate functional state of suprarenal glands.

Normal values (17-ketosteroids excretion per 24 hours):

- men – 12–18 mg/24 h
- women – 7–14 mg/24 h

The urine contains mainly two steroids – 17-hydroxysteroids and 17-ketosteroids – derived from the metabolism of glucocorticoids and mineralocorticoids. Androgens synthesized by gonads also contribute to the formation of 17-ketosteroids.

Addison's disease: impairment in adrenocortical function results in Addison's disease. This disorder is characterized by decreased blood glucose level (hypoglycemia, loss of weight, loss of appetite (anorexia), muscle weakness, impaired cardiac function, low blood pressure, decreased Na^+ and increased K^+ level in serum, increased susceptibility to stress etc.

Cushing's syndrome: Hyperfunction of adrenal cortex may be due to long term pharmacological use of steroids or tumor of adrenal cortex or tumor of pituitary. Cushing's syndrome is characterized by hyperglycemia (due to increased gluconeogenesis), fatigue, muscle wasting, edema, osteoporosis, negative nitrogen balance, hypertension, moon-face etc.

Experiment 2. Qualitative reaction on iodine in the solution of thyroxin.

Principle. Degradation of thyroxin molecules leads to potassium iodide formation. The released Iodine gives a blue color with starch.

$5\ KI + KIO_3 + 3\ H_2SO_4 \rightarrow 3\ I_2 + 3\ K_2SO_4 + 3\ H_2O$

I_2 + starch → blue color

Procedure:

1. Take a clean, dry tube and add 25 drops of thyroxin hydrolysate.
2. Add H_2SO_4 (10 %) mix the solution and add KIO_3 (10 %).
3. Add 5 drops of starch. Observe the formation of blue color.

Results:

Conclusion:

Clinical significance. Among the endocrine glands, thyroid is the most susceptible for hypo- or hyperfunction. Abnormalities of thyroid function are:

1) Hyperthyroidism (Grave's disease and diffuse toxic goiter)/
2) Hypothyroidism (cretinism, myxoedema and simple endemic goiter).

Goiter: Any abnormal increasing the size of the thyroid gland is known as goiter. Enlargement of thyroid gland is mostly to compensate the decreased synthesis of thyroid hormones and is associated with elevated TSH. Goiter is primarily due to a failure in the autoregulation of T_3 and T_4 synthesis. This may be caused by deficiency or excess of iodide.

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

Examples of Krock-1 tests

1. What are the groups of corticosteroids?
A. Glucocorticoids, mineralacorticoids
B. Glucocorticoids, estrogens
C. Mineralocorticoids, androgens
D. Corticosteroids, androgens
E. Glucocorticoids, progestogens

2. What is the most active glucocorticoid in humans?
A. Cortisol
B. Progesterone
C. Aldosterone
D. Corticosterone
E. Pregnenolone

3. What is the main biochemical mechanism of carbohydrate metabolism regulation in glucocorticoid action:
A. Activation of gluconeogenesis
B. Activation glycogenesis
C. Activation of glycolysis
D. Inhibition of gluconeogenesis
E. Activation of the pentose phosphate pathway

4. What are the main mechanisms controlling aldosterone synthesis?
A. Potassium concentration and activation of the renin-angiotensin system
B. Low levels of cortisol in the blood
C. Corticoliberin, corticotropin
D. Blood pressure and hypothalamic mechanisms
E. Concentration of calcium in the blood

5. What is the mechanism of an antiinflammatory action of glucocorticoids?
A. Inhibition of the activity of phospholipase A_2
B. Activation of antioxidant protection
C. Activation of synthesis of proinflammatory cytokines
D. Catabolic effect on the metabolism of proteins
E. The activation of phospholipase A_1

6. Some forms of pathology of the adrenal cortex are characterized by hyperpigmentation of the skin, general muscle weakness and wasting?
A. Hypofunction (Addison's disease)
B. Goiter
C. Hyperaldosteronism (Conn's disease)
D. Cushing's Disease
E. Increased synthesis and secretion of catecholamines

7. The main biological role of mineralocorticoid is:
A. Regulation of water-salt metabolism
B. Regulation of carbohydrate metabolism
C. Regulation of lipid metabolism
D. Regulation of defense mechanisms
E. Regulation of free radical oxidation processes

8. Which biologically active substance activates the synthesis and secretion of aldosterone?
A. Renin
B. Insulin
C. Proopiomelanocortin
D. Somatostatin
E. Cortisol

9. Which group of steroid hormones is regulated by the system «corticotroliberin-corticotropin»?
A. Glucocorticoids
B. Mineralocorticoid
C. Estrogens
D. Androgens
E. Cholecalciferol

10. Specify the pathology of the adrenal gland which is accompanied by hyponatremia and hyperkalemia:
A. Decreased mineralocorticoid production
B. Increased mineralocorticoid production
C. Inhibition of glucocorticoid synthesis
D. Hyperaldosteronism
E. Increased androgen synthesis

11. Increase of glucose concentration in the blood, decreased glucose tolerance, increased body mass index, hypertension and increased secretion of corticotrophin were found in a patient. What is the name of the pathological state?
A. Cushing's disease
B. Hyperaldosteronism
C. Cushing's syndrome
D. Addison's disease
E. Androsteroma

12. What is the action of glucocorticoids on the immune system?
A. Inhibition of the synthesis of antibodies and phagocytic activity of leukocytes
B. Activation of the synthesis of antibodies and phagocytic activity of leukocytes
C. Increasing of the cellular mechanisms of immune defence
D. Do not affect the immune system
E. Activation of the availability of amino acids for immunoglobulins

13. How is the production of hormones of the adrenal cortex and medulla layer changed under the influence of stress factors?
A. Increased synthesis and secretion of glucocorticoids and catecholamines
B. Increased synthesis and secretion of indolamine
C. Increased synthesis of progesterone and pregnenolone
D. Increased synthesis of aldosterone
E. Increased synthesis of histamine

14. The thyroid hormones T3 and T4 are synthesized in the follicular cells of the thyroid gland. From which of the following essential amino acids are the thyroid hormones synthesized?
A. Phenylalanine
B. Lysine
C. Methionine
D. Isoleucine
E. Valine

15. Which hormone concentration is determined for the diagnosis of Addison's disease?
A. Corticotropin, cortisol, aldosterone
B. Corticotropin, androgens, thyroxine
C. Corticotropin-releasing factor, aldosterone
D. Cortisol, angiotensin
E. Corticotropin, electrolytes

16. How does an excess of glucocorticoids affect the bone tissue?
A. Inhibition of collagen synthesis and mineralization
B. Activation of collagen synthesis and protein
C. Activation of fibroblasts
D. Activation of bone mineralization
E. Inhibition of the activity of osteoclasts

17. What is the effect of glucocorticoids on lipid metabolism?
A. Activation of lipolysis in adipose tissue
B. Activation of lipogenesis in adipose tissue
C. Increase of triglycerides in adipose tissue and blood
D. They potentiate the effects of insulin
E. They decrease blood levels of free fatty acids

18. What kind of violation of adrenal hormones production is indicated by high blood pressure, hypokalemia and hypernatremia?
A. Hyperaldosteronism (Conn's disease)
B. Hypofunction (Addison's disease)
C. Hyperactivity
D. Cushing's Disease
E. Increased synthesis and secretion of catecholamines

19. Physical and mental retardation are observed in a 5-year-old boy. Reduced secretion of what hormone can be assumed?
A. Thyroxine
B. Insulin
C. Growth hormone
D. Prolactine
E. Aldosterone

20. Under hyperthyroidism there is weight loss and fever. What biochemical processes are activated at this state?
A. Catabolism
B. Anabolism
C. Gluconeogenesis
D. Lipogenesis
E. Oxidative phosphorylation

21. Metyrapone is used to block the mitochondrial 11-β-hydroxylase in the corticosteroid synthetic pathway and is administered to evaluate hypothalamicpituitary-adrenal axis function. Which of the following results is most likely from this overnight diagnostic test in a healthy individual?
A. Increase in the levels of cortisol precursors
B. Feedback inhibition of cortisol biosynthesis
C. Decrease in ACTH levels
D. Inhibition of adenylate cyclase activity in adrenal cortical cells
E. Inhibition of pituitary function

22. Constant weakness, sweating, tachycardia, exophthalmos are observed in a patient. What biochemical tests are necessary to confirm the diagnosis?
A. Determination of T_3, T_4 concentration in blood
B. Determination of the daily urinary excretion of 17-keto steroids
C. Determination of the insulin concentration in the blood
D. Definition of the blood PTH concentration
E. Determination of blood glucagon concentration

23. What is the precursor of steroid hormones synthesis and the types of reactions that take place at different stages of their synthesis:
A. Cholesterol; hydroxylation, reduction, oxidation, reduction, condensation, isomerization
B. Triglycerides; hydroxylation, condensation, isomerization
C. Pregnenolone; isomerization, phosphorylation, oxidation, reduction, condensation, deamination
D. Calcitriol, hydroxylation, methylation, reduction, condensation, isomerization
E. Sphingosine; oxidative hydroxylation, methylation, reduction, condensation

24. A patient suffering from weakness, fatigue, nausea and vomiting was found to have low blood concentrations of Na^+ and Cl^- and high levels of serum K^+. Physical examination revealed a deep tanning of both exposed and unexposed parts of the body and dark pigmentation inside the mouth. The hyperpigmentation in this patient most likely resulted from which of the following?
A. Increased secretion of ACTH
B. Prolonged exposure of the patient to ultraviolet radiation
C. Excessive ingestion of β-carotenecontaining foods
D. Activation of melanocytes caused by medication side effects
E. Inhibition of plasma membrane Na^+, K^+-ATPase

25. Where specific steroid hormone receptor is located and what is the mechanism of steroid hormones interaction with target cells?

A. In the cytosol, where the hormone-receptor complex translocates to the nucleus and interacts with sensitive sites of nuclear chromatin DNA, activating the transcription of genes of relevant effector protein enzymes

B. On the surface of the plasma membrane of cells, where after the formation of hormone-receptor complex proteins signal through the transducers is transmitted to the secondary information mediators that ultimately leads to a change in the activity of the relevant enzyme systems

C. In the cytosol, where, after the activation of existing enzymes realized corresponding biochemical response

D. In the cell nucleus, where upon the interaction of hormones with sensitive sites activated enzyme DNA molecules leading to the activation of relevant biochemical processes

E. In the cytosol, there is a biochemical reaction wherein the hormonal signal after interaction with secondary signalling molecules

Topic 9.
Physiologically active eicosanoids

1. Objective: To know structure and biological properties of eicosanoids as physiologically active compounds of lipid origin.

2. Actuality of the theme: Eicosanoids are a family of compounds, precursors of which are eicosanoic (C20) fatty acids. Prostaglandins (PGs), thromboxanes (TXs) and leukotrienes (LTs) belong to them. They work as tissue hormones with various biological effects and also have wide clinical application.

Eicosanoids play an essential role in inflammatory and allergic processes. Aspirin, indometacin and other nonsteroidal anti-inflammatory drugs (NSAIDs) inhibit cyclooxigenase activity and also are applied as anti-inflammatory preparations.

Prostaglandins are for stimulation of childbirth, termination of pregnancy, for treatment of bronchial asthma.

3. Specific aims:

- ✓ To know general characteristic of eicosanoids.
- ✓ To know the scheme of biosynthesis of prostaglandins, thromboxanes, leukotrienes and enzymes of prostaglandin-synthase complex.
- ✓ To know the scheme of leukotriene synthesis.
- ✓ To be able to analyze biochemical basis of clinical application of eicosanoids.
- ✓ To explain anti-inflammatory effects of prostaglandin synthesis – aspirin and others NSAIDs.

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

Questions:	References:
1. **The general characteristics of eicosanoids:** ✓ Precursors of eicosanoid formation; ✓ Prostanoids, thromboxanes and leukotrienes, examples of structure of prostaglandins.	1. Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 644–645. 2. Gubsky Yu. Biological chemistry: textbook / edited by Yu. Gubsky. – Vinnytsia: Nova Knyha, 2018. – 2nd edition. – P. 346–347. 3. Lecture notes.
2. **The biosynthesis of prostaglandins:** ✓ Arachidonic acid – the precursor of eicosanoid synthesis (role of phospholipase A_2); ✓ Role of prostaglandin synthase complex in synthesis of prostaglandins and thromboxanes; ✓ Types of leukotrienes; ✓ Biosynthesis of leukotrienes (5-HPETE – leukotrienes).	1. Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 644–645, 648–648. 2. Gubsky Yu. Biological chemistry: textbook / edited by Yu. Gubsky. – Vinnytsia: Nova Knyha, 2018. – 2nd edition. – P. 347–349. 3. Lecture notes.
3. **Biochemical actions of prostaglandins:** ✓ Biological role of prostaglandins; ✓ Biological role of thromboxanes; ✓ Biological role of leukotrienes (role of slowly reacting substance in genesis of allergies); ✓ Eicosanoids as central mediators of inflammation (chemoattractors, vasodilators, stimulators of exudation, migrations and degranulation of leukocytes and phagocytosis); ✓ Clinical application of eicosanoids.	1. Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 646–648. 2. Gubsky Yu. Biological chemistry: textbook / edited by Yu. Gubsky. – Vinnytsia: Nova Knyha, 2018. – 2nd edition. – P. 347. 3. Lecture notes.
4. **Aspirin and other nonsteroidal antiinflammatory drugs as inhibitors of prostaglandin synthesis:** ✓ Non-steroidal anti-inflammatory drugs (NSAIDs) blockade cyclooxygenase and its consequences.	1. Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 646, 648. 2. Lecture notes.

5. Tasks for independent work and self-control
5.1. Describe different effects of hormones:
Endocrine effect

Paracrine effect

Autocrine effect

5.2. Indicate what eicosanoids are involved in:
1) smooth muscle contraction and relaxation

2) vasoconstriction or vasodilation

3) bronchoconstriction or bronchodilation

4) reproductive processes

5) digestion (gastric acid secretion)

6) platelet aggregation and thrombosis

7) salt and water balance

8) vascular permeability

9) inflammation

10) pain perception

11) lymphocyte proliferation

12) anaphylaxis (severe allergy)

5.3. Describe the mechanism of eicosanoids action (receptors, signal transduction pathways).

5.4. Complete the table «Inhibition of eicosanoids synthesis»

Inhibitors	Inhibitory enzyme	Characteristic of action and use as drugs
Corticosteroids (natural, synthetic)		
NSAIDs (aspirin, indomethacin etc.)		

6. Individual independent students work

Biological properties of eicosanoid.

Practical abilities.

1. To create a scheme of synthesis of prostaglandins, thromboxanes, leukotrienes .
2. To write structural formulas of prostaglandins, leukotrienes.

Examples of Krock-1 tests

1. Aspirin has anti-inflammatory effects by inhibiting an activity of cyclooxygenase. The concentration of what substances will be reduced in this case?
A. Prostaglandins
B. Catecholamines
C. Biogenic amines
D. Saturated fatty acids
E. Cholesterol

2. Glucocorticoid hormones inhibit enzyme eliminating arachidonic acid from phospholipids. What is the name of the enzyme?
A. Phospholipase A_2
B. Phospholipase A_1
C. Phospholipase B
D. Phospholipase C
E. Phospholipase D

3. What compound is a precursor in the synthesis of prostaglandins in the body?
A. Arachidonic acid
B. Palmitic acid
C. Linolenic acid
D. Oleic acid
E. Linoleic acid

4. Which type of fat-soluble vitamins arachidonic acid belongs to?
A. F
B. D
C. E
D. A
E. K

5. A 42-year-old man suffering from rheumatoid arthritis was prescribed aspirin that is an inhibitor of prostaglandin synthesis. Which acid is used for prostaglandins synthesis?
A. Arachidonic
B. Neuraminic
C. Linolenic
D. Linoleic
E. Propionic

6. Prednisolone has an anti-inflammatory effect due to the inhibition of the arachidonic acid release. What are the biologically active substances synthesized from arachidonic acid called?
A. Prostaglandins
B. Cholesterol
C. Uric acid
D. Heme
E. Urea

7. Doctor prescribed prostaglandin E_2 for the augmentation of labor. From what substance is it synthesized?
A. Arachidonic acid
B. Phosphatidic acid
C. Palmitic acid
D. Stearic acid
E. Glutamic acid

8. Long-term use of large doses of aspirin causes inhibition of prostaglandin synthesis by reducing the activity of the enzyme:
A. Cyclooxygenase
B. Peroxidase
C. 5-Lipoxygenase
D. Phospholipase A_2
E. Phosphodiesterase

9. For the prevention of atherosclerosis, coronary heart disease and cerebral circulatory disorders people should get 2-6 grams of essential polyunsaturated fatty acids. These acids are essential for the synthesis of:
A. Prostaglandins
B. Bile acids
C. Steroids
D. Vitamin D
E. Neurotransmitters

10. You prescribe ibuprofen to help reduce your patient's inflammation. Which of the following pathways is blocked as an anti-inflammatory mechanism of action of nonsteroidal anti-inflammatory drugs? [1]
A. Prostaglandin synthesis
B. Thromboxane synthesis
C. Leukotriene synthesis
D. All eicosanoid synthesis
E. Arachidonic acid release from the membrane

11. Arachidonic acid is a precursor of eicosanoids. Which group of substances it belongs to?
A. Polyunsaturated fatty acids
B. Keto acids
C. Saturated fatty acids
D. Monounsaturated fatty acids
E. Amino acids

12. Most of physiologically active eicosanoids are synthesized from:
A. Arachidonic, alpha- and gamma-linolenic acid
B. Arachidonic and palmitic acids
C. Palmitoleic and stearic acids
D. Arachidonic and palmitoleic acid
E. Alpha, gamma-linolenic and linoleic acids

13. Which group of eicosanoids has a noncyclic structure?
A. Leukotrienes
B. Thromboxanes
C. Prostaglandins
D. Prostacyclin
E. All of them

14. What is the metabolic pathway for the synthesis of prostaglandins called?
A. Cyclooxygenase
B. Pentose phosphate pathway
C. Glucuronate
D. Lipoxygenase
E. Glycolytic

15. What is the metabolic pathway for the synthesis of leukotrienes called?
A. Lipoxygenase
B. Pentose phosphate pathway
C. Glucuronate
D. Cyclooxygenase
E. Glycolytic

16. Specify the eicosanoid, promoting adhesion and platelet aggregation:
A. Thromboxane A_2
B. Prostacyclin I_2
C. Prostaglandin $F_{2\alpha}$
D. Leukotriene C_4
E. Leukotriene A

17. Which group of eicosanoids is called «slow-reacting substance of anaphylaxis»?
A. Leukotriene C_4, leukotriene E_4, leukotriene D_4
B. Prostacyclin, prostaglandin E_2, prostaglandin $F_{2\alpha}$
C. Prostaglandin $F_{2\alpha}$, prostaglandin D_2, thromboxane A_2
D. Leukotriene A_4, prostaglandin E_2, prostaglandin $F_{2\alpha}$
E. Thromboxane A_2, thromboxane B_2, leukotriene B_4

18. You have an asthmatic patient who is already on an inhaled steroid and albuterol, but is still having difficulty. You add montelukast to her regimen. Montelukast (Singulair) specifically blocks the product of which of the following metabolic pathways? [2]
A. Lipoxygenase
B. Cyclooxygenase
C. P_{450}
D. Cori cycle
E. TCA cycle

19. The coxibs, including celecoxib (Celebrex), are a recently developed class of nonsteroidal anti inflammatory drugs (NSAIDs). The coxibs show antiinflammatory action without affecting platelet function. These effects of the coxibs are best attributed to selective inhibition of which of the following?
A. The cyclooxygenase activity of the «inducible» prostaglandin H synthase isoform (PGHS-2)
B. The cyclooxygenase activity of the «basal» prostaglandin H synthase isoenzyme (PGHS-1)
C. The cytosolic isoenzyme of phospholipase A_2 (cPLA2)

20. Prostaglandins comprise a family of oxygenated lipid signaling molecules derived from polyunsaturated fatty acids such as arachidonic acid. They are involved in the regulation of a number of cellular processes. Some of the prostaglandins act to increase vasodilation and levels of cAMP in cells, whereas others increase vaso- and bronchoconstriction and smooth muscle contraction. In the conversion of arachidonic acid to prostaglandins, the oxygenation step is accomplished by the enzyme that synthesizes which of the following compounds?
A. Prostaglandin H_2

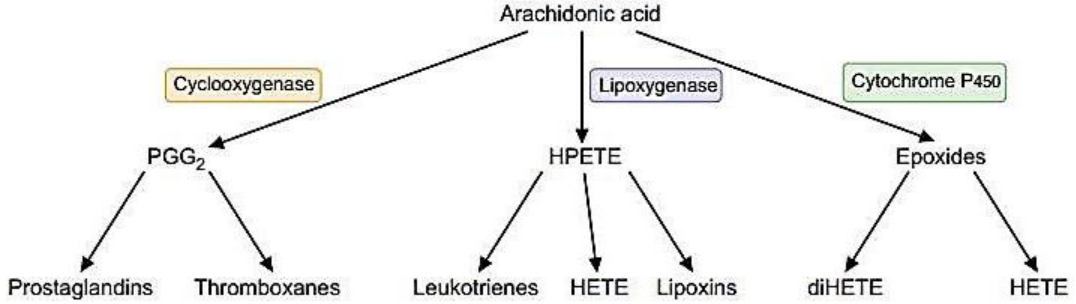
D. The microsomal isoenzyme of prostaglandin E synthase (mPGES-1)
E. Prostacyclin (PGI2) synthase (PGIS)

21. A 55-year-old man had been advised by his physician to take 81 mg of aspirin per day to reduce the risk of blood clots leading to a heart attack. The rationale for this treatment is which of the following? [3]
A. To reduce thromboxane synthesis
B. To reduce leukotriene synthesis
C. To reduce prostaglandin synthesis
D. To increase prostacyclin synthesis
E. To increase Lipoxin synthesis

B. Prostaglandin E_2
C. Prostaglandin $F_{2\alpha}$
D. Prostaglandin D_2
E. Prostaglandin I_2

22. Inhibitors specific for cyclooxygenase 2 (COX-2) were deemed more efficacious for certain conditions than inhibitors which blocked both COX-1 and COX-2 activities. This is due to which of the following? [4]
A. COX-2 is specifically induced during inflammation
B. Inhibiting COX-2 did not alter prostaglandin production
C. Inhibiting COX-1 increased the frequency of heart attacks
D. Specifically inhibiting COX-2 reduces the rate of heart attacks
E. COX-1 is inducible and only expressed during wound repair, while COX-2 is expressed constitutively

1 The answer is: Prostaglandin synthesis. Eicosanoids are potent regulators of cellular function. They are derived from arachidonic acid and are metabolized by three pathways: the cyclooxygenase pathway (prostaglandins and thromboxanes), lipoxygenase pathway (leukotrienes), and the cytochrome P450 pathway (epoxides). Nonsteroidalanti-inflammatory drugs (NSAIDs) do not block arachidonic acid release from the membrane (which would block all eicosanoid synthesis); however, they do interfere with the cyclooxygenase pathway. Prostaglandins affect inflammation, thromboxanes affect formation of blood clots, and leukotrienes affect bronchoconstriction and bronchodilatation. NSAIDs block prostaglandins as one of their anti-inflammatory mechanisms. Thus, while NSAIDS will block both prostaglandin and thromboxane synthesis, it is the blockage of prostaglandin synthesis which will block the inflammatory symptoms.



2 The answer is: Lipoxygenase. Montelukast is a leukotriene blocker. Leukotrienes are formed through the lipoxygenase pathway and affect bronchoconstriction and allergy pathways. The cyclooxygenase pathway produces prostaglandins and thromboxanes. The P450 pathway produces epoxides. The Cori cycle is related to gluconeogenesis (lactate transfer from the muscle to the liver), while the TCA cycle is utilized to oxidize acetyl-CoA to CO2 and H2O.

3 The answer is: To reduce thromboxane synthesis. Thromboxane A2 release from platelets is an essential element of forming blood clots, and aspirin will block prostaglandin, prostacyclin, and thromboxane synthesis It is the thromboxane inhibition which reduces the risk of blood clots. Leukotrienes and lipoxins require the enzyme lipoxygenase, which is not inhibited by aspirin.

4 The answer is: COX-2 is specifically induced during inflammation. COX-2 is induced during inflammatory conditions, while COX-1 is constitutively expressed. Thus, when an injury

occurs, and an immune response is mounted at the site of injury, COX-2 is induced in those cells to produce second messengers that play a role in mediating the pain response. Specifically inhibiting the COX-2 isozyme will block the production of those second messengers, without affecting the normal function of COX-1. Inhibiting COX-1 may reduce the frequency of heart attacks, and inhibiting COX-2 will block prostaglandin production via the cylcooxygenase. Recent data suggests that certain drugs that specifically block COX-2 have unwanted side effects, such as an increase in heart attacks.

Topic 10.
Research of nutrition biochemistry

1. Objective: to learn biochemical processes of digestion in stomach and intestines, methods of determination of constituents of gastric juice and to interpret obtained results; to learn the mechanisms of pathobiochemical processes, which develop during disorders of digestive system.

2. Actuality of the theme: As the prevalence of digestive system pathology grows annually, knowledge of pathochemical background of gastrointestinal diseases is essential for the correct diagnosis and treatment of these disorders. Qualitative and quantitative investigation of secrets of digestive glands (glands of stomach, pancreas) is an important diagnostic criterion in gastroenterology.

3. Specific aims:

- ✓ To interpret physiological requirements and energetic value of principal nutritional compounds: proteins, carbohydrates, lipids, vitamins, trace elements.
- ✓ To explain biochemical mechanisms of digestive processes and assimilation of nutritional products in normal conditions and in hereditary or acquired disorders in activity of proteolytic, amylolytic or lipolytic enzymes.
- ✓ To explain the development of main digestive disorders in certain diseases.

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

	Questions:	References:
1	**Requirements of human organism in nutrients – carbohydrates, lipids, proteins. Biological value of main nutrients. Well balanced nutrition. The content of nutrients in common dietary products.**	1. Gubsky Yu. Biological chemistry: textbook / edited by Yu. Gubsky. – Vinnytsia: Nova Knyha, 2018. – 2nd edition. – P. 350–351. 2. Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 506–515. 3. Lecture notes.
2.	**Characterization of digestion of proteins in stomach: mechanism of activation and specificity of certain enzymes (pepsin, gastrixin, rennin). Activation and stimulation of the secretion of enzymes.**	1. Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P.169–171. 2. Lecture notes.
3.	**Mechanism of production and role of chloride acid. Acidity of gastric juice and forms of its expression. Quantitative indices in health and pathology (due to pH-metry).**	1. Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 170, 463–464. 2. Lecture notes.
4.	**General characteristics of digestion of proteins in small intestine: proteolytical enzymes of pancreas and small intestine, mechanism of their activation and specificity of action. Mechanism of absorption of products of protein hydrolysis.**	1. Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 170–173. 2. Lecture notes.
5.	**Putrefaction of proteins in large intestine.**	1. Lecture notes.
6.	**Digestion of lipids in gut. Specificity of action of lypolitical enzymes, role of bile acids in lipids digestion. Absorption of lipids hydrolysis products.**	1. Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 173–177. 2. Lecture notes.
7.	**Digestion of carbohydrates in gut. Glycolytical enzymes. Mechanism of absorption of carbohydrates in gut.**	1. Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 166–169. 2. Lecture notes.
8.	**Regulation of digestion by GIT-system hormones.**	1. Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 449–451. 2. Lecture notes.
9.	**Changes in biochemical indices in disorders of stomach function and their diagnostic significance.**	1. Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 463. 2. Lecture notes.

10.	**Acute and chronic pancreatitis: mechanism of development, pathochemical characterization of the changes of secretory function of pancreas.**	1. Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 179, P.465. 2. Lecture notes.
11.	**Steatorrhea and its kinds: pancreatic (deficiency of lipase), hepatogenic (deficiency of bile in intestine), enterogenic (inhibition of lipolytic enzymes and triacylglycerides resynthesis in intestines).**	1. Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 177. 2. Lecture notes.
12.	**Hereditary enzymopathias of intestinal disaccharidases. Biochemical diagnostics of lactose intolerance.**	1. Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 169, 179. 2. Lecture notes.

5. Tasks for independent work and self-control

5.1. Not only the quantity, but also the quality, of the protein is vitally important. Proteins lacking or containing only small quantities of some of the essential amino acids are said to be of _____ biological value. For human beings, these are plant derived proteins. On contrary, animal proteins are considered to be _____ value nutrients.

5.2. Fill in the table the energetic value of 1 g of the following nutrients and their recommended daily requirements in grams and recommended percentage of daily kilocalories of every nutrient for an adult man with a body weight 75 kg.

Nutrient	Kilocalories in 1 g	Recommended daily need in g
Proteins		
Carbohydrates		
Lipids		

5.3. List the minerals

Macroelements	Microelements
The daily requirement:	**The daily requirement:**

5.4. Fill in the table, in which part of the gastrointestinal tract and with the involvement of which enzymes the following nutrients are digested:

Nutrient	Part of the gastrointestinal tract	Enzymes
Proteins		
Carbohydrates		
Lipids		

5.5. Give the characteristic of peptidases

Enzymes	Characteristic
Endopeptidases (proteases)	
Exopeptidases:	
1. Carboxypeptidases	
2. Aminopeptidases	

5.6. The investigation of the pancreatic gland secret showed the deficiency of the pancreatic inhibitor of trypsin. What is the danger of this condition?

5.7. Situational tasks

a) A patient was hospitalised with the complaints for diarrhoea after bread consumption. Objectively weight loss, dehydration of the organism, adynaemia, sleep disorders were evaluated. Coprogram (stool test) showed the domination of fatty acids and soap. The evaluation of blood serum showed the decreased content of calcium and phosphorus, decreased activity of the alkaline phosphatase, hypoglycaemia in the fasted state and plain glucose chart after sugar loading, acute decrease of the lipids content as well as free and bounded cholesterol. What disease may be suspected?

b) The clinical and laboratory examination of the patient evaluated the presence of the lactic acid in his gastric juice. What does it indicate? What should be recommended to the patient?

c) A patient manifests complaints for the pain in stomach, loss of appetite, general weakness. The laboratory investigation of urine evaluated the increased content of uropepsin. What do these changes indicate?

6. Individual independent students work

1. Infringement of protein digestion in the intestine.
2. Hereditary infringements of digestion of carbohydrates.
3. To create a scheme of digestion and transport of lipids.

Practice protocol №10 «____» _____ 20___

Experiment 1. Measurement of acidity.

Procedure.

Add indicators to 5,0 mL of gastric juice: 2 drops of dimethylaminoazobenzene and 2 drops of phenolphtalein and titrate with 0,1 M solution of NaOH.

Mark the volume of alkali before transition to yellow-orange color (free HCl, 1st point), to light yellow (2nd point, for calculation of bound HCl), to red color (3rd point), total acidity).

Calculation of acidity in mmol/l:

1st point – calculate amount of **free HCl**

$$A = \frac{X\ ml \times 1000 \times 0.1}{5}$$, where

X ml – volume of NaOH solution;

1000 – calculation for 1 L;

0,1 mol/l – molarity of NaOH.

2nd point is used for determination of **bound HCl**. Average of 2nd and 3rd points is total HCl. Bound HCl is determined as the difference between total and free HCl.

3rd point – **total acidity** (see principle of calculation in 1st point).

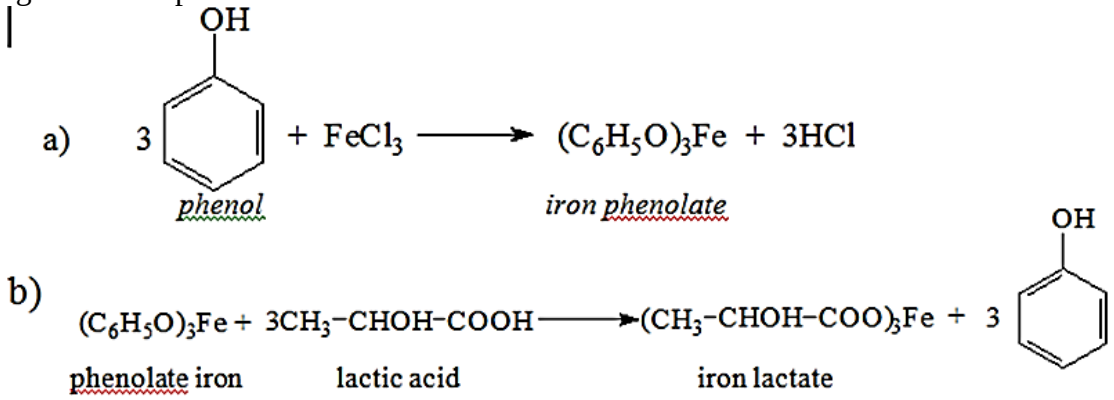
In norm gastric juice total acidity is 40-60 mmol/L, free HCl is 20-40 mmol/L, «debit-hour» of the total HCl – 2-6 mmol/hour, free HCl is 1-4,5 mmol/hour.

Results:

Conclusion:

Experiment 2. Detection of lactic acid (Uffelmann reaction).

Principle of the reaction. Violet colored solution of iron phenolate changes its color to yellow-green in the presence of lactic acid.



Procedure.

Add 2-3 drops of 3 % $FeCl_3$ solution to 5 ml of 1 % phenol solution. Violet color appears.

To this solution add several drops of tested gastric juice. The change of violet colour to yellow-green confirms the presence of lactic acid in gastric juice.

Results:

Conclusion:

Clinical and diagnostic significance. Lactic acid in stomach appears in the course of anaerobic glycolysis or lactic fermentation. This is an indirect proof of the absence or low concentration of HCl. The evaluation of lactic acid in gastric juice may be a sign of cancer as it is assumed, that lactic acid is overproduced by cancer cells due to intensive anaerobic glycolysis.

Experiment 3. Benzidine test for the detection of blood.

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 color.

Procedure.

Take 1-2 ml of tested gastric juice.

Add 4-5 drops of 10 % solution of benzidine in acetic acid.

Add 2-3 drops of 3 % H_2O_2.

The appearance of blue or greenish color due to oxidation of benzidine confirms the presence of blood in the investigated probe.

Results:

Conclusion:

Clinical and diagnostic significance. Benzidine test is informative for the detection of blood in biological specimens (gastric juice, urine, saliva, feces etc/) even in the case of very small or trace amount of blood in it. Blood in stomach may appear due to the bleeding from gastric mucosa damage, e.g. in gastric ulcer. It also may be caused by bleeding from oesophageal vein varicoses, in cases of nose or lung bleeding.

Experiment 4. Urease method for the detection of Helicobacter Pylori in gastric juice.

Principle. *H. pylori* can be detected due to the presence of urease, which is an enzyme that breaks down urea to ammonia and CO_2. Ammonia shifts pH of the solution to alkaline side and this is stated by the appearance of pink color in the presence of phenolphthalein.

Procedure.

Take 2 ml of gastric juice.

Add 1-2 ml of urea solution.

Add 2-3 droplets of phenolphthalein.

Leave the tubes for several minutes at room temperature and supervise the change of the colour.

The appearance of red color indicates the presence of urease, which may originate from *H. pylori*.

Results:

Conclusion:

Clinical and diagnostic significance. Due to the modern knowledge *H.pylori* is supposed to be the main ethiologic cause of peptic ulcer disease. The discovery of *H.pylori* as a causative agent of peptic ulcer disease was awarded a Nobel Prize in 1984 and changed the approaches to the treatment of this disease. Actually schemes of peptic ulcer disease treatment include antibacterial therapy, aimed at eradication of *H.pylori*, whereas in past years surgery was the most common treatment of this disorder.

H.pylori is detected in 92-100 % patients with duodenal ulcer, in about 90 % patients with gastritis, 70-90 % patients with gastric ulcer, approximately in 80 % cases of stomach cancer and in 40-70 % patients with dyspepsias without ulceration.

Thanks to flagellas, *Helicobacter* moves rapidly into the deep mucous layer of the stomach mucosa, being protected there from the harmful effect of *Hcl* and quickly produces colonies. Lipopolysaccharides of the membrane of *H.pylori* cause immune response and inflammation of the gastric mucosa. A significant role in pathogenicity of *H.pylori* belongs to the lytical enzymes it produces – proteases, mucinase, lipase and the most important of them - urease. The latter breaks down urea with the production of ammonia, which neutralizes *Hcl* in stomach, providing comfortable environment for the survival of *H.pylori*. Ammonia also contributes to the development of inflammation and damage of the gastric mucosa.

The report is checked up _____

(The signature of the teacher, date)

Examples of Krock-1 tests

1. Fats, proteins, carbohydrates, vitamins, mineral salts and water must be present in the daily diet of an adult healthy person. Indicate the amount of protein that provides a normal vital activity of the body:

A. 70-100 g
B. 50-60 g
C. 10-20 g
D. 40-50 g
E. 180-200 g

2. A 4-year-old child was delivered to the hospital with the signs of prolonged protein starvation such as edema, anemia, growth and mental retardation. What is the cause of edema development in a child?

A. Decreased synthesis of albumins
B. Decreased synthesis of globulins
C. Decreased hemoglobin synthesis
D. Decreased synthesis of lipoproteins
E. Decreased synthesis of glycoproteins

3. Violation of the digestion of proteins was diagnosed in a patient suffering from acute pancreatitis. The decreased synthesis and release of what enzyme caused such state?

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

4. Megaloblastic anemia is developed in a woman after subtotal resection of the stomach. The urinary excretion of what substance confirms the diagnosis?

A. Methylmalonate
B. Ketone bodies
C. Glucose
D. Urea
E. Uric acid

5. Coagulation of milk occurs in the stomach of infants. Which enzyme is involved in this process?
A. Rennin
B. Pepsin
C. Gastrin
D. Secretin
E. Lipase

6. Lactate has been revealed in the gastric juice of a patient. What is the cause of its appearance?
A. Insufficiency of HCl
B. Excess of HCl
C. Pepsin deficiency
D. Insufficiency of gastricsin
E. Rennin deficiency

7. Negative nitrogen balance was revealed during the examination of a 45-year-old patient being a long time on a vegetarian diet. What feature of the diet caused this state?
A. Insufficient amount of proteins
B. Insufficient amount of fats
C. A large amount of water
D. A large number of carbohydrates
E. Insufficient amount of vitamins

8. A 40-year-old man was hospitalised to the hospital. He was diagnosed with chronic gastritis. Infringement of digestion of what nutrients in stomach is a characteristic sign of this pathology?
A. Proteins
B. Phospholipids
C. Starch
D. Lactose
E. Triglycerides

9. A patient complains of weight loss and pain in the stomach after eating. The overall acidity is sharply reduced (hypoaciditis) in the analysis of gastric juice. Digestion of what food components is disturbed in the patient?
A. Proteins
B. Phospholipids
C. Neutral fats
D. Oligosaccharides
E. Starch

10. During the investigation of the secretory function of the patient's stomach it has been revealed the decreased concentration of hydrochloric acid in the gastric juice. The activity of which of the following enzymes has been decreased in this state?
A. Pepsin
B. Lipase
C. Hexokinase
D. Amylase
E. Carboxypeptidase

11. Under conditions of chronic pancreatitis there is a decrease in the synthesis and secretion of trypsin. What kind of digestion has been disrupted?
A. Proteins
B. Polysaccharides
C. Lipids
D. Nucleic acids
E. Fat soluble vitamins

12. The patient has a bad appetite and belching. The total acidity of the gastric juice is 10 units. What pathological state is the cause of such condition?
A. Hypoacidic gastritis
B. Hyperacidic gastritis
C. Acute pancreatitis
D. Hyperacidity
E. Stomach ulcer

13. Digestion of proteins in the stomach is the initial stage of protein digestion in the human digestive tract. Specify the enzymes that take part in the digestion of proteins in the stomach:
A. Pepsin and gastrixin
B. Trypsin and catheptins
C. Chymotrypsin and lysozyme
D. Enteropeptidase and elastase
E. Carboxypeptidase and aminopeptidase

14. In the human body chymotrypsinogen is secreted by the pancreas and converted into active chymotrypsin in the intestine by the partial proteolysis as a result of an action of the following enzyme:
A. Trypsin
B. Lipase
C. Pepsin
D. Aminopeptidase
E. Carboxypeptidase

15. The activation of which process in the cells of the stomach tumor is the most reliable reason for the appearance of lactic acid in the gastric juice?
A. Glycolysis
B. Pentose phosphate pathway
C. β-oxidation of fatty acids
D. Aerobic oxidation of glucose
E. Gluconeogenesis

16. The digestion of proteins has been disrupted in the stomach and small intestine of the patient. The deficiency of what enzymes caused the disorder?
A. Peptidases
B. Glycosidases
C. Lipases
D. Dehydrogenases
E. Transferases

17. The initial digestion of proteins occurs in the stomach. Which of the following enzymes splits the proteins of the connective tissue?
A. Pepsin A
B. Gastricsin
C. Rennin
D. Pepsin B
E. Enterokinase

18. Disaccharides are formed during the hydrolysis of polysaccharides. What kind of them is formed during the hydrolysis of starch?
A. Maltose
B. Cellulose
C. Lactose
D. Sucrose
E. Glycogen

19. Hydrochloric acid has a lot of functions in the stomach. Which function is not typical for hydrochloric acid?
A. It hydrolyses the peptide bonds of dicarboxylic amino acids
B. It provides swelling and denaturation of food proteins
C. It stimulates the secretion of enterokinase by the enterocytes of the duodenum
D. It promotes the conversion of pepsinogen into pepsin
E. It provides antibacterial properties of gastric juice

20. A 27-year-old patient has dermatitis, poor wound healing and poor eyesight for a long time. The patient did not eat fat with food but received a sufficient amount of carbohydrates and proteins. What is the possible cause of metabolic disorders in this case?
A. Insufficient intake of polyunsaturated fatty acids and fat-soluble vitamins
B. Low calorie diet
C. Insufficient intake of oleic acid and vitamins PP and C
D. Insufficient intake of saturated fatty acids
E. Insufficient intake of vitamins of group B

21. All proteolytic enzymes are divided into exo- and endopeptidases. What are exopeptidases?
A. Carboxypeptidase, aminopeptidase
B. Elastase, collagenase
C. Pepsin, trypsin
D. Chymotrypsin, carboxypeptidase
E. Aminopeptidase, elastase

22. Pepsin refers to proteolytic enzymes of gastric juice. The most optimal hydrolytic effect of this endopeptidase is on:
A. Denatured proteins
B. Protamines
C. Mucoproteins
D. Keratins
E. Histones

23. Pancreatic juice contains a full set of enzymes that are necessary for the digestion of proteins, lipids, carbohydrates. Choose the proteolytic enzymes of the pancreatic juice?
A. Chymotrypsin, trypsin, carboxypeptidases
B. Chymotrypsin, elastase, pepsin
C. Elastase, carboxypeptidases, gelatinase
D. Carboxypeptidases, rennin, lipase
E. Trypsin, chymotrypsin, rennin

24. A patient complains of weight loss and frequent diarrhoea, especially after eating fatty foods. Laboratory investigation revealed steatorrhoea, hypocholic stool. What it the most likely cause of the state?
A. Obstruction of the biliary tract
B. Inflammation of the small intestinal mucosa
C. Lipase insufficiency
D. Impaired phospholipase activity
E. Unbalanced diet

25. The activity of intestinal microorganisms ensures the breakdown of amino acids with the formation of toxic substances. Hydrogen sulphide and methyl mercaptan are formed due to the breakdown in the intestine of the following amino acids:
A. Cystine, cysteine, methionine
B. Tyrosine, tryptophan, phenylalanine
C. Glycine, threonine, lysine
D. Arginine, histidine, glycine
E. Proline, isoleucine, leucine

26. A patient complains of nausea and weakness after eating fatty foods. The signs of steatorrhea have been revealed. The cholesterol concentration in the blood is 9.2 mmol/L. The cause of this state is the deficiency of:
A. Bile acids
B. Triglycerides
C. Fatty acids
D. Phospholipids
E. Chylomicrons

27. The patient is diagnosed with an increased total gastric acidity. What is the normal total gastric acidity?
A. 40–60 mmol / L
B. 20–40 mmol / L
C. 30–50 mmol / L
D. 10–20 mmol / L
E. 60–80 mmol / L

28. Which of the following enzymes hydrolyses peptide bonds formed by carboxyl groups of aromatic amino acids?
A. Trypsin
B. Chymotrypsin
C. Procarboxypeptidase B
D. Enterokinase
E. Gastricin

29. Examination revealed lactic acid in the gastric juice of a patient. What method was used to determine lactic acid?
A. Uffelmann's reaction
B. Benzidine sample
C. The urease method
D. Reaction with resorcin
E. Fehling test

30. To digest fatty foods, one of the digestive secrets is needed. Which component directly takes part in the emulsification of fats?
A. Bile
B. Intestinal juice
C. Pancreatic juice
D. Saliva
E. Gastric juice

31. A patient complains of nausea and heartburn after eating fatty foods. Steatorrhea has been revealed. What is the cause of such state?
A. Insufficiency of bile acids
B. Increased release of lipase
C. Violation of the synthesis of trypsin
D. Amylase deficiency
E. Violation of the synthesis of phospholipase

32. The patient complains of discomfort after eating fatty foods. Undigested drops of fat are present in the stool. The cause of this state is the deficiency of:
A. Bile acids
B. Fatty acids
C. Chylomicrons
D. Triglycerides
E. Phospholipids

33. Examination revealed congestion of bile in the liver and stones in the gallbladder. Specify the main component of gallstones forming in this state:
A. Cholesterol
B. Triglycerides
C. Phospholipids
D. Protein
E. Mineral salts

34. Which of the following indicators characterizes the lithogenic properties of bile and indicates the high risk for stone formation?
A. The bile salt-cholesterol ratio
B. Cholesterol of bile
C. Bile acids
D. Billirubin of bile
E. Phospholipids

35. A patient suffering from cholelithiasis has been diagnosed with jaundice caused by the obstruction of the bile ducts. The disturbance of digestion of what substances has been observed in this state?
A. Lipids
B. Polysaccharides
C. Proteins
D. Nucleic acids
E. Disaccharides

36. Insufficient secretion of which enzyme contributes to incomplete digestion of fats in the gastrointestinal tract and the appearance of a large number of neutral fats in feces?
A. Pancreatic lipase
B. Phospholipase
C. Enterokinase
D. Monoacylglycerol lipase
E. Pepsin

37. A patient has been diagnosed with an acute pancreatitis. What drugs should a doctor prescribe to avoid the autolysis of pancreas?
A. Protease inhibitors
B. Protease activators
C. Trypsin
D. Chymotrypsin
E. Amylase

38. After the intake of fats, they are digested and absorbed in the intestine. What products of hydrolysis of fats are absorbed in the intestine?
A. 2-monoacylglycerols, fatty acids
B. Amino acids, fatty acids
C. Polypeptides, fatty acids
D. Monosaccharides, fatty acids
E. Lipoproteins, fatty acids

39. Coprological examination of a patient's feces revealed the presence of drops of neutral fat. The stool is acholic. The most reliable cause for such pathological changes is a violation of:
A. Bile flow to the intestine
B. Secretion of intestinal juice
C. Secretion of hydrochloric acid in the stomach
D. Secretion of pancreatic juice
E. Intestinal absorption processes

40. A patient suffering from chronic hepatitis has the signs of impaired digestion of fats (steatorrhea). What is the mechanism of its occurrence?
A. Decreased synthesis and secretion of bile acids
B. Decreased synthesis and secretion of cholesterol
C. Decreased trypsin activity
D. Malabsorption syndrome
E. Deficiency of fat-soluble vitamins

41. A 40-year-old patient has been diagnosed with obstruction of biliary tract. What kind of food causes steatorrhea in a patient?
A. Predominantly fats
B. Predominantly proteins
C. Fat deficiency
D. Preferably carbohydrates
E. Absence of proteins

42. Due to prolonged consumption of fatty foods alimentary hyperlipidemia has been developed in a patient caused by the increased content in the diet of:
A. Triglycerides
B. Cholesterol
C. Glycolipids
D. Chylomicrons
E. Phospholipids

43. What are the risk factors for cholelithiasis development?
A. Hypercholesterolemia, a decrease in the concentration of bile acids and phospholipids in bile, congestion of bile
B. Hypersecretion of epinephrine, increased concentration of high-density lipoproteins, increased thiolase and lipase activities, increased concentration of bile acids
C. Hypersecretion of thyroxine, an increase in the concentration of chylomicrons, a decrease in the activity of phospholipase and triglyceride lipase, an increase in the concentration of lecithin in the blood
D. An increase in the rate of biotransformation of cholesterol in bile acids, hypocholesterolemia, hypersecretion of adrenaline and thyrotropin
E. Reduction of the concentration of very low density lipoproteins, hypersecretion of sex hormones, decreased activities of biotin carboxylase and lipoprotein lipase

44. How can lipase activity be evaluated?
A. By the number of fatty acids formed as a result of hydrolysis
B. By the amount of pyruvic acid formation
C. By the ability of the enzyme to hydrolyze peptide bonds
D. By the ability to inhibit the reduction of fatty acids
E. By the ability to decolorize methylene blue upon reduction with aldehyde

45. A patient suffering from liver failure has been diagnosed with disruption of the formation and secretion of bile acids. The violation of absorption of which of the following vitamins occurs in the patient?
A. Vitamin E
B. Vitamin C
C. Vitamin B_6
D. Vitamin H
E. Vitamin B_{12}

Topic 11.
Research of biochemical functions of water-soluble vitamins

1. Objective: To learn the structure, principles of classification, functional significance of vitamins, vitaminoids, antivitamins, biologically active supplements. To master methods of qualitative and quantitative determination of water soluble vitamins.

2. Actuality of the theme: water-soluble vitamins are involved in metabolism as coenzymes and activators of many enzymatic and nonenzymatic processes. Disorders in vitamins turnover reduce intensity of energetic and plastic metabolism, what results in violations of the functions of several organs, decline of immunity to infectious diseases, loss of ability to adopt to unfavorable evironmerntal factors.

3. Specific aims:

- ✓ To interpret the role of water soluble vitamins and their precursors as nutritional components in metabolic and physiological processes.
- ✓ To evaluate the role of water soluble vitamins in metabolism, development of hypovitaminoses, their prevention and treatment.

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

	Questions:	References:
1.	**Vitamins as essential nutritional components. History of vitamins discovery and development of vitaminology:** ✓ History and nomenclature; ✓ Classification on vitamins; ✓ Synthesis of vitamins by intestinal bacteria; ✓ Vitamers.	1. Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 116–118. 2. Gubsky Yu. Biological chemistry: textbook / edited by Yu. Gubsky. – Vinnytsia: Nova Knyha, 2018. – 2nd edition. – P. 352. 3. Lecture notes.
2.	**Causes of exo- and endogenous hypo- and avitaminoses.**	1. Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 116–180 (see individually for each vitamin). 2. Gubsky Yu. Biological chemistry: textbook / edited by Yu. Gubsky. – Vinnytsia: Nova Knyha, 2018. – 2nd edition. – P. 354, 356, 357, 359, 360, 361, 363, 365, 368. 3. Lecture notes.
3.	**Vitamin B_1 and B_2: structure, biological function, sources, daily requirement. Symptoms of hypovitaminosis.**	1. Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 135–138. 2. Gubsky Yu. Biological chemistry: textbook / edited by Yu. Gubsky. – Vinnytsia: Nova Knyha, 2018. – 2nd edition. – P. 353–356. 3. Lecture notes.
4.	**Structure and properties of vitamin H and pantothenic acid. Their envolvement in metabolism, sources, daily requirement. Metabolic significance of CoA.**	Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 146–150. 2. Gubsky Yu. Biological chemistry: textbook / edited by Yu. Gubsky. – Vinnytsia: Nova Knyha, 2018. – 2nd edition. – P. 359–361. 3. Lecture notes.
5.	**Antianemic vitamins (B_{12}, folic acid): structure, biological function, sources, daily requirement. Symptoms of hypovitaminosis.**	1. Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 150–156. 2. Gubsky Yu. Biological chemistry: textbook / edited by Yu. Gubsky. – Vinnytsia: Nova Knyha, 2018. – 2nd edition. – P. 361–366. 3. Lecture notes.

6.	**Vitamins B_6 and PP, structure, biological function, sources, daily requirement. Symptoms of hypovitaminosis.**	1. Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 138–146. 2. Gubsky Yu. Biological chemistry: textbook / edited by Yu. Gubsky. – Vinnytsia: Nova Knyha, 2018. – 2nd edition. – P. 356–359. 3. Lecture notes.
7.	**Vitamin C and P: structure, biological function, sources, daily requirement. Functional interrelations between vitamin C and P, manifestations of insufficiency in human organism.**	1. Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 132–134. 2. Gubsky Yu. Biological chemistry: textbook / edited by Yu. Gubsky. – Vinnytsia: Nova Knyha, 2018. – 2nd edition. – P. 367–370. 3. Lecture notes.

5. Tasks for independent work and self-control

5.1. Complete the table

Vitamin	Chemistry	Recommended dietary allowance (RDA)	Dietary sources
Thiamine (B_1)			
Riboflavin (B_2)			
Niacin (nicotinic acid, PP, B_3)			
Pyridoxine (B_6)			
Biotin (H, B_7)			
Pantothenic acid (B_5)			
Folic acid (folacin)			
Cobalamin (B_{12})			
Ascorbic acid (C)			

5.2. Explain the biochemical functions of water soluble vitamins

Thiamine (B_1)

Coenzyme:

Biochemical functions (enzymes and reactions or metabolic pathways)

Deficiency symptoms

Riboflavin (B_2)

Coenzyme:

Biochemical functions (enzymes and reactions or metabolic pathways)

Deficiency symptoms

Niacin (nicotinic acid, PP, B_3)

Coenzyme:

Biochemical functions (enzymes and reactions or metabolic pathways)

Deficiency symptoms

Pyridoxine (B_6)

Coenzyme:

Biochemical functions (enzymes and reactions or metabolic pathways)

Deficiency symptoms

Biotin (H, B_7)

Coenzyme:

Biochemical functions (enzymes and reactions or metabolic pathways)

Deficiency symptoms

Pantothenic acid (B_5)

Coenzyme:

Biochemical functions (enzymes and reactions or metabolic pathways)

Deficiency symptoms

Folic acid (folacin)

Coenzyme:

Biochemical functions (enzymes and reactions or metabolic pathways)

Deficiency symptoms

Cobalamin (B_{12})

Coenzyme:

Biochemical functions (enzymes and reactions or metabolic pathways)

Deficiency symptoms

Ascorbic acid (C)

Coenzyme:

Biochemical functions (enzymes and reactions or metabolic pathways)

Deficiency symptoms

5.3. Situational tasks

a) Gross thiamine deficiency is most often associated with chronic alcoholism.
What possible causes?

What name of this syndrome?

Indicate the clinic symptoms of this syndrome.

b) Indicate the causes of B_{12} deficiency: 1) dietary deficiency; 2) malabsorption of dietary vitamin; 3) inherited defects. Explain why the strict vegetarians (vegans) are at risk of developing B_{12} deficiency.

Explain the mechanism of development of pernicious (megaloblastic/macrocytic) anemia. Why are the hemopoietic problems associated with a B_{12} deficiency identical to those observed in a folate deficiency?

Explain the mechanism of the neurologic dysfunction associated with a B_{12} deficiency.

c) A 30-year-old male goes to his dentist complaining of loosening teeth. Examination also reveals that his gums are swollen, purple, and spongy. The dentist also notes that the patient's fingers have multiple splinter hemorrhages near the distal ends of the nail and that a wound on the patient's forearm failed to heal properly. Select the vitamin that is most likely to be deficient.

6. Individual independent students work

Endogenous hypovitaminoses. Causes and mechanisms of development in diseases of digestive or cardiovascular systems.

Practice protocol №11 «____» _____ 20___

Experiment 1. Reduction of methylene blue by ascorbic acid (vitamin C).

Principle. Methylene blue is reduced by ascorbic acid to a colorless leuko-form.

Procedure.

Take 2 tubes and add one droplet of 0.01 % sol. of methylene blue and one droplet of 10 % sol. of Na_2CO_3 to each of them.

Add to the first tube several drops of the extract of canine rose fruits.

Add to the second tube several drops of water.

Heat the tubes above fire flame.

Observed decoloration of the first tube during heating due to reduction of dye.

Results:

Conclusion:

Clinical and diagnostic significance. The daily need for ascorbic acid is 80-100 mg. People, living in the conditions of hot or cold climate or working in factories with hot temperature inside have higher demands for vitamin C. Ascorbic acid is essential for the hydroxylation of proline and lysine, which compose collagen. Vitamin C also has outstanding antioxidant properties. The deficiency of vitamin C results in the disease, called scurvy, accompanied by fragility of small vessels, bleeding of gums etc.

Experiment 2. Diazoreaction for thiamine.

Principle. In an alkaline medium diazo reactive forms an orange complex with thiamine.

Procedure.

Add to diazoreactive consisting of 5 drops of 1 % solutionof sulfanilic acid and 5 drops of 5 % sodium nitrite, 1-2 drops of 5 % solution of thiamine and then add on the wall carefully 5–7 drops of 10 % sodium carbonate. Orange ring is formed at liquid-liquid interface.

Results:

Conclusion:

Experiment 3. Determine the content of ascorbic acid in the urine.

Principle. Ascorbic acid in acidic environment reduces molecular iodine, while its reduced form becomes oxidized. The appearance of a blue color indicates that all molecules of ascorbic acid from reduced forms are converted to oxidized ones and the first excessive drop of iodine solution gives a blue color in the presence of starch.

Procedure.

Add 5 ml of urine and 5 ml of 1 N HCl into the flask, pour 5 drops of starch solution. Titrate with 0.001 N iodine solution till formation of blue color which disappears in 30 seconds.

Calculation. According to the result of the titration daily excretion of ascorbic acid is calculated, taking into consideration the fact, that 1 ml of 0.001 N iodine solution corresponds to 0.5 μmol of ascorbic acid. The calculation is carried out in the following way:

$$\text{Quantity of ascorbic acid in } \mu\text{mol/day} = \frac{\text{a} \times 0.5 \times 1500}{5},$$

where

a – ml of 0.001 N iodine solution;

0.5 μmol ascorbic acid corresponding to 1 ml of 0.001 N J_2;

1500 – daily diuresis (ml);

5 – a volume of urine in the sample (ml).

284-568 μmol ascorbic acid can be excreted normally per day in the urine.

Results:

Conclusion:

The report is checked up _____

(The signature of the teacher, date)

Examples of Krock-1 tests

1. 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

2. The symptoms of pellagra (vitamin PP deficiency) are particularly pronounced in the patients obtaining a low protein diet, because one of the essential amino acids is a precursor of nicotinamide in the human body. What is this amino acid?

A. Tryptophan
B. Threonine
C. Arginine
D. Histidine
E. Lysine

3. A patient is diagnosed with pellagra. It is known that the patient ate corn and practically did not eat meat for a long time. Deficiency of which substrate led to this pathology?

A. Tryptophan
B. Tyrosin
C. Proline
D. Alanin
E. Histidin

4. 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 a_1
C. Cytochrome oxidase
D. Cytochrome b
E. Cytochrome c

5. Dermatitis, diarrhoea and dementia are observed in a patient. When collecting an anamnesis, it has been turned out that he was a vegetarian. The deficiency of which vitamin caused indicated disorders?
A. Vitamin PP
B. Vitamin B_1
C. Vitamin B_2
D. Vitamin H
E. Vitamin 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. Dermatitis, diarrhoea and dementia were revealed during medical examination of a patient. The absence of which vitamin caused such clinical symptoms?
A. Nicotinamide
B. Ascorbic acid
C. Folic acid
D. Biotin
E. Rutin

8. 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

9. The structural analogues 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. Muscle weakness, irritability, skin lesions, hair loss and anemia are observed in a patient under the tuberculosis treatment with isoniazid. The deficiency of which vitamin caused the disorders?
A. B_6
B. B_2
C. B_{12}
D. B_3
E. B_1

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. The patient has a decreased appetite, diarrhoea, pallor and dry skin, cyanosis of the lips, cheeks and hands. The tongue is shiny «lacquer», its edema is observed (teeth marks are visible around the edges). The deficiency of which vitamin is observed?
A. PP
B. B_2
C. B_1
D. B_3
E. B_{12}

14. A patient is suffering from the disrupted absorption of vitamin B_{12} after operative removal of a part of stomach. Anemia has been developed in the patient. Which factor is necessary for the absorption of this vitamin?
A. Castle's intrinsic factor
B. Gastrin
C. Hydrochloric acid
D. Pepsin
E. Folic acid

15. 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

16. 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

17. This vitamin is involved in all types of metabolism. Funicular myelosis and megaloblastic anemia are developed under its absence. What is this vitamin?
A. Cobalamin
B. Pyridoxine
C. Riboflavin
D. Biotin
E. Thiamine

18. Concentration of pyruvate is increased in the patient's blood. A significant amount of it is excreted in the urine. The deficiency of what vitamin is developed in a patient?
A. B_1
B. E
C. B_3
D. B_6
E. B_2

19. 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

20. The cause of pellagra can be preferential feeding of corn and reduction in the diet of products of animal origin. Absence of which amino acid in the diet leads to this pathology?
A. Tryptophan
B. Tyrosine
C. Proline
D. Alanine
E. Histidine

21. 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

22. 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

23. After the removal of the 2/3 of the patient's stomach it has been observed the decreased content of hemoglobin and red blood cells in the blood. The megaloblastic cells are appeared in the blood test. The deficiency of what vitamin leads to such changes?
A. B_{12}
B. C
C. P
D. B_6
E. PP

24. 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

25. Addison-Biermer disease is a malignant hyperchromic megaloblastic anemia. It is caused by the deficiency of vitamin B_{12}. Which microelement is a part of the vitamin?
A. Cobalt
B. Molybdenum
C. Zinc
D. Iron
E. Magnesium

26. A 55-year-old man suffering from alcoholic liver cirrhosis was diagnosed with megaloblastic anemia. The deficiency of what vitamin caused development of anemia?
A. Folate
B. Lipoic acid
C. Biotin
D. Riboflavin
E. Pantothenic acid

27. A worker of the poultry factory consuming 5 or more raw eggs every day complains of the weakness, drowsiness, pain in the muscles, hair loss and seborrhea. The deficiency of what vitamin is the cause of this state?
A. H (biotin)
B. C (ascorbic acid)
C. A (retinol)
D. B_1 (thiamine)
E. B_2 (riboflavin)

28. A 4-year-old child has been delivered to a hospital with symptoms of paresis of the lower limbs. It was revealed an anemia, high concentration of methylmalonate in the blood. The lack of what vitamin caused these symptoms?
A. Cobalamin
B. Pantothenic acid
C. Vitamin A
D. Niacin
E. Biotin

29. Hyperpyruvatemia was revealed in the patient. The deficiency of what vitamin is observed in the patient?
A. B_1
B. E
C. B_3
D. B_6
E. B_2

30. Which of the following vitamins provides a cofactor for transfer of one-carbon units?
A. Folate
B. Pyridoxine
C. Niacin
D. Riboflavin
E. Thiamin

31. A patient suffering from atrophic gastritis was diagnosed with megaloblastic anemia. The deficiency of which compound caused the development of anemia?
A. Gastromucoprotein (Castle factor)
B. Vitamin B_6
C. Vitamin B_1
D. Iron
E. Erythropoietin

32. Steatosis is caused by an accumulation of triacylglycerols in hepatocytes. What lipotropic substances prevent the development of liver steatosis?
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

33. Vitamins taking part in the biochemical processes of the body are synthesized by the normal microflora of the large intestine. What vitamins are synthesized primarily by microorganisms?
A. K, B_{12}
B. A, C
C. E, PP
D. B_1, B_2
E. B_6, E

34. A patient was diagnosed with the vitamin H (biotin) deficiency. The reduced activity of which of the following enzymes is observed in the patient?
A. Pyruvate carboxylase
B. Pyruvate decarboxylase
C. Alcoholdehydrogenase
D. Aminotransferase
E. Carbamoyl phosphate synthetase

35. 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. E, P, B_{15}
E. C, B_{12}, D, K

36. Which of the following vitamins is essential for fatty acid synthesis?
A. Biotin
B. Folate
C. Niacin
D. Ascorbic acid
E. Cobalamine

37. A 47-year-old patient suffering from tuberculosis of the upper lobe of the right lung received isoniazid in the combined therapy. After a while, the patient began to complain of a muscle weakness, decreased skin sensitivity, an impaired vision and coordination of movements. What vitamin should be used to eliminate these symptoms?
A. Vitamin B_6
B. Vitamin A
C. Vitamin D
D. Vitamin B_{12}
E. Vitamin C

38. Megaloblastic anemia has two most likely causes, deficiency of folate and deficiency of cobalamin. Often treatment of patients with cobalamin deficiency improves in terms of their hematologic features with treatment with folate but not in their neurologic symptoms. What is the most likely explanation for this?
A. Excess of folate blunts the trapping of folate as N5-methyltetrahydrofolate
B. Cobalamin deficiency is not serious
C. Folate in high concentrations can serve as a cofactor for the conversion of homocysteine to methionine
D.Excess of folate directly inhibits the destruction of red blood cells
E. Excess of folate stimulates erythropoietic tissues to synthesize cobalamin in situ

Topic 12.
Research of biochemical functions of fat-soluble vitamins

1. Objective: To learn the structure and functional significance of fat soluble vitamins, vitaminoids, antivitamins, biologically active supplements. To master methods of qualitative and quantitative determination of fat soluble vitamins.

2. Actuality of the theme: Fat-soluble vitamins – A, D, E, K – are components of nutrition, exogeneous hormons which stimulate synthesis of specific proteins in the organism. Vitamin E as the most powerful natural antioxidant inhibits peroxide oxidation of lipids as well as prevents oxidative modification of biomolecules (proteins, nucleic acids) and facilitates normal functioning of reproductive organs.

Polyunsaturated fatty acids (vitamin F) are components of phospholipids of cell membranes and are precursors of synthesis of physiologically active compounds – eicosanoids.

3. Specific aims:

- ✓ To explain bioregulatory (hormone-like) and antioxidant functions of fat-soluble vitamins A, E, K, F, D.
- ✓ To analyze the causes and molecular-biochemical mechanisms of pathological changes at hypo- and hypervitaminoses.

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

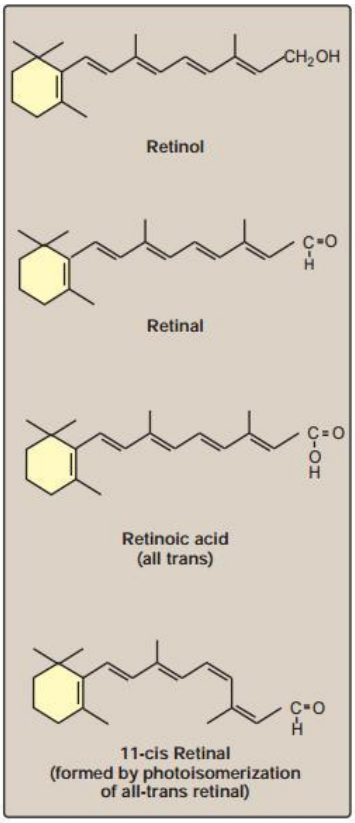
	Questions:	References:
1.	**Vitamins of D group: structure, biological function, nutritional sources, daily requirement. Symptoms of hypo- and hyper-vitaminosis, avitaminosis.**	1. Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 123–128. 2. Gubsky Yu. Biological chemistry: textbook / edited by Yu. Gubsky. – Vinnytsia: Nova Knyha, 2018. – 2nd edition. – P. 373–375. 3. Lecture notes.
2.	**Vitamin A: structure, biological function, sources, daily requirement. Symptoms of hypo- and hypervitaminosis.**	1. Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 118–123. 2. Gubsky Yu. Biological chemistry: textbook / edited by Yu. Gubsky. – Vinnytsia: Nova Knyha, 2018. – 2nd edition. – P. 370–373. 3. Lecture notes.
3.	**Vitamins E, F: structure, biological role, nutritional sources, mechanism of action, daily requirement. Symptoms of insufficiency, application in medicine.**	1. Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 128–129. 2. Gubsky Yu. Biological chemistry: textbook / edited by Yu. Gubsky. – Vinnytsia: Nova Knyha, 2018. – 2nd edition. – P. 375–377. 3. Lecture notes.
4.	**Antihemorrhagic vitamins (K_2, K_3) and their water soluble forms: structure, biological function, nutritional sources, mechanism of action, daily requirement, symptoms of insufficiency, application in medicine.**	1. Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 129–132. 2. Gubsky Yu. Biological chemistry: textbook / edited by Yu. Gubsky. – Vinnytsia: Nova Knyha, 2018. – 2nd edition. – P. 377–378. 3. Lecture notes.
5.	**Provitamins, antivitamins, mechanism of action and application in practical medicine.**	1. Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 156–159. 2. Lecture notes.
6.	**Vitaminoids: structure and biological activity.**	1. Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 156–159. 2. Lecture notes.

5. Tasks for independent work and self-control

5.1. Complete the table «Fat soluble vitamin functions»

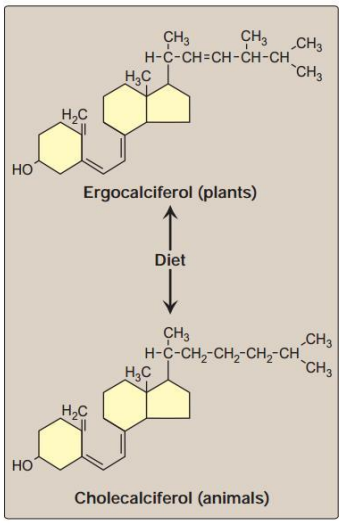
Fat soluble vitamin	Other names	Active form	Dietary sources
Vitamin A			
Vitamin D			
Vitamin E			
Vitamin K			

5.2. Which of the retinoids shown is required for vision? Which mediates the other actions of vitamin A?



5.3. How are the effects of vitamin A deficiency related to keratin, a fibrous protein that helps to prevent moisture loss in epithelia?

5.4. In addition to the preformed dietary precursors of active vitamin D shown, how else do humans obtain a precursor?



5.5. How and where is the active form of vitamin D generated? How is the process regulated?

5.6. What is the primary function of vitamin D?

5.7. What causes rickets and what are its effects?

5.8. Fill in the table «Fat soluble vitamin deficiencies and toxicities»

Fat soluble vitamin	Deficiency	Signs and symptoms	Toxicity (yes or no)
Vitamin A			
Vitamin D			
Vitamin E			
Vitamin K			

5.9. Complete the table «Biochemical functions of vitamin like compounds»

Vitamin like compounds	Formula	Biochemical functions
Choline		
Inositol		
Lipoic acid (thioctic acid)		
Para aminobenzoic acid (PABA)		

5.10. Find the antivitamins of the vitamins in the box below.

Aminopterin Avidin Deoxypyridoxine Desthiobiotin Dicumarol Galactoflavin Isoniazid Methotrexate Oxythiamine Pyrithiamine Sulfonilamide Thiaminase Trimethoprim Warfarin

Vitamin	Antivitamin(s)
Thiamine	
Riboflavin	
Niacin	
Biotin	
Folic acid (folacin)	
Vitamin K	
Para-aminobenzoic acid	

5.11. Situational tasks

a) Derivatives of vitamin A (retinoic acid) and D (calcitriol), like as steroid hormones, take part in the regulation of gene expression.

Where in the cell are their receptors located?

Indicate the mechanism of their action.

Give the examples of genes and corresponding proteins that are induced by retinoic acid and calcitriol.

b) The vitamin K-dependent carboxylase uses a reduced form of vitamin K (hydroquinone) as the electron donor and converts hydroquinone to an epoxide. Vitamin K epoxide is reduced, in two steps, back to its active hydroquinone form by the enzymes epoxide reductase and quinone reductase. Warfarin, synthetic analogue of vitamin K, inhibits these reductases and prevents γ-carboxylation of glutamate residues. The noncarboxylated blood clotting protein precursors increase in the blood, but they are unable to promote blood coagulation because they cannot bind calcium. Warfarin is a commonly used rat poison.

Explain why it is effective as a rat poison.

6. Individual independent students work

1. Biological properties of fat-soluble vitamins in metabolic processes are normal in pathologies.
2. Preparation for the control work.

Practice protocol №12 «____» _____ 20___

Experiment 1. Qualitative reaction for vitamin A (Drummond reaction).

Principle. Concentrated sulfuric acid takes water from retinol with the formation of colored products. Reaction is not specific.

Procedure.

Add 2 drops of 0.05 % oil solution of retinol in chloroform (1:5) to a dry test tube and 1 drop of concentrated sulfuric acid. A red-violet color appears which gradually becomes reddish-brown.

Results:

Conclusion:

Experiment 2. Reaction on retinol with ferric sulfate.

Principle. Retinol with ferric (III) sulfate in acidic conditions forms pinkish-red substance. Carotene gives greenish color in this reaction.

Procedure.

Add 2 drops of 0.05 % oil solution of retinol in chloroform (1:5) and 10 drops of glacial acetic acid saturated with ferric sulfate and 2 drops of concentrated sulfuric acid. A blue color appears which gradually turns into a pinkish-red.

Results:

Conclusion:

Experiment 3. Reaction on calciferol.

Principle. Interaction of vitamin D with aniline reagent causes the appearance of red color under heating

Procedure.

Add to a dry test tube 2 drops of cod liver oil and 10 drops of chloroform, then add 2 drops of aniline reagent continuously mixing with stirring rod. Gently heat and boil for 30 seconds with continuous stirring. In the presence of vitamin D yellow emulsion becomes green, then red.

Results:

Conclusion:

Experiment 4. Detection of vitamin E with the use of ferric chloride.

Principle. Alcohol solution of α-tocoferol is oxidized by ferric chloride to tocoferylquinone, which is of red colour.

Procedure.

Place 4-5 droplets of 0.1 % alcohol solution of tocoferol to a dry tube.

Add 0.5 ml of 1 % solution of ferric chloride.

Mix intensively the content of the tube.

Results:

Conclusion:

Clinical and diagnostic significance. Due to the modern outlook, the main function of tocopherols is to serve as antioxidants. Hydrogen atom in the molecule of α-tocopherol cooperates with the peroxide radicals of lipids, reducing them into hydroperoxides and thus breaks the chain reaction of peroxidation.

Vitamin E is also involved in the regulation of reproductive functions in males and females.

Daily need of vitamin E is 20 – 30 mg, its concentration in blood is 3.5 – 8.0 nmole/l.

Experiment 5. Reaction on naphthoquinone (vitamin K_1).

Principle. Vicasol has lemon-yellow colour in the presence of cysteine in alkaline medium.

Procedure. Put 5 drops of 0.05 % solution of vicasol on microscope slide, add 5 drops of 0.025 % solution of cysteine and 1 drop of 10 % sodium hydroxide. Lemon-yellow colour appears.

Results:

Conclusion:

Practical significance. Qualitative reactions on vitamins are based on colour reactions characteristic for certain chemical group that serves as a part of their structure. These reactions help to find vitamins in drugs, foods and medicinal plants. The principles underlying the qualitative reactions on vitamins are also used for developing methods for their quantitative determination.

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

Examples of Krock-1 tests

1. Many vegetables contain carotenes. Deficiency of which vitamin is eliminated by these plant pigments?
A. Retinol
B. Naphthoquinone
C. Riboflavin
D. Tocopherol
E. Calciferol

2. An antioxidant of natural origin is used in the treatment of periodontal disease. What is it?
A. Tocopherol
B. Thiamin
C. Gluconate
D. Pyridoxine
E. Choline

3. A patient has dry mucous membranes and hemeralopia. Deficiency of which vitamin leads to the appearance of such symptoms:
A. A
B. D
C. C
D. B
E. E

4. The patient has hemeralopia (night blindness). Which of the following substances should be prescribed to correct this state?
A. Carotene
B. Keratin
C. Creatine
D. Carnitine
E. Carnosine

5. An ophthalmologist has revealed an increased time of adaptation of the eyes to darkness and xerophthalmia in a patient. The deficiency of what vitamin caused such state?
A. A
B. E
C. C
D. K
E. D

6. The doctor revealed decreased mineralization of bone tissue in a child during the medical examination. The deficiency of which vitamin caused such state?
A. Calcipherol
B. Riboflavin
C. Tocopherol
D. Folic acid
E. Cobalamin

7. Vitamin A in complex with specific cytoreceptors penetrates into the nucleus, inducing transcription processes, stimulating the growth and differentiation of cells. This function is realized by the following form of vitamin A:
A. Trans-retinoic acid
B. Trans-retinal
C. Cis-retinal
D. Retinol
E. Carotene

8. A 4-month-old child has signs of rickets. Digestive disorders are not observed. The child gets enough sun. Within 2 months, the child received vitamin D_3 (cholecalciferol), but the rickets has been progressed. What is the cause of rickets in a child?
A. Impaired synthesis of calcitriol
B. A violation of the synthesis of parathyroid hormone
C. Violation of calcitonin synthesis
D. Violation of the synthesis of thyroxine
E. Disturbance of insulin synthesis

9. A doctor prescribed applications of vitamin A for a patient suffering from paradontosis. The activation of what process provides a therapeutic effect?
A. Growth and differentiation of cells
B. Hydroxylation of proline
C. Carboxylation of glutamic acid
D. Visual adaptation
E. Colored vision

10. The signs of rickets have been revealed in a child during medical examination. The deficiency of which vitamin caused the disease?
A. 1,25-Dihydroxycholecalciferol
B. Biotin
C. Tocopherol
D. Naphthoquinone
E. Retinol

11. The processes of Ca^{2+} absorption through the intestinal wall has been violated in a patient after cholecystectomy. The prescription of which vitamin will stimulate this process?
A. D_3
B. PP
C. C
D. B_{12}
E. K

12. The signs of rickets have been revealed in a 4-year-old child suffering from hereditary kidney disorder. The concentration of vitamin D in the blood is normal. What is the most likely cause of the rickets in a child?
A. Violation of calcitriol synthesis
B. Increased calcium excretion from the body
C. Hyperfunction of the parathyroid glands
D. Hypofunction of the parathyroid gland
E. Calcium deficiency in food

13. Due to post-translational changes in some blood clotting proteins, in particular prothrombin, they acquire the ability to bind calcium. What vitamin is involved in this process?
A. K
B. C
C. A
D. B_1
E. B_2

14. Inhibition of blood coagulation is observed in a patient suffering from obstruction of the biliary tract. The insufficient absorption of which vitamin causes this state?
A. K
B. E
C. C
D. D
E. A

15. Hemorrhagic syndrome has been developed in a newborn child. The medical examination revealed the hypovitaminosis of vitamin K. What is the biological role of vitamin K?
A. It is a cofactor of gamma-glutamatecarboxylase
B. It is a cofactor of prothrombin
C. It is a specific inhibitor of antithrombin
D. It affects the proteolytic activity of thrombin
E. It inhibits the synthesis of heparin

16. Blood clotting factors undergo the posttranslational modification involving vitamin K. It is a cofactor that participates in gammacarboxylation of blood clotting factors, which leads to an increase in the affinity of their molecules for calcium ions. Which amino acid is carboxylated in these proteins?
A. Glutamic acid
B. Valine
C. Serin
D. Phenylalanine
E. Arginine

17. It has been revealed decreased level of prothrombin in a patient taking anticoagulants of indirect action. What is the most likely cause of this state?
A. Deficiency of vitamin K
B. Deficiency of vitamin B_{12}
C. Decrease in the concentration of Ca^{2+}
D. Reduced amount of blood globulins
E. Deficiency of vitamin C

18. As a result of intoxication the synthesis of beta-lipoproteins has been disrupted in the patient. The absorption of what vitamin is impaired in this case?
A. Vitamin E
B. Vitamin C
C. Vitamin B_6
D. Vitamin H
E. Vitamin B_{12}

19. The activation of prooxidant systems increases the concentration of reactive oxygen species, which leads to the destruction of cell membranes. Antioxidants are used to prevent this damage. What is the most powerful natural antioxidant?
A. Alfa-tocopherol
B. Glucose
C. Vitamin D
D. Fatty acids
E. Glycerol

20. The deficiency of linoleic and linolenic acids in the body leads to immunodeficiency, skin damage, hair loss, delayed wound healing, thrombocytopenia and etc. The impaired synthesis of what biologically active substances most likely causes these symptoms?
A. Eicosanoids
B. Interleukins
C. Interferons
D. Catecholamines
E. Corticosteroids

21. A pregnant woman has several miscarriages in the anamnesis. She has been prescribed a therapy including vitamin preparations. Indicate the vitamin that must be prescribed without fail:
A. Alpha-tocopherol
B. Folic acid
C. Cyanocobalamin
D. Pyridoxine
E. Rutin

22. Arachidonic acid is an essential component of food. It is a precursor in the synthesis of biologically active substances. What compound is synthesized from arachidonic acid?
A. Prostaglandin E_1
B. Norepinephrine
C. Choline
D. Ethanolamine
E. Triiodothyronine

23. Some vitamins ensure the stability of biological membranes. What are they?
A. Tocopherol, retinol
B. Thiamin, folic acid
C. Biotin, cobalamin
D. Pantothenic acid, riboflavin
E. Riboflavin, ascorbic acid

24. The deficiency of which vitamin can cause the activation of lipid peroxidation process?
A. Vitamin E
B. Vitamin D
C. Vitamin K
D. Vitamin B_{12}
E. Vitamin B_6

25. A patient has been diagnosed with abetalipoproteinemia. A possible deficiency in which of the following vitamins could occur in this patient? [1]
A. Vitamin E
B. Vitamin B_2
C. Vitamin C
D. Vitamin B_1
E. Niacin

26. Which one of the fatty acids listed below can be synthesized by humans? [2]
A. Cis Δ5, 8, 13 C20:3
B. Cis Δ9, 12, 15 C18:3
C. Cis Δ9, 12 C18:2
D. Cis Δ5, 8, 11, 14 C20:4
E. Cis Δ10 C16:1

27. Calcitriol maintains physiological concentrations of calcium and phosphate in the blood plasma, ensuring the mineralization of bone tissue. What is the molecular mechanism of its action?
A. It causes the expression of genes for the synthesis of Ca^{2+}-binding and transporting proteins
B. It activates calcitonin synthesis in the thyroid gland
C. It activates the processing of proparathyroid hormone
D. It activates osteoblasts, leading to the mineralization of tissues
E. It activates the synthesis of cholecalciferol

28. A woman, who eats a standard meat-containing diet, has had one child born with a neural tube defect, and is considering becoming pregnant again. Blood work showed normal levels of B_{12} and total folic acid (specific type of folic acid not specified). One possible explanation for the woman's difficulties in her first pregnancy is a thermolabile variant of which of the following enzymes? [3]
A. N5,N10-methylenetetrahydrofolate reductase
B. Serine hydroxymethyl transferase
C. Ornithine transcarbamoylase
D. Phenylalanine hydroxylase
E. Tyrosine aminotransferase

29. A patient has had a series of blood clots, and has been placed on warfarin to reduce such incidents. Warfarin exerts its effect by blocking which of the following? [4]
A. Formation of γ-carboxyglutamate
B. Phospholipid synthesis
C. Clotting factor synthesis
D. Vitamin E activity
E. Platelet biogenesis

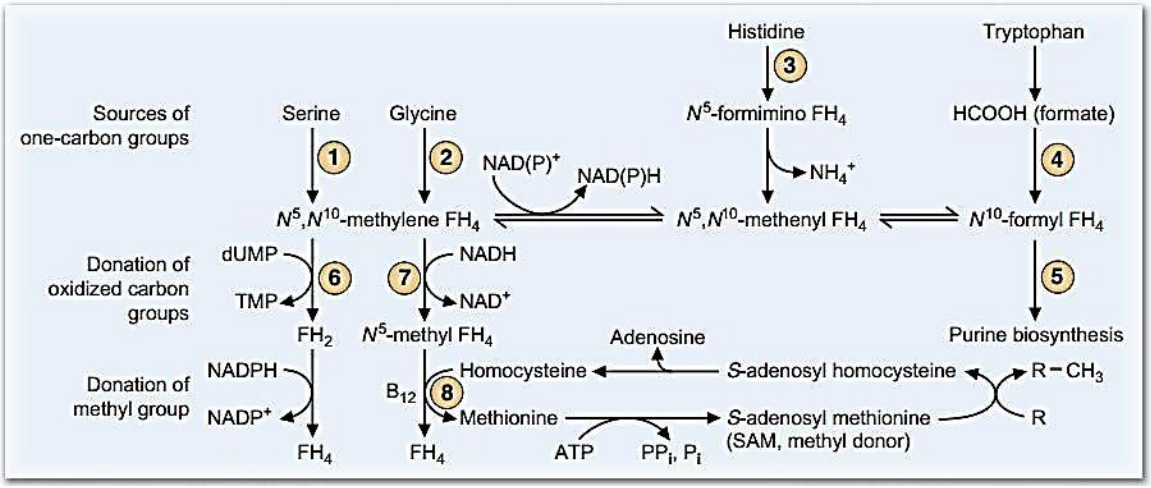
30. Considering the patient in the previous question, which food should be avoided in large quantities while the patient is on warfarin? [5]
A. Green leafy vegetables
B. Milk products
C. Trout
D. Orange-yellow vegetables
E. Steak

31. A 9-month-old child of strict vegan parents is brought to the pediatrician due to perceived muscle weakness. Due to their strict dietary beliefs, the child has not been given vitamin supplements. An image of the anterior of the knee reveals cupped and widened metaphyses. As the child is very fair skinned, the parents always cover up the child when they go outside such that minimal skin is exposed to the sun. In order to correct these problems the physician prescribes treatment with which of the following? [6]
A. Vitamin D
B. Vitamin K
C. Folic acid
D. Vitamin B_{12}
E. Vitamin E

1 The answer is: Vitamin E. Abetalipoproteinemia is a disorder in which neither nascent chylomicrons nor nascent VLDL can be produced due to a defect in the microsomal triglyceride transfer protein. Fat-soluble vitamins are delivered to tissues via chylomicrons; in the absence of chylomicron formation, the fat soluble vitamins will remain in the intestinal epithelial cell, or not even be absorbed from the intestinal tract. Vitamin E is believed to be a major antioxidant factor in cells. All of the other vitamins listed (B1, B2, C, and niacin) are water-soluble vitamins, and do not require chylomicron formation for vitamin delivery.

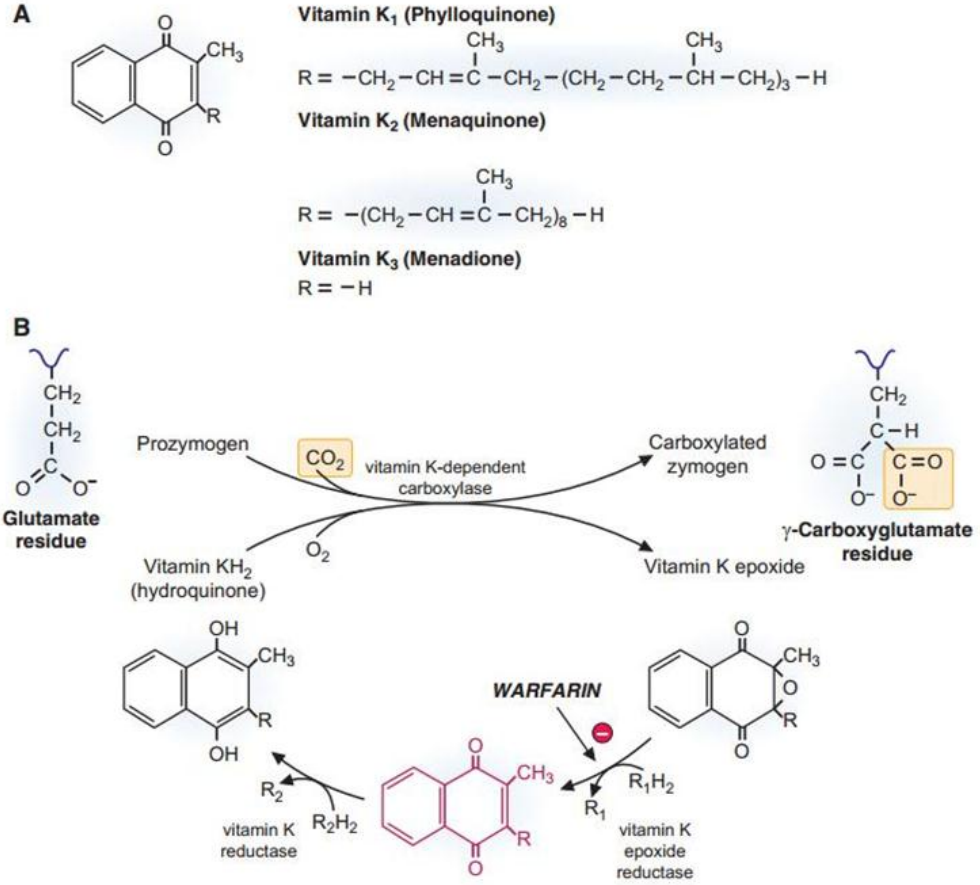
2 The answer is: cis Δ5, 8, 13 C20:3. Humans can synthesize fatty acids of the ω-7 series or higher, but not of ω-6 or lesser. This is due to the limitation of the desaturase system, which can only introduce double bonds at positions 4, 5, 6 and 9, in a substrate that contains at least 16 carbons. Start with C16:0 (palmitic acid), and add a double bond at position 9, creating a cis Δ9 C16:1. Elongate that fatty acid by two carbons, creating a cis Δ11 C18:1. Desaturate this 18-carbon fatty acid at position 6, creating a cis Δ6Δ11 C18:2, which is elongated by two carbons, producing a cis Δ8Δ13 C20:2. Desaturate this fatty acid at position 5 and the final product is obtained.

3 The answer is: N5,N10-methylenetetrahydrofolate reductase. A thermolabile (temperature-sensitive) N5,N10-methylene-THF reductase would reduce the amount of N5-methyl THF which can be formed (see the figure below; reaction 7 utilizes the N5,N10-methylene-THF reductase), and therefore reduce the amount of homocysteine converted to methionine (reaction 8 of the figure). This would lead to a reduction of S-adenosylmethionine levels, and hypomethylation in the nervous system, which may lead to altered gene expression and a neural tube defect. Elevated homocysteine would also be evident. This is a common mutation in the general population, and can be overcome by taking folic acid. An inactivating mutation in serine-hydroxymethyl transferase would reduce the major entry point of carbons into the THF pool, but there are other means to do this (reactions 2, 3, and 4), so a loss of this enzyme would not result in reduced S-adenosylmethionine levels in the nervous system. A defect in ornithine transcarbamoylase (a urea cycle enzyme) would not affect homocysteine and methionine metabolism. Similarly, mutations in phenylalanine hydroxylase, or tyrosine aminotransferase (enzymes involved in phenylalanine and tyrosine metabolism, respectively) would not affect homocysteine and methionine metabolism.



The sources of carbon for the tetrahydrofolate pool are indicated by reactions 1–4. The recipients of carbons from the THF pool are indicated by reactions 5–8. Reaction 7 is catalysed by the N5, N10-methylenetetrahydrofolate reductase, while reaction 8, which requires vitamin B12, is the methionine synthase reaction. Reaction 1 is catalysed by the serine hydroxymethyl transferase enzyme.

4 The answer is: formation of g-carboxyglutamate. Warfarin is a vitamin K antagonist, and blocks the regeneration of active vitamin K after it has participated in its reaction of creating a γ-carboxyglutamate residue. In the absence of this side-chain modification, clotting proteins cannot bind to platelets, and the clotting cascade is inhibited. Warfarin does not interfere with platelet synthesis, nor does it alter phospholipid biosynthesis. Warfarin does not alter the transcription or translation of the clotting factors, and has no relationship with vitamin E, which protects against radical damage within cells and tissues. Warfarin blocks the activity of vitamin K epoxide reductase.



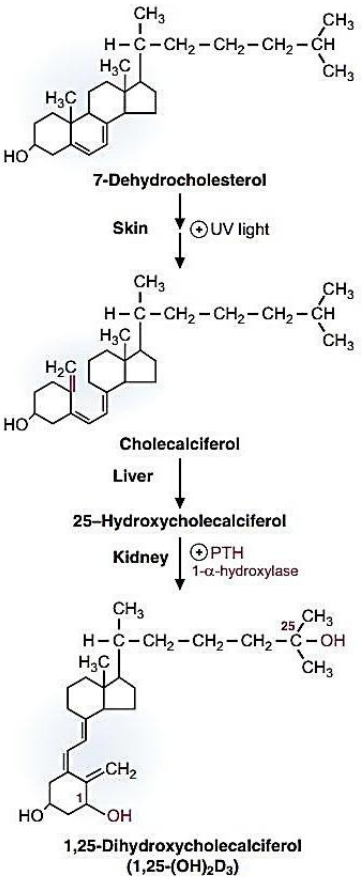
A: Structures of vitamin K derivatives. Phylloquinone is found in green leaves, and intestinal bacteria synthesize menaquinone. Humans convert menaquinone to a vitamin K active form.

B: Vitamin K-dependent formation of γ-carboxyglutamate residues. The vitamin K-dependent carboxylase uses a reduced form of vitamin K (KH2) as the electron donor and converts vitamin K to an epoxide. Vitamin K epoxide is reduced, in two steps, back to its active form by the enzymes vitamin K epoxide reductase and vitamin K reductase.

[5] The answer is: Green leafy vegetables. Green leafy vegetables contain large amounts of vitamin K, which would overcome the effects of the warfarin. The other foods listed are poor sources of vitamin K (milk is fortified with vitamin D, orange-yellow vegetables are high in vitamin A, trout, and beef are low in fat soluble vitamins, although organs, such as liver, would be high in vitamin K).

6 The answer is: Vitamin D. The child has rickets, which is due to a lack of vitamin D. Vitamin D is synthesized via a circuitous route, and due to the parents (and child's) diet, there is insufficient vitamin D for the child to form healthy bones. UV light is required to form the active form of vitamin D, and the child is also lacking exposure to sunlight. While, due to the diet, the child may become deficient for vitamin B12, lack of B12 does not lead to these symptoms. The symptoms are also not consistent with a lack of vitamin K (which would lead to bleeding problems), folic acid (which would lead to anemia), or E (loss of protection against oxidative radicals).

Synthesis of active vitamin D. 1,25-di(OH)2D3 is produced from 7-dehydrocholesterol, a precursor of cholesterol. In the skin, ultraviolet (UV) light produces cholecalciferol, which is hydroxylated at the 25th position in the liver and the 1st position in the kidney to form the active hormone.



Topic 13.
Investigation of blood functions: buffer systems, acid-base state. Pathological forms of hemoglobin

1. Objective: To learn biochemical composition of blood and to explain role of plasma proteins and diagnostic significance of their determination; to evaluate changes in content of final products of metabolism in blood. To conduct investigations of hemoglobin, to interpret functional significance of hemoglobin and its derivatives, to know about hemoglobinopathias and their prevalence in human population.

2. Actuality of theme: The respiratory function of red blood cells is provided by the presence of hemoglobin. Due to its ability to bind oxygen at high partial pressure and give back at low, the molecule of hemoglobin executes its main function as oxygen transporter: it binds oxygen in the capillaries of lung alveoli and gives it back to the tissues in venous capillaries. In addition to the transport of oxygen from lungs to the capillaries of peripheral tissues, hemoglobin plays also a substantial role in transport of carbon dioxide to the lungs.

In the diagnostics of the exigent states an important role belongs to the determination of the acid-basic state of blood, partial pressure of oxygen and carbon dioxide in blood, state of the blood buffer systems, ionic composition of extracellular liquid, osmolarity of biological liquids, dissociations of hemoglobin etc.

3. Specific aims:

- ✓ To explain mechanism of oxygen and CO_2 transport by hemoglobin in lung and tissue capillaries.
- ✓ Classification of molecular forms of hemoglobin in healthy adults and existence of pathological forms of hemoglobin, molecular mechanisms of their appearance.
- ✓ To learn the application of results of biochemical analysis of blood in evaluation of physiological or pathological state of a person.
- ✓ To explain molecular mechanisms of appearance of pathological forms of hemoglobin and changes in principally important indices of blood analysis for evaluation of functional state of a patient.

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

	Questions:	References:
1.	**Blood as internal medium of the body. Role of blood in homeostasis.**	1. Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 182. 2. Lecture notes.
2.	**The composition of whole blood, blood plasma and serum. Physical, chemical and biological properties of blood.**	1. Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 182. 2. Gubsky Yu. Biological chemistry: textbook / edited by Yu. Gubsky. – Vinnytsia: Nova Knyha, 2018. – 2nd edition. – P. 380–381. 3. Lecture notes.
3.	**Blood volume and its pH. Cellular elements of blood: red blood cells, white blood cells, blood platelets.**	1. Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 474–475. 2. Lecture notes.
4.	**Acid-base equilibrium of blood. Regulation of pH in biological fluids, disorders of acid-base equilibrium: metabolic and respiratory acidosis, metabolic and respiratory alkalosis, mechanisms of their development.**	1. Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 474–475, P.479–483. 2. Lecture notes.
5.	**Role of kidneys and lungs in support of acid-base equilibrium.**	1. Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 476–479. 2. Lecture notes.

6.	**Buffer systems of blood, their types, role of different buffer systems in providement of constant pH of blood.**	1. Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 475–477. 2. Lecture notes.
7.	**Humoral mechanisms of regulation of acid-base status.**	1. Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 469–470, P.444. 2. Lecture notes.
8.	**Hemoglobin, its structure, properties and molecular forms.**	1. Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 196–199, 202. 2. Gubsky Yu. Biological chemistry: textbook / edited by Yu. Gubsky. – Vinnytsia: Nova Knyha, 2018. – 2nd edition. – P. 382. 3. Lecture notes.
9.	**Pathology of hemoglobin: hemoglobinopathias and thalassemias.**	1. Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 202–208. 2. Gubsky Yu. Biological chemistry: textbook / edited by Yu. Gubsky. – Vinnytsia: Nova Knyha, 2018. – 2nd edition. – P. 384–388. 3. Lecture notes.
10.	**The function of grade of hemoglobin oxygenation dependently from partial pressure of oxygen, dissosiation curve of oxyhemoglobin, Bohr effect.**	1. Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 197–200. 2. Gubsky Yu. Biological chemistry: textbook / edited by Yu. Gubsky. – Vinnytsia: Nova Knyha, 2018. – 2nd edition. – P. 383–384. 3. Lecture notes.
11.	**Dependence of biochemical indexes of blood from metabolic processes in organism.**	1. Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 476–477, P.479–483. 2. Lecture notes.

5. Tasks for independent work and self-control

5.1. Indicate the composition of blood plasma and serum. What difference between plasma and serum?

5.2. Fill in the table «Biochemical and physiological functions of blood»

Function	Explanation
Transport function	
Maintenance of acid-base homeostasis	
Immune defense	
Clotting of the blood	

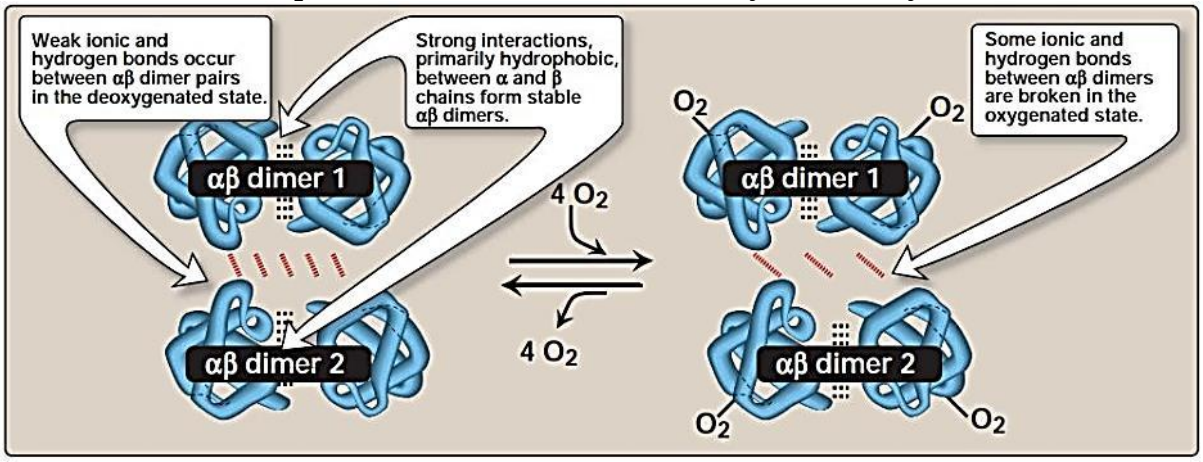
5.3. Explain the mechanisms of maintenance of blood pH

Mechanism	Explanation
Blood buffers: 1. Bicarbonate buffer	
2. Phosphate buffer	
3. Protein buffer	
Respiratory mechanism	
Renal mechanism	

5.4. Disorders of acid-base balance

Disorder	Cause	Clinical causes
Metabolic acidosis	Decrease in bicarbonate	
Respiratory acidosis		
Metabolic alkalosis		
Respiratory alkalosis		

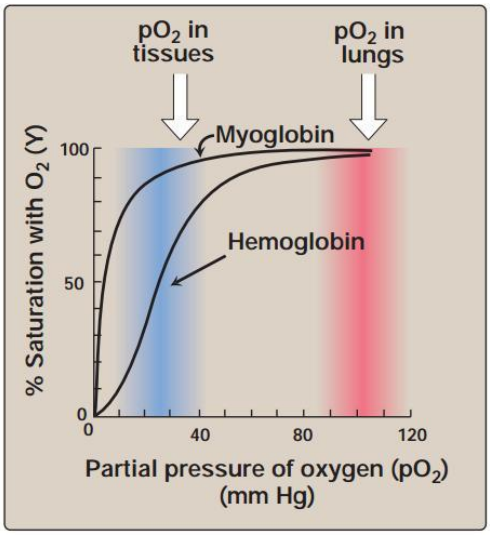
5.5. Which form of Hb (deoxygenated or oxygenated) is referred to as the R form? What determines the equilibrium concentrations of deoxyHb and oxyHb?



5.6. How does the structure of Hb change as O_2 binds to the heme Fe^{2+}?

5.7. What condition, characterized by a «chocolate cyanosis,» results from the oxidation of Fe^{2+} to Fe^{3+} in Hb?

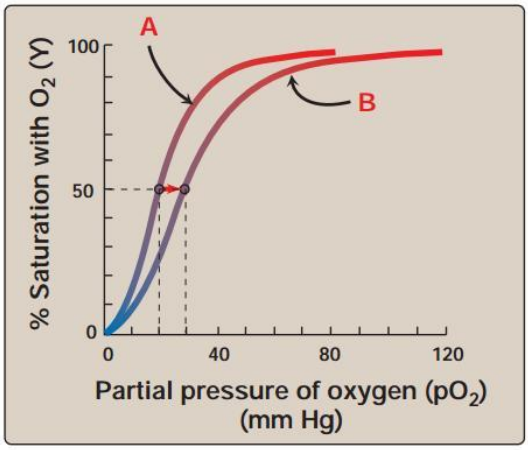
5.8. Use the figure to determine the approximate amount of O_2 that would be delivered by Mb and Hb when the pO_2 in the capillary bed is ~26 mm Hg.



5.9. Why is the O_2 -dissociation curve for Hb sigmoidal and that for Mb hyperbolic?

5.10. How might RBC production be altered to compensate for changes to Hb that result in an abnormally high affinity for O_2?

5.11. Which curve (A or B), as shown, represents the lower pH?



5.12. List two other allosteric effectors that, when increased, result in a rightward shift of the Hb O_2-dissociation curve. What does this shift reflect? Do these allosteric effectors stabilize the R or the T form of Hb?

5.13. How does the binding of CO_2 to Hb stabilize Hb's deoxygenated form?

5.14. What is the Bohr effect?

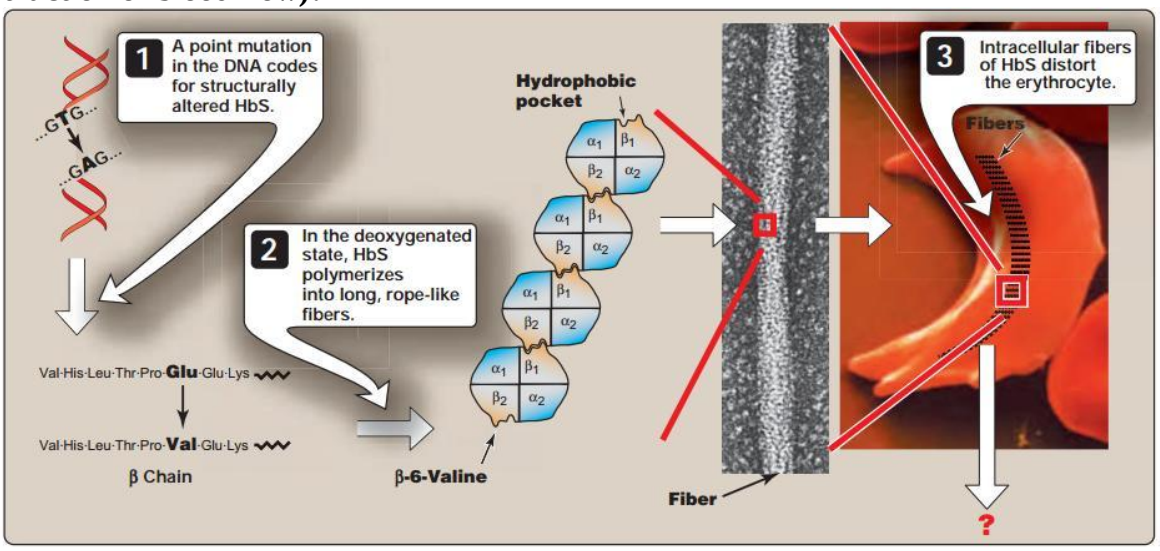
5.15. How does the subunit composition of HbF, as illustrated, influence the O_2 affinity of HbF?

Form	Chain composition
HbA	$\alpha_2\beta_2$
HbF	$\alpha_2\gamma_2$
HbA_2	$\alpha_2\delta_2$
HbA_{1c}	$\alpha_2\beta_2$-glucose

5.16. What form of Hb replaces HbF, and when does this occur?

5.17. What form of Hb is measured to assess glycemic control in individuals with diabetes?

5.18. How do the sickled RBCs illustrated cause infarction (cell/tissue death due to obstruction of blood flow)?



5.19. Which type of globin chain precipitates in β-thalassemia?

5.20. Is HbC disease a sickling or nonsickling disease? Why?

5.21. Situational tasks

a) A woman, age 70 years, activates her medical alert system and is transported to the hospital by ambulance. The patient tells she has a headache, feels weak, and is nauseated and drowsy. She vomited several times at home over the last few hours. She thinks she has the flu. Upon talking with her, a doctor discovered that she has been without power for 2 days due to a recent snowstorm and has been using a kerosene space heater to keep warm. He suspect CO poisoning, send a blood sample to the clinical laboratory for analysis, and begin O_2 therapy.

Why would CO poisoning cause an affected individual to feel weak and drowsy?

b) Severe diarrhoea is one of the most common causes of death in young children. One of the principal effects of diarrhoea is the excretion of large quantities of sodium bicarbonate.

In which direction does the bicarbonate buffer system shift under diarrhoea?

How is the resulting condition called?

6. Individual independent students work

1. Evaluation of condition of blood and its biochemical functions.
2. Disorders of acid-base equilibrium. Acidoses, alkaloses.

Practice protocol №13 «____» _____ 20___

Experiment 1. Quantitative determination of hemoglobin in the blood by hemoglobin cyanide method

Principle. Interaction of hemoglobin with potassium ferricyanide results in its oxidation to methemoglobin.

Procedure.

1. Add 0.02 ml of blood (diluted 25-fold) to 5 ml of transforming solution, mix well.
2. Determination is carried out in 15 minutes against the reagent blank (transforming solution). Determine the absorbance on a spectrophotometer at 540 nm in 1 cm thick cells.
3. Standard solution of hemoglobin is prepared in the same way as a sample of whole blood.
4. Hemoglobin content (B) in g/L is calculated in accordance with the formula:

$B = (D_{test} \times 150)/D_{st}$,

where

D_{test} – the optical density of the test sample,

D_{st} – the optical density of the standard sample,

150 – the hemoglobin content in the standard solution (g/L).

Results:

Conclusion:

Clinical and diagnostic significance. The normal hemoglobin content in the blood of males is 130-160 g/L and it is 120-140 g/L in the blood of females. Determination of hemoglobin is the necessary stage of the diagnosis of various diseases. The increase in blood hemoglobin content may be associated with the following conditions: 1) diseases accompanied by an increase in the level of red blood cells; 2) an increase in the oxygen capacity of blood caused by hypoxia in cardiovascular diseases, living in the highlands, obesity, smoking, and the formation of inactive hemoglobin; 3) dehydration; 4) stress and high physical activity, 5) tumors in the kidney, liver, CNS, ovaries; 6) dysfunction of blood vessels; 7) changes in hormonal levels (the increase in corticosteroids or androgens); 8) chemical poisoning by carbon monoxide, nitrates, or drugs; 9) burns. The decrease in blood hemoglobin level may be due to: 1) bleeding; 2) reduced level of red blood cells in different types of anemia (deficiency of vitamins B_9 and B_{12}, vitamin C, iron deficiency, intoxication or a disturbance of the kidney functioning, impaired maturation of red blood cells). Lower hemoglobin levels may be also caused by a variety of factors: malnutrition, disorders of the gastrointestinal tract; consumption of large quantities of tea, coffee and other products that inhibit iron absorption.

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

Examples of Krock-1 tests

1. Hypoxia been developed in a child during the first three months after birth manifested by dyspnea and cyanosis of the skin. The cause of this state is a violation of the replacement of fetal hemoglobin by:
A. Hemoglobin A
B. Hemoglobin F
C. Glycosylated hemoglobin
D. Methemoglobin
E. Hemoglobin M

2. The consumption of contaminated vegetables and fruit for a long time has led to the nitrates poisoning of a patient and the formation of blood derivative of hemoglobin:
A. Hb-Met
B. HbCO
C. HbO_2
D. HbCN
E. $HbCO_2$

3. A 19-year-old patient complains of general weakness, dizziness and fast fatigue. Hemoglobin of blood is 80 g/L, erythrocytes of a modified form are detected microscopically. What is the most likely cause of the state?
A. Sickle cell anemia
B. Hepatocellular jaundice
C. Porphyria
D. Obstructive jaundice
E. Addison's disease

4. A driver has been delivered to the hospital with symptoms of exhaust gas poisoning after the repair of the car in the garage. The concentration of what hemoglobin derivative is elevated in the blood?
A. Carboxyhemoglobin
B. Methemoglobin
C. Carbhemoglobin
D. Oxyhemoglobin
E. Glycated hemoglobin

5. Hemolysis of erythrocytes is developed after snake bite. The action of toxic snake venom leads to:
A. The formation of lysolecithin
B. Acidosis
C. Polyuria
D. Development of alkalosis
E. The formation of triglycerides

6. The determination of what type of hemoglobin in the blood is used for the diagnosis of diabetes mellitus?
A. Hb A_1C
B. Hb A_1
C. Hb F
D. Hb M
E. Hb S

7. Under the action of oxidizing agents (such as hydrogen peroxide, nitric oxide and etc.) hemoglobin containing Fe^{2+} is converted into a compound containing Fe^{3+} that is not able to carry oxygen. What is this compound called?
A. Methemoglobin
B. Carboxyhemoglobin
C. Carbhemoglobin
D. Oxyhemoglobin
E. Glycated hemoglobin

8. A patient with croupous pneumonia has very fast breathing. Skin is with a cyanotic shade. Blood pH is 7.3; the concentration of carbon dioxide in the blood is increased; acid excess (BE) is equal to – 3.4 mmol/L. What is acid-base disorder in the patient?
A. Respiratory acidosis
B. Metabolic alkalosis
C. Respiratory alkalosis
D. Metabolic acidosis
E. –

9. What is the major type of hemoglobin in adults?
A. Hb A_1
B. Hb S
C. Hb A_2
D. Hb F
E. Hb C

10. Which of the following pH values is characteristic for acidosis?
A. pH – 7.30
B. pH – 7.49
C. pH – 7.36
D. pH – 7.45
E. pH – 7.6

11. A doctor revealed severe complication in a patient with type 1 diabetes mellitus accompanied by violation of the acid-base balance. What metabolites cause the disorder in the patient?
A. Ketone bodies
B. Lactic acid
C. Hexuronic acids
D. Acidic phosphates
E. Citric acid

12. A patient suffering from renal failure has the following changes of acid-base balance: blood pH is 7.31, SB is 16 mmol/L, BE is - 3.4 mmol/L. What type of acid-base imbalance developed in the patient?
A. Metabolic acidosis
B. Respiratory acidosis
C. Metabolic alkalosis
D. Respiratory alkalosis
E. Acid-base balance is not changed

13. What is the most important blood plasma buffer system in the regulation of acid-base homeostasis?
A. Bicarbonate buffer
B. Protein buffer
C. Hemoglobin buffer
D. Phosphate buffer
E. Sulphate buffer

14. What metabolite plays the key role in the development of acidosis under hypoxic conditions?
A. Glutamic acid
B. Fatty acids
C. Succinic acid
D. Ketone bodies
E. Lactic acid

15. What form of acid-base disorder is characteristic for metabolic acidosis developed in a patient under hypoxic condition?
A. Accumulation of organic acids in the body
B. Accumulation of alkalis in the body
C. Violation of CO2 emission
D. Reduction of carbonic acid content
E. Loss of acidic compounds

16. In the lungs carbonic acid (H_2CO_3) is converted to form water and CO_2, which is eliminated in the expired air. Which enzyme catalyzes this reaction?
A. Carbonic anhydrase
B. Catalase
C. Peroxidase
D. Cytochrome oxidase
E. Cytochrome P-450

17. A 2-year old child was admitted to an emergency clinic with signs of poisoning by nitrates such as persistent cyanosis, shortness of breath, convulsions. What is the pathogenetic mechanism of symptoms in this case?
A. Formation of methemoglobin
B. Formation of glycated hemoglobin
C. Formation of carboxyhemoglobin
D. Formation of carbhemoglobin
E. Formation of oxyhemoglobin

18. A patient has decreased blood pH and bicarbonate ions content (decrease in blood alkaline reserve), the high content of lactic and pyruvic acid in the blood and urine. What is the type of acid-base disorder developed in the patient?
A. Metabolic acidosis
B. Respiratory acidosis
C. Metabolic alkalosis
D. Respiratory alkalosis
E. Respiratory alkalosis

19. Carbon monoxide poisoning typically occurs from breathing in too much carbon monoxide. The transport of oxygen by hemoglobin is disturbed from the lungs to the tissues. What is the derivative of hemoglobin formed in this case?
A. Carboxyhemoglobin
B. Oxyhemoglobin
C. Methemoglobin
D. Carbhemoglobin
E. Glycated hemoglobin

20. A worker of the manufacture of nitro compounds came to a doctor with complaints of dyspnea and rapid fatigue. Cyanosis of the lower extremities was revealed during the examination. What is the most probable cause of this state?
A. Enhanced methemoglobin formation
B. Vitamin C deficiency
C. Hypervitaminosis A
D. Fatty liver disease
E. Avitaminosis of B-complex vitamins

21. As a result of improper feeding, severe diarrhoea has been developed in an infant caused loss of bicarbonate. What form of disturbance of acid-base balance occurs in this case?
A. Metabolic acidosis
B. Metabolic alkalosis
C. Respiratory acidosis
D. Respiratory alkalosis
E. There will be no disturbances in the acid-base balance

22. As a result of an exhausting muscular work, the buffer capacity of blood has significantly decreased in a worker. The cause of this condition is the increase of acid in the blood: The accumulation of what acid is the cause of this state?
A. Lactate
B. Pyruvate
C. 1,3-biphosphoglycerate
D. Alpha-ketoglutarate
E. 3-phosphoglycerate

23. Activation of β-oxidation of fatty acids caused ketosis development in a patient suffering from diabetes mellitus. What acid-base disorder may be developed due to the excessive accumulation of ketone bodies in the blood?
A. Metabolic acidosis
B. Metabolic alkalosis
C. There will be no disturbances in the acid-base balance
D. Respiratory acidosis
E. Respiratory alkalosis

24. A patient with severe polytrauma was connected to the artificial respiration apparatus. After repeated studies of the parameters of acid-base balance, a decrease in the content of carbon dioxide in the blood and an increase in its release were established. What is the disturbance of acid-base balance in the patient?
A. Respiratory alkalosis
B. Respiratory acidosis
C. Metabolic alkalosis
D. Metabolic acidosis
E. Acid-base balance is not changed

25. A patient with double pneumonia has the following changes of acid-base balance: pH is 7.32; pCO_2 of blood plasma is 53 mm Hg; SB concentration is 18 mmol/L. What type of acid-base imbalance developed in the patient?
A. Respiratory acidosis
B. Metabolic acidosis
C. Metabolic alkalosis
D. Respiratory alkalosis
E. Acid-base balance is not changed

26. The release of oxygen from hemoglobin is enhanced when the pH is lowered, or when the hemoglobin is in the presence of an increased partial pressure of CO_2. What is the name of this effect?
A. Bohr effect
B. Pasteur effect
C. Warburg effect
D. Danysz effect
E. All-or-none effect

27. What type of the acid-base imbalance is characteristic for the uncompensated diabetes mellitus complicated by ketonemia and ketonuria?
A. Metabolic acidosis
B. Respiratory acidosis
C. Metabolic alkalosis
D. Respiratory alkalosis
E. Acid-base balance is not changed

28. What is a normal pH value of blood?
A. 7.35–7.45
B. 7.30–7.35
C. 7.00–7.30
D. 7.50–7.55
E. 7.60–7.65

Topic 14.
Studies of blood plasma proteins: proteins of the acute phaseof inflammation, lipoproteins, indicator enzymes

1. Objective: To learn about principal proteins of blood plasma and to interpret their quantities in diagnosis of hyper- and hypoproteinemias, dysproteinemias, paraproteinemias, hereditary diseases connected with deficient synthesis of distinct proteins.

2. Actuality of theme: In blood plasma over 100 proteins were described, which differ in their physical, chemical and functional properties. Among them there are transport proteins, enzymes, enzyme inhibitors, hormones, factors of coagulation, antibodies, anticoagulants, antioxidants et al.

Plasma proteins determine properties of plasma: colloid-osmotic pressure and permanent blood volume, viscidity of blood, support of permanent blood pH and function, namely protective, regulatory, termoregulatory, respiratory, trophic etc.

3. Specific aims:

- ✓ To perform fractionation of blood proteins with salting out method.
- ✓ To know about diagnostic significance of quantitative determination of acute phase proteins.
- ✓ To interpret molecular and biological mechanisms of changes in content of secretory and tissue enzymes in blood/
- ✓ To learn spectrophotometric method of determination of middle size molecules.
- ✓ To interpret obtained results and their application in diagnostic purposes.

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

	Questions:	References:
1.	**Principal groups of blood proteins, their composition and content in normal conditions and in pathology.**	1. Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 182–190. 2. Lecture notes.
2.	**Albumins and globulins. Resolution of blood plasma proteins by method of protein electrophoresis.**	1. Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 183–185. 2. Lecture notes.
3.	**Proteins of acute phase of inflammation: C-reactive protein (CRP), ceruloplasmin, haptoglobin, cryoglobulin, alpha-1 antitrypsin, alpha-2 macroglobulin, interferon, fibronectin, their diagnostic validity.**	1. Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 105, 186, 417–418. 2. Lecture notes.
4.	**Enzymes of blood plasma: genuine (secretory), excretory, indicatory (tissue) enzymes. Kallikrein-kinine and renin-angiotensin systems, their biological significance.**	1. Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 112–113, 472. 2. Lecture notes.
5.	**Diagnostic value of investigation of enzyme and isoenzyme activity in blood plasma: creatine kinase (CK), LDH, AST, ALT, amylase, lipase, cholinesterase.**	1. Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 112–113, 455–456. 2. Lecture notes.

5. Tasks for independent work and self-control

5.1. Fill in the table «Important plasma proteins and their major functions»

Protein	Major functions
Albumin	
α_1-Globulins	
α_1-Antitrypsin	
α_1-Lipoproteins (HDL)	
Retinol binding protein (RBP)	
Thyroxine binding globulin (TBG)	
Transcortin or cortisol binding protein (CBG)	
α_2-Globulins	
α_2-Macroglobulin	
Haptoglobins	
Prothrombin	
Ceruloplasmin	
β-Globulins	
β-Lipoproteins (LDL)	
Transferrin	
Hemopexin	
Plasminogen	
γ-Globulins	
Fibrinogen	
C-reactive protein (CRP)	

5.2. The albumin concentration of plasma is 40 to 50 g/l while that of total globulins is 20 to 40 g/l. The normal albumin/globulin (A/G) ratio is 1.5-2.0. The A/G ratio is lowered either due to decrease in albumin or increase in globulins, as found in the following conditions.

Condition	Cause
Decreased synthesis of albumin by liver	
Excretion of albumin into urine	
Increased production of globulins	

5.3. What changes of blood proteinogram will occur in the following disorders?

Liver failure

Acute rheumatic fever

Multiple myeloma

Renal insufficiency with nephrotic syndrome

5.4. Most enzymes measured in plasma release from cells as a result of damage to cell membrane. They have not a physiological function in plasma. Indicate the causes of pathological increases of activity in plasma of enzymes:

Mark («+») in the table which enzymes will be elevated in mentioned disorders.

Name of the enzyme	Myocardial infarction	Acute pancreatitis	Liver cirrhosis	Myopathies
Creatine kinase (CK) or creatine phosphokinase (CPK) isoenzymes				
Lactate dehydrogenase isoenzymes				
Alanine transaminase (ALT)				
Aspartate transaminse (AST)				
Amylase				
γ-Glutamyl transpeptidase (GGT)				
Cholinesterase				
Lipase				

5.5. Situational tasks

a) The results of biochemical analysis of women: ceruloplasmin concentration – 50 mmol/L (normal range 200-450 mmol/L [20-45 mg/dL]), serum copper – 8 μmol/L (normal range 13-19 μmol/L [80-120 μg/dL]), urinary excretion of copper – 2.2 mmol/ 24 h (normal range 2-3.9 μmol/24 h [13-25 μg/dL]), and a liver biopsy established the diagnosis of Wilson's disease.

What genetic defect results in Wilson's disease?

Explain why excessive amounts of copper accumulate in liver and brain that lead to their damage.

Indicate the treatment of Wilson's disease.

b) A 44-year-old woman was admitted to hospital because of weakness, anorexia, recurrent infections, bilateral leg edema, and breathlessness. Her plasma albumin concentration was 19 g/L and her urinary protein excretion 10 g/24 h (normal value <0.15 g/24 h). There was microscopic haematuria. Renal biopsy confirmed the diagnosis as glomerulonephritis.

Indicate the change of plasma albumin level.

Explain the mechanisms of the development of proteinuria and edema?

c) The patient have hemorrhagic stroke. In the blood found increased concentration of kinins. The doctor assigned to the patient kontrikal (protease inhibitor).

For inhibition of which proteases was made this appointment?

From what blood protein produced kinin?

Kinin. What is their biological role, give examples?

6. Individual independent students work

1. Biochemical bases of functioning of systems of regulation of blood pressure.
2. The use of antihypertensive drugs - angiotensin-converting enzyme inhibitors.

Practice protocol №14 «____» _____ 20___

Experiment 1. Fractionation of blood plasma proteins using a method of salting out: determination of albumins and globulins in blood plasma.

Principle. Proteins can be separated from the solution in the form of precipitates. There are many different ways to precipitate proteins. Precipitation of proteins using concentrated solutions of neutral salts (ammonium sulfate, sodium chloride, etc.) is called salting out. Most reactions of the salting out and reactions with alcohol, acetone are reversible, because protein precipitate can be dissolved again by diluting it with water or the concentration of salts may be reduced using dialysis.

Procedure.

Place 2.0 – 3.0 ml of blood serum to a test tube.

Add equal volume of saturated ammonia sulphate solution and mix well.

Observe the precipitation of globulins (50 % of the solution saturation).

Filtrate the precipitation.

Add some water to the precipitate, which remained on filtrating paper.

Boil obtained solution and observe the appearance of albumins precipitate.

Filtrate albumins precipitate, add some water and obtained filtrate divide into 2 tubes.

To the first tube add crystal ammonium sulphate till the complete saturation. Observe albumins precipitate.

Boil the content of the second tube, observe the appearance of albumins precipitate.

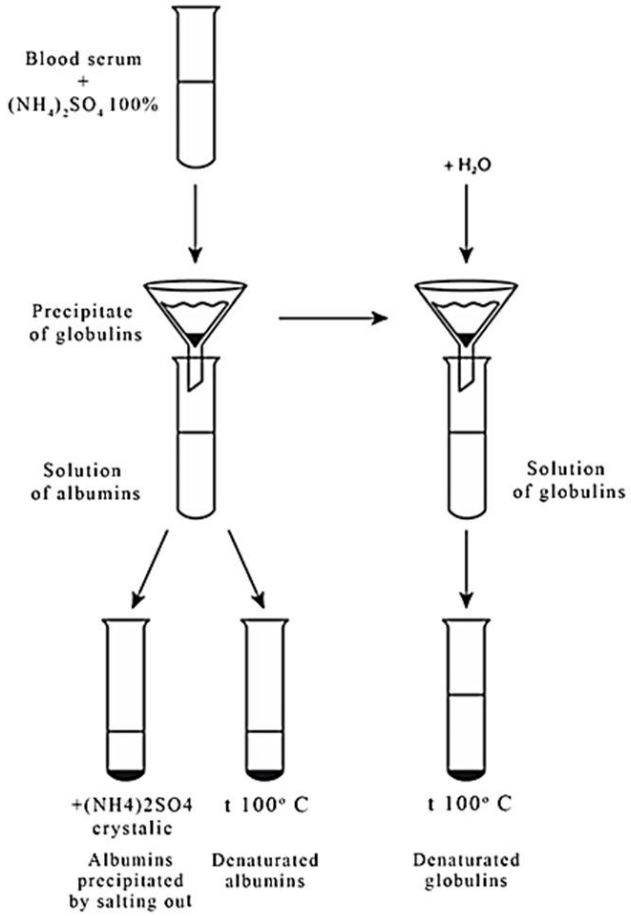


Fig. Scheme of the separation of albumins and globulins of blood using the method of salting out

Fraction separation of blood serum proteins

Name of the protein	Used salt	Level of saturation	Appearance of precipitate
Globulins			
Albumins			

Conclusion:

Clinical and diagnostic significance. Identification of certain fractions of serum proteins is important for clinical diagnosis. Normally blood albumin content is 40-45 g/l, globulin – 20-30 g/l. In various diseases of the liver (cirrhosis, hepatitis), in nephrosis, chronic diseases of the stomach, digestive tract tumors of blood, albumin concentration decreases. In acute infectious diseases, rheumatism concentration of α_2-globulins is increased. The concentration of β-globulins increases in hepatitis, multiple myeloma, and γ –globulins in chronic diseases, chronic polyarthritis.

Fractionation of proteins using a method of salting out is used in clinical practice for obtaining native proteins from biological fluids.

Experiment 2. Colloidal probes for blood protein condition (Weltman's test).

Principle. The method is based on the empirical observation that after addition of $CaCl_2$ solution to blood serum and subsequent heating, impairment of colloidal stability of serum proteins occurs and proteins are precipitated. The value of $CaCl_2$ concentration at which precipitation takes place is very important for the performance of this investigation.

Procedure.

Take 0.1 ml of blood serum and add 4.9 ml of distilled water to it, mix well.

Add 0.1 ml of 0.5 % $CaCl_2$, stir and carefully heat on a flame up to boiling.

Cool the tube is cooled and examine the appearance of obtained precipitate.

If no precipitate is found, additionally add 0.1 ml of $CaCl_2$ solution to the tested probe and boil again.

Repeat all the procedure until the appearance of visible precipitate.

The volume (in ml) of 0.5 % $CaCl_2$ used for the reaction of precipitation corresponds to the level of protein in plasma. Normally coagulation begins after addition of 0.4-0.5 ml of $CaCl_2$ solution.

Results:

Conclusion:

Clinical significance. The level of total protein in blood in healthy adults makes 65-85 g/l. The decrease of the level of total protein below 65 g/l is called hypoproteinemia, whereas increase of its content above 85 g/l is called hyperproteinemia. As well as its fractions is an important laboratory index commonly used in diagnosis of numerous diseases. Decrease of the protein content in blood (hypoproteinemia) occurs in kidney diseases, accompanied by nephritic syndrome, starvation, in patients after chemotherapy, irradiation, in malignancies, liver insufficiency. The increase of the level of total protein in blood occurs mainly in paraproteinemias, f.e. myeloma disease.

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

Examples of Krock-1 tests

1. Paper electrophoresis is widely used in clinical practice to separate blood plasma proteins into fractions. What are they?
A. Albumins, α_1-globulins, α_2-globulins, β-globulins, γ-globulins
B. Albumins, fibrinogen, α_2-globulins, β-globulins, γ-globulins
C. Albumins, α_2-macroglobulins, β-globulins, γ-globulins
D. Albumins, haptoglobin, α_1-globulins, α_2-globulins, γ-globulins
E. Albumins, immunoglobulins, transferrin, β-globulins, γ-globulins

2. Bradykinin is a mediator of inflammation. What are the symptoms of inflammation caused by the excessive formation of bradykinin?
A. Vasodilation, redness and pain
B. Decrease in blood pressure, pain, vasoconstriction
C. Inhibition of inflammation, increasing of blood pressure and vascular permeability
D. Activation of proteinase inhibitors, pain
E. Increase in blood pressure, pain, decreased vascular permeability

3. What are the main physiological functions of albumins?
A. They maintain blood oncotic pressure and carry out the transport function
B. They perform protective function
C. They inhibit proteinases and regulate vascular tension
D. They provide a non-specific resistance of the organism
E. They provide antioxidant defence of the body

4. A patient has been diagnosed with myocardial infarction. What is the most sensitive enzyme signalling of the cardiomyocytes damage in 2 hours after the onset of chest pain?
A. Creatine kinase-MB
B. Creatine kinase-BB
C. Aspartate aminotransferase
D. Lipase
E. Gamma-glutamyltransferase

5. What acute-phase protein performs a major role in the inhibition of proteolytic enzymes under the condition of the inflammatory process development?
A. α_1-antitrypsin
B. α_2-macroglobulin
C. Fibronectin
D. Ceruloplasmin
E. Haptoglobin

6. Estimate the indexes of blood plasma in the patient suffering from chronic renal failure, if the total protein content is 48 g/L and albumins are 28 g/L:
A. Hypoproteinemia
B. Dysproteinemia
C. Hyperproteinemia
D. Increased blood oncotic pressure
E. Paraproteinemia

7. Which component of the kallikrein-kinin system is the most strong vasodilator?
A. Bradykinin
B. Kallidin
C. Kallikrein
D. Prekallikrein
E. Lysine

8. What proteins of blood plasma are antibodies?
A. Immunoglobulins
B. Cryoglobulins
C. C-reactive protein
D. Kinins
E. Haptoglobin

9. The activity of which organ-specific enzyme is investigated for the diagnostics of acute pancreatitis?
A. Alpha-amylase
B. Alanine aminotransferase
C. Aspartate aminotransferase
D. Creatine phosphokinase
E. Collagenase

10. Level of C-reactive protein is increased in the blood of a patient. What is the diagnostic value of this indicator?
A. Level of the inflammatory process
B. Assessment of renal function
C. Diagnostics of immune deficiency
D. Diagnostics of hyperproteinemia
E. Diagnostics of paraproteinemia

11. What is a normal value of albumin/globulin ratio?
A. 1.5 – 2.0
B. 1.5 – 3.0
C. 10.0 – 15.0
D. 8.0 – 10.0
E. –

12. What is the most likely cause of severe hypoproteinemia?
A. Diseases of the liver
B. Diseases of the connective tissue
C. Mild infections
D. Hypothyroidism
E. Excessive protein intake

13. Estimate the indexes of blood plasma: total protein is 47 g/L and albumins are 30 g/L:
A. Total protein and albumins are decreased
B. Total protein and albumins are increased
C. Normal values of blood plasma indexes
D. Excess of dietary proteins
E. Dysproteinemia

14. Laboratory investigation of patient's blood revealed paraprotein and Bence-Jones protein in the patient's urine. What disease is characterized by these changes?
A. Multiple myeloma
B. Acute pyelonephritis
C. Chronic nephritis
D. Liver failure
E. Hypoxia

15. How does the protein ratio change in liver disease?
A. It decreases
B. It increases
C. It does not change
D. It exceeds the index of 1.5-2.0
E. -

16. What is the biological role of transferrin and ceruloplasmin?
A. Transport Fe^{2+} and Cu^{2+}
B. Transport of K^{+} and H^{+}
C. Transport of Ca^{2+}
D. Antibacterial action
E. Factors of immune resistance

17. Laboratory investigation of a patient's blood revealed that albumin content is moderately decreased, β-globulins are increased, γ-globulins are significantly increased. What group of diseases is this proteinogram characteristic for?
A. Chronic inflammation
B. Acute inflammation
C. Renal failure
D. Liver failure
E. Multiple myeloma

18. A 5-year-old child with signs of durative protein starvation has been admitted to the hospital. The signs were as follows: growth inhibition, anemia, edema, mental deficiency. What is the most likely cause of edema development?
A. Reduced synthesis of albumins
B. Reduced synthesis of globulins
C. Reduced synthesis of hemoglobin
D. Reduced synthesis of lipoproteins
E. Reduced synthesis of glycoproteins

19. A patient is diagnosed with the liver failure. Edemas are observed. What is the underlying mechanism of their formation?
A. The violation of albumin synthesis in hepatocytes, reduced blood oncotic pressure, the transition of water from vessels to the tissues and the development of oncotic edema
B. Increased blood oncotic pressure, the transition of water from vessels to the tissues and the development of oncotic edema
C. Reduced albumin synthesis in hepatocytes increases oncotic pressure and causes oncotic edema development
D. Violation of globulin synthesis in hepatocytes increases the flow of water into the tissues and causes the development of edema
E. Activation of albumin synthesis in hepatocytes

20. What are the acute phase proteins referring to the components of the system of nonspecific resistance of the organism?
A. C-reactive protein, α_1-antitrypsin, α_2-macroglobulin, cryoglobulin, proteins of the complement system, haptoglobin and ceruloplasmin
B. Albumins, complement system proteins, ceruloplasmin
C. Albumins, lipoproteins, proteins of the complement system and transferrin
D. Fibrinogen, C-reactive protein, aminoacids and proteins of the complement system
E. Albumin, phospholipids, cryoglobulin, haptoglobin

21. What enzymes are the most informative for the diagnosis of myocardial infarction?
A. Creatine kinase, aspartate aminotransferase, lactate dehydrogenase
B. Glutamate dehydrogenase, aspartate aminotransferase, glucokinase
C. Glucose-6-phosphate dehydrogenase, aspartate aminotransferase
D. Alpha-amylase, creatine kinase
E. Lipase, α-amylase, aspartate aminotransferase

Topic 15.
Research of nitrogen metabolism. End products: urea, uric acid, creatine, creatinine, amino acids

1. Objective: To know about nonprotein nitrogen containing blood compounds, which represent residual nitrogen, to learn methods of determination of residual nitrogen and middle size molecules in blood and to interpret obtained results in diagnosis of diseases.

2. Actuality of theme: Beside proteins in blood there are nitrogenous compounds of nonprotein character: urea, uric acid, creatinine, ammonium ion, indican, bilirubin, amino acids, peptides, creatine. Exept for the last three, they are end products of nitrogen metabolism. Nitrogen of the above-named substances is called «residual» as it is determined after sedimentation of proteins in a protein-free filtrate of blood.

There are two types of azotemias: retention and production azotemia. The retention azotemia results from infringement of nitrogen-exretion function of kidneys as well as from cardiovascular insufficiency. The production azotemia develops at hepatic insufficiency, at increased disintegration of proteins in the organism (malignant tumours, tuberculosis), etc.

Rest nitrogen is the important indicator of the state of protein metabolism. The detection of nonproteinous nitrogen and its separate components is conducted with the purpose of diagnosis of disorders of kidneys and liver functions, endogenous intoxication in pathology and estimation of kidney insufficiency degree.

3. Specific aims:

- ✓ To characterize the principal nonprotein nitrogen-containing compounds, their metabolic origin.
- ✓ To determine rest nitrogen in blood samples.
- ✓ To learn spectrophotometric method of determination of middle size molecules.
- ✓ To interpret obtained results and their application in diagnostic purposes.

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

Questions:	References:
1. **Definition of total and rest (or residual) nitrogen (RN) in blood. Non-protein nitrogen containing compounds of blood, their diagnostic significance:** ✓ Ammonia; ✓ Urea; ✓ Creatinine; ✓ Uric acid.	1. Popova L.D. Biochemistry: Manual for medical students or interns / L.D. Popova, A.V. Polikarpova – Kharkiv: KNMU, 2011. – p. 353–354. 2. Lecture notes.
2. **Nitrogen free organic and inorganic compounds of blood, their metabolic origin. Middle size molecules, their metabolic origin and diagnostic significance.**	1. Popova L.D. Biochemistry: Manual for medical students or interns / L.D. Popova, A.V. Polikarpova – Kharkiv: KNMU, 2011. – p. 354–355. 2. Lecture notes.
3. **Nitrogenemia, its kinds and causes of development, differentiation in clinical conditions.**	1. Popova L.D. Biochemistry: Manual for medical students or interns / L.D. Popova, A.V. Polikarpova – Kharkiv: KNMU, 2011. – p. 354. 2. Lecture notes.

5. Tasks for independent work and self-control

5.1. Fill in the blanks:

Total nitrogen of the blood includes _____

Residual (rest) nitrogen of the blood includes:

5.2. Fill in the table «Diagnostic significance non-protein nitrogen containing compounds of blood»

Compound	Concentration	Diseases	
		↑ level	↓ level
Ammonia			
Urea			
Creatinine			
Uric acid			

5.3. Match the nitrogen free organic compounds of blood with their concentration

1. Glucose
2. Lactate
3. Total lipids (whole blood)
4. Total lipids (plasma)
5. Free fatty acids (plasma)
6. Phospholipids
7. Cholesterol (total content)
8. Ketone bodies

A. 3.63-6.48 mmol/L
B. 3.8-6.7 g/L
C. 3.3-5.5 mmol/L
D. 0.1-0.6 mmol/L
E. 0.33-0.78 μmol/L
F. 400-800 mmol/L
G. 1.0-7.2 g/L
H. 2.2-4.0 g/L

5.4. Explain the diagnostic significance of ketone bodies and cholesterol.

5.5. Specify the causes of a certain states.

State	Causes
Hypernatriemia	
Hyponatriemia	
Hyperkalemia	
Hypokalemia	
Hypercalcemia	
Hypocalcemia	

5.6. Describe the kinds of nitrogenemia and causes of development.

Nitrogenemia	Description
I. Productive	
II. Retention	
1. Renal	
2. Extrarenal	

6. Individual independent students work

Investigtion of nitrogen metabolism and changes in the content of nitrogen-containing non-protein components of the blood.

Practice protocol №15 «____» _____ 20___

Experiment 1. Determination of rest nitrogen of blood (Bodansky's method).

Principle. The rest nitrogen of blood is determined in protein free supernatant or filtrate of blood after precipitation and elimination of proteins. Protein free filtrate is mineralized by sulfuric acid. In this case nitrogen of organic compounds is transformed into ammonia and is bound by sulfuric acid. Ammonium ion is determined with Nessler's reagent, which produces yellow-brown colored complex, and the intensity of color is proportional to ammonium ion concentration.

Procedure.

Pour 1.8 ml of water into a centrifuge tube and add 0.2 ml of blood, 0.3 ml of 10 % trichloroacetic acid solution and 0.2 ml 0.5 N sulphuric acid. Mix the content of the test tube with a glass rod, set the test tube aside for 20 min to provide precipitation of blood proteins. Then centrifuge for 10 min at 3,000 rpm. Transfer the supernatant liquid into a clean test tube. Pour 1 ml of protein-free centrifugate into another test tube, add 3 drops of concentrated sulphuric acid to it and 2 drops of 30 % hydrogen peroxide solution. Put the test tube for about 30 minutes on a sand bath for mineralization (up to full decoloration of solution).

All these manipulations hold using water instead blood for control sample.

Reagent	Test tube	Standard tube	Control tube
Blood mineralizate	0.5 ml	-	-
Standard solution of ammonia sulphate	-	0.5 ml	-
Distillate water (after mineralization)	-	-	0.5 ml
Distillate water	10 ml	10 ml	10 ml
50 % NaOH	6 drops	6 drops	6 drops
Nessler's reagent	0.5 ml	0.5 ml	0.5 ml
E =			

Measure the optical density of both probes in a colorimeter at blue light filter (590 nm) in 5 mm cuvette.

Determine the quantity of rest nitrogen in blood according to the following formula:

$C_{test} = C_{st} \times (E_{test} / E_{st}) = 21{,}42 \times E_{test} / E_{st}$ mmol/L,

where

C_{test} – concentration of nitrogen in test sample,

C_{st} – concentration of nitrogen in standard (0.2g/L),

E_{test} – extinction of tested sample,

E_{st} – extinction of standard sample.

Results:

Conclusion:

Diagnostic significance. In healthy person the content of rest nitrogen in blood corresponds to 14.3-25.0 mmol/L (20-35 mg %; 0.2-0.4 g/L). The increased level of residual nitrogen in blood is called **nitrogenemia**. Increase of rest nitrogen is observed in glomerulonephritis, pyelonephritis, tuberculosis, amyloidosis of kidneys. Extrarenal causes of nitrogenemia include disorders of hemodynamics and, subsequently, decrease in glomerular filtration in kidneys. An increase in rest nitrogen is observed also during the intensification of protein breakdown in malignant tumors, leukemias.

The report is checked up _____

(The signature of the teacher, date)

Examples of Krock-1 tests

1. The biochemical analysis of the blood is as follows: residual nitrogen – 48 mmol/L, urea – 15.3 mmol/L. Which organ is affected in this case?

A. Kidneys
B. Brain
C. Liver
D. Intestine
E. Spleen

2. The content of which component of the residual nitrogen is the greatest one and sharply increases under conditions of renal failure?

A. Urea
B. Peptides
C. Uric acid
D. Indicane
E. Bilirubin

3. What components of residual nitrogen fraction predominate in the blood under the conditions of productive azotemia?

A. Amino acids, nitrogen of ammonia
B. Lipids, carbohydrates
C. Ketone bodies, proteins
D. Porphyrins, bilirubin
E. Uric acid, choline

4. The process of urea synthesis has been disrupted in a patient. The pathology of which organ caused a disorder?

A. Liver
B. Kidneys
C. Brain
D. Muscles
E. Gall bladder

5. What components of residual nitrogen fraction predominate in the blood under the conditions of retention azotemia?
A. Urea, creatinine
B. Lipids, carbohydrates
C. Amino acids, urea
D. Porphyrin, bilirubin
E. Uric acid, choline

6. Which component of residual nitrogen is the most stable and clearly reflects the severity of renal failure?
A. Creatinine
B. Indicane
C. Urea
D. Uric acid
E. Creatine

7. After a course of treatment, the general condition and the biochemical parameters of urine of a patient suffering from chronic renal failure have been improved. Which biochemical indicator is the most informative for such conclusion?
A. Creatinine
B. Uric acid
C. Bilirubin
D. Sodium
E. Protein

8. The main part of nitrogen is excreted in the form of urea in humans. The decreased activity of what enzyme in the liver leads to inhibition of urea synthesis and the accumulation of ammonia in the blood and the tissues?
A. Carbamoyl phosphate synthetase
B. Aspartate aminotransferase
C. Urease
D. Amylase
E. Pepsin

9. Ammonia is a very toxic substance, especially for the nervous system. What substance is actively involved in the detoxification of ammonia in the brain tissue?
A. Glutamic acid
B. Lysine
C. Proline
D. Histidine
E. Alanine

10. A patient with extensive burns complains of vomiting and strong weakness. The residual nitrogen is 55 mmol/L. What type of azotemia is the most likely be suspected in the patient?
A. Productive azotemia
B. Latent azotemia
C. Retention decompensated azotemia
D. Retention subcompensated azotemia
E. Retention compensated azotemia

11. What is a normal value of urea in the blood?
A. 3.3 - 8.3 mmol/L
B. 3.0 - 4.0 mmol/l
C. 8.4 - 12.2 mmol/L
D. 10.0 - 13.0 mmol/L
E. More than 10.0 mmol/L

12. What is the daily creatinine excretion in the urine?
A. 1 - 2 g
B. 2 - 3 g
C. 3 - 4 g
D. 0,5 - 1 g
E. Over than 4.0 g

13. A high content of indican has been revealed in the patient's blood and urine that is an indicator of the activation of the putrefaction of proteins in the intestine. Which amino acid is the source of the indicane?
A. Tryptophan
B. Tyrosine
C. Proline
D. Arginine
E. Phenylalanine

14. A patient with signs of gout has been admitted to a hospital. What biochemical analysis should be used to clarify the diagnosis?
A. Determination of uric acid in the blood and urine
B. Determination of urea in the blood and urine
C. Determination of creatinine in the blood and urine
D. Determination of indican in the blood
E. Determination of amino acids in the blood

15. Brain injury caused increased production of ammonia. What amino acid is involved in the process of the temporary neutralization of ammonia?
A. Glutamic acid
B. Tyrosine
C. Valine
D. Tryptophan
E. Lysine

16. A decrease in blood residual (rest) nitrogen level was revealed in a patient with liver insufficiency. The diminished blood non-protein nitrogen was due to:
A. Urea
B. Ammonium
C. Amino acids
D. Bilirubin
E. Uric acid

17. Which level of residual nitrogen is normal for adults?
A. 14,3-25 mmol/L
B. 25-38 mmol/lL
C. 42,8-71,4 mmol/lL
D. 70-90 mmol/L
E. -

18. Which type of asotemia is observed as a result of dehydration?
A. Relative asotemia
B. Absolute asotemia
C. Retention asotemia
D. Extrarenal asotemia
E. Productive asotemia

19. A patient complains of vomiting, general weakness. Residual (rest) nitrogen in blood is 35 mmol/L, renal function is not affected. Which type of azotemia is developed?
A. Productive azotemia
B. Renal azotemia
C. Retention azotemia
D. Relative azotemia
E. -

20. Blood analysis revealed azotemia. The percentage of urea nitrogen in the (rest) residual blood nitrogen is significantly reduced. Which organ is affected?
A. Liver
B. Stomach
C. Kidney
D. Intestine
E. Heart

21. A patient suffering from chronic renal failure has an increase in the level of residual (rest) nitrogen to 35 mmol/L. More than half of its is urea. This type of azotemia is called:
A. Retention
B. Productive
C. Hepatic
D. Residual
E. Mixed

22. Rest (residual) nitrogen and urea were determined in the patient's blood. The proportion of urea in the residual nitrogen is significantly reduced. Which organ is affected?
A. Liver
B. Stomach
C. Kidney
D. Intestine
E. Heart

23. A 49-year-old patient complains of a sharp pain in small joints of the lower extremities. Objectively: local edema and fever. It was revealed an elevated level of C-reactive protein and α1-proteinase inhibitor in the blood. What biochemical blood test is required to clarify the diagnosis?
A. Determination of uric acid
B. Determination of urea
C. Determination of creatinine
D. Determination of creatine
E. Determination of hexuronic acids

24. The following biochemical parameters in the blood were revealed in a patient suffering from chronic glomerulonephritis for 5 years: urea concentration – 49 mmol/L, creatinine – 0.68 mmol/L, potassium – 6.1 mmol L, glucose – 3.2 mmol/L. There is a smell of ammonia from the oral cavity. The arterial pressure is 215/115 mm Hg. What pathology can the most probably be suspected in this case?
A. Chronic renal failure
B. Hyperammonemia
C. Hyperkalemia
D. Hypertension
E. Hypoglycemic coma

25. Which components of blood residual (rest) nitrogen fraction prevail in productive azotemia?
A. Amino acids, urea
B. Lipids, carbohydrates
C. Ketone bodies
D. Porphyrins, bilirubin
E. Glucose, lactate

Topic 16.
Research of coagulation and fibrinolytic blood systems

1. Objective: To learn the role of components of coagulation, anticoagulation and fibrinolytic systems of blood in provident of physiological state of blood as well as their role in development of atherosclerosis, hypertonic disease.

2. Actuality of theme: Blood coagulation is a complicated physiological and biochemical process, being the protective reaction of human body towards bleeding. Knowledge of biochemistry of coagulation, anticoagulation and fibrinolytic systems of blood is necessary for understanding the mechanisms of maintenance of the liquid state of blood in health and in numerous diseases, and also for their timely pharmacological correction.

3. Specific aims:

- ✓ To interpret biochemical principles of functioning of coagulation, anticoagulation and fibrinolytic systems of blood.
- ✓ To learn methods of investigation of coagulation and fibrinolytic systems of blood, to interpret obtained results.
- ✓ To know the role of components of coagulation, anticoagulation and fibrinolytic systems in development of intravascular disseminated coagulation syndrome, atherosclerosis and hypertonic disease.

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

Questions:	References:
1. **System of hemostasis, process and stages of hemostasis.**	1. Popova L.D. Biochemistry: Manual for medical students or interns / L.D. Popova, A.V. Polikarpova – Kharkiv: KNMU, 2011. – P. 375–376. 2. Lecture notes.
2. **Functional and biochemical characteristics of intrinsic and extrinsic blood coagulation pathways.**	1. Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 190–191. 2. Popova L.D. Biochemistry: Manual for medical students or interns / L.D. Popova, A.V. Polikarpova – Kharkiv: KNMU, 2011. – P. 382. 3. Gubsky Yu. Biological chemistry: textbook / edited by Yu. Gubsky. – Vinnytsia: Nova Knyha, 2018. – 2nd edition. – P. 391. 4. Lecture notes.
3. **Blood coagulation system; characteristics of coagulation factors. Cascade mechanism of activation and function of blood coagulation; intrinsic and extrinsic blood coagulation pathways. Role of vitamin K in reactions of hemocoagulation (carboxylation of glutamic acid residues, its role in Ca binding). Medical preparations as vitamin K agonists and antagonists.**	1. Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 190–193. 2. Popova L.D. Biochemistry: Manual for medical students or interns / L.D. Popova, A.V. Polikarpova – Kharkiv: KNMU, 2011. – P. 376–385. 3. Gubsky Yu. Biological chemistry: textbook / edited by Yu. Gubsky. – Vinnytsia: Nova Knyha, 2018. – 2nd edition. – P. 391–395. 4. Lecture notes.
4. **Hereditary disorders of hemocoagulation.**	1. Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 193. 2. Popova L.D. Biochemistry: Manual for medical students or interns / L.D. Popova, A.V. Polikarpova – Kharkiv: KNMU, 2011. – P. 385–386. 3. Gubsky Yu. Biological chemistry: textbook / edited by Yu. Gubsky. – Vinnytsia: Nova Knyha, 2018. – 2nd edition. – P. 395–397. 4. Lecture notes.

5.	**Anticoagulation system of blood, functional characteristics of its components – heparin, antithrombin III, citric acid, prostacycline. Role of vascular endothelium. Changes in biochemical characteristics of blood in prolong treatment with heparin.**	1. Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 191–193. 2. Popova L.D. Biochemistry: Manual for medical students or interns / L.D. Popova, A.V. Polikarpova – Kharkiv: KNMU, 2011. – P. 386–387. 3. Lecture notes.
6.	**Fibrinolytic system of blood: stages and factors of fibrinolysis. Pharmacological modulation of fibrinolytic process. Activators and inhibitors of plasmin.**	1. Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 192. 2. Popova L.D. Biochemistry: Manual for medical students or interns / L.D. Popova, A.V. Polikarpova – Kharkiv: KNMU, 2011. – P. 387–389. 3. Lecture notes.

5. Tasks for independent work and self-control

5.1. What is role of system of hemostasis?

5.2. System of hemostasis includes:

1. _____
2. _____
3. _____

5.3. Describe the stages of hemostasis?

5.4. How many types of thrombi are there? Describe them.

5.5. Compare the intrinsic and the extrinsic blood coagulation pathways.

a) Features of the intrinsic blood coagulation pathway:

b) Features of the extrinsic blood coagulation pathway:

5.6. List the participants of blood clotting:

1)

2)

3)

4)

5)

6)

7)

5.7. Properties of endothelium of blood vessel:

Anticlotting	**Procoagulant**

5.8. Explain the considerable role of platelets in process of coagulation. List three processes in the formation of a hemostatic plug.

5.9. List the platelet factors of blood clotting:

1 –

2 –

3 –

4 –

5 –

6 –

7 –

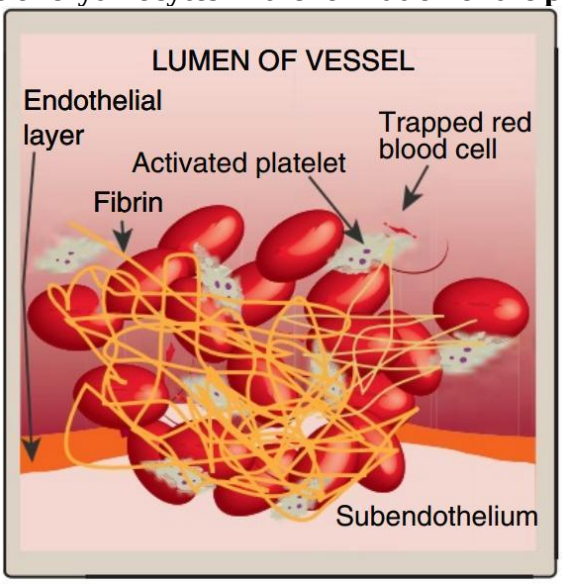
8 –

9 –

10 –

11 –

5.10. Explain the role of erythrocytes in the formation of the primary platelet plug.



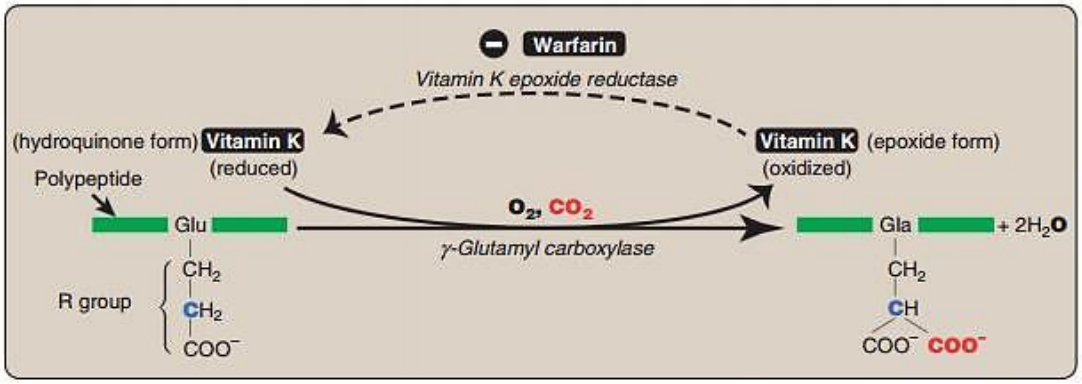
5.11. Explain the role of leukocytes in blood coagulation.

5.12. Blood clotting or coagulation is the body's major defense mechanism against blood loss. A blood clot is formed as a result of a series of reactions involving nearly 20 different substances, most of them being glycoproteins, synthesized by the liver.

Fill in the table «Factors of blood plasma»

Factor	Name	Features
I		
-	High-molecular-weight kininogen (Fitzgerald factor)	
-	Prekallikrein (Flethcher factor)	
-	Protein C	
-	Protein S	
-	Thrombomodulin	
-	Von Willebrand factor	

5.13. Which is role of vitamine K in blood clotting?



5.14. It is known that in the microcirculatory vessels with low blood pressure hemostasis is provided by the vascular-thrombocytic reactions. But in large vessels hemostasis can be achieved by the formation of fibrin thrombus. Explain why?

5.15. Coagulation hemostasis is the cascade of enzymatic reactions, which results in the polymerization of fibrinogen with formation of fibrin thrombus, as shown ((the active forms of the factors are represented in red with subscript 'a'). It involves three phases. Characterize them.

Phase I:

A) The extrinsic pathway:

B) The intrinsic pathway:

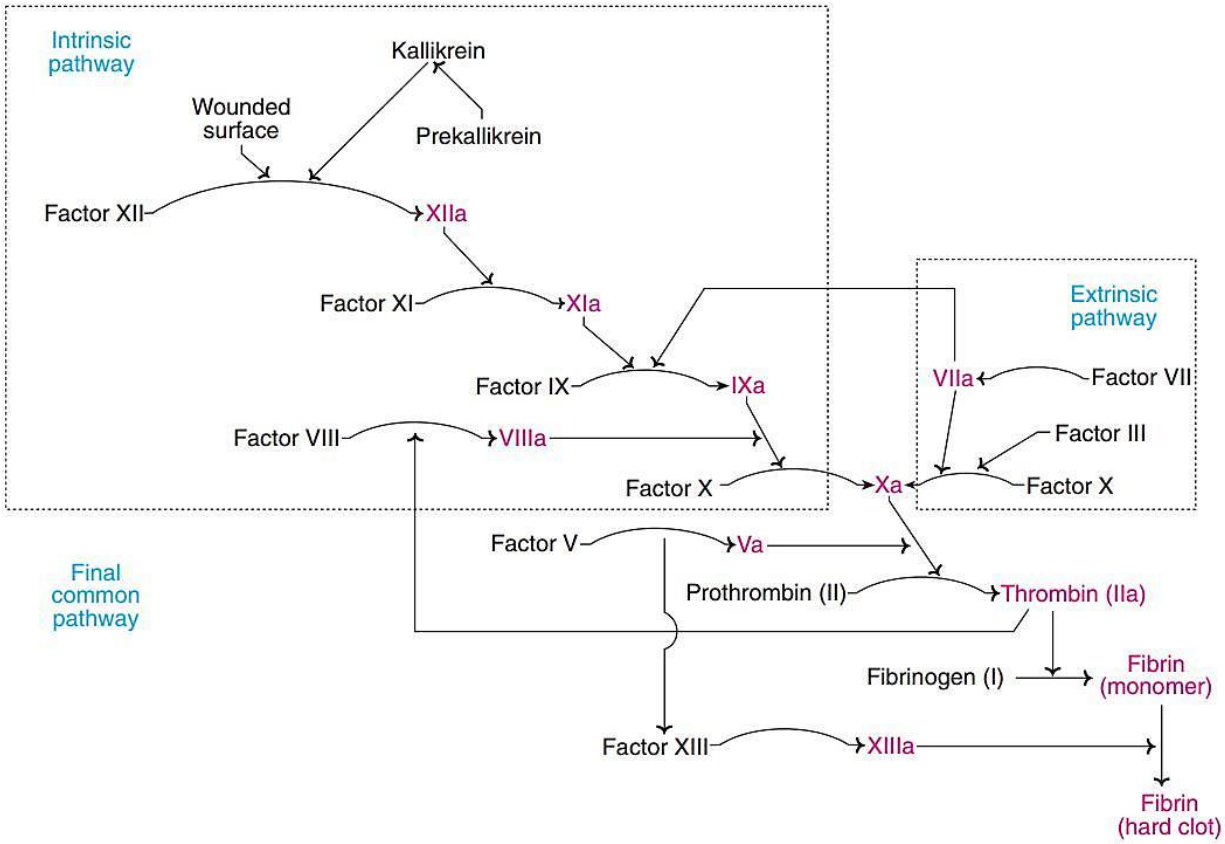
Phase II:

Phase III:

A) Stage 1:

B) Stage 2:

C) Stage 3:



5.16. Which protein of the clotting cascade plays a key role in platelet activation?

5.17. Why is aspirin considered an antiplatelet drug?

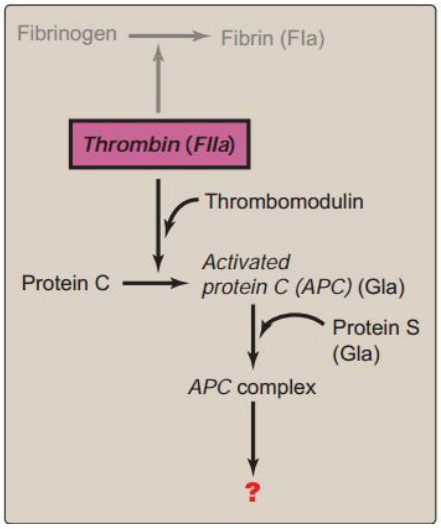
5.18. Fill in the table «The inherited coagulopathies»

Disorder	Cause
Hemophilia A (classical hemophilia)	
Hemophilia B (Christmas disease)	
Hemophilia C	
Von Willebrand's disease	
A-(hypo)-fibrinogenemia	
Disfibrinogenemias	

5.20. Several substances, known as anticoagulants, are in use to inhibit the blood clotting. Fill in the table «The primary anticoagulants»

Anticoagulant	Action
Antithrombin III	
α_2-Macroglobulin	
α_1-Antitrypsin	
Heparin	

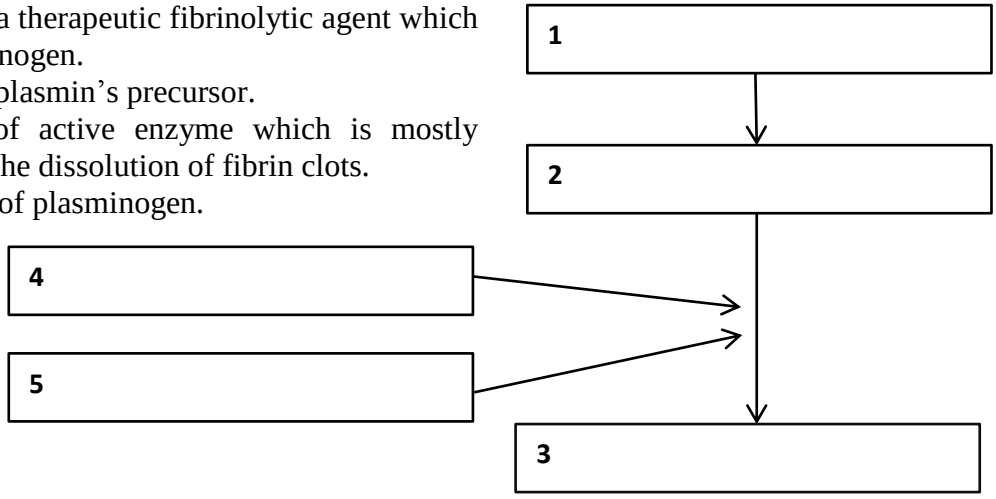
5.21. The secondary anticoagulants are produced during blood clotting and fibrinolysis. What is the function of the activated protein C complex (APC complex) shown? Complete the scheme.



5.22. Give examples of vitamin K antagonists. Explain the mechanism of their action.

5.23. Complete the scheme «Activation of plasmin»

1. The name of a therapeutic fibrinolytic agent which activates plasminogen.
2. The name of plasmin's precursor.
3. The name of active enzyme which is mostly responsible for the dissolution of fibrin clots.
4, 5. Activators of plasminogen.



6. Individual independent students work

1. Hereditary and acquired disorders of vascular-platelet and coagulation hemostasis.
2. Drugs that affect the processes of fibrinolysis.
3. Plasminogen activators and plasmin inhibitors.

Practice protocol №16 «____» _____ 20___

Experiment. Detection of vicasol with cysteine.

Principle. Solution of vicasol in alkaline medium in the presence of cystein turns into lemon yellow color.

Procedure.
Add 5-10 droplets of 0.05 % alcohol solution of vicasol into a tube.
Add 2.5 ml of NaOH.
Observe the appearance of lemon yellow color.
Results:

Conclusion:

Clinical and diagnostic significance. The most clarified function of vitamin K is its interrelationship with blood coagulation. Vitamin K is essential for the synthesis of blood coagulation factors, such as prothrombin (factor II), proconvertin (factor VII), Christmas factor (factor IX), Stuart's factor (factor X). Vitamin K contributes to the synthesis of the full molecule of prothronbin, providing its posttranslational modification.

Current scientific data also gives evidence that vitamin K as other fat soluble vitamins affects the state of cell membranes and subcellular structures, being the constituent of lipoproteins of these membranes.

Daily need in vitamin K makes 1-2 mg, its concentration in blood serum makes 400 – 600 nmol/l.

Deficiency of vitamin K in most cases is endogenous and results from the disturbance of its production in the intestine (use of antibiotics or sulphamides), impaired absorption due to the lack of bile production, obstruction of bile ducts, liver disorders. The use of antivitamins of vitamin K may also cause its deficiency. The signs of vitamin K deficiency include bleedings after small injuries, coagulopathies in newborns (before the appearance of appropriate microflora in the small intestine).

In medical practice the drugs of vitamin K are widely used and its water soluble analogueue – vicasol. These drugs are prescribed in pathologies, accompanied by hypoprothrombinemia and bleedings.

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

Examples of Krock-1 tests

1. Which blood coagulation factor levels are not decreased in liver diseases, which are accompanied by disturbance of protein synthesis?
A. Tissue thromboplastin
B. Stuart-Prower factor
C. Fibrinogen
D. Proconvertin
E. Proaccelerin

2. A-(hypo)-fibrinogenemia is diagnosed in patient with periodical severe bleeding due to complete uncapacity to coagulation. This disease is due to a deficiency of:
A. Fibrinogen
B. Proaccelerin
C. Thromboplastin
D. Proconvertin
E. -

3. Which action of heparin provides its wide application in clinical practice to prevent blood coagulation and to treat thromboses?
A. Activation of antithrombin III
B. Inhibition of antithrombin III
C. Binding factor IX
D. Stimulation of fibrin hydrolysis
E. -

4. Patient has deficiency of factor VII. Which stage of blood clotting is disturbed?
A. Conversion of factor X to Xa
B. Retraction of blood clot
C. Aggregation of thrombocytes
D. Conversion of prothrombin to thrombin
E. Conversion of fibrin monomer to fibrin polymer

5. Which conversion is catalysed by plasmin?
A. Fibrin to peptides
B. Fibrinogen to fibrin
C. Prothrombin to thrombin
D. Proconvertin to convertin
E. Plasminogen to plasmin

6. Which factor does not participate in intrinsic mechanism of blood coagulation?
A. III
B. XI
C. VIII
D. IXa
E. X

7. Boy has hemophilia A, which is manifected by prolonged bleeding even in small injury of vessels. Which blood clotting factor deficiency causes hemophilia A?
A. Antihemophilic globulin A
B. Prothrombin
C. Proaccelerin
D. Fibrinogen
E. Antihemophilic globulin B

8. Coumarin drugs inhibiting carboxylation of glutamate are used in clinic as anticoagulants. They are structural analogues of vitamin:
A. K
B. B
C. C
D. A
E. E

9. Cascade of reactions of thrombin formation may be realized by two pathways: extrinsic and intrinsic ones. Which stage are they combined in?
A. Factor X to factor Xa
B. Fibrinogen to fibrin
C. Prothrombin to thrombin
D. Factor V to Va
E. Factor IX to IXa

10. Hemophilia C is diagnosed in patient with increased bleeding. Which factor disturbed synthesis causes this form of hemophilia:
A. XI
B. VIII
C. IV
D. III
E. II

11. In experiments in vitro it is found, that kallikrein takes part in intrinsic pathway of blood coagulation. Which is its role?
A. Accelerates the conversion of factor XII to XIIa
B. Is necessary for initiation of intrinsic pathway of blood clotting
C. Participates in retraction of blood clot
D. Has transglutaminase activity
E. Initiates anticlotting system of blood

12. The formation of stable clot is impossible in inherited deficiency of factor XIII. Which is the role of factor XIII?
A. Participation in fibrin polymer formation
B. Participation in fibrin monomer formation
C. Participation in synthesis of fibrinogen in liver
D. Participation in the removal of fragments A and B from fibrinogen molecule
E. -

13. Urokinase was prescribed to patient with thrombophlebitis to dissolve thrombi. Which is the mechanism of this drug action?
A. Activation of plasminogen
B. Activation of antithrombin III
C. The increase of heparin synthesis
D. The increase of α2-macroglobulin level in blood
E. Disturbance of conversion of fibrin monomer to fibrin polymer

14. Thromboses and embolism of vessels are observed in patient. Those are due to inherited deficiency of blood plasma protein, which is the most potent inhibitor of blood clotting. Which is this protein?
A. Antithrombin III
B. α1-Antitrypsin
C. Fibrinogen
D. Factor VIII
E. Bradykinin

15. Calcium ions play the important role in blood coagulation. They activate certain factors of blood clotting by means of an attachment to sites of calcium binding. Which amino acid has major role in those sites?
A. γ-Carboxyglutamic acid
B. γ-Aminobutyric acid
C. Glucuronic acid
D. Glutamic acid
E. γ-Hydroxybutyric acid

16. Patient was transported to the clinic with suspiction on the myocardial infarction. For the prophylaxis of thrombogenesis he was prescribed a preparation of fibrinolysine (plazmin), which catalyse transformation...
A. Fibrine to peptides
B. Fibrinogen into fibrin
C. Protrombin into trombin
D. Proconvertin into convertin
E. Plasminogen into plasmin

17. Impossibility to formation of stable fibrin clot was found in patients with hemorrhage. Which factor deficiency causes this state?
A. XIII
B. XII
C. XI
D. X
E. Prothrombin

18. Which process results in activation of many factors of blood coagulation that preexist as zymogens?
A. Limited proteolysis
B. Amidination
C. Phosphorylation
D. Glycosylation
E. -

19. Intracardiac injection of imobilized streptokinase was made to patient with acute myocardial infarction. Which substance formation is stimulated by this drug?
A. Plasmin
B. Thrombin
C. Heparin
D. Prothrombin
E. Antithrombin

20. The patient has hemorrhages, the concentration of prothrombin is reduced in the blood. What vitamin deficiency led to a violation of the synthesis of this clotting factor?
A. K
B. A
C. D
D. C
E. E

21. In the patient's blood there was detected certainly high activity of protrombin, that is the threat of vessels thrombosis. What preparation should be used in this case?
A. Heparin
B. Potassium oxalate
C. Natrium citrate
D. Natrium oxalate
E. Ethylene diamine tetra acetate

22. A 16 year old boy after an illness has diminished function of protein synthesis in liver as a result of vitamin K deficiency. It will cause disturbance of:
A. Blood coagulation
B. Erythrocyte sedimentation rate
C. Anticoagulant generation
D. Erythropoietin secretion
E. Osmotic blood pressure

23. A 6-month-old child experienced frequent and intense subcutaneous hemorrhages. The appointment of a synthetic analogueue of vitamin K (vikasol) gave a positive effect. In the γ-carboxylation of glutamic acid, what protein of the blood coagulation system is this vitamin taking part in?
A. Prothrombin
B. Fibrinogen
C. Hageman factor
D. Antihemophilic globulin A
E. Rosenthal factor

24. A patient, who has been suffering for a long time from intestine disbacteriosis, has increased hemorrhaging caused by disruption of posttranslational modification of bloodcoagulation factors II, VII, IX, and X in the liver. What vitamin deficiency is the cause of this condition?
A. K
B. B_{12}
C. B_9
D. C
E. P

25. The patient complains of frequent bleeding from the gums. A deficiency of coagulation factor II (prothrombin) was found in the blood. What phase of blood coagulation is impaired in a person, above all?
A. Thrombin formation
B. The formation of prothrombinase
C. Formation of fibrin
D. Fibrinolysis
E. Clot retraction

26. As a result of posttranslative modifications some proteins taking part in blood coagulation, particularly prothrombin, become capable of calcium binding. The following vitamin takes part in this process:
A. K
B. C
C. A
D. B_1
E. B_2

27. A 37-year-old patient, with long-term use of antibiotics, has increased bleeding with minor injuries. In the blood - a decrease in activity II, VII, X blood clotting factors; lengthening of blood clotting time. What vitamin deficiency caused these changes?
A. Vitamin K
B. Vitamin A
C. Vitamin C
D. Vitamin D
E. Vitamin E

28. To prevent postoperative bleeding a 6 y.o. child was administered vicasol that is a synthetic analogueue of vitamin K. Name posttranslational changes of blood coagulation factors that will be activated by vicasol:
A. Carboxylation of glutamin acid
B. Phosphorylation of serine radicals
C. Partial proteolysis
D. Polymerization
E. Glycosylation

29. After implantation of a cardiac valve a young man constantly takes indirect anticoagulants. His state was complicated by hemorrhage. What substance content has decreased in blood?
A. Prothrombin
B. Haptoglobin
C. Heparin
D. Creatin
E. Ceruloplasmin

30. Activation of a number of hemostatic factors occurs through their joining with calcium ions. What structural component allows for adjoining of calcium ions?
A. Gamma-carboxyglutamic acid
B. Gamma-aminobutyric acid
C. Gamma-oxybutyric acid
D. Hydroxyproline
E. Monoamine-dicarboxylic acids

31. A few days before an operation a patient should be administered vitamin K or its synthetic analogueue Vicasol. Vitamin K takes part in the following posttranslational modification of the II, VII, IX, X blood clotting factors:
A. Carboxylation
B. Decarboxylation
C. Deamination
D. Transamination
E. Glycosylation

32. A newborn baby has numerous hemorrhages. Blood coagulation tests reveal increased prothrombin time. The child is most likely to have a disorder of the following biochemical process:
A. Production of gammacarboxyglutamate
B. Conversion of homocysteine to methionine
C. Conversion of methylmalonyl CoA to succinyl CoA
D. Degradation of glutathione
E. Hydroxylation of proline

33. Plasmic factors of blood coagulationare exposed to post-translational modification with the participation of vitamin K. It is necessary as a cofactor in the enzyme system of γ-carboxylation of protein factors of blood coagulation due to the increased affinity of their molecules with calcium ions. What amino acid is carboxylated in these proteins?
A. Glutamic
B. Valine
C. Serine
D. Phenylalanine
E. Arginine

34. Patient with the symptoms of the increased blood coagulation (thromboses, thrombophlebitis) was treated parenterally with an anticoagulant – heparin. However the speed blood coagulation did not decreased. The deficit of what proteinous factor of the anticoagulation system of blood is observed?
A. Antithrombin III
B. α_2-macroglobulin
C. α_1-inhibitor of proteinases
D. Antithromboplastin
E. Anticonvertin

Topic 17.
Investigation of biochemical regularities of the implementation of immune processes

1. Objective: To characterize biochemical components of immune system, to learn biochemical mechanisms of immunodeficiencies.

2. Actuality of theme: The immune system of the organism along with cellular and humoral mechanisms provides recognition, binding and destruction of antigens of both infectious and noninfectious origin. The immune system consists of the thymus, the spleen, lymphocytes of the bone marrow and blood. Numerous proteins and peptides (immunoglobulins, components of the complement system, hormones and mediators of immunity) take part in reactions of immunity along with lymphocytes. Knowledge of biochemical laws of immune processes is important for understanding mechanisms of immunodeficiency states that are developed at a damage of components of cellular or humoral immunity.

3. Specific aims:

- ✓ To characterize cellular and humoral factors of immune system.
- ✓ To explain mechanisms of development of immunodeficient states.

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

	Questions:	References:
1.	**General characteristic of immune system; cellular and humoral factors.**	1. Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 732–737. 2. Popova L.D. Biochemistry: Manual for medical students or interns / L.D. Popova, A.V. Polikarpova – Kharkiv: KNMU, 2011. – P. 393–395. 3. Lecture notes.
2.	**Immunoglobulins: structure, biological function, mechanisms of immunoglobulin synthesis. Characteristics of distinct immunoglobulin classes of human blood.**	1. Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 186–190. 2. Popova L.D. Biochemistry: Manual for medical students or interns / L.D. Popova, A.V. Polikarpova – Kharkiv: KNMU, 2011. – P. 407–411. 3. Lecture notes.
3.	**Mediators and hormones of immune system (interleukins, interferons, protein and peptide factors of cell growth and proliferation).**	1. Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 735. 2. Popova L.D. Biochemistry: Manual for medical students or interns / L.D. Popova, A.V. Polikarpova – Kharkiv: KNMU, 2011. – P. 403–406. 3. Lecture notes.
4.	**Factors of complement system. Classical and alternative pathways of complement activation.**	1. Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 734. 2. Popova L.D. Biochemistry: Manual for medical students or interns / L.D. Popova, A.V. Polikarpova – Kharkiv: KNMU, 2011. – P. 399–402. 3. Lecture notes.
5.	**Biochemical mechanisms of immunodeficiencies: primary (hereditary) and secondary immunodeficiencies; acquired immunodeficiency syndrome (AIDS).**	1. Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 736. 2. Popova L.D. Biochemistry: Manual for medical students or interns / L.D. Popova, A.V. Polikarpova – Kharkiv: KNMU, 2011. – P. 413–416. 3. Lecture notes.

5. Tasks for independent work and self-control

5.1. Describe two types of immunity.

a) Innate (non-specific) immunity:

b) Adaptive or acquired (specific) immunity:

5.2. List primary and secondary lymphoid organs.

A. Primary lymphoid organs:	B. Secondary lymphoid organs:

5.3. Compare the cells of immune system

Feature	B-lymphocytes	T-lymphocytes
The site of development and maturation		
Function		
Types		1) 2) 3)
Kind of factor (humoral or cellular)		

5.4. Fill in the table «The classes of immunoglobulins»

Type	H-Chain	L-Chain	Serum conc., g/L	Major function(s)
Ig G			10-15	
Ig A			1-5	
Ig M			1.5-3.5	
Ig D			0.03	
Ig E			0.0005	

5.5. Explain the function of the major histocompatibility complex (MHC).

5.6. It is known that cytokines are soluble mediators of the inflammatory and immune response. They are produced by a variety of cells and tissues, are active at concentrations between 10^{-9} – 10^{-15} molar. Cytokines can be grouped into families:

– Interleukins (ILs);
– Interferons (IFNs);
– Tumor necrosis factors (TNFs);
– Colony-stimulating factors;
– Transforming growth factors (TGFs).

Give the general characteristic of cytokines using appropriate points:

Nature:

Have effects (distance action):

Groups by principal effect:
1)

2)

3)

Therapeutic uses:

5.7. Describe the biological effects of different groups of cytokines.
A) Interleukins (IL-1-IL-18)

B) Interferons:
IFN-α and IFN-β

IFN-γ

C) Tumor necrosis factors:
TNF-α

TNF-β

D) Colony-stimulating factors

E) Transforming growth factors

5.8. The complement system brings about two sets of reactions:
1. Antibody dependent classical pathway.
2. Antibody independent alternative pathway.
Each one of the pathways consists of a series of reactions converting inactive precursors to active products by serine proteases which resembles blood coagulation.
Describe the both these pathways.

5.9. Match the categories of disorders of immune system with their characteristics:

1. Immunodeficiency disorders (primary or secondary)	**A.** The immune system overreacts in response to an antigen
2. Autoimmune disorders	**B.** The body's own immune system attacks its owntissues as foreign matter
3. Allergic disorders	**C.** Include lymphomas and leukemias, which are also types of blood cancer
4. Cancers of the immune system	**D.** Disbalances of functioning some chains of cellular or humoral immunity.

6. Individual independent students work

1. Analysis of disorders that occur in the functioning of the human immune system.
2. Primary and secondary immunodeficiencies.

Practice protocol №17 «____» _____ 20___

Experiment. Determination of circulating immune complexes (CIC).

Principle. Macromolecular immune complexes, which are circulating in blood plasma, are precipitated with polyethyleneglycole (PEG). The turbidity of samples is evaluated based on measurement of light absorption at 450 nm.

Procedure.

Add 2.7 ml of 0.1 M borate buffer (pH 8.4) and 0.3 ml of blood serum, diluted 3-fold by borate buffer into a control tube

Add 2.7 ml of 4 % solution of PEG_{6000} in borate buffer and 0.3 ml of 3-fold diluted blood serum into a test tube.

Leave both tubes at a room temperature for 1 hour.

After 1 hour measure the optical density of the solutions with the use of the spectrophotometer at wavelength 450 nm.

Calculate the difference in measured extinction of test and control tubes, multiply this value by 1,000 and compare with normal indices.

Results:

Conclusion:

Diagnostic significance. In health CIC level in blood corresponds to 30-100 arbitrary units. Immune complexes play an important role in of many diseases. Middle- and large sized CIC are the most dangerous, they can activate complement system, coagulation and kallicrein systems, which exhibit harmful effect on biological membranes and has cytopathogenic effect.

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

Examples of Krock-1 tests

1. In order to prevent rejection of the transplant after organ transplantation, it is mandatory to conduct a course of hormone therapy for immunosuppression. What hormones are used for this purpose?

A. Glucocorticoids
B. Mineralocorticoids
C. Sex hormones
D. Thyroid hormones
E. Catecholamines

2. Bruton's disease was diagnosed in a 5- year-old child. In the blood: absence of Blymphocytes and plasma cells. What changes will be observed in immunoglobulin content in the blood serum of the child?

A. Reduction of IgA, IgM
B. Increase of IgD, IgE
C. Increase of IgA, IgM
D. Reduction of IgD, IgE
E. There will be no changes

3. Protective function is one of the functions of saliva, in particular, providing the formation of local immunity of the mucous membrane due to the release of protein by parotid glands. What is the protein called?
A. Secretory immunoglobulin
B. Collagen
C. Elastin
D. Fibrinogen
E. Albumin

4. It has been obtained two positive results of the enzyme-linked immunosorbent assay (ELISA) during medical examination of a patient with AIDS. Which method should be used to avoid false-positive AIDS results?
A. Western blotting
B. Radioimmunoassay
C. Luminescent analysis
D. Immunofluorescence
E. Molecular hybridization

5. In humans universal antiviral agents are synthesized by lymphocytes and other cells in response to the virus entry. What are protein factors called?
A. Interferons
B. Interleukin-2 and 1β
C. Cytokines
D. Interleukin-4 and 6
E. Tumour necrosis factors

6. Electrophoretic study of the blood serum of a patient suffering from pneumonia showed an increase in one of the protein fractions. Determine it:
A. Gamma-globulins
B. Albumin
C. Alpha1-globulins
D. Alpha2-globulins
E. Beta-globulins

7. To prevent transplant rejection 6-mercaptopurine is administered. What mechanism of its action?
A. Inhibition of nucleic acid synthesis by immune cells
B. Inhibition of nucleic acid synthesis by cells of transplants
C. Inhibition of immunoglobulin synthesis by plasma cells
D. Inhibition of protein synthesis by cells of transplants
E. Inhibition of ATP synthesis in immune cells

8. A patient came to a doctor with complaints of exercise-induced dyspnea. Clinical examination revealed anemia and the presence of paraprotein in the gammaglobulin zone. What substance should be determined in the patient's urine to confirm multiple myeloma diagnosis?
A. Bence Jones protein
B. Bilirubin
C. Hemoglobin
D. Ceruloplasmin
E. Antitrypsin

9. What type of immunoglobulin dominates in the composition of saliva forming local immunity of the oral cavity?
A. Secretory immunoglobulin A
B. Immunoglobulin E
C. Immunoglobulin A
D. Immunoglobulin M
E. Immunoglobulin E

10. What fraction of globulins of blood plasma provides humoral immunity playing the role of antibodies?
A. γ -globulins
B. β -globulins
C. α_1-globulins
D. Cryoglobulins
E. α_1-macroglobulin

11. HBsAg was detected in the serum. Which disease this antigen is marker for?
A. Viral hepatitis
B. Tuberculosis
C. AIDS
D. Syphilis
E. Mononucleosis

12. Passive specific transplacental immunity is provided by:
A. Immunoglobulin G
B. Immunoglobulins M
C. Immunoglobulin A
D. Immunoglobulin D
E. Immunoglobulin E

13. Numerous plasma cells were found in the blood of a 16-year-old girl suffering from an autoimmune inflammation of the thyroid gland. The proliferation and differentiation of what blood cells caused increased number of plasma cells?
A. B cells
B. T-killers
C. Tissue basophils
D. T-suppressors
E. T-helper

14. A 12-year-old child has a high fever, a sore throat while swallowing, enlarged submandibular lymph nodes. The pediatrician diagnosed acute tonsillitis. What cytokines are involved in the countering of the inflammatory process?
A. g-immune IFN
B. Interleukin-2
C. a-leukocyte IFN
D. Tumour necrosis factor-α
E. Interleukin-3

15. What components of saliva and gingival fluid provide the local immunity of the oral cavity?
A. Lysozyme, immunoglobulins, leukocytes
B. Lactate dehydrogenase, glucuronidase
C. Lactic acid, urea, ammonia
D. Alkaline and acid phosphatase
E. Hyaluronidase, cathepsin D

16. Variability of immunoglobulins is determined by:
A. Recombination of DNA
B. DNA methylation
C. Repression of genes
D. Recombination of mRNA
E. Recombination of proteins

17. A 70-year-old patient with diagnosis of malignant tumour of the cecum complains of weakness, headache and fatigue. It is known that immunity is decreased in patients suffering from cancer. It is associated with an inhibition of adenilate desaminase activity in lymphocytes of patients. What process takes place with the participation of the enzyme?
A. Catabolism of the purine nucleotides
B. Catabolism of pyrimidine nucleotides
C. Synthesis of pyrimidine nucleotides
D. Synthesis of purine nucleotides
E. Reutilization of purine bases

18. A boy often began to suffer from respiratory diseases, stomatitis, pustular skin lesions in the second year of life. Even minor damage of the gums and mucous membranes complicated by a long flowing inflammation. It was revealed that all classes of immunoglobulins are practically absent in the blood. The decrease in the functional activity of which cell population underlies the described syndrome?
A. B-cells
B. NK-lymphocytes
C. T-lymphocytes
D. Neutrophiles
E. Macrophages

19. A patient has been delivered to the neurological department with cerebellar ataxia type. There was a complete absence of IgA and a low level of IgG in the patient's blood. Which congenital immunodeficiency state is in the patient?
A. Louis-Bar syndrome
B. DiGeorge Syndrome
C. Dissymunoglobulinemia
D. Bruton's disease
E. Swiss type agammaglobulinemia

20. A 3-month-old boy has congenital hypoplasia of the thymus gland, which is accompanied by frequent infections due to violations of the T-cell population, tetralogy of Fallot and cleft palate. What is this pathology called?
A. Di-George syndrome
B. Louis-Bar Syndrome
C. Dissymunoglobulinemia
D. Bruton's disease
E. Swiss type agammaglobulinemia

21. A 12-year-old patient was admitted to a hospital with diagnosis of infectious pneumonia. Frequent catarrhal diseases are in the anamnesis, which were characterized by a heavy course. A significant reduction of IgG concentration and a lack of B-lymphocytes were revealed in the patient's blood. What is the pathology in the patient?
A. Bruton's disease
B. Louis-Bar Syndrome
C. Dissymunoglobulinemia
D. DiGeorge syndrome
E. Swiss type agammaglobulinemia

22. In child, 5 years, Brutton disease has been examined, which showed hard proceeding of bacterial infections, absence of B-lymphocytes and plasmatic cells. Which changes in immunoglobulines content are observed in blood serum of child with immunodeficiency.
A. The decrease of Ig A, Ig M
B. The increase of Ig A, Ig M
C. The decrease of Ig D, Ig E
D. The increase of Ig D, Ig E
E. No changes

23. In humans immunoglobulins realize a humoral immune response to the entry of antigens. According to the chemical nature of the non-protein part, they refer to:
A. Glycoproteins
B. Lipoproteins
C. Nucleoproteins
D. Chromoproteins
E. Metalloproteins

24. A 25-year-old patient is diagnosed with AIDS. What cell populations are the most sensitive to the human immunodeficiency virus?
A. T-helper cells
B. Hepatocytes
C. Endothelial cells
D. Epitheliocytes
E. B-Lymphocytes

25. It was observed a significant decrease in the concentration of antibodies (IgG in 10 times, IgA and IgM in 100 times), some populations of lymphocytes and plasma cells in the patient's serum. What is the presumptive diagnosis?
A. B-cell insufficiency
B. T-cell insufficiency
C. Pathology of phagocytic cells
D. Pathology of the complement system
E. Secondary immunodeficiency

26. It is known that human immunodeficiency virus belongs to the family of retroviruses. Specify the main feature that characterizes this family:
A. The presence of the enzyme reverse transcriptase
B. Containing of minor RNA
C. Simple viruses infecting humans only
D. Nucleic acid is not integrated into the host genome
E. Containing DNA

27. Using skin allergy tests it was established the sensitization by an allergen of poplar fluff in a patient with bronchial asthma. Which factor of the immune system plays a main role in the development of bronchial asthma?
A. IgE
B. IgD
C. IgM
D. Interferon alpha
E. IgG

28. Immune reactions of organism are provided by high-specific interaction «antigen-antibody». Such specificity of immunoglobulins depends on their molecular structure. Which class of complex proteins they are involved to?
A. Glycoproteins
B. Metalloproteins
C. Chromoproteins
D. Lipoproteins
E. Nucleoproteins

29. Antigen presentation disfunction of immune cells was identified in a patient with clinical signs of primary immunodeficiency disorder. The defect in the structure of what cells can be assumed?
A. Macrophages, monocytes
B. T-lymphocytes
C. B-lymphocytes
D. Fibroblasts
E. Lymphocytes

30. The examination of several classes of immunoglobulines in birth period can be used as diagnostic test to verify the fetal infection. Which class of immunoglobulines can pass through placenta?
A. Ig G
B. Ig A
C. Ig M
D. Ig E
E. Ig D

31. Interleukine-2 is the cytokine, which is produced by mature T-helpers as result of their stimulation by antigen. It has a central place in interleukine regulation of immunity. Call the chemical nature of interleukine-2.
A. Glycoprotein
B. Lipoprotein
C. Simple protein
D. Phosphoprotein
E. Proteogycan

32. Tumor necrosis factors (TNF) cause lysis of several tumor cells of organism and they are observed as perspective anticancer drugs. One of them is lymphotoxin, protein, which has cytotoxic effect. Which cells produce lymphotoxin?
A. T-lymphocytes
B. B-lymphocytes
C. T- and B-lymphocytes
D. Macrophages
E. Monocytes

33. Immune system with help of cellular and humoral mechanisms provides the distinguishing, binding and destroying of antigens. The main classes of blood immunoglobulines, which realize humoral immune response, are.
A. Ig G and Ig M
B. Ig A and Ig E
C. Ig D and Ig A
D. Ig A and Ig M
E. Ig E and Ig D

34. In fever development the increase of «acute phase» proteins (ceruloplasmin, fibrinogen, C-reactive protein) is characteristic. Which mechanism of this is possible?
A. Stimulating influence of IL-1 to hepatocytes
B. Damage action of temperature to organism cells
C. Degranulation of tissue basophils
D. Proliferate action of IL-2 to T-lymphocytes
E. -

Topic 18.
Study of biliary function of the liver. Metabolism of bile pigments. Pathobiochemistry of jaundice

1. Objective: To learn biochemical pathways of heme metabolism. To interpret biochemical indices in blood and urine in sense of differential diagnosis of jaundices. To learn the principal functions of liver in metabolism of carbohydrates, lipids and simple and conjugated proteins as well as in biotransformation of toxic compounds.

2. Actuality of theme: The metabolism of bilirubin, which is the product of heme degradation occurs in a liver. Disorders in bilirubin metabolism result in jaundice, which is recognized with the use of biochemical tests in blood and urine. Liver plays a central role in general metabolism, supporting the stability of internal milieu of organism. The most important reactions of detoxification of endogenous and exogenous harmful substances are carried out in this organ.

3. Specific aims:

- ✓ To interpret principal biochemical processes in liver, i.e. metabolism of carbohydrates and lipids, protein biosynthesis, urea biosynthesis, metabolism of bile pigments and bile production.
- ✓ To analyze changes in biochemical indices of blood and urine (direct and indirect bilirubin) in recognition of types of jaundices.
- ✓ To explain the role of liver in regulation of glycemia (glycogenesis and glycogen breakdown, gluconeogenesis) in normal and pathological conditions.
- ✓ To explain biochemical principles of insufficiency of liver functions in chemical, biological or radiation damage of liver tissue.
- ✓ To learn methods of bilirubin determination in blood serum and to interpret obtained results.

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

Questions:	References:
1. **Homeostatic role of liver in human body. Involvement of liver in glucose turnover (glycogenesis and glycogen breakdown, gluconeogenesis), proteins and lipid metabolism.**	1. Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 453–454. 2. Popova L.D. Biochemistry: Manual for medical students or interns / L.D. Popova, A.V. Polikarpova – Kharkiv: KNMU, 2011. – P. 446–448. 3. Gubsky Yu. Biological chemistry: textbook / edited by Yu. Gubsky. – Vinnytsia: Nova Knyha, 2018. – 2nd edition. – P. 398–402. 4. Lecture notes.
2. **Role of liver in turnover of bile pigments. Bile-producing function of liver. Biochemical composition of bile.**	1. Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 214–216, P.453–456. 2. Popova L.D. Biochemistry: Manual for medical students or interns / L.D. Popova, A.V. Polikarpova – Kharkiv: KNMU, 2011. – P. 449. 3. Lecture notes.
3. **Hemoglobin catabolism: production of biliverdin, its transformation to bilirubin, synthesis of bilirubin diglucuronide and excretion with bile.**	1. Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 214–216. 2. Popova L.D. Biochemistry: Manual for medical students or interns / L.D. Popova, A.V. Polikarpova – Kharkiv: KNMU, 2011. – P. 363–366. 3. Gubsky Yu. Biological chemistry: textbook / edited by Yu. Gubsky. – Vinnytsia: Nova Knyha, 2018. – 2nd edition. – P. 403–406. 4. Lecture notes.

4. **Pathobiochemistry of jaundices; hemolytic (prehepatic), parenchimatous (hepatic), occlusive (posthepatic). Enzymatic congenital jaundices:** ✓ Crigler-Najjar syndrome as a consequence of insufficient activity of UDP-glucuronyl-transferase. ✓ Gilbert disease – pathology caused by combined disorder of synthesis of bilirubin diglucuronide and absorption of bilirubin from blood by liver cells («absorption jaundice). ✓ Dubin-Johnson syndrome – jaundice caused by disorder of transport of bilirubin diglucuronide from liver cells to bile («excretory jaundice»). Enzymatic jaundices of neonates and methods of their prevention.	1. Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 216–220. 2. Popova L.D. Biochemistry: Manual for medical students or interns / L.D. Popova, A.V. Polikarpova – Kharkiv: KNMU, 2011. – P. 367–370. 3. Gubsky Yu. Biological chemistry: textbook / edited by Yu. Gubsky. – Vinnytsia: Nova Knyha, 2018. – 2nd edition. – P. 407–408. 4. Lecture notes.

5. Tasks for independent work and self-control

5.1. Complete the table and mark the metabolic pathways that go only or principally in the liver.

	Metabolic pathways	Significance of these pathways, their products
Carbohydrates	1.Glycogenesis and glycogenolysis	
	2. Gluconeogenesis	
	3. Glycolysis + Krebs cycle + OP	
	4. Penthose phosphate pathway	
	5. Metabolism of fructose	
	6. Metabolism of galactose	
	7. Synthesis of galactose, mannose, sialic acid, glucuronic acid, amino sugars	
Lipids	1. Synthesis of fatty acids and TAG	
	2. Synthesis of cholesterol	
	3. Synthesis of phospholipids, production of VLDL	
	4. Oxidation of fatty acids	
	5. Synthesis of ketone bodies	
	6. Synthesis of bile acids	

Proteins	1. Synthesis and degradation of blood proteins, liver proteins	
Amino acids	2. Catabolism of amino acids	
	3. Conversions of amino acids into glucose, fatty acids, ketone bodies	
N-containing compounds	4. Synthesis of urea	
	5. Nucleotide synthesis (de novo)	
	6. Synthesis of heme	
	7. Synthesis of creatine	
	8. Synthesis of glutathione	

5.2. Biochemical liver function tests useful in detecting the presence of liver disease and in following its progress (monitoring the course of disease) but rarely provide a precise diagnosis since they reflect the basic pathological processes common to many conditions. The most frequently performed biochemical tests are the measurement in the plasma of: 1) albumin concentration, 2) bilirubin concentration, 3) activities of enzymes, 4) prothrombin time. Enzymes used in the assessment of hepatic function include: alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP, hepatic isoenzyme), γ-glutamyl transferase (GGT).

a) Plasma albumin concentration is insensitive indicator of liver diseases. Its concentration is usually normal in acute hepatitis. Low concentrations are characteristic of chronic liver disease. Explain why.

b) A raised plasma bilirubin concentration is a frequent but not invariable finding in patients with liver disease. Conjugated hyperbilirubinemia can result from hepatitis, intra- and extrahepatic cholestasis. What additional biochemical tests can help to the differential diagnosis of jaundice?

c) Increased aminotransferases activities reflect cell damage. Which of them, AST or ALT, is more specific to the liver?

d) Increased activity of what enzymes indicate the cholestasis (obstruction to the flow of bile)? Why?

e) An increase in the prothrombin time is often an early feature of acute liver disease, but may also reflect vitamin K deficiency. In such cases how can we determine the cause of prolonged prothrombin time?

5.3. Under physiologic conditions in the human adult, $1–2\times10^8$ erythrocytes are destroyed per hour. Thus, in 1 day, a 70-kg human turns over approximately 6 g of hemoglobin.

Indicate the tissues (cells, fluids) where go the conversions 1 -7.

Describe the fate of iron released during the degradation of heme.

Name the enzymes that catalyse reactions 2, 3, 5, 6. What of these enzymes are human and what are bacterial?

Describe the function of reaction 5.

Explain the changing colour of a bruise (hematoma).

1) Hb (also Mb, cytochromes, iron-containing enzymes) → heme + proteins→amino acids;

3) heme + O_2 → biliverdin + CO + Fe^{3+};

3) biliverdin + NADPH + H^+ → bilirubin + NADP;

4) bilirubin + albumin ↔ bilirubin-albumin;

5) bilirubin + 2 UDP-glucuronic acid → bilirubin diglucuronide + 2 UDP;

6) bilirubin diglucuronide → bilirubin → urobilinogen → stercobilin;

7) urobilinogen → urobilin.

5.4. Fill in the table «Bilirubin in the plasma»

Forms of bilirubin	Normal level in the serum (μmol/L, mg/dl)
Unconjugated (indirect, free) – bound with albumin	
Conjugated (direct) – bilirubin diglucuronide	
Total bilirubin	

5.5. When bilirubin in the blood exceeds 1 mg/dL (17.1 μmol/L), hyperbilirubinemia is observed. Hyperbilirubinemia may be due to the production of more bilirubin than the normal liver can excrete, or it may result from the failure of a damaged liver to excrete bilirubin produced in normal amounts, or a combination of these. In the absence of hepatic damage, obstruction of the excretory ducts of the liver – by preventing the excretion of bilirubin - will also cause hyperbilirubinemia. In all these situations, bilirubin accumulates in the blood, and when it reaches a certain concentration (approximately 2–2.5 mg/dL), it diffuses into the tissues, which then become yellow. That condition is called jaundice or icterus.

Fill in the table «Classification of jaundice»

Types of jaundice	Causes	Changes of level (↑ or ↓), presence or absence				
		Serum bilirubin		Urine bilirubin	Urine urobilin	Fecal stercobilin
		Uncon.	Conjug.			
Hemolytic (prehepatic)						
Hepatocellular (hepatic)						
Obstructive, (posthepatic)						
Physiological in newborns						

5.6. Situational task:

A massive rise in the plasma bilirubin in infants occurs in: 1) Rhesus blood group incompatibility, 2) prematurity, 3) birth trauma, 4) the Crigler-Najjar syndrome. If its concentration exceeds approximately 340 μmol/L (20 mg/dL) in infants, bilirubin uptake into the brain may cause severe, irreversible brain damage (kernicterus).

What form of bilirubin accumulates?

What possible mechanisms of bilirubin toxic action on the brain?

Explain why hyperbilirubinemia in jaundiced babies can be decreased by exposure to blue light (phototherapy) and by administration of phenobarbital.

6. Individual independent students work

Analysis of differential changes in biochemical parameters of blood and urine (free, conjugated bilirubin) to assess the pathobiochemistry of jaundice.

Practice protocol №18 «____» _____ 20___

Experiment 1. Determination of bilirubin in blood serum by the method of Jendrashik.

Principle of the method. Bilirubin reacts with diazoreagent with the formation of pink coloured azobilirubin. The intensity of colour is proportional to bilirubin concentration and can be measured with the use of photocolourimeter at 530-560 nm wavelength. Conjugated bilirubin reacts rapidly (direct reaction), non-conjugated bilirubin reacts slowly. In presence of accelerators (e.g. caffeine) non-conjugated bilirubin reacts as rapidly as conjugated one. For determination of total bilirubin (conjugated and free) buffer solutions with accelerators (caffeine) are used.

Procedure.

Analysis is carried out in accordance with the scheme:

Reagents (ml)	Control Tube 1	Standard Tube 2	Conjugated bilirubin Tube 3	Total bilirubin Tube 4
Blood serum	0.5	-	0.5	0.5
Standard solution of bilirubin	-	0.5	-	-
0.9 % solution of NaCl (saline)	0.25	-	1.75	-
Diazoreagent	-	0.25	0.25	0.25
Caffeine reagent	1.75	1.75	-	1.75
E =				

While performing the determination of total bilirubin, the solutions should stand for 20 min for the colour development; in the determination of bound bilirubin, this time is shortened to 5-10 min (if it is left to stand for a longer time, the free bilirubin begins to be involved in the colour reaction). At the end of the indicated time, measure the absorbance for each of three solutions against control probes on a photocolourimeter at wavelength of 520-560 nm (green light filter) in a 5 mm cuvette.

Calculation. The concentration of bilirubin is calculated using the formula:

$C_{test} = (C_{st} \times E_{test}) / E_{st}$,

where

C_{test} – concentration of bilirubin in test sample (μmol/L),

C_{st} – concentration of bilirubin in standard (10 μmol/L),

E_{test} – extinction of tested sample,

E_{st} – extinction of standard sample.

Unconjugated (free) bilirubin is calculated by the difference between the total and bound bilirubin concentrations. Calculate the total bilirubin and its fractions in the blood serum and make conclusions about the causes of possible variations of these parameters.

Results:

Conclusion:

Clinical and diagnostic significance. Normal values of total bilirubin concentration in blood serum are 0.1-1.2 mg % (8.5-20.5 μmol/l), non-conjugated – 0.1-1.0 mg % (1.7-17.1 μmol/l), conjugated – 0.05-0.25 mg % (0.86-4.3 μmol/l). High concentrations of bilirubin exhibit toxic effects, which are caused due to it photosensibilization properties. As a metabolite of protoporphyrine, a very active photosensibilizer, bilirubin transforms molecular oxygen to singlet form which is extremely active oxidant. Singlet oxygen induces damage of biological membranes, nucleic acids, proteins; it partially is expressed as hemolysis.

The increase in blood bilirubin concentration over 30 μmol/l causes the jaundice. The last one can be: hemolytic (prehepatic), parenchymatous (hepatic), occlusive (posthepatic, obturation of bile ducts).

In hemolytic jaundice liver is not able to transform all the quantity of free bilirubin in blood, which is produced due to increased hemolysis. In this case in blood is noted an increase in concentration of free (non-conjugated) bilirubin up to 100 μmol/l. This form of jaundice is characteristic to hemolytic and pernicious anemias.

In parenchimatous jaundice the conjugating capacity of liver cells is suppressed due to damage of liver cells. Production of bile is decreased, conjugated bilirubin partially returns back to blood. Bilirubinemia in this case is caused by increase in concentration of direct and indirect fractions of bilirubin. Parenchimatous jaundice is observed in fat degeneration of liver (steatosis), hepatitis (viral, toxic), cirrhosis of liver.

In occlusive jaundice bile is accumulated in bile ducts due to occlusion of bile duct with bile stones or tumor and bile is returned back to blood. Bilirubinemia in this type of jaundice is characterized by a marked increase of bilirubin level in blood (170-700 μmol/l) mainly due to conjugated fraction of bilirubin.

In neonates at first days of life jaundice is developed due to several causes. Bilirubin is not transformed to derivatives due to sterility of intestines and is intensively absorbed to blood from digestive tube. In neonates frequently is observed transient insufficiency of glucuronyl transferase, which causes the increase of nonconjugated bilirubin in blood.

Congenital disorders of bilirubin turnover and transport are observed in congenital jaundices, as such: Crigler-Najjar syndrome (absence or insufficiency of glucuronyl transferase), Gilbert disease (insufficiency of glucuronyl transferase and block of absorption of bilirubin from blood by liver cells – «absorption jaundice»); Dubin-Johnson syndrome (disorder of transport of bilirubin diglucuronide from liver cells to blood - «excretory jaundice»).

The report is checked up _____

(The signature of the teacher, date)

Examples of Krock-1 tests

1. Toxicity of ammonia (especially for brain) is due to its capacity to disturb the functioning of Krebs cycle as a result of the removal from cycle of:
A. Alfa-Ketoglutarate
B. Citrate
C. Malate
D. Succinate
E. Fumarate

2. A patient has yellow skin colour, dark urine, dark-yellow faeces. The concentration of what substance will be increased in the blood serum of the patient?
A. Unconjugated bilirubin
B. Conjugated bilirubin
C. Mesobilirubin
D. Verdoglobin
E. Biliverdin

3. Jaundice is developed in neonates during 5-6 days after birth. The insufficient activity of what enzyme is a cause of this disorder?
A. UDP-glucuronyl transferase
B. Porphobilinogen synthase
C. Aminolevulinate synthease
D. Heme oxygenase
E. Biliverdin reductase

4. A patient suffering from rheumatism was administered glucocorticoid therapy. What changes of carbohydrate metabolism in the liver can be expected?
A. Stimulation of gluconeogenesis
B. Stimulation of glycogenesis
C. Stimulation of glycogen hydrolysis
D. Stimulation of glycogen phosphorolysis
E. Increase of glycogen phosphorylase activity

5. A congenital liver disease was diagnosed in a patient, which is accompanied with high bilirubinemia due to an increase in free (nonconjugated) bilirubin. It was detected a trace activity of glucuronyl transferase using liver biopsia. What disease can be recognized?
A. Crigler-Najjar syndrome
B. Gilbert syndrome
C. Physiological jaundice
D. Dubin-Johnson syndrome
E. Wilson disease

6. It was detected a block in the transformation of bilirubin to bilirubin diglucuronide in a patient with jaundice. The concentration of indirect bilirubin in blood was markedly increased. What pathology can be suggested in a patient?
A. Pre-hepatic jaundice
B. Hepatic jaundice
C. Post-hepatic jaundice
D. Tumour of pancreas and occlusion of bile duct
E. Addison's disease

7. A 46-year-old woman suffering from cholelithiasis developed jaundice. Her urine became dark-yellow and feces became colourless. Blood serum will have the highest concentration of the following substance:
A. Conjugated bilirubin
B. Unconjugated bilirubin
C. Biliverdin
D. Mesobilirubin
E. Urobilinogen

8. Investigation of a patient indicated on inflammatory processes in gall bladder, alteration of colloidal stability and high risk of bile stones formation. What substance from listed below favours the formation of bile stones?
A. Cholesterol
B. Urates
C. Phosphates
D. Oxalates
E. Lecithine

9. A hereditary liver pathology was expected in 14-year-old patient. It was detected a high content of direct bilirubin in blood, deposition of melanin in liver due to alteration of bilirubin excretion to bile by liver cells. This state is characteristic to the next disease:
A. Dubin-Johnson syndrome
B. Crigler-Najjar syndrome
C. Gilbert disease
D. Physiological jaundice
E. Wilson disease

10. Examination of a patient revealed that dental hypoplasia was caused by a hypovitaminosis of vitamins A and D. These vitamins were administered perorally but they didn't have any medicinal effect. What is the probable cause of disturbed vitamin assimilation?
A. Bile acid deficiency
B. Hypochlorhydria
C. Hyperchlorhydria
D. Achylia
E. Achlorhydria

11. A man is resting after intensive physical effort. Which pathway of glucose metabolism is the most active at this time?
A. Gluconeogenesis from lactate
B. Glycolysis
C. Glycogenolysis
D. Breakdown of glycogen to glucose
E. Gluconeogenesis from amino acids

12. What type of reaction is used for the transformation of indirect bilirubin into direct bilirubin?
A. Conjugation
B. Oxidation
C. Hydrolysis
D. Reduction
E. Methylation

13. Fatty liver develops owing to the dietary deficiency or insufficient production of endogenous lipotropic factors in humans. What substances from listed below can be considered as lipotropic factor?
A. Choline
B. Fatty acids
C. Bilirubin
D. Triacylglycerols
E. Cholesterol

14. A diet enriched with lipotropic factors was recommended to obese patient with a risk of a fatty liver development. What nutritional component is the most important in the diet?
A. Methionine
B. Cholesterol
C. Vitamin C
D. Glycine
E. Glucose

15. Which of the following indicators characterizes the lithogenic properties of bile and indicates the onset of stone formation?
A. Holato-cholesterol coefficient
B. Cholesterol of bile
C. Bile acids
D. Bilirubin of bile
E. Phospholipids

16. A 40-year-old man was diagnosed with viral hepatitis. A blood test showed an increase in the activity of all of following enzymes, except:
A. Creatine kinase
B. Aspartate aminotransferases
C. Lactate dehydrogenase
D. Sorbitol dehydrogenase
E. Alanine aminotransferase

17. Edema is observed in a 39-year-old patient after a severe hepatitis. It was found in the patient's blood serum: albumins – 32 %, globulins – 68 %. What factor caused the development of edema?
A. Decreased oncotic blood pressure
B. Decrease in blood pressure
C. Violation of vascular permeability
D. Enhancement of proteolysis
E. Decreased synthesis of procoagulants

18. A patient suffering from chronic hepatitis has a positive galactose test. The content of glucose in the blood is 3.0 mmol/L. The violation of what biochemical mechanisms caused these changes?
A. Isomerization reactions of monosaccharides
B. Gluconeogenesis
S. Glycolysis
D. Glycogenolysis
E. Storage of glycogen

19. What digestive process is altered in case of obstruction of bile duct and arrest of bile excretion to intestines?
A. Hydrolysis of triglycerides
B. Absorption of carbohydrates
C. Hydrolysis of proteins
D. Hydrolysis of complex sugars and polysaccharides
E. Absorption of amino acids

20. A 20-year-old man was diagnosed with hereditary deficiency of UDP-glucoronyl transferase. The increased level of what blood index confirms the diagnosis?
A. Unconjugated bilirubin
B. Conjugated bilirubin
C. Urobilin
D. Stercobilinogen
E. Animal indican

21. What metabolite is used in hepatocytes for the synthesis of ketone bodies?
A. Acetyl-CoA
B. Amino acids
C. Fatty acids
D. Glucose
E. Glycerol

22. What is the leading mechanism of the hepatic encephalopathy development?
A. Elevated level of ammonia in the blood
B. Decreased synthesis of prothrombin
C. Insufficient synthesis of plasma proteins
D. Decreased synthesis of urea
E. Increased indole formation

23. The yellowness of a skin and mucous membranes are observed in a patient after hemotransfusion. The increased levels of total and indirect bilirubin in blood, urobilin in urine and stercobilin in feces are found. What is the type of jaundice in the patient?
A. Hemolytic
B. Hereditary
C. Obstructive
D. Parenchymatous
E. Neonatal jaundice

24. A patient complains of frequent diarrhoeas, especially after consumption of food, rich in fat, weight loss. Laboratory examination revealed steatorrhea; the feces were hypocholic. What might have caused such condition?
A. Obstruction of biliary tracts
B. lnflammation of mucous membrane of small intestine
C. Lack of pancreatic lipase
D. Lack of pancreatic phospholipase
E. Unbalanced diet

25. A patient suffering from chronic hepatitis has signs of impaired digestion of fats (steatorrhea). What is the mechanism of its occurrence?
A. Decreased synthesis and secretion of bile acids
B. Decreased cholesterol synthesis
C. Decreased synthesis of lipase
D. Decreased absorption of triacylglycerols
E. Insufficiency of fat-soluble vitamins

26. The patient was diagnosed with cirrhosis of the liver. Study of which of the listed substances that are excreted in the urine, can characterize the state of the antitoxic function of the liver?
A. Hippuric acid
B. Ammonium salts
C. Creatinine
D. Uric acid
E. Amino acids

27. The deficiency of which group of vitamins occurs under the conditions of chronic liver failure?
A. Fat-soluble vitamins
B. Vitamin B complex
C. Water-soluble vitamins
D. Vitamins A, C, E
E. Vitamin H

28. What form of enzymopathy often forms the basis of hemolytic jaundice?
A. Deficiency of glucose-6-phosphate dehydrogenase
B. Deficiency of alkaline phosphatase
C. Deficiency of aldolase
D. Alanine aminotransferase deficiency
E. Acid phosphatase deficiency

29. What components of bile in a result of the damage of hepatocytes contribute to the development of inflammation of the oral mucosa (stomatitis, gingivitis)?
A. Bile acids
B. Bilirubin
C. Phospholipids
D. Cholesterol
E. Calcium

30. After a long unbalanced diet steatosis of liver has been developed in a patient. What was the leading factor of its development?
A. Lack of lipotropic factors
B. Increased intake of neutral fats
C. Reduced VLDL synthesis
D. Insufficiency of secretion of fat-mobilizing hormones
E. Reduced synthesis of glycogen

31. A doctor prescribed a diet for the patient, enriched with products with a high content of lipotropic factors. The synthesis of what lipids is optimized by such diet?
A. Phospholipids
B. Triacylglycerides
C. Cholesterol
D. Unsaturated fatty acids
E. Saturated fatty acids

32. It was revealed that bilirubin content (indirect bilirubin) is increased in the blood of a newborn child and faeces were intensively coloured (the enhanced level of stercobilin). Bilirubin is not found in urine. Which type of jaundice is it?
A. Neonatal physiologic jaundice
B. Hemolytic
C. Obstructive
D. Inherited
E. Hepatocellular

33. What metabolite is needed for the formation of direct bilirubin in hepatocytes?
A. Glucuronic acid
B. Fructose
C. Glucose
D. Albumin
E. Glycine

34. Which protein transports hemoglobin to the reticuloendothelial system of the liver?
A. Transferrin
B. Albumin
C. Feritin
D. Haptoglobin
E. Ceruloplasmin

35. After a serious viral infection a 3-year-old child has repeated vomiting, loss of consciousness, convulsions. Examination revealed hyperammonemia. What may have caused changes of biochemical blood indices in the child?

A. Disorder of ammonia neutralization in the ornithine cycle
B. Activated processes of amino acid decarboxylation
C. Disorder of biogenic amine neutralization
D. Increased putrefaction of proteins in intestine
E. Inhibited activity of transamination enzymes

Topic 19.
Research of biochemical functions of a liver, disturbances at particular diseases. Microsomal oxidation

1. Objective: To learn main pathways of xenobiotics detoxification. To learn reactions of microsomal oxidation. To understand the mechanisms of development of drug tolerance in interrelationship with the processes of microsomal oxidation.

2. Actuality of the theme: Liver detoxifies endotoxins and xenobiotics, including drugs, which are foreign for the body and can change or damage the course of metabolic processes. At the same time drugs and medicinals are used in pathology in order to normalize metabolism and favour reconvalescence. Knowledge of kinetics of absorption, transport, distribution and metabolism of drugs in human body is necessary for the development of new pharmaceuticals with desired properties.

3. Specific aims:

- ✓ To explain biochemical mechanisms of functioning of detoxification systems in the liver,
- ✓ To explain reactions of microsomal oxidation and conjugation in biotransformation of xenobiotics and endogenic toxins.

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

Questions:	References:
1. **Detoxification function of liver; biotransformation of xenobiotics and endogenous toxins.**	1. Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 638–639. 2. Gubsky Yu. Biological chemistry: textbook / edited by Yu. Gubsky. – Vinnytsia: Nova Knyha, 2018. – 2nd edition. – P. 408–409. 3. Lecture notes.
2. **Types of reactions of biotransformation of foreign substances in liver.**	1. Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 639–642. 2. Popova L.D. Biochemistry: Manual for medical students or interns / L.D. Popova, A.V. Polikarpova – Kharkiv: KNMU, 2011. – P. 449–452. 3. Gubsky Yu. Biological chemistry: textbook / edited by Yu. Gubsky. – Vinnytsia: Nova Knyha, 2018. – 2nd edition. – P. 409–413. 4. Lecture notes.
3. **Reactions of microsomal oxidation; inducers and inhibitors of microsomal monoxygenases.**	1. Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P.639–642. 2. Popova L.D. Biochemistry: Manual for medical students or interns / L.D. Popova, A.V. Polikarpova – Kharkiv: KNMU, 2011. – P. 449–452. 3. Gubsky Yu. Biological chemistry: textbook / edited by Yu. Gubsky. – Vinnytsia: Nova Knyha, 2018. – 2nd edition. – P. 409–413. 4. Lecture notes.
4. **Conjugation reactions in hepatocytes: biochemical mechanisms, functional significance.**	1. Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 640–642. 2. Popova L.D. Biochemistry: Manual for medical students or interns / L.D. Popova, A.V. Polikarpova – Kharkiv: KNMU, 2011. – P. 451–452. 3. Gubsky Yu. Biological chemistry: textbook / edited by Yu. Gubsky. – Vinnytsia: Nova Knyha, 2018. – 2nd edition. – P. 411–413. 4. Lecture notes.

5.	**Electron transport chains of endoplasmic reticulum. Genetic polymorphismus and induction of biosynthesis of cytochrome P-450.**	1. Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 639–640. 2. Popova L.D. Biochemistry: Manual for medical students or interns / L.D. Popova, A.V. Polikarpova – Kharkiv: KNMU, 2011. – P. 449–451. 3. Gubsky Yu. Biological chemistry: textbook / edited by Yu. Gubsky. – Vinnytsia: Nova Knyha, 2018. – 2nd edition. – P. 410. 4. Lecture notes.
6.	**Ethanol metabolism.**	1. Popova L.D. Biochemistry: Manual for medical students or interns / L.D. Popova, A.V. Polikarpova – Kharkiv: KNMU, 2011. – P. 452–454. 2. Lecture notes.

5. Tasks for independent work and self-control

5.1. Steroid hormones and bilirubin as well as drugs, ethanol, and other xenobiotics are taken up by liver and inactivated and converted into highly polar metabolites. Explain the biological reason of this converting.

5.2. The detoxification of xenobiotics is accomplished through the activity of a group of enzymes with a broad spectrum of biologic activity. Because many of these substances are lipophilic, they are oxidized, hydroxylated, or hydrolysed by enzymes of phase I metabolism. After that exposed hydroxyl groups or other reactive sites can be used for conjugation reactions of phase II. The overall purpose of the two phases of metabolism of xenobiotics is to increase their water solubility and thus excretion from the body.

The reactions of phase I are oxidation (hydroxylation, epoxide formation, sulfoxide formation, dealkylation, deamination), reduction and hydrolysis.

Complete the schemes of reactions.

A. Oxidation:

Alcohols:

CH_3OH ⟶ (Methanol) ☐

C_2H_5OH ⟶ (Ethanol) ☐

$C_6H_5CH_2OH$ ⟶ (Benzyl alcohol) ☐

Aldehydes:

C_6H_5CHO ⟶ (Benzaldehyde) ☐

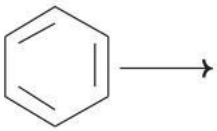
$C.Cl_3CHO$ ⟶ (Chloral) ☐

Amines and their derivatives

RCH_2NH_2 ⟶ [ ]
Aliphatic amine

$C_6H_5NH_2$ ⟶ [ ]
Aniline

Aromatic hydrocarbons


Benzene

⟶ [ ] + [ ] +

Sulfur compounds

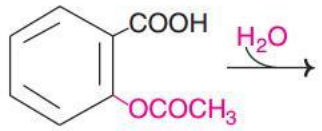
Organic sulfur ⟶ [ ] + [ ]

B. Reduction:

$CCl_3.CH(OH)_2$ ⟶ [ ]
Chloral

$C_6H_5NO_2$ ⟶ [ ]
Nitrobenzene

C. Hydrolysis:



Aspirin
(acetyl-
salicylic acid)

⟶ [ ] + [ ]

5.3. Most of the oxidation reactions of detoxification are catalysed by monooxygenase or cytochrome P450. This enzyme, also called mixed function oxidase, is associated with the microsomes. The enzymatic components of cytochrome P450 system (microsomal electron transport system) are the flavoprotein NADPH-cytochrome P450 reductase and cytochrome P450. Indicate the prosthetic group of both enzymes.

5.4. All of the oxidative reactions catalysed by cytochrome P450 may be viewed as hydroxylation reactions. Write the general equation of this reaction.

5.5. Reductase subunit uses NADPH as a substrate. Indicate the pathway that is the source of NADPH.

5.6. Several xenobiotics undergo detoxification by conjugation to produce less toxic and/or more easily excretable compounds. Conjugation is the process in which a foreign compound combines with a substance produced in the body. The process of conjugation may occur either directly or after the phase I reactions. At least 8 different conjugating agents have been identified in the body. Name these ones and write the examples of reactions.

1)

2)

3)

4)

5)

6)

7)

8)

5.7. Ethanol is a small molecule that is both lipid and water soluble. It is, therefore, readily absorbed from the intestine by passive diffusion. A small percentage of ingested ethanol (0-5 %) enters the mucosal cells of the upper GI tract (tongue, mouth, esophagus, and stomach), where it is metabolized. The remainder enters the blood. Of this, 85 to 98 % is metabolized in the liver, and only 2 to 10 % is excreted through the lungs or kidneys. Ethanol is oxidized in the liver to form acetate, which is converted to acetyl-CoA:

1) ethanol + NAD^+ → acetaldehyde + $NADH+H^+$

Enzyme: alcohol dehydrogenase (ADH)

2) acetaldehyde + NAD^+ → acetate + $NADH+H^+$

Enzyme: aldehyde dehydrogenase (ALDH)

3) acetate + ATP + CoA → acetyl-CoA + AMP + PPi

Enzyme: acetyl-CoA synthetase.

a) Most of the acetate generated in the liver from ethanol enters the blood. It is taken up and oxidized by other tissues, notably heart and skeletal muscle, which have a high concentration of the mitochondrial acetylCoA synthetase isoform. This isoenzyme is present in the mitochondrial matrix. It therefore generates acetylCoA that can directly enter the citric acid cycle and be oxidized to CO_2. Determine how many molecules of ATP are produced from 1 molecule of ethanol.

b) Explain the toxicity of ethanol.

5.8. Situational tasks:

a) The hemoproteins referred to as cytochrome P450 are so named because of the complexes they form with carbon monoxide. In the presence of CO, light is strongly absorbed at a wavelength of 450 nm.

Where in the cell are cytochromes P450 located?

What other cytochromes are present in the cell?

Indicate the likeness and difference of cytochrome P450 and mitochondrial cytochrome oxidase.

b) The drug isoniazid, used in the treatment of tuberculosis, is subject to acetylation. Polymorphic types of acetyltransferases exist, resulting in individuals who are classified as slow or fast acetylators, and influence the rate of clearance of drugs such as isoniazid from blood.

What individuals (fast or slow acetylators) are more subject to certain toxic effects of isoniazid?

Why?

6. Individual independent students work

1. Biochemical bases of detoxification processes of xenobiotics and endogenous toxins.
2. Assess liver detoxification function by biochemical parameters.

Practice protocol №19 «____» _____ 20___

Experiment 1. Determination of indican in urine (Obermeyer test).

Principle. Indican (animal indican) is potassium indoxyl sulfate. The method is based on transformation of indican into indoxyl during acid hydrolysis of the ester bond with a strong mineral acid (hydrochloric acid). At presence of thymol the obtained indoxyl is oxidized with ferric chloride to indigolignone (pinkish-violet).

Procedure.

Add 2 ml of 10 % solution of lead acetate ($Pb(CH_3COO)_2$) to 4 ml of urine for sedimentation of bile pigments, salts and other substances that interfere with the reaction.

Filter the obtained precipitate. Add 1 ml 5 % thymol solution in 96 % ethanol and 2 ml of Obermeyer reagent (0,4 g of ferric chloride $FeCl_3$ dissolved in 100 ml hydrochloric acid) to 2 ml of the filtrate. In 10 minutes add 1 ml of chloroform, mix thoroughly and observe formation of two layers: water on the top and chloroform in the bottom. The chloroformic layer is pinkish-violet.

The colour intensity increases to reddish-violet at indicanuria. It enables a semiquantitative estimation of results.

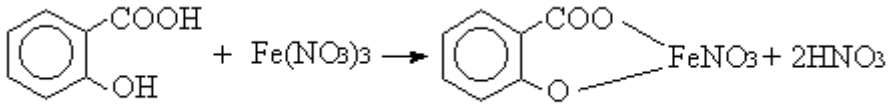
To obtain exact results, transfer the bottom chloroformic layer to another test tube with a pipette and add several drops of 20 % sodium tiosulfate solution. If indican is absent in urine the colour disappears after addition of sodium tiosulfate. At positive reaction, if indican is present in urine, the colour of the chloroform layer does not disappear after addition of sodium tiosulfate.

Results:

Conclusion:

Experiment 2. Detection of salicylic acid in biological fluids.

Salicylic acid is used in medicine in a form of sodium salts, ointments or chemical derivatives (aspirin) for the treatment of different inflammatory conditions, pain and fever relief, in skin diseases as well as with the purpose of prevention of blood thrombosis. Salicylic acid is excreted from the body partially in unchanged form, as well as in the form of conjugates with glycine or glucuronic acid. Metabolites of salicylic acid include O-hydroxybenzoylglucuronide and O-carboxyphenylglucuronide. In the course of hydroxylation 2,3-dihydroxybenzoic acid, 2,5-dihydroxybenzoic acid and 2,3,5-trihydroxybenzoic acid are also formed, which are eliminated with urine.



Procedure.

Add 4.5 ml of 0.55 % solution of iron nitrate in 0,04 N solution of nitric acid to 0.5 ml of urine.

Development of purple red colour indicates the presence of salicylic acid in tested sample.

Results:

Conclusion:

Side effects of xenobiotics oxidation, significance for medicine and pharmacotherapy. Consumption of some drugs may lead to a state of tolerance, in which there is a decrease in responsiveness to the medication. This phenomenon is caused by proliferation of smooth endoplasmic reticulum membrane as well as increase in total protein and microsomal protein, especially cytochrome P-450 per gram of liver. Cross-tolerance may also occur. The use of the anticoagulant warfarin is a classic example of such an interaction. When phenobarbital and warfarin are administered together, warfarin soon becomes ineffective. If phenobarbitol is withdrawn after warfarin dosage has been increased, there is an increased risk of internal haemorrhage in a patient. That is why medical personnel should be aware of such cases.

The report is checked up _____

(The signature of the teacher, date)

Examples of Krock-1 tests

1. A patient with alcohol-induced liver injury has an impairment of biotransformation of xenobiotics and endogenous toxic compounds. These changes are likely to be caused by hypoactivity of the following chromoprotein:
A. Cytochrome P-450
B. Hemoglobin
C. Cytochrome oxidase
D. Cytochrome b
E. Cytochrome c_1

2. A 50-year-old patient with the food poisoning is on a drip of 10 % glucose solution. It not only provides the body with necessary energy, but also performs the function of detoxification by the production of a metabolite that participates in the following conjugation reaction:
A. Glucuronidation
B. Sulfation
C. Methylation
D. Glycosylation
E. Hydroxylation

3. Neutralization of xenobiotics (drugs, epoxide, arenes, aldehydes, nitroderivatives and endogenous metabolites of estradiol, prostaglandins, leukotrienes) is realized in the liver by their conjugation with:
A. Glutathione
B. Aspartic acid
C. Creatine
D. S-adenosylmethionine
E. Phosphoadenosine

4. Study of conversion of a food colouring agent revealed that neutralization of this xenobiotic takes place only in one phase that is microsomal oxidation. Name a component of this phase:
A. Cytochrome P-450
B. Cytochrome b
C. Cytochrome c
D. Cytochrome a
E. Cytochrome oxidase

5. The universal biological system of the nonpolar substance oxidation (many of drugs, toxic substances, steroid hormones, cholesterol) is a microsomal oxidation. What cytochrome is included to the composition of the microsomal oxidation chain?
A. Cytochrome P-450
B. Cytochrome a_3
C. Cytochrome b
D. Cytochrome c
E. Cytochrome a

6. Anti-alcohol drug «teturam» is an inhibitor of aldehyde dehydrogenase. It is widely used in medical practice for prevention of alcoholism. The increasing of what metabolite in the blood causes aversion to alcohol?
A. Acetaldehyde
B. Ethanol
C. Malondialdehyde
D. Acetone
E. Methanol

7. Biological oxidation and neutralization of xenobiotics occurs due to heme-containing enzymes. Which metal is an indispensable component of these enzymes?
A. Fe
B. Mg
C. Zn
D. Co
E. Pb

8. A patient suffers from hepatocirrhosis. State of antitoxic liver function can be characterized by the examination of the following substance excreted by urine:
A. Hippuric acid
B. Ammonium salts
C. Creatinine
D. Uric acid
E. Amino acids

9. What is amino acid used for detoxification of benzoic acid to form hippuric acid in liver called?
A. Glycine
B. Alanine
C. Serine
D. Glutamic acid
E. Valine

10. What is an active form of sulphuric acid used in conjugation step of xenobiotics detoxification in liver called?
A. 3'-Phosphoadenosin-5'-phosphosulfate
B. UDP-sulfate
C. Sulfuryl-CoA
D. Guanosine sulfate
E. Thionyl chloride

11. A part of food protein is not digested in the gastrointestinal tract and is decomposed by bacterial enzymes in large intestine. This is defined as a putrifaction of protein. What substance detection in urine may serve as an indicator of the intensity of a putrifaction processes?
A. Animal indican
B. Protein
C. Urea
D. Creatine and creatinine
E. Urates

12. Patient with encephalopathy was admitted to the neurological department. Correlation of an increasing of encephalopathy and substances absorbed by the bloodstream from the intestine was revealed. What substances that are created in the intestine can cause endotoxemia?
A. Indole
B. Butyrate
C. Acetacetate
D. Biotin
E. Ornithine

13. Monooxygenase systems of liver cell endoplasmic reticulum contain cytochrome P-450, $NADPH_2$ cytochrome P-450 reductase, FAD and FMN-dependent enzymes. All these enzymes catalyse the following type of chemical reaction:
A. Hydroxylation
B. Acylation
C. Dehydrogenation
D. Methylation
E. Conjugation

14. Reactions of xenobiotics and endogenous toxins biotransformation in hepatocytes proceed or by oxidoreductive and hydrolytic conversions, or by conjugation. During the conjugation the compound, which should be detoxified, cannot bind the residue of:
A. Gluconic acid
B. Glucuronic acid
C. Sulfatic acid
D. Glycine
E. Glutathione

15. Development of resistance to drugs (drug tolerance) appears due to the following process:
A. Increase in the activity of microsomal oxidation
B. Decrease in the activity of mitochondrial oxidation
C. Increase in the activity of mitochondrial oxidation
D. Decrease in activity of microsomal oxidation
E. Activation of peroxide oxidation

16. The detoxification of xenobiotics (pharmaceuticals, epoxides, arenoxydes, aldehydes, nitro-derivatives and endogenous metabolites (estradiol, prostaglandines, leukotriens) will be realized in a liver by way of conjugation:
A. Glutathione
B. Aspartic acid
C. Glycine
D. S-adenosyl methionine
E. Phosphadenosine

17. Glucuronic acid used for conjugation reaction in detoxification of xenobiotics exists in the next active form:
A. UDP-glucuronate
B. Glucuronyl pyrophosphate
C. AMP-glucuronate
D. CDP-glucuronate
E. Glucuronyl-CoA

18. Which of the following reactions is used for the inactivation of cadaverine and putrescine in hepatocytes?
A. Hydroxylation
B. Reduction
C. Conjugation with glucuronic acid
D. Conjugation with glycine
E. Oxidation

19. A 2-day-old baby has yellowish skin and mucous membranes. This might be caused by the temporary deficiency of the following enzyme:
A. UDP-glucuronil transferase
B. Sulfotransferase
C. Heme synthetase
D. Hemoxygenase
E. Biliverdine reductase

20. The detoxification of natural metabolites and xenobiotics is disturbed in the patient's liver. The decrease of which chromoprotein activity can be reason of this?
A. Cytochrome P-450
B. Hemoglobin
C. Cytochromeoxidase
D. Cytochrome b
E. Cytochrome c_1

21. Jaundice treatment involves administration of barbiturates inducing the synthesis of UDP-glucuronyl transferase. A medicinal effect is caused by the production of:
A. Direct bilirubin
B. Indirect bilirubin
C. Biliverdin
D. Protoporphyrin
E. Heme

22. Patient has cirrhosis of liver. Investigation of which enumerated substances to be excreted with urine can characterize the state of a detoxification function of a liver?
A. Hippuric acid
B. Ammonius salts
C. Creatinine
D. Uric acid
E. Amino acids

23. What substance serves as a donor of hydrogen in reactions of microsomal oxidation?
A. $NADPH_2$
B. Ascorbic acid
C. Glucuronic acid
D. FAD
E. FMN

24. A patient is ill with hepatocirrhosis. State of antitoxic liver function can be characterized by examination of the following substance excreted by urine:
A. Hippuric acid
B. Uric acid
C. Ammonium salts
D. Amino acids
E. Creatinine

25. Examination of a chemical plant worker who had a poisoning revealed an increase in total bilirubin concentration at the expense of indirect fraction. Feces and urine are characterized by high stercobilin concentration. The level of direct bilirubin in the blood plasma is normal. What is type of jaundice in this case?
A. Hemolytic
B. Obstructive
C. Hepatic
D. Addison's disease
E. Neonatal physiologic jaundice

26. The symptoms of jaundice were appeared in newborn. The introducing of small doses of phenobarbital, which induces synthesis of UDP-glucuronyl transferase, has influenced meliorating of a state of the child. Which processes will be actuated under the influence of enzyme induced by phenobarbital?
A. Conjugation
B. Microsomal oxidation
C. Fabric breathing
D. Gluconeogenesis
E. Synthesizing glycogen

27. A 50-year-old man, who has been suffering for a long time from viral hepatitis, developed mental impairments, impairments of consciousness, and motor disturbances (tremor, ataxia, etc.). What is the mechanism of such condition?
A. Decreased detoxification function of the liver
B. Insufficient phagocytic function of stellate macrophages
C. Decreased synthesis of albumins and globulins in the liver
D. Disturbed lipid exchange in the liver
E. Alterations in the lipid composition of blood

28. A 50-year-old patient with food poisoning is on a drip of 10 % glucose solution. It not only provides the body with necessary energy, but also performs the function of detoxification by the production of a metabolite that participates in the following conjugation reaction:
A. Glucuronidation
B. Sulfation
C. Methylation
D. Glycosylation
E. Hydroxylation

29. Cytochrome P-450 belongs to the next class of enzymes:
A. Oxidoreductases
B. Transferases
C. Hydrolases
D. Lyases
E. Isomerases

Topic 20.
Investigation of water-salt and mineral exchanges

1. Objective: To learn processes of water and minerals turnover in human body. To learn methods of qualitative and quantitative determination of inorganic substances in blood serum and to interpret obtained results with diagnostic purpose and in treatment of some diseases.

2. Actuality of the theme. Water and minerals are of vital importance for normal functioning of human body. Changes in water and minerals turnover occur in different diseases, thus knowledge and understanding of these processes as well as their qualitative and quantitative determination is necessary for evaluation of water-mineral metabolism.

3. Specific aims:

- ✓ To interpret biochemical investigations of indices of different minerals and trace elements level in human body.
- ✓ Based on biochemical investigations to diagnose and distinguish different types of dehydration.
- ✓ To obtain skills in determination of the level of principal minerals in human body.

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

Questions:	References:
1. **Biological role of water and its distribution in human body. Water balance, its types.**	1. Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 468–470. 2. Popova L.D. Biochemistry: Manual for medical students or interns / L.D. Popova, A.V. Polikarpova – Kharkiv: KNMU, 2011. – P. 429–432. 3. Lecture notes.
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3. **Mechanism of Na,K-ATPase action and its regulation.**	1. Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 168, 653. 2 .Lecture notes.
4. **Regulation of water and mineral metabolism, its disorders. Dehydratation and rehydratation, biochemical mechanisms of their development.**	1. Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 472–474. 2. Popova L.D. Biochemistry: Manual for medical students or interns / L.D. Popova, A.V. Polikarpova – Kharkiv: KNMU, 2011. – P. 432–436. 3. Lecture notes.
5. **Biogenic elements, their classification, pathways of their providing.**	1. Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – 2006. – P. 403–422. 2. Lecture notes.
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7. **Water and mineral metabolism disorders.**	1. Popova L.D. Biochemistry: Manual for medical students or interns / L.D. Popova, A.V. Polikarpova – Kharkiv: KNMU, 2011. – P. 437–441. 2. Lecture notes.
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5. Tasks for independent work and self-control

5.1. Water is the solvent of life. Undoubtedly, water is more important than any other single compound to life. It is involved in several body functions.

a) Describe the structure and properties of a water molecule and explain why water is an ideal biologic solvent.

b) Describe the influence of water on the structure of biomolecules.

c) What pathway is the major source of metabolic water?

d) Explain the significance of water in the regulation of body temperature.

5.2. Water is the major body constituent. An adult human contains about 60 % water (men 55-70 %, women 45-60 %). The women and obese individuals have relatively less water which is due to the higher content of stored fat in an anhydrous form. A 70 kg normal man contains about 42 litres of water.

Indicate the volume of fluid compartments in the body based on an average 70-kg man:

1) intracellular:

2) extracellular (interstitial + blood plasma and transcellular fluids):

5.3. Ingestion of water is mainly controlled by a _____ centre located in the _____.

How is increasing in the osmolality of plasma causes increased water intake?

5.4. Water losses from the body are variable. There are four distinct routes for the elimination of water from the body. Describe these ones and their regulation.

1) Urine:

2) Skin:

3) Lungs:

4) Feces:

5.5. Water will move from a compartment with a low concentration of solutes (lower osmolality) to one with a higher concentration to achieve an equal osmolality on both sides of the membrane. Indicate the direction of water transport for such states:

1) hypoproteinemia

2) hyperglycemia

3) hyperaldosteronism

4) hypoaldosteronism

5.6. Electrolytes are the compounds which readily dissociate in solution and exist as ions i.e. positively and negatively charged particles. There is a marked difference in the concentration of electrolytes (cations and anions) between the extracellular and intracellular fluids.

Name the principal extracellular cation and the intracellular cation.

5.7. There are two ways of expressing the concentration of molecules with regard to the osmotic pressure. Give a definition of the concepts:

Osmolarity

Osmolality

5.8. The osmolality of plasma is in the range of 285-295 milliosmoles/kg. How is plasma osmolality computed for practical purposes?

5.9. The cells have a *high/low* intracellular K^+ concentration and a *high/low* Na^+ concentration. This is essentially needed for the survival of the cells. High cellular K^+ is required for _____ and for _____. Further, Na^+ and K^+ gradients across plasma membranes are needed for the _____.

5.10. Describe the structure and function of Na^+-K^+ ATPase. Name the its inhibitors.

5.11. Electrolyte and water balance are regulated together and the kidneys play a predominant role in this regard. The regulation is mostly achieved through the hormones aldosterone, ADH and renin-angiotensin Explain their action on electrolyte balance.

Hormone	Regulatory role
Aldosterone	
Antidiuretic hormone (ADH, vasopressin)	
Renin-angiotensin	
Atrial natriuretic factor (ANF)	

5.12. Indicate the causes and characteristic features of the disorders of water balance

The causes and characteristic features of dehydration	The causes and characteristic features of overhydration

5.13. The minerals are classified as principal elements and trace elements. The seven principal elements (macrominerals) constitute 60-80 % of the body's inorganic material. These are calcium, phosphorus, magnesium, sodium, potassium, chloride and sulfur. The principal elements are required in amounts greater than 100 mg/day.

Fill in the table «The major characteristics of principal elements (macroelements)»

Element	Major functions	Deficiency disease/symptoms	Major sources
Calcium			
Phosphorus			
Magnesium			
Sodium			
Potassium			
Chlorine			
Sulfur			

5.14. What causes of elevation and decrease of the following electrolytes you know and clinical signs of increased and decreased level of relevant macroelement in blood. Fill in the table:

Electrolyte, normal range	Causes of elevation	Causes of decrease
Sodium (135-145 mEq/L)		
Potassium (3.5-5.0 mEq/L)		
Calcium (8.5-10.5 mg/dL)		
Magnesium (1.5-2.5 mg/dL(		

5.15. The (microminerals) are required in amounts less than 100 mg/day. Fill in the table «The major characteristics of trace elements (microelements)»:

Element	Biochemical functions		Deficiency disease/symptoms
	Compounds containing TE	Major functions	
Iron	Hemoglobin Myoglobin Cytochromes Fe-S-proteins Catalase, peroxidases Transferrin Ferritin		
Cooper	Cytochrome oxidase Lysyl oxidase Tyrosinase Superoxide dismutase Ceruloplasmin		
Iodine	T_4, T_3 Thyreoglobulin		
Manganese	Pyruvate carboxylase Superoxide dismutase Glycosyltransferases Arginase		
Zinc	Carbonic anhydrase Carboxypeptidase Alcohol dehydrogenase Alkaline phosphatase Superoxide dismutase		
Molibdenum	Xanthine oxidase Aldehyde oxidase Sulfite oxidase		
Cobalt	Cobalamin		
Fluorine	Fluoroapatite		
Selenium	Glutathione peroxidase Selenocysteine		
Chromium	-		

5.16. Situational tasks:

a) A 6-year old boy had bone deformities such as bow legs and pigeon chest. He had a history of delayed eruption of teeth. On enquiring, the mother informed the physician that the boy had been on a strict vegetarian diet with low intake of milk as well as fats and oils. The following are the laboratory findings of this boy.

Parameter	Subject	Reference range
Calcium	8.1 mg/dl	9-11 mg/dl
Phosphate	2.5 mg/dl	3-4.5 mg/dl
Alkaline phosphatase	40 KAU/dl	3-13 KAU/dl

Make a diagnosis and explain its cause.

b) A 25-year old strict vegetarian woman, with two children aged 5 years and 3 years, complained of tiredness and appeared pale. In the recent few months, the woman had heavy and prolonged menstrual flow. Enquiries revealed that her consumption of milk and milk products was reasonably good, but leafy vegetables was low. The laboratory investigations showed that the woman's hematocrit was 28 % (reference range 40 % to 50 %), while her hemoglobin concentration was 8 g/dl (reference range 13-15 g/dl).

Make a diagnosis and explain its cause.

6. Individual independent students work

1. Human microelementosis.
2. Diagnostics of water-electrolyte composition.

Practice protocol №20 «____» _____ 20___

Experiment 1. Determination of calcium in urine (Sulkovitz probe).

Principle. The method is based on formation of insoluble Ca compounds in its increased quantity in urine. Sulkovitz reagent contains oxalic acid 2.5 g, ammonium oxalate – 2.5 g, conc. acetic acid – 5.0 ml, the volume is adjusted to 150 ml with water.

Procedure.

Place 5 ml of urine into the tube and add 2.5 ml of Sulkovitz reagent to it.

Observe the development of milky turbidity in a tube after 30 sec.

Make a conclusion, knowing that the quantity of calcium in urine is relevant to the intensiveness of the turbidity. If the content of calcium in urine is decreased – urine after adding Sulkovitz reagent remains transparent.

Results:

Conclusion:

Clinical and diagnostic significance. Calcium is an essential macroelement for the maintenance of homeostasis in human body, being a structural constituent of bones and involved in the processes of muscular contraction. Calcium turnover in human body is regulated by parathormon, derivatives of vitamin D3 and calcitonin. The normal level of calcium in blood is 2.2–2.6 mmol/l. The normal level of calcium in daily urine is 2.5-8.0 mmol/day. An increase or diminishing of the level of calcium ions in blood plasma can result in the development of different pathologic conditions. Pharmacologic remedies that contain salts of calcium have got wide application in different fields of medicine.

Experiment 2. Colorimetric method of potassium determination in blood serum. (Lasarev method)

Principle. Potassium ions in presence of Pb^{+2}, Cu^{+2} and NO^{2-} ions form water insoluble compound $K_2Pb[Cu(NO_2)_6]$, which can be dissolved in a mixture of rivanol and concentrated acetic acid. Optical density of solution is determined, which is proportional to the quantity of NO2 ions.

Procedure.

Add 1 ml of rivanol solution and 2 ml of concentrated acetic acid to the sediment of blood serum sample.

Stir the mixture and when sediment is dissolved adjust the volume of the solution to 25 ml with water.

Measure the optical density of the obtained solution at 540 nm (green filter). Estimate the level of potassium according to the formula:

K = A x 36 (mg %), where A is the value of extinction.

Results:

Conclusion:

Clinical and diagnostical significance.

Potassium is a main intracell cation. The normal level of potassium in blood serum makes 3.5–5.0 mmol/l. Decline of potassium level in blood (less than 3 mmol/l) results in general fatigue, weakness of legs, myalgias, disturbances of heart action, in severe cases violation of

breathing, paralysis and intussusception may occur. Hypopotassiemia may be caused by the use of diuretics, evacuates, after intensive vomiting, diarrhoea. Increase of the potassium level (hyperpotassiemia) mainly occurs in cases of kidney insufficiency and causes bradycardia, that in severe cases may lead to heart stoppage.

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

Examples of Krock-1 tests

1. The development of Addison-Biermer's disease (pernicious hyperchromic anemia) is due to a deficiency of vitamin B_{12}. Choose metal which is included to composition of this vitamin:
A. Cobalt
B. Zink
C. Molybdenum
D. Magnesium
E. Iron

2. The patient complains of thirst and polyuria. The urine analysis revealed: daily diuresis – 10 L; urine density – 1.001 (normal – 1.012-1.024). Which disease causes the indexes?
A. Diabetes incipidus
B. Steroid diabetes
C. Thyrotoxicosis
D. Acromegaly
E. Diabetes mellitus

3. Calcium ions are the second messengers in cells. They activate catabolism of glycogen interacting with:
A. Calmodulin
B. Calcitonin
C. Calciferol
D. Glutamine
E. Phospholipase C

4. In the patient the blood plasma level of potassium is 8 mmol/l (normal value – 3.6-5.3 mmol/l). That state is accompanied by:
A. Arrhythmia, heart stop
B. Tachycardia
C. The decrease of arterial pressure
D. The increase of arterial pressure
E. Bradycardia

5. Which is the normal blood calcium level (in mmol/l)?
A. 2.25–2.75
B. 1.75–2.00
C. 1.50–1.75
D. 3.0–4.5
E. 0.65–1.60

6. A patient has severe blood loss caused by an injury. What kind of dehydration will be observed in this particular case?
A. Iso-osmolar
B. Hyposmolar
C. Hyperosmolar
D. Normosmolar
E. –

7. A surgeon made an error to have removed patient's parathyroid gland while operating thyroid gland. The patient developed convulsions, tetany. Which element metabolism is disturbed?
A. Calcium
B. Magnesium
C. Potassium
D. Iron
E. Sodium

8. Osmotic pressure of a man's blood plasma is 350 mosmole/l (standard pressure is 300 mosmole/l). First of all it is due a high secretion of the following hormone:
A. Aldosteron
B. Adrenocorticotropin
C. Cortisol
D. Natriuretic
E. Vasopressin

9. A 50-year-old patient complains about general weakness, appetite loss and cardiac arrhythmia. The patient has muscle hypotonia, flaccid paralysis, weakened peristaltic activity of the bowels. Such condition might be caused by:
A. Hypokaliemia
B. Hypophosphatemia
C. Hyponatremia
D. Hypoproteinemia
E. Hyperkaliemia

10. A 35 y.o. patient who often consumes alcohol was treated with diuretics. There appeared serious muscle and heart weakness, vomiting, diarrhoea, AP- 100/60 mm Hg, depression. This condition is caused by intensified excretion with urine of:
A. Potassium
B. Phosphates
C. Chlorine
D. Sodium
E. Calcium

11. Goiter is a disease which is widely spread in some biogeochemical areas of the earth. Which element deficit causes this disease?
A. Iodine
B. Iron
C. Zinc
D. Copper
E. Cobalt

12. In liver of patient with iron deficient anemia the disturbance of formation of metalloprotein (the source of iron for heme synthesis) was revealed. Point this protein:
A. Ferritin
B. Transferrin
C. Hemosiderin
D. Myoglobin
E. Cytochrome c

13. As a result of increased permeability of the erythrocyte membrane in a patient with microspherocytic anaemia (Minkowsky-Shauffard disease) cells receive sodium ions and water. Erythrocytes take form of spherocytes and can be easily broken down. What is the leading mechanism of erythrocyte damage in this case?
A. Electrolytic osmotic
B. Nucleic
C. Calcium
D. Acidotic
E. Protein

14. Pathological changes of liver and brain were revealed in a 27-year-old-patient. Copper concentration is abruptly decreased in blood the plasma and increased in the urine. Wilson's disease was diagnosed. What enzyme activity in the blood serum should be examined to prove the diagnosis?
A. Ceruloplasmin
B. Xanthinoxidase
C. Alcoholdehydrogenase
D. Leucinaminopeptidase
E. Carboanhydrase

15. Examination of a 27-year-old patient revealed pathological changes in liver and brain. Blood plasma analysis revealed an abrupt decrease in the copper concentration, urine analysis revealed an increased copper concentration. The patient was diagnosed with Wilson's degeneration. To confirm the diagnosis it is necessary to study the activity of the following enzyme in blood serum:
A. Ceruloplasmin
B. Alcohol dehydrogenase
C. Leucine aminopeptidase
D. Carbonic anhydrase
E. Xanthine oxidase

16. A patient is 44 years old. Laboratory examination of his blood revealed that content of proteins in plasma was 40 g/l. What influence will be exerted on the transcapillary water exchange?
A. Filtration will be increased, reabsorption – decreased
B. Both filtration and reabsorption will be increased
C. Both filtration and reabsorption will be decreased
D. Filtration will be decreased, reabsorption – increased
E. Exchange will stay unchanged

17. Wilson's disease is a disorder of copper transport which leads to the accumulation of this metal in brain and liver cells. It is associated with a disturbance in the synthesis of the following protein:
A. Ceruloplasmin
B. Transcobalamin
C. Metallothionein
D. Haptoglobin
E. Siderophilin

18. A woman with intractable vomiting was admitted to the infectious disease ward. What changes of water-salt metabolism are likely to be observed?
A. Hypoosmolar dehydration
B. Isoosmolar dehydration
C. Hyperosmolar dehydration
D. Hypoosmolar hyperdehydration
E. Hyperosmolar hyperdehydration

19. After a surgery a 36-year-old woman was given an intravenous injection of concentrated albumin solution. This has induced intensified water movement in the following direction:
A. From the intercellular fluid to the capillaries
B. From the intercellular fluid to the cells
C. No changes of water movement will be observed
D. From the capillaries to the intercellular fluid
E. From the cells to the intercellular fluid

20. A man weighs 80 kg, after long physical activity his circulating blood volume is reduced down to 5,4 l, hematocrit makes up 50 %, whole blood protein is 80 g/l. These blood characteristics are determined first of all by:
A. Water loss with sweat
B. Increased number of erythrocytes
C. Increased protein concentration in plasm
D. Increased circulating blood volume
E. Increased dieresis

21. A 15 year old girl has pale skin, glossitis, gingivitis. Blood count: erythrocytes – 3.3×10^{12}/l, hemoglobin – 70 g/l, colour index – 0.5. Examination of blood smear revealed hypochromia, microcytosis, poikilocytosis. What type of anemia is it?
A. Iron-deficient
B. B_{12}-folic acid deficient
C. Sickle-cell
D. Hemolytic
E. Thalassemia

22. A 35 year old man consulted a dentist about reduced density of dental tissue, high fragility of teeth during eating solid food. This patient suffers the most probably from the deficiency of the following mineral element:
A. Calcium
B. Potassium
C. Sodium
D. Magnesium
E. Iron

23. Reduced activity of antioxidant enzymes enhances peroxidation of cell membrane lipids. The reduction of glutathione peroxidase activity is caused by the following microelement deficiency:
A. Selenium
B. Molybdenum
C. Cobalt
D. Manganese
E. Copper

24. A female patient with toxemia of pregnancy has hypersalivation resulting in a daily loss of 3–4 litres of saliva. What disorder of water-salt metabolism occurs in such cases?
A. Hyperosmolar hypohydration
B. Hypoosmolar hypohydration
C. Isoosmolar hypohydration
D. Hypokalemia
E. Hyponatremia

25. A patient who had been continuously taking drugs blocking the production of angiotensin II developed bradycardia and arrhythmia. A likely cause of these disorders is:
A. Hyperkalemia
B. Hypokalemia
C. Hypernatremia
D. Hypocalcemia
E. Hypercalcemia

26. An isolated heart of a mammal has had diastolic arrest in the process of perfusion with an ion-rich solution. Solution had excess of the following ions:
A. Potassium
B. Sodium
C. Magnesium
D. Chlorine
E. Calcium

27. After a person had drunk 1,5 liters of water, the amount of urine increased significantly, and its relative density decreased to 1,001. These changes are a result of decreased water reabsorption in the distal nephron portion due to reduced secretion of:
A. Vasopressin
B. Aldosterone
C. Angiotensin II
D. Renin
E. Prostaglandins

28. A 20-year-old patient complains of morbid thirst and hyperdiuresis (up to 10 l daily). Glucose concentration in blood is normal but it is absent in urine. The patient has been diagnosed with diabetes insipidus. What hormonal drug is the most appropriate for management of this disorder?
A. Antidiuretic hormone
B. Cortisol
C. Thyroxin
D. Oxytocin
E. Insulin

29. A patient has insufficient blood supply to the kidneys, which has caused the development of pressor effect due to the constriction of arterial resistance vessels. This is the result of the vessels being greatly affected by the following substance:
A. Angiotensin II
B. Angiotensinogen
C. Renin
D. Catecholamines
E. Norepinephrine

30. A 19-year-old young man has been examined in a nephrological hospital. Increased potassium content was detected in secondary urine of the patient. Such changes have been most likely caused by the increased secretion of the following hormone:
A. Aldosterone
B. Oxytocin
C. Adrenalin
D. Glucagon
E. Testosterone

31. A patient with pituitary tumour complains of increased daily diuresis (polyuria). Glucose concentration in blood plasma equals 4.8 mmol/l. What hormone can be the cause of this, if its secretion is disturbed?
A. Vasopressin
B. Aldosterone
C. Natriuretic hormone
D. Insulin
E. Angiotensin I

32. During perfusion of an isolated heart of a mammal with a high ion content solution the cardiac arrest in diastole occurred. The cardiac arrest was caused by the following ions present excessively in the solution:
A. Potassium
B. Sodium
C. Chlorine
D. Magnesium
E. Calcium

33. Examination of a patient revealed glycosuria and hyperglycemia. He complains of dry mouth, itchy skin, frequent urination, thirst. He has been diagnosed with diabetes mellitus. What is the cause of polyuria in this patient?
A. Increased urine osmotic pressure
B. Decreased plasma oncotic pressure
C. Increased filtration pressure
D. Decreased cardiac output
E. Increased plasma oncotic pressure

34. In human organism significant blood loss leads to decreased blood pressure, tachycardia, and weakness. Eventually the sensation of thirst appears. What hormone participates in the development of this sensation?
A. Angiotensin II
B. Adrenalin
C. Cortisol
D. Dopamine
E. Serotonin

35. A 35-year-old man has come to a dentist with complaints of decreased density of the dental tissue and increased brittleness of his teeth during consumption of hard food. Laboratory analysis measured Ca/P correlation in the enamel sample. What value of Ca/P indicates increased demineralization?
A. 0.9
B. 1.67
C. 1.85
D. 2.5
E. 1.5

36. A patient with hypochromic anemia has hair with split ends and suffers from hair loss. The nails are brittle. Gustatory sensations are affected. What is the mechanism of development of these symptoms?
A. Iron enzymes deficiency
B. Vitamin B_{12} deficiency
C. Low production of thyroid hormones
D. Low production of parathyroid hormone
E. Vitamin A deficiency

37. A woman with hypophyseal diabetes insipidus developed a water-mineral imbalance. What type of water-mineral imbalance develops in such cases?
A. Hyperosmolar dehydration
B. Hyperosmolar hyperhydration
C. Isoosmolar dehydration
D. Hypoosmolar dehydration
E. Hypoosmolar hyperhydration

38. A patient with hypertensic crisis has increased content of angiotensin II in blood. Angiotensin pressor effect is based on:
A. Contraction of arteriole muscles
B. Vasopressin production stimulation
C. Activation of biogenic amine synthesis
D. Prostaglandin hyperproduction
E. Activation of kinin-kallikrein system

39. A 30-year-old breastfeeding woman keeps to the diet that daily provides her with 1000 mg of calcium, 1300 mg of phosphorus, and 20 mg of iron. How should the daily dosages of minerals in this diet be adjusted?
A. Increase phosphorus intake
B. Decrease iron intake
C. Increase calcium intake
D. Decrease fluorine intake
E. Increase iron intake

Topic 21.
Normal and pathological components of urine

1. Objective: To know physical and chemical properties of urine; principal normal and pathological constituents of urine and pathways of their appearance. To be able to conduct biochemical analysis of urine and to interpret obtained results.

2. Actuality of the theme: A biochemical analysis of urine is one of the most important and commonly performed laboratory investigations in medical practice, that is very informative in complex diagnosis of numerous diseases and monitoring of effectiveness of treatment.

The renal (kidney) function is usually assessed by evaluating either the glomerular (clearance tests) or tubular function (urine concentration test). This is often guided by blood analysis (for urea, creatinine) and/or urine examination.

The clearance is defined as the volume of the plasma that would be completely cleared of a substance per minute. Inulin clearance represents glomerular filtration rate (GFR). Creatinine clearance and urea clearance tests are often used to assess renal function. A decrease in their clearance is an indication of renal damage.

Impairment in renal function is often associated with elevated concentration of blood urea, serum creatinine, decrease in osmolality and specific gravity of urine (by urine concentration test).

3. Specific aims:

- ✓ To interpret the biochemical mechanisms of water and minerals turnover regulation and role of kidneys in the formation of urine.
- ✓ To analyze the biochemical composition of urine in health and during pathological processes; to estimate the functional value of the final products of nitrogen turnover (urea, uric acid, creatinine) and products of detoxication (animal indican, hippuric acid), changes in their daily secretion.
- ✓ To analyze the state of health of a patient on a basis of biochemical parameters of blood and urine.

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

Questions:	References:
1. **Role of kidneys in regulation of volume, composition of electrolytes and acid-base equilibrium of biological fluids. Biochemical mechanisms of urine production (filtration, reabsorption, secretion and excretion). Characterization of renal clearance and renal threshold, their diagnostic significance.**	1. Popova L.D. Biochemistry: Manual for medical students or interns / L.D. Popova, A.V. Polikarpova – Kharkiv: KNMU, 2011. – P. 419-424. 2. Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 459-462. 3. Lecture notes.
2. **Physical and chemical properties of urine: volume, color, odor, transparency, acidity (pH), its dependence from diet. Role of kidneys and lungs in regulation of acid-base equilibrium. Ammoniogenesis.**	1. Popova L.D. Biochemistry: Manual for medical students or interns / L.D. Popova, A.V. Polikarpova – Kharkiv: KNMU, 2011. – P. 424-426. 2. Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 462-463, 766-767, 771. Lecture notes.
3. **Chemical composition of urine in health (organic and mineral components), their diagnostic significance and causes of changes of stable indices of urine analysis.**	1. Popova L.D. Biochemistry: Manual for medical students or interns / L.D. Popova, A.V. Polikarpova – Kharkiv: KNMU, 2011. – P. 426-427. 2. Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 460-463, 766-767, 771. Lecture notes.
4. **Pathobiochemistry of kidneys. Biochemical changes in acute renal insufficiency. Diagnostics of chronic renal insufficiency.**	1. Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 460-463, 766-767, 771. 3. Lecture notes.

5.	**Pathological constituents of urine – blood, protein, sugar, bile pigments, ketone bodies. Causes and pathways of their appearance in urine.**	1. Popova L.D. Biochemistry: Manual for medical students or interns / L.D. Popova, A.V. Polikarpova – Kharkiv: KNMU, 2011. – P. 427-428. 2. Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 460-463, 766-767, 771. 3. Lecture notes.
6.	**Glucosuria, galactosuria and pentosuria, causes of their development. Clinical significance of their detection.**	1. Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 674, 681, 277, 276. 2. Lecture notes.
7.	**Clinical significance of detection and determination of indicane, phenylpyruvic and homogentisic acids in urine.**	Lecture notes.
8.	**Clinical significance of detection and determination of ketone bodies in urine.**	1. Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 296. 2. Lecture notes.
9.	**Differentiation of jaundices on the appearance of bile acids and bile pigments in urine.**	Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 216-220.

5. Tasks for independent work and self-control

5.1. The major functions of the kidneys:

1) to excrete the waste,

2) to maintain the composition, osmolality, and volume of the extracellular fluid (ECF),

3) to control the acid-base balance,

4) hormone production.

Draw the nephron structure.

5.2. The blood supply to kidneys is relatively large. About 1200 ml of blood (650 ml plasma) passes through the kidneys every minute. From this, about 120 ml is filtered per minute by the kidneys and this is referred to as glomerular filtration rate (GFR). With a normal GFR (120 ml/min), the glomerular filtrate formed in an adult is about 170-180 L per day, out of which only 1-2 L is excreted as urine. Thus, more than 99 % of the glomerular filtrate is reabsorbed by the kidneys. The process of urine formation basically involves two steps - glomerular filtration and tubular reabsorption.

Glomerular filtration is a passive process that results in the formation of ultrafiltrate of blood. What factor determines the total filtration rate of the kidneys?

5.3. All constituents of plasma, with a molecular weight less than about 68 kDa, are passed through the filtration barrier into the filtrate. Therefore, the glomerular filtrate is almost similar in composition to plasma except that it is almost free of proteins. Filtration of larger molecules (proteins) through this barrier is limited by their size, shape, and electric charge. Compare the permeability of barrier for positively and negatively charged proteins with molecular weights lower than 68 kDa.

5.4. The renal tubules retain water and most of the soluble constituents of the glomerular filtrate by reabsorption. Under normal circumstances, all the glucose, amino acids, potassium and bicarbonate, and about 75 % of the sodium, is reabsorbed. This may occur either by passive or active process.

Describe the mechanisms of tubular reabsorption of glucose, amino acids, and ions.

5.5. Sodium is reabsorbed in the tubule by several mechanisms: through specific ion channels, in exchange for the potassium or hydrogen ion, and in cotransport with glucose, amino acids, phosphate, and other anions. Describe the mechanism of the control of the sodium reabsorption.

5.6. Indicate the mechanisms of water reabsorption in the different parts of the nephron. Describe the regulation of water reabsorption.

5.7. The excreted urine has an entirely different composition compared to glomerular filtrate from which it is derived. There are certain substances in the blood whose excretion in urine is dependent on their concentration. Such substances are referred to as renal threshold substances. At the normal concentration in the blood, they are completely reabsorbed by the kidneys, with a result that their excretion in urine is almost negligible. The renal threshold of a substance is defined as its concentration in blood (or plasma) beyond which it is excreted into urine. Indicate the renal threshold for glucose, ketone bodies, calcium.

5.8. Name the renal mechanisms for regulation of blood pH.

5.9. Write the equation of production of carbonic acid and bicarbonate. What enzyme catalyses the reaction of carbon dioxide hydration?

5.10. At normal rates of glomerular filtration, approximately 4.3 mol/24 h of bicarbonate are filtered by the renal glomeruli. The luminal surface of renal tubular cells is impermeable to bicarbonate. Explain how all the filtered bicarbonate is reabsorbed. Show the relationship between excretion of hydrogen ions and bicarbonate reabsorption.

5.11. In the lumen of the distal tubule, excreted hydrogen ions are buffered by the filtered phosphate ions and by ammonia synthesized by the renal tubular cell. Write the corresponding reactions.

5.12. Ammonia (NH_3), produced by the deamination of glutamine in renal tubular cells, diffuses freely through the luminal membrane and the hydrogen ion is trapped inside the tubular lumen as the ammonium ion (NH_4^+) to which the membrane is impermeable. What enzyme catalyses the deamination of glutamine? Write the equation of this reaction.

5.13. Acidification of the urine takes place in the distal convoluted tubules and in the collecting ducts. Hormone aldosterone facilitates hydrogen ions secretion. Explain how.

5.14. Creatinine is an excretory product derived from creatine phosphate (largely present in muscle). The excretion of creatinine is rather constant and is not influenced by body metabolism or dietary factors. Creatinine is filtered by the glomeruli and only marginally secreted by the tubules.

Where and how is creatinine produced?

5.15. The reference range for plasma creatinine in the adult population is 60-120 μmol/L (0.5-1.5 mg/dl). But the day-to-day variation in an individual is very small. What factor determines plasma creatinine concentration in separate individual?

5.16. A decrease in plasma creatinine concentration can occur as a result of starvation and in wasting diseases, immediately after surgery and in patients treated with corticosteroids. Explain why.

5.17. The one test of tubular function in widespread use is the urine concentration or dilution test. It involves the accurate measurement of specific osmolality or gravity of plasma and urine which depends on the concentration of solutes. Several measures are employed to concentrate urine and measure the specific osmolality. These include overnight water deprivation and administration of desmopressin, synthetic analogueue of vasopressin. The results of a fluid deprivation test help to investigate the causes of polyuria and polydipsia, cranial (pituitary or hypothalamic) or nephrogenic diabetes insipidus. Indicate the causes of these types of diabetes insipidus.

5.18. The small amount of low molecular weight protein that is filtered by the glomerulis normally absorbed by and catabolized in renal tubular cells. The presence of such proteins in urine can indicate renal tubular damage. Name the low molecular weight proteins that can be measure in urine.

What other compounds can be present in the urine due to tubular damage?

5.19. Fill in the table «Physico-chemacal characteristics of urine»

Characteristic	Normal state	Pathology state
Colour		
Odour		
Transparency		
Density (gravity)		
pH		
Sediment		

5.20. Fill in the table:

Pathologic state < 500ml and > 2000 ml	Causes
Pathologic polyuria	
Oligouria (diminished excretion of daily urine)	
Anuria	

5.21. Explain the terms:
Polyuria

Nycturia

5.22. Read the results of urine analyses, presented below and write which pathologies do you suspect, based on this data:

a) Daily diuresis – 2.0 L;
Weight – 1,025 g /L;
Ketone bodies ++;
Sugar – 3 g/L;
Protein – not revealed.

b) Daily diuresis – 9.0 L;
Weight – 1,000 g/L;
Sugar – not revealed;
Ketone bodies – not revealed;
Protein – not revealed.

c) Daily diuresis – 1.6 L;
Weight – 1,024 g /L;
Sugar – not revealed;
Ketone bodies – not revealed;
Protein – 0.033 g/L;
17-ketosteroids ++++.

d) Daily diuresis – 1.5 L;
Weight – 1,028 g /L;
Protein – 3 g/L
Sugar – not revealed;
Ketone bodies – not revealed.

5.23. Situational task:
A 20-year old man had generalized edema of the body with puffiness of the face in the mornings. His laboratory findings are given below:

Parameter	Subject	Reference range
Serum total proteins	4.5 g/dl	6-8 g/dl
albumin	1.5 g/dl	3.5-5 g/dl
globulins	3.0 g/dl	2.5-3.5 g/dl
Serum cholesterol	350 mg/dl	150-225 mg/dl
Blood urea	30 mg/d	15-40 mg/dl
Serum creatinine	1.2 mg/dl	0.5-1.5 mg/dl
Urinary proteins	15 g/day	< 100 mg/day

The serum electrophoresis of the subject showed a sharp and prominent α_2-globulin band.
Make a diagnosis and explain its cause.

6. Individual independent students work

Pathobiochemistry of kidneys. Clinical and biochemical changes at glomerulonephritis, amyloidosis, pyelonephritis, sharp and chronic kidney insufficiency.

Practice protocol №21 «____» _____ 20___

Biochemical analysis of urine

For the biochemical analysis a middle portion of urine from morning urination is used. Urine collection requires sterile conditions in order to avoid contamination with bacterias and flagellas. The urine analysis is conducted, beginning from the estimation of physical and chemical properties: amount, colour, smell, transparency, reaction (pH) and density of urine.

Experiment 1. Determination of physical and chemical properties of urine.

A. Urine volume.

Measure the volume of tested urine using graduated cylinder. The quantity of urine excreted in 24 hours is defined as diuresis.

Normally healthy person excretes daily in average 1,100-1,600 ml of urine. Increase in volume of urine excreted is called polyuria, decrease – oliguria, complete absence of urine – anuria.

B. Colour.

Estimate the colour of tested urine.

Normally urine has straw-yellow colour due to the presence of specific pigments - urochrome, urobilin, uroerythrin. In pathological conditions colour may be changed due to occurrence of bile pigments (bilirubinuria) or hemoglobin (hemoglobinuria, hematuria). Colour of urine also depends on diet.

C. Odour.

Estimate the odour of urine.

Usually urine has specific odour due to the presence of traces of volatile compounds. Ammonia odour appears in bladder inflammations, purulent – in gangrenous processes, fruit odour – in diabetes mellitus. Odour of urine also depends on diet.

D. Transparency.

Estimate the transparency of urine.

Normally fresh urine is transparent. During standing some sediment may appear due to formation of mucin clots. Turbidity may be caused by sediments of phosphates (in alkaline medium), urates (in acidic medium), mucin, cellular elements and microorganisms.

E. The acidity of urine (pH)

Using pH-meter or sensitive indicator papers determine pH of urine.

Normally urine has pH in ranges 4.5 – 8.0 and depends on a character of diet or metabolic disorders. Alkaline urine may occur in case of hyperacidities of gastric juice, in loss of acid equivalents in massive vomiting, in cystitis or pyelitis. Acidic reaction of urine occurs in diabetes mellitus, starvation (due to the presence of ketone bodies), in renal insufficiency. Highly acidic urine is estimated in gout and fever.

F. Urine density is measured with urometer with subsequent scale.

In health urine density makes 1,008 – 1,025 g/l.

Results:

Conclusion:

Experiment 2. Detection of protein in urine.

Principle. The detection of protein in urine is based on the precipitation of protein with sulfosalicylic acid.

Procedure.

To 2 ml of urine add 5 droplets of 20 % solution of sulfosalicylic acid.

If white precipitate or turbidity appears in a tube, it proves the presence of protein.

Results:

Conclusion:

Experiment 3. Detection of sugar in urine.

Principle. All mono- and disaccharides with a free hemiacetal hydroxyl possess reducing properties and reduce metal ions (silver, copper, bismuth) with degradation of carbon chain and polymerization. Reactions are performed after elimination of protein, as it is an interfering substance.

To 10-15 ml of urine add 10 droplets of 10 % acetic acid and boil.

Cool and separate the appeared sediment by filtration.

Test obtained filtrate for the presence of sugar using Trommer' reaction:

To 1-2 ml of test sample add 1 ml of 5 % NaOH and 2-3 drops of $CuSO_4$ solution.

Observe the formation of precipitate of $Cu(OH)_2$.

Boil the tube.

In case of presence of sugar in the tested sample the precipitate will become yellow during heating due to formation of coloured precipitate of cuprous hydroxide. In course of boiling the colour of the solution changes to red as a result of cuprous oxide formation.

Results:

Conclusion:

Experiment 4. Detection of blood (benzidine probe).
To 3 ml of urine add 2-3 droplets of 3 % H_2O_2 and 2-3 droplets of benzidine in acetic acid. In presence of blood the colour of the solution will change to green-blue.
Results:

Conclusion:

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

Examples of Krock-1 tests

1. After a person had drunk 1.5 litres of water, the amount of urine increased significantly, and its relative density decreased to 1,001. These changes are developed as a result of decreased water reabsorption in the distal nephron portion due to reduced secretion of:
A. Vasopressin
B. Aldosterone
C. Angiotensin II
D. Renin
E. Prostaglandins

2. The darkening of sclera, mucous membranes and ear bowels are observed in a suckling child. The urine is also developing a dark colour after exposure on air. Homogentisic acid is detected in blood and urine. What pathology can be suggested?
A. Alcaptonuria
B. Porphyria
C. Albinism
D. Cystinuria
E. Hemolytic anemia

3. A 25-year-old patient complains of morbid thirst and polyuria (up to 10 litres daily). Glucose concentration in blood is normal and it is absent in urine. The patient has been diagnosed with diabetes insipidus. What hormonal drug is the most appropriate for management of this disorder?
A. Vasopressin
B. Cortisol
C. Thyroxin
D. Oxytocin
E. Insulin

4. According to the results of glucose tolerance test a patient has no disorder of carbohydrate tolerance. Despite that glucose is detected in the patients' urine. The patient has been diagnosed with renal diabetes. What renal changes cause glucosuria in this case?
A. Decreased activity of glucose reabsorption enzymes
B. Increased activity of glucose reabsorption enzymes
C. Exceeded glucose reabsorption threshold
D. Increased glucose secretion
E. Increased glucose filtration

5. A child with renal insufficiency exhibits delayed teeth eruption. This is most likely caused by the abnormal formation of the following substance:
A. 1,25 $(OH)_2D_3$
B. Glycocyamine
C. Glutamate
D. Alfa-ketoglutarate
E. Hydroxylysine

6. A 15-year-old boy was diagnosed with alkaptonuria. After standing, his urine changes to a black colour. This disease is a hereditary disorder of:
A. Tyrosine metabolism
B. Alanine metabolism
C. Urea synthesis
D. Uric acid biosynthesis
E. Cysteine metabolism

7. Glucose concentration in a patient's blood is 15 mmol/L (reabsorption threshold is 10 mmol/L). What effect can be expected?
A. Glucosuria
B. Diuresis reduction
C. Reduced glucose reabsorption
D. Reduced vasopressin secretion
E. Reduced aldosterone secretion

8. What is the average range of fluctuations in the specific gravity of urine for healthy person being on normal diet?
A. 1,016 – 1,020
B. 1,008 – 1,010
C. 1,010 – 1,012
D. 1,013 – 1,015
E. 1,001 – 1,008

9. A patient has an insufficient blood supply to the kidneys, which has caused the development of pressor effect due to the constriction of arterial resistance vessels. This is the result of the vessels being greatly affected by the following substance:
A. Angiotensin II
B. Angiotensinogen
C. Renin
D. Catecholamines
E. Norepinephrine

10. Due to the use of poor-quality measles vaccine for preventive vaccination, a 1-yearold child developed an autoimmune renal injury. The urine was found to contain macromolecular proteins. What process of urine formation was disturbed?
A. Filtration
B. Reabsorption
C. Secretion
D. Reabsorption and secretion
E. Secretion and filtration

11. A 65-year-old man suffering from gout complains of kidney pain. Ultrasonic examination revealed kidney stones. An increased concentration of a certain substance can cause kidney stones formation. Name this substance:
A. Uric acid
B. Cholesterol
C. Bilirubin
D. Urea
E. Cystine

12. Atria of an experimental animal were super distended with blood, which resulted in decreased reabsorption of Na^+ and water in renal tubules. This can be explained by the influence of the following factor on kidneys:
A. Natriuretic hormone
B. Aldosterone
C. Renin
D. Angiotensin
E. Vasopressin

13. What index allows evaluate renal glomerular filtration?
A. Clearance of creatinine
B. Daily creatinine excretion in the urine
C. Proteinuria
D. The daily excretion of urea in the urine
E. Hematuria

14. Excretion of ammonium cations with urine is increased in the next condition:
A. Metabolic acidosis
B. Respiratory alkalosis
C. Hyperlipidemia
D. Hypoproteinemia
E. Obesity

15. A 55-year-old patient was diagnosed with acute glomerulonephritis. What is the basic mechanism of anemia development in this case?
A. Diminishing of erythropoetin production
B. Diminishing of glomerular filtration
C. Diminishing of kidney prostaglandines synthesis
D. Retentional azotemia
E. Diminishing of reabsorption in canaliculus

16. A patient with serious damage of muscular tissue was admitted to the traumatological department. What biochemical urine index will be increased in this case?
A. Creatinine
B. Lipids
C. Glucose
D. Mineral salts
E. Uric acid

17. It was revealed that blood glucose level in a patient is over the renal threshold. Polyuria is observed, as well as acidosis and ketonuria. What pathological state can be suggested?
A. Diabetes mellitus
B. Hypercorticism
C. Starvation
D. Addison disease
E. Hyperthyreosis

18. A 30-year-old woman has face edemata. Examination revealed proteinuria (5.87 g/L), hypoproteinemia, hyperlipidemia. What condition is the set of these symptoms typical for?
A. Nephrotic syndrome
B. Nephritic syndrome
C. Chronic pyelonephritis
D. Acute renal failure
E. Chronic renal failure

19. A child has an acute renal failure. What biochemical factor found in saliva can confirm this diagnosis?
A. Increase in urea concentration
B. Increase in glucose concentration
C. Decrease in glucose concentration
D. Increase in concentration of fatty acids
E. Decrease in nucleic acid concentration

20. What symptom indicates to the increased permeability of the glomerular membrane?
A. Proteinuria
B. Glucosuria
C. Aminoaciduria
D. Alkaptonuria
E. Pyuria

21. Osteoporosis has been developed in a patient suffering from chronic renal failure. What vitamin's metabolism infringement promotes this disorder?
A. Vitamin D
B. Vitamin E
C. Vitamin A
D. Vitamin K
E. Vitamin F

22. Arterial pH is 7.4; primary urine – 7.4; final urine – 5.8. Decrease in the pH of final urine is the result of the secretion of the following ions in the nephron tubules:
A. Hydrogen ions
B. Potassium ions
C. Hydrogen carbonate ions
D. Urea
E. Creatinine

23. What is the 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

24. What specific gravity of urine indicates to hypersthenuria?
A. 1,028–1,030
B. 1,010–1,012
C. 1,016–1,020
D. 1,020–1,022
E. 1,012–1,015

25. A patient who had been continuously taking drugs blocking the production of angiotensin II developed bradycardia and arrhythmia. A likely cause of these disorders is:
A. Hyperkalemia
B. Hypokalemia
C. Hypernatremia
D. Hypocalcemia
E. Hypercalcemia

26. A patient came to a doctor with complaints of frequent and excessive urination and thirst. Daily diuresis is 19 litres, density of urine is 1,001. What pathology can be suggested?
A. Diabetes insipidus
B. Steroid diabetes
C. Diabetes mellitus
D. Thyrotoxicosis
E. Addison's disease

27. A patient has oliguria caused by an acute renal failure. What daily amount of urine corresponds with this symptom?
A. 100–500 ml
B. 1,500–2,000 ml
C. 1,000–1,500 ml
D. 50–100 ml
E. 500–1,000 ml

28. The content of which component of the residual nitrogen sharply increases under the conditions of renal failure?
A. Urea
B. Peptides
C. Uric acid
D. Indicane
E. Bilirubin

29. Specify the normal rate of creatinine clearance:
A. 80–120 ml / min
B. 40–60 ml / min
C. 120–140 ml / min
D. Less than 40 ml / min
E. 60–80 ml / min

30. What test would you offer to determine the concentrating power of the kidney?
A. Zimnitsky test
B. Determination of urea excretion in the urine
C. Determination of urea in the blood
D. Determination of creatinine in the blood
E. Daily diuresis

31. Argininemia and argininuria are revealed in a patient. The amount of urea in the blood and urine is reduced. Deficiency of which enzyme is observed?
A. Arginase
B. Glutamate dehydrogenase
C. Ornithine carbamoyltransferase
D. Argininosuccinate synthetase
E. Tryptophan-5-monooxygenase

32. Complete obstruction of the common bile duct was revealed in a patient. What changes will be observed in the urine?
A. The appearance of direct bilirubin
B. Accumulation of indirect bilirubin
C. Absence of urobilin
D. The appearance of stercobilin
E. The appearance of fatty acids

33. A therapeutist has an appointment with a 40-year-old patient complaining of recurrent pain attacks in his hallux joints and their swelling. Urine analysis revealed its marked acidity and pink colour. What substances can cause such changes in the urine?

A. Uric acid salts
B. Chlorides
C. Ammonium salts
D. Calcium phosphate
E. Magnesium sulphate

34. A 57-year-old patient has been suffering from chronic glomerulonephritis for 5 years. The biochemical analysis of the blood is as follows: urea is 29 mmol/L, creatinine is 0.68 mmol/L, potassium is 6.1 mmol/L, glucose is 3.2 mmol/L. There is a smell of ammonia from the mouth. The arterial pressure is 215/115 mm Hg. Choose the form of violation for which these indicators are characteristic for?

A. Renal failure
B. Hyperammonemia
C. Hyperkalemia
D. Hypertension
E. Hypoglycaemic coma

35. Persistent arterial hypertension is observed in a patient suffering from chronic pyelonephritis. What biologically active substance initiates the development of the hypertension?

A. Renin
B. Erythropoietin
C. Endothelin
D. Norepinephrine
E. Aldosterone

Topic 22.
Investigation of biochemical functions of muscle tissue.

1. Objective: To learn the composition and peculiarities of metabolism in muscular tissue, its function in health and in pathologic conditions. To perform a quantitative determination of creatinine and creatine in urea and its diagnostic significance in certain diseases.

2. Actuality of theme: Muscular tissue has specific features of metabolism depending on age of a person, pathologic conditions, caused both by endogenous and exogenous factors, and that is why in diagnosis of diseases of muscles a special place belongs to biochemical investigations, f.i. determination of the excretion of creatine and creatinine in daily urine.

Muscle is the single largest tissue of the human body (30–40 % of body weight). It is composed of fibre cells into which myofibrils are embedded. Each myofibril contains alternating A and I bands. Sarcomere is the functional unit of muscle.

Actin, myosin, tropomyosin and troponin are the major contractile proteins found in muscles. The muscle contraction and relaxation occur due to the active involvement of these proteins. ATP is the immediate source of energy for muscle contraction.

3. Specific aims:

✓ To analyse biochemical composition of muscles and the role of proteins in formation of myocytes.

✓ To explain biochemical mechanisms of contraction and relaxation of muscular fiber.

✓ To analyse pathways of energetic support of muscular contraction, role of ATP and creatine phosphate in these processes.

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

Questions:	References:
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2. **Nitrogen-containing and nitrogen-free water soluble organic compounds: structure and functional significance. Molecular mechanisms of muscular contraction: modern data on interaction of muscular filaments. Role of Ca^{2+} ions in regulation of contraction and relax of striated and smooth muscles.**	1. Popova L.D. Biochemistry: Manual for medical students or interns / L.D. Popova, A.V. Polikarpova – Kharkiv: KNMU, 2011. – P. 478–479, 484–492. 2. Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 493–494. 3. Gubsky Yu. Biological chemistry: textbook / edited by Yu. Gubsky. – Vinnytsia: Nova Knyha, 2018. – 2nd edition. – P. 430–445. 4. Koolman J. Color Atlas of Biochemistry, Second edition / J. Koolman, K.H. Roehm. – Thieme. – 2005. – P. 332–435. 5. Lecture notes.
3. **Modern ideas on energetics of muscular contraction and relaxation. Macroergic compounds of muscles. Structure, production and role of ATP, creatine phosphate, creatine phosphokinases, sources of ATP in muscular cells; role of creatine phosphate in energetic supply of contraction.**	1. Popova L.D. Biochemistry: Manual for medical students or interns / L.D. Popova, A.V. Polikarpova – Kharkiv: KNMU, 2011. – P. 482–484. 2. Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 344, 490–495. 3. Gubsky Yu. Biological chemistry: textbook / edited by Yu. Gubsky. – Vinnytsia: Nova Knyha, 2018. – 2nd

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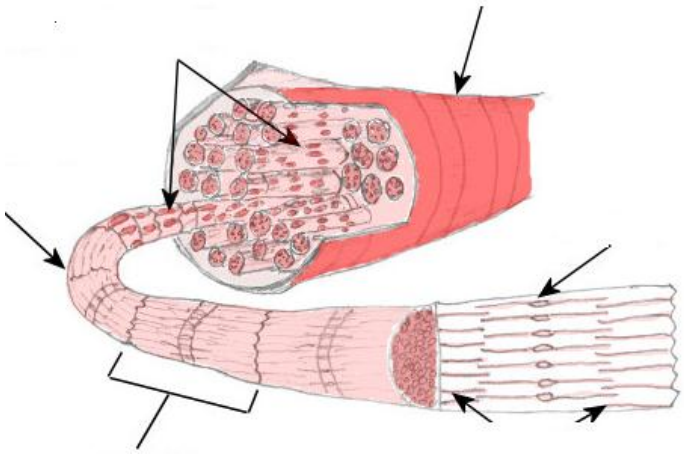
4.	**Features of cardial muscle metabolism and contraction.**	1. Popova L.D. Biochemistry: Manual for medical students or interns / L.D. Popova, A.V. Polikarpova – Kharkiv: KNMU, 2011. – P. 482, 488. 2. Lecture notes.
5.	**Biochemical changes of blood plasma enzymes in different periods of myocardial infarction and other heart diseases.**	1. Popova L.D. Biochemistry: Manual for medical students or interns / L.D. Popova, A.V. Polikarpova – Kharkiv: KNMU, 2011. – P. 492–494. 2. Satyanarayana U., Chakrapani U. «Biochemistry», Third Edition. – 2006. – P. 107–108, 112–113. 3. Lecture notes.
6.	**Peculiarities of smooth muscles function. Molecular mechanisms in regulation of tonus of vessels and bronchi.**	1. Popova L.D. Biochemistry: Manual for medical students or interns / L.D. Popova, A.V. Polikarpova – Kharkiv: KNMU, 2011. – P. 488–492. 2. Lecture notes.
7.	**Biochemical changes and diagnosis of muscular dystrophies.**	1. Popova L.D. Biochemistry: Manual for medical students or interns / L.D. Popova, A.V. Polikarpova – Kharkiv: KNMU, 2011. – P. 488–492. 2. Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 107–108. 3. Lecture notes.

5. Tasks for independent work and self-control

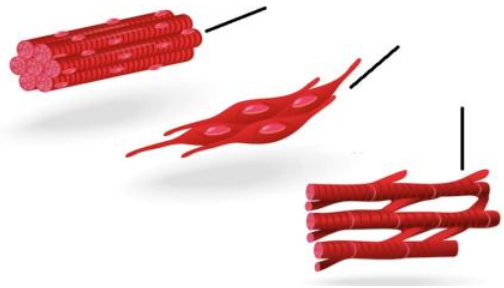
5.1. Fill in the table «Differences between skeletal, cardiac, and smooth muscle».

Features	Skeletal muscles	Cardiac muscles	Smooth muscle
Striated or no?			
Control of contraction (voluntarily or no)			
What factor initiates contraction (increases Ca^{2+} level in cytoplasm)?			
What sources of Ca^{2+} for contraction?			
Ca^{2+}-binding protein			
The rate of cross-bridges cycling			

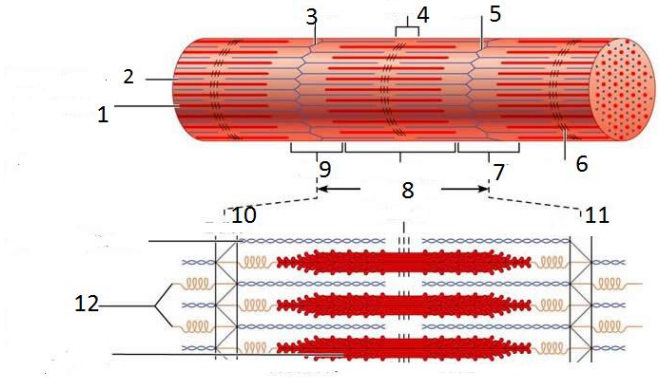
5.2. Sign the structural elements of the muscular fibre:



5.3. Sign and explain which fibres presented below belong to the smooth, skeletal and cardiac muscle tissue:



5.4. Sign the ultrastructural constituents of the muscle fibre, presented below:



5.5. Draw the scheme of the major biochemical events occurring during a cycle of muscle contraction and relaxation.

5.6. Cardiac muscle cells are designed for endurance and consistency. Indicate the peculiarities of heart muscle that allow such functioning.

5.7. If isolated cardiac muscle is deprived of Ca^{2+}, it ceases to beat within approximately 1 minute, whereas skeletal muscle can continue to contract without an extracellular source of Ca^{2+}. Explain this difference.

5.8. Cyclic AMP plays a more prominent role in cardiac than in skeletal muscle. It modulates intracellular levels of Ca^{2+} through the activation of protein kinases; these enzymes phosphorylate various transport proteins in the sarcolemma and sarcoplasmic reticulum and also in the troponin-tropomyosin regulatory complex, affecting intracellular levels of Ca^{2+} or responses to it. What hormones increase the level of cAMP, that lead to increased contraction of cardiac muscle (the inotropic effects on the heart)?

5.9. Smooth muscle, unlike skeletal and cardiac muscle, does not contain the troponin system. Describe the mechanism that initiates smooth muscle contraction.

5.10. It is known that the main feature of bronchial asthma is a spasm of smooth muscles of bronchioles. What causes manifestation of such symptoms?

5.11. Fill in the table timeline of biochemical markers of myocardial infarction

№	Marker	Timeline, hours	
		from	to
1.	Myoglobin		
2.	Troponin T		
3.	MB creatine phosphokinase (CPK)		
4.	Total creatine phosphokinase (CPK)		
5.	Aspartate aminotransferase (AST)		
6.	Lactate dehydrogenase ($LDH_{1,2}$)		
7.	Alanine aminotransferase (ALT)		

5.12. Skeletal and cardiac muscles can be affected by several disease processes, including trauma, ischemia, inflammation (myositis, polymyositis), acquired and genetic metabolic myopathies, including the muscular dystrophies. Complete the table:

Disease	Cause, defective protein	Mechanisms of disorder development, clinical symptoms
Duchenn muscular dystrophy	Mutations in the gene encoding the protein dystrophin	
Becker muscular dystrophy	Mutations in the gene encoding the protein dystrophin	
Malignant hyperthermia	Mutations in the gene encoding the Ca^{2+} release channel of SR +exposure to certain anesthetics	
Genetic metabolic myopathies, cardiomyopathies	Disorders of fatty acid oxidation, mitochondrial diseases; glycogen storage disease type V; mutations in genes encoding myosin, tropomyosin, troponins	
Acquired metabolic diseases	Hyper- and hypothyroidism, Cushing's syndrome; alcohol abuse	

5.13. Situational tasks

a) There are three isoenzymes of creatine kinase (CK): CK-MM, CK-MB, CK-BB. The CK normally present in plasma is mainly the CK-MM. Increases in plasma CK activity are usually the result of skeletal or cardiac muscle damage.

What changes are specific for:

1) skeletal muscle diseases, for example polymyositis, rhabdomyolysis, Duchenne muscular dystrophy;

2) myocardial infarction (MI) and other heart diseases?

Where is CK-BB present?

b) Sensitive early indicators of cardiac damage are myoglobin, troponins I and T. Concentration of cardiacspecific troponin T isoform in plasma increases within a few hours after a heart attack, peaks at up to 300 times normal plasma concentration, and may remain elevated for 1-2 weeks. An assay for a specific isoform in an adult heart, Tn-T2, is very sensitive for diagnosis of MI.

Explain why high level of myoglobin in plasma has not diagnostic significance but if myoglobin is not elevated within 2-6 hours after the onset of symptoms, MI did not occur.

c) A 55-year old man was brought to the hospital with severe chest pain, breathlessness and vomiting. He could be rushed to the city hospital 5 hours after the onset of chest pain. His blood was immediately drawn, and the laboratory data are given below:

Parameter (serum)	Subject	Reference range
Creatine phosphokinase (CPK)	410 IU/l	10-50 IU/l
Aspartate transaminase (AST)	67 IU/l	5-45 IU/l
Lactate dehydrogenase (LDH)	315 IU/l	50-200 IU/l

Further laboratory evaluation revealed that the isoenzymes CPK_2(MB) and LDH_1 were highly elevated.

Diagnosis:

d) Patient has vitamin E hypovitaminosis. How will it influence the function of muscles?

6. Individual independent students work

Molecular mechanisms of skeletal, smooth and cardiac muscle contraction. Comparative characteristic.

Practice protocol №22 «____» _____ 20___

Experiment. Quantitative determination of creatinine in urine after Folin method.

Principle. Creatinine combines with picric acid in alkaline medium and forms coloured complex (Jaffe reaction). The intensity of colour is proportional to creatinine quantity and is measured in photocolorimeter.

Procedure.

Analysis is carried out in accordance with the scheme:

Reagents (ml)	Control Tube 1	Standard Tube 2	Test Tube 3
Distilled water	0.1	-	-
Standard solution of creatinine	-	0.1	-
Urine	-	-	0.1
10 % NaOH	4 drops	4 drops	4 drops
2 % saturated solution of picric acid	0.15	0.15	0.15
Mix. After 5 min to every measuring test tube add distilled water that the total volume of the solution in each tube is 10 ml. *Mix the solutions and measure the intensity of colour using photocolorimeter in 5 mm cuvette at green light.*			
E=	-		

Calculation. The concentration of creatinine is calculated using the formula:

$X = (C_{st} \times E_{test}) / E_{st} \times 1.5$,

where

X – concentration of creatinine (g/day),

C_{st} – concentration of creatinine in standard (1 g/L),

E_{test} – extinction of tested sample,

E_{st} – extinction of standard sample,

1.5 – daily volume of urine, L

Results:

Conclusion:

Clinical and diagnostic significance. Normal daily excretion of creatinine is 8.8-17.7 mMole (1.0-2.0 g) in males and 7.1-15.9 mMoles (0.8-1.8 g) in females. The enhancement of creatinine excretion is observed in excessive ingestion of meat (exogenous source), under conditions of increased breakdown of proteins, in intensive physical efforts, diabetes mellitus and diabetes insipidus, infectious diseases, etc. Creatinine is not reabsorbed in kidney tubules, what permits to evaluate the glomerular filtration index.

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

Examples of Krock-1 tests

1. The intensive muscular work induced the significant decrease in the blood buffer capacity of the worker. Which metabolite induced this condition?
A. Lactate
B. 1,3-Diphosphoglycerate
C. Alfa-Ketoglutarate
D. 3-Phosphoglycerate
E. Pyruvate

2. Long physical work leads to overfatigue. This condition is accompanied by rigidity of the muscles. The lack of what substance in the muscles contributes to their rigidity?
A. ATP
B. ADP
C. AMP
D. Glucose
E. Glycogen

3. A patient suffering from progressive muscular dystrophy was administered the biochemical examination of urine. The presence of the large amount of what compound in urine can confirm the muscular pathology in this patient?
A. Creatine
B. Hippuric acid
C. Porphyrins
D. Urea
E. Creatinine

4. The change in the concentration of ions in the intracellular fluid occurs during the contraction and relaxation of muscles. The increased level of which of the cytosol ions of myocytes serves as a signal for muscle contraction?
A. Ca^{2+}
B. Mg^{2+}
C. Na^{+}
D. K^{+}
E. Cl^{-}

5. For muscles, which rapidly contracted, glycolysis is a quick way to generate energy (ATP) under oxygen deficiency. In how many seconds since the beginning of muscle contraction, the rate of glycolysis reaches a maximum?
A. 40–50
B. 20–30
C. 30–40
D. 60–70
E. 10–20

6. What effect of magnesium ions on the muscular tissue causes the wide use of the magnesium-containing drugs in clinical practice?
A. It decreases calcium concentration
B. It activates troponin complex
C. It increases calcium concentration
D. It enhances the nervous impulses transfer in the synapses
E. It increases ATP and phosphate concentration

7. Skeletal muscles and myocardium use different blood compounds as the substrates of oxidation. Which of the following compounds is utilized in myocardium but is not used by skeletal muscles?
A. Lactate
B. Glucose
C. Glycogen
D. Fatty acids
E. Ketone bodies

8. A 50-year-old man has been suffering from Duchenne muscular dystrophy for a long time. The change in the 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

9. The patient complains of acute chest pain. The doctor diagnosed myocardial infarction. An increase in the activity of which enzyme in the serum will confirm this diagnosis?
A. MB-Creatine phosphokinase
B. MM-Creatine phosphokinase
C. BB-Creatine phosphokinase
D. Amylase
E. Alanine aminotransferase

10. Which of the following clinical signs are not typical for the muscular tissue pathology?
A. Glucosuria
B. Myoglobulinuria
C. Creatinuria
D. Decrease of cAMP
E. Significant decrease of the content of carnosine and anserine

11. After short distance run untrained people develop delayed onset muscle soreness, resulting from lactate accumulation in muscles. The enhanced activity of what biochemical process in the organism may be associated with this condition?
A. Glycolysis
B. Glycogenesis
C. Pentose phosphate pathway
D. Gluconeogenesis
E. Lipogenesis

12. Long-term myocardial ischemia leads to necrosis and hyperenzymemia. Determining the activity of which enzymes in the blood is used in the clinic to diagnose myocardial infarction?
A. Creatine phosphokinase MB-isoform, AST, $LDH_{1,2}$
B. Succinate dehydrogenase, amylase, lipase
C. Arginase, urease, maltase
D. Nuclease, trypsin, chymotrypsin
E. Glycogen phosphorylase, glycogen synthase, malate dehydrogenase

13. The excessive intake of vitamin A is accompanied by an increased membrane permeability or membrane destruction with the release of the acid proteases and acid phosphatase from the lysosomes. The excretion of what muscular cell metabolite is indicative for their damage?
A. Creatine
B. Lactate
C. Pyruvate
D. Creatinine
E. Glucose

14. A suckling child was delivered to the clinic with signs of the damage of the muscles of the limbs and trunk. The examination evaluated carnitine deficiency in muscles. The disturbance of what process is the biochemical background for this pathology?
A. Fatty acids transport to mitochondria
B. Regulation of Ca^{2+} level in mitochondria
C. Substrate phosphorylation
D. Lactate utilization
E. Actin and myosin synthesis

15. Muscle contraction is provided by myofibrils of myocytes. What is the functional unit of myofibrils?
A. Sarcomer
B. Sarcoplasma
C. Sarcolema
D. Mitochondria
E. Sarcoplasmic reticulum

16. A 40-year-old man ran 10 km within 60 minutes. What changes of the energetic metabolism will occur in muscles?
A. The rate of fatty acid oxidation will increase
B. Proteolysis will increase
C. Gluconeogenesis will increase
D. Glycogenolysis will increase
E. Glycolysis will increase

17. Change in cytoplasm calcium ion concentration is the main biochemical regulator of the muscular contraction and relax. What component of the troponin system is activated by the increased calcium concentration?
A. Troponin C
B. Myosin
C. Actin
D. Troponin T
E. Troponin I

18. A patient presents high activity of $LDH_{1,2}$, aspartate aminotransferase, creatine phosphokinase. In what organ (organs) is the development of a pathological process the most probable?
A. In the heart muscle (initial stage of myocardium infarction)
B. In skeletal muscles (dystrophy, atrophy)
C. In kidneys and adrenals
D. In liver and kidneys
E. In connective tissue

19. Myofibrillar proteins are the proteins providing muscular contractions. Which of the following proteins exert ATP-ase activity?
A. Myosin
B. Actin
C. Troponin T
D. Troponin I
E. Troponin C

20. A 46 year woman complains of progressing Duchenne-type muscular dystrophy. Which enzyme activity changing is diagnostic test in this case?
A. Creatine phosphokinase
B. Lactate dehydrogenase
C. Pyruvate dehydrogenase
D. Glutamate dehydrogenase
E. Adenylylkinase

21. The large quantity of the MB-isoform of creatine kinase is evaluated in the blood of a patient with destructive changes of the muscular tissue. What is the most evident diagnosis?
A. Myocardial infarction
B. Muscular atrophy
C. Muscular dystrophy
D. Polymyositis
E. Duchenne disease

22. It has been revealed that intense physical exercise caused 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. Stearic acid

23. The preliminary diagnosis of a patient is myocardial infarction. Characteristic feature of this disease is an essential increase of the following in the blood:
A. Creatine phosphokinase
B. Catalase
C. Glucose-6-phosphatedehydrogenase
D. α-Amylase
E. Arginase

24. In a blood of the patient is revealed the magnification of activity of the MB-form of creatine phosphokinase and LDH1. Call a probable pathology.
A. Myocardial infarction
B. Hepatitis
C. Rheumatic disease
D. Pancreatitis
E. Cholecystitis

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

26. A worker has decreased buffer capacity of blood due to exhausting muscular work. An increase of what acid substance in the blood can cause this symptom?
A. Lactate
B. l,3-bisphosphoglycerate
C. Pyruvate
D. α-ketoglutarate
E. 3-phosphoglycerate

27. The high level of lactate dehydrogenase (LDH) isozymes concentration showed the increase of LDH_l and LDH_2 in a patient's blood plasma. Point out the most probable diagnosis:
A. Myocardial infarction
B. Acute pancreatitis
C. Skeletal muscle dystrophy
D. Viral hepatitis
E. Diabetes mellitus

28. Marked increase of activity of MB-forms of CPK (creatinephosphokinase) and LDHl was revealed by examination of the patient's blood. What is the most probab1e pathology?
A. Myocardial infarction
B. Hepatitis
C. Rheumatism
D. Pancreatitis
E. Cholecystitis

29. A 50-year-old woman diagnosed with cardiac infarction has been delivered into an intensive care ward. What enzyme will be the most active during the first two days?
A. Aspartate aminotransferase
B. Alanine aminotransferase
C. LDH_4
D. Alanine aminopeptidase
E. LDH_5

30. A traumatology unit received a patient with crushed muscular tissue. What biochemical indicator of urine will be raised in this case?
A. Creatinine
B. Total lipids
C. Glucose
D. Mineral salts
E. Uric acid

31. A patient has been delivered to a hospital with a provisional diagnosis of progressing muscle dystrophy. This diagnosis can be confirmed by the increased concentration of the following substance found in urine:
A. Creatine
B. Pyruvate
C. Carnosine
D. Troponin
E. Hydroxyproline

32. 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. Diabetes mellitus
D. Collagenosis
E. Diabetes insipidus

33. A 50-year-old driver complains about unbearable constricting pain behind the breastbone irradiating to the neck. The pain arose 2 hours ago. Objectively: the patient's condition is grave. He is pale. Heart's tones are decreased. Laboratory studies revealed high activity of creatine kinase and LDH_1. What disease are these symptoms typical for?
A. Acute myocardial infarction
B. Acute pancreatitis
C. Stenocardia
D. Cholelithiasis
E. Diabetes mellitus

34. What is the main energy source for the cardiac muscle?
A. Fatty acids
B. Amino acids
C. Lactic acid
D. Pyruvic acid
E. α-Ketoglutaric acid

Topic 23.
Investigation of biochemical functions of connective and bone tissues

1. Objective: To learn composition and metabolic peculiarities of connective tissue, its functioning in normal conditions and in pathology. To conduct a quantitative determination of hydroxyproline in urine for diagnosis of certain diseases.

2. Actuality of theme: An important problem of modern medicine is investigation of metabolic disorders in connective tissue in acquired and congenital diseases. Thus, it is necessary to learn and to know some biochemical tests used for in time diagnosis of these diseases and correct treatment.

The major proteins of connective tissue are collagen, elastin, fibrillin, laminin and proteoglycans. Among these, collagen is the most abundant, constituting one-third of the total body proteins. Type I mature collagen is a triple helical structure i.e. contains three polypeptide chains each with about 1000 amino acids. The repetitive amino acid sequence of collagen is $(Gly\text{-}X\text{-}Y)_n$. Glycine constitutes about $1/3^{rd}$ of the amino acids while X and Y represent other amino acids.

Keratins are structural proteins found in hair, skin, nails and horns. The strength of the keratins is directly related to the number of disulfide bonds.

3. Specific aims:

- ✓ To give morphological and biochemical description of the components of connective tissue (fibrillar structures, basic intercellular substance).
- ✓ To explain biochemical changes in the connective tissue in some pathological states (mucopolysaccharidosis, collagenosis).
- ✓ To master the method of quantitative determination of hydroxyproline in urine and explain obtained results.

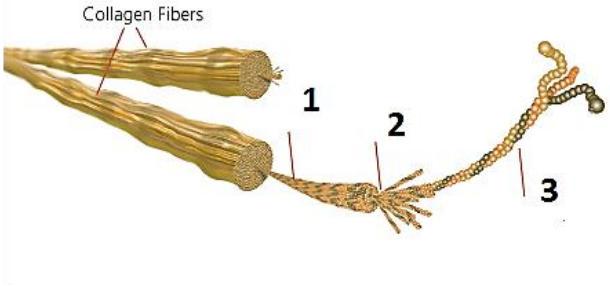
4. Reference card for the separate study of educational literature for the lesson preparation

Questions:	References:
1. **General characterization of morphology and biochemical composition of connective tissue. Biochemical composition of intercellular matrix of loose connective tissue: fibers (collagen, elastin, reticulin); amorphous material.**	1. Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 487–491. 2. Popova L.D. Biochemistry: Manual for medical students or interns / L.D. Popova, A.V. Polikarpova – Kharkiv: KNMU, 2011. – P. 496–497. 3. Gubsky Yu. Biological chemistry: textbook / edited by Yu. Gubsky. – Vinnytsia: Nova Knyha, 2018. – 2nd edition. – P. 446–450. 4. Lecture notes.
2. **Proteins of connective tissue fibers: collagen, elastin, glycoproteins and proteoglycans.**	1. Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 487–491. 2. Popova L.D. Biochemistry: Manual for medical students or interns / L.D. Popova, A.V. Polikarpova – Kharkiv: KNMU, 2011. – P. 497–500. 3. Gubsky Yu. Biological chemistry: textbook / edited by Yu. Gubsky. – Vinnytsia: Nova Knyha, 2018. – 2nd edition. – P. 450–457. 4. Lecture notes.
3. **Biosynthesis of collagen and formation of fibrillar structures.**	1. Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 488–489. 2. Popova L.D. Biochemistry: Manual for medical students or interns / L.D. Popova, A.V. Polikarpova – Kharkiv: KNMU, 2011. – P. 504–507. 3. Gubsky Yu. Biological chemistry: textbook / edited by Yu. Gubsky. – Vinnytsia: Nova Knyha, 2018. – 2nd edition. – P. 457–462. 4. Lecture notes.

4.	**Complex carbohydrates of amorphous matrix of connective tissue – glycosaminoglycans. Mechanisms of formation of intercellular matrix by molecules of glycosaminoglycans – (hyaluronic acid, chondroitin-, dermatan-, keratansulphates). Distribution of glycosaminoglycans in different human organs and tissues.**	1. Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 490. 2. Popova L.D. Biochemistry: Manual for medical students or interns / L.D. Popova, A.V. Polikarpova – Kharkiv: KNMU, 2011. – P. 500–503. 3. Lecture notes.
5.	**Biochemistry of the bone tissue. Features of chemical composition of bone. Features of metabolism in bone.**	1. Popova L.D. Biochemistry: Manual for medical students or interns / L.D. Popova, A.V. Polikarpova – Kharkiv: KNMU, 2011. – P. 503, 507–508. 2. Lecture notes.
6.	**Pathobiochemistry of connective tissue. Biochemical mechanisms of development of mucopolysaccharidoses and collagenoses, their biochemical diagnostics.**	1. Satyanarayana U., Chakrapani U. «Biochemistry», Fours Edition. – 2013. – P. 489. 2. Popova L.D. Biochemistry: Manual for medical students or interns / L.D. Popova, A.V. Polikarpova – Kharkiv: KNMU, 2011. – P. 509–513. 3. Gubsky Yu. Biological chemistry: textbook / edited by Yu. Gubsky. – Vinnytsia: Nova Knyha, 2018. – 2nd edition. – P. 462–465. 4. Lecture notes.

5. Tasks for independent work and self-control

5.1. Write the constituents of collagen fibers (marked with numbers), presented below:



5.2. Primary structure of collagen (tropocollagen): specific amino acid sequence $(\text{-Gly-X-Y-})_n$.

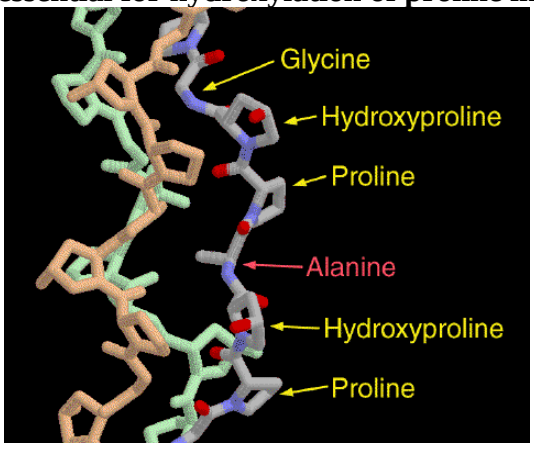
Secondary structure of collagen (tropocollagen): three left-handed α-helices → a right-handed triple helix (superhelix).

Write the formula of amino acid glycine and explain why glycine residues are at every third position of the polypeptide chain that is an absolute requirement for the formation of the triple helix.

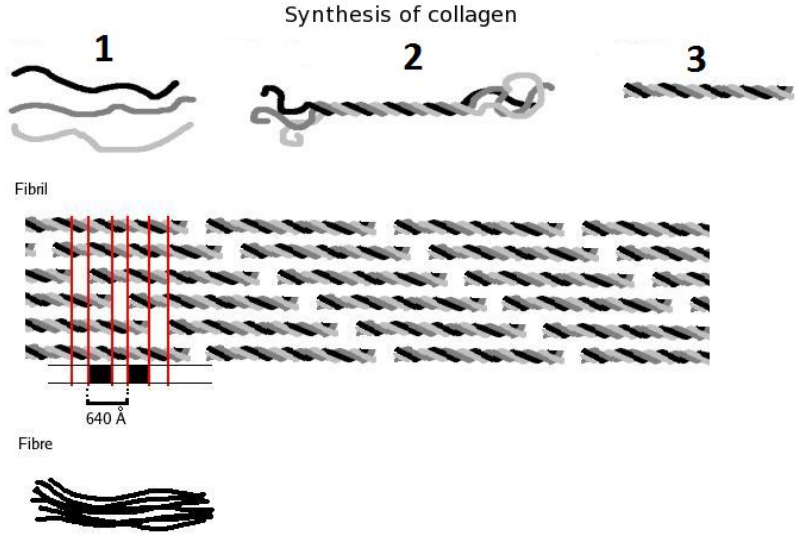
5.3. While X and Y can be any other amino acids, about 100 of the X positions are proline and about 100 of the Y positions are hydroxyproline. Write formula of proline and 4-hydroxyproline and explain what property do residues of these amino acids confer on the collagen molecule.

5.4. Collagen is initially synthesized as a larger precursor polypeptide, procollagen. After that some prolyl and lysyl residues of procollagen are hydroxylated. Name the enzymes and cofactors for hydroxylation.

5.5. Which vitamin is essential for hydroxylation of proline in the molecule of collagen?



5.6. Write biochemical intermediates (marked with numbers) of the process of collagen synthesis, presented below:



5.7. Collagen triple helices are stabilized by hydrogen bonds between residues in different polypeptide chains. What groups of amino acids residues participate in interchain hydrogen bonding?

5.8. In addition, glucosyl and galactosyl transferases attach glucosyl or galactosyl residues to the hydroxyl groups of specific hydroxylysyl residues. What possible function of carbohydrate groups in collagen molecule?

5.9. The procollagen molecule contains extension peptides at both its amino and carboxyl terminal ends, neither of which is present in mature collagen. Both extension peptides contain cysteine residues that form intrachain and interchain disulfide bonds. What possible function of the formation of disulfide bond?

5.10. Following secretion from the cell, extracellular enzymes procollagen aminoproteinase and carboxyproteinase remove the extension peptides at the amino and carboxyl terminal ends, respectively. Explain why these globular end peptides must be absent in mature collagen.

5.11. Collagen fibers are further stabilized by the formation of inter- and intrachain covalent cross-links. The first reaction in cross-link formation is catalyzed by the copper-containing enzyme lysyl oxidase, which converts lysine residues to the aldehyde allysine. The aldehyde then spontaneously reacts with other side chain aldehyde or amino groups to form cross-linked products (aldol condensation product or Schiff base) that are important for the tensile strength and rigidity of the collagen fibers. Write the equations of oxidative deamination of lysine residue and aldol condensation.

5.12. Fill the table «Genetic and acquired disorders of collagen biosynthesis and assembly into fibers»:

Disorder	Causes, mechanisms of disorder development
Scurvy	
Menkes syndrome	
Osteogenesis imperfecta	
Ehlers-Danlos syndromes	
Chondrodysplasias	
Alport syndrome	
Epidermolysis bullosa	

5.13. Fill in the table «Major properties of GAGs»:

GAG	Repeating units, bonds	Tissue location, function
Hyaluronic acid		
Chondroitin sulfate		
Dermatan sulfate		
Keratan sulfate		
Heparan sulfate		
Heparin		

5.14. Complete the table «The mucopolysaccharidoses»:

Disease	Defective enzyme	Accumulated products
Hurler	L-Iduronidase	
Hunter	Iduronate sulfatase	
Sanfilippo A	Heparan sulfamidase	
Sanfilippo B	N-Acetylglucosaminidase	
Sanfilippo D	N-Acetylglucosamine 6-sulfatase	
Maroteaux-Lamy	N-Acetylgalactosamine sulfatase	
Sly	β-Glucuronidase	
Morquio A	Galactosamine 6-sulfatase	
Morquio B	β-Galactosidase	

5.15. Situational task

Murein is a complex polymer that is the major structural feature of the cell walls of all bacreria responsible for the shape and rigidity of cell walls. It referred to as peptidoglycan that consists of three basic components: a backbone composed of disaccharide repeating units (N-acetyl-glucosamine and N-acetylmuramic acid), parallel tetrapeptide chains and series of peptide cross-bridges that form between the tetrapeptide chains.

What specific amino acids are present in murein peptides?

Indicate the function of bacterial cell walls.

6. Individual independent students work

1. Types of collagens. Age changes of collagen structures.
2. Collagen biosynthesis stages.

Practice protocol №23 «____» _____ 20___

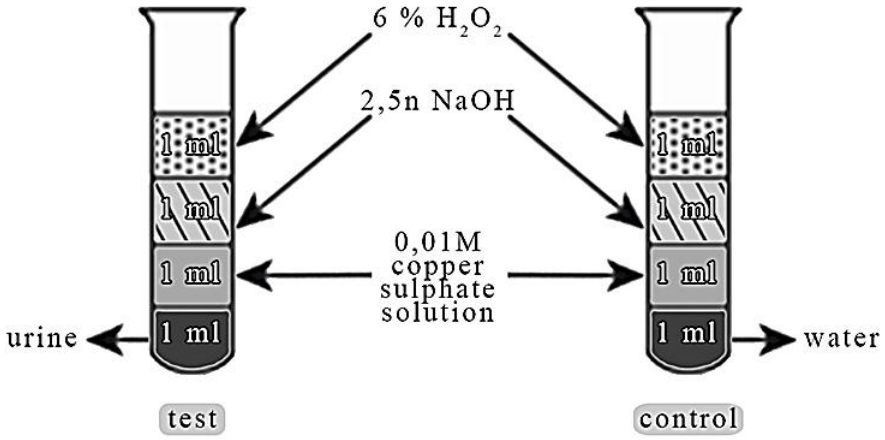
Experiment 1. Quantitative determination of hydroxyproline in urine.

Principle. Hydroxyproline is oxidized to the substance of pyrrole nature, which after condensation with Ehrlich reagent (p-dimethylaminobenzaldehyde) gives a product of pink colour. The intensity of colour is proportional to a concentration of hydroxyproline.

Procedure.

Into one tube add 1 ml of tested urine, into another tube – 1 ml of water.

To both tubes add 1 ml of 0.01 M solution of copper sulphate, 1 ml of 2.5 N NaOH, 1 ml of 6 % solution of hydrogen peroxide.



Stir the probes during 5 min.

Thereafter place the tubes into boiling water bath for 3 min.

Then cool the tubes in cold water, add 4 ml of 3 N solution of sulfuric acid and 1 ml of Ehrlich reagent.

Again heat the tubes on boiling water bath for 1 min and after cooling measure the optical density using a colorimeter at 500-560 nm (green filter) in 10 mm cuvette.

With the help of a calibrated diagram determine hydroxyproline level in the experimental sample and thereafter calculate the amount of hydroxyproline excreted with daily urine using a formula:

$$X = \frac{a_x \cdot V_{day}}{V_{exp}},$$

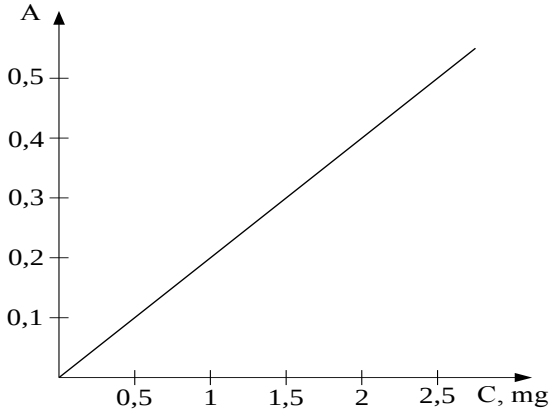
where

a – an amount of hydroxyproline, founded using a calibrated diagram;

V_{day} – a daily volume of urine, ml;

V_{exp} – a volume of urine taken for an analysis, ml.

Curve of dependence of optical density of hydroxyproline solution from its concentration



Results:

Conclusion:

Clinical and diagnostic significance. The quantity of hydroxyproline excreted with urine in in healthy adults in 24 hours is about 15-20 mg. In 10-20 years old persons about 200 mg of hydroxyproline is daily excreted. Increase in excretion of this amino acid up to 1 g/day is observed in collagenoses (rheumatism, rheumatoid arthritis, sclerodermia, dermatomyositis), in hyperparathyroidism, Paget's disease (generalized osteosclerosis). A substantial loss of hydroxyproline is observed in congenital hydroxyprolinemia, caused by deficiency of hydroxyproline oxidase.

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

Examples of Krock-1 tests

1. What class of glucosaminoglycans, due to the large number of carboxyl groups, binds large amounts of water and supports tissue turgor?
A. Hyaluronic acid
B. Dermatan sulfate
C. Keratan sulfate
D. Heparin
E. Chondroitin sulphate

2. Increased content of hydroxyproline in the blood and urine with lesions of joints and bones is observed due to increased catabolism of:
A. Collagen
B. Hyaluronic acid
C. Glycosaminoglycans
D. Proteoglycans
E. Elastin

3. A 60-year-old woman has the symptoms of a rheumatic arthritis. The increased level of which of the below mentioned indexes is the most considerable for confirmation of the diagnosis?
A. Common glucosaminoglycans
B. Lipoproteins
C. Acidic phosphatase
D. Total cholesterol
E. Alanine aminotransferase

4. It is known, that sinovial fluid diminishes abrasion of surfaces of joints. At rheumatic disease or arthritis its viscosity decreases owing to depolymerisation (breaking down) of:
A. Hyaluronic acid
B. Glycogen
C. Collagen
D. Heparin
E. Albumine

5. What hormones inhibit synthesis of proteoglycans and collagen in the connective tissue?
A. Glucocorticoids
B. Somatomedins
C. Somototropin
D. Insulin
E. Androgens

6. Influence of hypovitaminosis of vitamin C on the structure of collagen fibers caused by decreased activity of enzymes:
A. Lysyl hydroxylase, prolyl hydroxylase
B. Lysyl oxidase
C. Glycosil transferase
D. Procollagen peptidase
E. Collagenase

7. Hydroxyproline is an important amino acid for the collagen biosynthesis. Indicate vitamin which participates in the formation of this amino acid by hydroxylation of proline:
A. C
B. D
C. B_1
D. B_2
E. B_6

8. Collagen and elastin are fibrillar elements of connective tissue. Specify the amino acid, which is a component only of the collagen and its determination is used to diagnose several disorders of connective tissue:
A. Hydroxyproline
B. Cysteine
C. Glycine
D. Lysine
E. Hydroxylysine

9. Disturbance of the collagen structure occurs during the deficiency of vitamin C due to the fact that this vitamin is a cofactor of:
A. Lysyl hydroxylase, prolyl hydroxylase
B. Lysyl hydroxylase and collagenase
C. Glycosyltransferase
D. Procollagen peptidase
E. Collagenase

10. For the resorption of keloids hyaluronidase is used for the resorption of keloids. What biochemical process causes advantages of enzymotherapy?
A. Cleavage of hyaluronic acid
B. Cleavage of heparin
C. Cleavage of chondroitin sulfate
D. The synthesis of glucosaminoglycans
E. Cleavage of collagen

11. Insolubility of collagen and its metabolic resistance to various agents is caused by the amino acid composition and a special structure of the protein. Indicate amino acids that quantitatively dominate in the structure of collagen:
A. Glycine, proline
B. Methionine, serine
C. Phenylalanine, tyrosine
D. Cysteine, threonine
E. Arginine, histidine

12. The process of destruction of connective tissue due to collagenosis was observed. What laboratory tests should be assigning to a patient with a chronic form of collagenosis?
A. Content of hydroxyproline and hydroxylysine in blood serum and urine
B. The activity of LDH isoenzymes
C. The level of urates in the blood
D. C-reactive protein
E. Transaminase activity

13. A scar was formed after wound healing. What is the main component of this type of connective tissue?
A. Collagen
B. Elastin
C. Hyaluronic acid
D. Chondroitin sulfate
E. Keratan sulfate

14. Parodontitis is treated with calcium preparations and a hormone that stimulates tooth mineralization and inhibits tissue resorption. What is the hormone called?
A. Calcitonin
B. Parathormone
C. Adrenalin
D. Aldosterone
E. Thyroxine

15. It was revealed an increased concentration of hydroxyproline in the blood and urine in a 63-year-old woman suffering from rheumatism. What is the main reason of this state?
A. Degradation of collagen
B. Activation of prolyl hydroxylase
C. Renal impairment
D. Activation of cathepsins
E. –

16. Mucopolysaccharidoses are hereditary diseases that manifested by pathological changes during bones and joints formation. What urine index indicates this disease?
A. Excessive excretion of glucosaminoglycans
B. Excessive excretion of amino acids
C. Excessive excretion of lipids
D. Excessive excretion of glucose
E. Excessive excretion of proteins

17. A patient with burn disease is at the risk of blood clots formation. Thrombus formation may also take place in other diseases such as atherosclerosis, hypertension and myocardial infarction. What polysaccharide is used to prevent formation of blood clots?
A. Heparin
B. Amylose
C. Starch
D. Hyaluronic acid
E. Chondroitin-4-sulfate

18. Increased breaking of vessels, enamel and dentine destruction in scurvy patients are caused by a disorder of collagen maturing. What stage of modification of procollagen is disordered in this avitaminosis?
A. Hydroxylation of proline
B. Formation of polypeptide chains
C. Glycosylation of hydroxylysine residues
D. Removal of C-ended peptide from procollagen
E. Detaching of N-ended peptide

19. The cause of secondary osteoporosis in Cushing's disease is:
A. Excess of cortisol that inhibits proteosynthesis, which leads to proteolysis of osteoid
B. Excess of cortisol and disruption of cholecalciferol activation
C. Excess of cortisol and activation of proteosynthesis in osteoid
D. Excess of cortisol and increased urinary calcium excretion
E. Excess of cortisol and violation of calcium absorption

20. Parathyroid hormone stimulates calcium absorption in the intestine through the effect on the biosynthesis of calcitriol, which is an activator of calcium absorption. What is the molecular mechanism of calcitriol action?
A. It activates gene expression of Ca^{2+}-binding protein's synthesis
B. It activates synthesis of calcitonin in the thyroid gland
C. It activates processing pro-parathyroid hormone in parathyroid hormone
D. It activates synthesis of cholecalciferol
E. It activates alkaline phosphatase

21. A patient was hospitalised with an impaired vascular permeability. Specify the protein of connective tissue which is disturbed at such conditions:
A. Collagen
B. Myoglobin
C. Albumin
D. Tropomyosin
E. Ceruloplasmin

22. Examination of a patient revealed typical presentations of collagenosis. This pathology is characterized by an increase of the following urine index:
A. Hydroxyproline
B. Arginine
C. Glucose
D. Mineral salts
E. Ammonium salts

23. A 34-year-old patient has a history of periodontitis. As a result of increased collagen degradation, there is a significantly increased urinary excretion of one of the amino acids. Specify it:
A. Hydroxyproline
B. Valine
C. Alanine
D. Glycine
E. Serine

24. A 53-year-old patient is diagnosed with Paget's disease. The concentration of hydroxyproline in daily urine is sharply increased, which primarily means intensified disintegration of:
A. Collagen
B. Keratin
C. Albumin
D. Hemoglobin
E. Fibrinogen

25. Osteolaterism is characterized by a decrease in collagen strength caused by much less intensive formation of crosslinks in collagen fibrils. This phenomenon is caused by the low activity of the following enzyme:
A. Lysyl oxidase
B. Monoamine oxidase
C. Prolyl hydroxylase
D. Lysyl hydroxylase
E. Collagenase

26. Inherited diseases, such as mucopolysaccharidoses, are manifested in metabolic disorders of the connective tissue, bone and joint pathologies. The sign of this disease is the excessive urinary excretion of the following substance:
A. Glycosaminoglycans
B. Amino acids
C. Glucose
D. Lipids
E. Urea

27. Procollagen is a protein synthesized by fibroblasts of connective tissue. Then glycosylation process occurs. What carbohydrates bind to procollagen?
A. Galactose, glucose
B. Fructose, mannose
C. Ribose, deoxyribose
D. Arabinose, sucrose
E. Ribulose, xylulose

28. The insufficiency of which vitamin causes premature termination of bone growth associated with the impaired synthesis of chondroitin sulfate?
A. Vitamin A
B. Vitamin D
C. Vitamin E
D. Vitamin K
E. Vitamin C

29. A 10-year-old girl often experiences acute respiratory infections with multiple spotty hemorrhages in the places of clothes friction. What hypovitaminosis is present in the girl?
A. C
B. A
C. B_6
D. B_2
E. B_1

30. Parathyroid hormone regulates the level of calcium in the blood. Which of these effects implemented by hormone are characteristic for the bone tissue?
A. All of these
B. Reducing the synthesis of organic compounds
C. Inhibition of the citric acid cycle
D. Activation of acidic phosphatase
E. It promotes the formation of citric acid

31. What amino acids by polarity are few in elastin?
A. Polar
B. Nonpolar
C. Middle polar
D. Acidic
E. Alkaline

32. What form of bone tissue metabolism disorder develops in hypergonadism?
A. Osteopetrosis
B. Osteoporosis
C. Osteomalacia
D. Osteosclerosis
E. All of the above

33. Extremities fractures due to bone fragility were observed in the organism of a 9-year-old boy. Function of which endocrine organ is disturbed?
A. Parathyroid gland
B. Thyroid gland
C. Epiphysis
D. Adrenal gland
E. Pancreas

34. Specify metabolites that initiate periodontal bone mineralization:
A. Hydroxylysine, carboxyglutamate, phosphoserine
B. Glycine, alanine, valine
C. Triacylglycerols, cholesterol, sphingosine
D. Pyruvic acid, lactic acid
E. Phosphatidylcholine, phosphatidylethanolamine

35. What bioregulators are calcitropic hormones?
A. Calcitonin, parathyroid hormone
B. Adrenalin, glucagon
C. Thyroxine, insulin
D. Cortisol, aldosterone
E. Testosterone, estrogen

36. The mineralization and demineralization of bone tissue is controlled by hormones:
A. Calcitonin, parathyroid hormone
B. Insulin, glucagon
C. Thyroxine, cortisol
D. ACTH, growth hormone
E. Eicosanoids

37. There is osteoporosis in a patient with chronic renal failure. What vitamin's metabolism infringement promotes this disorder?
A. Vitamin D
B. Vitamin E
C. Vitamin A
D. Vitamin K
E. Vitamin B_1

38. In case of insufficiency of which vitamin observed premature termination of bone growth that is associated with the impaired synthesis of chondroitin sulfate:
A. Vitamin A
B. Vitamin D
C. Vitamin E
D. Vitamin K
E. Vitamin C

39. Which hormone causes mineralization of bone tissue under hypercalcemia?
A. Calcitonin
B. Melatonin
C. Insulin
D. Glucagon
E. Parathyroid hormone

40. What vitamins promote bone mineralization?
A. A C D
B. A K B_6
C. A B_3 C
D. A H D
E. B_1 C E

41. High level of what hormone leads to osteoporosis?
A. Parathyroid hormone
B. Calcitonin
C. Melatonin
D. Insulin
E. Glucagon

42. What hormone's high level leads to osteoporosis?
A. Cortisol
B. Calcitonin
C. Melatonin
D. Insulin
E. Glucagon

43. The softening of bones in rickets is caused by the deficiency of:
A. Cholecalciferol (D_3)
B. Pantothenic acid
C. Nicotiamide
D. Parathyroid hormone
E. Riboflavin

44. When the primary osteoporosis is developing at?
A. Menopause
B. Cushing's disease
C. Diabetes mellitus
D. Hyperparathyroidism
E. Cushing's syndrome

45. What form of bone tissue metabolism disorder develops in rickets?
A. Osteomalacia
B. Osteopetrosis
C. Osteosclerosis
D. Osteoporosis
E. All of the above

46. What form of bone tissue metabolism disorder develops in hyperparathyroidism?
A. Osteoporosis
B. Osteomalacia
C. Osteopetrosis
D. Osteosclerosis
E. All of these

47. What form of bone tissue metabolism disorder develops in Cushing's disease?
A. Osteoporosis
B. Osteomalacia
C. Osteopetrosis
D. Osteosclerosis
E. All of these

48. What enzyme is activated by parathyroid hormone in the kidney?
A. Alfa-1-hydroxylase of calcidiol
B. Alfa-24-hydroxylase of calcidiol
C. Alfa-25-hydroxylase of calcidiol
D. Alfa-25-hydroxylase of calciol
E. Alfa-24-hydroxylase of calciol

49. A patient has symptoms of active form of rheumatism. Clinical examination did not confirm this diagnosis. It should be also emphasized that rheumatism is the connective tissue damage, due to the destruction of heteropolysaccharides within glycoproteins. What biochemical blood and urine tests may be carried out to clarify the diagnosis?
A. Determine aminosugars, sialic acids
B. Determine glycoproteins, total nitrogen
C. Determine glucose, albumin, globulins
D. Glucocorticoids, 17-ketosteroids
E. Determine ketone bodies

50. Examination of a patient suffering from frequent haemorrhages in the inner organs and mucous membranes revealed proline and lysine being included in collagen fibres. Impairment of their hydroxylation is caused by the lack of the following vitamin:
A. C
B. E
C. K
D. A
E. D

Topic 24.
Biochemistry of the nervous system

1. Objective: To learn the composition and peculiarities of metabolism in nervous tissue, its functioning in health and in certain diseases. To use practically a method of cholinesterase activity determination.

2. Actuality of the theme: Brain and spinal cord are basic organs which provide the function of central nervous system in human body. Metabolism in nervous tissue has age dependent peculiarities and is involved in the mechanisms of development of different pathologies. In many diseases of nervous system and especially in stress situation it is important to determine the content not only of neurotransmitters, but also the activity of subsequent enzymes, for example, acetylcholinesterase in the blood serum.

3. Specific aims:

- ✓ To explain peculiarities of chemical composition of white and grey matter of brain.
- ✓ To analyse differences between cerebrospinal fluid and blood plasma.
- ✓ To interpret specific features of metabolism in brain tissue.
- ✓ To explain the role of neurotransmitters in regulation of functions of organs and cells.
- ✓ To analyse changes in cholinesterase activity in different diseases.

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

Questions:	References:
1. **Nerve tissue: general characteristics of structure and functions:** ✓ **Functions of nervous system;** ✓ **Central and peripheral nervous system.**	1. Gubsky Yu. Biological chemistry: textbook / edited by Yu. Gubsky. – Vinnytsia: Nova Knyha, 2018. – 2nd edition. – P. 466–469. 2. Koolman J. Color Atlas of Biochemistry, Second edition / J. Koolman, K.H. Roehm. – Thieme. – 2005. – P. 348. 3. Lecture notes.
2. **Features of biochemical composition: proteins, specific proteins of nervous tissue, lipids.**	1. Popova L.D. Biochemistry: Manual for medical students or interns / L.D. Popova, A.V. Polikarpova – Kharkiv: KNMU, 2011. – P. 497–500. 2. Lecture notes.
3. **Metabolism in nervous tissue:** ✓ **Features of carbohydrate metabolism;** ✓ **Features of lipid metabolism;** ✓ **Features of protein and amino acid metabolism;** ✓ **Features of energy metabolism.**	1. Popova L.D. Biochemistry: Manual for medical students or interns / L.D. Popova, A.V. Polikarpova – Kharkiv: KNMU, 2011. – P. 457–460. 2. Gubsky Yu. Biological chemistry: textbook / edited by Yu. Gubsky. – Vinnytsia: Nova Knyha, 2018. – 2nd edition. – P. 469–470. 3. Koolman J. Color Atlas of Biochemistry, Second edition / J. Koolman, K.H. Roehm. – Thieme. – 2005. – P. 356. 4. Lecture notes.
4. **Structure of nerve cells.**	1. Gubsky Yu. Biological chemistry: textbook / edited by Yu. Gubsky. – Vinnytsia: Nova Knyha, 2018. – 2nd edition. – P. 466–467. 2. Popova L.D. Biochemistry: Manual for medical students or interns / L.D. Popova, A.V. Polikarpova – Kharkiv: KNMU, 2011. – P. 460. 3. Koolman J. Color Atlas of Biochemistry, Second edition / J. Koolman, K.H. Roehm. – Thieme. – 2005. – P. 348. 4. Lecture notes.
5. **Resting and action potential.**	1. Popova L.D. Biochemistry: Manual for medical students or interns / L.D. Popova, A.V. Polikarpova – Kharkiv: KNMU, 2011. – P. 461–462. 2. Koolman J. Color Atlas of Biochemistry, Second edition / J. Koolman, K.H. Roehm. – Thieme. – 2005. – P. 350. 3. Lecture notes.

6. **Synaptic signal transmission:** ✓ **Synapses;** ✓ **Receptors for neurotransmitters.**	1. Popova L.D. Biochemistry: Manual for medical students or interns / L.D. Popova, A.V. Polikarpova – Kharkiv: KNMU, 2011. – P. 462–464. 2. Gubsky Yu. Biological chemistry: textbook / edited by Yu. Gubsky. – Vinnytsia: Nova Knyha, 2018. – 2nd edition. – P. 467–468, 470–483. 3. Koolman J. Color Atlas of Biochemistry, Second edition / J. Koolman, K.H. Roehm. – Thieme. – 2005. – P. 348. 4. Lecture notes.
7. **Biochemistry of neurotransmitters. Characteristic of some mediators and their receptors.**	1. Popova L.D. Biochemistry: Manual for medical students or interns / L.D. Popova, A.V. Polikarpova – Kharkiv: KNMU, 2011. – P. 464–472. 2. Gubsky Yu. Biological chemistry: textbook / edited by Yu. Gubsky. – Vinnytsia: Nova Knyha, 2018. – 2nd edition. – P. 470–483. 3. Koolman J. Color Atlas of Biochemistry, Second edition / J. Koolman, K.H. Roehm. – Thieme. – 2005. – P. 352–354. 4. Lecture notes.
8. **Drugs, neurotransmitters and synapses.**	1. Gubsky Yu. Biological chemistry: textbook / edited by Yu. Gubsky. – Vinnytsia: Nova Knyha, 2018. – 2nd edition. – P. 483–486. 2. Lecture notes.
9. **Pathobiology of mental disorders.**	1. Popova L.D. Biochemistry: Manual for medical students or interns / L.D. Popova, A.V. Polikarpova – Kharkiv: KNMU, 2011. – P. 472–474. 2. Lecture notes.

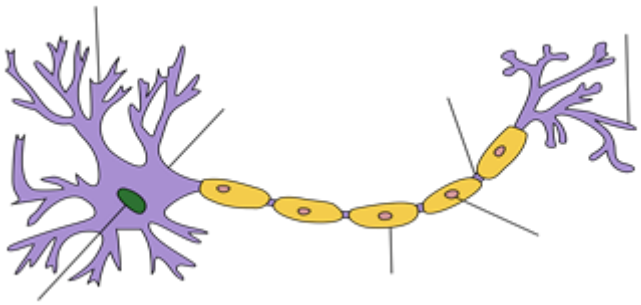
5. Tasks for independent work and self-control

5.1. Brain metabolism has a high requirement for glucose and oxygen. The brain lacks fuel stores (glycogen, fat) and hence requires a continuous supply of glucose from blood. It consumes about 120 g of glucose daily, which corresponds to an energy input of about 1760 kJ (420 kcal), accounting for some 60 % of the utilization of glucose by the whole body in the resting state. Name the major ways that require energy (ATP) in the brain.

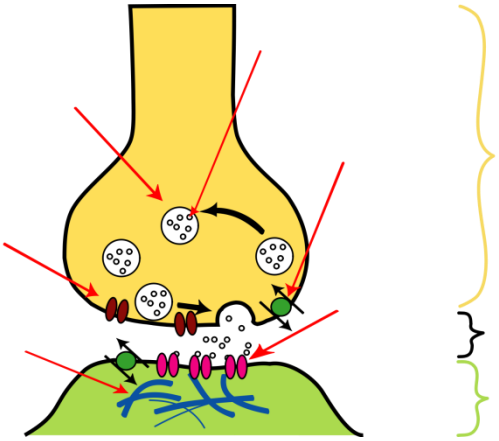
5.2. What compounds replace glucose as fuel for the brain during starvation? Can they totally substitute for glucose? Explain the answer.

5.3. Compose the scheme of glucose oxidation to end products in the brain. Calculate the ATP yield from 1 molecule of glucose.

5.4. Sign the structural parts of a neuron:



5.5. Sign the constituents of the synapse:



5.6. Indicate the criteria to be defined for true neurotransmitters (NTs).

5.7. Fill in the table «Small nitrogen-containing NTs»:

Neurotrans-mitter	Chemical nature	Precursor, synthesis (reaction, enzyme)	Degradation (reaction, enzyme)
Acetylcholine			
Dopamine			
Norepinephrine			
Epinephrine			
Serotonin			
Histamine			
Glutamate			
GABA			
Glycine			
Aspartate			
Nitric oxide			

5.8. Situational tasks:

a) Some neurodegenerative diseases (Alzheimer's disease, amyloidosis, prion diseases) are characterized by the deposition of pathologic insoluble fibrillar proteins that are resistant to proteolytic degradation. The different clinical presentations in each of these diseases results from differences in the function of the native protein and the site of deposition.

Explain the mechanism of the conversion of soluble native proteins into insoluble protein aggregates.

b) Parkinson's disease is a movement disorder caused by damage to brain structures called the basal ganglia and substantia nigra. The major clinical disturbances in Parkinson's disease (tremor, skeletal muscle rigidity, and difficulty in initiating movement) are a result of dopamine depletion, resulting from severe degeneration of dopaminergic neurons. The pathogenesis of Parkinson's disease is not well established and may be multifactorial. Excessive production of reactive oxygen species (superoxide, hydroxyl radical, NO and peroxynitrite anion) causes the neuronal damage. Describe the effects of reactive oxygen species and NO into lipids and proteins that result in cell death. Dopamine cannot penetrate the blood-brain barrier because its precursor L-DOPA is used to alleviate the symptoms of Parkinson's disease. Once L-DOPA is transported into appropriate nerve cells, it is converted to dopamine. What enzyme catalyses this reaction?

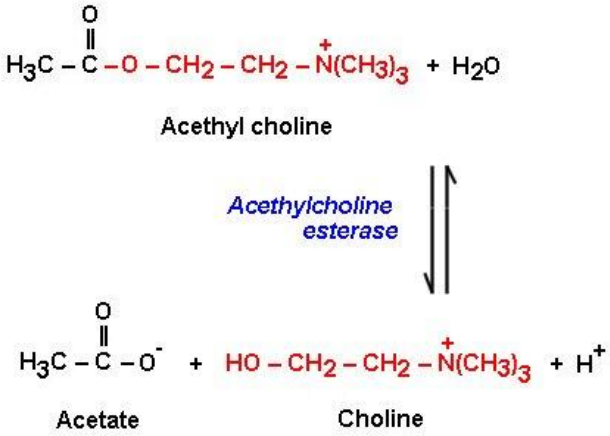
6. Individual independent students work

Features of neurotransmitter balance of the brain at stress

Practice protocol №24 «____» _____ 20___

Experiment 1. Determination of cholinesterase activity in blood serum by titrimetric method of Michel.

Principle. Acetylcholine is hydrolysed enzymatically with production of acetic acid, which is determined by titration with sodium hydroxide.



Procedure.

Take 2 tubes and add 1 ml of acetylcholine solution to each tube.

To test tube add 1 ml of tested serum.

To the second tube (control) add 1 ml of the same serum but inactivated by heating at 56°C during 30 min.

Then to each tube add 2-3 droplets of phenolphthaleine.

Titrate both solutions with 0.01 N NaOH till the appearance of pink colour.

Calculate the difference in volumes of NaOH, used for titration of test and control samples as follows:

$V = V_t - V_c$ (ml),

where

V_t and V_c – volumes of 0.01 N NaOH, expended for titration of test and control probes respectively.

Normal values correspond to 2-4 ml of 0.01 N NaOH used for titration of 1 ml of serum. Normal ranges of cholinesterase activity corresponds to 45-95 μmoles/sec × l.

Results:

Conclusion:

Clinical and diagnostic significance. Activity of cholinesterase in healthy individuals deviates in wide ranges, but is relatively constant in the same person. Cholinesterase is secreted by liver cells. Contrary to the majority of other enzymes its activity in blood is decreased in liver diseases due to alteration of biosynthesis of this enzyme in liver cells. Severe decrease in cholinesterase activity is observed in acute and chronic hepatitis, cirrhosis and malignant tumours of the liver.

Determination of cholinesterase activity is most frequently used as a prognostic index in cases of acute or chronic damage of liver parenchyma by phosphororganic toxins. The degree of enzymatic activity decrease corresponds to gravity and severity of liver cells damage.

Cholinesterase activity is slightly increased in some psychotic diseases, e.g. in maniacal-depressive psychosis, in stress conditions, in depression, in schizophrenia, during demyelinization of nerve trunks and fibers.

Significant increase in activity of cholinesterase in amniotic fluid may indicate on serious damage of nervous system of the fetus.

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

Examples of Krock-1 tests

1. What tissue has the highest sensitivity to the insufficient supply of oxygen?
A. Nervous
B. Muscle
C. Adipose
D. Epithelial
E. Bone

2. What metabolite is the main source of energy for brain?
A. Glucose
B. Fatty acids
C. Amino acids
D. Glycerol
E. Glycogen

3. What are the possible consequences of insulin overdose and a decrease in blood glucose to 2.5 mmol/L or lower?
A. Hypoglycemic coma
B. Hyperosmolar coma
C. Hyperketonemic coma
D. Hypoxic coma
E. –

4. What amino acids are the most widely represented in the structure of the nervous tissue?
A. Glutamic acid, aspartic acid
B. Glycine, proline
C. Histamine, tyrosine
D. Valine, Isoleucine
E. Phenylalanine, threonine

5. What type of complex lipids is neurospecific?
A. Gangliosides
B. Unsaturated fatty acids
C. Phosphatidylcholine
D. Phosphatidylserine
E. Saturated fatty acids

6. What amino acid is used in the nervous tissue for ammonia neutralisation?
A. Glutamic acid
B. Leucin
C. Proline
D. Valine
E. Glutamine

7. Which of the following functions of the brain needs the largest energy supply?
A. Generation and transmission of nerve impulses
B. Reduction of neurofilament
C. Synthesis of neurospecific lipids
D. Neutralization of ammonia
E. Synthesis of biogenic amines

8. Which inhibitory neurotransmitter is synthesized in the nervous tissue during decarboxylation of glutamic acid?
A. Gamma-aminobutyric acid (GABA)
B. Taurine
C. Glycine
D. Glutamine
E. Enkephalin

9. What amino acid is a source of the catecholamines formation?
A. Tyrosine
B. Tryptophan
C. Glutamic acid
D. Glutamine
E. Methionine

10. Which of the neurotransmitters is used as an anti-stress factor?
A. Gamma-aminobutyric acid
B. Glutamine
C. Norepinephrine
D. Dopamine
E. Acetylcholine

11. Which enzyme hydrolyses a neurotransmitter in the cholinergic synapses?
A. Acetylcholinesterase
B. Monoamine oxidase
C. Histaminase
D. Ceruloplasmin
E. Xanthine oxidase

12. A neurological disorder Parkinson's disease is associated with the underproduction of the following neurotransmitter:
A. Dopamine
B. Serotonin
C. GABA
D. Acetylcholine
E. Histamine

13. Which of the neuropeptides has morphinelike activity?
A. Dopamine
B. Norepinephrine
C. Enkephalin
D. Substance P
E. Neurotensin

14. Which enzyme catalyses the oxidative deamination of catecholamines?
A. Glutaminase
B. Glutamyltransferase
C. Cholinesterase
D. Acetylcholinesterase
E. Monoamine oxidase

15. What is the main pathway of brain metabolism providing it with energy?
A. Aerobic oxidation of glucose
B. Anaerobic oxidation of glucose
C. Gluconeogenesis
D. Oxidation of fatty acids
E. Oxidation of ketone bodies

16. Which neurotransmitter is synthesized from acetyl-CoA and cholin?
A. Acetylcholine
B. Serotonine
C. Dopamine
D. GABA
E. Norepinephrine

17. What structures of the brain are characterized by the highest intensity of gas exchange?
A. Cerebral cortex
B. White matter
C. Sympathetic nervous system
D. Parasympathetic parts of the nervous system
E. Synapses

18. Which substances can be used like a source of energy in the brain if the level of glucose is low in the blood?
A. Ketone bodies
B. Glucose
C. Fructose
D. Phospholipids
E. Fatty acids

19. What changes in the neurotransmitter balance are characteristic for stressful influences?
A. Increased synthesis and secretion of catecholamines
B. Increased synthesis of acetylcholine
C. Inhibition of catecholamine synthesis
D. Inhibition of endorphins synthesis
E. Inhibition of glycine synthesis

20. A patient with a craniocerebral trauma has regular epileptic seizures. The formation of what biogenic amine is violated in this condition?
A. Histamine
B. GABA
C. Adrenaline
D. Serotonin
E. Dopamine

21. Biogenic amines are used in psychiatry for treatment of a number of diseases. Which drug is the main inhibitory neurotransmitter?
A. Gamma-aminobutyric acid
B. Histamine
C. Serotonin
D. Dopamine
E. Taurine

22. Sense of fear in humans is caused by the synthesis of dioxiphenylalanine (DOPA) in the limbic system of the brain. What substance is DOPA formed from?
A. Tyrosine
B. Glutamic acid
C. Tryptophan
D. Lysine
E. 5-hydroxytryptophan

23. What is a neurochemical basis of using antidepressants in the treatment of depressive conditions?
A. Inhibition of monoamine oxidase activity
B. Increased concentration of acetylcholine in brain synapses
C. Increased concentration of GABA in brain synapses
D. Inhibition of cholinesterase activity in brain synapses
E. Increased activity of monoamine oxidase

24. The patient came to the doctor with complaints of dizziness, worsening of memory and periodic convulsions. It was found that the cause of such changes is the product of glutamic acid decarboxylation. Name it:
A. TPP
B. GTP
C. GABA
D. ATP
E. THF

25. The pharmacological effects of antidepressants are associated with the inhibition of an enzyme that catalyses the breakdown of such biogenic amines as norepinephrine and serotonin in the mitochondria of neurons of the brain. Which enzyme is involved in this process?
A. Monoamine oxidase
B. Transaminase
C. Decarboxylase
D. Peptidase
E. Lyase

26. Depression and emotional disorders are the result of the deficiency of dopamine, norepinephrine, serotonin and other biogenic amines in the brain. An increase in their content in synapses can be achieved due to antidepressants, which inhibit the enzyme:
A. Monoamine oxidase
B. Diaminooxydase
C. L-amino acid oxidase
D. D-amino acid oxidase
E. Phenylalanine-4-monooxygenase

27. Brain injury caused increased production of ammonia. What amino acid is involved in the removal of ammonia from this tissue?
A. Tyrosine
B. Lysine
C. Valine
D. Tryptophan
E. Glutamic acid

28. A patient has acute viral meningitis. What are changes of the protein composition in the liquor?
A. Increasing of the protein content
B. Reduction of the protein content
C. Protein content is unchanged
D. Advantageously increase of albumins
E. Increasing in the globulin content

29. A newborn has been observed epileptic seizures caused by a deficiency of vitamin B_6. This is due to a decrease of inhibitory neurotransmitter gamma-aminobutyric acid in the nervous tissue. Which enzyme's activity is reduced in this case?
A. Glutamate decarboxylase
B. Alanine aminotransferase
C. Glutamate dehydrogenase
D. Pyridoxal kinase
E. Glutamin synthetase

List of theoretical questions for the exam of 2, 3 semesters

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 D_3, 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 hypercholesterolemia

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.

41. Biochemical functions of nucleic acids and nucleotides. Formation of nucleic acid chain from nucleotides.

42. Nucleic acids: structure, properties. Primary structure of nucleic acids, polarity of polynucleotides, specific features of DNA and RNA structure. Secondary structure of DNA, role of hydrogen bonds in stabilization of secondary structure (Chargaff rules, Watson-Crick model), ant parallelism of chains.Tertiary structure of DNA. Physico-chemical properties of DNA: interaction with cationic ligads; hyperchromic effect; denaturation and renaturation of DNA.

43. Structure, properties and biological functions of RNA. Types of RNA: mRNA, tRNA, rRNA, snRNA; specific features of structure (secondary and tertiary) of different RNA types.

44. Biosynthesis of pyrimidine nucleotides: reactions, regulation. Orotic aciduria.

45. Biosynthesis of deoxyribonucleotides. Formation of thymidyl nucleotides; inhibitors of dTMP biosynthesis as antitumor drugs (structural analogues of dTMP, pterine derivatives).

46. Catabolism of purine nucleotides; hereditary disorders of uric acid metabolism. Biochemical background of hyperurucemia, gout, Lesch-Nyhan syndrome.

47. Biological significance of DNA replication. General scheme of DNA synthesis. Enzymes of DNA replication in prokaryots and eukaryots. Molecular mechanisms of DNA replication: topoisomerases, helicases, the significance of antiparallelism of DNA strains, Okazaki fragments. Stages of synthesis of daughter chains of DNA.

48. General scheme of transcription. Coding and noncoding DNA chains. RNA polymerases of prokaryots and eukaryots. Stages and emzymes of RNA synthesis. Markers of transcription: promotor, initiator, tetrmination segments of genome.

49. Processing as posttranscriptional modification of RNA. Antibiotics, which inhibit transcription.

50. Regulation of gene expression in prokaryots: scheme of regulation according to F.Jacob and J.Monod. Structure of Lac-operon of E.coli. structural and regulatory genes, promotor, operator, regulatory gene and production of protein repressors, repression and induction of Lac-operon function.

51. The genetic code, triplet structure, its properties. Table of genetic code.

52. Protein synthesis system of ribosomes. Components of protein synthesis system . Transfer RNA and amino acid activation. Aminoacyl-tRNA synthetases, second genetic code.

53. Stages and mechanisms of translation: initiation, elongation, termination. Initiating and terminating codons of mRNA. The role of protein factors of ribosomes in translation. Post-translational modification of peptide chains. Regulation of translation. Molecular mechanisms of translation control on example of globin synthesis.

54. The influence of biologically active compounds on translation. Antibiotics as inhibitors of transcription and translation in prokaryots and eukaryots, their biomedical application.

55. Hormones in a system of intercellular integration of physiological functions in human organism. Classification of hormones.

56. Mechanisms of hormonal action – amino acid derivatives, peptide and protein hormones, steroid hormones. Regulatory sites in DNA, which interacts with hormone-receptor complexes.

57. Messenger function of cyclic nucleotides, Ca/calmodulin system phosphoinositides. Serine, threonine and tyrosine proteinkinases in effectory response of the cell.

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

59. Regulation of calcium turnover by parathyroid hormone (PTH) and calcitonine (CT). Calcitriol: biosynthesis, the effect on intestinal absorption of calcium and phosphates. Calcitonine – structure, the effect upon calcium and phosphate turnover. Biochemical characterization of disorders in calcium metabolism (rickets, osteoporosis). Hypo- and hyper- parathyroidism. Distribution of calcium in the body, molecular forms of calcium in blood plasma. Role of bone tissue, intestines and kidneys in support of calcium homeostasis.

60. Hormones of pituitary gland, their role in regulation of endocrine glands function. Hormone family «growth hormone-prolactin-gonadotropins», pathology connected with disorders of GH, somatomedine, prolactin dysfunction.

61. Glycoprotein hormones of hypophysis – TSH, FSH, ICSH, prolactin. Proopiomelanocortine – products of processing of this hormone precursor, (ACTH, lipotropins, endorphins).

62. Hormones of neurohypophysis – oxitocine and vasopressin (ADH), pathology caused by disorder in ADH secretion.

63. Steroids of suprarenal glands. Glucocorticoids: cortisol, cortisone, corticosterone, their role in regulation of gluconeogenesis, anti-inflammatory effects. Itsenko-Cushing syndrome. Mineralocorticoids, role of aldosterone in regulation of water and mineral metabolism, Adison disease, aldosteronism.

64. Hormones of sexual glands. Estrogens – estradiol, estriol, estrone, physiological and biochemical effects, regulation of synthesis and secretion, connection with female month cycle. Androgens – testosterone, dihydrotestosterone, physiological and biochemical effects, regulation of biosynthesis and secretion.

65. Peculiarities of biochemical composition and metabolism of nervous tissue (chemical composition of brain, neurospecific proteins and lipids (gangliosides, cerebrosides, cholesterol). Energetic metabolism in human brain tissue.

66. Neurotransmitters (acetylcholine, noradrenalin, dopamine, serotonin, excitatory and inhibitory amino acids), fumctional role, mechanism of action. Receptors for neurotransmitters and physiologically active substances.

67. Requirements of human organism in nutrients – carbohydrates, lipids, proteins. Biological value of some nutrients. Rational nutrition.

68. Enzymes, biochemistry of digestion and absorption of carbohydrates in gastrointestinal tract.

69. Enzymes, biochemistry of digestion and absorption of proteins in gastrointestinal tract.

70. Enzymes, biochemistry of digestion and absorption of lipids in gastrointestinal tract.

71. Vitamins as essential nutritional components. History of vitamins discovery and development of vitaminology. Causes of exo- and endogenous hypo- and avitaminoses.

72. Vitamin B_1 and B_2, their structure, biological function, sources of supplement, daily requirement. Symptoms of hypovitaminosis.

73. Structure and properties of vitamin H and pantothenic acid. Their significance in metabolism, daily requirement.

74. Vitamins B_6 and PP, their structure, biological function, nutritional sources, daily requirement. Symptoms of hypovitaminosis.

75. Vitamin C and P, their structure, biological function, daily requirement, manifestations of insufficiency in human organism.

76. Vitamins of D group, their structure, biological function, nutritional sources, daily requirement. Symptoms of hypo- and hypervitaminosis, avitaminosis.

77. Vitamin A, its structure, biological function, nutritional sources, daily requirement. Symptoms of hypo- and hyper- vitaminosis.

78. Vitamins E, F, their structure, biological role, mechanism of action, daily requirement. Symptoms of insufficiency.

79. Antihemorrhagic vitamins (K group), their water soluble forms, structure, biological function, nutritional sources, mechanism of action, daily requirement, symptoms of insufficiency, application in medicine.

80. Hemoglobin, its structure, properties and molecular forms. Pathology of hemoglobin: hemoglobinopathias and thalassemias.

81. The function of hemoglobin oxygenation from partial pressure of oxygen, dissotiation curve of oxyhemoglobin, Bohr effect.

82. Acid-base equilibrium of blood. Regulation of pH in biological fluids, disorders of acid-base equilibrium: metabolic and respiratory acidosis, metabolic and respiratory alkalosis, mechanisms of their development.

83. Buffer systems of blood, their types, role of different buffer systems in providement of constant pH of blood.

84. Main groups of blood proteins, their composition and content in normal conditions and in pathology. Albumins and globulins. Resolution of blood plasma proteins by method of protein electrophoresis.

85. Proteins of acute phase of inflammation: C-reactive protein (CRP), ceruloplasmin, haptoglobin, cryoglobulin, alpha-1 antitrypsin, alpha-2 macroglobulin, interferon, fibronectin, their diagnostic validity.

86. Enzymes of blood plasma: genuine (secretory), excretory, indicatory (tissue) enzymes. Kallicrein-kinine and renin-angiotensine systems, their biological significance.

87. Definition of total and residual nitrogen in blood. Nonprotein nitrogen containing compounds of blood, their diagnostic significance. Nitrogenemia, its kinds and causes of development, differentiation in clinical conditions.

88. Functional and biochemical characteristics of intrinsic and extrinsic blood coagulation pathways.

89. Blood coagulation system; characteristics of coagulation factors.; intrinsic and extrinsic blood coagulation pathways. Role of vitamin K in reactions of hemocoagulation (carboxylation of glutamic acid residues, its role in Ca binding). Hereditary disorders of hemocoagulation.

90. Anticoagulation system of blood, functional characteristics of its components – heparin, antithrombin III, citric acid, prostacycline. Role of vascular endothelium.

91. Fibrinolytic system of blood: stages and factors of fibrinolysis. Medicinal influencing fibrinolytic process. Activators and inhibitors of plasmin.

92. Immunoglobulins: structure, biological function, mechanisms of immunoglobulin synthesis. Characteristics of distinct immunoglobulin classes of human blood.

93. Mediators and hormones of immune system; (interleukins, interferons, protein and peptide factors of cell growth and proliferation).

94. Role of liver in regulation of glycemia (glycogenesis and glycogen breakdown, gluconeogenesis), lipid metabolism, turnover of bile acids and bile pigments. Biochemical composition of bile.

95. Role of liver in turnover of bile pigments. Hemoglobin catabolism: production of biliverdin, its transformation to bilirubin, synthesis of bilirubin diglucuronide and excretion with bile. Pathobiochemistry of jaundices; hemolytic (prehepatic), parenchimatous (hepatic), occlusive (posthepatic). Enzymatic congenital jaundices.

96. Detoxification function of liver; biotransformation of xenobiotics and endogenous toxins.Types of reactions of biotransformation of foreign substances in liver.

97. Conjugation reactions in hepatocytes: biochemical mechanisms, functional significance.

98. Electron transport chains of endoplasmic reticulum. Genetic polymorphismus and induction of biosynthesis of cytochrome P-450.

99. Biological role of water and its distribution in human body. Water balance, its types. Regulation of water and mineral metabolism, its disorders. Dehydratation and rehydratation.

100. Biological role of macroelements, trace elements and ultramicroelements. Human microelementoses: endogenous and exogenous causes (technogenic, yatrogenic, etc.).

101. Role of kidneys in regulation of volume, composition of electrolytes and acid-base equilibrium of biological fluids. Biochemical mechanisms of urine production (filtration, reabsorption, secretion and excretion). Characterization of renal clearance and renal threshold, their diagnodtic significance.

102. Physico-chemical properties of urine: volume, color, odor, transparency, acidity (pH), its dependence from diet. Role of kidneys and lungs in regulation of acid-base equilibrium. Ammoniogenesis.

103. Pathological constituents of urine – blood, hemoglobin, creatin. Causes and pathways of their appearance in urine. Glucosuria, galactosuria and pentosuria, causes of their development. Clinical significance of their detection.

104. Clinical significance of detection and determination of indicane, phenylpyruvic and homogentisic acids, ketone bodies and bile pigments in urine.

105. Fine structure and biochemical composition of myocytes; structural organization of sarcomers. Myofibril proteins: myosine, actin, tropomyosine, troponine complex. Molecular organization of thick and thin filaments.

106. Nitrogen containing and nitrogen free water soluble organic compounds of muscules, their structure and functional significance. Molecular mechanisms of muscle contraction: modern data on interaction of muscle filaments. Role of Ca ions in regulation of contraction and relax of striated and smooth muscles.

107. Modern concept on energetics of muscle contraction and relaxation. Macroergic compounds of muscles. Structure, production and role of ATP, creatine phosphate, creatine phosphokinases, sources of ATP in muscle cell; role of creatine phosphate in energetic supply of contraction. Pathobiochemistry of muscles - myopathias.

108. Proteins of connective tissue fibers: collagen, elastin, glycoproteins and proteoglycans. Biosynthesis of collagen and formation of fibrilar structures.

109. Complex carbohydrates of amorphous matrix of connective tissue – glycosaminoglycans. Mechanisms of formation of intercellular matrix by molecules of glycosaminoglycans – (hyaluronic acid, chondroitin-, dermatan-, keratan- sulphates). Distribution of glycosaminoglycans in different human organs and tissues.

110. Pathobiochemistry of connective tissue. Biochemical mechanisms of development of mucopolysaccharidoses and collagenoses, their biochemical diagnostics.

Practical skills

1. Detection of proteins in the blood serum by biuret method. The principle of the method, clinical and diagnostic value.
2. Explain the basic principles of investigation of the salivary amylase (using the iodine test for starch and Trommer reaction).
3. Prove the relative specificity of saliva amylase. What other types of specificity characteristic of enzymes?
4. Determination of diastase of urine. The principle of the method, the rate of clinical and diagnostic value.
5. Inhibition of enzymes of TCA cycle by malonic acid.
6. Determination of glucose in the blood serum, normal glucose concentration in the blood.

7. Determination of the final product of anaerobic glycolysis - lactic acid - by Uffelman's reaction. The principle of the method.
8. Determination of acetone (ketone bodies) in urine. Identification of ketone bodies in blood and urine. Principles of the methods. The value of determination of ketone bodies in the blood and urine.
9. Determination of pyruvic acid in biological fluids with colorimetric method. Explain the principle of the method.
10. Quantitative determination of cholesterol in the blood serum. The principle of the method. What is normal value of cholesterol in the blood serum?
11. Determination of alanine aminotransferase and aspartate aminotransferase activities. The principle of the method. Clinical and diagnostic value of these enzymes.
12. Determination of urea in urine.
13. Determination of bile pigments in urine. Explain the pathway bile pigments production in the body.
14. Qualitative reaction on phenylpyruvate. Clinical and diagnostic importance of this test.
15. Determination of the main components of nucleoproteins. Explain principle of the method.
16. Determination of uric acid in biological fluids (blood, urine). Explain principle of the method.
17. Quantitative determination of vitamin C in urine. The principle of the method.
18. Determination of the creatine in blood serum and in the urine. Explain the principle of the method.
19. Polymerase chain reaction (PCR). Explain the principle of the method.
20. Physico-chemical properties of urine: volume, color, odor, transparency, acidity (pH), its dependence from diet.

APPENDIXES

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 componevts 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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3. Gubsky Yu. Biological chemistry: textbook / edited by Yu. Gubsky. – Vinnytsia : Nova Knyha, 2018. – 2nd edition. – 488 p.
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