

Ministry of Education and Science of Ukraine
Sumy State University
Medical Institute

5103
PRACTICAL TRAINING WORKBOOK
FOR BIOCHEMISTRY

for students of specialty 222 "Medicine"

In two parts

Part 1

Student of _____ group

(name)

Variant _____

Sumy
2021

Practical training workbook for biochemistry / compilers: S. A. Goncharova, L. I. Grebenik, L. O. Primova, N. V. Bozhko. – Sumy : Sumy State University, 2021. – 52 p.

**Status of credit-modular system
for study course "Biological Chemistry"
specialty: 222 "Medicine"**

Department of Biophysics, Biochemistry, Pharmacology and Biomolecular engineering

According to the statements "Rating assessment at the Medical Institute and the recommendations of the methodological Commission of the Medical Institute, the subject "Biological chemistry" for specialty "Medicine" contains module 1: "General regularities of metabolism. The metabolism of carbohydrates, lipids and its regulation. The metabolism of proteins. Molecular biology. Biochemistry of intercellular communications. Biochemistry of tissues and physiological functions", which consists of 4 content modules:

- III semester: 1) General patterns of metabolism.
2) Metabolism of carbohydrates, lipids and its regulation.
IV semester: 3) Metabolism of proteins. Molecular biology. Biochemistry of intercellular communications.
4) Biochemistry of tissues and physiological functions.

Knowledge of students is evaluated according to results:

- current progress;
- the final control.

Current students succeed.

Evaluation of knowledge of students in practical classes conducted according to the traditional system. At the end of the year is shown separately the value of the average marks for theory and testing. The average score that the student receives for the current *students succeed* according to the traditional system of evaluation is transferred into points in accordance with the requirements of credit-modular system (according to the conversion formulas of evaluation points). The maximum number of points for current educational activities of students for a year – 120.

The final control.

Final control is an exam; which students take after studying the entire course of biological chemistry (at the end of IV semester).

Evaluation of students' progress in each semester is following:

III semester: final result of the *current students succeeds* for the semester, the undifferentiated test that the student can pass under the following conditions:

- 1) absence of not worked off practical classes and lectures per semester;
- 2) a positive result of the test "Step-1" (III semester);
- 3) works with the exercises and workbook should have a positive mark;
- 4) the number of points for the current semester is not less than 72.0.

IV semester: final mark in this semester is the result of the exam, which includes material 1-4 content modules (III and IV semester).

For the exam students may be allowed providing:

- 1) absence of not worked off practical classes and lectures per semester;
- 2) a positive result of the test "Step-1" (IV semester);
- 3) works with the exercises and workbook should have a positive mark;
- 4) the number of points for current annual succeed – at least 72.0.

Students, who during the current students succeed during the year received less than 72.0, can raise the score to the minimum level through a retaking of the theoretical material from a mandatory list of questions ("List of questions to increase the score of current succeed" 140 questions). The maximum number of points that student can achieve for the exam is 80 points.

Evaluation in the discipline "Biological Chemistry" occurs according to the following scale

Current students' succeed (scores) for the year	Exam	Total for module (scores)	mark for the discipline
102–120	80 (5)	170–200	5 (Excellent/Full mark)
84.0–101.9	64.0 (4)	140–169.9	4 (Good)
72.0–83.9	48.0 (3)	120–139.9	3 (Fair/Passing grade)
less 72	0 (2)	less 120	2 (Failing grade)

List of compulsory practical skills in the discipline "Biological Chemistry"

The ultimate goals of the study course «Biological Chemistry» are that the student in his future professional activity should be able:

- ✓ To interpret the peculiarities of the physiological state of the organism and the development of pathological processes based on laboratory studies.
- ✓ To analyze the reactivity of carbohydrates, lipids, amino acids, which provides their functional properties and metabolic transformations in the body.
- ✓ To interpret the biochemical mechanisms of pathological processes in the human body and the principles of their correction.
- ✓ Explain the basic mechanisms of biochemical action and the principles of direct application of different classes of pharmacological agents.

Rules for using a workbook

Students should use the "Workbook for practical training for biochemistry" in the practical classes.

It should be used Methodical instructions when preparing for practical training, conducting laboratory work and completing a Workbook [1].

Each class in the Workbook has two types of tasks. Tasks related to the performance of laboratory work and tasks for independent work, which confirm the necessary level of preparation of students for practical training.

The experimental part of the work is performed by students in a practical class according to the algorithm of laboratory work and the method of its conducting [1]; the obtained results are recorded in the Workbook.

In preparation for the laboratory part of the practical training the student should pay attention to the following:

- students have the right to perform laboratory work with a clear understanding of the principles of methods and the main stages of conducting experiments;
- knowledge of the clinical diagnostic value (CDV) of the determination of certain substances in biological material is obligatory and involves the study of normal parameters and analysis of their changes in pathological conditions.

Laboratory work is considered completed if the student:

- confirmed the appropriate level of knowledge about the experiment;
- independently or with a group of students performed the experimental part and reported to the teacher about the result and explained it;
- formulated a protocol in the Workbook, in which he made the necessary calculations, recorded the results and the conclusions.

Students are required to complete the self-study assignments according to their version number. The teacher determines the amount of tasks students need to accomplish.

The teacher checks the quality of the tasks at each class makes comments and confirms the fact of enrollment with his signature.

At the end of the semester, it is compulsory to have all of enrolled work in the Workbook.

Topic: METHODS OF STUDING AMINO ACID COMPOSITION OF BIOLOGICAL LIQUIDS**Laboratory work “Color reactions of proteins and amino acids”****1. Biuret reaction (*Piotrovsky's reaction*)**

Principle of the method _____

2. *Ninhydrin reaction*

Principle of the method: _____

3. *Xanthoproteic reaction*

Principle of the method _____

4. *Adamkiewicz reaction*

Principle of the method _____

5. *Sulfur reaction (Fall's reaction)*

Principle of the method _____

6. *Nitroprusside reaction*

Principle of the method _____

Task 2 (performed in a practical training). For the specified mixture of amino acids and peptides, complete the results table and draw conclusions.

<i>Nº variant</i>	<i>The test fluid contains:</i>
1	Protein with the following amino acid composition: Glu-Ser-Met-Pro-Phe
2	A mixture of the following amino acids: Cys, Lys, Trp, Leu, Ile
3	The peptide with the following amino acid composition: Tir-Thr-Ser-Arg-Phe
4	Protein with the following amino acid composition: Ile-Val-Glu-Cys-Tyr
5	A mixture of the following amino acids: Pro, Trp, Met, Lys, Gis
6	The peptide with the following amino acid composition m: Arg-Phe-Pro-Tyr-Gly
7	Protein with the following amino acid composition: Glu-Lys-Met-Tyr-Cys
8	A mixture of the following amino acids: Asn, Ser, Gly, Trp, Phe
9	The peptide with the following amino acid composition: Gis-Phe-Val-Ala-Cys
10	Protein with the following amino acid composition: Glu-Tyr-Met-Pro-Phe
11	A mixture of the following amino acids: Met, Lys, Trp, Leu, Ile
12	The peptide with the following amino acid composition: Trp-Tre-Ser-Arg-Phe
13	Protein with the following amino acid composition: Leu-Val-Glu-Cys-Tyr
14	A mixture of the following amino acids: Pro, Trp, Met, Lys, Arg
15	The peptide with the following amino acid composition: Cys-Phe-Pro-Tyr-Gly
16	A mixture of the following amino acids: Asn, Met, Gly, Trp, Phe
17	The peptide with the following amino acid composition: Gis-Tyr-Val-Ala-Cys
18	Protein with the following amino acid composition: Glu-Trp-Met-Pro-Phe

Table of results for Task 2 (fill in according to your option number).

The name of the reaction	Observation (positive reaction "+"; negative reaction "-")	Conclusions	
		Amino acids and/or peptides and proteins detected by re- action	Overall conclusion
<i>Biuret</i>			
<i>Ninghydrin</i>			
<i>Xanthoproteic</i>			
<i>Adamkiewicz</i>			
<i>Foley's</i>			
<i>Nitroprusside</i>			

Laboratory work «Chromatographic methods of amino acid separation. Determination of amino acids by distributive chromatography».

Principle of the method: Separation chromatography is based on different solubility of the substances being separated in two partially mixed liquids.

Separation of amino acids by paper chromatography is performed to identify the amino acids present in solution. The method is based on different solubility of individual amino acids in two partially mixed liquids, one of which is water; the other is a water-saturated organic solvent (a mixture of butyl alcohol with acetic acid). The aqueous phase is stationary; in this case the precipitates are adsorbed on an inert carrier – cellulose, which in a saturated humid atmosphere (chromatographic chamber) holds up to 20 % of water while remaining externally dry. The mobile phase is a water-saturated organic solvent.

The mixture of amino acids is applied to a strip of chromatographic paper, the end of which is immersed in an organic solvent. The solvent rises on a strip of paper, dissolves the amino acids deposited on the paper and captures them behind them (Fig. 1). The speed of movement of amino acids on paper depends on the degree of their solubility in the mobile and stationary phases. The greater the solubility of the amino acid in the aqueous phase and the lower the organic solvent, the slower the amino acid moves on paper with the organic solvent.

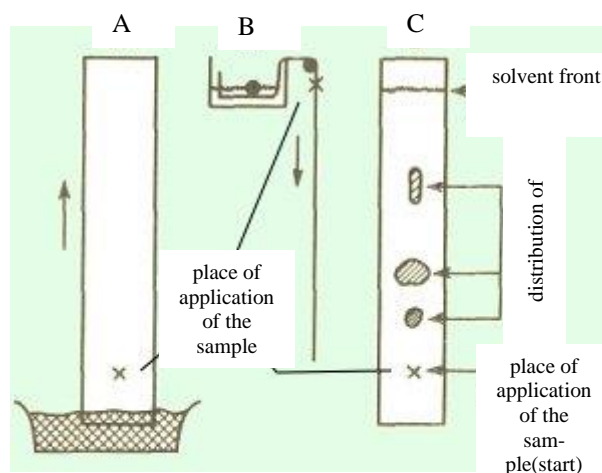


Fig. 1. Chromatography on paper:
A – Ascending chromatography;
B – Descending chromatography (side view);
C – Chromatogram with separated and colored substances.

As the solvent moves, the mixture of amino acids will separate, and those that dissolve better in the organic solvent move along the paper further. Those which dissolve in it worse, go from the place of application the shorter way.

Each amino acid is characterized by its own coefficient of movement speed - R_f , which is easy to calculate:

$$R_f = \frac{l_i}{L}, \text{ where}$$

l_i (cm) the distance moved by the amino acid;

L (cm) the distance moved by the solvent.

R_f constant for these conditions of experience. The values of R_f for some amino acids when separated by paper chromatography are shown in the table:

Amino acid	Solvent	Amino acid	Solvent
	n-butanol + acetic acid +H ₂ O		n-butanol + acetic acid +H ₂ O
Alanine	0.3	Tyrosine	0.45
Arginine	0.15	Phenylalanine	0.6
Asparagine	0.12	Cysteine	0.08
Valine	0.51	Glutamic acid	0.28
Glycine	0.23	Leucine	0.7
Isoleucine	0.67	Proline	0.34

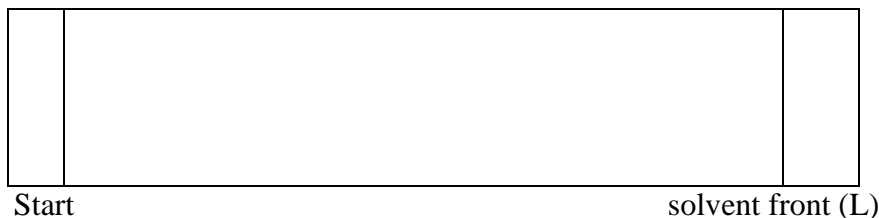
R_f is used in the identification of substances in their analysis by the method of distribution chromatography on paper. To do this, compare the R_f of the amino acids of the test mixture with the R_f of known standard amino acids.

Clinical and diagnostic value. Photocolorimetric and spectrophotometric methods are most commonly used for the quantitative determination of proteins in biological material, and in some cases photofelometric methods are used, as well as protein determination by total nitrogen content (azotometry).

Protein concentration in biological fluids (blood, urine, cerebrospinal fluid, exudates) is carried out in clinical biochemical laboratories for the diagnosis of the disease. Normally, the content of total protein in the serum is 65–85 g/l (6.5–8.5 g %) for adults, 56-85 g/l (5.6–8.5 g % for children up to 6 years) –0–, the normal content of amino acids in the blood plasma is about 21.4 mmol/l.

Task 3. The solvent front has moved from the starting point by 10 cm, when identifying the amino acid mixture by paper chromatography. After the paper was treated with ninhydrin, three blue spots appeared, the centers of which were distant from the starting point by a percentage equal to A, B, and C cm, respectively. Draw a chromatogram of the amino acid mixture (see Fig. 1C), calculate the R_f value and use the standard R_f values to indicate which amino acids are in the mixture (according to the number of your variant).

Variant	The distance from the starting line to the center of the spot (l_i , cm)			Variant	The distance from the starting line to the center of the spot (l_i , cm)		
	A	B	C		A	B	C
1	0.8	3	7	10	2.3	6.7	7
2	2.8	3.4	6.7	11	1.5	2.3	5.1
3	4.5	6	7	12	2.8	3	6.7
4	2.3	4.5	5.1	13	4.5	5.1	7
5	3	5.1	6.7	14	3.4	4.5	5.1
6	1.2	4.5	6	15	1.2	3	6
7	3.4	6	7	16	3.4	6.7	7
8	0.8	2.8	3.4	17	0.8	6	6.7
9	2.8	4.5	5.1	18	5.1	6	7



Calculations and conclusion:

Task 4 (performed in a practical session). Complete the table of results and outputs from the results of the virtual laboratory work "Color reactions to amino acids, peptides and proteins".

Sample	The results of reactions						The conclusion about the presence of amino acids and peptides
	Ninghyd- rin	Biuret	Foley's	Xantho- proteic	Nitro- prusside	Adam- kiewicz	
that invents							
1							
2							
3							
4							
5							
6							

Independent work of students:

Task 5. Complete the table according to the modern rational classification of amino acids

Group of amino acids	Amino acids	Average value pI
1. Nonpolar amino acids		
2. Polar uncharged amino acids		
3. Negative charged amino acids		
4. Positive charged amino acids		

Task 6. Write the structural formula of the peptide and name it (according to your variant number):

№ variant	Peptide	№ variant	Peptide	№ variant	Peptide
1	Ala-Asn-Lys	7	Phe-Met-Thr	13	Tre-Gly-Asn
2	Trp-Glu-Arg	8	Asp-Pro-Cys	14	Arg-Cys-Ser
3	Lys-Glu-Met	9	Leu-Trp-Ser	15	His-Ile-Phe
4	Ala-Trp-Gly	10	Val-Lys-Gly	16	Glu-Asn-Val
5	Val-Gly-Asn	11	Ser-Phe-Leu	17	Lys-Ala-Met
6	Leu-Ser-Glu	12	Ala-Cys-Tyr	18	Cys-Tre-Tyr

Rating and comments _____

The teacher's signature _____

Lesson 3

Date _____

Topic: PHYSICAL AND CHEMICAL PROPERTIES OF PROTEINS. METHODS OF PROTEIN EXTRACTION AND SEPARATION. CLASSIFICATION OF PROTEINS. CHARACTERISTICS OF SIMPLE PROTEINS AND NATURAL PEPTIDES

Laboratory work "Methods of protein extraction and separation."

1. Protein precipitation reactions (salting out).

The principle of the method _____

2. Irreversible sedimentation (denaturation) of proteins by salts of heavy metals, concentrated acids and heating.

2.1. Denaturation of proteins by salts of heavy metals.

The principle of the method _____

2.2. Denaturation of proteins by strong organic and mineral acids

The principle of the method _____

Record the results and conclusions for work 3 in the general table.

Substances / precipitation factors	Result	Conclusion
1	2	3
1. Protein reversible reactions (salting out). Separation of albumins and globulins.		
(NH ₄) ₂ SO ₄ , NaCl, Na ₂ SO ₄ , MgSO ₄		
2. Irreversible sedimentation (denaturation) of proteins by salts of heavy metals, concentrated acids and heating.		
2.1 Denaturation of heavy metals salts		
CuSO ₄ , Pb(NO ₃) ₂ , (CH ₃ COO) ₂ Pb, FeCl ₃		
2.2 Denaturation of proteins by strong organic and mineral acids		
HNO ₃ conc.		
H ₂ SO ₄ conc.		
CCl ₃ COOH, HO ₃ SC ₆ H ₅ COOH		

Laboratory work «The definition of gelatin pI »

The principle of the method _____

Record the results in the table and draw conclusions.

№ tube	pH medium	The degree of turbidity of the solution (-, +, ++)
1	5,6	
2	5,3	
3	5,0	
4	4,7	
5	4,4	
6	4,1	

Conclusion: _____

Independent work of students:

Task 7. At what pH values is the most appropriate electrophoretic fractionation of the protein mixture (explain !!!):

№ Variant	The composition of the protein mixture	Isoelectric point	№ Variant	The composition of the protein mixture	Isoelectric point
1	myosin	5.4	10	myosin	5.4
	hemoglobin	6.8		urease	5.0
2	urease	5.0	11	alkaline phosphatase	4.5
	hemoglobin	6.8		myosin	5.4
3	alkaline phosphatase	4.5	12	cytochrome C	10.65
	urease	5.0		hemoglobin	6.8
4	cytochrome C	10.65	13	pepsin	1.0
	hemoglobin	6.8		alkaline phosphatase	4.5
5	cytochrome C	10.65	14	pepsin	1.0
	myosin	5.4		myosin	5.4
6	cytochrome C	10.65	15	cytochrome C	10.65
	urease	5.0		transferrin	5.9
7	myosin	5.4	16	alkaline phosphatase	4.5
	hemoglobin	6.8		urease	5.0
8	urease	5.0	17	transferrin	5.9
	pepsin	1.0		pepsin	1.0
9	transferrin	5.9	18	hemoglobin	6.8
	hemoglobin	6.8		alkaline phosphatase	4.5

Task 8. Represent the electrophoregram of serum proteins under normal conditions. Specify 5 individual fractions of blood protein and their quantitative content.

Rating and comments _____

The teacher's signature _____

Topic. CLASSIFICATION, STRUCTURAL FEATURES AND RESEARCH METHODS OF COMPLEX PROTEINS

Laboratory work «Research methods of complex proteins»

1. Extraction of mucin from saliva and a qualitative test on the carbohydrate component

The principle of the method _____

2. Hydrolysis of yeast nucleoproteins and detection of a carbohydrate component

The principle of the method _____

Record the results in the table and draw conclusions.

<i>Design of experiment</i>	<i>Results</i>	<i>Conclusion</i>
1 Extraction of mucin from saliva and a qualitative test on the carbohydrate component		
Test with α -naphthol		
2 Hydrolysis of yeast nucleoproteins and detection of a carbohydrate component		
2.1 Test with α -naphthol		
2.2 Reaction to deoxyribose and ribose with difenylamine		

Independent work of students:

Task 9. Complete the "Classification of complex proteins" table:

The name of the class of complex proteins	Prosthetic groups	Representatives	Biological role
<i>1</i>	<i>2</i>	<i>3</i>	<i>4</i>

1	2	3	4

Task 10. Show the chemical (!!!) formulas of the compounds (according to your variant number):

№ variant	Formula	№ variant	Formula
1	adenine	10	deoxyadenosine
2	guanosine	11	thymidine
3	adenosine monophosphate	12	deoxycytidine
4	uridine	13	uracil
5	adenosine	14	deoxyguanosine
6	thymine	15	uridine monophosphate
7	deoxyadenosine monophosphate	16	cytosine
8	cytidine	17	thymine
9	guanine	18	deoxyguanosine monophosphate

Task 11. Draw a chemical formula (!!!) of a fragment of primary DNA (RNA) structure consisting of the following nucleotides (according to your variant number):

№ variant	Formula	№ variant	Formula
1	dAMP and dCMP	10	dAMP and dTMP
2	GMP and UMP	11	GMP and CMP
3	dGMP and dTMP	12	dGMP and dCMP
4	UMP and CMP	13	AMP and CMP
5	dTMP-dGMP	14	dTMP-dGMP
6	UMP and AMP	15	UMP and AMP
7	dAMP and dTMP	16	dAMP and dTMP
8	GMP and CMP	17	CMP and GMP
9	dCMP and dAMP	18	dGMP and dCMP

Rating and comments _____

The teacher's signature _____

Lesson 5

Date _____

Topic. STRUCTURE, PHYSICAL AND CHEMICAL PROPERTIES AND CLASSIFICATION OF ENZYMES. METHODS OF ENZYME ACTIVITY DEFINITION

Laboratory work «Determination of salivary amylase thermolability»

1. Amylase thermolability

The principle of the method _____

Record the results of the work in a table and draw conclusions.

№ tube	Enzyme	Substrate	Temperature	Result	
				Iodine test (+/-)	Trommer`s reaction (+/-)
1					
2					
3					

Conclusion: _____

2 The specificity of saliva amylase

The principle of the method _____

Record the results of the work in a table and draw conclusions.

№ tube	Enzyme	Substrate	Result of Trommer`s reaction (+/-)
1			
2			

Conclusion: _____

3 The pH influence on the amylase activity

The principle of the method _____

Record the results of the work in a table and draw conclusions.

Test tube №	Enzyme	Substrate	pH of medium	Result of Iodine test (+/-)
1				
2				
3				

Conclusion: _____

Independent work of students:

Task 12. Fill in the table "Classification of enzymes by structure"

Type of enzyme	Features of the structure		Examples of enzymes
	Presence of non-protein part (yes/no)	Enzymes of which class belong to this type	

Task 13. Fill in the table "Classification of enzymes by the type of reaction they catalyze"

№ class	Class of enzymes	Coenzyme (s)	Type of reaction	Examples of enzymes

Rating and comments _____

The teacher's signature _____

Lesson 6

Date _____

Topic. THE DEFINITION OF ENZYME ACTIVITY AND MECHANISM OF ENZYME ACTION. KINETICS OF ENZYME CATALYSIS. COFACTORS AND COENZYMATIC VITAMINS' FUNCTIONS IN THE CATALYTIC ACTIVITY OF ENZYMES

Laboratory work « The definition of the amylase activity in blood serum »

The principle of the method _____

Results: _____

Conclusion: _____

Diagnostic value of clinical test _____

Laboratory work « The definition of amylase activity in urine by Vol'gemut method»

The principle of the method _____

Parameter	Test tube №							
	1	2	3	4	5	6	7	8
The urine dilution								
Results of Iodine test (+/-)								

The calculation is performed by the formula

$$X = 1 \times 2 \times \text{urine dilution},$$

where 1 – quantity of urine, ml;

2 – quantity of 0.1% solution of starch, ml.

Conclusion: _____

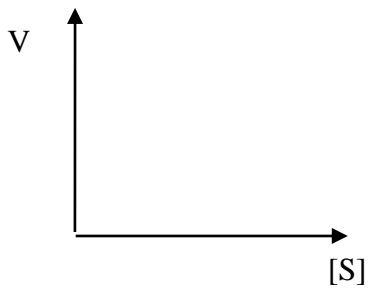
Diagnostic value of clinical test _____

Independent work of students:

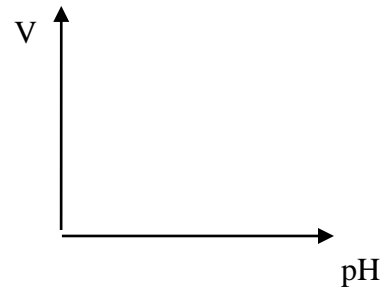
Task 14. Show a graph of the change in the energy of activation of a chemical reaction in the presence of the enzyme in the medium and without it. Analyze and explain why the changes are happening.

Task 15. Draw graphs of the rate of enzymatic reaction from:

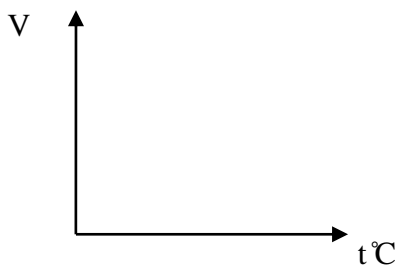
a) substrate concentration;



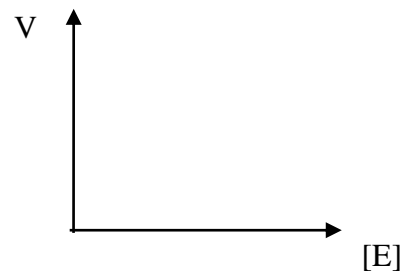
б) pH value;



в) temperature;



г) concentration of the enzyme.



In the graphs, indicate the optimum pH and temperature values, indicate the maximum enzyme activity value.

Task 16. Fill in the table "Clinical and diagnostic value of isoenzymes activity determination"

Enzyme	Isoenzymes form	The nature of the change in activity	Pathological conditions
LDH			
CK			

Task 17. Define the following terms:

Katal – _____

Unit – _____

Specific enzyme activity – _____

Task 18. While studying the enzyme's properties, an unknown substance was added to the enzyme-substrate system. As a result, the Michaelis constant increased 3 times. Explain what happened?

Task 19. Optimal conditions of action of chymotrypsin: pH = 7.8, T = 37 °C. When the pH was changed to 5, the enzyme activity decreased significantly. Explain why.

Task 20. Most body enzymes exhibit maximum activity at T = 37 °C. When the temperature is increased to 50 °C, the enzyme activity is significantly reduced. Explain why.

Rating and comments _____

The teacher's signature _____

Topic. REGULATION OF ENZYMATIC PROCESSES AND THE ANALYSIS OF THE ENZYME PATHOLOGY ORIGIN. MEDICAL ENZYMOLOGY.

Laboratory work «Effect of activators and inhibitors on salivary amylase activity»

The principle of the method _____

Write down the results of the experiment in a table and draw conclusions.

Tube content	Test tube №		
	1	2	3
Saliva			
1 % NaCl			
1 % CuSO ₄			
Starch			
Coloring with iodine (+/-)			

Conclusion:

Laboratory work «The definition of serum cholinesterase (pseudocholinesterase) activity»

The principle of the method _____

Result: _____

Conclusion: _____

Diagnostic value of clinical test _____

Independent work of students:

Task 21. Show the scheme of enzyme activity regulation by phosphorylation dephosphorylation and explain it. Give examples of such enzymes.

Explanation _____

Task 22. One way of regulating enzymatic processes is to regulate the catalytic activity of enzymes. Give a summary of each mechanism.

The mechanism of regulation	Characteristic	Examples of enzymes
Reverse covalent modification		
Allosteric regulation		
Partial proteolysis		
Involvement of cyclic nucleotides and regulatory proteins		

Rating and comments _____

The teacher's signature _____

Topic. METABOLISM: THE GENERAL CHARACTERISTICS. STAGES OF AEROBIC CATABOLISM. TISSUE RESPIRATION

Independent work of students:

Task 23. Explain the following definitions. Give examples of processes.

Catabolism – _____

Anabolism – _____

Amphibolism – _____

Anaplerotic reactions – _____

Task 24. Show a general scheme of the aerobic catabolism of carbohydrates, lipids and proteins. Mark the stages and specify their cellular location. Specify specific and general pathways for catabolism.

Task 25. Complete the table "Types of biological oxidation reactions"

Type of reaction	Scheme of reactions	Enzymes that catalyze this type of reaction	Coenzymes, cofactors	Examples of enzymes
Dehydrogenase				

Oxydase				
Oxygenase				
Hydroperoxi- dase				

Task 26. Determine what type of biological oxidation reaction the scheme belongs to (according to your variant):

№	Scheme of reactions	№	Scheme of reactions	№	Scheme of reactions
1	$\text{SH}_2 + \text{R} \rightarrow \text{S} + \text{RH}_2$	7	$\text{SH}_2 + \text{O}_2 \rightarrow \text{S} + \text{H}_2\text{O}_2$	13	$\text{SH}_2 + \text{R} \rightarrow \text{S} + \text{RH}_2$
2	$\text{SH}_2 + \Phi\text{AD} \rightarrow \text{S} + \Phi\text{ADH}_2$	8	$\text{SH}_2 + \text{HAD}^+ \rightarrow \text{S} + \text{HADH} + \text{H}^+$	14	$\text{SH}_2 + \text{O}_2 \rightarrow \text{S} + \text{H}_2\text{O}_2$
3	$\text{SH}_2 + \text{O}_2 \rightarrow \text{S} + \text{H}_2\text{O}$	9	$\text{Se}^- + \text{A} \rightarrow \text{S} + \text{Ae}^-$	15	$\text{SH}_2 + \text{HAD}^+ \rightarrow \text{S} + \text{HADH} + \text{H}^+$
4	$\text{SH}_2 + \text{HAD}\Phi^+ \rightarrow \text{S} + \text{HAD}\Phi\text{H} + \text{H}^+$	10	$\text{S} + \text{O}_2 \rightarrow \text{SO}_2$	16	$\text{SH}_2 + \Phi\text{AD} \rightarrow \text{S} + \Phi\text{ADH}_2$
5	$\text{SH}_2 + 1/2\text{O}_2 \rightarrow \text{S-OH}$	11	$\text{SH}_2 + \text{O}_2 + \text{H}_2\text{O} \rightarrow \text{S-OH} + \text{H}_2\text{O}_2$	17	$\text{SH}_2 + 1/2\text{O}_2 \rightarrow \text{S-OH}$
6	$\text{SH}_2 + \Phi\text{MH} \rightarrow \text{S} + \Phi\text{MHH}_2$	12	$\text{SH}_2 + \text{HAD}\Phi^+ \rightarrow \text{S} + \text{HAD}\Phi\text{H} + \text{H}^+$	18	$\text{Se}^- + \text{A} \rightarrow \text{S} + \text{Ae}^-$

Rating and comments _____

The teacher's signature _____

Topic. TCA CYCLE: GENERAL CHARACTERISTICS, REACTIONS, REGULATION, AND ENERGETIC BALANCE*Independent work of students:***Task 27. Fill in the table “TCA reactions”.**

№	Substrate (chemical structure)	Enzyme	Product (chemical structure)	Coenzyme
1				
2				
3				
4				
5				
6				
7				
8				

Task 28. Schematically depict the TCA; mark anaplerotic and amphibolic reactions for the citric acid cycle in the diagram.

Task 29. Complete table «TCA regulation»

Key enzyme	Activator	Inhibitor

Task 30. Calculate the maximum energy output of the Krebs cycle, taking into account oxidative phosphorylation when oxidizing three molecules of acetoacetate (ketone body). Acetoacetate is known to be cleaved to form 2 acetyl-CoA molecules.

Rating and comments _____

The teacher's signature _____

Topic. MECHANISMS OF BIOOXIDATION, OXIDATIVE PHOSPHORYLATION AND ATP SYNTHESIS. ELECTRON TRANSPORT CHAIN (ETC)

Laboratory work «Determination of blood catalase activity»

The principle of the method _____

Results: _____

Conclusion: _____

Diagnostic value of clinical test _____

Independent work of students:

Task 31. Complete the table "Biological oxidation enzymes in mitochondria"

№	Type of enzyme	Coenzyme, cofactor (their role in oxidation reactions and reduc- tion)	Reaction scheme	Example
1				
2				
3				
4				
5				

Task 32. Indicate the coefficient of oxidative phosphorylation of the oxidizing the substrate (according to your variant). Explain the answer.

№	Substrate		Substrate
1	α - ketoglutarate	4	Pyruvate
2	Isocitrate	5	Succinate
3	Malate	6	Acyl-CoA

Task 33. Complete the "Respiratory Chain Complexes" table

№	Respiratory Chain Complex (name)	Respiratory Chain Compound
I		
II		
III		
IV		

Rating and comments _____

The teacher's signature _____

Topic. BASIC PRINCIPLES OF CHEMIOSMOTIC THEORY. THE ANALYSIS OF ACTION OF INHIBITORS AND UNCOUPLERS OF THE OXIDATIVE PHOSPHORYLATION

Independent work of students:

Task 34. Schematically draw the respiratory chain and mark the points of coupling of electron transport with ATP synthesis.

Task 35. Using Task 34, designate the area of the respiratory chain that is affected by electron transport inhibitors, oxidative phosphorylation, and uncoupling. If you cannot use the scheme, please explain the mechanism of action of the specified compound below the task table (*according to your variant number*):

№ variant	Inhibitor/ uncoupling	№ variant	Inhibitor/ uncoupling	№ variant	Inhibitor/ uncoupling
1	rotenone	7	antimycin A.	13	amobarbital
2	cyanides	8	seconal	14	T ₃ , T ₄
3	malonate	9	carbon monoxide	15	cyanides
4	piericidin A	10	acetylsalicylic acid	16	dicumarin
5	2,4- dinitrophenol	11	amital	17	rotenone
6	hydrogen sulfide	12	oligomycin	18	anesthetics

Explanation _____

Rating and comments _____

The teacher's signature _____

Topic. EXAMINATION SUBMODULE 1 “BASIC ASPECTS OF METABOLISM”

The list of theoretical questions and practical skills for preparation for the submodule "Basic aspects of metabolism" is given in the guidelines [1].

Lesson 14

Topic. GLYCOLYSIS AS AN ANAEROBIC OXIDATION OF CARBOHYDRATES

Laboratory work «Reactions of mono- and disaccharides»

1. Qualitative determination of monosaccharides. Tromer’s reaction.

The principle of the method _____

Write the results in the table and draw conclusions.

№ tube	Tromer’s reaction	Conclusion
1		
2		
3		

2. Reaction of disaccharides. Barfoed’s test.

The principle of the method _____

Write the results in the table and draw conclusions.

№ tube	Result		Conclusion
	Tromer’s reaction	Barfoed’s test	
1			
2			

3. Determination of lactic acid concentration in blood serum by Boehner's method

The principle of the method _____

Results: _____

Conclusion: _____

Diagnostic value of clinical test _____

Independent work of students:

Task 36. Show the structural formulas of the following carbohydrates (according to your variant number):

№ variant	Carbohydrate	№ variant	Carbohydrate	№ variant	Carbohydrate
1	Deoxyribose	7	Glucose-6-phosphate	13	Lactose
2	Fructose-1,6-diphosphate	8	Glucuronic acid	14	Galactose-1-phosphate
3	Sucrose	9	Mannose	15	Fructose-6-phosphate
4	Galactose	10	Neuraminic acid	16	Ribose
5	Galactosamine	11	Ribose-5-phosphate	17	Glucosamine
6	Fructose	12	Maltose	18	Glucose

Task 37. Complete table “The glycolysis regulation”

The key enzyme	Activator	Inhibitor

Task 38. Draw a schematic of the glycolytic oxidoreduction reaction.

Rating and comments _____

The teacher's signature _____

Topic. AEROBIC GLUCOSE OXIDATION*Laboratory work «Quantitative determination of glucose in blood by glucose oxidase method»*

The principle of the method _____

Result: _____

Conclusion _____

Diagnostic value of clinical test _____

Independent work of students:

Task 39. Calculate the energy balance of complete aerobic oxidation of the substance (according to your variant number):

№ variant	Substance
1	1 glucose molecule (including malate-aspartate shuttle system)
2	1 molecule of glyceraldehyde-3-phosphate
3	monosaccharides formed by hydrolysis of 1 maltose molecule
4	1 molecule of dioxyacetone phosphate
5	1 molecule of pyruvate
6	1 lactate molecule in cardiomyocytes
7	2 molecules of pyruvate
8	1 glucose molecule (including glycerophosphate shuttle system)
9	2 molecules of glyceraldehyde-3-phosphate
10	monosaccharides formed by hydrolysis of 2 maltose molecules
11	2 molecules of dioxyacetone phosphate
12	monosaccharides formed during the hydrolysis of the maltose molecule
13	2 lactate molecules in cardiomyocytes
14	2 molecules of pyruvate
15	2 glucose molecules (including glycerophosphate shuttle system)
16	3 molecules of pyruvate
17	3 molecules of glyceraldehyde-3-phosphate
18	2 glucose molecule (including malate-aspartate shuttle system)

Calculation:

Task 40. Show a scheme of complete aerobic oxidation of a glucose molecule. Specify the stages and their cellular location.

Task 41. Outline the malate-aspartate shuttle mechanism of restorative equivalents NADH^+ transport into mitochondria and indicate the sequence of processes by marking them with numbers (where 1 is the first reaction of the process).

Task 42. Show a scheme that illustrates the change in glucose metabolism in the body of a chronic alcoholic when consuming a large amount of carbohydrates with food. For the correct answer, a deficiency of which vitamin is responsible for the increase in lactate production and the development of lactic acidosis should be established. Explain the answer.

Explanation _____

Rating and comments _____

The teacher's signature _____

Topic. CATABOLISM AND BIOSYNTHESIS OF GLYCOGEN. REGULATION OF GLYCOGEN METABOLISM. METABOLISM OF GLUCOCONJUGATES

Laboratory work «Properties of starch. Detection of glycogen in the liver »

1. The reaction of starch with iodine

The principle of the method _____

Result _____

Conclusion _____

2. The reducing properties of starch

Put results of the research in the table and conclusions.

Test tube №	Result (+/-)		Conclusions
	Lugol's test	Trommer's reaction	
1			
2			

Independent work of students:

Task 43. Show a scheme of a cascade mechanism for the activation of glycogenolysis by glucagon.

Task 44. Outline the cascade mechanism of glycogenesis inhibition by adrenaline.

Task 45. Fill in the table "Hormone regulation of glycogen metabolism in liver and muscle".

Organ	Hormone	Effect (↑ or ↓)		Mechanism of effect
		glycogenolysis	glycogenesis	
Muscles				
Liver				

Rating and comments _____

The teacher's signature _____

Topic. GLUCONEOGENESIS AND ALTERNATIVE PATHWAYS OF CARBOHYDRATE METABOLISM. DEFINITION METHODS OF GLUCOSE CONCENTRATION IN BLOOD

Laboratory work « The use of photometer “Glucophot” for the quantitative determination of glucose in blood»

Principle of the method _____

Result: _____

Conclusion _____

Independent work of students:

Task 46. Complete the reaction sequence fragment with the names of the missing components. Draw their structural formulas (according to your variant number):

№ variant	Reaction sequence fragment
1	Glucose → Glucose-6-phosphate → A → Fructose-1,6 - bisphosphate → B+C.
2	Pyruvate → A → Malat → A → PEP → B → 3-Phosphoglycerate → C
3	2 Phosphoglycerate → A → B → Lactate
4	Glycogen → A → Glucose -6- phosphate → B → 6- phosphate - gluconate → C
5	A → 2 Phosphoglycerate → B → Pyruvate → C
6	Pyruvate → A → PEP → B → 3-Phosphoglycerate → C
7	Fructose-1,6 - bisphosphate → A+B → 1,3-bisphoglycerate → C
8	Galactose → A → B → Glucose -6- phosphate → C
9	Gliceraldehyde-3- phosphate → A → B → 2 Phosphoglycerate → C
10	Glycogen → A → Glucose -6- phosphate → B → Fructose-1,6 - bisphosphate → C
11	A → Oxaloacetate → B → 2 Phosphoglycerate → C
12	A → 1,3-bisphoglycerate → B → C → PEP
13	A → 2 Phosphoglycerate → B → 1,3-bisphoglycerate → C
14	Glucose -6- phosphate → A → 6- phosphogluconate → B → Ribose-5-phosphate
15	1,3-bisphoglycerate → A → 2 Phosphoglycerate → B → Pyruvate → C

Substance A:

Substance B:

Substance C:

Task 47. Fill in the table “Regulation of gluconeogenesis”.

Irreversible glycolysis reaction	The name of the glycolysis enzyme	Glycolysis bypass	Gluconeogenesis enzyme	Regulation of enzyme activity	
				activators	inhibitors

Task 48. Fill in the table "Hereditary enzymes of the metabolism of certain carbohydrates".

Metabolism type	Pathology name	Defective enzyme	Changes in metabolism	Clinical manifestations
Pentose phosphate pathway				
Fructose metabolism				
Galactose metabolism				

Rating and comments _____

The teacher's signature _____

Lesson 18

Date _____

Topic. MECHANISMS OF METABOLIC AND HORMONAL REGULATION OF GLUCOSE METABOLISM AND ITS CONCENTRATION IN BLOOD. BIOCHEMISTRY OF DIABETES MELLITUS

Laboratory work «Quantitative determination of glucose in urine by the Althausen method. Sugar loading method»

1. Quantitative determination of glucose in urine by the Althausen method (in modification of K. A. Kost).

The principle of the method _____

Result: _____

Conclusion _____

Diagnostic value of clinical test _____

2. Glucose tolerance test (sugar loading test).

Task 49. Based on the results of the sugar loading, draw a glycolytic curve and conclude on the patient's health status (according to your variant number):

time, min	Variant	1	2	3	4	5	6	7	8	9
	Serum glucose concentration, mmol/l									
0	5.5	3.2	3	5	4	5.5	3.1	4.2	5.6	
30	6	3.8	4.8	6.5	5.5	6.5	3.2	4.6	6.6	
60	7	4.3	5.5	7	6	7.2	3.9	5.3	7.2	
90	9	5.5	6	8.5	7	9.2	5.2	6.2	8.3	
120	10	4.5	7	11	8	10.3	4.7	7.6	11.2	
150	8	4	5.5	10	6	8.6	4.4	5.5	10.8	
180	7.5	3	4.5	8	5.7	7.9	3.9	4.3	9	



Conclusion _____

Diagnostic value of clinical test _____

3. Definition of glucose-binding to haemoglobin (HbA_{1c}) and fructosamine

Diagnostic value of clinical test _____

Independent work of students:

Task 50. Explain how the activity of metabolic processes will change under these conditions (it is advisable to indicate the names of the key enzymes involved in regulation).

№ variant	Process of metabolism	Conditions	№ variant	Process of metabolism	Conditions
1	pentose phosphate pathway	consumption of carbohydrate food	10	pentose phosphate pathway	diabetes
2	glycogenolysis	starvation	11	glycogenolysis	consumption of carbohydrate food
3	Gluconeogenesis	diabetes	12	gluconeogenesis	glucocorticoid hyperproduction
4	pentose phosphate pathway	starvation	13	glycogenesis	stress
5	glycogenolysis	diabetes	14	glycogenesis	after eating
6	gluconeogenesis	starvation	15	gluconeogenesis	after eating of carbohydrate food
7	pentose phosphate pathway	insulinoma	16	pentose phosphate pathway	stress
8	glycogenolysis	stress	17	glycogenesis	diabetes
9	pentose phosphate pathway	physical activity	18	gluconeogenesis	physical activity

Task 51. Fill in the table "Pathological conditions accompanied by changes in blood glucose concentration"

Pathology	Changes in blood glucose concentration (↓,↑)	Explanation of the cause of the changes
Diabetes		
Insuloma		
Cushing's disease		
Hypothyroidism		
Acromegaly		
Von Gierke's disease		
Hyperthyroidism		

Rating and comments _____ **The teacher's signature** _____

Lesson 19

Date _____

**Topic. TESTS ON SITUATIONAL TASKS FROM “STEP-1”:
“BASIC ASPECTS OF METABOLISM”**

Use the literature for preparation for the class [2]:

Lesson 20

Date _____

**Topic. GENERAL CHARACTERISTICS OF LIPIDS. LIPIDS OF BIOMEMBRANES.
LIPOLYSIS AND ITS REGULATION.**

Laboratory work «Definition of total lipids in blood serum»

The principle of the method _____

Result: _____

Conclusion: _____

Diagnostic value of clinical test _____

Independent work of students.

Task 52. Draw the scheme of lipolysis regulation under the action of epinephrine (black) and insulin (red).

Task 53. Figure out the structure of diacylglycerol containing fatty acid (*according to your variant number*).

Variant №	Fatty acid	Variant №	Fatty acid	Variant №	Fatty acid
1	palmitic	4	stearic	7	linoleic
2	arachidonic	5	oleic	8	behenic (docosanoic)
3	lignoceric	6	palmitoleic	9	linolenic

Rating and comments _____

The teacher's signature _____

Lesson 20

Date _____

Topic. β -OXIDATION OF FATTY ACIDS. KETONE BODY METABOLISM RESEARCH.

Laboratory work « Qualitative determination of acetone and acetoacetate in urine»

1. Legal's test for acetone and acetoacetic acid

The principle of the method _____

2. The Gerhard's reaction on acetoacetic acid

The principle of the method _____

Enter the results in the table and draw conclusions.

Test tube №	Qualitative reaction		Conclusions
	Legal's test	The Gerhard's re- action	
1			
2			

Diagnostic value of clinical test _____

Independent work of students.

Task 54. Calculate the energy balance of the substance oxidation (*according to your variant number*)

Variant №	Substance	Variant №	Substance	Variant №	Substance
1	β -hydroxybutyrate	4	Stearic acid	7	Arachic acid
2	Lauric acid	5	Lignoceric (tetra-cosanoic) acid	8	Acetoacetate
3	Myristic acid	6	Palmitic acid	9	Behenic (docosa-noic) acid

Task 55. Draw the chemical reactions of the following transformations, name the appropriate enzymes (*according to your variant number*)

Variant №	Substance
1	Acetyl-CoA \rightarrow β -hydroxy- β -methyl-glutaryl-CoA
2	Stearyl-CoA \rightarrow Palmityl-CoA
3	Propionyl-CoA \rightarrow Succinyl-CoA
4	Acetyl-CoA \rightarrow Acetone
5	Glycerol \rightarrow Glyceraldehyde-3-p
6	Acetyl-CoA \rightarrow β -hydroxybutyrate
7	Palmityl-CoA \rightarrow Мірістил-КоА
8	β -Oxybutyrate \rightarrow Acetyl-CoA
9	Acetyl-CoA \rightarrow Acetoacetate

Task 56. Draw a scheme illustrating the synthesis of ketone bodies and their use in muscle.

Task 57. Show and explain a scheme that illustrates the increase in the production of ketone bodies in diabetes.

Rating and comments _____

The teacher's signature _____

Lesson 22

Date _____

**Topic. BIOSYNTHESIS OF FATTY ACIDS, TRIACYLGLYCEROLS AND COMPLEX LIPIDS.
DETERMINATION OF TOTAL PHOSPHOLIPID CONCENTRSTION IN BLOOD SERUM**

Laboratory work № «**Determination of total phospholipids in blood serum
according to the concentration of phosphate**»

The principle of the method _____

Result: _____

Conclusions: _____

Diagnostic value of clinical test _____

Independent work of students:

Task 58. Show and explain a scheme of shuttle system, which provides the first stage of palmitate synthesis.

Task 59. Show the sequence of biochemical transformations (*according to your variant number*)

Variant №	Biochemical transformations
1	phosphatidylethanolamine → phosphatidylcholine
2	phosphatidic acid → triacylglycerol
3	phosphatidylethanolamine → phosphatidylserine
4	phosphatidic acid → phosphatidylserine
5	ethanolamine → phosphatidylethanolamine
6	glycerol → phosphatidic acid
7	phosphatidylcholine → phosphatidylserine
8	choline → phosphatidylcholine
9	acetyl-CoA → malonyl-CoA
10	phosphatidic acid → phosphatidylserine
11	phosphatidic acid → phosphatidylethanolamine
12	glycerol-3-phosphate → phosphatidic acid
13	glycerol-3-phosphate → cardiolipin
14	phosphatidic acid → phosphatidyl inositol

Task 60. Fill in the table «Genetic anomalies of sphingolipid metabolism»

The name of sphingolipidoses	Molecular cause	Consequences

Rating and comments _____

The teacher's signature _____

Lesson 23

Date _____

**Topic. CHOLESTEROL BIOSYNTHESIS AND BIOTRANSFORMATION.
BLOOD LIPOPROTEINS.**

***Laboratory work «Determination of total blood cholesterol by Ilk method.
Qualitative reaction to bile acids»***

1. Determination of total blood cholesterol by Ilk method.

The principle of the method _____

Result: _____

Conclusion _____

Diagnostic value of clinical test _____

Independent work of students:

Task 61. Complete the table «Regulation of cholesterol synthesis ». Specify the features of regulation of key enzyme activity of cholesterol synthesis. (use tags: «+» – active, «-» – not active, ↑ – increased activity, ↓ – decreased activity).

Key enzyme of synthesis	Inhibitors	Covalent modification of enzyme		Hormonal regulation			
		phosphorylated form	dephosphorylated form	insulin	T ₃ ,T ₄	glucagon	glucocorticoids

Task 62. Draw the structural formula of the substance and explain its biological role (according to your variant number)

Variant №	Compound	Variant №	Compound
1	cholesterol	10	lysophosphatidylcholine
2	cholesterol ester which consists of oleic acid	11	cholesterol ester which includes palmitic acid
3	cholesterol ester which includes linoleic acid	12	cholesterol ester which includes palmitoleic acid
4	cholic acid	13	cholanic acid
5	glycocholic acid	14	deoxycholic acid
6	taurocholic acid	15	β-hydroxy-β-methylglutaryl-CoA
7	lecithin	16	mevalonate
8	lysolecithin	17	chenodeoxycholic acid
9	lithocholic acid	18	7-α-Hydroxycholesterol

Explanation _____

Task 63. Describe the chemistry of the formation of cholesterol ester in liver cells, which includes palmitic acid.

Task 64. Write the sequence of TAG resynthesis reactions in enterocytes. Indicate from which compound resynthesis begins. Explain why. Specify the names of enzymes.

Explanation _____

Task 65. Complete the table “Blood plasma lipoprotein classes”.

Name of the lipoproteins class		Place of synthesis	The biological role
According to density	according to the electrophoretic separation		

Rating and comments _____

The teacher's signature _____

Topic. METABOLISM IN ADIPOCYTES. METABOLISM OF GLYCEROL. BIOCHEMISTRY OF UNSATURATED FATTY ACIDS*Laboratory work «Definition of free fatty acids in blood serum »***Principle of the method** _____

_____**Result:** _____
_____**Conclusion:** _____
_____**Diagnostic value of clinical test** _____
_____*Independent work of students:*

Task 66. It is known that during fasting the ratio of insulin/contrainsular hormones decreases in the direction, first of all, of increasing the concentration of glucagon. Explain the changes in TAG metabolism in adipocytes under fasting conditions.

Task 67. Explain the features of metabolic changes in TAG metabolism in adipocytes in diabetes mellitus.

Task 68. Draw a scheme illustrating the use of glucose in the synthesis of TAG in adipocytes. Indicate (highlight) the four directions of glucose conversions that are necessary for lipogenesis.

Rating and comments _____**The teacher's signature** _____

**Topic. REGULATION AND DISORDERS OF LIPID METABOLISM.
INTERRELATION OF LIPID AND CARBOHYDRATE METABOLISM**

Laboratory work «Determination of β -lipoproteins (LDL) in blood»

Principle of the method _____

Result: _____

Conclusion: _____

Diagnostic value of clinical test _____

Independent work of students:

Task 69. Complete the table (\uparrow , \downarrow) "Hormonal regulation of lipid metabolism".

Hormone	Biochemical effects on lipid metabolism	
	lipolysis	lipogenesis
Insulin		
Prostaglandins		
Prolactin		
Epinephrin		
Glucagon		
Glucocorticoids		
Thyroid hormones		

Task 70. Draw a scheme that illustrates the factors regulating fat content of liver.

Task 71. Draw a scheme that illustrates the relationship between carbohydrate and lipid metabolism.

Task 72. Show a scheme that explains the increase in the synthesis of ketone bodies during fasting.

Rating and comments _____

The teacher's signature _____

Lesson 26–27

Date _____

Topic. EXAMINATION SUBMODULE 2 “BASIC ASPECTS OF METABOLISM. CARBOHYDRATE AND LIPID METABOLISM AND ITS REGULATION”

Use the literature for preparation for the class [1].

Literature

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2. Biochemical review questions for Step-1 examination of medical students (Part I) / L. A. Primova, L. I. Grebenic, I. V. Chorna, I. Yu. Vysotsky. – Sumy : SumSU, 2010. – 105 p.

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Електронне навчальне видання

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ДЛЯ ПРАКТИЧНИХ ЗАНЯТЬ ІЗ БІОЛОГІЧНОЇ ХІМІЇ**

для студентів спеціальності 222 «*Медицина*»

У двох частинах

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(Англійською мовою)

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