DANYLO HALYTSKYI
LVIV NATIONAL MEDICAL UNIVERSITY

DEPARTMENT OF BIOLOGICAL CHEMISTRY

Guide for inividual work on BIOLOGICAL CHEMISTRY

for students of pharmaceutical faculty

PART I

LVIV – 2017

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

Objective: Introduction to the assignments and methods of biochemical investigation; to learn specific methods of investigation of biologically active substances as well to make an acquaintance with instruments and devices used in biochemistry. To learn structure, classification and physico-chemical properties of proteins; to perform qualitative reactions on amino acids and quantitative determination of protein.

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

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

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

Specific aims:

- To know principal stages and regularities in origin and development of biochemistry as fundamental medical and biological science and educational discipline.
- To recognize priciples of methods of investigation of functional status in human body in health and disease.
- To interprete data of biochemical investigations and evaluate the status of selected metabolic pathways
- To determine optical density of colored solutions at distinct light wavelength using a photocolorimeter, to interpret obtained results properly.

GUIDE FOR THE INDEPENDENT STUDY OF THE TOPIC:

	Questions:	References:
1.	**The objectives and assignments of biochemistry and its principal trends and parts:** • Definition of biochemistry as a science; • Subject and object of biochemistry; • Major branches of biochemistry	1. Harper′s Biochemistry. 26th edition / R. K. Murray, Daryl K. Granner, Peter A. Mayes, Victor W. Rodwell, 2003.- P.1-4 2. Lecture notes.
2.	**A short history of biochemistry and its main periods.**	1. Lecture notes. 2. Dwb.unl.edu/Teacher/NSF/C10/C10Links/mills.edu/RESEARCH/FUTURES/JOHNB/biohistory.html

3.	**Methods in biochemical investigations:**	
	Optical methods in biochemical investigations: • Photoelectrocolorimetry; • Spectrometry; • Spectrophotometry; • Fluorescent analysis.	1. http://www.rmsc.nic.in/RHSDP%20Training%20Modules/Techniques%20In%20Biochemistry%20Lab.pdf P.17-22 2. Lecture notes.
	Electrophoresis: • Zonal and frontal electrophoresis; • Electrophoresis in different gels; • Isoelectrofocusing; • Immunoelectrophoresis.	1. Biochemistry. An Introduction/ Trudy McKee, James R. McKee , 1996.- P. S-3-S-4. 2. Lecture notes.
	Chromatography: • Ion-exchange chromatography; • Affinity chromatography; • Thin layer chromatography – TLC; • Gel filtration.	1. Biochemistry. An Introduction/ Trudy McKee, James R. McKee , 1996.- P. S-3 2. Lecture notes.
	Radioisotopic methods	1. Biochemistry. An Introduction/ Trudy McKee, James R. McKee , 1996.- P. S-10-S-11
	Enzyme immunoassays (ELISA)	1. Biochemistry. An Introduction/ Trudy McKee, James R. McKee , 1996.- P. S-11
4.	**General characterization of amino acids. Classification of amino acids:** • due to polarity; • due to structure; • due to biological properties. ***STUDENTS MUST KNOW STUCTURE OF ALL 20 PROTEINOGENIC AAs!!!***	1. Satyanarayana U., Chakrapani U. "Biochemistry", Third Edition. – 2006. – P. 40-49. 1. Harper′s Biochemistry. 26th edition / R. K. Murray, Daryl K. Granner, Peter A. Mayes, Victor W. Rodwell, 2003.- P.14-16. 2. Lecture notes.
5.	**Biologically active peptides:** glutathione; • hormones of hypophysis; • hormones of hypothalamus; • insulin **their significance and employment in medicine**	1. Satyanarayana U., Chakrapani U. "Biochemistry", Third Edition. – 2006. – P. 65-66. 2. Lecture notes
6.	• **Modern concept of structural levels in organization of**	1. Satyanarayana U., Chakrapani U. "Biochemistry", Third Edition. – 2006. –

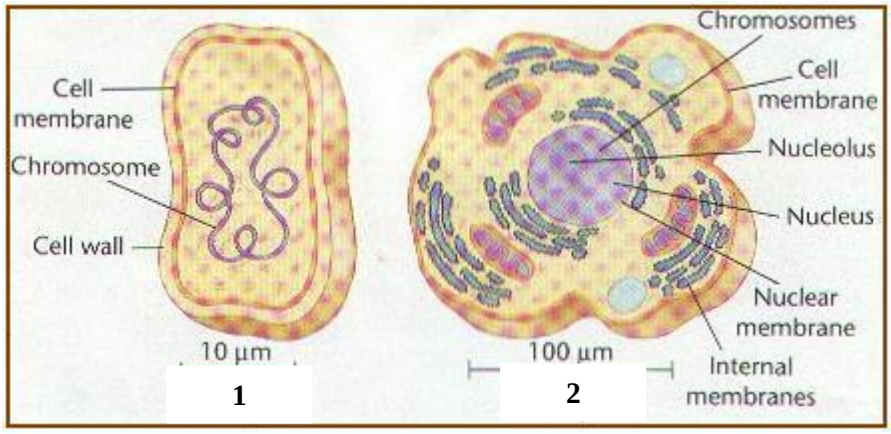
	protein molecule and types of chemical bonds in protein molecule. • **Physical and chemical properties of proteins.** • **Isoelectric point, proteins as amphoteric electrolytes.**	P. 52-59, 60, 2. Harper′s Biochemistry. 26th edition / R. K. Murray, Daryl K. Granner, Peter A. Mayes, Victor W. Rodwell, 2003.- P.14-16. 3. Lecture notes
7.	**Classification of proteins. Characterization of simple proteins**.	3. Satyanarayana U., Chakrapani U. "Biochemistry", Third Edition. – 2006. – P. 63-64. 4. Lecture notes
8.	**Conjugated proteins, their characteriristics**	1. Satyanarayana U., Chakrapani U. "Biochemistry", Third Edition. – 2006. – P. 64-65. 2. Lecture notes

COMPREHENSION QUESTIONS:

1. Select appropriate definitions of divisions of biochemistry:

	Divisions:		**Definition:**
1	Bioenergetics	A	the study of capabilities of the directed changes of nuclear genetic apparatus that has already found its application in the synthesis of biologically active compounds, hormones, enzymes, new species of plants and cloning of animals
2	**Molecular biology**	**B**	the study of the regulations of energy release, accumulation and utilization in biological systems;
3	**Biotechnology**	**C**	the study of vitamins, including their structures, modes of action, and function in maintaining body health
4	**Vitaminology**		the branch of science that deals with the biochemical nature and activity of enzymes
5	**Enzymology**	**D**	science of the regulations of preservation and realization of the genetic information

2. Which cell (1 or 2) on the picture bellow is eukaryotic one?

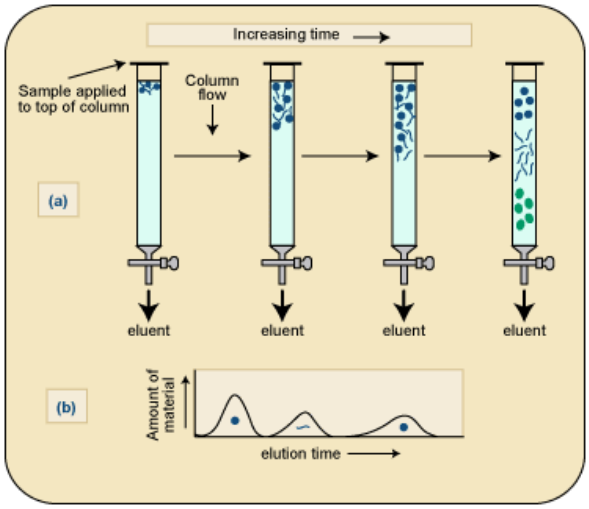


3. Select appropriate to year discovery and name of scientists:

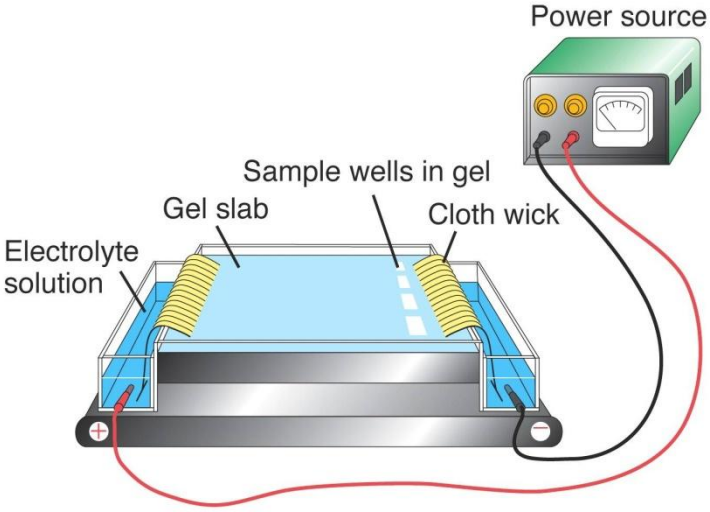
	Year		Names		Discovery
1	1937	A	Jonh Watson and Frensis Crick	I	proposed the α-helix and the β-pleated sheet structures for proteins
2	1950	B	Hans Krebs	II	determined 3-D structure of hemoglobin
3	1952	C	Erwin Chargaff	III	elucidated the citric acid cycle
4	1961	D	Linus Pauling and Robert Corey	IV	proposed the double helix for DNA
5	1953	E	François Jacob and Jacques Monod	V	elucidates the role of ATP in energy metabolism
6	1959	F	Marshal Nirenberg, H.Gobind Khorana, and Severo Ochoa	VI	propound the operon model
7	1914	G	Max Perutz	VI	completed the elucidation of the genetic code
8	1965	H	Fritz Lipmann	VII	published observation that A=T, G=C

4. Which of pictures correspond to methods:

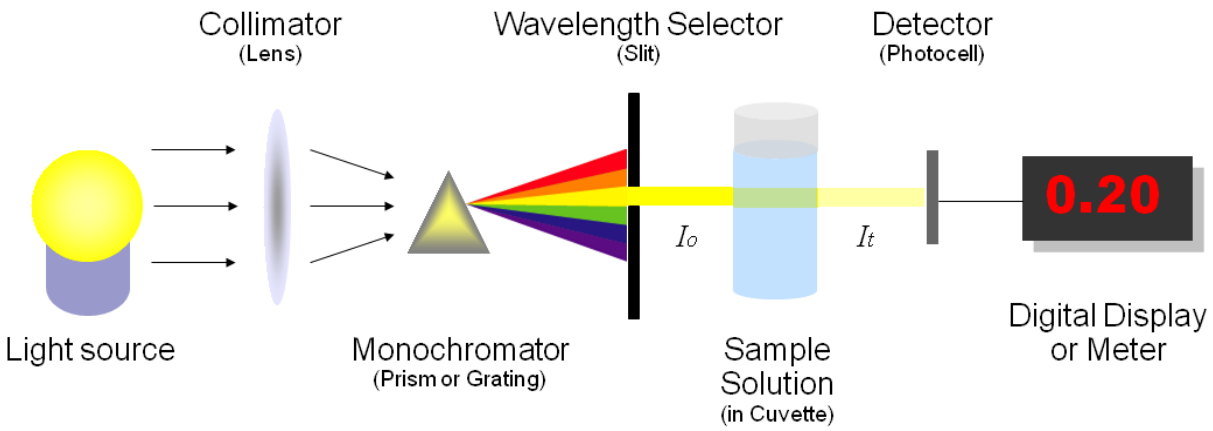
1. Electrophoresis
2. Spectrophotometry
3. Chromatography
4. ELISA


Increasing time
Sample applied to top of column
Column flow
(a)
eluent
eluent
eluent
eluent
(b)
Amount of material
elution time

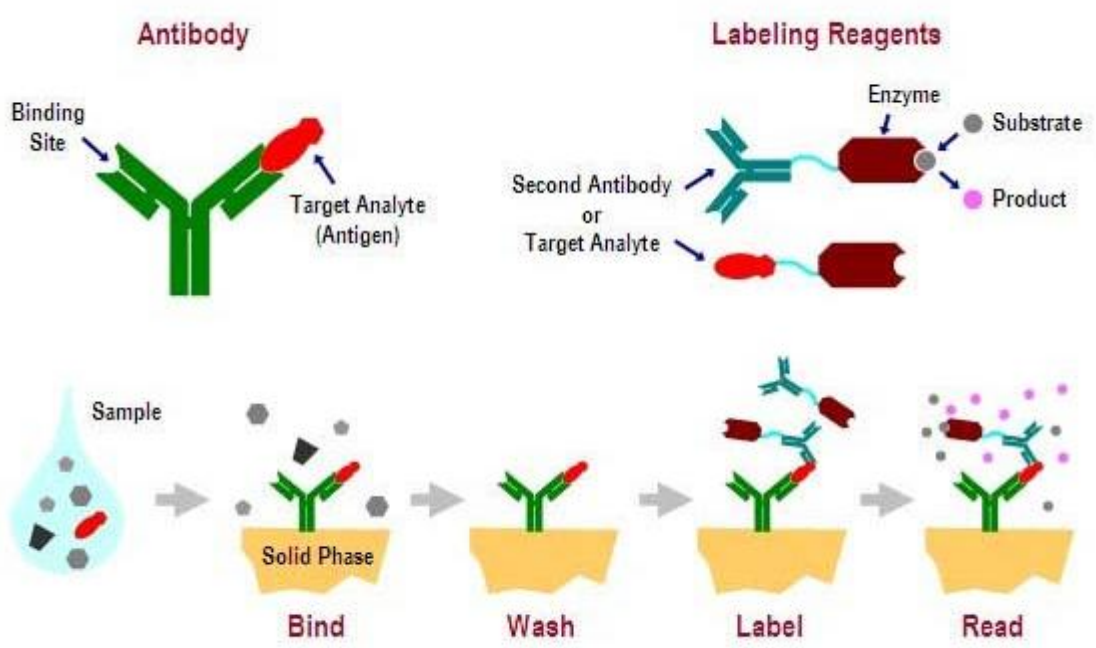
A


Power source
Sample wells in gel
Gel slab
Cloth wick
Electrolyte solution

B


Collimator
(Lens)
Wavelength Selector
(Slit)
Detector
(Photocell)
Io
It
0.20
Light source
Monochromator
(Prism or Grating)
Sample Solution
(in Cuvette)
Digital Display or Meter

C


Antibody
Binding Site
Target Analyte (Antigen)
Labeling Reagents
Enzyme
Substrate
Product
Second Antibody or Target Analyte
Sample
Solid Phase
Bind
Wash
Label
Read

D

EXAMPLES OF MULTI-CHOICE QUESTIONS:

1. Out of 200 different amino acids form in nature the number of amino acids present in protein:

A. 20
B. 25
C. 40
D. 35
E. 100

2. 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 messenger RNA

3. Determination of proportion between protein fractions in blood plasma or serum has an important clinical and diagnostic significance. The following routine method for obtaining results of this sort is most frequently used in clinical laboratories:

A. Salting out with neutral salts
B. Absorption chromatography
C. Precipitation with strong acids
D. Electrophoresis in agar gel or on acetyl-cellulose films
E. Immunoprecipitation

4. For determination of DNA synthesis in the cell usually is used measurement of incorporation of H^3-thymidine into cellular biopolymers. The next type of analysis is used in this specific case:

A. Radioisotope method
B. Polymerase chain reaction (PCR)
C. Electrophoresis
D. Radioimmunoassay
E. Affinity chromatography

5. In proteins the α-helix and β-pleated sheet are examples of:

A. Secondary structure
B. Primary structure
C. Tertiary structure
D. Quaternary structure
E. All of these

Individual independent students work

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

Additional literature:

1. Lehninger A. Principles of Biochemistry. – New York. – W.H.Freeman and Company. – 2005. – 1010 p.
2. Mardashko O.O., Yasinenko N.Y. Biochemistry. Texts of lectures.-Odessa. The Odessa State Medical University, 2003.-416p.

3. Devlin T.M., ed. Textbook of Biochemistry with Clinical Correlations, 5th ed. New York: Wiley-Liss, 2002.
4. Toy E.C., Seifert W. E., Strobel H.W., Harms K.P. "Case Files in Biochemistry. 2nd edition" – 2008. – 488 p.
5. MCQs / Prof. Sklyarov A.Ya., Lutsik M.D., Fomenko I.S., Klymyshin D.O., Nasadyuk C.M.. – 2012. – 308 p.
6. Copeland R. A. Enzymes: A Practical Introduction to Structure, Mechanism, and Data Analysis. - Wiley-VCH, Inc. – 2000. – 412 p.

Topic № 2. **Enzymes: structure, physico-chemical properties, classification and mechanism of action of enzymes. Methods of detection of enzymes in biological material.**

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

Specific objectives:

- *To interpret biochemical principles of structure and functioning of different classes of enzymes.*
- *On the basis of physical and chemical properties of enzymes as proteins to explain the dependence of enzymatic activity from pH of medium, temperature and other factors.*
- *To analyze values of the activity of enzymes in blood plasma in dependence from their localization in the cell, tissue or organ.*
- *To analyze methods of determination of enzymatic activity for an optimal application in clinical biochemistry.*

GUIDE FOR THE INDEPENDENT STUDY OF THE TOPIC:

	Questions:	**References:**
1.	**Enzymes: definition, properties of enzymes as biological catalysts, difference between enzymes and inorganic catalysts:** • Definition of enzymes. Biological importance of enzymes; • Common properties of enzymes and inorganic catalysts; • Different properties of enzymes and inorganic catalysts.	3. Satyanarayana U., Chakrapani U. "Biochemistry", Third Edition. – 2006. – P. 85. 4. Harper′s Biochemistry. 26th edition / R. K. Murray, Daryl K. Granner, Peter A. Mayes, Victor W. Rodwell, 2003.- P.49 5. Biochemistry. An Introduction/ Trudy McKee, James R. McKee , 1996.- P. 118 6. Lecture notes.
2.	**Nomenclature and classification of**	4. Satyanarayana U., Chakrapani U.

	enzymes: • Trival and systematic names of enzymes; • Classification of enzymes according IUB (six classes of enzymes with short characteristics and examples to each one)	"Biochemistry", Third Edition. – 2006. – P. 86-87 5. Harper′s Biochemistry. 26th edition / R. K. Murray, Daryl K. Granner, Peter A. Mayes, Victor W. Rodwell, 2003.- P.49-50 6. Biochemistry. An Introduction/ Trudy McKee, James R. McKee , 1996.- P. 120-121 7. Lecture notes. 8. http://www.chem.qmul.ac.uk/iubmb/enzyme/rules.html
3.	**Physico-chemical properties of enzymes (due to protein structure of enzymes):** • Solubility; • Molecular weight; • Isoelectric pH; • Denaturation.	3. Satyanarayana U., Chakrapani U. "Biochemistry", Third Edition. – 2006. – P. 60-63. 4. Lecture notes.
4.	**Simple and conjugated enzymes. Role of non-protein part of conjugated enzymes** • Prosthetic groups; • Coenzymes; • Cofactors.	1. Satyanarayana U., Chakrapani U. "Biochemistry", Third Edition. – 2006. – P. 87, 96-98. 2. Harper′s Biochemistry. 26th edition / R. K. Murray, Daryl K. Granner, Peter A. Mayes, Victor W. Rodwell, 2003.- P.50 3. Lecture notes.
5.	**Structure of enzymes: active centres and allosteric sites:** • Structure of active centre, its binding and catalytic sites; • Role of allosteric site	1. Satyanarayana U., Chakrapani U. "Biochemistry", Third Edition. – 2006. – P. 87, 91-92. 2. Lecture notes.
6.	**Levels of structural organization of enzymes. Multi-enzyme complexes their advantages.** • Levels of structural organization of enzymes (primary, secondary, tertiary and quaternary structure) • Multi-enzyme complexes, ensembles, polyfunctional enzymes and their advantages.	1. Satyanarayana U., Chakrapani U. "Biochemistry", Third Edition. – 2006. – P. 87, 96-98. 2. http://webcache.googleusercontent.com/search?q=cache:http://nsdl.niscair.res.in/bitstream/123456789/716/1/Introduction.pdf, P. 11-12
7.	**Specificity of enzymes:**	3. Satyanarayana U., Chakrapani U.

	• Stereo-specificity; • Reaction specificity; • Substrate specificity (relative, absolute and broad).	"Biochemistry", Third Edition. – 2006. – P. 95-96. 4. Lecture notes.
8.	**The localization of enzymes in cells (compartmentation) and organs.**	2. Satyanarayana U., Chakrapani U. "Biochemistry", Third Edition. – 2006. – P. 103. 3. Harper′s Biochemistry. 26th edition / R. K. Murray, Daryl K. Granner, Peter A. Mayes, Victor W. Rodwell, 2003.- P.56-57 4. Biochemistry. An Introduction/ Trudy McKee, James R. McKee , 1996.- P. 143 5. Lecture notes.

COMPREHENSION QUESTIONS:

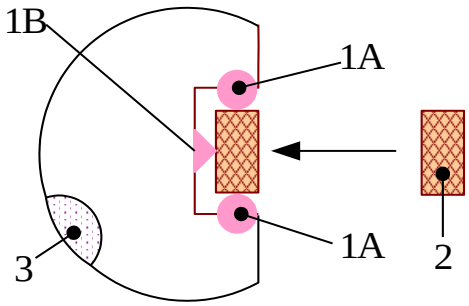
1. Choose from listed below: I-common and II-different properties of enzymes and inorganic catalysts of non-protein origin:

A. Ability to catalyze of only energy grounded reactions.
B. High specificity of action.
C. Inability to change the direction of reactions.
D. Ability to be regulated.
E. Inability to change the status of equilibrium in a reverse reaction, they only enhance its establishment.
F. They are not involved as component parts in the reaction end products, they release unchanged although it has been proved recently that some enzymes are subject to modifications and even disintegration in the process of a chemical reaction.
G. Catalysis of chemical processes only under "mild" conditions (the temperature is not high (about +37-40°C), pH of the medium 6-8, and pressure normal);
H. They do not waste in the process of catalysis.
I. Having been isolated from the organism they do not lose their ability to accomplish catalytic activity.

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

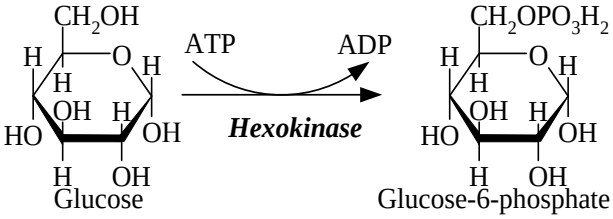
	Enzyme Class		**Reaction catalyzed**		**Example**
I	Oxidoreductases	A.	$A + B + ATP \rightarrow A\text{-}B + ADP$	1.	Phosphohexose isomerase
II	Transferases	B.	$A\text{-}B + H_2O \rightarrow AH + BOH$	2.	Glutamine synthetase
III	Hydrolases	C.	$AH_2 + B \rightarrow A + BH_2$	3.	Aldolase
IV	Lyases	D.	$A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

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

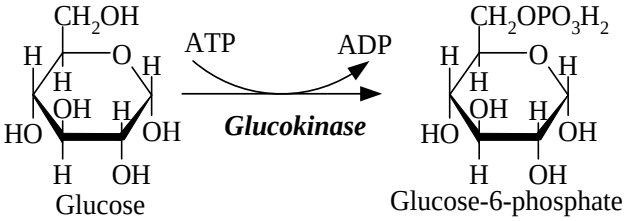


4. Choose the appropriate type of enzyme specificity (stereo-, relative, absolute, for each enzyme catalyzing the following reactions:

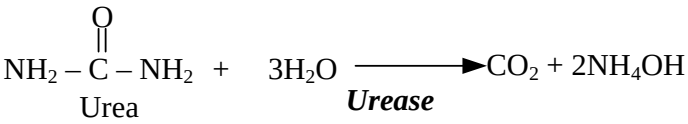
A.



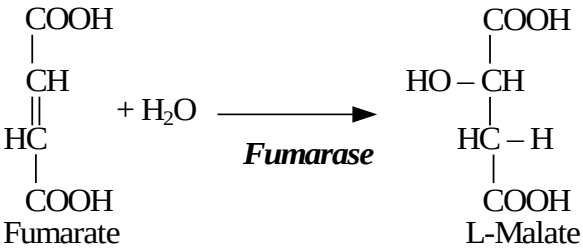
B.



C.



D.



5. Select proper enzymes to their compartmentation:

Organelle **Enzyme/metabolic pathway**

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

EXAMPLES OF MULTI-CHOICE QUESTIONS:

1. Chose a correct statement about common feature of enzymes and inorganic catalysts.

A. Acceleration of thermodynamically permitted reactions
B. Dependence of activity on pH of medium
C. High selectivity to type of catalyzed reaction
D. Specific dependence on substrate concentration
E. Dependence on the presence of cofactors

2. Enzymes accelerate the rate of reactions by:

A. Decreasing the energy of activation
B. Increasing the equilibrium constant of reactions
C. Increasing the energy of activation
D. Decreasing the free energy change of the reaction
E. Decreasing the equilibrium constant of reactions

3. Cytochrome c participates in transport of electrons in respiratory chain of the cell and is located in the next cellular compartment:

A. Mitochondria
B. Cytoplasm
C. Golgi vesicles
D. Nucleus
E. Lysosomes

4. The energy required to start an enzymatic reaction is called:

A. Activation energy
B. Chemical energy
C. Metabolic energy
D. Potential energy
E. Free energy

5. Chose from listed below enzymes ONE which exhibits selectivity to stereochemical epimers of substrate:

A. Urease
B. Aminopeptidase
C. Trypsin
D. Alcohol dehydrogenase
E. Lactate dehydrogenase

Individual independent students work

1. Multi-enzyme complexes and their advantages.
2. The employment of enzymes in biochemical investigations.

Additional literature:

1. Lehninger A. Principles of Biochemistry. – New York. – W.H.Freeman and Company. – 2005. – 1010 p.
2. Mardashko O.O., Yasinenko N.Y. Biochemistry. Texts of lectures.-Odessa. The Odessa State Medical University, 2003.-416p.
3. Devlin T.M., ed. Textbook of Biochemistry with Clinical Correlations, 5th ed. New York: Wiley-Liss, 2002.
4. Toy E.C., Seifert W. E., Strobel H.W., Harms K.P. "Case Files in Biochemistry. 2nd edition" – 2008. – 488 p.
5. MCQs / Prof. Sklyarov A.Ya., Lutsik M.D., Fomenko I.S., Klymyshin D.O., Nasadyuk C.M.. – 2012. – 308 p.
6. Copeland R. A. Enzymes: A Practical Introduction to Structure, Mechanism, and Data Analysis. - Wiley-VCH, Inc. – 2000. – 412 p.

Topic № 3. Kinetics of enzymatic reactions. Regulation of enzymatic activity, determination of enzymatic activity.

Objective: To show the catalytic role of enzymes on the basis of pancreatic proteinases and amylase activity measurements. To estimate clinical and diagnostic significance of determination of amylase activity in urine.

Actuality of the theme: Enzymes are biocatalysts with changeable activity, submitted to regulatory influences. The knowledge of kinetics of enzymatic reactions is important for the understanding of metabolic processes in cells, tissues and organs of human body.

Specific aims:

- To explain the mechanism of enzymatic action on basis of the affinity of enzyme to substrate and events which occur in enzyme-substrate complex.
- To interprete changes in activity of enzymes in biological fluids under conditions of different pathological processes.
- To explain the employment of inhibitors and activators of enzymes in cases of metabolic disorders or distinct pathology
- To analyse the mechanism of enzyme action using an example of chymotrypsin and acetylcholinesterase.

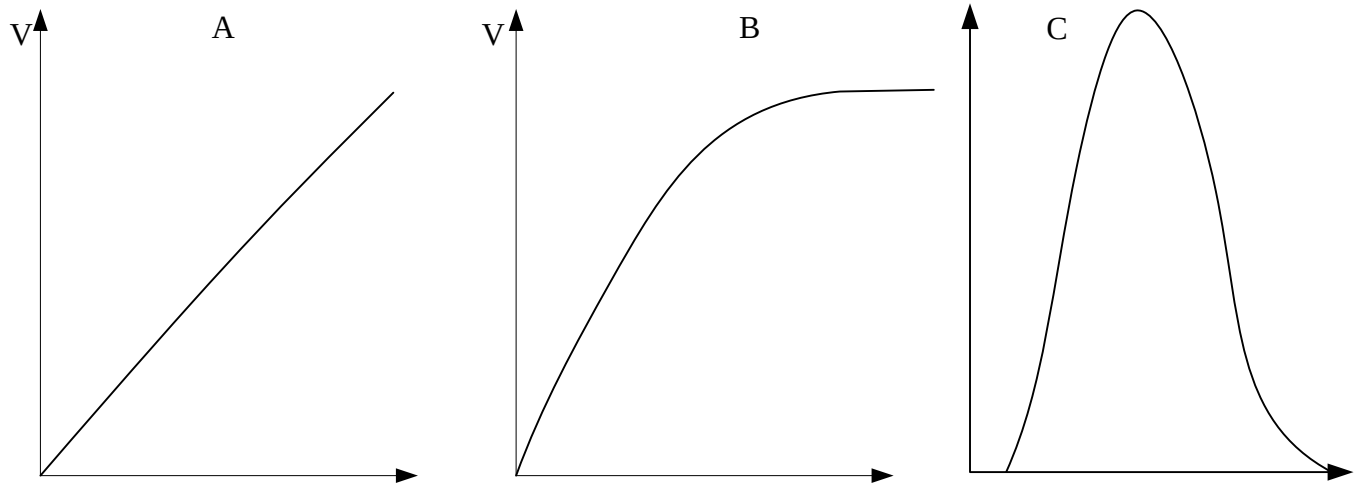
GUIDE FOR THE INDEPENDENT STUDY OF THE TOPIC:

1.	**Enzyme kinetics. Factors affecting enzymatic activity:** • definition of enzyme kinetics; • plot of dependence of enzymatic reaction velocity on concentration of enzyme (explain it); • plot of dependence of enzymatic reaction velocity on concentration of substrate (explain it); • plot of dependence of enzymatic reaction velocity on temperature (explain it); • plots of dependence of enzymatic reaction velocity on effect of pH for different enzymes (explain them).	1. Satyanarayana U., Chakrapani U. "Biochemistry", Third Edition. – 2006. – P. 88 - 90. 2. Harper′s Biochemistry. 26th edition / R. K. Murray, Daryl K. Granner, Peter A. Mayes, Victor W. Rodwell, 2003.- P.64 - 3. Biochemistry: the molecular basis of life, third edidion/ Trudy McKee, James R. McKee , 2003.- P. 167-170. 4. Lecture notes.
2.	**Michaelis-Menten kinetics:** • **Michaelis-Menten model of enzymatic reaction** • **Michaelis-Menten equation;** • **biological meaning of Michaelis constant.**	2. Satyanarayana U., Chakrapani U. "Biochemistry", Third Edition. – 2006. – P. 88-89 3. Harper′s Biochemistry. 26th edition / R. K. Murray, Daryl K. Granner, Peter A. Mayes, Victor W. Rodwell, 2003.- P.64-65 4. Biochemistry: the molecular basis of life, third edidion/ Trudy McKee, James R. McKee , 2003.- P. 169-171. 5. Lecture notes.
3.	**Lineweaver-Burk double-reciprocal plot**	1. Satyanarayana U., Chakrapani U. "Biochemistry", Third Edition. – 2006. – P. 60-63. 2. Biochemistry: the molecular basis of life, third edidion/ Trudy McKee, James R. McKee , 2003.- P. 172-173. 3. Harper′s Biochemistry. 26th edition / R. K. Murray, Daryl K. Granner, Peter A. Mayes, Victor W. Rodwell, 2003.- P.64-65
4.	**Mechanisms of enzyme catalysis:**	1. Satyanarayana U., Chakrapani U. "Biochemistry", Third Edition. – 2006. –

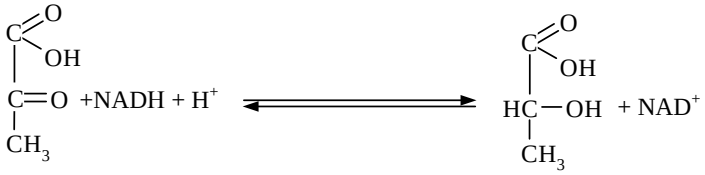
	• **acid-base catalysis;** • **substrate strain;** • **covalent catalysis;** • **entropy effect.**	P. 99-100. 2. Harper′s Biochemistry. 26th edition / R. K. Murray, Daryl K. Granner, Peter A. Mayes, Victor W. Rodwell, 2003.- P.51-53 3. Biochemistry: the molecular basis of life, third edidion/ Trudy McKee, James R. McKee , 2003.- P. 177-180.
5.	**Units of enzymatic activity:** • **katal;** • **International unit**	1. Satyanarayana U., Chakrapani U. "Biochemistry", Third Edition. – 2006. – P. 104-106. 2. Lecture notes.
6.	**Methods of enzymatic activity assays.**	Laboratory manual

COMPREHENSION QUESTIONS:

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



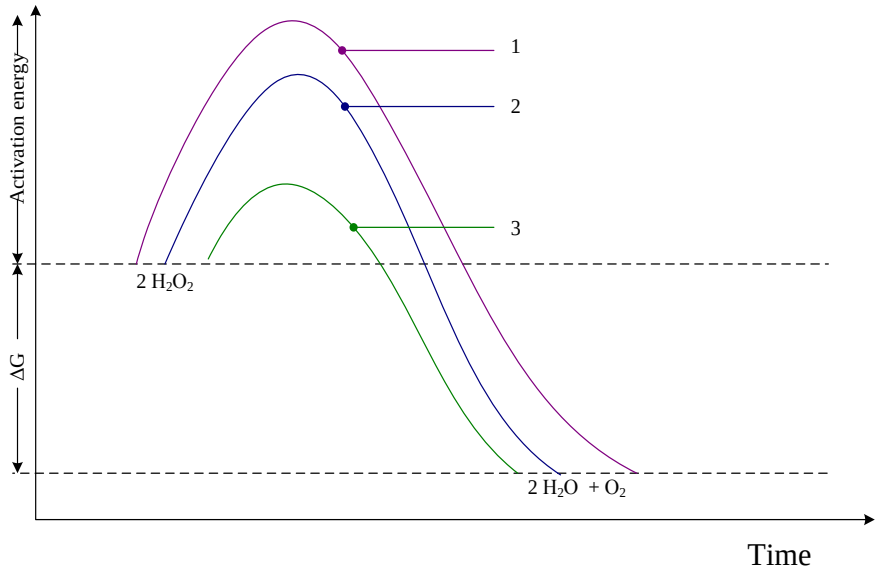
2. The muscle enzyme lactate dehydrogenase catalyzes the reaction:



Pyruvate Lactate

NADH and NAD^+ are the reduced and oxidized forms, respectively, of the coenzyrne NAD. Solutions of NADH, but not NAD^+, absorb light at 340 nm This property is used to determine the concentration of NADH in solution by measuring spectrophotometrically the amount of light absorbed at 340 nm by the solution Explain how these properties of NADH can be used to design a quantitative assay for lactate dehydrogenase.

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



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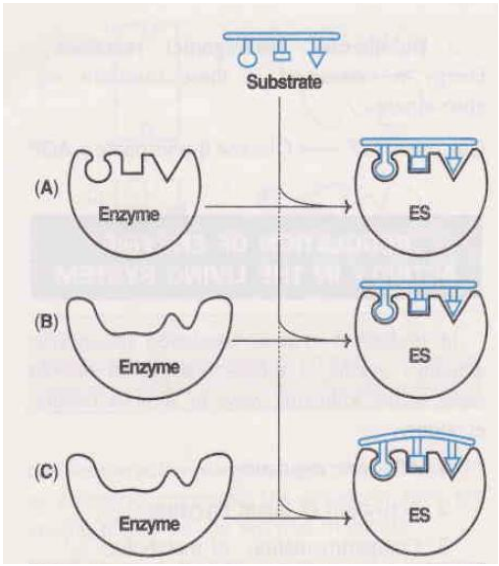
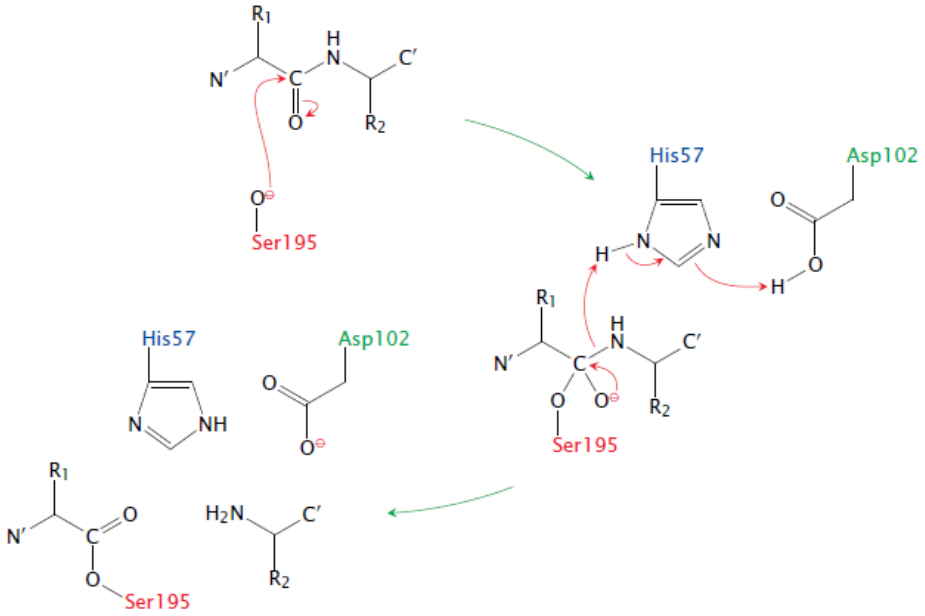
3. Which of the following corresponds to lock and key model, induced theory, substrate strain theory of enzyme catalysis?



5. Using the scheme of the catalytic mechanism of chymotrypsin action shown bellow explain what is the catalytic triad, and how does it work?



EXAMPLES OF MULTI-CHOICE QUESTIONS:

1. Michaelis-Menten constant corresponds to:

A. Substrate concentration at which reaction rate is half maximal
B. Optimal pH for activity of enzyme
C. Enzyme concentration, which provides half maximal velocity of reaction
D. Concentration of substrate, at which rate of reaction reach maximal value
E. Ionic strength of medium favoring maximal activity of enzyme

2. Michaelis-Menten constants of two enzymes are $1{,}3 \times 10^{-5}$ M/l and $2{,}3 \times 10^{-3}$ M/l subsequently. Indicate true statement about the affinity of these enzymes to substrate.

A. The first enzyme has higher affinity to substrate
B. The second enzyme has higher affinity to substrate
C. Enzymes possess equal affinity to substrate
D. For decision an information on concentration of enzyme is needed
E. Data are incomplete and it is impossible to draw a conclusion

3. Activity of many enzymes depends from the presence of free thiol groups in active center. What amino acid residue provides presence of these groups in enzyme molecule?

A. Cysteine
B. Lysine
C. Tryptophan
D. Methionine
E. Serine

4. Michaelis-Menten constant (K_m) reflects the next property of enzyme:

A. Affinity to substrate
B. Thermolability
C. Sensitivity to pH of medium
D. Affinity to a product of reaction
E. Sensitivity to competitive inhibitors

5. Enzymes requiring NAD as co-substrate can be assayed by measuring change in absorbance at:

A. 340 nm
B. 210 nm
C. 290 nm
D. 365 nm
E. 690 nm

Additional literature:

1. Lehninger A. Principles of Biochemistry. – New York. – W.H.Freeman and Company. – 2005. – 1010 p.
2. Mardashko O.O., Yasinenko N.Y. Biochemistry. Texts of lectures.-Odessa. The Odessa State Medical University, 2003.-416p.
3. Devlin T.M., ed. Textbook of Biochemistry with Clinical Correlations, 5th ed. New York: Wiley-Liss, 2002.
4. Toy E.C., Seifert W. E., Strobel H.W., Harms K.P. "Case Files in Biochemistry. 2nd edition" – 2008. – 488 p.
5. MCQs / Prof. Sklyarov A.Ya., Lutsik M.D., Fomenko I.S., Klymyshin D.O., Nasadyuk C.M.. – 2012. – 308 p.
6. Copeland R. A. Enzymes: A Practical Introduction to Structure, Mechanism, and Data Analysis. - Wiley-VCH, Inc. – 2000. – 412 p.

Topic № 4. Regulation of enzymatic activity and mechanisms of enzymopathias. Medical enzymology.

Objective: To learn the main principles of regulation of metabolic pathways, the consequences of alteration of enzymatic activity in the cell and employment of enzymes in medicine.

Actuality of the theme: Enzymes are biocatalysts with changeable activity, submitted to regulatory influences. Estimation of enzymatic activity is routinely used in laboratory investigations with diagnostic purposes. Enzymes are also employed as medicines and drugs in practical medicine.

Specific aims:

- To analyze pathways and mechanisms of regulation of enzymatic reactions as a background of metabolism in health and disease.
- To explain the application of activators and inhibitors of enzymes as medicines and pharmaceuticals for correction of metabolic disorders in pathology.
- To explain changes in metabolic pathways and accumulation of distinct metabolic intermediates in the inborn (hereditary) and acquired disorders of metabolism – enzymopathias.
- To analyze changes in activity of indicatory enzymes in blood plasma in pathology of distinct organs and tissues.

GUIDE FOR THE INDEPENDENT STUDY OF THE TOPIC:

	Questions:	References:
1.	**Enzyme inhibition:** • reversible (competitive, non-	1. Satyanarayana U., Chakrapani U. "Biochemistry", Third Edition. – 2006. –

	competitive); • irreversible. Each type of inhibition should be defined, explained, given examples, shown on plots.	P. 92-95. 2. Lehninger A. Principles of Biochemistry. – New York. – W.H.Freeman and Company. – 2005. – P. 201-205. 4. Lecture notes.
2.	**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.	1. Satyanarayana U., Chakrapani U. "Biochemistry", Third Edition. – 2006. – P. 100-103. 2. Harper′s Biochemistry. 26th edition / R. K. Murray, Daryl K. Granner, Peter A. Mayes, Victor W. Rodwell, 2003.- P.74-79. 3. Lehninger A. Principles of Biochemistry. – New York. – W.H.Freeman and Company. – 2005. – P. 220-228. 4. Lecture notes.
3.	**Control of enzymes synthesis:** • constitutive enzymes; • adaptive enzymes; • induction and repression.	1. Satyanarayana U., Chakrapani U. "Biochemistry", Third Edition. – 2006. – P. 104. 2. Harper′s Biochemistry. 26th edition / R. K. Murray, Daryl K. Granner, Peter A. Mayes, Victor W. Rodwell, 2003.- P.73-74. 3. Lecture notes.
5.	**Application of enzymes:** • enzymes as therapeutic agents; • enzymes as analytic agents; • immobilized enzymes.	1. Satyanarayana U., Chakrapani U. "Biochemistry", Third Edition. – 2006. – P. 105-106. 1. Biochemistry. An Introduction/ Trudy McKee, James R. McKee , 1996.- P. 145. 2. Lecture notes.
6.	**Diagnostical importance of enzymes** (plasma specific and non-plasma specific enzymes).	2. Satyanarayana U., Chakrapani U. "Biochemistry", Third Edition. – 2006. – P. 106-109. 3. Biochemistry. An Introduction/ Trudy McKee, James R. McKee , 1996.- P. 144-145. 4. Lecture notes.

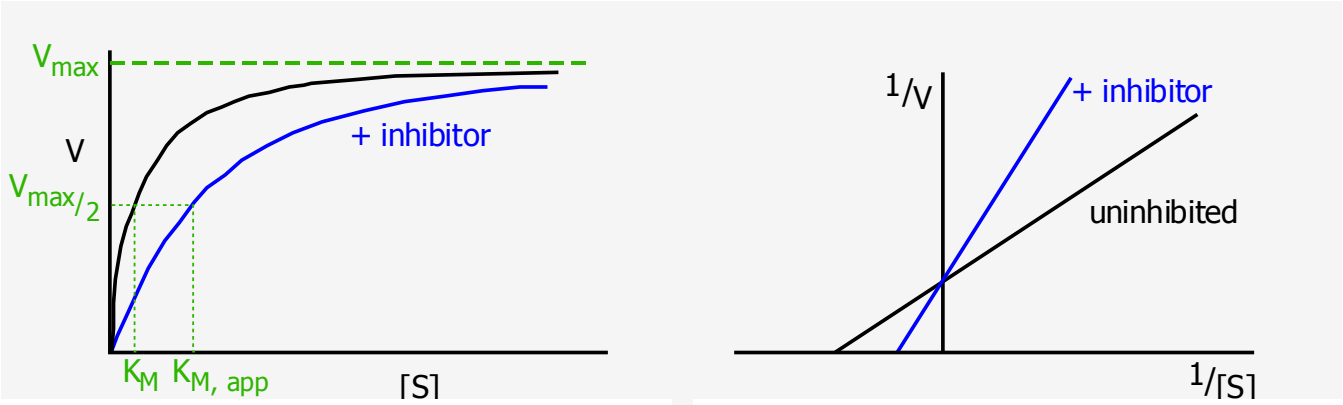
7.	**Changes in enzymatic activity of blood plasma and serum as diagnostic indexes (markers) of pathological processes in distinct organs:** • myocardial infarction; • acute pancreatitis; • liver diseases; • pathology of muscle tissue.	1. Satyanarayana U., Chakrapani U. "Biochemistry", Third Edition. – 2006. – P. 113. 2. Biochemistry. An Introduction/ Trudy McKee, James R. McKee , 1996.- P. 144-145. 3. Lecture notes.
8.	**Isoenzymes, their role in enzymodiagnostics.**	1. Satyanarayana U., Chakrapani U. "Biochemistry", Third Edition. – 2006. – P. 109-112.
9.	**Inborn (hereditary) and acquired metabolic defects, their clinical and laboratory diagnostics:** • Metabolic defects of amino acids metabolism (on example of phenylcotonuria, albinism, alcaptonuria) • Metabolic defects of carbohydrates metabolism (galactosemia) • Metabolic defects of lipids metabolism (lipid storage diseases)	Satyanarayana U., Chakrapani U. "Biochemistry", Third Edition. – 2006. – P. 351-352. Satyanarayana U., Chakrapani U. "Biochemistry", Third Edition. – 2006. – P. 277. 1. Satyanarayana U., Chakrapani U. "Biochemistry", Third Edition. – 2006. – P. 308.
10.	**Application of enzyme inhibitors as medicinal and drugs**: • acetylsalicylic acid • allopurinol • sulfonamides.	Satyanarayana U., Chakrapani U. "Biochemistry", Third Edition. – 2006. – P. 390, 396, 646.

COMPREHENSION QUESTIONS:

1. Select proper competitive inhibitor to its enzyme:

	Enzymes:		**Inhibitors:**
1	Xanthine oxidase	A	Ephedrine
2	Monoamine oxidase	B	Para aminobenzoic acd (PABA)
3	Dihydropteroate synthase	C	Aminopterin
4	Acetilcholine esterase	D	Allopurinol
5	Dihydrofolate reductase	E	Succinyl choline

2. Which of following pictures (1 and 2) represents reversible competitive and reversible noncompetitive inhibition?



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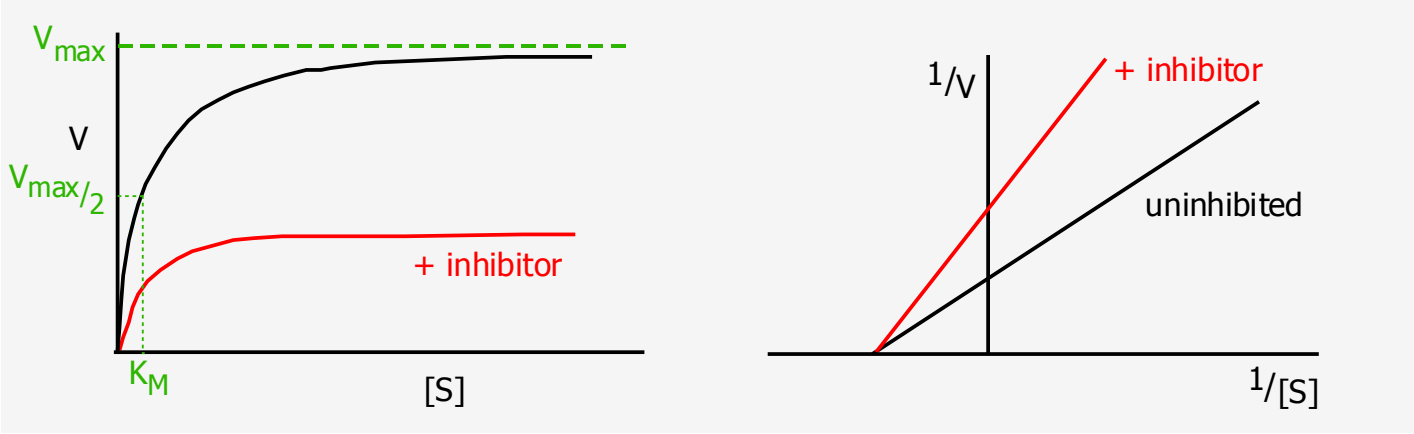

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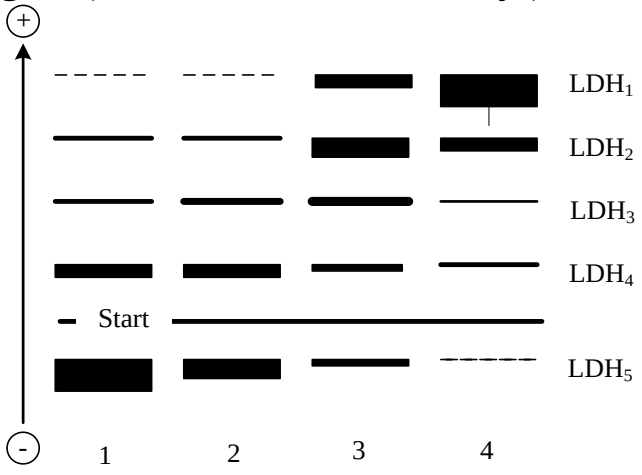
3. The inhibitor X decreases the activity of enzyme Y by 70 %. The increase of the concentration of the substrate S returns up to 60 % of Y activity. Which type of inhibition represents inhibitor X?

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



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

	Enzymes:		**Disease:**
1	Lactate Dehydrogenase-1	A	Acute pancreatitis
2	Alkaline phosphatase	B	Cancer of prostate gland
3	Acidic Phosphtase	C	Muscular dystrophy

4	Amylase	D	Myocardial infarction
5	Aldolase	E	Rickets

Examples of multi-choice questions:

1. In a patient the disorder of proteins digestion in stomach and small intestine is observed. What enzymes insufficiency cause this disorder?

A. Peptidases
B. Oxido-reductases
C. Amylases
D. Lipases
E. Aminotransferases

2. In diagnostics of myocardial infarction the next isoform of lactate dehydrogenase in blood has diagnostic significance:

A. H_4 (iso 1)
B. H_3M (iso 2)
C. H_2M_2 (iso 3)
D. HM_3 (iso 4)
E. M_4 (iso 5)

3. In diagnostics of an acute viral hepatitis estimation of the next enzymatic activity in blood serum is the most valuable:

A. Alanyl aminotransferase
B. Glutathion peroxidase
C. Creatine kinase
D. Amylase
E. Alkaline phosphatase

4. Different forms of lympholeukoses are effectively cured with enzyme preparation called:

A. Asparaginase
B. Plasmin
C. Tissue plasminogen activator (tPA)
D. Hyaluronidase
E. Streptokinase

5. A child manifests epileptic seizures caused by vitamin B_6 deficiency. This is conditioned by the decrease of the 7-aminobutyrate level in the nervous tissue which acts as an inhibiting neurotransmitter. The activity of which enzyme is decreased in this case?

A. Alanine aminotransferase.
B. Pyridoxal kinase.
C. Glutamate dehydrogenase.
D. Glutamate decarboxylase.
E. Glutamate synthetase

Additional literature:

1. Lehninger A. Principles of Biochemistry. – New York. – W.H.Freeman and Company. – 2005. – 1010 p.
2. Mardashko O.O., Yasinenko N.Y. Biochemistry. Texts of lectures.-Odessa. The Odessa State Medical University, 2003.-416p.
3. Devlin T.M., ed. Textbook of Biochemistry with Clinical Correlations, 5th ed. New York: Wiley-Liss, 2002.

4. Toy E.C., Seifert W. E., Strobel H.W., Harms K.P. "Case Files in Biochemistry. 2nd edition" – 2008. – 488 p.
5. MCQs / Prof. Sklyarov A.Ya., Lutsik M.D., Fomenko I.S., Klymyshin D.O., Nasadyuk C.M.. – 2012. – 308 p.
6. Copeland R. A. Enzymes: A Practical Introduction to Structure, Mechanism, and Data Analysis. - Wiley-VCH, Inc. – 2000. – 412 p.

Topic № 5. The role of cofactors, vitamins and their coenzyme forms in enzyme catalysis.

Objectives: To learn the structure, principles of classification and funtion of coenzymatic vitamins. To learn the methods of qualitative and quantitative determination of vitamins.

Actuality of the theme: Water soluble vitamin take part in metabolism as coenzymes and activators for many enzymatic reactions.

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

Specific aims:

- To interprete the role of vitamins and their biologically active derivatives in mechanism of catalysis by enzymes of different classes.
- To explain the application of antivitamins as inhibitors of enzymes in contagious diseases and in disorders of homeostasis.
- To explain the role of metals in mechanisms of enzymatic catalysis.
- To classify distinct groups of coenzymes according to their chemical nature and type of the reaction, which they catalyze.

GUIDE FOR THE INDEPENDENT STUDY OF THE TOPIC:

	Questions:	References:
1.	**Cofactors, coenzymes and prosthetic groups of enzymes** (to explain difference between tham and to gie examples).	1. Satyanarayana U., Chakrapani U. "Biochemistry", Third Edition. – 2006. – P. 96-97. 2. Harper′s Biochemistry. 26th edition / R. K. Murray, Daryl K. Granner, Peter A. Mayes, Victor W. Rodwell, 2003.- P.50 3. Lecture notes.
2.	**Role of metal ions in function of enzymes.**	1. Biochemistry. An Introduction/ Trudy McKee, James R. McKee , 1996.- P. 132-133. 2. Lecture notes.

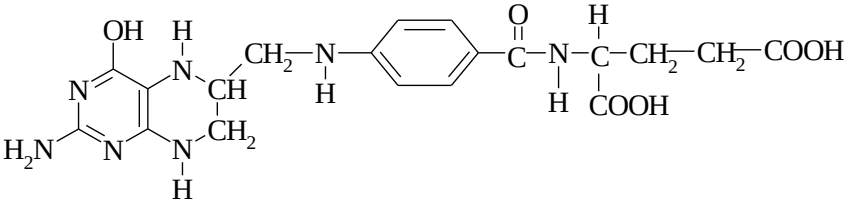
3.	**Classification of coenzymes due to their chemical nature and type of catalytic reaction.**	1. Lecture notes.
4.	**Coenzymes as transporters of hydrogen atoms and electrons** (chemical structure and short information about role of each coenzyme below): • NAD^+, $NADP^+$ coenzymes – derivatives of vitamin PP; • FAD, FMN coenzymes – derivatives of vitamin B_2 – riboflavin; • Role of vitamin C in oxidative-reductive reactions	1. Satyanarayana U., Chakrapani U. "Biochemistry", Third Edition. – 2006. – P. 132, 137-141. 2. Biochemistry. An Introduction/ Trudy McKee, James R. McKee , 1996.- P. 134-135. 3. Lecture notes.
5.	**Coenzymes as transporters of chemical groups** (chemical structure and short information about role of each coenzyme below): • pyridoxal phosphate; • HS-CoA – coenzyme of acylation; • lipoic acid; • THF – derivatives of folic acid	1. Satyanarayana U., Chakrapani U. "Biochemistry", Third Edition. – 2006. – P. 143 - 144, 148-151, 157-158. 2. Lecture notes.
6.	**Coenzymes of isomerisation, synthesis and cleavage of C-C bonds** (chemical structure and short information about role of each coenzyme below – except B_{12}): • thiamine pyrophosphate – coenzyme form of vitamin B_1; • biocytin – coenzyme form of vitamin H – biotin; • methylcobalamin and deoxyadenosylcobalamin – coenzyme forms of vitamin B_{12}	1. Satyanarayana U., Chakrapani U. "Biochemistry", Third Edition. – 2006. – P. 135, 146-147, 152-155. 2. Lecture notes.

COMPREHENSION QUESTIONS:

1. Find on the picture below following coenzymes:

- thiamine pyrophosphate
- FAD
- HS-CoA
- Methylcobalamin
- Ascorbic acid
- biocytin

- pyridoxal phosphate
- lipoic acid



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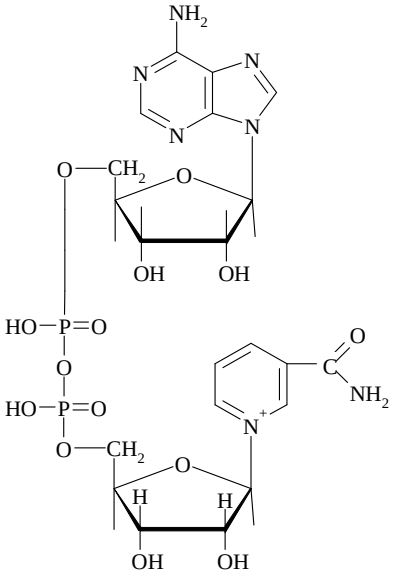

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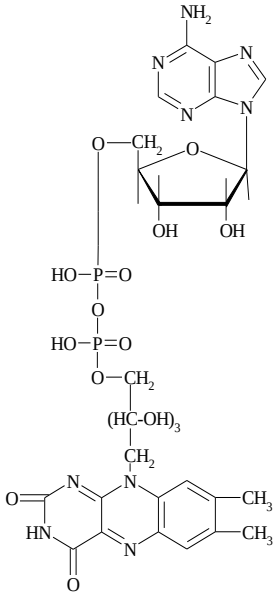

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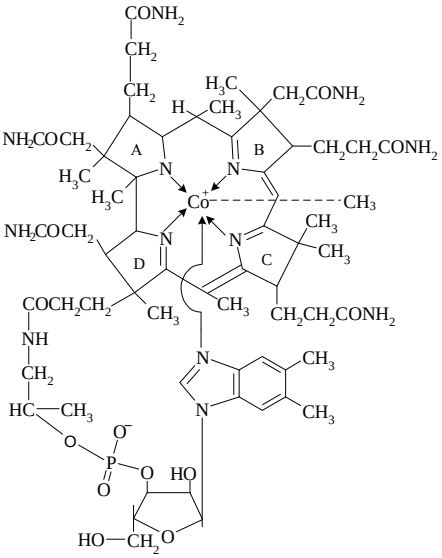

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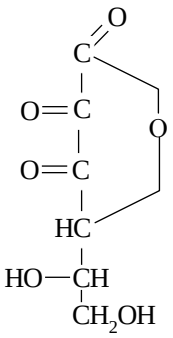

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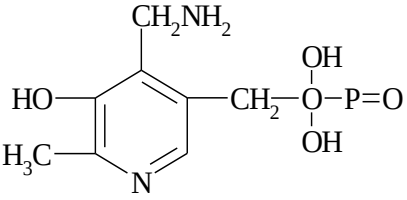

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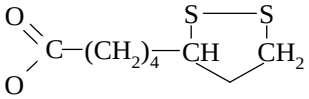

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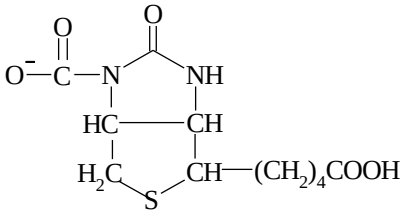

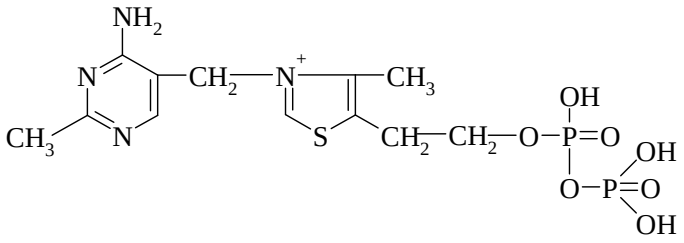

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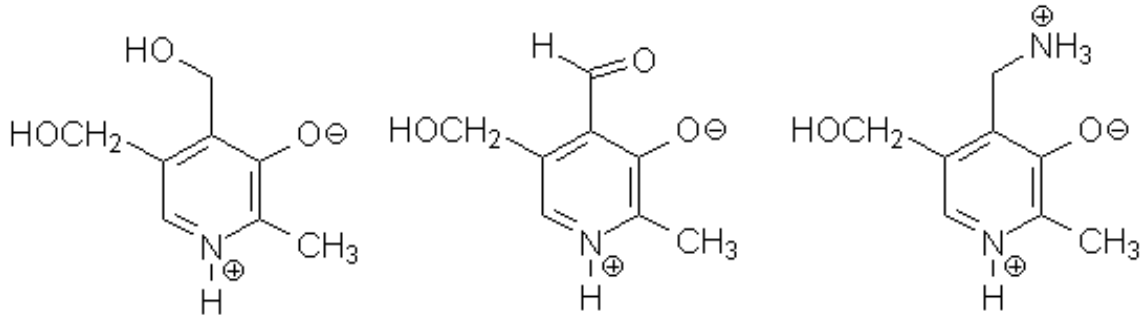
2. A 59-year-old male is brought to the emergency department by the EMS after a family member found him extremely confused and disoriented, with an unsteady gait and strange irregular eye movements. The patient has been known in the past to be a heavy drinker. He has no known medical problems and denies any other drug usage. On examination, he is afebrile with a pulse of 110 beats per minute and a normal blood pressure. He is extremely disoriented and agitated. Horizontal rapid eye movement on lateral gaze is noted bilaterally. His gait is very unsteady. The remainder of his examination is normal. The urine drug screen was negative and he had a positive blood alcohol level. The emergency room physician administers thiamine. What is the most likely diagnosis? What is importance of thiamine in biochemical reactions?

3. Select appropriate coenzyme forms for vitamins:

	Vitamin:		**Coenzyme:**
1	Thiamine (B1)	A	Biocytin
2	Pyrodoxine (B6)	B	Tetrahydrifolic acid
3	Nicotinic acid	C	Pyridoxalphosphate
4	Pantothenic acid	D	Thiamine pyrophosphate
5	Biotin	E	NAD and NADP
6.	Folic Acid	F	Coenzyme A

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



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EXAMPLES OF MULTI-CHOICE QUESTIONS:

1. There is an increase in the pyruvate level in the patient's blood and urine. What kind of avitaminosis developed in this case?

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

2. Pyridoxal phosphate was prescribed to a patient according to the clinical indication. For the correction of what biochemical pathway is this drug recommended?

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

3. After prolonged treatment of a patient with antibiotics, the suppression of intestinal microorganisms occurred. What kind of hypovitaminosis can resuit from this treatment?

A. B_6.
B. C.
C. A.
D. P.
E. D.

4. Malignant hyperchromc anemia, or Birmer's disease, is a pathological state caused by the deficiency of vitamin B_{12}. What chemical element is a constituent of the structure of this vitamin?

A. Cobalt.
B. Molybdenum.
C. Zinc.
D. Iron.
E. Magnesium.

CLINICAL CASE

A 59-year-old male is brought to the emergency department after a family member found him extremely confused and disoriented, with an unsteady gait and strange irregular eye movements. The patient has been known in the past to be a heavy drinker. He has no known medical problems and denies any other drug usage. On examination, he is afebrile with a pulse of 110 beats per minute and a normal blood pressure. He is extremely disoriented and agitated. Horizontal rapid eye movement on lateral gaze is noted bilaterally. His gait is very unsteady. The remainder of his examination is normal. The urine drug screen was negative and he had a positive blood alcohol level. The emergency room physician administers thiamine.

- **What is the most likely diagnosis?**
- **What is importance of thiamine in biochemical reactions?**

Additional literature:

1. Lehninger A. Principles of Biochemistry. – New York. – W.H.Freeman and Company. – 2005. – 1010 p.
2. Mardashko O.O., Yasinenko N.Y. Biochemistry. Texts of lectures.-Odessa. The Odessa State Medical University, 2003.-416p.
3. Devlin T.M., ed. Textbook of Biochemistry with Clinical Correlations, 5th ed. New York: Wiley-Liss, 2002.
4. Toy E.C., Seifert W. E., Strobel H.W., Harms K.P. "Case Files in Biochemistry. 2nd edition" – 2008. – 488 p.
5. MCQs / Prof. Sklyarov A.Ya., Lutsik M.D., Fomenko I.S., Klymyshin D.O., Nasadyuk C.M.. – 2012. – 308 p.

Topic № 6. **Metabolic pathways and bioenergetics. Tricarboxylic acid cycle and its regulation.**

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

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

Specific aims:

- To interprete biochemical principles of metabolic pathways: catabolic, anabolic, amphibolic pathways.
- To explain biochemical mechanisms of regulation of catabolic and anabolic reactions.
- To interprete biochemical principles of TCA cycle functioning and its anaplerotic reactions and their amphibolic sense.
- To explain biochemical regulatory mechanisms in TCA cycle and its principal position in turnover of matter and energy.

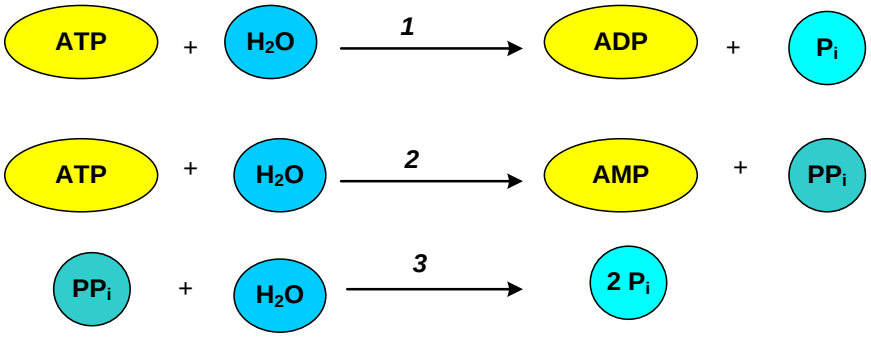
GUIDE FOR THE INDEPENDENT STUDY OF THE TOPIC:

	Questions:	**References:**
1.	**Conception of turnover of material and energy (metabolism). Characterization of catabolic, anabolic and amphibolic reactions and their significance.**	1. Satyanarayana U., Chakrapani U. "Biochemistry", Third Edition. – 2006. – P. 241-243. 2. Harper′s Biochemistry. 26th edition / R. K. Murray, Daryl K. Granner, Peter A. Mayes, Victor W. Rodwell, 2003.- P.122- 124. 4. Lecture notes.
2.	**Exergonic and endergonic biochemical reactions, role of ATP**	1. Satyanarayana U., Chakrapani U. "Biochemistry", Third Edition. – 2006. –

	and other macroergic phosphate containing compounds in their coupling.	P. 222-224. 2. Biochemistry. An Introduction/ Trudy McKee, James R. McKee , 1996.- P. 71-74. 3. Lecture notes.
3.	**Intracellular location of metabolic pathways, compartmentalization of metabolic reactions in the cell. Methods of investigation of metabolism.**	1. Satyanarayana U., Chakrapani U. "Biochemistry", Third Edition. – 2006. – P. 243. 2. Lecture notes.
4.	**Catabolic transformation of biomolecules:** proteins, carbohydrates, lipids, its characterization.	1. Satyanarayana U., Chakrapani U. "Biochemistry", Third Edition. – 2006. – P. 241-243. 2. Lecture notes.
5.	**The most important metabolites of amphibolic pathways in turnover of proteins, carbohydrates, lipids, their significance for integration of metabolism in the cell.**	1. Lecture notes.
6.	**Tricarboxylic acid (TCA) cycle:** • Cellular location of TCA cycle enzymes; • Sequence of TCA cycle reactions; • Characterization of enzymes and coenzymes participating TCA cycle; • Reactions of substrate phosphorylation in TCA cycle; • The effect of allosteric modulators upon TCA cycle reactions; • Energetic effect of TCA cycle.	1. Satyanarayana U., Chakrapani U. "Biochemistry", Third Edition. – 2006. – P.254 - 257. 2. Harper′s Biochemistry. 26th edition / R. K. Murray, Daryl K. Granner, Peter A. Mayes, Victor W. Rodwell, 2003.- P.130-135. 3. Biochemistry. An Introduction/ Trudy McKee, James R. McKee , 1996.- P. 218-227. 4. Lecture notes.
7	**Anaplerotic and amphibolic reactions of TCA cycle.**	1. Satyanarayana U., Chakrapani U. "Biochemistry", Third Edition. – 2006. – P. 257-258. 2. Lecture notes.

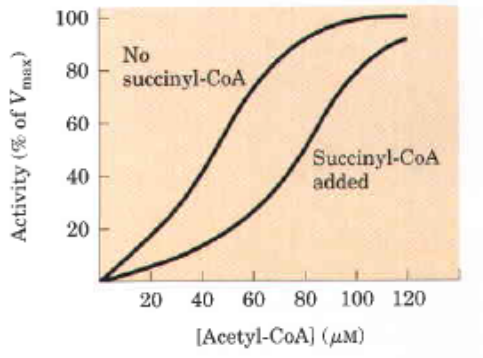
COMPREHENSION QUESTIONS:

1. ATP may be hydrolysed to form ADP and P_i (orthophosphate) or AMP and PP_i (pyrophosphate). Pyrophosphate may be subsequently hydrolyzed to orthophosphate releasing additional free energy. Note the ΔG^o values (1, 2 and 3), which are released in each reaction.



2. A postoperative patient on intravenous fluids develops lesions in the mouth (angular stomatitis). Urinalysis indicates an excretion of 15g riboflavin/mg creatinine, which is abnormally low. Which of the following TCA cycle enzymes is most likely to be affected.

3. In the presence of saturating amounts of oxaloacetate, the activity of citrate synthase from pig heart tissue shows a sigmoid dependence on the concentration of acetyl-CoA, as shown in the graph. When succinyl-CoA is added, the curve shifts to the right and the sigmoid dependence is morep ronounced.

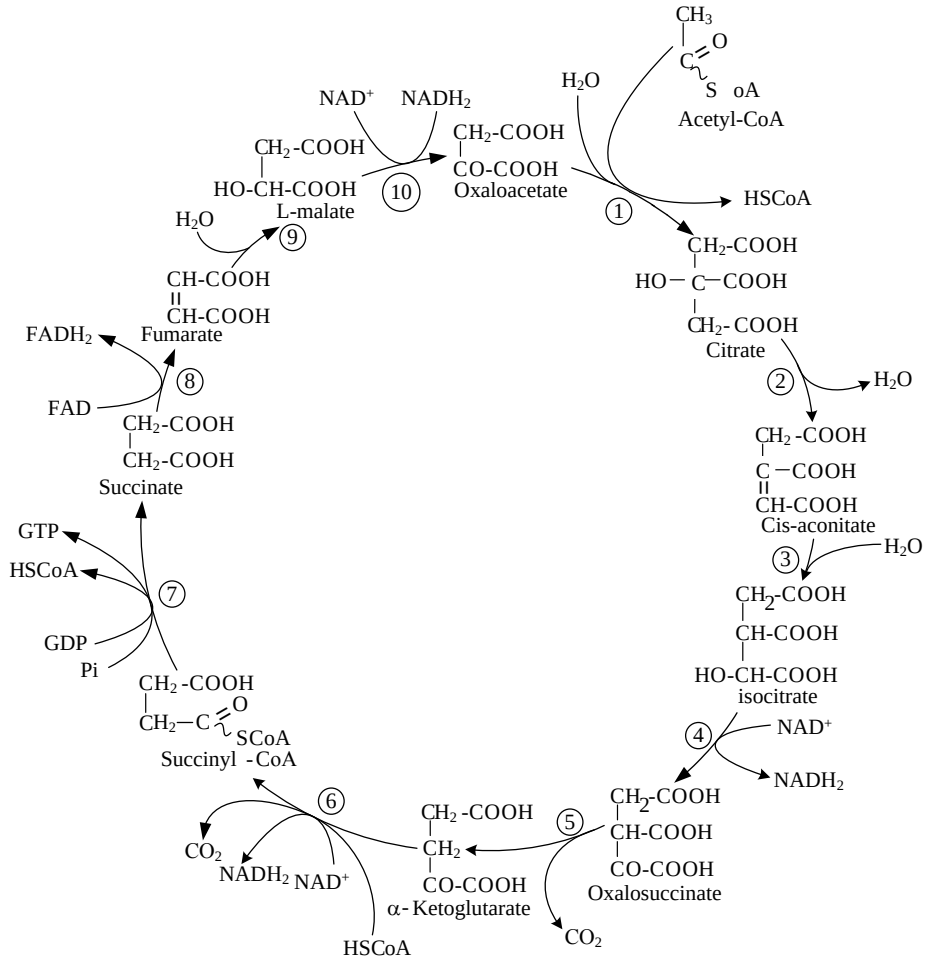


On the basis of these observations suggest how succinyl-CoA regulates the activity of citrate synthase. Why is succinyl-CoA an appropriate signal fo regulation of the citric acid cycle? How does the regulation of citrate synthase control the rate of cellular respiration in pig heart tissue?

4. After excessive drinking over an extended period of time while eating poorly, a middle-aged man is admitted to the hospital with "high output" heart failure. Which of the TCA enzymes is most likely inhibited?

5. There are many cases of human disease in which one or another enzyrne activity is lacking due to genetic mutation. However, cases in which individuals lack one of the enzymes of the citric acid cycle are extremely rare Why?

6. Name enzymes of citric acid cycle:



EXAMPLES OF MULTI-CHOICE QUESTIONS:

1. A patient was admitted into hospital with a diagnosis diabetes mellitus type I. In metabolic changes the decrease of oxaloacetate synthesis rate is detected. What metabolic passway is damaged as a result?

A. Tricarboxylic acid cycle
B. Glycolysis
C. Cholesterol biosynthesis
D. Glycogen mobilization
E. Urea synthesis

2. Mitochondria are subcellular organelles and are present in a cytoplasm of every cell exept mature red blood cells, bacteria, blue-green algae. What method is used principally for their isolation?

A. Differential centrifugation
B. Chromatography
C. Electrophoresis
D. Spectrophotometry
E. Gel-filtration

3. Enzymes of tricarboxylic acids cycle oxidize acetyl-CoA and produce 3 molecules of reduced NAD and one molecule of reduced FAD. Where are localized these enzymes?

A. In mitochondrial matrix
B. On plasma membrane
C. On external mitochondrial membrane
D. In cell cytoplasm

E. On inner mitochondrial membrane

4. Substrate phosphorylation is a process of phosphate residue transfer from macroergic donor substance to ADP or some other nucleoside diphosphate. What enzyme of tricarboxylic acid cycle participates in reaction of substrate phosphorylation?

A. Succinyl thiokinase
B. Citrate synthase
C. Succinate dehydrogenase
D. Fumarase
E. Alpha-ketoglutarate dehydrogenase complex

5. In a patient are manifested symptoms of intoxication with arsenic compounds. What metabolic process is damaged taking into account that arsen containing substances inactivate lipoic acid?

A. Oxidative decarboxylation of α-ketoglutarate
B. Fatty acids biosynthesis
C. Neutralization of superoxide anions
D. Coupling of oxidation and phopsphorylation
E. Microsomal oxidation

Additional literature:

1. Lehninger A. Principles of Biochemistry. – New York. – W.H.Freeman and Company. – 2005. – 1010 p.
2. Mardashko O.O., Yasinenko N.Y. Biochemistry. Texts of lectures.-Odessa. The Odessa State Medical University, 2003.-416p.
3. Devlin T.M., ed. Textbook of Biochemistry with Clinical Correlations, 5th ed. New York: Wiley-Liss, 2002.
4. Toy E.C., Seifert W. E., Strobel H.W., Harms K.P. "Case Files in Biochemistry. 2nd edition" – 2008. – 488 p.
5. MCQs / Prof. Sklyarov A.Ya., Lutsik M.D., Fomenko I.S., Klymyshin D.O., Nasadyuk C.M.. – 2012. – 308 p.

Topic № 7. Investigation of biological oxidation and oxidative phopshorylation. Mechanisms of ATP synthesis.

Objective: to learn general principles of enzymatic respiratory chain organization in mitochondria, distinct oxidoreductases and their functional significance in tissue respiration. To master the methods of investigation of the next oxidoreductases: phenol oxidase, aldehyde dehydrogenase and peroxidase.

Actuality of the theme: oxidoreductases catalyze reactions connected with transfer of electrons and protons and are in the background of macroergic compounds production. Investigation of their activity is necessary for detailed understanding of the mechanisms of tissue respiration and its changes in different functional status of the body.

Specific aims:

- To explain processes of biological oxidation of different substrates in the cell and reservation of released energy in a form of macroergic bonds of ATP.

- To analyze reactions of biological oxidation and their role in providement of fundamental biochemical processes in tissues.
- To explain the structural organization of electron transport chain and its macromolecular complexes.
- To interpret role of biological oxidation, tissue respiration and oxidative phosphorylation in generation of ATP in aerobic conditions.

GUIDE FOR THE INDEPENDENT STUDY OF THE TOPIC:

	Questions:	**References:**
1.	**Biological oxidation of substrates in cells**	7. Satyanarayana U., Chakrapani U. "Biochemistry", Third Edition. – 2006. – P. 224-225. 8. Harper′s Biochemistry. 26th edition / R. K. Murray, Daryl K. Granner, Peter A. Mayes, Victor W. Rodwell, 2003.- P.86
2.	**Types of reactions of biological oxidation and their functional significance:** • oxidases; • dehydrogenases; • peroxidases; • oxygenases (mono- and dioxygenases)	5. Satyanarayana U., Chakrapani U. "Biochemistry", Third Edition. – 2006. – P. 87, 235-236. 9. Harper′s Biochemistry. 26th edition / R. K. Murray, Daryl K. Granner, Peter A. Mayes, Victor W. Rodwell, 2003.- P.86-90 10. Lecture notes.
3.	**Pyridine dependent dehydrogenases:** • chemical structure of NAD^+ and $NADP^+$; • mechanism ol oxidation and reduction of NAD^+; • role of pyridine dependent dehydrogenases in reactions of oxidation and reduction.	6. Satyanarayana U., Chakrapani U. "Biochemistry", Third Edition. – 2006. – P. 140-141; 226-227. 7. Harper′s Biochemistry. 26th edition / R. K. Murray, Daryl K. Granner, Peter A. Mayes, Victor W. Rodwell, 2003.- P.87 8. Lecture notes.
4.	**Flavine dependent dehydrogenases**: • structure of FAD and FMN; • mechanism ol oxidation and reduction of NAD; • role of flavine dependent dehydrogenases in reactions of oxidation and reduction.	1. Satyanarayana U., Chakrapani U. "Biochemistry", Third Edition. – 2006. – P. 137-139, 227 2. Harper′s Biochemistry. 26th edition / R. K. Murray, Daryl K. Granner, Peter A. Mayes, Victor W. Rodwell, 2003.- P.87-88 3. Lecture notes.

5.	**Cytochromes and their role in tissue respiration.** Structure of their prosthetic group Cytochromes composition of respiratory chain	1. Satyanarayana U., Chakrapani U. "Biochemistry", Third Edition. – 2006. – P. 87, 91-92. 2. Lecture notes.
6.	**Molecular organization of electron transport chain of mitochondria** • constituents of respiratory chain in mitochondria • the sequence of electron transporters in respiratory chain • the significance of redox potentials in transport of electrons and protons	1. Satyanarayana U., Chakrapani U. "Biochemistry", Third Edition. – 2006. – P. 226-228. 2. Harper′s Biochemistry. 26th edition / R. K. Murray, Daryl K. Granner, Peter A. Mayes, Victor W. Rodwell, 2003.- P.92-94 3. Lecture notes.
7.	**Supramolecular complexes of respiratory chain in inner membrane of mitochondria –** five complexes, their names and constituents	1. Satyanarayana U., Chakrapani U. "Biochemistry", Third Edition. – 2006. – P. 87, 96-98. 2. Harper′s Biochemistry. 26th edition / R. K. Murray, Daryl K. Granner, Peter A. Mayes, Victor W. Rodwell, 2003.- P.50 3. Lecture notes.
8.	**Oxidative phosphorylation:** • **P/O ratio.** • **Sites of oxidative phosphorylation.** • **Energetics of oxidative phosphorylation** • **Chemical coupling hypothesis,** • **Chemiosmotic thery;** • **Inherited disorders of oxidative phosphorylation.**	1. Satyanarayana U., Chakrapani U. "Biochemistry", Third Edition. – 2006. – P. 228-232. 2. Harper′s Biochemistry. 26th edition / R. K. Murray, Daryl K. Granner, Peter A. Mayes, Victor W. Rodwell, 2003.- P.105-107
9.	**Molecular structure and principles of functioning of ATP-synthase.**	1. Satyanarayana U., Chakrapani U. "Biochemistry", Third Edition. – 2006. – P. 231. 2. Harper′s Biochemistry. 26th edition / R. K. Murray, Daryl K. Granner, Peter A. Mayes, Victor W. Rodwell, 2003.- P.106
10.	**Inhibitors of electron transport in a respiratory chain of mitochondria.** • **To draw the scheme of respiratory chain and to show**	1. Satyanarayana U., Chakrapani U. "Biochemistry", Third Edition. – 2006. – P. 232-233. 2. Harper′s Biochemistry. 26th edition / R.

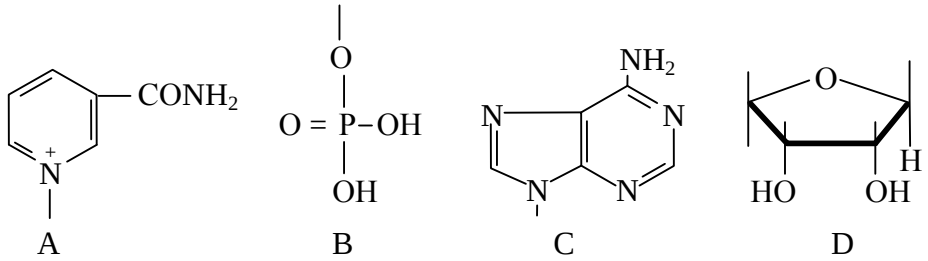
	three possible sites of action of inhibitors; • **Characteristics of inhibitors.**	K. Murray, Daryl K. Granner, Peter A. Mayes, Victor W. Rodwell, 2003.- P.107.
11	**Uncouplers of electron transport and oxidative phosphorylation in a respiratory chain of mitochondria:** • **Physiological and pathological uncouplers;** • **Significance of uncoupling**	1. Satyanarayana U., Chakrapani U. "Biochemistry", Third Edition. – 2006. – P. 233. 2. Harper′s Biochemistry. 26th edition / R. K. Murray, Daryl K. Granner, Peter A. Mayes, Victor W. Rodwell, 2003.- P.107.
12	**Modes of ATP biosynthesis in cells:** • **Oxidative phosphorilation;** • **Substrate level phosphorylation (examples of reactions).**	3. Satyanarayana U., Chakrapani U. "Biochemistry", Third Edition. – 2006. – P. 224. 4. Harper′s Biochemistry. 26th edition / R. K. Murray, Daryl K. Granner, Peter A. Mayes, Victor W. Rodwell, 2003.- P.91.
13	**Free radicals and mechanisms of their production and inactivation:** • **Sources and generation of free radicals;** • **Lipid peroxidation;** •	1. Satyanarayana U., Chakrapani U. "Biochemistry", Third Edition. – 2006. – P. 655-. 2. Harper′s Biochemistry. 26th edition / R. K. Murray, Daryl K. Granner, Peter A. Mayes, Victor W. Rodwell, 2003.- P.50

COMPREHENSION QUESTIONS:

1. Select appropriate type of biological oxidation:

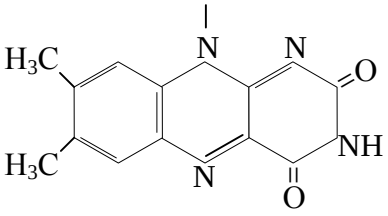
	Reaction		**Type**
1.	$SH_2 + R \rightarrow S + RH_2$	A	Dioxygenase
2.	$SH_2 + 1/2O_2 \rightarrow S - OH$	B	Oxidase
3.	$SH_2 + O_2 \rightarrow S + H_2O_2$	C	Dehydrogenase
4.	$S + O_2 \rightarrow SO_2$	D	Monooxygenase

2. Which component of the NAD^+ structure takes part in the binding of hydrogen?

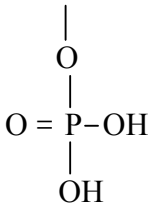


A B C D

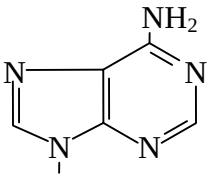
3. Which component of the FAD structure takes part in the binding of hydrogen?



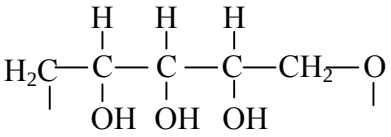
A



B

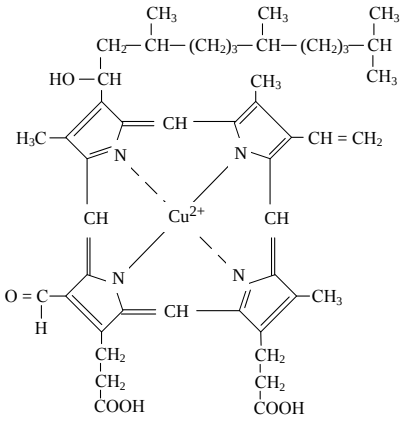


C

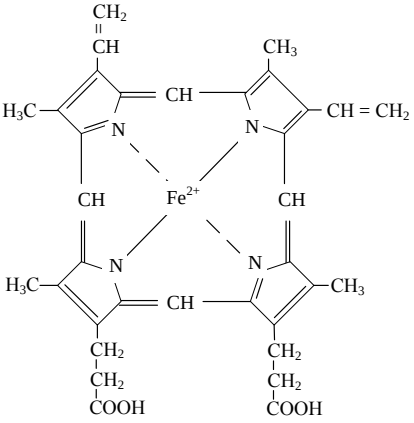


D

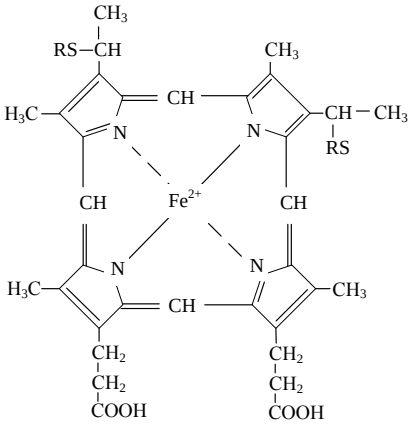
4. Choose cytochromes a, b and c from listed bellow:



A



B

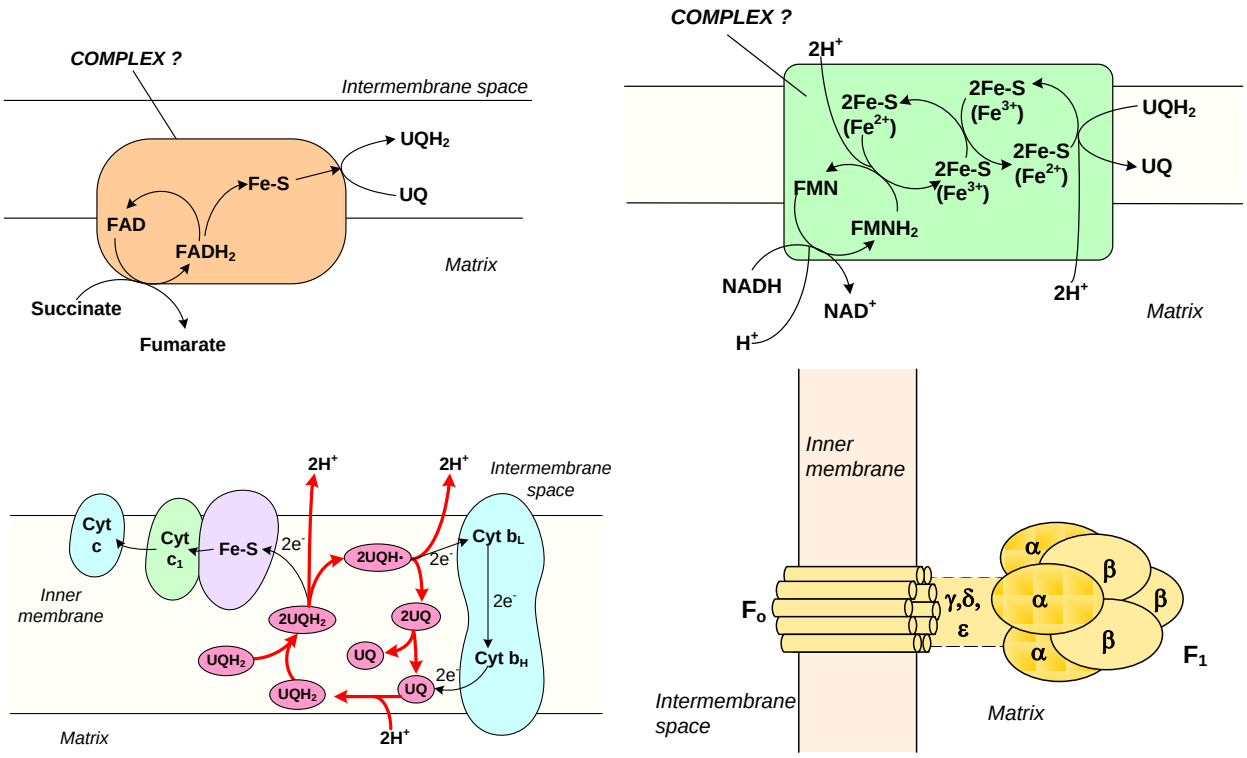


C

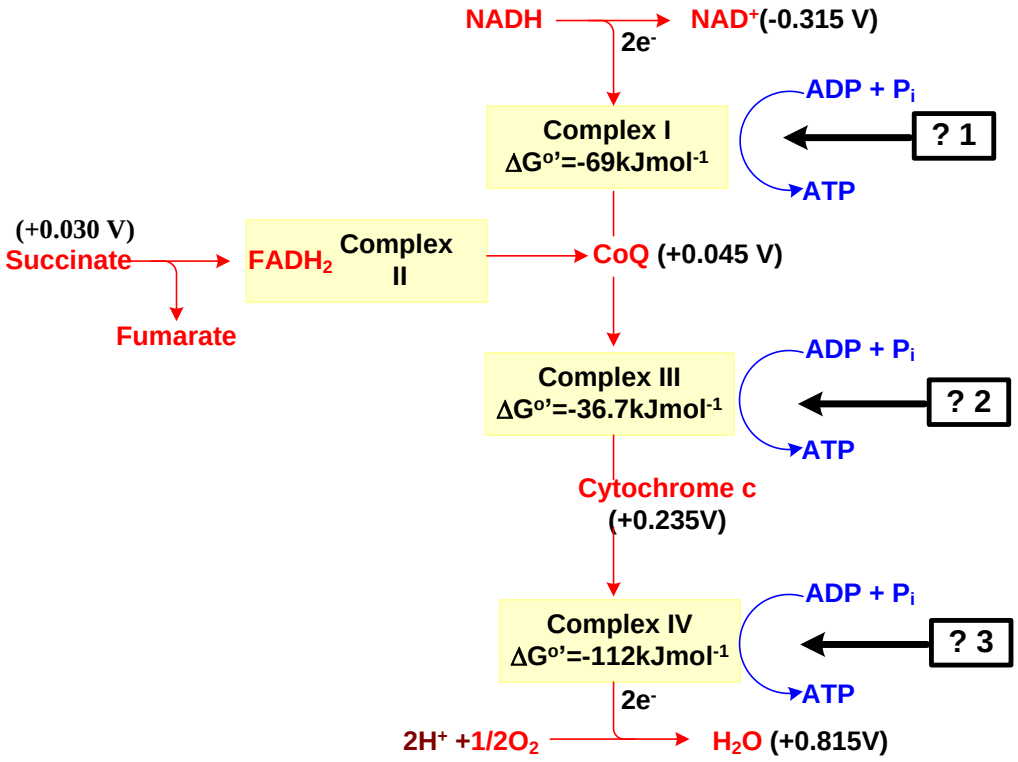
5. Choose the standard reduction potential for each redox pair of Electron-Transport Chain in mitochondria

	Redox pair		**$E^{o'}$ (Volts)**
1.	NAD^+/ NADH	A	+0.82
2.	FMN/$FMNH_2$	B	+0.10
3.	Succinate/α-ketoglutarate	C	+0.23
4.	FAD/ $FADH_2$	D	- 0.22
5.	$2H^+/H_2$	E	+ 0.07
6.	Coenzyme Q (ox/red)	F	- 0. 30
7.	Cytochrome b (Fe^{3+}/Fe^{2+})	G	+0.29
8.	Cytochrome c_1 (Fe^{3+}/Fe^{2+})	H	- 0.67
9.	Cytochrome c (Fe^{3+}/Fe^{2+})	I	- 0.42
10.	Cytochrome a (Fe^{3+}/Fe^{2+})	J	- 0.25
11.	½ O_2/H_2O	K	- 0.32

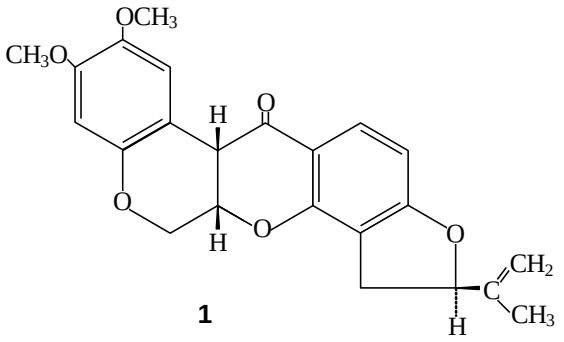
6. Name complexes shown at the pictures below:



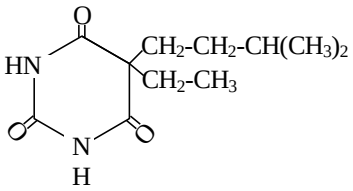
7. Several molecules are known to specifically inhibit the electron transport process. Used in conjunction with reduction potential measurements, inhibitors have been invaluable in the determination of the correct order of electron-transport chain components. Insert the corresponding inhibitors (at the scheme) to the sites of their action.



8. Name the respiratory inhibitors, shown below:



1



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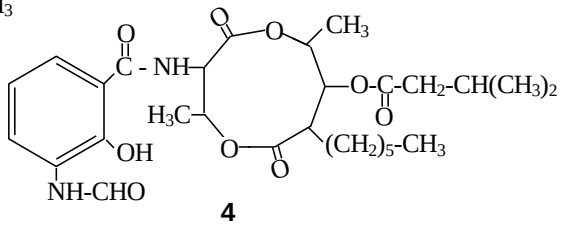

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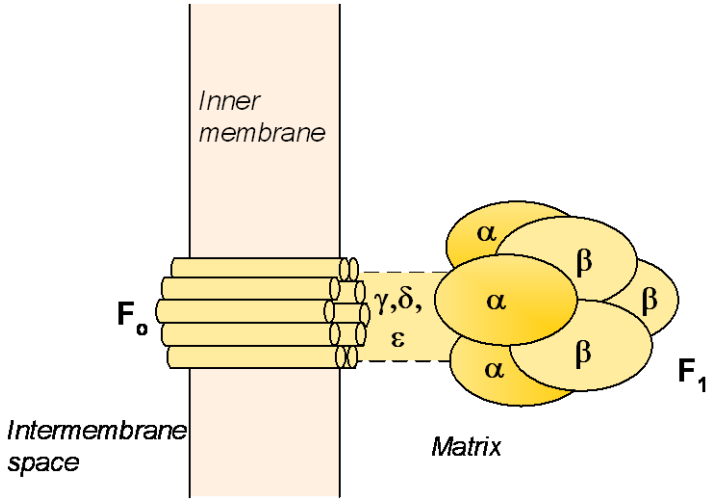
9. Explain the role of ATP-synthase components, shown below:



EXAMPLES OF MULTI-CHOICE QUESTIONS:

1. **The redox carriers are grouped into respiratory chain complex:**

A. In the inner mitochondrial membrane
B. In mitochondiral matrix
C. On the outer mitochondrial membrane
D. On the inner surface of outer mitochondrial membrane
E. Cytosol

2. The next enzyme complex serves as a point of entry for most of the electrons generated by the action of the citric acid cycle:.

A. Complex I
B. Complex II
C. Complex III
D. Complex IY
E. ATP synthase

3. An enzyme catalyzing oxidoreduction, using oxygen as hydrogen acceptor is:

A. Cytochrome oxidase
B. Lactate dehydrogenase
C. Malate dehydrogenase
D. Succinate dehydrogenase

E. Fumarase

4. Most of hydrogen atoms (protons and electrons) involved in process of tissue respiration are transported to the respiratory chain by the next compound:

A. $NADH+H^+$
B. $NADPH+H^+$
C. $FMNH_2$
D. $FADH_2$
E. Pyruvate

5. Dehydrogenases utilize, as coenzymes, all of the following except:

A. FH_4
B. $NADP^+$
C. FAD
D. NAD^+
E. FMN

CLINICAL CASE

A 68-year-old female in a hypertensive crisis is being treated in the intensive care unit (ICU) with intravenous nitroprusside for 48 hours. The patient's blood pressure was brought back down to normal levels; however, she was complaining of a burning sensation in her throat and mouth followed by nausea and vomiting, diaphoresis, agitation, and dyspnea. The nurse noticed a sweet almond smell in her breath. An arterial blood gas revealed a significant metabolic acidosis. A serum test suggests a metabolite of nitroprusside, thiocyanate, is at toxic levels.

- **What is the likely cause of her symptoms?**
- **What is the biochemical mechanism of this problem?**
- **What is the treatment for this condition?**

Additional literature:

1. Lehninger A. Principles of Biochemistry. – New York. – W.H.Freeman and Company. – 2005. – 1010 p.
2. Mardashko O.O., Yasinenko N.Y. Biochemistry. Texts of lectures.-Odessa. The Odessa State Medical University, 2003.-416p.
3. Devlin T.M., ed. Textbook of Biochemistry with Clinical Correlations, 5th ed. New York: Wiley-Liss, 2002.
4. MCQs / Prof. Sklyarov A.Ya., Lutsik M.D., Fomenko I.S., Klymyshin D.O., Nasadyuk C.M.. – 2012. – 308 p.

Topic № 8. Glycolysis – oxidation of carbohydrates in anaerobic conditions.

Objective: Carbohydrate metabolism plays an important role in providing an organism with energy. In process of glycolysis, as well as in alcohol fermentation, initially are produced phosphate esters of hexoses and trioses, which are further, oxidized with production of ATP. During phosphorylation of carbohydrate metabolites the concentration of inorganic phosphate in the medium is diminished and this permits to follow the process

of phosphorylation, resp. glycolysis. Concentration of lactate in blood increases after hard muscle exercises and in some diseases.

Specific objectives:

- *To interpret biochemical pathways of intracellular oxidation of glucose in anaerobic conditions*
- *To analyze peculiarities of glycolytic reactions, which occur with involvement of ATP*
- *To analyze peculiarities of substrate phosphorylation and production of ATP in glycolysis*
- *To interpret role of coenzymes and enzymes in glycolytic reactions*
- *To analyze regulatory mechanisms of glucose oxidation in anaerobic conditions*

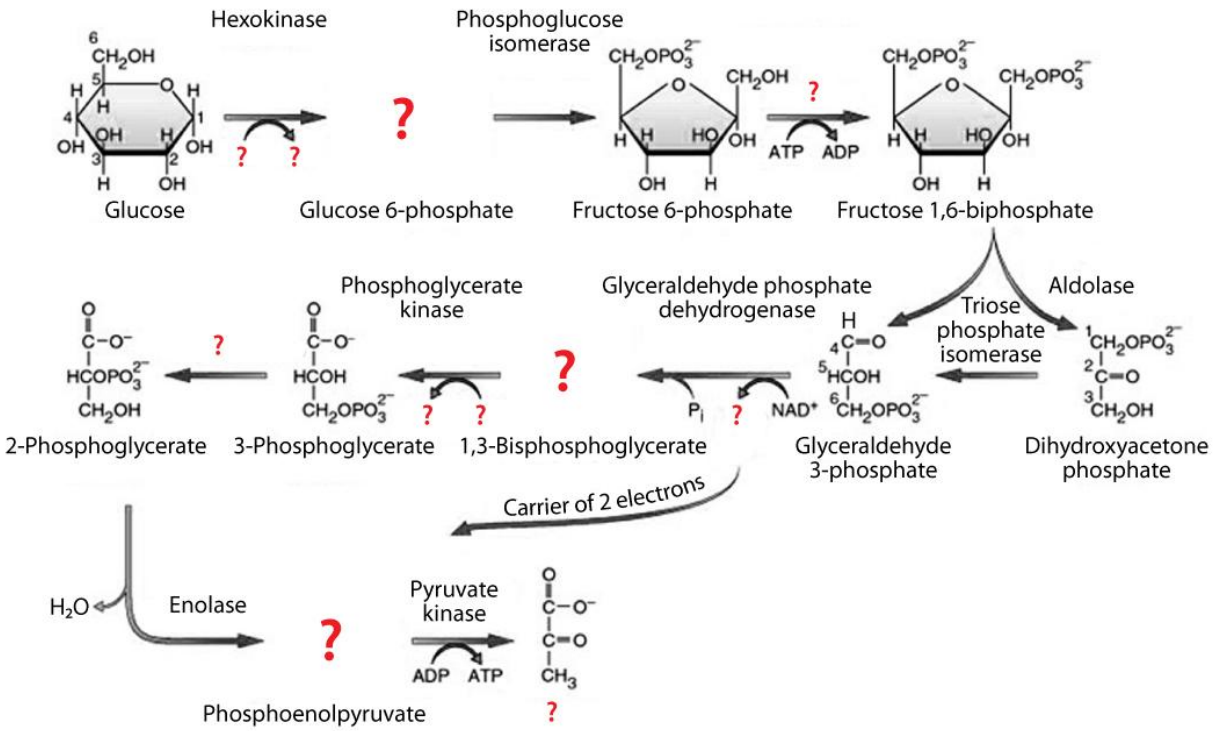
GUIDE FOR THE INDEPENDENT STUDY OF THE TOPIC:

Questions:	References:
1. **Glucose as an important metabolite in carbohydrate metabolism**: • Major pathways of carbohydrate metabolism; • Sources of glucose in the organism; • Entry of glucose into cells	1. Satyanarayana U., Chakrapani U. "Biochemistry", Third Edition. – 2006. – P. 244-245. 2. Biochemistry. The Molecular Bases of Life / Trudy McKee, James R. McKee, 2003. - P. 235 3. Lecture notes.
2. **Anaerobic oxidation of glucose:** • the sequence of reactions in glycolysis; • enzymatic reactions of anaerobic and aerobic glycolysis; • characterization of glycolytic reactions, which occur with utilization of energy; • characterization of enzymatic reactions of substrate phosphorylation in glycolysis; • mechanism of glycolytic oxido-reduction and reactions, which provide this process.	1. Satyanarayana U., Chakrapani U. "Biochemistry", Third Edition. – 2006. – P. 245-248. 2. Harper′s Biochemistry. 27th edition / R. K. Murray, Daryl K. Granner, Peter A. Mayes, Victor W. Rodwell, 2006.- P.151-154. 3. Biochemistry. The Molecular Bases of Life / Trudy McKee, James R. McKee, 2003. - P. 236-243. 4. Lecture notes.
3. **The role of lactate dehydrogenase (LDH) in glycolysis, mechanism of reaction and its peculiarities.** • Isoenzymes of LDH and their clinical diagnostic significance; • Conversion of pyruvate to lactate-signilicance;	1. Satyanarayana U., Chakrapani U. "Biochemistry", Third Edition. – 2006. – P. 248. 2. Harper′s Biochemistry. 27th edition / R. K. Murray, Daryl K. Granner, Peter A. Mayes, Victor W. Rodwell, 2006.- P.152. 3. Lecture notes.

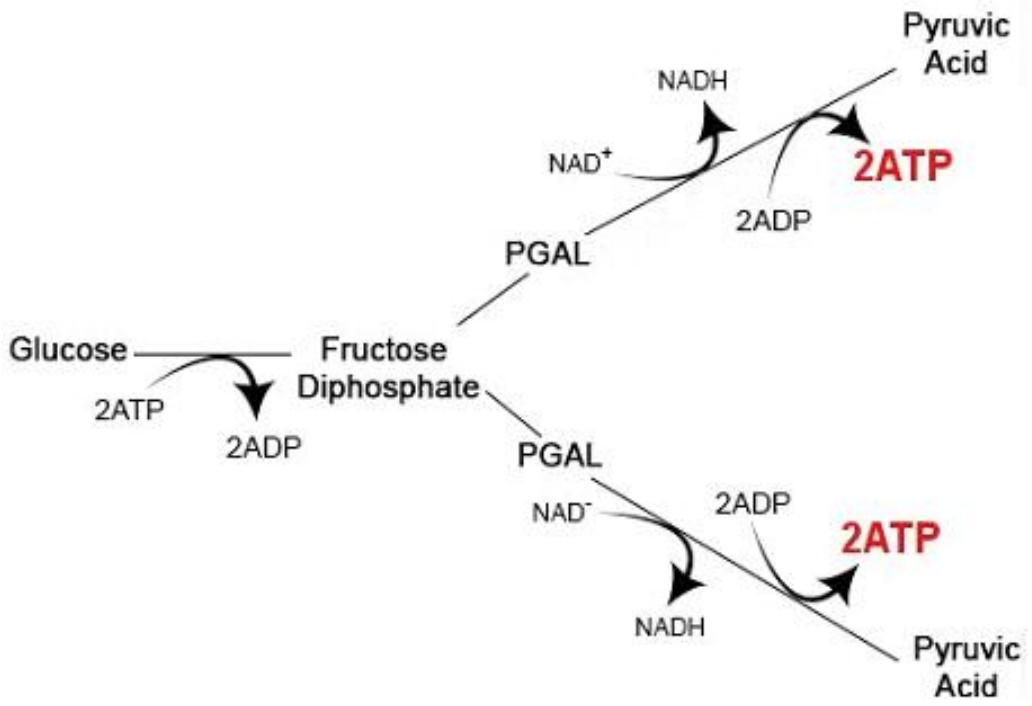
4.	**Mechanisms of regulation of the rate of reactions in anaerobic glycolysis** • Allosteric regulation of glycolysis; • Role of fructose 2,6-bisphosphate in glycolysis; • Irreversible steps in glycolysis.	1. Satyanarayana U., Chakrapani U. "Biochemistry", Third Edition. – 2006. – P. 250-251. 2. Biochemistry. The Molecular Bases of Life / Trudy McKee, James R. McKee, 2003. - P. 245-249. 3. Harper′s Biochemistry. 27th edition / R. K. Murray, Daryl K. Granner, Peter A. Mayes, Victor W. Rodwell, 2006.- P.154-155. 4. Lecture notes.
9.	**Energetic effect of anaerobic oxidation of glucose:** • Production of ATP in glycolysis	1. Satyanarayana U., Chakrapani U. "Biochemistry", Third Edition. – 2006. – P. 249. 2. Biochemistry. The Molecular Bases of Life / Trudy McKee, James R. McKee, 2003. - P. 245. 3. Harper′s Biochemistry. 27th edition / R. K. Murray, Daryl K. Granner, Peter A. Mayes, Victor W. Rodwell, 2006.- P.151-156-157. 4. Lecture notes.
6.	**Alcohol fermentation, common and different reactions in glycolysis and fermentation.** • Reactions of alcohol fermentation • Common and different reactions in glycolysis and fermentation.	1. Biochemistry. The Molecular Bases of Life / Trudy McKee, James R. McKee, 2003. - P. 244-245 2. Lecture notes.
7.	**Pasteur effect – switching over of anaerobic to aerobic oxidation of glucose, peculiarities of regulation:** • Regulation of Pasteur effect; • Enzymatic reactions of Pasteur effect.	1. Satyanarayana U., Chakrapani U. "Biochemistry", Third Edition. – 2006. – P. 251. 2. Lecture notes.

COMPREHENSION QUESTIONS:

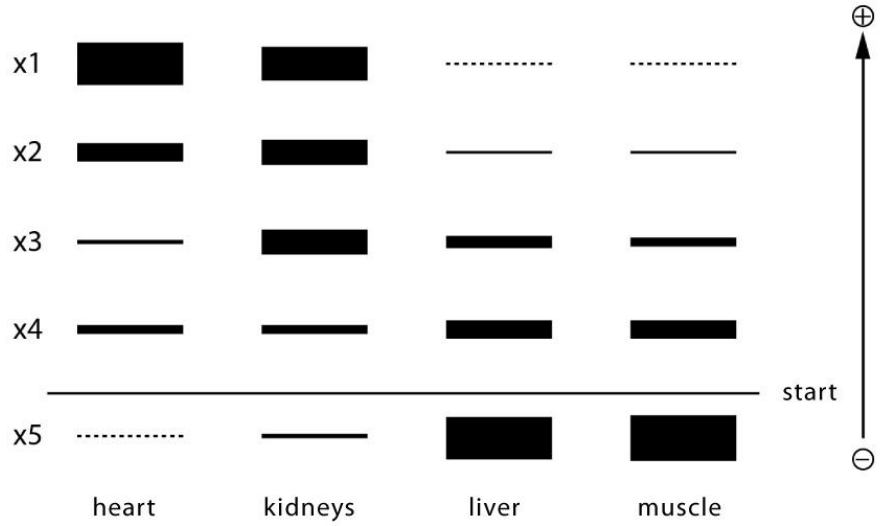
1. Fill in the blanks.



2. Indicate points of ATP generation in glycolysis and net production of ATP in anaerobic conditions; indicate enzymes of energy generation phase.



3. LDH has five distinct isoenzymes LDH1, LDH2, LDH3, LDH4 and LDH5. They can be separated by electrophoresis (cellulose or starch gel or agarose gel). LDH1 has more positive charge and fastest in electrophoretic mobility while LDH5 is the slowest. Put the correct izoforms of LDHs and explain diagnostic importance of LDH.



4. What substances are used for the synthesis of glucose in the human body during prolonged fasting or exhausting work? Explain your answer.

5. Explain the negative impact of excessive intake of carbohydrates on the human body. Which biochemical disorders it causes?

6. Examination of a patient revealed increased activity of LDH1, LDH2 and creatine. What human organ might be damaged?

EXAMPLES OF MULTI-CHOICE QUESTIONS:

1. **An untrained person who has not been practicing physical exercises for a long time complains of a muscle pain as a result of intensive manual work. What is the probable reason of the pain syndrome?**

A. Accumulation of lactate in muscles
B. Decreasing of lipids level in muscles
C. Increased disintegration of muscle proteins
D. Accumulation of creatinine in muscles
E. Increase of ATP level in muscles

2. The high speed sprint causes a feeling of pain in skeletal muscles of untrained people that occurs due to lactate accumulation. The activation of what biochemical process is it resulting from?

A. Glycolysis
B. Gluconeogenesis
C. Pentose phosphate pathway
D. Lipogenesis
E. Glycogenesis

3. A 7-year-old girl manifests obvious signs of anemia. Laboratory tests showed the deficiency of pyruvate kinase activity in erythrocytes. The disorder of what biochemical process is a major factor in the development of anemia?

A. Anaerobic glycolysis
B. Deamination of amino acids
C. Tissue respiration
D. Oxidative phosphrylation

E. Breaking up of peroxides

4. **During consumption of cakes or sweets in mixed saliva a transient increase in lactate level takes place. Activation of what biochemical process causes this effect?**

A. Anaerobic glycolysis
B. Tissue respiration
C. Aerobic glycilysis
D. Gluconeogenesis
E. Microsomal oxidation

5. **In yeast cells occurs a process which is similar to glycolysis- an alcohol fermentation. In course of this process through several stages from pyruvate is produced :**

A. Lactate
B. Ethanol
C. Acetaldehyde
D. Glyceraldehyde
E. Pyruvate

Individual independent students work

1. Disorders of carbohydrate metabolism and its pharmacological correction.
2. Principles of regulation of glucose metabolism. Characterization of regulatory enzymes in glycolysis and gluconeogenesis.

Additional literature:

1. Lehninger A. Principles of Biochemistry. – New York. – W.H.Freeman and Company. – 2005. – 1010 p.
2. Mardashko O.O., Yasinenko N.Y. Biochemistry. Texts of lectures.-Odessa. The Odessa State Medical University, 2003.-416p.
3. Devlin T.M., ed. Textbook of Biochemistry with Clinical Correlations, 5th ed. New York: Wiley-Liss, 2002.
4. Toy E.C., Seifert W. E., Strobel H.W., Harms K.P. "Case Files in Biochemistry. 2nd edition" – 2008. – 488 p.
5. MCQs / Prof. Sklyarov A.Ya., Lutsik M.D., Fomenko I.S., Klymyshin D.O., Nasadyuk C.M. – 2012. – 308 p.
6. Palmer M. "Human metabolism lecture notes" Ontario: Waterloo. – 2014. – 380 p.

Topic № 9. Glucose oxidation under aerobic conditions and alternative metabolic pathways of monosaccharides.

Metabolism of carbohydrates includes all complex process of carbohydrates transformation starting from digestion, absorption, transport and utilization in cells up to formation of end products – CO_2 and H_2O. In aerobic conditions pyruvate, as a product of glycolysis, releases CO_2 and is transformed to acetyl-CoA, which is further oxidized in tricarboxylic acid cycle (Crebs cycle) to CO_2 and H_2O. The rate of reactions in TCA cycle depends from requirements of the cell in ATP. Regulatory reactions in TCA cycle are

synthesis of citrate and oxidative decarboxylation of alpha-oxoglutarate, which are regulated by amount of ADP, succinyl-CoA and $NADH_2$.

Specific aims:

- To interpret mechanisms of monosaccharides transformation to final metabolic products and energetic effect in aerobic conditions
- To analyze structural and functional peculiarities of pyruvate dehydrogenase complex
- To explain the sequence of reactions in PPP and significance of this process
- To analyze metabolic pathways of fructose and galactose transformations in human body.

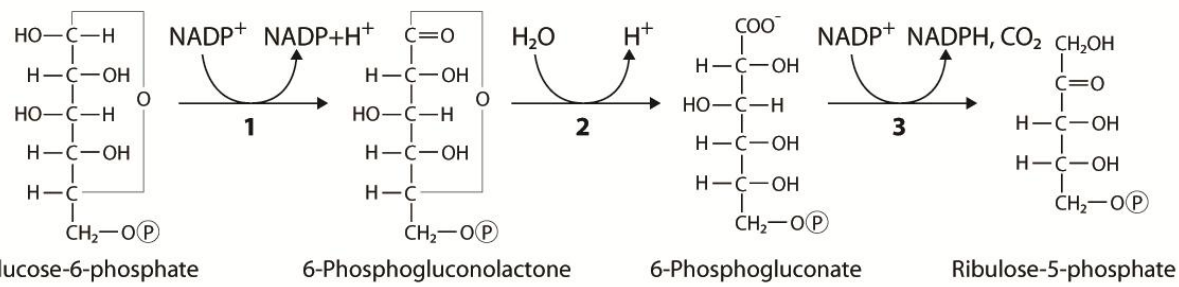
GUIDE FOR THE INDEPENDENT STUDY OF THE TOPIC:

	Questions:	
1.	**Stages of aerobic oxidation of glucose.**	1. Satyanarayana U., Chakrapani U. "Biochemistry", Third Edition. – 2006. – P. 251-253. 2. Harper′s Biochemistry. 27th edition / R. K. Murray, Daryl K. Granner, Peter A. Mayes, Victor W. Rodwell, 2006.- P.151-154. 3. Biochemistry. The Molecular Bases of Life / Trudy McKee, James R. McKee, 2003. - P. 236-243. 4. Lecture notes.
2.	**Oxidative decarboxylation of pyruvic acid.** • structure of multienzyme pyruvate dehydrogenase complex. • peculiarities of function of pyruvate tdehydrogenase complex. • mechanism of oxidative decarboxylation of pyruvate. • role of vitamins and coenzymes in transformation of pyruvate to acetyl-CoA.	1. Satyanarayana U., Chakrapani U. "Biochemistry", Third Edition. – 2006. – P. 252-253. 2. Harper′s Biochemistry. 27th edition / R. K. Murray, Daryl K. Granner, Peter A. Mayes, Victor W. Rodwell, 2006.- P.155-156. 3. Lecture notes.
3.	**Energetic effect of aerobic oxidation of glucose.** • Total ATP per mole of glucose under aerobic condition • Total ATP per mole of glucose under anaerobic o condition	1. Satyanarayana U., Chakrapani U. "Biochemistry", Third Edition. – 2006. – P. 249. 2. Biochemistry. The Molecular Bases of Life / Trudy McKee, James R. McKee, 2003. - P. 245. 3. Harper′s Biochemistry. 27th edition / R. K. Murray, Daryl K. Granner, Peter A. Mayes, Victor W. Rodwell, 2006.- P.156-

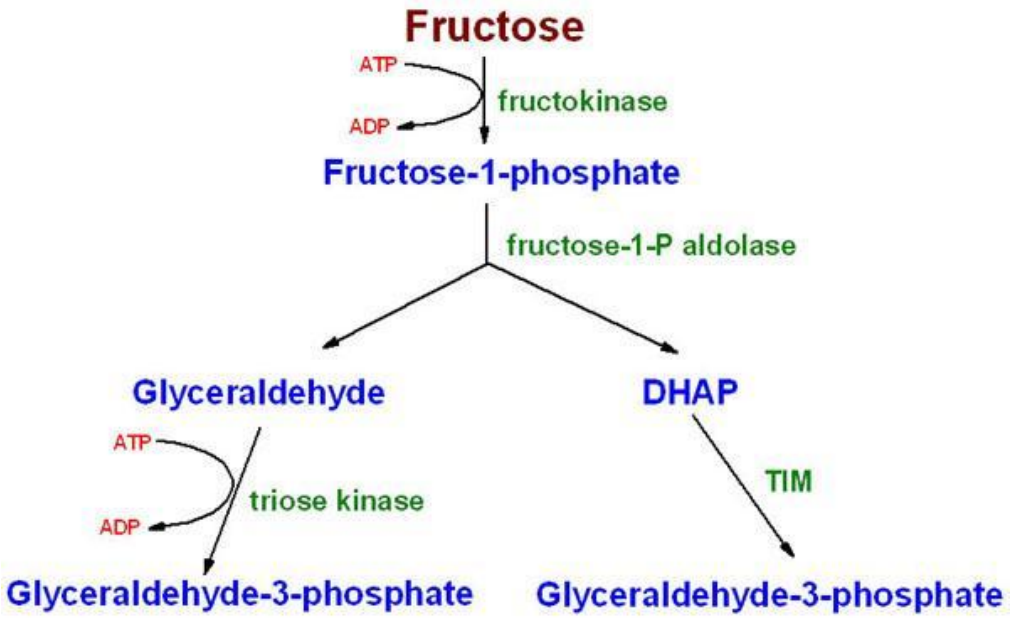
		157. 4. Lecture notes.
4.	**PPP and glucose utilization:** • scheme of reactions in oxidative and nonoxidative stages of PPP; • enzymes and coenzymes of PPP reactions; • biological significance of PPP; • disorders of PPP in red blood cells; • enzymopathias of glucose-6-phosphate dehydrogenase.	1. Satyanarayana U., Chakrapani U. "Biochemistry", Third Edition. – 2006. – P. 270-275. 2. Harper′s Biochemistry. 27th edition / R. K. Murray, Daryl K. Granner, Peter A. Mayes, Victor W. Rodwell, 2006.- P.177-180. 3. Biochemistry. The Molecular Bases of Life / Trudy McKee, James R. McKee, 2003. - P. 256-259. 4. Lecture notes.
5	**Enzymatic reactions of fructose turnover in human body. Hereditary enzymopathias of fructose metabolism.** • Reactions of fructose turnover; • Enzymopathias of fructose metabolism	1. Satyanarayana U., Chakrapani U. "Biochemistry", Third Edition. – 2006. – P. 87, 278-279. 2. Biochemistry. The Molecular Bases of Life / Trudy McKee, James R. McKee, 2003. - P. 260-261. 3. Harper′s Biochemistry. 27th edition / R. K. Murray, Daryl K. Granner, Peter A. Mayes, Victor W. Rodwell, 2006.- P.181. 4. Lecture notes.
6.	**Enzymatic reactions of galactose metabolism in human body. Hereditary enzymopathias of galactose metabolism.** • Reactions of galactose metabolism. • Enzymopathias of galactose metabolism	1. Biochemistry. The Molecular Bases of Life / Trudy McKee, James R. McKee, 2003. - P. 262. 2. Harper′s Biochemistry. 27th edition / R. K. Murray, Daryl K. Granner, Peter A. Mayes, Victor W. Rodwell, 2006.- P.186. 3. Lecture notes.

COMPREHENSION QUESTIONS:

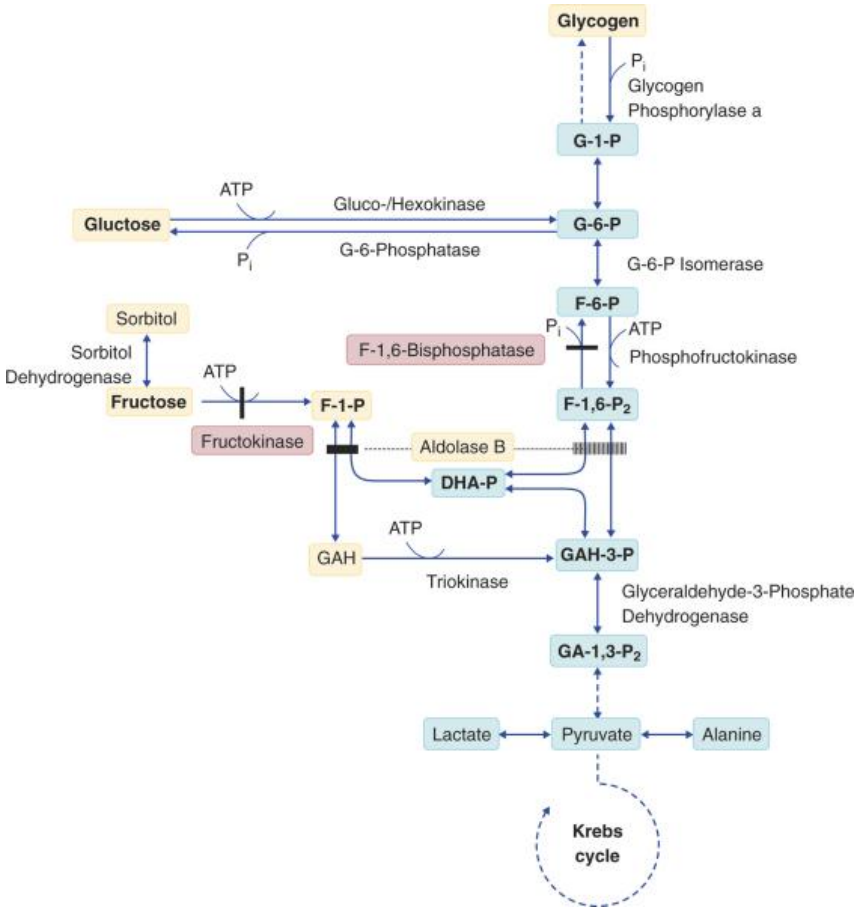
1. Indicate enzymes catalyzing the following reactions of HMP:



2. Name enzymes of fructose metabolism.



3. Defects in fructose metabolism are indicated with black blocks on a picture below. Characterize biochemical significance of fructose metabolism and the main reasons of essential fructosuria and hereditary fructose intolerance.



EXAMPLES OF MULTI-CHOICE QUESTIONS:

1. A newborn child with the signs of cataract, growth and mental retardation, who manifested vomiting and diarrhea, was brought to an emergency clinic. A presumptive diagnosis of galactosemia was made. The deficiency of what enzyme occurs in case of this disease?

A. Galactose-1-phosphate uridyl transferase.
B. Glucokinase.
C. UDP-galactose-4-epimerase.
D.Hexokinase.
E. Glucose-6-phosphate dehydrogenase

2. A cataract and fatty degeneration of the liver develop in the conditions of high galactose and low glucose level in blood. What disease do these symptoms testify to?

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

3. In a patient are manifested symptoms of intoxication with arsenic compounds. What metabolic process is damaged taking into account that arsenic containing substances inactivate lipoic acid?

A. Oxidative decarboxylation of pyruvate
B. Fatty acids biosynthesis
C. Neutralization of superoxide anions
D. Coupling of oxidation and phopsphorylation
E. Microsomal oxidation

4. A 2-year-old boy has the increase of liver and spleen sizes detected and eye cataract present. The total sugar level in blood is increased, but glucose tolerance is within the normal range. The inherited disturbance of the metabolism of what substance is the cause of the indicated state?

A. Galactose.
B. Fructose.
C. Glucose.
D. Maltose.
E. Saccharose.

5. Essential fructosuria is a hereditary disease, connected with disorders of fructose metabolism. The symptoms of lesions of liver and kidneys are manifested. This disease is caused by insufficiency of enzyme, which catalyze transformation of fructose to the next compound:

A. Fructoso-1-phosphate
B. Fructoso-6-phosphate
C. Fructoso-1,6-bisphosphate
D. Glucoso-6-phosphate
E. Glyceraldehyde phosphate

CLINICAL CASE

A 3-year-old boy is brought to the emergency department after several episodes of vomiting and lethargy. His pediatrician has been concerned about his failure to thrive and possible hepatic failure along with recurrent episodes of the vomiting and lethargy. After a careful history is taken, you observe that these episodes occur after ingestion of certain

types of food, especially high in fructose. His blood sugar was checked in the emergency department and was extremely low.

- **What is the most likely diagnosis?**
- **What is the biochemical basis for the clinical symptoms?**
- **What is the treatment of the disorder?**

Additional literature:

1. Lehninger A. Principles of Biochemistry. – New York. – W.H.Freeman and Company. – 2005. – 1010 p.
2. Mardashko O.O., Yasinenko N.Y. Biochemistry. Texts of lectures.-Odessa. The Odessa State Medical University, 2003.-416p.
3. Devlin T.M., ed. Textbook of Biochemistry with Clinical Correlations, 5th ed. New York: Wiley-Liss, 2002.
4. Toy E.C., Seifert W. E., Strobel H.W., Harms K.P. "Case Files in Biochemistry. 2nd edition" – 2008. – 488 p.
5. MCQs / Prof. Sklyarov A.Ya., Lutsik M.D., Fomenko I.S., Klymyshin D.O., Nasadyuk C.M.. – 2012. – 308 p.
6. Palmer M. "Human metabolism lecture notes" Ontario: Waterloo. – 2014. – 380 p.

Topic № 10. Catabolism and biosynthesis of glycogen. Regulation of glycogen metabolism Biosynthesis of glucose – gluconeogenesis

Glycogen is the storage form of glucose in animals, as is starch in plants. It is stored mostly in liver (6-8 %) and muscle (1-2 %). Due to more muscle mass, the quantity of glycogen in muscle (250 g) is about three times higher than that in the liver (75 g). Glycogen is stored as granules in the cytosol, where most of the enzymes of glycogen synthesis and breakdown are present.

Specific aims:

- To explain characteristic features of glycogen breakdown and biosynthesis.
- To analyze mechanisms of humoral regulation of glycogen metabolism in liver and muscles.
- To explain hereditary disorders of glycogen metabolism.
- To analyze specific features of gluconeogenesis reactions and substrates of this process.
- To explain and interpret regulatory mechanisms of gluconeogenesis.

GUIDE FOR THE INDEPENDENT STUDY OF THE TOPIC:

Questions:	References:
1. **Mechanism and peculiarities of enzymatic reactions of glycogenesis.**	1. Satyanarayana U., Chakrapani U. "Biochemistry", Third Edition. – 2006. – P. 263-265. 2. Harper′s Biochemistry. 27th edition / R. K.

		Murray, Daryl K. Granner, Peter A. Mayes, Victor W. Rodwell, 2006.- P. 159-161. 3. Biochemistry. The Molecular Bases of Life / Trudy McKee, James R. McKee, 2003. - P. 263-264. 4. Lecture notes.
2.	**Glycogenolysis.**	1. Satyanarayana U., Chakrapani U. "Biochemistry", Third Edition. – 2006. – P. 265-266. 2. Harper′s Biochemistry. 27th edition / R. K. Murray, Daryl K. Granner, Peter A. Mayes, Victor W. Rodwell, 2006.- P.167-169. 3. Biochemistry. The Molecular Bases of Life / Trudy McKee, James R. McKee, 2003. - P. 264-266. 4. Lecture notes.
3.	**Cascade mechanisms of ATP-dependent regulation of glycogen phosphorylase and glycogen synthase activities.**	1. Satyanarayana U., Chakrapani U. "Biochemistry", Third Edition. – 2006. – P. 266-268. 2. Biochemistry. The Molecular Bases of Life / Trudy McKee, James R. McKee, 2003. - P. 266-269. 3. Harper′s Biochemistry. 27th edition / R. K. Murray, Daryl K. Granner, Peter A. Mayes, Victor W. Rodwell, 2006.- P.164-165. 4. Lecture notes.
4.	**Peculiarities of hormonal regulation of glycogen metabolism in liver and muscles.**	1. Satyanarayana U., Chakrapani U. "Biochemistry", Third Edition. – 2006. – P. 266-268. 2. Biochemistry. The Molecular Bases of Life / Trudy McKee, James R. McKee, 2003. - P. 266-269. 3. Lecture notes.
5.	**Hereditary disorders in enzymes of glycogen synthesis and breakdown. Glycogenoses, aglycogenoses.**	1. Satyanarayana U., Chakrapani U. "Biochemistry", Third Edition. – 2006. – P. 269-270. 2. Harper′s Biochemistry. 27th edition / R. K. Murray, Daryl K. Granner, Peter A.

		Mayes, Victor W. Rodwell, 2006.- P.165-166. 3. Lecture notes.
6.	**Peculiarities of glycoconjugates metabolism.**	1. http://www.ncbi.nlm.nih.gov/books/NBK1956/ 2. Lecture notes.
7.	**Genetically determined disorders of glycoconjugate metabolism (glycosidoses), mucopolysaccharidosis, rheumatism.**	1. http://ghr.nlm.nih.gov/condition/mucopolysaccharidosis-type-i 2. http://emedicine.medscape.com/article/1258678-overview 3. http://www.medterms.com/script/main/art.asp?articlekey=11679 4. Lecture notes.
8.	**Metabolic pathways and substrates of gluconeogenesis.** • compartmentalization of enzymes, • biological significance of the process.	1. Satyanarayana U., Chakrapani U. "Biochemistry", Third Edition. – 2006. – P. 258-259. 2. Lecture notes.
9.	**Relations between glycolisis and gluconeogenesis (Cori cycle). Irreversible reactions of glycolysis and their shunt pathways. Glucose-lactate and glucose-alanine cycles.**	1. Satyanarayana U., Chakrapani U. "Biochemistry", Third Edition. – 2006. – P. 259-263. 2. Biochemistry. The Molecular Bases of Life / Trudy McKee, James R. McKee, 2003. - P. 252-256. 3. Harper′s Biochemistry. 27th edition / R. K. Murray, Daryl K. Granner, Peter A. Mayes, Victor W. Rodwell, 2006.- P.172-173. 4. Lecture notes.
10.	**Regulation of gluconeogenesis in human organism.**	1. Satyanarayana U., Chakrapani U. "Biochemistry", Third Edition. – 2006. – P. 262-263. 2. Biochemistry. The Molecular Bases of Life / Trudy McKee, James R. McKee, 2003. - P. 254-256. 3. Harper′s Biochemistry. 27th edition / R. K. Murray, Daryl K. Granner, Peter A. Mayes, Victor W. Rodwell, 2006.- P.172-175. 4. Lecture notes.

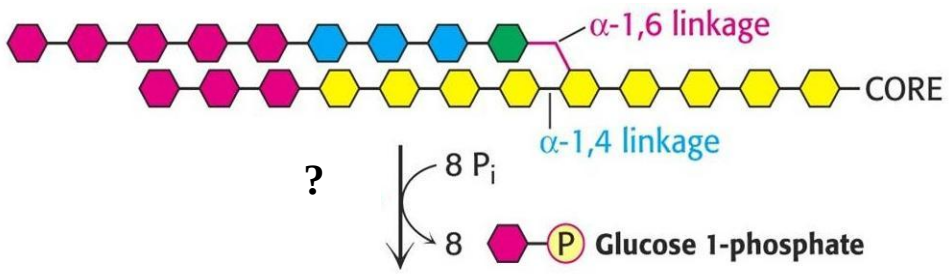
COMPREHENSION QUESTIONS:

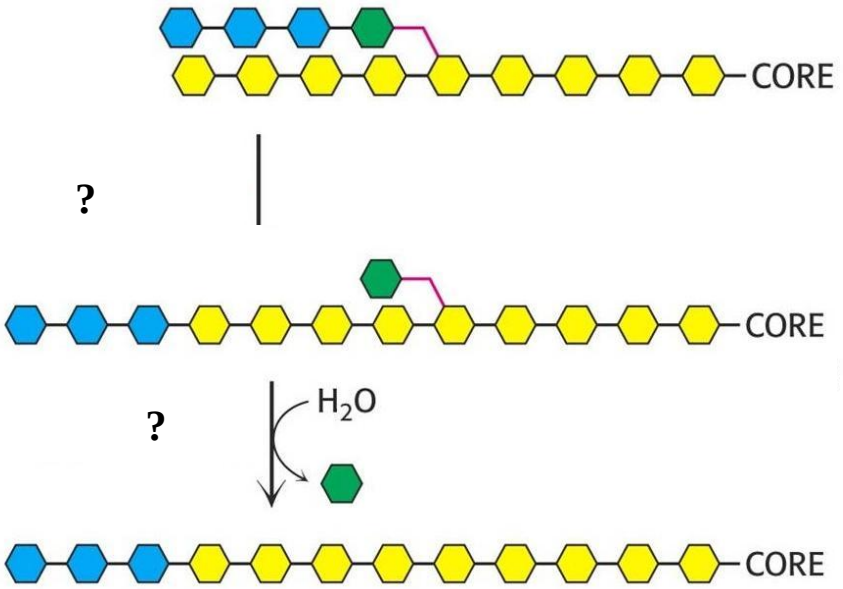
Fill in the blanks:

Type	Name	Enzyme defect	Organs involved	Characteristic features
I	Von Gierke's disease	Glucose 6 - phosphatase	Liver, kidney and intestine	Glycogen accumulation in hepatocytes and renal cells, inlarged liver and kidney, fasting hypoglycemia, lactic academia, ketosis, gouty arthrisis.
II	Pompe's disease	Lysosomal - 1.4 glucosidase (acid maltase)	All organs	**?**
III	Cori's disease (limit dextrinosis, Forbe's disease)	**?**	Liver, muscle, heart, leucocytes	**?**
IV	Anderson's disease (amylopectinose)	Glucosyl 4-6 transferase (?)	**?**	**?**
V	McArdle's diseas	**?**	Sceletal muscle	**?**
VI	Her's disease	**?**	Liver	**?**
VII	Tarul's disease	**?**	**?**	Muscle cramps due to exercise, blood lactate not elevated; hemolysis occurs

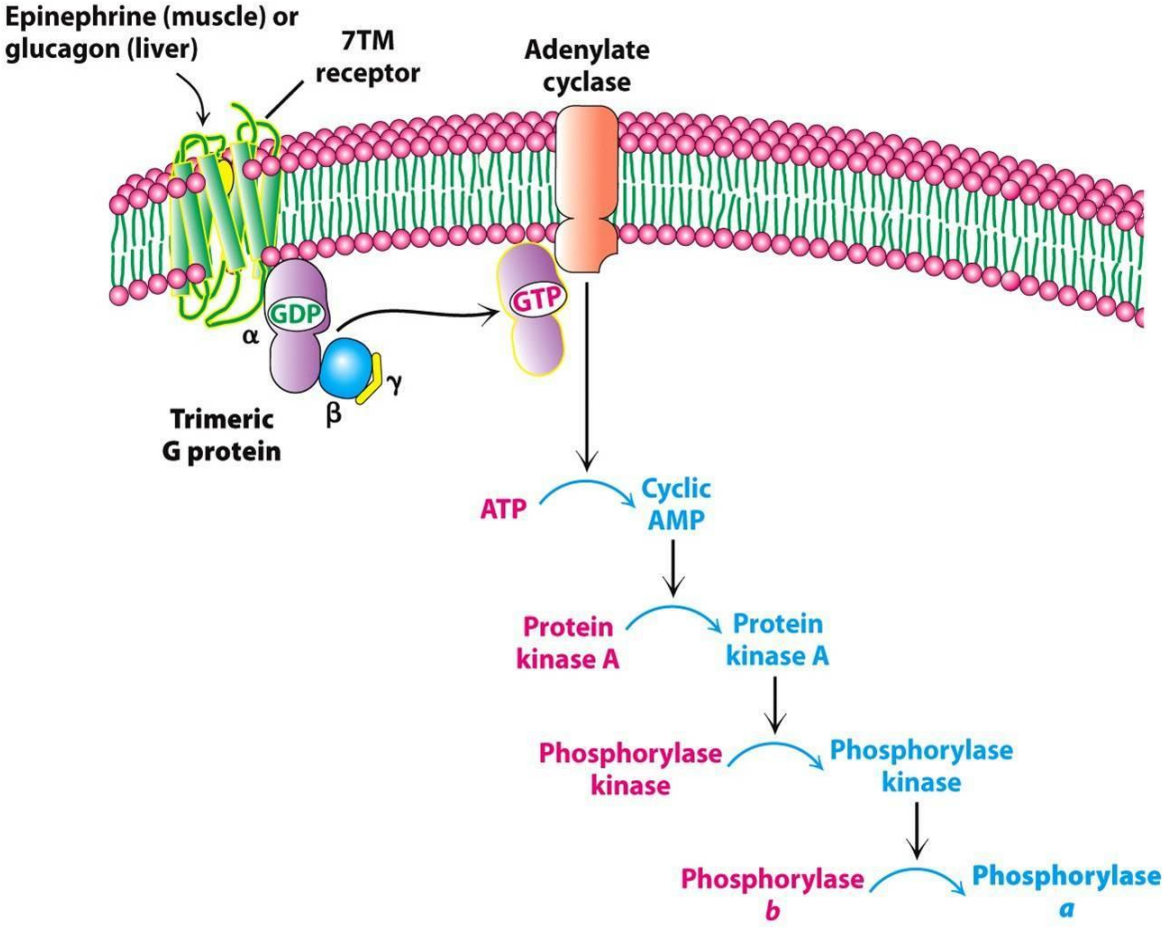
2. The degradation of stored glycogen in liver and muscle constitutes glycogenolysis. The pathways for the synthesis and degradation of glycogen are not reversible. An independent set of enzymes present in the cytosol carry out glycogenolysis. Glycogen is degraded by breaking α – l,4 - and α - 1,6 - glycosidic bonds.

Describe stages of glycogen degradation; indicate enzymes and coenzymes.





3. Characterize humoral regulation of glycogen degradation represented on the scheme above



EXAMPLES OF KROCK-1 TESTS

1. In a weak apathic infant an enlarged liver was detected, which in investigation of biopcia pieces showed an excess of glycogen. Blood glucose concentration is under the normal value. What may be the cause of this disease?

A. Lowered activity of glycogen phosphorylase in a liver
B. Lowered activity of glycogen synthase
C. Lowered activity of glucose 6-phosphate isomerase
D. Lowered activity of glucokinase
E. Deficiency of gene responsible for synthesis of glucose 1-phosphate uridyl transferase

2. During biochemical investigation of blood in a patient was detected hypoglycemia in fasting condition. Investigation of liver bioptates revealed the failure of glycogen synthesis. What enzyme deficiency may cause such status?

A. Glycogen synthase
B. Phosphorylase
C. Aldolase
D. Fructose bis-phosphatase
E. Pyruvate carboxylase

3. In an infant with point mutations in genes the absence of glucose 6-phosphatase, hypoglycemia and hepatomegalia were revealed. What disease is characterized by these symptoms?

A. Gierke disease
B. Adison disease
C. Parkinson disease
D. Cori disease
E. Mac Ardle disease

4. In a patient a lowering in ability to physical load was revealed, while in skeletal muscles the glycogen content was increased. The decrease in activity of what enzyme may cause this condition?

A. Glycogen phosphorylase
B. Phosphofructokinase
C. Glucose 6-phosphate dehydrogenase
D. Glycogen synthase
E. Glucose 6-phosphatase

5. What biochemical process is stimulated in the liver and kidneys of a patient exhausted by starvation?

A. Gluconeogenesis.
B. Synthesis of urea.
C. Synthesis of bilirubin.
D.Formation of hippuric acid.
E. Synthesis of uric acid.

Individual independent students work

1. Principles of regulation of glycogen biosynthesis and breakdown.
2. Hereditary disorders of synthesis and breakdown of glycogen and glycoconjugates.

Additional literature:

1. Lehninger A. Principles of Biochemistry. – New York. – W.H.Freeman and Company. – 2005. – 1010 p.

2. Mardashko O.O., Yasinenko N.Y. Biochemistry. Texts of lectures.-Odessa. The Odessa State Medical University, 2003.-416p.
3. Devlin T.M., ed. Textbook of Biochemistry with Clinical Correlations, 5th ed. New York: Wiley-Liss, 2002.
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6. Palmer M. "Human metabolism lecture notes" Ontario: Waterloo. – 2014. – 380 p.

Topic № 11. Studies of mechanisms of metabolic and hormonal regulation of carbohydrate metabolism. Diabetes mellitus.

Objective: Diabetes mellitus is a clinical condition characterized by increased blood glucose level (hyperglycemia) due to insufficient or inefficient insulin. In other words, insulin is either not produced in sufficient quantity or inefficient in its action on the target tissues. As a consequence, the blood glucose level is elevated which spills over into urine in diabetes mellitus. Determination of blood glucose level in clinical laboratory investigations is of great importance in diagnostics of diabetes mellitus and many other diseases and disorders.

Specific aims:

- To analyze the metabolic pathways of utilization of blood glucose
- To explain the role of hormones in maintenance of constant glucose level in blood
- To explain disorders in metabolism of carbohydrates in diabetes mellitus.

GUIDE FOR THE INDEPENDENT STUDY OF THE TOPIC:

	Questions:	References:
1.	**Principal carbohydrates of diet.** • Mechanisms of carbohydrates digestion in digestive tube. • Mechanisms of carbohydrate absorption.	9. Satyanarayana U., Chakrapani U. "Biochemistry", Third Edition. – 2006. – P. 244-245. 10. Biochemistry. The Molecular Bases of Life / Trudy McKee, James R. McKee, 2003. - P. 235. 11. Lecture notes.
2.	**Biochemical processes which provides the constant blood glucose level. Role of different pathways of carbohydrate metabolism in regulation of blood glucose level.** • Pathways of carbohydrate metabolism in regulation of blood glucose level.	11. Satyanarayana U., Chakrapani U. "Biochemistry", Third Edition. – 2006. – P. 674-676. 12. Biochemistry. The Molecular Bases of Life / Trudy McKee, James R. McKee, 2003. - P. 235. 13. Lecture notes.

	• Processes which provides the constant blood glucose level.	
3.	**Role of liver in carbohydrate metabolism.**	1. Satyanarayana U., Chakrapani U. "Biochemistry", Third Edition. – 2006. – P. 261-262. 2. Biochemistry. The Molecular Bases of Life / Trudy McKee, James R. McKee, 1996. - P. 252-256. 3. Lecture notes.
4.	**Hormonal regulation of carbohydrate metabolism:** • Insulin, its structure, mechanism of action, role in carbohydrate metabolism. • Adrenalin and glucagone, mechanism of their regulatory effects on carbohydrate metabolism. • Glucocorticoids, their effect on carbohydrate metabolism.	1. Satyanarayana U., Chakrapani U. "Biochemistry", Third Edition. – 2006. – P. 669-678. 2. Harper′s Biochemistry. 27th edition / R. K. Murray, Daryl K. Granner, Peter A. Mayes, Victor W. Rodwell, 2006.- P.173-175. 3. Biochemistry. The Molecular Bases of Life / Trudy McKee, James R. McKee, 2003. - P. 266-269. 4. Lecture notes.
5.	**Characterization of hypo- and hyperglycemia, glucosuria.** • Characterization of hypoglycemia • Characterization of hyperglycemia • Characterization of glucosuria.	1. Satyanarayana U., Chakrapani U. "Biochemistry", Third Edition. – 2006. – P. 678 - 679, 681. 2. Harper′s Biochemistry. 27th edition / R. K. Murray, Daryl K. Granner, Peter A. Mayes, Victor W. Rodwell, 2006.- P.173. 3. Lecture notes.
6.	**Insulin dependent and noninsulin dependent forms of diabetes mellitus.** • Insulin dependent forms of diabetes mellitus. Noninsulin dependent forms of diabetes mellitus.	1. Satyanarayana U., Chakrapani U. "Biochemistry", Third Edition. – 2006. – P. 679. 2. Lecture notes.
7.	**Characterization of metabolic disorders in diabetes mellitus**	1. Satyanarayana U., Chakrapani U. "Biochemistry", Third Edition. – 2006. – P. 678 - 679. 2. Lecture notes.
8.	**Biochemical tests for evaluation of conditions of patients with diabetes mellitus. Glucose tolerance test and its alteration in diabetes**	1. Satyanarayana U., Chakrapani U. "Biochemistry", Third Edition. – 2006. – P. 679 - 684. 2. Harper′s Biochemistry. 27th edition / R. K.

mellitus. Biochemical criteria of diabetes mellitus.

- Glucose tolerance test
- Biochemical criteria of diabetes mellitus.

Murray, Daryl K. Granner, Peter A. Mayes, Victor W. Rodwell, 2006.- P.175.
3. Lecture notes.

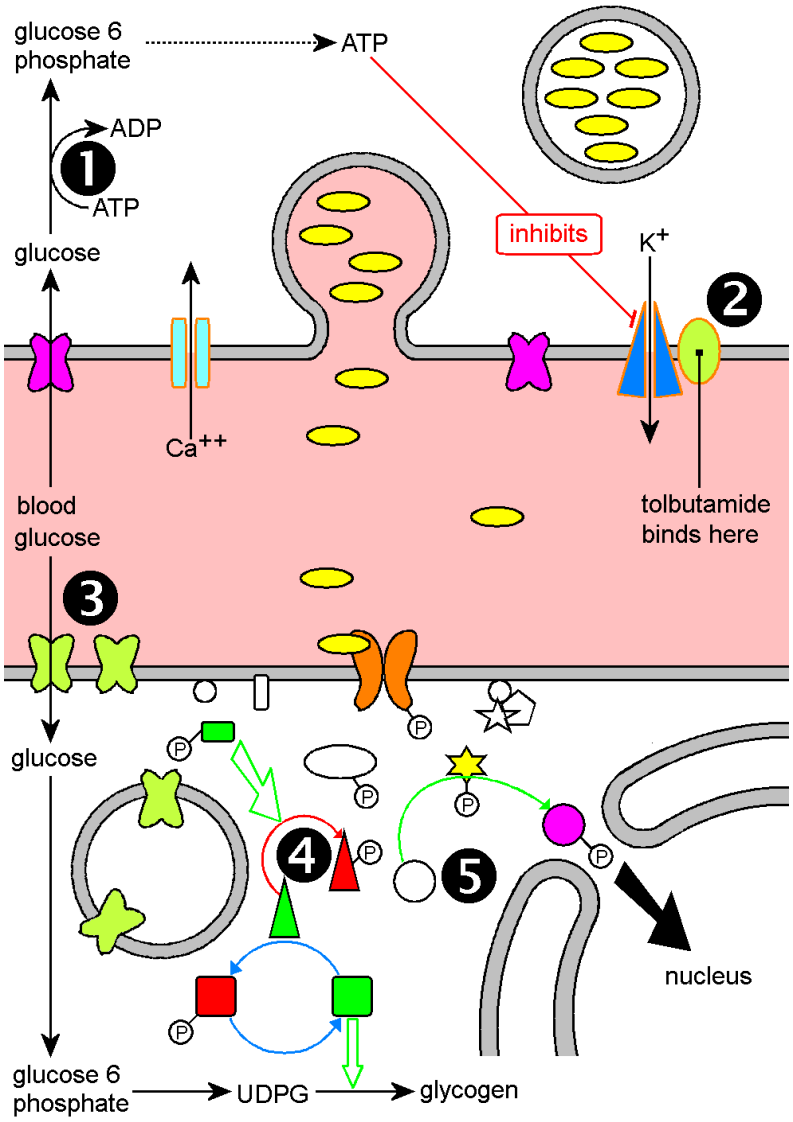
COMPREHENSION QUESTIONS:

1. Insulin plays a key role in the regulation of carbohydrate, lipid and protein metabolisms. Explain the net effect of insulin on the metabolic pathways represented above and effect on key enzymes.

№	Metaholism	Net effect	Effect on important enzyme(s)
1	Glycolysis	Increased	Glucokinase (↑) Phosphofructokinase (↑) Pyruvate kinaste (↑)
2	Gluconeogenesis		
3	Glycogenesis		
4	Glycogenolys		
5	HMPshunt		
6	Lipogenesis		
7	Lipolysis		
8	Ketogenesis		
9	Protein synthesis		
10	Protein degradation		

2. (a) Examine the figure below which depicts some of the systems that help to stabilize blood glucose concentration in humans. Select the most appropriate labels from the list provided that most accurately describe the signaling mechanisms. Each option may be used once, more than once or not at all.

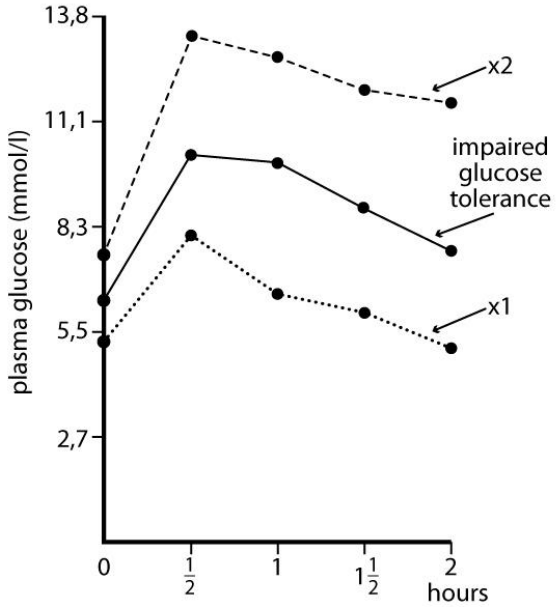
- *protein kinase A*
- *protein kinase B*
- *protein kinase C*
- *extracellular regulated kinase (ERK, MAPK)*
- *glucokinase*
- *GLUT2*
- *GLUT4*
- *glycogen phosphorylase*
- *glycogen synthase kinase*
- *heterotrimeric G protein*
- *hexokinase*
- *RAS*
- *sulphonyl urea receptor*



(b) Which component is which?

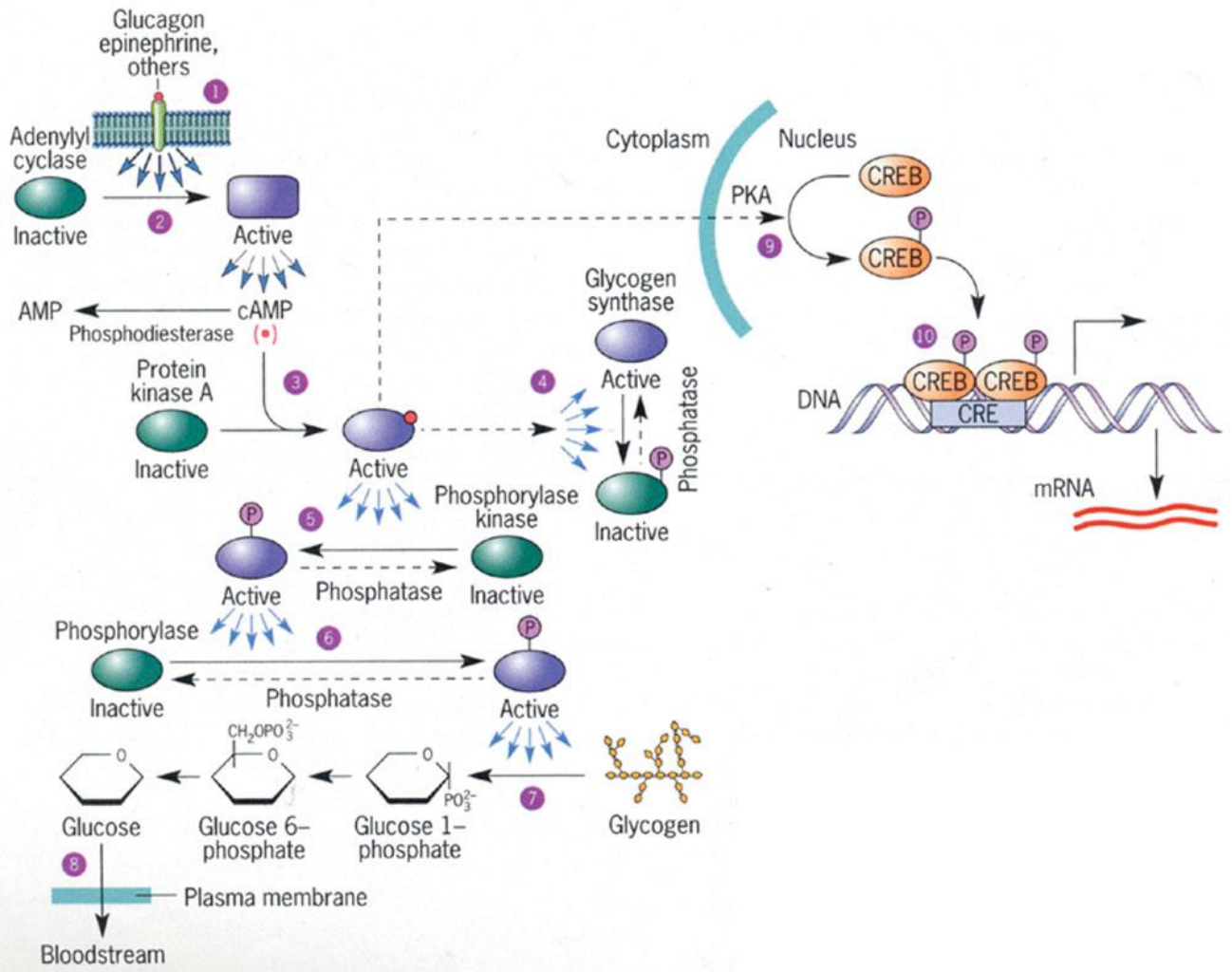
Cellular proteins:
1 –
2 –
3 –
4 –
5 –

3. Explain the results of glucose tolerance test for patient X1 and patient X2.



X1 –
X2 –

4. Second messenger mechanism is used by non lipid-soluble hormones, i.e protein hormones (eg. FSH, LH, insulin), as they cannot diffuse through the phospholipid bilayer by lipid/simple diffusion, hence their action upon target cells must be carried out practically indirectly. Characterize stages of mechanism of action of the hormones represented on a figure below.



EXAMPLES OF MULTICHOICE QUESTIONS

1. A 46-year-old woman complains of dryness in the oral cavity, thirst, frequent urination, general weakness. Biochemical research of the patient's blood showed hyperglycemia and hyperketonemia. Sugar and ketone bodies are revealed in the urine. Diffuse changes in myocardium are marked on the electrocardiogram. Make an assumptive diagnosis of the illness.

A. Diabetes insipidus.
B. Alimentary hyperglycemia.
C. Acute pancreatitis.
D. Diabetes mellitus.
E. Ischemic cardiomyopathy.

2. A patient was admitted to a hospital in comatose state. The accompanying mates explained that the patient loss his consciousness during the training on the last stage of marathon distance. What coma type can be recognized?

A. Hypoglycemic
B. Hyperglycemic

C. Hypovolemic
D. Hypothyroid
E. Hepatic

3. A patient addressed to physician with complaints on permanent thirst. In laboratory investigation it was revealed hyperglycemia, polyuria and increased content of 17-ketosteroids in urine. What disease is the most probable?

A. Steroid diabetes
B. Insulin dependent diabetes mellitus
C. Addison disease
D. Glycogenosis of the 1 type
E. Myxoedema

4. A 38-year-old man is receiving treatment for schizophrenia in hospital. Fhe initial levels of glucose, ketone bodies and urea in the blood are within the normal range. Shock therapy put into practice by regular insulin injections resulted in the development of the comatose state which improved the clinical status of the patient. What is the most probable cause of insulin coma?

A. Hypoglycemia.
B. Dehydratation of tissues.
C. Metabolic acidosis.
D. Ketonemia.
E. Hyperglycemia.

5. In patients' blood glucose level is over the renal threshold, polyuria is observed, as well as acidosis and ketonuria. What disease can be suggested?

A. Diabetes mellitus
B. Starvation
C. Hypercorticism
D. Addison disease
E. Hyperthyreosis

CLINICAL CASE

A 50-year-old women presents to your clinic with complaints of excessive thirst, fluid intake, and urination. She denies any urinary tract infection symptoms. She reports no medical problems, but has not seen a doctor in many years. On examination she is an obese female in no acute distress. Her physical exam is otherwise normal. The urinalysis revealed large glucose, and a serum random blood sugar level was 320 mg/dL.

- **What is the most likely diagnosis?**
- **What other organ systems can be involved with the disease?**
- **What is the biochemical basis of this disease?**

Additional literature:

1. Lehninger A. Principles of Biochemistry. – New York. – W.H.Freeman and Company. – 2005. – 1010 p.
2. Mardashko O.O., Yasinenko N.Y. Biochemistry. Texts of lectures.-Odessa. The Odessa State Medical University, 2003.-416p.
3. Devlin T.M., ed. Textbook of Biochemistry with Clinical Correlations, 5th ed. New York: Wiley-Liss, 2002.
4. Toy E.C., Seifert W. E., Strobel H.W., Harms K.P. "Case Files in Biochemistry. 2nd edition" – 2008. – 488 p.

5. MCQs / Prof. Sklyarov A.Ya., Lutsik M.D., Fomenko I.S., Klymyshin D.O., Nasadyuk C.M. – 2012. – 308 p.

Topic № 12. Catabolism and biosynthesis of triacylglycerols and phospholipids. Intracellular lipolysis and molecular mechanisms of its regulation.

Objective: to learn the processes of biosynthesis of phospholipids and triacylglycerols and the main pathways of intracellular metabolism of lipids. To learn the methods of determination of phospholipids concentration and activity of lipase and to interpret the obtained results.

Actuality of the theme: The knowledge of main pathways of intracellular metabolism of lipids under normal conditions and in pathology are necessary for medical students in further studies of general pathology, pharmacology and related clinical disciplines for correct interpretation of results of laboratory investigations and recognition of metabolic disorders in distinct cases.

Specific aims:

- To interpret biochemical function of simple and complex lipids in organism: their involvement in formation of structure and function of biological membranes, reserve and energetic significance, the role as precursors in biosynthesis of biologically active compounds of lipid nature.
- To explain the principal pathways of intracellular lipid metabolism.
- To explain enzymatic reactions of catabolism and biosynthesis of triacylglycerols.
- To interpret enzymatic reactions of synthesis of phospholipids and sphingolipids.
- To analyze the main pathways of lipid metabolism in human body in normal conditions and in pathology.
- To explain hormonal regulation of lipid metabolism.

GUIDE FOR THE INDEPENDENT STUDY OF THE TOPIC:

	Questions:	**References:**
1.	**Classification of lipids. Biological functions of simple and complex lipids in human body:** • reserve; • energetic; • thermoregulatory; • production of biologically active substances).	1. Satyanarayana U., Chakrapani U. "Biochemistry", Third Edition. – 2006. – P. 28-29. 2. Harper′s Biochemistry. 26th edition / R. K. Murray, Daryl K. Granner, Peter A. Mayes, Victor W. Rodwell, 2003.- P. 111-112. 3. Lecture notes.
2.	**Structure and role of fatty acids.** To write formulas of main saturated, unsaturated and polyunsaturated fatty acids.	1. Satyanarayana U., Chakrapani U. "Biochemistry", Third Edition. – 2006. – P. 29-31. 2. Harper′s Biochemistry. 26th edition / R. K. Murray, Daryl K. Granner, Peter A. Mayes, Victor W. Rodwell, 2003.- P.112-114.

		3. Lecture notes.
3.	**Triacylglycerols (TAG) their structure and properties.**	1. Satyanarayana U., Chakrapani U. "Biochemistry", Third Edition. – 2006. – P. 32 - 33. 2. Biochemistry. The Molecular Bases of Life / Trudy McKee, James R. McKee, 1996. - P. 114. 3. Lecture notes.
4.	**Structure and occurrence of main steroids**	1. Satyanarayana U., Chakrapani U. "Biochemistry", Third Edition. – 2006. – P. 37-38. 2. Harper′s Biochemistry. 26th edition / R. K. Murray, Daryl K. Granner, Peter A. Mayes, Victor W. Rodwell, 2003.- P. 117- 118.
5.	**Amphipathic lipids. Involvement of lipids in formation of structure and function of biological membranes. Liposomes.**	1. Satyanarayana U., Chakrapani U. "Biochemistry", Third Edition. – 2006. – P. 39 – 40. 2. Harper′s Biochemistry. 26th edition / R. K. Murray, Daryl K. Granner, Peter A. Mayes, Victor W. Rodwell, 2003.- P. 119- 120.
6.	**Digestion of lipids:** • digestion (action of lipases); • absorption (micelles); • re-synthesis and transport.	1. Lehninger A. Principles of Biochemistry. – New York. – W.H.Freeman and Company. – 2005. – P. 648-649. 2. Lecture notes.
7.	**Catabolism of triacylglycerols:** • characterization of intracellular lipolysis, its biological significance; • enzymatic reactions; • neurohumoral regulation of lipolysis: role of epinephrine, norepinephrine, glucagone, insulin; • energetic balance of triacylglycerol oxidation.	1. Satyanarayana U., Chakrapani U. "Biochemistry", Third Edition. – 2006. – P. 287. 2. Lehninger A. Principles of Biochemistry. – New York. – W.H.Freeman and Company. – 2005. – P. 649-650. 3. Lecture notes.
8.	**Biosynthesis of triacylglycerols and phospholipids, the significance of phosphatidic acid as a precursor.**	1. Satyanarayana U., Chakrapani U. "Biochemistry", Third Edition. – 2006. – P. 302-306. 2. Harper′s Biochemistry. 26th edition / R. K. Murray, Daryl K. Granner, Peter A. Mayes, Victor W. Rodwell, 2003.- P.

		199- 201. 3. Lecture notes.
9	**Metabolism of sphingolipids.** Genetic anomalies of sphingolipid metabolism – sphingolipidoses. Lysosomal diseases	1. Satyanarayana U., Chakrapani U. "Biochemistry", Third Edition. – 2006. – P. 307. 2. Harper′s Biochemistry. 26th edition / R. K. Murray, Daryl K. Granner, Peter A. Mayes, Victor W. Rodwell, 2003.- P. 201- 203. 3. Biochemistry: the molecular basis of life, third edidion/ Trudy McKee, James R. McKee , 2003.- P. 343-344.

COMPREHENSION QUESTIONS:

1. Complete the table (write the missed names or formulas of fatty acids):

	Common Name	Systematic name	Structure
1	Butyric acid	n-Butanoic acid	?
2	?	n-Hexa-decanaocicid	$CH_3(CH_2)_{14}COOH$
3	Steariac cid	?	$CH_3(CH_2)_{16}COOH$
4	Palmitoleic acid	cis9-Hexadeceancoid	?
5	?	cls-9-Octadecenaocicid	$CH_3(CH_2)_7CH=CH(CH_2)_7COOH$
6	Linoleic acid	cis, cis-9,12-Octadecadienoic acid	?
7	Arachidonic acid	All cls-5,8,11 Eicosatetranoic acid	?

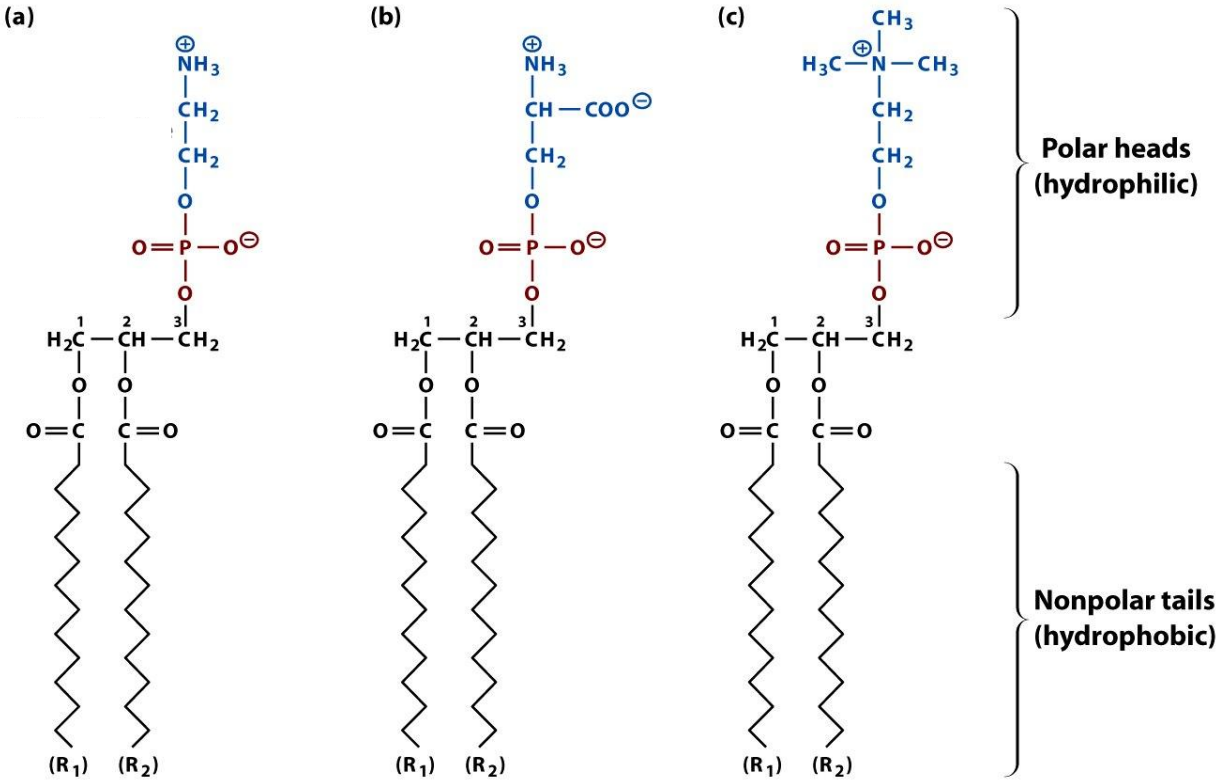
2. Triacylglycerols, with their hydrocarbon-like fatty acids, have the highest energy content of the major nutrients.

(a) If 15 % of the body mass of a 70 kg adult consists of triacylglycerols,what is the total available fuel reserve, in both kilojoules and kilocalories, in the form of triacylglycerols? Recall that 1.00 kcal : 4.18 kJ.

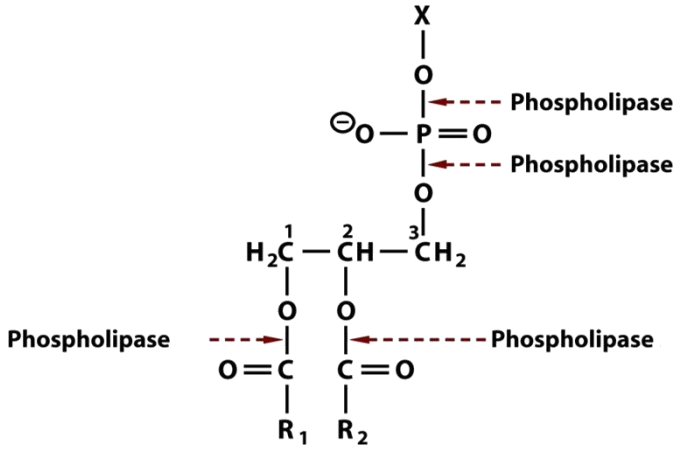
(b) If the basal energy requirement is approximately 8,400k J/day(2,000 kca/day), how long couldt his person survive if the oxidation of fatty acids stored as triacylglycerols were the only source of energy?

(c) What would be the weight loss in pounds per day under such starvation conditions(1 lb = 0.454kg)?

3. **Name phospholipids bellow (a, b, c):**



4. **Show the places for phospholipases A_1, A_2, C and D action. Which of them gives a lysophospholipid?**



5. **Complete the table (write the missed names, defective enzymes or symptoms of diseases):**

Disease	Missing/defective enzyme	Major storage compound	Symptoms
?	Sphingo-myelinase	Sphingomyelins	Liver and spleen enlargement, mental retardation

Farber's disease	?	Ceramide	Painful and progressively deformed joints, skin nodules, death within a few years
Gaucher's disease	β-Glucosidase	?	Liver and spleen enlargement, erosion of long bones, mental retardation in infantile form only
Krabbe's disease	β-Galactosidase	Galactocerebro-sides	?
?	Hexosaminidase A	Galactoside GM_2	Mental degradation, blidness, death by age 3
Fabry's disease	?	Ceramide Thihexoside	Skin rash, kidney failure, pain in lower extremities

EXAMPLES OF MULTICHOICE QUESTIONS

1. In patients suffering from diabetes mellitus an increase in a content of non esterified fatty acids (NEFA) in blood is observed. It may be caused by:

A. Increase in activity of triacylglycerol lipase
B. Stimulation of ketone bodies utilization
C. Activation of synthesis of apolipoproteins A_1 , A_2, A_3
D. Decrease in activity of phosphatidylcholine-cholesterol-acyltransferase in blood plasma
E. Accumulation in cytosol of palmitoyl-CoA

2. The essence of lipolysis, that is the mobilization of fatty acids from neutral fats depots, is an enzymatic process of hydrolysis of triacylglycerols to fatty acids and glycerol. Fatty acids that release during this process enter blood circulation and are transported as the components of:

A. Serum albumins..
B. Globulins.
C. HDL.
D. LDL
E. Chylomicrons.

3. Which one of the following statements about the absorption of lipids from the intestine is correct?

A. Dietary triacylglycerol is partially hydrolyzed and absorbed as free fatty acids and monoacyl glycerol
B Release of fatty acids from triacylglycerol in the intestine is inhibited by bile salts
C. Dietary triacylglycerol must be completely hydrotyzed to tree fatty acids and glycerol before absorption
D. Fatty acids that contain ten carbons or less are absorbed and enter the

circulation primarily via the lymphatic system

E. Formation of chylomicrons does not require protein synthesis in the intestinal mucosa.

4. After consumption of lipids in the body than begins their digestion and absorption in intestines. What products of lipid hydrolysis are absorbed in the intestine?

A. Monoacylglycerol, fatty acids
B. Amino acids
C. Polypeptides
D. Monosacharides
E. Lipoproteins

5. **Fabry's disease (one of sphingolipidoses) is an autosomal recessive disease. Major symptoms of this disease: skin rash, kidney failure, pain in lower extremities. It is caused by a deficiency of:**

A. α-Galactosidase A
B. Hexosaminidase A and B
C. G_{m1} Gangliosidase
D. Galactocerebrosidase
E. Ceraminase

CLINICAL CASES:

1. A Jewish couple of Eastern European descent presents to the clinic for prenatal counseling after their only child died early in childhood. The family could not remember the name of the disorder but said it was common in their ancestry. Their first child was normal at birth, a slightly larger than normal head circumference, an abnormal "eye finding," and a severe progressive neurologic disease with decreased motor skills and eventually death. The autopsy is consistent with Tay-Sachs disease.

- **What type of inheritance is this disorder?**
- **What is the biochemical cause of the disorder?**

2. A 9-year-old boy is brought to the ER by his parents after 2 days of worsening nausea/vomiting and abdominal pain. The abdominal pain is located in the epigastric region and radiates to his back. He has had several episodes of similar pain in the past but none quite as severe. His parents deny fever/chills and change in bowel habits. In the ER, the patient is afebrile and in moderate distress. Both the liver and spleen appear to be enlarged and he has epigastric tenderness. Several small yellow-white papules were noted on his back and buttocks. Laboratory tests reveal elevated amylase and lipase levels. On further questioning, the father reports having high triglyceride levels and several members of the mother's family have had early heart disease. Laboratory tests performed after hospitalization revealed elevated triglyceride levels and reduced lipoprotein lipase activity.

- **What is etiology of the boy's abdominal pain?**
- **What is the likely underlying biochemical disorder?**
- **What is the role of lipoprotein lipase?**

Additional literature:

1. Lehninger A. Principles of Biochemistry. – New York. – W.H.Freeman and Company. – 2005. – 1010 p.
2. Mardashko O.O., Yasinenko N.Y. Biochemistry. Texts of lectures.-Odessa. The Odessa State Medical University, 2003.-416p.

3. Devlin T.M., ed. Textbook of Biochemistry with Clinical Correlations, 5th ed. New York: Wiley-Liss, 2002.
4. Toy E.C., Seifert W. E., Strobel H.W., Harms K.P. "Case Files in Biochemistry. 2nd edition" – 2008. – 488 p.
5. MCQs / Prof. Sklyarov A.Ya., Lutsik M.D., Fomenko I.S., Klymyshin D.O., Nasadyuk C.M. – 2012. – 308 p.

Topic № 13. β –Oxidation and biosynthesis of fatty acids. Studies on metabolism of fatty acids and ketone bodies.

The aim of the lesson: To learn reactions of biosynthesis and oxidation of fatty acids. To know metabolic pathways of ketone bodies under normal conditions and in pathology and to determine their amount in urine.

Actuality of the theme: Oxidation of lipids, respectively fatty acids, as well as ketone bodies metabolism are important constituents of energetic metabolism in sense of providing tissues and cells with ATP. Determination of ketone bodies concentration in blood and in urine has important significance in diagnostics of several pathological processes.

Specific aims:

- To study reactions β -oxidation of long chain fatty acids.
- To interpret biosynthesis of long chain fatty acids and regulation of biosynthetic process on the level of acetyl-CoA-carboxylase and fatty acid synthetase.
- To analyze the metabolism of ketone bodies.
- To explain the mechanism of excessive accumulation of ketone bodies in diabetes mellitus and in starvation.

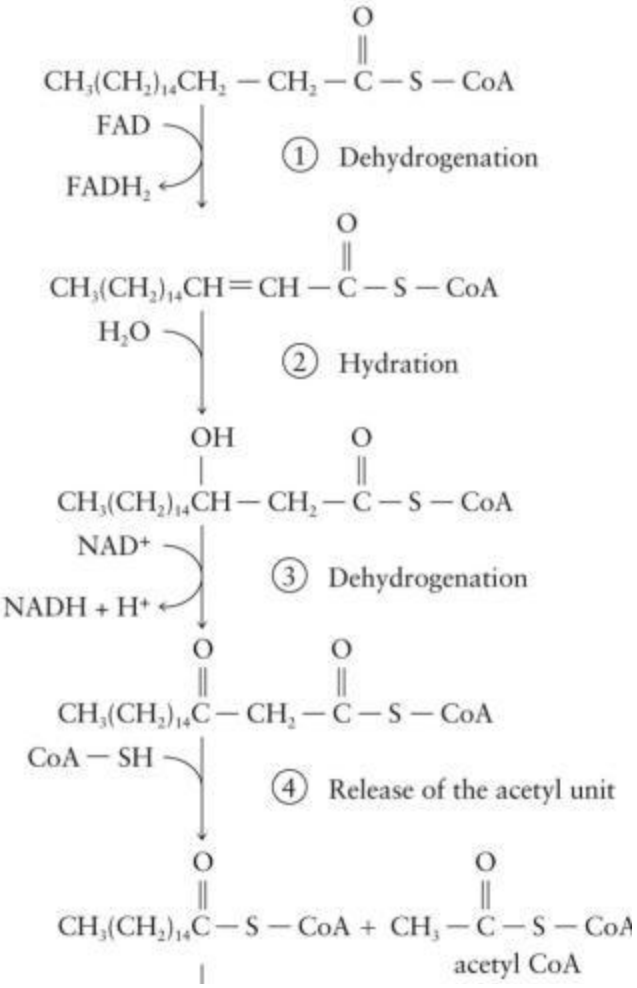
GUIDE FOR THE INDEPENDENT STUDY OF THE TOPIC:

	Questions:	References:
1.	**β –Oxidation of long chain fatty acids:** • localization of the process of β-oxidation of fatty acids; • activation of fatty acids, the role of carnitin in transport of fatty acids into mitochondria; • the sequence of enzymatic reactions in β –oxidation of fatty acids; • energetic balance of β – oxidation of fatty acids;	1. Satyanarayana U., Chakrapani U. "Biochemistry", Third Edition. – 2006. – P. 287-291. 2. Harper′s Biochemistry. 26th edition / R. K. Murray, Daryl K. Granner, Peter A. Mayes, Victor W. Rodwell, 2003.- P.180-187. 3. Biochemistry. The Molecular Bases of Life / Trudy McKee, James R. McKee, 2003. - P. 378-382. 4. Lecture notes.
2.	**Mechanism of glycerol oxidation:** • reactions; • bioenergetics.	1. Satyanarayana U., Chakrapani U. "Biochemistry", Third Edition. – 2006. – P. 287. 2. Lehninger A. Principles of Biochemistry. – New York. – W.H.Freeman and

		Company. – 2005. – P. 650. 3. Lecture notes.
3.	**Biosynthesis of long chain fatty acids:** • localization of biosynthesis of long chain fatty acids; • metabolic sources for biosynthesis of fatty acids; • stages in synthesis of saturated fatty acids; • characteristic of the synthetase of long chain fatty acids, the significance of acyl transporting protein and biotin; • sources of $NADPH_2$ for biosynthesis of long chain fatty acids; • the sequence of enzymatic reactions in biosynthesis of long chain fatty acids • regulation of biosynthetic process on level of acetyl-CoA-carboxylase and fatty acid synthetase; • elongation of carbon chain of fatty acids;	1. Satyanarayana U., Chakrapani U. "Biochemistry", Third Edition. – 2006. – P. 297-302. 2. Biochemistry. The Molecular Bases of Life / Trudy McKee, James R. McKee, 2003. - P. 387-394. 3. Lecture notes.
5.	**Metabolism of ketone bodies:** • enzymatic reactions of ketone bodies biosynthesis (ketogenesis); • reactions of ketone bodies utilization (ketolysis), energetic effect; • metabolism of ketone bodies in pathology. • mechanism of excessive accumulation of ketone bodies in diabetes mellitus and in starvation.	1. Satyanarayana U., Chakrapani U. "Biochemistry", Third Edition. – 2006. – P. 294-297. 2. Lehninger A. Principles of Biochemistry. – New York. – W.H.Freeman and Company. – 2005. – P. 666-667. 3. Biochemistry. The Molecular Bases of Life / Trudy McKee, James R. McKee, 2003. - P. 383-387. 4. Lecture notes.

COMPREHENSION QUESTIONS:

1. **Name enzymes of β–oxidation and explain net energetic effect of palmitoil-CoA oxidation:**

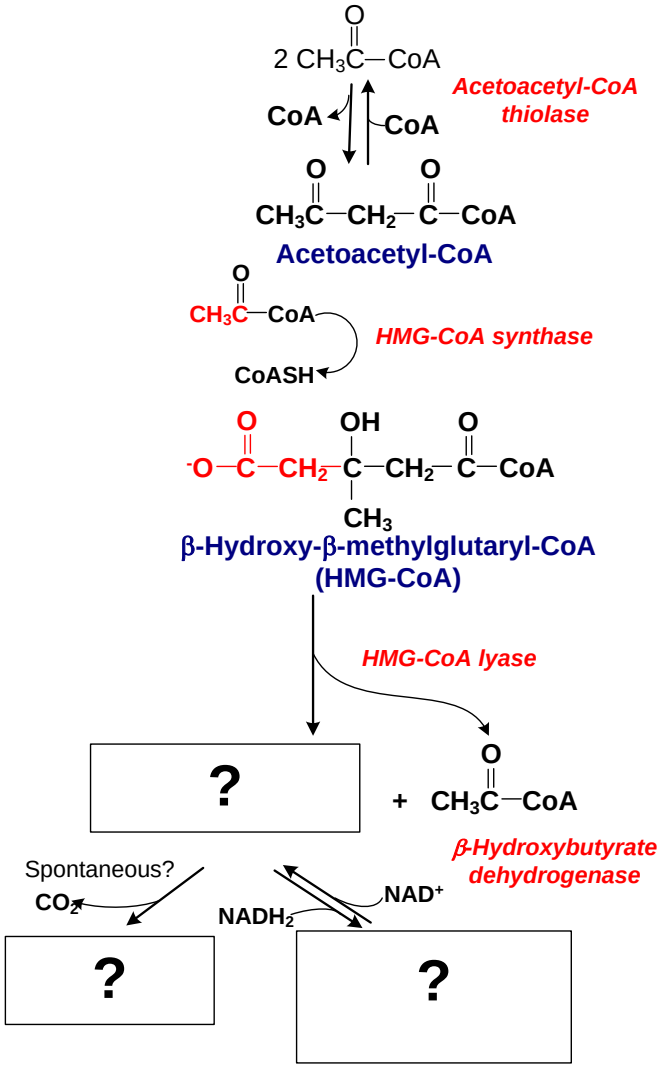


2. **How many cycles of β-oxidation are required for the complete oxidation of activated oleic acid, 18:1(Δ^9)?**

3. **An individual developed a condition characterized by progressive muscular weakness and aching muscle cramps. The ssymptoms were aggravated by fasting, exercise, and a high-fat diet. The homogenate of a skeletal muscle specimen from the patient oxidized added oleate more slowly than did control homogenates, consisting of muscle specimens from healthy individuals. When carnitine was added to the patient's muscle homogenate, the rate of oleate oxidation equaled that in the control homogenates, consisting of muscle specimens from healthy individuals. When carnitine was added to the patient's muscle homogenate, the rate of oleate oxidation equaled that in the control homogenates. The patient was diagnosed as having a carnitine deflciency.**

(a) Why did added carnitine increase the rate of oleate oxidation in the patient's muscle homogenate?
(b) Why were the patient's symptoms aggravated by fasting, exercise, and a high-fat diet?
(c) Suggest two possible reasons for the deficiency of muscle carnitine in this individual.

4. **Fill in the blanks:**



5. Determine the number of ATP that can be generated from the fatty acids in 1 mol of tristearin. (Tristearin is a triacylglycerol composed of glycerol esterified to three stearic acid moleculs)

EXAMPLES OF MULTI-CHOICE QUESTIONS:

1. A 1-year-old child was brought to a clinic with signs of muscle weakness. Through the inspection, the deficiency of carnitine in the muscles was determined. The biochemical mechanism of the development of this pathology consists in the disorder of the process of:

A. Transport of fatty acids into mitochondria.
B. Regulation of the level of Ca^{2+} in mitochondria.
C. Substrate level of phosphorylation.
D. Utilization of lactate.
E. Synthesis of actin and myosin.

2. Carnitine is recommended to a sportsman as a preparation that increases physical activity and improves achievements. What biochemical process is mostly activated under the action of carnitine?

A. Transport of fatty acids into mitochondria.
B. Ketone bodies synthesis.
C. Lipids synthesis.
D. Tissue respiration.
E. Steroid hormones synthesis.

3. Patients who suffer from severe diabetes and don't receive insulin have metabolic acidosis. This is caused by increased concentration of the following metabolites:

A. Ketone bodies
B. Fatty acids
C. Unsaturated fatty acids
D. Triacylglycerols
E. Cholesterol

4. In diabetes mellitus and starvation there is an increase of ketone bodies content in blood, which are utilized as energetic material by tissues. Note the substance which is used in ketone bodies synthesis.

A. Acetyl-CoA
B. Citrate
C. Succinyl-CoA
D. α–Ketoglutarate
E. Malate

5. Aerobic oxidation of substrates is typical of a cardiac muscle. Which of the following is the major oxidation substrate of a cardiac muscle?

A. Fatty acids
B. Triacylglycerols.
C. Glycerol.
D. Glucose
E. Amino acids.

Individual independent students work

1. Genetic defects in fatty acid-CoA dehydrogenases.

Additional literature:

1. Lehninger A. Principles of Biochemistry. – New York. – W.H.Freeman and Company. – 2005. – 1010 p.
2. Mardashko O.O., Yasinenko N.Y. Biochemistry. Texts of lectures.-Odessa. The Odessa State Medical University, 2003.-416p.
3. Devlin T.M., ed. Textbook of Biochemistry with Clinical Correlations, 5th ed. New York: Wiley-Liss, 2002.
4. MCQs / Prof. Sklyarov A.Ya., Lutsik M.D., Fomenko I.S., Klymyshin D.O., Nasadyuk C.M.. – 2012. – 308 p.
5. Palmer M. “Human metabolism lecture notes” Ontario: Waterloo. – 2014. – 380 p.

Topic № 14. Biosynthesis and biotransformation of cholesterol. Pathology of lipid metabolism: steatorrhea, atherosclerosis, obesity. Transport forms of lipids: lipoproteins of blood plasma.

Objective: To learn the pathways of cholesterol metabolism and principal disorders of lipid metabolism. To interpret free radical reactions, mechanisms of lipid peroxidation and its significance in biological processes in normal conditions and in pathology.

Actuality of the theme: Disorders in cholesterol biotransformation processes cause several diseases, such as atherosclerosis, obesity et al. In this connection the investigation of lipid metabolism indexes is obvious for diagnostics and treatment of different diseases.

Production of free radicals and products of peroxide oxidation of lipids are normal metabolic processes. In normal conditions the level of products of peroxide oxidation of lipids is regulated by antioxidative enzymatic system. Disorders in relations between activity of pro- and anti- oxidative systems is manifested in form of different pathological features. This makes necessary the investigation an evaluation of oxidative stress indexes in pathology.

Specific aims:

- To interpret stages of cholesterol biosynthesis.
- To explain regulation of cholesterol production in human body.
- To analyze pathways of cholesterol biotransformation: esterification, synthesis of bile acids, steroid hormones, vitamin D_3, excretion of cholesterol from the body.
- To interpret pathology of lipid metabolism: atherosclerosis, diabetes mellitus, obesity, steatorrhea.
- To explain processes of lipid peroxidation under normal conditions and in pathology.
- To interpret regulation of free radical reactions in human body.

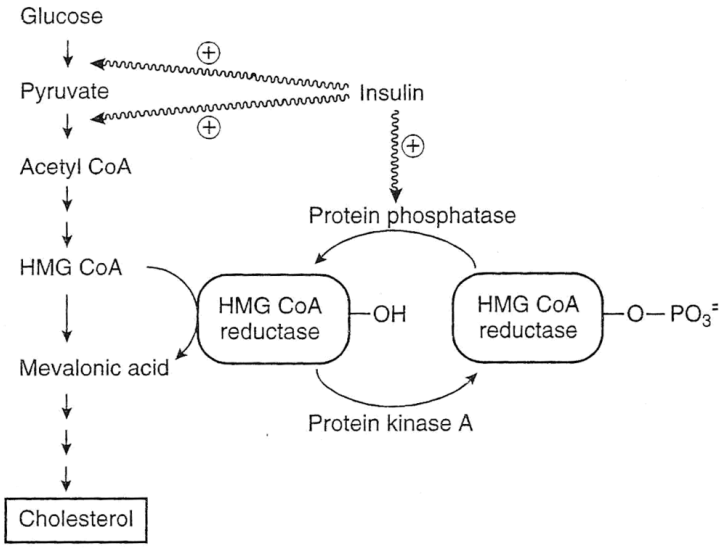
GUIDE FOR THE INDEPENDENT STUDY OF THE TOPIC:

	Questions:	References:
1.	**Biosynthesis of cholesterol in human body:** • localization of the process and its significance; • stages of cholesterol biosynthesis; • enzymatic reactions of biosynthesis of mevalonic acid; • regulation of cholesterol synthesis.	1. Satyanarayana U., Chakrapani U. "Biochemistry", Third Edition. – 2006. – P. 308-312. 2. Harper′s Biochemistry. 26th edition / R. K. Murray, Daryl K. Granner, Peter A. Mayes, Victor W. Rodwell, 2003.- P.219-224. 3. Lecture notes.
2.	**Pathways of cholesterol biotransformation** (esterification, production of bile acids and steroid hormones, synthesis of vitamin D_3, excretion from the body).	1. Satyanarayana U., Chakrapani U. "Biochemistry", Third Edition. – 2006. – P. 313-315. 2. Harper′s Biochemistry. 26th edition / R. K. Murray, Daryl K. Granner, Peter A. Mayes, Victor W. Rodwell, 2003.- P.225-

		227. 3. Lecture notes.
3.	**Atherosclerosis, mechanism of its development, role of genetic factors, hypercholesterolemia. Hypercolesterolemia in diabetes mellitus, myxoedema, obstructive jaundice, nephritic syndrome. Control of hypercholesterolemia**	1. Satyanarayana U., Chakrapani U. "Biochemistry", Third Edition. – 2006. – P.315-317, 326-327. 2. Lecture notes.
5.	**Lipoproteins: structure, classification, characteristics of apolipoproteins.**	1. Satyanarayana U., Chakrapani U. "Biochemistry", Third Edition. – 2006. – P. 317-318. 2. Lecture notes.
6.	**Metabolism of lipoproteins – a general view.**	1. Satyanarayana U., Chakrapani U. "Biochemistry", Third Edition. – 2006. – P.318-320. 2. Lecture notes.
7.	**Disorders of plasma lipoproteins** (classification of hyperlipoproteinemias, characteristics of hypolipoproteinemias.	1. Satyanarayana U., Chakrapani U. "Biochemistry", Third Edition. – 2006. – P.320-322. 2. Harper′s Biochemistry. 26th edition / R. K. Murray, Daryl K. Granner, Peter A. Mayes, Victor W. Rodwell, 2003.- P.227-229. 3. Lecture notes.
8.	**Fatty liver (steatosis), lipotropic factors.**	1. Satyanarayana U., Chakrapani U. "Biochemistry", Third Edition. – 2006. – P.322-325.
9.	**Pathological processes which leads to the development of obesity.**	1. Satyanarayana U., Chakrapani U. "Biochemistry", Third Edition. – 2006. – P.325-326.
10.	**Lipid peroxidation and mechanisms of antioxidant enzymatic system action:** • lipid peroxidation under normal conditions and in pathology. • regulation of free radical reactions in human body. • characterization of prooxidants and antioxidants, their significance in peroxide oxidation of lipids.	1. Satyanarayana U., Chakrapani U. "Biochemistry", Third Edition. – 2006. – P.656-657.

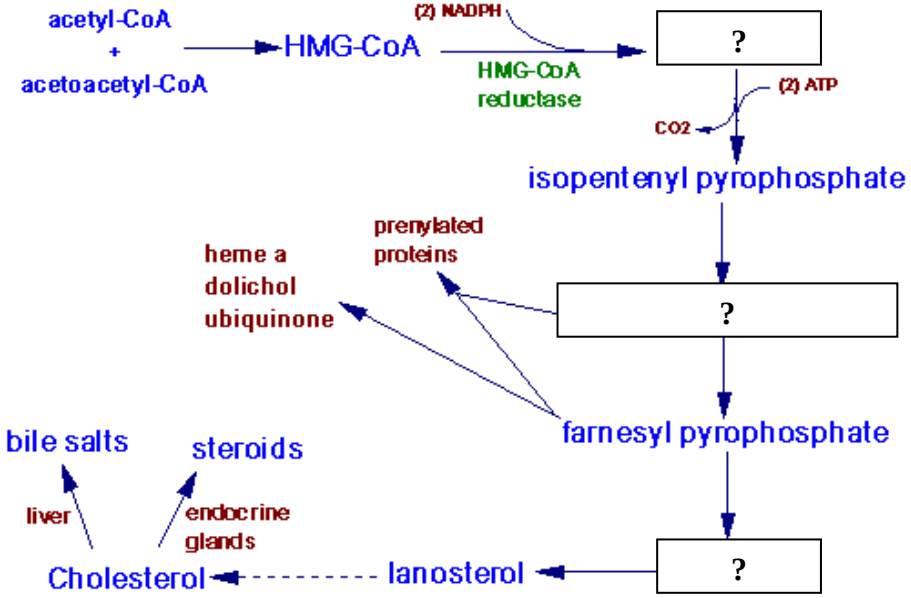
COMPREHENSION QUESTIONS:

1. **Explain the regulation of cholesterol biosynthesis on an example of HMG-CoA reductase regulation, shown bellow:**



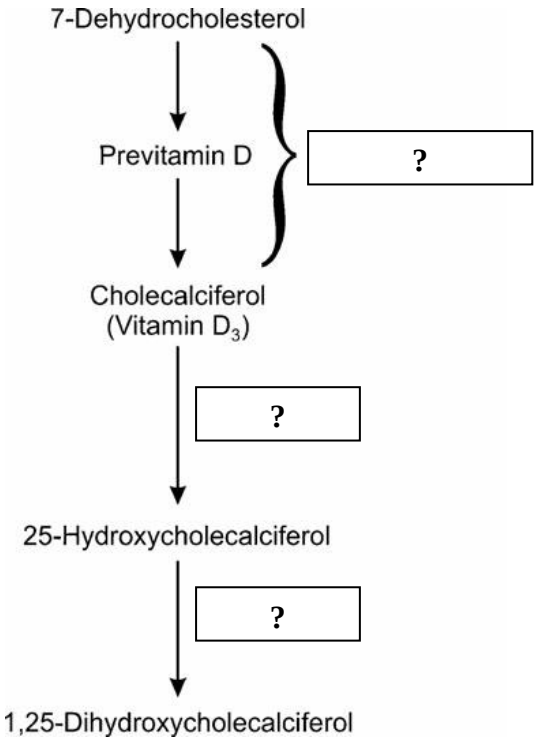
2. **A patient presents in your office with very high levels of serum cholesterol. He states that he has tried to follow the diet and exercise regimen you gave him last year. You decide that this patient would benefit from a drug such as Lipitor (atorvastatin). This class of drugs is effective in treating hypercholesterolemia because it has what effect?**

3. **Rite the missed names of cholesterol synthesis metabolites on the scheme bellow:**



4. **Although clinical trials have not yet been carried out to document benefits or side effects, some physicians have suggested that patient being trated with statins also take a supplement of coenzyme Q. Suggest a rational for this recommendation.**

5. **Indicate the location of stages of vitamin D synthesis from cholesterol.**



EXAMPLES OF MULTI-CHOICE QUESTIONS:

1. A patient suffers from arterial hypertension due to atherosclerotic injury of blood vessels. The consumption of what dietary lipid needs to be limited?

A. Cholesterol.
B. Oleic acid.
C. Lecithine.
D. Monooleateglycerol.
E. Phosphatidylserine.

2. Fats of phospholipids is disordered due to fat infiltration of the liver. Indicate which of the presented substances can enhance the process of methylation during phospholipids synthesis?

A. Methionine
B . Ascorbic acid
C. Glucose
D. Glycerin
E. Citrate

3. In a patient after investigation it was detected an increased content of low density lipoproteins in blood serum. What disease can be expected in this patient?

A. Atherosclerosis
B. Pneumonia
C. Gastritis
D. Acute pancreatitis
E. Kidney disease

4. A child 5 years old suffers from transient abdominal pains. Blood serum is turbid in fasting conditions. Cholesterol content – 4,3 mmoles/l, total lipids – 18 g/l. For precisement of diagnosis electrophoresis of blood lipoproteins is administered. What classes of lipoproteins are expected to be increased?

A. Chylomicrons
B. HDL
C. IDL
D. LDL
E. VLDL

5. In cases of complete or partial restriction of lipotropic factors in humans develops a fat degeneration of liver. What substances can be considered as lipotropic?

A. Choline
B. Pyridoxine
C. Fatty acids
D. Cholesterol
E. Triacylglycerols

CLINICAL CASES

1. A 48-year-old male presents to the clinic because of concerns about heart disease. He reports that his father died from a heart attack at age 46, and his older brother has also had a heart attack at age 46 but survived and is on medications for elevated cholesterol. The patient reports chest pain occasionally with ambulation around his house and is not able to climb stairs without significant chest pain and shortness of breath. The physical exam is normal, and the physician orders an electrocardiogram (ECG), exercise stress test, and blood work. The patient's cholesterol result comes back as 350 mg/dL (normal 200). The physician prescribes medication, which he states is directed at the ratelimiting step of cholesterol biosynthesis.

- **What is the rate-limiting step of cholesterol metabolism?**
- **What is the class of medication prescribed?**

2. A 49-year-old female presents to your clinic for follow-up after initiating a new medication (lovastatin) for her elevated cholesterol. She is currently without complaints and is feeling well. On repeat serum cholesterol screening, there is noted to be a decrease in the cholesterol level. The patient asks if she needs to continue the medication and what the potential side effects and benefits might be. Her physician explains that this medication inhibits the ratelimiting step and key enzyme in cholesterol biosynthesis.

- **What is the mechanism of action of this medication?**

Additional literature:

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3. Devlin T.M., ed. Textbook of Biochemistry with Clinical Correlations, 5th ed. New York: Wiley-Liss, 2002.
4. MCQs / Prof. Sklyarov A.Ya., Lutsik M.D., Fomenko I.S., Klymyshin D.O., Nasadyuk C.M. – 2012. – 308 p.
5. Palmer M. "Human metabolism lecture notes" Ontario: Waterloo. – 2014. – 380 p.

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