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# STRUCTURAL BIOCHEMISTRY

## Practical guide
## for Faculty of Dentistry students

**Student _____**

**Group nr. _____**

**Professor _____**

**CHISINAU, 2018**

**LESSON № 1** **Data _____**

## Introduction. The importance of biochemistry for medicine. Bioelements and biomolecules. Functional groups and types of chemical bonds specific for biomolecules

### The Initial level of knowledge

1. Chemical elements. Periodic table of chemical elements.

### Self-training questions Issues for training

1. Introduction to biochemistry. The importance of biochemistry for medical disciplines. Structural biochemistry and its goals.
2. Bioelements – organogenic and minerals. Content and overview of the main bioelements that make up the human body.
3. Biomolecules. Micromolecules, macromolecules and complex molecules.
4. Functional groups. Types of functional groups specific for biomolecules. Their general properties.
5. Types of chemical bonds specific for biomolecules. Their general properties.

### Tasks for individual work

1. Carbon in the unexcited state has the electronic configuration of the outer level (valence level) $2s^2 2p^2$ and in the compounds it can have the degree of oxidation +2, but in the excited state it has the electronic configuration $2s^1 2p^3$. How can schematically be shown the electron transition?

2. Draw the scheme of electronic interaction of ammonia with $H^+$ ion by donor-acceptor mechanism.

3. Place the molecule into the specific column of the table according to the chemical bond present in the molecule – $CO_2$, $H_2O$,HCl, $NH_4$, $O_2$, $N_2$, $Cl_2$, $CH_4$, NaCl, $NaH_2PO_4$, $Na_2CO_3$, protein, DNA.

| Covalent non-polar | Covalent polar | Ionic | Hydrogen |
|---|---|---|---|
| | | | |

**Self-assessment tests:**

**1. Which of the functional groups determines the acidic properties of biomolecules?**

a)–SH; b) $-NH_2$; c) $-CONH_2$ ; d) –COOH ; e) –OH

**2. Which of the functional groups determines basic properties of biomolecules?**

a) –SH; b) $-NH_2$ ; c) $-CONH_2$ ; d) –COOH ; e) –OH

**3. Which type of bond is produced between the atoms of the same kind or between the atoms with similar electronegativity?**

a) covalent polar; c) covalent non- polar; e) coordinative
b) hydrophobic; d) donor-acceptor;

**4. Which type of bond is produced between different atoms of non-metallic elements having different electronegativity?**

a) covalent polar; c) covalent non-polar; e) coordinative
b) ionic; d) donor-acceptor;

**5. What kind of chemical bond is formed as a result of ammonia interaction with hydrogen cations?**

a) covalent polar; c) covalent non-polar; e) hydrogen bond
b) ionic; d) donor-acceptor ;

**6. Select the correct statements regarding Van der Waals forces:**

a) relatively weak forces of attraction between neutral molecules;
b) relatively weak forces of rejection between neutral molecules;
c) forces of strong attraction between neutral molecules;
d) forces that stabilize the polar covalent bonds;
e) forces that stabilize the non-polar covalent bonds .

**7. Which pairs of atoms and functional groups will form ionic bonds?**

a) C and N; c) $COO^-$ and $NH_3^+$; e) $COO^-$ and $NH_2$.
b) $NH_2$ and $H^+$; d) $Cl^-$ and $Na^+$;

**8. Hydrogen bond is formed between:**

a) a partially positively charged hydrogen atom and partially negatively charged O, N or S
b) a partially negatively charged hydrogen atom and partially positively charged O, N or S
c) a positively charged hydrogen ion and partially negatively charged O, N or S
d) a partially positively charged hydrogen atom and partially negatively charged O, N or P
e) a positively charged hydrogen ion and negatively charged O or N.

**LESSON № 2 Data _____**

## Water structure, physical properties, ionization, ionic product and pH. Buffer solutions

### Experiment 1. Preparation of buffer solutions

**Task.** Prepare 20 ml of 0.1 mol/l acetic buffer with pH = 5.24 using 0.1 mol/l $CH_3COOH$ and 0.1 mol/l $CH_3COONa$ solutions. Dissociation constant of acetic acid is $3 \cdot 10^{-5}$.

**Background.** Buffer solutions can be prepared in two ways: 1) solutions of each components are prepared separately and after are mixed in one, 2.) one component of the buffer solution is dissolved in water and another component is added to the obtained solution. It is important to know the value of buffer solution $pK_a$ and to calculate solution pH using Henderson–Hasselbalch equation.

**Procedure**

1. Calculate how many milliliters of 0.1 mol/l CH3COONa and 0.1 mol/l CH3COOH are necessary to mix to obtain 20 ml 0.1 mol/l of acetic buffer with pH = 5.24.
2. Calculate the ratio of components using the equation $pH = pK_a + lg[salt]/[acid]$.
3. From the ratio of buffer mixture components results that it should contain X parts of salt and Y of acid, i.e. all 4 parts. So, the salt volume is equal to (20 • x) : 4, and acid volume is equal to (20 • y) : 4.
4. Check the pH of the prepared buffer solution using universal indicator paper strips or the pH-meter.
5. The report must contain the calculation of the ratio of the mixture buffer components.
6. Fill in the table:

| | |
|---|---|
| The ratio of the mixture buffer components | |
| Acid $V_a$ and salt $V_s$ values | |
| pH value | |
| **Conclusion** | |

**Experiment 2. Determination of blood serum buffer capacity.**

**Procedure.** 5 ml of blood serum with pH=7.4 are added to two test-tubes. In one test-tube are added 5 drops of phenolphthalein and solution is titrated with 0.1 mol/l NaOH till the appearance of the same color as color of reference solution that has pH = 9.4. In another test-tube methyl orange is added and solution is titrated with 0.1 mol/l HCl till the appearance of the same color as color of reference solution that has pH = 3.4.

Then the buffer capacity is calculated by acid and by base with equations (1) and (2).

$$B_{base} = \frac{n\,(NaOH)}{(pH_1 - pHs)\cdot Vs} = \frac{c(NaOH)\cdot V\,(NaOH)}{(pH_1 - pHs)\cdot Vs} \qquad (1)$$

$$B_{acid} = \frac{n(HCl)}{(pHs - pH_1)\cdot Vs} = \frac{c\,(HCl)\cdot V(HCl)}{(pHs - pH_1)\cdot Vs} \qquad (2)$$

where: $B_b$ – buffer capacity by base ;

$B_a$ – buffer capacity by acid;

n(HCl), n(NaOH) – quantity of added acid or base to 1 l buffer solution.

$pH_1$ and $pH_s$ – initial and final values of pH (till titration and after titration).

$V_s$ – the volume of blood serum;

V(NaOH), V(HCl) – the volumes of base and acid used for titration,

c(NaOH), c(HCl) – the concentration of base and acid consumed during titration.

In the conclusion compare the buffer capacity of blood serum by acid and by base and explain why the buffer capacity by acid is greater than the capacity by base.

**Results:**

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**Conclusion:**

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## Self-training questions

1. Theory of solutions.
2. Water – physical and chemical properties, role in the living organisms.
3. Electrolytic dissociation theory – basic concepts.
4. The main concepts of Bronsted-Lowry protolytic theory of acids and bases.
5. Water dissociation. The ionic product of water.
6. The notion of pH. Solution's pH and pOH methods of determination.
7. The buffer solutions. Principles of buffering. Henderson-Hasselbalch equation. Buffer capacity.
8. Body liquids pH level. Biologic buffer systems.

## Case study

1. Show the composition and the mechanism of action of the phosphate buffer system after addition of small amounts of strong acids or bases. The pH equation of phosphate buffer.

2. The content of hydrochloric acid ranges from 0.07 to 0.15% in normal gastric juice Calculate the range of pH change ignoring the interaction forces between the $H^+$ and $Cl^-$ ions.

3. To change the pH of 100 ml blood from 7.36 to 7.0 is necessary to add 36 ml 0.05 mol/l HCl sol. Calculate buffer capacity of the blood.

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**Self-assessment tests**

1. What abnormal physical properties of water are essential for a normal functioning of human organism?

a) low boiling point;
b) high boiling point;
c) big heat capacity;
d) low heat capacity;
e) high permittivity

2. Atoms of which elements in compounds can form hydrogen bonds with hydrogen?

a) carbon;
b) sulfur;
c) phosphorus;
d) oxygen;
e) nitrogen

3. What mixtures from the listed below are considered buffer systems?

a) sulfuric acid + sodium sulphate / $H_2SO_4 + Na_2SO_4$;
b) carbonic acid + sodium bicarbonate / $H_2CO_3 + NaHCO_3$;
c) acetic acid + sodium acetate / $CH_3COOH + CH_3COONa$;
d) acetic acid + ammonium acetate / $CH_3COOH + CH_3COONH_4$;
e) sodium dihydrogen phosphate + disodium hydrogen phosphate / $NaH_2PO_4 + Na_2HPO_4$

4. What factors affect buffer capacity?

a) the components of buffer system;
b) the strength of acids and bases;
c) the values of $pK_a$ and $pK_b$ of weak acids and bases;
d) the components concentration and their ratio;
e) the pH of buffer system.

5. The main blood buffer systems are:

a) hemoglobin – oxyhemoglobin;
b) ammonia buffer system;
c) hydrogen carbonate buffer system;
d) phosphate buffer system;
e) amino acid buffer system.

6. Select the correct statements considering the following two solutions: A. buffer with $H_2PO_4^-$ = 0,1 M, $HPO_4^{2-}$ = 0,2 M; B. buffer with $H_2PO_4^-$ = 0,15 M, $HPO_4^{2-}$ = 0,3 M.

a) solution A has a higher pH;
b) solution B has a higher pH;
c) both solution have the same pH;
d) solution A has a higher capacity;
e) solution B has a higher capacity.

7. Suppose we prepare a buffer with an acid HA, that has the pKa of 5. What is the pH when [A–]/[AH] = 10?

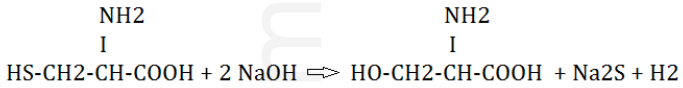
a) 1; b) 2; c) 3; d) 4; e) 5.

**LESSON № 3 Data _____**

**Amino acids – structure, classification and biomedical importance.**
**Primary structure of the proteins**

**Experiment №1.** Identification of amino acids that contained weak-bonded sulfur (Fol reaction)

**Method's principle:** Weakly bonded sulfur from proteins and peptides is eliminated by NaOH in the form of $Na_2S$, which interacts with $Na_2PbO_2$ to form insoluble PbS (dark brown or black precipitate).





**Note:** Fol reaction is negative for Met which contained stabile-bonded sulfur.

**Procedure:** Mix the reagents according to the table.

| № | Reagents | Test tube |
|---|---|---|
| 1 | Ovalbumine 1% | 5 drops |
| 2 | Fol reagent | 5 drops |
| ***Boil the solution for 1-2 min*** | | |

**Result:**_____
_____
**Conclusion:** _____
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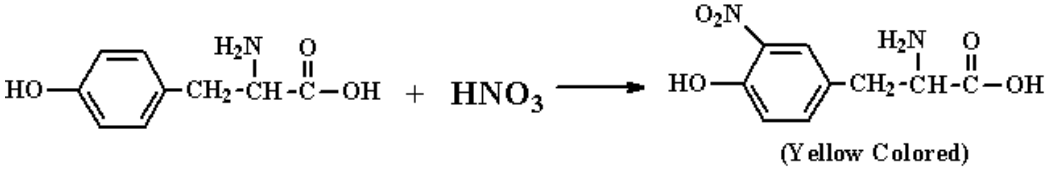
**Experiment № 2. Xantoproteic reaction**

**Method's principle:** Aromatic amino acids are nitrated while boiling with $HNO_3$ and the solution color terns yellow. Addition of alkali change the color in orange.




**Procedure:**

| № | Reagents | Test tube |
|---|---|---|
| 1 | Ovalbumine 1% | 5 drops |
| 2 | $HNO_3$ concentrated | 5 drops |
| Boil a few minutes | | |
| Cool the solution | | |
| 3 | NaOH 20% | 10 drops |

**Result:**_____

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**Conclusion:** _____

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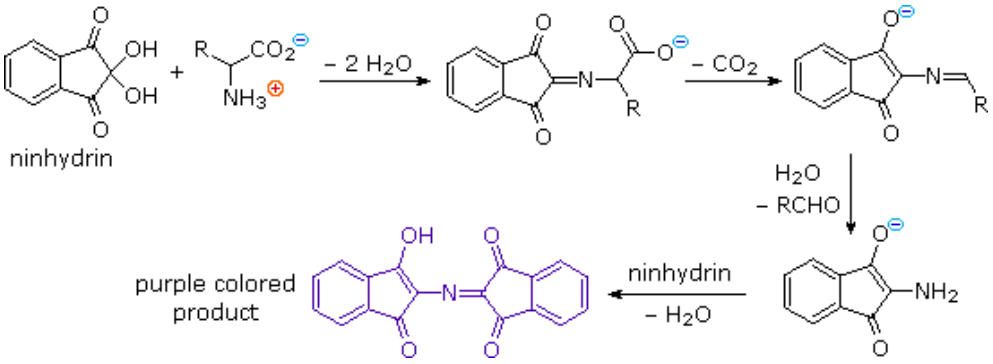
**Experiment №3. Ninhydrin reaction**

**Method's principle:** Ninhydrin reacts with α-amino groups of amino acids and proteins to form a blue-violet compound.




**Procedure:** Mix the reagents according to the table.

| № | Reagents | Test tube |
|---|---|---|
| 1 | Ovalbumine 1% | 5 drops |
| 2 | Ninhydrine 0,5% | 5 drops |
| Boil the solution for 1-2 min | | |

**Result:**_____

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**Conclusion:** _____

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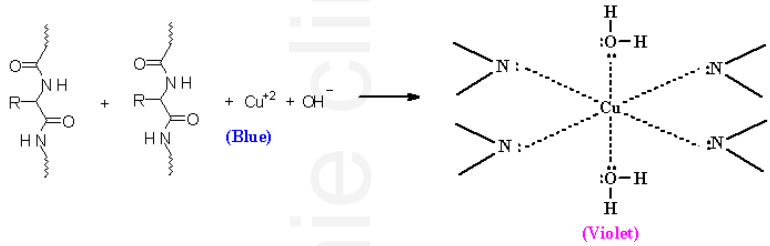
**Experiment №4. Biuret reaction**

**Method's principle:** Peptidic bonds react with $CuSO_4$ in alkaline conditions to form complex compounds colored in red-violet




**Procedure:**

| № | Reagents | Test tube |
|---|---|---|
| 1 | Ovalbumine 1% | 5 drops |
| 2 | NaOH 10% | 5 drops |
| 3 | $CuSO_4$ 1% | 2 drops |
| Shake thoroughly | | |

**Result:**_____

_____

**Conclusion:** _____

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**Self-training questions:**

1. Amino acids – role in the living organism. Proteinogenic and non-proteinogenic amino acids.
2. Classification of amino acids according to their chemical structure, physico-chemical and acid-base properties.
3. Amino acids properties – stereoisomery, solubility, acid-base properties.
4. Chemical properties of amino acids – reactions of carboxylation, decarboxylation, hydroxylation, deaminoation and transamination.
5. Polypeptide theory of the protein structure. Peptide bond properties. Name and reading the amino acids in peptides and proteins. N- and C- terminal amino acids.
6. Methods for determination of amino acid composition and sequence in the polypeptide chain.

**Case study:**

1. Divide the following amino acids in groups according to the biological classification: Thr, Cys, Phe, Gln, His, Met, Gly, Arg. Write a peptide that consists of essential amino acids. Give the definition of "essential amino acid", indicate the amino acids that belong to this group and the sources of these amino acids? In which proteins are all the essential amino acids present?

2. Divide the proteinogene amino acids accoding to their physico-chemical properties. Fill in the table.

| Hydrophobic amino acids | Hydrophilic neutral amino acids | Hydrophilic basic amino acids | Hydrophilic acid amino acids |
|---|---|---|---|
| | | | |

3. Write the structure and show the role of the following non-proteinogenic amino acids.

| Amino-acid | Structure | Role |
|---|---|---|
| Beta-alanine | | |
| Homocysteine | | |
| Gamma-aminobutiric acid | | |
| DOPA – dioxyphenylalanine | | |
| Ornithine | | |

4. To which electode the following amino acids will move to ain solution with acid, neutral and basic pH?

| Amino acid | pH < 7.0 | pH = 7.0 | pH > 7.0 |
|---|---|---|---|
| Alanine | | | |
| Serine | | | |
| Lysine | | | |
| Aspartic acid | | | |
| Glutamine | | | |

5.Write the reaction of glutamate side chain carboxylation in $Ca^{2+}$-binding proteins. What is the biologic importance of the reaction?

6. Write the reactions of histidine and glutamate decarboxylation. What is the biologic importance of these reactions?

5. Write the reactions of alanine and aspartic acid transamination with alpha-ketoglutarate. What is the biologic importance of the reaction?

6. Write the following tripeptides: Lys-Val-Pro; Glu-Pro-Arg; Pro-Asp-His. Highlight classic and atypic peptide bonds and show their differences.

**Tests for self assessment:**

**1. What groups of amino acids are present in proteins?**

a) hydroxy amino acids
b) homocyclic amino acids
c) beta-amino acids
d) diamino dicarboxylic amino acids
e) D-amino acids

**2. What cyclic structures are present in amino acids encountered in the proteins and to which amino-acid side chain they belong?**

| | |
|---|---|
| a) purine | phenylalanine |
| b) indole | triptophane |
| c) imidazole | histidine |
| d) pyrimidine | proline |
| e) skatole | tyrosine |

**3. Select the correct paris – amino acid – functional group specific for the side chain:**

| | |
|---|---|
| a) arginine | guanidino |
| b) alanine | thio |
| c) tyrosine | phenyl |
| d) cysteine | hydroxy |
| e) triptophane | indole |

**4. Select non-polar hydrophobic amino acids:**

a) Ser b) Val c) Ile d) Trp e) Glu.

**5. Select the basic amino acids:**

a) Ala b) Ser c) Tyr d) Gln e) Lys

**6. Select the correct statements regarding serine:**

a) is a hydroxy amino acid
b) its isoelectric point is in basic pH
c) is a "non-essential" amino acid
d) is an "essential" amino acid
e) in a solution with pH=4 migrates to the anode.

**7. Select the correct statements regarding arginine:**

a) at pH = 3 has a negative charge
b) its isoelectric pH (pI) is in alkaline media
c) in hydroxylated form is present in collagen structure
d) is an amino acid
e) has a guanidine group in its structure.

**8. Which statements are correct about methionine?**

a) is a derivative of butanoic acid
b) contains weak-bonded sulfur
c) at pH = 7 migrates to the cathode
d) in solutions with a pH less than pI it migrates to the anode;
e) is a non-essential amino acid.

**9. Which bond is specific for the primary structure of proteins?**

a) hydrogen
b) peptide
c) esther
d) ionic
e) disulphide

**LESSON № 4** **Data _____**

## Protein structure and function. Classification of proteins. General characteristic of simple and conjugated proteins

### Experiment 1. Chromatographycal identification of the amino acids

**Method's principle:** Amino acids have different distributive coefficient in water and in organic solvent (butanol). Amino acid's velocity of migration is directly proportional to their solubility in butanol.

**Procedure:**

1. Mark the take-off line on the chromatographycal paper with a pencil.
2. Pipette a drop of amino acids mixture in the middle of the starting line (the diameter of the spot must be less then 5 mm), dry the spot.
3. Introduce the chromatographycal strip in a vessel with the solvents mixture (water-butanol). The strip must be in vertical position and not touch the vessel.
4. Take the strip out of the vessel with the solvents after 90 min, mark the distance passed by the solvent (use only pencil) and dry the chromatogram (10 min at 70-100ºC).
5. Pass the strip through 0,1-0,2% ninhydrin solution and dry it at 100ºC. On the strip will develop several coloured spots. Each spot correspond to one amino acid.
6. Measure the following distances:
   a – from the take-off line to the middle of each spot;
   b – from the take-off line to the solvent's front.

**Calculation:** the distributive coefficients for each amino acid is calculated according to the next formula: $\mathbf{R_f = a/b.}$

The amino acids are identified according to the standard Rf table.

| **Standard Rf values** | | | | **Draw the obtained chromatogram and mark the take-off line, distances „a" and „b".** | **Measure the distances „a" and „b". Calculate Rf,** |
|---|---|---|---|---|---|
| **Amino acid** | **Rf** | **Amino acid** | **Rf** | | |
| Histidine | 0.11 | Cysteine | 0.40 | | |
| Glutamine | 0.13 | Proline | 0.43 | | |
| Lysine | 0.14 | Tyrosine | 0.45 | | |
| Arginine | 0.20 | Asparagine | 0.50 | | |
| Aspartic acid | 0.24 | Methionine | 0.55 | | |
| Glycine | 0.26 | Valine | 0.61 | | |
| Serine | 0.27 | Triptophane | 0.66 | | |
| Glutamic acid | 0.30 | Phenilalanine | 0.68 | | |
| Threonine | 0.35 | Isoleucine | 0.72 | | |
| Alanine | 0.38 | Leucine | 0.73 | | |

***Conclusion*: Which amino acids are present in the solution?**

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**Clinical significance:** This method allows to determine which amino acids and in what amount are present in different biological samples. Assays of different biological samples for amino acid's content and composition are indispensable in clinical diagnosis of numerous hereditary errors of metabolism, liver, kidneys diseases etc.

**Self-training questions:**

1. Protein levels of structural organization: primary, secondary, tertiary and quaternary structures, general description. Chemical bonds that stabilize each structural level. Basic notions about protein structural domains.
2. Proteins classification.
3. Simple proteins: albumins and histones – general characteristic, structural peculiarities. Biologic role.
4. Conjugated (complex) proteins: nucleo-, phospho-, lipo-, glyco-, chromo- and metalloproteins; their general characteristic.
5. Globular proteins: hemoglobin – structure and biologic role.
6. Fibrillar proteins: collagen and elastin – peculiarities of amino acids composition and structure. Biologic role.
7. $Ca^{2+}$-binding proteins: clotting factors, Ca2+-ATPase, calmodulin and collagen. Peculiarities of the amino acid composition that ensure calcium fixation. Biomedical role.

**Case study:**

1. What change of the hemoglobin structure is characteristic for HbS that causes sickle cell anemia? What repercussions has this change of the primary structure on the upper structural levels of the protein, its function and state of the erythrocytes?

2. Collagen, calmodulin, plasma coagulation factors II, VII, IX and X, etc. are $Ca^{2+}$-binding proteins. What is the common structural property of these proteins? What vitamin ensures this property? What are the sources of the vitamin? What are the roles of these proteins in the body?

Write the reaction in which is involved this vitamin.

3. Fill in the table:

| | | |
|---|---|---|
| This is the deffinition of | **The sequence of amino acids in the polypep-tide chain, that is genetically determined** | |
| This is the deffinition of | **Is formed due to the interaction of α-carboxyl group of one amino acid with α-amino group of the next amino acid** | |
| What structure has this property? | **Coplanarity** | |
| What chemical bond has this property? | **resonance formes** (keto or enol) | |
| Write the structure which will ilustrate the statement | **trans position** of the substituents in respect to the link C-N | |
| What chemical bond has | **the capacity to form hydrogen bonds** | ***Example:*** each peptide group can form two hydrogen bonds |

4. What are the similarities and differences of hemoglobin and myoglobin structure and function? What type of hypoxia develops in hemoglobin deficiency? What is the normal level of hemoglobin in the blood?

**Tests for self-assessment:**

1. **Select the correct statements about the secondary structure of the proteins:**
   a) is the arrangement into an ordered structure of the polypeptidic chain
   b) it is determined by hydrophobic and ionic interactions
   c) can be alpha-helix and beta-structure
   d) it is stabilized by hydrogen bonding
   e) it is stabilized by peptide bonds

2. **Select the correct statements about the secondary structure of the proteins:**
   a) occurs due to ionic bonding of the adjacent polypeptide chains
   b) has minimal and maximal periodicity
   c) can be both – alpha-helix and beta-structure
   d) appears due to hydrogen bond formation within a single chain and between adjacent chains
   e) it is stabilised also by disulfide bonds that are formed between Met radicals.

**3. Select the correct statements about the alfa-helix:**
   a) predominates in fibrillar protein's molecules
   b) possess helical symmetry
   c) radicals of amino acids are involved in the formation of thebalpha-helix
   d) the hydrogen bonds are formed between the groups –C=O and -NH that belong to the same polypeptide chain
   e) the hydrogen bonds are formed between the groups –C=O and -NH that belong to the different polypeptide chain

4. **Select the correct statements about the tertiary structure:**

a) functions are based on conformational changes
b) the domains are part of these structure
c) the domains determine special protein functions
d) it is possible the interaction between radicals AA-Cys-Cys
e) it is possible the interaction between radicals AA-Ser - Ser

**5. Select the pair of amino acids which side chains can form hydrogen bonds:**

a) Lys Leu
b) Phe Val
c) Asp Ala
d) Ser Cys
e) Asn Thr

**6. Select the pair of amino acids which side chains can form ionic bonds:**

a) Lys Glu
b) Trp Ile
c) Asp Arg
d) Gln Val
e) His Met

7. **Select the correct statements about the cuaternary structure of proteins:**

a) is the organization of subunits in a single functional protein molecule
b) is formed due to the non-covalent bonds between the contact surfaces of the domains
c) is rigid and stable
d) is formed due to covalent bonding
e) is not destroyed by denaturation

8. **Select the correct statements about the cuaternary structure of proteins:**

a) assembly of the molecule goes through the stage of intermediate compounds
b) is favored by hydrophobic interactions between the radicals of amino acids
c) functioning of proteins is correlated with the movement domeniior
d) is specific for hemoglobin
e) is specific for myoglobin

**9. Select the oligomers:**

a) hemoglobin (Hb)
b) myoglobin
c) LDH (lactate dehydrogenase)
d) immunoglobulins
e) creatine

**10. Select the correct statement about collagen:**

a) the polypeptide chain shows classical alpha helix conformation
b) contains a lot of cystein
c) is present only intracellular
d) the structural unit of collagen is tropocollagen
e) tropocollagen units are connected by weak, non-covalent bonds in collagen fibers

**LESSON № 5** **Data _____**

## Physico-chemical properties of proteins. The purification and analysis of proteins

### Experiment №1: Dialysis

**Method's principle:** Dialysis (from Greek διάλυσις, *diàlysis*, "dissolution"; from διά, *dià*, "*through*", and λύσις, *lỳsis*, "loosening or splitting") is a method of separation on the basis of molecular size. Small molecules can be removed from solutions because they pass through semi permeable membrane. Proteins are larger than the pores of the membrane and don't cross it.

**Procedure:**

1. Mix in a flask 20 ml sol of ovalbumine and 20 drops of saturated solution of $(NH_4)_2SO_4$.
2. Put the solution in a cellophane bag and immersed it in a glass full of distillated water.
3. After 60 min pull out the bag and transfer the solution into a test-tube. Identify protein and $(NH_4)_2SO_4$ in both solutions.
4. The presence of proteins is determined by biuretic reaction (see Theme nr. 1).
5. The presence of $(NH_4)_2SO_4$ is determined with $BaCl_2$. To 5 drops of experimental solution add 3-4 drops of 5% sol. $BaCl_2$. Formation of insoluble $BaSO_4$ certified the presence of $SO_4^{2-}$.

$$\mathbf{BaCl_2 + (NH_4)_2SO_4 \rightarrow BaSO_4\downarrow + 2\ NH_4Cl}$$

6. Fill in the table:

| Solution | Compunds present befor dialysis | DIALYSIS | Biuretic reaction | Reaction with $BaCl_2$ | What compound – the protein or $SO_4^{2-}$, is present in the solutionn? |
|---|---|---|---|---|---|
| from the bag | Protein (ovalbumine) $(NH_4)_2SO_4$ | | | | |
| from the glass | $H_2O$ | | | | |

***Clinical value:*** In medical practice dialysis is a method used to remove excess water, other normal compunds and wastes from the blood of the patients whose kidneys have lost their functions. Dailysis can be temporary in persons with acute kidey failure or who are waiting for transplant and chronic if the transplant is not indicated or possible.

***Conclusion*:** _____
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**Self-training questions:**

1. Molecular mass of the proteins. General notions about the most important methods for determining protein mass – ultracentrifugation, chromatography and mass spectroscopy.
2. Amphoteric properties of the protein. The electric charge of the protein. Factors that determine the electric charge of the protein. Isoelectric point and state. Electrophoresis – principle and biomedical use. Electrophoresis of blood plasma proteins.
3. The solubility of the proteins according to the conformation of the molecule and the amino acid composition, solution pH and temperture. Colloidal solutions of proteins. Factors that stabilize the protein colloidal solution. States of the colloidal solutions: sol, gel, xerogel.
4. Denaturation of proteins, agents causing denaturation. Structural changes in denaturated proteins. Biomedical role.
5. Methods of protein separation, purification and analysis: salting, dialysis, electrophoresis and chromatography (ion exchange chromatography, size exclusion chromatography/gel filtration chromatography and affinity chromatography). Method's principle and biomedical importance.

**Case study:**

1. Write tripeptides with the isoelectic point (pI) in acidic, neutral and basic pH.

2. Divide into groups according to their solubility the following proteins: albumin, hemoglobin, keratin, transferrin, IgM, fibrin, prothrombin, collagen. What factors influence the solubility of these proteins?

3. Albumins and histones are dissolved in a solution. Select buffer solution that should be used for precipitation of each protein: Sol. 1, pH=4.0; Sol. 2 pH=7.0; Sol. 3 pH=11.0. What is the mechanism of separation of individual proteins from the mixture by this method?

4. A solution containing a mixture of albumins and globulins is treated with ammonium sulfate ($[NH_4]_2SO_4$) to semisaturation, and then to saturation. What effect will have this treatment on the solubility of albumins and globulins? What is the mechanism of action of $[NH_4]_2SO_4$ on the proteins from the solution?

5. Which electrode albumins and histones will move to at pH=7,0, if the starting point is in the middle between anode and cathode? Explain.

6. What method of those listed below can be used to purify fluids (serum, plasma, lymph) of harmful micro molecular substances: denaturation, hydrolysis, electrophoresis, dialysis, affinity chromatography? Describe the principle of the method and its clinical usefulness.

7. It is necessary to separate and purify certain enzymes from animal or plant sources for the production of enzyme drugs. Which of the listed below methods is the most quick and efficient one for the separation and purification of enzymes: denaturation, hydrolysis, electrophoresis, dialysis, affinity chromatography? Describe the principle of the method.

8. What character – hydrophilic or hydrophobic, has the fragment -Gly-Ser-Asn-Trp-Tyr- from the primary structure of a protein? Where is located these sequence in the 3D structure of the protein – on the surfice of the molecule or inside? Explain. Write the structure of the sequence.

**Tests for self assessment:**

1. **Select the pH range that correspond to the isoelectric point of the peptide –Arg-His-Lys-Ala:**
   a) 1,5 – 3,0 b) 3,0 – 4,5 c) 4,5 – 6,0 d) 6,0 – 7,5 e)7,5 – 9,0

2. **Select the correct statements about the protein that contains 10% Arg, 26% Lys, 13% Val, 35% Pro, 8% Ala, 8% Gly, at physiologic pH:**
   a) moves to the anode
   b) moves to the cathode
   c) has a positive net charge
   d) has a negative net charge
   e) does not have charge

3. **Select the correct statement about protein solubility:**
   a) fibrillar proteins are well soluble in pure water
   b) depends on the electric charge and aqueous membrane
   c) depends on the type of the solvent and its temperature
   d) fibrillar proteins are better soluble then globular one
   e) is maximum at the isoelectric point

4. **Select the factors that ensure the stability of the protein in the solution:**
   a) the aqueous membrane (MA), which is formed due to the hydration of the hydrophilic functional groups
   b) the electric charge, that depends on the pH of the solution
   c) electric charge, that depends on the hydrophobic radicals of amino acids
   d) the aqueous membrane (MA), which is formed due to the hydration of hydrophobic functional groups
   e) the electrical charge of the "N"- and "C"-terminal amino acids

5. **What can determine the precipitation of proteins?**
   a) aqueous membrane damage
   b) neutralization of the electric charge
   c) bringing the basic protein to the isoelectric state by the addition of acid
   d) bringing the acid protein to the isoelectric state by the addition of base
   e) aqueous membrane damage by removing fixed water

6. **Select the correct statements about salting:**
   a) is the hydration of the protein molecule
   b) is the dehydration of the protein molecule
   c) is an irreversible process
   d) is fenomenon of micromolecules passing through the semipermeable membrane
   e) destroys the tertiary structure of the protein

7. **What statements about the denatured protein molecule are correct?**
   a) primary structure is destroyed
   b) the biological activity is increased
   c) tertiary and quaternary structures are destroyed
   d) the peptide bonds are destoyed
   e) does not change the native state of the molecule

**8. What properties are specific for protein colloidal solution?**

a) increased capacity of diffusion
b) increased viscosity
c) low diffusion capacity
d) optical properties
e) reduced viscosity.

**LESSON № 6** **Data _____**

## Test on Chapter I – Chemistry of proteins

1. Bioelements – organogenic and minerals. Content and overview of the main bioelements that make up the human body. Biomolecules.
2. Functional groups. Types of functional groups specific for biomolecules. Their general feature.
3. Types of chemical bonds specific for biomolecules. Their general feature.
4. Water – physical and chemical properties, role in the living organisms.
5. Electrolytic dissociation theory – basic concepts.
6. The main concepts of Bronsted-Lowry protolytic theory of acids and bases.
7. Water dissociation. The ionic product of water.
8. The notion of pH. Solution's pH and pOH methods of determination.
9. The buffer solutions. Principles of buffering. Henderson-Hasselbalch equation. Buffer capacity.
10. Body liquids pH level. Biologic buffer systems (bicarbonate, phosphate).
11. Amino acids – role in the living organisms. Amino acids properties – stereoisomery, solubility, acid-base properties.
12. Classification of amino acids according to their chemical structure, physico-chemical and acid-base properties.
13. Chemical properties of amino acids – reactions of carboxylation, decarboxylation, hydroxylation and transamination.
14. Polypeptide theory of the protein structure. Peptide bond properties. Name and reading the amino acids in peptides and proteins. N- and C- terminal amino acids.
15. Methods for determination of amino acid composition and sequence in the polypeptide chain.
16. Levels of structural organization of the protein molecule. The primary structure of the protein. Inherited modifications of the primary structure ( sickle cell anemia). Protein secondary structure: types, bonds that stabilize secondary structure .
17. Levels of structural organization of the protein molecule . The tertiary structure of the protein. Types of intramolecular bonds in the protein. Quaternary structure of the protein. Cooperative changes of protomers conformation (on the example of hemoglobin and myoglobin ). Term " domain " .
18. Globular and fibrillar proteins (hemoglobin, collagen) - peculiarities of conformational and physico-chemical properties .
19. Calcium-binding proteins - collagen, calmodulin, blood clotting factors . Peculiarities of amino acid composition resulting calcium fixation . The role of these proteins in the body.
20. The biological role of proteins. Albumin, globulins, histones – their features and biological role. Methods for determination and separation of plasma proteins.

21. Classes of conjugated proteins. General features of chromoproteins, metaloproteins, nucleoproteins, phosphoproteins, glycoproteins and lipoproteins, their biological role, examples.
22. Physico-chemical properties of proteins. The solubility of the proteins. Factors that influence solubility. Colloidal solutions of proteins, their properties, stabilizing factors . Salting out. Dialysis of proteins.
23. Electro-chemical properties of proteins. Factors determining the charge of the proteins. Isoelectric state and point. Protein electrophoresis.
24. Protein denaturation and renaturation. Denaturation factors.

**LESSON №7** **Data _____**

**Nucleic acids – classification, structure and role.**
**Nitrogenous bases, nucleosides and nucleotides – structure and nomenclature**

**Experiment 1: Molisch's reaction** (named after Austrian botanist Hans Molisch)
**Method's principle:** is based on the dehydration of the ribose by sulfuric acid to produce an aldehyde (furfural), which condenses with two molecules of thymol, resulting in a red- or purple-colored compound.
**Procedure:**

| № | Reagents | Test tube |
|---|---|---|
| 1. | Yeast hydrolyzate | 10 drops |
| 2. | Thymol sol. 1% | 3 drops |
| Thoroughly shake the solution. | | |
| Pour the $H_2SO_4$ solution onto the test tube wall. | | |
| 3. | $H_2SO_4$ concentr. | 20-30 drops |

**Rezult:** _____
_____
**Conclusion:** _____
_____

**Experiment 2: Molybdenum reaction (phosphoric acid identification)**
**Method's principle:** When phosphoric acid is treated with molybdenum reagent a yellow sediment is produced.
**Procedure:**

| № | Reagents | Test tube |
|---|---|---|
| 1. | Yeast hydrolyzate | 10 drops |
| 2. | Molybdenum reagent | 20 drops |
| Boil the solution. | | |
| Cool it in flowing water | | |

**Rezult:** _____
_____
**Conclusion:** _____
_____

**Self-training questions:**

1. Types of nucleic acids, functions and cell location.
2. Chemical composition of nucleic acids: nitrogenous bases, pentoses and phosphate.
3. Nucleosides and nucleotides – structure and functions.
4. Primary structure of DNA. Polynucleotide chain. Phosphodiester bonds.
5. Secondary and tertiary structure of DNA. DNA double helix – Watson-Crick model (types B, A and Z). Levels of compaction of DNA molecule in prokaryotes (nucleoid) and eukaryotes (nucleosomes and solenoid).
6. RNA primary, secondary and tertiary structures. Peculiarities of tRNA, mRNA and rRNA structure.

**Case study:**

1. Write the formulas of the following nucleotides 5´-thymidyl acid, 5´-uridyl acid, 5´-adenyl acid and 5´-deoxyadenyl acid. Which of these nucleotides belong to DNA and RNA? Show the glycosidic and esther bonds.

2. Write in your notebook the chemical structure of the polynucleotide sequence dT-dC-dG-dA. Which nucleic acid this sequence belongs to? What will be the electric charge of

given nucleic acid at physiological pH? What is the role of this electrical charge for nucleosome formation?

3. Write in your notebook the chemical structure of the polynucleotide sequence G-A-U-C. Which nucleic acid this sequence belongs to? Show the bonds specific for this structure. Is it possible hydrogen bonds formation in RNA? Draw and explain in which cases this is possible.

4. Show the complementary interaction of cytosine with the corresponding nitrogenous base.

5. Show the structure of the DNA fragment that is transcribed if known that RNA contains the anticodon UGC.

6. Which is the sequence of the anticodone of the tRNA that carry the amino acid encoded by the codone UCG from mRNA. Which pyrimidine base is complementar to guanine? Show how hydrogen bonds between these nitrogenous bases are formed.

7. Represent the structural formula of a DNA fragment that contains two pairs of complementary nitrogenous bases.

8. Write the structure of ATP, ADP and AMP. What are ATP functions?

**Tests for self-assessment:**

**1. Select the structural elements of DNA:**

a) dehydrouracyl
b) deoxyribose
c) phosphate
d) thymine
e) ribosylthymine

**2. Select types of chemical bonds that are present in DNA:**

a) peptidic
b) 3',5'-phosphodiester
c) N-glycosidic
d) ionic interactions
e) hydrogen bonds

**3. Select the correct statements regarding the structure of DNA:**

a) is double stranded
b) strands are parallel
c) nitrogenous bases are located inside the double helix
d) strands are linked between them by phosphodiester bonds
e) strands can not be separated at high temperature

**4. Select correct statements about DNA:**

a) classic „B" form of DNA contains 10 base pairs per turn
b) „V" form of DNA contains 11 base pairs per turn
c) „Z" form contains 12 base pairs per turn and is left-handed spiral
d) for all DNA is specific the ratio G+C/A+T=1
e) mainly is located in the cytosol

**5. Select correct statements about nucleosome:**

a) histone proteins are organized in octamers - double set of H4,H3,H2A,H2B;
b) octamere is wrapped by double DNA ring length of 146 nucleotides;
c) are located in mitochondria
d) is a form of RNA supra organization
e) are molecules of extra chromosomal DNA

**6. Select the correct statements regarding RNA:**

a) the molecule is mainly double-stranded
b) nucleotide composition correspond to complemetatity law
c) the RNA quantity in the cell is constant
d) it is a single-stranded polyribonucleotide
e) the specific nucleotides are ATP, GTP, TTP and CTP

**7. Select the correct statements regarding mRNA:**

a) are very heterogenous molecules
b) each gene has a correcponding mRNA molecule
c) are synthesized in the cytoplasm
d) contains methylated guanine at the 5´-end
c) it is constantly attached to the ribosomes

**8. Select the correct statements regarding tRNA:**

a) the 5´-end contains the CCA triplet
b) contains many minor nitrogenous bases
c) the amino acid is attached to the 3´-end
d) contains 75-90 nucleotides
e) 3´-end contains the anticodone

**9. Select the correct statements regarding tRNA:**

a) are molecules with similar structure and shape
b) the secondary structure of all tRNA has the shape of the clover leaf
c) all molecules are free in the cell
d) are structural elements of the ribosomes
e) are completely double-stranded molecules

**LESSON № 8** **Data _____**

## Carbohydrates: classification and biological role. Monosacchirides – structure, isomerism, properties and biomedical importance

**Experiment № 1:** Glucose identification by Trommer reaction.
**Method's principle:** When glucose is treated with $Cu(OH)_2$ in alkaline solution, a brick-red precipitate of $Cu_2O$ is formed.

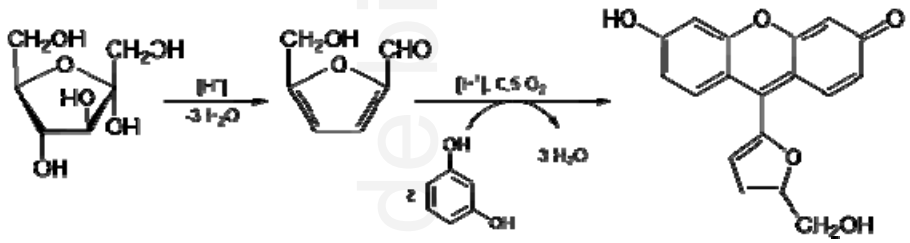
$$CuSO_4 + 2NaOH \rightarrow Na_2SO_4 + Cu(OH)_2 \text{ blue}$$
$$\text{Glucose} + 2Cu(OH)_2 \rightarrow \text{gluconic acid} + H_2O + 2CuOH \text{ yellow}$$
$$2CuOH \rightarrow H_2O + Cu_2O \text{ brick-red}$$

**Procedure:**

| № | Reagents | Test-tube |
|---|---|---|
| 1 | glucose | 2 drops |
| 2 | 10% NaOH | 6 drops |
| 3 | 2% $CuSO_4$ | 1 drop |
| 4 | $H_2O$ | 3-4 drops |
| 5 | The colour of the solution | |
| 6 | Heat carefull the mixture | |
| 7 | The colour of the solution | |

**Rezult:** _____
_____
**Conclusion:** _____
_____

**Experiment № 2: Fructose identification by Seliwanoff's test**
**Method's principle:** Seliwanoff's test is a test which separates aldose and ketose sugars. Keto sugars are dehydrated by concentrated acids (HCl) to yield furfurals or their subsidiaries which react with resorcinol in Seliwanoff reagent to yield a cherry-red complex. When added to aldoses, a slower forming pink color is seen.



**Procedure:**

| № | Reagents | Test-tubes | |
|---|---|---|---|
| | | I | II |
| 1 | Resorcin pouder | 1-2 grains | 1-2 grains |
| 2 | HCl concentr. | 2 drops | 2 drops |
| 3 | Fructose 0.5% | 2 drops | 2 drops |
| 4 | Glucose 0.5% | 2 drops | 2 drops |
| 5 | Boil few minutes | | |
| 6 | The colour of the solution | | |

**Rezult:** _____

_____

**Conclusion:** _____

_____

**Self-training questions:**

1. The biological role of carbohydrates.
2. Classification and structure of the carbohydrates.
3. Structure and properties of the main monosaccharides (glyceraldehyde, dihydroxyacetone, ribose, deoxyribose, glucose, galactose, fructose).
4. Stereoisomerism of monosaccharides: enantiomers, D- and L- stereoisomers, diastereolsomers and epimers.
5. Linear and cyclic forms of the monosaccharides. Closed ring structure of 5 or more carbon atoms (pyranose and furanose rings) monosaccharides. Haworth projections. The role and properties of the hemiacetal hydroxyl, notions of α- and β-anomers.
6. Important chemical reactions of monosaccharides (formation of phosphoric esters, N- and O-glycosides, oxidation and reduction).
7. Ascorbic acid (vitamin C) structure, synthesis and role.
8. Aminated carbohydrates: glucosamine, galactosamine and sialic acid – structure, formation and biologic role.

**Case study:**

1. Which are the similarities and differences between glyceraldehyde and dihydroxyacetone?

| | Glyceraldehyde | Dihydroxyacetone |
|---|---|---|
| Similarities | | |
| Differences | | |

2. Write the structure of the following compounds and show the difference in their structures:

| D-glucose | L-glucose | Type of isomerism |
|---|---|---|
| | | |

| α-D-glucopyranose | β-D-glucopyranose | Type of isomerism |
|---|---|---|
| | | |
| α-D-glucopyranose | α-D-galactopyranose | Type of isomerism |
| | | |

3. Write the structure of the D-glucose, D-galactose, D-ribose and D-fructose anomers.

4. Write the reaction of glucose-6-phosphate formation in the organism.

5. Write the reactions of D-glucose and D-galactose oxidation till gluconic and glucuronic acids.

6. Ascorbic acid (vitamin C) is synthesized from D-glucose and is the γ-lactone of the 2-oxo-L-gulonic acid. Write the scheme of vit. C synthesis from D-glucose. For which organisms is this synthetic pathway specific? What is the biologic role of the vitamin?

**Tests for self-assessment:**

**1. Functions of carbohydrates**

a) energetic function
b) to maintain oncotic pressure
c) are emulsifiers
d) are constituents of connective tissue and nucleic acids
e) transport

**2. Choose carbohydrates that are present in the human body :**

a) amylose
b) glucose
c) glycogen
d) ribose, deoxyribose
e) amylopectin

**3. Select the correct statements about glucose:**

a) is a polysaccharide
b) is a ketose
c) is an aldohexose
d) has 2 anomers – alpha and beta
e) does not have asymmetric carbons

**4. Select the correct statements about fructose:**

a) is an aldopentose
b) is an aldohexose
c) is a ketopentose
d) is a glucose isomer
e) is the main monosacchride from the blood

**5. Select the metabolic active form of glucose:**

a) glucosamine
b) acetyl-glucose
c) phosphoric esther
d) methyl glucose
e) glucose sulfate

**6. Which statement is characterizing the monosaccharides?**

a) all monosaccharides can produce intramolecular hemiacetals
b) are polyhydroxycarbonyl compounds
c) are classified in essential and non-essential
d) all monosaccharides have anomers
e) can be hydrolysed

**7. Choose the correct statements regarding the stereoisomerism of monosaccharides:**

a) enantiomers do not possess optical properties
b) diastereoisomers differ in the configuration of all chiral carbon atoms
c) epimers are diastereoisomers characterized by the configuration of one asymmetric atom
d) D-mannose is the epimer of D-glucose at the second carbon atom
e) open forms of monosaccharides exist in the form of α- and β-anomers

**8. Indicate the process (2) during which these derivatives of carbohydrates are formed (1) – draw arrows:**

a) sorbitol
b) glucuronic and iduronic acids
c) mannitol
d) glucose-6-phosphate
e) deoxyribose

oxidation of glucose
reduction of glucose and fructose
reduction of ribose
reduction of mannose and fructose
esterification of glucose

**9. Select the structural elements of the neuraminic acid:**

a) acetic acid
b) pyruvic acid
c) galactosamine
d) glucuronic acid
e) mannosamine

**LESSON № 9** **Data _____**

## Olygo and polysaccharides – structure, properties and biomedical importance

**Experiment №1: Demonstration of the reducing properties of carbohydrates (Fehling reaction)**

**Method's principle:** When monosaccharides are treated with $Cu(OH)_2$ in alkaline medium, a brick-red precipitate of $Cu_2O$ is formed:

$$CuSO_4 + 2NaOH \rightarrow Na_2SO_4 + Cu(OH)_2 \text{ blue}$$

$$\text{Glucose} + 2Cu(OH)_2 \rightarrow \text{gluconic acid} + H_2O + 2CuOH \text{ yellow}$$

$$2CuOH \rightarrow H_2O + Cu_2O \text{ brick-red}$$

**Procedure:**

| № | Reagents | Test-tubes | | | | |
|---|---|---|---|---|---|---|
| | | I | II | III | IV | V |
| 1 | 4-5 drops of | **glucose** | **Fructose** | **succrose** | **starch** | **pathologi-cal urine** |
| 2 | Fehling solution | 4-5 drops | 4-5 drops | 4-5 drops | 4-5 drops | 4-5 drops |
| 3 | Boil the mixtures | | | | | |
| 4 | The colour of the solution | | | | | |

**Conclusion:** _____

_____

### Self-training questions:

1. Classification and structure of the olygosaccharides – reducing and non-reducing disaccharides (maltose, lactose and sucrose). Biomedical role.
2. Classification, structure, properties and biomedical role of the polysaccharides:
   a) **homo**polysaccharides (glycogen, starch, cellulose)
   b) **hetero**polysaccharides (hyaluronic acid, chondroitin-sulfate and heparin).

### Case study:

1. Sucrose does not have two anomer forms. Why?

2. What is the only difference in the structure of starch and cellulose? How this difference reflects the properties of the corresponding polysaccharides?

3. Ruminants use cellulose as food, but most mammals can not use it. Explain why. Write the structure of the structural unit of cellulose.

4. Trehalose – the non-reducing disaccharide that consist of 2 molecules of α-D-glucose, is a structural element of the toxin produced by many microorganisms. Write the structure of this disaccharide.

5. Which heteropolysaccharide consists of β-D-glucuronic acid and N-acetyl- β-D-glucosamine linked by a β (1→3) glycosidic bond? Write its structure and explain its biomedical role.

**Tests for self-assessment:**

1. **Select the structural element of maltose:**
   a) alpha-glucose c) alpha-galactose e) alfa-ribose
   b) beta-glucose d) beta-fructose

2. **Select the structural elements of sucrose:**
   a) alpha-glucose c) alpha-galactose e) alfa-ribose
   b) beta-glucose d) beta-fructose

3. **Select the structural elements of lactose:**
   a) alpha-glucose c) beta-galactose e) beta-ribose
   b) beta-glucose d) alfa-fructose

4. **Select the carbohydrates that contain alpha-glucose:**
   a) glycogen c) cellulose e) sucrose
   b) starch d) lactose

5. **Select the carbohydrates that contain beta-glucose:**
   a) glycogen c) cellulose e) sucrose
   b) starch d) lactose

6. **Select the correct statements about homoglycans:**
   a) structural unit of cellulose is maltose
   b) cellulose is a polysaccharide that predominates in plants
   c) starch is composed of alpha-glucose
   d) glycogen is composed of beta-glucose
   e) α-1,6-glycosidic bonds predominate in the structure of cellulose.

7. **Select the correct statements regarding glycogen:**
   a) is deposited in scheletal muscles
   b) is deposited in adipose tissue
   c) is deposited in the liver
   d) is used to maintain the normal level of blood glucose
   e) it is not produced in the human cells

8. **Select the chemical bonds that are specific for starch (amylose+amylopectin) and glycogen:**
   a) α (1→4) c) α (1→6) e) non of above
   b) β (1→4) d) β (1→6)

9. **Select the correct statements about heteroglycans:**
   a) main forms are amylase and amylopectin
   b) hyaluronic acid consists of D-glucuronic acid and N-acetylglucosamine
   c) in hyaluronic acid the monomers are linked by β-(1→4) and β-(1→3) glycosidic bonds
   d) there are 6 forms of chondoritin sulfate
   e) are important structural elements of the connective tissue

**10. Heparin - select the correct statement regarding the compound:**

a) is a mineral component of the blood plasma
b) is a protein
c) is a direct anticoagulant
d) is a clotting factor
e) is a fibrinolytic factor

**LESSON № 10 Data _____**

## Water-soluble vitamins: $B_1$, $B_2$, B6, PP, biotin, pantothenic, folic and ascorbic acids and $B_{12}$ – structure and coenzyme function

**Experiment №1: Vitamin C (ascorbic acid) level assay in the urine**

**Method's principle:** Vitamin C has the property of reducing 2,6-dichlorophenol-indophenol (2,6-DCPIP), which leads to the change in the color of the solution**.**

ascorbic acid + oxidized 2,6-DCPIP → dihydroascorbic acid + reduced 2,6-DCPIP
(blue color) (pink color)

**Procedure:**

| Nr. | Reagents | Test-tube |
|---|---|---|
| 1 | Urine | 10 ml |
| 2 | $H_2O$ dist. | 10 ml |
| 3 | 10% HCl sol. | 20 drops |
| 4 | Initial color of the solution | |
| 5 | Titrate with 0,001N 2,6-DCPIP solution | |
| 6 | Final color of the solution | |

**Calculation:** The amount of vitamin C in the urine is determined by the formula:

C (mg / 24ore) = (0.088 • A • C) / B

where: 0,088 - conversion factor;

A is the amount of 2,6-DCPIP used to titrate the sample;
B - the amount of urine used in the experiment (10 ml);
C - daily diuresis (on average - 1500 ml in men and 1200 ml in women).

**Clinical significance:** Humans cannot make vitamin C (ascorbic acid or ascorbate) and must obtain it through the food or supplements. Vitamin C disappears from the urine early in blood or tissue depletion. Plasma levels fall next and tissue levels (such as in leukocytes and platelets) are the last to fall. Vitamin C levels in the body of 1500 mgs or less will result in no urinary excretion of vitamin C. However, certain medications such as aspirin, aminopyrine, barbiturates, hydantoins and paraldehyde as well as cold or heat stress are known to increase the excretion of vitamin C in the urine.

**Rezult:** _____
_____
**Conclusion:** _____
_____

**Self-training questions:**

1. Classification and biomedical role of vitamins.
2. Water-soluble vitamins $B_1$, $B_2$, B6, PP, biotin, pantothenic, folic and ascorbic acids and $B_{12}$:
    a) structure;
    b) coenzymes derivatives of these vitamins;
    c) metabolic function of the coenzymes derivatives of these vitamins;
    d) recommended daily allowances (RDAs) and food sources;
    e) hypo- and hypervitaminoses – causes, metabolic disorders and clinical signs.

**Case study:**

1. Fill in the table as in the example:

| **Vitamin** | **Name** | **Coenzyme** | **Biologic role** | **Hypovitaminosis Main clinical signs** |
|---|---|---|---|---|
| **$B_1$** | Thiamine | Thiamin pyrophosphate (TPP) | Coenzyme of the enzymes that catalyze the oxidative decarboxylation of the α-keto acids | Beriberi, mental depression, mental confusion, peripheral neuropathy, ataxia, loss of eye coordination |
| **$B_2$** | | | | |
| **$B_6$** | | | | |
| **PP ($B_3$)** | | | | |
| **H** | | | | |
| **$B_5$** | | | | |
| **$B_9$** | | | | |

| | | | | |
|---|---|---|---|---|
| **$B_{12}$** | | | | |
| **C** | | | | |

**Tests for self-assessment:**

**1. Select the functions of the vitamins:**

a) energetic
b) structural
c) transport
d) coenzyme
e) genetic

**2. Select the chemical compounds that can be coenzymes:**

a) nucleotides
b) proteins
c) hem
d) peptides
e) triglycerides

**3. Select the coenzymes that contain adenosine monophosphates:**

a) pyridoxal phosphate
b) FMN
c) FAD
d) $NAD^+$
e) thyamine pyrophosphate

**4. Select the coenzymes of the dehydrogenases:**

a) pyridoxal phosphate
b) pyridoxamine phosphate
c) thyamine pyrophosphate
d) FAD
e) $NAD^+$

**5. Select the coenzymes involved in the transfer of the amino group:**

a) folic acid
b) FAD
c) $NAD^+$
d) pantothenic acid
e) pyridoxal phosphate

**6. Select the coenzymes involved in the transfer of the one carbon groups:**

a) folic acid
b) FAD
c) $NAD^+$
d) pantothenic acid
e) pyridoxal phosphate

**7. Select the reactions in which can be involved biotin:**

a) carboxylation
b) decarboxylation
c) transcarboxylation
d) tranamination
e) dehydrogenation

**8. Select the correct statemetns regarding Vit. $B_{12}$:**

a) contains cobalt ion
b) participate in protein synthesis reactions
c) participate in the oxidation of fatty acids with odd number of carbons
d) participate in the oxidation of valine, isoleucine, methionine, and threonine
e) is predominantly present in food of plant origin

**LESSON № 11 Data _____**

**Concluding test on Chapters „Nucleic Acids structure and function", „Carbohydrates structure, properties and functions" and „Water soluble vitamins"**

1. Types and functions of nucleic acids.
2. Structure of nitrogenous bases, nucleosides, nucleotides and cyclic nucleotides. Chemical bonds specific for the nucleotides. Biological role of nucleotides.
3. Structure of DNA – double helix. Watson-Crick model. Types B, A and Z of double helix.
4. Levels of DNA molecule compaction in prokaryotes (nucleoid) and eukaryotes (nucleosomes, chromatin and chromosomes).
5. General characteristics and biological role of carbohydrates.
6. Classification and functions of carbohydrates.
7. Structure of carbohydrates:
   a) monosaccharides (glyceraldehyde, dihydroxyacetone, ribose, deoxyribose, glucose, galactose, mannose, fructose);
   b) disaccharides (maltose, lactose, sucrose);
   c) homopolysaccharides (glycogen, starch, cellulose);
   d) heteropolysaccharides (hyaluronic acid, chondroitin sulfate and heparin).
9. Stereoisomerism of monosaccharides: enantiomers, D- and L- stereoisomers, diastereolsomers and epimers.
10. Linear and cyclic forms of the monosaccharides. Closed ring structure of 5 or more carbon atoms (pyranose and furanose rings) monosaccharides. Haworth projections. The role and properties of the hemiacetal hydroxyl, notions of α- and β-anomers.
11. Important chemical reactions of monosaccharides (formation of phosphoric esters, N- and O-glycosides, oxidation and reduction). Ascorbic acid (vitamin C) structure and role.
12. Formation of amino-carbohydrates (glucosamine and sialic acid), biologic role.
13. Classification of the olygosaccharides – reducing and non-reducing disaccharides (maltose, lactose and sucrose). Biomedical role.
14. Classification, properties and biomedical role of the polysaccharides:
    a) homopolysaccharides (glycogen, starch, cellulose)
    b) heteropolysaccharides (hyaluronic acid, chondroitin-sulfate and heparin).
14. Classification and biomedical role of vitamins.
15. Water-soluble vitamins $B_1$, $B_2$, B6, PP, biotin, pantothenic, folic and ascorbic acids and $B_{12}$:
    a) structure;

b) coenzymes derivatives of these vitamins;
c) metabolic function of the coenzymes derivatives of these vitamins;
d) recommended daily allowances (RDAs) and food sources;
e) hypo- and hypervitaminoses – causes, metabolic disorders and clinical signs.

**LESSON № 12 Data _____**

## Lipids – classification, structure, physico-chemical properties, biological role. Biological membranes

**Experiment №1: Formation of insoluble calcium salts of fatty acids**
**Method's principle:** The experiment is based on the following reaction.

$$2CH_3\text{-}(CH_2)n\text{-}COOH + CaCl_2 \rightarrow (CH_3\text{-}(CH_2)n\text{-}COO)_2Ca + HCl$$

**Procedure:**

| № | Reagents | Test-tubes |
|---|---|---|
| 1. | Soap solution | 5 drops |
| 2. | $CaCl_2$ sol. | 1 drop |

**Write the reaction of the calcium salt of stearic acid formation.**

**Rezult:** _____
_____
**Conclusion:** _____
_____

**Experiment №2: The solubility of calcium soap in acetic acid**
**Method's principle:** The experiment is based on the following reaction.

$$(CH_3\text{-}(CH_2)n\text{-}COO)_2Ca + CH_3COOH \rightarrow 2CH_3\text{-}(CH_2)n\text{-}COOH + (CH_3COO)_2Ca$$

**Procedure:**

| № | Reagents | Test-tubes |
|---|---|---|
| 1. | Use the precipitate obtained in the previous esperiment. | |
| 2. | $CH_3COOH$ 2M | 1 drop |

**Rezult:** _____
_____
**Conclusion:** _____
_____

**Self-training questions:**

1. Biological functions of lipids.
2. Classification of lipids (structural, functional, according physico-chemical properties).
3. Saturated and unsaturated fatty acids. Structure, physico-chemical properties, representatives. Biomedical role.
4. Triglycerides – structure, physico-chemical properties and biomedical role.
5. Glycerophospholipids: phosphatidylserines, phosphatidylethanolamines (cephalins), phosphatidylcholines (lecithins), phosphatidylinositols – structure, physico-chemical properties and biomedical role.
6. Sphingomyelins – structure, physico-chemical properties and biomedical role.
7. Glycolipids: galacto- and glucocerebrosides, sulphatides, gangliosides – structure, physico-chemical properties and biomedical role.

**Case study:**

1. Vegetable oils are liquid triglycerides and animal fats - solid triglycerides. Write a triacylglycerol structure present in oil and a triacylglycerol present in animal fat. Give their names.

2. What fatty acids are essential for the human body? Write their structure. What are the main food sources of the essential fatty acids?

3. What are the products of the basic hydrolysis of phosphatidylcholine that contains stearic and oleic acids? Write the reaction of the hydrolysis process.

4. Write the reactions of the acid hydrolysis of the following triglycerides: dioleostearine, linoleodioleine and dipalmitostearine.

5. Write the structures of phosphatidylcholine that contains palmitic and oleic acids and of phosphatidylehtanolamine that contains linoleic and stearic acids. Show the polar and non-polar parts of the molecules.

6. Write the the structures of sphingolipids (ceramide and sphingomyeline) and cerebroside that contain the following fatty acids – palmitic, stearic, oleic and arachidonic.

**Tests for self-assessment:**

1. **Select the fatty acids that are essential humans:**
   a) lignoceric
   b) oleic
   c) palmitoleic
   d) linolenic
   e) linoleic

2. **Select the monoenic fatty acids (with one double bond):**
   a) palmitic
   b) oleic
   c) palmitoleic
   d) linolenic
   e) linoleic

3. **Select the polyenic fatty acids (with two or more double bonds):**
   a) arahidonic
   b) oleic
   c) palmitoleic
   d) linolenic
   e) linoleic

4. **Select the correct statements regarding triglycerides :**
   a) are constituents of biological membranes
   b) are esters of glycerol and fatty acids
   c) represent a form of energy storage
   d) are soluble in water
   e) are derivatives of phosphatidic acid

5. **Select the compound from which the phospholipids are synthesized:**
   a) glycerol phosphate
   b) phosphatidic acid
   c) phosphoric acid
   d) triglycerides
   e) ceramides

6. **Select the polar lipids from the following:**
   a) phosphatidylcholine
   b) phosphatidylserine
   c) phosphatidylethanolamine
   d) phosphatidyinositol
   e) triglycerides

7. **Select the lipids that can produce bilayers:**
   a) triglycerides
   b) phosphatidylserine
   c) phosphatidyl-ethanolamine
   d) phosphatidyinositol
   e) ceramides

8. **Select the correct statements about phosphatidylcholine and phosphatidylethanolamine:**
   a) are representatives of waxes
   b) are the main components of cell membranes
   c) represent a form of energy storage
   d) are derivatives of phosphatidic acid
   e) have different electric charge

9. **Select the correct statements about sphingosine:**
   a) is a saturated dihydroxy aminoalcool
   b) is a component of sphingomyeline
   c) is a component of glycolipids
   d) is a constituent of glycerophospholipids
   e) doesn't enter in the composition of the ceramide

10. **Select the correct statements about cerebrosides:**
    a) don't contain sphingosine
    b) contain a beta-galactose or a beta-glucose bound to ceramide
    c) contain oligosaccharides
    d) sulfatides are a class of sulfated cerebrosides
    e) the white matter of the brain contains cerebrosides in large amounts

11. **Select the correct statements about gangliosides:**
    a) contain several residues of glycerol
    b) contain N-acetylneuraminic acid (NANA)
    c) contain only glucose in the oligosaccharide
    d) contain sulfate residues linked to galactose
    e) are situated on the inner surface of membranes

**LESSON № 13** **Data _____**

## Cholesterol and its derivatives. Steroid hormones. Bile acids

**Experiment №1: Emulsification capacity of the byle acids**

**Method's principle:** Emulsification is the process of making an emulsion, allowing fat and water to mix, by breaking down of large fat globules into smaller, uniformly distributed

particles. Can be accomplished in the small intestine through the action of bile acids, which lower tension at the watter-lipids surface due to polar properties.

**Procedure:**

| Reactive | I test-tube | II test-tube |
|---|---|---|
| Oil | 1 drop | 1 drop |
| Water | 5 drops | 5 drops |
| Bile | - | 5 drops |
| Shake the test-tubes. | | |
| How long last the emulsion | | |

**Rezult:** _____

_____

**Conclusion:** _____

_____

**Experiment №2: Identification of bile acids (Pettencoffer reaction)**

**Method's principle:** When sucrose is treated with concentrated $H_2SO_4$ oxymethylfurfurol is formed. It reacts with bile acid to generate a red-violet complex compound.

**Procedure:** Put into a test-tube:

| Reactive | |
|---|---|
| Bile | 2 drops |
| Sucrose 20% | 2 drops |
| Shake the test-tube. | |
| Concentrated $H_2SO_4$ | 5-6 drops |
| Wait 2-3 min for the result. | |

**Rezult:** _____

_____

**Conclusion:** _____

_____

**Self-training questions:**

1. Steran. Cholesterol and cholesterides. Structure, physico-chemical properties and biomedical role.
2. Steroid hormones: glucocorticoids, mineralocorticoids, estrogens, gestagens and androgens – structure and functions.
3. Bile acids: cholic, taurocholic and glycocolic acids – structure, properties and biomedical role.

**Case study:**

1. Write the structures of the hydrocarbons from which derive the main classes of steroids – cholestan, pregnan, androstan, estran and cholan.

2. Write the structures and describe the main functions of the following steroid hormones – cortisol, aldosterone, estradiol, progesterone and testosterone.

3. Bile acids are synthesized in the liver cells and conjugated with glycine and taurine producing the the corresponding amides. Write the reactions of glycocholic and taurocholic acids synthesis. What is the biological significance of bile acids conjugation?

**Tests for self-assessment:**

**1. Select the correct statements regarding cholesterol:**

a) is the precursor of steroid hormones
b) is hydrophobic
c) is a storage lipid
d) enter into the composition of biological membranes
e) is the precursor of all fat-soluble vitamins

**2. Select the correct statements about the chemical properties of cholesterol:**

a) is an acid
b) is a simple lipid
c) is an alcohol
d) is an ether
e) is an ester

**3. Choose the correct statements about bile acids:**

a) contain 28 carbon atoms
b) are non-polar compouns
c) are synthesized from cholesterol
d) are synthesized from phosphatidic acid
e) can emulsify dietary fats

**4. Indicate by arrow the correct pairs: hormone - place of its synthesis:**

| | |
|---|---|
| a) cortisol | yellow body |
| b) aldosterone | ovarian follicles |
| c) testosterone | cortex of the adrenal glands |
| d) estradiol | testis Leydig cells |
| e) progesterone | |

**5. Choose the correct statements about cortisol:**

a) is synthesized in the liver
b) it is a derivative of pregnan
c) is a mineralocorticoid
d) participates in the regulation of blood glucose level
e) contains 27 carbon atoms

**6. Choose the right statements regarding aldosterone:**

a) it is synthesized in the cortex of the adrenal glands
b) it is a derivative of androstane
c) contains 18 carbon atoms
d) is a glucocorticoid
e) regulates water and electrolytes metabolism

**7. Regarding progesterone, the following statements are true:**

a) contains an aromatic ring
b) it is synthesized in the yellow body
c) it is synthesized from cholesterol
d) it is used in menopausal hormone replacement therapy
e) regulates carbohydrate metabolism

**LESSON № 14** **Data _____**

**Fat soluble vitamins – A, D, E and K**

**Experiment №1: Identification of vitamins D (Rosenheim reaction)**

**Method's principle:** When vitamins D are treated with concentrated trichloroacetic acid ($CCl_3COOH$) a red compound is formed. The color of the compound turns blue in time.

**Procedure:** Put into a test-tube:

| Reactive | |
|---|---|
| Vitamin D sol. | 5 drops |
| $CCl_3COOH$ conc. | 5 drops |
| Shake the test-tube. | |

**Rezult:** _____
_____

**Conclusion:** _____
_____

**Experiment №2: Identification of vitamins A**

**Method's principle:** When vitamins A are treated with concentrated trichloroacetic acid ($CCl_3COOH$) a yellow compound is formed. The color of the compound turns blue in time.

**Procedure:** Put into a test-tube:

| Reactive | |
|---|---|
| Vitamin A sol. | 5 drops |
| $CCl_3COOH$ conc. | 10 drops |
| Shake the test-tube. | |

**Rezult:** _____

_____

**Conclusion:** _____

_____

**Self-training questions:**

1. Vitamins D: cholecalciferol and ergocalciferol – structure, synthesis and biologic role.
2. Calcitriol – structure, synthesis and biologic role.
3. Isoprenoids. B-caroten. Fat soluble vitamins: A, E and K – structure and and biologic role.

**Case study:**

1. Specify schematically the pathway of cholesterol conversion to vitamin D3 (cholecalciferol). What is the biological significance of vitamin D?

2. Write the structure of β-carotene and show the fragments of isoprene in its structure. Why is β-carotene orange?

3. Which compounds are called carotenoids? Write the structure of β-caroten, retinol and retinal and show the isoprene fragments. What are the functions of vitamin A?

4. Write the structure and describe the role of α-tocopherol.

5. Write ths tructures of vitamin $K_2$ and its structural analog – vicasol. What functions have these chemical compounds?

**Tests for self-assessment:**

**1. Select the correct statements about fat soluble vitamins:**

a) all can be synthesized in the human organism
b) representatives are folic, pantothenic and ascorbic acids
c) representatives are vitamins A, E, D and K
d) are delivered by the food
e) are structural elements of the membranes

**2. Select the correct statements about vitamin D:**

a) is synthesized in the skin form cholesterol
b) the active form or the vitamin is calcitriol
c) calcitriol is produced by dehydrogenation in the liver and kidneys
d) the vitamin can not be deposited in the human organism
e) one of its functions is to regulate calcium and phosphate metabolism

**3. Choose the right statement about the transformation of vitamin D:**

a) vitamin D is hydroxylated in the liver and kidneys
b) vitamin D is hydroxylated in muscles and bones
c) the active form of vitamin D is calcitriol
d) active form of vitamin D is cholecalciferol
e) calcitriol is synthesized in the skin by hydroxylation of cholesterol

**4. Calcitriol – select the correct statements:**

a) is synthesized by 2 reactions of hydroxylation of vitamin D in the liver and kidneys
b) it is a plant form of vitamin D
c) regulates the level of glucose in the blood
d) regulates the level of sodium and potassium in the blood
e) has an anticoagulant effect

**5. Select the correct statements about vitamin A:**

a) has 2 forms – β-caroten and retinol
b) has 3 forms – retinol, retinal and retinoic acid
c) β-caroten is the vitamin precursor
d) regulate sodium and potassium homeostasis
e) is involved in the vision processes

**6. Select the correct statements about vitamin E:**

a) has antioxidant function
b) is an anticoagulant
c) regulates mineral metabolism
d) has several forms, the most active is α-tocopherol
e) is an alcohol

**7. Select the correct statements about vitamin K:**

a) can not be synthesized in the human organism
b) is produced by the bacteria in the large intestine
c) has anticoagulant function
d) participate in the synthesis of the calcium binding proteins
e) vicasol is the plant form of the vitamin

**LESSON № 15** **Data _____**

## Biological membranes. Chemical composition, structural-functional organization, properties and functions. Membrane transport

### Self-training questions:

1. Biological membranes.
   a) The biological and medical role
   b) Chemical composition – lipids, proteins, carbohydrates. Their functional role.
   c) Structural and functional organization – fluid-mosaic model of Singer-Nicolson
   d) The properties of membranes: fluidity, motility, selective permeability, asymmetry, self-assembling and self-repairer.
   e) Structural and functional diversity and specificity.
2. Membrane transport:
   a) passive transport:
      - simple diffusion;
      - facilitated diffusion - glucose transporters (GLUT), anion exchangers;
      - channel type alpha and beta (structural features).
   b) active transport:
      - primary ($Na^+$, $K^+$-ATPase, $Ca^{2+}$-ATPase, ABC-transporters);
      - secondary (amino acid transporters, glucose).
   c) diseases caused by deficiency of membrane channels and transporters.

### Case study:

1. Write the structures of membrane lipids and show the polar and non-polar parts.

2. List the monosaccharides from the glycocalix. Which chemical bonds appear between the sugars and proteins?

3. Schematically represent the biological membrane – lipid bilayer, periferic, integral and transmembrane proteins, glicocalix.

4. What are the differences between the cytoplasmic membrane of a normal cell and a cancer cell?

**Tests for self assessment:**

**1. Select the chemical bonds between the membrane proteins and lipids:**

a) hydrophobic nteractions
b) ionic bonds
c) hydrogen bonds
d) peptide bonds
e) disulfide bonds

**2. Select the correct statements about the carbohydrates in biological membranes (glycocalix):**

a) are located on both sides of the membrane
b) bind to lipids and membrane proteins non-covalently only
c) have a catalytic function
d) determine the selective permeability of the membranes
e) are responsible for the intercellular interaction and adhesion

**3. Select the lipids present in biological membranes:**

a) triacylglycerols
b) glycerophospholipids
c) cholesterol esters
d) sphingomyelins
e) gangliosides

**4. Select the correct statements about the proteins of biological membranes:**

a) are located only in the outer layer of the plasma membrane
b) can be peripheral, integral and transmembrane
c) have no mobility
d) perform the role of intercellular interaction
e) can be linked by covalent bonds with the carbohydrates from the membrane surface

**5. Select the substances which cross the plasma membrane by sodium simport:**

a) proteins
b) amino acids
c) triglycerides
d) cholesterol
e) glucose

**6. Select the substances that pass through the membrane by simple diffusion:**

a) water
b) amino acids
c) $Ca^{2+}$
d) cholesterol
e) $CO_2$

**7. Select substances that are transported through the cell membrane by translocases (facilitated diffusion):**

a) $K^+$
b) ammonia
c) triglycerides
d) oxygen
e) glucose

**8. Select the substances that are transported through the membrane by ATP-ases (primary-active transport):**

a) proteins
b) nitrogen
c) $H^+$
d) glucose
e) $Na^+$

**9. Select the substances that are transported through the cell membrane with the participation of $Na^+$-dependent transports (secondary active transport):**

a) proteins
b) amino acids
c) triglycerides
d) cholesterol
e) glucose

**LESSON № 16 Data _____**

## Concluding test on Chapters „Lipids", „Biological membranes"

1. Biological functions of lipids.
2. Classification of lipids (structural, functional, according physico-chemical properties).
3. Saturated and unsaturated fatty acids. Structure, physico-chemical properties, representatives. Biomedical role.
4. Triglycerides – structure, physico-chemical properties and biomedical role.
5. Glycerophospholipids: phosphatidylserines, phosphatidylethanolamines (cephalins), phosphatidylcholines (lecithins), phosphatidylinositols – structure, physico-chemical properties and biomedical role.
6. Sphingomyelins – structure, physico-chemical properties and biomedical role.
7. Glycolipids: galacto- and glucocerebrosides, sulphatides, gangliosides – structure, physico-chemical properties and biomedical role.
8. Steran. Cholesterol and cholesterides. Structure, physico-chemical properties and biomedical role.
9. Steroid hormones: glucocorticoids, mineralocorticoids, estrogens, gestagens and androgens – structure and functions.
10. Bile acids: cholic, taurocholic and glycocolic acids – structure, properties and biomedical role.
11. Vitamins D: cholecalciferol and ergocalciferol – structure and biologic role. Calcitriol – structure and biologic role.
12. Isoprenoids. B-caroten. Fat soluble vitamins: A, E and K – structure and and biologic role.
13. Biological membranes.
    a) The biological and medical role
    b) Chemical composition – lipids, proteins, carbohydrates. Their functional role.
    c) Structural and functional organization – fluid-mosaic model of Singer-Nicolson
    d) The properties of membranes: fluidity, motility, selective permeability, asymmetry, self-assembling and self-repairer.
    e) Structural and functional diversity and specificity.
14. Membrane transport:
    a) passive transport:
        - simple diffusion;
        - facilitated diffusion - glucose transporters (GLUT), anion exchangers;
        - channel type alpha and beta (structural features).
    b) active transport:
        - primary ($Na^+$, $K^+$-ATPase, $Ca^{2+}$-ATPase, ABC-transporters);
        - secondary (amino acid transporters, glucose).
    c) diseases caused by deficiency of membrane channels and transporters.