

MINISTRY OF HEALTH OF UKRAINE
ODESA NATIONAL MEDICAL UNIVERSITY
Department of Medical Biology and Chemistry

CONFIRMED BY
Vice-rector for scientific and
pedagogical work
Eduard BURLACHKIVSKIY

“ 1 ” 2024

**METHODOLOGICAL DEVELOPMENT
TO THE INDEPENDENT WORK
ON THE EDUCATIONAL DISCIPLINE**

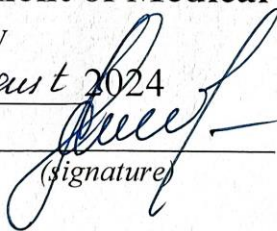
<u>Faculty, course</u>	<u>International faculty, 1,2 year</u>
<u>Specialty</u>	<u>221 "Dentistry"</u>
<u>Academic discipline</u>	<u>Biological and Bioorganic chemistry</u>

The program was approved:

at a meeting of **the Department of Medical biology and chemistry** Odesa
National Medical University

Minute № 1 dated 26 august 2024

Head of Department _____


(signature)

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Practical class № 1

Topic: Classification, nomenclature, isomerism of bioorganic compounds. Nature of chemical bonds.

Actuality of theme: In medical universities, chemistry is a fundamental general theoretical discipline. Knowledge of chemistry largely determines the theoretical basis of a highly qualified doctor or a physician working in the field of medical science. Chemistry lays the physical and chemical basis for the study of the functioning of biological systems of different levels of organization, determines the possibility of approaching the molecular level of the processes occurring in the body in normal and various pathologies.

Aims: To form systematic knowledge about the structure of organic molecules, their classification, the influence of individual groups of atoms on chemical properties, as well as on the biological role of organic compounds. Explain the mechanisms of homogeneous and heterogeneous bond breaking. Understand the influence of substituents on the reactivity of organic compounds. Master the mechanism of organic reactions necessary to determine the behavior of compounds and the preliminary orientation of possible products, which is important in both synthetic chemistry and organic medicinal chemistry.

Basic concepts: organic chemistry, bioorganic chemistry, Butlerov's theory, classification of organic compounds, types of reagents, electronic effects, inductive and mesomeric effects, types of organic reactions.

Equipment: department laboratory

Plan and organizational structure of the lecture:

1. The subject and objectives of organic chemistry.
2. Classification of organic compounds.
3. Carbon chains, hydrocarbon radicals, functional groups, homologous series.
4. Nomenclature of organic compounds: trivial, rational, IUPAC.
5. Structural isomerism.

The higher education applicant should be able to and know:

- a) electronic structure of biogenic elements: C, H, O, N, S;
- b) homologous series of classes of organic compounds;
- c) the structure of organic compounds;
- d) give names to organic compounds according to modern systematic nomenclature;
- e) structure of organic molecules; types of chemical bonds in organic molecules, hybridization of bonds.

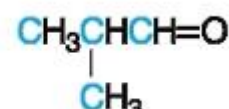
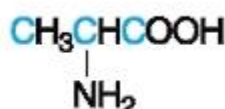
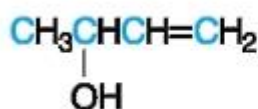
Content of the practical class

Organic compounds are classified according to the following features:

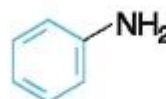
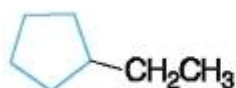
- a structure of molecular framework (sometimes called a molecular skeleton);
- the presence of functional groups in a molecule.

1.1. Classification According to the Molecular Framework

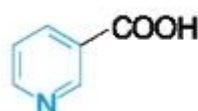
Organic compounds are subdivided into the following groups. Acyclic compounds. They have unbranched or branched carbon chain, but no rings. In the examples below, the first two represent compounds with unbranched carbon chain, whereas the third one is a compound with a branched chain:



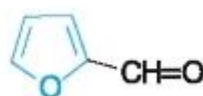
Carbocyclic compounds. They contain a ring (or rings) of carbon atoms only. The ring may contain multiple bonds and may have side carbon chains.



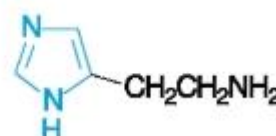
Heterocyclic compounds. They contain a cyclic skeleton having at least one heteroatom, an atom that is not carbon. The most common heteroatoms are nitrogen, oxygen, or sulfur. More than one heteroatom may be present and these atoms may be identical or different. The structures of some natural heterocyclic compounds are presented below:



nicotinic acid
(vitamin PP)



furfural
(responsible for the odour
of rye-bread)



histamine
(biologically important amine)

1.2. Classification According to Functional Groups

Hydrocarbons are parent compounds in organic chemistry, which, according to their name, consist of only carbon and hydrogen atoms. Most organic molecules involve *functional groups*, i. e. an atom or a group of atoms of non-hydrocarbon origin that determine chemical properties of a compound. Indeed, chemical changes occur in most reactions at the functional group whereas the molecular framework remains unchanged. Thus, the knowledge of properties of the functional groups will greatly help in the study of organic chemistry.

Organic compounds are divided into classes depending on the functional groups present. Some of the main functional groups and classes are listed in Table 1.

Table 1.

Some of the functional groups and the corresponding classes of organic compounds

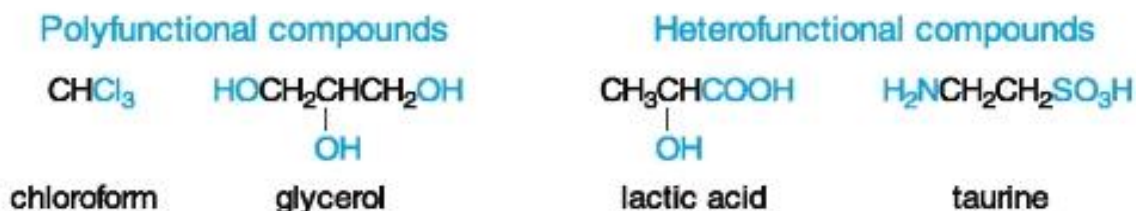
Functional group		Name of the class	General formula of the class*
formula	name		
–	–	Hydrocarbons**	R–H
–F, –Cl, –Br, –I (–Hal)	Fluorine, chlorine, etc. (halogens)	Halogen compounds	R–Hal
–OH	Hydroxyl	Alcohols, phenols	R–OH Ar–OH
–O–	Oxy	Ethers	R–O–R'
–SH	Mercapto	Thiols	R–SH
–NH ₂	Amino	Amines***	R–NH ₂
	Nitro	Nitro compounds	R–NO ₂
	Carbonyl	Aldehydes, ketones	R–CH=O R–C(O)–R'
	Carboxyl	Carboxylic acids	R–COOH
	Sulfo	Sulfonic acids	R–SO ₃ H

* The symbol R is usually used for any hydrocarbon radical, the symbol Ar - for an aromatic radical only.

** Multiple bonds in unsaturated compounds are sometimes related to the functional groups.

*** Only primary ones are shown.

Molecules with one functional group belong to *monofunctional* compounds. *Polyfunctional* compounds contain several identical functional groups, for example, chloroform and glycerol. Molecules with different functional groups are considered as *heterofunctional* compounds, they may be related to several classes. For example, lactic acid is both an alcohol and a carboxylic acid. Similarly, taurine belongs both to amines and sulfonic acids.



Classification characteristics form a foundation of the systematic chemical nomenclature of organic compounds.

NOMENCLATURE

At the earliest stage of organic chemistry, each new compound was usually named on the basis of its source (caffeine – from coffee-beans, urea – from urine) or its evident properties (glycerol and glucose – from the Greek *glykys*, sweet). Such names are known as trivial or common names. Trade names are widely used in pharmacy and medicine indicating some pharmaceutical effect (anesthesin, sarcolysin). Trivial and trade names are very convenient because of their brevity, but they give no information about the structure of a compound and cannot be systematized. Some trivial names went out of use with time; others have shown their viability and are used now in the systematic nomenclature.

Systematic nomenclature is an arrangement of terms that describes complete structure of organic molecules.

The first systematic nomenclature appeared as far back as 1892 (Geneva Rules). It was then perfected by a commission of the International Union of Pure and Applied Chemistry (IUPAC) and is known now as the IUPAC rules or the IUPAC nomenclature.

1.3. General Principles of the IUPAC Nomenclature

To minimize confusion the following terms are used in the present rules.

Parent name: a part of the name used for the formation of a particular name according to the appointed rules. For example, the name *ethanol* is derived from ethane. The parent name may be both systematic.

Characteristic group: this term is practically equal to the term functional group, for example, the amino group $-\text{NH}_2$, the carbonyl group $>\text{C}=\text{O}$, the oxo group $=\text{O}$, the carboxyl group $-\text{COOH}$.

Principal (senior) group: the characteristic group chosen for expression as a suffix in a particular name. This group has no other advantages over remainder groups.

Substituent: any atom or group replacing hydrogen of a parent compound.

Radical: a part of a molecule that remains after removal of one or more hydrogen atoms from it. For example, the radicals, such as methyl, CH_3- , and methylene, $-\text{CH}_2-$, are derived from methane, CH_4 .

Locant: a numeral or a letter showing a position of a substituent or a multiple bond in a parent structure.

Multiplying affix: syllables di-, tri-, tetra-, etc., which are used to indicate a set of identical substituents or multiple bonds.

Nomenclature Systems. There are eight basic nomenclature systems from which the most versatile and common therefore is the *substitutive* nomenclature. The next in prevalence is

the *radicofunctional* nomenclature. These two nomenclatures, especially the former, will be considered in greater detail.

Substitutive nomenclature. The particular name of a polyfunctional compound represents a complex word that consists of a root (parent name), a suffix (principal group), and prefixes (other substituents). Fig.1 demonstrates this approach.

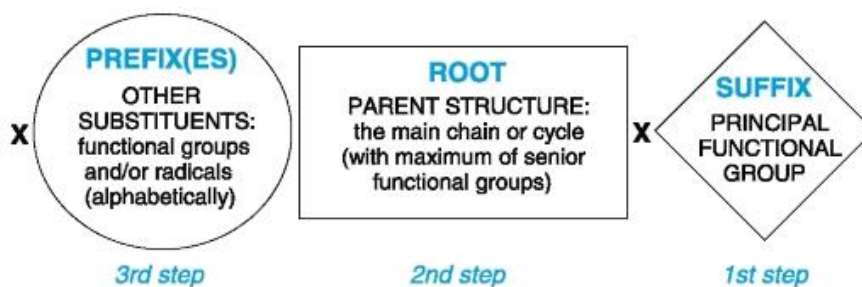


Figure 1. The scheme for constructing the IUPAC substitutive name. The symbol \times represents multiplying affix(es).

There are two types of characteristic groups. One type is designated in a name only as prefixes. Nitro group, halogens, and some other groups belong to this type; they are listed in the lower part of Table 2.

Most of characteristic groups (the upper part of Table 2, beyond the coloured line) may be cited either as suffixes or as prefixes. But only one kind of group (principal group) is to be cited as a suffix. Within these groups, a conventional order of priority has been established (Table 2). It means the principal group is that which characterizes the class occurring as high as possible in Table 2. All other characteristic groups are then cited as prefixes. Multiplying affixes and locants are added as necessary.

Radicofunctional nomenclature. The principles of the radicofunctional nomenclature are identical with those of the substitutive nomenclature except that suffixes are never used. Instead of the principal group being named as a suffix, the class name of a compound is expressed as one word and the remainder of the molecule as another.

Table 2.

Suffixes and prefixes used for some important groups in the substitutive nomenclature IUPAC (in order of decreasing priority)

Class	formula*	Characteristic group	
		prefix	suffix
Carboxylic acids	-COOH	—	-oic acid
	-COOH	carboxy-	-carboxylic acid
Sulfonic acids	-SO ₃ H	sulfo-	-sulfonic acid
Salts of carboxylic acids	-COOM	—	metal*...-oate
	-COOM	—	metal*...-carboxylate
Acid anhydrides	-C(O)-O-C(O)-	—	-oic anhydride
Esters	-COOR	—	R*...-oate
	-COOR	R-oxycarbonyl-	R*...-carboxylate
Acid halides (on example of chloride)	-C(O)Cl	—	-oyl chloride
	-C(O)Cl	chloroformyl-	-carbonyl chloride
Amides	-C(O)NH ₂	—	-amide
	-C(O)NH ₂	carbamoyl-	-carboxamide
Nitriles	-C≡N	—	-nitrile
	-C≡N	cyano-	-carbonitrile
Aldehydes	-CH=O	oxo-	-al
	-CH=O	formyl-	-carbaldehyde
Ketones	>C=O	oxo-	-one
Alcohols	-OH	hydroxy-	-ol
Phenols	-OH	hydroxy-	-ol***
Thiols	-SH	mercapto-	-thiol
Amines	-NH ₂	amino-	-amine
Imines	=NH	imino-	-imine
Ethers	-O-	...-oxy-****	—
Sulfides	-S-	...-thio-****	—
Halogen derivatives (on example of chloride)	-Cl	chloro-	—
Nitro compounds	-NO ₂	nitro-	—

* Coloured carbon atoms are included in the name of parent structure and not in the suffix or prefix.

** Should be added in front of the name.

*** Phenols have usually common names.

**** Used only with the name of radical R, e. g. ROalkoxy- or RSalkylthio-.

This type of nomenclature is the most convenient one for such classes as ethers, sulfides, amines, and halogen compounds, especially for the compounds with simple radicals.

1.4. General Principles of Forming a Systematic Name

The formation of a name for a chemical compound usually involves the following steps in the order given below.

Step 1. From the nature of the compound determine the most pertinent type of nomenclature (substitutive, radicofunctional, or else).

Step 2. Determine the kind of characteristic group for use as the principal group, if any. It is this group that stipulates then the choice of a parent structure and its numbering.

Step 3. Determine the parent structure (principal chain or parent ring system²). When in an acyclic compound there is a choice for principal chain, the following criteria are applied successively, in the order listed, until a decision is reached:

- a) the maximum number of substituents of the highest priority from Table 2.2;
- b) the maximum number of double and triple bonds considered together;
- c) the maximum length of the chain;
- d) the maximum number of substituents cited as prefixes.

Step 4. Name the parent structure and the principal group(s).

Step 5. Determine and name prefixes.

Step 6. Complete the numbering. The starting point and direction of numbering must be chosen so as to give the lowest number to the principal group. If this rule does not effect the choice, a principle of the *lowest locants* is used. It means that the locants of substituents must then be as low as possible.

Step 7. Assemble the partial names (according to steps 4 and 5) into a complete name, using the alphabetic, but not numerical, order. Multiplying affixes are not included in this order. Position of locants is not strictly determined by the IUPAC rules. In this respect, there are alternative versions in different languages and, what is more, there are some differences between American and British chemical English! But usually, locants are placed in front of prefixes and a suffix. Finally, locants are separated from each other by commas and separated from the letters by hyphens. All parts of a name are written without space, except for the word acid in the name of carboxylic acids.

1.5. Names of Parent Structures

Parent structures are presented either by an open carbon chain, or carbocyclic framework, or heterocyclic one.

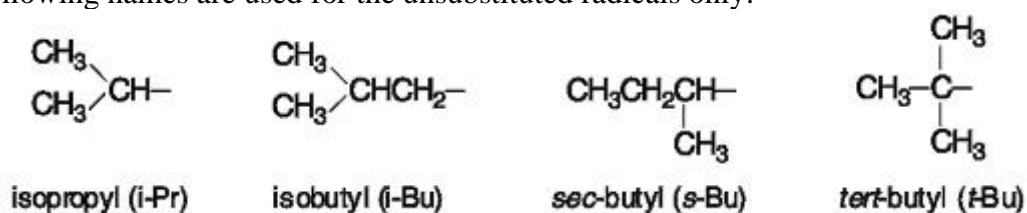
Acyclic hydrocarbons. The generic name of saturated acyclic hydrocarbons (unbranched or branched) is *alkanes*. The first four saturated unbranched acyclic hydrocarbons are called methane, ethane, propane, and butane. Names of the higher members of this series consist of a numerical term (Greek mainly), followed by -ane with elision of the terminal -a from the numerical term. Examples are given in Table 2.3.

Table 3.

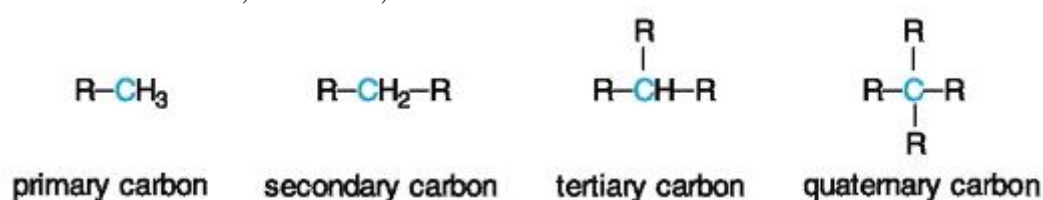
Straight-chain saturated hydrocarbons and alkyl radicals

Alkanes		Alkyl radicals	
formula	name	formula	name
CH ₄	Methane	CH ₃ -	Methyl
C ₂ H ₆	Ethane	CH ₃ CH ₂ -	Ethyl
C ₃ H ₈	Propane	CH ₃ CH ₂ CH ₂ -	Propyl
C ₄ H ₁₀	Butane	CH ₃ (CH ₂) ₃ -	Butyl
C ₅ H ₁₂	Pentane	CH ₃ (CH ₂) ₄ -	Pentyl
C ₆ H ₁₄	Hexane	CH ₃ (CH ₂) ₅ -	Hexyl
C ₇ H ₁₆	Heptane	CH ₃ (CH ₂) ₆ -	Heptyl
C ₈ H ₁₈	Octane	CH ₃ (CH ₂) ₇ -	Octyl
C ₉ H ₂₀	Nonane	CH ₃ (CH ₂) ₈ -	Nonyl
C ₁₀ H ₂₂	Decane	CH ₃ (CH ₂) ₉ -	Decyl
C ₁₁ H ₂₄	Undecane	CH ₃ (CH ₂) ₁₀ -	Undecyl
C ₁₂ H ₂₆	Dodecane	CH ₃ (CH ₂) ₁₁ -	Dodecyl
C ₁₆ H ₃₄	Hexadecane	CH ₃ (CH ₂) ₁₅ -	Hexadecyl
C ₁₈ H ₃₈	Octadecane	CH ₃ (CH ₂) ₁₇ -	Octadecyl
C ₂₀ H ₄₂	Eicosane	CH ₃ (CH ₂) ₁₉ -	Eicosyl

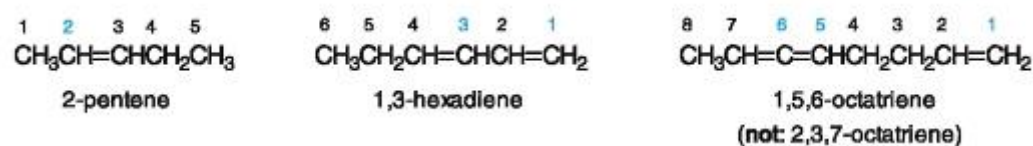
Univalent radicals derived from these hydrocarbons by removal of a hydrogen atom from a terminal carbon are named by replacing the suffix -ane in the name of the hydrocarbon by -yl (Table 2.3). The following names are used for the unsubstituted radicals only:



The prefixes *sec* (secondary) and *tert* (tertiary) refer to the degree of alkyl substitution at a carbon atom. There are four types of carbons that differ in their alkyl environment. If the carbon atom is bonded to only one carbon, the former is referred to as a *primary* carbon. A *secondary* carbon has two other carbons bonded to it, and so on, as shown below:



Unsaturated unbranched hydrocarbons having one double bond are named by replacing the suffix -ane in the name of the corresponding alkane by the suffix -ene. If there are two or more double bonds, the suffix will be -adiene, -atriene, and so on. The chain is so numbered as to give the lowest possible numbers to the double bonds; only the lower locant is cited in the name.



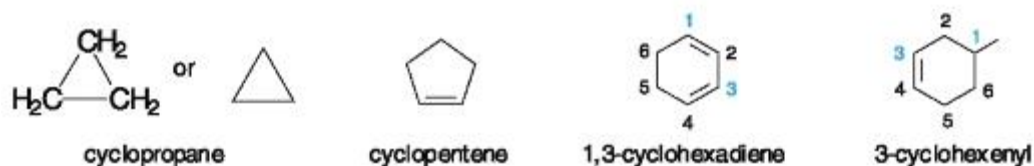
The generic name of unsaturated hydrocarbons with one triple bond is *alkynes*. Unbranched compounds of this series are named similarly to alkenes but using the suffix -yne. The position of the triple bond is indicated in the same way as for alkenes.

The following non-systematic names are retained: ethylene for $\text{CH}_2=\text{CH}_2$, acetylene for $\text{CH}\equiv\text{CH}$, and isoprene for $\text{CH}_2=\text{C}(\text{CH}_3)-\text{CH}=\text{CH}_2$, as well as vinyl for the radical $\text{CH}_2=\text{CH}\cdot$ and allyl for the radical $\text{CH}_2=\text{CHCH}_2\cdot$.

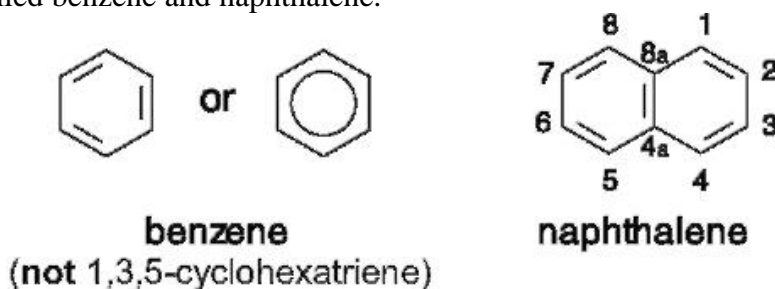
It should be noted that the numbering system described above is applicable only to hydrocarbons (including a branched chain) and their derivatives with non-principal characteristic groups, such as halogens or a nitro group. The numbering of a chain may be changed in the presence of principal characteristic groups.

Cyclic hydrocarbons. The names of saturated monocyclic hydrocarbons (with no side chains) are formed by adding the prefix *cyclo-* to the name of unbranched alkane with the same number of carbon atoms. The generic name of these hydrocarbons (with or without side chains) is *cycloalkanes*.

Unsaturated monocyclic hydrocarbons are named and numbered similarly to acyclic analogues. This also concerns univalent radicals derived from cyclic hydrocarbons. The carbon with the free valence is numbered as 1 (but this locant may be omitted) regardless of a characteristic group present in the radical.

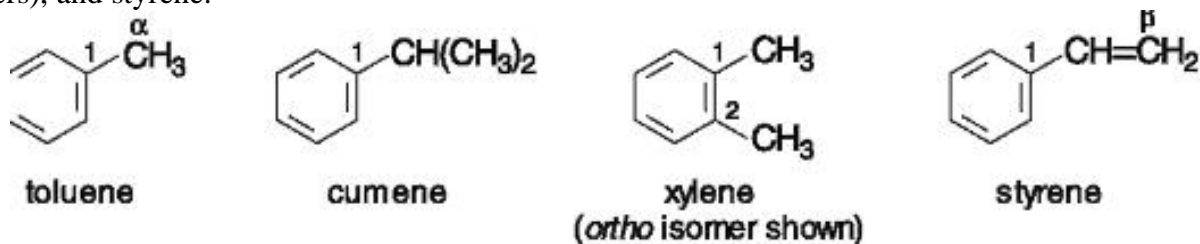


The generic name of mono and polycyclic aromatic hydrocarbons is *arenes*. The simplest representatives are called benzene and naphthalene.

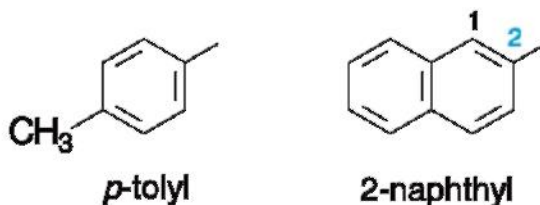


The numbering in the benzene ring is applied only to derivatives having more than one substituent. The symbols *o-* (*ortho*), *m-* (*meta*), and *p-* (*para*) may be used in place of 1,2-, 1,3-, and 1,4-, respectively, when only two substituents are present.

Several aromatic hydrocarbons with side chains may be used as parent structures for the names of compounds having non-principal characteristic groups. These are toluene, cumene, xylenes (three isomers), and styrene:

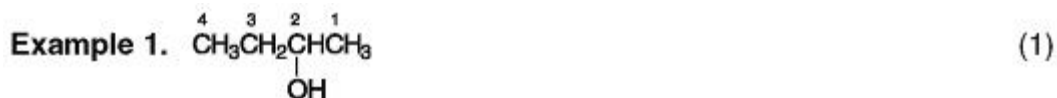


The most widespread aromatic radicals are phenyl, $\text{C}_6\text{H}_5\cdot$; benzyl, $\text{C}_6\text{H}_5\text{CH}_2\cdot$; tolyl (*ortho*, *meta*, and *para* isomers) derived from toluene; and naphthyl (1- and 2-isomers) - from naphthalene, for example:



1.6. Examples of Constructing the Systematic Names

Naming of simple hydrocarbons and monofunctional compounds encounters usually few problems because the basic principles of nomenclature are logical and easy to understand. Difficulties arise with polyand heterofunctional compounds. Examples given below illustrate how the IUPAC rules (the substitutive names unless otherwise stated) are applied for a particular compound of different classes.



The name of the alcohol (1) is 2-butanol. The only carbon chain consists of four atoms (butane); the principal characteristic group (OH) is expressed by the suffix -ol. We number the chain from the right, starting closest to the OH group.

The radicofunctional name of the compound is *sec*-butyl alcohol, which is derived from the name of the radical (*sec*-butyl) and the name of a class. It should be noted that the names such as *sec*-butanol, as well as isopropanol and *tert*-butanol are incorrect because there are no hydrocarbons *sec*-butane, isopropane, and *tert*-butane to which the suffix -ol can be added.



The main chain in the ether (2) is a three-carbon chain of propane. The group CH₃O- attached to C-2 represents a combined substituent methyl + oxy = methyloxy, or shortly methoxy that may be used only as a prefix. Together this gives the name 2-methoxypropane.

The radicofunctional name of the compound (2) is isopropyl methyl ether (two radicals are set in alphabetic order), which seems to be more convenient than the substitutive one.



The name of the secondary amine (3) is N-methyl-1-propanamine. The locant *N* means that substitution with methyl group was performed at nitrogen atom but not at carbon (as is usually in the substitutive nomenclature).

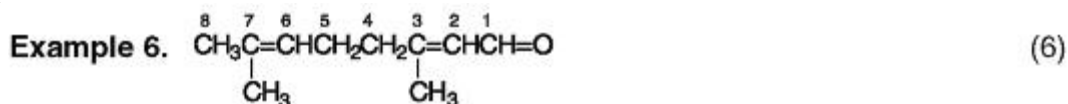
Using the radicofunctional nomenclature we get simpler name - methylpropylamine.



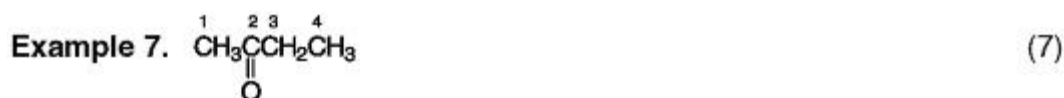
The systematic name of isovaleric acid, the compound (4), employed in pharmacy is 3-methylbutanoic acid. The suffix -oic acid is used because carbon of the carboxyl group is a member of the parent name (butane).



The name of the compound (5) is 3-cyclohexenecarboxylic acid (*not* 3-cyclohexenoic acid). Carbon of the carboxyl group cannot be a member of the cyclic parent structure (cyclohexene) in this example. The cycle is always numbered, starting from a carbon to which the principal characteristic group is attached.



The systematic name of citral, the compound (6), a component of lemon oil used in treatment of eye-diseases, is 3,7-dimethyl-2,6-octadienal. It should be mentioned here that the locant 1 is never used for suffixes -al and -oic acid.

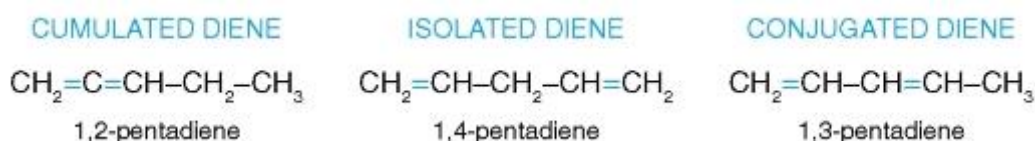


The compound (7) is named simply butanone. Pay attention to the absence of a locant for the oxo group. The atoms C-2 and C-3 are identical whereas the oxo group attached to C-1 gives rise not to a ketone, but an aldehyde.

The radicofunctional name of the compound (7) is ethyl methyl ketone.

REACTIVITY OF ORGANIC COMPOUNDS

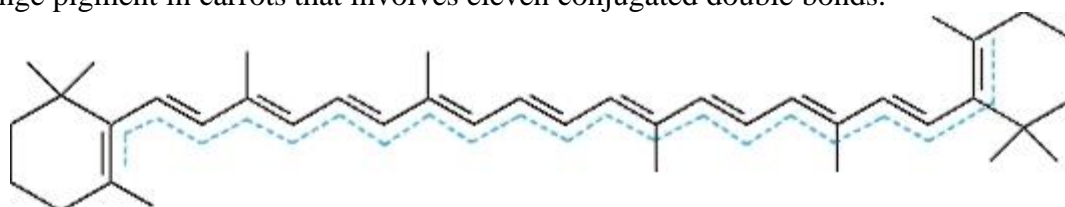
There are many organic compounds whose molecules contain more than one multiple (double or triple) bonds. According to mutual arrangement, the multiple bonds of polyunsaturated compounds are classified as being *cumulated*, *isolated*, and *conjugated*. These will be considered by the simplest examples, namely, alkadienes that are usually referred to simply as *dienes*.



The term *cumulated* means that one carbon participates in two double bonds; in other words, these bonds follow one after another. This type of bonding occurs very rarely among the natural products. If at least one saturated carbon atom intervenes between the double bonds of a diene, these bonds are regarded as isolated. Thus, 1,2-butadiene is an example of cumulated dienes, and 1,4-pentadiene represents isolated dienes.

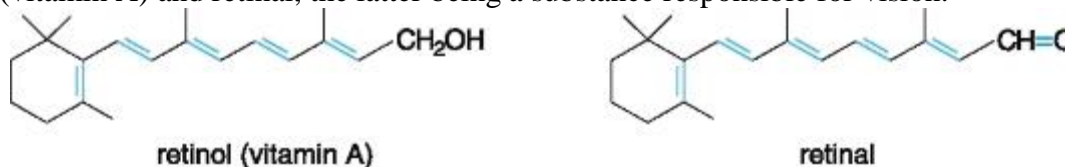
1.1. CONJUGATION AS STABILIZING FACTOR OF MOLECULES

The most interesting are dienes with conjugated double bonds, i. e. 1,3-dienes where the double and single bonds alternate in the chain. There are many polyunsaturated compounds with conjugated double bonds, which play an important role in nature and biology. For example, β -carotene is a yellow-orange pigment in carrots that involves eleven conjugated double bonds:



β -carotene
(conjugation chain is underlined in colour)

Other examples of highly conjugated systems among biologically active compounds are retinol (vitamin A) and retinal, the latter being a substance responsible for vision.

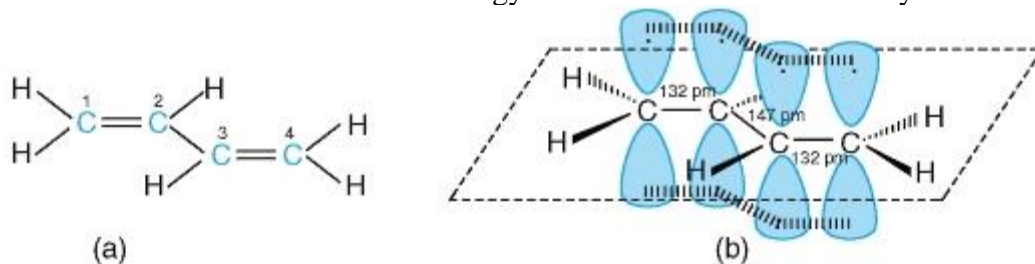


π, π Conjugation

Conjugated dienes are similar to nonconjugated dienes (and alkenes) in many but not all of their chemical properties. The former are somewhat more stable than nonconjugated dienes. An explanation for the higher stability of conjugated dienes can be given in describing molecular orbitals of the conjugated systems by example of 1,3-butadiene, a simplest conjugated diene (Fig. 1, a).

All carbons in the molecule are sp^2 -hybridized, which results in the *delocalization* of π electrons in the bonding molecular orbitals. According to this standpoint, p orbitals of the central carbon atoms are overlapped as well (Fig. 1, b). It should be stressed here that the interaction between unhybridized p orbitals of the atoms C-2 and C-3 can only occur when the molecule is planar. This

provides for parallel arrangement of the p orbitals and favours the effective orbital overlap. Delocalization of electron cloud leads to lower-energy orbitals and increased stability of the molecule.



Another observational proof for the peculiar nature of conjugated dienes arises from data on bond lengths (Fig. 1, *b*). Both double bonds of conjugated dienes are slightly longer than those of ethylene, whereas the central C-C bond of 1,3-butadiene is considerably shorter than the single bond of ethane. This signifies that the C-2-C-3 bond of 1,3-butadiene has an intermediate value (147 pm) between a pure single bond (154 pm) and a pure double bond (133 pm) and possesses, therefore, a partial double-bond character.

The type of orbital interaction when the p orbitals are delocalized over the entire π system is called π,π conjugation.

p,π Conjugation

Another type of conjugation exists in compounds with a fragment $>C=CH-X$, where \times is an atom possessing a lone pair of electrons, namely, oxygen of the hydroxyl and alkoxy groups, nitrogen of the amino group, or halogens. In this case three orbitals are delocalized, two p orbitals of the double bond and one p orbital of the atom \times (Fig..2).

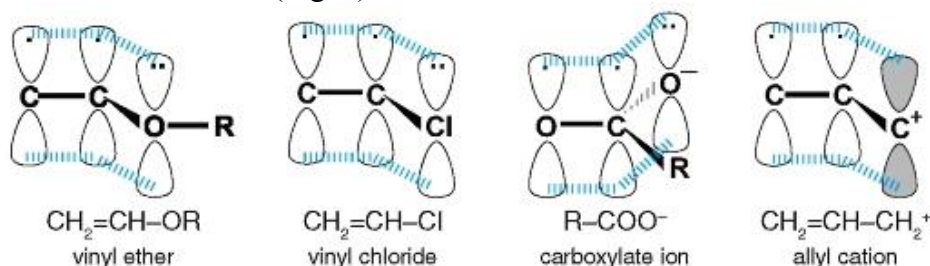


Figure 2. p,π -conjugated systems.

The overlap of a p orbital on an atom adjacent to a double bond is called p,π conjugation.

p,π -Conjugated systems can be either neutral compounds, or free radicals, or ions (both cations and anions). An allylic cation is exemplified in Fig..2 showing conjugation of an empty p orbital of positively charged carbon with p orbitals of the double bond. We can now more accurately describe a conjugated system as follows:

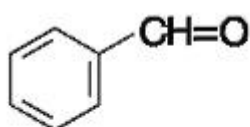
The conjugated system is one that consists of an extended series of overlapping p orbitals.

As we will see in further chapters, this phenomenon explains not only extra stability, but also specific chemical properties of conjugated molecules, ions, or radicals. The concept of conjugation is extremely useful in *understanding* chemical and biochemical processes.

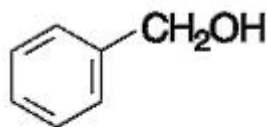
Problem 2. State a type of conjugation (the π,π or p,π), if any, in the following molecules: (a) $CH_3CH_2-CH=O$; (b) $CH_2=CH-O-CH_3$; (c) $CH_3CH=CHCH_2CH=O$; (d) $CH_2=CH-CH_2OH$.

3.2. AROMATICITY

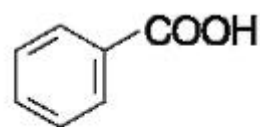
The term *aromatic* was originally applied to relatively simple compounds because of their specific odours. Most of the first aromatic compounds were isolated from balsams, resins, or essential oils, for example, benzaldehyde from bitter almonds, benzyl alcohol and benzoic acid from gum benzoin, and vanillin from vanilla



benzaldehyde



benzyl alcohol

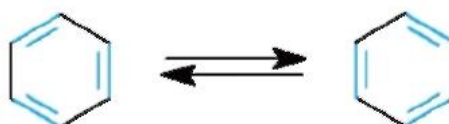


benzoic acid

All these compounds and many other flavoured substances are derivatives of the parent hydrocarbon *benzene*, C_6H_6 . Nowadays, however, we use the term *aromatic compounds* not because of their odour, but because of their specific reactivity that can be explained by electronic structure of benzene.

1.2. Benzene

Benzene, according to the molecular formula C_6H_6 , must be a highly unsaturated compound and we might expect a substantial similarity in the reactivity of benzene and alkenes. However, as we will see in Chapter 7, these hydrocarbons differ markedly in their reactivity. Nevertheless, F.A. Kekule (in 1865) proposed the first structure of benzene as a cyclic compound with three double bonds. To explain chemical diversity of benzene and unsaturated hydrocarbons, Kekule suggested equilibrium between two forms of benzene that results in *rapid* exchange of the positions of the double bonds:



the Kekulé structures for benzene

1.3. Modern Theories of the Structure of Benzene

Physical measurements show that benzene is a flat, symmetrical molecule with a shape of regular hexagon with all carbon-carbon bond lengths of 140 pm (compare this value with bond lengths of 147 and 134 pm for the C-C and C=C bonds in conjugated dienes, respectively). All carbons are sp^2 -hybridized, as in ethylene. Two sp^2 orbitals form σ bonds with adjacent carbons and the third sp^2 orbital of each carbon forms the C-H bond (Fig. 3, a). In addition, each carbon has a p orbital containing the fourth valence electron. All six p orbitals are perpendicular to the plane of the six-membered carbon framework (Fig. 3, b).

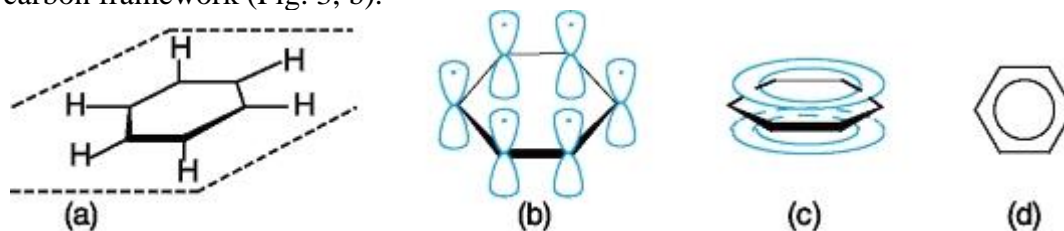


Figure 3. Benzene structure: a planar σ skeleton (a), p orbital view (b), cyclic conjugation (c), symbolic representation (d). The hydrogen atoms in (b)-(d) are omitted for clarity.

ELECTRONIC EFFECTS IN ORGANIC MOLECULES

As it has been shown earlier, the main types of chemical bonds are either covalent or ionic. We generally meet with covalent bonds in organic compounds because their atoms do not differ greatly in electronegativity.

1.4. Polar and Nonpolar Covalent Bonds

When two atoms of the same electronegativity are combined together the bonding electrons are shared equally forming a *nonpolar* covalent bond. The simplest examples are molecules of hydrogen, oxygen, halogens. But, if two atoms of different electronegativity form a covalent bond, the bonding electrons are not shared equally. In this case electrons are attracted somewhat more strongly by one

atom (which is more electronegative) than by the other. Such a bond, in which electron distribution is unsymmetrical, is called a *polar* covalent bond.

The hydrogen chloride molecule provides an example of a compound with the polar covalent bond. A chlorine atom is more electronegative than hydrogen atom (their electronegativities are 3.0 and 2.1, respectively; see Table 1). This means that the bonding electron pair is shifted to chlorine, which becomes partially negatively charged with respect to the hydrogen atom. A short *arrow*, instead of a dash, is used to indicate the direction of polarity. By convention, electrons move in the direction of the arrow. Partial charges designated as δ^+ or δ^- (should be read «delta plus» or «delta minus») may be shown by the examples of the hydrogen chloride and water molecules:



The C-H bond, which is so common in organic compounds, is relatively nonpolar because carbon and hydrogen have similar electronegativities. Another typical bond in organic chemistry, i. e. a carbon-carbon bond, is also nonpolar when two identical carbons form the bond. The carbons identity means the same type of hybridization or similar environment. For example, the carbon-carbon bond is nonpolar in ethane, $\text{CH}_3\text{-CH}_3$, in ethylene, $\text{CH}_2=\text{CH}_2$, or in ethylene glycol, $\text{HOCH}_2\text{-CH}_2\text{OH}$. There are, however, many instances of polar carbon-carbon bonds to be discussed later.

Other elements occurring in organic compounds are mostly situated in right side of the periodic table. Therefore, such elements as oxygen, nitrogen, and halogens are more electronegative than carbon (Table 1.3). Thus, the electrons in C-O, C-N, and C-Cl bonds are attracted stronger by a more electronegative atom, as it is shown in chloromethane and methanol molecules (the O-H bond in the latter is polarized too, of course).



There are few compounds having a bond between carbon and a less electronegative atom (except for hydrogen), e. g. metal. Inverse polarization of the carbon-metal bond is observed in so-called organometallic compounds. A well-known example is tetraethyllead, $(\text{C}_2\text{H}_5)_4\text{Pb}$, an antiknock additive in leaded petrol.

Localized π bonds of functional groups containing a double or triple bond, such as $>\text{C}=\text{O}$, $>\text{C}=\text{N}$, or $-\text{C}\equiv\text{N}$, are more readily polarized than the corresponding ordinary bonds (C-O or C-N). This can be explained by the fact that π electrons are less tightly held by the nuclei (recall the lateral overlap in the π bond formation). *Curved arrows* are used to show the shifting of the π electrons, as it is demonstrated in the following examples



The curved arrow is always drawn alongside of the bond starting at its middle (the initial position of electrons).

1.5. Inductive Effect

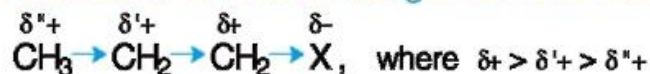
The presence of a polar σ or π bond in an organic molecule results in polarization of neighbouring sites.

The shifting of electrons in a bond in response to electronegativity of nearby atoms is called the inductive effect.

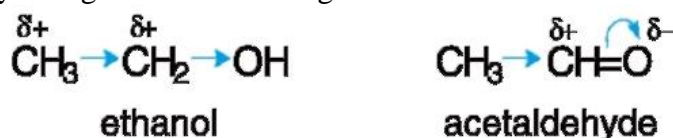
The inductive effect symbolized by the letter *I* can be *electron-withdrawing* (negative, designated *-I*) or *electron-donating* (positive, designated *+I*). In the first case electron density at the

nearby site is decreased, in the second case it is increased. The inductive effect extends to include three (maximum four) bonds owing to low polarizability of C-C bonds. The effect of a substituent is the strongest on the neighbouring atom decreasing along the carbon chain in the following way:

The $-I$ effect of an electronegative substituent X



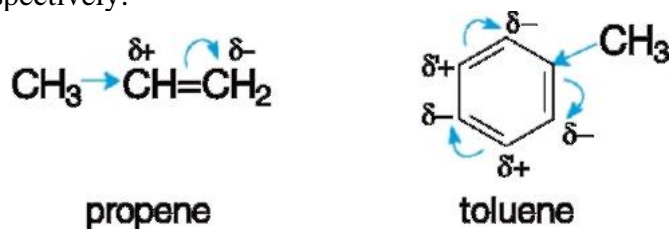
The inductive effect is only a qualitative characteristic of a substituent since it is very difficult to take into consideration all particulars in electron density distribution. Nevertheless, functional groups with double-bonded oxygen reveal a certain strong $-I$ effect. Thus, a partial positive charge on the atom C-1 in acetaldehyde is greater than a charge on the atom C-1 in ethanol:



Other substituents that have strong $-I$ effect are a sulfonyl group, $-\text{SO}_3\text{H}$, a cyano group, $-\text{C}\equiv\text{N}$, a nitro group, $-\text{NO}_2$, and a carboxyl group, $-\text{COOH}$.

Problem 4. Using arrow symbolism show the distribution of electron density in the following molecules: (a) ethylamine, (b) dimethyl ether, (c) propanoic acid, (d) acetone (propanone).

The positive inductive effect is rarely to be observed. It may be attributed to methyl (or other alkyl) group, if only this group is attached to sp^2 -hybridized carbon. The polarity of the C-C bond in this case is accounted for the difference in electronegativity of the sp^3 - and sp^2 -hybridized carbons. So we can say that the methyl group in propene or toluene has the $+I$ effect in regard to the double bond or the benzene ring, respectively:



The $+I$ effect of the methyl group results in appearance of partial charges on carbons of the double bond in propene and on carbons of the benzene ring. It should be noted that the electron-donating methyl group increases electron density on *all* carbon atoms but mainly on the atom C-1 in propene or the atoms C-2, C-4, and C-6 (*ortho* and *para* positions) of the benzene ring according to the arrow head, regardless of the signs δ^+ used. This means the symbols δ^+ and δ^- are relative.

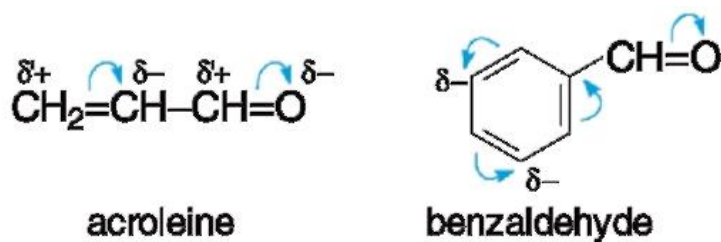
1.6. Mesomeric Effect

A more pronounced electronic effect is observed in molecules having conjugated fragments. The polar effect of a substituent extends in this case through the entire system of conjugation.

The shifting of electron density caused by a substituent in conjugated system through p orbital overlap is called the mesomeric (or resonance) effect.

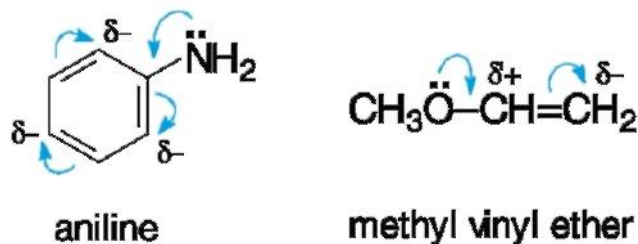
The mesomeric effect symbolized by the letter M can be, like the inductive effect, electron-donating (designated $+M$) or electron-withdrawing (designated $-M$). Let us consider the mesomeric effect in different π,π - and p,π -conjugated systems.

Acrolein (propenal) and benzaldehyde represent π,π -conjugated systems. The aldehyde group in these compounds withdraws the electron density from the C=C double bond or the benzene ring, respectively:



The -M effect of the functional group leads to decreased electron density on all the carbons of the remaining part of the molecule in comparison with unsubstituted compounds (ethylene and benzene). But less electron deficient are the atom C-2 in acroleine as well as the atoms C-3 and C-5 (both *meta* positions) in benzaldehyde as it follows from the heads of the arrows.

The positive mesomeric effect is observed in most p,π -conjugated systems, where a substituent with a lone pair of electrons donates electrons to the neighbouring benzene ring or a π bond. It is demonstrated in the following examples:



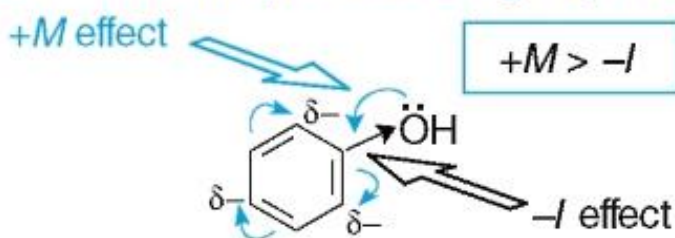
It should be mentioned once more that electron density is increased on all carbons of the benzene ring in aniline as well as on both double-bonded carbons of the unsaturated ether.

As is evident from the above, the mesomeric effect of a substituent can only be observed in the conjugated systems. The substituents with +M effect have an atom that possesses a lone pair of electrons and is *directly* attached to an aromatic ring or to a double bond. Examples of these substituents are hydroxyl, amino, alkoxy (-OR) groups, substituted amino group (-NHR, -NR₂), and halogens.

Conversely, the substituents with -M effect have the general structure -X=Y, where the atom Y is a more electronegative atom than the X one. Many important functional groups fit in this category, namely, a carbonyl group of aldehydes and ketones, a carboxyl group, a nitro group, a sulfonyl group, a cyano group (a single representative with a triple bond).

It may seem surprising that hydroxyl, amino and similar groups belong to electron-donating substituents. Indeed, both oxygen and nitrogen are highly electronegative and would be supposed to inductively withdraw electron density from the aromatic ring (or double bond). In reality, the electron-donating (+M) effect through p,π conjugation is stronger, than the electron-withdrawing (-I) effect through a σ bond.

Electron-donation of the phenolic OH group in conjugation



Problem 5. Indicate the electronic effect of the methoxyl group in anisole, C₆H₅OCH₃. Show partial charges on all carbons of the benzene ring. Compare electron density on the cyclic carbons in anisole and benzene.

Thus, these substituents (with the exception of halogens) possess a net electron-donating property (Table 3.1).

Table 1.

Electronic effects of substituents

Substituent		Electronic effects (I or/and M)		Electron donor (D) or withdrawer (W) (at sp ² -hybridized carbon)
Name	Formula			
Alkyl	CH ₃ , C ₂ H ₅ , etc.	+I	no M	D
Amino	NH ₂ , NHR, NR ₂	-I	+M	D
Hydroxyl	OH	-I	+M	D
Alkoxy	OR	-I	+M	D
Halogens	Cl, Br, I	-I	+M	W
Nitro	NO ₂	-I	-M	W
Carboxyl	COOH	-I	-M	W
Carbonyl	>C=O	-I	-M	W
Cyano	-C≡N	-I	-M	W
Sulfo	SO ₃ H	-I	-M	W

Control materials for the final stage of the class.

Questions to check the final level of knowledge:

1. Bioorganic chemistry as a science: definition, subject and objectives, sections, research methods. Value in the system of higher medical education.
2. Classification of organic compounds by the structure of the carbon radical and the nature of functional groups.
3. Nomenclature of organic compounds: trivial, rational, international. Principles of formation of names of organic compounds according to IUPAC nomenclature: substituents, radical-functional.
4. Nature of chemical bonding in organic compounds: hybridization of orbitals, electronic structure of carbon compounds.
5. Spatial structure of bioorganic compounds: stereochemical formulas. Stereoisomers: geometric, optical.
6. Types of reactions in bioorganic chemistry: classification by result (direction) and mechanism of reaction. Examples.

Literature

Basic:

1. Biological and Bioorganic Chemistry: Bioorganic Chemistry: textbook / B.S. Zimenkovsky, V.A. Muzychenko, I.V. Nizhenkovska, G.O. Syrova. — 3rd edition – 2020. – 288 p.
2. Biological and Bioorganic Chemistry. Biological Chemistry: textbook / Yu.I. Gubsky, I.V. Nizhenkovska, M.M. Korda et al. — 2nd edition – 2021 – 544 p.
3. Bioorganic Chemistry. Rineyskaya O.N. textbook. – 2018. – 174 p.
4. Baynes J., Dominiczak M. Medical Biochemistry. 5th Edition. Elsevier, 2018. 712 p.

Additional:

5. Satyanarayana U. Biochemistry. 5th edition. India 2020. – 777 p.
6. Lehninger. Principles of Biochemistry. 7th edition. NY, United States. 2017.
7. William Marshall, Marta Lapsley, Andrew Day, Kate Shipman. Clinical Chemistry. Elsevier, 2020. 432 p.

Електронні інформаційні ресурси:

1. <https://info.odmu.edu.ua/chair/biology/>
2. <http://libblog.odmu.edu.ua/>
3. <https://moodle.odmu.edu.ua/login/index.php>

Practical class № 2

Topic: Types of chemical reactions. Study of the reactivity of alkanes, alkenes, arenes.

Actuality of theme: Relevance of the topic: Organic medicinal chemistry is one of the scientific disciplines that includes the basic principles of pharmaceuticals, biology and medicine. The main idea of this science is the identification, discovery, development and production of biologically active compounds with the further study of their metabolism at the molecular level and the creation of the structure-activity relationship. All medicinal substances are extracted from natural and artificial raw materials. Resources for the synthesis of organic drugs and auxiliary materials: natural gas, oil, coal, wood and shale. Oil products are valuable raw materials for the synthesis of hydrocarbons, which are the main types of intermediates for the production of full-fledged drugs from organic substances. Vaseline, paraffin and petroleum jelly, which is widely used in medicine, are extracted from oil. Hormone-based drugs are made from animal resources. Various living microorganisms are used for the production of antibiotics. Extremely important are semi-synthetic antibiotics, which are artificial secondary materials for antibiotics that are produced separately from microorganisms (e.g. penicillins). Semi-synthetic agents are also used to produce other categories of drugs: alkaloids, vitamins, hormones. The connection between chemistry and medicine, which originated in ancient times, continues to exist today. The synthesis of new drugs continues, which can play a key role in the treatment of currently incurable diseases. Science continues to develop, and perhaps in the future there will be a cure for such terrible diseases as cancer and AIDS. Without chemistry, medicine would not have reached the heights it is at today.

Aims: To form fundamental knowledge of the main classes of organic chemistry with their further application. To show how deeply chemistry is connected with medicine and everyday life. Necessary for the full assimilation of general chemistry, typical and complex tasks in their content have a professional medical orientation.

Basic concepts: organic chemistry, bioorganic chemistry, reactivity of alkanes, alkenes, arenes.

Equipment: department laboratory

Plan and organizational structure of the lecture:

1. Alkanes. The corresponding series. Structural isomerism, nomenclature. Chemical properties (S_R -reactions).
2. Classification: alkenes, alkynes, alkadienes, types of dienes. Structural and spatial isomerism. Nomenclature. Addition reactions: A_E , A_N reactions.
3. Aromatic hydrocarbons (arenes). Benzenes and their homologues. Isomerism of benzene homologues: ortho-, meta- and para-isomers. Nomenclature.
4. General characteristics of the reactivity of benzene. Reactions of benzene, confirming the non-boundary character, their features.
5. SE -reactions of benzene: halogenation, nitration, sulfation, alkylation, acylation.

Content of the topic

Alkanes. Cycloalkanes

Alkanes are compounds of carbon with hydrogen, in the molecules of which carbon atoms are connected by a single bond (saturated hydrocarbons). The general formula for the homological series of alkanes is C_nH_{2n+2} . The radical resulting from the detachment of one hydrogen atom from a saturated hydrocarbon molecule is called an alkyl, the general formula of alkyls is C_nH_{2n+1} .

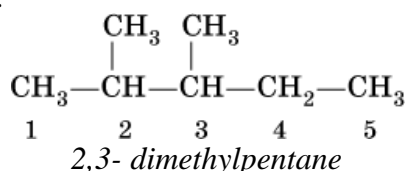
The first four members of a number of alkanes have historically established names.

Molecular formula	Name	Root	Hydrocarbon substituent formula	Name
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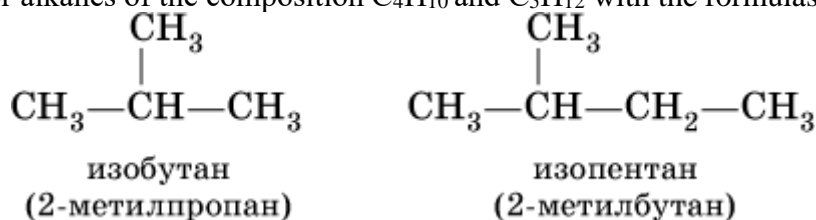
CH ₄	methane	meth-	CH ₃	methyl
C ₂ H ₆	ethane	eth-	C ₂ H ₅	ethyl
C ₃ H ₈	propane	prop-	C ₃ H ₇	propyl
C ₄ H ₁₀	butane	but-	C ₄ H ₉	butyl
C ₅ H ₁₂	pentane	pent-	C ₅ H ₁₁	pentyl
C ₆ H ₁₄	hexane	hex-	C ₆ H ₁₃	hexyl
C ₇ H ₁₆	heptane	hept-	C ₇ H ₁₅	heptyl
C ₈ H ₁₈	octane	oct-	C ₈ H ₁₇	octyl
C ₉ H ₂₀	nonane	non-	C ₉ H ₁₉	nonyl
C ₁₀ H ₂₂	decane	dec-	C ₁₀ H ₂₁	decyl

To give names to alkanes:

1. Choose the longest carbon chain and get the name base.
2. Number the chain so that the substituents get the lowest numbers.
3. In the name, the arabic numerals indicate the position of the deputies, and the prefixes *di* - 2, *tri* - 3, *tetra* - 4, etc. - the number of identical substituents.
4. If there are different substituents, their names are arranged alphabetically, that is, for example, first ethyl, and then methyl.



For some branched saturated hydrocarbons, traditional names are used, along with systematic ones, for example, for alkanes of the composition C₄H₁₀ and C₅H₁₂ with the formulas:

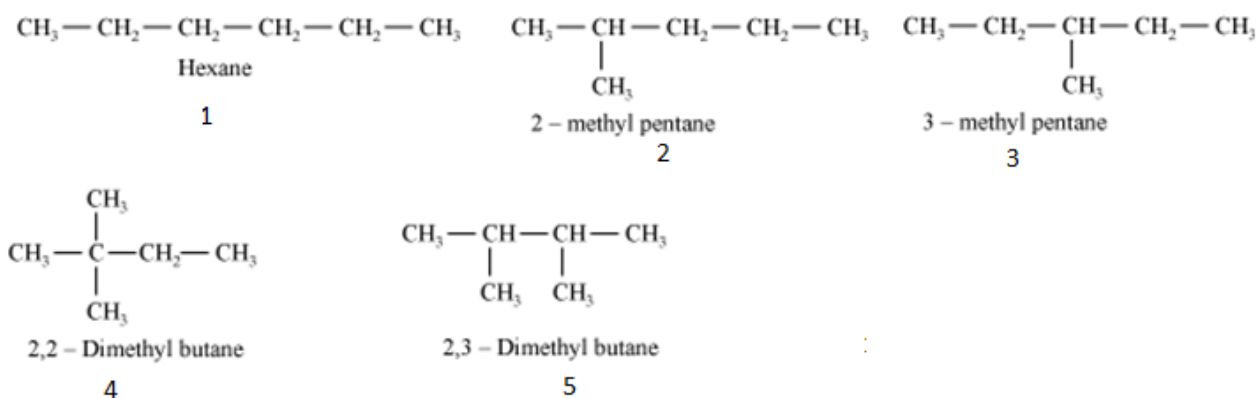


Physical properties

Under standard conditions, the first alkanes — methane, ethane, propane, and butane (C₁—C₄) — are gases without color and odor, sparingly soluble in water. Subsequent homologues (C₅—C₁₅) are liquids (at 20 °C), higher homologues (C₁₆ and higher) are solids.

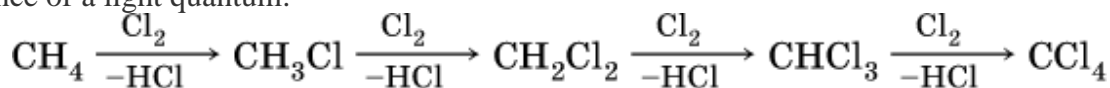
Isomers

Alkanes are non-cyclic saturated hydrocarbons. Molecules are long or branched carbon chains. Homologous alkanes can form isomers. The more carbon atoms, the more variants of isomers. The first three alkanes (methane, ethane, propane) do not form isomers. Butane, pentane, hexane have only structural isomers. Butane has two: n-butane and isobutane. Pentane forms n-pentane, isopentane, neopentane. Hexane has five isomers: n-hexane, isohexane, 3-methylpentane, diisopropyl, neohexane. For example, hexane and its isomers.

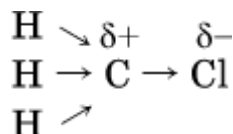


In alkanes, carbon atomic orbitals have sp^3 hybridization; four electron clouds of the carbon atom are directed to the vertices of the tetrahedron at angles of 109.5° . The covalent bonds formed by each carbon atom in the alkanes are not polar.

Therefore, alkanes, which are comparatively inert substances, enter only substitution reactions proceeding with a symmetric (radical) cleavage of C – H bonds. These reactions usually occur in harsh conditions (high temperature, lighting). As a result, it becomes possible to replace hydrogen with halogen (Cl, Br) and nitro group (NO_2), for example, when methane is treated with chlorine in the presence of a light quantum:



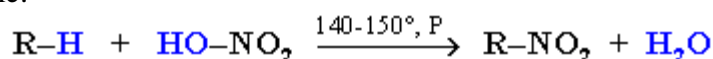
The second and subsequent stages of the reaction proceed more easily than the first, due to a shift in the electron density to the chlorine atom: and an increase in the mobility of the remaining hydrogen atoms.



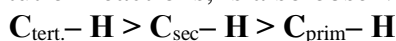
Product names: CH_3Cl - chloromethane, CH_2Cl_2 - dichloromethane, CHCl_3 - trichloromethane (*chloroform*), CCl_4 - carbon tetrachloride (*carbon tetrachloride*).

The Konovalov's reaction. Alkanes react with diluted nitric acid under heating and pressure. As a result, the hydrogen atom is replaced by the remainder of nitric acid - the nitro group NO_2 . This reaction is called the nitration reaction, and the products of the reaction are called nitro compounds.

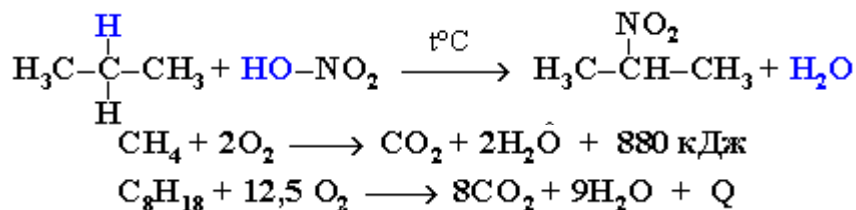
The reaction scheme:



When nitration of alkanes, the order of the reactivity of C-H bonds, characteristic of radical substitution reactions, is also observed:



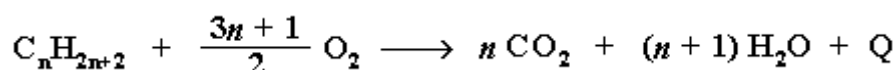
For example:



Combustion and oxidation.

The process of burning hydrocarbons is widely used to generate energy (in internal combustion engines, in thermal power plants, etc.).

The equation of the reaction of combustion of alkanes in general form:



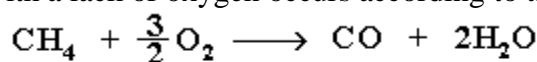
From this equation it follows that with an increase in the number of carbon atoms (n) in the alkane, the amount of oxygen necessary for its complete oxidation increases.

The lower homologues (methane, ethane, propane, butane) form explosive mixtures with air, which must be taken into account when using them.

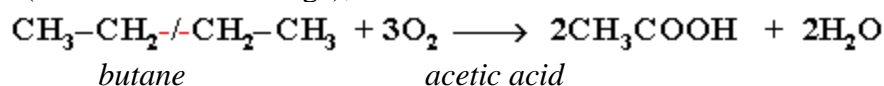
During the combustion of higher alkanes (n >> 1), the oxygen contained in the air may not be enough for their complete oxidation to CO₂. Then the products of partial oxidation are formed:

- *carbon monoxide* CO (oxidation state of carbon +2),
- *carbon black* (fine carbon, zero oxidation state). Therefore, higher alkanes burn in the air with a smoky flame, and the emission of toxic carbon monoxide (odorless and colorless) is dangerous to humans.

Combustion of methane with a lack of oxygen occurs according to the equations:

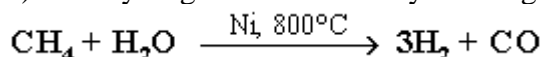


The last reaction is used in industry to produce carbon black from natural gas containing 80-97% methane. Partial oxidation of alkanes at a relatively low temperature and with the use of catalysts is accompanied by the breaking of only part of the C – C and C – H bonds and is used to produce valuable products: carboxylic acids, ketones, aldehydes, alcohols. For example, with incomplete oxidation of butane (C2 – C3 bond cleavage), acetic acid is obtained:



Higher alkanes (n > 25) under the influence of atmospheric oxygen in the liquid phase in the presence of manganese salts turn into a mixture of carboxylic acids with an average chain length of C₁₂–C₁₈, which are used to obtain detergents and surfactants.

The reaction of methane interaction with water vapor is of great importance, as a result of which a mixture of carbon monoxide (II) with hydrogen is formed - "synthesis gas":



This reaction is used to produce hydrogen. Synthesis gas serves as a raw material for the production of various hydrocarbons.

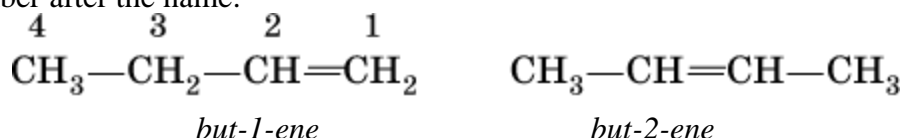
Alkenes. Alkadienes

Alkenes (*olefins*) are hydrocarbons whose molecules contain carbon atoms interconnected by a double bond (*unsaturated hydrocarbons of a number of ethylene*). The simplest representative is ethylene C₂H₄, the general formula for the homologous series of ethylene hydrocarbons C_nH_{2n}.

The systematic *names* of olefins are derived from the roots of the names of alkanes with the replacement of the suffix **-ane** to **-ene**:

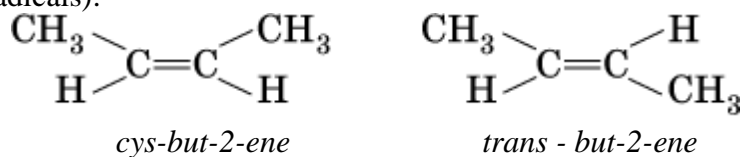
C ₂ H ₄ - Ethene	C ₆ H ₁₂ - Hexene
C ₃ H ₆ - Propene	C ₇ H ₁₄ - Heptene
C ₄ H ₈ - Butene	C ₈ H ₁₆ - Octene
C ₅ H ₁₀ - Pentene	C ₉ H ₁₈ - Nonene

The position of the double bond C = C in the isomers of the structure (starting from C₄ alkene) indicates the number after the name:



Another type of isomerism in unsaturated hydrocarbons, in addition to structural isomerism, is because the carbon atoms forming the double bond are in the sp² hybrid state; The π-component of the C=C double bond and the C–H double bond lie in the same plane at an angle of 120° to each other, and the σ component of the C=C double bond is an electron cloud elongated in the direction

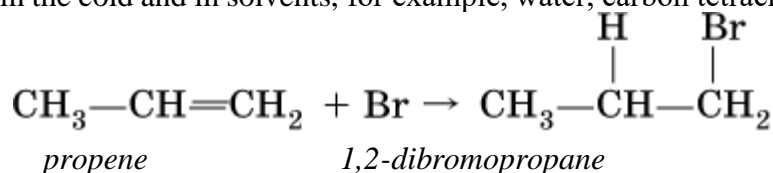
perpendicular to the plane of the σ -bonds. The consequence of this structure of alkenes is the possibility of geometric isomerism (or cis-trans isomerism) depending on the position of the substituents (atoms or radicals):



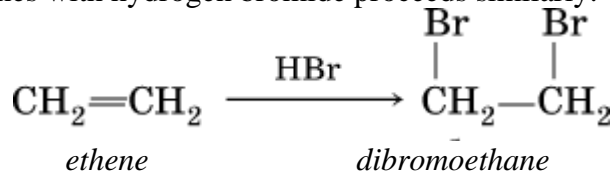
(*cis* - from lat. "nearby, on one side", *trans* - from lat. "opposite, on opposite sides").

Alkenes $C_2 - C_4$ at room temperature are colorless gases with a faint smell of oil, sparingly soluble in water; $C_5 - C_{18}$ alkenes are liquids; C_{19} alkenes and higher are solids.

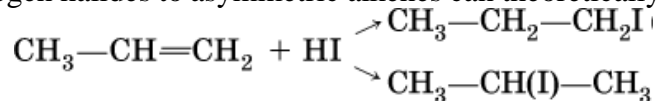
The most important chemical properties of alkenes are determined by the fact that, due to the lower strength of the π -bond (as compared to the σ -bond), it easily breaks, as a result of which addition reactions occur and saturated organic compounds are formed. As a rule, such reactions proceed under mild conditions, often in the cold and in solvents, for example, water, carbon tetrachloride CCl_4 , etc.:



The interaction of alkenes with hydrogen bromide proceeds similarly:



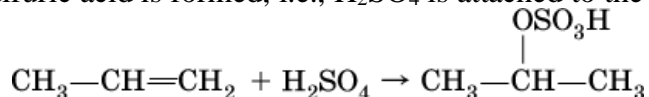
The addition of hydrogen halides to asymmetric alkenes can theoretically lead to two products:



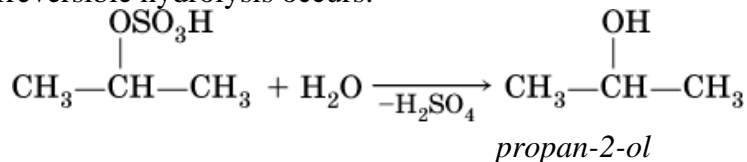
According to Markovnikov's rule, the addition of hydrogen halides to asymmetric alkenes proceeds so that the hydrogen is directed to the carbon atom, which already contains a larger number of hydrogen atoms. In the above reaction, the product will be 2-iodopropane $\text{CH}_3\text{CH}(\text{I})\text{CH}_3$.

According to Markovnikov's rule, a hydration reaction also takes place, i.e., a water addition reaction in the presence of sulfuric acid. It occurs in two stages:

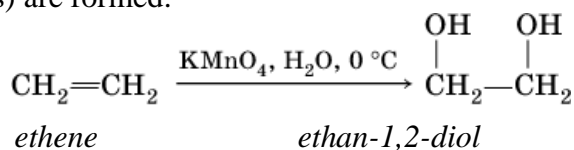
- a) first, alkylsulfuric acid is formed, i.e., H_2SO_4 is attached to the alkene:



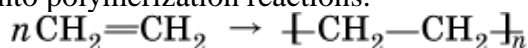
- b) then its irreversible hydrolysis occurs:



Alkenes discolor a solution of potassium permanganate in the cold in a neutral environment, and glycols (dihydric alcohols) are formed:



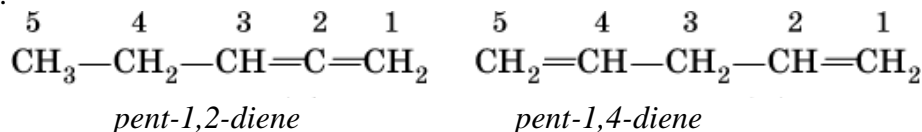
Alkenes are able to enter into polymerization reactions:



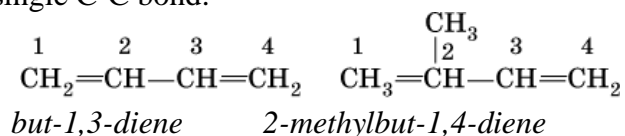
Qualitative reactions to alkenes - discoloration of bromine water and KMnO_4 solution (reaction equations, see above).

Alkadienes are unsaturated hydrocarbons whose molecules contain two $\text{C}=\text{C}$ bonds. The general formula of alkadienes is $\text{C}_n\text{H}_{2n-2}$ ($n > 3$), the formula coincides with that for alkynes.

Examples:



The conjugated dienes have the great practical importance, in the molecules of which the $\text{C}=\text{C}$ bonds are separated by a single $\text{C}-\text{C}$ bond:



Alkynes

Alkynes are hydrocarbons with a $\text{C}\equiv\text{C}$ **triple bond** in molecules (unsaturated hydrocarbons of the acetylene series). The simplest representative of this series is acetylene C_2H_2 , the general formula of alkynes is $\text{C}_n\text{H}_{2n-2}$ (for $n > 2$).

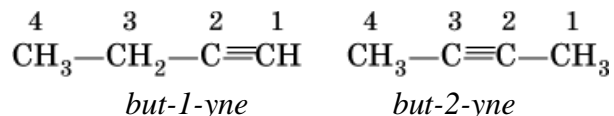
The names of the simplest alkynes:

C_2H_2 - ethyne (traditionally: acetylene)

C_3H_4 - propyne (methylacetylene)

C_4H_6 - butyne

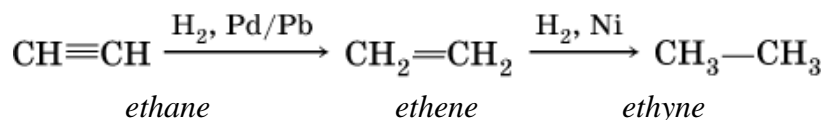
Butyne isomers:



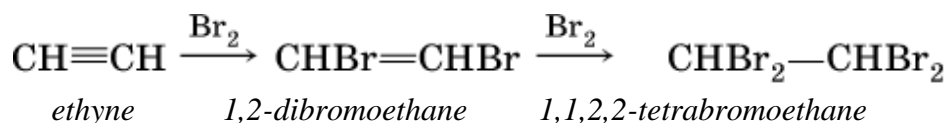
Acetylene, propyne and butin-1 are colorless gases at room temperature, butin-2 is a low-boiling liquid, has a slight "ethereal" odor.

In alkynes, the carbon atomic orbitals of the triple bond have sp hybridization (linear structure). The presence of two π -bonds determines their chemical properties, in particular, their high ability to reactions of stepwise addition of hydrogen, chlorine, bromine, hydrogen halides, water:

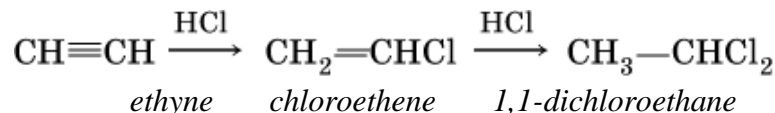
a)



b)

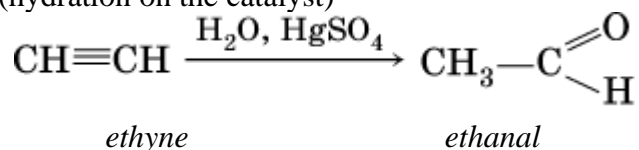


c)

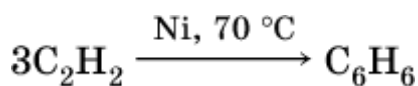


(the addition of HCl to chloroethene occurs according to the Markovnikov rule; chloroethene is traditionally called chlorovinyl or vinyl chloride);

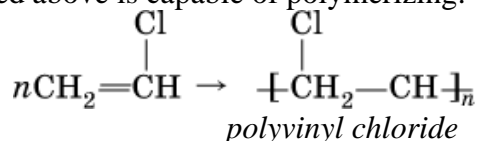
d) Kucherov reaction (hydration on the catalyst)



During the cyclization of acetylene, benzene is formed:



The vinyl chloride mentioned above is capable of polymerizing:

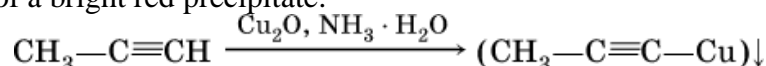


Polyvinyl chloride (PVC) - a polymer, the basis of plastic, fibers and films, used in the manufacture of pipes, artificial leather, electrical insulation, foams.

Qualitative reactions:

1) for alkynes of any structure – discoloration of the KMnO_4 solution; most often, the carbon chain breaks at the site of the triple bond (with alkenes);

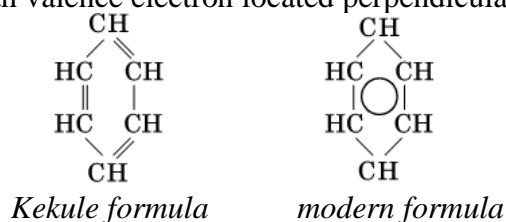
2) to alkynes with a terminal triple bond – substitution of the terminal hydrogen atom by copper (I) with the formation of a bright red precipitate:



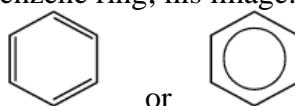
Arenas

Arenas are unsaturated hydrocarbons that can be considered as derivatives of the simplest of them – C_6H_6 benzene. The general formula of hydrocarbons is the homologous series of benzene $\text{C}_n\text{H}_{2n-6}$.

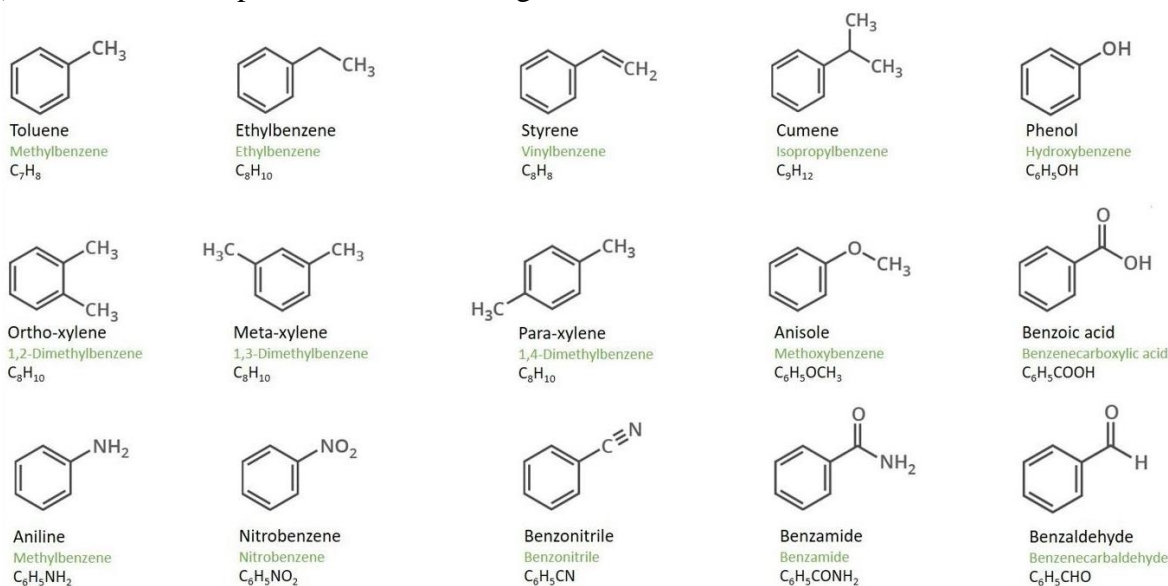
In the benzene molecule, all carbon atoms are in sp^2 hybridization, each carbon atom is connected in one plane by σ bonds with two other carbon atoms and one hydrogen atom. The carbon atom still has a cloud of the fourth valence electron located perpendicular to the plane.



The Kekule formula is often used in cases where it is necessary to more clearly visualize the reaction with the participation of the C_6 benzene ring; his image:



In both formulas, C ring atoms and H atoms not participating in the reaction are omitted (for brevity). Some of the simplest benzene homologues are:

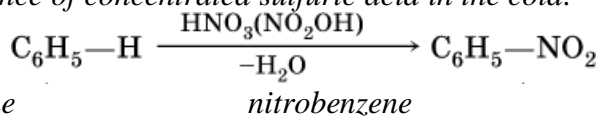


The C_6H_5 benzene radical is called *phenyl*, the $\text{C}_6\text{H}_5\text{CH}_2$ toluene radical is called *benzyl*.

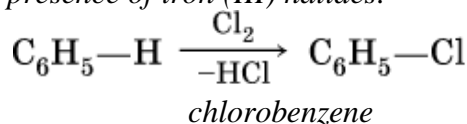
Benzene and its closest homologues are fluids without color, but with a characteristic odor, have a wide range of liquid state. Almost insoluble in water, but mix well with each other and with other organic solvents. Benzene vapor is highly toxic.

Despite the formal unsaturation, benzene is highly resistant to heat and oxidation (only the side chain is oxidized in benzene homologs). Substitution reactions are characteristic of benzene:

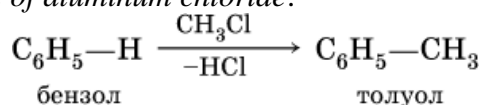
a) nitration in the presence of concentrated sulfuric acid in the cold:



b) halogenation in the presence of iron (III) halides:



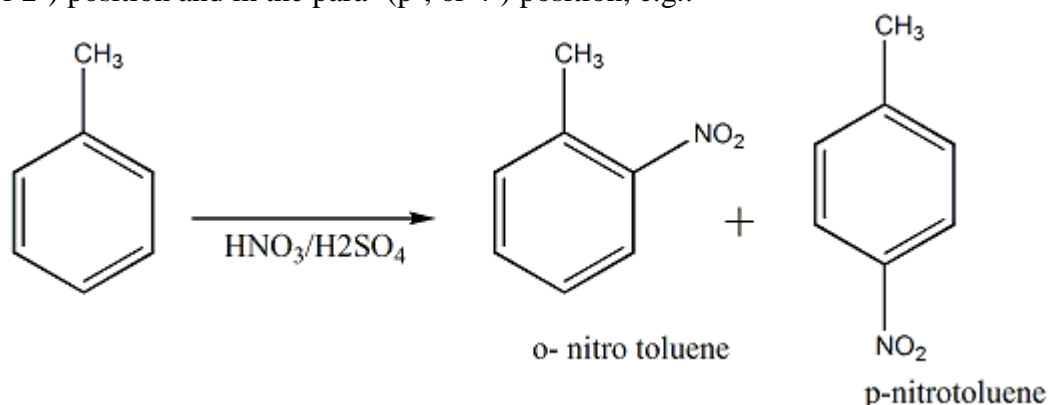
c) alkylation in the presence of aluminum chloride:



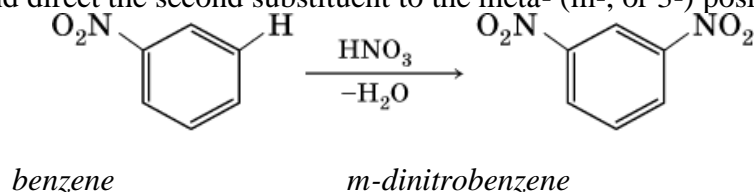
The special nature of the unsaturation of benzene and its homologs is illustrated by these chemical properties and is called the "aromatic" nature.

In benzene derivatives, the atom or group that replaces the hydrogen rings and the benzene ring itself affect each other. By the nature of the influence, they distinguish:

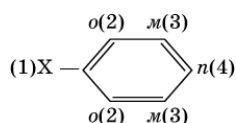
1) substituents of the first kind are -Cl, Br, I, CH₃, C_nH_{2n+1}, OH and NH₂. They facilitate the reactions of further substitution and direct the second substituent with respect to themselves in the ortho- (o-, or 2-) position and in the para- (p-, or 4-) position, e.g.:



2) Type II substituents — NO₂, C(H)O, COOH and CN. They complicate the reactions of further substitution and direct the second substituent to the meta- (m-, or 3-) position, for example:

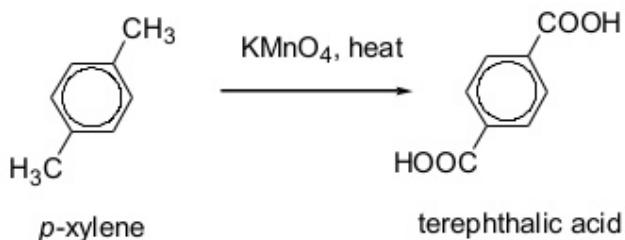
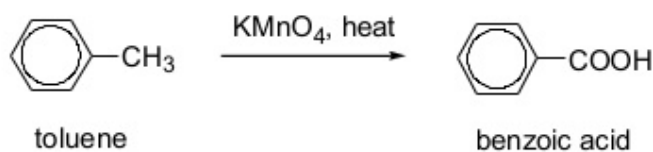


Obviously, there are two ortho positions next to the first substituent X, two meta positions separated from the first substituent by one carbon ring, and only one para position through two carbon atoms of the benzene ring:

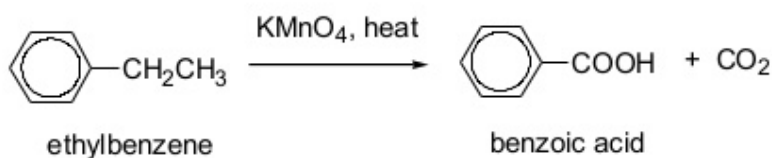


It has already been noted that benzene is resistant to oxidation even under the influence of strong oxidizing agents. Homologues of benzene with one side radical enter into oxidation reactions

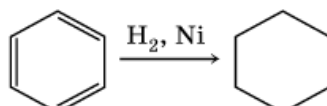
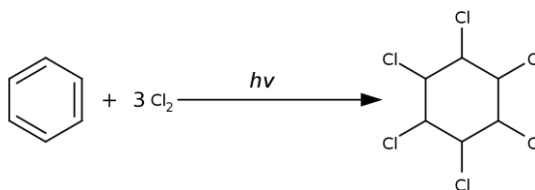
only due to the radical; at the same time, whatever its length, the whole chain is split off, except for the carbon atom closest to the ring (it creates a carboxyl group):



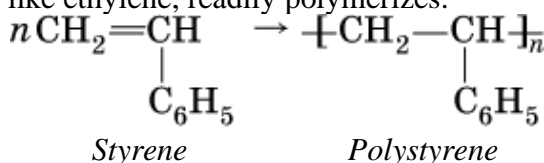
note: aromatic acids only!



Under severe conditions, benzene enters into the addition reaction:



Styrene $\text{C}_6\text{H}_5\text{—CH=CH}_2$, like ethylene, readily polymerizes:



Polystyrene is a thermoplastic plastic (thermoplastic), a transparent material that softens at temperatures above 80 °C. It is used for the manufacture of insulation of electrical wires, disposable tableware, packing mass (foam plastic).

Control materials for the final stage of the class.

Questions to check the final level of knowledge:

1. Chemical properties of alkanes.
2. Chemical properties of alkenes.
3. Qualitative reactions for the determination of alkenes.
4. Polymerization reaction.
5. Chemical properties of alkynes.
6. Substitution reactions in benzene.

Literature

Basic:

1. Biological and Bioorganic Chemistry: Bioorganic Chemistry: textbook / B.S. Zimenkovsky, V.A. Muzychenko, I.V. Nizhenkovska, G.O. Syrova. — 3rd edition – 2020. – 288 p.
2. Biological and Bioorganic Chemistry. Biological Chemistry: textbook / Yu.I. Gubsky, I.V. Nizhenkovska, M.M. Korda et al. — 2nd edition – 2021 – 544 p.
3. Bioorganic Chemistry. Rineyskaya O.N. textbook. – 2018. – 174 p.
4. Baynes J., Dominiczak M. Medical Biochemistry. 5th Edition. Elsevier, 2018. 712 p.

Additional:

1. Satyanarayana U. Biochemistry. 5th edition. India 2020. – 777 p.
2. Lehninger. Principles of Biochemistry. 7th edition. NY, United States. 2017.
3. William Marshall, Marta Lapsley, Andrew Day, Kate Shipman. Clinical Chemistry. Elsevier, 2020. 432 p.

Електронні інформаційні ресурси:

1. <https://info.odmu.edu.ua/chair/biology/>-
2. <http://libblog.odmu.edu.ua/>
3. <https://moodle.odmu.edu.ua/login/index.php>

Practical class № 3

Topic: Structure and properties of hydroxo- and oxo-compounds. Biologically active hydroxo- and oxo-compounds.

Relevance of the topic: The materials of the topic have an important role in the study of the course of bioorganic, biological chemistry and pharmacology. Hydroxyl and carbonyl groups are present in many biomolecules - carbohydrates, lipids, natural amino-, hydroxy-, keto acids, vitamins, steroid hormones, heterocyclic compounds. Their reactivity determines the biotransformation of digestive products, drug metabolism, oxidative catabolism of carbohydrates, lipids and other compounds in the presence of oxygen.

Goal: to have an idea of the classification, isomerism, reactivity of oxygen-containing bioorganic compounds. To learn the structure of alcohols, phenols, aldehydes and ketones. Understand the biological role of these compounds, their use in medical practice and their effect on the human body.

Equipment: department laboratory

Plan and organizational structure of the class:

1. Classification, nomenclature and isomerism of alcohols and phenols.
2. Comparative characteristics of acid properties of alcohols and phenols.
3. Chemical properties of alcohols (dehydration reactions of alcohols, nucleophilic substitution reactions in alcohols (S_N), features of oxidation reactions of primary, secondary and tertiary alcohols, esterification reactions).
4. Electrophilic substitution reactions in phenols (S_E).
5. Nomenclature and isomerism of aldehydes and ketones.
6. Nucleophilic addition reactions to oxo compounds.
7. Aldol condensation and its importance for carbon chain elongation.
8. Oxidation of aldehydes and ketones.
9. Medico-biological significance of hydroxo- and oxo-compounds.

The higher education applicant should know and be able to:

- a) classification of oxygen-containing organic compounds;

- b) show the electronic effects in the molecules of hydroxo- and oxo-compounds, determine the reaction centers, types of bond breaking and the electronic nature of the resulting particles;
- c) types of chemical reactions depending on the conditions of the process and the nature of the reagent;
- d) explain the difference between acid-base properties of alcohols and phenols;
- e) chemical properties of hydroxo- and oxo-compounds;
- f) perform qualitative reactions that are characteristic of hydroxo- and oxo-compounds.

Content of the topic

1.1. Alcohols, phenols.

Alcohols and phenols are compounds that have a hydroxyl group, -OH, which is attached to a saturated carbon in alcohols or to an aromatic ring in phenols. They have the general formulas R-OH and Ar-OH, respectively.

Compounds of these classes occur widely in nature. A hydroxyl group is literally the most widespread functional group of naturally occurring compounds. The simplest alcohols have many industrial applications as solvents and raw materials. Ethanol is present in alcoholic drinks.

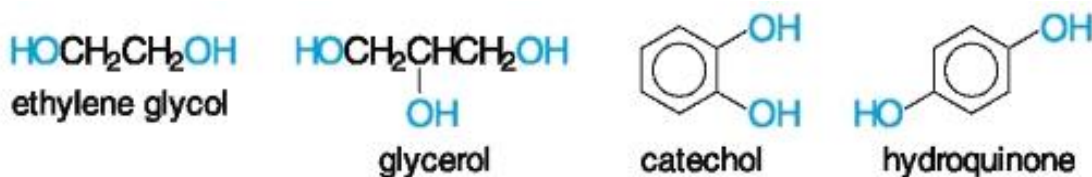
Thiols are sulfur analogues of alcohols of the general formula R-SH; their functional group is a mercapto group, -SH.

Classification and Nomenclature

Several classifications of alcohols exist. According to the first one, alcohols are classified as primary, secondary, or tertiary, depending on the number of organic groups bonded to the hydroxyl-bearing carbon atom. Another classification takes into account the nature of a hydrocarbon part of the alcohol molecule. According to this, alcohols fall into saturated, unsaturated, or aromatic ones (an aromatic ring in the latter is not directly bonded to the hydroxyl group). A peculiar type of unsaturated alcohols represents *enols*, unstable compounds which contain the hydroxyl group attached to an sp^2 -hybridized carbon, i. e. a fragment =CH-OH. The examples below show various types of alcohols:

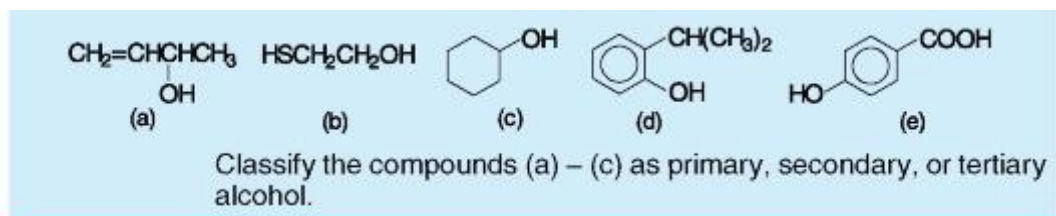
Primary	Secondary	Tertiary	Unsaturated	Aromatic	Enolic
$(\text{CH}_3)_2\text{CHCH}_2\text{OH}$	$(\text{CH}_3)_2\text{CHOH}$	$(\text{CH}_3)_3\text{COH}$	$\text{CH}_2=\text{CHCH}_2\text{OH}$	$\text{C}_6\text{H}_5\text{CH}_2\text{OH}$	$[\text{CH}_2=\text{CHOH}]$
2-methyl-1-propanol (isobutyl alcohol)	2-propanol (isopropyl alcohol)	2-methyl-2-propanol (tert-butyl alcohol)	2-propen-1-ol (allyl alcohol)	phenylmethanol (benzyl alcohol)	ethenol (vinyl alcohol)

There are also alcohols and phenols that have two or more hydroxyl groups. Alcohols containing two hydroxyl groups are classified as *glycols*. The generic systematic name *diol* is used for dihydroxyl alcohols. The most known examples and trivial names accepted in the IUPAC system are given below:



In the IUPAC substitutive nomenclature of alcohols, the hydroxyl group is indicated by the suffix -ol, with elision of terminal -e (if present) from the name of the parent compound. The radicofunctional nomenclature is also used to relatively simple alcohols as shown in the above examples (in parentheses). As regard to nomenclature of phenols, the word phenol is the name both of a class of compounds and of a specific compound $\text{C}_6\text{H}_5\text{OH}$. The hydroxyl group is named as the prefix hydroxy- when it occurs in a heterofunctional molecule with substituents of higher priority (Table 2).

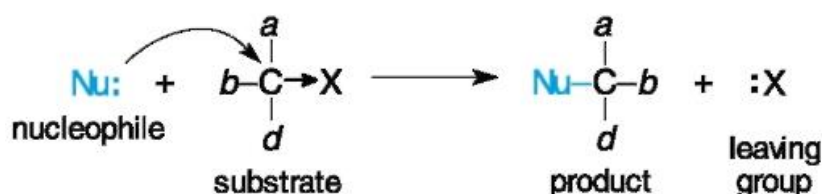
The same principles of classification and nomenclature are applied to thiols.



1.2. Nucleophilic Substitution Reactions

Nucleophilic substitution at saturated carbon is one of the simplest and, at the same time, the most important type of organic reactions. It may also be characterized as the *alkylation* reaction with reference to the nucleophile. Many reactions of biological importance represent nucleophilic substitution.

In this reaction, one covalent bond is broken, and a new bond is formed. The reaction can be expressed in the following general equation, where *a*, *b*, *d*, and \times are atoms or groups attached to the electrophilic carbon:



In the overall transformation, the C-X bond is ruptured in such a way that a pair of electrons that formed the bond becomes associated with a group \times called the *leaving group*. The nucleophile possesses an unshared (non-bonding) pair of electrons and uses them to form a new bond to the carbon.

A typical nucleophilic substitution reaction is alkaline hydrolysis of halides, to give an alcohol and a halide ion, for example:

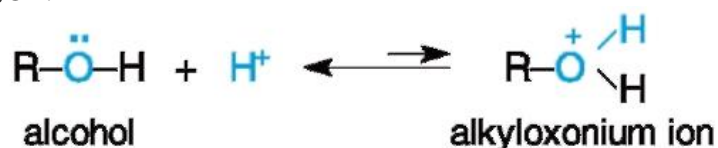


In this reaction a nucleophilic hydroxide ion attacks the substrate (methyl bromide) and expels a bromide ion as the leaving group.

Nucleophiles and Substrates. Compounds of different classes may be considered as nucleophiles. Both neutral molecules and anions can serve as nucleophilic reagents. They are classified according to the kind of an atom that forms a new bond to the electrophilic carbon. The most common nucleophiles are oxygen, nitrogen, sulfur, halogen, and carbon nucleophiles. Table 1 lists some nucleophilic reagents and shows a vast variety of products that they form in the reaction with alkyl halides. It should be noted once more that aryl halides and vinyl halides undergo this type of nucleophilic substitution with difficulty, if at all.

1.3. Acidic and Basic Properties

Acidic properties of alcohols, phenols and thiols were discussed quite enough in Chapter 5. In the same chapter these classes, especially thiols and phenols, were shown to be weak Bronstedt bases. Nevertheless, alcohols are reversibly protonated by strong acids to give a salt-like product, analogous to the hydronium ion, H_3O^+ :



This interaction is the first step in two important reactions of alcohols, namely, nucleophilic substitution and elimination, which will be considered in the following sections.

Being weakly acidic and weakly basic, alcohols, phenols and thiols belong to *amphoteric* compounds which, like water, form hydrogen bonds. The most pronounced intermolecular hydrogen bonding is in alcohols.

Hydrogen bonds account for exceptionally high boiling points of alcohols. Table 2 demonstrates boiling points of some lowest alcohols in comparison with those of alkanes, halogenoalkanes, and thiols. The molecules of a close size occupy one line in the table, yet alcohols have the boiling points by 20-60 °C higher than thiols, by 40-80 °C higher than chloroalkanes, and by 80-150 °C higher than alkanes.

Due to hydrogen bonding to water molecules, the lower alcohols (C₁-C₃ and *tert*-butyl alcohol) are completely miscible with it. Phenols show appreciable solubility in water. Solubility in water gradually decreases as the hydrocarbon portion of the molecule lengthens. Such compounds become less polar that results in better solubility in hydrocarbons and other nonpolar solvents¹. Water solubility of organic substances is of interest to us since water is the medium of the cells of all living organisms.

Table 2.

Boiling points of alkanes, chloroalkanes, alcohols, and thiols R-Z (°C)

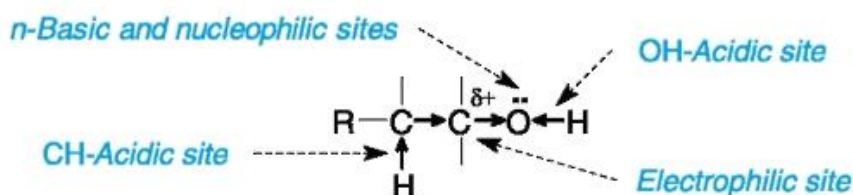
Alkyl group R	Alkane (Z = CH ₃)	Chloroalkane (Z = Cl)	Alcohol (Z = OH)	Thiol (Z = SH)
CH ₃ -	-88.6	-23.8	64.5	6.0
CH ₃ CH ₂ -	-42.1	12.3	78.4	35.0
CH ₃ CH ₂ CH ₂ -	-0.5	46.6	97.4	67.6
(CH ₃) ₂ CH-	-11.7	35.7	82.4	52.6
CH ₃ CH ₂ CH ₂ CH ₂ -	36.1	78.4	117.4	98.6

It was well known to ancient Greeks that *similar subjects dissolve similar subjects*.

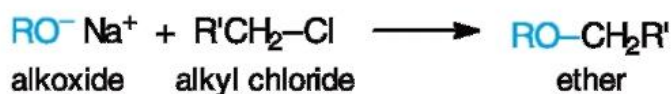
1.4. Electrophilic and Nucleophilic Properties

Though the functional groups of alcohols, phenols and thiols are similar in many respects, electrophilic and nucleophilic properties of these classes differ substantially and, therefore, are considered for each class separately.

Alcohols. In addition to the OH-acidic and basic sites discussed in the previous section, alcohols possess an electrophilic, nucleophilic, and CH-acidic sites shown below:

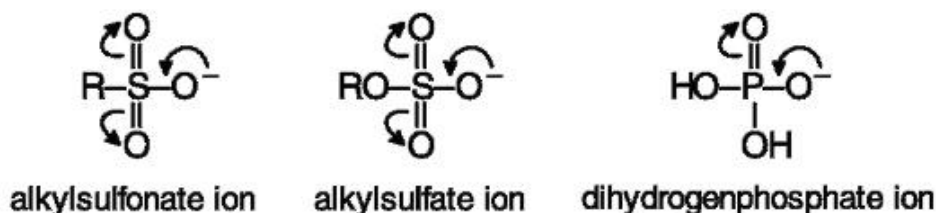


Alcohols are weak nucleophiles owing to high electronegativity of the oxygen atom. An alkoxide ion is a much stronger nucleophile than an alcohol; it reacts with electrophilic substrates such as alkyl halides to form substitution products called *ethers*. This is one of the oldest reactions in organic chemistry known as the *Williamson synthesis* (1851).



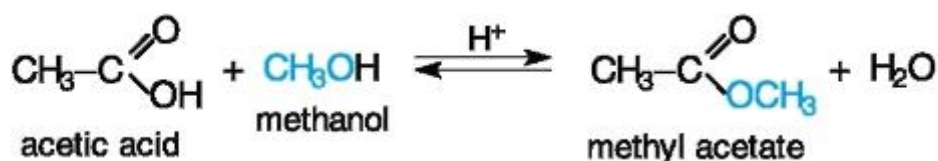
Primary alkyl halides work best, since competitive elimination of hydrogen halide can occur with secondary and tertiary halides (as shown in Example 3).

Besides halide ions there are other good leaving groups such as alkyl and aryl sulfonates RSO_3^- , alkyl sulphates ROSO_3^- , and dihydrogenphosphate H_2PO_4^- . The three anions are well stabilized due to conjugation:

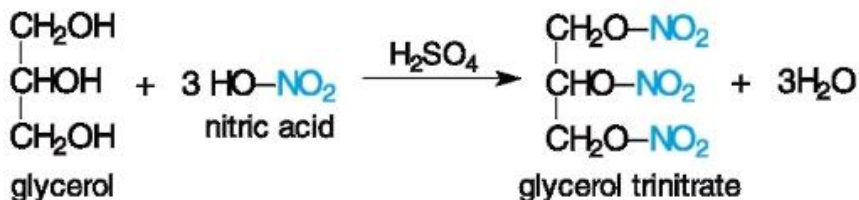


Sulfates and sulfonates are mostly used in laboratory practice; the phosphate ion is an excellent leaving group in biological substrates.

Alcohols as nucleophiles react with carboxylic acids and their derivatives by replacing the OH group of an acid to form *esters*. With respect to an alcohol this reaction is classified as *acylation*, or introduction of an *acyl group*, $\text{R}-\text{C}(\text{O})-$. Only the principal equation is given here, the details of the reaction are dealt.

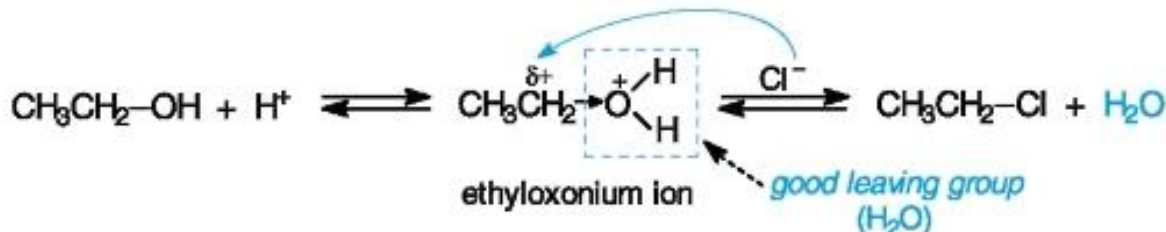


Alcohols also react with oxygen-containing inorganic acids (sulfuric, nitric, nitrous, boric), for example:



Glycerol trinitrate is, perhaps, the most known inorganic ester. It is a powerful explosive, the main brisant component of dynamite invented by Alfred Nobel (1865). Glycerol trinitrate is also used in medicine as a vasodilator.

Like haloalkanes, alcohols are potential substrates in reactions with nucleophilic reagents. However, one must keep in mind that the hydroxyl group is a bad leaving group. Nevertheless, it is possible to substitute the OH group after its conversion into protonated (oxonium) form. Thus the protonated substrate is much more reactive than the neutral alcohol. A product is readily formed because of expelling a better leaving group, a water molecule.

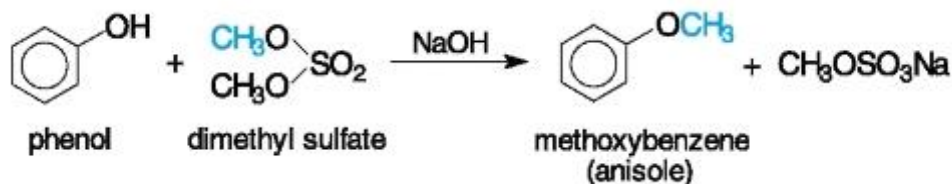


Primary alcohols undergo such substitution for alkyl halides. The reaction proceeds slowly on heating an alcohol with concentrated hydrochloric acid. Tertiary alcohols, on the contrary, easily react at room temperature to give the corresponding halide.

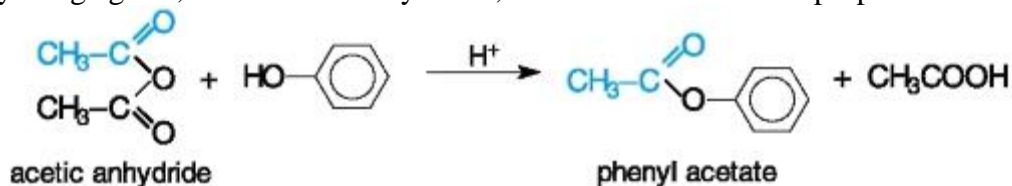


1.5. Phenols.

Nucleophilic strength of phenols is even lower than that of alcohols in a neutral medium. But in alkaline media phenols are easily converted into phenoxide ions that possess an increased nucleophilic activity. Thus phenols can be alkylated with alkyl halides or other alkylating agents, for example:



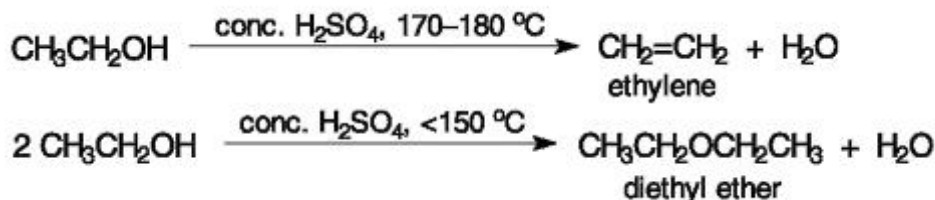
In contrast to alcohols, phenols do not form esters in the reaction with carboxylic acids. More powerful acylating agents, such as acid anhydrides, have been used for this purpose:



Again in contrast to alcohols, in phenols the C-O bond is difficult to break and replace the hydroxyl group, for instance, by a chlorine atom. The reason is that this bond is about 50 kJ/mol stronger than the Csp³-O bond in alcohols. This can be explained by *p*, π conjugation of the oxygen lone pair of electrons with the benzene ring.

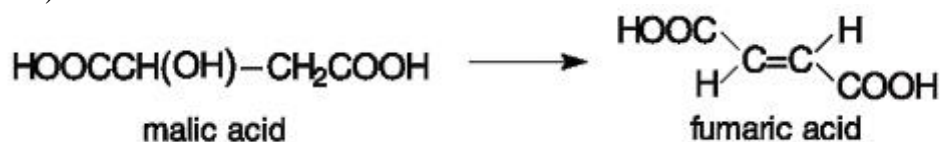
1.6. Elimination Reactions

Alcohols can eliminate a water molecule on heating with a strong acid to yield alkenes. The reaction is classified as *dehydration*. Ethers, as by-products of a competitive substitution reaction, are produced only in a small amount. Ethers are, however, the main products when the reaction is carried out at lower temperatures. For example:



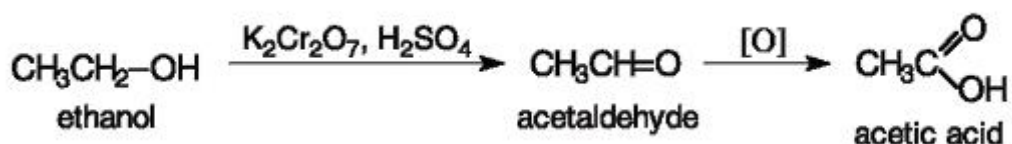
Acid-catalyzed dehydration usually follows the Zaitsev's rule and yields predominantly the more substituted alkene. The ease of alcohol dehydration is the same as the order of carbocation stability, i. e. primary < secondary < tertiary.

Elimination is a well-known *in vivo* reaction. For example, enzymic dehydration of malic acid (a hydroxy acid) results in the formation of fumaric acid:

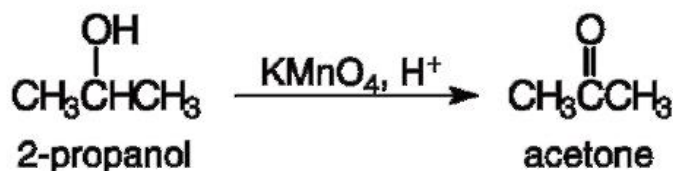


1.7. Oxidation Reactions

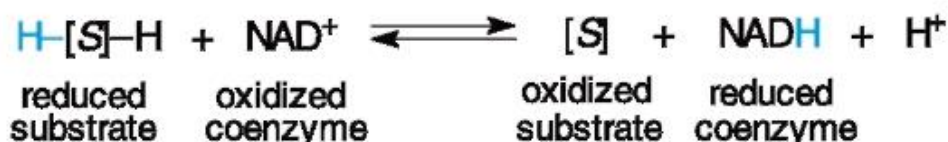
Oxidation of alcohols. Primary and secondary alcohols can be oxidized with potassium permanganate or potassium dichromate, K₂Cr₂O₇, in a dilute acid. Primary alcohols form aldehydes, but further oxidation to carboxylic acids usually occurs:



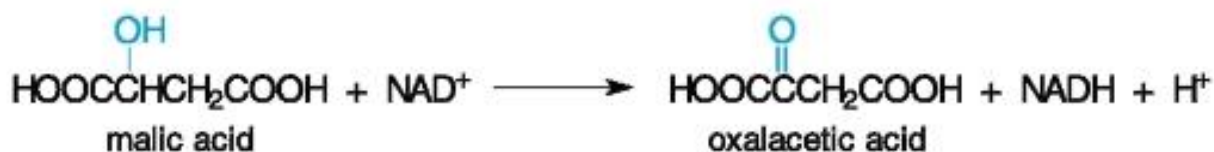
Secondary alcohols are oxidized to ketones. Further oxidation to carboxylic acid does not occur except under severe conditions. Tertiary alcohols are normally resistant to oxidation.



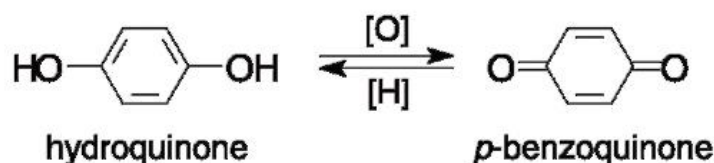
In plants and animals, similar oxidations are accomplished enzymically with a coenzyme *nicotinamide adenine dinucleotide*, abbreviated NAD⁺, and its phosphate, NADP⁺. The role of NAD⁺ formally consists in abstraction of a hydride ion from a substrate schematically shown as H[S]H:



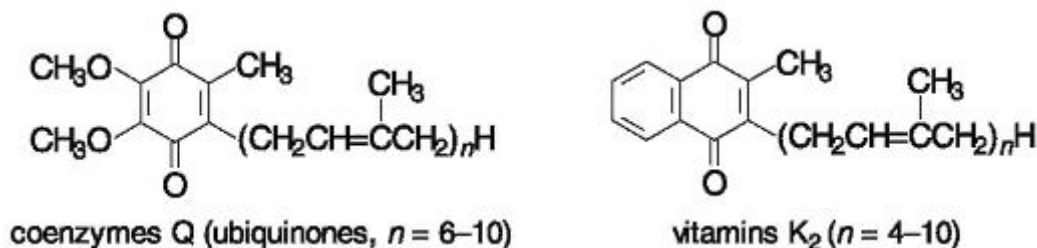
Oxidation of malic acid into oxalacetic acid (an oxo acid) with NAD⁺ in the Krebs cycle is an example of numerous biochemical oxidation reactions:



Oxidation of phenols. Phenolic compounds, especially those containing two or more hydroxyl groups, are susceptible to oxidation, even by atmospheric oxygen. Aromatic 1,2- and 1,4-dihydroxyl compounds are oxidized to cyclic unsaturated diketone known as *quinones*. The most significant property of quinones is their reversible reduction to the corresponding dihydroxybenzenes.



Quinones of many kinds are important compounds both because of their prevalence in nature as the products of plant and animal metabolism and because of their use in medicine. A group of quinones called *coenzymes Q* (or *ubiquinones* which are so named because of their ubiquitous, or widespread occurrence in nature) serves as electron-carriers (oxidizing agents) to mediate the respiration process. Vitamins of group K, which are required for the normal clotting of blood, represent derivatives of 1,4-naphthoquinone.

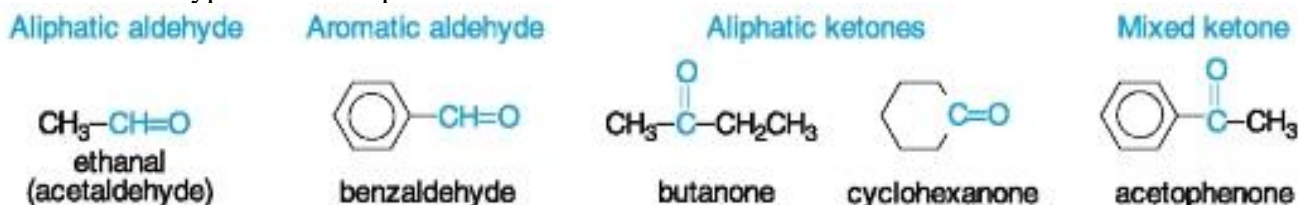


1.8. GENERAL CHARACTERISTICS OF ALDEHYDES AND KETONES

Chemical properties of aldehydes and ketones are very similar regardless of their specific structure. It is no wonder that both of the classes are considered together.

Classification and Nomenclature

Aldehydes may be classified as either aliphatic or aromatic depending on the R substituent. The only exception is the simplest representative - formaldehyde, $\text{HCH}=\text{O}$, where R is hydrogen in the general formula of aldehydes. Ketones are classified in a similar way, except for existence of cyclic ketones and a mixed type of the compounds.



In the IUPAC nomenclature system, the aldehyde group in aliphatic compounds is indicated by the suffix -al, with elision of terminal -e from the name of the parent hydrocarbon. Note that carbon of the aldehyde group is a part of the hydrocarbon structure. The parent name benzaldehyde is used for aromatic aldehydes. Many aldehydes have semi-trivial names derived from the corresponding carboxylic acids and accepted by the IUPAC nomenclature, for example *acetaldehyde* - from *acetic acid* (as shown above), *butyraldehyde*, $\text{CH}_3\text{CH}_2\text{CH}_2\text{CH}=\text{O}$ - from *butyric acid* (C_4 acid), and so on.

Ketones are named (see examples above) using the suffix -one which, actually, designates a group $=\text{O}$ called the *oxo group*. If higher functional group is present in the same molecule, the prefix oxo- is used for the group $=\text{O}$. The simplest ketone $\text{CH}_3\text{C}(\text{O})\text{CH}_3$ has the trivial name acetone.

Electronic Structure of the Carbonyl Group

Both carbon and oxygen atoms are sp^2 -hybridized in the carbonyl group. Like a $\text{C}=\text{C}$ double bond, a carbon-oxygen double bond represents a combination of a σ bond, formed by overlap of hybrid orbitals, and a π bond, formed by lateral overlap of unhybridized p orbitals (Fig. 1, a). Three σ bonds of the carbon atom, as well as two unshared electron pairs on the oxygen atom (shown in the figure by dots), lie in the same plane with bond angles of approximately 120° .

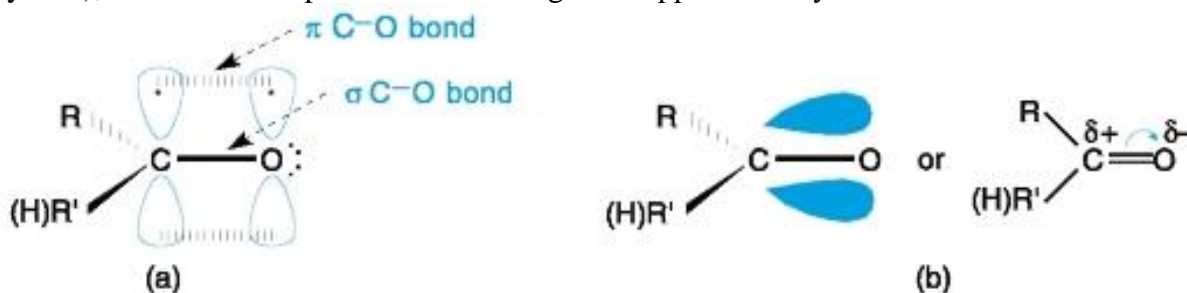
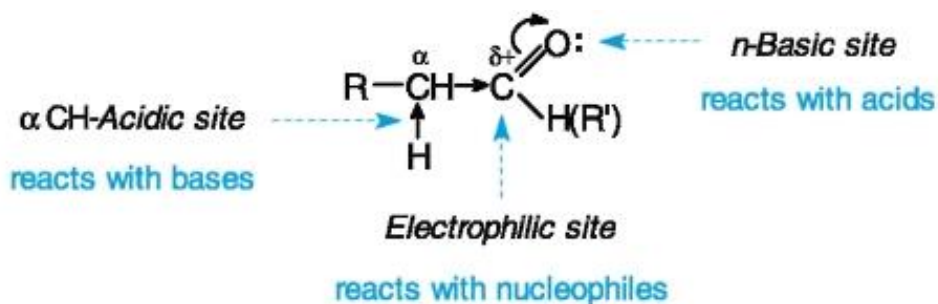


Figure 1. Bonding in the carbonyl group: the p orbital overlap (a) and polarization of the $\text{C}=\text{O}$ bond (b).

Unlike alkenes, the $\text{C}=\text{O}$ double bond, especially the π bond, is highly polarized because oxygen is much more electronegative than carbon. Thus, the carbonyl carbon carries a partial positive charge and, conversely, the oxygen atom carries a partial negative charge (Fig. 1, b).

Several reaction sites can be emphasized as a consequence of the carbonyl group polarization:

- the carbonyl carbon as an electrophilic site which can be attacked by nucleophiles;
- the oxygen atom as a weak n -basic site that can be protonated with strong acids;
- the α CH-acidic site is a weak acidic site that can be deprotonated with strong bases.



Additional reaction sites may arise when a double bond or an aromatic ring is present in the hydrocarbon portion of a carbonyl compound.

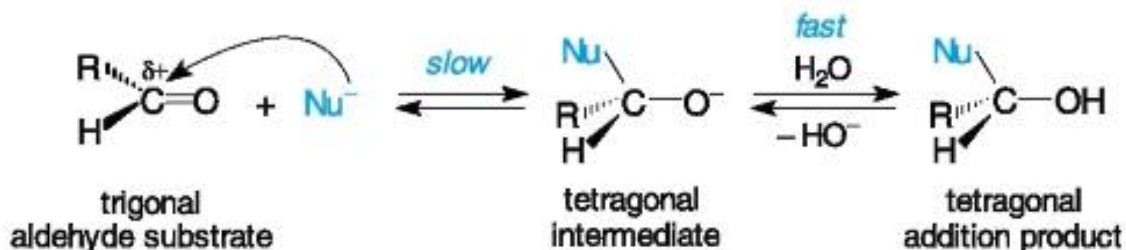
Aldehydes and ketones possess very weak acidity and basicity, therefore they cannot form intermolecular hydrogen bonds. Carbonyl compounds are more volatile than the corresponding alcohols. Compare, for example, boiling points of propanal (49 °C), acetone (56 °C), and 1-propanol (97 °C).

1.9. Nucleophilic addition reactions

Carbonyl compounds are susceptible to be attacked by nucleophiles. They undergo nucleophilic addition (A_N) reactions rather than substitution because they have very bad potential leaving group attached to the carbonyl carbon atom, i. e. anions H^- or R^- . The nucleophilic addition is the most important reaction of aldehydes and ketones.

A nucleophile that attacks the electrophilic carbon can be either negatively charged ($Nu:^-$) or neutral ($Nu:$). A hydroxide ion, alkoxide ions RO^- , and a hydride ion H^- are the examples of charged nucleophiles. To the neutral nucleophiles there belong water, alcohols, ammonia, and amines.

A nucleophile approaches the carbon atom from a direction approximately perpendicular to the plane of the carbonyl group. In the first, slow step of the reaction, the nucleophile uses its electron pair to form a new C-Nu bond. This step is accompanied by carbon rehybridization from sp^2 to sp^3 , resulting in a tetrahedral alkoxide ion intermediate. When the reaction is carried out in a protic solvent such as water or alcohols, the reaction is completed (in the second, fast step) by addition of a proton to the negatively charged oxygen.



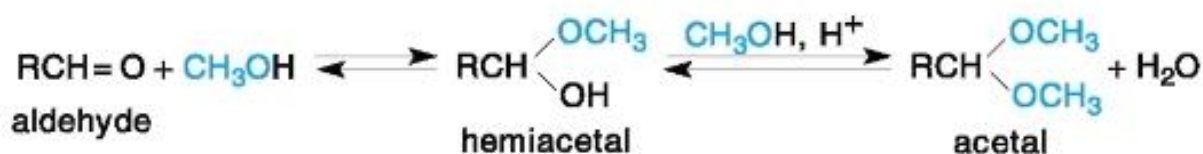
In general, aldehydes are somewhat more reactive than ketones towards nucleophiles because organic groups R' in ketones are both larger and more electron donating than the hydrogen atom in aldehydes. Thus, the carbonyl carbon is more hindered in ketones, and the partial positive charge on this atom is also reduced. For the same reasons aromatic aldehydes and ketones are less reactive than their aliphatic counterparts.

1.10. Addition of Alcohols: Hemiacetal and Acetal Formation

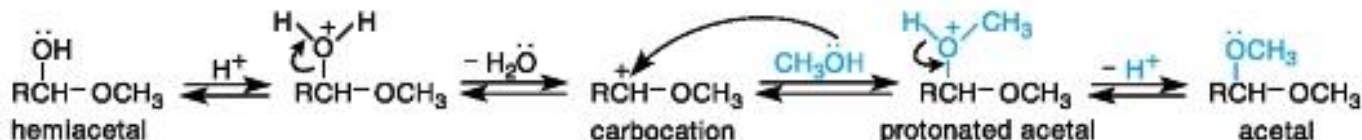
Aldehydes and some ketones react reversibly with alcohols to yield stepwise *hemiacetals* and *acetals*.

Hemiacetals are compounds in which a hydroxyl group and an alkoxy group are attached to the same carbon. Acetals have two alkoxy groups at the same carbon.

The overall transformation of an aldehyde into an acetal involve two steps: a) nucleophilic addition of an alcohol and b) nucleophilic substitution of the OH group for an alkoxy group of the bond alcohol molecule. Most open-chain hemiacetals are not sufficiently stable to be isolated.

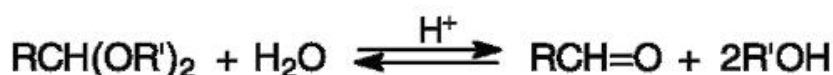


An acid catalyst is required in the second step of the reaction since alcohols are weak nucleophiles and the hydroxyl group is a poor leaving group.



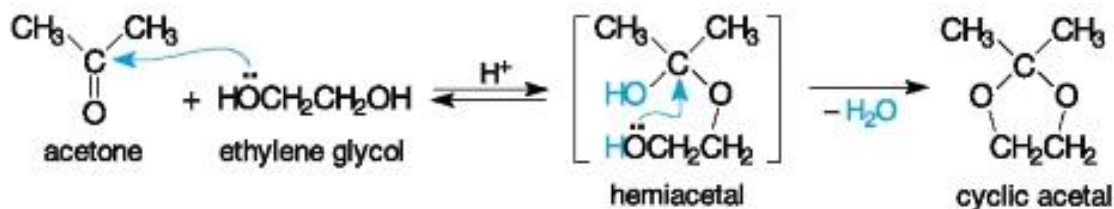
Protonation converts the hemiacetal hydroxyl into a good leaving group, a water molecule. Dehydration yields an intermediate carbocation, that reacts with excess alcohol to give a protonated acetal. Loss of a proton results in a neutral acetal product.

Note that all the steps of acetal formation are reversible. To drive the process in the forward direction it is necessary to use a large excess of an alcohol or to remove water, a product of the forward reaction (remember one of the Le Chatelier's principles). On the other hand, the reverse reaction is favoured when acetal is treated with a large excess of water in the presence of an acid. This reaction, called acetal hydrolysis, results in the formation of the parent carbonyl compound and the alcohol component. Thus, acetals are inert to bases, but they are acid-sensitive and therefore can be hydrolyzed in acidic media.



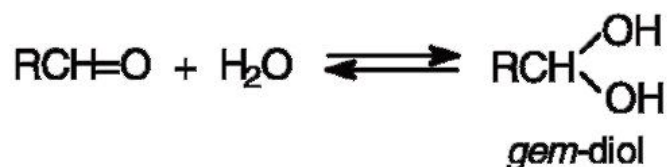
Example 5. Write the steps for the acid-catalyzed reaction of acetone with ethylene glycol (1,2-ethanediol).

Solution. In the first step an intermediate hemiacetal is formed in a usual way. The second step represents the *intramolecular* nucleophilic reaction, which is more probable than reaction between two molecules (of the hemiacetal and the second mole of the glycol). Thus, a product is a cyclic acetal:



Addition of Water: Hydration

Water, like alcohols, is an oxygen nucleophile and can react with some aldehydes and ketones by nucleophilic addition to yield so called hydrated forms of aldehydes, or gem-diols (1,1-diols). The reaction is reversible and hydrates of most aldehydes and ketones cannot be isolated from aqueous solution because they readily eliminate water to regenerate the carbonyl compound.

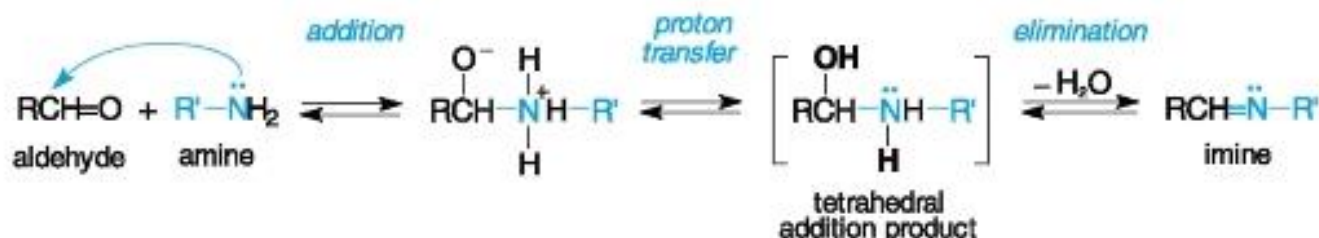


The equilibrium position depends on the structure of a compound. Formaldehyde, for example, exists in aqueous solution almost completely (over 99.9%) as the hydrate, $\text{CH}_2(\text{OH})_2$. Acetaldehyde is approximately half hydrated in water, while acetone does not form a hydrate, though it is completely soluble in water due to hydrogen bonding.

Electron-withdrawing substituents in a hydrocarbon portion increase stability of the hydrated form of a carbonyl compound. Thus, trichloroacetaldehyde (chloral) forms a stable crystalline hydrate, $\text{CCl}_3\text{CH}(\text{OH})_2$, which is used in medicine as a sedative and soporific.

Addition of Nitrogen Nucleophiles: Imines and Related Compounds

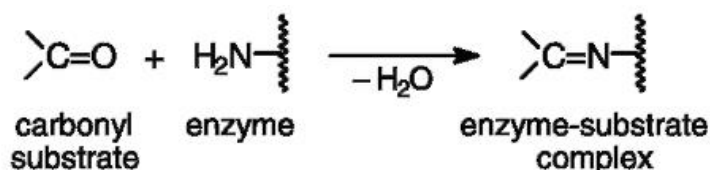
Amines and some related compounds (see below) act as nitrogen nucleophiles towards the carbonyl carbon. Primary amines, for example, add to aldehydes and ketones to yield N-substituted *imines*. The overall process involves two successive reactions, nucleophilic addition and elimination.



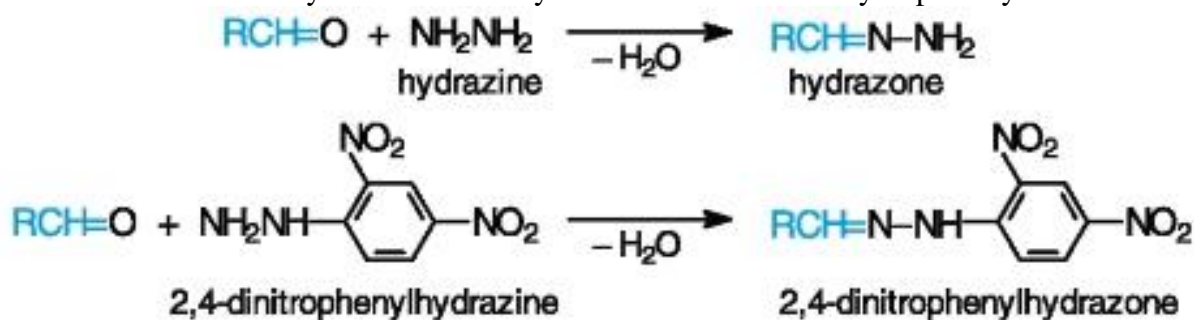
Nucleophilic attack on the carbonyl carbon by the lone-pair of electrons of an amine leads, after a proton transfer, to the tetrahedral addition product, a geminal amino alcohol that is similar to a hemiacetal. Such substances are normally unstable and cannot be isolated. They eliminate water to form a product with a carbon-nitrogen double bond.

It should be noted that free imines, i. e. compounds with a fragment $>\text{C}=\text{NH}$, are usually unstable because of subsequent condensation reactions. On the contrary, if the radical R or R' in the above general formula is an aryl group, such imines called *Schiff's bases* are quite stable.

Imine compounds are important intermediates in a number of biochemical transformations and in biosynthesis of amino acids (Chapter 14). Imine formation represents sometimes an interaction mode in binding carbonyl compounds to the amino groups of proteins, including enzymes, as it is shown below in a generalized form:



In the laboratory, reactions of this type are useful in analytic and synthetic procedures. From other organic and inorganic compounds containing an amino group the most widely used are hydrazine and its derivatives. They react with aldehydes and ketones similarly to primary amines.



The products of these reactions are usually crystalline insoluble solids that have characteristic melting points. They are applied for isolation and identification of liquid aldehydes and ketones. Imines and hydrazones can be subjected to acid-catalyzed or alkaline hydrolysis with formation of the initial compounds.

Control materials for the final stage of the class.

Questions to check the final level of knowledge:

1. Structure of the most important classes of bioorganic compounds by the nature of functional groups: alcohols, phenols, thiols, aldehydes, ketones.

2. Carbonyl compounds in bioorganic chemistry. Chemical properties and biomedical significance of aldehydes and ketones.
3. Classification of alcohols.
4. Chemical properties of alcohols.
5. Substitution reactions in phenols.
6. Chemical properties of aldehydes and ketones.

Literature

Basic:

1. Biological and Bioorganic Chemistry: Bioorganic Chemistry: textbook / B.S. Zimenkovsky, V.A. Muzychenko, I.V. Nizhenkovska, G.O. Syrova. — 3rd edition – 2020. – 288 p.
2. Biological and Bioorganic Chemistry. Biological Chemistry: textbook / Yu.I. Gubsky, I.V. Nizhenkovska, M.M. Korda et al. — 2nd edition – 2021 – 544 p.
3. Bioorganic Chemistry. Rineyskaya O.N. textbook. – 2018. – 174 p.
4. Baynes J., Dominiczak M. Medical Biochemistry. 5th Edition. Elsevier, 2018. 712 p.

Additional:

1. Satyanarayana U. Biochemistry. 5th edition. India 2020. – 777 p.
2. Lehninger. Principles of Biochemistry. 7th edition. NY, United States. 2017.
3. William Marshall, Marta Lapsley, Andrew Day, Kate Shipman. Clinical Chemistry. Elsevier, 2020. 432 p.

Електронні інформаційні ресурси:

1. <https://info.odmu.edu.ua/chair/biology/>-
2. <http://libblog.odmu.edu.ua/>
3. <https://moodle.odmu.edu.ua/login/index.php>

Practical class № 4

Topic: Study of the reactivity of carboxylic acids. The use of carboxylic acids in medicine. Structure, reactivity and biological significance of heterofunctional derivatives of carboxylic acids (hydroxy acids, keto acids and phenolic acids).

Relevance of the topic: The materials of the topic have an important role in the study of the course of bioorganic, biological chemistry, pharmacology. The medical and biological importance of carboxylic acids is that many of them are metabolites and are present in various plant and animal biological environments. Acetic acid and its derivatives are the structural synthetic unit from which many complex biomolecules (steroid hormones, lipids, etc.) are built. Higher fatty acids, saturated and unsaturated, are components of simple and complex saponifiable lipids. Carboxyl group is a part of such important biomolecules as oxy-, keto- and amino acids. Nicotinic acid is a provitamin of vitamin PP (nicotinamide). Aromatic monocarboxylic acids (benzoic, phenylacetic, cinnamic acids) are used in the synthesis of fragrances and medicines. Dicarboxylic acids and their derivatives are also widely used in organic synthesis (in particular, sodium malonic ester is a starting substance in the synthesis of heterocyclic compounds and drugs). In analytical practice, phenolphthalein, a product of condensation of aromatic dicarboxylic phthalic acid anhydride with phenol, is used as an acid-base indicator.

Aims: To have an idea of the classification of bioorganic compounds. To learn the structure of carboxylic acids. In the process of conducting chemical reactions to learn the reactivity of carboxylic acids in biological systems. To master the skills of conducting qualitative reactions to carboxylic acids (Fehling reaction; iodoform reaction to acetone). Based on knowledge of the structure and

reactivity of carboxylic acids to form an idea of the peculiarities of human metabolism and the need to use this knowledge to substantiate the pathogenesis of diseases (pathochemistry).

Basic concepts: structure of carboxylic group, carboxylic acid, classification of carboxylic acids, nomenclature, isomerism, chemical properties of carboxylic acids.

Equipment: department laboratory

Plan and organizational structure of the class:

1. Nomenclature of carboxylic acids.
2. Classification of carboxylic acids.
3. Isomerism (structural and optical) of carboxylic acids.
4. Homologous series of saturated monobasic carboxylic acids. The structure of the carboxyl group.
5. Hydroxy acids. Oxo acids.
6. Aromatic acids and their derivatives.
7. Chemical properties of carboxylic acids and their derivatives.

The higher education applicant should know and be able to:

1. Protolytic theory of acids and bases.
2. The electronic structure of the carboxyl group. Explain its influence on the course of a chemical reaction with a hydrocarbon radical.
3. Compare acidic properties of organic compounds depending on the nature of electronic effects of the substituent.
4. Explain the CH acidity of the α -carbon atom.
5. The main types of chemical reactions of carboxylic acids.
6. Show the electron density distribution in the aromatic nucleus and explain the deactivating and orienting effect of the carboxyl group in electrophilic substitution reactions.
7. Biological importance of carboxylic acids.

Content of the topic

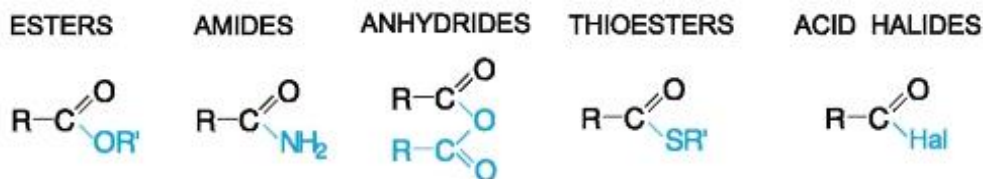
CARBOXYLIC ACIDS AND THEIR DERIVATIVES

Carboxylic acids were among the earliest organic compounds studied by chemists. The Swedish pharmaceutical chemist C.W. Scheele was the first to discover many carboxylic acids at the end of the 18th century, well before the theory of the chemical structure had been developed.

Not only carboxylic acids themselves but also their functional derivatives are of chemical and biochemical interest.

Carboxylic acids are compounds of the general formula RCOOH that have the carboxyl group -COOH as a functional group. Acid derivatives have the general formula RC(O)Z, where Z is a substituent containing an unshared electron pair.

The most important acid derivatives are esters, amides, anhydrides, thioesters, and acid halides. Acid derivatives are called so because all of them can be derived from carboxylic acids and can be hydrolyzed to regenerate carboxylic acids.



GENERAL CHARACTERISTICS OF CARBOXYLIC ACIDS

Carboxylic acids and their numerous derivatives form a family of compounds whose chemistry is exclusively varied. It is no wonder because we observe various types of functions in their molecules.

1.1. Classification and Nomenclature

Carboxylic acids may be classified similarly to aldehydes, i. e. as aliphatic or aromatic ones. The classification signs may be extended with unsaturated, heterocyclic, and dicarboxylic acids. These types are exemplified below and presented in Table 1.

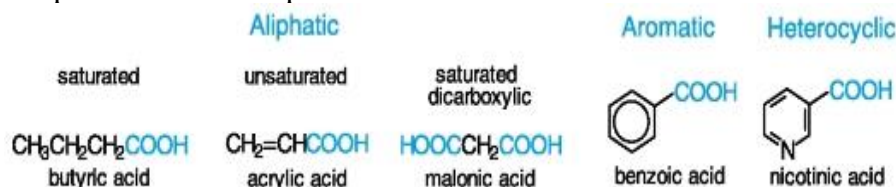
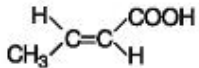
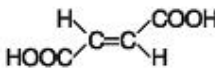
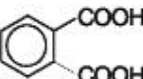
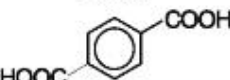


Table 1.

Names and some physical constants of selected carboxylic acids

Trivial name*	Systematic name*	Formula	Melting point, °C	Boiling point, °C	pK _a **
Monocarboxylic					
Formic	Methanoic	HCOOH	8	101	3.8
Acetic	Ethanoic	CH ₃ COOH	17	118	4.8
Propionic	Propanoic	CH ₃ CH ₂ COOH	-21	141	4.9
Butyric	Butanoic	CH ₃ (CH ₂) ₂ COOH	-5	164	4.8
Valeric	Pentanoic	CH ₃ (CH ₂) ₃ COOH	-34	185	4.8
Isovaleric	3-Methylbutanoic	(CH ₃) ₂ CHCH ₂ COOH	-29	177	4.8
Stearic	Octadecanoic	CH ₃ (CH ₂) ₁₆ COOH	70	376	
Acrylic	Propenoic	CH ₂ =CHCOOH	12	141	4.2
Crotonic	<i>trans</i> -2-Butenoic		71	185	4.7
Benzoic	Benzenecarboxylic	C ₆ H ₅ COOH	122	249	4.2
Dicarboxylic					
Oxalic	Ethanedioic	HOOC-COOH	189		1.2; 4.2
Malonic	Propanedioic	HOOCCH ₂ COOH	136***		2.8; 5.7
Succinic	Butanedioic	HOOCCH ₂ CH ₂ COOH	186	300	4.2; 5.5
Glutaric	Pentanedioic	HOOC(CH ₂) ₃ COOH	98	303***, 200 (20 Torr)	4.3; 5.4
Fumaric	<i>trans</i> -Butenedioic		296***	165 (1.7 Torr)	3.0; 4.4
Phthalic	1,2-Benzenedicarboxylic		200***		3.0; 5.4
Terephthalic	1,4-Benzenedicarboxylic		427***		3.5; 4.5

* The word *acid* is omitted in the table.

** For dicarboxylic acids the first number denotes pK_{a1}, the second one - pK_{a2}.

*** Melts or boils with decomposition.

For historical reasons mentioned above, many carboxylic acids received their trivial names long ago. Many of them (and all the names listed in Table 1) are allowed, and even recommended by the IUPAC rules, including the names of acid derivatives (Table 2). Trivial names are preferably used in the biochemical literature.

Table 2.

Trivial names of carboxylic acid derivatives

Number of carbons in a chain	Acyl group $RC(O)-$	Anion or a basis of the ester	Amide
Monocarboxylic			
1	Formyl	Formate	Formamide
2	Acetyl	Acetate	Acetamide
3	Propionyl	Propionate	Propionamide
4	Butyryl	Butirate	Butyramide
5	Valeryl	Valerate	Valeramide
–	Benzoyl	Benzoate	Benzamide
Dicarboxylic*			
2	Oxalyl	Oxalate	Oxalamide
3	Malonyl	Malonate	Malonamide
4	Succinyl	Succinate	Succinamide
5	Glutaryl	Glutarate	Glutaramide

* For derivatives on both carboxyl groups.

In the IUPAC substitutive nomenclature, the carboxyl group in aliphatic representatives is indicated by the suffix **-oic** with the addition of the word **acid**. When the carbon atom of the COOH group is not a part of the parent structure, the ending **-carboxylic acid** is added. Acyl groups $RC(O)-$ are named from the corresponding acids by changing the suffix **-ic** of the common or systematic name to the suffix **-yl**, for example, acetyl or ethanoyl for the group $CH_3C(O)-$ (see Table 2).

Table 2 will also be useful in constructing the names of carboxylic acid derivatives.

1.2. Electronic Structure of the Carboxyl Group

Although the carboxyl group appears to be a simple combination of the hydroxyl and carbonyl groups, the interaction between them generates some unique properties.

The carboxyl group represents a planar ρ, π -conjugated system, in which a lone pair of electrons of the hydroxyl oxygen overlaps with p orbitals of the $C=O$ double bond (Fig. 1). This is confirmed by the data on carbon-oxygen bond lengths in related compounds.

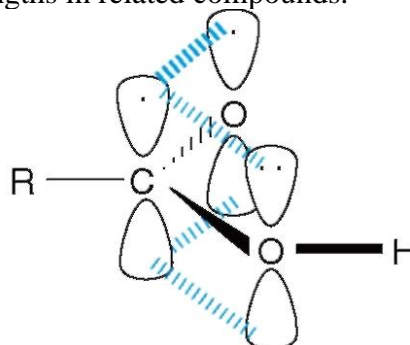
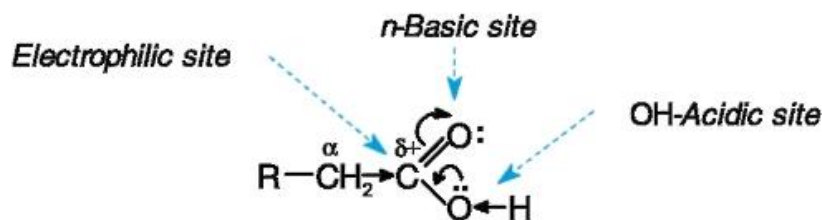


Figure 1. Orbital overlap in carboxylic acids.

The following reactive sites can be pointed out in the carboxylic acid molecule:

- the OH-acidic site that reacts with bases by deprotonation;
- the carbonyl carbon as an electrophilic site which can be attacked by nucleophiles by substitution;
- the oxygen atom as a weak *n*-basic site that can be protonated with strong acids.

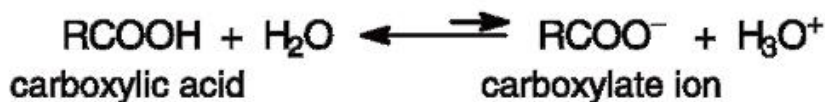


Similarly to other classes of organic compounds, additional reaction sites may arise in the hydrocarbon portion of a molecule.

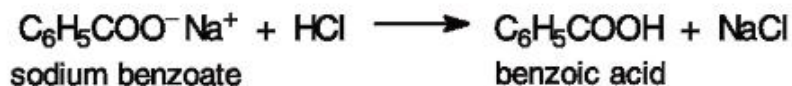
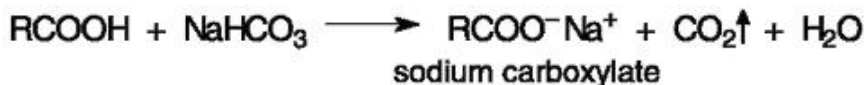
The α -hydrogen virtually is not acidic in carboxylic acids because a stronger acidic site is present. When the carboxyl group is ionized the α -hydrogen loses acidity completely. However α -CH acidity becomes appreciable in acid derivatives, for example, in esters.

1.3. ACIDIC PROPERTIES

As their name suggests, carboxylic acids are *acidic*. Conjugation in the carboxyl group increases acidity of a carboxylic acid as compared with other OH-acids such as alcohols and phenols. Carboxylic acids are among the strongest acidic compounds in organic chemistry (only sulphonic acids, RSO_3H , exceed them in acidity). They partly dissociate in water, giving a carboxylate ion and a hydronium ion:

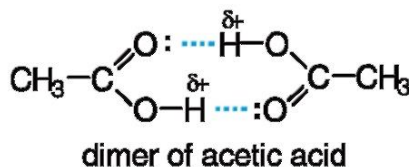


Carboxylic acids react readily with metal hydroxides to form salts. They also displace weaker acids from their salts to give metal carboxylate salts. The reaction serves as a simple visible test for carboxylic acids by a gas evolution. Carboxylate salts are converted into carboxylic acids on treatment with strong mineral acids.



Thus, carboxylic acids exist in acidic media only in an unionized form (RCOOH) whereas in alkaline media they are always ionized (RCOO^-).

Conjugation within the carbonyl group increases not only acidity of a compound but also basic properties of the double-bonded oxygen as compared with that of carbonyl compounds. This explains the fact that carboxylic acids exist normally in an associated form with strong intermolecular hydrogen bonds between the basic and OH-acidic sites. For example, acetic acid exists as a dimer, even in a vapour phase.



Hydrogen bonding with water also explains the complete water-solubility of the first four liquid monocarboxylic acids (including butyric acid) and the good solubility of the first four solid dicarboxylic acids (including glutaric acid).

Acid strength. Most monocarboxylic acids of an aliphatic or aromatic series are acids of moderate strength with pK_a values in the range from 4 to 5.

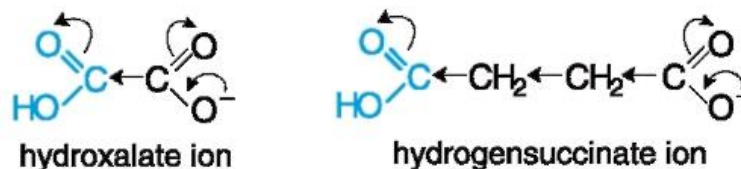
We turn to this question in the following example.

Example 1. How are the following facts explained?

(I) The pK_{a1} for all dicarboxylic acids in Table 9.1 are higher than the pK_a for monocarboxylic acids with the same number of carbons.

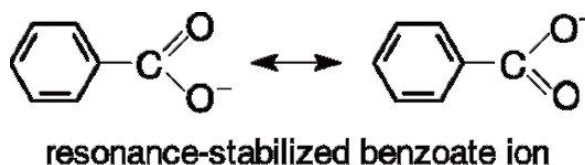
(II) The acidity of the dicarboxylic acids decreases with the length of a carbon chain.

Solution. (I) The carboxyl group is an electron-withdrawing group. The inductive effect of such substituent tends to spread the negative charge in a carboxylate ion over more atoms and thus stabilizes an anion. Thus in dicarboxylic acids the second carboxyl group (unionized) increases the acidity of the other.



(II) The inductive effect falls off rapidly with distance. The carboxyl group exerts much smaller -I effect in the hydrogensuccinate ion as compared with the hydroxalate ion. Succinic acid therefore only slightly exceeds in acidity monocarboxylic acids.

It might seem unexpected that a substituent affects stability of a carboxylate ion only inductively. (Recall delocalization of a negative charge in a phenoxide ion through p, π conjugation.) Conjugation of the oxygen lone pair of electrons is possible only with a $C=O$ double bond regardless of other bonds present. Likewise, it is impossible to draw a contributing resonance structure, using other but oxygen negatively charged atoms. Any attempt to do this with the benzoate ion will be unsuccessful.



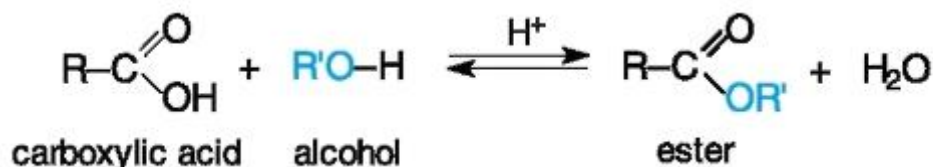
For this reason substitution in benzoic acid slightly affects acidity, depending on electron-donating or electron-withdrawing character of the substituent. For the same reason oxalic acid is the strongest unsubstituted carboxylic acid. The strongest of all carboxylic acids is trifluoroacetic acid, CF_3COOH , pK_a 0.2.

1.4. NUCLEOPHILIC SUBSTITUTION AT ACYL CARBON

Nucleophilic substitution is, perhaps, the most common and important reaction of carboxylic acids and their derivatives. In carboxylic acids a partial positive charge (δ^+) on the carboxyl carbon is decreased compared to that of aldehydes and ketones. This means, in general, that carboxylic acids are less reactive towards nucleophilic reagents than carbonyl compounds are. Moreover, a hydroxyl group belongs to poor leaving groups. However, it can be modified or transformed into other functions, which are good leaving groups. This approach is realized in the reaction of carboxylic acids with alcohols to form esters.

Esterification of Carboxylic Acids

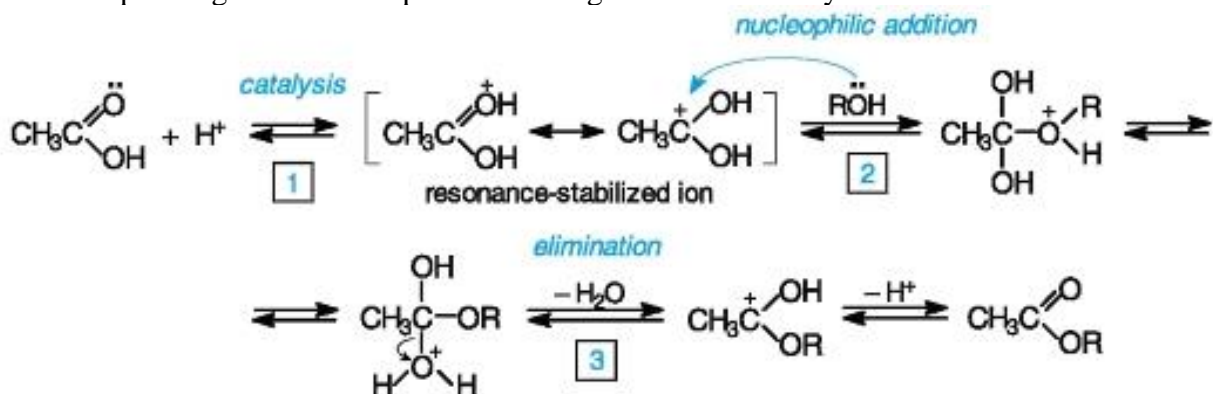
On heating a carboxylic acid with an alcohol in the presence of an acid catalyst (usually anhydrous H_2SO_4 or gaseous HCl), reversible ester formation occurs.



This reaction is called the *Fischer esterification*. The application of large excess of one of the reactants or removal of the ester or/and water can shift the equilibrium to the right.

The esterification mechanism. There are three main steps in a reaction mechanism. In the first step, protonation of a carboxylic acid increases the positive charge on the carboxyl carbon to give a resonance-stabilized cation. In the second, addition step an alcohol (as a nucleophile) attacks the

carbocation with the formation of a new C-O bond. Then, after proton migration, water is eliminated. Finally, loss of a proton gives the ester product and regenerates the catalyst.



Thus, the overall reaction is outwardly similar to nucleophilic substitution that occurs by the $\text{S}_{\text{N}}2$ mechanism. But as it is seen from the reaction mechanism, esterification is not a direct substitution, rather it is addition-elimination. Since the net result of the reaction is substitution (OR for OH), the reaction is referred to as *nucleophilic acyl substitution*.

Acidic hydrolysis of esters is a reverse reaction to the ester formation. Esters can also be hydrolyzed with alkalis.

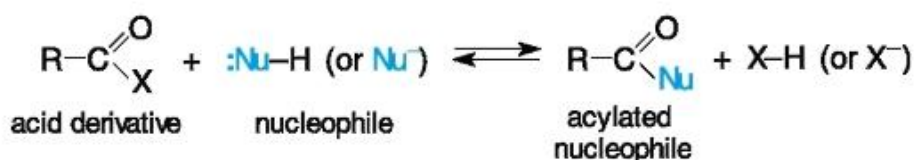


Alkaline hydrolysis is called *saponification* (from the Latin *sapo* - soap) because this type of reaction has been used and is used now to make soaps (alkali metal salts of long-chain acids) from fats. Saponification is an irreversible reaction, and at least one equivalent of an alkali is required.

1.5. Acylation Reactions with Carboxylic Acid Derivatives

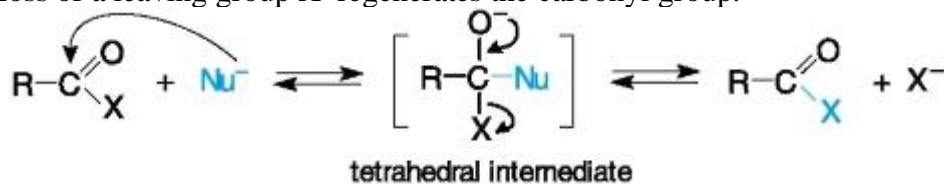
Numerous acid derivatives are known, but we will be concerned only with five of them: esters, amides, thioesters, anhydrides, and acid halides. Esters and amides occur widely in nature; anhydrides and, especially, acid halides are creatures of the laboratory chemists because of their high reactivity.

The general reaction can be presented as follows (where X is a nucleophilic group):

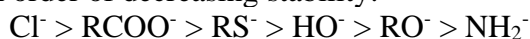


These reactions involving common nucleophiles, such as water, alcohols, ammonia, and amines, are usually designated hydrolysis, alcoholysis, ammonolysis, and aminolysis, respectively. With regard to nucleophiles, transformations of this kind are often referred to as acylation reactions, i. e. the acyl group is transferred from the group X in the acid derivative to nucleophile in the product. The term *acyl transfer* is used for such reactions in biochemistry.

Acylation reaction proceeds by a two-step addition-elimination pathway through a tetrahedral intermediate. Loss of a leaving group X^- regenerates the carbonyl group.



Relative reactivity of acid derivatives depends on stability of their leaving groups. The following anions are arranged in order of decreasing stability:



For this reason, the reactivity order in acylation reactions for carboxylic acids and their derivatives is as follows:



This means that it is easy to transform a more reactive acid derivative into a less reactive one. Analysis of the reactivity order leads to the following conclusion shown in Fig. 2.

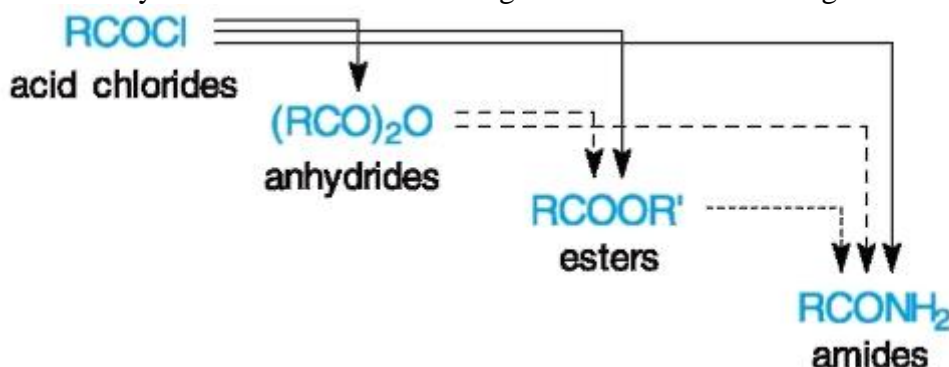
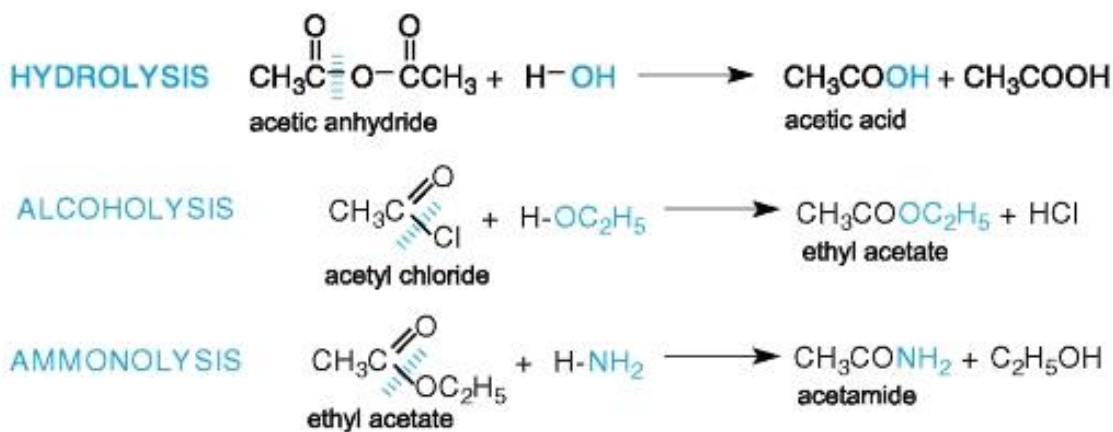
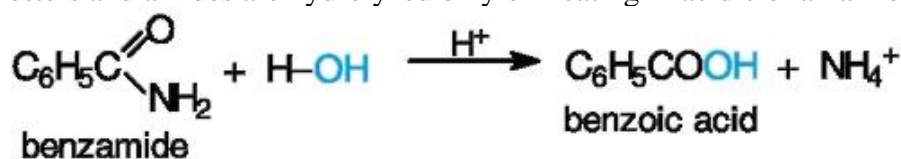


Figure 2. Interconversions of carboxylic acid derivatives.

Some typical nucleophilic substitution reactions are illustrated below with various derivatives of acetic acid:

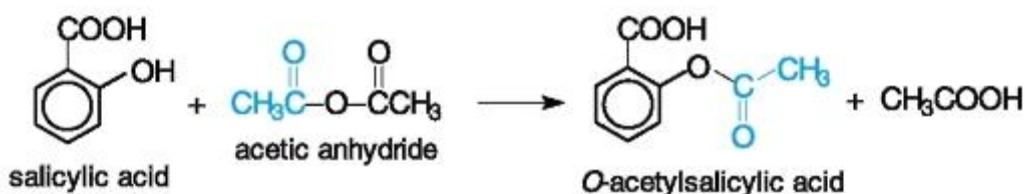


All acid derivatives can be hydrolyzed. Acid halides and anhydrides undergo hydrolysis most readily, whereas esters and amides are hydrolyzed only on heating in acidic or alkaline medium.



Amides are acid derivatives that resist to hydrolysis most. The reason is that the amino group is a very poor leaving group.

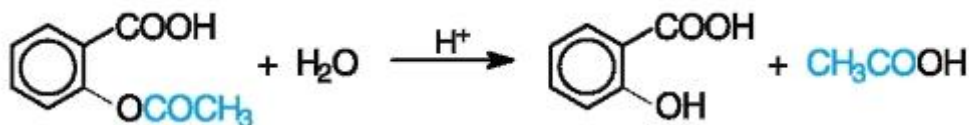
Many drugs are esters or amides from the chemical point of view. For example, aspirin (O-acetylsalicylic acid) is an ester manufactured from salicylic acid (2-hydroxybenzoic acid). The phenolic hydroxyl group undergoes acetylation in this reaction:



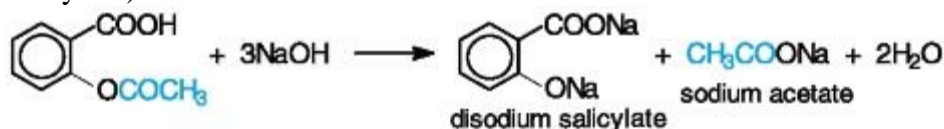
Aspirin can be hydrolyzed under acidic or alkaline conditions. That is why we should always bear in mind the possibility of ester hydrolysis in the acidic medium of the stomach or in the alkaline medium of the intestines.

Example 2. Write equations for the acidic and alkaline hydrolyses of aspirin.

Solution. Acid-catalyzed hydrolysis yields, as usual, constituents of the ester, i. e. acetic acid plus salicylic acid as a phenolic component.



Alkaline hydrolysis results in the same principal products but in the ionized form (one should recall phenolic acidity too).

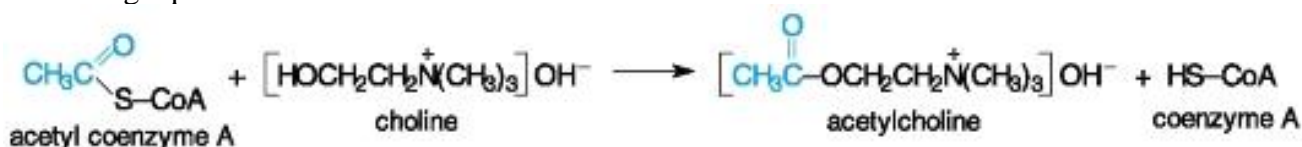


Two other esters of salicylic acid are known as remedies, they are the phenyl ester (the trade name Salol) and the methyl ester.

Problem 4. Write the structural formulas for: (a) phenyl salicylate; (b) methyl salicylate. Show how methyl salicylate can be prepared from salicylic acid.

Esters are among the most widespread of all natural substances. Many simple esters are responsible for the pleasant odour of fruits and flowers. For example, pentyl acetate, $\text{CH}_3\text{COO}(\text{CH}_2)_4\text{CH}_3$, is a constituent of banana oil; octyl acetate, $\text{CH}_3\text{COO}(\text{CH}_2)_7\text{CH}_3$, has been isolated from orange oil; butyl butyrate, $\text{CH}_3\text{CH}_2\text{CH}_2\text{COO}(\text{CH}_2)_3\text{CH}_3$, has been found in pineapple oil; benzyl acetate, $\text{CH}_3\text{COOCH}_2\text{C}_6\text{H}_5$ smells of jasmine. As we will see further, fats and oils from animal and vegetable sources are also esters.

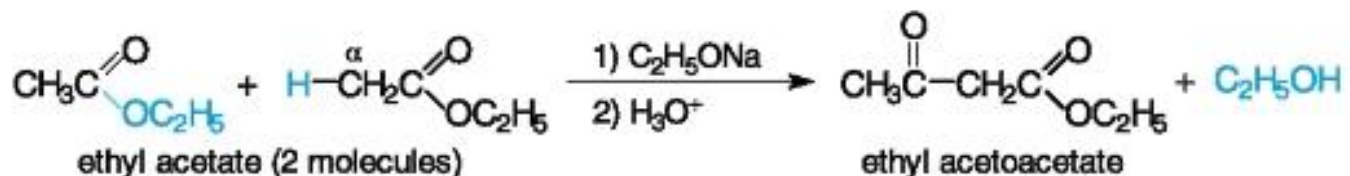
Ester formation is an important type of reactions, which take place in living matter. Acyl halides and anhydrides are too reactive to be cell constituents because they are rapidly hydrolyzed by water. The best acylating agents *in vivo* are thioesters, acid derivatives of moderate reactivity. An example is *acetyl coenzyme A* (Sec. 8.2.3) usually written in a shortened form $\text{CH}_3\text{C}(\text{O})\text{S-CoA}$. Acetyl coenzyme A serves as an acylating agent in the enzymic transformation of choline into acetylcholine by the following equation:



Acetylcholine is then hydrolyzed in the cell. The direct and back reactions make up acetylcholine cycle that is the basis of the nervous conductivity.

1.6. ESTER CONDENSATION

Esters, like aldehydes and ketones, are weakly acidic when an α -hydrogen is present in a molecule. A reversible condensation reaction occurs on treatment of an ester with a strong base to give a β -keto ester, for example:



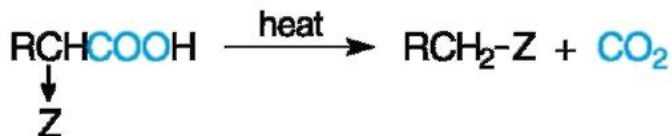
This reaction called the *Claisen condensation* is based on CH-acidic properties of the α -hydrogen thus resembling the aldol condensation in many respects.

The Claisen condensation is very important both in nature and in the laboratory. Like the aldol condensation, it provides lengthening carbon chain in the biosynthesis of many natural products. For example, long-chain fatty acids, constituents of natural fats and oils, are produced biosynthetically from the simple two-carbon precursor, acetic acid, in the form of acetyl coenzyme A. It undergoes the Claisen condensation to form acetoacetyl CoA, a four-carbon unit that is similar to the product in

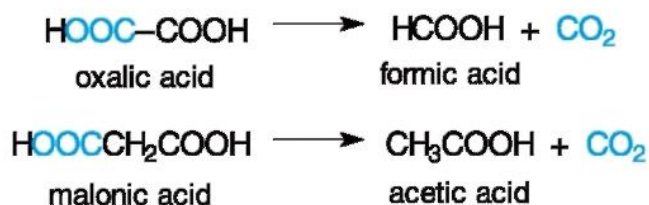
the example above. Thus, in eight steps (which involve additional transformations) two acetic acid units combine into a butyric acid unit. Further repetition of the cycle yields a six-carbon unit, and so on.

1.7. DECARBOXYLATION OF CARBOXYLIC ACIDS

Decarboxylation, or the loss of carbon dioxide, is one of important transformations of a carboxyl group. Monocarboxylic unsubstituted acids resist decarboxylation and can eliminate carbon dioxide only under severe conditions or by means of specific reagents. Electron-withdrawing substituents at α -carbon (Z is $-\text{COOH}$, $-\text{CO-R}'$, $-\text{C}\equiv\text{N}$, $-\text{NO}_2$ in the equation below) facilitate decarboxylation.



For this reason, oxalic and malonic acids eliminate carbon dioxide on moderate heating (140-150 °C) thus converting to mono carboxylic acids having one less carbon atom:



Succinic and glutaric acids, dicarboxylic acids with a longer chain, are not decarboxylated on heating. Instead, they lose the water molecule on heating to give *cyclic anhydrides* with a stable five or six-membered ring, respectively; the same applies to phthalic acid.

Control materials for the final stage of the class.

Questions to check the final level of knowledge:

1. Carboxylic acids in bioorganic chemistry: structure and chemical properties; functional derivatives of carboxylic acids.
2. Structure and properties of dicarboxylic acids: oxalic, malonic, succinic, glutaric, fumaric.
3. Features of chemical properties of heterofunctional derivatives of carboxylic acids - hydroxy acids, oxo acids, phenol acids.

Literature

Basic:

1. Biological and Bioorganic Chemistry: Bioorganic Chemistry: textbook / B.S. Zimenkovsky, V.A. Muzychenko, I.V. Nizhenkovska, G.O. Syrova. — 3rd edition – 2020. – 288 p.
2. Biological and Bioorganic Chemistry. Biological Chemistry: textbook / Yu.I. Gubsky, I.V. Nizhenkovska, M.M. Korda et al. — 2nd edition – 2021 – 544 p.
3. Bioorganic Chemistry. Rineyskaya O.N. textbook. – 2018. – 174 p.
4. Baynes J., Dominiczak M. Medical Biochemistry. 5th Edition. Elsevier, 2018. 712 p.

Additional:

1. Satyanarayana U. Biochemistry. 5th edition. India 2020. – 777 p.
2. Lehninger. Principles of Biochemistry. 7th edition. NY, United States. 2017.
3. William Marshall, Marta Lapsley, Andrew Day, Kate Shipman. Clinical Chemistry. Elsevier, 2020. 432 p.

Електронні інформаційні ресурси:

1. <https://info.odmu.edu.ua/chair/biology/>

2. <http://libblog.odmu.edu.ua/>
3. <https://moodle.odmu.edu.ua/login/index.php>

Practical class № 5

Topic: Study of the properties of natural fatty acids. Triglycerides.

Relevance of the topic: Lipids, along with other organic compounds, are the structural basis of all living organisms and play an important role in the processes of life. Lipids perform plastic, energy, regulatory, protective functions. Fats are an essential component of food. Knowledge of the structure, chemical properties of lipids is necessary for further study of their biological functions in the courses of biological chemistry, physiology. Violation of lipid metabolism affects the general condition of the body. Knowledge of the general properties of lipids (saponifiable and unsaponifiable) will allow future doctors to understand the pathogenesis of diseases, correctly diagnose it and solve the issues of drug therapy.

Goals: to form systematic knowledge about the structure, chemical properties and biological role of simple saponifiable lipids - triacylglycerols and their structural components as a chemical basis for studying the structure of biological membranes and lipid metabolism.

Equipment: department laboratory

Plan and organizational structure of the class:

1. The main structural components of lipids.
2. Classification of lipids and their biological functions.
3. Features of the structure of the structural components of saponifiable lipids (triacylglycerols): Fats (saturated and unsaturated) and alcohols (polyatomic and monatomic).
4. Conditions of obtaining and hydrolysis of triacylglycerols.
5. Chemical properties of lipids.
6. Fats, oils, waxes. Their consistency and chemical properties.
7. Analytical characteristics of fats.
8. Surface-active properties of lipids, the diffuse structure of their molecules.

The higher education applicant should know and be able to:

- structure and properties of the double bond; π -diastereomers (geometric cis-trans isomerism);
- mechanism of electrophilic addition of AE and nucleophilic substitution of SN;
- chemical properties of esters (acid and alkaline hydrolysis);
- conformation of open chains of carbon atoms.

Content of the topic

Lipids (from the Greek *lipos* - fat) are compounds of vegetable or animal origin that are characterized by their solubility properties¹. They are practically insoluble in water but highly soluble in nonpolar organic solvents. Lipids can be extracted from cells and tissues by organic solvents, such as chloroform, ether, or hydrocarbons.

Lipids vary considerably in their chemical structure. In general they are considered as derivatives of long-chain carboxylic acids. The distinguishing feature of lipids lies in their *biphilic* properties, resulting from the presence in their molecules polar (hydrophilic) and non-polar (hydrophobic) regions. Thus, lipids have an affinity both to water and to non-aqueous phase.

1.1. CLASSIFICATION

Lipids are classified as either simple (two-component) or complex ones; the latter consist of three or more components (Table 12.1). Simple lipids represent esters in which carboxylic acids acylate trihydroxylic alcohol glycerol (as in fats and oils) or long-chain alcohols (as in waxes). Lipids of this group give only alcohols and carboxylic acids on hydrolysis.

Complex lipids may contain other components such as a substituted phosphate group (as in phosphatides) or carbohydrate units (as in glycolipids). Moreover, the amino alcohol sphingosine (instead of glycerol) is a constituent of the sphingolipid group.

Table 1.

<i>Classification of lipids</i>				
<i>Simple</i>		<i>Complex</i>		
<i>waxes</i>	<i>fats and oils*</i>	<i>phosphatides</i>	<i>glycolipids</i>	<i>sphingolipids</i>
RCOOR'	$\begin{array}{l} \text{CH}_2\text{O}-\text{CO}-\text{R} \\ \\ \text{CHO}-\text{CO}-\text{R}' \\ \\ \text{CH}_2\text{O}-\text{CO}-\text{R}'' \end{array}$	$\begin{array}{l} \text{CH}_2\text{O}-\text{CO}-\text{R} \\ \\ \text{CHO}-\text{CO}-\text{R}' \\ \\ \text{CH}_2\text{O}-\text{P}(\text{O})-\text{OR}'' \\ \\ \text{OH} \end{array}$ <p>R' = aminoalkyl</p>	$\begin{array}{l} \text{CH}_2\text{O}-\text{CO}-\text{R} \\ \\ \text{CHO}-\text{CO}-\text{R}' \\ \\ \text{CH}_2\text{O}-\text{X} \end{array}$ <p>X = sugar residue</p>	$\begin{array}{l} \text{CH}=\text{CH}(\text{CH}_2)_{12}\text{CH}_3 \\ \\ \text{CHOH} \\ \\ \text{CHNH}-\text{CO}-\text{R} \\ \\ \text{CH}_2\text{O}-\text{X} \end{array}$ <p>X = phosphate or sugar residue</p>

* Unstated R's are saturated or unsaturated long carbon chains.

¹ Note that this definition is based on a physical property (solubility) differing from that used for other classes. The latter were defined on the basis of their structures.

1.2. STRUCTURAL COMPONENTS OF LIPIDS

There are two obligatory components in all groups of lipids: long-chain carboxylic acids and alcohols.

1.2.1. Fatty Acids

Many of the most common carboxylic acids were first obtained from natural sources, particularly from fats and oils. Consequently, they are called *fatty acids*. Over 200 fatty acids are known today, that differ in the chain length, degree of unsaturation, degree of branching, and even in the presence of additional functional groups. Some of the commonly occurring fatty acids are listed in Table 2.

Table 2.

Common fatty acids

Code symbol *	Structure	Common name	Melting point, °C
Saturated			
C _{4:0}	CH ₃ (CH ₂) ₂ COOH	Butyric	-5
C _{12:0}	CH ₃ (CH ₂) ₁₀ COOH	Lauric	44
C _{14:0}	CH ₃ (CH ₂) ₁₂ COOH	Myristic	54
C _{16:0}	CH ₃ (CH ₂) ₁₄ COOH	Palmitic	63
C _{18:0}	CH ₃ (CH ₂) ₁₆ COOH	Stearic	70
C _{20:0}	CH ₃ (CH ₂) ₁₈ COOH	Arachidic	75
Unsaturated**			
C _{16:1}	CH ₃ (CH ₂) ₅ CH=CH(CH ₂) ₇ COOH	Palmitoleic	32
C _{18:1}	CH ₃ (CH ₂) ₇ CH=CH(CH ₂) ₇ COOH	Oleic	13
C _{18:2}	CH ₃ (CH ₂) ₄ CH=CHCH ₂ CH=CH(CH ₂) ₇ COOH	Linoleic	-5
C _{18:3}	CH ₃ CH ₂ CH=CHCH ₂ CH=CHCH ₂ CH=CH(CH ₂) ₇ COOH	Linolenic	-12
C _{20:4}	CH ₃ (CH ₂) ₄ (CH=CHCH ₂) ₄ CH ₂ CH ₂ COOH	Arachidonic	-50

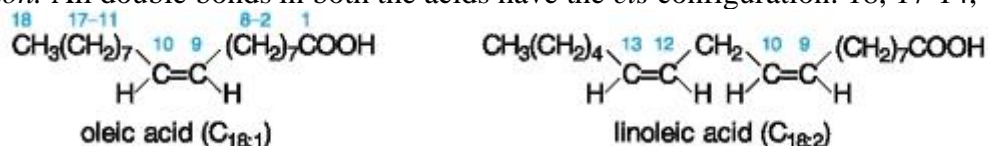
* The first number represents total carbons, the second - the number of the C=C double bonds.

** All double bonds have the *cis* configuration.

In spite of a big variety of fatty acids, most of them are unbranched and contain an *even* number of carbon atoms. This is a consequence of their biosynthesis from the two-carbon fragment of acetyl coenzyme A. The most widespread acids are those containing 16 and 18 carbon atoms. Unsaturated fatty acids usually have the *cis* (or *Z*) configuration and *are not* conjugated.

Example 1. Write the structures of oleic and linoleic acids, showing the geometry at each double bond.

Solution. All double bonds in both the acids have the *cis* configuration: 18, 17-11, 11, 8-2 1



Saturated fatty acids and saturated portions of unsaturated acids exist in more stable staggered conformation because in this conformation the carbon atoms are as remote from one another as possible. Thus, the preferred conformation of saturated acids is fully extended, whereas a carbon chain in unsaturated acids makes a bend at the position of the *cis* double bond (Fig. 1).

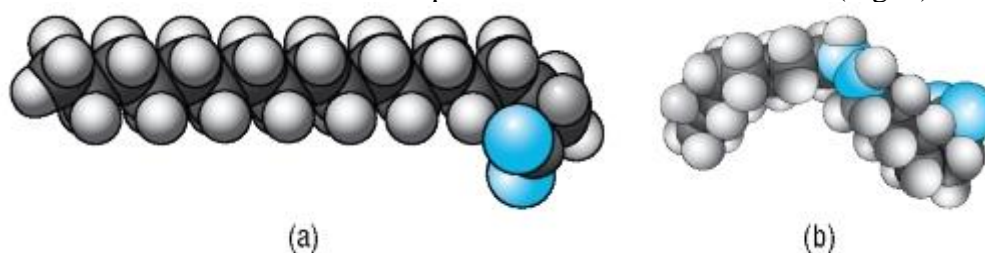
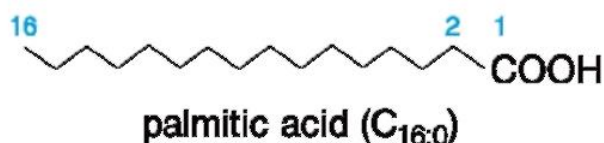


Figure 1. Space-filling models of stearic acid (a) and oleic acid (b). The oxygen atoms are coloured, the carbons of the double bond are pale coloured in (b).

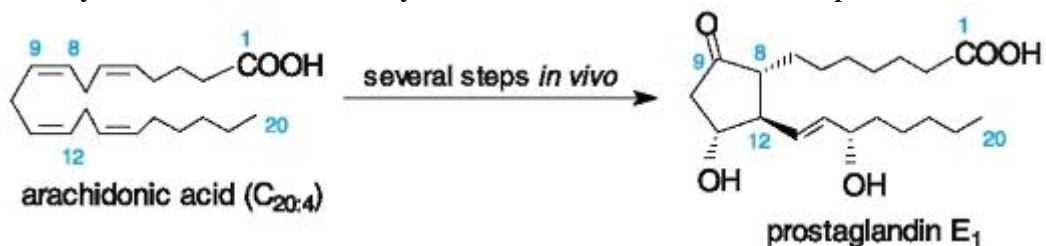
It is convenient to represent the carbon chain of fatty acids by the zigzag line in a skeletal formula that corresponds to a staggered conformation, for example:



Certain polyunsaturated fatty acids are termed *essential* because their absence in the human diet leads to some dysfunction. These acids are also known to be precursors of *prostaglandins*, a family of physiologically potent lipid acids.

Prostaglandins. This name originates from the fact that prostaglandins were first thought to be produced by the prostate gland. It is known today that prostaglandins are widely distributed in many human tissues but in minute amounts. Most of them being in very small concentration possess a powerful physiological activity of a broad spectrum.

Prostaglandins are C₂₀ carboxylic acids related to the unsaturated fatty acids. They contain a five-membered ring with two long side chains and differ from one another only in the number of hydroxyl and oxo groups present and in a degree of unsaturation. Prostaglandins are biosynthesized by oxidation and cyclization of the C_{20:4} fatty acid, arachidonic acid, for example:



Problem 1. Write equations for reactions of oleic acid with: (a) aqueous sodium hydroxide; (b) bromine in CCl₄; (c) alkaline solution of KMnO₄. Name the products obtained.

1.2.2. Alcohols

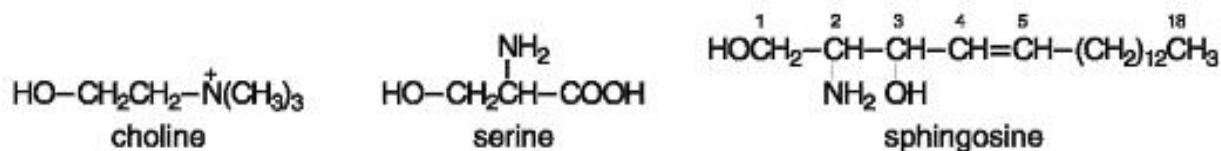
Several types of alcohols may serve as lipid components:

- monohydric long-chain alcohols;
- polyhydric alcohols;
- amino alcohols.

The first group is mainly presented in waxes that contain linear saturated alcohols with 16 and more carbons, such as cetyl alcohol (C₁₆) and melissyl alcohol (C₃₀).

The main representative of polyhydric alcohols is *glycerol*, though other polyols are sometimes encountered.

A group of amino alcohols includes *colamine* (2-aminoethanol), *choline*, an amino acid *serine*, and a long-chain unsaturated alcohol *sphingosine*.



1.3. SIMPLE LIPIDS

1.3.1. Waxes

Waxes are mixtures of esters formed by fatty acids and long-chain alcohols.

Both fatty acids and alcohols (usually monohydric alcohols) may each contain from 12 to 46 carbon atoms. Waxes are natural protective coatings on the skin, fur, or feathers of animals and on the leaves and fruits of plants. These solid substances are insoluble in water and in cold ethanol. *Beeswax* is largely myricyl palmitate, C₁₅H₃₁COOC₃₀H₆₁.

Spermaceti, a flavoured substance from the head of the sperm whale, is mainly cetyl palmitate, C₁₅H₃₁COOC₁₆H₃₃.

It was much used in cosmetics until it was interdicted in the 1970's to save the whales population.

Waxes are generally harder and less greasy than fats. They find important applications in the production of cosmetics, ointments, and other pharmaceutical preparations.

1.3.2. Fats and Oils

Fats and oils, along with carbohydrates and proteins, constitute a class of foodstuffs. We consume fats every day with milk, butter, animal fat, vegetable oil, and many other foods. Fats are

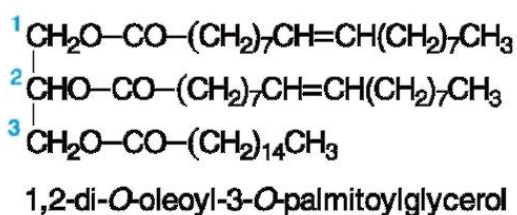
the greatest and most concentrated storehouse of energy for animals. (Plants, on the contrary, usually store their energy in the form of starch.) Enough fat can be accumulated to last a person for months. Besides, fats and oils find application in various industries for manufacturing soap, paint, margarine, etc.

Fats and oils are mixtures of esters formed by fatty acids and glycerol.

These esters are known as *triacylglycerols*; the older term *triglycerides* is also used but it is not accepted by the IUPAC nomenclature.

Fats and oils have the same principal structure (Table 1) in which all three OH groups of glycerol are esterified with the same or different fatty acids. The esters that have two or three different fatty acid residues are referred to as *mixed* triacylglycerols.

Systematic names of triacylglycerols are formed from glycerol as its *O*-acyl derivatives. This is exemplified as follows:



The R radicals of fatty acids may be written in a shortened form such as C₁₅H₃₁ for palmitic acid, C₁₇H₃₅ - for stearic acid, C₁₇H₃₃ - for oleic acid, C₁₇H₃₁ - for linoleic acid, and C₁₇H₂₉ - for linolenic acid.

A particular fat or oil consists not of a single triacylglycerol, but of a multi-component mixture (up to 20) of glycerol esters. Therefore, the composition of a fat or oil is usually expressed as the percentage of various fatty acids obtained from it by hydrolysis. Fatty acid composition of some fats and oils is given in Table 3.

Table 3.

Fatty acid composition of some fats and oils (% by mass)

Source	Saturated acids					Unsaturated acids		
	≤C ₁₀	C ₁₂	C ₁₄	C ₁₆	C ₁₈	C _{18:1}	C _{18:2}	C _{18:3}
Animal fats								
Butter	10–13	2–5	8–15	25–30	9–13	20–35	2–5	–
Lard	–	–	1–2	25–30	13–18	45–50	6–9	0–1
Beef tallow	–	–	2–5	24–34	15–25	35–45	2–5	0–1
Human fat	7	1	3	25	8	46	10	–
Vegetable oils								
Olive	–	–	1	5–10	2–4	70–84	7–17	–
Corn	–	–	1–2	7–10	3–4	30–45	41–50	–
Sunflower	–	–	1	6–9	2–5	24–40	45–70	1
Soybean	–	–	1–2	5–10	3–7	20–30	45–60	5–10
Peanut	–	–	–	6–11	25	40–60	20–35	–
Coconut	12–15	40–50	15–20	9–12	2–4	6–9	1	–

Now the question arises: Why are some triacylglycerols solids (fats) and the other are liquids (oils)? To answer this question, let us analyze the data in Tables 2 and 3. Unsaturated fatty acids have lower melting points than their saturated counterparts do, because the double bond (or bonds) present in the unsaturated fatty acids prevents the molecules from packing together as tightly in the crystal lattice as the saturated acids do. As a result, the forces between the molecules of unsaturated fatty

acids in a crystal are weaker than those between the molecules of saturated fatty acids, and this accounts for the lower melting points.

This is also true for triacylglycerols. Vegetable oils generally contain a high proportion of low-melting unsaturated acids in comparison to saturated ones than animal fats do, thus oils are liquids at room temperature¹. A high percentage of saturated fatty acids generally confers a semisolid or solid character of the fat.

Coconut oil and cocoa-bean oil, not listed in the table, are exceptions. Both of them contain a high proportion of high-melting saturated fatty acids and are therefore solids. This means that there is no strict definition for fats and oils. Fats are usually referred to as solid triacylglycerols, while oils are liquid ones. Sometimes they are classified according to their natural sources: fats are triacylglycerols of animal origin and oils are those of plants.

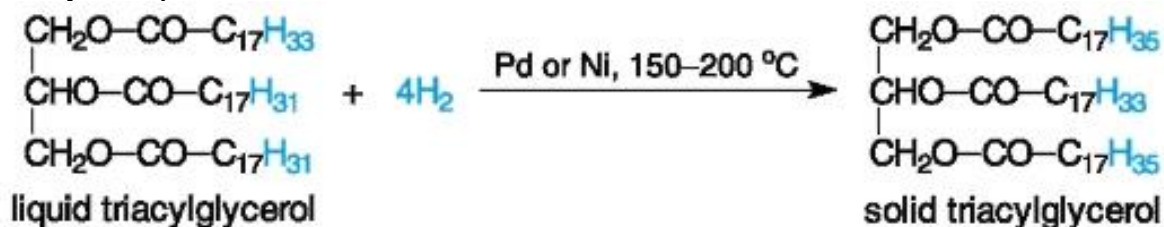
The degree of unsaturation in any fat or oil is conveniently expressed by *iodine value* (or iodine number). This is the mass of iodine in grams taken up by 100 g of a fat or oil. The method is based on electrophilic addition of iodine to the C=C double bond.



The higher the iodine value, the greater the degree of unsaturation. Oils tend to have iodine numbers above 70, whereas those of fats are generally below 70. For example, butter has the iodine value of 25-30, olive oil - of 80-85, corn and sunflower oils - of 120-130, and linseed oil, one of the most unsaturated oils, - of 180-185.

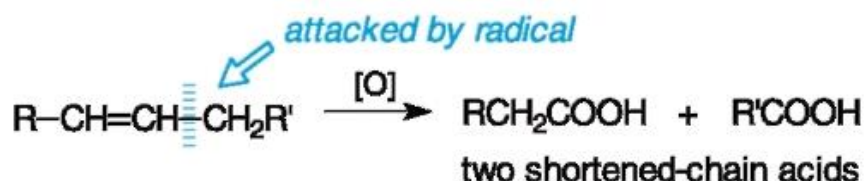
Problem 2. Triacylglycerols from olive oil and corn oil consist of approximately equal amounts of saturated (8-14%) and of unsaturated (86-92%) fatty acids, but their iodine values differ considerably. In the same order the freezing points of these oils decrease. Explain these facts.

Hydrogenation of oils. Because solid fats are often easier to use than liquid oils, the conversion of vegetable oils to solids by hydrogenation of some double bonds in the unsaturated fatty acid chains is done commercially on a large scale. *Margarine* and solid cooking fats are manufactured by hydrogenating vegetable oils until the proper consistency is obtained. A principal equation of the reaction may be expressed as follows:



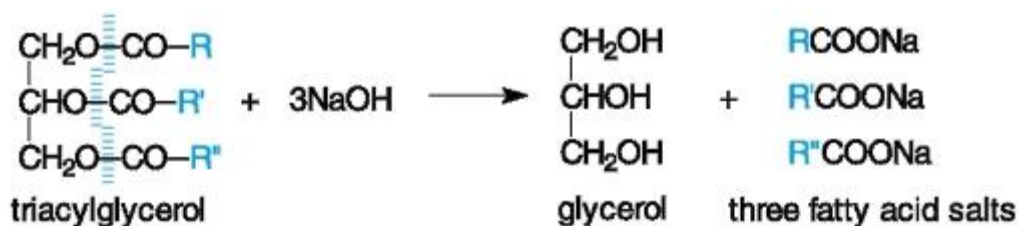
The process of hydrogenation of oils to fats is referred to as *hardening* of vegetable oils. Saturated fats have been implicated in arterial disease, and unsaturated fats have been thought to have a beneficial effect in preventing arterial deposits. The use of liquid oils in human diet has therefore increased.

Oxidation of triacylglycerols. This is one of the most important reactions of unsaturated triacylglycerols, including their transformation *in vivo*. Oxidation with molecular oxygen proceeds as a free radical process in which the radical HO' or HOO' attacks a CH₂ group neighboring to the C=C fragment. The process may be shown schematically as follows:



This reaction leads to rancidity of fats and oils because short-chain fatty acids have an unpleasant odor. In living cells the oxidation results in destroying cell membranes.

Hydrolysis of triacylglycerols. Either acid or base can catalyze hydrolysis of triacylglycerols in the same manner as with other esters. When heated with aqueous alkali, fats and oils yield glycerol and fatty acids (as salts):

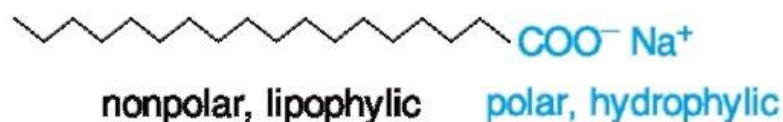


Such sodium (and potassium) salts are called *soaps*, from which the term *saponification* is derived. It is general practice to describe the alkaline hydrolysis of an ester as saponification even when the product is not soap.

1.3.3. Soaps and Detergents

One of the most ancient organic reactions is the boiling of lard with solution of caustic soda (NaOH) to produce soap. Modern commercial soaps contain perfumes and other additives to enhance their attractiveness.

Soap molecules have two dissimilar ends: a hydrocarbon chain that is non-polar and hydrophobic (repelled by water) but lipophilic (attracted to fats), and carboxylate salt end, which has just the opposite properties and is polar and hydrophilic.



One of the undesirable properties of soaps is that they form insoluble salts with calcium, magnesium, or ferric ions present in hard water. For this reason one of the most useful innovations in cleansing has been the development of synthetic detergents. They have desirable properties of ordinary soaps but form water-soluble salts with the ions of hard water. Widely used detergents are alkylated benzenesulfonates, $\text{R}-\text{C}_6\text{H}_4\text{SO}_3\text{Na}$, and alkyl sulfates, $\text{RO}-\text{SO}_3\text{Na}$ ($\text{R} = \text{C}_{10}-\text{C}_{14}$ alkyl).

Control materials for the final stage of the class.

Questions to check the final level of knowledge:

1. Structural components of lipids.
2. Features of the structure and properties of higher fatty acids.
3. Classification of lipids.
4. Preparation of lipids.
5. Chemical properties of lipids.
6. Biological role of simple lipids.

Literature

Basic:

1. Biological and Bioorganic Chemistry: Bioorganic Chemistry: textbook / B.S. Zimenkovsky, V.A. Muzychenko, I.V. Nizhenkovska, G.O. Syrova. — 3rd edition – 2020. – 288 p.
2. Biological and Bioorganic Chemistry. Biological Chemistry: textbook / Yu.I. Gubsky, I.V. Nizhenkovska, M.M. Korda et al. — 2nd edition – 2021 – 544 p.
3. Bioorganic Chemistry. Rineyskaya O.N. textbook. – 2018. – 174 p.
4. Baynes J., Dominiczak M. Medical Biochemistry. 5th Edition. Elsevier, 2018. 712 p.

Additional:

1. Satyanarayana U. Biochemistry. 5th edition. India 2020. – 777 p.
2. Lehninger. Principles of Biochemistry. 7th edition. NY, United States. 2017.

3. William Marshall, Marta Lapsley, Andrew Day, Kate Shipman. Clinical Chemistry. Elsevier, 2020. 432 p.

Електронні інформаційні ресурси:

1. [https://info.odmu.edu.ua/chair/biology/-](https://info.odmu.edu.ua/chair/biology/)
2. <http://libblog.odmu.edu.ua/>
3. <https://moodle.odmu.edu.ua/login/index.php>

Practical class № 6

Topic: Phospholipids. Biological significance and structure of the lipid component of biomembranes.

Relevance of the topic: Phospholipids are complex saponifiable lipids that are found in all living cells. They are important components of biological membranes and nervous tissue. As part of blood lipoproteins, they participate in the transport of fats, fatty acids and cholesterol. Knowledge of the properties of phospholipids is necessary for further study of such disciplines as biochemistry, pharmacology.

Goals: to form systematic knowledge about the structure, chemical properties and biological role of complex omilic lipids - phospholipids and their structural components as a chemical basis for studying the structure of biological membranes and lipid metabolism.

Equipment: department laboratory

Plan and organizational structure of the class:

1. Classification of complex lipids.
2. Amino alcohols - structural components of phospholipids.
3. Phospholipids. Features of the structure. Hydrolysis.
4. Sphingolipids.
5. Glycolipids.
6. Biological role of phospholipids.

The higher education applicant should know and be able to:

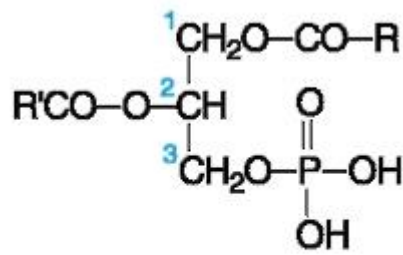
- mechanism of esterification and hydrolysis reactions;
- biologically important fatty acids;
- basic structural components of lipids;
- conditions of obtaining and hydrolysis of triglycerols;
- fats, oils, waxes. Their consistency and chemical properties;
- analytical characteristics of fats;
- surface-active properties of lipids, diffusivity, structure of their molecules.

Content of the topic

1.1. Phospholipids

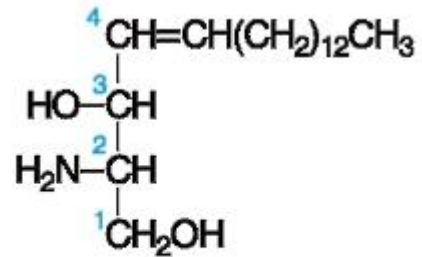
Phospholipids form a class of great biochemical importance; they constitute about 40% of cell membranes (the remaining being proteins). There are two main types of phospholipids: *phosphatides* (or glycerophospholipids) and *phosphosphingolipids*.

Phosphatides are derivatives of *phosphatidic acid* which is related structurally to triacylglycerols, except that one of the three acyl groups is replaced by phosphoric acid residue. Phosphosphinglipids are phosphorylated and *N*-acylated derivatives of sphingosine. Note that the formulas below indicate the stereochemistry of the chiral centres, i. e. the *R* configuration at C-2 in phosphatidic acid and the *2S,3R* configuration in sphingosine.



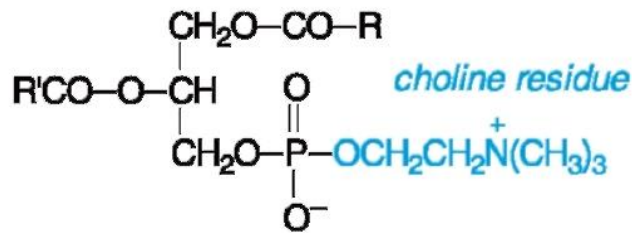
phosphatidic acid

(R and R' are fatty acid radicals)



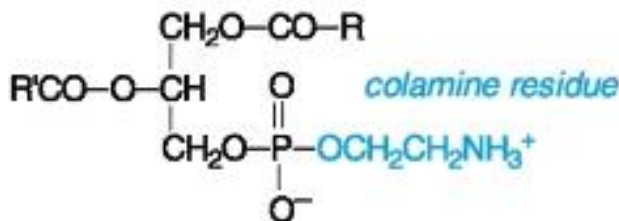
sphingosine

In phosphatides, the phosphate group is also linked by a separate ester bond to an amino alcohol. *Lecithins*, or phosphatidylcholines, are the most important representatives of phospholipids of higher animals and plants. They constitute up to 50% of total phospholipids.

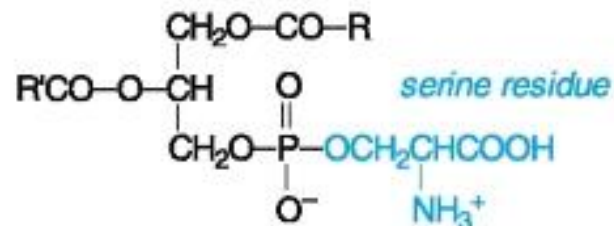


lecithin

Cephalins (from the Greek *kephalikos* - head) are isolated from the brain and spinal tissues. They form another important group of phospholipids in which nitrogenous component is colamine or serine.

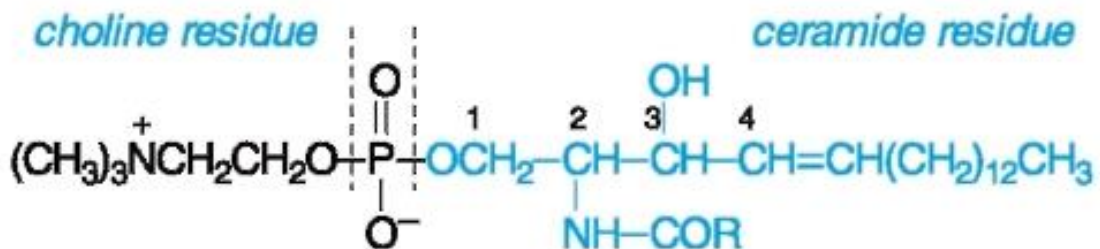


phosphatidylcolamine



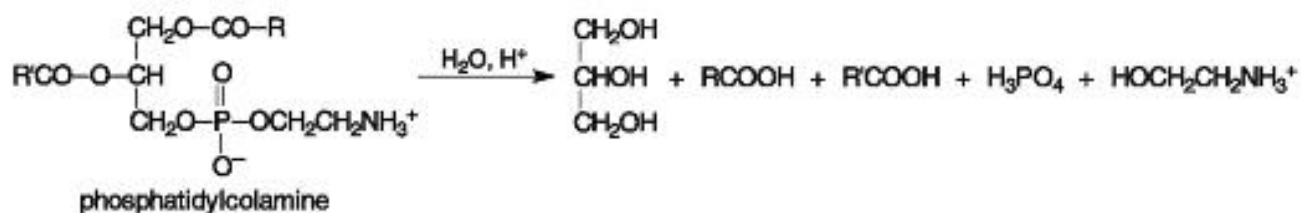
phosphatidylserine

Sphingomyelin, first found in the nervous tissues, is another example of cell membrane phospholipids. Its molecule consists of three parts: choline, phosphate, and *ceramide*; the latter is sphingosine acylated at the amino group with long-chain acids (mainly C_{24:0} and C_{24:1}).



sphingomyelin

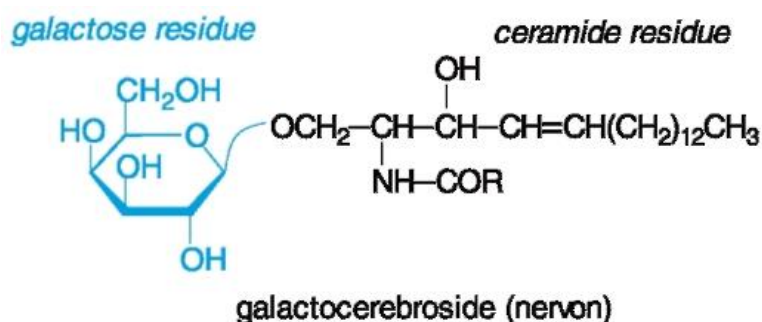
Like esters, phospholipids can be hydrolyzed under acidic or alkaline conditions. For example, complete hydrolysis of phosphatides releases fatty acids, glycerol, phosphoric acid, and choline, colamine, or serine, respectively.



Problem 1. Write the structures of the products that would be obtained by saponification of lecithin containing oleic and stearic acid residues.

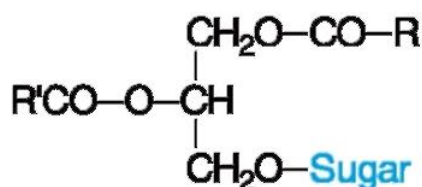
1.2. Glycolipids

The most important glycolipids are *glycosphingolipids*, compounds in which monoor oligosaccharide is linked by a glycosidic bond to the terminal hydroxyl group of the ceramide molecule. Monoand oligosaccharide derivatives are called *cerebrosides* and *gangliosides*, respectively. The sugar in cerebrosides is mainly D-galactose in the β anomeric form as shown below. An oligosaccharide chain in gangliosides may contain 4 to 10 various monosaccharide units.



Both types of glycosphingolipids were first isolated from the brain and it is reflected in their names. Then they were found in other tissues.

Glycolipids of the glycerol type are also known but they are not widespread. They are present in microorganisms, higher plants, and nerve tissues of mammals. Structurally, they are glycosylated 1,2-di-*O*-acylglycerols, as shown in the general formula where a sugar unit may be D-glucose, D-galactose, an amino sugar, or a disaccharide:



1,2-di-*O*-acyl-3-*O*-glycosylglycerol

A number of important functions have been found for complex lipids. Like soaps, phospholipids and glycolipids have a long, non-polar hydrocarbon tail bound to a polar head such as a phosphate group or sugar units. Cell membranes are composed mostly of phospholipids arranged in a *lipid bilayer* with polar heads exposed outside. Proteins are incorporated in the bilayer.

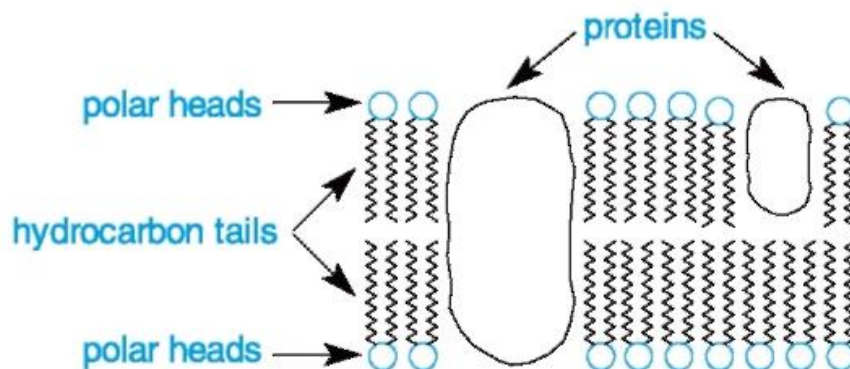
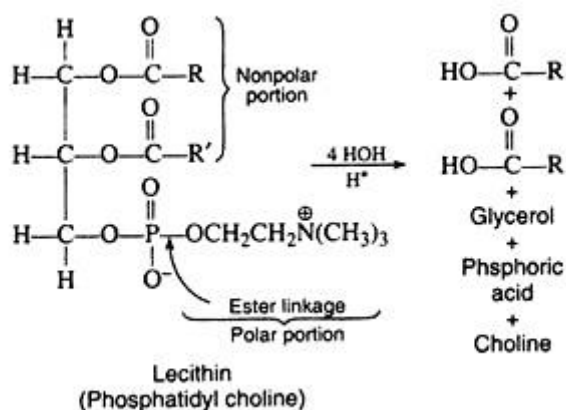


Figure 1. Schematic diagram of the lipid bilayer in the cell membrane.

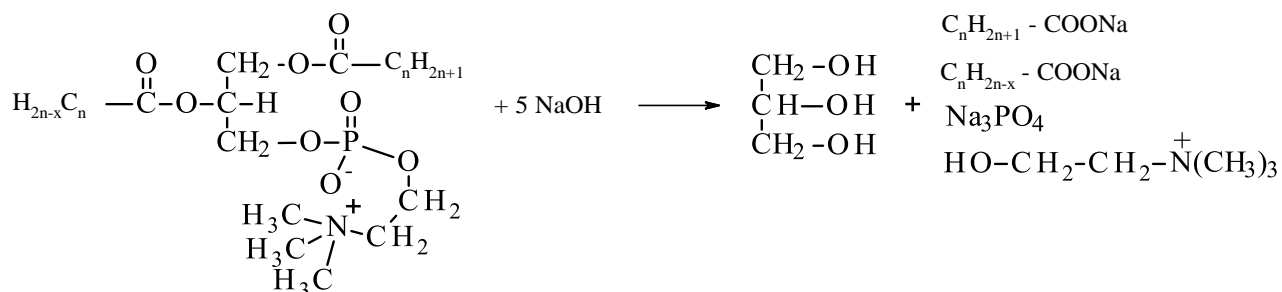
Complex lipids play special roles in secretory processes, in ion transport, and in selective permeability of cell membranes.

Chemical properties of phospholipids

a. Acidic hydrolysis



b. Alkaline hydrolysis



Control materials for the final stage of the class.

Questions to check the final level of knowledge:

1. Structural components of phospholipids.
2. Stage-by-stage production of phospholipids.
3. Reaction of acid hydrolysis of mullet, lecithin, phosphatidylserine.
4. Reaction of alkaline hydrolysis of mullet, lecithin, phosphatidylserine.

Literature

Basic:

1. Biological and Bioorganic Chemistry: Bioorganic Chemistry: textbook / B.S. Zimenkovsky, V.A. Muzychenko, I.V. Nizhenkovska, G.O. Syrova. — 3rd edition – 2020. – 288 p.
2. Biological and Bioorganic Chemistry. Biological Chemistry: textbook / Yu.I. Gubsky, I.V. Nizhenkovska, M.M. Korda et al. — 2nd edition – 2021 – 544 p.
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4. Baynes J., Dominiczak M. Medical Biochemistry. 5th Edition. Elsevier, 2018. 712 p.

Additional:

1. Satyanarayana U. Biochemistry. 5th edition. India 2020. – 777 p.
2. Lehninger. Principles of Biochemistry. 7th edition. NY, United States. 2017.
3. William Marshall, Marta Lapsley, Andrew Day, Kate Shipman. Clinical Chemistry. Elsevier, 2020. 432 p.

Електронні інформаційні ресурси:

1. <https://info.odmu.edu.ua/chair/biology/>-
2. <http://libblog.odmu.edu.ua/>
3. <https://moodle.odmu.edu.ua/login/index.php>

Practical class № 7

Topic: Carbohydrates. Structure and chemical properties of monosaccharides.

Relevance of the topic: The materials of the topic play a very important role in the study of bioorganic, biological chemistry, pharmacology. Carbohydrates are part of the cells and tissues of all plant and animal organisms. By weight, carbohydrates make up the bulk of organic matter on Earth. In nature they are of great importance as a source of energy in metabolic processes (in plants - starch, in animal organisms - glycogen), they are structural components of cell walls of plants (cellulose), bacteria (muramine) and constituent elements of vital substances (nucleic acids, coenzymes, vitamins). Some carbohydrates and their derivatives are used as medicines.

Goals: to strengthen the knowledge of the principles of stereochemical structure and chemical properties of monosaccharides and the ability to conduct qualitative reactions to identify the most important monosaccharides.

Basic concepts: optical isomerism, monosaccharide, glucose, fructose, mannose, galactose, ribose, deoxyribose.

Equipment: department laboratory

Plan and organizational structure of the class:

1. Classification of carbohydrates. Isomerism. Tautomeric forms of monosaccharides. Mutarotation.
2. Chemical reactions of monosaccharides with the participation of carbonyl group. Redox reactions as qualitative reactions for the detection of aldehyde group.
3. Formation of glycosides, their role in the construction of oligo- and polysaccharides, nucleosides, nucleotides and nucleic acids.
4. Phosphorus esters of glucose and fructose, their importance in metabolic transformations of carbohydrates.
5. Ascorbic acid as a derivative of hexoses, biological role of vitamin C.

The higher education applicant should know and be able to

- 1) classification of monosaccharides depending on the chain length and nature of the carbonyl group;
- 2) nomenclature of carbohydrates;
- 3) the phenomenon of optical isomerism, chirality, enantiomers, diastereomers;
- 4) D- and L- stereochemical series of carbohydrates;
- 5) cyclo-oxotautomerism of saccharides; pyranose, furanose;
- 6) anomerism in the series of monoses - α - and β - forms; the phenomenon of mutarotation;
- 7) the most important chemical properties of carbohydrates.

Content of the topic

CARBOHYDRATES. MONOSACCHARIDES

Carbohydrates are among the most abundant substances on the Earth and perform many vital functions in both plants and animals. The most common of these are cellulose, starch, and various sugars. Cellulose is the main structural component of plants, used to construct rigid cell walls, fibres, and woody tissue. Starch is the chief form for storing carbohydrates for later use as a food or energy source. In higher animals, the sugar glucose is an essential component of blood. Ribose and 2-deoxyribose are constituents of genetic material. Other carbohydrates are important components of coenzymes, antibiotics, cartilage, the shells of crustaceans, and bacteria cell walls. For the last decades a definite role played by carbohydrates in the processes of cell recognition and immunity was established.

Carbohydrates being the initial products of photosynthesis (from carbon dioxide and water) represent a certain «bridge» between inorganic and organic substances.

The term *carbohydrates*, which has been used since the mid-nineteen century, derives historically from the observation that many compounds in this class (for example ribose, $C_5H_{10}O_5$, glucose, $C_6H_{12}O_6$, and sucrose, $C_{12}H_{22}O_{11}$) have the empirical formula $C_x(H_2O)_y$ and are formally regarded as «hydrates of carbon». However many important compounds related to carbohydrates, such as deoxyribose, $C_5H_{10}O_4$, uronic acids, and amino sugars (see further in this chapter), are not covered by this definition. Nevertheless, this term is steadily employed along with the equivalent names *saccharides* or simply *sugars*.

It is difficult enough to give a clear definition of carbohydrates, because they include very different types of compounds, from small molecules with several carbon atoms to polymers whose molecular mass amounts to millions. Sometimes carbohydrates are defined as «polyhydroxy aldehydes, polyhydroxy ketones, or substances that give such compounds on hydrolysis». But in this book we have never used chemical properties in the definition of classes. It would be better therefore to divide carbohydrates into three big groups (or sub-classes), namely, monosaccharides, oligosaccharides, and polysaccharides, and to study them separately.

Monosaccharides are usually crystalline sweet substances. They are readily soluble in water and insoluble in ethanol, ether, and other solvents of low polarity. The most common definition of monosaccharides is the following:

Monosaccharides are polyhydroxy carbonyl compounds, namely, polyhydroxy aldehydes or polyhydroxy ketones.

Although this definition focuses on the main functional groups of monosaccharides, it is not entirely satisfactory too. We will soon see that the carbonyl group is virtually absent in monosaccharides.

1.1. Classification, Stereoisomerism, and Nomenclature

Monosaccharides can be classified as *aldoses* or *ketoses* depending on whether they contain an aldehyde or ketone group. They can also be classified according to the number of carbon atoms they contain. A *pentose*, for example, contains five carbons, a *hexose* is a six-carbon monosaccharide. These are the most widespread monosaccharides though trioses (C_3), tetroses (C_4), heptoses (C_7) etc. also exist. A combination of the two classifications gives rise, for example, to aldopentoses, ketohexoses, and so on (Fig. 1). Thus ribose (Fig. 1, a) is an aldopentose, i. e. it has five carbons and an aldehyde group, and fructose (Fig. 1, d) is a ketohexose (six carbons and a ketone group).

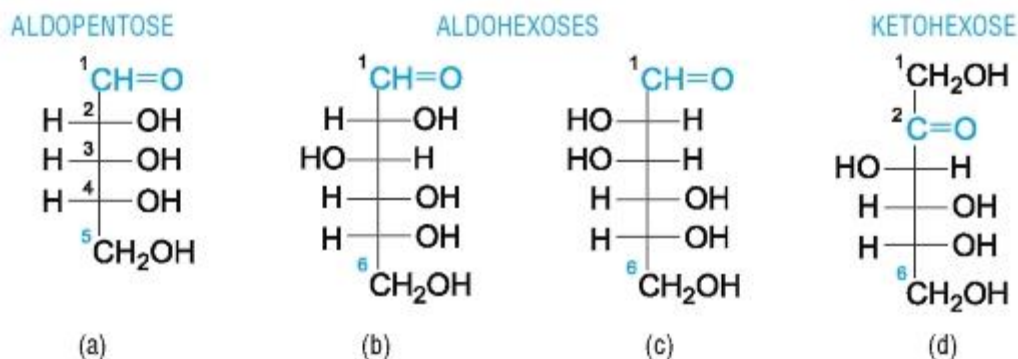


Figure 1. Classification of monosaccharides.

Monosaccharides have an abundance of chiral centres. Indeed, all carbons except for a carbonyl one and one of a terminal unit CH_2OH are chiral carbons. In other words, each carbon of the fragment $>\text{CHOH}$ is asymmetric one. The Fischer projection formulas are very convenient for studying stereoisomerism of monosaccharides. Let us consider the monosaccharides presented in Fig. 1.

Example 1. How many stereoisomers are possible for each compound from (a) to (d) in Fig. 1?

Solution. It is easy to calculate the number of asymmetrical carbon atoms and the number of stereoisomers of each type of monosaccharides, which is 2^n .

The compounds (a) and (d) have three chiral centers each: C-2, C-3, and C-4 for (a), and C-3, C-4, and C-5 for (d); thus each compound can exist as eight (2^3) stereoisomers, i. e. four pairs of enantiomers.

Sixteen (2^4) stereoisomers, i. e. eight enantiomeric pairs, are possible for both (b) and (c) since each of them contains four chiral carbons, C-2, C-3, C-4, and C-5.

If the chiral carbon *farthest* from the carbonyl group has the D configuration (hydroxyl on the right), a compound is a D monosaccharide. If the remote carbon has the L configuration, the compound as a whole is an L monosaccharide. Notice that designations D and L refer *only* to the configuration of the highest numbered (or the lowest in the Fischer projection) chiral carbon. They are not used for other chiral centres.

Each monosaccharide gets a definite name according to the combination of chirality of *all* centres.

The IUPAC nomenclature is based upon the trivial names with the suffix *-ose*. Figure 2 shows the Fischer projections for all aldoses through hexoses for the D series. Starting with D-glyceraldehyde, one chiral fragment CHOH (shown by the bold type) is inserted just after the aldehyde carbon. In each step, the new chiral centre can have the OH group at the right or at the left in the Fischer projection.

Two stereoisomers that differ in configuration at only one chiral centre are called *epimers*. For example, D-glucose and D-mannose (Fig. 2) are epimers since they have the opposite configuration only at C-2. Note that D-glucose and D-mannose are diastereomers but not enantiomers. D-Fructose is neither diastereomer nor enantiomer but constitutional isomer of D-glucose.

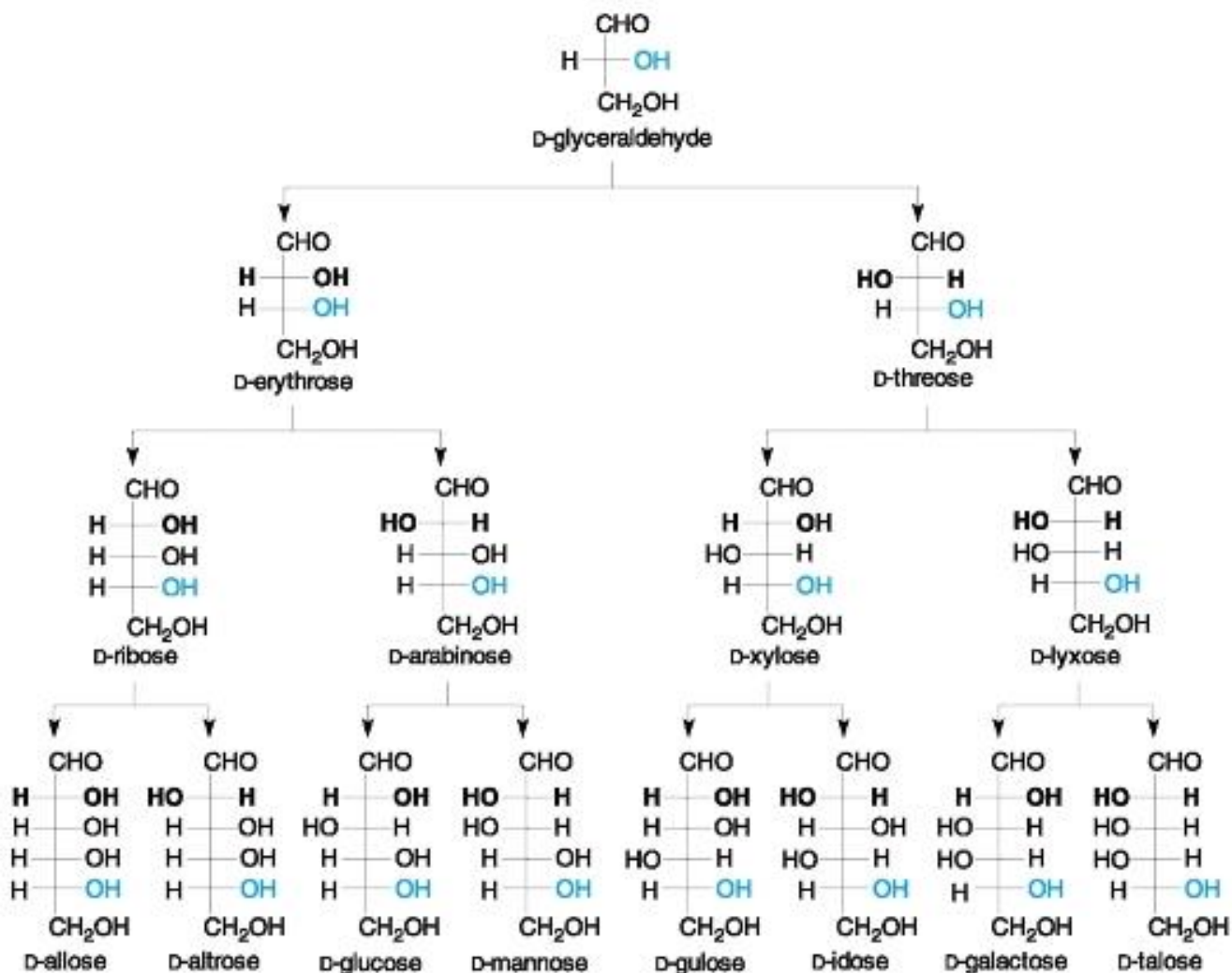


Figure 2. The family of the D-aldoses with up to six carbon atoms. The bold-faced H and OH show appearance of a new diastereomeric pair with additional carbon.

As you can see, all monosaccharides shown in Fig. 14.2 belong to the d series. In nature most monosaccharides, in fact, are d-sugars, but natural l-sugars are known as well. Here it should be stressed once more:

The D,L notation has no direct relationship to the sign of optical rotation of a given sugar.

Thus three of eight D-aldohexoses, three of four D-aldopentoses, and both D-aldotetroses are levorotatory substances. D-Fructose is also a levorotatory sugar, its first name was *levulose*. Moreover, Kekule suggested for D-glucose the name *dextrorse* because of its dextrorotation. The two terms remain in use in the German chemical literature up to the present time.

Only a few monosaccharides from Fig. 2 are virtually important, and their structures should be remembered. These are aldohexoses d-glucose, d-galactose, and d-mannose due to their wide occurrence in nature. The structures of the latter two sugars are easy to memorize once the structure of glucose is learned, since they are epimers of glucose. Mannose differs from glucose in configuration at C-2, galactose - at C-4.

The structure of ribose is very easy to memorize because all its OH groups are on the same side (for d-ribose on the right). Another aldopentose, xylose, is epimeric to ribose at C-3.

Finally, the most important ketose, d-fructose, has the same configuration at C-3, C-4, and C-5 as d-glucose.

Deoxy sugars - the absence of a hydroxyl group (or groups);

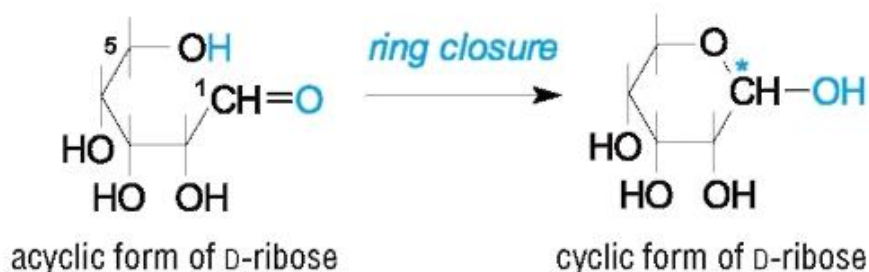
Amino sugars - replacement of an OH group (or groups) by the NH₂ and their N-acetylated derivatives group or -NHCOCH₃ group;

Uronic acids - the atom C-6 is oxidized to a carboxyl group;
 Aldonic acids - the atom C-1 is oxidized to a carboxyl group;
 Aldaric acids - both C-1 and C-6 are oxidized to carboxyl groups;
 Alditols - a carbonyl group is reduced to an alcoholic group.
 Specific examples of such unusual monosaccharides will be given hereafter.

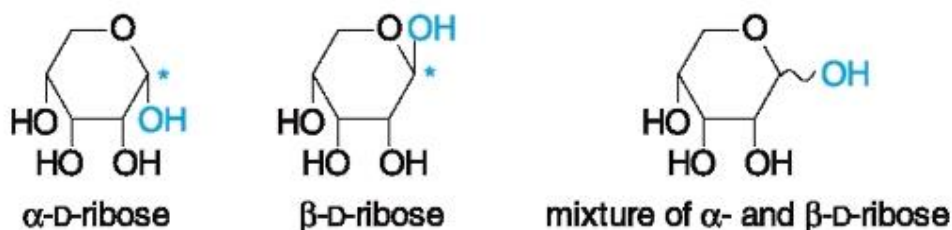
1.2. The Cyclic Hemiacetal Structures

Although the structures of monosaccharides described so far are consistent with much of their chemistry, they do not respond to some physical and chemical properties of the compounds. It is time to examine the true monosaccharide structures.

Recall that γ - and δ -hydroxy aldehydes readily form cyclic hemiacetals. The same is true of monosaccharides. The following scheme shows how the chain in D-ribose can be arranged so that the OH group on C-5 comes within reacting distance of the aldehyde carbon (C-1). Interaction of the two functional groups results in the cyclic, six-membered hemiacetal forms of the monosaccharide (most H symbols are not shown for simplicity; both the hydrogens and bonds to them will be omitted hereafter).



Anomeric centre. In the acyclic form of an aldose, the C-1 atom is achiral, but it becomes chiral in the cyclic structures. Thus, two hemiacetal structures are possible, differing in the configuration at the new chiral centre. This centre is called the anomeric centre, a new OH group at C-1 is also called anomeric (or hemiacetal) hydroxyl group. Two compounds that differ only in their configuration at the anomeric centre are said to be *anomers*. Anomers are attributed to the α or β type, depending on the position of the OH group (see below).

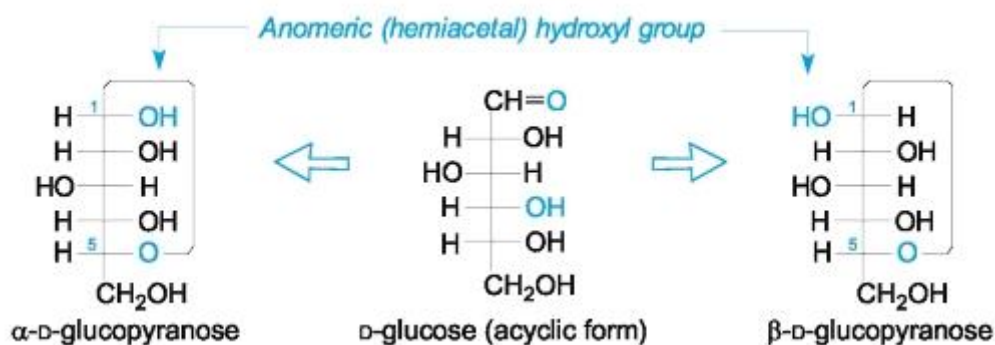


The anomeric mixture of monosaccharides can be depicted by a single structural formula with a wavy bond, like \sim OH (as above).

Conventions for writing cyclic monosaccharide structures. The six-membered cyclic form of most monosaccharides is the preferred structure. These structures are called *pyranose* forms, after the six-membered oxygen heterocycle *pyran*. Thus, the full names of the anomeric forms of D-ribose in the example above are α -D-ribopyranose and β -D-ribopyranose.

Three types of formulas commonly represent the cyclic structures of monosaccharides. *Conformational* formulas that will be discussed later give the closest representation of the true molecular geometry and shapes, but it is a little difficult to draw them. Two other types are the *Fischer projection formulas* and *Haworth formulas*.

The Fischer projection formulas demonstrate visually configurations at each asymmetric carbons. They can be adopted to show cyclic structures on the example of D-glucose:

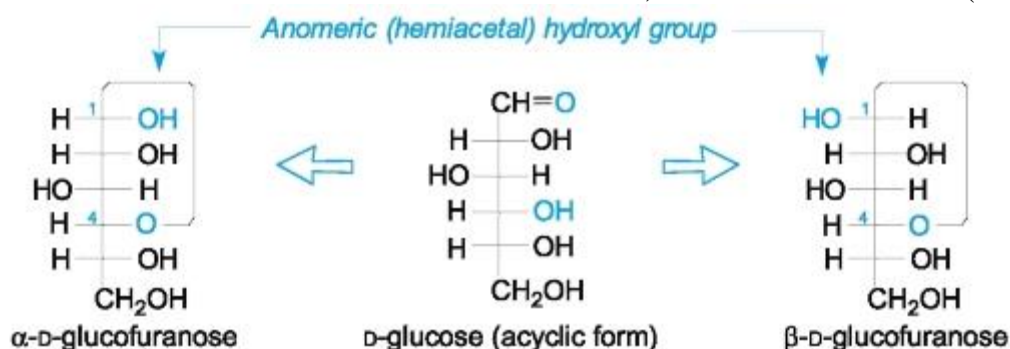


In the centre of the drawing we have the open-chain aldehyde form (sometimes designated **aldehydo-** or *al-*) of the monosaccharide. It is shown at the right and left that the OH group at C-5 is a part of hemiacetal structure. In the α -anomer the C-1 atom has the same configuration as configurational carbon (C-5 in hexoses and C-4 in pentoses), i. e. the anomeric OH group is at the right for D-sugars.

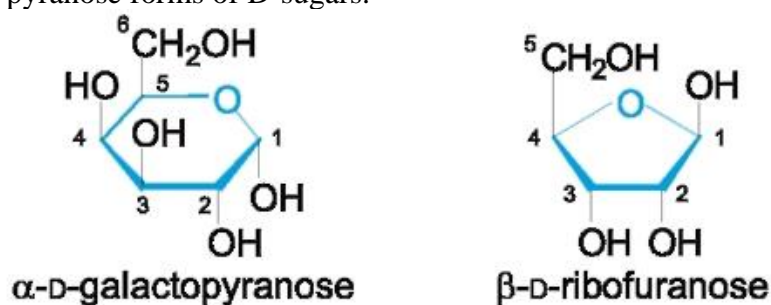
Although the Fischer conversion is fairly easy to use for cyclic structures, it contains long and distorted bonds that connect C-1 and C-5 through the ring oxygen.

There is another possibility for the cyclization. If the hydroxyl group at C-4 of an aldose participates in the cycle formation, the cyclic product represents a five-membered ring called a *furanose*, after the parent heterocycle *furan*.

¹ They are named after the British chemist W.N. Haworth, the Nobel Prize winner (1937).



The Haworth formulas that appeared in the 1920's use planar hexagon or pentagon to represent the cyclic structures, pyranose and furanose, respectively. The monosaccharide is depicted with the carbon chain horizontally, the anomeric C-1 atom being to the right. The cyclic oxygen is then depicted as being formed behind the plane of the paper. The ring is therefore located in a plane perpendicular to the plane of the paper and the groups attached to the carbons are above and below the ring. The groups, including the anomeric hydroxyl, which occur to the right in the Fischer projection then appear below the ring plane. The atom C-6 (usually CH₂OH group) will always be above the plane in the pyranose forms of D-sugars.



The Haworth perspective formulas clearly show the configuration at each chiral centre, but it should be kept in mind that they do not correspond to true geometry of the monosaccharide molecule either.

Mutarotation. Tautomerism of monosaccharides.

Let us consider the following experimental data. If D-glucose is crystallized from aqueous ethanol, the pure α form is obtained. On crystallization of D-glucose from acetic acid, another product was isolated, the β form. Both forms of D-glucose are *diastereomers* since they differ only in the configuration at C-1. Being diastereomers, the α and β forms have different physical properties such as melting point, specific rotation, solubility, etc.

The two forms of D-glucose interconvert in solution (Fig. 3). For example, if the pure α anomer is dissolved in water, the specific rotation decreases with time from an initial value of $+112^\circ$ to an equilibrium value of $+53^\circ$. On the other hand, the same experiment with the pure β anomer results in a gradual increase of specific rotation from an initial $+19^\circ$ to the same equilibrium value of $+53^\circ$. This phenomenon is known as *mutarotation* (mutation of rotation) and can be explained by the slow conversion of the α - and β -pyranose forms into the 36:64 equilibrium mixture (contribution of furanose forms may be ignored for D-glucose).

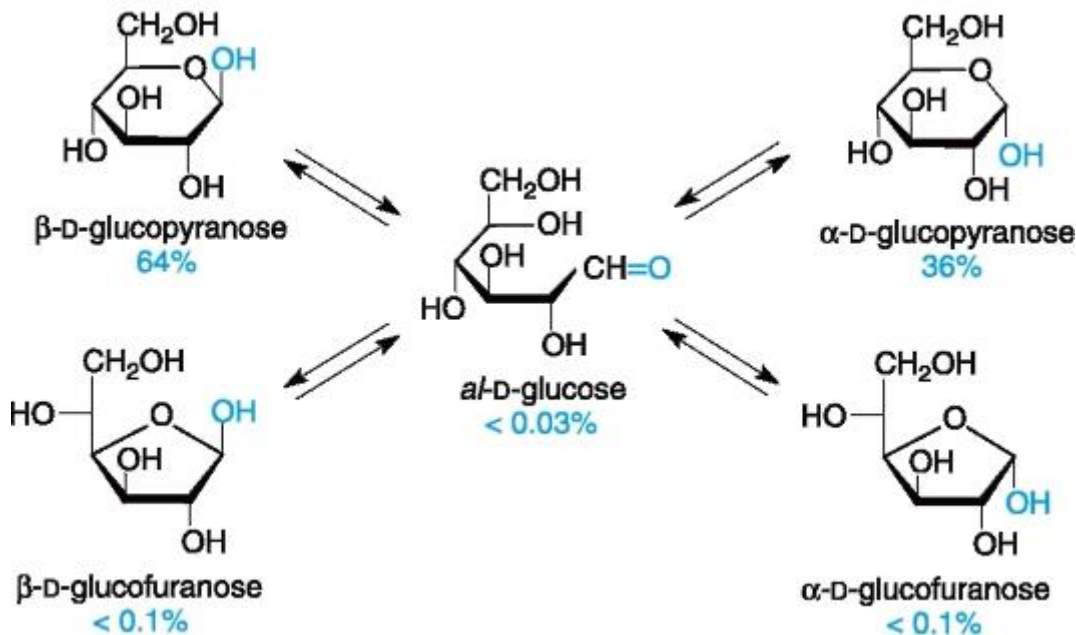


Figure 3. A tautomeric equilibrium of D-glucose.

Starting with either pure anomeric form, the ring opens to form an acyclic form, which then recycles to give both the α and β forms. Finally, an equilibrium mixture of five tautomers is obtained. At equilibrium, an aqueous solution of D-glucose contains approximately 64% of the β -pyranose form, 36% of the α -pyranose form, less than 0.1% of both furanose forms, and less than 0.03% of the open-chain form.

Interconversions of different forms of monosaccharides in a solution is called ring-chain tautomerism (or cyclo-oxo tautomerism).

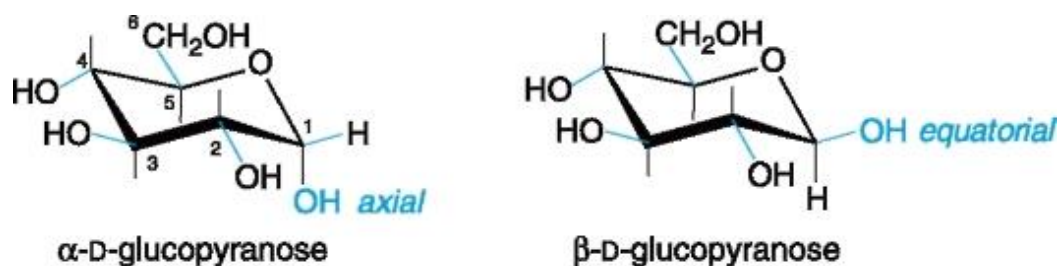
The ratio of tautomers in solution is specific for each monosaccharide. As we have seen, D-glucose in neutral solution at room temperature consists of over 99.9% of pyranose forms. D-Galactose and D-xylose have almost the same ratio of tautomers as D-glucose, whereas the α -pyranose form is predominant (68%) for D-mannose and the ratio between pyranose and furanose forms for D-ribose is about 3:1 in the same solution. Since any monosaccharide, especially in the crystalline form, is virtually a cyclic compound but not a carbonyl compound, a new definition for monosaccharide can be suggested:

Monosaccharides are cyclic hemiacetals of polyhydroxy carbonyl compounds.

Conformations.

Finally, the most real structures for monosaccharides are conformational ones. It is known that a six-membered ring is nonplanar, but has the *chair* conformation, as being more stable. The same is true of the pyranose ring.

Let us look at the conformations of α - and β -D-glucopyranose, in which the CH_2OH group and all OH groups (except for the hemiacetal OH in the α form) are in stable *equatorial* positions:

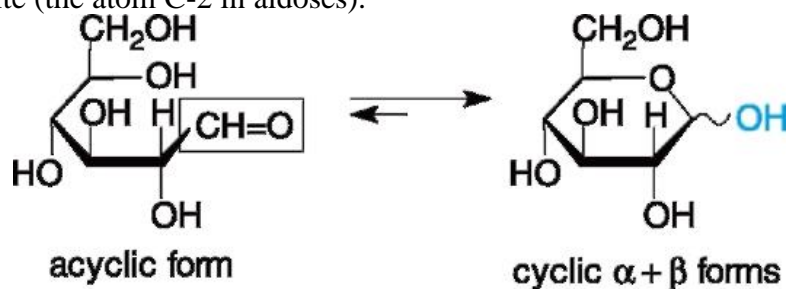


Such arrangement of substituents in the ring accounts for the predominance of the β anomer over the α anomer in an equilibrium mixture of D-glucopyranose. Besides, this makes it clear why D-glucose is the most stable aldohexose. Indeed, any hexose, except for D-glucose, has at least one OH group oriented axially that diminishes its stability. Conformation of monosaccharides is very important for the space structure of polysaccharide chains.

1.3. Chemical Properties

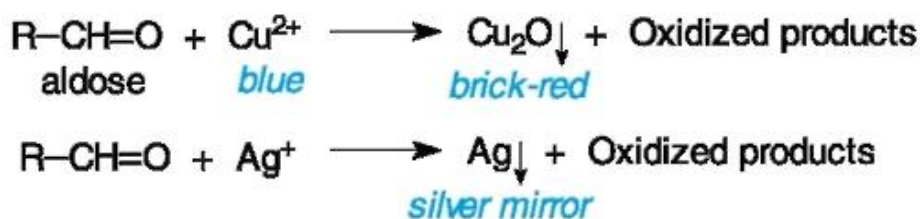
Monosaccharides are compounds of very high reactivity due to their polyand heterofunctionality. The following reactive sites can be noted in the monosaccharide molecule:

- the carbonyl group of an acyclic form shown in a rectangle;
- the hemiacetal hydroxyl group shown in colour;
- alcoholic hydroxyl groups (the remaining hydroxyls);
- a CH-acidic site (the atom C-2 in aldoses).



Let us consider all of them.

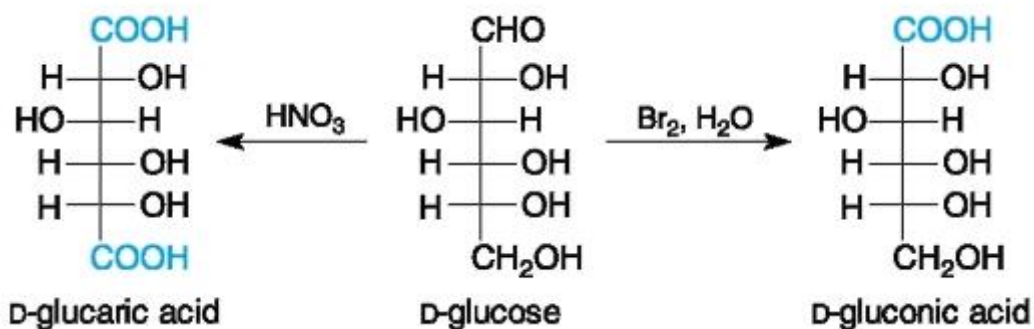
Reactions of the carbonyl group. In spite of a very low concentration of an open-chain form in a tautomeric mixture of monosaccharides, some reactions of monosaccharides as aldehydes can be carried out. For example, aldoses react with Tollens' reagent, Fehling's reagent, or Benedict's reagent (Cu^{2+} complexed with citrate ion) to yield reducing metal species and an intricate mixture of oxidized products:



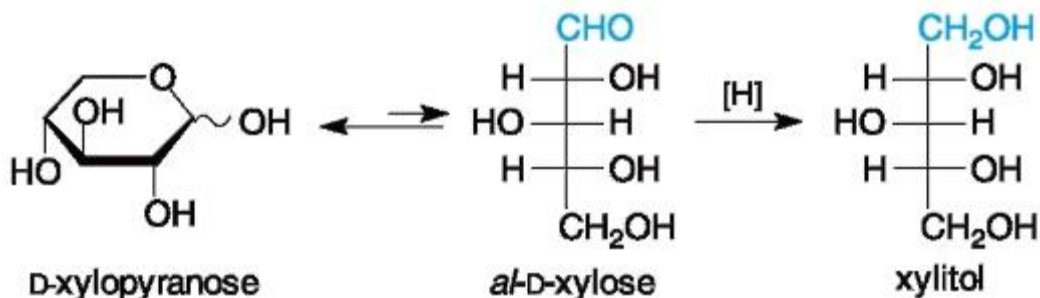
For this reason aldoses are classified as *reducing sugars*. It might be somewhat surprising that ketoses are also oxidized by the mentioned ions (recall that ketones do not normally reduce these reagents). Explanation is that ketoses readily convert into the isomeric aldoses in basic solution.

The above reactions are used as diagnostic tools in quantitative determination of glucose in blood and urine.

Mild oxidation of aldoses with bromine water affects only the aldehyde group giving rise to *aldonic acids* (particular names are D-gluconic acid, D-galactonic acid, etc.). Stronger oxidants, such as dilute nitric acid, attack both the aldehyde group and the primary alcoholic group to form dicarboxylic acids known as *aldaric acids*.



Reduction of the carbonyl group into the CH₂OH fragment gives *alditols*, for example:

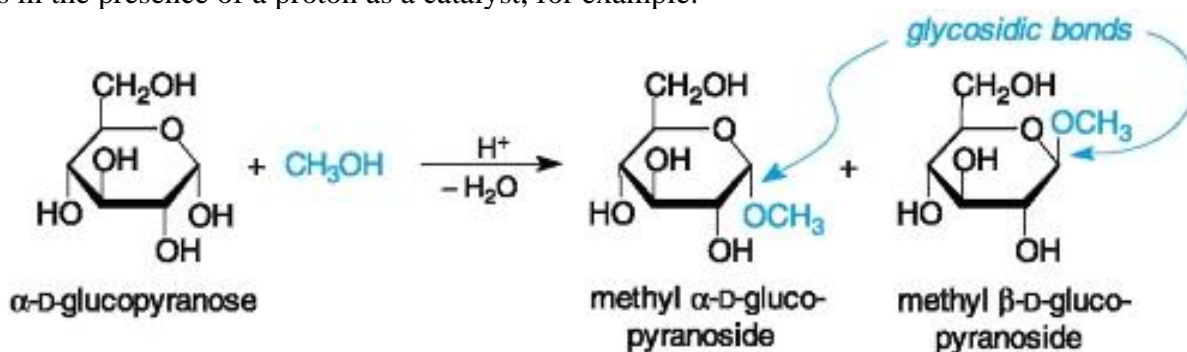


Some of them, such as xylitol and D-glucitol (the old name *sorbitol*, from D-glucose), are used as a sugar substitute for diabetics. Alditols are not involved in biochemical cycles because they do not belong to classical monosaccharides by their structure (they do not contain a carbonyl group).

Reactions of the hemiacetal hydroxyl.

This reactive site causes perhaps the most important chemical properties of monosaccharides. In plants and animals monosaccharides are rarely found in a free state, but mainly as acetals. (Here you should recollect the formation of an acetal in the reaction of an aldehyde with an alcohol through an intermediate hemiacetal).

A similar reaction takes place between a monosaccharide and lower alcohols under anhydrous conditions in the presence of a proton as a catalyst, for example:

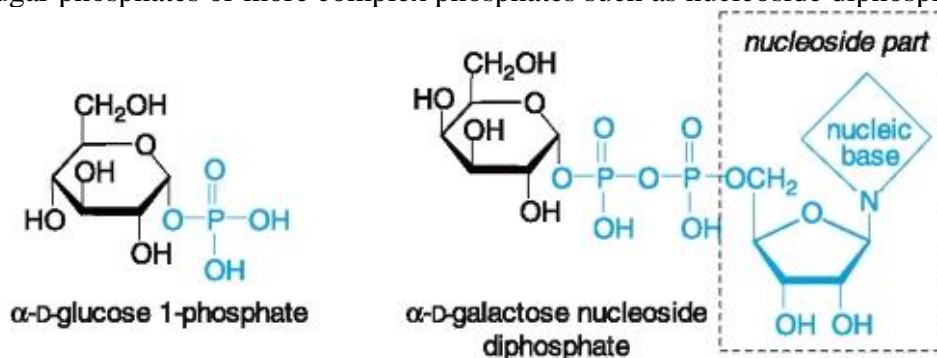


The method is known as the *Fischer glycosidation reaction* (1893). This is a familiar nucleophilic substitution reaction, in which a catalyst (H⁺) converts the OH group at C-1 into a good leaving group (a water molecule).

It is interesting that the classic reaction of acetal formation was also devised by E. Fischer but several years later after his working out the glycosidation reaction.

The resulting sugar acetals are called *glycosides*. Particular glycosides are named from the respective monosaccharides, using the suffix -oside: glucosides from glucose, ribosides - from ribose, etc.; a name of the R group is placed in front the full name of a glycoside (see examples in the text). The bond from C-1 to the OR group of an alcohol is called the *glycosidic bond*, and the OR unit of the glycoside is called an *aglycone*.

Glycosides are also formed in living systems. Substrates in the reactions that occur in the organisms are sugar phosphates or more complex phosphates such as nucleoside diphosphates:

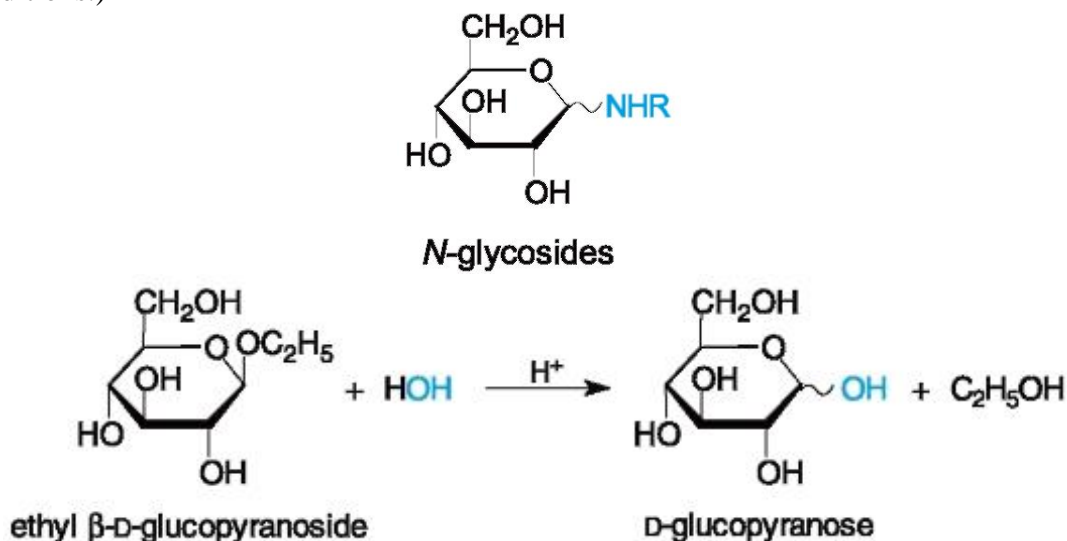


The main feature is that a phosphate and a nucleoside diphosphate (shown in colour) are excellent leaving groups.

In biological systems monosaccharides form glycosidic bonds with an alcoholic OH group of another monosaccharide molecule thus giving a disaccharide, and so until a polysaccharide is formed.

The so-called *N-glycosides* relate to glycosides as their nitrogen analogues. *N-Glycosides* of D-ribose and 2-deoxy-D-ribose represent structural blocks of nucleic acids (RNA and DNA) and will be considered in Chapter 17.

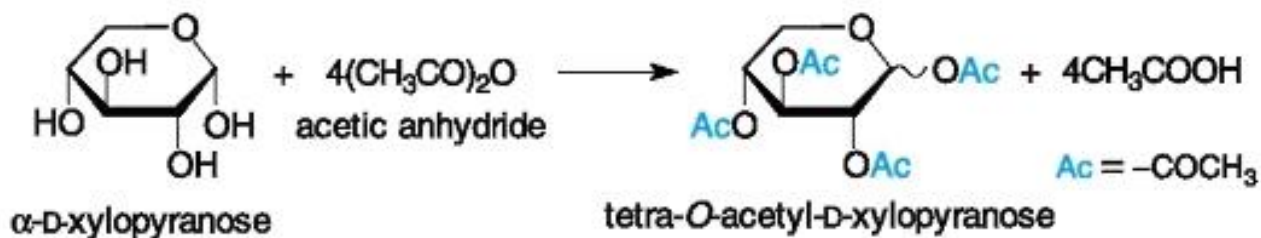
The most important property of glycosides is their hydrolysis in acidic solution, whereas in dilute alkaline solution glycosides are quite stable. (Compare this with the behaviour of acetals under similar conditions.)



This reaction is reversed to the formation of glycosides.

Reactions of alcoholic hydroxyls.

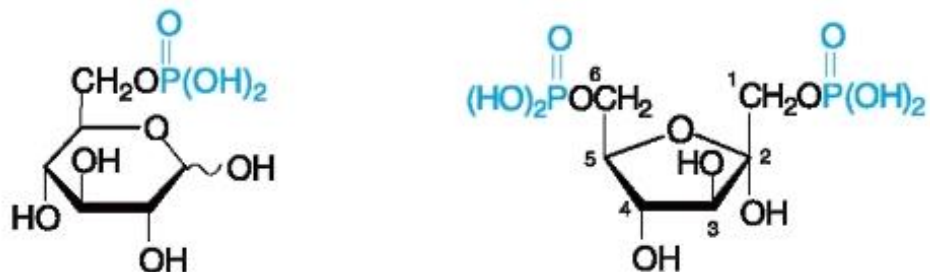
Being polyhydroxylic compounds, monosaccharides can result in ester and ether formation. Sugar esters are formed in the reaction of monosaccharides with acylating agents such as acyl halides or acid anhydrides, for example:



In biological transformations of carbohydrates, inorganic esters, namely, sugar phosphates, are very important.

Example 2. Draw the Haworth formulas for D-glucose 6-phosphate and α -D-fructose 1,6-diphosphate, both formed in the glycolysis process.

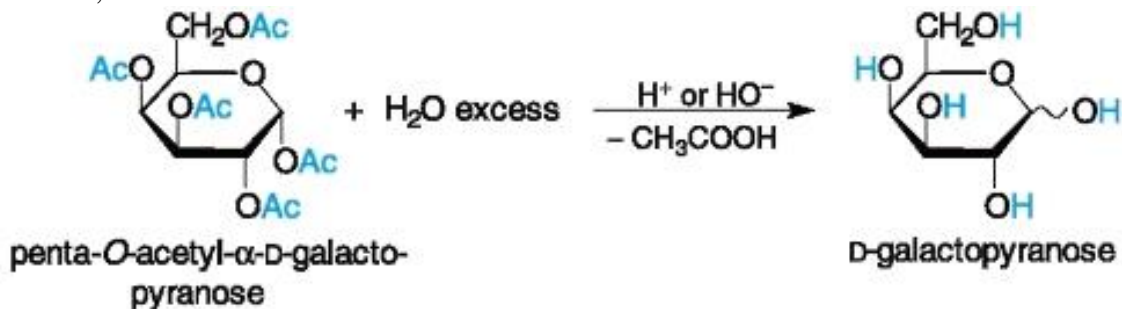
Solution. The structure of D-glucose 6-phosphate is very similar to that of 1-phosphate (see above). D-Fructose can exist in two cyclic forms, pyranose and furanose. 1,6-Diphosphate can be formed only from the furanose form that has the OH group at C-6 (to make sure of it draw the Haworth formula for D-fructopyranose).



D-glucopyranose 6-phosphate

α -D-fructofuranose 1,6-diphosphate

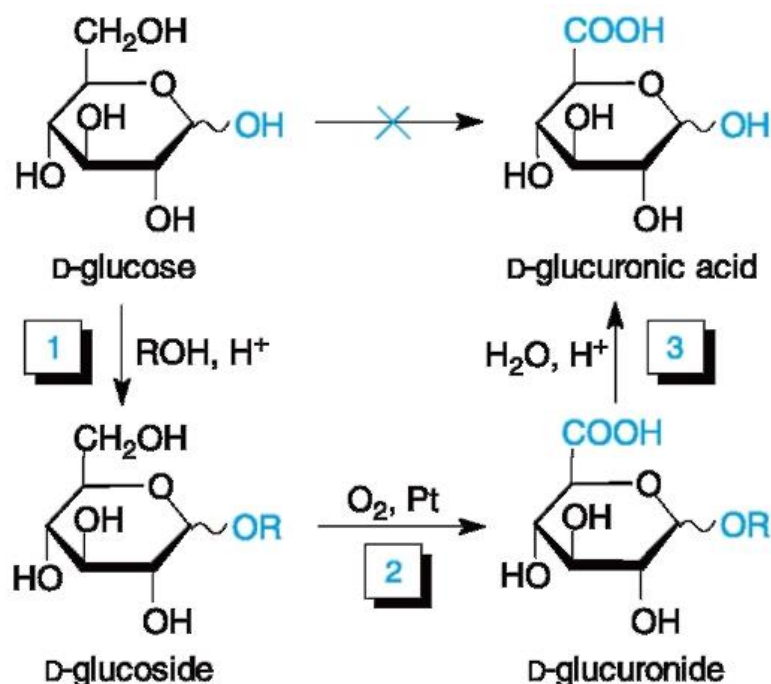
Monosaccharide esters can be hydrolyzed in an acidic or alkaline medium to the corresponding acid and alcohol, in our case it will be a monosaccharide:



penta-O-acetyl- α -D-galactopyranose

D-galactopyranose

As has already been shown, primary and secondary alcohols can be oxidized into carboxylic acids or ketones, respectively. Primary alcohols are oxidized easier than secondary ones. However, the atom C-1 (in the potential aldehyde group of an aldose) is the most sensitive site to oxidation. Therefore, if we want to oxidize a primary alcoholic group (the atom C-6 in hexoses), it is necessary to «protect» the hemiacetal group, for instance, by means of glycoside formation (step 1 in the scheme below).

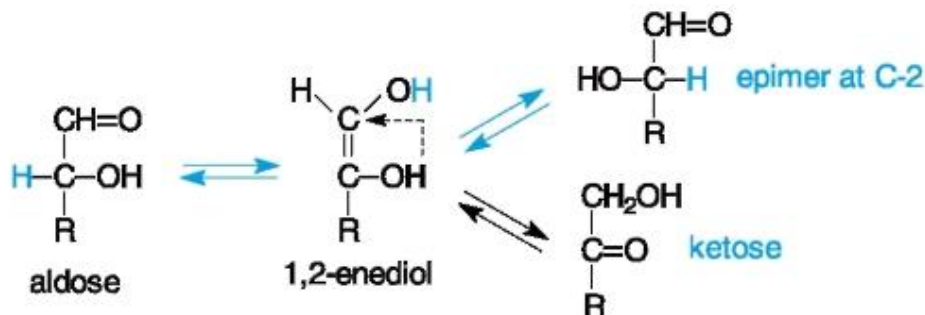


Subsequent catalytic oxidation of the primary CH₂OH group in the glycoside gives a uronic acid glycoside (step 2), which then is hydrolyzed (step 3) to a *uronic acid*.

In living systems, a phosphate group at C-1 is used as a protection in the oxidation step. Uronic acids are components of connective tissue polysaccharides.

CH-Acidic site. This site is the C-2 atom of aldoses and the C-1 and C-3 atoms of ketoses, i. e. nearest to the carbonyl group.

Aldoses isomerize partly in basic solution at room temperature to give an equilibrium mixture of an epimeric at C-2 aldose and a ketose. Isomerization is thought to proceed through an intermediate 1,2-enediol.



D-Glucose thus yields a mixture with D-fructose (29%) and D-mannose (1%) on storage in calcium hydroxide solution. Such isomerization occurs also in an acidic medium, but slower, and can be catalyzed by enzymes in living systems.

Control materials for the final stage of the class.

Questions to check the final level of knowledge:

1. Classification of carbohydrates.
2. Isomerism - structural, structural, interclass.
3. D- and L-row.
4. Cyclo-oxo-tautomerism of monosaccharides - glucose, mannose, galactose, fructose, ribose and deoxyribose.
5. Oxidation and reduction reactions of monosaccharides.

Literature

Basic:

1. Biological and Bioorganic Chemistry: Bioorganic Chemistry: textbook / B.S. Zimenkovsky, V.A. Muzychenko, I.V. Nizhenkovska, G.O. Syrova. — 3rd edition – 2020. – 288 p.
2. Biological and Bioorganic Chemistry. Biological Chemistry: textbook / Yu.I. Gubsky, I.V. Nizhenkovska, M.M. Korda et al. — 2nd edition – 2021 – 544 p.
3. Bioorganic Chemistry. Rineyskaya O.N. textbook. – 2018. – 174 p.
4. Baynes J., Dominiczak M. Medical Biochemistry. 5th Edition. Elsevier, 2018. 712 p.

Additional:

1. Satyanarayana U. Biochemistry. 5th edition. India 2020. – 777 p.
2. Lehninger. Principles of Biochemistry. 7th edition. NY, United States. 2017.
3. William Marshall, Marta Lapsley, Andrew Day, Kate Shipman. Clinical Chemistry. Elsevier, 2020. 432 p.

Електронні інформаційні ресурси:

1. <https://info.odmu.edu.ua/chair/biology/>
2. <http://libblog.odmu.edu.ua/>
3. <https://moodle.odmu.edu.ua/login/index.php>

Practical class № 8

Topic: Structure and functions of di- and polysaccharides.

Relevance of the topic: Oligosaccharides are carbohydrates that contain in the molecule from two to ten identical or different monosaccharide residues. According to the number of such residues distinguish disaccharides, trisaccharides, tetrasaccharides, etc. Disaccharides are formed by the condensation of two molecules of monosaccharides with the splitting of water and are O-glycosides (full acetals). But, although the methods of synthesis of disaccharides are known, they are practically obtained from natural sources. The most famous representatives of disaccharides are sucrose, lactose, maltose, cellobiose. Given their role in the vital activity of the body, it is necessary to know the features of their structure and reactivity.

Goals: To study the most important representatives of reducing and irreducible saccharides, their cyclo-oxo-tautomeric forms, bond type and properties.

Equipment: department laboratory

Plan and organizational structure of the class:

1. Classification of carbohydrates. Isomerism. Tautomeric forms of monosaccharides. Mutarotations.

2. Chemical reactions of monosaccharides with the participation of carbonyl group. Redox reactions as qualitative reactions for the determination of the aldehyde group.

3. Formation of glycosides, their role in the construction of oligo- and polysaccharides, nucleosides, nucleotides and nucleic acids.

4. Phosphorus esters of glucose and fructose, their importance in metabolic transformations of carbohydrates.

The higher education applicant must know and be able to

1) the main representatives of natural disaccharides - maltose, lactose, cellobiose, sucrose - their composition, nomenclature;

2) structure of the starch molecule, starch fraction;

3) type of bonds in the starch molecule, qualitative reaction to starch;

4) glycogen, structure, type of bond, fiber derivatives.

Content of the practical class

Along with monosaccharides, other sugars that consist of several monosaccharide units are also present in the vegetable and animal sources. These are oligosaccharides, most frequently encountered are *disaccharides*.

Disaccharides are compounds that consist of two monosaccharide units linked together by a glycosidic bond between the anomeric carbon of one unit and the hydroxyl oxygen of another monosaccharide.

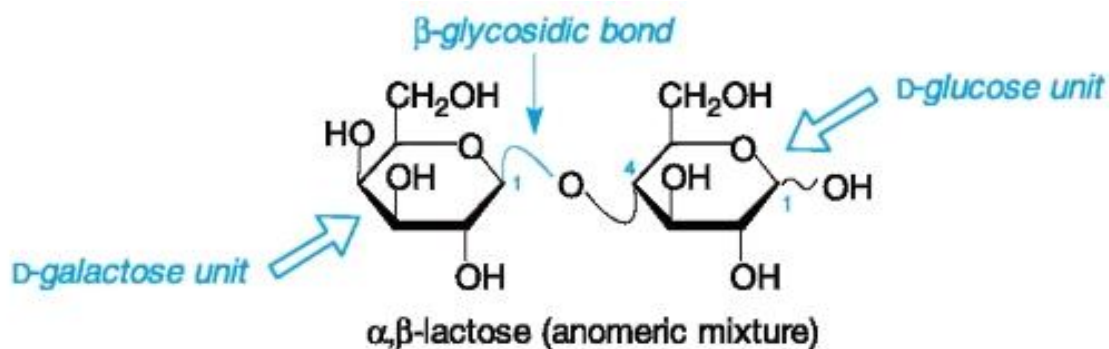
Two types of disaccharides (like all oligosaccharides) can be distinguished, namely reducing and nonreducing compounds. They differ in the mode of bonding monosaccharide units.

Most of the naturally occurring oligosaccharides have well established common names (for example, cellobiose, maltose, lactose, and sucrose) which were assigned before their complete structures were known.

1.1. Reducing Disaccharides

By definition, one hemiacetal hydroxyl group is preserved in the molecule of a reducing disaccharide. It becomes clear on consideration of the structure of *lactose*.

Lactose, or milk sugar, is a sugar found in human milk (6-8%) and, to a lesser degree, in cow's milk (about 4%). The monosaccharide composition of lactose is determined by the results of its acidic hydrolysis that gives equal amounts of D-glucose and D-galactose. Chemical and enzymic analyses showed that the anomeric carbon of the galactose unit has the β configuration and is linked to the OH group at C-4 of the glucose unit:



Note that the OH group at C-1 of the glucose unit in lactose is the hemiacetal one. Naturally, both cyclic hemiacetal forms exist in solution at equilibrium with an open-chain aldehyde form as shown in Fig. 1.

Lactose and other reducing disaccharides, like monosaccharides, give the positive Tollens' and Fehling's tests.

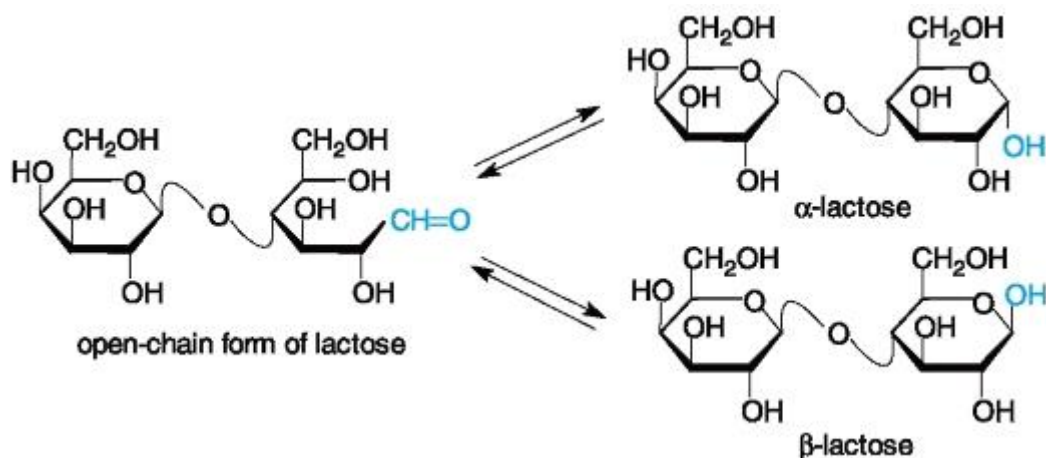


Figure 1. A tautomeric equilibrium of lactose.

Systematic nomenclature of disaccharides. Reducing disaccharides are in principle *O*-substituted derivatives of a monosaccharide to which another (nonreducing) monosaccharide unit is bound by a glycosidic bond. This substituent is generally called glycosyl, in particular, glucosyl or to be more specific β -D-glucopyranosyl depending on its complete stereochemistry. Thus the systematic name for α -lactose is 4-O-(β -D-galactopyranosyl)- α -Dglucopyranose.

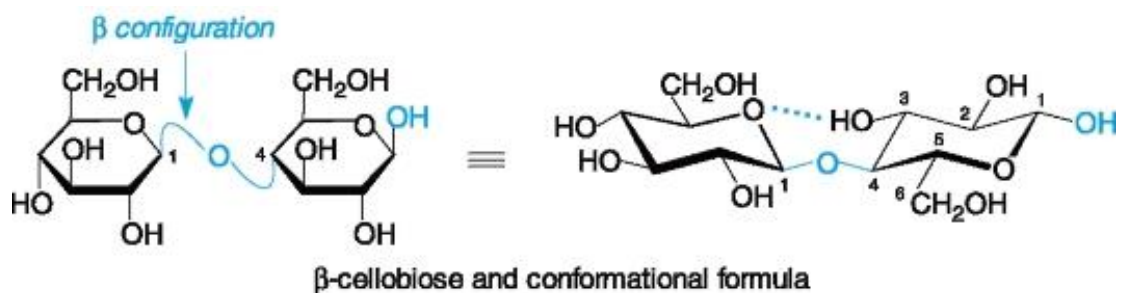
Alternatively, α -lactose may be named as O- β -D-galactopyranosyl-(1 \rightarrow 4)- α -D-glucopyranose, where locants in parentheses indicate the respective positions involved in the glycosidic bond. The locants are separated by an arrow pointing from the glycosyl carbon to the hydroxylic carbon involved. This method is especially useful for naming longer oligosaccharides. It may be simplified if a three-letter abbreviation is applied for monosaccharide units, for example, β -D-Galp-(1 \rightarrow 4)- α -D-Glcp, where the italicized letter p designates the pyranose ring.

The most common monosaccharides are abbreviated as follows: *Glc* - for glucose, *Gal* - for galactose, *Man* - for mannose, *Rib* - for ribose, *Fru* - for fructose, etc.

Cellobiose is a disaccharide that gives on hydrolysis only D-glucose. Hence, cellobiose consists of two linked glucose units.

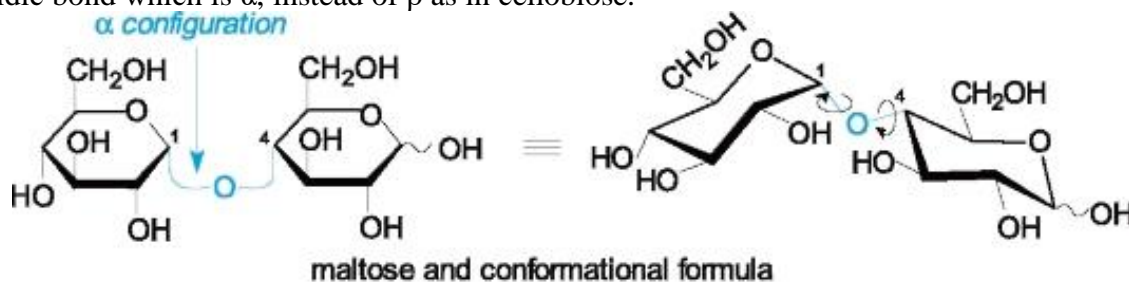
Example 1. Draw the structural formula for β -cellobiose, taking into account its systematic name α -D-glucopyranosyl-(1 \rightarrow 4)- β -D-glucopyranose.

Solution. According to this name, the anomeric carbon of a nonreducing unit is linked to the hydroxyl group at C-4 of the other (reducing) unit and has the β configuration. Both units are pyranoses. The second symbol β in the name designates the configuration of the atom C-1 in the reducing unit.



In the conformational formula of cellobiose (see above), the oxygen atoms of each pyranose ring (but not glucose units as a whole) are reflection symmetric. This is a result of intramolecular hydrogen bonding between the OH group at C-3 and the cyclic oxygen of the left glucose unit.

Maltose, or malt sugar, is a disaccharide obtained by partial hydrolysis of starch or glycogen, and represents a stereoisomer of cellobiose. Maltose differs from cellobiose only in configuration of a glycosidic bond which is α , instead of β as in cellobiose.

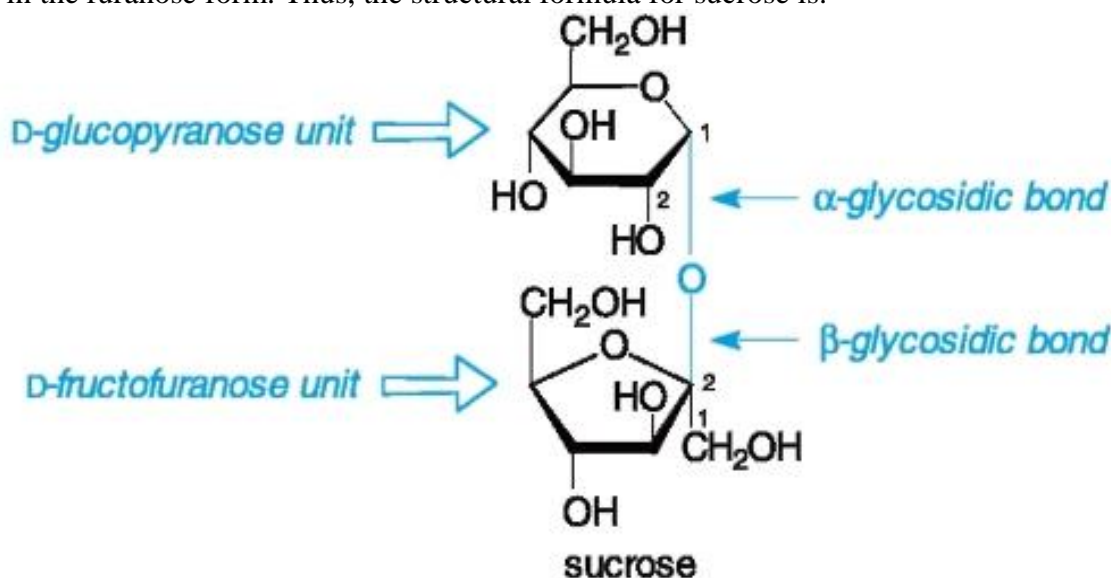


Spatial structure of maltose is more flexible as compared with that of cellobiose owing to free rotation around two C-O bonds. One of the possible conformations of maltose is shown above.

1.2. Nonreducing Disaccharides

Only one representative of nonreducing disaccharides will be considered - *sucrose*, but it is perhaps the most abundant and important of all disaccharides. Sucrose is an ordinary table sugar which is produced commercially from sugar cane or sugar beets in the amount of over 100 million tons annually in the world. It occurs in all photosynthetic plants.

Hydrolysis of sucrose gives equal amounts of D-glucose and D-fructose. The principal difference of sucrose from the aforesaid disaccharides is that the anomeric carbons of both monosaccharide units are involved in the glycosidic bond. Namely, the atom C-1 of the glucose unit is linked to the atom C-2 of the fructose unit through an oxygen bridge. Note that the fructose unit is present in the furanose form. Thus, the structural formula for sucrose is:



Both anomeric carbons in sucrose are linked; therefore no hemiacetal group remains in either monosaccharide unit. An acyclic form is impossible for sucrose, therefore this disaccharide cannot

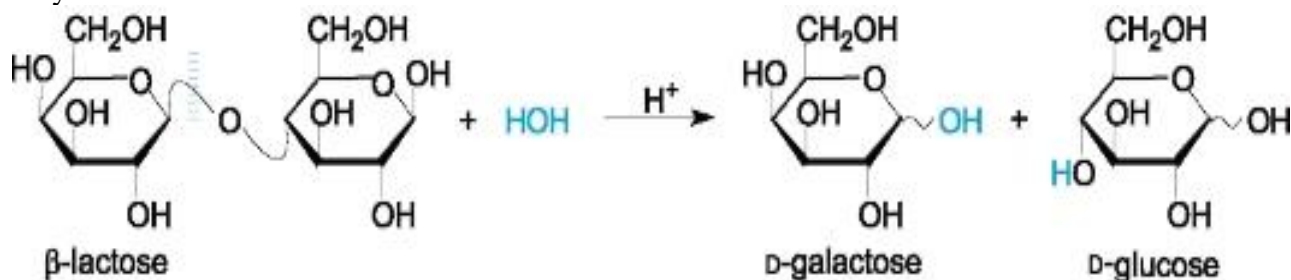
mutarotate. It therefore is referred to as a nonreducing disaccharide. In its chemical nature sucrose belongs rather to glycosides.

It should be kept in mind, however, that sucrose reduces the Tollens' and Fehling's reagents to a certain degree. This unexpected phenomenon is caused by partial hydrolysis of furanosides in an alkaline medium (not to mention an acidic medium). For this reason, sucrose is not a typical nonreducing disaccharide because it gives many other colour reactions characteristic of monosaccharides.

1.3. Chemical Properties

Many properties of oligosaccharides are very similar to those of monosaccharides. Reducing disaccharides can be oxidized and reduced. All disaccharides being polyhydroxyl compounds can produce corresponding esters.

Oligosaccharides differ from monosaccharides in the possibility to be hydrolyzed under acidic conditions resembling monosaccharide glycosides in this respect. This is illustrated by the reaction of hydrolysis of lactose:



Note that the products are not β anomeric monosaccharides but their tautomeric mixtures.

1.4. Polysaccharides

Polysaccharides constitute the dominant mass of organic matter in the Earth's biosphere. They have three important functions in the living organisms, such as an energy source, as structural components of cells and tissues, and as protective substances.

Polysaccharides are high-molecular carbohydrates built up of many monosaccharide units and therefore possess a high molecular weight. A principal structure of polysaccharides is similar to that of reducing oligosaccharides, i. e. one monosaccharide unit is bound with the next unit by means of glycosidic linkage. These units may form a linear chain or branched chains. Since polysaccharides have no free anomeric hydroxyls (except for only one at the end of the long chain), they are nonreducing compounds and do not mutarotate. Polysaccharides differ from simple sugars in some other characteristics. They do not have a sweet taste, and most of them are insoluble in water.

The monosaccharides occurring mostly in polysaccharide structures are D-glucose and, to a lesser extent, D-galactose, D-mannose, D-xylose, D-glucuronic acid, D-glucosamine, D-galactosamine and the N-acetates of these amino sugars. In spite of such a big variety of monosaccharides involved in polysaccharide structures, the latter are very regular in their constitution, in contrast to proteins, for instance.

There are two types of polysaccharides:

- *homopolysaccharides* - compounds built up from identical monosaccharide units;
- *heteropolysaccharides* - compounds consisting of different monosaccharide units.

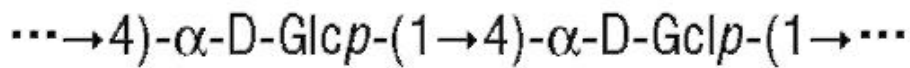
1.4.1. Homopolysaccharides

The most widespread homopolysaccharides are those composed of glucose units only and called *glucans*. The examples are starch, glycogen, and cellulose whose structures will be considered in this section.

Starch.

This is the principal food reserve polysaccharide of plants, a major component of cereals, corn, and potatoes. In fact, starch consists of two fractions: *amylose* and *amylopectin*.

Amylose is the water-soluble linear polymer, composed of a chain of up to 3,000 glucose units joined by the $\alpha(1\rightarrow4)$ -glycosidic bonds. It constitutes about 20-25% of starch depending on its source. The abbreviated structure of amylose can be written as follows:



Amylose has helically ordered conformation. Six glucose units constitute a coil of the helix. Amylose forms crystalline complexes (so-called inclusion compounds, or clathrates) with iodine and some polar compounds, in which «guest» molecules penetrate into a helix channel with axial disposition. Such a complex with iodine is deep-blue coloured, and its formation is used as the test for detection of both starch and iodine; this is the so-called *iodine-starch test*.

Amylopectin represents the water-insoluble fraction of starch which is a branched polysaccharide with the $\alpha(1 \rightarrow 4)$ bonds and $\alpha(1 \rightarrow 6)$ bond in a branching point (Fig. 2).

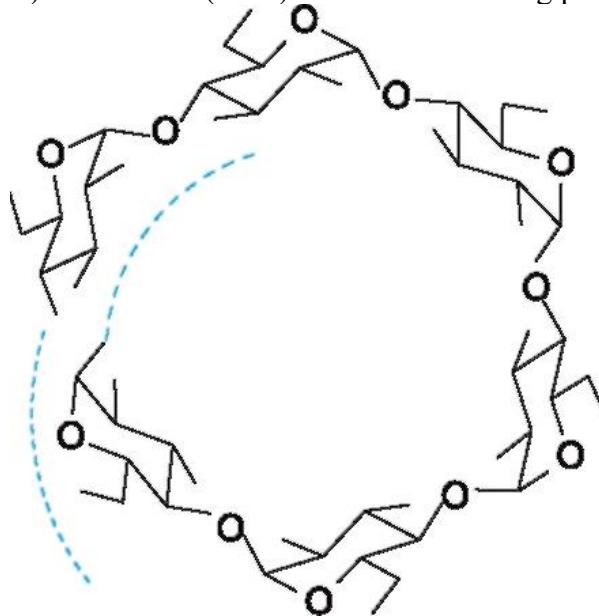


Figure 2. The helical structure of the amylose fragment.

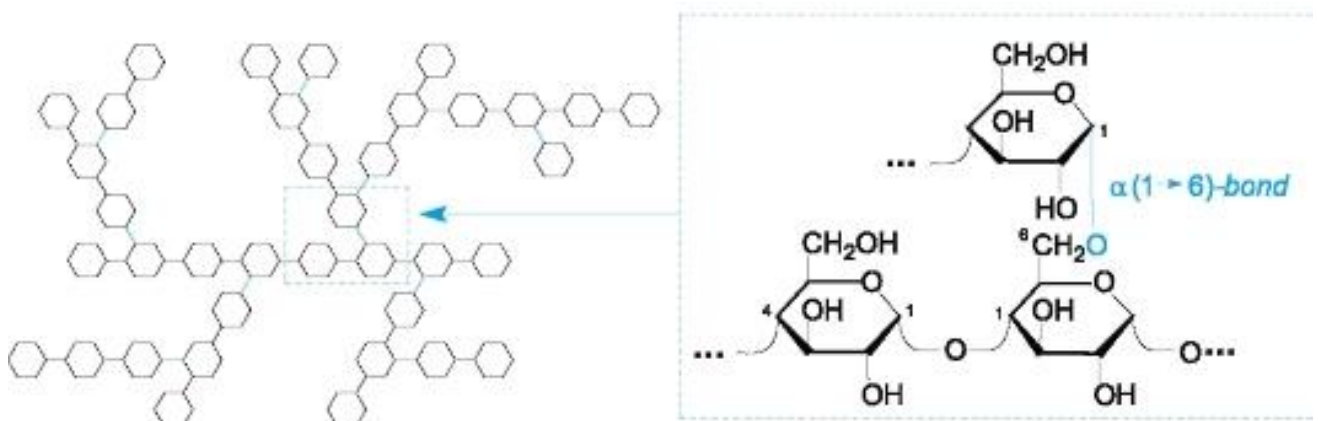


Figure 3. Schematic representation of the amylopectin fragment. The hexagons represent $\alpha\text{-D-glucose}$ units, the black dashes symbolize $(1 \rightarrow 4)$ bonds, the coloured dashes - $(1 \rightarrow 6)$ bonds.

Amylopectin has an average molecular mass about $10^6\text{-}10^7$ (tens of thousands of units). The branching of the chain occurs about every 20 to 30 glucose units. Thus, amylopectin is organized in hundreds of relatively short chains and has a compact shape.

Animals have enzymes called *amylases* that cleave (hydrolyze) the α -glucosidic bonds of a starchy food such as bread, corn, or potatoes. The initial product of hydrolysis is the *disaccharide* maltose which is further hydrolyzed to glucose in the intestinal tract. In the laboratory, starch can be hydrolyzed either partially to shorter polysaccharides called *dextrins* or completely to yield glucose.

Glycogen.

This is a branched polymer of D-glucose with the molecular weight up to tens of millions. Its structure is very similar to that of amylopectin (Fig. 3), differing in a higher degree of branching. The

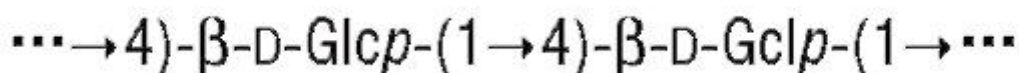
branching in glycogen occurs about every 10-12 glucose units in the outer chains or even 3-4 units in the inner chains.

Glycogen is the storage form of glucose in animals. It is found in the liver and muscle tissues. When glycogen is hydrolyzed in the animal body, it forms glucose to help maintain the normal sugar content of the blood.

Cellulose.

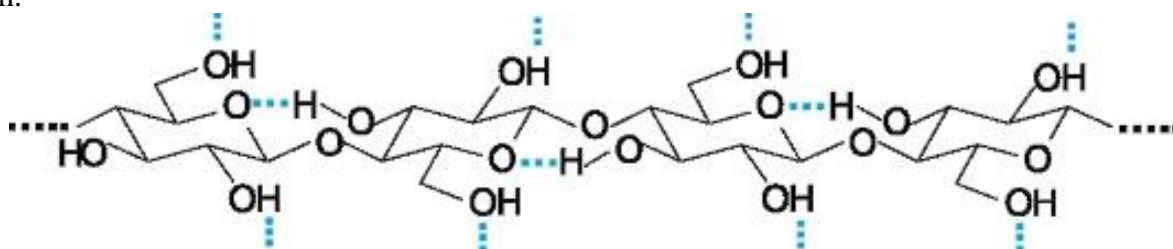
The most widespread organic substance on the Earth is cellulose. This polysaccharide occurs in various plants as a cell-wall material. Wood contains about 55% of cellulose, cotton and flax being almost pure (over 98%) cellulose.

Like amylose, cellulose is composed of a straight chain of D-glucose units linked by the (1 - 4)-glycosidic bonds and numbers in 10,000 units. The main difference between the two glucans is in the configuration of the glycosidic bonds, in amylose it is α , while in cellulose it is β as shown in the abbreviated form:

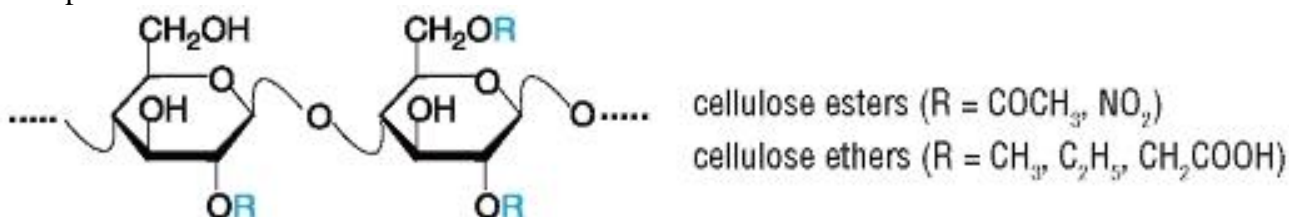


This stereochemical difference is, however, crucial for the biological fate of both the polysaccharides. Humans do not contain enzymes that catalyze the hydrolysis of the β -glucosidic bond; such enzymes are present in many bacteria. Ruminants (cows, for example) can digest grass because they have the necessary microorganisms in their digestive system. We can eat bread and potatoes but not wood and grass.

In the linear conformation of the cellulose chain, the OH groups at C-3 are involved in intermolecular hydrogen bonding like in cellobiose, and two remaining OH groups form intermolecular hydrogen bonds with hydroxyls of the adjacent chains (not shown in the drawing below). Such rigid structure of cellulose ensures its insolubility in water and considerable mechanical strength.



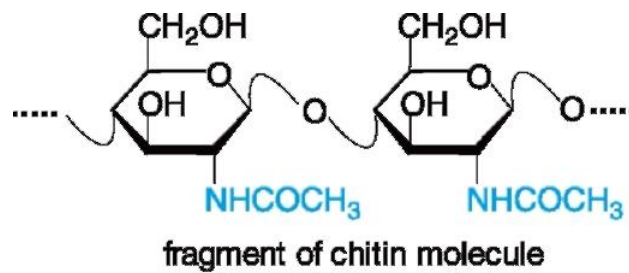
Cellulose is a readily available material for the preparation of commercially important derivatives. Hydroxyl groups of the polysaccharide are subjected to chemical modifications to give rise to cellulose esters (acetate and nitrates) and ethers (alkyl and carboxymethyl derivatives) with random disposition of the R substituents:



These derivatives are used in manufacturing textiles, fibers, coatings, plastics, and cosmetics.

Chitin.

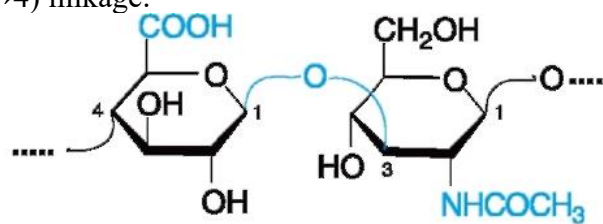
The repeating unit of this homopolysaccharide is N-acetyl-D-glucosamine (more precisely 2-acetamido-2-deoxy-D-glucose), the glucose analogue in which the OH group at C-2 is replaced by the acetamido group, -NHCOCH₃. In all other respects, the structure of chitin is similar to that of cellulose. Like cellulose in plants, chitin is a hard constructive polysaccharide; it forms the shells of crustaceans and insects.



1.5. Heteropolysaccharides

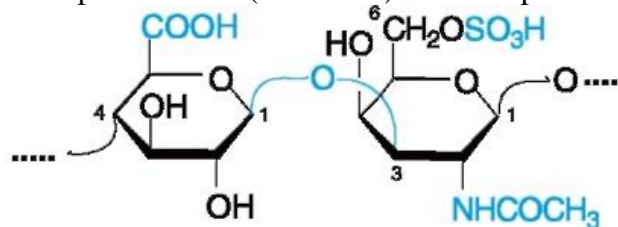
In living systems a significant role belongs to heteropolysaccharides that are composed mostly of repeating disaccharide blocks. Only two examples from numerous animal and plant heteropolysaccharides are presented in this section.

Hyaluronic acid is a structural polysaccharide found in higher animals. It is an essential component of the ground substances (or intercellular cement) of connective tissue. It has a high viscosity and a molecular weight in tens of millions. The molecule consists of repeating blocks of D-glucuronic acid and N-acetyl-D-glucosamine joined by the $\beta(1\rightarrow3)$ linkage. The disaccharide blocks, in turn, are joined by the $\beta(1\rightarrow4)$ linkage:



repeating disaccharide block of hyaluronic acid

Chondroitin sulfates are heteropolysaccharides important to the structure of the cartilage, skin, tendons, cornea, and heart valves. Their structures are similar to hyaluronic acid except that N-acetyl-D-galactosamine sulfated at the position O-6 (see below) or O-4 replaces N-acetyl-D-glucosamine:



repeating disaccharide block of chondroitin 6-sulfate

1.6. CARBOHYDRATES ON CELL SURFACES

Oligoand polysaccharide chains are often a component of complex biopolymers. When carbohydrate chains are linked covalently by O- or N-glycosidic bond with a protein molecule such biopolymers are called *glycoproteins*. Sites of binding are shown in Fig. 4.

Relatively short oligosaccharide chains of glycoproteins act as biochemical labels on cell surfaces. Immunoglobulins and so-called blood-group substances (antigens) belong to glycoproteins. In the schematical structure (Fig. 5), a great number of carbohydrate chains (up to 80% by weight) are bound to the protein chain like bristles in a brush for cleaning bottles.

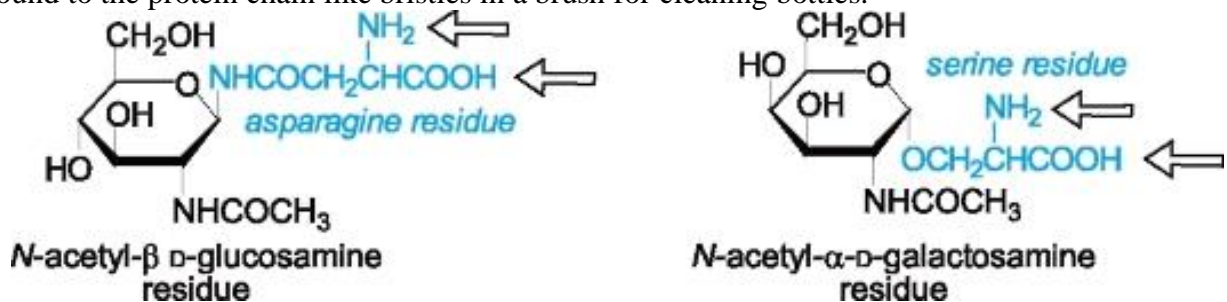


Figure 4. Sites of binding carbohydrate chains to protein: N-acetyl-D-glucosamine to asparagines (left) and N-acetyl-D-galactosamine to serine (right). Continuations of a protein chain are shown by arrows.

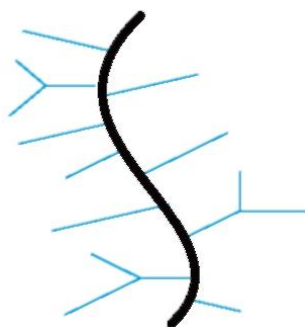
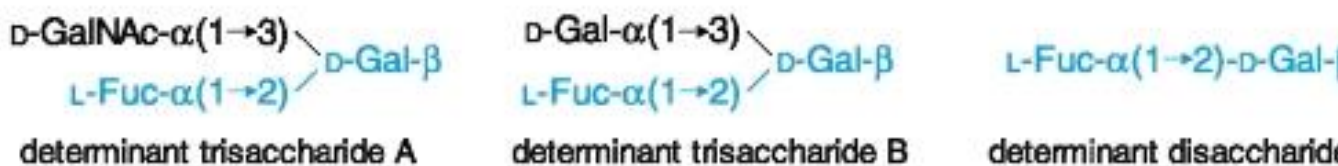


Figure 5. Schematic representation of glycoprotein molecule. Oligosaccharide chains are shown in colour.

The potentiality for cellular identification is exemplified by the structure proposed for oligosaccharide portions of glycoproteins responsible for the human blood-group antigens.

It is well known that human blood cannot be transfused successfully from one person to another unless it is of the proper type. This fact can be explained by the interaction of complementary proteins and glycoproteins on the cell surfaces. Erythrocytes thus carry surface-bound glycoproteins that identify them as belonging to the A, B, or H blood groups (the latter is sometimes called the «zero» group).

Each blood group in the ABH (or ABO) system is characterized by the definite oligosaccharide sequence at the nonreducing end of a carbohydrate chain called *antigenic determinants*. They contain usually no more than four monosaccharide units, for example:



Additional abbreviations of the monosaccharide units are also used: GalNAc - for N-acetyl-D-galactosamine and Fuc - for the deoxy sugar fucose that is 6-deoxygalactose. Note that fucose is presented as the L sugar.

It is easy to see that all three blood-group determinants contain the disaccharide sequence shown above in colour. Interesting *in vitro* experiments were performed in the 1970's. The treatment of red blood cells of the B group with galactosidase, an enzyme that splits off the terminal D-galactose unit from the oligosaccharide chain, gave a new antigenic determinant of the H group. Thus, the B blood group was enzymically transformed into the H group.

This example demonstrates that the role of carbohydrates is not limited by the traditional function as energy sources and as structural materials. Elucidation of the role of carbohydrates in cell recognition is under active investigation.

Control materials for the final stage of the class.

Questions to check the final level of knowledge:

- Classification of disaccharides.
- Cyclo-oxo-tautomerism of disaccharides.
- Oxidation reaction of reducing disaccharides.
- Classification of polysaccharides.
- Hydrolysis reactions of di- and polysaccharides.

Literature

Basic:

1. Biological and Bioorganic Chemistry: Bioorganic Chemistry: textbook / B.S. Zimenkovsky, V.A. Muzychenko, I.V. Nizhenkovska, G.O. Syrova. — 3rd edition – 2020. – 288 p.
2. Biological and Bioorganic Chemistry. Biological Chemistry: textbook / Yu.I. Gubsky, I.V. Nizhenkovska, M.M. Korda et al. — 2nd edition – 2021 – 544 p.
3. Bioorganic Chemistry. Rineyskaya O.N. textbook. – 2018. – 174 p.
4. Construction features, chemical properties and the biological role of carbohydrates. Ia.F. Burdina, A.V. Grekova, S.V. Shcherbakov, T.A. Sidelnikova, K.V. Bevziuk. Teaching aid. Odesa, 2017. – 44 p.
5. Baynes J., Dominiczak M. Medical Biochemistry. 5th Edition. Elsevier, 2018. 712 p.
6. Lipids: classification, structural features, properties and biological role. Ia.F. Burdina, A.V. Grekova, S.V. Shcherbakov, T.A. Sidelnikova. Teaching aid. Odesa, 2017. – p. 32.

Additional:

1. Satyanarayana U. Biochemistry. 5th edition. India 2020. – 777 p.
2. Lehninger. Principles of Biochemistry. 7th edition. NY, United States. 2017.
3. William Marshall, Marta Lapsley, Andrew Day, Kate Shipman. Clinical Chemistry. Elsevier, 2020. 432 p.

Електронні інформаційні ресурси:

1. <https://info.odmu.edu.ua/chair/biology/>-
2. <http://libblog.odmu.edu.ua/>
3. <https://moodle.odmu.edu.ua/login/index.php>

Practical class № 9

Topic: Chemical properties of carbohydrates. Qualitative reactions for the determination of carbohydrates.

Relevance of the topic: Carbohydrates are widespread in nature and play an important role in human life. In all processes of life there are complex chains of chemical transformations. The carbohydrate molecule consists of two functional groups - alcohol and aldehyde (ketone). Most chemical reactions occur with the participation of these groups. Therefore, knowledge of the reactions of oxidation, reduction, formation of esters and esters is necessary to understand the processes that occur in the body. The reactivity of semi-acetal hydroxyl has a number of features. The conditions under which the process of glycoside formation and hydrolysis takes place are important characteristics for understanding metabolic processes, which will be considered in depth by higher education applicants in the course of biochemistry.

Goals: to master the skills of identifying carbohydrate solutions (mono-, di- and polysaccharides). Distinguish between reducing and non-reducing disaccharides.

Equipment: department laboratory

Plan and organizational structure of the class:

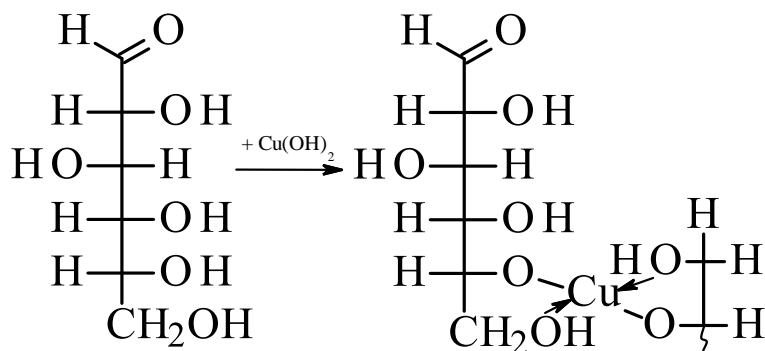
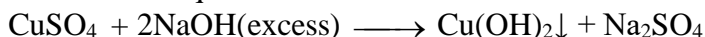
1. Qualitative reactions on multi-atomicity of alcohols.
2. Qualitative reactions of aldehyde group opening.
3. Selivanov reaction on ketones.
4. Qualitative reactions of disaccharides.
5. Reaction of sucrose hydrolysis.
6. Qualitative reaction to starch.

Content of the practical class

Laboratory procedure

1. Reactions of monosaccharides as polyhydric alcohols.

Place in a test tube 1 drop of 0.5% glucose solution and 6 drops of sodium hydroxide. Add 2–3 drops of solution of copper (II) sulfate to the mixture. The resulting precipitate of the cuprum hydroxide is immediately dissolved and a dark blue transparent is formed (the reaction takes place at room temperature). Write the reaction equation.

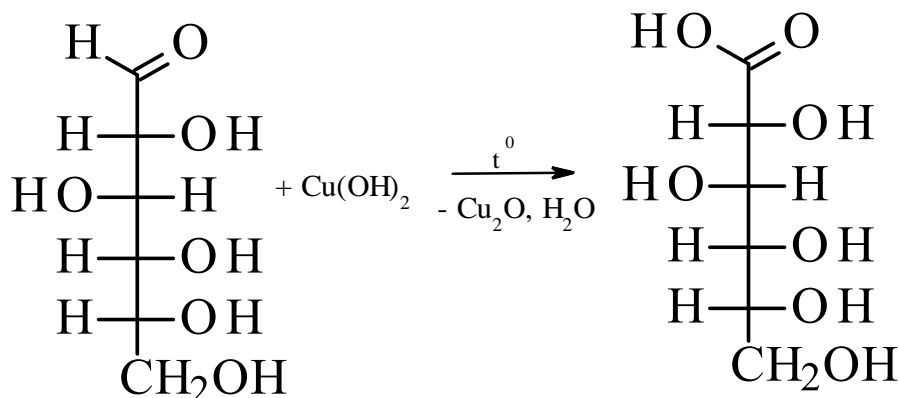


D-glucose

blue transparent solution of copper gluconate (chelate complex)

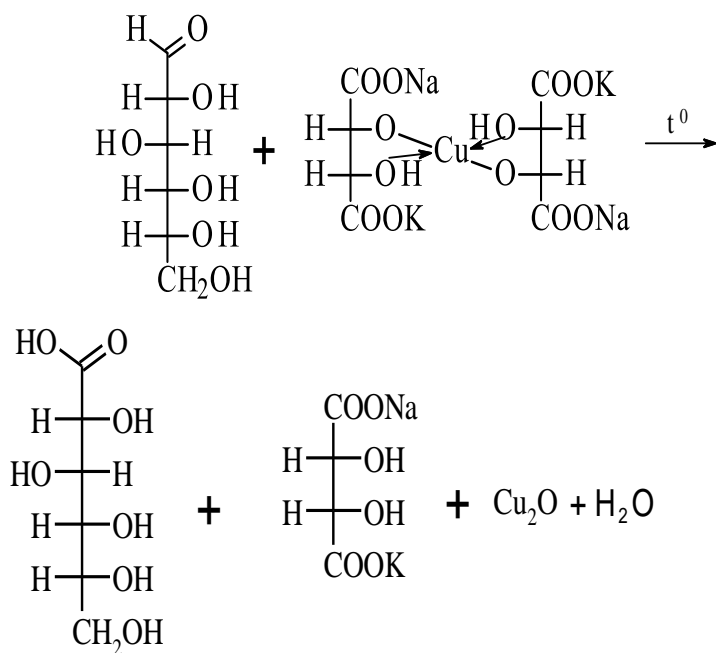
2. Oxidation of glucose with copper (II) hydroxide in an alkaline solution (Trommer reaction).

Add 5 drops of 1% glucose solution to the test-tube, thadd 1 drop of 10% sodium hydroxide solution and 1 drop of 1% solution of copper (II) sulfate. Heat on a water bath until a brick-red precipitate forms (Cu_2O).



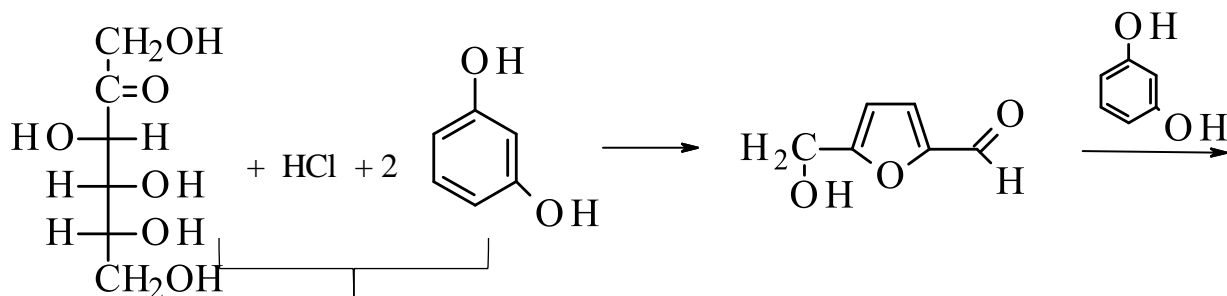
3. Oxidation of glucose by Fehling's reagent.

In a test tube, put 5 drops of 1% glucose solution and 1 drop of Fehling's reagent. Carefully heat on a spirit lamp or water bath. Observe the discolouration and a brick-red precipitate Cu_2O formation. Write the reaction equation.

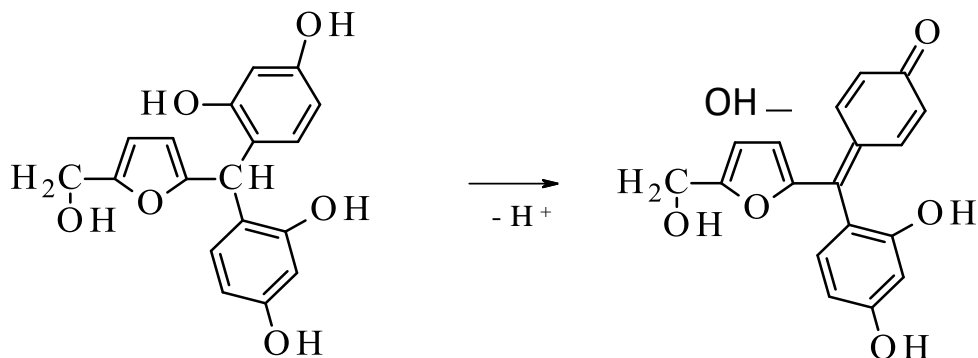


4. Selivanov's ketosis reaction.

Place 2 drops of Selivanov's solution and 1 drop of fructose solution into a test tube. Heat the tube. Notice the discolouration and vine red colour solution formation. Write the reaction equation.



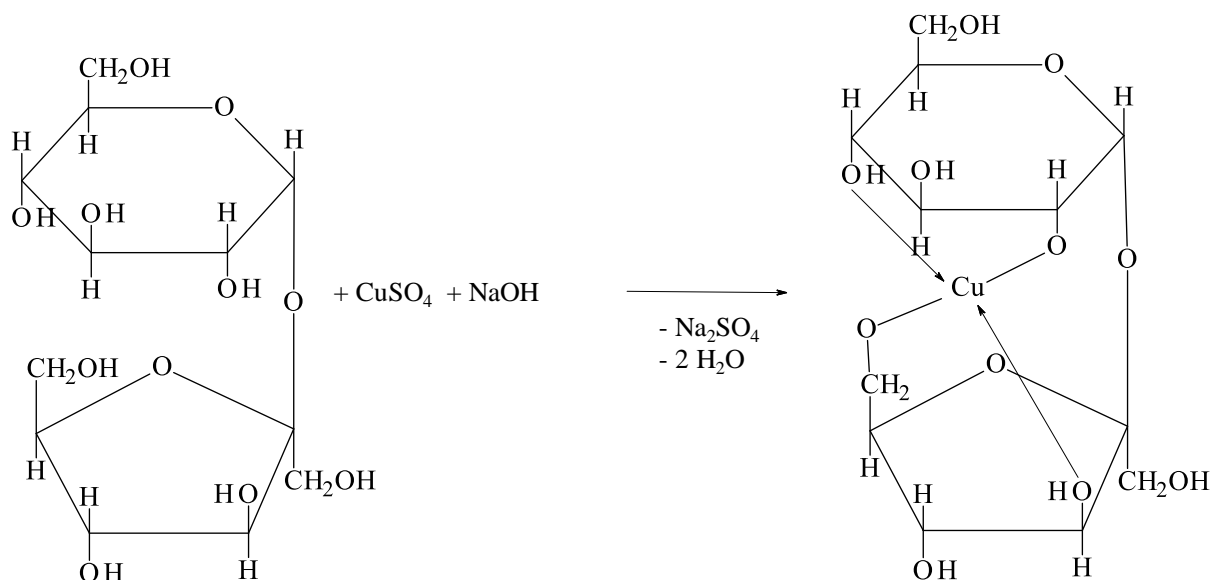
D-fructose Selivanov Reagent



Wine red color

5. Evidence for the presence of hydroxyl groups in bioses.

Place 1 drop of sucrose solution and 6 drops of 1 mol / 1 sodium hydroxide solution into the test tube. Add 1 drop of solution of copper (II) sulfate (II). Instead of a precipitate of copper (II) hydroxide, a transparent solution of the dark blue chelate sugar complex is obtained.

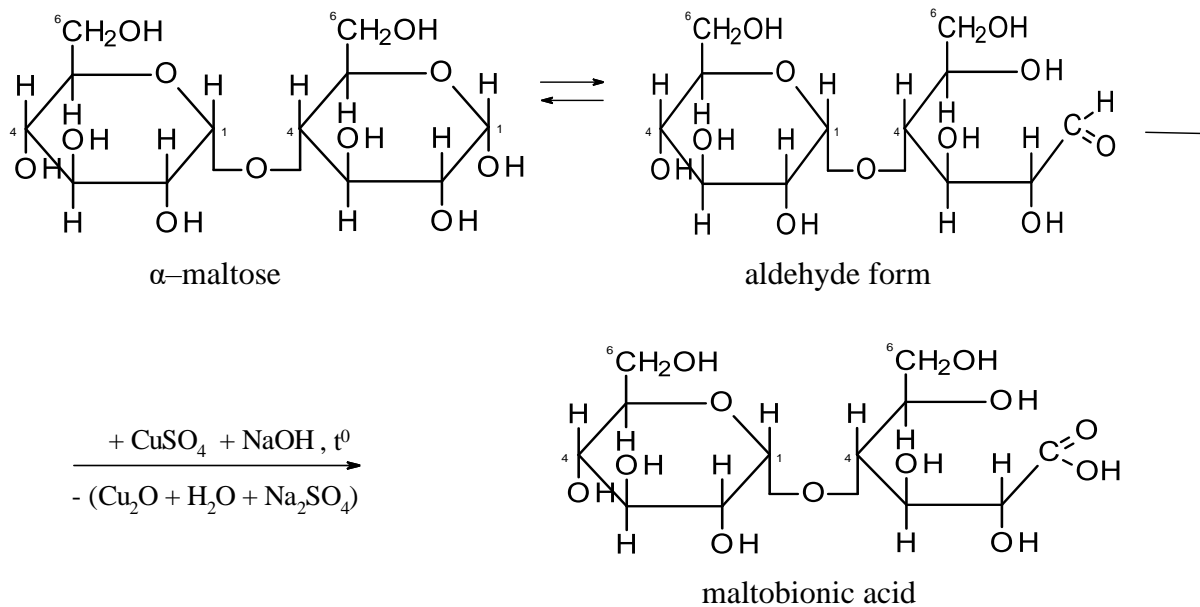


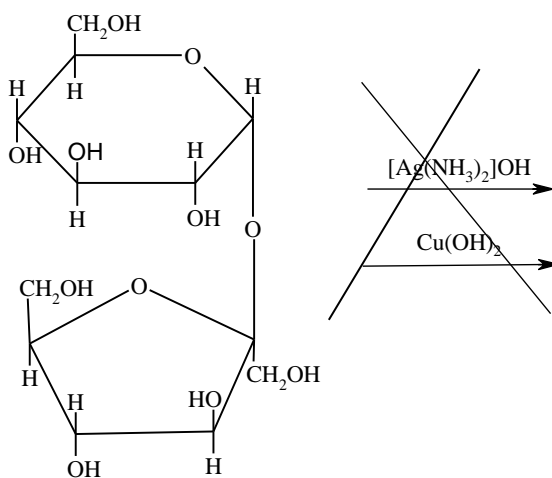
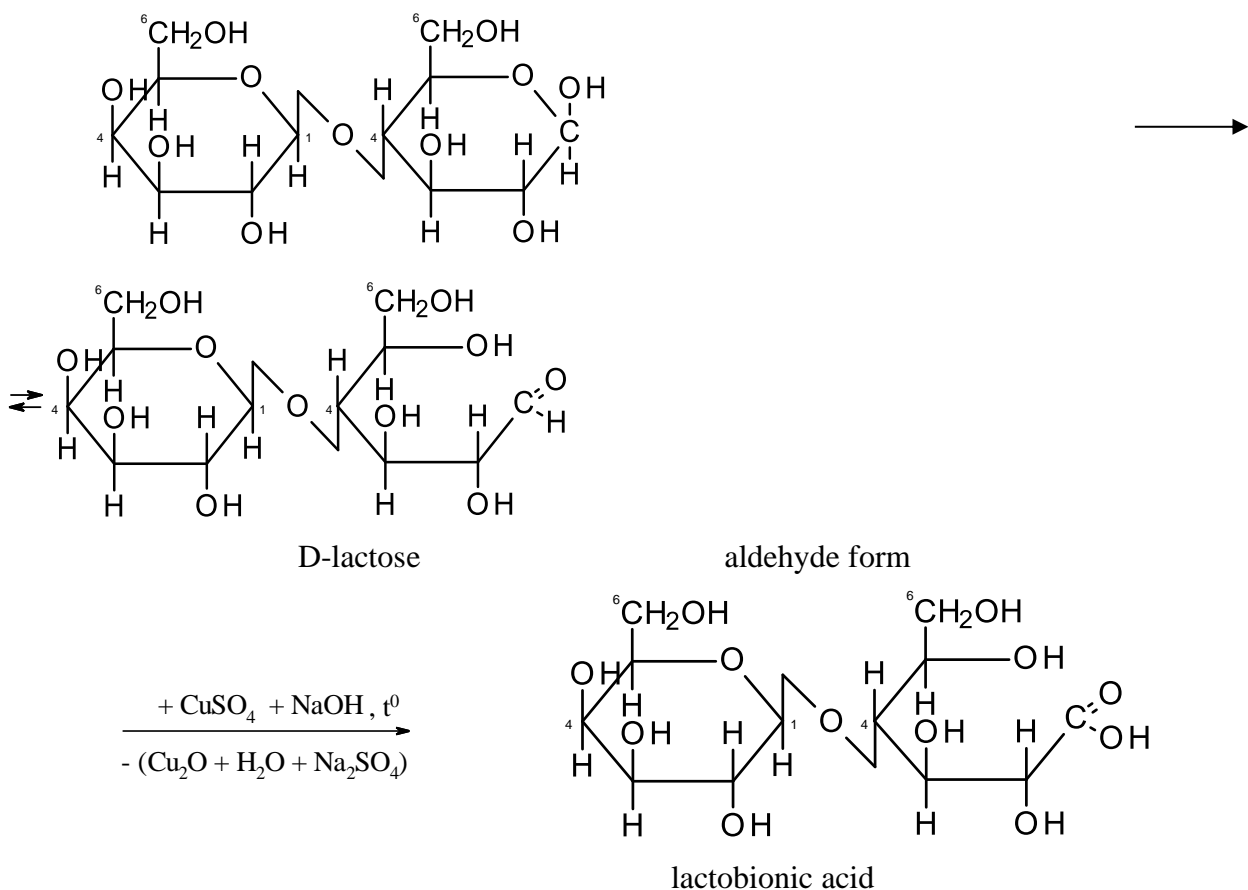
copper saccharate (transparent blue solution)

6. Test reaction of reducing disaccharides.

Place 5 drops of lactose solution into one test tube, into the second one 5 drops of the maltose solution, into the third 1–5 drops of sucrose solution. Add one drop of Fehling's reagent to all tubes and simultaneously dip them into a water bath. As soon as you see a discoloration in at least one test tube, remove all the test tubes.

Explain the appearance of brick-red sediment in test tubes with lactose and maltose and its absence in a test tube with sucrose.

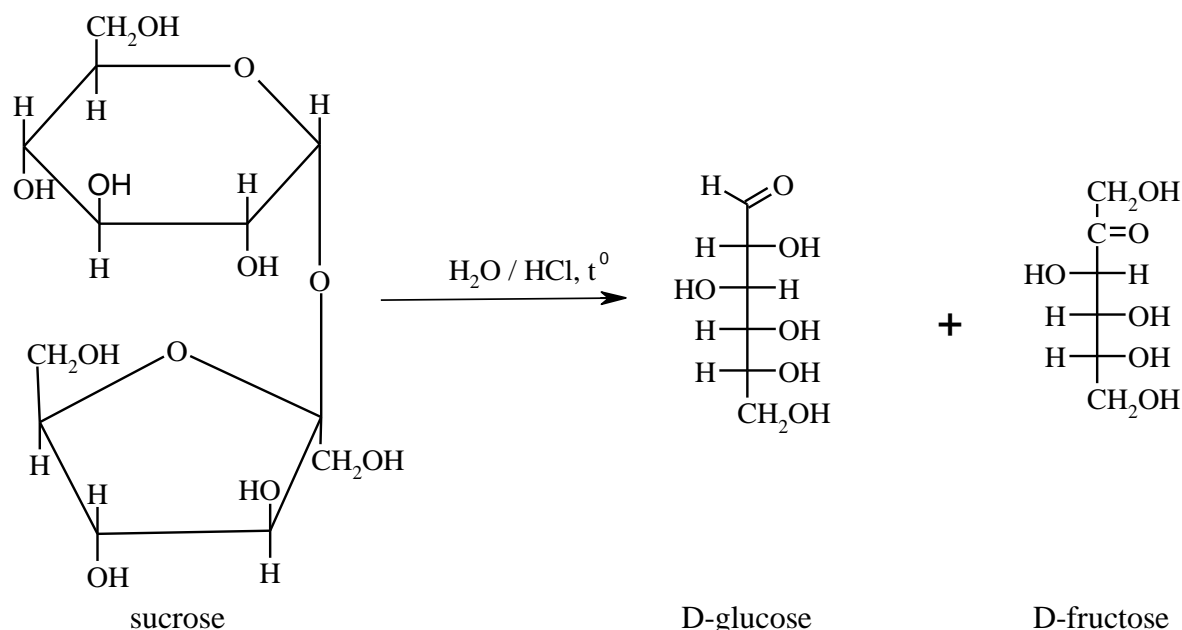




7. Hydrolysis of sucrose.

Into a test tube add 5 drops of 1% sucrose solution and 5 drops of hydrochloric acid solution. Heat the test tube on a water bath (during 15-20 minutes). After this, the contents of the tube are divided into two parts and make the reaction:

- a) with a Fehling's reagent (evidence of the presence of an aldehyde group);
- b) with Selivanov's reagent (qualitative reaction of ketosis).



D-glucose + Fehling`s reagent \rightarrow Cu_2O + dation products.

D-fructose + Selivanov`s Reagent \rightarrow reaction products

8. Test reaction of starch.

Place 5 drops of starch paste solution and 2 drops of iodine solution into a test tube. Appears blue-black color, which disappears when heated and reappears upon cooling. Explain this phenomenon.



Control materials for the final stage of the class.

Questions to check the final level of knowledge:

Qualitative reaction to the polyatomicity of mono- and disaccharides.

Qualitative reaction on the aldehyde group of monosaccharides.

Oxidation reactions of monosaccharides.

Test for reducing disaccharides.

Reaction of sucrose hydrolysis.

Qualitative reaction to starch.

Literature

Basic:

1. Biological and Bioorganic Chemistry: Bioorganic Chemistry: textbook / B.S. Zimenkovsky, V.A. Muzychenko, I.V. Nizhenkovska, G.O. Syrova. — 3rd edition – 2020. – 288 p.
2. Biological and Bioorganic Chemistry. Biological Chemistry: textbook / Yu.I. Gubsky, I.V. Nizhenkovska, M.M. Korda et al. — 2nd edition – 2021 – 544 p.
3. Bioorganic Chemistry. Rineyskaya O.N. textbook. – 2018. – 174 p.
4. Construction features, chemical properties and the biological role of carbohydrates. Ia.F. Burdina, A.V. Grekova, S.V. Shcherbakov, T.A. Sidelnikova, K.V. Bevziuk. Teaching aid. Odesa, 2017. – 44 p.
5. Baynes J., Dominiczak M. Medical Biochemistry. 5th Edition. Elsevier, 2018. 712 p.
6. Lipids: classification, structural features, properties and biological role. Ia.F. Burdina, A.V. Grekova, S.V. Shcherbakov, T.A. Sidelnikova. Teaching aid. Odesa, 2017. – p. 32.

Additional:

4. Satyanarayana U. Biochemistry. 5th edition. India 2020. – 777 p.

5. Lehninger. Principles of Biochemistry. 7th edition. NY, United States. 2017.
6. William Marshall, Marta Lapsley, Andrew Day, Kate Shipman. Clinical Chemistry. Elsevier, 2020. 432 p.

Електронні інформаційні ресурси:

1. [https://info.odmu.edu.ua/chair/biology/-](https://info.odmu.edu.ua/chair/biology/)
2. <http://libblog.odmu.edu.ua/>
3. <https://moodle.odmu.edu.ua/login/index.php>

Practical class № 10

Topic: Study of amino acid composition of proteins and peptides.

Relevance of the topic: Proteins are high molecular weight natural polymers that are built from α -amino acids connected by peptide bonds. More than two hundred years of history of protein chemistry is filled with endless improvement of experimental methods and is rich in various theoretical concepts. A significant contribution to the development of protein chemistry was made by such scientists as O.Y. Danilevsky, M.D. Zelinsky, E. Fischer, T. Kurtzius, M. Bergman, F. Senger and others. Protein chemistry has always combined the ideas and methods of biology, medicine, chemistry, physics. Proteins create the material basis of the chemical activity of the cell. It is conventionally believed that peptides contain up to 100 α -amino acids in a molecule and have a molecular weight of about 1000, and proteins contain more than 100 α -amino acid residues. The biological activity of proteins is closely related to the high level of organization of the molecule. Therefore, living organisms synthesize proteins of the required conformation.

Goals: To develop knowledge of the structure and chemical properties of the most important α -amino acids in vivo and in vitro.

Basic concepts: amino acid, amino acid production, amino acid deamination, amino acid decarboxylation, polypeptide bond formation.

Equipment: department laboratory

Plan and organizational structure of the class:

1. Classification, nomenclature and isomerism of alcohols and phenols.
2. Comparative characteristics of acidic properties of alcohols and phenols.
3. Chemical properties of alcohols (dehydration reactions of alcohols, nucleophilic substitution reactions in alcohols (S_N), features of oxidation reactions of primary, secondary and tertiary alcohols, esterification reactions).
4. Electrophilic substitution reactions in phenols (S_E).
5. Nomenclature and isomerism of aldehydes and ketones.
6. Nucleophilic addition reactions to oxo compounds.
7. Aldol condensation and its importance for carbon chain elongation.
8. Oxidation of aldehydes and ketones.
9. Medico-biological significance of hydroxo- and oxo-compounds.

The higher education applicant should know and be able to:

- 1) the structure of amino acids;
- 2) structural and optical isomerism;
- 3) reactions by functional groups;
- 4) describe the amphotericity of amino acids by chemical reactions.

Content of the practical class

Peptides and proteins are polymeric compounds composed of amino acid units. It is difficult to overemphasize the role of proteins in living matter. They are the principal components of muscles, skin, hair, and blood. Proteins are constituents of antibodies and hemoglobin, having protective and transport functions. Many antibiotics and hormones are proteins, and all enzymes are proteins only. Therefore it is no wonder that the term *proteins* originates from the Greek *protos* the first.

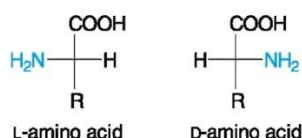
In this chapter, we will mainly discuss the structure, chemical and some biochemical properties of amino acids. We will next consider briefly the structure and properties of peptides, and, finally, the main features of a protein structure.

From various types of these heterofunctional compounds, α -amino acids are the most important in biological processes being the building blocks of proteins. Recall that α -amino acids are carboxylic acids with an amino group attached to the α -carbon atom; they may be represented by the general formula $RCH(NH_2)COOH$. Only α -amino acids will be the target of our consideration so the symbol α will further be omitted.

Hydrolysis of most animal proteins produces about twenty different amino acids listed in Table 1. With the exception of glycine ($R = H$ in the general formula), all amino acids have four different groups attached to the α -carbon. This carbon is chiral, and two enantiomeric forms of each amino acid are therefore possible. Most natural amino acids belong to the same stereoisomeric family, namely, to the L series. D-Amino acids are also known in nature, but only as constituents of certain antibiotics and of proteins of bacterial cell walls. The generalized formulas for enantiomers are given in the Fischer projections.

1. α -AMINO ACIDS

Structure and Classification



Amino acids are known by their trivial names, which are accepted by the IUPAC nomenclature (Table 1). They also have a three-letter abbreviation (mostly the first three letters are used), which are useful for writing the formulas of peptides and proteins. Systematic names of amino acids are, of course, possible but they are never used.

Table 1.

Common amino acids $RCH(NH_2)COOH$ found in proteins

Trivial name	Abbreviation**	Structure of R	Isoelectric point
Glycine	Gly	H-	6.0
Alanine	Ala	CH ₃ -	6.0
Valine*	Val	(CH ₃) ₂ CH-	6.0
Leucine*	Leu	(CH ₃) ₂ CHCH ₂ -	6.0
Isoleucine*	Ile	CH ₃ CH ₂ CH(CH ₃)-	6.0
Phenylalanine*	Phe	-CH ₂ -	5.5
Serine	Ser	HOCH ₂ -	5.7
Threonine*	Thr	CH ₃ CH(OH)-	5.6
Tyrosine	Tyr	HO--CH ₂ -	5.7
Aspartic acid	Asp	HOOCCH ₂ -	2.8
Glutamic acid	Glu	HOOCCH ₂ CH ₂ -	3.2
Asparagine	Asn	H ₂ NCOCH ₂ -	5.4
Glutamine	Gln	H ₂ NCOCH ₂ CH ₂ -	5.7
Lysine*	Lys	H ₂ N(CH ₂) ₄ -	9.6
Arginine	Arg	-NHCH ₂ CH ₂ CH ₂ -	10.8
Cysteine	Cys	HSCH ₂ -	5.0
Methionine*	Met	CH ₃ SCH ₂ CH ₂ -	5.7
Histidine	His	-CH ₂ -	7.5
Tryptophan*	Trp	-CH ₂ -	5.9
Proline***	Pro		6.3

* Essential amino acids.

** It may be applied only for shortened formulas of peptides.

*** Full structure is presented.

Problem 1. Draw the Fischer projections for the following amino acids: (a) L-cysteine; (b) D-glutamine; (c) L-serine; (d) L-valine. Assign the configuration of the compounds (a) and (c), using the *R,S* system.

Amino acids can be classified as neutral, acidic, or basic, depending on the nature of their side chain, the R substituent. Most amino acids have the neutral R's. Two amino acids (aspartic and glutamic acids) have an extra carboxyl group and are acidic. Three compounds (lysine, arginine, and histidine) have an extra basic function in the side chains and are referred to as basic amino acids. In the largest group of neutral amino acids, there are compounds with polar groups in the R substituent, such as the OH groups of serine and threonine or even ionizable groups, such as a phenolic OH group of tyrosine and the SH group of cysteine.

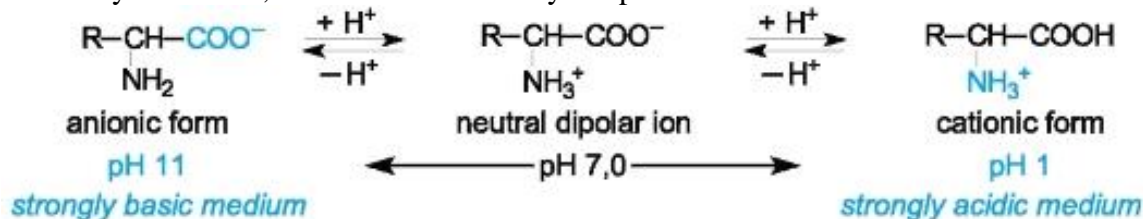
Alternatively, amino acids can be subdivided into several groups on the basis of the structural features of their side chains. These are aliphatic (the first five in the table), aromatic (phenylalanine and tyrosine), heterocyclic, hydroxyl-containing, and sulfur-containing amino acids (find representatives of the three last groups in the Table 1 on your own).

From a biological point of view, *essential amino acids* (Table 1) stand out because they, in contrast to other amino acids, cannot be synthesized in sufficient quantity by adult humans and therefore must be obtained from dietary sources. The lack of some essential amino acids in the diet can lead to severe deficiency diseases.

2. Chemical Properties

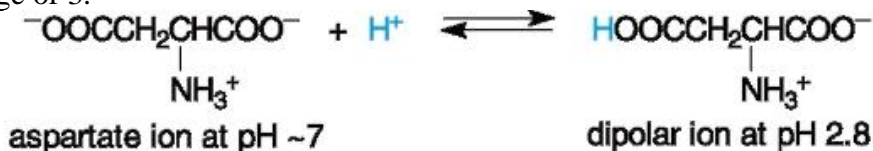
The acid-base properties. As has already been shown, amino acids have a dipolar ion structure since they contain both an acidic group (COOH) and a basic group (NH₂) within the same molecule. The dipolar structure is in agreement with the salt-like properties of amino acids, which are crystalline compounds with high melting points (in the range of 220-340 °C) and are much better soluble in water than in organic solvents.

The predominant form of an amino acid in solution depends on the pH of the solution and on the nature of the amino acid (i. e. the R group in the general formula). In acidic solutions all amino acids exist mainly as cations; in basic solutions they are present as anions.



At some intermediate pH, the amino acid is present in an electrically neutral form. At this pH, called the *isoelectric point* (pI), the amino acid exists almost exclusively in the dipolar form. The isoelectric point depends on the structure of an amino acid. Neutral amino acids have isoelectric points in the pH range of 5.0-6.3 (the pI values are listed in Table 1).

Aspartic and glutamic acids contain an extra carboxyl group and at neutral pH values they are mainly present in anionic form. To convert this anion into a neutral dipolar ion (in other words to reach the pI) some quantity of an acid must be added. Thus, the isoelectric point of dicarboxylic amino acids is in the range of 3.



For a similar reason, the isoelectric points of basic amino acids are in the basic region of pH.

Due to amphoteric nature of amino acids they are able to neutralize small quantities of acids or bases, thus maintaining a constant pH of the solution. Such compounds are termed *buffers* and are used in biochemical investigations.

Problem 2. Illustrate by equations the acid-base behaviour of the amino acids (a) - (c) in reactions with excess hydrochloric acid and of the amino acids (d) - (f) with excess sodium hydroxide:

(a) aspartic acid

(d) proline

(f) tyrosine

(b) cysteine

(e) serine

(c) lysine

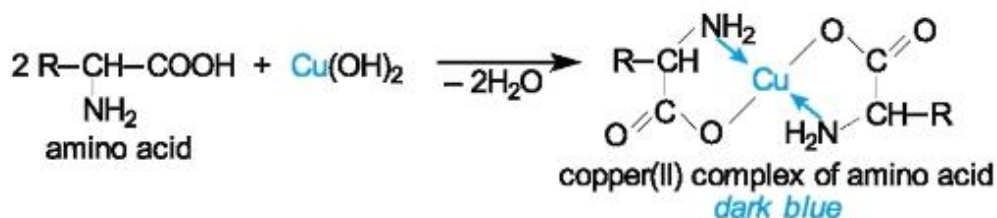
General characteristics of functional group reactivity. Amino acids being heterofunctional compounds show the chemical behavior that would be expected due to the presence of the amino and carboxyl groups. They can be *N*-acylated or *N*-alkylated with participation of their amino group. They can also be esterified or transformed into amides and other carboxylic acid derivatives. Some reactions involve additional functional groups of amino acids present in the side chain. Generally, these reactions have been considered in the previous chapters.

The reactions that can be useful in the amino acid analysis and identification are of interest in this section.

Complexing properties.

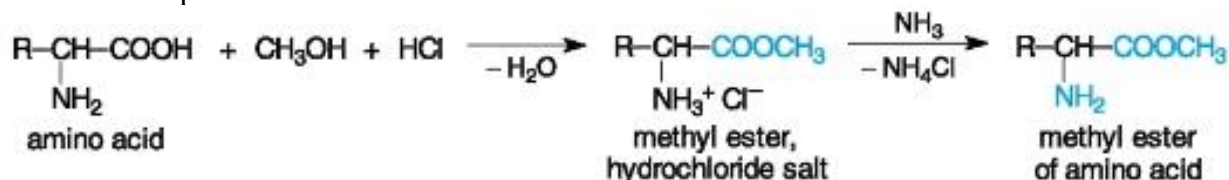
Like 1,2-diols and α -amino alcohols, α -amino acids form complex salts with some metal ions with the formation of dark blue coloured solution.

These are another characteristic of heterofunctional compounds. Their complexing (or chelating) ability is based on a tendency to form a stable five or six-membered cycle in the reaction with some metal ions (especially with Cu^{2+} and Ni^{2+}). For example, insoluble copper(II) hydroxide reacts with 1,2-diols with the formation of dark blue coloured solution.



Esters formation.

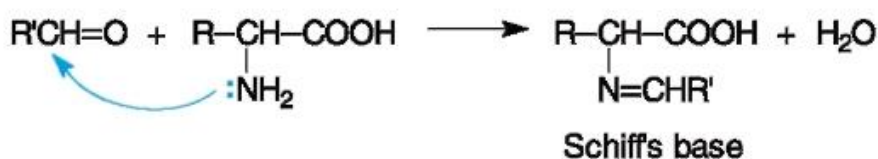
The familiar Fischer esterification of an amino acid yields an ester as a salt. A strong acid acts in this reaction not only as a catalyst but in the first place as an acid that converts an amino acid into protonated (cationic) form. An appropriate base (as ammonia in the example below) should be used in conversion of the product into a free base ester.



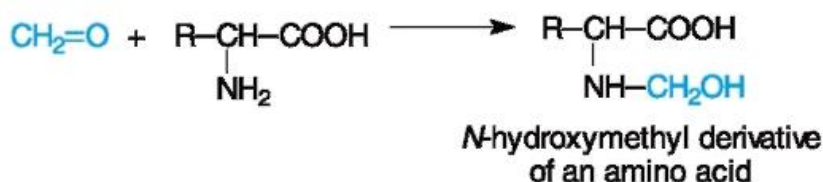
Amino acid esters, in contrast to amino acids themselves, are relatively volatile derivatives that can be distilled (sometimes in vacuum). This property is used in analysis of amino acid mixtures. Besides, amino acid esters are important intermediates in peptide synthesis.

Chemical synthesis of peptides is perhaps the most exciting field of the chemistry of amino acids over the whole 20th century; however, this question is beyond the scope of our course.

Reaction with carbonyl compounds. Most of carbonyl compounds react with the amino group of an amino acid giving Schiff's bases. Such derivatives of some amino acids can be analyzed by spectral methods.



Formaldehyde reacts with amines and amino acids to form quite stable addition products (without elimination of water) in accordance with the equation:



This reaction leads to the blocking of the amino group of an amino acid. In such a form an amino acid cannot exist as a dipolar ion because of low basicity of the nitrogen atom, and it is possible to determine its quantity by the titration method. This procedure is known as the *Sorensen method*.

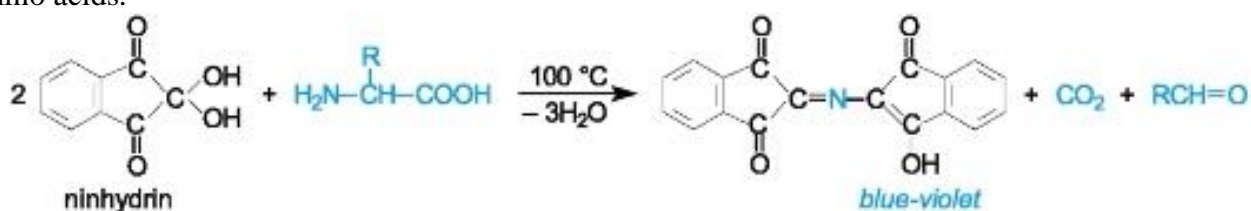
Reaction with nitrous acid. Amino acids, like primary aliphatic amines, rapidly react with nitrous acid, HNO_2 , producing alcohols and molecular nitrogen.



Measuring the volume of the nitrogen evolved it is possible to determine the quantity of amino groups in the tested sample of an amino acid. The reaction is a background of the *Van Slyke method*.

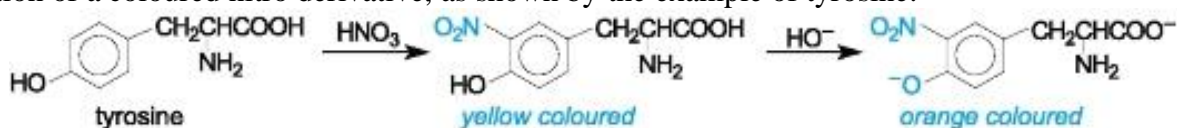
Problem 3. Write equations for reactions of alanine with: (a) methanolic hydrogen chloride; (b) formaldehyde; (c) nitrous acid. Name the products obtained.

Ninhydrin reaction. α -Amino acids (and primary amines) react with triketone ninhydrin to form blue-violet coloured product. Reaction is extremely sensitive and used in chromatographic analysis of amino acids.



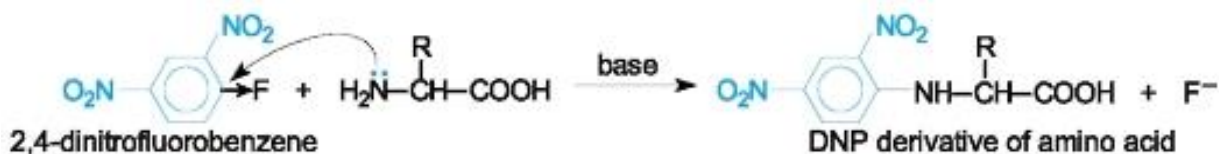
The reaction is also useful in criminalistics for visualization of fingerprints.

Xanthoproteic reaction. This reaction permits detection of aromatic amino acids: tyrosine, phenylalanine, histidine, and tryptophan. It is based on the nitration of the aromatic ring with the formation of a coloured nitro derivative, as shown by the example of tyrosine:



The colouration becomes deeper in the presence of an alkali, which ionizes the phenolic hydroxyl group.

Reaction with 2,4-dinitrofluorobenzene. The nucleophilic amino group of amino acids reacts with 2,4-dinitrofluorobenzene in mildly basic solution to give a yellow 2,4-dinitrophenyl (DNP) derivative:



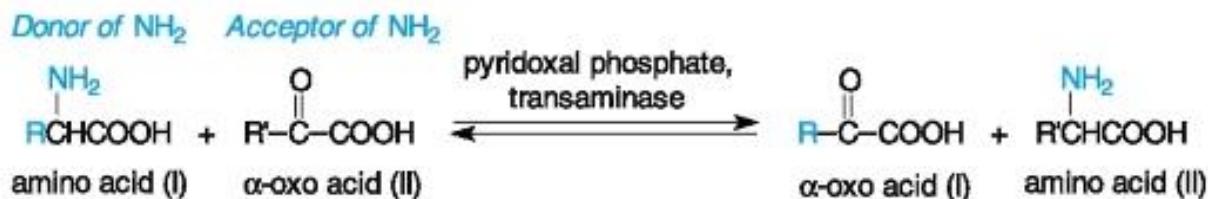
The reaction represents an example of nucleophilic aromatic substitution which is fairly rare in aromatic compounds. Two strong electron-withdrawing nitro groups facilitate substitution of the fluorine atom.

The method is mainly used in analysis of amino acids that compose a peptide molecule.

Biologically Important Reactions

A number of reactions of amino acids occurring in biological systems proceed with the assistance of a coenzyme *pyridoxal phosphate*.

Transamination. This enzyme-catalyzed reaction is one of the most important metabolic transformations of amino acids. On the other hand, the transamination represents a pathway in which most of the naturally occurring α -amino acids are biosynthesised from α -oxo acids, according to the following general equation:

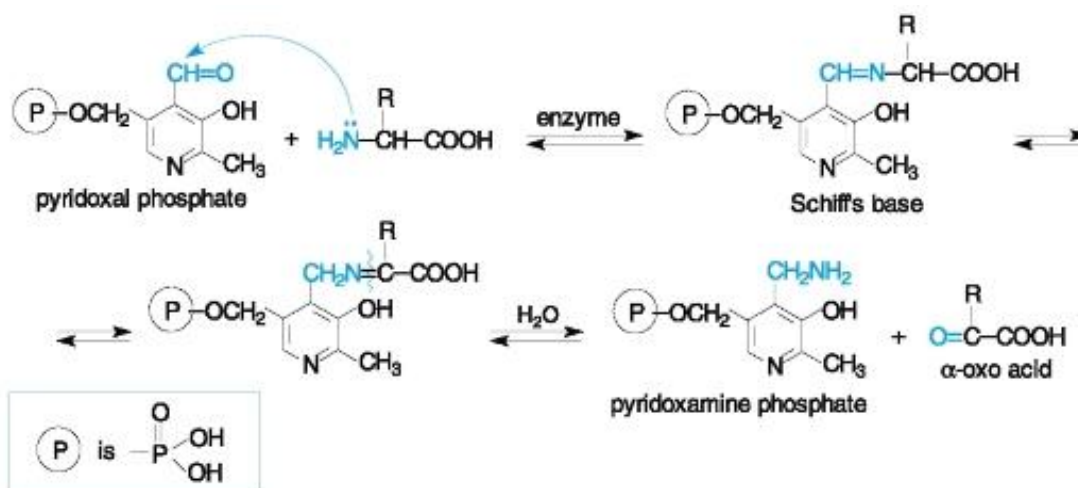


Example 1. Glutamic acid is produced in the organism along with oxalacetic acid. Which are their precursors in the transamination reaction?

Solution. Glutamic acid can be formed from the corresponding C₅ dicarboxylic oxo acid, i. e. 2-oxoglutaric acid. The precursor of oxalacetic acid is the corresponding C₄ dicarboxylic amino acid, i. e. aspartic acid.



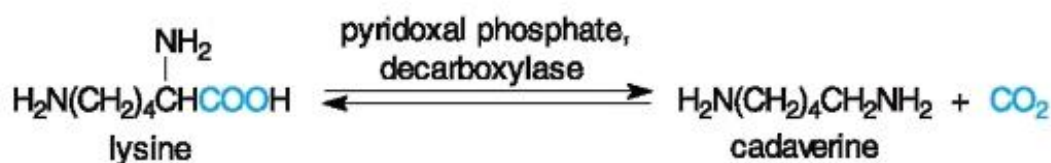
In one of the initial steps of the reaction, pyridoxal phosphate (as an aldehyde) reacts with the NH₂ group of an amino acid to form a Schiff's base. One of the possible transformations of the Schiff's base involves its hydrolysis that results in the formation of an α -oxo acid and pyridoxamine phosphate as shown below:



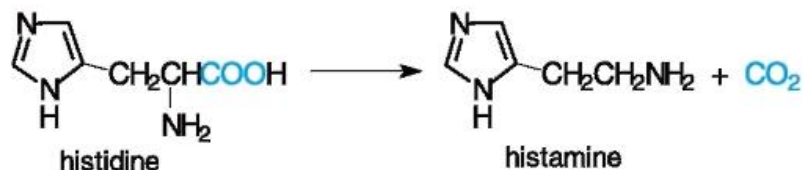
Pyridoxamine phosphate can react with another oxo acid in the reversed direction with the formation of another amino acid and regeneration of pyridoxal phosphate. A general equation shows that pyridoxamine phosphate functions as a reversible carrier of the amino group from an amino acid to an oxo acid.

Problem 4. Write an equation for enzymic transamination between 2-oxopentanedioic acid and alanine. Name the products of the reaction.

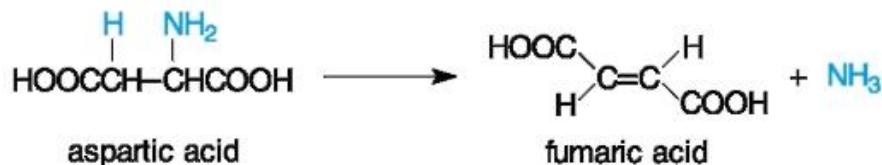
Decarboxylation. This reaction also proceeds with the participation of pyridoxal phosphate and leads to the formation of naturally occurring amines. The simple diamines putrescine (1,4-butanediamine) and cadaverine (1,5-pentanediamine) occur (as their names suggest) in decomposing animal matter. Cadaverine is the decarboxylation product of lysine:



A similar reaction with histidine gives the biogenous amine, histamine.



Deamination. Two types of the enzymic deamination (i. e. removal of an amino group) are known for amino acids. The first one is the *non-oxidative* deamination that takes place without the use of oxygen and leads to the formation of α,β -unsaturated carboxylic acids.



Another type of the reaction is the *oxidative* deamination which is a two-step process. The first step represents the enzymic oxidation of an amino acid into an intermediate α -imino acid in the presence of a coenzyme NAD^+ . Subsequent hydrolysis yields an α -oxo acid.

Control materials for the final stage of the class.

Questions to check the final level of knowledge:

1. Classification of amino acids.
2. Biologically important amino acids.
3. Reactions for the production of amino acids.
4. Decarboxylation reactions in vivo and in vitro.
5. Deamination reactions in vivo and in vitro.
6. Reactions of peptide bond formation.

Literature

Basic:

1. Biological and Bioorganic Chemistry: Bioorganic Chemistry: textbook / B.S. Zimenkovsky, V.A. Muzychenko, I.V. Nizhenkovska, G.O. Syrova. — 3rd edition – 2020. – 288 p.
2. Biological and Bioorganic Chemistry. Biological Chemistry: textbook / Yu.I. Gubsky, I.V. Nizhenkovska, M.M. Korda et al. — 2nd edition – 2021 – 544 p.
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3. William Marshall, Marta Lapsley, Andrew Day, Kate Shipman. Clinical Chemistry. Elsevier, 2020. 432 p.

Електронні інформаційні ресурси:

1. <https://info.odmu.edu.ua/chair/biology/>-
2. <http://libblog.odmu.edu.ua/>
3. <https://moodle.odmu.edu.ua/login/index.php>

Practical class № 11

Topic: Organization of the structure of proteins. Physicochemical properties of proteins. Precipitation reactions of proteins. Denaturation. Qualitative reactions for the determination of amino acids and proteins.

Relevance of the topic: The importance of amino acids is very great, because protein substances are composed of amino acid residues. More than 180 different amino acids are found in the cells and tissues of living organisms, but only 20 (α -amino acids) of them serve as the building blocks of peptides and proteins of all organisms (therefore they are called protein amino acids). The sequence of these amino acids in proteins is encoded in the nucleotide sequence of the corresponding genes. Proteins are found in the nucleus and protoplasm of all animals and in plant cells. Muscle, supporting and other tissues are built of proteins, with their help substances necessary for the body are transported, for example, oxygen from the lungs to the tissues; numerous biochemical processes are catalyzed.

Aims: To form knowledge about the structural organization of protein molecules for further study of biological functions of proteins at the molecular level. To form knowledge about the structure and reactivity of amino acids and be able to determine the type of amino acids using qualitative reactions.

Basic concepts: primary, secondary, tertiary structure of protein, qualitative reactions for the determination of amino acids, qualitative reactions for the determination of peptide bonds.

Equipment: department laboratory

Plan and organizational structure of the class:

1. Biological role of proteins in the body.
2. Physicochemical properties of proteins.
3. Levels of protein structure. Types of bonds.
4. Solubilization of proteins. Denaturation.
5. Qualitative reactions to amino acids, peptides, proteins.
6. Factors of stability of proteins in colloidal solutions. Mechanism of protein precipitation.
7. Denaturation of proteins. Types of denaturation and factors that cause it. Renaturation.
8. Qualitative reactions for the detection of proteins and amino acids (laboratory work).

The higher education applicant should know and be able to:

- 1) structural and optical isomerism of amino acids;
- 2) reactivity of amino acids;
- 3) biologically important reactions involving amino acids;
- 4) distinguish between the levels of protein structure;
- 5) perform analytical reactions for the determination of amino acids in solution.

Content of the topic

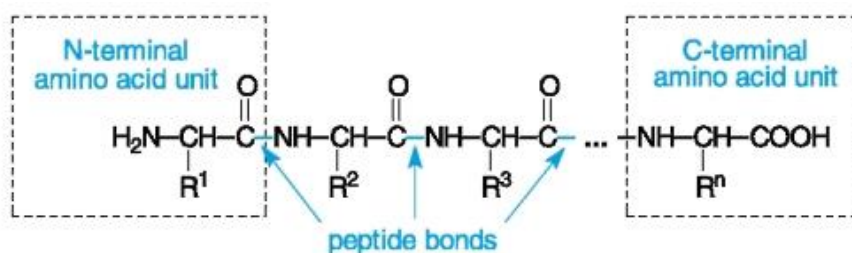
PEPTIDES AND PROTEINS

Peptides and proteins are compounds composed of many amino acids joined to one another through amide bonds called *peptide bonds*. Two terms are generally used for compounds made up of long sequences of amino acids: peptides and proteins.

The term *protein* is usually applied to naturally occurring polyamides that are derived from amino acids and have molecular mass greater than 10,000 (about 100 amino acid residues). The term *peptide* is used for natural or synthetic substances with a molecular mass less than 10,000. This limit is relative, of course. Peptides and proteins are very similar in their principal structure, but proteins, because of their size, have more intricate, three-dimensional arrangement inherent in them than peptides do.

1. Primary Structure

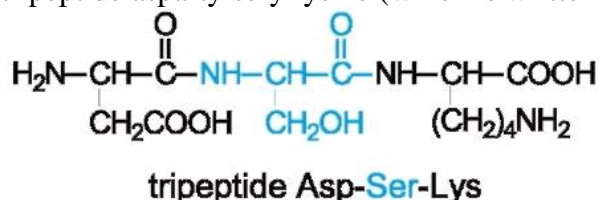
In the early 20th century, E. Fischer postulated that in peptides and proteins, peptide bonds are formed between the amino group of one amino acid and the carboxyl group of another as shown below:



If an amino acid contains two carboxyl groups or two amino groups, the α -amino and α -carboxyl groups are usually involved in the bonding.

By convention, the peptide chain is always written with the amino acid having a free NH_2 group at the left and the amino acid with a free COOH group at the right. These amino acids are called the *N-terminal* and *C-terminal* ones, respectively.

Formulas for peptides are often written in the abbreviated form for each amino acid listed in Table 1, starting with the *N*-terminus. Peptides are named as *N*-substituted *C*-terminal amino acids, using the suffixyl for the amino acid substituents; for example: glycyl, aspartyl and glutamyl - for aspartic and glutamic acids, asparaginyl and glutaminyl - for amides. Thus, Asp-Ser-Lys is the shortened formula for the tripeptide aspartylserlylsine (which is written without hyphens):



A sequence of amino acids in the chain signifies the primary structure of peptides and proteins.

Problem 5. Draw the structural formulas for each of the following peptides: (a) glutaminylserine; (b) alanylphenylalanine; (c) Glu-His-Gly. Give the name of the latter peptide.

2. Secondary Structure

Proteins and long-chain peptides might be expected to have rather amorphous, or floppy structure. But many peptides and some proteins have been isolated in crystalline form that indicates well-defined shapes of their molecules. The shapes seem to be quite regular even in solution. To understand this, let us consider some structural features of peptide chains.

Examination of a peptide group geometry (Fig. 1, a) shows that the amide C-N bond (132 pm long) is much shorter than the usual C-N single bond (147 pm; compare with 127 pm for the C=N double bond), while the C=O bond is slightly longer (124 pm) than that of carbonyl compounds (121 pm). All bond angles around the nitrogen atom are practically 120° , which is typical of sp^2 hybridization. All the data are the result of ρ, π conjugation or resonance in the amide group (Fig. 1, b and c).

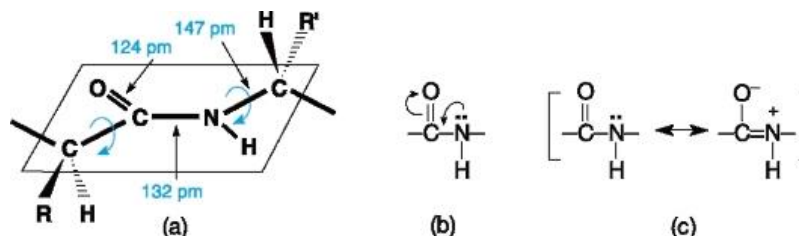


Figure 1. Peptide group: planar structure (a); ρ, π conjugation (b); resonance hybrid (c).

As a consequence, the C-N bond in the peptide group has a considerable double bond character and the peptide group is *planar*. But two adjacent peptide groups are not coplanar because of rotation about the other single bonds in the chain, i. e. the C-C and N-C bonds (Fig. 2). Thus, a peptide chain represents the series of planar portions divided by «joints», which are -CHR fragments.

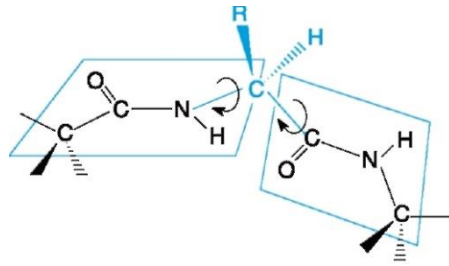
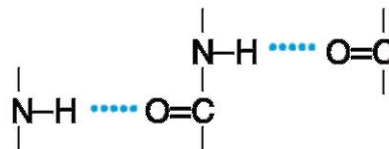


Figure 2. Planar portions and a -CHR joint of the peptide chain.

Such geometry and restricted rotation of the peptide bond govern a definite shape of proteins. The actual proteins are more often coiled or folded to give helical or compact, often globular, molecules. The binding forces that give rise to the unique conformational characteristics of individual proteins are of several kinds.

Hydrogen bonding. The presence of the recurring -NHCO unit along the linear polypeptide chain provides for a structural organization common to the majority of proteins. The drawing represents parts of the same chain, or parts of separate chains. Note that the acidic site is the NH fragment and the basic site is the oxygen of the carbonyl group.



The most distinctive structural consequence of hydrogen bonding is a coiling of the peptide chain about itself into a structure like a spiral staircase, which is called an α -helix (Fig. 3). The helix has a pitch of 0.54 nm, or about 3.6 amino acid units, and is maintained by hydrogen bonds that are approximately parallel with the axis of the helix. The bulky R groups of the amino acid residues are arranged outward, thus avoiding steric interaction between such R groups.

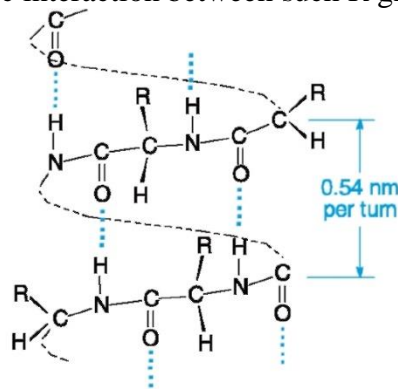


Figure 3. Hydrogen bonds (coloured dotted lines) in a segment of a polypeptide α -helix.

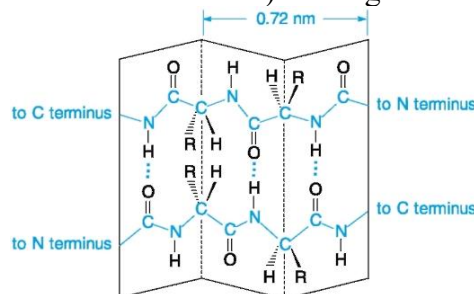


Figure4. A segment of a pleated sheet structure (hydrogen bonds and peptide core are shown in colour).

Another type of hydrogen bonding is realized in a *pleated sheet* arrangement of the peptide chain (Fig. 4). In this case, some portions of the peptide chain lie side by side in opposite directions and form *interchain* hydrogen bonds. Such an arrangement is possible only in proteins having a high content of amino acid with small R groups. Otherwise, there will be appreciable steric repulsion between the R groups on adjacent chains. The pleated sheet structure is found in the structural protein

β -keratin, obtained from silk fibroin, in which about 50% of the amino acid units are glycine (R = H) and over 20% are alanine (R = CH₃).

Of two spatial organizations of the peptide chains, helical and pleated, the α helix is a more common structure for the above reason.

The secondary structure of proteins is defined by hydrogen bonding between peptide groups of the chain.

3. Tertiary Structure

So far we have considered the protein backbone regardless of the nature of their constituents. Different R groups of amino acids also affect the mode of spatial arrangement of proteins. The tertiary structure of proteins is their overall three-dimensional shape that arises from further folding of the polypeptide chain.

Disulfide linkages. Oxidative coupling of two SH groups of cysteine results in a disulfide bond (-S-S-) formation. This transformation can join two portions of a protein chain to form a loop or ring (Fig. 5), or can join two polypeptide chains together. The disulfide bond can be broken with reducing agents.

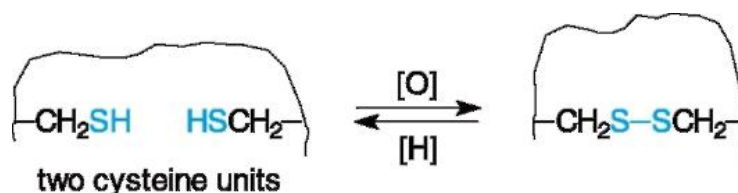


Figure 5. Disulfide bond formation and cleavage.

Dipolar interactions. Ionized amino and carboxyl groups, suitably disposed along the polypeptide chain, can give rise to electrostatic attraction between different segments of one chain or between separate chains (Fig. 6). The most ionogenic groups are the extra carboxyl groups of aspartic and glutamic acids that exist in a deprotonated form (-COO⁻) at physiological pH's, whereas the basic functional groups of lysine and arginine are protonated in the same medium.

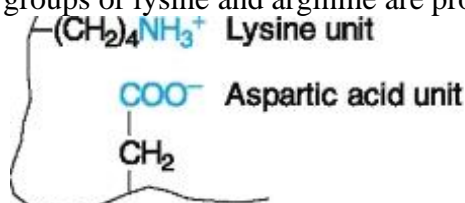


Figure 6. Electrostatic interaction

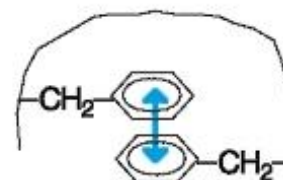


Figure 7. Hydrophobic interaction between ionized groups. between phenylalanine residues

Hydrophobic interactions. Attractive van der Waals' forces arise between nonpolar R groups of globular proteins. These proteins tend to expose their polar (hydrophilic) groups to the aqueous environment and to dispose a maximum of nonpolar (hydrophobic) groups within the molecule (Fig. 7). As it follows from Table 1, phenylalanine and amino acids with alkyl R groups are responsible for hydrophobic interaction.

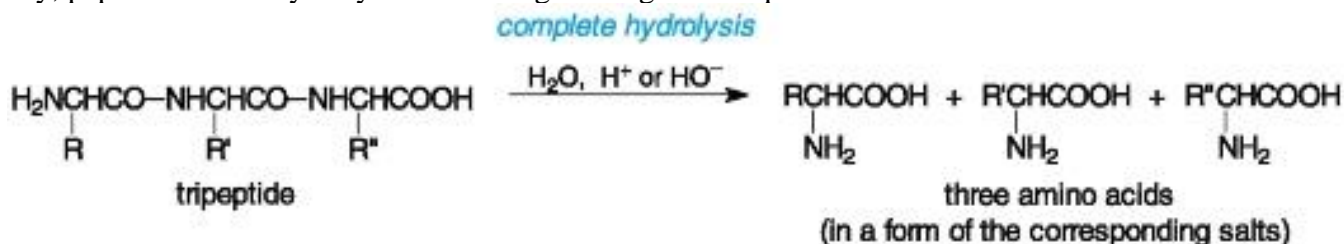
In concluding consideration of spatial organization of polypeptide chains, it should be noticed that the shape of the chain depends strongly on both amino acid content and amino acid sequence of a macromolecule.

Proteins are classified as *globular* and *fibrous* ones according to their shapes. The former are of roughly spherical shape and are generally soluble in water. The most important representatives of these are enzymes. Fibrous proteins have a roughly linear shape and are water-soluble. They represent a constructive material of skin, hair, muscle, connective tissue, silk fibroin, etc.

4. Hydrolysis of Peptides and Proteins

Of all the chemical properties of peptides and proteins, in this section we shall only touch upon complete and partial hydrolyses. The former leads to amino acid components of the peptide, the latter - to a mixture of shortened peptides. Complete hydrolysis is a way to estimating amino acid content of peptides and proteins.

As has already been shown, amides undergo hydrolysis in an acidic or alkaline medium. Similarly, peptides can be hydrolyzed according to the general equation:



The alkaline hydrolysis is rarely applied in the peptide chemistry because of instability of some amino acids on heating with strong alkalis. The acid-catalyzed hydrolysis is preferably used¹ but it is not free of shortcomings too. They consist in the complete destroying tryptophan molecule and the loss of information about asparagine and glutamine that are also hydrolyzed into the corresponding acids.

In living systems, proteins are hydrolyzed enzymically. That problem is beyond the scope of this book.

QUALITATIVE REACTIONS OF DETECTION PROTEINS AND AMINO ACIDS

Technique of laboratory work

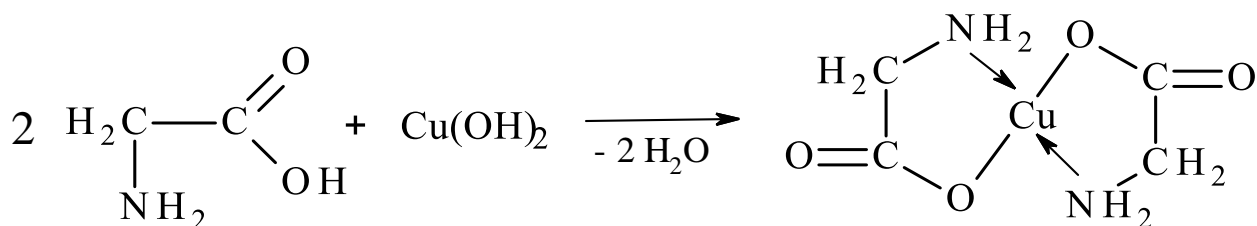
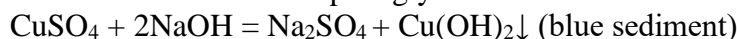
1. Study of amphoteric properties of amino acids using the indicator (methylrot):

Place in three tubes 3 drops of glycine, aspartic acid and lysine solution. Add 1 drop of indicator to each tube. Note the change in the color of the indicator in each tube.

<i>Amino acid</i>	<i>pH</i>	<i>Solution color</i>
glycine	~7	yellow
aspartic acid	<7	crimson
lysine	>7	yellow-green

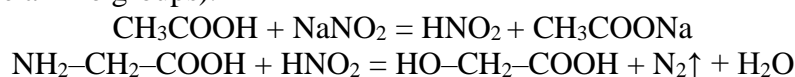
2. Formation of a complex glycine salt

Add 3-4 drops of sulfate copper(II) and 4 drops of sodium hydroxide to the tube. Observe the formation of a light blue precipitate of copper(II) hydroxide, which dissolves when a glycine solution is added, forming a clear blue solution of the complex glycine salt.



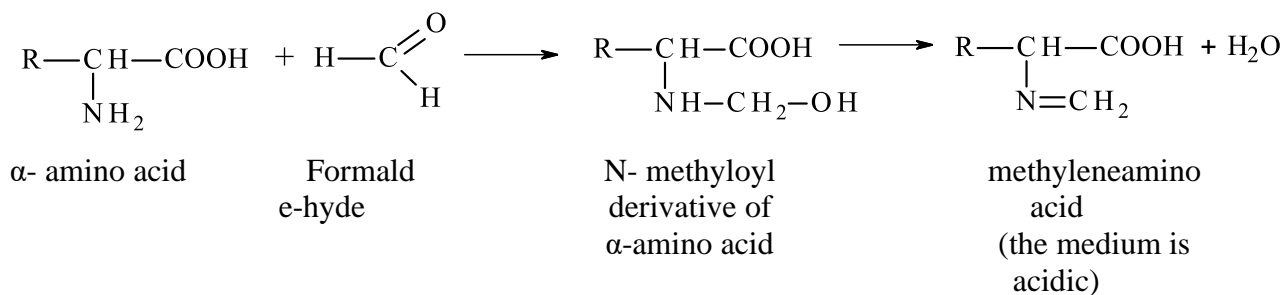
3. The reaction of glycine with nitrous acid. Van Slyk reaction.

In the tube, add 5 drops of glycine solution and a volume-equal sodium nitrite solution. Add 2 drops of concentrated acetic acid and gently mix the mixture. Observe the release of gas. (The reaction is used to quantify free amino groups).



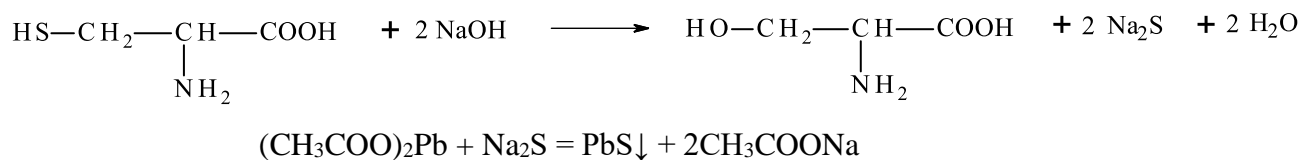
4. The reaction of glycine with formaldehyde. Serensen's reaction.

To the solution of amino acid (glycine) 0.5 ml add 2 drops of methyl red indicator. Since the reaction medium is neutral, the solution will turn yellow. Add the formalin solution dropwise until pink coloration appears, indicating the appearance of a free carboxyl group.



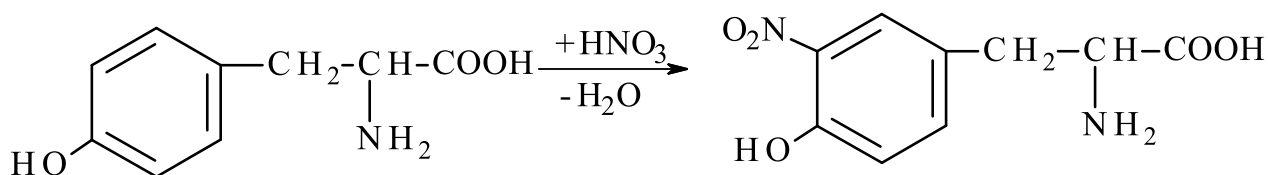
5. Color reaction to cysteine. Fole's reaction.

Add 5 drops of cysteine solution to the test tube. Add 2 drops of 10% sodium hydroxide solution, heat the mixture to boiling. And then add 2 drops of lead acetate solution. Observe the formation of lead-sulphide sediment of gray-black color



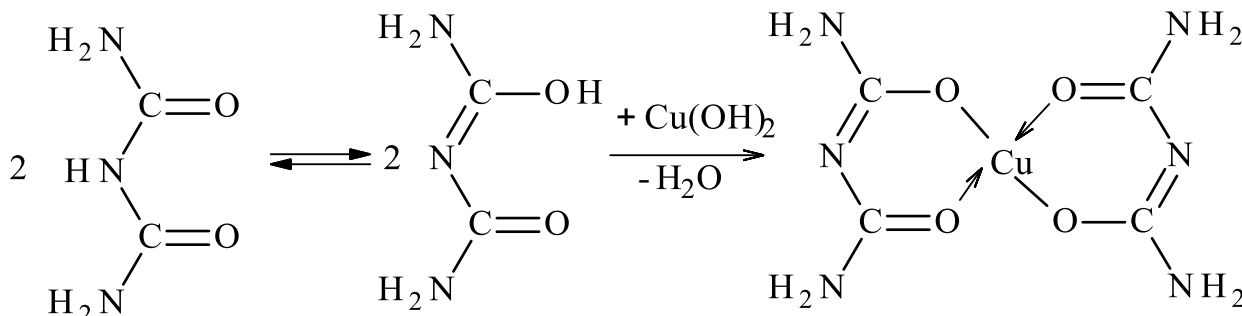
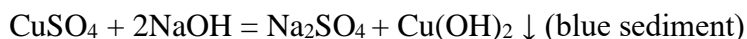
6. Xantoprotein reaction to aromatic amino acids.

Add 6 drops of tyrosine or egg white to the tube and add 3 drops of concentrated nitric acid. Heat the mixture until yellow.



7. Biuret reaction to peptide bonds.

To the protein solution, add 2 drops of sulfate sulfate and 2 drops of sodium hydroxide. Appears violet color.



Control materials for the final stage of the class.

Questions to check the final level of knowledge:

1. Levels of structural organization of proteins.
2. Types of bonds that are characteristic of different levels of protein structure.
3. Qualitative response to amphoteric amino acids.
4. Qualitative reaction to aromatic amino acids.

5. Qualitative reaction to sulfur-containing amino acids.
6. Qualitative reaction to peptide bonds.

Literature

Basic:

1. Biological and Bioorganic Chemistry: Bioorganic Chemistry: textbook / B.S. Zimenkovsky, V.A. Muzychenko, I.V. Nizhenkovska, G.O. Syrova. — 3rd edition – 2020. – 288 p.
2. Biological and Bioorganic Chemistry. Biological Chemistry: textbook / Yu.I. Gubsky, I.V. Nizhenkovska, M.M. Korda et al. — 2nd edition – 2021 – 544 p.
3. Bioorganic Chemistry. Rineyskaya O.N. textbook. – 2018. – 174 p.
4. Baynes J., Dominiczak M. Medical Biochemistry. 5th Edition. Elsevier, 2018. 712 p.

Additional:

1. Satyanarayana U. Biochemistry. 5th edition. India 2020. – 777 p.
2. Lehninger. Principles of Biochemistry. 7th edition. NY, United States. 2017.
3. William Marshall, Marta Lapsley, Andrew Day, Kate Shipman. Clinical Chemistry. Elsevier, 2020. 432 p.

Електронні інформаційні ресурси:

1. [https://info.odmu.edu.ua/chair/biology/-](https://info.odmu.edu.ua/chair/biology/)
2. <http://libblog.odmu.edu.ua/>
3. <https://moodle.odmu.edu.ua/login/index.php>

Practical class № 12

Topic: Classification, structure and importance of biologically active five-membered heterocyclic compounds with one and two heteroatoms. Biologically important six-membered heterocycles with one and two heteroatoms. Condensed heterocycles.

Relevance of the topic: The great importance of heterocyclic compounds is that they are the basis of many natural biologically active substances and drugs. Suffice it to say that of the most well-known and widely used drugs of natural and synthetic origin, more than 62% are heterocyclic compounds. Among the six-membered heterocycles with two heteroatoms a special place is occupied by hydroxy- and amino derivatives of pyrimidine - components of nucleic acids (uracil, thymine, cytosine), as well as 2,4,6-trihydropyrimidine - barbituric acid. Its derivatives, so-called barbiturates, are used as hypnotics and anticonvulsants.

Objective: To form knowledge about the structure and features of chemical behavior of five-membered heterocyclic compounds with biological activity. To form knowledge about the structure and features of chemical behavior of six-membered heterocyclic compounds with biological activity.

Basic concepts: pyridine, nicotinic acid, isonicotinic acid, pyrimidine, uracil, thymine, cytosine, adenine, guanine, barbituric acid, uric acid, lactic-lactam tautomerism.

Equipment: Laboratory of the department

Plan and organizational structure of the class:

1. Classification of heterocycles by cycle size, number and type of heteroatoms.
2. Structure and properties of pyrrole, furan and thiophene.
3. Non-benzoin aromatic systems. Biomedical importance of tetrapyrrole compounds: porphine, porphyrins, heme.
4. Indole, thiophene, pyrazole and their derivatives as drugs (vitamin H, analgin, amidopyrine, antipyrine).
5. Imidazole and its derivatives (histidine and histamine).

6. Five-membered heterocycles with two heteroatoms.

The higher education applicant should know and be able to:

1. The electronic structure of the atom of nitrogen, sulfur, oxygen.
2. Reactions of electrophilic and nucleophilic substitution.
3. Types of chemical bonds. Hydrogen bonding.
4. Acidity and basicity of organic compounds.
5. The phenomenon of tautomerism.

Content of the topic

BIOLOGICALLY ACTIVE HETEROCYCLIC COMPOUNDS

Heterocycles form the largest group of organic compounds, and many have important biological properties. Most natural substances as well as synthetic and natural drugs contain heterocyclic fragments. More precisely, about two thirds of all organic compounds and over three quarters of drugs belong to heterocyclic compounds.

The main heteroatom that occurs in heterocyclic compounds is nitrogen, but there are also rings containing oxygen and sulfur.

In previous chapters we have encountered some compounds with the oxygen or nitrogen atom in a cycle. Formally, cyclic anhydrides, lactones, lactides, and lactams are heterocycles. But the mentioned compounds have the same chemistry as their open-chain counterparts: lactones and ordinary esters behave similarly, as well as lactams and ordinary amides, and so on. These cyclic compounds undergo many reactions with ring opening that is not typical of «genuine» heterocycles, therefore they do not relate to heterocyclic compounds.

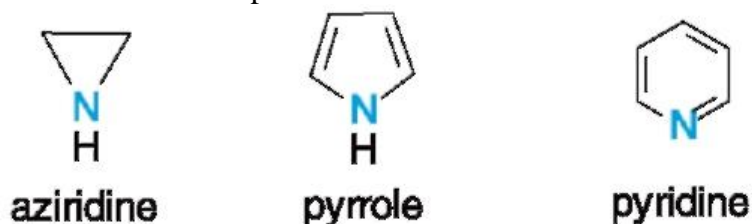
GENERAL CHARACTERISTICS OF HETEROCYCLIC SYSTEMS

1. Classification

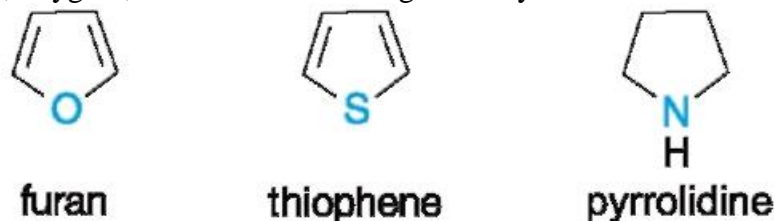
There is no unified classification of heterocyclic compounds because of their big variety (tens of thousands of types are known at present). They are usually classified in accordance with the following features of their skeleton.

According to the ring size.

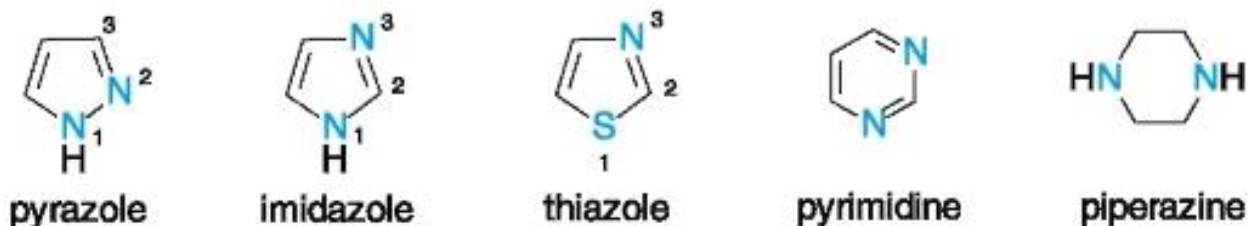
There are mainly three-, four-, five-, six-, and sevenmembered heterocycles. Out of them, fiveand six-membered are the most widespread.



According to the heteroatom incorporated in a ring. As has already been said, the most important are nitrogen-, oxygen-, and sulfur-containing heterocycles.

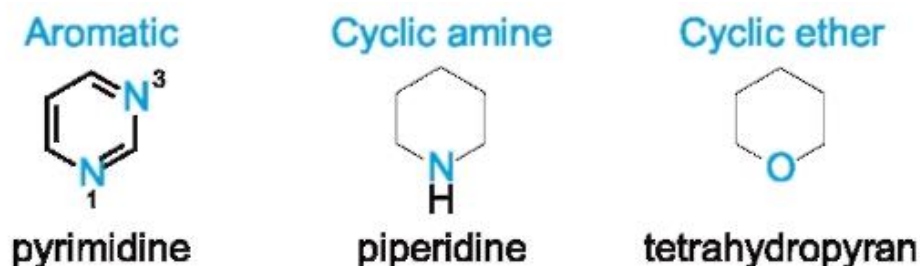


According to the number of heteroatoms and their mutual arrangement in a ring. The most common are compounds with one or two heteroatoms, but cycles with more heteroatoms are also known. Various combinations of heteroatoms are possible (for example, two nitrogens, nitrogen and oxygen, etc.), and heteroatoms can occupy the 1 and 2, 1 and 3, and 1 and 4 positions.



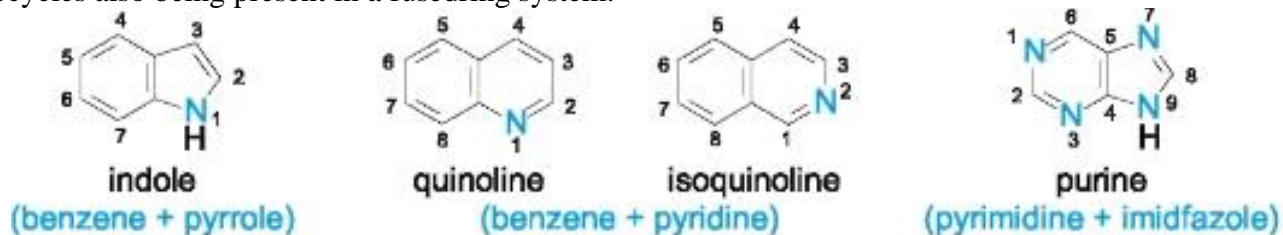
According to a degree of unsaturation of a ring.

Heterocycles can be divided into two subgroups: aromatic and nonaromatic (fully or partly saturated). The former are much more important, therefore the main attention in this chapter will be devoted to them. Besides, fully saturated heterocycles are similar to their acyclic analogues, i. e. secondary amines, ethers, or sulfides.



According to the number of rings.

In addition to monocyclic compounds, heterocycles with fused rings are well known too, carbocycles also being present in a fused ring system.



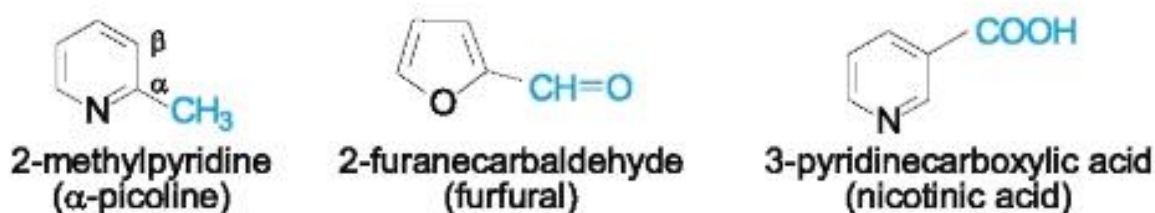
2. Nomenclature

Systematic nomenclature of heterocycles was developed at the end of the 19th century. It is known as *Hantzsch-Widman system* (see below). Nevertheless, several decades of trivial and semi-trivial names of heterocycles are widely used according to the IUPAC recommendations.

The position of a single heteroatom determines the numbering in monocyclic compound (but not in fused heterocycles). When the same heteroatom occurs twice or more, the numbering is chosen to give the lowest locants to the heteroatoms (see pyrimidine). In compounds with two nitrogens having a different configuration (see pyrazole and imidazole) the lowest locant gets the –NH fragment. When different heteroatoms are present, the priority of atoms in numbering is O > S > N (see thiazole).

The numbering in fused bicyclic compounds begins from the atom next to rings fusion in the direction to a heteroatom (see quinoline and isoquinoline). However, there are many exceptions to this rule, as, for example, in purine that retains historical numbering.

The general principles of substitutive nomenclature should be applied in systematic names of heterocycle derivatives, for example (trivial names are given in parentheses):



Hantzsch-Widman system.

Monocyclic compounds containing one or more heteroatoms in a three to ten-membered ring are named by combining the appropriate *prefix* (or prefixes) that denotes a heteroatom with a *stem* that indicates a ring size and its unsaturation (Table 1).

For the most widespread heteroatoms the following prefixes are used: *aza-* for nitrogen, *oxa-* for oxygen, and *thia-* for sulfur (eliding terminal a where necessary for euphony). Thus, monocyclic heterocycles shown above will be named as follows: *oxole* (furan), *thiole* (thiophene), *azole* (pyrrol), *1,3-diazole* (imidazole), and *azine* (pyridine).

Table 1.

The stems for designation of a ring size in the Hantzsch-Widman system

Number of atoms in the ring	Rings containing nitrogen		Rings containing no nitrogen	
	unsaturated*	saturated	unsaturated*	saturated
3	-irine	-iridine	-irene	-irane
4	-ete	-etidine	-ete	-etane
5	-ole	-olidine	-ole	-olane
6	-ine	**	-in	-ane
7	-epine	**	-epin	-epane
8	-ocine	**	-ocin	-ocane

* Corresponding to the maximum number of non-cumulative double bonds.

** Expressed by addition of the prefix perhydroto the name of the respective unsaturated compound.

GENERAL ASPECTS OF REACTIVITY OF AROMATIC HETEROCYCLES

Two heterocycles, pyridine and pyrrole, are of fundamental importance for a reason that will be considered in the next section.

1. Aromaticity of Pyridine and Pyrrole

Pyridine.

This six-membered heterocycle has a structure similar to that of benzene, except that one CH fragment of the benzene ring is replaced by a nitrogen atom (Fig. 1, a).

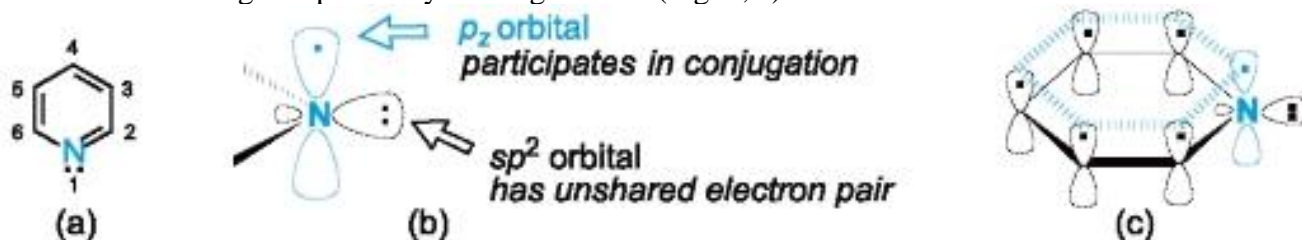


Figure 1. The pyridine molecule: the structure (a), the pyridine-type nitrogen atom (b), and the orbital picture (c). The orbital overlap below the ring and all hydrogen atoms are omitted for clarity.

Nitrogen in pyridine is sp^2 -hybridized (as well as all carbons). The two hybrid orbitals of nitrogen, each with one electron, overlap sp^2 orbitals of the C-2 and C-6 atoms to form two σ bonds (these orbitals are not shown in Fig. 1, b). The remaining hybrid orbital of nitrogen possesses an unshared electron pair and does not form a bond. The unhybridized p orbital of nitrogen (with one electron) is perpendicular to the plane of the ring and overlaps the p orbitals of carbons to form aromatic six π -electron cloud (Fig. 16.1, c). Otherwise, pyridine is very like benzene in its π -electron configuration, i. e. pyridine is *isoelectronic* to benzene. Thus, pyridine is in accord with all criteria of aromaticity:

- all atoms in the cycle are sp^2 -hybridized,
- it represents a planar compound with cyclic conjugation,
- it obeys the Huckel's rule because it is a six π -electron system.

The conjugation energy in pyridine is sufficiently high (117 kJ/mol) but markedly less than that of benzene (151 kJ/mol). The difference is caused by the presence of the electronegative nitrogen atom that distorts complete uniformity of the π -electron cloud. This results in decreasing electronic density on the carbon atoms. Pyridine is referred to as π -deficient heterocycles for this reason.

Pyrrole.

Five-membered heterocycle pyrrole contains all the carbons and the nitrogen atom in sp^2 -hybridized state (Fig. 2, a).

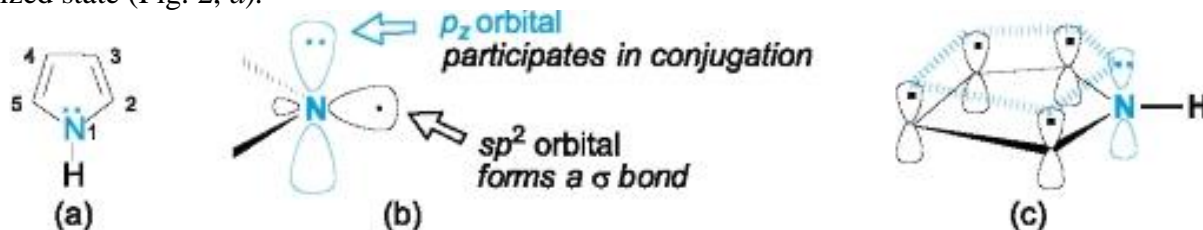


Figure 2. The pyrrole molecule: the structure (a), the pyrrole-type nitrogen atom (b), and the orbital picture (c). The orbital overlap below the ring and all C-H bonds are omitted for clarity.

All three hybrid orbitals on nitrogen form three σ bonds, two orbitals (not shown in Fig. 2, b) - with the atoms C-2 and C-5 and the third orbital - with hydrogen. The nitrogen lone pair of electrons occupies unhybridized p orbital and forms, together with four p orbitals on carbons, a delocalized π -electron cloud (Fig. 2, c). Pyrrole is therefore an aromatic compound.

In contrast to pyridine, pyrrole is said to be a π -excessive (or π -rich) heterocycle because *six* π electrons of the aromatic system spread over *five* atoms of the cycle. In other words, each p orbital contains more than one electron.

Finally, pay attention to different roles of the nitrogen atoms in pyridine and pyrrole. Nitrogen in pyridine contributes only one π electron to the aromatic sextet, while nitrogen in pyrrole contributes two p electrons (the lone pair) to the aromatic sextet.

Example 1. Prove that imidazole is an aromatic heterocycle. Draw its orbital picture, showing all p orbitals and all lone-pairs of electrons.

Solution. The atom N-1 is pyrrole-type nitrogen that has a lone pair of electrons on its unhybridized p orbital (Fig. 3). The atom N-3 is pyridine-type nitrogen with one electron on a p orbital.

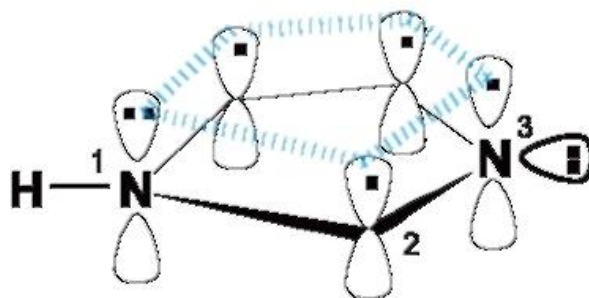


Figure 3. Orbital picture of imidazole. The orbital overlap below the ring and all C-H bonds are omitted for clarity.

As a result of such difference in the nitrogen electron demand, pyridine and pyrrole behave in a different manner with respect to various chemical reagents.

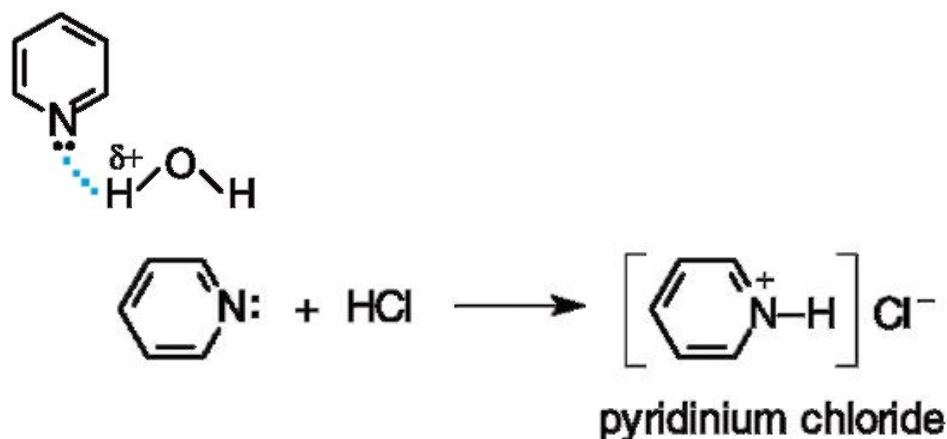
Problem 1. Which of the following compounds represent aromatic heterocycles: (a) furan; (b) piperidine; (c) pyrimidine; (d) quinoline. Explain the reason for your choice.

2. Basicity and Acidity

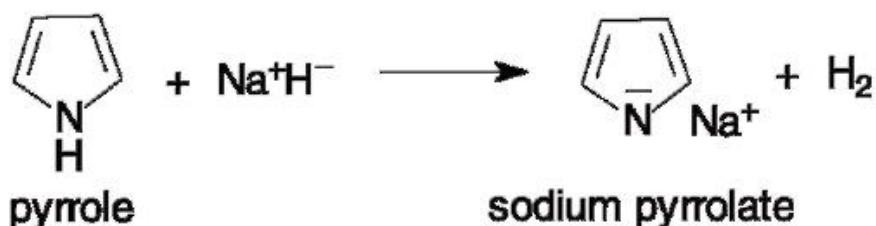
In pyridine, the lone pair of electrons on the nitrogen atom is *not* a part of the aromatic π -electron system but occupies an sp^2 orbital in the ring plane (Fig. 1). Consequently, pyridine can donate this lone pair of electrons for accepting a proton and for hydrogen bonding with water. Unlike benzene, it is completely miscible with water.

Pyridine forms pyridinium salts in reaction with acids. It is a weakly basic compound, with pK_{BH^+} 5.2, which is comparable with the value of 4.6 for aniline. Pyridine is however much less basic

than ammonia and aliphatic amines because the electronegativity of an sp^2 -hybridized nitrogen is greater than that of an sp^3 -hybridized nitrogen in ammonia and in aliphatic amines.



In pyrrole on the contrary, the nitrogen lone pair of electrons is an essential part of the aromatic π system. Pyrrole is an extremely weak base; it has a pK_{BH^+} of -3.8, about 10^9 times weaker than pyridine. It can be protonated on carbon rather than on the nitrogen atom. Protonation of pyrrole would destroy the aromatic system, therefore it belongs to so-called *acidophobic* heterocycles. At the same time, pyrrole possesses a marked NH-acidity (pK_a 17.5 that is in the range of alcohol acidity). Thus, it can be deprotonated like alcohols in reactions with strong bases, for example, with sodium hydride:



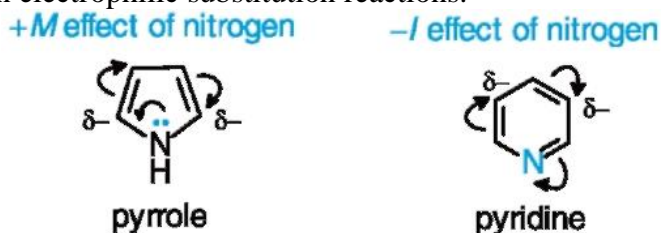
Note that both pyridinium and pyrrolate salts retain aromatic character.

Problem 2. Only one of two nitrogens in pyrazole is quite basic. Show which one it is, and explain the reason for your choice.

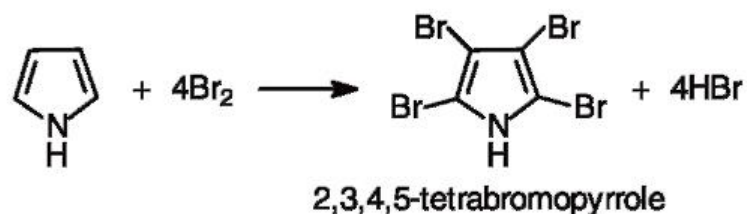
3. Substitution Reactions in Heterocycles

Electrophilic substitution is a typical reaction not only for aromatic hydrocarbons but also for aromatic heterocycles. The influence of a heteroatom on the reactivity of a heterocycle is similar to that of a substituent in the benzene ring.

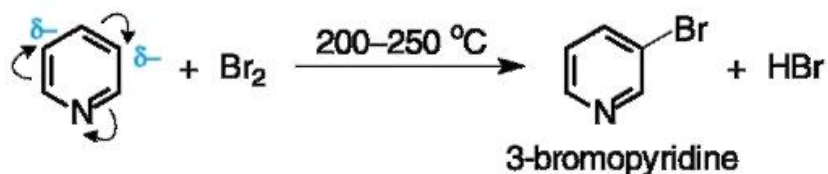
In pyrrole, the donating effect of the nitrogen lone pair of electrons increases the electron density on the ring carbons, thus increasing reactivity of the heterocycle towards electrophiles. In pyridine, on the contrary, the pyridine-type nitrogen is an electron-withdrawing atom which decreases the electron density on the carbons (especially at the 2, 4, and 6 positions), thus making the heterocycle less reactive in electrophilic substitution reactions.



A difference in reactivity of pyrrole and pyridine is demonstrated in their bromination reactions. Pyrrole reacts readily with bromine at low temperature to yield the corresponding tetrabromo derivative:

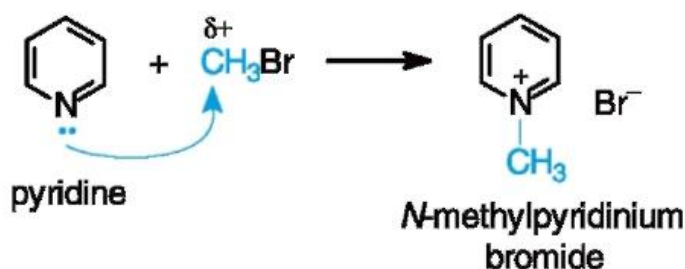


Bromination of pyridine can be carried out under drastic conditions in a low yield where a free-radical mechanism may operate. Electrophilic substitution nearly always takes place at the 3 position according to the bond polarization.

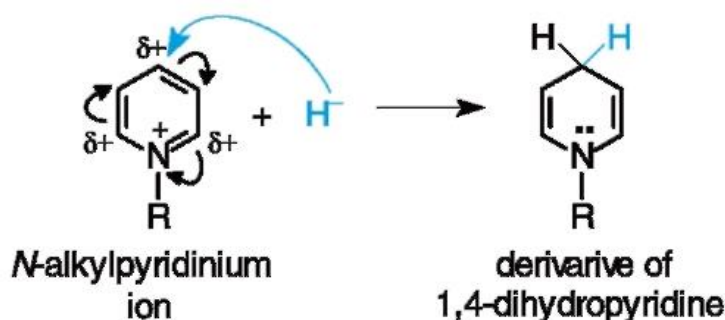


Another factor decreasing the reactivity of pyridine in electrophilic substitution reactions is acid-base complexation between the basic nitrogen and the attacking electrophile, which may be often a proton. This results in the formation of a positive charge on the ring, further deactivating it. Thus, pyridine does not undergo Friedel-Crafts alkylation and acylation.

Pyridine exhibits nucleophilic properties in the reaction with electrophiles. It behaves as tertiary amine and forms quaternary pyridinium salts when reacting with alkyl halides.



The aromatic ring of pyridinium salts is susceptible to nucleophilic attack owing to its high electron-deficiency. For example, a strongly nucleophilic hydride ion reacts with an alkylpyridinium salt by addition, producing non-aromatic 1,4-dihydropyridine derivative (attack of the 2 position is also possible):



The pyridinium ion is thus reduced, i. e. it acts as an oxidant. This ion is a part of the NAD⁺ molecule, one of the most important coenzymes in biological oxidations. So the above equation represents a simplified version of the biological reaction.

FIVE-MEMBERED RINGS WITH ONE NITROGEN

The pyrrole ring is a structural component of several biologically important compounds. It is often found as a fused-ring system indole. The latter is usually biosynthesized from the protein amino acid tryptophan. Indole itself and its 3-methyl derivative, skatole, are the decay products of proteins (both contribute to the odour of feces).

Tryptophan is decarboxylated *in vivo* to give tryptamine. Many compounds that contain the tryptamine skeleton have an effect on the brain and nervous system. For example, serotonin (5-

hydroxytryptamine) is a neurotransmitter and vasoconstrictor active in the central nervous system. A disturbance in its metabolism leads to schizophrenia.



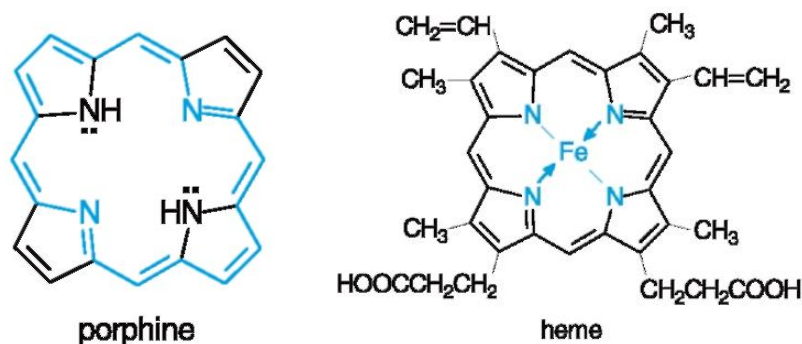
The structure of saturated pyrrole called pyrrolidine is a fragment of the wellknown alkaloid nicotine.

The pyrrole ring is a structural component of many vitally important compounds called *porphyrins*. Their parent structure is porphine, a tetrapyrrolic macrocyclic system. Porphine represents a flat symmetrical molecule in which four pyrrole rings are linked by one-carbon bridges. It forms a conjugated system of eighteen π electrons shown in colour and is, therefore, aromatic.

Note that 18 is the Huckel's number when $n = 4$. The formula of porphine represents one of the resonance contributing structures. Total amount of electrons in conjugation is 26, it also being the Huckel's number.

Aromaticity of porphine is confirmed by its high conjugation energy which amounts to 840 kJ/mol. All the porphyrins (substituted porphines with various side chains at the pyrrole rings) are exceptionally stable compounds that decompose at about 500 °C.

Porphyrins form metallic complexes in which two NH hydrogens are absent and each of the four nitrogens is bound to metal in the middle of the structure. The best known of these is heme, the iron(II)-porphyrin complex that imparts the red colour to blood. Heme is a constituent of the complex protein hemoglobin responsible for binding molecular oxygen in the process of respiration.



Another example of tetrapyrrolic compounds is chlorophyll, the green plant pigment essential for photosynthesis. It represents a magnesium-porphyrin complex.

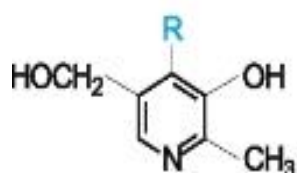
SIX-MEMBERED RINGS WITH ONE HETEROATOM

This group of heterocyclic compounds is the oldest one. Pyridine was discovered in the mid-19th century and its structure was established in 1869, shortly after the Kekule structure of benzene had been suggested.

1. Nitrogen-Containing Heterocycles

The main parent compounds of this type are pyridine and fused-ring heterocyclic systems of quinoline and isoquinoline. They are found in a small quantity in coal tar. Pyridine is a toxic liquid with unpleasant odour.

All these rings are constituents of many naturally occurring compounds and numerous drugs. Some physiologically important pyridine derivatives are related to vitamin B₆. This is a relatively simple pyridine derivative. The R group at C-4 may be a fragment of an alcohol, aldehyde, or amine. The vitamin functions as a coenzyme in the interconversions of oxo carboxylic acids and amino acids.



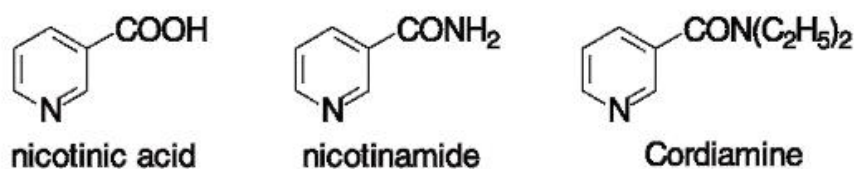
vitamin B₆:
 R = CH₂OH, pyridoxin
 R = CH=O, pyridoxal and its phosphate
 R = CH₂NH₂, pyridoxamin

Problem 3. Which of the following statements corresponds to the structure and properties of pyridoxal?

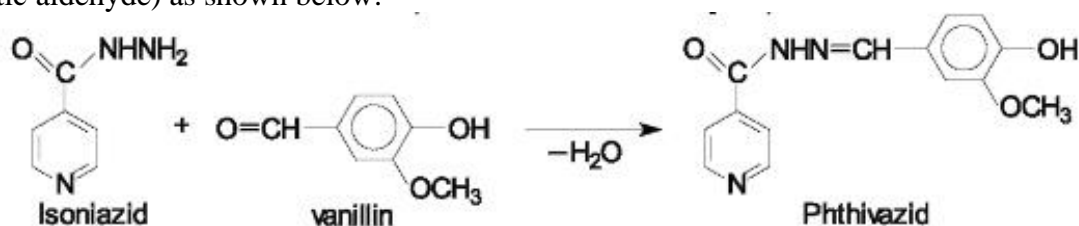
- (a) the unshared electrons of the nitrogen are involved in conjugation to form aromatic sextet;
- (b) it contains the pyrrole ring system;
- (c) it forms a salt with hydrochloric acid;
- (d) it forms a salt with potassium hydroxide, disposing both hydroxyl groups;
- (e) it reacts with primary amines to give a Schiff's base.

Write equations for reactions, if they take place.

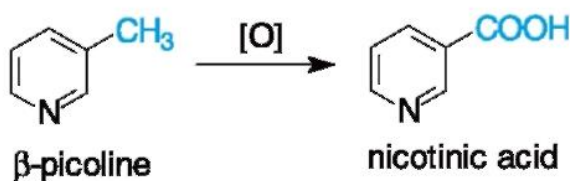
Two pyridine derivatives which relate to vitamin PP are nicotinic acid (Niacin) and its amide (Niacinamide). A substituted nicotinamide Cordiamine (Niacetamide) is used as a stimulator of the central nervous system.



Other examples of pyridine containing drugs are hydrazide derivatives of isonicotinic acid (4-pyridinecarboxylic acid). They are known as tuberculostatic drugs Isoniazid (Tubazid) and Phthivazid. The latter is prepared from Isoniazide by a familiar nucleophilic reaction with vanillin (an aromatic aldehyde) as shown below:

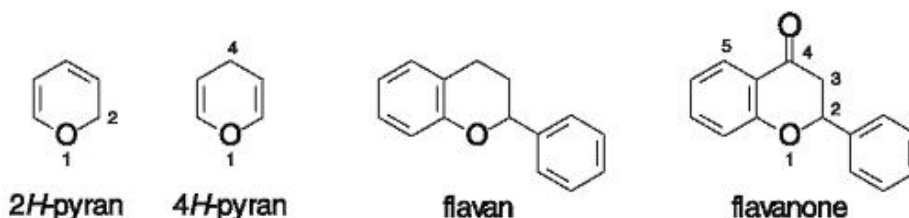


Nicotinic acid first obtained by oxidation of nicotine is now produced from available β -picoline (3-methylpyridine) and other 3-alkylpyridines also by oxidation, for example:



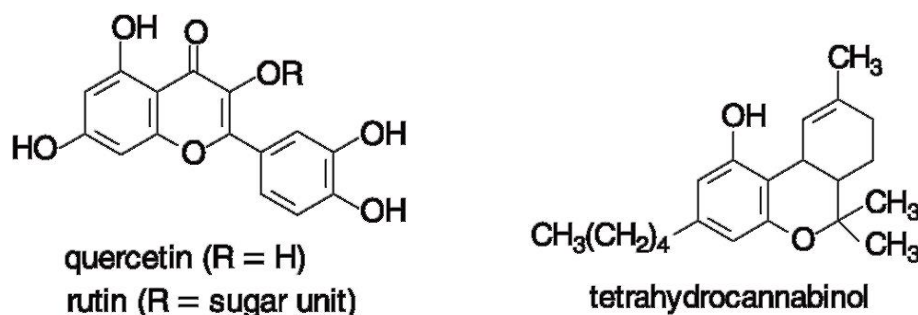
2. Oxygen-Containing Heterocycles

Compounds consisting of oxygen-containing heterocycles are widespread in nature; there are, first of all, cyclic forms of carbohydrates. Unsaturated six-membered representatives - 2/ -pyran and 4/ -pyran are inherently unstable; moreover the former is at present unknown. Nevertheless, a group of the naturally occurring pyran derivatives called *flavonoids* has attracted attention of chemists and biochemists in the last decades. Basic structures of flavonoids are polycyclic compounds flavan and its 4-oxo derivative flavanone, which may also contain the C-2-C-3 double bond (not shown below):



A characteristic feature of flavonoids is the presence of several hydroxyl groups (up to six) in all three rings, some of which are often bound to sugar units.

Flavonoids occur ubiquitously in plants and foods. It is estimated that the mean human intake of all flavonoids is about 100-150 mg per day. Many flavonoids possess multiple biological activities, including cardiovascular, anticarcinogenic, anti-inflammatory, immune-stimulating effects, some of them are used in medicine as, for example, quercetin (Quertin) and its sugar analogue rutin (Rutosid).



It is known today that flavonoids take the protective role in the human body, saving the latter from destructive action of free radicals.

One compound containing a fused pyran ring system is of no benefit to humans; this is tetrahydrocannabinol, a psychotropic component of marijuana.

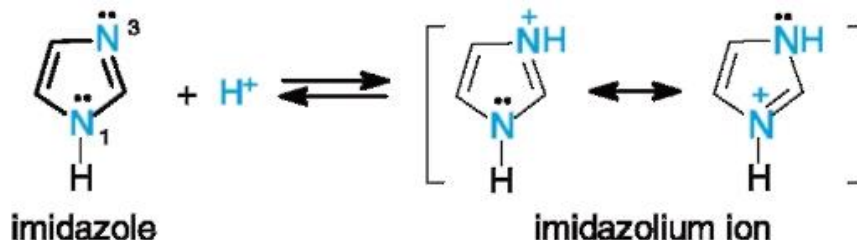
The symbol H («indicated hydrogen») is used for accurate defining the position of the double bonds in compounds with the maximum number of non-cumulative double bonds.

RINGS WITH MORE THAN ONE HETEROATOM

Four important heterocyclic ring systems with more than one heteroatom are imidazole, pyrazole, pyrimidine, and purine. Other combinations of heteroatoms are known, of course, but we will be concerned with only nitrogen-containing aromatic heterocycles.

1. Imidazole and Pyrazole

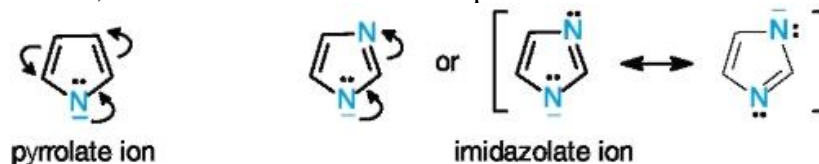
Imidazole is a little like pyrrole in the following way: the unshared electron pair on the N-1 atom is delocalized and is a part of the aromatic six π -electron system (Fig..1). But the unshared electron pair on the N-3 atom is available for protonation. The pK_{BH^+} of imidazole is 7.0, so it is about 100 times more basic than pyridine and about 10^{11} times more basic than pyrrole. The positive charge in protonated imidazole can be delocalized over both nitrogens through resonance:



The similar consideration may be applied to pyrazole, which is an isomer of imidazole. The N-1 atom (pyrrole-type) represents the acidic site in both compounds whereas the C=N nitrogen (pyridine-type) is the basic site.

Example 2. As we have just seen, imidazole is much more basic than pyrrole. At the same time, imidazole is stronger as an acid too. Suggest an explanation for these facts.

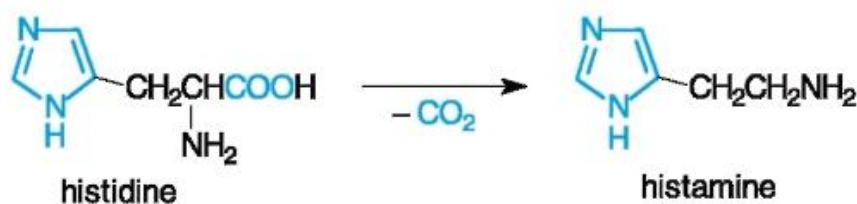
Solution. It should be reemphasized that the best way for discussing acidity is to compare stability of conjugate bases, i. e. anions obtained after deprotonation of both heterocycles.



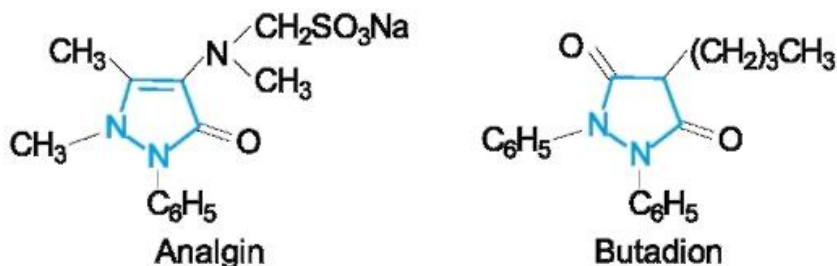
The imidazolite ion is better stabilized due to the presence of the second, electron-withdrawing nitrogen atom. In terms of the resonance theory, this anion represents two equal contributing structures. Indeed, the pK_a of imidazole is 14.2 and that of pyrrole is 17.5. Thus, imidazole is an amphoteric compound.

The imidazole unit is a part of the protein amino acid histidine. In some enzymic reactions, histidine situated on the active site of an enzyme can realize either acidic or basic catalysis; both are a consequence of amphoteric properties of imidazole.

The imidazole derivative histamine that relates to biogenous amines is produced on decarboxylation of histidine in living systems.



Pyrazole itself and its derivatives do not occur in nature, but the pyrazole skeleton is present in some analgesics and antipyretics, for example, in Analgin (Dipyrone) and Butadion (Phenylbutazone), that have many other synonyms.

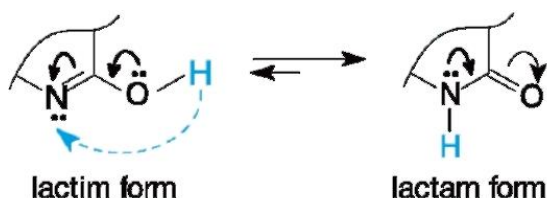


2. Pyrimidine Derivatives

The most important pyrimidine derivatives are uracil, thymine, and cytosine called nucleic bases (or heterocyclic bases) since they are constituents of nucleic acids.



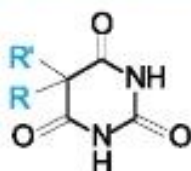
The nucleic bases are capable of existing in several tautomeric forms. A new kind of tautomerism arises for nitrogen-containing heterocycles that have the OH group attached to the C=N fragment of a ring system. This phenomenon is called *lactim-lactam tautomerism*, and tautomers are known as the *lactim form* and *lactam form*. The lactam tautomers are usually the predominant forms at equilibrium.



Problem 4. Draw the lactim tautomeric form for (a) uracil and (b) cytosine.

A group of compounds called *barbiturates*, whose usage ranges from mild sedatives to hypnotics and anesthetics, are also pyrimidine derivatives. Examples include Barbital (Veronal), the first synthetic soporific, Phenobarbital (Luminal), and Amobarbital (Amytal).

General formula of barbiturates



Barbituric acid

R R'

H H

Barbital

C₂H₅ C₂H₅

Phenobarbital

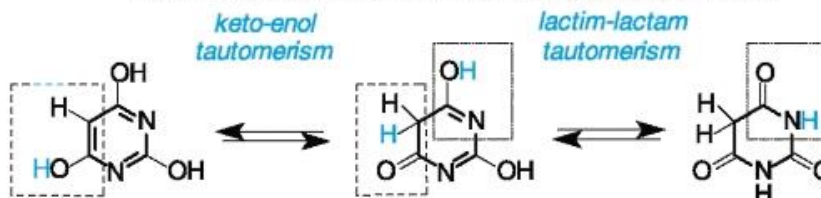
C₂H₅ C₆H₅

Amobarbital

C₂H₅ CH₂CH₂CH(CH₃)₂

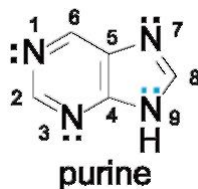
Barbituric acid exists in solution as a mixture of several tautomeric forms: the keto and enol forms (keto-enol tautomerism), and the lactim and lactam forms (not all the forms are given below):

TAUTOMERIC FORMS OF BARBITURIC ACID



3. Purine Derivatives

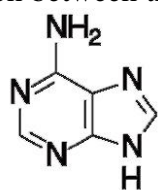
Purine represents a combination of two aromatic heterocycles imidazole and pyrimidine. Three of four nitrogens (N-1, N-3, and N-7) in the purine molecule are pyridine-type and the remaining (N-9) is a pyrrole-type nitrogen.



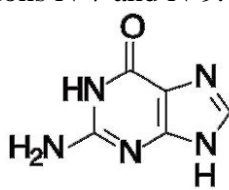
Purine is an aromatic compound because it has a cyclic system of conjugation with a *p* orbital on each atom and contains 10 π electrons (the Huckel's number). Each *p* orbital of five carbons and each of three pyridine-type nitrogens contributes one π electron to the aromatic system, and the pyrrole-type nitrogen contributes two *p* electrons.

Purine itself is not found in nature. Its two derivatives, adenine and guanine, represent purine components of nucleic acids in addition to three pyrimidine nucleic bases.

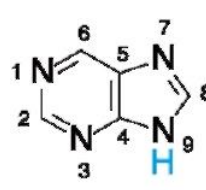
Purine and its derivatives are subjected to prototropic tautomerism caused by hydrogen migration between the positions N-7 and N-9.



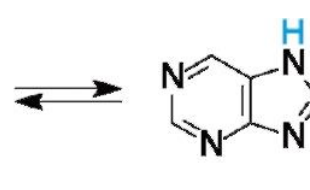
adenine



guanine



9H-purine



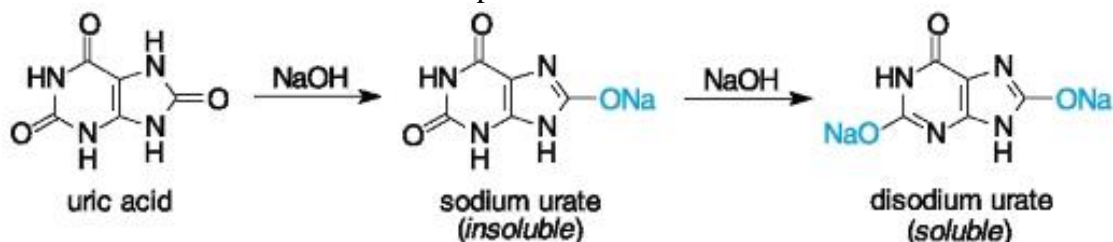
7H-purine

Crystalline purine is the 9 tautomer as well as adenine and guanine incorporated into nucleic acids. Lactim-lactam tautomerism is possible for guanine.

Three hydroxylated purines are the products of nucleic acid metabolism. These are uric acid (2,6,8-trihydroxypurine), the final metabolite, xanthine (2,6-dihydroxypurine), and hypoxanthine (6-hydroxypurine), shown below in the most stable tautomeric forms:

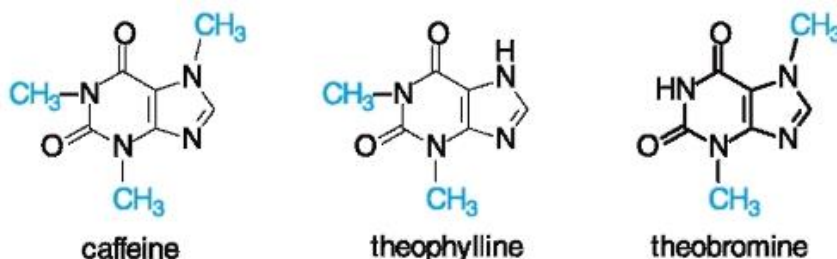


Water-insoluble uric acid as a relatively strong acid (pK_{a1} 5.7, pK_{a2} 10.3) reacts with alkalis to form two series of salts called *urates*, for example:



Insoluble urates can be deposited in the joints and tendons as «stones» (or calculi) in some disorder in the human body.

Naturally occurring purine derivatives are N-methylated xanthines such as caffeine (present in coffee, tea, and cola beverages), theophylline (also present in coffee beans and tea leaves), and theobromine (found in cocoa).



Caffeine is known as a stimulator of the central nervous system; two other xanthines are also used in medicine. All the three compounds are sometimes assigned to alkaloids.

ALKALOIDS

Alkaloids are nitrogen-containing, mostly heterocyclic compounds that produce striking physiological effects on animals.

Effects of alkaloids vary greatly from one compound to another. The term *alkaloid* originates from the fact that these substances are «alkali-like», i. e. they react with acids to form soluble salts. Moreover, alkaloids are present in plants as salts with organic acids (oxalic, malic, citric, and others).

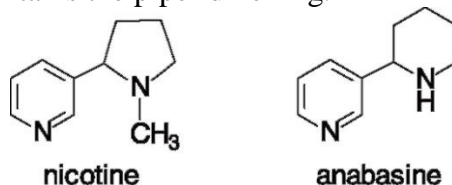
Common names are usually used for alkaloids because of the complexity of their structures. These names often reflect the botanical source of the substance. The alkaloid papaverine, for example, was isolated from the opium poppy, *Papaver somniferum*; the alkaloid cocaine – from *Erythroxylon coca*, etc. Sometimes the names of alkaloids are eccentric: the name of the another opium alkaloid morphine came from Morpheus, the Greek god of dreams; the name of the tobacco alkaloid nicotine came from J. Nicot, a French diplomat who brought tobacco seeds to France in 1560. Names of most alkaloids have the ending **-ine** that shows the amine nature of the compounds.

Over five thousand alkaloids are known today. The old classification of alkaloids used phylogenetic features, for example, tobacco alkaloids, coca alkaloids, and so on. The modern classification is based on the structure of a basic heterocyclic system though more than one heterocycle may be present.

Only a few selected examples are considered in this section with minimal information on biological or pharmacological activity. Stereochemistry in all presented structures is omitted for simplicity but it should be remembered that almost all alkaloids are optically active compounds.

1. Pyridine Alkaloids.

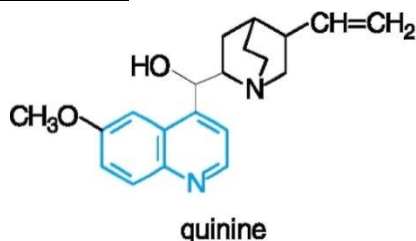
The major alkaloid of the tobacco leaves is nicotine, one of the simplest of all alkaloids, that contains, besides the pyridine ring, also the pyrrolidine ring. Its isomer anabasin found in the Central Asian plant *Anabasis aphylla* L. contains the piperidine ring.



The two alkaloids are toxic to humans (the effect of nicotine is well-known). It is interesting that anabasin as a hydrochloride salt is advised as the aid for breaking the habit of smoking. Both compounds are used as agricultural insecticides and as raw materials for producing nicotinic acid by oxidation.

Problem 5. Nicotine is present in tobacco as a salt with organic acids. Which of the two nitrogens of the nicotine molecule is more basic? Draw the structure of nicotine hydroxalate.

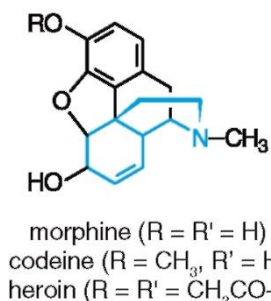
2. Quinoline and Isoquinoline Alkaloids



Quinine is the most known and the oldest alkaloid of the quinoline group. It contains an additional and unusual heterocyclic ring system – *quinuclidine* (non-coloured in the drawing). Quinine was isolated from cinchona bark in 1820 but the South American natives had used a decoction of the bark for centuries before this. For many long years quinine was a single remedy for malaria.

The group of isoquinoline alkaloids amounts to over 1000 substances which are located in about thirty plant families. We will be concerned with only several most known alkaloids of the opium poppy.

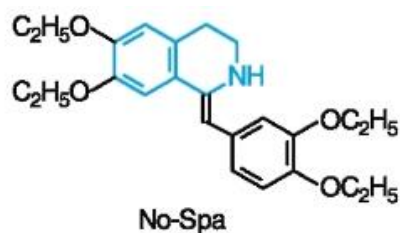
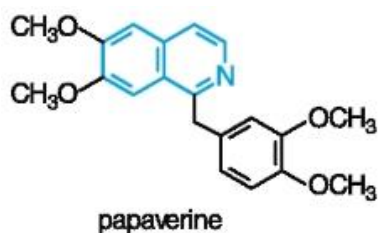
Morphine is the main component of opium and was first isolated from it in 1806. It was the first alkaloid obtained in a pure form, but its highly complicated structure was deduced only in 1920's and finally confirmed by independent masterly synthesis in 1952. Morphine remains one of the strongest analgesics though opium was used in ancient Egypt. Unfortunately, it has many disadvantages, and we will not discuss pharmacological and social aspects of morphine applications.



The author of this book attempted to apply it but unsuccessfully.

The monomethyl ether of morphine, another component of opium, called *codeine* is useful as an anticough agent. The synthetic diacetyl derivative of morphine is the dolefully known narcotic heroin.

Papaverine mentioned above is a relatively simple isoquinoline derivative which is used as an antispasmodic medicine. A synthetic drug No-Spa (Drotaverine) has a similar structure but a more pronounced pharmacological effect.



Control materials for the final stage of the class.

Questions to check the final level of knowledge:

1. Classification of six-membered heterocycles.
2. Derivatives of pyrimidine - uracil, thymine, cytosine.
3. Lactyl-lactam tautomerism of uracil, thymine, cytosine.
4. Purine derivatives - adenine and guanine.
5. Prototropic tautomerism of uracil.
6. Lactim-lactam tautomerism of guanine.
7. Tautomeric forms of barbituric and uric acids.

Literature

Basic:

1. Biological and Bioorganic Chemistry: Bioorganic Chemistry: textbook / B.S. Zimenkovsky, V.A. Muzychenko, I.V. Nizhenkovska, G.O. Syrova. — 3rd edition – 2020. – 288 p.
2. Biological and Bioorganic Chemistry. Biological Chemistry: textbook / Yu.I. Gubsky, I.V. Nizhenkovska, M.M. Korda et al. — 2nd edition – 2021 – 544 p.
3. Bioorganic Chemistry. Rineyskaya O.N. textbook. – 2018. – 174 p.
4. Baynes J., Dominiczak M. Medical Biochemistry. 5th Edition. Elsevier, 2018. 712 p.

Additional:

1. Satyanarayana U. Biochemistry. 5th edition. India 2020. – 777 p.
2. Lehninger. Principles of Biochemistry. 7th edition. NY, United States. 2017.
3. William Marshall, Marta Lapsley, Andrew Day, Kate Shipman. Clinical Chemistry. Elsevier, 2020. 432 p.

Електронні інформаційні ресурси:

1. <https://info.odmu.edu.ua/chair/biology/>
2. <http://libblog.odmu.edu.ua/>
3. <https://moodle.odmu.edu.ua/login/index.php>

Practical class № 13

Topic: Structure and biological role of nucleosides, nucleotides. Structure and biological role of nucleic acids

Relevance of the topic: Nucleic acids are natural biopolymers, monomers of which are mononucleotides. Mononucleotides are composed of heterocyclic bases of pyrimidine or purine series linked to a carbohydrate fragment esterified with orthophosphoric acid by N-nucleoside bond. Nucleic acids play a major role in the transmission of genetic information and in the control of protein synthesis..

Objective: to form and consolidate knowledge of the principles of structure, chemical properties of nucleic acids and their monomers - nucleotides to understand their biosynthesis and biological role in the body.

Basic concepts: nucleotide, nucleoside, nucleic acid, DNA, RNA.

Equipment: Laboratory of the department

Plan and organizational structure of the class:

1. Nucleosides and nucleotides as products of incomplete hydrolysis of nucleic acids. Nucleosides as medicinal products.

2. Structure of nucleotides - complex components of nucleic acids (AMP, GMP, UMP, CMP, TMP). Structure and importance of 3',5' - c-AMP, its role in the action of hormones on cells.

3. Phosphorylated derivatives of nucleotides, biological significance of ADP and ATP. Participation of nucleotides in the structure of coenzymes. Mechanism of action of coenzyme NAD.

4. Nucleic acids - polynucleotides, biopolymers that store, transmit hereditary information and participate in protein biosynthesis.

5. Types of RNA: m-RNA, r-RNA, t-RNA, their structural organization and biological role.

6. Structure and biochemical functions of DNA. Differences in the structure and functions of DNA and RNA.

The higher education applicant should know:

1. Features of the structure of pyrimidine and purine bases.

2. Cyclo-oxo-tautomerism of carbohydrates: D-ribose and 2-deoxy-D-ribose.

3. Formation of glycosidic bonds.

4. Nature and properties of glycosidic and ester bonds, their relation to the hydrolysis reaction.

5. Lactim-lactam tautomerism of heterocyclic bases.

Content of the practical class

NUCLEOTIDES AND NUCLEIC ACIDS

Nucleic acids, in addition to polysaccharides and proteins, represent the third type of biopolymers. They occur in all living systems, playing an exclusive role in the biosynthesis of proteins and in the transmission of hereditary characteristics. The nucleic acids are the chemical carriers of the genetic code, which prescribes the specific amino acid sequences in proteins.

Two types of nucleic acids, namely *ribonucleic acids* (RNA) and *deoxyribonucleic acids* (DNA), differ in their structure and biological functions. In this chapter, the main attention will be paid to the structure and some chemical properties for better understanding biochemical behaviour of these biopolymers.

1. CONSTITUENTS OF NUCLEIC ACIDS

Just as polysaccharides are polymers made of monosaccharide units and as proteins are polymers made of amino acid units, nucleic acids are high molecular compounds that consist of building blocks called *nucleotides*. Nucleotides can be produced on enzyme-catalyzed hydrolysis of a nucleic acid (Fig. 1). The nucleotide, in its turn, can be hydrolyzed to yield a *nucleoside* and phosphoric acid. Each nucleoside can finally be cleaved into a sugar (pentose) and a pyrimidine or a purine nucleic base.

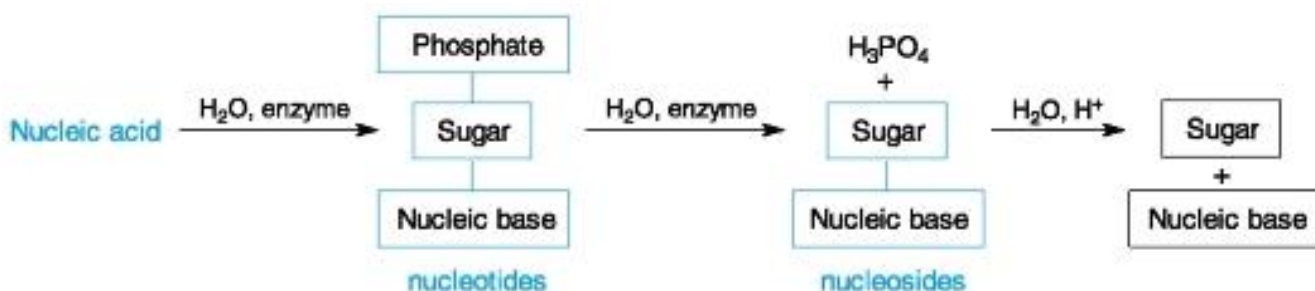
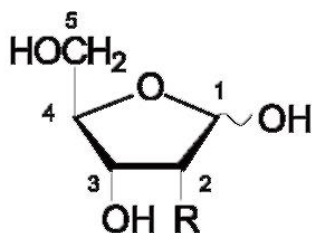


Figure 1. The principal scheme of splitting of nucleic acids. The rectangles designate constituents of a product.

The sugar component of RNA is D-ribose, whereas DNA contains 2-deoxy-D-ribose, which lacks the hydroxyl group at C-2 (the names *ribose* and *deoxyribose* will be used hereafter). Both pentoses are in the furanose form.



D-ribose (R = OH)

2-deoxy-D-ribose (R = H)

These terms are usually used in the singular, but they designate a great number of various molecules of RNA or DNA.

1.1. Structure of Nucleosides and Nucleotides

Nucleic bases represent the hydroxy or (and) amino derivatives of purine or pyrimidine. The most common nucleic bases are adenine, guanine, and cytosine (from both RNA and DNA), uracil (from RNA), and thymine (from DNA). For convenience they are often abbreviated to their three initial letters as shown below. Thus each type of nucleic acids involves four nucleic bases, and DNA differs from RNA not only in its sugar content but also in one nucleic base (thymine instead of uracil, the former is the 5-methyl analogue of the latter).



Note the hydroxy derivatives are exclusively presented in the lactam tautomeric form.

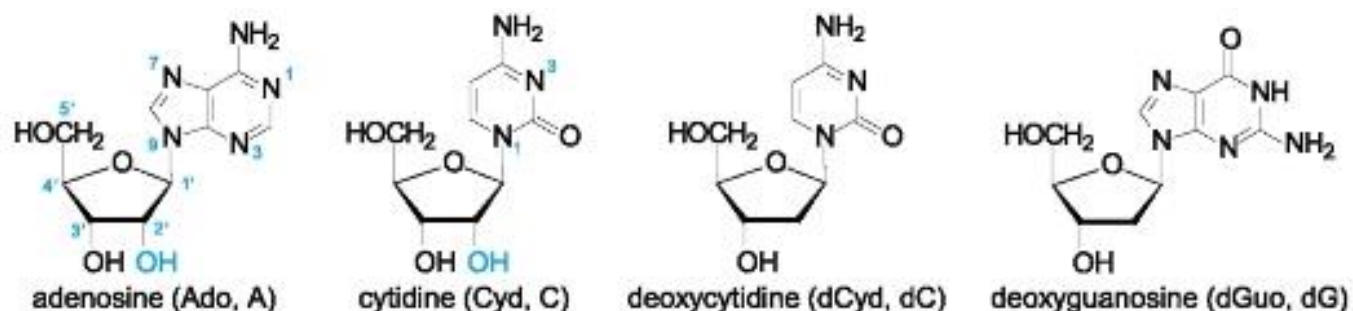
In nucleic acids, a nucleic base is connected to the anomeric carbon of the sugar by N-glycosidic bond to form a nucleoside.

Nucleosides are N-glycosides of nucleic bases and D-ribose or 2-deoxy-D-ribose.

The pyrimidine bases are linked at the N-1 atom and the purine bases at the N-9 atom; the configuration of the glycosidic bond is β in all naturally occurring nucleosides.

Nucleosides are named by replacing the suffixine in the name of the corresponding nucleic base with the suffixosine for the purine nucleosides or -idine for the pyrimidine nucleosides. Nucleosides containing deoxyribose get the prefix deoxy- to the name of the respective ribonucleoside. Nucleoside constituents are numbered independently like their components, except that primes are added to the numbers for the sugar unit.

For a long time, thymine nucleosides were named with some deviation from the above rule. Thus, the name «thymidine» was used for the deoxyriboside (since thymine is a component of DNA only) and the name «ribothymidine» meant the corresponding riboside. This exception was recently abandoned but the old names can be encountered in the literature.

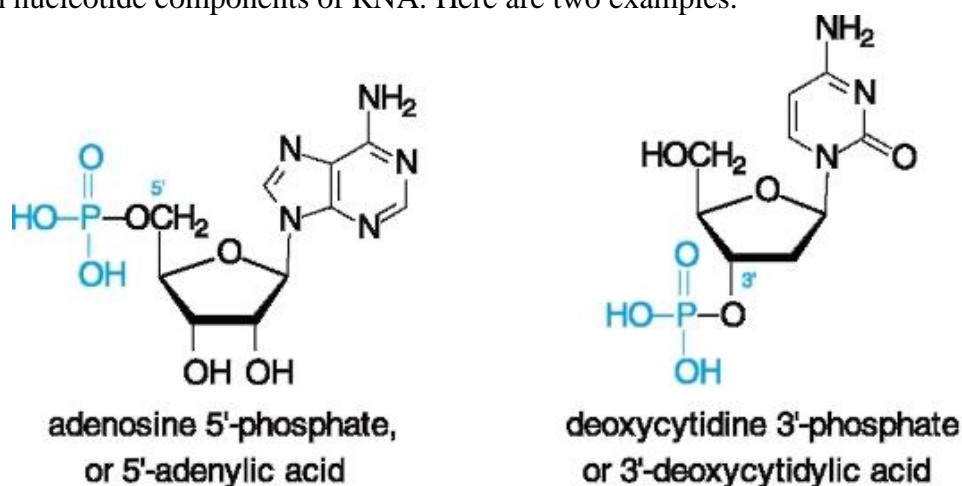


A three-letter abbreviations of nucleosides differ from those of nucleic bases in the last letter as shown in the examples above. One-letter symbols (A, C, G, T, U) are used for nucleoside *residues* in more complex structures. The letter d should be added in front to the abbreviation of deoxyribonucleosides.

Polymeric chains of nucleic acids are composed of phosphorylated nucleosides. Such nucleoside derivatives are also components of nucleotide coenzymes.

Nucleotides are phosphate esters of nucleosides.

Three ribonucleoside phosphates and two deoxyribonucleoside phosphates are possible according to the numbers of hydroxyl groups in the ribose unit. But only hydroxyls at C-3' and C-5' are esterified in nucleotide components of RNA. Here are two examples:



Nucleotides are named as the 3'- or 5'-phosphate esters of a nucleoside. Alternative names use the ending *-ylic acid* in place of *-ine* in the name of the parent nucleic base, as shown above. Nucleotide names are often abbreviated like corresponding nucleosides with additional small letter **p**, which is placed in front for 5'-phosphates and after the one-letter abbreviation for 3'-phosphates. In the biochemical literature, the abbreviation MP¹ stands for 5'-monophosphate, for example, AMP means adenosine 5'-phosphate. The complete names of nucleosides and nucleotides are given in Table 1.

Table 1.

Names of nucleosides and nucleotides

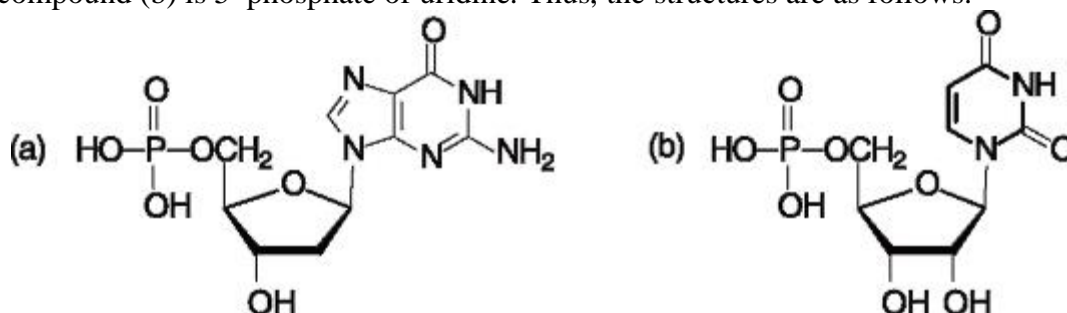
Nucleic base	Source	Nucleoside	Nucleotide		
			name	abbreviations	
Adenine	RNA	Adenosine	Adenosine 5'-phosphate	pA	AMP
	DNA	Deoxyadenosine	Deoxyadenosine 5'-phosphate	pdA	dAMP
Guanine	RNA	Guanosine	Guanosine 5'-phosphate	pG	GMP
	DNA	Deoxyguanosine	Deoxyguanosine 5'-phosphate	pdG	dGMP
Cytosine	RNA	Cytidine	Cytidine 5'-phosphate	pC	CMP
	DNA	Deoxycytidine	Deoxycytidine 5'-phosphate	pdC	dCMP
Uracil	RNA	Uridine	Uridine 5'-phosphate	pU	UMP
Thymine	DNA	Deoxythymidine	Deoxythymidine 5'-phosphate	pdT	dTMP

As we will see later, some nucleotides are diphosphates (esters of diphosphoric acid) or triphosphates (esters of triphosphoric acid) that are abbreviated DP or TP, respectively.

Example 1. Draw the structures for (a) dGMP and (b) 5'-uridylic acid.

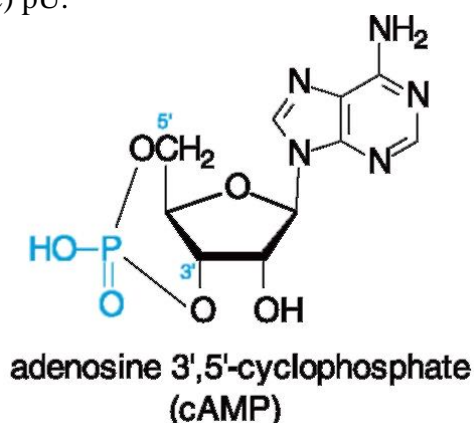
Solution. The letter *d* in the compound (a) indicates that this is a deoxynucleotide. The letter G which stands for the base is guanine, and the MP designates a monophosphate.

The compound (b) is 5'-phosphate of uridine. Thus, the structures are as follows:



Nucleosides can form cyclic phosphates, compounds in which two hydroxyls of the pentose (usually at C-3' and C-5') are esterified simultaneously by one molecule of phosphoric acid. Such cyclic phosphates are not, of course, components of nucleic acids but some of them are important in biological processes. For example, adenosine 3',5'-cyclophosphate is a mediator of a certain hormonal activity.

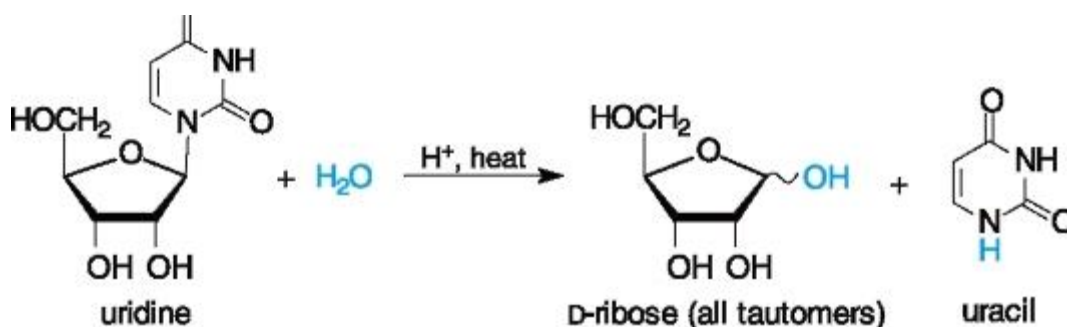
Problem 1. Draw the full structures of the following and give full names for the abbreviated compounds: (a) uridine; (b) dA; (c) pU.



1.2. Some Chemical Properties of Nucleosides and Nucleotides

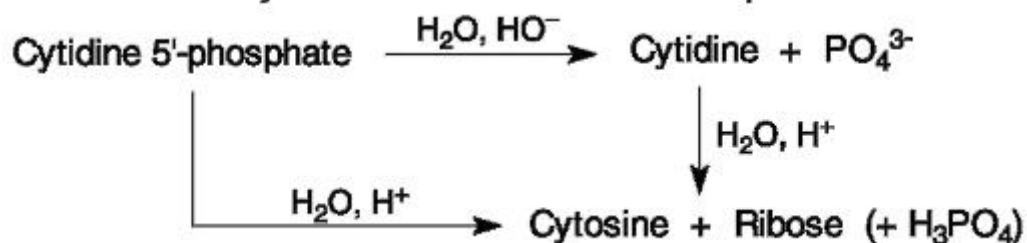
Hydrolysis.

Like other glycosides, nucleosides are quite stable in an alkaline medium but they can be hydrolyzed in an acidic medium or by enzymes to the pentose sugar and the nucleic base, for example:



It should be noticed that N-glycosides are more resistant to the acid-catalyzed hydrolysis than ordinary O-glycosides and that deoxyribonucleosides undergo splitting much easier than ribonucleosides. Besides, deoxyribose is subjected to further transformation under acidic conditions and cannot be identified in hydrolysis products.

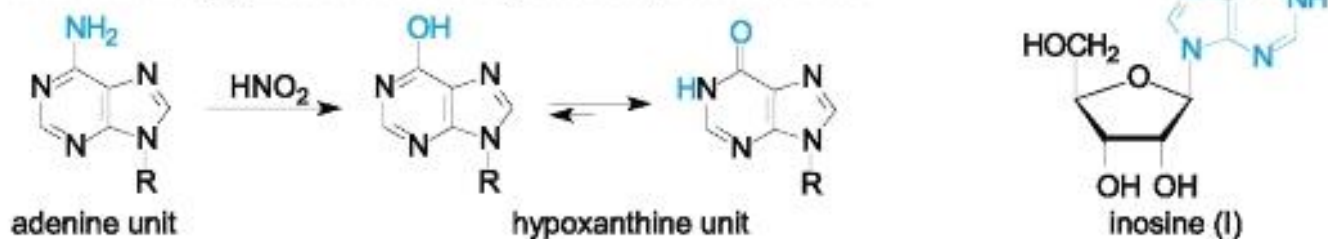
Complete acidic hydrolysis of nucleotides result in splitting both the phosphate ester bond and N-glycosidic bond (with the above mentioned limitation concerning deoxyribose). Nucleotides can also be hydrolyzed stepwise, first to a nucleoside and then to nucleoside components as shown above. The first step is performed under alkaline conditions that retain the N-glycosidic bond unaffected. These transformations are illustrated only with the names of compounds:



Problem 2. Write structural formulas for all compounds in the above scheme.

Modification of nucleic bases. Three nucleic bases – adenine, guanine, and cytosine - contain an amino group and, like amines, can react with nitrous acid. When, for example, an adenine fragment is treated with nitrous acid, it is converted into a hypoxanthine derivative. Similarly, guanine is deaminated to give xanthine (2,6-dihydroxypurine), and cytosine is converted into uracil. By the way, hypoxanthine is a nucleic base of some intact RNA's; its nucleoside has the trivial name inosine (abbreviated I).

R is a remaining part of a nucleoside, nucleotide, or nucleic acid



If deamination reaction takes place in living cells, it results in changing structure of nucleic acids thus affecting the genetic system. In other words, this reaction causes mutations, and nitrous acid is one of the most potent chemical mutagens.

Other known reactions that involve various functional groups of nucleic bases can be used for modification. These are alkylation, acylation, halogenation, oxidation, reactions with aldehydes and hydrazine. When applied to nucleic acids, these modifications help in determination of biopolymer structure and in genetic investigations.

2. PRIMARY STRUCTURE OF NUCLEIC ACIDS

Nucleotides link together in nucleic acids by forming a phosphate ester bond between the phosphate group of one nucleotide and the hydroxyl group of a sugar unit (ribose or deoxyribose) of another nucleotide. The overall structure of the nucleic acid is a macromolecule with a backbone of

pentose components alternated with phosphate groups and with a nucleic base attached to each sugar unit (Fig. 2). The backbone of a DNA molecule differs from that of an RNA only in the absence of the -OH group at C-2 of the ribose unit. The end of the polymeric chain that has a free hydroxyl at C-5' is called the *5' end*, and the other end (with a free OH at C-3') is called the *3' end*. Each end of the chain can be phosphorylated.

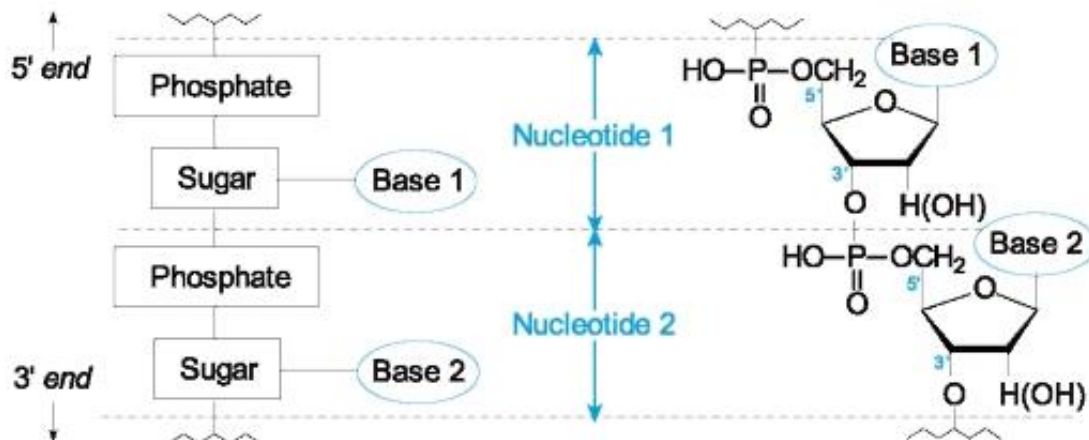
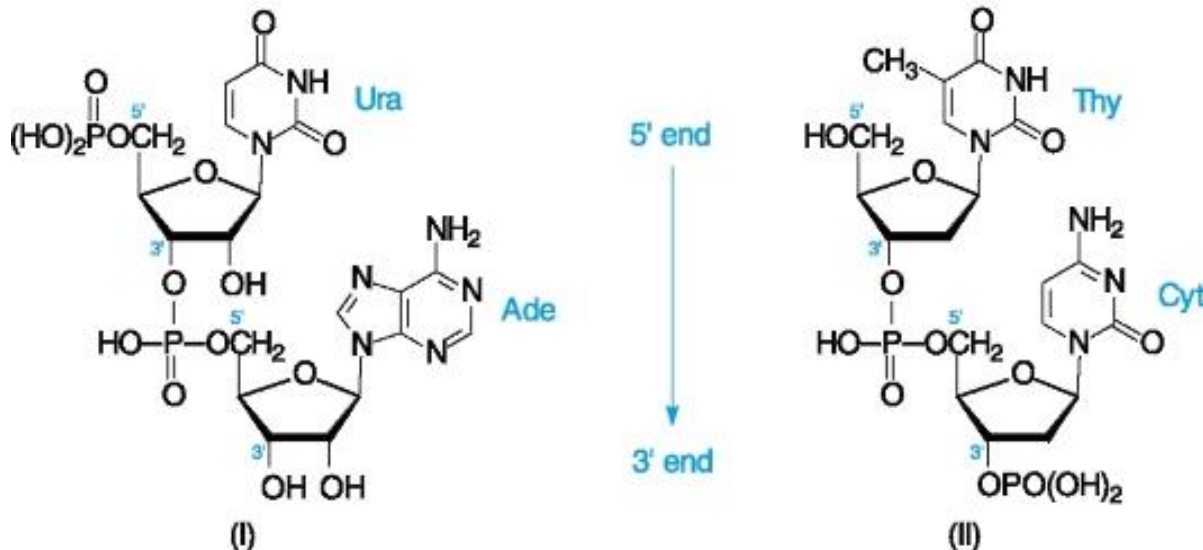


Figure 2. Generalized structure of nucleic acids.

Thus, RNA and DNA differ slightly in their backbones and nucleic base contents. Like proteins, each nucleic acid has a specific sequence of monomeric units in a polymeric chain.

A sequence in which nucleotides are bound in a chain is the primary structure of nucleic acids.

The nucleotide sequence in an oligoor polynucleotide is written by starting at the 5' end and identifying the nucleic bases in the direction of the 3' end. For convenience, all abbreviations given above may be used. Two examples illustrate this mode of representation. Thus, the dinucleotide (I) may be written in a short form as pUpA, and the dinucleotide (II) as d(TpCp).



For longer nucleotide chains, the letter *p* may be used only once designating phosphate group on either 5' or 3' end. The letter *d* may also be omitted when it is obvious that a nucleotide is a component of DNA (remember that thymine is present only in DNA). Thus, the above compound (I) may simply be written as pU-A, and the compound (II) as TpCp or T-Cp.

Problem 3. Draw the full structure of the trinucleotide U-C-Gp.

The problem of sequencing nucleic acids is much more difficult than that of sequencing proteins because of extremely high molecular mass (or long chain) of most nucleic acids. The shortest of nucleic acids is *transfer RNA* (tRNA) that consists of about 70 to 95 nucleotide units, whereas the smallest DNA contains over 5,000 nucleotide units. The primary structures of hundreds of tRNA have become known since 1965. Further progress in nucleic acid sequencing has been striking.

The DNA sequencing is a powerful method applied in forensic medicine. Most of human DNA varies extremely from person to person and is characteristic of the individual (except for identical twins). The method known as *DNA profiling*, or DNA fingerprinting, is, indeed, similar to ordinary fingerprinting since it allows identifying a person. A paramount importance of the method is that it needs a negligible amount of the DNA sample (a few micrograms).

A discussion of the genetic code is not the purpose of this book (this is the object of biochemistry).

3. SECONDARY STRUCTURE OF NUCLEIC ACIDS

The notion expressed in the heading is principally similar to that of proteins.

The secondary structure of nucleic acids is a spatial organization of a macromolecule that is defined by hydrogen bonding between nucleotide components of a chain (or chains).

Despite the fact that DNA molecules are much longer than that of RNA, the spatial arrangement of DNA was established first. A result of great importance in deducing the secondary structure of DNA was the finding that the ratio of adenine to thymine and of guanine to cytosine was very close to unity. On the basis of this fact and other physical evidence, J. Watson and F. Crick in 1953 made a revolutionary but now a classical proposal for the secondary structure of DNA supported shortly after by the X-ray data of M. Wilkins. The most important features of the Watson-Crick model are the following:

- DNA consists of two polynucleotide chains (strands) coiled around a common axis in a *double helix*;
- the two strands are right-handed and run in opposite directions with regard to their 3' and 5' ends;
- the nucleic bases lie inside the helix and form *complementary pairs* by strong hydrogen bonds to each other; adenine is always paired with thymine, and guanine is always paired with cytosine;
- the helix is about 2.0 nm in diameter; the pitch of the helix is 3.4 nm and exactly ten nucleotide pairs are in each full turn.

Fig. 3 illustrates the above features the main of which is complementarity of the nucleic bases: A-T and G-C. This means when an adenine residue occurs in one strand, a thymine residue is opposite in the other strand. Likewise, whenever a guanine base occurs in one strand, a cytosine base appears opposite in the other strand. And vice versa. Thus the two strands of the double helix are complementary but not identical; they are said to be *anti-parallel*.

The American biochemist James D. Watson, the English biochemist Francis H.C. Crick, and the English biophysicist Maurice H.F. Wilkins won the Nobel Prize for physiology and medicine (1962) for this discovery and establishing the hereditary role of DNA.

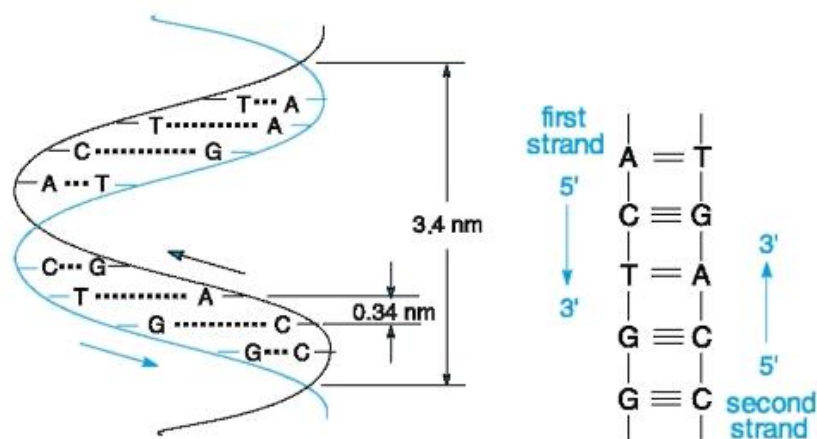


Figure 3. Schematic representations of the DNA double helix. The letters A, C, G, and T designate bases but not nucleosides. In the left drawing the dots indicate hydrogen bonds.

The A-T pair form two hydrogen bonds and the G-C pair is joined by three hydrogen bonds (Fig. 4). The geometry of both pairs is almost identical. Note that acidic sites in the hydrogen bonding

are either an NH₂ group or NH fragment of a lactam form of thymine and guanine, while basic sites are either the pyridine-type nitrogen or the oxygen atom.

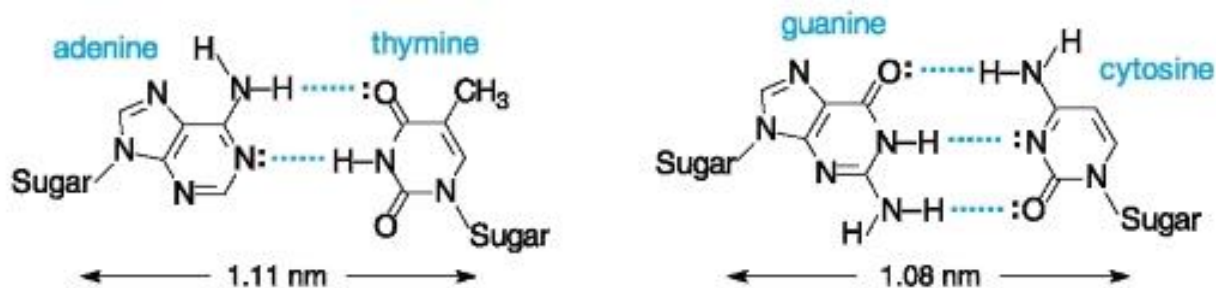


Figure 4. Hydrogen bonding in pairs A-T (two bonds) and C-G (three bonds).

Problem 4. What nucleotide sequence on one strand of DNA is complementary to the sequence ATACCTG (written from 5' to 3') on the other strand?

The principal difference in the structure of RNA is that they consist of only one polynucleotide chain. RNA has less rigid spatial arrangement when compared to that of DNA. Transfer RNA is the most studied of all RNA's because of its low molecular weight. The secondary structures of many tRNA's have been determined but it is too complicated to be presented here.

A typical tRNA has roughly the shape of a cloverleaf, as shown in Fig. 5 for tRNA that transports the amino acid alanine. The molecule is folded into several loops or stems by means of base pairing along the chain. Note that adenine forms the complementary pair A-U, which has the same geometry as the pair A-T in DNA.

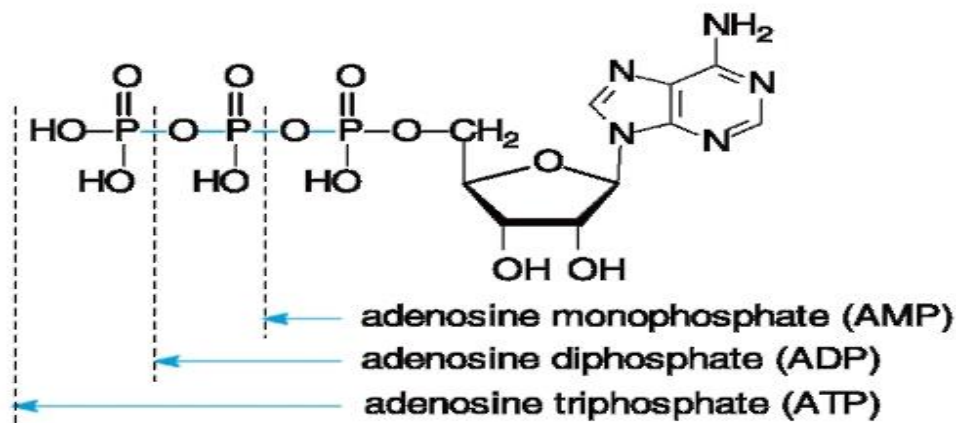
One stem of the molecule always terminates in the sequence CCA to which a specific amino acid is bonded through the free 3'-OH group of the terminal adenosine. Each tRNA also contains in one of the loops a sequence of three nucleotides (triplet) called an *anticodon*. The latter is highly important because it allows the tRNA to bind with the complementary nucleotide triplet of mRNA called a *codon*. Thus each codon (and anticodon) corresponds to only one amino acid constituting a genetic message.

4. NUCLEOSIDE PHOSPHATES IN BIOLOGICAL PROCESSES

Nucleotides are important not only because of their participation in biopolymers (RNA and DNA) but also because of other biological functions such as energy storage (ATP and ADP) and enzyme cofactors (NAD⁺, NADH, their phosphates, acetyl coenzyme A, and others).

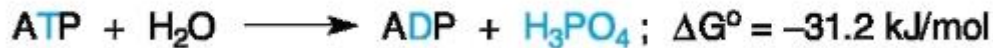
4.1. Nucleoside Polyphosphates.

In living cells nucleosides exist in several different phosphate forms. The most important are the 5'-phosphates of adenosine - AMP, ADP, and ATP, shown below¹:



The bond P-O in AMP is a familiar ester bond, while the bonds P-O-P in ADP and ATP (coloured in the above structures) are phosphoric anhydride bonds. When the anhydride bond is hydrolyzed, a large amount of energy is released (compare the value given below with -12.5 kJ/mol

for hydrolysis of the ester bond in aMp). For this reason, ATP and ADP are referred to as *high energy* compounds.



The biosynthesis of nucleoside triphosphates proceeds by phosphorylation of the low energy monoor diphosphate precursors. For example, ATP may be synthesized from ADP with either inorganic phosphate or another high energy triphosphate such as guanosine triphosphate (GTP). The energy liberated during oxidation of glucose and other substrates is used in the endothermic process.



ATP can transfer its potential energy to various biochemically important compounds. For example, the initial step in the glycolysis process involves phosphorylation of glucose that results in the formation of glucose 6-phosphate.

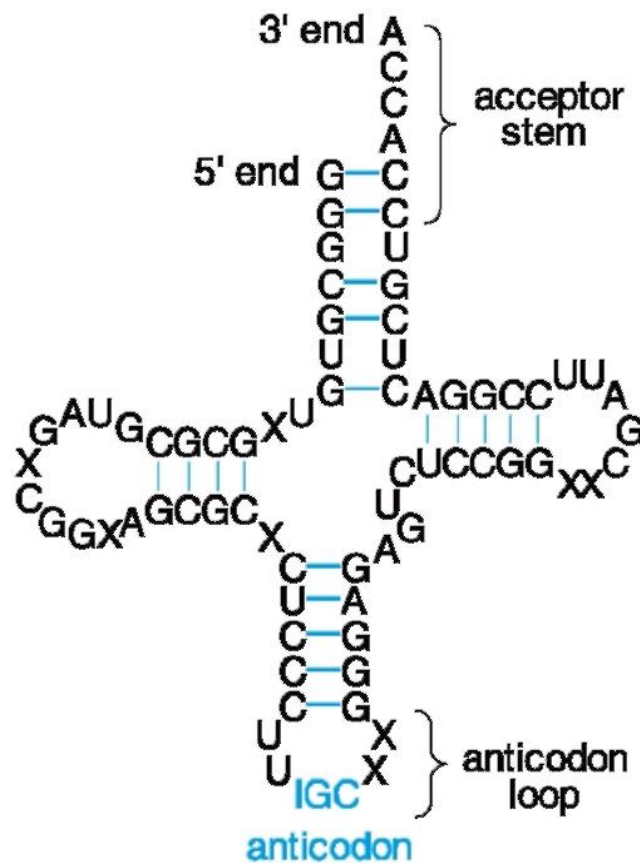
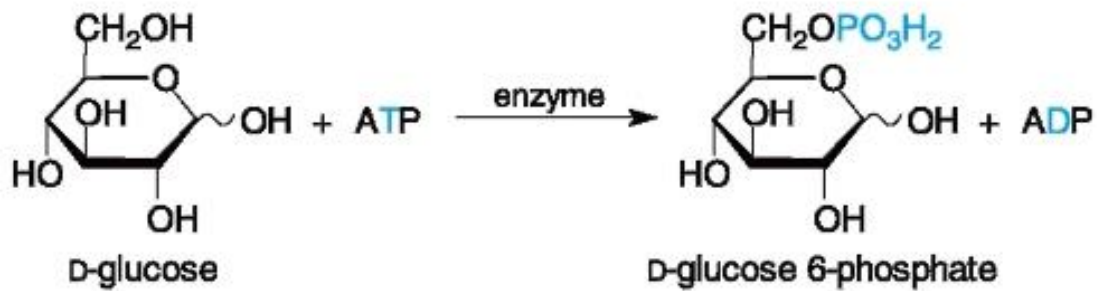
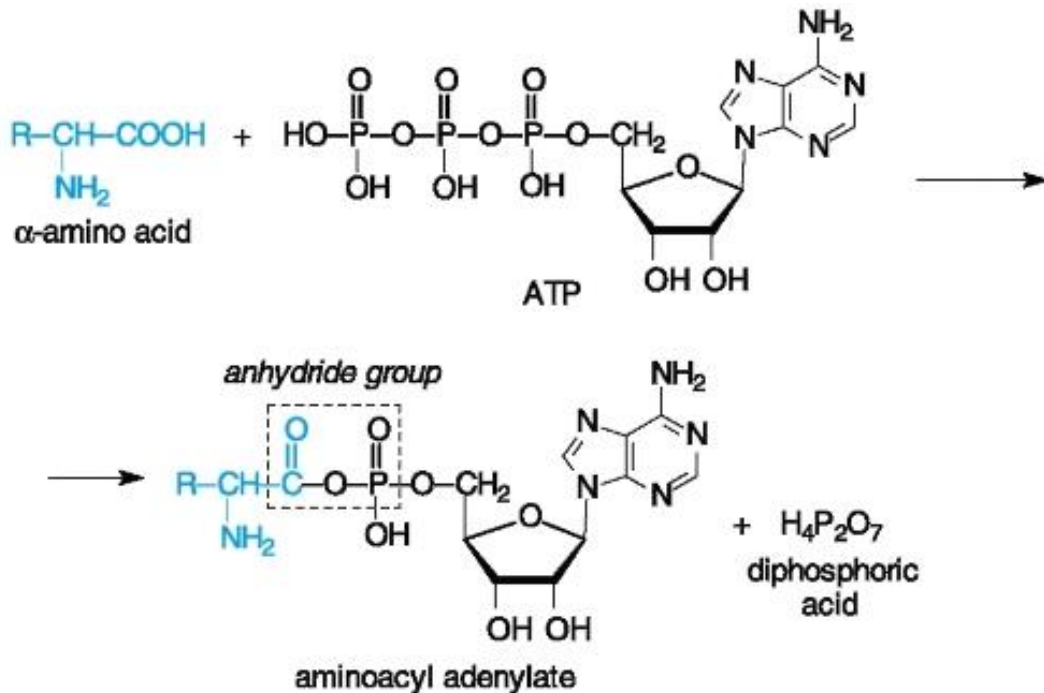


Figure 5. Structure of alanine tRNA from yeast. X's designate other nucleosides with unusual bases. Hydrogen bonds are shown by coloured dashes.

¹ In the biochemical literature, nucleoside phosphates, as well as other organic phosphates, are represented in an ionized form since they lose at least one hydroxylic proton at physiological pH (about 7).



A further example demonstrates the energy transfer from ATP to an α -amino acid in the form of an energy-rich anhydride bond. Phosphorylation of the amino acid results in the formation of a mixed anhydride called an *aminoacyl adenylate*, thus making the amino acid more reactive in protein biosynthesis.



The aminoacyl adenylate then reacts by acylation with the 3'-OH group at the 3'-terminal nucleotide of the specific tRNA (Fig. 4). Finally, the linked amino acid is transported to the ribosome where a peptide bond is synthesized.

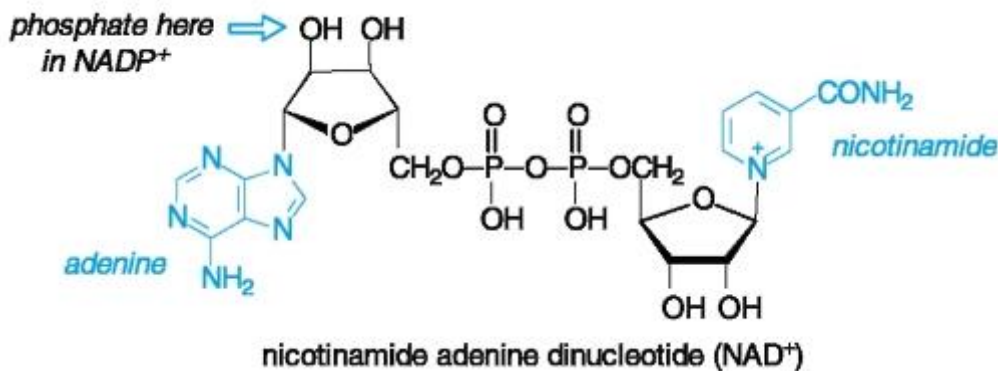


Problem 5. Which structural features are present in the alanyl adenylate molecule?

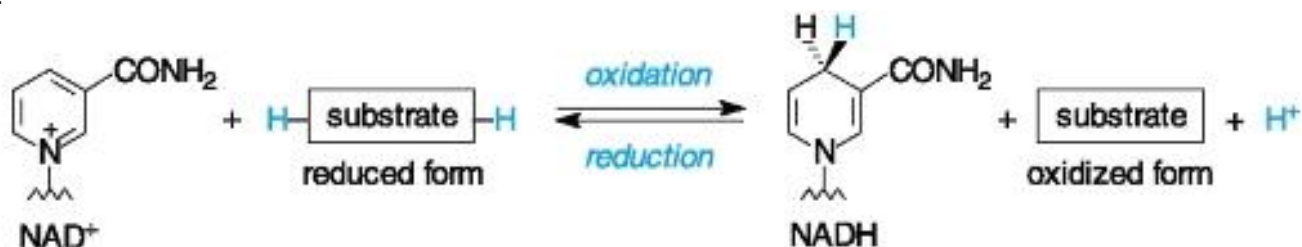
- (a) an amide bond;
- (b) an ester bond;
- (c) an *N*-glycosidic bond;
- (d) an anhydride group;
- (e) a pyrimidine nucleic base.

4.2. Nucleotide Coenzymes

Several coenzymes, that contain nucleotides in their structures, participate in various biochemical processes. Nicotinamide adenine dinucleotide (NAD⁺) and its phosphate (NADP⁺) are coenzymes involved in oxidation-reduction reactions.

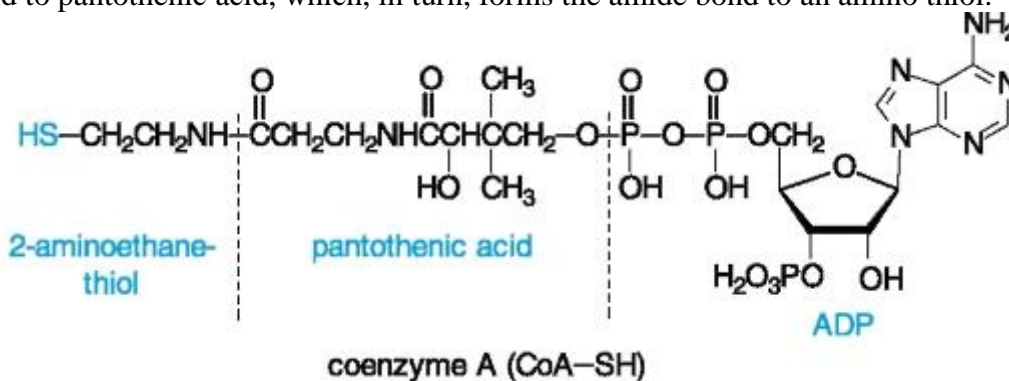


The function of NAD⁺ and NADP⁺ as oxidants consists in formal abstraction of the hydride ion, H⁻, from an organic substrate by the pyridinium ion of the nicotinamide part. In the reaction catalyzed by an enzyme *dehydrogenase*, both the coenzymes are converted to their reduced forms, NADH or NADPH, in which the pyridinium unit is reduced to 1,4-dihydropyridine. The reverse reaction occurs when NADH or NADPH reduces a substrate (only a changeable part of the coenzymes is shown below):

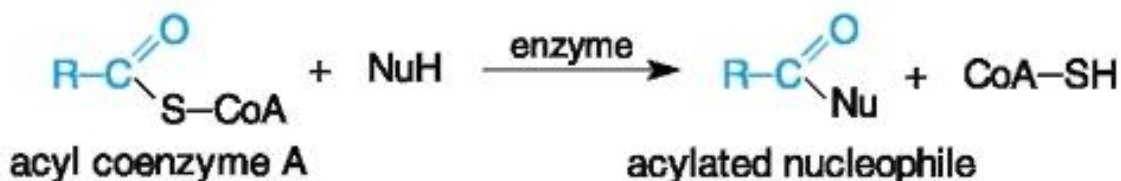


The most typical reactions of this kind such as oxidation of alcohols and hydroxy acids to corresponding carbonyl compounds, as well as reduction of a carbonyl group to hydroxyl group.

Coenzyme A (CoA-SH or simply CoA) is another example of the complex nucleotide coenzymes. Its molecule consists of three parts: ADP (with additional phosphate group), linked by the ester bond to pantothenic acid, which, in turn, forms the amide bond to an amino thiol.



The important part of this rather complicated structure is the SH group that can be converted to thioester group to give an acylated derivative of the formula RC(O)SCoA. The latter reacts with many nucleophiles transferring an acyl group, as shown in the general equation:



The most important CoA derivative is acetyl coenzyme A, which serves as a universal acetyl-transfer agent in the cell. It is also a donor of a twocarbon block in the aldol and Claisen condensations.

Control materials for the final stage of the class.

Questions to check the final level of knowledge:

1. Features of the structure of nucleotides.
2. Features of the structure of nucleosides.
3. Structural components of nucleic acids.
4. Levels of structural organization of nucleic acids.
5. DNA and RNA.

Literature

Basic:

1. Biological and Bioorganic Chemistry: Bioorganic Chemistry: textbook / B.S. Zimenkovsky, V.A. Muzychenko, I.V. Nizhenkovska, G.O. Syrova. — 3rd edition – 2020. – 288 p.
2. Biological and Bioorganic Chemistry. Biological Chemistry: textbook / Yu.I. Gubsky, I.V. Nizhenkovska, M.M. Korda et al. — 2nd edition – 2021 – 544 p.
3. Bioorganic Chemistry. Rineyskaya O.N. textbook. – 2018. – 174 p.
4. Baynes J., Dominiczak M. Medical Biochemistry. 5th Edition. Elsevier, 2018. 712 p.

Additional:

1. Satyanarayana U. Biochemistry. 5th edition. India 2020. – 777 p.
2. Lehninger. Principles of Biochemistry. 7th edition. NY, United States. 2017.
3. William Marshall, Marta Lapsley, Andrew Day, Kate Shipman. Clinical Chemistry. Elsevier, 2020. 432 p.

Електронні інформаційні ресурси:

1. <https://info.odmu.edu.ua/chair/biology/>-
2. <http://libblog.odmu.edu.ua/>
3. <https://moodle.odmu.edu.ua/login/index.php>

Practical class №14

Topic: General characteristics, properties of enzymes. The protein nature of enzymes. Active, allosteric enzyme centers. Regulation of enzyme activity. Enzyme activators and inhibitors. Coenzymes, their role in catalysis. Coenzyme functions of vitamins. International classification and nomenclature of enzymes. Proenzymes. Activation of proenzymes, role in metabolism.

Goal: Learning basic laboratory methodsdetermination of the structure of enzymes

Basic concepts:enzymes, amino acids, peptide, ester, glycosidic bonds, hydrolysis, food digestion

Equipment: Laboratory of the department

Plan:

1. Organizational activities (greetings, verification of those present, announcement of the topic, purpose of the class, motivation of higher education seekers to study the topic).
2. Control of the reference level of knowledge.

The higher education applicant should know:

- the chemical nature of enzymes, their structure, biological role and basic properties of proteins as biocatalysts;
- types of chemical reactions that form the basis of the International Classification of Enzymes and the principles of their nomenclature;

- characteristics of individual classes of enzymes and give examples of each class of enzymes;
- meaning of four-digit codes of enzymes;
- specificity and types of enzyme specificity;
- the role of active and allosteric centers in the action of enzymes;
- principles of enzyme activity regulation;
- the mechanism of action of activators and inhibitors on the activity of enzymes (for example, amylase of saliva);
- diagnostic value of determination of amylase activity in urine (for pancreatitis).

The higher education applicant should be able to:

- determine the chemical nature of enzymes using reactions to proteins and amino acids
- determine the activity of amylase (diastase) in biological fluids (saliva and urine) in the presence of activators and inhibitors;
- trace and evaluate the action of amylase activators and inhibitors.

Questions to check basic knowledge on the topic of the class:

- Stages of development of the teaching about enzymes
- The role of domestic scientists in enzymology
- Chemical nature of enzymes
- Evidence of the chemical structure of enzymes
- Types of connection in the structure of proteins
- Levels of structure in protein molecules
- Principles of protein detection in solutions
- What are the main types of chemical reactions you know from the bioorganic chemistry course
- Which compounds are characterized by peptide, ester, and glycosidic bonds
- Chemistry of the biuret reaction
- Foley reaction principle
- What determines the speed of chemical reactions
- Factors affecting the rate of an enzymatic reaction
- Activation energy of chemical reactions
- Mechanism of acceleration of chemical reactions by enzymes

3. Formation of professional skills and abilities.

3.1 Demonstration and practical work «Determining the structure of the enzyme using the biuret reaction».

Recommendations for performing tasks.

Principle of the method: discovery of peptide bonds in proteins and peptides. These substances form a red-violet complex with copper sulfate in an alkaline environment.

Procedure: Pour 0.5 ml of the test solution into a test tube, add 0.5 ml of 10% NaOH solution and 1-2 drops of 1% CuSO₄ solution and mix. In the presence of protein, a red-purple color appears.

Conclusion: the appearance of a red-violet color indicates the presence of peptide bonds in the enzyme molecule, that is, its protein nature.

Requirements for work results.

Enter the obtained data and calculations into the workbook.

Make medical and biological conclusions.

3.2 Demonstration and practical work "Foley's reaction to sulfur-containing amino acids".

Recommendations for performing tasks.

The principle of the method: when boiling with alkalis, sulfur, which is separated from sulfur-containing amino acids, forms sodium sulfide, which with lead acetate gives a dark precipitate of lead sulfide.

Procedure: 5-6 drops of 30% NaOH solution and 1-2 drops of lead acetate are added to 0.5 ml of the test solution. The contents of the test tube are boiled. With a positive reaction to sulfur, the liquid in the test tube darkens.

Conclusion: the structure of the enzyme contains sulfur-containing amino acids.

Requirements for work results.

Enter the obtained data and calculations into the workbook.

Make medical and biological conclusions.

3.3 Demonstration and practical work "Determination of salivary amylase activity in the presence of activator and inhibitor".

Recommendations for performing tasks.

The principle of the method: the amylase activity of saliva and urine is determined by the amount of substrate (starch) broken down by 1 ml of saliva or urine over a set period of time (30 minutes), and is based on finding the maximum dissolution at which the investigated liquid breaks down starch to the stage of red coloration with iodine (formation of erythroextrins). Sodium chloride accelerates the cleavage of starch under the action of amylase. Copper sulfate slows down the action of amylase. Normally, human urine contains little amylase. The amount of amylase in urine increases with pancreatitis, parotitis, orchitis.

Progress:

a) Preparation of a number of dilutions of saliva. Prepare three rows of tubes of 10 each. Pour 1 ml of distilled water from a burette into all test tubes. Add 1 ml of saliva, diluted 10 times, to test tubes under №1. Stir the liquid. Transfer 1 ml of the resulting saliva solution to test tube #2. Stir. Transfer 1 ml of liquid from the test tubes under №2 to the test tube under №3, etc. Pour 1 ml of liquid from test tubes under №10. 3 rows of tubes with diluted saliva were obtained, in each subsequent tube the concentration of the enzyme is 2 times lower than in the previous one.

b) Addition of activator and inhibitor. Add 1 ml of water to 10 test tubes of the 1st row. This series will serve as a control against which amylase activity in the presence of activator and inhibitor is compared. Add 1 ml to the second row of test tubes. 0.85% NaCl solution, which is an amylase activator. In the third row of test tubes, add 1 ml of CuSO₄ solution, which inhibits the action of amylase.

c) Preparation of enzyme-substrate mixture. Pour 2 ml of starch solution into all test tubes and mix quickly. Start with test tube 10, that is, with the highest dilution of saliva, where the enzyme concentration is the lowest.

d) Incubation. Place all 30 tubes for 30 minutes in a water bath at 37°C. Add 2 drops of iodine (Lugol's solution) to each test tube, mix and observe the color range from yellow to blue.

Conclusion: In the test tube, where the liquid is blue, the cleavage of starch did not occur. Sufficient cleavage occurs, for example, in test tube №5, where saliva is diluted 320 times, that is, 1/320 ml of saliva splits 2 ml of 0.1% starch solution, and 1 ml of undiluted saliva splits X ml of starch.

$$X = 320 \cdot \frac{2}{1} = 640 \text{ од.}$$

This is depicted as:

$$A \text{ (амілазна активність)} = \frac{37^0}{30} = 640 \text{ од.}$$

Requirements for work results.

Enter the obtained data and calculations into the workbook.

Make medical and biological conclusions.

Control materials for the final stage of the class.

Questions to check the final level of knowledge:

- Common and divergent structures of simple and complex enzymes
- Chemical nature of cofactor, apoenzyme, holoenzyme
- Enzyme activators
- Enzyme inhibitors. Reversible and irreversible inhibition
- Allosteric centers of enzymes
- The nature of allosteric effectors
- Methods of determination of enzyme activity
- Enzyme activity units
- The structure of the active center of enzymes
- Chemistry of determination of amylase activity in biological fluids

Test tasks.

1 What are enzymes?

- a) inorganic cell catalysts
- c) organic compounds that can trigger new reactions
- c) inhibitors of chemical reactions
- +d) biological catalysts of protein nature
- e) reaction substrates

2 What is catalysis?

- a) inhibition of the rate of chemical reactions
- c) acceleration of chemical reactions contrary to the laws of thermodynamics
- +c) change in the speed of chemical reactions under the influence of catalysts
- d) the ability of enzymes to initiate reactions contrary to the laws of thermodynamics
- f) the course of a chemical reaction without an enzyme

3 What causes the high specificity of enzymes?

- +a) the unique structure of the apoenzyme
- c) the unique structure of the coenzyme
- c) conformational and electrostatic complementarity between the substrate and the enzyme
- d) conformational and electrostatic complementarity between coenzyme and apoenzyme
- f) the unique structure of the cofactor

4 What is the absolute specificity of an enzyme?

- +a) the ability to catalyze the transformation of only one substrate
- c) the ability to catalyze the breaking of one specific bond
- c) the ability to catalyze the transformation of one specific group of substrates
- d) the ability to catalyze the transformation of all substrates
- e) the ability to catalyze the transformation of the general group of stereoisomers

5 What is the relative specificity of enzymes?

- a) the ability to catalyze the transformation of a certain group of substrates
- c) the ability to catalyze the transformation of only one substrate
- +c) the ability to catalyze the transformation of a group of substrates with a certain type of

bonds,

- d) the ability to catalyze the transformation of the general group of stereoisomers
- f) decrease in enzyme activity

6 What is an apoenzyme?

- a) vitamins
- +c) protein part of complex enzymes
- c) hormones
- d) enzyme cofactors
- f) nucleotides

7 What is the prosthetic group of a two-component enzyme?

- a) non-protein part of the enzyme that easily dissociates from the complex with the apoenzyme

- c) vitamins
 - c) the protein part of the enzyme
 - +d) non-protein part of the enzyme, which is strongly (covalently) connected to the apoenzyme
 - f) hormones
- 8 What are the main functions of apoenzyme?
- a) enhances the catalytic activity of the non-protein part of the enzyme
 - +c) determines the specificity of enzyme action
 - c) participates in the act of catalysis and stabilizes the enzyme
 - d) is responsible for the connection between the coenzyme and the substrate
 - f) is in direct contact with the substrate
- 9 What is the active site of an enzyme?
- a) active region of the non-protein part of the enzyme
 - +c) part of the enzyme molecule on which the substrate is converted
 - c) a unique combination of active sites of apo- and coenzyme
 - d) the region of the apoenzyme responsible for contact with the substrate
 - f) coenzyme site
- 10 What temperature is optimal for the action of most enzymes?
- a) 28-32 °C
 - +c) 37-43 °C
 - c) 45-50 °C
 - d) 50-60 °C
 - f) 90-100 °C
- 11 What is the optimal pH value for the action of pepsin?
- a) pH 4.5-5.0
 - c) pH 6.8-7.0
 - c) pH 7.5-8.5
 - +d) pH 1.5-2.5
 - e) pH 9.3 10.5
- 12 What criterion is the basis for the classification of enzymes?
- a) chemical nature of enzymes
 - c) chemical nature of substrates
 - +c) type of catalyzed reaction
 - d) the type of chemical bond for which the enzymatic reaction is carried out
 - f) chemical nature of inhibitors
- 13 How many classes are enzymes divided into?
- a) by four
 - +c) by six
 - c) by twelve
 - d) by two
 - f) by nine
- 14 What reactions are catalyzed by oxidoreductases?
- a) transfer of groups, radicals, etc.
 - c) dehydration
 - +c) redox
 - d) isomerization
 - f) synthesis
- 15 Which enzymes belong to the second class?
- a) aldolase, aminotransferase, lipase
 - +c) aminotransferase, phosphotransferase, CoA-transferase
 - c) pepsin, trypsin, carbonic anhydrase
 - d) aspartate decarboxylase, ATPase, fumarate hydratase
 - e) hexokinase, pepsin, pyruvate decarboxylase

- 16 How are enzymes classified according to modern classification and nomenclature?
- +a) for classes, subclasses, subsubclasses, where each enzyme has a serial number
 - c) on classes and subclasses, where each enzyme has a serial number
 - c) into groups and subgroups, where each enzyme has a serial number
 - d) into types and subtypes, where each enzyme has a serial number
 - f) only for subclasses

4. Summing up.

5. List of recommended literature (main, additional, electronic information resources):

Main:

1. Gubsky Yu.I., I.V. Nizhenkovska, Korda M.M. Biological and Bioorganic Chemistry: in 2 books. Book 2. Biological Chemistry: textbook. 2021. 544 p.
2. Satyanarayana U. Biochemistry. 5th edition. India 2020. 777 p.
3. Lehninger. Principles of Biochemistry. 7th edition. NY, United States. 2017.
4. Jeremy M. Berg, John L. Tymoczko, Gregory J. Gatto. Biochemistry. 8th Revised edition. 2015.
5. Lippincott Illustrated Reviews: Biochemistry. Philadelphia :Wolters Kluwer, 2017. 560 p.
6. Baynes J., Dominiczak M. Medical Biochemistry. 5th Edition. Elsevier, 2018. 712 p.
7. Donald Voet, Judith G. Voet, Charlott W. Pratt. Fundamentals of Biochemistry: Life at the Molecular Level. ISBN: 978-1-118-91840-1 February 2016, 1184 P.

Additional:

1. William Marshall, Marta Lapsley, Andrew Day, Kate Shipman. Clinical Chemistry. Elsevier, 2020. 432 p.
2. Harper's Illustrated Biochemistry / V.W. Rodwell, D.A. Bender, K.M. Botham et al. – Mc Graw Hill Education, 2015. – 817 p.

Electronic information resources:

1. [https://info.odmu.edu.ua/chair/biology/-](https://info.odmu.edu.ua/chair/biology/)
2. <http://libblog.odmu.edu.ua/>
3. <https://moodle.odmu.edu.ua/login/index.php>

Practical class №15

Topic: Basic theories of biocatalysis. Kinetics of catalysis. Methods of qualitative and quantitative determination of enzymes. Intracellular localization of enzymes. Use of enzymes in the clinic (fundamentals of medical enzymology). Enzyme diagnostics and enzyme therapy. Enzymopathies.

Goal: Study of the basics of the theory of biocatalysis, kinetics of enzymatic reactions and the use of these data in medical enzymology

Basic concepts: biocatalysis, dissociation constant, Michaelis-Menten constant, dependence of enzymatic reaction rate on substrate concentration, isoenzymes, enzyme diagnostics, enzyme therapy, hereditary enzymopathies

Equipment: Laboratory of the department

Plan:

1. Organizational activities (greetings, verification of those present, announcement of the topic, purpose of the class, motivation of higher education seekers to study the topic).
2. Control of the reference level of knowledge.

The higher education applicant should know:

- cellular organization and interrelationship of enzymes;
- peculiarities of action of individual forms of enzymes and their importance in tissue metabolism;
- the role of isozyme spectra in metabolism and their significance in enzymodiagnosics;
- basics of enzyme diagnostics and enzyme therapy;
- enzyme systems that are damaged in the most common enzyme diseases.

The higher education applicant should be able to:

- to isolate cell organelles and blood components by centrifugation;
- make an assumption about a possible pathology based on the analysis of enzyme activity.

Questions to check basic knowledge on the topic of the class:

- Cellular organization of enzymes;
- The role of membranes in biocatalysis;
- Enzymodiagnosics and its role in medical practice;
- Enzyme therapy and its significance in medicine;
- Isoenzymes, their importance in biocatalysis;
- Organ specificity of LDH isozymes;
- The principle of detection of LDH isozymes.



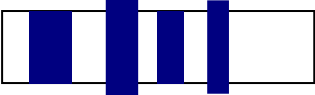

3. Formation of professional skills and abilities.

3.1 Evaluation of blood enzymograms of healthy and patients with diseases of the cardiovascular system and liver.

Recommendations for performing tasks.

Principle of the method: As a result of differences in the primary structure of the apoenzyme, isoenzymes have different affinity for the substrate and different physicochemical properties, in particular, mobility in an electric field. Therefore, they are separated by electrophoresis in an agar or polyacrylamide gel, incubated in a substrate-coenzyme mixture, and then stained using special dyes.

Procedure: 0.01 ml of biological fluid (blood serum, homogenate) is applied to the strip of polyacrylamide gel, the gel is placed in an electric field for 2 hours, and then incubated in a mixture containing buffer, substrate, coenzyme, phenazine metasulfate and nitroblue tetrazolium. After incubation, the electrophoregram is subjected to densitometry.

LDH isozyme spectrum of skeletal muscles	
LDH isoenzyme spectrum of the myocardium	
Isoenzyme spectrum of serum LDH in liver lesions	
Isoenzyme spectrum of LDH in blood serum during myocardial infarction	

Conclusion: The LDH isoenzyme spectrum of skeletal muscles and myocardium is significantly different: LDH1 and LDH2, which function in aerobic conditions, prevail in the myocardium, and LDH5 and LDH4, which function in anaerobic conditions, prevail in skeletal muscles. When various

organs are damaged, LDH isoenzymes are released into the blood, which prevail in the affected tissue due to impaired permeability of plasma membranes.

Requirements for work results.

Enter the obtained data into the workbook.

Make medical and biological conclusions.

3.2 Demonstration and practical work "Observation of the kinetics of lipase action on milk fat. The influence of bile on lipase activity".

Recommendations for performing tasks.

Principle of the method: The speed of the enzymatic reaction can be determined by the amount of substrate split per unit of time. To study the kinetics of the reaction in separate portions of the enzyme-substrate mixture, which contains fat and lipase, the amount of acids formed is determined by determining the time interval. The results of the determination are expressed graphically. The graph shows that the hydrolysis of fat proceeds quickly in the first 15 minutes of incubation, then stops completely at the end. This course of the process is caused by a constant decrease in the amount of the substrate and an increase in the cleavage products. When bile is added to the sample, lipase is activated, and fat hydrolysis proceeds at a faster rate.

Progress:

a) Preparation of enzyme-substrate mixture. Measure 10 ml of boiled diluted milk 1:10 and 1 ml of extract from the pancreas, which contains lipase, into 2 chemical beakers of 50 ml each. Add 1 ml of water to one of the glasses, bile to the second. Mix the liquid in the glasses. Take 2 ml of the mixture from each glass, transfer it to a 50 ml flask, add 1-2 drops of phenolphthalein solution, titrate with a 0.01 N solution to a faint pink color. Mark the volume (ml). Write in the table.

b) Incubation with sampling for 15-30-45 minutes. Place the mixture left in the glass in a water bath at a temperature of 37°C. Every 15 minutes, take 2 ml of the mixture and titrate with 0.01 N NaOH solution. Record the titration time and amount of alkali in the table.

c) Graphic representation of fat hydrolysis. From the obtained data for determining the amount of alkali neutralized by acids formed from milk at different stages of incubation, plot a graph, put the time of incubation on the abscissa axis, in min. (15-45) on the ordinate axis - volume of alkali (ml) of each sample.

Incubation time (min.)	Volume of alkali used for titration (ml)	
	Sample without bile	Bile test
0		
15		
30		
45		

Medical and biological evaluation of the obtained results.

In a sample with bile, fat hydrolysis proceeds faster than without it. Based on the evaluation of the curves on the graph, the activating role of bile was noted in the conclusions.

Requirements for work results.

Enter the obtained data and calculations into the workbook.

Make medical and biological conclusions.

3.3 Demonstration and practical work "Observation of the kinetics of lipase action on milk fat. The influence of bile on lipase activity".

Recommendations for performing tasks.

Principle of the method: The speed of the enzymatic reaction can be determined by the amount of substrate split per unit of time. To study the kinetics of the reaction in separate portions of the enzyme-substrate mixture, which contains fat and lipase, the amount of acids formed is determined by determining the time interval. The results of the determination are expressed graphically. The graph shows that the hydrolysis of fat proceeds quickly in the first 15 minutes of incubation, then

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Incubation time (min.)	Volume of alkali used for titration (ml)	
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0		
15		
30		
45		

Medical and biological evaluation of the obtained results.

In a sample with bile, fat hydrolysis proceeds faster than without it. Based on the evaluation of the curves on the graph, the activating role of bile was noted in the conclusions.

Requirements for work results.

Enter the obtained data and calculations into the workbook.

Make medical and biological conclusions.

Control materials for the final stage of the class.

Questions to check the final level of knowledge:

- Compartmentalization of enzymes
- Enzymopathies of carbohydrate metabolism
- Enzymopathies of amino acid metabolism
- Enzymatic parameters that can be used to detect myocardial damage
- Enzyme indicators that can be used to detect liver damage
- Enzyme diagnosis of muscular dystrophies
- Enzyme therapy for hereditary pathology

Test tasks.

1 What is catalysis?

- a) inhibition of the rate of chemical reactions
- c) acceleration of chemical reactions contrary to the laws of thermodynamics
- +c) change in the speed of chemical reactions under the influence of catalysts
- d) the ability of enzymes to initiate reactions contrary to the laws of thermodynamics
- f) the course of a chemical reaction without an enzyme

2 What temperature is optimal for the action of most enzymes?

- a) 28-32 °C
- +c) 37 – 43 °C
- c) 45-50 °C

d) 50-60 °C

f) 90-100 °C

3 What is the optimal pH value for the action of pepsin?

a) pH 4.5-5.0

c) pH 6.8-7.0

c) pH 7.5-8.5

+d) pH 1.5-2.5

f) pH 9.3-10.5

4 What is the Michaelis constant?

a) a value equal to the concentration of the enzyme at which the reaction rate is maximal

c) ratio of enzyme and substrate concentrations

c) a value equal to the concentration of the reaction product at which the reaction rate is half of the maximum

+d) a value equal to the concentration of the substrate at which the reaction rate is half the maximum

e) a value equal to the concentration of the enzyme at which the reaction rate is half the maximum

5 What mechanisms of lowering the energy barrier occur during enzymatic reactions?

a) formation of additional covalent bonds between apo- and coenzyme

+c) formation of an intermediate enzyme-substrate complex

c) participation of macroergic compounds in enzymatic catalysis

d) reducing the area of the contact area between the enzyme and the substrate

f) participation of additional compounds in enzymatic catalysis

6 What are the stages of the catalytic action of enzymes?

a) initial and terminal

c) preparatory, initial, working and terminal

+c) attachment of the substrate to the enzyme, conversion of the substrate, cleavage of the final products of the reaction from the enzyme

d) activation of the substrate-enzyme complex of substrate conversion, cleavage of the modified enzyme from the substrate or reaction product

e) preparatory and terminal

7 The child was diagnosed with galactosemia. The concentration of glucose in the blood does not change significantly. Deficiency of which enzyme causes this disease?

+a) galactose-1-phosphate-uridylyltransferases

c) amyl-1,6-glucosidases

c) phosphoglucomutase

d) galactokinase

e) glucokinase

8 Cataract (clouding of the lens), mental retardation, enlarged liver, galactosemia, galactosuria were found in the sick child. Which enzyme is deficient in the child's body?

+a) galactose-1-phosphate-uridylyltransferases

c) galactokinase

c) sorbitol dehydrogenase

d) phosphoglucomutase

f) amyl-1,6-glucosidases

9 In a patient with acute pancreatitis, the activity of one of the specified enzymes is sharply increased in the blood and urine, which confirms the diagnosis of the disease:

+a) α -amylase

c) pepsin

c) dipeptidase

d) sucrase

e) hexokinase

10 Name the enzyme, the determination of which in the blood is the most informative in the first hours after the occurrence of a myocardial infarction:

- +a) creatine phosphokinase
- c) alanine aminotransferase
- c) pyruvate decarboxylase
- d) diastasis
- f) dihydrolipoyltransacetylase

11 Name the enzyme whose determination in the blood is the most informative in Botkin's disease:

- a) creatine phosphokinase
- +c) ornithine carbomoyltransferase
- c) lactate dehydrogenase
- d) diastasis
- f) pepsin

12 Trasilol and Kontrikal are used to treat pancreatitis. What biochemical processes do these drugs affect?

- +a) reduce the activity of proteinases
- c) increase the activity of proteinases
- c) increase amylase activity
- d) reduce amylase activity
- f) increase the activity of amylo-1,6-glucosidase

13 Congenital oligophrenia is accompanied by inhibition of the conversion of phenylalanine into tyrosine. A sign of the disease is the accumulation of organic acids in the body:

- +a) Phenylpyruvate
- c) Lemon
- c) Pyruvinogradnoi
- d) Dairy
- f) Glutamine

14 An increase in LDH activity is characteristic of heart and liver diseases. What additional research should be conducted to clarify the localization of the pathological process?

- +a) Determination of LDH isozymes
- c) Determination of amylase activity
- c) Determination of alkaline phosphatase activity
- d) Determination of creatine kinase isoenzymes
- f) Determination of aminotransferase activity

15 An increase in the activity of which enzyme can be observed in a patient with an early stage of muscular dystrophy:

- +a) Creatine kinases
- c) Alanine aminotransferases
- c) Collagenases
- d) Hyaluronidase
- f) Glutaminase

16. The patient was diagnosed with toxic hepatitis, which arose against the background of the use of drugs. The activity of which serum enzymes should be determined to confirm this diagnosis?

- +a) Ornithine carbomoyltransferases
- c) Creatine phosphokinase
- c) Pyruvate dehydrogenase
- d) Maltases
- f) Malate dehydrogenase

17 When prostate cancer metastasizes to other tissues, acid phosphatase activity increases in the serum. What class does this enzyme belong to?

- +a) Hydrolases

- c) Oxidoreductases
- c) Transferases
- d) Lyases
- f) Synthetases

18 The patient complains of pain in the chest area that is not relieved by nitroglycerin, weakness, increased sweating. He has cyanosis of the lips, pallor of the skin, and bradycardia. 4 hours have passed since the beginning of the anginal attack. Determining the activity of which enzyme will make it possible to make a diagnosis of myocardial infarction?

- +a) Creatine kinases (MV)
- c) LDH4
- c) LDH5
- d) ASAT
- f) AIAT

19 The patient, 55 years old, was hospitalized in the infectious department with a diagnosis of viral hepatitis. An increase in the activity of which organ-specific liver enzyme will be observed in the patient's blood serum?

- +a) Sorbitol dehydrogenase
- c) Acid phosphatases
- c) Lactate dehydrogenase
- d) Cholinesterases
- f) Creatine kinases

20 The temperature at which the enzyme denatures:

- a) 0 °C
- +c) 80-100 °C
- c) 20-30 °C
- d) 30-40 °C
- e) 92 °C

4. Summary:

5. List of recommended literature (main, additional, electronic information resources):

Main:

1. Gubsky Yu.I., I.V. Nizhenkovska, Korda M.M. Biological and Bioorganic Chemistry: in 2 books. Book 2. Biological Chemistry: textbook. 2021. 544 p.
2. Satyanarayana U. Biochemistry. 5th edition. India 2020. 777 p.
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7. Donald Voet, Judith G. Voet, Charlott W. Pratt. Fundamentals of Biochemistry: Life at the Molecular Level. ISBN: 978-1-118-91840-1 February 2016, 1184 P.

Additional:

1. William Marshall, Marta Lapsley, Andrew Day, Kate Shipman. Clinical Chemistry. Elsevier, 2020. 432 p.
2. Harper's Illustrated Biochemistry / V.W. Rodwell, D.A. Bender, K.M. Botham et al. – Mc Graw Hill Education, 2015. – 817 p.

Electronic information resources:

1. [https://info.odmu.edu.ua/chair/biology/-](https://info.odmu.edu.ua/chair/biology/)
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3. <https://moodle.odmu.edu.ua/login/index.php>

Practical class №16

Topic: Specific and general pathways of catabolism. Citric acid cycle (CAC). Sequence of reactions and characteristics of enzymes. Biological significance of CAC. Bioenergetics of significance. Anaplerotic and amphibolic reactions of CAC.

Goal: to study the general ways of catabolism of biomolecules in living cells, as well as the sequence of reactions and the biological significance of the cycle of tricarboxylic acids as a universal way of oxidative catabolism of biomolecules

Basic concepts: metabolism, catabolism, anabolism, CTC enzymes, anaplerotic reactions, amphibolic reactions

Equipment: Laboratory of the department

Plan:

1. Organizational activities (greetings, verification of those present, announcement of the topic, purpose of the class, motivation of higher education seekers to study the topic).
2. Control of the reference level of knowledge.

The higher education applicant should know:

- interpret patterns of biochemical metabolic features of catabolic, anabolic and amphibolic metabolic pathways;
- to analyze the regularities of the functioning of the tricarboxylic acid cycle and the mechanism of its regulation;
- explain the structure and significance of the pyruvate and α -ketoglutarate dehydrogenase complex;
- to reveal the essence and meaning of anaplerotic reactions of CTC.

The higher education applicant should be able to:

- write the reactions of the cycle of tricarboxylic acids;
- calculate the energy balance of the CTC;
- give examples of anaplerotic and amphibolic reactions of CTC.

Questions to check basic knowledge on the topic of the class:

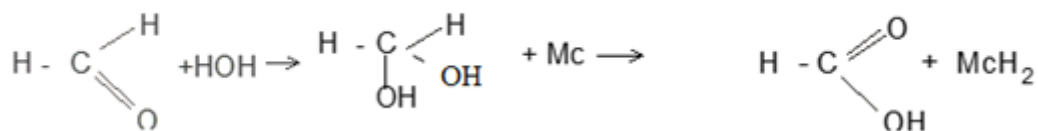
- Write the formulas of pyruvic, α -ketoglutaric and oxalic-acetic acids.
- Write the formulas of citric, isocitric, cis-aconic and oxalic-succinic acids.
- Write the formulas of succinic, fumaric and malic acids.

3. Formation of professional skills and abilities.

3.1 Demonstration and practical work «Detection of milk dehydrogenase».

Recommendations for performing tasks.

The principle of the method is that if you take formaldehyde as the oxidation substrate (hydrogen donor) and methylene blue as the hydrogen acceptor, then under the action of milk dehydrogenase, formic aldehyde will be oxidized by removing hydrogen, which is attached to methylene blue, being restored to a colorless compound. In the form of a scheme of reactions that take place in this case, it can be depicted as follows:



Reagents:

1. 1% formaldehyde solution.
2. Methylene blue solution.
3. Milk.

Equipment:

1. Test tubes.
2. Pipettes.
3. Electric tiles.

The main stages of the work:

- a) pour 4-5 ml of milk into 2 test tubes;
- b) boil the contents of the second test tube;
- c) add 8-10 drops of formaldehyde solution and 1-2 drops of methylene blue solution to both test tubes;
- d) mix the contents of both test tubes, place them in a water bath (at 37°C).

After a few minutes, observe the coloration of methylene blue in the first tube and its absence in the second tube.

Requirements for work results.

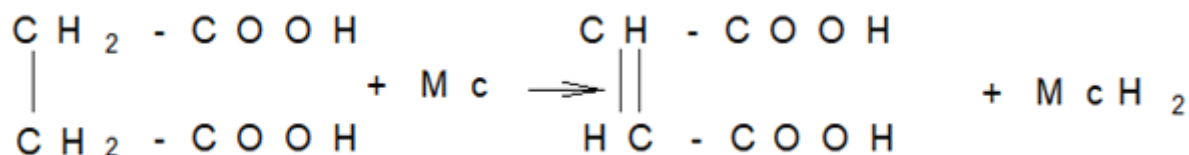
Enter the obtained data and calculations into the workbook.

Make medical and biological conclusions.

3.2 Demonstration and practical work "Detection of succinate dehydrogenase in muscles".

Recommendations for performing tasks.

The principle of the method is that succinate dehydrogenase catalyzes the oxidation of succinic acid to fumaric acid. In the presence of succinate dehydrogenase, succinic acid and methylene blue, the oxidation of succinic acid and the reduction of methylene blue occur. At the same time, the latter is discolored. The reaction proceeds according to the following scheme:



Reagents:

1. Muscle porridge.
2. Neutralized solution of succinic acid.
3. Methylene blue solution.

Equipment:

1. Test tubes.
2. Pipettes.
3. Electric tiles.

The main stages of the work:

- a) pour 4-5 ml of muscle slurry (muscle homogenate) into 2 test tubes. Add 0.5 ml of neutralized succinic acid solution to the first test tube. Add 2 drops of methylene blue solution to both test tubes;
- b) mix the contents of both test tubes and place them in a water bath (at 37°C).

After a few minutes, observe the discoloration of methylene blue in the 1st test tube and its absence in the 2nd test tube. After decolorization, shake the 1st test tube strongly, the blue color will appear again due to the oxidation of methylene blue.

Requirements for work results.

Enter the obtained data into the workbook.
Make medical and biological conclusions.

Control materials for the final stage of the class.

Questions to check the final level of knowledge:

1. Exchange of substances (metabolism) - general patterns of catabolic and anabolic processes.
2. Common stages of intracellular catabolism of biomolecules: proteins, carbohydrates, lipids.
3. Cycle of tricarboxylic acids. Localization, sequence of enzymatic reactions, significance in metabolism.
4. Energy balance of the cycle of tricarboxylic acids. Physiological significance of CTC reactions.
5. Amphibolic pathways and anaplerotic reactions. Examples.

Test tasks.

- 1 The concentration of which metabolite affects the work of CTC?
 - a) ADP
 - b) Glucose
 - c) Oxaloacetate
 - d) Alanine
 - e) Phospholipases
- 2 In the course of CTC reactions, CO₂ is formed, the amount of which is:
 - a) 2
 - b) 1
 - c) 5
 - d) 3
 - e) 4
- 3 In the reactions of CTC, reduced flavin dehydrogenases are formed, which can lead to the synthesis of:
 - a) 2 ATP
 - b) 1 ATP
 - c) 3 ATP
 - d) 5 ATP
 - e) 4 GTF
- 4 In the reactions of CTC, reduced pyrimidine dehydrogenases are formed, which can lead to the synthesis of:
 - a) 9 ATP
 - b) 7 ATP
 - c) 3 ATP
 - d) 5 ATP
 - e) 2 GTF
- 5 CTC reactions ensure the synthesis of macroergic compounds (ATP and GTP). At the same time, the number of these molecules, the synthesis of which is not related to the work of the electron transport chain, is:
 - a) 1
 - b) 7
 - c) 3
 - d) 2
 - e) 5

6 A 10-year-old child has Weber's optic neuropathy, the cause of which is a decrease in the activity of NADH-dehydrogenase of the respiratory chain of mitochondria in the cells of the optic nerve. The development of neuropathy is associated with a violation of which process?

- a) Oxidative phosphorylation
- b) Glycolysis
- c) Cycle of three carboxylic acids
- d) ketogenesis
- e) oxidation of fatty acids

7 The main energy process in the body is the cycle of three carboxylic acids, discovered in the 1930s. Outstanding biochemist, Nobel Prize laureate:

- a) H. Krebsom
- b) D. Sumner
- c) O. Warburg
- d) P. Mitchell
- e) O. Meyerhof

8 During the oxidation of carbohydrates, lipids, and proteins, a large amount of energy is generated, the main part of which is synthesized in the cycle of three carboxylic acids from acetyl-CoA. How many molecules of ATP are formed when one molecule of acetyl-CoA is oxidized?

- a) 12
- b) 24
- c) 38
- d) 1
- e) 3

9 During the oxidation of acetyl-CoA in the cycle of three carboxylic acids, many enzymes, which include non-protein substances, take part. Name the non-protein substance that is necessary for the conversion of succinic acid into fumaric acid:

- a) FAD
- b) OVER
- c) FMN
- d) TPF
- e) Coenzyme Q

10 A decrease in the activity of enzymes of the Krebs cycle, which occurs as a result of the influence of alcohol on the body, can cause the development of hypoxia:

- a) Fabric
- b) Respiratory
- c) Hypoxic
- d) Circulatory
- e) Chemical

11 A low level of which of the metabolites listed below causes inhibition of the Krebs cycle and increased ketogenesis in hepatocytes under the condition of limited utilization of carbohydrates:

- a) Oxaloacetate
- b) Acetyl-CoA
- c) ATP
- d) Fatty acids
- e) ADP

12 Anabolism processes are characterized by:

- a) Breakdown of metabolites into simpler compounds
- b) Convergence of metabolic pathways
- c) Formation of ATP molecules
- d) Formation of various organ- and tissue-specific biomolecules
- e) Formation of joint metabolites

thirteen Uncharacteristic for the second stage of catabolism:

- a) Entry of monomers from the gastrointestinal tract into the blood and tissue cells
- b) Formation of key metabolites
- c) Formation of endogenous water
- d) The release of the energy of chemical bonds in the form of high-energy hydrogen
- e) Entry into cells of monomers that are formed as a result of intracellular catabolism of own carbohydrates, lipids and proteins

14 In the third phase of catabolism:

- a) Completion of cleavage of key metabolites
- b) Formation of final products CO₂ and H₂O
- c) Energy release
- d) Inclusion of the Krebs cycle – a common cyclic metabolic pathway
- e) All answers are correct

15 Substrates of biological oxidation can be:

- a) Starch, glycogen, triacylglycerols, lactose food
- b) Own proteins of blood and tissues
- c) Nucleic acids and other high-molecular compounds
- d) Amino acids, glucose, fatty acids, alcohols, oxyacids, etc.
- e) Cellulose

16 At all stages of biological oxidation:

- a) Catabolic transformation of substrates
- b) The release of the same amount of free energy
- c) Air oxygen consumption
- d) Consumption of carbon dioxide
- e) Release of various amounts of free energy

17 In the Krebs cycle, oxidation undergoes:

- a) A central key metabolite
- b) Glucose
- c) Pyruvic acid
- d) Glycerol
- e) Amino acid

18 The biological functions of CTC are:

- a) Maintenance of the physiological concentration of PVC in the cell
- b) Formation of substrates for gluconeogenesis
- c) Formation of high-energy hydrogen in the form of four portions of reduced cofactors
- d) Formation of endogenous water
- e) Formation of biologically active substances

19 To increase the performance of athlete K.'s training, the doctor recommended taking citric acid or products containing it, because it:

- a) CTC substrate
- b) The source of the starting material for the synthesis of fatty acids
- c) Fatty acid synthesis activator
- d) Activator of gluconeogenesis
- e) Inhibitor of glycolysis

20 A patient with atrophic gastritis has a sharply reduced content of erythrocytes in the blood. The biochemical mechanism of the development of this pathology is a violation of the central nervous system. The concentration of which metabolite affects the work of CTC?

- a) ADP
- b) Glucose
- c) Oxaloacetate
- d) Alanine
- e) Phospholipases

21 A woman with signs of metabolic acidosis was brought to the hospital. In the course of CTC reactions, CO₂ is formed, the amount of which is:

- a) 2
- b) 1
- c) 5
- d) 3
- e) 6

22 The biochemical basis of the increase in the content of ketone bodies in pathological conditions is a decrease in the degree of utilization of acetyl-CoA in the central nervous system due to a violation of carbohydrate metabolism. This is due to the outflow from the CTC:

- a) Oxaloacetate
- b) Ketoglutarate
- c) Fumarate
- d) Malatu
- e) Succinate

23 Which carboxylic acid - an intermediate product of CTC - takes part in the regulation of blood calcium transport level?

- a) Citrate
- b) Isocitrate
- c) Oxaloacetate
- d) Succinate
- e) α -ketoglutarate

24 Which of the components of CTC forms tightly bound complexes with calcium in dentin and takes part in the processes of mineralization and demineralization?

- a) Citrate
- b) Isocitrate
- c) Oxaloacetate
- d) Succinate
- e) Malat

25 The central intermediate product of all exchanges (proteins, lipids, carbohydrates) is:

- a) Acetyl-CoA
- b) Succinyl-CoA
- c) Oxaloacetate
- d) Pyruvate
- e) Citrate

26 A patient suffering from diabetes has ketonuria and ketonemia. Indicate which of the substances listed below is a precursor of ketone bodies?

- a) Acetyl CoA
- b) Oxaloacetate
- c) Unsaturated fatty acids
- d) Alpha-ketoglutarate
- e) Cholesterol

27 In the brain, ammonia, which is formed during the deamination of amino acids and amines, binds to alpha-ketoglutaric and glutamic acids. Therefore, the toxic effect of ammonia on the central nervous system is due to the suppression of:

- a) Cycle of three carboxylic acids
- b) Ornithine cycle of urea formation
- c) Pentose phosphate cycle
- d) Glycolysis
- e) Gluconeogenesis

28 In the human body, CTC reactions provide the synthesis of macroergic compounds (ATP and GTP). At the same time, the number of these molecules, the synthesis of which is connected with the work of the electrolyte transport chain, is:

- a) 1
- b) 3
- c) 5
- d) 2
- e) 4

29 During the oxidation of acetyl-CoA in the CTC, many enzymes, which include non-protein substances, take part. Name the non-protein substance that is necessary for the conversion of succinic acid into fumaric acid.

- a) FAD
- b) OVER
- c) FMN
- d) TPF
- e) Coenzyme Q

30 In the tissues of a healthy person, in the reactions of CTC, reduced pyrimidine dehydrogenases are formed, which can lead to the synthesis of:

- a) 9 ATP
- b) 7 ATP
- c) 3 ATP
- d) 6 ATP
- e) 2 ATP

31 Name the common end product of the second stage of catabolism of carbohydrates, lipids, and proteins:

- a) Acetyl CoA
- b) Pyruvate
- c) Citrate
- d) Acyl-CoA
- e) ATP

32 The biochemical basis of the increase in the number of ketone bodies in pathological conditions is a decrease in the degree of utilization of acetyl-CoA in the central nervous system due to a violation of carbohydrate metabolism. This is due to the leakage from the CTC:

- a) Oxaloacetate
- b) Ketoglutarate
- c) Fumarate
- d) Malatu
- e) Succinate

33 During the oxidation of carbohydrates, lipids, and proteins, a large amount of energy is generated, the main part of which is synthesized in the central nervous system from acetyl-CoA. How many molecules of ATP are formed when one molecule of acetyl-CoA is oxidized:

- a) 12
- b) 24
- c) 36
- d) 4
- e) 2

34 Anaplerotic reactions are:

- a) Reactions that increase the concentration of CTC substrates, forming them from intermediates of other metabolic pathways
- b) Reactions that use CTC substrates for the formation of intermediates necessary for biosynthetic processes
- c) Nucleotide decay reactions

- d) Reactions of heme biosynthesis
- e) Reactions of protein biosynthesis.

4. Summary:

5. List of recommended literature (main, additional, electronic information resources):

Main:

1. Gubsky Yu.I., I.V. Nizhenkovska, Korda M.M. Biological and Bioorganic Chemistry: in 2 books. Book 2. Biological Chemistry: textbook. 2021. 544 p.
2. Satyanarayana U. Biochemistry. 5th edition. India 2020. 777 p.
3. Lehninger. Principles of Biochemistry. 7th edition. NY, United States. 2017.
4. Jeremy M. Berg, John L. Tymoczko, Gregory J. Gatto. Biochemistry. 8th Revised edition. 2015.
5. Lippincott Illustrated Reviews: Biochemistry. Philadelphia :Wolters Kluwer, 2017. 560 p.
6. Baynes J., Dominiczak M. Medical Biochemistry. 5th Edition. Elsevier, 2018. 712 p.
7. Donald Voet, Judith G. Voet, Charlott W. Pratt. Fundamentals of Biochemistry: Life at the Molecular Level. ISBN: 978-1-118-91840-1 February 2016, 1184 P.

Additional:

1. William Marshall, Marta Lapsley, Andrew Day, Kate Shipman. Clinical Chemistry. Elsevier, 2020. 432 p.
2. Harper's Illustrated Biochemistry / V.W. Rodwell, D.A. Bender, K.M. Botham et al. – Mc Graw Hill Education, 2015. – 817 p.

Electronic information resources:

1. [https://info.odmu.edu.ua/chair/biology/-](https://info.odmu.edu.ua/chair/biology/)
2. <http://libblog.odmu.edu.ua/>
3. <https://moodle.odmu.edu.ua/login/index.php>

Practical class №17

Topic: The structure of mitochondria. Molecular organization of Electron transport chain: components; their redox-potential, molecular complexes of the inner mitochondria membranes. Oxidative phosphorylation in the respiratory chain. High energy compounds. Peroxide and microsomal oxidation. Antioxidant system.

Goal: learn the basic principles of the mitochondrial respiratory chain, the role of redox enzymes in tissue respiration and the effect on the electron transport chain of biologically active and toxic substances

Basic concepts: biological oxidation, tissue respiration, oxidative phosphorylation, macroergic compounds

Equipment: Laboratory of the department

Plan:

1. Organizational activities (greetings, verification of those present, announcement of the topic, purpose of the class, motivation of higher education seekers to study the topic).
2. Control of the reference level of knowledge.

Requirements for theoretical readiness of higher education applicants to perform practical classes:

The higher education applicant should know:

- explain the structure of the respiratory chain and the purpose of its main links (enzymes, coenzymes);
- analyze the structure and biological role of the respiratory chain; to explain the formation mechanism and biological role of redox potential in the respiratory chain;
- to analyze the mechanisms of action of medicines, biologically active and toxic substances in the processes of tissue respiration;
- the structure of the atom;
- active forms of oxygen;
- lipid peroxidation processes;
- "lines of defense" against free radicals;
- characteristics of monooxygenase reactions;
- characteristics of the microsomal oxidation system.

The higher education applicant should be able to:

- reproduce the scheme of transfer of protons and electrons in the respiratory chain;
- indicate the conjugation points of oxidation and phosphorylation;
- write reactions of formation of free radicals;
- sequence of microsomal oxidation enzymes.

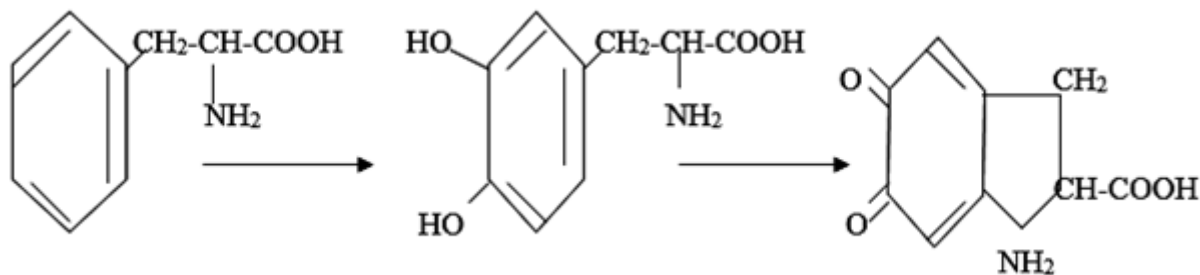
Questions to check basic knowledge on the topic of the class:

1. Write the formula for NADP+
2. Write the formula for ATP.
3. Define free radicals.
4. Localization and components of the microsomal oxidation chain.
5. Write the formula of vitamin PP. Which coenzymes does it include?
6. Write the formula for vitamin B2. Which coenzymes does it include?
7. Write the formula for OVER.
8. Write the formulas of FAD and FMN.
9. Which complex proteins include iron?
3. Formation of professional skills and abilities.

3.1 Demonstration and practical work «Discovery of oxidase (tyrosinase) in potatoes».

Recommendations for performing tasks.

The principle of the method consists in catalyzing oxidation of substrates by free oxygen by oxidases. Tyrosinase is the most typical oxidase, found in potatoes and in animal organisms. It catalyzes the oxidation of tyrosine with its transformation into dark substances similar to pigments (melanins). Oxidation occurs in several stages:



In the human body, tyrosinase catalyzes the transformation of adrenaline into the pigment adrenochrome.

Reagents:

1. Tyrosine solution.
2. Potatoes.

Equipment:

1. Pipettes.

The main stages of the work:

Apply a few drops of tyrosine solution to the potato slice and leave it until the end of the class. A red color is observed on the cut of the potato.

Requirements for work results.

Enter the obtained data into the workbook.

Make medical and biological conclusions.

3.2 Demonstration and practical work "Discovery of peroxidase in horseradish extract".

Recommendations for performing tasks.

The principle of the method consists in peroxidase catalyzing the oxidation of phenols, polyphenols, aromatic amines due to peroxide oxygen. Peroxidases are found in plants (especially abundant in horseradish) and animal organisms. Hemoglobin, myoglobin, cytochromes have weak peroxidase activity.

Reagents:

1. 10% alcohol solution of guava resin.
2. 2% hydrogen peroxide.
3. Extract from horseradish.
4. Distilled water.

Equipment:

1. Test tubes.
2. Pipettes.

The main stages of the work:

a) put 5 drops of 10% alcohol solution of guava resin and 5 drops of 2% hydrogen peroxide into two test tubes;

b) add 5 drops of fresh horseradish extract to the first test tube;

c) add 5 drops of water to the second test tube;

d) mix the contents of the test tubes.

A change in color is observed. Pine resin, being oxidized, acquires a blue color.

Requirements for work results.

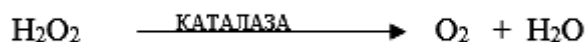
Enter the obtained data into the workbook.

Make medical and biological conclusions.

3.3 Demonstration and practical work "Discovery of catalase in blood".

Recommendations for performing tasks.

The principle of the method consists in catalase of blood catalyzing the decomposition of H₂O₂ into O₂ and H₂O:



The biological role of catalase is to neutralize hydrogen peroxide.

Reagents:

1. Citrate blood.
2. 2% hydrogen peroxide solution.

Equipment:

1. Test tubes.
2. Pipettes.

The main stages of the work:

Pour 10-15 drops of 2% hydrogen peroxide and one drop of blood into the test tube.

Foaming of the liquid due to the violent release of oxygen bubbles is observed.

Requirements for work results.

Enter the obtained data into the workbook.

Make medical and biological conclusions.

3.4 Demonstration and practical work "Quantification of blood catalase (catalase number) according to Bach and Zubkova.

Recommendations for performing tasks.

The catalase number is the number of milligrams of H₂O₂ that is decomposed by one microliter of the examined blood (1 μl = 1 mm³).

The principle of determining the catalase number is based on the following reaction:



that is, the amount of decomposed peroxide is judged by the difference in the amount of potassium permanganate spent on titration before and after the action of catalase.

The main stages of the work:

Preparation of the basic blood solution (1:1000). Pour about 10 ml of distilled water into a 100 ml measuring flask. Add 0.1 ml of the tested blood to the flask with a micro pipette, having previously wiped the tip of the capillary from the blood that has stuck to the outside. Rinse the pipette with liquid from the volumetric flask. Add distilled water to the volumetric flask up to the mark. Note the time when the blood was diluted.

Preparation of enzyme-substrate mixture. Pour 7-8 ml of distilled water into two conical flasks. Add 1 ml of the basic blood solution to both flasks. Boil for 2 minutes. the contents of the first flask for the destruction of catalase. Let both flasks stand at room temperature for 30 minutes, counting from the moment of blood dilution.

Incubation at room temperature. Add exactly 2 ml of H₂O₂ solution to each flask and leave again for 30 minutes.

The interaction of the remaining peroxide with sulfuric acid. Add 4-5 ml of H₂SO₄ solution to each flask.

Determination of the amount of H₂SO₄ that remained in the flask. Titrate the contents of each flask with 0.1N KMnO₄ solution until a pink color appears.

Catalase decomposes part of H₂O₂. Therefore, more KMnO₄ solution will be used for the titration of the contents of the second flask than for the titration of the contents of the first flask, where the catalase was destroyed by boiling. This difference is multiplied by 1.7 and the catalase number of the examined blood is obtained.

Medical and biological evaluation of the obtained results. Normally, the catalase number is 12-20. The content of catalase in the blood decreases in some diseases (cancer, anemia, tuberculosis).

Requirements for work results.

Enter the obtained data into the workbook.

Make medical and biological conclusions.

Control materials for the final stage of the class.

Questions to check the final level of knowledge:

1. Definition of the concept of tissue respiration.
2. Action of nicotinamide enzymes. Write the chemistry of the process of converting the oxidized form of NAD into the reduced form.
3. Action of flavin enzymes. Write the chemistry of the process of converting the oxidized form of FAD into the reduced form.
4. The action of coenzyme A. Write the chemistry of the process of converting the oxidized form of ubiquinone into the reduced form.
5. Action of cytochromes. Schematically depict the process of electron transfer through the cytochrome system to oxygen.
6. Electron transfer from cytochrome oxidase to oxygen.
7. The interaction of highly reactive oxygen O₂-with 2H⁺ protons.

8. Values of redox potentials. Their value in determining the sequence of enzymes of the respiratory chain.

9. Draw a diagram of the main, longer and shorter respiratory chains.

Test tasks.

1 A 10-year-old child has Weber's optic neuropathy, the cause of which is a decrease in the activity of NADH-dehydrogenase of the respiratory chain of mitochondria in the cells of the optic nerve. The development of neuropathy is associated with a violation of which process?

- a) Oxidative phosphorylation
- b) Glycolysis
- c) Cycle of three carboxylic acids
- d) ketogenesis
- e) oxidation of fatty acids

2 In all living nature, including the human body, the main macroergic compound is:

- a) ATP
- b) Creatine phosphate
- c) Phosphoenolpyruvate
- d) Acetyl-CoA
- e) Diphosphoglycerate

3 The patient underwent a long course of treatment with the antibiotic oligomycin. What kind of metabolic disorder can this cause?

- a) Inhibition of ATP synthesis
- b) Increase in ATP synthesis
- c) Inhibition of tissue respiration
- d) Inhibition of NAD-dependent dehydrogenase
- e) Inhibition of cytochromes

4 A patient in a state of acute hypoxia after hydrogen sulfide poisoning was taken to the hospital. What is one of the possible mechanisms of action of this gas on the body?

- a) Inhibits tissue respiration in mitochondria
- b) Inhibits dehydrogenation reactions
- c) Inhibits CTC
- d) It separates tissue respiration and phosphorylation
- e) Inhibits glycolysis

5 The organelles that belong to the "energy stations" of the cell are:

- a) Mitochondria
- b) Lysosomes
- c) Plasma membrane
- d) Golgi apparatus
- e) Ribosomes

6 In the bilipid layer of membranes, the protein molecule is fixed with the help of bonds:

- a) Electrostatic and hydrophobic
- b) Peptide and their disulfide
- c) Hydrophobic and ionic
- d) Ionic and hydrogen
- e) Hydrogen and disulfide

7 In the human body, compounds that contain macroergic connections are represented:

- a) Succinate
- b) 3-phosphoglycerate
- c) 2-phosphoglycerol
- d) pyruvate
- e) ATP, GTF, UTF

8 Substrates of biological oxidation can be:

- a) Starch, glycogen, triacylglycerols, lactose food
 - b) Own proteins of blood and tissues
 - c) Nucleic acids and other high-molecular compounds
 - d) Amino acids, glucose, fatty acids, alcohols, oxyacids, etc.
 - e) Cellulose
- 9 At all stages of biological oxidation:
- a) Catabolic transformation of substrates
 - b) The release of the same amount of free energy
 - c) Air oxygen consumption
 - d) Consumption of carbon dioxide
 - e) Release of various amounts of free energy
- 10 In the process of tissue respiration, the following occurs:
- a) Oxidation of reduced cofactors
 - b) Transport of hydrogen (H^+ and e^-) from oxidizing substrates to air O_2
 - c) Complete oxygen recovery
 - d) Formation of the final product of biological oxidation - endogenous water
 - e) All answers are correct
- 11 Tissue respiration is associated with processes:
- a) Oxidative decarboxylation
 - b) Substrate phosphorylation
 - c) Transdeamination
 - d) Oxidative phosphorylation
 - e) glycolysis
- 12 The number of areas of conjugation in tissue respiration depends on:
- a) From the oxidizing substrate
 - b) From the amount of energy released at each transfer of an electron along the electron transport chain
 - c) From the supply of tissue with oxygen
 - d) From the supply of tissue with carbon dioxide
 - e) From the total amount of energy released
- thirteen Separating substances:
- a) Do not affect the transfer of electrons in mitochondria
 - b) Contribute to the formation of heat
 - c) Suppress the synthesis of ATP from ADP and inorganic phosphate
 - d) Prevent the emergence of a gradient of hydrogen ion concentration between the two sides of the mitochondrial membrane
 - e) All answers are correct
- 14 The intensity of tissue respiration in mitochondria depends on:
- a) Exclusively from the amount of oxidation substrate
 - b) From the concentration of phosphoric acid
 - c) From the concentration of H^+
 - d) From the concentration of OH^-
 - e) From the relationship
- 15 The most intense redox processes are:
- a) In the cytoplasm
 - b) In mitochondria
 - c) In lysosomes
 - d) In ribosomes
 - e) In EPR
- 16 Manifestations of polyneuritis with hypovitaminosis B1 are mainly the result of impaired energy supply to the brain due to:
- a) Reduction of oxidative decarboxylation of keto acids

- b) Decreased substrate phosphorylation
- c) Deficiency of ATP
- d) Low creatine kinase activity
- e) High activity of adenylate cyclase

17 Acrychin is prescribed for enterobiosis - a structural analog of vitamin B2. Violation of the synthesis of which enzymes takes place under the action of this drug?

- a) FAD-dependent dehydrogenases
- b) Cytochrome oxidases
- c) Peptidase
- d) NAD-dependent dehydrogenases
- e) Aminotransferase

4. Summary:

5. List of recommended literature (main, additional, electronic information resources):

Main:

1. Gubsky Yu.I., I.V. Nizhenkovska, Korda M.M. Biological and Bioorganic Chemistry: in 2 books. Book 2. Biological Chemistry: textbook. 2021. 544 p.
2. Satyanarayana U. Biochemistry. 5th edition. India 2020. 777 p.
3. Lehninger. Principles of Biochemistry. 7th edition. NY, United States. 2017.
4. Jeremy M. Berg, John L. Tymoczko, Gregory J. Gatto. Biochemistry. 8th Revised edition. 2015.
5. Lippincott Illustrated Reviews: Biochemistry. Philadelphia :Wolters Kluwer, 2017. 560 p.
6. Baynes J., Dominiczak M. Medical Biochemistry. 5th Edition. Elsevier, 2018. 712 p.
7. Donald Voet, Judith G. Voet, Charlott W. Pratt. Fundamentals of Biochemistry: Life at the Molecular Level. ISBN: 978-1-118-91840-1 February 2016, 1184 P.

Additional:

1. William Marshall, Marta Lapsley, Andrew Day, Kate Shipman. Clinical Chemistry. Elsevier, 2020. 432 p.
2. Harper's Illustrated Biochemistry / V.W. Rodwell, D.A. Bender, K.M. Botham et al. – Mc Graw Hill Education, 2015. – 817 p.

Electronic information resources:

1. <https://info.odmu.edu.ua/chair/biology/>-
2. <http://libblog.odmu.edu.ua/>
3. <https://moodle.odmu.edu.ua/login/index.php>

Practical class №18

Topic: Intracellular catabolism of glucose. Glycolysis: reactions. Comparison of glycolysis and alcoholic fermentation. Glycogenolysis, regulation. Differences from glycolysis. Stages of aerobic oxidation of glucose: oxidative decarboxylation of pyruvate. Bioenergetics of the process. Alternative pathways of monosaccharide metabolism. The pentose phosphate pathway of glucose oxidation: scheme, biological significance, features of functioning in various tissues. Metabolic pathways of fructose and galactose conversion: hereditary enzymopathies of their metabolism.

Goal: ATintroducing higher education applicants to the biological role and main ways of converting carbohydrates in the human body, glucose oxidation by glycolysis, the differences between glycolysis and alcoholic fermentation

Basic concepts: carbohydrates, glycolysis, glycolytic oxidoreduction, alcoholic fermentation, aerobic glycolysis, oxidative decarboxylation of pyruvate, glycogenolysis

Equipment: laboratory of the department.

Plan:

1. Organizational measures: greetings, verification of those present, announcement of the topic, purpose of the class, motivation

2. Control of the reference level of knowledge

2.1. Requirements for theoretical readiness of higher education applicants:

1. What compounds are carbohydrates?
2. Which formulas can be used to represent the structure of glucose and fructose
3. Classification of carbohydrates.
4. Types of isomerism characteristic of representatives of the class of carbohydrates.
5. What is an asymmetric carbon atom, how is it designated? Optical isomerism.
6. What is glycolysis?
7. In which compartment of the cell does the process of glycolysis take place?
8. What end products are formed in the process of glycolysis?
9. Draw up the energy balance of anaerobic glycolysis.
10. What is the biological role of the glycolysis process?
11. How does the oxidation of NADH.H⁺, formed in the sixth reaction of glycolysis, take place under anaerobic conditions?

2.2. Questions to check basic knowledge on the topic of the class:

1. Write the sequence of glycolysis reactions.
2. Specify the reactions of ATP formation in anaerobic conditions.
3. How does the process of alcoholic fermentation differ from glycolysis.
4. Write the sequence of fermentation reactions.
5. In which body tissues does glycolysis occur.
6. Name the key enzyme of glycolysis.
7. Name the test for glycolysis. Name the sample for fermentation.
8. Name the end products of anaerobic glycolysis.
9. Name the methods by which the end products of anaerobic glycolysis can be detected.
10. What is glycolytic redox.
11. What enzymes and coenzymes are part of the pyruvate dehydrogenase complex
12. Sequence of reactions of complex oxidative decarboxylation of pyruvate
13. Regulation of the activity of the pyruvate dehydrogenase complex
14. Distinguish between glycolysis and glycogenolysis
15. Features of glycogenolysis in the liver and skeletal muscles
16. Regulation of glycogenolysis.
17. Calculate the energy balance of aerobic glycolysis and glycogenolysis

3. Formation of professional skills and abilities: demonstration and practical work.

3.1. Content of demonstration and practical work

- 3.1.1. Determination of "in vitro" process of glycolysis, study of final products.
- 3.1.2. Sample for alcoholic fermentation.
- 3.1.3. Determination of pyruvate content in blood serum

3.2. Implementation recommendations:

3.2.1.1. Preparation of the reaction mixture:

Animal muscles (1 g) are ground in a mortar with glass sand, adding 2-3 ml of phosphate buffer with glucose content. Pour 3 ml of the same buffer into two test tubes. 1 ml of 20% THO solution is added to the first test tube (control) to precipitate proteins and end the enzymatic processes. Add 0.5 ml of muscle slurry to both test tubes and close them with stoppers.

2. Thermostat:

Both test tubes are placed in a thermostat for 45 to 30 minutes^{at}C. Before the end of incubation, prepare two test tubes.

3. Carrying out the Uffelman reaction:

10 drops of 1% solution of Uffelman's reagent are poured into the prepared test tubes. The contents of the incubation tubes are filtered through a cotton filter, after which 0.5 ml of the filtrate is added dropwise to Uffelman's reagent. A change in coloration from purple to yellow-green in the test sample is observed.

3.2.2.1. Preparation of the reaction mixture:

1 g of baker's yeast is ground in a mortar while constantly adding a 5% glucose solution (about 30 ml). The resulting liquid is introduced into the fermentation tube in such a way that the upper knee is completely filled, and a little liquid remains in the area of expansion.

2. Thermostating the sample:

The fermentation tube is placed in the thermostat for 40 minutes.

3. Observation of fermentation:

Observe CO₂ release; it is necessary to add 2-3 ml of 10% solution of NaOH to the tube and tightly close the opening of the tube with the pad of the thumb, after which the tube is turned over several times, stirring its content. CO₂ is absorbed by alkali and the pad of the finger is sucked into the opening of the tube.

4. Detection of ethanol:

To detect ethanol, a part of the contents of the tube is taken (3-5 ml), filtered through a cotton filter, the pH is checked (if the filtrate is acidic, it is alkalized with NaOH to a weak alkaline reaction), a few drops of iodine solution are added and heated slightly. A characteristic smell of iodoform is formed.

3.2.2.3. Principle of the method:

After precipitation of serum proteins, pyruvic acid without protein filtrate reacts in an acidic environment with 2,4-DNFH, forming the corresponding hydrazone. After the addition of alkali to the incubation medium, a characteristic color develops, the intensity of which is proportional to the concentration of pyruvate, which is estimated from a standard solution containing 5.0 µg of pyruvate in 1 ml. The method is not specific enough, since hydrazones are also formed by other keto compounds, but since the increase in the inhibition level of hydrazones can occur mainly due to pyruvate, it can be used for diagnostic purposes.

Progress of work. 1. Obtaining the researched material:

0.7 ml of H is added to 0.3 ml of blood serum₂Oh, mix, add 1 ml of 10% THO. Stir and after 2-3 minutes centrifuge at 1500 rpm for 15 minutes. Take two test tubes, mark the experiment on one, and the control on the second.

Experiment

1 ml of centrifuge

0.5 ml of 2,4-DNFH solution

CONTROL

1 ml of pyruvate No

0.5 ml of 2,4-DNFH solution

leave for 20 minutes at room temperature in a dark place

After 10 minutes, colorimeter against water. Measurements are carried out with experimental and control samples. The obtained measurements are entered in the experiment protocol and the calculation is carried out according to the formula:

$$X_h = \frac{S_{st}}{S_{est}} \times Est$$

E samples

CONCLUSION AND MEDICO-BIOLOGICAL ASSESSMENT OF RESEARCH RESULTS

Summary table of data on lactate and pyruvate

c	Metaboli State	Lactate mmol/l		Pyruvate mmol/l		Arterial-venous difference
		Artery	vei n	artery	Vei n	
	NORM	0.55	0. 80	50.0	70.0	Lactate-0.3 mmol/l; Pyruvate-20.0

					mmol/l	
A	HYPOXI Lung	0.70 20	1. 1.	150.0	120. 0	Lactate-0.50 mmol/l; Pyruvate-30 mmol/l
	average	2.15 70	1. 1.	200.0	160. 0	Lactate-0.65 mmol/l Pyruvate-0.40 mmol/l
	Heavy or more (death- case)	3.60 50 and more	2. 2.	240.0 or more	200. 0 or more	Lactate-1.10 mmol/l Pyruvate-0.40 mmol/l

3.3. Requirements for work results: in a notebook for demonstration and practical work, write down the method of work performance, the results of observation. Provide a medico-biological evaluation of the obtained results. Draw conclusions.

3.4. Control materials for the final stage of the class: test tasks for the topic:

1. The erythrocyte needs energy in the form of ATP for its vital activity. What process provides this cell with the required amount of ATP?

- A Anaerobic glycolysis
- B Aerobic oxidation of glucose
- C The pentose cycle
- D Beta-oxidation of fatty acids
- E Cycle of tricarboxylic acids

2. Anaerobic breakdown of glucose to lactic acid is regulated by appropriate enzymes. Specify which enzyme is the main regulator of this process?

- A Enolase
- B Glucosel-6-phosphate isomerase
- C Aldolase
- D Phosphofructokinase
- E Lactate dehydrogenase

3. The concentration of glucose in the blood plasma of a healthy person is within the following limits:

- A 3.5-5.5 mmol/l
- B 2-4 mmol/l
- C 10-25 mmol/l
- D 6-9.5 mmol/l
- E 1-2 mmol/l

4. What substance is the main source of energy for brain tissue?

- A Glycerin
- B Fatty acids
- C Glucose
- D Amino acids
- E Lactic acid

5. During short-distance running, untrained people experience muscle wasting due to the accumulation of lactate. Indicate the strengthening of which biochemical process this may be associated with.

- A Glycogenesis.

- B Gluconeogenesis.
- C pentose phosphate pathway.
- D Lipogenesis.
- E Glycolysis.

6. A 7-year-old girl has obvious signs of anemia. Laboratory established deficiency of pyruvate kinase in erythrocytes. Disruption of which process plays the main role in the development of anemia in a girl?

- A Anaerobic glycolysis
- B Oxidative phosphorylation
- C Tissue respiration
- D Decomposition of peroxides
- E Deamination of amino acids

7. A large number of metabolites of glucose oxidation are dissolved in the cytoplasm of myocytes. Name one of them, directly converted to lactate.

- A Oxaloacetate
- B Pyruvate
- C Glycerophosphate.
- D Glucose-6-phosphate.
- E Fructose-6-phosphate.

8. During long-distance running, the skeletal muscles of a trained person use glucose to obtain ATP energy for muscle contraction. Indicate the main process of utilization of glucose under these conditions.

- A Anaerobic chandnumberandwith.
- B Aerobic chandnumberandwith.
- C Seeandcogenolandwith.
- D Gluconeogenesis.
- E Glycogenesis.

9. Carbohydrates, especially sucrose, play a significant role in the spread of dental caries, especially in childhood. Which of the carbohydrate conversion pathways is accompanied by the formation of acids that leads to dentin demineralization?

- A. Gluconeogenesis
- B. The pentose cycle
- C. Glycolysis
- D. Synthesis of fatty acids
- E. Cholesterol synthesis

10. After long-term physical exertion during the physical education class, the higher education applicants developed muscle weakness. The cause of its occurrence was the accumulation of lactic acid in the skeletal muscles. It was formed after activation in the body of higher education applicants:

- A Lipolysis.
- B Gluconeogenesis.
- C Pentose phosphate cycle.
- D Glycolysis.
- E Glycogenesis.

11. The patient was taken to a medical institution in a comatose state. From the words of the attendants, it was found that the patient fainted during training at the final stage of the marathon distance. Who is most likely to be suspected of

- A. Hyperglycemic.
- B. Hypoglycemic.
- C. Acidotic.
- D. Hypothyroidism.
- E. Pechinkova.

12. In the process of glycolysis, hexose D-fructose-1,6-diphosphate is synthesized, from which two trioses are subsequently formed: dioxyacetone phosphate and glyceraldehyde-3-phosphate. The enzyme that catalyzes this reaction is:

- A. Fructose-1,6-diphosphate aldolase
- B. Phosphohexose isomerase
- S. Triosephosphatisomerase
- D. Phosphofructose isomerase
- E. Enolase

13. Patient A. was in an unconscious state after a brain injury. During the examination, an increase in the content of lactic acid and pyruvic acid in the blood serum was established, the pH of the blood was 7.2. These indicators indicate a violation of which metabolic process?

- A Strengthening of aerobic glycolysis
- In Inhibition of gluconeogenesis
- C Enhancement of gluconeogenesis
- D Enhancement of anaerobic oxidation of glucose
- E Enhancement of glycogenolysis

14. A 42-year-old man ate a large portion of spaghetti, a piece of cake, and drank a glass of sweet tea. The activity of which enzyme of hepatocytes is activated to the greatest extent after consumption of high-carbohydrate food?

- A. Hexokinase
- B. Glucose-6-phosphatase
- S. Glucose-6-phosphate dehydrogenase
- D. Glycogen phosphorylase
- E. Beta-galactosidase

4. Summary of the class. Assessment.

5. List of recommended literature

Main:

1. Gubsky Yu.I., I.V. Nizhenkovska, Korda M.M. Biological and Bioorganic Chemistry: in 2 books. Book 2. Biological Chemistry: textbook. 2021. 544 p.
2. Satyanarayana U. Biochemistry. 5th edition. India 2020. 777 p.
3. Lehninger. Principles of Biochemistry. 7th edition. NY, United States. 2017.
4. Jeremy M. Berg, John L. Tymoczko, Gregory J. Gatto. Biochemistry. 8th Revised edition. 2015.
5. Lippincott Illustrated Reviews: Biochemistry. Philadelphia :Wolters Kluwer, 2017. 560 p.
6. Baynes J., Dominiczak M. Medical Biochemistry. 5th Edition. Elsevier, 2018. 712 p.
7. Donald Voet, Judith G. Voet, Charlott W. Pratt. Fundamentals of Biochemistry: Life at the Molecular Level. ISBN: 978-1-118-91840-1 February 2016, 1184 P.

Additional:

1. William Marshall, Marta Lapsley, Andrew Day, Kate Shipman. Clinical Chemistry. Elsevier, 2020. 432 p.
2. Harper's Illustrated Biochemistry / V.W. Rodwell, D.A. Bender, K.M. Botham et al. – Mc Graw Hill Education, 2015. – 817 p.

Electronic information resources:

1. <https://info.odmu.edu.ua/chair/biology/>-
2. <http://libblog.odmu.edu.ua/>
3. <https://moodle.odmu.edu.ua/login/index.php>

Practical class №19

Topic: Glucose biosynthesis: physiological significance, reactions, regulatory enzymes. Substrates of gluconeogenesis. Glucose-lactate and glucose-alanine cycles. Regulation of glucose metabolism. Glucosemia: normal state and its disorders. Glycogen biosynthesis. Regulation of carbohydrate metabolism. Genetic disorders of glycogen metabolism of (glycogen storage diseases).

Goal:Formation of systemic knowledge on ways of glucose metabolism in the human body. Study of molecular mechanisms of glycogenesis and gluconeogenesis and their regulation

Basic concepts:Glycogenesis, gluconeogenesis, glucose-lactate, glucose-alanine cycles, glycogenoses.

Equipment:laboratory of the department.

Plan:

1. Organizational measures:greetings, verification of those present, announcement of the topic, purpose of the class, motivation

2. Control of the reference level of knowledge

2.1. Requirements for higher education applicants' theoretical readiness:

1. General ideas about the mechanism of glucose delivery to cells
2. Glycolysis: reactions, enzymes. Irreversible reactions of glycolysis
3. How dangerous is the accumulation of lactate? The mechanism of reutilization of lactate, which is formed in the process of anaerobic glycolysis. Corey cycle.

4. The concept of normo-, hyper- and hypoglycemia

5. Glycogen, structure and functions

6. Glycogenolysis. Regulation of the process of glycogen splitting in the human body.

2.2. Questions to check basic knowledge on the topic of the class:

1. What enzymes and coenzymes are involved in gluconeogenesis?

2. What is the biological significance of gluconeogenesis?

3. What mechanisms of gluconeogenesis regulation do you know?

4. Why is blood glucose determined?

5. Glycogenesis. Reactions, enzymes, process regulation.

6. Glycogenoses. Types. Causes of occurrence.

7. Diabetes.

3. Formation of professional skills and abilities:demonstration and practical work.

3.1. Content of demonstration and practical work

3.1.1.Determination of glucose content in the blood by the Hagedorn-Jensen method.

3.2. Implementation recommendations:

3.2.1.Principle of the method:

With the help of the Hagedorn-Jensen method, not only glucose is determined in the blood, but also some other reducing substances (for example, uric acid, glutathione, creatine). At the same time, the total regenerative capacity in the blood is taken for "sugar". The method is based on the ability of glucose in a protein-free blood filtrate in an alkaline medium when heated to reduce red blood salt ($K_4Fe(CN)_6$);

Due to the reversibility of this reaction, $K_4Fe(CN)_6$ under the action of zinc sulfate ($ZnSO_4$) is converted into an insoluble salt $K_2Zn_3(Fe(CN)_6)_2$, which is taken in excess and its residue unused in the reaction is determined iodometrically in an acidic medium (for example, in the presence of acetic acid), titrating the amount of iodine formed with sodium thiosulfate.

Starch is used as an indicator for molecular iodine. Glucose content is calculated according to a special table. The table is compiled in such a way that it shows the amount of sodium thiosulfate used for the titration of iodine, and therefore the excess (K₄Fe(CN)₆), corresponds to the number of milligrams of glucose that reacted in the reaction.

The main stages of the work:

1. 1 ml of 0.1N NaOH is placed in two test tubes. 0.1 ml of blood is added to one of them (sample), and 0.1 ml of distilled water is added to the other (control).

Then add 5 ml of 0.45% ZnSO₄, place in a boiling bath for 2-3 minutes, then filter through a cotton swab inserted into the funnel.

2. Add 2 ml of 0.005 N alkaline solution of K₃Fe(CH)₆ to the filtrate, boil in a water bath for 15 minutes.

3. The mixture is cooled and 3 ml of solution (ZnSO₄ + KJ + NaCl) is added, and then 2 ml of CH₃COOH and 2 drops of starch (1% p-p) are added

4. The released iodine is titrated with a 0.005N solution of sodium thiosulfate until the blue color disappears.

5. Calculation of results is carried out using a table

Hypo-sul-fit in junior	0.00	0.01	0.02	0.03	0.04	0.05	0.06	0.07	0.08	0.09
0.0	0.358	0.382	0.379	0.376	0.373	0.370	0.367	0.364	0.361	0.358
0.1	0.355	0.352	0.350	0.348	0.345	0.343	0.341	0.338	0.336	0.333
0.2	0.331	0.329	0.327	0.325	0.323	0.321	0.318	0.316	0.314	0.312
0.3	0.310	0.308	0.306	0.304	0.302	0.300	0.298	0.296	0.294	0.292
0.4	0.290	0.288	0.286	0.284	0.282	0.280	0.278	0.276	0.274	0.272
0.5	0.270	0.268	0.266	0.264	0.262	0.260	0.259	0.257	0.255	0.253
0.6	0.251	0.249	0.247	0.245	0.243	0.241	0.240	0.238	0.236	0.234
0.7	0.232	0.230	0.228	0.226	0.224	0.222	0.221	0.219	0.217	0.215
0.8	0.213	0.211	0.209	0.208	0.206	0.204	0.202	0.200	0.199	0.197
0.9	0.195	0.193	0.191	0.190	0.188	0.186	0.184	0.182	0.181	0.179
1.0	0.177	0.175	0.173	0.172	0.170	0.168	0.166	0.164	0.163	0.161
1.1	0.159	0.157	0.155	0.154	0.152	0.150	0.148	0.146	0.145	0.143
1.2	0.141	0.139	0.138	0.136	0.134	0.132	0.131	0.129	0.127	0.125
1.3	0.124	0.122	0.120	0.119	0.117	0.115	0.113	0.111	0.110	0.108
1.4	0.106	0.104	0.102	0.101	0.099	0.097	0.095	0.093	0.092	0.090
1.5	0.088	0.086	0.084	0.083	0.081	0.079	0.077	0.075	0.074	0.072
1.6	0.070	0.068	0.066	0.065	0.063	0.061	0.059	0.057	0.056	0.054
1.7	0.052	0.050	0.048	0.046	0.045	0.043	0.041	0.039	0.038	0.036
1.8	0.034	0.032	0.031	0.029	0.027	0.025	0.024	0.022	0.020	0.019
1.9	0.017	0.015	0.014	0.012	0.010	0.008	0.007	0.005	0.003	0.002

3.1.2.Determination of glycogen content in the liver.

3.2.2.Principle of the method:

Determination of glycogen is carried out with the help of Lugol's solution, and is also confirmed by its hydrolysis to glucose under the action of saliva amylase.

The main stages of the work:

1. Filtrate production:

2.5 grams of liver are mixed in a porcelain mortar, to which 4 ml of dist. H₂O and grind with glass sand. The homogenate is quantitatively transferred to a flask and 20 ml of dist. H₂O, after which the sample is boiled in a water bath for 20-30 minutes. For more complete precipitation, add 5-10 drops of 1% acetic acid solution to the boiling liquid. The protein precipitate is separated by filtering

through a paper filter moistened with water.

2. Carrying out qualitative reactions:

For this, take 4 test tubes and number them with a glass pencil:

№1 control,

№2 glycogen deposition,

№3 digestion of glycogen,

№4 pure glycogen.

no	1	2	3	4
1.	H ₂ O dist.- 5 drops.	Filtrate-5 drops.	Filtrate-5 drops.	Filtrate-5 drops.
2	Lugol's solution - 2 drops.	2. sulfuric acid	2. saliva (1:10)-2 drops.	2. Fehling's solution - 2 drops. They heat up
3	-	3. Lugol's solution - 2 drops.	3. Lugol's solution - 2 drops.	-

A change in color is observed

3.3. Requirements for work results: in a notebook for demonstration and practical work, write down the method of work performance, the results of observation. Provide a medico-biological evaluation of the obtained results. Draw conclusions.

3.4. Control materials for the final stage of the class: test tasks for the topic:

1. In a patient exhausted by starvation, the process in the liver and kidneys intensifies:

- A Bilirubin synthesis
- B Urea synthesis
- C Gluconeogenesis
- D Formation of hippuric acid
- E Uric acid synthesis

2. For the synthesis of polysaccharide chains of glycogen, a precursor - the active form of glucose - is used. The direct donor of glucose residues in the process of glycogen synthesis is:

- A ADP-glucose
- B glucose-1-phostat
- C UDF-glucose
- D glucose - 6-phosphate
- E glucose-3 phosphate

3. When there is a lack of blood circulation during intense muscle work, lactic acid accumulates in the muscle as a result of anaerobic glycolysis. What is her future fate?

- A Involved in gluconeogenesis in the liver
- B It is removed through the kidneys with urine
- C It is used in the muscle for the synthesis of amino acids
- D It is used by tissues for the synthesis of ketone bodies
- E It is used in tissues for the synthesis of fatty acids

4. A characteristic sign of glycogenosis type V (McArdle's disease) is muscle pain during physical work. Congenital deficiency of which enzyme causes this pathology?

- A Glycogen synthases
- In Glucose-6-phosphatase
- C Glycogen phosphorylase

- D Amylo-1,6-glycosidases
- E Lysosomal glycosidase

5. In Itsenko-Cushing's disease (hyperfunction of the adrenal cortex with increased production of corticosteroids), hyperglycemia occurs. What process is stimulated in this case?

- A Gluconeogenesis
- B Glycogen phosphorolysis
- C Krebs cycle
- D Pentose phosphate pathway of glucose oxidation
- E Glycolysis

6. As a result of prolonged starvation in the human body, reserves of carbohydrates quickly disappear. Which of the metabolic processes restores the glucose content in the blood?

- A Glycogenolysis
- B Anaerobic glycolysis
- C Aerobic glycolysis
- D Gluconeogenesis
- E Pentophosphate pathway

7. A 40-year-old woman with complaints of thirst and increased appetite is being treated in the endocrinology department with a diagnosis of diabetes. What pathological components were found during the laboratory examination of the patient's urine?

- A Glucose, ketone bodies
- B Protein, amino acids
- C Protein, creatine
- D Bilirubin, urobilin
- E Blood

8. With a chronic overdose of glucocorticoids, the patient develops hyperglycemia. Name the process of carbohydrate metabolism due to which the concentration of glucose increases:

- A Aerobic glycolysis
- B Seeandcogenolandwith
- C Gluconeogenesis
- D Pentose phosphate cycle
- E Seeandcogenesis

9. A 40-year-old woman has Itsenko-Cushing's disease - steroid diabetes. During biochemical examination: hyperglycemia, hypochloremia. Which of the following processes is activated first?

- A Gluconeogenesis
- B Glycogenolysis
- C Reabsorption of glucose
- D Transport of glucose into the cell
- E Glycolysis

10. In the patient's blood, the glucose content on an empty stomach is 5.6 mmol/l, after 1 hour after the sugar load - 13.8 mmol/l, and after 3 hours - 9.2 mmol/l. Such indicators are likely for:

- A Hidden form of diabetes
- B A healthy person
- C Thyrotoxicosis
- D Itsenko-Cushing diseases
- E Acromegaly

11. In a patient with a diagnosis of Itsenko-Cushing's disease (hyperproduction of the adrenal cortex), an increased concentration of glucose, ketone bodies, and sodium was determined in the blood. What biochemical mechanism is the leading cause of hyperglycemia?

- A Glycogenesis
- B Gluconeogenesis
- C Glycogenolysis
- D Glycolysis
- E Aerobic glycolysis

12. A one-year-old child lags behind his peers in mental development. In the morning: vomiting, convulsions, loss of consciousness. Fasting hypoglycemia in the blood. Which enzyme defect is this associated with?

- A. Glycogen synthases
- B. Phosphorylases
- C. Arginases
- D. Sucrase
- E. Lactases

13. A patient with signs of acute alcohol poisoning was brought to the clinic. What changes in carbohydrate metabolism are characteristic of this condition

- A. The rate of gluconeogenesis decreases in the liver
- B. Glycogen breakdown increases in the liver
- C. Anaerobic breakdown of glucose prevails in muscles
- D. Gluconeogenesis increases in the liver
- E. Aerobic breakdown of glucose increases in muscles

14. As a result of prolonged fasting, hypoglycemia occurs, which is aggravated by alcohol, because it slows down:

- And Lipolysis
- In Glycolysis
- C Glycogenolysis
- D Gluconeogenesis
- E Proteolysis

4. Summary of the class. Assessment.

5. List of recommended literature

Main:

1. Gubsky Yu.I., I.V. Nizhenkovska, Korda M.M. Biological and Bioorganic Chemistry: in 2 books. Book 2. Biological Chemistry: textbook. 2021. 544 p.
2. Satyanarayana U. Biochemistry. 5th edition. India 2020. 777 p.
3. Lehninger. Principles of Biochemistry. 7th edition. NY, United States. 2017.
4. Jeremy M. Berg, John L. Tymoczko, Gregory J. Gatto. Biochemistry. 8th Revised edition. 2015.
5. Lippincott Illustrated Reviews: Biochemistry. Philadelphia :Wolters Kluwer, 2017. 560 p.
6. Baynes J., Dominiczak M. Medical Biochemistry. 5th Edition. Elsevier, 2018. 712 p.
7. Donald Voet, Judith G. Voet, Charlott W. Pratt. Fundamentals of Biochemistry: Life at the Molecular Level. ISBN: 978-1-118-91840-1 February 2016, 1184 P.

Additional:

1. William Marshall, Marta Lapsley, Andrew Day, Kate Shipman. Clinical Chemistry. Elsevier, 2020. 432 p.

2. Harper's Illustrated Biochemistry / V.W. Rodwell, D.A. Bender, K.M. Botham et al. – Mc Graw Hill Education, 2015. – 817 p.

Electronic information resources:

1. [https://info.odmu.edu.ua/chair/biology/-](https://info.odmu.edu.ua/chair/biology/)
2. <http://libblog.odmu.edu.ua/>
3. <https://moodle.odmu.edu.ua/login/index.php>

Practical class №20,21

Topic: Intermediate control for the semester

Goal: To determine the level of higher education applicants' assimilation of knowledge for the semester

Equipment: Laboratory of the department

Plan:

1. Organizational activities (greetings, verification of those present, announcement of the topic, purpose of the class, motivation of higher education seekers to study the topic).

2. List of questions for preparation for control:

Theoretical basis of the future and reaction properties of bioorganic compounds.

1. Bioorganic chemistry as a science: significance, subject and knowledge, sections, research methods. Significance in the system of high medical coverage.
2. Classification of organic compounds based on the carbon radical and the nature of the functional groups.
3. The most important classes of bioorganic compounds based on the nature of functional groups: alcohols, phenols, thiols, aldehydes, ketones, carboxylic acids, folded ethers, amides, nitrogen compounds, etc.
4. Nomenclature of organic ideas: trivial, rational, international. The principles of establishing the names of organic structures according to the IUPAC nomenclature: mixed, radical-functional.
5. The nature of the chemical binder in organic compounds: hybridization of orbit, electronic structure of semi-carbon.
6. Expanse of bioorganic properties: stereochemical formulas; configuration and conformation. Stereoisomers: geometric, optical, rotational (conformers).
7. Optical isomerism; chirality of organic molecules. D/L- and R/S-stereochemical nomenclatures. Enantiomers and diastereoisomers of bioorganic compounds. The connection between spacious living and physiological activity.
8. Types of reactions in bioorganic chemistry: classification based on the result (directness) and the reaction mechanism. Apply it.
9. Carbonyl compounds in bioorganic chemistry. Chemical power and biomedical significance of aldehydes and ketones.
10. Carboxylic acids in bioorganic chemistry: natural and chemical power; functional derivatives of carboxylic acids (anhydrides, amides, folding esters). Decarboxylation reactions.
11. Structure and the power of dicarboxylic acids: oxalic, malonic, succinic, glutaric, fumaric.
12. Lipids: identification, classification. Fatty acids: palmitic, stearic, oleic, linoleic, linolenic, arachidonic. Sorry lipids. Triacylglycerols (neutral fats): structure, physiological significance, hydrolysis.
13. Folding lipids. Phospholipids: phosphatidic acid, phosphatidylethanolamine, phosphatidylcholine, phosphatidylserine. Sphingolipids. Glycolipids. The role of folding lipids in everyday biomembranes.

14. Amen: nomenklatura, authorities. Biomedical significance of biogenic amines (adrenaline, norepinephrine, dopamine, tryptamine, serotonin, histamine) and polyamines (putrescine, cadaverine).
15. Amino alcohols: structure, power. Biomedically important are ethanol amine (cola mine), choline, acetylcholine.
16. Hydroxy acids in bioorganic chemistry: the presence and power of monocarboxylic (lactic and hydroxybutyric), dicarboxylic (malic, tartaric) hydroxy acids.
 α -Amino acids, peptides, proteins.
17. Amino acids: amino acids, stereoisomerism, chemical power. Biomedical significance of L-a-amino acids. Reactions of biochemical transformations of amino acids: deamination, transamination, decarboxylation.
18. Amino acid storage of proteins and peptides; classification of natural L-a-amino acids. Chemical and physical-chemical power of proteinogenic amino acids. Ninhydrin reaction, its significance in the analysis of amino acids.
19. Proteins and peptides: identification, classification, biological functions. Types of bonds between amino acid residues in protein molecules. Peptide link: composition, structure; Biuret reaction.
20. Levels of structural organization of proteins: primary, secondary, tertiary and quaternary structures. Oligomeric proteins.
21. Physico-chemical properties of proteins; Ex molecular weight. Denaturation of proteins. Structure and function of carbohydrates.
22. Carbohydrates: identification, classification. Monosaccharides (aldoses and ketoses; trioses, tetrosities, pentoses, hexoses, heptoses), biomedical significance of several representatives.
23. Monosaccharides: pentose (ribose, 2-deoxyribose, xylose), hexose (glucose, galactose, manose, fructose) - Structure, properties. Clear reactions to glucose.
24. The power of similar monosaccharides. Amino derivatives: glucosamine, galactosamine. Uronic acids. L-Ascorbic acid (vitamin C). Monosaccharide renewal products: sorbitol, mannitol.
25. Oligosaccharides: power, power. Disaccharides (sucrose, lactose, maltose), their biomedical value.
26. Polysaccharides. Homopolysaccharides: starch, glycogen, cellulose, dextrin-structure, hydrolysis, biomedical value. A clear reaction to starch.
27. Heteropolysaccharides: meaning, structure. The potential and biomedical significance of glycosaminoglycans (mucopolysaccharides) – hyaluronic acid, chondroitin sulfates, heparin. Biologically active heterocyclic compounds. Nucleosides, nucleotides, nucleic acids.
28. Five-membered heterocycles with one heteroatom (pyrole, furan, thiophene). Biomedical significance of tetrapyrollic compounds: porphins, porphyrins, heme.
29. Indol and its derivatives: tryptophan and reactions of tryptamine and serotonin; Indoxyl, skatole, skatol are important in the processes of protein rotting into the intestines.
30. Five-membered heterocycles with two nitrogen heteroatoms. Pyrazol, pirazolone; Similar treatments for pirazolone-5 as medicinal agents (antipyrine, amidopirine, analgin). Imidazole and its derivatives: histidine, histamine.
31. Five-membered heterocycles with two different heteroatoms: thiazole, oxazole. Thiazole is a structural component of the thiamine molecule (vitamin B1).
32. Six-membered heterocycles with a nitrogen atom: pyridine. Nicotinamide (vitamin PP.) is a storage component of oxidatively derived pyridine coenzymes. Pyridoxine and molecular forms of vitamin B6.
33. Six-membered heterocycles with two nitrogen atoms. Diazines: pyrimidine, pirazine, pyridazine. Nitrogen bases - similar pyrimidines (uracil, cytosine, thymine).
34. Related substances as medicinal uses: 5-fluorouracil, potassium orotate. Barbituric acid; barbiturates as an anesthetic and against epileptic symptoms (phenobarbital, veronal).
35. Purin and it's derivates. Amino-like purines (adenine, guanine), their tautomeric forms; biochemical significance in the concentration of nucleotides and coenzymes.
36. Hydroxy-based purines: hypoxanthine, xanthine, uric acid. Methylated xanthine compounds

- (caffeine, theophylline, theobromine) are physiologically active and act on the central nervous and cardiovascular system.
37. Nucleosides, nucleotides. Nitrogens are the basis of the purine and pyrimidine series, which are part of the stock of natural nucleotides. Minor nitrogenous bases.
 38. Nucleosides. Nucleotides as phosphorylated derivatives of nucleosides (nucleoside mono-, di- and triphosphates). Nomenclature of nucleosides and nucleotides as components of RNA and DNA.
 39. What are the biochemical functions of strong nucleotides: coenzyme nucleotides; cyclic nucleotides 3',5'-cAMP and 3',5'-cGMP.
 40. Nucleic acids (deoxyribonucleic acids, ribonucleic acids) as polynucleotides. Polarity of polynucleotide chains of DNA and RNA.
 41. Structure and the power of DNA; nucleotide storage, complementarity of nitrogenous bases. The primary, secondary and tertiary structure of DNA. RNA: Structure, types of RNA and their role in protein biosynthesis.
 42. Vitamins: their characteristics; understanding about the coenzyme action of vitamins. Structure and the power of vitamins B1, B2, B6, PP.
- Introduction into biochemistry. Biochemical components of cells.'
43. Biological chemistry (biochemistry) as a science. The place of biochemistry among other medical and biological disciplines. History of biochemistry; development of biochemical research in Ukraine.
 44. Objects of education and training in biochemistry. The role of biochemistry in the established molecular mechanisms of the pathogenesis of human illness is evident.
 45. Relationship between biochemistry and other biomedical sciences. Medical biochemistry. Clinical biochemistry. Biochemical laboratory diagnostics.
 46. Biochemical components of cellulose, their biochemical functions. Class of biomolecules. Hierarchy of biomolecules, their relationships.
- Enzymes and coenzymes. Regulation of metabolism.
47. Enzymes: value; the power of enzymes as biological catalysts.
 48. Classification and nomenclature of enzymes, characteristics of certain classes of enzymes.
 49. What are the mechanisms of enzymes. Active and allosteric (regulatory) center.
 50. Cofactors and coenzyme. Due to the power of coenzymes, vitamins are precursors in the biosynthesis of coenzymes. Coenzymes: types of reactions that catalyze other classes of coenzymes.
 51. Isoenzymes, features of their functioning, significance in the diagnosis of illness.
 52. Mechanisms of action and kinetics of enzymatic reactions: duration of liquid reaction, substrate concentration, pH and temperature. Principles and methods for identifying enzymes in biological objects. One type of activity and number of enzymes.
 53. Activators and inhibitors of enzymes: applications and mechanisms of action.
 54. Types of enzyme inhibition: turnover (competitive, non-competitive) and non-turnover inhibition.
 55. Regulation of enzymatic processes. Paths and mechanisms of regulation: allosteric enzymes; covalent modification of enzymes. Cyclic nucleotides (cAMP, cGMP) as regulators of enzymatic reactions and biological functions of cells.
 56. Enzymopathies - problems (surges) in the metabolism of carbohydrates, amino acids, porphyrins, purines.
 57. Enzymodiagnosis of pathological processes and illness.
 58. Enzymotherapy - stagnation of enzymes, their activators and inhibitors in medicine.
- Metabolism fundamentals. Citric acid cycle.
59. Metabolism (metabolism) - the underlying patterns of catabolic and anabolic processes.
 60. Complex stages of internal cellular catabolism of biomolecules: proteins, carbohydrates, lipids.
 61. Tricarboxylic acid cycle. Localization, sequence of enzymatic reactions, significance in the metabolism of speech. Energy balance of the tricarboxylic acid cycle. Physiological significance of the TCA reaction.
- Molecular basis of bioenergetics.
62. Reactions of biological oxidation; types of reactions (dehydrogenase, oxidase, oxygenase) and

their biological significance. Respiratory chain.

63. Enzymes of biological oxidation in mitochondria: pyridine-, flavin-dehydrogenases, cytochromes. Sequence of components of the mitochondrial membrane. Molecular complexes of the inner membranes of mitochondria.

64. Oxide phosphorylation: points of conjugation of electron transport and phosphorylation, coefficient of oxide phosphorylation

65. Chemiosmotic theory of oxide phosphorylation, mitochondrial ATP synthetase.

66. Inhibitors of electron transport and inhibitors of oxide phosphorylation.

67. Microsomal oxidation: cytochrome P-450; molecular organization of electron transfer chain.

Metabolism of carbohydrates and its regulation.

68. Aerobic and anaerobic oxidation of glucose, natural characteristics of processes.

69. Anaerobic oxidation of glucose. Sequence of reaction of enzymes to glycolysis.

70. Aerobic oxidation of glucose. Stages of transformation of glucose to CO₂ and H₂O. Oxidation decarboxylation of pyruvate. Enzymes, coenzymes and sequence of reactions in a multienzyme complex.

71. Glycolytic oxidoreduction: substrate phosphorylation and nutrient mechanisms of glycolytic NADH oxidation.

72. The bioenergetics of aerobic and anaerobic glucose oxidation, the Pasteur effect, are consistent.

73. Phosphorolytic pathway for the breakdown of glycogen in liver and meat. Regulation of glycogen phosphorylase activity.

74. Glycogen biosynthesis: enzymatic reactions, physiological significance. Regulation of glycogen synthase activity.

75. Mechanisms of reciprocal regulation of glycogenolysis and glycogenesis through cascade cAMP-dependent phosphorylation of enzyme proteins. The role of adrenaline, glucagon and insulin in the hormonal regulation of glycogen exchange in meat and liver.

76. Genetic disorders of glycogen metabolism (glycogenosis, aglycogenosis).

77. Gluconeogenesis: substrates, enzymes and physiological significance of the process. Glucose-lactate (Coria cycle) and glucose-alanine cycles.

78. Blood glucose (glucosemia): normoglycemia, hypoglycemia, glucosuria. Blood diabetes is a pathology of glucose metabolism. Hormonal regulation of blood glucose concentration and exchange.

79. Pentose phosphate pathway of glucose oxidation: scheme of the process and biological significance.

80. Metabolic pathways for the transformation of fructose and galactose; hereditary disorders of metabolism.

Lipid metabolism and its regulation.

81. Catabolism of triacylglycerols in adipocytes of adipose tissue: sequence of reactions, mechanisms of regulation of triglyceride lipase activity. Neurohumoral regulation of lipolysis with the participation of adrenaline, norepinephrine, glucagon and insulin).

82. Reactions of oxidation of fatty acids (β-oxidation); the role of carnitine in the transport of fatty acids in mitochondria. Energetic activity of the oxidation of fatty acids in cells.

83. Oxidation of glycerol: enzymatic reactions, bioenergetics.

84. Ketone bodies. The reactions of biosynthesis and utilization of ketone bodies are of physiological significance. Disruption of the exchange of ketone bodies due to pathology (diabetes of the blood, fasting).

85. Biosynthesis of saturated fatty acids: reactions to the biosynthesis of saturated fatty acids (palmite) and regulation of the process. Biosynthesis of unsaturated fatty acids in the human body.

86. Biosynthesis of triacylglycerols and phosphoglycerides. Metabolism of sphingolipids. Genetic abnormalities in the metabolism of sphingolipides - sphingolipidoses.

87. Biosynthesis of cholesterol: reaction scheme, regulation of cholesterol synthesis. Ways of biotransformation of cholesterol: esterification; the release of urinary acids, steroid hormones, vitamin D₃.

88. Circulatory transport and deposition of lipids in adipose tissue. Lipoprotein lipase in endothelium.

Blood plasma lipoproteins: lipid and protein (apoprotein) storage. Hyper-lipoproteinemia.

89. Pathologies of lipid metabolism: atherosclerosis, obesity, blood diabetes.

3. Summing up.

4. List of recommended literature (main, additional, electronic information resources):

Main:

1. Gubsky Yu.I., I.V. Nizhenkovska, Korda M.M. Biological and Bioorganic Chemistry: in 2 books. Book 2. Biological Chemistry: textbook. 2021. 544 p.
2. Satyanarayana U. Biochemistry. 5th edition. India 2020. 777 p.
3. Lehninger. Principles of Biochemistry. 7th edition. NY, United States. 2017.
4. Jeremy M. Berg, John L. Tymoczko, Gregory J. Gatto. Biochemistry. 8th Revised edition. 2015.
5. Lippincott Illustrated Reviews: Biochemistry. Philadelphia :Wolters Kluwer, 2017. 560 p.
6. Baynes J., Dominiczak M. Medical Biochemistry. 5th Edition. Elsevier, 2018. 712 p.
7. Donald Voet, Judith G. Voet, Charlott W. Pratt. Fundamentals of Biochemistry: Life at the Molecular Level. ISBN: 978-1-118-91840-1 February 2016, 1184 P.

Additional:

1. William Marshall, Marta Lapsley, Andrew Day, Kate Shipman. Clinical Chemistry. Elsevier, 2020. 432 p.
2. Harper's Illustrated Biochemistry / V.W. Rodwell, D.A. Bender, K.M. Botham et al. – Mc Graw Hill Education, 2015. – 817 p.

Electronic information resources:

1. [https://info.odmu.edu.ua/chair/biology/-](https://info.odmu.edu.ua/chair/biology/)
2. <http://libblog.odmu.edu.ua/>
3. <https://moodle.odmu.edu.ua/login/index.php>

Practical class №22

Topic: The role of lipids in the structure and functions of biological membranes. Molecular mechanisms of lipolysis regulation. Tissue, intracellular metabolism of lipids. Oxidation of fatty acids and glycerol. Energy yield from fatty acid β -oxidation.

Goal:Formation of systemic knowledge on the structure of biomembranes, their role in cell protection, transmission of nerve impulses, creation of intercellular contacts. Study of molecular mechanisms of lipolysis and its regulation.

Basic concepts:lipids, biomembranes, lipolysis, hormonal mechanisms of lipolysis regulation.

Equipment:laboratory of the department.

Plan:

1. Organizational measures:greetings, verification of those present, announcement of the topic, purpose of the class, motivation

2. Control of the reference level of knowledge

2.1. Requirements for higher education applicants' theoretical readiness:

1. Define lipids as esters.
2. Alcohols included in the structure of lipids (glycerol, myricyl alcohol, cholesterol)
3. Higher fatty acids are structural components of lipids. Saturated and unsaturated higher fatty acids. Give their formulas.
4. What is the biological role of polyunsaturated fatty acids in the body.
5. Classification of lipids (simple, complex, derivatives of lipids). Give the formulas of simple

and complex lipids.

6. Fats (neutral - triglycerides, charged - phospholipids). Features of the structure of solid fats and liquid oils.

2.2. Questions to check basic knowledge on the topic of the class:

1. Biological role of lipids and higher fatty acids in the body.
2. Classification, chemical structure of lipids.
3. Types, functions and chemical composition of biomembranes.
4. Lipolysis Hormonal regulation of lipolysis
5. Enzymatic digestion of lipids.
6. Modern ideas about the mechanisms of absorption of lipids from the intestines into the blood and lymph. The role of bile acids in the absorption of lipid digestion products.

3. Formation of professional skills and abilities:demonstration and practical work.

3.1.1 Content of demonstration and practical work

Observation of the effect of bile on fat emulsification.

3.2.1 Implementation recommendations:

Measure 1 ml of water into 4 test tubes. Add 2 drops of oil to the first test tube, 2 drops of 0.5% baking soda and 2 drops of oil to the second, 1 ml of protein and 4 drops of oil to the third. In the fourth - 1 ml of bile and 2 drops of oil. Mix the contents of the test tubes by vigorous shaking.

Note: bile lowers the surface tension of fat droplets, forming stable emulsions.

3.1.2 Content of demonstration and practical work

Observation of the effect of pancreatic lipase on milk fat with and without bile.

3.2.2 Implementation recommendations:

Pour 1 ml of milk into 3 test tubes. Add 0.5 ml of water to the first and second. Add 0.5 ml of bile to the first test tube. Shake all the test tubes, add 2 drops of an alcoholic solution of phenolphthalein and an aqueous solution of KHSO₃ to each of them until it turns pale pink. Place all tubes in a thermostat at 37°C for 30 min. draw a conclusion

Note: phenolphthalein will become discolored when the pH of the medium changes due to the formation of fatty acids during the splitting of milk fat by lipase.

3.3. Requirements for work results:in a notebook for demonstration and practical work, write down the method of work performance, the results of observation. Provide a medico-biological evaluation of the obtained results. Draw conclusions.

3.4. Control materials for the final stage of the class:test tasks for the topic:

1. What lipids make up the basis of fat depots in the body?
A And steroids
B In phospholipids
C With triglycerols
D sphingolipids
E glycolipids
2. Which enzyme catalyzes the hydrolysis of triglycerides in the intestinal cavity?
A And monoglyceride lipases
B In acetylcholinesterase
C transacetylase
D lipase
E phospholipase
3. High lipase activity was detected in the gastric juice of a 6-month-old child. What is the optimal pH of this enzyme?
A- 7.8
B - 5.5

- C - 3.2
- D - 1.5
- E - 9.5

4. What pancreatic enzyme is activated by bile acids?

And proelastase

In lipase

C oligo-1-6-glucosidase

D trypsinogen

E chymotrypsinogen

5. Which protein of the pancreas takes part in the emulsification of fats?

And elastase

In trypsin

C collagenase

D chymotrypsin

E colipase

6. Which of the following substances are surface active and take part in the emulsification of fats?

And glycosidases

In bicarbonates

With bile acids

D proteases

E glycosaminoglycans

7. Deficiency of which enzyme is most often the cause of incomplete digestion of fats in the gastrointestinal tract and an increase in the amount of neutral fat in feces?

And enterokinase

In intestinal lipase

With pancreatic lipase

D gastric lipase

E liver lipase

8. In the bilipid layer of membranes, a protein molecule is fixed with the help of bonds:

And hydrophobic and ionic

In peptide and disulfide

With electrostatic, hydrophobic

D ionic and hydrogen

E of hydrogen and disulfide

9. The gradual process of lipolysis in adipocytes is enzymatic. Which of these enzymes controls the slowest stage of lipolysis?

And diglyceridlipase

In triglyceride lipase

C monoglyceride lipase

D glycerol acyltransferase

E phosphotidat phosphatase

10. The activity of tissue triglyceride lipase is regulated by hormones

And vasopressin, oxytocin

In adrenaline, insulin, glucagon, somatotropin
C cortisol, corticosterone
D thyroxine, triiodothyronine
E prostaglandins

11. A patient with pulmonary emphysema was prescribed oxygen treatment, which will probably lead to an increase in the activity of oxygen radicals in the body. What can this lead to?

And destruction of membrane phospholipids
In increasing the number of membranes
With a violation of the structure of protein components of membranes
D increase of transport proteins on membranes
E oxidation of cholesterol.

12. The doctor recommended a bile preparation to the patient to improve the digestion of fatty food. What components of this drug are involved in the emulsification of fats?

And diglycerides;
Cholesterol and its esters;
C higher fatty acids;
D bilirubin, glucuronides;
E bile acids.

4. Summary of the class. Assessment.

5. List of recommended literature

Main:

1. Gubsky Yu.I., I.V. Nizhenkovska, Korda M.M. Biological and Bioorganic Chemistry: in 2 books. Book 2. Biological Chemistry: textbook. 2021. 544 p.
2. Satyanarayana U. Biochemistry. 5th edition. India 2020. 777 p.
3. Lehninger. Principles of Biochemistry. 7th edition. NY, United States. 2017.
4. Jeremy M. Berg, John L. Tymoczko, Gregory J. Gatto. Biochemistry. 8th Revised edition. 2015.
5. Lippincott Illustrated Reviews: Biochemistry. Philadelphia :Wolters Kluwer, 2017. 560 p.
6. Baynes J., Dominiczak M. Medical Biochemistry. 5th Edition. Elsevier, 2018. 712 p.
7. Donald Voet, Judith G. Voet, Charlott W. Pratt. Fundamentals of Biochemistry: Life at the Molecular Level. ISBN: 978-1-118-91840-1 February 2016, 1184 P.

Additional:

1. William Marshall, Marta Lapsley, Andrew Day, Kate Shipman. Clinical Chemistry. Elsevier, 2020. 432 p.
2. Harper's Illustrated Biochemistry / V.W. Rodwell, D.A. Bender, K.M. Botham et al. – Mc Graw Hill Education, 2015. – 817 p.

Electronic information resources:

1. <https://info.odmu.edu.ua/chair/biology/>-
2. <http://libblog.odmu.edu.ua/>
3. <https://moodle.odmu.edu.ua/login/index.php>

Practical class №23

Topic: Biosynthesis of glycerol, fatty acids and glycerides. Formation of phospholipids. Structure, biological role and metabolism of cholesterol. Cholesterol biosynthesis. Disorders of lipid metabolism. Lipoproteins, structure and functions. Metabolism of acetoacetic acid. Ketone bodies.

Goal:Formation of systemic knowledge on intracellular lipid exchange. Study of the molecular mechanisms of the biosynthesis of VLKA, glycerol, triglycerides and phospholipids.

Basic concepts:higher fatty carboxylic acids, biosynthesis, glycerol, triglycerides, phospholipids.

Equipment:laboratory of the department.

Plan:

1. Organizational measures:greetings, verification of those present, announcement of the topic, purpose of the class, motivation

2. Control of the reference level of knowledge

2.1. Requirements for theoretical readiness of higher education applicants:

1. What components are necessary for the biosynthesis of simple fat - triglyceride?
2. What is the name of the active form of glycerin? Write the formula.
3. What is the active form of higher fatty acids called?
4. Name the sources of glycerol formation in the body?
5. What components are included in the composition of phospholipids - lecithin and kephalin?

2.2. Questions to check basic knowledge on the topic of the class:

1. Explain the necessity and mechanism of acetyl-CoA transport from mitochondria to cytoplasm.

2. Write the equation for the formation of malonyl-CoA, explain the role of active CO₂ in this process.

3. Structure of the multienzyme complex "synthase of higher fatty acids".

4. Write the stages of biosynthesis of a saturated higher fatty acid:

A) binding of acetyl-CoA to APB;

B) binding of malonyl-CoA to APB;

C) condensation of acyl from malonyl derivatives on APB;

D) reduction of β -keto-butyril-APB;

D) dehydration of β -hydroxybutyril-APB;

E) reduction of enoyl-APB followed by elongation of the butyril-APB carbon chain.

5. Write the equation of the enzymatic reactions of the formation of the active form of glycerol in adipose tissue and in the liver.

6. Write the reaction of the synthesis of phosphatidic acid followed by the formation of triglyceride.

7. Phospholipid synthesis reactions.

3. Formation of professional skills and abilities:demonstration and practical work.

3.1. Content of demonstration and practical work

3.1.1Determination of total lipids in blood serum by the method of Bang.

3.1.2.Qualitative reactions to acetone (iodoform, nitroprusside)

3.1.3.Quantitative determination of acetone in urine by the Rudogi method

3.1.4.Qualitative response to the presence of cholesterol in the brain

3.2. Implementation recommendations:

The principle of the method

3.2.1 Determination of total lipids in blood serum is carried out after their extraction with alcohol and ether. At further stages of determination, lipids are subjected to oxidation with potassium dichromate followed by iodometric titration of dichromate, sodium hyposulfite solution in test and control samples. Based on the difference between the number of milliliters of sodium hyposulfite used for the titration of control and experimental samples (K - D), the formula calculates the amount of total lipids in the blood, which normally ranges from 350 to 800 mg/100 ml, or 3.5 to 8.0 g/l serum.

The main stages of work performance.

1. Extraction of lipids according to Bloor: add 0.2 ml of blood serum drop by drop to 4 ml of alcohol with ether /3:1/. Heat in a sand bath for 15 minutes. filter through a paper filter, which is washed with 1 ml of a mixture of alcohol and ether, and bring the volume to 15 ml.

Notes: the normal concentration of total lipids is 3.5 - 8.0 g per liter of blood serum or 350-800 mg/per 100 ml of serum.

Pour 2.5 ml of filtrate into a test tube, corresponding to 0.1 ml of blood serum, add 1.0 ml of 1% caustic sodium solution and heat in a sand bath until the ether smell disappears. The control sample instead of blood serum contains 1.5 ml of water and 1 ml of 1% sodium hydroxide solution.

Note: All of the above is performed by the educational laboratory.

2. Oxidation of lipids with dichromate. After removing the ether, add 2 ml of 0.1N potassium dichromate solution and 3 drops of concentrated sulfuric acid to the filtrate. Place the test tube in a boiling water bath for 15 minutes, after heating carefully add water to the test tube, transfer all the liquid to a flask and add up to 100 ml of distilled water.

3. Iodometric titration of dichromate. Add 2 ml of potassium iodide solution, 2 drops of 1% starch solution, and titrate the contents of the flasks with 0.01N sodium hyposulfite solution to a pale green color. In parallel with the test sample, put a control, where instead of blood serum, take 1.5 ml of water and 1 ml of 1.0% caustic sodium solution. The difference between the control and the experiment divided by 2.45 corresponds to the amount in mg of lipids in the blood.

Counter - experiment

Calculation: $\frac{\text{---} \cdot 10,000 \text{ g/l}}{2.45}$

Make a calculation and give a medical - biological assessment of the result.

Medical and biological evaluation of the obtained results.

An increase in the content of total lipids in the blood (hyperlipemia) is a physiological phenomenon that occurs 1-3 hours after eating. This is the so-called alimentary hyperlipemia, which is temporary in healthy people.

In diabetes, hyperglycemia occurs simultaneously with hyperlipemia (i.e. 500 - 1000 mg or more / 100 ml)

In acute hepatitis, hyperlipemia is a constant phenomenon, especially in the presence of jaundice.

In acute and chronic nephritis accompanied by edema, the amount of lipids in the blood is increased.

With lipid nephrosis, the increase in the amount of lipids in the blood reaches 1000 mg or more /100 ml.

With chronic malnutrition and starvation, the amount of total lipids in the blood is increased due to the mobilization of fat from fat depots.

3.2.2. Lieben's iodoform test. Pour 2 ml of the tested urine into the test tube, add 1 ml of 10% caustic potassium solution and 6 drops of Lugol's solution. Mix the contents of the tube by shaking. Make a conclusion.

Notes:

In the presence of acetone, the solution becomes cloudy and a light yellow precipitate falls out.

Rother's nitroprusside test. Pour 2 ml of the tested urine into the test tube, add 0.5 ml of sodium nitroprusside and 0.5 ml of saturated ammonium sulfate solution. Mix the contents of the test tube by shaking and carefully pour 2 ml of ammonia solution along the wall. Watch. Make a conclusion.

Notes:

In the presence of acetone, a purple ring /coloring/ appears at the liquid boundary.

3.2.3. Pour 1 ml of saturated sodium chloride solution into 6 test tubes (except the first one), and 2 ml of the tested urine into the first one. From the first tube, transfer 1 ml to the second, and from the second to the third, etc., take 1 ml from the last. Thus, in each subsequent test tube, the

amount of urine will be 2 times less than in the previous one. That is, we will obtain a number of dilutions of 2, 4, 8, 16 and 32 times. Then add 0.5 ml of sodium nitroprusside solution and 1 ml of saturated ammonium sulfate solution to all test tubes and carefully layer 2 ml of ammonia solution; determine the layering time. Note in which test tube a purple ring appeared before the end of 4 min. Do the calculation, taking into account the dilution of urine in this test tube. Rate the result.

Notes

The time of appearance of color depends on the concentration of acetone. At a concentration of 0.85 mg%, a colored ring appears between 3.5-4 min. after ammonia layering.

Medical and biological evaluation of the obtained results

Acetone and other acetone bodies, if they are present in urine in detectable quantities, are pathological components of urine. If acetone is present in the urine, it means that it also contains other acetone bodies (acetoacetic and beta-hydroxybutyric acids). This condition - acetonuria, ketonuria - indicates a violation of lipid metabolism. During the intensive splitting of fats in the body (in the case of diabetes), a significant number of acetyl coenzyme-A molecules accumulate, which do not have time to be oxidized in the cycle of tricarboxylic acids to end products. The conditions for the transformation of acetoacetyl-CoA through an intermediate product to acetoacetic, beta-hydroxybutyric acids and acetone are created. Acetonuria is observed in severe forms of diabetes with impaired carbohydrate and lipid metabolism.

3.2.4. Brain tissue is rich in cholesterol, which is a cyclic, monounsaturated alcohol. During the interaction of sulfuric acid with cholesterol, its dehydration occurs, i.e. splitting of H₂O, as a result of which cholesterol turns into an unsaturated cyclic hydrocarbon of red-brown color.

Sequence of actions. Cholesterol is extracted from the brain by grinding a piece of tissue in a mortar, with the addition of 2 ml of chloroform.

Filter the resulting homogenate into a test tube through cotton wool.

To the obtained chloroform extract containing cholesterol, add (Caution!) 2 ml of conc. sulfuric acid (H₂SO₄).

Carefully mix the liquids. Let stand.

After settling and placement of liquids, observe the appearance of a red-brown color in the upper layer of the liquid, while the lower layer remains yellowish-green, the lower layer is occupied by sulfuric acid.

Medical and biological evaluation of the obtained results

Qualitative determination of cholesterol in the brain tissue confirms the known data that in the human body the most cholesterol is in the nervous tissue (myelin sheath) and in the cortex of the adrenal glands.

Human tissues contain about 140 g of cholesterol. Part of the cholesterol in the tissue is esterified by VHL, mainly oleic. Cholesterol esters are, as a rule, its stored or transport form. 2/3 of plasma lipoprotein cholesterol is esterified in blood, and the same amount of cholesterol is esterified in adrenal cells. In most other organs and tissues, cholesterol esters make up a smaller part of it (in the liver, for example, only 20-25%).

The cholesterol fund in the body is created due to food cholesterol (about 0.3 g per day). When eating plant-based food, low in cholesterol, the biosynthesis of cholesterol in the body is of leading importance.

3.2. Implementation recommendations:

3.3. Requirements for work results: in a notebook for demonstration and practical work, write down the method of work performance, the results of observation. Provide a medico-biological evaluation of the obtained results. Draw conclusions.

3.4. Control materials for the final stage of the class: test tasks for the topic:

1. What substances transport acetyl-CoA from the mitochondria to the cytoplasm?

And citrate, carnitine

In malonyl-CoA, butyryl-CoA

C malate, lactate

- D acyl-CoA, acetoacetyl - CoA
- E carnosine, anserine

2. A 65-year-old patient with signs of general obesity and the risk of fatty liver dystrophy is recommended a diet enriched with lipotropic substances, among which the content in products is important:

- A methionine
- B cholesterol
- C glucose
- D vitamin C
- E glycine

3. What process is the source of NADPH₂ for the synthesis of VHL?

- And mitochondrial oxidation
- In the pentose phosphate pathway of glucose oxidation
- C oxidation of pyruvate to acetyl-CoA
- D glucose oxidation to lactate
- E oxidation of ketoglutarate

4. In which tissue does the biosynthesis of triglycerides occur?

- And muscles
- In the intestines
- C adipose tissue
- D liver
- E mammary gland during lactation

5. Name the enzyme that catalyzes the formation of the active form of glycerol only in the liver.

- And dioxyacetone phosphate dehydrogenase
- B glycerol phosphate dehydrogenase;
- C glycerol phosphate transferase;
- D glycerol acyltransferase;
- E glycerol kinase.

6. Fatty degeneration of the liver develops in the absence or insufficient formation of lipotropic factors in the human body. Which of the following substances can be classified as lipotropic?

- A Cholesterol
- B Holin
- C Triacylglycerides
- D Fatty acids
- E Riboflavin

7. Which enzyme of biosynthesis of VLDL is biotin-containing:

- And acetyl-transacetylase
- In acetyl-CoA-carboxylase
- C β -ketoacyl-APB reductase
- D β -hydroxybutyryl-APB dehydratase
- E enoyl-APB reductase

8. When the substance A labeled by carbon is administered to experimental rats, the label is incorporated into glycerophospholipids and triglycerides, thus A is a common precursor in the biosynthesis of these lipids. Name the substance A.

And methionine;
In ethanolamine;
C choline; D cytosine triphosphate;
E phosphatidic acid.

9. The drug "Heptral", which is used for liver diseases, contains S-adenosine methionine. In what processes is this active amino acid involved?

And heme synthesis
In the synthesis of bile acids
Synthesis of triacylglycerols
D synthesis of cholesterol
E synthesis of phospholipids

10. Excessive consumption of carbohydrates (600 g per day), which exceeds the energy needs of a 28-year-old person, will be accompanied by the activation of:

A lipolysis
B lipogenesis
C of glycolysis
D gluconeogenesis
E β -oxidation of fatty acids

11. Fatty degeneration of the liver during starvation and diabetes develops because hepatocytes:

And the intake of fatty acids from adipose tissue increases
Fatty acid oxidation decreases
C reduces the formation of ketone bodies from fatty acids
And the synthesis of triacylglycerols decreases
And the formation of high-density lipoproteins decreases

12. Biosynthesis of fatty acids is constantly taking place in the body. Which of the listed substances is the main source of their biosynthesis?

A succinyl-CoA
B glucose-6-phosphate
C acetyl-CoA
D acyladenylate
E aminoacyladenylate

13. The patient consumes several raw eggs daily, which contain the antivitamin biotin-avidin. What disorders in lipid metabolism can occur in this case?

A biosynthesis of cholesterol
B biosynthesis of fatty acids
C oxidation of glycerol
D absorption of lipids
E transport of lipids in the blood

14. The doctor recommended a patient with coronary heart disease to use fats containing polyunsaturated higher fatty acids. What components of biomembranes are synthesized with the participation of these substances?

A phospholipids
B cholesterol
C proteins
D glycolipids
E lipoproteins

15. In the human body, both triacylglycerols [neutral fats] and glycerophospholipids are synthesized from the same precursor, namely from:

- A acetic acid.
- B orotic acid.
- C phosphatidic acid.
- D lipoic acid.
- E malonic acid.

4. Summary of the class. Assessment.

5. List of recommended literature

Main:

1. Gubsky Yu.I., I.V. Nizhenkovska, Korda M.M. Biological and Bioorganic Chemistry: in 2 books. Book 2. Biological Chemistry: textbook. 2021. 544 p.
2. Satyanarayana U. Biochemistry. 5th edition. India 2020. 777 p.
3. Lehninger. Principles of Biochemistry. 7th edition. NY, United States. 2017.
4. Jeremy M. Berg, John L. Tymoczko, Gregory J. Gatto. Biochemistry. 8th Revised edition. 2015.
5. Lippincott Illustrated Reviews: Biochemistry. Philadelphia :Wolters Kluwer, 2017. 560 p.
6. Baynes J., Dominiczak M. Medical Biochemistry. 5th Edition. Elsevier, 2018. 712 p.
7. Donald Voet, Judith G. Voet, Charlott W. Pratt. Fundamentals of Biochemistry: Life at the Molecular Level. ISBN: 978-1-118-91840-1 February 2016, 1184 P.

Additional:

1. William Marshall, Marta Lapsley, Andrew Day, Kate Shipman. Clinical Chemistry. Elsevier, 2020. 432 p.
2. Harper's Illustrated Biochemistry / V.W. Rodwell, D.A. Bender, K.M. Botham et al. – Mc Graw Hill Education, 2015. – 817 p.

Electronic information resources:

1. <https://info.odmu.edu.ua/chair/biology/>-
2. <http://libblog.odmu.edu.ua/>
3. <https://moodle.odmu.edu.ua/login/index.php>

Practical class №24.

Topic: Ways of formation and maintenance of the pool of amino acids in the body. Transport of amino acids into cells. Deamination of amino acids. Mechanism of indirect deamination of L-amino acids. Decarboxylation of amino acids: enzymes, physiological significance. Oxidation of biogenic amines. Transamination. Biochemical significance, mechanisms of action of aminotransferases. Diagnostic value of determination of aminotransferases in blood serum.

Goal: Learn about deamination reactions, which are the central link in the intracellular metabolism of amino acids. As a result of deamination processes, ammonia is formed - a toxic substance that is subject to temporary and final detoxification processes and is excreted in the urine in the form of end products of nitrogenous metabolism, one of which are ammonium salts. During the decarboxylation of amino acids, biogenic amines are formed, which are mediators of the central nervous system and have a hormonal effect.

Basic concepts: pool of amino acids, transport of amino acids through the biological membrane, deamination, decarboxylation.

Equipment: Laboratory of the department

Plan:

1. Organizational measures (greetings, verification of those present, announcement of the topic, purpose of the class, motivation of higher education seekers to study the topic).

2. Control of the reference level of knowledge.

The higher education applicant should know :

- the composition of the biological membrane
- replaceable and essential amino acids
- α -ketoglutarate shuttle mechanism
- mechanism of decarboxylation
- The role of pyridoxal phosphate in metabolic processes.

The higher education applicant should be able to:

- write structural formulas of amino acids
- explain the role of glutathione in the transport of amino acids
- which are central nervous system mediators
- indicate reactions of amino acid metabolism
- explain the role of biogenic amines
- explain the role of PALF in the metabolism of amino acids
- know the shuttle mechanisms of pyruvate and α -ketoglutarate

Questions to check basic knowledge on the topic of the class:

- structural formula of pyruvate
- structural formula of α -ketoglutarate
- functions of catecholamines
- structural formulas of 20 amino acids
- biological role of aminoxylotes in the human body
- the difference between replaceable and essential amino acids

3. Formation of professional skills:

3.1 Demonstration and practical work: Quantitative determination of ammonia nitrogen (ammonium salts) in urine by the Model method.

Recommendations for performing tasks

Principle of the method:

Nessler's reagent forms an orange-colored complex salt with ammonium salts, the color intensity is proportional to the amount of ammonium salts, therefore a colorimetric method of determination is used.

Progress:

Pour 0.5 ml of 10-fold diluted urine into one test tube, and 0.5 ml of standard $(\text{NH}_4)_2\text{SO}_4$ solution containing 0.025 mg of nitrogen into the second. Add 0.5 ml of distilled water and 0.5 ml of ferrous salt to both test tubes. Mix, add 0.1 ml of Nessler's reagent to both test tubes.

Colorimetry on the FEK (photoelectrocolorimeter) with a green light filter (wavelength 500-600 nm) in a cuvette with a layer thickness of 1 cm against water.

The calculation is made according to the formula:

$$C_x = \frac{C_{cr} \times E_x}{E_{cr}}, \text{ where}$$

C_{cr} - 0.025 mg of nitrogen

E_{cr} - optical density of the standard sample

E_x - optical density of the test sample

C_x - the nitrogen content of 0.5 ml of urine diluted 10 times.

When completed, dilution and daily diuresis (D) are counted. According to the norm, the composition of nitrogen salts in urine is 0.5-1.2 g per day.

Clinical and diagnostic value of the method:

The amount of ammonium salts in the urine increases in chronic and severe forms of diabetes accompanied by acidosis, diffuse liver diseases with impaired urea synthesis. The amount of ammonium salts decreases with a plant-based diet and kidney disease

Make medical and biological conclusions.

Requirements for work results.

Enter the obtained data and calculations into the workbook.

Make medical and biological conclusions.

Test tasks:

A 7-year-old child was brought to the emergency hospital in a state of allergic shock, which developed after a wasp bite. The blood concentration of histamine is increased. As a result of which reaction is this amine formed?

- A - restoration;
- B - dehydrogenation;
- C - deamination;
- D - hydrolysis;
- E* - decarboxylation.

A 24-year-old patient was injected with glutamic acid to treat epilepsy. The therapeutic effect in this disease is due not to glutamate itself, but to the product of its decarboxylation.

- A - adrenaline;
- B* - GABA;
- C - histamine;
- D - serotonin;
- E - dopamine.

3. A 32-year-old man was diagnosed with acute radiation sickness. A sharp decrease in the level of serotonin in platelets was established in the laboratory. The most possible reason for a decrease in platelet serotonin is a violation of the decarboxylation process:

- A - serine;
- B - histidine;
- C - pyruvic acid;
- D - tyrosine;
- E* - 5 - oxytryptophan.

The patient, who is in the gastroenterology department, is prescribed a histamine test. For what purpose is histamine administered to the patient?

- A - to study the secretory function of the stomach;
- B - to stimulate digestion of lipids in the intestine;
- C - to assess the activity of proteolytic enzymes of the pancreas;
- D - for activation of limited proteolysis in the intestine;
- E* - for studying the nitrogen balance.

5. There are several ways of neutralizing ammonia in the body, but there are specific ones for individual organs. What is the path of neutralization of ammonia typical for brain cells?

- A* - formation of glutamine;
- B - formation of urea;
- C - formation of asparagine;
- D - formation of ammonium ion;
- E - the formation of a fold.

4. Summary.

5. List of recommended literature (main, additional, electronic information resources):

Main:

1. Gubsky Yu.I., I.V. Nizhenkovska, Korda M.M. Biological and Bioorganic Chemistry: in 2 books. Book 2. Biological Chemistry: textbook. 2021. 544 p.
2. Satyanarayana U. Biochemistry. 5th edition. India 2020. 777 p.
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Additional:

1. William Marshall, Marta Lapsley, Andrew Day, Kate Shipman. Clinical Chemistry. Elsevier, 2020. 432 p.
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Electronic information resources:

1. [https://info.odmu.edu.ua/chair/biology/-](https://info.odmu.edu.ua/chair/biology/)
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Practical class № 25.

Topic:Ammonia metabolism in the human body. Urea. Ways of ammonia formation. Ammonia toxicity and mechanisms of its neutralization. Transport forms of ammonia (glutamine and asparagine). Urea biosynthesis: enzyme reactions, genetic abnormalities.

Goal:To study the main mechanism of ammonia detoxification in the human body, urea is the main end product of nitrogen metabolism.

Basic concepts:ornithine cycle, ammonia toxicity, ways of its neutralization, glutamine, asparagine.

Equipment:Laboratory of the department

Plan:

1. Organizational measures (greetings, verification of those present, announcement of the topic, purpose of the class, motivation of higher education seekers to study the topic).

2. Control of the reference level of knowledge.

The higher education applicant should know :

- the amount of ammonia formed during the deamination of amino acids
- blood urea level
- transport forms of ammonia
- reasons for increasing or decreasing the level of urea in liquids
- urinary function of the liver
- excretory function of the kidneys
- state of protein metabolism
- the role of urinary function

The higher education applicant should be able to:

- write the reactions of the ornithine cycle
- write the formula of carbomoyl phosphate
- explain the difference between urea and urine
- write the formula of the vitamin present in the reaction with carbomoyl phosphate
- write reactions with consumption of ATP

Questions to check basic knowledge on the topic of the class:

- The role of dicarboxylic acids in the processes of binding and transport of ammonia in the blood

- A compound formed from ammonia and carbon dioxide in the presence of ATP
- What vitamin is necessary for the functioning of carbamoyl phosphate synthetase
- Reaction catalyzed by ornithine carbamoyltransferase.
- The reaction of formation of citrulline, arginine in the process of urea biosynthesis.
- The energy of how many ATP molecules is spent on the synthesis of one molecule of urea
- Under which pathological conditions can the synthesis of urea increase
- What lesions of the liver lead to a decrease in its urea-forming function

3. Formation of professional skills:

3.1 Demonstration and practical work: "Quantitative determination of urea in urine"

Recommendations for performing tasks.

Content of laboratory work

Principle of the method: urea forms a red complex with diacetyl monooxime in the presence of Fe³⁺ ions and thiosemicarbazide, and its concentration is determined by the intensity of the color.

Progress: solutions of diacetyl monoxide, biological fluid or physiological solution and thiosemicarbazid are measured sequentially in test tubes, in accordance with the table. Urine must be diluted 30 or 100 times before starting the analysis, multiply the obtained results by the dilution factor.

Measured liquid	Experimental	Sample calibration	empty
Diacetyl monooxime	1.0 ml	1.0 ml	1.0 ml
Biological fluid	-	0.01 ml	-
Calibration solution of urea	-	0.01 ml	-
Physiological solution	-	-	0.01 ml
Thiosemicurea solution	1.0 ml	1.0 ml	1.0 ml

The test tubes are covered with foil, the contents are mixed and simultaneously placed in a boiling water bath for exactly 10 minutes. Then the test tubes are cooled under a stream of cold water. Then colorimetry is performed on a photoelectrocolorimeter.

The concentration of urea is calculated according to the formula:

Ex.

$C = \frac{Ex}{Ekal} \cdot 8.32 \text{ mmol/l}$, where

Ekal

C - concentration of urea; Ex. - optical density of the test sample; Ekal - optical density of the calibration sample.

Norm: blood - 2.5-8.3 mmol/l, urine - 330-580 mmol/l.

Clinical and diagnostic value of the method

The content of urea in the blood serum of healthy people is 3.3-8.3 mmol/l (20-50 mg%). A decrease in this indicator is observed in parenchymal hepatitis, cirrhosis and liver dystrophy, which are accompanied by a sharp decrease in urea biosynthesis, as well as during pregnancy and eclampsia.

An increase in the content of urea in the blood serum is one of the main signs of nephritis and tuberculosis of the kidneys, but it is also observed with increased breakdown of proteins in the body, with loss of fluid (dehydration, vomiting, diarrhea), with sepsis, fever, and excess protein nutrition.

Requirements for work results.

Enter the obtained data and calculations into the workbook.

Make medical and biological conclusions.

Test tasks.

How is ammonia neutralized in the body?

- A - is partially used to neutralize acids;
- B - by synthesis of urea;
- C - in the form of amides;
- D - goes to the synthesis of ammonium salts;
- E* - in all the indicated ways.

How much urea is formed per day in an adult?

- A* - 25-35 g (depending on the amount of proteins in the diet);
- B - 10-35 g;
- C - 35-50 g;
- D - 50-75 g;
- E - 2-5 g.

Ammonia is a very poisonous substance, especially for the nervous system. Which compound is particularly active in neutralizing ammonia in brain tissues?

- A. Lysine.
- B. Glutamic acid
- C. Proline.
- D. Histidine
- E.* Alanine

Citrulline and a high level of ammonia are determined in the urine of newborns. The formation of which substance is most likely disturbed?

- A. Ammonia.
- B*. Uric acid.
- C. Urea.
- D. Creatinine.
- E. Creatine

4. Summary.

5. List of recommended literature (main, additional, electronic information resources):

Main:

1. Gubsky Yu.I., I.V. Nizhenkovska, Korda M.M. Biological and Bioorganic Chemistry: in 2 books. Book 2. Biological Chemistry: textbook. 2021. 544 p.
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Additional:

1. William Marshall, Marta Lapsley, Andrew Day, Kate Shipman. Clinical Chemistry. Elsevier, 2020. 432 p.
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2. <http://libblog.odmu.edu.ua/>
3. <https://moodle.odmu.edu.ua/login/index.php>
4. <http://moz.gov.ua> – Ministry of Health of Ukraine
5. www.who.int - World Health Organization
6. www.dec.gov.ua/mtd/home/ - State Expert Center of the Ministry of Health of Ukraine
7. <http://bma.org.uk> - British Medical Association
8. www.gmc-uk.org - General Medical Council (GMC)

Topic: Ways of formation and maintenance of the pool of amino acids in the body. Transport of amino acids into cells. Deamination of amino acids. Mechanism of indirect deamination of L-amino acids. Decarboxylation of amino acids: enzymes, physiological significance. Oxidation of biogenic amines. Transamination. Biochemical significance, mechanisms of action of aminotransferases. Diagnostic value of determination of aminotransferases in blood serum. Ammonia metabolism in the human body. Urea. Ways of ammonia formation. Ammonia toxicity and mechanisms of its neutralization. Transport forms of ammonia (glutamine and asparagine). Urea biosynthesis: enzyme reactions, genetic anomalies.

Goal: Learn about deamination reactions, which are the central link in the intracellular metabolism of amino acids. As a result of deamination processes, ammonia is formed - a toxic substance that is subject to temporary and final detoxification processes and is excreted in the urine in the form of end products of nitrogenous metabolism, one of which are ammonium salts. During the decarboxylation of amino acids, biogenic amines are formed, which are mediators of the central nervous system and have a hormonal effect. Know the effect of alanine aminotransferase (ALT) and aspartate aminotransferase (AST), which is widely used in the clinic for early diagnosis of hepatitis of various etiologies and early diagnosis of myocardial infarction. Transamination reactions are a central link in the metabolism of amino acids, controlling the metabolism of proteins and carbohydrates, as well as lipids.

Basic concepts: pool of amino acids, transport of amino acids through the biological membrane, deamination, decarboxylation.

Equipment: Laboratory of the department

Plan:

1. Organizational measures (greetings, verification of those present, announcement of the topic, purpose of the class, motivation of higher education seekers to study the topic).

2. Control of the reference level of knowledge.

The higher education applicant should know:

- the amount of ammonia formed during the deamination of amino acids
- blood urea level
- transport forms of ammonia
- reasons for increasing or decreasing the level of urea in liquids
- urinary function of the liver
- excretory function of the kidneys
- state of protein metabolism
- the role of urinary function

- the composition of the biological membrane
- replaceable and essential amino acids
- α -ketoglutarate shuttle mechanism
- mechanism of decarboxylation
- The role of pyridoxal phosphate in metabolic processes.

The higher education applicant should be able to:

- write structural formulas of amino acids
- explain the role of glutathione in the transport of amino acids
- which are central nervous system mediators
- indicate reactions of amino acid metabolism
- explain the role of biogenic amines
- explain the role of PALF in the metabolism of amino acids
- know the shuttle mechanisms of pyruvate and α -ketoglutarate

Questions to check basic knowledge on the topic of the class:

- structural formula of pyruvate
- structural formula of α -ketoglutarate
- functions of catecholamines
- structural formulas of 20 amino acids
- biological role of aminoxylores in the human body
- the difference between replaceable and essential amino acids

3. Formation of professional skills:

3.1 Demonstration and practical work: Quantitative determination of ammonia nitrogen (ammonium salts) in urine by the Model method.

Recommendations for performing tasks

Principle of the method:

Nessler's reagent forms an orange-colored complex salt with ammonium salts, the color intensity is proportional to the amount of ammonium salts, therefore a colorimetric method of determination is used.

Progress:

Pour 0.5 ml of 10-fold diluted urine into one test tube, and 0.5 ml of standard $(\text{NH}_4)_2\text{SO}_4$ solution containing 0.025 mg of nitrogen into the second. Add 0.5 ml of distilled water and 0.5 ml of ferrous salt to both test tubes. Mix, add 0.1 ml of Nessler's reagent to both test tubes.

Colorimetry on the FEK (photoelectrocolorimeter) with a green light filter (wavelength 500-600 nm) in a cuvette with a layer thickness of 1 cm against water.

The calculation is made according to the formula:

$$C_x = \frac{C_{cr} \times E_x}{E_{cr}}, \text{ where}$$

C_{cr} - 0.025 mg of nitrogen

E_{cr} - optical density of the standard sample

E_x - optical density of the test sample

C_x - the nitrogen content of 0.5 ml of urine diluted 10 times.

When completed, dilution and daily diuresis (D) are counted. According to the norm, the composition of nitrogen salts in urine is 0.5-1.2 g per day.

Clinical and diagnostic value of the method:

The amount of ammonium salts in the urine increases in chronic and severe forms of diabetes accompanied by acidosis, diffuse liver diseases with impaired urea synthesis. The amount of ammonium salts decreases with a plant-based diet and kidney disease.

Make medical and biological conclusions.

Requirements for work results.

Enter the obtained data and calculations into the workbook.

Make medical and biological conclusions.

3.2. Demonstration and practical work "Detection of alanine aminotransferase (AlAT) in normal and pathological blood serum."

Recommendations for completing tasks:

The method is based on the study of the activity of alanine aminotransferase expressed in micromoles of pyruvic acid, which was formed during the incubation of 1 ml of serum for 1 hour at 37°C according to the formula: $x=C*2*10$, where C is micromoles of pyruvate, which is found using a calibration curve, 10 is the conversion factor for 1 ml of serum, 2 is the conversion factor for 1 hour of incubation. Normally, the activity of alanine aminotransferase is equal to 0.1-0.68 $\mu\text{M/h}$, the activity of alanine aminotransferase is 0.1-0.45 $\mu\text{M/h}$.

Progress.

Measure 0.5 ml of 1% solution into two test tubes α - acid glutarate, 0.5 ml of 1% analin solution and 1 ml of 0.1% KHSO_3 solution. Add 0.5 ml of the patient's serum to one test tube. Mix, place both test tubes for 30 minutes in a thermostat at a temperature of 37°C.

After incubation, add 0.5 ml of 2,4-dinitrophenylhydrazine solution and 0.5 ml of 0.4 N NaOH solution to each test tube, mix. Give color. If the activity of alanine aminotransferase is low, the color will be pale, if it is high, it will be dark.

We measure color on a photoelectrocolorimeter (PEKi) with a green light filter (wavelength 500-560 nm) and find the optical density. Then we calculate the activity of alanine aminotransferase with the help of a calibration curve according to the formula given earlier

Medical and biological evaluation of the obtained results.

The activity of analin and aspartate aminotransferase in the blood increases in diseases that penetrate with necrosis and tissue damage - mainly the heart muscle and liver. During a myocardial infarction, AST activity reaches its maximum after 6-12 hours, and ALT activity rises less noticeably. With infectious hepatitis, we observe the second picture - the activity of alanine aminotransferase increases much more noticeably than the activity of aspartame aminotransferase. Therefore, the aminotransferase test is a valuable diagnostic test.

Requirements for work results.

Enter the obtained data and calculations into the workbook.

Make medical and biological conclusions.

3.3 Demonstration and practical work: "Quantitative determination of urea in urine"

Recommendations for performing tasks.

Content of laboratory work

Principle of the method: urea forms a red complex with diacetyl monooxime in the presence of Fe^{3+} ions and thiosemicarbazide, and its concentration is determined by the intensity of the color.

Progress: solutions of diacetyl monoxide, biological fluid or physiological solution and thiosemicarbazid are measured sequentially in test tubes, in accordance with the table. Urine must be diluted 30 or 100 times before starting the analysis, multiply the obtained results by the dilution factor.

Measured liquid	Experimental	Sample calibration	empty
Diacetyl monooxime	1.0 ml	1.0 ml	1.0 ml
Biological fluid	-	0.01 ml	-
Calibration solution of urea	-	0.01 ml	-
Physiological solution	-	-	0.01 ml
Thiosemicurea solution	1.0 ml	1.0 ml	1.0 ml

The test tubes are covered with foil, the contents are mixed and simultaneously placed in a boiling water bath for exactly 10 minutes. Then the test tubes are cooled under a stream of cold water. Then colorimetry is performed on a photoelectrocolorimeter.

The concentration of urea is calculated according to the formula:

Ex.

$C = \text{-----} 8.32 \text{ mmol/l}$, where

Ekal

C - concentration of urea; Ex. - optical density of the test sample; Ekal - optical density of the calibration sample.

Norm: blood - 2.5-8.3 mmol/l, urine - 330-580 mmol/l.

Clinical and diagnostic value of the method

The content of urea in the blood serum of healthy people is 3.3-8.3 mmol/l (20-50 mg%). A decrease in this indicator is observed in parenchymal hepatitis, cirrhosis and liver dystrophy, which are accompanied by a sharp decrease in urea biosynthesis, as well as during pregnancy and eclampsia.

An increase in the content of urea in the blood serum is one of the main signs of nephritis and tuberculosis of the kidneys, but it is also observed with increased breakdown of proteins in the body, with loss of fluid (dehydration, vomiting, diarrhea), with sepsis, fever, and excess protein nutrition.

Requirements for work results.

Enter the obtained data and calculations into the workbook.

Make medical and biological conclusions.

Test tasks.

What is the difference between reamination and deamination?

A* – transfer of amino groups from an amino acid to a keto acid;

B – there is no difference – amino acid by deamination to keto acid;

C - in the organism of higher animals and humans, peramination does not occur; islot

D – by transferring the amino group to asparagine and glutamine with the formation of amides;

E - the formation of hydrochloric acids.

Reamination processes provide all processes, with the exception of:

A* – binding of ammonia;

B – deamination of a number of amino acids;

C – transamination;

D – synthesis of individual (replaceable) amino acids;

E - formation of ammonia.

The vitamin takes part in transamination processes:

A – ascorbic acid;

B – thiamine;

C – biotin;

D* – pyridoxamine;

E is routine.

ALT activity is slightly elevated. What additional sign will help establish a patient with gallstone disease, and not hepatitis?

A – transketolosis;

B – cholinesterosis;

C – glycogen synthetase;

D* – alkaline phosphatase;

E - arginase.

The patient complained of nausea, increased fatigue. When examining blood serum, the activity of ALT was 2.3 mmol/hour, LDH was 14 mmol/hour, and the content of LDH-5 was increased. Previous diagnosis:

A – gastritis;

B – myocardial infarction;

C – gallstone disease;

D* – hepatitis;

E - glomerulonephritis.

For a long time, the patient experienced chest pain radiating under the left scapula, and was admitted to the clinic due to the deterioration of his health. In the study of blood serum, the activity of AST-1.2 mmol/h.L, LDH-16 mmol/h.L, increased content of LDH-1. What disease can you think of?

- A – gastritis;
- B* – myocardial infarction;
- C – gallstone disease;
- D – hepatitis;
- E - glomerulonephritis.

Test tasks:

A 7-year-old child was brought to the emergency hospital in a state of allergic shock, which developed after a wasp bite. The blood concentration of histamine is increased. As a result of which reaction is this amine formed?

- A - restoration;
- B - dehydrogenation;
- C - deamination;
- D - hydrolysis;
- E* - decarboxylation.

A 24-year-old patient was injected with glutamic acid to treat epilepsy. The therapeutic effect in this disease is due not to glutamate itself, but to the product of its decarboxylation.

- A - adrenaline;
- B* - GABA;
- C - histamine;
- D - serotonin;
- E - dopamine.

3. A 32-year-old man was diagnosed with acute radiation sickness. A sharp decrease in the level of serotonin in platelets was established in the laboratory. The most possible reason for a decrease in platelet serotonin is a violation of the decarboxylation process:

- A - serine;
- B - histidine;
- C - pyruvic acid;
- D - tyrosine;
- E* - 5 - oxytryptophan.

The patient, who is in the gastroenterology department, is prescribed a histamine test. For what purpose is histamine administered to the patient?

- A - to study the secretory function of the stomach;
- B - to stimulate digestion of lipids in the intestine;
- C - to assess the activity of proteolytic enzymes of the pancreas;
- D - for activation of limited proteolysis in the intestine;
- E* - for studying the nitrogen balance.

5. There are several ways of neutralizing ammonia in the body, but there are specific ones for individual organs. What is the path of neutralization of ammonia typical for brain cells?

- A* - formation of glutamine;
- B - formation of urea;
- C - formation of asparagine;
- D- formation of ammonium ion;
- E - the formation of a fold.

4. Summary.

5. List of recommended literature (main, additional, electronic information resources):

Main:

1. Gubsky Yu.I., I.V. Nizhenkovska, Korda M.M. Biological and Bioorganic Chemistry: in 2 books. Book 2. Biological Chemistry: textbook. 2021. 544 p.
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3. <https://moodle.odmu.edu.ua/login/index.php>

Practical class №26.

Topic: Ways of metabolism of the nitrogen-free skeleton of amino acids in the human body. Glycogenic and ketogenic amino acids. Specialized ways of exchange of acyclic amino acids. Glutathione, its role in the exchange of organic peroxides. Arginine exchange, biological role of nitric oxide, NO-synthase. Features of branched-chain amino acid metabolism: participation of coenzyme forms of vitamin B12 in amino acid metabolism. Metabolic pathways of cyclic amino acids. Hereditary enzymopathies of cyclic and acyclic amino acid metabolism.

Goal:to investigate the ways of metabolism of the nitrogen-free skeleton of amino acids, to know their biological significance, the function of glitaton, the metabolism of cyclic amino acids.

Basic concepts:ways of exchange of acyclic and cyclic amino acids, synthesis of catecholamines, creatine, creatinine.

Equipment:Laboratory of the department

Plan:

1. Organizational measures (greetings, verification of those present, announcement of the topic, purpose of the class, motivation of higher education seekers to study the topic).

2. Control of the reference level of knowledge.

The higher education applicant should know:

- inclusion of amino acids in CTC
- structural formula of glutamine
- synthesis of glycine
- functions in serine and glycine metabolism
- threonine metabolism
- functions of individual nitrogenous and nonnitrogenous amino acids
- pathologies of amino acid metabolism

The higher education applicant should be able to:

- describe maple syrup disease
- know the concept of cystinuria

- describe phenylketonuria
- violation of amino acid metabolism in albinism
- sources of tryptophan
- Explain the metabolic schemes of transformations of individual amino acids
- To characterize the chemistry of creatine synthesis and cleavage
- Determine creatinine in blood serum
- Interpret results regarding serum creatinine concentration

Questions to check basic knowledge on the topic of the class:

- Specialized ways of exchange of acyclic amino acids. Exchange of glycine and serine.
- The role of folate in the transfer of one-carbon radicals. Dehydrofolate reductase inhibitors.
- Exchange of sulfur-containing amino acids: methylation reactions; creatine synthesis.
- Glutathione, its role in the exchange of organic peroxides.
- Features of the exchange of amino acids with branched chains.
- Participation of coenzyme forms of vitamin B12 and biotin in amino acid metabolism.
- Arginine exchange; biological role of nitric oxide, NO-synthase.
- Specialized pathways of cyclic amino acid metabolism.

3. Formation of professional skills:

3.1. Demonstration and practical work: "Reaction to phenylpyruvic acid (Fehling's test)"

Recommendations for performing tasks.

Principle of the method. Phenylpyruvic acid forms a blue-green complex compound with trivalent ferrum ions.

Procedure. Add 8-10 drops of 5% FeCl₃ solution to 2 ml of freshly filtered urine. In the presence of phenylpyruvic acid in the urine, a blue-green color appears after 30-60 seconds, which gradually disappears within 5-30 minutes, depending on the concentration of phenylpyruvic acid in the urine.

Clinical and diagnostic significance. Congenital absence of the enzyme phenylalanine-4-monooxygenase in the liver of children leads to the blocking of the oxidation of phenylalanine to tyrosine and, accordingly, all subsequent metabolic transformations of tyrosine. Accumulation of phenylalanine and its breakdown products, including phenylpyruvic acid, in the blood and tissues causes intoxication of the body. The consequence of this is a violation of the normal development of the brain and severe nervous disorders. The diagnostic criterion of this hereditary disease is the increased content of phenylalanine in the blood, the presence of phenylpyruvic acid in the urine. Normally, the average concentration of phenylalanine in the blood of children is: up to 1 month - 0.133 mmol/l, from 1 month to 1 year - 0.095 mmol/l, from 1 year to 14 years - 0.115 mmol/l.

The test for phenylpyruvic acid can be performed on filter paper. A strip of filter paper is moistened with urine, dried in air and a drop of 10% FeCl₃ solution is applied. A positive test gives a blue-green color. A similar test can be performed on a dry or wet baby diaper.

Requirements for work results.

Enter the obtained data and calculations into the workbook.

Make medical and biological conclusions.

Test tasks:

The patient has disturbed sleep, there is a weakening of the activity of inhibitory processes in the central nervous system, which is associated with a violation of the formation of gamma-aminobutyric acid. What substance is the precursor of GABA?

- A Histidine
- B Tryptophan
- C Methionine
- D Valin
- E Glutamate

When forming the tertiary structure of most proteins, nonpolar amino acid residues form the inner hydrophobic part of the globule. Name one of these hydrophobic

amino acids.

- A valine
- B lysine
- C arginine
- D glutamic acid
- E aspartic acid

The hormone of local action, histamine, is produced in the lungs, digestive system, and skin.

He is

vasodilator. Indicate which compound it is as a result of decarboxylation is formed:

- A Histidine
- B Valina
- C Alanine
- D Serena
- E Threonine

In the process of decarboxylation of 5-hydroxytryptophan, a biogenic amine is formed, which has

vasoconstrictor effect. Name this biogenic amine.

- A serotonin
- B histamine
- C gamma-aminobutyric acid
- D putrescine
- E cadaverine

In a man who suffers from chronic intestinal obstruction, the decay of proteins increases in the large intestine. What toxic substance is formed in this case with tryptophan:

- A Indole
- B Bilirubin
- C Lactate
- D Creatine
- E Glucose

The patient warned that the use of painkillers can cause allergic shock. An increase in the amount of which biogenic amine in the blood can be the cause such a state?

- A Histamine;
- B GABA;
- C Cadaverine;
- D Dopamine;
- E Putrescin

L-DOPA and its derivatives are used to treat Parkinson's disease. From which Amino acids form this substance?

- A Tyrosine
- B Asparagine
- C Glutamate
- D Tryptophan
- E Arginine

In the course of histidine catabolism, a biogenic amine is formed, which has a significant vasodilating effect. Specify this substance.

- A Histamine
- B Serotonin
- C DOFA
- D Thyroxine

E Dopamine

The patient has pronounced allergic symptoms: rashes on the body, swelling of the face, itching. From the increase in the formation of which biogenic amine is it related to?

A Histamine

B Serotonin

C adrenaline

D Norepinephrine

E to Holin

The structure of the lateral radical is the basis of the structural classification of amino acids. Which of the listed amino acids belongs to diaminomono-carbonic ones?

A Lysine

B Proline

C Valin

D Leucine

E Methionine

4. Summary.

5. List of recommended literature (main, additional, electronic information resources):

Main:

1. Gubsky Yu.I., I.V. Nizhenkovska, Korda M.M. Biological and Bioorganic Chemistry: in 2 books. Book 2. Biological Chemistry: textbook. 2021. 544 p.
2. Satyanarayana U. Biochemistry. 5th edition. India 2020. 777 p.
3. Lehninger. Principles of Biochemistry. 7th edition. NY, United States. 2017.
4. Jeremy M. Berg, John L. Tymoczko, Gregory J. Gatto. Biochemistry. 8th Revised edition. 2015.
5. Lippincott Illustrated Reviews: Biochemistry. Philadelphia :Wolters Kluwer, 2017. 560 p.
6. Baynes J., Dominiczak M. Medical Biochemistry. 5th Edition. Elsevier, 2018. 712 p.
7. Donald Voet, Judith G. Voet, Charlott W. Pratt. Fundamentals of Biochemistry: Life at the Molecular Level. ISBN: 978-1-118-91840-1 February 2016, 1184 P.

Additional:

1. William Marshall, Marta Lapsley, Andrew Day, Kate Shipman. Clinical Chemistry. Elsevier, 2020. 432 p.
2. Harper's Illustrated Biochemistry / V.W. Rodwell, D.A. Bender, K.M. Botham et al. – Mc Graw Hill Education, 2015. – 817 p.

Electronic information resources:

1. [https://info.odmu.edu.ua/chair/biology/-](https://info.odmu.edu.ua/chair/biology/)
2. <http://libblog.odmu.edu.ua/>
3. <https://moodle.odmu.edu.ua/login/index.php>

Practical class №27

Topic: Nucleotides' metabolism in tissues: purine and pyrimidine nucleotides catabolism. Disorders of purine metabolism (gout). Purine and pyrimidine nucleotides biosynthesis. Regulation of nucleotide biosynthesis. Deoxyribonucleotide biosynthesis. Formation of thymidine nucleotides: dTMP biosynthesis inhibitors as antitumor agents.

Goal: providing knowledge about the biological role of nucleic acids and nucleoproteins as carriers of genetic information, Studying the processes of nucleotide synthesis and the mechanisms of their regulation, using the acquired knowledge to understand the principles of inhibition of tumor

processes _____

Basic concepts: nucleoproteins, nucleic acids and their levels of organization, mononucleotides, end products of purine and pyrimidine metabolism, disorders of purine metabolism, synthesis of nucleotides, regulation of synthesis, deoxyribonucleotides, synthesis inhibitors as antitumor agents.

Equipment: _____ Laboratory of the department _____

Plan:

1. Organizational activities (greetings, verification of those present, announcement of the topic, purpose of the class, motivation of higher education seekers to study the topic).

2. Control of the reference level of knowledge.

The higher education applicant should know:

- the structure of nucleoproteins and nucleic acids, their localization in the cell,
- structure of constituent parts of nucleic acids - mononucleotides (DNA and RNA)
- formation of end products of purine and pyrimidine metabolism (urea and uric acid)
- disorder of purine metabolism (in gout)
- synthesis processes of purine nucleotides to inosinic acid, and then the formation of adenylyl and guanylyl nucleotides.
- synthesis processes of pyrimidine nucleotides with features of deoxyribonucleotide formation
- mechanisms of regulation of synthesis of purine and pyrimidine nucleotides.
- inhibitors of deoxyribonucleotide biosynthesis (dTMP) as antitumor agents.

The higher education applicant should be able to:

- whoLkandsleep to determine artandArt urinaryeatacid in urineand
- understand the synthesis schemes of purine and pyrimidine nucleotides.
- to determine inhibitors of deoxyribonucleotide synthesis.
- distinguish the causes of Lesch-Nyhan disease.

Questions to check basic knowledge on the topic of the class:

- What are nucleoproteins and what do they consist of?
- How are nucleosides and mononucleotides different?
- What simple proteins are part of nucleoproteins?
- How do you form the name of a nucleotide?
- What are the functions of DNA and RNA in the body?
- What is the role of nucleic acids (DNA, RNA) in protein biosynthesis (general provisions)?
- What nitrogenous bases are in the composition of nucleic acids?
- Define complementarity
- Chargaff rules
- What carbohydrates are part of DNA, RNA?
- Types of RNA and their functions.
- What amino acids and other substances are used for the synthesis of nitrogenous bases:
 - a) purine; b) pyrimidine?
- What is the difference between purine mononucleotides and pyrimidine mononucleotides?
- Where does the synthesis of nucleic acids take place?
- Name the sources of synthesis of purine bases of nucleotides.
- Name the sources of synthesis of pyrimidine bases of nucleotides
- Biosynthesis of mononucleotides (scheme).

3. Formation of professional skills and abilities.

3.1 Demonstration and practical work «Quantitative determination of uric acid in urine».

Recommendations for performing tasks.

Principle of the method: discovery of uric acid salts in urine by titration with potassium permanganate.

Procedure: Measure 50 ml of urine into a flask, add 12 ml of uranium-ammonium reagent for precipitation of mucin and phosphates. Leave for 10-15 minutes, filter. Measure 60 ml of filtrate, add 5 ml of 25% ammonia to the flask and leave for 24 hours. for precipitation of uric acid salt. **WARNING!** This stage of work is performed by the laboratory! After 24 hours (on the second day) higher education applicants will receive samples with precipitates of uric acid salt and continue working with them.

After receiving a sample with sediment, decant the solution, and collect the sediment on a filter, wash it 2 times with 10% ammonium sulfate, and transfer the filter together with the sediment to a flask for titration. Add 15-20 ml of distilled water and 5 ml of concentrated sulfuric acid H₂SO₄. Titrate the contents of the flask with potassium permanganate KMnO₄ (0.02N) to a pink color that does not disappear within 10 seconds.

Calculation example:

For example, 2 ml of KMnO₄ solution was used for sample titration, 1 ml of KMnO₄ corresponds to 1.5 mg of uric acid, and 2 ml - x. Then $x = (2 \times 1.5):1 = 3$ mg of uric acid in the sample (in 50 ml). Calculate daily diuresis: 3 mg of uric acid in 50 ml, and x mg - in 1200 ml, $x = 360$ mg/day.

The norm is 0.3-0.5-1.2 g of uric acid per day.

Conclusion:

The presence of uric acid in terms of daily diuresis normally should not exceed 1.2 g. If this value is exceeded, it is a pathology of the breakdown of purine nucleotides.

Requirements for work results.

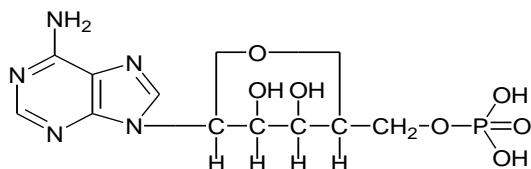
Enter the obtained data and calculations into the workbook.

Make medical and biological conclusions.

Control materials for the final stage of the class.

Questions to check the final level of knowledge:

- Give the full name of the substance, which is abbreviated as UTF.
- Write the name of the substance denoted as GDF.
- Write the nucleotide consisting of AMP and uridine acid.
- What is the name of a nucleotide whose molecule contains 2,6-dihydroxypyrimidine?
- Name the nucleotide:



- Which nucleotide contains the nitrogenous base of 2-amino-6-hydroxypurine?
- What are minor nitrogenous bases?
- Name Chargaff's rules.
- What are the options for adding phosphoric acid to nucleosides?
- The phenomenon of transformation.
- Hypochromic effect; denaturation and renaturation of DNA.
- Free nucleotides and their participation in metabolic processes.
- Methods of nucleic acid research.

- Compare the reactions of sequential cleavage of AMP and HMF.
- Reactions of microsomal oxidation in the process of catabolism of purine nucleotides.
- Gout: causes and symptoms of the disease.
- Lesch-Nyhan syndrome: describe the symptoms and causes of the disease.
- Scheme of conversion of cytidyl nucleotide by cleavage to ammonia.
- Compare the processes of splitting uracil and thymine.
- Name the sources of synthesis of purine bases of nucleotides.
- Give the scheme of the synthesis of purine nucleotides.
- Give the reactions of the biosynthesis of pyrimidine nucleotides.
- How are UTF and CTF formed?
- How is the synthesis of purine nucleotides regulated?
- What enzymes regulate the synthesis of pyrimidine nucleotides?
- How are thymidyl nucleotides formed?
- Explain the mechanism of conversion of ribonucleotides to deoxyribonucleotides.
- Explain the causes of Lesch-Nyhan disease.

Test tasks.

1. A 37-year-old man came to the therapist with complaints of periodic intense pain attacks in the joints of the big toe and their swelling. When analyzing urine, its sharply acidic character and pink color were established. Such changes in urine can be associated with the presence of what substances?

- A * Uric acid salts
- B Chlorides
- C Ammonium salts
- D Calcium phosphate
- E Magnesium sulfate

2. For the treatment of gout, the patient was prescribed allopurinol, a structural analogue of hypoxanthine, which led to an increase in the excretion of hypoxanthine in the urine. What process is blocked at the same time?

- A * Formation of uric acid
- B Spare path of synthesis of purine nucleotides
- C The main path of synthesis of purine nucleotides
- D Urea synthesis
- E Decay of pyrimidine nucleotides

3. A 48-year-old patient turned to the doctor with complaints of severe pain, swelling, redness in the joints, temperature rise to 38°C. A high content of urates was found in the blood. A possible cause of this condition may be a metabolic disorder:

- A * Purinov
- B Collagen
- C Cholesterol
- D Pyrimidines
- E Carbohydrates

4. A 46-year-old patient turned to the doctor with a complaint of pain in the joints, which worsens on the eve of a change in weather. An increase in the concentration of uric acid was detected in the blood. Increased decay of which substance is the most likely cause of the disease?

- A * AMP
- B TsMF
- C UTF
- D UMF
- E TMF

5. The patient has increased uric acid content in the blood, which is clinically

confirmed by a pain syndrome due to the deposition of urates in the joints. As a result of which process is this acid formed?

- A * Decay of purine nucleotides
- B Decay of pyrimidine nucleotides
- C Heme catabolism
- D Cleavage of proteins
- E Recycling of purine bases

6. Nitrous acid is formed in the body from nitrates, nitrites and nitrosamines, which causes the oxidative deamination of the nitrogenous bases of nucleotides. This can lead to a point mutation-substitution of cytosine to:

- A.* Uracil
- B.Guanine
- C.Timin
- D.Adenine
- E.Inosine

7. A 37-year-old man came to the therapist with complaints of periodic intense pain attacks in the joints of the big toe and their swelling. When analyzing urine, its sharply acidic character and pink color were established. With the presence of which substances can be

- A.* Uric acid salts
- B.Chlorides
- C.Ammonium salts
- D.Calcium phosphate
- E.Magnesium sulfate

8. The patient's joints are enlarged and painful. The patient's blood has an elevated level of urates. What is this pathology called?

- A.*Gout
- B.Rickets
- C.Scurvy
- D.Pellagra
- E.Caries

9. The patient has pain in small joints, the joints are enlarged. There is an increased content of urates in the blood serum. The exchange of what substances

- A.* Purinov
- B.Amino acids
- C.Disaccharides
- D.Pyrimidines
- E.Glycerin

10. A patient with suspected gout was admitted to the clinic. What biochemical analysis should be prescribed to clarify the diagnosis?

- A.* Determination of uric acid in blood and urine
- B.Determination of urea in blood and urine
- C.Determination of creatine in the blood
- D.Determination of uricase activity in blood
- E.Determination of amino acids in blood

11. On the basis of laboratory analysis, the diagnosis of gout was confirmed in the patient. What analysis was carried out for the production

- A.* Determination of uric acid in blood and urine
- B.Determination of creatinine in urine
- C.Determination of residual nitrogen in the blood
- D.Determination of urea in blood and urine
- E.Determination of ammonia in urine

12. A 46-year-old patient turned to the doctor with a complaint of pain in the joints, which worsens on the eve of a change in weather. An increase in the concentration of uric acid was detected in the blood. Enhanced decay of which substance is the most likely cause

- A.*AMP
- B.TsMF
- C.UTF
- D.UMF
- E.TMF

thirteen. An 8-year-old boy has Lesch-Nyhan disease. The concentration of uric acid in the blood is increased. Indicate the violation of which process is the cause of this hereditary disease.

- A.*Decomposition of purine nucleotides
- B.Synthesis of purine nucleotides
- C.Synthesis of pyrimidine nucleotides
- D.Decay of pyrimidine nucleotides
- E.Formation of deoxyribonucleotides

14. Methotrexate, a structural analogue of folic acid, is prescribed for the treatment of malignant tumors, which is a competitive inhibitor of dihydrofolate reductase and therefore suppresses

- A.* Nucleotides
- B.Monosaccharides
- C.Fatty acids
- D.Glycerophosphatides
- E.Glycogen

15. Pterin derivatives - aminopterin and metatrexate - are competitive inhibitors of dihydrofolate reductase, as a result of which they inhibit the regeneration of tetrahydrofolic acid from dihydrofolate. These drugs lead to inhibition of the intermolecular transport of one-carbon groups. The biosynthesis of which nucleotide is inhibited in this case?

- A.* dTMF
- B.IMF
- C.UMF
- D.OMF
- E.AMP

16. The structural analogue of glutamine, the antibiotic azaserine, a powerful inhibitor of purine nucleotide synthesis, was prescribed to the patient for tumor chemotherapy. What type of inhibition is characteristic of this drug?

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

17. The child has delayed growth and mental development, a large amount of orotic acid is excreted in the urine. This hereditary disease develops as a result

- A.*Synthesis of pyrimidine nucleotides
- B.Decay of pyrimidine nucleotides
- C.Synthesis of purine nucleotides
- D.Breakdown of purine nucleotides
- E.Conversion of ribonucleotides into deoxyribonucleotides

18. A 58-year-old man underwent surgery for prostate cancer. After 3 months, he underwent a course of radiation and chemotherapy. The complex of medicines included 5-fluorodeoxyuridine - a thymidylate synthase inhibitor. The synthesis of which substance is primarily blocked under the action of this drug?

- A.+ DNA

- B.i-RNA
- C.p-RNA
- D.t-RNA
- E.Squirrel

19. For the treatment of malignant tumors, methotrexate is prescribed, a structural analog of folic acid, which is a competitive inhibitor of dihydrofolate reductase and therefore suppresses

- A.* Nucleotides
- B.Monosaccharides
- C.Fatty acids
- D.Glycerophosphatides
- E.Glycogen

20. Methotrexate, a structural analogue of folic acid, is prescribed for the treatment of malignant tumors, which is a competitive inhibitor of dihydrofolate reductase and therefore suppresses the synthesis of nucleic acids at the level of:

- A.*Synthesis of mononucleotides
- B.Replications
- C.Transcriptions
- D.Reparations
- E.Processing

21. With hereditary orotaciduria, the excretion of orotic acid is many times higher than the norm. The synthesis of which substances will be disturbed in this pathology?

- A.*Pyrimidine nucleotides
- B.Purine nucleotides
- C.Biogenic amines
- D.Uric acid
- E.Urea

4. Summing up.

5. List of recommended literature (main, additional, electronic information resources):

Main:

1. Gubsky Yu.I., I.V. Nizhenkovska, Korda M.M. Biological and Bioorganic Chemistry: in 2 books. Book 2. Biological Chemistry: textbook. 2021. 544 p.
2. Satyanarayana U. Biochemistry. 5th edition. India 2020. 777 p.
3. Lehninger. Principles of Biochemistry. 7th edition. NY, United States. 2017.
4. Jeremy M. Berg, John L. Tymoczko, Gregory J. Gatto. Biochemistry. 8th Revised edition. 2015.
5. Lippincott Illustrated Reviews: Biochemistry. Philadelphia :Wolters Kluwer, 2017. 560 p.
6. Baynes J., Dominiczak M. Medical Biochemistry. 5th Edition. Elsevier, 2018. 712 p.
7. Donald Voet, Judith G. Voet, Charlott W. Pratt. Fundamentals of Biochemistry: Life at the Molecular Level. ISBN: 978-1-118-91840-1 February 2016, 1184 P.

Additional:

1. William Marshall, Marta Lapsley, Andrew Day, Kate Shipman. Clinical Chemistry. Elsevier, 2020. 432 p.
2. Harper's Illustrated Biochemistry / V.W. Rodwell, D.A. Bender, K.M. Botham et al. – Mc Graw Hill Education, 2015. – 817 p.

Electronic information resources:

1. <https://info.odmu.edu.ua/chair/biology/>-

2. <http://libblog.odmu.edu.ua/>
3. <https://moodle.odmu.edu.ua/login/index.php>

Practical class №28

Topic: Biosynthesis of nucleic acids. Molecular mechanisms of DNA replication. Stages of synthesis of daughter chains of DNA molecules. Molecular mechanisms of transcription. RNA synthesis stages and enzymes. Processing as a post-transcriptional modification of RNA. Antibiotics as transcription inhibitors. Protein biosynthesis in ribosomes. Genetic code: triplet code structure, its properties. Post-translational modification of peptide chains. Regulation of translation. Regulation of gene expression. Mechanisms of DNA mutations and repairs. Obtaining recombinant DNA, transgenic proteins.

Goal: Studying the mechanisms of DNA and RNA synthesis, to study the processes of protein synthesis from amino acids, translation of information from the language of the genetic code, regulation of translation and post-translational transformations of peptide chains. Get information about the possibility of gene expression, the mechanisms of mutations and the formation of recombinant DNA.

Basic concepts: replication, repair, transcription, splicing, keeping, processing, genetic code, translation, regulation of gene expression, mutations, recombinant DNA.

Equipment: Laboratory of the department

Plan:

1. Organizational activities (greetings, verification of those present, announcement of the topic, purpose of the class, motivation of higher education seekers to study the topic).
2. Control of the reference level of knowledge.

The higher education applicant should know:

- structural differences between DNA and RNA;
- the role of DNA and RNA in the storage and transfer of genetic information;
- stages of synthesis of daughter chains of the DNA molecule;
- mechanisms of matrix (programmed) RNA biosynthesis;
- antibiotics capable of inhibiting the transcription process;
- processes of post-transcriptional modification of RNA;
- mechanisms of matrix (programmed) biosynthesis of nucleic acids, as the basis for understanding matrix biosynthesis of proteins;
- mechanisms of matrix synthesis of proteins and its regulation;
- effects of physiologically active compounds on protein synthesis mechanisms.

The higher education applicant should be able to:

- explain the meaning of using the genetic code to encode amino acids;
- explain the stages of broadcasting;
- Give examples of the influence of physiologically active compounds on the stages of protein synthesis.

Questions to check basic knowledge on the topic of the class:

- What nitrogenous bases (as part of nucleic acids) do you know?
- What carbohydrates are part of DNA, RNA?
- Localization of RNA and DNA in cells.
- What is the difference between DNA and RNA?
- Define complementarity.
- Name the types of RNA.

- What are the functions of DNA and RNA in the body?
- Ribosomal protein-synthesizing system
- Aminoacyl-t-RNA synthetases.
- Genetic code, its properties.
- Explain the meaning of the terms: transcription, translation, codon, anticodon.
- Explain the stages of biosynthesis: recognition, initiation, elongation, termination, informosomes.
- Formation of higher levels of the protein structure.
- Molecular mechanisms of protein biosynthesis - Stages of translation.
- How is protein biosynthesis regulated?
- Functional organization of a gene: what is a gene-regulator, gene-operator, structural gene, cistron, operon?
- Mechanisms of protein biosynthesis regulation.
- Violation of protein biosynthesis. Molecular mutations as the primary source of genetic changes.
- Influence of physiologically active compounds on translation processes.
- Antibiotics - translation inhibitors, their biomedical application.

3. Formation of professional skills and abilities.

Control materials for the final stage of the class.

Questions to check the final level of knowledge:

- What is the role of nucleic acids (DNA, RNA) in protein biosynthesis (general provisions)?
- What is meant by matrix biosynthesis of nucleic acids and proteins?
- Where does the synthesis of nucleic acids take place?
- From what and how does DNA biosynthesis occur? Name the main stages, draw a general scheme of DNA biosynthesis; possible options.
- Molecular mechanisms of DNA reduplication.
- What regularity underlies the structure of copies of polynucleotide chains?
- What do DNA replication errors lead to?
- From what and how does RNA biosynthesis occur? Name the main stages, possible variants of biosynthesis: state the general scheme of RNA biosynthesis.
- The process of "maturation" of RNA (splicing, processing). The role of enzymes in this process (nucleases, RNA ligases).
- Describe the functions of the most important groups of nucleases (DNAases, exodeoxyribonucleases, polynucleotide phosphorylases, restriction enzymes, etc.).
- What regularity underlies the structure of copies of polynucleotide chains?
- How is protein biosynthesis induced?
- How is repression of protein biosynthesis carried out?
- Violation of protein biosynthesis: what are enzymopathies, hemoglobinopathies, hemoglobinoses?
- Hereditary diseases, as one of the manifestations of biochemical polymorphism, ideas about the biochemical mechanisms of hereditary molecular diseases (examples).
- Name the causes of: phenylpyruvic oligophrenia (phenylketonuria), alkaptonuria, galactosemia, albinism.
- Name antibiotics - inhibitors of protein synthesis in prokaryotes, determine their role in the treatment of infections.
- What is the role of diphtheria toxin in inhibition of protein synthesis?
- Individual features of the antigenic composition of organisms as the basis of tissue (transplantation) incompatibility.
- Regulation of protein biosynthesis.
- Influence of physiologically active compounds on translation processes.
- Antibiotics - translation inhibitors, their biomedical application.

- Differential activity of genes as a mechanism of cell differentiation in ontogenesis.
- Molecular mutations as the primary source of genetic variability.
- Biochemical polymorphism of proteins (on examples of hemoglobins and isozymes).
- What are mutations?
- What types of mutations can you name?
- Gene, genomic, chromosomal mutations.
- Mutagens.
- Mechanisms of DNA repair.
- Recombinant DNA.
- Genetic engineering.

Test tasks.

1. In experimental studies, it was established that steroid hormones affect protein synthesis.

Indicate which stage of this process they influence.

A. *Synthesis of specific m-RNAs.

B. Synthesis of ATP.

C. Synthesis of specific t-RNAs.

D. GTP synthesis.

E. Synthesis of specific r-RNAs.

2. The blood of the child and the alleged father was submitted for forensic examination to establish paternity. Specify which chemical components must be identified in the test blood.

A. * DNA.

B. t-RNA.

C. p-RNA.

D. m-RNA.

E. my-RNA.

3. In patients with xeroderma pigmentosum, the skin is extremely sensitive to sunlight, skin cancer may develop. The reason is a hereditary deficiency of the enzyme UV-endonuclease. As a result of this defect, the process is disrupted:

A. *DNA repairs

B. DNA replication

C. Transcriptions

D. Reverse transcription

E. Broadcasts

4. In the experiment, it was shown that ultraviolet-irradiated skin cells of patients with xeroderma pigmentosum restore the native structure of DNA more slowly than cells of normal people due to a defect in the repair enzyme. Choose the enzyme of this process.

A. * Endonuclease

B. RNA ligase

C. Primaza

D. DNA polymerase Sh

E. DNA gyrase

5. The antibiotic rifomycin, which is used in the treatment of tuberculosis, affects certain biochemical processes. Name them.

A. *Inhibits RNA polymerase at the initiation stage

B. Inhibits DNA polymerase at the initiation stage

C. Inhibits DNA ligase

D. Inhibits aminoacyl RNA synthetase

6. Quinolones are used to treat urogenital infections - DNA gyrase enzyme inhibitors. Indicate which process is disrupted by quinolones in the first place.

A. *replication

B. reparation

- C. gene amplification
- D. recombination of genes
- E. reverse transcription

7. A 58-year-old man underwent surgery for prostate cancer. After 3 months, he underwent a course of radiation and chemotherapy. The complex of medicines included 5-fluorodeoxyuridine - a thymidylate synthase inhibitor. The synthesis of which substance is primarily blocked under the action of this drug?

- A. DNA
- B. i-RNA
- C. p-RNA
- D. t-RNA
- E. Squirrel

8. When poisoned with amanitin - the poison of the pale toadstool, RNA polymerase B(II) is blocked. At the same time, it stops:

- A. *Synthesis of mRNA
- B. Synthesis of tRNA
- C. Reverse transcription
- D. Synthesis of primers
- E. Maturation of mRNA.

9. Patients with xeroderma pigmentosum are characterized by an abnormally high sensitivity to ultraviolet light, the result of which is skin cancer, due to the inability of enzyme systems to restore damage to the hereditary apparatus of cells. What process is this pathology associated with?

- A. * DNA repair
- B. Gene conversion
- C. DNA recombination
- D. Gene complementation
- E. DNA reduplication

10. A 40-year-old woman was hospitalized in serious condition with symptoms of Amanita phalloides (pale toadstool) poisoning. It is known that one of the toxins of these mushrooms blocks the synthesis of mRNA precursors. This toxin:

- A. * amanitin
- B. actinomycin
- C. taurine
- D. ribophorin
- E. bicuculline

11. A 57-year-old patient diagnosed with viral hepatitis was prescribed interferon. The antiviral effect of this drug is based on:

- A - *Suppression of the translation of virus envelope proteins
- B - Inhibition of viral DNA replication
- C - Activation of the complement system
- D - Formation of antiviral antibodies
- E - Inhibition of the synthesis of transforming growth factors.

12. For the formation of a transport form of amino acids for protein synthesis on ribosomes, it is necessary:

- A - *Aminoacyl-tRNA synthetase
- In - GTF
- C - m-RNA
- D - ribosome
- E - revertase

13. The transport form of AK for protein synthesis on ribosomes acts as:

- A - *aa tRNA
- B - Aminoaceladenylate

- C – S-adenosylmethionine
- D - phosphoadenosine phosphosulfate (FAPS)
- E – Aminoacyladenylate

14. The intensity of gene expression is controlled by a developed system of transcription regulation signals. Effective activating elements of such a system are specific DNA sequences, which are called:

- A - *Enhancers
- B - Silencers
- C - Repressors
- D - Operators
- E – Inductors

15. The human genetic apparatus contains about 30,000 genes, and the number of antibody variants reaches millions. What mechanism is used for the formation of new genes responsible for the synthesis of such a large number of antibodies?

- A - * Gene recombination
- B – Amplification of genes
- C – DNA replication
- D - DNA repair
- E – Formation of Okazaki fragments

16. Development of the method in view and lenience gene and in and with 'is dna eath in new ones combination and nalyah became new band O and mand we do achievement genetic ex and gin. For with 'is dna two chain DNA, as and view and flax and and with pand of them body and z mand in, eath processed:

- 2. A - * restriction endonuclease
- 3. B - lyase
- 4. C - helicase
- 5. D - transferase
- 6. E - synthetase.

4. Pipounding pand bag and in:

5. List of recommended literature (main, additional, electronic information resources):

Main:

1. Gubsky Yu.I., I.V. Nizhenkovska, Korda M.M. Biological and Bioorganic Chemistry: in 2 books. Book 2. Biological Chemistry: textbook. 2021. 544 p.
2. Satyanarayana U. Biochemistry. 5th edition. India 2020. 777 p.
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6. Baynes J., Dominiczak M. Medical Biochemistry. 5th Edition. Elsevier, 2018. 712 p.
7. Donald Voet, Judith G. Voet, Charlott W. Pratt. Fundamentals of Biochemistry: Life at the Molecular Level. ISBN: 978-1-118-91840-1 February 2016, 1184 P.

Additional:

1. William Marshall, Marta Lapsley, Andrew Day, Kate Shipman. Clinical Chemistry. Elsevier, 2020. 432 p.

2. Harper's Illustrated Biochemistry / V.W. Rodwell, D.A. Bender, K.M. Botham et al. – Mc Graw Hill Education, 2015. – 817 p.

Electronic information resources:

1. [https://info.odmu.edu.ua/chair/biology/-](https://info.odmu.edu.ua/chair/biology/)
2. <http://libblog.odmu.edu.ua/>
3. <https://moodle.odmu.edu.ua/login/index.php>

Practical class №29.

Topic: Hormones general concept. Classification, mechanisms of action of hormones on target cells. Hormones of the hypothalamus and pituitary gland hormones. Thyroid and parathyroid glands hormones. Structure and synthesis of thyroid hormones. Thyroid gland pathology. Regulation of phosphorus-calcium metabolism. Metabolic disorders of calcium homeostasis. Demonstration-practical work: Study of the nature of hormones using the biuret reaction. Determination of iodine in the thyroid gland.

Goal: To study and be able to characterize the hormones of the hypothalamic-pituitary system according to the following plan: 1) the name of the hormone; 2) place of synthesis; 3) features of the structure; 4) mechanism of action, biological role; 5) violation of synthesis.

Basic concepts: hormones, classification of hormones, protein nature of hormones, the mechanism of action of hormones depends on their nature.

Equipment: Laboratory of the department

Plan:

1. Organizational measures (greetings, verification of those present, announcement of the topic, purpose of the class, motivation of higher education seekers to study the topic).

2. Control of the reference level of knowledge.

The higher education applicant should know:

- General ideas about hormones and other signaling molecules;
- Properties of hormones and features of endocrine system functioning;
- Classification of hormones: 1) by the city of synthesis, 2) by chemical nature, 3) by ensuring and maintaining homeostasis, 4) by primary contact with the cell.

- To study the concept of receptors, their structure, localization and interaction with hormones

The higher education applicant should be able to:

- hormones of the hypothalamic-pituitary system
- the name of the hormone
- place of synthesis
- features of the structure
- mechanism of action, biological role
- violation of synthesis

Questions to check basic knowledge on the topic of the class:

- General characteristics of hormones, the role of hormones in the system of intercellular integration of human body functions.

- Hormone research methods.
- Properties of hormones and features of endocrine system functioning.
- Classification of hormones by the city of synthesis, chemical nature, provision and maintenance of homeostasis.
- Name groups of hormones and representatives of each of them.
- Mechanisms of action of hormones of protein-peptide nature and derivatives of amino acids.
- Biochemical systems of intracellular transmission of hormonal signals: G-proteins, secondary messengers (cAMP, cGMP, Ca²⁺/calmodulin, IP₃, DAG).
- Adenylate cyclase messenger system. Structure of ATP and cyclic 3',5'-AMP.

- Hormones of the hypothalamus - liberins and statins. Their structure and role in neurohumoral regulation.
- Hormones of the anterior lobe of the pituitary gland. Pathological processes associated with a violation of their synthesis.
- Group "growth hormone (somatotropin) - prolactin - chorionic somatomammotropin"; pathological processes associated with a violation of their functions.
- A group of glycoproteins - tropic hormones of the pituitary gland (thyrotropin, gonadotropins).
- Vasopressin and oxytocin: structure, biological functions. Pathology associated with a violation of vasopressin production.

3. Formation of professional skills:

3.1 Demonstration and practical work:

Study of the nature of hormones using the biuret reaction.

Recommendations for performing tasks.

Principle of the method: discovery of peptide bonds in proteins and peptides. These substances form a red-violet complex with copper sulfate in an alkaline environment.

Procedure: Pour 0.5 ml of the test solution into a test tube, add 0.5 ml of 10% NaOH solution and 1-2 drops of 1% CuSO₄ solution and mix. In the presence of protein, a red-purple color appears.

Conclusion: the appearance of a red-violet color indicates the presence of peptide bonds in the enzyme molecule, that is, its protein nature.

Requirements for work results.

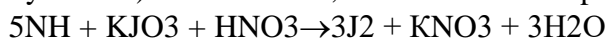
Enter the obtained data and calculations into the workbook.

Make medical and biological conclusions.

3.2 Demonstration and practical work "Iodine determination in the thyroid gland".

Recommendations for completing tasks:

The method is based on the separation during acid hydrolysis of thyroid hormones (iodothyronines) of iodide acid, which reacts with potassium iodate to release free iodine:



In chloroform, iodine has a purple color.

Progress. Place several crystals of thyroidin in a test tube, add 10 drops of concentrated nitric acid and heat for 3-5 minutes in a water bath. Then add 20 drops of 10% potassium iodate solution. Mix the contents and cool. Add 15 drops of chloroform to the test tube, mix by shaking. Color development is observed.

Designing the work: fill in the table

Hormones	Place of synthesis	Chemical structure	Qualitative response	Reaction mechanism	Color
Insulin					
Iodothyronine					

In clinical and biological laboratories, methods of qualitative and quantitative analysis are widely used to determine hormones in biological material for the purpose of diagnosis and prognosis of various endocrine diseases.

Requirements for work results.

Enter the obtained data and calculations into the workbook.

Make medical and biological conclusions.

Test tasks.

What hormone stimulates the activity of the enzyme adenylate cyclase?

- A. Adrenaline
- B. Aldosterone
- S. Testosterone
- D. Progesterone
- E. Calcitriol

In which glands are steroid hormones synthesized?

- A. *Shield-like*
- V. *Pidzhudzskova*
- S. *Adrenal cortex*
- D. *The cerebral part of the adrenal glands*

What hormone regulates the function of the thyroid gland?

- A. Tyroliberin
- V. Transkortin
- S. Cortisol
- D. Somatoliberin
- E. Somatotropin

What hormone regulates water balance and osmotic pressure of blood plasma, stimulates contraction of smooth muscles?

- A. Prolactin
- B. Somatostatin
- S. Corticoliberin
- D. Vasopressin
- E. Glucagon

For the purpose of analgesia, a substance that imitates the effects of morphine, but is synthesized in the central nervous system, can be used. Name this substance.

- A. Somatoliberin
- B. Oxytocin
- S. Vasopressin
- D. Calcitonin
- E. Endorphin

The patient has a headache, changes in appearance (increasing the size of the limbs, brow ridges, nose, tongue), hoarse voice, memory impairment. The disease started about three years ago. The reason for this condition can be:

- A. Aldosterone deficiency
- B. Glucagon deficiency
- S. Thyroxine deficiency
- D. Hyperproduction of somatotropin
- E. Hyperproduction of corticosteroids

Products of hydrolysis and modification of some proteins are biologically active substances - hormones. Lipotropin, corticotropin, melanotropin and endorphins are formed from which protein in the pituitary gland?

- A. Proopiomelanocortin
- B. Neuroalbumin
- S. Neurostromin
- D. Neuroglobulin
- E. Thyroglobulin

Products of hydrolysis and modification of some proteins are biologically active substances - hormones. Lipotropin, corticotropin, melanotropin and endorphins are formed from which protein in the pituitary gland?

- A. Proopiomelanocortin
- B. Neuroalbumin

- S. Neurostromin
- D. Neuroglobulin
- E. Thyroglobulin

Ca²⁺ ions play the role of secondary messengers in cells. They are activators of a number of processes if they interact with:

- A. Calcitonin
- V. Calmodulin
- S. Calciferol
- D. Myosin kinase
- E. Phosphorylase S

After a cerebral hemorrhage with damage to the hypothalamic nuclei, the patient developed diabetes insipidus, which is accompanied by polyuria as a result of:

- A. Hypoglycemia
- B. Reduction of reabsorption of potassium ions
- S. Acceleration of glomerular filtration
- D. Hyperglycemia E. Reduction of water reabsorption

Which of the listed hormones is hydrophilic and does not require a special transport protein:

- A. Dihydrotestosterone
- B. Progesterone
- S. Paratyryn
- D. Aldosterone
- E. Estradiol

The husband was diagnosed with angina pectoris. A phosphodiesterase inhibitor is included in the complex of drugs prescribed to the patient. The concentration of which substance in the heart muscle will increase?

- A. AMF
- V. GMF
- S. cAMP
- D. ADP
- E. ATP

The patient turned to the doctor with complaints of frequent and excessive urination, thirst. Urinalysis revealed: daily urine output – 19 L, urine density – 1.001. For which disease are these indicators characteristic?

- A. Steroid diabetes
- B. Diabetes mellitus
- S. Thyrotoxicosis
- D. Diabetes insipidus
- E. Addison's disease

The boy is being examined in the hospital for short stature. Over the past two years, it has grown by only 3 cm. This condition is caused by the lack of which hormone?

- A. Somatotropin
- B. Corticotropin
- S. Gonadotropin
- D. Thyrotropin
- E. Parathyroid hormone

4. Summary.

5. List of recommended literature(main, additional, electronic information resources):

Main:

1. Gubsky Yu.I., I.V. Nizhenkovska, Korda M.M. Biological and Bioorganic Chemistry: in 2 books. Book 2. Biological Chemistry: textbook. 2021. 544 p.
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2. <http://libblog.odmu.edu.ua/>
3. <https://moodle.odmu.edu.ua/login/index.php>

Practical class №30.

Topic: Steroid hormones. Hormones of the adrenal cortex and gonads. Their structure and biochemical mechanisms of action.

Goal: characterize hormones of steroid nature, mechanism of action, structures of hormones, biological role

Basic concepts: steroid hormones, nomenclature, classification, synthesis scheme, hormones of the adrenal cortex (C21-steroids) - cortisol, corticosterone, aldosterone, biochemical effects of corticosteroids.

Equipment: Laboratory of the department

Plan:

1. Organizational activities (greetings, verification of those present, announcement of the topic, purpose of the class, motivation of higher education seekers to study the topic).

2. Control of the reference level of knowledge.

The higher education applicant should know:

- adrenal cortex hormones
- glucocorticoids
- Itsenko-Cushing syndrome
- the role of aldosterone in the regulation of water-salt metabolism; aldosteronism
- Female sex hormones: estrogens - estradiol, progesterone physiological and biochemical effects, regulation of synthesis and secretion
- Male sex hormones (androgens) – testosterone, dihydrotestosterone, regulation of synthesis and secretion

Questions to check basic knowledge on the topic of the class:

- Mechanism of action of steroid hormones
- Structural formulas of steroid hormones

- Steroid hormones of the adrenal cortex (C21-steroids) – glucocorticoids and mineralocorticoids; structure, properties.

- Female sex hormones: estrogens, progesterone. Physiological and biochemical effects; connection with the phases of the ovulatory cycle

- Male sex hormones (C19-steroids). Physiological and biochemical effects of androgens; regulation of synthesis and secretion.

- Catecholamines (adrenaline, norepinephrine, dopamine): structure, biosynthesis, physiological effects, biochemical mechanisms of action.

3. Formation of professional skills and abilities.

3.1. *Refractometric determination of protein in blood serum*

Work progress: At the beginning, the refractive index of distilled water is determined, with the correct setting of the device, the refractive index is 1.3330. To do this, open the chamber and apply 1-2 drops of water to the lower half of the prism so that it completely wets the chamber. The camera is closed and the sharpness of the eyepiece is set so that the field of vision and sight lines are clearly visible. If the chiaroscuro boundary is blurred, clarity is achieved by rotating the dispersion limb screw. The chiaroscuro boundary is set at the point of intersection of the scale with the three sighting lines of the eyepiece. Then lift the upper half of the chamber, wipe both prisms and apply 2 drops of blood serum. At the same time, the boundary of chiaroscuro shifts, the sight lines of the eyepiece are set on it, and the refractive index is taken on the reference scale.

Calculation of protein percentage by refractive index:

Refractive index	Protein in blood serum in %	Refractive index	Protein in blood serum in %
1.33705	0.63	1.34575	3.68
1.33743	0.86	1.34612	5.90
1.33781	1.08	1.34650	6,12
1.33820	1.30	1.34687	6.34
1.33858	1.52	1.34724	6.55
1.33896	1.74	1.34761	6.77
1.33934	1.96	1.34798	6.98
1.33972	2.18	1.34836	7.20
1.34000	2.40	1.34873	7.42
1.34048	2.62	1.34910	7.63
1.34086	2.84	1.34947	7.85
1.34124	3.06	1.34984	8.06
1.34162	3.28	1.35021	8.28
1.34199	3.50	1.35058	8.49
1.34237	3.72	1.35095	8.71
1.34275	3.94	; 1.35132	8.92
1.34313	4.16	1.35169	9,14

Requirements for work results.

Enter the obtained data and calculations into the workbook.
Make medical and biological conclusions

Control materials for the final stage of the class:

Questions to check the final level of knowledge

- Mechanism of action of estrogens
- Biological significance of steroid hormones
- Mechanism of action of androgens
- Synthesis of catecholamines
- Biological value of aldosterone, cortisol
- Glucocorticoids
- Structural formulas of steroid hormones

Test tasks

Arachidonic acid as an irreplaceable component of food is a precursor of biologically active substances. What compounds are synthesized from it?

- A. Ethanolamine
- V. Kholin
- S. Norepinephrine
- D. Prostaglandin E1
- E. Triiodothyronine

A patient complained of constant thirst to the doctor. Hyperglycemia, polyuria, and increased content of 17-ketosteroids in urine were established. What disease is possible?

- A. Steroid diabetes
- B. Non-insulin-dependent diabetes
- S. Myksedema
- D. Glycogenosis of type I
- E. Addison's disease

Hyperglycemia occurs in Itsenko-Cushing's disease. What process is stimulated in this case?

- A. Glycolysis
- B. Phosphorolysis of glycogen
- S. Krebs cycle
- D. Pentose phosphate pathway of glucose oxidation
- E. Gluconeogenesis

What hormone stimulates the synthesis of corticosteroids?

- A. Parathyroid hormone
- B. Thyrotropin
- S. Corticoliberin
- D. Calcitonin
- E. Corticosterone

The patient was found to have hypernatremia, hypervolemia, and hypokalemia. What is the possible cause of this condition?

- A. Hyperaldosteronism
- B. Hypoaldosteronism
- S. Addison's disease
- D. Based's disease
- E. Diabetes

Taking oral contraceptives containing sex hormones inhibits the secretion of pituitary hormones. The secretion of which of the following hormones is inhibited at the same time?

- A. Vasopressin
- B. Somatotropin
- S. Oxytocin
- D. Follitropin
- E. Corticotropin

Testosterone and its analogues increase the mass of skeletal muscles, which allows them to be used for the treatment of dystrophies. This action of the hormone is determined by the interaction with which cell substrate?

- A. Membrane receptors
- B. Ribosomes
- S. Nuclear receptors
- D. Transcription activator proteins
- E. Chromatin

The patient, who has been taking glucocorticoids for a long time, had an exacerbation of the disease, a decrease in blood pressure, and weakness as a result of the withdrawal of the drug. What explains this?

- A. Occurrence of adrenal insufficiency
- B. Cumulation
- C. Addiction to the drug
- D. Hyperproduction of ACTH
- E. Sensitization

The patient has been taking glucocorticoids for a long time. After abrupt withdrawal of the drug, he complains of myalgia, increased fatigue, emotional instability, headache. Glucocorticoid withdrawal syndrome developed. What drugs are prescribed to correct this condition?

- A. AKTG
- B. Glucocorticoids
- S. Mineralocorticoids
- D. Adrenaline
- E. Corticosteroids

A patient with Itsenko-Cushing syndrome has an increased cortisol content in the blood. Which endocrine gland pathology is it connected with?

- A. The cortical part of the adrenal glands
- B. The cerebral part of the adrenal glands
- S. Pancreas
- D. Hypophysis
- E. Thyroid gland

A man who has been in a state of stress for a long time has a significantly increased content of 17-ketosteroids in his urine, which primarily indicates increased secretion:

- A. Estradiol
- B. Aldosterone
- S. Adrenaline
- D. Cortisol
- E. Progesterone

The woman showed signs of virilism (hair growth on the body, irregular menstrual cycle). Hyperproduction of which hormone can cause such a condition?

- A. Estriola
- B. Testosterone
- S. Relaxin
- D. Oxytocin
- E. Prolactin

The woman has a "moon-shaped" face, obesity of the upper part of the body, stretch marks on the front abdominal wall, hirsutism. The urine has an elevated level of 17-oxyketosteroids. Such manifestations are characteristic of:

- A. Pheochromocytomas
- B. Kon's syndrome
- S. Itsenko-Cushing syndrome
- D. Primary hypoadosteronism
- E. Secondary hyperaldosteronism

A patient suffering from Itsenko-Cushing's disease was consulted about excess body weight. The survey revealed that the energy value of the consumed food is 1700-1900 kcal/day. What is the leading cause of obesity in this case?

- A. Hypodynamia
- B. Insufficiency of insulin
- C. Excess insulin
- D. Insufficiency of glucocorticoids
- E. An excess of glucocorticoids

Glucocorticoids and nonsteroidal anti-inflammatory drugs are widely used in practical medicine. One of the negative consequences of long-term glucocorticoid therapy is:

- A. Polyuria
- B. Hyponatremia
- S. Hyperkalemia
- D. Osteoporosis
- E. Hypotension

4. Summary:

5. List of recommended literature(main, additional, electronic information resources):

Main:

1. Gubsky Yu.I., I.V. Nizhenkovska, Korda M.M. Biological and Bioorganic Chemistry: in 2 books. Book 2. Biological Chemistry: textbook. 2021. 544 p.
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3. <https://moodle.odmu.edu.ua/login/index.php>

Practical class №31.

Topic: Pancreas and adrenal medulla hormones. Chemical structure and mechanism of action. Hormonal regulation of blood sugar. Local hormones, their structure, biological role. Hormones of the digestive tract.

Goal: to study the mechanism of action of insulin, glucagon, the mechanism of their action, diabetes mellitus, the stimulating effect of insulin.

Basic concepts: metabolism, digestion of carbohydrates, lipids and proteins, hypoglycemia, hyperglycemia.

Equipment: Laboratory of the department

Plan:

1. Organizational activities (greetings, verification of those present, announcement of the topic, purpose of the class, motivation of higher education seekers to study the topic).

2. Control of the reference level of knowledge.

The higher education applicant should know:

- Mechanisms of influence of insulin and glucagon on metabolism
- Insulin: structure
- Synthesis of insulin
- Influence of insulin on the metabolism of carbohydrates, lipids and proteins
- The difference between glucagon and insulin
- The nature of gastrointestinal hormones, their mechanism of action on target cells.
- Origin and mechanism of action of leukotrienes.
- Enzyme cyclooxygenase, what reactions does it catalyze.
- Features of clinical manifestations of functional and organic diseases of the esophagus, stomach and duodenum.
- The concept of enterohormone.
- Groups of gastrointestinal hormones.
- The concept of gastroinhibitory polypeptide.

The higher education applicant should be able to:

- Mechanism of insulin synthesis
- The structure of the tyrosine kinase receptor
- The influence of pancreatic hormones on the metabolism of biopolymers
- Hypoglycemia, index

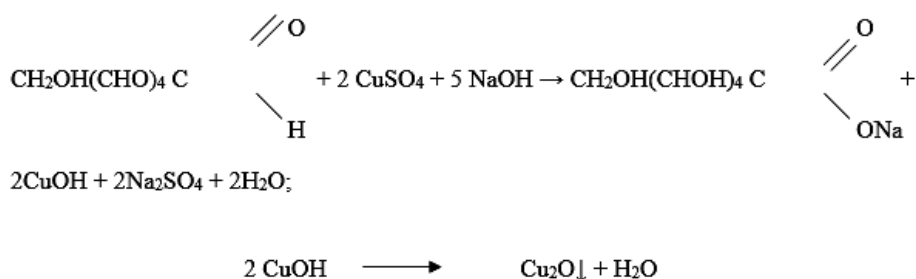
Questions to check basic knowledge on the topic of the class:

- insulin: structure,
- biosynthesis and secretion of pancreatic hormones
- influence on the metabolism of carbohydrates, lipids, proteins.
- growth-stimulating effects of insulin.
- be able to explain the work of the insulin receptor
- types of diabetes
- Methods of research of gastrointestinal hormones
- Pathology of violation of the action of gastrointestinal tract hormones
- subtract secretin from gastrin
- describe the effect of eicosinoids
- to calculate the action of prostaglandins from prostocyclins.

3. Formation of professional skills.

3.1 Demonstration and practical work: "Determination of sugar in urine by Fehling's reagent"

Principle of the method: In Fehling's reagent, copper (P) ions are in the form of a complex compound with tartrates. This reaction for glucose in its general form can be represented by equations:



The advantage of Fehling's reagent is that copper does not fall out in the form of copper (II) oxide when the reagent is in excess.

Procedure: 1 ml of urine and 1 ml of Fehling's reagent are introduced into the test tube. The mixture is stirred and heated to boiling. In the presence of sugar in the urine, a brick-red precipitate of copper (I) oxide appears.

Requirements for work results.

Enter the obtained data and calculations into the workbook.

Make medical and biological conclusions.

Test tasks:

What hormone stimulates glycogen biosynthesis and enhances anabolic processes?

- A. Adrenaline
- B. Norepinephrine
- S. Cholecystokinin
- D. Insulin
- E. Thyroxine

Glucose is used by its transport from the extracellular space through the plasma membrane into cells. This process is stimulated by the hormone:

- A. Insulin
- B. Glucagon
- S. Thyroxine
- D. Aldosterone
- E. Adrenaline

The patient is in a state of hypoglycemic coma. An overdose of which hormone can lead to such a situation?

- A. Insulin
- B. Cortisol
- S. Somatotropin
- D. Progesterone
- E. Corticotropin

The parents of a five-year-old child turned to the hospital. During the examination, it was found: retardation in mental development and growth, the child is sedentary. General exchange is reduced. What disease does the child have?

- A. Cretinism
- B. Lesch-Nyhan syndrome
- S. Phenylketonuria
- D. Hyperparathyroidism
- E. Endemic goiter

Insulin, like other hormones of a protein-peptide nature, has receptors on the surface of the cytoplasmic membrane. Name the mechanism of realization of the effect of insulin in target cells?

- A. Adenylate cyclase messenger system
- B. Guanylate cyclase messenger system
- C. Protein kinase cascade
- D. Phosphoinositide messenger system
- F. All answers are correct

The patient mistakenly took an excess dose of thyroxine. What changes in the secretion of thyroliberin and thyrotropin will this lead to?

- A. The secretion of hormones will increase
- A. There will be no change in secretion
- C. Secretion of thyroliberin will increase, thyrotropin will decrease
- D. Secretion of thyrotropin will increase, and thyroliberin will decrease
- F. Hormone secretion will decrease

Acetylsalicylic acid is used in the treatment of rheumatism. For what process does acetylsalicylic acid affect?

- A Synthesis of prostaglandins
- B Breakdown of glucose
- C Glycogen synthesis
- D Synthesis of amino acids
- E Fat breakdown

Various biologically active compounds are involved in blood pressure regulation. Which can peptides entering the blood affect the tone of blood vessels?

- A Quinines
- B Leukotrienes
- C Enkephalins
- D Iodothyronine
- E Endorphins

The anti-inflammatory effect of a number of drugs is due to inhibition of the release of arachidonic acid. This acid is a precursor of which biologically active substances?

- A Prostaglandins
- B Uric acid
- C Urea
- D Hema
- E Cholesterol

The exchange of arachidonic acid is accompanied by the formation of biologically active compounds. Name one of them that is formed with the participation of the lipooxygenase pathway.

- A Leukotrienes.
- B Quinines.
- C Catecholamines.
- D Bile acids.
- E Steroids.

Nonsteroidal anti-inflammatory drugs are used in medical practice for treatment rheumatoid arthritis, osteoarthritis, inflammatory connective tissue diseases.

The activity of which enzyme is inhibited by these drugs?

- A cyclooxygenase
- B hexokinase
- C succinate dehydrogenase
- D aminotransferases
- E xanthine oxidase

The patient was prescribed aspirin as an anti-inflammatory agent that inhibits production prostaglandins What enzyme is blocked at the same time?

- A Cyclooxygenase
- B Monooxygenase
- C Dioxygenase
- D Lipoxygenase
- E Peroxidase

Indomethacin is an active nonsteroidal anti-inflammatory drug that used in medical practice for the treatment of rheumatoid arthritis, osteoarthritis, inflammatory diseases of connective tissue. Which process slows down indomethacin?

- A synthesis of prostaglandins
- B formation of kinins
- C formation of angiotensin II
- D synthesis of amino acids
- E synthesis of purines

Steroid hormones activate the synthesis of the inhibitor of phospholipase A2, in connection with which their anti-inflammatory action consists in inhibiting the synthesis:

- A Prostaglandins
- B Kallikrein
- C bradykinin
- D Kininogens
- E Histamine

Biosynthesis of prostaglandins begins with the release of arachidonic acid from phosphoglycerides. What enzyme catalyzes this process?

- A Phospholipase A2
- B Cholesterolesterase
- C Sphingomyelinase
- D Triacylglyceride lipase
- E Lipoprotein lipase

A patient with rheumatism was prescribed prednisolone. The anti-inflammatory effect is due to the release of arachidonic acid. This acid is a precursor of which biologically active substances?

- A Prostaglandins
- B Urea
- C Hemu
- D Uric acid
- E Cholesterol

During the utilization of arachidonic acid on the cyclooxygenase pathway, biologically active substances are formed. Will indicate them.

- A Prostaglandins
- B Interferons
- C Biogenic amines
- D Somatomedins
- E Insulin-like growth factors

In the therapy of chronic inflammatory processes, a number of drugs are used. Indicate which of them reversibly inhibits cyclooxygenase of arachidonic acid

- A Indomethacin
- B Antimycin
- C Vikasol
- D Carnitine
- E Cholecalciferol

The activity of cyclooxygenase can be inhibited by the use of certain drugs. Which of them irreversibly inhibits the action of this enzyme?

- A Aspirin

- B Insulin
- C Oligomycin
- D Allopurinol
- E Aminalon

4. Summary:

5. List of recommended literature (main, additional, electronic information resources):

The main one:

1. Biological and bioorganic chemistry: In 2 books. Book 2: Biological chemistry: Textbook for med. University of the IV R.A. 2nd ed., ed. Approved by the Ministry of Education and Culture / Ed. Yu.I. Gubskiy, I.V. Nizhenkovskaya. K., 2017. 544 p.
2. Biological and bioorganic chemistry: In 2 books. Book 1: Bioorganic chemistry: Textbook for med. University of the IV R.A. 2nd ed., ed. Approved by the Ministry of Education and Culture / Ed. B.S. Zimenkovsky, I.V. Nizhenkovskaya. K., 2017. 272 p.
3. Ya.I. Gonskyi, T.P. Maksymchuk, Human biochemistry. Textbook. Ternopil: Ukrmedknyga, 2020. 736 p.

Additional:

1. Biological chemistry: textbook / O.Ya. Sklyarov, N.V. Apron, T.I. Bondarchuk. Ternopil: TDMU, 2020. 706 p.
2. William Marshall, Martha Lapsley, Andrew Day, Kate Shipman. Clinical Chemistry. Elsevier, 2020. 432
3. Medical Biochemistry/ Baynes J., Dominiczak M.. Saunders, Elsevier, 2018 712 p.
4. Lippincott Illustrated Reviews: Biochemistry/Ferrier D. Philadelphia: Wolters Kluwer, 2017. 560 p.

Electronic information resources:

1. <https://info.odmu.edu.ua/chair/biology/>-
2. <http://libblog.odmu.edu.ua/>
3. <https://moodle.odmu.edu.ua/login/index.php>

Practical class №32

Topic: Digestion of carbohydrates, lipids, proteins, nucleoproteins in the gastrointestinal tract. Enzymes, biochemical mechanisms. Chemical composition of gastric and intestinal juice. Hereditary disturbances of digestion. Biochemical characteristics and classification of vitamins. Water-soluble vitamins B1, B2, B6, PP. Their coenzyme role and symptoms of hypovitaminosis.

Goal: To inform higher education applicants that nutrition is a necessary prerequisite for human life, which ensures normal metabolism, the dynamic state of all biomolecules, cellular and extracellular structures. To study the mechanism of action and biological role of this group of vitamins.

Show the possibility of using them in practical medicine.

To acquaint higher education applicants with the coenzyme functions of vitamins B1, B2, B6, RR.

To teach higher education applicants to carry out qualitative determination of vitamins B1, B2, B6, RR.

Study of coenzyme forms of vitamins and their role in the catalytic activity of enzymes.

Basic concepts:–digestion of food nutrients, nutrients, components of normal nutrition; biological value of certain nutrients, vitamins, coenzymes, prosthetic group

Equipment: Laboratory of the department

Plan:

1. Organizational activities (greetings, verification of those present, announcement of the topic, purpose of the class, motivation of higher education seekers to study the topic).

2. Control of the reference level of knowledge.

The higher education applicant should know:

–organization of the digestive tract,
–the enzyme spectrum and optimal pH values in certain departments of the digestive tract,
–end products of digestion of carbohydrates, lipids, nucleoproteins and proteins in the small intestine.

–what vitamins are, their biological role for the body
–chemical composition and structure of vitamins;
–symptoms and consequences of hypovitaminosis B1, B2, B6, PP.

The higher education applicant should be able to:

–determine all forms of acidity of gastric juice, make a medical-biological conclusion and predict the type of pathology under which the acidity of gastric juice will increase or decrease.

Questions to check basic knowledge on the topic of the class:

1. Components of normal human nutrition. Macrocomponents, microcomponents.

2. The needs of the human body in nutritional compounds.

3. Digestion of nutrients.

4. Coenzyme function of vitamins

5. Causes of hypovitaminosis B1, B2, B6, RR.

1. Symptoms of hypovitaminosis B1, B2, B6, PP and their consequences for the body.

3. Formation of professional skills and abilities.

3.1 Demonstration and practical work «*Determination of all forms of acidity of gastric juice*». *Recommendations for performing tasks.*

Principle of the method: Quantitative determination of the acidity of gastric juice is performed by titrating a portion of the filtered juice with 0.1 n NaOH with indicators, taking into account the amount of alkali used for titration (alkali neutralization). Distinguish between total acidity, total HCl, free and bound HCl.

Procedure: Measure 5 ml of filtered gastric juice into a flask, add 2 drops of dimethylaminoazobenzene and 2 drops of phenolphthalein. Titrate with 0.1 N NaOH until an orange color appears. Note the amount of alkali used for titration (V1). Continue the titration to a lemon-yellow color, note the amount of alkali (V2) that went into the titration, count from zero. Continue the titration until a pink color, note the amount of alkali (V3), count from zero.

Calculation:

V1 – corresponds to free HCl;

V2 is auxiliary, used for calculation. Corresponds to total HCl:

$$\frac{V_2 + V_3}{2}$$

V3 corresponds to total acidity.

Acidity values are determined by the formula:

$$X = \frac{V (\text{мл}) \cdot 1000 \cdot 0,1}{5}, \text{ ммоль/л}$$

Bound HCl is found by the difference between total and free HCl.

Normally in adults: free HCl – 20-40 mmol/l;
total HCl – 30-50 mmol/l;
bound HCl – 10-20 mmol/l;
total acidity - 40-60 mmol/l.

Conclusion: The obtained result should be evaluated from the point of view of belonging to normal parameters of acidity of gastric juice or differences from them. Based on the received data, propose a diagnosis.

Requirements for work results.

Enter the obtained data and calculations into the workbook.

Make medical and biological conclusions.

3.2. Demonstrations just now-practical work "Qualitative reactions to vitamins B1, B2, B6, PP".

Recommendations for performing tasks.

A. Qualitative determination of thiamine

The principle of the method: during oxidation, thiamine turns into thiochrome, which has the ability to fluoresce blue in ultraviolet light.

Work progress: 1. Oxidation of thiamine to thiochrome:

Pour 0.5 ml of 5% thiamine solution into the test tube, then 1.0 ml of 5% ferric cyanide and 2.0 ml of 10% NaOH solution. Mix thoroughly and leave for 10 minutes.

2. *Extraction of thiochrome:*

After 10 minutes, 1.0 ml of isobutyl alcohol is added to the test tube, shaken and allowed to settle for 5 minutes.

3. *Registration of indicators and conclusion:*

The test tube is brought to the source of ultraviolet light. A solution of thiochrome in isobutyl alcohol fluoresces blue.

B. Qualitative determination of riboflavin

Principle of the method:

In ultraviolet light, riboflavin is able to fluoresce in a yellow-green color. When it is reduced with sodium hyposulfite, it loses this property.

The main stages of work performance.

1. *Preparation of material for research:*

Take 2 test tubes and label them "experiment" and "control". Pour 1.0 ml of 0.02% riboflavin solution into both test tubes.

2. *Restoration of riboflavin:*

A few crystals of sodium hyposulfite are added to the test tube marked "experiment". The solution turns from bright yellow to pale yellow.

3. *Comparative fluorometry:*

Both test tubes are raised to the source of ultraviolet light. Reconstituted riboflavin in a test tube does not fluoresce under ultraviolet light.

B. Qualitative determination of pyridoxine

Principle of the method:

If iron chloride is added to the pyridoxine solution, a red color of the complex salt appears, similar to red iron phenolate.

The main stages of work performance.

1. Preparation of material for research:

Pour 0.5 ml of 5% pyridoxine solution into the test tube.

2. Carrying out the reaction:

Pour 0.5 ml of 5% ferric chloride solution into the test tube and shake it. The mixture turns red.

G. Qualitative determination of vitamin RR

Principle of the method:

Nicotinic acid, when heated with a solution of copper acetic acid, forms a blue soluble precipitate of the copper salt of nicotinic acid.

The main stages of work performance.

1. Preparation of material for research:

Pour 1.0 ml of 1% nicotinic acid solution into the test tube.

2. Formation of the copper salt of nicotinic acid:

Add 1.0 ml of 5% copper acetic acid solution to the nicotinic acid solution. They stir. They heat up. A poorly soluble blue precipitate of the copper salt of nicotinic acid is formed.

Requirements for work results.

Enter the obtained data and calculations into the workbook.

Make medical and biological conclusions.

Control materials for the final stage of the class.

Questions to check the final level of knowledge:

- Participation of nicotinamide coenzymes in catalysis
- Participation of flavin coenzymes in catalysis
- Participation of pyridoxal coenzymes in catalysis
-

Questions to check the final level of knowledge:

1. Representatives of water-soluble vitamins.
2. Structure, physical and chemical properties of vitamin B1 (thiamine). Coenzyme form of thiamine. Mechanism of action and biological role of thiamine. Avitaminosis and hypovitaminosis. Use in medical practice.
3. Structure, physical and chemical properties of vitamin B2 (riboflavin). Coenzyme form of vitamin B2. Mechanism of action and biological role. Avitaminosis and hypovitaminosis. Use in medical practice.
4. Structure, physical and chemical properties of vitamin B6 (pyridoxine). Coenzyme forms of pyridoxine. Mechanism of action and biological role of pyridoxine. Avitaminosis and hypovitaminosis.
5. Structure, physical and chemical properties of vitamin PP (niacin, nicotinic acid, nicotinamide). Coenzyme forms of vitamin PP. Mechanism of action and biological role of niacin. Avitaminosis and hypovitaminosis.

Control materials for the final stage of the class.

Questions to check the final level of knowledge:

1. Biochemistry of human nutrition: components and nutrients of normal nutrition.

2. Digestion and biological value of carbohydrates. Enzymes of the stomach and intestines.
3. Digestion and biological value of lipids. Enzymes of the stomach and intestines.
4. Digestion and biological value of proteins. Enzymes of the stomach and intestines.
5. Digestion of nucleoproteins.
6. Disruption of digestion of certain nutrients in the stomach and intestines. Hereditary enzymopathies of digestive processes.
7. Microelements in human nutrition. Biological functions of individual trace elements; manifestations of trace element deficiency.
8. Coenzyme function of vitamins
9. Structure of the most common coenzymes
10. Causes of hypovitaminosis B1, B2, B6, RR.
11. Symptoms of hypovitaminosis B1, B2, B6, PP and their consequences for the body.

Test tasks.

1. Substances in the digestive system undergo certain changes. Enzymes of which class mainly carry out enteral transformations?
 - A *Hydrolases
 - B Oxidoreductases
 - C Transferases
 - D Lyases
 - E Ligases

2. The most important phase of the breakdown of starch and glycogen occurs in duodenum under the action of pancreatic enzymes. What enzyme splits (-1,4-glycosidic bonds in these molecules?
 - A * alpha-amylase
 - B Amylo-1,6-glycosidase
 - C aldolase
 - D hexokinase
 - E maltase

3. In a patient with complaints of poor appetite, weight loss, pain in the epigastric area, the analysis of gastric juice showed the presence of achillea. What does this term mean?
 - A * Absence of free NSI and pepsin.
 - In the absence of free NSI
 - C Lack of acidity
 - D Absence of free and bound NSI
 - E Absence of gastromucoprotein

4. The patient complains of loss of appetite, weight loss, unpleasant belching, pain and heaviness in the epigastric area. Analysis of gastric juice showed: total acidity 20 mM/l, free HCl absent, bound HCl 18 mM/l, lactic acid present. What disease can be assumed?
 - A *Stomach tumor
 - In Gastric ulcer disease
 - C Hyperacid gastritis
 - D. Hypoacidic gastritis
 - E Ulcerative disease of the duodenum

5. The patient notes periodic pains in the epigastric area, heartburn. When examining the gastric juice, hyperacidity was established. Which of the given data corresponds to this condition?
 - A * 80 mM/l

- In 40 mM/l
- With 60 mM/l
- D 25 mM/l
- E 55 mM/l

6. A large amount of fluoride was found in the water of the river, which is the source of the city's water supply. What pathology can develop among the inhabitants of this city?

- A *Fluorosis
- In Thyrotoxic goiter
- C Juvenile current
- D Caries
- It's beri-beri

7. A patient with hypofunction of the pancreas is prescribed pancreatin. When is it most rational to take this drug?

- A *Before food
- In After eating
- C During meals
- D Before going to bed
- E Any time

8. At the final stage of protein breakdown, trace elements Zn, Mn, Mg, Co play an important role, which:

- A * They increase the activity of peptidases
- Contribute to the absorption of proteins
- C They reduce the activity of peptidases
- D They inhibit the absorption of proteins
- E Inactivate peptidases

9. The patient has impaired protein digestion in the stomach and small intestine. This process is caused by a deficiency of which enzymes?

- A Peptidase
- B Synthetase
- C Amylase
- D Lipase
- E Transferase

10. Pancreatic enzymes are secreted in the duodenum in an inactive state. Specify which enzyme activates trypsinogen.

- A Enterokinase
- B Gastrixin
- C Lipase
- D Pepsin
- E Elastase

11. The child complains of a toothache. The dentist diagnosed caries enamel damage. The amount of which mineral substances is decreasing in the region carious damage:

- A * Phosphorus, fluorine, calcium;
- B Sodium, calcium, potassium;
- C Potassium, phosphorus, fluorine;
- D Magnesium, fluorine, calcium;

E Phosphorus, magnesium, potassium.

12. A woman, an employee of a confectionery shop, turned to a dentist. The patient drew attention to increased sensitivity to caries. For the purpose of remineralizing therapy, the doctor prescribed fluoride preparations. What is the role of fluoride in this therapy?

- A *Increase in the formation of fluorine apatite;
- B Increased permeability of enamel;
- C Suppression of alcoholic fermentation;
- D Decreased synthesis of proteoglycans;
- E Activation of salivary proteases.

13. The patient is hyperacidic. Name the hormone that stimulates the secretion of HCl and pepsinogen in the stomach.

- A Gastrin
- B Insulin
- C Somatotropin
- D secretin
- E Glucagon

14. Parenteral nutrition is recommended for a patient with a damaged esophagus. Indicate which of the indicated pharmaceutical preparations is a hydrolyzate of amino acids?

- A Hydrolysin
- B Asparkam
- C Rheopolyglukin
- D Polyglukin
- E Panangin

15. To obtain amylase enzyme in its pure form from the pancreas of animals, the method of affinity chromatography with a ligand attached to the carrier is used. Which of the following substances is used as a ligand?

- A Starch
- B Cellulose
- C Lactose
- D Sucrose
- E Glucose

16. In the patient, a stone in the common bile duct blocked the flow of bile to the intestine. Indigestion of what substances is observed?

- A Zhiriv
- B Proteins
- C Water-soluble vitamins
- D Microelements
- E Carbohydrates

17. The patient was diagnosed with achlohydria. This leads to a decrease in the activity of which enzyme?

- A Pepsin
- B Trypsin
- C Chymotrypsin
- D Elastase
- E Aminopeptidases

18. The drug tannin is used in practical medicine as an astringent for acute and chronic intestinal diseases. The astringent effect of tannin is related to its ability to:

- A Denature proteins
- B Hydrolyze proteins
- C Renature proteins
- D Salt the proteins
- E Oxidize proteins

19. In the patient, as a result of the study, a violation of protein digestion in the stomach and small intestine was revealed. Lack of which enzymes leads to such a violation?

- A Peptidases
- B Transferases
- C Amylase
- D Lipases
- E Oxidoreductases

20. Nucleosidases and nucleotidases of the gastrointestinal tract catalyze the hydrolysis of nucleic acids and mononucleotides, as well as those medicinal substances that have the following chemical bond in the molecule:

- A Phosphodiester
- B Hydrogen
- C Peptide
- D Glycoside
- E Amid

21. In children, the pH of gastric juice ranges from 4.0 to 5.0. Name the enzyme of gastric juice that is active under these conditions.

- A Renin
- B Pepsin
- C Chymotrypsin
- D Trypsin
- E Elastase

22. According to the result of the analysis of the patient's saliva, it was established that the pH is 8.0, i.e. shifted to the alkaline side. This state of saliva contributes to:

- A * Formation of tartar;
- B Development of caries;
- C Development of fluorosis;
- D Development of tooth tissue hyperplasia;
- E Development of tooth tissue hypoplasia.

23. The patient was prescribed a bile preparation to improve the digestion of fatty food. What are the components?

Does this drug take part in the emulsification of fats?

- A Bile acids;
- B Cholesterol;
- C Diglycerides;
- D Amino acids;
- E Higher fatty acids.

24. The pancreas secretes an enzyme capable of hydrolyzing α -1,4-glycosidic bonds in the glycogen molecule. Point to this enzyme.

- A α -Amylase
- B Phosphatase;
- C Enterokinase
- D Chymotrypsin
- E Lysozyme.

25. With exocrine insufficiency of the pancreas sometimes with the drug "festal", which contains pancreatic enzymes, for improvement Bile acid preparations are recommended for digestion. For what purpose is such an additive used?

- A *For emulsifying fats
- B To activate proteolytic enzymes
- C For activation of β -amylase
- D To stimulate the secretion of pancreatic juice
- E To stimulate intestinal peristalsis

26. Inhibition of the synthesis of bile acids from cholesterol in the experimental liver animals led to impaired digestion of lipids. What is the role of these acids in lipid digestion?

- A *Emulsify food lipids
- B Participate in resynthesis of lipids
- C They are part of LDL
- D Maintain an alkaline environment in the intestines
- E Activate the formation of chylomicrons

Test tasks.

27. Inactivation of vitamin PP and its coenzyme forms is carried out by methylation to N-methylnicotinamide, which is excreted in the urine. Therefore, with the long-term appointment of vitamin PP in high doses, the following dietary recommendation is appropriate:

- A *Enrichment of the diet with proteins rich in methionine
- B Enriching the diet with proteins rich in tryptophan
- C Enrichment of the diet with unsaturated fatty acids
- D Enrichment of the diet with carbohydrates
- E Sharp restriction of carbohydrates

28. Vitamin derivatives are part of the coenzymes of the respiratory chain. NAD is the coenzyme form of which vitamin?

- A *B3
- B B6
- C B1
- D B2
- E B3

29. Tuberculosis patients take a drug that is an anti-vitamin of nicotinic acid. Specify this substance.

- A *Isoniazid
- B Sulfanilamide
- C Akrichin
- D Isoriboflavin
- E Oxythiamine

30. The patient complains of lack of appetite, nausea, abdominal pain, diarrhea, headache, memory impairment. Dermatitis is observed in the neck and face. Which vitamin is deficient?

- A *Vitamin B5
- B Vitamin B1
- C Vitamin B3
- D Vitamin B2
- E Vitamin B6

31. A patient with a severe form of diarrhea, dermatitis and dementia was prescribed vitamin PP. State the role of vitamin PP in metabolism.

- A *Participation in redox processes
- B Participation in the hydrolysis of peptide bonds
- C Participation in isomerization reactions
- D Participation in oxygen transport
- E Participation in the formation of peptide bonds

32. Determination of the activity of some transaminases is widely used in medical practice for the purpose of diagnosing damage to internal organs. The cofactor of these enzymes is the active form of the vitamin

- A *B6
- B B1
- C B2
- D B12
- E RR

33. Determination of the activity of alanine aminotransferase (ALT) and aspartate aminotransferase (AsT) is widely used in medical practice for the purpose of diagnosing damage to internal organs. The prosthetic group of these enzymes is the coenzyme form of which vitamin?

- A *B6
- B B1
- C WITH
- D B5
- E R

34. Insufficiency of which vitamin causes a decrease in the activity of aminotransferases and decarboxylases?

- A *B6
- B B12
- C B2
- D B3
- E B15

35. The patient has dry lips, cracks and "crusts" in the corners of the mouth, a bright red tongue, seborrheic dermatitis of the nasolabial folds, photophobia and conjunctivitis. What vitamin deficiency is this associated with?

- A *Riboflavin
- B Cholecalciferol
- C Cobalamin
- D Pyridoxine
- E Ascorbic acid

36. The patient has neurasthenic syndrome, diarrhea, dermatitis. What vitamin deficiency is this associated with?

- A *Nicotinic acid
- B Vitamin D
- C Vitamin K
- D Vitamin B12
- E Folic acid

37. A compound containing isoalloxazine was found in the patient's urine cycle. What is this compound?

- A *Vitamin B2;
- B Vitamin B5;
- C Vitamin B6
- D Vitamin B1
- E Vitamin B3

38. In clinical practice, the drug isoniazid is used, which is competitively included in the coenzyme structure, which cannot participate in redox processes, and this leads to the cessation of growth of Koch's bacillus. Specify which enzyme systems are inhibited:

- A *NAD-dependent enzymes
- B FAD-dependent enzymes
- C CoQ
- D Cytochrome c
- E Cytochrome a1

39. A 38-year-old patient suffering from chronic alcoholism has edema, muscle atrophy, cardiovascular insufficiency, and peripheral nerve pain. Such symptoms are caused by the lack of which vitamin in the body?

- A *vitamin B1
- B vitamin A
- C vitamin E
- D vitamin B6
- E vitamin K

40. The patient has pain along the course of peripheral nerves. This can be caused by a lack of which vitamin?

- A *vitamin B1
- B vitamin A
- C vitamin E
- D vitamin B12
- E vitamin K

41. Redness of the mucous membrane of the mouth, cracks in the corners and lips of the patient, peeling of the skin, dryness and inflammation of the conjunctiva on the face, sprouting of the vascular mesh into the cornea. The probable cause of this pathology is a lack of vitamin:

- A *B2
- B WITH
- C IS
- D K
- E D

42. Nicotinic acid amide plays an important role in metabolism. What a disease

occurs with his hypovitaminosis?

- A *Pellagra
- B Rickets
- C Anemia
- D Xerophthalmia
- E Beri-Beri

43. Pyridine-dependent dehydrogenases act as the primary hydrogen acceptor during tissue respiration. Which of the vitamins is necessary for the formation of the corresponding coenzyme (NAD⁺)?

- A * Vitamin PP
- B Vitamin C
- C Vitamin B1
- D Vitamin B2
- E Vitamin B6

44. Vitamins must be included in a person's diet. Which one is used to treat pellagra?

- A *Vitamin B5
- B Vitamin B1
- C Vitamin C
- D Vitamin A
- E Vitamin D

45. For the treatment of heart diseases, the drug cocarboxylase is used. This drug is the coenzyme form of which vitamin?

- A * B1
- B B6
- C B12
- D WITH
- E R

46. Water-soluble vitamins in the body are converted into coenzyme forms. Coenzyme form of which vitamin is thiamine diphosphate (TDP)?

- A * vitamin B1
- B vitamin B2
- C vitamin C
- D vitamin B6
- E vitamin B12

47. The biochemical function of water-soluble vitamins depends on their ability to transform into coenzyme forms. What coenzyme form can vitamin B2 (riboflavin) convert into:

- A * FMN (flavin mononucleotide)
- B NAD⁺ (nicotinamide adenine dinucleotide)
- C TMF (thiamine monophosphate)
- D TDF (thiamine diphosphate)
- E PALF (pyridoxal phosphate)

48. A 30-year-old man with pulmonary tuberculosis is prescribed isoniazid. Insufficiency of which vitamin can develop as a result of long-term use of this one drug?

- A * Pyridoxine
- B Tocopherol

- C Cobalamin
- D Ergocalciferol
- E Retinol

4. Summing up.

5. List of recommended literature (main, additional, electronic information resources):

Main:

1. Gubsky Yu.I., I.V. Nizhenkovska, Korda M.M. Biological and Bioorganic Chemistry: in 2 books. Book 2. Biological Chemistry: textbook. 2021. 544 p.
2. Satyanarayana U. Biochemistry. 5th edition. India 2020. 777 p.
3. Lehninger. Principles of Biochemistry. 7th edition. NY, United States. 2017.
4. Jeremy M. Berg, John L. Tymoczko, Gregory J. Gatto. Biochemistry. 8th Revised edition. 2015.
5. Lippincott Illustrated Reviews: Biochemistry. Philadelphia :Wolters Kluwer, 2017. 560 p.
6. Baynes J., Dominiczak M. Medical Biochemistry. 5th Edition. Elsevier, 2018. 712 p.
7. Donald Voet, Judith G. Voet, Charlott W. Pratt. Fundamentals of Biochemistry: Life at the Molecular Level. ISBN: 978-1-118-91840-1 February 2016, 1184 P.

Additional:

1. William Marshall, Marta Lapsley, Andrew Day, Kate Shipman. Clinical Chemistry. Elsevier, 2020. 432 p.
2. Harper's Illustrated Biochemistry / V.W. Rodwell, D.A. Bender, K.M. Botham et al. – Mc Graw Hill Education, 2015. – 817 p.

Electronic information resources:

1. [https://info.odmu.edu.ua/chair/biology/-](https://info.odmu.edu.ua/chair/biology/)
2. <http://libblog.odmu.edu.ua/>
3. <https://moodle.odmu.edu.ua/login/index.php>

Practical class №33

Topic: Water-soluble vitamins C, biotin, folic acid, B12, pantothenic acid. Structure, biological role, hypovitaminosis.

Goal: To make higher education applicants aware of the importance of studying the mechanism of action and biological role of this group of vitamins. Show the possibility of using them in practical medicine.

To acquaint higher education applicants with the peculiarities of the coenzyme functions of the vitamins biotin, folic acid, vitamin B12, with the peculiarities of the influence of vitamin C on metabolic processes.

To teach higher education applicants the quantitative determination of vitamin C in plant objects.

Basic concepts: _____ vitamins, coenzyme functions, symptoms of hypo-vitaminosis, antivitamins

Equipment: _____ Laboratory of the department

Plan:

1. Organizational activities (greetings, verification of those present, announcement of the topic, purpose of the class, motivation of higher education seekers to study the topic).
2. Control of the reference level of knowledge.

The higher education applicant should know:

- structural formulas of vitamins Bc, C, B12, P, H.
- the role of these vitamins in metabolic processes as coenzymes or prosthetic groups.
- causes, symptoms and consequences of hypovitaminosis.

The higher education applicant should be able to:

- to determine the amount of vitamin C in a plant object.

Questions to check basic knowledge on the topic of the class:

1. Which water-soluble vitamins are coenzymes?
2. Sources of vitamin C, daily requirement. What does vitamin C cause?
3. Coenzymes-nucleotides.
4. Coenzymes-phosphorus esters of vitamins.

3. Formation of professional skills and abilities.

3.1 Demonstration and practical work «Quantitative determination of vitamin C in products by the Tillmans method.

Recommendations for performing tasks.

Principle of the method: Determination of the content of vitamin C (ascorbic acid) is based on its reaction with 2,6-dichlorophenolindophenol. Using the change in color, based on the amount of the reagent spent on the oxidation of vitamin C, it is possible to determine its amount in the studied object.

Progress:

1. Preparation of material for research.

Take 1.0 g of rose hips or needles, grind it in a porcelain mortar, then add 10.0 ml of 2% hydrochloric acid. Quickly filter the hood to a dry flask.

2. Determination of content by titration.

Measure 3.0 ml of the filtrate into a conical flask and titrate with a 0.001 N solution of the sodium salt of 2,6-dichlorophenolindophenol to a pale pink color that does not disappear within 30 seconds.

The calculation is carried out according to the following formula:

$$C = , \text{ where } \frac{M \cdot 0,088 \cdot 10}{A \cdot B}$$

C is the concentration of vitamin C in the solution;

M is the amount of 2,6-dichlorophenolindophenol used for titration;

0.088 – the amount of vitamin C that binds 1 ml of 2,6-dichlorophenolindophenol;

10 – the number of hoods;

A is the amount of extract taken for titration;

B is the amount of researched material in

Conclusion: with the help of the described method, it is possible to determine the content of vitamin C in plant objects.

Requirements for work results.

Enter the obtained data into the workbook.
Make medical and biological conclusions.

Control materials for the final stage of the class.

Questions to check the final level of knowledge:

- 1. Physico-chemical characteristics of folic acid, biological role and mechanism of action.
- 2. Physico-chemical characteristics of biotin, biological role and mechanism of action.
- 3. Physico-chemical characteristics of vitamin C, biological role and mechanism of action.

The main signs of hypovitaminosis.

- 4. Structure, physical and chemical properties, biological role, mechanism of action of vitamin P.
- 5. Structure, physical and chemical properties, biological role, mechanism of action of vitamin B12.
- 6. Structure, physical and chemical properties, biological role, mechanism of action of pantothenic acid.
- 7. Hypovitaminosis of the above-mentioned vitamins.

Test tasks.

1. Insufficiency of which vitamin leads to the development of hypercoagulation syndrome?

A *S

In B12

S A

D B6

E D2

2. The doctor diagnosed the patient with scurvy. This disease was a manifestation of which pathological condition associated with improper nutrition?

A * Nutritional imbalance

In Malnutrition

C Overeating

D Stomach ulcer

E Helminthosis

3. The mechanism of antimicrobial action of sulfonamide drugs is based on their structural similarity with:

A * Para-aminobenzoic acid

In Glutamic acid

With folic acid

D Nucleic acid

E Antibiotics

4. In the process of biotransformation of the anesthetic drug novocaine, para-aminobenzoic acid is formed. Therefore, with the combined use of novocaine and sulfonamides, the following is most likely to occur:

A *Decreasing the antimicrobial effect of sulfonamides

In Strengthening the antimicrobial action of sulfonamides

C Reduction of the anesthetic effect of novocaine

D Strengthening of the anesthetic effect of novocaine

E Decrease in the pharmacological activity of both drugs

5. Lack and absence of ascorbic acid in food cause various diseases. Name the main one of them.

- A Scurvy
- B Rickets
- C Beri-Beri
- D Pellagra
- E Gout

6. Complex proteins in the human body perform various functions. Which glycoprotein contained in raw egg white disrupts the absorption of vitamin H and can lead to acute biotin deficiency?

- A Avidin
- B Fibrinogen
- C Hemoglobin
- D Interferon
- E Caseinogen

7. The formation of collagen in the human body requires the hydroxylation of proline, which occurs with the participation of prolyl hydroxylase. What substance activates this process:

- A Ascorbic acid
- B OVER
- C FAD
- D Biotin
- E Pyridoxine phosphate

8. The patient was diagnosed with pernicious anemia. Indicate which vitamin deficiency this may be due to.

- A B12
- B B3
- C B2
- D WITH
- E IS

9. Vitamins, when used simultaneously, can enhance each other's effects. Which of the vitamins potentiates the antihyaluronidase activity of vitamin P?

- A *Vitamin C
- B Vitamin B1
- C Vitamin D
- D Vitamin A
- E Vitamin B2

10. Certain conditions are necessary for the assimilation of vitamins by organisms. For the absorption of which vitamin, the presence of Castle's factor (a glycoprotein produced by the lining cells of the gastric mucosa) is necessary?

- A *Vitamin B12
- B Vitamin C
- C Vitamin B5
- D Vitamin E
- E Vitamin B6

11. A patient with angina was prescribed a sulfonamide drug, the antimicrobial action of which is due to a violation of the synthesis of folic acid. With which substance do sulfonamides compete for the active center of the enzyme?

- A * Para-aminobenzoic acid
- B Ubiquinone
- C Succinate
- D Glutamic acid
- E Citric acid

12. Bacteriostatic sulfonamides suppress the synthesis of nucleotides, nucleic acids and proteins in microbial cells, but in pharmacological doses they do not affect the synthesis of these substances in the human body. This difference is due to the fact that eukaryotic cells:

- A *Folic acid is not synthesized
- B Para-aminobenzoic acid is not synthesized
- C Nucleotides are not synthesized
- D Impermeable to sulfonamides
- E Very quickly inactivate sulfonamides

13. Antimicrobial sulfonamide drugs are metabolized in the body by acetylation. At the same time, their bacteriostatic action:

- A *Lost
- B is growing
- C Does not change
- D It changes to toxic in the human body
- E Intensifies

14. The patient has megaloblastic anemia (Addison-Birmer disease). It is advisable to treat with vitamin B12 in combination with the following drug:

- A *Folic acid
- B Lipoic acid
- C Ascorbic acid
- D Nicotinic acid
- E Pangamic acid

15. The patient consumes several raw eggs daily, which contain the anti-vitamin of biotin - avidin. What disorders in lipid metabolism can occur in this case?

- A *Biosynthesis of fatty acids
- B Cholesterol biosynthesis
- C Oxidation of glycerol
- D Absorption of lipids
- E Transport of lipids in the blood

16. Bacterial cells use folic acid to synthesize a certain vitamin, the derivatives of which are coenzymes of a number of important bacterial enzymes. Sulfanilamide drugs block the formation of these coenzymes, as they are antivitamins:

- A * Para-aminobenzoic acid
- B Pyridoxine
- C Nicotinic acid
- D Riboflavin
- E to Holin

17. In a patient with complaints of pain in the stomach, a decrease in the secretory function of the stomach, which was accompanied by anemia, was established during the biochemical examination. Indicate which of the vitamins has an anti-anemic effect:

- A * Cobalamin
- B Tocopherol
- C Retinol
- D Thiamine
- E Nicotinic acid

18. Some vitamins inhibit the formation of lipid peroxides in cells membranes and ensure the stability of biological membranes. Specify one of vitamins that have this effect.

- A * ascorbic acid
- B naphthoquinone
- C cholecalciferol
- D pantothenic acid
- E folacin

19. In a patient with frequent bleeding in the internal organs and mucous membranes in the composition

proline and lysine were found in collagen fibers. Lack of which vitamin leads to violation of their hydroxylation.

- A *Vitamin C
- B Vitamin E
- C Vitamin K
- D Vitamin A
- E Vitamin B1

20. The parents of a 10-year-old boy went to the doctor with a complaint about stunted growth. During the examination, the doctor found changes in the mucous membranes and suspicion of malignant anemia. He suggested that this disease is associated with vitamin deficiency. Indicate which vitamin deficiency can lead to

the development of such a condition?

- A * Folic acid
- B Nicotinic acid
- C Orotic acid
- D to Holin
- E Arachidonic acid

21. The result of a violation of which biochemical reaction is the appearance of hemorrhages at scurvy disease.

- A *hydroxylation of proline
- B glucose phosphorylation
- C dehydrogenation of isocitric acid
- D isomerization of phosphodioxycetone
- E deamination of glutamic acid

22. A patient with tuberculosis was treated with isoniazid (tuberculostatic drug). Later, he developed signs of dermatitis, diarrhea, and damage to the central nervous system. Which vitamin should be prescribed for this patient?

- A *vitamin RR
- B vitamin C

- C lipoic acid
- D vitamin A
- E vitamin B1.

23. In medical practice, sulfonamide drugs are used, which are antimetabolites of paraaminobenzoic acid, which is synthesized by microorganisms. Indicate the synthesis of which vitamin is blocked.

- A * Folic acid
- B Pangamic acid
- C Orotic acid
- D Nicotinic acid
- E Ascorbic acid

24. The patient complains of bleeding gums, spot hemorrhages. which vitamin preparation can be used in this case?

- A * Askorutin
- B Thiamine hydrochloride
- C Cyanocobalamin
- D Nicotinic acid
- E Pyridoxine hydrochloride

25. Most vitamins undergo transformation in the human body. What vitamin takes part in the formation of coenzyme of acetylation (CoASH)?

- A *Vitamin B3
- B Vitamin C
- C Vitamin D
- D Vitamin A
- E Vitamin K

26. A 50-year-old patient developed hypovitaminosis C as a result of long-term irrational nutrition. A decrease in the activity of which enzyme is the basis of connective tissue damage in this pathology?

- A *Proline hydroxylases
- B Alanine aminotransferases
- C Pyruvate carboxylase
- D Tryptophan hydroxylase
- E Glutaminase

27. The enzyme hyaluronidase splits hyaluronic acid, as a result of which intercellular permeability increases. Which vitamin helps to strengthen the walls of blood vessels and inhibits the activity of hyaluronidase?

- A * Vitamin R
- B Vitamin A
- C Vitamin B1
- D Vitamin B2
- E Vitamin D

4. Summary:

5. List of recommended literature (main, additional, electronic information resources):

Main:

1. Gubsky Yu.I., I.V. Nizhenkovska, Korda M.M. Biological and Bioorganic Chemistry: in 2 books. Book 2. Biological Chemistry: textbook. 2021. 544 p.
2. Satyanarayana U. Biochemistry. 5th edition. India 2020. 777 p.
3. Lehninger. Principles of Biochemistry. 7th edition. NY, United States. 2017.
4. Jeremy M. Berg, John L. Tymoczko, Gregory J. Gatto. Biochemistry. 8th Revised edition. 2015.
5. Lippincott Illustrated Reviews: Biochemistry. Philadelphia :Wolters Kluwer, 2017. 560 p.
6. Baynes J., Dominiczak M. Medical Biochemistry. 5th Edition. Elsevier, 2018. 712 p.
7. Donald Voet, Judith G. Voet, Charlott W. Pratt. Fundamentals of Biochemistry: Life at the Molecular Level. ISBN: 978-1-118-91840-1 February 2016, 1184 P.

Additional:

1. William Marshall, Marta Lapsley, Andrew Day, Kate Shipman. Clinical Chemistry. Elsevier, 2020. 432 p.
2. Harper's Illustrated Biochemistry / V.W. Rodwell, D.A. Bender, K.M. Botham et al. – Mc Graw Hill Education, 2015. – 817 p.

Electronic information resources:

1. [https://info.odmu.edu.ua/chair/biology/-](https://info.odmu.edu.ua/chair/biology/)
2. <http://libblog.odmu.edu.ua/>
3. <https://moodle.odmu.edu.ua/login/index.php>

Practical class №34

Topic: **Fat-soluble vitamins. Vitamins of groups A, D, E, K. Structure, biological role. Hypo- and hypervitaminosis.**

Goal: __To study the mechanism of action and biological role of this group of vitamins. Show the possibility of using them in practical medicine.

higher education applicants should study and know the structure, biochemical characteristics of fat-soluble vitamins dissolved in fats A, D, E, K, know the mechanisms of action of these vitamins and signs of hypovitaminosis and hypervitaminosis of vitamins A, D, K.

Teach higher education applicants the qualitative determination of vitamins A, D, E, K.

Basic concepts: fat-soluble vitamins, hypo- and hypervitaminosis, causes, consequences, symptoms

Equipment: Laboratory of the department

Plan:

1. Organizational activities (greetings, verification of those present, announcement of the topic, purpose of the class, motivation of higher education seekers to study the topic).
2. Control of the reference level of knowledge.

The higher education applicant should know:

- know the role of each of the vitamins listed above in metabolism

The higher education applicant should be able to:

i. write the formulas of vitamins A, D, E, K;

- to qualitatively determine the vitamins in the studied sample.

-

Questions to check basic knowledge on the topic of the class:

–1. Characteristics of fat-soluble vitamins.

- 2. What are carotenoids.
- 3. The role of bile acids in the absorption of fat-soluble vitamins.
- 4. Participation of fat-soluble vitamins in metabolism.

3. Formation of professional skills and abilities.

3.1 Demonstration and practical work «*Qualitative reactions to fat-soluble vitamins A, D, E, K (Vikasol)*».

Recommendations for performing tasks.

A. Qualitative determination of vitamin A

Principle of the method: If chloroform and concentrated sulfuric acid are added to fish oil containing vitamin A, a purple ring forms at the interface between the two liquids.

The main stages of work performance.

1. *Preparation of the hood:*

2 drops of fish oil are dripped into the test tube, and then 5 drops of chloroform. They shake

2. *Formation of a colored complex:*

Add 0.5 ml of concentrated sulfuric acid to the obtained extract. Mix carefully. A purple ring appears at the interface between the two liquids, which then turns brown. The appearance of such a ring indicates that the solution contains vitamin A.

B. Qualitative determination of vitamin D

Principle of the method: When vitamin D interacts with hydrochloric acid aniline, a red color is observed.

The main stages of work performance.

1. *Preparation of the reaction mixture:*

Pour 0.5 ml of fish oil into a dry test tube, then add 1.0 ml of aniline hydrochloric acid solution.

2. *Boiling:*

The contents of the test tube are heated to boiling with constant stirring and boiled for 30 seconds. The yellow emulsion first acquires a dirty-green, and then a brown-red color. This indicates that the solution contains vitamin D.

B. Qualitative determination of vitamin E

Principle of the method: When an alcoholic solution of tocopherol reacts with concentrated nitric acid, the reaction mixture turns red.

The main stages of work performance.

Pour 0.5 ml of an alcoholic tocopherol solution into a dry test tube and add 1.0 ml of concentrated nitric acid. As a result of the reaction, a product of quinoid nature is formed, which gives a red color. This color indicates the presence of vitamin E in the solution.

D. Qualitative determination of vitamin K

Principle of the method: If cysteine and NaOH are added to a solution containing vitamin K, the solution will acquire a lemon-yellow color.

The main stages of work performance.

Pour 0.5 ml of Vikasol into the test tube, then add 0.5 ml of cysteine and one drop of 10% NaOH solution.

In the presence of cysteine, the Vikasol solution in an alkaline environment acquires a lemon-yellow color.

Requirements for work results.

Enter the obtained data and calculations into the workbook.

Make medical and biological conclusions.

Control materials for the final stage of the class.

Questions to check the final level of knowledge:

1. Vitamin A, its structure, forms in the body, biological role and mechanism of action. Avitaminosis, hypovitaminosis, hypervitaminosis A.
2. Vitamin D, its structure, biological role and mechanism of action on mineral metabolism. Avitaminosis, hypovitaminosis, hypervitaminosis D.
3. Vitamin E, its chemical nature, biological role and mechanism of action. Hypovitaminosis E.
4. Vitamin K, its structure, biological role and mechanism of action on blood coagulation processes. Hypovitaminosis K, signs of hemophilia.
5. Hypervitaminoses. Toxic effect of large doses of vitamins.

Test tasks.

1 The doctor recommends the patient to take Vikasol under conditions of increased risk of bleeding. What vitamin is this drug an analogue of?

- A *Vitamin K
- B Vitamin A
- C Vitamin B5
- D Vitamin B12
- E Vitamin B6

2. Administration of dicoumarol into the body causes a sharp decrease in blood clotting factors. Dicoumarol is an antivitamin of which vitamin?

- A *Vitamin K
- B Vitamin C
- C Vitamin B2
- D Vitamin E
- E Vitamin R

3. The patient suffers from thrombophlebitis. Which of the vitamins that enhances the synthesis of blood coagulation factors can provoke an exacerbation of this disease?

- A * vitamin K
- B vitamin E
- C vitamin B2
- D vitamin D
- E vitamin B1

4. In the large intestine, microorganisms synthesize vitamins that participate in the biochemical processes of the body. What vitamins are mainly synthesized by microflora?

- A *K, B12
- B A, S
- C E, RR
- D B1, B2
- E B6, E

5. A patient with chronic hepatitis has bleeding gums, hemorrhages in the skin even with a minor injury. These manifestations are most likely associated with a violation of the metabolism of which vitamin?

- A *K
- B D
- C IS
- D RR
- E IN

6. Vitamin D is necessary for the formation of the bone system of the fetus during intrauterine development. This vitamin is a derivative of which chemical compound?

- A Cholesterol;
- B Glycerol;
- C Sphingosine;
- D Inositol;
- E Ethanol.

7. Some vitamins ensure the stability of biological membranes. Name one of the vitamins that has this effect.

- A * tocopherol
- B naphthoquinone
- C cholecalciferol
- D pantothenic acid
- E riboflavin

8. Vitamin A quickly oxidizes in the air, which leads to the loss of biological activity. Which component of food products mainly prevents oxidation of the vitamin?

- A *tocopherol
- B nicotinic acid
- C Table salt
- D White
- E Fat

9. Deficiency of which vitamin will most likely cause activation of lipid peroxidation processes?

- A *Vitamin E
- B Vitamin D
- C Vitamin K
- D Vitamin B12
- E Vitamin B6

10. An elderly woman complains of impaired vision at dusk. Which of the listed vitamins should be prescribed in this case?

- A *AND
- B WITH
- C IS
- D D
- E RR

11. The patient was hospitalized with intestinal bleeding. What drug should be included in the treatment regimen?

- A Vikasol
- B Sulfanilamide
- C Cocarboxylase
- D Aspirin
- E Riboflavin

12. The state of the patient's antioxidant system was assessed based on the determination of the content of one of the endogenous antioxidants. Which one exactly?

- A *Alpha-tocopherol
- B Trivalent Ferum
- C Ornithine
- D Hydrogen peroxide
- E Cholecalciferol

13. The ophthalmologist found that the patient had an increase in the time it took for the eye to get used to the dark. The lack of which vitamin can be the cause of such a symptom?

- A * vitamin A
- B vitamin C
- C vitamin K
- D vitamin B1
- E vitamin B6

14. 20 minutes after the skin cut, the woman noticed that the wound did not stop bleeding. Deficiency of which vitamin causes this condition?

- A * vitamin K;
- B vitamin A;
- C vitamin D;
- D vitamin E;
- E vitamin B12.

15. A woman contacted a pediatrician about the ill health of her 8-month-old child: sweating, increased size of the relative, delay in teething. Which drug should be prescribed first?

- A *Cholecalciferol;
- B Cyanocobalamin;
- C Calcium gluconate;
- D Thiamine bromide;
- E Calcium pangamate

16. The child did not receive vitamin D in a timely manner. After a while, everyone appeared

symptoms of rickets. A decrease in the activity of which blood enzyme is observed in this case?

- A *Alkaline phosphatase;
- B Acid phosphatase;
- C Alpha amylases;
- D Choline esterase;
- E Creatine kinases.

17. During the examination of a one-year-old child, the doctor paid attention to the late eruption of teeth, their incorrect location. The lack of which vitamin can cause such a condition?

- A *Vitamin A;
- B Vitamin C;
- C Vitamin E;

- D Vitamin D;
- E vitamin B2.

18. During the examination of the patient's oral cavity, the dentist determined dryness mucous membrane, numerous erosions. The lack of which vitamin caused these phenomena?

- A *Vitamin A;
- B Vitamin K;
- C Vitamin P;
- D Vitamin H;
- E Vitamin PP.

19. In the last month of pregnancy, the doctor recommends that women take Vikasol according to the scheme. Which vitamin is it an analogue of?

- A *Vitamin K
- B Vitamin B12
- C Vitamin B5
- D Vitamin B6
- E Vitamin A

20. Vitamins are the amines of life. What vitamin in the body is formed from provitamin beta-carotene?

- A *A1
- B B1
- C B12
- D WITH
- E D

21. With long-term use of the sulfonamide drug, the patient's microbial flora in the intestine was disrupted. During the examination in the polyclinic, small point hemorrhages were found on the skin. Their reason is most likely:

- A *Deficiency of vitamin K in the body
- B Lack of vitamin C in the body
- C Enterocolitis (inflammation of the mucous membrane of the small and large intestine)
- D Liver disease
- E Hereditary defect of blood coagulation factors

22. A patient with cirrhosis of the liver is noted to have impaired vision at dusk. Which of the following is the most likely cause?

- A * Violation of absorption of vitamin A in the intestine
- B Insufficient supply of vitamin A with food products
- C Excessive intake of vitamin A with food products
- D Violation of trans-retinal conversion into cis-retinal
- E Violation of rhodopsin synthesis

23. To stimulate the activity of the blood coagulation system, vitamin K is used. Its effect is based on participation in the process:

- A *Carboxylation of amino acid residues of blood coagulation factors II VII, IX and X
- B Glycosylation of amino acid residues of blood coagulation factors II VII, IX and X
- C Phosphorylation of amino acid residues of blood coagulation factors II VII, IX and X
- D Deamination of amino acid residues of blood coagulation factors II VII, IX and X
- E Limited proteolysis of amino acid residues of blood coagulation factors II VII, IX and X

X

24. As a result of intoxication, the patient has impaired synthesis of beta-lipoproteins. This is accompanied by malabsorption:

- A *Vitamin E.
- B Vitamin C.
- C Vitamin B6.
- D Vitamin H.
- E Vitamin B12.

25. The patient complains of loss of appetite, headache, bad sleep. Inflammation of the eyes, hair loss, and general exhaustion of the body were noted. It is known from the anamnesis that the patient took fish oil. What disease can be suspected?

- A *Hypervitaminosis of vitamin D
- B Hypovitaminosis of vitamin D
- C Hypervitaminosis of vitamin A
- D Vitamin A hypovitaminosis
- E Hypervitaminosis of vitamin F

26. Dicoumarol is prescribed for the prevention of thrombosis. Antivitamin of which vitamin is it?

- A *K
- B D
- C C
- D A
- E RR

27. A patient with symptoms of increased blood coagulation has been taking anticoagulant drugs - coumarol derivatives for a long time. As a result, the patient developed signs of bleeding. The appointment of which vitamin will quickly and effectively eliminate unwanted complications?

- A *K
- B AND
- C D
- D WITH
- E IS

28. Under the influence of ultraviolet rays and ionizing radiation, active forms of oxygen are formed in the body. Substances exhibiting antioxidant properties are used to stabilize redox reactions. Specify them.

- A *Vitamin E
- B Vitamin B12
- C Vitamin B2
- D Vitamin B6
- E Vitamin B1

29. A 35-year-old patient was prescribed vikasol (a synthetic analogue of vitamin K) in the preoperative period. What is the mechanism of antihemorrhagic action of this drug?

- A * Stimulation of prothrombin synthesis
- B Activation of plasminogen
- C Stimulation of tissue thromboplastin synthesis
- D Activation of the Hageman factor (XII)
- E Activation of the complement system

30. Antivitamins are substances of various structures that limit the use of vitamins in the body and have the opposite effect. Specify among the listed substances the anti-vitamin of vitamin K:

- A *Dicumarol
- B Deoxyipyridoxine
- C Aminopterin
- D Sulfapyridazine
- E Isoniazid

31. A certain vitamin is necessary for the normal development and functioning of the reproductive system in humans. Specify it.

- A *IS
- B WITH
- C N
- D D
- E AND

32. The process of blood coagulation cannot normally be carried out without the participation of certain vitamins. What vitamin is involved in this process?

- A *vitamin K
- B vitamin B6
- C vitamin C
- D vitamin E
- E vitamin D

33. The child was given vitamin D in a dose of 50 mg/day to prevent rickets, which led to the appearance of signs of vitamin intoxication. Select the signs of hypervitaminosis D

- A * Bone demineralization
- B "Chicken Blindness"
- C Anemia
- D Violation of blood coagulation
- E Gout

34. Vitamins regulate various biochemical processes. What vitamin ensures the conversion of prothrombin to thrombin?

- A *Vitamin K
- B Vitamin A
- C Vitamin E
- D Vitamin B1
- E Vitamin D

35. A man suffering from enterocolitis was treated for a long time with the sulfonamide drug Phthalazol. At the last examination in the polyclinic, small point hemorrhages were found on the patient's skin. Their reason is most likely:

- A * lack of vitamin K in the body
- B Insufficiency of vitamin C in the body
- C Enterocolitis (inflammation of the mucous membrane of the small intestine)
- D Liver disease
- E Hereditary defect of blood coagulation factors

36. The blood coagulation process normally cannot be carried out without the presence of certain vitamins. What vitamin is involved in this process?

- And *vitamin K

B vitamin B6
C vitamin C
D vitamin E
And vitamin D

4. Summary:

5. List of recommended literature (main, additional, electronic information resources):

Main:

1. Gubsky Yu.I., I.V. Nizhenkovska, Korda M.M. Biological and Bioorganic Chemistry: in 2 books. Book 2. Biological Chemistry: textbook. 2021. 544 p.
2. Satyanarayana U. Biochemistry. 5th edition. India 2020. 777 p.
3. Lehninger. Principles of Biochemistry. 7th edition. NY, United States. 2017.
4. Jeremy M. Berg, John L. Tymoczko, Gregory J. Gatto. Biochemistry. 8th Revised edition. 2015.
5. Lippincott Illustrated Reviews: Biochemistry. Philadelphia :Wolters Kluwer, 2017. 560 p.
6. Baynes J., Dominiczak M. Medical Biochemistry. 5th Edition. Elsevier, 2018. 712 p.
7. Donald Voet, Judith G. Voet, Charlott W. Pratt. Fundamentals of Biochemistry: Life at the Molecular Level. ISBN: 978-1-118-91840-1 February 2016, 1184 P.

Additional:

1. William Marshall, Marta Lapsley, Andrew Day, Kate Shipman. Clinical Chemistry. Elsevier, 2020. 432 p.
2. Harper's Illustrated Biochemistry / V.W. Rodwell, D.A. Bender, K.M. Botham et al. – Mc Graw Hill Education, 2015. – 817 p.

Electronic information resources:

1. <https://info.odmu.edu.ua/chair/biology/>-
2. <http://libblog.odmu.edu.ua/>
3. <https://moodle.odmu.edu.ua/login/index.php>

Practical class №35

Topic: Biochemical characteristics and functions of blood. Biochemical composition of blood plasma. Characteristics of protein fractions of blood. Characteristics of non-protein substances of blood plasma. Residual nitrogen of blood, its components. Diagnostic value of residual nitrogen determination in blood. Lipid transport forms - plasma lipoproteins. Types of hyperlipoproteinemia. The role of lipoproteins in the development of atherosclerosis. Osmotic pressure and acid-base state of blood. Blood buffer systems, hormonal regulation mechanisms, lung and kidney function. Blood respiratory function. Hemoglobin, structure, synthesis in the body. Role in the transport of oxygen and carbon dioxide. Abnormal hemoglobin.

Goal: To study theoretical material on blood biochemistry: fractions of plasma and serum proteins, classification of enzymes and their value for differential diagnosis of pathology, acute phase proteins and the value of their determination in clinical diagnosis. Be able to determine the total protein in blood serum by the biuret method and explain the diagnostic value of quantitative determination of protein in blood serum.

Basic concepts: albumins, globulins, fibrinogen, lipoproteins, residual nitrogen, blood buffer systems

Equipment: Laboratory of the department

Plan:

1. Organizational activities (greetings, verification of those present, announcement of the topic, purpose of the class, motivation of higher education seekers to study the topic).
2. Control of the reference level of knowledge.

The higher education applicant should know:

- functions and composition of blood;
- components of residual blood nitrogen;
- structure and function of lipoproteins;
- the role of lipoproteins in the development of atherosclerosis;
- mechanisms of regulation of the acid-base state with the participation of individual organs and types of violations and mechanisms of compensation of the acid-base state.

The higher education applicant should be able to:

- evaluate the main biochemical indicators of blood composition in healthy people and in a number of diseases;
- to quantitatively determine the total protein of the blood plasma, individual fractions of proteins and to know their biochemical characteristics and biological significance for the body.

Questions to check basic knowledge on the topic of the class:

1. Comparative characteristics of the chemical composition of plasma and blood serum in the norm.
2. Biochemical functions of blood proteins. Characteristics of the main fractions of blood plasma proteins (albumin, α -, β -, γ -globulins). The concept of hypo-, hyper-, para- and dysproteinemia. Dyslipoproteinemia.
3. Clinical and biochemical characteristics of proteins of the acute phase of inflammation.
4. Classification of blood plasma enzymes, their use in the diagnosis of diseases.
5. Blood buffer systems. Violation of acid-base balance: types of acidosis and alkalosis, mechanisms of their occurrence.
6. The main organic non-protein nitrogen-containing and nitrogen-free components of plasma, characteristics and significance of determination in pathology.
7. General ideas about the mineral composition of blood plasma in normal and pathological conditions.

3. Formation of professional skills and abilities.

3.1 Demonstration and practical work «Quantitative determination of blood proteins by the biuret method».

Recommendations for performing tasks.

The principle of the method is that alkaline solutions of proteins and peptides, thanks to peptide bonds, become colored like biuret when a solution of copper sulfate is added.

Progress. To 0.1 ml of serum, add 8 ml of a 4.8% solution of lye, 3 ml of a 20% solution of copper sulfate. Centrifuge at 3000 rpm. 5 minutes. Colorimeter on FEK with a green light filter against the mixture in which the serum is replaced by water.

A red-violet color appears, the intensity of which is directly proportional to the amount of protein. Multiply the optical density by 12 to find a quantitative representation of the protein content (%).

Requirements for work results.

Enter the obtained data and calculations into the workbook.

Make medical and biological conclusions.

3.2 Demonstration and practical work "Refractometric determination of protein in serum".

Recommendations for performing tasks.

The principle of the method consists in the protein's ability to increase the refractive index of light passing through the solution.

Progress. Place 2-3 drops of water between the prisms and install a zero shunt on the 1.322 section of the light refraction scale. Place 2-3 drops of serum instead of the eyepiece, move the handle of the eyepiece along the scale until the viewing scale aligns with the border of the dark and light parts of the field of vision. According to the table The flight determines the appropriate amount of protein.

Requirements for work results.

Enter the obtained data and calculations into the workbook.

Make medical and biological conclusions.

3.3 Demonstration and practical work "Fractionation of blood serum proteins by the method of salting out".

Recommendations for performing tasks.

The principle of the method is the ability of proteins to coagulate at different concentrations of salts, depending on the molecular weight of the proteins.

Progress. Add an equal volume of saturated ammonium sulfate solution to 3 ml of serum. A precipitate of globulins falls out. They filter. Ammonium sulfate is added to the filtrate until saturation. A precipitate of albumin falls out. The precipitate of albumins and globulins is dissolved in 4 ml of water, a biuret reaction is carried out and the content of globulins and albumins is determined.

Medical and biological evaluation of the obtained results

Hypoproteinemia can be caused by a lack of protein in the diet, a violation of the processes of digestion and absorption of proteins, a violation of protein synthesis (for example, when the liver is damaged), protein loss during acute and chronic bleeding, kidney damage.

Hyperproteinemia can be absolute - with an increase in the protein content in the blood plasma - for example, an increase in the number of γ -globulins in infectious diseases, the appearance of abnormal proteins in the blood in myeloma, in macroglobulinemia. Loss of water in the body (diarrhea, vomiting, extensive burns) can lead to an increase in plasma protein, that is, to relative hyperproteinemia.

Requirements for work results.

Enter the obtained data and calculations into the workbook.

Make medical and biological conclusions.

3.4. Demonstration and practical work «Determination of osmotic resistance of erythrocytes".

Recommendations for performing tasks.

The principle of the method is based on the quantitative determination of the degree of hemolysis in hypotonic solutions of sodium chloride, in which swelling and hemolysis of erythrocytes are known to occur

Progress. Pre-prepared working solutions of sodium chloride of different concentrations: 1%; 0.85%; 0.75%; 0.70%; 0.65%; 0.60%; 0.55%; 0.50%); 0.45%; 0.40%; 0.35%; 0.30%; 0.20% and 0.10%. Working solutions of sodium chloride are poured into 14 centrifuge tubes (5.0 ml each).

Take 1.5 ml of venous blood in a sterile test tube with heparin, mix and add 0.02 ml of heparinized blood to each of 14 centrifuge tubes with sodium chloride working solutions. The tubes are left for 1 h at room temperature, and then centrifuged (5 min at 2000 rpm). The supernatant from each test tube is examined on a photoelectrocolorimeter. The supernatant liquid from the test tube containing 1% sodium chloride solution is used as a blank sample. Determine the percentage (degree) of hemolysis, assuming 100% hemolysis in a test tube with 0.1% sodium chloride solution.

Hemolysis can also be determined visually by the color of the supernatant. In the case of complete hemolysis of erythrocytes, intense red-lacquer staining of the supernatant liquid is noticeable, while the beginning of hemolysis (its minimum degree) is determined by a light pink color (when hemolysis is visually determined, the amount of working solution in the test tube should be less than 1.0 ml).

Normally, the beginning of hemolysis is noted at a concentration of 0.50–0.45%, and complete hemolysis — at a 0.40–0.35% sodium chloride solution.

Medical and biological evaluation of the obtained results

In some hemolytic anemias (congenital microspherocytic, autoimmune hemolytic, etc.), a decrease in osmotic resistance is observed: hemolysis begins at a concentration of sodium chloride solution of 0.55–0.70% and ends at a concentration of 0.40–0.45% (Fig. 7.17 , b).

An increase in the osmotic resistance of erythrocytes occurs in mechanical jaundice, thalassemia, and hemoglobinosis.

Requirements for work results.

Enter the obtained data and calculations into the workbook.

Make medical and biological conclusions.

3.5 Demonstration and practical work «Determination of the hemoglobin content in the blood".

Recommendations for performing tasks.

The principle of the method consists in transforming blood hemoglobin into hydrochloric acid hematin and comparing the obtained color with the standard available in the device. Sali's hemometer is used for determination. It consists of two sealed tubes with a standard colored liquid (1% solution of hematin hydrochloride in glycerol) containing 16.67 g% hemoglobin (16.67 g per 100 ml of blood). Between them is a graduated test tube with two scales. One - with distributions from 0 to 23 is used to determine hemoglobin in grams per 100 ml of blood, that is, in grams as a percentage; another scale with divisions from 0 to 140 shows hemoglobin units (hemoglobin percentage).

Progress. A 0.1N solution of hydrochloric acid is poured into a graduated test tube located in the middle slot up to the beginning of the scale. Then, from the injection site on the flesh of the finger, Sally collects blood up to the mark of 0.02 ml (20 mm³) with a pipette, sucking it through the mouth through a rubber tube with a glass mouthpiece attached to the upper end of the pipette. The tip of the pipette is wiped from the blood and lowered into a test tube with hydrochloric acid, carefully blowing out the contents so that air bubbles do not form. Hitting the bottom of the test tube with a finger, thoroughly mix the blood and leave it for 5 minutes. for the formation of hydrochloric acid hematin. During this time, blood is collected for another part of the analysis. After this time, distilled water is added to the test tube drop by drop, stirring with a glass rod until the color of the solution of the tested blood completely equals the color of the standard liquid.

Requirements for work results.

Enter the obtained data and calculations into the workbook.

Make medical and biological conclusions.

Medical and biological evaluation of the obtained results

Normally, the hemoglobin content in gram percentages in men varies from 13.3 to 18 g%, in women - from 11.7 to 15.8 g% (on average 13.7); in units (percentages) in men - from 80 to 108 units, in women - from 70 to 95 units.

Control materials for the final stage of the class.

Questions to check the final level of knowledge:

1. List and explain the main functions of blood.
2. Chemical composition of blood plasma.
3. What is the difference between plasma and blood serum? Methods of obtaining them.

4. Specify the structure of the protein fractions of the blood and their role in the vital activity of the body.
5. Pathological conditions associated with quantitative and qualitative changes in blood plasma proteins.
6. Blood plasma enzymes. Diagnostic value of determination of blood plasma enzymes.
7. Non-protein organic blood compounds.
8. Nitrogen-containing compounds. Residual (rest-nitrogen) blood plasma. Its components.
9. Methods of determining the level of residual blood nitrogen.
10. Hyperazotemia. Types and causes of occurrence.
11. Nitrogen-free compounds.
12. Inorganic components of blood plasma.

Test tasks.

Plasma includes mineral salts. What physical and chemical properties of blood are due to their presence?

- And osmotic pressure
- In oncotic pressure
- With an active blood reaction
- D blood viscosity
- E SHOE

The buffer systems of the blood support the constancy of its acid-alkaline balance. Which substances of one of the buffer systems have amphoteric properties?

- And plasma proteins
- In the bicarbonate system
- C hemoglobin
- D phosphate system
- E electrolytes

Cyanides are strong poisons for the human body. Indicate which compound will be the best to connect them?

- A Methemoglobin
- B Carboxyhemoglobin
- C Carbhemoglobin
- D Oxyhemoglobin
- E Hem

A diabetic patient has a hyperglycemic coma as a result of long-term elevated blood glucose. What is the most likely mechanism for the development of such a condition?

- And * Ketonemia. A change in blood pH and, as a result, a decrease in the affinity of Hb to O₂.
- B Increased BBB to glucose.
- Coma due to high glucose content in brain neurons
- D Decrease in neurocirculation
- E Acidosis. Methemoglobinemia due to a change in the valence of Fe under the influence of a high concentration of glucose and the formation of a stable compound Hb with O₂.

Liver diseases (hepatitis, cirrhosis, tumor) lead to all the following disorders of protein metabolism except:

- A * Hyper-alpha₂-globulinemia
- In hypoalbuminemia
- With Hemorrhage

D Hyperaminoacidemia
E of Azotemia

A number of biochemical tests (samples) are used to diagnose liver diseases. Which of the following pathological conditions is most likely indicated by an increase in the concentration of alpha-fetoprotein in the blood plasma.

- A * Liver cancer
- In Cirrhosis of the liver
- C Viral hepatitis
- D Cholestasis
- E Fatty infiltration of the liver

Which of the following conditions develops when a large volume of 5% glucose solution is introduced.

- A * Hypoosmolar hyperhydration
- B. Hypoosmolar dehydration
- C Isoosmolar dehydration
- D. Hyperosmolar dehydration
- E Hyperosmolar hyperhydration

All of the pathological conditions listed below can be accompanied by hyperosmolar dehydration except:

- A * Burns
- In Hyperaldosteronism
- C Heart failure
- D Jade
- E Diabetes

The formation of carbonic acid from CO₂ occurs in the presence of the enzyme carbonic anhydrase. Where does this process take place?

- And in erythrocytes
- In leukocytes
- C in plasma proteins
- D in platelets
- E in plasma

For the prevention and therapy of radiation sickness, among various radioprotectors, sodium nitrite is used, which causes the development of hypoxia. What is the mechanism of its hypoxic action:

- A * Stimulates the formation of methemoglobin
- Stimulates the breakdown of hemoglobin
- C Inhibits the dissociation of oxyhemoglobin
- D Inhibits the activity of tissue respiration enzymes
- E Activates lipid peroxidation

4. Summing up.

5. List of recommended literature (main, additional, electronic information resources):

Main:

1. Gubsky Yu.I., I.V. Nizhenkovska, Korda M.M. Biological and Bioorganic Chemistry: in 2 books. Book 2. Biological Chemistry: textbook. 2021. 544 p.

2. Satyanarayana U. Biochemistry. 5th edition. India 2020. 777 p.
3. Lehninger. Principles of Biochemistry. 7th edition. NY, United States. 2017.
4. Jeremy M. Berg, John L. Tymoczko, Gregory J. Gatto. Biochemistry. 8th Revised edition. 2015.
5. Lippincott Illustrated Reviews: Biochemistry. Philadelphia :Wolters Kluwer, 2017. 560 p.
6. Baynes J., Dominiczak M. Medical Biochemistry. 5th Edition. Elsevier, 2018. 712 p.
7. Donald Voet, Judith G. Voet, Charlott W. Pratt. Fundamentals of Biochemistry: Life at the Molecular Level. ISBN: 978-1-118-91840-1 February 2016, 1184 P.

Additional:

1. William Marshall, Marta Lapsley, Andrew Day, Kate Shipman. Clinical Chemistry. Elsevier, 2020. 432 p.
2. Harper's Illustrated Biochemistry / V.W. Rodwell, D.A. Bender, K.M. Botham et al. – Mc Graw Hill Education, 2015. – 817 p.

Electronic information resources:

1. [https://info.odmu.edu.ua/chair/biology/-](https://info.odmu.edu.ua/chair/biology/)
2. <http://libblog.odmu.edu.ua/>
3. <https://moodle.odmu.edu.ua/login/index.php>

Practical class №36

Topic: Biochemistry of coagulation, anticoagulation and fibrinolytic blood systems. Functional and biochemical characteristics of the homeostasis system in the human body: coagulation and vascular-platelet hemostasis. Blood coagulation system, characteristics of individual components (coagulation factors). Mechanisms of coagulation. Blood clotting system, anticoagulants. The role of vitamin K in coagulation reactions. Hereditary disorders of the blood coagulation system.

Goal: to study the mechanisms of blood coagulation and anticoagulation, disorders in the functioning of these processes. evaluate the molecular structure of coagulants and anticoagulants, the mechanisms of hemophilia of various genesis

Basic concepts: blood coagulation system, external coagulation pathway, internal coagulation pathway, anticoagulation system of blood, fibrinolytic system of blood, anticoagulants, hemophilia, CVD syndrome

Equipment: Laboratory of the department

Plan:

1. Organizational activities (greetings, verification of those present, announcement of the topic, purpose of the class, motivation of higher education seekers to study the topic).
2. Control of the reference level of knowledge.

The higher education applicant should know:

- molecular mechanisms of blood coagulation and anticoagulation systems, the role of the liver in this process.
- molecular structure of coagulants and anticoagulants, mechanisms of hemophilia of various genesis.

The higher education applicant should be able to:

- determine biochemical indicators of the state of the coagulation system and give them a medical and biological assessment.

Questions to check basic knowledge on the topic of the class:

1. Composition and properties of erythrocytes and platelets;
2. Chemical composition of blood plasma;
3. Physico-chemical properties of proteins;
4. Glycosaminoglycans. Their structure and functions;
5. Blood plasma enzymes.

3. Formation of professional skills and abilities.

3.1 Demonstration and practical work «Determination of the amount of fibrinogen in blood plasma».

Recommendations for performing tasks.

Progress. 0.1 ml of 5% calcium chloride solution is added to 1 ml of clear plasma. Fibrin is wound on a stick, dried with filter paper and weighed. The weight of fibrin is multiplied by a factor of 22.2 and expressed in mg%. Normally, the amount of fibrinogen in blood plasma is 200-400 mg%.

Requirements for work results.

Enter the obtained data and calculations into the workbook.

Make medical and biological conclusions.

3.2 Demonstration and practical work "Determination of prothrombin time".

Recommendations for performing tasks.

The principle of the method. With excess thromboplastin and optimal calcium content, the time of clot formation in plasma depends on the activity of II, VII, IX, X factors.

Progress. Add 0.1 ml of 1% thrombolastin solution to 0.1 ml of plasma, incubate for 1 minute and add 0.1 ml of 0.025 M calcium chloride solution, turn on the stopwatch until a dense clot forms. A dense clot is formed. Time is expressed in seconds.

$$\text{PAK} = \frac{A}{B} \times 100\%$$

A - the time of a healthy person (20 seconds)

In - the time of a sick person

Requirements for work results.

Enter the obtained data and calculations into the workbook.

Make medical and biological conclusions.

3.3 Demonstration and practical work "Definition of plasma recalcification".

Recommendations for performing tasks.

Progress. Blood is collected in a centrifuge tube with 1.34% sodium oxalate in a ratio of 9:1 and centrifuged for 10 minutes. at 1500 rpm. 0.2 ml of 0.025 M calcium chloride and 0.1 ml of physiological solution are mixed in a test tube and placed in a water bath. Then add 0.1 ml of plasma and start the stopwatch. Clotting is complete if blood does not flow when the tube is inverted. Normally, the recalcification time is 60-120 seconds. when fibrin threads appear.

Medical and biological evaluation of the obtained results

Violations of coagulation hemostasis can be genetically determined and acquired. Plasma recalcification time increases in hemophilia A, Hagemann's defect, factor VII deficiency. Is prothrombin activity reduced in hemophilia B, factor VII deficiency? V, X. With hypofibrinogenemia, all coagulation tests are sharply increased. In case of enteropathy and damaged liver, hypovitaminosis of vitamin K, deficiency of factors I, II, IX, X, XI, XII is possible.

Requirements for work results.

Enter the obtained data and calculations into the workbook.

Make medical and biological conclusions.

Questions to check the final level of knowledge:

1. What are the phases of blood coagulation?
2. Where are blood coagulation factors located and how are they affected?
3. How many blood clotting factors found in plasma and erythrocytes have been studied?
4. What is the process of autocatalysis on the example of activation of coagulation factors?
5. What factors of plasma and platelets take part in the 1st phase of blood coagulation?
6. What factors of plasma and platelets take part in the II phase of blood coagulation?
7. What factors of plasma and platelets take part in the III phase of blood coagulation?
8. The absence of which factors causes hemophilia A and which stages of coagulation are disrupted?
9. The absence of which factors causes hemophilia B and which stages of coagulation are disrupted?
10. Why is Vikasol administered to patients with bleeding?
11. What systems make up the anti-coagulation system?
12. What is the mechanism of action of heparin?
13. How is the liquid state of the blood in the vessels ensured?
14. What antivitamin are used to strengthen the anticoagulation system and their mechanism of action?
15. Medicines affecting fibrinolysis processes.

Test tasks.

A patient with thrombophlebitis is prescribed complex therapy, which affects various stages of thrombus formation. Which of the mentioned means helps restoration of vascular patency?

- A. Neodicumarin
- B. Fibrinolysin
- C. Acetylsalicylic acid
- D. Dipyridamole
- E. Heparin

A patient suffering from a streptococcal infection developed hemorrhagic diathesis. What is the cause of increased bleeding?

- A Increased fibrinolysis
- Lack of vitamin A
- C Increase in the amount of kallikrein in the blood plasma
- D Increase in the amount of heparin in the blood plasma
- E Lack of vitamin C

Heparin is used to prevent blood clotting. What class of complex proteins does it belong to?

- A Glycoprotein
- In Metalloprotein
- C Hemoprotein
- D Lipoprotein
- E Phosphoprotein

In a patient with a streptococcal infection, diffuse bleeding was observed after tooth extraction, which is a consequence of:

- A Activation of fibrinolysis
- In Violation of the coagulation system
- With hypovitaminosis of vitamin K
- D Insufficiency of anticoagulants
- E Violation of calcium metabolism

4. Summing up.

5. List of recommended literature (main, additional, electronic information resources):

Main:

1. Gubsky Yu.I., I.V. Nizhenkovska, Korda M.M. Biological and Bioorganic Chemistry: in 2 books. Book 2. Biological Chemistry: textbook. 2021. 544 p.
2. Satyanarayana U. Biochemistry. 5th edition. India 2020. 777 p.
3. Lehninger. Principles of Biochemistry. 7th edition. NY, United States. 2017.
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6. Baynes J., Dominiczak M. Medical Biochemistry. 5th Edition. Elsevier, 2018. 712 p.
7. Donald Voet, Judith G. Voet, Charlott W. Pratt. Fundamentals of Biochemistry: Life at the Molecular Level. ISBN: 978-1-118-91840-1 February 2016, 1184 P.

Additional:

1. William Marshall, Marta Lapsley, Andrew Day, Kate Shipman. Clinical Chemistry. Elsevier, 2020. 432 p.
2. Harper's Illustrated Biochemistry / V.W. Rodwell, D.A. Bender, K.M. Botham et al. – Mc Graw Hill Education, 2015. – 817 p.

Electronic information resources:

1. <https://info.odmu.edu.ua/chair/biology/>-
2. <http://libblog.odmu.edu.ua/>
3. <https://moodle.odmu.edu.ua/login/index.php>

Practical class №37

Topic: Biochemical functions of the liver, its role in the metabolism of carbohydrates, lipids, and proteins. Hemoglobin breakdown. The role of the liver in the exchange of bile pigments. Pathobiochemistry of jaundice, hereditary (enzymatic) jaundice. Detoxification function of the liver: biotransformation of xenobiotics and endogenous toxins. Types of reactions of biotransformations of foreign chemical compounds. Reaction of microsomal oxidation, inducers and inhibitors of microsomal monooxidases. Conjugation reactions in hepatocytes: biochemical mechanisms, functional significance.

Goal: to study the main biochemical functions of the liver, its role in the exchange of proteins, carbohydrates, and lipids; to learn the main stages of the breakdown of hemoglobin in the body with the formation of bile pigments, to be able to carry out quantitative and qualitative determination of bilirubin in serum and give it a medical and biological assessment. To interpret the mechanisms of biotransformation of xenobiotics and endogenous toxins. To study theoretical material on the biochemistry of the immune system.

Basic concepts: total bilirubin, conjugated bilirubin, unconjugated bilirubin, UDF-glucuronyltransferase, hemolytic jaundice, parenchymal jaundice, obstructive jaundice; microsomal oxidation, cytochrome P-450, animal indican, hippuric acid, immunoglobulins, cytokines

Equipment: Laboratory of the department

Plan:

1. Organizational activities (greetings, verification of those present, announcement of the topic, purpose of the class, motivation of higher education seekers to study the topic).
2. Control of the reference level of knowledge.

The higher education applicant should know:

- the main biochemical functions of the liver, its role in the exchange of proteins, carbohydrates, and lipids;
- the main stages of the breakdown of hemoglobin in the body with the formation of bile pigments;
- the main types of jaundice;
- the main mechanisms of neutralization of xenobiotics and endogenous toxins;
- cellular and biochemical organization of the immune system; mediators and hormones of the immune system;
- biochemical components and mechanisms of complement system activation; biochemical mechanisms of immunodeficiency states.

The higher education applicant should be able to:

- carry out quantitative and qualitative determination of bilirubin in serum and give it a medical and biological assessment;
- determine hippuric acid and indican in urine;
- assess the state of the body's immune system.

Questions to check basic knowledge on the topic of the class:

1. List and explain the main functions of the liver.
2. Microsomal oxidation: cytochrome P-450; molecular organization of the electron transport chain. Biological significance.
3. Formation of peroxides. Antioxidant systems.
4. Structure and functions of blood plasma γ -globulins.
5. Formed elements of blood. Structure and functions.
6. Anatomical and physiological features of the lymphoid system.
7. The main ways of metabolism of carbohydrates, lipids and proteins;
8. Structural and functional features of erythrocytes;
9. List the metabolic features of erythrocytes;
10. The structure of hemoglobin and its synthesis in the human body. Abnormal types of hemoglobin.

3. Formation of professional skills and abilities.

3.1 Demonstration and practical work «Determination of total, direct and indirect bilirubin in blood serum».

Recommendations for performing tasks.

The principle of the method is that the direct bilirubin of the blood serum gives a pink color when the diazo reagent is added; indirect bilirubin reacts with the diazo reagent only after adding caffeine. The color intensity is proportional to the amount of bilirubin.

Progress. a) determination of total bilirubin: add 3.5 ml of caffeine reagent and 0.5 ml of diazo reagent to 1 ml of serum - a pink color appears, after 5 minutes the resulting mixture is placed in a SF cuvette with a layer thickness of 1 cm with a green light filter against the compensation solution, containing 1 ml of serum, 3.5 ml of caffeine solution and 0.5 ml of water.

Calculation: $C = 6.34 \times E - 0.05$ (C - bilirubin concentration)

b) determination of direct bilirubin: to 1 ml of serum add 3.5 ml of physiological solution and 0.5 ml of diazo reagent and 0.5 ml of diazo reagent - a pink color appears, the following stages are similar to point a)

c) the concentration of indirect bilirubin is determined by the difference between total and direct bilirubin.

Medical and biological evaluation of the obtained results

An increase in indirect bilirubin in the blood is observed with hepatic jaundice - hemolytic anemias of various etiologies, as well as posthepatic hyperbilirubinemia, jaundice of newborns.

With mechanical jaundice, direct bilirubin is increased in the blood. In parenchymal jaundice, an increase in both direct and indirect bilirubin is observed.

Requirements for work results.

Enter the obtained data and calculations into the workbook.

Make medical and biological conclusions.

3.2 Demonstration and practical work "Determination of hippuric acid and indican in urine".

Recommendations for performing tasks.

The principle of the method is that alkaline solutions of proteins and peptides, thanks to peptide bonds, become colored like biuret when a solution of copper sulfate is added.

Progress. To 0.1 ml of serum, add 8 ml of a 4.8% solution of lye, 3 ml of a 20% solution of copper sulfate. Centrifuge at 3000 rpm. 5 minutes. Colorimeter on FEK with a green light filter against the mixture in which the serum is replaced by water.

A red-violet color appears, the intensity of which is directly proportional to the amount of protein. Multiply the optical density by 12 to find a quantitative representation of the protein content (%).

Requirements for work results.

Enter the obtained data and calculations into the workbook.

Make medical and biological conclusions.

3.3 Demonstration and practical work "Determination of indican in urine".

Recommendations for performing tasks.

The principle of the method consists in the transformation of indican into indoxysulphuric acid and its subsequent oxidation (with iron chloride or potassium permanganate) to blue or red indigo.

Progress. Mix 8-10 ml of urine with an equal volume of hydrochloric acid, add 1-2 ml of chloroform and 1-2 drops of potassium permanganate. Close the test tube and invert several times without shaking.

In the presence of indican, chloroform turns blue or pink.

Note: In the presence of iodide salts in the urine, chloroform also gives a pink color. In this case, a hyposulfite crystal is added. The disappearance of the pink color of chloroform indicates the presence of iodide salts. In the presence of indican, the pink color does not disappear.

Medical and biological evaluation of the obtained results

In normal urine, indican is contained in a small amount, which is not detected by ordinary quality tests. Turkey anuria occurs with intense decay of protein substances in the large intestine (colitis, colon abscess, peritonitis, constipation), as well as with increased breakdown of proteins in the body (tumor, emphysema, abscesses, pulmonary tuberculosis).

Requirements for work results.

Enter the obtained data and calculations into the workbook.

Make medical and biological conclusions.

Questions to check the final level of knowledge:

1. List and explain the main functions of the liver.
2. What role does the liver play in carbohydrate metabolism?
3. What role does the liver play in lipid metabolism?
4. What role does the liver play in protein metabolism?
5. What are the main stages of hemoglobin breakdown?
6. What forms of bilirubin are formed when hemoglobin breaks down?
7. What indicators of pigment metabolism change:
 - a) with hemolytic jaundice?
 - b) with parenchymal jaundice?

- c) with obstructive jaundice?
8. What are Gelber, Dubin-Johnson and Crigler-Nayar syndromes related to?
 9. Diagnostic value of determination of bound and free bilirubin in jaundice of various etiology.
 10. List the compounds that have adverse, toxic effects on both individual cells and the higher organism as a whole.
 11. Name the types of biotransformation reactions of xenobiotics and endogenous toxins.
 12. What role do microsomal and peroxidation reactions play in detoxification of toxic substances?
 13. What role do conjugation reactions play in the detoxification of xenobiotics?
 14. Name the most common conjugation reactions?
 15. Name the main classes of lymphocytes. Their structure and functions.
 16. Name the main factors of the non-specific immune system.
 17. Name the main classes of immunoglobulins. Their structure and functions.
 18. The main classes and biological role of cytokines.
 19. Molecular mechanisms of antiviral action of interferons.
 20. Tumor necrosis factors, colony-stimulating factors and transforming growth factors. Their biological role.
 21. Biological organization and ways of activation of the complement system.
 22. Mechanisms of impaired functioning of the human immune system. Primary and secondary immunodeficiencies.

Test tasks.

1. A patient with signs of acute poisoning was admitted to the hospital. A high content of methemoglobin was found in the blood. Which of the following compounds led to this:
 - A. - Lead salts
 - B. - Alkaloids
 - C. - Tetrachloromethane
 - +D. - Nitrates
 - E. - Radionuclides

2. A child born prematurely has hypoglycemia in the first days due to:
 - +A. - Deficiency of gluconeogenesis enzymes
 - B. - Violation of glycogen synthesis
 - C. - Violation of glycolysis
 - D. - Hyperinsulinemia
 - E. - Uncoupling of tissue respiration and oxidative phosphorylation

3. A 56-year-old patient complains of general weakness, nausea, and poor appetite. Yellowish skin, hyperbilirubinemia (direct bilirubin), light, foamy urine, acholic stools are noted. For which condition are these changes most characteristic?
 - A. - Dubin-Johnson syndrome
 - B. - Parenchymal jaundice
 - +C. - Obstructive jaundice
 - D. - Hemolytic jaundice
 - E. - Gilbert's disease

4. Patient F., 44 years old, notes pain in the right hypochondrium after minor physical exertion, sometimes at rest, periodic nausea, loss of appetite, swelling of the legs and trunk. During a biochemical blood test: total bilirubin 88.4 $\mu\text{M/l}$, indirect 58 $\mu\text{M/l}$, direct 30.4 $\mu\text{M/l}$, AlAT - 22.4 $\mu\text{mol/h/ml}$, AsAT-14.7 $\mu\text{mol/h/ml}$, total protein 35 g/l, albumins 15 g/l, globulins 20 g/l, K-2.2 mM/l, Na-1-8 mM/l. The patient probably has:
 - A. - Hepatic jaundice

- +B. - Cirrhosis
- C. - Obstructive jaundice
- D. - Chronic pancreatitis
- E. - Chronic cholecystitis

5. A patient was admitted to the infectious department with complaints of itching, jaundice of the skin and mucous membranes. Laboratory: increased concentration of bilirubin in blood serum due to direct, acholic stool. Determining the activity of which serum enzyme will allow to confirm the clinical signs of cholestasis in this patient?

- A. - Aspartate aminotransferase
- B. - Creatine phosphokinase
- C. - Lactate dehydrogenase
- D. - Alpha amylase
- +E. - Gamma-glutamyltranspeptidase

6. The baby has an unstable light yellow color of the skin, icteric sclera. Laboratory: anemia is not determined, hyperbilirubinemia, mainly due to the fraction of unconjugated bilirubin. Diagnosis: Gilbert's syndrome. Which enzyme defect is one of the causes of this pathology?

- A. - Glycogen synthases
- B. - Alanine aminotransferases
- C. - Glucose-6-phosphatases
- +D. - UDP-glucuronyltransferase
- E. - Biliverdin reductases

7. A 52-year-old female patient has been bothered by attacks of pain in the right hypochondrium for the past few days after eating fatty food. Yellowing of sclera and skin, acholic stool, "beer-colored" urine is visually determined. The presence of which substance in the patient's urine caused the dark color of the urine in obstructive jaundice?

- A. - Ketone bodies
- +B. - Bilirubin glucuronides
- C. - Urobilin
- D. - Glucose
- E. - Stercobilin

8. In a patient with liver disease, there is no urobilinogen in the urine in the presence of bilirubin, this is due to a violation of:

- +A. - The influx of bile into the intestines
- B. - Formation of direct bilirubin
- C. - Kidney function
- D. - Conversion of bilirubin in the intestine
- E. - Formation of stercobilin

9. Wilson-Konovalov disease (hepatocerebral degeneration) is accompanied by a decrease in the concentration of free copper in blood serum, as well as the level of:

- A. - Transferrin
- B. - Albumin
- +C. - Ceruloplasmin
- D. - C-reactive protein
- E. - Fibrinogen

10. Indicate which of the following biochemical blood parameters is most important for confirming the diagnosis of liver cirrhosis?

- +A. - Hypoalbuminemia
- B. - Hypercholesterolemia
- C. - Hyperglycemia
- D. - Hypoglycemia
- E. – Hyperglobulinemia

11. Yellowing of the skin is observed in a newborn. Specify the blood index, the increase of which led to such a condition:

- A. - Creatine
- B. - Urea
- C. - Direct bilirubin
- D. - Uric acid
- +E. – Indirect bilirubin

12. Hyperbilirubinemia with an increase in the conjugated form was found in the patient. The thymol test is normal, a slight increase in the activity of alanine aminotransferase is noted. Choose a possible diagnosis.

- A. - Viral hepatitis
- +B. - Mechanical jaundice
- C. - Hemolytic jaundice
- D. - Acute cholecystitis
- E. – Polyarthritis

13. In jaundice, the content of total bilirubin in the blood is increased due to indirect bilirubin, in the feces and urine there is a high content of stercobilin. Name the type of jaundice.

- A. - Jaundice of newborns
- B. - Biliary
- C. - Mechanical
- D. - Hemolytic
- +E. - Parenchymatous

14. In which jaundice hyperbilirubinemia is not accompanied by bilirubinuria?

- A. - Terminal
- B. - Parenchymatous
- C. - Obturational
- +D. - Hemolytic
- E. – Mixed

15. With hemolytic jaundice, the level of direct bilirubin:

- +A. - It is growing
- B. - Does not change
- C. - Decreases
- D. - Not defined
- E. – It fluctuates

Neutralization of toxic substances and inactivation of biologically active substances in hepatocytes is carried out by various reactions. Sulfonamides are converted by which of the following reactions?

- A * Acetylation
- In Demining
- C Conjugation with glucuronic acid
- D Oxidation

E Conjugation with glycine

Drug metabolism in hepatocytes is carried out mainly:

- A *In the endoplasmic reticulum
- In the plasma membrane
- C In the core
- D In mitochondria
- E In lysosomes

In the process of microsomal oxidation, binding and transformation of the substrate is carried out by:

- A * Cytochrome P450
- In Flavoprotein
- C Iron-containing non-heme protein
- D NADP
- E OVER

Neutralization of toxic and inactivation of biologically active substances in hepatocytes is carried out in different ways. How is benzoic acid neutralized?

- A Conjugation with glycine
- B Restoration
- C Methylation
- D Oxidation
- E Acetylation

In a boy with intestinal obstruction, the urinary excretion of indican, which is formed in the liver as a result of the reaction of conjugation of indoxyl with:

- A with phosphoadenosine phosphosulfate
- B Glycine
- C Glutathione
- D Acetyl-Co A
- E Taurine

Neutralization of toxic substances and inactivation of biologically active substances in hepatocytes is carried out by various reactions. Barbiturates are converted by which of the following reactions?

- A Oxidation
- B Deamination
- C Acetylation
- D Restoration
- E Conjugation with glycine

4. Summing up.

5. List of recommended literature (main, additional, electronic information resources):

Main:

1. Gubsky Yu.I., I.V. Nizhenkovska, Korda M.M. Biological and Bioorganic Chemistry: in 2 books. Book 2. Biological Chemistry: textbook. 2021. 544 p.
2. Satyanarayana U. Biochemistry. 5th edition. India 2020. 777 p.
3. Lehninger. Principles of Biochemistry. 7th edition. NY, United States. 2017.

4. Jeremy M. Berg, John L. Tymoczko, Gregory J. Gatto. Biochemistry. 8th Revised edition. 2015.
5. Lippincott Illustrated Reviews: Biochemistry. Philadelphia :Wolters Kluwer, 2017. 560 p.
6. Baynes J., Dominiczak M. Medical Biochemistry. 5th Edition. Elsevier, 2018. 712 p.
7. Donald Voet, Judith G. Voet, Charlott W. Pratt. Fundamentals of Biochemistry: Life at the Molecular Level. ISBN: 978-1-118-91840-1 February 2016, 1184 P.

Additional:

1. William Marshall, Marta Lapsley, Andrew Day, Kate Shipman. Clinical Chemistry. Elsevier, 2020. 432 p.
2. Harper's Illustrated Biochemistry / V.W. Rodwell, D.A. Bender, K.M. Botham et al. – Mc Graw Hill Education, 2015. – 817 p.

Electronic information resources:

1. [https://info.odmu.edu.ua/chair/biology/-](https://info.odmu.edu.ua/chair/biology/)
2. <http://libblog.odmu.edu.ua/>
3. <https://moodle.odmu.edu.ua/login/index.php>

Practical class №38

Topic: General characteristics of the morphology and biochemical composition of connective tissue. Biochemical structure of the intercellular substance of loose fibrous connective tissue: fibers (collagenous, reticular, elastic), the main amorphous substance. Proteins of connective tissue fibers: collagen, elastin. Collagen biosynthesis and formation of fibrillar structures. Complex carbohydrates of the main amorphous matrix of connective tissue: glycosaminoglycans (mucopolysaccharides), proteoglycans. Mechanisms of participation of glycosaminoglycan molecules. Pathochemistry of connective tissue. Biochemical mechanisms of mucopolysaccharidoses and collagenoses, their clinical and biochemical diagnosis.

Goal: Learn the peculiarities of metabolism in connective tissue

Basic concepts: collagen, elastin, glycosaminoglycans, proteoglycans, collagenases

Equipment: Laboratory of the department

Plan:

1. Organizational activities (greetings, verification of those present, announcement of the topic, purpose of the class, motivation of higher education seekers to study the topic).
2. Control of the reference level of knowledge.

The higher education applicant should know:

- structural organization of connective tissue;
- peculiarities of the structure and mechanisms of synthesis of the main proteins of the connective tissue;
- features of the structure of the carbohydrate component of proteoglycans;
- age-related features of the connective tissue structure;
- connective tissue pathology.

The higher education applicant should be able to:

- evaluate the functional state of the body's connective tissue.

Questions to check basic knowledge on the topic of the class:

1. Features of the morphology of connective tissue:
2. Formation and biological role of connective tissue cells.

3. Formation of professional skills and abilities.

Functions and composition of connective tissue

- 1) Protective: mechanical barrier, immune protection
- 2) Supporting: bones, cartilage, tendons, fascia
- 3) Plastic, reparative (scars)
- 4) Trophic (metabolic)
- 5) Depot of fats, vitamins, hormones, pigments (melanins, porphyrins, hemosiderin, bilirubin).

Extracellular matrix: cellular elements, collagen fibers, elastic fibers, intercellular substance: proteoglycans, glycoproteins

Connective tissue cells: constant: fibroblasts, reticulocytes, chondrocytes, osteoblasts (synthesis of biopolymers (collagen, proteoglycans), enzymes, cytokines); histiocytes (macrophages), tissue basophils.

Collagen fibers are filamentous formations consisting of collagens.

Reticular fibers - form three-dimensional meshes; a bundle of microfibrils wrapped in a shell of glycoproteins and proteoglycans.

Elastic fibers from elastin - a glycoprotein, a feature of the primary structure - many residues of glycine, valine, proline. Mature elastin is a fibrillar protein, consisting of tropoelastin connected by covalent bonds. U3 is formed from tropoelastin. In the intercellular matrix, lysine residues are oxidized to allisine, which form cross-links, as well as desmosine and isodesmosine from lysine. Due to this, they are combined by stable covalent bonds.

Fibrillin is a glycoprotein necessary for the formation of elastic fibers of connective tissue.

Proteoglycans are molecular complexes that ensure high viscosity of the main substance of the connective tissue. Polysaccharide chains are connected to a polypeptide fragment of a proteoglycan.

Glycosaminoglycans are heteropolysaccharides, the monosaccharide components of which are hexuronic acid, N-acetyl derivatives of hexosamines.

Glycoproteins-adhesive proteins:

- fibronectin "molecular glue"
- cell adhesion
- participation in blood clotting, binds fibrin
- participation in phagocytosis
- laminin
- interaction with basal membranes, cells

Collagen is a fibrillar protein, glycoprotein

Type I – bones, skin, tendons, cornea (90%)

II - articular cartilage

III - vessel walls

IV – basement membrane

Gives fabrics strength and elasticity

Collagen molecule = tropocollagen - Helix of 3 protein chains of 2 types (α_1 and α_2) of glycine, proline and oxyproline, a lot of lysine and oxylysine. Carbohydrates (glu, gala) are attached to oxylysine. Pyridine crosslinks (between lysine and oxylysine residues).

Synthesis of collagen

They are synthesized on ribosomes bound to the membranes of the endoplasmic reticulum, in the cells of the fibroblastic series of connective tissue.

1. Synthesis of procollagen.

2. Reaction of hydroxylation of proline and lysine residues catalyzed by proline and lysine hydroxylase. Molecular oxygen and alpha-ketoglutaric acid are needed as substrates for enzyme action, and Fe²⁺ ion and ascorbic acid are needed as cofactors.

3. Galactose and glucose are added to part of the oxylysine and oxyproline residues. The glycosylation reaction is catalyzed by lycosyltransferases in the tubules of the granular endoplasmic

reticulum, where procollagen polypeptide chains enter.

4. Polypeptide chains form a three-stranded helix, which is facilitated by the formation of disulfide bonds between the chains at the C-ends

5. Procollagen is secreted in vesicles from the cell into the intercellular space. Under the action of procollagenpeptidases, the final propeptides are cleaved.

6. The formed tropocollagen molecules form fibrils, which are stitched together by transverse covalent bonds. Collagen-bound proteoglycans contribute to the structural organization of collagen fibers.

Questions to check the final level of knowledge:

1. Biological role of connective tissue.
2. Basic elements of connective tissue.
3. Features of the structure and amino acid composition of collagen.
4. The main structural unit of collagen.
5. Mechanisms of collagen synthesis. Scheme of the formation of a mature collagen fiber.
6. The biological role of vitamin C in the formation of mature collagen.
7. Features of the structure of elastin. Its biological role.
8. Features of the structure and biological role of proteoglycans.
9. Features of the structure of glycosaminoglycans.
10. The main representatives of glycosaminoglycans, their structure and functions.
11. Age-related features of the connective tissue structure.
12. Pathological conditions associated with impaired functioning of connective tissue.

Test tasks.

A 30-year-old woman has been sick for about a year, since the time when they first appeared pain in the joints, their swelling, redness of the skin over them.

Previous diagnosis - rheumatoid arthritis. One of the probable reasons this disease has a change in the protein structure of the connective tissue:

- A. Myosin
- B. Mucin
- S. Collagen
- D. Troponin
- E. Ovalbumin

Increased fragility of blood vessels, destruction of enamel and dentine of teeth patients with scurvy are largely due to impaired maturation

collagen What stage of procollagen modification is violated in this case vitamin deficiency?

- A. Removal of C-terminal peptide from procollagen
- B. Glycosylation of hydroxylysine residues
- C. Cleavage of the N-terminal peptide
- D. Formation of polypeptide chains
- E. Hydroxylation of proline

In patients with collagenosis, the process of destruction of connective tissue takes place. This is confirmed by an increase in the blood:

- A. Creatine and creatinine content
- B. Contains oxyproline and oxylysine
- C. Activities of transaminases
- D. Contains urates
- E. Activities of LDH isozymes

Fibrillar elements of connective tissue include collagen, elastin and reticulin. Specify the amino acid that is included only in composition of collagen, and its determination in biological fluids used to diagnose connective tissue diseases.

- A. Lysine
- B. Proline
- C. Glycine
- D. Phenylalanine
- E. Hydroxyproline

A 63-year-old woman has symptoms of rheumatoid arthritis. Level up which of the blood indicators listed below will be the most significant to confirm the diagnosis?

- A. Acid phosphatase
- B. Total glycosaminoglycans
- C. N-glycosidases
- D. Lipoproteins
- E. Total cholesterol

4. Summing up.

5. List of recommended literature (main, additional, electronic information resources):

Main:

1. Gubsky Yu.I., I.V. Nizhenkovska, Korda M.M. Biological and Bioorganic Chemistry: in 2 books. Book 2. Biological Chemistry: textbook. 2021. 544 p.
2. Satyanarayana U. Biochemistry. 5th edition. India 2020. 777 p.
3. Lehninger. Principles of Biochemistry. 7th edition. NY, United States. 2017.
4. Jeremy M. Berg, John L. Tymoczko, Gregory J. Gatto. Biochemistry. 8th Revised edition. 2015.
5. Lippincott Illustrated Reviews: Biochemistry. Philadelphia :Wolters Kluwer, 2017. 560 p.
6. Baynes J., Dominiczak M. Medical Biochemistry. 5th Edition. Elsevier, 2018. 712 p.
7. Donald Voet, Judith G. Voet, Charlott W. Pratt. Fundamentals of Biochemistry: Life at the Molecular Level. ISBN: 978-1-118-91840-1 February 2016, 1184 P.

Additional:

1. William Marshall, Marta Lapsley, Andrew Day, Kate Shipman. Clinical Chemistry. Elsevier, 2020. 432 p.
2. Harper's Illustrated Biochemistry / V.W. Rodwell, D.A. Bender, K.M. Botham et al. – Mc Graw Hill Education, 2015. – 817 p.

Electronic information resources:

1. <https://info.odmu.edu.ua/chair/biology/>
2. <http://libblog.odmu.edu.ua/>
3. <https://moodle.odmu.edu.ua/login/index.php>

Practical class №39

Topic: Chemical composition of tooth tissue: organic (collagenous, non-collagenous proteins, carbohydrates, lipids, nucleic acids) and mineral components. Mineral metabolism in tooth tissue: mineralization, demineralization, remineralization (specific proteins and enzymes,

role of citric acid in calcium metabolism). Chemical composition and biological functions of saliva. Features of mineralization role of saliva in the tooth tissue. Saliva enzymes, their role in digestion. Determination of proteins and enzymes in human saliva under normal and pathological conditions. Regulation of salivation, biochemical basis of salivation disorders.

Goal: To learn biochemical processes in tooth tissues

Basic concepts: enamel, cement, dentin, fluorapatite, hydroxyapatite, alkaline phosphatase, amylase

Equipment: Laboratory of the department

Plan:

1. Organizational activities (greetings, verification of those present, announcement of the topic, purpose of the class, motivation of higher education seekers to study the topic).

2. Control of the reference level of knowledge.

The higher education applicant should know:

- Organic components of tooth tissues;
- Inorganic components of tooth tissues;
- Saliva enzymes

Questions to check basic knowledge on the topic of the class:

1. Enamel, chemical composition (organic and inorganic components), biological role.
2. Dentin, chemical composition (organic and inorganic components), biological role.
3. Cement, chemical composition (organic and inorganic components), biological role.
4. Pulp, chemical composition (organic and inorganic components), biological role.
5. Biochemical mechanisms of enamel mineralization. Initiators of mineralization. Features of enamel mineralization and the difference between dentin and cementum. Remineralization.
6. Metabolism in tooth tissues.
7. Hormonal regulation of metabolism in tooth tissues.
8. The role of vitamins in the metabolism of tooth tissues.

3. Formation of professional skills and abilities.

Dental caries is the process of destruction of the hard tissues of the tooth, the basis of which is demineralization and softening, followed by the formation of a defect (cavity). The theory of the development of caries: demineralization of hard tissues of the tooth under the influence of organic acids formed in the process of vital activity of microorganisms.

Common cariogenic factors: unhealthy diet; violation of metabolic processes (during the period of formation and maturation of tooth tissues); the influence of adverse factors on the body; heredity (violation of the chemical composition of the tooth).

Local factors: dental plaque; violation of the composition and properties of saliva; violation of the biochemical composition and resistance of tooth tissues; the state of the pulp and maxillofacial apparatus during the period of laying, development and eruption of teeth.

Acidogenic effect of food products and drinks: sugar, candies, ice cream, Coca-Cola, etc.

Cariogenic effect of microorganisms: *Str. mutans*, *Str. salivatorius*, *Str. sanguis*.

Periodontitis - inflammation of the periodontium - a complex of tissues that surround and hold the tooth: gums, periodontium, bone tissue of the alveoli of the tooth.

Etiology: a) dental plaque microorganisms (1 mg of plaque substance – 500×10^{11} microorganisms, more than 70% streptococci; 15% *Veylonella*, *Neisseria*; lactobacilli, staphylococci, etc.); b) state of cellular and humoral immunity; c) neurotrophic and vascular disorders (microcirculatory); d) local factors: plaque and calculus, incorrect bite, change in the chemical composition of saliva).

Biochemical bases of changes: metabolic disorders in the soft and bone tissues of the periodontium; depolymerization of collagen and non-collagen proteins of connective tissue

structures; resorption of the alveolar process; hypoxia of periodontal tissues; activation of phosphofructokinase, strengthening of glycolysis processes; reduction of oxidation-reduction processes; increase in lactate concentration; a change in the acid-alkaline state; local acidosis; reduction of RNA and protein synthesis (reduction of plastic processes); violation of membrane transport of ions; release of hydrolytic enzymes (collagenase, elastase, acid phosphatase) from lysosomes; endogenous intoxication; increased proteolysis (increased free amino acids); increase in pro-inflammatory cytokines - interleukins 1 β , 6, 8 and tumor necrosis factor alpha; autoimmune processes; violation of neurohumoral regulation; change in the ratio of osteogenesis and osteolysis; decrease in synthesis and increase in breakdown of collagen and proteoglycans (excretion of oxyproline and uronic acids with urine); inclusion of apoptosis; dysproteinemia, an increase in citrate in the blood (over 156 $\mu\text{mol/l}$); acidosis, etc.).

Sialosis – reactive changes in the salivary glands to the action of various pathogenic factors; caused by a violation of saliva secretion, endocrine regulation of salivary gland metabolism (diabetes, hypothyroidism).

Sialoadenitis – inflammation of the salivary glands; origin: viral or bacterial (endemic mumps, herpes); hyposalivation

Sialolithiasis - the formation of stones in the ducts of the salivary glands; stone composition: inorganic salts, epithelium, bacteria, fungi.

Questions to check the final level of knowledge:

1. Biological role, physicochemical properties and chemical composition of saliva.
2. The clinical value of saliva research.
3. Enamel: inorganic component; organic matrix; peculiarities of metabolism in enamel.
4. Dentin, cementum, tooth pulp.
5. Biochemical mechanisms of the development of the main diseases of the oral cavity (caries, periodontitis, sialoadenitis, sialosis).

1. During the examination of the patients, the dentist noted that many of them had tooth enamel without shine, with porcelain-like and pigmented spots. Individual patients have single and multiple enamel defects in the form of colorless or pigmented erosions. Excessive intake of what substance in the body led to the development of such changes in the teeth?

- A. Fluor
- B. Calcium
- S. Kaliya
- D. Magnesium
- E. Sodium

2. During the treatment of periodontitis, the patient was prescribed a fat-soluble vitamin preparation that takes an active part in redox processes in the body. Antioxidant is a growth factor, antixerophthalmic, ensures normal vision. In dental practice, it is used to accelerate epithelization in diseases of the mucous membrane of the oral cavity and periodontal tissues. Identify this drug:

- A. Retinol acetate
- B. Ergocalciferol
- C. Tocopherol acetate
- D. Vikasol
- E. Cyanocobalamin

3. The main means of increasing enamel resistance include fluoridation. The mechanism of anticarious action of fluoride is related to:

- A. Synthesis of fluorapatite
- B. Synthesis of hydroxyapatite
- C. Demineralization of the tooth

- D. –
- E. Chlorapatite synthesis

4. Which enzyme has a demineralizing effect - enhances the splitting of mineral components of tooth tissues?

- A. Acid phosphatase
- B. Glucose-6-phosphatase
- C. Alkaline phosphatase
- D. Glycogen phosphorylase
- E. Phosphotransferase

5. Which enzyme has a demineralizing effect - enhances the splitting of mineral components of tooth tissues?

- A. Acid phosphatase
- B. Alkaline phosphatase
- C. Glucose-6-phosphatase
- D. Glycogen phosphorylase
- E. Phosphotransferase

6. With periodontitis, the patient was prescribed a fat-soluble vitamin preparation that takes an active part in redox processes in the body. Antioxidant is a growth factor, antixerophthalmic, ensures normal vision. In dental practice, it is used to accelerate epithelization in diseases of mucous membranes and periodontitis. Identify this drug:

- A. Retinol acetate
- B. Ergocalciferol
- C. Tocopherol acetate
- D. Vikasol
- E. Cyanocobalamin

7. The child has been diagnosed with acute kidney failure. What biochemical indicator of saliva can be used to confirm this?

- A By increasing the content of urea in saliva
 - By increasing the glucose content in saliva
 - With a decrease in the content of glucose in saliva
 - D By increasing the content of high-density lipoproteins
 - E A decrease in the content of nucleic acids
4. Summing up.

5. List of recommended literature (main, additional, electronic information resources):

Main:

1. Gubsky Yu.I., I.V. Nizhenkovska, Korda M.M. Biological and Bioorganic Chemistry: in 2 books. Book 2. Biological Chemistry: textbook. 2021. 544 p.
2. Satyanarayana U. Biochemistry. 5th edition. India 2020. 777 p.
3. Lehninger. Principles of Biochemistry. 7th edition. NY, United States. 2017.
4. Jeremy M. Berg, John L. Tymoczko, Gregory J. Gatto. Biochemistry. 8th Revised edition. 2015.
5. Lippincott Illustrated Reviews: Biochemistry. Philadelphia :Wolters Kluwer, 2017. 560 p.
6. Baynes J., Dominiczak M. Medical Biochemistry. 5th Edition. Elsevier, 2018. 712 p.
7. Donald Voet, Judith G. Voet, Charlott W. Pratt. Fundamentals of Biochemistry: Life at the Molecular Level. ISBN: 978-1-118-91840-1 February 2016, 1184 P.

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Electronic information resources:

1. [https://info.odmu.edu.ua/chair/biology/-](https://info.odmu.edu.ua/chair/biology/)
2. <http://libblog.odmu.edu.ua/>
3. <https://moodle.odmu.edu.ua/login/index.php>

Practical class №40

Topic: Muscle biochemistry. Features of the chemical composition and metabolism in muscles. Molecular mechanisms of muscle contraction. Bioenergetics of muscle tissue: sources of ATP in muscles. Features of the biochemical composition and metabolism of the nervous system. Biochemical composition of the brain. Energy metabolism of the human brain, the value of aerobic oxidation of glucose. Neurotransmitters: acetylcholine, norepinephrine, dopamine, serotonin. Molecular basis of bioelectrical processes on neuron membranes.

Goal: Learn the peculiarities of metabolism in muscle tissue

Basic concepts: sarcomere, actin, myosin, tropomyosin, troponin, creatine phosphate, adenylate kinase

Equipment: Laboratory of the department

Plan:

1. Organizational activities (greetings, verification of those present, announcement of the topic, purpose of the class, motivation of higher education seekers to study the topic).
2. Control of the reference level of knowledge.

The higher education applicant should know:

- molecular organization of muscle tissue and the structure of the sarcomere;
- structure and functions of the main proteins of muscle tissue;
- mechanisms of muscle contraction and relaxation;
- energy sources for muscle contraction;
- biochemical diagnosis of muscle tissue pathology.

The higher education applicant should be able to:

- determine the content of creatine and creatinine in blood and urine and be able to correctly interpret the obtained results.

Questions to check basic knowledge on the topic of the class:

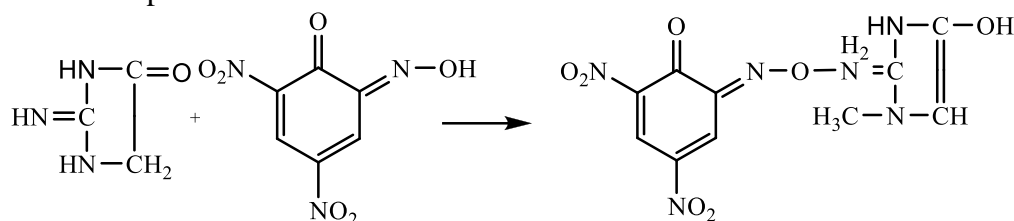
1. Anaerobic glycolysis;
2. Krebs cycle;
3. Tissue respiration and oxidative phosphorylation;
4. Mechanisms of formation and exchange of creatine and creatinine.

3. Formation of professional skills and abilities.

3.1 Demonstration and practical work «Determination of the level of creatinine and creatine in urine".

Recommendations for performing tasks.

The principle of the method. The detection and quantification of creatinine is based on the color reaction of creatinine in an alkaline medium with picric acid, resulting in the formation of yellow-brown creatinine picrate



The mass concentration of creatinine in the test mixture can be calculated from the colorimetrically determined color intensity of the control (standard 1% potassium dichromate solution, which corresponds to the color of 2.5 mg of creatinine in 250 ml of solution) and the test sample. When determining the mass concentration of creatine in urine, the concentration of creatinine in it is first determined. Then creatine is converted into creatinine by boiling urine in an acidic medium and the creatinine content is also determined. The amount of creatine in the urine is calculated based on the difference between the second and first determinations, taking into account the ratio of creatinine to creatine.

Progress. Determination of the mass concentration of creatinine and creatine in urine is carried out in two portions. In one of them, the creatinine content is immediately determined. For this purpose, 2.0 ml of filtered urine, 5 ml of saturated picric acid solution, 2 ml of 10% sodium hydroxide solution are added to the volumetric flask and thoroughly mixed. After 10 minutes, the contents of the flask are brought to a volume of 250 ml with distilled water and colorimeted. The mass concentration of creatinine in urine (mg/ml) is calculated using the formula

$$C1 = D1Q / D2V$$

where D1 and D2 are optical density values of the standard potassium dichromate solution and the test solution, respectively; Q is the mass of creatinine in 250 ml of solution, equivalent in color to a 1% standard solution of potassium dichromate (2.5 mg); V is the volume of filtered urine taken for analysis (2 ml).

In another portion of urine, creatine is converted into creatinine and the amount of creatinine formed is determined. To do this, add 2 ml of the studied urine and 5 ml of a saturated picric acid solution to a 250 ml flask. Note the level of liquid in the flask, add another 100 ml of distilled water and boil for one hour. If during this time the liquid in the flask boils above the level of the mark, distilled water is added, if less, the liquid is evaporated to the mark.

After cooling, add 2 ml of 10% sodium hydroxide solution to the flask, after 10 minutes pour into a 250 ml volumetric flask, add distilled water to the 250 ml mark and colorimeter. The mass concentration of creatinine in the second portion of urine is calculated using the same formula as the first.

The difference between the values in the second and first portions, taking into account the conversion factor of creatinine into creatine, is equal to the content of the mass concentration of creatine in the examined urine

$$C = (C2 - C1) \times K$$

where C1 and C2 are the mass concentration of creatinine in the first and second portions of urine, respectively; K is the conversion factor of creatinine into creatine (1.16). The coefficient of 1.16 is obtained as follows: the molecular weight of creatine is 131, and the molecular weight of creatinine is 113; $131 : 113 = 1.16$.

Medical and biological evaluation of the obtained results

The amount of creatinine that is excreted in the urine of adults during the day ranges from 1.5 to 2.4 g. An increase in the amount of creatinine in the urine is observed during intense physical work, during fevers, etc.

There is no creatine in normal adult urine. Creatinuria occurs in cases associated with increased tissue breakdown, for example, with postpartum involution of the uterus. Children's urine always contains both creatine and creatinine.

Requirements for work results.

Enter the obtained data and calculations into the workbook.
Make medical and biological conclusions.

Questions to check the final level of knowledge:

1. What are the types of muscle tissue?
2. What body functions does muscle tissue take part in?
3. What subcellular formations of muscles do you know?
4. What is ER sarcoma? Its structure.
5. What types of muscle tissue proteins do you know? Their structure and functions
6. Structural and biological role of anserine and carnosine in the functioning of muscle tissue.
7. Muscle tissue enzymes. Diagnostic value of determination of muscle tissue enzymes.
8. Molecular mechanisms of contraction and relaxation.
9. Sources of energy for muscle contraction depending on the intensity of physical activity.
10. Pathology of muscle tissue.

Test tasks.

A patient with progressive muscular dystrophy was treated
biochemical examination of urine. The appearance of which substance in large quantities in
urine can confirm muscle disease in this patient?

- A. Porphyrins
- B. Hippuric acid
- C. Urea
- D. Creatinine
- E. Creatine

A patient with a crush injury was brought to the traumatology department
muscle tissue. What will be the biochemical indicator of urine in this case
enlarged?

- A. Mineral salts
- B. Glucose
- C. Total lipids
- D. Uric acid
- E. Creatinine

When examining the patient's blood, a significant increase was found
activity of MB-forms of CPK (creatine phosphokinase) and LDH-1. Which one
the most likely pathology?

- A. Myocardial infarction
- B. Pancreatitis
- S. Hepatitis
- D. Rheumatism
- E. Cholecystitis

The patient has muscle atony. Name the muscle enzyme
tissue, the activity of which may be reduced in such a condition.

- A. γ -Glutamyltransferase
- B. Catalase
- C. Amylase
- D. Creatine phosphokinase
- E. Transketolase

A 47-year-old man was admitted to the intensive care unit diagnosis of myocardial infarction. Which of the fractions of lactate dehydrogenase (LDH) will prevail in the blood serum during the first two days?

- A. LDH 3
- B. LDH5
- C. LDH1
- D. LDH4
- E. LDH2

As a result of the exhausting muscular work, the employee has a lot decreased blood buffer capacity. The arrival of which acidic substance can this phenomenon be explained in the blood?

- A. Pyruvate
- B. Lactate
- C. 3-phosphoglycerate
- D. 1,3-bisphosphoglycerate
- E. alpha-ketoglutarate

An increase in the activity of LDH1, LDH2, AST was found in the patient's blood, creatine kinase. In which organ of the patient is the most likely development pathological process?

- A. Pancreas
- B. Skeletal muscles
- C. Liver
- D. Kidneys
- E. Heart

A 46-year-old patient has been suffering from progressive muscular dystrophy for a long time Duchenne dystrophy. A change in the level of an enzyme in the blood diagnostic test in this case?

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

An 18-year-old boy was diagnosed with muscular dystrophy. Increase in blood serum content of which substance is most likely with this pathology?

- A. Myoglobin
- B. Alanine
- C. Creatine
- D. Myosin
- E. Lactate

4. Summing up.

5. List of recommended literature (main, additional, electronic information resources):

Main:

1. Gubsky Yu.I., I.V. Nizhenkovska, Korda M.M. Biological and Bioorganic Chemistry: in 2 books. Book 2. Biological Chemistry: textbook. 2021. 544 p.

2. Satyanarayana U. Biochemistry. 5th edition. India 2020. 777 p.
3. Lehninger. Principles of Biochemistry. 7th edition. NY, United States. 2017.
4. Jeremy M. Berg, John L. Tymoczko, Gregory J. Gatto. Biochemistry. 8th Revised edition. 2015.
5. Lippincott Illustrated Reviews: Biochemistry. Philadelphia :Wolters Kluwer, 2017. 560 p.
6. Baynes J., Dominiczak M. Medical Biochemistry. 5th Edition. Elsevier, 2018. 712 p.
7. Donald Voet, Judith G. Voet, Charlott W. Pratt. Fundamentals of Biochemistry: Life at the Molecular Level. ISBN: 978-1-118-91840-1 February 2016, 1184 P.

Additional:

1. William Marshall, Marta Lapsley, Andrew Day, Kate Shipman. Clinical Chemistry. Elsevier, 2020. 432 p.
2. Harper's Illustrated Biochemistry / V.W. Rodwell, D.A. Bender, K.M. Botham et al. – Mc Graw Hill Education, 2015. – 817 p.

Electronic information resources:

1. [https://info.odmu.edu.ua/chair/biology/-](https://info.odmu.edu.ua/chair/biology/)
2. <http://libblog.odmu.edu.ua/>
3. <https://moodle.odmu.edu.ua/login/index.php>

Practical class №41

Topic: Kidney role in body fluids electrolyte composition and pH regulation. Biochemical mechanisms of the urine formation of the kidneys. Pathobiochemistry of kidneys. Biochemical composition of human urine in normal conditions and under conditions of pathological processes, nephrolithiasis. Clinical and diagnostic significance of urine composition analysis.

Goal: Learn the peculiarities of metabolism in the kidneys

Basic concepts: primary urine, secondary urine, urea, clearance, ACE inhibitors

Equipment: Laboratory of the department

Plan:

1. Organizational activities (greetings, verification of those present, announcement of the topic, purpose of the class, motivation of higher education seekers to study the topic).
2. Control of the reference level of knowledge.

The higher education applicant should know:

- kidney function;
- peculiarities of metabolism in the kidneys;
- the role of the kidneys in maintaining acid-base balance;
- biochemical mechanisms of the urinary function of the kidneys;
- the biochemical composition of human urine in normal conditions and under the conditions of pathological processes;
- clinical and diagnostic value of urine composition analysis;
- hormones involved in the regulation of water-salt metabolism and kidney functions
- the principle of action of hypotensive drugs - angiotensin-converting enzyme inhibitors

The higher education applicant should be able to:

- perform qualitative reactions to protein in urine;
- determine the amount of protein in urine;
- determine the amount of glucose in the urine.

Questions to check basic knowledge on the topic of the class:

1. The structure of the nephron.
 2. The mechanism of urine formation: filtration, reabsorption, secretion.
 3. pH of biological fluids in the body.
 4. What are the functions of the kidneys?
 5. What are the organs of excretion other than the kidneys do you know?
 6. What is urine?
 7. How much urine is released per day from the body of a healthy person?
 8. What is diuresis?
 9. What is the ratio between daily water consumption and diuresis in the norm?
 10. Hormones of the hypothalamic-pituitary system and the cortex of the adrenal glands, which take part in the regulation of water-salt metabolism.
 11. Components of the renin-angiotensin system and its role in maintaining blood pressure.
 12. Natriuretic factors of the atrium and other tissues
3. Formation of professional skills and abilities.

3.1 Demonstration and practical work «Qualitative determination of protein in urine (heating and acid precipitation».

Recommendations for performing tasks.

Progress

A) Boiling test: urine is tested in advance using litmus. If the urine has an acidic reaction, then it (2-3 ml) is immediately boiled in a test tube, and if the urine has an alkaline reaction, then it is first acidified according to litmus, adding 1% acetic acid solution drop by drop. In the presence of protein during boiling, turbidity or a precipitate of coagulated proteins is formed, which does not dissolve during repeated boiling after adding 3-5 drops of 10% acetic acid to the liquid.

B) Precipitation of protein with concentrated nitric acid (Heller's test): about 1 ml of concentrated nitric acid is poured into a test tube and urine is carefully poured from a pipette along the wall of the test tube. In the presence of protein, a white amorphous layer or turbidity, the so-called protein ring, forms at the boundary of both liquids. In the absence of protein in the urine, a colored transparent ring appears on the border between the two liquids, caused by a change in the pigments of the urine under the influence of nitric acid.

C) Precipitation of protein with sulfosalicylic acid: 2-3 drops of freshly prepared 20% solution of sulfosalicylic acid are added to 1-2 ml of urine. In the presence of protein in the urine, a white precipitate or turbidity is formed.

Requirements for work results.

Enter the obtained data and calculations into the workbook.

Make medical and biological conclusions.

3.2 Demonstration and practical work "Quantitative determination of protein by the Branderg-Stolnikov method".

Recommendations for performing tasks.

The principle of the method. The method is based on an experimentally established fact: the appearance of a barely noticeable protein ring in the Heller test occurs between the second and third minutes at a urine concentration of 0.0033% protein, i.e. 0.033 g/l. By successively diluting urine and layering it on nitric acid, such a maximum dilution of urine is achieved that a ring appears between the second and third minutes. Multiply the dilution by 0.033 g/l and get the protein content in the urine.

Progress. A Heller's test is performed with normal and pathological urine, for which 20 drops of concentrated nitric acid are placed in a test tube and the urine is carefully layered with a pipette. If the urine contains protein, then after 2-3 minutes. a white turbidity in the form of a ring is formed. Urine with a positive Heller test is used for protein quantification, for which a urine dilution is

prepared. Pour 2 ml of distilled water into five test tubes. Add 2 ml of urine to the first, mix and take 2 ml of the mixture, transfer to the second, etc. Discard 2 ml of the collected liquid from the last test tube. Urine diluted 2, 4, 8, 16, 32 times is obtained. 2 ml of concentrated nitric acid is measured into the other five test tubes and a suitable sample of diluted urine is carefully layered on the acid using a pipette. They note the maximum dilution of urine, in which a cloudy white ring appears between the second and third minutes. The found urine dilution is multiplied by 0.033 g/l. For example, a ring of denatured protein was formed in the fourth test tube, where the dilution is equal to 16. Therefore, the protein content in the test urine is $0.033 \times 16 = 0.548$ g/l.

Medical and biological evaluation of the obtained results

Normal urine does not contain protein because it is unable to pass through the capillary walls. The appearance of protein in the urine indicates kidney disease. Protein can appear in the urine either as a result of a pathological change in the permeability of the capillary walls, when they begin to pass protein into the urine, or during inflammatory processes in the kidneys. For example, protein in the urine appears in glomerulonephritis (that is, inflammation of the glomeruli of the kidneys, when their permeability increases), in case of heart failure, sometimes during pregnancy.

3.3 Demonstration and practical work "Quantitative determination of glucose in urine using a polarimeter".

Recommendations for performing tasks.

Principle of the method: glucose rotates the plane of the polarized beam to the right. The amount of glucose in the urine is determined by the angle of rotation.

Progress. Determination is carried out strictly according to the instructions for the polarimeter. The polarimeter tube is filled with filtered urine (without air bubbles), covered with ground glass, screwed tightly, wiped dry and inserted into the device. Determination is carried out 2-3 minutes after filling the tube, because fluctuations of liquid particles interfere with the study. When the color or intensity of illumination of a part of the field of view changes, the fields are equalized by the rotation of the disk and the angle of deviation of the polarized beam is determined, which is expressed in degrees of the scale of the device. With a tube length of 18.94 cm, the deflection angle of 10 corresponds to 1% glucose; if the length of the tube is 9.47 cm, then the obtained results are multiplied by 2, if the length is 4.74 cm, then by 4. After the work is finished, the urine is poured out, the tube and the glass of the polarimeter are washed with distilled water and dried.

Requirements for work results.

Enter the obtained data and calculations into the workbook.

Make medical and biological conclusions.

Questions to check the final level of knowledge:

1. Biochemical processes in the cortical and medullary layers of the kidneys.
2. Glomerular filtration.
3. Reabsorption and secretion.
4. Involvement of kidneys in regulation of volume, electrolyte composition and pH of body fluids.
5. Specify the acidity of urine. What buffer system plays an important role in maintaining the constancy of urine pH?
6. Amount, color, smell, transparency, reaction of normal urine.
7. Clinical and diagnostic value of quantitative and qualitative analysis of urine.
8. How much urea, uric acid, creatinine is excreted in the urine per day?
9. How much residual nitrogen is in urine?
10. What is Creatine Ratio?
11. What causes the active reaction of normal urine?
12. How many chlorides, sulfates, phosphates are excreted in the urine per day, their sources of origin.

4. Summing up.

5. List of recommended literature (main, additional, electronic information resources):

Main:

1. Gubsky Yu.I., I.V. Nizhenkovska, Korda M.M. Biological and Bioorganic Chemistry: in 2 books. Book 2. Biological Chemistry: textbook. 2021. 544 p.
2. Satyanarayana U. Biochemistry. 5th edition. India 2020. 777 p.
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6. Baynes J., Dominiczak M. Medical Biochemistry. 5th Edition. Elsevier, 2018. 712 p.
7. Donald Voet, Judith G. Voet, Charlott W. Pratt. Fundamentals of Biochemistry: Life at the Molecular Level. ISBN: 978-1-118-91840-1 February 2016, 1184 P.

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Electronic information resources:

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3. <https://moodle.odmu.edu.ua/login/index.php>

Practical class №42, 43

Topic: Intermediate control for the semester

Goal: Determine the level of assimilation of knowledge for the semester

Equipment: Laboratory of the department

Plan:

1. Organizational activities (greetings, verification of those present, announcement of the topic, purpose of the class, motivation of higher education seekers to study the topic).
2. List of questions for preparation for control:
 1. The pool of the amino acids in the body. Routes for transport and utilization of amino acids in tissues. Research on the metabolism of carbon skeletons of amino acids in the human body. Glucogenic and ketogenic amino acids.
 2. Direct and indirect deamination of high-grade L-amino acids in tissues. Transamination of amino acids: reactions and their biochemical significance, mechanisms of aminotransferases. Decarboxylation of L-amino acids in the human body. Physiological significance of the creation of products. Oxidation of biogenic amines.
 3. Ways of creation and release of ammonia in the body. Biosynthesis of meat: sequence of enzyme reactions in biosynthesis, genetic abnormalities of enzymes in the fruit cycle.
 4. Biosynthesis and biological role of creatine and creatine phosphate. Glutathione: natural, biosynthesis and biological functions of glutathione.

5. Specialized routes to the metabolism of cyclic amino acids - phenylalanine and tyrosine. Decreased enzyme metabolism of cyclic amino acids - phenylalanine and tyrosine. Exchange of cyclic amino acid tryptophan and its spasmopathic enzyme.
6. Nitrogen bases, nucleosides and nucleotides as the composite components of the nucleic acids. Minor nitrogen bases and nucleotides. Free nucleotides: ATP, NAD⁺, NADP⁺, FAD, FMN, CTP, UTP, 3',5'-cAMP, 3',5'-cGMP, their biochemical functions.
7. Nucleic acids. General characteristics of DNA and RNA, their biological importance in the storage and the transfer of genetic information. Features of DNA and RNA primary structure. Chemical bonds, which are responsible for the formation of nucleic acids primary structure. Secondary structure of DNA, role of hydrogen bonds in its formation (Chargaff's rules, Watson-Crick model), anti-parallelity of strands. Tertiary structure of DNA. Physical and chemical properties of DNA, interaction with cation ligands, formation of nucleosomes.
8. Molecular organization of nuclear chromatin of eukaryotes; nucleosome organization, histone and non-histone proteins. Nucleoproteins: structure, biological functions.
9. Structure, properties and biological functions of RNA. Types of RNA: m-RNA, t-RNA, r-RNA. Features of the different type of RNA structural organization.
10. Biosynthesis of purine nucleotides; scheme of IMP synthesis reactions. Formation of AMP and GMP from IMP, mechanisms of regulation. Catabolism of purine nucleotides, hereditary disturbances of the uric acid metabolism.
11. Biosynthesis of pyrimidine nucleotides; scheme of reactions, regulation of synthesis. Biosynthesis of deoxyribonucleotides. Formation of the thymidine nucleotides. Inhibitors of TMP synthesis as anti-cancer medicines. Scheme of the pyrimidine nucleotide catabolism.
12. Replication of DNA, its biological importance, and semiconservative mechanism of replication. Sequence of the steps and DNA replication enzymes in prokaryotes and eukaryotes.
13. RNA transcription: prokaryotes and eukaryotes RNA-polymerases, signals of transcription: promoter, initiator and terminator fragments of genome. Processing and post-translational modification of synthesized RNA.
14. Genetic (biologic) code, triplet structure and properties. Transport RNA and transportation of amino acids. Amino acyl-tRNA-synthetases. Steps and mechanism of translation (protein synthesis) in ribosomes: initiation, elongation and termination.
15. Post-translational modification of peptide chains. Regulation of translation. Inhibitors of transcription and translation in prokaryotes and eukaryotes. Antibiotics and interferons, they use in medicine. Diphtheria toxin.
16. Regulation of prokaryote gene expression: regulatory and structural fragments of lactose, Lac-operon, gene regulator, promoter, operator.
17. Gene engineering: construction of recombinant DNA, gene cloning. Genetic engineering of enzymes, hormones, interferons, etc.
18. Mutations: genome, chromosome, gene. Mechanisms of mutagen activity; role of the induced mutations in the origin of the enzymopathology and hereditary human diseases. Biological importance and mechanisms of DNA reparations. Reparation of UV-induced gene mutations: xeroderma pigmentosum.
19. Hormones and their general characteristics. Role of hormones and other bioregulators in the system of the intracellular integration of the human organism functions.
20. Classification of hormones and bioregulators in correspondence of structure and mechanisms of hormone activity.
21. Reaction of the target cells on the hormone action. Membrane (ionotropic, metabotropic) and cytosol receptors. Biochemical systems of the hormonal signals intracellular transfer: G-proteins, and secondary messengers cAMP, Ca²⁺-calmodulin, inositol-3-phosphate, and diacylglycerol. Molecular cell mechanisms of the steroid and thyroid hormone activity.
22. Neuropeptides of hypothalamus. Liberins and statins, their mechanisms of activity and biologic role.

23. Hormones of the anterior pituitary gland: somatotropin (GH), prolactin. pathological processes associated with impaired functions of these hormones. Hormones of the posterior pituitary gland. Vasopressin and oxytocin: biological, biological functions.
24. Insulin: structure, biosynthesis and secretion. Mechanism of insulin activity on the carbohydrate metabolism. Mechanism of insulin activity on the lipid metabolism. Mechanism of insulin activity on the protein and nucleotide metabolism. Glucagon and its mechanisms of activity on the carbohydrate and lipid metabolism.
25. Thyroid hormones, their structures, biological effects of T3 and T4. Disturbances of metabolic processes due to hypo- and hyperthyreosis.
26. Epinephrine, norepinephrine, dopamine, their structure, biosynthesis, physiological effects, biochemical mechanisms of activity. Pathological processes related to the disturbances of hormone functions.
27. Steroid hormones of the suprarenal glands (C21-steroids), glucocorticoids and mineralocorticoids, their structures and properties. Mechanisms of glucocorticoids activity on the carbohydrate and lipid metabolism.
28. Female sex hormones estrogens, progesterone. Physiological and biochemical effects, related to the ovulation cycle phases. Male sex hormones (C19-steroids). Physiological and biochemical effects of androgens, regulation of synthesis and secretion.
29. Eicosanoids: biological, biological and pharmacological influences. Aspirin and other non-steroidal anti-inflammatory agents as inhibitors of prostaglandin synthesis.
30. Biochemistry of human nutrition, the food components and nutrients, biological value of certain nutrients. Mechanisms of conversion of nutrients, proteins, carbohydrates, and lipids, in the digestive tract. The saliva, stomach and intestine enzymes. Digestion disorders of certain nutrients in the stomach and intestines, and hereditary enzymopathies of digestive processes. Microelements in human nutrition. Biological functions of certain microelements, and microelement deficiency manifestations.
31. Vitamins in human nutrition. Water-soluble and fat-soluble vitamins; exogenous and endogenous causes of vitamin deficiency.
32. Vitamin B1 (thiamin): structure, biological properties, mechanism of action, the sources, daily need. Vitamin B2 (riboflavin): structure, biological properties, mechanism of action, the sources, daily need. Vitamin PP (nicotinic acid, nicotinamide): structure, biological properties, mechanism of action, manifestations of deficiency, sources, the daily need.
33. Vitamin B6 (pyridoxine): structure, biological properties, mechanism of action, the sources, the daily need. Vitamin B12 (cobalamin): biological properties, mechanism of action, manifestations of deficiency, sources, the daily need. Vitamin Bc (Folic Acid): biological properties, mechanism of action, the sources, the daily need.
34. Vitamin H (biotin): biological properties, mechanism of action, the sources, the daily need. Vitamin B3 (pantothenic acid): biological properties, mechanism of action, the sources, and the daily need. Vitamin C (ascorbic acid): structure, biological properties, mechanism of action, manifestations of deficiency, sources, the daily need. Vitamin P (flavonoids): structure, biological properties, mechanism of action, manifestations of deficiency, sources, the daily need.
35. Vitamin A (retinol, retinal, retinoic acid): biological properties, mechanism of action, manifestations of deficiency, sources, the daily need. Vitamin D3 (cholecalciferol): biological properties, mechanism of action, manifestations of deficiency, sources, the daily need.
36. Vitamin K (phylloquinone, farnohinon): biological properties, mechanism of action, manifestations of deficiency, sources, the daily need. Vitamin E (α -tocopherol): biological properties, mechanism of action, manifestations of deficiency, sources, the daily need.
37. Biochemical and physiological functions of blood in the human body. Respiratory function of erythrocytes. Hemoglobin: mechanisms of its' participation in the transport of oxygen and carbon dioxide. Types and pathological forms of human hemoglobin.

38. Buffers of the blood system. Disturbance of the acid-base balance in the body (metabolic and respiratory acidosis, alkalosis). Biochemical storage of human blood. Blood plasma proteins and their clinical and biochemical characteristics.
39. Blood plasma enzymes; significance in enzyme diagnosis of diseases of organs and tissues. Kallikrein-kinin system of blood and tissues. Medicines - antagonists of kinin formation.
40. Non-protein organic compounds of blood plasma. Inorganic components of plasma.
41. Biochemical and functional characteristics of the hemostatic system. Glottal blood system; characteristics of other factors; mechanisms of functioning of the cascade system of laryngeal blood. The role of vitamin K in coagulation reactions; medicinal properties - agonists and antagonists of vitamin K. Anticoagulant blood system; characteristics of anticoagulants. Recession of the process of laryngeal blood. Fibrinolytic blood system. Medicines that influence the process of fibrinolysis.
42. Immunoglobulins; biochemical characteristics of individual classes of human immunoglobulins. Mediators and hormones of the immune system: interleukins; interferons; protein-peptide factors of cell growth and proliferation regulation. Complement system; biochemical components of the human complement system; classical and alternative ways of activation. Biochemical mechanisms of immunodeficiency states.
43. Biochemical functions of the liver: carbohydrate, protein-synthesizing, urea-forming, bile-forming, regulation of blood lipid composition.
44. Detoxification function of the liver; types of biotransformation reactions of xenobiotics and endogenous toxins. Conjugation reactions in hepatocytes: biochemical mechanisms, functional significance.
45. Reactions of microsomal oxidation. Cytochrome P-450; electron transport chains in the membranes of the endoplasmic reticulum of hepatocytes.
46. Metabolism of porphyrins: heme structure; Scheme of biosynthesis reactions of protoporphyrin IX and heme. Hereditary disorders of porphyrin biosynthesis, types of porphyrias.
47. The role of the liver in the exchange of bile pigments. Pathobiochemistry and types of jaundice; biochemical diagnosis of jaundice; hereditary (enzymatic) jaundice. Catabolism of hemoglobin and heme (scheme); formation and structure of bile pigments.
48. Chemical composition of saliva, functions. Features of the mineralizing function of saliva. Salivary enzymes, role in digestion. The role of saliva in the supply of Ca and phosphates to enamel. Differences in composition and biological significance of oral fluid and saliva from salivary gland ducts.
49. Peculiarities of the chemical composition of the tooth (enamel). Ways of getting substances to tooth enamel. Tooth enamel proteins, the role of mineralization. Crystals of fluorapatite, hydroxyapatite, physical and chemical properties, biological role of mineralization. Chemical composition of tooth dentin. Non-collagenous tooth proteins, features of amino acid composition, role in mineralization.
50. The influence of nutrition on the state of teeth, the role of carbohydrates, proteins, trace elements and vitamins. The role of refined food carbohydrates on enamel demineralization.
51. Chemical composition of bone. Bone proteins, role in mineralization. Collagen proteins of teeth and bones. Chemical structure and role. The role of citric acid in calcium metabolism. Theory of bone and tooth mineralization. The role of Ca²⁺ binding elements-proteins, phosphates and citric acid in mineralization.
52. The influence of vitamins on the condition and metabolism in the tissues of the oral cavity and teeth. Hormones affecting metabolism in mineralized tissues - calcitonin, parathyroid hormone, somatotrophic hormone.
53. Microelements of fluorine, strontium, etc., their biological significance for the state of teeth and bones.
54. Macroelements: calcium, phosphorus, role in tooth and bone tissue exchange.
55. Water-salt exchange in the body. Intracellular and extracellular water; exchange of water, sodium, potassium. The role of the kidneys in regulating the volume, electrolyte composition and pH of body fluids. Biochemical mechanisms of the urinary function of the kidneys. Biochemical

composition of human urine in normal conditions and under conditions of development of pathological processes. Clinical and diagnostic value of urine composition analysis.

56. Renin-angiotensin system of kidneys. Hypotensive drugs - angiotensin-converting enzyme inhibitors.

57. Biochemical composition of muscles. Myofibril proteins: myosin, actin, tropomyosin, troponin. Molecular mechanisms of muscle contraction. Bioenergetics of muscle tissue.

58. Biochemistry of the nervous system. Energy exchange in the human brain. Value of aerobic oxidation of glucose; changes in the conditions of physiological sleep and anesthesia.

59. Biochemistry of neurotransmitters; receptors of neurotransmitters and physiologically active compounds. Brain peptidergic system: opioid peptides, opioid peptide receptors. Disorders of the exchange of brain mediators and modulators in mental disorders. Neurochemical mechanisms of action of psychotropic drugs.

3. Summing up.

4. List of recommended literature (main, additional, electronic information resources):

Main:

1. Gubsky Yu.I., I.V. Nizhenkovska, Korda M.M. Biological and Bioorganic Chemistry: in 2 books. Book 2. Biological Chemistry: textbook. 2021. 544 p.

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