Liliia Kalachniuk, Viktor Tomchuk

BIOCHEMISTRY OF ANIMALS WITH THE BASICS OF PHYSICAL AND COLLOID CHEMISTRY

Study guide

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Reviewers:

Savchuk O., Doctor of Biological Sciences, Professor, Head of the Biochemistry Department, ESC "Institute of Biology and Medicine" of Taras Shevchenko National University of Kyiv

Kuchmerovska T., Doctor of Biological Sciences, Professor, Palladin Institute of Biochemistry of the National Academy of Sciences of Ukraine

Klepko A., Doctor of Biological Sciences, Associate Professor, Head of the General Ecology, Radiobiology and Life Safety Department, National University of Life and Environmental Sciences of Ukraine

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The content of the study guide corresponds to the working program of the discipline "Biochemistry of animals with the basics of physical and colloid chemistry".

For the purpose to improve theoretical and practical use of knowledge in biochemistry for students of the faculty of veterinary medicine, the study guide provides information on the basics of physical and colloid chemistry, physical and chemical methods in biochemistry, static biochemistry, the regulatory influence of inorganic and organic substances on metabolism, dynamic and functional biochemistry, metabolism and its reflection in biochemical indicators

For the training of specialists in specialty 211 "Veterinary Medicine".

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03041 Kyiv, Heroiv Oborony Str., 15.

INFORMATION ABOUT THE AUTHORS

Kalachniuk Liliia

Doctor of Biological Sciences, Professor of the Department of Biochemistry and Physiology of Animals named after Academician M.F. Gulyi of the National University of Life and Environmental Sciences of Ukraine, academician of the National Academy of Sciences of Higher Education of Ukraine, Laureate of the State Prize of Ukraine in Science and Technology. Teaches the disciplines "Biochemistry of animals with the basics of physical and colloid chemistry" (in Ukrainian and English), " New tendencies in biological research of bioresources (scientific seminars)", "Molecular mechanisms of metabolism regulation", "Biochemistry of blood", "The latest methods in biological research". Scientific interests are related to the study of mechanisms of regulation of metabolism in the organism of animals under the influence of factors of different natures. Author of more than 270 scientific and educational works, including 4 monographs, 1 textbook, 6 study guides, 4 author certificates, and 6 patents. Email addresses: lilkalachnyuk@gmail.com, kalachnyuk_liliya@nubip.edu.ua

Tomchuk Viktor

Doctor of Veterinary Sciences, Professor, Head of the Department of Biochemistry and Physiology of Animals named after Academician M.F. Gulyi of the National University of Life and Environmental Sciences of Ukraine, Academician of National Higher Education Academy of Sciences of Ukraine, winner of the S. Z. Gzhytskyi prize, Honored Scientist and Pedagogical Worker of NULES of Ukraine. Teaches the discipline "Veterinary Clinical Biochemistry". Scientific interests are related to the study of molecular mechanisms of regulation of metabolic processes in the animal body. The author of more than 350 scientific and educational works, including 6 monographs, 9 textbooks and study guides, 2 terminological dictionaries, 10 patents, and 9 author's certificates. Email address: tomchuk_viktor@ukr.net

INTRODUCTION

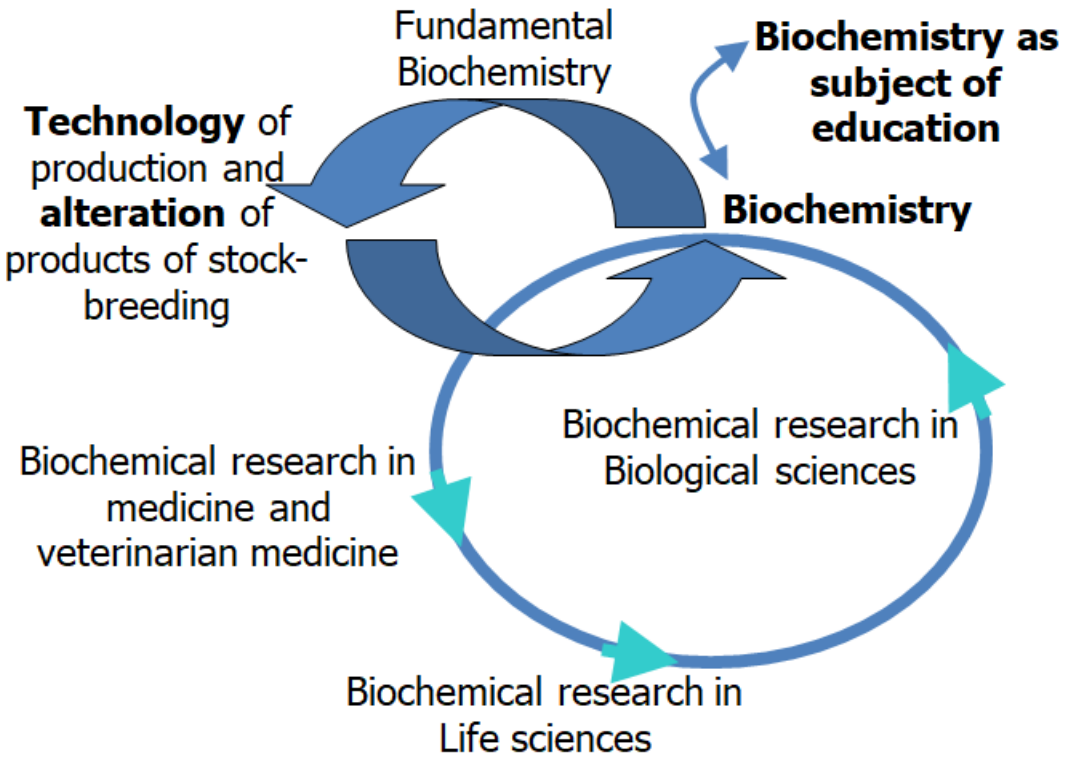
«Biochemistry of Animals with the Basics of Physical and Colloid Chemistry» is a mandatory component of the educational program «Veterinary Medicine», which provides basic concepts about the chemical composition of animals, classification, and functions of proteins, carbohydrates, lipids, minerals, enzymes, hormones, and vitamins. The main ways of biochemical processes that provide homeostasis, energy balance, growth and development of animals are subject to study. Significant attention paid to the study of the biochemical composition of biological fluids and tissues of animals and the processes that occur in them. The study of the discipline «Biochemistry of Animals with the Basics of Physical and Colloid Chemistry» provides the acquisition of such general competencies as: 1) the ability to abstract thinking, analysis, and synthesis; 2) the ability to apply knowledge in practical situations; 3) knowledge and understanding of the subject area and profession; 5) the ability to communicate in a foreign language. The study of the discipline «Biochemistry of Animals with the Basics of Physical and Colloid Chemistry» provides the mastery of such professional competencies as: 1) the ability to establish the features of the structure and functioning of cells, tissues, organs, their systems and apparatus of the animal body; 2) the ability to use tools, special devices, instruments, laboratory equipment and other technical means to carry out the necessary manipulations during professional activities; 3) the ability to follow the rules of labor protection, asepsis and antiseptics during professional activities, the ability to use modern knowledge of the laws of biochemistry of different species of animals to effectively manage the treatment of animals and the use of medicines.

Discipline "Biochemistry of animals with the basics of physical and colloid chemistry" studies the chemical composition of living organisms and the structural structure of biomolecules, their transformation in the processes of life and regulation in animals at the cellular, organ and the whole organism level, based on the basics of physical and colloid chemistry and physicochemical methods of biochemical research.

The aim of the course "Biochemistry of animals with the basics of physical and colloid chemistry" is to form in students a holistic system of knowledge about the chemical composition of living organisms, physicochemical and biological properties of natural compounds, basic metabolic pathways, mechanisms of regulation and relationship of biochemical transformations. master the theoretical foundations of metabolic processes and their regulation in animals and practical skills of their study.

The subject of the discipline "Biochemistry of animals with the basics of physical and colloid chemistry" are metabolic processes that occur in animals, and their regulation to correct them in case of pathological changes in the body.

Place of biochemistry (as study subject) in the system of biological education, its role for technology of production and alteration of products of stock-breeding



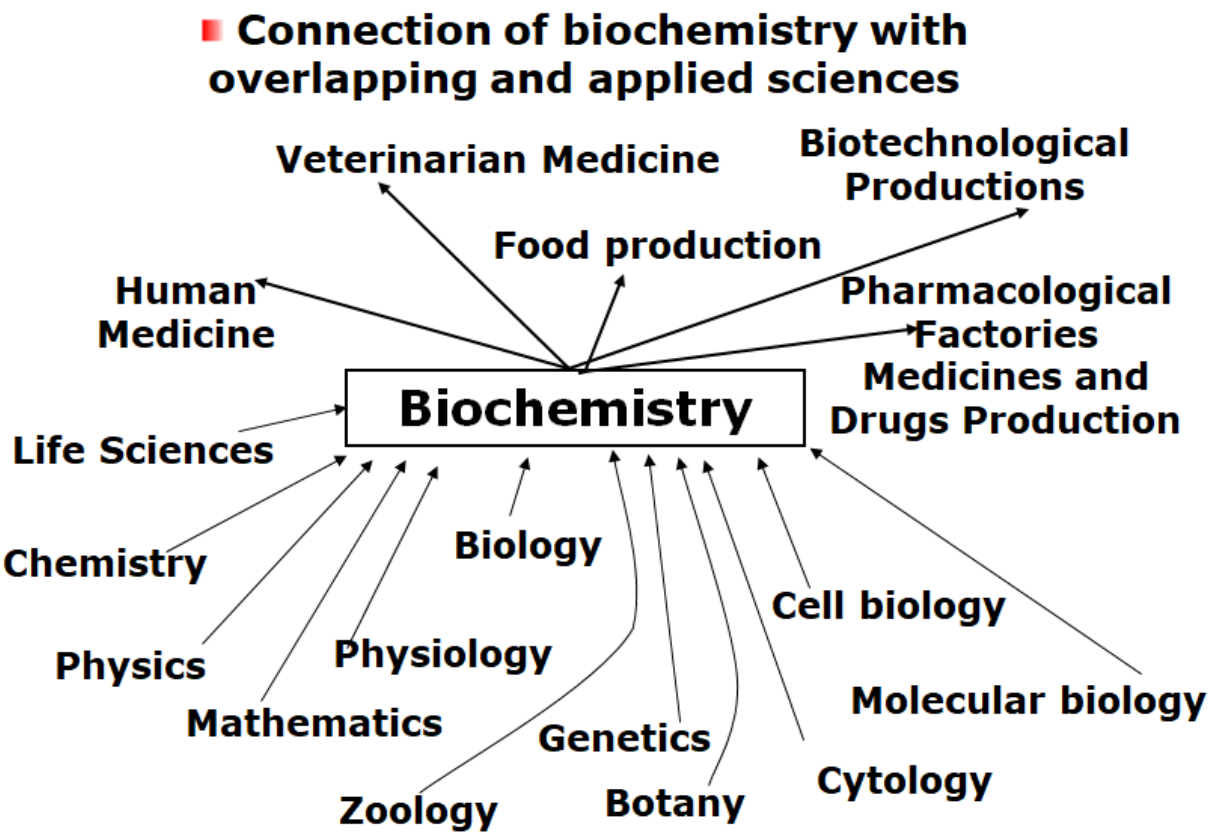
The course "Biochemistry of animals with the basics of physical and colloid chemistry" (BA BPCC) studies the basics of organisms, namely: the structure, physicochemical and biological properties of substances, their metabolism and regulation and changes in metabolic processes with both feed and drugs to strengthen health and productivity of animals. Hence, first it is important to study the biochemistry of animals and the individual foundations of physical and colloid chemistry.

The BA BPCC course harmoniously combines animal biochemistry and the basics of physical and colloid chemistry. Updates in physical and colloid chemistry are essential for understanding the biochemical processes that occur in animals.

Physical chemistry is a branch of science that studies chemical phenomena and processes based on general principles of physics using physical experimental methods.

Colloid chemistry is the physics-chemistry of dispersed systems and surface phenomena.

Biochemistry is the science of the chemical composition of living organisms and the chemical processes that ensure their existence. Biochemistry is one of the leading disciplines that shape the worldview of the future doctor (veterinarian) and his professional knowledge, namely: metabolism and its regulation; metabolic changes in pathology; biochemical methods of diagnosis and treatment of diseases; control over the course of conditions and the effectiveness of treatment; development of new drugs and treatments.



In the direction of studying organisms, biochemistry divided into static (chemical nature and properties of substances); dynamic (transformation of substances up to the final products of metabolism); functional (chemical transformations at the level of the organism, organ, cell, and subcellular components).

Both authors mutually collaborate in writing all chapters of this Study Guide: Liliia Kalachniuk was responsible for chapters 1.2, 2.1, 2.2, and Viktor Tomchuk – 1.1, 1.3, 2.3.

PART 1

Chapter 1.1
FUNDAMENTALS OF PHYSICAL AND COLLOID CHEMISTRY

The purpose of this section of the course "Biochemistry of animals with the Basics of Physical and Colloid Chemistry" is to provide students with theoretical knowledge of physical and colloid chemistry and some experiments in lab classes.

The following topics will be considered in this section:

Topic 1. pH: methods of determination
Topic 2. Buffer solutions
Topic 3. Osmosis, osmotic pressure in biological systems
Topic 4. Sorption and biological phenomena
Topic 5. Colloid solutions. High-molecular compounds of biological liquids

Physical chemistry is a branch of science that studies chemical phenomena (especially biochemical transformations) and processes based on the general principles of physics using physical experimental methods.

Since all living organisms (from bacteria to mammals) are dispersed systems, it is important to study colloid chemistry - the science of dispersed systems and surface phenomena.

Chemical phenomena are extremely diverse, especially in biological systems, but they all obey the general laws, the study of which is the subject of physical chemistry. Physical processes, heat transfer, absorption, or release of energy, etc. accompanies chemical phenomena. On the other hand, physical processes cause chemical phenomena. Thus, physical chemistry studies the relationship of chemical processes and physical phenomena that accompany them and establishes patterns between the chemical composition, structure of substances, and their properties.

Physical chemistry exists on the border between chemistry and physics because it studies the laws of interconversion of chemical and physical forms of motion of matter. Using theoretical and experimental methods of both sciences, as well as their methods, physical chemistry establishes the laws of chemical processes and the conditions for achieving chemical equilibrium.

To theoretically generalize experimental material and create a coherent system of ideas about the properties of substances and the laws of chemical processes in physical chemistry uses three independent methods of theoretical physics: quantum mechanical, statistical, and thermodynamic, the latter two are especially important in studying biochemical processes occurring in living organisms.

The basis of chemical thermodynamics is the application of thermodynamics to chemical phenomena biochemical processes in biosystems. Thermodynamics is based on three postulate laws, based on which the rest of its provisions can be obtained experimentally.

The first law of thermodynamics is directly related to the law of conservation of energy and allows you to calculate the thermal balances of various chemical and biochemical processes.

The law on the possibility of spontaneous processes is the second law of thermodynamics and based on it, it is possible to predict under what external conditions the process is possible, and in what direction it will take place.

The third law of thermodynamics is the law of the absolute value of entropy, it allows us to calculate the equilibrium constant of chemical reactions, and hence the maximum possible yield of the reaction product.

The subject of chemical thermodynamics is the application of the laws of classical thermodynamics to chemical and physicochemical phenomena; it considers thermal effects in chemical reactions, phase transitions of individual substances and mixtures, and chemical equilibria. The object of study in thermodynamics is the thermodynamic system. A system is a single body or group of interacting bodies that are, or imaginary separated from the environment. The environment is everything that surrounds the system and is in direct or indirect contact with it. It is believed that the environment is so large that the return or receipt of heat does not change its temperature. Depending on the nature of interaction with the environment, there are open, closed, and isolated systems.

Biochemical processes rage in living organisms (which are open systems that can exchange energy and matter with the environment). Cells, tissues, and organisms are heterogeneous (heterogeneous) systems because they consist of several phases, where the phase is a set of homogeneous parts of a heterogeneous system, characterized by a certain composition, the same chemical, physical and thermodynamic properties, and separated from other parts of the distribution surface. The set of all physical and chemical properties of the system is called the state of the system. The state of the system is characterized by thermodynamic parameters. The system parameter is a quantity that quantifies the system. Parameters that can be measured directly are called basic system state parameters. State parameters that cannot be directly measured (internal energy, enthalpy, entropy, thermodynamic potentials) are considered functions of the basic state parameters. The process is any change in the parameters of the system, namely temperature, volume, and pressure.

All biological processes can be divided into spontaneous (natural) and non-spontaneous. Spontaneous processes are processes that do not require energy from the outside (for example, the transfer of heat from a hotter to a less hot body). Non-spontaneous processes are processes that occur at the expense of energy, for example, synthetic processes in biosystems are carried out at the expense of the energy of the organism.

It is known that the animal's body contains 66% water, and a living cell - 85%. Water as a universal solvent is the basic liquid of living nature, so it is important to know and understand its physicochemical properties and significance, and role in the biochemical processes of a living organism.

1.1.1. *Topic* pH: METHODS OF DETERMINATION

pH, value for the body. pH scale

According to Branstad's theory, water is an ampholyte

$$H\text{-}O\text{-}H \rightarrow H^+ + OH^- \text{ and } H^+ + H\text{-}O\text{-}H \rightarrow H_3O^+$$

This reaction is called autoprotolysis of water:

$$H_2O + H_2O \leftrightarrow H_3O^+ + OH^- \text{ or } 2H_2O \leftrightarrow H_3O^+ + OH^-$$

Quantitatively, the autoprotolysis of water is characterized by the ionic product of water.

Hydrogen bond

$$H\text{-}O\text{-}H \ldots H\text{-}O\text{-}H \rightarrow H_3O^+ + OH^-, \text{ де}$$

$[H_3O^+]$ -concentration of hydroxonium ions (protons),

$[OH^-]$ -concentration of hydroxide ions.

$[H_3O^+]$ or $[OH^-]$ in 1 liter of pure water at 25 ° C is equal to $1 \cdot 10^{-7}$ mol

$H\text{-}O\text{-}H \rightarrow H^+ + OH^-$.

$K_{dis} = [[H^+] \cdot [OH^-]) / [H_2O]$

The concentration of H_2O in 1 liter of clean water at 25 ° C

$C(H_2O) = 1000$ (g / l) / 18 (g / mol) = (55.5 mol / l) = 55.5 M = const

Ionic product of water (Kw)

$K_{dis} = ([10^{-7}] \cdot) [10^{-7}]) / 55{,}5 = 1.8 \cdot 10^{-16}$ (mol / l)

$K_w = 55.5 \cdot K_{dis} = [H^+] \cdot [OH^-] = 10^{-14}$ (mol^2 / l^2)

The value of the ionic product of water is constant and it depends only on temperature. As the temperature increases, the dissociation of water, as in most compounds, increases, so the ionic product of water increases.

$$pK_w = pH + pOH = 14$$

The practical value of the ionic product of water is large, for example, at a known acidity (alkalinity) of any solution (i.e., at a known concentration of $[H^+]$ or $[OH^-]$) to find, respectively, the concentration of $[OH^-]$ or $[H^+]$. However, in most cases, for convenience, they do not use absolute values of concentrations but taken with the opposite sign of their decimal logarithms - respectively, hydrogen (pH) and hydroxyl (pOH).

Since K_w is a constant, when acid (H^+ ions) is added to the solution, the concentration of OH^- hydroxide ions will decrease and vice versa. In a neutral medium $[H^+] = [OH^-]$ = mol / l. At a concentration of $[H^+] > 10^{-7}$ mol / l (respectively, a concentration of $[OH^-] < 10^{-7}$ mol / l) the medium will be acidic; At a concentration of $[OH^-] > 10^{-7}$ mol / l (respectively, a concentration of $[H^+] < 10^{-7}$ mol / l) - alkaline.

Hydrogen index (pH) is the negative decimal logarithm of the concentration of hydrogen ions:

$$pH = -\log 10 [H^+] = -\lg [H^+]$$

For example: $[H^+] = 10^{-3}$ M → pH = 3

Read more: $pH = -(\lg 10^{-3}) = -(-3) = 3$, pOH = 14-3 = 11

The media depending on the pH value are:

strongly acidic pH = 0 - 3.0

(gastric juice → pH = 1 - 2; 0.1 M HCl → pH = 1.0)
weakly acidic pH = 4.0 - 6.9
(milk → pH = 6.6 - 6.9; tomato juice → pH = 4.0-5.0)
neutral - pH = 7.0
slightly alkaline pH = 7.1 - 10.0
(blood → pH = 7.35 - 7.45; sea water → pH ~ 8.0)
strongly alkaline pH = 11 - 14
(25% ammonia → pH = 11.8; 0.1 M NaOH → pH = 13.0)

The reaction of body fluids is acidic when $[H^+]$ predominates $[OH^-]$, and alkaline - if vice versa.

Some pH values	
Substance	**pH**
Gastric juice	1.0 - 2.0
The skin of a healthy person	5.5
Saliva	6.35 - 6.85
Milk	6.6 - 6.9
Clean water	7.0
Blood	7.36 - 7.44
Cerebrospinal fluid	7.35 - 7.8
Bile	7.3-8.0
Seawater	8.0

In the blood of healthy animals, the reaction is slightly alkaline, and the pH is in the range: in cows - 7.35-7.45, sheep - 7.46-7.52, horses - 7.30-7.50, pigs - 7.44-7.47. In humans, blood pH is 7.36-7.42.

Determination of pH of solutions by colorimetric (or indicator) method. The basis of the colorimetric method is that the indicators, depending on the reaction medium, show the color of undissociated molecules or a combination of colors of undissociated molecules and anions. Since the indicators at different pH values have different colors, scales of standards are built.

Phenolphthalein at pH below 8.0 - colorless, and above 8.0 becomes crimson
Methyl orange to pH 3.0 is red, and above 3.0 - yellow
Litmus up to pH 6.0 is red, and above 6.0 - blue

Characteristics of the most used indicators

Name	The pH range of the color transition	Colour within the specified pH range
Thymol blue (first transition)	1.2-2.8	Red-yellow
Resol red (first transition)	1.9-3.1	Orange-yellow
Methyl orange	3.1-4.4	Red-orange
Bromophenol blue	3.0-4.6	Yellow-blue

Methyl red	4.2-6.2	Red-yellow
Bromothymol blue	6.0-7.6	Yellow-blue
Phenolic red	6.4-8.0	Yellow-red
Cresol red (second transition)	7.4-9.0	Amber-yellow-purple-red
Phenolphthalein	8.2-10.0	Colorless-crimson-red
Thymolphthalein	9.3-10.5	Colorless blue
Alizarin yellow	10.1-12.1	Yellow-purple

Universal indicator paper - colorimetric methods

Potentiometric methods of analysis

Potentiometric methods of analysis are based on the dependence of the electrode potential on the composition of the solution in which it is immersed.

A metal indifferent conductor immersed in a solution acquires a certain potential if an equilibrium process of electron transfer takes place in the solution between two forms of substances - redox vapor.

Classification of electrodes. Analytical signal.

According to the mechanism of potential on the electrodes, the latter ones are divided into electron exchange and ion exchange.

In electron exchange electrodes, the potential arises due to the exchange of electrons between the metal and the solution through the interface. They, in turn, are divided into electrodes of I, II, and III kind and indifferent.

Electrodes of the first kind are metals (Pt, Ag, Cu, Cd, Au, Ir) in the form of a plate or wire, immersed in a solution of a well-soluble salt of this metal. The potential of the electrode of the first kind depends on the activity of the metal ions of which the electrode consists in solution.

Electrodes of the second kind are metals immersed in a saturated solution of insoluble salt of this metal over salt anions. (silver wire immersed in a solution of potassium chloride in the presence of solid silver chloride. Scheme of the following electrode: Ag, AgCl $\mid Cl^-$, K^+). The potential of the electrode of the second kind depends on the activity of the anion of insoluble metal salt in the solution.

Type III electrodes are metals immersed in a solution saturated for two sparingly soluble salts with a common anion. The first salt contains an electrode metal cation, the second - another cation. For example, a silver wire immersed in a solution of $Cd(NO_3)_2$ in the presence of solid Ag_2S and CdS. The scheme of such an electrode: Ag, Ag_2S, CdS $\mid Cd^{2+}$, NO_3^-)

A glass electrode is widely used to determine pH. This is a glass tube, at the end of which there is a ball with a very thin wall, made of special glass with high

electrical conductivity (for example, composition: SiO2 - 64, Na_2O - 30, CaO - 3, MgO - 3%).

The ball is filled with a solution of hydrochloric acid, into which a silver chloride electrode is inserted. When soaked in water, the surface cations of silicates are exchanged for hydrogen ions and the surface acquires the properties of silica gel, which can dissociate.

Due to dissociation, the glass surface receives a charge that depends on the activity of hydrogen ions in the solution. This charge causes the appearance of a certain potential of the electrode surface. The composition of the internal solution is constant, so the potential of the glass electrode linearly depends on the pH of the solution in which it is immersed.

The glass electrode is characterized by compactness, a wide range of linearity (pH from 1 to 13), is not poisoned, is not sensitive to oxidants and reducing agents, equilibrium is quickly established.

The disadvantages of the glass electrode are fragility, high internal resistance. In a strongly alkaline medium, the potential of the glass electrode may depend on the presence in the solution of high concentrations of ions of some alkali metals, which is due to the ion-exchange properties of the glass surface.

Determination of pH of solutions by the electrometric (or potentiometric) method. Measurement of the electromotive force between the electrodes (the potential of one of which is known, and the other is used for equilibration) is the basis of the electrometric method of determining pH. To determine the pH by this method, pH meters (ion meters) are used.

pH-meter (ion meter)

LAB-CLASS

1. Determination of pH of solutions by colorimetric (or indicator) method

The basis of the colorimetric method is that the indicators, depending on the reaction of the medium, show color not dissociated molecules or a combination of colors observed not dissociated molecules and anions. Based on what indicators at different pH values have different color scales are based on standards.

Progress of experiment. First, it is necessary to determine the approximate pH of the test solution using a universal indicator piece of paper, put a piece of paper on the strip drop of 1 and 2 by means of determining the color scale pH. Ante gives grounds selection indicator.

Indicator	Limits of pH indicator (color display)	
Methyl red	from 3.6 (red)	from 5.8 (yellow)
Bromothymol blue	From 5.8 (yellow)	From 7.6 (blue)

Further, 6 drops of the appropriate indicator are added in a test tube, measure at10 ml, mix well and the coloring is corresponding to the pH value of the appropriate scale of standards.

2. Determination of pH of solutions by electrometric (or potentiometric) method

Measurement of electromotive force between the electrodes (the potential of one of which is known, and the other is used for balancing) is the basis of

electrometric method of determining pH. To determine the pH by this method using devices pH meters (ionometry).

Progress of experiment. Following the main provisions of work with the pH-meter, you should measure the pH of the solution in its volume (50 - 70 ml), which is poured into a glass with a volume of 100 ml.

3. pH measurement in biological samples

Biological samples	Indicator method	Electrometric method

…

Control questions, tasks and exercises for the section «pH: METHODS OF DETERMINATION**»**

1. What means and devices can be used to determine the pH of the solution?
2. Here are some examples of pH values for biological samples.
3. What is the value of $[H^+]$ and $[OH^-]$ at neutral pH.
4. Define the ionic product of water and indicate why it is equal at 25° C
5. Calculate pH if the concentration of the H^+ ions is equal 10^{-4}M
6. Calculate the pH of the solution, if the concentration of OH^- ions is equal 10^{-5}M.

1.1.2. ***Topic*** BUFFER SOLUTIONS

Buffer solutions, their components. Blood buffer systems

As a result of chemical reactions, Hydrogen ions can be formed and consumed. Experimental research in chemistry, biology, medicine, and industrial production needs to ensure a stable pH environment. For the process to take place at a constant pH value, buffer systems are introduced into the solution, which keeps the pH of the environment practically unchanged.

Physiological fluids of the body are characterized by a constant pH value and are regulated by two mechanisms: physiological (i.e., involving the organs - kidneys, liver, lungs, intestines) and physicochemical (under the action of buffer systems). Buffer systems are in blood plasma (hydrogen carbonate, phosphate, protein), and erythrocytes (hemoglobin). In cells, urine, and digestive gland secretions, the phosphate buffer system is the most important.

Buffer systems are solutions that can maintain a constant concentration of hydrogen ions, that is, the pH value of the medium, when small amounts of acid or alkali are added to them or when they are diluted.

Buffer capacity. For example, when the acetate buffer is diluted 100 times, its pH increases from 4.62 to 4.74, that is, by 0.12. Such changes in pH should be considered when working with blood in the process of its biochemical studies, since a change in blood pH even by 0.2-0.3 units leads to serious pathological disorders. Dependence of the buffer capacity on the ratio of components of the buffer solution. The ability of buffer solutions to maintain a constant pH value when diluted or

when acids or alkalis are added is limited. A quantitative measure of the stability of buffer systems to maintain a constant pH value is the value of the buffer capacity.

The buffer capacity (B) is the number of mole equivalents of a strong acid or a strong base that must be added to one liter of buffer solution to change its pH by one unit.

$$B=(C\cdot V)/(\Delta pH\cdot V_{buf}),$$

where C is the acid or base concentration in mol/l; V – a volume of added electrolyte (l), V_{buf} – a volume of buffer solution (l), ΔpH – pH change.

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Buffer systems of the organism. The human body has special mechanisms for coordinating physiological and biochemical processes. The body itself can maintain and coordinate at a certain level the content of various substances (gases, water, electrolytes, metal, and hydrogen ions, bioligands, etc.). Such coordination is called homeostasis (as proposed by K. Kenon).

The action of buffer systems (physicochemical mechanisms) enables the body, as an open thermodynamic system, to implement Le Chatalier's principle, namely: to actively counteract the influence of external factors aimed at changing the acidity of its physiological fluids - blood, bile, urine, secretions of internal glands, etc.

For example, the protein buffer system operates in the cells and tissues of the body. Its composition mainly includes albumins. The mechanism of action is explained by the amphoteric properties of amino acids. In an aqueous solution, glycine (Gly) exists in the form of a bipolar ion (zwitter ion), the concentration of which significantly exceeds the concentration of non-ionized molecules.

$$H_2N\text{-}CH_2\text{-}COOH \leftrightarrow {}^{+}H_3N\text{-}CH_2\text{-}COO^{-}$$

$$H_2N\text{-}CH_2\text{-}COOH + \text{“acid”} \leftrightarrow +H_3N\text{-}CH_2\text{-}COOH$$

$$H_2N\text{-}CH_2\text{-}COOH + \text{“base”} \leftrightarrow H_2N\text{-}CH_2\text{-}COO^{-}$$

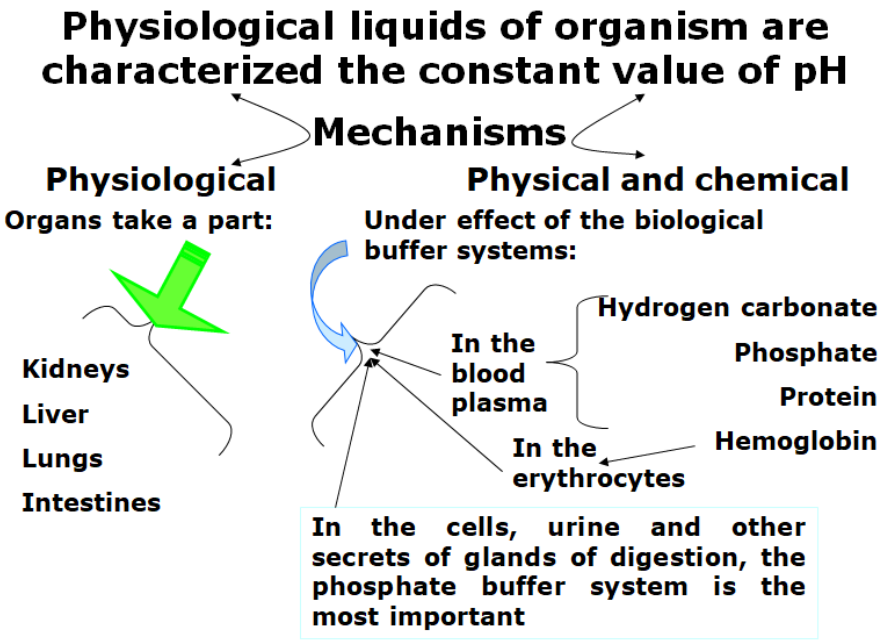
The hemoglobin buffer system operates in erythrocytes (75% - a share in ensuring the buffer capacity of blood).

A type of protein buffers

$$H\mathit{Hb} + O_2 \leftrightarrow H\mathit{Hb}O_2$$

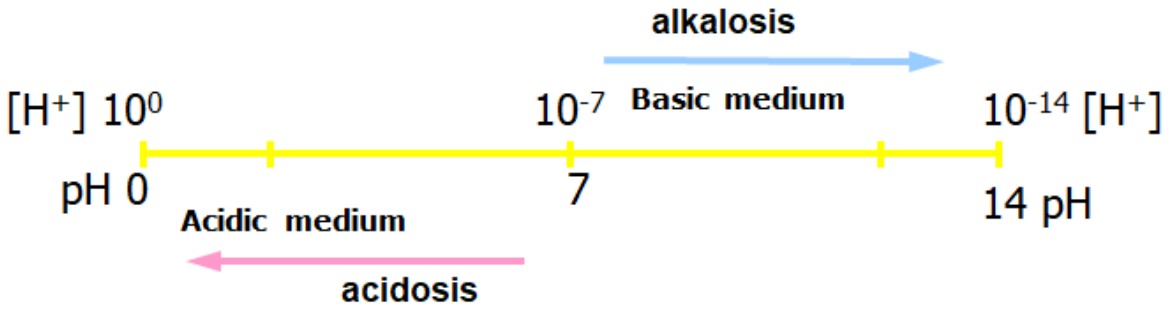
hemoglobin oxyhemoglobin

During normal functioning of the body pH levels, depending on the individual characteristics of each person varies in the range (7.25 - 7.44). Mean blood pH is 7.36, which is optimal for multiple enzyme systems, course hydrolysis reactions, protolysis and redox processes involving H^+ and OH^-.



The acid-base state (ABS) is a set of physicochemical and physiological processes that determine the relative constancy of the hydrogen indicator (pH) of the body's internal environment.

The constancy of the pH of the body's internal environment is a necessary condition for the normal course of vital processes.



Offset the acid-base status of blood in the direction of increasing concentration of hydrogen ions is called acidosis, and towards reducing their concentration - alkalosis.

In clinical practice, the acid-base status of the body expressed in units that are marked BE* (base excess).

Under normal conditions, the body of ABS BE = 0 ± 3, if the value of BE is ± (3-5), the state of the body considered normal stress, at BE ± (6-9) - disturbing, ± (10-14) - threatening, and if ± 14 then - critical.

LAB-CLASS

1. Preparation of acetate buffer and calculation of it's the pH

pH buffer solution can be defined as colorimetric, as electrometric method. When we know the ratio of components in the buffer system, the pH of buffer solution calculates using the formula:

pH = - {lgK_{dis} + lg ([acid] / [salt])}, where K_{dis} - corresponding acid dissociation constant, and ([acid] / [salt]) - the ratio of the volume of acid and salt solution of equal concentration. For acetic acid $K_{dis} = 1.8 \cdot 10^{-5}$.

lg1 = 0; lg2 = 0.3; lg3 = 0.48; lg4 = 0.6; lg5 = 0,69; lg6 = 0.78; lg7 = 0.85; lg8 = 0.9; lg9 = 0.95; lg1,8 = 0.26; $lg10^{-7}$= -7; $lg10^{-5}$ = -5

Progress of experiment. To prepare the acetate buffer using 4, 5 or 9 tubes (depending on the allotted time for this experiment), a few drops of methyl red and an appropriate amount of acetic acid and sodium acetate (according to Table 1) are added, and well mixed in every tube.

pH is determined for each tube's contents on a scale of standards. The data recorde in Table 1.

pH is also determined by calculation, making appropriate entries in the table. Example of calculating the pH of acetate buffer (for sample №1):

pH = - {lgK_{dis} + lg ([acid] / [salt])} = - (lg1,8 $\cdot$ 10^{-5} + lg (9/1)) = - (lg1,8 + $lg10^{-5}$ + lg9 - lg1) = - (0.26 - 5 + 0.95 - 0) = -0.26 + 5 - 0.95 + 0 = 5 - 1.21 = 3.79

Table 1. Determination of acetate buffer pH

№ sample	0,1 M p-н CH_3COOH, ml	0,1 M p-н CH_3COONa, ml	pH (colorimetrically)	pH (by calculation)
1	9	1		
2	8	2		
3	7	3		
4	6	4		
5	5	5		
6	4	6		
7	3	7		
8	2	8		
9	1	9		

2. Preparation of phosphate buffer and calculation of its pH

pH buffer solution can be defined as colorimetric, as electrometric method. When we know the ratio of components in the buffer system, the pH of buffer solution calculates using the formula:

pH = - {lgK_{dis} + lg ([acid] / [salt])}, where K_{dis} - corresponding acid dissociation constant, and ([acid] / [salt]) - the ratio of the volume of acid and salt solution of equal concentration. For phosphoric acid $K_{dis} = 2 \cdot 10^{-7}$.

lg1 = 0; lg2 = 0.3; lg3 = 0.48; lg4 = 0.6; lg5 = 0.69; lg6 = 0.78; lg7 = 0.85; lg8 = 0.9; lg9 = 0.95; lg1.8 = 0.26; $lg10^{-7} = -7$; $lg10^{-5} = -5$

Progress of experiment. To prepare the phosphate buffer using 4, 5 or 9 tubes (depending on the allotted time for this experiment), a few drops of methyl red and an appropriate amount of **KH_2PO_4** and **Na_2HPO_4** (according to Table 2) are added and well mixed in every tube.

pH is determined for each tube contents on a scale standard. The data record in Table 2.

pH is also determined by calculation, making appropriate entries in the table. Example of calculating the pH of phosphate buffer (for sample №1):

$pH = - \{lgK_{dis} + lg ([acid] / [salt])\} = - (lg2 \cdot 10^{-7} + lg (9/1)) = - (lg2 + lg10^{-7} + lg9 - lg1) = - (0, 3 - 7 + 0.95 - 0) = -0.3 + 7 - 0.95 + 0 = 7 - 1.25 = 5.75$

Table 2. Determination of phosphate buffer pH

№ sample	0,15 M p-н KH_2PO_4, ml	0,15 M p-н Na_2HPO_4, ml	pH (colorimetrically)	pH (by calculation)
1	9	1		
2	8	2		
3	7	3		
4	6	4		
5	5	5		
6	4	6		
7	3	7		
8	2	8		
9	1	9		

3. Determination of buffer capacity

The buffer capacity (quantitative characterization of buffer solutions) is determined by the number of gram equivalents of acid or alkali to be added to 1 liter of the solution to change the pH by on.

a) acetate buffer

Progress of experiment. Into a graduated cylinder, it is poured 37.5 ml of 0.2 M acetic acid and 2.5 ml of 0.2 M sodium acetate.

In the test-tube, a few drops of methyl red and 10 ml of the prepared acetate buffer are added. It is necessary to determine the pH with the colorimetric method.

In a glass, it is poured 5 ml of acetate buffer and 2 drops of phenolphthalein and titrated by 0,1N of NaOH solution to the appearance of a light pink color (this is pH 9 according to the colored pH-scale).

Buffer capacity is calculated by the following formula:

$B = (mol\ eq\ NaOH) / (pH_2 - pH_1) = (N \cdot V_2) / ((pH_2 - pH_1) \cdot V_1)$,

where V_1- the volume of the output buffer, V_2- the used amount of alkali or acid for titration, N - normality of alkali or acid, pH_1 and pH_2 – the investigated pH values of buffer before and after titration.

b) serum.

Progress of experiment. In a glass, it is necessary to pour 1 ml of serum, add 1 - 2 drops of phenolphthalein, and titrating 0,1N of NaOH solution to the appearance of a light pink color (it responds pH 9). The results of titration buffer capacity are calculated as in the previous experiment.

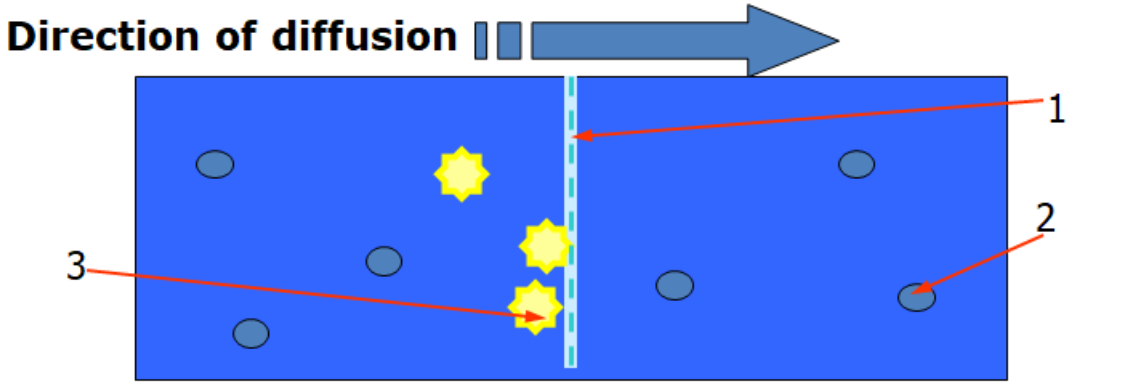
Control questions, tasks and exercises for the section «BUFFER SOLUTIONS**»**

1. What solutions are buffers?
2. What are the most important buffer systems in mammals?
3. What caused buffering properties of proteins?
4. What factors does the buffer capacity depend on?
5. Write/draw a scheme of the mechanism of action of protein buffer solution.
6. Value / role of blood buffer systems.

1.1.3. Topic OSMOSIS, OSMOTIC PRESSURE IN BIOLOGICAL SYSTEMS

Diffusion. Osmosis. Osmotic pressure

Diffusion is a spontaneous process of equalizing the concentration of a substance in the entire volume of the solution, caused by the thermal movement of the particles of the dissolved substance and the solvent. Diffusion occurs from a solution with a higher concentration of a dissolved substance into a solution with a lower concentration of this substance.



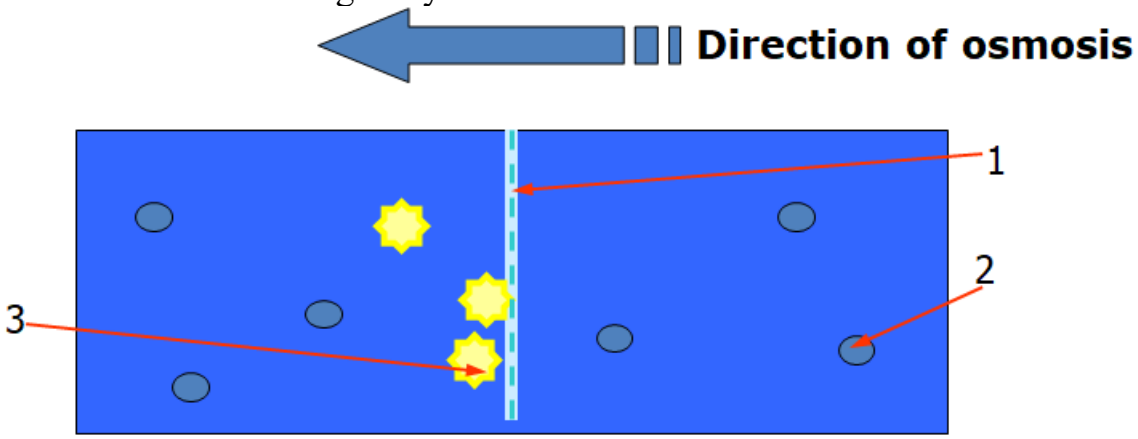
1 – a permeable membrane; 2 – molecules of solvent; 3 – particles of solute

Osmosis is the one-way diffusion of solvent molecules through a semipermeable membrane from a solution with a lower concentration to a solution with a higher concentration. As a result of the diffusion of the solvent from the

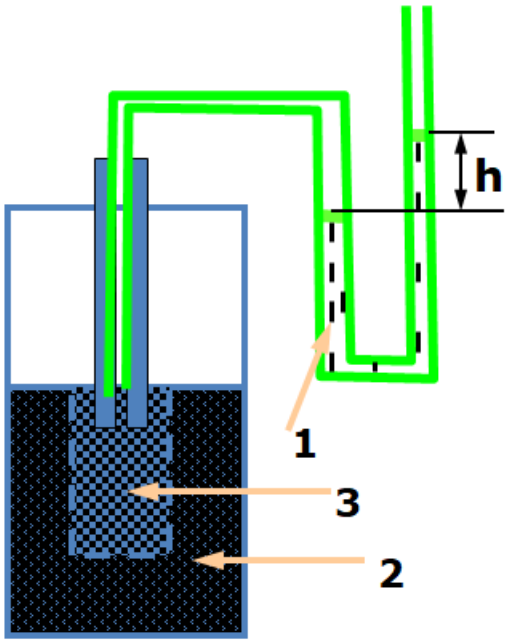
outer vessel into the osmometer, the liquid level in the tube will rise, which will create an excess hydrostatic pressure p

$$p = h\rho g,$$

where h is the excess liquid column; ρ is the density (density) of the liquid: g is the acceleration of the Earth's gravity.



1 – a semipermeable membrane; 2 – molecules of solvent; 3 – particles of solute



Scheme of osmometer :

1- manometer; 2- container with solution, 3- container with solvents

The combined Mendeleev–Clapeyron equation of the gaseous state:

$$pV = \nu RT = (mRT)/M$$

The equation of the osmotic pressure of Van't Hoff solution:

$$\pi = (mRT)/(MV) = CMRT,$$

where π is the osmotic pressure of the solution, kPa; CM - molar concentration of the solution, mol/l; R is the universal gas constant {8.314 J/(mol•K)}; T is absolute temperature, K.

Van't Hoff's law: the osmotic pressure of a solution is directly proportional to its molar concentration and absolute temperature. The value of the osmotic pressure of the solution depends on the osmotic concentration (osmomolality)

$$\pi = (mRT)/(MV) = C_{osm}RT,$$

where π– osmotic pressure of solution, kPa; C_M- molar concentration of solution, mol/L; *R*- universal gas constant {8.314 J/(mol·K)}; T – absolute temperature, K.

Biological fluids are aqueous solutions of many mineral and organic substances (p=constant). Blood plasma p = 770 – 821 kPa (7.6 – 8.1 atm), where blood p is determined by Na^+ and Cl^- ions (by 60%), proteins – to a lesser extent.

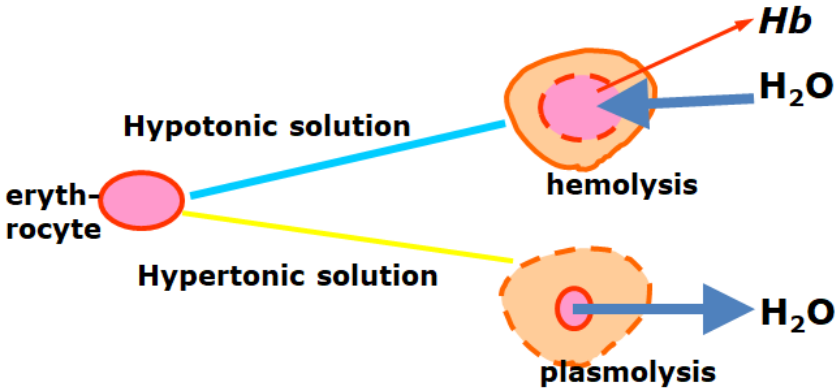
The pressure created by high-molecular biologically active compounds is called oncotic pressure. It is 0.5% of the total osmotic pressure (3.04-4.05 kPa or 0.03-0.04 atm); 80% is determined by albumins; since p of lymph ~ 1.33 kPa, p of blood ~ 4 kPa, water flows from the lymph into the blood due to the pressure difference.

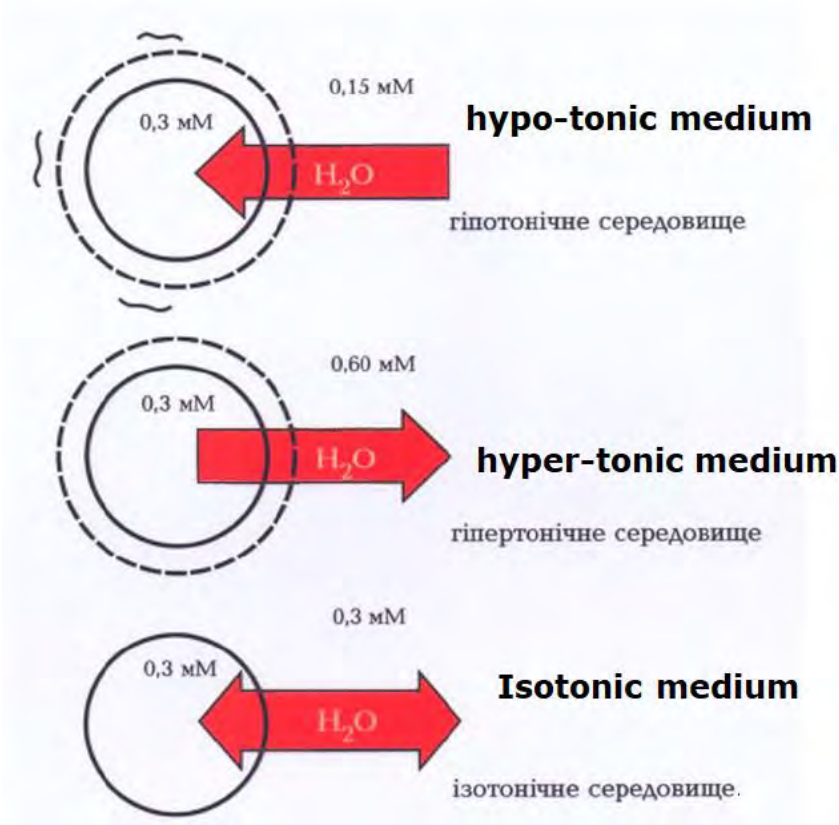
Hypotonic, hypertonic, and isotonic solutions.

If the osmotic pressure of one solution is greater than the other, then the first solution is hypertonic, and when the opposite is the case, it is hypotonic. Solutions with the same osmotic pressure are called isotonic.

Isotonicity is important for infusion solutions. In clinical practice: isotonic solutions have an osmotic pressure equal to blood plasma p = 7.7-8.1 atm or 0.85-0.9% sodium chloride, or 4.5-5% glucose solution. Physiological solutions are similar in composition to sea water.

Hemolysis and plasmolysis of erythrocytes.





A cell as osmometer (Cited: Мусил Я., Новакова О., Кунц К. Современная биохимия в схемах - М.: Мир, 1981. - с. 176.)

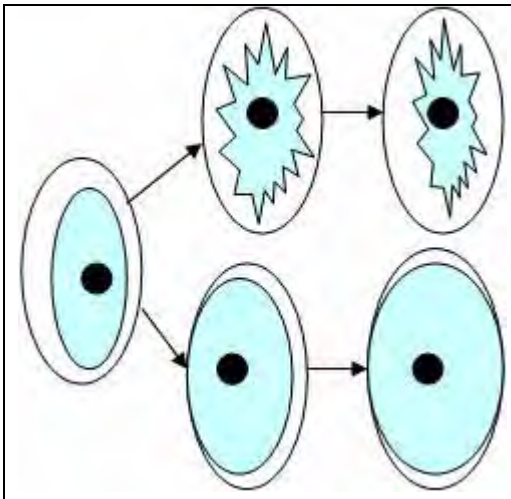
The destruction of the erythrocyte membrane when hypotonic solutions are introduced into the blood plasma is accompanied by the release of hemoglobin into the plasma, called hemolysis ("lacquered blood"). The phenomenon of erythrocyte shrinkage when hypertonic solutions are introduced into the blood plasma is called plasmolysis.

LAB-CLASS

1. The influence of solutions with different osmotic pressure in cells

Progress of experiment. In three test tubes, it is necessary to pour 2 ml with appropriate concentrations of NaCl: (1) 10% - a hypertonic solution; (2) 0.89% - isotonic (saline) solution; (3) 0.1% - hypotonic solution.

In each tube, it should be added a piece of film coating onions. The contents of each tube are stirred with a glass rod for 5 minutes, take the film from one tube on a glass slide, cover with cover glass, and examined under a microscope at high magnification. The same do with the content of the tubes 2 and 3. Observation record, conclude the experiment and explain the scheme below.

 Scheme	Explanation:

2. Osmotic pressure in red blood cells

The study of changes in erythrocyte osmotic pressure in solutions of varying concentrations of NaCl and their effects on cells.

Progress of experiment. In three test tubes, it is poured 2 ml of solutions with appropriate concentrations of NaCl: (1) 10%− hypertonic solution; (2) 0,89%− isotonic (saline) solution; (3) 0,1%−hypotonic solution. Then, in each tube, it is added 2 drops of blood, which were heparinized or processed by sodium citrate. The contents of each tube are stirred with a glass rod and 1 drop of taken out of the tube and put it on a slide (then coated by the cover glass) and examined it under a microscope at high magnification. The same one should be done with the content of the tubes 2 and 3.

3. The phenomenon of blood osmosis caused by changes in osmotic pressure

Progress of experiment. The different concentrations of NaCl are exactly pre-prepared solutions before the experiment. In numbered test tubes, the solution of NaCl and drops of blood are mixed according to the scheme-table (see below):

# test tube	1	2	3	4	5
% sol. NaCl	0	0,2	0,85	1	4
Volume, sol. NaCl, ml	5	5	5	5	5
citrate blood	2-3drops	2-3 drops	2-3 drops	2-3drops	2-3 drops
vigorous shaking					
What color does the tube has after 1 h?					

Control questions, tasks and exercises for the section «OSMOSIS, OSMOTIC PRESSURE IN BIOLOGICAL SYSTEMS**»**

1. Define osmosis and diffusion

2. What is the difference between osmosis and diffusion? What do these phenomena have in common?
3. Provide examples of osmosis in biological systems.
4. Explain the hemolysis of erythrocytes.
5. What is cell plasmolysis?
6. Which (hypo-, iso- or hypertonic) solutions can be administered intravenously? Why?

1.1.4. ***Topic*** SORPTION AND BIOLOGICAL PHENOMENA

Sorption, types of sorption. Chemical and physical nature of sorption. Adsorption, types of adsorption

All cases of absorption of gases and vapors by solid bodies are called sorption. If this process occurs only on the surface, then it is called adsorption, and when the substance that is absorbed by the surface diffuses inside the absorbing substance and is distributed throughout its volume, then this phenomenon is called absorption.

A solid body on the surface of which adsorption occurs is called an adsorbent, and a substance that is adsorbed is called an adsorbate.

Desorption is the removal of adsorbed molecules from the surface of the adsorbent. The rate of adsorption decreases with time, and the rate of desorption increases.

Depending on the nature of adsorption forces, two types of adsorptions are distinguished: physical and chemical (chemosorption).

Physical adsorption is caused by the action of the force fields of the surface molecules of the adsorbent, while the molecules of the adsorbate do not lose their individuality. This process occurs only on certain areas of the adsorbent (adsorption centers) - protrusions, nodes of crystal lattices, which have excess surface energy. Physical adsorption is characterized by the following factors: fast reversibility of adsorption-desorption processes, absence of stoichiometric ratios when determining adsorption, and decrease of adsorption with increasing temperature.

Chemical adsorption is caused by interactions between the surface molecules of the adsorbent and the adsorbate. Chemical compounds are formed on the surface of the adsorbent and individual molecules lose their individuality. Chemisorption is characterized by irreversibility, a thermal effect close to the energy of the formation of chemical bonds (40-120 kJ/mol), and an increase in adsorption with increasing temperature (in contrast to physical adsorption).

LAB-CLASS

1. The adsorption capacity of sorbents relative to electrolytes, dyes, and sol

Progress of experiment. In 5 test tubes, 5 ml of colored solutions (1% $CuSO_4$, 0.5% $K_2Cr_2O_7$, a solution of fuchsine, eosin, $Fe(OH)_3$ sol) are poured. 0.2 g of activated carbon or sorbent "X" is added to each solution. The mixture is

repeatedly shaken for 3 minutes and filtered. The results of the observations have to be recorded and explained.

Adsorption capacity of sorbents

Substances	**1 % $CuSO_4$**	**0.5% $K_2Cr_2O_7$**	**fuchsine**	**eosin**	**$Fe(OH)_3$ sol**
color / intensity					
after adding to the solution and activated carbon filter					
color/intensity filtrate (+)					
after adding to the solution and filtering sorbent X					
color/intensity filtrate (+)					

2. Adsorption of coal

Progress of experiment. In two test tubes, it is added 5 ml of 0.07% solution of $Pb(NO_3)_2$, then one of them (1) to leave as the control sample. 0.2 grams of coal added to the other (2) tube, shaken for 5 min, and filtered. Several drops of 0.5% solution of $K_2Cr_2O_7$ are added to the source solution of $Pb(NO_3)_2$ (sample 1) and the filtrate (sample 2). It is necessary to compare samples for the presence of lead before adsorption and after.

3. Sorption and desorption processes

Progress of experiment. In a test tube, it is added 5 ml of methylene blue, 0.2 grams of coal, and shaken for 5 min. Then the mixture is filtered, and observations are recorded too. The used filter paper with sorbent (coal) must be put in another dry tube. Few drops of ethanol are put on the used filter paper. Observation of desorption phenomenon: dye elution from the used filter paper with sorbent (coal) and the eluate color is blue. Give an explanation.

4. Adsorption of fuchsine on the glass and its desorption

Progress of experiment. In the flask, a 1% aqueous solution of fuchsine is poured for its adsorption for 10-15 minutes. After that, the flask carefully is washed by water until it becomes colorless. For observation of desorption of fuchsine, 15-20 ml of ethanol are poured into the flask and then shaken for 2 minutes. Ethanol has to be painted pink and red because of desorption of fuchsine.

Control questions, tasks and exercises for the section «SORPTION AND BIOLOGICAL PHENOMENA**»**

1. Define adsorption.
2. How is absorption different from adsorption?
3. What is the phenomenon opposite to adsorption called and why?
4. What types of sorption do you know?
5. Common and distinctive features of physical and chemical adsorption.
6. Where and why are surfactants adsorbed in water?

1.1.5. ***Topic*** COLLOID SOLUTIONS. HIGH-MOLECULAR COMPOUNDS OF BIOLOGICAL LIQUIDS

Colloid chemistry

Colloid (colloidal) chemistry is the physics-chemistry of dispersed systems and surface phenomena. The subject of colloid chemistry is the study of properties of substances in the high-dispersed state and surface phenomena in dispersed systems.

In 18 and 19 centuries, the foundations of colloidal chemistry were emerging. The founder of colloidal chemistry was the Scottish scientist T. Graham (Thomas Graham, 1805-1869):

crystalloids ← substances → colloids

Colloids are substances that do not crystallize from solutions but form amorphous precipitates, their solutions do not diffuse well (hydroxides of some metals, starch, destrins).

Studies of colloidal processes were carried out by M. Lomonosov in production of colored glass, 18th century, F. Reiss in electrokinetic phenomena, J. Berzelius in instability, optical phenomena, and M. Faraday in production of colloidal crystals.

A colloidal system contains two distinct phases: the dispersed phase (or internal phase) and the continuous phase (or dispersion medium). A colloidal system can be solid, liquid, or gaseous. Milk is an emulsified colloid of liquid oil globules dispersed within the liquid-water boundary. Dispersed systems are characterized only by relative aggregate stability.

Biological objects are such systems: liquid dispersion medium (water) and dispersed phase (proteins, carbohydrates, nucleic acids). Some biological objects with different particle size are dispersed systems. Cell of living organism is heterogeneous colloidal system created by high-molecular and low-molecular substances.

Tissue is a heterogeneous system of higher degree: dispersed phase (cells), dispersion medium (the fluid around cells). Knowing the properties and peculiarities of colloidal-dispersed systems is necessary to understanding very complex processes of organisms' life activity. Importance is the development of cell models, living membranes, neuron fibers, oxygen transportation, etc.

Classification of dispersed systems according to their structure:

- Free-dispersed systems are systems without any structure (suspensions, emulsions, sols), in which particles of the dispersed phase are not connected into one continuous web and they are able to move independently in dispersion medium under Brownian motion effect or under of force of Earth gravity.
- Associated dispersed systems are characterized that particles create spatial structure. Therefore, they are not able to move freely (gels, capillary-porous bodies or diaphragm, membranes, sol-gel)

Obtaining the dispersed systems:

- By dispersion way (crushing solid and liquid substances into smaller ones in a certain medium; peptization conditionally belongs to dispersion technique, peptization is chemically dispersing by peptizing agent: solvent, electrolyte solution, surface-active substance solution
- By condensation way (creation from particles of the dispersed phase, molecules, atoms, or ions; physical and chemical condensation)

Purification of dispersed systems off over-excess of electrolytes and other low-molecular dashes (which decrease system stability very much) carries out by dialysis or ultra-filtration.

Ultrafiltration is filtration under overpressure through ultrafilters made from a semipermeable membrane.

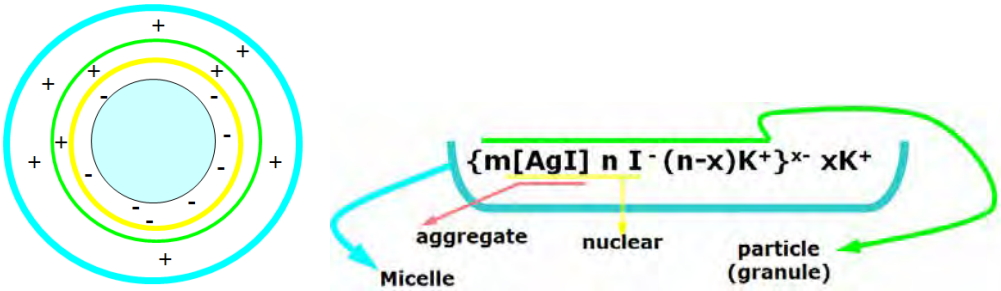
Ultrafiltration is used not only to remove low molecular weight impurities but also for concentrating systems.

The use of membranes with defined pore size allows for dividing the disperse phase into fractions according to particle size and identifying these dimensions. In this way, it was found the size of some viruses and bacteriophages.

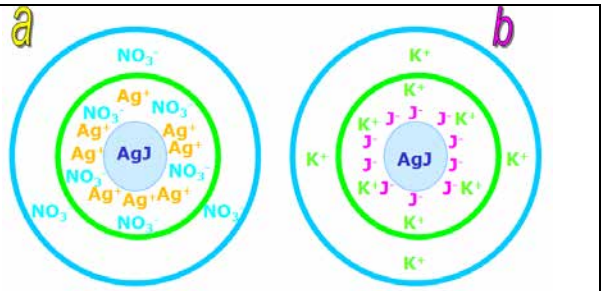
Electrical properties of dispersed systems

The electrical properties of colloidal systems are caused by the availability of an electrical double layer of ions on the surface of particles of the dispersed phase. The electrical double layer could be formed because of selective adsorption of one of the ions of electrolyte, which is in solution, on account of surface dissociation of functional groups or orientation of polar molecules on the inter-phases border. The electrical properties of colloidal systems are caused by the availability of an electrical double layer of ions on the surface of particles of the dispersed phase.

The electrical double layer could be formed because of selective adsorption of one of the ions of electrolyte, which is in solution, on account of surface dissociation of functional groups or orientation of polar molecules on the inter-phases border. The theory of the double electrical layer by Stern does explain the structure of particles of the dispersed phase of the colloidal system.



Micelle of AgI under excess of KI (where m – number of molecules AgI; n – number of the potential-determining Iodine-ions; (n-x) – number of Potassium-ions in adsorption layer; x – number of Potassium-ions in diffusive layer).

	**Structure of micelles of sol Argentum iodide with positive (a) and negative (b) charge of granule**

Sol is formed of micelles and intermicelles fluid. Micelle is the structural colloidal unit, intermicelles fluid is a dispersion medium, that separates micelles and consists of electrolyte, non-electrolyte, and surface-active substances. Micelle is a crystal of dispersed phase with a rounding double electrical layer. The solid phase (which is inside of micelle) is called aggregate, aggregate with potential-determining ions – the nucleus of the micelle. The nucleus with an adsorption layer forms a particle or granule, which is rounded by a diffusive layer.

LAB-CLASS

1. Obtaining colloidal solutions by dispersion

The basic principle of obtaining colloid dispersing agents are grinding through Mills, ultrasound, agitation, dissolution, electric spraying, and others.

Progress of experiment. 3 - 5 ml of water are added in the test tube and heated till gets boiled. Few pieces of gelatin are added by gently boiling water and gently stirred until it gets dissolved. The resultant gelatin sol turns into a sol with a blue tint. The cooling gelatin sol loses mobility and turns into the jelly-form if its concentration is high. It should be noted the external difference between sol and jelly. Sol-transformation into the jelly-form is due to an increase in viscosity and slower Brownian motion of particles.

2. Preparation of colloids via condensation

The atoms or molecules are combined into units that reach the size of colloidal particles thus formed from molecular colloidal solutions. The basis of chemical condensation is a variety of chemical reactions, oxidation, reduction, hydrolysis, etc.

2.1. Preparation of sol iron (III) hydroxide by hydrolysis

The method is based on the hydrolysis of iron (III) chloride, which is a salt of strong acid and weak base: $FeCI_3 + 3H_2O = Fe(OH)_3 + 3HCI$

Some iron hydroxide molecules enter into a chemical reaction with HCl:

$Fe(OH)_3 + 3HCI = FeOCI_3 + 2H_2O$

The formed salt dissociates into ions, which serve as the basis for the formation of adsorption and diffusion layers of iron hydroxide micelles:

$FeOCI = FeO^+ + CI^-$.

Colloidal particles of iron (III) hydroxide (adsorbed ions on the surface of the solution) acquire the aggregate stability. The structure of colloidal particles of iron (III) hydroxide

$$\{m[Fe(OH)_3]\ nFe^{3+}3(n-x)Cl^-\}^{3x+}3xCl^-$$

core

adsorption layer

diffusion layer

_____granule_____

_____micelles _____

Progress of experiment. 3-4 ml of water are poured into two test tubes. In one of the tubes, water is heated till it's boiled, then a few drops of 2% solution of iron chloride added carefully on the tube's wall for longer heat it (sample 1). At the same time, iron chloride is also added to the test tube with cold water (sample 2). The results of the observations must be recorded and explained.

2.2. Preparation of Prussian blue sol

The interaction of the "yellow blood salt" (potassium hexacyanoferrate) with iron chloride produces a new substance - Prussian blue. Prussian blue is condensed into colloidal particles that stabilized in solution by potassium hexacyanoferrate molecules:

$3K_4[Fe(CN)_6] + 4FeCl_3 \rightarrow 12KCl + Fe_4[Fe(CN)_6]_3$

Alkalis decompose Prussian blue, with the formation of iron (III) hydroxide:

$Fe_4[Fe(CN)_6]_3 + 12\ KOH = 4Fe(OH)_3 + 3Fe_4[Fe(CN)_6]$

Progress of experiment. 4-5 ml of 0.1% solution of "yellow blood salt" are poured in the test tube and 1-2 drops of 2% solution of iron chloride add. The resultant sol is called salt Prussian blue.

3. Properties of colloidal solutions

3.1. Coagulation of colloidal solutions

Coagulation is a process of association of colloidal particles into larger aggregates, which is accompanied by turbidity, change in color, and form sediment in colloidal solutions.

Progress of experiment. Few crystals of sodium sulfate are added into the test tube with sol of iron (III) hydroxide from the previous experiment. Pay attention to the color change and transparency of iron (III) hydroxide sol.

4. Irreversible coagulation of organic colloids

Irreversible coagulation of colloidal solutions is observed when they are added to the electrolytes, ions of which are adsorbed on the colloidal particles to form insoluble precipitates with them.

Progress of experiment. 1 ml of 2% protein solution is poured in three test tubes, and then it is added 1-2 drops of 5% copper sulfate (to the 1st tube), 0.5% lead acetate (to the 2nd tube), and 3% silver nitrate (to the 3d tube) for next

precipitation observation in all tubes. Then, 5.8 ml of water is added to every tube, mixed thoroughly, and watched the precipitate of protein.

Control questions, tasks and exercises for the section «COLLOID SOLUTIONS. HIGH-MOLECULAR COMPOUNDS OF BIOLOGICAL LIQUIDS»

1. What solutions are called colloidal?
2. What substances stabilize colloidal solutions?
3. What are the ways to get colloidal solutions?
4. Give explanations colloidal protection.
5. Draw a diagram of iron (III) hydroxide micelle
6. Draw a diagram of the micelle of argentum iodide with a positive charge of granule

Generalized conclusions

to chapter 1 "FUNDAMENTALS OF PHYSICAL AND COLLOID CHEMISTRY"

1. The importance of knowing the pH value of the medium and its methodical approaches are presented.
2. Briefly describe buffer solutions, their components, blood buffer systems, and their role in the vital activity of living organisms
3. The phenomenon of diffusion, osmosis, and the value of osmotic pressure in biological systems are described, in particular for the functioning of erythrocytes.
4. Describing the phenomena of sorption-desorption, the characteristics of physical and chemical adsorption are given with the addition of vivid experimental examples.
5. Colloidal solutions are described from the point of view of dispersed systems, and high molecular components of biological fluids are considered.

Chapter 1.2
PHYSICAL and CHEMICAL METHODS OF RESEARCH IN BIOCHEMISTRY

The purpose of this section of the course "Biochemistry of animals with the Basics of Physical and Colloid Chemistry" is to provide students with theoretical knowledge of physical and chemical methods of research in biochemistry and some experiments in lab classes.

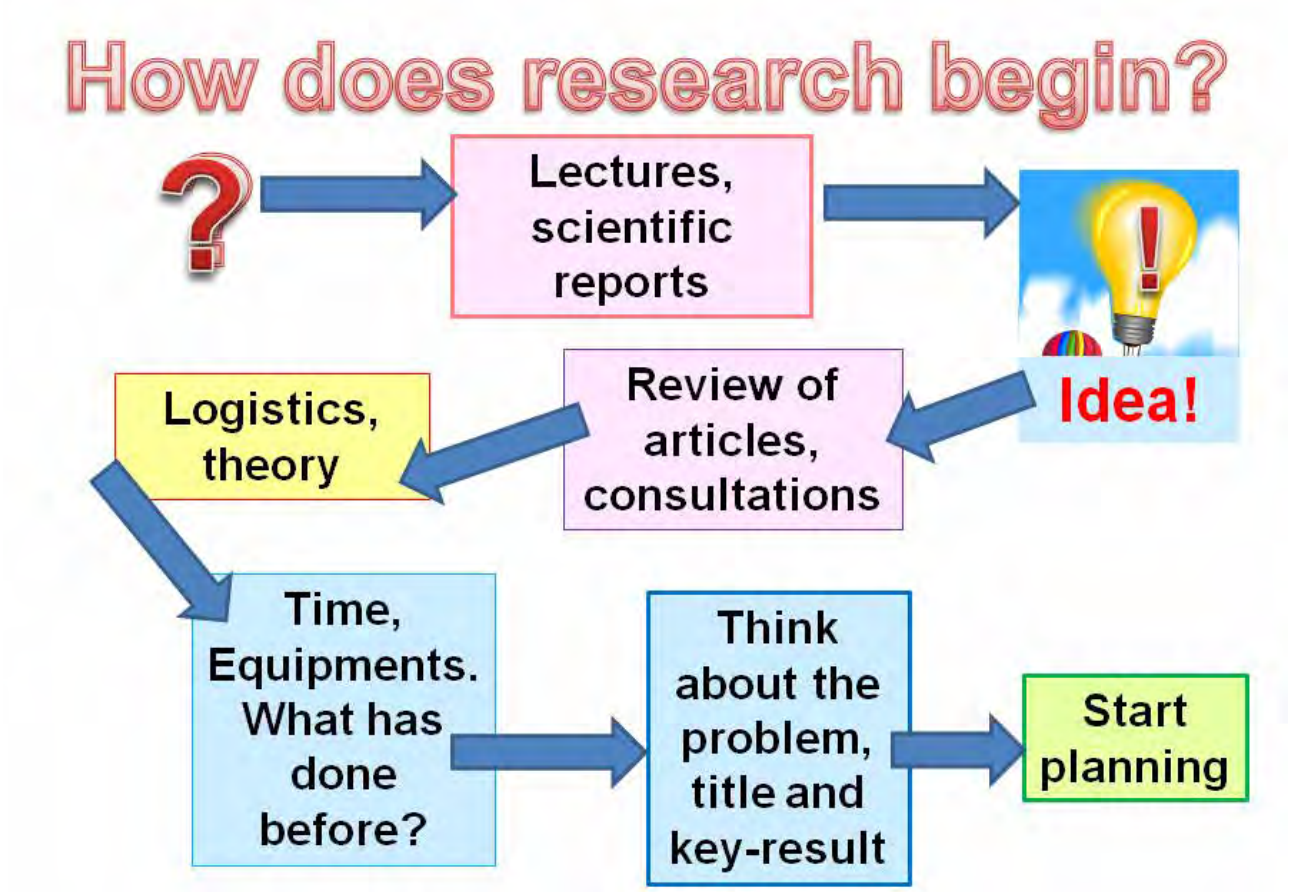
The following topics will be considered in this section:

Topic 1. Spectrophotometric and photo electrocolorimetricmethods

Topic 2. Centrifugation

Topic 3. Fundamentals of electrophoretic separation of high molecular compounds

Topic 4. Chromatographic methods



Adaptated: ***Dr. Graham Basten*** **Introduction to Scientific Research Projects.** – 2010. Ventus Publishing ApS. - 51 p.)

Methods of study of metabolism

Levels of research:

- the whole organism (balance studies - from the food to the final decay products)
- tissues (individual organs, blood - a mirror of health)

- cells
- subcellular compartments

Methods of research:
methods for fractionation; centrifugation, differential centrifugation, ultracentrifugation; sedimentation, radioactive isotopes, immunological techniques, enzyme kinetics, sequencing; gas, gas-liquid and liquid chromatography, etc.

Physic and chemical fundamentals of methods of investigation in biochemistry

Physic and chemical fundamentals of spectral analysis of compounds (spectrophotometric and colorimetric methods); separation of substances by differential centrifugation, chromatographic and electrophoretic methods, sequencing macromolecular compounds, X-ray analysis, etc. Examples of determining the unknown concentration of a substance.

Physic and chemical methods of analysis are united in group of methods based on the dependence of physical properties of matter from its nature.

The value of physical property, which is functionally related to the concentration or mass of the component, which is determined, is the analytical signal.

In physic and chemical methods of analysis as an analytical signal use intensity, current strength, electrical conductivity, the potential difference.

Physic and chemical methods of analysis can include chemical conversion of the compound, which is determined, the dissolution of the sample, the concentration of the component, which is analyzed masking substances that interfere, etc.

The important physical and chemical methods of analysis include: spectroscopy (e.g., luminescence analysis, spectral analysis, nephelometry and turbidimetry, etc.) based on the study of the emission and absorption of radiation in various areas of the spectrum, electrochemical methods based on measuring the electrical properties of substances (voltammetry, conductometry, coulometry, potentiometry, etc.) chromatography (e.g., gas chromatography, liquid chromatography, TLC, ion exchange chromatography).

Widely used methods based on measuring the rate of chemical reactions (kinetic methods of analysis), thermal effects of reactions (Thermometric titration or calorimetry), as well as the separation of ions in a magnetic field (mass spectrometry).

When the physic and chemical methods of analysis using a special, rather complicated instrumentation, so these methods are called instrumental. In carrying out physic and chemical methods of analysis, it's used a special enough complicated measuring equipment, so these methods are called instrumental.

Many modern devices are equipped with computers that allows you to find the optimal conditions of analysis (e.g., to determine the spectral area of obtainment of accurate results in the analysis of mixtures of colored substances, perform calculations, etc.).

In almost all physical and chemical methods of analysis, the methods of direct measurement and titration are used.

2.1. ***Topic*** SPECTROPHOTOMETRIC AND PHOTOELECTROCOLORIMETRIC METHODS

Spectrophotometry and Photo-colorimetry

Optical absorption analysis methods based on the use of combined law light absorption of Bouguer-Lambert-Beer:

$$\lg(I/I_0)=\chi cl,$$

where I_0 – intensity of light that falls on a solution substance; I – intensity of light that has passed through a solution of the substance; χ – index of absorption of solution; c – concentration of test solution; l – thickness of the layer of solution.

$\lg(I/I_0) = D$. index of absorption of solution (χ) is constant for each substance at a certain length of light. It is equal to the optical density of the solution with a layer thickness of 1 cm and the concentration expressing 1 mol / l. If the concentration is expressed in mol / l, then denoted by ε and called the molar extinction coefficient:

$$D=\varepsilon cl.$$

Photo-colorimetry

The method is based on measuring the absorption non-monochromatic light that passes through the colored investigated solution.

Non-monochromatic radiation with a narrow range of wavelengths is obtained by using filters.

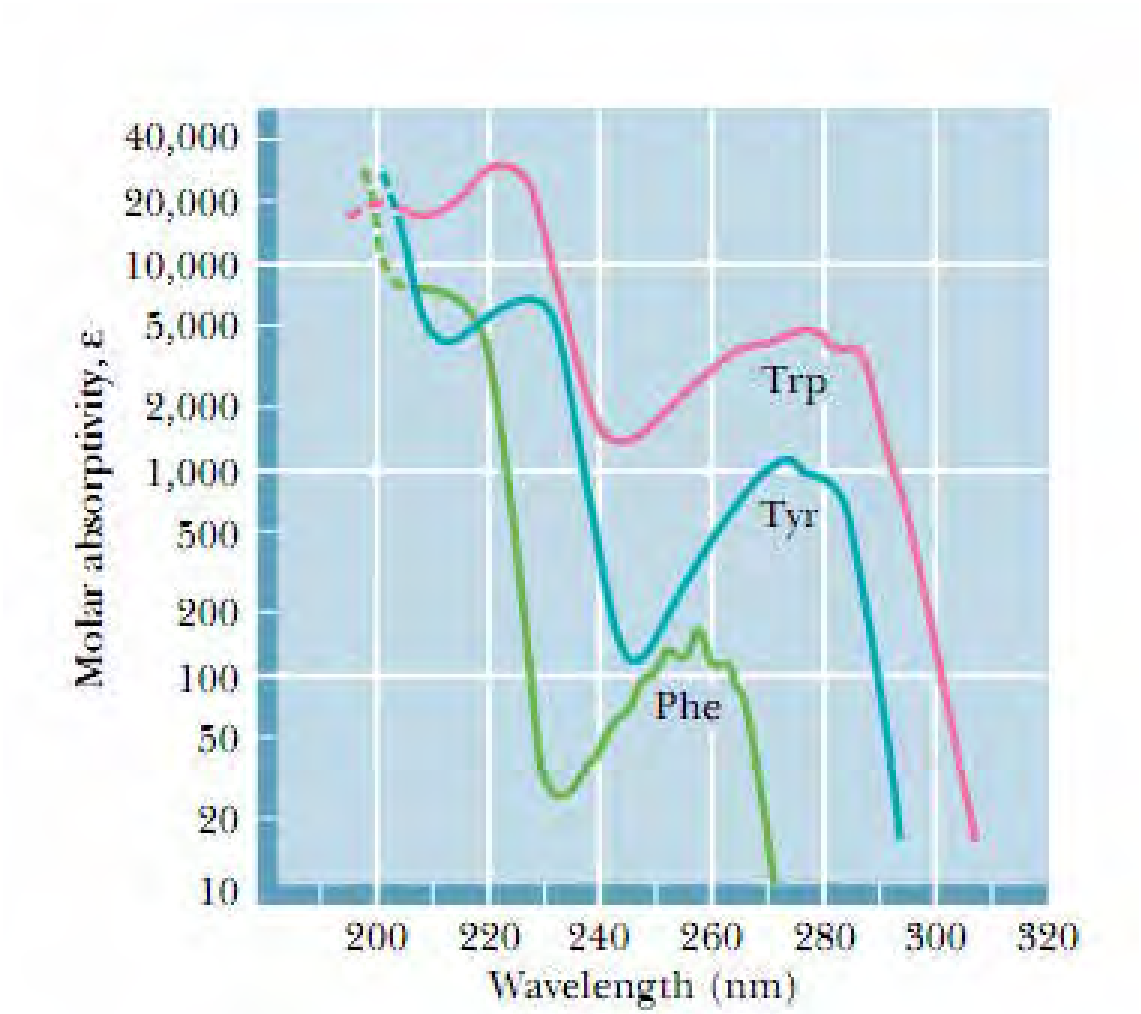
The intensity of light passing through the test solution is measured by the magnitude of electric current, which occurs in photocell.

Scale indicator graduated in the values of optical density D and the values of light transmission coefficient T. Value $T= I/I_0$.

Spectrophotometry

Epoch™
Micro-plate spectrophotometer

λ 200-999 nm (possibility of change by 1-nm step by step)



The ultraviolet absorption spectra of the aromatic amino acids at pH 6. (From Wetlaufer, D.B., 1962. Ultraviolet spectra of proteins and amino acids. Advances in Protein Chemistry 17:303–390.)

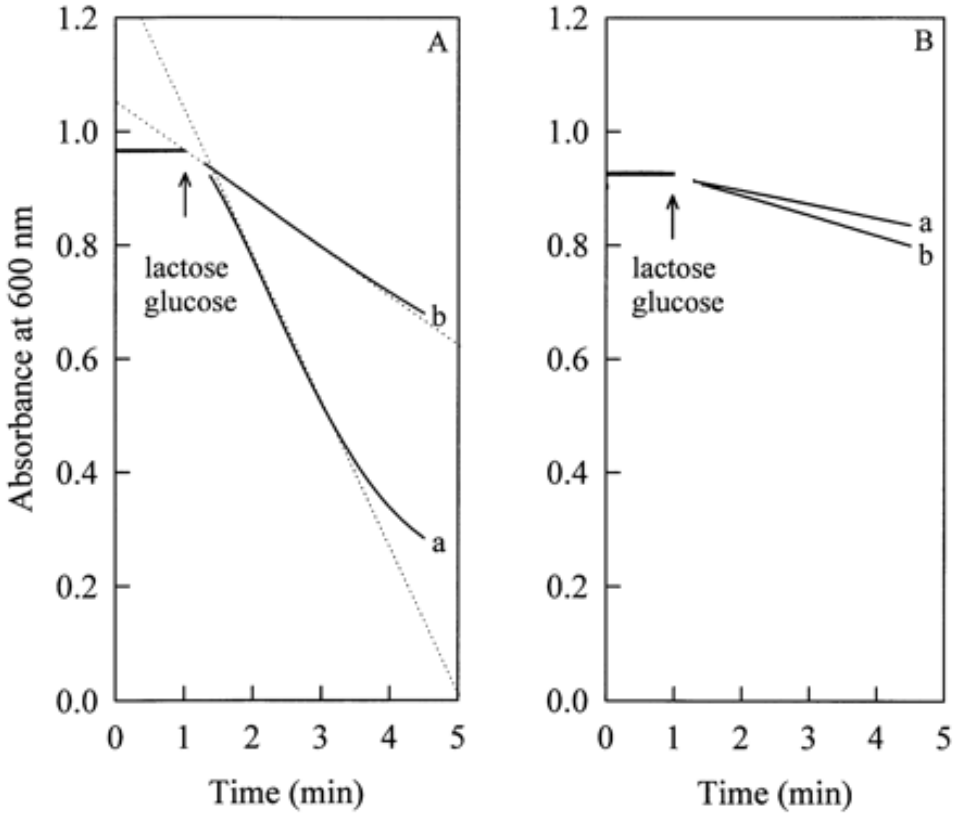
Spectrophotometry is different from photo-colorimetry that analysis carried out by the absorption of substances monochromatic radiation in the ultraviolet, visible, and infrared regions of the spectrum.

Spectrophotometric determination based on the law of Bouguer-Lambert-Beer, but unlike photo colorimetric studies can analyze not only colored but also colorless solutions, performing in the latter case the measurements in the ultraviolet or infrared regions of the spectrum.

One of the tasks spectrophotometric methods is quantitative determination which is characterized the absorption of this substance of monochromatic radiation of different wavelengths.

These values can be used to quantify the characteristics of the substance and to quantify in solution or in a mixture with other substances.

In connection with the division of the electromagnetic spectrum by wavelength into certain areas, is called Spectrophotometry in the infrared, visible and ultraviolet regioni.



Change in absorbance versus time that characterizes the flow of certain processes.

In the ultraviolet and visible area, electronic spectra of molecules are appear and in the infrared - vibrational spectra.

In modern biochemical studies, spectral methods are widely used.

Among optical methods, the most accessible and therefore most common is visible and ultraviolet (UV) spectrophotometry, which allows for a relatively uncomplicated equipment quick and precise quantitative analysis of substances.

White Horse Scientific has announced the European launch of DNAMaster, a microvolume nucleic acid and protein analyser.

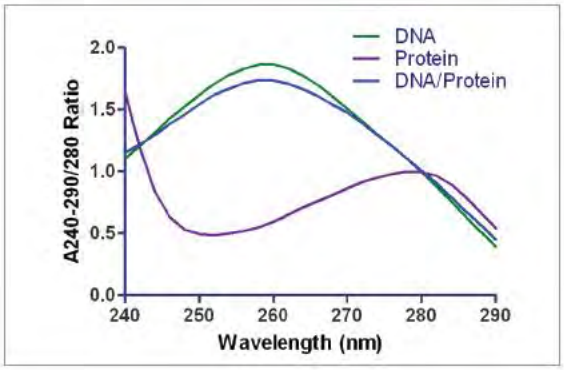
DNAMaster is designed for the measurement and analysis of precious samples by requiring only microvolume quantities.

It boasts a wavelength range from 200 to 900nm, an absorbance range between 0 - 4 O.D. and a wavelength accuracy of 1nm.

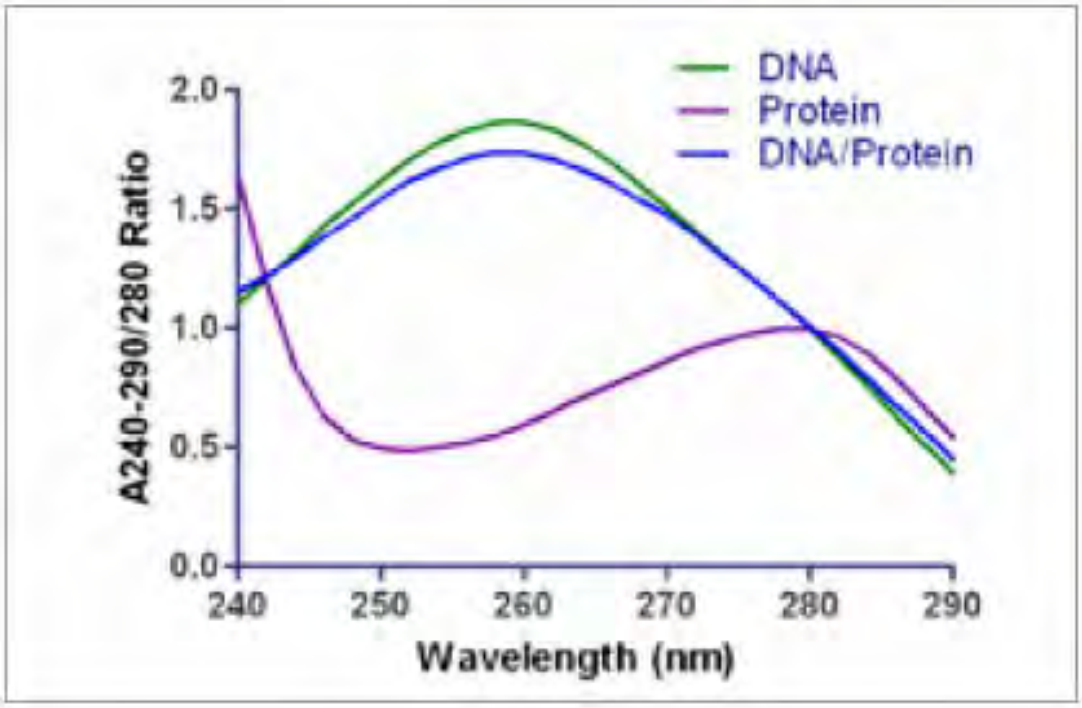
BioTek Get a Better Reaction.

Spectrophotometric Nucleic Acid Quantification

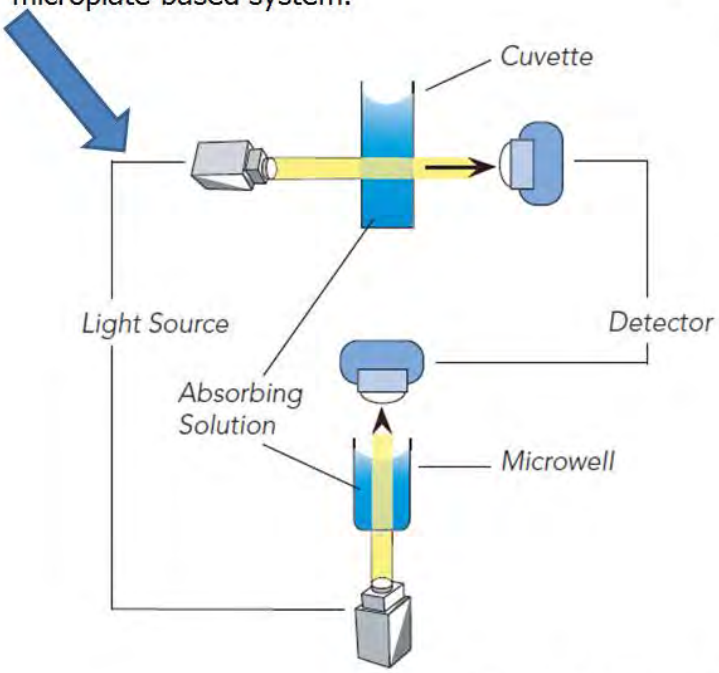
in Microplates and Micro-Volumes



Typical absorbance spectrum for DNA, RNA and protein, indicating the peak at about 260 nm for DNA and RNA and the peak at about 280 nm for protein.



Comparison of the fixed 1 cm path length of light in cuvette based system and the variable vertical light path of microplate based system.



BioTek's patented BioCell allows fixed 1 cm path length readings in most of BioTek's microplate spectrophotometers. Up to 8 BioCells can be measured at a time.

The Take3 plate from BioTek has up to 48 sample locations for 2 µL nucleic acid samples.

*Commonly accepted extinction coefficients at known concentration. * Based on a 1 cm path length.*

Nucleic Acid Type	Average Extinction Coefficient $(\mu g/mL)^{-1}$ cm^{-1}	Concentration (µg/mL) if OD=1*
Double-stranded DNA	0.020	50
Single-stranded DNA	0.027	37
Single-stranded RNA	0.025	40

Approximate purity based on A260/A280 ratio. Note that specific nucleic acids are not clearly distinguished from each other using this ratio.

Nucleic Acid Type	Approximate A_{260}/A_{280} Ratio
Pure DNA	2.0
Pure RNA	1.8
Pure Protein	0.57

LAB-CLASS

Optical absorption analysis methods based on the use of combined law light absorption of Bouguer-Lambert-Beer (Beer–Lambert–Bouguer law):

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These values can be used to quantify the characteristics of the substance and quantify it in a solution or in a mixture with other substances.

In connection with the division of the electromagnetic spectrum by wavelength into certain areas, is called Spectrophotometry in the infrared, visible, and ultraviolet regions.

Construction of a calibration graph to determine the protein concentration with biuret reagent

When an alkaline solution of copper is added to a protein solution, a purple color develops, the intensity of which is proportional to the protein concentration. The presence of peptide bonds in proteins determines the development of color. The

biuret reaction is characteristic not only of proteins but also of peptides (with at least two peptide bonds).

0.2, 0.4, 0.6 and 1.0 ml of albumin solution (10 mg / ml) are added to five tubes, respectively. Makeup to 1 ml with distilled water.

To 1 ml of the test-sample, 4 ml of biuret reagent are added (see below). The contents of the tubes are stirred and left for 30 min at room temperature. The color intensity is determined on a spectrophotometer at a wavelength in the range of 540-650 nm or on a photocolorimeter using appropriate light filters. Measurements are performed against a “blank” sample in which the same components are present, except for the protein solution.

The obtained data are plotted graphically, plotting on the ordinate axis the value of the optical density, and on the abscissa axis - the protein concentration corresponding to this value.

It should be noted that peptides, tris, sucrose, and bile pigments give color in the biuret reaction, and ammonium salts, tris, sucrose, and glycerol affect the color formed by proteins. Lipids and detergents cause turbidity of the solution.

Biuret reagent: to 250 ml of distilled water add 0.75 g of copper sulfate ($CuSO_4 \cdot 5H_2O$) and 3 g of sodium-potassium tartrate ($NaKC_4H_4O_6 \cdot 4H_2O$), then with vigorous stirring - 150 ml of 10% NaOH solution (free of sodium carbonate) and 1 g of KI. The total volume of the solution is adjusted to 1 liter with water. The reagent is stored in plastic containers or in glassware covered with paraffin inside.

Calibration graph (above)

Control questions, tasks and exercises for the section

«SPECTROPHOTOMETRIC AND PHOTOELECTROCOLORIMETRIC METHODS»

1. Where are spectrophotometric and photoelectrocolorimetric methods used?
2. Common and different peculiarities/characteristics of spectrophotometry and colorimetry.
3. Give examples of these methods used in the study of biochemical processes that take place in the animal organism
4. Give the scheme of the experiment "Quantitative determination of protein"
5. How do spectrophotometric measurement methods differ from photocolorimetric ones?
6. What is the theoretical basis of adsorption optical methods of analysis?

1.2. ***Topic*** CENTRIFUGATION

Sedimentation

In all dispersed systems with no ability to chaotic motion of particles, the latter due to the gravitational field will gradually subside until completely fall out as sediment.

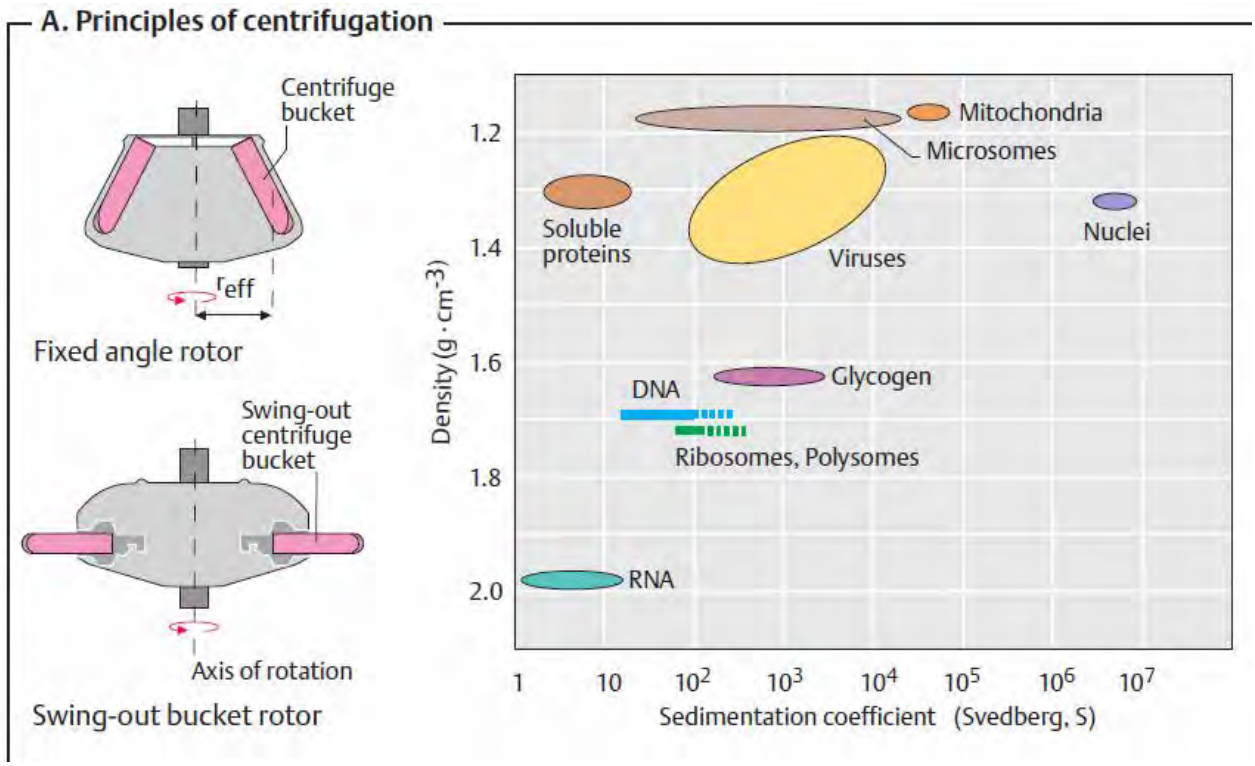
The process of sedimentation of the dispersed phase under gravity is called sedimentation and a system (where these processes are carried out) is referred to as kinetically unstable.

Centrifugation

There are **preparative** and **analytic** ***centrifugations.***

In biochemical investigation, **preparative centrifugation** is used for purification of biological material, subcellular compartments, some molecules (DNA, proteins, polysaccharides etc.) in the purpose to study their structure and biological activity.

Analytic centrifugation is used for separation of emulsions and thin-dispersed suspensions in the purpose to study sedimentation peculiarities macromolecules and other purified preparations of cellular organelles.



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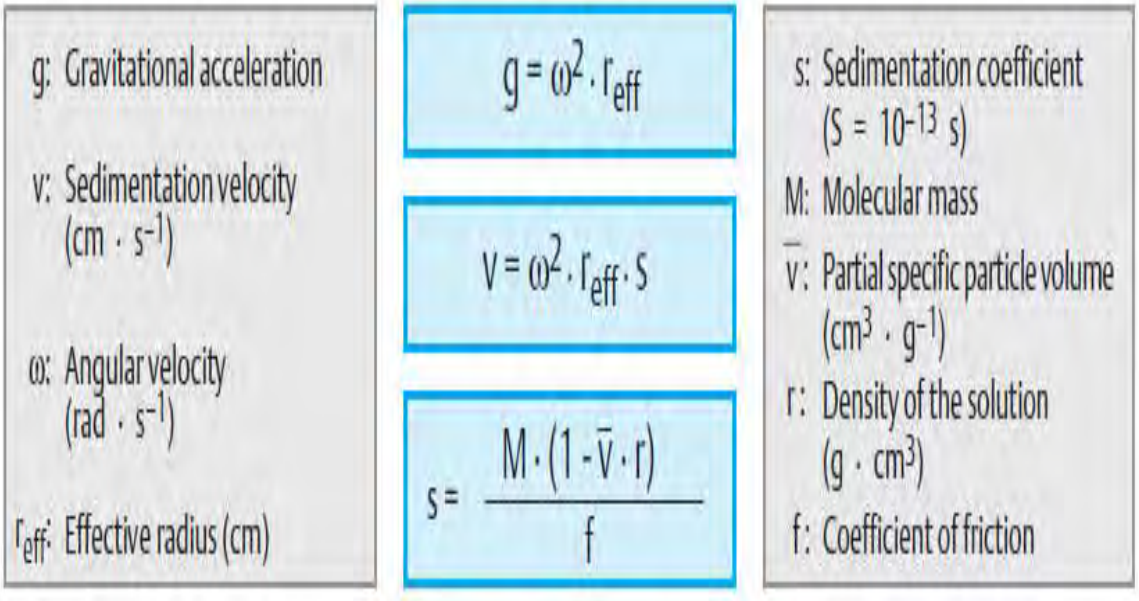
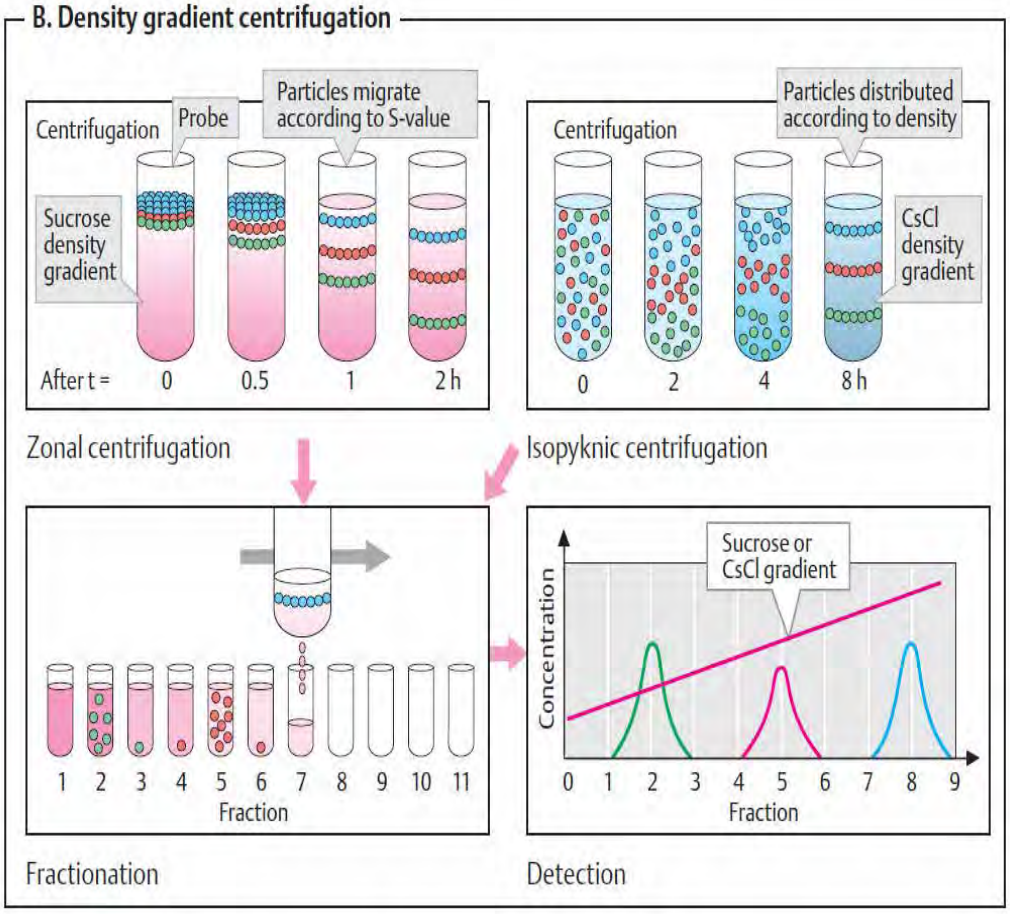
The velocity (v) of particle sedimentation during centrifugation depends on the angular velocity ω of the rotor, its effective radius (r_{eff}, the distance from the axis of rotation), and the particle's sedimentation properties. These properties are expressed as the **sedimentation coefficient** S (1 Svedberg, = 10^{-13} s). The sedimentation coefficient depends on the mass M of the particle, its shape (expressed as the coefficient of friction, f), and its density (expressed as the reciprocal density v–, "partial specific volume"). **g=9.81 m·s²**



Scheme of centrifuge

Density gradient centrifugation

Density gradient centrifugation is used to separate macromolecules that differ only slightly in size or density. Two techniques are commonly used. These techniques are zonal and isopyknic centrifugations.



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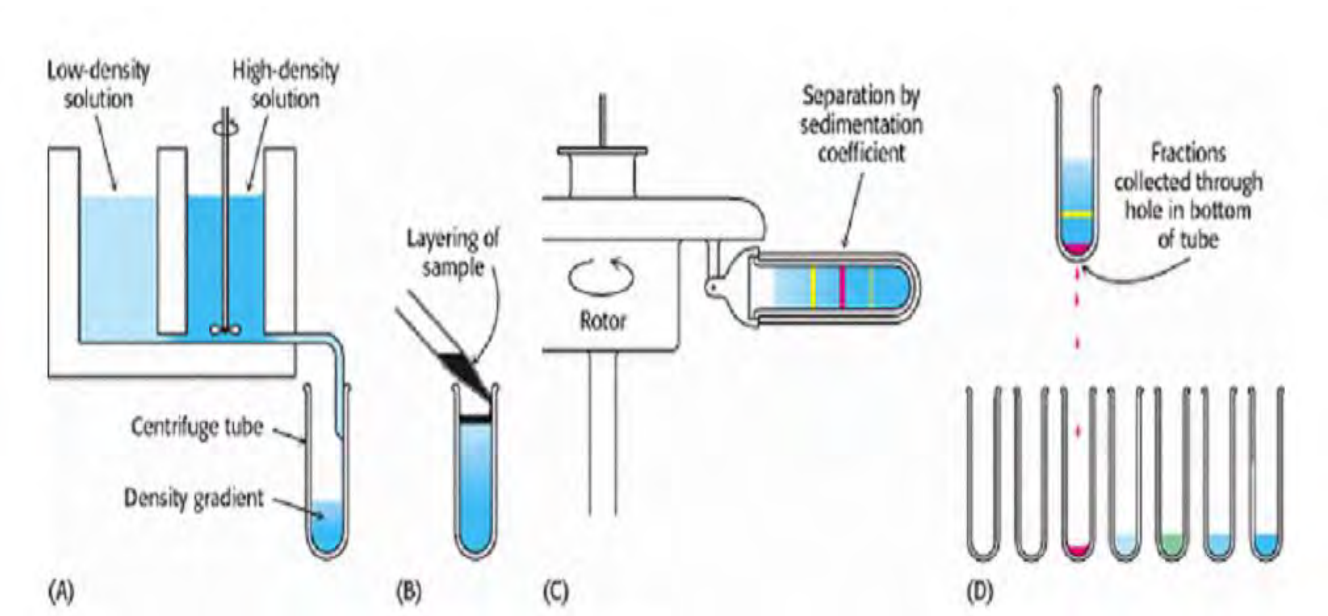
Zonal Centrifugation

Zonal Centrifugation. The steps are as follows: (A) form a density gradient, (B) layer the sample on top of the gradient, (C) place the tube in a swinging-bucket rotor and centrifuge it, and (D) collect the samples. [After D. Freifelder, Physical Biochemistry, 2d ed. (W. H. Freeman and Company, 1982), p. 397.]



Ultracentrifugation

Method of ultracentrifugation is separation of particles (possibly, less than - 10^{-7}м) with different mass under influence of the out-center force. The method used for fractionation the dispersed systems, for example, in medical and biological

studies in the purpose of separation of biopolymers, viruses, subcellular components.

Differential Centrifugation

Differential Centrifugation. Cells are disrupted in a homogenizer and the resulting mixture, called the homogenate, is centrifuged in a step-by-step fashion of increasing centrifugal force. The denser material will form a pellet at lower centrifugal force than will the less-dense material. The isolated fractions can be used for further purification.

Subcellular structures, which are isolated by fractionation tissue homogenates using method of differential centrifugation

The sedimentation coefficient S (1 Svedberg, = 10^{-13} s)	Fraction	Composition of fraction, marker enzyme
600 g	pellet - nucleus	Cellular nuclei, DNA- and RNA-polymerases
10 000 g	pellet - mitochondrial	Mitochondria, enzymes of TCAC*, biological oxidation and oxidative phosphorylation
12 - 16 000 g	pellet - lysosomal	Acidic hydrolase
100 000 g	pellet - microsomal	Vesicles of endoplasmic reticulum, ribosomes, enzymes of oxidative hydroxylation, protein biosynthesis,
100 000 g	Supernatant	Soluble enzymes of cytosol

*TCAC -Three-carboxylic acids cycle (Kreb's cycle)

The metabolic processes take place in the appropriate compartments:

In nucleus, there are DNA replications, RNA transcriptions.

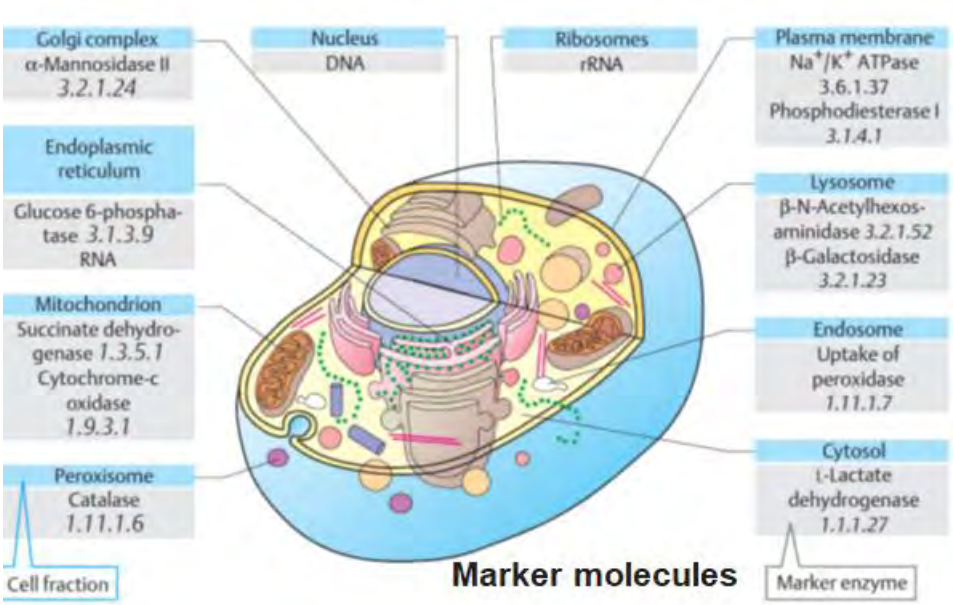
In mitochondria, there are reactions of TCAC (Krebs' cycle), β-oxidation of fatty acids, electrons transport and oxide phosphorylation.

In cytoplasm, there are reactions of glycolysis, gluconeogenesis, synthesis of fatty acids, metabolism of amino acids, etc.

On ribosomes, there are reactions of synthesis of protein (peptide bonds formation).

In lysosomes, there are reactions of hydrolysis of biopolymers (protein, nucleic acids. They are originated from the cell or there are exogenous ones which got in through endocytosis)

In the membranes of endoplasmic reticulum, cytochrome P-450 dependent processes of oxide hydroxylation of hydrophobic substrates (reaction of microsomal oxidation).



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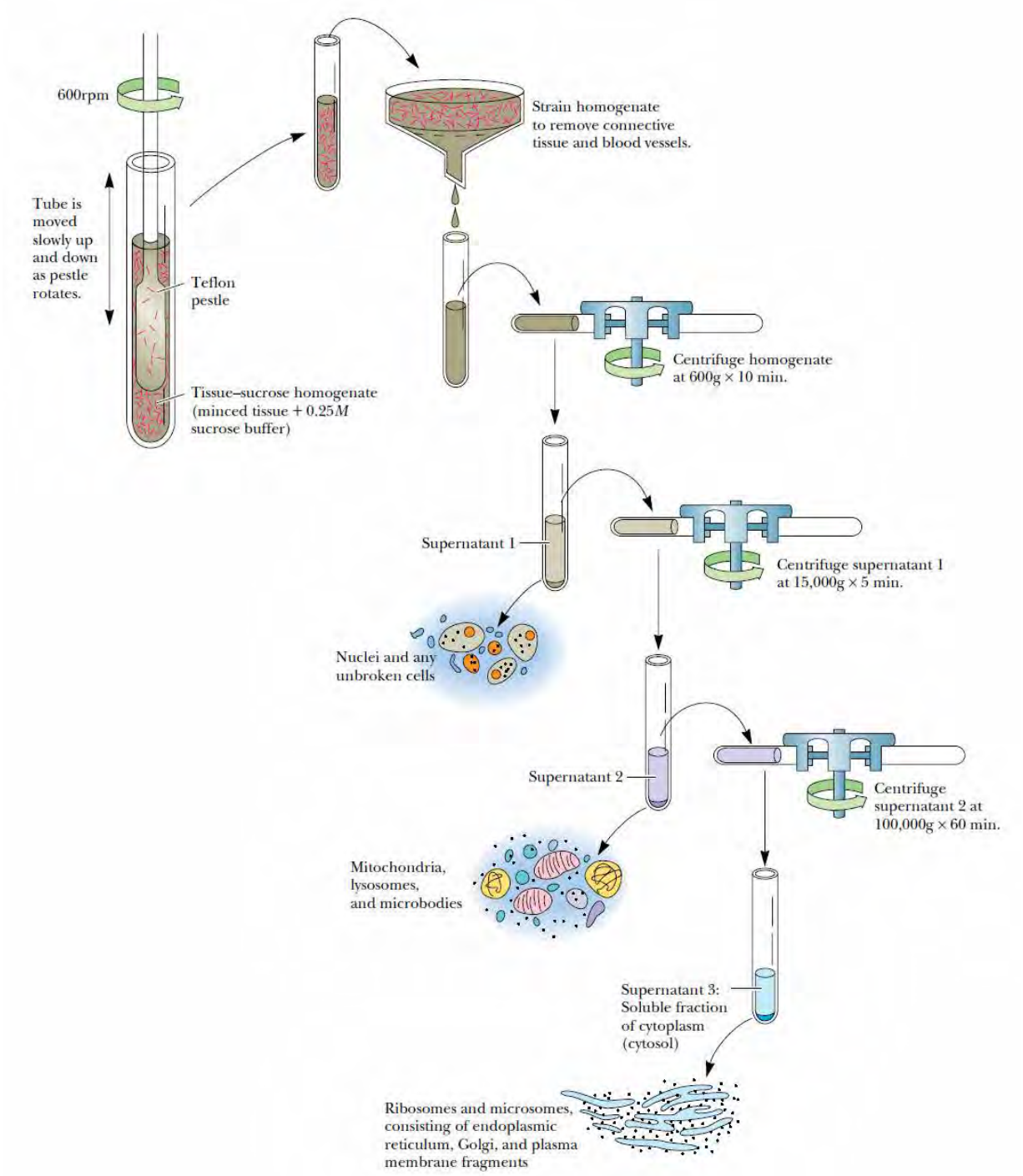
Nuclei already sediment at low accelerations that can be achieved with bench-top centrifuges. Decanting the residue (the "supernatant") and carefully suspending the sediment (or "pellet") in an isotonicmedium yields a fraction that is enriched with nuclei. However, this fraction may still contain other cellular components as contaminants- e. g., fragments of the cytoskeleton. Particles that are smaller and less dense than the nuclei can be obtained by step-bystep acceleration of the gravity on the supernatant left over from the first centrifugation. However, this requires very powerful centrifuges (high-speed centrifuges and ultracentrifuges). The sequence in which the fractions are obtained is: **mitochondria, membrane vesicles**, and **ribosomes**. Finally, the supernatant from the last centrifugation contains the **cytosol** with the cell's soluble components, in addition to the buffer. The isolation steps are carried out at low temperatures on principle (usually 0–5°C), to slow down degradation reactions - e. g., due to released enzymes and other influencing factors. The addition of thiols and chelating agents protects functional SH groups from oxidation. Isolated cell organelles quickly lose their biological activity despite these precautions. Nevertheless, it is possible by working carefully to isolate mitochondria that will still take up substrates for a few hours in the test tube and produce ATP via oxidative phosphorylation.

Marker molecules. During cell fractionation, it is very important to analyze the purity of the fractions obtained. Whether or not the intended organelle is present in a particular fraction, and whether or not the fraction contains other components, can be determined by analyzing characteristic **marker molecules.** These are molecules that occur exclusively or predominantly in one type of organelle. For example, the activity of organelle-specific enzymes **(marker enzymes)** is often assessed. The distribution of marker enzymes in the cell reflects the compartmentation of the processes they catalyze. These reactions are discussed in greater detail here under the specific organelles.



Fractionation of a cell extract by differential centrifugation. It is possible to separate organelles and subcellular particles in a centrifuge because their inherent size and

density differences give them different rates of sedimentation in an applied centrifugal field. Nuclei are pelleted in relatively weak centrifugal fields, mitochondria in somewhat stronger fields, whereas very strong centrifugal fields are necessary to pellet ribosomes and fragments of the endomembrane system.

Tabletop-Centrifuge in Lab

Tabletop-Centrifuge in Lab

High-speed and refrigerated centrifuge

Ultra-centrifuge with ultra-speed, refrigeration, and vacuum system

Ultrafiltration

Ultrafiltration (filtration under over-pressure through ultra-filter prepared of semi-permeable membrane) is used for pressing out low molecular admixtures and for system-concentrating.

For example, sizes of some viruses and bacteriophages were determined under separation of the dispersed phase into fractions depending on particles size with usage of membrane with definite size of pore.

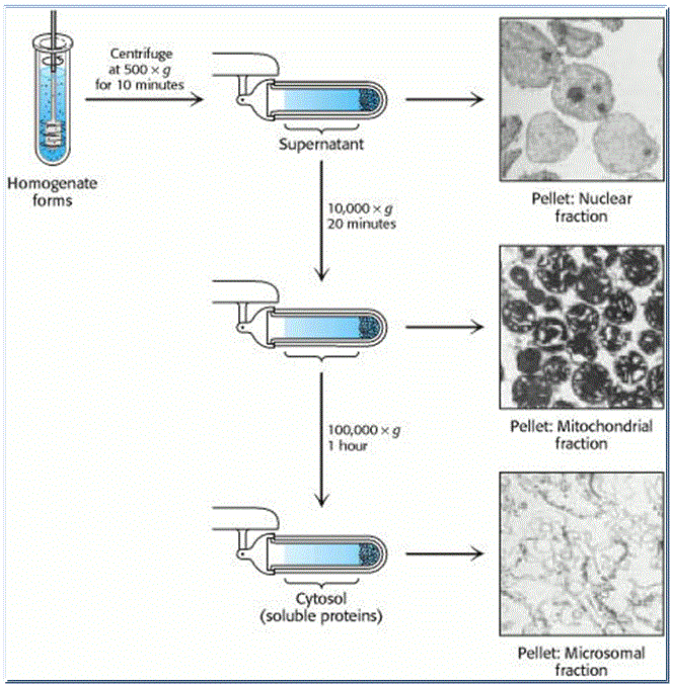
LAB-CLASS

There is *preparative and analytical centrifugation*.

In biochemical studies, *preparative centrifugation* is used to purify biological material: subcellular organelles, individual macromolecules (DNA, proteins, polysaccharides, etc.) in order to study their structure and biological activity.

Analytical centrifugation is used to separate emulsions and fine suspensions in the study of sedimentation properties of macromolecules and other purified preparations of cellular organelles.

1. Preparation of cell fractions by centrifugation (model experiment)



Centrifugation scheme (by *Berg J.M., Tymoczko J.L., Stryer L.* Biochemistry. – New York: W H Freeman; 2002. 1515 p.)

After analyzing the scheme above, try to make a short action plan for the centrifugation protocol (how many times to centrifuge and on which centrifuges) in the case of (1) DNA testing, (2) mitochondrial enzymes, and (3) macromolecular compounds present in microsomes.

2. Preparation of blood plasma

The principle of the method. The method is based on the distribution of blood into fractions by centrifugation.

Equipment and reagents. Centrifuge. Clean dry centrifuge tubes. Thermostat. Thin elastic wire. Blood. Heparin, a synthetic drug (1%). EDTA solution (Trilon B), 10%). Sodium citrate. Sodium oxalate.

Progress of experiment. The main prerequisite for obtaining blood plasma is to prevent its coagulation. For this purpose, an anticoagulant is pre-introduced into

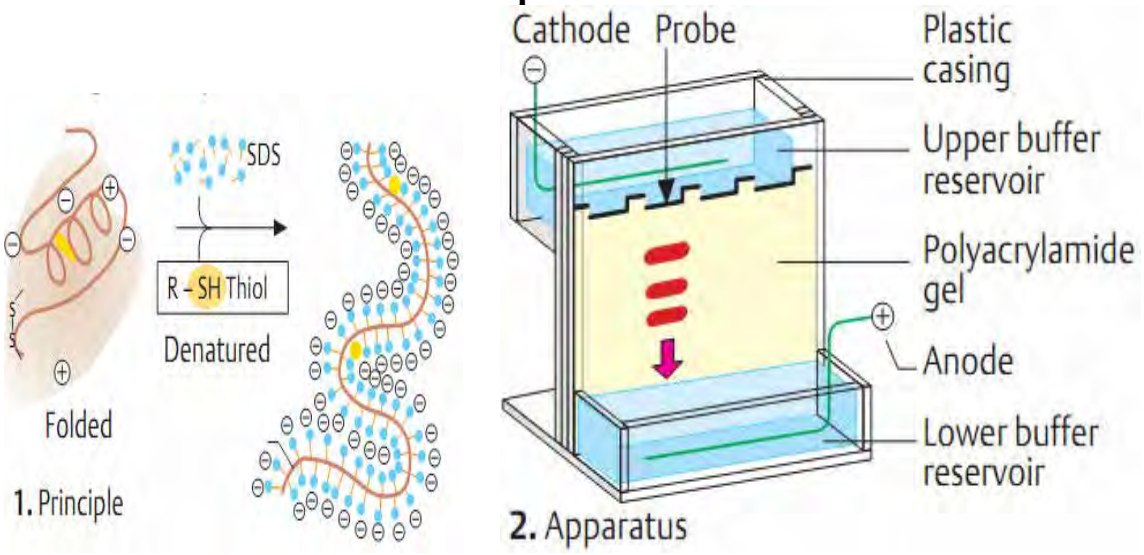
the blood plasma test tube. The following amounts of anticoagulants are taken for 5-20 ml of blood: 1-2 drops (0.05-0.1 ml) of heparin solution (synthetic drug), (1%), 3-4 drops of EDTA solution (Trilon B, 10%), 15 -20mg, sodium citrate or 15-20mg sodium oxalate. Blood samples with anticoagulant are centrifuged for 20-30 minutes at 2000-3000 rpm. The clear yellow-straw liquid at the top of the tube is blood plasma. Blood plasma is stored and used in the same way as serum. All stages of obtaining blood plasma are carried out at temperatures below 10° C, which contributes to the sustainability of most of its biochemical parameters.

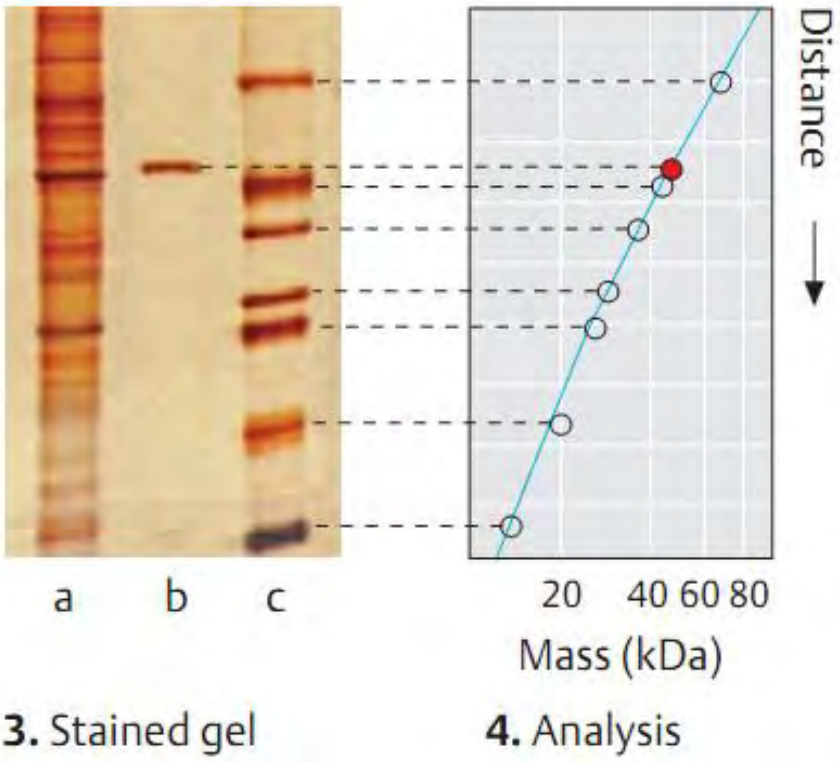
Control questions, tasks and exercises for the section
«CENTRIFUGATION»

1. What is the purpose of preparative centrifugation?
2. What is the purpose of analytical centrifugation?
3. What centrifuges are used for analytical centrifugation?
4. Indicate which steps of centrifugation should be performed to obtain the microsomal fraction of cells.
5. What is the general difference between a conventional centrifuge and an ultracentrifuge?
6. When is centrifugation used in veterinary medicine?

2.2. ***Topic*** FUNDAMENTALS OF ELECTROPHORETIC SEPARATION OF HIGH MOLECULAR COMPOUNDS

Electrophoresis





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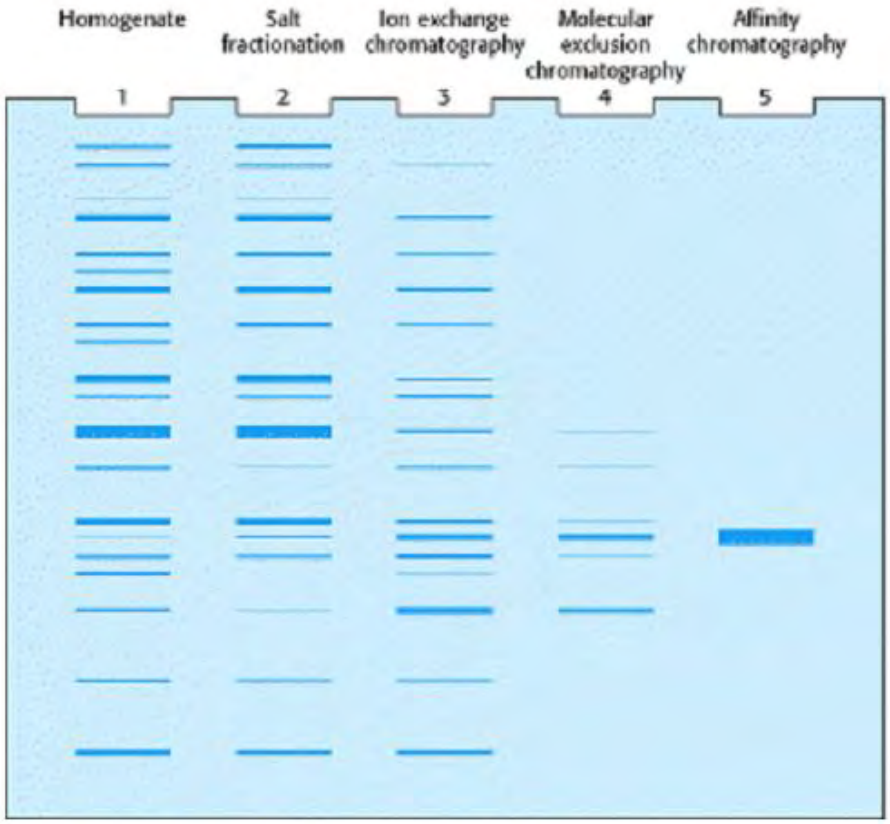

Electrophoretic Analysis of a Protein Purification. The purification scheme in Table 4.1 was analyzed by SDS-PAGE. Each lane contained 50mg of sample. The

effectiveness of the purification can be seen as the band for the protein of interest becomes more prominent relative to other bands.

Quantification of a purification protocol for a fictitious protein

Step	Total protein (mg)	Total activity (units)	Specific activity, (units mg^{-1}	Yield (%)	Purification level
Homogenization	15,000	150,000	10	100	1
Salt fractionation	4,600	138,000	30	92	3
Ion-exchange chromatography	1,278	115,500	90	77	9
Molecular exclusion chromatography	68.8	75,000	1,100	50	110
Affinity chromatography	1.75	52,500	30,000	35	3,000

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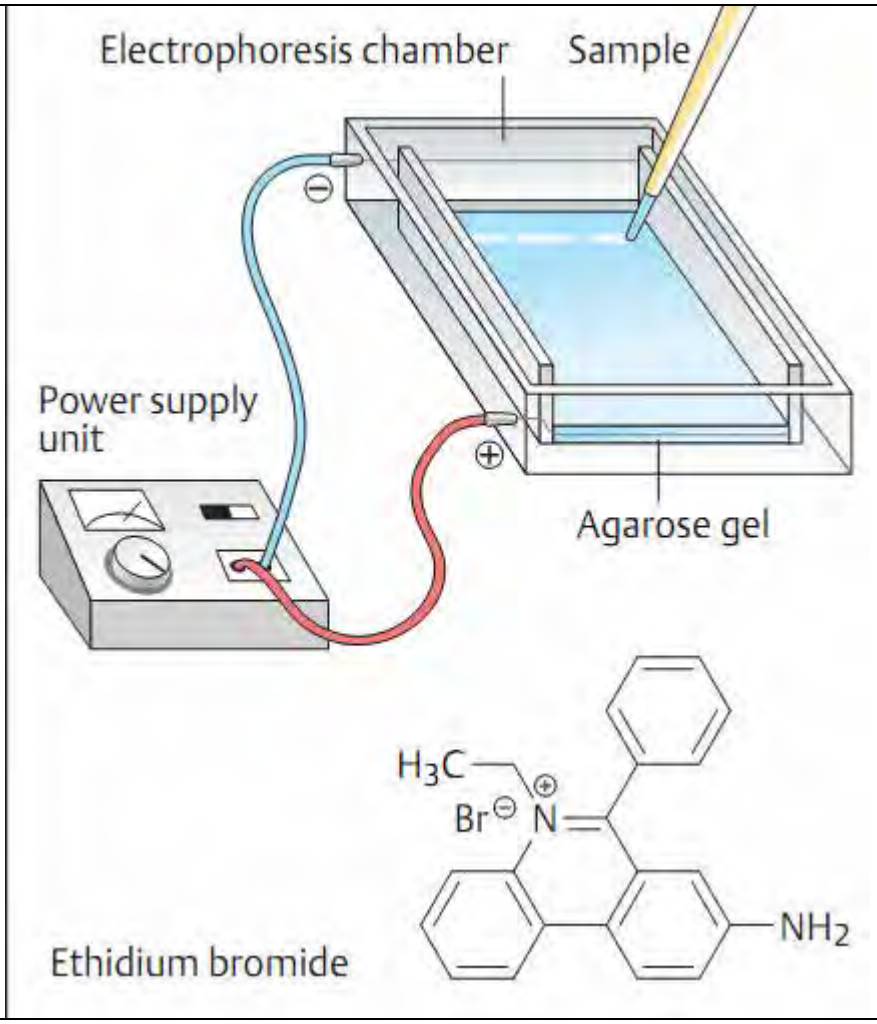
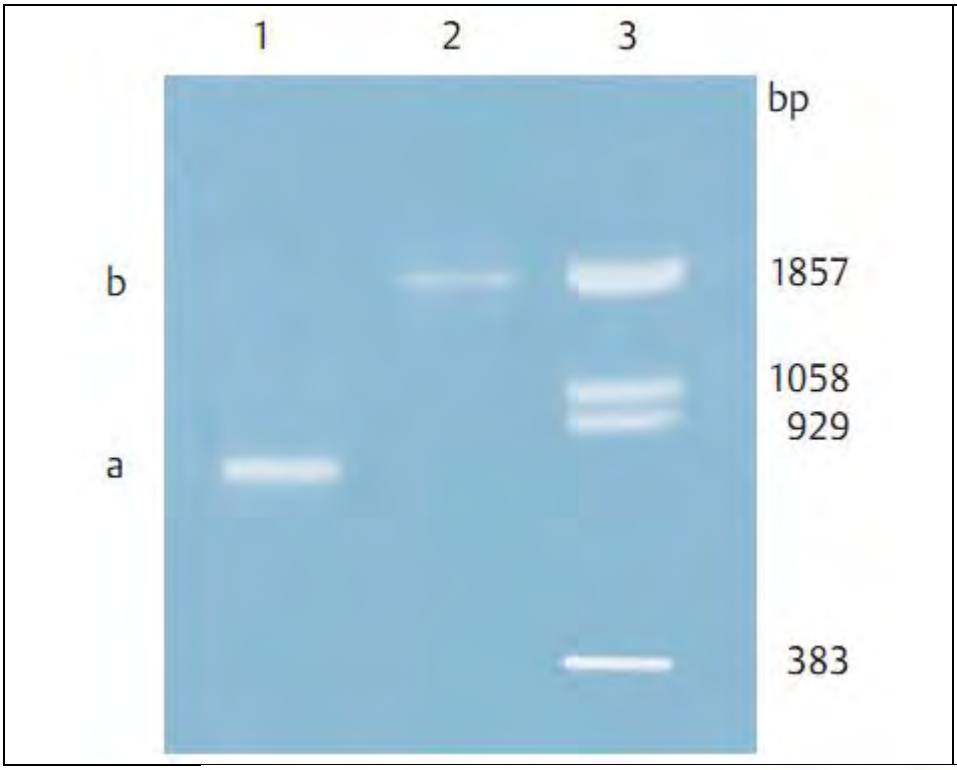
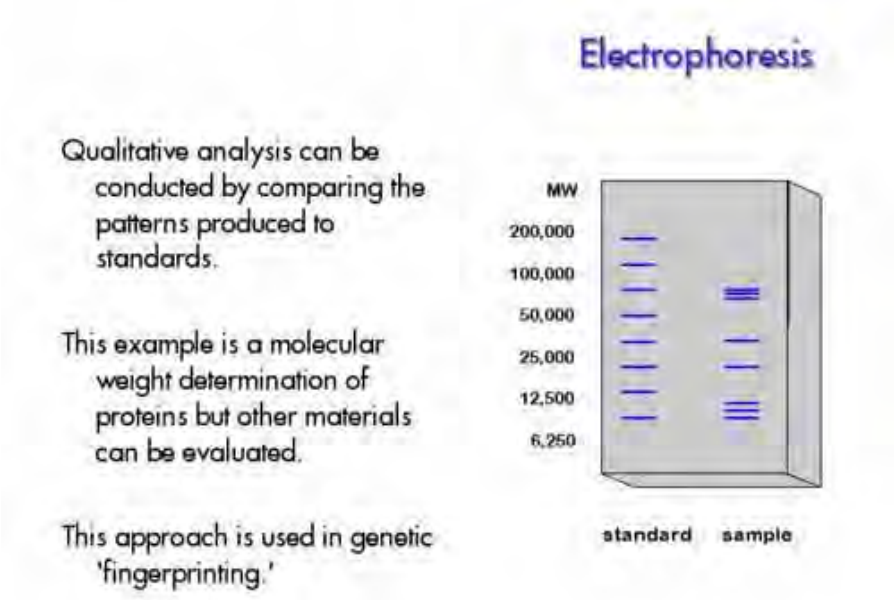


Scheme of electrophoresis in agarose-gel



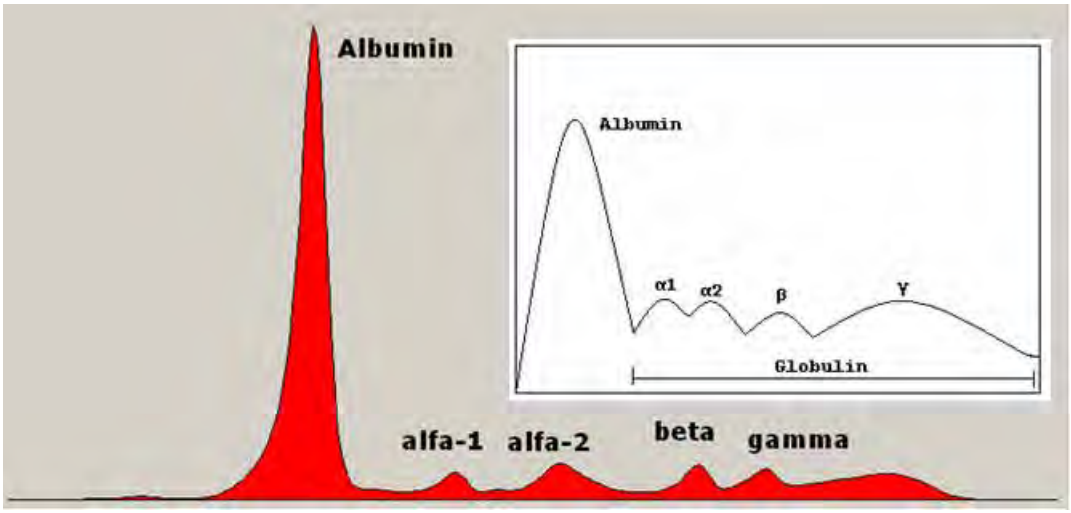
Staining agarose gel by Ethidium bromide for detecting DNA fragments (a, b) in UV-light (bp – base pare). Gel-tracks: 1 – DNA fragment a; 2 – DNA fragment b; 3 – DNA markers (lengh 300-2000 base pares.)



Molecules, which have different electric charges, spatial configuration, molecular mass (size), are separated by **electrophoretic method in the liquids** using some gels or other carrier of separation.

Serum protein electrophoresis

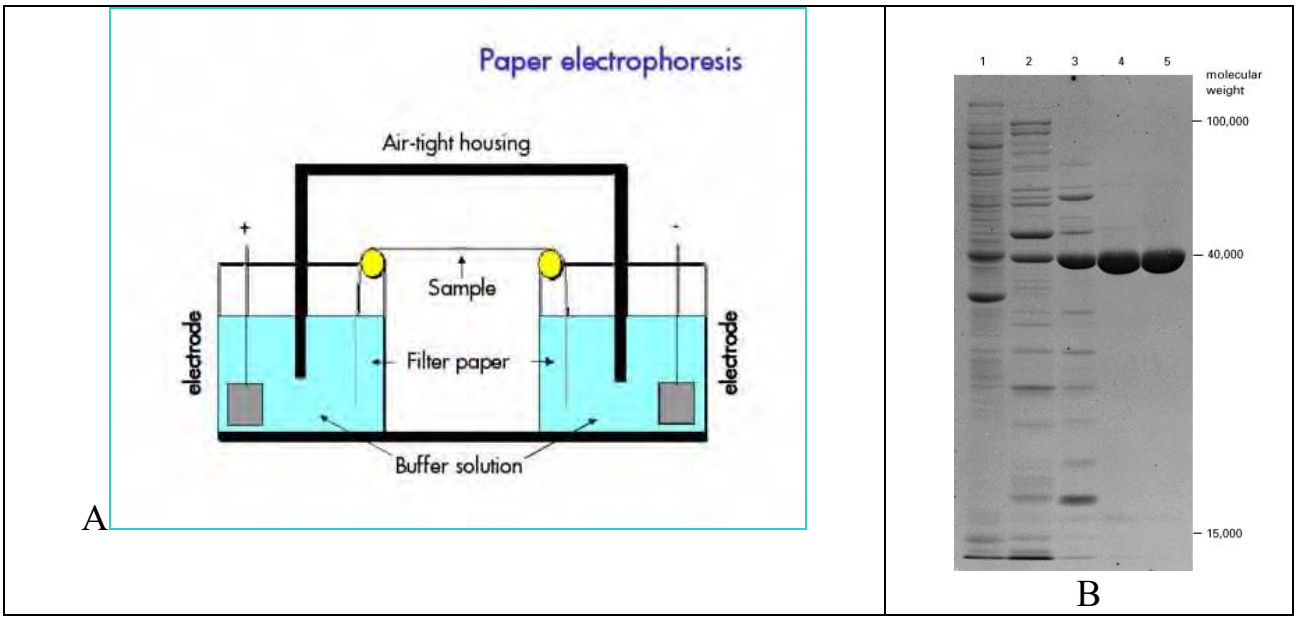
Serum protein electrophoresis (SPEP) is a laboratory test that examines specific proteins in the blood called globulins. Blood must first be collected, usually into an airtight vial or syringe.



Electrophoresis is a laboratory technique where the blood serum (the fluid portion of the blood after the blood has clotted) is placed on special paper treated with agarose gel and exposed to an electric current to separate the serum protein components into five classifications by size and electrical charge, those being serum albumin, alpha-1 globulins, alpha-2 globulins, beta globulins, and gamma globulins.

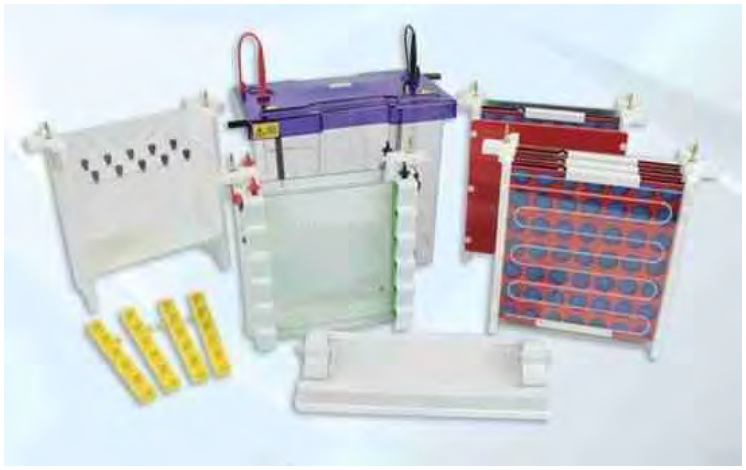
The albumin fraction also includes *transthyretin* (prealbumin), which together with other proteins transports the hormone thyroxine and its metabolites.

Schematic representation of a protein electrophoresis gel

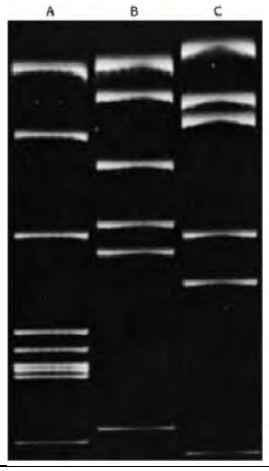


A) Scheme of paper electrophoresis
B) Electrophoregramme

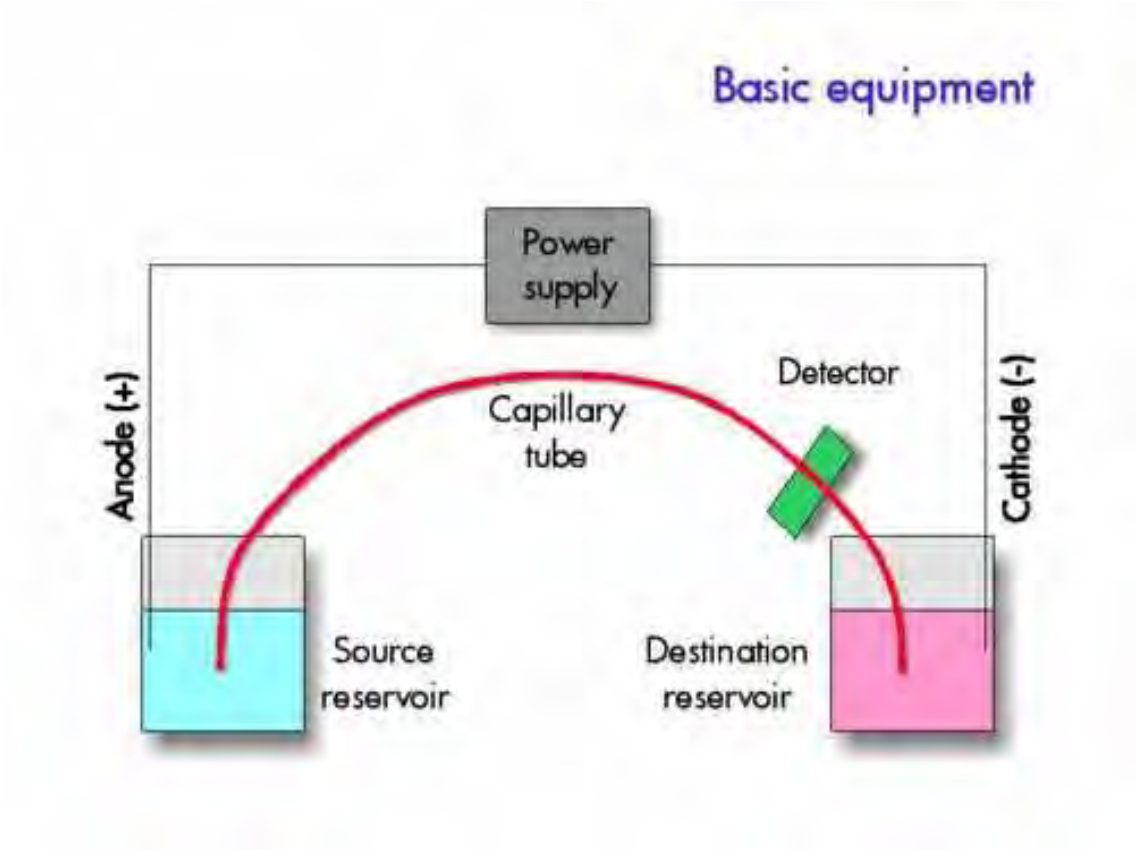
C



D



C) Set of vertical gel-electrophoresis
D) Gel Electrophoresis Pattern of a Restriction Digest. This gel shows the fragments produced by cleaving SV40 DNA with each of three restriction enzymes. These fragments were made fluorescent by staining the gel with ethidium bromide



Capillary tubes

Capillary tube

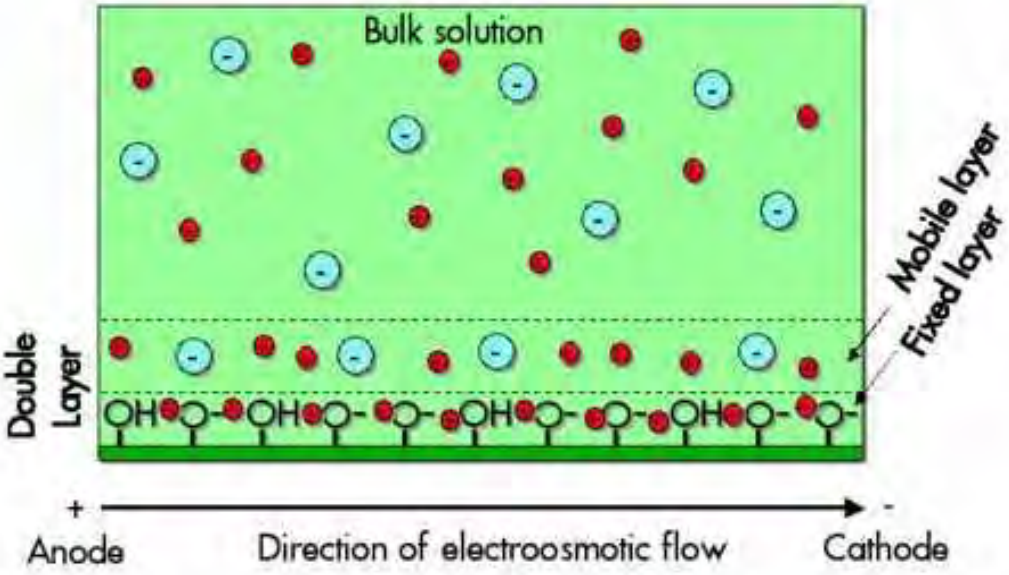
ID typically 25-75 μm.

Length varies based on application but is normally in the 20-50 cm range.

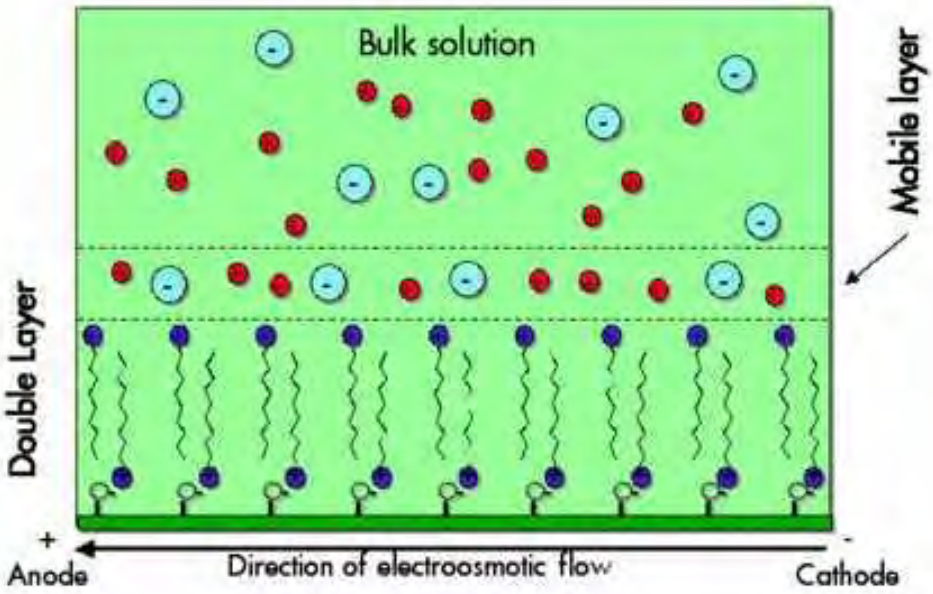
The small bore and thickness of the silica are important. When a current is applied, this leads to Joule heating.

Using a small ID and having a thick wall reduces this problem.

Double layer



Capillary zone electrophoresis

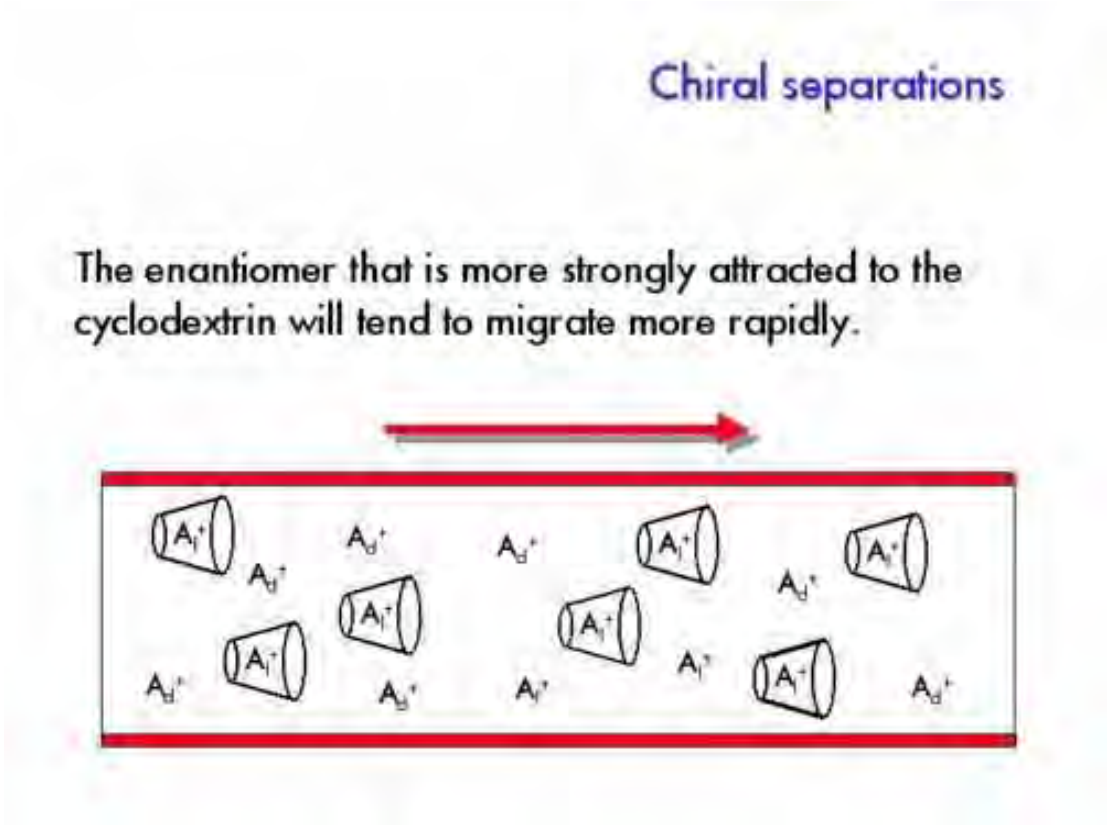


Chiral separations

It is possible to separate enantiomers using CE.

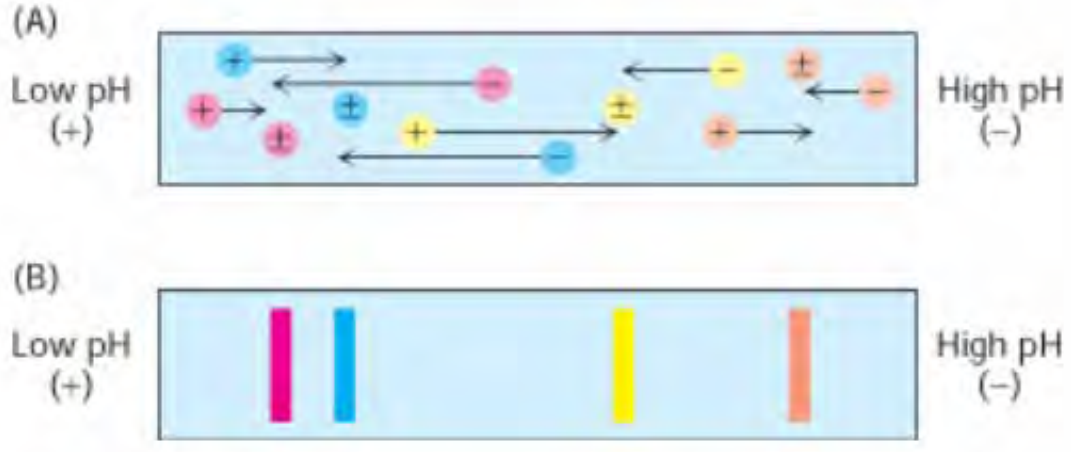
The most common approach is to add cyclodextrins into the buffer solution (a 1-100 mM).

Common cyclodextrins include: β-cyclodextrin, chemically modified cyclodextrans like dimethylated or hydroxypropylated forms.



Isoelectric Focusing

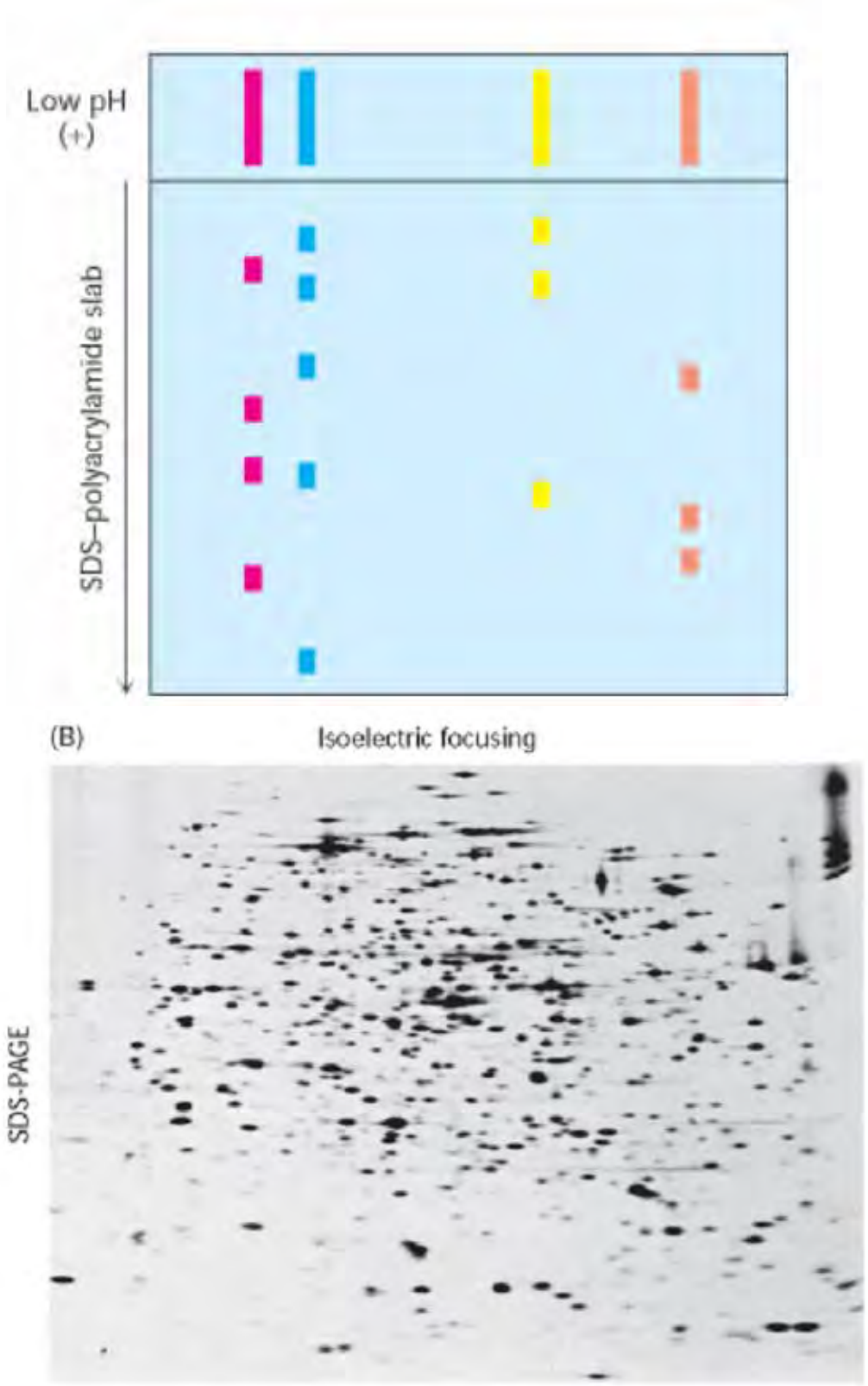
The **Principle of Isoelectric Focusing**. A pH gradient is established in a gel before loading the sample. (A) The sample is loaded, and voltage is applied. The proteins will migrate to their isoelectric pH, the location at which they have no net charge. (B) The proteins form bands that can be excised and used for further experimentation

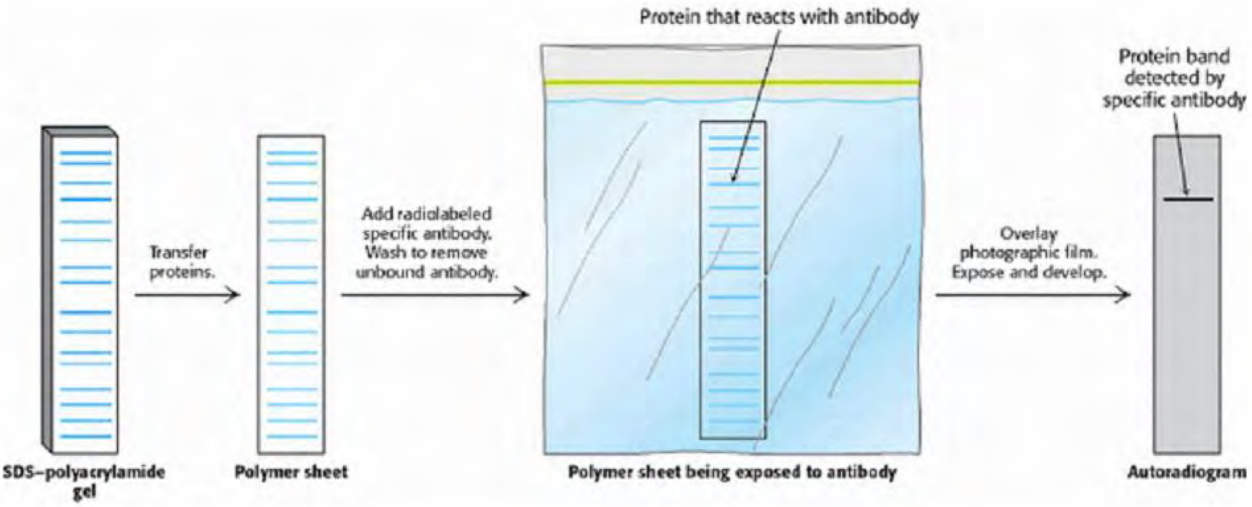


Two-Dimensional Gel Electrophoresis

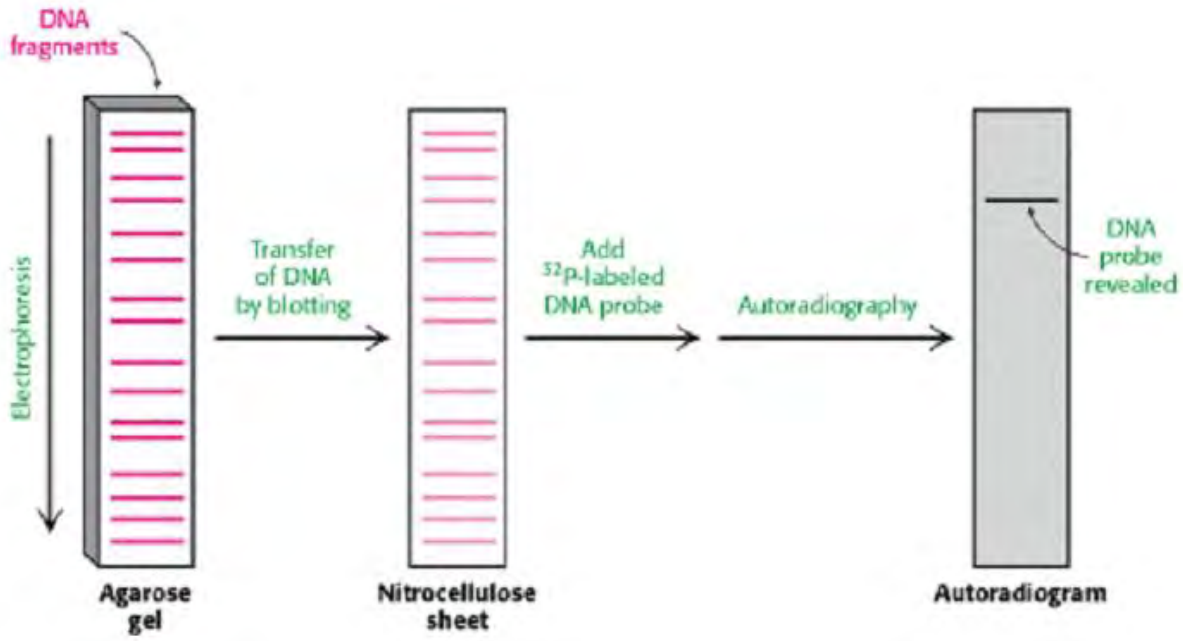
Two-Dimensional Gel Electrophoresis. (A) A protein sample is initially fractionated in one dimension by isoelectric focusing as described. The isoelectric focusing gel is then attached to an SDS-polyacrylamide gel, and electrophoresis is

performed in the second dimension, perpendicular to the original separation. Proteins with the same pI are now separated based on mass. (B) Proteins from E. coli were separated by two-dimensional gel electrophoresis, resolving more than a thousand different proteins. The proteins were first separated according to their isoelectric pH in the horizontal direction and then by their apparent mass in the vertical direction



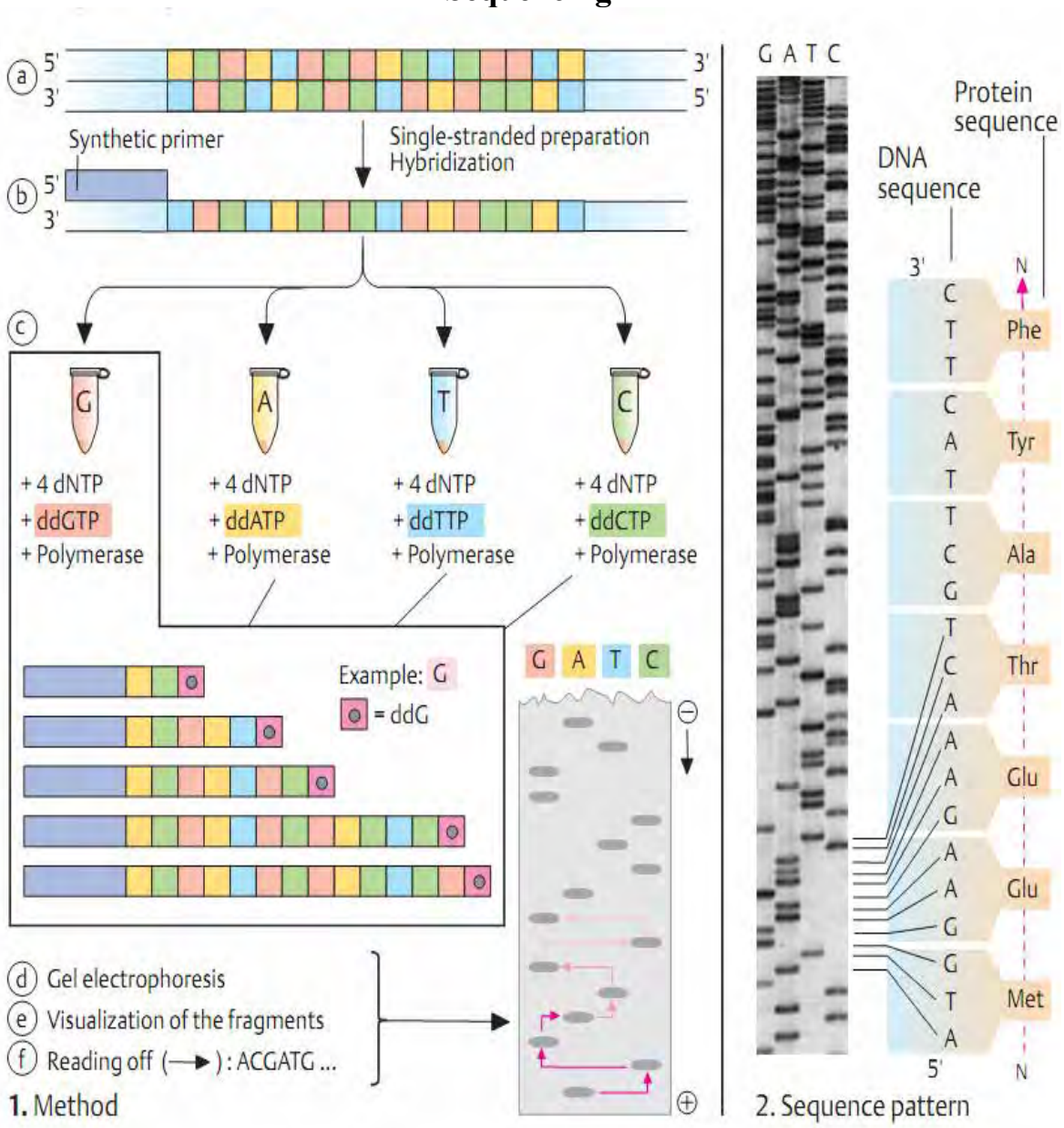


Western Blotting. Proteins on an SDS-polyacrylamide gel are transferred to a polymer sheet and stained with radioactive antibody. A band corresponding to the protein to which the antibody binds appears in the autoradiogram

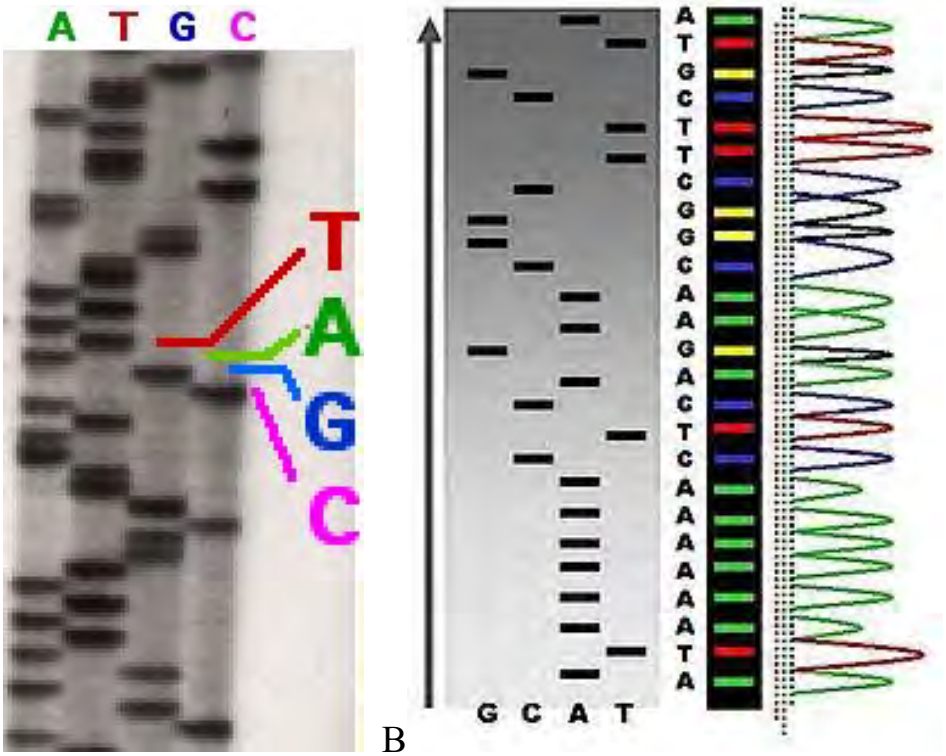


Southern Blotting. A DNA fragment containing a specific sequence can be identified by separating a mixture of fragments by electrophoresis, transferring them to nitrocellulose, and hybridizing with a ^{32}P-labeled probe complementary to the sequence. The fragment containing the sequence is then visualized by autoradiography.

Sequencing



Maxam-Gilbert sequencing requires radioactive labeling at one 5' end of the DNA and purification of the DNA fragment to be sequenced. Chemical treatment then generates breaks at a small proportion of one or two of the four nucleotide bases in each of four reactions (G, A+G, C, C+T). The concentration of the modifying chemicals is controlled to introduce on average one modification per DNA molecule. Thus, a series of labeled fragments is generated, from the radiolabeled end to the first "cut" site in each molecule. The fragments in the four reactions are electrophoresed side by side in denaturing acrylamide gels for size separation. To visualize the fragments, the gel is exposed to X-ray film for autoradiography, yielding a series of dark bands each corresponding to a radiolabeled DNA fragment, from which the sequence may be inferred.



A B

A) Part of a radioactively labelled sequencing gel
B) An example of the results of automated chain-termination DNA sequencing

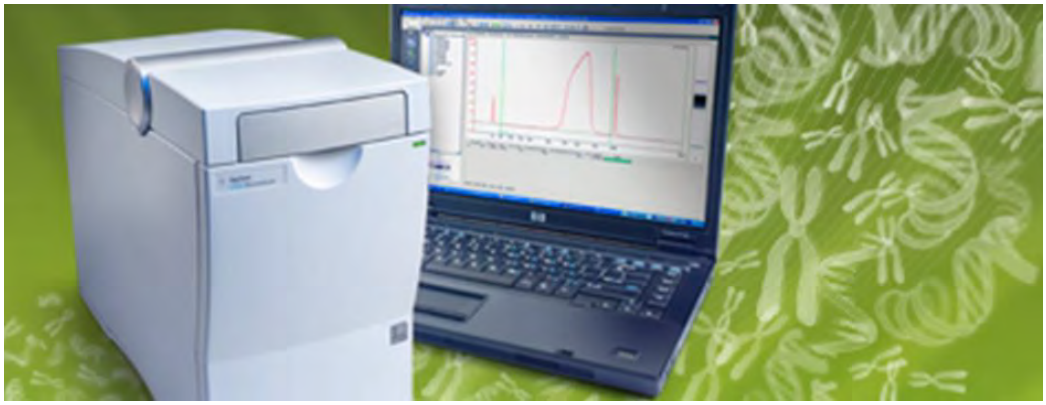
«NanoPlus» is used photon-corelation spectroscopy and method of electrophoretic light scattering techniques for determination of size of particles in range from 0.6 nm to10 μm.

Automated electrophoresis

Agilent 2100 Bioanalyzer System

Electrophoresis is used to separate, quantify, enrich and purify biomolecules which differ in their electrical charge or polarity. Agilent offers innovating electrophoretic separation solutions. The Agilent 2100 Bioanalyzer system analyzes

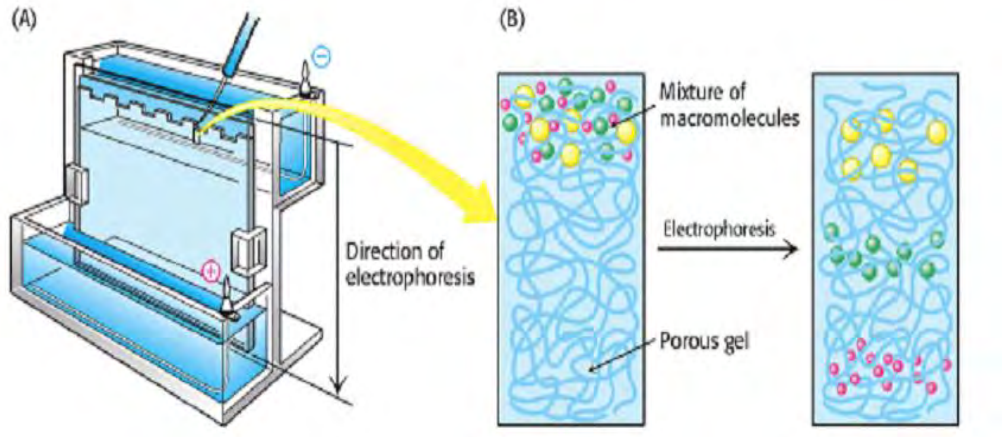
biomolecules or cells in microfluidic networks of channels and wells etched into glass chips.



The Agilent 2200 TapeStation system exhibits 96 well plate compatibility while performing rapid automated DNA, RNA, and protein electrophoresis. The Agilent 3100 OFFGEL Fractionator resolves proteins or peptides by isoelectric point with liquid-phase recovery to achieve high resolution pI-based fractionation. The Agilent 7100 Capillary Electrophoresis system is the most sensitive CE system on the market and seamlessly integrates with Agilent's MS systems.

LAB-CLASS

Electrophoresis is the movement of colloidal particles caused by an external electric field.



Scheme of electrophoresis in SDS-PAAG (here and further adapted for *Berg J.M., Tymoczko J.L., Stryer L.* Biochemistry. – New York: W H Freeman; 2002. 1515 p)

The electrophoretic method is used to separate in liquids using certain carriers of the molecule, which differ in the magnitude of electric charge, spatial configuration, molecular weight (size).

In biochemical studies, electrophoresis is used to separate different molecules in order to identify and quantify them, as well as to establish the molecular weight and changes in the composition of molecules when replacing charged groups with uncharged and vice versa.

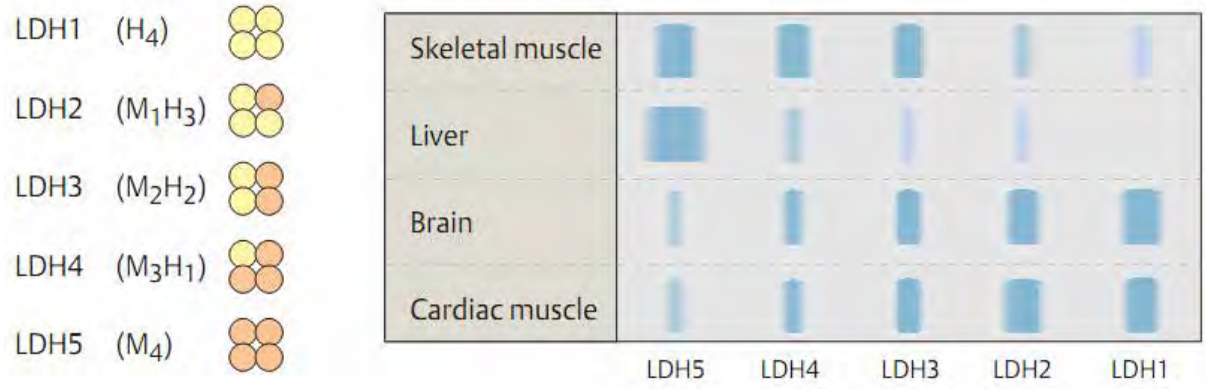
Zonal electrophoresis is widely used in biochemical studies due to its high resolution, high speed, the ability to separate various substances: proteins, nucleic acids, carbohydrates, lipids, inorganic compounds. The essence of the method is that the process of electrophoresis occurs in the pores of solid material, which are homogeneous. The material itself is mechanically and chemically stable, insoluble in a wide range of pH values, and has low adsorption and electroosmotic capacity.

Ordinary chromatographic paper, cellulose acetate, as well as starch, agarose and agar gels, etc. can be used for electrophoresis.

Polyacrylamide gels (SDS) are the most common in biochemical studies due to the following characteristics: 1) the size can be changed in a wide range; 2) they can be used with a variety of buffers; 3) they have very low adsorption and electroosmosis; 4) the distribution is fast and with wide separation.

Polyacrylamide gels are obtained by polymerization of acrylamide (CH_2 = CH-CO-NH_2) with N, N'-methylenediacrylamide [CH_2(NHCOOH = CH_2)$_2$] in the presence of a catalyst of 0.1 to 0.3% ammonium persulfate, 0.1 to 0 , 3% β-dimethylaminopropionitrile, etc. Polyacrylamide gels are prepared with a total acrylamide content of 3% (pores have a diameter of 0.5 nm) to 30% (pores 0.2 nm).

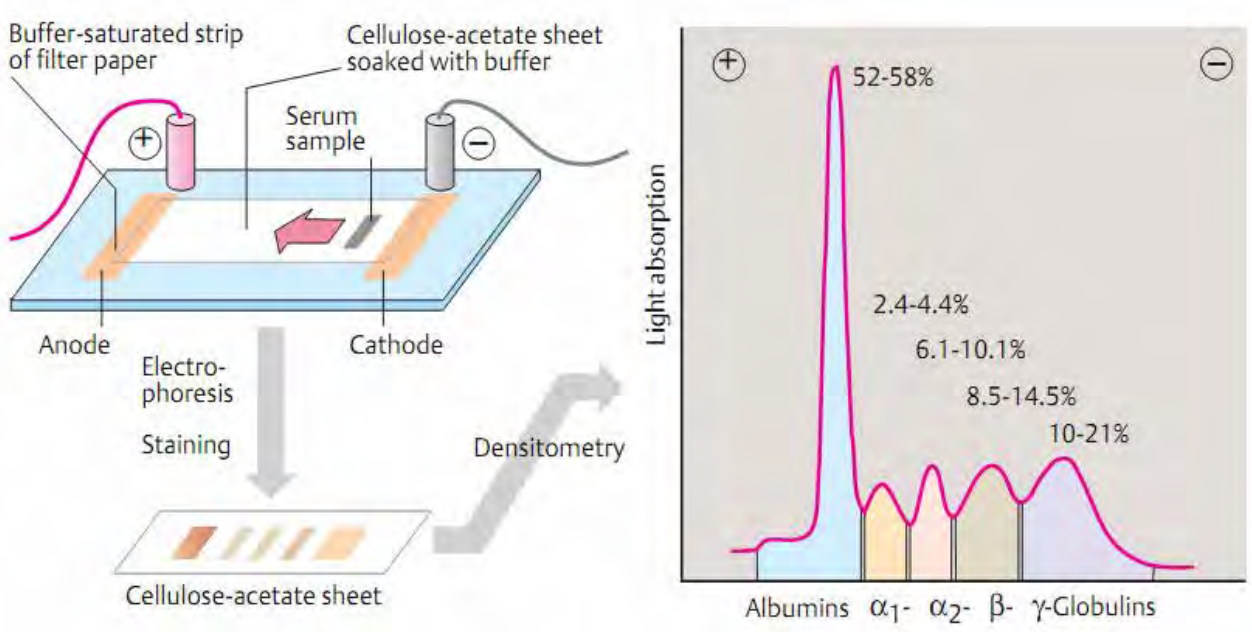
Electrophoresis in the SDS-PAAG is performed in vertical tubes or on plates.



Scheme 1. Electrophoresis of lactate dehydrogenase isozymes (LDH1, LDH2, LDH3, LDH4, LDH5). Skeletal muscle – скелетний м'яз, liver – печінка, brain – мозок, cardiac muscle – серцевий м'яз (Cited: *Кольман Я., Рем К.-Г.* Наглядная биохимия. 3-е изд.: Пер. с нем. М.: Мир, 2009. – 469 с)

1. It should be concluded about the conditions of electrophoretic separation of lactate dehydrogenase isozymes (LDH, scheme 1): which gel (PAAG or agarose, vertical or horizontal, tubules or plate), as well as the predominance of certain isozymes in skeletal and cardiac muscles, liver and brain.

Determination of serum protein fractions by electrophoresis (Cited: *Кольман Я., Рем К.-Г.* Наглядная биохимия. 3-е изд.: Пер. с нем. М.: Мир, 2009. 469 с**)**



Try to describe briefly the process of electrophoretic separation of serum protein fractions (shown in the diagram above) and its results.

Serum protein fractions determination with the turbidimetric method

Since phosphate solutions of a certain concentration precipitate with the formation of a very fine suspension of different serum protein fractions, the degree of turbidity of the solutions (measured photo electro colorimetrically) can determine the protein concentration in the test material.

Progress of experiment. In a tripod, it is necessary to put seven test tubes (V = 10 ml), marking them № 0, 1, 2, 3, 4, 5, and 6. In test tubes: № 0 pour 10 ml of water, №№ 1, 2, 3, 4, and 5 - 5 ml of respectively diluted solutions (I, II, III, IV, and V) of phosphate buffer (see below). 0.5 ml of serum, 0.75 ml of distilled water, and 3.75 ml of basic phosphate buffer solution are added to test tube № 6, stirring the contents by gentle inversion 5-6 times and preventing the formation of air bubbles.

In test tubes: №№ 1, 2, 3, 4, and 5 transfer 0.5 ml of the resulting mixture in № 6, and in № 0 - 1 ml. The contents of each tube were carefully mixed and after 15 minutes the extinction was measured at 630 nm in 10 mm cuvettes. The solution № 0 is a control sample, which exposes the zero point of the device.

It should be noted that the contents of the tubes should be carefully mixed again before the determination.

Calculation:

1) $D_{albumin}=D_1 - D_2$;
2) $D_{\alpha 1} =D_2 - D_3$;
3) $D_{\alpha 2} =D_3 - D_4$;
4) $D_{\beta} =D_4 - D_5$;
5) $D_{\gamma} =D_5$.

Calculate the content of each protein fraction in relative and absolute percent.

Example:

D_1= 78; D_2=35; D_3=30; D_4 =24 i D_5 =13.
From here $D_{albumin}$=78-35=43, $D_{\alpha 1}$ =35-30=5, $D_{\alpha 2}$ =30-24=6, D_{β}=24-13=11, D_{γ}=13.
$\sum D_{protein\ fractions}$=78 take for 100%.
Determine the% content of each protein fraction.
Albumin: x=(100·43)/78=55,1%
α1: x=(100·5)/78=6,4%
α2: x=(100·6)/78=14,1%
β: x=(100·11)/78=7,7%
γ: x=(100·13)/78=16,7%. The obtained values are characteristic of the norm, which is the same in the case of determining the fractions of serum proteins by electrophoresis.

Basic phosphate buffer (3,347 M, pH 6,5): Pour 400 ml of distilled water into a beaker in which 33.5 g of sodium hydroxide, 226.8 g of potassium dihydrogen phosphate (KH_2PO_4) are dissolved, shaking until completely dissolved. The solution is cooled to room temperature and made up to the mark in a volumetric flask (V = 500 ml) or to a mass of 667.5 g.

Working solutions of phosphate buffer:

Solution I (3.084 M). Transfer 92.51 ml (123.5 g) of basic phosphate buffer to a 100 ml flask and add to the mark with distilled water.

Solution II (2,496 M). Transfer 74.91 ml (100 g) of basic phosphate buffer to a 100 ml flask and add to the mark with distilled water.

Solution III (2.359 M). Transfer 59.18 ml (94.5 g) of basic phosphate buffer to a 100 ml flask and add to the mark with distilled water.

Solution IV (1.959 M). Transfer 48.68 ml (78.5 g) of basic phosphate buffer to a 100 ml flask and add to the mark with distilled water.

Solution V (1.622 M). Transfer 48.45 ml (65 g) of basic phosphate buffer to a 100 ml flask and add to the mark with distilled water.

Control questions, tasks and exercises for the section «FUNDAMENTALS OF ELECTROPHORETIC SEPARATION OF HIGH MOLECULAR COMPOUNDS**»**

1. How are molecules separated by electrophoresis?
2. What factors affect the mobility of molecules during electrophoresis?
3. Where is the zonal electrophoresis method used?
4. What are the differences between vertical and horizontal electrophoresis? For what purpose are they used?
5. Is it possible to use electrophoresis to find out the molecular weight of a test compound?
6. Is it possible to decipher the sequence of a DNA or RNA fragment by electrophoresis?

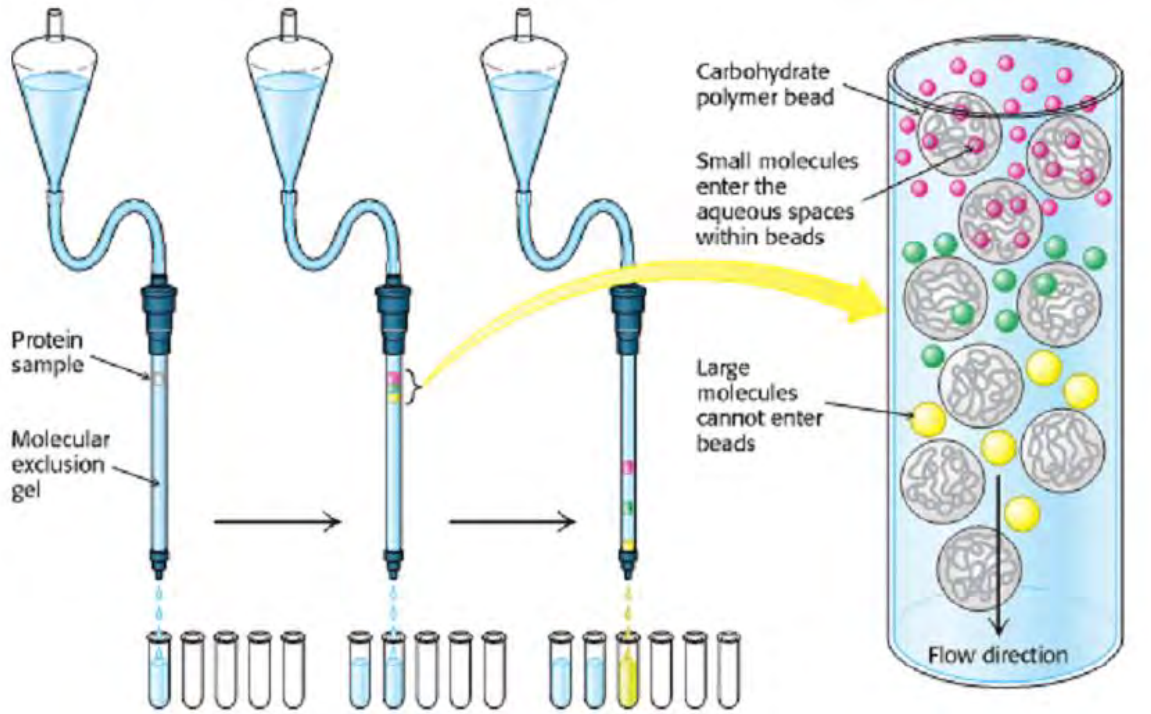
1.2.4. ***Topic*** CHROMATOGRAPHIC METHODS

Paper chromatography is one of the modifications of the chromatographic method proposed by the Ukrainian scientist M. Tsvet in 1903.

Thin layer chromatography is used to separate components of a plant extract, illustrating the experiment with plant pigments that gave chromatography its name

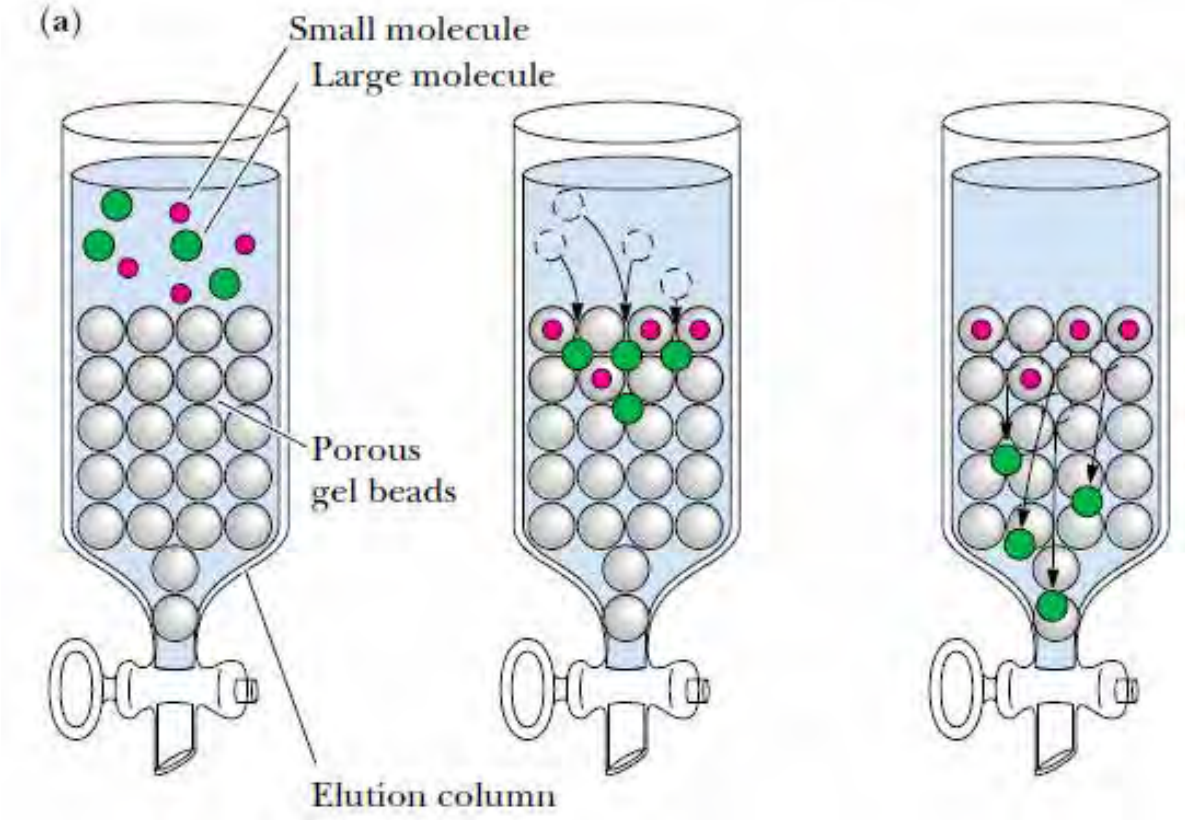
Gel Filtration Chromatography. A mixture of proteins in a small volume is applied to a column filled with porous beads. Because large proteins cannot enter the internal volume of the beads, they emerge sooner than do small ones.

into the surrounding medium.

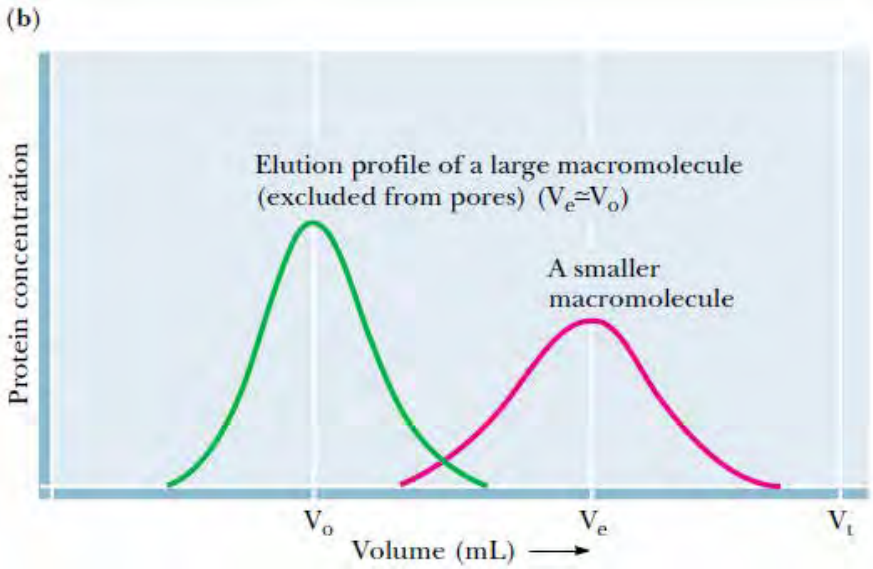


Size Exclusion Chromatography. *Size exclusion chromatography* is also known as *gel filtration chromatography* or *molecular sieve chromatography.* In this method, fine, porous beads are packed into a chromatography column. The beads are composed of dextran polymers
(*Sephadex*), agarose (*Sepharose*), or polyacrylamide (*Sephacryl* or *BioGel P*). The pore sizes of these beads approximate the dimensions of macromolecules. The total

bed volume of the packed chromatography column, Vt, is equal to the volume outside the porous beads (Vo) plus the volume inside the beads (Vi) plus the volume actually occupied by the bead material (Vg): Vt Vo Vi Vg. (Vg is typically less than 1% of Vt and can be conveniently ignoredin most applications.)



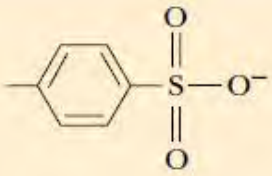
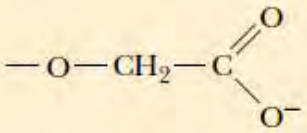
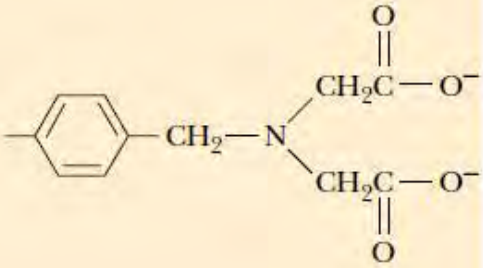
A gel filtration chromatogrphy column. Larger molecules are excluded from the gel beads and emerge from the column sooner than smaller molecules, whose migration is retarded because they can enter the beads.

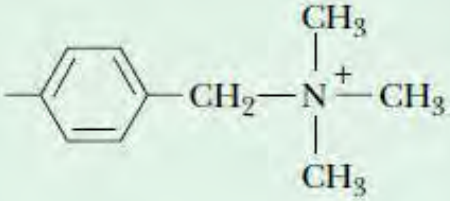
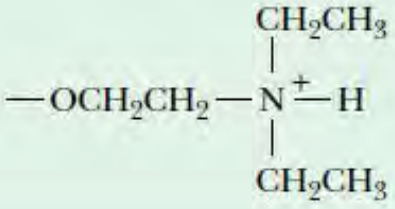


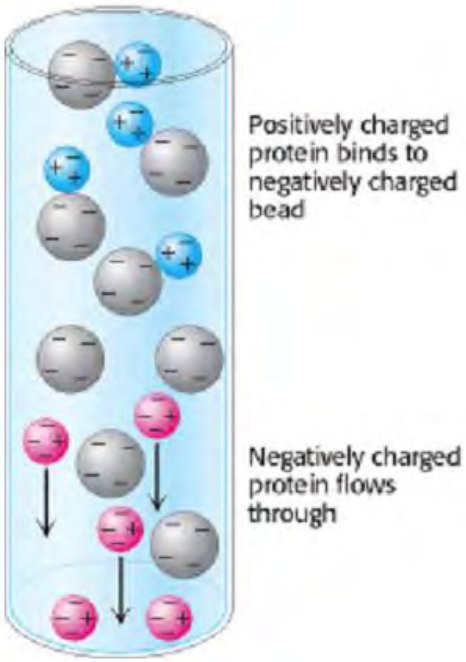
[1] *Protein Techniques,* these methods are also applicable to other macromolecules such as nucleic acids

Ion-Exchange Chromatography

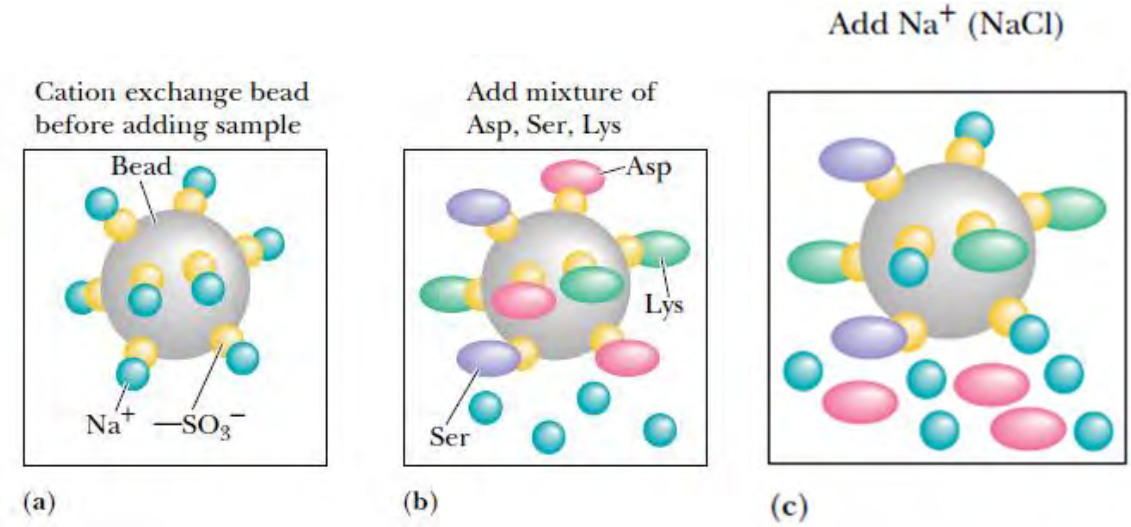
Cation (a) and **anion** (b) **exchange resins commonly used for biochemical separations**

(a) Cation Exchange Media	Structure
Strongly acidic, polystyrene resin (Dowex–50)	
Weakly acidic, carboxymethyl (CM) cellulose	
Weakly acidic, chelating, polystyrene resin (Chelex–100)	

(b) Anion Exchange Media	Structure
Strongly basic, polystyrene resin (Dowex–1)	
Weakly basic, diethylaminoethyl (DEAE) cellulose	

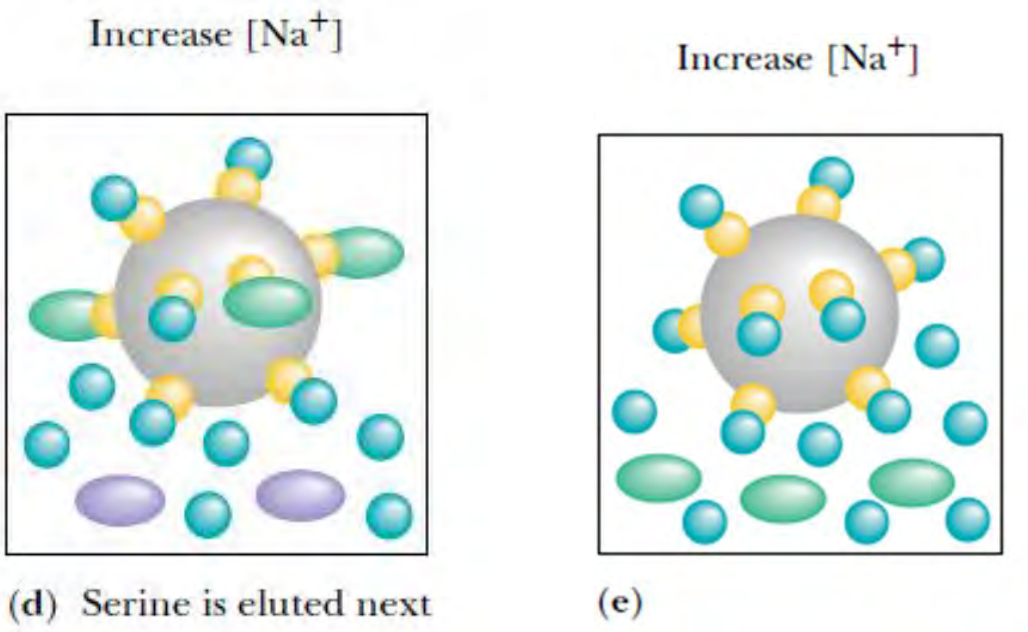


Ion-Exchange Chromatography. This technique separates proteins mainly according to their net charge



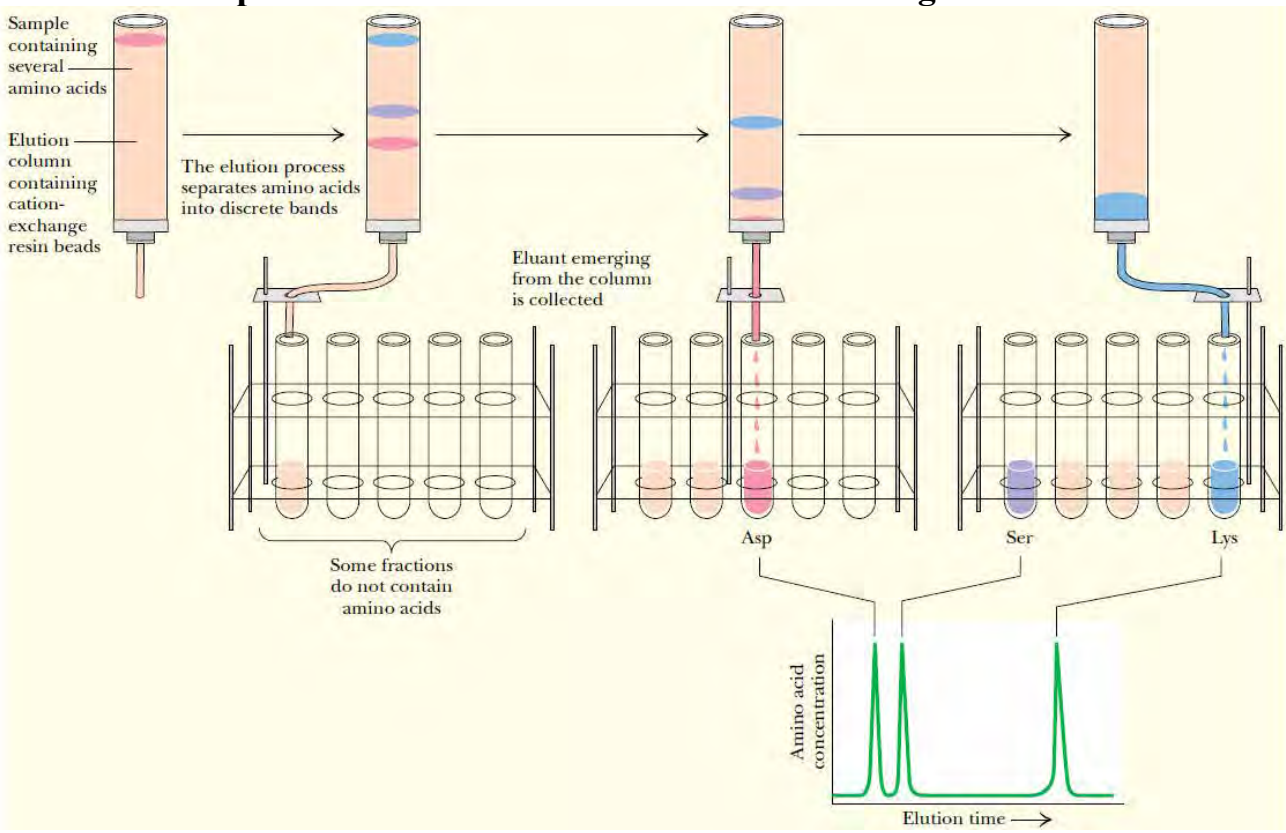
Operation of a cation exchange column, separating a mixture of **Asp, Ser,** and **Lys**. (a) The cation exchange resin in the beginning, Na form. (b) A mixture of Asp, Ser, and Lys is added to the column containing the resin. (c) A gradient of the eluting salt (e.g., NaCl) is added to the column. Asp, the least positively charged amino acid, is eluted first.

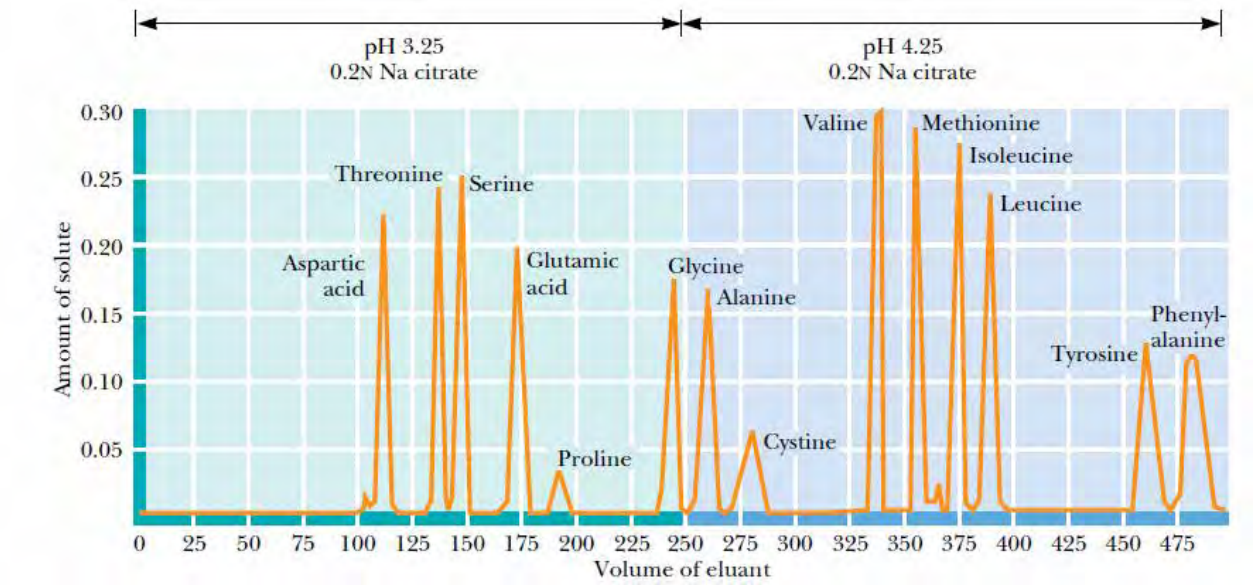
Asp, the least positively charged amino acid, is eluted first



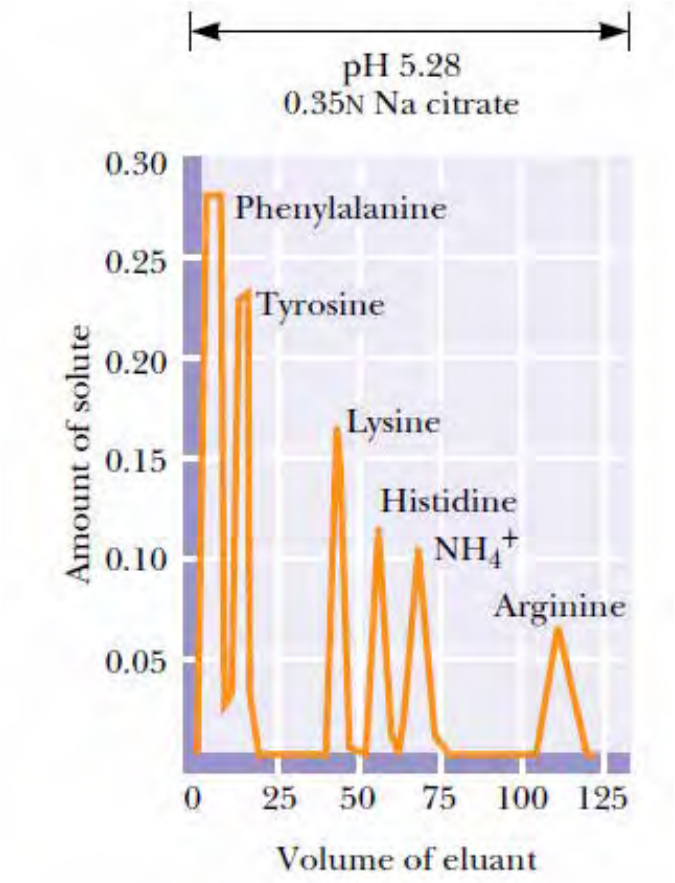
Lysine, the most positively charged amino acid, is eluted last.

The separation of amino acids on a cation exchange column

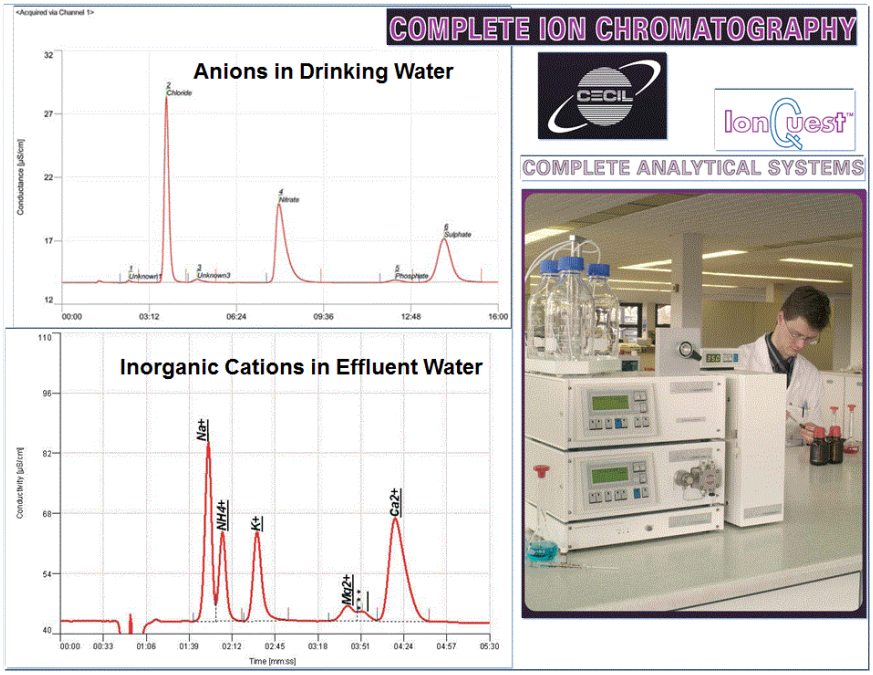




Chromatographic fractionation of a synthetic mixture of amino acids on ion exchange columns using Amberlite IR-120, a sulfonated polystyrene resin similar to Dowex-50. (*Adapted from Moore, S., Spackman, D., and Stein, W., 1958. Chromatography of amino acids on sulfonated polystyrene resins.* Analytical Chemistry *30*:*1185–1190.)*

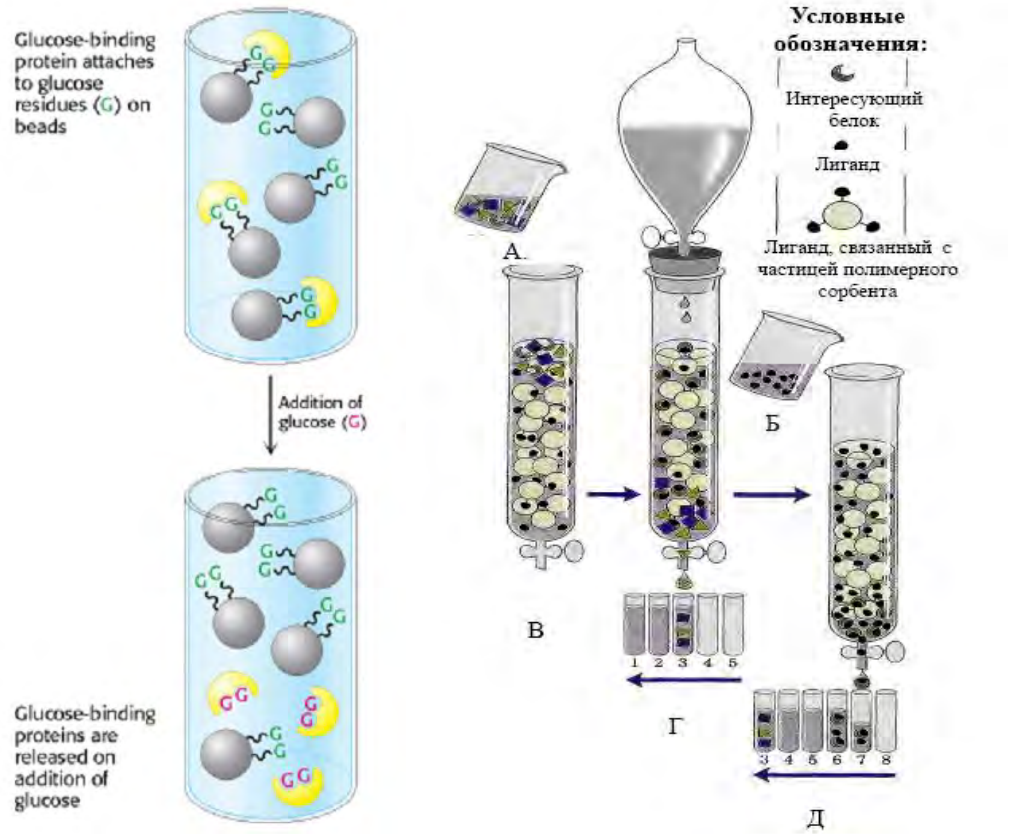


Chromatographic fractionation of a synthetic mixture of amino acids on ion exchange columns using Amberlite IR-120, a sulfonated polystyrene resin similar to Dowex-50. A second column with different buffer conditions is used to resolve the basic amino acids. (*Adapted from Moore, S., Spackman, D., and Stein, W., 1958. Chromatography of amino acids on sulfonated polystyrene resins.* Analytical Chemistry *30*:*1185–1190.)*



Affinity Chromatography

Affinity chromatography of concanavalin A (shown in yellow) on a solid support containing covalently attached glucose residues (G)

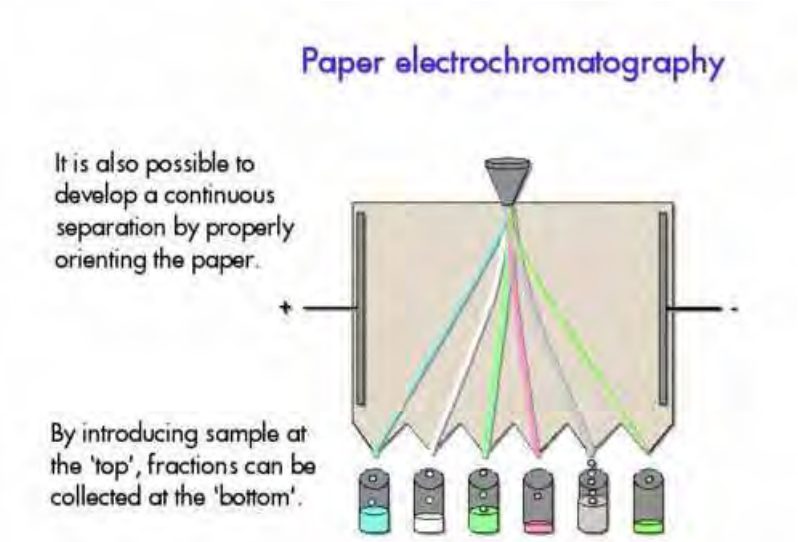


The method is based on ability of proteins to bind tightly with different molecules using non-covalent bonds. This method is used for isolation and purification of enzymes, immunoglobulins, receptor-proteins.

Paper chromatography

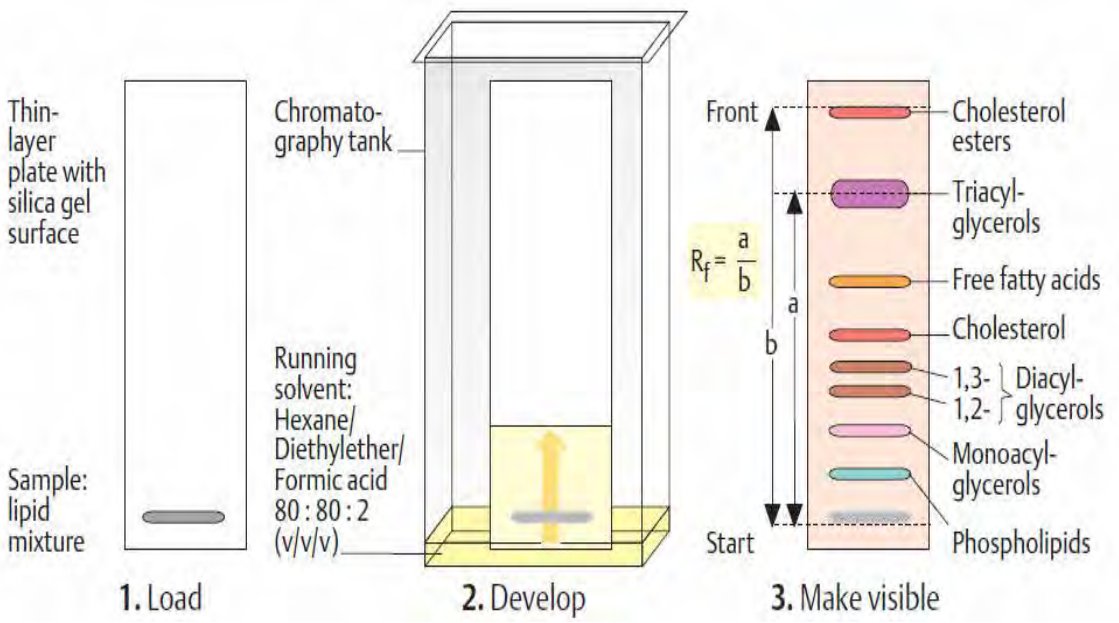
Paper chromatography is a technique that involves placing a small dot or line of sample solution onto a strip of chromatography paper. The paper is placed in a jar containing a shallow layer of solvent and sealed. As the solvent rises through the paper, it meets the sample mixture, which starts to travel up the paper with the solvent. This paper is made of cellulose, a polar substance, and the compounds within the mixture travel farther if they are non-polar. More polar substances bond with the cellulose paper more quickly, and therefore do not travel as far.

Scheme of chromatography of dyes.

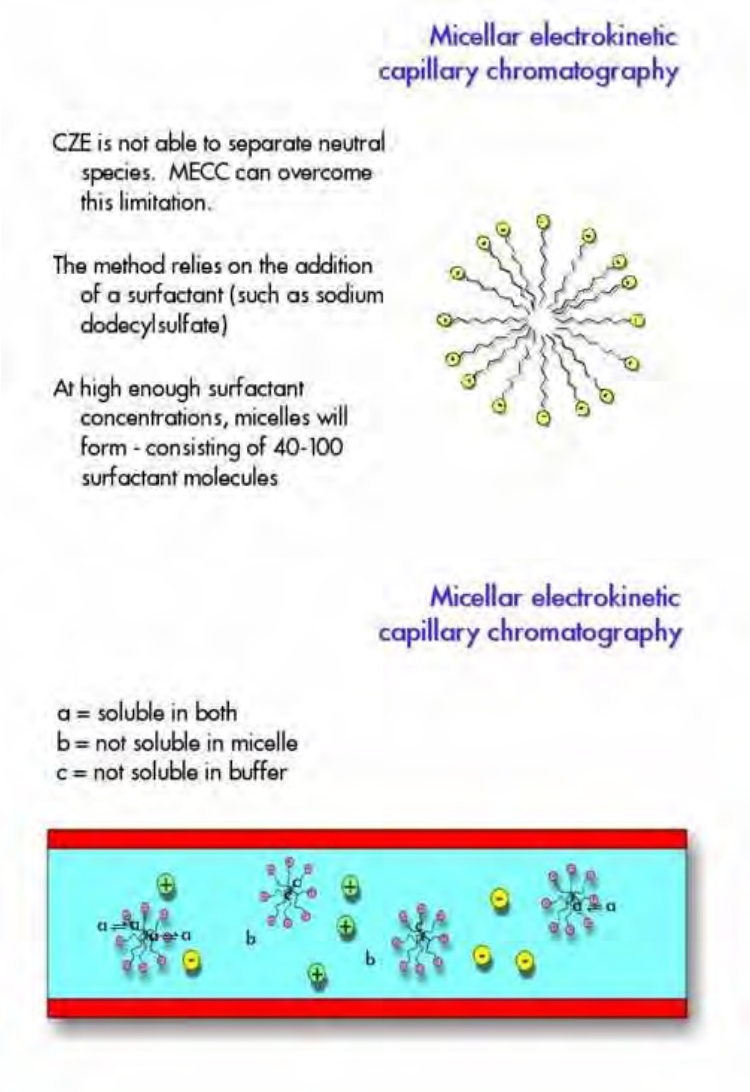


Thin layer chromatography (TLC)

Thin layer chromatography (TLC) is a widely employed laboratory technique and is like paper chromatography. However, instead of using a stationary phase of paper, it involves a stationary phase of a thin layer of adsorbent like silica gel, alumina, or cellulose on a flat, inert substrate. Compared to paper, it has the advantage of faster runs, better separations, and the choice between different adsorbents. For even better resolution and to allow for quantification, high-performance TLC can be used.



Cited: ***Jan Koolman, Klaus-Heinrich Roehm*** **Color Atlas of Biochemistry**. Second edition revised and enlarged. 2005. Thieme Stuttgart New York. – 476 p



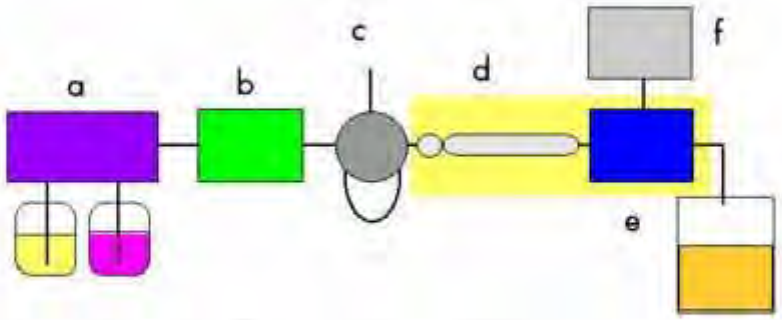
Liquid chromatography (LC) and high-performance liquid chromatography (HPLC).

Chromatographic system

Liquid chromatography (LC) is a separation technique in which the mobile phase is a liquid. Liquid chromatography can be carried out either in a column or a plane. Present day liquid chromatography that generally utilizes very small packing particles and a relatively high pressure is referred to as high performance liquid chromatography (HPLC).

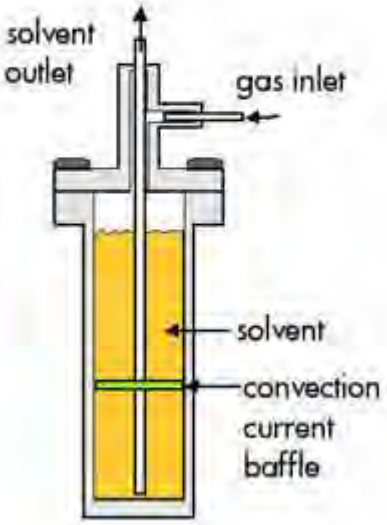
In HPLC the sample is forced by a liquid at high pressure (the mobile phase) through a column that is packed with a stationary phase composed of irregularly or spherically shaped particles, a porous monolithic layer, or a porous membrane. HPLC is historically divided into two different sub-classes based on the polarity of the mobile and stationary phases. Methods in which the stationary phase is more polar than the mobile phase (e.g., toluene as the mobile phase, silica as the stationary phase) are termed normal phase liquid chromatography (NPLC) and the opposite (e.g., water-methanol mixture as the mobile phase and C18 = octadecyl silyl as the stationary phase) is termed reversed phase liquid chromatography (RPLC). Ironically the "normal phase" has fewer applications and RPLC is therefore used considerably more.

Basic HPLC equipment



a - gradient controller
b - pump/ dampning system
c - sample introduction
d - column/ precolumn
e - detector
f - data output

Direct pressure pump

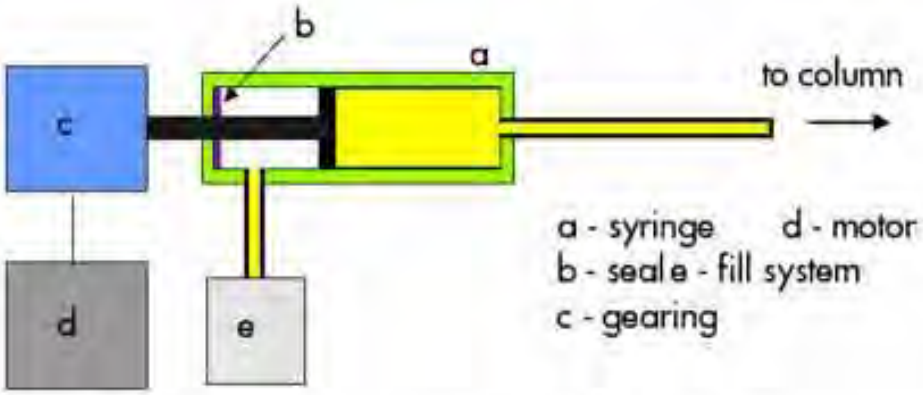


Gas pressure is applied from an external gas tank using a high pressure regulator.

For this system

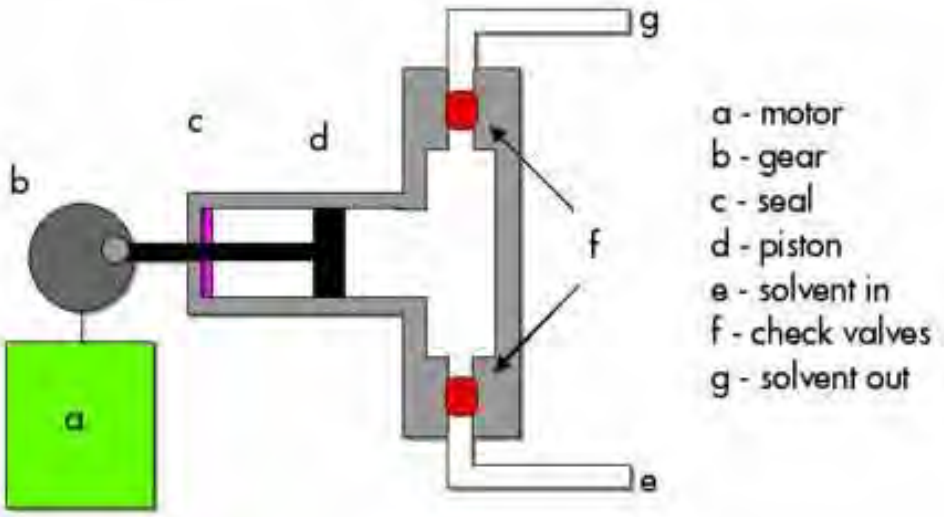
- No pressure pulses are produced.
- The solvent reservoir is limited.
- Low cost system
- Major problem is introduction of gas into the solvent

Motor driven syringe



Another non-pulsating system with a limited reservoir. Stepper motor/gear system allows for very fine flow control.

Reciprocating pump

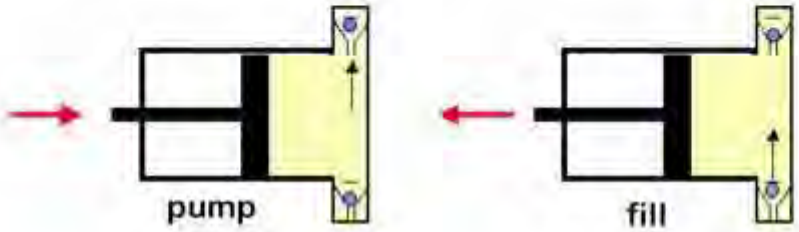


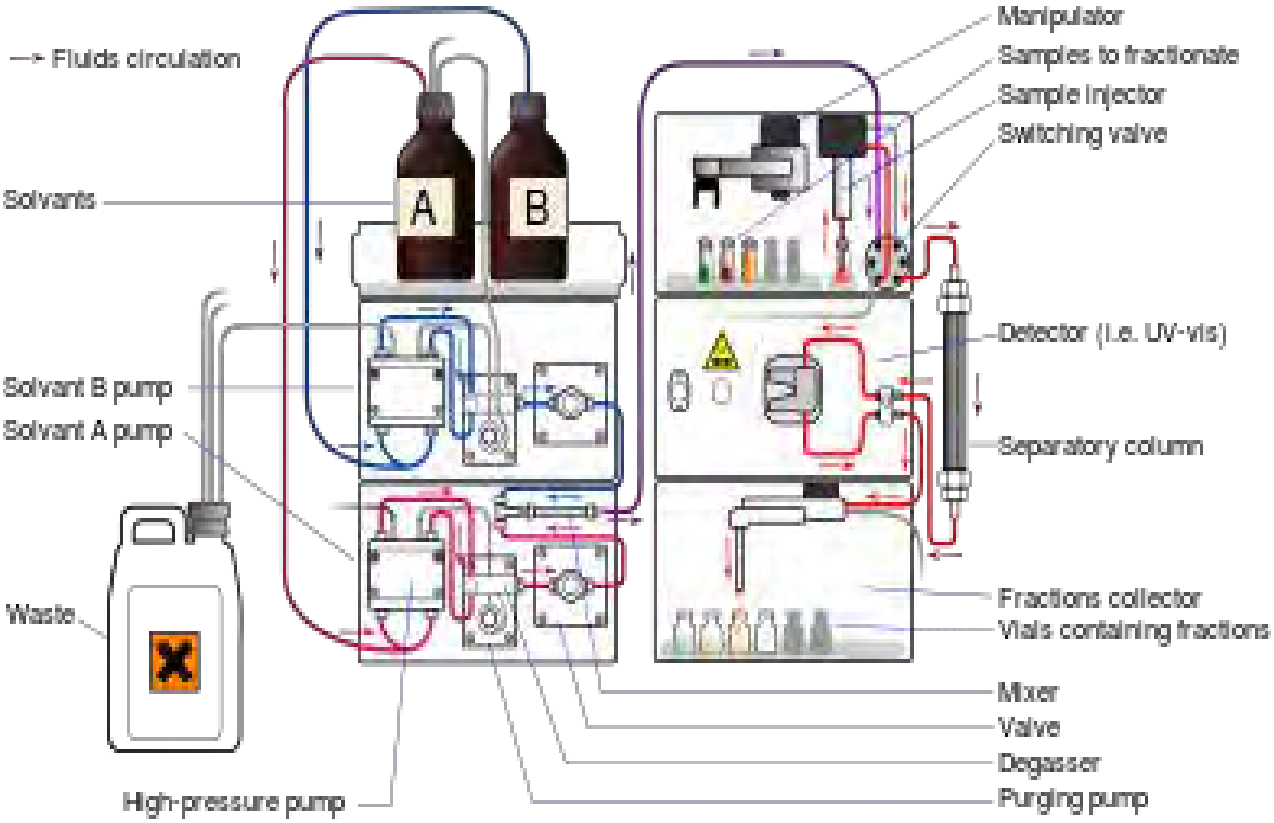
Reciprocating pump

One of the most common type of systems.

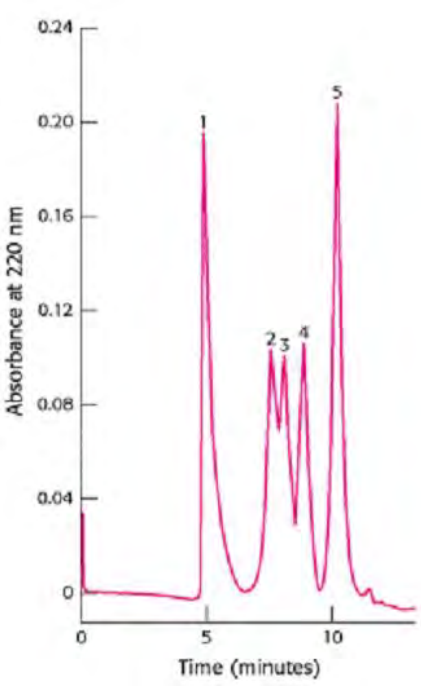
Unlimited reservoir system but expensive.

Another problem is that it produces variable pressure - must reverse stroke to refill.





Preparative HPLC apparatus



High-Pressure Liquid Chromatography (HPLC). Gel filtration by HPLC clearly defines the individual proteins because of its greater resolving power: (1) thyroglobulin (669 kd), (2) catalase (232 kd), (3) bovine serum albumin (67 kd), (4) ovalbumin (43 kd), and (5) ribonuclease (13.4 kd). [After K. J. Wilson and T. D. Schlabach. In Current Protocols in Molecular Biology, vol. 2, suppl. 41, F. M. Ausbel, R. Brent, R. E. Kingston, D. D. Moore, J. G. Seidman, J. A. Smith, and K. Struhl, Eds. (Wiley, 1998), p. 10.14.1.]

Gas chromatography (GC),

Chromatogrphy-columns for GC and HPLC

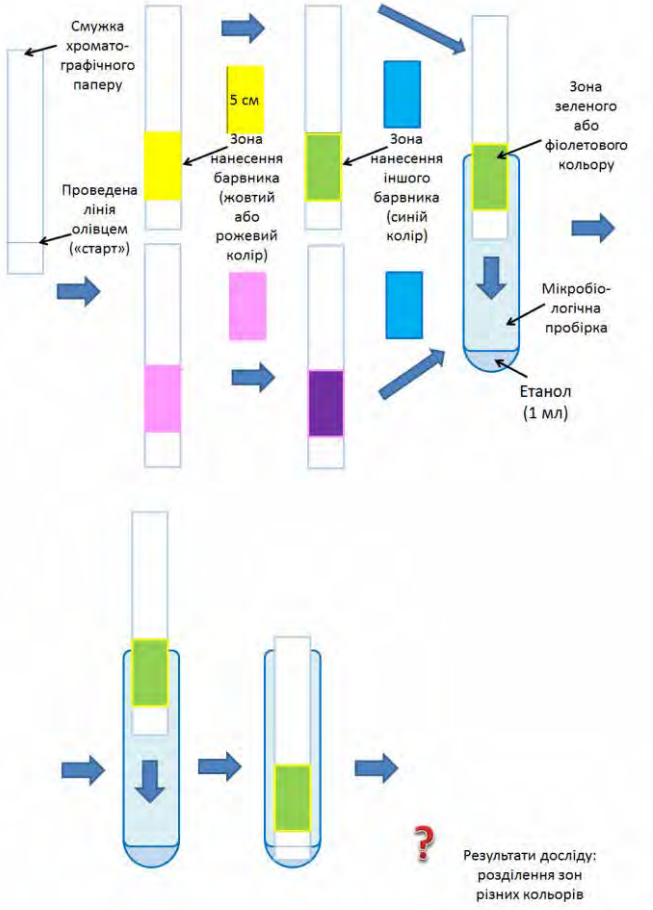
Chromato-grapher

Gas chromatography (GC), also sometimes known as gas-liquid chromatography, (GLC), is a separation technique in which the mobile phase is a gas. Gas chromatography is always carried out in a column, which is typically "packed" or "capillary" (see below).

Gas chromatography is based on a partition equilibrium of analyte between a solid stationary phase (often a liquid silicone-based material) and a mobile gas (most often helium). The stationary phase is adhered to the inside of a small-diameter glass tube (a capillary column) or a solid matrix inside a larger metal tube (a packed column). It is widely used in analytical chemistry; though the high temperatures used in GC make it unsuitable for high molecular weight biopolymers or proteins (heat denatures them), frequently encountered in biochemistry, it is well suited for use in the petrochemical, environmental monitoring and remediation, and industrial chemical fields. It is also used extensively in chemistry research.

LAB-CLASS

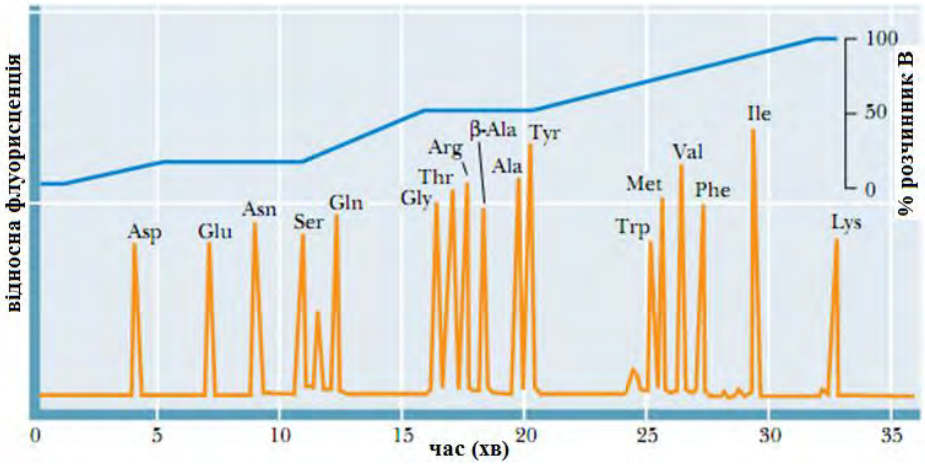
1. Separation of a mixture of dyes by paper chromatography



The scheme of the chromatography of dyes

3. Model experiment HPLC-separation of amino acids

With knowledge of chromatography-method, try to explain steps of amino acids separation1



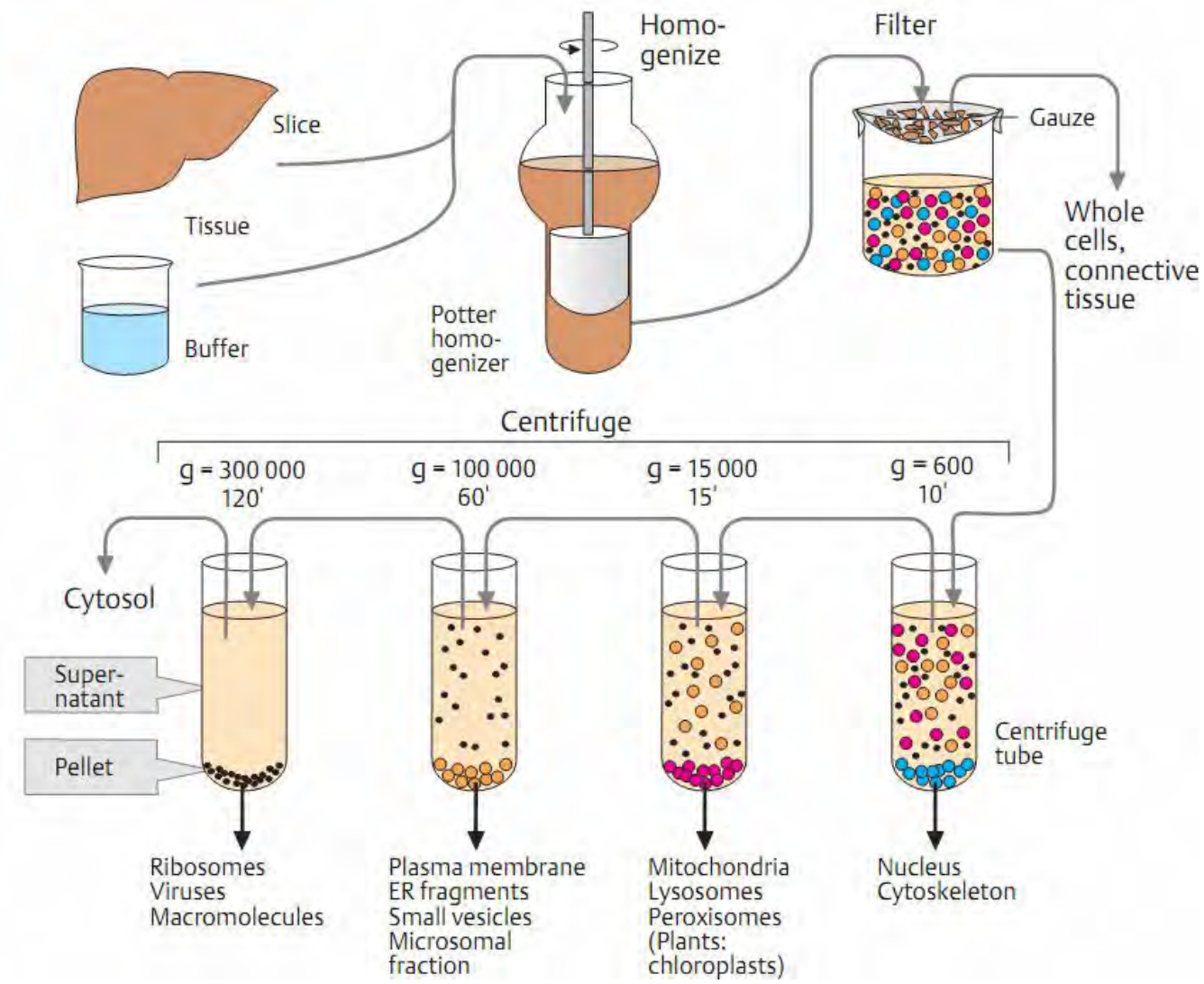
HPLC (high-performance liquid chromatography) chromatogram of amino acids employing precolumn derivatization with OPA (*o*-phthaldialdehyde). Chromatography was carried out on an Ultra sphere ODS column using a complex tetrahydrofuran : methanol : 0.05 *M* sodium acetate (pH 5.9) 1:19:80 to methanol : 0.05 *M* sodium acetate (pH 5.9) 4:1 gradient at a flow rate of 1.7 mL/min. *(Adapted from Jones, B. N., Pääbo, S., and Stein, S., 1981. Amino acid analysis and enzymic sequence determination of peptides by an improved* o*-phthaldialdehyde precolumn labeling procedure.* Journal of Liquid Chromatography *4*:*56–586.)*

Control questions, tasks and exercises for the section «CHROMATOGRAPHIC METHODS»

1. What is the purpose of using chromatographic methods in biochemical research?
2. What are the possibilities of chromatography on paper? What substance was first separated using this method?
3. What are the differences between gel filtration and ion exchanger chromatography?
4. Give examples of separation by chromatography.
5. Briefly describe the separation of molecules by ion exchange chromatography.
6. Briefly describe the separation of molecules by gel filtration.

Example of biochemical study

Preparative isolation of protein
Homogenization of tissue



Proteins Must Be Released from the Cell to Be Purified

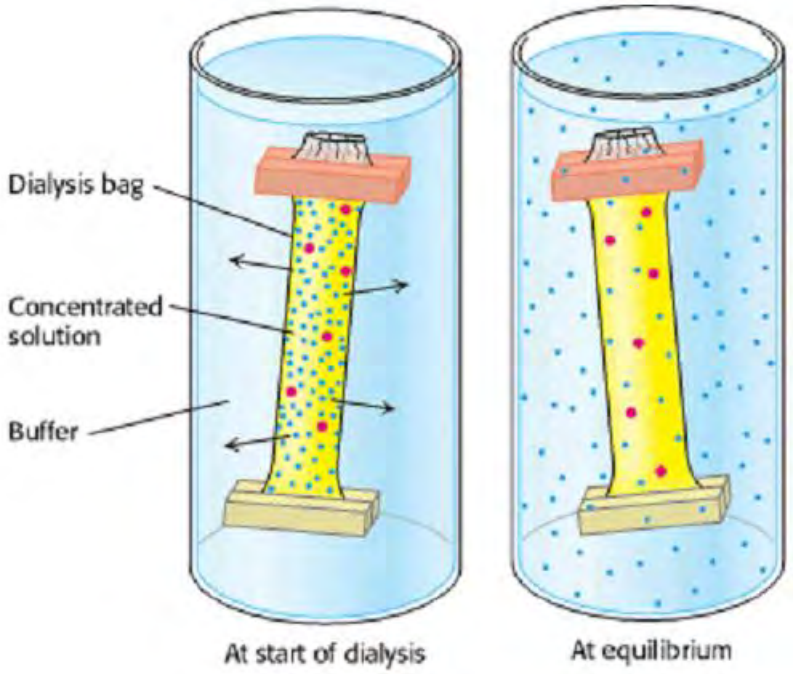
Having found an assay and chosen a source of protein, we must now fractionate the cell into components and determine which component is enriched in the protein of interest. Such fractionation schemes are developed by trial and error, on the basis of previous experience.

In the first step, a *homogenate* is formed by disrupting the cell membrane, and the mixture is fractionated by centrifugation, yielding a dense pellet of heavy material at the bottom of the centrifuge tube and a lighter supernatant above.

The supernatant is again centrifuged at a greater force to yield yet another pellet and supernatant. The procedure, called ***differential centrifugation*****,** yields several fractions of decreasing density, each still containing hundreds of different proteins, which are subsequently assayed for the activity being purified.

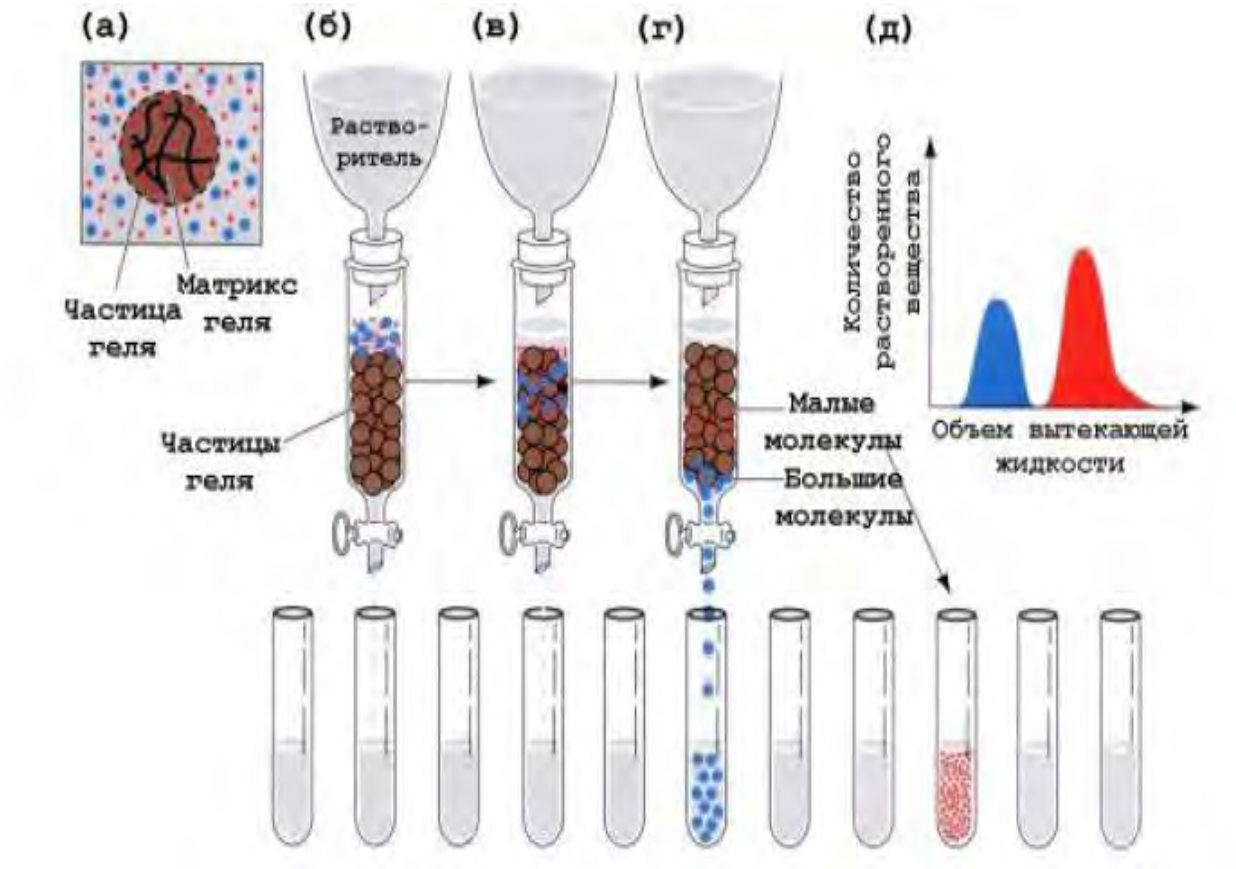
Usually, one fraction will be enriched for such activity, and it then serves as the source of material to which more discriminating purification techniques are applied.

Salt-precipitation of protein. *Dialysis.*



Dialysis. Protein molecules (red; bigger) are retained within the dialysis bag, whereas small molecules (blue; smaller) diffuse.

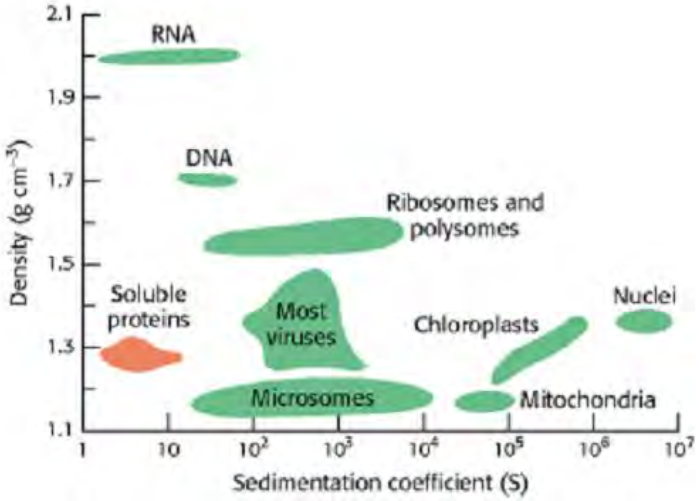
Proteins can be separated from small molecules by *dialysis* through a semipermeable membrane, such as a cellulose membrane with pores. Molecules having dimensions significantly greater than the pore diameter are retained inside the dialysis bag, whereas smaller molecules and ions traverse the pores of such a membrane and emerge in the dialysate outside the bag. This technique is useful for removing a salt or other small molecule, but it will not distinguish between proteins effectively.



Scheme of gel-chromatography

In biochemical studies preparative centrifugation is used for purification of biological material: subcellular organelles of individual macromolecules (DNA, proteins, polysaccharides, etc.) in order to study their structure and biological activity.

Steps of centrifugation



Density and Sedimentation Coefficients of Cellular Components. [After L. J. Kleinsmith and V. M. Kish, Principles of Cell, and Molecular Biology, 2d ed. (Harper Collins, 1995), p. 138.]

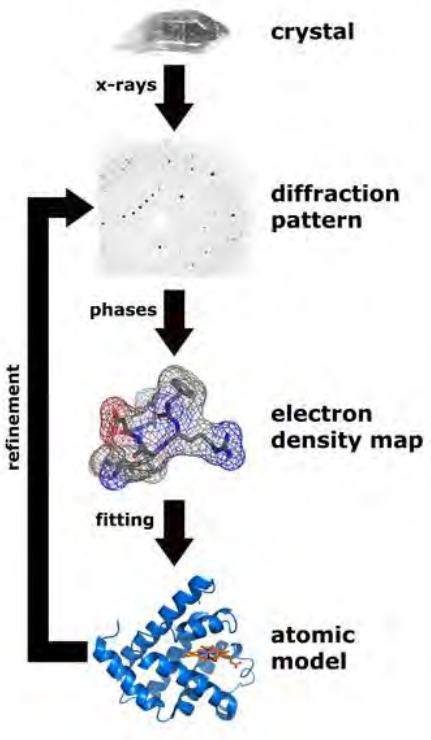
After steps of isolation and purification, biological sample can be studied by different techniques (some of them find above: sequencing, X-ray analysis, etc.).

The purified water is used in biochemical laboratories.

Water purification

X-ray analysis

X-ray crystallography (analysis) is the method of investigation of substantial structure in basis of which is phenomenon of diffraction of X-ray radiation on three-dimensional crystal lattices.



The Purification of Proteins Is an Essential First Step in Understanding Their Function

An adage of biochemistry is, never waste pure thoughts on an impure protein. Starting from pure proteins, we can determine amino acid sequences and

evolutionary relationships between proteins in diverse organisms and we can investigate a protein's biochemical function.

Moreover, crystals of the protein may be grown from pure protein, and from such crystals we can obtain x-ray data that will provide us with a picture of the protein's tertiary structure the actual *functional* unit.

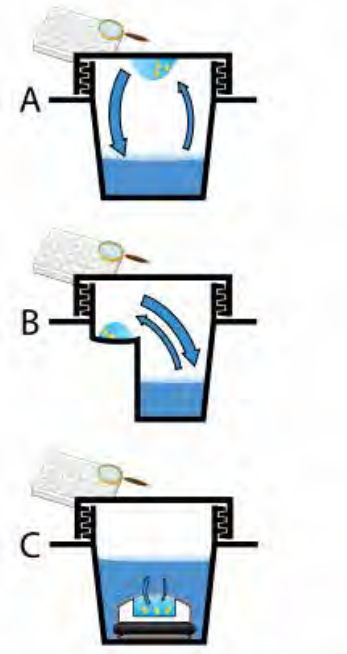
(1)

(2)

(1) A representation of the 3D structure of myoglobin showing coloured *alpha* helices.

(2) Crystal of protein is under microscope. Crystals for X-ray analysis have size from 0.1 to 1 mm.

Ribbon diagram of the structure of myoglobin, showing colored alpha helices. Such proteins are long, linear molecules with thousands of atoms; yet the relative position of each atom has been determined with sub-atomic resolution by X-ray crystallography. Since it is difficult to visualize all the atoms at once, the ribbon shows the rough path of the protein polymer from its N-terminus (blue) to its C-terminus (red).

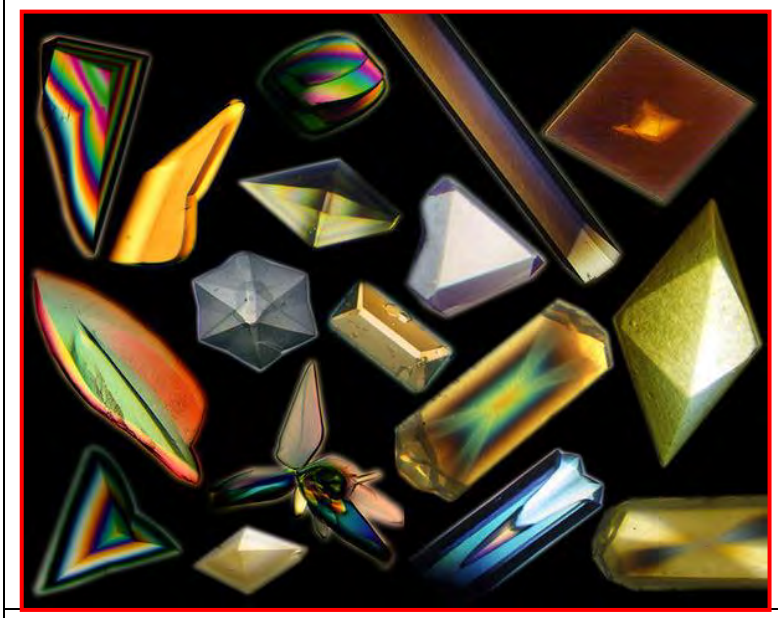


Three methods of preparing crystals, *A: Hanging drop. B: Sitting drop. C: Microdialysis*

Myoglobin and hemoglobin were the first to have their structure solved by X-ray crystallography. Max Perutz and Sir John Cowdery Kendrew got for that Nobel Prize in Chemistry in 1962.

Protein crystals are almost always grown in solution. The most common approach is to lower the solubility of its component molecules very gradually; if this is done too quickly, the molecules will precipitate from solution, forming a useless dust or amorphous gel on the bottom of the container. Crystal growth in solution is characterized by two steps:*nucleation* of a microscopic crystallite (possibly having only 100 molecules), followed by *growth* of that crystallite, ideally to a diffraction-quality crystal.

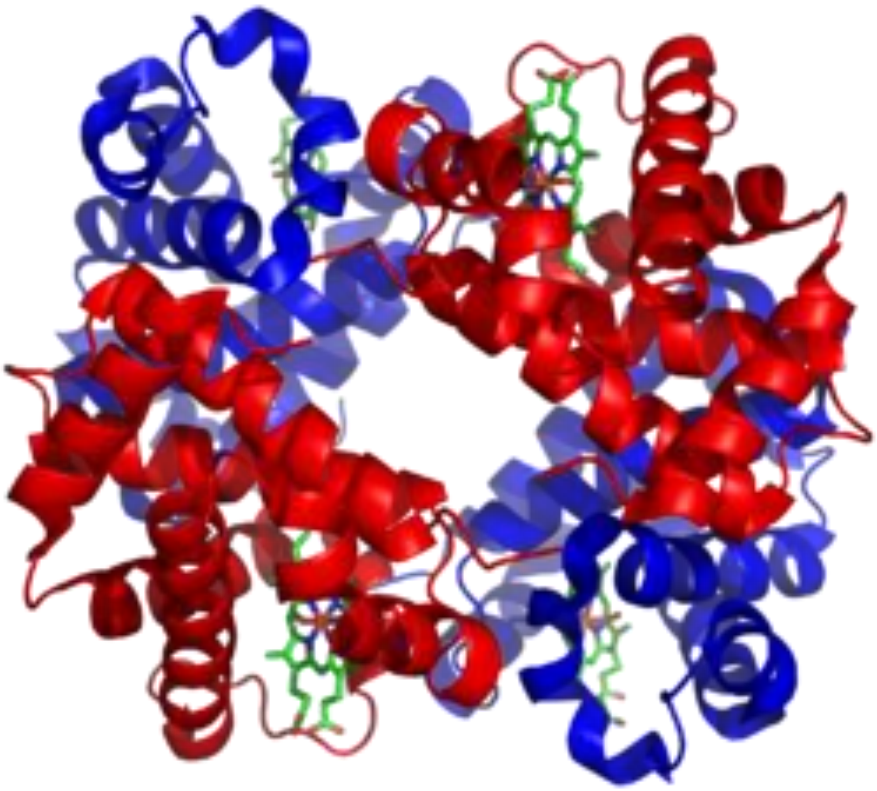
Crystallization of Myoglobin



Protein crystals grown in space.

Protein crystals (dimension 0.1 to 1 mm) grown for crystallography. Photo was made in polarized light.

Model of high molecular substance (HMS; hemoglobin) was obtained using study of its three-dimensional structure by X-ray analysis.



Who was Nobel Prize for this work? (Look for answer above).

Generalized conclusions

to chapter 1.2 “PHYSICAL and CHEMICAL METHODS OF RESEARCH IN BIOCHEMISTRY”

1. Presented common view of theoretical knowledge about the Spectrophotometric and photo electrocolorimetric methods with exanples of tools and data of Spectrophotometric and photo electrocolorimetric aanalyses.
2. Charaterized characteristics of centrifuges and approaches of separation of the high molecular substances with explanations use in lab
3. Given fundamentals of electrophoretic separation of high molecular compounds and examples of electrophoresis apparatus with different possibility of separation.
4. Represented different kinds of chromatography and their use in practice.

Chapter 1.3
STATIC BIOCHEMISTRY

The purpose of this section of the course "Biochemistry of animals with the Basics of Physical and Colloid Chemistry" is to provide students with theoretical knowledge of physical and colloid chemistry and some experiments in lab classes.

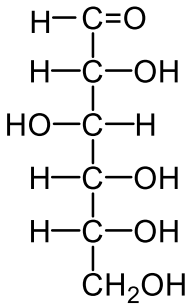
The following topics will be considered in this section:

Topic 1. Carbohydrates: monosaccharides, disaccharides
Topic 2. Carbohydrates: polysaccharides
Topic 3. Lipids and their components
Topic 4. Amino acids.
Topic 5. Acid amides.
Topic 6. Proteins
Topic 7. Nucleic acids and their components

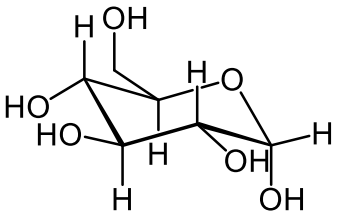
The objects of biochemical investigation are compounds of different classes of organic substances: carbohydrates, lipids, proteins, nucleic acids.

1.3.1. ***Topic*** CARBOHYDRATES: MONOSACCHARIDES, DISACCHARIDES

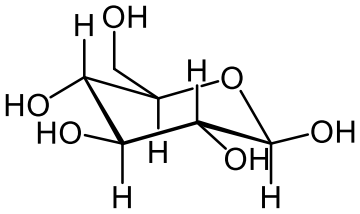
Carbohydrates



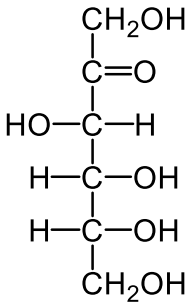
D-glucose



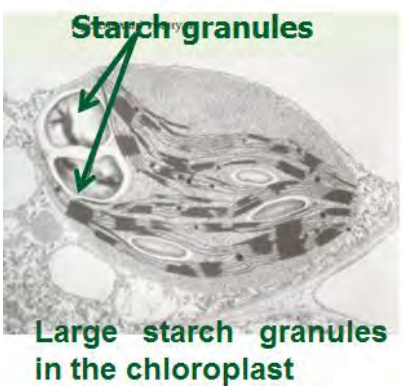
α,D-glucose

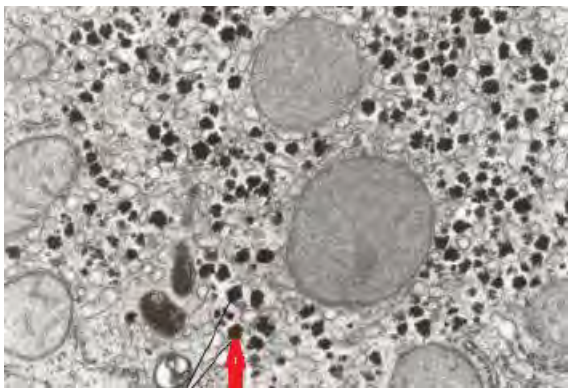


β,D-glucose



D-fructose





Granules of glycogen in the hepatocyte. Granules are formed in the cytosol and size of granule is small (~0,1 μm) comparing to starch size.

Carbohydrates (glucides, glycides) are united into group of organic substances, molecules of which mainly include three chemical elements: Carbon, Hydrogen and Oxygen. Common formula of carbohydrates $C_n(H_2O)_m$.

Carbohydrates are product of assimilation of Carbon dioxide by green plants and photosynthetic microorganisms.

Classification of carbohydrates

Simple carbohydrates (Monosaccharides)

- Aldoses (glucose)
- Ketoses (fructose)
- Products of substitution of one or several hydroxyls: by Hydrogen (ramnose), amino-group (glucose-amine), simple saccharides with atoms Sulfur or Nitrogen etc.
- Trioses (glyceraldehydes, dioxiaceton)
- Tetroses (threose, erythrose)
- Pentoses (ribose, desoxyribose, xylose)
- Hexoses (glucose, fructose)
- Heptoses (sedoheptulose)

Complex carbohydrates

Oligosaccharides (disaccharides: Lactose, sucrose, tregalose, cellobiose)

Polysaccharides /glycans/

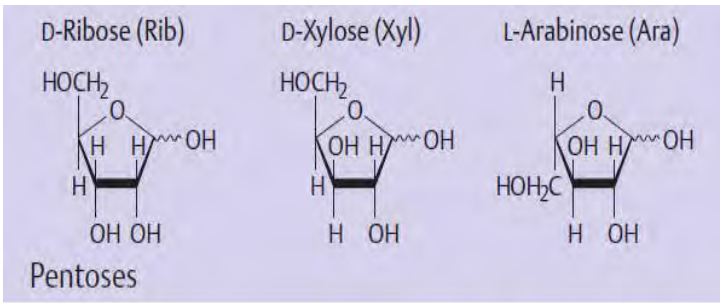
Homopolysaccharides (*pentosans*, hexosans: starch, cellulose, glycogen, inulin, chitin, pectin substances)

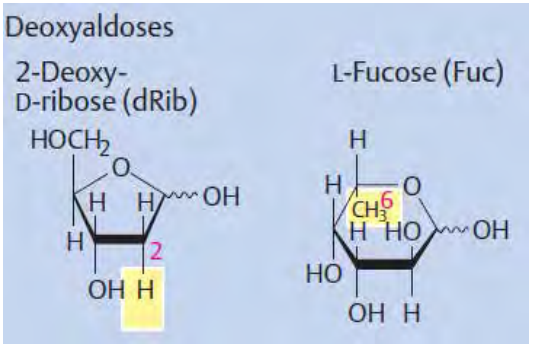
Heteropolysaccharides

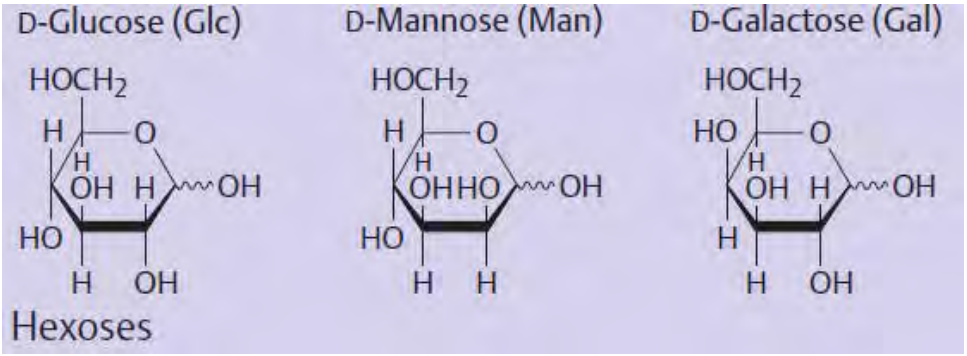
glycosaminoglycans: acidic (chondroitin sulfuric acid, hialouronic acid, heparin, dermatin, keratin) and neutral (their molecules include remains of neuramine and sialic/сіалових acids)

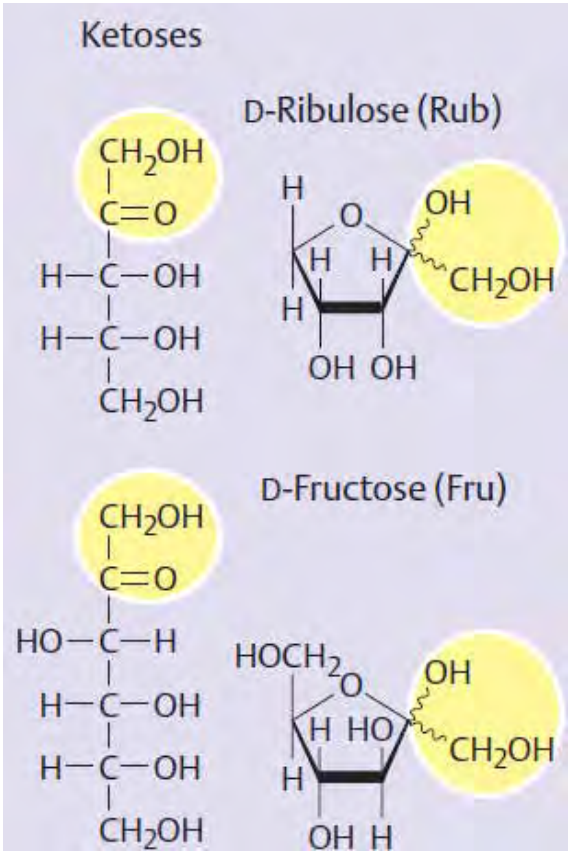
Glycopolysaccharides (pectin substances, agar-agar, gummy Arabic, dextrin, polysaccharide complexes of microorganisms)

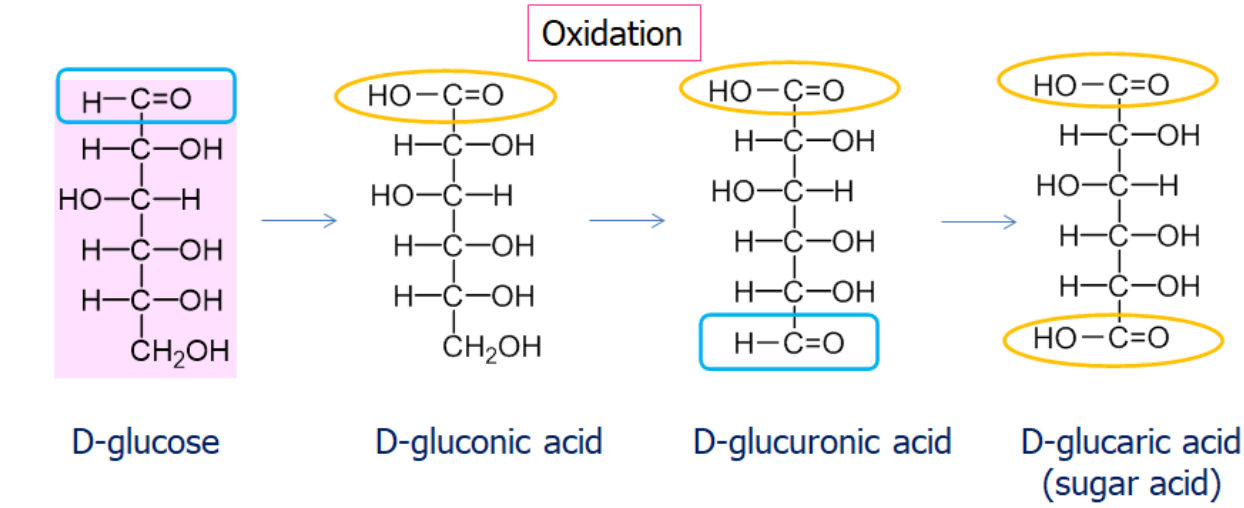
Aldoses

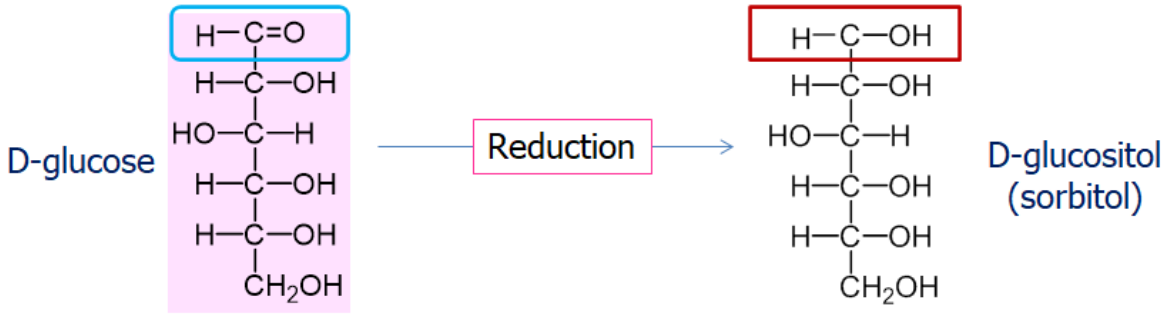


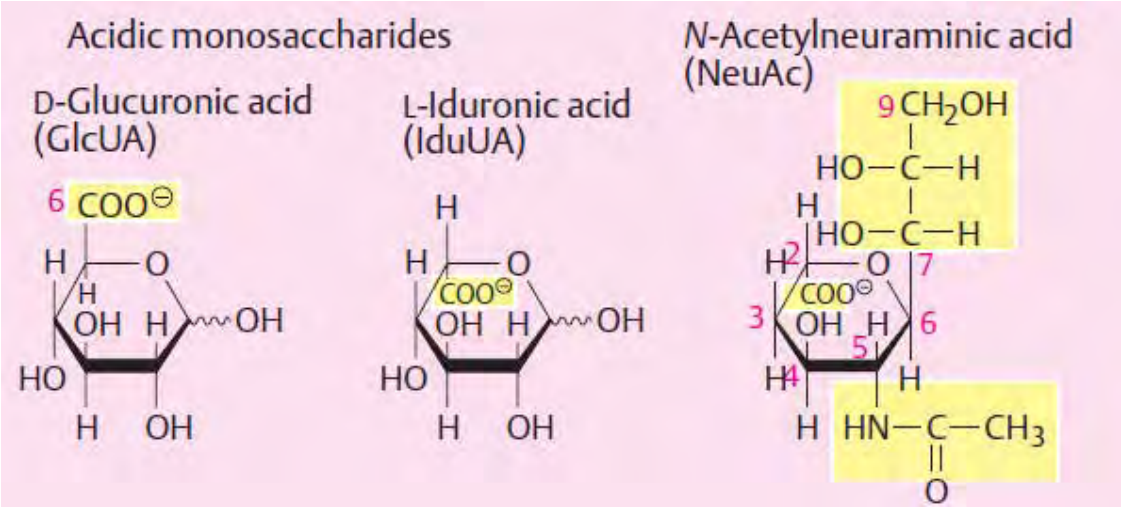




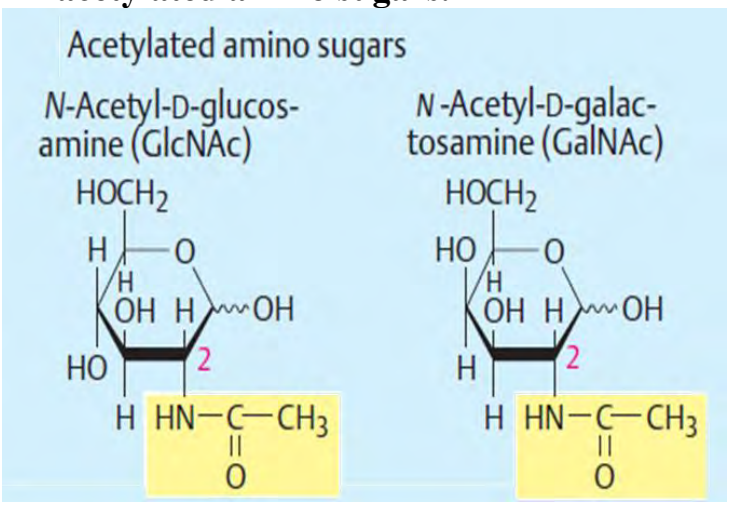

Ketoses
D-Ribulose (Rub)
D-Fructose (Fru)


Oxidation
D-glucose
D-gluconic acid
D-glucuronic acid
D-glucaric acid
(sugar acid)


D-glucose
Reduction
D-glucositol
(sorbitol)

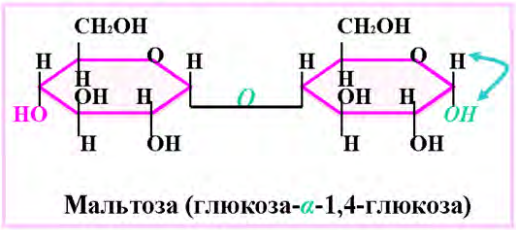


Acidic monosaccharides are found in glycoproteins. They are typical constituents of the glycosaminoglycans found in connective tissue. Other components of glycoproteins are the **acetylated amino sugars.**



disaccharide

4-O-α-glucopyranosil-D-glucose is disaccharide which can reduce



Maltose (*malt* sugar) is soluble in water very good. In free state, it is in germinated grains of cereals (*проросле зерно злаків*), nectar (*нектар*).

Maltose is fermented by yeast, it is a component of many nutritious media in microbiological practice.

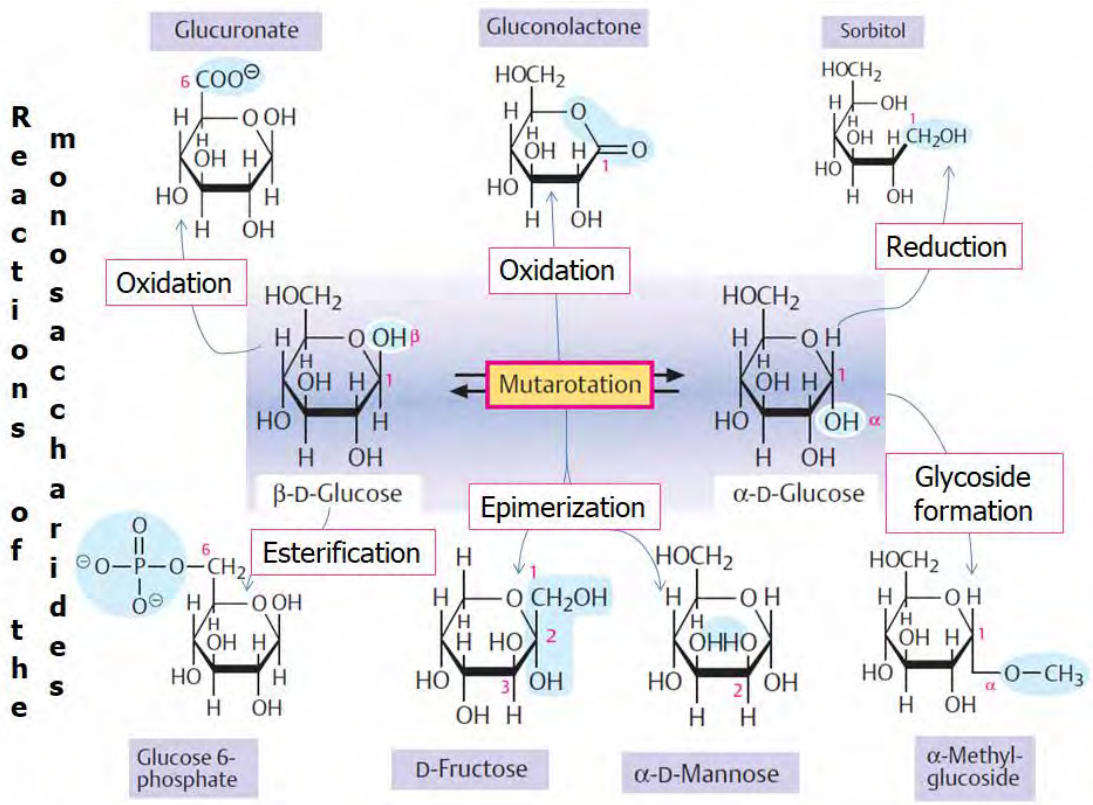
Glucose is produced after its acidic or fermentative hydrolysis.

It is obtained during partial hydrolysis of starch under effect of amylase of malt (*солод*).

Starch → dextrins → maltose → glucose

$(C_6H_{10}O_5)_n \rightarrow (C_6H_{10}O_5)_x \rightarrow y\ C_{12}H_{22}O_{11} \rightarrow n\ C_6H_{10}O_5$

Disaccharides are maltose, lactose (monomers: glucose and galactose), saccharose (monomers: glucose and fructose), and trehalose (monomers: 2 glucoses).



Reactions of monosaccharides

LAB-CLASS

1. **Detection of hydroxyl groups in monosaccharides**

Monosaccharides mainly belong to aldehyde or keto-polyhydric alcohol. Thus, the monosaccharides can form alcoholates due to the fact that in its molecule there are OH groups.

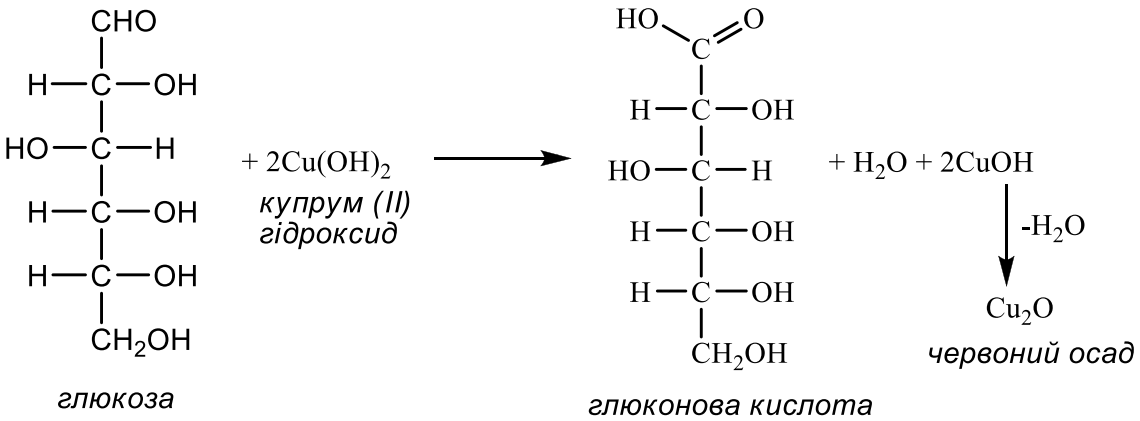
Progress of experiment. It is necessary to pour 3 ml of 5% glucose into a test tube, then add solutions: 1 ml of 30% NaOH and few drops of 1% $CuSO_4$. The copper (II) hydroxide formed reacts with glucose; the solution turns blue, which is due to the formation of a colored complex of glucose and copper alkoxide.

2. **Reactions of oxidation of carbohydrates with aldehyde group and reduction of metal ions in alkaline medium (Trommer's test)**

Metal ions can be reduced through the carbohydrate molecule has free aldehyde groups that are easily oxidized. For example, in the Trommer reaction, glucose interacts with $Cu(OH)_2$ (blue) while warming up Cu^{2+} is reduced, forming a CuOH (yellow precipitate) or Cu_2O (red precipitate).

In alkaline solution, monosaccharides, oxidizing, can reduce Cu^{+}, Cu^{2+}, Bi^{3+}, Ag^{+} consisting of oxides or hydroxides corresponding to free metals.

This established a number of ways qualitative and quantitative determination of carbohydrates.



Progress of experiment. Two tubes are filled with: (1) 3 ml of 1% glucose solution and (2- "control for excess presence of CuSO4") 3 ml of water, then 1 ml of 10% sodium hydroxide solution and 1% solution of copper sulfate solution are added to both tubes. In the presence of glucose, the formed precipitate of copper (II) hydroxide dissolves, turning the liquid blue. The upper layer of liquid is heated to boiling. The appearance of a yellow, then red precipitate indicates the oxidation of glucose and the reduction of copper.

During the Trommer reaction, the following copper compounds can be formed:

Copper (II) hydroxide (Cu (OH) 2) is blue

Copper (I) hydroxide (CuOH) - yellow

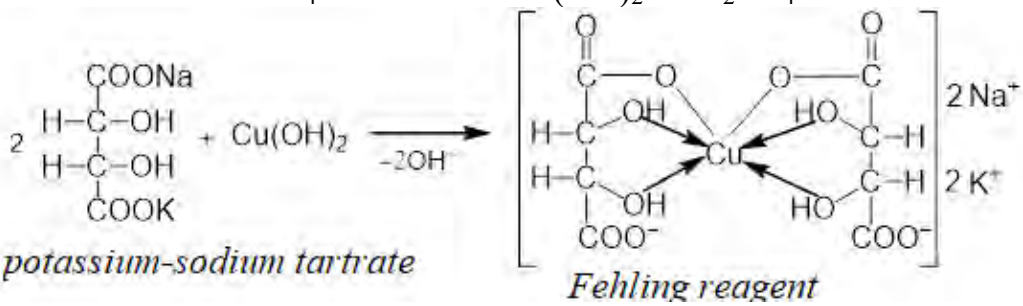
Copper (I) oxide (Cu2O) is red

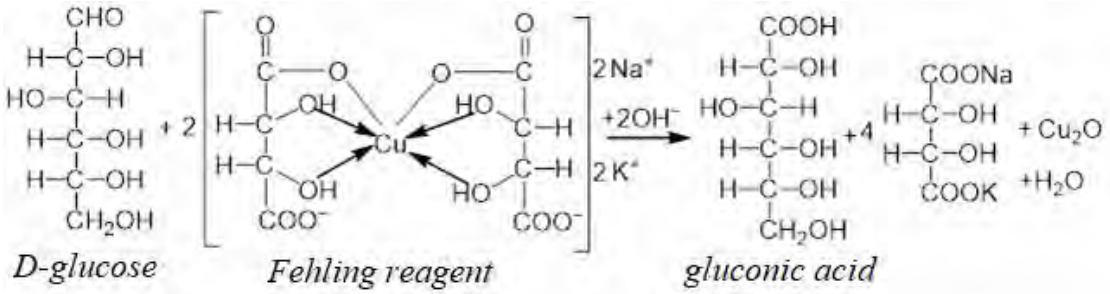
Copper (II) oxide (CuO) - black

3. Quantitative determination of glucose in solution by the Fehling method

Principle Fehling reaction is the oxidation of glucose and reduction due to this divalent copper into monovalent or metallic copper. Fehling reagent (alkaline solution copper complex Rochelle salt) by mixing equal amounts of a solution of copper sulfate (Fehling 1) and an alkaline solution of Rochelle salt (potassium-sodium tartrate; Fehling 2).

$$CuSO_4 + 2NaOH = Cu(OH)_2 + Na_2SO_4$$





Progress of experiment. In a flask of 100 ml, it is poured 10 ml of Fehling reagent (prepared by mixing 5ml of the Fehling-solution-1 and 5ml of the Fehling-solution-2), then 40 ml of water are added, and heated to boiling. In boiling liquid, the test solution of glucose slowly introduces, stirring constantly the inner of the flask. The reaction is considered complete if fully precipitate copper (I) oxide and liquid over sediment will be transparent.

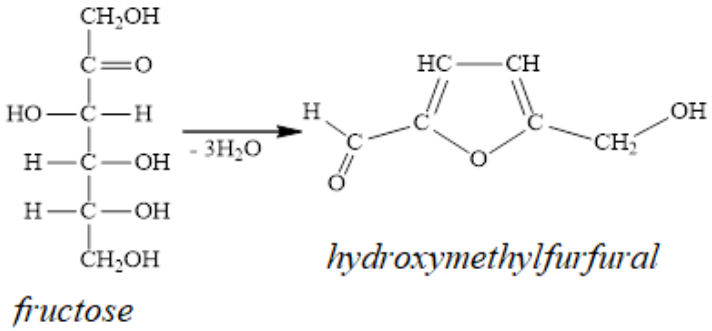
Interest glucose (in the test solution) is calculated based on the fact that 1 ml of working reagent Fehling restores 0.005 grams of glucose. Hence, used in the experiment Fehling reagent (10 ml), restores 0.05 g of glucose. For example, the restoration of Fehling reagent used 10 ml of glucose solution, ie 10 ml solution contains 0.05 g of glucose and 100 ml is

x =0,05 *100 / 10 = 5/10 = 0.5%

Answer: The solution taken for the experiment contains 0.5% glucose.

4. **Selivanov's reaction to ketohexose**

This is a qualitative reaction to carbohydrates with a keto group (ketose), fructose, sucrose, fructans. The reaction is based on the formation of an unstable substance that originated from fructose when it heated with acid; it is called hydroxymethylfurfural, which with resorcin (m-Dihydroxybenzene) gives a cherry-red color.



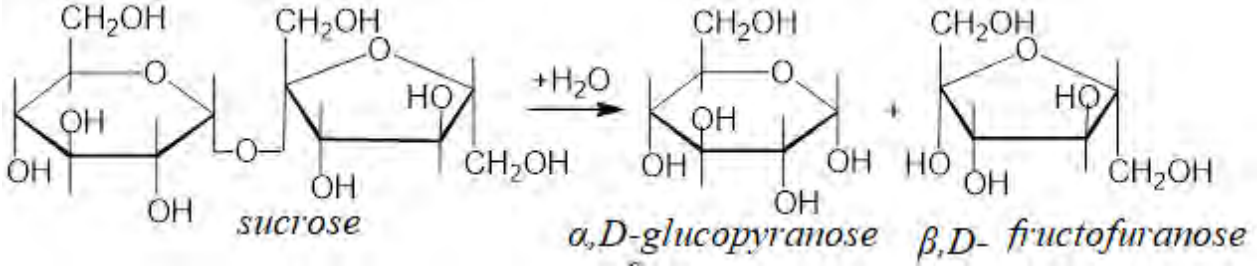
Progress of experiment. In a test tube with 2-3 ml of 1% solution of fructose, it is added the same volume of concentrated hydrochloric acid and then, a few crystals of resorcin are added. The test tube with the liquid is heated for 1-2 minutes in a boiling water bath. Observe a change of color of the solution.

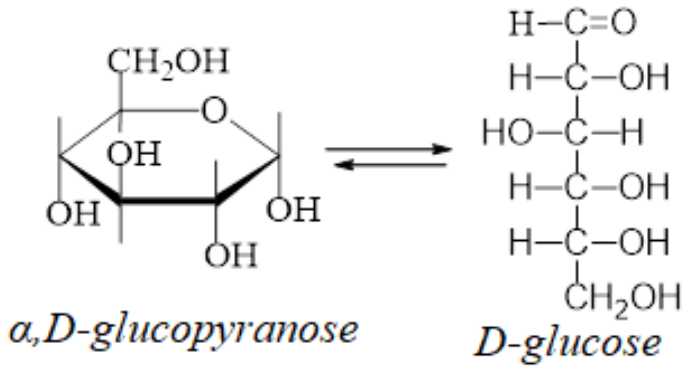
5. **Reactions to sucrose. Reduction of metal ions**

This reaction demonstrates the lack of ability of sucrose to reduce metal ions, due to the bound state of the carbonyl groups of hexoses, which form its molecule.

Progress of experiment. In two test tubes, it is poured 2-3 ml of sucrose solution. In one of them, a few drops of 2.5% hydrochloric acid are added, stirred and boiled for 3-5 minutes on open heat. The liquid is then cooled and neutralized (litmus paper is monitored) by adding 10% sodium hydroxide solution. Trommer's reaction is carried out with both samples (boiled and unboiled).

A negative Trommer reaction in the first test tube indicates the absence of free carbonyl groups in sucrose. A positive Trommer reaction in the second (boiled) test tube - the formation of a red precipitate indicates that boiling hydrolyzed sucrose and released carbonyl groups.



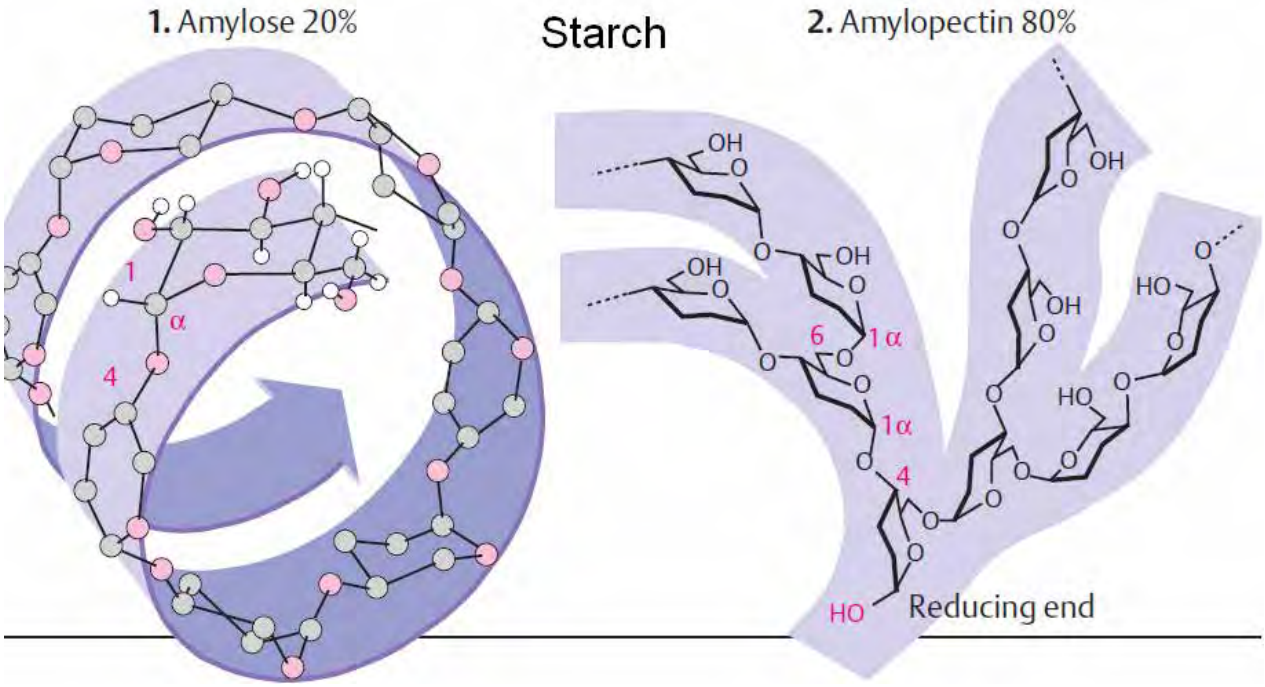


Control questions, tasks and exercises for the section
«CARBOHYDRATES: MONOSACCHARIDES, DISACCHARIDES»

1. Draw the structural formula of sucrose._
2. Give the structural formulas of glucose and fructose, their common and distinctive features.
3. What reactions are characteristic for the determination of aldehydes?
4. The value of glucose for living organisms (animals, humans).
5. What are the chemical reactions of monosaccharides and disaccharides? Give examples.
6. Write the structural formulas of lactose, maltose, cellobiose.

1.3.2. ***Topic*** CARBOHYDRATES: POLYSACCHARIDES

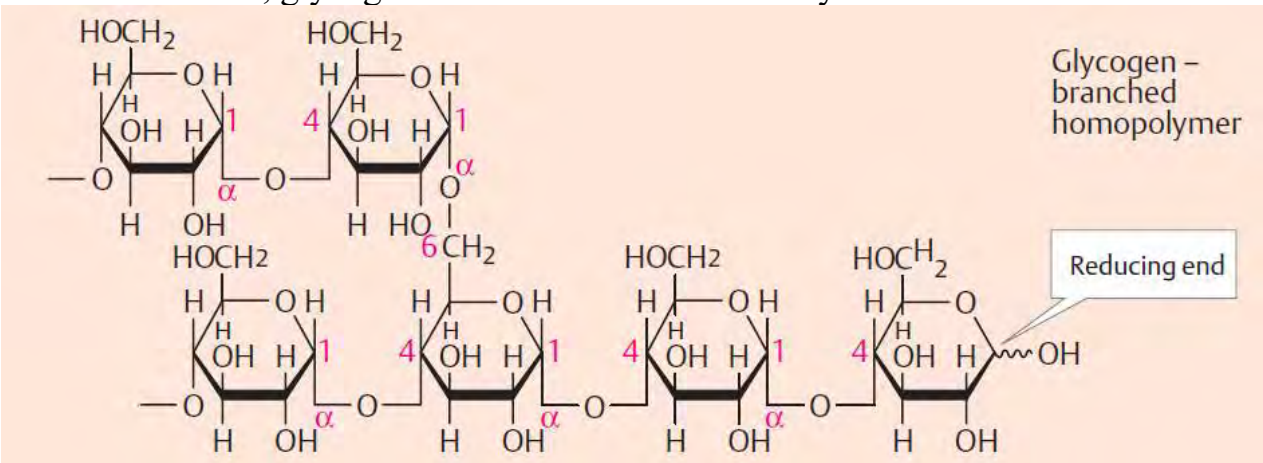
Starch is polysaccharide of plants which is synthesized in the cellular organelles (chloroplasts and aminoplasts) of plant leaves in a result of processes of photosynthesis. Starch is accumulated as seeds with different shape.

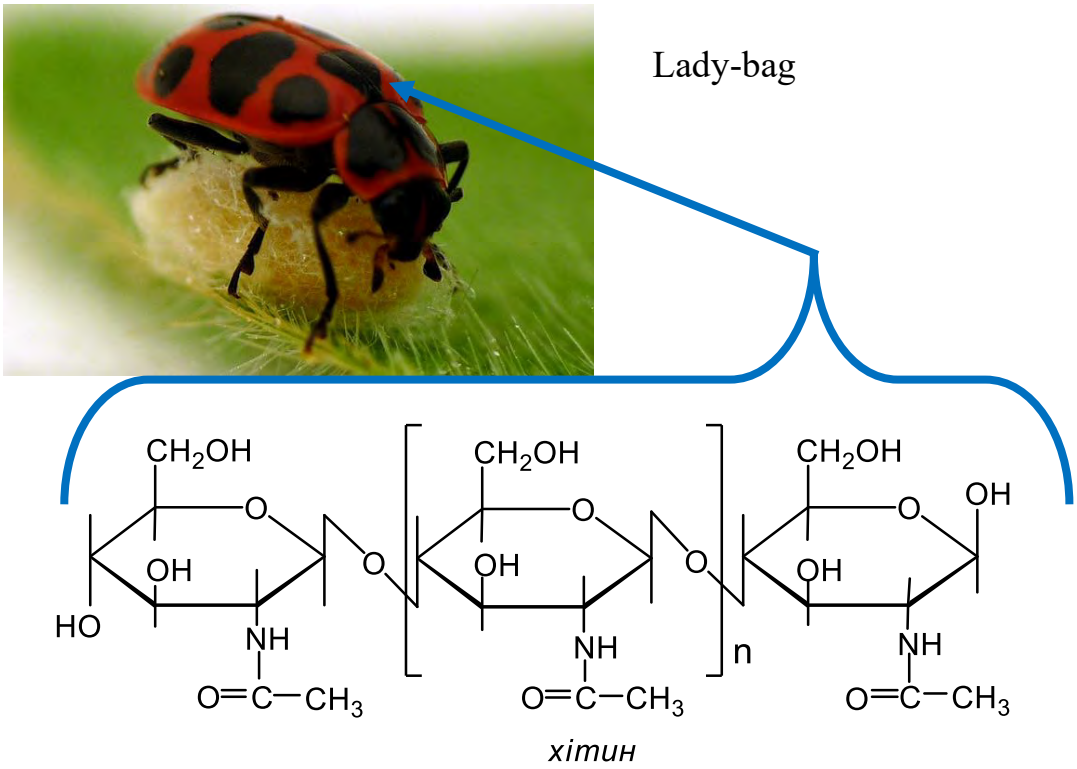


Starch is a mixture of two polysaccharides: linear polymer (amylose which is constructed by residues α-D-glucopyranose, that is bonded 1 → 4 bonds; 10 –30 %) and brunched-chain polymer (amylopectin, in which residues of α-D-glucopyranose are bonding by 1→4 and 1→6 bonds; 70 – 90 %)

Glycogen is called “animal starch”. Its molecule is consisted of *400 thousand* to *50 million* residues of glucose. The highest content of glycogen is in the hepatic cells (0,2 – 2 % of total dry mass), skeleton muscles (0,2 – 2 %).

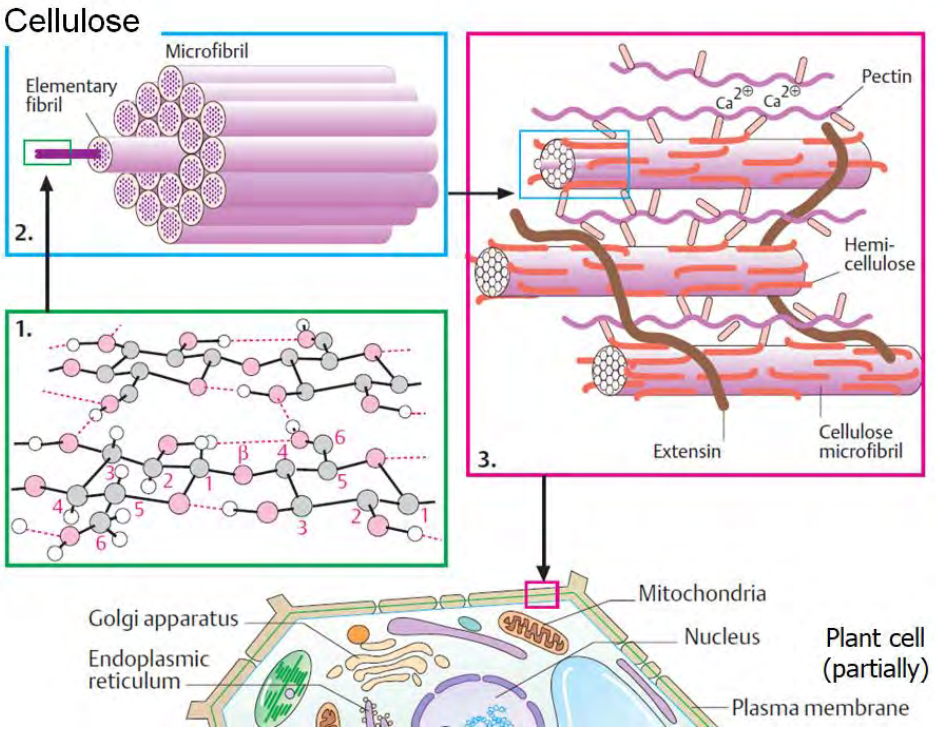
Sometimes, glycogen can be in mushrooms and yeast.





Chitin

Chitin, a homopolymer from β1→4-linked *N*-acetylglucosamine, is the most important structural substance in insect and crustacean shells and is thus the most common animal polysaccharide. It also occurs in the cell wall of fungi.



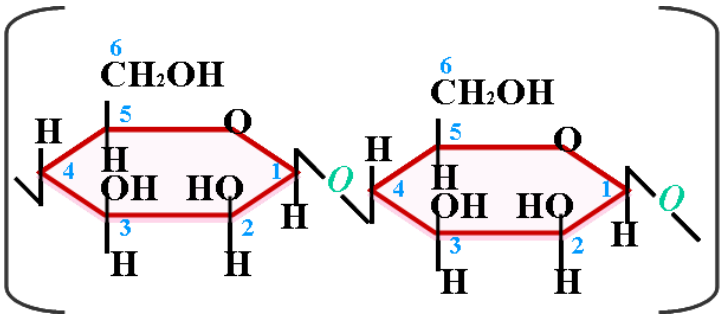
Cellulose is polysaccharide. All cellular walls of plants are constructed from the polysaccharide.

From natural raw material, cellulose is prepared by reagents that dissolve and demolish non-cellulose components (lignin etc.) under conditions (105 – 180 °C; higher pressure) with use sulfate, sulfite and basic (alkaline) boiling.

In microorganisms, cellulose is hydrolyzed by ferments (cellulase and cellobiase) or under effect of mineral acids (HCl, H_2SO_4):

Cellulose → amiloid → cellobiose → glucose

In the higher animals, including humans, cellulose is **indigestible**, but important as **roughage**. Many herbivores (e. g., the ruminants) have symbiotic unicellular organisms in their digestive tracts that break down cellulose and make it digestible by the host.



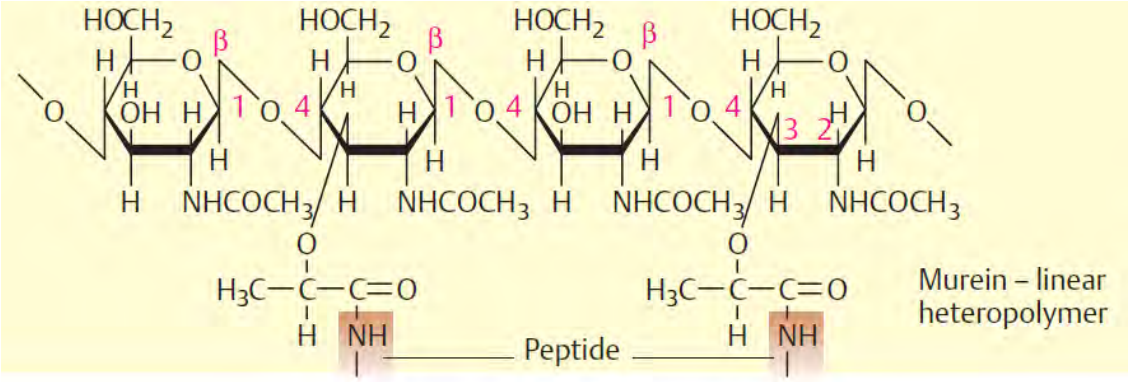
Bonding by β(1→4)–bonds of mannanopyranose in mannan oligosaccharide obtained from nuts of elephant's palm-tree

Mannan-oligosaccharide (which is obtained from nut of *elephant's* palm-tree) is consisted of residues of mannopyranose. Commonly, type of bond between monomers in mannan oligosaccharide depends on kind of source of plant or microbial origin (seaweeds, needles timber, peels of citruses, yeasts, fungi, etc. They are also in the content of blood, saliva, mucus intestine/gut, fluid of joints, hormones, glycolipids, glycoproteins.

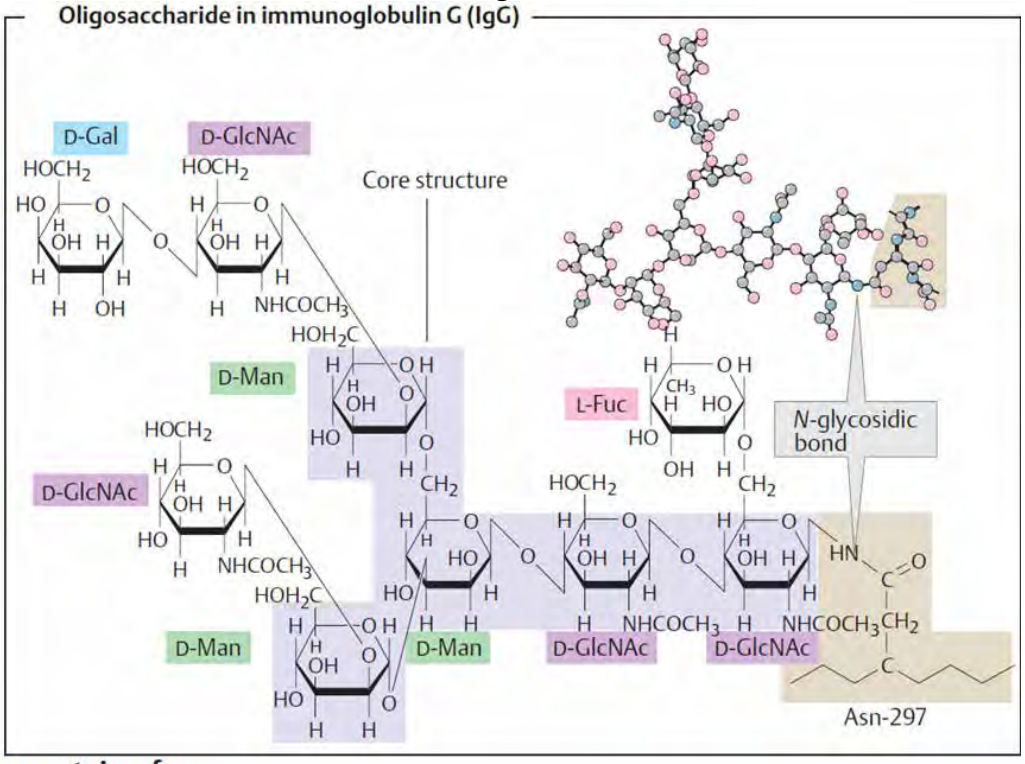
The other polysaccharides are fructans.

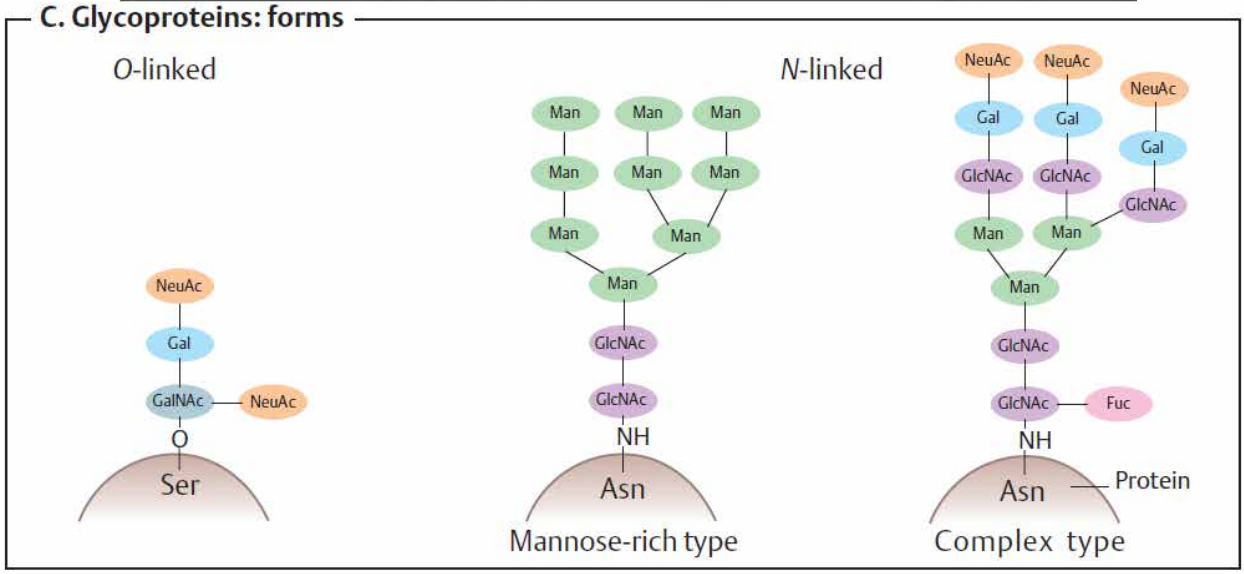
The important role of fructans of plant and bacterial origin is in keeping high metabolic ability of microorganisms-symbionts in digestive system of ruminants.

Inulin, a fructose polymer, is used as a starch substitute in diabetics' dietary products. In addition, it serves as a test substance for measuring renal clearance

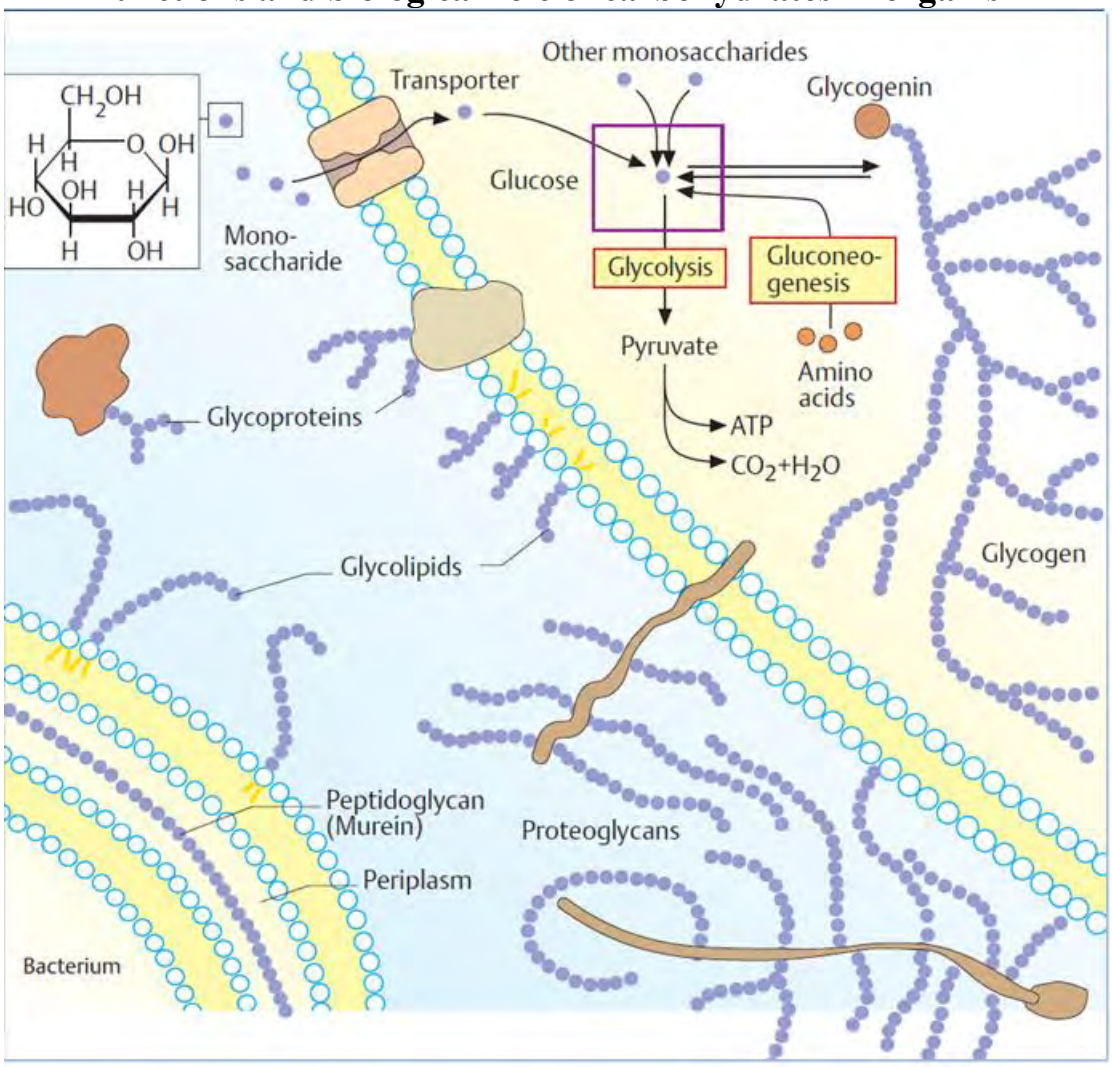


The linear heteroglycan **murein**, a structural polysaccharide that stabilizes the cell walls of bacteria, has a more complex structure.





Functions and biological role of carbohydrates in organism



LAB-CLASS

1. Color reactions to starch

A characteristic reaction to starch is the appearance of a blue color from a solution of iodine in potassium iodide due to the formation of a complex of starch with iodine.

Progress of experiment. In two test tubes, make 2-3 ml of 1% starch solution, add one drop of Lugol's solution (J_2 + KJ). The liquid in each tube turns blue, which disappears when heated and reappears when cooled. Therefore, iodine tests should be performed only with cold solutions of polysaccharides.

2. Colloidal properties of starch

Starch grains of different origins differ in size, shape and structure. They are insoluble in cold water, but swell in hot water and give a thick solution of a polymer called starch paste, similar in properties to colloids.

Progress of experiment. In two test tubes, 1% starch solution (2-3 ml) is poured. Crystalline $(NH_4)_2SO_4$ is added to the test tube (1) until fully saturated. A precipitate of starch (salting) is formed over the undissolved ammonium sulfate. In

a test tube (2), to the starch solution, ethanol is added and observed the formation of a precipitate (starch precipitates).

3. **Acid hydrolysis of starch**

Starch does not show pronounced reducing properties, but when heated with concentrated mineral acid, it is hydrolyzed to dextrins of varying complexity and glucose monosaccharide.

Progress of experiment. In a test tube, it is poured 3-5 ml of a 1% solution of starch and a few drops of concentrated hydrochloric acid. Stir well and boil on an open fire. Aliquots for the qualitative reaction on starch are taken before acid hydrolysis (0 min) and (2, 4, 6, 10, 15, 20, and 30 min from the beginning of hydrolysis). Example of a selection of an aliquot for a qualitative reaction on starch: after 2 minutes after the start of boiling, take a sample to react with iodine. Pipette a few drops of the solution into a test tube containing 5-6 ml of distilled water, and add 1-2 drops of Lugol's solution here. Purple color is formed, which indicates the presence of amylodextrins in the solution, etc.

Dependence of the level of acid hydrolysis of starch on its duration

Time, min	0	2	4	6	10	15	20	30
The color of the product of a qualitative reaction to starch								
The level of acid hydrolysis (–or +)								

The hydrolyzed starch solution is neutralized with 10% sodium hydroxide solution (verifying by litmus), the Fehling reagent is added and heated. Observe a yellow precipitate of CuOH or red Cu_2O precipitates (after glucose test).

Control questions, tasks and exercises for the section «CARBOHYDRATES: POLYSACCHARIDES»

1. Give examples of polysaccharides and their presence in a living organism.
2. What monomers are polysaccharides?
3. How can starch be detected?
4. Describe homopolysaccharides, give examples
5. How do heteropolysaccharides differ from homopolysaccharides?
6. What are the functions of polysaccharides in a living cell?

1.3.3. ***Topic*** LIPIDS AND THEIR COMPONENTS

Lipids are organic substances. Their main characteristics are insolubility in water and in other polar solvents and an ability to dissolve in non-polar (hydrophobic) liquids.

Non-polar solvents: diethyl ether, dichlorethan, chloroform.

Non-polar solvents are used for lipid extraction from biological objects (blood, tissues, food-products etc.)

According to chemical structure, lipids are esters of high molecular carbonic (fatty) acids and alcohols (glycerol, sphinhosine, cholesterols etc).

Complex lipids also include residues of phosphoric acid, nitrogenous alcohols (*cholamine*, cholin), carbohydrates.

A **biological membrane** or **biomembrane** is an enclosing or separating membrane that acts as a selective barrier, within or around a cell. It consist of a lipid bilayer with embedded proteins that may constitute close to 50% of membrane content. The cellular membranes should not be confused with isolating tissues formed by layers of cells, such as mucous and basement membranes.

Membranes in cells typically define enclosed spaces or compartments in which cells may maintain a chemical or biochemical environment that differs from the outside. For example, the membrane around peroxisomes shields the rest of the cell from peroxides, and the cell membrane separates a cell from its surrounding medium.

Most organelles are defined by such membranes, and are called "membrane-bound" organelles.

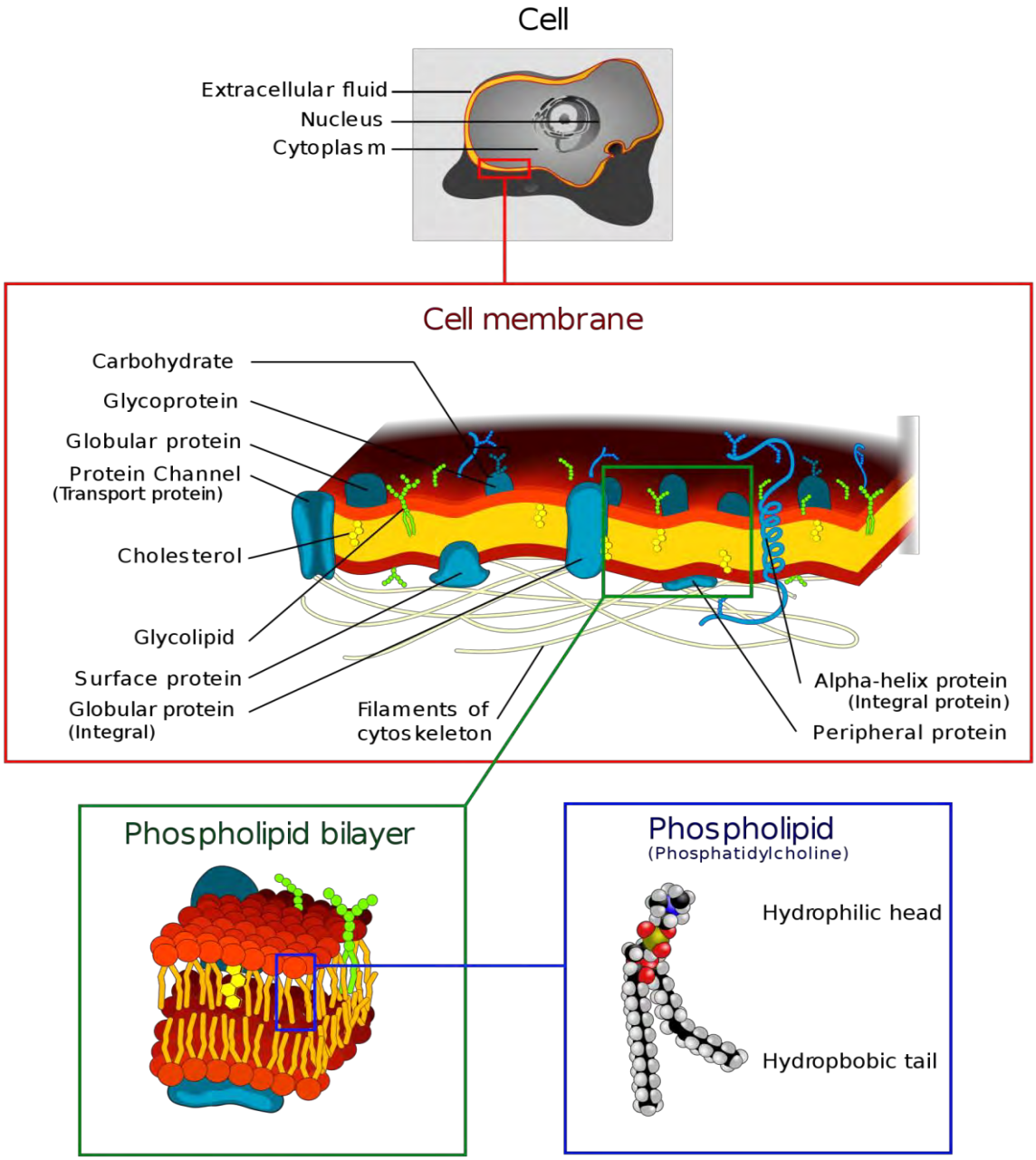
Probably the most important feature of a biomembrane is that it is a selectively permeable structure. This means that the size, charge, and other chemical properties of the atoms and molecules attempting to cross it will determine whether they succeed in doing so. Selective permeability is essential for effective separation of a cell or organelle from its surroundings. Biological membranes also have certain mechanical or elastic properties. Particles that are required for cellular function but are unable to diffuse freely across a membrane enter through a membrane transport protein or are taken in by means of endocytosis.

The **cell membrane** is a biological membrane that separates the interior of all cells from the outside environment.

The cell membrane is selectively-permeable to ions and organic molecules and controls the movement of substances in and out of cells. It consists of the phospholipid bilayer with embedded proteins.

Cell membranes are involved in a variety of cellular processes such as cell adhesion, ion conductivity and cell signaling and serve as the attachment surface for the extracellular glycocalyx and cell wall and intracellular cytoskeleton.

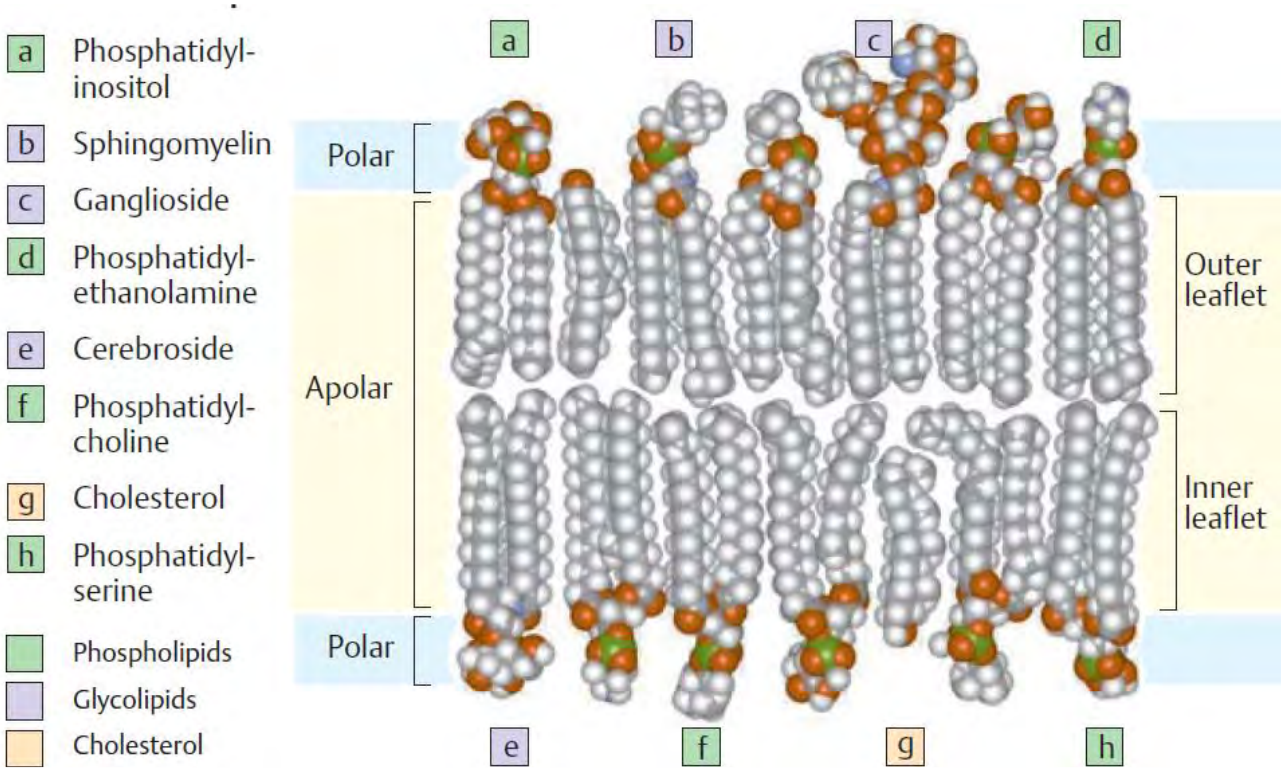
Many types of specialized plasma membranes can separate cell from external environment: apical, basolateral, presynaptic and postsynaptic ones, membranes of flagella, cilia, microvillus, filopodia and lamellipodia, the sarcolemma of muscle cells, as well as specialized myelin and dendritic spine membranes of neurons.



Plasma membranes can also form different types of "supramembrane" structures such as caveola, postsynaptic density, podosome, invadopodium, desmosome, hemidesmosome, focal adhesion, and cell junctions.

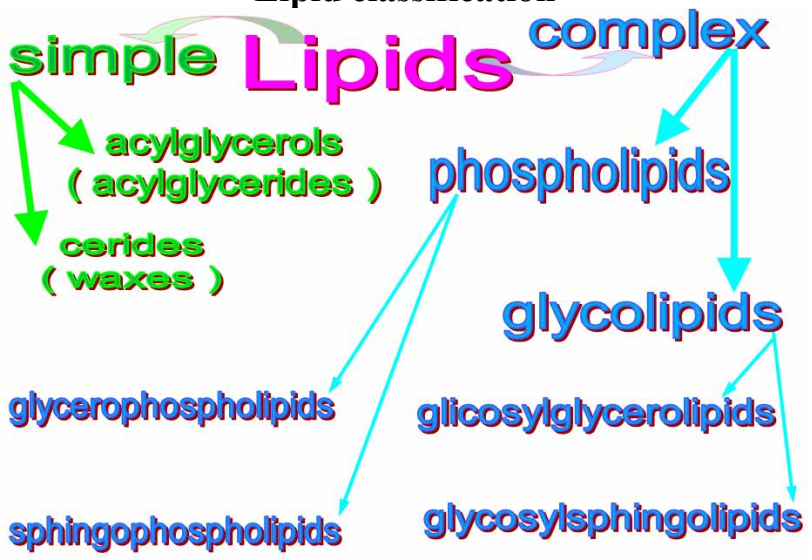
These types of membranes differ in lipid and protein composition.

The **phospholipids** are the most important group of membrane lipids. They include *phosphatidylcholine* (lecithin), *phosphatidylethanolamine*, *phosphatidylserine, phosphatidylinositol,* and *sphingomyelin*. In addition, membranes in animal cells also contain **cholesterol** (with the exception of inner mitochondrial membranes). **Glycolipids** (a *ganglioside* is shown here) are mainly found on the outside of the plasma membrane. Together with the *glycoproteins,* they form the exterior coating of the cell (the *glycocalyx*).



Cited: ***Jan Koolman, Klaus-Heinrich Roehm*** **Color Atlas of Biochemistry**. Second edition revised and enlarged. 2005. Thieme Stuttgart New York. – 476 p

Lipid classification



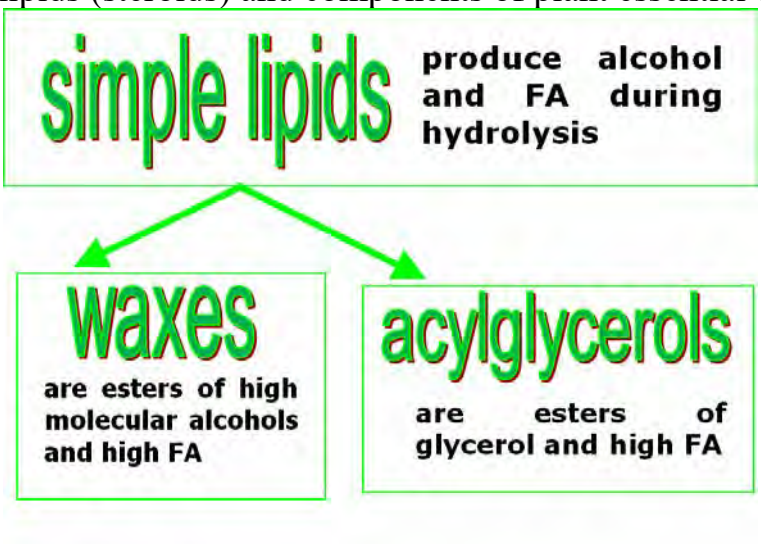
The mentioned classes of lipid compounds include the so-called saponificated lipids which are capable to form alcohols and carboxylic acids during acid or alkaline hydrolysis in the case of simple lipids and additional substances (phosphoric acid, nitrogen compounds, mono- and oligosaccharides) in the case of complex lipids.

Saponification

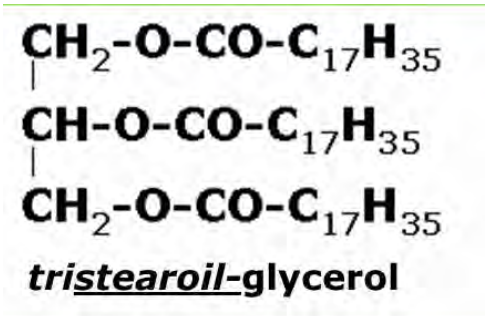
When fatty acids are required in free form for further analysis, lipids (presented as glycerides, glycerophosphatides, glycosyldiglycerides, sterides or waxes) are firstly hydrolyzed in alkaline medium allowing to extract also the unsaponifiable material if present in the crude lipid mixture (sterols, alcohols, hydrocarbons, pigments, vitamins etc.).

Glycosphingolipids are poorly hydrolyzed with the described procedure but, if any contribution of these complex lipids is to be avoided, a mild saponification process must be adopted.

Depending on physico-chemical properties of the lipids, they are also various unsoponificated lipids (steroids) and components of plant essential oils (terpenes).



- **Triacylglycerol (TAG; neutral fats; which is consisted of glycerol and the same fatty acids) is called simple.**
- **TAG (in composition of which are different FA) are called mixed. Natural TAG are mixed**



Waxes

For instance, waxes of animal origin: *bee wax* ($C_{15}H_{31}C(O)O\text{-}C_{30}H_{61}$ – ester of palmitic acid and myricilic alcohol), lanolin *of sheep wool*, *spermaceti* ($C_{15}H_{31}C(O)O\text{-}C_{16}H_{33}$ – ester of palmitic acid and cetylic alcohol) *from whale tissues*, which are used in pharmacy for preparing ointments, creams, in production of cosmetics.

Plant fats which include mainly remains of unsaturated FA are more often are liquid (oils) – sunflower, *linen*, etc.

It's important to know that some of animal fats (fish fat) are liquid and some of plant fats (*coconut* butter) are solid.

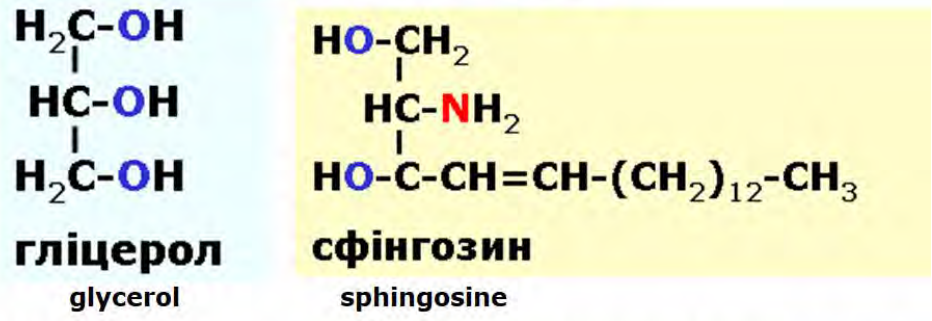
The degree of unsaturation of different fats is determined by ***Iodine number***, which is equal to Iodine mass (g) that is bonded with 100 g of investigative fat.

Under interaction with TAG, molecules of J_2 interact with unsaturated bonds of residues of FA, that's why increasing value of Iodine number corresponds to higher degree of unsaturation of FA including in composition of this TAG.

Content of FA in TAG of fatty tissue of human

Fatty acids	Content, %	Temperature of melting,°C
Saturated FA		
Myristic ($C_{14:0}$)	3	+54,4
Palmitic ($C_{16:0}$)	20	+62,8
Stearic ($C_{18:0}$)	5	+69,6
Unsaturated FA		
Palmitooleic ($C_{16:1}$)	5	+ 10
Oleic ($C_{18:1}$)	55-60	+13
Linoleic ($C_{18:2}$)	10	- 11,0
Arachidonic ($C_{20:4}$)	0,2	- 49,5

alcohols



High molecular alcohols are included into content of waxes: *cetylic* ($C_{16}H_{33}OH$), ***myricilic*** ($C_{30}H_{61}OH$)

fatty acids (FA)

Saturated FA ($C_nH_{2n+1}COOH$):

$C_{12:0}$	lauric (dodecanoic) acid	$C_{11}H_{23}COOH$
$C_{14:0}$	myristic (tetradecanoic) acid	$C_{13}H_{27}COOH$
$C_{16:0}$	palmitic (hexadecanoic) acid	$C_{15}H_{31}COOH$
$C_{18:0}$	stearic (octadecanoic) acid	$C_{17}H_{35}COOH$
$C_{20:0}$	arachidic (eycosanoic) acid	$C_{19}H_{39}COOH$

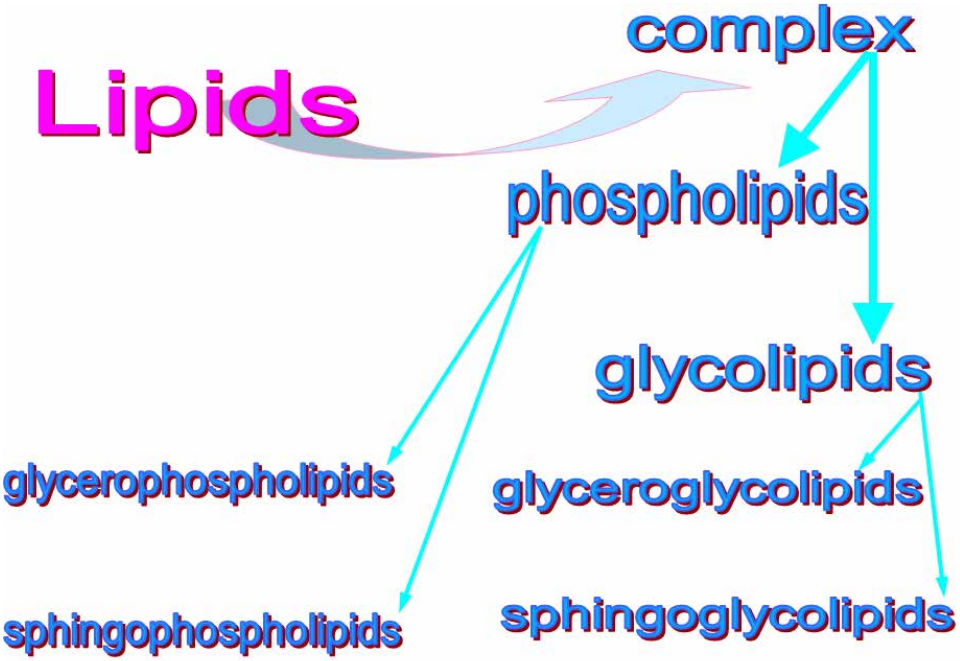
Unsaturated FA

Monoenic acids ($C_nH_{2n-1}COOH$):

$C_{16:1}$	palmitooleic (*cis*-hexadecen-10-ic) acid	$C_{15}H_{29}COOH$
$C_{18:1}$	oleic (*cis*-octadecen-9-ic) acid	$C_{17}H_{33}COOH$

Polyenic acids ($C_nH_{2n-3(5,7)}COOH$):

$C_{18:2}$	linoleic (*cis*-,*cis*-octadecadien-9,12-ic) acid	$C_{17}H_{31}COOH$
$C_{18:3}$	linolenic (*cis*-, *cis*-,*cis*-octadecatrien-9,12,15-ic) acid	$C_{17}H_{29}COOH$
$C_{20:4}$	arachidonic (*cis*-,*cis*-,*cis*-,*cis*-eicosatetraen-5,8,11,14-ic) acid	$C_{19}H_{31}COOH$

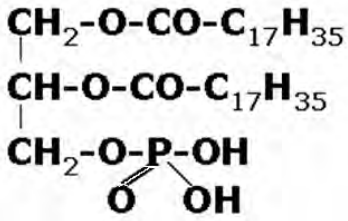


Complex lipids are hydrolyzed into alcohol (glycerol, sphingosine, inositol), FA and phosphoric acid, amino alcohol, carbohydrates, etc.

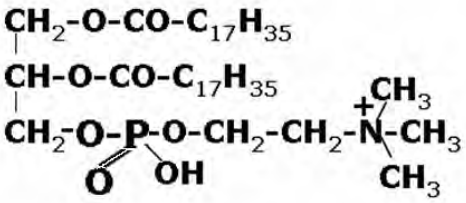
They are polar, amphiphilic substances, most of them are responsible for structural functions as compartments of biological membranes.

Glycerophospholipids **a**re esters of glycerol and high FA which are derivative of phosphatidic acid bonded *by residue of phosphoric acid and OH-group of amino alcohols* (*choline; ethanol amine* or *cholamine*) and *oxyamino acid serine*.

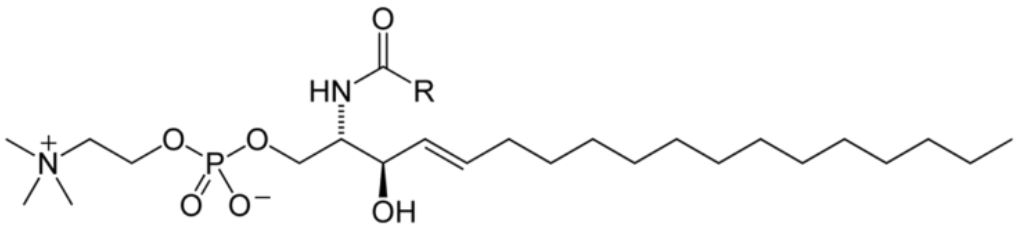
phosphatidic acid



***alpha*-lecithin**



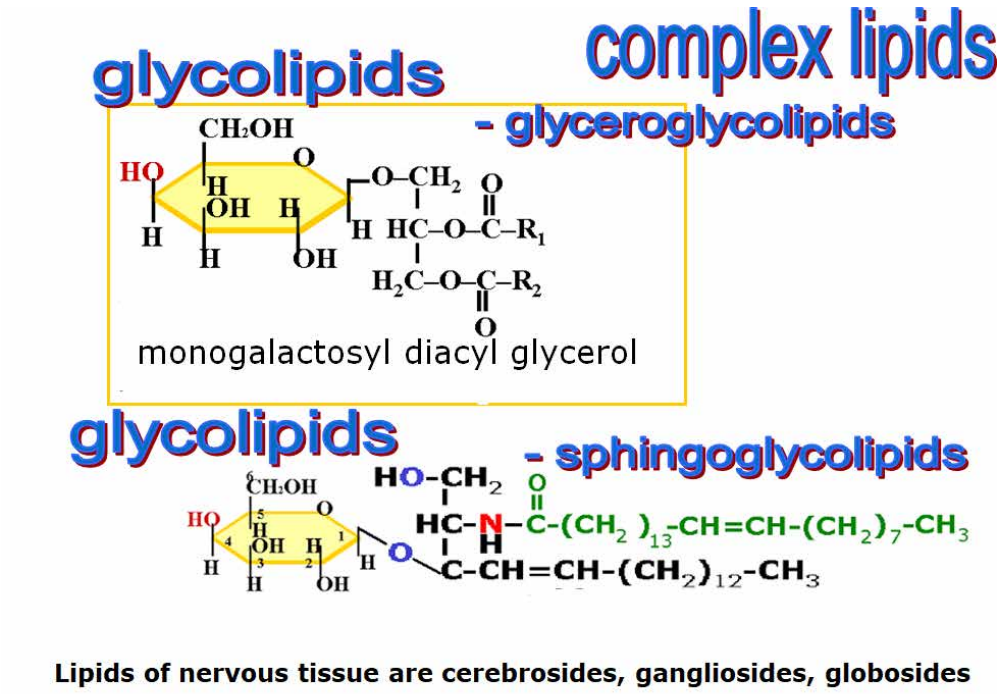
Sphingomyelin



The major phosphosphingolipids of mammals are sphingomyelins (ceramide phosphocholines), whereas insects contain mainly ceramide phosphoethanolamines and fungi have phytoceramide phosphoinositols and mannose-containing headgroups.

The glycosphingolipids are a diverse family of molecules composed of one or more sugar residues linked via a glycosidic bond to the sphingoid base. Examples

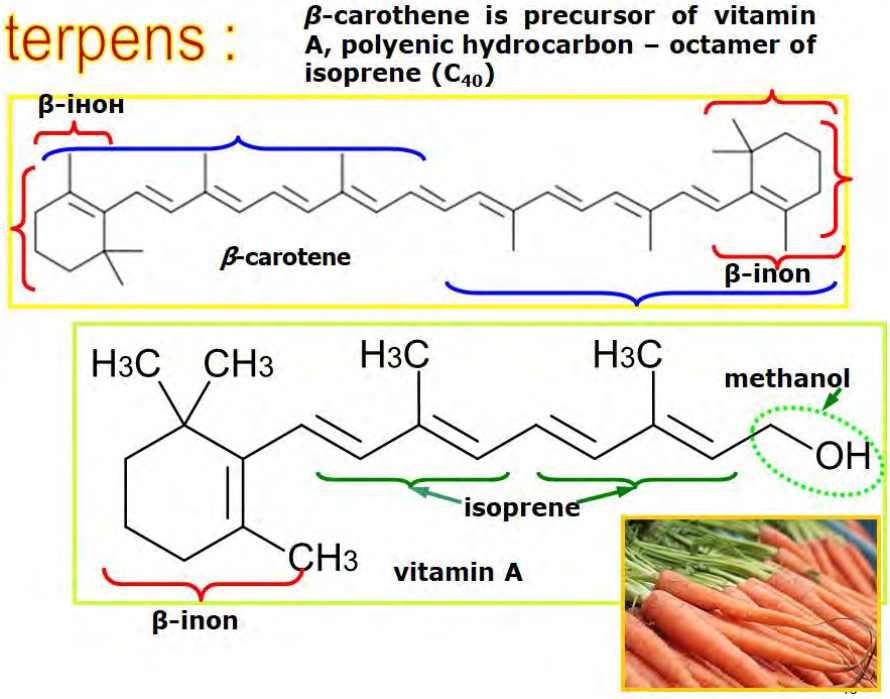
of these are the simple and complex glycosphingolipids such as cerebrosides and gangliosides.



Lipids of nervous tissue are cerebrosides, gangliosides, globosides

Terpens are belonged to organic substances which are unsaturated hydrocarbons. In the basis of their structure, there are residues of molecule isoprene (C_5H_8; 2-methylbutadiene-1,3) that are repeated (isoprenoids)

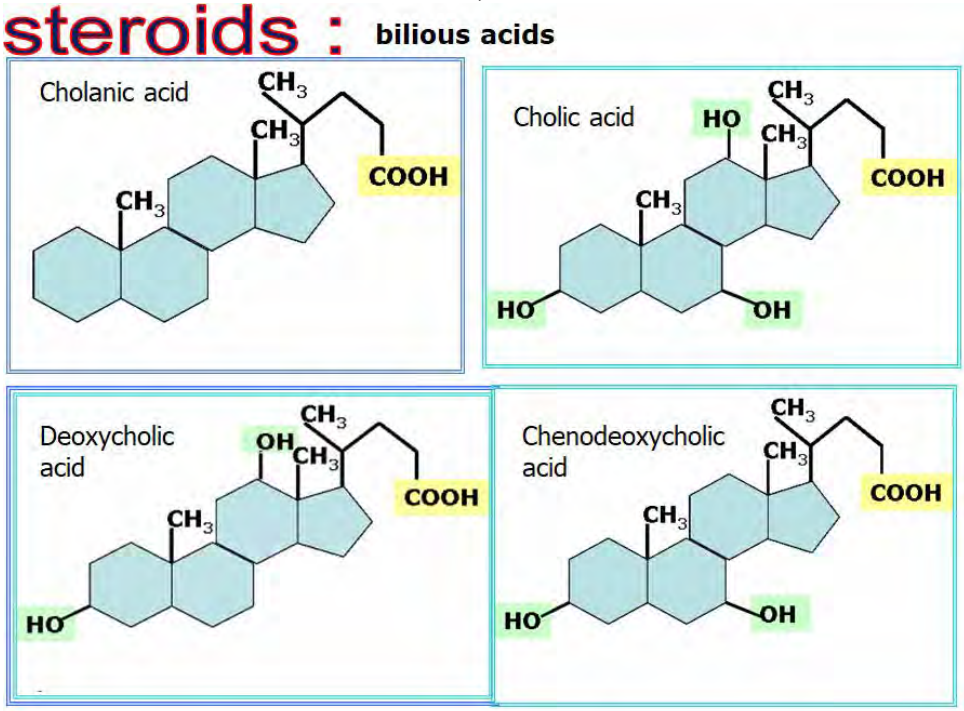
Carothenoids (*β*-carothene, vitamin A) are also biologically important derivatives of terpens, and vitamin E is derivative of diterpens.



Steroids are class of organic substances which are derevative of steran (cyclopentan perhydrofenantren). Cyclopentan perhydrofenantren (steran) is consisted of three fully hydrogenated inlinear condensed cyclohexane rings (A, B, C) and cyclopentan core (D).

Steroids according to biological properties, are belonged to low molecular regulators of metabolic processes and of physiological functions of organism.

There are vitamins of group D (D_2 and D_3), steroidal hormones of adrenal cortex, male and female sexual hormones, bilious acids.



Functions	Metabolism
Regulative (hormones of lipid nature); **Protective** (protective structure for internal organs, under skin fat); **Heat isolation** (under skin fatty cells); **Composition of vitamins** (D, E), plant pigments; **Energetic** (1 g = 39 kJ)	**Macromolecules of fats→digestion tract→glycerol, FA(→lymph)→blood →fatty deposition →synthe- tic processes**

LAB-CLASS

1. Solubility of fats in different solvents

Progress of experiment. In two test tubes, pour 2 - 3 ml of ethanol (1) and water (2). Add a few drops of oil to each tube, shake and observe the result of the

experiment. Similarly, a few drops of heated lard are added to the other two tubes (1a, 2a) with the appropriate solvents, shaken and observed. All four tubes are placed for 3-5 minutes in a boiling water bath. Enter the results in the table.

Solubility of fats in different solvents

Samples	1		2	
	before boiling	**after boiling**	**before boiling**	**after boiling**
Solubility				
Samples	1a		2a	
	before boiling	**after boiling**	**before boiling**	**after boiling**
Solubility				

2. The process of saponification of fats

Progress of experiment. Add 1 ml of fish oil (0.5 g of animal fat) and 5 ml of 30% sodium hydroxide solution to the test tube. Heat to boiling. Observe the formation of soap.

3. Soap hydrolysis

Progress of experiment. In a test tube, pour 5-6 drops of an alcoholic solution of soap and one drop of phenolphthalein. The mixture is not colored. After that, constantly shaking the tube, add drops of distilled water. Observe the appearance of color.

4. Detection of unsaturated fatty acids (FA) in fats

Progress of experiment. In a test tube, pour 1 ml of fat solution and one drop of 1% iodine solution. Shake.

5. Oxidation of unsaturated fats

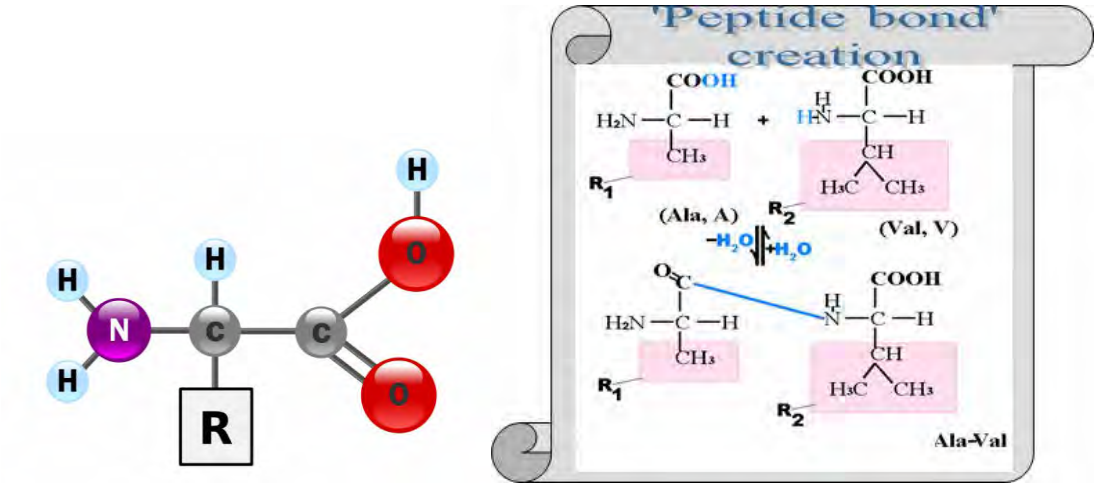
Progress of experiment. In a test tube, pour 2-3 ml of vegetable oil, 1-2 ml of 1% aqueous solution of potassium permanganate, the mixture is shaken. Observe the formation of a brown precipitate (MnO_2) and discoloration of the mixture.

Control questions, tasks and exercises for the section «LIPIDS AND THEIR COMPONENTS»

1. Give examples of complex lipids.
2. Name the constituent components of triacylglycerols (TAG).
3. What reactions can TAGs have? Give examples.
4. Write the structural formula of tripalmitylglycerol.
5. Write the structural formula of triacylglycerol, which has stearic acid residues in the α- and α'-positions, and palmitoleic in the β-position.
6. What chemical reactions can unsaturated fatty acids enter into in contrast to saturated ones?

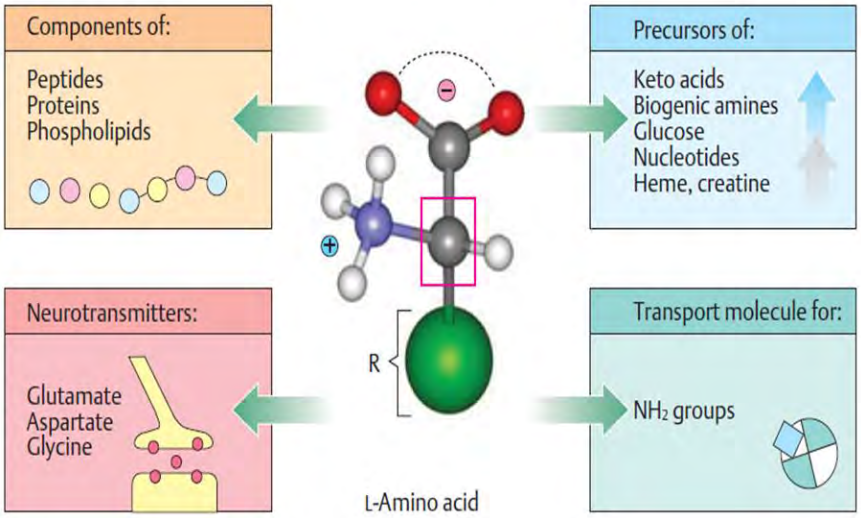
1.3.4. Topic AMINO ACIDS

Amino acids are molecules containing an amine group, a carboxylic acid group and a side chain that varies between different amino acids.

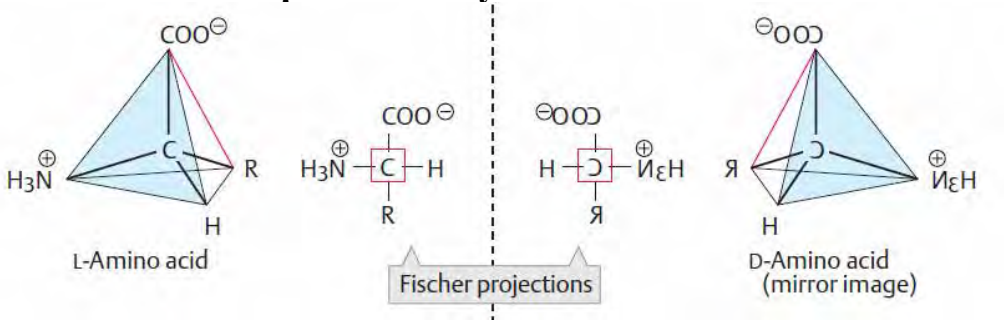


These molecules contain the key elements of carbon, hydrogen, oxygen, and nitrogen. These molecules are particularly important in biochemistry, where this term usually refers to alpha-amino acids with the general formula $H_2NCH(R)COOH$, where R is an organic substituent.

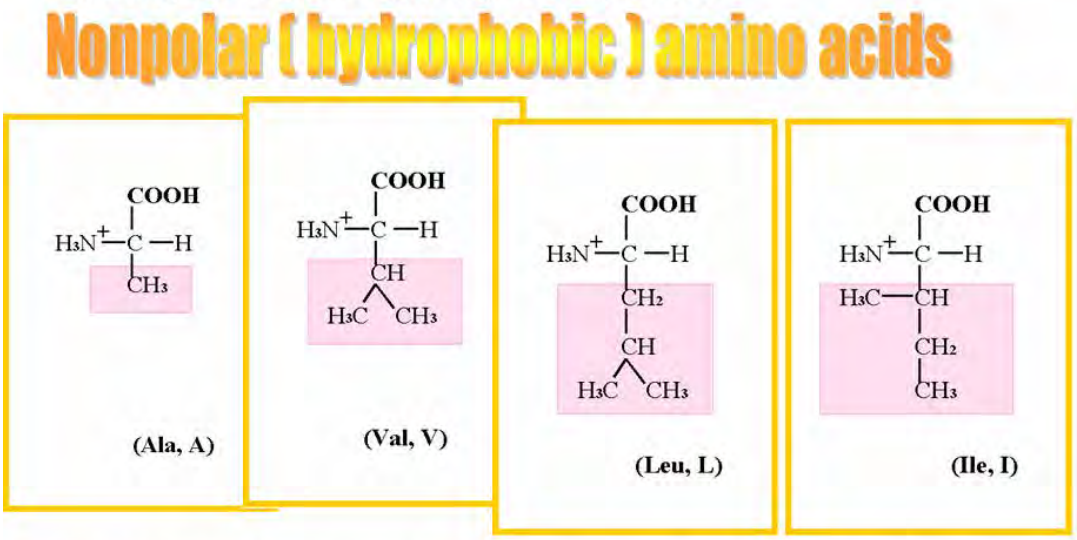
Amino acids: functions



Optical activity of amino acids

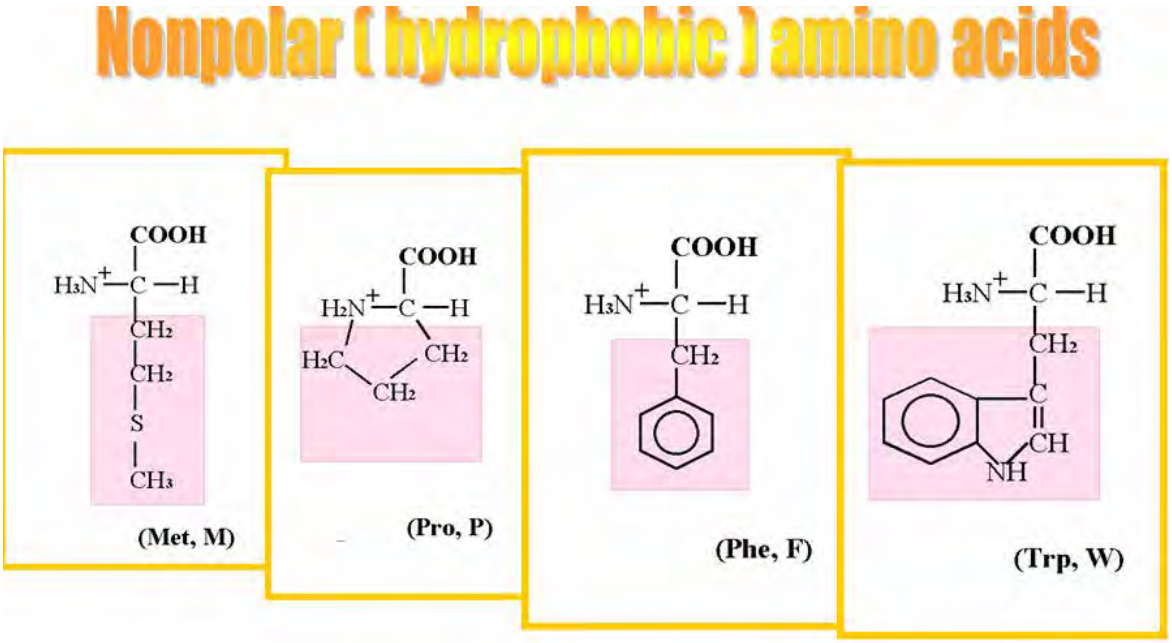


Amino acids are divided into 4 groups depending on properties of their radicals: hydrophobic (non-polar), hydrophilic (polar), acidic, basic.



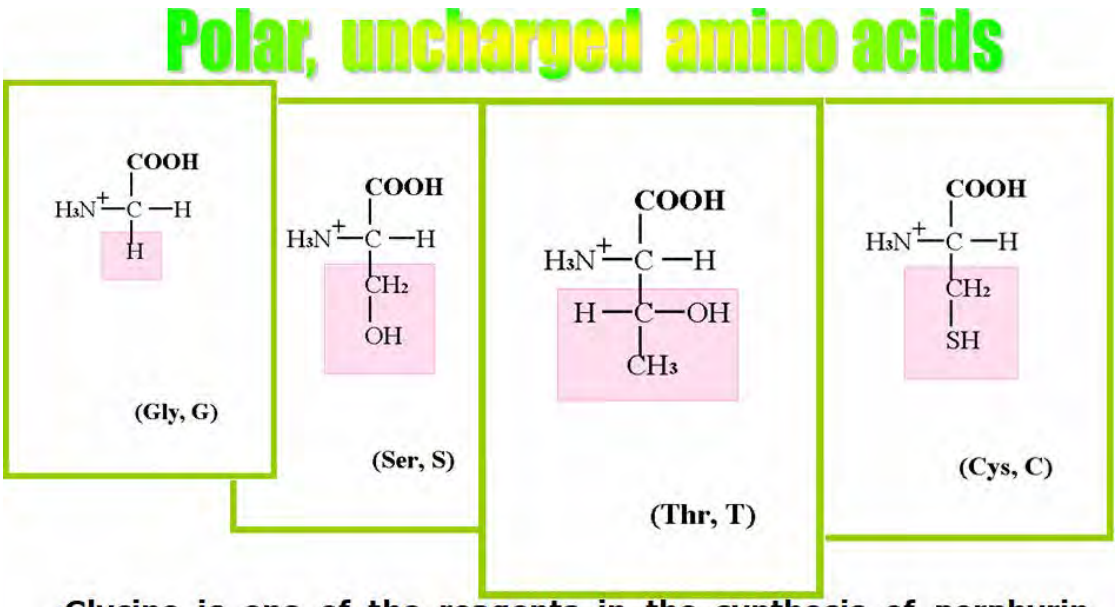
Besides protein synthesis, amino acids perform many other important biological functions in the animal body

alanine (Ala), valine (Val), leucine (Leu), isoleucine (Ile)



Tryptophan is a precursor to the neurotransmitter serotonin

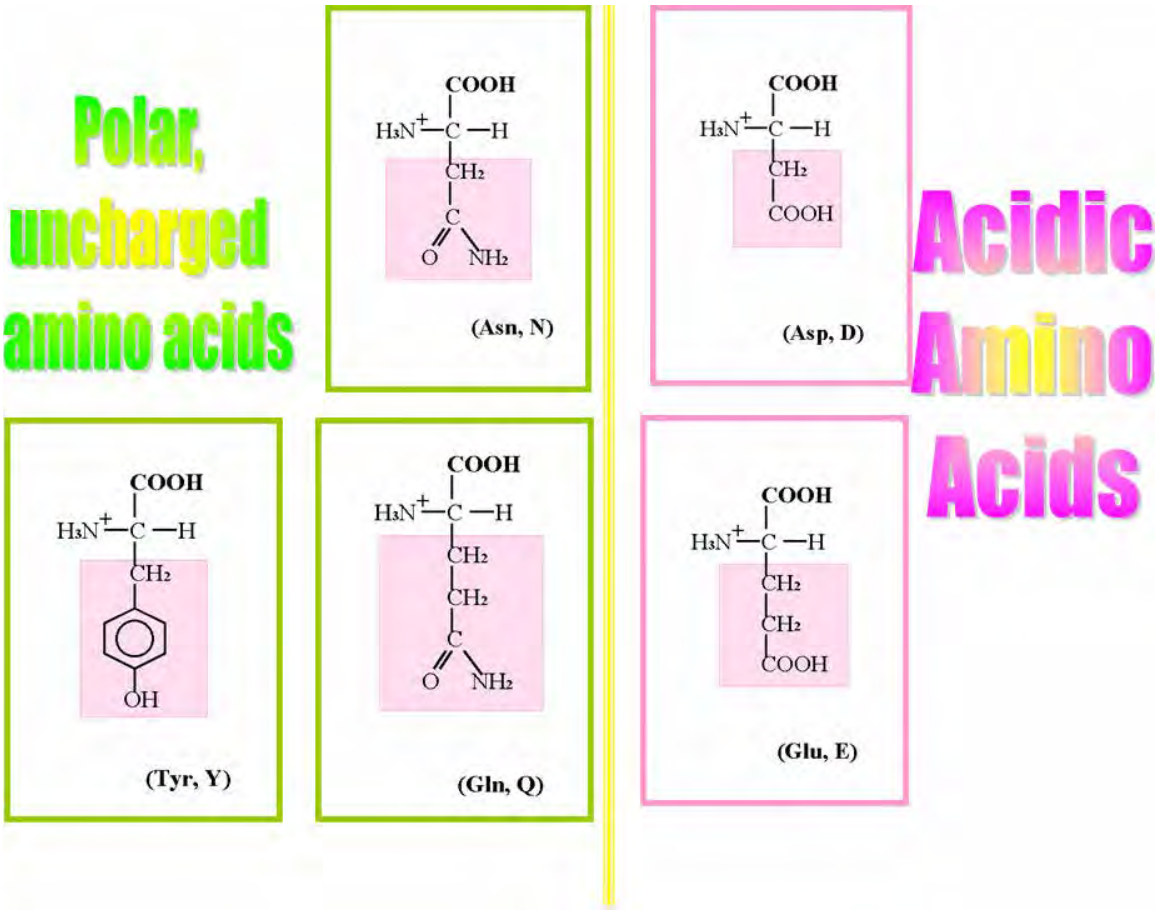
methionine (Met), proline (Pro), phenylalanine (Phe), tryptophan (Trp)



•Glycine is one of the reagents in the synthesis of porphyrin (such as respiratory pigment heme)

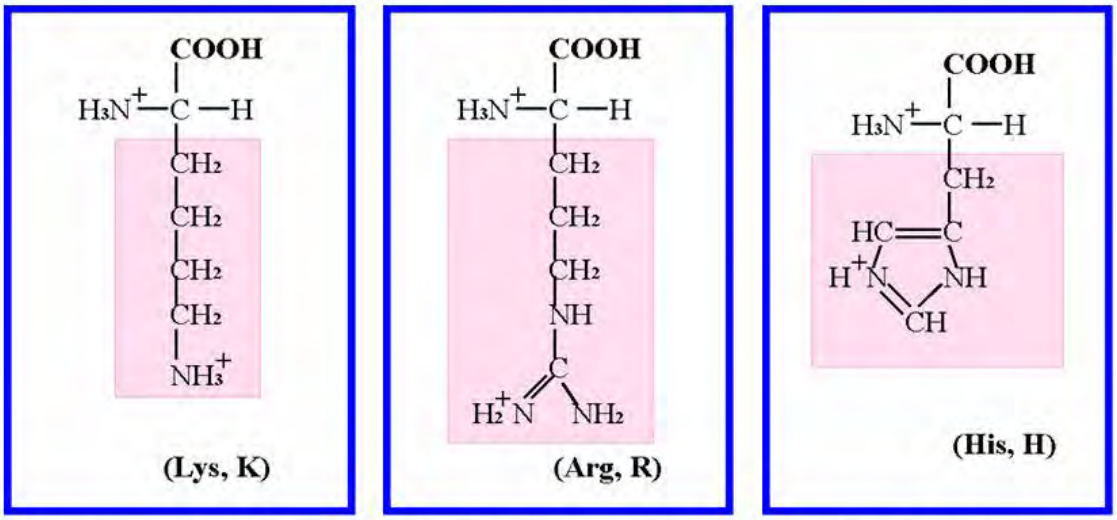
•Glycine and glutamate (glutamic acid anion) are monomers of the proteins and also used as neurotransmitters in the nervous transmission via chemical synapses.

glycine (Gly), serine (Ser), threonine (Thr), cysteine (Cys)



asparagine (Asn), tyrosine (Tyr), glutamine (Gln)
aspartic acid (Asp), glutamic acid (Glu)

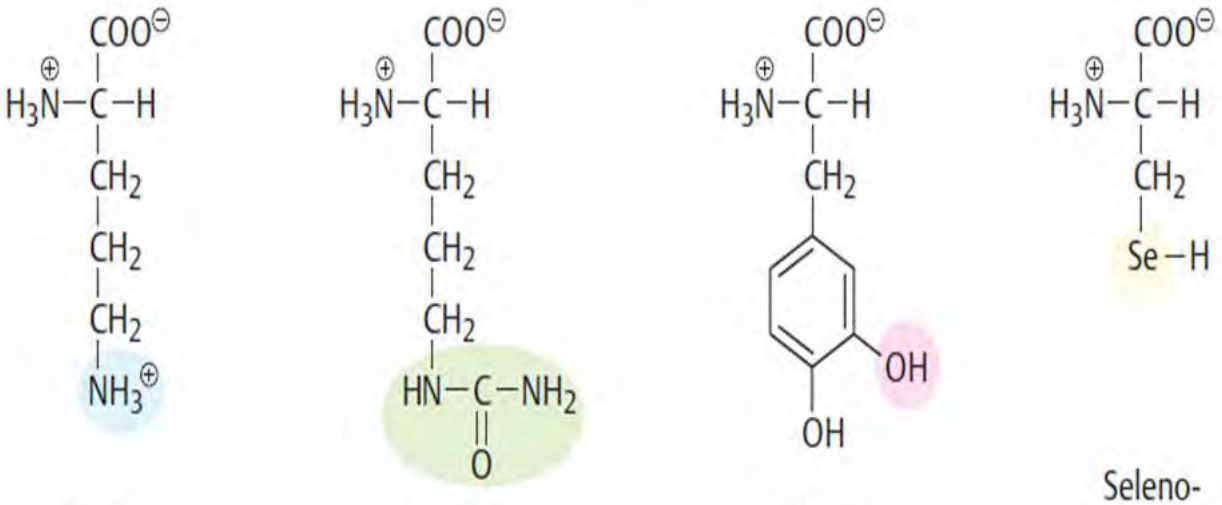
Basic Amino Acids



Also important are biologically and non-standard amino acid, GABA (gamma-aminobutyric acid - another neurotransmitter), carnitine (used to transport lipids in the cell), ornithine, citrulline, homocysteine, hydroxyproline, hydroxylysine, sarcosine etc.

lysine (Lys), arginine (Arg), histidine (His)

Rare amino acids



dopamine

The transmitter *dopamine* is also a precursor for the catecholamines epinephrine and norepinephrine

LAB-CLASS

Separation of a mixture of amino acids by paper chromatography

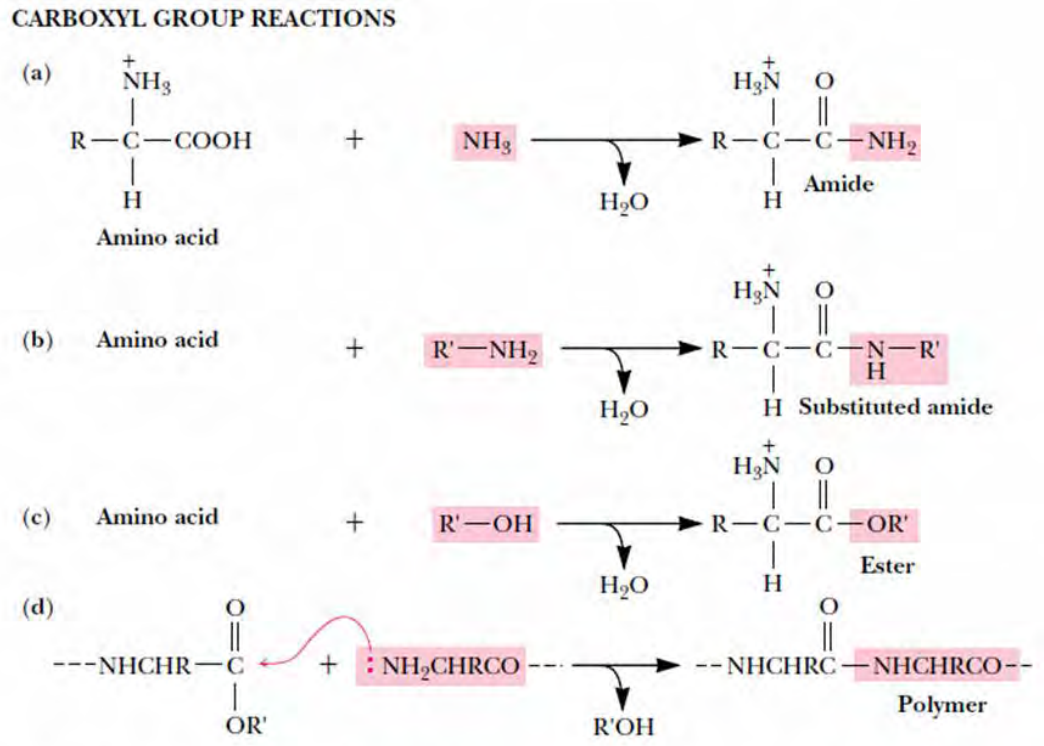
For example, a drop of a solution of a mixture of amino acids is applied to the filter paper. After drying, the paper is introduced into the solvent medium of the chromatographic chamber. Solvents under the action of capillary forces gradually move on the paper, capturing the separators of the substance. Some of them due to different structures and properties are held differently by the paper and therefore move at different speeds. After the front of the solvent moves a certain distance, the paper in the case of amino acids is dried and spots of amino acids are detected with a solution of ninhydrin, evaluating their separation, and by chromatography of dyes we observe their separation.

Progress of experiment. In the center of the chromatographic paper with a graphite pencil draw a circle with a radius of 1.5 cm and apply a drop of a solution of a mixture of amino acids and amino acids-witnesses (1) or dyes (2) (each sample separately at a distance of 1 cm). After drying the paper, a wick is inserted into the central hole of the paper and introduced into the chromatographic chamber with solvents (butanol, acetic acid, and water in a ratio of 4: 1: 5). The chromatography is carried out for 30-45 minutes, following the front of the solvent so as not to go beyond the chromatographic paper. In the case of amino acid chromatography (1), the paper is sprayed with ninhydrin reagent, calcined to 100 ° C and the location of amino acids is concluded. In the case of dye chromatography (2), the Rf of each dye is observed compared to the solvent front (Rf = a / v, where a is the distance from the start to the end of the dye front, b is the distance from the start to the end of the solvent front, namely ethanol).

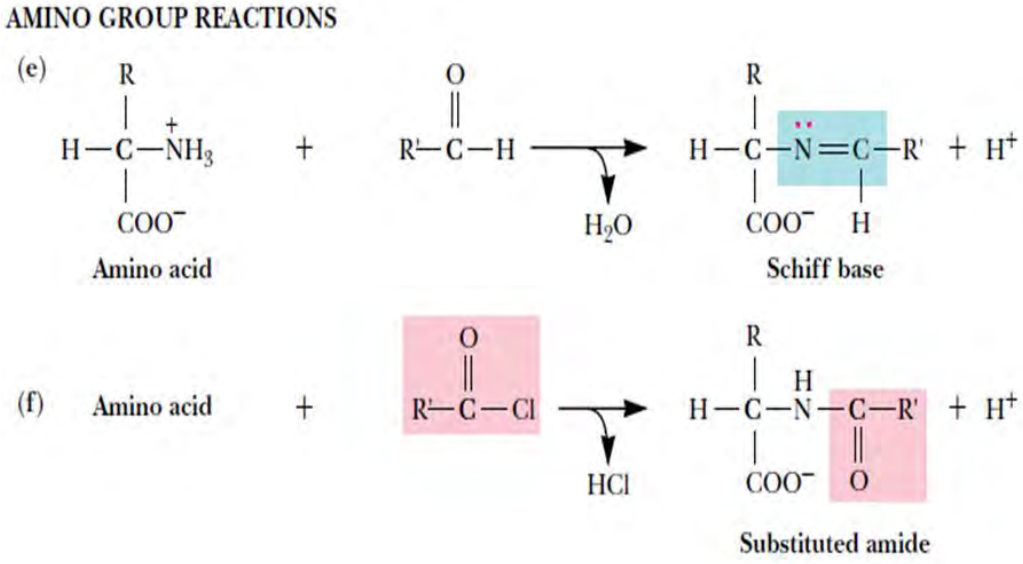
Control questions, tasks and exercises for the section «AMINO ACIDS**»**

1. What functional groups are part of amino acids?
2. Give examples of monoaminomonocarboxylic acids.
3. Give examples of monoaminodicarboxylic acids.
4. Write the structural formulas of phenylalanine, tyrosine, tryptophan.
5. Describe the qualitative reactions to Phe, Tyr and Trp? How can you prove that in the oligopeptide of these amino acids is present Trp?
6. Write the structural formulas of sulfur-containing amino acids? Their role?

1.3.5. ***Topic*** ACID AMIDES



Typical reactions of the common amino acids



Typical reactions of the common amino acids

LAB-CLASS

Urea and its properties

Amides are derivatives of acids that are formed by the substitution of hydroxyl in their carboxyl group by amino groups. Amides are neutral substances because the alkaline nature of the amino group is significantly reduced due to the

associated acid residue. Amides form salts (for example, with strong acids). They are easily hydrolyzed to form the corresponding acids and ammonia.

1. Obtaining nitric acid salt of urea

Urea exhibits weakly alkaline properties, which are easily detected by its ability to give salts that are sparingly soluble in water. Because the alkaline properties of the amide group in urea are weak, urea gives salt with only one molecule of nitric acid.

Progress of experiment. Make 1-2 drops of concentrated urea solution in a test tube and check the reaction to litmus. Then, add 1-2 drops of nitric acid, mix thoroughly and observe the release of sediment.

2. Decomposition of urea when heated. Biuret reaction

When urea is heated above the melting point, it decomposes with the release of ammonia and the formation of biuret

$$2CO(NH_2)_2 \rightarrow NH_3 + (NH_2)CO(NH)CO(NH_2)$$

In an alkaline environment, biuret with copper salts forms a complex compound colored pink-violet due to the presence in it of the group - (CO) -NH- with a peptide (amide) bond.

It should be noted that a positive biuret reaction is given by many compounds with the above group, in particular proteins and polypeptides.

Progress of experiment. Put a small amount of urea in a dry test tube on the tip of a spatula and heat gently over a flame of alcohol. Initially, urea melts from the release of ammonia, and the alloy then gradually hardens. The tube is cooled, add 5-10 drops of water and heated to dissolve the alloy more completely. Then add 2-3 drops of 2N. sodium hydroxide solution, 1-2 drops of 0.1 N. solution of copper sulfate, and observe the color change of the solution.

Control questions, tasks and exercises for the section «ACID AMIDES**»**

1. What nitrogen-containing substances do you know?
2. Give an example of acid amides
3. Write the structural formula of biuret.
4. Write the structure of urea. Where is it formed in the body of animals?
5. Write the structural formulas of asparagine and glutamine.
6. Write the structural formula of carbamic acid. What acid amide is it? What is common and different in the structure of carbamic acid and urea?

1.3.6. ***Topic*** PROTEINS

Classification of protein depending on degree of organization

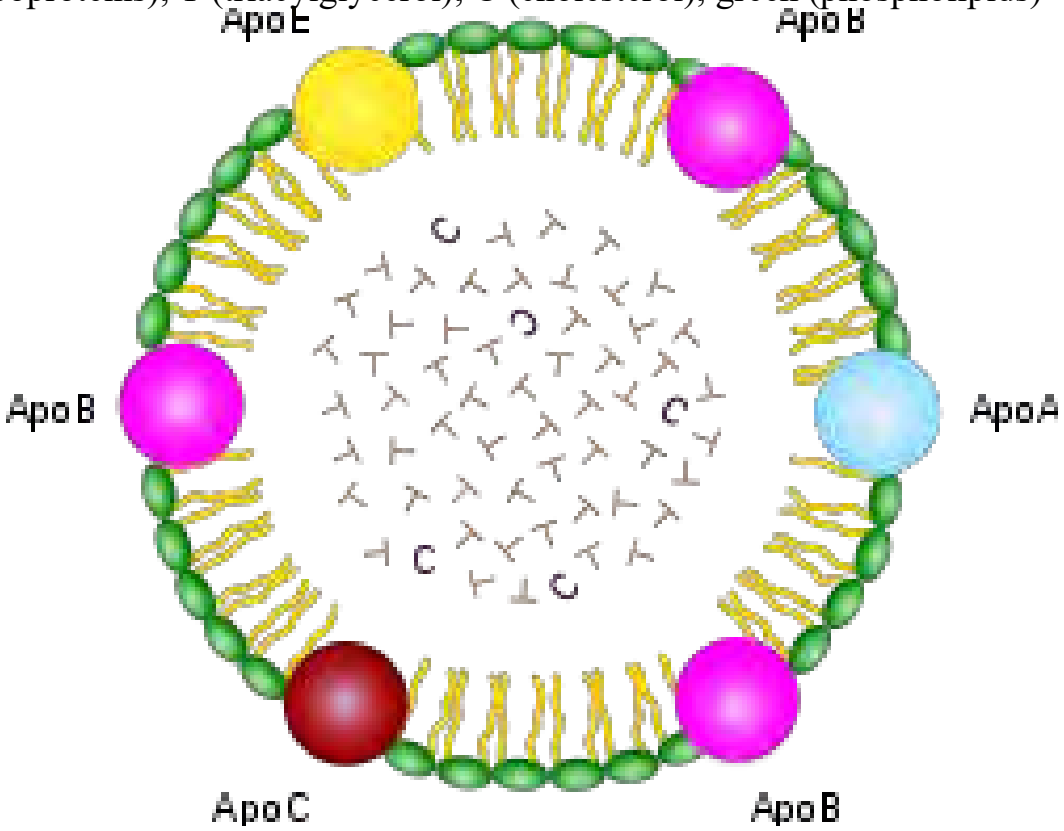
simple proteins

- **Albumin: lactalbumin, serum albumin, lehumelin (peas) leykozyn (wheat)**
- **Globulin, serum (α-, β-, γ-), milk and egg globulin; myosin, thyroglobulin, neyrohlobulin, neyrostromin, edestyn hemp hlitsynin soybeans, beans phaseoline**
- Histones
- Protamine - in the nucleoprotein fish sperm in the tissues of parenchymal organs (liver, spleen, kidney) and endocrine glands
- Prolamine: gliadin wheat, rye, barley hordeyin, zeyin corn, oats averyn, oryzyn rice, sorghum caferin
- Glutelines - feed and food proteins
- *Sklero*proteins: collagen, keratin, elastin, fibroin

Conjugated proteins

- Glycoproteins: proteoglycan
- Lipoproteins: HDL (α-lipoprotein), LDL (β-lipoprotein)
- Hemoproteins: Hb, myoglobin, cytochrome c, catalase, nitrate reductase, ammonium oxidase
- Nucleoproteins: ribosome, tobacco mosaic virus, adenovirus, AIDS virus
- Flavoproteins: succinate and NADH degydrogenases
- Phosphoproteins: cazein, glycogen phosphorylase a,
- Metalloproteins: ferritin, alcohol dehydrogenase, cytochrome oxidase, nitogenase, pyruvate carboxylase

Lipoprotein structure (chylomicron) ApoA, ApoB, ApoC, ApoE (apolipoproteins); T (triacylglycerol); C (cholesterol); green (phospholipids)



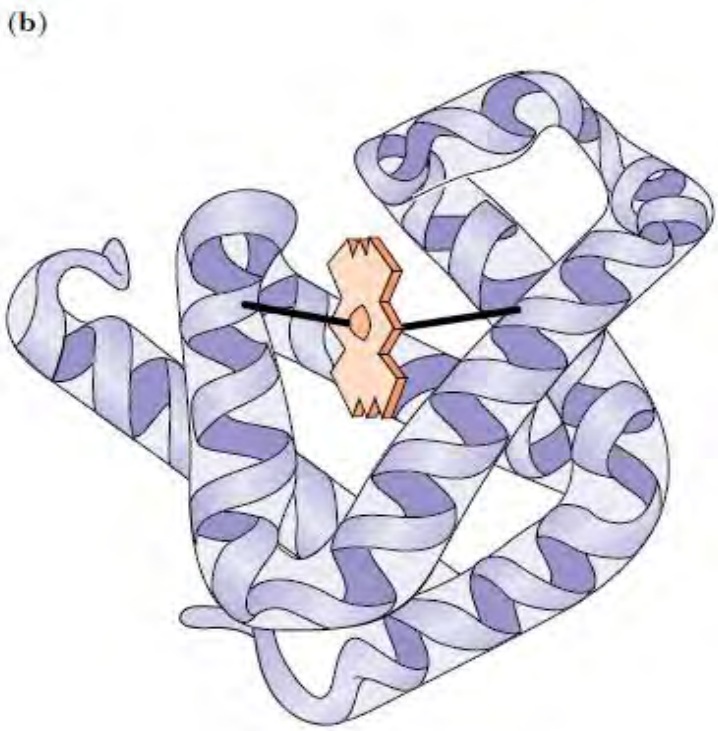
Proteins

Proteins within the membrane are key to the functioning of the overall membrane. These proteins mainly transport chemicals and information across the membrane. Every membrane has a varying degree of protein content. Proteins can be in the form of peripheral or integral. Different types of biological membranes have diverse lipid and protein compositions. The content of membranes defines their physical and biological properties. Some components of membranes play a key role in medicine, such as the efflux pumps that pump drugs out of a cell.

(a) Proteins having structural roles in cells are typically fibrous and often water insoluble. Collagen is a good example. Collagen is composed of three polypeptide chains that intertwine. (*a, b, Irving Geis, cited according to Garrett and Grisham BIOCHEMISTRY*).

Collagen, a fibrous protein

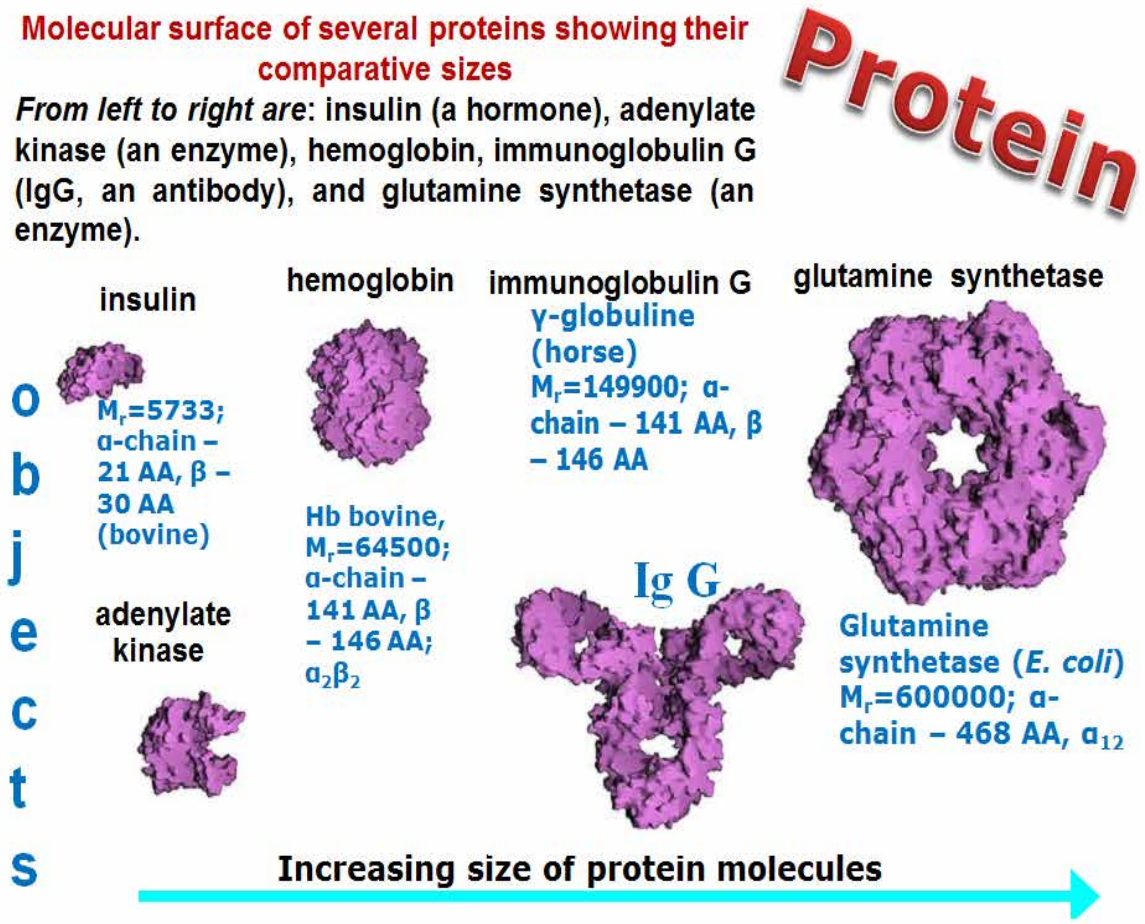
(b) Soluble proteins serving metabolic functions can be characterized as compactly folded globular molecules, such as myoglobin. The folding pattern puts hydrophilic amino acid side chains on the outside and buries hydrophobic side chains in the interior, making the protein highly water soluble.



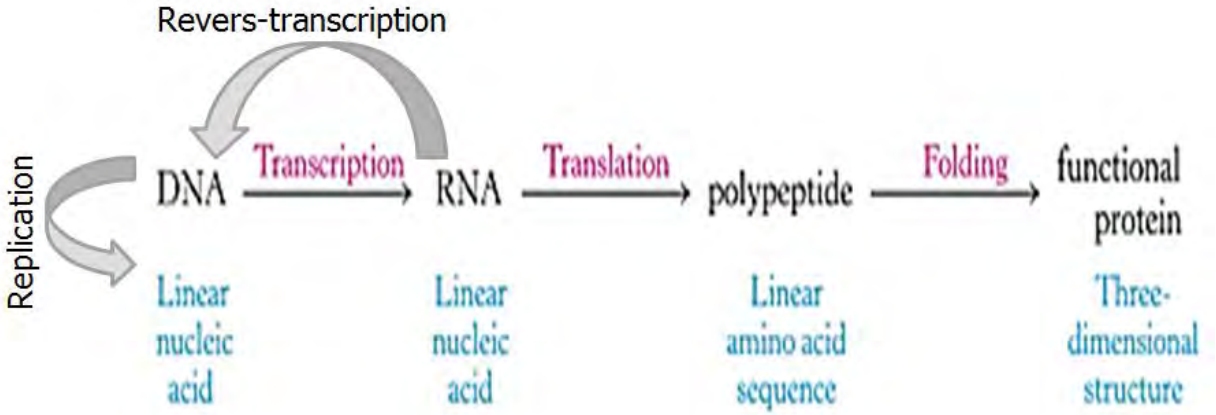
Myoglobin, a globular protein

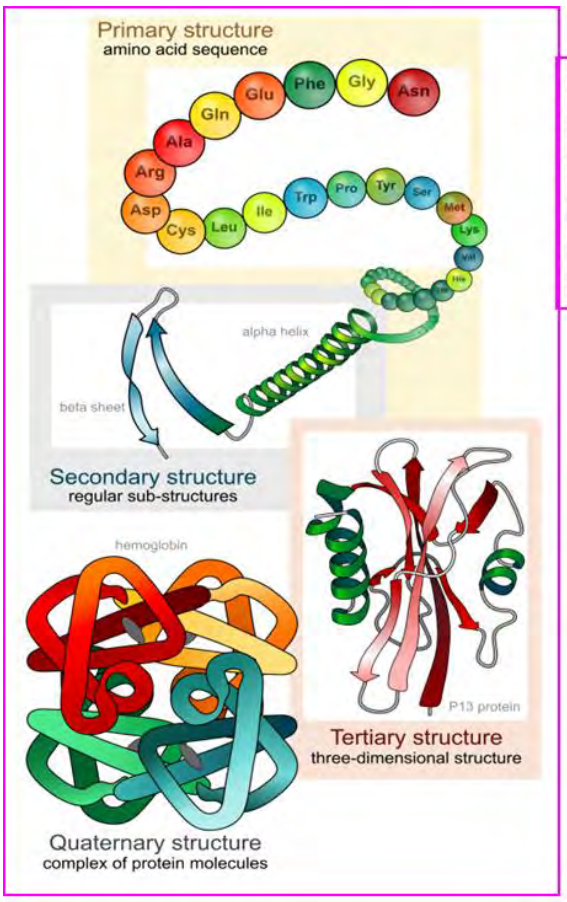
Molecular surface of several proteins showing their comparative sizes

From left to right are: insulin (a hormone), adenylate kinase (an enzyme), hemoglobin, immunoglobulin G (IgG, an antibody), and glutamine synthetase (an enzyme).

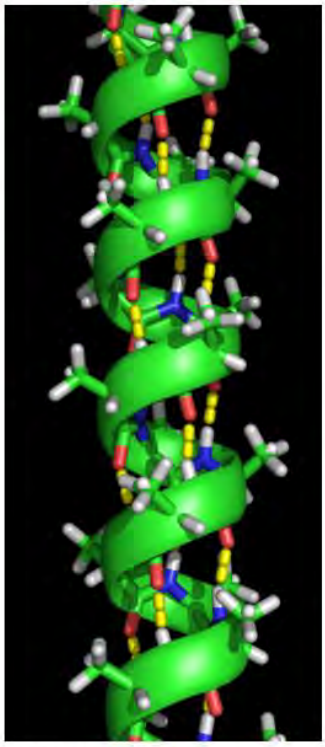


There is the central dogma of molecular biology:



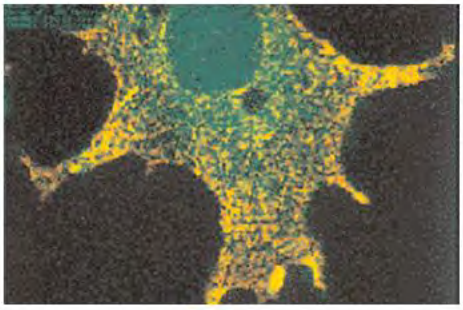


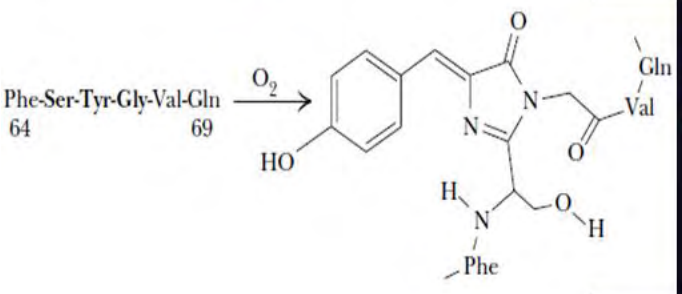
Protein structure, from primary to quaternary structure

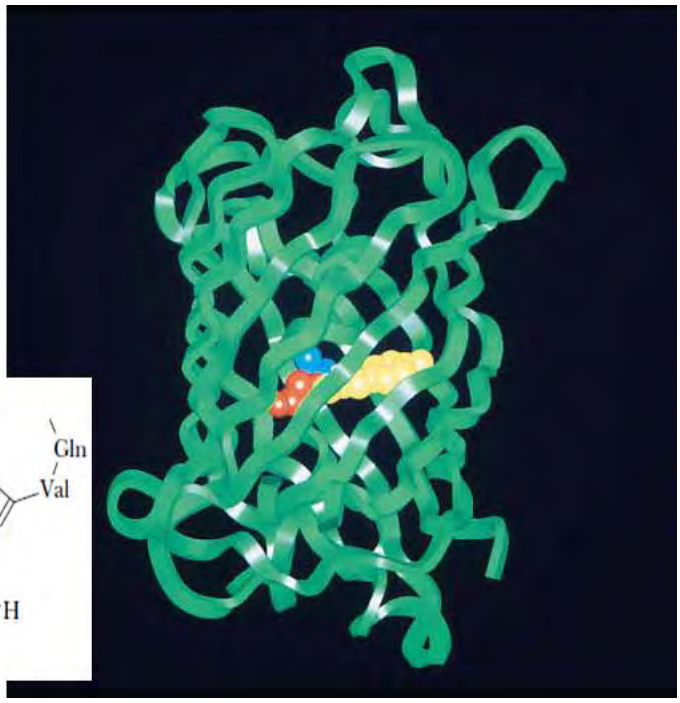


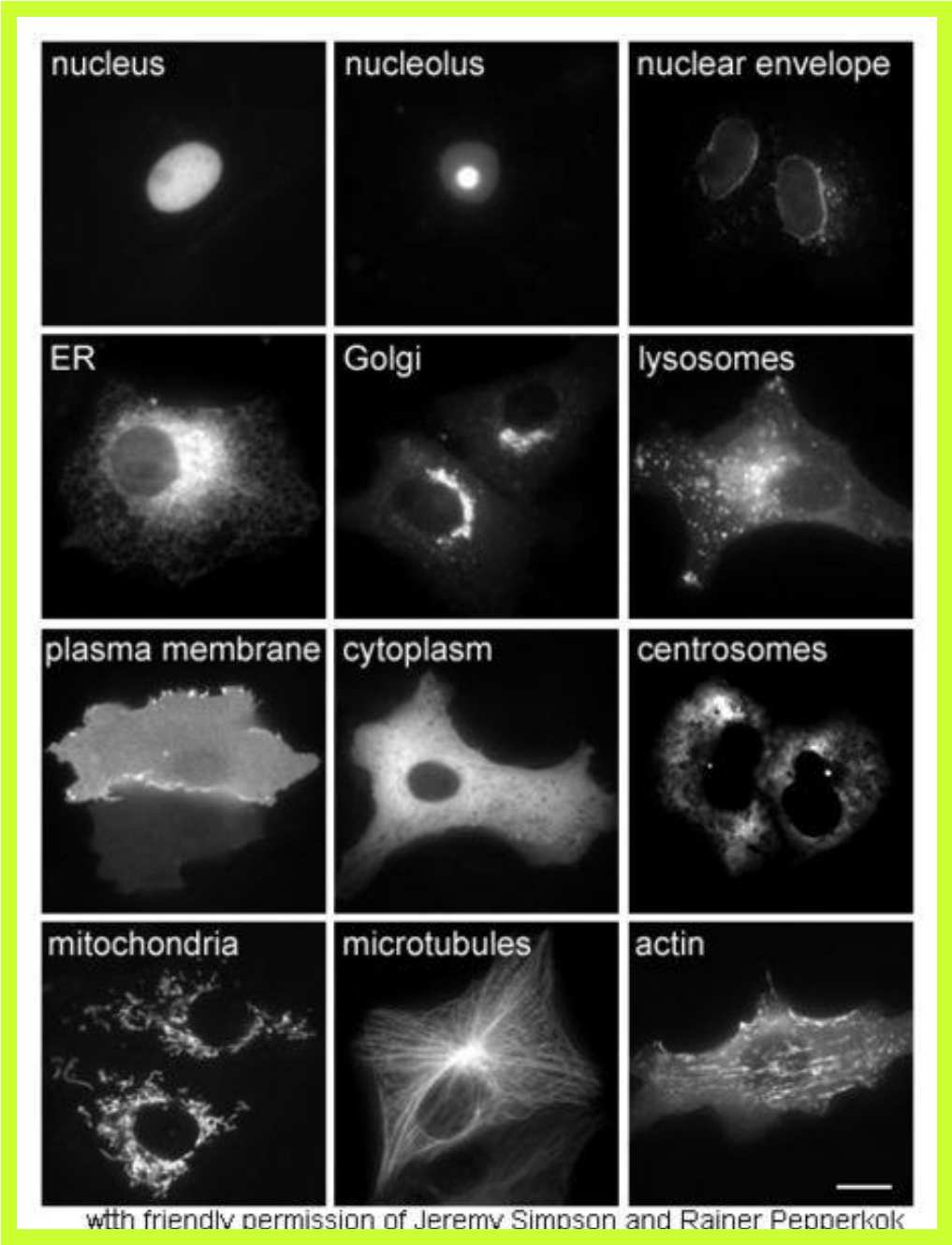
An *alpha*-helix with hydrogen bonds (yellow dots)

Autocatalytic oxidation of GFP amino acids leads to the chromophore shown on the left. The green fluorescence requires further interactions of the chromophore with other parts of the protein







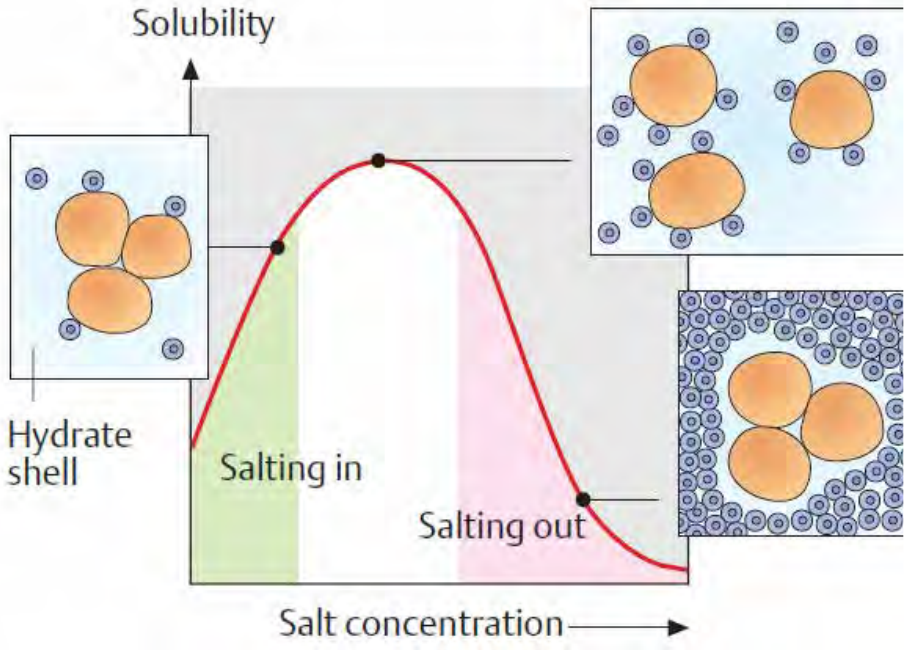


Proteins in different cellular compartments and structures tagged with green fluorescent protein - GFP (here, white).

LAB-CLASS

1. Reverse reactions of protein precipitation

Reverse precipitation of proteins is a reaction in which the protein can be converted back into a solution while maintaining its initial properties (neutral salts of alkali and alkaline earth metals, alcohol, acetone).



Salt-precipitation of protein

Scheme of precipitation of ammonium sulfate proteins (Cited: *Кольман Я., Рем К.-Г.* Наглядная биохимия. 3-е изд.: Пер. с нем. М.: Мир, 2009. – 469 с.)

1.1. Ammonium sulfate salting

Progress of experiment. Pour 2 ml of protein solution into a test tube and gradually add ammonium sulfate until the protein precipitates. The precipitate is separated from the solution by filtration. The presence of protein in the precipitate and filtrate is checked by means of a biuret reaction.

1.2. Proteins precipitation by alcohol

Progress of experiment. Pour 1 ml of protein solution into two test tubes, add a few NaCl crystals and shake. Slowly add 1 ml of ethanol to one test tube, 1 ml of 10% alcohol solution to another, and vigorously shake the test tubes with the contents. After a few minutes in the first test-tube precipitate, which is separated from the solution by filtration. The presence of protein in the precipitate and filtrate is checked by means of a biuret reaction.

2. Irreversible protein precipitation reactions

In reactions of irreversible precipitation of proteins, there are profound changes in the structure of the protein or its denaturation (under the influence of salts of heavy metals, mineral, and organic acids, reagents for alkaloids, temperature).

2.1. Precipitation of proteins by salts of heavy metals

Progress of experiment. Pour 1 ml of protein solution in two test tubes, then, add 1-2 drops of 5% solution of copper (II) sulfate (into the1st tube), and 0.5% solution of lead (II) acetate (into the 2nd tube). After a few minutes, a precipitate falls out in both test tubes.

2.2. Precipitation of proteins by reagents on alkaloids (tannin, potassium hexacyanoferrate, and picric acid)

Progress of experiment. Pour 1 ml of protein solution in three tubes and add 1-2 drops of 1% acetic acid solution (for acidification). Then, in (1st) test tube, add 5-6 drops of saturated tannin solution, in (2nd) test tube - saturated picric acid solution, and in (3d) - potassium hexacyanoferrate - $K_4[Fe(CN)_6]$. Observe precipitation of proteins in all tubes.

2.3. Precipitation of proteins by concentrated inorganic acids

Concentrated inorganic acids (except phosphate) cause precipitation of proteins from solution. The appearance of sediment is caused by dehydration of colloidal protein particles, reduction of charge, the formation of protein salts. Excess mineral acids (except nitric) dissolve the protein precipitate due to hydrolysis. The reaction with nitric acid is used in diagnostic urine tests.

Progress of experiment. In three tubes, pour 1 ml of protein solution and add 5-6 drops: hydrochloric (into the 1st tube), sulfuric (into the 2nd tube) and nitric acids (into the 3d tube). A protein precipitate falls out. The precipitate dissolves in the case of an excess of hydrochloric and sulfuric acids.

2.4. Precipitation of proteins by organic acids

Organic acids precipitate proteins in different ways. Particularly sensitive and specific reactions to protein are its precipitation with trichloroacetic, perchloric and sulfasalicylic acids. Given that these acids do not precipitate the breakdown products of proteins (peptides, amino acids, acid amides, urea, uric acid, etc.), they are used for the separate determination of protein and non-protein nitrogen in tissues.

Progress of experiment. In two test tubes, pour 1 ml of protein solution and add: to (1) 4-5 drops of 10% solution of trichloroacetic acid, and to (2) - 4-5 drops of 20% solution of sulfosalicylic acid. Protein precipitates in both tubes.

2.5. Precipitation of proteins by boiling

When boiled in a neutral or weakly acidic environment, denaturation of the protein is observed, followed by its precipitation. In strongly acidic and alkaline solutions, the protein molecule is recharged, and the denatured protein remains in solution. While the addition of electrolytes, which remove the electric charge, there is a precipitation of proteins.

Progress of experiment. In 5 test tubes, pour 1 ml of protein solution. The contents of the 1st tube are boiled, after which a protein precipitate is formed. In the 2nd test-tube, add 3 drops of 2% acetic acid solution and heat to boiling, then there is a more intense formation of sediment. In the 3d test-tube, add 15 drops of 2% acetic acid solution and boil, no precipitation is observed. In the 4th test-tube, add 15 drops of 2% acetic acid solution and 2 drops of saturated sodium chloride solution, after which the sample is boiled, the formation of a precipitate is observed. In the 5th test-tube, add 10-15 drops of 10% sodium hydroxide solution and boil, no precipitate.

3. Physical and chemical properties of proteins

The isoelectric point is the pH value at which the total charge of the protein is zero. At the isoelectric point, proteins are the least stable and easily precipitate.

3.1. Determination of the isoelectric point (pI) of a protein

Since specific proteins have certain pI values, this is used to fractionate proteins from the mixture, creating the appropriate pH values of the medium. The proteins are precipitated sequentially, and the protein precipitates are separated each time. This method of separating a mixture of proteins is called the method of isoelectric precipitation.

Progress of experiment. The corresponding components according to the table (see below) are added to each of the 5 tubes.

After mixing the components after 30 minutes, observe the intensity of turbidity of the solution in each tube. Weak turbidity is indicated by (+), moderate - (++), strong - (+++). The pH in the test-tube, where is the highest coagulation intensity, corresponds to the isoelectric point of this protein.

The scheme and the results of the experiment

Components	Tubes				
	1	**2**	**3**	**4**	**5**
0.1 M solution of acetic acid, ml	1.8	1.4	1.0	0.6	0.2
0.1 M sodium acetate, ml	0.2	0.6	1.0	1.4	1.8
pH	3.8	4.4	4.7	5.1	5.7
0.5% gelatin solution, ml	1.0	1.0	1.0	1.0	1.0
Ethanol	4.0	4.0	4.0	4.0	4.0
The observed intensity coagulation					

Control questions, tasks and exercises for the section «PROTEINS**»**

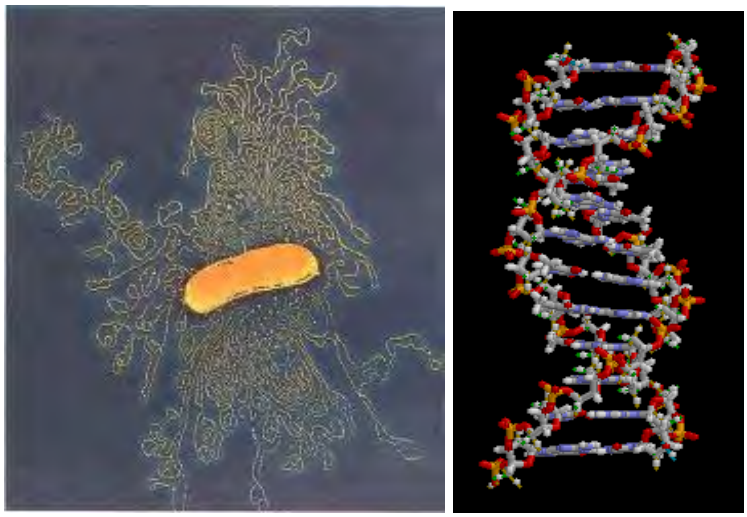
1. How does the primary structure of a protein differ from the primary structure of polysaccharides?
2. What is common and different in the structural organization of simple and complex proteins?
3. Give examples of globular and fibrillar proteins.
4. What role do electrolytes play in protein precipitation?
5. Is it possible to separate proteins from polysaccharides during their isolation and purification?
6. What is observed when salting-out proteins with ammonium sulfate? What is the purpose of such protein precipitation?

1.3.7. ***Topic*** NUCLEIC ACIDS AND THEIR COMPONENTS

Nucleic Acids

Nucleic acids are biological molecules essential for life and include DNA (deoxyribonucleic acid) and RNA (ribonucleic acid).

Nucleic acid types differ in the structure of the sugar in their nucleotides - DNA contains 2-deoxyribose while RNA contains ribose (where the only difference is the presence of a hydroxyl group). Also, the nitrogenous bases found in the two nucleic acid types are different: adenine, cytosine, and guanine are found in both RNA and DNA, while thymine only occurs in DNA and uracil only occurs in RNA. Other rare nucleic acid bases can occur, for example inosine in strands of mature transfer RNA.

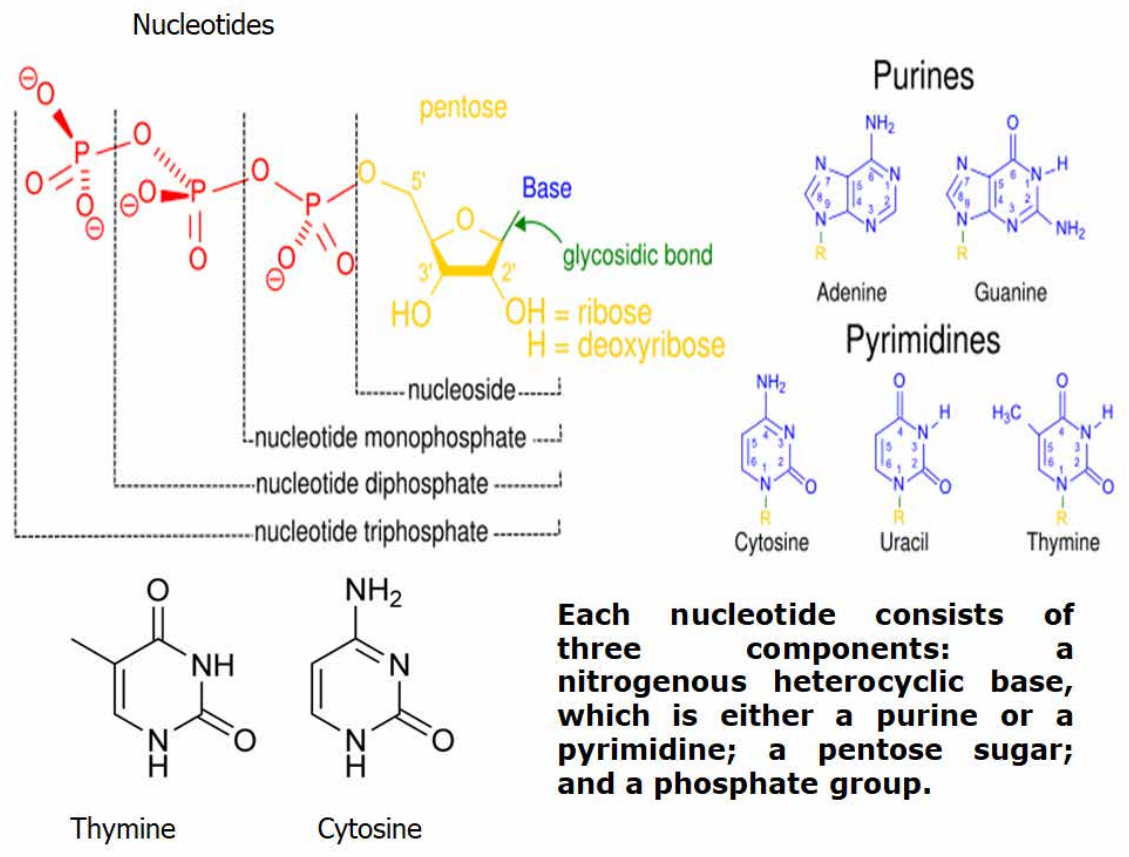


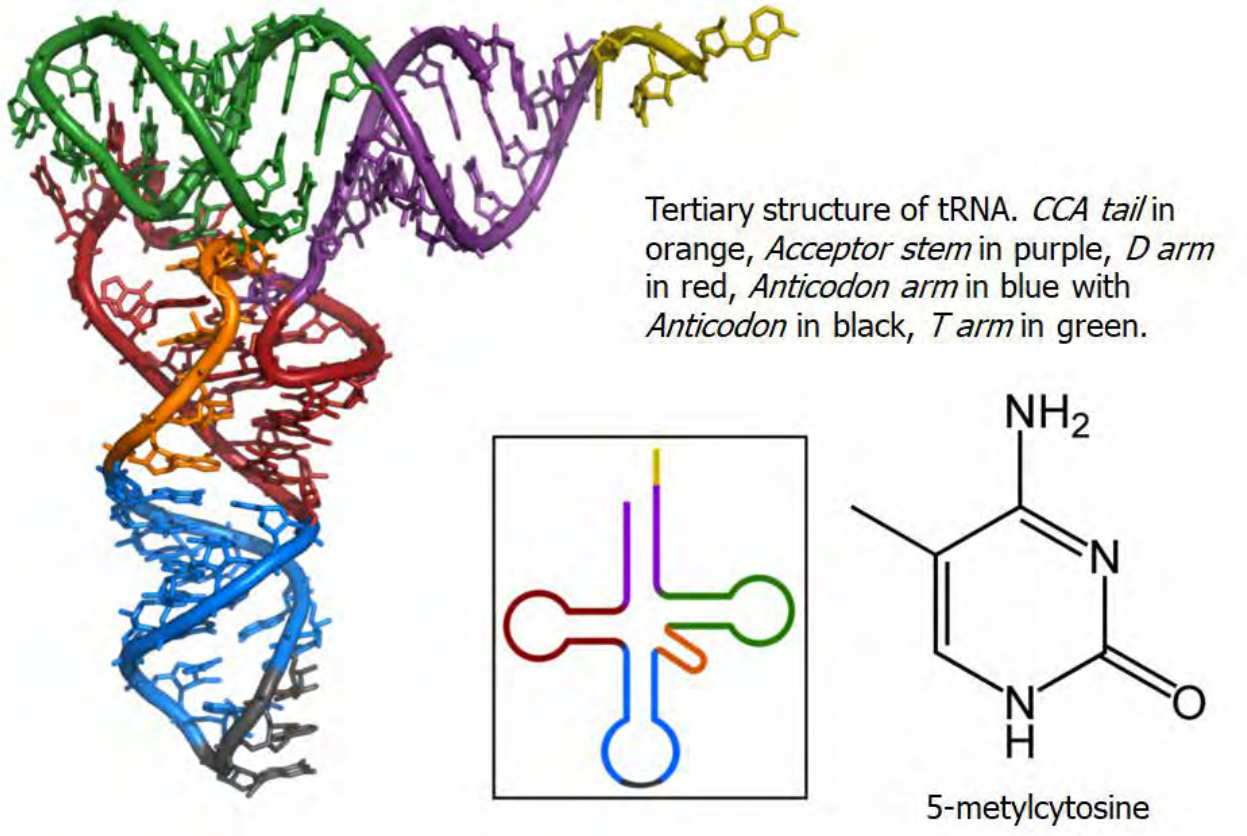
Electron Micrograph of Part of the *E. coli* genome – *cytation* : *Berg J.M., Tymoczko J.L., Stryer L.* Biochemistry. – New York: W H Freeman; 2002. 1515 p**]**

Kilobase (kb). A unit of length equal to 1000 base pairs of a double-stranded nucleic acid molecule (or 1000 bases of a single-stranded molecule).
One kilobase of double-stranded DNA has a contour length of 0.34 μm and a mass of about 660 kd.

Nucleic acids are **polymers** of repeating units (called **monomers**). Specifically, nucleic acids are long chains of nucleotide monomers connected by covalent chemical bonds. RNA molecules may comprise as few as 75 or more than 5,000 nucleotides, while a DNA molecule may comprise more than 1,000,000 nucleotide units.

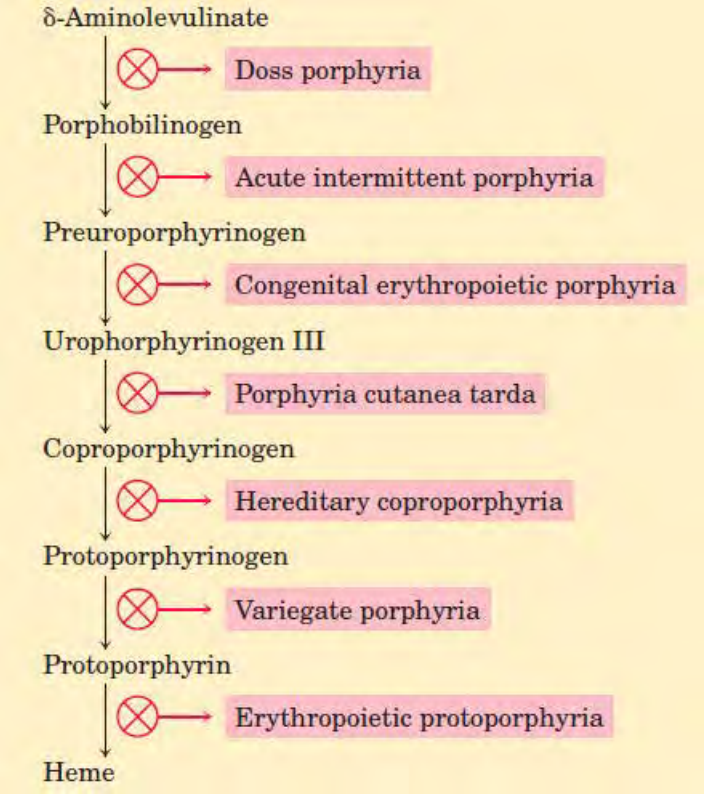
A **nucleotide** is a chemical compound comprising the union of three molecular components: a nitrogen-containing base, a pentose (five-carbon) sugar, and one or more phosphate groups. One phosphate group per nucleotide is standard for the nucleotides that make up DNA and RNA. Both the base and the pentose in a nucleotide are a cyclic and hence stable molecule whose core is at least one closed ring of atoms, with bases having one or two nitrogen atoms in a ring of carbon atoms and sugars having one oxygen in a ring of carbon atoms.



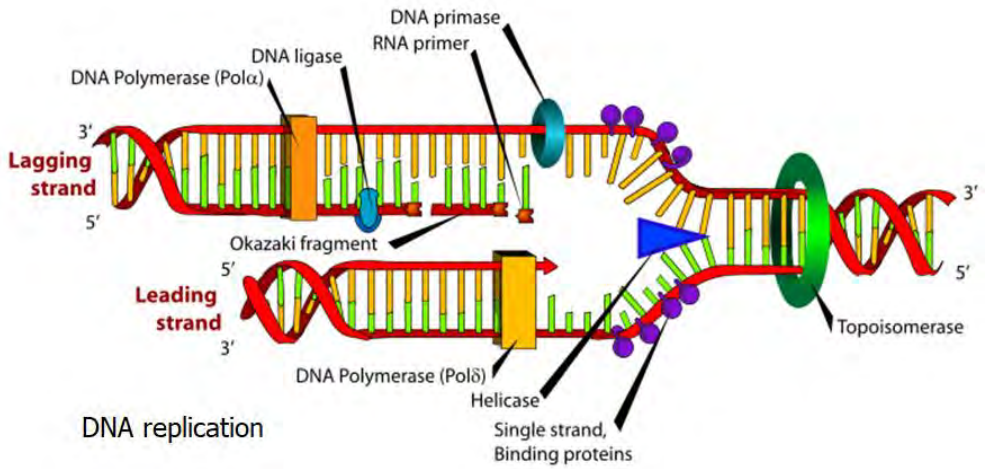


Biochemistry of Kings and Vampires. Porphyries are a group of genetic diseases in which, because of defects in enzymes of the biosynthetic pathway from glycine to porphyrins, specific porphyrin precursors accumulate in erythrocytes, body fluids, and the liver. The most common form is acute intermittent porphyria.

Most affected individuals are heterozygotes and are usually asymptomatic, because the single copy of the normal gene provides a sufficient level of enzyme function. However, certain nutritional or environmental factors (as yet poorly understood) can cause a buildup of δ–aminolevulinate and porphobilinogen, leading to attacks of acute abdominal pain and neurological dysfunction. King George III, British monarch during the American Revolution, suffered several episodes of apparent madness that tarnished the record of this otherwise accomplished man. The symptoms of his condition suggest that George III suffered from acute intermittent porphyria.



Steps of heme formation (**Lehninger Principles of Biochemistry** (Fourth Edition) ***David L. Nelson (U. of Wisconsin–Madison) Michael M. Cox (U. of Wisconsin–Madison)*** W. H. Freeman Publishers. 2004.-1124p.)



One of the rarer **porphyrias** results in ***an accumulation of uroporphyrinogen I***, an abnormal isomer of a protoporphyrin precursor. **This compound stains the urine red, causes the teeth to fluoresce strongly in ultraviolet light, and makes the skin abnormally sensitive to sunlight**.

Many individuals with this porphyria are anemic because insufficient heme is synthesized. This **genetic condition** may have given rise to the vampire myths of folk legend. The symptoms of most porphyria are now readily controlled with dietary changes or the administration of heme or heme derivatives.

LAB-CLASS

1. Isolation of deoxyribonucleoprotein

Nuclear proteins have a characteristic feature, namely the ability to form very viscous solutions in concentrated salt solutions and insolubility in dilute saline solutions. They are precipitated from saline solutions in the form of threads.

Progress of experiment. 2-3 g of liver tissue is ground in a porcelain mortar with an equal amount of quartz sand, first, add 5 ml of chilled 2 M sodium chloride solution. Rubbing is continued for another 10-15 minutes in a mortar, which is pre-cooled and is in an ice bath. The homogenate is transferred into centrifuge tubes and centrifuged for 20-25 minutes at 2000-3000 rpm. Measure the volume of the centrifuge, pour it into six times the volume of water with a thin stream, slowly stirring the liquid with a wooden stick.

If the nucleoprotein strands are not formed, and there was a flaky precipitate, you need to allow the precipitate to settle, carefully drain all the clear liquid, and the residue is centrifuged again (20-25 minutes at 2000-3000 rpm). The precipitate after centrifugation is examined for the content of the components of the nucleoprotein.

2. Hydrolysis of nucleoprotein

When nucleoproteins are boiled with dilute acid, they are broken down into protein and nucleic acid, which breaks down into individual mononucleotides, and then purine bases (adenine and guanine) and phosphoric acid are cleaved. The protein is partially hydrolyzed to low molecular weight peptides and amino acids. In the hydrolyzate, determine protein, purine bases, pentose, and phosphoric acid. Pyrimidine nucleotides are not hydrolyzed under these conditions.

Progress of experiment. The nucleoprotein precipitate is transferred to a hydrolysis flask and 15 ml of a 5% sulfuric acid solution is added. The flask is capped and refluxed at a low boil for one hour. After cooling, the hydrolyzate is filtered and used to analyze the hydrolysis products.

3. Detection of DNA in deoxyribonucleoprotein

Since deoxyribose is an integral part of the DNA nucleotides of deoxyribose, the presence of DNA can be judged from a positive reaction to deoxyribose with diphenylamine. The interaction of deoxyribose with diphenylamine produces a product that has a blue color.

While ribose and RNA with diphenylamine give a green color.

Progress of experiment. A small portion of the deoxyribonucleoprotein precipitate is transferred to a test tube and dissolved in 1 ml of 0.4% sodium hydroxide solution. Then add an equal volume of diphenylamine reagent and place it in a boiling water bath for 15-20 minutes. Observe the blue product of the reaction.

4. Detection of pentoses

Since ribose and deoxyribose are aldopentoses, they are detected as aldose by Fehling's reagent (alkaline solution of a complex compound of copper and tartaric acid salts). When heated, aldose with Fehling's reagent Cu^{2+} is reduced to Cu^{+} (CuOH - yellow or Cu_2O - red).

Progress of experiment. 0.5-1 ml of hydrolyzate is added to the test tube, neutralized with alkali solution, an equal volume of Fehling's reagent is added, stirred, and heated almost to boiling. Observe the change in color of the contents of the tube from bright blue to yellow or red.

5. Detection of purine bases

The reaction is based on the fact that during it Argentum salts of purine bases are formed, which precipitate.

Progress of experiment. Add 2 ml of nucleoprotein hydrolyzate to the test tube, add a few drops of concentrated ammonia solution to the alkaline reaction, then add 0.5 ml of Argentum ammonia solution $[Ag(NH_3)_2]OH$ (which is prepared as follows: 5% ammonia solution is added dropwise to 1% Argentum nitrate solution, first a brown precipitate appears, and with further addition and shaking of the ammonia solution the precipitate dissolves and the solution becomes completely transparent and colorless). A brown lamellar precipitate of Argentum salts of purine bases is formed.

6. Determination of phosphoric acid in the hydrolyzate

Phosphate acid is detected by the formation of phosphorus-molybdenum blue by the reaction of the hydrolyzate with ammonium molybdate in sulfuric acid and in the presence of ascorbic acid.

Progress of experiment. Add 2 ml of nucleoprotein hydrolyzate to the test tube, add 3 ml of a 2.5% solution of ammonium molybdate in 5 M sulfuric acid and 1 ml of freshly prepared 0.4% ascorbic acid solution. The mixture is stirred and heated to 40-50° C. The color of phosphorus-molybdenum blue develops.

Control questions, tasks and exercises for the section «NUCLEIC ACIDS AND THEIR COMPONENTS»

1. What nucleic acids are present in the cell?
2. How the structure of DNA differs from the structure of RNA?
3. What pentoses are part of the nucleotides of DNA and RNA?
4. What nucleotides are part of DNA and RNA?

5. Why the basis for the determination of DNA in deoxyribonucleoprotein was taken by the reaction, which detects deoxypentoses?
6. Write the structural formula of adenosine and thymine.

Generalized conclusions

to chapter 1.3 “STATIC BIOCHEMISTRY” which is devoted to study the compounds of different classes of organic substances: carbohydrates, lipids, proteins, nucleic acids

1. Presented common view of classification of carbohydrates, structure and peculiarities of some monosaccharides and disaccharides, typical chemical reactions for monosaccharides and experiments of qualitative and quantitative determination of carbohydrates and their functional groups.
2. Characterized structure and peculiarities of some polysaccharides and their role for living cell and whole organisms. Presented lab experiments for study of polysaccharides.
3. Characterized structure and functions of lipids and their components. Presented typical lab experiments for the study peculiarities of lipid compounds.
4. Presented common view of amino acids, their structure, and peculiarities, typical detection reaction of amino acids using paper chromatography.
5. Characterized typical chemical reactions of acid amides formation and other chemical reactions for amino acids, and lab experiments for the study of urea and its properties.
6. Characterized structural organization and peculiarities of proteins and their role for living organisms. Presented lab experiments for study of proteins and their physical and chemical characteristics.
7. Presented common view of role and structural organization of nucleic acids, lab experiments for DNA isolation and detection of its components.

PART 2

Chapter 2.1

REGULATORY EFFECTS OF INORGANIC AND ORGANIC SUBSTANCES ON THE METABOLISM

The purpose of this section of the course "Biochemistry of animals with the Basics of Physical and Colloid Chemistry" is to provide students with theoretical knowledge of physical and colloid chemistry and some experiments in lab classes.

The following topics will be considered in this section:

Topic 1. Biological membranes. Water and minerals in animals and plants, biochemistry of transmembrane transfers of minerals and organic substances

Topic 2. Vitamins. Coenzymes

Topic 3. Enzymes and their kinetic properties

Topic 4. Hormones and mechanisms of their influence on metabolic processes

Topic 2.1.1. BIOLOGICAL MEMBRANES. WATER AND MINERALS IN ANIMAL AND PLANTS, BIOCHEMISTRY OF TRANSMEMBRANE TRANSFERS OF MINERAL AND ORGANIC SUBSTANCES

Biological membranes and transmembrane transfer of substances

A **biological membrane** or **biomembrane** is an enclosing or separating membrane that acts as a selective barrier, within or around a cell. It consists of a lipid bilayer with embedded proteins that may constitute close to 50% of membrane content. The cellular membranes should not be confused with isolating tissues formed by layers of cells, such as mucous and basement membranes.

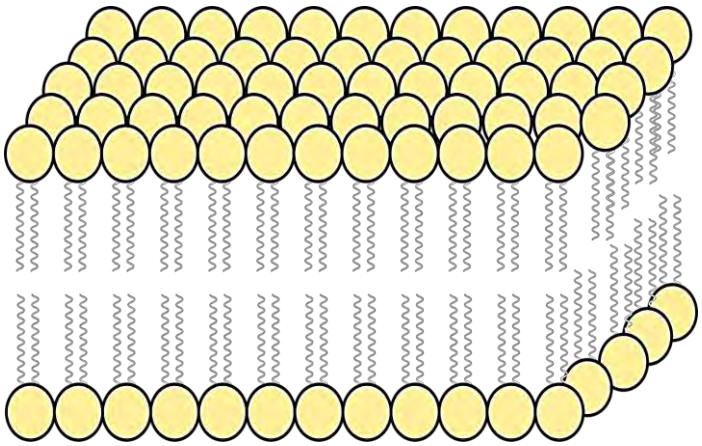
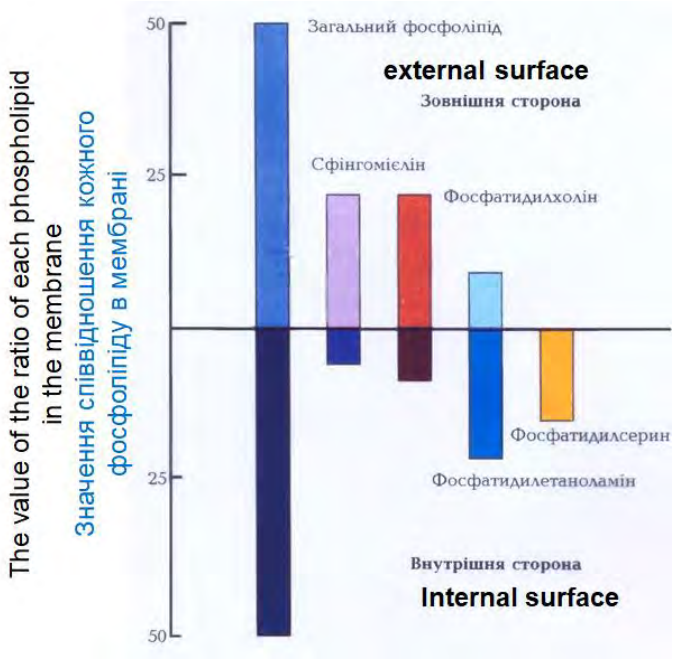
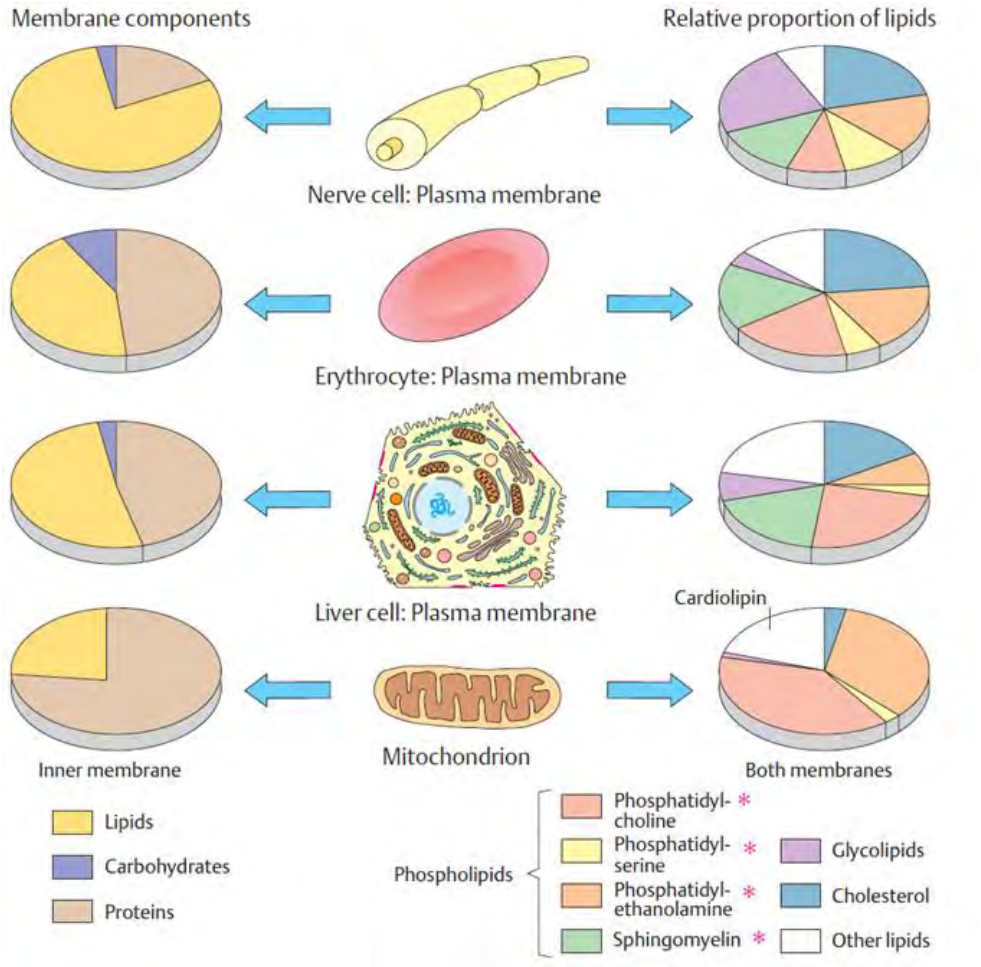


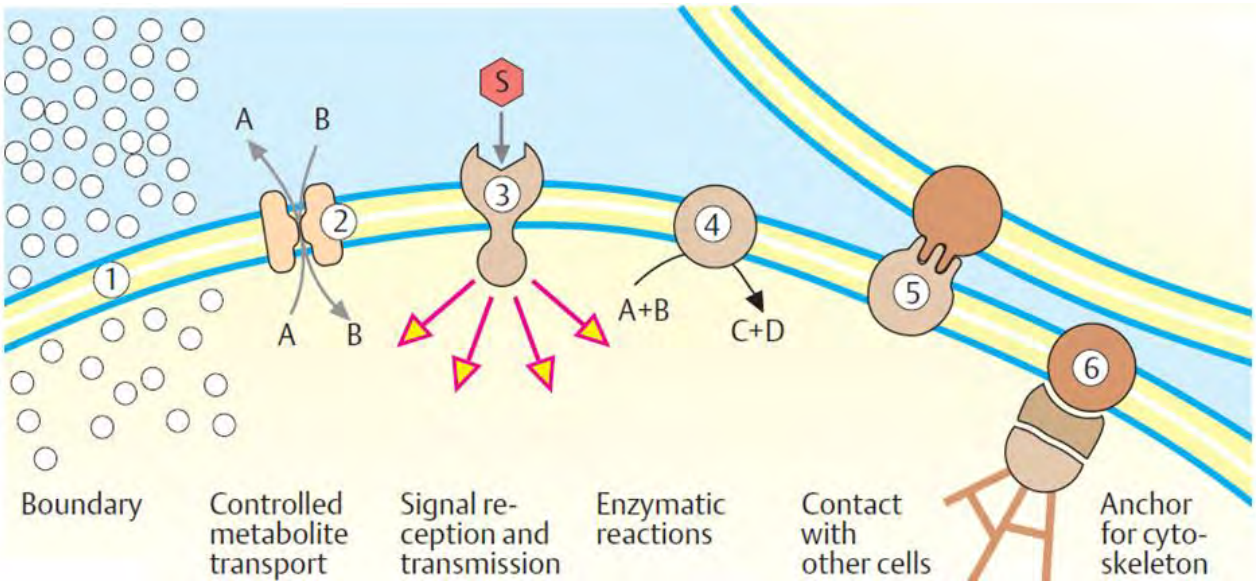
Diagram of the arrangement of amphipathic lipid molecules to form a lipid bilayerю Location of amphipathic lipid molecules that form the lipid bilayer. Yellow polar heads, separated by gray hydrophobic tails, border on aqueous cytosolic and extracellular media.



Distribution of phospholipids between internal and external surfaces of erythrocyte membrane in the human (Textbook of biochemistry: with clinical correlations / edited by Thomas M. Devlin. - 4th ed. - p. 194.)



Composition of membranes (Cited: *Кольман Я., Рем К.-Г.* Наглядная биохимия, 2009.)



Functions of membranes (Cited: *Кольман Я., Рем К.-Г.* Наглядная биохимия, 2009.)

Erythrocyte membrane - frozen in water (split by sublimation of water). In electronic photography, membrane proteins look like bulges of various sizes and shapes.

David L. Nelson, Michael M. Cox. Lehninger principles of biochemistry: 3rd ed. - New York, 2000. - P. 396

Elemental membrane model

The inner part of the membrane is formed by two-layer structures of polar lipids oriented inward by their aliphatic non-polar chain, which forms a continuous lipid phase. Hydrophobic interactions are observed between aliphatic chains and non-polar parts of other membrane components. Although each such interaction is quite weak, in total they provide the strength of lipid layers and the stability of lipid membranes. Further organization of lipid membranes is determined by the properties of proteins located on the surface of the membrane, the nature of which is determined by the genetic code. The protein part of the membranes often has a β-keratin structure and is associated with phospholipid layers of both hydrophobic and polar bonds, which also involve various ions and water molecules.

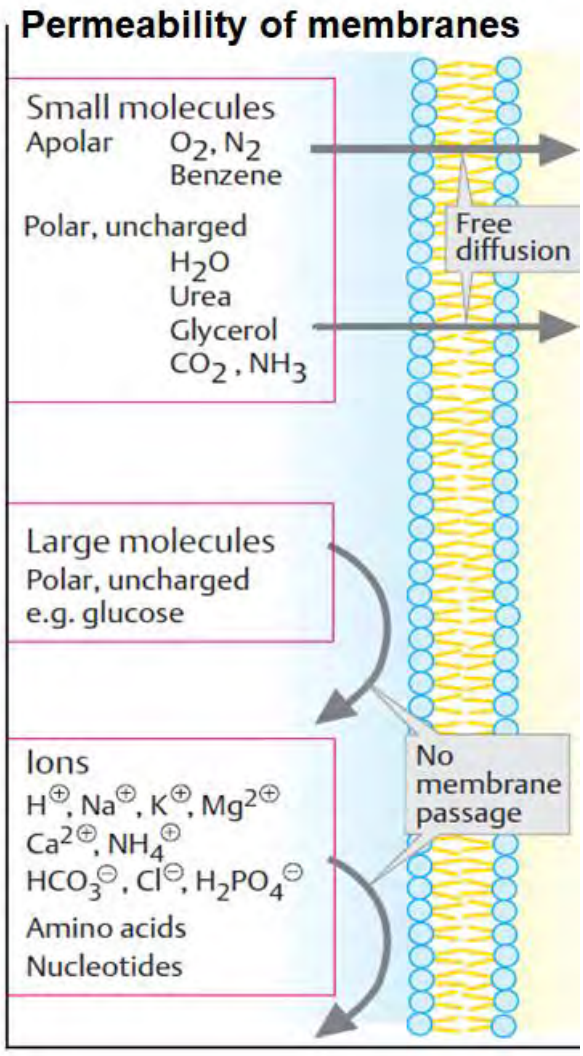
Osmosis is the unilateral diffusion of solvent molecules through a semipermeable membrane from a solution with a lower concentration to a solution with a higher concentration (see PART 1).

Osmotic pressure is due to a decrease in the chemical potential of the solvent in the presence of the solute. If the osmotic pressure of one solution is greater than another, then the first solution is hypertonic, and if vice versa - then hypotonic. Solutions with the same osmotic pressure are called isotonic. Isotonicity is important for infusion solutions. In clinical practice: isotonic solutions have an osmotic pressure equal to “p” plasma = 7.7 - 8.1 atmospheres or 0.85-0.9% sodium

chloride, or 4.5-5% glucose solution. Saline solutions are similar in composition to seawater.

Transmembrane transfer of matter

Types of transport of substances across the membrane:
1. Simple diffusion
2. Passive transport (facilitated and exchange diffusion)
3. Active transport (primary and secondary transport).
4. Endocytosis and exocytosis. Two types of endocytosis: phagocytosis and pinocytosis.

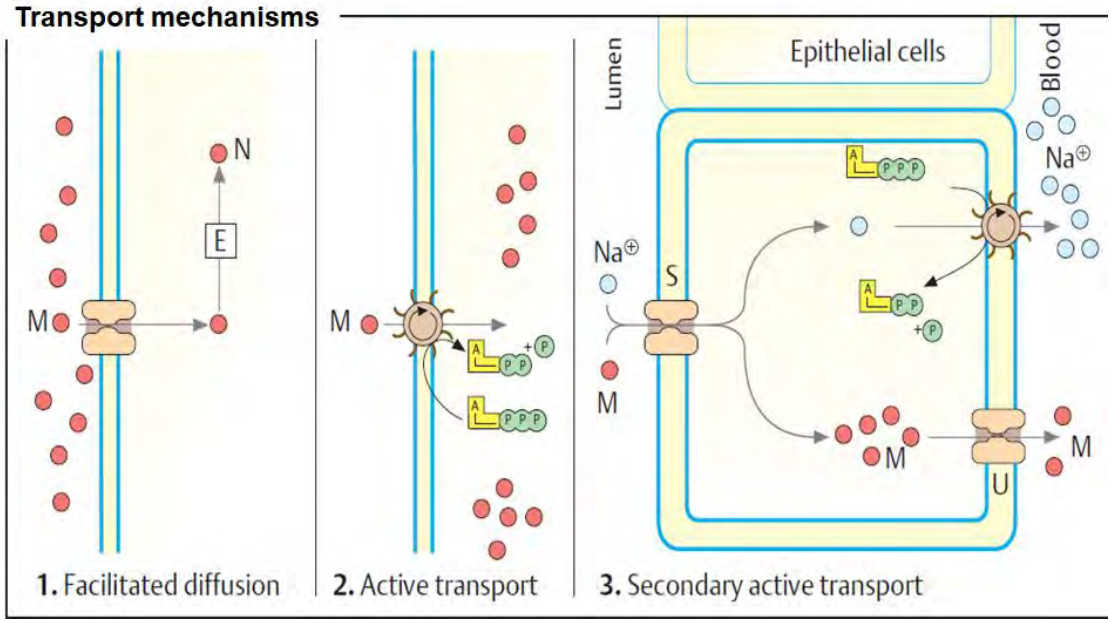


Diffusion is a spontaneous process of equalization of the concentration of a substance in the entire volume of the solution, due to the thermal motion of the particles of the solute and the solvent. Diffusion occurs from a solution with a higher concentration of solute into a solution with a lower concentration of this substance (see PART 1).

Passive transport - the transfer of matter by a concentration gradient without energy consumption, but with the help of special means: facilitated and exchange diffusion.

Facilitated diffusion is carried out: a) with the help of carrier proteins - translocase, permease; b) ion channels (pores that are formed by proteins), such as ion channels of the membranes of nerve and muscle cells for K +, Na +, Ca2 +; ionophores - the antibiotic valinomycin carries K + across mitochondrial membranes.

Exchange diffusion is carried out by an antiport mechanism, when one substance is exchanged for another, moving along a concentration gradient, such as the exchange of K + for Na +.



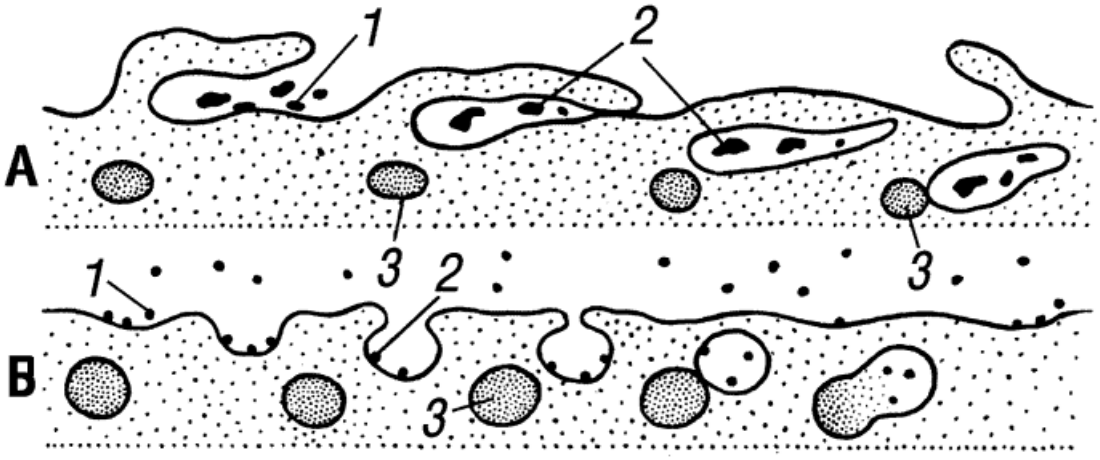
3. **Active transport** - the transfer of substances against the concentration gradient or electrochemical gradient with energy consumption. There is primary and secondary transport.

During primary transport, energy is expended by the direct transfer of substances across the membrane. This is how ATPases (ion pumps) work, which use ATP energy, such as Na +, K + -ATPase, which works on the principle of antiport, pumping Na + from the cell, and K + - inside the cells. Ca2 + -ATPase transports Ca2 + to its depot.

Secondary active transport is symport and antiport. Simport is the transport of one substance along a concentration gradient of another: for example, the absorption of glucose and amino acids in the intestine due to the Na + gradient. Antiport is the transport of a gradient of one substance against a gradient of concentration of another.

4. Endocytosis and exocytosis - the transfer of molecules across membranes together with part of the plasma membrane (in endocytosis - in the middle of the cell, in exocytosis - outside).

There are two types of endocytosis: phagocytosis - the capture and absorption of large particles and pinocytosis - the absorption of fluid droplets.



A - endocytosis with the transition of enzymes with liposomes in the formed vesicles with absorbed substance; B - endocytosis with the fusion of vesicles with absorbed substance with lysosomes. 1 - sedimentation of the biopolymer on the surface of the cell membrane; 2 - immersion of particles in the cytoplasm; 3 - primary lysosomes.

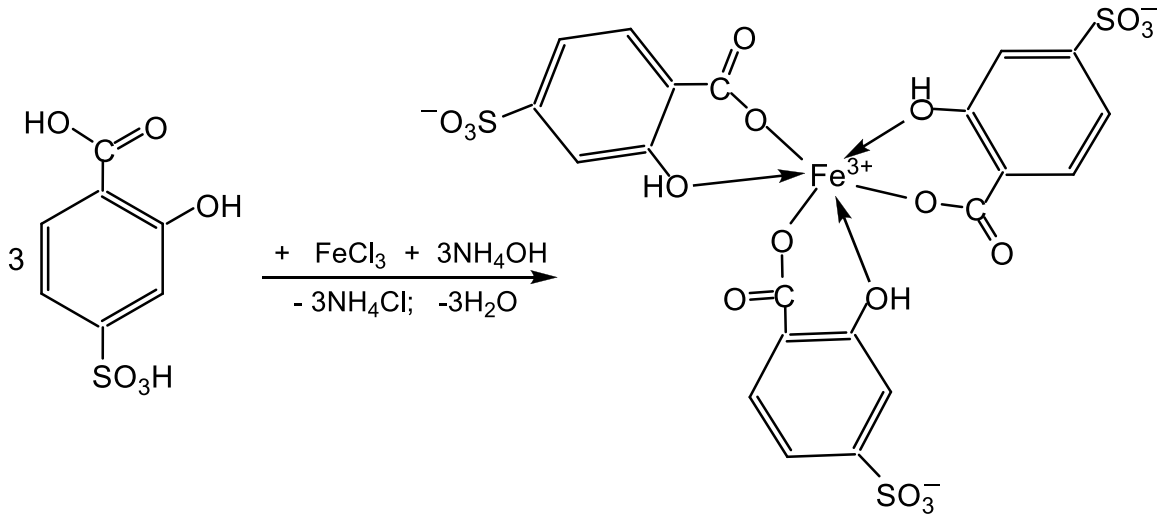
LAB-CLASS

1. **Quantitative determination of the content of iron and magnesium in tissues**

The principle of the method. The test material is dried and then burned in a microwave or muffle furnace. The obtained mineral residue is dissolved in hydrochloric acid. Next, Mg^{2+} is precipitated as magnesium ammonium phosphate. The solubility of magnesium ammonium phosphate is less than that of magnesium oxalate.

$$MgCl_2 + NH_4H_2PO_4 + 2NH_4OH = MgNH_4PO_4 + 2NH_4Cl + 2H_2O$$

Iron remains in solution as a water-soluble complex with sulfosalicylic acid:



The precipitate of magnesium ammonium phosphate is separated by filtration and washed from impurities. The filtrate is collected in a volumetric flask, adjusted

to a certain volume and spectrophotometrically or colorimetrically with a light filter with a wavelength of 440 nm.

The precipitate is transferred to a titration flask, heated to remove excess ammonia and determined by Mg2 + volume method. To determine Mg2 + the precipitate is dissolved by adding a known volume of titrated sulfuric acid solution:

$$MgNH_4PO_4 + H_2SO_4 = MgSO_4 + NH_4H_2PO_4,$$

Excess sulfuric acid is titrated with sodium hydroxide solution in the presence of a combined indicator, which is a mixture of methyl red with methylene blue. The indicator solution is prepared in distilled water without alcohol.

Equipment and reagents. Spectrophotometer or photoelectrocolorimeter. Drying cabinet. Microwave or muffle furnace. Tile. Porcelain cup. Porcelain crucible. Filter. Conical flask of 50 ml. Clock glass or funnel, the hole of which is closed with moistened cotton wool. Microburette. Measuring flask for 100 ml. Tight filter. Glass wand. Tripod with test tubes. Muscles of animals. Hydrochloric acid solution (1: 1). Phosphate-oxalic-sulfosalicylic reagent. Concentrated ammonia solution. Distilled water. $NH_4Fe(SO_4)_2$ standard solution containing 0.2 mg Fe^{3+} in 1 ml solution. Ammonia solution (1:10). Argentum nitrate (1%). Nitric acids. Sulfuric acid (c (1/2) = 0.1 mol / l). Mixed indicator. Sodium hydroxide (0.1 mol / l). Potassium permanganate solution (c (1/5) = 0.05 mol / l).

1.1. Determination of iron

Progress of experiment. The test sample should contain from 2 to 15 mg of Mg^{2+}. Muscles of animals weighing 20-25 g are carefully crushed and dried in a cup in an oven at 100 ° C. The dried material is ground again, transferred to a porcelain crucible, placed in a microwave or muffle furnace and the sample is burned at 450-500 ° C. After burning, 1 ml of distilled water is added to the residue in the crucible, dried and calcined again for 15-20 minutes. until complete combustion. There should be no black coal particles in the residue. If they are, then add water again, dry it and calcine. When complete mineralization has taken place, the crucible is cooled, 1 ml of distilled water and 2 ml of hydrochloric acid solution (1: 1) are added, mixed, filtered into a 50 ml conical flask, and the filter is washed with a small amount of distilled water. In the filtrate determine Mg^{2+}, Fe^{3+}. To do this, add 10 ml of phosphate-oxalic-sulfosalicylic reagent, heat to 70-80° C, remove from the plate, and slowly stirring the neutralizing acid drops with concentrated ammonia solution until turbid. The contents of the flask are then heated for 5 minutes, another 1 ml of concentrated ammonia solution is added dropwise and left until the temperature drops to about 40° C. Then add 5 ml of concentrated ammonia solution again, close the flask with a watch glass or funnel, the opening of which is closed with moistened cotton wool, and leave for 10 hours for complete precipitation of Mg^{2+}. The contents of the flask are then filtered into a 100 ml volumetric flask through a tight filter and the filter is washed with an ammonia solution (1:10) until the flask is almost filled to the brim. Then the solution in the flask is adjusted with distilled water to the line, stirred. The solution contains a complex compound of iron with sulfosalicylic acid. The resulting solution is spectrophotometrically (colorimetric) with a light filter with a wavelength of 440 nm against water. Based on the obtained value of the optical density on the

calibration graph determine the content of iron in the sample and list its content in the muscles in mg%. 1,2,3,5,7,10ml of a standard solution of iron-ammonium alum $NH_4Fe(SO_4)_2$ containing 0.2 mg of Fe^{3+} in 1 ml of solution is added sequentially to 100 ml volumetric flasks. Then, 10 ml of phosphate-oxalic-sulfosalicylic reagent, 6 ml of concentrated ammonia solution were added to each flask, made up to the mark with distilled water, and the optical density was measured as in the experiment. A calibration graph is plotted by plotting the Fe^{3+} content in the sample on the abscissa axis and the optical density of the solution on the ordinate axis.

The sensitivity of the method is 1.5–15 μg / cm^3 Fe^{3+}.

1.2. Determination of Magnesium

Progress of experiment. The precipitate of magnesium ammonium phosphate on the filter is washed with an ammonia solution (1:10) until Cl^- ions are detected in the filtrate. To check for completeness of washing, collect 5 ml of filtrate in a test tube, acidify with nitric acid and add 1 drop of Argentum nitrate (1%). The filtrate should not become cloudy. The filter with the precipitate is then removed from the funnel, torn into pieces, placed in a conical flask in which the precipitation was carried out, and dried until complete removal of moisture and ammonia. Then add 20 ml of distilled water, 20 ml of titrated sulfuric acid solution (c (1/2) = 0.1 mol / l) to the flask and heat to boiling, stirring and kneading the precipitate with a glass rod. After cooling, the rod is removed, rinsed with water, it is added to the flask two drops of a solution of mixed indicator, and the excess sulfuric acid is titrated with sodium hydroxide solution (0.1 mol / l) to the blue color of the solution. Separately titrate 20 ml of sulfuric acid solution (c (1/2) = 0.1 mol / l) with sodium hydroxide solution (0.1 mol / l) and determine the volume of alkali solution used to titrate this volume of sulfuric acid. From the obtained data calculate the content of Mg^{2+} in the test material by the formula:

$$X = \frac{M \cdot C \cdot (V_1 - V_2) \cdot 100}{m}, \text{ where}$$

X – content Mg^{2+} in the investigated object, mg %

C – molar concentration of the equivalent solution NaOH, mol/l;

V_1 – volume of solution of NaOH (0,1 моль/л), spent on titration of 20 ml of solution of H_2SO_4 (c ($^1/_2$)= 0,1 mol/l), ml;

V_2 – volume of solution of NaOH (0,1 моль/л), spent on titration of sulfuric acid, remaining in the test solution after interaction with $MgNH_4PO_4$, ml;

m- the mass of the studied tissue of the fish, г;

M - molar mass equivalent Mg^{2+} – 12,15 g/mol.

3. Quantitative determination of chlorides in the blood

The principle of the method. Chloride ions do form sparingly soluble Argentum chloride with Argentum ions. The solubility of AgCl in water is $1 \cdot 10^{-5}$M. To determine Cl^- ions in biological objects, this method must release them from proteins and carbohydrates, which can also bind Ag^+ ions. Protein-free blood filtrate is titrated with a solution of Argentum nitrate (0.01M) in the presence of potassium chromate in the test solution as an indicator. The solubility of Ag_2CrO_4 in water is

$7 \cdot 10^{-5}$ M. When titrating a solution containing Cl^- and CrO_4^{2-} ions, a white AgCl precipitate will first form. After titration of Cl ions, even an extra drop of the $AgNO_3$ solution will lead to the formation of a brick-red Ag_2CrO_4 precipitate.

The purpose of the work. Master the method of determining chlorides in the blood. To deepen knowledge about the role of chlorine in animal life.

Equipment and reagents. Water bath. Test tubes. Filter. Zinc sulfate solution (0.45%). Sodium hydroxide solution (0.1M). Potassium chromate solution (5%). A solution of Argentum nitrate (0.01M). Blood. Distilled water.

Progress of experiment. In two test tubes (experimental and control), make 5 ml of zinc sulfate solution (0.45%) and 1 ml of sodium hydroxide solution (0.1 M). Then 0.1 ml of blood is added to the first test tube. Both tubes are placed in a boiling water bath for 3 minutes. In this case, proteins are precipitated in the first test tube, and insoluble zinc hydroxide is still formed in both test tubes.

The contents of both tubes are filtered, the precipitates on the filters are washed twice with 5 ml of distilled water. To the filtrates add two drops of potassium chromate solution (5%) and titrate with a solution of Argentum nitrate (0.01M) until a barely noticeable brick-red color.

Calculations of the content of Cl^- ions in the blood are calculated by the formula:

$$C = \frac{(V_1 - V_2) \cdot T \cdot 1000}{V_3 \cdot mM}$$, where

C – the concentration of chloride ions in the blood, mM;
V_1 – the volume of Argentum nitrate solution (c = 0.01M), which was spent on titration of the test sample, ml;
V_2 – the volume of Argentum nitrate solution (c = 0.01M), which was spent on titration of the control sample, ml;
V_3 – the volume of blood taken for analysis, ml;
T – the titer of Argentum nitrate solution (0.01M) for chloride ion, mg/ml (T=0,355mg/ml);
mM – millimolar mass of chloride ion (35,5 mg/mol).

Control questions, tasks and exercises for the section «BIOLOGICAL MEMBRANES. WATER AND MINERALS IN ANIMAL AND PLANTS, BIOCHEMISTRY OF TRANSMEMBRANE TRANSFERS OF MINERAL AND ORGANIC SUBSTANCES»

1. Give examples of chemical elements related to macro-, micro- and ultramicroelements, and indicate their role in the life of the organism
2. How is the regulation of phosphorus-calcium metabolism in animals?
3. The value of Potassium, Sodium, and Chlorine in animals.
4. Biological role: a) iron, b) copper, c) zink
5. What is the purpose of atomic adsorption spectral analysis?
6. What is the role of Cobalt in the life of organisms? Cobalt is a component of (1) and belongs to the (2) -elements

(1)	(2)
A) vitamin B12 B) ATP C) lecithin D) sphingosine E) thiamine	F) macro- G) micro- J) ultramicro-

Topic **2.1.2** VITAMINS. COENZYMES

Metabolic regulators: vitamins. General characteristics. Vitaminology. Fat-soluble and water-soluble vitamins

Water-soluble vitamins, for example B1 (thiamine); B2 (riboflavin); B3 (nicotinamide or vitamin PP); B5 (pantothenic acid); B6 (pyridoxal); B8 (H or biotin); B9 (folic acid), B12 (cyanocobalamin).

Vitamins (vita - Latin life) are low molecular weight organic substances of various chemical nature, which are vital for a living organism in very small quantities compared to basic foods.

The doctrine of vitamins - vitaminology - is now allocated to independent science.

General biological properties of vitamins

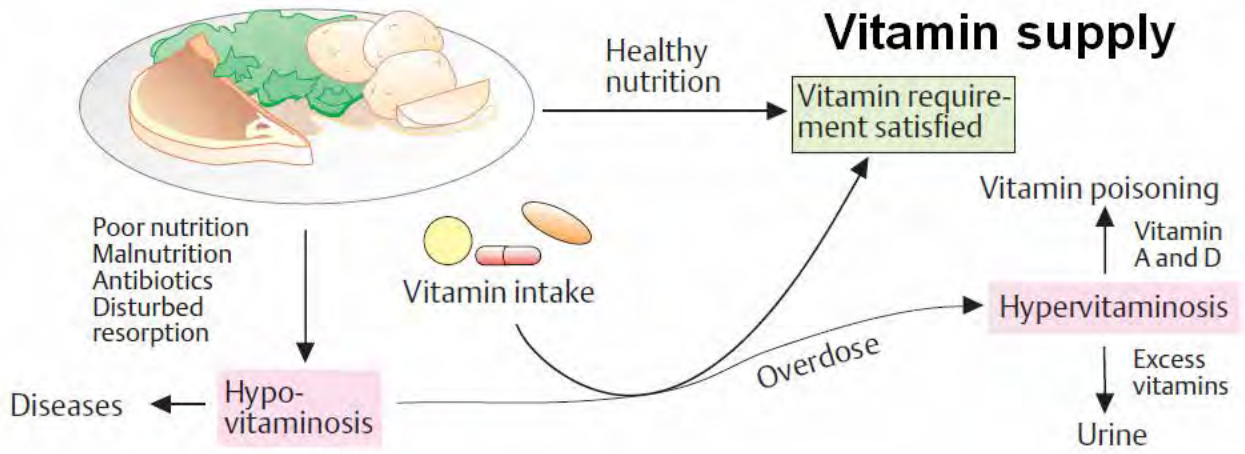
• Vitamins are not synthesized or insufficiently synthesized in the human body, so they are indispensable factors in nutrition.

• Vitamins generally cannot be a plastic material or a source of energy for the body.

• Vitamins show their biological activity in very small doses (mcg, mg) and are essential for all life processes.

• Insufficient intake or impaired absorption of certain vitamins causes the development of pathological conditions in the form of specific hypo- and avitaminosis.

• In high doses, vitamins can be used for therapeutic purposes as powerful non-specific pharmacological agents.

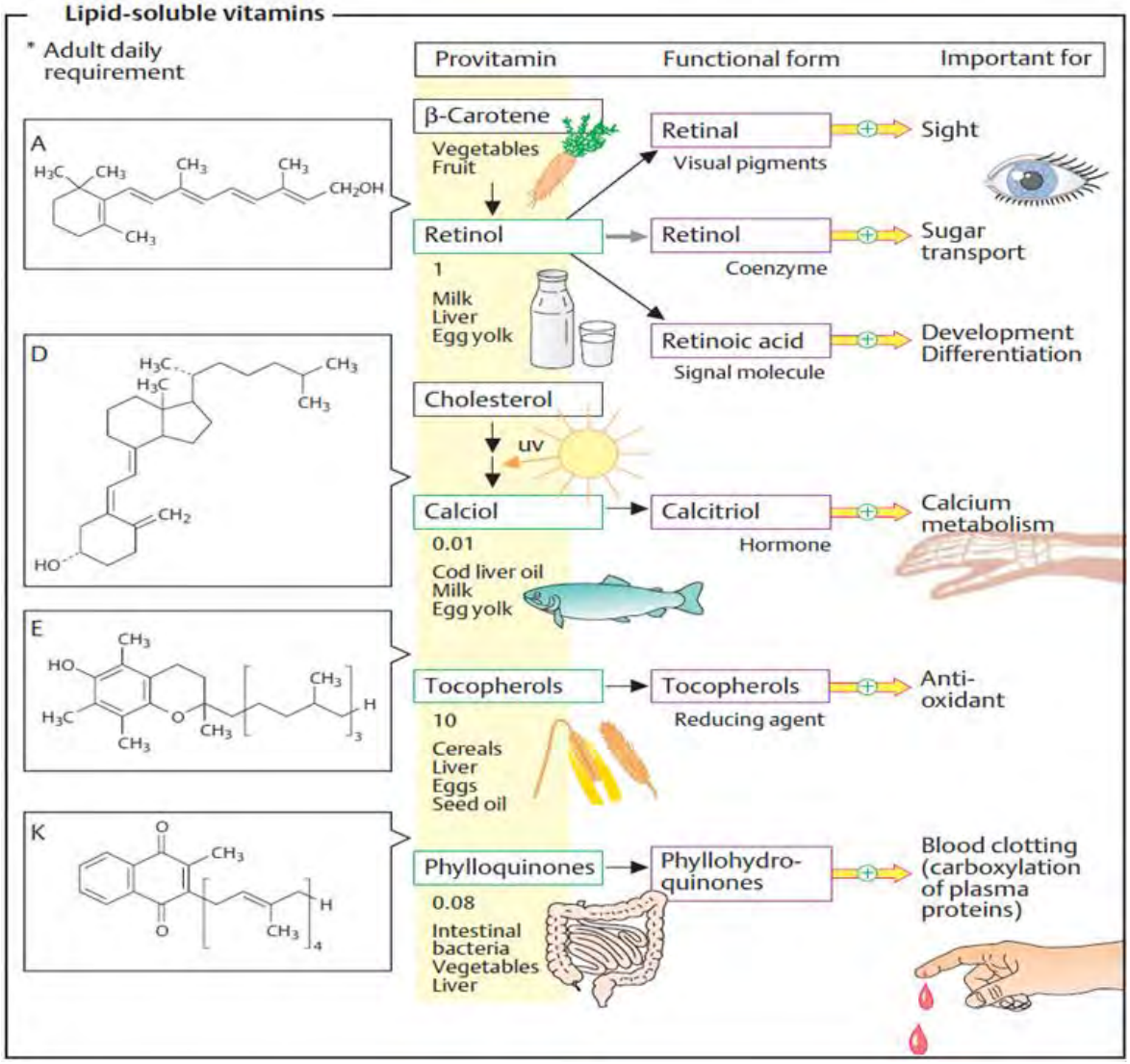


Remember: For example, ascorbic acid (vitamin C) is a vitamin for humans, but not for most animals (for example, in humans and guinea pigs scurvy or scurvy develops with insufficient intake of vitamin C, and in rats, rabbits - no), and biotin and vitamin D are necessary for human nutrition only under certain circumstances.

There are two main types of classification of vitamins: physical and chemical. According to the physical classification, all vitamins are divided into two groups on the basis of solubility in fats or water: fat-soluble and water-soluble. Chemical classification is based on the nature of the structure of molecules: aliphatic, alicyclic, aromatic, heterocyclic vitamins.

In the following classification of vitamins, in addition to the letter designation, the main biological effect is indicated in parentheses, with the prefix "anti", which indicates the ability of this vitamin to prevent or eliminate the development of the disease; the following is the nomenclature chemical name of each vitamin.

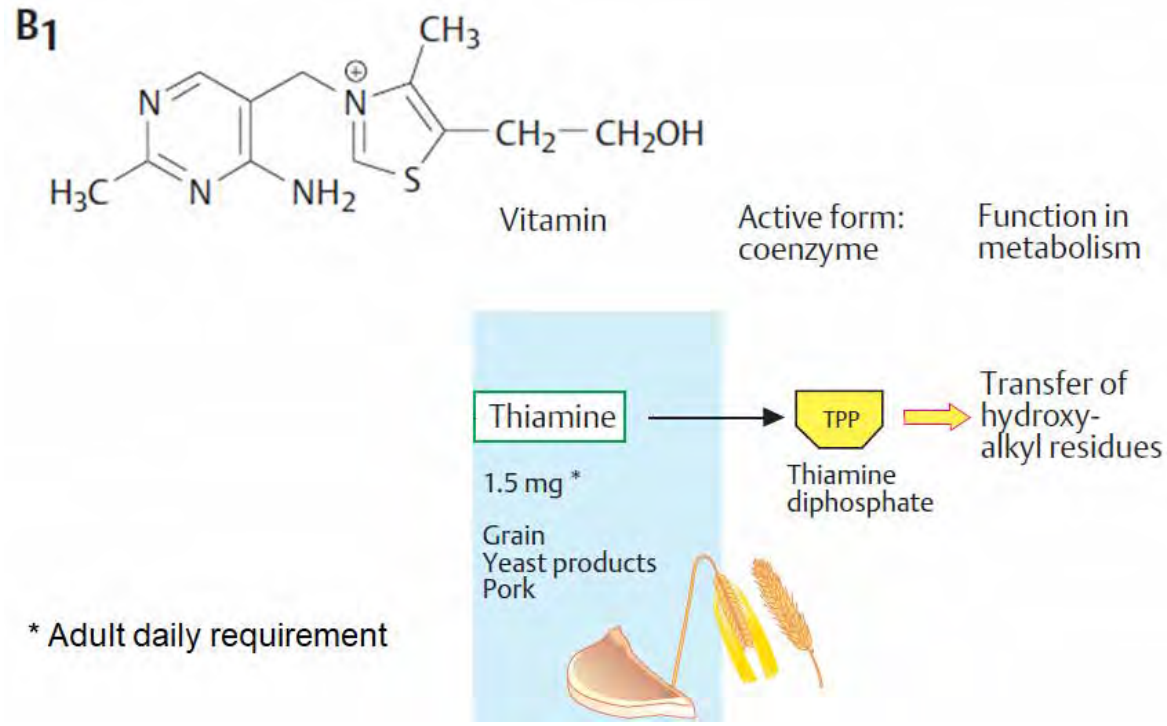
Fat-soluble vitamins: vitamin A (anti-xerophthalmic, growth vitamin), retinol; vitamin D (antirachitic), calciferol; vitamin E (anti-sterile, vitamin reproduction), tocopherols; vitamin K (antihemorrhagic), naphthoquinones; vitamin F (unsaturated Fatty Acids (FA): linoleic, linolenic, arachidonic).



Vitamin A is necessary for cell growth and reproduction, normal activity of the visual organ promotes normal metabolism. At A-beriberi first of all the organ of sight is struck. Resistance to infectious diseases decreases. The visual pigment in some mammals, birds, and reptiles is pink and is called rhodopsin. Visual pigments contain vitamin A aldehyde, a protein-bound retinal. Vitamin A1 is part of rhodopsin. Vitamin A is one of the few vitamins that can accumulate in the body. Vitamin A contains cyclic unsaturated alcohols with many interconnected double bonds.

Vitamin A has several vitamins: A1, A2, A3, which exist in the form of a number of geometric isomers. Vitamin A2 differs slightly in structure from vitamin A1 in that it has not one but two double bonds in its β-ionone ring.

Water-soluble vitamins: vitamin B1 (anti-neurite, aneurine), thiamine; vitamin B2 (growth vitamin), riboflavin; vitamin B3 (antidermatitis), pantothenic acid; vitamin B5 (PP; anti=pelargic), nicotinamide, niacin, nicotinic acid; vitamin B6 (antidermatitis; adermin), pyridoxine; vitamin B8 (H; antiseborrheic), biotin; vitamin B9 (Sun), folic acid; vitamin B12 (anti-anemic), cyanocobalamin; vitamin C (anti-zinc, antiscorbutic), ascorbic acid; vitamin P (capillary-strengthening, permeability vitamin), blood bioflavonoids and regulates vascular permeability; vitamin U (antiulcer).



Vitamin B1 (thiamine) was one of the first vitamins discovered by science. The study of the vitamin is associated with beriberi, a disease common in Southeast Asia. Millions of people have died from a strange seizure associated with the disease - polyneuritis. In ancient times, many generations of Chinese knew that a decoction of rice bran cures beriberi, but this information has not spread. In 1893, the Dutch physician Aikman, who worked for Fr. Java, experimentally caused

convulsions in chickens, feeding them white polished rice, which was consumed by the local population. Aikman also showed that cramps disappear quickly when rice extract is added to the feed. At first, he believed that white rice contained a toxic compound that was neutralized by some substance from the bran. He later concluded that rice bran contained the necessary nutrient. The structure of thiamine, first isolated in the crystalline state by Jansen in the Netherlands and Windaus in Germany, was established by R.R. Williams and his colleagues.

By chemical nature, thiamine is a derivative of thiazole (4-methyl-5-oxy-ethyl thiazole) and pyrimidine (2-methyl-5-oxy methyl-6-amino-pyrimidine).
The thiamine molecule consists of residues of two components - a pyrimidine derivative (2-methyl, 5-oxy methyl, 6-aminopyrimidine) and a thiazole derivative (4-methyl, 5-oxy-ethyl thiazole).

In the following form, i.e. in the form of a salt of a four-substituted ammonium base (thiamine chloride), vitamin B1 exists in an acidic environment.

Vitamin – Coenzyme form

Tiamine (vitamin B1) – Tiamine pyrophosphate
Niacin (nicotinic acid) – NADP+, NAD+
Ribophlavin (vitamin B2) – FAD, FMN
Pantothenic acid – Coenzyme A
Pyridoxal, pyridoxine, pyridoxamine or vitamine B6 – Pyridoxal phosphate
Cobalamin or vitamin B12 – 5'-Deoxyadenosylcobalamin Metylcobalamin
Biotin – Biotin-lysine complexes (byocytin)
Lipoic acid – Lipoil-lysine complexes (lipoamide)
Folic acid – Tetrahydrofolate

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Fat-soluble vitamins, their detection, and role

1. Qualitative reactions to vitamin A

Equipment and materials: tripod with test tubes, pipettes, rubber pears.

Reagents: 10% oil solution in chloroform, 10% fish oil solution in chloroform, 1% iron chloride solution, concentrated H_2SO_4.

1.1. Test with iron chloride

Progress of experiment. A solution of fish oil in chloroform is poured into the 1st test tube, a solution of oil in chloroform - into the 2nd test tube, and, then, a few drops of a solution of iron chloride are added into both test-tubes.

In the presence of vitamin A, the solution turns bright green.

1.2. Test with sulfuric acid

Progress of experiment. A solution of fish oil in chloroform is poured into the 1st test tube, a solution of oil in chloroform - into the 2nd test tube, and, then, it

is added 1-2 ml of concentrated sulfuric acid. In the presence of vitamin A, a blue color appears, which gradually turns purple, and then reddish-brown.

2. Qualitative reactions to vitamin E.
Test with nitric acid

Vitamin E with concentrated nitric acid is oxidized to form o-quinone, which turns the color of the solution into the red.

Equipment and materials: tripod with test tubes, pipettes, rubber bulbs, water bath.

Reagents: concentrated nitric acid, oil solution of vitamin E.

Progress of experiment. In two test tubes, pour 2-3 drops of oil solution of vitamin E. In the first test tube, add 1-2 ml of water, in the second - the same amount of concentrated nitric acid. Both tubes are heated in a boiling water bath for 10 minutes. In the test tube with nitric acid, the oil solution of the vitamin turns red.

Water-soluble vitamins, their role as coenzymes

1. Qualitative reaction for vitamin C (ascorbic acid)

Progress of experiment. There are 2 test-tubes (1 - experimental and 2 - control). In the 1st test-tube, it is necessary to add 5 drops of vitamin C, 1 drop of 10% solution of NaOH, and 1 drop of 5% solution of $K_3[Fe(CN)_6]$. Mix them well and add 3 drops of 10% solution of HCl and 1 drop of 1% $FeCl_3$ solution. The sediment of the Prussian blue falls.

Perform the same reaction by adding distilled water instead of vitamin C solution in the 2nd test-tube (control). In this case, the Prussian blue is not formed.

4. Quantitative determination of vitamin C

The principle of the method is that vitamin C, oxidizing reduces 2,6-dichlorophenolindophenol, which is converted into a colorless compound.

Progress of experiment. Weigh 20 g of the test substance (apples, horseradish, potatoes), crumble, grind in a porcelain mortar with 20 ml of 2% HCl solution. After that, the contents are transferred into a measuring cylinder, rinsed with the same solution, and adjusted to a volume of 40 ml. The mixture is filtered. Measure 10 ml of the filtrate into a beaker and titrate with 0,001 N a solution of 2,6-dichlorophenolindophenol to a pink color which does not disappear for 30 seconds. The amount of vitamin C is calculated by the formula:

$$C = X \times 0{,}088 \times 20 \text{мг\%}$$, where:

C – the amount of vitamin C per 100 g of substance;
X – the number of ml of 0.001 N 2,6-dichlorophenolindophenol spent on titration;
0,088 - the amount of vitamin C is equivalent to 1 ml of indicator;
20 – conversion factor per 100 g of the test substance.

Control questions, tasks and exercises for the section «VITAMINS. COENZYMES»

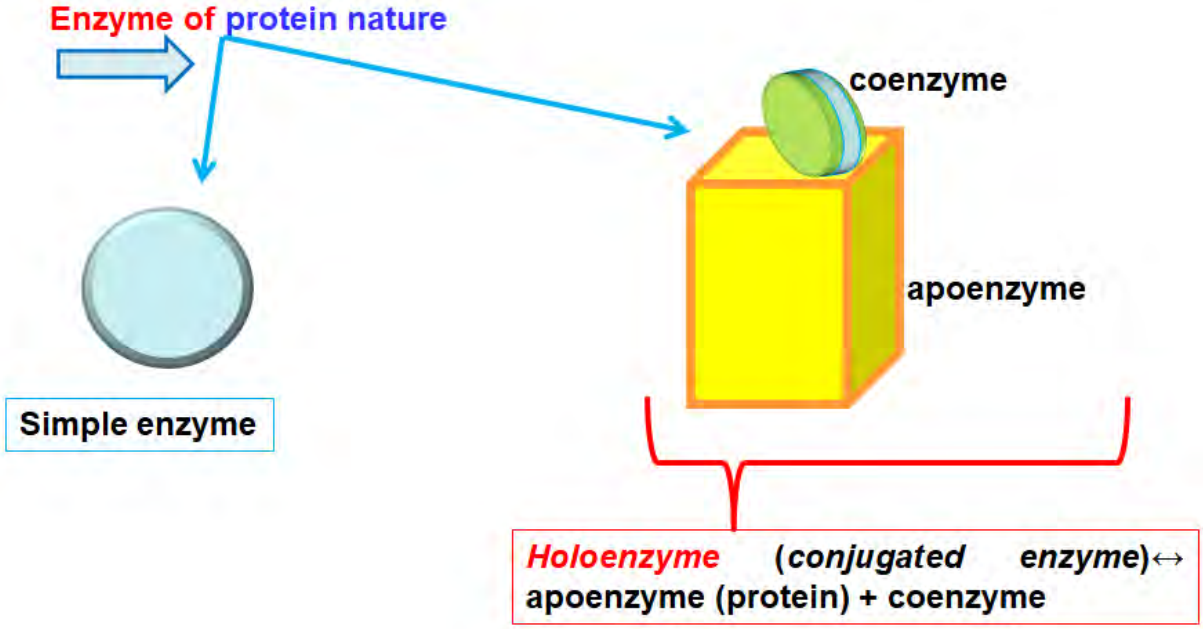
1. What organic compounds are called vitamins? Classification of vitamins.
2. Give examples of fat-soluble vitamins and their importance for animal life

3. Give examples of water-soluble vitamins and their role for enzyme activity
4. Give a diagram of the transformation of vitamin A in the visual process.
5. In what processes in the body is involved ubiquinone?
6. Vitamins B2 and B6. Indicate the names of enzyme reactions in which these vitamins are involved.

2.1.3. ***Topic*** ENZYMES AND THEIR KINETIC PROPERTIES

Living organism seethes with metabolic activity. Thousands of chemical reactions are proceeding very rapidly at any given instant within all living cells. Virtually all of these transformations are mediated by enzymes, proteins (and occasionally RNA) specialized to catalyze metabolic reactions.

Enzymes are generally globular proteins and range from just 62 amino acid residues in size, for the monomer of 4-oxalocrotonate tautomerase to over 2,500 residues in the animal fatty acid synthase.



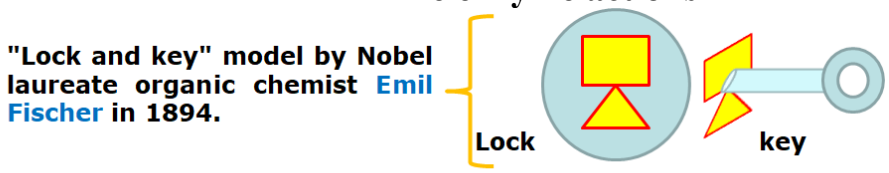
Most enzymes are much larger than the substrates they act on, and only a small portion of the enzyme (around 3–4 amino acids) is directly involved in catalysis.

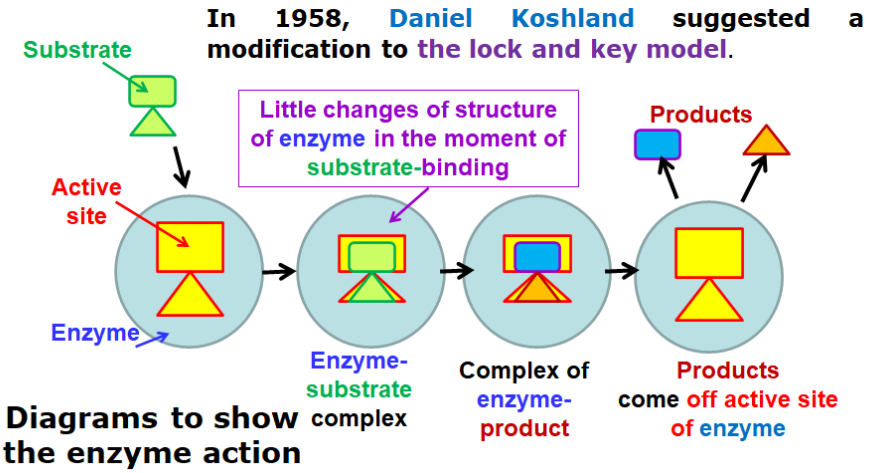
The region that contains these catalytic residues, binds the substrate, and then carries out the reaction is known as the active site.

Enzymes can also contain sites that bind cofactors (ions of metals and coenzymes), which are needed for catalysis.

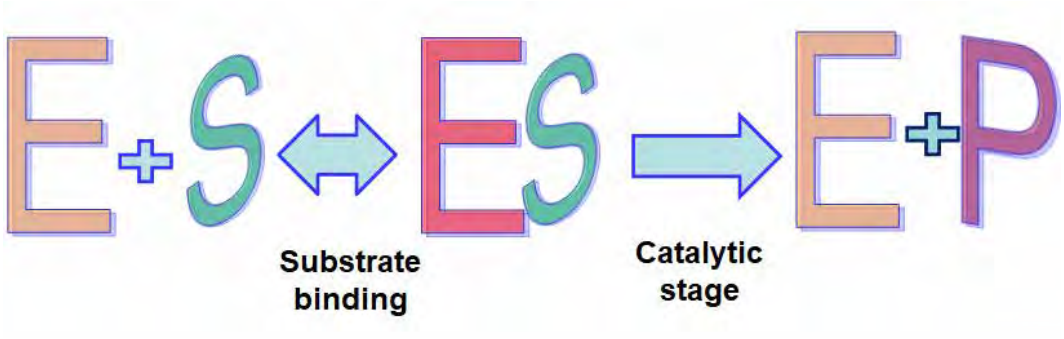
Some enzymes also have binding sites for small molecules, which are often direct or indirect products or substrates of the reaction catalyzed.

The enzyme actions





Mechanism for a single substrate enzyme catalyzed reaction. The enzyme (E) binds a substrate (S) and produces a product (P).



The International Union of Biochemistry and Molecular Biology have developed a nomenclature for enzymes, the EC numbers; each enzyme is described by a sequence of four numbers preceded by "EC".

The first number broadly classifies the enzyme based on its mechanism.

The top-level classification is

EC 1 *Oxidoreductases*: catalyze oxidation/reduction reactions

$CH_3CH_2OH + NAD^+ \rightarrow \rightarrow (CH_3)CHO + NADH + H^+$

OXIDOREDUCTASE

(ALCOHOL DEHYDROGENASE)

EC 2 *Transferases*: transfer a functional group (*e.g.* a methyl or phosphate group)

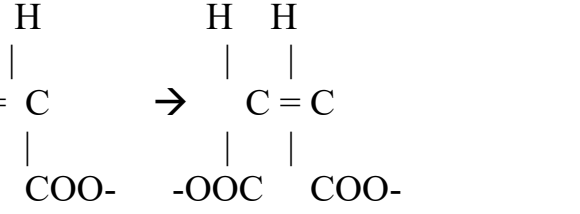
GLUCOSE +ATP→→ GLUCOSE-6-PHOSPHATE + ADP

TRANSFERASE (HEXOKINASE)

EC 3 *Hydrolases*: catalyze the hydrolysis of various bonds
$ATP + H_2O \rightarrow ADP + P_i$
HYDROLASE (ATPase)

EC 4 *Lyases*: cleave various bonds by means other than hydrolysis and oxidation
$CH_3(CO)COO^- + H^+ \rightarrow CH_3CHO + CO_2$
(PYRUVATE) (ACETALDEHYDE)
LYASE (PYRUVATE DECARBOXYLASE)

EC 5 *Isomerases*: catalyze isomerization changes within a single molecule



(6) ISOMERASE (MALEATE ISOMERASE)

EC 6 *Ligases*: join two molecules with covalent bonds.
$CH_3(CO)COO^- + ATP + CO_2 \rightarrow$
(PYRUVATE)
$\rightarrow ADP + P_i + -OOC\text{-}CH_2(CO)COO^-$
(OXALOACETATE)
LIGASE (PYRUVATE CARBOXYLASE)

EC 7 *Translocases* are a separate class of enzymes that catalyze the transfer of ions or molecules across membranes or their separation in membranes (for example, carnitine-acylcarnitine translocase or carnitine-acylcarnitine transporter).

The terms used in Enzymology

Katal ("**kat**") corresponds to the number of catalyst that can convert 1 mole of substrate to product in 1 second (s^{-1}).

International unit enzymatic activity (**IU**) can be converted into catal through the following equations
1 kat = 1 mol of substrate $\cdot s^{-1} = 60$ mol $\cdot min^{-1} = 60 \cdot 10^6$ μmol $\cdot min^{-1} = 6 \times 10^7$ IU
1 IU = 1 μmol $\cdot min^{-1} = (1/60)$ μmol $\cdot s^{-1} = (1/60)$ μkat = 16,67 nkat

Rotation speed = (number of mol of substrate converted) / min

The **international activity unit** (IU) = (number of enzyme that catalyzes the conversion of one micromole of substrate) / min

Specific activity = (number of "kat") / (amount of active protein, mg)

Molar activity = (number of "kat") / (number of mol of enzyme)

Determination of enzymatic activity

It's often measured by the relative velocity of the transformation

Substrate → Products

1 unit of activity - is the amount of enzyme protein nature, which makes one micromole of substrate per 1 min at 25 ° C and optimum pH

1 unit of specific activity - the number of units per mg of the protein (for example, 37μmol/min/mg of protein)

1 unit of molecular activity - the number of units per micromole of purified enzyme (for example, 12 units / μmol of enzyme)

Kinetic peculiarities

Enzymes are the catalysts of biological systems, and they are extremely efficient as catalysts.

In fact, typically, an enzyme accelerates the rate of a reaction by factors of at least a million compared to the rate of the same reaction in the absence of the enzyme.

Most biological reactions do not occur at perceptible rates in the absence of enzymes.

Enzyme kinetics is the study of the chemical reactions that are catalyzed by enzymes.

In enzyme kinetics, **the reaction rate** is measured and the effects of varying the conditions of the reaction investigated.

Studying an enzyme's kinetics in this way can reveal *the catalytic mechanism of this enzyme*, *its role in metabolism*, how its activity is controlled, and how a drug or a poison might inhibit the enzyme.

There are **energies of the stages of a chemical reaction**. Substrates need a lot of energy to reach a transition state, which then decays into products. The enzyme stabilizes the transition state, reducing the energy needed to form products.

Enzymes are usually protein molecules that manipulate other molecules - the enzymes' substrates.

These target molecules bind to an enzyme's active site and are transformed into products through a series of steps known as the enzymatic mechanism.

These mechanisms can be divided into *single-substrate* and *multiple-substrate* mechanisms.

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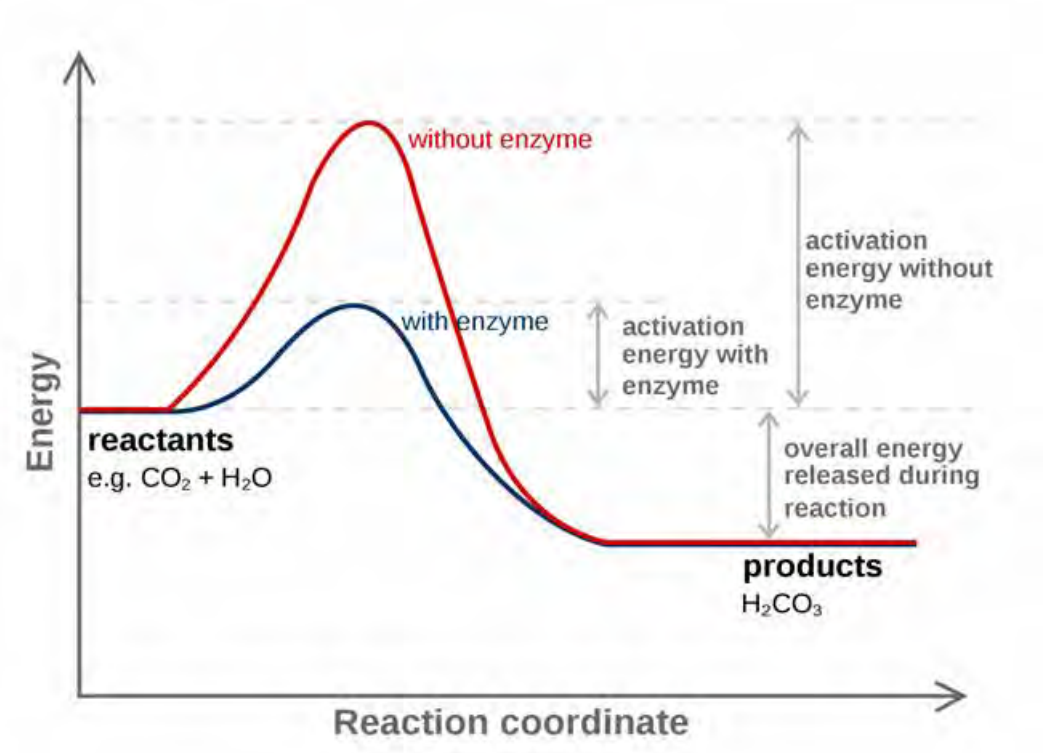

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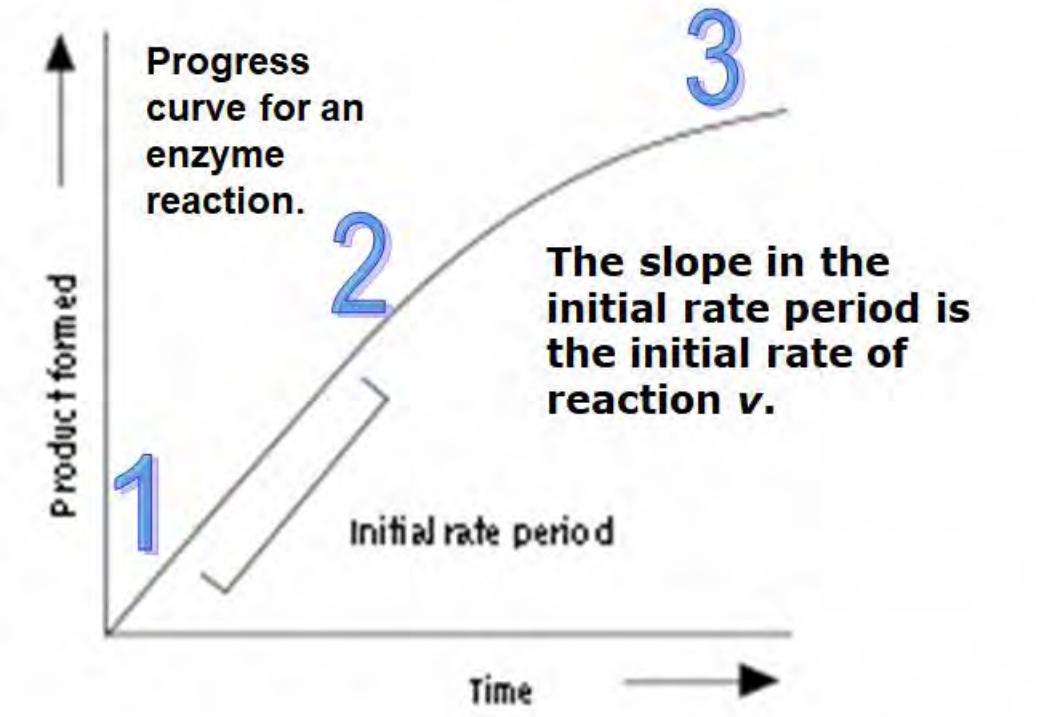
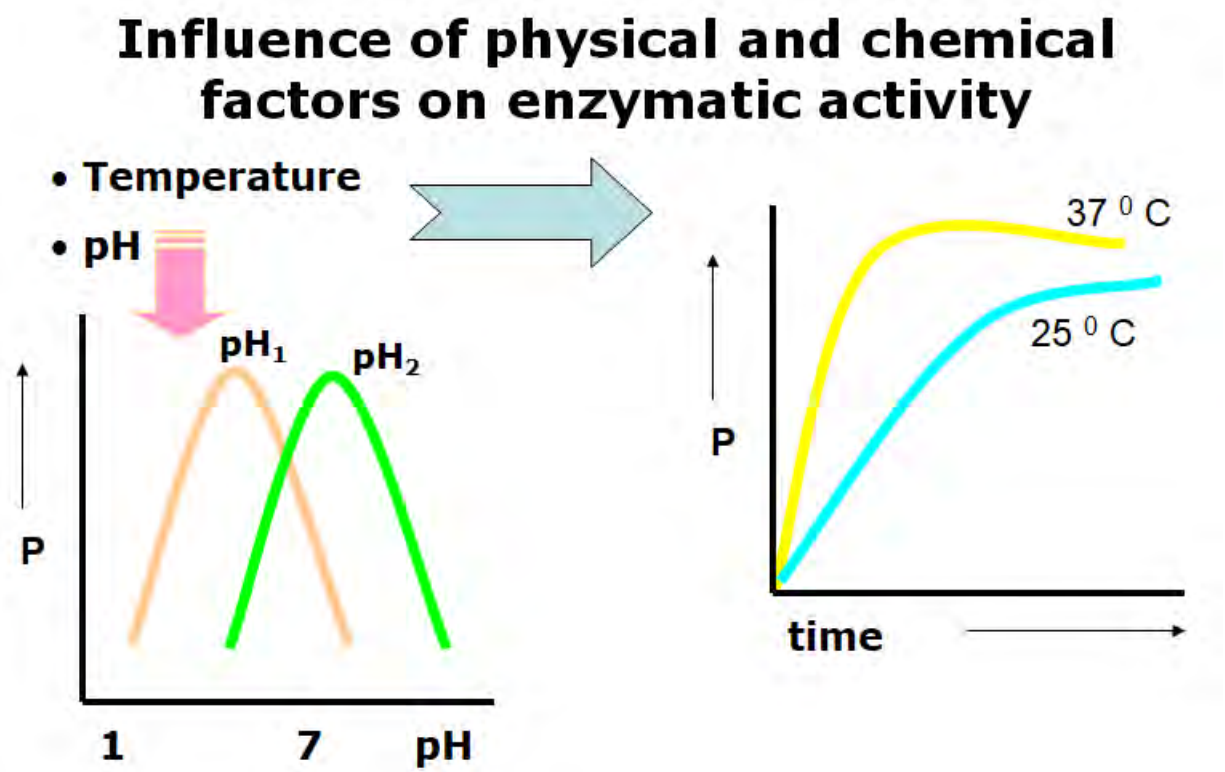
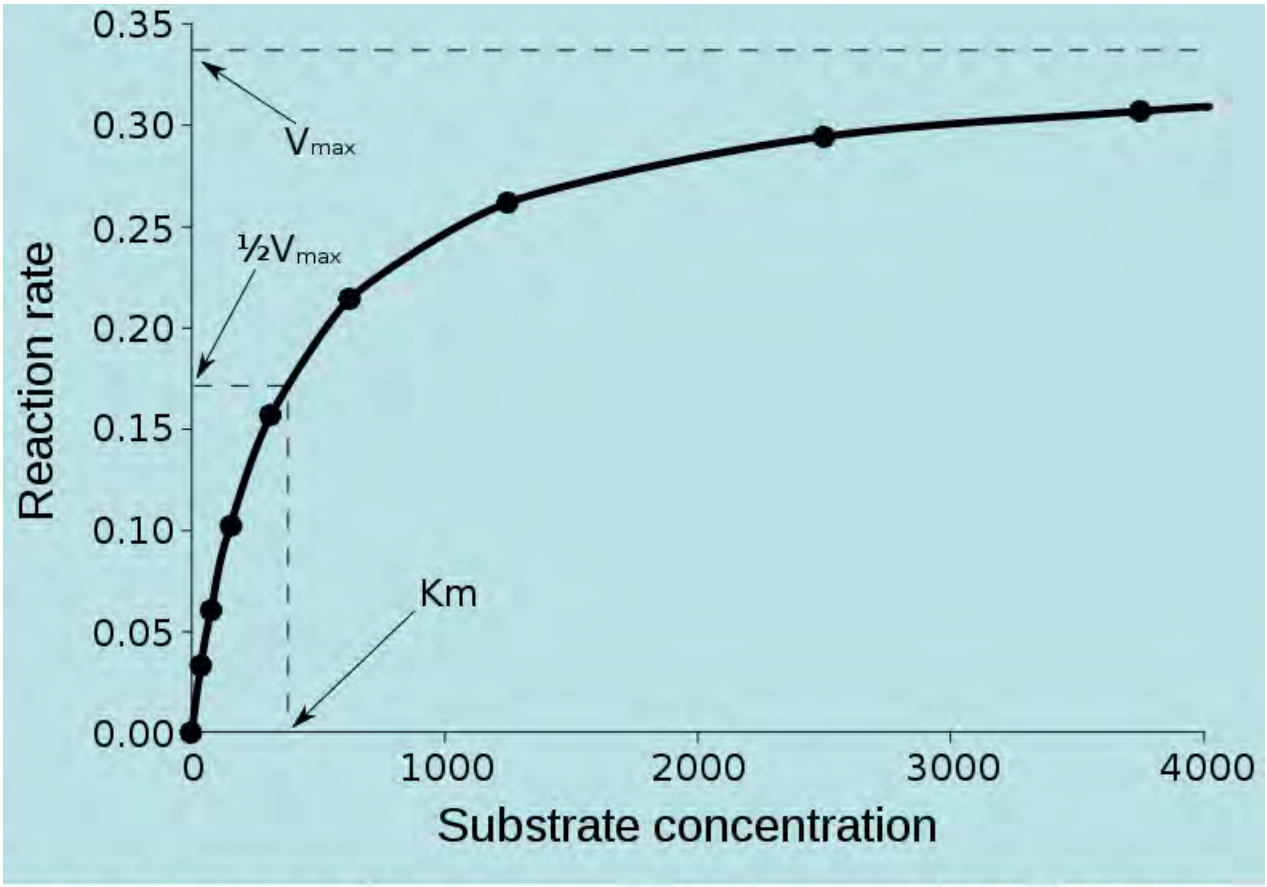


The Michaelis–Menten equation describes how this slope varies with the concentration of substrate



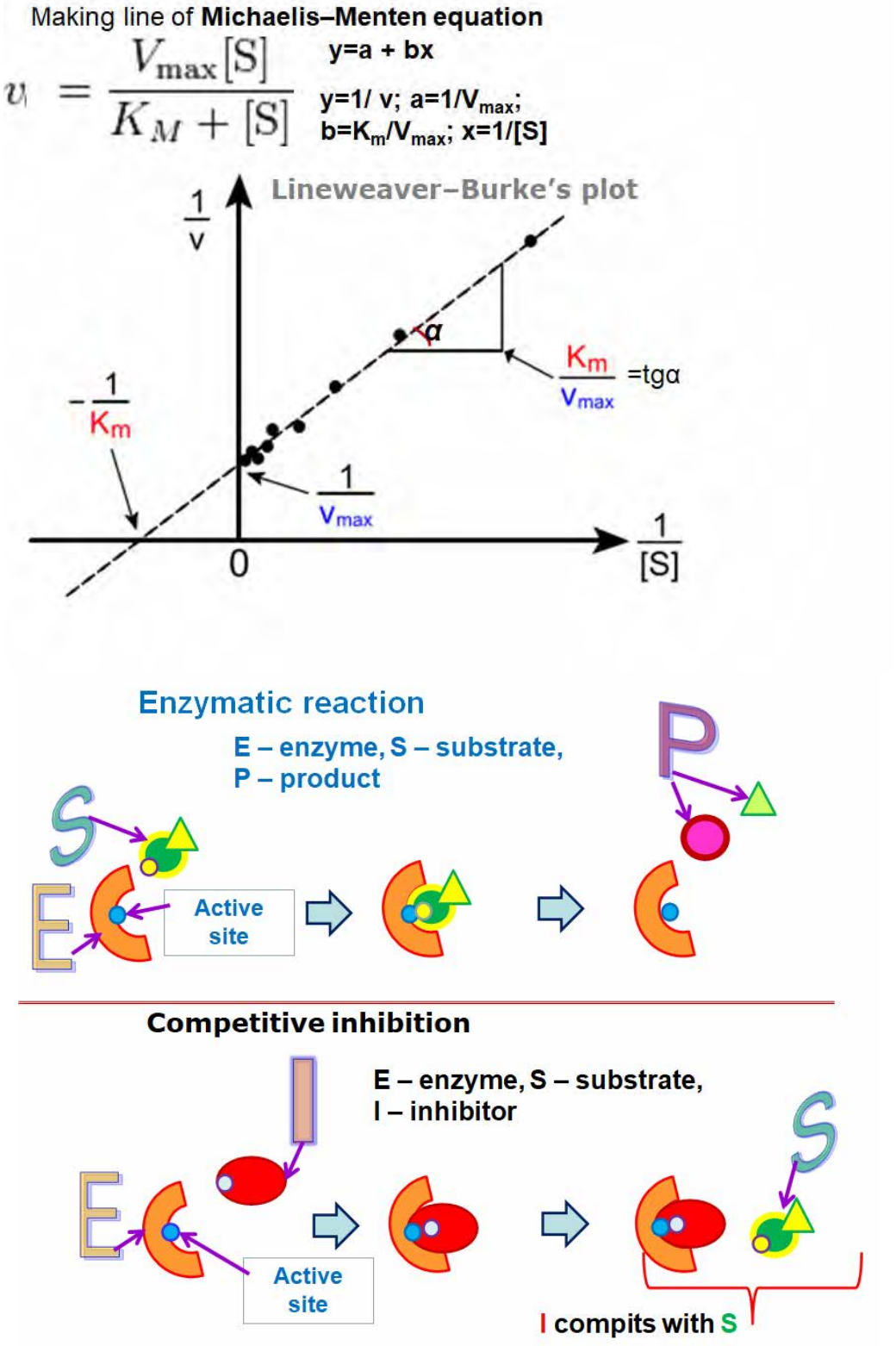


Saturation curve for an enzyme showing the relation between the concentration of substrate and rate

The study of enzyme kinetics is important for two basic reasons.

Firstly, it helps explain how enzymes work, and secondly, it helps predict how enzymes behave in living organisms.

The kinetic constants defined below, K_M and V_{max}, are critical to attempts to understand how enzymes work together to control metabolism.



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1. The effect of temperature on amylase activity

Equipment and materials: tripod, test tubes, glass with ice, alcohol, water bath with thermometer, pipettes, saliva.

Progress of experiment. In four test-tubes (№ 1, 2, 3, 4), pour 5 ml of starch solution. Then, add 2-3 ml of diluted saliva in test tubes № 1, 2, 3. Add 2-3 ml of pre-boiled saliva to the fourth tube. Stir the contents of each tube. To incubate for 10 minutes-period, place the 1st test-tube in a glass with ice (0° C), the 2nd test-tube is left in a tripod at room temperature, the 3d and 4th test-tubes are placed in a water bath at 38-40° C, and after that, they are cooled.

1-2 drops of Lugol's solution are added to all test tubes. Pour some solution from test tube № 1 to react with iodine. In the first test tube, the solution should turn blue, in the second - purple or red-brown, in the third test tube - yellow, in the fourth - blue. If you place a test tube № 1 in a water bath at 38-40 ° C for 10 minutes, you can see the results of the hydrolysis of starch (the blue color of the sample will disappear). Thus, the above result demonstrates the functioning of the enzyme, the activity of which was inhibited by low temperature

2. The effect of the medium pH on amylase activity

Reagents: saliva (amylase source), 0.2% starch solution, 1 N hydrochloric acid solution, Lugol's solution.

Equipment and materials: tripod, test tubes, glass sticks, pipettes.

Progress of experiment. In two test tubes, pour 5 ml of 0.2% starch solution. In the first test-tube, add 1 ml of 1N hydrochloric acid solution and in the second test-tube - the same amount of water. Pour 1 ml of saliva into two test tubes, mix and leave for 20 minutes. Then, add 5 drops of Lugol's solution into two test-tubes and mix. In the first test tube, the color of the solution does not change due to the fact that amylase is inactivated hydrochloric acid.

3. The specificity of action of enzymes (amylase)

Reagents: saliva, 0.5% starch solution, 2% sucrose solution, Fehling's reagent [preparation method is described below: CARBOHYDRATE METABOLITES: 4. Quantitative determination of glucose in solution by the Fehling method]

Equipment and materials: tripod, test tubes, pipettes, water bath, thermometer, glass sticks.

Progress of experiment. In two test-tubes, pour 1-1.5 ml of saliva, add 1-2 ml of starch in the first test-tube, sucrose - in the second test-tube, mix well. All tubes are placed for 15 minutes in a water bath at a temperature of 38 ° C, after which the Fehling reaction is carried out with the contents of the test tubes.

4. Determination of amylase activity

The principle of the method. Quantitative determination of salivary amylase activity is that saliva is diluted in a certain sequence, then add the same amount of

starch solution, find the lowest content of the enzyme that completely hydrolyzes the entire amount of added starch, next, it's recalculating per 1 ml of saliva.

Reagents: saliva, 0.1% starch solution, Lugol's solution.

Equipment and materials: tripod, test tubes, pipettes, water bath, thermometer, glass sticks.

Progress of experiment. It is necessary to take 10 test tubes and number them. In the first two tubes make 1 ml of amylase (saliva), then in all tubes except the first, add 1 ml of distilled water. Then from the second tube transfer 1 ml contained in the third tube, mix well with a glass rod, take 1 ml of the mixture and transfer to the fourth tube, etc. to the last test tube. From the tenth tube, 1 ml of the mixture is poured.

The result is a series of test tubes with the appropriate amount of saliva:

##test tubes	1	2	3	4	5	6	7	8	9	10
The amount of amylase (saliva), ml	1.0	0.5	0.25	0.125	0.0625	0.0312	0.0156	0.0078	0.0039	0.00195

Then 5 ml of 0.1% starch solution is added to each tube. All tubes are incubated in a water bath for 30 minutes at 37 ° C. After 30 minutes of incubation, all tubes are cooled. In each test tube, add 5 drops of Lugol's solution, mixing well with a glass stick.

The appearance of yellow color in vitro indicates a complete breakdown of starch. Red indicates the presence of dextrins, blue - the presence of starch.

Amylase activity is determined by the number of ml of 0.1% starch solution, which can hydrolyze 1 ml of undiluted saliva at a temperature of 37 ° C for 30 minutes.

$$C = 5/x$$

where: 5 - the number of ml of 0.1% hydrolyzed starch solution;

x is the amount of amylase (saliva), ml.

5. Influence of activators and inhibitors on enzyme activity

The activity of salivary amylase is investigated in the presence of compounds that have the properties of positive and negative effectors.

Equipment and materials: tripod, test tubes, pipettes, water bath, thermometer.

Reagents: diluted saliva, 1% starch solution, 1% NaCl solution, 1% $CuSO_4$ solution, Lugol's solution.

Progress of experiment. In two test tubes, pour 4-5 ml of starch solution, in a test tube # 1 - 1-2 ml of NaCl solution, in a test tube # 2 - 1-2 ml of $CuSO_4$ solution. 1-2 ml of diluted saliva are added to both tubes, the contents of the tubes are mixed and the tubes are incubated for 10 minutes in the thermostat at 37 ˚C.

Then, add 1 drop of Lugol's reagent to both tubes. Observations recorded in a notebook show the effect of sodium chloride and copper sulfate on amylase activity:

# test tube	Enzyme	Effector	Substrate	Incubation at 37 ˚C	Color after iodine staining
1	amilase	NaCl	starch	10 min	
2	amilase	$CuSO_4$	starch	10 min	

6. Kinetic properties of amylase activity

The principle of the method. The substrate content is estimated during the hydrolysis of starch by amylase at appropriate time intervals (1 min, 5 min, 10 min, 15 min, 20 min, 30 min, 40 min) by adding a few drops of Lugol's solution. The experiment uses the lowest content of the enzyme (experiment 4), which completely hydrolyzes the entire amount of added starch.

Reagents: saliva, 0.1% of starch solution, Lugol's solution.

Equipment and materials: tripod, test tubes, pipettes, water bath, thermometer, glass sticks.

Progress of experiment. Number of 8 tubes, to which added 1 ml of amylase solution (saliva; Experiment 4).

Table

## of test tubes	0	1	2	3	4	5	6	7
Time, min	0	1	5	10	15	20	30	40
%, starch content	100							
Color of the obtained products	blue							

Then 5 ml of 0.1% of starch solution are added to each tube. All tubes are incubated in a water bath for an appropriate period of time at 37 ° C, then the appropriate tube is cooled and add 5 drops of Lugol's solution, stirring well with a glass stick. Enter the results in the table above.

1.1.3. *Control questions, tasks and exercises for the section «*ENZYMES AND THEIR KINETIC PROPERTIES*»*

1. What organic substances are called enzymes?
2. What are the basic principles of classification and nomenclature of enzymes?
3. What is common and different in enzymes and inorganic catalysts for their action on the conversion of compounds?
4. What isoenzymes do you know? What role do they play in the metabolic processes of animals?
5. Give the differences between enzymes:
    a) hydrolases and hydratases;

b) phosphatases and phosphorylases;
c) exopeptidases and endopeptidases;
d) pepsin and cathepsin;
e) trypsin and chymotrypsin;
f) trypsin and trypsinogen.

6. Specify the correspondence of the performed function for a certain enzyme:

1. Pepsin	A. Sterol hydrolysis
2. Cholesterol esterase	B. Hydrolysis of carbohydrates
3. Amylase	C. The main enzyme of gastric juice
4. Urease	D. Hydrolysis of urea to ammonia and CO_2
5. Thrombokinase	E. Thrombin formation from prothrombin

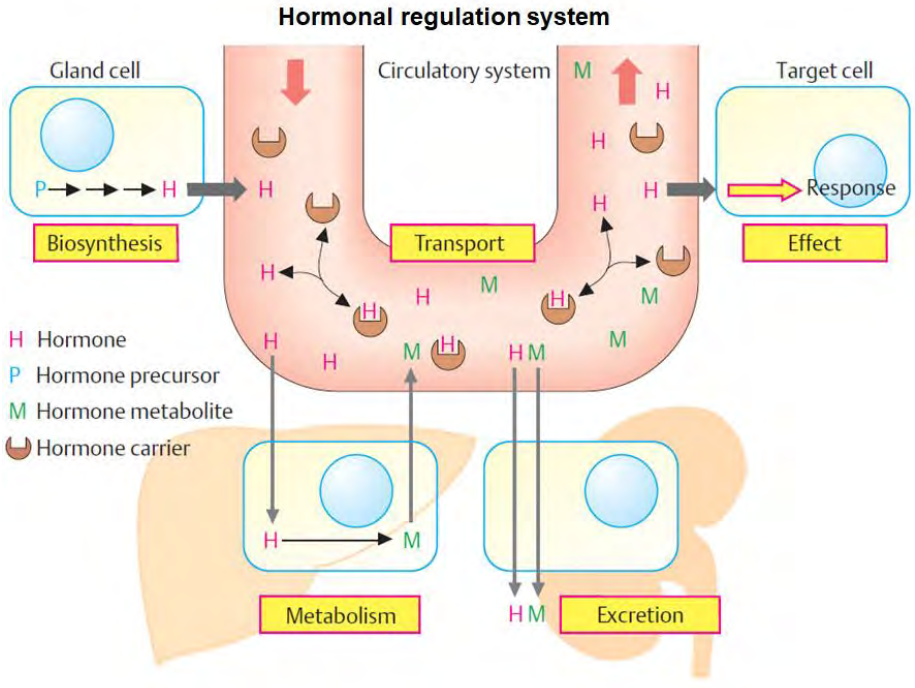
2.1.4. Topic HORMONES AND MECHANISMS THEIR INFLUNCES ON METABOLIC PROCESSES

Hormones are chemicals released by a cell or a gland in one part of the body which send out messages that affect cells in other parts of the organism.

Only a small amount of hormone is required to alter cell metabolism. In essence, it is a chemical messenger that transports a signal from one cell to another.

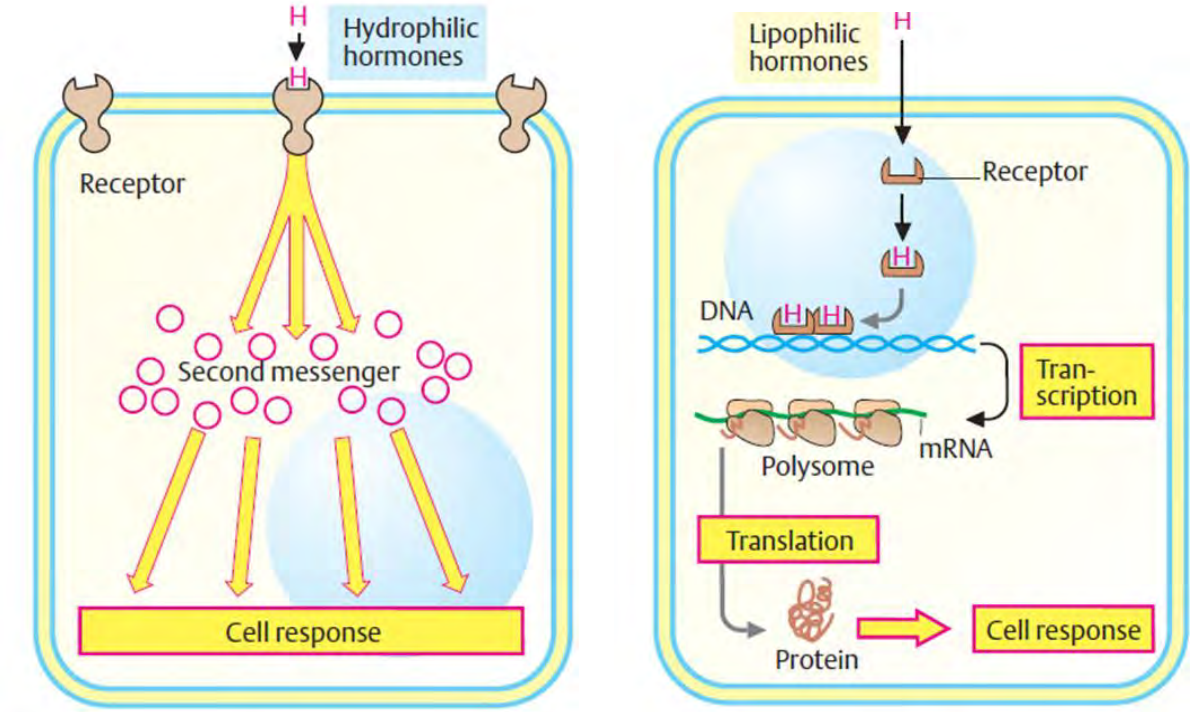
All multi-cellular organisms produce hormones; plant hormones are also called phytohormones.

Hormones in animals are often transported in the blood. Cells respond to a hormone when they express a specific receptor for that hormone. The hormone binds to the receptor protein, resulting in the activation of a signal transduction mechanism that ultimately leads to cell type-specific responses.



Principles of hormone action

The group of **hydrophilic hormones** consists of hormones derived from amino acids, as well as peptide hormones and proteohormones. Their *receptors* are in the plasma membrane. Binding of the hormone to this type of receptor triggers a signal that is transmitted to the interior of the cell, where it controls the processes that allow the hormone signal to take effect (**signal transduction**).



Lipophilic hormones, which include the steroid hormones, thyroxine, and retinoic acid, bind to a specific *receptor protein* inside their target cells. The complex formed by the hormone and the receptor then influences *transcription* of specific genes in the cell nucleus.

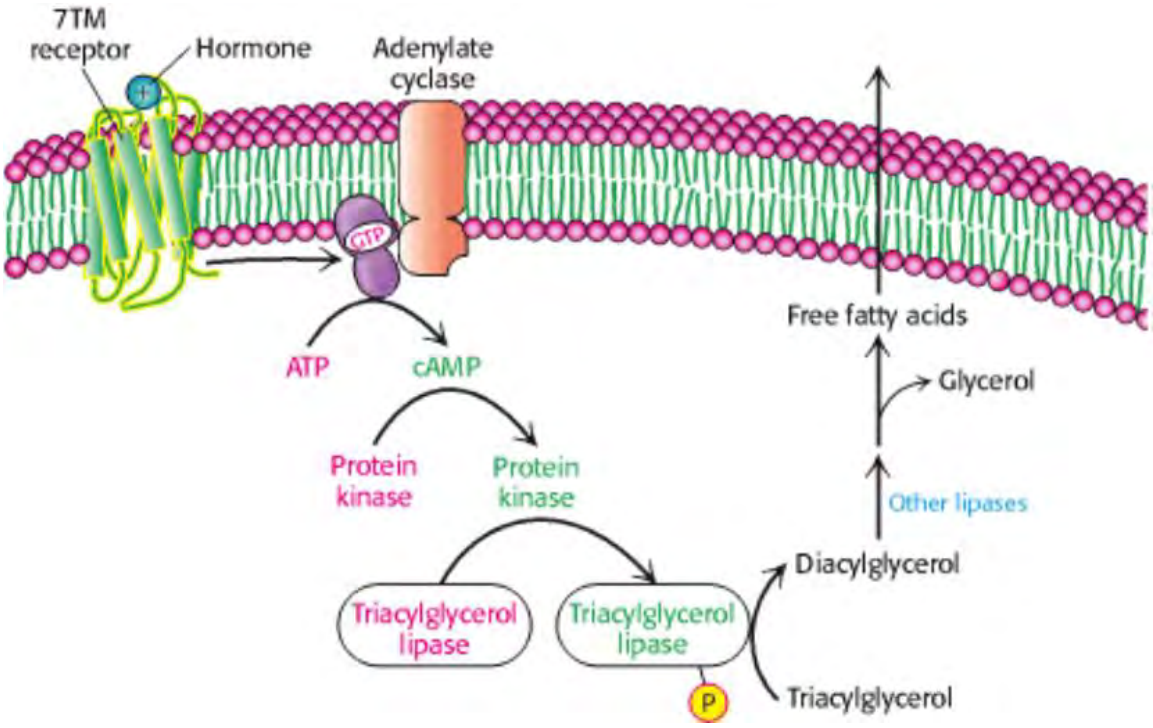
Regulation of lipolysis

Hormones (that affect lipolytic processes) are divided into 2 groups:

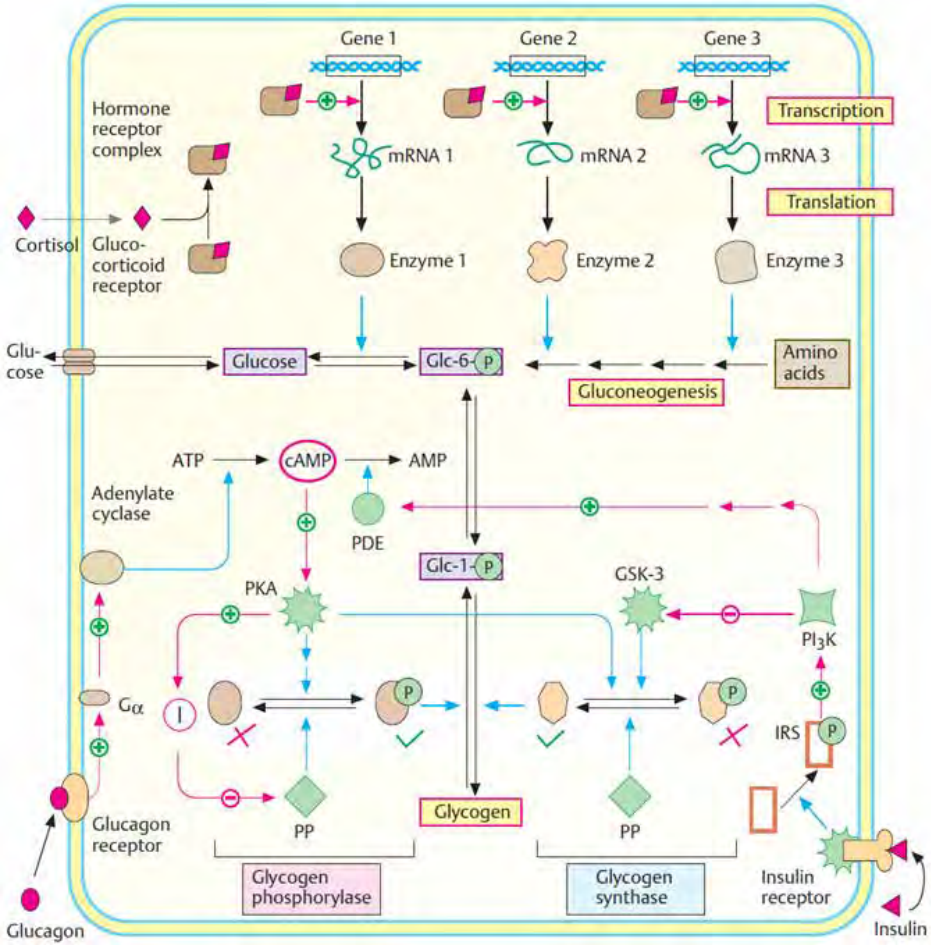
1. Hormones of direct action (adrenaline, glucagon, somatotropin, insulin)
2. Hormones of mediated action (thyroid hormones, glucocorticoids, sex hormones, leptin)

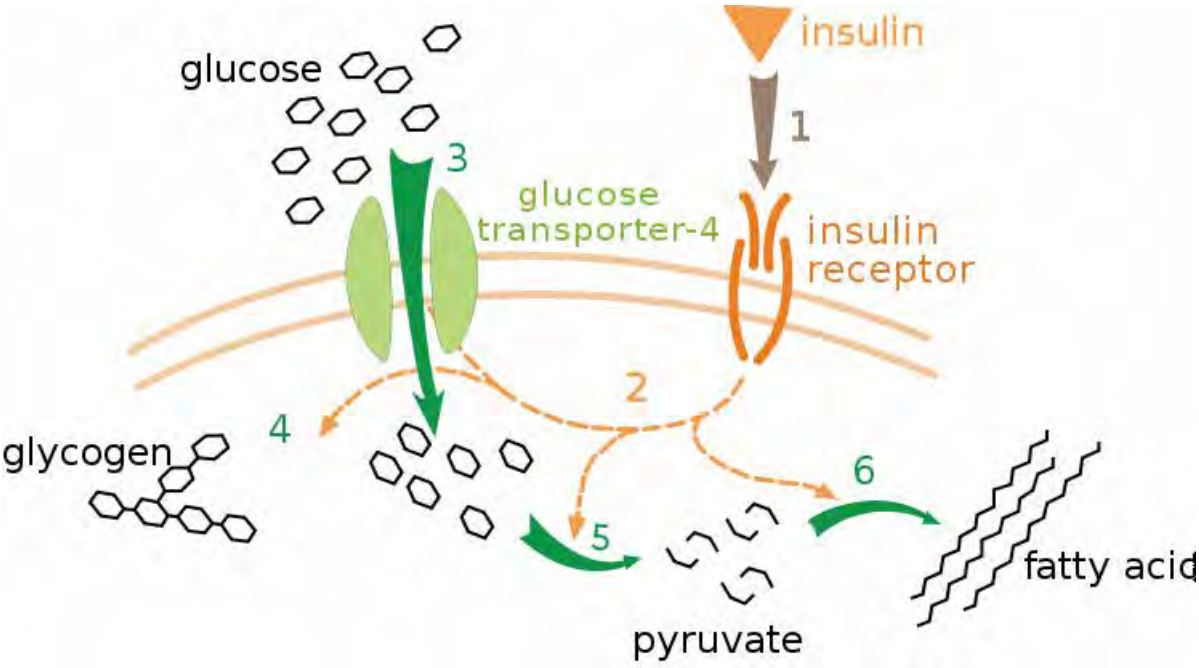
Epinephrine, glucagon, somatotropin, sex hormones, thyroxin and triiodothyronine, leptin stimulate lipolysis in adipocytes, and insulin is suppressed.

Mobilization of Triacylglycerols. Triacylglycerols in adipose tissue are converted into free fatty acids and glycerol for release into the bloodstream in response to hormonal signals. A hormone-sensitive lipase initiates the process.

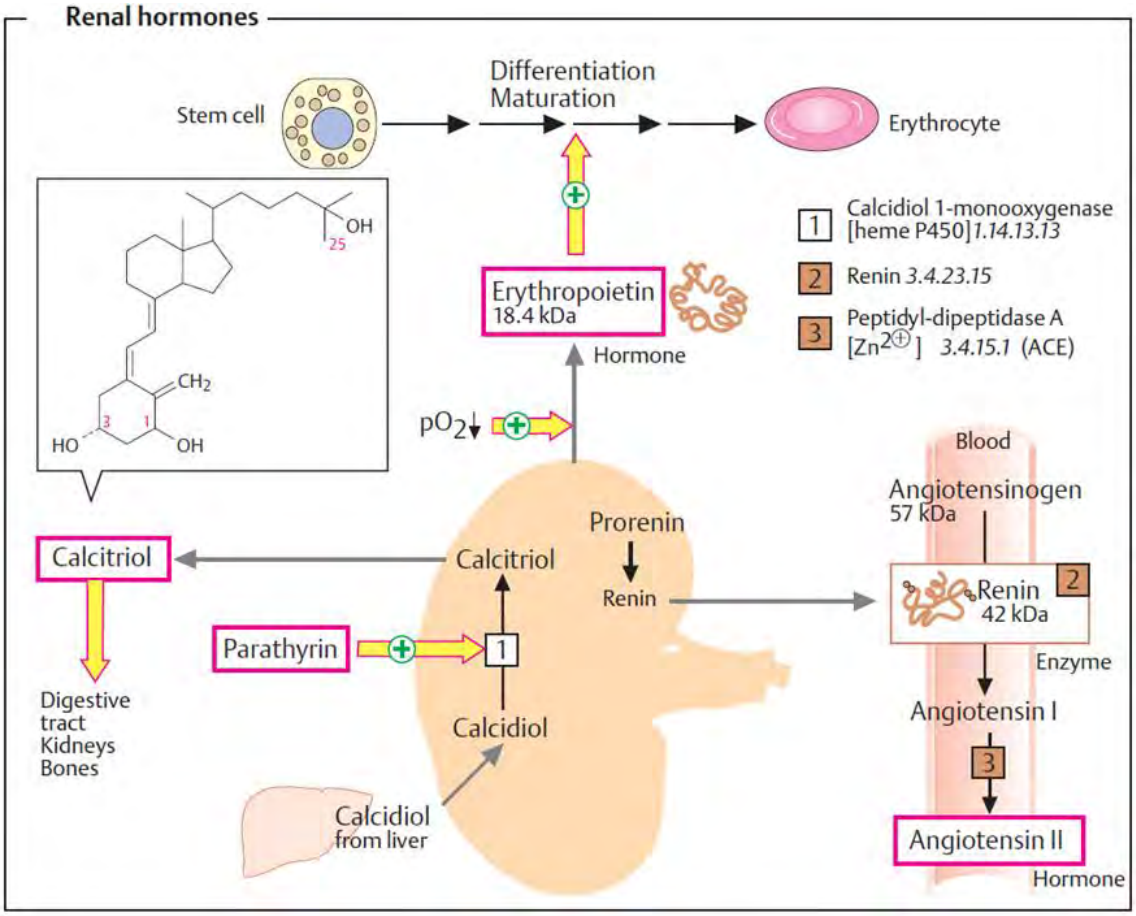


Hormonal regulation of glucose metabolism in the liver

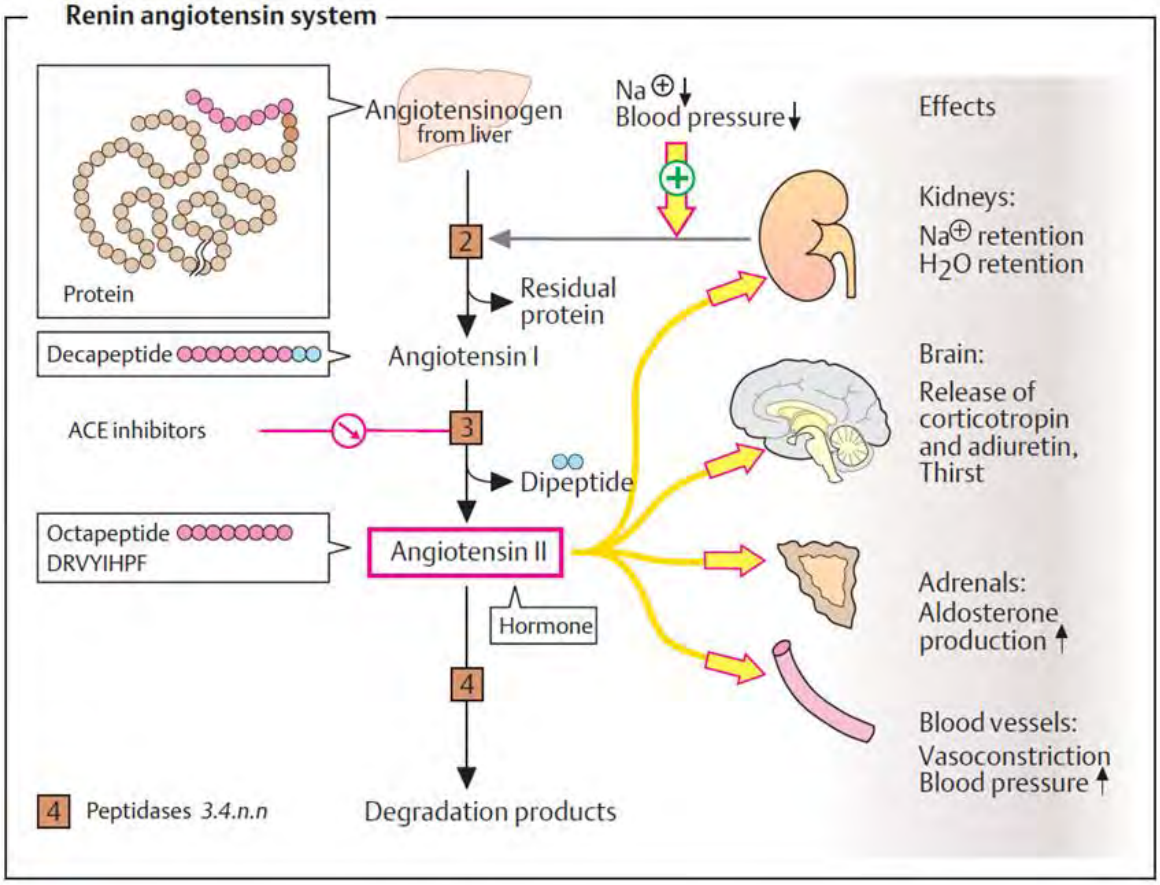




Insulin effect on glucose uptake and metabolism. Insulin binds to its receptor (1), which starts many proteins activation cascades (2). These include translocation of Glut-4 transporter to the plasma membrane and influx of glucose (3), glycogen synthesis (4), glycolysis (5) and fatty acid synthesis (6).



Renal hormones (erythropoietin and calcitriol; hormone angiotensin II and releasing the enzyme *renin*)



LAB-CLASS

1. Qualitative reactions to hormones

1. 1. Qualitative reactions to epinephrine

The principle of the method. The formation of colored products of epinephrine oxidation underlies these qualitative reactions.

The purpose of the work. Learn to determine the presence of epinephrine in a solution using a test with iodine and iron (III) chloride.

Equipment and reagents. Tripod with test tubes. Pipettes. Alcohol burner. Adrenaline, aqueous solution. Iodine solution (0.1 M). A solution of iron (III) chloride (3%). NaOH solution (4%).

1.1.1. Test with iodine

Progress of experiment. Add 1 ml of aqueous epinephrine solution to the test tube and add a drop of iodine solution, then heat and observe how to develop the red or pink color of the solution.

1.1.2. Test with iron (III) chloride

Progress of experiment. In a test tube, it is necessary to add 1 ml of an aqueous solution of epinephrine, then add a drop of a solution of iron (III) chloride. The liquid turns green, which turns red when alkali is added.

1.1.3. Qualitative reactions to insulin

The principle of the method. In an alkaline environment, the polypeptides form complex salts of copper, which have a purple color. The first reaction (biuret)

is due to the presence of a peptide bond, which confirms the protein nature of insulin. The second reaction (to sulfur-containing amino acids) is based on the interaction of these amino acids with alkalis when heated. In this case, sulfur is cleaved from amino acids in the form of hydrogen sulfide, which is found by reaction with lead acetate.

The purpose of the work. Learn to determine the presence of insulin in the test solution using a biuret reaction and a reaction for sulfur-containing amino acids.

Equipment and reagents. Tripod with test tubes. Pipettes. Insulin, solution. NaOH solution (ω=10%). Solution of $CuSO_4$, (ω=1%). The solution of lead acetate (ω=0,5%).

1.1.3.1. Biuret reaction for insulin

Progress of experiment. To 1-2 ml of insulin solution, add an equal volume of sodium hydroxide solution and 1-2 drops of copper sulfate solution. In the test tube, to observe developing a purple color.

1.1.3.2. Reaction for sulfur-containing amino acids

Progress of experiment. An equal volume of sodium hydroxide solution is added to 1-2 ml of insulin solution and heated to boiling. Then, add 2-3 drops of lead acetate solution. A brown or black color appears in the test tube.

2. Quantitative reactions to hormones

2.1. Quantitative determination of epinephrine by colorimetric method

The principle of the method. The method is based on the colorimetric determination of the intensity of the blue color that occurs when adrenaline interacts with Folin's reagent.

The purpose of the work. To master the method of colorimetric determination of epinephrine in solution by reaction with Folin's reagent, to find out the biological role of the hormone.

Equipment and reagents. Photoelectrocolorimeter. Calibrated test tubes of 10 ml. Standard solution of epinephrine. The studied biological fluid. Sodium carbonate solution (10%). Folin's reagent.

Progress of experiment. In two calibrated test tubes of 10 ml, it is added: in the first tube - 1 ml of the standard solution of epinephrine, containing 0.04 mg in 1 ml; in the second tube - 1 ml of the investigated solution. Next, 4 ml of sodium carbonate solution (10%) and 0.5 ml of Folin's reagent are added to each tube. Then, it is necessary to mix the contents of the tubes. The liquid gradually turns blue, reaching its greatest intensity after 5 minutes. The volume of the liquid in both tubes is adjusted to 10 ml with sodium carbonate solution (10%) and mixed. Color both solutions in cuvettes (l = 10 mm) with a light filter with a wavelength of 670 nm against control on Reagents (contains all components except adrenaline solution, total volume - 10 ml). The adrenaline content in the sample is calculated by the formula:

$$m = \frac{E_1}{E} \cdot a \text{ , where}$$

M - the mass of epinephrine in the sample, mg;
a - mass of adrenaline in a standard solution, mg;
E - an extinction of standard solution;
E_1 - the extinction of the test solution.

Calculate the adrenaline content in 1 ml of the test solution

Control questions, tasks and exercises for the section «HORMONES AND MECHANISMS THEIR INFLUNCES ON METABOLIC PROCESSES**»**

1. What organic substances are called hormones?
2. How are hormones classified by chemical nature?
3. What mechanisms of action of hormones do you know?
4. Where are insulin and glucagon produced and what metabolic processes do they affect?
5. What hormones and how do they affect the increase in blood glucose?
6. What hormones and how do they affect the decrease of glucose concentration in the blood?
7. How does insulin affect protein metabolism?

a) activates the synthesis of proteins and nucleic acids;
b) inhibits the synthesis of proteins and nucleic acids;
c) reduces the permeability of membranes to amino acids;
d) activates the conversion of amino acids into glucose;
e) activates the conversion of amino acids into lipids.

Generalized conclusions

to chapter 2.1. “STATIC BIOCHEMISTRY” which is devoted to study regulatory effects of inorganic and organic substances on the metabolism

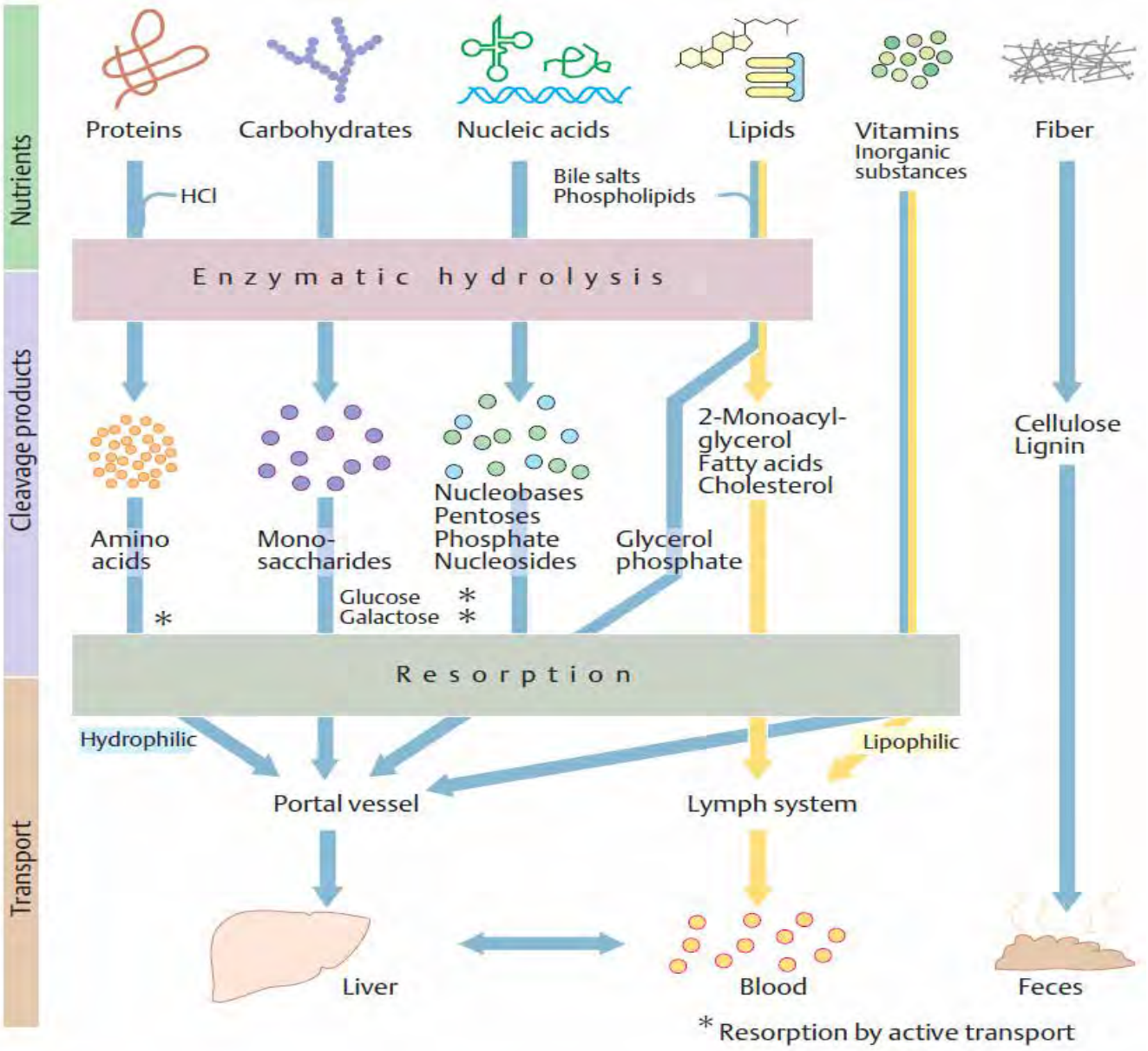
1. Presented common view on structure, and components of biological membranes. Characterized water and minerals in animals and plants, biochemistry of transmembrane transfers of minerals and organic substance as well as lab experiments of detection of some ions.
2. Given classification of vitamins based on their solubility and some representatives. Presented list of water-soluble vitamins and their coenzymes forms
3. Characterized enzymes as bio catalyzers in the view of their nature, structure, typical one substrate reaction and their kinetic properties as well as lab experiments of study pH, temperature, activators and inhibitors influence on the typical chemical reactions that happened in a living organism and enzymatic activity determination and other characteristics detection.
4. Presented hormones as regulators of metabolic processes, especially carbohydrates and lipid metabolism, and the mechanisms of their influence on metabolic processes. Charactarised also the best easy way of hormones qualitative and quantitative detection.

Chapter 2.2
DYNAMIC AND FUNCTIONAL BIOCHEMISTRY

The purpose of this section of the course "Biochemistry of animals with the Basics of Physical and Colloid Chemistry" is to provide students with theoretical knowledge of dynamic and functional biochemistry, and some experiments in lab classes.

The following topics will be considered in this section:

Topic 1. Carbohydrate metabolism, its intermediates
Topic 2. Biological oxidation of substances and their energy exchange
Topic 3. Lipid metabolism and its intermediates
Topic 4. Characteristics of protein exchange intermediates (I)
Topic 5. Characteristics of protein exchange intermediates (II)
Topic 6. Components of nucleic acids



Nutrients

	Quantity in body (kg)	Energy content kJ · g⁻¹ (kcal · g⁻¹)	Daily requirement (g) a	b	c	General function in metabolism	Essential constituents
Proteins	10	17 (4.1)	♂ 37 ♀ 29	55 45	92 75	Supplier of amino acids Energy source Daily requirement in mg per kg body weight	Essential amino acids:: Val (14) Leu (16) Ile (12) Lys (12) Phe (16) Trp (3) Met (10) Thr (8) Cys and His stimulate growth
Carbohydrates	1	17 (4.1)	0	390	240-310	General source of energy (glucose) Energy reserve (glycogen) Roughage (cellulose) Supporting substances (bones, cartilage, mucus)	Non-essential nutritional constituent
Fats	10-15	39 (9.3)	10	80	130	General energy source Most important energy reserve Solvent for vitamins Supplier of essential fatty acids	Polyunsaturated fatty acids: Linoleic acid Linolenic acid Arachidonic acid (together 10 g/day)
Water	35-40	0	2400	–	–	Solvent Cellular building block Dielectric Reaction partner Temperature regulator	
Minerals	3	0				Building blocks Electrolytes Cofactors of enzymes	Macrominerals Microminerals (trace elements)
Vitamins	–	–				Often precursors of coenzymes	Lipid-soluble vitamins Water-soluble vitamins

a: Minimum daily requirement b: Recommended daily intake c: Actual daily intake in industrialized nations

Enzymatic hydrolysis in the digestive tract breaks down foodstuffs into their resorbable components.

Resorption of the cleavage products takes place primarily in the small intestine.

Only ethanol and short–chain fatty acids are already resorbed to some extent in the stomach.

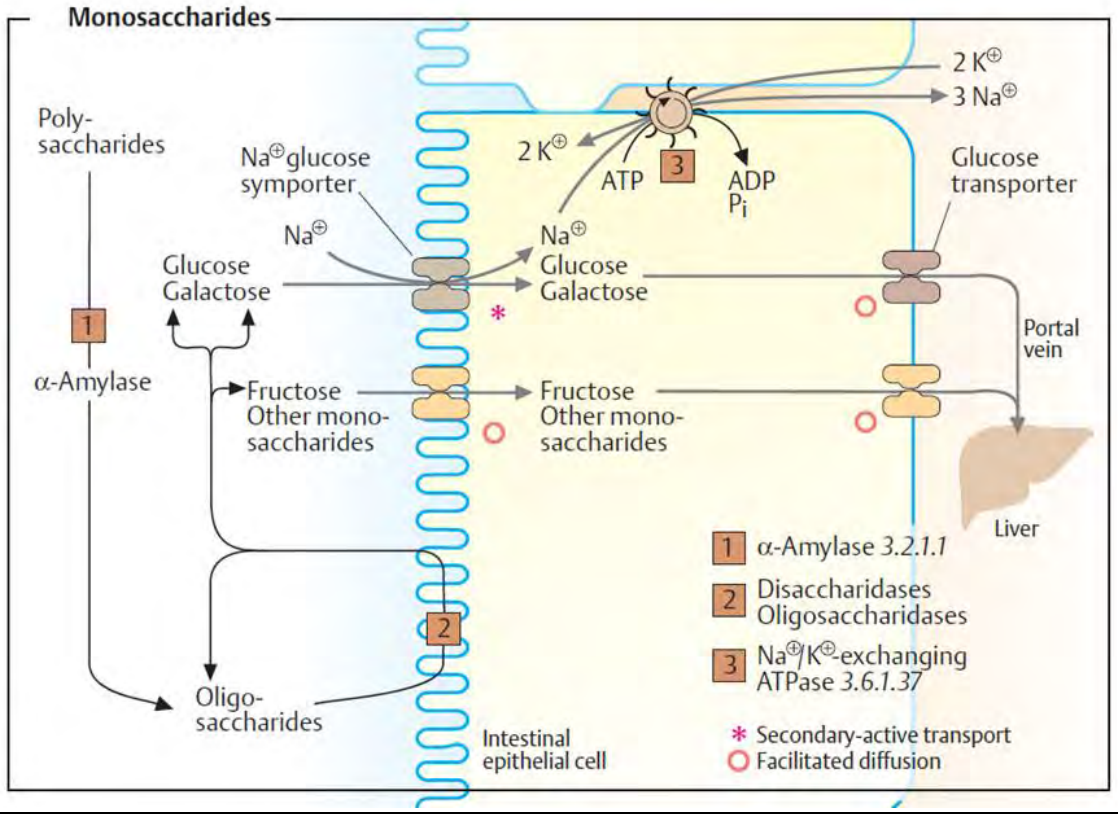
The resorption process is facilitated by the large inner surface of the intestine, with its brush–border cells.

Lipophilic molecules penetrate the plasma membrane of the mucosal cells by simple diffusion, whereas polar molecules require *transporters* (facilitated diffusion).

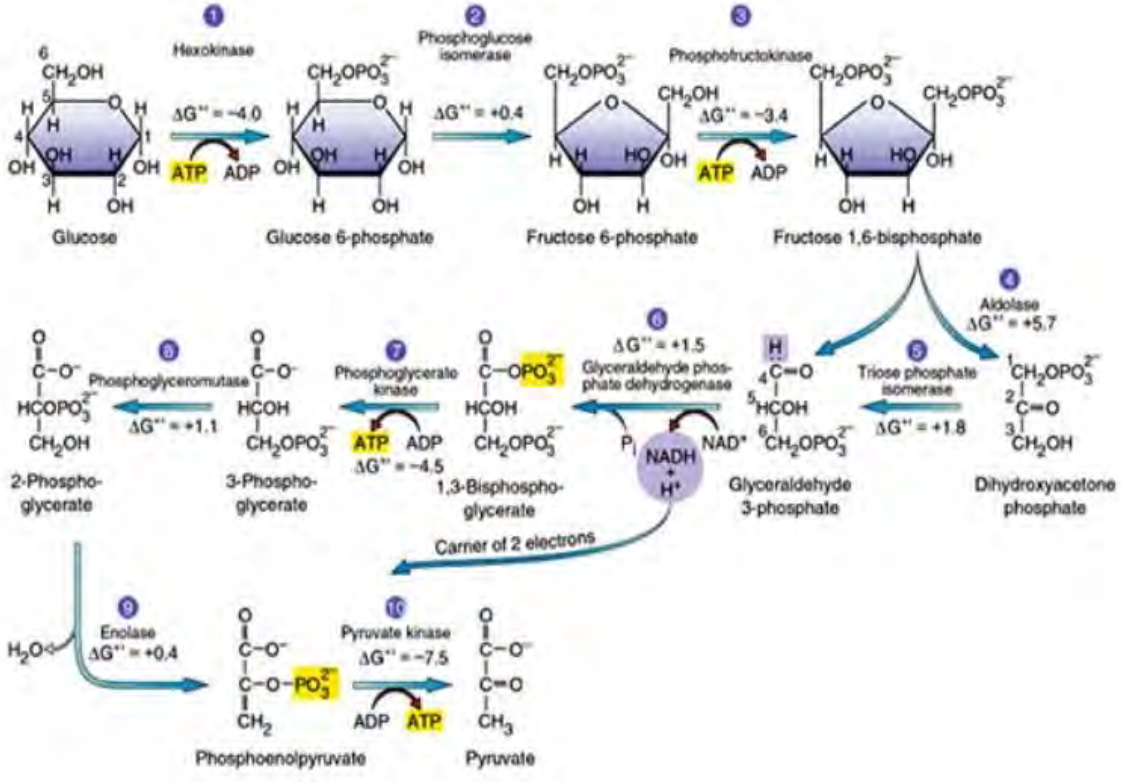
In many cases, carrier-mediated cotransport with Na+ ions can be observed. In this case, the difference in the concentration of the sodium ions (high in the intestinal lumen and low in the mucosal cells) drives the import of nutrients against a concentration gradient (secondary active transport).

Failure of carrier systems in the gastrointestinal tract can result in diseases.

2.2.1. Topic CARBOHYDRATE METABOLISM, ITS INTERMEDIATES

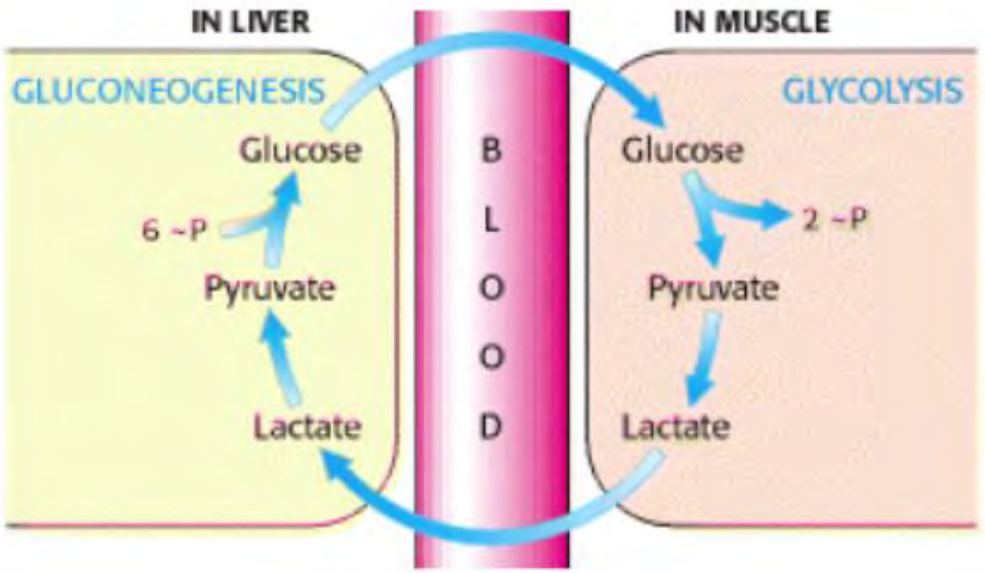


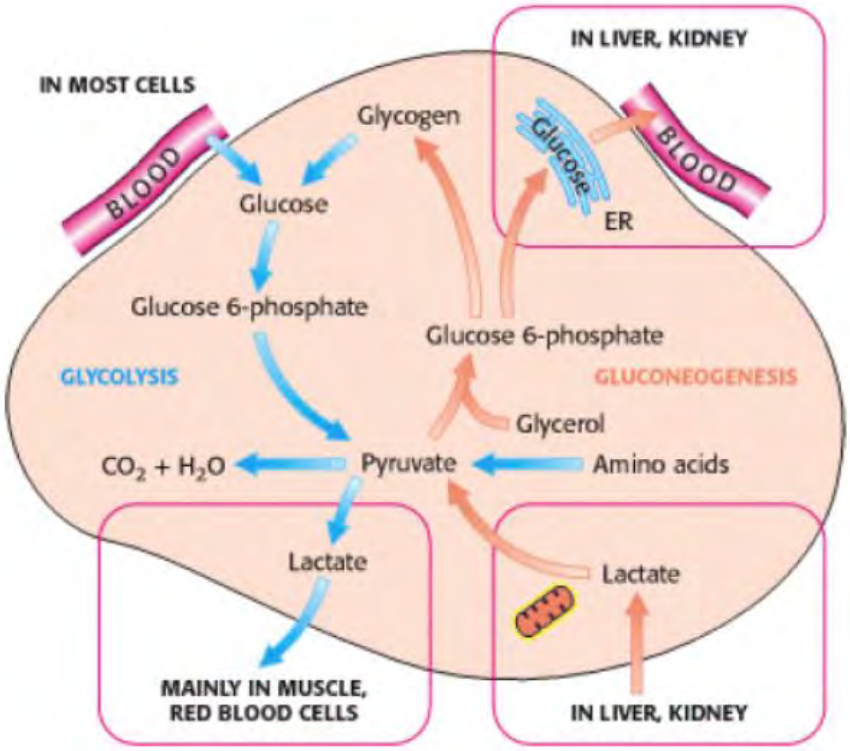
Monosaccharides absorption



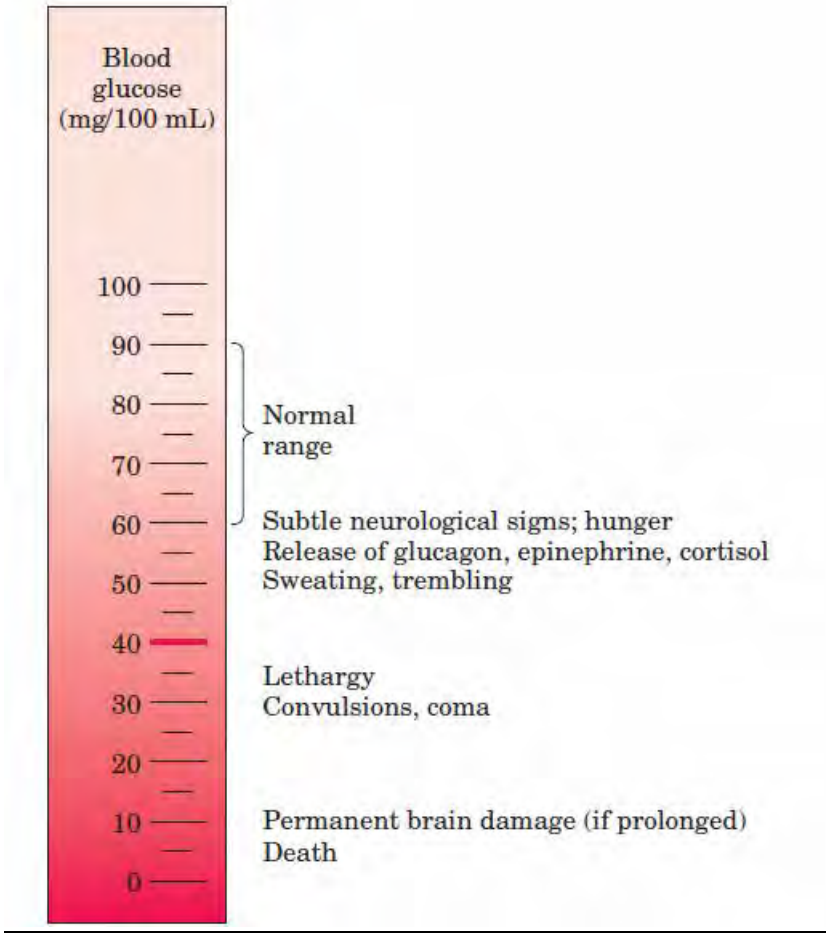
Glycolysis

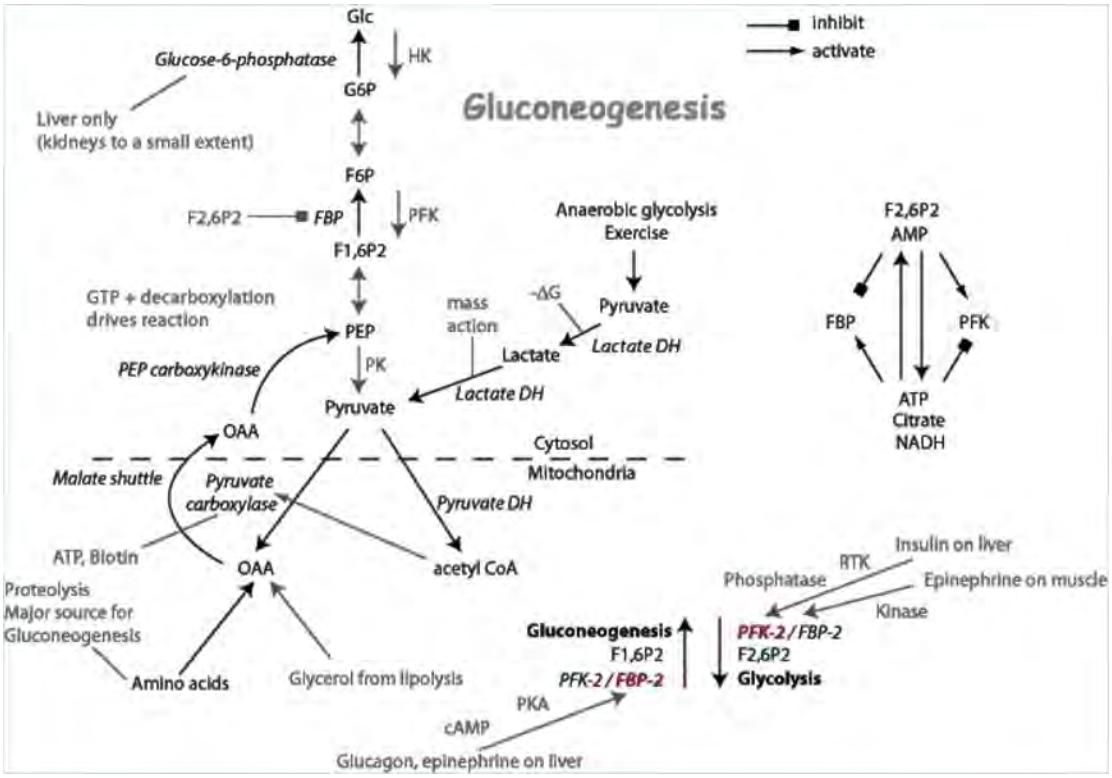
Lactate formed by active muscle is converted into glucose by the liver. This cycle shifts part of the metabolic burden of active muscle to the liver.





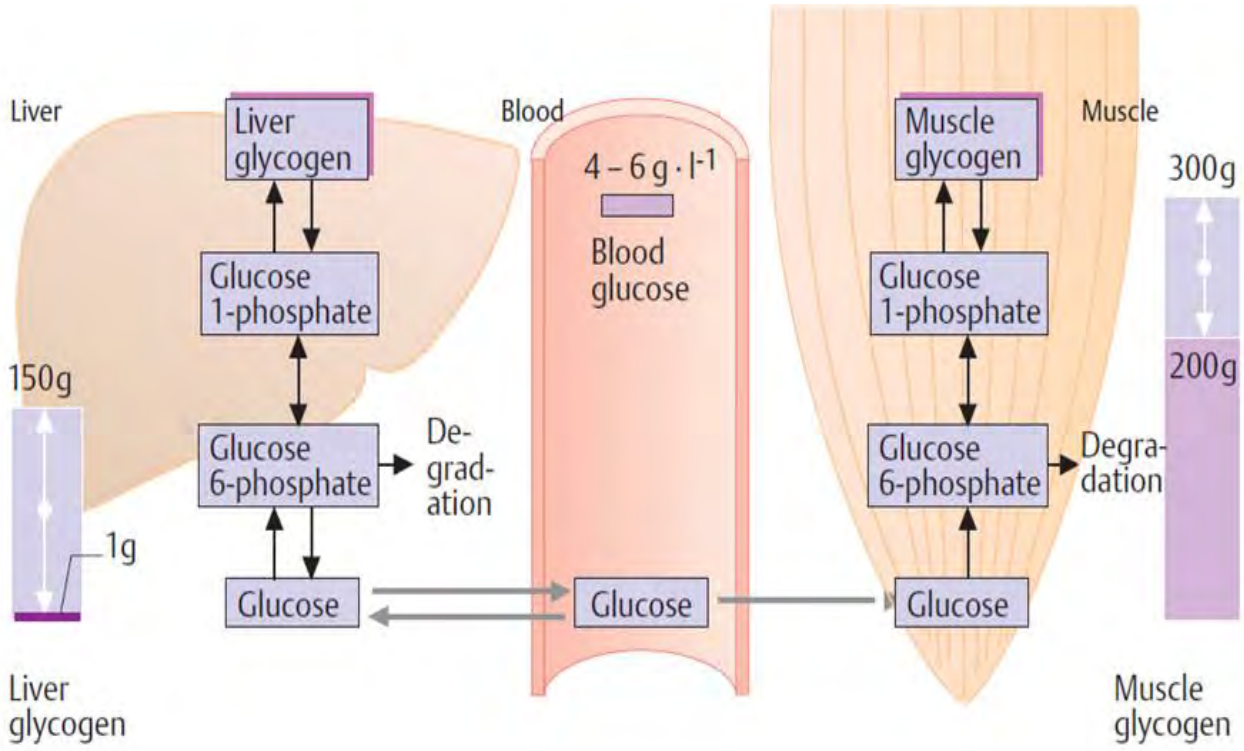
Glycolysis and gluconeogenesis are coordinated, in a tissue-specific fashion, to ensure that the glucose-dependent energy needs of all cells are met.

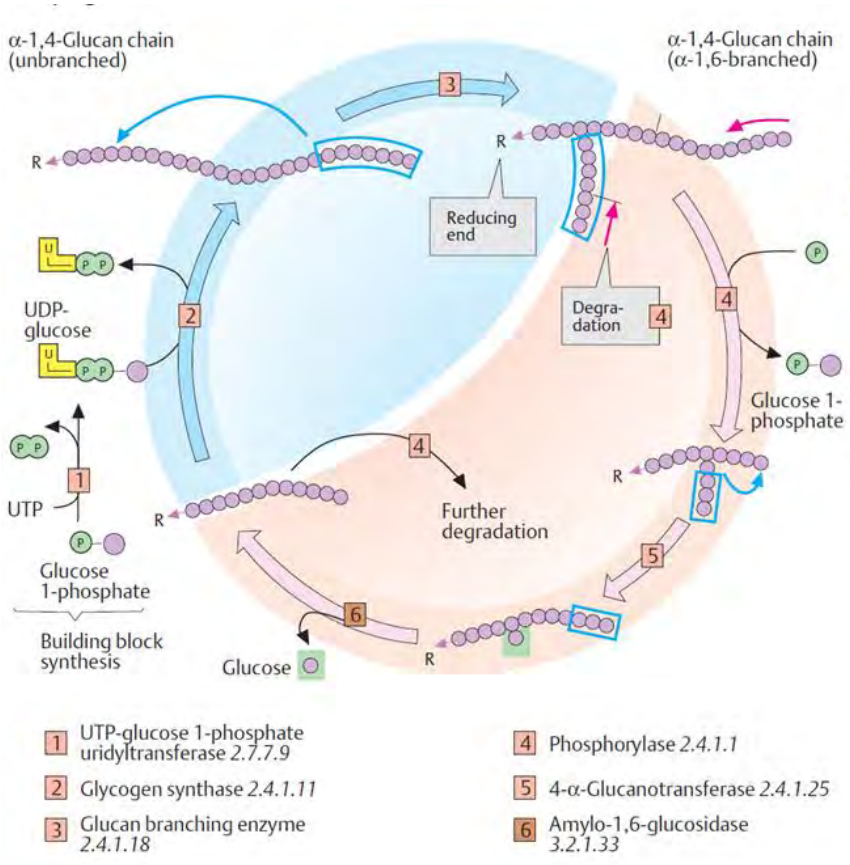




Gluconeogenesis pathway and its regulation

Glycogen balance and its metabolism

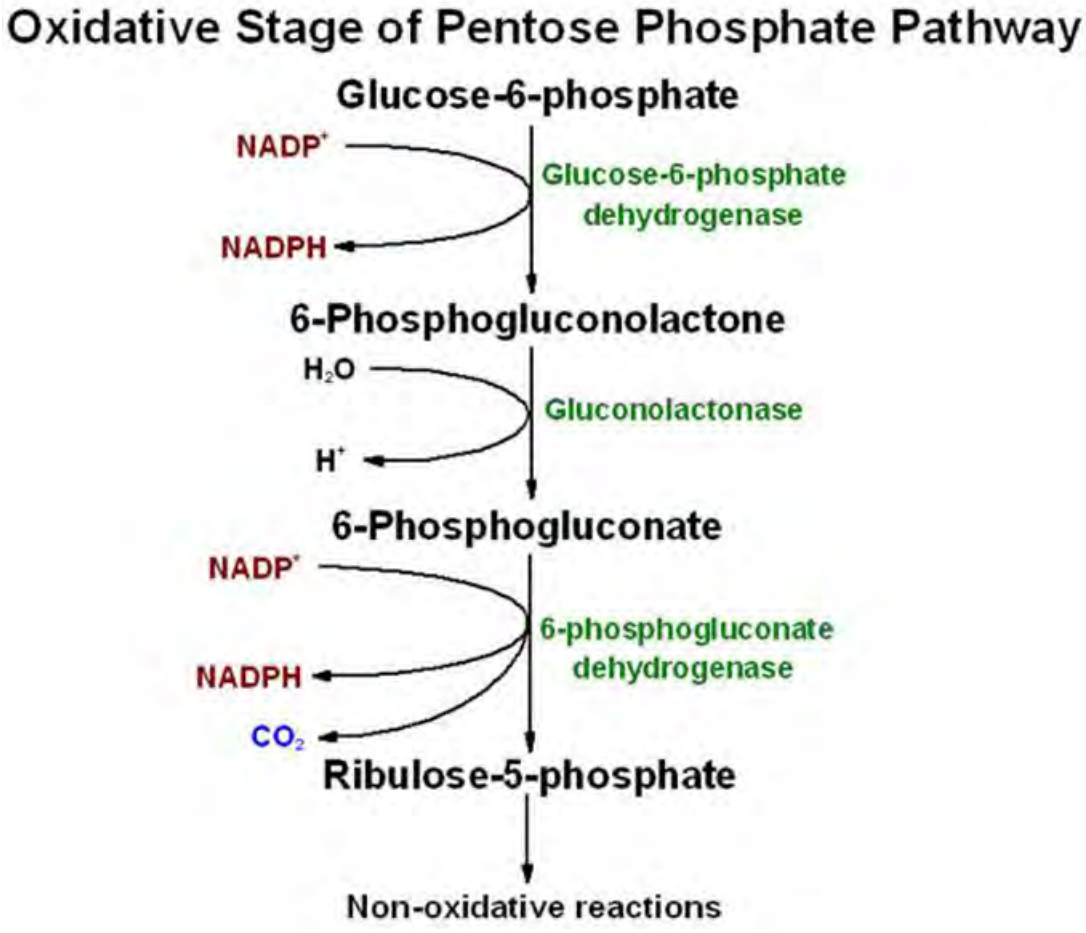


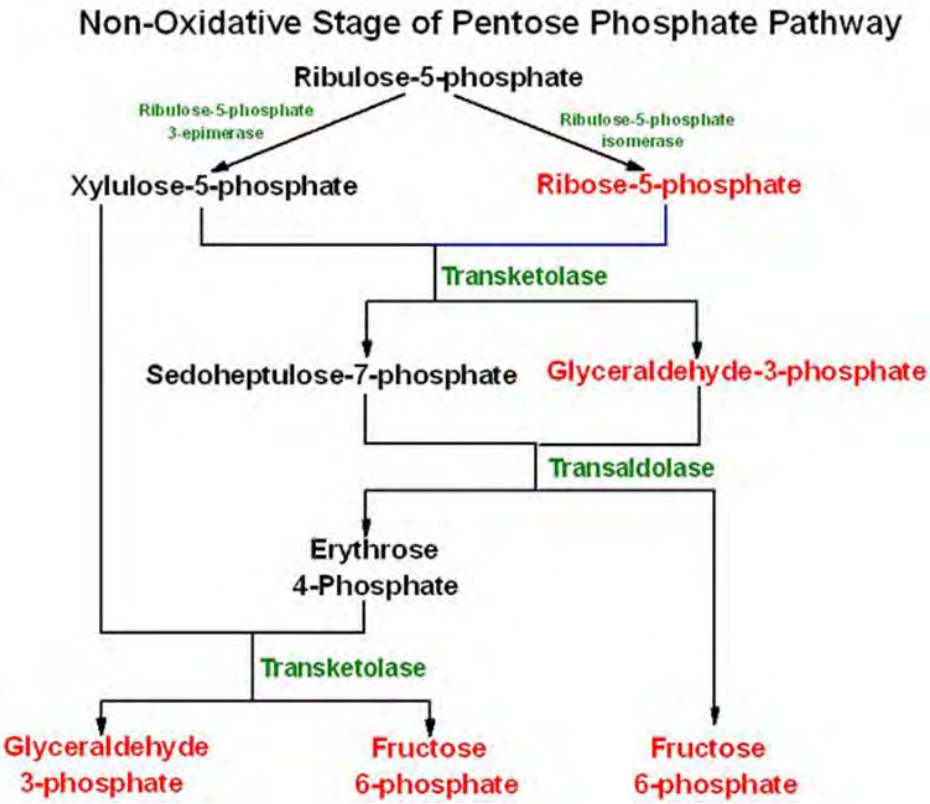


Glycogen metabolism

Koolman, Color Atlas of Biochemistry, 2nd edition © 2005 Thieme

Pentose phosphate pathway (oxidative and non-oxidative stages)





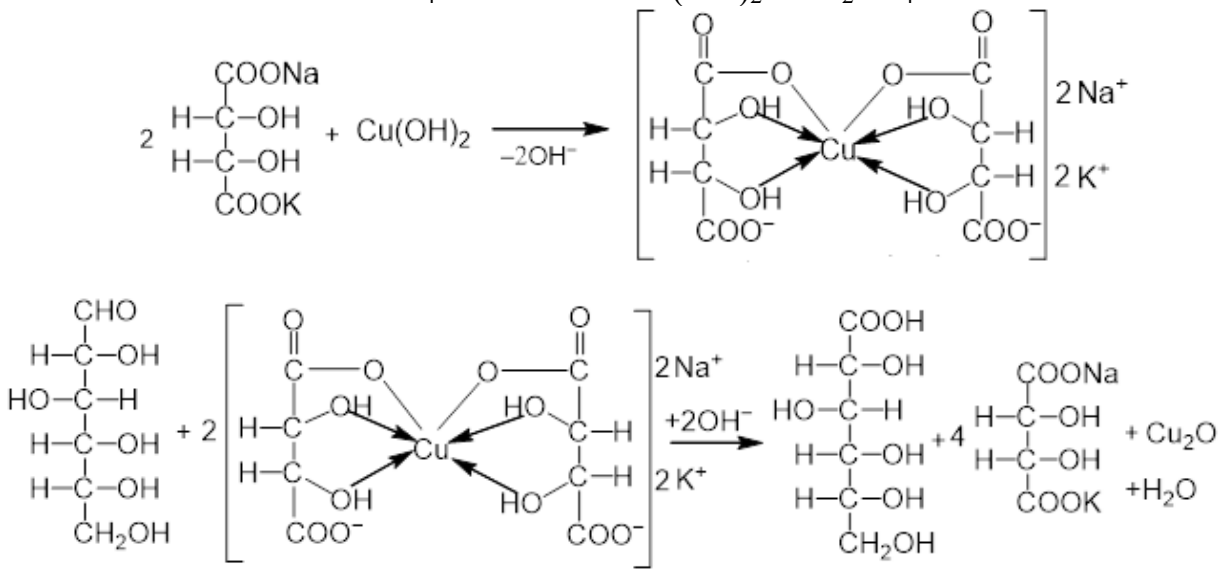
LAB-CLASS

Investigation of the properties of mono- and disaccharides

1. Quantitative determination of glucose in bio-sample by the Fehling method

The principle of the method. The principle of the Fehling reaction is to oxidize glucose and reduce due to this divalent copper to monovalent or metallic. The Fehling's reagent used in the reaction is an alkaline solution of a copper complex of Rochelle salt (Potassium sodium tartrate tetrahydrate). It is obtained by mixing equal amounts of a solution of copper sulfate (Fehling 1) and an alkaline solution of Rochelle salt (Fehling 2).

$$CuSO_4 + 2NaOH = Cu(OH)_2 + Na_2SO_4$$



Devices and materials: heating devices (alcohol burner, electric stove); 100 ml flasks; pipette dispensers with volumes of 0.02-0.5 ml; glass pipettes graduated by 1-5 ml; burettes; measuring cylinders; test tubes; glass sticks.

Reagents: 1% glucose solution; ***Fehling's solution 1*** (in 1 liter of solution, it is contained 69.278 g of $CuSO_4$, chemical pure); ***Fehling's solution 2*** (prepared by dissolving in the water when heated 346 g of crystalline Rochelle salt (Na, $KC_4H_4O_6 \cdot 4H_2O$), to which 140 g of NaOH are added and (after cooling) it is adjusted to 1 liter with distilled water)

Progress of experiment. Pour 10 ml of Fehling's reagent (5 ml of ***Fehling's 1*** and ***Fehling's 2*** solutions) into a 100 ml flask, add 40 ml of water and heat to boiling. Slowly add the test sugar solution to the boiling liquid from the burette, stirring the contents of the flask constantly. The reaction is considered complete if the red precipitate of copper oxide completely falls off and the liquid above the precipitate is clear.

Calculation. The percentage of glucose in the test solution is calculated based on the fact that 1 ml of Fehling's working reagent reduces 0.005 g of glucose. Then, 10 ml of Fehling's reagent used in the experiment will reduce 0.05 g of glucose.

For example, 10 ml of glucose solution was used to reduce Fehling's reagent. That is, 10 ml of solution contains 0.05 g of glucose, and 100 ml - x

$$x = \frac{0.05 \cdot 100}{10} = \frac{5}{10} = 0.5\ \%$$

Answer: The solution taken for the experiment contains 0.5% glucose.

Glucose content is normal in the blood of humans, animals and birds

Glucose content	*human*	cattle	small cattle	horse	pig	dog	cat	rabbit	birds
(mmol/l)	3.5-6.0	2.5-4.2	2.8-4.4	4.0-6.5	4,7-8.3	3.6-6.5	4.0-10.0	2.2-6.1	4.9-7.8

2. Reaction for disaccharides. Barfed's reaction

The principle of the method: Barfed's reaction allows you to quickly distinguish monosaccharides from disaccharides of the maltose type, which have reducing properties (lactose, maltose, cellobiose). It is based on the fact that the reducing properties of monosaccharides are also preserved in an acidic environment, while disaccharides reduce metals only in an alkaline environment. When disaccharides interact with Barfed's reagent, the red precipitate of copper oxide does not appear immediately, but only after some time (15-20 minutes) after their hydrolytic decomposition, which is catalyzed by acids.

Progress of experiment: 1 ml of Barfed's reagent (copper acetate + acetic acid) is poured into two test tubes. In the first tube add 1 ml of 1% glucose solution. In the second test tube - the same 1% lactose solution. The tube is shaken and placed on a boiling water bath. In a test tube with glucose after 2-3 minutes, a red precipitate of copper oxide appears, while in a test tube with a disaccharide the reduction reaction is observed only after 15-20 minutes of incubation (about 100˚C) in a water bath.

Note. The 1st and 2nd reactions are performed as experimental - determine the presence of mono- and disaccharides in biological fluids, which are provided in several tubes. The result is recorded in a table and make the appropriate Conclusions.

# of test tube	Results	
	Trommer's reaction	Barfed's reaction

Investigation of the properties of polysaccharides

1 Detection of glycogen in the liver

The principle of the method: glycogen, like starch, forms colored compounds with iodine (starch - dark blue, glycogen - red-brown).

Progress of experiment: 0.5 g of the fresh-frozen liver is placed in a glass, crushed with scissors, poured 4 ml of boiled distilled water, transferred to a test tube, and boiled for 2-3 minutes (to inactivate enzymes). Then the contents of the tube are poured into a porcelain mortar and grind until smooth. This homogenate is diluted with 1 ml of distilled water, transferred to a test tube, and boiled in a water bath for 20 minutes, adding water dropwise as the liquid boils.

For more complete precipitation of proteins, the boiling liquid is acidified with 5-10 drops of 1% acetic acid solution.

The protein precipitate is separated by filtration using a water-soaked paper filter. 2-3 drops of Lugol's solution are added to the filtrate. In the presence of glycogen in the test material, the solution acquires a characteristic color.

2. Isolation of glycogen by the Pfluger method

The principle of the method. The liver or muscle tissue is just boiled with an equal volume of KOH solution. At the same time proteins of fabric are hydrolyzed, and glycogen passes to a solution from which then is precipitated by ethanol.

Equipment and reagents. Centrifuge tubes. Tripod. Centrifuge. Water bath. A glass of ice. Pipettes. Potassium hydroxide solution (15% and 60%). Alcohol. Hydrochloric acid solution (2.5%). Sodium hydroxide solution (10%). Fehling's reagent.

Progress of experiment. Pour 2 ml of KOH solution into a centrifuge tube; 1.5-2 g of tissue are placed there. The test tube is placed for an hour in a boiling water bath, often shaken. An hour later, the test tube is removed from the bath, cooled and 8-10 ml of ethanol are added to it. Glycogen precipitates. The tube is placed in a centrifuge and centrifuged. The liquid is drained. The precipitate is dissolved in 2 ml of KOH solution (15%) and 8-10 ml of ethanol are added again. Glycogen precipitates again. Centrifuge and drain the liquid over the precipitate.

3. Glycogen hydrolysis

The principle of the method. When glycogen is hydrolyzed by boiling for 5-10 minutes with dilute hydrochloric acid, the final product is glucose, which is then tested with Fehling's reagent.

Equipment and reagents. Heating devices. Tripod with test tubes. Glycogen precipitate from experiment №1. Hydrochloric acid solution (2.5%). Sodium hydroxide solution (10%). Fehling's reagent.

Progress of experiment. The glycogen precipitate is dissolved in 3-4 ml of the hydrochloric acid solution and boiled for 15-20 minutes. Cool and neutralize with alkali solution, add Fehling's reagent, and boil. The test should be positive for glucose.

Determination of glycogen content in tissues

The principle of the method. Tissue analysis for glycogen includes the dissolution of tissues in a hot solution of alkali, precipitation of glycogen with alcohol, acid hydrolysis of glycogen, and quantification of glucose formed. When

hot alkali acts on tissues, hydrolysis of α- (1 → 4) - and α- (1 → 6) - bonds in glycogen molecules is almost non-existent.

Preliminary hydrolysis of glycogen is not required for the determination of glycogen by anthrone. In the presence of concentrated sulfuric acid, glucose or its residues in glycogen with anthrone give products that have a blue color. The intensity of this color determines the content of glucose or glycogen in the test tissue.

Equipment and reagents. Photoelectrocolorimeter. Centrifuge. Centrifuge tubes. Water bath. Measuring flask for 50 ml. Pipette with a wide spout. Fish liver or muscles. Test tubes. Potassium hydroxide (30%). Sodium sulfate (10%). Ethanol. Distilled water.

Progress of experiment. Freshly isolated fish tissue (liver, muscle) weighing 500 mg is added to a test tube with 2 ml of hot potassium hydroxide solution (30%). The tube is heated for 30-60 minutes in a boiling water bath with frequent stirring. Then cool and add to the contents of the tube 0.2 ml of sodium sulfate solution (10%) and 5 ml of ethanol. The mixture is mixed well, leave until the next day in the refrigerator. The precipitate formed is then separated by centrifugation for 40 minutes. at 7000 rpm The supernatant is discarded and the precipitate is dissolved in 2 ml of water. To the resulting solution was added 4 ml of ethanol, stirred and left for 30 minutes. in the refrigerator. The precipitate is separated by centrifugation, the supernatant is discarded. The precipitate formed is again precipitated with alcohol. After the third glycogen redeposition, the supernatant is removed. The glycogen precipitate is dissolved in water, quantitatively transferred from a test tube into a 50 ml volumetric flask, made up to the mark with water, mixed.

In two test tubes (two replicates of the experiment) make 0.5 ml of the obtained test solution of glycogen, and the other two (two replicates of control on reagents) - 0.5 ml of water. 5 ml of anthrone reagent is added to all tubes. The reagent must be added quickly so that the jet of reagent enters the center of the sample. To do this, use a pipette with a wide spout. The mixture is immediately mixed thoroughly and the tubes are placed in a water bath at room temperature for 10-15 minutes, and then transferred to a boiling water bath for 15 minutes. When heating, make sure that no water gets into the test tubes. Otherwise, the contents of the tubes may become cloudy, which will interfere with colorimetry. After heating, the tubes are quickly cooled in running water and left in the dark for 30 minutes. Develops a blue color.

The stained solutions are colorimetric with a light filter with $\lambda = 620$ nm (red light filter) in cuvettes with $l = 5$ mm against control on reagents.

To determine the glycogen content in the sample build a calibration graph. To do this, prepare a stock standard glucose solution containing 200 mg of glucose in 500 ml of saturated benzoic acid solution. 1 ml of this solution contains 400 μg of glucose. By diluting the initial glucose solution with a saturated benzoic acid solution, a series of solutions with a glucose content of 1 ml 400 is obtained; 300; 200; 100; 50 μg.

Next, 0.5 ml of the obtained solutions are taken, 5 ml of anthrone reagent are added and the same operations are performed as with the test samples. On the abscissa axis plot the glucose content in the sample, and on the ordinate axis - the optical density of the solutions.

According to the calibration graph, the content of glucose, which is part of the polysaccharide chain of glycogen, is found. To convert to the glycogen content in the sample, the result is multiplied by 0.9, because the relative molecular weight of the glucose residue in the glycogen molecule is 162.1, glucose - 180.1, then 161.1 divided by 180.1 = 0.8999, or 0.9.

The glycogen content in the test tissue is calculated by the formula:

$$X = \frac{a \cdot V \cdot 100}{m \cdot V_1 1 \cdot 10^6}, \text{ where}$$

X - glycogen content in the test tissue,%;

a - glycogen content in the test sample, μg;

V is the total volume of glycogen solution isolated from the studied tissue mass, ml;

V_1 - aliquot volume of glycogen solution taken for reaction with anthrone, ml;

m is the mass of tissue taken for analysis, g;

100 - conversion into interest;

$1 \cdot 10^6$ - conversion from micrograms to grams.

Control questions, tasks and exercises for the section «CARBOHYDRATE METABOLISM, ITS INTERMEDIATES»

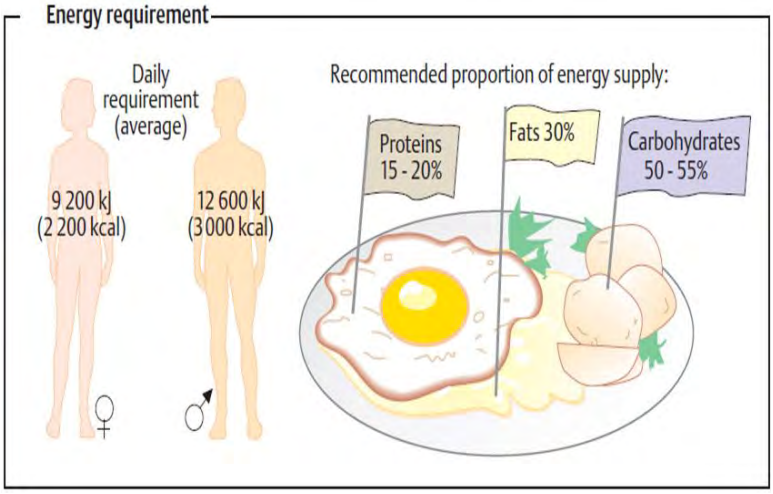
1. Natural monosaccharides belong to which row? [A) L - row b) D - row]. Give examples of suitable carbohydrates and their structural formulas.
2. Given the division of carbohydrates into aldehyde- and keto-containing polyhydric alcohols, give examples of qualitative reactions on their functional groups:
3. What diseases occur when carbohydrate metabolism is disturbed? Give examples.
4. From the suggested statements, choose the correct ones (give arguments about your choice):
    a) in glycolysis, the conjugation of ATP synthesis with oxidation occurs at the stage of the action of aldolase on fructose-1,6-diphosphate;
    b) the conversion of 1,3-diphosphoglyceric acid into 3-phospho-glyceric acid is accompanied by the synthesis of the ATP molecule;
    c) the conversion of 2-phosphoenolpyruvate to pyruvate occurs with the transfer of phosphoryl to ADP with the formation of ATP;
    d) polysaccharides - macromolecular compounds containing monosaccharide residues of only one species;
    e) the products of hydrolysis of many polysaccharides are hexoses and their derivatives;

f) by chemical structure disaccharides are glycosides of monosaccharides, aglycones of which are monosaccharide residues.

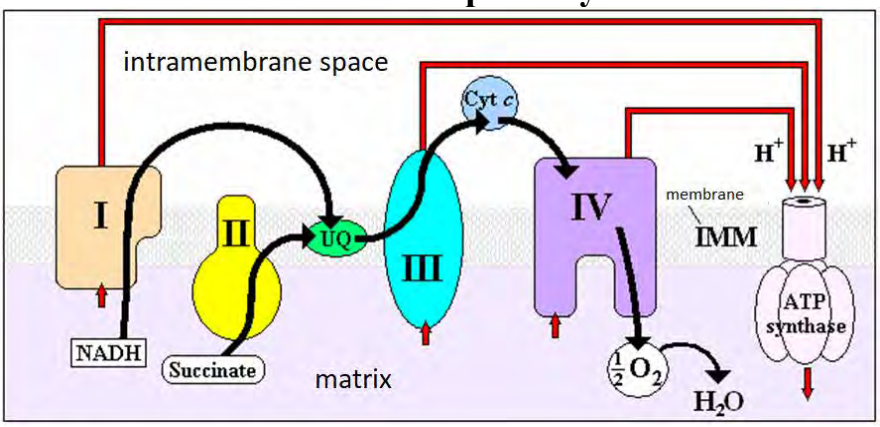
5. Write the equation of the reactions of conversion of D-glucose to pyruvic acid in a way that involves the enzyme - phosphofructokinase
6. The function of carbohydrates is:
   a) protective
   b) reserve
   c) structural
   d) energetical
   e) catalytic. Explain the answer with examples.

2.2.2. ***Topic*** BIOLOGICAL OXIDATION OF SUBSTANCES AND THEIR ENERGY EXCHANGE

Energy requirement and recommended proportion of energy supply

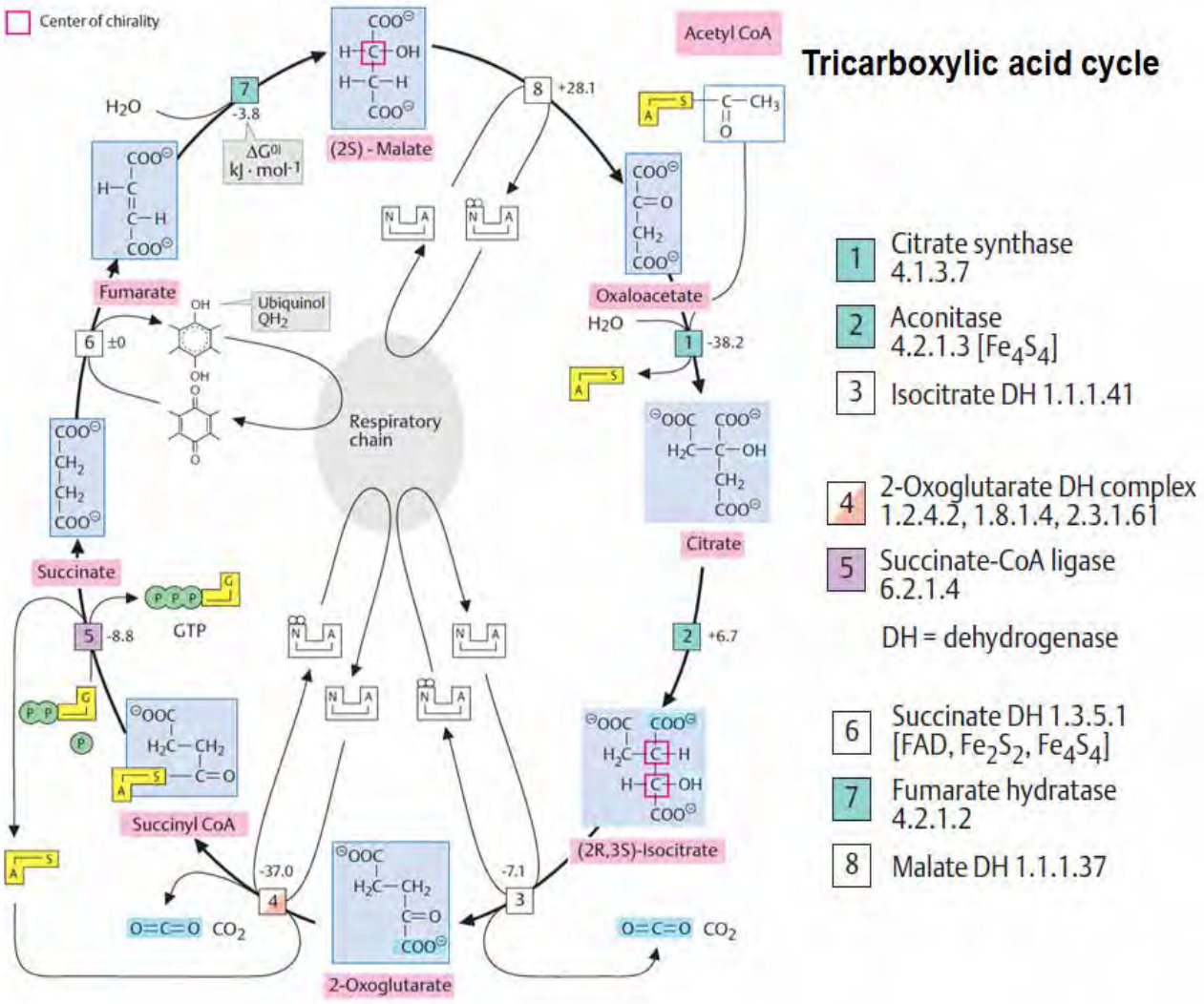


Scheme of the respiratory chain

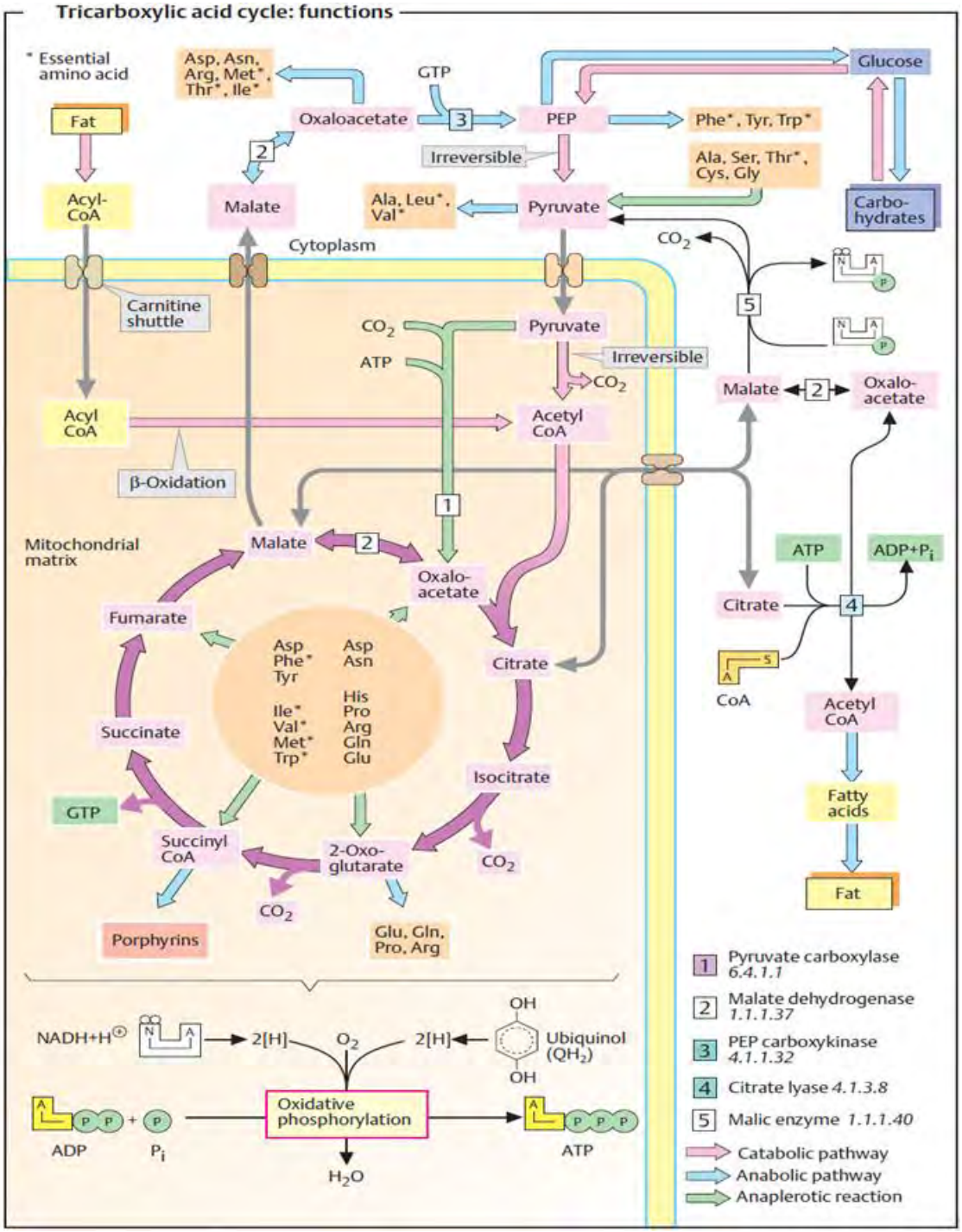


The tricarboxylic acids cycle (citric acid cycle, or Krebs' cycle, or TCAC) is cyclic sequence of enzymatic reactions resulting in acetyl-CoA (CH_3-CO~S-CoA) product of catabolism of main metabolic fuel (carbohydrates, fats, amino acids),

oxidized to CO_2 with the formation of H^+ used to reduce primary acceptor respiratory chain of mitochondria that are nicotine amide nucleotides or flavines coenzymes

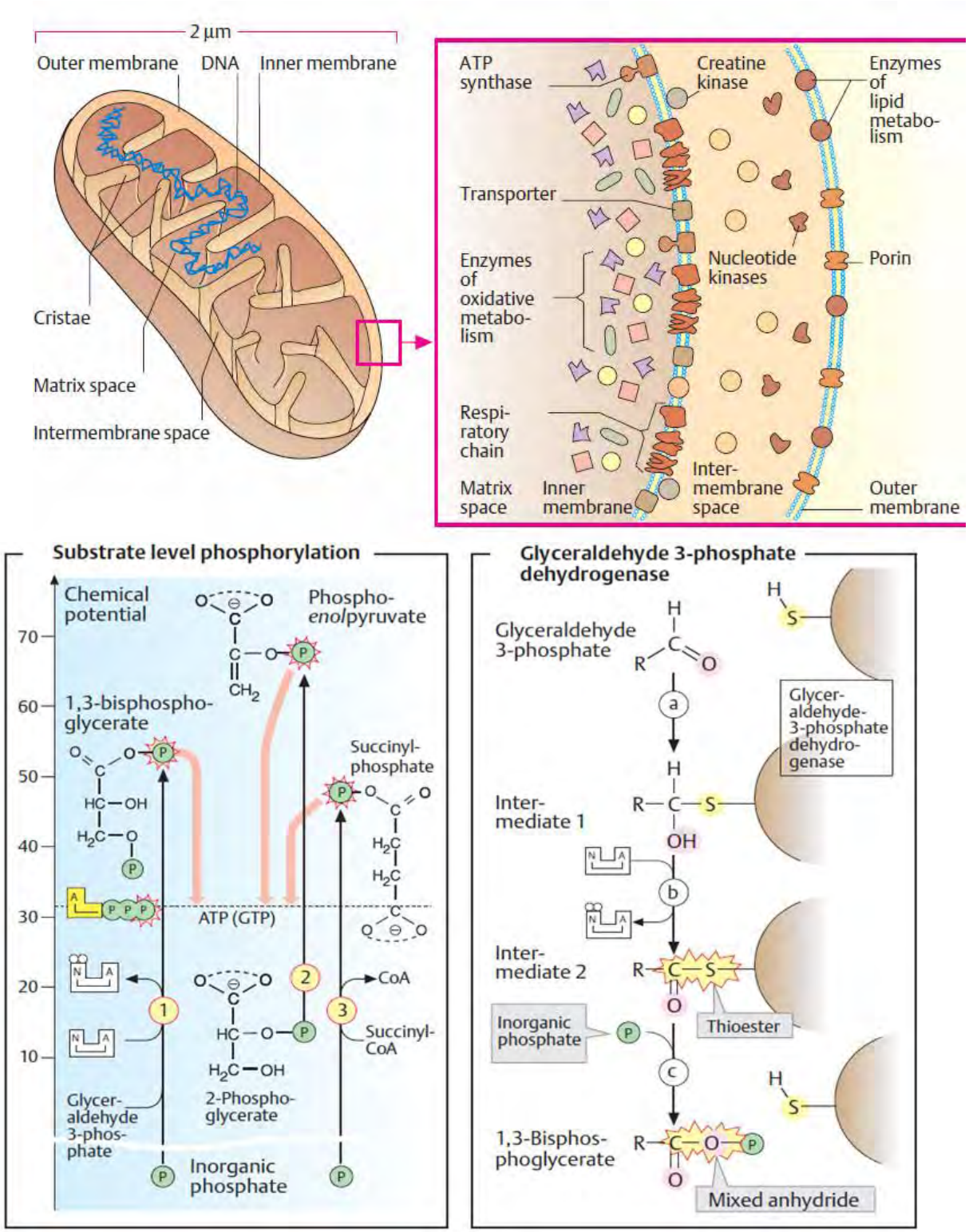


Reactions of **the tricarboxylic acids cycle (**TCAC**)** and the enzymes (which catalyzed them) are localized in the matrix and inner membrane of mitochondria. They are functionally and biochemically conjugate with mitochondrial electron transport chains that are used to reduce Oxygen atoms provided with the reduced equivalents of NADH (NADH $+H^+$) and $FADH_2$ or $FMNH_2$ and form of ATP during oxidative phosphorylation.

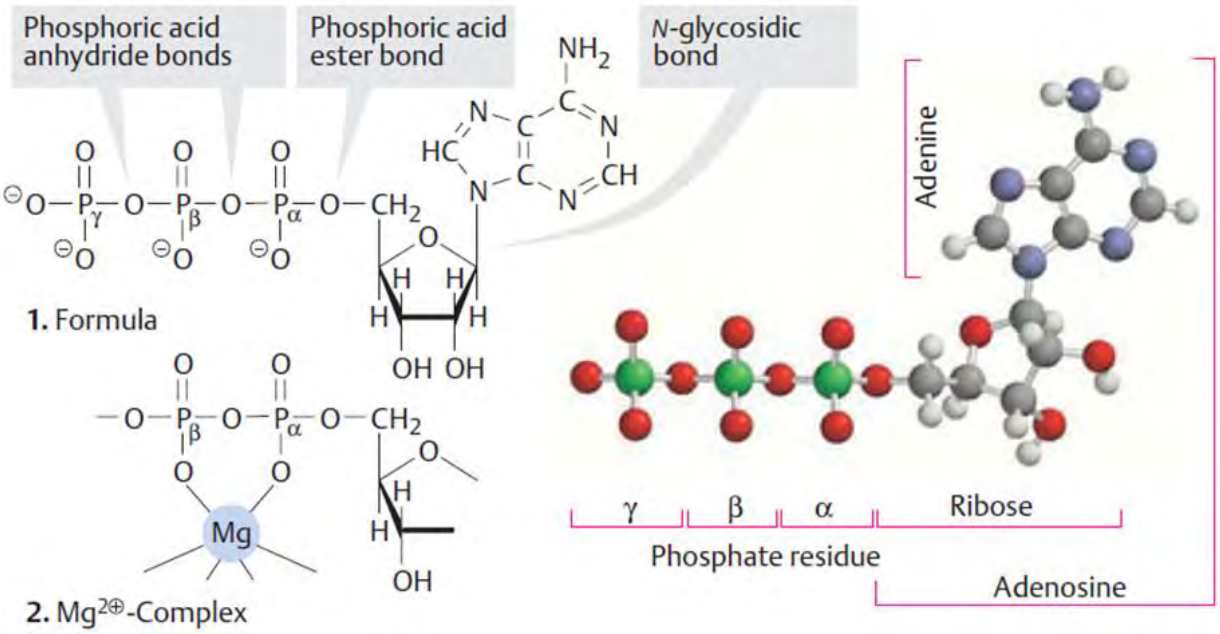


Tissue respiration is the process of absorption of tissues of oxygen and carbon dioxide secretion.

Oxidative phosphorylation is ATP synthesis, which is associated with tissue respiration.

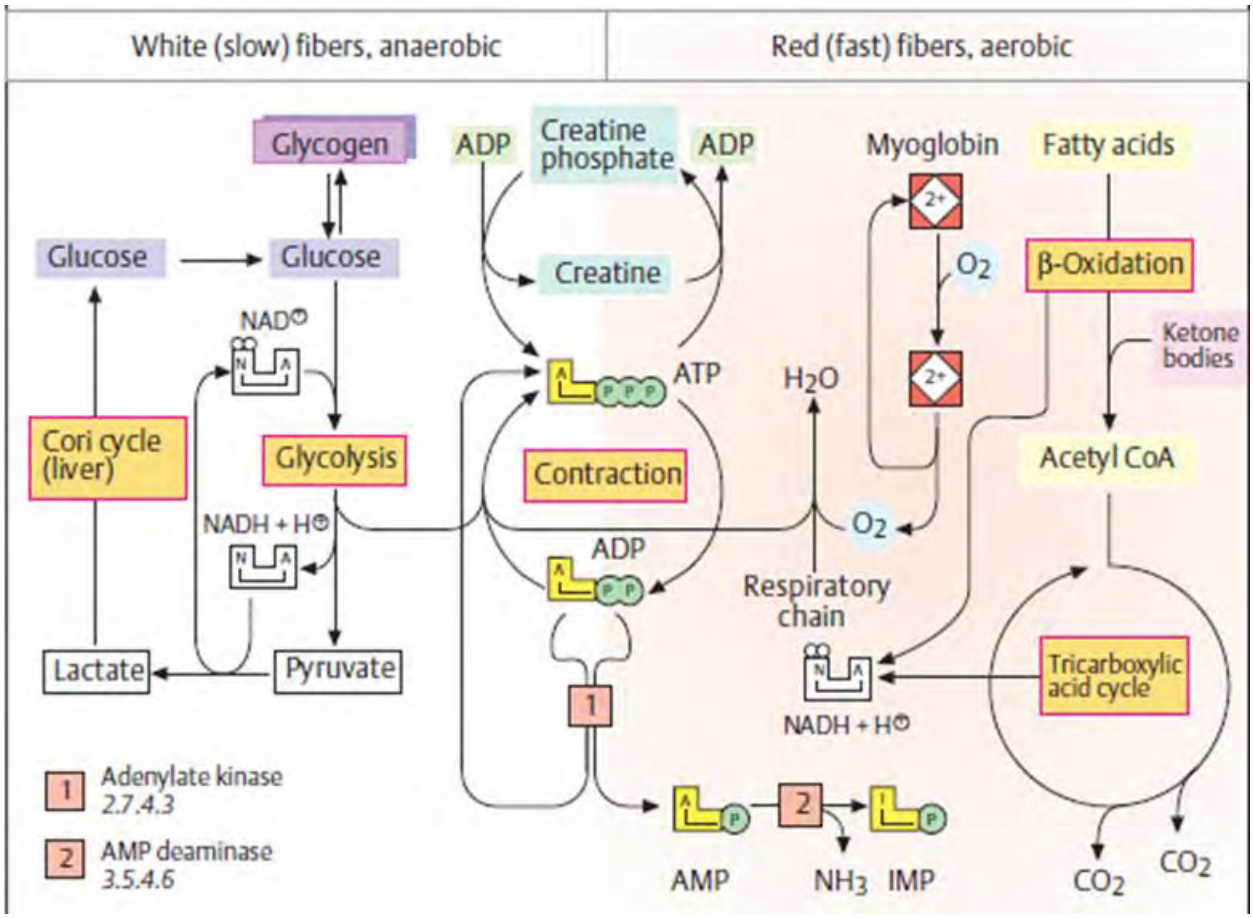


Substrate phosphorylation is ATP synthesis, which is not associated with tissue respiration (ATP synthesis from other ‘macro-ergic’ compounds)



ATP structure

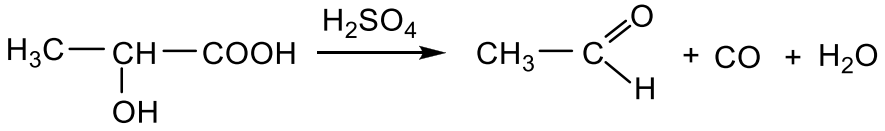
Energy metabolism in the white and red muscle fibers



LAB-CLASS

1. Quantitative determination of lactic acid in the serum of animals by colorimetric method

The principle of the method. Lactic acid when heated with sulfuric acid is converted into acetaldehyde, which reacts with hydroquinone. A red-brown compound is formed.



Equipment and reagents. Photoelectrocolorimeter. The measuring tube of 10-15 ml with a ground glass stopper. Serum. Centrifuge or filter. Glass sticks. Water bath. Metaphosphate acid. Copper sulfate (20% and 6%). Calcium hydroxide. Concentrated sulfuric acid. Ice water. Anhydrous zinc lactate. An alcoholic solution of hydroquinone (20%).

Progress of experiment. In a measuring tube of 10-15 ml with a ground glass stopper, make 1 cm^3 of blood serum, about 5 ml of water, 1 ml of metaphosphate acid for protein precipitation. Adjust to 10 ml with water and mix. After a few minutes, centrifuge or filter.

To precipitate carbohydrates, 1 ml of copper sulfate solution (20%), approximately 1 g of calcium hydroxide are added to the filtrate and shaken vigorously at once. If the light blue color due to the formation of $Cu(OH)_2$ does not appear, then $Ca(OH)_2$ should be added. The mixture is allowed to stand for 30 minutes, shaking occasionally, and then centrifuged or filtered.

Take 1 ml of the supernatant or filtrate in a volumetric tube with a ground glass stopper, add 0.1 ml of copper sulfate solution (6%), add exactly 6 ml of concentrated sulfuric acid dropwise under cooling with ice water, and stir with a glass rod. The closed tube is placed for 5 minutes in a boiling water bath and then cooled in cold water to 10-15 °C. 0.1 ml of an alcoholic solution of hydroquinone (20%) is added to the necessarily cooled solution, mixed well and the test tube is placed in a boiling water bath for 15 minutes. After that, the contents of the tube are cooled to room temperature and colorimetric with a light filter with a wavelength of 465nm (blue light filter) in cuvettes with an optical layer thickness of 10mm against water.

To quantify lactic acid in the sample build a calibration graph. To do this, first prepare a stock solution of lactic acid. Dissolve 1.3516 g of anhydrous zinc lactate or another salt in 10 ml of water, add 0.5 ml of concentrated sulfuric acid, and adjust to 1 liter with water. To prepare a standard solution of lactic acid, take 10 ml of the initial solution and adjust to 1000 ml with water. 1 ml of this solution contains 10 mcg of lactic acid.

0.2 is added to the test tubes; 0.4; 0.6; 0.8; 1.0; 1.2 ml of standard lactate solution corresponding to lactic acid content 2; 4; 6; 8; 10; 12 μg and treated as serum samples. A calibration graph is plotted by plotting the lactate content in the

standard solution sample on the abscissa axis and the optical density value on the ordinate axis. According to the calibration, the schedule determines the content of lactic acid in the test sample of serum.

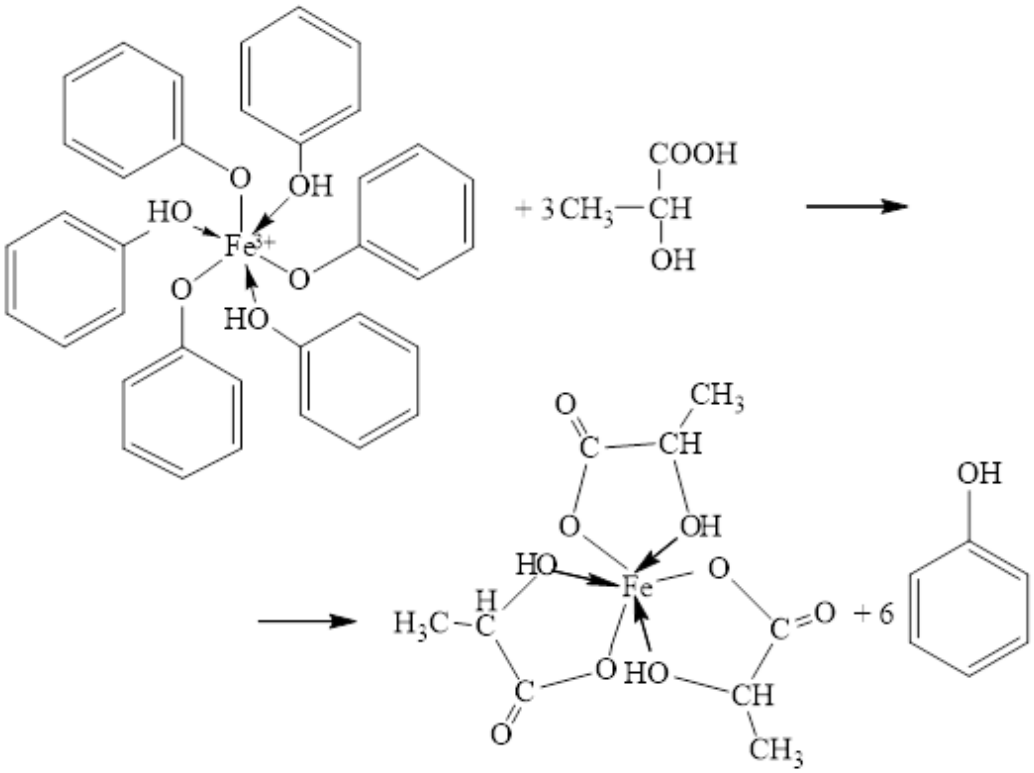
The content of lactate in the serum is determined by the formula:

$$C = \frac{a \cdot 1000}{V \cdot mM}, \text{ where}$$

C - the content of lactic acid in the serum, mM;
a - the content of lactic acid in the serum sample taken for analysis, mg;
V - the volume of serum taken for analysis, ml;
mM - millimolar mass of lactic acid, mg/mol.

2. Detection of lactic acid in muscles

The principle of the method. At the interaction of complex iron phenolate of violet color with lactic acid, the complex iron lactate of yellow-green color is formed.



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Equipment and reagents. Meat grinder. Porcelain mortar. Gauze. Folded paper filter. Tripod with test tubes. The aqueous solution of phenol (1%). Iron (III) chloride solution. Lactic acid solution (0.5%). Distilled water. Muscle tissue.

Progress of experiment. The muscle tissue is crushed with a meat grinder, then 2-3 g of it is rubbed with 5-7 ml of water in a porcelain mortar. The resulting muscle slurry is filtered through a double layer of gauze. The filtrate is boiled for 1 minute, cooled, filtered through a folded paper filter.

Make 5 ml of an aqueous solution of phenol (1%) in three tubes and add a solution of ferric chloride dropwise to each of them until an intense purple color

appears. Then 1 ml of lactic acid solution (0.5%) is added to the first test tube, an extract of muscle tissue is added to the second tube, and 1 ml of water is added to the third tube. The contents of the tubes are mixed. In the first and second test tubes, the purple color turns yellow-green, indicating the presence of lactic acid; in the third test tube the color of the solution does not change.

3. Determination of muscle adenosine triphosphatase activity

The principle of the method. Myosin exhibits adenosine triphosphatase activity, catalyzing the hydrolysis of ATP with the formation of ADP and inorganic phosphate:

$$ATP + H_2O \rightarrow ADP + H_3PO_4$$

ATPase activity of myosin is manifested only in the presence of salts. Ca^{2+} and Mg^{2+} ions have the greatest effect on the ATPase activity of myosin. The significant role of myosin light chains in providing enzymatic activity. Ca^{2+} ions are thought to be allosteric regulators and increase myosin ATPase activity, whereas Mg^{2+} ions inhibit it.

The ATPase activity of myosin is determined by the amount of inorganic phosphate produced by the hydrolysis of ATP. Inorganic phosphate is determined by the Fiske-Subbarou method.

Equipment and reagents. Photoelectrocolorimeter. Filters. The fresh muscle tissue of animals or fish. Sucrose solution (0.25 M). Measuring test tubes. Trichloroacetic acid (20%). Buffer mixture (pH 7.4). Magnesium sulfate (0.05M). Sodium adenosine triphosphate (0.02 M). Distilled water. Ammonium molybdate (2.5%) in sulfuric acid ($c(^1/_2)$= 5.0 M). The freshly prepared solution of ascorbic acid (0.4%). Phosphate standard solution (KH_2PO_4 or Na_2HPO_4) containing 0.025 mg of Phosphorus in 1 ml.

Progress of experiment. The fresh muscle tissue weighing 100 mg was carefully ground and homogenized with 5 ml of sucrose solution (0.25 M).

In two measuring tubes (experimental and control), add 2 ml of the obtained homogenate. Immediately add 1 ml of trichloroacetic acid solution (20%) to the control tube. In both tubes, add 1 ml of buffer mixture (pH 7.4), 1 drop of magnesium sulfate solution (0.05M), 0.25 ml of sodium adenosine triphosphate solution (0.02M), incubate for 30 minutes. After incubation, 1 ml of trichloroacetic acid (20%) was immediately added to the test tube to stop the enzymatic reaction. The volume of the liquid in the test tubes is adjusted to 5 ml with distilled water, mixed and filtered into other test tubes.

To determine the inorganic phosphate formed during the hydrolysis of ATP by ATPase, transfer 1 ml of the filtrate to 10 ml measuring tubes, add 2 ml of a solution of ammonium molybdate (2.5%) in sulfuric acid ($c(^1/_2)$=5.0 M) and 1 ml of freshly prepared solution of ascorbic acid (0.4%). The sample volume is adjusted to 10 ml, mixed, and after 30 minutes colorimetric with a light filter with λ = 590 nm in cuvettes with ℓ = 10 mm against control on reagents. The control contains all the ingredients of the mixture, except for a solution of sodium adenosine triphosphate.

The phosphorus content of inorganic phosphate in the experimental and control samples is determined according to the calibration schedule. To build it in a

series of tubes make, respectively, 0.5; 1.0; 1.5; 2.0; 2.5; 3.0; 3.5; 4.0; 4.5; 5.0 ml of standard phosphate solution (KH_2PO_4 or Na_2HPO_4) containing 0.025 mg of Phosphorus in 1 ml. And then carry out operations on the formation of phosphorus-molybdenum blue as in the determination of inorganic phosphate, which is formed by hydrolysis of ATP. Phosphorus content in standard samples is plotted on the abscissa axis, and the optical density of phosphorus-molybdenum blue solutions is plotted on the ordinate axis.

Muscle ATPase activity is expressed by the number of micromoles of ATP that can be hydrolyzed by an enzyme contained in 1 g of tissue per minute.

Calculations are performed according to the formula:

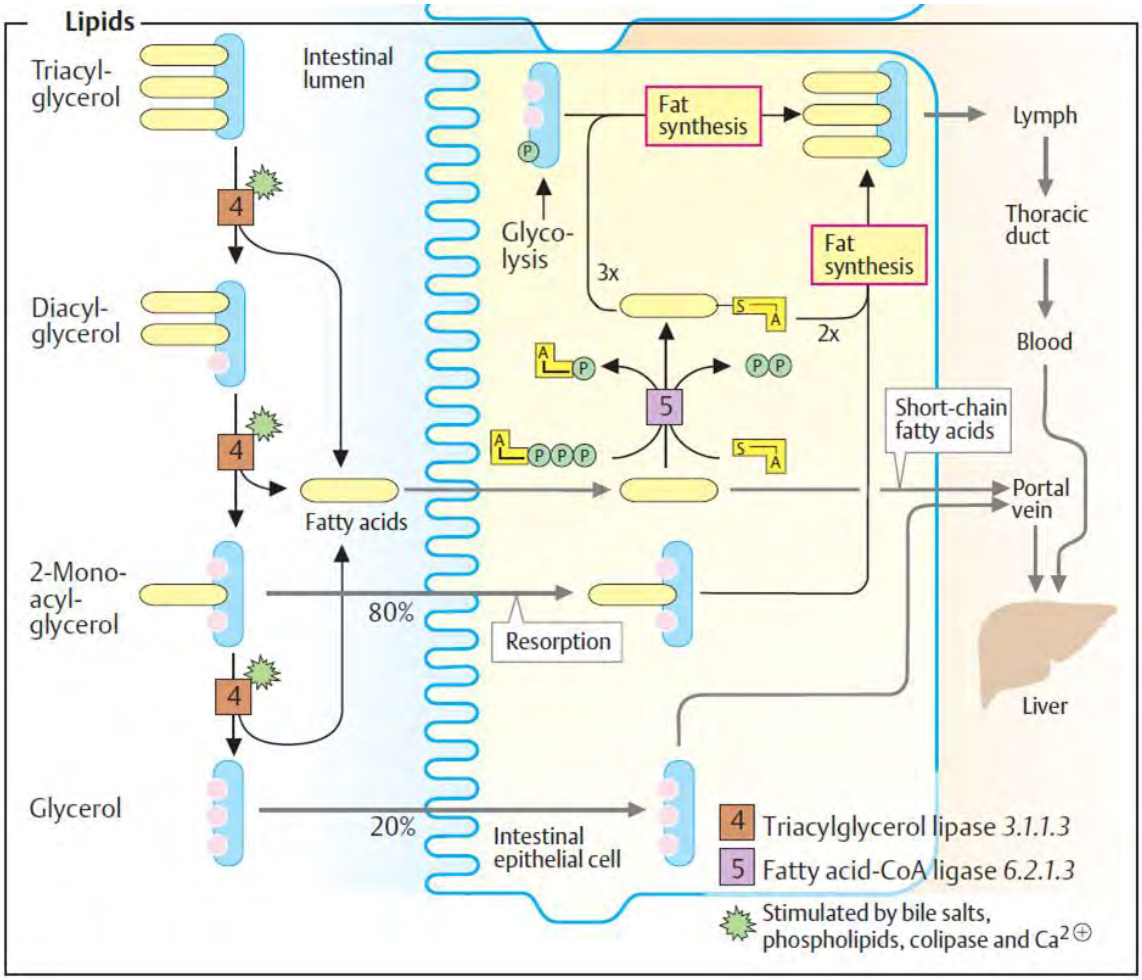
$$E = \frac{(a - b) \cdot V}{m \cdot t \cdot \mu M}, \text{ where}$$

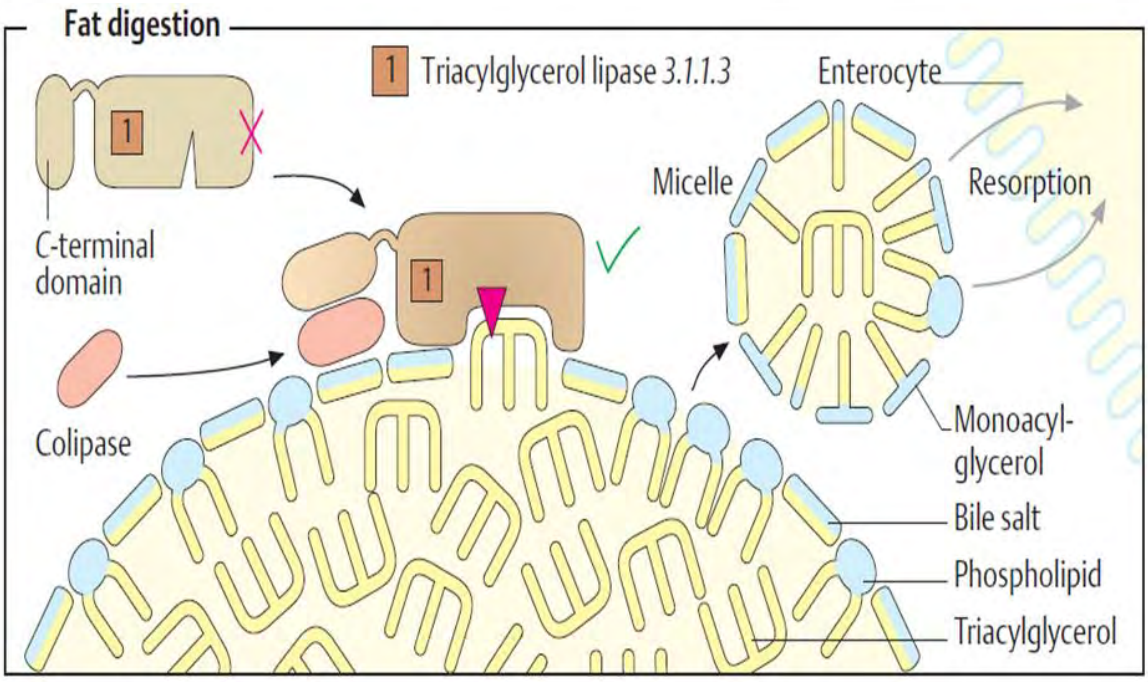
E - the activity of actomyosin ATPase, μmol P / g / 1 min;
a - Phosphorus content in the test sample, found on the calibration graph, μg / ml;
b - Phosphorus content in the control sample, found on the calibration graph, μg/ml;
V - the volume of the filtrate after the enzymatic reaction, ml;
μM - the micromolar mass of Phosphorus, μg/mol;
m - the mass of muscle tissue taken for research, g;
t - the time of the enzymatic reaction, min.

Control questions, tasks and exercises for the section «BIOLOGICAL OXIDATION OF SUBSTANCES AND THEIR ENERGY EXCHANGE**»**

1. What do you know about macroergic compounds?
2. What role does lactic acid play in energy metabolism?
3. The role of ATP in muscle contraction.
4. Describe the cycle of muscle contraction and the role of macroergic compounds?
5. How creatine phosphate is used in muscles?
6. Which ions stimulate the ATPase activity of actomyosin?

2.2.3. ***Topic*** LIPID METABOLISM AND ITS INTERMEDIATES

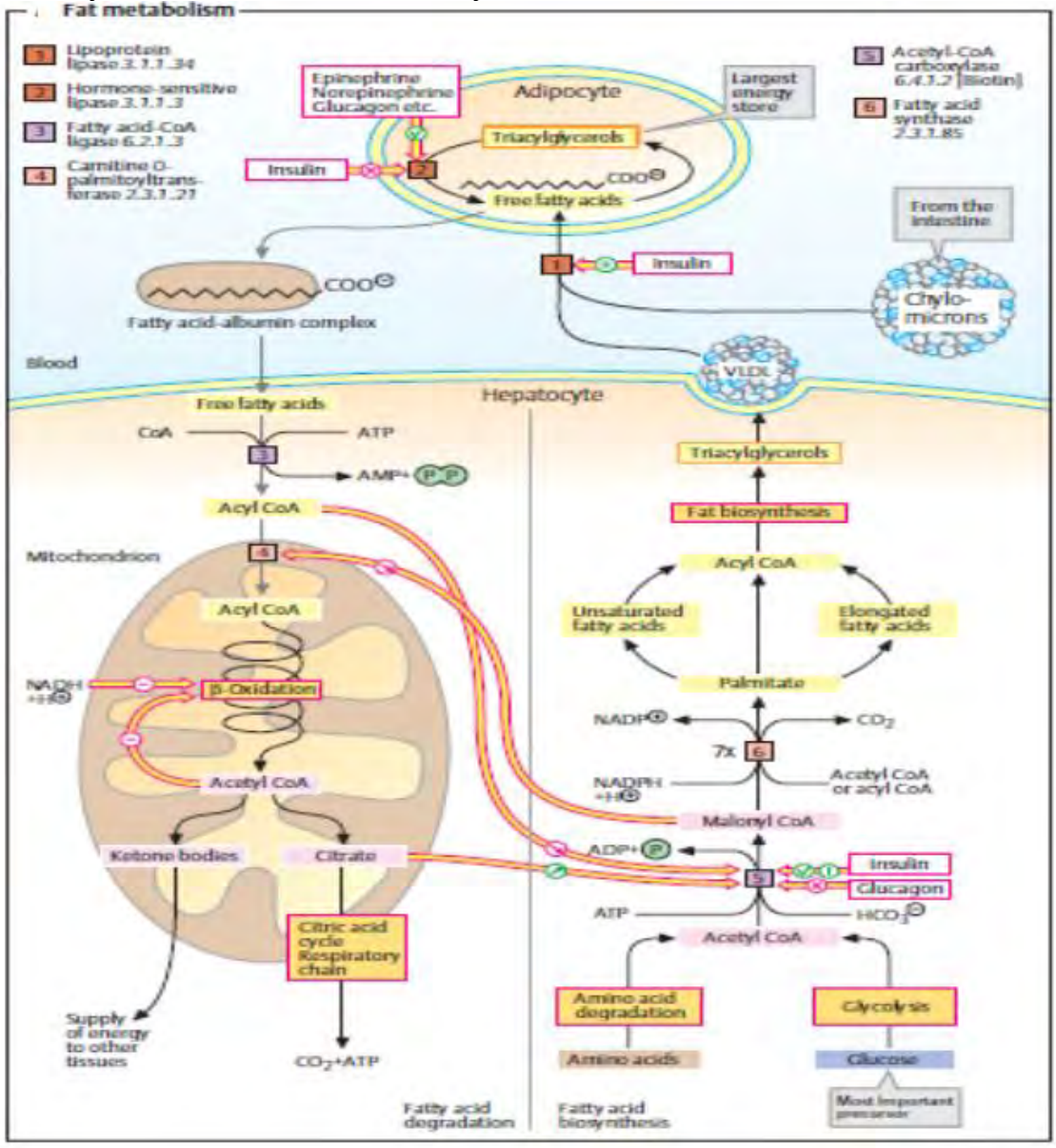




About 50% of TAG hydrolysis products are absorbed in the form of monoacyl-glycerols (by simple diffusion or foam pynocytosis).

- Short-chain FA (less than 12 Carbon atoms) - simple diffusion.
- Long-chain FA and cholesterol - in the form of complexes with bile acids.

'Cholein' complexes in the intestine wall break up, bile acids released through the blood enter the liver, where, together with bile, they are released into the intestine again (there is intestinal and liver recirculation of bile acids). Within a day of such cycles can be from 4 to 10 cycles.

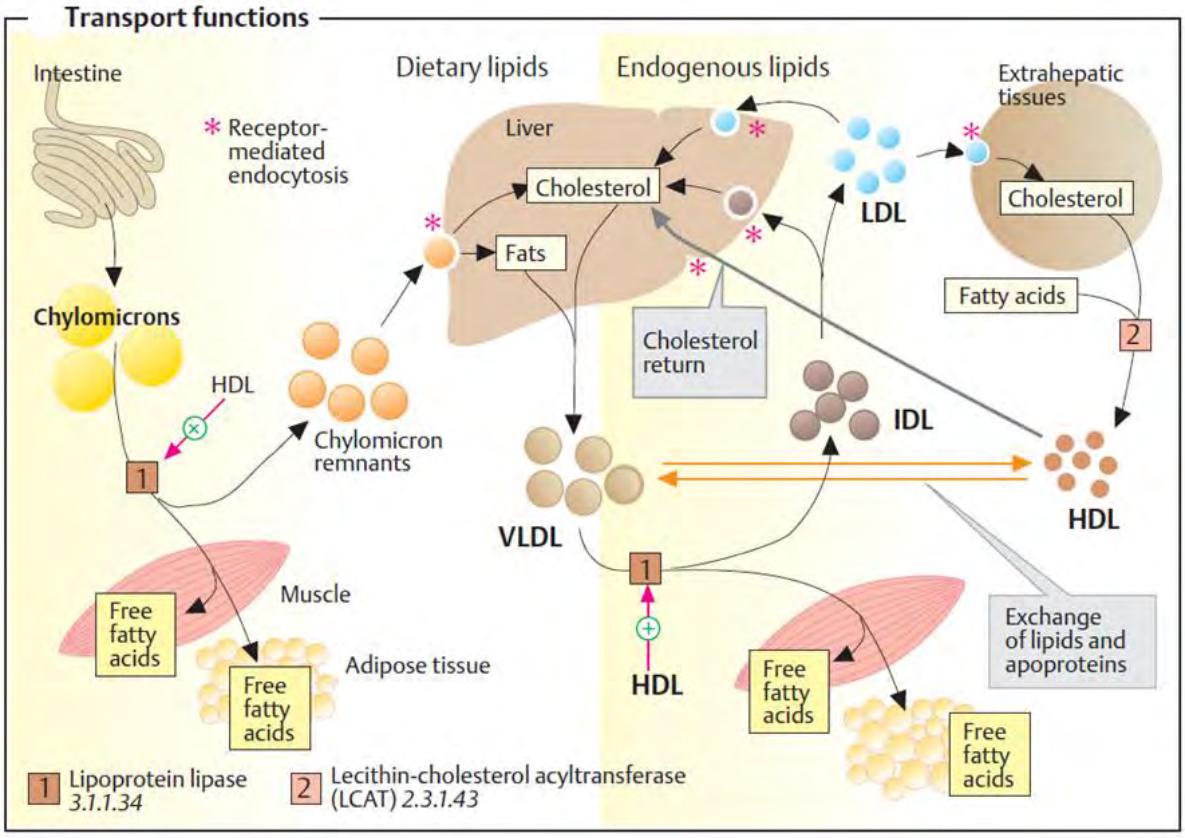


FA, glycerol and monoacylglycerols, which have been absorbed into the intestinal wall, are undergoing resynthesis (repeated synthesis of TAG or other lipids) that occurs in the liver, adipose tissue, and other organs.

Fatty acid degradation is the process in which fatty acids are broken down into their metabolites, in the end generating acetyl-CoA, the entry molecule for the citric acid cycle, the main energy supply of animals.

It includes three major steps:

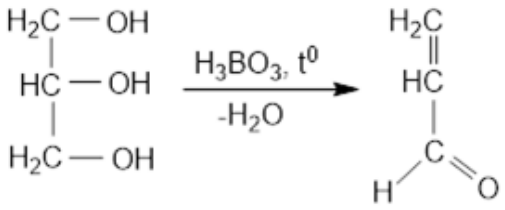
1. Lipolysis of and release from adipose tissue
2. Activation and transport into mitochondria
3. β-oxidation



LAB-CLASS

1. Detection of fats. Acrolein test

The principle of the method. Fats give a characteristic oil stain. With a glass stick, a drop of fat is applied to a piece of paper. There is a sebaceous spot that does not disappear when heated. The reaction for the presence of fat may be an acrolein test. When fat is heated with water-removing substances, such as $KNSO_4$, NaHSO3, or H_3BO_3, very corrosive acrolein vapors are formed, which are formed from glycerol due to the cleavage of the last two water molecules:



Equipment and reagents. Tripod with test tubes. Vegetable oil. Animal fat. Fish Oil. Wax. Mineral oil. Crystalline $KHSO_4$ or $NaHSO_3$.

Progress of experiment. Take two dry test tubes. In one of them add a few drops of vegetable oil or fish oil, in the other - animal fat. Dry powder KSO_4 or

$NaHSO_3$ is added to each tube. Stir and heat gently. There is a sharp smell of acrolein. The same reaction is carried out with wax or mineral oils. The obtained results are entered in the table:

The results of the acrolein test

# of test tube	Test tube contents	Result (positive or negative)
1	Beef fat	
2	Fish Oil	
3	Sunflower oil	
4	Wax	
5	Mineral oil	

2. Determination of iodine number of fat

The determination of the iodine number is based on the reaction of joining iodine at the site of the rupture of double bonds in unsaturated fatty acids. Unreacted iodine is titrated with sodium hyposulfite.

Material for research: butter, vegetable oil, solid fat.

Laboratory glassware and equipment: 50 ml flasks with ground corks; pipette dispensers; glass pipettes; burettes; laboratory scales; foil or cellophane.

Reagents: 96° ethyl alcohol; 0.1 N of alcoholic solution of iodine; 0.1 N of sodium hyposulfite solution ($Na_2S_2O_3$); 1% of starch solution.

Progress of experiment. Weigh 0.2 g of fat on cellophane or foil, place it in a flask, add 10 ml of 96° ethyl alcohol and 10 ml of 0.1 N of alcoholic solution of iodine. The contents of the flask are mixed well and left for 15 minutes. Titrate with 0.1 N sodium hyposulfite solution until light yellow color appears. Add 1 ml of starch and continue the titration until the discoloration**.**

Calculation of iodine number of fat:

$$X = \frac{a \times 0{,}01269 \times 100}{0{,}2}$$

where:

X - the iodine number of the studied fat;

a - the number of ml of 0.1 N sodium hyposulfite solution, which went to the titration of the test sample;

0.2 - the number of grams of fat taken for research;

0.01269 - the number of grams of iodine corresponding to 1 ml of 0.1 N of sodium hyposulfite solution.

The iodine value of some fats and oils varies within the following limits:

Beef - 27 - 47, mutton - 31 - 46, pork - 46 - 66, dog - 56 - 67, sunflower oil - 129 - 136, hemp oil - 145 - 162, linseed oil - 175 --201.

3. Determination of acid number of fat

Material for research: vegetable oil or solid fat.

Laboratory glassware and materials: glass conical flasks of 100 ml; pipette dispensers; burettes; laboratory scales; cellophane or foil.

Reagents: 0.1 N of KOH solution; 96° ethyl alcohol; 0.1% of an alcohol solution of phenolphthalein.

Progress of experiment. Weigh 1 g of fat on cellophane or foil and place it in a flask. Add 5 ml of 96% ethyl alcohol and 2-3 drops of 0.1% of phenolphthalein solution. The contents of the flask are mixed well and titrated with 0.1 N of KOH solution until pink.

Calculation of the acid number of fat:

1 ml of 0.1 N of KOH solution corresponds to 5.6 mg of KOH dry matter. Then, the number of KOH in milligrams, which went to the titration of free fatty acids in 1 g of fat will be equal to

$$C = A \times 5.6$$

where:

C - the acid number of the studied fat;

A - the amount of 0.1 N of KOH solution, which went to the titration of the sample.

The acid number of fresh fat of different varieties should not exceed 1.2 - 3.5.

4. Qualitative reaction for bile acids

The principle of the method: when bile acid interacts with oxi methyl furfurol, which is formed from sucrose under the action of concentrated sulfuric acid, a red-violet color appears.

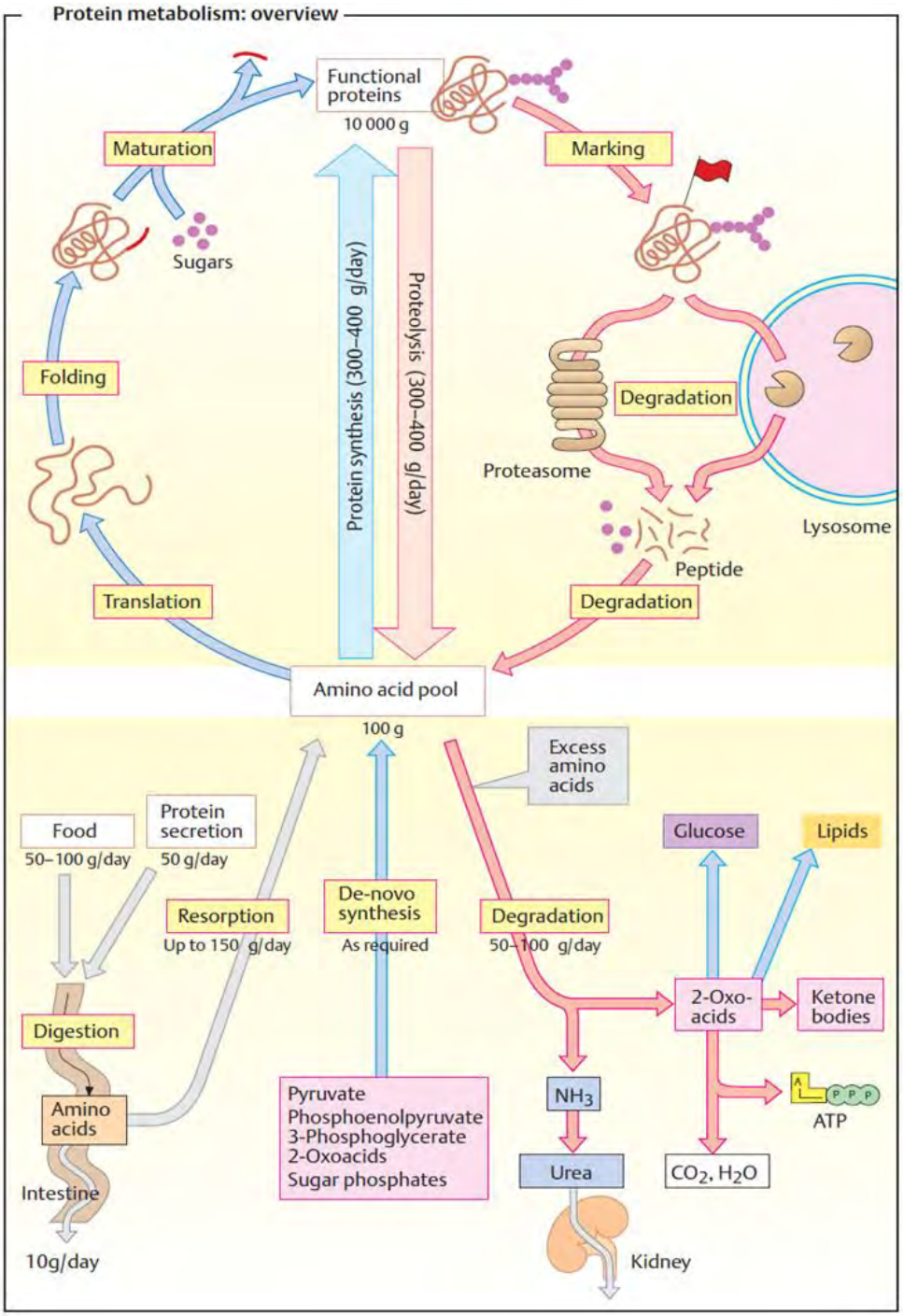
Progress of experiment: on a dry glass put 2 drops of bile, 2 drops of 20% of sucrose solution, and mix thoroughly with a glass stick. Then, add 7 drops of concentrated H_2SO_4, mix with the same stick. After 2-3 minutes, a red-violet color appears, which gradually acquires a red-violet color.

Control questions, tasks and exercises for the section «LIPID METABOLISM AND ITS INTERMEDIATES»

1. What organic compounds are called lipids?
2. What is the importance of lipids for the body and their energy value?
3. Give a definition of the iodine and acid numbers of fat. What is the purpose of their determination? Do you know what other constants that characterize the quality of fats?
4. It is necessary to choose acids that are essential for the human body:
    a) Lipoic, stearic, palmitic;
    b) Oleic, linoleic, linolenic;
    c) Palmitic, stearic, arachidonic;
    d) Arachidonic, linoleic, linolenic;
    e) Butyric, oleic, linoleic.
5. Write the equation of the first two reactions of conversion of linoleic acid before its oxidation by the mechanism of β-oxidation.

6. Write the equation of the reaction of synthesis of phosphatidylethanolamine from palmitoyl glycerol and CDP-ethanolamine with the participation of ethanolamine phosphotransferase.

2.2.4. ***Topic*** CHARACTERISTICS OF PROTEIN EXCHANGE INTERMEDIATES (I)

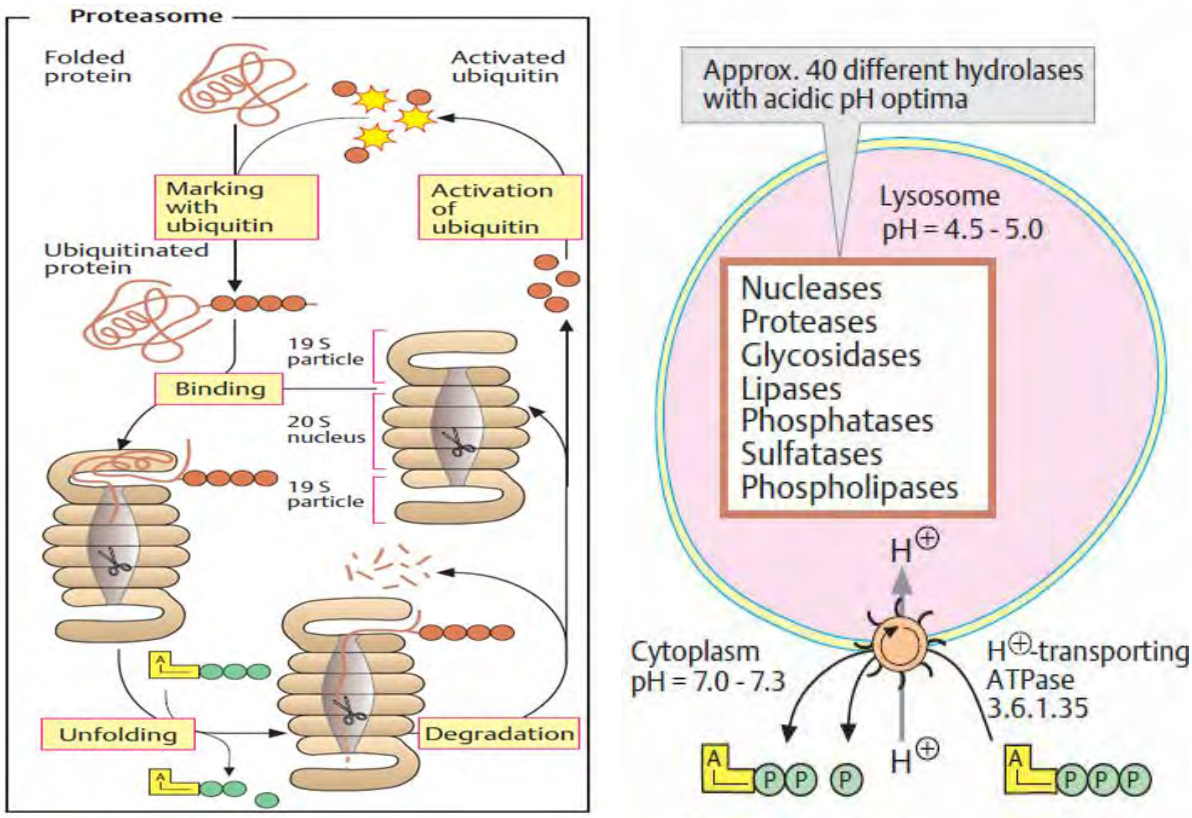


Proteins taken up in food are initially broken down in the gastrointestinal tract into amino acids, which are resorbed and distributed in the organism via the blood.

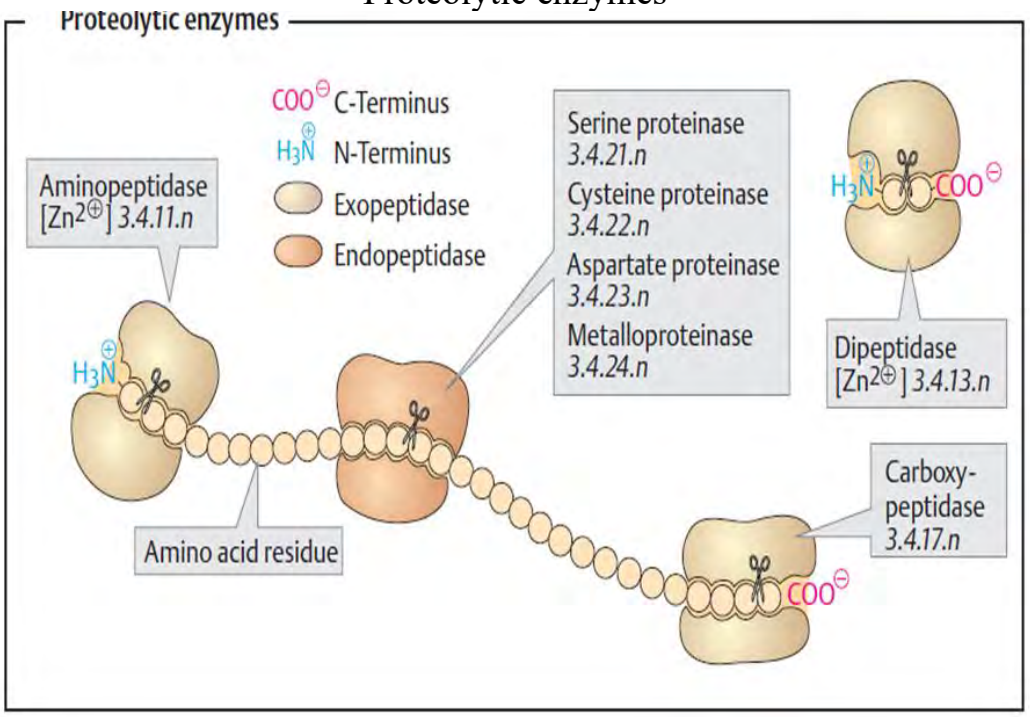
The human body is not capable of synthesizing 8–10 of the 20 proteinogenic amino acids it requires.

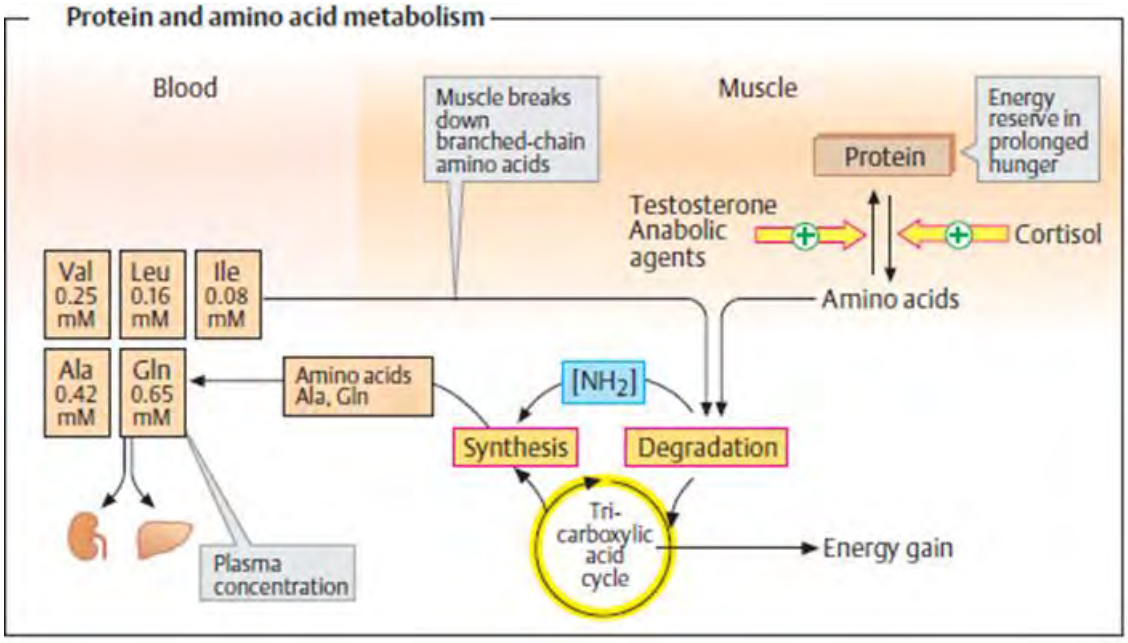
These amino acids are **essential and** must be supplied from food.

Protein degradation in proteosome and lysosome



Proteolytic enzymes





LAB-CLASS

1. Influence of temperature and reaction of the environment on the solubility of proteins

To number 5 tubes, each of which has 0.5 ml of 1% of a solution of egg white (or blood plasma). Alternately conduct the following experiments:

a) A solution of protein in №1 tube is heated to boiling. The liquid is cloudy. Conclude.

b) A solution of protein in №2 tube is heated to boiling and added 1 drop of 1% acetic acid solution for acidification. When defending the protein precipitates in the form of flakes. Conclude.

c) To the protein solution of test tube № 3, add 0.5 ml of 1% acetic acid solution to create a strongly acidic environment. When the liquid boils, no precipitate forms. Conclude.

d) To the protein solution of test tube № 4, add 0.5 ml of 1% acetic acid solution and 2 drops of saturated NaCl solution. The mixture is heated. A white precipitate of protein in the form of flakes. Conclude.

e) To the protein solution of test tube № 5, add 2 drops of 10% NaOH solution. When boiling the liquid precipitate is not formed. Conclude.

1. Physical and chemical properties of proteins

1.1. Determination of the isoelectric point of a protein

The principle of the method. Since specific proteins have certain pI values, this is used to fractionate proteins from the mixture, creating the appropriate pH values of the medium. The proteins are precipitated sequentially and the protein precipitates are separated each time. This method of separating a mixture of proteins is called the method of isoelectric precipitation.

Isoelectric points of animal blood proteins are mostly 5.5… 5.8. In order to prevent the transition of these proteins as a result of acidosis to an isoelectric state and to coagulate, which is very dangerous for the body, there are blood buffer systems that keep the blood pH around 7.4. At this pH value, acidic blood proteins have negative charges and are far from the pI values, which guarantees their normal functioning.

Equipment and reagents. Water bath. Flask of 200 ml. Test tubes. White. Sodium acetate (0.1M). Acetic acid (0.1M). Distilled water. Crystalline casein.

Progress of experiment. You can take any protein for research. Here is an example of determining the isoelectric point of the casein protein.

Prepare a solution of casein (0.1%) in sodium acetate solution

(0.1 M). To do this, 5 ml of sodium acetate solution (0.1 M) is added to crystalline casein weighing 0.2 g, and casein is dissolved by weakly heating the flask in a water bath.

After dissolving the casein, adjust to 200 ml with sodium acetate solution of the same concentration and mix.

Next, take 5 tubes and make each component according to the table (see below).

After mixing the components after 30 minutes, observe the intensity of turbidity of the solution in each tube. Weak turbidity is indicated by (+), moderate - (++), strong - (+++). the pH in vitro, where the highest coagulation intensity, corresponds to the isoelectric point of this protein.

The results of the experiment

Components	Tubes				
	1	2	3	4	5
Acetic acid solution (0.1M), ml	0.25	0.50	1.0	2.0	4.0
Distilled water, ml	8.75	8.5	8.0	7.0	5.0
A solution of casein (0.1%) in a solution of sodium acetate (0.1M), ml	1	1	1	1	1
pH of the mixture	5.3	5.0	4.7	4.4	4.1
The observed intensity of coagulation					

1.2. Protein precipitation reactions

The principle of the method. In an aqueous solution, protein molecules are charged and hydrated, which ensures the stability of protein solutions. At high concentrations of salts, the ions of which are also hydrated, the destruction of the aqueous shells of protein molecules occurs as a result of competition for salt ions for water. In addition, salt ions are adsorbed on the surface of the protein molecule resulting in a decrease in the charge of the protein molecule, protein particles are less repelled, stick together, precipitate.

2.2.1. Precipitation of proteins by organic acids

a) ***Protein precipitation with trichloroacetic acid***

To 0.5 ml of the plasma of blood add 0.5 ml of 10% solution of trichloroacetic acid. Protein precipitation falls out.

b) ***Protein precipitation by picric acid***

To 0.5 ml of the plasma of blood add 1-2 drops of acetic acid (for acidification) and 0.5 ml of saturated picric acid solution. Protein precipitate falls out.

2.2.2. Organic solvents precipitation of proteins

a) ***Ethanol precipitation of proteins***

To 0.5 ml of the plasma of blood, add 1.5-2.0 ml of ethanol. The solution is cloudy. Add 1 drop of saturated NaCl solution. After 5-15 min, a protein precipitate falls out.

b) ***Acetone precipitation of proteins***

To 0.5 ml of the plasma of blood, add 1.5-2.0 ml of acetone. The solution is cloudy. Add 1 drop of saturated NaCl solution. A protein precipitate falls out.

2. Quantitative determination of protein by the Lowry method

The principle of the method. The intensity of the color of the solution, which appears as a result of at least two reactions to the protein: the biuret reaction and the Folin reaction with tyrosine and cysteine residues in the composition of protein molecules, is measured by the Lowry method. Folin's reagent is a mixture of phosphotungstic and phosphomolybdic acids. With the participation of protein, it is the reduction of these acids. Their reduced forms have an intense blue color. It is believed that the reduction reaction involves complex compounds of copper, which occur when the protein interacts in an alkaline environment with copper hydroxide. The Lowry method is extremely sensitive and allows us to determine the protein in a highly diluted solution, where its number is only tens of micrograms and is usually used to determine proteins in the eluates from the columns during fractionation on ion exchange resins and Sephadex. According to the Lowry method, the solution is determined not by the protein itself, but by the result of its reductive action on phosphotungstic and phosphomolybdic acids.

Equipment and reagents. Photoelectrocolorimeter. Protein solution. Solution A: a solution of sodium carbonate (2%) in a solution of sodium hydroxide (0.1M). Solution B: a solution of copper sulfate (0.5%) in a solution of sodium citrate (1%) or sodium or potassium tartrate (3.33%). Folin's reagent.

Progress of experiment. Prepare a solution for the biuret reaction. To do this, mix 49 ml of solution A with 1 ml of solution B before determining. To 1 ml of test protein solution containing 10 to 100 μg of protein, add 4 ml of a mixture of solutions A and B. Stir and leave for 10 minutes. at room temperature. Then 0.4 ml of Folin's reagent is quickly pipetted, mixed vigorously, and left for 30-90 minutes. for the development of color. The yellow color of Folin's reagent gradually turns blue. The optical density of the solution is determined on a photo electro colorimeter at a wavelength of 750 nm in a cell with l = 10 mm.

The protein content in the test sample is determined by a calibration graph, which is built on a solution of any pure protein of exactly known concentration. It is better to use a solution of the protein or a mixture of proteins, which is determined by the Lowry method. To do this, prepare a series of solutions of pure protein, 1 ml

of which contains from 20 to 400 μg of protein. The standard protein for the calibration graph is dissolved in sodium hydroxide solution (0.1M). A series of solutions are prepared by diluting the original concentrated protein solution (400 μg in 1 ml) to the required values. Lowry reaction was performed at least 3 times with each of these solutions using the same volumes of reagents as above (5.4 ml). According to the obtained data, a graph is constructed - a calibration curve. The values of the optical density are plotted on the ordinate, and the protein content in the sample is plotted on the abscissa.

Having determined the optical density of the test protein solution after the Lowry reaction, the calibration graph is drawn from a certain value of the optical density straight, parallel to the abscissa axis to the intersection with the calibration curve and lowered perpendicular to the abscissa axis. Thus determine the protein content in the sample of the test solution. Make calculations of the protein content per 100 ml of the test solution.

The advantages of Lowry protein determination are the short detection time compared to other methods and extremely high sensitivity, which makes it possible to determine trace amounts of protein. The disadvantages are that this method can determine only water-soluble proteins and the blue color of the solution is not stable enough (which reduces the accuracy of measurement).

Control questions, tasks and exercises for the section «CHARACTERISTICS OF PROTEIN EXCHANGE INTERMEDIATES (I)»

1. Describe the levels of structural organization of the protein molecule.
2. What determines the nutritional value of proteins?
3. What is common and different for globular and fibrillar proteins? Give examples.
4. From the following statements, determine the correct ones:
    a) in the formation of hemoglobin molecules, the combination of protomers into a multimer is carried out by self-assembly;
    b) the hemoglobin molecule has one hemin group;
    c) from the four hemoglobin polypeptide chains, two are exactly the same;
    d) each subunit of the hemoglobin molecule in its tertiary structure is similar to the myoglobin molecule.
5. Calculate the relative molecular weight of the protein-enzyme dihydroorotate dehydrogenase, the molecule of which includes 2 iron atoms with a content of 0.18%.
6. At what pH values can be most effectively separated by electrophoresis the following protein mixtures (Note. The pI values of these proteins are as follows: serum albumin - 4.9; hemoglobin - 6.8; myoglobin - 7.0; chymotrypsinogen - 9.5; urease - 5.0):
    a) serum albumin and hemoglobin;
    b) myoglobin and chymotrypsinogen;
    c) hemoglobin and urease?

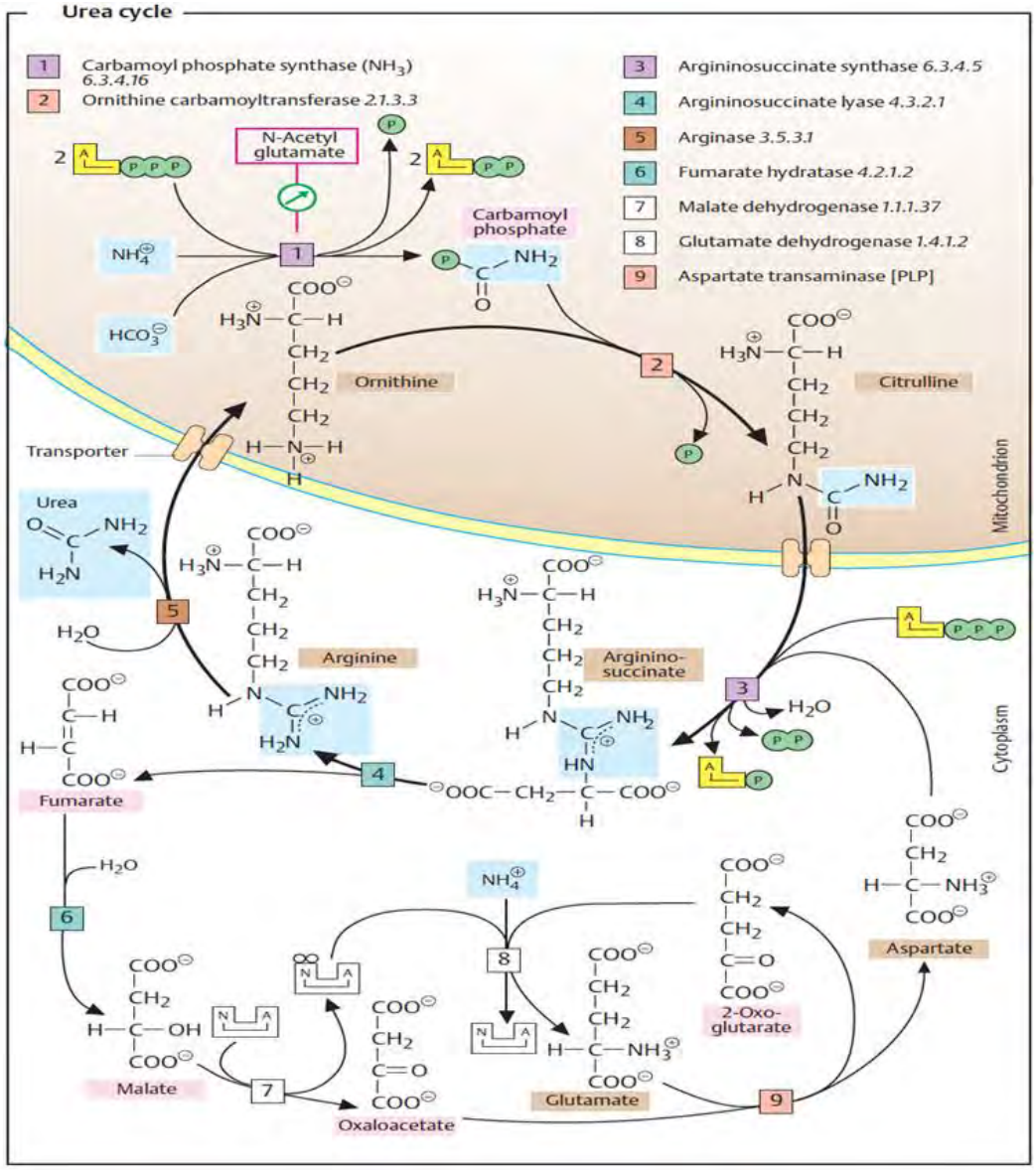
2.2.5. Topic CHARACTERISTICS OF PROTEIN EXCHANGE INTERMEDIATES (II)

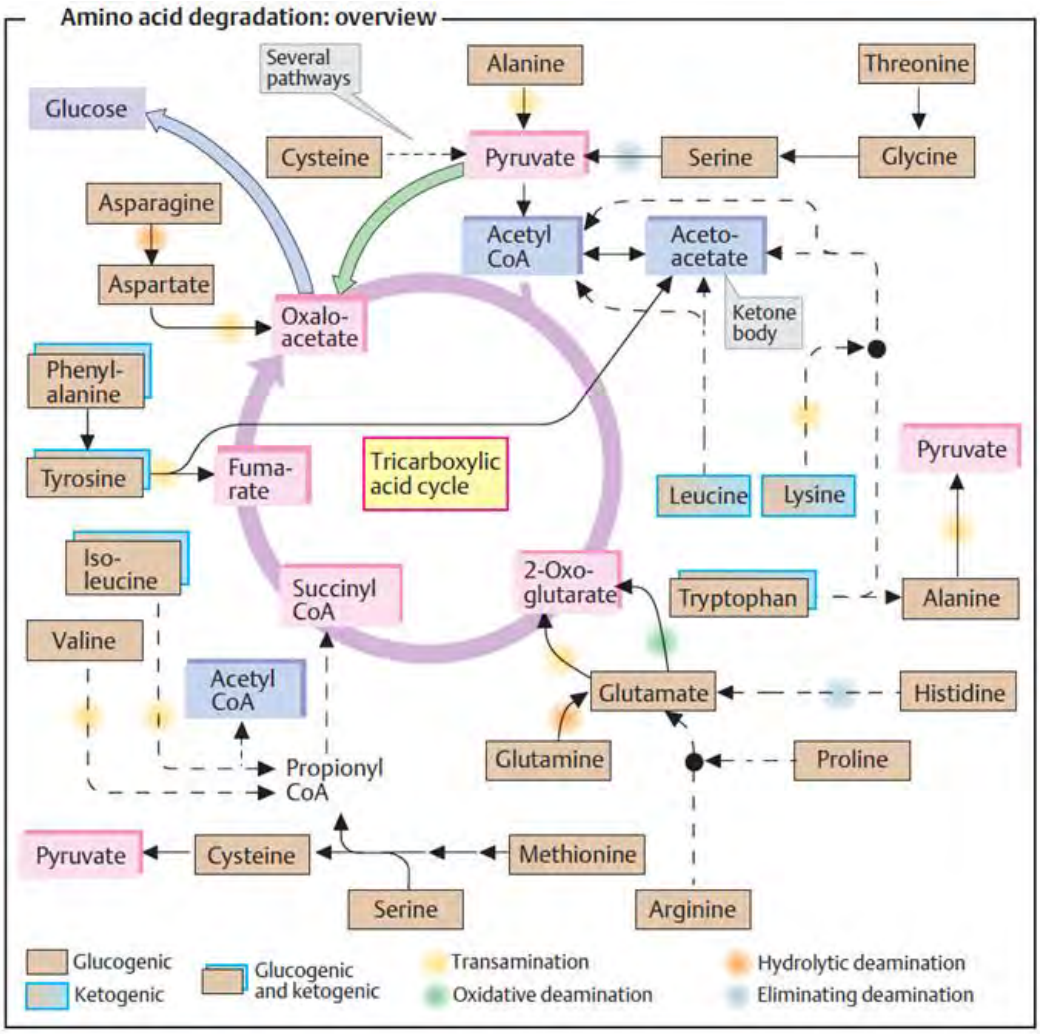
Protein degradation is initiated by *proteinases*— by pepsins in the stomach and by trypsin, chymotrypsin, and elastase in the small intestine.

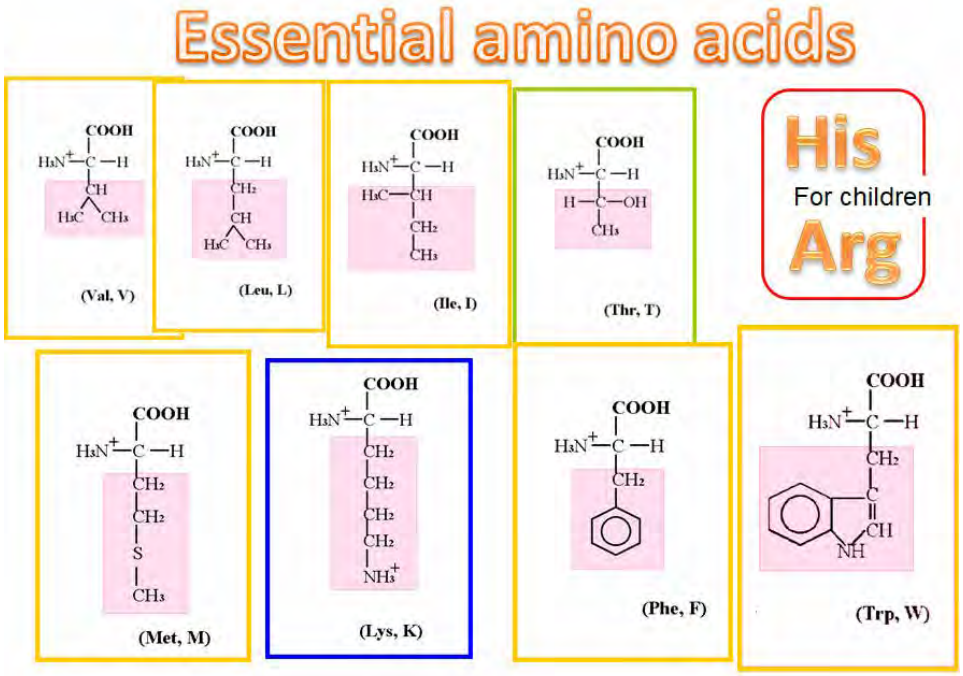
The resulting peptides are then further hydrolyzed by various *peptidases* into amino acids.

Individual amino acid groups have ***group–specific amino acid transporters***, some of which transport the amino acids into the enterocytes in cotransport with Na^+ ions (**secondary active transport**), while others transport them in an Na^+–independent manner through **facilitated diffusion**.

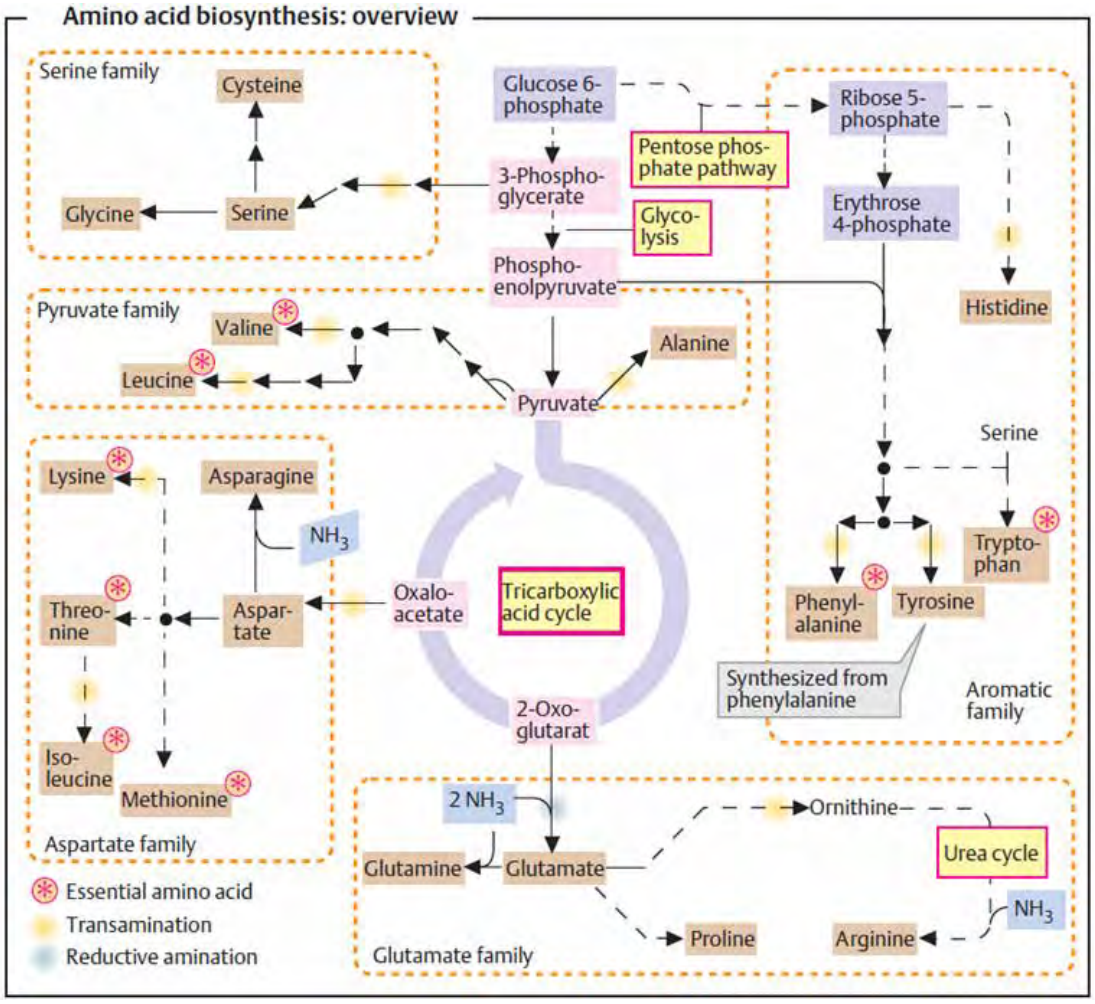
Small peptides can also be taken up.



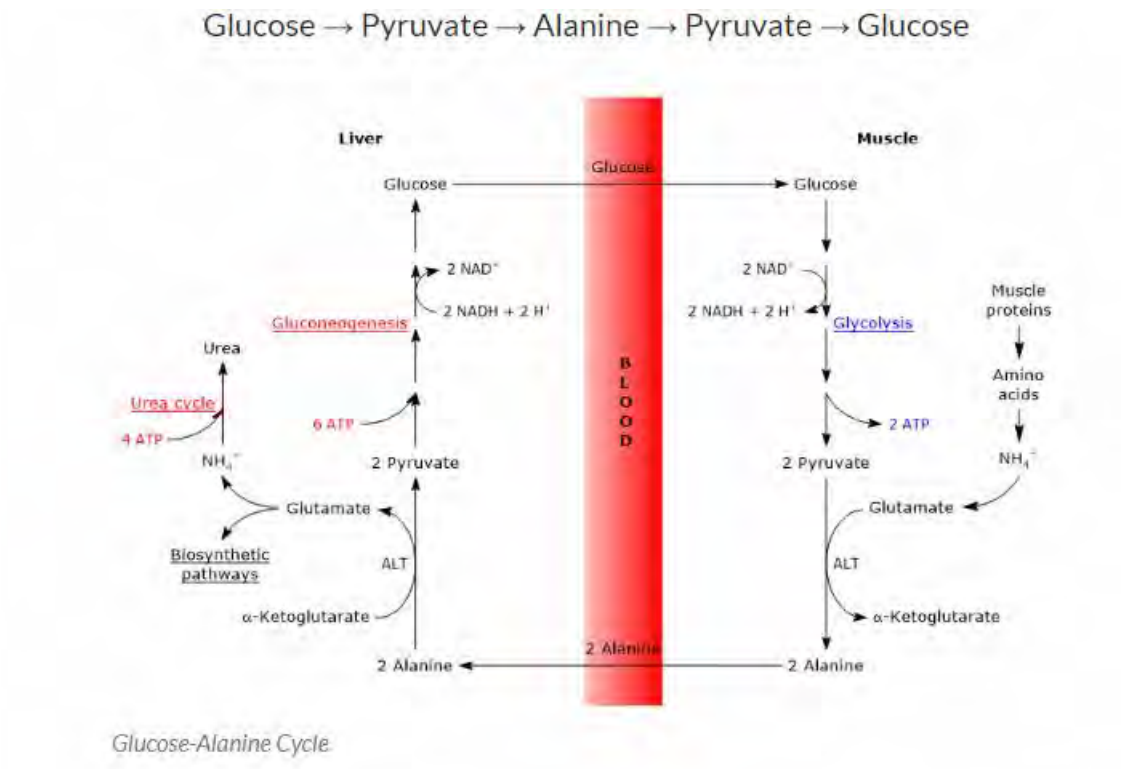




Valine, leucine, isoleucine, threonine, methionine, lysine, phenylalanine, and tryptophan are essential amino acids. Histidine and arginine are essential amino acids for children.



Glucose-Alanine Cycle



LAB-CLASS

1. Qualitative reactions to aromatic amino acids

1.1. Reaction for phenylalanine, tyrosine, and tryptophan

The reaction makes it possible to detect cyclic amino acids in the protein molecule - phenylalanine, tyrosine, and tryptophan. The xanthoprotein reaction is caused by cyclic amino acids present in the protein molecule, the aromatic rings of which are nitrated and as a result, yellow-colored nitro derivatives of proteins are formed. Tyrosine when heated turns into nitrotyrosine, from which under the influence of alkali ammonium salt is formed, which has a quinoid group.

Laboratory glassware and materials: test tubes, glass pipettes, glass alcohol burners.

Reagents: 0.1% of phenol solution, concentrated HNO_3, protein solution, 20% of NaOH solution or ammonia.

Progress of experiment. Control reaction for phenol (as a model experiment): pour 2 ml of phenol solution into a test tube, add 1-2 ml of concentrated nitric acid, heat gently. Due to heating, the reaction mixture turns yellow.

Pour 2 ml of protein solution into the test tube and add 6-10 drops of concentrated nitric acid. Under the influence of acid, a precipitate of protein appears, which turns yellow when heated. The tube is then cooled and the excess sodium hydroxide or ammonia solution is carefully added. In this case, the yellow color turns orange.

1.2. Reaction for tyrosine

If tyrosine is present in the protein molecule (which has a phenolic group in its structure), then when heated with Millon's reagent, this group gives a dark red color. The chemistry of the reaction is reduced to the formation of nitrotyrosine, which joining Argentum when heated, turns into a red salt. Almost all proteins give a reaction with Millon's reagent because they contain the amino acid tyrosine.

Laboratory glassware and materials: test tubes, glass pipettes, glass alcohol burners.

Reagents: solutions of protein and gelatin (1%); 0.1% phenol solution; Millon's reagent: 40 g of Argentum is dissolved in 57 ml of concentrated HNO_3 first at room temperature and then at low heat in a water bath, then the resulting solution is diluted with two volumes of water, stood for a while and decanted (to prepare under a fume hood!)

Progress of experiment. The *control* sample (as a model experiment): 2 ml of phenol solution and 1 ml of Millon's reagent solution are poured into a test tube, heated slowly, resulting in a pink color of the reaction mixture. *Test* samples: 1) in a test tube, pour 2 ml of protein solution and add 6-8 drops of Millon's reagent. First, a precipitate of protein appears, which turns red when heated. Millon's reagent should be carefully added (avoiding its excess), as excess nitric acid can stimulate the xanthoprotein reaction (yellow color of the reaction mixture, which masks the reaction for tyrosine); 2) similarly carry out the reaction with a solution of gelatin (usually the reaction of Millon with gelatin is negative. Why?)

1.3. Reaction for tryptophan

The principle of the reaction is that under the influence of sulfuric acid is the hydrolysis of sucrose to monosaccharides, which are dehydrated, turning into oxy methyl furfurol. Tryptophan, which reacts with oxy methyl furfurol, forms a complex colored in cherry red.

Laboratory glassware and materials: test tubes, glass pipettes.

Reagents: protein solution, 10% of sucrose solution, concentrated H_2SO_4.

Progress of experiment. Pour 1 ml of protein solution into a test tube and add 2 drops of sucrose solution. Then pipette 1 ml of concentrated sulfuric acid. A cherry-red ring-shaped color appears at the interface.

1.4. Reaction for thioamino acids

The molecules of most proteins include sulfur-containing amino acids - cysteine, cystine and methionine. When heated with alkali, sulfur is cleaved from these amino acids, which is detected in the reaction with lead acetate.

1. $$\begin{array}{l} CH_2SH \\ | \\ CHNH_2 \\ | \\ COOH \end{array} + NaOH \rightarrow \begin{array}{l} CH_2OH \\ | \\ CHNH_2 \\ | \\ COOH \end{array} + Na_2S + H_2O$$

2. $Na_2S + (CH_3 - COOH)_2Pb \rightarrow 2CH_3 - COONa + PbS$

Laboratory glassware and materials: test tubes, glass pipettes.

Reagents: undiluted egg white, 20% of NaOH solution, 0.5% of $PbSO_4$ solution.

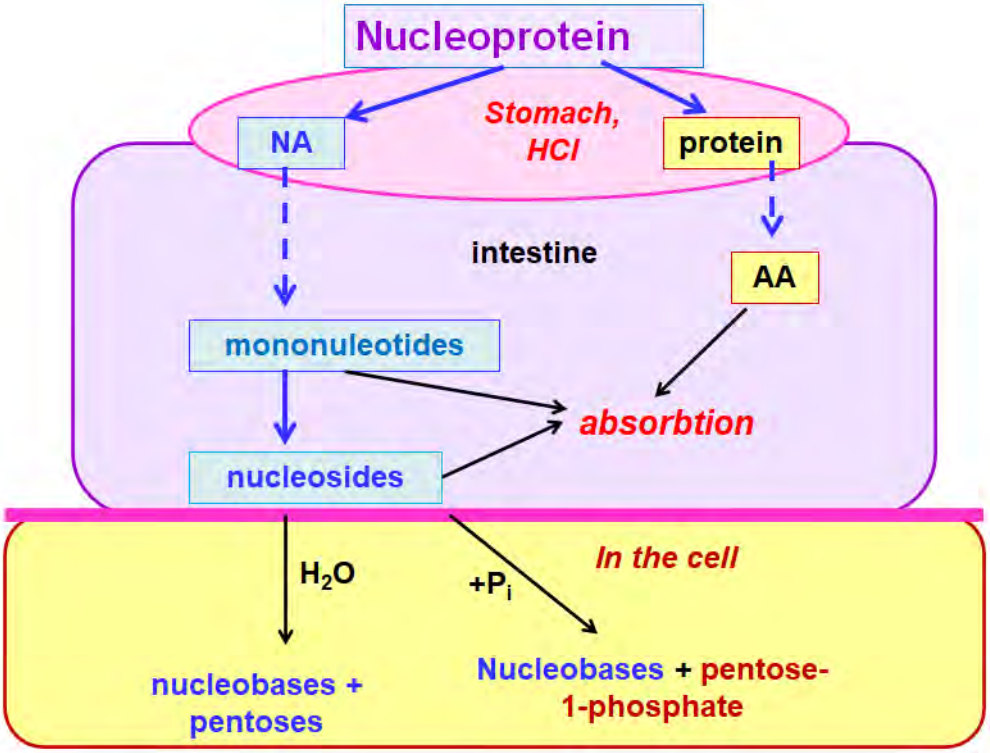
Progress of experiment. Pour 1-2 ml of protein into a test tube and add an equal volume of sodium hydroxide, heat to boiling, and add 1-2 drops of lead acetate. Observe the gradual darkening of the solution.

Control questions, tasks and exercises for the section «CHARACTERISTICS OF PROTEIN EXCHANGE INTERMEDIATES (II)»

1. What functional groups are part of amino acids?
2. Which amino acids are essential and nonessential?
3. Which amino acids belong to Sulfur-containing? What is their role?
4. Define the terms - alkaptonuria, phenylketonuria, cystinuria.
5. What functions of glutamic acid in animals are?
6. Justify the statement:
   a) the location of amino acids in polypeptide chains is natural; b) proteins are characterized by extremely high specificity of the primary structure; c) all amino acids found in proteins and peptides belong to the L-series; d) the presence in protein molecules of other covalent bonds, other than peptide, is a very rare phenomenon

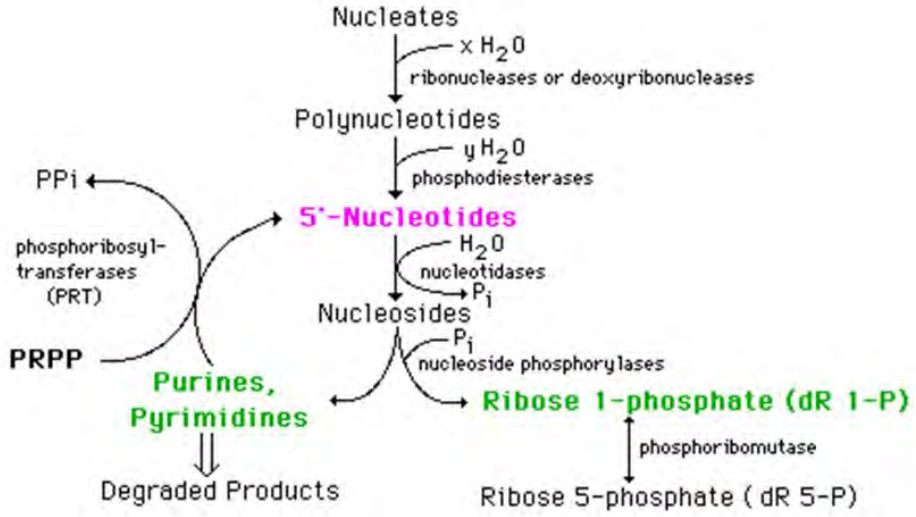
2.2.6. ***Topic*** COMPONENTS OF NUCLEIC ACIDS

Scheme of digestion and absorption of nucleoproteins in the gastrointestinal tract

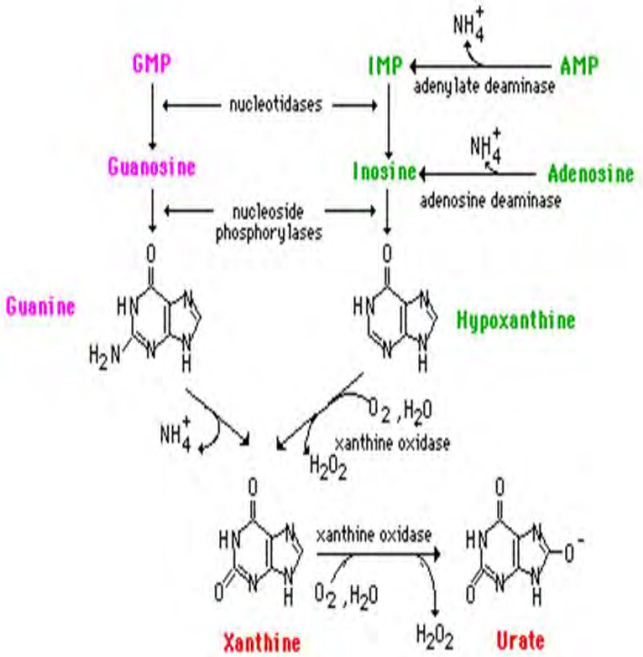


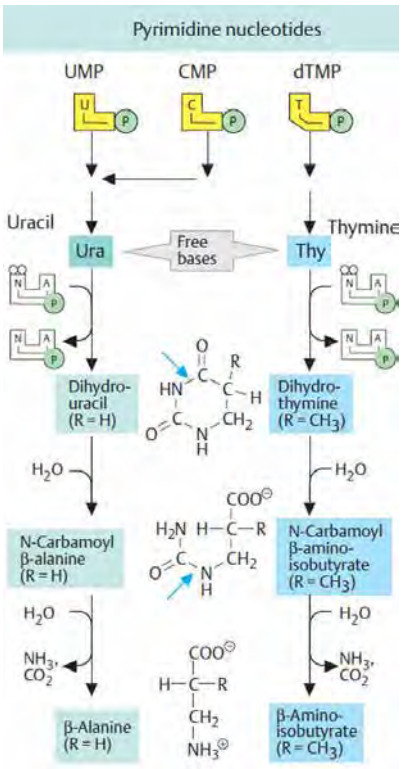
In the stomach, nucleoproteins, by the action of enzymes and chloride acid, "break up" into polypeptides (which are first in the stomach, and then, in the intestine, they are cleaved by appropriate enzymes to amino acids) and nucleic acids.

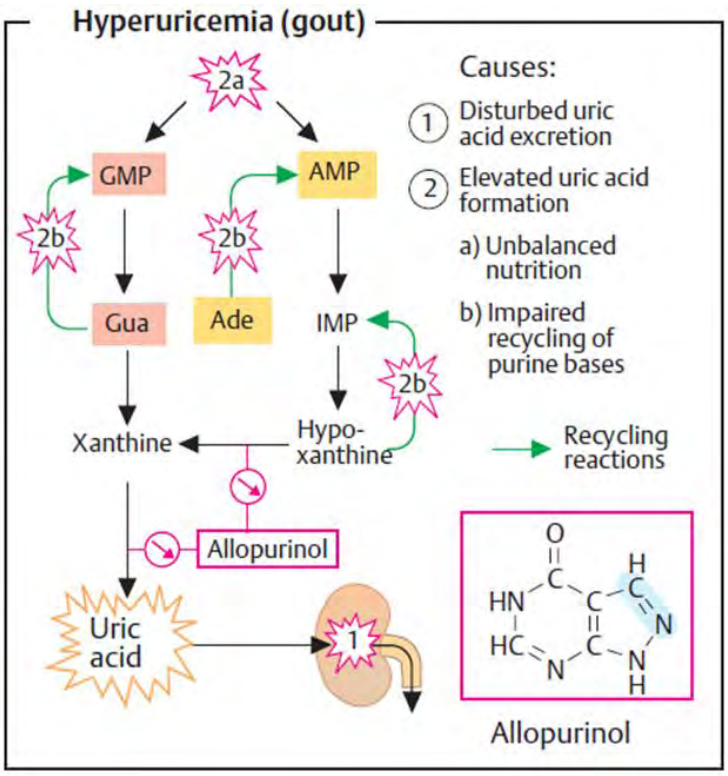
The way of degradation of nucleates and polynucleotides



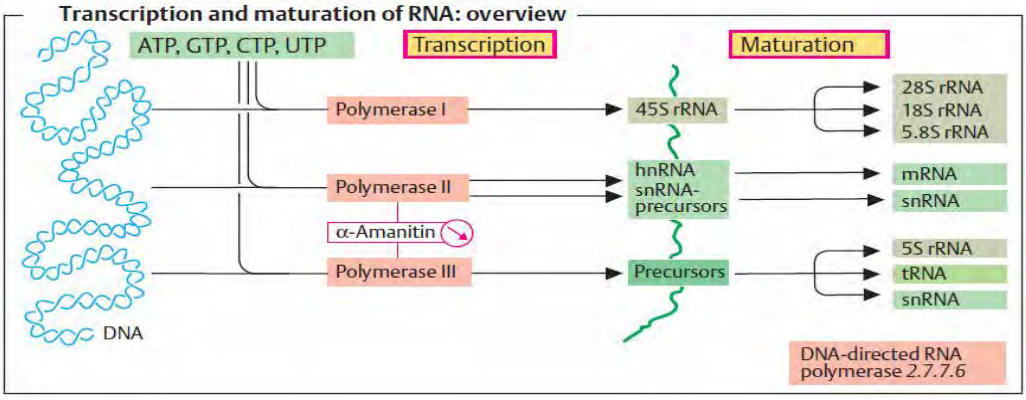
In the small intestine, nucleic acids under the influence of DNA-ases and RNA-ases of pancreatic juice are hydrolyzed to oligonucleotides, which break down into mononucleotides with the involvement of phosphodiesterases of the intestinal mucosa. Nucleotidases or phosphatases cleaved mononucleotide to phosphoric acid and nucleosides, which are cleaved by nucleosidases to the pentose and nucleobase, which are absorbed by the mucous membrane of the small intestine and transferred into the blood. Purine and pyrimidine nucleotides degradation



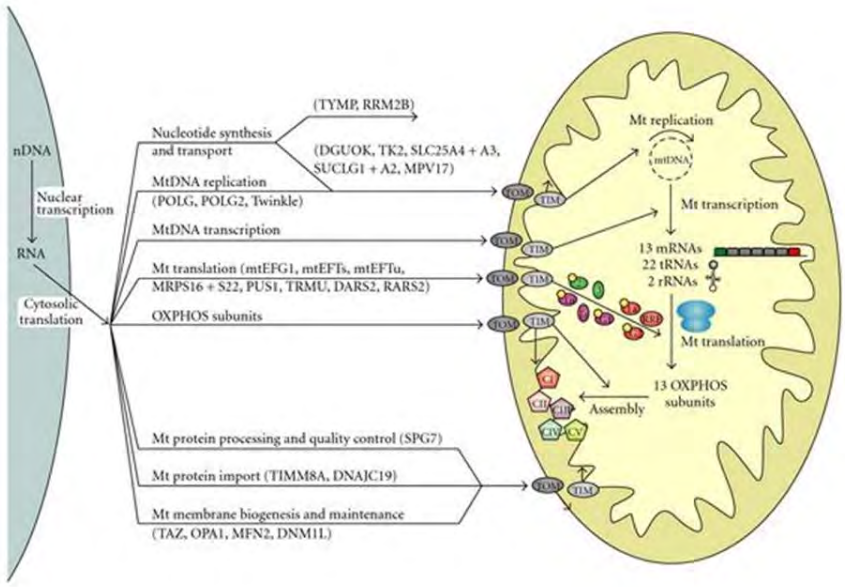


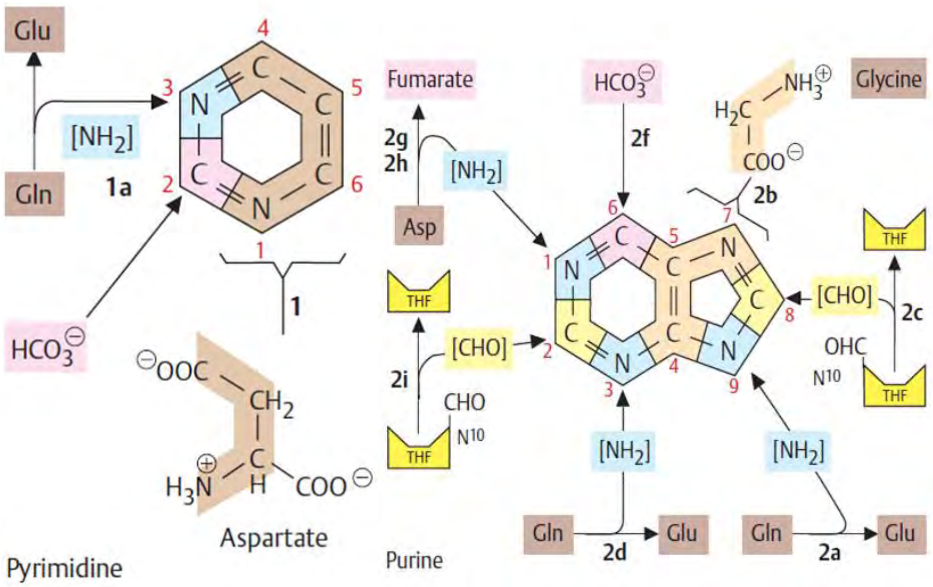


Transcription and maturation of RNA



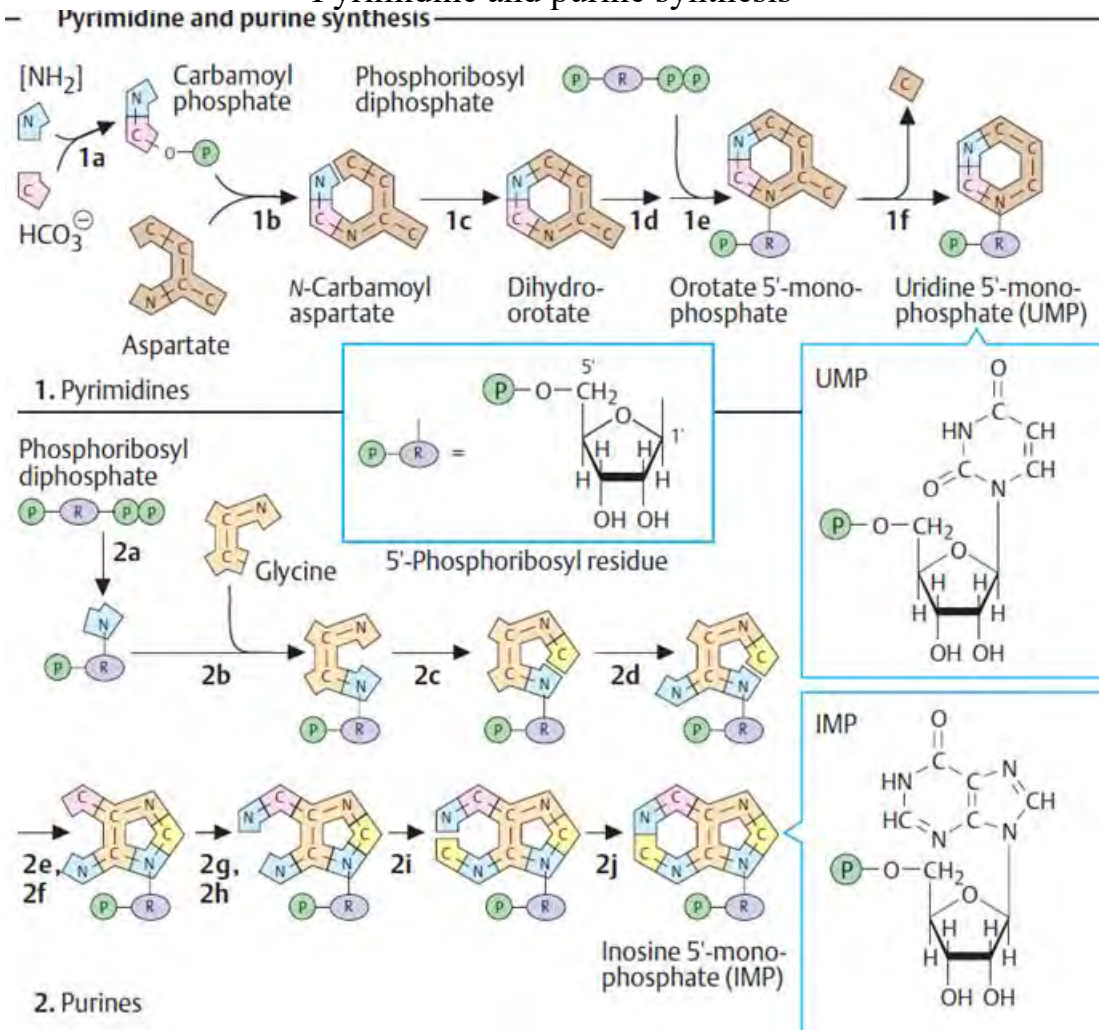
In the organelles (mitochondria and chloroplasts), there are DNA, RNA too.



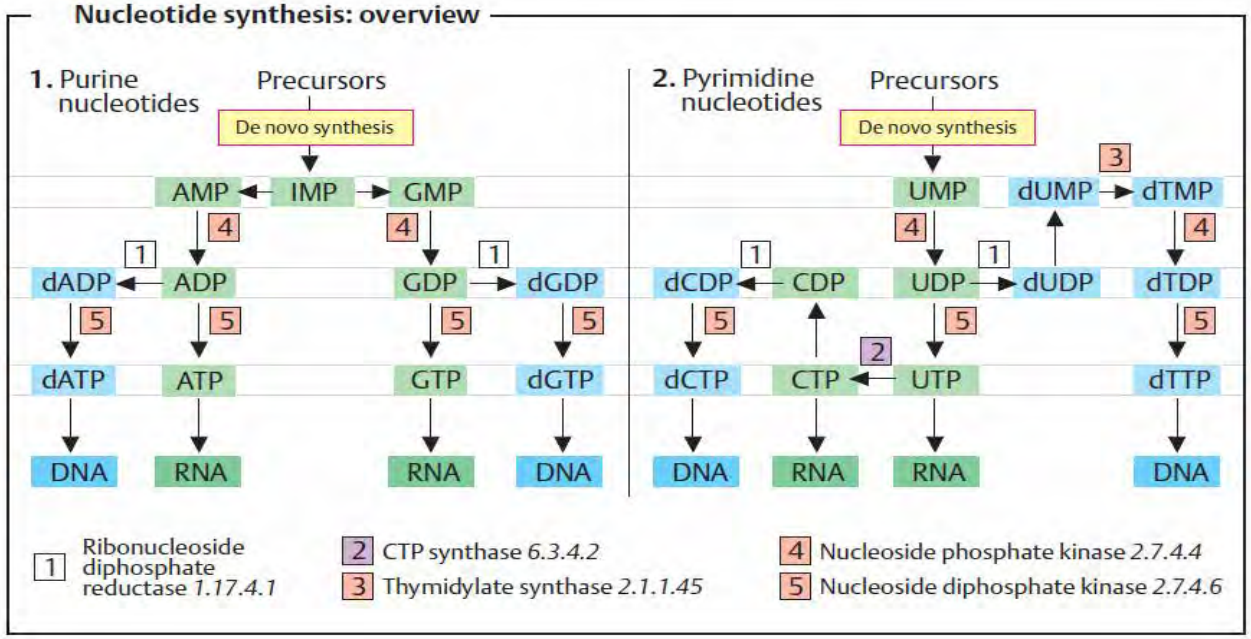


The synthesis of pyrimidine and purine rings

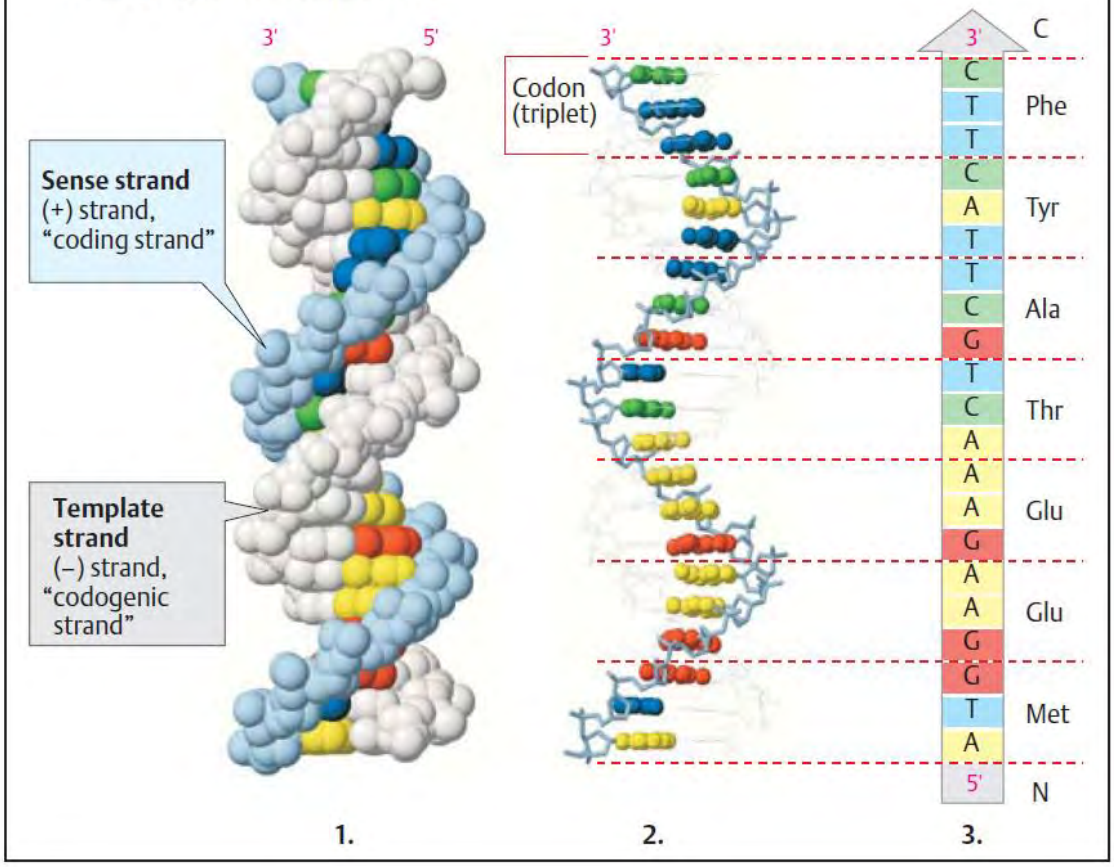
Pyrimidine and purine synthesis



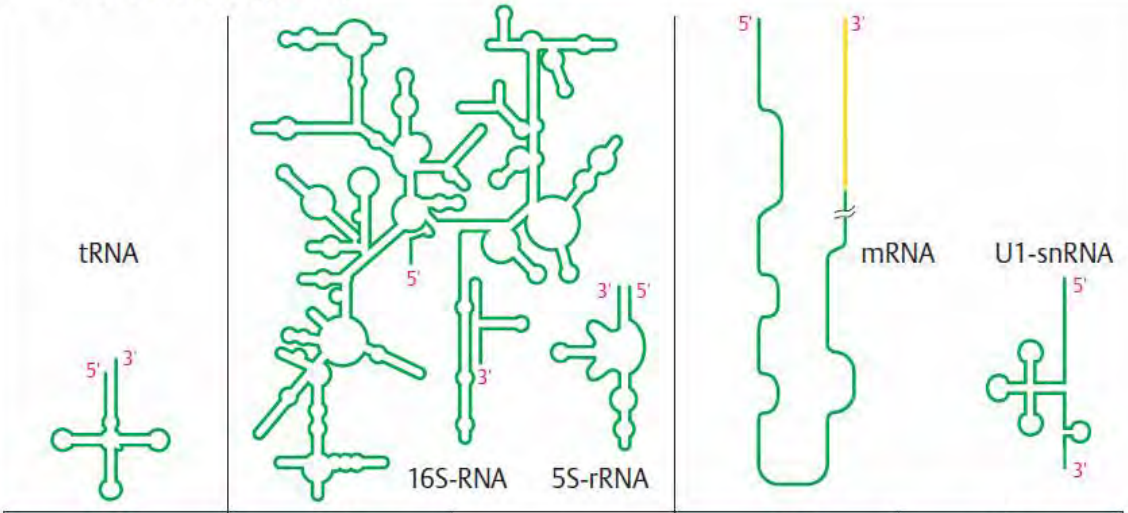
Nucleotide synthesis



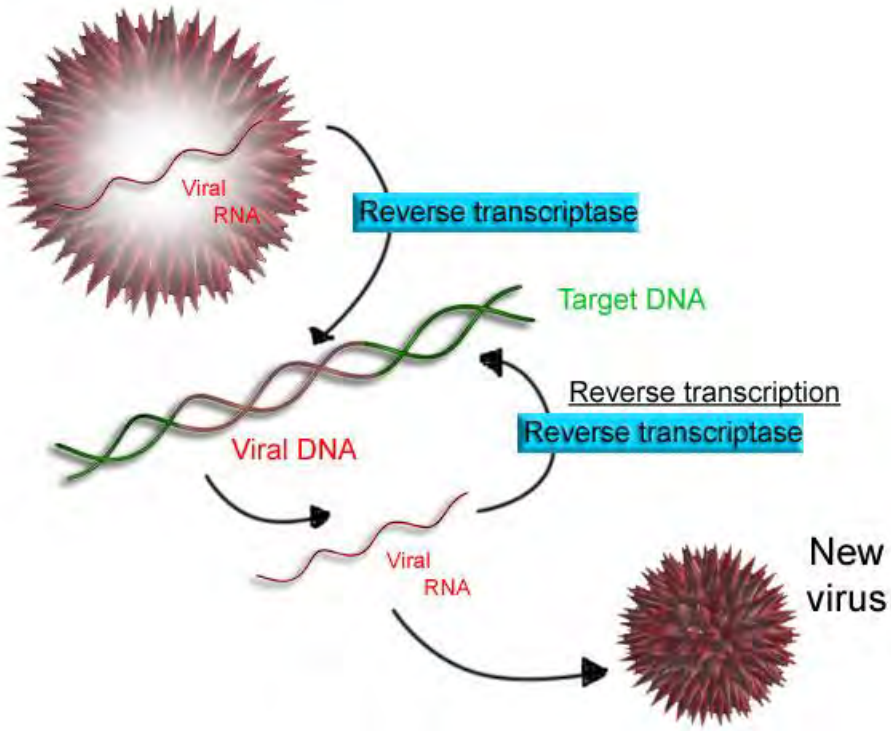
Coding of genetic information

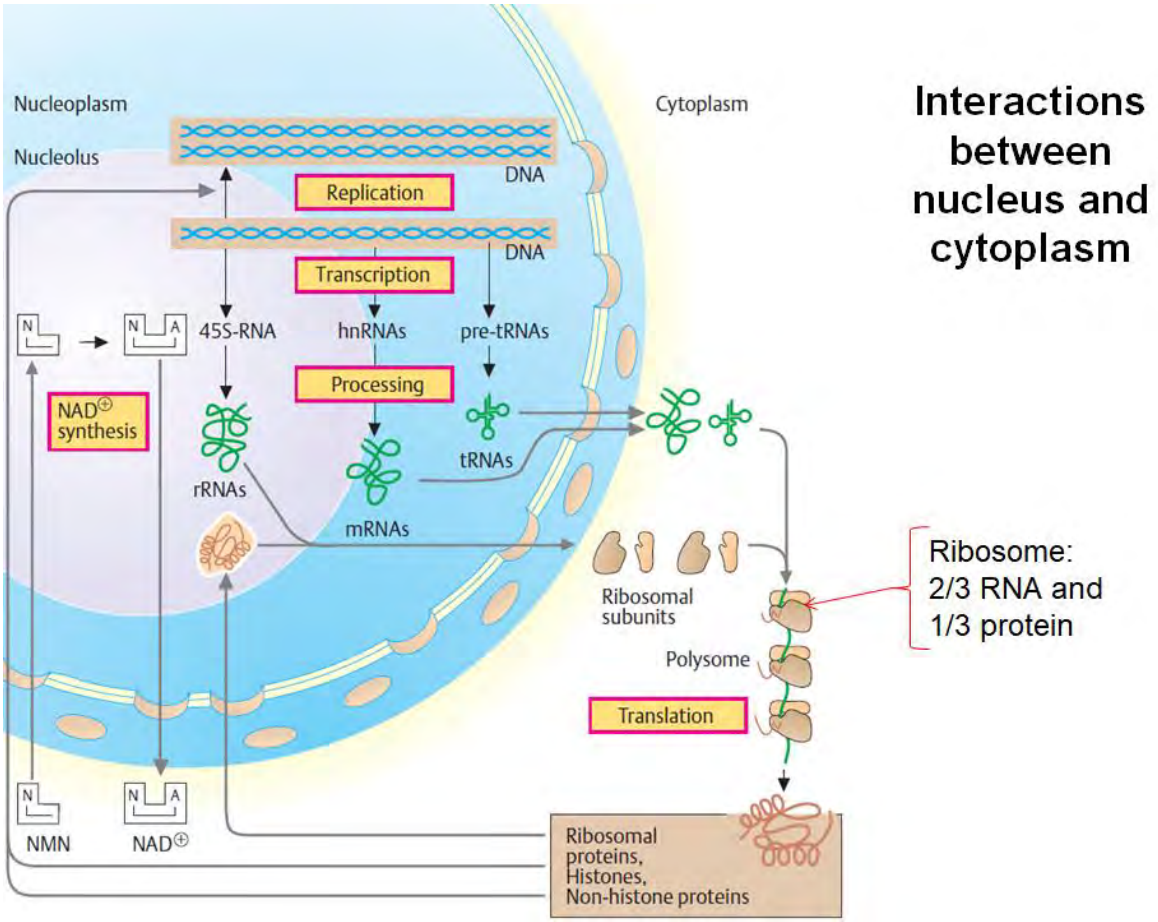


Ribonucleic acids (RNAs)



tRNA	rRNA	Type	mRNA	snRNA
>50	4	Species per cell	> 1000	~ 10
74 - 95	120 - 5000	Length (b)	400 - 6000	100 - 300
10-20%	80%	Proportion	5%	< 1%
Long	Long	Lifespan	Short	Long
Translation	Translation	Function	Translation	Splicing





Interactions between nucleus and cytoplasm

LAB-CLASS

Isolation of nucleoproteins from the liver of animals and investigation of their chemical composition

1. 1. Isolation of deoxyribonucleoprotein

The principle of the method. A characteristic feature of nuclear proteins is their ability to form very viscous solutions in concentrated salt solutions and insolubility in dilute saline solutions.

When precipitated from saline solutions, nuclear proteins fall out in the form of filaments.

Equipment and reagents. Centrifuge. Centrifuge tubes. Porcelain mortar. Liver. Quartz sand. Sodium chloride (2.0 M).

Progress of experiment. The liver of a fish weighing 2-3 g is ground in a porcelain mortar with an equal amount of quartz sand, first, add 5 ml of cooled sodium chloride solution (2.0 M). Rubbing is continued for another 10-15 minutes. in a mortar cooled with ice. The homogenate is transferred into centrifuge tubes and centrifuged for 15 minutes at 300 rpm Measure the volume of the centrifuge, pour it into six times the volume of water with a thin stream, slowly stirring the liquid with a wooden stick.

If the nucleoprotein strands are not formed, and a flaky precipitate was found, it is necessary to allow the precipitate to settle, carefully drain all the clear liquid from it, and centrifuge the residue. The precipitate after centrifugation is examined for the content of the components of the nucleoprotein.

1.2. Hydrolysis of nucleoprotein

The principle of the method. When nucleoproteins are boiled with dilute acid, they are broken down into protein and nucleic acid, which breaks down into individual mononucleotides, and then purine bases (adenine and guanine) and phosphoric acid are cleaved. The protein is partially hydrolyzed to low molecular weight peptides and amino acids. Protein (optional), purine bases, pentose, and phosphoric acid are determined in the hydrolyzate. Pyrimidine nucleotides are not hydrolyzed under these conditions.

The purpose of the work. Learn to hydrolyze the nucleoprotein.

Equipment and reagents. Flask for hydrolysis. Cork with a reflux condenser. Filter. Sulfuric acid (5%).

Progress of experiment. The nucleoprotein precipitate is transferred to a hydrolysis flask and 15 ml of sulfuric acid solution (5%) is added. The flask is capped and refluxed by hydrolysis at a low boil for one hour. After cooling, the hydrolyzate is filtered and used to analyze the hydrolysis products.

1.3. Detection of DNA in deoxyribonucleoprotein

The principle of the method. DNA nucleotides contain deoxyribose, which can be detected by reaction with diphenylamine. The interaction of deoxyribose with diphenylamine produces a product that has a blue color. Ribose and RNA with diphenylamine give a green color.

Equipment and reagents. Test tubes. Water bath. Sodium hydroxide (0.4%). Diphenylamine reagent.

Progress of experiment. A small amount of deoxyribonucleoprotein precipitate is transferred to a test tube and dissolved in 1 ml of sodium hydroxide (0.4%). Add an equal volume of diphenylamine reagent and place it in a boiling water bath for 15-20 minutes. There is a blue color.

2. Nucleic acids

Reagents: fresh or dry baker's yeast, 10% H_2SO_4 solution, 10% and 30% NaOH solutions, 1% $CuSO_4$ solution, 1% AgNO3 solution, concentrated ammonia solution, 30% NaOH solution, Fehling's reagent (I and II), 0.5% solution of phloroglucinol in concentrated HCl, molybdenum reagent (solution of ammonium molybdate in nitric acid).

At a temperature of 100 °C, in the presence of sulfuric acid, nucleoproteins are broken down (hydrolyzed) into a number of compounds (hydrolyzate): polypeptides, purine and pyrimidine bases, carbohydrates, phosphoric acid.

2.1. Test on purine bases

To 0.5 ml of the yeast hydrolyzate, add 1-2 drops of concentrated ammonia solution for neutralization and 0.5 ml of 1% AgNO3 solution. When defending, a "loose" brown precipitate falls out due to the formation of silver compounds of purine bases.

2.2. Qualitative reactions to the carbohydrate component

2.2.1. Trommer test. To 5 drops of the yeast hydrolyzate, add 5 drops of 30% NaOH solution and 2-3 drops of 1% $CuSO_4$ solution until the disappearing turbidity of $Cu(OH)_2$. When heated to boiling, a yellow precipitate of copper oxide hydrate ($Cu(OH)_2$) or a red precipitate of copper oxide (Cu_2O).

2.2.2. Felling's test. To 5 drops of the yeast hydrolyzate, add 5-7 drops of Fehling's reagent (equal volumes of Fehling-1 and Fehling-2 reagents), mix, and heat to boiling. A precipitate of $Cu(OH)_2$ or Cu_2O precipitates.

2.3. Qualitative reaction for the presence of phosphoric acid

Molybdenum test. To 0.5 ml of the yeast hydrolyzate, add 1 ml of molybdenum reagent and boil. When the test tube is cooled with running water, a lemon-yellow crystalline precipitate falls out due to the formation of ammonium phosphorus-molybdate.

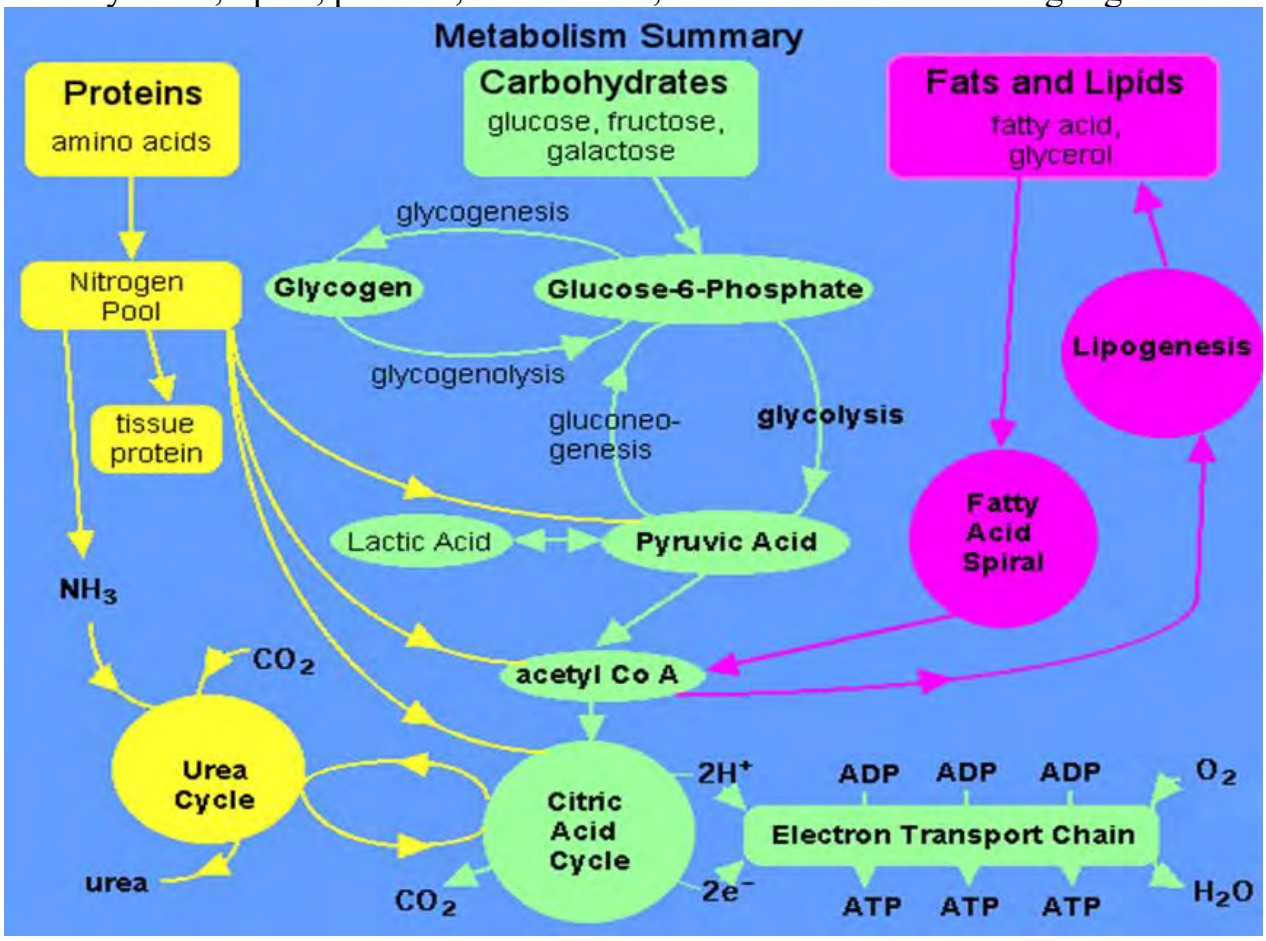
Control questions, tasks and exercises for the section «COMPONENTS OF NUCLEIC ACIDS»

1. What is the biological significance of nucleoproteins in animals and humans?
2. How the structure of DNA differs from the structure of RNA?
3. Which of the following diseases is associated with the pathology of nucleic acid metabolism? a) rickets; b) gout; c) scurvy; d) diabetes mellitus; e) osteoporosis.

4. What hormones stimulate the synthesis of nucleic acids?
5. What happens to pro-mRNA during splicing (RNA "maturation")?
6. Where are mainly purine and pyrimidine bases "neutralized" in the body of mammals?

Generalized conclusions

to chapter 2.2. “DYNAMIC AND FUNCTIONAL BIOCHEMISTRY” which is devoted to study dynamic and functional biochemistry: metabolic processes of carbohydrates, lipids, proteins, amino acids, and nucleic acids in living organisms.



Summary of metabolism of carbohydrates, lipids, proteins and nucleic acids is presented by common scheme.

1. Presented main characteristics of carbohydrates metabolism and schemes of their pathways of transformation as well as lab-class experiments of intermediates study. Shown common view on investigation of the properties of monosaccharides, disaccharides, and polysaccharides as well as lab experiments of glycogen isolation, its hydrolysis, and lab-determination of glycogen content in tissues.
2. Characterized biological oxidation of substances and their energy exchange and presented schemes the Kreb’s cycle, respiration chain, the

other ones devoted to energy exchange as well as lab-experiments detection and quantitative determinaton of lactic acids.

3. Given main characteristics of lipids metabolism and schemes of their pathways of transformation as well as lab-class experiments of intermediates study, especially fat detection, determination of iodine and acid numbers, and qualitative bile acids determination
4. Presented characteristics of protein exchange intermediates (I - mainly protein) and schemes of their pathways of transformation as well as lab-class experiments of intermediates study.
5. Presented characteristics of protein exchange intermediates (II - mainly amino acids) and schemes of their pathways of transformation as well as lab-class experiments of intermediates study.
6. Presented main characteristics of nucleic acids metabolism and schemes of their pathways of transformation as well as lab-class experiments of intermediates study.

Chapter 2.3.
METABOLISM AND ITS REFLECTION IN THE BIOCHEMICAL PARAMETERS

The purpose of this section of the course "Biochemistry of animals with the Basics of Physical and Colloid Chemistry" is to provide students with theoretical knowledge of dynamic and functional biochemistry, and some experiments in lab classes.

The following topics will be considered in this section:

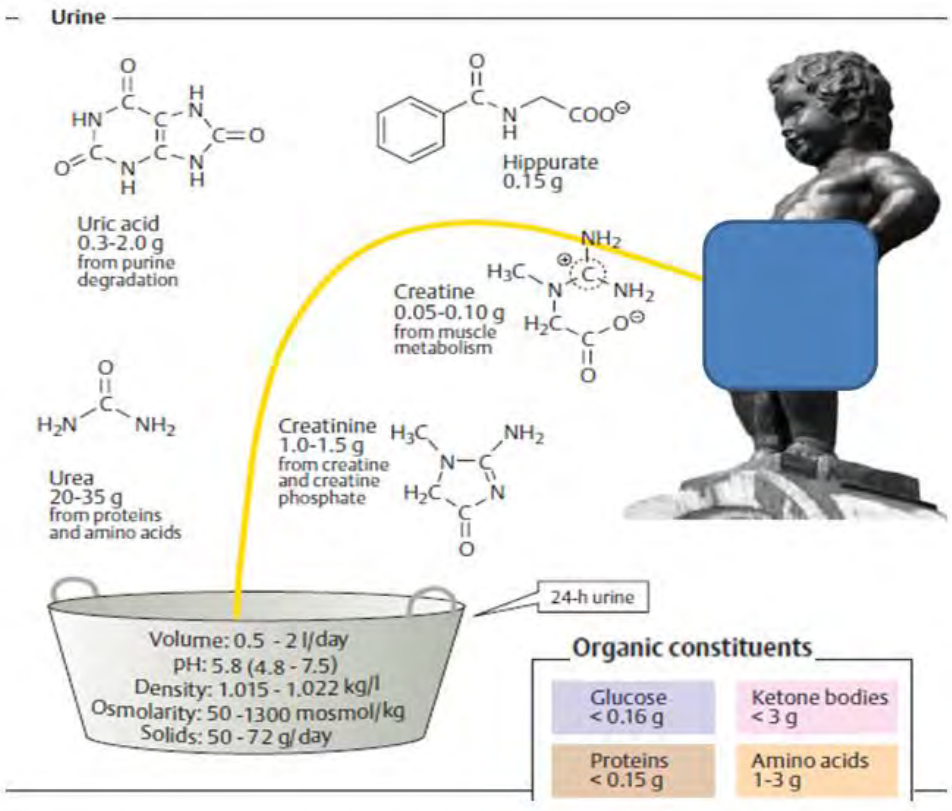
Topic 1. Certain biochemical indicators of biological liquids

2.3.1.1. Certain biochemical indicators of biological liquids. I. Biochemical indicators of urine

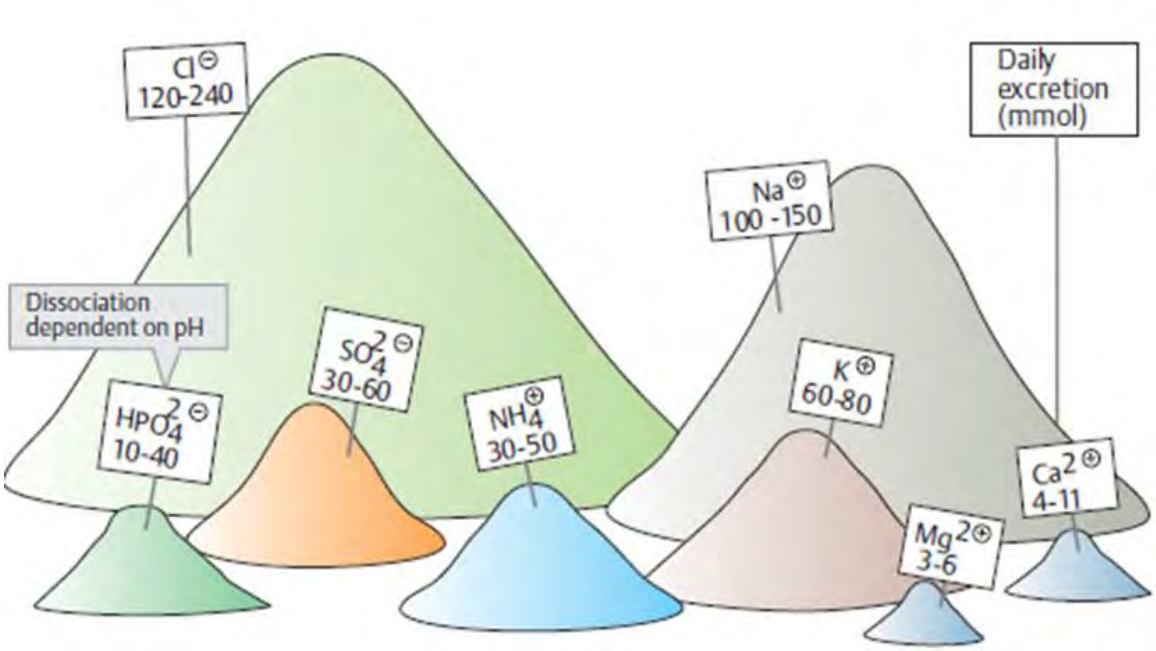
2.3.1.2. Certain biochemical indicators of biological liquids. II. Biochemical indicators of milk

2.3.1. ***Topic*** CERTAIN BIOCHEMICAL INDICATORS OF BIOLOGICAL LIQUIDS

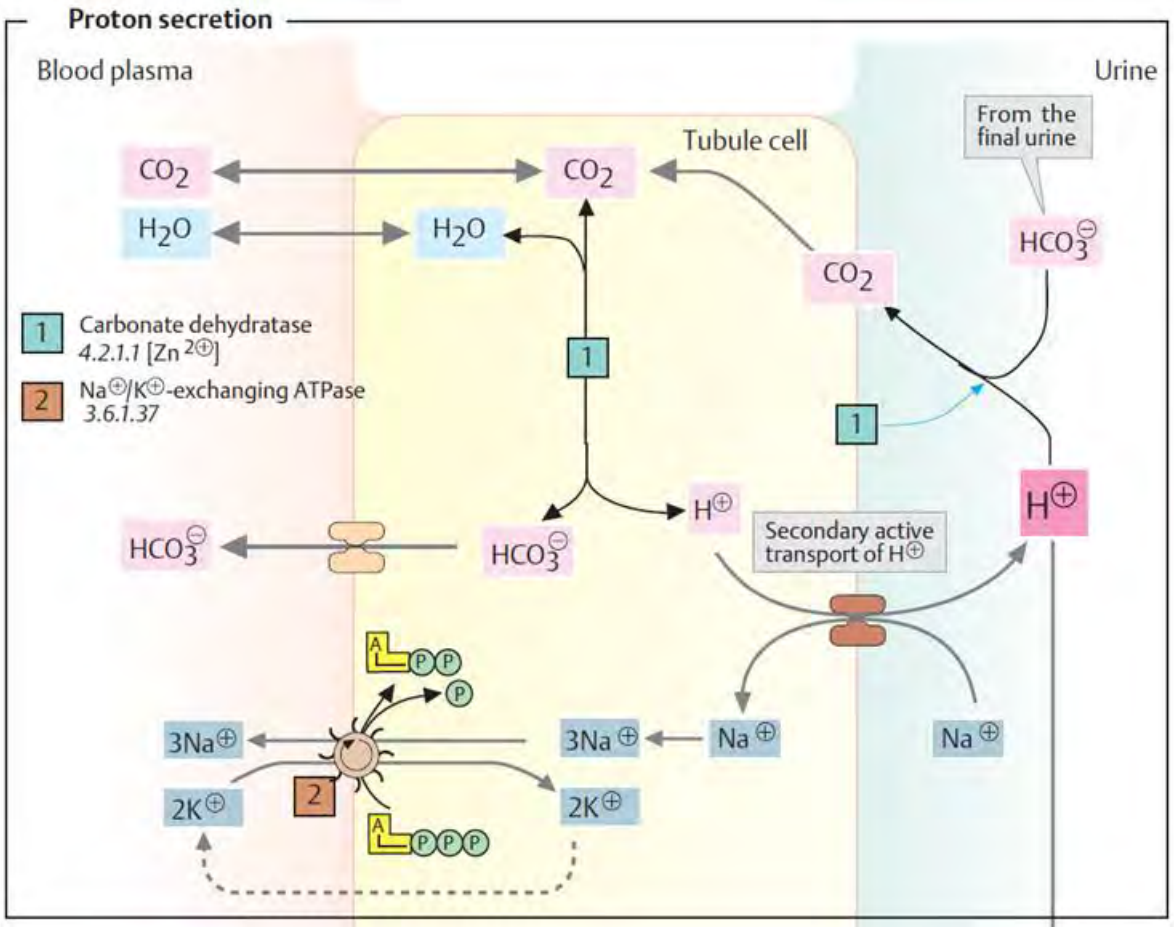
I BIOCHEMICAL INDICATORS OF URINE



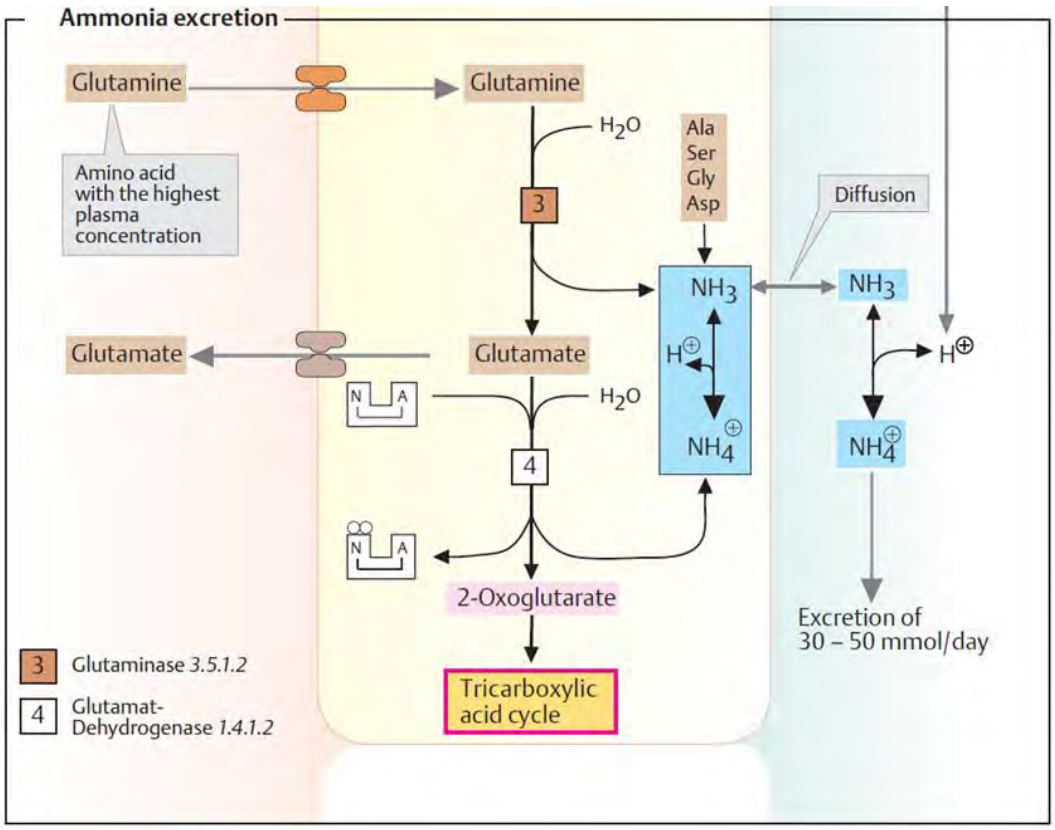
Organic components of urine



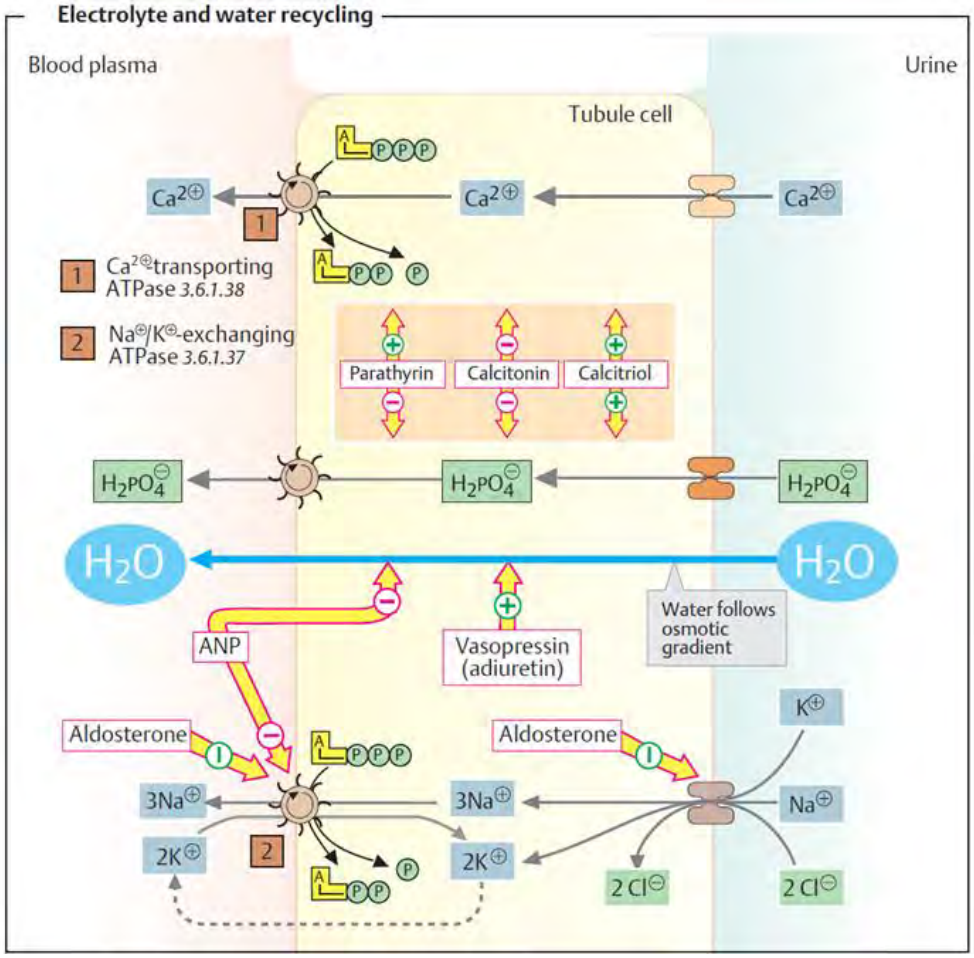
Inorganic components of urine

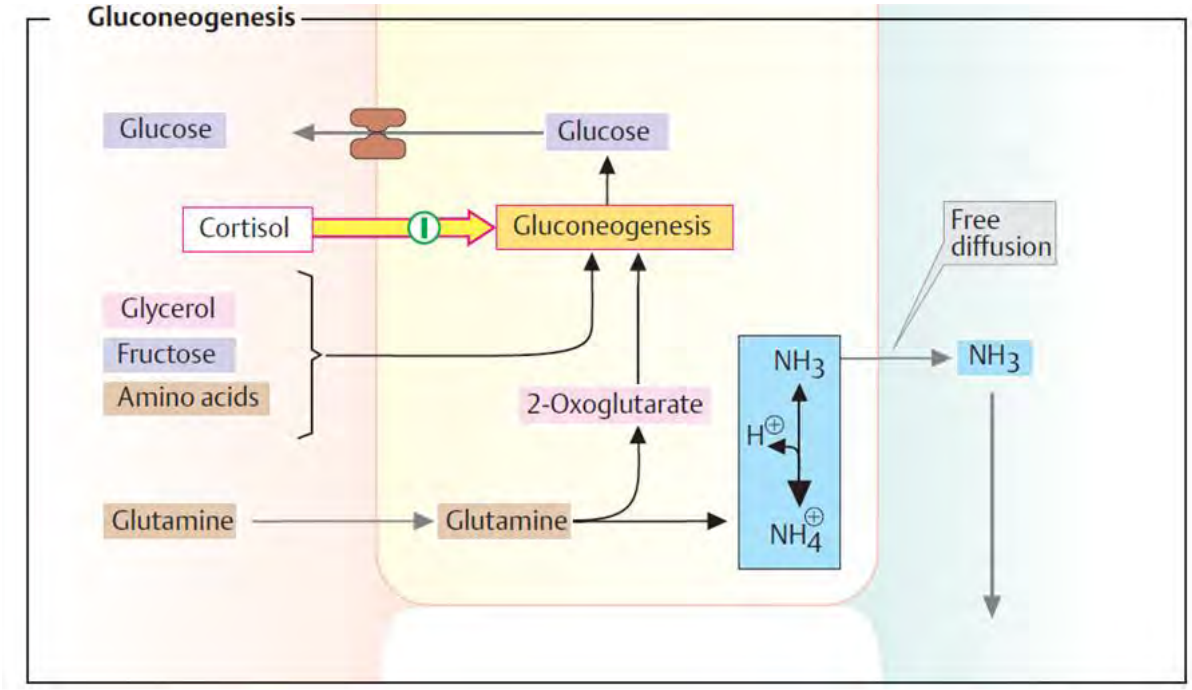


Proton secretion and ammonia excretion are in presented objects (blood plasma, tubule cell, urine)



Electrolyte and water recycling are in biological objects (blood plasma, tubule cell, urine)

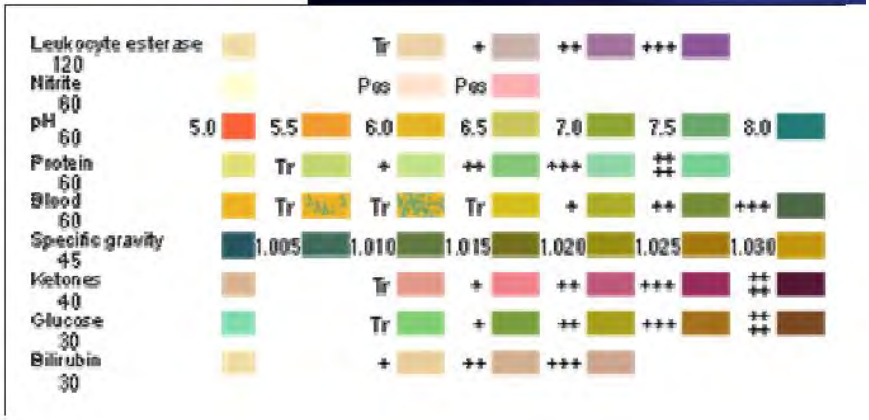




Gluconeogenesis is in biological objects (blood plasma, tubule cell, urine)

Dipstick analysis

Dipstick analysis is an easy an convenient method for the detection of leukocytes, nitrite, protein, blood, ketone bodies, glucose and bilirubin in urine and for the determination of urine pH and specific gravity. A "**dipstick**" is a paper strip with patches impregnated with chemicals that undergo a color change when certain constituents of urine are present in a certain concentration. The strip is dipped into the urine sample, and after the appropriate number of seconds, the color change is compared to a standard chart to determine urine constituents.



Dipstick analysis of urine

LAB-CLASS

1. Determination of color, transparency, odor, specific gravity, pH, acidity and alkalinity of urine

The principle of the method. Determination of color, transparency, odor, specific gravity, and pH of urine is carried out taking into account these indicators in healthy animals of different species during normal feeding and maintenance.

Reagents: animal urine, litmus paper, potassium oxalate (powder), 0.1 N KOH solution or NaOH; 0.1 N HCl solution, 1% alcohol solution of phenolphthalein, 1% sodium alizarin acid solution, universal paper indicator.

Equipment: cylinders of 250 ml, urometers, thermometers, beakers (V = 100 ml), pH meter.

Progress of experiment. 150 - 200 ml of urine is poured into the cylinder. Give to defend. Remains of foam are removed with filter paper. Write in a notebook the nature of urine color, transparency, the odor of urine. Determine the temperature of urine with a thermometer, and then lower the urometer into the cylinder. When the urometer stops at a certain height, the meniscus is determined by the specific gravity and the temperature is adjusted.

For every 3 ° C of urine temperature above 15 °C, one unit must be added to the last figure of specific gravity, and for temperatures below 15 °C, one unit must be subtracted for every 3 ° difference in urine temperature.

The urine reaction can be determined using blue or red litmus paper or using a universal indicator. More precisely, the pH of urine is determined using a pH meter.

The specific weight of urine in different species of animals

Horse – 1.025 – 1.060, Cattle – 1.025 – 1.050, Small cattle – 1.020 – 1.070, pig – 1.010 – 1.040.

2. Quantitative determination of urine acidity

Progress of experiment. Pour 25 ml of urine into a beaker (V = 100 ml), add 15-20 g of potassium oxalate, shake well and titrate with 0.1 N KOH or NaOH solution to a pale pink color in the presence of phenolphthalein indicator (2-3 drops).

To determine the acidity of urine in units of hydrochloric acid, the number of milliliters of KOH or NaOH that went to the titration - multiplied by 0.00365 - is the amount of HCl in grams, which corresponds to 1.0 ml of 0.1 N solution of KOH or NaOH.

3. Qualitative reactions to creatinine

Reagents: saturated picric acid solution (12 g / 1 l), 10% sodium hydroxide solution, 3% sodium nitroprusside solution, 5% acetic acid solution, urine.

Equipment: test tubes, pipettes, tripods.

a) Reaction with picric acid

Progress of experiment. Pour 2 ml of urine into a test tube and add 5-6 drops of 10% sodium hydroxide solution, add 3-4 drops of picric acid. The contents of the test tube are colored orange.

b) Reaction with sodium nitroprusside

Progress of experiment. Pour 2 ml of urine into a test tube, alkalize with 5-6 drops of sodium hydroxide and add 3 drops of sodium nitroprusside. The liquid in the test tube turns red, which gradually turns yellow. When it's acidified with acetic acid, the transition from red to yellow is significantly accelerated. This distinguishes the reaction for creatinine from the reaction for acetone. If you add acetic acid, in the presence of acetone in the urine, the red color turns to cherry, not yellow.

4. Determination of inorganic components of normal urine

4.1 Determination of chlorides

The principle of the method. Chlorides in urine are determined by the Mohr method, which is based on the precipitation of chlorides with a titrated solution of silver nitrate ($AgNO_3$) in the presence of an indicator of potassium chromic acid (K_2CrO_4). If there is a protein in the urine, it must be precipitated before the experiment.

Reagents: 5% solution of potassium chromate, 0.01 n solution of Argentum nitrate (1.6989 g of chemically pure Argentum nitrate is dissolved in a volumetric flask of 1.0 liter in double-distilled water). The titer is determined by 0.01 N sodium chloride solution. A 5% solution of potassium dichromate or sodium dichromate (K_2CrO_4 or Na_2CrO_4) is used as an indicator. 1.0 ml of Argentum nitrate precipitates 0.01 g of sodium chloride.

Equipment: burettes, conical flasks for titration, pipettes for 2-10-50 ml; measuring flasks per 100 ml; funnels with filters, dry flasks.

Progress of experiment. Pour 2.0 ml of test urine into the flask, add 1-2 drops of 5% potassium dichromate solution and titrate from the burette with a titrated solution of Argentum nitrate until a pale pink color appears (Ag_2CrO_4).

The amount of sodium chloride (in grams) in the daily amount of urine is equal to:

$$\frac{0{,}01 \cdot a}{в} \cdot V,$$

where:

a - the number of milliliters of titrated $AgNO_3$ solution;

в - the amount of urine taken for analysis;

4.2. Determination of sulfates

The principle of the method. To determine the sulfates and ether sulfuric acids of urine use the method of precipitation of sulfuric acid in the form of insoluble barium sulfate.

$$Na_2SO_4 + BaCl_2 \longrightarrow 2\ NaCl + BaSO_4$$

To release sulfuric acid from ether sulfuric acids, they are hydrolyzed by heating with hydrochloric acid. If there is protein in the urine, it is pre-precipitated by boiling with acetic acid.

Reagents: 10% acetic acid solution, 5% barium chloride solution, 10% hydrochloric acid solution, urine.

Equipment: test tubes, pipettes for 2 - 5 ml, funnels, filters, water baths.

Progress of experiment. Pour 5 ml of urine into a test tube and add 2-3 drops of 1% acetic acid solution. Then add 5 ml of 5% barium chloride solution. In the presence of sulfates precipitates barium sulfate ($BaSO_4$).

The precipitate is filtered off. To the filtrate, add 3 ml of 10% hydrochloric acid solution. The tube with the reaction mixture is boiled for 15 minutes in a water bath and gradually cooled under running cold water. After cooling the tubes, observe the formation of a precipitate of barium sulfate due to the hydrolysis of sulfuric acid ethers.

4.3. Determination of phosphates

The principle of the method. To determine phosphates use their ability to form with ammonium molybdate in the nitric acid medium when heated, a yellow sparingly soluble complex compound - ammonium phosphomolybdate - $(NH_4)_3PO_4 \cdot 12MoO_3$.

Reagents: 5% nitric acid solution, molybdenum reagent (7.5 g of ammonium molybdate) is dissolved in 100 ml of water and adjusted with 100 ml of 32% nitric acid solution (specific gravity 1.2). Complete dissolution of ammonium molybdate occurs after the addition of nitric acid.

Equipment: test tubes, tripods, pipettes for 2-5 ml, funnels, filters, glass alcohol burners.

Progress of experiment. Pour 1 ml of molybdenum reagent into a test tube, heat to boiling, add 2 drops of urine and observe the formation of a yellow crystalline precipitate.

The precipitate is insoluble in nitric acid, but soluble in NH_4OH.

5. Determination of pathological components of urine

Reagents: urine, concentrated HNO_3, concentrated H_2SO_4, concentrated CH_3COOH, saturated NaCl solution, 10% CH_3COOH solution, 10% NaOH solution, 1% CuSO4 solution, 10% of sugar solution, 10% of sodium nitroprusside solution, concentrated ammonia solution, Lugol's solution (2.0 g of potassium iodide + 1 g of crystalline iodine, mix well in 10 ml of water and adjusted to 300 ml with distilled water).

Equipment: tripods, test tubes, pipettes, funnels, filters, glass alcohol burners.

5.1. Tests to protein

a) Pour 3 ml of urine into a test tube, add 3 drops of 10% CH_3COOH solution, and 1 ml of saturated NaCl solution. When boiled, flakes of protein appear.

b) Pour 1 ml of concentrated nitric acid into a test tube and pipette 1 - 2 ml of filtered urine with a pipette. In the presence of protein, a protein ring appears at the boundary between the two liquids.

c) Pour 3 ml of urine into a test tube, add 0.5 ml of 10% NaOH solution, and heat to boiling. Boiling phosphates capture hemoglobin and the precipitate turns red.

5.2. Sugar test

To 3 ml of urine add 2 drops of 1% $CuSO_4$ solution and 0.5 ml of 10% NaOH solution, heat to boiling. In the presence of sugar, a red precipitate of Cu_2O falls out.

5.3. Tests for bile pigments

a) Pour 1 ml of concentrated HNO_3 into a test tube and carefully layer 1 ml of urine on the wall. On the border of two liquids, appear rings: green, blue, purple.

b) Pour 10 ml of urine into a test tube and add 2-3 ml of 10% sugar solution and shake until foaming. Concentrated H_2SO_4 is then added dropwise. In the presence of bile acids, the foam turns purple-red.

5.4. Tests on ketone bodies

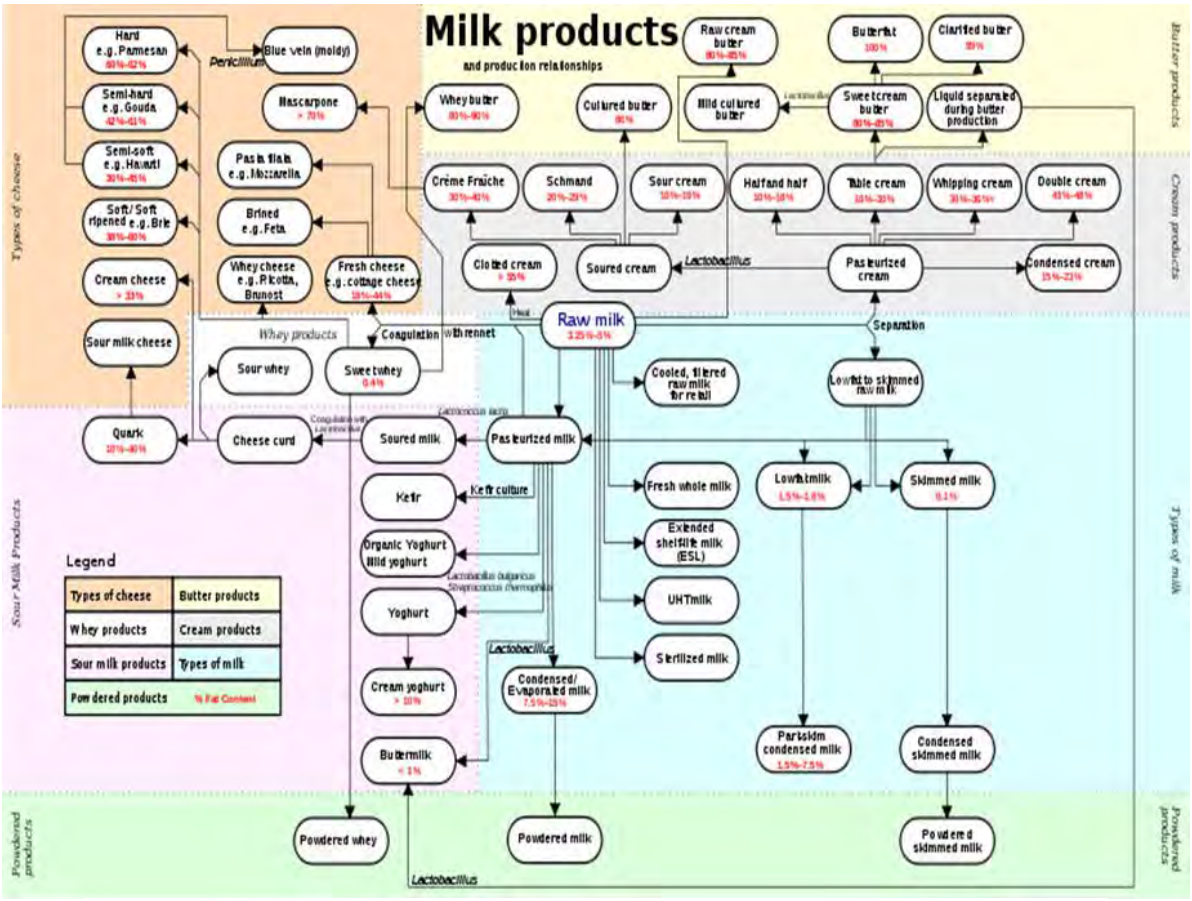
a) Pour 5 ml of urine into a test tube, add a few drops of Lugol's solution, and a few drops of 10% NaOH solution. In the presence of acetone, a yellow precipitate of iodoform is formed, which has a specific odor.

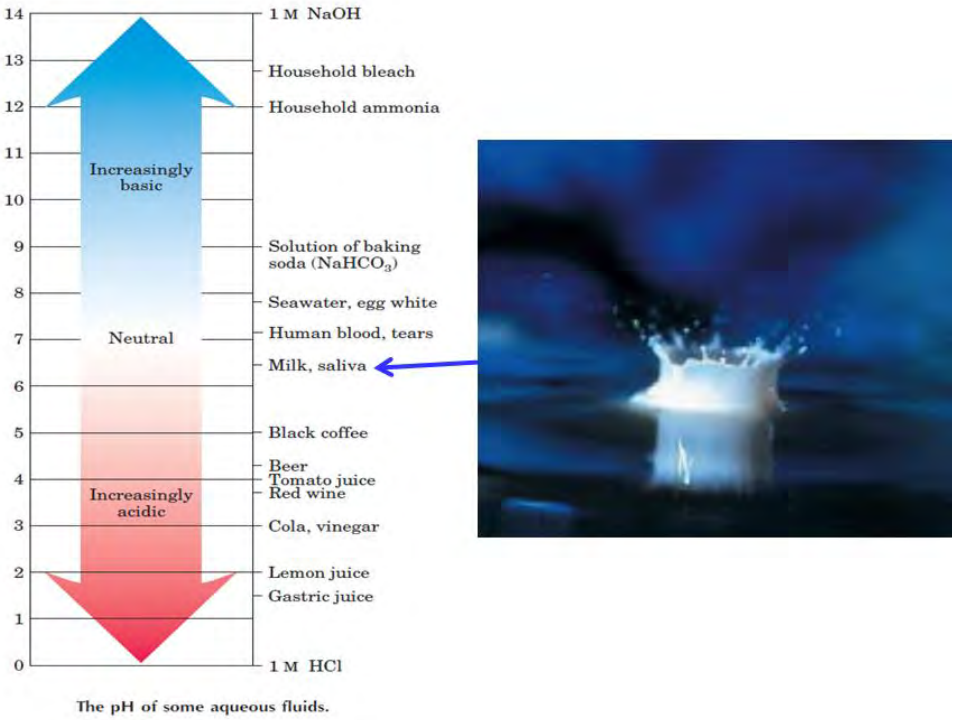
b) Pour 5 ml of urine into a test tube, add 1 ml of concentrated CH3COOH, and 0.5 ml of freshly prepared 10% sodium nitroprusside solution. The tube is shaken and then carefully layered on the wall with 1 ml of concentrated ammonia solution. In the presence of acetone, a purple ring is formed.

Control questions, tasks and exercises for the section «CERTAIN BIOCHEMICAL INDICATORS OF BIOLOGICAL LIQUIDS. (I) BIOCHEMICAL INDICATORS OF URINE»

1. What is the daily amount of urine excretion by different species of animals?
2. What is the chemistry of urine formation?
3. What are the main indicators of physicochemical properties of urine and their clinical significance?
4. Specific component of poultry urine is a) hyaluronic acid; b) uric acid; c) glutamic acid; d) ornithuric acid; e) hippuric acid; f) phenaceturic acid
5. The appearance of ketone bodies in the urine is called: a) anuria; b) oliguria; c) polyuria; d) ketonuria?
6. Which compound in the urine increases with gout? a) allantoin; b) glucose; c) uric acid; d) creatine
   Explain the choice of answer.

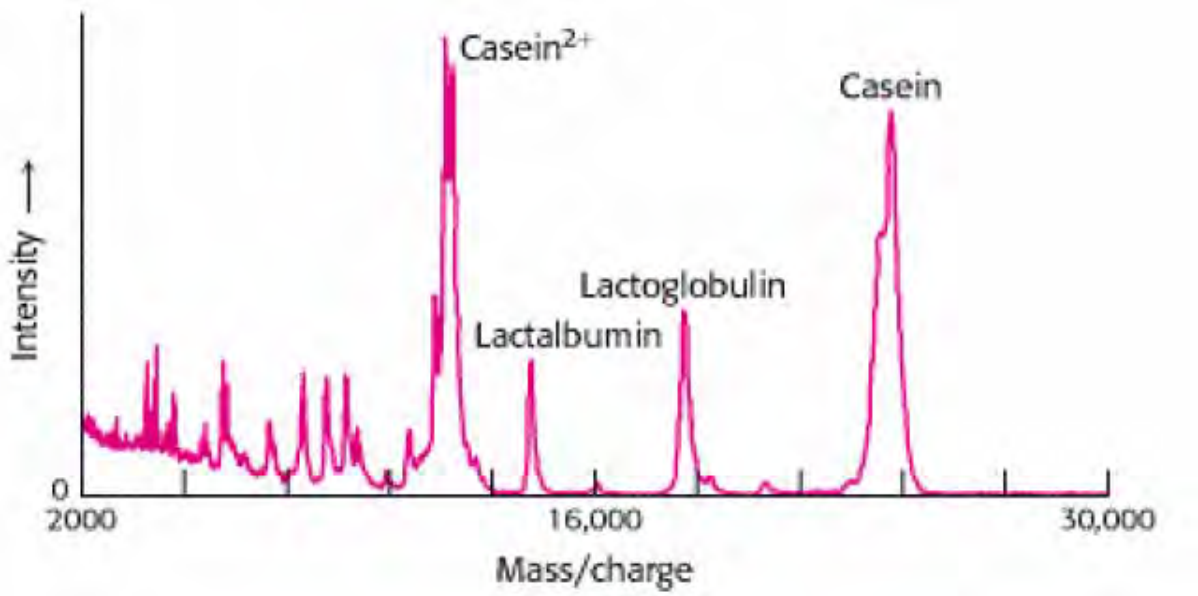
2.3.2. ***Topic*** CERTAIN BIOCHEMICAL INDICATORS OF BIOLOGICAL LIQUIDS
II BIOCHEMICAL INDICATORS OF MILK



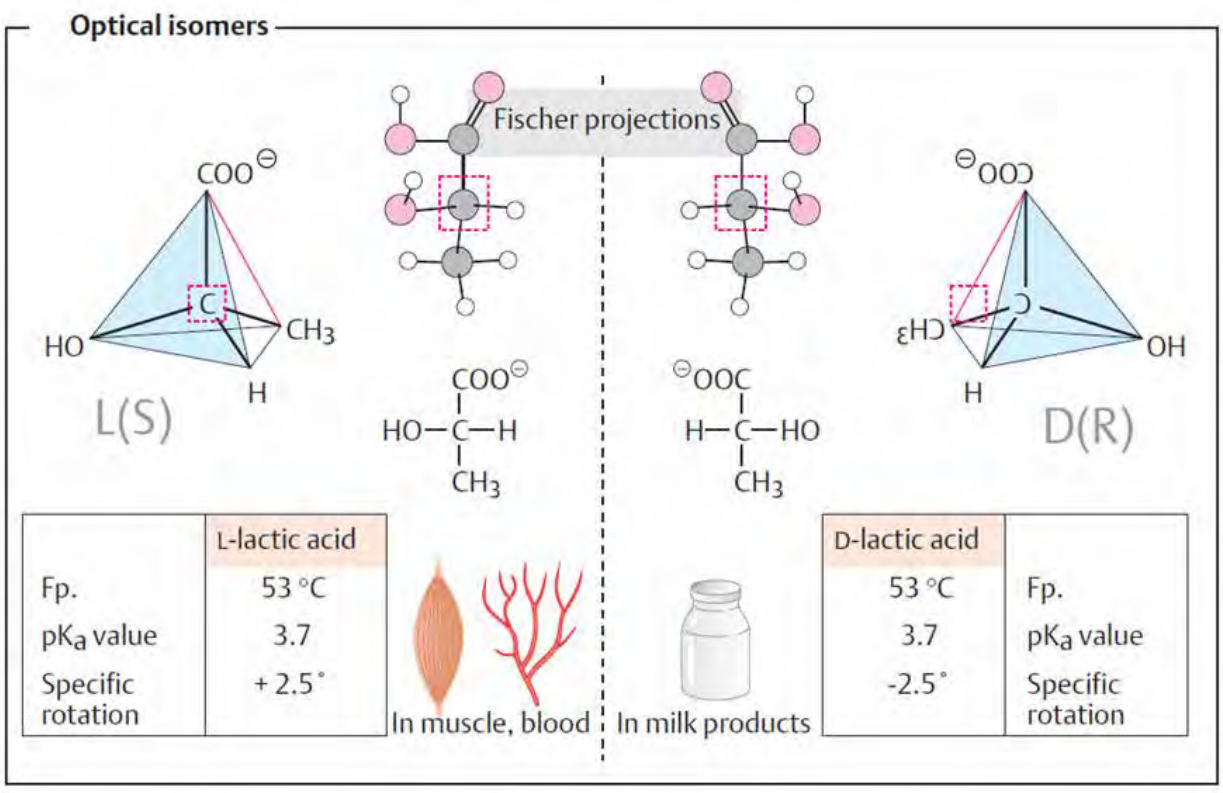


The pH of some aqueous fluids.

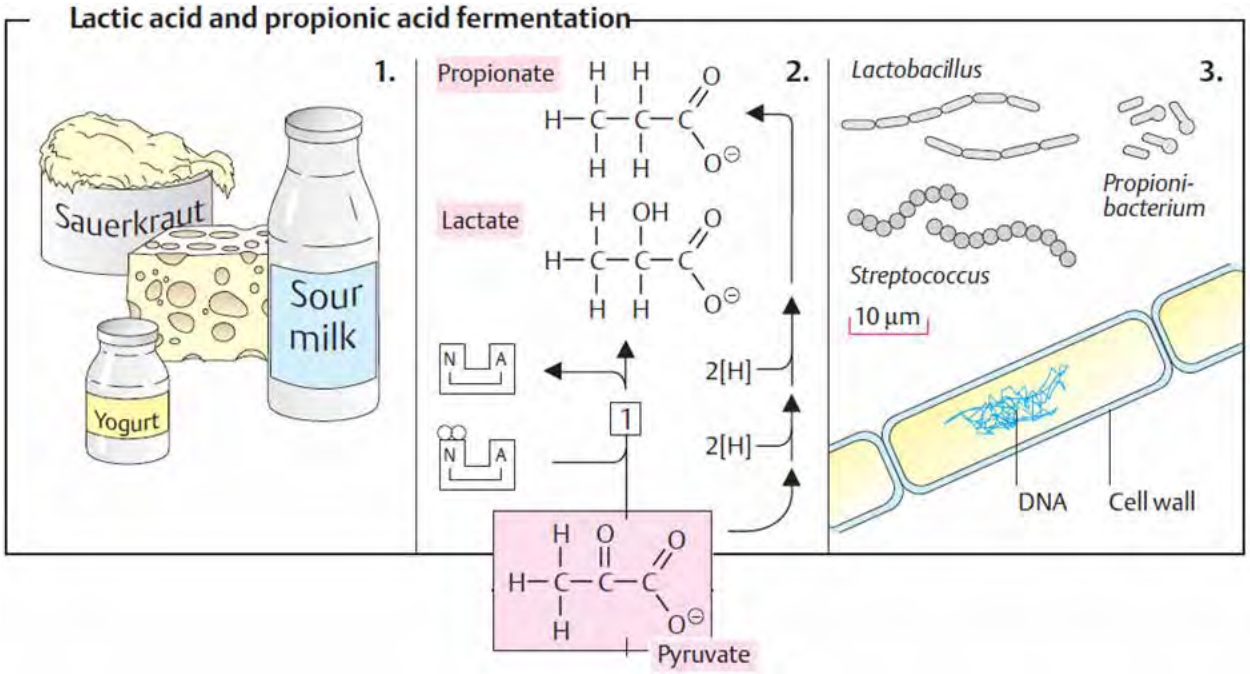
Milk products and the pH of some aqueous fluids



The protein components of milk are revealed by the technique of MALDI-TOF mass spectrometry, which separates molecules based on their mass to charge ratio.



Optical isomers of lactic acid in muscle, blood, and milk products



Lactic acid and propionic acid fermentation

LAB-CLASS

1. Isolation whey milk/colostrum

The procedure for obtaining whey or colostrum requires:

a) separation of fat; b) coagulation of casein; c) precipitation of the casein.

Progress of experiment. Milk (or colostrum) is poured into centrifuge tubes and placed for 6-8 hours at a temperature of 4-10 ° C and then centrifuged for 15-20 minutes at 2000-3000 rpm. Carefully remove the crust of fat with a spatula.

Caseinogen is separated from milk in the form of casein when exposed to milk by acids (acetic, hydrochloric). To 5 ml of skim milk in the previous procedure dropwise and with constant stirring add 2% acetic acid solution. White flakes of casein, insoluble in water, fall out. Completion of caseinogen coagulation is indicated by the absence of flake formation with the addition of acetic acid.

By centrifugation for 15 minutes at 3000 rpm, precipitate casein, select the top layer of serum, which is neutralized with 0.1N NaOH solution to pH 7.0, fixing the pH value with a pH meter. When calculating the biochemical parameters of milk (or colostrum), take into account the degree of dilution, which was at the stage of coagulation or neutralization.

2. Precipitation and isolation of casein

Progress of experiment. Dilute 25-30 ml of milk in a glass or flask with 3-4 volumes of water and add to the solution dropwise (with stirring) 0.1% acetic acid solution to stop the loss of white precipitate of casein, which also captures fats. Add acid very carefully, because with its excess casein dissolves easily. Filter the precipitate, rinse thoroughly on the filter 2-3 times with water.

3. Determination of milk acidity

Fresh milk binds a small amount of alkali. It depends on the presence of proteins and monosubstituted phosphoric acid salts, which exhibit weakly acidic properties.

Determining the acidity of milk is of great practical importance for assessing its freshness.

During storage of milk, lactic acid fermentation takes place in it, as a result of which lactic acid accumulates.

The principle of the method. The acidity of milk is determined in degrees Turner (° T). Turner degrees show the number of milliliters of decinormal (0.1 n) alkali solution that neutralizes 100 ml of milk, fixing the color change of phenolphthalein.

Reagents: 0.1N sodium hydroxide solution, phenolphthalein, milk.

Equipment: 100 ml flasks, 10 ml and 20.0 ml pipettes, burettes.

Progress of experiment. Pour 10 ml of milk into the flask (V = 100 ml), then 20.0 ml of distilled water, and 2-3 drops of phenolphthalein. From the burette add dropwise, stirring constantly, 0.1 N sodium hydroxide solution until a pale pink color that does not disappear within one minute. To calculate the number of ml of alkali that went to neutralize 100 ml of milk, the obtained data are multiplied by 10.

The acidity of fresh milk ranges from 15 to 16 ° T, at 24-27 ° T the milk changes in taste and smell, it coagulates when boiled. Milk self-coagulation occurs at 60 - 65 ° T.

4. Qualitative analysis of the chemical composition of milk

Reagents: milk, 2% acetic acid solution, 1% copper sulfate solution, 10% sodium hydroxide solution, 1% sodium carbonate solution, biuret reagent, saturated ammonium sulfate solution, 2N nitric acid solution, 1% Argentum nitrate solution, 2 n acid chloride solution, 5% barium chloride solution, 2.5% ammonium molybdate solution (2.5 ammonium molybdate is dissolved in 50 ml of 5 N sulfuric acid solution and adjust to 100 ml with distilled water).

Equipment: test tubes, tripods, funnels, filters, alcohol or gas burners.

Progress of experiment.

Determination of casein. To 2 ml of milk in a test tube diluted with water 1: 4, add dropwise 2% acetic acid solution until a white precipitate.

The contents of the tubes are filtered and the precipitate on the filter is washed 2-3 times with distilled water.

To the washed casein on the filter, add 2-3 drops of 1% $CuSO_4$ solution and a few drops of 10% NaOH solution. A purple color appears (biuret reaction). The casein content in cow's milk, on average, is about 2.8%.

Determination of albumins and globulins. The filtrate remaining after precipitation of casein, acidic from the addition of acetic acid used to precipitate casein, is heated to boiling, and a 1% solution of Na_2CO_3 is added dropwise to the boiling mixture until slightly acidic, checking with litmus paper. Albumins and globulins precipitate. The liquid is filtered, washed and the biuret reaction is carried out with the precipitate (see the task of determining casein).

The content of albumin and globulins in cow's milk, on average, about 0.7%.

Determination of lactose. Take 1-2 ml of the 2nd filtrate remaining after the release of albumin and globulins, add 2-3 drops of 1% solution of $CuSO_4$, and a few drops of 10% NaOH. The mixture is heated. A red precipitate of Cu_2O precipitates. The sugar content in the milk of cows averages 4.6%.

Determination of calcium. Take 0.5 ml of the 2nd milk filtrate and acidify it with a 2% solution of acetic acid. Then, 5-6 drops of saturated ammonium sulfate solution are added to the test tube. Calcium precipitates. The calcium content in the milk of cows is on average 0.12%.

Determination of chlorides. Take 0.5 ml of the 2nd milk filtrate and acidify with 2 N HNO_3 solution. Then add a few drops of 1% $AgNO_3$ solution to the contained tube. Chloride precipitates. The chloride content in cow's milk is, on average, about 0.11%.

Determination of phosphates. To 0.5 ml of the 2nd milk-filtrate add 2N HNO_3 solution to a slightly acidic reaction. An equal volume of 2.5% ammonium molybdate solution is then added. A yellow precipitate of phosphates falls out. The content of phosphates in the milk of cows is, on average, about 0.10%.

Determination of sulfates. Take 0.5 ml of the 2nd milk filtrate and acidify with 2 N HCl solution, and then add a few drops of 5% BaCl2 solution. A precipitate of sulfates precipitates. The content of sulfates in the milk of cows is, on average, 0.18 g / l.

Determination of milk oxidoreductase. Milk oxidoreductase is determined by the Chardinger reaction. Formaldehyde or acetaldehyde and a few drops of methylene blue solution are added to the milk. Leave in a thermostat or water bath at a temperature of 37-38 ° C. Discoloration due to aldehyde oxidation by reduction of methylene blue to a colorless compound is observed. This reaction is used to distinguish raw milk from boiled milk because when boiled, oxidoreductase (the enzyme Chardinger - lactate oxidoreductase) is destroyed.

Control questions, tasks and exercises for the section «CERTAIN BIOCHEMICAL INDICATORS OF BIOLOGICAL LIQUIDS. (II) BIOCHEMICAL INDICATORS OF MILK**»**

1. What is the chemical composition of milk in different species of animals?
2. Colostrum and its physical and chemical, biological properties, compared with milk?
3. How and where are the main chemical components of cow's milk synthesized?
4. What is the importance of colostral immunity?
5. In the formation of the shell of fat globules involved: a) L - lactalbumin; b) L - casein; c) lactose; d) phospholipids; e) caseinate-calcium phosphate complex?
6. The main milk protein is a) α-casein; b) α-lactalbumin, c) heparin, d) immunoglobulins?

Generalized conclusions

to chapter 2.3. “METABOLISM ANDITS REFLECTION IN THE BIOCHEMICAL PARAMETERS” which is devoted to study biochemical indicators of metabolic processes in living organisms.

1. Characterized certain biochemical indicators of biological liquids. I. Biochemical indicators of urine theoretically and experimentally.
2. Characterized certain biochemical indicators of biological liquids. I. Biochemical indicators of milk theoretically and experimentally.

Additions

BRIEF INSTRUCTIONS OF THE RULES OF WORK AND SAFETY TECHNIQUES IN THE BIOCHEMICAL LABORATORY

To safely perform laboratory work, the following rules should be followed:

- Before conducting laboratory work, students are required to theoretically prepare for the topic using the recommended literature.
- A student can work in the laboratory only in the presence of a teacher or laboratory assistant.
- In the laboratory, students should observe silence, cleanliness, the order of placement and arrangement of equipment, apparatus, and reagents. It is forbidden to take reagents out of the room, to transfer them from under a fume cupboard.
- All experiments with toxic substances or volatile compounds with an unpleasant odor should be carried out in a fume hood.
- When heating test tubes, the open part of the test tube should be directed away from yourself and the people around you. The test tube must be held with a special holder.
- Do not cover the tube with your finger while mixing the liquid.
- When working with concentrated acids, they should be poured into the water in small portions. Protective goggles and rubber gloves should be worn when working with concentrated acid and alkali solutions.
- Do not taste the reagents, do not pipette an unknown compound into your mouth, as it may be poisonous. The emitted gases should be studied from a distance, slightly directing the flow of air from the test tube to yourself. Avoid the deep breathing of gases or vapors.
- Reagents should be stored in a closed container with a label indicating the name, formula, and concentration of the substance.
- It is forbidden to pour the remains of solutions containing concentrated acids into the washbasin sinks. They should be poured into special containers located in the fume cupboard or next to the sink.
- Work with substances that irritate the respiratory system or have a strong odor, which should be under a fume cupboard.
- Bulk reagents should be collected with special spoons or a spatula.
- During working in the laboratory, it is strictly forbidden to leave working units and electric heaters unattended.
- Do not throw used filters, paper, cotton wool, broken test tubes, or broken glass into the washbasin.
- In the case of a fire, disconnect the circuit breakers immediately, remove flammable substances to a safe place, extinguish the flame with a fire extinguisher, or it cover with sand or asbestos.
- When leaving the laboratory, you should be sure to turn off the electric heaters, and close the water tap, and the fume cupboard.

PECULIARITIES OF SAMPLING OF DIFFERENT BIO OBJECTS FOR BIOCHEMICAL RESEARCH

The natural habitat of living organisms does an imprint on their anatomy, physiology, and biochemistry. Chemical processes that occur in different organisms are adapted to their stay in the appropriate environment. For example, unlike mammals, fish are cold-blooded organisms and their metabolism depends on the ambient temperature. In fish, the anatomical heterogeneity of the chemical composition is very clear.

The main objects of biochemical studies are various tissues, biological fluids (blood, plasma, serum, lymph, rarely - other body fluids: cerebrospinal fluid, intra-articular fluid, etc.,) and excrements: urine, bile, saliva, gastric and intestinal juice, feces, sweat, milk, semen.

It is recommended to take blood in the morning before feeding and before exercising. Blood tests are performed after 8 to 12 hours of fasting and for the determination of triacylglycerols, it is necessary to maintain a 10 to 12-hour interval after eating.

The state of the body affects the concentration of total protein, albumin, creatine, cholesterol, triacylglycerols, the activity of alkaline phosphatase, aspartate aminotransferase and other components of plasma. The content of these substances and the activity of enzymes significantly increase when changing the position of the body from horizontal to vertical and, conversely, decrease - in the horizontal. The maximum change is characteristic of the level of total protein, enzyme activity (11%) and calcium content (3 - 4%).

When taking blood by venipuncture, the time of compression of the vessels by the tourniquet should be kept to a minimum, as compression of the vessel causes stasis and hypoxia, as well as a shift in the distribution of certain substances (cholesterol, potassium, sodium, calcium, etc.) between blood cells and its liquid part.

To prevent hemolysis, blood should be taken with a dry syringe, dry needle (disposable), in a dry test tube and under sterile conditions. If the blood collected by the syringe is transferred to a test tube, this procedure is performed slowly to prevent foaming of the blood.

Hemolyzed serum and plasma are not recommended for analysis, except in some cases (for example, hemolysates can be used to determine the activity of fructosomonophosphataldolase, glucose and some other tests).

Tissue samples should be taken from only one pre-selected location on the animal's body so that they can then be compared. You need to separate the connective tissue from the muscle. A thin film of connective tissue binds adjacent cells together, and the gaps between them are filled with intercellular fluid. Thus, any muscle sample will have some connective tissue, not counting the collagen that is in the membranes of muscle cells. As a result, the prepared muscles contain 0.2-0.4% hydroxyproline, an amino acid present only in collagen membranes. For example, different parts of a fish's body contain different amounts of minerals. Thus, it was found that the content of Na^+ in the tail is higher, and K^+ is lower than

in other parts of the fillet. But in some fish, there is a higher content of Na^+ and K^+ in the tail. Glycogen levels, for example, increase from the head to the middle of the body and then decrease in the tail in the muscles of Gadus morhua and Auxis tapeinosoma. These data suggest that it is necessary to indicate the place of sampling for analysis and the type of organisms studied.

Conditionally clean muscle tissue is also heterogeneous. Although the cell wall is relatively thin, it also consists of a completely different substance than the cell contents - mainly collagen fibers. Such heterogeneity can also be eliminated by dissection, although there may be a very small sample. When sampling, it is also important to consider features such as the different diameters of individual muscle cells.

Significant differences are also observed in the biochemical parameters of the white and red muscles of organisms. Therefore, mixing white and red muscles in one sample of muscle tissue is not allowed. Even the same muscles differ in chemical composition but are located on the surface and depth of the animal's body.

With the exception of blood, almost all animal tissues are taken for analysis after their death. To obtain values clothe se to the physiological parameters of a certain indicator, try to take a sample immediately after the slaughter of animals, birds or fish, but even this can not ensure the reliability of the result. The content of protein, water, lipids, vitamins does not change under stress, so it can be determined in the selected biological material. If fresh material is not available to identify the main components, you can use frozen material. The content of protein and lipids is also easily determined in tissue samples that were stored in a frozen state.

It should be borne in mind that the content of many chemicals in the body at rest is not constant, but changes under the influence of endogenous factors, environmental factors, depending on the age of the animal or the nature of its nutrition and maintenance.

METHODS OF DETERMINING CONCENTRATIONS OF SUBSTANCES, THEIR UNITS

Units of concentrations according to the System of International Units (SI; SIU)

	Base unit	Derived units
Molar concentration	mol / l	mmol / l, μmol / l, nmol / l
Molal concentration	mol / kg	mmol / kg, μmol / kg, nmol / kg
Molal part	mol / mol	mmol / mol, μmol / mol, nmol / mol
Mass concentration	kg / l	g / l, mg / l, μg / l, ng / l
The mass ratio	kg / kg	g / kg, mg / kg, μg / kg, ng / kg
Volume ratio	l / l	ml / l, μl / l

Different ways of expressing concentrations

■ **Milligram-percentage (mg-%) - the amount of substance (in mg) in 100 g of solution**

■ **Millionth (ppm, ppm)**

1 million -1 = 1 · 10-4%, i.e. 0.0001%

1 ppm = 0.1 mg-% (solution)

1 ppm = 1 μg / ml = 1 mg / ml

When the molecular weight of the substance is unknown, it is better to use a percentage (percentage) concentration.

■ Mass percentage (wt.%)
w / w - the amount of substance in grams per 100 g of solution;
w / v - the amount of substance in grams in 100 ml of solution

■ Volume percentage (% by volume)
v / v - the amount of substance in milliliters in 100 ml of solution

■ Molar concentration = n2: V · 1000 = (g2: M2): V · 1000, where
n_2 is the number of moles of solute in V ml of solution,
g_2 is the mass of solute in grams,
M_2 is the mass of the substance, which is numerically equal to its molecular mass
Molar (1 M) solution (mol / l) contains 1 mol of solute in 1 l of solution.

■ Normal concentration = r_2: V · 1000 = (g_2 · ν: M_2): V · 1000, where
r_2 is the number of gram equivalents of solute in V ml of solution,
ν is the factor that connects the number of moles and the number of gram equivalents of the substance; it is numerically equal to the inverse of the basicity (atomicity) of the acid (base), the number of electrons that are transferred or received by one molecule in redox processes or the formal valence of electrons
Normal (1 N.) solution (g-eq./l) contains 1 g-eq. solute in 1 liter of solution

■ Molal concentration = n_2: $g_1 \cdot 100 = (g_2 : M_2) : g_1 \cdot 1000$, where
g_2 is the mass of solute in grams,
n_2 is the number of moles of solute in g1 g of solvent.
Molar (1 ml) solution (mol / kg) contains 1 mol of solute in 1 kg of solvent.
Mass concentration - a commonly used unit for molality in chemistry is mol/kg. A solution of concentration 1 mol/kg is also sometimes denoted as 1 molal.

The basic SI units

Meter (m), Kilogram (kg), Second (s),
Ampere (A), Kelvin (K), Mole (mole)

Derived SI units are formed as follows: a) multiplication by a factor 10^3

Prefix	Symbol	Factor	Prefix	Symbol	Factor
exa	E	10^{18}	mili	m	10^{-3}
peta	P	10^{15}	micro	μ	10^{-6}
tera	T	10^{12}	nano	n	10^{-9}
giga	G	10^{9}	piko	p	10^{-12}
mega	M	10^{6}	femto	f	10^{-15}
kilo	k	10^{3}	atto	a	10^{-18}

B) in some cases by multiplication by a factor 10^1

Prefix	Symbol	factor
hecto	h	10^2
deka	da	10^1
deci	d	10^{-1}
centi	c	10^{-2}

The ratio of extrasystem units of mass and units of SI

	kg	**g**	**ounce**	**pound**
1 kg kilogram (CI)	**1**	**10^{-3}**	**32,2**	**2,2046**
1 g gram	**10^{-3}**	**1**	**$32,2 \cdot 10^{-2}$**	**$2,2046 \cdot 10^{-3}$**
1 oz.. (oz. apoth.) ounce of pharmacy	**$31,1035 \cdot 10^{-3}$**	**31,1035**	**1**	**$68,5715 \cdot 10^{-3}$**
1 p (lb) poud	**0,45359236**	**$4,5359 \cdot 10^2$**	**14,6**	**1**

The unit of mass is the kilogram (kg)
It is recommended the derived units: Mg, g, mg, μg

Test question, the tests set to determine the level assimilation of knowledge by students.

Part 1

Biochemistry (common):

1. Metabolic processes of microorganisms in rumen and their influence on living ability of cows are one of the subject of study … (of what biochemistry)
2. Static / Dynamic / Functional biochemistry does study...
3. Who got Nobel Prize for discovery of cytric acid cycle or tricarboxylic acids cycle in 1953?

4. The indicated below substances are belong to …
5. It's necessary to choose right definition of process presented on picture below...

Basics of Physical and Colloid Chemistry:

6. How many grams of glucose ($C_6H_{12}O_6$) and water is necessary for preparing 100 g with mass fraction of glucose 10 %?
7. What solution is isotonic? Solution is isotonic when …
8. What index is measured with pH-meter?
9. What is the 1st law of Raul about?
10. Please, choose the right phrase about protective power of some colloids

Methods:

11. Spectral characteristics of nucleoside are determined with what device?
12. What is possibility of centrifugation? Please, give characteristic of the method.
13. In medico-biological investigation, what is used in the purpose to separate biopolymers, viruses and subcellular elements?
14. General description of electrophoretic method.
15. What is possibility of electrophoresis? Please, give characteristic of the method.
16. What is possibility of gel-filtration?
17. Common characteristics of ion-exchanged chromatography.
18. Common characteristics paper chromatography.

Carbohydrates:

19. What is monomer of polysaccharide?
20. Monomer(s) of maltose is/are …
21. After fermentation, the disaccharide (see below) is hydrolyzed into two monomers. It's necessary to choose right equation of disaccharide hydrolysis.
22. Monomer(s) of starch is/are …
23. What significance/role of starch is?
24. It's necessary to choose polysaccharide which has mainly monomer fructose
25. Monomer of mannan-polysaccharides is…

Lipids:

26. What saturated fatty acids do you know?
27. What significance/role of saturated acids is?
28. What unsaturated fatty acids do you know?
29. Tristearoil glycerol is composed of …
30. What significance/role of sphingolipids are?

Proteins:

31. Structural organization of protein (common characteristics)
32. What is monomer of protein?
33. Primary structure of protein (common characteristics)
34. The first spatial structure of hemoglobin and myoglobin were determined with X-ray analysis by scientists who got Nobel Prize. These scientists are John Kendrew and …
35. What amino acids are essential?
36. What significance/role of essential amino acids are?

Nucleic Acids:

37. What is monomer of nucleic acid?
38. What purine nucleobases do you know?
39. Primary structure of nucleic acid (common characteristics)
40. What difference between DNA and RNA?
41. What fuctions of DNA do you kow?
42. Synthesis of ribonucleic acid (common characteristics)
43. According to the principle of complementarities, it's necessary to calculate the molar content of uracil in DNA when there are 158 mol of adenine.
44. Replication of DNA sequence of GGTTGCCAA results …
45. What common characteristics of replication are?
46. According to the principle of complementarities, it's necessary to calculate the molar content of thymine in DNA when there are 632 mol of adenine.

Part 2

1. Formation of ethanol amine (in the activated form)
2. Build right chain of reactions of the urea cycle from citrulline
3. How does somatotropin influence gluconeogenesis in the liver?
4. Functional biochemistry does study...
5. Glycerol oxidation: glycerol phosphokinase reaction (it's necessary to choose substrates and products).
6. What peculiarities of baby-digestions (it's about enzymes in the different compartments of digestion-tract?
7. It's necessary to write names of substrates, products and enzyme of the reaction represented below (You can make this task if you know: what pathway is represented by one reaction here).
8. Lactic acid fermentation: under fermentation by Bact. lactis, lactic acid is

formed from glucose: $C_6H_{12}O_6$ -> 2 CH_3-CHOH-COOH;

9. ... is compartment of such enzymes as carboanhydrase, uricase, urease, aldolase, and phosphatase.
10. What enzyme does digestion of proteins?
11. Glyceraldehyde-3-phosphate dehydrogenase reaction of glycolysis:
12. (NAD-dependent) isocitric acid reaction of TCAC:
13. Enzymes can be consisted of what class of organic substances? Enzymes can be consisted of…
14. What function do biological membranes in animal organism?
15. What element takes part in the process of oxidative phosphorylation?
16. It's necessary to choose a name of biological active substance which is catalyzed by chemical processes in the organism
17. It's necessary to choose substrates and products of the carbamoyl phosphate synthase reaction of the urea cycle (substrates, products)
18. What is the second irreversible reaction of glycolysis (according to the name of enzyme)?
19. Formation of choline (in the activated form):
20. Build right chain of reactions of the TCAC starting from a-ketoglutarate:
21. All pointed out carboxylic acids belong to …
22. Cortisol (which is belonged to glucocorticoids) ... gluconeogenesis.
23. What peculiarities of digestions in 'mature' animals (it's about enzymes in the different compartments of digestion-tract)?
24. It is necessary to choose names of substrates, products and enzyme of the reaction represented below (You can make this task if you know: what pathway is represented by one reaction here; substrates, products, enzyme, coenzyme).
25. Synthesis of TAG. It's necessary to choose right reaction of glycerol-3-phosphate formation by glycerol phosphokinase (substrate, products).
26. Catabolism of the sterol esters by enzymes (It's necessary to choose typical reaction with the pointed-out substrates, products and enzyme).
27. Substrate phosphorylation in the TCAC is carried at step of transformation of…
28. Alcoholic fermentation and its brief characteristics.
29. Role of Sodium in the animal life:
30. Glycolysis. It's necessary to choose names of substrates and products of the reaction catalyzed by 'phosphoglycerate kinase'
31. The reaction of the TCAC is catalyzed by multi-enzyme complex of α-ketoglutarate dehydrogenase (substrates and products):
32. Biological role of vitamins.
33. Biological role of fat-soluble vitamins.
34. Biological role of water-soluble vitamins.
35. Biological role of vitamin A.
36. Formation of histamine is ...
37. What enzymes do digestions of polysaccharides?

38. What reaction does catalyze by lyase?
39. β-oxidation of fatty acids: what is going on in reaction catalized by carnithine acyltransferase I?
40. In the humane organism, it's founded Cu-deficit that causes lowering enzyme activity of…
41. Role of choleic acid in digestion of lipids:
42. Role of fat-soluble vitamins, especially vitamin F.
43. Localisation of processes of β-oxidation (activation of FA and actually their β-oxidation):
44. Under transamination of pyruvate, it is formed such important substrate of gluconeogenesis as ...
45. Distraction of phospholipids: it's necessary to give characteristic of reaction that catalize by phospholipase D (substrates, products).
46. How it could be lowered the glucose level in the dog's blood (under conditions of diabetus melitus)?
47. Lactic acid fermentation: under fermentation by Bact. lactis, lactic acid is formed from glucose: $C_6H_{,2}0_6$ 2 CH_3-CHOH-COOH;
48. What enzyme does digestion of proteins?
49. Glyceraldehyde-3-phosphate dehydrogenase reaction of glycolysis:
50. (NAD-dependent) isocitric acid reaction of TCAC:
51. Role of Selenium in the animal life:
52. What is biological importance of glucose-lactate cycle?
53. Carotenoids are precursors of ... vitamin
54. What is remarkable characteristic of urease?
55. What enzymes do digestion of oligosaccharides?
56. What is "a main purpose" of ornithine cycle?
57. Under transamination of oxaloacetate, it is formed such important substrate of gluconeogenesis as ...
58. What reaction is catalyzed by isomerase?
59. Enzymes of cellular respiration are mainly localized in ...
60. Butyric acid fermentation:

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- Basic

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1. Ukrainian Biochemical Journal http://ua.ukrbiochemjournal.org/
2. Animal Biology http://aminbiol.com.ua/
2. US National Library of Medicine, National Institutes of Health https://www.ncbi.nlm.nih.gov/pubmed/
3. National Library of Ukraine named after VI Vernadsky [Electronic resource]: [Website]. Electronic data. Kyiv: NBUV, 2013-2015. Access mode: www.nbuv.gov.ua.
4. Wikipedia https://uk.wikipedia.org/wiki/
5. EEC "Biochemistry of animals with the basics of physical and colloid chemistry" (full term in English) https://elearn.nubip.edu.ua/course/view.php?id=686
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