BASIC METHODS OF BIOCHEMICAL RESEARCH

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Annotation: The article provides information on the main methods of biochemical research and the essence of their content.

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Because the object of biochemistry is a living organism, complex methods are used to extract a substance from it. For normal physicochemical analysis, a number of additional processes will be required. The procedure for the separation of substances from biological material is as follows:

1. Homogenization.

2. Ultracentrifugation.

3. Extraction.

4. Analysis (re-extraction, heat treatment, dialysis, sedimentation, electrophoresis, chromatography).

The method of isolation and analysis depends on the properties of biological substances. In the study of isolated biological materials, the structure and physical and chemical properties of substances are studied in accordance with the purpose. In their quantification, various methods of physical, physicochemical, chemical analysis, as well as quantum-mechanical calculations of the electronic shell of the separated compound are used. The methods used should allow to preserve the natural structure of biological substances.

Biological material for biochemical research can be easily obtained under

experimental conditions. In the clinic, this possibility is relatively limited. In pharmacy, animal tissues and drugs are used as biological material.

Plasma, serum, and other biological fluids are water-soluble mixtures of various natural substances. Since enzymes are composed of complex proteins by nature, no modifiers are added to biological fluids in their determination, and they are diluted when the concentration of the enzyme is high. If the enzyme in the biological fluid catalyzes and prevents the detection of the test substance, the activity of the enzyme is stopped with the appropriate reagent. Typically, acetic acid, nitric acid, phosphorus tungsten, sulfuric acid or thermal action are used for this purpose. Along with enzymes, other proteins are precipitated.

Homogenization. If biochemical studies are performed on organelles or their fragments, such as membranes, located in the cell, tissue, organ components, then the cell or tissue must first be crushed. To do this, the method of mechanical disintegration of tissue using a homogenizer is often used. The homogenizer is similar to a glass beaker and comes in different sizes. This glass is filled with scissors and a medium of liquid (usually sucrose, potassium chloride), which maintains the integrity of the extracted cells. As a result of the rotation of the homogenizer handle in the beaker with the help of an electric current, the cell membrane breaks down, the structural fragments separate. Under normal conditions, the homogenate tissue is crushed in a porcelain mortar with glass powder or quartz sand.

Homogenates consist of a complex mixture of tissues, cell fragments of which differ in size, shape, chemical structure. To conduct biochemical research, a number of physicochemical methods are used in their distribution and separation by molecular weight.

Centrifugation is a method of separating heavy parts of a liquid from light parts by centrifugal force. The heavier particles in the mixture settle first. Once separated, the supernatant can be re-centrifuged at high speed to separate the other particles. Centrifugation for the detection of components in the mixture is called preparative centrifugation, which is used for the separation of trace

elements in the blood, sedimentation and separation of cells in the urine, and other purposes.

The maximum rotation speed of small centrifuges used in clinical and biochemical laboratories does not exceed 6000 per minute. Special biochemical research is called analytical centrifugation due to the use of high-speed (up to 70,000 revolutions per minute) ultracentrifuges to determine the molecular weight of proteins and nucleic acids and to separate particles of different densities.

The settling velocity of a particle is measured by the increase in centrifugal force and is expressed in units of g (gravitational constant 980 cm • s-2). In practice, g is constructed according to the instructions given in the nomogram of each ultrasonic centrifuge. For example, blood-forming elements precipitate when centrifuged at 300-400 g for 20-30 minutes, and so on. Differential centrifugation separates subcellular parts - nucleus, mitochondria, lysosomes, microsomes and others.

Methods of analysis. Electrophoresis. Electrophoresis is the distribution of charged particles under the influence of an external electric field. Electrophoresis is a modern method used in biological experiments, in clinical medicine for the analysis of blood proteins and peptides, especially serum. Charged particles move at different speeds in the electric field, depending on the size and size of the charge, which leads to their separation and distribution.

There are two main types of electrophoresis, divided into frontal and zonal methods, the latter of which are more common. In this case, the protein solution is placed in a buffer solution in the form of a thin layer. During electrophoresis, various protein molecules are divided into separate fractions. These fractions are easily separated by separation. The basis of zonal electrophoresis is filter paper tape, acetylcellulose, starch powder, agar, polyacrylamide gel and other materials.

Currently, special polyacrylamide gel electrophoresis is widely used in biological and medical research.

The foregram of the fractions is held in a 10 V solution of kumassi blue, bromphenol blue or amidoshwart for 20-30 minutes, and the amount is

determined by the thickness of the dye, ie the binding of different proteins to the dye. is directly proportional to the amount of this protein.

Chromatography. Chromatography studies the distribution of various compounds into their components. There are four main types of chromatography.

a) Information column chromatography - this method is used in the distribution of soluble substances in ionic form. The method differs from the stationary and mobile phases, the stationary phase is based on organic polymers - resins, consisting of ion exchangers.

b) In liquid chromatography, microscopic particles are used instead of the stationary phase. This method has a high speed and can distribute almost any combination in a short time.

c) In disseminated chromatography, the substances in the mixture are divided into their individual components depending on the size of the radicals, the presence of functional (hydrophilic) groups, different solubility in mobile and inactive solutions.

A drop of the test substance is dropped 1 cm above the lower limit of the chromatographic paper strip, and the moving solution is placed in a chromatographic chamber, usually containing an organic solution. A substance saturated with water vapor in the chamber atmosphere forms a motionless (polar) solution. The moving solution moves upwards with the hydrophobic substance, and the hydrophilic substances remain at the start because they are dissolved in water. After staining the substances in the chromatogram, the individual Rfs of each are measured or identified with a standard substance.

Optical methods. In photocolorimetric analysis, the degree of clarity (concentration) of the color of the test solution is compared with the color of a previously known standard solution. Colorimetric determination uses the reaction of a measured substance to form a colored compound with another substance. The intensity of the color of the resulting solution is directly proportional to the amount of dye. The higher the color intensity, the higher the optical density. To determine the graphical relationship, the amount of substance in mol '/ l (S) is placed on the

abscissa axis, and the optical density (D) of the solution is placed on the ordinate axis. To apply the method, there must be a proportional relationship between D and C. Spectrophotometric analysis determines the absorption of light by a substance in solution or solid medium at a certain wavelength. Using a spectrophotometer, it is possible to work in the visible part of the spectrum (600 to 1100 nm), in the ultraviolet part of the spectrum (220 to 650 nm).

In addition to chemical, physicochemical, mathematical, physiological methods to study the structure, exchange and functions of biological compounds, biological chemistry has its own method of research - the method of enzymatic analysis. This method is widely used in applied medicine, various fields of pharmacy and science, as well as in the national economy.

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