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DIAPHONIZATION AS A METHOD OF MODERN MORPHOLOGICAL RESEARCH

ДІАФОНІЗАЦІЯ ЯК МЕТОД СУЧАСНИХ МОРФОЛОГІЧНИХ ДОСЛІДЖЕНЬ

Резюме. У статті розглянуто діафонізацію як один із методів морфологічних досліджень у процесі вивчення кровопостачання мозку. Проаналізовано історію впровадження цього методу, сфери його застосування, класифіковано відповідно до об'єктів дослідження. Нами виділено переваги застосування методу діафонізації для виконання досліджень і з освітньою метою. Також на основі стандартного протоколу проведення діафонізації створено оновлений його варіант, адаптований саме для вивчення мозкового кровопостачання. У вдосконаленому нами протоколі змінено пропорції відповідних речовин, виключено чи якісно або кількісно змінено певні етапи виготовлення препарату; зміни вносилися з урахуванням гістологічної та гістохімічної специфіки тканин головного мозку та з метою максимального вдосконалення видимості відповідних структур у препараті. Окрім опису експериментального дослідження та зміненого протоколу, нами окреслено окремі способи застосування власне готових препаратів у навчальному процесі здобувачів освіти, а також їх використання у колаборації з сучасним навчальними системами тривимірної анатомії (Artec Space Spider scanner, Anatomage virtual anatomical table та ін.) та інші перспективи наукових спроб.
Ключові слова: діафонізація, артеріальні взаємозв'язки, кровопостачання мозку, 3D моделі, морфологічні особливості, порівняльна анатомія.

One of the urgent medical and biological problems is the study and establishment of signs of comparative anatomy of the arterial relations of the human brain stem of adult people and some laboratory animals. Phylogenetic changes in the structure of the human body and the features of the blood supply of various organs and systems objectively deserve special attention for the improvement of the scientific overview of the morphological features of the blood supply of the human brain and some laboratory animals and the further usage of research results in scientific experiments, educational projects and clinical modeling of pathologies and diseases, related to the blood supply of the brain. Such significance of this vector of research, first of all, requires qualitative improvement, optimization and modernization of research methods taking into account some anatomical, histological, biochemical, technical, etc. factors, influencing the results of experiments and of the research in general. Despite the fact that the variability of the distribution and individual variability of vessels, the values of the diameters of arteries are described in many works, in the available to us literature there is no data about the changes in some anatomical characteristics of arteries in comparative anatomy relations of adult people and some laboratory animals.

Purpose of the research. The study was carried out to clarify some specifics and to classify the ways of using the diaphonization as a modern morphological method, to remark the ways of using diarhonized preparations in the educational process using some modern technologies. Aim of the experiment is to identify the sources of blood supply, relationships and anastomoses of the arteries of the brain of humans and animals.

Material and methods. To conduct our experimental study, a brain was taken from a laboratory corpse purebred white rat aged 5 months. The rat's corpse was taken away the vivarium of KhNMU under the agreement on scientific cooperation. The research method was used in compliance with the rights of experimental animals, in accordance with the legislation in the law of Ukraine and international ethical requirements and does not violate ethical norms in science and standards of conducting biomedical research. The study was conducted at the Department of Human Anatomy and Physiology named after Doctor of Medicine, Prof. Y. R. Synelnikov of H. S. Skovoroda Kharkiv National Pedagogical University.

Results of the research. Modern scientific and technical progress has contributed to the appearance of a significant number of methods for researching the vascular system of the brain. However, their small number makes it possible to see a complete picture of their mutual locations both on the surface and inside the organ without violating the integrity and in accordance with their real topography. One of the modern research methods, which makes it possible, is diaphonization.

For the first time, the diaphonization technique was invented in 1977 by German scientists G. Dingerkus and L. D. Uhler and became known as the «cleaning and staining» method. Gradual further changes in the technique of its implementation contributed to the modification and improvement of the method. In general, diaphonization consists in creating peculiar models of body parts or fairly small organisms with the help of stepwise exposure of various substances (mainly proteolytic enzymes to achieve the transparency of certain structures and dyes to color others) on the samples [1]. In modern scientific studies, unfortunately, diaphonization is rarely used and is more known and popular as a form of art, as a way of making extraordinary decorative objects, using the bodies of various animals, and not for research works with scientific and educational purposes.

Considering the scientific vector of the usage of diaphonization, we can distinguish three directions: botanical, zoological and odontological.

In botany, diaphonization is used to identify specific structures (trichomes, localization of stomata) in the epidermis of plants [2, 3]. The method of conducting such research includes the collection of plants, the isolation of vegetative organs (mainly leaves) and further processing. The obtained samples after diaphonization are analyzed by mathematical modeling to obtain digitized results for the purpose of their further analysis. Thus, diaphonization in the context of botanical studies is one of the ways of the complex study of plant structures.

Using of diaphonization in zoology involves the usage of this method mainly for the study of bone and cartilaginous structures of vertebrates (reptiles, amphibians, fish, small mammals, etc.) as one of the leading methods of studying the skeleton without violating the integrity of the skeletal system for its full study. [4-6]. In this context diaphonization makes it possible to study age-related changes in the structure of the skeleton and to identify pathological changes and general patterns of development associated with the processes of bone calcification. For example, with the help of the diaphonization method, it becomes possible to detect not only ontogenetic disorders in the development of the musculoskeletal system of animals, but also the consequences of metabolic disorders (primarily calcium metabolism) by detecting the difference in staining of samples taken from animals with congenital pathologies or experimentally removed organs. At the same time, the diaphonization method is defined as one of the most effective and more appropriate for this type of research than X-ray studies and computer tomography.

Odontological vector of the usage of diaphonization consists in studying the root canal of the tooth with its help for the analysis of morphological features. The technology of this type of research involves the preparation of a diaphonized sample and its further study by the method of observation under a binocular magnifier and the naked eye and description, mainly for scientific and educational purposes. This direction of diaphonization was practically used as one of the methods of preparing future dentists in institutions of higher education. The success of this experience allows us to consider diaphonization as a promising method for the production of educational anatomical preparations [7].

In our opinion, diaphonization is a promising and relevant scientific method, which has a number of features that are advantages for a more qualitative study of body structures, including blood vessels, their topographical relations both in the whole body and in individual organs. Firstly, an important factor is the clarity of the structures on the preparations made in this way, which is especially important for their detection among other structures at the stage of studying their localization and structure. Secondly, it is important to study the integrity and undisturbed location of blood

vessels close to their real course in a living organism. Preparations, which were made with the diaphonization method make it possible to see the structures of an organ or organism «in situ», which excludes the possibility of partial distortion of the real topography, structure and structure of certain parts that are lost during normal preparation. Thirdly, we consider the safety factor to be one of the most important: using of a ready-made preparation for the study of certain structures does not involve direct contact with substances that can directly or indirectly harm the researcher's health, for example, formalin vapors during classical research.

For the completeness of our research, we suggested to start by considering the standard technique of diaphonization [8], which we took as a basis for the further formation of an updated protocol, optimized taking into account the characteristics of brain tissues. So, this technique consists of two stages. At the first stage, the soft tissues are «cleaned» by immersing them in a solution of trypsin – a digestive enzyme that slowly destroys the soft membranes of the sample, with the help of which we achieve the desired transparency for the visibility of colored structures. Afterwards, alizarin red and alcian blue are most often used for coloring. Presenting the diaphonization process in the form of a protocol, we highlight the following stages:

1. Sample preparation.
2. Fixation by 10% formalin solution.
3. Ablution.
4. Cartilage staining (Alcian Blue).
5. Dehydration (carrying out through alcohols 30, 50, 70, 90, 96.6% concentrated.
6. Decomposition by trypsin.
7. Bone staining (alizarin red).
8. Enlightment (0.5 KOH + glycerin (1:3) + peroxide of hydrogen (1:100)).
9. Place the samples in this bath for 7 days (fig. 1).

Fig. 1. Diaphonized preparations

In order to achieve the aim of clarifying the brain substance with the diaphonization method, we made some changes to the standard protocol of the technique. These changes were primarily determined by the specificity of nervous tissue, which has its own characteristics related to connective tissue and muscle tissue. This specificity consists in the elemental composition and ratio of inorganic and organic substances in the corresponding tissues. Actually, glia and myelin of nervous tissue are very difficult to undergo the process of chemical enlightenment, which requires special physical and chemical influences. Therefore, in order to achieve the goal of the study, we experimentally made some changes in the diaphonization technique, having previously learned the standard protocol and adapted it according to the histological specificity of the studied organ (fig. 2).

Fig. 2. Naked rat's brain

Our research protocol (methodology of brain diaphonation with arteries filled with tinted latex) is as follows:

1. Sample preparation consists in filling the arterial vessels of the brain with the special substances. The consistency of these dyes, the quality of their coloring and the coagulation time are optimal for working with our samples. Tinted acrylic latex (3060 LBS, SYNTHOS DWORY). Acrylic paint for silk, batik Decola (red color) was used as a dye. Arteries were filled with a disposable 2.0 ml syringe through a KD-FIX catheter, G 18 1.3 x 45 mm, which was previously fixed with a ligature. The tinted latex was injected through the carotid and vertebral arteries (fig. 3).

Fig. 3. Filling the blood vessels with dyes

2. Fresh brain was not fixed with formalin.
3. Ablution was also not carried out.

4. Our research object does not contain cartilaginous tissue, therefore, accordingly, staining was also not carried out (Alcian Blue was used in the primary protocol).

5. Accordingly, without taking into account the missed stages, the next stage is dehydration, which is actually passing through alcohols. In our variation, the step-by-step passage of the sample through alcohols was shortened compared to the classical method: we used alcohols of 50%, 70%, and 96.6% concentrations.

6. As for the trypsin digestion step, we also optimized the method of its implementation. In particular, we doubled its amount and periodically heated it in a water bath at temperature of 60^{O}C (fig. 4).

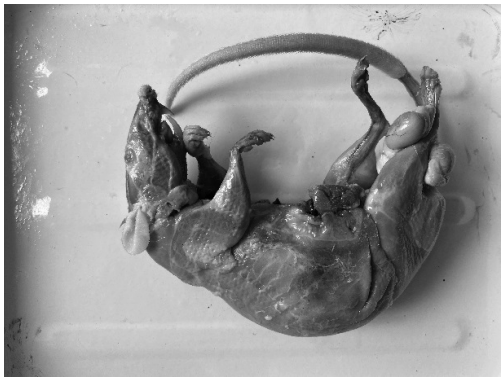

Fig. 4. Rat's preparation in the process of diaphonization

7. Bone staining with alizarin red dye was not carried out, since the material we studied does not contain bone tissue.

8. We also made the necessary corrections regarding the stage of enlightment of the sample: to improve the obtained result, it was carried out with double the amount of KOH, glycerin and hydrogen peroxide and with an increased duration, namely for 10 days (fig. 5).

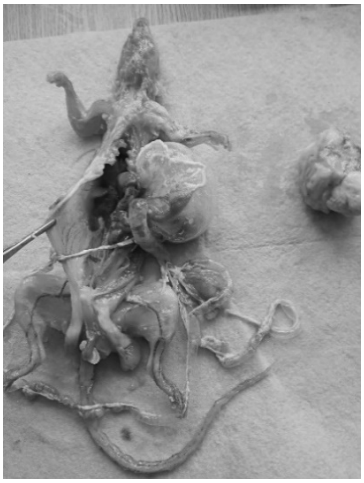

Fig. 5. Sample preparation process

9. After that, the preparation was placed in a 20% glycerin solution for permanent storage (fig. 6).

Fig. 6. Preparation placed for permanent storage

On the obtained preparation of the diaphonized brain of a laboratory white rat, it becomes possible to detect the contours of arteries filled with dyed latex, their branches and the relations between them both on the surface and in the depth of the brain substance, which determines the effectiveness of the theoretically and experimentally justified changes to the standard protocol (fig. 7).

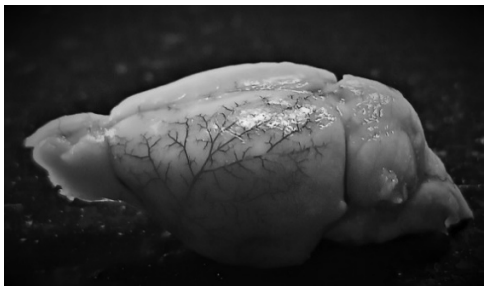

Fig. 7. Preparation of the brain (outside vessels are visible)

A separate vector of using diaphonized preparations can be considered in their using with the help of modern technologies, which is promising both for scientific research and for full-fledged training of students. 3D models can be created from the obtained preparations using the Artec Space Spider scanner, which will make it possible to study the arteries of the brain using the capabilities of Artec3d program and to get printed 3d models based on the center of 3D technologies of the Educational and Scientific Institute of Education Quality of Kharkiv National Medical University, as well as to add research materials to the database of simulation preparations in the system of the Anatomage virtual anatomical table, which can be used while studying comparative anatomy of the nervous and vascular systems. Such broad possibilities for the usage of diaphonized preparations are another confirmation of the relevance and perspective of this method of research and the need for further development in this direction (fig. 8).

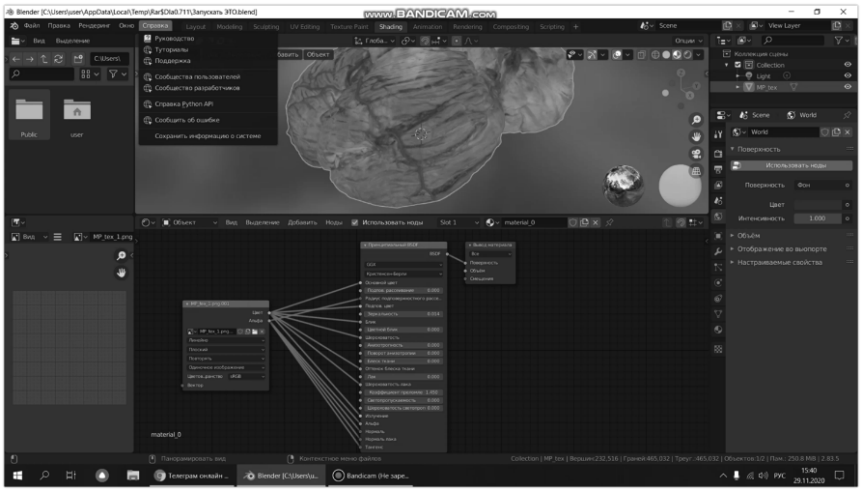

Fig. 8. Process of preparation scanning

Conclusion. Diaphonisation as one of the morphological methods of research is observed in the vectors of studing the blood supply of the brain and also in different vectors of its usage. Difference between classical method of diaphonization and method of brain vessels diaphonization are discussed and the changes in the protocol are justificated according to some histological and morphological specifics. Odontological, botanical, zoological ways of using diaphonization are analysed. Advantages and modern ways of using of obtained diaphonized preparations in collaboration with 3D modeling and educational equipment (Artec Space Spider scanner, Anatomage virtual anatomical table, etc.) and other persrectives of using of diaphonization method are sketched.

Perspectives of further research. Using of the diaphonization method with some introduced by us changes, allows getting preparations of enlightened brain with blood vessels, which has been proven experimentally. It gives possibility to use ready-made preparations for a more detailed and holistic study arterial relations in the blood supply of the brain, namely its stem parts. Also, on the basis of the obtained preparations is that this method can be used to make biomechanical models of the arteries of the brain stem with various variants of their configuration and branching of both the brains of laboratory animals and humans.

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Abstract. In the article diaphonization is discussed as one of the methods of morphological research in the direction of the study of blood supply to the brain. The history of the research of this method is analyzed, the areas of its application are classified according to the research objects. We have highlighted the advantages of using the diaphonization method for research and educational purposes. Also basing on the standard diaphonization protocol, an updated version of it was created, adapted specifically for the study of cerebral blood supply. In the protocol improved by us, the proportions of the relevant substances have been changed, certain stages of the preparation have been excluded or quantitatively changed; changes were made taking into account the histological and histochemical specificity of brain tissues and with the aim of maximally improving the visibility of the corresponding structures in the preparation. In addition to the description of the experimental study and the modified protocol, we have outlined some separate methods of using ready-made preparations in the educational process of students, as well as their usage in collaboration with modern educational systems of three-anatomy (Artec Space Spider scanner, Anatomage virtual anatomical table, etc.) and others research perspectives.

Key words: diaphonization, arterial relations, blood supply of the brain, 3D models, morphological features, comparative anatomy.

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