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REVIEW ARTICLE

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HISTORY OF ORIGIN, ADVANTAGES AND DISADVANTAGES, VECTORS OF APPLICATION OF THE DIAPHONIZATION METHOD: CURRENT STATE OF THE PROBLEM

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ABSTRACT

The article deals with the method of diaphonization, which consists in clarification and staining of tissues, organs and whole organisms for long-term storage in the form of preparations as one of the modern methods of morphological research and, in general, a powerful tool for studying the internal structure and topography of organisms. The authors have analyzed the history of the diaphonization method, its advantages and disadvantages. Its advantages include the ability to study the internal anatomy without destroying the object, detailing structures, preserving samples for further research, and the possibility of combining with other modern research methods. Despite its advantages, the diaphonization method also has certain limitations and disadvantages (possible deformation of structures, loss of color, time and space complexity, and limitations in application to certain types of organisms). With its three main application vectors (clinical, experimental, and botanical), the diaphonization method is an essential tool for researchers in many fields, which continues to evolve and find new applications, improving the overall scientific picture of understanding the structure of organisms and contributing to scientific progress. The article also highlights the importance and prospects of combining the diaphonization method with other techniques to determine a more accurate picture of the relative location and structural features of certain tissues, organs, and systems.

KEY WORDS: diaphonization, morphology, history of origin, advantages and disadvantages, clinical medicine, experimental medicine, botany

INTRODUCTION

It is a common fact that methods of morphological research can be rightly considered the basis for many branches of medicine and other natural sciences as a way to analyze the structure and its normal or pathological variants in organisms. Due to this, it is a constantly relevant issue to modernize, update and improve the existing methods of morphological research, their further development and improvement, taking into accounts the characteristics of research objects and the possibilities of modern scientific progress. Among a number of currently available methods of morphological research, we consider the method of diaphonization to be one of the most noteworthy as a relevant and modern method that can be used at different levels and in combination with other modern methods to form a more complete anatomical picture and obtain new information about the object of study [1-3].

AIM

The purpose of the study was to analyze the history of the origin, advantages and disadvantages, vectors of application of the diaphonization method based on the literature data.

MATERIALS AND METHODS

The articles were searched in open electronic databases of scientific literature. The following words and phrases were used for the search: "diaphonization", "history of diaphonization", "advantages of diaphonization", "disadvantages of diaphonization", "vectors of diaphonization application", "diaphonization in clinical medicine", "diaphonization in experimental medicine", "diaphonization in botany". The methods of abstraction, generalization, induction, deduction, explanation, and classification were used in our literature review.

REVIEW AND DISCUSSION

Diaphonization is a method that consists in reducing or "removing" the optical color of the objects under study in order to achieve their transparency while maintaining their morphological structure, which is mainly studied in the finished sample [4] (Fig. 1). Another name for the

Fig. 1. Newborn guinea pig after diaphonization.

diaphonization method is the "cleaning and staining method", which actually explains the essence of the method, which consists in enzymatic decolorization of the preparation and subsequent staining of its bone and cartilage structures with dyes. Sample preparation, proportions of working solutions, processing time and method of storage are determined by the characteristics of the tissues and the ratio of their various types in the preparation. Currently, small laboratory vertebrates are most often used for research using this technique, because due to their size and tissue characteristics, diaphonization on this type of preparation takes less time and is more effective (transparency is more expressed and the structures under study are more visible) compared to larger organisms.

The discovery and application of this method was an important step for research on normal and pathological anatomy. The method of diaphonization developed by Schultze in 1897 has been improved more than once by many scientists [5]. For example, in 1991, the German anatomist Spaltegolz proposed one of the methods of achieving transparency of anatomical specimens based on optical laws [6]. Its essence was to achieve transparency by placing previously prepared preparations in a solution with a refractive index equal to that of the tissue or organ under study. Also, the method of diaphonization, which can now be considered a classic, was developed in 1977 by scientists G. Dingerkus and L.D. Uhler [3]. It is according to this methodology that the essence of diaphonization is defined and updated protocols are still being created to take into account the characteristics of the tissues under study.

We have identified three main vectors of application of diaphonization (clinical, experimental and botanical), each of them having its own characteristics, prospects and features. It is worth noting that this classification is based on existing scientific publications describing the use of diaphonization for the study of certain objects [7].

The use of diaphonization for the preparation of tooth samples for the study of their tubular-root system was started by Robertson [8], who improved the current Spaltegolz protocol, taking into account the characteristics of tooth tissues. This technique is considered to be the basic one for research in clinical odontology and is currently being actively improved and widely used by scientists. With its help, by decalcification, purification and staining of preparations, the visibility of the system of roots and tubules of the tooth is achieved with the naked eye. Favorable factors for this are the small size and relatively easy accessibility of human teeth. Finished preparations, in addition to macroscopic studies, can also be examined under a microscope, combining diaphonization with various types of microscopy [7].

The mentioned above method has also become the basis for some other methods of dental examination, the

attractiveness of which, similar to the classical method of diaphonization, is the ability to accurately preserve the original location of canals, roots and cavities of the tooth without changing their topographic features. In addition, this method is often combined with modern technologies, such as electron microscopy, computed tomography, etc. The use of the diaphonization method in clinical practice allows to identify the topographic features of tooth parts, which is especially important for morphological studies or in the process of teaching students, as well as in clinical practice when working with extracted teeth. Accordingly, determining the relative position of canals, roots and cavities of the tooth, especially in atypical variants of their topography, can be useful in diagnosing disorders not only of a particular tooth but also of the entire dentition or jaws [8, 9].

Among the promising applications of diaphonization, it should also be mentioned its use in para-endodontic surgery, i.e. an alternative method to endodontic surgery for research, for example, leakage during filling. In this case, it is the use of diaphonization that simplifies the visualization of the tooth structure and the assessment of dye leakage between different materials in the root canal. In this case, the tooth to be examined is prepared: dentin is removed and old fillings, if any, are removed, and a dye or contrast solution is applied to the boundaries of the dentin and filling material to help highlight the boundary between these materials. To analyze the internal structure of the root and dye leakage, diaphonization is used in combination with special light that is transmitted through the tooth, and its intensity varies depending on the properties of the materials. In this case, the image of the internal anatomy of the root and the place of the dye leakage can be displayed on a screen or other visual device. This allows the dentist to see the structure of the root canal and measure the leakage rate at the interface between dentin and filling material. The data obtained in this way can be analyzed by the dentist to determine the quality of the filling, identify possible leaks that may require additional treatment, and improve the overall diagnosis and treatment of dental problems.

As for the experimental vector of diaphonization, as already mentioned, it is mainly used for studies of small laboratory animals (mice, rats, guinea pigs, rabbits), and the object of study is the corpses of such animals. Here it is important to mention the importance of the bioethical aspect in this context: it is significant that the method of diaphonization can be used on the bodies of animals that died in a "natural" way, which does not require causing harm and suffering to animals, i.e., violation of bioethics and rules for the use of laboratory animals in research [7]. Animals that have died as a result of diseases can be used for research, which allows them to be used in pathomorphological studies. At the same time, the bodies of animals that did not die as a result of disease can be used to create preparations intended to study normal anatomy and morphology, for example, as a kind of standard for comparing the corresponding structures in normal and pathological conditions.

According to the scientific literature available to us, the most common objects of experimental research using diaphonization are nervous, bone, cartilage structures and blood vessels [2, 10-12]. In particular, when studying bone and cartilage structures in laboratory animals, it is possible to investigate the degree of ossification of certain bone structures [11]. Also, based on the degree of ossification of the structures under study, it is possible to determine, for example, the age of the animal, or to diagnose calcification disorders, which can, accordingly, contribute to the diagnosis of disorders of not only osteogenesis but also hormonal and vitamin metabolism [12]. Such studies can be conditionally attributed to microscopic studies, while morphological changes in the topography of certain bone structures (as seen with the naked eye) are macroscopic. Among the studies on this topic, we can, for example, mention the work of Atanasoff and others. [5], where young male sturgeons are the object of study. Thus, using the diaphonization method and by combining it with computed tomography to obtain more accurate data, it is possible not only to identify certain pathologies in bony fish, but also to determine the causes of their occurrence, which, for example, may be due to changes in the diet of fish, their migration, and the influence of various environmental factors (temperature, water salinity, etc.). By analyzing the latter, it is hypothetically possible to determine and predict the effects of environmental changes on fish populations, which determines the use of the diaphonization method as an interdisciplinary research method that is relevant not only in the field of zoology and botany but also on a more global level.

Among the significant studies on the application of the diaphonization method in experimental practice, one should highlight the work devoted to the study of the characteristics of the auditory tube in pigs [13]. The object of study in this work is primarily non-standard, due to which the possibilities of diaphonization as a method of morphological research are expanded. The author also noted the high accuracy of diaphonization, which allows to notice the smallest morphological studies of the auditory canal without violating its integrity and surrounding structures, which is especially important given its relative topographic "inaccessibility".

Angiological studies by diaphonization primarily involve combining this method with methods of filling vessels, because the tissues of organs, the blood supply of which is to be investigated, enlightened by diaphonization, act as a kind of background for the vessels, which can be made more visually contrasting and visible by filling them with appropriate substances, the choice of which depends on the purpose and further course of the experiment [3]. Thus, it becomes possible to visualize for further research the peculiarities of branching and relative location of vessels, as well as their pathological disorders (ischemia, thrombosis, aneurysms, etc.). The results of such studies can be used not only for zoological research, but also in the field of experimental medicine. In the latter case, we mean, for example, the idea of using small (i.e., more

accessible for research by diaphonization) animals as a kind of model for understanding the mechanisms of angiotherapy methods under development in order to avoid the risks of unforeseen complications due to the use of the corresponding method in humans without prior experimental testing [14, 15]. In addition, the ability to observe the effectiveness of a particular vascular treatment method on the drug can contribute to the development of new methods or the improvement of existing ones. At the same time, the diaphonization method, due to the possibility of volumetric visualization without the use of screens and artificial changes in the location of vessels, is the most visual for such studies.

The study of blood vessels by combining diaphonization and computed tomography is also effective and promising, as it contributes not only to the study of blood vessels in vitro, but also to achieving greater depth of penetration and resolution [10], as well as to the digitalization and digitization of the results obtained, which will facilitate the dissemination of experimental results and expand the boundaries of scientific discussion. Thus, the architecture of blood vessels can be studied more comprehensively, which contributes to the understanding of physiological and pathological processes in organs and systems of the body.

In general, the experimental vector of diaphonization has connections with various fields of science other than anatomy and morphology. Due to the fact that this method allows to study organs, bones, cartilage and other structures without the use of invasive procedures, it becomes possible to compare the anatomical features of different species and study their changes, for example, through the prism of evolution [16]. The study of anatomical features by means of diaphonization can help to reveal the adaptation and specialization of organs and tissues associated with certain environmental conditions [17]. Thus, the study of anatomy using diaphonization can help to establish more accurate differences between different species and groups of animals, which can be useful for their classification and systematics in paleontology, etiology, etc. In addition, diaphonization can help study the development of animal embryos and fetuses at different stages. This can help establish the timeframe for the development of various tissues and organs. Also, diaphonization can be used to study the functional aspects of animal tissues and organs, primarily, for example, blood vessels or nervous structures [4, 10]. In addition, as mentioned above, diaphonization can be used in medical research to create models for studying the anatomical aspects of animals in order to conditionally project the results onto the human body to improve diagnostic and treatment methods [18, 19].

As for the botanical vector of using diaphonization, it, like the other vectors, allows us to study the internal structure of plants in greater detail and without damaging the samples, which is important for various aspects of botanical research. That is, in botany, diaphonization can help study the internal structure of plant leaves and stems, including cellular structures, vessels, fibers, and other components [20]. There are a number of studies on the use of diaphonization in botanical research, which describe in detail the differential differences of this area of application of the method and describe the technology and results of specific studies [21-23]. In general, it is worth noting that the study of the internal structure of plants using diaphonization helps to understand how they adapt to different environmental conditions, and also allows us to monitor the development and differentiation of tissues during different stages of plant growth. In general, in botany, the method is used to solve various scientific problems and deepen understanding of the functioning of plant systems.

By analyzing and testing the existing protocols for conducting experiments using the diaphonization method [4, 7], we were able to identify certain functional and general features of the diaphonization method, which define it as a multifunctional, relevant, and promising method of morphological research. Among them, for example, it is worth noting the relative atraumatic nature of the method, which allows for research in general without violating the integrity of the object and without physically destroying it for future study [3]. In addition, under the right conditions, finished preparations have a fairly long shelf life, which facilitates their use for scientific and educational purposes. This is also facilitated by the rather high detail of the objects prepared using the diaphonization method. It makes it possible to study quite small structures (vessels, nerves, small organs) with a fairly high level of visualization, moreover, in a three-dimensional format, which is especially important for future physicians to understand the topographic and spatial relations of certain structures and parts in the body [1, 8].

The advantages of the research technology itself include cost-effectiveness, which is ensured by the relative availability of materials required by the research protocol. In addition, the use of complex and highly specialized equipment is not required to obtain finished products, which also simplifies the methodology. However, combining diaphonization with other technologies, including, for example, digital technologies, significantly expands the effectiveness and prospects of research. Important features of the method include its relative versatility, i.e., the ability to process organisms of different classes (fish, amphibians, reptiles, mammals), which is, however, limited by the size of the research object. Also, the diaphonization method is a relatively safe research method that does not require contact with hazardous substances or harmful fumes for a long time. The finished products are stored in closed containers and do not require direct contact with the product. This greatly simplifies the visualization of the finished product, makes it possible to use the method for educational purposes, and protects the researcher from deterioration of health during the preparation and dissection of samples.

However, like any research method, the diaphonization method has certain drawbacks. Among them are color loss, possible changes in tissue structure, which, although insignificant, can distort the results of research, which, however, requires further study and experimental

confirmation. Another peculiarity is the static nature of the method, i.e., the impossibility of using it to study the dynamic manifestations of the organism, which can, however, be studied by comparing similar samples. Also, diaphonization can be limited for large organisms or organisms with a complex structure, such as large and dense bones or exoskeletons, which narrows the choice of research objects. However, these shortcomings, in our opinion, are only certain features of the method that should be taken into account when conducting experiments using it to achieve the highest quality and most accurate results.

The success of diaphonization may depend on the type and concentration of solutions, the duration of its implementation, and the storage conditions of the drugs used, which may require appropriate optimization and testing [2]. However, in this regard, special attention should also be paid to the plasticity of the method, i.e. the possibility of changing it in order to optimize it in accordance with the histochemical characteristics of the tissues or organism that is the object of study. This creates really significant prospects for further development and improvement of the diaphonization method, which we see as one of our scientific tasks.

CONCLUSIONS

The method of diaphonization has a long history, which testifies to its importance in the study of anatomy and morphology of various tissues, organs, systems, and organisms. From early attempts to isolate internal organs and achieve their transparency to the modern use of chemical solutions, the diaphonization method continues to evolve and improve. The diaphonization method is extremely useful for scientific research in many fields. Its advantages include the ability to study internal anatomy without destroying the object, detailing structures, preserving samples for further research, and the ability to combine with other modern research methods. Despite its advantages, the diaphonization method also has certain limitations and disadvantages (possible deformation of structures, loss of color, time and space complexity, and limitations in application to certain types of organisms). With three main vectors of application (clinical, experimental and botanical), the diaphonization method is an important tool for researchers in many fields, which continues to evolve and find new applications, improving the overall scientific picture of understanding the structure of organisms and contributing to scientific progress.

PROSPECTS FOR FURTHER RESEARCH

The prospect for further research is, firstly, the application of the diaphonization method together with the methods of filling the vessels in order to study the anatomy and topography of the vessels supplying the brain in humans and some experimental animals; secondly, determination of the histological and histochemical features of various tissues of humans and experimental animals after the application of the diaphonization technique; thirdly, determination of the anatomical features of the jaws in experimental animals in normal conditions, in modeling fractures and contractures.

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CONFLICT OF INTEREST
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* Contribution: A – Work concept and design, B – Data collection and analysis, C – Responsibility for statistical analysis, D – Writing the article, E – Critical review, F – Final approval.