Review Article

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Staining of Nervous Tissue. Methodological Foundations

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ABSTRACT

Nervous tissue (neurons and neuroglia) are frequent objects of scientific research. Classical methods of nerve cell staining are the fundamental basis on which the design of a broad study of cerebral and cerebrospinal pathology can be built. This article provides an overview of methods for staining nerve cells.

Keywords: Neurons; Nervous System; Histology; Staining

Review

Nervous tissue (neurons and neuroglia) are frequent objects of scientific research. Classical methods of nerve cell staining are the fundamental basis on which the design of a broad study of cerebral and cerebrospinal pathology can be built. This article provides an overview of methods for staining nerve cells.

Painting by Nissl

Nissl staining is proposed for chromatophilic substance in the form of lumps or grains and nuclei and from the side of gliose tissue. For the study, small pieces are taken, no thicker than 0.3 cm. The best nerve cells. The condition of these parts of the cell allows us to judge the nature of changes and deviations from the normal equivalent picture of it. At the same time, the Nissl method reveals changes in alcohol fixation (96°), lasting from 5 days to 1.5 months. Alcohol needs to be changed several times. The longer the pieces are kept in alcohol, the better they are decreased, and in this regard, better results are achieved later when painting. Formalin fixation is allowed, but it is worse [1,2]. Only cell iodine and paraffin sections are suitable for painting, and the thinner the better. The most used paints are toluidine blue, thionine and cresylviolet in aqueous solutions (on distillated water); the first two in a dilution of 1: 1000 and the last - 0.5%. Favorskiy recommends using coloring solutions that have a cooking prescription of at least 3 months [3,4].

Painting technique:

A. Heating the slices in the paint over the flame of the alcohol lamp until bubbles appear (on the glass);

B. Rinsing in plain water;

C. Differentiation with pure 96° alcohol or aniline (the latter is prepared by mixing 1 part of aniline oil with 9-10 parts of 96° alcohol; aniline alcohol can be stored in dark flasks for some time, but it is better to cook it fresh every time);

D. Enlightenment of sections (kajeput oil, colorless turpentine, xylene);

E. Balsam (Canadian, fir).

During painting, the following points should be kept in mind.

a. Freshly filtered paints are used. To avoid misunderstandings associated with the use of dense varieties of filter paper, which

retain a lot of paint, you need to use thin ash-free filters (round). In the absence of such, the paint is carefully drained from the flask (without shaking).

b. Celloidine slices are heated in paint on a watch glass; paraffin - directly on the object (of course, after de-waxing), on which the paint is poured. Heating is carried out very carefully, until the first bubbles appear on the glass (hour or subject).

The slices should be heavily repainted. If there is insufficient painting, reheating is allowed. After heating, to achieve greater colorization, it is not a bad idea to leave the slices in the paint for some more time (at room temperature), for 0.5-1 hour and even overnight. With such a delay, the cell iodine slices remain in the same watch glasses in which the heating was carried out, and the paraffin ones - in Petri dishes (on matches, cut down) or in flat cups. It is difficult to give precise instructions about time here, since the quality and demonstrativeness of the coloring are determined by many factors, such as the coloring power of a particular paint, fixation, filling, and most importantly - which department of the nervous system. It is necessary to adapt somewhat in these cases, and then the most advantageous technique will be revealed, i.e. is it possible to limit yourself to one heating, is it necessary to combine this heating with subsequent leaving in the cold (more precisely, at room temperature) or, finally, it is possible to completely abandon heating and it will be enough to limit yourself to only one staining at room temperature for more or less a long time (up to a day). They also use a thermostat with a temperature of 37° C. In particular, nodes from the solar plexus are very well stained with toluidine blue and without heating, especially on paraffin sections, in terms of 2 hours to a day [5].

c. Differentiation is the most crucial moment. It is also necessary to adapt to it. The intensity of the cross-section painting will determine whether it is enough to do with one 96° alcohol or a stronger differentiating agent - aniline alcohol - is required.

Differentiation of 96° with alcohol is relatively slow and sometimes takes many minutes or even tens of minutes (depending on the degree of painting). Aniline alcohol differentiates very quickly within a few minutes. Differentiation is carried out under the control of a microscope. The cut currently discolors and becomes pale blue. When the details of the cells (nuclei, granularity, protoplasm) have clearly appeared, differentiation is stopped, the cut is quickly squeezed out with filter paper folded in 3-4 layers, and kaeput oil is poured on it (and in the absence of such, a simple colorless turpentine). Maintain the cut until enlightenment, if on- , before - re-drain and pour oil (turpentine), combining with squeezing with filter paper. After enlightenment, the cut is thoroughly washed with xylene (pouring again). Thorough washing with xylene is necessary to completely remove the remnants of aniline oil (in the case of aniline alcohol) and turpentine, otherwise the cut is diffusely painted over after a few days (from the extracted paint). Finally, it is possible to do not only without kaeput oil, but also without turpentine, using, along with the differentiating agents, also absolute alcohol. Finishing the differentiation with absolute alcohol, the cut is dried with filter paper and treated with xylene. Re-pouring xylene and squeezing the slice, they quickly achieve its enlightenment. Strictly speaking, absolute alcohol is more desirable for celloidine sections, as for paraffin sections, they, as thinner, are easily illuminated by xylene alone and after 96° alcohol (with pressing.). Then they are enclosed in a balm. The preparations gradually fade, so it is better to store in the dark. Toluidine blue is the most practical.

The Gros - Bilshevsky - Lavrentiev Method (for Peripheral Nerves and Nerve Endings)

Preparation of Ammonia Silver: To any amount of 20% silver nitrate, 25% ammonia 2 is added drop by drop until the resulting brown-brown precipitate is dissolved with strong stirring and further addition of ammonia. It is necessary to catch the moment when from one added drop the entire precipitate will dissolve. It is impossible to pour a single extra drop of ammonia, so it is better to bring the dissolution of the precipitate only to the stage of opalescence of the solution.

Painting Technique:

A. Fixation of the Pieces in the AFA Mixture for 1 Hour.

Liquid composition: 20% neutral formalin (formalin neutralization - see page 9), 96° alcohol and 1% arsenic acid solution.

All the components of the mixture are taken equally. Arsenic acid dissolves poorly, so it is prepared in advance, about a week in advance, or put the solution in a thermostat at 37° for 2-3 days. The whole mixture is prepared as needed.

B. Additional fixation of the pieces in 20% neutral formalin - from a few days to 3 weeks. Optimal terms of fixation in formalin are established experimentally. So, for better impregnation of nerve endings in the heart and bladder, short terms (2-4 days) are most advantageous, and for nerve plexuses of smooth muscles, it is desirable to stay in formalin for 10 to 20 days.

a) When working with such surrogates of celloidine as film, one cannot be afraid of its dissolution from the use of absolute alcohol, at least during the insignificant time required for differentiation.

b) Weaker solutions of ammonia, as a rule, lead to failures. In any case, the delay of the material in neutral formalin for more than 1 month has a bad effect on impregnation. After fixing, the pieces are quickly rinsed in distilled water.

c) Obtaining slices by freezing. The thickness of the slices is determined by the objectives of the study and varies quite widely (from 20 to 100 microns, and on average from 30 to 50 microns).

d) The slices from the knife of the freezing microtome are transferred to the same water in which the piece was washed.

(The amount of washing water is approximately 100.0-200.0 ml D per 1 small piece). .

e) After a short washing in distilled water (1-2 min.), the slices are transferred to a 20% solution of silver nitrate for 5-10 minutes (can be kept up to 30 min.). More precisely, the residence time in the solution of silver nitrate is established after several samples. If, with further manipulations, it turns out that the slices are impregnated very quickly, then the processing time in silver nitrate is reduced (to 1-2 minutes) and, conversely, with poor impregnation, it is increased to 1 hour.

f) From silver nitrate, the slices are transferred directly (bypassing water) into 20% neutral formalin, prepared on tap water and poured into 4-5 small cups (or jars). The slice is quickly transferred from one cup to another, as the turbidity forms around it. In the last cup, the turbidity should not leave. The whole procedure through formalin takes about 10 minutes.

g) From the last portion of formalin, the slice enters an opalescent solution of ammonium silver (preparation - see above). Such a slightly cloudy solution is poured on a watch glass (of such dimensions that it fits freely on the microscope table). Before transferring a slice to this solution, a few more drops of 25% ammonia are added to it (about 1 drop per 1.0 ml of solution). The process of impregnation of fibers is observed at low magnification of the microscope. Initially, the slice turns yellow, then nerve fibers and cells begin to appear, which first turn brownish, and then black. At the same time, the nuclei of other tissues can also be impregnated, but always much weaker than nerve fibers and cells.In the case when the nuclei of other tissues are impregnated very sharply and first of all, this indicates an insufficient number of drops of ammonia added to ammonia silver. Therefore, when conducting the next cut, ammonia alcohol is added to the ammonia silver solution (of course, a new portion is taken) by a few drops more (2-3 drops) than it was the first time.

h) When microscopic examination shows a sufficiently sharp impregnation of nerve elements, the slice is transferred to diluted ammonia (1 part 25% ammonia and 2 parts distilled water) for 10-15 minutes (to stop impregnation).

i) Thoroughly wash the slices in several portions of distilled water for several hours (can be left overnight).

j) Gilding in weak solutions of chlorine gold (approximately 1-2 drops of 1% solution of chlorine gold per 1.0 ml of distilled water). The slices are kept for 0.5-1 hour or longer, until they acquire a gray color.

k) Rinse in tap water.

l) Hyposulfite solution - 5-10 minutes.

m) Thoroughly washed in tap water, repeatedly changing it, for several hours.

n) Washed slices are optionally tinted with any paints.

o) Dehydration, enlightenment, and imprisonment in Canadian balm.

With this method of silvering, the quality of formalin, water, silver, and the purity of dishes are extremely important. The technique is difficult and requires some experience in the sense of clarifying certain stages of silvering in the process of work. They only work with glass needles.

The Bilshovsky Method (for the Central Nervous System and Autonomic Nodes (Cells and Fibers)

Preparation of Ammonia Silver: Take any amount of 10% silver nitrate solution (5.0 ml is enough for a small number of slices) and add strong ammonia (25%) to it drop by drop. First, a brown suspended precipitate (silver oxide hydrate) is formed, which then, with the subsequent addition of ammonia, begins to dissolve, soluble ammonium silver is formed. Our alcohol is poured drop by drop and very carefully, the test tube or beaker in which it is usually produced is constantly shaken. The precipitate gradually dissolves, passing through the opalescence stage of the solution. It is very important not to pour a single extra drop of ammonia. About 15 drops of 25% ammonia will be required for 5.0 ml of 10% silver nitrate solution. The criterion for a properly prepared solution of ammonium silver is a very weak smell of ammonia. If the latter is accidentally poured and the ammonia silver has a pungent smell of ammonia, then either a few small crystals or a few drops of a solution of silver nitrate should be added to it. Again, turbidity is formed, which is again carefully dissolved with ammonia. An excess of ammonia in an ammonia silver solution is undesirable because dissolved ammonia silver easily decomposes in an alkaline environment, which can lead to contamination of the drug by precipitation.

It is not necessary to filter the ammonia silver prepared in this way.

Painting Technique:

A. Fixation in 15-20% formalin, preferably neutral. The slices are frozen and as thin as possible, no thicker than 10-12 microns (frozen slices give the best results).

B. First, the slices are placed in 96° alcohol (for the purpose of degreasing) for 2-3-5 minutes, then washed in water, first tap water (in a large cup) - 5-10 minutes. and then in the destilled- 5 min. and longer (the longer, the better).

C. From distilled water, the slices are transferred to a cup with a 10% solution of silver nitrate and placed in a thermostat at a temperature of 37-40° for 15-25 minutes. (If you must use an electrified drying cabinet).

D. By means of light bulbs located inside it, then the cup with silver nitrate, where the slices lie, is wrapped in black paper).

E. After silver nitrate, the slices are quickly rinsed in distilled water and transferred to a freshly prepared solution of ammonium silver for 15-20 minutes (already at normal room temperature). Here the slices turn yellow. It is necessary to pay attention to the fact that rinsing in distilled water before ammonia silver should be fast (3-5 seconds), but at the same time sufficient, and therefore it is recommended to rinse only one slice at a time. With simultaneous rinsing on a needle of several slices at once, as is often done, there may remain unwashed silver nitrate in their folds, which then in ammonia silver will lead to the formation of turbidity (silver oxide hydrate) and to contamination of the drug by precipitation.

F. After ammonia silver, the slices (one at a time) are quickly rinsed in distilled water (3-5 seconds) and placed in 5% formalin "a distilled water for 1-2 minutes. In formalin, the slices immediately turn dark brown - 'silver is restored.

G. Then the slices are thoroughly rinsed in tap water and transferred to a 5% aqueous solution of hyposulfite "a distilled water for 5-10 minutes or more to remove the unrecoverable silver.

H. Rinse again in tap water (10-15 minutes). Monitor the quality of impregnation under a microscope.

Thick slices can look very dark and rough. In this case, they are kept in hyposulfite for a longer time, 0.5-1 hour or more, monitoring every 20-30 minutes, until the maximum possible clarity of the drug. The treatment in hyposulfite can thus be considered as a kind of differentiation. It is impossible to leave it in it for a very long time, impregnation weakens sharply. After hyposulfite and washing in water, the cut is carried out through alcohols, carbol-xylene (or some other illuminating agent), xylene and enclosed in a balm. There is no need to finish painting the preparations. If possible, the sections are gilded (after reduction in formalin and rinsing in water) in a weak or strong solution of chlorine gold After gilding, rinsing in tap water, treatment in 5% hyposulfite solution, etc. Gilded preparations are gray or grayish purple, not gilded - brownish-yellow. Gilded preparations are better and more demonstrative.

The general scheme of silver impregnation is thus presented in the following form:

a. Alcohol 96°-3-5 min.

b. Water is simple and then distilled for 5-10 minutes.

c. 10% solution of silver nitrate, in the thermostat (in the dark) - 15-25 min.

d. Distilled water - 3-5 seconds.

e. Ammonia silver -15-20 min.

f. Distilled water - 3-5 sec.

g. Formalin 5% -1-2 min.

h. Tap water - 3-5 min.

i. Gilding - 3 - 5 min.

j. Tap water

k. Hyposulfite 5% - 5-10 min.

l. Tap water, thorough washing.

m. Alcohols, carbolxylene (or other brightening agent), xylene, balm.

Work with glass needles.

The Kahal - Favorsky Method

This method is proposed for the impregnation of nerve cells and fibers. Impregnation occurs in a piece [6].

For fixation, take small pieces, no thicker than 0.3-0.5 cm.

A. Fixation during the day in 80° alcohol, to which either formic acid is added (when working with pieces of nervous tissue or other, but loose objects), or strong (or icy) acetic acid (with dense objects and when they mean mainly nerve fibers). Both acids are added at the rate of 30 drops for every 50.0 ml of 80° alcohol.

B. Carefully prune the pieces (up to 0.2-0.3 cm) and transfer to 85° alcohol for 24 hours.

C. A mixture of 96° alcohol with 25% ammonia (1.0 ml of ammonia is added for every 50.0 ml of ethyl alcohol) - 2 days.

D. The pieces are transferred to distilled water, in which they are kept until they sink.

E. Dried with filter paper and placed in pyridine for 1-2 days (the thicker the piece, the longer).

F. Washed in running water for 24 hours.

G. The pieces are placed in 3% silver nitrate for a period of one to three weeks, preferably in a thermostat at a temperature of 37° (in the latter case, they are kept for up to 10 days). The silver solution is changed several times (2-3 times).

H. Quickly rinsed in distilled water and transferred to the developer of the following composition: distilled water - 100.0 ml, pyrogallic acid-2.0-4.0 g, neutral formalin-10.0 ml. Keep 1-2 days. The process of manifestation must necessarily go in the light.

I. It is carried out through alcohols and poured into celliodine. To avoid excessive extraction of silver compounds through alcohols and liquid celliodine, the pieces are carried out quickly, approximately in the following terms: 70-80° alcohol - 0.5- 1 hour, 96° alcohol - 3 hours, absolute alcohol - overnight, liquid celliodine - 8-24 hours, thick celliodine - 1 day. Seal in a clean portion of thick celliodine for 2-3 days.

J. Obtaining slices and conventional wiring through alcohols,

carbol-xylene, xylene, and encapsulation in Canadian or fir balsam.

When shifting the pieces, use glass objects [6].

Chapter XIII

Amyloid Staining:

A. Staining With Gentian Violet or Methylviolet

A very beautiful demonstrative coloring based on metachromasia. It is produced on frozen slices obtained from a fresh or a piece fixed in absolute alcohol (in the latter case after thorough washing in running water for several hours) [7]. It is possible to color even after formalin fixation.

B. Painting technique:

a. Frozen slices are transferred for 1-2 minutes into a 1% aqueous solution of gentian violet (or methyl violet).

b. Rinse in water and differentiate in 1% acetic acid for 0.5-1 min., until the cut becomes pale purple.

c. Rinse in clean water for several minutes.

d. A slice is caught on a slide and enclosed in a drop of saturated aqueous solution of potassium acetic acid (it can also be enclosed in glycerin, but then the meta-chromatic staining soon disappears).

e. Cover glass. They are fixed with paraffin or Mendeleev putty. The amyloid substance turns reddish-purple, other tissues turn blue [7-10].

Coloring with a Kongorot

A widespread method. Coloring works well both after alcohol and after formalin fixation and on any slices. Colored preparations allow for the conclusion of a Canadian balm and are preserved for a long time [9,11].

A. Painting technique:

a. The slices from the water are transferred to a 1% aqueous solution of kongorot (prepared on distilled water) for 1-2-3 minutes.

b. Washed in plain water for 1-2 minutes.

c. Differentiate (in a cup or directly on the slide) 70-80° with alcohol 0.5-2 min., and sometimes more, until the drug turns from bright red to slightly pink. With a sharp repainting , it is necessary to differentiate up to 10-15 minutes . It is good to differentiate under the control of a microscope.

d. Wash in plain water for 1-2 minutes.

e. The cut is tinted with some alum hematoxylin (Boehmer, Delafield) or hematein and washed again in water for 2-3 minutes.

f. Dehydrate the cut 96° alcohol.

g. Enlighten in carbol-xylene or creosote, washed with xylene, and enclosed in balsam (Canadian, fir).

Amyloid turns red [12,13]. Thus, the data presented in the article will serve as a fundamental basis for further study of cerebral and cerebrospinal pathology, the development of effective methods for diagnosing and correcting disorders of the nervous system.

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