A MODIFICATION OF PETERS' METHOD AS APPLIED TO CYTOLOGICAL INVESTIGATIONS

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UDC 611.8-018.82-086

To detect the clearly defined network of fibers as well as the fine histochemical features of nerve cells in the same paraffin section a combined approach is suggested including Peters' impregnation method and Brachet's histochemical method. A scheme of additional treatment of the paraffin sections for the test was drawn up. Analysis of the results showed that the additional treatment of the sections not only allows the two techniques to be combined, but also appreciably improves the quality of impregnation; in particular, the axons of the nerve cells can be seen almost completely.

Among the many methods used by neurohistologists, an important place is occupied by impregnation, by means of which the specific features of neurons and their processes can be analyzed. However, the various methods of silver impregnation of nerve tissue do not always yield constant results. The difficulties arising with these tests are due both to complex physicochemical changes which lie at the basis of impregnation and the widely differing properties of nervous structures as a result of differences in their development and the variability of their functional state. Analysis of the pictures of nerve tissue in specimens impregnated with silver may therefore not always be complete, and it demands a critical approach.

To increase the reliability of impregnation methods, and to obtain more complete morphological pictures, an extremely promising method is to combine impregnation with selective methods of staining nerve tissue. This not only yields good quality survey preparations but also enables the fine structure and histochemical properties of the nerve cells to be studied. The aim of such methods is to detect both the clearly defined network of fibers and also the fine cytological details of the nerve cells on the same section.

Having regard to the demands of the problem, the choice of Peters' silver impregnation method was made [3]. Among the impregnation methods requiring formalin fixation and embedding in paraffin wax, in the writers' view this method is one of the best for it gives comparatively stable results (impregnation takes place at a definite range of pH, from 8.2 to 8.3), and in addition it allows the selective action of the silver ions, so that the processes of the nerve cells are impregnated but not their bodies. This selectivity of the impregnated effect of Peters' method is evidently attributable to the electrocolloidal properties of the cells. This hypothesis is confirmed by experimental observations [2, 4] showing that during staining of the cells in fixed preparations, under conditions similar to the reaction in Peters' method, the basophilic elements of the cytoplasm and nucleus become electrically positive, as the result of which adsorption of silver ions does not take place on the surface of the cell bodies.

To detect the bodies of the nerve cells in impregnated sections, these are then stained by various dyes: cresyl violet, thionine, methylene blue, or hematoxylin. In addition to the additional counterstaining, the writers propose the use of methylene blue–pyronine as in Brachet's method [1] for additional staining of the nerve cells. This method is one of the most specific and differential of those used to study the fine structure and histochemical properties of the nerve cell and its components, and it is particularly successful in sections fixed with Carnoy's fluid. Because of the two-color stain, the RNA and DNA revealed by this method

Laboratory of Neuronal Structure of the Brain, Institute of the Brain, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR B. N. Klosovskii.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 75, No. 4, pp. 122-124, April, 1973. Original article submitted June 9, 1972.

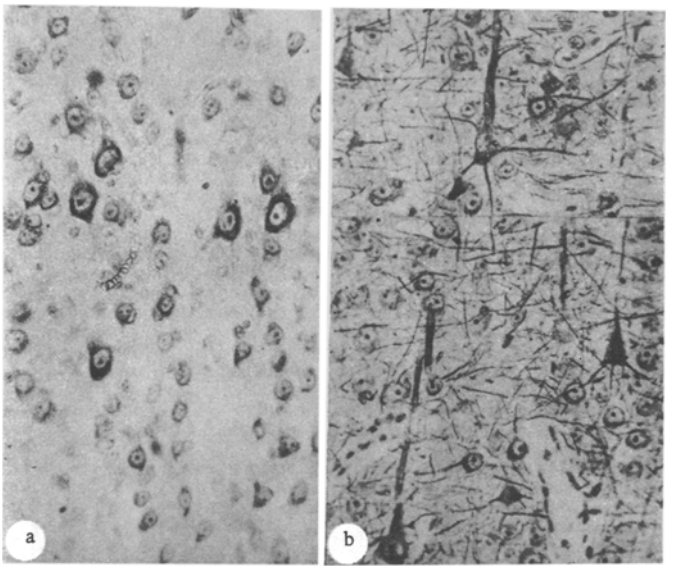

Fig. 1. Temporal cortex of the cat's brain, layer IV: a) Brachet's method; b) Peters' method with counterstaining by Brachet's method, 280×.

can be clearly detected in the cytoplasm and nucleus of the nerve cell. Bearing in mind that Peters' and Brachet's methods had many advantages and allowed tests to be carried out on thin paraffin sections, the writers concluded that it was essential to combine these methods. However, on the attempt to impregnate sections fixed with Carnoy's fluid by Peters' method, negative results were obtained (Fig. 1). During empirical selection of optimal conditions for impregnation and further staining, a scheme of preparation of the nerve tissue was evolved that was based on treatment of the dewaxed sections before impregnation with 20% neutral formalin solution and placing the sections before staining in alcohol acidified to pH 4.8.

Analysis of the sections obtained by this scheme showed that this additional treatment of the sections not only enabled the two methods with different fixing fluids to be combined, but that it also appreciably improved the quality of impregnation; in particular, the axons of the nerve cells could be seen almost completely (Fig. 1a, b).

Description of the method. Fixation. Small pieces of brain are fixed in Carnoy's fluid and embedded in paraffin wax. Sections are cut to a thickness of 10 to 30 μ, fixed with albumin to the slide, dewaxed, rinsed in distilled water, and immersed for 2-3 h in 20% neutral formalin solution.

Impregnation. After rinsing in distilled water the sections are placed for 15 h in the following mixture: to 200 ml of a 0.5% solution of dry crystalline egg albumin with 2-3 drops 5% ammonia (pH of mixture 8.2-8.3) 7.5 ml of 2% silver nitrate solution is added, aiming to obtain a mixture with opalescent properties and not allowing precipitation. The sections are then carefully washed in several portions of distilled water for 5 min and transferred for 10 min into a mixture of 1% hydroquinone solution, made up in a 10% solution of crystalline sodium sulfite ($Na_2SO_3 \cdot 6H_2O$), after which they are again washed in tap water for 10 min and rinsed in distilled water. The sections are then placed in 0.2% gold chloride solution for 10 min. After washing in distilled water they are transferred to 1% oxalic acid solution for 10-15 min. They are then washed in tap water for 5 min, rinsed in distilled water, and immersed in 5% hyposulfite solution for 10 min.

Staining. After rinsing in distilled water the sections are transferred for 1 h into 70° alcohol, acidified with acetic acid to pH 4.8, rinsed in distilled water, and differentiated in acetone, from which they are carried through acetone–xylol (1:1) into xylol and mounted in balsam.

It is considered that this combined method, in which impregnation is combined with histochemical staining, not only allows morphological survey pictures of cells and fibers to be obtained in thin paraffin sections under normal conditions, but also allows their changes to be studied during development and under pathological conditions.

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