METHODS

A Method for Staining of Semithin Sections of the Brain

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A double-color method for differentiated staining of the perikaryon and neuropile without resin removal is proposed.

Key Words: *morphological study of the brain; semithin section staining*

Analysis of semithin sections is an obligatory step of correct electron microscopic study in modern morphology, which, in addition to its accessory role in electron microscopy, is significant by itself. Due to good preservation of structures in technological preparation of the material for electron microscopy and little thickness (0.5-2 μ) of the section, it is possible to obtain higher resolution in comparison with common histological methods.

The stains do not or poorly penetrate into epoxy resins, and therefore the number of methods for staining sections involving the use of resins is limited. Toluidine blue staining is used most often [1,2]. This method gives quite satisfactory results with the majority of tissues, including the peripheral nerves [4,5], and for this reason it has been used for a long time. However, some problems in studies of the CNS cannot be solved by examining the sections stained with toluidine blue. Toluidine blue clearly shows the cell cytoplasm in many tissues, but not in the brain: the perikaryon fuses with the neuropile. Only in sharply pronounced disease (diffuse tigrolysis) the perikaryon is seen against the background of the neuropile, but the staining method suitable only for rough destruction cannot satisfy morphologists.

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Attempts at solving these problems by using multicolor stains, which are used after the resin dissolution, were described. However, the problem is complicated by the fact that the resin is removed with concentrated alkali solutions, which remove the sections from the slides together with the resin. For this reason the methods involving resin dissolution are not widely used [3].

In our study of brain cortex regeneration we faced the problem of detecting fused neurons (dikaryons) and evaluating cell size and cytoplasm structure. All this could be realized only on condition of differentiation between the perikaryon and neuropile, which we did by using our method for staining semithin sections.

MATERIALS AND METHODS

The material (rat brain) was fixed by perfusion with 2.5% glutaraldehyde solution in phosphate buffer (pH 7.4) with 4.5% sucrose. After perfusion, the brain was removed from the skull and left in the fixative of the same composition for 24 h in a refrigerator. Fragments of the cortex (1 mm^3) were then cut out and left in the same fixative for another 24 h, after which were postfixed in osmium tetroxide by the standard method and embedded in araldite. Since our aim was to examine the maximum volume of the tissue, we sliced 3-μ sections,

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but the proposed method can be used for staining semithin sections of any thickness.

The slices were transferred from the ultramicrotome into a droplet of distilled water on a slide. Before the droplet dried, the slides were transferred to a plate for manipulations with semithin sections from the ultramicrotome kit. The temperature of the plate did not provide sufficiently strong fixation of the sections on the slide, and therefore after water dried, the slides were additionally warmed for 2-3 sec in the flame of an alcohol burner. The epoxy resin was not removed from the sections. We used ethanol solutions of stains which did not form a droplet above the slice (like water), but spread over the slide. In order to prevent leakage, a piece of filter paper covering the section or group of sections, was placed onto them and a droplet of stain solution was pipetted on this paper. Two saturated filtered ethanol solutions were used: methylene blue and basic fuchsin.

The staining was carried out on a plate. A droplet of methylene blue was pipetted onto filter paper first. The ethanol solution rapidly evaporated on the hot plate and therefore had to be added 2 or 3 times, so that the duration of staining was 10-12 sec. The slides with the sections were then washed in tap water and then in distilled water. After drying on the plate, fuchsine staining is carried out similarly, but for just 5-6 sec. The duration of staining can be modulated in order to obtain the desired color and intensity of staining.

The sections stained with one or both stains can be differentiated with ethanol. The color of the sections seems to depend on the amount of osmium bound to the structures. The center of the section, where osmium penetrates less, is stronger stained with fuchsine. However, the objects of interest (perikaryons) are discernible in the red center and at the green-violet periphery of the section. Hence, the proposed staining method has solved our problems and can be useful in all cases when the neuron cytoplasm is to be examined.

RESULTS

The aim of our study was to detect dikaryons in the cortical stroke area and to evaluate their density. Figure 1 shows a cortical section stained with toluidine blue. Close disposition of two nuclei (seeming to be located directly in the neuropile) just suggests that it is a dikaryon, as the cytoplasm and neuropile are not differentiated and hence, we cannot say for sure what is there between the nuclei: if it is the cytoplasm — we see a dikaryon, if the neuropile — there are two separate cells.

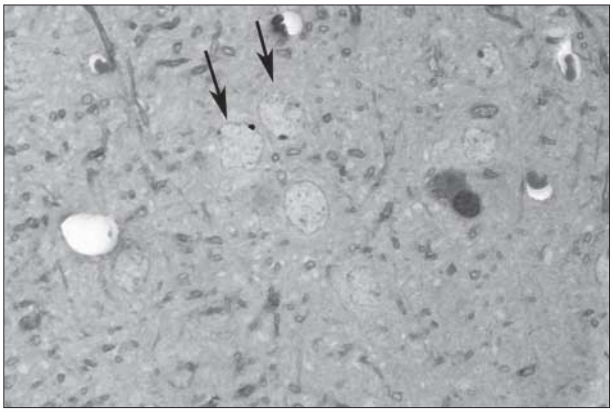

Fig. 1. Semithin section of prefrontal cortex stained with toluidine blue (×600). Arrows show the nuclei.

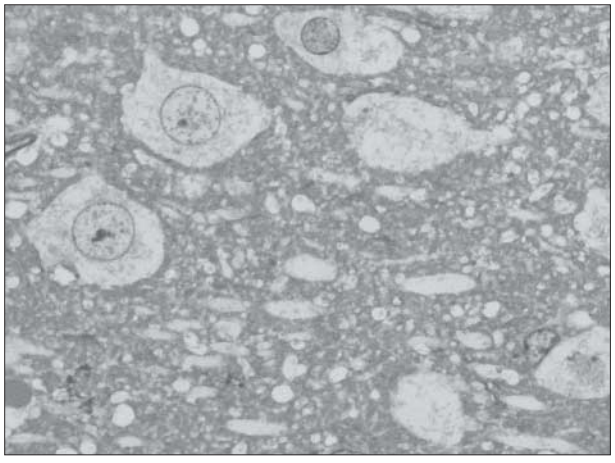

Fig. 2. Semithin section of prefrontal cortex stained with toluidine blue, 7 days after ischemic stroke (×600).

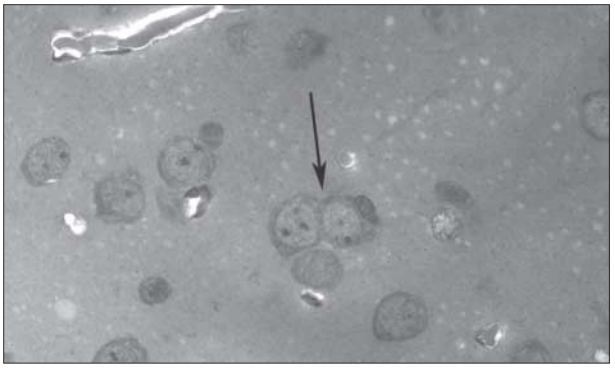

Fig. 3. Semithin section of prefrontal cortex stained with alcohol solutions of methylene blue and basic fuchsine, 2 months after ischemic stroke. Arrow shows a dikaryon (both nuclei in the same cytoplasm; ×600).

The perikaryon stained with toluidine blue is clearly seen in hypoxic destruction of the majority of cytoplasmic structures: sharply manifest chromatolysis makes the cytoplasm, containing almost no ultrastructures, clearly seen against the background of the neuropile (Fig. 2). However, this case just

emphasizes the narrow sphere of toluidine blue staining application in neuromorphology.

The proposed method of double staining with methylene blue and Fuchsine without removal of the resin detects the neuronal cytoplasm, due to which it is possible to identify the dikaryon, because the cytoplasm of this binuclear cell is clearly visualized (Fig. 3). Despite recovery of the cytoplasmic ultrastructures 2 months after ischemic stroke, this cell is clearly seen against the background of the neuropile.

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