METHOD OF DETECTING FATTY ACIDS IN NERVE FIBERS

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A modification of Fischler's method is described, enabling fatty acids in nerve fibers to be detected more accurately.

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Initially, to detect fatty acids in nerve fibers we used Fischler's method [1] without modification. However, having used this method for a long period of time we became aware of certain disadvantages: differences between results depending on times of fixation and time taken to wash copper acetate from the sections; lithium hematoxylin does not always give reliable results; the process of differentiation requires a more accurate specification; the copper salts of the fatty acids formed during the procedure are readily soluble in water and aqueous solutions, so that all specimens which we prepared initially and which were mounted by Fischler's method in glycerol-gelatin proved unsuitable. We give below details of the method after introduction of certain modifications.

Main Solutions: 1) lithium hematoxylin. Solution A: 10% hematoxylin solution in absolute alcohol. Solution B: 0.5% solution lithium acetate. Immediately before use, equal parts of solutions A and B are mixed. 2) Differential mixture: 5 g borax, 5 g potassium ferricyanide, 200 ml distilled water. 3) Saturated solution of copper sulfate or copper acetate (copper nitrate is unsuitable).

DESCRIPTION OF METHOD

Fig. 1. Part of the nervous apparatus of the sinus hair of the skin of an albino mouse. Variant of method with sections treated by means of copper acetate mordant. Objective 20, ocular 10.

1) Fixation of material in 12% magnesium or calcium formalin for 1-5 days (if the fixation time is increased the results are less satisfactory, although a positive reaction may be obtained). 2) Preparations of frozen sections 15-30 μ in thickness. 3) Washing sections in distilled water for 10 min. 4) Treatment of sections in a saturated aqueous solution of copper sulfate or acetate mordant at 37° for 24 (or possibly up to 48) h. 5) Washing sections in distilled water for not more than 2-5 min. 6) Staining in lithium hematoxylin for 20-30 min. 7) Differentiation of sections without washing in two portions of a borax-potassium ferricyanide mixture to give a selective blue coloration of the nerve fibers. Decoloration of the sections takes place more rapidly in the first portion of the mixture. Differentiation in watch glasses under microscopic control. 8) Washing sections in one portion of distilled water for not more than 5-10 sec and transfer without delay into 96% alcohol. 9) Mounting sections in balsam (in benzene) after passage, through 96% alcohol, carbol-benzene, and benzene.

Control sections are preliminary extracted for 24 h in a 1:1 mixture of alcohol and ether, and then treated with copper sulfate, stained, and differentiated. Nerve fibers are not stained the specific blue color in the control section.

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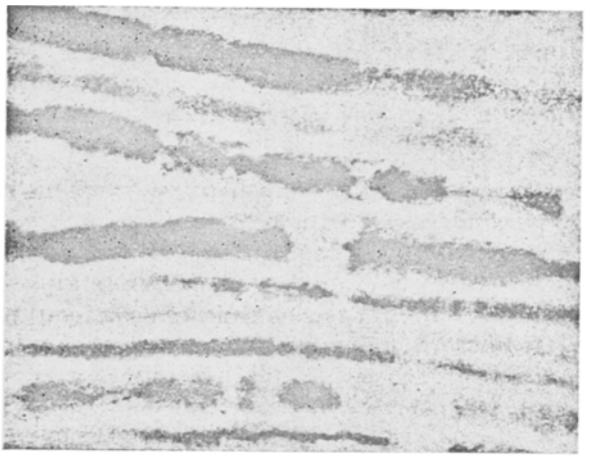

Fig. 2. Sciatic nerve of a cat. Axons most intensively stained, membranes least intensively; region of node and cleft not stained. Variant of method using copper sulfate. Objective 60 (immersion), ocular 10.

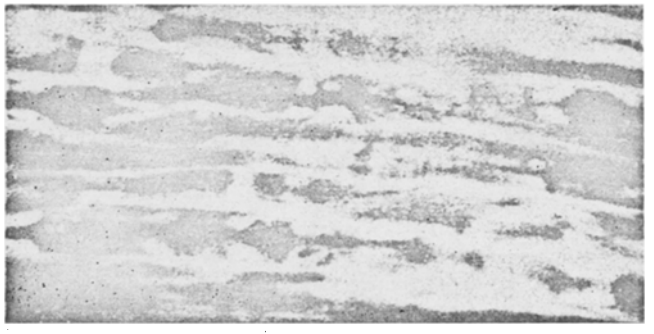

Fig. 3. Sciatic nerve of a cat. Axons stained most intensively, membranes least; region of node (including axon) and cleft not stained. Variant of method using copper sulfate. Objective 60 (immersion), ocular 10.

RESULTS

The axons of the nerve fibers are most intensively stained a dark blue color; the myelin sheath is less intensively stained; the Schmidt–Lantermann clefts and the region of the nodes of Ranvier are not stained (Fig. 1-3).

LITERATURE CITED

1. A. G. E. Pearce, Histochemistry, Theoretical and Applied [Russian translation], Moscow (1962), pp. 287, 783.