SILVER IMPREGNATION OF GLIA AND NERVE FIBERS IN PARAFFIN SECTIONS AFTER FORMALIN FIXATION *

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Silver impregnations of nerve fibers and glia comprise a formidable number of long and tedious processes. Many of these methods give beautiful results, but, as they require special fixatives or lengthy and tedious pretreatment, they are impracticable for use in the majority of laboratories where formalin is the routine fixative. Foot [1, 2] has obtained satisfactory glial impregnation in frozen sections after fixation in neutral formalin and, with his work as a starting point, I have endeavored to devise a method that would be applicable to paraffin sections as well.

Using the silver diammino hydroxid recommended by Foot,[2, 3, 4] as a result of his tests of the work of Kubie and Davidson [5] and his formol-sodium carbonate reducer, I experimented with paraffin sections after formalin fixation. The experiments included application on the slide, before impregnation, of reagents advocated as sensitizers of glia to silver. Ammonium bromid,[6] ammonium hydroxid,[7] hydrobromic acid,[7] pyridin,[3] Carnoy's fluid,[8] and other reagents that have been of value in neurological methods were tried without success. Del Río-Hortega's [9] results with formalin-uranium nitrate fixation preparatory to silver impregnation of frozen sections led me to precede silver impregnation of formalin-fixed paraffin sections by treatment with uranium nitrate. A differential reaction to silver was immediately apparent. There was no precipitate deposited on the slide and the sections showed argentation of the fibrillar elements; nerve fibers, fibrillar astrocytes, reticulum and collagen were impregnated. After uranium nitrate, silver staining was so intense that it became necessary to use the silver diammino hydroxid at room temperature rather than at 50° C, and to reduce the time from 30 minutes to 20 seconds. Uranium nitrate in 1 per cent solution proved most satisfactory, but exposure of more than 5 seconds inhibited impregnation. The other nitrates were of no value.

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The previous experiments were repeated, introducing the additional step of uranium nitrate, because sharper definition of nerve fibers, more complete staining of glial elements, a clearer background, and an elimination of reticulum and collagen were desirable. Hydrobromic acid accomplished the desired results with the greatest consistency and proved of especial importance as a differentiating factor. Substitution of uranium nitrate for the sodium carbonate of Foot's reducer further accentuated the glial fibers. The gold toning of Foot's revised Variant 3 [4] (1: 500 gold chloride, 10 minutes; formalin 5 cc., oxalic acid 0.5 gm., water 100 cc., 10 minutes; 5 per cent sodium thiosulphate, 10 minutes; wash in tap water after each step) tended to clear the background and, where glial fibers showed granular deposits of silver, to give them a more solid and fibrillar appearance. However, the improvement was not consistent and, as it failed to differentiate glia from nerve fibers, it is not included as an essential feature of the stain.

All experiments were carried out on material that had been fixed in unneutralized formalin from two to twenty-four hours after death or removal.

Technique

Fixation and Embedding: Fix tissues in 10 per cent formalin, wash in tap water, dehydrate in alcohol, clear in chloroform, and embed in paraffin.

Bromuration: Pass paraffin sections through xylol and graded alcohols, rinse in distilled water, and place in 34 per cent hydrobromic acid for 30 minutes.

Sensitization: Wash in distilled water 10 to 20 seconds and flood the slide with 1 per cent uranium nitrate (sodium free) for 5 seconds or less.

Impregnation: Wash in distilled water 10 to 20 seconds and place for 20 seconds in silver diammino hydroxid:

To 5 cc. of 10.2 per cent silver nitrate add ammonium hydroxid drop by drop until the precipitate which forms is dissolved. Add 5 cc. of 3.1 per cent sodium hydroxid and just dissolve the resulting precipitate with a few drops of ammonium hydroxid. Make the solution up to 50 cc. with distilled water.

Reduction: Wash in distilled water 2 seconds and agitate each

slide separately in the following reducing solution until it ceases to give off a brown cloud:

Distilled water 50 cc., 40 per cent neutral formalin (neutralized with magnesium carbonate) 0.5 cc., 1 per cent uranium nitrate 1.5 cc.

Counterstaining and Mounting: Wash in distilled water, counterstain with eosin, dehydrate in alcohol, clear in xylol and mount in Canada balsam. Argentation frequently allows hematoxylin to be used as a nuclear stain, but bluing must take place in tap water, as ammonia dissolves the silver.

Distilled water is used in the preparation of all solutions. The uranium nitrate solution and the 10.2 per cent silver nitrate keep indefinitely and the silver diammino hydroxid keeps for a week or more in amber, glass-stoppered bottles. The impregnating and reducing solutions retain their activity in Coplin jars for two days. The hydrobromic acid may be kept in a Coplin jar and used repeatedly for an indefinite time. It is important that the ammonium hydroxid be kept in a well stoppered bottle.

Results

Ganglion cells, nerve fibers, glia cells and their processes are black. Tissues fixed twenty-four hours after death or removal show excellent impregnation of nerve fibers and fibrous astrocytes, but the processes of protoplasmic astrocytes and oligondendroglia cannot be demonstrated when more than six hours have elapsed before fixation. In fresh fixed tissue all the fibers are sharply defined, but tend to become granular when fixation is less prompt. Although differentiation between nerve fibers and glia must be on a morphological basis, the method has the advantage of being quick, simple, and applicable to paraffin sections of formalin-fixed tissue.

REFERENCES

1. Foot, N. C. Suggestions for staining tumors of spongioblastic origin. *Am. J. Path.*, 1929, **5**, 215.

2. Foot, N. C. Comments on the impregnation of neuroglia with ammoniacal silver salts. *Am. J. Path.*, 1929, **5**, 223.

3. Foot, N. C. On the silver impregnation of melanotic tumors. *Am. J. Path.*, 1931, **7**, 619.

4. Foot, N. C., and Foot, E. B. A technique of silver impregnation for general laboratory purposes. *Am. J. Path.*, 1932, **8**, 245.

5. Kubie, L. S., and Davidson, D. The ammoniacal silver solutions used in neuropathology; their staining properties, chemistry, and methods of preparation. *Arch. Neurol. & Psychiat.*, 1928, **19**, 888.

6. Cajal, Ramon y. Gold chloride sublimate method for neuroglia astrocytes. Handbook of Microscopical Technique. C. E. McClung, New York, 1929, 367.

7. Globus, J. H. The Cajal and Hortega glia staining methods. A new step in preparation of formaldehyde-fixed material. *Arch. Neurol. & Psychiat.*, 1927, **18**, 263.

8. Campbell, H. A differential stain for nerve fibres. *The Military Surgeon*, 1922, **51**, 11.

9. del Río-Hortega, P. Mitochondria and gliosomes. The Microtomists' Vade-mecum. A. B. Lee, Philadelphia, 1928, Ed. 8, 644.

DESCRIPTION OF PLATE

PLATE 123

FIG. 1. Fibrous astrocytes with pedicles to a blood vessel in a medulla fixed twenty-four hours after death. × 600.

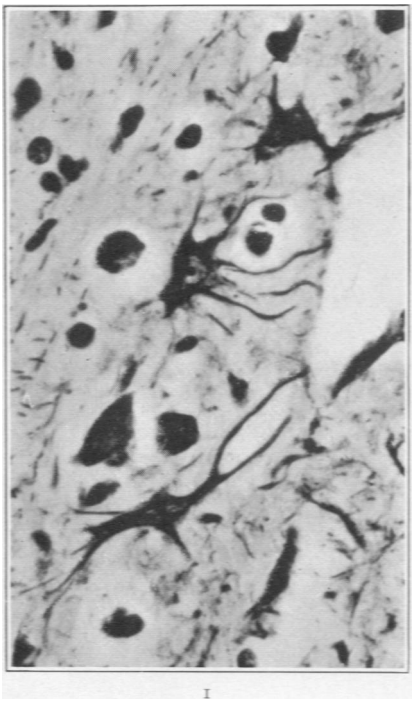
FIG. 2. Large astrocytes around a vessel in cerebral arteriosclerosis. The tissue was fixed twenty-four hours after death. × 475.

FIG. 3. Oligodendroglia in a cerebral cortex fixed six hours after death. × 600.

FIG. 4. Fibrous astocytoma fixed two hours after death. × 600.

FIG. 5. Fibrous astrocytes in a spinal cord fixed twenty-four hours after death. Nerve fibers appear in cross-section. × 475.

FIG. 6. Bipolar cells in a spongioblastoma multiforme fixed ten hours after death. × 475.



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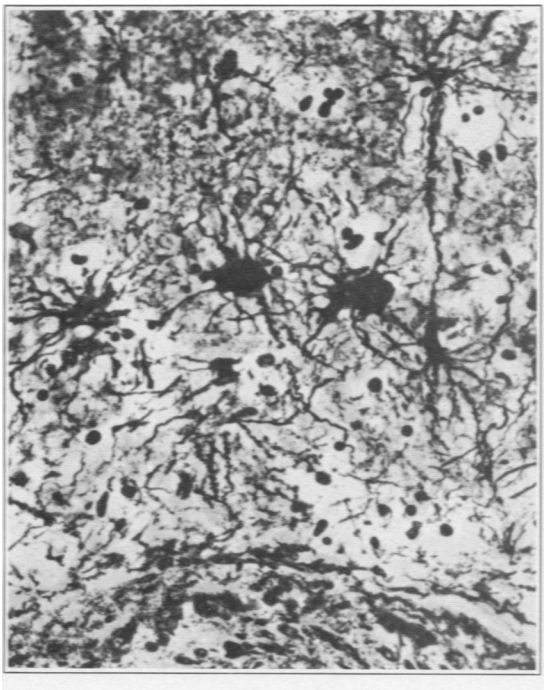

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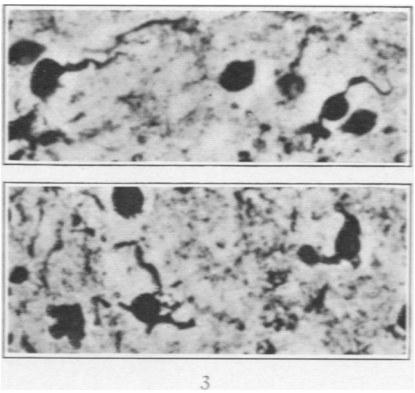

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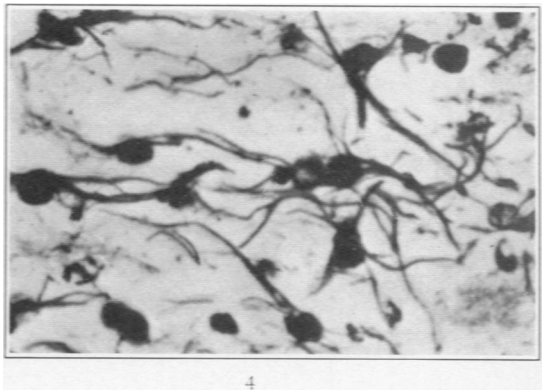

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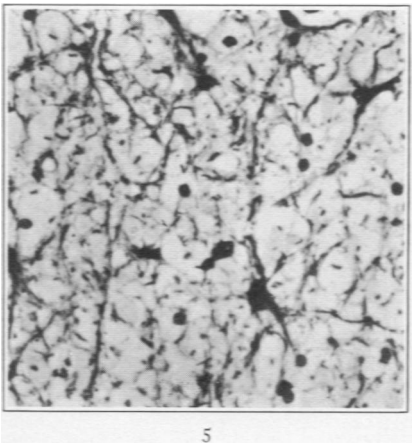

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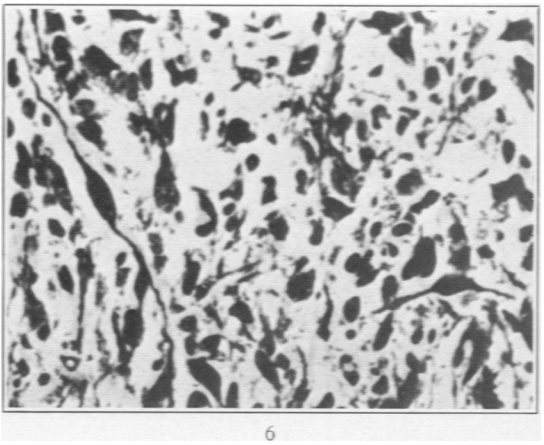

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Wilder

Silver Impregnation of Glia and Nerve Fibers