A SILVER CARBONATE STAINING METHOD FOR OLIGODENDROCYTES AND MICROGLIA FOR ROUTINE USE *

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The use of silver staining methods for oligodendrocytes and microglia is not widespread in pathological laboratories. Two of the reasons have been the necessity of special fixation and the variability of the results. A small addition to Penfield's [1] second modification of del Río Hortega's silver carbonate stain has given fairly uniform results on formalin-fixed central nervous system tissue. With attention to some details of time of fixation, as commented on below, successful and differential stains have been obtained quite regularly in our hands. The method used is simple and elastic, and lends itself to routine neuropathological use.

METHOD

1. The tissue is fixed by immersion in 10 per cent formalin.

2. Frozen sections are cut at 20 to 25 μ and are placed in distilled water, to which about 20 drops of strong ammonia are added for each 100 cc.

3. The sections remain in this dilute ammonia solution for a few minutes if recently fixed, to overnight for material that has been in formalin for several weeks.

4. Without washing, the sections are passed into Globus' [2] 10 per cent hydrobromic acid solution (10 cc. of 40 per cent hydrobromic acid to 90 cc. of distilled water), and are placed in the incubator at 37° C. for 1 hour.

5. The sections are then passed through 2 changes of distilled water.

6. Place the sections in 5 per cent sodium carbonate solution. After they are in the solution, approximately an equal quantity of a 5 per cent solution of ammonium alum (aluminum and ammonium sulphate) is added. This causes a white flocculent precipitate of aluminum hydroxide. The sections remain in this

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solution for 1 hour, although they may stay in this solution 2 to 3 days, if not convenient to stain sooner.

7. Wash well by passing through 2 changes of distilled water, gently opening out the sections, which will be found to be pliable and easily manipulated.

8. Stain in Hortega's strong silver carbonate solution until the sections turn a deep grayish brown on reduction, usually 2 to 5 minutes. (To 5 cc. of 10 per cent silver nitrate, add 20 cc. of 5 per cent sodium carbonate; then add ammonia, 28 per cent, drop by drop until the precipitate is just dissolved; filter and add 20 cc. of distilled water.)

9. Pass the sections directly to 1 per cent formalin, where they are agitated by blowing on them until reduction is complete.

10. Wash in distilled water.

11. Tone in a 1:500 yellow gold chloride solution, removing the sections just as soon as they are a uniform gray color.

12. Fix in a 5 per cent sodium thiosulphate solution about 3 to 5 minutes.

13. Wash in distilled water.

14. Tease sections onto a slide, blot with filter paper, dehydrate, clear, and mount in balsam.

There is a differential staining of oligodendrocytes and microglia. The oligodendrocytes are best obtained just at the time the tissue is well fixed in 10 per cent formalin, about 2 days fixation for spinal cord, and 4 to 5 days for the brain. Microglia are stained better after a somewhat longer fixation. There are no maximum or minimum times for fixation that can be given. However, the longer the tissue has been in formalin, the longer the sections should remain in the dilute ammonia water (Step 3).

The use of the sodium carbonate-ammonia alum mordant seems to aid in differential staining of the cytoplasm and processes of these cells. The other elements are a smooth gray in color and usually so lightly stained that they serve as an identifiable background in good contrast to the stained cells. The nuclei of the oligodendrocytes are not stained, and the processes may be stained a gray-black, which reveals details of structure such as gliosomes. The nuclei of the microglia take a light gray stain in contrast with the grayish black staining of the processes.

It should be noted that pathological alterations in these cells

render them more easily stained. Swollen oligodendrocytes, rod cells and compound granular corpuscles will stain well and smoothly after many weeks in formalin.

SUMMARY

A modification of Penfield's method for silver staining of oligodendrocytes and microglia is reported. The method allows use of the formalin-fixed tissue and is easily applied. It has given good results in our hands, and it is believed this method should be of value in a neuropathological laboratory.

REFERENCES

1. Penfield, Wilder, and Cone, William. Neuroglia and microglia (the metallic methods). Handbook of Microscopical Technique, McClung, C. E., Ed. Paul B. Hoeber, Inc., New York, 1937, 489–521.
2. Globus, J. H. Cajal and Hortega staining methods. A new step in the preparation of formaldehyde-fixed material. *Arch. Neurol. & Psychiat.*, 1927, **18**, 263–271.

DESCRIPTION OF PLATE

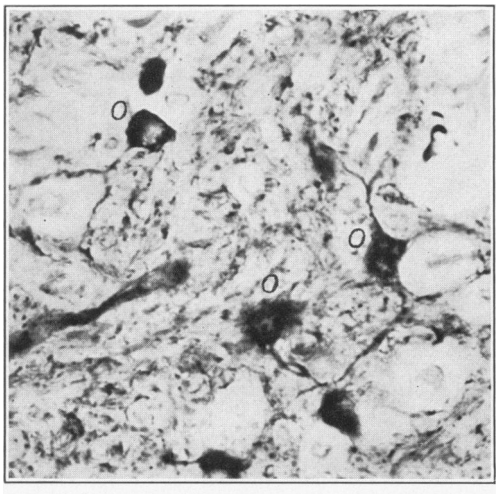
PLATE 61

FIG. 1. Normal oligodendrocytes "O" in a cross section of the spinal cord of a cat. Note the delicate processes winding about the myelin sheaths. Tissue fixed in 10 per cent formalin for 1 day.

FIG. 2. Normal oligodendrocytes in the medulla of a cat. One delicate process "P" is in focus for most of its length. Tissue fixed in 10 per cent formalin for 3 days.

FIG. 3. Normal microglia in the cerebral cortex of a monkey. A swollen oligodendrocyte is also seen. Tissue fixed in 10 per cent formalin for 2 weeks.

FIG. 4. Two rod cells "R" in a section of human cerebral cortex in general paresis. Several swollen oligodendrocytes "O" are also seen. Tissue fixed in 10 per cent formalin for 28 weeks.



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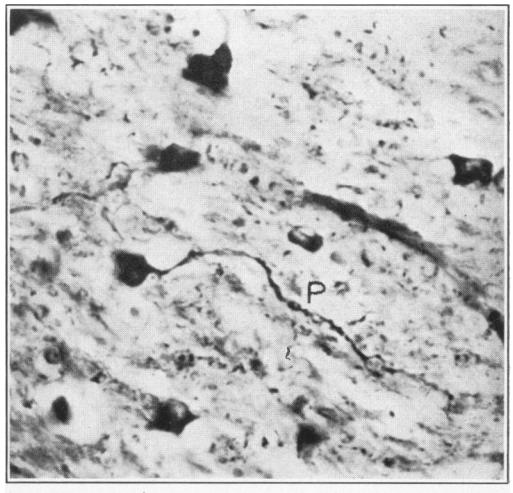

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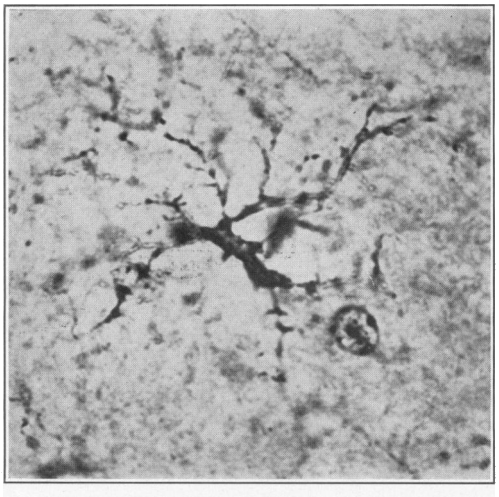

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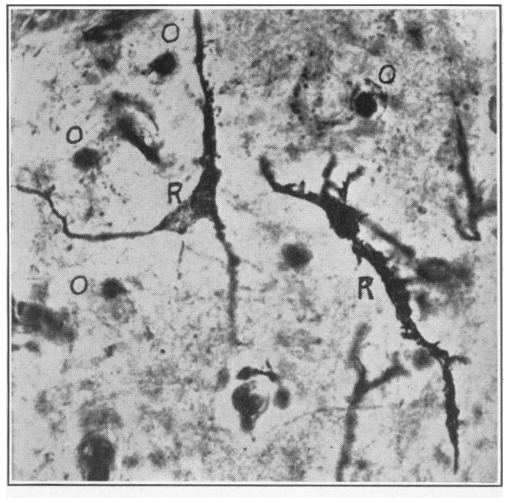

4

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Silver Carbonate Staining Method