THE IMPREGNATION OF GLIOMA SECTIONS FROM PARAFFINATED TISSUE WITH HORTega'S SILVER CARBONATE (LITHIUM) SOLUTION

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The staining methods of S. Ramon y Cajal and his pupils, P. del Rio Horta and N. Achucarro, have been used by Bailey and Cushing, Penfield, Globus and Strauss, as well as others in studying neuropathology. From their work and the contributions of Mallory, Spiller, Rosenthal, Ribbert, and others, there has developed a working classification of the glioma growths. In a histologic study of the "glioma cases" from the neurosurgical service of Dr. Ernest Sachs ¹ several years ago, considerable difficulty was encountered in staining glioma sections cut from old tissue. Inasmuch as the microscopic diagnosis depended somewhat upon individual cell study, a method for staining the glial and neural elements in their entirety was desirable.

In a number of the cases the only available tissue was that which had been removed at operation for diagnosis. This tissue, fixed either in Zenker’s fluid or formalin, had been embedded in paraffin. Sections cut from these blocks and stained according to the modification described here gave very satisfactory microscopic detail. It was then decided to embed tissue in paraffin that had been long preserved in solutions which did not permit specific gold or silver impregnation. The procedure was subsequently extended to include tissue which did not lend itself to satisfactory frozen sectioning. Clear cut pictures of the individual cells and their processes were possible in every instance, though in some sections rather trying repetition was necessary before satisfactory sections were obtained.

Since these early experiences and subsequent satisfactory experiences, there have been several requests for the technic thus employed. There have been one or two favorable reports, and from Dorothy Russel, of London, there comes a report that the method, with some slight modification, has been used in studying

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The cells dominating the sections of these tumors, as might be expected, impregnate with the same ease and rapidity as the more differentiated forms of neural cells.

The variable cellular components and the characteristic blood vessel changes are clearly depicted and make excellent sections for individual cell study without the employment of several staining methods.

other pathological lesions of the nervous system. The technic is as follows:

1. Section the tissue as thin as possible from the paraffin blocks.
2. Mount with glycerine and egg albumin.
3. Incubate over night at 37°.
4. Xylol, 3 to 5 minutes.
FIG. 3. FORMALIN-FIXED TISSUE WHICH HAD BEEN EMBEDDED IN PARAFFIN FOR EIGHT YEARS. DIAGNOSIS: SPONGIOBLASTOMA POLARE (?)

The component cells in their entirety did not lend themselves to several of the staining methods, and it was only after sections were stained with this modification that a detailed study was possible.

FIG. 4. FORMALIN-FIXED TISSUE WHICH HAD BEEN EMBEDDED IN PARAFFIN FOR THREE MONTHS. DIAGNOSIS: OLIGODENDROGLIOMA

The fine filaments which traverse the cytoplasm between the nucleus and the cell margin impregnate constantly with this modification. The astrocytes, which in some sections are numerous, stain much darker than the dominating oligoglia.

5. Xyrol, 3 to 5 minutes.
6. Xyrol and absolute alcohol, 3 to 5 minutes.
7. Absolute alcohol, 3 to 5 minutes.
8. Alcohol 95%, 3 to 5 minutes.
9. Alcohol 95%, wash.
FIG. 5. Formalin-ammonium-bromide-fixed tissue which was immediately embedded in paraffin. Diagnosis: Ependymoma

The long processes of the younger ependymal cells are seen to fill the perivascular spaces. These spaces in routine staining appear as clear areas about the blood vessels.

FIG. 6. Kaiserling-preserved tissue which was paraffined and from which sections were made eighteen months later

The tendency to reproduce the medullary canal is seen in the low powered magnification. The neural elements with their characteristic affinity for silver impregnation can be studied in detail with higher magnification.

10. Mordant:
   Silver nitrate (2 per cent solution) . . . . . oz. i.
   Pyridine ........................................... gtts. x.
   Alcohol ............................................ gtts. xv.
   At 60° C. until sections are a yellow color.

11. Alcohol 95%, wash.
12. Impregnate:
   Silver carbonate (lithium)\(^2\). oz. i.
   Pyridine. gtts. x.
   Alcohol. gtts. xv.
   At 60° C. until sections are an amber color.
13. Wash rapidly in 95% alcohol.
14. Reduce in 10% neutral formalin.
15. Wash in distilled water.
16. Tone in gold chloride (1:500 solution).
   At 60° C. until sections are a grayish-red.
17. Fix in hyposulphite (5% solution).
18. Wash in distilled water.
19. Dehydrate in 95% alcohol.
22. Mount.

If the tissue has been "Zenker fixed" before the slides are put into alcohol, they should be treated with Gram’s iodine as usual. Any excess of precipitated silver may be removed by allowing the slides to remain over night in xylol, to which has been added a few drops of pyridine. The neural and glial elements do not impregnate as heavily as the connective-tissue constituents, the former staining a pinkish-red as contrasted with the fibroblastic elements which stain a dark brown. Sections thus stained make very excellent preparations for microphotography.

\(^2\) Hortega’s silver carbonate (lithium) solution:
   Silver nitrate 10% .................. 5 c.c.
   Lithium carbonate 0.5% .................. 20 c.c.
   Ammonium hydroxide q.s. to dissolve precipitant. Distilled water
   add .......................... 75 c.c.