A FURTHER MODIFICATION OF DEL RÍO-HORTEGA'S METHOD OF STAINING OLIGODENDROGLIA *

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This modification has been worked out with untiring enthusiasm in our laboratory by the senior technician, Mr. Edward Dockrill. In recognition of this fact it is proposed that the method be called Dockrill's Modification of the silver carbonate method for oligodendroglia.

The method is particularly reliable for staining the oligodendrocytes of the spinal cord, brain stem and cerebral white matter, where other methods are less often successful. It also stains the *sheath* of *Schwann cells* on the peripheral nerves selectively.

FIXATION

Fresh tissue should be fixed in the following solution for 2 hours or up to 1 or 2 days.

Fixative (F. U. P. I.)	Formalin (40 per cent commercial)	20 cc.
	Urea	4 gm.
	Potassium iodide	6 gm.
	Water (doubly distilled)	80 cc.

Cut sections at about 15 microns on the freezing microtome and place in distilled water.

STAINING METHOD †

1. *Wash* in two dishes of distilled water, the first containing 10 drops of ammonia.

2. *Stain* in undiluted silver carbonate ‡ from 1 minute to 1½ hours.

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† The numbers correspond with those in Text-Figure 1.

‡ The solution is del Río-Hortega's undiluted ammoniacal silver carbonate made up carefully as follows:

Solution of silver nitrate (Merck) 10 per cent	5 cc.
Solution of sodium carbonate (pure) 5 per cent	20 cc.
Ammonium hydroxide (sufficient to dissolve precipitate).	

The ammonium hydroxide, as indicated above, should be added drop by drop until the precipitate is just dissolved, stirring the solution all the while. Finally, filter and place in a dark bottle, where it will keep for long periods.

3. *Wash* rapidly in 60 per cent alcohol.* The section should be carried through with a small angulated glass rod so as to allow all of it to be washed equally, without wasting time. If the section is wrinkled or folded, the alcohol will produce a patchy result.

4. *Reduce* by passing sections directly into 1 per cent formalin.

5. *Wash* in distilled water.

6. *Tone* by placing sections in gold chloride toning bath † 10 or 15 minutes until they become purple-gray in color.

7. *Fix* in 5 per cent hyposulphite of soda for ½ minute or more until sections are flexible.

8. *Wash* in water.

9. *Dehydrate* in dishes of graded alcohol followed by clearing in carbol-xylol-creosote.‡

10. *Mount* on slide in Canada balsam.

Best results as a rule are obtained by leaving tissues for 5 to 20 hours in the fixative which, for the sake of brevity, is called F. U. P. I. or fupi. But the staining capacity may be revived in an overfixed subject by placing sections in 4 per cent urea overnight and then passing them directly into the silver bath for an hour or less. If the subject has been fixed by preliminary carotid injection, it is better to try for oligodendroglia within 2 hours or less after the block has been placed in fixative.

To stain oligodendroglia in sections of the *spinal cord, optic nerve* and *retina,* place small fresh pieces in the above fixative to which has been added 4 gm. of chloral hydrate. Our best results were obtained by leaving fresh blocks of tissue from 2 to 4 days in this fixative. Cut sections and proceed as above.

Creditable staining of oligodendrocytes has been obtained from old formol material in the following manner: Blocks were cut and placed in 15 per cent ammonia water for 24 hours. They were then washed in running tap water overnight and placed in the F. U. P. I. fixative for a week. Sections were then cut and left in 4 per cent urea overnight and stained as before. This applies to brain tissue. We have had no marked success with old formalin-fixed spinal cords.

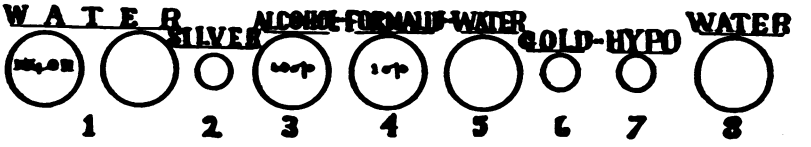
* Commercial alcohol (95 per cent) usually contains some impurity. When diluted there appears a slight opalescence. This is prejudicial to the success of the staining. For this reason we have always used absolute alcohol in preparing the 60 per cent.

† Toning bath: Gold chloride (yellow) ..... 1 gm.
Distilled water ..... 500 cc.

‡ Carbolic acid 10 cc., creosote 10 cc., xylol 80 cc.

RESULTS

More complete staining of the oligodendrocytes in the white matter of the brain and spinal cord may be obtained by this modification of del Río-Hortega's method (Figs. 1 and 2), although the results in the gray matter are less delicate and satisfactory than by the original method of that author [1] or the modification for microglia and oligodendroglia by Penfield.[1]



TEXT-FIGURE 1

Order of staining procedure from left to right. Numbers correspond with the text.

The recently described method of del Río-Hortega,[2] which is a modification of Golgi's chrome silver method, occasionally gives results which for complete staining of the oligodendrocyte expansions are unequalled. But the results are so unequal and the staining so powdery as to make it so far of little use for routine work.

The method described here has been found particularly useful by Dr. Cone in staining the ologodendroglia of the retina, nerve head and optic nerves. Microglia is also stained with varying success by this method.

REFERENCES

1. Penfield, W., and Cone, W. Neuroglia and Microglia (the metallic methods), chapter in McClung's Handbook of Microscopical Technique, Philadelphia, 1929, 359–388.

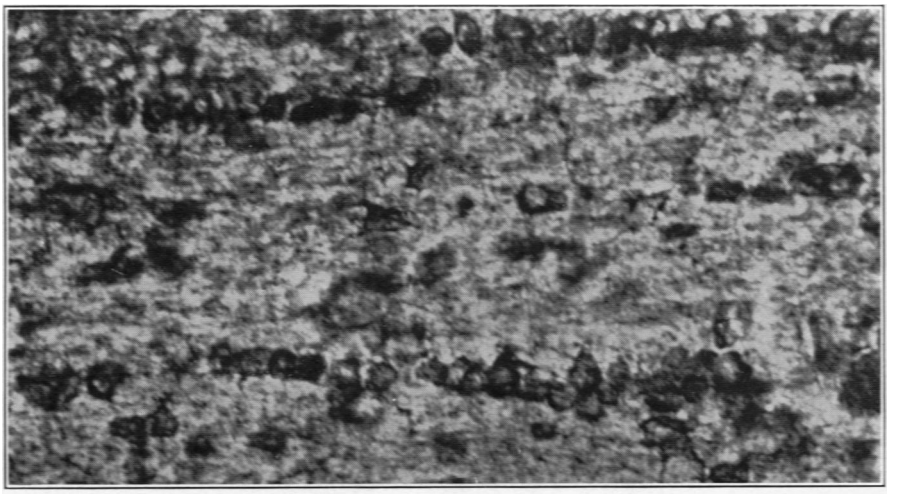
2. Río-Hortega, P. del. Tercera aportacion al conocimiento morfologico e enterpretacion funcional de la oligodendroglia. *Mem. d. l. R. Soc. Espan. d. Hist. Nat.*, 1928, 14, 1.

DESCRIPTION OF PLATE

PLATE 95

FIG. 1. Rows of oligodendrocytes in the cerebral white matter of a dog; normal.

FIG. 2. Higher magnification of oligodendrocytes from the white matter of the same animal.



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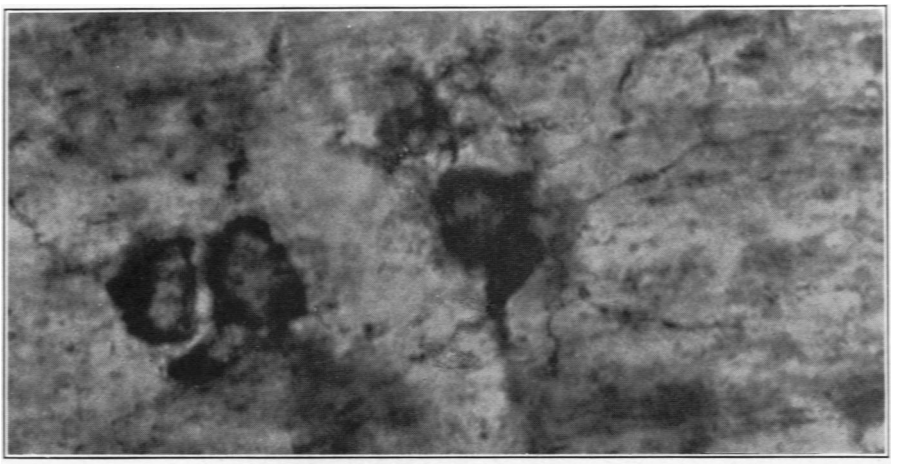

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