Method of silver impregnation for nervous tissue embedded in paraffin

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The aim of the silver impregnation methods for paraffin-embedded sections of nervous tissue is to combine finesse of histological detail with an easy method of processing.

The following method is presented here because of its rapidity, reliability, staining quality, and utility as a general purpose stain. The basic technique, without the variations, was devised by Fincher (1932) on the basis of Hortega's silver carbonate impregnation method for astrocytes and its purpose was to stain cerebral tumours.

METHOD

1 Paraffin sections, of 5 to 8 μ for pathological studies and of 10 to 20 μ for anatomical studies, are mounted on albuminized slides a few hours before use. The sections are then slowly brought through xylol (5 minutes), absolute alcohol (5 minutes), and alcohol 95° (5 minutes), and washed in distilled water.

2 Mordant in the following solution at 60°C. until yellow.

Silver nitrate 2% 30 ml.
Pyridine 10 drops
Absolute alcohol 15 drops

This usually takes 30 minutes. I prefer not to pre-warm my solution. Prolonged mordanting gives too dark a picture.

3 Wash quickly in 95° alcohol.

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A simple constant-infusion apparatus—continued.

At present two of these models are being used for the injection of tritiated thymidine into rats at a rate of 0.5 ml. per 25 hours (Foot, 1963).

The advantages of the present device are its simplicity, cheapness, accuracy, 'foolproofness', and the minute amounts that can be injected. Its working can also be checked easily by observing the rotation of the micrometer head. Its disadvantage is that a separate motor is required for each different speed but these are cheap and can be changed in a few moments.

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REFERENCES

Foot, E. C. (1963). *Nature (Lond.)*, 198, 297.

4 Impregnate in the following solution at 60°C. until a dark amber colour. If the solution has been pre-warmed this takes from 15 to 30 minutes; if not it may take up to an hour, at which time the section should be taken out no matter what colour it is.

Hortega's strong silver carbonate

(lithium or sodium)^a 30 ml.

Pyridine 10 drops

Absolute alcohol 15 drops

5 Wash rapidly in 95° alcohol.

6 Reduce in 10% formol for one to two minutes.

7 Wash in distilled water.

8 If desired tone in a 0.2% gold chloride solution.

9 Wash in distilled water.

10 Leave in a 2% oxalic acid solution for a few minutes until the sections become reddish. This step accentuates the staining of axones.

11 Fix, if desired, in a 5% solution of sodium thio-sulphate.

12 Wash in distilled water, dehydrate, clear, and mount in balsam.

^a*Lithium carbonate solution* To 5 ml. 10% silver nitrate add 20 ml. of a saturated solution of lithium carbonate and then drop by drop add 28% ammonia water barely to dissolve the precipitate. Bring volume to 75 ml. with distilled water and filter. Keep in brown bottle.

Sodium carbonate solution To 5 ml. 10% silver nitrate add 20 ml. 5% sodium carbonate solution and then drop by drop add 28% ammonia water barely to dissolve the precipitate. Add about 20 ml. distilled water and filter, bringing volume to 45 ml. Keep in brown bottle.

FIG. 1. Normal Purkinje cells, and horizontal fibres of basket cells. Variant 4 $\times$ 120 (mordanting, one hour, and impregnation one hour).

TABLE I
PROCEDURE FOR VARIANTS OF THE METHOD

Step	Variant	1	2	3	4	5	6
1		Mount on albuminized slides, leave to dry for 12 hours and deparaffinize					
2		Mordant in 2% silver nitrate solution until yellow, usually about 30 minutes, then wash in 95° alcohol.					
3		Impregnate with Hortega's silver carbonate (lithium) solution.			Impregnate with Hortega's silver carbonate (sodium) solution		
4		Reduce in 10% formol, then wash in water			Reduce in 10% formol, then wash in water		
5		Fix in 5% hypo	Tone in 0.2% AuCl	Tone in 0.2% AuCl	Fix in 5% hypo	Tone in 0.2% AuCl	Tone in 0.2% AuCl
6		Clear and mount	Fix in 5% hypo	Wash in water then leave in 2% oxalic acid	Clear and mount	Fix in 5% hypo	Wash in water then leave in 2% oxalic acid
7			Dehydrate, clear, and mount	Fix in 5% hypo		Dehydrate, clear, and mount	Fix in 5% hypo
8				Dehydrate, clear, and mount			Dehydrate, clear, and mount

FIG. 2. Hypertrophied oligodendrocytes in cerebral white matter. Variant 2 $\times$ 400 (mordanting 45 minutes; impregnation 45 minutes).

FIG. 3. Climbing fibres ascending through the granular layer and surrounding the base of the Purkinje cells. Above are seen the horizontal fibres of the basket cells. Variant 2 $\times$ 400 (mordanting half an hour; impregnation half an hour).

VARIANTS ON THE METHOD

There are a number of variants to this technique which when employed bring out different morphological features of the section (Table I). These are divided into two groups, the first one uses Hortega's strong lithium silver carbonate solution, the second Hortega's strong

FIG. 3

sodium silver carbonate solution. Thus six sections can be placed in a Coplin jar and then carried on through the various steps until the sections have been mordanted (step 2). Then three sections are impregnated with the lithium silver carbonate solution (variants 1, 2, 3) and the others through the sodium silver carbonate solution (variants 4, 5, 6). Variants 1 and 4 are reduced in 10% formol and mounted in balsam. Variants 2 and 5 are

FIG. 4. *Nerve fibre coursing through muscle fibres. Variant 3 $\times 400$ (mordanting half an hour; impregnation half an hour).*

FIG. 5. *The meshwork of reticulin in a meningioma. Variant 5 $\times 120$ (mordanting for one hour; impregnation 40 minutes).*

FIG. 6. *Isomorphic gliosis in the cerebellum. Variant 6 $\times 120$ (mordanting for half an hour; impregnation for half an hour).*

reduced in formol and toned with gold chloride. Variants 3 and 6 are reduced in formol, toned in gold chloride, washed in 2% oxalic acid, dehydrated and mounted in balsam.

Variants 1 and 4, the untuned sections, show well the axons and glial fibres (Fig. 1). Variant 2 shows the glia (Fig. 2), the more delicate fibrillary systems (Fig. 3), and neurons. Variant 3 shows the axons well, and has been useful in showing the nerve fibres coursing through muscle (Fig. 4). Variant 5 is a reticulin stain (Fig. 5).

Variant 6 also shows the reticulin, as reddish fibres against a grey background, and gliosis (Fig. 6). With all these variants the staining may benefit from varying the times for mordanting and impregnating, and the reducing formol solution can be diluted to 1%, 5%, or 20%.

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REFERENCES

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