A MODIFICATION OF RANSON'S SILVER NITRATE METHOD FOR DEMONSTRATING AXIS CYLINDERS

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Of all the impregnation methods available for the histological study of the peripheral nervous system, the technique of Ranson(1) is perhaps one of the most valuable to the average worker in that it combines comparative simplicity with a tolerable certainty of a good result, and is in addition applicable to the making of serial paraffin sections. The method was originally designed to show the non-myelinated fibres in mixed nerves, but under suitable conditions nerve endings of all kinds can also be well demonstrated, as well as nerve cells and myelinated fibres.

The great disadvantage of the technique, however, is the necessity for fixation in absolute alcohol and ammonia: this treatment renders certain tissues extremely brittle and difficult to cut in serial section, and it also limits the application of the method to tissues obtained in fresh condition. An investigation was accordingly made of the various stages in the process in an endeavour to find a modification that would overcome these difficulties and yet give the uniformly satisfactory results of the original method, thus increasing its range of usefulness.

Ranson's original method

This is as follows:

Fix small pieces of tissue for 48 hours in absolute alcohol, 99 parts; concentrated ammonia, 1 part.
Rinse in distilled water.
Transfer to pyridine for 24 hours.
Wash in many changes of distilled water for 24 hours.
Transfer to 2 per cent. $AgNO_3$, in the dark, at 35° C., for 3 days.
Rinse in distilled water.
Reduce for 1 day in 4 per cent. pyrogallol in 5 per cent. formalin.
Wash in water.
Dehydrate, clear, and embed in paraffin wax.

(*a*) *Fixation.*

Of the common fixatives other than alcohol none gave satisfactory results except formalin. Fixation in the usual way with 7 per cent. formalin in tap water is not in any way detrimental to the subsequent processes, provided that the tissue is after fixation very thoroughly washed in running tap water for at least 12 hours. This point is important because uniform impregnation with silver salts is much helped by a slight degree of alkalinity. Excellent results were obtained by this means even with human brain tissue that had been lying in formalin for 6 months, the washing in this case being extended to 24 hours. After washing, the tissue may be put straight into pyridine, as an intervening treatment with absolute alcohol and ammonia does not seem to have any beneficial effect. The addition of ammonia to the fixing formalin solution is of no apparent value.

(*b*) *Action of pyridine.*

The treatment with pyridine is essential because it prevents the impregnation of nuclei, of connective tissue fibres, and of capillaries, and should be carried out for 1–3 days according to the size and density of the tissue. Fixation by pyridine alone was not found to be very adequate: it causes much shrinkage and in addition renders the tissues extremely brittle.

Before treating with $AgNO_3$ it is essential that *all* pyridine should be removed by distilled water, and this can only be effected by giving repeated washings with fresh distilled water for about 24 hours.

(*c*) *Impregnation with* $AgNO_3$.

The duration of the treatment with 2 per cent. $AgNO_3$ is not very important, but it should be not less than 3 days and as a rule not more than 5 days, and must be carried out in the dark at 37° C. If tissues are left in $AgNO_3$ for as long as 7 or 8 days there is a tendency for a precipitate to appear in them.

(*d*) *Reduction.*

After washing, reduction in the pyrogallol-formalin mixture should be carried out for at least 6 hours, and preferably for 10 hours or even longer for large blocks: if the solution becomes dark or cloudy it is important that the tissues should be transferred to fresh fluid. Reduction should not be accelerated by warming, as this causes blackening and hardening of the outside of the block and consequent lack of reduction inside.

There does not seem to be any particular reason for using 5 per cent. formalin, as reduction with 4 per cent. pyrogallol in distilled water is quite effective, and treatment with the formalin alone (even in a strength of 10 per cent.) gives very incomplete reduction.

(*e*) *After treatment.*

Dehydration should be carried out rapidly as the tissues are apt to become brittle in alcohol. Paraffin sections should be not more than 10 μ thick. If the yellow colour of the background is objected to, sections can be toned in the usual way with 0·2 per cent. $AuCl_3$, but the resulting picture does not show the nerve fibres and endings in quite such sharp contrast as before. The material can be embedded in celloidin in the usual way if this is preferred to paraffin.

Application of the method

(1) *Nerve fibres in muscle.*

Blocks of tissue should not be more than 3–4 mm. thick. Fixation in formalin renders the muscle less brittle than does absolute alcohol and is therefore more suitable for serial work, and the impregnation is equally good in both cases. Motor nerve endings, and sensory spindles and tendon organs, are sharply picked out in sections, and show best if the muscle fibres are cut longitudinally, this plane of section being also the most useful for following the distribution of nerve fibres. Incidentally the detailed structure of the muscle fibres is also shown exceedingly well.

(2) *Cerebellum and cerebrum.*

Fixation with absolute alcohol shortens the process, but does not seem to have any other real advantage over formalin. Sections obtained by this method compare quite favourably with successful Bielschowsky preparations, and the technique is much shorter and simpler, and serial sections are easily accomplished. The whole brain of a human 36-week foetus was treated by this method, after fixation in formalin: the smaller blocks were embedded in paraffin wax, and the larger in celloidin: the detail was found to be very good in both series.

(3) *Whole brain.*

As shown by Hüber and Guild(2) Ranson's method is applicable to whole brains of small animals. A good method is to expose the dorsal surface of the brain and fix *in situ*, dissecting out later. Excellent results were obtained with rabbit foetuses of about 16 days and with new-born kittens, the brains being cut in serial sections of 10–12 μ thickness.

(4) *Whole embryos.*

Whole embryos up to about 30 mm. can be impregnated quite successfully, but if decalcification is necessary de Castro's method(3) (chloral hydrate modification) is preferable, although Hüber and Guild obtained good results with 7 per cent. HNO_3 after alcohol fixation. A human embryo of about 60 mm. was cut into four blocks after fixation in absolute alcohol had been carried out for 2 hours, and the blocks were then treated by the standard Ranson technique, impregnation and reduction being excellent throughout. When a variety of tissues, such as those of a foetus, is being dealt with the pyridine treatment should last not less than 2 days. Developing bone present in such embryos is also impregnated and appears black.

Summary

A modification is given of Ranson's $AgNO_3$ method for the staining of axis cylinders which increases its range of usefulness.

REFERENCES

(1) Ranson, S. W. (1914). *Anat. Anzeig.* Bd. xlvi, S. 522.
(2) Hüber, G. C. and Guild, S. R. (1913). *Anat. Record,* vol. vii, p. 253.
(3) de Castro, F. (1926). *Travaux du Laboratoire de Recherches biologiques de l'Université de Madrid,* t. xxiii, fasc. 4, p. 427.