should be carried out on other results. Between 0.5% and 1% of samples show a result between 4 and 6 mg/100 ml. These babies should be retested at about 14 days when most levels will have fallen to normal.

Antibiotics

The effect of antibiotics can occasionally be seen in Guthrie testing but, provided the samples have been autoclaved, levels of phenylalanine above 4 mg/100 ml will not be masked. Further details can be found in the paper by Newman and Starr (1971).

Commercial PKU Reagents and Equipment

All of the reagents mentioned in this paper may be purchased commercially. Care should be taken to ensure that any standards purchased from commercial sources are prepared on paper the same as that used for collecting samples. Standards made from aqueous solutions of phenylalanine are not comparable with those made in whole blood. A list of suppliers is appended whose products have been found to be satisfactory.

Suppliers

$\frac{1}{4}$ in. HAND PUNCH Maun Industries Ltd, Moor Lane, Mansfield, Notts.

PLASTIC TRAYS B.B.L., Empire Way, Wembley, Middlesex

METAL AND GLASS ASSAY PLATES Mast Laboratories Ltd, 38 Queensland Street, Liverpool 7

PKU AGAR Oxoid Ltd, Southwark Bridge Road, London, SE1

INHIBITOR β -2-THIENYLALANINE Sigma Ltd, 12 Lettice Street, London, SW6

REFERENCE STANDARDS AND SPORES

Sets of reference standards will be sent at regular intervals to screening laboratories on application direct to: Dr R. L. Newman, Regional Infant Screening Service, Pathology Department, Queen Mary's Hospital for Children, Carshalton, Surrey (Tel: 01-643-3300). Spores may be obtained from the same source.

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A rapid silver impregnation technique for oligodendrocytes, microglia, and astrocytes

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Marshall (1956) described the arrangement of the cells of the reticular tissue throughout the body using silver impregnation methods based on those of Rio-Hortega. The method he used for the demonstration of metallophilic cells in frozen or paraffin wax-embedded sections is a simple one and is based on the Weil-Davenport modification (Weil and Davenport, 1933; Weil, 1946) of Stern's method (1932) for demonstrating astrocytes and oligodendrocytes in celloidin sections. We have found it to be of particular use in tissues of the central nervous system as a rapid and reliable method which can be employed to give useful impregnation of astrocytes, oligodendrocytes, and microglia. Minor variations of staining technique will heighten the degree of impregnation obtained in any one cell type. The method may also be used for the demonstration of microglia in celloidin-embedded sections.

Method

PARAFFIN SECTIONS

Cut sections of 15-20 μ thickness and transfer directly into two successive xylol baths. Place sections in absolute alcohol then in 50% alcohol. Wash twice in distilled water.

FROZEN SECTIONS

Cut sections of formalin-fixed material of 20-25 μ thickness. Wash twice in distilled water. Impregnate sections in the silver bath for a variable time; usually three or four seconds is sufficient but often the shortest possible time is preferable. Transfer the sections rapidly to the formalin bath, moving them continuously while reduction takes place (approximately 30 seconds). When reduction is complete the sections should be an even, reddish brown colour. Wash the sections twice in distilled water and fix in 5% sodium thiosulphate. Wash the sections twice in distilled water, fix on to glass slides, dehydrate, clear, and mount.

Received for publication 27 October 1970.

Fig. 1 A blood vessel with oligodendrocytes. Frozen section, $\times 650$.

Fig. 3 Astrocytes in a reticulum cell sarcoma. Frozen section, $\times 200$.

Fig. 2 Hypertrophic microglia. Frozen section, $\times 400$.

Fig. 4 Astrocytes in a cerebral abscess. Paraffin section, $\times 400$.

Reagents

SILVER REAGENT

To 2 ml of 0.88 ammonia is added fresh 5% silver nitrate solution until a slight permanent turbidity is formed.

REDUCING SOLUTION

Formaldehyde (37%) is diluted to 3% with distilled water.

FIXING SOLUTION

Sodium thiosulphate (5%) in distilled water.

All solutions should be made up in freshly distilled water in chemically clean glassware.

The colour of the silver reagent should be orange/brown and not grey or black.

The formalin bath should be replaced after every second section is reduced or precipitates will form.

Sections may be impregnated more than once providing they are passed through distilled water before and after re-impregnating and reducing.

Frozen sections left in 10% ammonia solution for at least two hours before impregnation usually give better results.

Results

We have compared our results with those obtained with Cajal's gold sublimate, Hortega's triple impregnation, Penfield's method for microglia and oligodendrocytes, Gallyas' method for microglia (Gallyas, 1963), the tannin-silver method of Achucarro and the silver iodate method of Duckett and Triggs (Duckett, 1965).

The advantage of astrocytes staining in paraffin-embedded material makes this method more useful than either Cajal's gold sublimate or Rio-Hortega's triple impregnation methods in certain cases. With

tumours of the central nervous system clear staining of astrocytes is often possible simultaneously with the associated microglia. Comparison with the microglial methods of Penfield and Gallyas is often more than favourable. The Gallyas method is not sufficiently selective and gives marked staining of vessels, while Penfield's method will not always stain microglial processes. The method of Duckett and Triggs does not stain microglia. Oligodendrocytes are often difficult to stain well whatever method is used; for best results the shortest possible time should elapse between the removal of the tissue and its fixation. Under optimum conditions this method will stain the fine processes of oligodendrocytes equally as well as that of Penfield. Using both normal and pathological material we found that our results compared favourably with those of the more time-consuming methods. This method is to be specially recommended where facilities for using other techniques, or experience with them, are lacking.

I should like to thank Professor W. H. McMenemey and Dr R. O. Barnard for their help and advice. This method was developed under a grant from The British Empire Cancer Campaign.

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