TECHNICAL METHOD

A FAST GREEN METHOD FOR STAINING RED CELLS

BY

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In a study of the vascular pattern of the human pituitary body carried out by one of us (Xueréb, 1953) the benzidine methods (Lepehne, 1919; Pickworth, 1934) of staining red cells were chiefly used. Satisfactory results were obtained, but the use of these methods is accompanied by two well-known disadvantages: the stained preparations gradually fade and the technique cannot be applied to old, formalin-fixed material. In order to overcome these difficulties the method of fuchsin staining (Eros, 1941) was also used, but it did not yield satisfactory results when applied to the pituitary body as a whole. We therefore substituted various other dyes for the acid fuchsin, and good results were obtained by the use of fast green. The following staining procedure was evolved using a dilute aqueous solution of this dye.

The pituitary bodies were fixed in 10% formol saline for a period varying from two to 10 weeks, and the staining method was applied to frozen sections ranging in thickness from 100 to 120 μ . The sections are washed in distilled water and then immersed in an aqueous solution of fast green (5 drops of a stock 2% solution of fast green to 50 ml. distilled water) for 30 to 45 minutes. The sections are then differentiated in a phenol-lithium carbonate solution (5 drops of 0.5% lithium carbonate solution to 50 ml. of 1% phenol). This stage is controlled under the microscope and the differentiation allowed to proceed until the network of blood vessels becomes apparent (15–30 minutes). After washing in distilled water the sections are dehydrated in absolute alcohol, cleared in a terpineol-xylol mixture (3 parts terpineol to 2 parts xylol) and mounted in dammar. In the finished preparations the red blood cells appear dark green against a colourless background.

The pituitary preparations have now retained the intensity of the stain for two years. Equally satisfactory results were obtained from tissues preserved in formol saline for as long as 12 months.

These advantages outweigh the fact that the fast green method is somewhat time consuming owing to microscopic control of differentiation. It is, however, more rapid than the acid fuchsin method.

FIG. 1.—Fast green preparation showing the vascular pattern in the various components of the pituitary body (female, aged 76 years). Sagittal section through neural lobe (top), posterior part of pars distalis (bottom) and infundibular stem (centre). Note relatively sparse, delicate vessels in the circular area of chromophobe adenoma (lower bottom) contrasting with the surrounding dense network of sinusoids ($\times 12$).

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When the latter technique was applied to the pituitary body it was found that by careful differentiation either the vessels within its epithelial or those within its neural portions could be shown clearly. With the fast green method, however, it was possible to make preparations in which the vessels of both adeno- and neuro-hypophysis stand out equally well.

Lendrum's (1949) kiton red method was given an extensive trial on thin and thick paraffin and frozen sections of the human pituitary body, and the following conclusions were drawn: (1) Fast green is better than kiton red for staining the blood vessels in thick frozen sections (120 μ) and thick paraffin sections (20 μ). The fast green adheres more tenaciously to the red blood cells and consequently allows better differentiation from the densely cellular background. (2) Fast green is as good a method as kiton red when applied to

thin paraffin (7 μ) and thin frozen (12 μ) sections. (3) Differentiation is quicker with fast green.

The fast green method of staining red cells has proved useful in determining the arrangement of the blood vessels in all the components of the hypophysis cerebri, not only under normal conditions but also in areas affected by disease processes (Fig. 1). This method has also given us good results with renal tissues, and is now being adapted for the study of the cerebral capillary bed.

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