Coloration of the Golgi–Nissl Network in a Vertebrate Neurone by Sudan Black

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With one plate (fig. 1)

SUMMARY

Though it is generally considered that the cytoplasmic inclusions, commonly described as 'Golgi apparatus', contain phospholipid, the routine histochemical tests for lipid do not reveal the apparatus in the neurone of vertebrates. A technique for colouring the apparatus in the neurones of vertebrates with Sudan black is described in this paper.

IT is often assumed that the cytoplasmic inclusions generally described as Golgi apparatus contain phospholipid, which reduces osmium tetroxide or silver nitrate in the routine Golgi techniques. In the neurones of vertebrates, however, the standard histochemical tests for lipid, namely Sudan black and acid haematein, do not ordinarily reveal the characteristic 'apparatus' of Golgi (Casselman and Baker, 1955; Malhotra, 1959), even after powerful unmasking agents (Clayton, 1959) have been used in fixation (David and Brown, 1961; Malhotra, 1961).

In this paper a technique for colouring the apparatus in the neurones of vertebrates with Sudan black is described. This technique is essentially the same as one devised by Dr. O. L. Thomas (1948), who kindly sent me a preparation of a dorsal root ganglion of the kitten prepared by his method. The neurones possessed a Sudanophil inclusion very similar in appearance to the recticular apparatus of Golgi. The method used by myself is as follows:

(1) Fix in Helly's (1903) fluid for about 18 h.
(2) Postchrome in a saturated solution of potassium dichromate for about 36 h at 37° C.
(3) Wash in running water for several hours.
(4) Dehydrate and embed in paraffin.
(5) Colour the sections for ½ h in a saturated solution of Sudan black in 70% ethanol. (Thomas recommends colouring for ½ h at 60° C, but room temperature seems equally effective.)
(6) Rinse in 50% ethanol and bring sections to distilled water.
(7) Mount in Farrants's medium.

Dorsal root ganglia of the mouse were fixed and coloured with Sudan black according to this method. A reticulate object in the neurones, resembling in form and distribution the apparatus as shown by Golgi impregnation methods, is coloured with Sudan black. No such structure could be seen in these neur-

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ones when Sudan black was used on gelatine sections of dorsal root ganglia fixed in formaldehyde / calcium or formaldehyde / cadmium chloride (Malhotra, 1961).

When paraffin sections coloured with Sudan black are placed in 70% ethanol, the colour is not readily removed from the network. These observations would suggest that the colouring might possibly be due to some acidic substance and was not necessarily associated with the presence of lipid, since Sudan black is capable of acting as a weak basic dye (Baker, 1958; Casselman, 1959). Nevertheless, there is strong reason for believing that a lipid component of the network is blackened, because acetylated Sudan black, which cannot act as a dye (Casselman, 1954, 1959), is equally effective. It would thus appear that the lipid, which is ordinarly masked (probably by protein) in the object under investigation, becomes freely available when Thomas's technique is used; and it can then be demonstrated by the use of Sudan black.

It has recently been suggested (Malhotra, 1959; David and others, 1960) that the apparatus of Golgi in the neurones of vertebrates results from a deposit of silver or osmium on the basiphil reticulum of Nissl. It is therefore instructive to decolorize a Sudan black preparation that shows the typical network in the neurones and then stain the same section with a basic dye. The results of such an experiment are illustrated in fig. 1, A, B. It is clearly indicated that the same cytoplasmic inclusion is revealed by both Sudan black and basic dyes. In electron micrographs the object, corresponding to the basiphil network, is seen to be made up of membraneous endoplasmic reticulum and the small ribonucleoprotein particles associated with it (Hess, 1955; Palay and Palade, 1955; Palay, 1956; Young, 1956; Malhotra and Meek, 1960). These ribonucleoprotein particles are presumably the components that take up basic dyes (in the Nissl technique), while colouring with Sudan black is more likely to be associated with the membraneous endoplasmic reticulum, on which silver nitrate or osmium tetroxide is reduced in the Golgi techniques (Malhotra, 1959; Malhotra and Meek, 1960).

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FIG. 1 (plate). A, dorsal root ganglion cells of the mouse, prepared by fixation in Helly's fluid, postchroming, and colouring with Sudan black.

B, the same cells as illustrated in A after decolorizing and then staining with the basic dye, cresyl violet. Note that the same cytoplasmic inclusions are revealed by both Sudan black and cresyl violet; compare *a* and *a*, *b* and *b*, and so on.

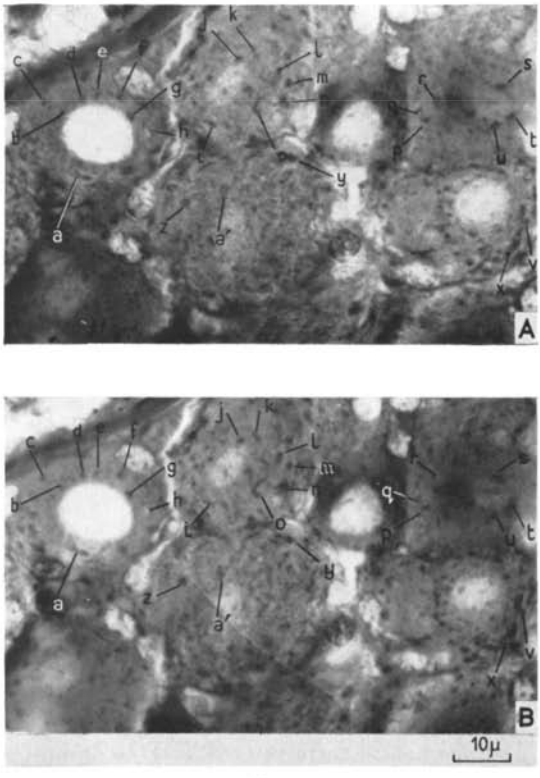

FIG. 1

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