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A SIMPLE SAMPLING TECHNIQUE FOR OBTAINING REPRESENTATIVE ELECTRON MICROGRAPHS FROM BRAIN FRACTIONS

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Abstract

Subcellular fractions of brain tissue were placed on and adhered to the surface of millipore filters in a commercially available holder. Subcellular particles forming a thin layer on the filters in an even distribution could be readily processed for electron microscopic evaluation.

It is expected of an electron microscopic study that it will assess with accuracy not only the distribution of particles derived from subcellular fractions, but also the proportions of various components being present in the original fraction. However, because of certain inherent difficulties such as the different sedimentation coefficient of particles and the extremely small volume of a thin section, it is hard to obtain representative electron micrographs by using conventional electron microscopic processing from small pellets of the brain fractions.

We report here that, after collecting subcellular particles in an easy way on the surface of millipore filters, a small but still representative volume of brain fractions can be readily processed for electron microscopic investigation.

Materials and Methods

Subcellular fractions from rat brain were prepared according to the procedure of Joó and Karnushina (1973), and elaborated for the isolation of brain capillaries. After density gradient centrifugation, four fractions (from A to D) were separately collected. D fraction, the capillary-rich pellet, was suspended in 2.0 ml of 0.25 M sucrose. Then 100 µl of each fractions (A—D) was diluted with a buffered aldehyde mixture of high osmolarity (Karnovsky, 1965) to 1.0 ml. These suspensions were placed separately on millipore filters of 0.22 µ pore size and adhered to the surface in a holder with an inner diameter of 13 mm (Analysenfilterhalter, Millipore GmbH) by a water pump at 40—60 Hgmm vacuum. Filters were post-fixed in the same fixative for 2 hours at 4 °C. After overnight washing in the buffer used to make up the fixative, the filters were osmicated for 1 hour in buffered 1.0 per cent solution (Millonig, 1961) and dehydrated in graded ethanols. The filters were dissolved in propylene oxide, resulting in a thin layer of particles to be embedded flat. Sections were cut on a Porter-Blum ultramicrotome in a plane parallel to the direction of sedimentation and stained by lead citrate. Photographs were taken on a JEOL 100B electron microscope equipped with a side entry goniometer.

Results and discussion

Figures 1 and 2 show the electron microscopic appearance of the entire thickness of the fractions. In order to orientate the thin layer of fractions in the electron microscope, a BSR specimen holder was used, rendering possible rotation up to 180°. The surface facing the millipore filter, upon which particles were accumulated in a random distribution, could be easily recognized. The fine granular network, seen occasionally, may have resulted from soluble proteins. Fine structural features, characteristic of the myelin, synaptosomes, dendritic profiles and capillaries, could be readily identified.

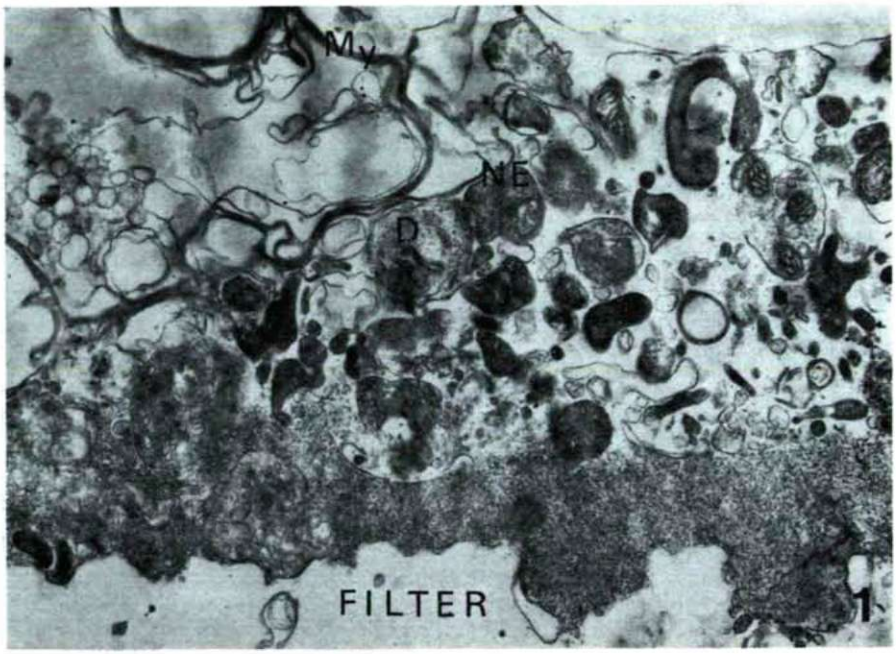

Fig. 1. A characteristic detail from the B (nerve ending) fraction. NE = nerve ending, D = dendrite, My = myelin. x25 000.

Millipore filters have already been introduced in electron microscopy by BAUDHUIN et al (1967) for the preparation of very thin pellicles of packed particles. To accelerate the filtration of suspensions derived from rat liver, a special Filterfuge assembly was built and gas pressure of 3.6 kg cm^{-2} was applied over the solution.

It is well-known that the interpretation of biochemical and physiological experiments involving subcellular fractions depends considerably on the purity of the fractions (COTMAN and FRANSBERG, 1970; GROVE et al, 1973). The main advantage of the simple sampling technique described here is that, apart from providing representative electron micrographs of fractions, it allows the carrying out of rapid quantitative evaluations on the relative concentration of an organelle in certain brain fractions.

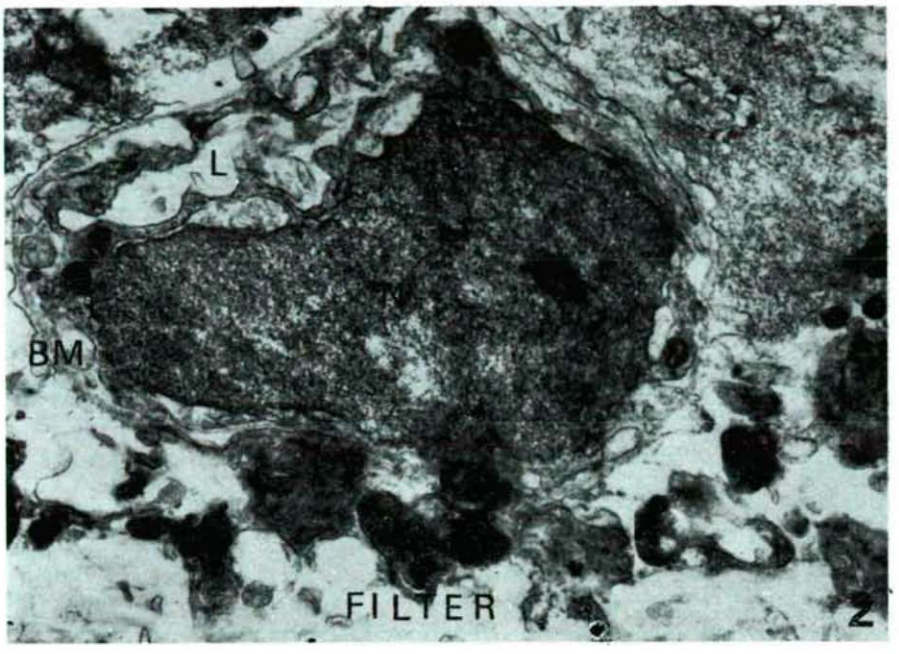

Fig. 2. Fine structural appearance of a capillary in the D fraction. N = nucleus, L = lumen, BM = = basement membrane. x18 000.

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