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Summary

For the isolation of chloroplasts in non-aqueous media the following organic solvents are suitable: pentane, hexane, heptane, petroleum ether and carbon tetrachloride. These solvents cause a minimum loss of lipids.

The loss of lipids and some lipid components of frozen-dried and ground shoots from *Elodea canadensis* during the isolation of chloroplasts in non-aqueous media (petroleum ether b. r. 60–80°C and petroleum ether-carbon tetrachloride mixtures) is reported.

PRO EXPERIMENTIS

Preparation of Subcellular Particles from Brain Tissue by a Filtration Technique[1]

The chemistry and physiology of subcellular particles have been extensively studied during the past several decades. These particles are usually isolated by differential centrifugation of tissue homogenates[2]. The present communication describes the isolation of two particulate subcellular fractions from homogenates of brain tissue by means of Millipore filters[3], which are microscopic and submicroscopic sieves of cellulosic membranes with pores of precise, specified sizes. The particles in these two fractions have approximate size ranges, swelling properties and at least two biochemical properties that are similar to those of the large granules, prepared by differential centrifugation, to which the term 'mitochondria' is frequently applied.

Millipore filters have had several applications in the biological field. For example, they have been employed in the concentration of bacteria and viruses, which are in the size range of several classes of subcellular particles.

Methods. All operations in preparation of the subcellular fractions were carried out at 0°–4°C. 1 g of mixed grey and white matter from the cerebral hemispheres of Sprague-Dawley male rats was placed in sufficient 0·25 *M* sucrose solution to make the final volume 10 ml, and homogenization effected in a Dounce all glass homogenizer by 10 strokes with the loosely fitting plunger and 5 strokes with the tightly fitting plunger. The homogenate was passed successively through electrodeposited nickel screens[4] of approximately 40 and 20 μ pore sizes. These filtrations removed tissue debris, whole cells and many nuclei. This is essential to avoid clogging of the filters with small pore size. Next the filtrate was passed through Millipore filter SM (pore size 5·0 μ ± 1·2 μ)[5]. Nuclei, red cells, and some of the largest rod shaped mitochondria were removed at this step. The filtrate was then passed through Millipore filter SS (pore size 3 μ ± 0·9 μ). This filtrate will be designated SSF. The residue on the SS filter disc was recovered by removing the disc from the filter holder, turning it upside down, replacing it in the holder and running 2 ml of 0·25 *M* sucrose through the filtration apparatus, under suction. This resuspended residue is designated SSR. SSF was now put through the Millipore filter AA (pore size 0·80 μ, ± 0·05 μ). The residue on the disc, designated AAR, was removed as described previously.

Suction of minus 20–26 inches mercury vacuum was used at each filtration step. 4·7 cm filter discs were employed. The XX20 047 000 Hydrosol standard filter holder manufactured by the Millipore Corporation was used for support of the filter discs. The filter discs were placed directly on the wire screen. At 0°–4°C ambient temperature, great care must be exercised in manipulation to avoid producing minute cracks in the Millipore filters, especially when they are dry. Suction must be applied gently for the same reason. Failure to observe these precautions may lead to badly contaminated fractions.

Adenosine triphosphatase and nitrogen determinations were done by methods similar to those previously reported from this laboratory[6]. Cytochrome *c* oxidase determinations were done spectrophotometrically by the method of COOPERSTEIN and LAZAROW[7].

Phase microscopy was used in optical study of the fractions. Sizes of the particles were estimated by means of a filar micrometer. Because of Brownian movement of the particles, these estimates are very difficult to make with accuracy and should be regarded as only rough approximations.

Swelling of the particles was studied by observing changes in their size under direct microscopic observation. Solutions of sucrose with osmotic strengths lower than that of the standard 0·25 *M* sucrose suspension media were run beneath the cover slip and changes in particle size were noted.

For the purposes of comparison with filtration fractions SSR and AAR, the subcellular fraction that sediments after centrifugation for 15 min at 12000 × gravity – the 'mitochondrial' fraction – was prepared from rat brain homogenate according to procedures previously described[6]. The fraction was not washed. It will be referred to as 12G15[6].

Results. The two fractions SSR and AAR contain spherical, comma, and rod shaped particles morphologically indistinguishable in the phase microscope from the particles seen in the 12G15 or 'mitochondrial' fraction prepared by differential centrifugation. There was not a clear cut separation of particles with respect to size in the two fractions. In SSR there was a preponderance of the larger particles from *approximately* 2·5 to 4·0 μ in size. However, many of the smaller particles which are the main constituents of AAR were present in SSR. Rarely, a red cell was encountered in SSR. AAR consisted largely

[1] We are grateful to the David Baird Grant in Neurological Research of the United Cerebral Palsy Research and Educational Foundation, Inc. and to the National Institute for Neurological Diseases and Blindness, National Institutes of Health, United States Public Health Service (Grant B-305 C), for generous support of this work.

[2] R. R. BENSLEY and N. L. HOERR, Anat. Rec. *60*, 449 (1934).

[3] Can be obtained from the Millipore Corporation, Bedford, Massachusetts. In the early phase of our work some filters were supplied gratis. We are also indebted to their staff for helpful advice.

[4] Kindly supplied gratis by the Pyramid Screen Corporation, Brookline, Massachusetts.

[5] Details of calibration and standardization of these filters can be obtained from the Millipore Corporation.

[6] W. K. JORDAN and R. MARCH, J. Histochem. Cytochem. *4*, 301 (1956).

[7] S. J. COOPERSTEIN and A. LAZAROW, J. biol. Chem. *189*, 665 (1951).

of smaller spheres and very thin rods ranging from *approximately* 1·0 to 2·0 μ. Some of the larger particles seen in SSR were also in AAR. Some very small particles less than 0·8 μ were also seen in AAR.

The sizes of particles in the 12G15 fraction were from 1 μ to 4 μ, which is the range reported by BRODY and BAIN[8] in their original description of the microscopic characteristics of this fraction. We observed some particles in this fraction which were less than 1 μ. The microscopic observations of BRODY and BAIN were done on washed 12G15 fractions, which may account for this difference.

Lowering of osmotic strength of the ambient liquid medium produced swelling in most of the particles in both filtration fractions.

Aging the filtration fractions for periods of from 1–8 h at 4°C induced crescent formation in many of the particles.

The swelling properties and crescent formation of 12G15 particles were similar to those of fractions AAR and SSR.

In the Table are summarized the results of observations on the adenosine triphosphatase and cytochrome c oxidase activities of these two fractions. For comparison, similar observations on fraction 12G15 prepared by differential centrifugation are reported. As can be seen, the specific activities of these two biochemical reactions are in the same range in all three particle groups.

The specific activity of ATPase is expressed as the micrograms of phosphorus released per h per mg of nitrogen. The specific activity of cytochrome *c* oxidase is expressed as the decrease in $\log_{10}$ of the concentration of ferrocytochrome c/min/mg of nitrogen. Designations of the fractions are defined in the text. Results are the means of 4 experiments

Fraction	ATPase	Cytochrome *c* Oxidase
12G15	3257	28.2
SSR	3536	19·7
AAR	3994	21·8

Preparation of the particles by filtration could be effected in about half the time required by the differential centrifugation technique. The final yield was less than that obtained by centrifugation.

Discussion. By means of filtration, two fractions of subcellular granules can be isolated from brain homogenates that resemble in morphological, swelling, and two biochemical properties, the 'mitochondrial' fraction obtained by differential centrifugation. This technique requires less time than isolation by centrifugation, and hence the particles prepared by the filtration procedures are exposed to unnatural conditions for a shorter period before study than are those prepared in the centrifuge. In view of the well known 'aging' effect on biochemical and other properties of the particles, this may prove advantageous in certain types of investigation. For this reason, and because of the simplicity of the equipment required, the filtration technique for preparation of subcellular granules may serve as a useful alternative to the differential centrifugation procedure under some cricumstances. Fractions SSR and AAR can be used singly or in combination.

With the 4·7 cm filter discs and with 10 ml of a 10% homogenate, separation of particles in respect to size is not precise, after one filtration. This is apparently the result of several factors. The larger particles doubtless pile up on the upper surface of the filter and trap the smaller particles remaining in the suspension above the filter. Also, some of the rod shaped particles probably slip through the filter sideways. We have tried repeated filtration with the 4·7 cm filters, analogous with repeated washings in the differential centrifugation procedure, and this does lead to much sharper resolution of particle size. However, with the 4·7 cm filters and with the quantity of homogenate used the reduction in yield with repeated filtrations is too great to provide preparations of practical value for most biochemical determinations.

The Millipore filters are available in sizes up to 60 cm by 130 cm. We have under construction a filter holder that will accommodate the larger filters and also will incorporate features making it more suitable for cell fractionation work than the present commercially available holders.

The initial impression we have derived from our experience with these filters is that with the equipment presently available they may have a definite, though limited, use in biochemical cytology. However, after improvement of apparatus and refinement of technique they may prove to have a wider applicability.

In addition to investigations directed toward improvement in methods for preparing the 'mitochondrial' fraction, studies on the preparation of other subcellular fractions by Millipore filters are in progress in our laboratory.

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Zusammenfassung

Es wird eine Technik für die Isolierung von subzellulären Partikeln in der Grössenordnung von 1–4 μ («Mitochondrien»-Fraktion) von Gehirnhomogenaten mittels Millipore-Filter beschrieben. Die Partikel zeigen morphologische Quellung und haben mindestens zwei biochemische Eigenschaften, ähnlich denjenigen von durch differentiale Zentrifugation gewonnenen «Mitochondrien»-Fraktionen. Diese Technik kann als alternative Methode zur Präparierung von subzellulären Grossteilchen-Fraktionen herangezogen werden.

[8] T. M. BRODY and J. A. BAIN, J. biol. Chem. *195*, 685 (1952).

CORRIGENDUM

R. JAQUES und R. MEIER: *Über eine Strahlenschutzwirkung von Apresolin und C. 5864-Su (2-Octahydro-1-azocinyl-äthyl-guanidin)*. Exper. vol. XVI, fasc. 2, p. 75. (1960).

Aus Versehen wurde im obigen Titel «azocynil» gedruckt, statt «azocinyl».