

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 BAIN⁸ 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 circumstances. 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».