USE OF A MICROCENTRIFUGE FOR PREPARATION OF ISOLATED MITOCHONDRIA AND CELL SUSPENSIONS FOR ELECTRON MICROSCOPY

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INTRODUCTION

The special problems of preparing suspensions of cell fractions such as mitochondria for electron microscopy are largely eliminated once the fixed organelles are gathered into a cohesive pellet. These pellets may then be handled in the manner conventional for blocks of tissue (1, 2). The use of very small samples (*e.g.*, the mitochondrial yield from 4 mg of rat liver) has been made possible by the use of a small, high speed centrifuge only recently available.

Customary glass or cellulose nitrate (Lusteroid) centrifuge tubes and related apparatus have been awkward to use for the preparation of samples for electron microscopy. Small conical glass tubes may require specially made adapters to fit them to heads for larger tubes. In addition, until the mitochondria are aggregated into a cohesive pellet, changes of solution must be tediously accomplished by repeated centrifugation, decantation, and resuspension of the sample in the new solution. In order to handle the final pellet as a tissue block, the packing must be tight enough to minimize erosion of the sample during the subsequent solution changes. When cellulose nitrate tubes are used, packing of small samples is inefficient, because the smallest of these tubes have rounded bottoms. The flat or concave pellets embedded in these tubes present a broad face to the microtome knife. Finally, where the amount of material available for electron microscopy is greatly limited, as in correlated biochemical and morphological studies, this sort of pellet may be useless (3).

What is needed, then, is a method of accumulating a small volume of mitochondria (or other dispersed particulates) into a near cylindrical or conical pellet whose shape may be maintained from fixation through hardening of the embedding medium and whose long axis may be then oriented perpendicular to the microtome knife. This would minimize wastage through trimming of the block of embedding medium and would be especially advantageous when many sections of a relatively small volume are desired.

THE METHOD

These aims can be achieved with none of the aforementioned inconveniences by means of a high speed centrifuge equipped with disposable polyethylene tubes of 0.4 ml capacity. These are translucent and conical. The centrifuge used in this study was the Microfuge made by Beckman/Instruments Inc., Spinco Division (Palo Alto, California).[1] The speed is fixed at 15,000 RPM (about 10,000 *g*). The capacity of the centrifuge is 20 tubes.

Fig. 1 is a scheme of a typical procedure, a description of which follows. A small sample of a pellet of isolated rat liver mitochondria is transferred on the tip of a Pasteur (capillary) pipette or microspatula to a microcentrifuge tube filled to about two-thirds with the fixative. The slurry of mitochondria is reaggregated into a manageable pellet by 5 minutes of centrifugation. After 45 to 60 minutes of fixation, the fixative is withdrawn with a Pasteur pipette and the tubes are cut transversely just above the top of the pellet. The large top part of the tube is discarded; the lower, very small part is carefully cut just under the bottom of

[1] A similar centrifuge and accessories are made by Coleman Instruments, Inc., Maywood, Illinois.

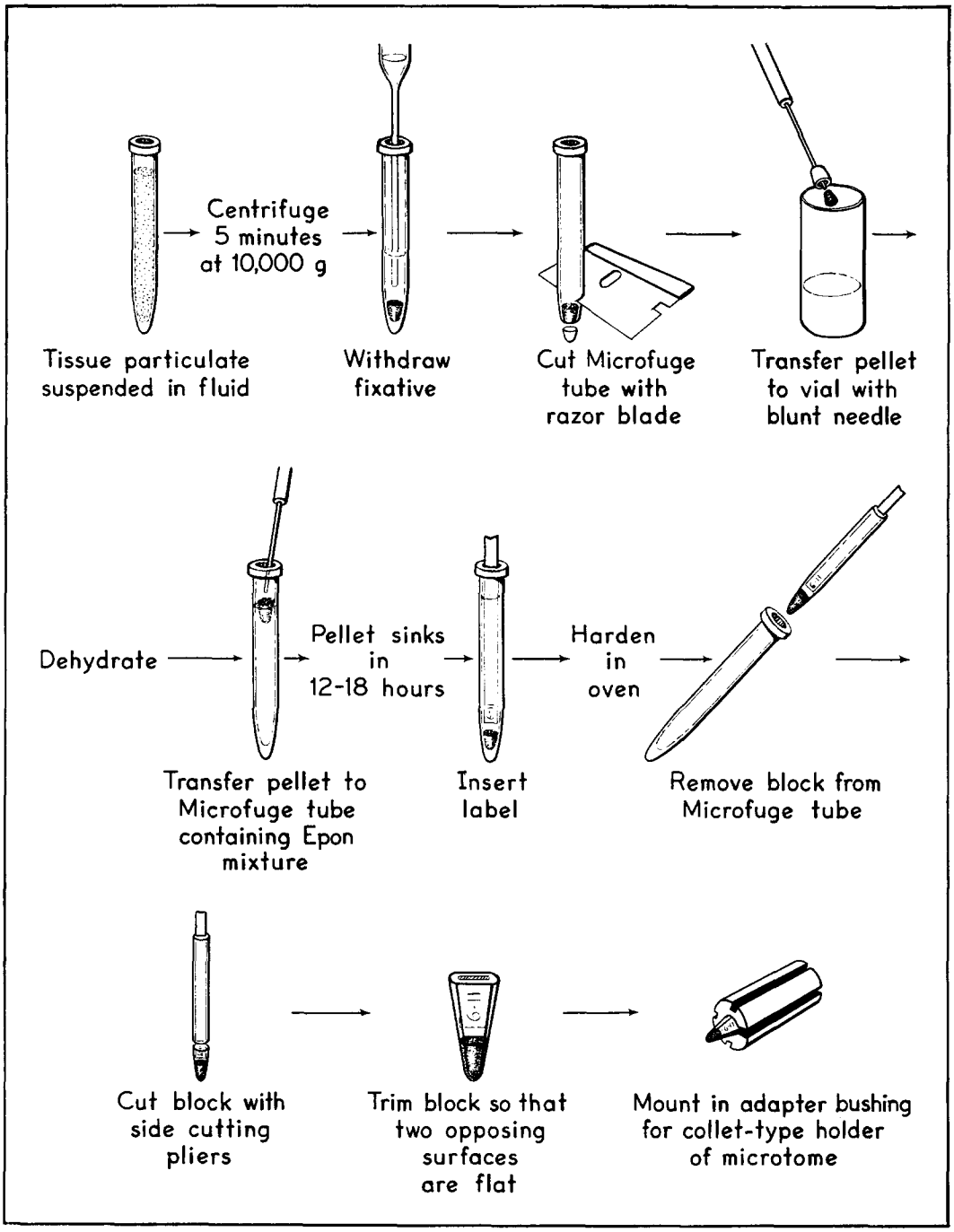
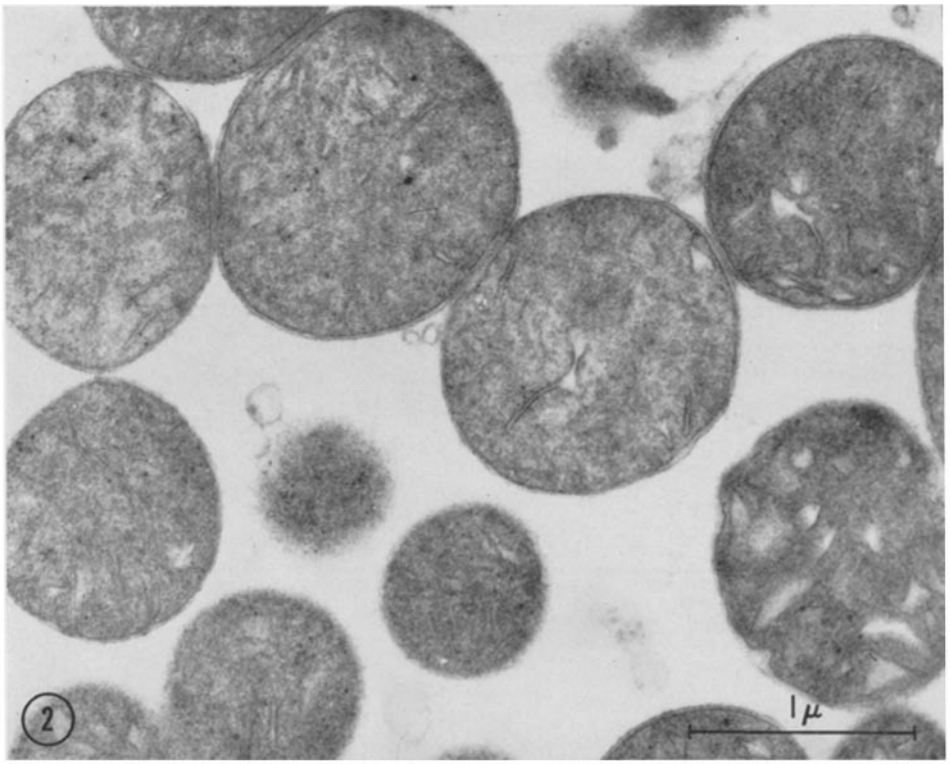
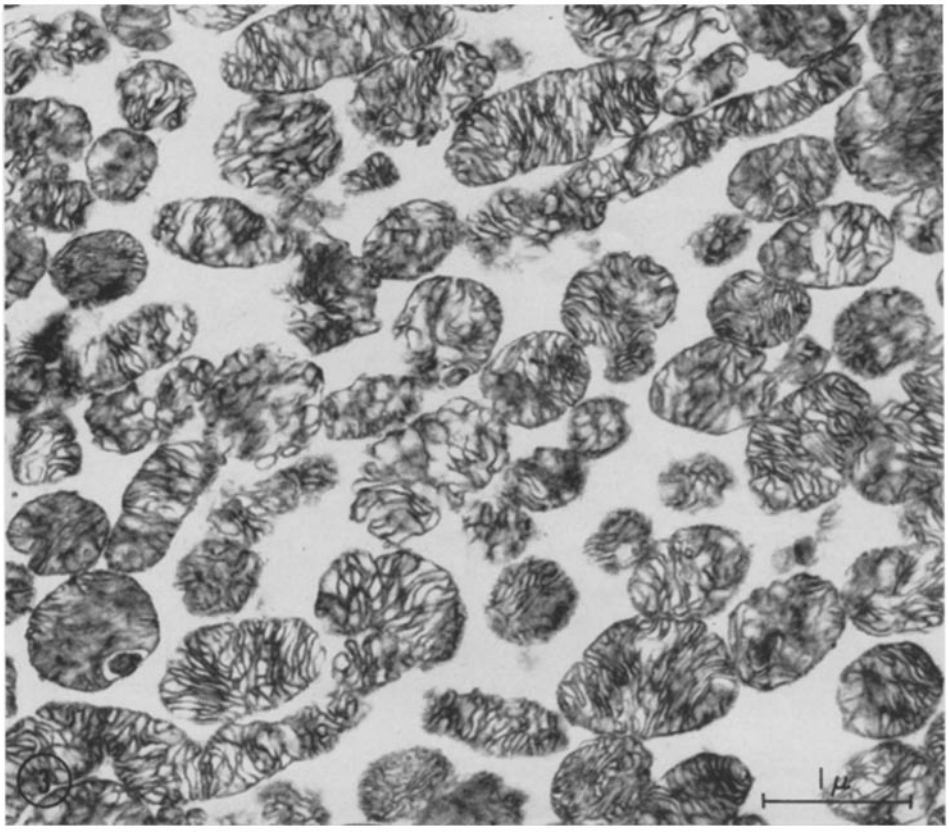


FIGURE 1 Schematic representation of microcentrifugal method for processing material for electron microscopy.


2
1 μ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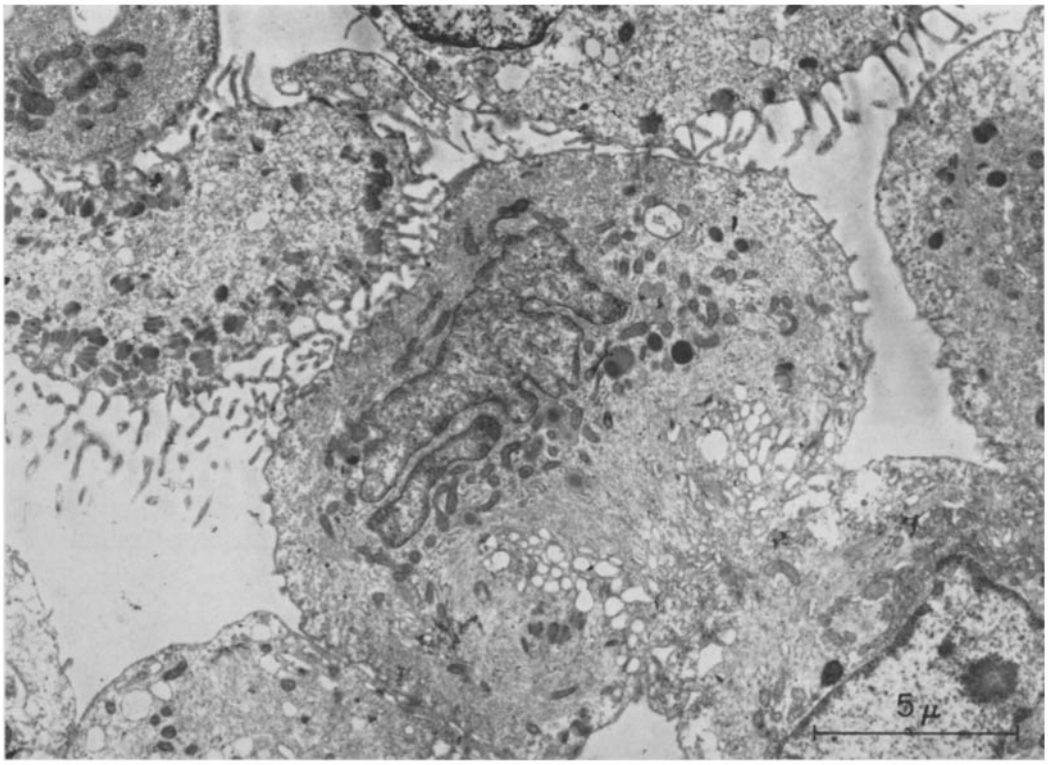

FIGURE 4 Dissociated epithelial cells of the toad urinary bladder showing retention of microvilli. × 5400.

the pellet. A wooden toothpick or blunt stainless steel needle poked into the lower, smaller hole dislodges the pellet, which is allowed to drop into a vial containing 70 per cent alcohol. During further dehydration, the pellet is handled as if it were a tissue block.[2] Finally, the pellet is transferred from the vial to Luft's Epon embedding medium of medium hardness (4) in a microcentrifuge tube (or a gelatin capsule). The pellet sinks to the bottom of the tube overnight. A narrow label is inserted lengthwise into the tube, the code number close to the pellet. The tube and its contents are then placed in an oven at 60°C for 3 days. The hardened Epon block is removed from the polyethylene tube (the latter is easily cut with a razor blade). The small tip of this long, pointed block contains the pellet of mitochondria originally fixed.[3] With a side cutting pliers, the block is now cut just above the code number on the embedded

[2] Solution changes in microcentrifuge tubes containing an unresuspended pellet result in blocks which disintegrate on cutting. Since we have been unable to resuspend the pellet intact in the original microcentrifuge tube in which fixation occurs, the pellet is transferred to a vial.

[3] The larger the pellet, the softer the tip of the block. Probably the ratio of embedding medium mass to tissue mass is more important in the use of these pointed blocks than in the use of blocks formed in gelatin capsules. Although most of the softer blocks have sectioned well, it seems advisable to work with pellets of less than 1 mm^3.

FIGURE 2 Isolated rat liver mitochondria showing inner and outer membranes and cristae. × 30,100.

FIGURE 3 Isolated pigeon heart sarcosomes showing preservation of elongate forms. × 19,400.

label. Little trimming is required before sectioning. To avoid circular sections, however, a slight shaving of opposite sides is advisable; sections then adhere to each other as they float off the knife. The trimmed block is inserted into a specially made longitudinally grooved metal sleeve, which in turn fits into the jaws of the $\frac{3}{16}$-inch collet-type holder of the Porter-Blum microtome (Ivan Sorvall, Inc., Norwalk, Connecticut).

RESULTS

Electron micrographs of material prepared by this procedure are shown in Fig. 2 (RCA EMU-3E), Fig. 3 (Philips EM-75B), and Fig. 4 (RCA EMU-3E). Fig. 2 shows rat liver mitochondria which were isolated essentially as described by Malamed and Recknagel (5) and then fixed in 1 per cent OsO_4 in veronal-acetate buffer, pH 7.3–7.5 (6). The outer and inner membranes and the cristae of the mitochondria are clearly seen. Fig. 3 shows pigeon heart sarcosomes isolated by Dr. Ann E. Bresler according to the method of Chance and Hagihara (7), and then fixed in 1 per cent OsO_4 in veronal-acetate buffer, pH 7.3–7.5, made up in the isolation medium. The preservation of their elongate form is noteworthy. The microcentrifugal processing method has been used also for a non-mitochondrial cell fraction and for whole cells. Fig. 4 shows dissociated epithelial cells of the toad urinary bladder prepared by Dr. Richard M. Hays. These were fixed in 1 per cent OsO_4 in amphibian Ringer's solution with bicarbonate, pH 7.8. The microvilli previously described *in situ* (8, 9) are clearly seen in the dissociated epithelial cells. Although the Microfuge tubes offer little advantage over vials and gelatin capsules for the preparation of whole tissue, they have been used conveniently for very small blocks of beef neurohypophysis.

SUMMARY

A convenient microcentrifugal method for preparing very small samples of isolated mitochondria or whole cells for electron microscopy is described. Fixation, packing of the mitochondria into a cohesive pellet, and embedding are accomplished in 0.4-ml conical polyethylene tubes.

The substance of this paper was presented at the Fifth International Congress for Electron Microscopy in Philadelphia, September 3, 1962 (10).

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