A Modification of the 'Hermann PO' Method for Mitochondria

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With one plate (fig. 1)

SUMMARY

Tissues are fixed in Hermann's fluid, postosmicated at 34° C, and embedded in *n*-butyl methacrylate. Sections are cut with an oblique knife on a sliding microtome, attached to glass slides with albumen, and mounted in DPX or Canada balsam.

Mitochondria are shown in black against a colourless, transparent cytoplasm. They retain their original form. Preparations made in this way are superior to those made by the original Hermann PO technique in the remarkable clarity of the mitochondria and the absence of distortion in the tissue as a whole.

It is confirmed that the presence of acetic acid at 5% in the fixative in no way damages the mitochondria.

HERMANN'S fluid is an excellent fixative for mitochondria, provided that the tissue is postosmicated; and if the postosmication is carried out for several days at 34° C, the mitochondria are blackened (Baker, 1957). This is probably the easiest and most satisfactory way of making mitochondrial preparations for light microscopy. Filamentous mitochondria retain their form.

In the original technique, paraffin sections were used. It occurred to me to try embedding instead in methacrylate. The blackening of the mitochondria was not affected, and the smaller degree of general shrinkage of the tissue resulted in even better preparations.

Dr. J. R. Baker and I have devised new methods of cutting methacrylate sections for light microscopy, and of attaching them to glass slides. These methods have only been briefly mentioned in print (Baker, 1960). It is thought that a fuller account of them might be of use to others and this is presented here as a part of the new technique.

The following fluids are required:

1. Hermann's fluid (1889). This is most easily made up thus:

Distilled water	0·8 ml
Chloroplatinic acid, 5% aq	0·3 ml
Osmium tetroxide, 2% aq	0·4 ml
Acetic acid, 20% aq	0·5 ml

2. Osmium tetroxide, 2% aq, for postosmication.
3. *n*-butyl methacrylate for embedding, prepared as for electron-microscopy.
4. Adhesive albumen (Baker and Jordan, 1953), for attaching sections to slides.
5. Ethyl acetate, for the solution of methacrylate polymer.

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The technique is carried out as follows:

I. Fix pieces of tissue not more than 3 mm thick in Hermann's fluid for 24 h.

II. Wash in running water (or repeated changes) for 24 h. Give a final wash ($\frac{1}{4}$ h) in distilled water.

III. Postosmicate for 3, 4, or 5 days at 34° C. The best period must be discovered by experiment. (Postosmication at 37° C gives much less satisfactory preparations.)

IV. Wash for several hours in running water (or repeated changes).

V. Embed in methacrylate, by the same process as is used in electron microscopy. Since larger pieces are used than in electron microscopy, the period in the monomer should be extended (up to 24 h, with several changes).

VI. Attach the block to a sliding microtome by means of a metal chuck especially made to hold it. (If the polymer is particularly hard, the block may be soaked overnight in 70% ethanol.)

VII. Cut sections at 3 to 5μ in the same way as collodion sections are cut, that is, with an oblique plano-concave knife (concave surface upwards); but the knife should be tilted at a more oblique angle than for cutting collodion sections. Flood the knife with 70% ethanol. The sections may be stored in 70% ethanol.

VIII. Transfer the sections to distilled water.

IX. Attach the sections to glass slides exactly as though they were paraffin sections, by the use of adhesive albumen (Baker and Jordan, 1953).

X. When the sections are dry, put the slides in ethyl acetate, and leave them until the methacrylate has dissolved.

XI. Bring the slides through absolute ethanol to xylene, and mount in DPX or Canada balsam.

No claim is made that the method is specific for mitochondria. For instance, the chromophil part of the acroblast is blackened (fig. 1, D, *ac*). In most tissues, however, the mitochondria are the chief objects that are blackened. The zymogen granules of the pancreas, which are deeply coloured by most mitochondrial methods, are untouched, and indeed are almost invisible (fig. 1, A). The form of the mitochondria is remarkably well preserved, as the photomicrographs show (fig. 1). They are quite black against a colourless, transparent cytoplasm. It has not been found possible to obtain a distinctive

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FIG. 1 (plate). All four photomicrographs represent tissues treated by the Hermann / PO / methacrylate technique.

A, pancreas of mouse (exocrine cells).

B, intestinal epithelium of mouse.

C, testis of mouse. The labelled cell is a primary spermatocyte.

D, testis of cricket (*Acheta domesticus*).

ac, acroblast; *ap*, apical group of mitochondria; *bas*, basal group of mitochondria; *fb*, free border of intestinal epithelium; *lum*, lumen of intestine; *mit*, mitochondrion, mitochondria; *Neb*, mitochondrial Nebenkern; *nuc*, nucleus.

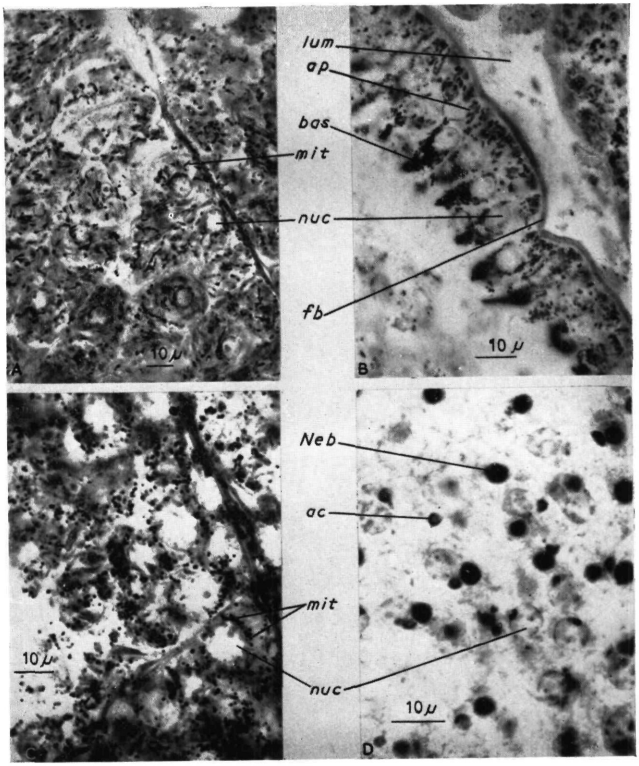

FIG. 1

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dyeing of chromatin, and it is best to leave the sections unstained. The nuclei can be distinguished, though neither the nuclear membrane nor the chromatin is blackened. The best part of the preparation is often a little below the surface, and for this reason very minute pieces should not be used as a general rule. The intestinal epithelium of mammals, however, shows mitochondria well, even if directly exposed to the fixative. The general fixation of the tissue is good, and there is little distortion by uneven shrinkage.

There are a few tissues (notably mammalian liver) that are rendered brittle by the Hermann PO technique, and do not give good methacrylate sections.

The success of this technique proves conclusively once again that mitochondria are not damaged (far less destroyed) by the inclusion of acetic acid at 5% in a fixative, provided that the tissue is postosmicated before being subjected to the action of lipid solvents.

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