MOUNTING OF ULTRATHIN SECTIONS WITH THE AID OF AN ELECTROSTATIC FIELD

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During the development of new methods for preparing ultrathin sections of unembedded tissue for electron microscopy (1, 2, 3, 5), the problem came up of placing the sections on the grid. Many experiments showed that floating the sections on water could result in numerous perforations, tearing of cellular structure, and extraction of water-soluble substances. With intensely fixed tissue, *e.g.* classical double fixation with aldehyde and osmium tetroxide, these artefacts were relatively rare and insignificant. However, they became very pronounced with mild (*e.g.* 2 per cent formaldehyde) or no fixation. Further investigations indicated that the dry transport of sections onto the grid could eliminate these problems.

PROCEDURE

We observed that ultrathin sections can be moved in an electrostatic field. A horizontal and very dense field at the knife's edge of a microtome seemed appropriate. Preliminary studies showed that, using the MT-1 Porter-Blum microtome, the sections were mostly moved in the same direction towards the specimen (block) holder, regardless of the polarization. We fastened an electrode on the specimen holder parallel to and vertically over the tissue block. With each turn of the microtome the electrode and the tissue block moved as one unit. The tip of the electrode, passing at a very small, adjustable distance from the knife's edge, concentrated the electrostatic field and exerted a maximum attracting force on the sections (fig. 1).

The electric charge (positive or negative) was produced by a direct current generator which delivered up to 10,000 volts. Since a static high tension and no current was needed for the transport of the tissues, it was safe to employ a generator supplying not more than 5 milliamperes, thus avoiding the danger of severe electrocution. Furthermore, the microtome was grounded by means of a wire connected to a water pipe.

The loading generator may be connected either to the electrode (3) or preferably to the knife. We achieved the best working conditions and the strongest attraction of the ultrathin sections when we connected the electrical potential to a copper fitting encasing the glass knife (Fig. 1). Arcing or loss of charge could be eliminated by protecting the copper fitting with a suitably constructed, insulating plastic holder (Fig. 1 A and B). Small knives of 45° were prepared from 2-cm wide glass bars with a LKB glass knife-maker and were held in place by compressing the plastic holder from the outside. The electrode, forming the other pole of the electrostatic field, was grounded. The end facing the knife had a diameter of 2 to 3 mm corresponding to the size of the grid. To insure a maximum density of the electrostatic field, the whole unit, including the electrode and the specimen holder, was insulated, excepting the tip of the electrode.

The supply of the high voltage to the copper plate was controlled by a pedal, placed in such a way that in the "on" position the copper plate was connected with the generator, and in the "off" position the copper plate unloaded itself quickly and safely through the ground (Fig. 1). This setup enabled us either to give short impulses at the moment the electrode passed the knife or to produce a continuous high tension if needed.

The grid, either a standard 200-mesh grid or, even better, a grid with a single large hole in the center, was covered with an uncarbonized formvar membrane and was placed onto the tip of the electrode. A small drop of water on the back of the grid held it in place. The drop of water had to be replaced every few minutes to prevent the dried-out grid from falling off the electrode. Because of its semipermeability, the formvar membrane became slightly moist on the side to which the sections were attracted. This moisture caused the individual sections to unfold partially and

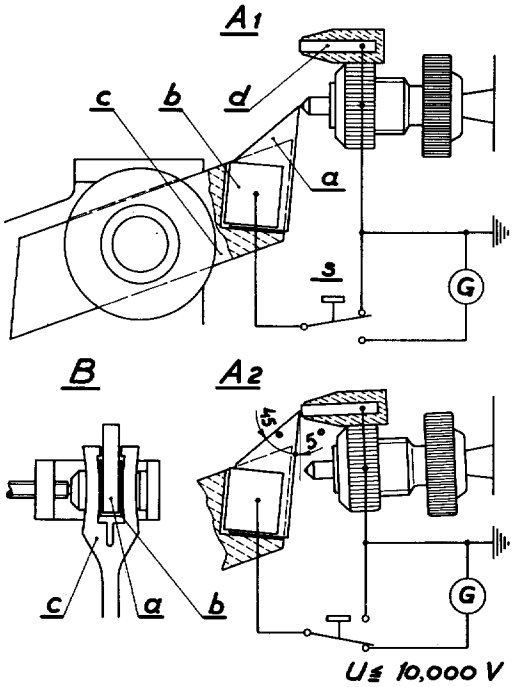

FIGURE 1 Procedure for dry transport of ultrathin sections with the aid of an electrostatic field. A 1 and 2, diagrams of the classical specimen and knife holder of the MT-1 Porter-Blum microtome. With an insulating knife holder (*c*), a small glass knife (*a*) is fastened on the microtome (see B, cross-section of this knife holder). The loading generator (*G*) is connected with a copper plate (*b*) which surrounds the knife. With a small drop of water adhering to the back of the grid, the grid is stuck on the uninsulated tip of the grounded electrode (*d*). When the electrode tip (*d*) moves opposite the knife's edge (*a*), the section, swinging in the air while adhering with one side to the knife's edge, jumps onto the grid. The supply of high voltage (*U*, Fig. A 2) has to be modified from one instance to another. By pressing down on the pedal (*s*), it is possible to give a short high voltage impulse at the moment of the electrode passage, or the loading generator may be left continuously connected to enable a continuous quick cutting.

to adhere. From time to time the number of sections was checked with the light microscope. Adherence of the specimens on the formvar membrane was improved by subsequently floating the grid (sections on the top and membrane at the bottom) for 15 to 30 seconds on distilled water or for a few minutes on the staining solution.

In order to eliminate air currents and to control humidity, the microtome was placed in a plastic chamber with two holes allowing manipulations from the outside. The most favorable relative humidity was 50 to 70 per cent, and was kept constant with either P_2O_5 or water vapor. When the humidity was too high, the ultrathin sections tended to roll up and adhere too strongly to the knife's edge before they were moved in the electrostatic field. On the other hand, if the air became too dry, the sections could no longer be moved in the electrostatic field. With correct humidity the section adhered with one side to the knife's edge, swinging in the air without curling. As the electrode point moved opposite the knife's edge, the section jumped onto the grid.

The charge distribution with our set-up on the MT-1 Porter-Blum microtome was very complex, and conditions may be different for another microtome.

MATERIAL

In our experiments with rat tissues, we used non-embedded pancreas, kidney and liver. The preparation of the tissues has been described elsewhere (3) and is only briefly summarized here.

1. Fixation was carried out for 1 hour at 0 to 4°C in 2 per cent formaldehyde in phosphate-buffered saline (0.02 M, pH 7.2), or by exposing the tissue for 1 hour to vapors of 35 to 40 per cent formaldehyde at 20°C.
2. The tissues were washed with phosphate-buffered saline at 0 to 4°C for 5 minutes. (This can be omitted, if fixation occurred over vapors.)
3. The water in the tissue was replaced with liquid nitrous oxide (N_2O) in a pressure chamber at 35 atm for 24 hours at 0°C.
4. The tissues were dried by evacuating the nitrous oxide at 0°C.
5. The dried tissues (blocks approximately 1 mm^3) were placed on the microtome using a modified needle holder or gelatine capsules filled with methylmethacrylate ("Palacos" Kulzer and Co., Bad Homburg von der Höhe, Germany) which polymerizes in a few minutes at room temperature, thus preventing its penetration into the tissue.
6. The sections were stained with 2.5 per cent uranyl acetate in 50 per cent alcohol for 5 minutes.

RESULTS

The dry-sectioning enabled us to use mildly fixed (or even chemically unfixed) tissue without embedding. We obtained fairly good, reproducible sections. Fig. 2 shows a portion of exocrine rat pancreas, fixed for 1 hour in a 2 per cent formaldehyde solution. The section is thin, and the nucleus and the structures within the cytoplasm are well preserved. Fig. 3 shows part of a rat liver cell,

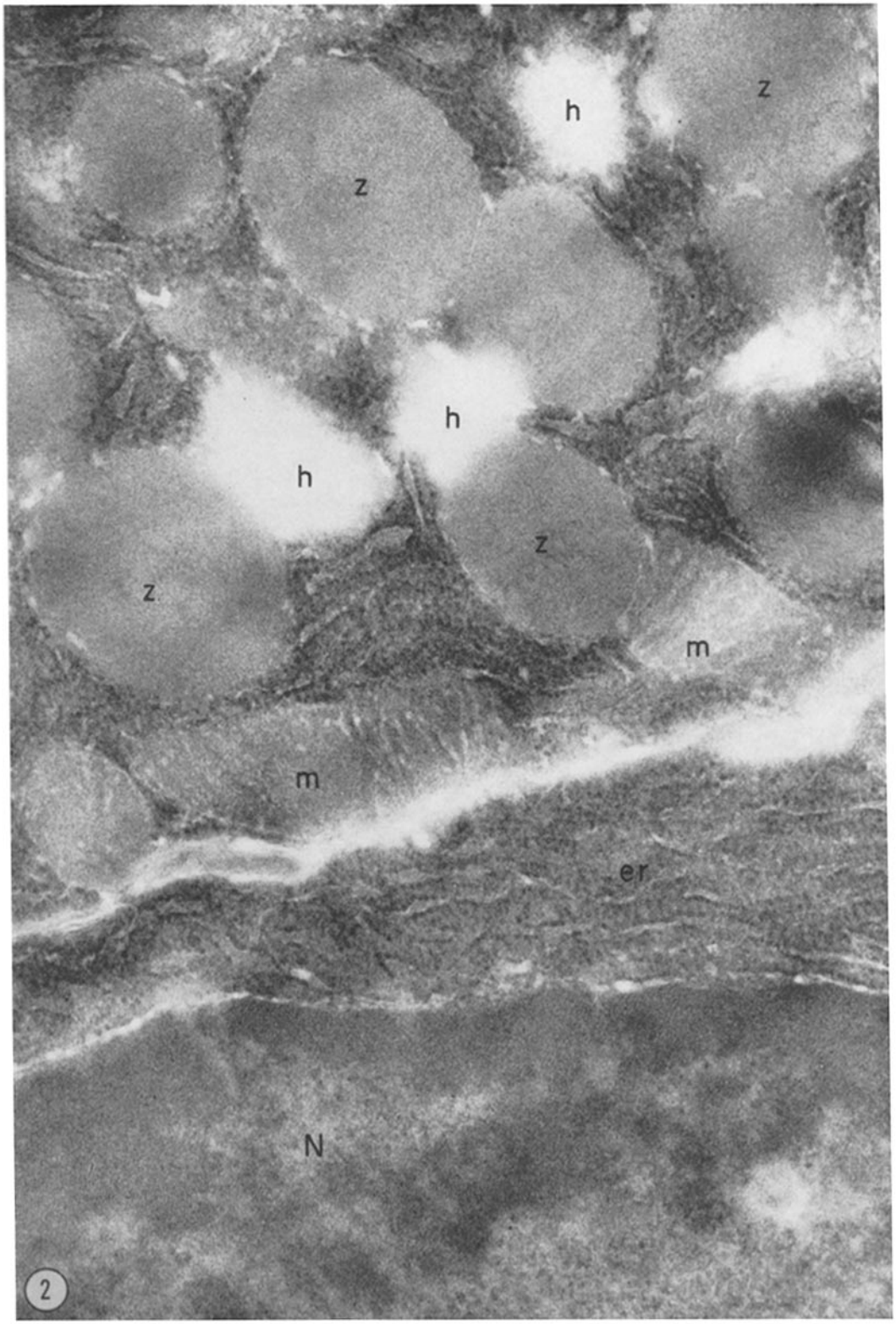

FIGURE 2 Section of exocrine pancreas of the rat without embedding material. Portion of a nucleus (*N*), zymogen granules (*z*), mitochondria (*m*) and ergastoplasm (*er*). Cutting artefact (*h*). Mild fixation in buffered 2 per cent formaldehyde. × 80,000.

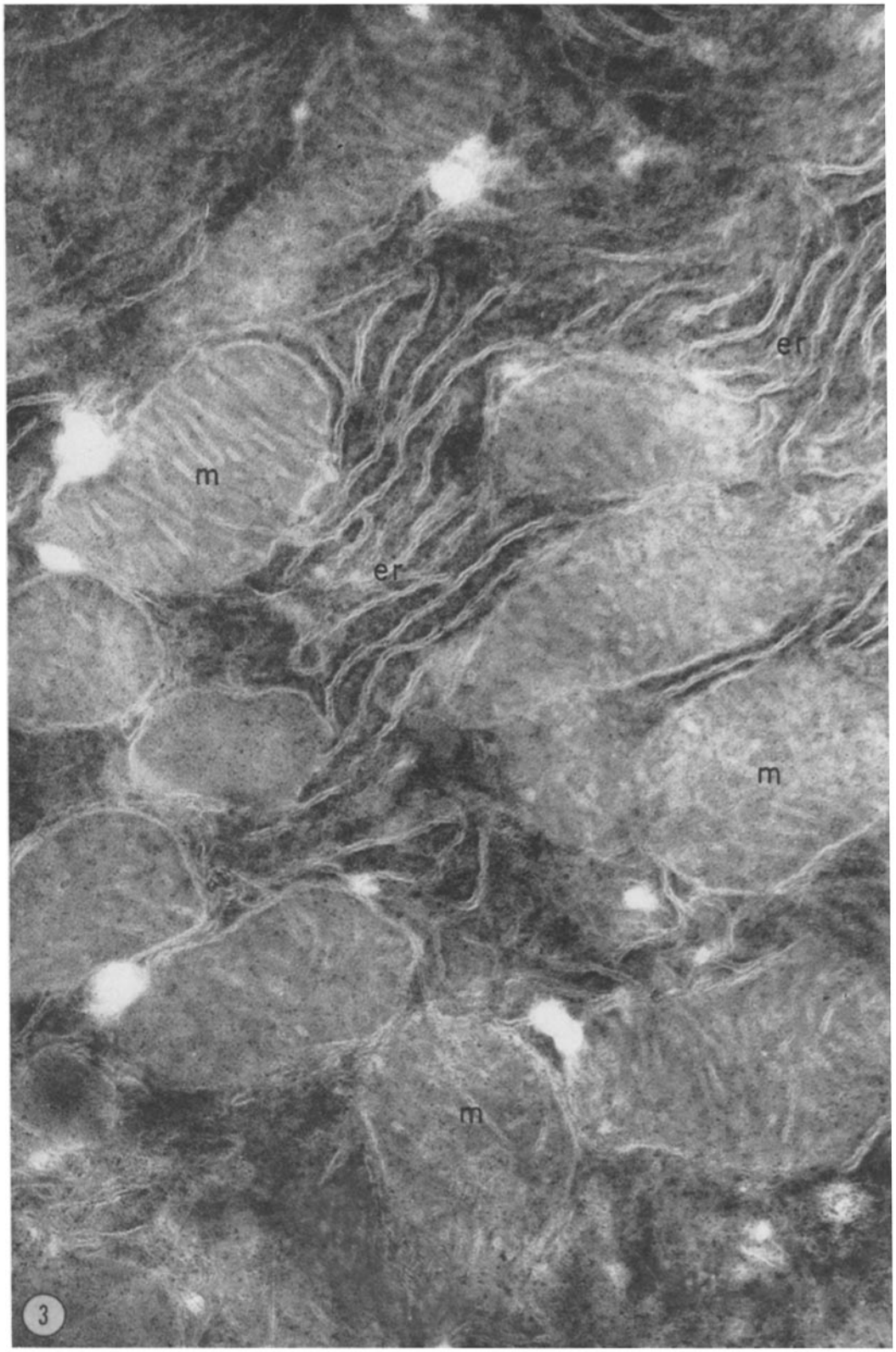

FIGURE 3 Section of liver cell of the rat without embedding material. Cytoplasm with mitochondria (*m*) and ergastoplasm (*er*). Fixation in vapors of 40 per cent formaldehyde. × 60,000.

fixed with formalin vapors, which contains mitochondria and ergastoplasm.

Advantages of the dry transport of ultrathin sections were that (*a*) extraction of water-soluble substances was minimal, and (*b*) formation of holes, tearing, and structural disturbance, which always took place when mildly fixed ultrathin sections without embedding material were floated on water, were reduced. There were, however, still irregularities caused by the knife (see *h* in Fig. 2).

The disadvantage of the dry section transport was that only a comparatively small part of the section was sufficiently spread out. Folding was frequent. Large sections on the knife were often torn by the attracting electrostatic forces. Therefore, the pieces on the grid were often small. In order to get well spread areas, it was necessary to cover the grid with many sections. With the microtome with properly adjusted electrode and a tissue which was easy to cut, the covering of the grid took 10 to 15 minutes.

Dry transport of ultrathin sections is proposed for sectioning mildly fixed, non-embedded biological material (1, 3). In mineralogy, dry sectioning could be applied to the study of water-soluble materials.

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