A SIMPLE SECTIONING TECHNIQUE FOR ELECTRON MICROSCOPY

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Since the electron microscope has been applied to biological problems, it has been seriously required by histologists and cytologists to get tissue sections thin enough to permit the penetration of the electron beam without destruction of the sections. After the first description in 1939 concerning this problem by Ardenne, who tried to get thin sections by the use of the thinnest portion of wedge-shaped sections, many attempts had been made for the solution of this problem. In 1948 Pease and Baker succeeded in constant production of the sections as thin as 0.2 microns using the Spencer rotary microtome with the altered microtome-advancing mechanism. In the next year Newman, Borskyo and Swerdlow succeeded by the use of thermal expansion apparatus as the advancing mechanism and the resin as the embedding material. Thereafter, many devices have been made, especially in America, and now, it seems to be not difficult to obtain thin sections for electron microscopic observation. However, these methods for thin sectioning, which are performed chiefly in America, require still some special apparatus and material. In our country, in which these are not easily available, many difficulties are felt to introduce these thin sectioning techniques. For this reason, we have tried to get thin sections for electron microscopic observation using the ordinary microtome and embedding materials, and we have produced a reasonably good result in the preparation of sections thin enough to permit the electron microscopic observation with several animal tissues. It is the purpose of this paper to describe this thin sectioning techniques in detail.

TECHNIQUES

Small tissue blocks are fixed and dehydrated according to ordinary cytological techniques and embedded in 64–5°C M.P. paraffin.

For sectioning, the ordinary sliding microtome (Jung or Schanze type) is used. The paraffin block containing the tissue is fastened on wooden block and the excess of paraffin surrounding the tissue block is cut off thoroughly, then the block is fixed to the specimen holder of the microtome.
Instead of ordinary microtome knife, we have used “Valet” razor blade attached to blade holder which is designed to resemble the ordinary microtome knife in size. The knife is fastened to the knife holder of the microtome and the specimen is adjusted to the knife moving the specimen with the aid of feed screw of the microtome up to the cutting position. Then the knife is stopped close by the specimen and an icebag is placed both on the specimen and the knife. After about 5 minutes of cooling down, the icebag is removed and the water drops gathered on the surface of the specimen and the knife edge are wiped off with clean dry gauze. Because the specimen shrinks by the cooling down, no slice of specimen is made on the cutting stroke of the knife. Then the specimen is moved slowly up to the knife with the aid of feed screw of the microtome until the first slice is made. Then a drop of 70% ethyl alcohol is put on the blade adjacent to the knife edge. A few seconds after the first cut, a thin slice is cut off from the specimen on the cutting stroke of the knife without moving the specimen up to the knife, because the surface of the specimen is moved a bit up to the knife by the effect of thermal expansion of the specimen itself. The slice cut off floats onto the surface of the alcohol dropped on the blade. Thus, on each cutting stroke with the interval of a few seconds, a thin slice of the specimen is cut off and floated onto the surface of alcohol, until the temperature of the specimen reaches near the room temperature.

After several cuts the knife is detached from the knife holder of the microtome and the knife edge is immersed into the water warmed to about 50°C slowly, then the sections float up on to the surface of it, because alcohol dissolves rapidly into the water, and flatten out by the softening of paraffin on the warmed water. With the procedure just described above, folding of the sections is reasonably avoidable. The techniques of the mounting of the sections are performed according to the description of Newman, Borysko and Swerdlow. The sections are floated onto clean microscopic cover glass and allowed to dry flat. The embedding media are then dissolved out by placing the cover glass in xylene or amyl acetate. 1% solution of celloidin in amyl acetate is allowed to flow over the cover glass bearing the tissue, which is then permitted to dry at room temperature. The celloidin film containing the section is floated from the cover glass onto water, and the specimen-mounting screen for the electron microscope are placed over the area of the film containing the section in the usual manner.

By the techniques described above it is difficult to obtain the thin sections in continuous layer and this is the most weak point of this techniques, which is
said to be eliminated by the method described by Pease and Baker or by Newman, Borysko and Swerdlow. But the techniques require no special apparatus or material and it is not difficult to get skilled in them to whom experienced in the use of the sliding microtome. Therefore, these techniques seem to be adoptable also for the laboratories of small scale.

Various materials have been employed by many authors as the embedding material for ultra-thin sectioning, but the most excellent one seems to be polymerized n-butyl methacrylate proposed by Newman, Borysko and Swerdlow. Because this synthetic resin is not available to us, we have used paraffin (64–5°C M.P.) as the embedding material. About some tissues such as liver, kidney and spleen, which possess uniformly parenchymatous consistency, it is not difficult to get sufficiently thin sections from the specimen embedded in paraffin (figs. 1, 2, 5, 6), but for the tissues rich in fibrous elements paraffin embedding seems to be not suitable. As seen in the electron micrographs (figs. 3 and 4) of pancreatic exocrine cells, the secretion granules which are thought to have much denser consistency than the matrix, are apparently moved from their original position during the sectioning. Such a dislocation of granules with higher consistency engraved in the media of lesser consistency seems to be unavoidable, when the specimen is embedded in relatively soft and fragile material such as paraffin. We used also the collodion-paraffin double embedding method proposed by Pease and Baker, but the specimens prepared by this method were too hard for sectioning by our techniques. Some modifications of this collodion-paraffin embedding method are now attempted.

It is obvious that the cells receive profound alteration in their fine structures during the fixation and that, as far as fixation is concerned, electron microscopy requires more exact criterion than light microscopy. Tissues observed by us are fixed in Champy’s fixative for 24 hours according to the ordinary cytological techniques. The tissue blocks are then cut into pieces a few millimeter on the side after the removal of the superficial zone, in which the fine structures of cells appear to be strongly disturbed by the first attack of fixative. Formalin fixation is also performed in some instances. Apparent differences are seen between electron micrographs of the tissue fixed in Champy’s fixative and those fixed in 10% formalin solution and the fine structures of the cells seem to be less distinctly preserved in the specimen fixed in the latter fixative than those fixed in the former. It seems to be note-worthy that some differences are seen among the electron micrographs of the tissue sections obtained from the same kind of
organs despite that they are fixed and prepared in the same condition. Fig. 1 and 2 are the electron micrographs of the normal mouse liver cells taken from different animals, fixed in Champy's fixative and prepared in the same manner. A distinct difference of the cell contents is clearly demonstrated, when these two electron micrographs are compared. In Fig. 1 mitochondria are demonstrated as a structure which possess a definite limiting membrane and some filamentous inner structures in its ground substance, while in Fig. 2 mitochondria are seen as dark, homogenous granules or rods showing no specific inner structure. It could not be denied that these structural differences between these two samples are attributable to the vital conditions of the liver cells at the time of fixation, but the possibility that they are produced by some delicate methodical variations during the preparation, especially fixation, of the specimen might be more significant. Palade claimed that the structural details of mitochondria of the rat liver cell were most clearly demonstrated when the tissue blocks were fixed in 1% OsO4 solution buffered at pH 7.3–7.5, and in the specimens fixed in unbuffered OsO4 mitochondria appeared as homogeneous dark granules without distinct inner structure. It must be careful that the effect of pH of fixative upon the structural alteration of the cytological constituents must be seriously considered for analysis of the electron micrographs of tissue sections, but it is also obvious from Fig. 1 and 2 that many factors other than pH of fixative play an important role for the structural alteration of cytological constituents during fixation. Moreover, it seems to be realized that a variety of fixatives must be selected for the fixation of various tissues.

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EXPLANATION OF PLATES

All figures are electron micrographs showing sections of tissue blocks immersed in Champy’s fixative for 24 hours, embedded in 64–5°C M.P. paraffin and sectioned by the method described above. The sectioned material is examined in a Shimazu SM-IB type electron microscope at an original magnification of 1500–2000× and enlarged thereafter photographically, as desired.
Fig. 1. Electron micrograph of the normal mouse liver. Four dark strives running obliquely in the picture are the folding of the section. Parts of the cross sections of six liver cells are seen. Each cell is limited by the definite cell membrane. A nucleus (n) is seen in left upper part and two are seen in both right and left lower part of the picture, respectively. The nuclear membrane is distinct. Dense, almost homogenous granules in the nuclei are thought to be nucleoli (nl). Nuclear content shows foamy character. In the ground substance of the cytoplasm many small vacuoles are present and the ground substance itself is composed of very fine granular material. Numerous round structures measuring 0.5–1.0 microns in diameter seen in the cytoplasm are thought to be mitochondria (m) cross sectioned. They possess a definite limiting membrane and their content shows fine granular appearance. In some instances, fine filamentous structures are apparently observed in mitochondrial ground substance arranged radially against the limiting membrane (mf). Among the mitochondria considerable number of the round dense granules measured 0.1–0.2 microns in diameter are seen scattered about in the cytoplasm, which seem to coincide with the lipid inclusions (l) described in Palade's paper. Moreover, groups of filamentous structures measured 30–50 millimicrons in thickness (f) can be seen embedded in the ground substance. They are distributed irregularly in the cytoplasm, but some of them appear to adhere to the mitochondria (f').

Fig. 2. Section from the normal mouse liver taken from an animal other than that of Fig. 1. A distinct difference is observed between Fig. 1 and 2 concerning the character of mitochondria (m). The mitochondria show no limiting membrane and inner structure, but they are demonstrated as dense homogenous granules or rods in this figure. Therefore, they are difficult to distinguish from the so-called lipid inclusions. Another structures show almost the same characteristics as seen in Fig. 1.

In this figure two sinusoids (s) are seen, one in cross section and the other in longitudinal section. Two Kupffer cells (K) with elongated nucleus are seen located in latter one.
Fig. 3. Electron micrograph of the section of the pancreatic exocrine cells obtained from normal guinea pig. The acinar structure is well preserved. Numerous dense round granules with definite outline scattered in the central portions of the acini appear to be secretion granules (sg). In the basal portion of the cells a few round or rodlike structures are seen which show lesser density than secretion granules. They are thought to be mitochondria (m). Among the mitochondria numerous filamentous structures (f) are observed showing the tendency to arrange parallel to each other. The ground substance of the cytoplasm shows foamy appearance.

Fig. 4. Three adjacent cells obtained from the same specimen as in Fig. 3 are shown. In the apical part of the cells, in which the secretion granules (sg) are numerous, the destruction of the cytoplasm is conspicuous. Such a destruction seems to be attributable to the technical imperfections described above. In the basal part of the middle and right cells in the picture some fine canalicular structures (ca) can be seen embedded in cytoplasmic ground substance, while in the left cell numerous filamentous structures (f) are clearly observed arranging parallel in apico-basal direction.
Fig. 5. Electron micrograph of the kidney obtained from normal guinea pig. A part of the renal tubulus sectioned obliquely is shown. The basement membrane (bm) of the tubulus is demonstrated as a dense thick line without specific structure. Cytoplasmic constituents are gathered together to the basal part of the cells and in their apical part (ap) the ground substance is strongly vacuolated. The lumen of the renal tubulus is not shown in this figure. The mitochondria (m) are limited from the matrix by somewhat thicker membrane. Numerous dense, homogeneous granules, which vary considerably in size, are present. These are thought to be identical with so-called lipid inclusions (1). A group of numerous filamentous structures (f) is seen in the intermediate part of cytoplasm. It is probably due to unsatisfactory deparaffinization that a part of this structure is obscured as if it is clouded over.

Fig. 6. A longitudinal section of a renal tubulus of the rat kidney. Parts of the epithelial linings sectioned longitudinally are seen in the upper and lower half of the picture. Between these two linings runs longitudinally a zone (lz) composed of somewhat homogeneous material. Embedded in this homogeneous material, numerous fine filaments (f) are detectable, which are arranged parallel at an approximately right angle to the longitudinal axis of the renal tubulus. In this figure mitochondria (m) are shown as dense homogeneous granules that they are difficult to distinguish from so-called lipid inclusions.