

The Application of Polyethylene Glycol (PEG) to Electron Microscopy

JOHN J. WOLOSEWICK

Department of Anatomy, University of Illinois at the Medical Center, Chicago, Illinois 60612

ABSTRACT The cytoplasm of cells from a variety of tissues has been viewed in sections (0.25–1 μm) devoid of any embedding resin. Glutaraldehyde- and osmium tetroxide-fixed tissues were infiltrated and embedded in a water-miscible wax, polyethylene glycol (PEG), and subsequently sectioned on dry glass or diamond knives. The PEG matrix was removed and the sections were placed on Formvar-carbon-polylysine-coated grids, dehydrated, dried by the critical-point method, and observed in either the high- or low-voltage electron microscope. Stereoscopic views of cells devoid of embedding resin present an image of cell ultrastructure unobscured by electron-scattering resins similar to the image of whole, unembedded critical-point-dried or freeze-dried cultured cells observed by transmission electron microscopy. All organelles, including the cytoskeletal structures, are identified and appear not to have been damaged during processing, although membrane components appear somewhat less distinct. The absence of an embedding matrix eliminates the need for additional staining to increase contrast, unlike the situation with specimens embedded in standard electron-scattering resins. The PEG technique thus appears to be a valuable adjunct to conventional methods for ultrastructural analysis.

During the past five years we have focused our attention on the appearance of the cytoplasm of whole, unembedded cultured cells observed with the transmission electron microscope at both high and low voltages (3, 7, 11–13). Stereo-electron microscopy of whole cultured cells fixed by a variety of techniques, and dried either by the critical-point method or by freeze-drying, has revealed a cytoskeleton of microtrabeculae, microfilaments, and microtubules (13). The microtrabeculae seen in these preparations have been interpreted as the fixed and dried ultrastructural correlates to a nonrandom, nonhomogeneous cytoplasmic ground substance present in the living state.

In the thin plastic section, fine, feathery structures project from the surface of the microtubules and form elements of the cytoplasm. Similar strands extend around clusters of ribosomes. These fine projections hold the same relation to all of the major components of the cytoplasm as do the microtrabeculae. Accordingly, we have hypothesized these fine structures to be the thin-section equivalents to the microtrabeculae of whole, unembedded cells (13).

To test this hypothesis, as well as to extend our studies on the cytoplasmic ground substance, cells of solid tissues were fixed, dehydrated, and embedded in an extractable embedding, polyethylene glycol (PEG). Thick and thin sections were cut, divested of their PEG matrix, and dried by the critical-point process, as for whole cells. These extracted sections present the same aspect of cell ultrastructure to the electron beam as whole, fixed, and dried cultured cells. In this manner a more complete imaging of the structured components of cells

is achieved from sections than can be obtained from conventional epoxy resin-embedded cells.

MATERIALS AND METHODS

6- to 8-wk-old mice were lightly anesthetized by intraperitoneal injection of Nembutal (sodium pentobarbital, Abbott Laboratories, North Chicago, Ill.) and fixed by whole-body vascular perfusion with 2% glutaraldehyde (Polysciences Inc., Warrington, Pa.) in 0.1 M sodium cacodylate, pH 7.2 (Sigma Chemical Co., St. Louis, Mo.). Samples of cerebral cortex, liver, spleen, skeletal muscle, and testis were removed 15 min after perfusion and minced in the fixative into $\sim 1 \text{ mm}^3$ pieces; the fixation was continued for a total of 2 h at room temperature. The tissue blocks then were rinsed three times (10 min each change) in 0.2 M sodium cacodylate (pH 7.2), exposed for 1 h to 1% osmium tetroxide (Sigma Chemical Co.) in 0.1 M sodium cacodylate (pH 7.2), rinsed well in buffer, and transferred to distilled water.

In addition, samples of mouse mammary tumor and human blood cells (isolated by Ficoll-Hypaque gradients according to published procedures [4]) were fixed by immersion in buffered glutaraldehyde, minced into small pieces, and processed as described above. After exposure to osmium tetroxide, the blood cells were washed in distilled water and invested in warm 2% agar. This was allowed to solidify and the small pieces were processed as tissue blocks.

The samples were processed through PEG by one of the following procedures.

PROCEDURE 1: Tissue blocks were placed into 4-dram vials filled with warm (55°–60°C) 50% PEG (vol/vol, mol wt 4,000 or 6,000, Sigma Chemical Co.) in distilled water. The vials were placed in a temperature-regulated shaker bath maintained at 55°–60°C, and agitated during infiltration (1–8 h). The blocks were removed from the 50% PEG, drained of excess PEG on warm filter paper, transferred to vials containing 70% PEG (vol/vol in distilled water), and infiltrated on the shaker bath for 1–8 h. The process was repeated twice more with 100% molten PEG.

PROCEDURE 2: Tissue blocks were dehydrated in increasing concentrations of ethyl alcohol (15, 25, 50, 70, 95, and 100%), using 15 min for each concentration. The samples were transferred to a 50% solution of PEG in 100% ethyl alcohol (vol/vol) prepared by mixing equal volumes of the liquid PEG and ethyl alcohol. The samples were infiltrated for 1–8 h as described above, or in a 60°C oven.

The blocks were removed from the 50/50 PEG-alcohol solution, transferred to jars containing 70% PEG in 100% ethyl alcohol (vol/vol), and infiltrated with 100% molten PEG.

The samples were embedded in PEG contained in either well-dried dried-gelatin capsules or small aluminum foil boats. The embedding procedure consisted of filling the gelatin capsules or boats with molten PEG and transferring individual blocks to the capsules or boats. The samples were kept in a 60°C oven until the blocks sank to the bottom, at which point each capsule or boat was removed individually from the oven with forceps and quickly immersed in swirling liquid nitrogen. Some samples were allowed to cool slowly at room temperature.

The top of the gelatin capsule or the aluminum foil was removed, exposing the blackened tissue. Solidified PEG is brittle, hence each tissue block was cut out and mounted on wooden dowels with dental wax, or Pyseal (Fisher Scientific Co., Fairlawn, N. J.), before trimming and sectioning.

The blocks trimmed easily with sharp, degreased razor blades. Block faces as large as 2–4 mm² have been sectioned, although smaller blocks (e.g., 0.5–1 mm²) were handled more readily. The blocks were sectioned on dry glass or diamond knives in either a Sorvall MT-1 or MT-2B ultramicrotome (DuPont Instruments-Sorvall, Newtown, Conn.). Knife angles and speed of sectioning needed to be adjusted for each tissue until full, relatively wrinkle-free sections resulted. In some instances a cooling stream of CO₂ (dry ice held in a metal sieve) was directed onto the block. This facilitated sectioning, but caution was exercised so as to prevent water vapor from condensing on the knife edge.

All of the PEG sections were mounted onto Formvar-coated (Formvar, 0.25–0.7%, E. F. Fullam Inc., Schenectady, N. Y.), carbon-stabilized copper grids that had been treated with poly-L-lysine (0.1% in distilled water; mol wt 50,000–300,000; Sigma Chemical Co.) similar to the method of Mazia et al. (5). Best results were obtained with large mesh grids (e.g., 50- or 100-mesh or slot-type grids). It was also necessary to allow the grids to dry completely, and frequently to apply a second coat of poly-L-lysine before mounting of the sections. Mounting and removal of the PEG from the sections was accomplished in one of the following ways. (a) Short ribbons of sections were transferred from the knife edge to a well of a porcelain spot plate, and the sections were covered with either a solution of 95% ethyl alcohol containing 5 g of PEG, or 50% PEG in distilled water (wt/vol). Each coated grid was held vertical in the solution and, while viewed with the dissecting microscope, individual sections were moved into position with the camel hair. The section was then spread gently onto the Formvar surface between the grid bars. (b) A coated grid was held in self-clamping forceps and placed on the viewing stage of a dissecting microscope. A short ribbon of sections was carefully placed on the grid with a fine hair. A small drop (10 μl) of the alcoholic PEG or the 50% aqueous PEG solution was placed on the grid, and the sections were spread gently onto the Formvar surface.

When aqueous PEG was used, the grids were gradually rehydrated to distilled water and transferred to a submerged multiple grid holder (e.g., one from Tousimis Research Corp., Rockville, Md.). The holder (and grids) was transferred quickly, and without draining, to a 50-ml beaker filled with distilled water and dehydrated in increasing concentrations of ethyl alcohol. This procedure consisted of slowly adding 25-ml volumes of the alcohol (15, 25, 50, 70, 95, and 100%) to the beaker and aspirating the fluid to a level just above the grids. Another 25-ml volume of the solvent was added and the process was repeated. 100 ml of each concentration was used for the dehydration. After the final change of ethanol, the holder was transferred to the bomb of the critical-point apparatus (Tousimis Research Corp. or Dupont Instruments-Sorvall) filled with ethanol and dried from liquid CO₂ by the critical-point process (1).

For those sections mounted in alcoholic PEG, the grids were transferred to 95% ethyl alcohol and then placed in the multiple grid holder (submerged in the 95% ethyl alcohol), exchanged twice more with absolute ethyl alcohol, and dried by the critical-point process.

The dried grids were stabilized by evaporating carbon on both sides (Denton Vacuum Inc., Cherry Hill, N. J.) and stored over silica gel in a glass dessicator until ready for viewing in either the high- or low-voltage electron microscope.

In addition to specimens prepared by the procedures detailed above, comparable samples of glutaraldehyde-perfused mouse tissues were prepared for conventional epoxy embedding. After perfusion, tissue samples were minced in the fixative, carried through osmium tetroxide, rinsed in distilled water, dehydrated in ethyl alcohol, infiltrated, and embedded in Epon. Similarly, monolayer cultures of WI-38 were fixed and processed for electron microscopy by methods published previously (13).

RESULTS

Some of the typical features of transmission electron microscope images obtained from epoxy-embedded cells are illustrated in Fig. 1. Cytoskeletal structures (microtubules and microfilaments), cisternae of the endoplasmic reticulum, and ribosomes are recognized readily in this image of a cultured

WI-38 cell. Closer examination of high-magnification images reveals structures of smaller dimensions (5 nm in diameter and less) apparently contiguous with the cisternae of the ER, free polysomes, the surfaces of microtubules and microfilaments (arrows, Fig. 1). The limits of these structures are not defined as well as those of the membrane and cytoskeletal structures. Further examination of these sections also reveals that the resin is electron-translucent, not electron-transparent; thus the resin itself exhibits a granular substructure. The organelles are seen distinctly as a result of electron-scattering contrast; that is, the high atomic number compounds used to "stain" the section greatly increase the differential scattering of the electrons of these structures over that of the embedding resin. Very small structures, however, may not possess the thickness nor density (even after staining) to be contrasted against the resin. The majority of these small structures are obscured in the resin section, with little chance of being imaged distinctly. In contrast (and as seen below), small structures in sections of cells devoid of embedding resins are imaged easily in the transmission electron microscope, even at high accelerating voltages, without additional stains to increase contrast.

The PEG-embedded tissues can be sectioned readily at 0.25 μm. Fig. 2, showing a small part of a section (0.25-μm thick) of mouse spleen, illustrates the quality of preservation and retention of integrity of sections after the PEG is removed. Sections of well-fixed tissues remain intact whether they are taken from a relatively compact cell-rich tissue as spleen or liver, or from more loosely organized tissues such as testis or mammary tumor. These sections, supported completely on Formvar-carbon films, are stable under low- and high-voltage beams, provided the sections are firmly attached to the film. Loosely attached sections frequently show instability and shrinkage distortion.

The absence of equivalent detail and contrast in epoxy-embedded specimens is demonstrated in Fig. 3. This sample of mouse spleen (0.25-μm thick) was fixed in the same way (including osmium) as the sample in Fig. 2, and viewed (as for

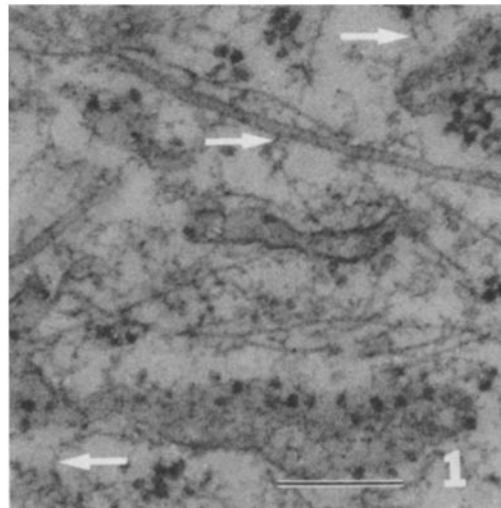


FIGURE 1 Image of a thin section of an epoxy-embedded human fibroblast (WI-38). What may be equivalent to the microtrabeculae of whole-cell preparations is seen in this section as feathery strands (arrows) associated with the surfaces of the ER, polysomes, microtubules, and microfilaments. The epoxy embedment scatters electrons sufficiently, giving the fuzzy appearance to the lattice trabeculae, as well as the granular character to the background. This section was stained with 4% aqueous uranyl acetate for 20 min and with lead citrate for 3 min. Bar, 0.2 μm. × 80,000.

Fig. 2) without additional staining. The sections (i.e., Figs. 2 and 3) were examined under identical conditions. That is, the same objective-aperture size ($30\ \mu\text{m}$), beam intensity, and photographic emulsion were used to record each image. Similarly, the same grade of photographic paper was used to print each micrograph. The only difference between these specimens is that the section in Fig. 3 has epoxy resin present, and the

one in Fig. 2 is devoid of any embedding matrix.

We have examined a variety of cells and tissues, each exhibiting typical organelles in their normal, *in situ*, three-dimensional positions. Examined at higher magnifications, and in stereo, sections devoid of embedding matrices yield images of the cytoplasm of cells similar in design to that seen in whole, unembedded critical-point dried cells (Fig. 4; for comparison,

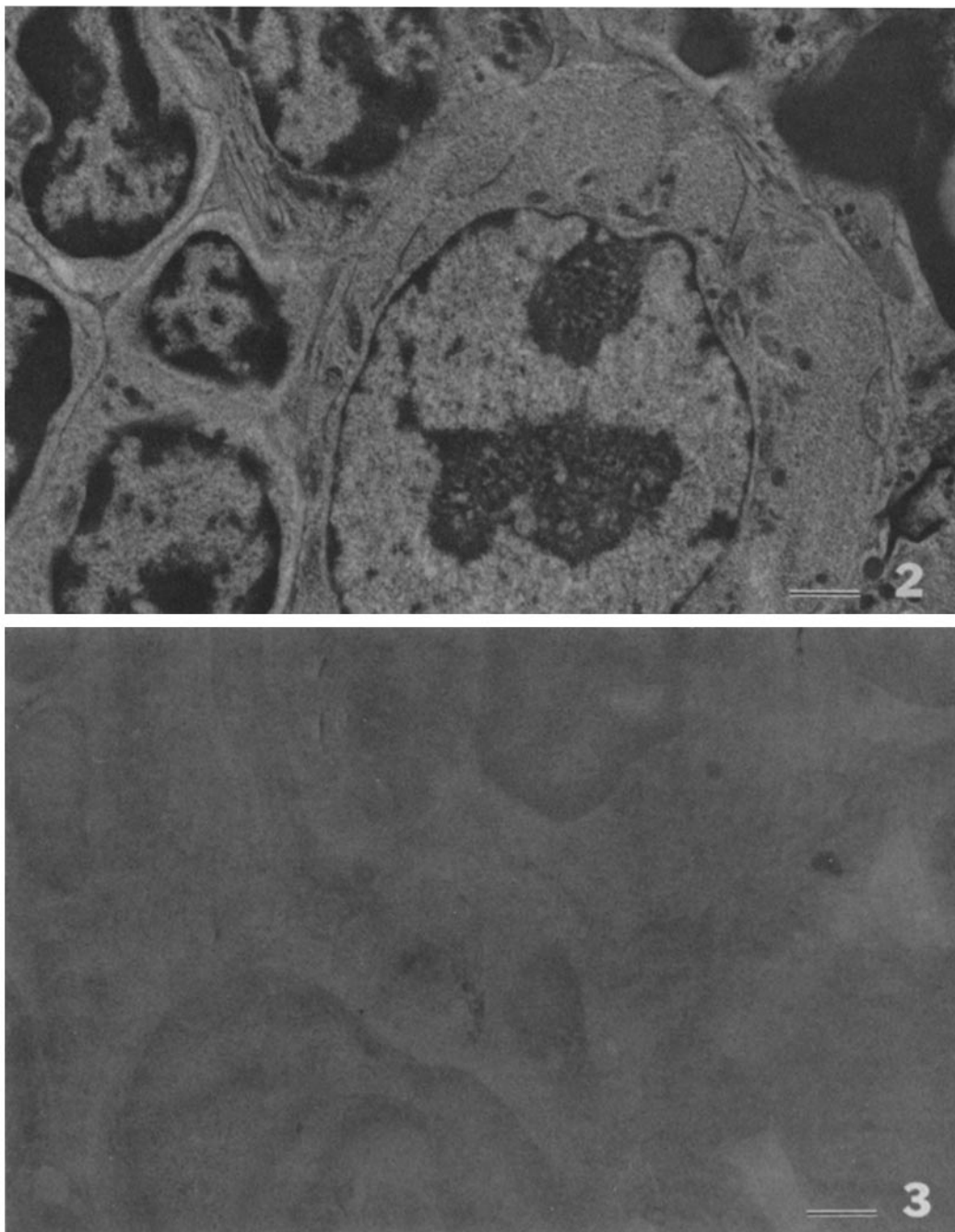


FIGURE 2 A $0.25\text{-}\mu\text{m}$ -thick section of PEG-embedded mouse spleen. The image resembles a well-stained conventional (epoxy-embedded) specimen. However, no stain (except osmium postfixation) was used. The absence of the embedding resin provides, as it does with whole-cell preparations, increased contrast in thick specimens examined with the high-voltage electron microscope. Bar, $1\ \mu\text{m}$. $\times 10,000$.

FIGURE 3 A $0.25\text{-}\mu\text{m}$ -thick section of epoxy-embedded mouse spleen. The presence of an electron-scattering matrix (e.g., Epon) obscures most of the detail and contrast of the specimen even at low magnifications. Indeed, it is most difficult to see structures in the section at high accelerating voltages; at higher magnifications (above $\times 20,000$) it is virtually impossible to discern any detail of cell ultrastructure. As with the material in Fig. 2, no additional staining was used except for the osmium postfixation. Bar, $1\ \mu\text{m}$. $\times 10,000$.

see references 7 and 11–13). Fig. 4 shows a portion of the nucleus and cytoplasm of a human small lymphocyte, as well as a portion of an erythrocyte. Here, the absence of any embedding matrix permits the detection of the fixed elements of the cytoplasm in their entirety. Included in this section is a portion of the nucleus of the lymphocyte. In most respects the nucleoplasm in these preparations resembles the fibrillar structure evident in conventional images derived from thin sections of epoxy-embedded cells. The heterochromatin is too dense for effective observation in 0.25- μm -thick sections, although the euchromatin areas are filled with a loosely organized lattice. It is also important to notice the homogeneous nature of the erythrocyte. Although the erythrocyte and lymphocyte were fixed and processed under identical conditions, the erythrocyte shows little evidence of a similar lattice structure. The apparent strands between the cells are the remnants of the agar investment.

Fig. 5 shows, at low magnification, a section of a telophase cell of mouse mammary tumor devoid of any embedding matrix. The daughter nuclei have reformed and interpolar microtubules are visible. We have examined a number of stages of mitosis and, at higher magnification, spindle microtubules consistently have elements of the lattice contiguous with their surfaces. Fig. 6 shows a glancing section through a portion of a mitotic spindle. Broad trabeculae (6–10 nm) are seen impinging on the microtubules. Lattice elements appear to surround the spindle fibers as well as to form cross-bridges between microtubules. Also of interest is the apparent alignment of

lattice trabeculae normal to the microtubules. The significance of this remains uncertain and further observations are warranted. Nonetheless, the absence of embedding resin permits a view of the structures surrounding the spindle tubules that is not attainable in epoxy-embedded cells.

Other characteristic microtubule arrays are preserved by these techniques (e.g., the manchette microtubules and axonemes of developing spermatids). Fig. 7 shows a 0.2- μm -thick section of a portion of the manchette, as observed and photographed with the 100-kV electron microscope. The lumina of those microtubules parallel to the electron beam are imaged clearly, as are the strands of the lattice. Indeed, the microtubules are cross-bridged by the lattice trabeculae. Cross-bridges have been visualized in thin sections of epoxy-embedded testis (10), but in the section devoid of resin the bridging appears far more distinct.

Cross-bridges between microtubules in spermatid tails are preserved by these techniques. Fig. 8 shows the radial spokes and interdoublet links clearly at various levels along the length of the axonemes. The tubule lumina here appear occluded, although most often microtubule lumina are open as in Fig. 7.

DISCUSSION

It has been recognized for a number of years that plastic embedding resins scatter electrons sufficiently to obscure any object embedded in them that has the same, or closely similar, scattering properties as the resin. To increase the contrast between structures with similar scattering properties, objective

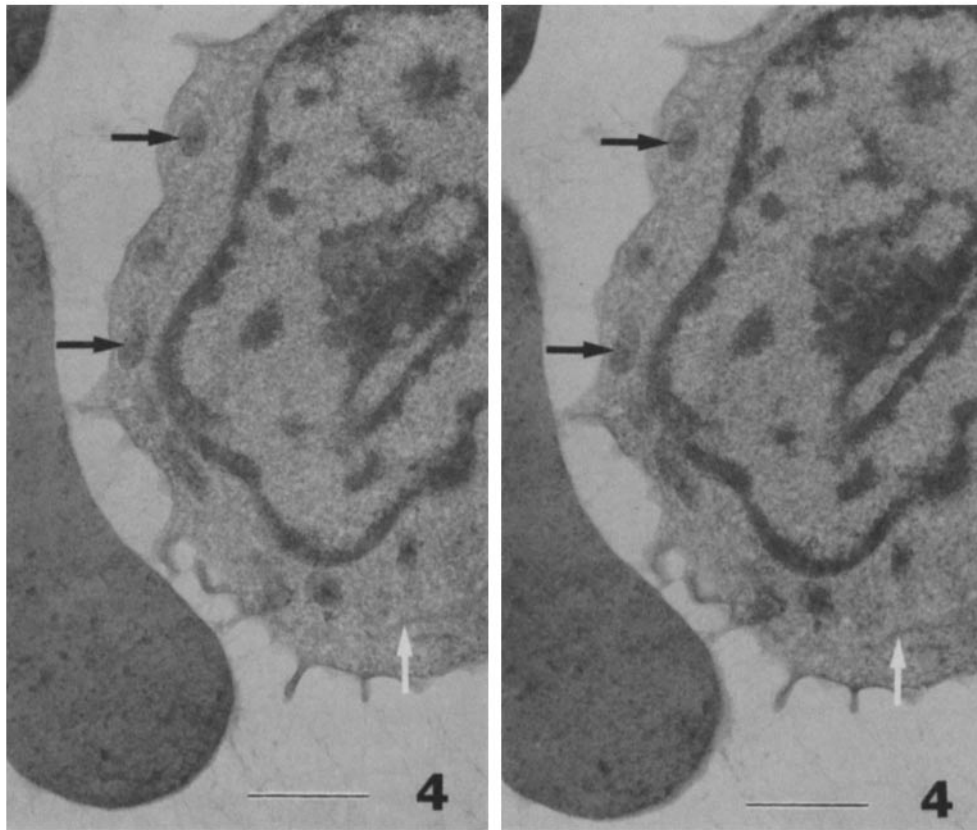


FIGURE 4 Stereo image of a lymphocyte and erythrocyte from circulating human blood. In this image of a 0.25- μm -thick section devoid of embedding matrix, the euchromatin and heterochromatin of the nucleus, small mitochondria (black arrows) and a profile of the ER (white arrow) are identified easily. With the aid of a small binocular stereoscope, the three-dimensional lattice filling the cytoplasm can be seen even at this intermediate magnification. In contrast, the erythrocyte shows little evidence of a similar lattice. Prepared according to procedure 1; matrix removed in aqueous PEG. Examined at 1,000 kV. 20° total tilt. Bar, 1 μm . $\times 16,000$.

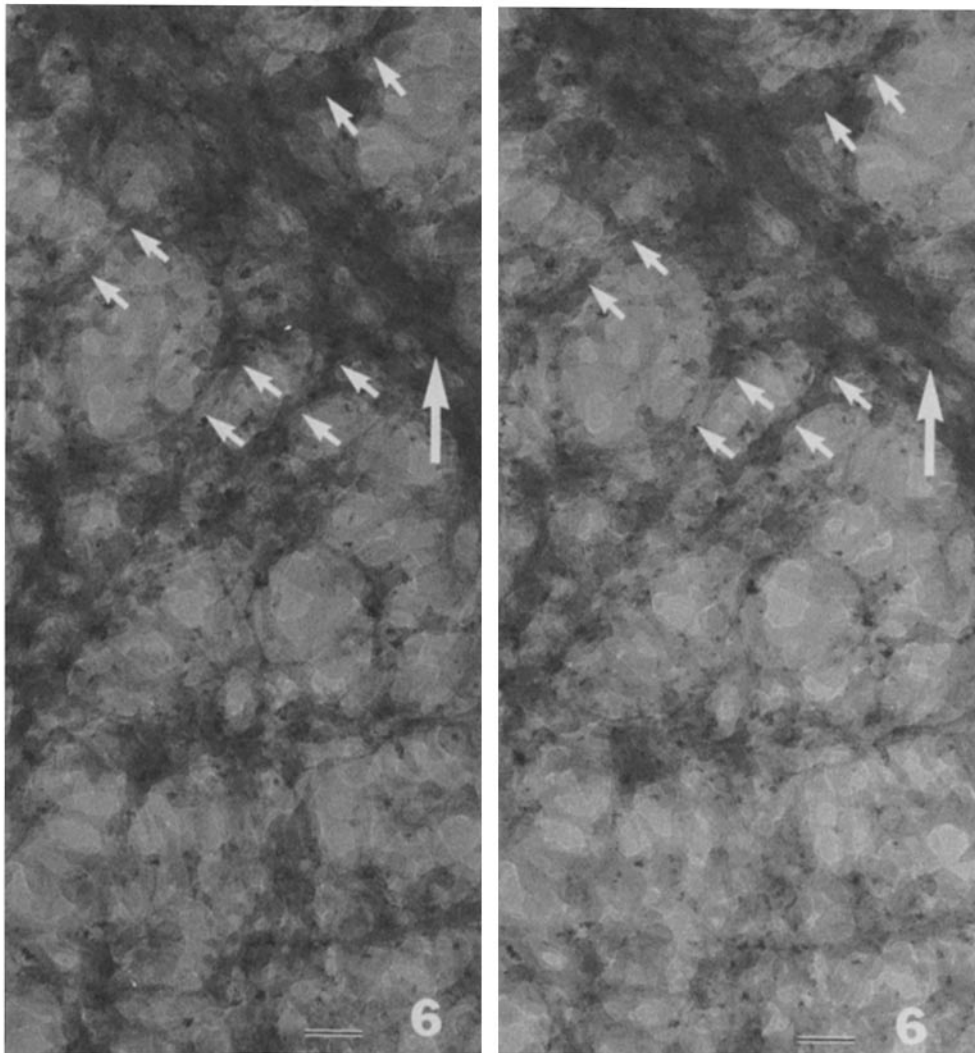
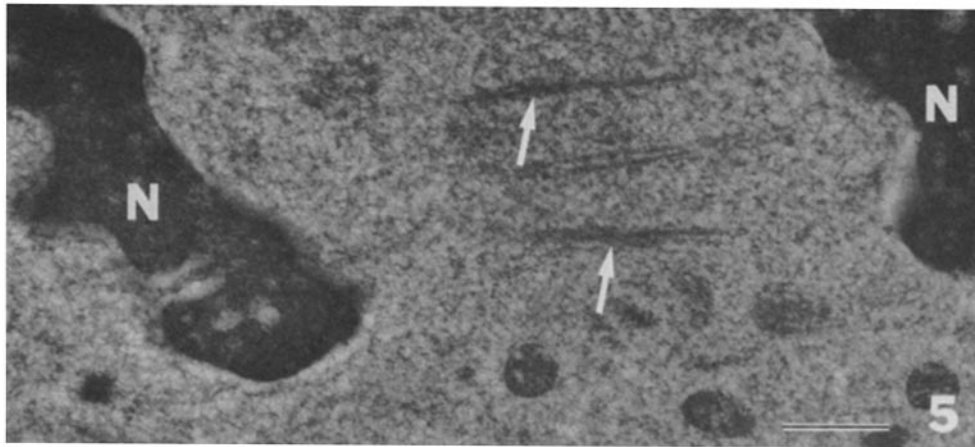


FIGURE 5 Image of a telophase cell from a mouse mammary tumor. This low-magnification image of a 0.25- μm -thick section shows clearly the re-formed daughter nuclei (N), a few mitochondria, the interpolar fibers (arrows), and the dense cytoplasm. This and other stages of mitosis are identified readily in this tumor. Prepared according to procedure 2; matrix removed in alcoholic PEG. Examined at 1,000 kV. Bar, 1 μm . $\times 14,000$.

FIGURE 6 Stereo image of a portion of the mitotic apparatus from a mouse mammary tumor cell. In this grazing section (0.25 μm) through the mitotic apparatus, the overlapping microtubules (large arrow) appear to be enveloped in the structures of the lattice. The lattice trabeculae form a continuum in this mitotic cell very similar to that seen in whole cultured cells. However, in this image there is evidence of alignment of the trabeculae. The small arrows point to the array of trabeculae that appear normal to the longitudinal axis of the spindle fibers. The small, dense flakes of material within the trabeculae are most likely precipitates of osmium. Even extensive washing failed to remove the densities. Prepared according to procedure 2; matrix removed in alcoholic PEG. 8° total tilt. Examined at 1,000 kV. Bar, 50 nm. $\times 150,000$.

apertures of suitably small size were introduced and are now standard features of all electron microscopes. For the same reason, heavy atom staining of specimens has been used routinely since their introduction (8, 9). If the electron-scattering resins are absent, specimen contrast is increased greatly even at high (1,000 kV) accelerating voltages. The numerous published images of critical-point-dried cultured cells examined by transmission electron microscopy unequivocally substantiate this statement (3, 7, and 11–13). The removal of epoxy embedding resins from sections is difficult. Methacrylates can be removed easily by inert solvents, but this results in considerable damage

to cell ultrastructure. PEG, however, appears to be acceptable as an extractable embedding medium, as it can be removed readily in a variety of common solvents without alteration of structure.

The use of 6,000 mol wt PEG was determined empirically. Lower molecular weight PEG formulations (e.g., mol wt 1,500) are too soft for good sectioning although they melt at lower temperatures. Higher molecular weight PEG compounds (e.g., mol wt 20,000) were not tried to any great extent, because they must be melted at higher temperatures, are considerably more viscous, and would require longer infiltration times. However,

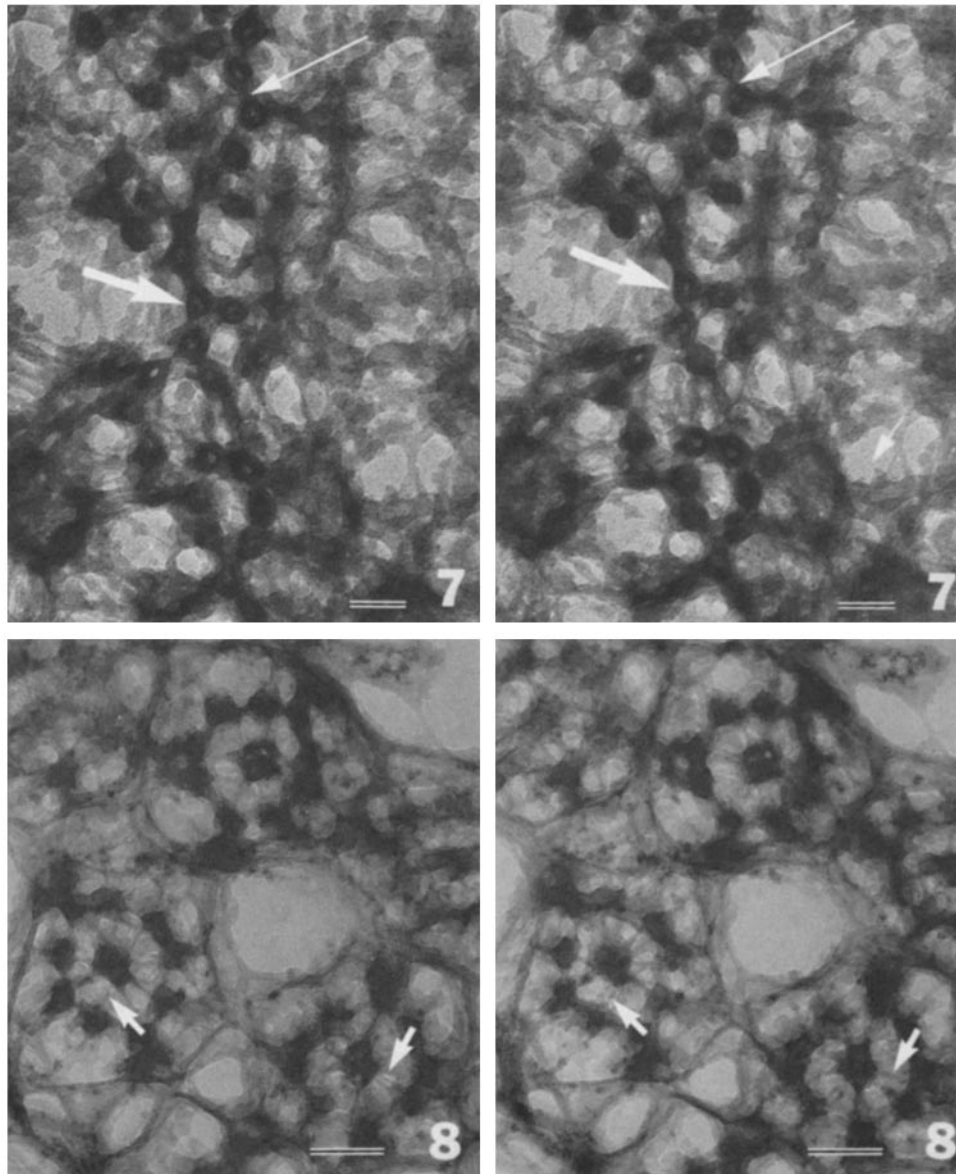


FIGURE 7 Stereo image of a portion of the manchette from a developing step-12 mouse spermatid. This 0.2- μ m-thick section (examined at 100 kV) shows transverse sections of the manchette microtubules (the large arrow points to one microtubule) and the lattice-trabeculae. The contrast is increased greatly at lower voltages; thus, the microtubule lumina as well as the trabeculae linking the microtubules (small arrow) are very distinct. Prepared according to procedure 1; matrix removed in ethyl alcohol. 7° total tilt. Bar, 50 μ m. \times 150,000.

FIGURE 8 Stereo image of flagellar axonemes from the mouse testis. It is remarkable that the 0.25- μ m segments of these axonemes remain intact and almost parallel to the electron beam. Although the lumina of the tubules in this section appear occluded, the regularity of the radial spokes (arrows) and interdoublet links is apparent. Those segments of the plasma membrane parallel to the beam are also imaged distinctly. Prepared according to procedure 1; matrix removed in ethyl alcohol. 9° total tilt. Examined at 1,000 kV. Bar, 50 nm. \times 100,000.

these compounds may be suitable for some samples and may allow thinner sectioning.

Two dehydration schemes are presented in this report. Procedure 1 used PEG as the dehydration fluid. This appeared to be adequate for most tissues, although in some cells (e.g., Sertoli's cells) large lipid droplets did not remain intact. This is explicable because PEG compounds, although soluble in water, alcohols, and glycols, are insoluble in fats, oils, and paraffins. This was noted also by Blank (2) while using PEG as an embedment for light microscopy. To impregnate samples with large amounts of lipids, Blank first placed samples in a fat solvent (acetone)-PEG solution. We felt a more logical approach would be to dehydrate samples in ethyl alcohol (procedure 2). Because of the thinness of the PEG sections, it was felt that procedure 2 would better keep in place those structures containing lipids. Indeed, this appears to be true for the samples sectioned thus far. In either procedure the samples are embedded in pure PEG.

PEG blocks, especially those rapidly cooled, are brittle. Therefore, it was advantageous to remount the samples for trimming. Many sectioning procedures were tried. At first, it seemed reasonable to section onto distilled water, and this produced sections of uniform thinness as judged by interference colors. Because the PEG is dissolved quickly, it was thought that the capillary action of the water through the section would protect the exposed surface of the section from air-drying. This was not the case, for, when examined, such sections showed air-drying damage. We have also experimented with sectioning into various solutions of lower surface tension to minimize the chance of air-drying. Dilute detergent solutions (e.g., Triton X-100), alcohols, alcohols + PEG, mineral oil, petroleum ether, dimethyl sulfoxide, all seem to allow good sectioning. However, considerable difficulty was encountered in recovering the sections from the fluid.

The easiest and most reliable method for producing and recovering a large number of sections was the dry-sectioning method. Both glass and diamond knives produced good sections. Best results were obtained with diamond knives upon blocks rapidly cooled at the embedding stage. Glass knives were discarded, as with epoxy sectioning, whereas diamond knives were cleaned with orange wood sticks, using distilled water or alcoholic solutions. Some compression of the sections occurred during sectioning (as with epoxy sections) but the sections decompressed to their initial dimensions when the matrix was dissolved. The sections were handled easily with fine camel hairs although caution was needed to prevent tearing of individual sections.

A number of techniques have been tested, including rehydration with low concentrations of PEG, with dilute detergent solutions, and with alcoholic PEG. With the latter method, the removal was rapid, and there appeared to be far less distortion of the sample.

The PEG technique, although not new to light microscopy (2), now offers to electron microscopy of solid tissues the same enhancement in contrast and resolution that the whole, unembedded cultured cell approach provides (11-13). Beyond the morphological information the technique will provide, the procedure lends itself to additional applications. For example, it is not unreasonable to expect that aldehyde-fixed tissues will be processed for immunological or histochemical localizations. Indeed, some investigators have used PEG embedding for

immunochemical studies, although critical-point drying was not employed after removal of the PEG (6). Such sections also can be readily investigated by scanning electron microscope methods (Becker et al., manuscript in preparation).

Important advantages of the technique are that it is rapid, relatively simple, and does not require special equipment. Other methods of preparing critical-point-dried sections from frozen samples have met with success (14), but the necessary equipment is costly and not generally available to most laboratories. Finally, critical-point sections can be viewed in conventional, 100-kV electron microscopes, although there will be an obvious loss of resolution as compared with view in the high-voltage electron microscope. However, slightly thinner sections can be cut (e.g., 0.1-0.07 μm) and recovered, thus permitting better resolution in lower voltage instruments.

The image of the cytoplasm that this technique provides strikingly resembles the image of the cytoplasm of whole, unembedded critical-point-dried cells observed by transmission electron microscopy. We have previously discussed the artifact question as it relates to whole, critical-point-dried cells (13). In this respect it is important to note the presence of the lattice structure in cells *in situ*, and the similarities and dissimilarities of the lattice structure between cultured cells and cells *in situ*. The lattice appears to be structured similarly in all cells examined thus far; that is, trabeculae are linked to other trabeculae, to polysomes, and to membrane- and nonmembrane-bounded organelles. The membranous components appear somewhat indistinct, as in whole-cell preparations. This may be a consequence of the electron-scattering properties of the material.

This study was supported in part by grants from the American Cancer Society Illinois Division, Inc. (no. 80-4) to J. J. W. and the National Institutes of Health (6M 28397) to J. J. W. and (RR-00592) to K. R. Porter.

Received for publication 21 February 1980, and in revised form 12 May 1980.

REFERENCES

1. Anderson, T. F. 1951. Techniques for the preservation of three-dimensional structure in preparing specimens for the electron microscope. *Trans. N. Y. Acad. Sci.* (Ser. II). 13:130-134.
2. Blank, H. 1949. A rapid embedding technic for histologic sections employing a water soluble wax. *J. Invest. Dermatol.* 12:95-99.
3. Buckley, I. K. 1975. Three-dimensional fine structure of cultured cells: Possible implications for subcellular motility. *Tissue & Cell.* 7:51-72.
4. English, D., and B. R. Anderson. 1974. Single step separation of red blood cells, granulocytes, and mononuclear leukocytes on discontinuous density gradients of Ficoll-Hypaque. *J. Immunol. Methods.* 5:249-252.
5. Mazia, D., G. Schatten, and W. S. Sale. 1975. Adhesion of cells to surfaces coated with polylysine. Application to electron microscopy. *J. Cell Biol.* 66:198-200.
6. Mazurkiewicz, J. E., and P. K. Nakane. 1972. Light and electron microscopic localization of antigens in tissues embedded in polyethylene glycol with a peroxidase labeled antibody method. *J. Histochem. Cytochem.* 20:969-974.
7. Porter, K. R. 1976. Introduction. Motility in Cells. *Cold Spring Harbor Conf. Cell Proliferation.* 3(Book A):1-28.
8. Reynolds, E. S. 1963. The use of lead citrate at high pH as an electron opaque stain in electron microscopy. *J. Cell Biol.* 17:208-212.
9. Watson, M. L. 1958. Staining of tissue sections for electron microscopy with heavy metals. *J. Biophys. Biochem. Cytol.* 4:475-479.
10. Wolosewick, J. J., and J. H. D. Bryan. 1977. Ultrastructural characterization of the manchette microtubules in the seminiferous epithelium of the mouse. *Am. J. Anat.* 150:301-332.
11. Wolosewick, J. J., and K. R. Porter. 1976. Stereo-high voltage electron microscopy of whole cells of the human diploid line, WI-38. *Am. J. Anat.* 147:303-324.
12. Wolosewick, J. J., and K. R. Porter. 1977. Observations on the morphological heterogeneity of WI-38 cells. *Am. J. Anat.* 149:197-226.
13. Wolosewick, J. J., and K. R. Porter. 1979. The microtrabecular lattice of the cytoplasmic ground substance: Artifact or reality. *J. Cell Biol.* 82:114-139.
14. Yamada, E., and H. Watanabe. 1977. High voltage electron microscopy of critical point dried cryosection. Proc. Fifth International Conference on High Voltage Electron Microscopy, Kyoto. 339-342.