

## FRACTOGRAPHIC STUDIES OF PLASTIC-EMBEDDED CELLS BY SCANNING ELECTRON MICROSCOPY

WALTER J. HUMPHREYS, TOMASZ J. WODZICKI, and JEROME J. PAULIN. From the Department of Zoology, and the Tree Physiology Laboratory, School of Forest Resources, University of Georgia, Athens, Georgia 30601. Dr. Wodzicki's present address is the Faculty of Forestry, Central University of Agriculture, Warsaw, Poland.

Three-dimensional views of cell structures at high magnifications made possible by the scanning electron microscope (SEM) are becoming increasingly useful to biologists. Methods that have proved useful for stabilizing biological soft tissue for observation in the SEM include the critical point method (1, 2), freeze drying, freeze fracturing followed by freeze drying (4, 9, 10), observation of specimens frozen and fractured in the SEM specimen stage (7), and applied glycerol substitutions (8). Viewing animal cells embedded and fractured in plastic is a convenient alternative for studying both extracellular and intracellular structures. The method permits a direct comparison of structures seen by the SEM with structures viewed by transmission electron microscopy in ultrathin sections cut from the same embedment in the same block.

### MATERIALS AND METHODS

Unfertilized eggs of the polychete worm, *Mercierella enigmatica*, were spontaneously shed when the animals

were pulled from their tubes and placed in individual containers of seawater. These eggs and cells of *Tetrahymena pyriformis* were double fixed in glutaraldehyde and osmium tetroxide, concentrated into pellets by mild centrifugation, embedded in Epon (5) and Maraglas (3), respectively, and sectioned for transmission electron microscopy. The same blocks from which the thin sections were cut were then fractured in planes passing through the pellets of embedded cells. With the embedded material facing upwards, fractures were made under a dissecting microscope just below the trimmed and sectioned faces of the blocks. In the region of the embedded pellet of cells, a sharp razor blade was used to cut horizontally into the side of the block for a depth of 1 mm or less. The razor blade edge was then flicked upward against the plastic at a right angle to the plane of the cut so that ahead of the razor blade edge the plastic was caused to fracture through the mass of embedded cells. If the fracturing is done inside a transparent plastic bag, the chip of plastic fractured from the block can be easily recovered. The surfaces exposed by the fracture, either on the piece of plastic that chipped away, or on the

---

FIGURE 1 Transmission electron micrograph of a thin section cut from a pellet of unfertilized eggs of *Mercierella enigmatica* embedded in Epon. Tips of microvilli (*MV*) on the egg surface project beyond the outer surface of the vitelline coat (*V*) that envelops the egg. *GV*, germinal vesicle; *Y*, yolk granule; *CG*, cortical granules.  $\times 3,200$ .

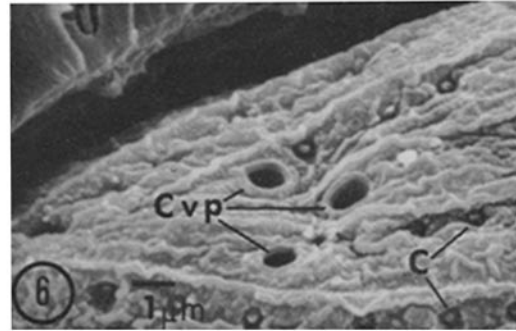
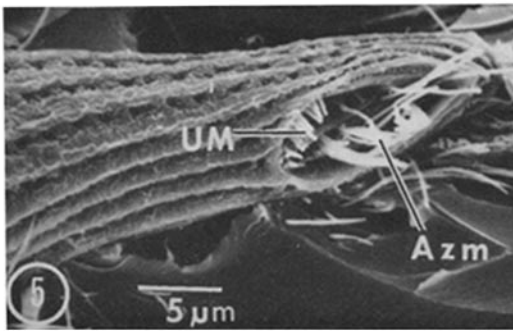
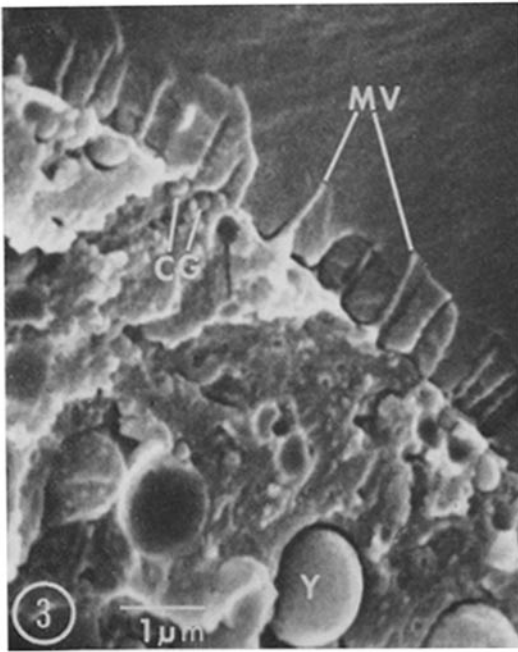
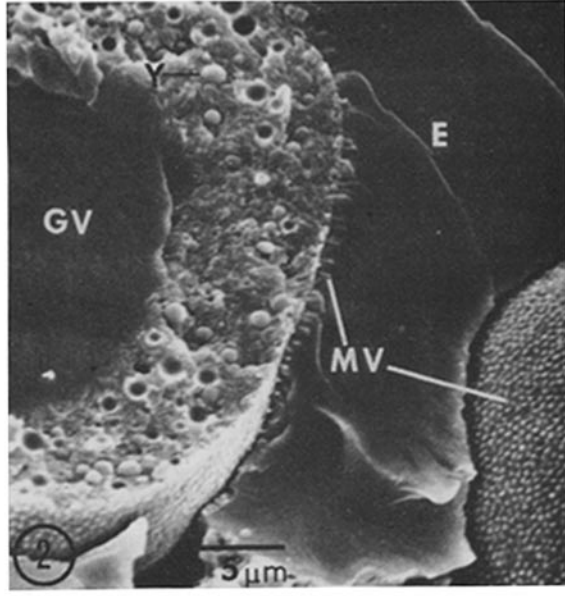
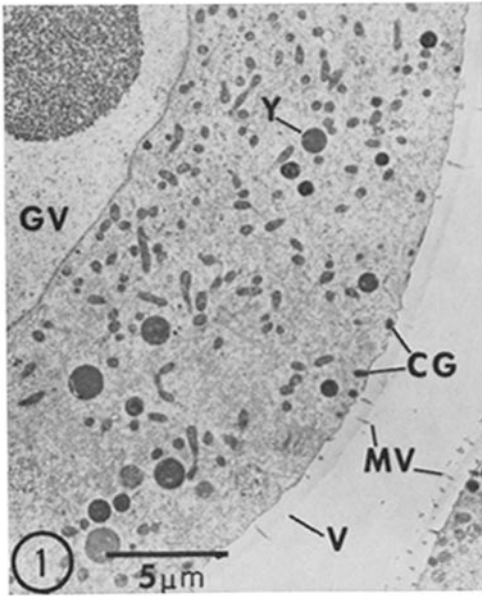
FIGURE 2 SEM of a fracture plane through an egg embedded in the same block from which the section shown in Fig. 1 was cut. Structures such as the germinal vesicle (*GV*), yolk (*Y*), and microvilli (*MV*) may be compared in both preparations. *E*, cured Epon in which the eggs were embedded.  $\times 2,200$ .

FIGURE 3 SEM of a fracture similar to that shown in Fig. 2, at a higher magnification. Cortical granules (*CG*), yolk (*Y*), and microvilli (*MV*) are easily identifiable.  $\times 11,000$ .

FIGURE 4 SEM of a fracture plane through a pellet of *Tetrahymena pyriformis* embedded in Maraglas (*M*). Large surfaces of the protozoan pellicles are exposed. Ciliary meridians ( *Cm*) are clearly visible.  $\times 1,000$ .

FIGURE 5 SEM of a different region of the same preparation shown in Fig. 4. The buccal cavity of *Tetrahymena pyriformis* with cilia of the undulating membrane (*UM*) and of the adoral zone (*Azm*) of membranelles is shown.  $\times 2,200$ .

FIGURE 6 SEM of a different region of the same preparation shown in Fig. 4. Surface features of the pellicle of *Tetrahymena pyriformis* that are shown include contractile vacuole pores (*Cvp*) and the bases of cilia (*C*) broken away when the embedment was fractured.  $\times 5,600$ .



piece of plastic from which it was chipped, were oriented for examination by attaching the trimmed pieces of fractured plastic to specimen stubs with a droplet of silver-conducting paint. After the paint was dry the specimens were coated by vaporizing a layer of gold 200–300 Å thick onto them in a vacuum evaporator. These fractured and coated surfaces were then viewed and photographed with a Kent Cambridge Mark II A SEM.

## RESULTS AND DISCUSSION

Fig. 2 is a fractograph through a pellet of Epon-embedded eggs of *Mercierella*. The Epon fractured in a plane that exposed both a cross-sectional view and a surface view of the same egg. Structures inside the egg can be identified and compared with similar structures in the transmission electron micrograph in Fig. 1, which is part of a thin section of another egg cut from the same block before it was fractured. In Fig. 2 the germinal vesicle (GV) is distinctive in appearance because of its uniform texture. Some yolk particles (Y) protrude from the fractured surface. Other yolk particles were scooped free of the surface, leaving pits. Microvilli (MV) are clearly visible in both preparations. In Fig. 1 a vitelline coat (V) is clearly visible as a distinct envelope surrounding the egg. Microvilli (MV) extend through the coat, and their tips extend somewhat beyond its outer surface. A surface view of another egg in the lower right-hand corner of Fig. 2 shows only the tips of the microvilli (MV) extending outward through the vitelline coat. The same is seen in the surface view at the lower part of the fractured egg in the same figure. Fig. 3 is a higher magnification of the same type of fracture shown in Fig. 2. Microvilli (MV), or replicas of microvilli fractured away from the plastic, are visible in greater detail, and cortical granules (CG) can be identified. Figs. 4–6 are scanning electron micrographs of surfaces exposed by fracturing through a pellet of *Tetrahymena pyriformis* cells embedded in Maraglas. A glance at Fig. 4 indicates that the preferential plane of fracture is along the interface of the cell's pellicle and the Maraglas matrix (M). Thus large surfaces of the pellicle are exposed. In some cases entire cells are pulled free of the plastic. Most of the cilia are sheared off, but otherwise, surface detail on the pellicle is quite good. Ciliary meridians (Cm) are prominent. In Fig. 5 the Maraglas apparently was pulled out of the buccal cavity, leaving substantial lengths of ciliary axonemes extending from the undulating membrane (Um) and the adoral zone

(Azm) of the membranelles. In Fig. 6, contractile vacuole pores (Cvp) are shown in considerable detail. Plastic appears to have pulled out of the pores during fracturing, permitting the SEM to image even the sides of the walls of the pores.

While the technique works well with embedded pellets of individual animal cells such as egg cells or protozoan cells, application of the technique for the study of soft animal tissue such as kidney has not as yet been very successful. On the other hand, the method has been very useful for the study of plastic-embedded plant tissue. This is due to the fact that fracturing occurs predominantly at the interfaces of cytoplasm and cell walls. Fig. 7, for example, is a fractograph of pine stem tissue embedded in Maraglas. Much cell wall material was fractured away, exposing large areas of plasmalemma surfaces on which clusters of plasmodesmata (P) fractured in cross section appear as small pits. In other areas where protoplasts were torn away, exposing the inner surfaces of the cell walls, pit fields with remnants of plasmodesmata fractured in cross section may be seen. Such preparations promise to be useful in studying plasmalemma surfaces and cytoplasmic continuity between plant cells.

The fracturing away of plastic from surfaces of embedded structures occurs on a fine scale in routine ultramicrotomy. Maser et al. (6) have demonstrated with transmission electron microscopy and platinum shadowing that even very thin epoxy sections that show silver interference colors may have a considerable amount of three-dimensional relief that results from fracturing, rather than smooth cutting, of embedded specimens. Collagen fibrils, for example, observed projecting upwards from the surface of the sections were not smoothly cut, but they were apparently pulled and fractured away from the adjacent plastic. Such preparations are valuable for the study of much finer three-dimensional detail than is possible with the limited resolution obtained by scanning electron microscopy of the more gross fractures used in our study.

Fracturing of plastic-embedded specimens permits an easy way to obtain three-dimensional views of large areas of specimens which can be compared with results obtained from thin sections cut from the same embedment. Specimens embedded and used even years ago for transmission electron microscopy can now be fractured and studied with this method and the results correlated with earlier findings. It is also quite possible to cut thin sections

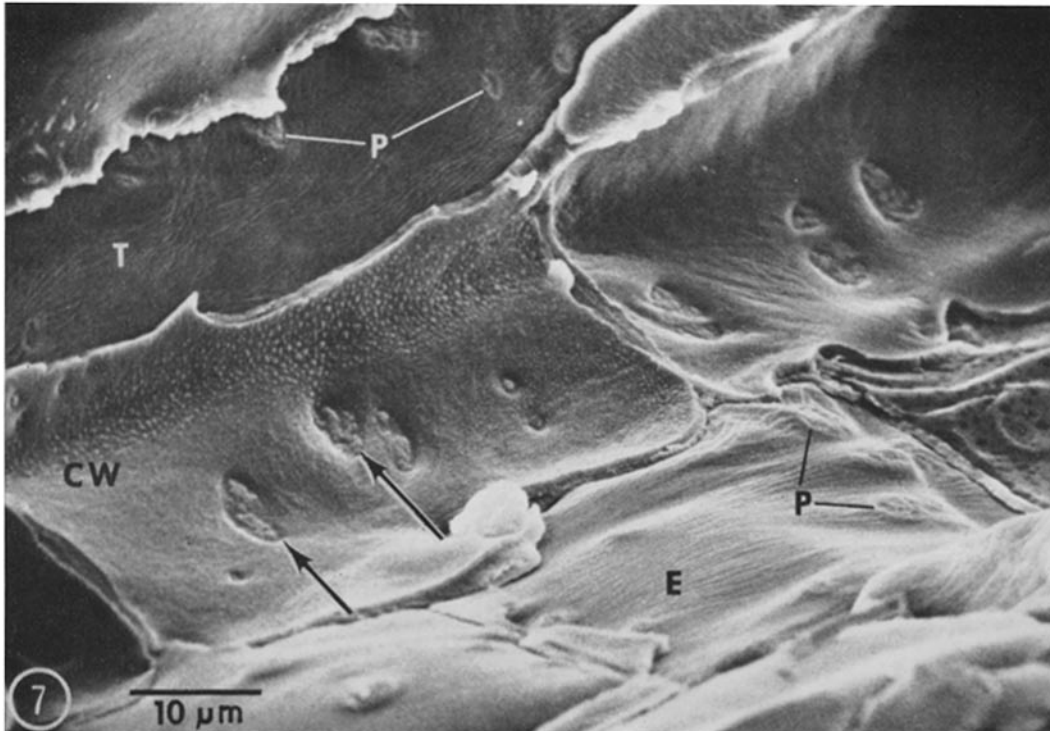


FIGURE 7. Axial resin canal in the differentiating xylem of pine stem (*Pinus echinatus* Mill.) embedded and fractured in Araldite. The exposure is radial. *T*, outer plasmalemma surface of a differentiating tracheid; *E*, outer plasmalemma surface of an epithelial cell; *P*, clusters of plasmodesmata fractured in cross section; *CW*, inner surface of the cell wall of an epithelial cell or the parenchyma cell accompanying the resin canal. Pit fields with remnants of plasmodesmata are seen at arrows.  $\times 1,700$ .

at right angles to the fracture plane studied, either before or after viewing the specimen in the SEM, if positive identification of a particular portion of the fractograph warrants thin sectioning of the specimen.

Within the limits of the resolution of the SEM, the results are similar to those obtained by freeze-fracture replication. No systematic studies of the quality of the fractures relating to factors such as the consistency of the embedment, the temperature at which the embedment is fractured, etc., have yet been made. Using fractured specimens such as shown in Figs. 2 and 3, electron microprobe analysis could be profitably employed to determine the elemental content of certain exposed cellular constituents.

The authors thank Mrs. Janet Johnson for her superb technical assistance and Ms. Beverly Higdon for photographic assistance.

Received for publication 5 September 1972, and in revised form 6 October 1972.

#### 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.* 13:130.
2. COHEN, A. L., D. P. MARLOW, and G. E. GARNER. 1968. A rapid critical point method using fluorocarbons ("Freons") as intermediate and transitional fluids. *J. Microsc.* 7:331.
3. FREEMAN, J. A., and B. O. SPURLOCK. 1962. A new epoxy embedment for electron microscopy. *J. Cell. Biol.* 13:437.
4. HUMPHREYS, W. J., and T. J. WODZICKI. 1972. Methods for viewing by scanning electron microscopy the interior organization of protoplasts of plant cells. Proceedings 30th Annual

- Meeting of the Electron Microscope Society of America, Los Angeles. 238.
5. LUFT, J. H. 1961. Improvements in epoxy resin embedding methods. *J. Biophys. Biochem. Cytol.* **9**:409.
  6. MASER, M. D., T. P. OBRIEN, and M. E. MCCULLY. 1967. Shadowing of ultrathin sections of epoxy embedded tissues as an aid in three-dimensional interpretation. *J. Microsc.* **6**:305.
  7. MCALEAR, J. H., L. GERMINARIO, and R. FUCCI. 1971. Retinal elements revealed by SEM cryomicrodissection. Proceedings 29th Annual Meeting of the Electron Microscope Society of America, Boston. 446.
  8. PANESSA, B. J., and J. F. GENARRO, JR. 1972. Preparation of fragile botanical tissues and examination of intracellular contents by S.E.M. Proceedings 5th Scanning Electron Microscope Symposium, Chicago. 328.
  9. WODZICKI, T. J., and W. J. HUMPHREYS. 1972. Cytodifferentiation of maturing plant tracheids: the final stage. *Tissue and Cell.* **4**:527.
  10. WODZICKI, T., J., and W. J. HUMPHREYS. 1972. Maturing pine tracheids. Organization of intravacuolar cytoplasm. *J. Cell Biol.* **56**:263.