1973

Osmium-Fixed and Epon-Embedded Whole Mounts of Delicate Specimens

Cavey, Michael J.; Cloney, Richard A.

Blackwell Publishing

Michael J. Cavey, Richard A. Cloney "Osmium-Fixed and Epon-Embedded Whole Mounts of Delicate Specimens" Trans. Amer. Micros. Soc., 92(1): 148-151 1973
http://hdl.handle.net/1880/43426
journal article

OSMIUM-FIXED AND EPON-EMBEDDED WHOLE MOUNTS OF DELICATE SPECIMENS[1]

MICHAEL J. CAVEY and RICHARD A. CLONEY
Department of Zoology, University of Washington, Seattle, Washington 98195

CAVEY, M. J. & CLONEY, R. A. 1973. Osmium-fixed and Epon-embedded whole mounts of delicate specimens. *Trans. Amer. Micros. Soc.*, 92: 148–151. An osmium fixative and an epoxy mountant were used to prepare delicate organisms and tissues as whole mounts for light microscopy. Fine structural details are well preserved by the technique, and common artifacts of whole mount preparation are largely eliminated. The final specimens are suitable as bright field objects or as phase/quasi-phase objects.

In the preparation of whole mounts of delicate organisms and tissues, conventional methods of fixation, staining, and mounting are sometimes inadequate. Fine details of structure are often lost and extensive artifacts may be introduced. The use of osmium tetroxide as a fixative and an epoxy resin as the mounting medium (see Ores, 1971) can circumvent these problems. The finished whole mounts are suitable for low-power bright field microscopy if specimens are left in the osmium tetroxide until the desired contrast is attained. With minimal fixation times, the specimens are excellent for high-resolution phase, polarization, or Nomarski differential interference microscopy. Techniques will be presented for the preparation of whole mounts of invertebrate embryos and larvae, protozoans, large pieces of excised skin or mesentery, and cells in tissue culture.

MATERIALS AND METHODS

Developing embryos or larvae are pipetted from the culture dishes into centrifuge tubes and allowed to settle. Motile specimens can be gently spun to the bottom of the tube with a hand centrifuge. Excess culture medium is decanted, and the operation is repeated until sufficient specimens are concentrated in a small volume of liquid. Protozoa are handled similarly.

Excised pieces of skin, mesentery, or other membranous tissue can be stretched over and pinned to the cut end of a short length of Tygon tubing before fixation. Cells in tissue culture are processed in situ in plastic culture dishes, or the cells may be grown on coverglasses to simplify handling.

Osmium tetroxide buffered with sodium bicarbonate (Wood & Luft, 1965) has consistently yielded good preparations. A 2.5% sodium bicarbonate solution

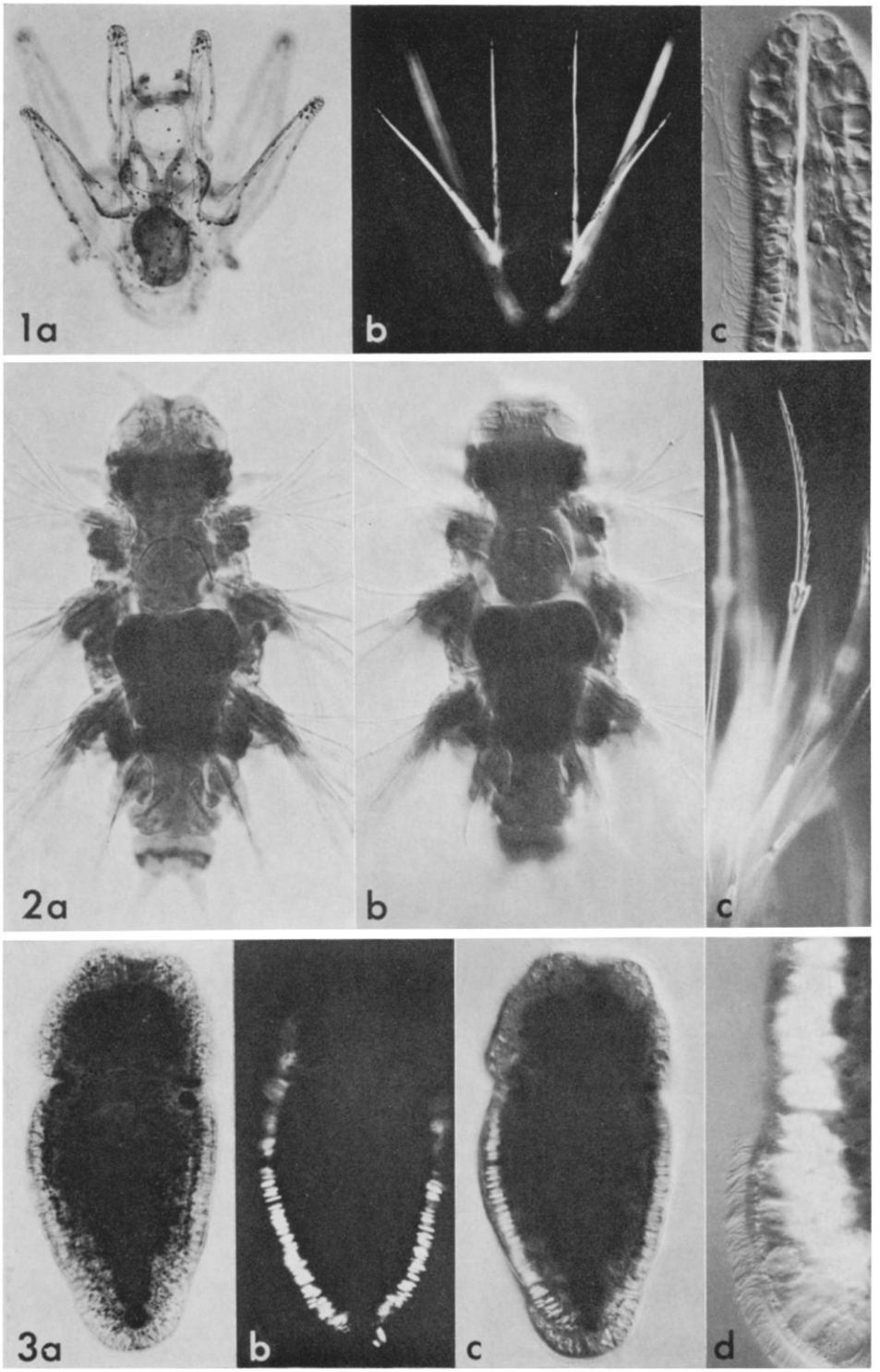
[1] This work was supported in part by NIH Developmental Biology Training Grant No. 5-TO1-HD00266 to the University of Washington. Rita M. Gustus kindly supplied the nectochaetal larvae used in Figure 2, and Bruce J. Crawford offered helpful suggestions for the preparation of cells in tissue culture.

→

FIG. 1. Whole mount of an echinopluteus larva of the sea urchin *Strongylocentrotus franciscanus*. a, bright field microscopy. × 70. b, polarization microscopy. × 70. c, Nomarski differential interference microscopy of an arm. × 445.

FIG. 2. Whole mount of a nectochaetal larva of the annelid *Nereis vexillosa*. a, bright field microscopy. × 165. b, Nomarski differential interference microscopy. × 165. c, Nomarski differential interference microscopy of a seta. × 495.

FIG. 3. Whole mount of a veliger larva of the chiton *Mopalia ciliata*. a, bright field microscopy. × 175. b, polarization microscopy. × 175. c, Nomarski differential interference microscopy. × 175. d, Nomarski differential interference microscopy of the foot. × 545.


1a
b
c
2a
b
c
3a
b
c
d

is prepared immediately before use and adjusted to pH 7.2 with 1N HCL. The buffer solution is diluted with an equal volume of 4% osmium tetroxide and the combination is chilled in an ice bath. The bicarbonate buffer was selected because it is relatively inexpensive and easy to prepare; any conventional buffer used with osmium is acceptable. Filtered sea water is a suitable substitute for the buffer when preparing marine organisms, and chilled culture medium is often acceptable when preparing cells in tissue culture. Primary fixation with glutaraldehyde should be avoided, since the specimens tend to become too dark upon post-fixation with osmium.

The chilled fixative is added to the container with the concentrated specimens, and the container is then sealed and placed in the ice bath. If specimens are primarily intended for bright field microscopy, their contrast is periodically monitored with a dissection microscope until the desired contrast is attained (generally 10–30 min, depending on the organism, the tissue or cell type, the stage of development or differentiation, specimen thickness, and so forth). If specimens are intended for phase, polarization, or differential interference microscopy, fixation time (and hence contrast) is kept to a minimum (ca. 5–10 min). The reaction is stopped by pipetting off the fixative and rinsing the specimens with distilled water for 10 sec. (Pieces of skin or mesentery are detached from the tubing at this point, cut into smaller pieces if necessary, and removed to a glass container for the remaining steps.)

Following the distilled water rinse, specimens are dehydrated in a graded series of ethanol at room temperature (30%, 50%, 70% for 5 min each; 95%, 100% for 10 min each with two changes) and then transferred to propylene oxide for 15 min with three changes. (Plastic culture dishes begin to dissolve when in contact with the propylene oxide, and cultured cells become detached. The cellular colonies are carefully lifted from the bottom of the dish with forceps and spatula and placed in a glass container before continuing.)

The epoxy resin mixture routinely used has been 7 parts of Epon A (Epon 812[2], 62 ml; dodecenyl succinic anhydride[2], 100 ml) and 3 parts of Epon B (Epon 812, 100 ml; nadic methyl anhydride[2], 89 ml) containing 1.5% 2,4,6-tri(dimethylaminomethyl)phenol[3] (Luft, 1961). The complete resin mixture can be kept frozen in sealed ampules for several months without serious deterioration. The resin is allowed to come to room temperature before opening the ampule. After the final change of propylene oxide, a 1:1 mixture of resin and propylene oxide is added to the container with the specimens; the container is gently rotated to effect complete mixing and to prevent unnecessary specimen damage. After 1 hr of infiltration, the proportion of resin to propylene oxide is increased to 3:1 and the specimens are infiltrated for an additional 1–2 hr. The 3:1 combination is then removed and replaced with pure resin, in which the specimens are held for 3–6 hr at room temperature. This final step in the infiltration permits the residual propylene oxide in the specimens to evaporate before mounting; if sufficient time is not allotted, the propylene oxide will create bubbles which become trapped under the coverglass as the resin polymerizes.

Microscope slides are cleaned with acid alcohol and thoroughly dried before use. Embryos, larvae, or protozoans are placed on the slide in a drop of resin with a large bore pipette. Pieces of skin, mesentery, or cellular colonies are placed on a slide with forceps and spatula. Additional resin is added as needed, and a coverglass is positioned, being careful not to introduce air bubbles under it. The

---

[2] Obtained from E. V. Roberts & Associates, Inc., Plastics and Chemicals Division, 9601 Jefferson Boulevard, Culver City, California 90231.

[3] Obtained from Polysciences, Inc., Paul Valley Industrial Park, Warrington, Pennsylvania 18976.

microscope slides are laid flat in a 90 C oven for 2 hr or in a 60 C oven for 12 hr to polymerize the epoxy resin.

Examples of invertebrate larvae prepared in the manner described above are shown in Figures 1–3, where the efficacy of the technique is evaluated with different types of light microscopy. The echinopluteus larva of the sea urchin is relatively transparent, the nectochaetal larva of the annelid contains a moderate amount of yolk, and the veliger larva of the chiton is heavily pigmented.

Discussion

The preceding method is best applied to relatively transparent embryos, larvae, and protozoans. Specimens containing large quantities of lipid or yolk are usually less satisfactory because of the great osmiophilia of these materials. Heavy pigmentation also obscures details.

In compiling a permanent record of many stages of embryonic development, the method can be simplified. Aliquots of embryos can be collected at intervals from a culture, fixed, dehydrated, and held in the 70% ethanol. The aliquots are then pooled and further processed together.

Shrinkage of delicate specimens is minimized with the technique. Use of the epoxy mountant often eliminates the need for coverglass supports; damage to the specimens by the coverglass as the resin polymerizes is not a serious problem. The final preparations can be stored indefinitely.

Resolution with these whole mounts is excellent due, in part, to the relatively high refractive index of the epoxy resin mixture. Measurements of the refractive index of our resin mixture show it to be 1.491, which is in close agreement with the value of 1.488 reported by Ores (1971) for a mixture of different proportions.

The Nomarski differential interference microscope is designed to provide excellent contrast and high resolution of phase or quasi-phase objects, such as these whole mounts. The microscope is easily adjusted to enhance the contrast of amplitude or mixed phase-amplitude objects. The depth of field is very small, owing to the large numerical apertures used, and permits "optical sectioning" of whole mounts (Allen et al., 1969).

Literature Cited

Allen, R. D., David, G. B. & Nomarski, G. 1969. The Zeiss-Nomarski differential interference equipment for transmitted-light microscopy. *Z. Wiss. Mikros. Mikros. Tech.*, 69: 193–221.

Luft, J. H. 1961. Improvements in epoxy resin embedding methods. *J. Biophys. Biochem. Cytol.*, 9: 409–414.

Ores, R. O. 1971. Advantages of epoxy resin as a mounting medium for light microscopy. *Stain Tech.*, 46: 315–317.

Wood, R. L. & Luft, J. H. 1965. The influence of buffer systems on fixation with osmium tetroxide. *J. Ultrastr. Res.*, 12: 22–45.