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TECHNICAL NOTE:

SERIAL SECTIONS WITH PLASTIC-EMBEDDED TISSUE[1]

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ABSTRACT

For embedding, sectioning and examination of small samples of tissue such as (but not limited to) the ovaries of insects, plastic embedment offers considerable advantages over the more classical paraffin wax embedment. However, in many cases it is hard to maintain plastic sections in serial order. Here we describe a simple method, which overcomes this problem.
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INTRODUCTION

The purpose of this note is to acquaint tissue and cell biologists who have not had experience in plastic embedding with the advantages in tissue quality, convenience and simplicity of this technique. When tissue samples to be examined are inherently small, plastic embedment offers significant advantages over paraffin embedment. For instance, internal organs of many species of insects may themselves be nearly microscopic in size. The ovaries of a female *Drosophila* are only 600 μm long, 250 μm wide, and the male testes are even smaller. Attempts to embed any such organs or tissue samples of similar size in paraffin can be frustrating. Such small samples cannot easily be followed, placed, nor oriented as molten paraffin is added to a mold. Presently, embedding materials including molds, resins, and hardening agents, all specifically intended for light microscopy examination of tissue, have been developed from those used for preparation of tissue to be examined by transmission electron microscopy. With plastic embedment, polymerization of the transparent resin is sufficiently slow to allow the sample to be carefully positioned and oriented after it has been placed in activated resin within a mold, and this can even be done under a stereomicroscope. Tissue may then be successfully cut from blocks with a face of 2 mm^2 or smaller. Furthermore, while paraffin embedment works well for examination of whole tissues, plastic embedment is far superior if cellular details need to be preserved.

One drawback often encountered with plastic embedment is difficulty in keeping successive sections in serial order. With paraffin embedment, seriality of successive sections is easily achieved, as the trailing edge of each paraffin section tends to adhere to the leading edge of the next, forming long ribbons. When plastic blocks are cut, the sections do not form ribbons and thus each section often needs to be individually transferred to distilled H_2O on a slide or warming bath. There they will spread and flatten. However, upon being placed on the surface of a H_2O bath, as sections flatten and decompress, we find that they spin rapidly and move over the H_2O surface in a random manner. Thus, sequential sections may come to rest far from each other. Nor can sections be easily manipulated via probe, forceps, or any other such tools. When touched by even glass probes, the sections tend to fold and/or adhere to the probe. As the H_2O evaporates, sections dry against the ETOH-cleaned glass surface of the slide, but are distributed randomly rather than in sequential order (Figure 1A).

In the past, several strategies have been employed to avoid this problem. One solution was to create slides with small wells into which the sections could be manipulated (Aweele, 1976). Royer (1988) suggested coating the "leading" and "trailing" surfaces of each block with contact cement, causing the sections to adhere to each other. Alternatively, a water-filled trough attached to the microtome blade has been used to receive and trap the sections, much as would be done for EM thin sections (Wali and Jagadeesh, 1988). When sections were subsequently to be subjected to immunohistochemical staining, Guitteny et al. (1988) recommend placing each section in a separate vial. In two additional methods, loss of seriality was not a problem because of the size of the sections; in one case sections were 5cm × 5cm (Randall et al., 1988) in another instance the sections were cut 25 μm thick and then examined via confocal scanning microscopy (Tosney and Landmesser, 1986).

MATERIALS AND METHODS

JB-4 resin system, molding cups, and block holders were purchased from Electron Microscopy Sciences (Hatfield,

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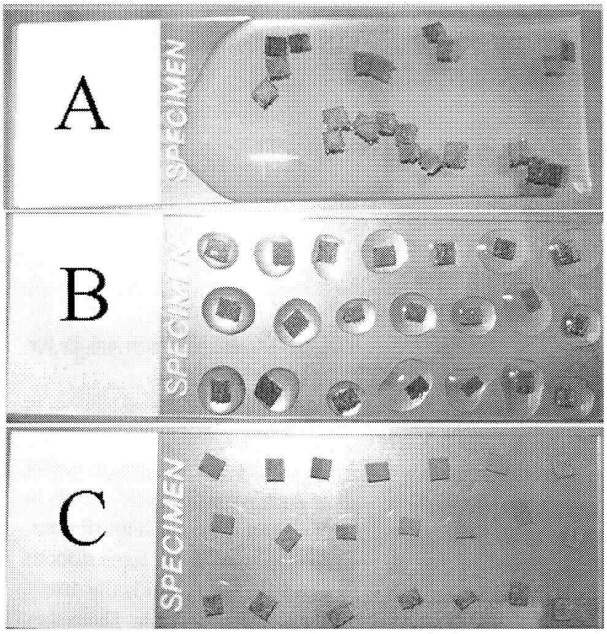

Figure 1. Avoidance of random scattering of sections by use of multiple drops. Sections mounted in a single large body of H2O become randomly oriented (A), while those in a series of small drops remain in sequential order as they dry and adhere to slide (B and C)

PA). Instructions that accompany the resin system are detailed and easily followed. As with most resins for tissue embedment, JB-4 is hydrophilic and thus does not require total removal of all water from the specimen. In our experiments, the tissue was fixed in Carnoy's acid alcohol. After 1–2 hrs fixation, tissue was transferred to 70% ETOH and washed three times (can be stored in 70% ETOH for several days). Tissue was then washed 3 times for 15 min each in 80% ETOH, then 3 times for 15 min each in 95% ETOH. The tissue was then transferred to JB-4 infiltration medium in a 6 × 12 × 5 mm mold and left overnight. Under a stereomicroscope, the tissue, now thoroughly infiltrated with resin, was positioned in one corner of the mold and oriented as desired. New medium with the hardening agent (catalyzing medium) was then added to just overfill the mold. The position of the specimen was checked, adjusted if needed, and a plastic block holder (which also protects the well and provides it with the necessary anaerobic environment) was placed into the socket above the well. The catalyzed resin was left to polymerize overnight at 4°C. Sections were cut on a standard microtome using steel knives. If specimen material occupied only a small portion of the block face, the unoccupied regions could be cut away, making eventual examination of the tissue more convenient.

On the surface of an ETOH-cleaned slide, a series of small drops of distilled H_2O were placed sequentially in rows. This could be done with either a Pasture pipette, or with a pipettor set to deliver between 5–10 µl. We did not find the volume of each drop to effect the results. As individual sections were cut from the block they were placed sequentially, each in its own drop (Figure 1B). The water was allowed to evaporate on a warming tray (Figure 1C). At 40°C the water dried and sections adhered in about 10 min. while several sections in a single large drop required over 30 min. Schoenwolf and Chandler (1983) mention a similar strategy in a paper focused primarily upon the mechanics of plastic embedding. A 24 × 60 mm cover slip was added and the sections were examined with phase contrast optics. If tissue has been pre-stained, or contains fluorophores, or is to be examined with phase contrast optics, there is no need for post-section staining. Yet resolution of small details of tissue and intracellular structures remains excellent.

RESULTS

As an example, we have used insect ovarian follicles, each consisting of an oocyte and surrounding epithelial cells; in many species, these occur in long "chains" called ovarioles. In each ovariole there are sequentially positioned follicles, each older than the one immediately proximal to it. It is often desirable to cut such ovarioles longitudinally, making position in the mold of great importance. Figure 2 shows sequential sections of two plastic embedded insect ovarian follicles sectioned 4µm thick, and photographed with a 20X, 0.45 N.A. Phase Contrast objective.

DISCUSSION

There are several advantages favouring embedment of small samples in plastic. First, the position and orientation of the tissue can be followed throughout the process. Second, preservation of tissue and intracellular structural details are preserved far better than when embedded in paraffin. Third, by the strategy described here, seriality of sections is easily preserved. Fourth, drying time by which the water-floated tissue becomes adherent to the slide is greatly reduced.

In paraffin embedding, assurance of position and orientation of very small specimens is nearly impossible. With plastic embedding, the resin does not harden at once, but remains both fluid and transparent after surrounding the specimen. Thus, the ability to position and re-position a specimen; even to determine this with a stereomicroscope, is a major advantage. For small specimens, the combination of ease with which the specimens could be positioned and the clarity with which cellular level detail was preserved demonstrated that this was clearly a superior method of preparation.

Stereological measurements in which z-axis measurements depend upon accurate examination of serial sections require that the order of sections on the slide be maintained. By placing each successive section in its own drop, this was assured. The series of sections shown in Figure 2 demonstrate the preservation of seriality easily achieved with this technique.

While "East-West" orientation of sections is maintained,

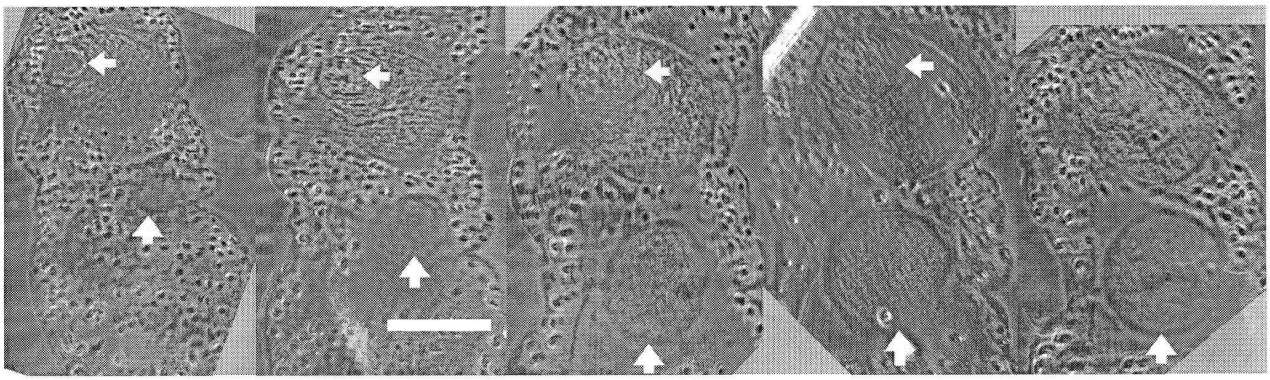

Figure 2. Phase Contrast micrographs of five serial longitudinal sections from a block containing an insect ovariole. Shown are two developing insect oocytes and their surrounding epithelial cells. The horizontal arrows indicate the germinal vesicle of oocyte V-2, which extended from section 1.3 (first row, third section of that slide) through section 2.5 (second row, fifth section). The vertical arrows indicate the sequential appearance of oocyte V-1. Dark spots are the nuclei of follicle epithelial cells. Scale bar = 100μm.

this is not true for "North-South" orientation. However, micrographs of sections can be rotated to a common orientation, as was done here, and the "left-right" positions remain consistent and correct. Alternatively, the camera may be rotated, allowing the sections to be studied in consistent orientation on a monitor.

The ease with which sections can be confidently identified in their proper sequence is the most useful feature of placing them in separate small drops. Occasionally, when cutting sections 1–5 μm thick, a section may be irretrievably lost; and this can easily be recorded during the process of sectioning a block. The precise section and where it would have appeared in serial sections can thus be included in the data. Stereological measurements require accurate 3-D information (Howard and Reed, 1998), and it is for such measurements that this technique would be of particular importance. In such cases, the procedure described here is simple, rapid and effective.

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