

A METHOD FOR SEQUENTIAL HIGH RESOLUTION LIGHT AND ELECTRON MICROSCOPY OF SELECTED AREAS OF THE SAME MATERIAL

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INTRODUCTION

There is need for a simple and reliable technique for re-embedding and thin sectioning of thicker sections (previously examined with light microscopy) for electron microscopy. Because of the small area and extreme thinness of sections for electron microscopy, the sampling error of sections taken at random from a tissue mass can be very large. Furthermore, even if some of the random sections do contain the area of interest, its orientation may be unsatisfactory. Searching for a particular small area within a large block by thin sectioning is laborious; and methods in which alternating thick and thin sections are prepared are tedious, and may not be adequate for a

particular purpose. A method of selecting specific cells or areas of cells to be thin sectioned in known orientation is essential. Several authors have described techniques for doing this (Grimley, 1965; Woodcock and Bell, 1967; Reingold et al., 1970; Mogenson, 1971; Campbell and Hermans, 1972; and Springer, 1973). All have certain drawbacks: for instance, the techniques of Grimley (1965), Woodcock and Bell (1967), and Mogenson (1971) require specially built equipment; the technique of Reingold et al. (1970) requires special plastic slides; the technique of Campbell and Hermans (1972) requires excessive handling of sections; while the method of Springer (1973) makes photography of thick sections difficult.

The technique described below permits rapid location of specific cells, optimal light microscopy of these cells (either stained or unstained), and simple re-embedding and sectioning of selected areas of the cells in the same orientation as the original thick section.

METHODS

The material to be examined is fixed, dehydrated and embedded after standard electron microscopy procedures. We routinely embed in an Epon-Araldite embedding mixture (Mollenhauer, mixture no. 1, 1964). Although not used extensively, limited trials using the Epon mixture of Luft (1961) and the Araldite mixture of Luft (1961) suggest that these embedding materials will work equally well. Extremely hard blocks will make the production of sections of greater than 2 μm thickness difficult. After polymerization, the block is faced with a razor blade in the normal fashion. Freehand (razor blade) sections may be cut and examined while mounted in immersion oil to get near the desired area. The block face (area to be thick sectioned) may be very large by normal ultramicrotomy standards—sections up to 4 mm \times 4 mm are routinely prepared. Thick sections are cut on a dry (no flotation liquid) glass knife on a standard ultramicrotome. Sections of 2–8 μm are the easiest to work with. Each section is removed from the knife with watchmakers' forceps and placed on a drop of water on a slide and dried on a hot plate or slide warmer at 50°C.

SLIDE TREATMENT

We have found that with slides of certain manufacturers, there are problems when trying to remove the re-embedded section. To avoid this, the slides should be sprayed before use with Dupont Spray Slip, (E. I. Dupont de Nemours and Co., Wilmington, Del.) a dry lubricant available at hardware stores. This leaves an opaque, white deposit which is polished off by breathing on the slide and then wiping it clean. Spray Slip contains a fluorocarbon telomer, of which enough remains after wiping to permit easy removal of the re-embedded section. This step can be avoided if a trial indicates that the slides to be used do not exhibit this removal problem. In our experience, slides from Carolina Biological Supply Co., Burlington, N. C. work well without treatment.

LIGHT MICROSCOPY

After the sections are dried down they may be stained for light microscopy using standard stains for plastic-embedded material (Davie, 1970). Staining with methylene blue, azure II, or toluidine blue has no apparent effect on subsequent electron microscopy. In general, however, osmium-fixed or -postfixed tissue does not require staining if examined with phase contrast.

After staining, if it is needed, a small drop of embedding mixture is placed on each section. This should be the same type of resin used in the initial embedding. It is convenient to keep frozen resin in disposable syringes for just this purpose—when needed, a syringe can be thawed and used without the bother of mixing fresh resin.

Cover slips are then placed on the drops of resin and gently pressed down. The material can now be examined. Since the refractive indices of the sections and the unpolymerized resin are similar, any small scratches present are rendered invisible. The sections are photographed if desired. Since the resin will polymerize slowly at room temperature, the slides should be frozen if more than 12 h is going to elapse before re-embedding. Sections that are not going to be re-embedded can simply be put in an oven and polymerized, making permanent slides.

RE-EMBEDDING

After examination and photography by light microscopy, the sections selected for electron microscopy are re-embedded. The cover slips are removed by gently prying them off with a razor blade. The sections will remain attached to the slide. A BEEM embedding capsule filled with embedding mixture is inverted over each section. The slide with capsules is placed in an oven at 60–80°C and polymerized. It is useful to include a piece of paper in each capsule with the section number on it. After polymerization the cooled blocks can be broken from the treated slide quite easily, leaving the section flat in the end of the block. With untreated slides, blocks are broken more easily from glass slides while still warm. Very rarely, a block may break when removal is attempted, leaving the section and a small amount of polymerized plastic attached to the slide. If this occurs, another filled capsule is polymerized over the broken plastic, and the process repeated.

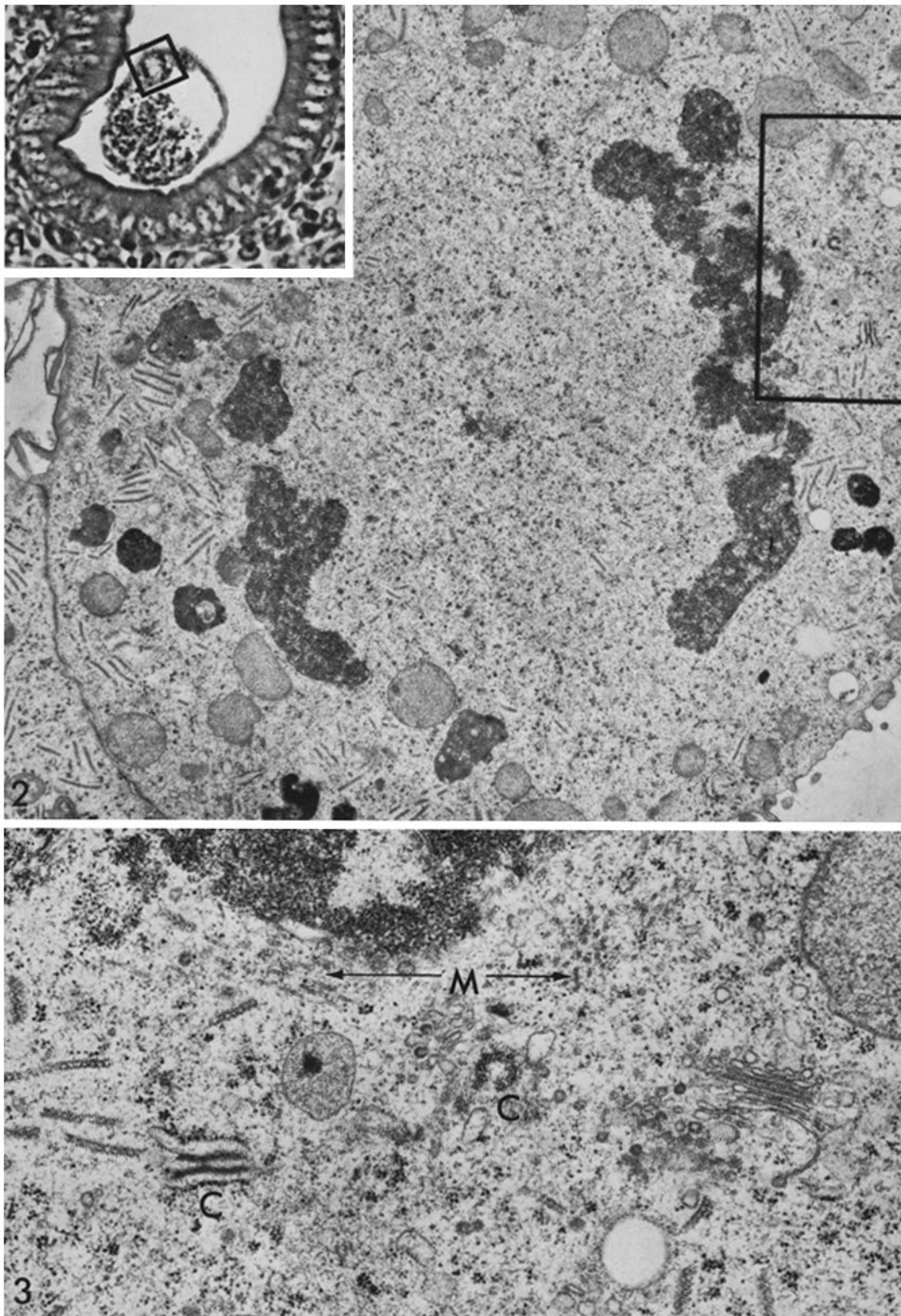


FIGURE 1 An unstained 3 μm section (Epon-Araldite) of a golden hamster blastocyst just before implantation. The partitioned area is that of a trophoblast cell in the process of dividing. $\times 625$.

FIGURE 2 The section in Fig. 1 was re-embedded in Epon-Araldite and thin sectioned for electron microscopy. The trophoblast cell is the same cell as that partitioned above. $\times 8,670$.

FIGURE 3 A higher magnification of the partitioned area in Fig. 2 demonstrating that the re-embedding technique has not had an adverse effect upon the fine structure. Centrioles (*C*) and microtubules (*M*) are evident in association with the dividing chromosomes. $\times 27,700$.

PREPARING THE RE-EMBEDDED SECTION FOR THIN SECTIONING

In most cases a pyramid of a size suitable for thin sectioning can now be made with the aid of a dissecting scope which will afford sufficiently good images to select the desired area. However, if it is necessary to make the pyramid over very small regions of the section which are not visible in a dissecting scope, a compound scope can be used by sawing the end of the block flat and placing it on a slide, section side up. A drop of immersion oil between the slide and the block will improve the illumination. The desired area can now be easily located, especially if photographs or sketches were made previously. The area is marked for facing by using a diamond-tipped slide marker (Zeiss no. 46-29-60, Carl Zeiss, Inc. New York, N. Y.) which replaces an objective lens and can be used to make a small mark on the block. Four such marks are made to delineate the corners of the pyramid which is then made under a dissecting scope using the marks for orientation.

THIN SECTIONING

Thin sectioning is done in a normal manner using glass or diamond knives. The only precaution to be observed is that since the re-embedded section is very thin, it is necessary to orient the block as precisely parallel to the knife edge as possible, and to approach cautiously to avoid cutting a thick section. It is also possible to cut sections perpendicular or at another angle to the original plane of cutting by remounting the block. This might be useful in the case of very small objects which are completely included in the thick section, and could also be used to study effects of stains or other agents on the surface of the section. Interestingly, we find that re-embedded material sections somewhat more easily than the same material in its original embedding.

SUMMARY

Following is a summary for re-embedding thick epoxy-resin sections for electron microscopy. (a)

Fix and embed tissues with conventional techniques. (b) Section material at 2–8 μm , floating sections on water on treated or untreated glass slides and dry on a slide warmer. (c) Mount with a cover slip and embedding resin for observation and photography under phase optics. (d) Re-embed section with an inverted resin-filled BEEM capsule and polymerize. (e) Remove block from glass slide and resection for electron microscopy.

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