POLYESTER-METHACRYLATE EMBEDMENTS FOR ELECTRON MICROSCOPY

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ABSTRACT

It has been found that tissues fixed for electron microscopy and dehydrated in acetone can be embedded in mixtures of n-butyl methacrylate and polyester resin. Activation with 1 per cent tert-butyl hydroperoxide followed by 12 to 48 hours at 60°C produces blocks that section well with glass knives. The ribbons are cleared of methacrylate by heat (200-250°C for 1 hour) and/or immersion in organic solvents (CCl₄, acetone-ether). After removal of the methacrylate the residual polyester matrix provides thermostable and insoluble support for the tissue. Its insolubility permits staining by immersion of cleared preparations in organic solvents carrying heavy metal compounds in solution. Clearing by heat stabilizes section-grid relationships. The removal of volatile materials by clearing substantially reduces contamination of both specimen and microscope. Tissue fine structure is well preserved in these preparations.

INTRODUCTION

The plastic embedding media used for electron microscopy now include methacrylates (5, 16), epoxy resins (2, 6, 8, 9, 15), and polyester resins (10, 11, 22). Each kind of embedment has special characteristics that affect its usefulness to the microscopist. Methacrylates cut readily but are thermolabile, and in their use damage may result to tissue fine structure unless special precautions are taken (22, 24). Both epoxy resins (Araldite, Epon) and polyesters (Vestopal-W, Rigolac) are thermostable and can withstand the heat of the electron beam without membrane support. But they infiltrate tissue less readily, are difficult to cut, and sometimes present contrast problems (6, 18). They are likely to be irregular in their responses to routine manipulations, although a recent publication has reported elimination of these difficulties in Araldite and Epon (15). It is evident that both advantages and disadvantages attend the use of any one of the generally known embedding media (18).

Polyester resins possess two characteristics of special interest to the microscopist. First, their monomers have a wide range of miscibility with other substances. Second, their polymers form a reticular matrix that is thermostable and insoluble in organic solvents (1, 3, 7, 22). Their miscibility is shared with the methacrylates (whose polymers are neither thermostable nor insoluble) and their resistant matrix with the epoxy resins (whose monomers have limited miscibilities). Furthermore, the polyester matrix is sufficiently resistant to heat and organic solvents to permit mounted ultrathin sections to be “cleared” of volatile and soluble substances (13). These cleared preparations are then stainable by heavy metal compounds dissolved in organic solvents (13).

It seemed reasonable to expect that the interesting properties of methacrylate and polyester might be retained in mixtures of the two. This paper reports the results of experiments performed to investigate this possibility.
FIGURE 1
This field, from mouse testis, illustrates small parts of a spermatid in spermiogenesis, whose dark cytoplasm contrasts with that of an adjacent Sertoli cell (S). It was fixed in KMnO₄ (14), dehydrated in acetone, and embedded in 60 per cent Selectron 5003 and 40 per cent n-butyl methacrylate. The sections were mounted on a 200 mesh copper grid covered with a carbon-Formvar membrane. The grid was cleared by immersion in CC₁₄ for 1½ hours and then in acetone-ether with lead acetate crystals for 7 hours. No heat was used. The section is thick, as indicated by the obliquity of the membranes in the endoplasmic reticulum (ER) and the Golgi zone (G). The mitochondria (M) show no signs of compression. × 20,000.

FIGURE 2
The acinar cells of monkey pancreas are often characterized by “open” cisternae of endoplasmic reticulum (ER). The cisternae in the cell at the lower right are more nearly closed. This tissue was fixed in OsO₄ (17, 20) and postfixed overnight in neutral buffered 10 per cent formalin. The water used to dilute the formalin contained 0.1 per cent uranyl nitrate. The tissue was dehydrated in acetone and embedded in a mixture containing 10 per cent Selectron 5214, 50 per cent Selectron 5003, and 40 per cent n-butyl methacrylate. The sections, mounted on a 500 mesh nickel grid without membrane, were cleared by heating slowly to 225°C, and held at this temperature for 1½ hours. The grid was then immersed in CC₁₄ with lead acetate crystals for 26½ hrs. The long axes of the nucleus (N), mitochondria (M), and zymogen granules (Z) are parallel, indicating some cutting compression. × 20,000.
This parenchymal cell from mouse liver was routinely fixed in KMnO₄ (14) but was dehydrated by immersion in glycol methacrylate overnight. It was passed to a mixture containing 60 per cent Selectron 5003, 20 per cent n-butyl methacrylate, and 20 per cent glycol methacrylate, in which it was embedded. The 400 mesh grid, on which the sections were mounted without membrane, was first cleared by heating slowly to 240°C. This temperature was held for 2 hours and the grid was then immersed for 42 hours in acetone-ether. No stain was used. The structural integrity of nucleus (N), mitochondria (M), and cytoplasmic vacuoles (V) is well preserved. X 75,000.

PROCEDURE AND RESULTS

Two kinds of polyester, Selectron 5003 (hard) and 5214 (soft), were mixed with n-butyl methacrylate in various proportions. It was found that as much as 50 per cent n-butyl methacrylate could be used without critically affecting the stability of mounted sections during clearing procedures or during actual use in the microscope. The formulas described below were found to be the most useful.

A mixture containing 60 per cent Selectron 5003 and 40 per cent n-butyl methacrylate yields blocks which are hard and brittle but which can be sectioned with care. Cutting compression is usually absent (Fig. 1). Sections from these blocks clear well even if thick. Better cutting quality results from the addition of some Selectron 5214 to make a mixture containing 10 per cent 5214, 50 per cent 5003, and 40 per cent n-butyl methacrylate. Some cutting compression is noticeable but is slight (Fig. 2). There is no noticeable change in clearing properties or thermal stability. Equal parts of Selectron 5003 and n-butyl methacrylate polymerize to blocks whose cutting qualities resemble those of conventional mixtures of methacrylates. There is some thermal instability in the

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1 "Selectron" is a proprietary name for 17 types of polyester resin manufactured by the Pittsburgh Plate Glass Co., Pittsburgh, Pennsylvania. Retail sales of 1 gallon cans (minimum) are usually handled by local paint stores operating through Plastic Sales, Paint and Brush Division of this company.
mounted sections but this can be overcome by
using fine mesh grids (500) or membrane support.

Tissue may be fixed by any of the usual methods.
No special precautions are required for KMnO₄
(14) and formalin (12). OsO₄ fixation (17, 20)
sometimes inhibits curing, and the most successful
preventive measure is postfixation in 10 per cent
neutral buffered formalin for 2 hours or longer.
Very small pieces of tissue are less likely to cause
trouble.

Specimens are dehydrated in acetone, in
concentrations of 30, 60, and 90 per cent, for ½ hour
each. All the water used to dilute the acetone con-
tains 0.1 per cent uranyl nitrate (or acetate) by
weight to reduce polymerization damage (23).
One hour in pure acetone dried over Drierite and
filtered is sufficient to complete the dehydration.
It is followed by 1 hour in 50 per cent acetone and
50 per cent monomer mixture. One hour in the
pure uncatalyzed monomer mixture is customary
but prolonged infiltration does no harm. The
actual embedding mixture is catalyzed with 1
per cent tert-butyl hydroperoxide, but the concen-
tration may be varied from 0.5 to 2 per cent.
Polymerization takes place in 12 to 48 hours at
60°C. Nitrogen atmosphere (19) is helpful at this
stage. The trimmed blocks are cut with a glass
knife, the cutting angle of which should be as close
as possible to 45°. A very slow cutting motion is
used. Usually no membrane support is required if
400 or 500 mesh grids are used.

Dehydration with the water-soluble plastic
glycol methacrylate (21) has received limited
trial.² This compound mixes readily with n-butyl
methacrylate and the Selectrons. The tissue illus-
trated in Fig. 3 was passed from glycol metha-
crylate into a mixture containing 60 per cent
Selectron 5003, 20 per cent n-butyl methacrylate,
and 20 per cent glycol methacrylate, in which it
was embedded. The other technical procedures
were routine. These blocks section well and
respond characteristically to clearing and staining
techniques.

Freshly mounted sections of Selectron-metha-
crylate embedments can often be used in the micro-

² A 200 gm sample of glycol methacrylate was
received from CHEMAPOL, Praha, Czechoslovakia,
through the courtesy of Mr. J. Šedina. The same
compound, manufactured by Rohm and Haas Co.,
Philadelphia, and marketed by their Special Prod-
ucts Division as 2-hydroxyethyl methacrylate
(Hema), has also been used.

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specimen quality for electron microscopy results from removal of its volatile fraction during clearing by heat. For example, when gross blocks of polyester-methacrylate are heat-cleared, it becomes evident that each ingredient of the final block retains the characteristics of its own polymer. Clearing by heat causes discs of Selectron-methacrylate (2 × 7 mm) to lose considerable weight. The percent weight loss is determined by the relative amounts of methacrylate (90 per cent volatile) and polyester (ca. 10 per cent volatile). These discs do not change in size or shape. The heat drives off volatile substances, chiefly methacrylate, but leaves most of the polyester in place (4). Essentially the same changes must occur to produce the observed improvement in contrast that takes place when mounted sections are cleared by heat. The clearing process can be further extended by immersing heat-cleared grids in organic solvents. Any residual materials soluble in the organic compounds used are thereby removed. The tissue can also be stained at this stage if heavy metal compounds are dissolved in the fluid. During all these operations the polyester matrix remains undisturbed because of its resistance to heat and organic solvents. It therefore continues to provide support for the tissues.

The characteristics of the polyester-methacrylate embedding technique may be summarized as follows. Tissue sections remain unharmed after removal of as much as 50 per cent of the plastic. They are then readily susceptible to chemical influences upon immersion in a suitable fluid. Unwanted solvent action may be avoided (during early preparation) by dehydration in water-soluble methacrylate. Considered together, these characteristics present a wide spectrum of possible approaches to the analysis of ultrastructure.

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