NEW EMBEDDING METHOD FOR CELL SUSPENSIONS

R. F. BAKER and H. E. PEARSON. From the Departments of Medical Microbiology and Public Health, School of Medicine, University of Southern California, Los Angeles, and the Laboratory Division, Los Angeles County General Hospital

The electron microscopy of sections of isolated cells, such as tissue culture cells, white cells, or red blood cells, presents special problems in handling and orientation.

When it is desired to retain the original relationship between cells, as, for example, tissue culture cells growing on a glass surface, it is necessary to process and embed the cells *in situ* (1, 2). One of the disadvantages of such a procedure is that a monolayer of cells is then presented at the block surface and it is difficult to achieve an adequate sampling from such a distribution of cells. This is a major disadvantage in the case of a virus-infected cell population where it is desirable to view a large number of cells.

The alternate method commonly used is that of centrifugation of the cells into a pellet which is then treated like a block of tissue (3, 4). This

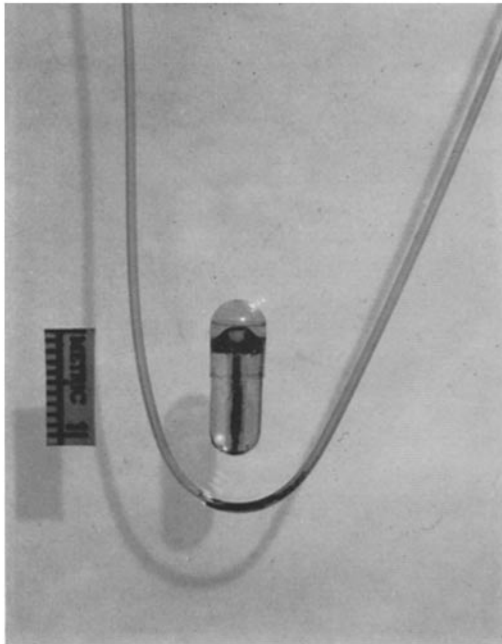

FIGURE 1

U-shaped length of polyethylene tubing which contains cells embedded in methacrylate. To give the necessary support for ultrathin sectioning short segments of this tubing are re-embedded in No. 00 gelatin capsules, as shown in center.

method is apt to be very inefficient, however, since it is usually necessary to throw away much of the sample in trimming the block, even though the smaller sizes of gelatin capsule are used for the final embedding.

The method of embedding described here was evolved when the problem of quantitatively sampling a virus-infected cell population had to be faced. It was desired to cut a known number of thin sections from a group of cells, then the same number of sections from an entirely different group of cells, and repeat this process many times in a systematic fashion.

Throughout the steps of fixation, dehydration, and impregnation with plastic monomer, the cells are contained in a Pasteur pipette which had been sealed at the tip. Low speed centrifugation holds the cells at the tip while changes of fluid are effected through the open end, ending with prepolymerized plastic. The cells are transferred from the glass pipette to a length of polyethylene surgical tubing which had been fitted to a hypodermic syringe. An appropriate size of tubing[1] is 0.57 mm. inside diameter, 0.92 mm. outside diameter. The tubing is then taken off the syringe and put into a centrifuge tube after being bent into a U-shape. The free ends are secured to the upper end of the centrifuge tube with Scotch tape. The cells are brought to the bottom of the U by centrifugation, after which the ends are sealed with a hot needle. Polymerization by ultraviolet or heat follows. When polymerization is complete the polyethylene tubing is cut into short lengths, and each length is re-embedded in a gelatin capsule to give needed support for sectioning.

The end result is a column of cells several mm. long and slightly more than 0.5 mm. in diameter embedded parallel to the long axis of a plastic cylinder, as seen in Fig. 1. The final trimmed block may include the polyethylene if desired, since polyethylene is easily cut with a diamond knife. In any event the cells are readily visualized and trimming and sectioning may be done with efficiency. Adaptations of the method to cover the case of a few cells or even one pre-selected cell will readily suggest themselves.

[1] Intramedic No. PE-50 polyethylene tubing, Clay-Adams, New York City.

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