A Device for Embedding Tissue Culture Preparations Grown on Coverslips. By Mitsugu Nishiura* and S. R. S. Rangan. (*From the Indian Cancer Research Center, Parel, Bombay, India.*)‡

Borysko and Sapranauskas (1) have described a technique for the electron microscope study of thin sections of cells cultured *in vitro*. Similar studies have been carried out for smear preparations by Gay (2). A modification of the above method has been described by Howatson and Almeida (3) for the study of cells grown *in vitro* and sectioned in a plane parallel to the glass surface to which the cells are attached. In this method the lower half of gelatin capsules containing prepolymerised monomer of the "right viscosity" are inverted over selected fields of cells grown in Petri dishes and the plastic hardened in an incubator. A further slight modification has been effected by Latta (4) in another experimental study of cultured cells by placing small rings of solid methacrylate around the cell colonies. The well thus created is filled with liquid methacrylate and the sample is polymerised.

In this laboratory cells grown on coverslips have been processed for electron microscopy according to the method of Howatson and Almeida (3). Coverslips, with cells on their surface, are placed on microscope slides and held in place with a drop of monomer. Gelatin capsules containing prepolymerised monomer are inverted over selected areas and hardened in an incubator.

The inversion of the gelatin capsules with the monomer in them has been found to be difficult unless the prepolymerised monomer is highly viscous. To overcome this difficulty, and to permit the use of only moderately viscous or unprepolymerised monomer, a special stand has been designed which facilitates the inversion process.

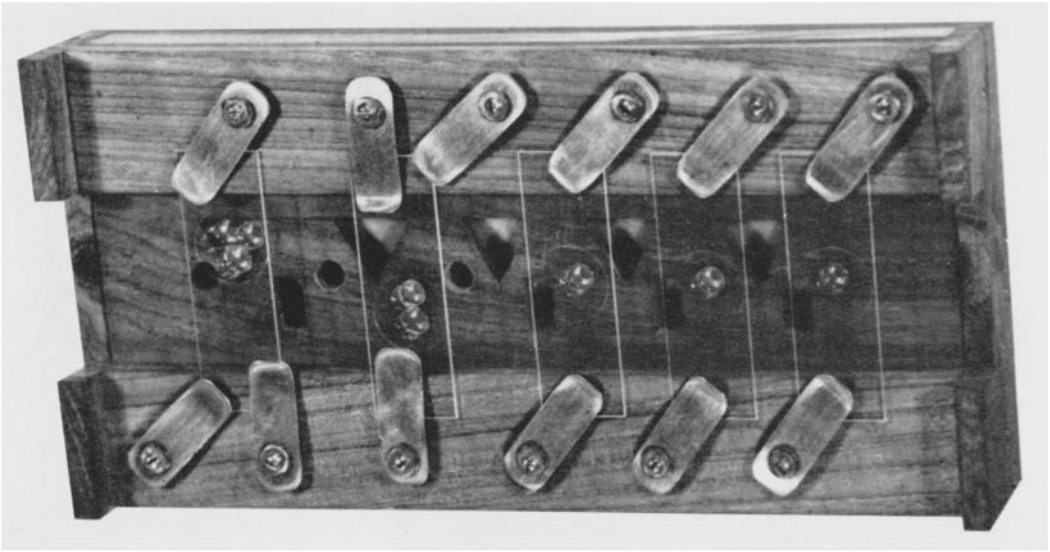

Fig. 1. Actual model of the stand.

Description of the Stand and Method:

A sectional representation of the stand is shown in the accompanying Diagram 1, while Fig. 1 depicts an actual model of the stand in use in the laboratory.

Essentially, the stand consists of a wooden base (*A*) with holes to fit snugly the bottom half of gelatin capsules No. 00 (*G*). The closed end of the capsule rests against a soft cushion of sponge rubber (*B*) stuck to a second piece of wood (*C*). Both parts of the base are secured tightly in position by supporting pieces of wood (*D*). Clamping springs (*S*) which hold the ends of the microscope slides (*M*) against the top surface (*E*) of the stand

* Permanent address: Leprosy Research Laboratory, Kyoto University, Japan.

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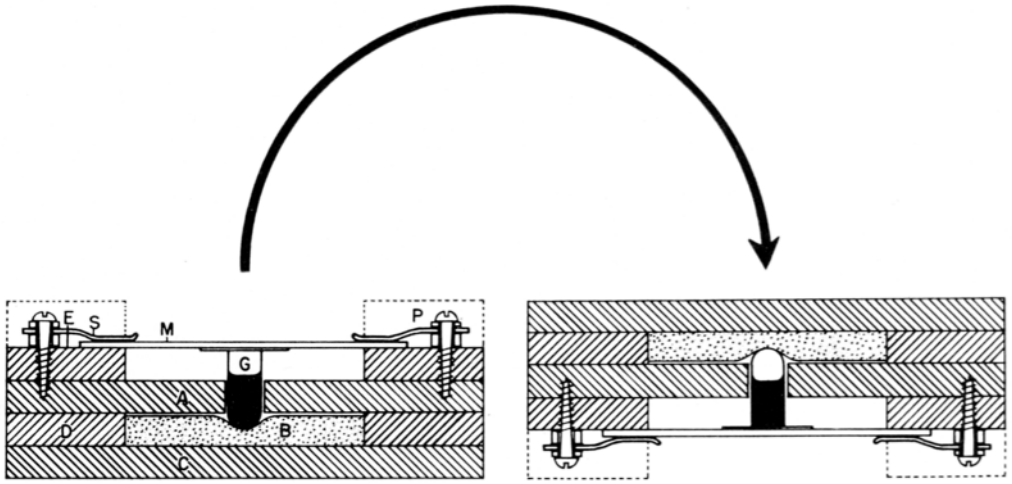

DIAGRAM. 1. Cross-section of the stand.

are provided. It is essential of course that the gelatin capsules protrude slightly above the top surface of the stand before the slides are clamped against them. Supporting projections (*P*) at the ends enable the stand to rest evenly on inversion.

When the apparatus is used, the gelatin capsules are filled to about 2/3rd their volume with the embedding monomer. Glass coverslips on which the cells have been cultured and processed are attached to microscope slides with a drop of monomer. The cells on the coverslips are kept wet with the addition of a few drops of monomer and cell groups are selected under the dissecting microscope. The desired areas, while under microscope observation, are then inverted over the gelatin capsules and the microscope slides are clamped in position with the spring clamps. The soft spring action of the sponge inside the stand keeps the capsule tightly pressed against the coverslip on the microscope slide. When the stand is inverted, the liquid monomer in the capsule covers the cells in the selected fields. Since the monomer is not of a high viscosity, air bubbles are avoided entirely during polymerisation. The monomer is polymerised in the usual manner inside an incubator. If holes in the base are made the proper shapes (Fig. 1) 2 or 3 blocks can be polymerised simultaneously from a single coverslip preparation of cultured cells. The hardened blocks are readily detached from the coverslip after cooling the microscope slides for about 5 minutes in the ice cubicle of a refrigerator. It is necessary to have the coverslip adhering to the microscope slide to prevent it from breaking when the hardened blocks are detached from it. If the drop of monomer placed between the coverslip and slide for this purpose should evaporate during polymerisation, the coverslip can be reattached with paraffin wax, before trying to remove the blocks.

REFERENCES

1. Borysko, E., and Sapranauskas, P., A new technique for comparative phase-contrast and electron microscope studies of cells grown in tissue culture, with an evaluation of the technique by means of time-lapse cinemicrographs, *Bull. Johns Hopkins Hospital*, 1954, **95**, 68.
2. Gay, H., Serial sections of smears for electron microscopy, *Stain Technol.*, 1955, **30**, 239.
3. Howatson, A. F., and Almeida, J. D., A method for the study of cultured cells by thin sectioning and electron microscopy, *J. Biophysic. and Biochem. Cytol.*, 1958, **4**, 115.
4. Latta, H., A cellular reaction to antibody in tissue culture studied with the electron microscope, *J. Biophysic. and Biochem. Cytol.*, 1959, **5**, 405.