

is to be regarded as a virus inactivator or an inhibitor to host infection as reported by Stanley for trypsin<sup>3</sup> has not been fully determined. Since the inhibitory effects on infectivity is instantaneous it suggests that the inactivation of the virus in the instance of the two above organisms grown in culture can not be attributed to decomposition or digestion. Efforts at precipitation and purification have not been successful. The substance may be concentrated appreciably by evaporation of the culture medium, the inactivator being tolerant to drying and to heat, partly withstanding 100° C. for several hours. In a dried broth culture the product is normally active at 0.1 per cent. water solution and retains most of its activity at 0.01 per cent. concentration. A normal concentration will completely inactivate an equal volume of undiluted extract of ordinary

tobacco mosaic. It is equally effective on several other plant viruses. The inactivator passes bacterial proof filters but with considerable reduction in potency. The substance retains its activity for months in either the original culture, a heat-sterilized culture or in a state of desiccation. It will also withstand high concentrations of alcohol, chloroform, mercuric chloride and charcoal. The substance differs strikingly from the *Phytolacca* juice and trypsin inhibitors in several respects, as for example in its ability to tolerate higher temperatures. There is therefore probably no chemical relationship between these peculiar virus inhibitors.

JAMES JOHNSON

WISCONSIN AGRICULTURAL EXPERIMENT  
STATION AND BUREAU OF PLANT INDUSTRY,  
U. S. DEPARTMENT OF AGRICULTURE

## SCIENTIFIC APPARATUS AND LABORATORY METHODS

### THE USE OF PLASTIC MATERIALS FOR OPERATIONS ON AMPHIBIAN EMBRYOS

OPERATIONS on amphibian embryos are usually carried out in black wax dishes, while grafts are held in place by means of glass rod "bridges."<sup>1</sup> When these devices are employed for transplantation experiments on the hind limb of *Amblystoma*,<sup>2</sup> however, it is usually difficult to hold the embryos firmly since the wax can not be molded about them *in situ*; nor is it always possible to adjust the glass bridges to the proper contact and pressure for good healing.

The following materials and devices have been developed<sup>3</sup> to provide more adequate equipment for operations on *Amblystoma* embryos. They have been used successfully, with minor improvements, for five seasons and have been found adaptable to a wide variety of experiments with both anurans<sup>4</sup> and urodeles.

#### A. OPERATING DISHES

Various types of waxes and modeling clays have been tested to find a non-toxic material which can be molded readily and into which pins can be inserted easily and firmly. Most waxes are unsuitable for these purposes, although colorless "Rainbow Wax" (American Art Clay Company) works easily but lacks the firmness of clays. Stone<sup>5</sup> also reports satisfactory results from molding white refined beeswax. On the other hand, almost all clay modeling materials can be

molded readily under the solution used for operating and are sufficiently firm, but many of them are toxic, especially those with noticeable odor or color.

To date, uncolored or gray Permoplast Modeling Clay (made of China clay, petrolatum and glycerine—American Art Clay Company, Indianapolis, Ind.) has been found most satisfactory for retaining the embryos without injury and for holding the graft covers securely during the progress of healing.

The operating dish is made by filling a Stender preparation dish of 50 or 60 mm diameter about half full of the modeling material. In performing the operation a depression is made in the plastic material by means of a blunt glass stylus, while the embryo lies alongside for comparison of size and shape. The embryo is then inserted into the cavity and the material pressed around it, or tiny spurs rolled up over it by means of a spear-shaped needle or glass rod. When the embryo is ready to be removed, it may be freed without injury by pushing or scooping away the surrounding material.

#### B. GRAFT COVERS

Graft covers with pin supports have been devised to hold the transplanted tissue in place during healing. The cover proper is made of some clear transparent plastic sheet material such as du Pont's Lucite, Plastacele or Pyralin of .090 inch to .125 inch thickness. The pin supports are fashioned from small gold-plated safety pins (Fig. 1, A) or large (No. 7) non-corrosive insect pins. The safety pins are preferable, however, since the clasps form convenient "handles."

The graft covers are made by sawing a piece of the sheeting into small rectangular blocks of about  $\frac{1}{2}$  inch x  $\frac{3}{8}$  inch each. A fine hole is then drilled through each end of the block, using a No. 70 or 71 wire gauge drill,

<sup>3</sup> W. M. Stanley, *Phytopath.*, 24: 1055-1085, 1934.

<sup>1</sup> R. G. Harrison, *Jour. Exp. Zool.*, 32: 6, 1921.

<sup>2</sup> W. A. Stultz, *Jour. Exp. Zool.*, 72: 330, 1936.

<sup>3</sup> W. A. Stultz, *Anat. Rec.*, 64: Suppl. no. 1, 43, 1935.

<sup>4</sup> K. A. Youngstrom, *Jour. Comp. Neur.*, 68: 353, 1938.

<sup>5</sup> L. S. Stone, *Proc. Soc. Exp. Biol. and Med.*, 31: 1084, 1934.

depending on the diameter of the pin. The edges of the block are then smoothed with a sharp blade, but care should be taken not to mar the upper and lower surfaces where clear visibility is necessary. The block may also be trimmed away to form a surface of contact of any shape or size desired.

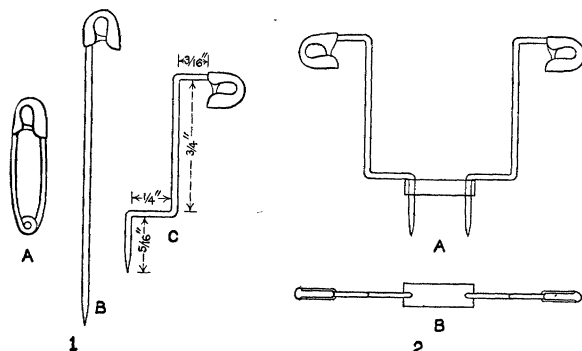


FIG. 1. Graft cover support made from small safety pin (A) straightened out (B) and bent at three points (C).  $\times 1$ .

FIG. 2. Completed graft cover viewed from the side (A) and from above (B).  $\times 1$ .

The supports are made by first straightening out the safety pins (Fig. 1, B). Each pin is then bent to form three alternating right angles (Fig. 1, C). The pins are then driven tightly through the holes in the block (Fig. 2). They may also be cemented in place.

The graft cover is used by centering it over the transplant and pressing the pin points into the plastic material until the cover rests evenly on the graft with sufficient pressure to hold it in place during healing.

This type of graft cover has the advantages of affording clear vision and of being easily manipulated, perfectly adjustable and of standard construction so that any one cover may be used for any graft, irrespective of the size or position of the embryo or the location of the transplant.

WALTER ALVA STULTZ

THE UNIVERSITY OF VERMONT  
COLLEGE OF MEDICINE

### A CULTURE SLIDE FOR DARK-FIELD MICROSCOPY

THE slide has a central circular stage about 3 mm in diameter which is surrounded by a circular moat approximately 0.9 mm deep and 4.5 mm wide at the top. The wall slopes away from the central stage so that the stage is at the upper surface of a truncated cone with a broad base. At a slight distance from the outer margin of the moat, a second narrow and shallow groove is cut in the glass, entirely encircling the moat. The surface of the central stage, the glass surface between the moat and the encircling groove, and the glass surface outside the groove are all on the

same level so that a thin coverslip applied to the slide leaves only a capillary space between the under surface of the coverslip and the surface of the stage, while there remains a considerable space in the moat for culture medium to supply pabulum. The outer circular groove guards against contact of this medium with the vaseline or paraffin used for sealing the edge of the coverslip.

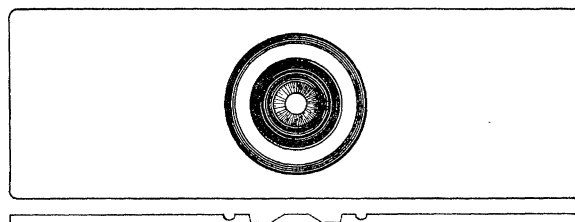


FIG. 1.

The upper drawing shows the pattern of the face of the slide. Below it is the pattern of the profile view of the median longitudinal section.

The measurements and the level of the stage may be varied in different models to meet special needs. At the present time two models are available. One of these is 1.15 to 1.20 mm thick for use with the Zeiss cardioid condenser and the paraboloid condenser of the Spencer Lens Company, and the other is 1.30 to 1.40 mm thick for use with the paraboloid condenser of Bausch and Lomb.

The slides are being made by C. A. Hausser and Son and may be obtained through Arthur H. Thomas Company, 230 South Seventh Street, Philadelphia, Penna.

WARD J. MACNEAL  
ANNE BLEVINS

THE DEPARTMENT OF PATHOLOGY  
AND BACTERIOLOGY,  
NEW YORK POST-GRADUATE  
MEDICAL SCHOOL AND HOSPITAL,  
COLUMBIA UNIVERSITY

### BOOKS RECEIVED

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- EVE, A. S. and D. A. KEYS. *Applied Geophysics in the Search for Minerals*. Third edition. Pp. x + 316. Illustrated. Cambridge University Press, Macmillan. \$4.25.
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