

Shivertaker, L.W. Department of Pathology, West Virginia University School of Medicine, Morgantown, West Virginia. The microinjection of *Drosophila* larvae.

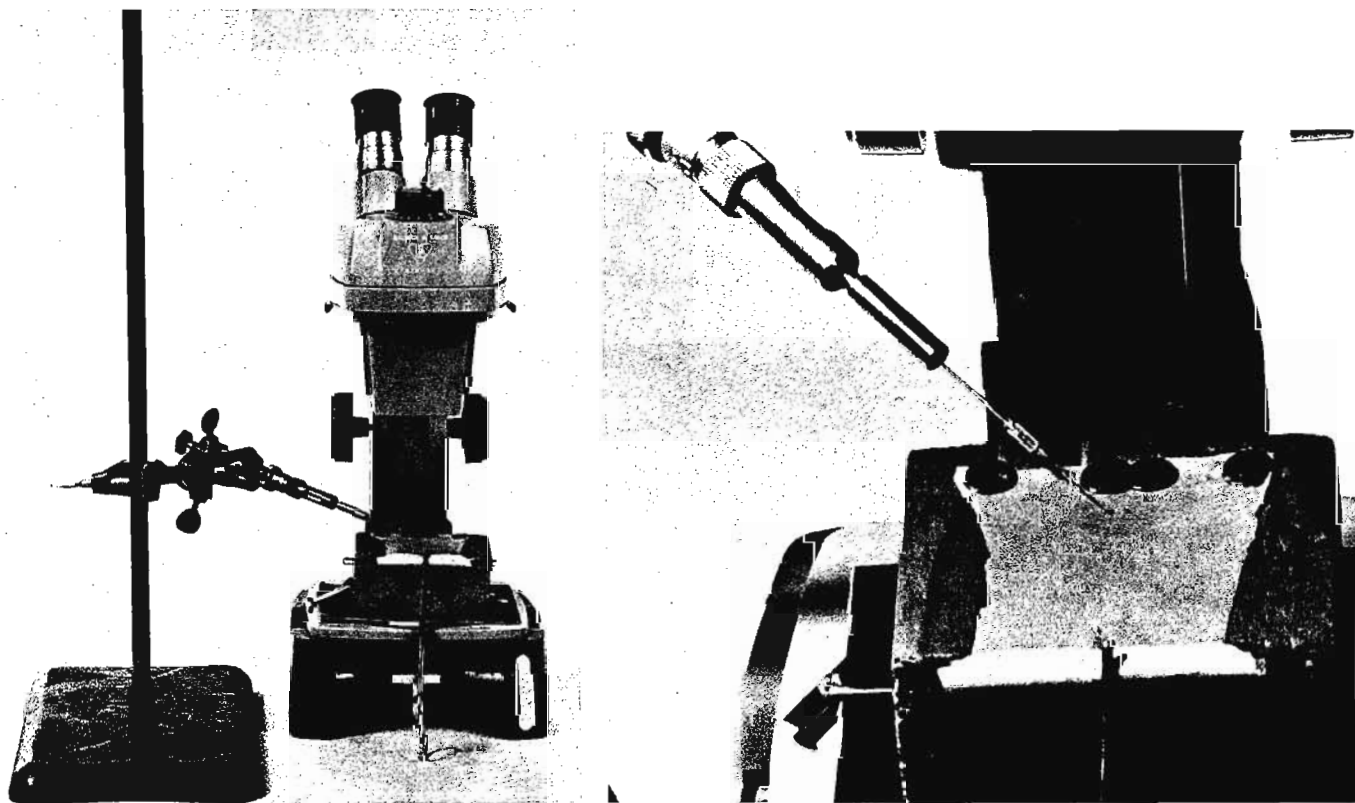
A simple, accurate, microinjection technique is described for the injection of small species such as larvae. This method renders a considerably higher survival than previously described techniques.

Several lines of evidence suggest that the overall pattern of metabolism, growth, and differentiation are greatly influenced by a variety of exogenous agents. In the use of small species, the introduction of experimental compounds by injection methods has been fraught with many technical difficulties. This investigation was undertaken to develop a simple, accurate microinjection technique. The method has been used for the larvae of *D. melanogaster*, but can be adapted for any small species.

Prepupal larvae of *D. melanogaster* grown on a banana-agar-yeast medium at 27°C. were utilized. The apparatus consisted basically of three parts: (1) a wooden stand, (2) a microinjecting syringe, (3) a dissecting microscope.

A modification of Burdette's method (1965) is used to steady the larvae during injection. A small piece of rubber sheeting is stretched across a hole in a wooden stand. A small perforation of selected size made in the rubber sheeting serves to hold the larva firmly but without injury. The rubber is fastened by one end to the wooden stand by thumbtacks. The loose end is clamped by a hemostat to allow easy stretching of the rubber sheeting. The larva is placed vertically through the rubber sheeting with its ventral surface toward the needle. This method allows adequate manipulation of the larva.

Figure (1): The relationship of the injecting needle to the wooden stand



The injection apparatus consists of two parts: (1) a glass needle, and (2) a spring-loaded microinjecting syringe. The needle is made by drawing a pyrex capillary tube, 1 mm in diameter, to a fine point by the use of an automatic pipette puller (Dave Kopf Vertical Pipette Puller - model 700 B using an electric coil). If the needles are sili-conized (Siliclad: Clay-Adams Co.) before bevelling, penetration appears to be less traumatic. The point is bevelled and sharpened with fingernail clippers or similar biting instruments,

which give a clean sharp break. The external diameter at the tip ranges from 40-78 micra. This type of needle is much less traumatic than the 30 gauge needle used by Burdette (1965). With a series of polyethylene adapters, the needle is connected to the spring-loaded microinjecting syringe (Hamilton Co., Whittier, California) which delivers from 0.1 lambda to 10 lambda of solution. The apparatus is positioned as shown in Figure 1. The wooden stand is placed on the stage of the dissecting microscope. The microinjecting syringe is then fastened securely to a ring stand by a clamp. The larva is manipulated by teasing needles and positioned by moving the wooden stand instead of the syringe. The larva is then brought to the needle and the tip of the needle is allowed to enter the posterior ventrolateral portion of the larva. Care must be taken to avoid breaking the injecting needle by keeping it straight with the teasing needles. After the tip of the injecting needle enters the larva, a spring loaded plunger of the syringe is released and the required amount of solution is injected. Effectiveness of the injections can be demonstrated by the use of methylene blue. Rapid diffusion throughout the larva is observed with little or no leakage of the dye. With practice, injections can be done every three to five minutes.

Mortality of the injection procedure occurs within the first four hours after injection. Once the larva enters full pupation after injection, death, if it occurs, is probably a result of the solution injected and not the technique employed. In one series, 135 larvae were injected of which 104 larvae entered pupation. This method, therefore, renders a survival rate of 77%.

Reference: Burdette, W. and R. Anderson, Genetics, 51: 625 (1965). I appreciate the assistance of Enid Gilbert, M.D., Warren Pistey, M.D., and Judith R. Hildebrandt, Ph.D. The work was supported by an Institutional Research Grant, School of Medicine and Dentistry, West Virginia University, and the Lederle Foundation.

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Doane, W.W. Yale University, New Haven, Connecticut. A quick and easy method for rearing large quantities of 'clean' larvae.

When handling large numbers of larvae for biochemical studies or genetic screening programs, it is desirable for their ages to be synchronized and for them to be free of food debris at harvest time. We have been using a quick, easy method for rearing 'clean' larvae that elimin-

ates the need for cooked food medium or agar and yet provides an adequate diet, judging from larval weight and the minimum in its variability when larval ages are properly timed.

Larvae are simply reared on an aqueous paste of brewer's yeast (20%) and cane sugar (10%) that is spread over a 1/4 to 1/2 inch thick foam plastic pad ('plastafoam', or polyurethane of the sort sold for mattress pads). This sits in the bottom of a plastic box or crisper. A ventilation hole at one end of the box is closed with a cotton plug. The paste is made by mixing sterile, aqueous stocks of yeast and sugar. To 100 ml of paste, 1.1 ml of stock Tegasept (10 g/100 ml 95% ETOH) may then be added. However, putrefaction of the yeast occurs when Tegasept is used as inhibitor. Therefore, to prevent an unpleasant odor, use of the mixture of phosphoric and propionic acids described by Lewis (DIS 34: 117) is recommended. Stock food paste stores well under refrigeration; plastic pads may be cleaned, autoclaved and reused. For sterile rearing conditions, additional nutrients may be added to supplement the partially degraded yeast.

Pairs of mature, well-fed adults are released into food boxes for egg deposition over given time intervals; the number depends on the box size and species used. They are removed through the hole in the container by means of a tube attached to a vacuum cleaner and equipped with a cotton gauze bag for catching flies. The same adults may be used repeatedly. The number of larvae that develop cannot be precisely predicted, but should the food appear to be running out, more paste may be poured or spread onto the foam pad.

This method is particularly successful for rearing larvae of *D. hydei* which tend to restrict themselves to the upper layers of ordinary culture media. The larvae feed mostly on the surfaces of the plastic foam and are easily washed off with water at the time of sacrifice into a collecting vessel. Excess food and waste products are removed by repeated rinses with water which are decanted off, leaving the clean larvae in the bottom of the vessel. Pouring the last rinse water with larvae through a mesh may prove useful before blotting them dry. For *D. melanogaster*, whose larvae are considerably smaller than those of *D. hydei*, the method should prove most useful in harvesting late third instar larvae on the verge of puparium formation. The plastic box may be inverted with the food pad placed on the lid and the larvae collected from the walls of the bottom section as they climb up.

(Supported by NSF Grant GB 7106.)