

THE PREPARATIVE ISOLATION OF IMAGINAL DISCS FROM LARVAE OF *DROSOPHILA MELANOGASTER*

J. W. FRISTROM and H. K. MITCHELL. From the Division of Biology, California Institute of Technology, Pasadena. Dr. Fristrom's present address is Department of Genetics, University of California, Berkeley

Imaginal discs of insects have long been known to be embryonic *Anlagen* (1). Indeed, in *Drosophila*, the external structures in the head and thoracic regions, as well as some in the abdominal region, develop from imaginal discs (2). Discs develop normally when transplanted into larvae, and Schneider (3) has recently shown that eye discs develop normally and become pigmented in a defined medium. Thus, for the study of morphogenesis, imaginal discs offer a convenient tool. However, such studies have previously been ham-

pered by the requirement for tedious manual dissection of discs. The present paper outlines a procedure for isolating large numbers of discs without dissection.

MATERIALS AND METHODS

Ficoll, a polymer (M.W. ~400,000) produced from sucrose and epichlorohydrin, was obtained from Pharmacia Fine Chemicals, New Market, New Jersey, and exhaustively dialyzed against deionized water and then lyophilized before use. Silk sifting cloth was obtained from Tobler, Ernst, and Traber, Inc., New

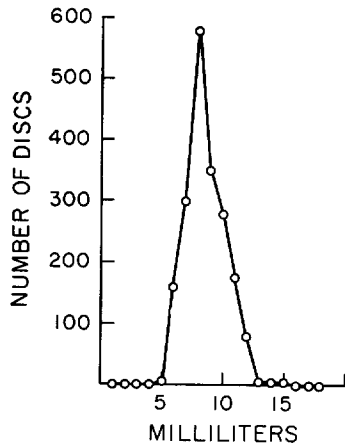


FIGURE 1 Distribution of discs recovered from Ficoll gradient.

York. The *Drosophila* Ringer's solution described by Ephrussi and Beadle was used (4). Continuous 16-ml gradients were prepared with a double mixing chamber similar to one described by Mikeš (5). Large numbers of larvae were obtained using the mass culture technique of Mitchell and Mitchell (6). Late third instar larvae were used for disc isolation and were obtained by washing the sides of the container with distilled water.

DISC ISOLATION PROCEDURE: The entire procedure for disc preparation is carried out in the cold (ca. 5°C). Late third instar larvae (15- to 20-gm wet weight) are ground in a meat grinder (75 to 100 mesh, model 4-E, Quaker City Mill Co., Philadelphia) by washing the larvae through the grinder in 200 ml of Ringer's solution. The homogenate is then washed through two wire screens with 1.43-mm and 0.5-mm openings, respectively, and then through No. 5 silk sifting cloth. The final volume of the washings is about 400 ml. The discs are allowed to settle for 10 minutes and the excess fluid is decanted. The mixture of discs and other tissues is then transferred to a 50-ml beaker, resuspended in Ringer's solution, and allowed to settle for 1 to 2 minutes, after which

excess fluid along with tissue debris is removed with an aspirator. The procedure is repeated until the supernatant becomes relatively free of debris (about 10 times). The material is then transferred to a cellulose nitrate centrifuge tube (16 x 120 mm) and sedimented at 800 RPM in an International clinical centrifuge. The supernatant is decanted and the discs are resuspended in 2 ml of 14 per cent Ficoll (w/v) made up in Ringer's solution. The suspension is then carefully layered onto the upper phase of tubes containing 2 ml of 19 per cent Ficoll (lower phase) and 7 ml of 14 per cent Ficoll (upper phase). The tubes are spun at 900 RPM for 2 to 3 minutes. The discs move to the interface between the two phases and may be recovered with a Pasteur pipette. They are then returned to the 50-ml beaker and washed 3 more times with Ringer's solution, and are again suspended in 2 ml of 14 per cent Ficoll. The Ficoll suspension is layered on top of a 16-ml gradient running from 24 to 14 per cent Ficoll, and then spun at 1,100 RPM in the clinical centrifuge for 10 to 15 minutes. The discs are too large to allow convenient collection of drops using the standard pin hole method, but it is possible to recover them with a pipette. If 1-ml samples are removed carefully from the top of the tube, the distribution of discs is found to be similar to that depicted in Fig. 1. There is no apparent differential distribution of discs along the gradient.

The discs, as may be seen in Fig. 2 which shows a representative sample of isolated material, are over 95 per cent free of other tissue contaminants and are recovered with 10 to 20 per cent efficiency. The morphology of the discs is normal (Figs. 3 to 5). The two appendages attached to the antenna disc (Fig. 5) have not been previously described to the authors' knowledge. The appearance of these structures in preparations is rare. Attempts to identify them by dissection have revealed the presence of similar appendages attached to the antenna discs and closely associated with the larval mouth parts. Frequently, eye discs are recovered detached from antenna discs (Fig. 2). Transplantation studies on eye discs to 80-hour larval hosts have shown that they are capable of developing normally (Table I).

TABLE I
Transplantation of Eye Discs to 80-Hour Larvae

	No. of larvae	No. of pupae	No. of imagoes	No. of discs	Imagoes with discs	Larvae with discs
Exp.	78	41	23	20 + 1*	per cent 86.9	per cent 25.6
Control	20	11	9	3	33.3	15.0

* 1 imago had 2 differentiated eye discs.

Both eye and eye-antenna discs were used; however, the imagoes were scored only for the presence of pigmented eyes. In the control, discs were isolated by dissection.

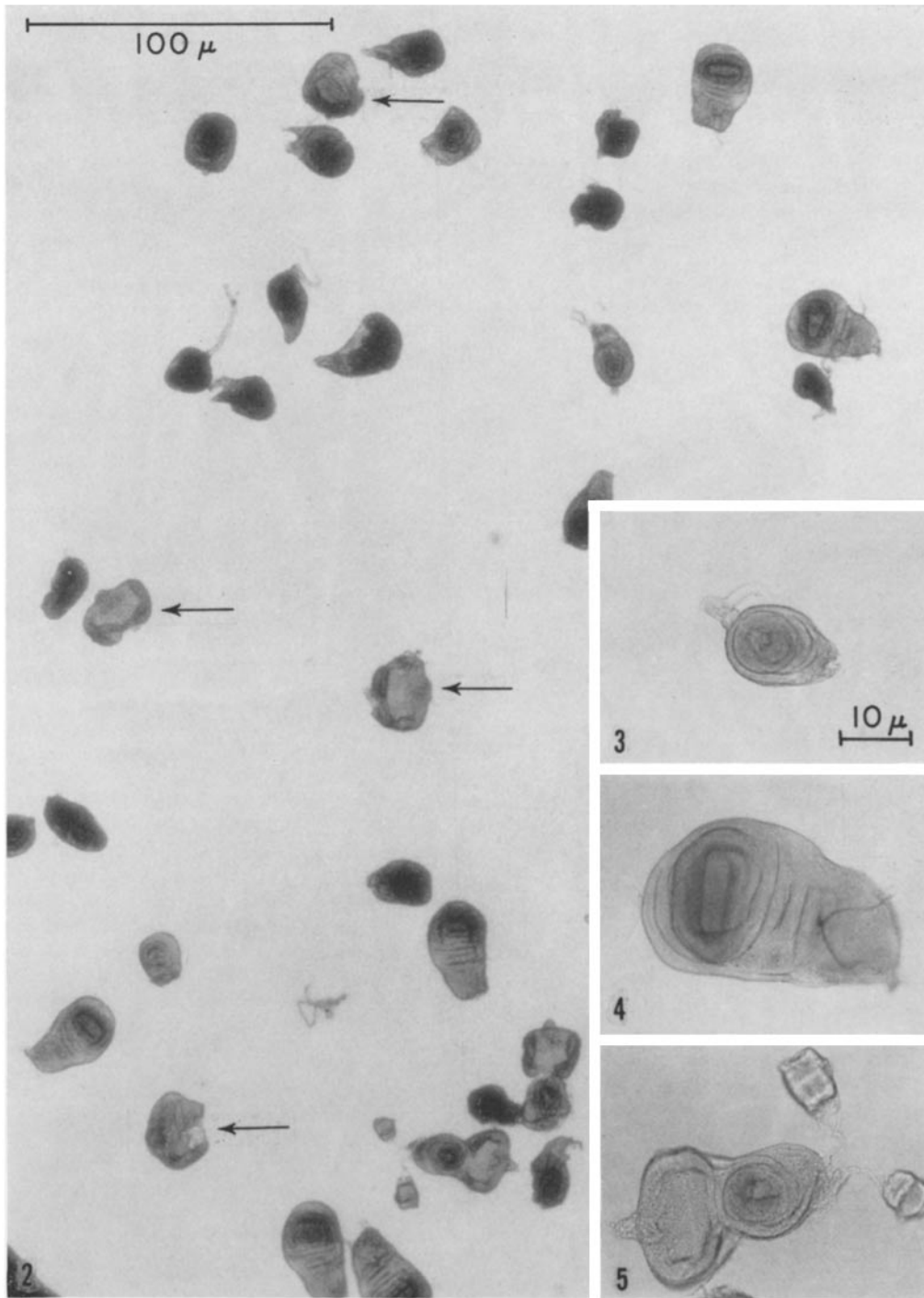


FIGURE 2 Representative sample of discs from preparation. Arrows indicate eye discs recovered independently of antenna discs.

FIGURE 3 Leg disc.

FIGURE 4 Wing disc.

FIGURE 5 Eye-antenna disc showing attached appendages.

DISCUSSION

The preparation of imaginal discs described here takes less than 2 hours and hence offers a convenient method for isolation. Since any number of larvae may be used, there is no limitation on the number of discs obtainable in a single preparation. The transplantability of the eye discs indicates that they are biologically intact and hence should serve as satisfactory material for morphogenetic studies. Although transplantation studies on other kinds of discs have not been performed, they should also be satisfactory. Attempts have been made to use sucrose instead of Ficoll for the

gradients. However, when sucrose is used the discs clump and undergo severe and rapid morphological deterioration. It appears, therefore, that sucrose cannot be substituted for Ficoll in the isolation procedure.

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