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Hill, North Carolina. The preparative
mass isolation of melanotic pseudotumors
from larvae of *Drosophila melanogaster*.

Prior to initiating a series of experiments
designed to investigate patterns of protein
synthesis in *Drosophila melanotic* pseudotumors,
it was necessary to devise a technique for the
mass isolation of experimental material. The
procedure to be described was based on that of
Fristrom and Mitchell (1965) and Fristrom and

Heinze (1968) who have succeeded in isolating 90-93% pure preparations of *Drosophila* imaginal discs. Although the present technique was utilized for the collection of tumors from deep orange-lethal (dor^1 I-0.3±) larvae, it should be useful for any strain in which tumors appear in late larval stages and are free-floating in the larval hemocoel rather than being associated with various larval organs.

(1) Culture techniques and collection of larvae: 50 to 75 grams of larvae are required for this operation. Since the dor^1 melanotic masses are larger and more numerous in female larvae than in males, and because this phenotype is associated with a recessive third instar lethal, a special cross is required. Males of the genotype $dor^1/Dp(1:Y)67g24-2$ ($Dp(1:Y)67g24-2$ representing a special Y chromosome to which a short segment of X carrying the wildtype allele of *dor* has been translocated) are crossed to $dor^1/FM3$ females. Any method, suitable for the collection of large numbers of larvae (50 to 75 gram batches) can be used. In our laboratory the above cross is made in 1/2 pint milk bottles on standard cornmeal-agar medium which has been lightly seeded with dry yeast. Ten pairs of parents are used per bottle and permitted to lay eggs for four days before clearing. Larvae are collected by washing the walls of the culture bottles and surface of the food with distilled water; dor^1 larvae characteristically crawl out of the food onto the sides of the bottles as their normal sibs are pupating. Third instar larvae are cleaned of contaminating younger larvae, pupae, dead imagoes and food debris by the following method. The larval suspension is poured into a glass column, 40cm x 6.5cm OD. The column is filled with 12-15% w/v sucrose solution in which the larvae float while food and other debris and sediment can be removed from the bottom of the column. Pupal and adult contaminants are removed by adding distilled water to the layer of larvae causing adults and pupae to float to the surface where they may be aspirated off. Subsequent operations are performed in the cold (0-6°C).

(2) Preparation of homogenates: Larvae are ground in batches of 50-75 grams per 200 to 250 ml of cold Ephrussi-Beadle ringer's solution in a semi-micro Waring blender at full speed for 2 minutes. After grinding, the brei is filtered through a metal screen with 0.5mm openings. The filtrate is saved and the material remaining on the screen is reground in 150ml of ringer's solution and refiltered.

(3) Washing of material: The combined filtrates of step #2 are placed in a 600ml beaker, brought to a total volume of about 500ml with additional ringer's solution, and stirred vigorously for about 30 seconds. After stirring, tumors and mouthparts settle quickly to the bottom and can be easily followed visually because of their black color. The various non-tumorous debris is aspirated from the top of the suspension after 2-3 minutes. Aspiration is continued until about 200ml of suspension remains. This process of resuspension, stirring, and settling followed by aspiration is repeated three times.

(4) Centrifugation: After washing is complete, the pseudotumor suspension (about 200ml total volume) is again stirred vigorously, divided among eight 30ml glass centrifuge tubes and spun at 10,000 x g in a refrigerated centrifuge for 15 minutes. After centrifugation all but 1-1.5ml of the supernatant from each of the tubes is discarded. The pellets are resuspended by vigorous mixing on a vortex mixer, and each is layered on the top of a discontinuous sucrose density gradient tube (2.5ml each of 50, 60, 70, and 80% w/v reagent grade sucrose per 15ml centrifuge tube) and spun for 3 minutes (or as necessary) at 1350 rpm in an International Clinical centrifuge with model #213 head. Larval debris bands on the top of the gradient tubes or at the interfaces between the 50-60% and 60-70% steps. The pseudotumors band at the interface between the 70-80% steps. They may be gently removed with a Pasteur pipette, washed in buffer and frozen under liquid nitrogen in a number of protective solutions until needed. The preparations are estimated to be about 90% pure, the major source of contamination being mouthparts. Starting from 50 grams of larvae 50-60 mg of pseudotumors (wet weight) are routinely obtained.

References: Fristrom, J.W. and H.K. Mitchell 1965 The preparative isolation of imaginal discs from larvae of *Drosophila melanogaster*. J. Cell Biol. 27: 445-448; Fristrom, J.W. and W. Heinze 1968 The preparative isolation of imaginal discs. DIS 43: 186.

(Aided by an Institutional Grant of the American Cancer Society.)