Anderson, B.A.S. University of Oregon, Eugene, Oregon*. Mass isolation of fat body tissue from Drosophila larvae.

A method has been developed (drawing on the work of Boyd, Berendes and Boyd, 1968) for isolation of fat body tissue from D. melanogaster and D. hydei. Mid to late third instar larvae are placed on a watch glass or glass plate over ice. A rotary circular blade (a pizza cutter or noodle cutter) is used to cut the larvae quickly into two or three sections each. Larvae are then washed into a beaker with Drosophila Ringer's solution, pH 7.2 (0.01 M Tris-HCl), containing 1 to 1 1/2% Ficoll. After stirring for fifteen minutes the suspension is allowed to stand for five to ten minutes. Large fragments of fat body rise to the top and can be removed by pipetting or decanting. repetition of the stirring and settling is sometimes necessary.

All steps are carried out at 2-5°C. All glassware is siliconized.

Fragments of fat body attached to testes or salivary glands sink in the settling step.

Use of higher concentrations of Ficoll led to contamination of fat body tissue with Malpighean tubules and tracheae. Omission of Ficoll reduced the extent to which fat body was concentrated in the surface layer.

Fat body tissue thus isolated was almost entirely free of other tissue. It was compared by acrylamide gel electrophoresis to fat body tissue isolated by dissection. All major bands were present, though two bands, D and E (see Research Note, this issue), were somewhat diminished in relative intensity. Preliminary work indicates that this fat body tissue is active in incorporation of ^{14}C amino acids into protein.

Reference: Boyd, Berendes, and J. Boyd, 1968 J. of Cell Biol. 38: 369-376.

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Posch, N.A. University of California, Los Angeles, California*. Effective means of eliminating bacterial contamination in Drosophila culture media.

One of the studies conducted in this laboratory required the raising of Drosophila melanogaster cultures with no live yeast added to the media, one a cornmeal-molasses-agar medium and the other a banana-corn syrup-agar medium (General Biological Supply House, Chicago, Illinois). Both media contained mold inhibitors. The usual procedure of using sterile media and sterile glass bottles was followed. Within three generations, bacterial contamination was present in 40% of the cultures. The organism was identified simply as a gram-negative bacillus and produced a dark brown color in both types of media, although the contamination, as judged by color, appeared more severe in the banana medium (pH 4.8), than in the cornmeal medium (pH 3.4). Many larvae failed to pupate. Adult viability was slightly reduced.

Several measures were taken to eliminate the contamination. Enough concentrated water solution of merthiolate (sodium ethylmercurithiosalicylate) was added to each medium immediately after boiling so that each medium contained 0.01% (W/V) merthiolate. This eliminated the bacteria, but also the flies.

Benzalkonium chloride (Winthrop: Zephiran chloride), a germicidal solution commonly used in hospitals, was added to hot media so that the media contained 0.08% (W/V) of the chemical. The bacterial contamination was not alleviated by the presence of Zephiran chloride.

A combination of antibiotics was added to the hot (~70°C) media prior to dispensing, specifically, Squibb potassium Penicillin G, 100 units/ml medium, and Squibb Streptomycin sulfate, 0.2 mg/ml medium. Adult flies from contaminated cultures were placed in the bottles containing the antibiotic supplemented media. No bacterial growth appeared. Larval viability was not affected and adult fecundity was good. From these cultures, using sterile technique, it was possible to raise several subsequent contamination-free generations in media with no antibiotic content.

It is important to point out, however, that we also observed that addition of live yeast to the surface of the media just prior to the introduction of contaminated flies resulted in clean cultures. No form of bacterial contamination ever occurred in our cultures when live yeast had been added to the surface of the medium. It is therefore advisable to use live yeast in all cultures, unless the experiment demands otherwise, from a bactericidal point of view as well as a nutritional one.

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