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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. melano-*
gaster 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 Mal-
pighian 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 dim-
inished in relative intensity. Preliminary work indicates that this fat body tissue is active
in incorporation of ¹⁴C amino acids into protein.

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

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Minnesota.

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 pro-
duced 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 immedi-
ately 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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