Malich, C. W. NASA Ames Research Center, Moffett Field, Calif. Thin window holder for particle irradiation of Drosophila.

The finite range of energetic charged particles and their variation in linear energy transfer with penetration require special techniques for the treatment of Drosophila. A simple holder has been devised which makes irradiation convenient and uniform for particles having ranges between a few millimeters and a few centimeters. A circular well 1" diam x 0.5 mm deep (for flies averaging 1.0 mm thick) is machined into a lucite disk 1 3/4" diam x 1/2" thick. Eight ventilation grooves 0.015" x 0.015" are milled from this depression to the outside. A cover is made by cementing a thin window over a circular hole 1 1/4" diam in a lucite disk 1 3/4" diam x 1/4" thick. We use Mylar of thickness 0.001" for the window because it is quite strong, handles well and will give a flat surface readily. The window is attached by brushing a small amount of acrylic cement onto the lucite and gently dropping a square of selected Mylar in place. This is trimmed after drying under pressure. John E. Neff of the NASA Ames Research Center suggests stretching 1/2 mil Mylar on an optical flat of glass and cementing this to the plastic ring with Eastman 910 adhesive, to give a very flat, taut window.

In use, flies are lightly etherized and placed dorsally in the depression as close together as desired. The window is placed over the flies carefully and taped to the back piece. The flies are slightly compressed, holding them in place and minimizing the depth of the germ cells and its variation from fly to fly. Air is gently blown through the chamber every few minutes with a hypodermic syringe or rubber bulb until the flies recover from the anesthetic. Ventilation is adequate, since Rosemarie Binnard at the NASA Ames Research Center found no evidence of an oxygen effect in X-ray induced mutations when comparing standard gelatin capsules to these holders. Drosophila will live in them for several hours without forced ventilation, dying on longer containment from apparent dessication. Modification of the holders for continuous flow of moist air (or other gases) is simple. Keying of the two pieces to prevent rotation, as done by Jane Duffy at the Institute for Cancer Research, is recommended to prevent damage to the flies during assembly.

The thick lucite construction is adequate for most particle irradiations. The nuclear properties of $H^1$ and $C^{12}$ minimize neutron production and bremstralungen, and tests have shown that such secondary reactions contribute less than 1% to the dose in these holders. It is important to have the diameter of the window larger than the beam, so that forward scattering will not contribute to the dose. A double thin window holder has been constructed for comparison, and is preferable for highly penetrating radiations. The double window design is also better for use with X-rays, since the back scatter from thick lucite significantly increases the dose absorbed from the incident radiation and its effective linear energy transfer. We are developing a more sophisticated design which will improve ventilation, increase compression of the flies and permit partial body irradiation. This should be useful with UV as well as charged particles, but the simplicity of the present design makes it preferred for routine work.

Hendrix, Nina, and Elizabeth Ehrlich. University of Oregon. A method for treating bacterial contamination of Drosophila cultures with antibiotics.

It was observed in our laboratory that the medium of some cultures turned a dark, reddish-brown color after several days, while others remained the light, yellow-brown color of the original new food. We had also noticed bacterial contamination in cultures sent to us from various laboratories throughout the country including the two major stock centers. Because in some of the cultures with this discoloration marked decreases in productivity were found, it was decided to try to identify the cause of the change in color.

By allowing flies from the darkly colored bottles to walk on a Petri dish containing new food and then removing them, it was found that the same dark color appeared on this food. From these dark areas sub-cultures were made with the advice of Dr. Priscilla Kilbourn of the University of Oregon, Department of Biology and using standard bacteriological identification methods, we found the organism apparently responsible for the discoloration to be of the Genus Achromobacter. It was a short, small, Gram-negative rod-shaped bacterium. This genus can be found in soil and water.

A sensitivity test was done using discs of broad-spectrum antibiotics and of sulfa drugs. Two drugs - dihydrostreptomycin and tetracycline - were found to have the best inhibitory effects on the bacterial growth.

For purposes of decontaminating the cultures, these two antibiotics were incorporated simultaneously into the regular fly media in the following proportions:

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| dihydrostreptomycin sulfate (Upjohn Co.) | 100 micrograms/ml. food |
| tetracycline hydrochloride (Tetracyn Phizer) | 30 micrograms/ml. food |

The procedure for decontamination is as follows: contaminated flies are put onto antibiotic food in bottles or vials. After a good number of eggs has been laid, all the parents are removed. The eggs are allowed to mature on the antibiotic food. After hatching, these new flies are transferred again to plain food without antibiotics, preferably without etherizing. If it is necessary to select the flies, care should be taken to clean brushes, etherizers, plates, etc., with 70% ethanol to prevent recontamination. The plain food cultures are then incubated and observed to see if the discoloration appears again.

Because the action of the antibiotics used may have unknown and potentially undesirable genetic effects, the drugs probably should not be used for very special stocks. The treatment should be completed in one generation.

We have found this method very satisfactory for ridding most of our cultures of all contamination. The productivity of the cultures is much improved with this treatment.

Gallo, A. J. Govêrno do Estado de São Paulo, Brazil. An apparatus for filling vials.

We saw this apparatus in a bacgeriological laboratory (Instituto Adolfo Lutz-São José do Rio Prêto) and we are using it with success in our own laboratory. It is specially useful because we can fill all vials with a standard quantity without dirtying the walls of the tubes. The vial is placed under the apparatus and gets filled when one presses the Mohr pinchcock. Only a wooden basis, a funnel, a piece of latex, a piece of glass tube and a Mohr pinchcock are required. According to the picture, it may be made in a proper size.