Thompson, C. F., Jr., and F. J. Gottlieb. University of Pittsburgh, Pittsburgh, Pennsylvania. A method for obtaining large numbers of accurately timed pupae.

The following method enables the collection of large numbers of pupae of very accurately known age. In order to do so, very heavy cultures of larvae are necessary. The food of David (D.I.S. 36:128) allows for extremely heavy cultures, particularly if a large number of parents are used and if several cc. of thick yeast suspension are added to the culture with the parents and again when the parents are removed several days before collection. As larvae prepare to pupate, they crawl on the side of the bottle if no dry paper is available to them. If five or six bottles are set up, larvae will pupate at a rate of up to two per minute for several days. Even though the cultures are very heavy the mature larvae are larger than those grown on normal corn-meal-molasses medium and they show no noticeable effects of crowding. At the beginning of collection, all pupae are removed or pushed down into the food from the side of the bottle. After an appropriate interval, pupae are collected from the bottles in the same order in which they were cleared of pupae, using a stiff moist brush such as Grumbacher's No.1271-F or 626-B. Thus the time of pupation of the collected pupae is the midpoint of the interval between collections plus or minus one half of the interval. With practice, collections can be done at a rate of one bottle a minute. The minimum interval between collections is a function of the number of bottles, i.e., six minutes for a six bottle series. In such a case, the order of the bottles must not be changed and an even rate of collection must be maintained so that the interval between collections from the same bottle is constant. The initiation of pupation is determined on the basis of three criteria: immobility, spiracle eversion and shape characteristic of pupae. Any individual which does not meet all three criteria is discarded. As they are collected, pupae are transferred to a small petri dish 5 cm. in diameter in which a moistened piece of filter paper has been placed. The petri dishes are sequentially numbered and all pupae collected within a minute interval are grouped together. Thus it is easy to determine the age of the groups of pupae by referring to the number of the petri dish. Occasionally, an animal which meets all three criteria will "revert" back to a larva and crawl away as a result of being transferred to the petri dish. It will frequently pupate later. To avoid including these animals, all pupae are placed on a small area marked in the center of the filterpaper. Any "revertants" will crawl off this area and pupate outside of it. Such a pupa is easily spotted and removed later. (Work supported by Public Health Service Research Grant GM11084, from the National Institute of General Medical Sciences.)

Thompson, C. F., Jr. University of Pittsburgh, Pittsburgh, Pa. A method for collecting accurately timed larvae.

The following method of obtaining accurately timed larvae, using the event of egg hatching as the base time, avoids many of the problems associated with techniques which use the time of egg collection as the base.

Eggs are collected by any convenient means. We prefer the method using Norite blackened, yeasted food on a paper milk bottle cap which fits the standard half pint milk bottles. After eggs have been laid, they are transferred under a dissecting microscope with a spear point probe, brush or fine jewelers' forceps and lined up on a small block (about a $cm^2$) of nutrient agar which rests on a square (about 2 $cm^2$) of black polyethylene tape folded over so that no stickly surface is exposed. The tape containing the agar block is placed in a petri dish containing the same nutrient agar, to insure a moist atmosphere, and the dish is covered. The eggs are observed periodically and as the larvae hatch they are collected for whatever use they may be required. The beginning of larval life is thus the midpoint of the interval since the previous collection of larvae from that block plus or minus one half of the length of that interval. The process, though seemingly laborious, is quite efficient as virtually every egg provides a timed specimen. If eggs are initially collected over a short period of time the majority will hatch within a subsequent two to three hour period although the entire range of hatching time may be quite wide. The black tape serves several purposes. It is a convenient means of handling the agar block and it provides a dark background against which the eggs and larvae are easily observed. If the tape is somewhat wider and longer than

the block, it provides a dry barrier which keeps the larvae on the block where they can easily be retrieved. Those few which do cross the barrier can usually be retrieved from the medium in the petri dish. The newly hatched larvae usually do not burrow for the first half hour after hatching and if they do, they can easily be seen in the semi transparent medium. All larvae are removed during each observation period and empty egg cases are also counted and removed to insure that all newly hatched larvae have been accounted for. It is possible by this method to watch several hundred eggs continuously and collect the larvae as they hatch. If a small interval between observations is permissible, many more blocks of eggs can be observed. The black tape cannot be replaced by cardboard or paper since these materials will curl when they come in contact with the moist agar. (Work supported by Public Health Service Research Grant GM11084, from the National Institute of General Medical Sciences.)

Corwin, H. O. and P. D. DeMarco. University of Pittsburgh, Pittsburgh, Pennsylvania. Technique for feeding chemical mutagens to adult Drosophila.

Adult male Drosophila do not survive multiple injections of chemical mutagens. In experiments where more than one mutagen is to be introduced into the adult fly at separate time intervals, feeding is necessary. The agent to be introduced must be dissolved in a medium that will stimulate fly feeding but will not induce chemical or physical alterations in the agent. The quantity and type of filter paper placed at the bottom of the shell vial, which acts as a reservoir for the mutagen solution, is also important. It must be sufficiently absorbent to remain moist, but it must not retain an excessive amount of solution or else the flies will drown. An additional problem was encountered during feeding experiments. Plugs had to be removed and replaced in the feeding vials every time new solution was added. Flies were often lost or squashed between the cotton plug and the side of the vial during the above process. The following method, adapted from the feeding technique introduced by Pelecanos and Alderson (DIS 37:116, 1963), is designed to solve the above problems.

Different concentrations of chemical mutagens were fed to Canton-S wild type males. The adult males had been aged 12-24 hours. The various concentrations of mutagens were dissolved in a 5% glucose solution. Groups of ten male flies were treated in 20 dram shell vials. The bottom of each vial was lined with three thicknesses of Whatman filter paper #3 cut to the diameter of the vials. A pasteur pipette, inserted through the cotton plug until it touched the filter paper, served as a permanent delivery tube for the different treatment solutions. This arrangement allowed for the addition of fresh solutions without the removal of the cotton plug. After the initial saturation of the filter paper, only five drops of solution every ten to twelve hours were required to keep the filter paper moist. A single agent was administered over a 48 hour period. When two agents were administered, the simple glucose medium sustained the male flies over the total 96 hour feeding period and no decrease in progeny production per male was noted following this treatment. The treated males were then tested for specific visible mutations induced at the dumpy locus. All agents fed by means of this above technique demonstrated mutation frequencies equal to or exceeding those frequencies obtained when these same agents were introduced by means of the injection technique.

Mittler, S. Northern Illinois University, DeKalb, Illinois. A rapid method for removing and discarding adult Drosophila.

For several years we have used with much success a small portable tank vacuum cleaner to remove and discard flies from bottles prior to collecting virgins. The open end of the bottle is tapped at a 45° angle on to a rubber mat about an inch from the end of the vacuum tube. The tube is not inserted into the bottle because air rushing in along the sides will cause some Drosophila to stick to the food. The cleaning attachment on the end of the flexible tube is not used, and of course, the opening is plugged after use.