Clancy, C. W. Two methods of ligating Drosophila larvae. Larvae of Drosophila may be ligated with fine, human hair at any desired segment by placing two surgical-dressing forceps on a table under a wide field binocular microscope in such a manner that the jaws support between them a previously prepared loop of hair. The loop, into which the etherized larva is placed by means of a small camel's hair brush moistened with saline solution, lies in the center of the binocular field dipping into a small drop of saline lying on a thick glass slide. If the larva has been properly etherized, i.e., quickly and sufficiently, it will relax and extend itself the moment the saline solution touches it. Orientation with respect to the segment at which ligature is desired is made with the brush or by moving the forceps holding the loop. The surface tension of the drop of water tends to hold the larva and aids in placing the loop.

In case the exact position of the ligature need not be determined at the time of tying, an even simpler and more rapid procedure may be carried out that eliminates both the binocular and the forceps. A heavy glass rod (1.0 cm. in diameter and 25.0 cm. long) is clamped to a ring stand by one end so that the opposite or free end extends horizontally toward the hands of the operator can rest comfortably on the table and at the same time hold the loop of hair at the same level as the rod. The larva, etherized, relaxed, and extended, is first placed in a small drop of saline on the upper surface of the rod. Surface tension of the water holds the larva and enables one to slip the loop around it and tighten the knot in the desired position.

In connection with a preliminary study of pupation, several hundred larvae have been tied by the above two methods. When tied shortly before pupation the larvae survive and go through preliminary pupation changes.

Ephrussi, Boris and G. W. Beadle A technic of transplantation for Drosophila anlagen. A technic of transplantation in Drosophila has been elaborated. By means of this technic imaginal discs of eyes, legs, wings and ovaries can be successfully implanted into larvae shortly before pupation.

The actual technic consists of the injection of the desired organ into the body cavity of a larva by means of a glass micro-pipette connected with the capillary tube and syringe of the standard Chambers' micro-manipulator. The pipette is made in drawing out with a micro-burner a glass capillary with an external diameter of about 0.7 mm. and a wall thickness of about 0.1 mm. to a finer capillary shaft of an external diameter from 0.1 to 0.16 mm. The bore of the shaft should be from 0.06 to 0.12 mm. The length of the shaft should be about 2-3 mm. At its base a constriction is made in the bore by heating with a horizontal micro-flame. The function of this

constriction is to block the tissue in the shaft, thus preventing its entering the larger part of the pipette, and also to act as resistance to the flow of liquid through the pipette. The pipette should have a very sharp point, which can be obtained by grinding on a fine-grained hone.

In our experiments the dissection of the donor-larva is made free-hand in a drop of 0.7% physiological salt solution. The host larvae are anesthetized on a glass slide in a simple glass vessel. After they are etherized and extended, they are moistened with a drip of Ringer, which tends to produce more nearly complete extension. They are then dried with filter paper and after a few minutes adhere to the slide.

The injection is made free-hand under a binocular microscope. Our equipment consists of two binoculars arranged so that they can be used from opposite sides of a narrow table. Two persons cooperate in the operation: one holding the larvae with a blunt curved metal needle, the other making the actual injection. (The complete description of the technic will appear in the American Naturalist.)

Beadle, G. W. Pigmentation of Malpighian tubes in larvae of D. melanogaster.

During the course of work on transplantation, it has been noticed that the Malpighian tubes of larvae of certain eye-color mutants are pale yellow or practically colorless, while those of wild-type larvae are distinctly yellow. Thus, ca, car, cm, g^2, lt, pp, rb, and w larvae appear to have very little or no pigment in the tubes. It is clear that these, and probably many more mutants, can be classified in the larval stage. To do this, it is not ordinarily necessary to dissect the larvae; the color can be seen through the body wall. The ability to identify particular mutant types in the larval stages obviously can be used to advantage in cytological studies, transplantation, etc.

Hoover, Margaret E. Some uses of Beadle's Malpighian tubes technique.

Although we have worked in a preliminary way for only a short time with Beadle's suggested technique utilizing the colors of the Malpighian tubes, it seems to offer interesting and important possibilities. The difference between yellow and white tubes can be easily seen in three-day old larvae and with practice the distinction can be made in younger ones. Careful examination with good strong lighting is necessary but the difference can be seen with accuracy. Such a technique can be put to good use in cases where an X-chromosome carrying a deficiency is balanced against dl-49 carrying garnet eye color. If ♂ carrying the deficiency die during larval life, the exact extent of survival can be accurately determined by isolating and observing the ♂ larvae with yellow Malpighian tubes. Moreover, by first observing the Malpighian tubes of the ♀ larvae used in making salivary gland chromosome preparations of such deficiencies, the use of ♂♀ not carrying the deficiency can be avoided.