Ellison, J.R. University of Oregon, Eugene. An improved method for electron microscope preparation of salivary gland polytene chromosomes.

The following technique allows both high resolution phase contrast light microscopy and electron microscopy to be performed on the same cell. The salivary gland is dissected out in saline and placed in a drop of a mixture of 1 part acetic acid, 2 parts lactic acid, and 2 parts water on a silicone coated slide and allowed to stand for 3 minutes. The gland is covered with a silicone coated cover slip and squashed. The slide may be surveyed at this time using phase contrast microscopy. The slide is then frozen in liquid nitrogen and the cover slip is removed. Immediately the slide is immersed in 95% ethanol (5 min.). The slide is then placed in a mixture of equal parts of absolute ethanol and acetone (5 min.), followed by 100% acetone (5 min.). The slide is then stained with acetone saturated with uranyl acetate (5-10 min.). The slide is rinsed in 100% acetone (1 min.) and put into a 20% epon/acetone mixture (5 min.). Epon is then applied over the squash with a 10 ml. disposable syringe. Broken pieces of slides are used as spacers and another slide is placed on top of the epon (fig. 1). The epon is allowed to polymerize for 48 hours at 60°C. The slide is then placed on a hot plate at about 200°C. with the slide

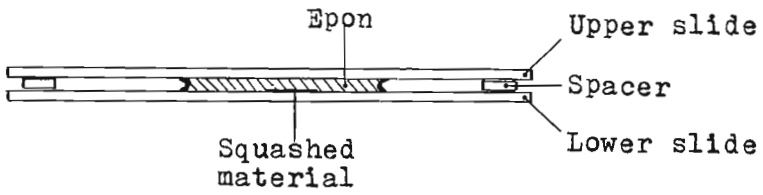

Fig. 1

on which the squash was made against the hot plate. Immediately the slides are pried apart by inserting a small screwdriver between the slides and gently twisting. The epon will separate from the lower slide taking the squash material with it. The cells are now on the surface of the plastic and can be observed with phase contrast optics. Desired cells can be marked with either a diamond stylus or ink objective marker. If oil immersion microscopy is desired, glycerine may be placed on the epon and a cover slip applied. Immersion oil can then be used on top of the cover slip. The glycerine washes off and does not interfere with subsequent sectioning. The selected cells are then cut out and glued (with epoxy glue) on to 1 cm. segments of 8mm plastic rods, and sectioned.

R. Nöthiger. Zoological Institute, University of Zürich, Switzerland. Sucrose density separation - a method for collecting large numbers of Drosophila larvae.

Materials required: a separatory funnel (1000 ml, with a valve opening of 4-6 mm) fixed to a ringstand, a long glass rod, a brush, a piece of fine meshed nylon cloth, solution of ca. 20% sucrose in water.

Pour sucrose solution into populated food container, stir with brush and bring larvae "into solution". Pour the suspension of larvae, corn meal, and perhaps dead carcasses and empty pupal cases into separatory funnel. Add slowly a little water, stir with the glass rod until corn meal sinks to the bottom and the rest floats. Bigger pieces of corn meal are crushed with the glass rod. Now open valve and release cornmeal fraction. Repeat this washing procedure, if necessary. Then add water: larvae will sink to the bottom, carcasses and empty pupal cases will float. Now release larvae onto nylon mesh, wash if desired, and collect.

This method is especially useful for collecting young larvae. Development after this treatment is normal.

The essentials of this method have been brought to my attention by Drs. F. Ratty and R. Rinehart of San Diego State College, California.