Granholm, N.A. Department of Biology, University of Oregon. Studies of selected spermatocytes in the light and electron microscope.

A technique is available which enables one to observe living Drosophila spermatocytes in vitro and to recover the observed cells for electron microscopy. The technique is patterned closely after one reported by Brinkley and Nicklas for grasshopper spermatocytes (1968).

Pupal testes are dissected under series 11-14 Halocarbon Oil (Halocarbon Products Corp., 82 Burlews Court, Hackensack, N.J.). An intact testis is freed of adhering fat and transfered to a drop of Halocarbon Oil on a clean but otherwise untreated #1 coverslip. The testis is cut using small dissecting knives and smeared over an area of the coverslip, thereby expelling the cells from the testis and spreading the cells into a single-cell layer. If care is taken to spread the cells evenly, keeping the cell layer intact, the cells will adhere to the coverslip. Enough oil is then added such that the coverslip will remain slightly above the surface of the glass slide onto which it is inverted. The coverslip is ringed with VALAP (vaseline + lanoline + paraffin wax with a 50°C melting point, in the proportions 1:1:1) (Mole-Bajer and Bajer, 1968) to hold it in place. Cells are selected and photographed. Cells in such a preparation have been followed from diakinesis through completion of the first meiotic division.

It is necessary to separate the cells from the oil for fixation. This is accomplished through quick-freezing the slide-coverslip preparation in pentane-isopentane (ca. 1:1) cooled in a liquid nitrogen bath. The preparation is then quickly transfered from the pentane-isopentane into the liquid nitrogen. The coverslip is carefully lifted vertically from the slide by prying one corner with a scapel; this is done while the preparation is immersed in liquid nitrogen. It is very important that the transfer of the coverslip into the fixative (3% glutaraldehyde in 0.1 M phosphate buffer, pH 7.2) is done as quickly as possible. The remainder of the schedule is routine: fix 30 min. in glutaraldehyde; wash in buffer 30 min.; postfix in 2% OsO_4 in 0.1 M phosphate buffer, pH 7.2; rapid dehydration in an alcohol series followed by propylene oxide (PO). The coverslip is removed from PO and the surface bearing the cells is quickly flooded with epoxy which has been mixed with PO (ca. 2 epoxy:1 PO). The coverlip is kept at room temperature overnight and then cured at 60°C for at least 24 hours.

The cured plastic with coverslip can now be cemented to a glass slide (Eastman 910 Adhesive; Eastman Chemical Products, Inc., Kingsport, Tenn.). The preparation is examined and the desired cells located. The coverslip is removed by placing the cemented preparation on a block of dry ice for ca. 10 min. and carefully prying the coverslip with a razor blade. In general, the coverslip will be removed in a few large fragments. The plastic may now be divided, while cemented to the slide, to recover the cells individually. It is technically difficult to separate more than 3-4 cells. Initial rough trimming is easily done at this time also. Desired portions of the plastic are removed from the slide and cemented to clear plastic pegs for final trimming and sectioning. It is possible to examine, using either a high power dissecting scope or a compound microscope, the plastic during sectioning in order to determine when one has sectioned the cell completely.

The technique can be used to recover intact, fixed cells for further histochemical studies, also.

References: Brinkley, B.R. and R. Bruce Nicklas. Ultrastructure of the meiotic spindle of grasshopper spermatocytes after chromosome micromanipulation. J. Cell Biol., 39 (2) part 2, 16A (1968). Mole-Bajer, J. and A. Bajer. Studies of selected endosperm cells with the light and electron microscope: The technique. La Cellule 67, 257 (1968).

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Kirschbaum, Werner F. Argentine Catholic University, Faculty of Agrarian Sciences, Buenos Aires, Argentina. Fast sexing of larvae.

A successful method for sexing a high number of Drosophila larvae and of other diptera consists in placing them in a drop of water between two slides and looking at them in this motionless condition with a stereoscopic microscope using transparent illumination. Several larvae can be placed in a row on each slide and the right position to inspect the gonads of each larvae can be accomplished by moving gently the upper slide to the sides.