Kirschbaum, W.F. and H.G. Fromme.
Comisión Nacional de Energía Atómica, Buenos Aires, Argentina. Method of obtaining sections for electron microscopy of squashed salivary gland chromosomes.

A method of obtaining sections for electron microscopy of squashed Drosophila melanogaster salivary gland chromosomes, that we consider to have the following advantages, was developed: 1) Practically with one squash of embedded polytene chromosomes enough material is obtained to study a problem and enough prepared chromosomes left to repeat or pursue further studies; 2) The possibility of selecting, by optical microscopy, the chromosomes or regions of chromosomes already embedded, that are to be studied by electron microscopy; 3) To obtain sections parallel to the chromosome axis of a whole polytene chromosome or a special region that contains several chromosomes.

The procedure is as follows: a) Squash of salivary gland chromosomes with lactic acid orcein using siliconed slide and a cover slip with a carbon film; b) Remove cover slip with liquid N, discard slide; c) 95% ethanol saturated with uranyl acetate for 10 min; d) Wash with 95% ethanol for 10 min; e) 100% ethanol for 10 min; f) 100% ethanol and propylene oxide (1:1) for 10 min; g) Propylene oxide for 10 min; h) Propylene oxide and Epon (1:1) for 10 min; i) 3 passages in liquid Epon for 2 min each; j) 12 hours in liquid Epon in a refrigerator at 4°C; k) Fix cover slip with Epon on a 2 mm thickness plate of Epon; l) Once the Epon is polymerized, remove cover slip with liquid N; m) Select desired chromosome or region of chromosomes with optical microscope and cut the chosen piece with a fine saw; n) Fix plate piece with chromosomes facing up to the block; good care has to be taken that the plane of the chromosomes is perpendicular to the block axis.

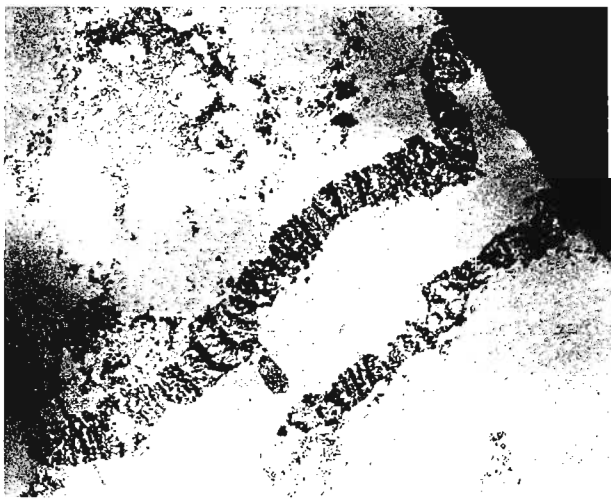

Figure 1. Section of a single whole U-shaped chromosome, which is partially covered by the grid; scanning electronmicrograph x 1540 magnification.

Figure 2. Detail of chromosome x 16000 magnification.

Good squashes and stained chromosomes are obtained using lacto-acetic orcein which makes it easy to choose the right ones already included in the Epon plate, with the optical microscope. It acts also as a good fixative and gives a good resolution to study the banding pattern of the polytene chromosomes. We found that a careful embedding of the chromosomes was very important and came to the conclusion that best sections for this type of study are those of around 90 mμ thickness.