

Stalker, H. D. Washington University, St. Louis, Missouri. Techniques for improving salivary chromosome preparations.

in lactic-acetic-orcein, and squashed on siliconed slides. The staining time is adjusted so as to give preparations that are a little too light to be studied with comfort. The preparations are then heated gently (placed on a black table top approximately 9" from a gooseneck lamp with a 100 watt bulb) for three to ten minutes. During the heating process the slides are inspected about once a minute. The heating causes the bands to darken and become thinner, thus increasing the contrast and improving the fine detail. This technique seems to improve all good squashes; it has little effect on poor ones. A second consideration in preparing satisfactory squashes is the time of day: many species produce satisfactory squashes in the morning, but not in the afternoon, or vice versa. Even closely related species may differ in this characteristic.

For many (possibly all) species, the fine banding detail can be greatly improved by controlled heating. Glands are dissected in 40 - 60% acetic acid (the concentration depending on the species), stained

Danieli, G. A. and E. Rodinò. University of Padua, Italy. A homogeneous medium for studies of labelled precursors incorporation in growing larvae.

A problem in metabolism studies approached by autoradiographic technique is the procedure used in labelled precursors administration. In small sized animals there is the possibility of microinjection, but the extreme accuracy

required by this procedure may lead to significant errors in final concentration of the isotope in the organism. Therefore many investigators, working on *Drosophila* salivary gland metabolism, choose incubation in vitro of isolated organs (Arnold, 1965; Plaut, 1966) or labelled precursors administration directly in the food (Taylor et al., 1955; McMaster et al. 1957, 1959, 1960; Woods et al., 1961). In the latter method, however, the normal food is scarcely suitable for experimental approach, because it is not homogeneous and hence leading to a nonrandom distribution (very important, in fact, in short term incubations) and a decrease of the culture synchronization.

In order to overcome these disadvantages we tried to obtain a medium as homogeneous as possible. We found that the most suitable and successful medium is a thick dead yeast suspension. In our experiments the labelled precursor was H^3 -Thymidine and was administered to larvae from a fairly uniform culture at different stages as follows:

In a heat killed yeast suspension (50 gr. in 100 ml.) the isotope (Schwarz-Biores. sp.act. 19 mC/mM) was dissolved to a final concentration 8.3 μ C/ml. This solution was mixed vigorously by magnetic stirring for 2 minutes at room temperature. In order to maintain exactly the same feeding conditions throughout the entire time of the experiment, an adequate number of containers were filled with the isotope solution and at once frozen on dry ice and stored at -30°C until used. The larvae (ten per point), collected from a synchronized culture, were thoroughly washed in Ephrussi's Ringer solution containing antibiotics and then incubated in 1 ml. of tritiated yeast suspension for 8 hours at 24°C . After incubation the larvae, washed in Ringer solution containing excess unlabelled Thymidine, were transferred to normal food (Bakker, 1962) for growth until the late third instar.

Autoradiography of squashed salivary chromosomes were made using Kodak NTB 2 bulk emulsion, exposed for 2 weeks, developed in Kodak D 19, fixed and then stained with acetic orcein or toluidine blue.

The figures 1 and 2 show two microphotographs of these preparations.

References: Arnold, G. 1965, *J. Morph.*, 116:65; Bakker, K. 1962, *Archiv. Néerl. Zool.* tome 14:200; McMaster-Kaye, R. J. 1957, *Bioch. Bioph. Cytol.*, 4:5; IDEM 1959 *ibidem*, 5:461; IDEM 1960 *ibidem*, 8:56; Plaut, W. et al. 1966, *J. Mol. Biol.*, 16:85; Taylor, J. H. et al. 1955, *Exptl. Cell Res.*, 9:460; Woods, P. S. et al. 1961, *Proc. Nat. Acad. Sci.*, 46:1486.