

prevent the cells being crushed properly.

Place slide, with cover glass well centered, on lower half of folded paper towel and drop upper half over the cover. Gentle pressure on top half, without side slip, removes excess of aceto-carmin, leaving only faint tinge of pink on slide and the glands partly spread and crushed. Complete the spreading and crushing, with slide on white plate on stage, by vigorous sweeps with side of needle tip, held nearly parallel to surface, holding corners of cover with fingers to avoid all side slippage. If too much fluid has been left in slide the spread chromosomes will later change their place rather than stick where they are spread. The thin film of albumin makes a soft bed which saves the chromosomes from being crushed, as would easily occur between bare slide and cover glass, and causes practically 100% adherence to the slide.

The fresh preparations are now "ripened" by storage in saturated 95% alcohol vapor over night. A large glass vessel 6" deep is lined on bottom and sides with several layers of filter or towel paper, 95% alcohol poured in until the paper is melted and a little excess liquid is left over on the bottom. The slides are stood on end around the sides and the whole covered tightly. This vapor method of replacement of aceto-carmin (or other liquid under a cover glass) by alcohol gives a slow and perfect dehydration with a minimum of shrinkage. Vapor condenses on the upper end of the slide and the displaced liquid seeps off at the bottom.

After ripening and dehydration (the material also sticks to slide better after ripening in alcohol vapor) the slides are immersed in 95% alcohol, where they may be left indefinitely. The covers may detach spontaneously; but they can be easily removed by placing the slide in a shallow Petrie dish (J.C. Li), covering with 95% alcohol and holding 2 rear corners of cover (against slippage) with fingers, while a flat thin point of bent needle is slipped like a wedge under opposite edge. The slide is left in the alcohol while the cover is cleaned of traces of tissue. The slide is drained briefly, laid on its back and 2 drips of Euparal (thinned slightly with thinner or with 100% alcohol) dropped on the glands. The cover is put on swiftly (to avoid drying and also condensation of moisture) and air bubbles avoided. The excess of thin Euparal is squeezed out by folded paper which absorbs it as it emerges (avoid slippage while pressing). After drying of slide, clean off excess of Euparal with cloth barely moistened in 95% alcohol. Slides continue to clear and improve, and are fully equal to balsam slides, without the time and expense and shrinkage involved in treatments with absolute and Xylol.

Schultz, J. Notes on methods for salivary chromosomes.

females which are transferred to fresh 1/2 pint culture-bottles daily. Several such transferred pairs provide the required numbers of uniformly well-developed larvae. The larvae should be

Larvae used for salivary gland preparations should optimally be from single

raised in a cool place, preferably at 19° cold room, and the culture medium should be enriched with yeast (sterilized) or extra live yeast (1/8-1/16 cake of Fleischmanns yeast) sprinkled on the food surface when the larvae are half grown. Absorbent paper should be provided for the adult larvae to emerge upon, and the easiest way to collect larvae is to pull out this paper and pick off the fully grown individuals.

Glands dissected at practically zero temperature and with the fixation started at that temperature seem to give sharper detail and less "capsulation" of the banding than material dissected and fixed at room temperature. The Ringers solution (or 0.73% Na Cl solution) and the aceto-carmin solution should first be chilled to near zero by standing on ice. The larvae also should have an ice treatment of five or more minutes before dissection. The dissection and first stages of fixation should be carried out on a depression slide held at low temperature (for details of procedure see notes by Bridges). After the fixation has proceeded for five or more minutes at the low temperature, the remainder of the fixation can be carried out at either room temperature or higher. With higher temperatures less time is needed for the fixation and staining to reach a suitable stage.

Mounting in Euperal, directly from 95% alcohol, has been found perfectly satisfactory if done rapidly in a dry atmosphere.

Bauer, Hans    Notes on permanent preparations of salivary gland chromosomes.

Perfect attachment of smeared cells and chromosomes to the mounting slide can be obtained by

use of a film of albumen on the slide. The albumen solution is made by mixing together 100 cc distilled water, 25 gm powdered egg albumen (Merck) and 0.5 gm thymol. After the mixture has stood several days and the undissolved albumen has settled, the top clear portion is decanted for use. A drop of albumen solution is spread evenly and thinly over the whole slide by scraping with the end of a second slide whose edge should be unchipped. The albumen film must be thoroughly dry before use! For use, the dried albuminized slide is put on a level place and three drops of aceto-carmin spread evenly over its entire surface. The stained glands are placed in this fluid film, covered with a cover glass (air bubbles must be avoided) and crushed by pressure.

The gradual replacement of the aceto-carmin by 95% alcohol can be made by the vapor method of Bridges (see above) or by putting the slide in a staining jar filled with alcohol only high enough to cover 1 or 2 mm of the lower edge of the cover glass. After half an hour the slides are ready to be transferred to another jar filled completely with 95% alcohol. Here the cover slip usually detaches itself after a short time; otherwise it can be removed by needles. Mounting is done according to the methods described above by Bridges.

In cases of too strong stainability of the cytoplasm by aceto-carmin, the Foulgon method is advisable (Foulgon, R.,