

raised in a cool place, preferably a 19° cold room, and the culture medium should be enriched with yeast (sterilized) or extra live yeast (1/8-1/16 cake of Fleischmann's 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 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 Euparal, 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.,

1926, Handbuch der biol. Arbeitsmethoden 5: 2,2 Hälfte. - Bauer, H., 1932, Zeits.f.Zellf.u.mikr.Anat. 15. - The receipts are to found in the last edition of Lee's Vademecum.) Slides are prepared by the aceto-carmin method to the stage of detached cover glasses; or slides already mounted in euparal can be soaked in 95% alcohol till the cover glasses detach. The slides are then transferred through 70% alcohol and distilled water into chrom-formol for postfixation (3 parts of 1% chromic acid and 1 part of formalin); duration 10-20 hours (overnight). Thereafter the slides are washed for 10 minutes in running water and, after transfer through distilled water and cold normal H Cl solution, hydrolyzed for 30-40 minutes in normal H Cl warmed to 60° C. on a water bath. After hydrolysis they are transferred through cold normal H Cl and distilled water into the fuchsin-sulphurous acid for 1-3 hours. After the staining the slides are washed in a large jar with tap water, moving them backward and forward several times (washing in SO<sub>2</sub>-water is not necessary); the side of the slide carrying the objects should always be turned in the direction of the movement. Then they are transferred through distilled water and 70% alcohol into 95% alcohol. There they should remain until the red color in the albumen layer, caused by the decomposition of the fuchsin-sulphurous acid, is completely removed. Then the slides are mounted in euparal, or, through absolute alcohol and xylol, in Canada balsam. Only the chromatic structures (containing thymus nucleic acid) are stained; the staining is not quite as intense as that by aceto-carmin and does not seem any sharper or less diffuse in the demonstration of the banding. During the postfixation the chromosomes, due to shrinkage, become thinner.

These methods are applicable also to ganglia, ovaries and testes.

Lawrence, Elizabeth Gay (Carnegie Institution of Washington, Baltimore, Md.) Note on the use of dioxan in making permanent aceto-carmin preparations.

At the suggestion of Mr. C. H. Miller of this laboratory, dioxan has been tested as a medium to replace alcohol and xylol in making permanent

smears. Only preliminary tests have been made so no detailed method has yet been worked out. The general procedure, however, seems simple and the preliminary results very satisfactory.

Cover slips are soaked off in dioxan, then the material is rinsed in dioxan for a few seconds and mounted directly in balsam. Dioxan is relatively inexpensive. It may be procured from the Carbide & Carbon Chemicals Corporation, Carbide & Carbon Building, 30 E. 42nd Street, New York City, and presumably from Grubler in Germany. Its use has been described in the following papers:

"The Use of Dioxan in the Embedding of Microscopic Objects," by Heinz Graupner and Arnold Weissberger, Zool. Instit. & Chem. Lab. of the Univ. of Leipzig; Zool. Anzeiger, Bd. 96:204-206, 1931.

"The Use of Solutions in Dioxan as Fixatives for Frozen Sections", by Heinz Graupner and Arnold Weissberger; Zool. Anzeiger, Bd. 102:39-44, 4 abb., 1933.