tained, either by sedimentation or filtration. No iron is added at any time.

(2) The glands are dissected out and stained in deep depression slides for not longer than fifteen to twenty minutes. If left for a longer period the chromosomes become fragile and cannot be well stretched.

(3) The glands are mounted on a microscope slide and washed with fresh aceto-carmine to remove any debris that may be present. They are then covered with a square coverslip and the excess fluid taken up with blotting paper.

(4) The chromosomes are then spread by pressing on the coverslip with a dissecting needly. The coverslip must not be allowed to slide or the nuclei will be rolled into dense useless masses. This is easily prevented by pressing firmly on one corner of the coverslip with the finger. This step is executed under a dissecting microscope so that each nucleus may be observed and adequately spread.

(5) A saturated solution of carmine in glycerine is then put around the coverslip and a piece of absorbent paper placed against one edge of the coverslip. The slide is then put away until the glycerine has displaced all the aceto-carmine under the coverslip, usually over night. The glycero-carmine solution is prepared by dissolving the carmine in warm glycerine and then filtering. If ordinary glycerine is used in this step, the preparation will fade after a few days.

(6) The slide is immersed in alcohol to remove the excess glycerine and then blotted. It is then sealed with balsam, gum mastic-paraffin or any other suitable seal.

By this technique it has been possible to mount three hundred glands in two days and have them all preserved in excellent condition for observation. After one month there has been no noticeable change in the chromosomes. Furthermore, it is possible by this technique to stretch the chromosomes a great deal without breaking them.

If it is desired to mount the glands in balsam, the slides may be allowed to stand with the glycerine for a day or two longer. The coverslip can then be readily slipped off, or pried off with a fine needle, or floated off in absolute alcohol. It is then cleared in clove oil and xylol and mounted in balsam. The loss of material by this technique is much less than with the method of removing the coverslip in aceto-carmine.

Bridges, Calvin B. Current method for permanent aceto-carmine smears. Trial of various modifications of the methods for making temporary and then permanent preparations of smeared cells from salivary glands and other tissues have been carried out by various workers here. The method is so uniformly reliable and yields permanents of such high quality that it is no longer customary to carry out even preliminary examinations on temporary mounts. Permanents are essential for continual rechecking of the banding in each case as new information or material raises questions.

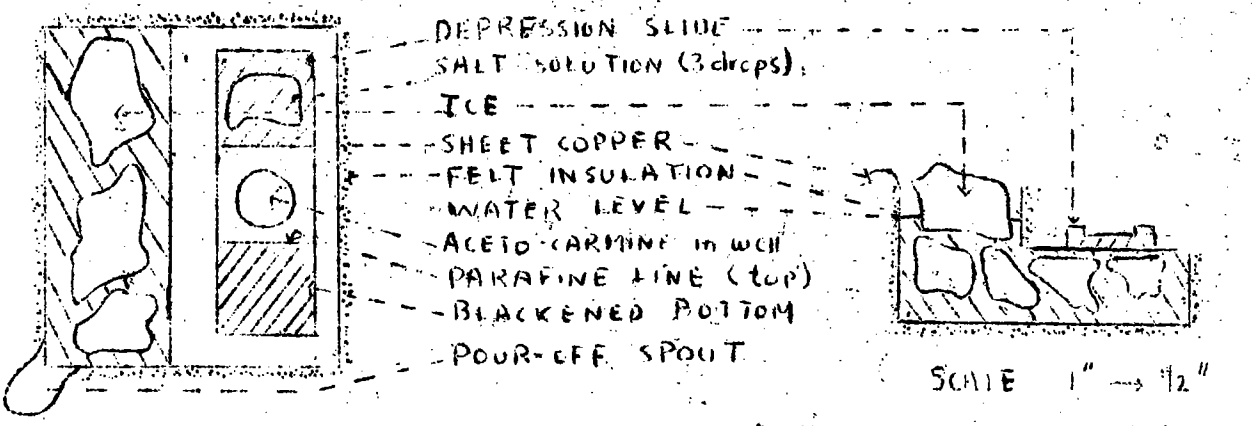
A prime condition of success is that the larvae be uniformly and thoroughly overfed with yeast and raised at a low temperature (see notes by J. Schultz). Fully adult but still unpupated larvae should be used, since degeneration of the cells starts immediately with pupation. Female larvae should be selected and males discarded, since in males the chromosomes, especially the X, are paler in stain and less turgid than in females. Best are females with one or two extra Y-chromosomes, where the extra "inert" material leads to plump easily-stained chromosomes. Attached-XY females are used as the source of an extra Y whenever it is otherwise desirable to make an outcross of a balanced autosomal stock (to get heterozygotes freed from the balancer). The giant attached-X females, formerly recommended, are no longer used in preference to other attached-X females, since the extra large chromosomes found in giant females are too resistant and elastic to stretch well in the smearing. Female larvae can be distinguished from males by their tiny colorless transparent spherical ovaries attached to the yellowish-opaque lateral fat-bodies between the third and fourth branches of the trachae, counting from the rear. Male larvae have large ellipsoidal testes each embedded in the fat body.

Dissection and fixation of the salivary glands at near-zero temperatures seems to give better detail and less capsulation of the banding (see notes by Schultz). For piercing the larvae and other materials, a large low-sided basin or tray is filled with cracked ice. In the ice are bedded salt cellars (black) for prechilling the larvae, bottles of physiological salt solution and of concentrated aceto-carmine and special depression slides for the dissection and fixing. The depressing slide (see figure below) is 1" x 3" x 1/4", with a 5/8" circular well, with vertical walls, 1/8" deep. The upper surface of the slide is finely ground. The end regions are marked off for dissecting spaces by lines drawn in melted paraffin to keep the salt solution from running into the well of the slide. The bottom of the slide, under these end regions, not under the well, is painted black for contrast to the larvae.

The larvae are put in the dry depressions of the chilled saltcellars for about five minutes before dissection. Because of the chilling they stop crawling about and are much easier to handle and dissect. For keeping the larvae and the liquids chilled during dissection and the first stages of fixing, a special insulated iced platform (see figure below) can be made of soldered sheet copper with felt covering, of a size to fit conveniently on the stage of a dissecting microscope.

The interior of the dissecting platform is filled with ice at the time the tray is filled. The platform is put on the dissecting microscope stage so that the light comes from the far side along the ledge. The well is filled nearly level full with the chilled aceto-carmine. (Aceto-carmine can be made by boiling for an hour 45% acetic acid with an excess of carmine powder (Coleman and Bell is good) in a flask with reflux condenser, and

decanting for use the clear liquid after cooling and thorough settling). On the ground glass surface at the far end of the slide are put three drops of salt solution for the dissecting bath.



In dissecting (at 15 diameters magnification) cut off anterior end of larva, as close behind mouth hooks as possible, by pressure with side of needle tip against non-slip ground glass surface, while holding larva with another needle. From cut-open end emerge first the pair of salivary glands and then other organs. The glands (transparent club-shaped bodies attached to each other by the short branched duct which led to the base of the mouth hooks) are cleared from the fat bodies and picked up together on the tip of a needle and immersed under the surface of the aceto-carmine in the well.

In case the glands break apart they can be made to stick together on the needle tip, turning this upside down and then immersing under the aceto-carmine for 30 seconds before scraping them off. Up to about 16 pairs of glands may be fixed together in one well. Avoid excessive iron (from the needles) in the early stages of fixation. After the required number are in the well, the slide may be set aside at room temperature or higher for continuing the fixation at a faster rate. Short fixations before the smearing are liable to result in pale chromosomes and distortion and tearing through adhesions of the still soft material of the strands. Fixation from 1 to 2 hours seems best. Longer than about 4 hours may lead to fragmentation of the chromosomes upon smearing. With long fixation refill the well as the acetic acid evaporates.

To make mounts, put albuminized slide (see notes of Baur) on white plate on stage of dissecting scope. Put 3 drops concentrated aceto-carmine on slide and spread evenly over albumin surface. Four pairs of glands may be put in a row on the slide and covered with a 22 x 40 mm. cover. Slides should not be over 1.20 mm. thick and covers not over .16 mm. Avoid bubles in lowering cover in place! The albuminized slides must have been protected against dust and lint while drying and during storage and the covers carefully cleaned, otherwise any particle would

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-carmine, 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-carmine (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 side 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. Larvae used for salivary gland preparations should optimally be from single 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