Cytological technique

Painter, T.S. Aceto-carmine technique for salivary chromosomes. The writer has worked almost exclusively with temporary aceto-carmine mounts and the commments given below apply only to that type of preparation.

Much difficulty has been experienced in getting a good iron aceto-carmine stain. Powdered carmines, from many different sources, have been tried including the certified product of Coleman and Bell, Grübler's Karmin Rubrum Opticum, and Carmine 40 from several sources. The trouble has been either that the stain would not take or was not selective enough or else it would not keep, at ordinary room temperature here in Texas for more than a few days without going bad. At present we are using some uncertified carmine manufactured by Coleman and Bell and are obtaining a satisfactory stain in the following way. In one flask an excess of carmine is simmered, under a reflex condenser, in 40 per cent acetic acid, for an hour or two, when it is cooled and filtered. In another flask carmine is boiled in 60 per cent acetic acid similarly, and after cooling is filtered. A trace of ferric acetate is added to both stock solutions. From time to time, as needed, the two stains are mixed in equal proportions. We are unable to explain why this procedure gives a good stain but it works.

The salivary glands are dissected out in Ringer's solution (cold-blooded or frog formula) and transferred to a clean slide with a pipette. The Ringer's is quickly removed with a pipette and iron-aceto-carmine is flowed over the glands from one side. After a few moments the first stain is removed and fresh stain is added in considerable excess. The slide is put to one side and allowed to stand until a little of the carmine begins to precipitate around the edge, a matter of 15 to 25 minutes depending on atmospheric conditions. A cover slip is now placed over the glands, and the excess stain removed with a pipette and filter paper. Being careful not to let the coverslip move, the preparation is next blotted with a good deal of pressure, a process which usually frees the individual nuclei from the surrounding cytoplasm. Under a dissecting binocular, the individual nuclei are crushed with a blunt needle, by the pressure applied locally to the cover slip, and then after blotting the slide once more to remove all traces of the stain the coverslip is sealed with vaseline or melted paraffin.

The type of light filter used for the examination of preparations is the BG 7 optical glass filter put out by Zeiss.

Marshak, A. A rapid method for making permanent mounts of Drosophila salivary gland chromosomes. (1) A saturated solution of aceto-carmine is prepared by boiling carmine in a 45 per cent aqueous solution of glacial acetic acid for several hours. A reflux condenser is attached to the flask containing the solution in order to prevent changes in concentration by evaporation. A clear dark red solution is ob-

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.