

satisfactory preparations could be obtained with most of the other stains, but the best results were from the use of a 1 per cent. aqueous solution of crystal violet, 84 per cent. dye content. The staining was carried out in the cold for two minutes. The slide was then washed with 20 per cent. copper sulfate in the usual way and blotted dry. Better differentiation was obtained by this procedure than by any other method tested. An increase of the staining time did not improve the results obtained.

The procedure given above has been tried out in class with practically no failures, a condition which rarely prevailed with the original method of Hiss.

In view of the fact that the method here given does not require steaming in order to secure satisfactory results in a short period of time, it is felt that it is to some extent an improvement over the earlier method. An added advantage is to be found in the fact that the staining solution is the same as the primary stain of the Kopeloff and Beerman modification of the Gram stain, this being one of the methods recommended in the "Manual of Methods" of the Society of American Bacteriologists.²

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PREPARATION OF BEE SLIDES

THE following combination of methods has been found very satisfactory for clearing and mounting the chitinous skeletons of insects. It is particularly adaptable to the preparation of the head and mouth parts, the legs, or total mounts of bees, when they are to be used for the gross study of the skeletal structures.

When preparing mounts of each of the three types of legs and the head, the desired number of each are removed and each group tied up in small cheesecloth bags so that they may be handled more easily during the first part of the process.

The bags are placed in a small porcelain dish and covered with a solution of 20 per cent. potassium hydroxide, and boiled for 15 to 30 minutes.¹ As the water evaporates more of the solution is added so that the concentration is increased during the boiling. Remove the bags and wash in running water for 12 hours. Pressing the bags gently and then releasing them several times at two or three hour intervals assists greatly in thoroughly washing out the cavities in the skeletons.

When washed, the parts are bleached to the shade best suited for study. For bleaching Mayer's Chlo-

rine Method² is used. For the bee preparations the following proportions were found to give best results: Concentrated HCl 3 cc, 70 per cent. alcohol 10 cc. This is put in a small vial and to it is added potassium chlorate, a few crystals at a time. The parts to be bleached are put in the solution and left until the desired shade is obtained. More of the potassium chlorate is added each time the liberation of chlorine ceases. The parts can be bleached to a creamy white and stained, but it has been found quite as satisfactory to bleach until the color is a light tan, no staining being necessary. It is advisable to remove the parts from the bags and treat a few at a time so that the amount of bleaching can be regulated.

When the parts are removed from the bleach they must be handled with care until after hardening. Wash in four or five changes of distilled water for 30 minutes. The heads are now placed on a slide in a drop of water and the mouth parts arranged under a lens. A second slide is placed on top and the two pressed together to flatten the head. See that the mouth parts are not disarranged, and then put a rubber band or clip around both slides and place in 80 per cent. alcohol for one hour. Drain and put in 95 per cent. for one hour. The parts are now hardened in position and can be removed from the slides and placed in absolute alcohol. The other parts which do not need flattening are carried through the same procedure all together. Use two changes of absolute, one hour and two hours, then clear in clove oil for 24 hours. Mount in balsam.

Total bees can be fixed in the same manner and suitably arranged before hardening. By careful pressing the total bee or bee's head can be flattened so that it is no thicker than a No. 1 cover-glass.

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CHEMICAL TREATMENTS TO SHORTEN THE REST PERIOD OF TREE SEEDS

IN the past three years it has been demonstrated that the dormant seeds of sugar maple, Norway maple and the acorns of black oak and red oak can be stimulated into germination by treatments with solutions of thiourea and ethylene chlorhydrin and by the vapors of ethylene chlorhydrin. While full details of these investigations will be published later it is thought desirable to make available the methods which have given the best results to date.

With sugar maple and Norway maple seeds, immersion of the seeds in a 3 per cent. solution of thiourea for 1 minute proved to be the most successful treatment. The solution was drained off the seeds and the bottle stoppered and the seeds allowed to

² "Manual of Methods for Pure Culture Study of Bacteria," Society of American Bacteriologists, Geneva, 1928.

¹ Mayer, "Chlorine Method." McClung, "Microscopical Technique," p. 478, and Guyer, 1st ed. p. 45.

² KOH for softening. Kingsbury and Johannsen, "Histological Technique," p. 130, par. 313. Lee, "Vade Mecum," 7th ed. par. 551.