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 Chlorine Method[2] 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.

stand for a day before planting. Immersing the seeds in 3 and 6 per cent. solutions of ethylene chlorhydrin (made by mixing 6 and 12 milliliters, respectively, of ethylene chlorhydrin, technical, with 194 and 188 milliliters of water) also was effective. The seeds were immersed for a minute, the solution poured off of them and the bottle stoppered for twenty-four hours before planting.

With black and red oak acorns consistently good results have been obtained by subjecting 50 or 100 acorns in a liter wide-mouth bottle to the vapors of four milliliters of ethylene chlorhydrin, technical, for twenty-four hours. The chemical was placed on a five-inch square of cheesecloth suspended from the stopper. This treatment initiated germination of acorns gathered in October within four weeks and within ten weeks more than 70 per cent. had germinated while the acorns not treated showed 1 per cent. or no germination. Immersion of these acorns in a 3 per cent. solution of thiourea for 15 minutes was effective but slower than the ethylene chlorhydrin vapor treatment. Germination in acorns treated with thiourea solution did not start until the seventh to tenth week after treatment.

It is not claimed that the procedures described are the best methods of hastening dormant tree seeds into germination with chemicals since much more work needs to be done upon the most effective concentrations of the chemicals and the most effective time periods of treatment but these chemicals do give a new mode of attack upon dormancy in seeds. The results reported are based on tests made with more than 9,000 maple seeds and 5,000 acorns.

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SPECIAL ARTICLES

THE BIOLOGICAL EFFECT OF HIGH VOLTAGE X-RAYS

FOR many years radiologists have debated the question whether equal doses of X-rays of different wave-lengths produce the same or different quantitative biological effects, a problem of practical importance in therapy. The chief obstacles in deciding the matter were the lack of a standard unit of X-ray intensity, the lack of adequate measuring apparatus and the highly variable character of the biological materials which were used in making the tests. The adoption of a standard unit, the Roentgen, now permits an accurate definition of dosage, where previously none was possible. The question now is this: When a definite number of r units, measured by an air ionization chamber, is delivered to a suitable material, will the amount of effect which is produced vary with the wave-length of the beam, that is, with the voltage.

An almost ideal biological material consists of the eggs of the wild fruit fly, Drosophila. These eggs when freshly laid are comparatively sensitive, and are remarkably uniform in response. Different strains of wild flies are apparently equally radiosensitive. Therefore one may make experiments with them anywhere with the assurance that his results will be comparable with those obtained by other workers at other places.

A long series of tests[1] with carefully measured beams produced at constant potentials shows that the mortality curve has an asymmetrical sigmoid shape. These tests were made with different wave-lengths, (0.20, 0.50 and 0.70 A. U.), that is, with hard, medium and soft X-rays. In each instance the results showed that the quality of the beam has no effect on the mortality rate; it is the intensity which is the deciding factor. Furthermore, the course of the curve is the same in each case. From such a curve we can determine how many r units are required to kill any percentage of eggs in a sample.

The method may be reversed.[2] By knowing how long a dose is needed to kill, say 50 per cent. of the eggs, we can estimate the intensity with considerable accuracy. Half the eggs are killed by 180 r units. If 10 minutes are required to kill this proportion, the intensity was 18 r/min.

The wave-lengths employed lay within the range of ordinary radiotherapeutic practice, that is, they were produced at potentials of 50 to 180 KV. But now that machines capable of running at much higher voltages are being developed it is necessary to determine whether a definite dose of these very short waves is biologically equivalent to an equal dose of longer waves. We have recently made this test at the California Institute of Technology where a tube which operates at 550 KV is in use.[3]

In these experiments the X-rays were filtered through 6 mm of steel, the emergent beam having an effective wave-length of 0.04 A. U. Ionization tests showed that at the point where the eggs were exposed the intensity was 15 r/min. This includes a small amount of scatter from the walls of the room, amounting to perhaps 1 r/min. The eggs were given 120, 180 and 240 units. From the curve we should expect the percentages of eggs killed to be 22, 50 and 67 per cent. The actual results, which are averages of many tests

[1] Packard, C., *J. Cancer Res.*, 1927, 11, 1.

[2] Packard, C., *J. Cancer Res.*, 1927, 11, 282.

[3] Lauritsen, C. C. and B. Cassen, *Phys. Rev.*, 1930, 36, 988.