

The crucial test would, of course, consist in the determination of color-temperatures of groups of stars in open clusters, where all the members are known to be at the same distance. If the hypothesis of Miss Payne is correct, the more luminous stars should show more pronounced reddening than the less luminous stars of the same spectral types. If Trumpler is right, the amount of reddening should be the same for all stars. Miss Payne mentions in this connection the work of Balanovsky and states that there is some evidence in his results favoring her point of view, but she states that "quantitative estimates of the temperature can not be made from his discussion."

Incidentally it may be noted that there is a correlation between reddening of B-type stars and intensity of the interstellar calcium lines, and, contrary to the statement of Miss Payne's book on page 120, there is a very pronounced concentration of red B-type stars in the very region where some of the strongest interstellar lines are observed (in the constellation Cepheus and in adjoining regions). But this does not mean that the reddening may be caused by the calcium itself: the amount of matter, in the form of ionized calcium, in the line of sight between the observer and some of the most distant stars is not more than is contained in one cubic centimeter of air at normal pressure and temperature. It is clear that so small an amount of matter could never produce the enormous amount of reddening observed by Trumpler and by others.

Whatever the outcome of this extremely interesting problem may be, we are left with the unsatisfactory state of our present temperature scale. Future work will have to deal with this side of astrophysics, and will have to devise new methods by which this scale may be ascertained. If the reddening should turn out to be due to interstellar absorption, then there would be no reason to question the radiation laws. The nearer stars would give us more nearly correct temperatures than the more distant stars, and an extrapolation should enable us to get the energy-distribu-

tion for zero distance. If, however, space reddening is not present, the matter would be more complicated. Perhaps the study of the Stark effect in stellar spectra may help to establish another function of temperature and pressure. It should then be possible from this and from the ionization formula to evaluate pressure and temperature independently. Even now it is possible to say, from the Stark effect alone, that pressures of the order of  $10^{-13}$  atm. are not possible, and that consequently the energy-distributions can not be taken at their face value. An independent determination of the temperature scale could perhaps be obtained by a method similar to the one used by Adams and Russell in 1928.

Speaking of the ionic Stark effect, it is of interest to note that Miss Payne finds evidence of its existence, at least in spectral class A. The question might justly be asked: if ionic Stark effect is present, is it permissible to apply the Unsöld formula to the evaluation of the numbers of atoms? Strictly speaking the absorption coefficient in a line affected by Stark effect is not that given by the classical theory (as was pointed out by Unsöld) and the formulae which may be used for lines produced by radiation damping are not applicable. But it is fairly safe to say that the discrepancy will not be a serious one and that the numbers obtained will at least be comparable to those that would apply in the case of no Stark effect.

The last part, "Analysis of Stellar Atmospheres," gives a short summary of the observational results described in the preceding chapters and discusses them, rather briefly, in the light of the "generalized" ionization equations of Milne.

There are many useful tables in the book. A complete list of O stars, a catalogue of stars showing the so-called c-characteristic in their spectra, and a list of Cepheid variables add greatly to the value of the volume.

O. STRUVE

YERKES OBSERVATORY,  
WILLIAMS BAY, WISCONSIN

## SCIENTIFIC APPARATUS AND LABORATORY METHODS

### A NOTE ON CAPSULE STAINING

SOME difficulty in securing good results is frequently encountered in the routine laboratory exercise on staining capsules by the well-known method of Hiss.<sup>1</sup> In an effort to make this procedure more adaptable to class use several variations have been tried and a series of dyes compared by various procedures. The dyes tested were as follows: crystal vio-

<sup>1</sup> P. H. Hiss, Jr., "A Contribution to the Physiological Differentiation of *Pneumococcus* and *Streptococcus*, and to Methods of Staining Capsules," *Jour. Exper. Med.*, 6, 317, 1905.

let, 84 per cent., crystal violet, 92 per cent., crystal violet (dye content not stated), methyl green, gentian violet, methyl violet 1 B, methyl violet 2 B (two brands) and aniline violet. The organisms were *pneumococcus* from the peritoneal cavity of an infected mouse and *Klebsiella pneumoniae* from serum agar slants. Thin smears were made without the use of a diluent; the films were allowed to dry in the air and stained without fixation.

Methyl green was not found to be a satisfactory stain by any of the procedures tested. More or less

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.<sup>2</sup>

E. E. ANTHONY, JR.

DEPARTMENT OF BACTERIOLOGY,  
UNIVERSITY OF TEXAS

#### 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.<sup>1</sup> 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 Method<sup>2</sup> 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.

L. S. ROWELL

UNIVERSITY OF VERMONT

#### 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

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

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

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