Prof. Charles W. Davis, of Jackson, Tenn., and Prof. George R. Mayfield, of Nashville, Tenn.

The Knoxville Scientific Club entertained the members of the academy at noon on Friday at the Andrew Johnson Hotel. At the dinner on Friday evening, President J. D. Hoskins, of the University of Tennessee, welcomed the members of the academy and Dr. H. A. Morgan of The Tennessee Valley Authority, made an address.

On Saturday afternoon there was an excursion to Norris and the Norris Dam. Sunday morning there were field trips to Mount LeConte and to the bird sanctuary near Knoxville. The Indiana Academy of Science was represented at the meeting by Dr. Stanley A. Cain, professor of botany in the University of Indiana, and the Virginia Academy of Science by Dr. Arthur Bevan, state geologist of Virginia. Dr. Cain read a paper on "Beech-Maple Forests"; Dr. Bevan, a paper on "Recent Work on the Stratigraphy of the Appalachian Valley of Virginia."

JOHN T. MCGILL,
Secretary

SCIENTIFIC APPARATUS AND LABORATORY METHODS

FISHING COLONIES FROM A GELATIN FILM CULTURE

CERTAIN kinds of soil bacteria form in agar or gelatin plate culture very minute punctiform surface colonies which can hardly be discerned by the naked eye. Such colonies can easily be picked out under the microscope from a gelatin film culture, which can be prepared as follows: Test tubes containing about 1.5 cc of melted gelatin are inoculated and, after mixing contents, are once or twice rotated in an oblique position under the cold water tap so that only a thin layer of gelatin coats about two thirds of the inner surface of the test tube, while the rest of the gelatin is allowed to solidify into a slope. After twenty-four to forty-eight hours of incubation at room temperature the colonies can be examined and picked off under the microscope.

To prevent melting of the gelatin a simple cooling apparatus can be made in the following manner: Rubber tubing of about one-inch diameter is filled with cold water, closed on both ends with rubber stoppers, bent in U-shape, bound with two rubber bands and fixed on the microscope stage by means of two other rubber bands; between the arms of the tubing is placed the test tube containing the gelatin film culture. The tubing holds the test tube so firmly that the left hand is left free during the fishing and transplanting of the colonies.

PROCEDURE

Select by means of the microscope a colony of the gelatin film. Take out the cotton plug and put it into a sterile Petri dish. Flame the neck of the test tube. Put into it a sterile platinum wire, the end of which is bent into a small hook. Take care not to touch the wall of the test tube. Push the wire in until its end shades the optical field of the objective. Approach the wire carefully, while looking further through the microscope to the selected colony. After touching it remove the wire carefully and leave its end inside the neck of the test tube, about 2 cm from the top. Take with the left hand a test tube containing a medium to be inoculated; approach it to the right hand, which holds the wire; take off the plug with the little and next finger taking care that during that operation the end of the wire in the neck of the test tube does not touch its wall. Take off the wire and put its end immediately into the test tube and inoculate the medium while approaching the test tube to the flame. Sterilize the neck of the test tube and plug it. Select another colony and transfer it in the same way.

If solid medium is used for inoculation of the picked-out colonies, several of them may be transferred into the same test tube or into a Petri dish.

In this way one can transfer safely hundreds of colonies without any danger of contamination.

Finally take the cotton plug from the Petri dish, flame it and plug the test tube with the original culture and save it if necessary.

The above described gelatin film culture is a modification of Esmarch's "Roll culture" method[1] which has become obsolete in modern bacteriological technique chiefly on account of the low melting point of gelatin, notwithstanding that it is a much cleaner technique than a plate culture.

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"USE OF SODIUM DIETHYLDITHIOCARBAMATE IN THE DETERMINATION OF MINUTE AMOUNTS OF COPPER"

FOLLOWING the suggestion of Callan and Henderson[1] that the "carbamate reagent" (sodium diethyldithiocarbamate) could be successfully used in the determination of minute amounts of copper colorimetrically, they and other workers have been considerably troubled in certain instances by turbidities developing in the solution after the addition of the reagent. This

[1] *Ztschr. f. Hygiene*, I: 293, 1886.
[1] Callan and Henderson, *Analyst*, 54: 650, 1929.

made the subsequent procedure of matching the colors difficult and often decidedly unreliable. Thus, an otherwise delicate and accurate method of analysis became under certain circumstances, wholly unreliable.

In some recent work in this laboratory,[2,3] the use of the reagent was often accompanied by pronounced turbidities, and in instances involving relatively large amounts of copper, some actual precipitation resulted. An attempt was made, therefore, to remedy this situation. Considering that the turbidity and precipitation were the result of the coagulation of a colloid, it was at once suggested that some suitable protective agent might solve the problem. With this in view, gum tragacanth and gelatin were used and found to solve the difficulty. Both these colloids were equally effective and satisfactory. Having found a way out of the situation, no other colloids were studied. Undoubtedly there are others quite as suitable for the purpose.

The following outline is offered as furnishing conditions under which satisfactory results may be obtained as indicated by the experience of the writers with the modified method. When the final volume of the carbamate mixture is somewhat less than five cc there is sufficient volume for matching colors in a microcolorimeter, or block comparator. In the latter, small calibrated fermentation tubes or Wassermann tubes are useful. The various proportions used were as follows: 1 to 2 cc of the unknown copper solution; 1 cc of the gelatin or gum tragacanth solution, freshly filtered; 1 to 2 cc of copper free water, depending upon the volume of the unknown used; and 0.6 cc of the carbamate reagent (conc. 0.1 per cent.). The total volume is thus kept at 4.6 cc.

When graduated pipettes are used in the measurements, the errors obtained in matching a series of ten standards (0.01–0.10 mg) against a duplicate set of standards were found to lie between 0.0001 and 0.0020 mg of copper. If a microburette is used with a similar set of standards, the errors between the observed and calculated values lie between 0.0001 and 0.0010 mg of copper. The accuracy may be improved by selecting the range of standards lying between 0.01–0.04 mg, where the errors were found to be less, namely, 0.0000 to 0.0006 mg of copper.

A preliminary determination on the unknown will show the dilution necessary to make it fall within the more accurate range, *i.e.,* 0.005 to 0.04 mg of copper in one cc of the solution.

The reduction in the amount of the unknown, as described above, makes it possible to begin with an amount of original material containing from 0.05 to 0.40 mg of copper and subsequently dilute the volume of the unknown to 10 cc. This volume is ample for making from four to nine determinations, depending upon the copper concentration.

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SPECIAL ARTICLES

THE THIRD MAJOR MECHANICAL FACTOR IN THE CIRCULATION OF THE BLOOD[1]

NEARLY 25 years ago during studies on the heart and the circulation of the blood Henderson[2] was led to the opinion that besides the heart and the vasomotor nervous control of the blood vessels there must be a third factor in the circulation. It is the factor that sends the blood back to the heart through the veins. Without it the blood would stagnate in the tissues instead of returning to the heart. To the factor that insures the venous return to the heart he gave the name of the "Venopressor Mechanism."

In spite of a vast deal of work no one has succeeded in defining just what the venopressor mechanism is. It is important to know: for it is the failure of this third mechanical factor in the circulation, rather than that of either the heart or vasomotor system, that causes the weakened circulation of the blood following illness and the extreme depression of the circulation in surgical shock.

There have been many attempts to explain the variations of the venous return and their regulation by contraction or relaxation of the veins. The vasomotor nervous system influences veins as well as arteries. But no explanation of the venous return under vasomotor regulation that is mechanically satisfying has been developed. Experimentally it was found by Henderson and Harvey[3] that, if the entire vasomotor mechanism is stimulated by an injection of adrenalin, both arterial and venous pressures rise. But if the injection of this vasomotor stimulant is continued, only the arterial pressure is maintained; venous pressure falls again to its former level. Clinically also adrenalin fails to restore a de-

[2] Moseley and Rohwer, "The Determination of Minute Amounts of Copper," unpublished thesis, 1933, Tulane University.

[3] Moore and Moseley, "Examination of Oyster Liquors for Copper," unpublished report, 1933, Tulane University.

[1] Read before the National Academy of Sciences on April 24.

[2] Y. Henderson, *Am. Jour. Physiol.*, 27: 152, 1910.

[3] Y. Henderson and S. C. Harvey, *Am. Jour. Physiol.*, 46: 533, 1918.