Professor Osborn. Founded less than four decades ago, the department has risen in that space of time to an unchallenged position of leadership in the field, while throughout the country there are few paleontologists who have not at some time or other been connected with this institution, few museums which have not been greatly influenced by the example set by the American Museum. The present monograph is a lasting monument to Professor Osborn's work in paleontology; but a still more enduring testimony to his labors for paleontology will be the American Museum's work and the inspiration it will continue to give to workers in the field of vertebrate history.

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# SCIENTIFIC APPARATUS AND LABORATORY METHODS

## A SIMPLE METHOD FOR THE GERMINATION OF OOSPORES OF SCLEROSPORA GRAMINICOLA

THE germination of oospores of *Selerospora graminicola* has been a subject of interest since the time of Schroeter.[1] However, no one except Magnus[1] has succeeded in germinating oospores, prior to the writer's recent investigation.[2,3] In further studies in the department of plant pathology, University of Nebraska, a simple method for the successful germination of oospores has been devised. Briefly, the method consists of placing a layer of moistened cotton in the two parts of a Petri dish. Then a small piece of moist filter-paper on which small amounts of oospore powder are placed is put upon the surface of the moist cotton in such a way that the filter-paper will partly, but not entirely, touch the cotton. Both the cotton and filter-paper must be drained of excess moisture before the oospores are added to the dish. It is essential that the space between the two layers of cotton in the dish be about one half the height of the dish. Small blocks of 2 per cent. agar-agar, on which the oospores are scattered over the surface just as the agar is hardening, can be substituted for the filter-paper. One difficulty encountered when moist filter-paper is used is that the oospores on the periphery of the mass germinate earlier and better than those in the mass.

The time required for germination is markedly different at different temperatures. For instance, the time required for germinating at 35° C. is 22 to 40 hours; at 30° C., 24 to 45 hours; at 25° C., 30 to 48 hours; at 20° C., 42 to 60 hours; at 15° C., three to four and one half days, and at 10° C., nine to ten days. The percentage of germination, of course, varies with the temperature and also to a great extent with the source and age of the oospores. Therefore, it is advisable that oospores from different sources and ages be tested. Germination has been obtained within a range of 10° to 35° C. The optimum temperature appears to be near 20° C., although in previous experiments a higher optimum temperature over a short period of time was reported.[3]

[1] J. Schroeter, *Hedwigia*, 18: 83–87, 1879.
[2] M. Hiura, *Agriculture and Horticulture* (Japan), 4: 11–20, 1929.
[3] M. Hiura, *Jour. Plant Protect.* (Japan), 16, 5 pp., 1929.

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## A PRACTICAL FLAGELLA AND CAPSULE STAIN FOR BACTERIA

THE following method of flagella and capsule staining is offered as a contribution both to the teacher of bacteriology and to the technician in the laboratory. It is rapid, simple and dependable. It has been especially designed for staining *Bacillus proteus vulgaris* and *Bacillus subtilis*, common soil species, and the various members of the colon-typhoid group. The procedure is as follows.

(1) Make a thin smear of 15 to 24 hour agar growth of bacteria in a loopful of water on a clean slide. Air-dry. Do not heat. (2) Cover with mordant (5 per cent. tannic acid, 3 parts; 10 per cent. ferric chloride, 1 part) for two minutes. (3) Put seven drops of mordant in a small receptacle and add 1 drop of Ziehl-Neelsen carbol fuchsin stain. Mix. Add 1 drop of concentrated hydrochloric acid. Mix. Add 1 drop of concentrated formaldehyde. Mix. (4) Pour off mordant from slide and cover smear with the mixture prepared in (3). Apply seven minutes. (5) Wash smear in running water. (6) Cover with Ziehl-Neelsen carbol fuchsin stain (Basic fuchsin, 10 grams; ethyl alcohol, 95 per cent., 100 cc; phenol, 5 per cent. aqueous, 1000 cc) and *gently* steam for one half minute. (7) Remove stain with running water. (8) Blot and examine.

The following precautions are in order.

(*a*) Take only a minute portion of the agar growth. Do not use semidry agar. For best results add the agar growth to a drop of water on a slide, stir, and let stand for five minutes, permitting individual bacteria to become detached from the agar mass; then spread loopful on another slide and work with this second slide. (*b*) The mordant will keep indefinitely and so can be prepared in quantity. The mixture

prepared in (3) should be used fresh. (*c*) Filtered mordant and stain yield better preparations than unfiltered materials. (*d*) A small variation in ferric chloride content of mordant affects the depth of color of the flagella. (*e*) If a tube containing 2 cc of water be heavily inoculated with agar growth, it will supply hundreds of flagella smears over a period of two days.

The flagella stain described is a capsule stain as well. It stains the capsules of such organisms as *Diplococcus pneumoniae, Streptococcus fecalis* and Friedlander's bacillus when these are grown in broth.

It also stains the capsules of pneumococci recovered from the peritoneal exudate of white mice. The following procedure is recommended for staining the exudate. (1) Spread a loopful of the exudate in a loopful of water on slide. Undiluted exudate may be used, omitting the water. (2) Apply mordant described in (2) for ten seconds. (3) Wash in running water. (4) Apply cold diluted carbol fuchsin stain for ten seconds. (5) Wash with water, blot and examine.

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# SPECIAL ARTICLES

## AN ATTEMPT TO PRODUCE MUTATIONS BY THE USE OF ELECTRICITY

THE calculations made recently by Muller and Mott-Smith[1] indicate that high frequency radiations are not the only cause of mutations. It is, therefore, desirable that a further search be made for other causes. On account of its wide distribution in nature, its wave properties and the fact that it travels at enormous speeds, electricity, and especially high frequency electricity, offers a good field for investigation in this connection.

Two tests have been conducted at the Agricultural and Mechanical College of Texas to determine whether or not mutations can be produced with electricity. The organism used in these experiments was *Drosophila melanogaster*. The well-known C1B method of Muller was adopted. It offers an excellent technique for studying any new agency as a possible causative factor in the production of mutations.

In the first experiment, which was conducted in 1928, the flies were treated in a field between two concentric copper cylinders. It was found necessary to cover one end of the opening with cheesecloth and to pass a strong current of air through the space between the cylinders to remove the gases produced by the electricity. Otherwise the flies were killed by the gases. The peak voltage was 33,000 volts at 60 cycles, giving a voltage gradient from 25,000 volts per cm at the surface of the inner cylinder to 7,000 volts per cm at the inner surface of the outer cylinder. Treatments for various lengths of time from one minute to thirty minutes were given.

The treatment had very obvious immediate effects on the flies. Some were killed. Those which were not killed were so affected that nearly all lost control of themselves. The legs usually became tangled. A fly so affected would lie on its side apparently trying to untangle its legs. Some of the flies recovered in a few minutes and became normal in their actions. Others required as long as twenty-four hours in which to recover their equilibrium. Still others died without ever becoming normal again. Some of those which did recover were sterile.

A total of 172 daughters of treated males were mated. Not a single case of a lethal mutation was observed.

The progeny of these females, that is, the $F_2$ generation from the treated flies, was examined in detail for visible effects. A white-eyed female was found in one of the cultures. This was not a contamination, because this fly was gray whereas the only stock of white-eyed flies in the laboratory at that time was yellow. Several peculiar variations in wing size and shape were noted. An example is the blister wing occurring as the left wing of one female. This wing stood out from the body, had six veins instead of the normal four and had a blistered or bubble-like area covering about one sixth of the wing.

These results were not conclusive in either direction. Enough effects were observed, however, to warrant the repetition of the experiment on a larger scale.

This was done in the spring of 1930. The adult males were treated this time in an electrostatic field of a potential equal to the breaking-down point of air, or 30,000 volts per cm, a total of 225,000 volts at a frequency of an oscillating current of 1,225,000 cycles per second. Care was taken to prevent the current from breaking over.

The flies were held in the field confined in small cheesecloth bags. An attempt was made to hold the flies in gelatin capsules while treating them. However, the current was observed to go around the capsule, hence the adoption of the cheesecloth bags.

One minute was the longest time it was found practical to expose the flies in this field. This is the length

[1] H. J. Muller and L. M. Mott-Smith, *Proc. Nat. Acad. Sci.*, 16: 277–285, 1930.