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.

of treatment that was used. This treatment killed half the flies exposed to it and rendered still others useless for breeding.

Sixty-nine fertile matings were made with treated males. Ten C1B daughters from each male were mated in individual cultures, making 690 matings producing 100,000 flies which were observed for lethal mutations. In no case where large numbers of progeny were produced were any lethal mutations observed. Three matings showed no males, but each of these produced only two or three females, hence showed nothing significant.

Thus far the results are of such a nature as to indicate that very probably mutations can not be produced by the use of electricity, at least of the particular kinds used in these experiments.

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THE FECUNDITY OF THE OYSTER[1]

IT is a well-known fact that many marine invertebrates, especially those that discharge the eggs into the water where fertilization outside of the organism occurs, produce large numbers of sex cells during a spawning season. The estimation of the total number of eggs developed in a single female is of certain scientific interest, but unfortunately it presents considerable difficulties. In the case of the oyster, which is known to be extremely prolific, the attempts to determine the number of eggs produced by one adult female were made by Möbius[2] in 1883 and Brooks[3] in 1880. Möbius's method consisted in weighing first the whole mass of the embryos which were scraped by means of a small brush from the gills of the female, then in weighing and counting the number of embryos in a small portion of it. He estimated that the average number of embryos in each of five full-grown *Ostrea edulis* from Schleswig-Holstein was 1,012,955. This figure is less than that given by Eyton,[4] whose estimate was 1,800,000. Brooks estimated the number of eggs in the American oyster, *Ostrea virginica*, by determining the total volume of eggs washed out of the ovary and by measuring the dimensions of eggs. He arrived at the conclusion that an oyster of average size developed more than 9,000,000 eggs. An unusually large oyster, according to his computation, would possibly produce 60,000,000 eggs in one summer. Nelson[5] thinks that a large oyster, "if fat the preceding spring, undoubtedly would mature from 50,000,000 to 60,000,000 eggs in a season."

During the course of the experiments on the spawning of oysters in which the writer was engaged during last summer and fall opportunity presented itself to enumerate the eggs laid by *O. virginica* and *O. gigas*. Experiments with the American oysters were carried out at Woods Hole; those with the Japanese species (*O. gigas*) were made at the Hopkins Marine Station, Pacific Grove, California. Japanese oysters were shipped from Samish Bay, Puget Sound, to Pacific Grove where they were kept for about a month in the laboratory tanks. *Ostrea gigas* grows very well in Samish Bay, but in spite of good development of the gonads, fails to spawn there.

Female oysters, placed in twenty-liter glass tanks filled with sea water, were stimulated to spawn, and kymograph tracings of the spawning reaction, which is characterized by the rhythmical contraction of the adductor muscle, were obtained. After the reaction was over, the water in the tank was stirred with a powerful electric stirrer and a 100 cc sample was taken. Eggs, killed by addition of a few drops of 1 per cent. osmic acid, were counted, using the Sedgwick

NUMBER OF EGGS DISCHARGED AND DURATION OF SPAWNING REACTION OF *O. virginica* AND *O. gigas*

Oyster No.	Length cms	Width cms	Date 1929	Temp. ° C.	Duration of reaction, minutes	Number of contractions	Average number of eggs per contraction, millions	Total number of eggs discharged in one spawning period, millions
O. virginica			July					
292	13.3	10.5	23	22.5	61	56	1.26	70.3
295	9.2	7.0	24	24.0	36	57	0.53	30.3
299	11.2	8.0	24	23.0	70	75	0.20	15.0
302	9.4	6.6	25	25.0	70	135	0.85	114.8
O. gigas			Oct.					
J–2	15.2	6.9	2	25.0	23	31	1.34	41.5
J–2	15.2	6.9	9	30.0	23	47	0.83	39.0
J–2	15.2	6.9	19	30.0	19	44	0.26	11.4
J–16	9.5	6.1	20	27.5	15	.....	.....	30.4
J–20–1*	11.6	6.8			59	121		
J–20–2*	10.9	6.2						
J–20–3*	11.2	6.8	22	25.3				55.8**
J–20–4*	12.0	4.8						
J–20–5*	10.8	7.4						

* Five females were kept together; kymograph tracing obtained from one oyster only.

** Average per female; total number discharged by five oysters, 278.8 millions.

[1] Published by permission of the U. S. Commissioner of Fisheries.

[2] K. Möbius, "The Oyster and Oyster Culture," Appendix H to the Report of the Commissioner of Fisheries for 1880, pp. 681–747, 1883.

[3] W. K. Brooks, "Development of the American Oyster," Johns Hopkins University, Studies from the Biological Laboratory, No. IV, p. 81, 1880.

[4] T. C. Eyton, "History of the Oyster and Oyster Fisheries," London, 1858. Quoted from Brooks, *loc. cit.*

[5] T. C. Nelson, "Aids to Successful Oyster Culture," New Jersey Agricultural Experiment Stations, 1921, Bulletin 351, p. 59, 1921.