

the specimens of squid be immersed in nicotine solution for 1 minute. They are then put into the solution of camphor. Camphor spasms take place, after which the animals lie quiet for several minutes. At the end of the required latent period¹ nicotine spasms occur just as typically as in animals treated with nicotine alone.

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A method of standardizing bacterial suspensions.

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If a wire loop is thrust down into a suspension of bacteria in a test tube, and viewed by looking down into the mouth of the tube, the depth at which the loop disappears will be determined by the opacity of the supervening suspension. If, however, a second suspension of the same organism containing half as many bacteria per cubic centimeter is similarly examined, or if an equal amount of the diluent is added to the original suspension, and the "depth of disappearance" again measured it will be found to be less than twice as great as in the original suspension. In other words, the observed depths of disappearance are not in proportion to the bacterial concentrations or the corresponding volumes.

This discrepancy is due to the presence in each reading of a constant which is apparently related to the size and opacity of the individual organisms. It is found that this constant may be eliminated, thus bringing the opacity observations into inverse ratio with the corresponding bacterial concentrations, and a *corrected* reading (the observed reading minus the constant) for any suspension may be obtained by making two readings at different dilutions of the suspension, and substituting the observed values in the following equation:

$$A = \frac{\text{vol } a (b - a)}{\text{vol } b - \text{vol } a},$$

¹Moore, A. R., *J. Gen. Physiol.*, 1919, I, 505.

in which

A = the corrected reading for the first volume of the suspension = $(a - \text{constant})$

a = first observed reading.

vol a = first volume of the suspension on which reading a is made.

b = second observed reading.

vol b = second volume of the suspension (diluted), on which reading b is made.

A concrete example will illustrate the method. In 4 c.c. (vol a) of a given suspension the loop disappears 1.2 cm. below the meniscus (reading a). The suspension is diluted to 10 c.c. (vol b). The loop now disappears 2.7 cm. below the meniscus (reading b). Then the corrected reading for the opacity of the suspension (A), is $\frac{4(2.7 - 1.2)}{10 - 4}$ or 1.0 cm.

Such a *corrected* reading may be directly compared with corrected readings on other suspensions of the same organism, since the corrected readings on such suspensions stand in inverse ratio to their bacterial concentrations. Thus, if two suspensions of the same organism are to be compared and the corrected reading for one is half that for the other, the first suspension contains twice as many bacteria as the second. If the actual bacterial count is required, a standard for the given organism must first be established by the correlation of several corrected readings with the corresponding counts, obtained by the usual methods. Thereafter the concentration of the organism per cubic centimeter in suspensions under examination is obtained by inverse proportion:

$$\frac{\text{The required count}}{\text{The standard count}} = \frac{\text{The standard corrected opacity}}{\text{The given corrected opacity}}.$$

The simplest possible instrument for making the determinations consists of a piece of 18 gauge nichrome, chromel, or black iron wire about 20 cm. long, bent into a small loop at right angles to one end, and with the other end thrust through a cork, near one side, so that a view may be obtained past the cork into an ordinary test tube, 1.6 X 16 cm. on whose lip it rests. The sterile test tube is partly filled with a measured quantity of the suspension, and the wire loop raised or lowered through the cork until the

point is found at which the loop just disappears from view. The distance of the loop below the meniscus is then measured with a centimeter scale laid along the tube, giving the first reading. A second reading is obtained after the addition of a measured amount of the diluent, and the data required for substitution in the equation for the corrected reading is at hand. The readings and calculations require but two or three minutes. In the zone of most accurate measurement, with suspensions of such opacity that the loop disappears between 1 and 4 cm. below the surface, repeated readings may be made with a variation of about one millimeter, an error of less than 10 per cent. The wire loop, which alone comes in contact with the bacteria, may be flamed after each determination, thus reducing the danger of contamination to a minimum.

A more complete explanation of the method, with a description of the more convenient and accurate instrument shown at the meeting will appear in a forthcoming number of the *Journal of Experimental Medicine*.

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Some studies on the surface layer in the living egg cell.

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The results recorded here were obtained through the use of Barber's mechanical pipette holder somewhat modified for microdissection purposes.

The cells experimented upon were the egg cells of the starfish and of the sea urchin. The eggs, which are somewhat over 1/10 of a millimeter in diameter, were placed in a drop of sea water hanging from the roof of a moist chamber. The microscopically fine tips of the glass dissecting needles projected into the moist chamber and up into the hanging drop. By manipulation of the screws of the mechanical pipette holder the cells in the hanging drop could be dissected with considerable accuracy and an estimate ascertained of their physical consistency. Detailed accounts