The results of these measurements showed that the compound membrane of skin plus chorion was capable of producing concentration potentials much greater than those produced by the chorion alone. The average of the chorion values was 19.4 mv. with a maximum of 40.5 mv., as compared with the figures across chorion plus skin, which averaged 55.2 mv. and reached an extreme of 114.6 mv. For both sets of values, with change to the dilute solution, the inside of the egg became more negative to the outside. If it is permissible to interpret these results in the same manner as those of Michaelis on simpler membranes, the negative sign of the concentration potential indicates a relatively greater impermeability of the membranes to anions than to cations. And its greater magnitude in the case of the 2 membranes together, than in that of the chorion alone, furnishes evidence that the differential permeability is less pronounced in the chorion than in the system as a whole.

That the relative impermeability to anions of the system as a whole is largely due to the skin is indicated by measurements of electrical resistance. When allowance was made for the resistance of the remainder of the system, the net resistance of the chorion to direct current was found in 23 experiments to range from 7,000 to 92,000 ohms. On the other hand, the resistance of the chorion plus the skin was 300,000 ohms, or more, in all cases. Since resistances in series are summed, it would appear that the resistance of the skin alone was, in these cases, at least 208,000 ohms.

3957

Preservation of Luminous Bacteria in Absence of Oxygen.

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In the course of experiments on the respiration of the luminous bacterium, *Bacillus fischeri,*[1] it became necessary to determine whether or not this organism was a facultative anaerobe, a characteristic assigned by Beijerinck[2] to one species of luminous bacteria.

The luminescence of luminous bacteria is absolutely dependent on the presence of a partial pressure of oxygen in the medium or

[1] Migula, W., *System der Bakterien.* Jena, 1900.

[2] Beijerinck, M. W., *Arch. Neerlandaises,* 1889, T. 23, 416.

the atmosphere in which they grow.[3, 4] If all oxygen is removed from the medium, and from the atmosphere above the medium after inoculation, the organisms may be allowed to remain in quite anaerobic conditions. Preparations were made for the maintenance of luminous bacteria in absence of oxygen as follows:

A number of experimental tubes were made by narrowing the middle of the ordinary bacteriological culture tubes to facilitate sealing off after inoculation. Nutrient media was placed in the sterile tubes and the whole autoclaved. By means of short and long glass tubes passed through the tightly-fitting rubber stopper replacing the cotton plugs in the tubes, pure hydrogen was passed over the medium and out again. (It is necessary that this hydrogen be passed over hot platinized asbestos contained in a heated quartz tube and be carried to the preparation through lead tubing to prevent diffusion of oxygen into the system.) The medium was boiled to eliminate oxygen while the pure hydrogen passed through the system, cooled as a slant with the pure hydrogen passing over the medium, quickly inoculated with a vigorous growth of the luminous bacteria and replaced in the system so that the pure hydrogen might again replace all air present. With the pure hydrogen still passing rapidly through the system, the tube was then sealed off at the narrow neck, allowing the inoculation to remain in an atmosphere of pure hydrogen, and on an oxygen-free medium. A number of tubes prepared in this manner were set aside with two opened to the air and plugged with sterile cotton to serve as controls.

Within 12 hours an abundant growth with brilliant glow appeared in the control tubes, where air was allowed to replace the hydrogen atmosphere. After from 48 to 72 hours no growth had occurred in the tubes where inoculations were maintained in an atmosphere of pure hydrogen, but on opening these at various intervals a good growth with brilliant luminescence appeared within 12 hours. Inoculations made in the above manner on oxygen free-media and sealed in atmospheres of pure hydrogen have been kept for periods exceeding 2 months (64 days) before opening, and have always regained luminescence and have begun to grow abundantly within 12 hours after the tubes were opened to an atmosphere of air. The inoculation itself has been in every case preserved and kept moist, yet no actual growth occurs so long as an atmosphere of pure hydrogen is maintained above the medium. The property of luminescence is quickly regained on readmission to the normal atmosphere, no drying having impaired the vigor of the inoculation, and

[3] Harvey, E. N., "The Nature of Animal Light," Philadelphia, 1920, p. 67.

[4] Harvey, E. N., *Physiol. Rev.*, 1924, iv, 4, 639.

a complete new growth may shortly be obtained for reinoculation.

It has been shown that the amount of oxygen necessary for just visible luminescence in a suspension of luminous bacteria in sea-water is only of .0053 mm. of mercury pressure.[5] The amount of oxygen in tubes sealed in pure hydrogen with nutrient medium out of which dissolved gases have been driven by boiling, must be very minute indeed.

It has been demonstrated that oxygen is necessary for the growth of this species of luminous bacterium, *(B. fischeri)* as well as for the maintenance of luminescence. The very fact that the glow reappeared in the experimental tubes in each case only after several hours of exposure to an atmosphere containing a partial pressure of oxygen, indicated that it was necessary for growth to occur and permit luminescence over a larger area of medium before it became visible to the observer.

The above method is also suggested as a means of storage and transport of the living luminous bacteria where one is especially anxious that luminescence reappear quickly. The inoculation itself while in pure hydrogen will lie dormant on the nutrient medium until again exposed to an atmosphere containing a partial pressure of oxygen sufficient for growth and luminescence, whereupon both occur, and a vigorous culture of the organisms will cover the surface of the medium.

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The Presence of an Unknown Factor in Serum Which Influences Calcification.

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It has been shown by Shipley[1] and the observation has been confirmed by Shipley, Howland and Kramer[2] that calcification of slices of cartilage and bone from a rickety animal takes place *in vitro* within 48 hours when the slices are immersed in normal blood serum at 37° C.

In the course of a series of experiments upon calcification from

[5] Harvey and Morrison, *J. Gen. Physiol.*, 1923, vi, 13.

[1] Shipley, P. G., *Johns Hopkins Hosp. Bull.*, 1924, xxxv, 304.

[2] Shipley, Kramer and Howland, *Trans. Am. Ped. Soc.*, 1925, xxxvii, 36; *Am. J. Dis. Child.*, 1925, xxx, 37; *Biochem. J.*, 1926, xx, 379.