THE EFFECTS OF LIGHT ON LUMINOUS BACTERIA.

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It is a well known fact that sunlight and artificial light inhibit the luminescence of some luminous animals, notably Ctenophores. I have recently discovered (1925) that a glowing mixture of *Cypridina* luciferin and luciferase in a test-tube will have its luminescence suppressed by light from a carbon arc, and it is possible to show that the inhibitory effect is exerted upon the luciferin and not upon the luciferase. I am inclined to regard this phenomenon as possibly of more general occurrence than heretofore believed and of fundamental significance in connection with bioluminescence. One is reminded of the action of red and infra-red radiation in suppressing the phosphorescence of ZnS and other phosphors.

The question arises whether luminous bacteria show any suppression of luminescence in light, that might be compared with inhibition in Ctenophores or in *Cypridina*. Suchsland (1898) found no effect of sunlight on luminous *Bacterium phosphorescens*, kept under glass and water to prevent warming. McKenney (1902) kept cultures of *Bacillus phosphorescens* in darkness, alternate daylight and darkness, and exposed continuously to a 16 c.p. electric lamp at 2 feet during a period of 48 hours. He found no differences in intensity of luminescence in the three cultures.

On the other hand Lode (1908) reports that luminous *Vibrio rumpel* is very sensitive to sunlight and Beijerinck (1915) finds *Photobacter splendidum* killed by direct sunlight. It is well known (Beijerinck (1915) and Gerretsen (1915)) that ultra-violet radiation from a quartz mercury lamp will kill luminous bacteria, although the luminescence does not immediately disappear as a result of the exposure. The ultra-violet light produces changes in the organism which ultimately lead to its death, together with failure of luminescence.

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The above mentioned experiments have not been carried out in the proper manner to demonstrate an inhibiting effect of light which may disappear quickly. The sort of suppression we are interested in is an immediate inhibition of luminescence after radiation, with possible recovery in the dark, *i.e.* a direct effect of light upon the luminous reaction proceeding within the luminous bacteria, not an effect of light on the growth of the organism or a lethal effect finally resulting in death. Accordingly, the following experiments have been devised to demonstrate any immediate inhibitory effect of light upon luminescence in bacteria.

The light from a carbon arc (soft cored 13 mm. diameter carbons, at right angles, using 15 amperes at 55 volts = 825 watts) in a dark house, after passing through 60 mm. water, is condensed to a slightly converging beam by a lens 135 mm. in diameter. The beam passes through a black tube with a screen at the end containing a slit 8 mm. wide × 20 mm. long, so that all light is excluded from the dark room except a narrow band, 8 mm. × 20 mm. in whose path a small test-tube of luminescent bacterial emulsion may be placed. The illumination in the region of the test-tube is about 15,000 foot candles, much greater than sunlight at noon in summer (10,000 foot candles), but the light had passed through glass so that all deleterious ultraviolet rays were removed. A camera shutter for rapid screening of the beam was placed before the test-tube so that it could be examined very quickly after exposure. Since the beam is narrow (8 mm.) only a narrow area of the test-tube need be exposed to light, the portions above and below the beam remaining in comparative darkness. Thus we have the opportunity of examining contiguous areas of bacteria one of which has been illuminated and the other not, a condition which should bring out any changes in luminescence intensity which may appear. The bacterium used was *Bacterium phosphorescens*[1] isolated from fish by Mr. T. F. Morrison, to whom I am deeply indebted for culturing the organism. The emulsion of the organisms in sea water was made so dilute that it was not necessary to bubble air through the test-tube containing them during the course of an experi-

[1] The experiments were repeated with another form isolated by me and gave identical results.

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ment. There was enough oxygen dissolved in the sea water to maintain the luminescence.

Emulsions of luminous bacteria exposed to 15,000 foot candles, as described above, for periods, of time varying from 15 seconds to 4 minutes, show no trace of inhibition when examined perhaps $\frac{1}{5}$ of a second after the illumination was cut off. They also show no change in light intensity when exposed in a quartz test-tube to a narrow pencil of light from a quartz mercury arc (Cooper-Hewett 72 volts, 3.9 amperes) at a distance of 15 cm. As *Cypridina* luminescence is inhibited under the same conditions in a few seconds, we must conclude that luminous bacteria show no suppression of luminescence as a result of illumination.

Thinking that light might bring about a suppression of luminescence of very short duration in bacteria, I have examined the organisms in

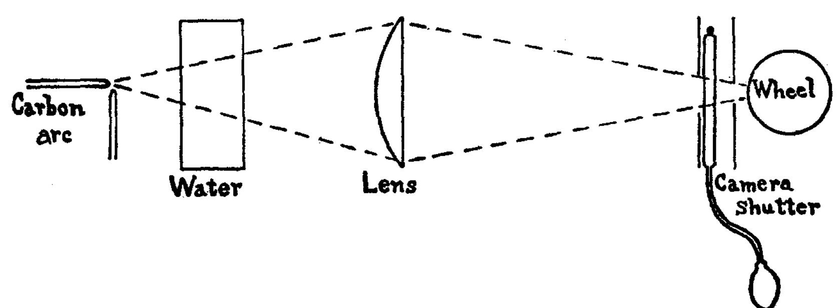

FIG. 1.

a phosphoroscope of the revolving disc type, first described by Becquerel and used in somewhat modified form by many others since (see Andrews, 1920). Fig. 1 shows the general arrangement of the apparatus. Light from a small carbon arc in a dark house is made parallel by a quartz lens, screened and diaphragmed, and allowed to strike the edge of a flat-faced wheel as a circle of light, 6 mm. in diameter. The soft cored, 8 mm. diameter carbons used about 200 watts and gave about 5,700 foot candles on the wheel. If white paper is wound on the wheel and the wheel, attached to the shaft of a motor, is rapidly rotated, one can see very clearly the phosphorescence of the paper by examining the rotating disc through a window from the side opposite to that which the light beam strikes. The paper and many

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other substances luminesce for a considerable time after illumination, so that when we examine the luminous bacteria on the wheel it is necessary to place them on some non-phosphorescent material. Black felt cloth was finally selected as a proper medium, wound on the wheel and painted with a dense emulsion of luminous bacteria to form a band about 18 mm. wide. When the wheel is rapidly rotated many of the bacteria are thrown off by centrifugal force but enough of them remain to present a luminous band when viewed through the window. As the beam of light is 6 mm. in diameter and strikes the middle of the luminous band, 18 mm. wide, any changes in luminescence intensity of the bacteria should be plainly apparent in the center of the luminous field.

I have failed to detect any effect of illumination on the luminescence of the bacteria. The wheel revolved about 100 times per second and the bacteria were examined on the opposite side of the wheel to the one illuminated, so that the time between illumination and examination is about $\frac{1}{200}$ second. Therefore, any inhibiting effect of light must disappear in less than $\frac{1}{200}$ second.

As the illuminated area is 6 mm. across and the circumference of the rotating wheel is about 144 mm. the bacteria are illuminated only $\frac{6}{144}$, or $\frac{1}{24}$ of the time. Assuming that light would affect the luminous bacteria according to the Bunsen-Roscoe law (effect proportional to illumination $\times$ time), they were really exposed to an illumination of $\frac{5,500}{24}$ or 229 foot candles. In later experiments an illumination of 15,000 foot candles was used, so that the effective illumination during rotation was $\frac{15,000}{24} = 625$ foot candles. No effect could be observed in the bacteria after 1.5 minutes exposure. Again assuming the Bunsen-Roscoe law to hold, our exposure in this experiment is $625 \times 90 = 56,250$ foot candle seconds, amply sufficient to suppress the inhibition of *Cypridina* luminescence.

CONCLUSION.

A conservative statement would therefore be that luminous bacteria show no changes in luminescence as a result of illumination by 625 foot candles for 1.5 minutes when examined $\frac{1}{200}$ of a second after exposure, and none as the result of illumination by 15,000 foot candles for 6 minutes when examined $\frac{1}{5}$ of a second after exposure.

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