THE INHIBITION OF CYPRIDINA LUMINESCENCE BY LIGHT.

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Since Allman (1862) reported that Ctenophores would exhibit no luminescence in daylight, a similar inhibition of luminescence in light has been described for Noctiluca (Massart, 1893), Ceratium (Zacharias, 1905), Ptychodera (Crozier), Pelagia (Heymans and Moore, 1923-24), Renilla (Parker, 1920), and some other forms.

Most luminous animals shun the light. We might therefore expect to find an adaptive mechanism in animals, especially in such marine forms as are stimulated to luminescence by the agitation of the waves, by which luminescence would be prevented in daylight, thus saving luminous material.

In the intact Ctenophores, Moore (1923-24) believes the principal action of light to be through local photoreceptor nerves whose stimulation by light converts a preluminous material, A, into D, a non-luminescent product. In the dark, mechanical stimulation of tactile receptors would have converted A to L, the luminescent material, and the Ctenophore would shine. As the luminous slime of Ctenophores, removed by dragging the meridional canals over filter paper (Moore, 1923-24) will also have its luminescence suppressed by light, as well as filtered extracts of the animal (Harvey, 1924-25) in which nerve connections are surely severed, we must conclude that light may also suppress luminescence by a direct effect on the luminous cells and cell fragments.

In the ostracod crustacean, Cypridina, a fluid is secreted from gland cells near the mouth. In contact with the dissolved oxygen of the sea water this fluid luminesces. It contains two substances, luciferin and luciferase, both necessary for luminescence. It should
be noted that, once projected into the sea water, the luminous fluid is never again recovered by the animal. We should therefore expect that if Cypridina is to conserve luminous material adaptively there should be an inhibitory effect of light on the secretory mechanism. I do not know whether light inhibits the secretion of luminous material or not, but I have recently noted a rapid inhibitory effect of light on the luminescent secretion itself. One wonders what the significance of this inhibition can be. There is no doubt that in this case light acts directly on the luminescent reaction with no possibility of nerve influence. Moreover, it is a simple matter to determine which of the two substances, luciferin or luciferase, is affected by illumination.

The experiments are carried out in the following manner. 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 solution 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 ultra-violet 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 two contiguous areas of luminescent solution, one of which has been illuminated and the other not, and any change in intensity of luminescence may be easily observed.

By mixing large amounts of Cypridina luciferin with a small amount of Cypridina luciferase, the resultant solution will emit a plainly visible continuous luminescence whose intensity falls off only very slowly over a period of 10 to 15 minutes. Such a glowing fluid in a small test-tube partly exposed to the beam from the carbon arc will have the luminescence completely suppressed in the exposed region in a few
seconds. In fact one may notice a slight inhibitory effect of the arc in 1.5 seconds, which is unmistakable in 2 seconds and marked in 3 seconds. If the tube, partly exposed to the light beam, with consequent suppression of luminescence, is allowed to stand in the dark, the luminescence again reappears somewhat in the suppressed region, but never becomes as bright as the luminescence in the unexposed parts of the tube. The recovery of luminescence is not due to diffusion of more unexposed material into the illuminated area, as a tube of glowing luciferin and luciferase completely exposed to illumination until its luminescence is practically extinguished will partially recover in the dark. The effect of light is thus reversible, at least in part.

Not only is it possible to demonstrate the inhibitory effect of light on luminescent material in solution, quite apart from cells, but one may show very simply that light affects the luciferin and not the luciferase. Two tubes of luciferin are prepared, one completely exposed to the carbon arc for several seconds, and the other kept in darkness. When luciferase is now added simultaneously to these tubes in the dark, the exposed tube gives only a faint light while the unexposed one gives a bright luminescence. The converse experiment, exposure of a tube of luciferase to the carbon arc while a second tube of luciferase is kept in the dark, results in the emission of an equally bright luminescence from each tube on the addition of luciferin in the dark.

If the beam of light is allowed to pass through a Nicol prism before striking the test-tube, its inhibitory effect is reduced. That is not surprising since the Nicol transmits only 38 to 40 per cent of the light. I am inclined to attribute the reduction in effectiveness to the absorption and not to the polarization of the light, although I have as yet made no special experiments to determine if one may explain the effect quantitatively by absorption in the Nicol prism.

The far ultra-violet (wave-lengths shorter than 3,000 Å. u.) plays no particular part in the inhibition. A quartz test-tube of luminescent luciferin and luciferase is only very slightly inhibited after 2½ minutes exposure to the light from an iron spark1 at 3 cm. distance. And the

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1 A disruptive condenser discharge between iron terminals. The apparatus is described by Andrews (Andrews, W. S., *Gen. Elec. Rev.*, 1916, April). It is very convenient for demonstrating ultra-violet fluorescence. The instrument was loaned to me by Mr. Andrews to whom I express my sincere thanks.
inhibition is just as marked when a piece of window glass (opaque to wave lengths less than 3,200 Å. u.) is inserted in the path of the light. Negative effects with far ultra-violet are further seen by studies with the mercury arc. The quartz mercury arc (an old Cooper-Hewett using 3.9 amperes at 72 volts = 280 watts) at a distance of 15 cm. gives as good an inhibition of Cypridina luminescence after 15 seconds in a glass test-tube as in a quartz test-tube. Moreover, if we screen out the near ultra-violet by a chlorine-bromine gas filter\(^2\) in a quartz vessel, which lets pass only red, yellow, green, blue (4,358 Å. u.), and far ultra-violet we find only slight inhibition in 90 seconds exposure. The chlorine-bromine filter is especially transparent to the region between 2,550 and 2,650 Å. u. and red, yellow, and green, but opaque to violet (4,078 Å. u. and 4,046 Å. u.) and near ultra-violet (between 4,000 and 3,000 Å. u.). As I shall presently show that red, yellow, and green light has no inhibiting effect, the slight amount of inhibition with the chlorine-bromine filter must be due to the 4,358 Å. u. line.

Since the far ultra-violet is ineffective in inhibiting Cypridina luminescence, I have made a study of inhibition in carbon arc light filtered through various glass screens whose transmission is known. The results are tabulated below. For convenience the test-tubes of luminescent luciferin were exposed exactly 15 seconds to an illumination of approximately 8,600 foot candles. Without any filter this illumination appreciably inhibits luminescence in about 2 seconds. The experiments all indicate that light from 4,600 to 3,800 Å. u. is the effective light and that green-blue, green, yellow, red, and infra-red are without action.

For instance the No. 8 filter allows over 75 per cent of all red, yellow, green, and green-blue wave-lengths to pass and 10 per cent of 4,700 Å. u. but only 1.6 per cent of 4,600 Å. u. and none of 4,500 Å. u. The No. 8 filter lets through no light that will inhibit Cypridina luminescence under the conditions above mentioned. The No. 3 filter differs from No. 8 only in allowing 40 per cent of 4,600 Å. u., 4 per cent of 4,500 Å. u., and no 4,400 Å. u. to pass. Nevertheless we get inhibi-

\(^2\) Kindly loaned to me by the Hanovia Chemical and Manufacturing Company to whom I express my sincere thanks.
tion of *Cypridina* luminescence behind this filter. Therefore, I place the longer wave-length limit about 4,600 Å. u.

On the other hand, No. 18, the ultra-violet filter, which lets pass a narrow band of ultra-violet with a maximum of about 20 per cent

### TABLE I.

**Inhibition of *Cypridina* Luminescence by Light Passing through Wratten Filters.**

<table>
<thead>
<tr>
<th>Filter</th>
<th>Maximum transmission (figures in Å),</th>
<th>Inhibition of luminescence</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. 2</td>
<td>Absorbs ultra-violet; transmits all visible.</td>
<td>+</td>
</tr>
<tr>
<td>3 yellow</td>
<td>“ violet to 450; “ rest.</td>
<td>-</td>
</tr>
<tr>
<td>8 “</td>
<td>“ “ 460; “ “</td>
<td>-</td>
</tr>
<tr>
<td>15 “</td>
<td>“ “ and blue to 510; transmits rest.</td>
<td>-</td>
</tr>
<tr>
<td>17 “</td>
<td>“ “ transmits much 350, 10 per cent of 388, and much 470 to red end.</td>
<td>-</td>
</tr>
<tr>
<td>18 ultra-violet.</td>
<td>Absorbs all but ultra-violet around 350.</td>
<td>-</td>
</tr>
<tr>
<td>22 orange.</td>
<td>“ blue end to 550; transmits rest.</td>
<td>-</td>
</tr>
<tr>
<td>25 red.</td>
<td>“ “ “ 590; “ “</td>
<td>-</td>
</tr>
<tr>
<td>29 “</td>
<td>“ “ “ 610; “ “</td>
<td>-</td>
</tr>
<tr>
<td>36 violet.</td>
<td>Transmits 18 per cent around 410; absorbs rest.</td>
<td>+</td>
</tr>
<tr>
<td>50 blue.</td>
<td>“ 14 “ “ 460; “ “</td>
<td>-</td>
</tr>
<tr>
<td>54 green.</td>
<td>“ 0.93 “ “ 550; “ “</td>
<td>-</td>
</tr>
<tr>
<td>55 “</td>
<td>“ 72 “ “ 520; “ “</td>
<td>-</td>
</tr>
<tr>
<td>61 “</td>
<td>“ 52 “ “ 530; “ “</td>
<td>-</td>
</tr>
<tr>
<td>62 “</td>
<td>“ 14 “ “ 530; “ “</td>
<td>-</td>
</tr>
<tr>
<td>70 red.</td>
<td>“ red to 560; “ “</td>
<td>-</td>
</tr>
<tr>
<td>71 “</td>
<td>“ “ “ 610; “ “</td>
<td>-</td>
</tr>
<tr>
<td>72 orange.</td>
<td>“ 4 per cent around 610; “ “</td>
<td>-</td>
</tr>
<tr>
<td>73 yellow-green.</td>
<td>“ 8 “ “ 570; “ “</td>
<td>-</td>
</tr>
<tr>
<td>74 green.</td>
<td>“ 15 “ “ 530; “ “</td>
<td>-</td>
</tr>
<tr>
<td>75 blue-green.</td>
<td>“ 19 “ “ 490; “ “</td>
<td>-</td>
</tr>
<tr>
<td>76 violet.</td>
<td>“ 9.5 “ “ 440; “ “</td>
<td>-</td>
</tr>
<tr>
<td>88 infra-red.</td>
<td>“ infra-red and 5 per cent of 700; “ “</td>
<td>-</td>
</tr>
<tr>
<td>G 584 J copper blue.</td>
<td>Strong transmission in blue.</td>
<td>+</td>
</tr>
<tr>
<td>“ 585 L blue-purple.</td>
<td>“ 85 per cent at 380; 80 per cent at 400.</td>
<td>+</td>
</tr>
<tr>
<td>“ 586 A W “</td>
<td>Like G 586 A but very dense.</td>
<td>-</td>
</tr>
</tbody>
</table>

Very slight after 1½ minutes exposure.

Transmission at 3,500 Å. u. completely prevents inhibition of *Cypridina* luminescence by arc light, while No. 36 which lets pass a narrow band of violet with 18.7 per cent maximum transmission at 4,000 Å. u. allows a little inhibitory light to pass. No. 17 transmits 10
per cent of 3,800 Å. u. and gives some inhibition. The shorter wave-
length limit must be somewhere near 3,800 Å. u.

Infra-red radiation which has so marked an effect in inhibiting the
phosphorescence of ZnS, CaS and other phosphors, does not inhibit
Cypridina luminescence, as the experiment with No. 88 filter indicates,
and other tests which I have made using iodine in carbon disulfide
to absorb the visible.

Cypridina emits light over a spectral range from 4,150 to 6,500
Å. u. and it will be seen that some of these wavelengths coincide
with those we have just found to have an inhibiting effect upon lumi-
nescence. We may ask whether Cypridina luminescence could pos-
sibly inhibit itself. When one considers the difference in illumination
between the carbon arc and the Cypridina luminescence, a detectable
self-inhibition seems hardly likely, but it was thought worth while to
test the matter. Accordingly two small test-tubes, A and B were
fixed within two larger test-tubes and the latter filled with a glowing
mixture of luciferin and luciferase. In one of the small test-tubes
(A) water was placed, and in the other (B) Cypridina luciferin. At
the proper time Cypridina luciferase was added to B so as to produce
a bright luminescence which lasted for about 15 seconds and was then
completely quenched by adding acid to the B tube. Thus the lumines-
cent mixture in the large test-tube surrounding B had been exposed to
Cypridina luminescence while the luminescent mixture surrounding A
had not. However, both luminescent mixtures were observed to be
equally bright, so I conclude that any self-inhibition of luminescence
by Cypridina light is too slight to be detected.

Finally I have thought it worth while to test the inhibiting effect
of light on other chemiluminescences. Using the large carbon arc
light providing about 15,000 foot candles, I have observed no quench-
ing effect on the chemiluminescence of phosphorus in air (1 minute
exposure), lophin in hot alkaline alcohol (2 minutes exposure), pyrogal-
lol + H₂O₂ oxidized with hemoglobin (10 seconds exposure), or chlor-
phenylmagnesium bromide³ in air (2 minutes exposure). The latter
compound was placed in a watch-glass and the beam directed on its
surface by a silver-under-glass mirror. The illumination was not
15,000 foot candles but possibly 85 per cent of this.

³ A sample of this compound was kindly presented to me by Dr. R. T. Dufford
of the University of Missouri.
CONCLUSIONS.

The luminescence of *Cypridina* luciferin-luciferase solution is inhibited by illumination from a carbon arc of 15,000 foot candles in between 1 and 2 seconds. The blue to violet rays are the effective ones, the limits lying somewhere around 4,600 Å. u. to 3,800 Å. u. The luciferin, not the luciferase, is the substance affected by the light. The effect is partially reversible in the dark. The chemiluminescences obtained by oxidizing phosphorus, lophin, and chlorphenylmagnesium bromide are not inhibited by light under the above conditions.

BIBLIOGRAPHY.