THE PHOTOLYSIS OF THE LUMINESCENT GRANULES OF EUCHARIS MULTICORNIS.

BY A. R. MOORE.*

(From the Physiological Laboratory of Rutgers University, New Brunswick, and the Zoological Station, Naples, Italy.)

(Received for publication, December 2, 1925.)

In former papers it has been shown that the luminescence of ctenophores is inhibited by light according to the Bunsen-Roscoe photochemical law; namely, the effect of the light is proportional to the product of intensity by time. The quantity of light necessary to inhibit luminescence in Mnemiopsis leidyi was found to be 4776 meter candle minutes, in Beroe (sp.) 57,285 m.c.min., in Beroe forskali approximately 50,000 m.c.min., and in Cestus veneris 1167 m.c.min.

In the paper on Mnemiopsis I called attention to the fact that the luminescent material spread upon filter paper could be inactivated by light. Subsequently Harvey showed that such material prepared by making a suspension of it in sea water could be inactivated by light, and that the reaction was to a certain extent reversible. Just recently Harvey has found that light inhibits the luminescence of Cypridina photogenic material, and that the substance acted upon is luciferin and not luciferase. The amount of the light of a carbon arc required to inhibit Cypridina luminescence is 80 to 150 m.c.min. Luciferin is therefore enormously more sensitive to light than are any of the ctenophores or their photogenic material with which I have worked.

It seemed to me desirable to follow step by step the photolytic reaction by which light inactivates the luminescent substance of

* Occupant of the Table of the American Association for the Advancement of Science, Naples, 1925.

3 Moore, A. R., Arch. sc. biol., 1926, viii (in press).
ctenophores. I chose for this purpose *Eucharis multicornis*, for the reason that a large amount of material can be obtained from each animal, and the light which the material gives out when mixed with tap water is brilliant blue-green.\(^6\)

In practice a large specimen of *Eucharis* which had been dark-adapted was slipped into an Erlenmeyer flask and shaken. There resulted a suspension of photogenic granules which passed readily through rapid filter paper. The filtered suspension was very stable and could not be thrown down with an ordinary centrifuge. If 50 cc. of this suspension were mixed with 200 cc. of tap water a bright light developed which lasted 5 to 7 seconds. The amount of light was usually sufficient to cause a deep blackening of a photographic plate (Cappelli's extra rapid). But if the suspension of photogenic granules was exposed for an hour to a light of approximately 800 m.c., the luminescence which then developed as a result of mixing the suspension with tap water was very faint, producing little or no effect on the photographic film. In this way it was found possible to obtain photographic records of the luminescent activity of the suspension of photogenic granules, and thus to follow step by step the photolytic decomposition of this material. Reference should be made to the beautiful work of Amberson\(^7\) who studied the dynamics of the luminescent reaction of *Cypridina* material. Since this reaction lasts but a few seconds, Amberson had to use cinema films in order to follow the process step by step. My problem was a wholly different one, since I was studying the destructive action of light on photogenic substance, and desired a record of the sum of the light which the unchanged photogenic substance could produce. It was therefore only necessary for me to secure a photographic record of the sum of the luminescence produced by the reaction of any particular sample.

This was accomplished in the following way. 300 cc. of a suspension of the photogenic granules of *Eucharis* were exposed in a beaker to the action of a 200 c.p.\(^8\) lamp placed at a vertical distance of 50 cm.

---


\(^8\) This value is only approximate, but since the same lamp was used in all the experiments, the results are strictly comparable.
The depth of the solution was approximately 2 cm. The suspension was quite transparent and there was no stirring except momentarily before taking a sample. From time to time, after thorough agitation, a 50 cc. sample was withdrawn in a pipette and taken to the dark room. Here a rectangular dish had been prepared containing 200 cc. of tap water, and with a sensitive photographic plate, film side down, resting on top. The suspension was poured rapidly into the dish, and after the resulting luminescent glow had subsided completely, the exposed photographic plate was removed, numbered, covered, and kept until the end of the experimental series. The plates used in any given series were obtained from the same box in order that the sensitized films might be as uniform as possible. As soon as a series of exposures had been completed all six of the plates were put into a large tray and developed together for the same length of time. Hydroquinone developer was used. The processes of fixing, washing, and drying were carried out in a manner as nearly identical as possible for all the plates of a series.

Each plate, then, constituted a record of the amount of light given off by the suspension to which it had been exposed. The amount of light given off by a suspension is the indicator of the unchanged photogenic molecules present at that time in the suspension, i.e. \( a - x \) in chemical notation where \( a = \) amount of material present at the start and \( x = \) amount changed in the time \( t \). The next step was to obtain numbers which would denote the degree of blackening of each photographic plate in a series. Now it is known that the opacity of the developed photographic film is proportional to the quantity of light causing the opacity. This relation is assumed since for every increase in log intensity there is an equal increase in log opacity. By means of a Lummer-Brodhun photometer\(^6\) the opacity of the plates was measured. Four readings were made with each plate and the results averaged. The opacity, which is the reciprocal of the transparence of the plate to the photometer light, is proportional to the intensity of the light found necessary to match the constant standard lamp. The opacity of the plate which recorded the amount of light produced by the 50 cc. of suspension which had not been

\(^6\) I owe my thanks to Professor George Winchester of the Department of Physics for the loan of this apparatus.
subjected to illumination is taken as equal to 100 in each series, and the opacity of the remaining plates of the series calculated as percentages of this.

In suspensions derived from different individuals there was a considerable range of variation. In some cases the luminescence was too intense, with the result that some of the experimental plates as well as the control showed maximum blackening. In others the luminescence was too faint, consequently the resulting photographic films were very thin, and differences within the series were slight. Now, for moderate intensities of light the relation between intensity and photochemical action is perfectly regular; namely, density (log opacity) is proportional to log intensity. But this relationship does not hold for very weak nor for very strong light. For this reason two classes of series of plates were rejected: (1) those in which the films were too dense, (2) those in which the films were too faint. Only those have been considered in which there is an easily measurable difference between the control and any other plate of the series, which means that the velocity of the photolytic reaction was approximately the same in all the series considered.

Two series were run to determine the validity of the Bunsen-Roscoe law for the photolytic action of light on the photogenic granules. Three intensities of light were used with corresponding times of exposure so that in each case the quantity of light (intensity \( \times \) time) \( = 24,000 \) m.c.min., thus assuming the Bunsen-Roscoe rule to hold, in which case the experimental plates should be identical in opacity.

The results indicate with a fair degree of consistency that the Bunsen-Roscoe law holds just as it does for the intact animals of the ctenophore group. It will be noted that the higher intensity is more effective. Further experiments will be necessary to show

<table>
<thead>
<tr>
<th>Intensity</th>
<th>Time</th>
<th>Series A, opacity ( a - x )</th>
<th>Series B, opacity ( a - x )</th>
</tr>
</thead>
<tbody>
<tr>
<td>3200</td>
<td>7.5</td>
<td>52</td>
<td>46</td>
</tr>
<tr>
<td>1600</td>
<td>15</td>
<td>47</td>
<td>58</td>
</tr>
<tr>
<td>800</td>
<td>30</td>
<td>56</td>
<td>60</td>
</tr>
</tbody>
</table>
whether this represents a constant deviation from the rule. With live animals the deviation is in the opposite sense; i.e., the high intensities are less effective.

The next relation to be investigated was the effect of temperature on the photolytic reaction. Five different temperatures were chosen. Each lot of 50 cc. of suspension of photogenic granules was put into a separate dish and kept at the desired temperature during the exposure to light. In each case the material was exposed to 3200 m.c. for 10 minutes. The suspensions were brought to the same temperature, 18°C., before being mixed with tap water to produce the luminescent reaction. This is necessary since the reaction producing luminescence presumably has a high temperature coefficient. Table II shows the results of two series.

**TABLE II.**

<table>
<thead>
<tr>
<th>Temperature (°C.)</th>
<th>Series A, opacity</th>
<th>Series B, opacity</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>42</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>57</td>
<td>50*</td>
</tr>
<tr>
<td>12</td>
<td>59</td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>50</td>
<td>50*</td>
</tr>
<tr>
<td>22</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Values in Series B are 69 and 76 but reduced for purposes of comparison, by putting 50 for 76, which gives 45 instead of 69.

Since in both series the amount of change is greatest at the lower temperature, it therefore follows that a rise in temperature does not increase the efficiency of the photolyzing light. This failure of temperature to affect the velocity of the photolytic process is characteristic of photochemical reactions.

It was next attempted to determine the order of the reaction. The intensity of the photolyzing light was kept constant at 800 m.c., and the time varied. In all, four series were completed.

In each of these series there was an easily measurable difference between any two plates of the series. Since the sensitive films were

---

of identical depth the velocities were approximately the same in all four series. Values for identical time intervals were averaged. Use of the graphic criterion showed the reaction to conform fairly consistently to the rule for a reaction of the first order. According to this \( \log (a - x) \) plotted against time yields a straight line. Fig. 1 shows how nearly the observed values conform to the rule. The curve in the figure is constructed from the values of \( x \) (the amount

![Graph showing the progress of the reaction]

**Fig. 1.** The graphs represent the progress of the reaction by which light destroys the photogenic material of *Eucharis multicorns*. The intensity of the photolyzing light is constant and the time of exposure in minutes is shown on the abscissa. The ordinates on the right represent the values of \( \log (a - x) \). \( a - x \) is the quantity of photogenic material left unchanged by the light, and is proportional to the opacity of the photographic records. The experimental points are indicated by + and fall approximately on a straight line, thus proving the reaction to be of the first order. The ordinates on the left represent values of \( x \) (the amount of photogenic material destroyed by the light in time \( t \)). Assuming the reaction to be of the first order the theoretical values of \( x \) plotted against time yield the curve. The dots indicate average experimental determinations.
of material destroyed by the light) plotted against time. This curve has the form characteristic of a first order reaction.

The arithmetical values are shown in Table III. The values of the constant $K$ calculated for a monomolecular reaction are shown in the last column. It will be seen that except for the first value of $K$, which is not included in the average, the variations from the mean are not great.

The usual significance should attach to the fact that the photolysis of the photogenic material of *Eucharis* follows the course of a first order reaction. This means that equal amounts (per cents) of unchanged material are destroyed in equal time intervals, and it does not necessarily follow that we are concerned with a simple reaction such as the hydrolysis of a single kind of molecule. Evidence collected from the study of photochemical reactions of molecules of known structure shows that chemical change is proportional to the "chemically" absorbed light, and that the absorption "order" and reaction "order" are identical; i.e., the order is always 1.\(^{12}\)

For physiology the interest in these studies lies in the fact that in the inhibition of luminescence by light we have a class of phenomena

\(^{12}\) Sheppard,\(^{11}\) p. 218.
PHOTOLYSIS OF LUMINESCENT GRANULES

which demonstrably conform to the rules of photochemistry, namely the Bunsen-Roscoe law, the reaction order of known photochemical processes, and the absence of a temperature effect.

CONCLUSIONS.

1. By means of a photographic method a study was made of the photolytic effect of light on the luminescent granules of *Eucharis multicornis*.

2. The photolytic reaction conforms to the Bunsen-Roscoe law.

3. The velocity of the photolytic reaction is not increased by a rise in temperature.

4. The photolytic reaction proceeds as a first order reaction.