

LUMINESCENCE IN MNEMIOPSIS.*

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The Ctenophore, *Mnemiopsis leidyi*, is a southward ranging form in New Jersey waters, occurring annually in greater or less numbers in Barnegat Bay.¹ The animal is provided with eight rows of paddle plates (Fig. 1) and underlying each row of plates is a

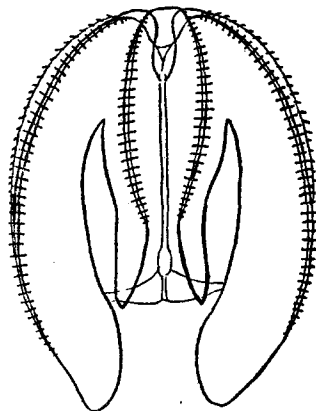


FIG. 1. Drawing of *Mnemiopsis*. The rows of paddle plates show where luminescence appears when a dark adapted animal is stimulated.

* The preliminary experiments of this paper were made in May, 1919, in the house boat of Dr. T. C. Nelson, then stationed at Tuckerton, New Jersey. During that season *Mnemiopsis* was very abundant in the waters of Barnegat Bay, but only rarely were specimens found in the 3 succeeding years. During the summer and fall of 1923, however, *Mnemiopsis* occurred again in great numbers (T. C. Nelson, Report of New Jersey Experiment Station, 1923). It thus became possible to finish some of the work begun in 1919. I am very glad to take this occasion to thank Dr. Nelson for giving me the use of his house boat laboratory and for much assistance in securing material both in 1919 and during the past season.

¹ Nelson, T. C., *Anat. Rec.*, 1923, xxvi, 381.

corresponding row of luminescent organs. When the dark adapted animal is stimulated mechanically the luminescent organs respond with a glow of blue-green light, so that in the dark the outline of the individual is defined by eight meridians of light. This reaction is inhibited completely if the animal has been exposed to sunlight for a few minutes. In the dark, after a time, the power of luminescence is recovered, and Peters² found that the recovery process is facilitated by mechanical agitation.

The Physiology of Luminescence.

It can be shown that the luminescence reaction is normally under the control of the nervous system. If a dark adapted animal be touched at one point with a needle, this may result in luminescence spreading over the entire body along each of the eight meridians. Hence the action of mechanical stimulation cannot be directly on the luminescent material but upon a receptor from which the impulse is transmitted to the luminescent organs by conducting paths. This corresponds to the mechanism underlying general luminescence in *Pelagia*.³

Moreover, the receptors for mechanical stimulation of the luminescent organs are limited in their distribution to the eight rows of paddle plates, because, while a touch with a glass needle applied to a meridian causes an immediate glow along that particular row of paddle plates, similar stimulation applied to the intermeridian does not result in luminescence unless the pressure applied is sufficient to cause some deformation of the meridian. On the other hand, contact stimulation *at any point* whatever causes cessation of movement of the swimming plates and closure of the oral lobes. It is therefore clear that while the swimming plates and muscles are innervated by the general nerve-net system, the luminescent organs are limited in their innervation to the receptor cells and conducting tissue of the eight meridians. The experiment also proves that luminescence does not depend upon excitation of contractile tissue.

² Peters, A. W., *J. Exp. Zool.*, 1905, ii, 103.

³ Heymans, C., and Moore, A. R., *Compt. rend. Soc. biol.*, 1923, lxxxix, 430.
Heymans, C., and Moore, A. R., *J. Gen. Physiol.*, 1923-24, vi, 273.

The luminescent material may be studied apart from the animal by means of luminescent paper obtained in the same way as a similar indicator paper was prepared from the medusa *Pelagia noctiluca*.³ This was done by rolling the animal over a piece of filter paper. It is necessary to be sure there are no large pieces of tissue left on the paper since these contain intact cells which are stimulated by NaCl. Such indicator paper does not glow in the dark until rubbed, or wet with an appropriate solution. Solutions of pure salts isosmotic with the sea water of Barnegat Bay, namely of concentration $M/4$, show characteristic action on the indicator paper. All solutions were brought to pH 7.7. It was further found what variations in pH between pH 6 and 8 were without effect on the reaction. The indicator glows in solutions of K_2SO_4 , KCl, $CaCl_2$, $SrCl_2$, $MgSO_4$ but does not glow in solutions of NaCl and $MgCl_2$. That $CaCl_2$ and $SrCl_2$ should act in a stimulatory sense and NaCl should not is worthy of note since this is a result opposite to that obtained in the case of nerve and muscle with these two salts. On the other hand if entire animals are immersed in NaCl solution the resulting hyperirritability of the tissues causes the animal to emit spontaneous flashes of light. This experiment adds confirmation to the view expressed above that in the animal the luminescent material is under nervous control.

While the power of luminescence of the animal is suppressed in a very few minutes with a light of high intensity, the luminescent properties of the luminescent substance are not destroyed by the same treatment. An individual in which light of 681 c. p. suppressed luminescence in 8 minutes, yielded luminescent material which showed no deterioration after 30 minutes exposure to the same light. This is shown by the fact that the paper glows when rubbed or torn or put into KCl solution. However, exposure for a few minutes to direct sunlight or artificial illumination long continued does destroy the luminescent properties of the indicator paper. These facts suggest that in the suppression of luminescence *in the animal* the light acts not directly on the luminescent substance itself, but indirectly through a photoreceptor cell and nervous connections. The situation is analogous to that of mechanical stimulation. Weak mechanical stimulation of the animal causes the luminescent reaction, but vigorous rubbing is necessary to cause the luminescent material to glow.

Similarly, luminescence in the animal is suppressed by a quantity of light which is not sufficient to affect the luminescent material on filter paper.

The character of the photoreceptor connections can be shown by illuminating one part of a large animal. This results in suppression of luminescence only in the area of the animal which has been subject to illumination. Thus if the aboral half has been illuminated with sufficient quantity of light, mechanical stimulation of a meridian anywhere results in luminescence of the oral half alone. Hence there can be no extensive linear connection between the photoreceptors and the luminescent cells.

An unusual relation of luminescence to temperature was discovered. If animals living at a temperature, of 20°C. be cooled to 9° no luminescence is at once elicitable. This was noted by Peters. But if the cooling is continued to 3°C. of course no luminescence is obtainable although the paddle plates continue beating.⁴ (This shows that the motion of the plates and luminescence are independent variables.) If now the temperature is raised to 7°C. luminescence appears upon stimulation. Furthermore, if the animals are kept a few hours at 3°C. luminescence occurs regularly on stimulation at that temperature.

Inhibition of Luminescence by Light.

It was next attempted to determine the relation between the intensity of illumination and the time required for the suppression of luminescence. The procedure was as follows: Each animal was put into a beaker containing sea water to a depth of 1 cm. and then dark adapted for 30 minutes. The experiment was made by exposing the dark adapted animals in groups of three to a light of known intensity until no luminescence was observable when the light was extinguished and the dish rotated. Observations were always made with dark adapted eyes. The point of extinction was determined by testing the animal every 2 minutes when such time had elapsed that it was judged the exposure was very nearly sufficient. It was always possible to approximate this time because the intensity of the luminescence obtainable decreases with continued exposure to illumination. The time required

⁴ I have seen the paddle plates beating at a temperature of -0.6°C .

for suppression was taken as the mean between the time of the last reading at which luminescence appeared and the next reading which showed complete suppression. After this point had been found the animal was again kept in darkness 30 minutes at the end of which time its power of luminescence was completely restored and another determination could be made. Each individual showed consistent readings but there were considerable differences between individuals in the time necessary for the light at a given intensity to suppress luminescence (Table I). The fact that each individual yields consistent results for the five intensities is further proof that the luminescent material reacts in a closed system. Animals which had been freshly brought into the

TABLE I.

Intensity.	Time.						Average time.
	A	B	C	D	E	F	
<i>meter candles</i>	<i>min.</i>	<i>min.</i>	<i>min.</i>	<i>min.</i>	<i>min.</i>	<i>min.</i>	<i>min.</i>
170	23	17	16	17	17	19	18.2
305	17	13	11	11	18	12	13.7
436	8	6	6	8	11	7	7.7
681	7	3	5	2	12	4	5.5
1,210	5	1	6	2	7	2	3.8

A, B, C, D, E, F, denote the six individuals used in this experiment.

laboratory required a longer time for suppression of luminescence with light of a given intensity than those which had been kept in the laboratory several days. As a source of light a tungsten lamp of 109 c.p. was used. The lamp was clamped to a tall iron stand and five different intensities were obtained by fixing the light at distances of 80, 60, 50, 40, and 30 cm. which yielded intensities of 170, 305, 436, 681, 1,210 m.c., respectively. The temperature was 20–21°C.

When the suppression time in minutes for each intensity had been determined for a given series of animals the average time for each intensity was calculated. These average values in turn form the basis for the mean values shown in the plot (Fig. 2, Table II). With freshly collected animals it was impracticable to obtain readings for the lowest intensity of light since the exposure time was too long, but with animals which had been kept in dishes in the laboratory for

several days all the exposure times were shorter, and such animals were always used for the lower intensities. On the other hand, for the high intensities only fresh animals were employed in order that the exposure times might be as long as possible, thus increasing the accu-

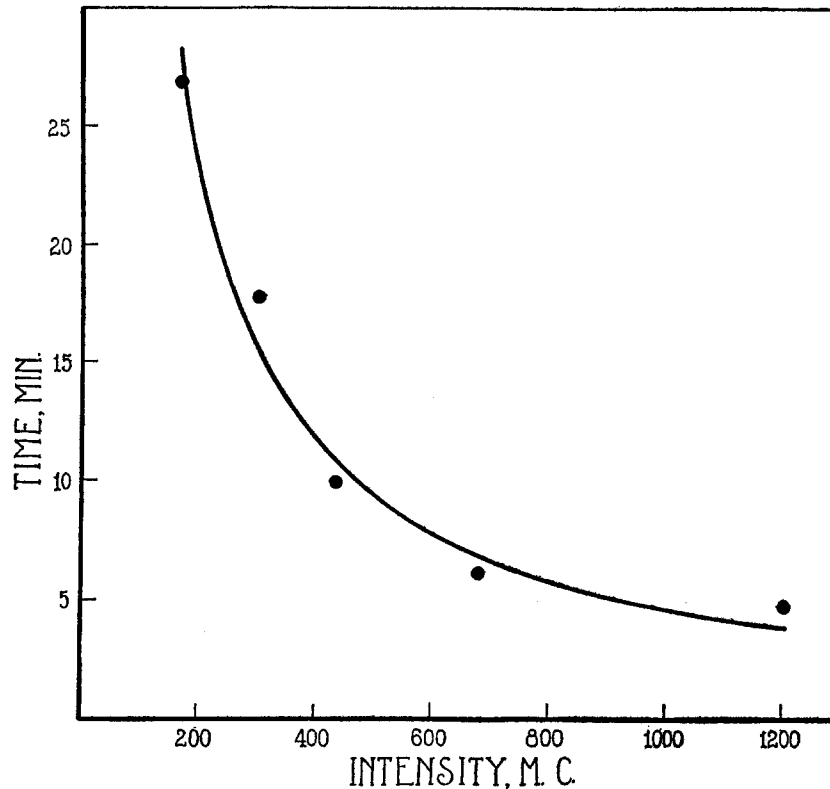


FIG. 2. Abscissæ represent intensities of light (I) and ordinates the average time (t) in minutes required at the intensity given to suppress luminescence. In the equation $I \times t = K$, $K = 4,776$, average value, calculated values of t are obtained for each intensity by substituting this value of K and the values of the intensities in the equation. From the calculated values of t the curve is drawn; circles indicate average observed values of t for corresponding intensities of light.

racy of the measurements. It turned out that while the curves yielded by the different experiments were parallel, those obtained from the animals kept in the laboratory for a day or more were always displaced toward the x -axis. In order to construct the curve (Fig. 2), it was

necessary to reduce all sets of measurements to the same standard of value. This was done by setting the time at intensity 436 m.c. in each series as 10 minutes and by simple proportion calculating all the other exposure times of the series. The method used in calculating the mean of the averages may be illustrated by the following example. There were three series of experiments made with 305 m.c. intensity, two involved six animals, and one, nine animals. The average exposure time for the first series (six animals) was 17.8 minutes; for the second (nine animals), 19.2 minutes; and for the third (six animals), 15.8 minutes.

Hence,

$$\begin{aligned} 6 \times 17.8 &= 106.8 \\ 9 \times 19.2 &= 172.8 \\ 6 \times 15.8 &= 94.8 \\ \text{Total animals 21} &\overline{)374.4} \end{aligned}$$

17.8 mean exposure time at 305 m.c.

TABLE II.

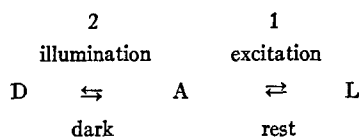
Intensity.	No. of animals.	Time.	K
<i>meter candles</i>		<i>min.</i>	
170	12	26.9	4,573
305	21	17.8	5,429
436	30	10.0	4,360
681	35	6.2	4,222
1,210	20	4.8	5,808
Average.....			4,776

In similar fashion the mean exposure time for each of the five intensities was calculated. The results are summarized in Table II. Each exposure time multiplied by the corresponding intensity yields a value for *K* which is fairly constant for the series and gives an average value for *K* of 4,776. Since the product of intensity and time is a constant, given the same end-point, it follows that the Bunsen-Roscoe law holds for the effect of light on the luminescence reaction in *Mnemiopsis*. This relation proves the photochemical nature of the action of light in this form.

Theoretical Considerations.

The foregoing experiments were made with a view to getting an insight into the physiology of the luminescent reaction in *Mnemiopsis*.⁵ While the picture is at present by no means complete it may be of some value to present a view of the process which the experiments up to this time seem to support. In order to account for the results of illumination in suppressing luminescence it is necessary to suppose that the photoreceptor cells are connected with luminescent cells in the simplest possible fashion, in which there are neither linear nor lateral branches. The assumption that light acts on photoreceptor cells and not on luminescent material in suppressing luminescence in the animal is based on the fact that the luminescence reaction is more delicately responsive to the action of light in the animal than in the indicator paper. Since, then, the light acts on the animal only locally, in suppressing luminescence, the photoreceptors cannot have appreciable lateral nervous connections. As to the effects of mechanical stimulation, tactile receptors for luminescence occur only along the lines of the paddle plates and are connected with a conducting system only along this line; there are no intermeridional branches. In contrast to this limited conducting arrangement for the luminescence reaction, the tactile receptors for muscular and ciliary action possess, through the nerve net, two or three dimensional connections with all parts of the organism.

If we consider the reaction system of the luminescent material as it acts in the animal we may provisionally adopt the following scheme.



It is assumed that the system is a closed one. The evidence indicates that the reactions are simultaneous and of the first class, namely side reactions,⁶ in which *A* is the luminescent substance in the resting,

⁵ Moore, A. R., *Proc. Soc. Exp. Biol. and Med.*, 1923, xxi, 52.

⁶ Lewis, W. C. McC., *A system of physical chemistry*, London and New York, 1920, i, 398.

dark adapted animal, L is the light giving form of the substance, formation of which is catalyzed by stimulation, D is a decomposition product, formation of which is facilitated by illumination of sufficient intensity and duration. Such a scheme makes intelligible the experiment of Peters² in which he found that stimulation hastens the recovery of luminescence in an animal which had previously been light adapted, since excitation removes A from the system as rapidly as it is formed and thus facilitates the "dark" reaction. Likewise the "rest" reaction goes on even under strong illumination. It is evident that D cannot be directly converted into L , and it also seems probable that L cannot be changed into D without passing through the intermediary stage A .

If we assume that both reactions involving the decomposition of A are set up by nervous impulses, then each luminescent organ must receive two types of innervation. A nerve impulse arriving from a tactile receptor sets going reaction 1 and luminescence results, but an impulse coming from the light receptor causes A to decompose (reaction 2) into an altogether different substance, D , which cannot give rise to luminescence until it has been reformed into substance A . The luminescent substance thus receives double innervation and the character of the decomposition is determined by the type of nerve fiber stimulated.

CONCLUSIONS.

1. In the dark adapted *Mnemiopsis*, mechanical stimulation causes luminescence along the eight rows of paddle plates. The tactile receptors for this reaction lie only in the paddle plate rows, and are connected only longitudinally along these rows.

2. The tactile receptors for ciliary and muscular movement are distributed generally over the surface and are connected by a nerve net.

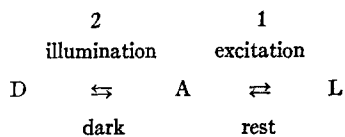
3. Luminescence may occur at 3°C. provided the animal has been kept sufficiently long at that temperature. Ciliary action goes on at - 0.6°C.

4. Luminescent paper made by spreading the luminescent secretion of *Mnemiopsis* on filter paper, yields the following effects. The paper shows luminescence in solutions of K_2SO_4 , KCl , $MgSO_4$, $SrCl_2$,

CaCl_2 ; no luminescence in NaCl , MgCl_2 . Changes in pH value of salt solutions between pH 6 and 8 do not affect the phenomenon. Illumination of the paper with strong light for longer time than necessary to suppress luminescence in the living animal has no effect on the subsequent luminescence of the paper. Hence in the animal, light affects luminescence through the photoreceptor system; the nervous system carries the impulse to the luminescent organs.

5. The power of luminescence of the animal is suppressed by sufficiently intense light, the relation between the intensity and the time requisite being expressed by the equation for the Bunsen-Roscoe photochemical law, namely, $I \cdot t = K$.

6. It is suggested that the reaction scheme involved in luminescence is of the following form



in which A is the luminescent substance in the resting, dark adapted animal, L is the light-giving decomposition product, and D is a product which does not yield light.

7. The luminescent substance receives double innervation and the character of the decomposition is determined by the type of nerve fiber stimulated.