LUMINESCENCE IN PELAGIA NOCTILUCA.

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Pelagia noctiluca is a medusa found in the Mediterranean during the spring months. This animal shows a diurnal rhythm in its luminescence. Early in the evening it begins to give brillant light in response to mechanical stimulation. If the animal is weakly stimulated, as by a mere touch, the area of luminescence is confined to the region stimulated (local luminescence); but if the stroke is a vigorous one, the whole bell and tentacles respond with a glow which may last for some seconds (general luminescence) (1).

Rhythmical Pulsations and General Luminescence.

As in the case of other medusæ, *Pelagia* pulsates rhythmically, and the rate of the rhythm is quickened by stimulation. The phenomena of general luminescence and pulsation, as will be shown, are both under the control of the nervous system and in each case the reaction depends upon the ionic composition of the fluid in which the animal is immersed. As to the rhythmical contractions of medusæ, numerous studies have been made showing the dependence of contractions upon the ionic content of the surrounding fluid: Loeb on *Gonionemus* (2) and *Polyorchis* (3), Herbst on *Obelia* (4), Mayer on *Cassiopeia* (5), Bethe on *Rhizostoma* (6). If *Pelagia* be placed in artificial sea water (van't Hoff's solution) containing solutions of pure salts isosmotic with the sea water of Naples Bay (0.6 M) in the following proportions, NaCl 100, $CaCl_2$ 1.5, KCl 2.2, $MgCl_2$ 7.8, $MgSO_4$ 3.8 plus 20 cg. $NaHCO_3$ per 1,000 cc. of solution, the medusa lives and reacts just as in ordinary sea water. But if one of the cations is omitted from the solution the conduct of the animal with regard to pulsations and luminescence is entirely altered.

1. In a solution from which $CaCl_2$ is omitted the pulsations of *Pelagia* stop in 3 to 5 minutes. At the same time general luminescence fails, so that when the animal is strongly stimulated light appears only in the area of contact. If now the proper amount of $CaCl_2$ be added to the solution or if the animal be returned to artificial sea water, the beats reappear within 90 seconds and simultaneously the power of general luminescence is restored. It must, therefore, be concluded that $CaCl_2$ is necessary to the conduction of impulses both for the muscular beats and for general luminescence.

2. If the solution lacks K ions, after 10 minutes the pulsations stop in the diastolic phase and at the same time the power of general luminescence is lost. When the animal is replaced in sea water or in complete van't Hoff's solution the beats return within 65 seconds and general luminescence is reestablished in 140 seconds. These results prove that K like Ca is necessary for normal beats and for the conduction of the impulse for general luminescence.

3. In a solution containing no Mg salts, *Pelagia* shows great acceleration of beat, and after 11 minutes stops in systole with spasmodic fibrillation. During this time automatic flashes of light appear and, if the body is touched, the whole surface breaks into light and glows for some seconds. This condition persisted for at least an hour and a quarter, at the end of which time the observations were discontinued. Since the absence of Mg results in a condition of hyperirritability both with regard to rhythmical contractions and general luminescence, it follows that Mg ions must act to decrease irritability. The locus of the action of Mg on the luminescence reaction is some part of the nervous system, since an excess of Mg inhibits general luminescence, but does not affect local luminescence.

Inhibition of Luminescence by Light.

The fact of nightly periodicity in the luminescence of *Pelagia* suggests the possibility of inhibiting luminescence at night by means of artificial illumination. Such a relation has been shown to exist in other forms, (Allman (7), Peters (8), Heinemann (9)). It was found that exposure to light of the carbon arc resulted in inhibition of general luminescence, but did not affect local luminescence. These results suggest that strong illumination acts on the nervous mechan-

ism to inhibit general luminescence, but exercises no direct effect on the luminescent organs themselves, since local luminescence persists. It should be noted, however, that during the day excitation of *Pelagia* fails to elicit local luminescence even after 5 hours in the dark room.

It was next attempted to determine whether inhibition of general luminescence by light depended upon the absolute intensity of the light alone or upon the quantity of light (intensity time). Two series of experiments were completed with different individuals. The animal was put into a rectangular glass box filled with sea water, and the preparation placed in such a position that it lay directly in the path of the rays of a carbon arc of 1,000 c.p. The specimen was always tested for general luminescence before being subjected to illumination. If found to be properly sensitive, light from the arc

TABLE I.

Intensities.	A Time.	B Time.
meter candles	*sec.*	*sec.*
4,000	22	180
1,000	52	480
250	150	720
62.5	600	

was admitted by removing a screen of black cloth. After an exposure for a certain number of seconds the arc was extinguished and the animal tested for general luminescence. If local responses alone were obtainable then it was considered that sufficient illumination had been given, and another trial with a shorter exposure was next made, but only after the specimen had been allowed to recover its power of general luminescence by remaining in the dark; this usually took 6 to 10 minutes. The table gives approximate values for the times of exposure which are required at the intensities stated to suppress general luminescence. The data obtainable were not sufficiently extensive to warrant further mathematical treatment.

The figures show that inhibition of general luminescence by light may be regarded as a function of the quantity of light received by the organism, and that individual *Pelagia* differ in their sensitivity to light.

Reaction of Luminescent Material.

In the course of the experiments it was found that at night *Pelagia* threw off quantities of luminescent material (1) which stuck to hands or towel and which would glow brightly if rubbed or if washed with fresh water. Use was made of this fact to prepare an indicator paper. This was done by rolling a *Pelagia* on filter paper, which was, as a result, saturated with luminescent material and if kept moist would continue to react for an hour or longer. Normally this indicator paper showed no light, but when torn, rubbed or put into proper solutions it glowed with light.[1]

TABLE II.

$MgSO_4$	18	16	14	12	10	8	6	4	4	2	
$MgCl_2$	2	4	6	8	10	12	14	16	16	18	
Result	S	S	S	S	W	W	W	W	0	0	
$BaCl_2$	10	8	6	4	2	$SrCl_2$	10	8	6	4	2
$MgCl_2$	10	12	14	16	18	$MgCl_2$	10	12	14	16	18
Result	W	W	W	0	0	Result	W	0	0	0	0
KCl	10	8	6	4	2	KCl	10	8	6	4	2
$MgCl_2$	10	12	14	16	18	NaCl	10	12	14	16	18
Result	M	M	W	W	0	Result	W	W	W	0	0

Letters indicate comparative intensities of light evoked. W = weak, M = medium, S = strong, 0 = no light.

When a piece of luminescent test paper was put into sea water no lasting glow was obtained, nor did the paper show luminescence when put into pure cane-sugar solution of concentration 1.1 M (isosmotic with the sea water of Naples Bay). This fact suggests two things: (1) that where the ions are balanced the luminescence reaction does not occur, and (2) in the absence of ions there is no reaction. Pure solutions of certain salts, however, in concentrations isosmotic with sea water, namely 0.6 M, caused the indicator paper to glow brightly for several seconds or even minutes. At the outset, we found that solutions of pure salts were effective in activating the paper in the following order as to brightness, $MgSO_4$, K_2SO_4, Na_3 citrate, KCl,

[1] The Ctenophores, *Eucharis multicornus* and *Beroë ovata*, and the Anthozoan, *Pennatula phosphorea*, yield material which behaves similarly.

$BaCl_2$, $SrCl_2$, $CaCl_2$, and LiCl while NaCl and $MgCl_2$ caused no luminescence of the test paper. It can further be shown that $MgCl_2$ and NaCl act as inhibitors to the reaction since if present in sufficient quantity they suppress the positive action of $MgSO_4$, KCl, $BaCl_2$ and $SrCl_2$. In the following experiments the solutions were made up to 20 cc. and the relative quantities of the two salts altered as indicated in Table II.

The salt solutions used were approximately neutral in reaction, but it was not possible to determine their exact pH values on account of lack of facilities in the Naples laboratory. However, the matter has been checked by one of us, since his return to America, using the luminescent material from *Mnemiopsis*. This behaves similarly to the material obtained from *Pelagia*. It was found that salt solutions of pH 7.7 caused characteristic effects on the luminescent material. Luminescence of the indicator paper occurs in solutions of K_2SO_4, KCl, $MgSO_4$, $CaCl_2$, but does not take place in NaCl and $MgCl_2$. Alterations in the pH values of the salt solutions between pH 6.0 and 8.0 are without effect on the phenomenon.

Since NaCl and $MgCl_2$ are present in sea water in sufficient concentrations to inhibit the activity of KCl and $MgSO_4$, the luminescent material of *Pelagia* does not glow in sea water.

Alkalies of concentration N/10 made up in isosmotic cane-sugar solution caused luminescence while mineral acids N/10 inhibited the action of $MgSO_4$ and KCl (10). Ammonia in concentration 0.027 N to 0.9 N made up in sucrose solution 1.1 M caused strong luminescence for 10 minutes at 20°.

In order to gain further information on the nature of the luminescence reaction, we determined the temperature coefficients for the reaction in $MgSO_4$ and in ammonia 0.9 N. The procedure consisted in bringing the solution to the required temperature in a water bath, then a piece of indicator paper was introduced into the solution and the time between the introduction of the paper, and the cessation of light was measured with a stop-watch. Experiments 1 and 2 were done with $MgSO_4$ solution 0.6 M and Experiment 3 with ammonia in sugar solution, the sugar solution being of concentration 1.1 M, and the ammonia 0.9 M.

TABLE III.

Experiment 1.			Experiment 2.			Experiment 3.		
Temperature of solution.	Duration of light.	Temperature coefficient.	Temperature of solution.	Duration of light.	Temperature coefficient.	Temperature of solution.	Duration of light.	Temperature coefficient.
°C.	sec.	Q_{10}	°C.	sec.	Q_{10}	°C.	sec.	Q_{10}
5	305	1.6	8	108	1.8	10	640	1.1
15	188		18	65	1.4	20	593	3.7
20	121	1.8	28	42	2.6	30	160	2.0
25	105	5.0	38	16	1.2	40	81	2.3
30	24	2.8	48	13		50	35	(9.0)*
35	37	1.4				60	4	
40	17							
47	6							
Average.....		2.52	Average.....		1.75	Average.....		2.27

* Very little light given, the magnitude of the temperature coefficient shows the destructive action of high temperature to be predominant. This figure is therefore omitted from the calculations.

The temperature coefficient for the reaction is thus about 2 for a difference in temperature of 10°C. This conforms to the van't Hoff-Arrhenius rule for chemical reactions and suggests that in the case of luminescence we have to do with a chemical reaction. This conclusion is in harmony with the work of Amberson (11) who studied the decay curve of luciferin and found the luminescence reaction to follow the course of a monomolecular reaction.

Harvey, as a result of his work on luminous bacteria, suggests that luminescence and cytolysis may be related. In the case of *Pelagia* such a view seems plausible since water and hypotonic solutions cause the luminescent material to glow. But several facts serve to indicate that cytolysis and luminescence in *Pelagia*, are not conditioned by similar reactions.

(1) Raising the temperature of the sea water in which luminescent material is immersed does not cause the luminescent reaction although such a procedure does produce cytolysis of egg cells, blood corpuscles, etc.

(2) Solutions of pure salts in the concentration used to cause luminescence, do not produce cytolysis.

CONCLUSIONS.[2]

1. Ca and K condition the irritability of *Pelagia* both in regard to rhythmical contractions and general luminescence. If either ion is omitted from the solution conduction of stimuli for pulsations and luminescence does not occur, although local responses still persist.

2. When Mg is omitted from the solution, *Pelagia* shows hyperirritability with respect to rhythmical contraction and general luminescence. This is referable to the unantagonized action of K and Ca ions.

3. Exposure to the carbon arc suppresses general luminescence, the effect depending upon the quantity of light *i.e.* intensity $\times$ time of exposure.

4. The luminescent material secreted by *Pelagia* is inactive in sea water, but when put into salt solutions is activated by some of them. The efficiency of the salts, measured by brightness of light, is in the following order: $MgSO_4$, K_2SO_4, Na_3 citrate, KCl, $BaCl_2$, $SrCl_2$, $CaCl_2$, and LiCl while NaCl and $MgCl_2$ act as inhibitors.

5. Acidity inhibits the reaction, alkalinity promotes it. NH_4OH in concentrations 0.27 N to 0.9 N causes luminescence for 10 minutes at 20°.

6. The average temperature coefficient for the reaction of the luminescent substance when activated by ammonia or $MgSO_4$ is 2.18 for a temperature interval of 10°C.

[2] A number of the facts which we have determined regarding the luminescence of *Pelagia* incidentally have a bearing on the views put forth by Pierantoni (12) and Zirpolo (13) according to which luminescence in Metazoa is caused by symbiotic luminous bacteria (16). Such a view is untenable as regards *Pelagia* since (1) this animal repeatedly excretes within a very short time enormous quantities of luminous material, a result which can come only from a process akin to glandular secretion. (2) It is impossible that bacteria and living cells in general could continue to live and function for 10 minutes in 0.9 N solution of ammonia, yet the luminescent material of *Pelagia* glows brilliantly in such a solution for 10 minutes at 20°, and for 8 minutes in 0.1 N NaOH. In fact, E. N. Harvey (10) finds that a hydroxyl concentration of N/1,000 is sufficient to extinguish the light of luminescent bacteria in 10 minutes and E. B. Harvey (15) reports that the luminescence of *Noctiluca* is extinguished in 2 minutes by N/250 NaOH. (3) General luminescence in *Pelagia* is under the control of the nervous system whereas Harvey finds that luminous bacteria living symbiotically on fish, glow continuously and are not affected in this respect through the nervous system (14).

7. The luminescence reaction cannot be the result of cytolysis, because (*a*) raising the temperature of sea water in which luminous material is immersed does not cause luminescence, although sufficient to produce cytolysis. (*b*) The salt solutions used in our experiments to cause luminescence, do not act cytolytically on cells in general.

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