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The Physiological Mechanism of Nematocyst Responses in Sea Anemone

VIII. Photodynamic Triggering of the Nematocysts *in situ*[1]

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As was shown in a previous paper by one of the writers (Yanagita, 1960), the large nematocysts (microbasic p-mastigophores) on the acontium of *Diadumene luciae*[3] discharge their stinging threads in response to contact of a prey animal as well as of human skin surface. It was also found that K^+ or NH_4^+ ions in the external medium or an electric current flowing across the acontial surface have a similar effect of exciting the nettling mechanism.

Since the latter group of facts was suggestive of the ordinary type of "excitable membrane" being involved in the present system, it seemed interesting to see if photodynamic treatment of the nematocyst-bearing structure of acontium may have an effect of activating that system. Apart from those cells and tissues which contain naturally a photosensitizing pigment, an ordinary photic stimulus is known to be effective on a variety of excitable systems including vertebrate muscle (Lippay, 1959 and 1930; Lippay and Wechsler, 1930; Lillie, Hinrichs and Kosman, 1935; Kosman, 1938). Though Stieve (1958) was able to deal with only a decreased excitability as the photodynamic effect on frog sciatic fibres, stimulation of the same nerve by this means has long been one of the laboratory course stuffs familiar to the physiology students (See, e. g., Yamamoto, 1949). One of the recent examples of the photodynamic excitation was afforded by Okajima (1961) on the ciliary and myoneme responses in the ciliate, *Spirostomum*.

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3) A new generic name, *Haliplanella*, was created for this species by Hand (1956), whereas Dr. Cutress of the Smithsonian Institution recommends reapplication of Carlgren's old name, *Aiptasiomorpha*.

Material and Method

Diadumene luciae used in this study was collected on the rocky shore next to the campus of Misaki Marine Biological Station and kept in the writers' laboratory in Tokyo, in large battery jars of circulating and self-filtering sea water. They were fed from time to time with living brandlings (*Allolobophora* sp.).

Pieces of acontial filaments, about one centimeter in length, were clipped off from the anemone and kept in a dish of sea water. A piece was placed in a small pool of sea water on a glass slide containing the photodynamic dystuff (eosin, S. S. Konishi and Co., Tokyo, almost exclusively) in dilution, and, simultaneously, illuminated obliquely from top with a light beam from an ordinary low-voltage microscope illuminating lamp. No light filter was used. A diversion from this procedure, which consisted in staining the material previously in darkness for 5 to 10 minutes and then bringing it to the illumination, was tried on occasion. In another group of tests, modified sea water such as to be described below was used as the solvent for the dye, in place of natural sea water. The acontium was kept watched during the period of immersion and illumination, under a low power of microscope, for nematocyst response, if any.

Parallel sets of observations were carried out with isolated nematocyst preparations, which were obtained in the way to be described below.

The intensity of illumination was determined by means of "Etalon" Photo-electric Lux-o-meter PH-II (maximum sensitivity : 2 lux). All the experiments were performed at uncontrolled room temperature in summer, which varied around 30° C.

Results

The effect of the concentration of dye

When varied concentrations, C, of eosin solution (in sea water) were tested for their effect on the nematocysts-in-acontium system with the intensity of illumination, I, fixed at 40 klx, the results obtained were as shown by a typical set of data which is given in Table I. Each figure in this table represents average from repeated tests, while all the tests for this set of data are derived from acontia of one and the same specimen of anemone. (The parenthesized entries mean uncertain occurrences.)

At eosin concentrations above 6×10^{-4} M, there was a marked response of nematocyst explosin (discharge of the thread) starting all along the piece of acontium within two minutes of the immersion and illumination, and it went on for a few minutes. The explosin was

Table I. Nematocyst response of acontia of *Diadumene luciae* to illumination of 40 klx in different srengths of eosin solution (in sea water)

Concentration of eosin, C, in M	Nematocyst response		
	Reaction time, t, in min.	Time for the response to be finished, in min.	Form of response
2.4×10^{-3}	0—1	3	Extrusion plus explosion
1.2×10^{-3}	1	3	„
6×10^{-4}	2	5	Extrusion (plus explosion)
1.2×10^{-4}	5*	8*	Extrusion only
6×10^{-5}	5	10	„
1.2×10^{-5}	15*	20*	„
6×10^{-6}	30	40	„
1.2×10^{-6}	40	60	(Extrusion only)
1.2×10^{-7}	—	—	No response

accompanied by extrusion (slipping out) of the capsule bodies from the surface of acontium. As the eosin concentration was lowered from this, the effect was not only progressive prolongation of the reaction (i. e., illumination) time, t, but also a gradual shift of the form of response from "extrusion with explosion" to "extrusion only". The threshold concentration for response was to be located at somewhere between 10^{-6} and 10^{-7} M.

The data obtained by illuminating the acontium previously in darkness (see above) were also included in Table I, as marked with asterisks. As may be seen, the illumination time required for the response was practically unchanged from that derived from the former procedure, indicating that the time taken for the staining process itself was relatively short.

The observed values for C and for t included in Table I will give a straight line rather nicely, when plotted on a coordinate system with logarithmic scales on both axes. This means that an empirical formula of the type:

$$C\times t^{p}=k\,, \tag{1}$$

applies to the present case. The value of p will come out as 1.7 to 1.8, by graphical means, while $k=20$ against the t value in seconds.

The effect of the intensity of illumination

The effect of varying intensity of illumination down from 40 klx on the nematocyst excitation by photodynamic means is illustrated by a representative set of data derived from a single anemone specimen, which are summarized in Table II. The eosin strength used was 1.2×10^{-4}M (0.01%) throughout this set of tests. The illumination was

Table II. Nematocyst response of acontia of *Diadumene luciae* to different intensities of illumination in 1.2×10^{-4} M eosin solution (in sea water)

Illumination, I, in klx	Nematocyst response (extrusion)		
	Reaction time, t, in min.	Total amount	Slipping out of individual capsules
40	5	++	Complete
20	7	++	Complete
10	10	++	Less complete
5	20	++	Incomplete
1	60	+	,,

started at the same time as the staining. Corresponding to the relative weakness of stain as well as of illumination used, the response of nematocysts here consisted in the extrusion of capsules without explosion in all the cases, but the reaction time went much prolonged with decreasing intensity of illumination. Besides, the extrusion became less and less in number as well as in individual completeness (see Table II), until the threshold intensity was reached somewhere below 1 klx.

The I and t values from Table II may well be fitted into the equation:

$$I\times t^{p}=k\,, \tag{2}$$

instead of the Bunsen-Roscoe reciprocity law ($I\times t=k$). The value of p is to be estimated graphically as 1.5, whereas $k=5200$ against the t values measured in seconds.

The tests with the isolated nematocysts

The isolated nematocysts were obtained from the acontia utilizing the extrusion response toward a salt-free environment (1 M glycerol solution in deionized water) (see Yanagita, 1959a, b, c). They were re-suspended in eosin solution prepared with 1 M glycerol solution as the solvent (sea water could not be used because of its explosion-eliciting effect on naked nematocysts), and illuminated on the microscope stage with an intensity up to 40 klx. No explosion was observed at all, indicating that the response described above of the nematocysts-in-acontium system is the one which is mediated by the living matrix surrounding the nematocysts.

The effect of lowered Cl^- ion concentration in the external medium

In view of the role ascribed to the external Cl^- ions in those forms of nematocyst response which have so far been studied (see Yanagita, 1959c and 1960), the effect of the external media containing less Cl^- ions than normal sea water was studied as to the photo-

dynamic excitation of the nematocysts-in-acontium complex. The chloride-poor media used for this purpose (M/20 and M/40 Cl^-) were prepared by simply mixing normal (natural) sea water with isotonic (1 M) glycerol solution in ratio of 9 : 1 and 19 : 1, respectively. They were used unbuffered.

When the acontia were immersed in 1.0×10^{-4}M eosin solution which had been made with either one of these mixtures, and illuminated with 40 klx, the results were something against the expectation; in addition to the capsule extrusion, there was a marked amount of explosion (nearly in hundred per cent of the extruded capsules) elicited from the acontium under the given conditions. Since the response would have consisted in "extrusion only" in normal sea water under comparable strength of stain and illumination (see Tables I and II), the scarceness of Cl^- ions in the exterior was to be looked upon as having an effect of facilitating (and not preventing) the explosion. A similar series of tests was made also with stain and illumination strengths still lower than these, i. e., 10^{-5}M$\times$5 klx. The Cl^--poor media were found to render even such a weak photodynamic treatment (which would have been hardly effective in inducing any response at all in normal sea water) effective enough to elicit some nematocyst explosion from the acontium.

The effect of photodynamic dyes other than eosin

So far, a limited amount of preliminary tests were performed only with Bengal rose (Tokushu Chem. Co., Tokyo) and with Methylene blue (E. Merck A. G., Darmstadt). Methylene blue was found to be barely effective at a concentration of 0.02% (6.5×10^{-4}M) in causing some extrusion (but no explosion) to take place from the acontia in response to a photic stimulation with 40 klx. With Bengal rose (0.1% corresponds to 1.0×10^{-3}M), the record obtained was as shown in Table III.

Table III. Nematocyst response of acontia of *Diadumene luciae* to illumination of 40 klx in different strengths of Bengal rose solution (in sea water)

Concentration of Bengal rose, in M	Nematocyst response	
	Reaction time, in min.	Form of response
1.0×10^{-3}	3	Extrusion only
1.0×10^{-4}	5	〃
1.0×10^{-5}	15	〃
1.0×10^{-6}	25	(Extrusion only)

Consideration

That the photodynamic action affects primarily the cell surface has been demonstrated rather amply, e. g., by Tappeiner (1909) and Blum (1941) for vertebrate red cells, by Kosman and Lillie (1935) for vertebrate muscle, and by Dognon (1927) and Okajima (1961) for ciliates. This is seemingly the case also in the present system, in view, particularly, of the apparent shortness of the time required for immersion in dye solution to be effective. Such a situation may lead quite naturally to an interpretation of the present findings along the line of the "chloride ion hypothesis" of nematocyst responses, which has been proposed by one of the writers (Yanagita, 1959c).

The first two sets of data (Tables I and II) presented above, as combined with the results from the tests with the nematocysts in isolation, may well indicate that the photodynamic effect causes a certain kind of consistency change ("loosening") in the surface structure of the epithelial cells (see Yanagita and Wada, 1959), thus giving rise primarily just to the extrusion response of nematocyst capsules. The explosion may be considered as merely a secondary event here (like in the cases of other modes of exciting the nematocysts-in-acontium system), which will follow the extrusion, though in momentary sequence, with the result of bringing the nematocysts into contact with the external medium (normally, sea water) with its explosion-eliciting ion contents.

The reason why the explosion fails in the lower effective ranges of C and of I may be sought for in decreased *abruptness* of the individual process of extrusion, and, consequently, of exposure to the external medium, under these conditions. As a matter of fact, the individual capsules were seen to slip out more slowly from the surface of acontium when in response to weaker C and/or I, besides their prolonged reaction time. On the other hand, it is a fact established from the previous study (Yanagita, 1959b) that isolated nematocysts from *Diadumene* acontia become readily adapted to Cl^- ions through previous contact with subthreshold Cl^- concentration, so as to have their sensitivity to the same ions diminished.

According to Okajima (1961), there is an optimum concentration of stain (1×10^{-4}M eosin, at an unspecified intensity of illumination) to give the maximum effect of photic stimulation on *Spirostomum* cilia, supposedly due to a depressing influence of the dye penetrating deeper into the cell. Such an optimum could not be found in the present case. It is interesting to note that the value of the constant p in the equation (1) as well as (2) has turned out rather close to those found by Okajima for the ciliary (and also myoneme) response in *Spirostomum*.

Up to the foregoing, the chloride hypothesis seems to hold well in the case of photodynamic excitation. On the other hand, the results derived from the use of the chloride-poor media may require some special explanation. Though Cl^- ion level in these media was still well above the threshold for inducing "spontaneous" extrusion from the acontium, which is known to be about M/100 (Yanagita, 1959c), it is possible to suppose that they were high enough already to affect somehow the surface properties of acontium so as to make it more sensitive to photodynamic stimulation. (There was some indication that the immersion in M/60 Cl^--medium had an effect of inducing "spontaneous" extrusion from an eosin-stained acontium already in dim room light.) If this change involves an enhanced *abruptness* of the nematocyst extrusion which is to take place as the response of a photo-sensitized acontium to a photic stimulus, it might possibly favour a higher rate of explosin as was actually observed among the nematocysts extruded on coming into contact with the external medium.

As a matter of fact, however, it will have to be admitted that the nematocysts own sensitivity toward external Cl^- ions has been raised extraordinarily high in these cases, to make them react to a Cl^- concentration as low as M/40, instead of M/10 which is the threshold as is usually found (Yanagita, 1959b). It would be interesting to imagine that this value of M/40 may represent an original level of sensitivity of the nematocysts *in situ*, before any desensitization has taken place due to adaptation in the course of isolating procedure. On the other hand, information is still lacking as to whether there is any alteration in the Cl^- sensitivity of isolated nematocysts under the influence of eosin staining. This matter seems worth inquiring, because a chemical like ethanol is known to *sensitize* the isolated nematocysts toward Cl^- ions (Yanagita, 1959b). Verification of these possibilities has to be left for investigations in future.

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Summary

Photodynamic sensitization of the acontium of *Diadumene luciae* was found to have an effect of making its nematocysts respond to an ordinary light stimulus and discharge their stinging threads. Exposure of the acontium to an illumination of 40 klx in 6×10^{-4}M eosin solution in sea water was sufficient to cause the nematocyst explosion, while the sensitization weaker than that tends to result in capsule extrusion without explosion. Nematocysts in isolation were found insusceptible to photodynamic activation.

The results were understandable along the line of the chloride ion hypothesis of nematocyst response, though some additional explanation seemed necessary as to the effect found of Cl^--poor media which facilitate the explosion response to a photodynamic stimulus.

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