THE RESPONSE MECHANISM OF NEMATOCYSTS: THE CASE OF FRESHWATER HYDRA

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THE RESPONSE MECHANISM OF NEMATOCYSTS: THE CASE OF FRESHWATER HYDRA[1),2)]

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Though many pieces of work have appeared during recent years on the coelenterate nematocysts (cnidae), applying modern tools of research and producing novel informations, the central problem of physiological response mechanism of cnidae seems to remain unfurthered. Several years ago, the present author (YANAGITA, 1960a) proposed a hypothesis on that mechanism on the basis of observations and experiments with one type of cnidae, microbasic p-mastigophores, of the acontial filaments of the sea anemone *Diadumene luciae.*[3)] In this hypothesis, the unit system of response as an independent effector is interpreted to be consisting of two trigger devices, the outer and the inner, which act one after the other through mediation of a certain coupling agent. The outer trigger was shown experimentally to possess the responsive characteristics of usual excitable membrane, and is suggested to correspond morphologically to the surface membrane of the epithelial cell or of the cnidoblast which harbors the cnida (*cf.* WESTFALL, 1965). The inner trigger device is represented by the cnida itself, which is embedded beneath the living cell surface as a lifeless collagenous structure (organelle) secreted in the cnidoblast (*cf.* BLANQUET and LENHOFF, 1966).

As for the coupling agent mentioned above, however, the author's hypothesis with the anemone cnida proposes the chloride ions in external sea water, which, upon the action of the outer trigger, *i.e.*, the "excitation", come in contact with the internal cnida to pull the inner trigger. It was obvious that this proposition could

1) Contributions from the Marine Biological Station of Asamushi, Aomori Ken, No. 370

2) Substantial part of this work was done while the author was staying at Dr. A.L. Burnett's laboratory of the Western Reserve University, Cleveland, Ohio. The author expresses gratitude to Dr. Burnett and his laboratory members for their hospitality and help in many ways.

3) HAND (1956) has created a new genus *Haliplanella* for this species, whereas CUTRESS (personal communication) suggests to revive the old generic name of CARLGREN, *Aiptasiomorpha.*

not apply, at least just as it was, to the cnidae of any freshwater form. Such a situation has prompted the author to attempt some preliminary inquiries with the cnidae of the freshwater hydra.

EXPERIMENTAL

The material was chiefly the stenoteles (penetrants) of the tentacles of the giant European hydra *Hydra pirardi*[1] cultured in the "BVC" medium as modified from the original prescription by LOOMIS and LENHOFF (1956): 1.18 mM $NaHCO_3$, 0.15 mM disodium versene, and 1.35 mM $CaCl_2$ in distilled water. The other types of tentacle cnidae, desmonemes and isorhizas, were taken notice of only on occasion. The behavior of cnidae was studied *in situ* (*i.e.*, as embedded in the tentacle) as well as in isolation.

The responsive behavior of the nematocysts in situ. There are already confirmations by a number of authors (JONES, 1947; KLINE, 1961; BURNETT, DAVIDSON, and WIERNICK, 1963) that the cnidae of the hydratentacles explode (discharge their threads) in response to electric shocks. In the present study, therefore, possible efficacy of externally applied electrolyte ions in provoking the same response was examined. The test solutions were applied to flood over the whole hydra body or the excised tentacle crown placed on a glass slide with a minimum amount of the culture water, and inspection was made for an effect under the microscope. The overall result was similar to that which is to be obtained with the cnidae of *Diadumune* acontia (YANAGITA, 1960a), K^+ and NH_4^+ representing the group of explosion-provoking ions. RbCl and CsCl were also included in the present tests and were found to be equally effective, while LiCl, similarly tested, had no effect. The threshold concentration of all these effective ions were found to be below 50 mM,[2] in contrast to the rather higher levels required by the anemone cnidae *in situ*.

It is important here to note that the response to these ions was prevented reversibly by the previous treatment of the preparation in solution of anaesthetics such as urethane. Such anaesthetizability of the exploding response *in situ* had long been known as to the more natural forms of response of hydra cnidae including the one toward the contact of a prey (JONES, 1947; BOUCHET, 1961) and indicates the involvement of a usual excitatory process. Whereas both acid and alkali solutions provoke explosion from the *Diadumene* acontium as well as in the isolated cnidae (YANAGITA and WADA, 1953), it was found with the present

1) *Hydra pseudoligactis* was also tried on occasion, and no different behavior of the cnidae was noticed from that in *H. pirardi* in so far as the features dealt with in the present paper are concerned.

2) According to the estimation by KOBLICK and YU-TU (1967), the internal osmotic concentration of gastrodermal cells of *Chlorohydra viridissima* may be equivalent to 30 to 100 mM NaCl.

material that only the acid (HCl) was effective in the *in situ* conditions. This is interesting in reference to the fact to be mentioned below that only the alkali (NaOH) had the effect on the cnidae in isolation.

Dilute saponin solutions were found to have no steadfast effect of provoking explosion *in situ*, unlike in the case of the anemone (PANTIN, 1942; YANAGITA, 1960b). In these solutions, however, the epithelial "battery" cells swelled out remarkably leaving their contained cnidoblasts behind, and, after they had bursted and vanished eventually, there remained the cnidoblasts (still unexploded) exposed on the tentacle surfaces. This has offered a convenient method of exposing and isolating cnidobalsts, as will be mentioned later.

The responsive behavior of the nematocysts and cnidoblasts in isolation. In the case of the microbasic p-mastigophores of *Diadumene* acontia, a very convenient technique for obtaining isolated preparation of cnidae was that of utilizing the "extrusion" response to a salt-poor medium (YANAGITA, 1959b). It is, however, self-evident that the same method is not applicable to the present material of freshwater source, so that some more forcible measures had to be taken.

Brief sonication of whole hydra bodies in a small vial of culture water or of distilled water had been known to be sufficient for disintegrating their tissues with denuded, but unexploded, cnidae sedimented at the bottom in abundance.[1] The cnidae thus isolated, however, had been believed to be already functionless toward any known kind of explosion-eliciting agents. Then, it was just in the natural course of things for the present author to take up the task of testing for possible efficacy of those electrolyte anions which had been known to be capable of triggering the explosion of the cnidae isolated from *Diadumene* acontia (YANAGITA, 1959a). As the result of such tests which were made preliminarily on the hydra cnidae isolated through sonication, it was found that a number of anions including Cl^- actually elicited their explosion at a strength of order of 30 to 50 mM. Choline chloride solution also proved to be effective much like NaCl, etc. Within the rather limited range of anions so far tested, some discrepancies as to the effective anion species from the results with the anemone cnidae were to be noticed as shown in Table 1.

It is particularly interesting to note that HPO_4^{2-} ions had a quite remarkable effect of eliciting explosion of isolated cnidae (all the four types) from hydra, while $H_2PO_4^-$ were of no effect at all. Another difference from the anemone cnidae appeared to exist as regards the pH effect; though concentrated NaOH solutions elicited the explosion of isolated cnidae, 100 mM HCl solution (despite of its high Cl^- content) failed to show such an effect, in contrast to its efficacy on the cnidae *in situ* (see above).

1) The author owes to Mr. Richard Davidso an kind orientation into this matter.

Table 1 Threshold concentrations of anions for triggering explosion of the isolated cnidae from *Diadumene* and *Hydra*. The anions are aligned according as the classical lyotropic series of Hofmeister except for those parenthetized, which are given the place arbitrarily. The data for *Diadumene* are taken from YANAGITA (1959a).

Anions	*Diadumene* p-mastigophores	*Hydra* stenoteles
SCN^-	60 mM	<50 mM
(HCO_3^-)	60	—
I^-	120	—
ClO_3^-*	120	—
NO_3^-	100	—
Br^-	120-250	—
Cl^-	120-250	<50
$Acetate^-$	502	—
$(Butyrate^-)$	250	—
$(H_2PO_4^-)$	330	∞
HPO_4^{2-}	30-60	<30
(SO_3^{2-})	30	—
SO_4^{2-}	∞	<30
$Tartrate^{2-}$	∞	—
$(Oxalate^{2-})$	30-60	—
$Citrate^{3-}$	30	—

* Tested only as the potassium salt.

All these ionic effects were insusceptible to anaesthesia also in the present material, indicating their nature as of a direct physicochemical event.

The results were apparently the same as those obtained with the naked cnidae when the anion tests were made on the preparations of isolated cnidoblasts obtained with the saponin treatment of tentacles described above. Further, such cnidoblasts were already responseless toward the K^+ ions, etc., unlike the cnidoblasts *in situ*. These facts lead one to suspect physiological invalidity of the "Plasmamantel" of the cells, at least after their removal from the original places in the tentacle.

The effects of dehydration and re-hydration treatment of the tentacle. It had long been known that dehydration followed by re-hydration of cnida-bearing tissues is quite effective means of demonstrating the discharge of coelenterate cnidae (JACOBSOHN, 1912; WEILL, 1925, 1926; JONES, 1947). For this reason, the presence of water has been claimed to be an essential factor for the event of explosion.

The present author tried the dehydration and re-hydration experiment on the hydra tentacles by simply immersing them in absolute ethanol on a watch glass and then allowing this evaporate. When a small amount of distilled water was applied to the tentacles which had thus been dried up, there was an abundant discharge of stenotle and other cnida threads from the surfaces. Though the discharge of stenoteles was rather abnormal in appearance in that the eversion

of threads proceeded much slower and the threads everted assumed a coiling shape, the result seemed to only confirm the conventional findings. However, when the ethanol treatment was repeated once more prior to the re-hydration so as to remove salts from the tissue, there was practically no discharge of threads on re-hydration. Further, if a solution of, say, 50 mM NaCl, was used instead of distilled water for re-hydration, the discharge ensued invariably even under the latter conditions. These results indicate inadequacy of pure water for eliciting the explosion and the necessity of the presence of a certain strength of electrolyte, of which there was evidence here again that the anionic moiety was active.

DISCUSSION

From the results so far presented it may be stated that the overall situation is alike between the hydra cnidae and the *Diadumene* ones except for the role to be ascribed to the chloride ions from the external environment in the response mechanism of the latter. In particular, the conditions for excitation to be provoked are much the same in both cases, in spite of the presence in hydra cnidae of the cnidocil structure. In the case of hydra cnidae, however, which operate in fresh water as the external environment, the coupler agent already mentioned has to be sought for in some internal factor. Thus, for instance, if there is intracellular some inter release (instead of intrusion from exterior) of a sufficient amount of anions, say, HPO_4^{2-}, in the cytoplasm at the distal ("stoppered") end of cnida capsule in a moment of excitation, it would be a just logical inference from the results given above (see Table 1) that it must lead necessarily to the explosion of that cnida. It is interesting to note in this connection that, according to LENTZ and BURNETT (1961), there is localization of certain phosphatases to be detected cytochemically in the said region of hydra cnidoblasts, and, further, external application of substrate and inhibitor substances for the phosphatase activities to the tentacle causes sensitization and desensitization, respectively, of the cnida response system toward mechanical contact from the exterior (LENTZ and BURNETT, 1962).

The fact that the isolated cnidae can be made explode by a mere contact with anions indicate also in the present material that all the energy needed for the event of explosion is preformed or built in within the cnidae themselves at maturity. Therefore, an energy-giving agent such as ATP, which LENTZ and BURNETT apparently suspect to play a role in explosion, might be eliminated from the scheme of mechanism, though for the intracellular process of "cnidogenesis" such should probably be called for. In this sense, the author would agree with JONES (1947), who considers that the excitation of cnidoblast merely triggers the explosion of cnida and that an active contractile response of cytoplasm which he assumes to take place does not supply the bulk energy required by the explosion process. However, his hypothetical cnidoblast contraction which should cause the

operculum to fall so as to release the cnida capsule to shrink freely is now unnecessary; if some intracellular ionic change that triggers is to be expected to exist for any myoid contraction to occur in coupling with the excitation, it would be superfluous to assume such a contraction to intervene in the present mechanism, now that it has been shown that an ionic change may directly trigger the final event of cnida explosion.[1)]