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NEMATOCYST INACTIVATION DURING FEEDING IN *HYDRA LITTORALIS*

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SUMMARY

Hydra littoralis uses stenotele nematocysts for prey-killing. Unused stenoteles are retained when prey-killing ceases. This inactivation results from an accumulation of soluble factors in the surrounding medium, and ends when hydra are placed in fresh culture solution. The inactivation factors are most probably released from the hydra during nematocyst discharge since stenotele activity is reduced by KCl solutions containing nematocyst factors and is enhanced by prey homogenate.

INTRODUCTION

Nematocysts are used by cnidarians for feeding, defence, aggression and locomotion. These harpoon-like organelles are discharged in response to specific chemical and mechanical stimulation (Pantin, 1942; Lubbock, 1979) of an external compound ciliary structure, the cnidocil (Mariscal & Bigger, 1976). Little is known of the physiological mechanisms of discharge, and it is not known whether nematocysts are independent effectors, or if their response can be altered by the host or the environment (see reviews by Picken & Skaer, 1966; Mariscal, 1974).

One situation in which 'control' of discharge might occur is following heavy feeding, when the ingestion of prey ceases. Previous studies of hydra have indicated that prey-killing nematocysts (stenoteles) remain unused upon 'satiation', thus conserving these complex structures: it has been suggested that hydra modify the responses of nematocysts after stimulation of stretch receptors in gut cells (Burnett, Lentz & Warren, 1960), or as a result of sensing prey factors in the gut (Smith, Oshida & Bode, 1974). Feeding sea anemones are known to inhibit the activity of their nematocysts (Mariscal, 1973; Sandberg, Kanciruk & Mariscal, 1971).

We present evidence that nematocysts of the brown hydra, *Hydra littoralis*, are inactivated after feeding, as a result of an accumulation of inhibitory substances in the environment. These factors seem to be released from the hydra during nematocyst discharge.

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MATERIALS AND METHODS

Hydra littoralis were purchased from the Carolina Biological Supply Co., Burlington, N. C., cultured in M-solution (Muscatine & Lenhoff, 1965) and kept in covered plastic boxes at 21 ± 1 °C under a 12/12 h light-dark photoperiod. The hydra were fed excess *Artemia salina* nauplii daily but were starved for 24 h prior to experimental use. After feeding, the culture solutions were changed and the boxes wiped clean of debris.

All experiments were performed in small round dishes 10 mm in diameter; each dish contained 1·5 ml of test solution and one or two hydra, depending on the experiment. Unless otherwise stated, we used 'adult' hydra, i.e. hydra with a single bud which lacked tentacles. The dishes were covered at all times to prevent evaporation except during feeding manipulations. The hydra were observed with a Wild dissecting microscope at 6× to 50×.

Feeding experiments and assay of stenotele activity

Experimental hydra were placed in test dishes and left undisturbed for 30 min before use to allow them to attach and expand their tentacles. Extensively washed *Artemia* nauplii were then presented singly *via* a flame-drawn Pasteur pipette. Each nauplius was repeatedly released near the tentacles until the shrimp swam into a tentacle. Freshly hatched nauplii were obtained every hour for feeding tests.

We defined the responses of hydra to offered shrimp as follows (Smith *et al.* 1974). A shrimp that struck a tentacle was either (1) killed and swallowed, (2) killed and not swallowed or (3) not killed. Of the last category, some were captured (adhered to a tentacle) and some were not. Escaped shrimp had to swim normally for 60 s after contact or release to be considered not killed. Since it was often difficult to distinguish slight tentacle contacts from near misses, positive contacts with four different shrimp were required for a 'never captured' designation.

All experimental hydra were presented with shrimp by this regimen until three consecutive 'not killed' responses occurred. At this time the stenoteles were considered to be non-functional or 'inactive'. The total number of shrimp killed prior to inactivation of stenoteles (responses 1 and 2 above) was defined as the 'killing response' for a given hydra.

Killing responses of buds and parents

We first determined if prey-killing by parent or an attached bud could affect prey-killing by the other hydranth. Two groups of hydra having one mature bud with tentacles were put in individual dishes. 'Mature' buds lacked a gut continuous with the parent under 50×; these buds often detached 2–4 h after experimentation. The parent or bud was offered shrimp until inactivation occurred, then the other hydranth (bud or parent) was similarly fed. During digestion of the larvae, we saw no ingested material pass between the two hydranths, confirming the separation of gut cavities.

In other experiments buds were allowed to detach before feeding. Two hours after the last bud had detached, solutions were changed, the hydra were left undisturbed for 30 min, and then preferential feeding of either parent or bud was begun. After the first hydranth had fed to inactivation, the killing response of the second hydranth was test

Inactivation of hypostome-tentacle preparations

To determine if stenotele inactivation could occur without a functional gut, we prepared hydra as follows. Eighteen expanded adult hydra were cut just below the level of the tentacles, and the pieces of hypostome with attached tentacles left in M-solution for 1 h while the cut surfaces closed over. The solutions were changed and feeding began 30 min later. These preparations readily captured and killed shrimp, but only one or two shrimp could be swallowed due to the limited gut volume. We saw no evidence that these shrimp were digested; enzymatic gland cells do not occur in the hypostomal gastroderm (Rose & Burnett, 1968). Killed shrimp that were not swallowed either remained on the tentacles or were released.

The effects of prey fluid on the killing response

These experiments were designed to test whether prey fluids at concentrations similar to those occurring during a feeding bout could alter nematocyst activity. To estimate the amount of protein which accumulated in media containing feeding hydra, we calculated the difference in protein content between culture solution which had contained fed hydra and that of unfed hydra (1·5 ml per hydra). Unfed hydra were kept in these solutions for the time required for hydra to complete feeding, as a control for sloughed hydra tissue. The mean increase in protein, as determined by the method of Lowry, Rosebrough, Farr & Randall (1951), was 5·8 μg per hydra. Chilled homogenates of washed *Artemia* were centrifuged ('clarified') at 12 000 ***g*** for 30 min, and samples (9·8 μl) of the supernatant equivalent to the net protein increase due to feeding were added to dishes of unfed test hydra. Controls received an equal volume of culture solution. Assays of killing responses of both groups were begun 5 min later.

Preparation of nematocyst factors

To obtain substances from nematocysts which might influence the killing response, we induced stenotele discharge by electric shock (Smith *et al.* 1974) and by ultrasonication (Wilby, 1976). In neither case could we induce discharge without extensive tissue damage.

As an alternative approach, we tested homogenates of hydra tissue which were either rich or poor in the types of nematocysts used in feeding. These included single tentacles, five tentacles, hypostomes with five attached tentacles, or peduncles (the region below the budding zone, including the pedal disc). Tissues from 24-h starved adult hydra were homogenized individually in 1·5 ml of M-solution; adult hydra were transferred directly to these solutions and tested 5 min later.

We also obtained nematocyst factors by inducing discharge with KCl. Five adult hydra were placed on separate glass slides in a drop of M-solution; residual fluid was replaced with 2 μl of 100 mmol l^{-1} KCl, sufficient to cover the hydra. Immediately, large quantities of nematocysts were discharged. When discharge appeared maximal (2–5 min), we transferred the KCl-nematocyst solutions to 5 ml of distilled water, rinsed each hydra three times with drops of distilled water, rinsed the slides after removal of hydra and pooled the rinses. Stock solutions of salts other than KCl were added and the final volume adjusted to yield 10 ml of M-solution (final $[K^+]$:

100 μmol l^{-1}) containing the nematocyst discharge solutions of five hydra. Ad hydra were tested 5 min after immersion in 1·5 ml of the nematocyst solution. Each test solution contained the discharge of nematocysts from 0·75 hydra.

Other experiments are described in the Results section.

Statistical analyses

Differences in the mean killing responses of two groups of hydra were tested with the two-sample *t*-test, and groups of percentage data were tested with the Mann-Whitney nonparametric test (Campbell, 1974). We found daily variation in the killing responses of control hydra; therefore, we made statistical comparisons only between animals tested on the same day.

RESULTS

Which nematocysts does hydra use during feeding?

Our observations confirm that only two types of nematocysts are used during feeding, as reported by Ewer (1947). Desmonemes entwine around prey setae and bristles during capture, and stenoteles pierce the prey and inject toxin.

We fed single *Artemia* nauplii to twenty hydra, then carefully removed the shrimp with watchmaker's forceps. The shrimp were examined with phase contrast optics (200×); the various types of nematocyst were easily distinguished with Mariscal's (1974) key. In most cases we observed 5–10 desmoneme nematocysts coiled around shrimp projections and 2–4 stenoteles with threads that had pierced the prey. We also observed discharged stenoteles which had fired but did not penetrate the exoskeleton. We never observed the use of isorhizas, although during an extensive feeding bout some of these, particularly the defensive holotrichs, might be discharged.

The loss of stenotele activity during feeding

Stenotele activity in *H. littoralis* declined gradually with increased prey-killing (Table 1). The stenoteles were most responsive to the first 5–15 shrimp that were offered: prey were killed immediately upon contact. As an additional 15–25 shrimp were killed, the responsiveness of the stenoteles declined; shrimp were readily caught,

Table 1. *Loss of stenotele response in adult* Hydra littoralis *during continual killing of* Artemia *nauplii*

Cumulative number of prey killed	Stenotele response
0–15	Stenoteles most responsive; prey killed immediately
20–40	Stenoteles less responsive; prey caught, struggle and then are killed
30–80	Stenoteles intermittently responsive; captured prey escape or are killed
—	Stenoteles inactive; captured prey escape or are never captured

Each hydra tested in 1·5 ml of M-solution.

but struggled for <15 s before being killed. When 25–35 shrimp had been ingested, hydra became satiated and no further ingestion occurred; additional shrimp were killed, but these were not moved to the mouth and were eventually released by the tentacles.

The stenoteles were intermittently responsive during the next phase. Some shrimp were caught and killed, remaining attached to the tentacles. Others escaped, and some of these survived. During this period the hydra could kill an additional 10–40 shrimp, after which no further stenotele activity could be detected; all shrimp which then struck tentacles were either caught and escaped or were never caught.

Stenotele activity and concomitant prey-killing under our conditions usually ceased after a hydra had killed 30–80 shrimp (Table 1), although we have observed as many as 110 shrimp killed by a single hydra. Desmonemes also became less active as prey-killing continued, because shrimp were often not captured during the final phase.

Stenotele depletion or inactivation?

To determine if the inability of fed hydra to kill additional prey resulted from the depletion of stenoteles, we compared the discharge of stenoteles in inactivated and unfed hydra as elicited with acidified methylene blue (Sherman & Sherman, 1976). Discharge in fed hydra was approximately one-half that of unfed hydra ($50{\cdot}2 \pm 17{\cdot}3$ *vs* $95{\cdot}3 \pm 31{\cdot}9$, $N = 6$ for each; $P < 0{\cdot}01$). This suggests that hydra retain one-half of their functional stenoteles at inactivation, although the response of stenoteles to methylene blue may differ from that to prey stimuli. The recovery experiments (see below) provide additional evidence for inactivation or inhibition, as do the various manipulations which affected the killing responses of hydra throughout this study.

Killing responses of attached buds and parents: does inactivation require the presence of food in the gut?

Table 2 shows the number of shrimp killed by attached 'mature' buds (see Methods) and parents before or after feeding of the other hydranth. The results show that prior feeding by parents decreased the mean response of buds ($P < 0{\cdot}005$), and that prior feeding by buds decreased the responses of parents ($P < 0{\cdot}025$). However, buds always killed fewer shrimp than did parents, and the relative effects of prior feeding were greater for buds. When parents fed, the killing response of buds decreased by 83·2 %, while in the converse experiment, parental responses were reduced by only 31·9 % ($P < 0{\cdot}05$, Mann-Whitney).

As the digestive cavities of parents and buds were not continuous, killing responses of affected hydranths must have been influenced by factors other than the presence of prey in the gut cavity. Two alternatives are that neural signals were propagated from the fed hydranth across the bud attachment zone, and that prey-killing and/or the external release of digestive products by one hydranth altered the chemical environment of the other. When this experiment was repeated using separated buds and parents (Table 2), prey-killing by the parents decreased the responses of buds ($P < 0{\cdot}005$), but prey-killing by buds had no effect on parental hydranths ($P > 0{\cdot}10$). The lowered bud response in this experiment must have been due to changes in the external solution, rather than to neural signals between polyps.

Table 2. *The decrease in killing response of attached buds and detached buds, and the parents, after feeding of the other hydranth to inactivation*

Experiment	Killing response ($\bar{X} \pm$ S.D.)	P(t-test)	% Decrease
Attached buds and parents:			
(A) Killing responses of buds:			
Bud fed first (8)	10·1 ± 4·6	<0·005	83·2
Parent fed, then bud (6)	1·7 ± 0·9		
(B) Killing responses of parents:			
Parent fed first (6)	63·0 ± 16·4	<0·025	31·9
Bud fed, then parent (8)	42·9 ± 13·3		
Detached buds and parents:			
(A) Killing responses of buds:			
Bud fed first (10)	17·4 ± 5·2	<0·005	48·3
Parent fed, then bud (6)	9·0 ± 3·3		
(B) Killing responses of parents:			
Parent fed first (6)	49·0 ± 15·4	NS	6·1
Bud fed, then parent	42·9 ± 12·3		

Sample sizes in parentheses
NS, not significant.

Media exchange experiments

Effects of 'used' culture solutions

To test the hypothesis that changes in the external environment during feeding caused stenotele inactivation, unfed hydra were tested in solutions in which other hydra had fed. In one series of experiments hydra were fed to inactivation in test dishes, dead shrimp were removed, and the solution ('used') was transferred to an unfed hydra which was tested 15 min later. The killing responses of the group in 'used' solution were significantly lower than those which were fed in fresh solution (Table 3; $P < 0{\cdot}001$). In other experiments we obtained 'highly used' solution by mass feeding of >100 hydra to inactivation in 25 ml of M-solution. The effects of 'highly used' solution on the killing responses of unfed hydra (an 82 % decrease; $P < 0{\cdot}001$) were greater than those of 'used' solution (63·3 %; Table 3), suggesting a greater accumulation of inactivating substance(s) in 'highly used' solution.

Table 3. *Decreases in the killing response of adult hydra fed in 'used' and 'highly used' solution*

	Killing response ($\bar{X} \pm$ S.D.)	P*	% Decrease
Controls in fresh M (15)	39·8 ± 11·4	<0·001	63·3
Experimentals in 'used' M (15)	14·6 ± 14·5		
Controls in fresh M (5)	59·4 ± 12·5	<0·001	81·5
Experimentals in 'highly used' M (4)	11·0 ± 10·2		

Sample sizes in parentheses.
* t-test, controls *vs* experimentals.

Recovery experiments

The previous experiments provided evidence that externally accumulating substances produce stenotele inactivation. In converse experiments we transferred inactivated hydra to fresh M-solution; inactivated controls were replaced in the solution in which they had been fed. The killing responses of all hydra were assayed 15 min later. Although the initial killing responses of the two groups were similar ($74 \cdot 6 \pm 16 \cdot 9$ *vs* $68 \cdot 8 \pm 17 \cdot 7$; $N = 5$, $P > 0 \cdot 6$), the group which was returned to fresh M-solution killed an additional $20 \cdot 6 \pm 8 \cdot 4$ shrimp; those which were re-immersed in their 'used' solutions killed only $4 \cdot 0 \pm 3 \cdot 8$ more shrimp ($N = 5$ for each; $P < 0 \cdot 02$). Within 15 min, the hydra in fresh solution recovered 26·7 % of their original killing response, while those which remained in 'used' solution regained only 5·4 % ($P < 0 \cdot 02$; Mann-Whitney).

The killing response in larger volumes

If factors which accumulate in the culture solution inactivate hydra stenoteles, killing responses in larger volumes of medium should be greater. We tested hydra in large (15 ml) and normal (1·5 ml) volumes of M-solution; smaller volumes were not practical. The killing response was nearly twice as great in the larger volume ($83 \cdot 3 + 7 \cdot 1$ *vs* $46 \cdot 6 + 13 \cdot 0$; $N = 5$, $P < 0 \cdot 005$).

Both the media exchange and dilution experiments clearly show that substances accumulate in the external medium of a fed hydra which inhibit the action of stenotele nematocysts, and provide additional evidence that the cessation of prey-killing is due to stenotele inactivation, rather than to depletion. The recovery of stenotele activity within 15 min would be unlikely if used nematocysts had to be replaced before additional killing could occur, and the feeding volume should have no effect on the killing response if depletion were the cause of cessation.

Inactivation of hypostome-tentacle preparations

To determine if the gut of hydra was the source of inactivating compounds and to investigate the site of their effect (i.e. ectoderm *vs* endoderm), we performed experiments with 'gut-less' adult hydra, lacking body columns. All 18 of these hypostome-tentacle preparations killed shrimp (killing response: $14 \cdot 4 \pm 7 \cdot 3$) and killed additional shrimp after transfer to fresh media. The inactivation of stenoteles occurred in the absence of digestion or significant ingestion (see Materials and Methods). In all cases the killing responses of these preparations were less than those of normal adult hydra.

Effects of prey homogenate on the killing response

If compounds present in prey fluids were responsible for the inactivation of nematocysts, then prey homogenate should reduce the killing response. However, the addition of a clarified homogenate of *Artemia* (equivalent in protein content to that accumulating in the environment of a fed hydra) actually increased the killing response by 35 % (Table 4).

In a related experiment, we tested the effects of crude *Artemia* homogenates, prepared by homogenizing 1 ml of washed, wet-packed larvae and filtering the homogenate through bolting silk. 160–170 μl of this crude preparation was added to

Table 4. *Killing responses of adult hydra in crude and clarified (12 000* g *supernatants) homogenates of whole* Artemia *larvae, homogenates of hydra tissue, and in KCl-induced nematocyst discharge solutions*

	Killing response ($\bar{X}$ ± S.D.)	*P*	% Change
Clarified *Artemia* homogenate (7)	60·1 ± 20·0	<0·05	+ 35
Controls (7)	81·1 ± 17·6		
Crude *Artemia* homogenate (9)	16·1 ± 12·1	<0·01	− 53
Controls (9)	34·3 ± 10·7		
Tissue homogenates:			
Single tentacles (5)	0·0	<0·001	—100
Controls (5)	23·8 ± 16·2		
Five tentacles (5)	0·0	<0·001	—100
Controls (5)	23·8 ± 16·2		
Hypostome-tentacles (8)	0·5 ± 0·8	<0·001	− 98
Controls (8)	21·3 ± 13·0		
Peduncles (8)	52·0 ± 25·0	NS	—
Controls (8)	35·0 ± 13·4		
Nematocyst discharge solution (8)	58·4 ± 13·2	<0·02	− 29
Controls (8)	81·9 ± 18·2		

Sample sizes in parentheses; statistical significance from paired *t*-tests.
NS, not significant.

test hydra, while controls received only M-solution. After behaviour associated with the feeding response subsided (15–30 min), we assayed the killing responses and found that crude homogenate decreased the response by 53 % (Table 4). Observation under 200× revealed that the crude homogenate elicited appreciable nematocyst discharge (75–100 stenoteles and 10–20 desmonemes per hydra), while no discharge occurred in hydra in either M-solution or when 10 μl of the clarified homogenate was added. It appears that the lowered killing responses in crude homogenate could have been due to the release of nematocyst factors into the medium.

Effects of nematocyst factors on nematocyst activity

Table 4 summarizes the effects of homogenate of nematocyst-poor or nematocyst-rich tissue on the killing response. Stenotele activity was negligible when hydra were tested in homogenates of tentacles (one or five), or of hypostome plus tentacles. The number of shrimp killed by hydra tested in nematocyst-poor homogenates of peduncles did not differ significantly from controls in M-solution ($P > 0{\cdot}2$).

The nematocyst solutions obtained by KCl-induced discharge also decreased the killing response of adult hydra (58·4 ± 13·2 for hydra in toxin solution, 81·9 ± 18·2 for controls; $N = 8$, $0{\cdot}02 > P > 0{\cdot}01$). KCl treatment seemed to discharge only stenoteles and desmonemes in great numbers, with most discharge occurring along the tentacles; discharged holotrichous and atrichous isorhizas were not seen. The killing responses in the nematocyst discharge solutions (1·5 ml, equivalent to 0·75 KCl-treated hydra) decreased by 29 % (Table 4).

DISCUSSION

Nematocyst inactivation and feeding responses

Lenhoff (1974) characterized feeding behaviour in hydra as occurring in three phases: (1) the capture and killing of prey after encounters with tentacles; (2) the contraction and bending of tentacles and opening of the mouth; (3) the ingestion of prey following contact with the mouth. One possible source of confusion of our work with that of others is the distinction between the first phase and the second, commonly termed the 'feeding response'. 'Feeding responses' in cnidarians refers to the behaviour which occurs between the capture of prey and ingestion. They are generally responses to particular amino acids or small peptides (e.g. reduced glutathione [GSH] in hydra, Loomis, 1955; proline and GSH in the scleractinian *Cyphastrea ocellina*, Mariscal & Lenhoff, 1968). The relationship between prey capture and subsequent behaviour is usually interpreted as follows: penetrant nematocysts pierce the body wall of the prey, releasing body fluids which contain activators of feeding responses. An alternative suggestion is that nematocysts themselves release activating compounds when they are fired (Burnett, Davidson & Wiernik, 1963). Our experiments are concerned only with discharge of penetrant stenotele nematocysts ('killing response'), and not with the ensuing feeding responses. Thus, studies such as those of Burnett *et al.* (1963), which have demonstrated that nematocyst discharge may stimulate the *feeding response* in hydra, are not contradictory to our finding that discharge of nematocysts by one hydra inhibits discharge in another.

Nematocyst inactivation and accumulated factors in the media

Previous workers concluded that inactivation of hydra nematocysts after feeding resulted from either a distended gut (Burnett *et al.* 1960) or the perception of prey-produced metabolites or ions by gut cells (Smith *et al.* 1974). Our experiments with 'used' and 'highly used' media and hypostome-tentacle preparations show that inactivation can occur without food in the gut, or even without a gut at all. These results and those involving detached buds and recovery provide good evidence that the external accumulation of metabolites during feeding produces inactivation; the comparison 'used' *vs* 'highly used' media and the experiments with large and small media volumes and with buds imply dose-dependency. This explanation can be applied to many of the previous results. For example, Smith *et al.* (1974) fed one head of a two-headed graft and produced partial inactivation of the other head, and concluded that material in the common gut cavity was responsible for the effect. As buds without connections to parents can be similarly inhibited (Table 2), their results could reasonably be explained as due to external factors. The experiments of previous authors involving the injection of *Artemia* homogenates into the gut *via* the mouth possibly caused the discharge of nematocysts by disturbing tentacles or the premature release of prey fluids; material released during nematocyst discharge is a possible source of inactivating substances.

The site of action of these factors appears to be the tentacle or hypostome surface, since the hypostome-tentacle preparations can be inactivated. Whether the effect is localized to the nematocyst 'trigger' (the cnidocil), to other structures or is generalized over the external surface remains to be determined.

The source of the accumulating factors

The source of the inactivating factor(s) is probably associated with nematocysts and not with prey fluids, since prey homogenate stimulated nematocyst activity, and nematocyst discharge solution was inhibitory (Table 4). Pantin's (1942) classic experiments also showed that prey homogenate stimulated nematocyst response in *Anemonia sulcata*, and Burnett *et al.* (1963) showed that a number of compounds likely to be present in prey fluids stimulated stenotele discharge in hydra. However, our interpretation is equivocal for the following reasons: (1) the *Artemia* extracts which we tested probably were not qualitatively identical with prey fluids which are released during prey capture; (2) the homogenates of tentacles which we used as a source of 'nematocyst-rich' tissue (Table 4) would contain substance which would come from sources other than nematocysts, and it is known that anemone tissue lacking nematocysts can be toxic (Burnett & Carlton, 1977); (3) the KCl solutions which we used to discharge nematocysts possibly caused the release of other compounds from hydra tissue. Critical evidence would be the effects of purified nematocyst substances on the killing response and the demonstration of these substances in the environment surrounding feeding hydra. Unfortunately, we have been frustrated in our attempts to isolate pure preparations of unfired nematocysts in bulk using detergents, salt solutions, sucrose gradients and other approaches (e.g. Blanquet, 1970; Wilby, 1976; Burnett & Carlton, 1977).

Burnett *et al.* (1963) reported experiments which suggested that discharged nematocysts were sources of reduced glutathione (GSH) or other compounds which induce the feeding response (mouth-opening and tentacle contraction) in hydra (Loomis, 1955). We have found that GSH in concentrations which elicit the feeding response (1–10 μmol l^{-1}) has no effect on the killing response. To our knowledge GSH has neither been detected in nematocysts, nor has been shown to affect nematocyst function. Burnett *et al.* (1963) also found that GSH had no effect on stenotele discharge.

External inactivation versus *control by the hydra*

Several of our observations indicate that hydra have some control over the response of their nematocysts. We failed to inactivate completely nematocysts of hydra exposed to 'used' or 'highly used' media, even after 15 min of exposure (Table 3); complete inactivation might be expected if inactivation were due solely to external factors. Hypostome-tentacle preparations always killed fewer prey than whole hydra (14 *vs* 30–80). This indicates that the body column plays a role in nematocyst discharge, as it does in some sea anemones (Davenport, Ross & Sutton, 1961; Ross & Sutton, 1964; Conklin & Mariscal, 1976). Finally, inactivated hydra recovered only 26·7 % of their original killing response after transfer to fresh culture solution. Unless some 'used' culture solution remained in the environment or some inactivating substances remained bound to receptor sites, this also suggests that inactivation is not solely controlled by the concentration of 'factors' in the medium.

We found some evidence that the effect of inactivation is concentration-dependent, as a ten-fold increase in the testing volume produced a doubling of the killing response. A possible reason why the difference was not greater is the chemical microenvironme

surrounding a hydra. Loomis (1961) demonstrated the existence of a 'halo' zone, a concentration gradient of metabolites and ions released from hydra. Lenhoff (1965) discussed how such a microenvironment affects the GSH-mediated feeding response, and proposed an 'ultramicroenvironment', a region of 50–150 nm resulting from the distribution of charged moities on the surface. The accumulation of nematocyst factors in the microenvironment during discharge might account for our results.

It is clear that further experimentation is needed to determine if the hydra plays an active role in nematocyst inactivation. As our results demonstrate that external substances which accumulate during feeding affect the nematocyst response, and possibly that these substances are associated with the nematocysts themselves, future studies on feeding and nematocyst function in cnidarians should address such possibilities.

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