Reference: *Biol. Bull.*, **147**: 186–202. (August, 1974)

INHIBITION OF NEMATOCYST DISCHARGE IN HYDRA FED TO REPLETION [1]

SCOTT SMITH, JAMES OSHIDA AND HANS BODE

Department of Developmental and Cell Biology, School of Biological Sciences, University of California, Irvine, California 92664

The nematocysts of coelenterates are commonly assumed to be independent effectors; that is, each nematocyst discharges in direct response to environmental stimuli, usually a combination of mechanical and chemical factors provided by the prey (*e.g.* Pantin, 1942; Picken and Skaer, 1966). Though *stimulation* of discharge is apparently not influenced by the animal itself, there is some evidence that *inhibition* of nematocyst discharge is. The sea anemone, *Calliactis parasitica,* will use the nematocysts of the tentacles to make a preliminary adhesion to a whelk shell. Once a solid attachment is formed by the pedal disk, the tentacles will not react to a second whelk shell indicating an inhibition of discharge (Davenport, Ross and Sutton, 1961). Ross and Sutton (1964) demonstrated that the tentacles of the sea anemone, *Stomphia,* will not capture prey when swimming which, though not strictly proven, suggests a control of nematocyst discharge. More recently, Sandburg, Kanciruk and Mariscal (1971) have shown that the anemone, *Calliactis tricolor,* greatly reduces nematocyst discharge upon feeding to repletion. Similarly, hydra will no longer capture shrimp larvae after feeding to repletion (Hyman, 1940; Burnett, Lentz and Warren, 1960) although Burnett *et al.* (1960) did not attribute this phenomenon to the inhibition of nematocyst discharge. In this report, we have extended the description of nematocyst discharge behavior of hydra fed to repletion. The results indicate that inhibition of discharge does exist and is probably due to a rise in concentration of a metabolite(s).

Materials and Methods

Hydra attenuata were used in all experiments. They were maintained in M solution (Lenoff and Brown, 1970), fed daily with *Artemia* nauplii, and washed 6–8 hours after feeding.

All feeding experiments were conducted with adult hydra having 1–2 buds. Each hydra was placed in 1 ml (in some experiments) or 10 ml (in others) of M solution. The hydra was offered a given number of shrimp, 1–10, depending on the experiment, which were placed repeatedly within the ring of tentacles until the animal caught them. Several minutes later (6–20), the number of dead and alive shrimp remaining outside the hydra was counted under a dissecting microscope (25 ×). From these numbers, the number killed and ingested was determined. After removal of the uningested shrimp, another group of shrimp (the same number as before) was offered. This procedure was repeated until the hydra would no longer ingest or kill shrimp, and the number captured was greatly reduced.

[1] This work was supported by grants from the National Science Foundation (GB-29284) and the National Institute for Child Health and Development (HD-08086-01).

Inhibition of the killing response, or stenotele discharge, was defined as five consecutive rejections of offered shrimp. A rejection was defined as a repeated contact (> 4) of one tentacle or three contacts of at least two tentacles by a shrimp which elicited neither a capture nor a killing response. Also, shrimp which were captured, escaped, and were still swimming normally 30 seconds thereafter were considered as rejected. At least two different shrimp had to be involved in the five consecutive rejections. Shrimp which contacted the tentacles and were not captured, but became immobile within 5–10 seconds were counted as killed.

For grafting experiments, hydra were bisected above the budding region, and the two apical halves were grated together by threading the pieces, back-to-back, on 12 lb nylon fishline (Stren, 0.013 in diameter). Sleeves of polyethylene tubing were placed at either end of the graft to prevent migration of individual pieces. After 60 minutes, the graft was removed to M solution and used in feeding experiments 4–7 hours later.

Nematocysts were discharged from the tentacles by electric shock as follows. Hydra were placed in M solution containing $2 \cdot 10^{-4}$ M fructose-1, 6-diphosphate (which enhances stenotele discharge; Lentz and Barnett, 1962) in a leucite chamber fitted with silver electrodes at two ends. Single pulses of current (40V, 5 mAmp, 13 msec duration) were delivered every 5 seconds by a Grass SD6 Student Stimulator. The percentage of stenoteles discharged was measured by counting the number of stenoteles in the tentacles before and after shocking as follows. Animals were placed in a drop of M solution on a glass slide, flattened slightly but not killed with a coverslip, and the visible stenoteles on the upper half of each tentacle counted with phase microscopy. The shocking procedure resulted in the discharge of 60% of the stenoteles in 10 minutes, and 90% in 40 minutes. By 40 minutes, about 50% of the other three nematocyst types were estimated to be discharged. The animals were not damaged by this procedure and were used for experiments 60 minutes after treatment.

Dilute and concentrated homogenates of *Artemia* were prepared as follows. Specimens of *Artemia* were centrifuged at 2000 *g* for 2 minutes. The loosely pelleted animals were transferred to a glass tissue homogenizer and homogenized. The homogenate was centrifuged at 300 *g* for 5 minutes to remove the skeletal parts. The resulting supernatant was termed dilute homogenate. The concentrated homogenate was prepared by pouring a thick suspension of *Artemia* onto a filter paper funnel to remove the fluid. The wet mass of shrimp was homogenized and the skeletal parts removed as before. Either homogenate was introduced into the gastral cavity of a hydra with a 10 μl Hamilton syringe tipped with a polyethylene needle. Normally, two injections spaced 15 minutes apart were made. After another 30 minutes, the ability of the animal to capture and kill shrimp was tested.

Results

Pattern of inhibition of nematocyst discharge during feeding

To put the nematocyst discharge behavior during feeding in context, a description of the capture, killing and ingestion of prey by hydra is necessary. Prey (usually *Artemia* nauplii in the laboratory) swimming close to the tentacles contact the long cnidocils of the desmonemes and cause discharge of these nematocysts. Each nematocyst everts a short, thick thread which wraps itself around any rod-

like object, such as, a spine on the swimming appendage of a shrimp. The trapped, struggling shrimp contacts the tentacle repeatedly and very soon hits the shorter cnidocil of a stenotele nematocyte. The nematocyst is discharged, everting a hollow thread which penetrates the shrimp, and a toxin is injected (Hessinger, Lenhoff and Kahan, 1973; Shapiro, 1968) which almost instantly paralyzes the shrimp, and probably kills it. Thereafter, the *Artemia* is transported to the hypostome by the tentacle and ingested. After taking in a number of shrimp by repeating this process several times, ingestion ceases. The number of shrimp ingested varies from day to day but for a group of animals of similar size is reasonably constant on a given day.

Since it is the stenotele and desmoneme nematocysts which are involved in feeding, the pattern of their discharge during the entire feeding process was first examined. Individual one-day starved hydra were placed in 1 ml M solution and a single *Artemia* placed within the crown of tentacles and the response noted. This was repeated until the animal no longer ingested or killed the shrimp larvae. A typical pattern with three or four stages emerged. At first, the starved animals quickly captured, killed, and ingested the shrimp. In contrast to earlier observations (Burnett *et al.*, 1960), shrimp were caught and killed on an out-stretched tentacle. Curling of the tentacle after capture to aid in bringing the shrimp in contact with a stenotele cnidocil did not seem to be necessary. After the ingestion of a number of shrimp, specimens of *Artemia,* were caught and killed but not ingested. Later shrimp were captured but not killed and eventually escaped. Such shrimp frequently struggled violently for up to two minutes making innumerable contacts with one or more tentacles before escaping. They were found to be swimming normally at least thirty minutes later indicating that they had not been struck by a stenotele nematocyst. This behavior indicates that discharge of the stenotele nematocyst had become inhibited. Finally, a stage was reached where shrimp could contact several tentacles in succession without capture. Though this was not carefully documented, and inhibition was not as complete as for stenotele discharge, a distinct inhibition of desmoneme discharge was apparent.

The behavior, or feeding activity, of six animals is shown in Figure 1 (solid line) in which each of the responses is given a numerical value: 3 = capture, kill and ingestion; 2 = capture, kill, but no ingestion; 1 = capture and escape; and 0 = no capture. The pronounced decreasing trend, which is typical, clearly depicts the change in response of the animal (= change in nematocyst discharge behavior) during the course of feeding. The increasing cessation of killing indicates that stenotele discharge becomes increasingly inhibited. The decreased activity and inhibition of nematocyst discharge is not due to a factor accumulating in the medium because the same behavior is obtained if the medium is changed after every five shrimp offered, or if the experiment is carried out in fifty ml of M solution. As a second measure of the increasing inhibition of stenotele discharge, the time required to kill a shrimp after capture was determined for each consecutive shrimp caught and killed by the animal. With increasing number of shrimp killed and ingested, the time of killing increases implying an increasing inhibition of stenotele discharge or a raising of the threshold for stenotele discharge.

Burnett *et al.* (1960) also found that after feeding to repletion killing of shrimp ceased and their capture occurred far less frequently than at the beginning of feeding

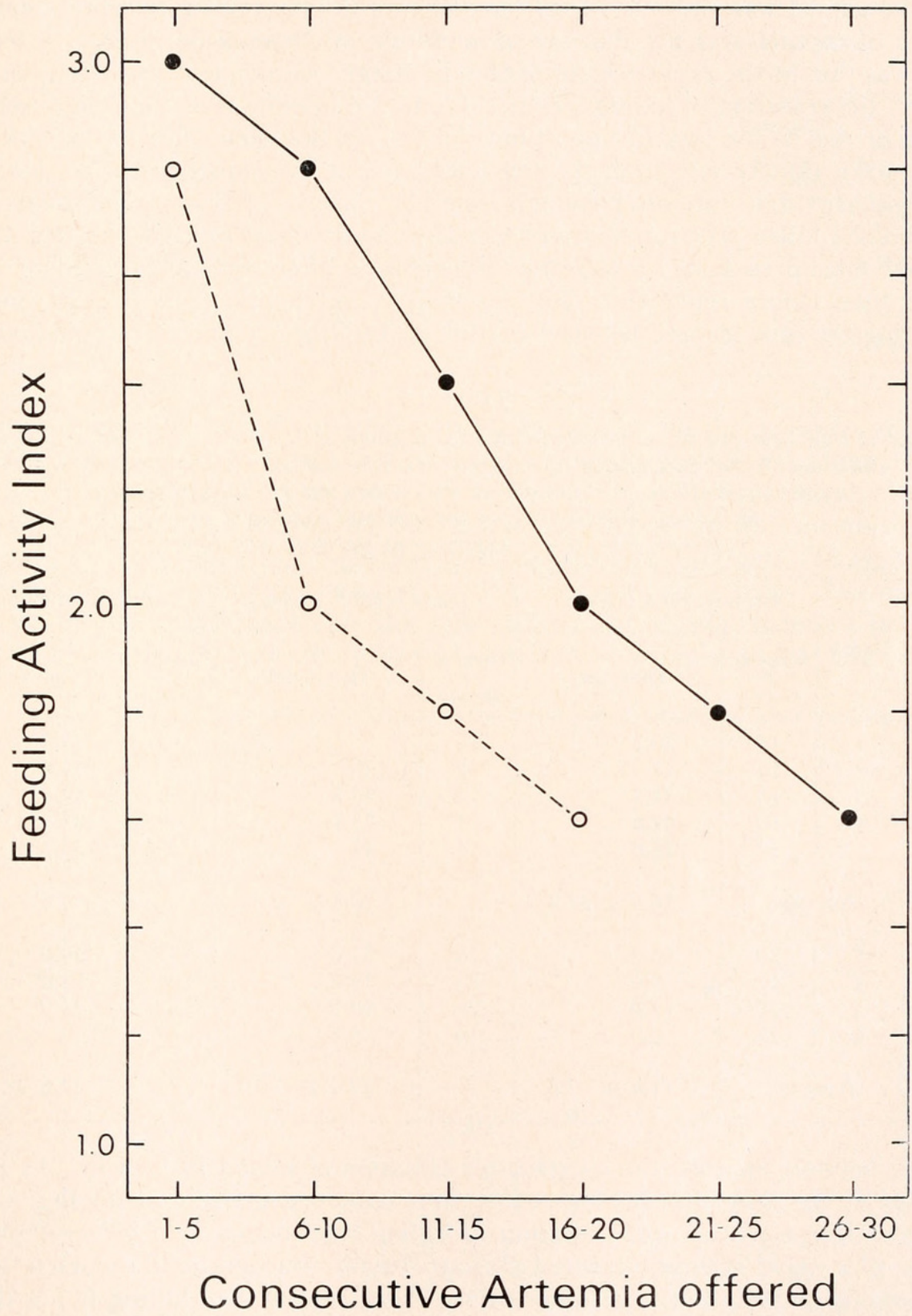

FIGURE 1. Quantitation of feeding behavior of hydra expressed as the feeding activity index. Individual hydra were offered one *Artemia* at a time. The response of the hydra to each *Artemia* was given a numerical value, the feeding activity index: 3.0 = capture, kill and ingestion of the shrimp; 2.0 = capture, kill, but no ingestion; 1.0 = capture and escape. Each point represents the average value of responses to five consecutive shrimp/hydra and the average of six hydra. Normal animals expressed by (filled circles); animals with a truncated gastral cavity by (open circles).

when the animal was starved. They have offered the interesting interpretation that the lack of capture was not due to the inhibition of desmoneme discharge, but instead, was due to the rapid release of the discharged desmoneme from the tentacle. Further, the cessation of killing was not due to the inhibition of stenotele discharge but rather due to the lack of opportunity of fully active stenoteles to fire since the shrimp were rapidly lost from the tentacles. Normally nematocysts are discarded after discharge, but only after enough time has elapsed (2–5 minutes) so that the captured and killed prey can be ingested. If a rapid release existed, then the time a captured shrimp remained attached to the tentacle after repletion should be much shorter than before repletion. This possibility was examined in *H. attenuata* by measuring the time elapsed between capture and kill before and after repletion, and

TABLE I

Length of time captured Artemia *remain attached to tentacles at three stage of feeding activity. Individual hydra were offered four shrimp at a time and the time elapsed between capture and kill or escape measured with a stop watch. Each time value is the average elapsed time for the indicated number of shrimp captured. The error is expressed as the mean deviation*

Expt.	Hydra	Capture, kill & ingestion		Capture, kill & release		Capture & escape	
		Number of shrimp	Time (sec)	Number of shrimp	Time (min)	Number of shrimp	Time (min)
1	1	15	20.7	—	—	3	38.3
	2	14	47.9	1	130	5	47.4
	3	13	54.5	2	36.5	5	44.8
	4	10	42.6	7	74.7	7	185.3
	5	12	128.6	1	42	7	38.3
	Average		58.9 ± 27.9		70.8 ± 31.5		70.8 ± 45.8
2	1	20	16.3	16	31.6	4	60.8
	2	11	9.6	23	19.4	5	11.0
	3	20	11.8	20	40.5	4	29.0
	4	12	8.0	30	24.6	—	—
	Average		11.4 ± 2.6		29.0 ± 7.0		33.6 ± 18.1

the time between capture and escape after cessation of killing had set in. As shown in Table I, the time that a captured shrimp remained actively struggling on the tentacle after repletion and cessation of killing was longer than before repletion. Instead of a rapid release, captured shrimp remain attached to the tentacle longer after feeding to repletion in *H. attenuata*. Thus, cessation of killing is not due to lack of contact of the shrimp with active stenoteles, but due to the inhibition of stenotele discharge. Similarly, lack of capture sometime after feeding to repletion is probably due to cessation of desmoneme discharge instead of a rapid release of discharged desmonemes.

Three other aspects of the nematocyst discharge pattern were examined. Repletion in the animal could be a response simply to the number of shrimp ingested, or it could involve a time factor. Thus, cessation of ingestion may set

TABLE II

Effect of the amount of time elapsed on the total number of Artemia *ingested. Error is the mean deviation*

Feeding regime	Expt.	Number of hydra	Average number of shrimp ingested
One shrimp at a time	1	8	6.4 ± 2.6
Two shrimp, a 1 hour pause, then one shrimp at a time		7	9.3 ± 2.9
Four shrimp, a 1 hour pause, then one shrimp at a time		7	9.1 ± 1.0
Fed to repletion, a 2 hour pause, fed again until repletion	2	12	18.4 ± 5.4
Fed 6 shrimp, a 2 hour pause, fed until repletion		14	16.6 ± 2.7

in after feeding for a certain time period, regardless of the number of shrimp ingested. To decide between these alternatives, animals were fed under different regimes in two experiments as shown in Table II. In each experiment, the hydra reached repletion after having ingested a similar number of shrimp even though the total period of time until repletion was reached was different. Thus, the time interval from the onset of feeding to repletion does not play a role. The difference between the two experiments in the total number of shrimp ingested per hydra reflects the day-to-day variability in the number of shrimp a hydra will consume.

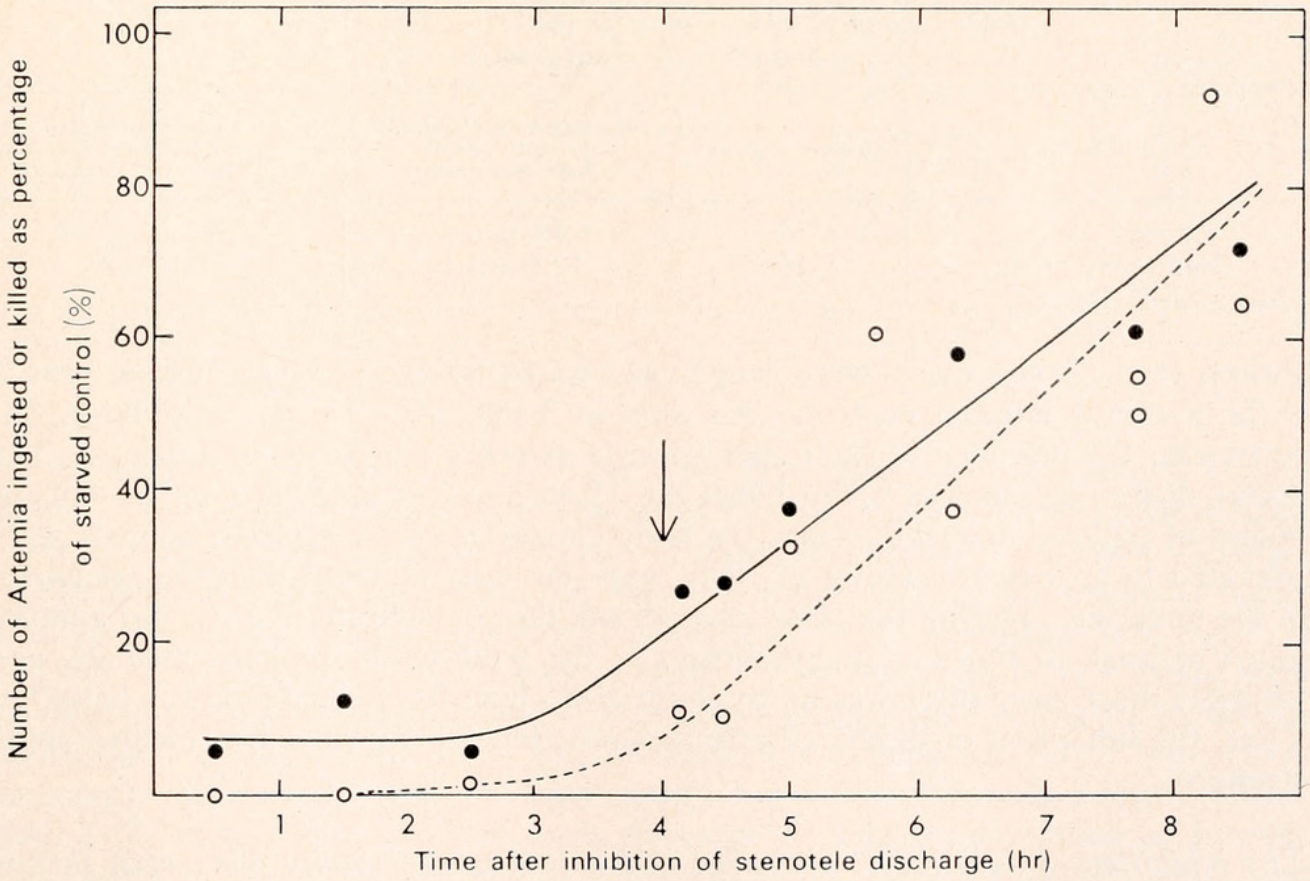

FIGURE 2. Kinetics of recovery from inhibition of stenotele discharge. The results of two experiments are presented together. Each point is the average of 4–6 hydra. The two curves are the percentage of *Artemia* killed (filled circles) and the percentage ingested (open circles) compared to starved controls.

How long stenotele discharge remains inhibited, once inhibition was reached, was also investigated. Animals were fed to inhibition. Then, at intervals over a period of several hours, a group (different group for each interval) of animals was tested for their ability to ingest and kill shrimp. They were offered shrimp until cessation of killing set in. As shown in Figure 2, the animals do not ingest any and kill very few shrimp until they regurgitate the remainder of their previous meal, which occurs about four hours after feeding to repletion. Thereafter, the recovery to the state of a starved animal requires an additional four to five hours. A pronounced change from severe inhibition to a state of some killing and ingestion takes place shortly after regurgitation.

Finally, the question arose as to whether or not the stenoteles mounted along the body column, which are not involved in the feeding process, were also inhibited from discharging after feeding to repletion. These stenoteles will also kill shrimp upon contact but do so without previous capture since there are no desmonemes mounted on the body column. The behavior of these stenoteles was examined by offering shrimp to the bodies of starved and repleted animals and then measuring the time required for the body to kill 15 shrimp. In the process of offering the

TABLE III

Effect of feeding to repletion on the discharge of stenoteles mounted on the body column. Each hydra was offered about 120 specimens of Artemia *and the time required for the body to kill 15 shrimp measured. The number of contacts of shrimp with the body and tentacles were measured on average as 20 contacts/min for the tentacles and 12 contacts/min for the body. The error is expressed as the mean deviation*

State of feeding	Number of hydra	Average time required for body to kill 15 *Artemia* (min)	Number of shrimp killed by tentacles in the time required for body to kill 15 shrimp
Starved	5	5.4 ± 2.5	~40
Fed to repletion	14	19.5 ± 4.1	4.9 ± 2.0

shrimp to the body, many were caught and killed by the tentacles, which proved to be a useful internal control. As seen in Table III, there is a striking difference in the behavior of body and tentacle stenoteles in repleted animals. The bodies of one-day starved hydra killed the 15 shrimp 3–4 times faster than did the bodies of repleted animals. Thus, the body stenoteles of the repleted animals were inhibited to an extent. However, they were far less inhibited than the stenoteles of the tentacles. During the same time period, the stenoteles of the starved animals killed at least 10 times as many shrimp as the tentacles of repleted animals, and probably more since there was no room for more than forty shrimp on the tentacles. Thus, the inhibition of discharge affects those stenoteles involved in feeding more strongly.

The inhibition of nematocyst discharge is due to a gastral cavity filled with shrimp

The foregoing experiments indicate that the ability to discharge stenoteles and desmonemes is sharply inhibited in animals fed to repletion. The inability to kill more shrimp could be due merely to an exhaustion of fully mature stenoteles or an

inhibitory feedback effect caused by discharging a given number of the stenoteles in the tentacles. A lack of mature stenoteles seems unlikely in the light of an experiment by Burnett *et al.* (1960) which we corroborated. If the captured and killed shrimp are removed from the tentacles, thus preventing ingestion, a hydra will kill at least twice as many than if allowed to ingest them. Thus, the tentacles contain at least twice as many mature stenoteles as are used at the time of inhibition of killing. Also, there is no apparent feedback on discharge due to a loss of the stenoteles.

The availability and feedback arguments were examined more directly in the following experiments. First, the percentage of stenoteles used to feed to inhibition was determined by counting the number of stenoteles in the tentacles before and after feeding as described in Materials and Methods. As shown in Table IV, 21%

TABLE IV

Percentage of stenoteles discharged by animals fed to inhibition. All errors are expressed as the mean deviation

Hydra	Number of *Artemia*		Average number of stenoteles/½ tentacle*		Percentage of stenoteles discharged (%)
	Killed	Ingested	Before feeding	After inhibition	
1	30	12	93.6 ± 30.9	64.2 ± 7.4	31.4
2	27	16	53.6 ± 9.1	52.0 ± 9.4	3.0
3	27	15	66.2 ± 10.9	50.8 ± 12.5	23.2
4	23	10	63.2 ± 12.7	34.6 ± 6.4	45.2
5	20	11	56.8 ± 6.6	39.8 ± 7.3	30.0
6	28	23	66.6 ± 12.7	47.4 ± 4.7	28.8
7	21	17	53.0 ± 11.2	45.2 ± 15.0	14.7
8	23	16	51.2 ± 7.6	35.7 ± 6.8	30.3
9	26	18	55.0 ± 10.2	51.0 ± 13.2	7.3
10	15	10	34.0 ± 7.3	33.6 ± 11.7	0.1
				Average	21.1 ± 12.1

* Each value is the average of 4–6 ½-tentacles.

of the stenoteles had been used. Sixteen per cent were used in a second experiment. If these percentages represented either the number of available mature stenoteles or the percentage of stenoteles discharged required to achieve inhibition of discharge by a feedback mechanism, then removal of 50% of the stenoteles before feeding should completely prevent killing of prey. This possibility was tested as follows. Two groups of hydra were selected. The stenoteles of one group were counted with the coverslip method. Then, both groups were subjected to electric shock, as described in Materials and Methods, for various periods of time (5–15 minutes). The group of animals previously counted were counted again. More than 60% of the stenoteles had been discharged. The second group of animals was placed in M solution, allowed to recover from the treatment for two hours, and then offered shrimp. As shown in Table V, they killed and ingested a normal number of shrimp. These results argue against availability of mature stenoteles and feedback due to a number of discharged stenoteles as the cause of the observed

TABLE V

Effect of large numbers of discharged stenoteles on feeding behavior. Errors are expressed as the mean deviation

Expt.	Length of electric shock treatment (min)	Animals assayed for feeding behavior			Animals assayed for number of stenoteles after electric shock	
		No. Hydra	Average number of shrimp		Number of hydra	Percentage of stenoteles remaining (%)
			Killed	Ingested		
1	0	4	29.5 ± 1.5	19.0 ± 0.5	—	(100)*
	10	4	27.5 ± 2.2	17.5 ± 2.2	3	36.4
	15	4	25.0 ± 3.0	20.0 ± 3.0	3	21.9
2	0	4	14.8 ± 1.7	12.0 ± 1.5	—	(100)*
	5	4	17.2 ± 4.8	14.5 ± 3.2	1	87.8
	10	4	10.8 ± 3.2	9.2 ± 4.8	1	42.0
	15	4	11.0 ± 2.5	8.0 ± 1.5	1	43.2

* 100% was assumed, not measured. Percentage of stenoteles remaining

$$= \left(\sum_{i=1}^{N} \frac{\text{Stenoteles}/\frac{1}{2}\ \text{Tentacle After}}{\text{Stenoteles}/\frac{1}{2}\ \text{Tentacle Before}} \Big/ \text{N} \right) \times 100$$

cessation of killing and support the view that stenotele discharge has become inhibited.

To obtain evidence that the inhibition of discharge was directly related to the gastral cavity filled with shrimp, the following pair of experiments were carried out. If a filled gastral cavity is related to inhibition, then an animal with a smaller gastral cavity should ingest fewer shrimp and display the observed pattern of nematocyst discharge inhibition (Fig. 1) after a smaller number of offered shrimp than a normal animal. To test this possibility, animals were bisected beneath the ring of tentacles, the apical part allowed to heal for 24 hours, and then offered *Artemia* one at a time. As expected these smaller animals ingested fewer shrimp compared to normal animals (Table VI), and they displayed the cessation of killing and capturing behavior much earlier than the normal animals [compare smaller animals (dotted line) with normal animals (solid line) in Fig. 1]. These results indicate that the filled volume, but not the absolute number of shrimp, is significant.

In a second experiment, two one-day starved animals were bisected in the mid-gastric region and grafted together as shown in Figure 3. The graft was allowed

TABLE VI

Number of shrimp ingested as a function of the size of the gastral cavity; Group A = animals bisected beneath ring of tentacles and allowed to heal; Group C = control. Errors expressed as the mean deviation

Expt.	# Hydra	Group A	Group C
1	12	2.9 ± 0.6	26.9 ± 2.4
2	12	4.5 ± 0.7	13.0 ± 1.5
3	8	5.3 ± 0.8	24.0 ± 1.1

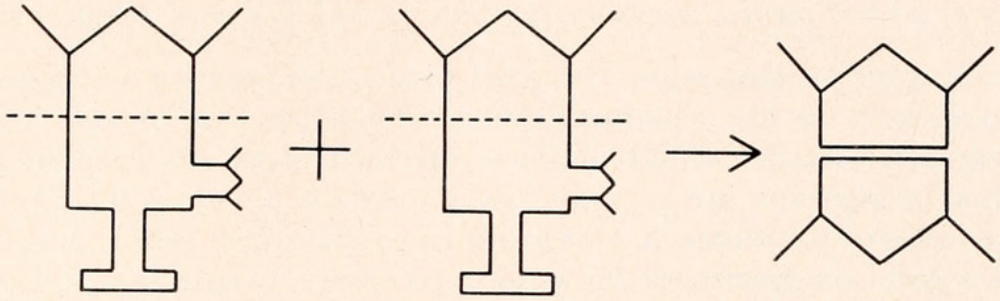

FIGURE 3. Grafting procedure for double-headed animals.

to heal (which requires about 1-½ hours; Bibb and Campbell, 1973) and then fed 4–7 hours later. In these experiments, the two heads had a common gastral cavity; that is, a wall had not yet formed between the two halves. If both heads of a starved animal were offered shrimp simultaneously, both killed and ingested shrimp in equal amounts indicating that the graft combination had no effect on nematocyst discharge in either head. If one head was fed to repletion and inhibition of killing, and then, the second head fed, the second head ingested essentially no shrimp and captured or killed ¼–⅕ as many as the first head (Table VII). A similar effect was found, but not as pronounced if a wall partitioning the cavity existed. This indicates the inhibition need not be transmitted through the gastral cavity alone. Clearly, the presence of the shrimp in the gut caused the inhibition of the discharge of stenotele nematocysts in the second head.

These results taken together with those of the earlier experiments provide more evidence that inhibition of stenotele discharge is the reason the killing of shrimp ceases after feeding to repletion and that a filled gastral cavity is in some way responsible for the inhibition of stenotele discharge. This result is not unexpected since animals normally cease eating when their stomachs are full.

TABLE VII

Effect of filled gastral cavity on the feeding behavior of the second head of a two-headed animal. Errors expressed as the mean deviation

Animal	Time after graft (hrs)	First head: number of shrimp			Second head: number of shrimp		
		Captured	Killed	Ingested	Captured	Killed	Ingested
1	4	15	13	11	10	7	0
2	4	9	7	3	9	3	0
3	4	16	14	9	4	2	0
4	6	21	17	11	2	2	1
5	6	24	20	13	5	4	0
6	6	18	18	8	3	1	0
7	6	19	19	14	2	1	0
8	6	26	23	12	6	2	1
9	6	20	20	7	6	4	0
10	6	26	24	10	3	1	0
11	7	21	17	10	3	2	0
12	7	11	11	5	3	3	1
13	7	15	9	3	3	3	0
Average		18.5 ± 4.2	16.3 ± 4.2	8.9 ± 3.0	4.5 ± 2.0	2.7 ± 1.2	0.23 ± 0.35

Properties of a filled gastral cavity responsible for inhibition of nematocyst discharge

The next question that arose was what kind of information is transmitted from filled gastral cavity to the nematocysts in the tentacles. In animals higher on the evolutionary scale, several different kinds of mechanisms involved in satiation or the cessation of ingestion are known to exist. One is based on the physical distention of the walls of the stomach (Janowitz and Grossman, 1949), which in several species of insects, is monitored by stretch receptors (Finlayson and Lowenstein, 1958). A second is based on the levels of metabolites, such as glucose, in the blood (Mayer and Thomas, 1967). As another, Mook (1963) has suggested the involvement of osmoreceptors in the stomach. Also, controls of ingestion related to the physical process of eating have been shown to exist (Grossman, 1955; LeMagnen, 1971). In analogy with other animals cessation of ingestion and inhibition of nematocyst discharge in hydra may be based on one or more of these mechanisms.

TABLE VIII

Effect of extreme distention of the body wall on feeding behavior. Feeding was initiated 0–25 minutes after injection. The variable starting time had no effect. The errors are expressed as the mean deviation

State of body wall	Number of hydra	Number of shrimp		
		Captured	Killed	Ingested
Control	3	12.0 ± 2.7	11.3 ± 3.1	9.0 ± 0.7
Distended	7	14.3 ± 4.1	11.1 ± 3.0	8.6 ± 1.8

To test the idea that the hydra recognizes physical distension of the body wall, an air bubble (1.0–1.5 μl) was injected into the gastral cavity of a hydra with a microliter syringe tipped with a polyethylene needle. The bubble, with a volume 10 times greater than the empty gastric cavity (0.1 μl, Bode, unpublished results), extended the walls more than those of an animal fed to repletion. The hydra was then offered 3–4 *Artemia* at a time and the response noted. The results Table VIII) indicate that the hydra behaved as a starved animal killing and ingesting as many shrimp as a one-day starved animal. With the increasing number of shrimp ingested, the bubble decreased in volume, but the total extended volume of the gastral cavity remained unchanged. These results indicate that severe distention of the body walls surrounding the gastral cavity has no effect on killing, hence on stenotele discharge in the tentacles. Thus, it is unlikely that the nature of the formation is due to mechanical stretching. Burnett *et al.* (1960) carried out a similar experiment by injecting glass beads into the gastral cavity and reported that the animals killed half as many shrimp as did a set of control animals. The discrepancy between the two results may be more apparent than real for the experimental animals of Burnett *et al.* (1960) were compared with control animals tested in a previous experiment, and as noted above, the number of shrimp killed and ingested can vary greatly from day to day.

An observation made in an experiment described earlier supports the conclusion that the distended body wall of the gastral cavity is not responsible for the

inhibition of stenotele discharge. Animals fed to repletion have a fully extended gastral cavity. Four hours later, shortly before regurgitation of the remaining food, the volume of the gastric cavity had returned close to that of a one-day starved animal, yet the animal killed very few of the offered shrimp; that is, stenotele discharge was still inhibited to a large degree. If simple physical distention of the body wall were related to inhibition of stenotele discharge, we would have expected the extent of stenotele discharge, we would have expected the extent of stenotele discharge to be more like that of a starved animal than like a repleted one.

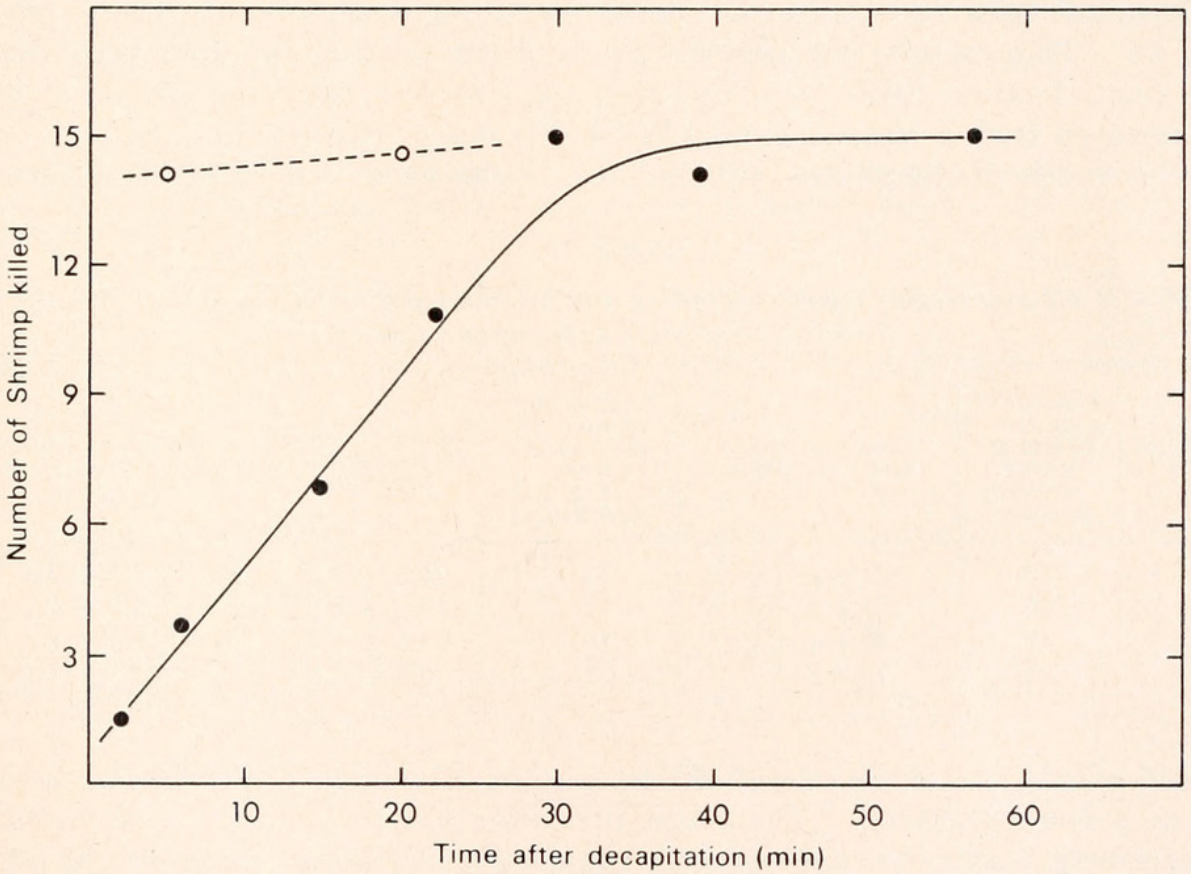

FIGURE 4. Decay of inhibition of stenotele discharge in severed heads. At each time interval after severance, a group (3–5) of hydra were offered 15 shrimp and the number killed within two minutes noted. The two curves are for heads of repleted animals (filled circles) and for heads of starved animals (open circles).

A related possibility concerns "neck formation" in the animal. Some time after feeding to repletion, the portion of the body column directly beneath the ring of tentacles elongates, that is, stretches, becoming very narrow like a neck (Blanquet and Lenhoff (1968). A stretch receptor located in this region could monitor ingestion and hence be involved in the control of nematocyst discharge. However, this is unlikely because neck formation takes place after inhibition of stenotele discharge sets in.

Since physical distention of the body walls is probably not involved, the inhibition of stenotele discharge may be due to the accumulation of a specific metabolite somewhere in the animal or to a general osmotic effect due to the accumulation of ions and metabolites in the gastral cavity. If so, gradual changes in inhibition

might be expected as the level of (say) the metabolite rose or fell in the animal. The slow recovery from inhibition by animals after regurgitation of the remainder of the previous meal is consistent with this view, as are the three following experiments.

Removing the head from the filled distended body of a repleted hydra releases the inhibition of stenotele discharge to some extent after 15 minutes (Burnett *et al.,* 1960). This was examined more fully by decapitating hydra, and thereafter, perioically offering 15 shrimp to each of a group of severed heads (a different group was used for each time point) and noting how many were killed within two minutes. The head of a starved animal killed *Artemia* immediately after severance and behaved as an intact animal by killing essentially all shrimp offered (Fig. 4). Thus, simply the removal of the head, as was expected from earlier work (*e.g.,* Pantin, 1942), had no effect on stenotele discharge. In contrast, a head removed from a repleted animal killed only one or two shrimp in two minutes. Hence, stenotele discharge was still inhibited. With increasing time after severance,

TABLE IX

Effect of different feeding regimes on the lag time between repletion and cessation of killing. Errors are expressed as the mean deviation

Number of hydra	Number of shrimp offered at a time before repletion	Time required to feed to repletion	Number of shrimp offered at a time after repletion	Lag time between repletion and inhibition	Number of shrimp killed after repletion
5	10	25.0 ± 1.6	10	20.0 ± 3.2	9.2 ± 1.4
5	10	27.2 ± 5.0	2	19.0 ± 6.8	5.8 ± 2.2
5	2	67.4 ± 5.1	10	13.6 ± 3.7	13.2 ± 6.4
4	2	93.5 ± 8.2	2	6.5 ± 3.8	2.8 ± 1.6

the head killed more of the offered shrimp, and by 30 minutes, behaved as a severed head of a control animal. The result indicates that the inhibition of discharge decays slowly which is consistent with the gradual loss of a metabolite by either degradation or leakage.

The lag time between cessation of ingestion and inhibition is also of this general length suggesting that the inhibition of stenotele discharge sets in gradually. If the lag time is due to a gradual rise in the concentration of a metabolite, then the feeding regime (the number of shrimp offered and ingested per time interval) may affect the length of the lag time. To examine this, hydra were fed either two or ten shrimp at a time until repletion. As was expected (Table IX), the time required to reach repletion was three times longer for animals fed two shrimp at a time than for those fed ten at a time since repletion is a function of a gastral cavity filled with shrimp. Thereafter, animals fed to repletion of both groups were offered shrimp at short intervals until killing ceased. The lag time was, indeed, affected by the initial feeding regime. Animals fed to repletion by offering shrimp ten at a time had a lag of ~ 20 minutes compared to ~ 10 minutes for those offered two at a time (Table IX). The difference in lag times is consistent with the view that a certain amount of metabolism must take place before the concentration of some metabolite reaches levels which inhibit stenotele discharge.

Hydra fed ten shrimp at a time reach repletion in 25 minutes, but the digestion must continue another twenty minutes before the concentration is high enough. Animals fed shrimp two at a time require longer to reach repletion ($\sim$75 minutes) and, therefore, have more time to accumulate a metabolite(s). This would explain why the lag time between repletion and inhibition of killing is shorter in this case.

Further, if the lag time is a function of metabolism, the length of the lag time should be independent of the number of shrimp killed after repletion has set in. This point was examined in the same experiment. As shown in Table IX, animals fed to repletion by either regime were then offered either two or ten shrimp at short intervals until killing ceased. Those hydra offered 10 shrimp at a time killed twice as many as those offered two at a time indicating that the numbers of shrimp killed after repletion have no effect on the lag time.

TABLE X

Effect of two concentrations of homogenates of Artemia *injected into the gastral cavity on the feeding behavior. Errors are expressed as the mean deviation*

Expt.	Injected solution	Number of hydra	Number of shrimp		Feeding activity of experimental animals as compared to controls (%)	
			Killed	Ingested	Killed	Ingested
1	M solution	5	20.8 ± 3.8	8.2 ± 2.6		
	Dilute homogenate	5	14.8 ± 4.7	2.0 ± 1.6	71	24
2	M solution	4	17.5 ± 3.0	7.5 ± 1.8		
	Dilute homogenate	4	9.5 ± 4.0	0	54	0
3	M solution	5	20.2 ± 1.4	8.8 ± 0.7		
	Concentrated homogenate	7	5.1 ± 1.9	0	25	0
4	No injection	4	22.5 ± 2.0	10.2 ± 1.8		
	M solution	6	20.8 ± 2.6	9.5 ± 2.5		
	Concentrated homogenate	7	7.4 ± 2.5	0	35	0

Finally, if inhibition of stenotele discharge is due to a metabolite either obtained directly from the *Artemia* or by metabolizing a component of the *Artemia,* then the degree of inhibition may be a function of the concentration of the original metabolite in the gastral cavity. This was tested as follows. Two different concentrations of *Artemia* homogenate were prepared as described in Materials and Methods. Two injections, 15 minutes apart, of either homogenate into the gastral cavity yielded hydra that had the extended appearance of animals fed to repletion. Thirty minutes later, these animals were offered shrimp four at a time and their ability to kill and ingest the shrimp analyzed in the usual manner. As shown in Table X, the dilute homogenate inhibited killing by only 30–50% as compared to controls injected with M solution, whereas the concentrated homogenate inhibited ingestion completely and inhibited killing by 65–75%. In experiment 4, some animals that had not been injected were also tested, and it is evident that injection as such has no effect on the ingestion and killing behavior. These results are consistent with the hypothesis that a metabolite(s) is involved and supports an experiment carried out by Burnett *et al.* (1960). They injected homogenates of starved hydra and hydra repleted with shrimp and found that the latter, but not the former,

reduced the killing of shrimp. The fact that the dilute homogenate, estimated to be 5–10 times less concentrated than the concentrated homogenate, is significantly less effective suggests that the metabolite(s) must be present in rather high concentration. Also, these results underscore the earlier result that the distended body walls are not responsible for the inhibition of nematocyst discharge since the dilute and concentrated homogenates extended the body walls to the same degree but have different effects on the killing activity.

Discussion

The results presented above indicate that stenotele, and to a lesser extent desmoneme, discharge can be inhibited by a hydra fed to repletion. This adds to the increasing body of evidence (see Picken and Skaer, 1966) that nematocytes are not completely independent effectors. It has been suggested (Burnett *et al.*, 1960) that the inhibition in hydra is more apparent than real because after an animal has fed to repletion, desmonemes discharged while contacting shrimp are released so rapidly that contact with the still active stenoteles is avoided. For *H. attenuata,* the length of time between discharge and release of desmonemes does not change after reaching repletion, yet the killing of *Artemia* ceases, and, in time, far fewer are captured indicating that the discharge of stenoteles and desmonemes has become inhibited.

This effect is not due to a lack of available mature stenoteles and desmonemes nor due to feedback inhibition based on the number of nematocysts discharged for the following reasons: (a) the animal will kill more shrimp than normal if prevented from ingesting captured and killed shrimp; (b) removal of more than half the available stenoteles has no effect on feeding whereas feeding to repletion requires less than 20% of the stenoteles; (c) animals with a truncated gastral cavity but normal set of tentacles will capture and kill far fewer shrimp than an animal with a normal-sized gastral cavity; (d) if the first head of a two-headed animal with a common gastral cavity is fed to repletion and inhibition of killing, the second head will kill very few shrimp even though it has a normal complement of stenoteles in the tentacles. All of these experiments strongly support the original indications that stenotele discharge is inhibited after feeding to repletion.

Other observations suggest that there are a variety of circumstances in which a hydra will not capture and kill shrimp, and indicating an inhibition of nematocyst discharge. Hydra subjected to electrical shock will not capture shrimp for 30 minutes after treatment. Animals squashed mildly under a coverslip will not feed for 60 minutes thereafter. Similarly, in a stock culture, some animals will feed normally and others will not feed at all. These observations are understandable in the teleological sense that a "sick" hydra or one fed to repletion has no need for food and, hence, will inhibit the discharge of nematocysts involved in obtaining food. In this connection, it is interesting that stenoteles mounted on the body column which are not involved in feeding are inhibited far less ($\sim$ 5 times less) than those mounted in the tentacles. This suggests that the mechanism of inhibition is somewhat specific for tentacle stenoteles. It is not known if the discharge of the two types of isorhizas are subject to inhibition.

As to possible mechanisms underlying the inhibition of stenotele and desmoneme discharge, some information has been obtained. The experiments with two-headed

animals and those with truncated gastral cavity [(c) and (d) in an earlier paragraph of the discussion] demonstrate that inhibition is correlated with a gastral cavity filled with shrimp, but is independent of the absolute number of shrimp ingested. Though an animal with a filled gastral cavity has greatly distended body ways, suggesting physical distention as the mechanism, this is not the case. Animals fed to repletion 3 to 4 hours previously no longer have a distended gastral cavity, yet, stenotele discharge was still severely inhibited. Also, distending the body walls with an air bubble does not cause inhibition and, in fact, has no effect on feeding at all. Finally, two different concentrations of *Artemia* homogenate injected into the gastral cavity, though distending the body walls to the same extent, had markedly different effects on the number of shrimp killed and ingested during subsequent feeding.

The results of other experiments are consistent with a metabolite(s) as the basis of the mechanism of inhibition. The lag time between cessation of ingestion and inhibition of stenotele discharge is of the order of 10–20 minutes while the decay time of inhibition in severed heads is about 30 minutes. These gradual processes are much too long to be based on mechanisms involving the nervous system but are consistent with slow rise or fall in concentration of a metabolite. Further, the fact that the concentrated *Artemia* homogenate was far more effective than the dilute homogenate in inhibiting stenotele discharge indicates not only that the *Artemia* provided some component that caused inhibition but that the component must be in relatively high concentration. These data suggest that the *Artemia* provides a (or several) specific metabolies(s) that is directly involved or is altered by the hydra and then involved in the inhibition of nematocyst discharge. The results are also consistent with an inhibition based on an osmotic effect due to a general rise in all metabolite and ion concentrations.

The logical extention of this work is to determine whether inhibition is due to a general osmotic effect or due to a threshold concentration of a specific metabolite(s). The answer to this question will help clarify whether or not the basic mechanisms involved in satiation in hydra and more highly evolved animals are similar.

Summary

1) The pattern of nematocyst discharge was observed in hydra fed shrimp until repletion. Some time after cessation of ingestion stenotele discharge ceases, and at a later time desmoneme discharge is greatly diminished. Lack of capture of shrimp is not due to a rapid release of desmonemes in animals fed to repletion.

2) Stenotele discharge remains severely inhibited until regurgitation of the remainder of the previous meal. Thereafter, stenotele discharge becomes progressively less inhibited and is normal 4–5 hours later.

3) Discharge of stenotele mounted on the body column is partially inhibited in animals fed to repletion, but far less inhibited than for the stenoteles in the tentacles.

4) Cessation of stenotele discharge after feeding to repletion is not due to the absence of mature stenoteles, as there are at least twice as many mature stenoteles present in the tentacles as are needed to feed to repletion.

5) Inhibition of stenotele discharge is correlated with a gastral cavity filled with shrimp, but is not correlated with the absolute number of shrimp ingested.

6) Physical distention of the gastral cavity due to the ingested shrimp is not the cause of inhbition of stenotele discharge.

7) The changes in inhibition of stenotele discharge are gradual. Stenoteles in heads severed from animals fed to repletion recover from inhibition of discharge in thirty minutes. The lag time between cessation of ingestion and inhibition of discharge is ten–twenty minutes.

8) A concentrated homogenate of *Artemia* injected into the gastral cavity inhibits stenotele discharge to a greater extent than does a dilute homogenate.

9) The results indicate that hydra can inhibit stenotele and desmoneme discharge, and that a metabolite(s), obtained from the *Artemia* is involved in the inhibition.

LITERATURE CITED

Bibb, C., and R. D. Campbell, 1973. Tissue healing and septate desmosome formation in hydra. Tissue and Cell, **5(1)**: 23–35.

Blanquet, R. S., and H. M. Lenhoff, 1968. Tyrosine enteroreceptor of hydra. Its function in eliciting a behavior modification. Science, **159**: 633–634.

Burnett, A. L., T. Lentz and M. Warren, 1960. The nematocyst of hydra. I. The question of control of the nematocyst discharge reaction by fully-fed hydra. *Ann. Soc. Roy. Zool. Belg.*, **90**: 247–267.

Davenport, D., D. M. Ross and L. Sutton, 1961. The remote control of nematocyst discharge in the attachment of *Calliactis parasitica* to shells of hermit crabs. *Vie Milieu*, **12**: 197–209.

Finlayson, L. H., and O. Lowenstein, 1958. The structure and function of abdominal stretch receptors in insects. *Proc. Roy. Soc. London Series B*, **148**: 433–449.

Grossman, M. I., 1955. Integration of current views on the regulation of hunger and appetite. *Ann. New York Acad. Sci.*, **63**: 76–89.

Hessinger, D., H. M. Lenhoff and L. B. Kahan, 1973. Haemolytic, phospholipase A and nerve-affecting activities of sea anemone nematocyst venom. *Nature New Biology*, **241**: 125–127.

Hyman, L. H., 1940. *The Invertebrates: Protozoa Through Ctenophora.* New York, McGraw Hill Book Co.

Janowitz, H., and M. I. Grossman, 1949. Some factors affecting the food intake of normal dogs and dogs with esophagostomy and gastric fistula. *Amer. J. Physiol.*, **159**: 143–148.

LeMagnen, J., 1971. Advances in studies on the physiological control and regulation of food intake. *Progr. Physiol. Psychol.*, **4**: 204–261.

Lenhoff, H. M., and R. D. Brown, 1970. Mass culture of hydra: an improved method and its application to other aquatic invertebrates. *Laboratory Animals*, **4**: 139–154.

Lentz, T. L., and R. J. Barnett, 1962. The effect of enzyme substrates and pharmacological agents on nematocyst discharge. *J. Exp. Zool.*, **149**: 33–38.

Mayer, J., and D. W. Thomas, 1967. Regulation of food intake and obesity. *Science*, **156**: 328–337.

Mook, D. G., 1963. Oral and postingestional determinants of the intake of various solutions in rats with esophageal fistulas. *J. Comp. Physiol. Psychol.*, **56**: 645–659.

Pantin, C. F. A., 1942. The excitation of nematocysts. *J. Exp. Biol.*, **19**: 294–310.

Picken, L. E. R., and R. J. Skaer, 1966. A review of researches on nematocysts. Pages 19–50 in W. J. Rees, Ed., *The Cnidaria and Their Evolution.* Academic Press, New York.

Ross, D. M., and L. Sutton, 1964. Inhibition of the swimming response by food and of nematocyst discharge during swimming in the sea anemone *Stomphia coccinea*. *J. Exp. Biol.*, **41**: 751–757.

Sandberg, D. M., P. Kanciruk and R. N. Mariscal, 1971. Inhibition of nematocyst discharge correlated with feeding in a sea anemone, *Calliactis tricolor* (Leseur). *Nature*, **232**: 263–264.

Shapiro, B., 1968. Purification of a toxin from tentacles of the anemone *Condylactis gigantea Toxicon*, **5**: 253.