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THE NEMATOCYST OF HYDRA (Part I).

THE QUESTION OF CONTROL OF THE NEMATOCYST DISCHARGE REACTION BY FULLY FED HYDRA

by

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DEDICATION.

The author would like to dedicate the following series of papers to Dr. John M. Anderson who was the author's major professor during his graduate career, and who provided him with an atmosphere which was entirely conducive to the pursuit of free research.

Introduction.

One of the most structurally complex and certainly one of the most enigmatic organelles in the animal kingdom is the nematocyst of coelenterates. For nearly a century hosts of scientists, too numerous to mention, have concentrated their attentions on the mode of formation, the migration pathways, the mechanism of discharge, and the chemical nature of these unusual structures which are found only in the phylum Coelenterata. Today, none of these subjects of investigation has been resolved to any degree of satisfaction. There are three theories in existence today which attempt to explain the mechanism of nematocyst discharge; the subject of migration path-

ways, i.e., how does a nematocyst reach its final destination in the tentacles, is explained by two competent but conflicting schools. The mode of formation of a nematocyst remains almost completely unknown, and the chemical nature of the nematocyst capsule plus its enclosed contents is still a subject of research, although LENHOFF et al. (1957) have contributed greatly to our understanding of the chemical constituents of the capsule.

The present studies will report mainly a series of investigations into subjects of the control of discharge, the maturation process, and the migration pathways of nematocysts in hydra. It cannot be hoped that a single investigation will answer in detail all of the problems concerned with these baffling processes; however; it is desired that such a study will add to our present knowledge of the nematocyst and will stimulate further investigation into the nature of this complex organelle.

The authors feel that it would be repetitious to give an account of the morphology of the four nematocyst types found in hydra, because several excellent descriptions of their gross structure (WEILL, 1934; HYMAN, 1940) and their fine structure (SEMAL-VAN GANSEN, 1954; BOUILLON et al., 1958; CHAPMAN and TILNEY, 1959 a, 1959 b) already exist in the literature. The classification of nematocysts used in the following reports will be based on the classification of HYMAN (1940—after WEILL, 1934).

The mechanism which controls the discharge of nematocysts has been a subject of controversy for many decades. Since each of the nematocysts of hydra contain a prominent « trigger » or cnidocil which projects from the nematocyst to the exterior of the cnidoblast cell or nematocyte, it was naturally thought that this structure must be influential in the discharge process. However, as HYMAN (1940) cautions, although the cnidocil may be responsible for initiating the discharge, the mechanism involved is not a simple one of the cnidocil eliciting the discharge reaction simply because it has been mechanically stimulated. HYMAN brings attention to the commensal ciliates which are frequently found moving over the surface of hydra; these animals apparently never discharge nematocysts in spite of the fact that they run over and even bend the cnidocils in their search for food. Furthermore, the nematocyst is able to discharge after it has been completely removed from the cnidoblast cell and is no longer in contact with the cnidocil, but this must not be taken as a positive indication that the cnidocil does

not control the eversion of nematocysts when the nematocyst is inside a cnidoblast cell.

The author has repeatedly attempted to discharge nematocysts by stimulating the cnidocils mechanically with pieces of lens tissue, fine glass rods, hair, cotton thread, etc., but has met with virtually no success. When the same manuever was attempted with an isolated thoracic appendage of the brine shrimp, *Artemia salina,* there was a prompt discharge of « grappling nematocysts » or desmonemes. On the other hand, *Artemia* « juice » (obtaining by squeezing several hundred animals through fine bolting cloth) when flooded over the tentacles of a hydra causes relatively no eversion of nematocysts. This evidence is offered in support of HYMAN's (1940) statement, « apparently the cnidocils react primarily to the general mechanical features of objects as texture and shape and secondarily to their chemical emanations ». PANTIN (1942 a, 1942 b) has further demonstrated that some nematocysts which do not discharge after stimulation of the tentacles with a glass rod will discharge if the glass rod is applied in the presence of food juices. It appears that some chemical or chemicals from the food extract has lowered the threshhold for mechanical stimulation.

EWER (1947) presents a fine account of the stimuli required to discharge the different types of nematocysts in hydra. For instance, he found that the atrichous isorhizas, presumably functioning in locomotory processes, discharge when the tentacle comes into contact with a smooth surface such as a glass slide. Furthermore, he has shown that the presence of a food extract from copepods will inhibit the discharge of atrichous isorhizas while augmenting the discharge of stenoteles. EWER speculated that a chemical in the food might bring about the solation of the cnidocils of one type of nematocyst while effecting the gelation of another type at the same time. Solation would tend to make the cnidocil more supple and make it possible for it to deform the capsule and unlatch the operculum.

The authors have conducted a simple experiment, the results of which, contradict EWER's theory. It was found that if hydra are kept in the cold (4° C) for 1 ½ hours, they are able to hold prey, such as *Artemia,* to their tentacles, but are not able to kill the prey. Microscopic examination of the *Artemia* has revealed that although several desmonemes have been released from the hydra's nematocysts batteries, stenotele discharge was

altogether lacking. After five minutes when the water surrounding the hydra has been warmed slightly, the *Artemia* which previously were wriggling actively on the tentacles are immediately killed. At this time their bodies are found to be pierced with numerous stenoteles. These results cannot be explained in the light of EWER's theory which would interpret the prevention of stenotele discharge as being effected by a solation of the cnidocil; normally reduced temperatures would bring about a gelation of the cnidocil.

It is obvious that there is a different threshhold of stimulation for the desmoneme and stenotele. After these preliminary experiments with « cold » hydra, the authors came to the conclusion that these reduced temperatures were either inhibiting some nervous reaction in the hydra or blocking a chemical reaction in the cnidocil or nematocyst membrane. Today it is generally assumed that the nematocyst is an independent effector and is not dependent on the nervous system for its eversion. When an *Artemia* or *Daphnia* strikes the tentacle of a hydra, nematocysts are discharged only at the point of contact; there is never a general discharge throughout the tentacle. CHAPMAN and TILNEY (1959 a), employing the electron microscope, have not been able to demonstrate the presence of nerves in contact with the nematocyst capsule. IWANZOFF (1896) and PARKER and VAN ALSTYNE (1932) strongly supported the hypothesis that the reactions of the nematocyst were non-nervous. Further support came from PANTIN and PANTIN (1943), EWER (1947), PICKEN (1953), and ROBSON (1953) who believe that a local stimulation of the cnidocil is responsible for the discharge.

However, several early investigators, notably CHUN (1881), LEDENFIELD (1887), and MURBACH (1893) believed that there was a nervous innervation of the nematocyst. More recently, GLASER and SPARROW (1909) and JONES (1947) have shown that narcotization of the cnidoblast cell prevents the discharge of its enclosed nematocyst; thus, the nematocyte itself becomes important as a regulator of the discharge mechanism.

Thus, we have two major schools, each with radically different theories, to explain the mechanism controlling discharge, and within these schools there is also a vast disagreement as to the precise manner in which this mechanism operates. The problem becomes even more complex when one considers HYMAN's (1940) statement which states that neither school explains the fact that when coelenterates are satiated with food

the nematocysts apparently fail to explode against the usual food animals.

It was this statement of HYMAN's that stimulated the present experiments. If a fully fed coelenterate does not discharge nematocysts after rich feeding, then it appears that the animal is able to exert some control upon the process of nematocyst discharge. On the other hand, if the nematocyst is truly an independent effector it should discharge whenever it is properly stimulated, and, theoretically, it should be possible to completely eliminate a hydra's nematocyst reserve by flooding the tentacles with food animals over a prolonged period. Some of the experiments conducted yielded results which are not completely understandable at the present time, but the results will be listed in this paper in their entirety with the hope that other investigators may have more plausible explanations for observations that have baffled us.

MATERIALS AND METHODS.

The animals used for these experiments were specimens of *Pelmatohydra oligactis* (Pallas), originally obtained from Ward's Natural Science Establishment, Rochester, New York. They were cultured by the thousands in our laboratories after the methods of LOOMIS and LENHOFF (1956) with modifications by BURNETT (1959). Approximately 400-500 animals were kept in each of 7 finger bowls, 4 ½ inches in diameter.

One bowl of starved animals was available at all times, so that a ready supply would be available for experimental work. The bowl was replenished daily from stock animals just before feeding time; thus, the starved animal culture contained individuals which had not fed for at least twenty-four hours.

During the course of these experiments it was necessary to inject the gastrovascular cavities of several hydra with various chemicals and food extracts. To accomplish this, a modified syringe was employed. A standard ¼ cc tubercular syringe was attached to a 2 foot metal stand. A 24 G, ¾ inch needle was placed on the syringe, and a 12 inch piece of neoprene tubing (size — I.D., .030"; O.D. .048") was placed on the needle. The connection was sealed with nail polish to insure an air tight fit. A special needle was constructed to pass through the mouth of the hydra. A glass capillary tube (size .8 - 1.1 mm diameter) was drawn out at both ends until fine points were

made. One end was placed in the free end of the plastic tubing while the other end was forced through the center of the hypostome of the animal to be injected (See Fig. 1). Following this procedure, anything with a liquid consistency can be injected into or withdrawn from the gastrovascular cavity.

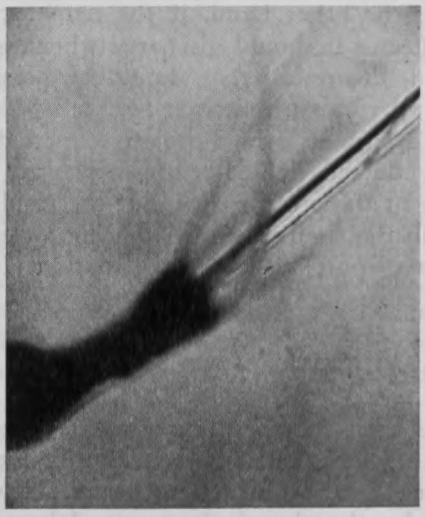

FIG. 1.
This photograph reveals the magnitude of a capillary needle designed for introducing substances into or removing substances from the gastrovascular cavity of a hydra.

In those cases where it was necessary to inject hydra with the body fluids of another animal such as *Artemia,* the following procedure was employed. The material to be injected was placed in the barrel of a 1 cc syringe. A piece of bolting cloth (mesh size 200/inch) was wrapped around the end of the barrel; the plunger was then placed in the normal opening and pushed through forcing the material in the barrel through the bolting cloth. *Artemia,* when subjected to this procedure, were squeezed into a fine paste which could easily be drawn into the injection needle. In order to facilitate the passage of material through the cloth, the normally drawn out end of the syringe was broken off before it narrowed to a thin tip.

During the course of these experiments several starved hydra were filled with a non-digestible material in order to simulate mechanical conditions of a hydra which had just devoured

several *Artemia*. To accomplish this, glass beads were fed to starved animals in the following manner. Capillary tubes (size .8 - 1.1 mm diameter) were drawn to a fine point. When cooled, the tip of each tube was placed into a Bunsen burner flame; after a second's contact with the flame, a small glass bead was formed at the end of the thin tube. This bead, along with a small stalk of the tube, was broken off. The stalk attached to the bead served as a useful handle for the attachment of microforceps when the bead was fed to the hydra.

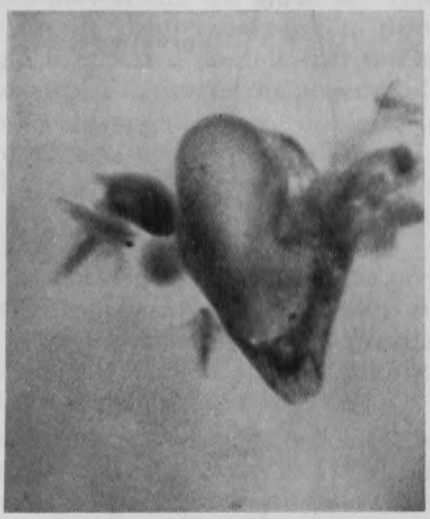

FIG. 2.
Photograph of a hydra which has devoured a single large glass bead. The animal was first stimulated with a solution of reduced glutathione.

The glass beads were then dipped in a weak solution of reduced glutathione. Reduced glutathione causes hydra to open its mouth and begin a feeding reaction. The animals, under this stimulus, will attempt to ingest anything that comes into contact with the hypostome (LOOMIS, 1956). When the beads were placed into contact with the hypostome of hydra, the mouth opened and the bead was promptly ingested. If the investigator is patient, hydra will devour several beads over the period of a half-hour and will stretch to enormous proportions (See Fig. 2).

Various amino acids were also injected into the gastrovascular cavities of approximately 100 animals in order to determine

whether the digested products of proteins were influential in inhibiting nematocyst discharge. Each amino acid tested was injected in high concentrations into the guts of five animals.

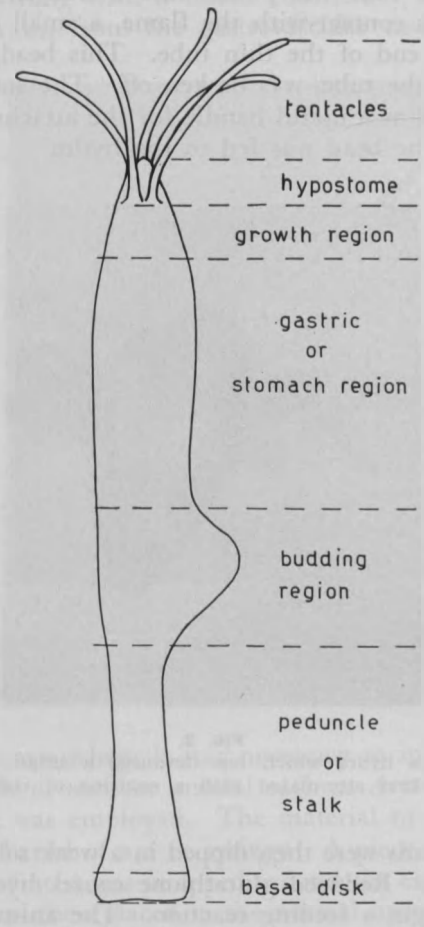

FIG. 3.
A schematic illustration revealing the 7 body regions of a normal hydra.

The amino acids employed were d-1 threonine, d-1 valine, d-1 leucine, d-1 iso-leucine, d-1 methionine, d-1 phenylalanine, 1-histidine, d-1 tryptophan, d-1 lysine, 1-tyrosine, 1-cysteine, glycine, 1-alanine, d-1 serine, d-1 aspartic acid, d-1 glutamic acid, 1-proline, 1-argenine. These chemicals may all be obtained from General Biochemicals Inc., Laboratory Park, Chagrin Falls, Ohio. Fresh solutions of the chemicals were prepared immediately before use.

There will be many references in the following series of papers to the 7 body regions of hydra, described in detail by BURNETT (1959). Figure 3 indicates the position of these regions in the body of a hydra.

OBSERVATIONS AND DISCUSSION.

Several simple experiments were conducted in an effort to determine why a fully fed hydra apparently fails to discharge nematocysts when prey strikes the tentacles. Hydra, when fed daily, normally devour about twenty-five to thirty *Artemia* in a single feeding. It appeared possible that mechanical stretching of the gastrovascular cavity, or the presence of digested food materials in the cavity might be responsible for the inhibition of nematocyst discharge in the fully fed animal. In order to test this hypothesis, ten hydra were flooded individually with 10-15 *Artemia*. As soon as a few *Artemia* were killed, they were removed from the tentacles with microforceps. It was found that if a hydra was not allowed to ingest the prey after it had killed the victim; hydra was capable of killing as many as 115 brine shrimp. After this time, there is no further nematocyst discharge; clearly the hydra were capable of killing many more *Artemia* than they were capable of ingesting.

In order to further demonstrate whether the presence of *Artemia* in the gastrovascular cavity inhibited nematocyst discharge, the following experiment was conducted. Thirty hydra were fed with *Artemia* until their gastrovascular cavities were full and no additional *Artemia* adhered to the tentacles. Then the tentacles and hypostomes were excised from 15 animals while the remaining 15 animals served as controls. After 15 minutes, additional *Artemia* were offered to the excised distal portions and to the control animals. It was found that the tentacles which had been separated from the gastric region took an average of 8 additional *Artemia* while the tentacles which were still in contact with the gastric region via the gastrovascular cavity took an average of one additional *Artemia*. The experiment was repeated with another group of 30 animals, and on this occasion excised portions took an average of 6 animals, unexcised portions, one animal.

These results support HYMAN's (1940) statement that a fully fed animal is inhibited from firing off excess nematocysts, for if the food is removed from the gastrovascular cavity of a hydra

immediately after feeding there is a further discharge of nematocysts and several additional *Artemia* are killed. It was decided to determine whether the inhibition of nematocyst discharge after feeding resulted from a simple mechanical stretching of the gastrovascular cavity or was chemical in origin. Presumably a break-down product resulting from extracellular digestion, or possibly a substance, such as an enzyme, secreted by glandular cells of the gastrodermis during digestion, could be transported via the gastrovascular cavity to the tentacle where the substance would be able to exert its inhibitory action upon nematocysts located in this region.

In an attempt to analyze the effect of pressure alone on the digestive cavity after feeding, glass beads were fed to 30 hydra until the animals were swelled to enormous proportions (See Fig. 3). Glass was chosen because it is inert chemically and should have no effect upon normal digestive processes such as the stimulation of enzyme secretion etc. After ingestion of the glass beads the hydra killed an average of 45 additional *Artemia*. Obviously, pressure exerted on the walls of the gastro-vascular cavity does not prevent nematocyst discharge, but it must be remembered that a single hydra normally kills over 100 *Artemia* if the prey is not allowed to pass into the gastrovascular cavity. The fact that hydra filled with glass beads were capable of killing only half this number takes on significance in the light of this observation. In addition, another very important observation was made during the course of these experiments. It was noticed that hydra, when filled with glass beads, were not capable of holding the prey to the tentacles once the prey had been captured. If a hydra, which has captured many *Artemia,* is picked up with microforceps and shaken slightly, the *Artemia* fall from the tentacles to the bottom of the bowl. If this manuever is repeated with a normal animal, it is found that it is nearly impossible to dislodge the *Artemia* unless they are forcibly pulled from the tentacles with microforceps. The significance of this observation will be elaborated in another section of this paper.

Another set of experiments were undertaken in order to determine whether the presence of partially digested food materials in the gastrovascular cavity is capable of inhibiting nematocyst discharge. Several hydra were allowed to feed until their digestive cavities were filled and no further brine shrimp were ingested. The time required for this process is usually 30-60 minutes.

These fully fed animals were then strained through bolting cloth into a fine paste. The paste was taken into the needle of the injection apparatus, previously described, and injected into the gastrovascular cavities of 10 starving animals. In order to eliminate variables in this experiment, and insure that the results were due solely to the introduction of digested food into the gastrovascular cavity of the 10 starving animals, several starved hydra were similarly strained into a paste, and the paste was then injected into the digestive cavities of 5 animals which had not fed for at least 24 hours. The results of these experiments can be seen in Table I and II.

TABLE I.

Hydra	*Number of* Artemia *Killed*	*Time between Injection and Feeding*
1	6	4 minutes
2	5	7 minutes
3	2	30 seconds
4	3	30 seconds
5	3	30 seconds
6	5	30 seconds
7	2	30 seconds
8	6	30 seconds
9	1	30 seconds
10	0	30 seconds

TABLE I. — This table indicates the number of *Artemia* killed by starved hydra after the digestive cavities of these hydra had been injected with a paste containing the tissues of hydra plus the tissues of partially digested *Artemia*.

TABLE II.

Hydra	*Number of* Artemia *Killed*	*Time between Injection and Feeding*
1	25	30 seconds
2	27	30 seconds
3	30	30 seconds
4	30	30 seconds
5	28	30 seconds

TABLE II. — This table indicates the number of *Artemia* killed by starved hydra after the digestive cavities of these hydra had been injected with a paste containing the tissues of hydra but containing no tissues of partially digested *Artemia*.

It appears obvious from the foregoing experiment that the presence of digested food materials in the stomach region of

hydra has an inhibitive effect on the number of *Artemia* that will be killed by a starving hydra. It might also be mentioned that the number of *Artemia* killed in Tables I and II is also a record, at least roughly, of the number of *Artemia* that were ingested after the killing was complete. It will be further noticed that the inhibitive effect registered by the introduction of digestive materials in the gastrovascular cavity is an immediate one since less than a minute usually elapsed between injection time and feeding time. An explanation for this immediate action will be offered later in this paper. Finally, attention should be paid to the fact that although hydra which contained no *Artemia* in their digestive tracts devoured a normal amount of *Artemia*, the total number of *Artemia* killed was far below the number that a normal animal is capable of killing. It will be remembered that a hydra can kill over 100 *Artemia* if the *Artemia* are not allowed to pass into the digestive tract after they have been immobilized.

To further test the effect of partially digested food materials on the feeding reaction, several hundred *Artemia* were digested with a .4 % trypsin solution at 37° C for one hour. The *Artemia* were crushed before being placed into the enzymatic solution in order to allow the trypsin to attack the internal tissues more readily. After cooling, the digested material was injected into starved hydra which were subsequently offered living *Artemia*. The results of this experiment are tabulated in Table III.

TABLE III.

Hydra	*Number of* Artemia *Taken*	*Time between Injection and Feeding*
1	6	30 seconds
2	10	30 seconds
3	10	30 seconds
4	10	30 seconds
5	8	30 seconds
6	6	30 seconds
7	6	30 seconds
8	7	30 seconds
9	7	30 seconds
10	7	30 seconds

TABLE III. — The above table indicates the number of *Artemia* killed by starved hydra after the digestive cavities of these hydra have been injected with a paste containing tissues of *Artemia* which had been digested by trypsin at 37° C for one hour.

Clearly, there is a reduction in the number of *Artemia* taken by starving animals containing trypsin digested *Artemia* in their gastrovascular cavities as compared to the amount taken by normal starving hydra. However, it will be seen that the number of *Artemia* taken per hydra is slightly above that taken by animals which were injected with an « *Artemia* paste » removed from the gastrovascular cavities of living animals.

It appeared therefore, that some product of digestion, after passing via the gastrovascular cavity to the tentacles, was able to inhibit nematocyst discharge to a great degree. It was conceivable that certain amino-acids resulting from protein digestion were influential in the process, but none of the nineteen amino acids mentioned in the section, Materials and Methods, were effective after they had been introduced into the digestive cavities of starving animals. This is not surprising when one considers that there is very little food breakdown in the gastrovascular cavity of hydra. Burnett (1959) has shown that hydra ingests large protein droplets directly into its tissues via large digestive cells, and most of the protein digestion that occurs in the animal is intracellular. In order to test whether the inhibitive action was due to fat droplets in the cavity, peanut oil was injected into the cavities of ten hydra. These animals all killed great numbers of *Artemia* after the injection was complete.

A single observation recorded early in the course of the present investigation prompted the authors to repeat earlier feeding experiments. It will be remembered that a hydra, when filled with glass beads, is able to capture and kill several *Artemia,* but is unable to hold the prey to the tentacles. It appeared possible that hydra, after devouring several *Artemia,* might exhibit a similar behavior. Several hydra which had not fed for twenty-four hours were flooded with *Artemia.* These animals were observed steadily for a period of two hours under a binocular dissecting microscope giving a magnification of 20 ×. It was noticed that once the gastrovascular cavity of an animal was full of food the mouth opening closed. *Artemia* which were still clinging to the tentacles of the hydra at this time began to fall to the bottom of the dish. In several cases the hydra rubbed their tentacles over one another or over the surface of the dish in what appeared to be an obvious attempt to dislodge the *Artemia.* After the prey had been dislodged, further *Artemia* which came into contact with the tentacles appeared to

swim away unharmed. A superficial observation of this behavior would induce an investigator to assume that hydra was no longer discharging its nematocysts. However, it was noticed that several *Artemia* which struck the tentacles and swam away, apparently unharmed, were capable of only swimming a few millimeters. After this time they would sink to the bottom of the dish either completely paralyzed or twitching slightly. When these *Artemia* were examined under an oil immersion objective, their bodies were found to be pierced with one, sometimes two, stenoteles. Other individuals were seen to come into contact with the tentacles, struggle a few seconds, then swim away completely unharmed. Finally, numerous *Artemia* contacted the tentacles and were immediately killed, but these animals did not adhere to the tentacles. After a minute or so the *Artemia* would be dislodged, often without active movements on the part of the hydra.

Thus, it was realized that a hydra is perfectly able to discharge its nematocysts after it is fully fed. Again, it must be stressed that this process is very easily overlooked since the hydra does not retain the *Artemia* for a long period of time.

Furthermore, a fully fed hydra exhibits a behavior pattern quite unlike that of a normal animal. Normally, when a starved hydra receives a stimulus indicating that prey has contacted the tentacles, the tentacle containing the prey quickly bends into a loop in such a manner that the struggling prey is quickly brought into contact with other nematocyst batteries. Several additional stenoteles are fired off and in a matter of seconds the prey is completely immobilized. A fully fed animal, however, remains perfectly still after the prey has contacted the tentacles. If an *Artemia* contacts a tentacle in an area where there are no stenoteles, i.e., an area which has had its stenotele supply depleted by previous contact with *Artemia* in this region, the *Artemia*, after struggling for a few seconds with the grappling desmonemes, is capable of swimming away unharmed. Other *Artemia* striking the tentacles may cause the discharge of one or two stenoteles at the point of contact, since, however, the animal is not pulled into contact with more nematocyst batteries, it often has the power to break from the tentacles, and may be quite distant from the hydra when paralysis ensues. Other *Artemia* receive a sufficient dose of poison from the stenoteles to be killed on the point of contact with the tentacles. Close examination with a water immersion objective reveals that cnido-

blast cells containing the discharged nematocysts are slowly squeezed out from the epidermal layer and released entirely from the tissues of the hydra. The *Artemia,* plus its attached stenoteles then sinks to the bottom of the dish. The mechanism which controls this release of cnidoblast cells is not known at the present.

It has been shown that most hydra are capable of killing or partially immobilizing as many as 45-50 *Artemia* after full feeding. The time required for this process may be as long as one and a half hours. It has been concluded that hydra preserves its nematocyst supply after full feeding simply by not responding physically when prey comes into contact with its tentacles.

FIG. 4.

Photograph showing two hydra at different intervals during the feeding process. Note that when then animal is full of food there is a great centrifugal stretching in the area just under the hypostome. The animal which is half full of food does not show this stretching.

Further investigation into this matter has revealed that once a hydra's mouth closes after full feeding, it cannot be induced to open again. Fully fed hydra were placed in dilute solutions of reduced glutathione along with starved control individuals.

Loomis (1956) has demonstrated that this chemical is released from the body cavities of hydra's prey through the hole produced by the stenotele after it penetrates the tissues of the prey. When a hydra receives a reduced glutathione stimulus, it responds by opening its mouth and attempting to devour anything that comes into contact with the hypostome. The tentacles squirm actively during this period and repeatedly are brought towards the mouth opening exhibiting hydra's typical « feeding reaction ». Fully fed animals, however, never open their mouths after a reduced glutathione stimulus, whereas starved control animals open their mouths immediately. Moreover, there is no tentacle response from fully fed animals after a glutathione stimulus.

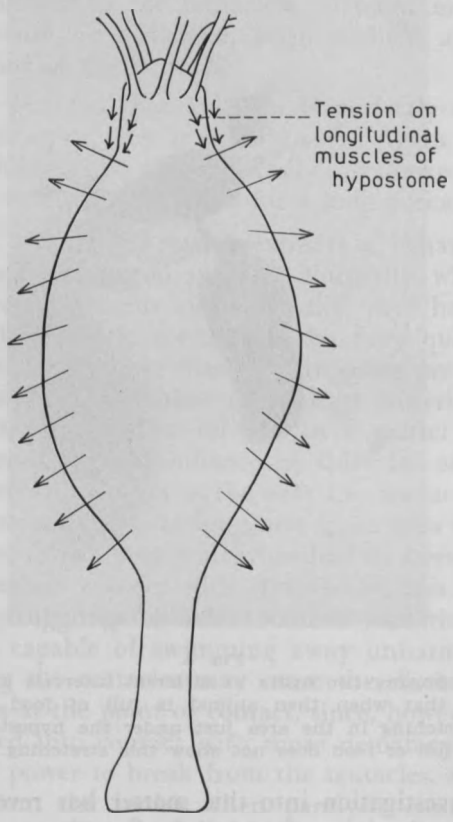

Fig. 5.

This figure is an attempt to illustrate the force exerted upon the walls of the gastrovascular cavity of an animal which has recently fed. The pressure in the lower regions would exert a tension upon the longitudinal muscles surrounding the mouth thus preventing them from contracting.

The mechanism which inhibits a fully fed animal from opening its mouth appears to be a simple one. Once the gastrovascular cavity of a hydra has been swelled by the intake of food materials, there is a great pressure exerted against the walls of the cavity. This pressure makes it impossible for the mouth to open because the longitudinal muscles surrounding the mouth are not able to overcome the resistance of the pressure acting centrifugally on the walls just proximal to the mouth opening. Longitudinal muscles located in the hypostome open the mouth by contracting. Anything which would tend to stretch the muscle fibers would prevent mouth opening. Figures 4 and 5 represent an attempt to illustrate this idea.

Dr. Brien at Brussels University has suggested another theory which explains the inability of the mouth to open. It is quite possible that the mouth of hydra is not opened by a contraction of longitudinal muscles surrounding the mouth, but rather by a relaxation of circular muscles in the same region. Dr. Brien has suggested that during the swelling of the gastrovascular cavity after rich feeding, impulses may be set up in the ectodermal « nerve net » of hydra which stimulate the circular muscles surrounding the mouth to contract. These forces would act antagonistically to any force which normally would cause to mouth to open. Since the relationship existing between the nervous and muscular systems of hydra have never been clearly defined, it is difficult at the present to determine which of the two muscles layers is more influential in the opening and closing of the mouth of hydra.

In order to further confirm these hypotheses, several fully fed hydra were placed in a glutathione solution. None of these animals was able to open its mouth. The regions distal to the growth region were then severed in 5 animals. These animals immediately opened their mouths while the remaining intact animals showed no response. Furthermore, the animals which opened their mouths began to capture additional *Artemia* immediately; the tentacles responded in a manner identical with that of normal starving animals.

The pressure exerted against the walls of the gastrovascular cavity after feeding is not entirely due to the fact that food materials are pressing against the body wall of the animal. Once protein materials, neutral fats, etc. are liberated from the tissues of the partially digested prey, there is a great intake of water into the gastrovascular cavity which causes the hydra to

« bloat ». It appears that during digestion the tissues of the hydra act as a kind of semi-permeable membrane which allows water to pass into the cavity faster than it can escape. The swelling or bloating resulting from the water intake causes the mouth to close. Once the mouth closes, tentacle movement stops, and any *Artemia* that are adhering to the tentacles are quickly dislodged. The inactivity of the tentacles when prey contacts them is not easily explainable. Perhaps, an increased pressure in their cavities, similar to the pressure produced in the cavity of the gastric region, prevents the longitudinal muscles of the tentacle from contracting freely.

In the light of recent findings it is now possible to interpret the observations recorded after feeding hydra glass beads and the paste consisting of the partially digested tissues of *Artemia*. Injection of digested products of ingested *Artemia* into the gastrovascular cavity of a hydra caused the hydra to swell through a rapid intake of water into the digestive cavity. This swelling essentially prevented further mouth opening and further manipulation of food materials by the tentacles. Hence, fewer *Artemia* than normal adhered to and were killed by the tentacles. The fact that a few of these *Artemia* were actually ingested can readily be explained by the fact that it was impossible by the injection of a food extract to exactly simulate the conditions of a fully fed hydra. Swelling after injection procedures was probably not as great as one would find in a normal, previously starved animal. It is quite likely that many more *Artemia* were killed in these experiments than is recorded in Table I, however, at this time the investigators were not aware that many *Artemia* were capable of swimming away from a hydra after they had been pierced by a stenotele, and that several *Artemia* were soon dislodged from the tentacles after they had been killed.

Hydra which had been fed with glass beads had a normal nematocyst supply and were capable of killing many additional *Artemia*. The swelling of the gastrovascular cavity undoubtedly produced a small effect in curtailing tentacle activity but it must be remembered that the swelling produced by feeding an animal with glass beads is not a uniform one. The animals were grossly distorted, and there was not an equal pressure exerted on all of the walls of the gastrovascular cavity. Many of these animals were capable of opening their mouths and ingesting several *Artemia*. As long as *Artemia* were passing

into the mouth, the tentacles were actively engaged in the feeding reaction. When the mouth opening was finally closed, i.e., after several *Artemia* had been ingested, the few dozen *Artemia* still adhering to the tentacles were quickly dislodged.

Thus, it has been established that hydra is not able to prevent nematocyst discharge completely after rich feeding. The animal is merely capable of reducing the number of nematocysts discharged. Again, it appears that the nematocyst of hydra is truly an independent effector.

The mechanism which causes a nematocyst to discharge is at the present unknown. The fact that this mechanism is in no way connected with the nervous system can readily be demonstrated by placing hydra in a solution containing an anaesthetic. If hydra are placed in solutions of 1 % chloretone or 10 % alcohol they are quickly paralyzed. Pinching the body or tentacles with microforceps causes no response whatsoever. However, if these hydra are flooded with *Artemia* they are capable of killing great numbers of these animals.

The authors hold the belief that the nematocyst of hydra is controlled by the cnidocil. Examination of the tentacle of a living hydra under oil immersion reveals that the cnidocils of desmonemes are much longer and thinner than are the cnidocils of stenoteles. Prey contacting the tentacles of a hydra will stimulate the cnidocils of desmonemes before those of stenoteles. If the cnidocil is indeed the « trigger » of the nematocyst then it would be expected that desmonemes will be discharged before stenoteles when the prey has contacted the tentacles. This proves to be the case. *Artemia* striking the tentacles are always captured before they are killed. Desmonemes hold the *Artemia* to the tentacles. The *Artemia* invariably struggles and during the struggling process the stenotele is discharged. The efforts of the *Artemia* to dislodge itself from the tentacles presumably brings the body of the *Artemia* into contact with the short, blunt cnidocils of the stenoteles.

It was noted in the Introduction that hydra which have been subjected to temperatures of 4° C for one and a half hours are capable of discharging desmonemes when prey contacts the tentacles, but are not capable of discharging stenoteles. The authors are not able to explain these observations at the present time, however they feel that cold temperatures are not exerting their inhibition through the nervous system, but by interfering with a chemical mechanism in the cnidocil proper. More evidence will be necessary to elucidate these findings.

Before concluding, the authors would like to caution any investigator who repeats any of the foregoing experiments on hydra. The strain of *Pelmatohydra oligactis* that we have employed in the United States contained approximately 100 stenoteles per tentacle. Thus, a hydra which has killed 100 *Artemia* (probably employing about three stenoteles per killing) has greatly depleted its supply of nematocysts. Any further killing will occur only if an *Artemia* strikes the tentacle in an area where a stenotele is located; in other words, it must be a « direct hit ». The strain of hydra that the senior author is presently studying in Belgium contains approximately 500 stenoteles per tentacle. Preliminary experiments have shown that these animals can easily kill as many as 250-300 *Artemia* during a period of one and a half hours. Therefore, the numbers of *Artemia* killed by a hydra in the present experiments are relative, and the number may greatly differ between different strains of animals.

Summary and Conclusion.

It has been shown, contrary to popular belief, that a fully fed hydra is capable of discharging nematocysts and subduing prey. However, once the hydra are fully fed there is no attempt on the part of the hydra to ingest prey which has been killed after the gastrovascular cavity of the hydra is filled. At this time the mouth is incapable of opening due to the internal pressure exerted on the walls of the gastrovascular cavity. This pressure results from a large intake of water into the gastrovascular cavity during the early phases of digestion. It has been demonstrated that *Artemia* which are killed by the hydra after full feeding are quickly dislodged from the tentacles, and many *Artemia* are able to recover from the initial contact with the hydra because the tentacles are inactive at this time. The inactivity of the tentacles after full feeding by the hydra is interpreted as a means whereby a fully fed animal preserves its nematocyst supply.

An injection apparatus designed for removing and introducing substances from or into the gastrovascular cavity is described. Also, a method is described whereby it is possible to feed hydra substances which are inert chemically, but which serve to mimic the physical state of a fully fed animal.

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