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THE MORPHODYNAMICS AND ACTIONS OF STENOTELE NEMATOCYSTS IN HYDRA

BY

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ABSTRACT

Trembley (1744) and Baker (1743) were the first to have suggested the presence of nematocysts in the tentacles of hydra and other members of the *Cnidaria,* although these remarkable scientists did not recognize the function of these cellular organelles which subsequently were revealed to be of an exceptionally high structural and functional complexity serving food capture, defense, locomotion and other functions.

With special reference to the stenotele nematocysts of the freshwater hydra, this paper gives a survey of the present state of knowledge concerning the ultrastructure, and the explosive discharge of these cellular projectiles as well as their actions upon prey organisms.

RÉSUMÉ

Bien qu'ignorant leurs fonctions, Trembley (1744) et Baker (1743) ont été les premiers à découvrir indépendamment la présence de nématocystes dans les tentacules de l'hydre d'eau douce.

Par la suite ces composants cellulaires qui sont propres à tous les cnidaires, se révélèrent d'être des organelles d'un degré exceptionnel de complexité structurelle et fonctionnelle mise au service de la capture de proies, de la défense, de la locomotion et d'autres fonctions encore.

En se basant particulièrement sur les sténothèles de l'hydre cet article donne une vue d'ensemble sur l'état actuel de connaissances concernant l'ultrastructure, la morphodynamique et l'énergétique de l'explosion spectaculaire de ces projectiles cellulaires ainsi que des effets de ceux-ci lors de la capture de la proie.

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INTRODUCTION

In the first "Mémoir" of his magnificent monograph dedicated to the fresh-water polyp hydra Abraham Trembley (1744) gives an accurate description of this polypoid organism. On pages 61 to 63 he draws attention to the mobile tentacles (Figure 1) with which these animals catch their prey, mostly small crustaceans (copepods, cladocerans), insect larvae and annelids (*Stylaria, Tubifex*). Amongst other things Trembley (1744, p. 61) writes: "Quand le bras est parvenu à un certain degré d'extension, sa superficie n'est plus que parsemée de boutons... Ces *boutons* se forment par la réunion de plusieurs *grains*" (When the tentacle has reached a certain degree of extension its surface is covered with beads... These beads represent a conglomerate of several granules.) Trembley, herewith, had seen and described what we know now to be the numerous stinging capsules (cnidocysts, nematocysts) with which the tentacles of hydra, like those of all other Cnidaria are equipped. Almost simultaneously Baker (1743) had discovered these structures in hydra.

Later, these cnidocysts were revealed to be found in a particular cell-type, a cell-type which has reached the highest standard of structural and functional complexity at a cellular level found in the animal kingdom. These cells are called nematocytes (cnidocytes).

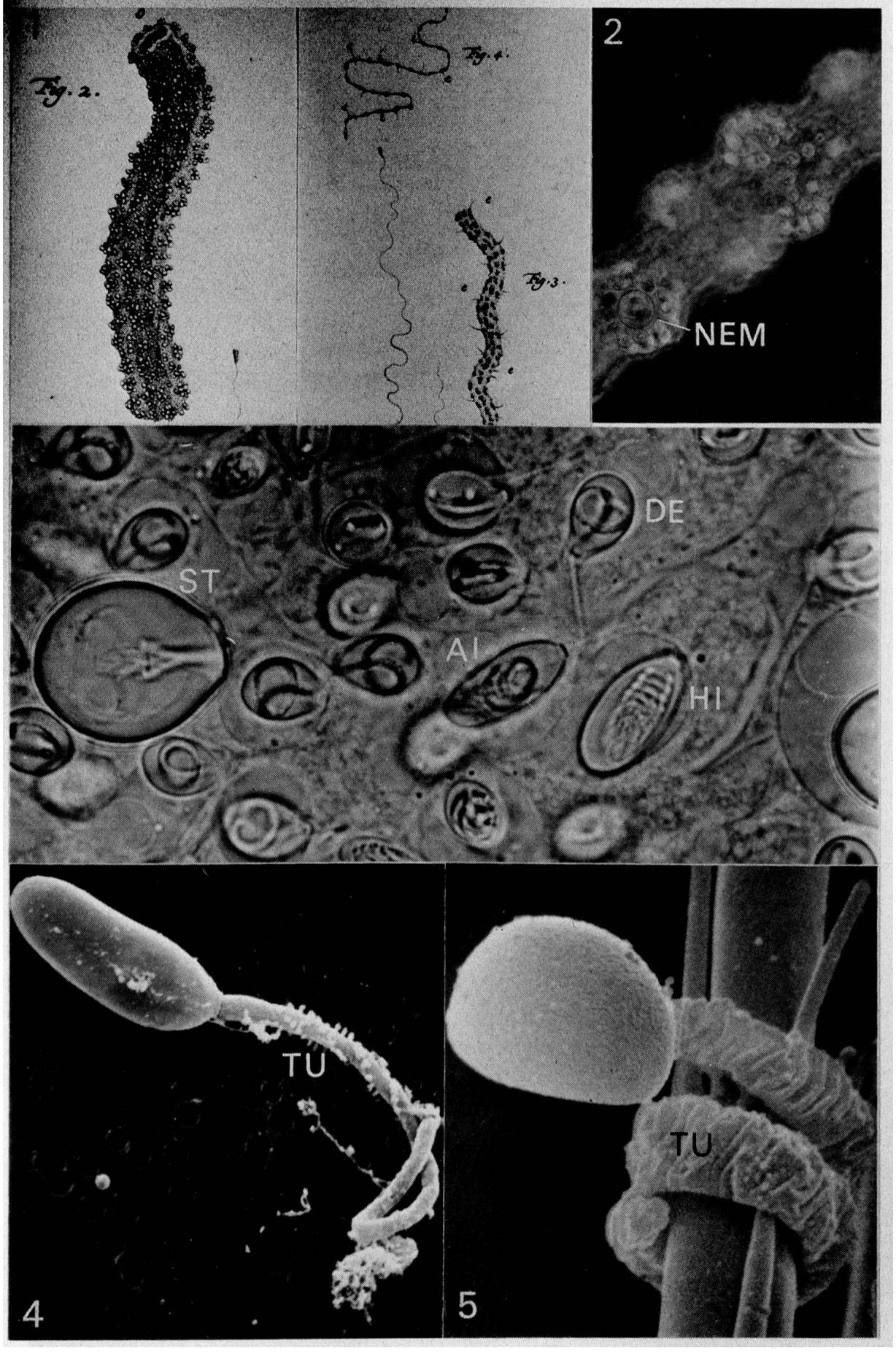
In these cells the conspicuous component, Trembley's "grains", is a spherical or oblong capsule (nematocyst, cnidocyst). It is closely associated with a triggering device consisting of a stereocilium (cnidocil) which is surrounded by a crown of rod-like structures. With his modest optical equipment Trembley seems even to have seen these hair-like cilia (Figure 1). "Les espèces de poils... se remarquent dans un bras de Polype étendu, lorsqu'on l'expose à une forte lentille du microscope. Ils paroissent transparens" (p. 62). ("With a powerful magnifying lens hairs of a sort can be seen on an extended tentacle. They appear to be transparent"). These hairs, however, could also be evaginated tubules of discharged cysts.

In the tentacle of hydra 19 to 25 of these nematocytes are embedded within an ectodermal epithelial cell (Figure 2) which Trembley had described as "boutons".

Upon collision of the prey with the cnidocil the mechanical stimulus is, in a yet not understood manner, transmitted to the cyst which explodes and discharges its tubular content to the outside (Figure 20). The numerous types of cysts (Weill, 1930; Werner, 1965; Mariscal, 1974) serve various functions.

FIG. 1-5.

1. — Engravings from Trembley's monograph of hydra (1744) representing contracted and extended tentacles of hydra. 2. — Section of a semi-contracted tentacle with nematocyte batteries (NEM. 375×). 3. — Squashed tentacle of hydra featuring the 4 different types of nematocysts (DE = desmoneme; HI = holotrichous isorhiza; AI = atrichous isorhiza; ST = stenotele; 1730×). 4. — SEM-photograph of a discharged isorhiza (3270×). 5. — SEM-photograph of a discharged desmoneme the tubule (TU) of which is coiled around bristles of a prey organism *(Artemia)* (9350×).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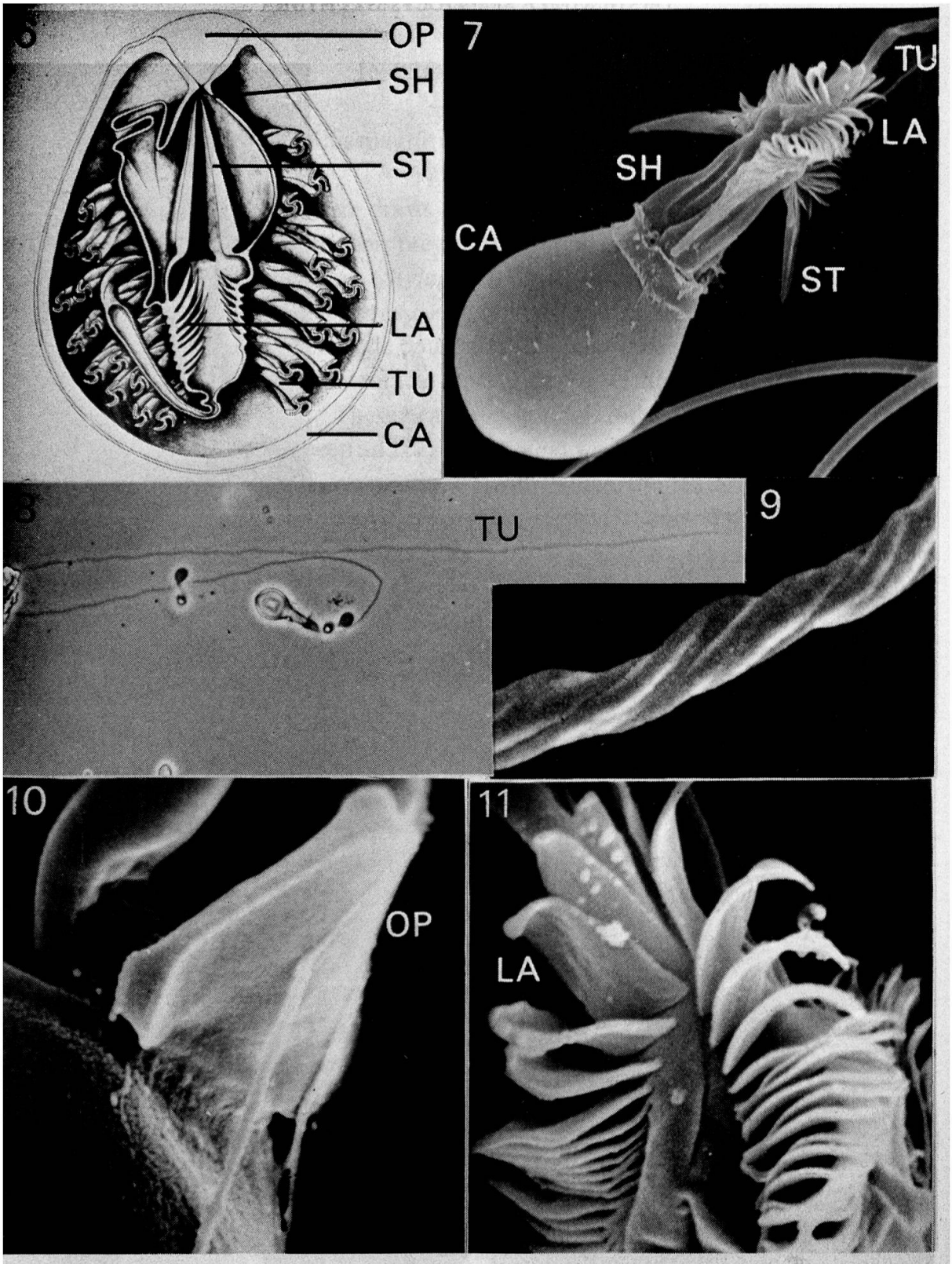


FIG. 6-11.

5. — Longitudinal section through an undischarged stenotele as reconstructed from and drawn after numerous TEM sections (3600×). 7. — SEM picture of a discharged stenotele of which only a short section of the evaginated tubule is seen (2750×). 8. — Phase contrast picture of an exploded stenotele showing the tubule in its full length (375×). 9. — Portion of an evaginated stenotele tubule as seen with the SEM (12 770×). 10. — Flipped-open triangular operculum of a discharged stenotele (SEM, 11 750×). 11. — Close view (SEM) of two rows of lamellae inserted into the conical part of an evaginated shaft (14 000×). (Abbreviations to Figs 6-11: CA = capsule, cyst; LA = lamellae; OP = operculum; SH = shaft; ST = stylet; TU = tubule).

The members of the genus *Hydra (Hydrozoa)* are provided with 4 different kinds of nematocytes which constitute the only reliable taxonomic criterion for the identification of the various species of the genus *Hydra* (Holstein, 1985). The atrichous and holotrichous *isorhizas* (Figure 3) extrude a sticky tubule (Figure 4) which serves to fasten the polyp's tentacles to a substrate, when it moves by means of somersault, as this mode of locomotion has also been accurately described by Trembley.

The remaining two types, the *stenoteles* (Figures 3, 6, 7) and *desmonemes* (Figures 3, 5) are both engaged in the process of food capture. The stenotele, the most elaborate of the 4 types, is a poison-loaded projectile which after breaking through the target's integument (Figure 18) introduces paralysing and fatal toxins into the struggling prey. The task of the more numerous desmonemes consists in preventing the stricken prey from falling off the tentacles. For this purpose their ejected short but robust tubules are tightly coiled around bristles, setae and other appendices of the prey (Figure 5).

Trembley (1744) had carefully observed how hydra catches its prey and how firmly the latter sticks to the tentacles once it has been nettled. For understandable reasons, however, he failed to recognize any functional connection between these events and the "grains" he had detected in the tentacle.

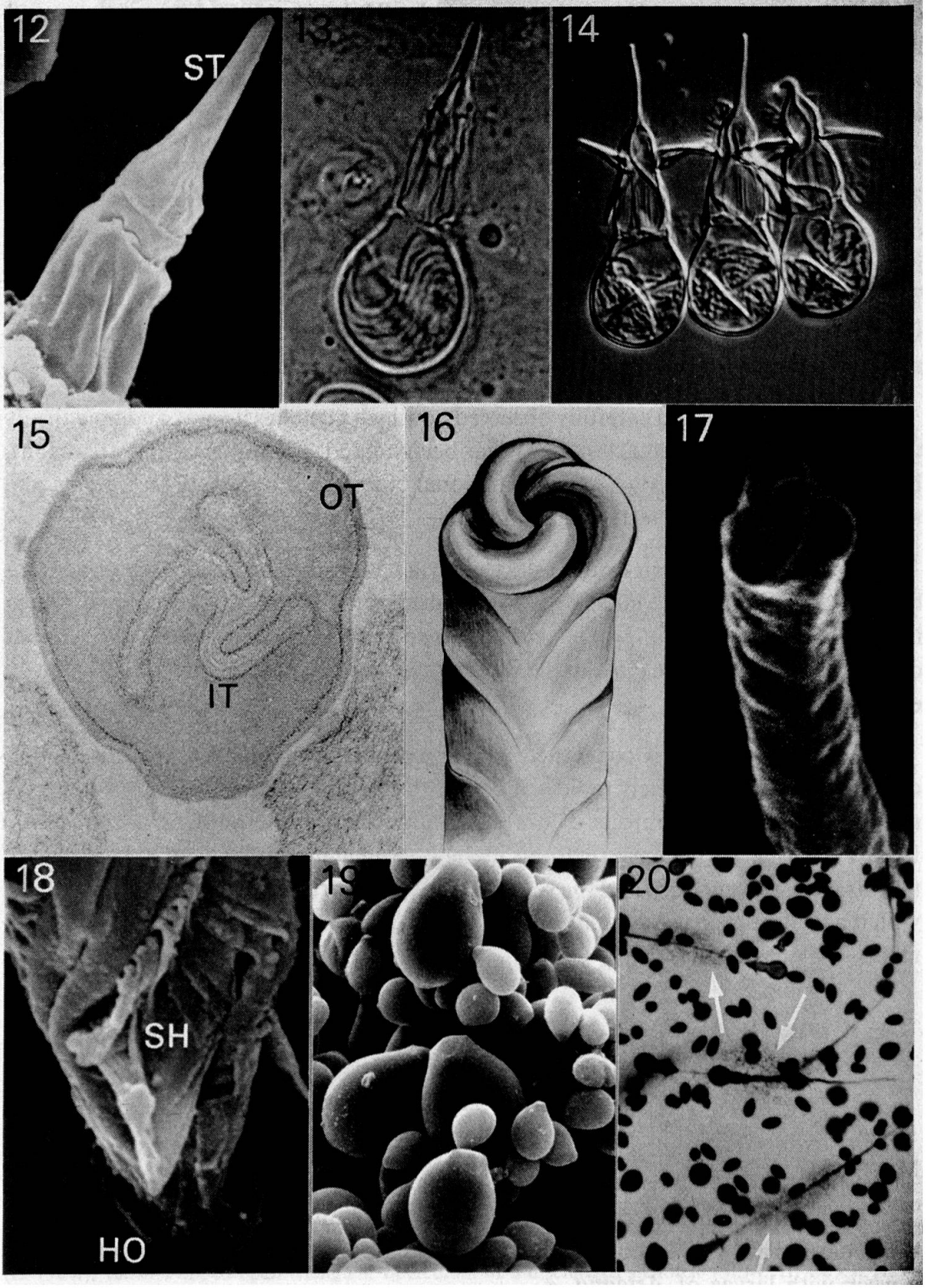
Since then, the nematocytes of the Cnidaria have gained attention of a great number of investigators (see Mariscal, 1974) because of the fascinating complexity which is proper to these cells but also because the encounter with stinging cells of a number of marine species may be most unpleasant or even fatal to human beings (Williamson et al., 1980).

The present paper is concerned with the process by which the cyst discharges, a process which is extremely rapid and is known to occur within fractions of a second (Reisinger, 1937). The objects of our studies are the stenoteles of *Hydra attenuata* Pall. (Figures 6, 7). The investigations include a cinematographical analysis of stenotele discharge (Figure 21), and the search for dynamic principles and forces associated with it as well as a close examination of the action of stenoteles upon the prey's integument.

MATERIALS AND METHODS

MATERIAL

Hydra attenuata Pall. collected 1962 in the river Limmat in Zürich (Tardent, 1966) were cultured at 18° C in Loomis' solution (Loomis and Lenhoff, 1956) and fed twice a week with larvae of *Artemia.*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METHODS

a) Triggering of stenotele cysts

For investigating the process of discharge either by discharging the cysts into gels of various degrees of viscosity (Figures 12-14) or for cinematographical purposes (Figure 21) the stenoteles were triggered electrically (24 V; continuous current; Tardent and Holstein, 1982; Holstein and Tardent, 1984).

For investigating the impact of stenoteles on food organisms either newly hatched *Artemia* or small fragments (2 × 2 mm) of daphnia carapaces were exposed to the polyp's tentacles. Before being swallowed the objects were snatched from the tentacles and processed for SEM examination (Tardent et al., 1980).

b) Electron microscopy

Details about the preparation of undischarged and discharged stenoteles for TEM- and SEM-examination have been given in Tardent et al. (1980) and Tardent and Holstein (1982).

For the morphological analysis of discharged cysts (Figures 4, 7) whole hydra were treated for 3 h with a solution made of 3 parts of 2.5% SDS and 4 parts of 0.1 M NaOH. For concentrating the isolated and cleaned stenoteles the solution was centrifuged (4000 rpm, 5 min) and washed in 0.05 M PIPES buffer. The sediment was transferred to nucleopore filters (0.2 µm; Gutman) which previously had been treated with 0.01% L-polylysine.

SEM examination of undischarged cysts (Figure 19) was performed on isolated and washed specimens. The latter were fixed in a 2.5% glutaraldehyde solution containing 0.05 M cacodylate buffer, which was also used for postfixation in 2% OsO_4. The 100% acetone solution used for dehydration and containing the specimens was directly dribbed on stubs that were air-dried and gold-coated.

FIG. 12-20.

12. — Partially discharged stenoteles after their electrical triggering into gels (SEM, 2700×). 13. — (Phase-contrast, 1400×) represent *phase b* with the stylets (ST) joined to an arrow-head. 14. — (Nomarsky, 1100×) corresponds to the *phase d* with the tubule about to be evaginated (cf. Figure 21). 15. — Cross-section (TEM) through a partially evaginated tubule (cf. Figures 16, 17). The cross-section of the outer tubule (OT) is cylindrical, that of the inner tubule (IT) propellar shaped. Note the inversion of the tubules walls (62 550×). 16-17. — Drawing and SEM picture (23 490×) of the front-end of an evaginating tubule. 18. — Tip of the shaft (SH) of a discharged stenotele which has poked a hole (HO) into the cuticule of a daphnia. The tubule has already evaginated into and through the hole (13 980×). 19. — Isolated and cleaned undischarged cysts of stenotele, desmoneme, and isorhiza as they are used for the biochemical analysis of their contents (980×). 20. — Isolated nematocysts stained with Toluidine blue and triggered artificially with mercaptoethanol. Note the stained granular material (arrow) diffusing away from the tubules of the discharged stenoteles (245×).

All SEM observations including the X-ray spectral analysis (Figure 23) of single cysts were performed with a Cambridge S-4 stereoscan equipped with EDAX. Specimens selected for the X-ray analysis were dribbed on carbon coated teflon-stubs and sputtered with carbon.

RESULTS AND DISCUSSION

Dimensions and architecture of the stenotele

The average outer diameter of an undischarged stenotele cyst (Figure 6) measures 13.3 ± 0.5 μm, its length 16.6 ± 0.7 μm. The corresponding values for discharged cysts (Figure 7) are 10.6 ± 0.6 μm and 13.6 ± 0.8 μm respectively. The 3-layered cyst's wall (Holstein, 1981) is 0.33 ± 0.08 μm and in spite of the reduction of the capsule's volume (Figure 22) this value is not subjected to changes during discharge.

The tubular content of the cyst consists of 3 main sections (Figures 6, 7): the thin walled basal portion of the shaft ("neck"), the short conical shaft-section which is provided with 3 rows of triangular lamellae (Figure 11) of which the proximal-most is drawn out to a long stylet (av. length: 8.6 ± 1.0 μm). During the phases b and c of the process of discharge these 3 stylets are joined and form an arrow-head (Figures 12, 13, 21). The third part consists of a long (470.7 ± 77.4 μm) and slender (Diam. 0.8-0.9 μm) tubule (Figures 8, 9), which, in the cyst, is coiled 11 to 15 times around the centrally located shaft. This tubule which is twisted around its axis (Figure 6) features—in its state of relaxation—a peculiar cross-section resembling a 3-bladed propeller (Figure 15). Whether this tubule, which is practically without an inner lumen, has an open or closed end remains to be ascertained.

Morphodynamics, speed and chronology of stenotele discharge

Jointly, the method of triggering stenoteles into gels (Tardent and Holstein, 1982) and the high-speed cinematographical analysis (Holstein and Tardent, 1984) provided us with the necessary data for a chronological reconstruction of the events taking place during discharge of the cyst.

The time between the onset of the electrical pulse (duration 30 μsec) and the complete extrusion of the capsule's tubular content is in the order of 3 msec. The process of discharge (Figures 13, 14, 21) can be subdivided into the following 4 main phases (Holstein and Tardent, 1984):

Phase a: The delay between the onset of the electrical stimulus and the actual initiation of discharge, which is marked by the flipping open of the cyst's triangular operculum (Figure 10), varied between 0.025 and 0.25 msec (n = 20). During this period the volume of the capsule increases 10% (Figure 22).

FIG. 21. — High-speed film sequence (40 000 frames per second) of the electrically triggered free discharge of a stenotele, including the *phases a-d* (exposure time of each frame = 10 µsec; intervals between two frames = 15 µsec). Note that the ejection of the stylets has occurred between two frames. The film was made with a Hycam 16 mm/120 m high-speed camera (Redlake City Corporation) which was coupled with a Zeiss Universal microscope (for details see Holstein and Tardent, 1984).

Phase b is initiated by the operculum flipping open, thereby permitting the basal part of the shaft with the 3 joined stylets (Figures 12, 13) to be catapulted out of the cyst in less than 10 µsec. In doing so the tip of the arrow-head covers a distance of about 20 µm at an average velocity of 2 $m \cdot sec^{-1}$ for which a calculated constant acceleration of 40.000 g is required.

During this crucial phase of the process, i.e., when under normal circumstances the projectile drives a hole into the integument of the prey (Figure 18), the cyst loses about 22% of its original volume (Figure 22).

Phase c: For about 150 µsec discharge is arrested, allowing the stylets to withdraw from the opening in the target and to flip back (Figures 7, 14). This movement makes way for the extrusion of the lamellae-bearing conical part of the shaft as well as for that of the tubule.

Phase d: The extrusion of these structures is a true evagination by which the tubular wall is turned inside out like the fingers of an inverted glove (Figures 15-17). During this process the cross-section of the already evaginated part of the tubule assumes temporarily a cylindrical shape (Figures 15-17), a measure which reduces considerably the friction between the walls of the outer and inner tubule (Figure 15, Tardent and Holstein, 1982). Yet, this plausible phenomenon creates a particular problem at the tip of the evaginating tubule, where the typical propeller-shaped cross-section converts into a circular one (Figures 16, 17) and where the tubule temporarily features an untwisted structure. However, as soon as the tubule is fully evaginated it returns to its twisted state (Figure 9) and typical cross-section. Both seem to represent the relaxed state of the tubule.

As compared to the previous phases tubule evagination is the slowest event of the whole process during which the cyst's volume is reduced to about half of the original value (Figure 22).

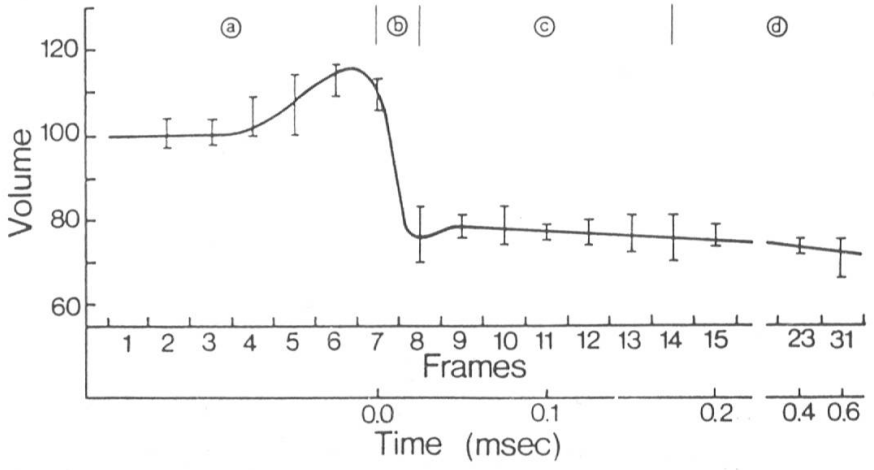

FIG. 22. — Average volumetric changes (as percentages of the initial volumes) performed by 5 stenotele capsules (cyst only) during discharge *(phases a-d).* The measurements were taken from high-speed film sequences such as shown in Figure 21.

The energetics of nematocyst discharge

The cinematographical analysis of stenotele discharge (Figure 21) and the volumetric data resulting from it (Figure 22) offer a new insight into the kind of forces required for this spectacular exocytosis. So far, three fundamentally different hypotheses related to this problem have been expressed (cf. Mariscal, 1974):

a) The "*contractile hypothesis*" (Toppe, 1910; Müller, 1950; Chapman and Tilney, 1959; Mattern et al., 1965; Westfall, 1965; Cormier and Hessinger, 1980) postulates that the cyst has a cytomuscular envelope, the contraction of which is supposed to cause the extrusion of the cyst's contents.

In our case, at least, the enaction of such cytomuscular forces appears to be unlikely for the following reasons: So far we have failed to detect the presence of such a cytomuscular envelope (Tardent and Holstein, 1982). Furthermore, in *phase b* of discharge (Figure 22) the surface of the cyst shrinks by 22% in less than 10 µsec. This means that contracting elements would have to shorten by 11% and, thus, the speed of their contraction would have to be at least 500 times faster than that of the fastest striated muscles (Close, 1965; Holstein and Tardent, 1984).

b) According to the "*tension hypothesis*" (Jones, 1947; Carré, 1980) the necessary forces are intrinsic to the wall of the cyst and that of the tubule. This mechanical energy would have to be built up during cnidogenesis in concert with the intracellular assembly of the cyst and its components (Holstein, 1980, 1981). At discharge, it would be released like the energies of a tightened spring. Considering the exceptional speed (10 µsec) at which the proximal part of the shaft and the stylets are ejected from the capsule (Figure 21) we assume that this particular phase (b) of discharge, at least, is enacted by such "tension forces". The latter are expected to implicate a restructuration of the cyst's wall (Tardent and Holstein, 1982). Indeed, although during this phase the cyst loses 22% of its initial volume (Figure 22); unexpectedly the thickness of the cyst's wall remains the same, thus suggesting the occurrence of such restructuration processes.

It has been claimed that the evagination of the nematocyst tubule should also be due to the release of mechanical energy stored in the tubule wall because in siphonophores the latter is twisted within the undischarged cyst but becomes permanently cylindrical upon discharge (Carré, 1980).

In the stenoteles of hydra, however, following discharge the tubule returns again to the twisted state (Figure 9) which actually seems to be its state of relaxation. For tubule evagination we, therefore, postulate another principle which is the essence of the following third hypothesis.

c) The "*osmotic hypothesis*" (Schneider, 1900; Glaser and Sparrow, 1909; Picken, 1953, 1957; Robson, 1953; Lubbock and Amos, 1981; Lubbock et al., 1981) claims that the contents of the triggered cyst are being placed under internal pressure

due to osmotically caused intake of water and/or ions. In hydra stenoteles this influx of water in connection with discharge can be visualized as follows: Whereas undischarged cysts of a live tentacle which was immersed in a methylene-blue solution hardly pick up the dye, the latter penetrates readily into an electrically triggered capsule; this finding indicates that in the course of discharge liquid has penetrated into the capsule. This, however, is only true for cysts which are still part of a live nematocyst cell, since isolated and cleaned undischarged capsules (Figure 19) are not refractory as to the uptake of dyes (Figure 20). Water intake must therefore not be mediated and controlled by the cyst's wall, but by live structures probably situated outside the latter. Whether this function is taken care of by a membrane enveloping the cyst or by the nematocyte's plasma membrane is not yet known.

Figure 22 shows that during *phase a* of the process the volume of the still undischarged cyst increases by about 10%. This swelling is most probably due to an initial influx of water that occurs within 0.025 to 0.25 μsec and which probably forces the cyst's operculum to flip open (Figure 10). While *phase b* seems to be enacted by "tension forces" (see above), the last *phase (d),* which includes the evagination of the long tubule, is most probably subjected to osmotic forces and is brought about by a swelling of the cyst's matrix. It has been shown that the nematocysts of sea anemones (*Anthozoa*) contain considerable amount of calcium ions, which are released by the triggered capsule (Mariscal, 1980; Lubbock and Amos, 1981; Lubbock et al., 1981; Gupta and Hall, 1984). This may in its turn initiate the rapid influx of water. Since Ca^{++} seem to be able to diffuse freely through the cyst's wall, in the resting capsule it must be bound to proteins. This implicates the existence of yet another type of release mechanism. Our investigations with hydra related to this subject have yielded the following observations:

(1) Electrically triggered stenotele discharge can indeed be inhibited by raising the concentration of external Ca^{++} to a level of 0.25 M, thus, confirming the findings of Lubbock and Amos (1981) in sea anemones.

(2) Our X-ray spectrophotometry performed on SEM preparations of isolated, undischarged cysts of stenoteles, desmonemes and isorhizas (Figure 23) has revealed the presence of considerable amounts of Ca^{++} and of even greater quantities of Mg^{++}. The sum of these divalent ions inside the capsule exceeds the value of 1 molar as revealed by a flanking chemical study. The concentration of Ca^{++} and Mg^{++} correspond to 0.4 and 0.8 μmol. per mg dry-weight respectively (unpublished data). Johnson and Lenhoff (1958) had already found that the cysts of hydra contain unusual quantities of calcium.

These findings raise a number of questions such as: Are the Mg-ions like the Ca-ions involved in regulating osmotic changes as suggested by Lubbock and Amos (1981)? Are all these cations inside the resting capsule bound to proteins and, if so, to what kind of proteins? Investigations dedicated to these problems are in course.

At present, there is sufficient evidence for concluding that alternatively two kinds of energy sources, the osmotically generated forces and the tension forces are involved in this process.

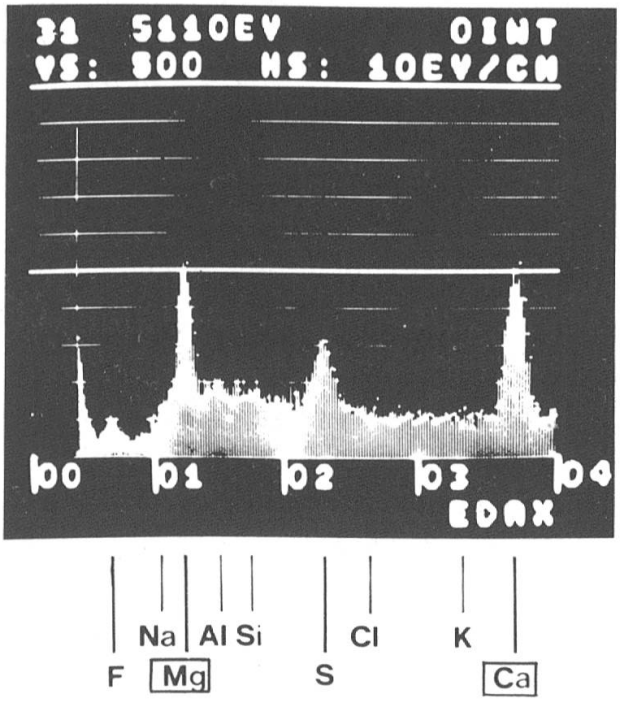

FIG. 23. — X-ray spectrophotogram (EDAX) of an isolated, cleaned undischarged stenotele cyst (cf. Figure 19). Undischarged desmonemes and isorhizas yielded exactly the same spectrum with pronounced peaks for Ca^{++} and Mg^{++}.

The impact and action of the stenoteles on their targets

TEM-pictures of objects, such as whole larvae of *Artemia* and cuticular fragments of other small crustaceans (Figure 18) which had been brought in contact with tentacles of hydra, clearly demonstrate that the joined stylets of the exploding stenotele (*phase b,* Figures 12, 13) poke a small hole into the target (Figure 18). It is through this hole that the evaginating tubule (phase d) can penetrate into the prey's body. This armour-breaking performance is due exclusively to the mechanical impact of the ejected stylets. An additional enzymatic dissolution of the armour can be excluded becaused of the speed (10 µsec) at which this event occurs and because no detectable amounts of chitinase were found to be present within the stenotele cyst (Klug et al., 1984).

Since the tip of the fully ejected stylets (Figure 21) is only 20 μm above the cyst's opening, i.e. above the surface of the tentacle, a successful perforation is possible only when the tentacle has established an intimate contact with the prey's integument. This contact must last about 2 msec in order to allow the successful introduction of the poison from the tubule into the prey; this length of time includes the lag period following the stimulus, the production of the opening by the stylets and the subsequent evagination through this opening of the first section of the tubule. If we assume that a struggling *Artemia* moves at a speed of 1 mm/sec i.e. 1 μm/sec, the possible displacement by the prey during the required critical period (2 msec) would be in the neglectable order of 2 μm, thus, giving the exploding stenotele ample time for the successful fulfillment of its task.

We have failed to detect a particular device by which the stenotele could be held in place or anchored to the hole which it has produced. In this connection the "spines" of the conical part of the shaft, which actually turned out to be lamellae (Figure 11), seem not to act as barbed hooks, because in pictures of SEM of discharged stenoteles which have successfully hit a target, these lamellae have never been found to be inserted in the openings produced by the stylets (Figure 18). Their function may consist in separating the joined stylets and in forcing them sidewards such as to clear the way for the evaginating tubule. In the light of this interpretation the entering of the tubule into the narrow opening (Figure 18) appears to be hazardous and susceptible to occasional failures. According to our observations, however, one or two successfully penetrating stenotele tubules have proved to be sufficient for paralyzing and killing successfully a prey as large as a mosquito larva *(Aedes aegyptii).*

It is not known how the evaginating tubule behaves and proceeds inside the prey organism once it has penetrated through the opening in the integument. It is to be expected that its evagination is stopped when the tip meets a solid obstacle, as it does when it is artificially triggered into gels (Figure 13, 14). However, this would not necessarily constitute a functional failure with respect to the successful application of the toxins since the stenotele of hydra does not act like a syringe which releases the toxins at the tip of its "needle" (i.e. tubule) whenever the latter is completely evaginated. Should it act in this manner a considerable shorter tubule would equally meet the requirements of the syringe principle. There is evidence (Figure 20) that the cyst's liquid content and the substances dissolved in it are more or less continuously released all along the evaginating tubule. Such a mechanical spreading of the toxins by the tubule which races through the prey's body appears to be more efficient than a passive diffusion from the tip of the completely evaginated tubule.

However, the questions as to how and where the toxins are stored within the tubule and how they are released from it remains a matter of speculations. These take into consideration the 3 following possibilities:

(1) In the undischarged cyst (Figure 6) the active substances are located within the

narrow lumen of the tubule. During discharge while the tubule's inside is turned out, they are set free at the tip, as indicated in Figure 24a.

(2) As in the first mentioned possibility the toxins are contained within the lumen of the undischarged tubule but enclosed in a mucus-like matrix. Upon evagination this matrix now covers the outside of the tubule and gradually releases its soluble content.

(3) The toxins are contained in the free spaces of the undischarged cyst. Upon discharge they are either released through small openings located in the wall of the tubule or they diffuse freely through the latter (Figure 24b).

So far, examinations of TEMS of partly evaginated tubules (Figures 16, 17) of hydra and *Craspedacusta* (unpublished) have failed to reveal the presence of such openings. When cysts stained heavily with toluidine blue are triggered to discharge by means of reducing agents (mercaptoethanol), the dye contained in the cyst is released all along the evaginating tubule (Figure 20). This observation, however, could testify in favour of any one of the 3 considered principles. In other experiments (unpublished) stenoteles were discharged into gels (Tardent and Holstein, 1982), which subsequently were stained with various reagents ("stain-all; Coomassie-blue"). All these preparations clearly indicate that stainable material diffuses away from the

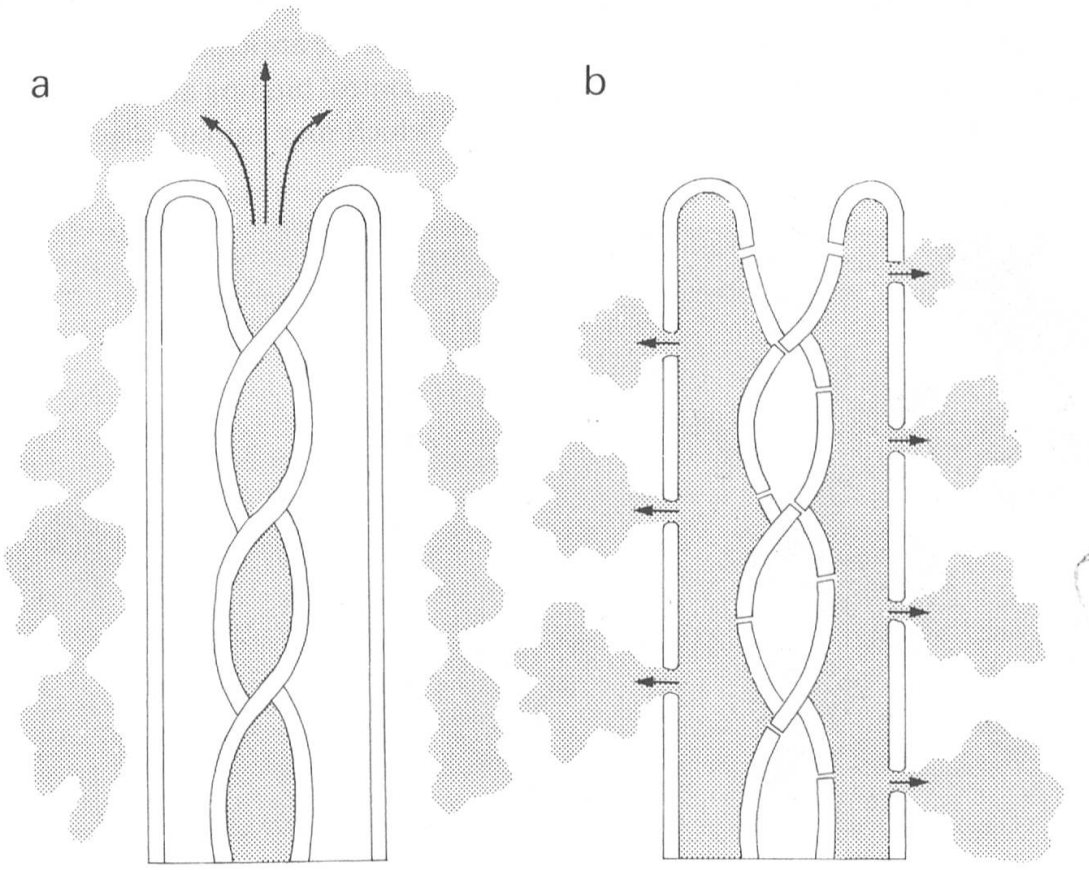

FIG. 24. — Schematical drawing of two possible modes by which toxins (stippled areas) contained in the stenotele cyst could be released by the evaginating tubule.

surfaces of both the stylets and the discharged tubule, thus, speaking in favour of the second hypothesis (see above). At the present state of knowledge, either one of the mentioned principles meets facts which testify in its favour or are contradictory.

CONCLUDING REMARKS

The ultrastructure of stenoteles of hydra and morphodynamics and chronology of their explosive discharge are now fairly well understood. These investigations have revealed a most remarkable dynamic process, the speed of which exceeds other processes which were known so far to occur at the level of cell organelles. This finding has raised a number of problems which are about to be tackled experimentally. They are concerned with the mechanisms involved in natural and artificial triggering of discharge and the role played by the cnidocil apparatus on one hand and the nervous system on the other.

They ask for more information about the nature of and forces which enact this explosive process, and for the mechanisms by which they are generated either during the morphogenesis of the cyst or following the triggering of the functional cyst.

In this connection more information is needed about the nature and functions of the soluble components contained within the cysts. We anticipate finding 3 categories of components: (1) toxins, (2) proteins which control binding and release of Ca^{++} and Mg^{++} and, possibly (3) digestive enzymes which may initiate digestion of the

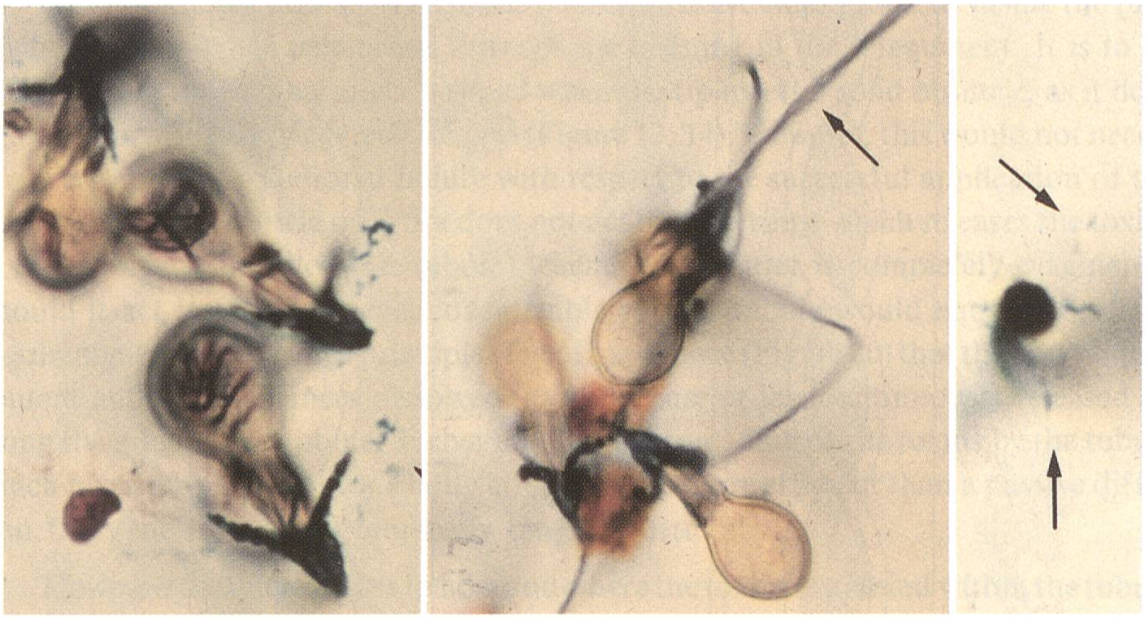

FIG. 25. — Partly and fully evaginated stenoteles which had been electrically triggered into a gel (cf. Figures 12-14) and subsequently stained with "stain-all". Note the stained stylets and tubules and the stainable material which has diffused away from these structures.

prey before it has been swallowed by the polyp. This latter expectation is not unjustified since preliminary electrophoretical analysis (unpublished) has shown the presence of at least 35 different proteins and peptides to be present within the capsule.

Another project deals with the identification and characterization of the toxic substances contained in the stenoteles as well as their physiological effects upon prey organisms.

Although hydra is a simple multicellular organism, a fact which had already been recognized and appreciated by Abraham Trembley, it is, as it was 200 years ago, still challenging the developmental and cellular biologists with new problems to investigate.

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