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The Morphogenesis of Nematocytes in *Hydra* and *Forskålia*: An Ultrastructural Study

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The morphogenesis of nematocytes in *Hydra attenuata* and *Forskålia* sp. involves the complex processes of capsule formation, growth, and differentiation; establishment of nematocyst thread regions within the external tube and invagination; development of pericentriolar processes and the cnidocil-associated apparatus; and the differentiation of the armament and operculum. Several important morphological questions are clarified in the present ultrastructural study. Throughout all stages of capsule development, including the earliest one observed, the two main structural layers, sklera and propria, are present in addition to the unit membrane. The external tube structure is directly proportional to the mature nematocyst thread and is encircled by a basket of microtubules the number of which is also dependent on size. The microtubules seem to be nucleated by the pair of centrioles or its pericentriolar processes. The wall of the external tube consists of three layers and the controversial invagination process is shown to be actually a modified invagination since only the middle layer invaginates. Nematocyte development overall is characterized by a close correlation between the formation of the nematocyst and the formation of the sensory apparatus.

The first papers on the morphogenesis of nematocysts appeared soon after the discovery of these organelles (Moebius, 1866; Jickeli, 1882). Many contradictory theories have appeared in the literature, and even the application of electron microscopy and modern histochemical techniques has not yet resolved the controversy.

The most discussed structure in the morphogenesis of nematocysts is the so-called external tube, described for the first time by Jickeli (1882). It arises from the capsule and coils up in the cytoplasm of the developing cell. The existence of the external tube has been ignored by numerous investigators, but since Westfall's examination (1966) there is no doubt that it is a normally occurring step in nematocyst morphogenesis. Its function, however, has been interpreted in quite different ways. There are those who hold the view that the external tube invaginates along its entire length to become the nematocyst thread, whereas others have denied that such an invagination occurs and described the nematocyst thread as a newly differentiated product arising from the matrix of the external tube and capsule.

A second major feature of nematocytes is the sensory apparatus. Within the Hydrozoa this is a modified cilium, the cnidocil. This structure is also poorly understood and no investigation has ever been published concerning its development or the development of its associated structures.

In the present study I have examined the differentiation of both of these main structures in Hydrozoan nematocytes in *Hydra attenuata* and *Forskålia* sp. On the ultrastructural level I will describe capsule formation, growth and organization of the external tube, the controversial invagination process, and the main events of maturation. The study of the invagination process is the first time such a detailed morphological description has been accomplished. This paper further intends to illustrate the relationship between the morphogenesis of the nematocyst itself and the morphogenesis of the sensory apparatus of the cell, the cnidocil.

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MATERIALS AND METHODS

Cultures of *Hydra attenuata* were maintained by the method of Loomis and Lenhoff (1956). For TEM fixation the specimens were chilled down to 4°C for 1 hr. and double-fixed with 3.5% glutaraldehyde (2–3 hr) and with 1% osmium-tetroxide (1–2 hr). The fixatives were buffered with 0.05, 0.075, or 0.1 *M* phosphate, cacodylate, or collidine buffer (pH 7.2). The best results were obtained in *Hydra attenuata* by the use of 0.05 *M* collidine buffer.

Specimens of *Forskålia sp.* were collected in the Gulf of Pozuoli near Naples and chilled down to 4°C for 4 hr before fixation. Buffering with 0.075 cacodylate buffer (pH 7.3) gave the best results in *Forskålia sp.*

All specimens were embedded in Epon-Araldite with propylene oxide as an infiltration solvent or in ERL-4206 (Spurr, 1969) with ethanol as an infiltration solvent.

Serial sections were made with a Reichert OM-U 3 or a Reichert Ultracut microtome, stained with uranyl acetate and lead acetate, and examined with a Zeiss EM-9-S 2 electron microscope.

RESULTS

Formation of the Capsule

The capsules investigated in the present study have a diameter of about 0.5 μm and are spherical during the earliest stage of development. At this very early stage it is possible to distinguish the wall layers and the matrix of the nematocyst (Fig. 1). The outer layer, named the sklera, is more electron dense with a thickness of about 0.12 μm, whereas the inner layer, the propria, appears more electron lucent, having a width of 0.09 μm (Fig. 1). The two main wall layers of the capsule can be seen in all developmental stages of the nematocysts investigated. Both structural layers of the capsule are enclosed by an additional layer, the unit membrane (7.5 nm).

The matrix consists of homogeneous, lightly granulated material. Later on 30-nm-thick electron-dense granules can be distinguished in the matrix. These granules have the tendency to form large aggregates (Figs. 1, 2).

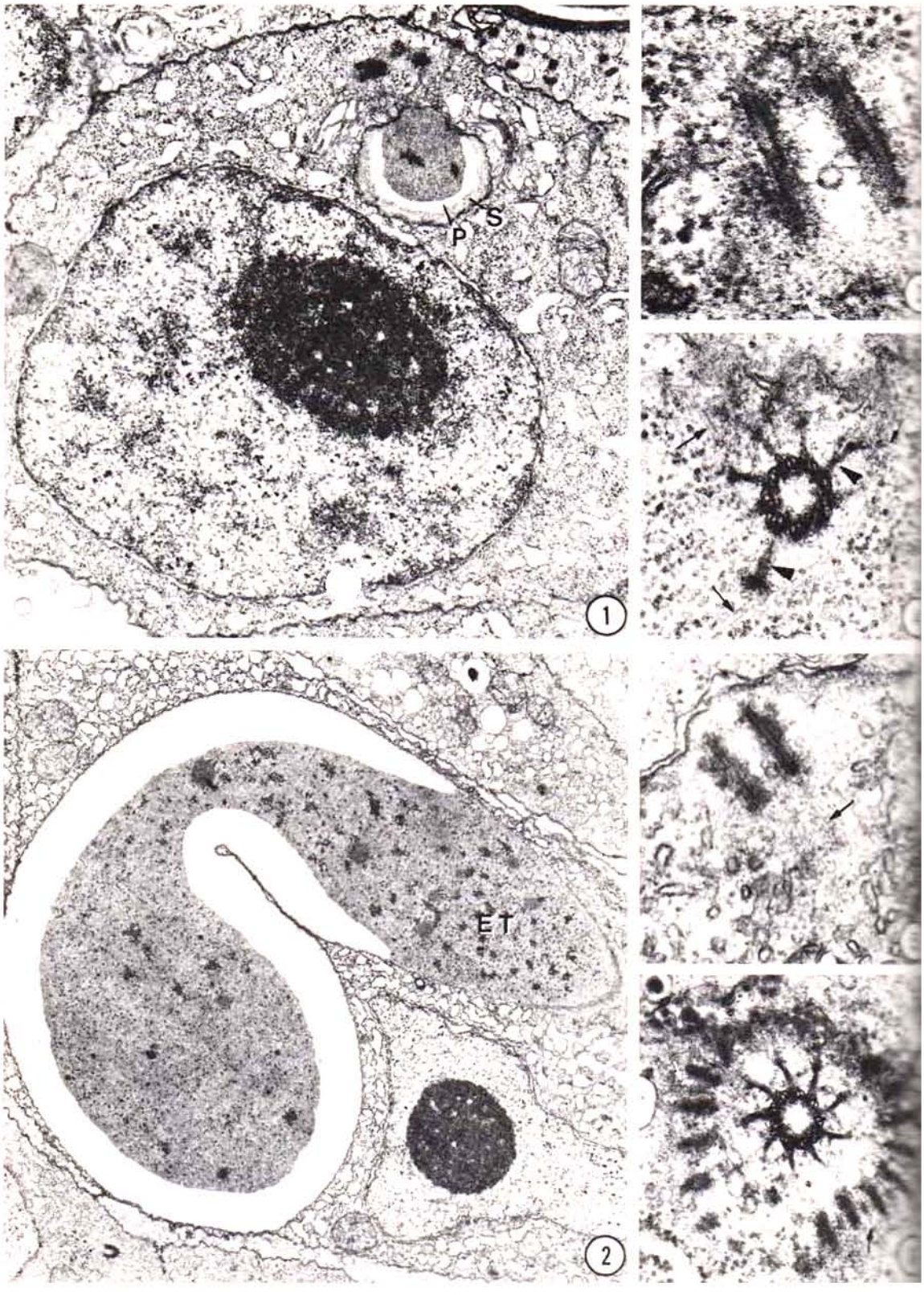
Located distally to the tip of the capsule there is a pair of centrioles (Figs. 1, 7). The two centrioles are positioned at an angle of 90° to one another and are 0.4 μm long. In contrast to later stages (Fig. 4) they do not possess pericentriolar structures (Fig. 3). In the center of the centriole a clear vacuole can be found (Fig. 3). Microtubules could not be observed at this early stage.

Phase of Growth

The phase of growth is characterized by a general increase in volume of the capsule and the nematocyte. Three separate modes of development take place simultaneously: (a) secretion of material from different cell organelles; (b) establishment of the different regions of the mature nematocyst thread; (c) development of the centriole-associated pericentriolar processes and the cnidocil-associated structures.

(a) Secretory process. In the simplest example studied, the growing capsule and external tube (ET) are surrounded by a circle of microtubules, the Golgi apparatus, and the endoplasmic reticulum. The microtubules are separated from the capsule and the ET by a distance of 12 nm (Fig. 8, inset, and Fig. 10), and the distance between each microtubule is 14 nm (Fig. 8, inset). From the wall of the microtubules fine, 4-nm thin appendages radiate into the cytoplasm. Microtubules encircling the external tube first appear with the tip of the outgrowing tube. Near the tip of the external tube the ring-like pattern of microtubule arrangement is interrupted. The interruption is accompanied by a club-shaped thickening of the external tube. Rather than lying strictly parallel to the long axis of the ET, the microtubules are arranged around it in a spiral (Fig. 12). The number of microtubules encircling the tube varies depending in general on the type of nematocyst and the different regions of the prospective thread. In stenoteles the number of microtubules ranges from 50 in the prospective terminal thread region to more than 150 in the prospective shaft region.

The stacks of the Golgi apparatus are thin, cupped, and partially enclose the ET in a semicircle. The cisternae that are situated nearest to the tube are enlarged and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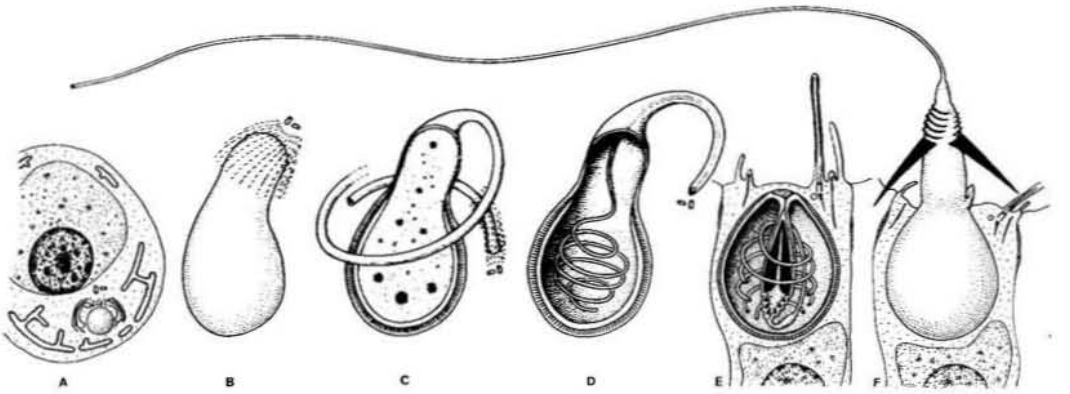

FIG. 7. Schematic drawing of different steps in nematocyst morphogenesis. (A) Early nematocyte; (B) formation of the shaft region in a stenotele; (C) formation of the shaft region in a stenotele; (D) invagination process; (E) mature nematocyst (stenotele); (F) exploded stenotele. Note the close relationship between external tube, microtubules, and the pair of centrioles in (B) and (C). In (D) the electron-dense cap and the centrioles can be seen at the tip of the invaginating external tube. Cell components are only illustrated in (A), (E), and (F). The formation of the cnidocil-associated apparatus is omitted.

expanded reaching a width of 1.8 μm and a length of 1.3 μm (Figs. 8, 11, 12). They possess irregularly shaped granules (ca. 75 nm in diameter) which stain heavily and which are very similar to granules observed in the matrix of the upper region of the external tube. Due to the presence of these granules the matrix of the upper region of the ET closely resembles the matrix of the expanded cisternae. Within the tube itself the upper region matrix is less electron dense than that of the rest of the tube. In association with these large spheres there are numerous smaller vesicles (50 nm in diameter) which are also concentrated around the tip of the outgrowing tube. A fusion of these vesicles with the growing tip is quite common (Fig. 13), whereas a connection of the expanded cisternae with the tip of the ET was never observed.

The ET, the microtubules, and the Golgi apparatus are all surrounded by an elaborate endoplasmic reticulum. Around the growing tip the smooth ER is concentrated, whereas the ribosomal ER fills the whole cell (Figs. 8, 11, 12). The rough ER displays the tendency to form large expanded spheres and the transition is much less continous than in the above-mentioned cisternae (Fig. 9). Located between the smooth

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FIG. 1. Longitudinal section of a very early stage of a nematocyst capsule of *Hydra attenuata*. The two wall layers are the sklera (S) and propria (P). The pair of centrioles is located at the tip of the capsule. × 20 000.

FIG. 2. Longitudinal section of a developing stenotele of *Hydra attenuata* showing the formation of the external tube (shaft region) (ET). × 9000.

FIG. 3. Centriole of a very early nematocyte in longitudinal section with vacuole (*Hydra attenuata*). × 100 000.

FIG. 4. Centriole of a later secretory stage with striated pericentriolar structures (arrowheads). Arrows indicate microtubules which appear to radiate from the button-shaped, terminal thickenings of the processes. Slightly tangential section (*Hydra attenuata*). × 63 000.

FIG. 5. Longitudinal section of a centriole with microtubules at its base (arrow) in *Hydra attenuata*. × 52 000.

FIG. 6. Centriole of a late secretory stage with pericentriolar structures and a developing cnidocil-associated apparatus in cross section (*Hydra attenuata*). The arrows indicate microtubules running between and parallel to the rods. × 52 000.

ER and the Golgi apparatus are 60-nm large vesicles which seem to be derived by constriction from the smooth endoplasmic reticulum (Fig. 11).

(b) Establishment of the regions of the nematocyst thread. The growth of the external tube is by no means isomorphous throughout its total length. These regional differences also exhibit a wide range of variation between the different types of nematocysts investigated. The most simple mode of growth can be observed in desmonemes and isorhizas. Here the diameter of the ET shows no alterations and is constant from the base to the tip. Heteronemes are nematocysts with a broadened proximal region of the nematocyst thread, and in all heteroneme types investigated, especially in the stenoteles, regions of different diameter can be most distinctly observed in the ET. This indicates that the presumptive shaft region in the stenoteles develops first (Figs. 2, 7B) and is followed by the presumptive terminal thread region (Figs. 7C, 10). This characteristic of a varying ET diameter presents a reliable distinguishing mark for different nematocyst types during morphogenesis.

(c) Development of pericentriolar processes and cnidocil-associated apparatus. The development of the pericentriolar processes and the cnidocil-associated apparatus occurs concurrently with the growth phase of the capsule and the external tube. The pericentriolar processes arise around the distal centriole during the growth of the capsule (Fig. 4) and form a circle of nine electron-dense spokes around the centriole. At its base each spoke is linked with one triplet at an angle of 90° and is embedded in an electron-dense matrix (Figs. 4, 6). At their tips these nine spokes or processes end in button-shaped thickenings with a diameter of 30 nm. Microtubules can be observed at the base of the centrioles and are probably associated with the distal end of the pericentriolar processes (Figs. 4, 5). Two rootlets are formed in connection with the other centriole, but only one is well defined. These rootlets have a length of 1.1 μm, and are broader in the middle region than at either end.

Cnidocil-associated structures first appear as 25-nm-thick, separate electron-dense masses which form around the centrioles. Later they elongate (with no change in diameter) in the direction of the capsule and assume their typical rod-like shape. Microtubules are situated exactly equidistant between the rods and run parallel to their long axis for a distance of 15 nm (Fig. 6). The cnidocil-associated apparatus at this stage already has the typical arrangement of the mature apparatus. In the case of stenoteles, the rods later enclose both the centrioles and the underlying capsule (Fig. 17). Rods in desmonemes and isorhizas differentiate to enclose only the centrioles.

Phase of Differentiation

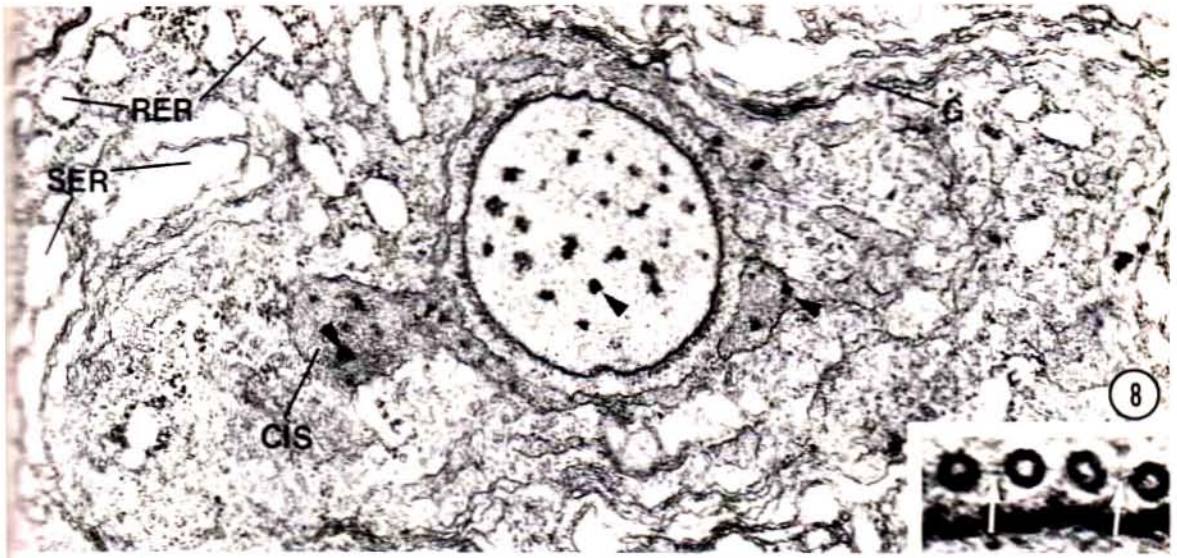
Microtubules can be observed only in the growth region of the capsule and the external tube. Here the ET is bounded by a single-unit membrane. Nearer to the capsule, in the more proximal region of the tube, the microtubules disappear and it is possible to identify two additional layers comprising

FIG. 8. In this region rough endoplasmic reticulum (RER), smooth endoplasmic reticulum (SER), several Golgi apparatus (G), and the enlarged cisternae (CIS) can be observed in *Hydra attenuata* (cross section). The enlarged cisternae contain similar electron-dense granules (arrowheads) as the external tube. The inset shows the bridges between the microtubules (arrows). Fig. 8, × 28 000; inset, × 200 000.

FIG. 9. The regularly lamellated rough endoplasmic reticulum forms expanded sacks (SAC). The arrow indicates the formation of transitional vesicles (*Forskålia sp.*, cross section). × 49 000.

FIG. 10. Cross section of the tip region of the external tube in *Hydra attenuata* with the circle of microtubules. × 49 000.

FIG. 11. The enlarged cisternae are separated from the Golgi stacks; the space between them is filled with numerous vesicles (*Forskålia sp.*, cross section). × 34 000.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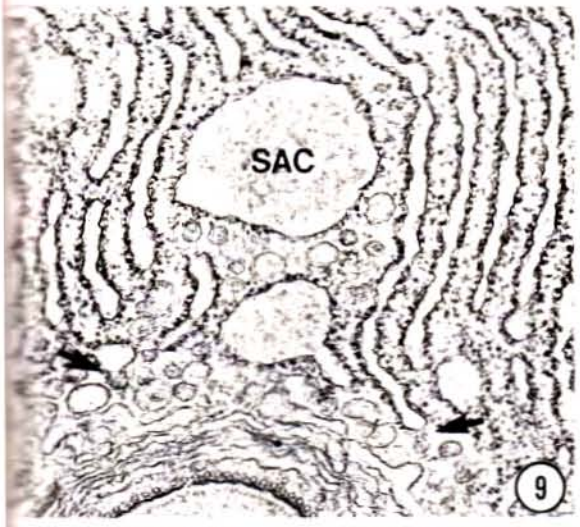
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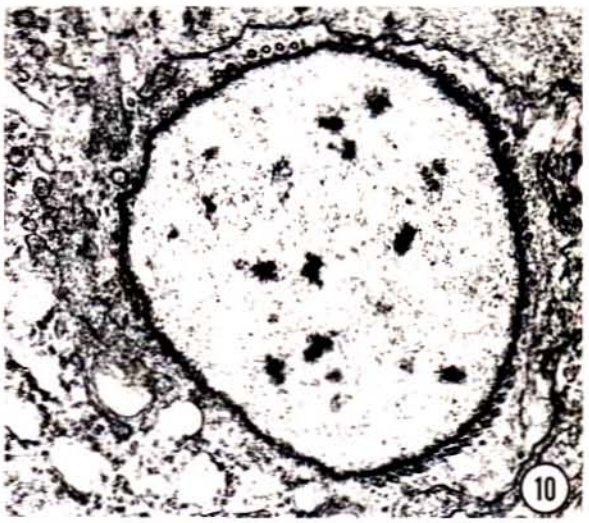
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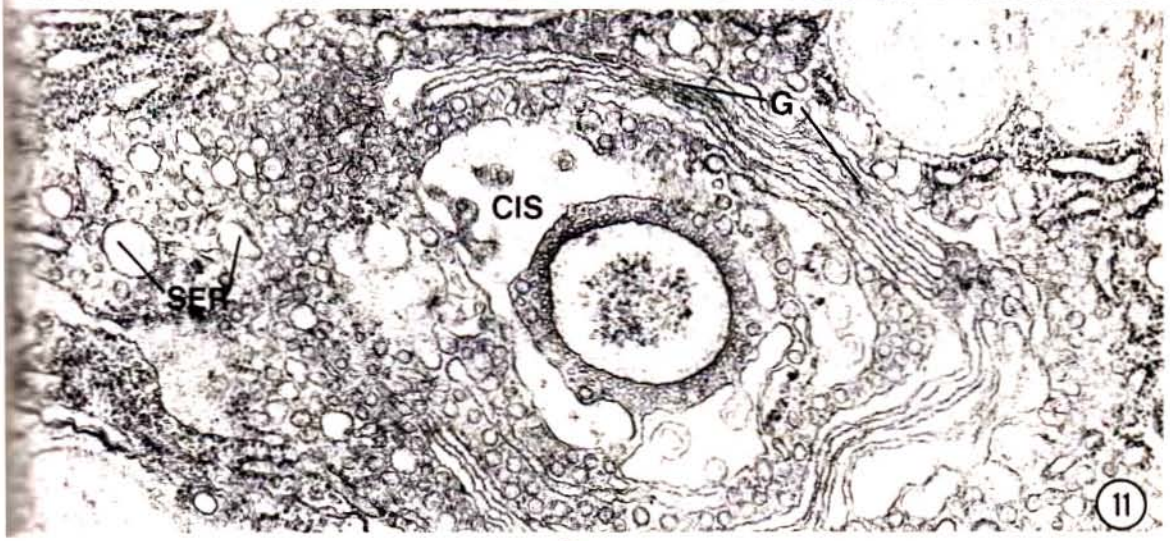
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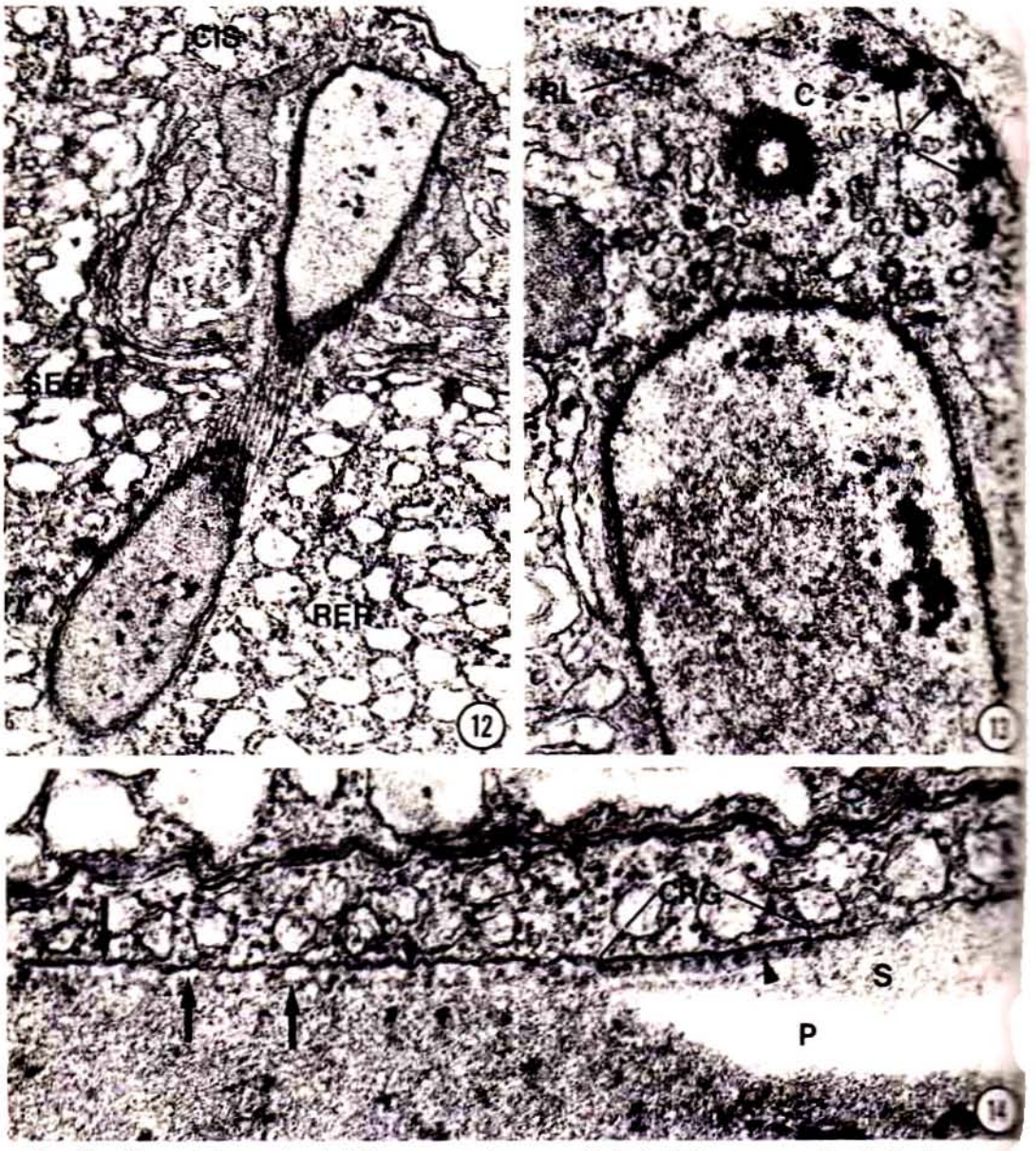

FIG. 12. Microtubules run in spiral-like course to the external tube in *Hydra attenuata* (longitudinal section) × 23 000.

FIG. 13. Numerous, small vesicles are situated close to the tip of the outgrowing external tube. One of the two centrioles (C) with its rootlet (RL) and the rods of the developing cnidocil-associated apparatus are situated distally to the tip of the tube (*Hydra attenuata*, longitudinal section). × 56 000.

FIG. 14. The three-layered external tube wall (arrows indicate the three layers) with enclosed electron-dense granules (arrowhead) in longitudinal section (*Hydra attenuata*). The region of contact to the capsule wall stains more darkly than adjacent regions (CRG). × 63 000.

the wall. Thus the wall of the ET in all developing nematocysts of *Hydra attenuata* and *Forskålia sp.* ultimately has three layers (Fig. 14). The outer layer is the already existing membrane, the middle layer is clearly electron lucent and 20–25 nm thick

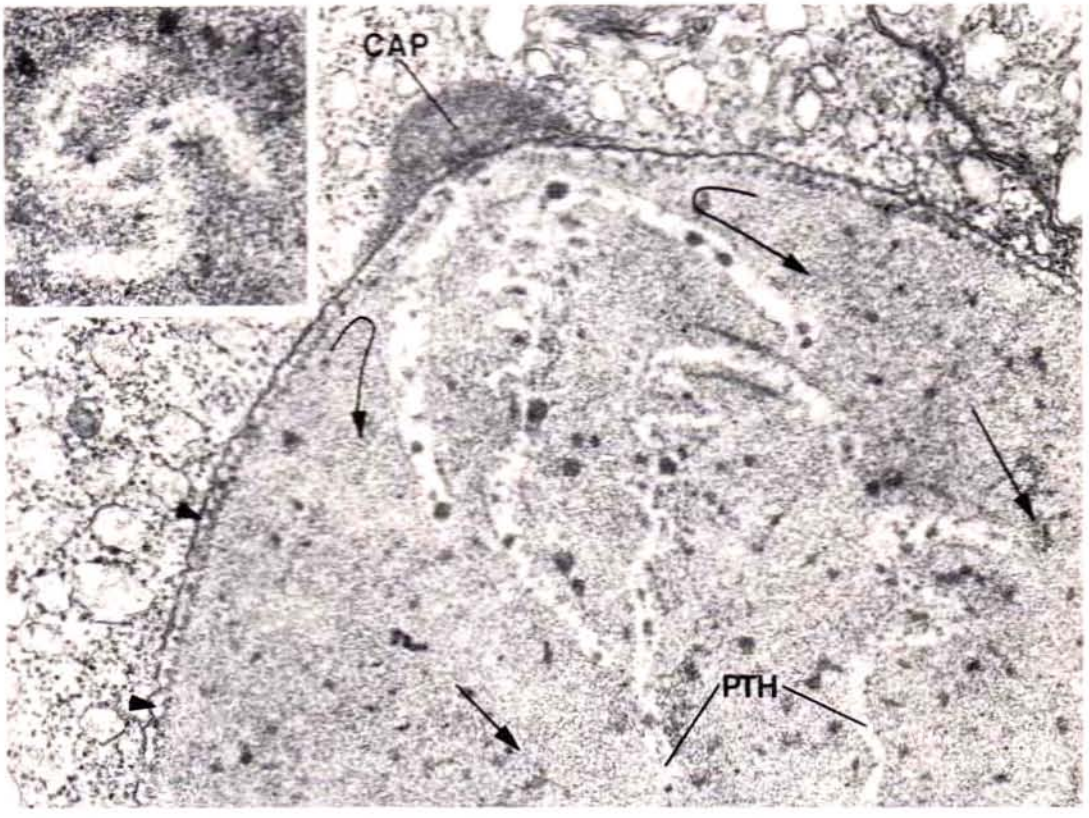

FIG. 15. Longitudinal section of the invaginating external tube (stenotele of *Hydra attenuata*). The electron-lucent middle layer (arrows) can be continuously followed from the wall of the external tube into the matrix of the tube. The tip is covered by the electron-dense cap (CAP). The invaginated middle layer is the presumptive nematocyst thread (PTH). Inset shows the cross section of the triply pleated, presumptive nematocyst thread (terminal region in a stenotele of *Hydra attenuata*). Fig. 15, × 42 000; inset, × 66 000.

and the inner layer is electron dense and 15–20 nm thick. Electron-dense particles are incorporated into the middle layer. The formation of two additional layers starts at the point where the external tube joins the capsule and proceeds in a distal direction. There is no indication that the middle or inner layer could be the continuation of either of the capsule wall layers, the propria or sklera, although there may be contact with the sklera. The outer membrane is the only layer which is common to both the external tube and the capsule (Fig. 14).

Phase of Invagination

The phase of growth and differentiation is followed by the invagination process (Fig. 7D) which actually involves only the middle layer of the external tube wall. This layer can be continuously observed as it invaginates into the tube and can be easily distinguished from the surrounding matrix of the ET and the capsule (Figs. 15, 16). The middle layer is preserved in its structure and measures a constant 20 nm as it protrudes into the interior of the tube. In this investigation the invaginated middle layer is termed the "presumptive nematocyst thread." The presumptive, as well as the mature nematocyst thread, is a hollow structure, but it possesses a much smaller lumen than the external tube. In stenoteles, in the area which later becomes the terminal thread region, the sides of the wall are

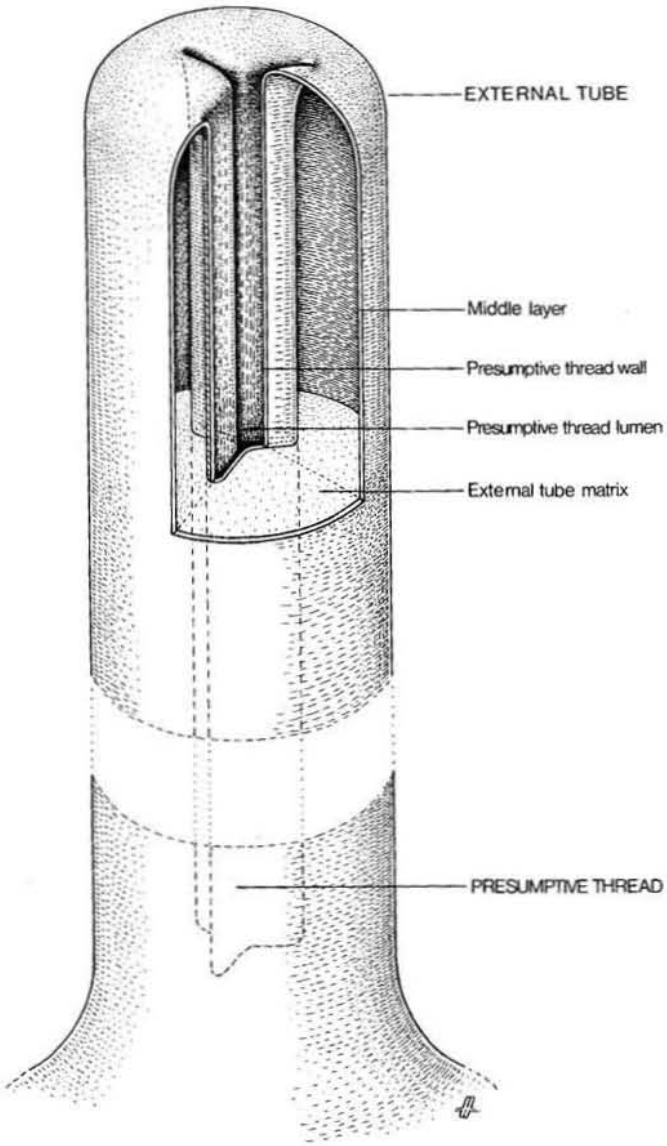

FIG. 16. Schematic drawing of the invagination process. Only the middle layer is shown; the electron-dense layer and the electron-dense cap are not illustrated.

juxtaposed and the presumptive nematocyst thread assumes a triply pleated appearance (Fig. 15, inset, and Fig. 16). During invagination the ET retains its circular shape. The partly invaginated, inner, electron-dense layer can sometimes be found in contact with the invaginated middle layer. In *Hydra attenuata* this layer has been observed only near the tip and does not seem to be preserved after invagination. In *Forskalia sp.* this layer seems to be more stable and is observed in later stages.

The outer layer, the membrane of the ET, does not invaginate. It covers the area of invagination and changes at the tip into electron-dense material which forms a cap over the tip of the invaginating tube (Figs. 7D, 15). The cap is not limited by a mem-

brane and can be distinguished from the surrounding cytoplasm by its greater electron density and homogeneity. As invagination progresses the cap becomes larger. These observations lead to the conclusion that the external tube is closed during the entire invagination process and remains separated from the surrounding cytoplasm.

During invagination all of the matrix of the external tube is displaced into the capsule. At the same time the volume of the capsule increases, but without significant change of its proportions. The propria diminishes in width by only 15% during this extension.

Phase of Maturation

Maturation is defined in this study as all those processes which occur after invagination of the external tube and which lead to the formation of a ripe, functional nematocyst. This developmental phase can be subdivided into three processes which usually overlap in time: (a) differentiation of the armament and operculum; (b) maturation of the capsule components; and (c) maturation of the cnidocil-associated apparatus.

(a) Differentiation of the armament and operculum. Immediately after the external tube is completely invaginated spines differentiate from the electron-dense material which fills the lumen of the presumptive nematocyst thread. The spines insert along the presumptive thread at inpocketings which formed in the differentiation phase. The earliest visible precursors of the spines already possess a multilayered substructure (Figs. 20, 23). Different nematocyst types show considerable differences in spine development, but these will be discussed elsewhere.

The middle layer of the ET up to this point in development was anchored with the other two layers to the membrane as well as partly to the sklera of the capsule wall. During maturation these relationships change and a new connection between the capsule and the nematocyst thread forms. The invaginated middle layer lies between the developing operculum and the propria (Fig. 20). In this zone the propria progressively fuses with the middle layer (=presumptive nematocyst thread) to form a new, solid connection between the capsule and the nematocyst thread (Figs. 21, 22).

The operculum itself shows fine striations during the time the spines are differentiating (Fig. 20), but these striations are not visible in later stages. At the beginning of operculum development electron-dense material lies immediately above the capsule. This material represents the remainder of the electron-dense cap which covered the tip of the external tube during invagination (Fig. 20).

(b) Maturation of the capsule components. As soon as the spines are formed, the wall of the presumptive thread becomes denser and more distinct from the surrounding matrix. A fine but intensively staining layer, 20 nm wide, accumulates on the outer side of the broadened middle layer (Figs. 20–22). This electron-dense layer first appears in the transitional region between capsule and thread, and later it can also be observed adjacent to the propria (Figs. 20–22). Because it is the innermost layer of the capsule wall, it is termed the interna. At this point the final wall substructure of capsule and nematocyst thread is established. The capsule wall consists of three layers: the outer sklera, the middle propria, and the inner interna. The nematocyst thread consists of two layers: the former middle layer of the external tube and the outer interna. Capsule and nematocyst thread are linked by the interna and the fused propria-middle layer (Fig. 24).

The morphological changes of the matrix material include the disintegration of the homogeneous material and the disappearance of the electron-dense droplets. Fully mature stenoteles and desmonemes have a pronounced electron-lucent matrix (Fig. 24); in contrast, the matrix of holotriches is uniformly electron dense (Fig. 25).

(c) Development of the cnidocil-associ-

ated apparatus. The cnidocil-associated apparatus undergoes maturation before the nematocysts migrate to the tentacles. The rods have elongated and can be observed extending parallel to the long axis of the capsule (Fig. 18). The stereocilia begin to develop in close association with the elongation of the rods. They first appear adjacent to the distal end of the rods as limited filamentous masses and are connected to the rods by a homogeneous material. These initial steps of stereocilia formation appear only in the maturation phase, in contrast to the rods which are already present during the growth phase.

DISCUSSION

Based on the results presented here, two main processes can be distinguished in the morphogenesis of nematocytes: (a) the development of the nematocyst itself by means of a complicated secretory process, and (b) the development of a sensory apparatus unique to the nematocyte. Both processes are coordinated with each other.

Early stages have been described using TEM methods by Slautterback and Fawcett (1959), Chapman and Tilney (1959b), Westfall (1966), Lentz (1965), Davis (1969), Carré (1972), and Bouillon and Massin (1974). These authors held the view that the capsule is a secretory product of the Golgi apparatus, and there has been some confusion concerning the involvement of other cell organelles and the identification of the structures which constitute the earliest stage of capsule formation.

Schneider (1900) distinguished early stages of the capsule from other vesicles in the cell by using a special stain. Recently the capsule wall has been described by Guenzl (1968) as consisting of elastin-like material. In the present investigation the earliest stages observed (when the capsule is less than 0.5 μm in diameter) already possess the two main wall layers. It should be pointed out that these layers can be recognized in all papers which have been published on nematocyst morphogenesis. It appears quite uncertain that the nematocyst capsule can be descended from an enlarged Golgi vesicle as assumed in the literature. Instead of this hypothesis, I suggest that even the earliest stages are a secretory product of different, segregated components.

Westfall proposed in 1966 that microtubules encircling the external tube prevent the fusion of Golgi vesicles with the external tube wall and Slautterback (1963) assumed that these microtubules are involved in the transport of small molecules. I found

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FIG. 17. Tangential section of the cnidocil-associated apparatus in a stenotele of *Hydra attenuata*. × 36 000.

FIG. 18. Cross section of a desmoneme in *Hydra attenuata*. The rods and forming stereocilia (SC) are linked by homogeneous material of medium electron density. × 21 000.

FIG. 19. Cnidocil-associated apparatus in *Forskålia sp.* in cross section. Arrowheads indicate the two rootlets, arrows indicate the microtubules between the rods. × 21 000.

FIGS.20–22. Development of operculum and formation of a new linkage between nematocyst thread and capsule wall in *Hydra attenuata*.

FIG. 20. The presumptive thread wall lies between the propria and the operculum (O). Adjacent to the thread wall the electron-dense layer (DL) differentiates. At the tip of the capsule a remainder of the electron-dense cap is situated (arrowheads). Within the lumen of the thread the spines (SP) differentiate. × 20 000.

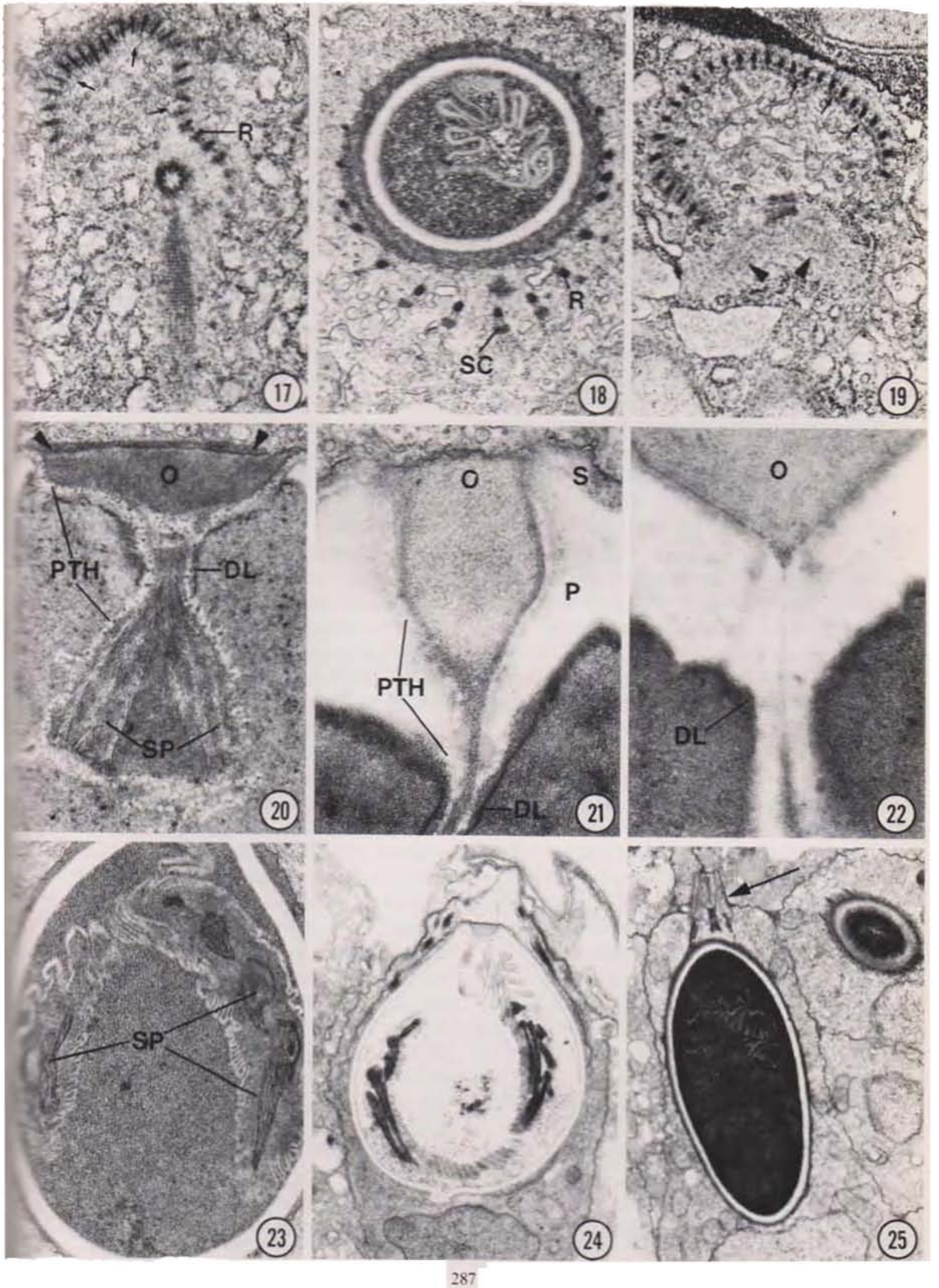
FIG. 21. Fusion between propria and embryonic thread wall (=middle layer) in an advanced state (longitudinal section). × 51 000.

FIG. 22. Fused presumptive thread wall and propria with the typical striations in a longitudinal section. × 47 000.

FIG. 23. The multilayered substructure of developing spines in a desmoneme (*Hydra attenuata*). × 20 000.

FIG. 24. Mature desmoneme of *Hydra attenuata* with homogeneous spines and the two-layered wall of the nematocyst thread. × 10 000.

FIG. 25. Mature holotriche of *Hydra attenuata* with outgrowing cnidocil (arrow). × 8000.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that there is a correlation between the number of microtubules around the ET and the tube's diameter. The thicker the ET, the more microtubules are present around it. The distance between two adjacent microtubules is constantly 14 nm; and, in favorably oriented sections, one can recognize the electron-dense bridges between the microtubules. These observations indicate a possible morphogenetic function of the microtubules in that the number and circular arrangement of microtubules may determine the diameter of the external tube. The question of what determines the number and pattern of microtubules is still unresolved in this as well as in other systems. The problem exists as to whether this could be determined by a specific interaction of the bridges, by the centriole itself, or by both (see review, Dustin, 1978).

The varying diameter of the external tube was reported for the first time by Nussbaum (1887) and later by Schneider (1900); however no significance was given to this observation until recently (Carré and Carré, 1973). Differentiation of the invaginated external tube into regions of different diameter, as it has been postulated by Skaer (1973) on *Rosacea cymbiformis*, was not observed in the present study.

Skaer (1973) also described details of the external tube wall construction. Based on observations made at the tip region he described three layers having a total thickness of 92 nm. These consisted of a middle layer of 12 nm, an outer layer, and an inner layer of 40 nm each. My observations also indicate that the tube wall consists of three layers. However, only a single unit membrane is present in the tip region. The three layers of the more proximal wall consist of the unit membrane, a middle layer 20–25 nm thick, and an electron-dense inner layer 15–20 nm thick.

Perhaps the most important and controversial event in nematocyst morphogenesis is the invagination of the external tube. This is actually a modified invagination, since only the middle layer of the ET wall invaginates and not the complete tube wall as assumed by Jickeli (1882), Nussbaum (1887), Murbach (1894), Iwanzoff (1896a,b), Schneider (1900), Lipin (1911), Slautterback and Fawcett (1959), Slautterback (1961, 1963), Carré (1972, 1974), Carré and Carré (1973), and Raikova (1978). The inner layer does not appear to be stable, and the outer membrane condenses into an electron-dense cap, which covers the tip of the invaginating tube and encloses it throughout the whole process of invagination.

On the other hand, Will (1910, 1926), Ewald (1915), Westfall (1966), Guenzl (1968, 1972), Bouillon and Massin (1974), Ivester (1977), and Germer and Huendgen (1980) presumed that the ET is lost, either by assimilation into the capsular region or by resorption into the cytoplasm. Following this theory, the nematocyst thread would be newly formed independently of the external tube. The fact that the invaginated middle layer, the presumptive nematocyst thread (interpreted by Westfall as the new "internal thread"), has a completely different appearance from the three-layered external tube wall may explain why such a hypothesis could persist even after the use of TEM.

The forces affecting the invagination of the external tube remain unclear. In this process the total matrix has to be transported into the capsule with a speed of 0.75 μm/min (Holstein, 1980a, b). There are some indications that contractile proteins, which are involved in cell motility in general, could be involved in this process. The hypothesis presented by the early light microscopists of a negative pressure effect seems to be highly improbable since I observed no changes in the density of the matrix material during invagination. This is in contrast to the distinct changes which were observed during the maturation phase.

The present observations further indicate that the formation of spines and operculum seem to be physically connected. The de-

veloping operculum is continuous with the forming armament and also has a morphological similarity to the material which comprises the spines. Two different assumptions are found in the literature. Iwanzoff (1896b), Schneider (1900), Will (1910), and Guenzl (1968) believed that the operculum was a product of the surrounding cytoplasm. Until now no evidence has been found that the Golgi apparatus or the endoplasmic reticulum directly form this structure. Ewald (1915) and Westfall (1966) thought that the operculum was a part of the capsule wall. However, this mode of formation can be excluded because the developing operculum is always clearly separated from the surrounding capsule wall by the invaginated middle layer (=presumptive nematocyst thread wall). In addition there are no structural similarities between the capsule wall and the operculum.

It is possible that material from the electron-dense cap may participate in formation of the operculum. This seems likely since this cap covers the tip of the ET in all stages of invagination but is no longer present once the operculum is fully formed.

A most interesting result of the present investigation is the finding of a definite correlation between nematocyst morphogenesis and the development of the cnidocil and its associated apparatus. In the literature only isolated observations on this subject exist (Mattern *et al.*, 1965; Westfall, 1966; Bouillon and Massin, 1974) and a direct relationship between the development of the two main structures of a nematocyte has not been considered. Mattern *et al.*, (1965) have suggested an inducing potency of the centrioles in the formation of the capsule, but I have presented evidence of an intimate relationship between the pair of centrioles and the developing nematocyst throughout the entire process of nematocyte morphogenesis.

The function of the centrioles seems to be twofold. First, they act as the microtubule-organizing center for growth and shaping of the external tube and capsule. Second, they induce the formation of the sensory apparatus of the cell, the cnidocil and its associated structures. The function of the centriolar region as an organizing center for microtubules is generally accepted (Dustin, 1978), but different hypotheses exist to explain which of the centriolar structures might be responsible for the nucleation of microtubules (see for review, Dustin, 1978; Telzer and Rosenbaum, 1979; Pepper and Brinkley, 1979; Schmidt and Hoeltgen, 1980). It is possible that the nine pericentriolar, spoke-like processes could act as a special region nucleating the microtubules. This is indicated by the contact of microtubules with the button-shaped thickenings of the ends of these processes. Further studies on this question are necessary.

One additional point to be made concerns the terminology for the cnidocil and its associated apparatus. I propose that the term cnidocil be used to name only the ciliary structure of the sensory apparatus of the nematocyte. This is a modified cilium, and it has associated stereocilia which have been previously included in the term cnidocil (Slautterback, 1967; Bouillon and Lévi, 1967). The stereocilia should be considered together with the rods as the cnidocil-associated apparatus.

The literature contains many studies on nematocytes with diverse results and conclusions. In this paper an attempt has been made to explain some of the discrepancies and to present new information which is necessary for understanding the structure and morphogenesis of one of the most complex organelles known, the nematocyst.

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Note added in proof. The morphogenesis of hematocysts in Anthozoa (Octocorallia) will be described by H. Schmidt (*Helgolaender Wiss. Meeresunters.*, in press, 1981).

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