# ELECTRON MICROSCOPE OBSERVATIONS ON THE STRUCTURE AND DISCHARGE OF THE STENOTELE OF HYDRA

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ABSTRACT

Sections of the stenotele type of nematocyst of *Chlorohydra hadleyi* have revealed that the stenotele, upon firing, completely everts its stylets and spines and the long, thin tubule, much as the eversion of the tubule of the nematocyst of the jewel anemone (Picken, 1953; Robson, 1953). Alternative mechanisms for supplying the energy necessary to forcefully discharge the stenotele contents are discussed as well as the possible significance of several regions containing highly ordered periodic structure. The origin of nematocysts as kinetosomal derivatives is discussed as a possibility suggested by the symmetry of the stenotele contents and the structure, location, and function of the cnidocil.

## INTRODUCTION

The mechanism of discharge of the large holotrichous glutinant type of nematocyst of the jewel anemone, *Corynactis viridis*, appears to be well established as resulting from eversion of this tubular organelle activated by progressive swelling within the nematocyst capsule (Picken, 1953; Robson, 1953).

However, some of the more recent views regarding the method of discharge of the stenotele of hydra (Chapman, 1961; Slautterback, 1961) leave the mechanism of discharge and the means of extension of the fine tubule in some doubt.

Electron micrographs will be presented which support the view that the entire process of extrusion involves eversion of the stylets, the spines, and the long thin tubule. Other electron micrographs appear compatible with the hypothesis that the mechanism of eversion involves hydrostatic pressure.

## MATERIALS AND METHODS

Specimens of *Chlorohydra hadleyi* were fixed in 6 per cent glutaraldehyde and postfixed in 1 per cent osmium tetroxide buffered with McEwen's saline. One part of 2 per cent osmium tetroxide was added to one part of McEwen's saline buffered to pH 7.4 (McEwen, 1956). Dehydration was accomplished by ethanol and propylene oxide, and cells were embedded in Maraglas (Spurlock *et al.*, 1963). Sections, cut in a Porter-Blum microtome, were stained with lead citrate (Reynolds, 1963) and examined in an RCA EMU 3 E microscope.

## RESULTS

Fig. 1 depicts a mature, or nearly mature, stenotele. Just beneath the operculum, the tips of the stylets of the stenotele are observed. Micrographs of Slautterback (1961) and Chapman (1961) show the stenotele to contain three stylets, two of

FIGURE 1   A longitudinal section through a stenotele. *O*, operculum; *S*, stylet; *SS*, spines; *T*, tubule; *H*, head of tubule; *CP*, capsule; *M*, matrix; *SM*, stenotele membrane (invaginated capsular wall, Chapman, 1961); *G*, glycogen granules. × 29,000.
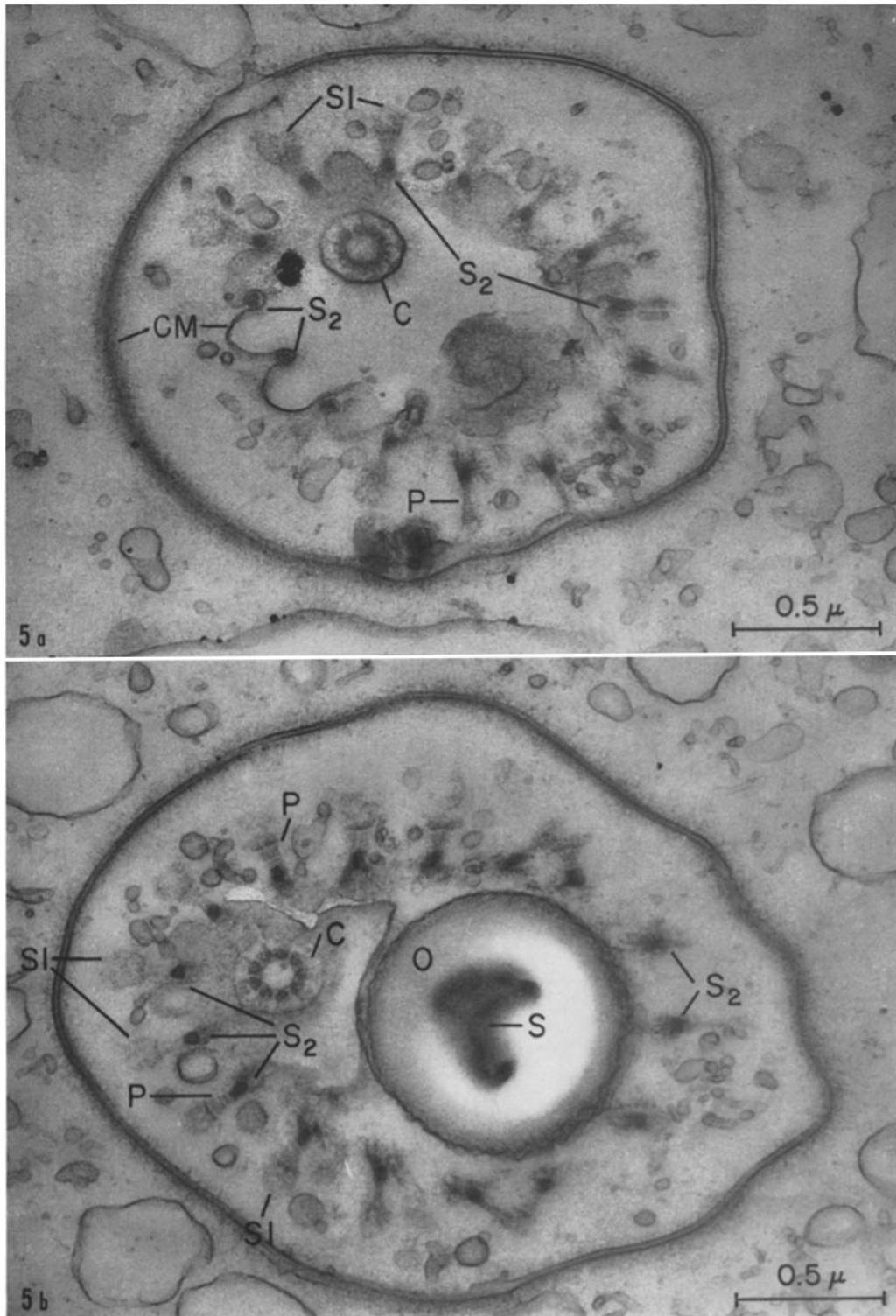
FIGURE 2   An oblique section through the cnidocil (C) region of a nematocyst. Outer long supports (S₁) and inner, short, dense supports (S₂) are seen along with a cell membrane fold (F) derived from adjacent cells. A periodicity of 100 A is visible in the desmosomes (D). × 58,500.
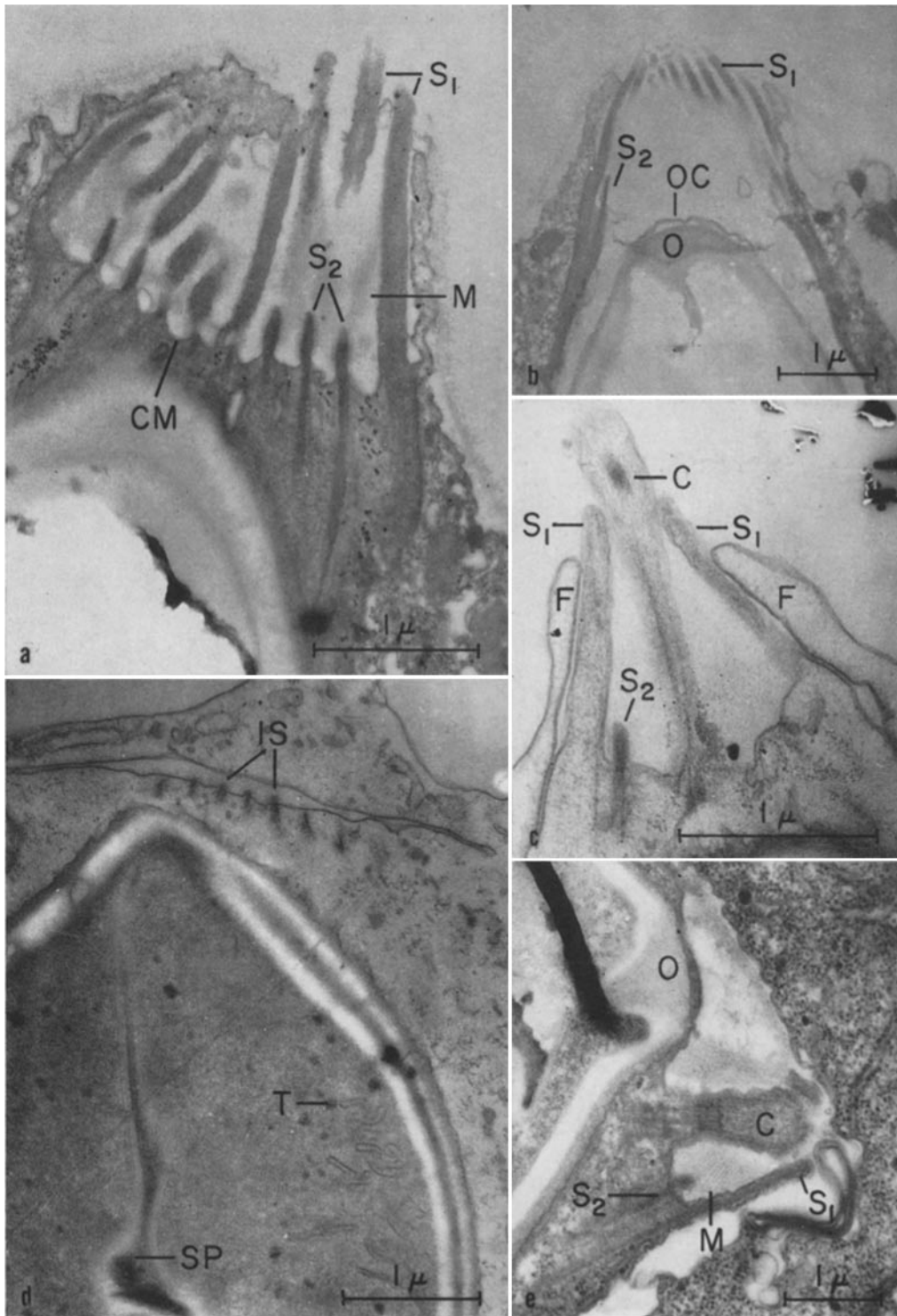
FIGURES 3 *a* to 3 *e*   Longitudinal views of the cnidocil region. Figs. 3 *d* and 3 *e* are of immature nemato-cysts. *CM*, cell membrane; *OC*, membranous opercular cap; *O*, operculum; $S_1$, external supports; $S_2$, internal supports; *C*, cnidocil; *IS*, immature supports; *M*, light matrix, with periodic structure; *F*, membranous fold; *SP*, developing stenotele spines. Fig. 3 *a*, × 28,000; Fig. 3 *b*, × 17,000; Fig. 3 *c*, × 33,500; Fig. 3 *d*, × 23,000; Fig. 3 *e*, × 16,500.
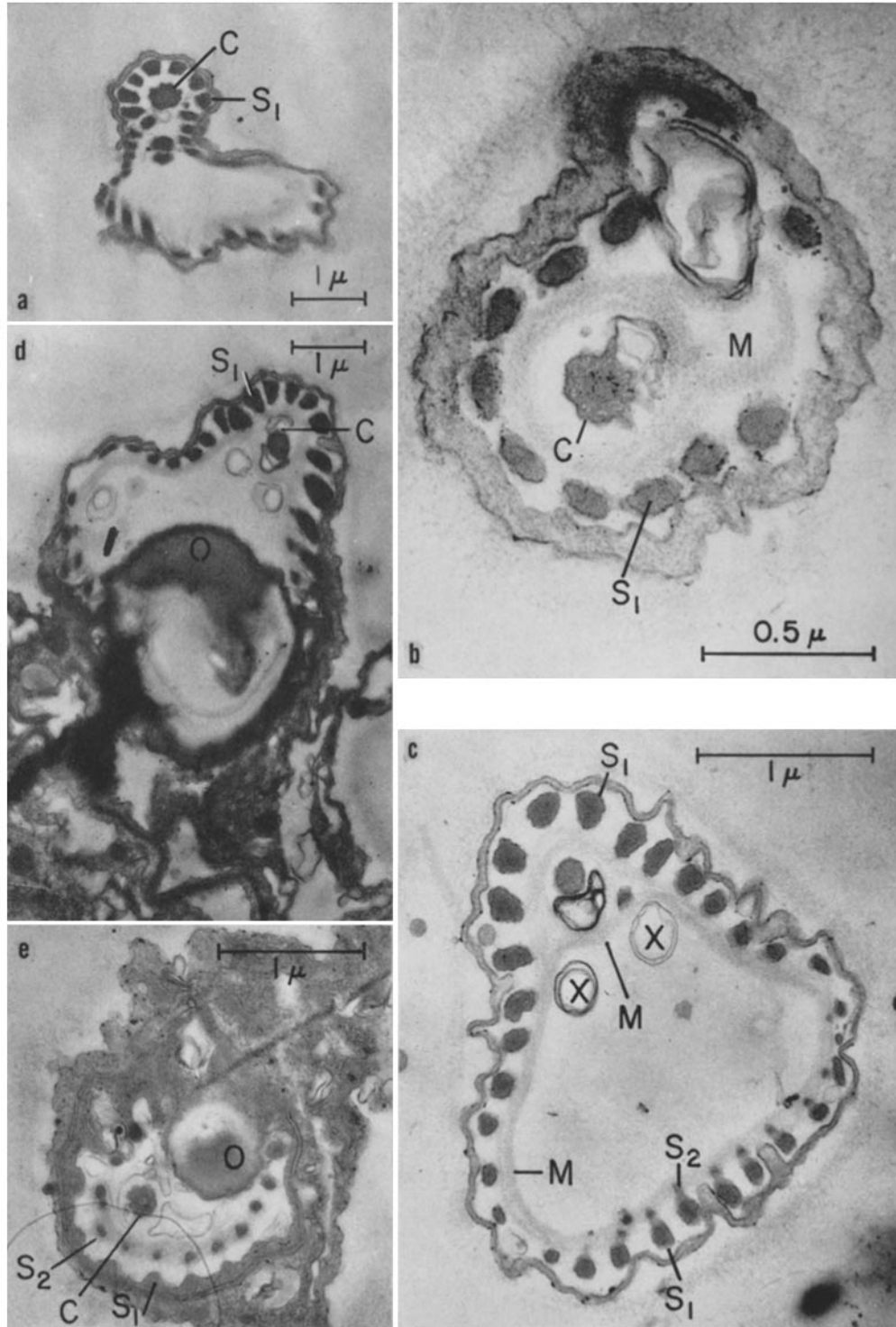
FIGURES 4 $a$ to 4 $e$   Progressively deeper cross-sections through the cnidocil ($C$), showing outer supports ($S_1$), the light matrix ($M$), inner supports ($S_2$), and double membrane structures ($X$) of unknown significance. Fig. 4 $a$, $\times$ 12,500; Fig. 4 $b$, $\times$ 57, 500; Fig. 4 $c$, $\times$ 29,000; Fig. 4 $d$, $\times$ 12,000; Fig. 4 $e$, $\times$ 24,500.

C. F. T. MATTERN, H. D. PARK, AND W. A. DANIEL   *Nematocyst of Hydra*   625

FIGURES 5 *a* to 5 *d*   Cross-sections of stenotele nematocysts at varying levels.

FIGURE 5 *a*   A cross-section distal to the operculum. The invaginated cell membrane (*CM*) is tightly applied to the inner circumferential supports.

FIGURE 5 *b*   A section including part of the operculum and stylets (*S*). Both sections show the ninefold symmetry of the cnidocil (*C*), the inner circumferential supports (*S₂*) connected by a periodic structure (*P*) to the outer circumferential supports (*S₁*).
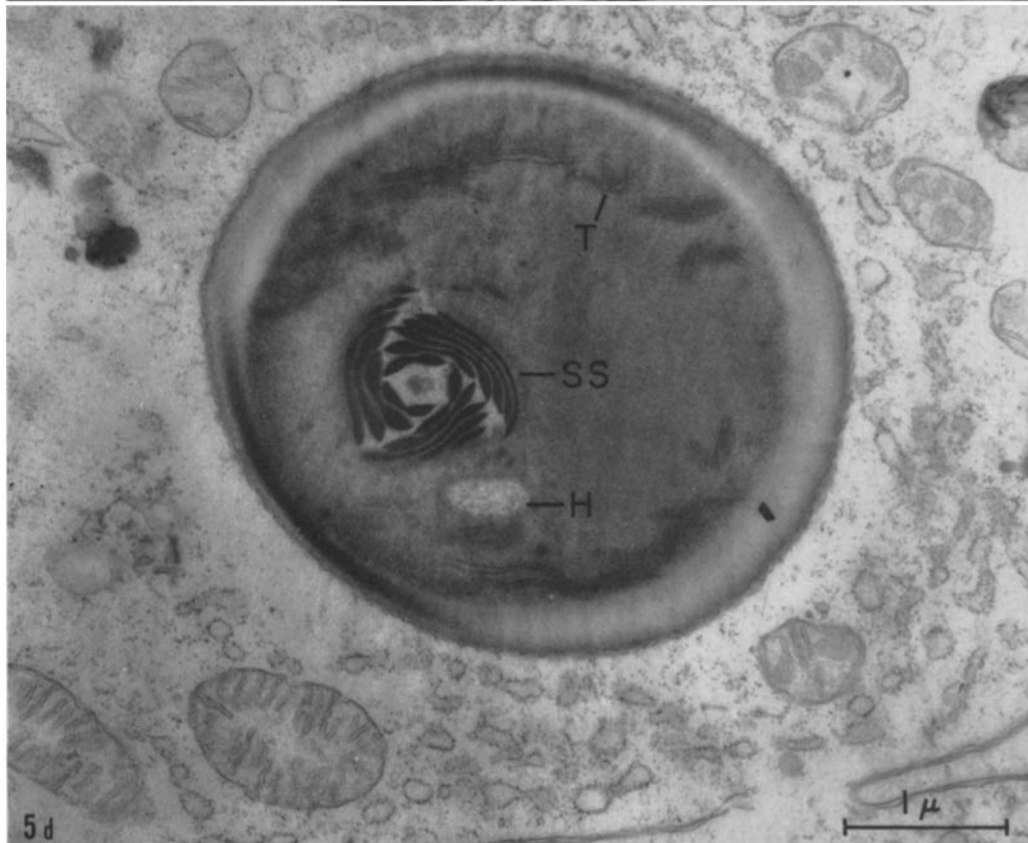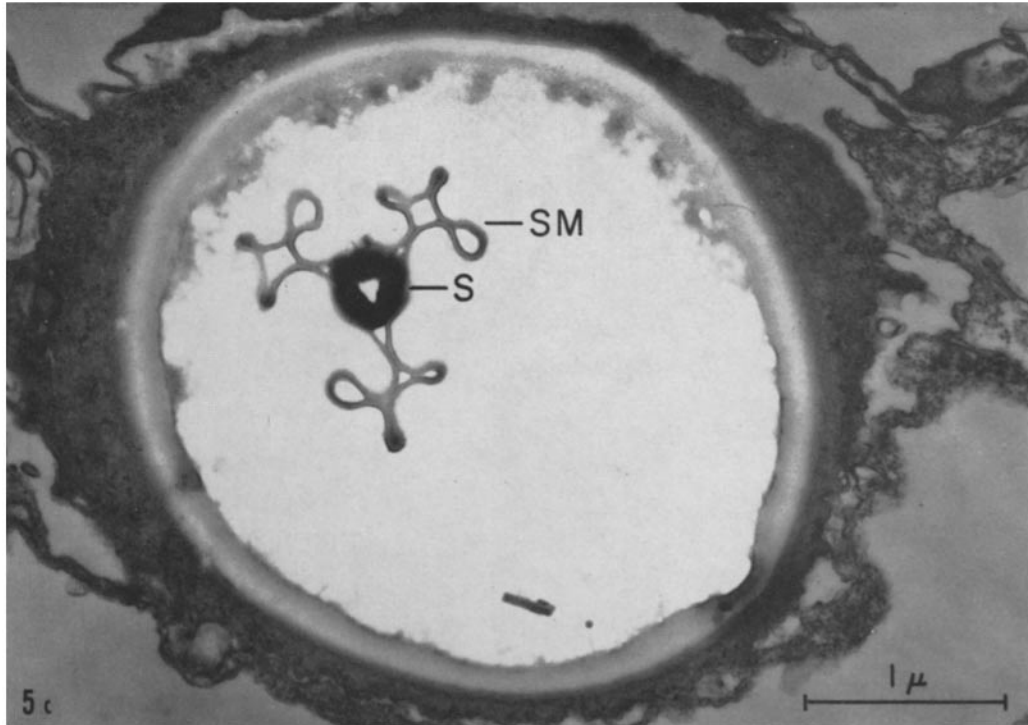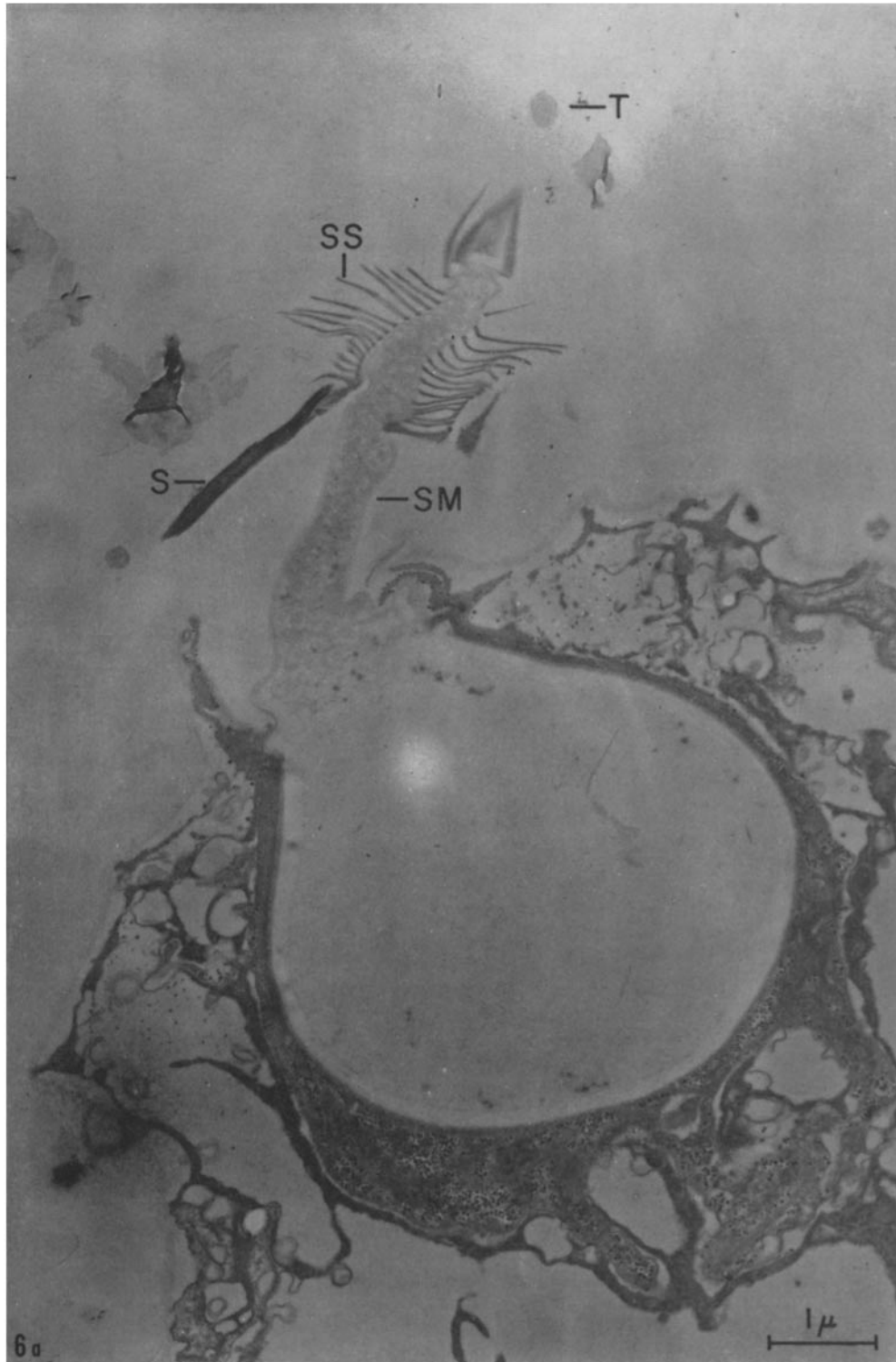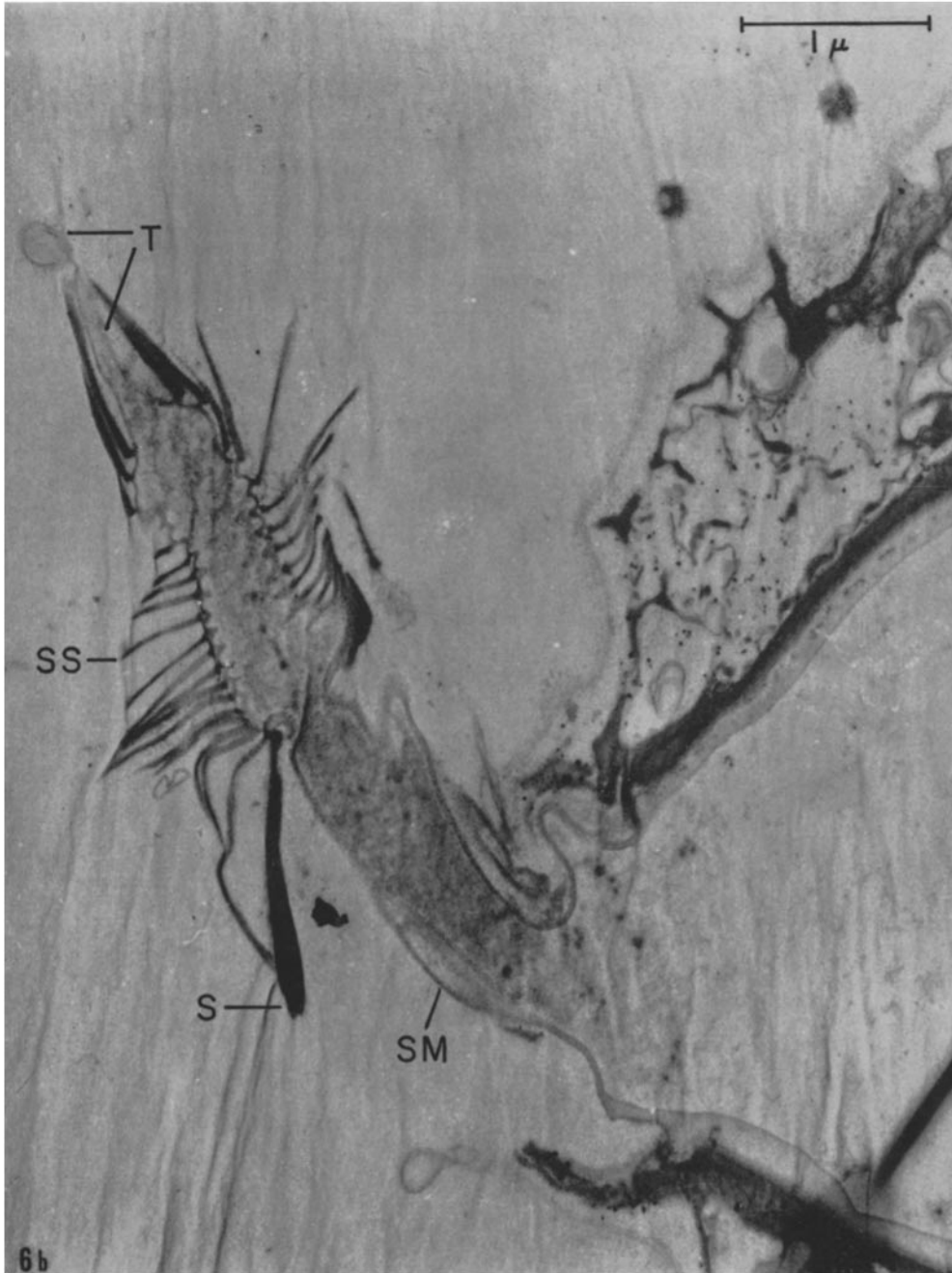
FIGURE 5 c  A section through the stylets (S) showing the threefold (pseudo-ninefold) symmetry of the stenotele membrane (SM).

FIGURE 5 d  This section is through the spine (SS) region of the stenotele, demonstrating the small tubule (T) and head of the tubule (H). Fig. 5 a and b, × 50,500; Fig. 5 c, × 30,000; Fig. 5 d,× 24,500.

FIGURES 6 a and 6 b    Different sections through the same fired nematocyst. In them may be seen a stylet (S), numerous small spines (SS), and the everted stenotele membrane (SM) now forming the "harpoon rope." The matrix material is no longer present and the tubule (T) has been ejected and may be seen near the point of the everted stenotele. In Fig. 6 b may be seen a narrow tubule continuous with the membrane to which the spines are attached and extending proximally between the spines at the tip of the everted small spines. This appears to be the beginning of the everted fine tubule. Fig. 6 a, × 18,000; Fig. 6 b, × 29,000.

which are seen in Fig. 1. Proximal to the stylets are a large number of small spines which end at the stenotele membrane in a slight enlargement generally associated with "waves" in the membrane. One additional structure of note is the coil of the fine tubule. In cross-section the tubule is seen folded into a triple-"bladed" structure. This nematocyst contains a tubular coil consisting of at least nine full turns, not including that portion which connects the coil with the dilated head of the tubule and, presumably, ultimately to the base (spine end) of the stenotele. The nematocyst capsule and operculum are visible, but the cnidocil is not seen in this section. The location of the cnidocil just lateral to the operculum was clearly shown by Chapman and Tilney (1959a and b) and by Slautterback (1961).

An oblique section through a cnidocil is seen in Fig. 2. Desmosomal regions on either side of the cell are seen to consist of a regular periodic structure of about 100 A. It is not known if this ordered structure is related to that of epidermal septate desmosomes of *Pelamatohydra* as shown by Wood (1961). Outer and inner supporting structures (Chapman and Tilney, 1959 a, Chapman, 1961) and the cnidocil are also visible, but the nematocyst proper does not appear at this level.

Figs. 3 a to 3 e are longitudinal views of the cnidocil region. It is not possible to identify the type of nematocyst in all of these micrographs. Fig. 3 a, in conjunction with Figs. 4, 5 a, and 5 b, shows a rather uniform termination of the internal supports just above the cell membrane. Figure 3 b shows a membranous cap over the opercu'um as well as the distal convergence of the long external supports. Fig. 3 c is a similar view showing the close approximation of the tips of the outer supports to the cnidocil proper. Fig. 3 d shows an immature stenotele with an early differentiation of supports at the cell surface. In Fig. 3 e, another immature nematocyst; a periodic structure of about 88 A spacing is seen in the light matrix internal to the developing outer supports and into which the inner supports protrude (see also the matrix in Fig. 3 a). The electron opacity of this matrix is similar to that of the developing operculum.

Figs. 4 a to 4 e are progressively deeper cross-sections through the cnidocil area. Fig. 4 a, probably the most distal view, shows an indistinct cnidocil region surrounded immediately by nine closely applied outer supports (also in Fig. 4 b).

Additional supports are visible in the membrane-bounded region away from the cnidocil. In Fig. 4 b a relatively electron-transparent (light) matrix, having a periodic structure as in Fig. 3 e, is observed internal to the outer supports and surrounding the cnidocil proper. The doublet-nine structure of the cnidocil can barely be distinguished. Fig. 4 c is a somewhat oblique section, being deeper at the bottom of the figure than at the top. Some of the inner, shorter, supporting structures now appear in the light matrix. It is apparent that the outer supports diverge as they course deeper. Fig. 4 d is a still more oblique section encompassing part of the operculum as well as the cnidocil and supporting structures. Fig. 4 e, also oblique, shows the termination of the outer supports as they become fused with the peripheral cell cytoplasm.

Figs. 5 a through 5 d show cross-sections of stenoteles at several levels. The first, Fig. 5 a, appears to be just distal to the operculum. Fig. 5 b presents a section including part of the operculum and the stylets. The ninefold symmetry of the cnidocil is clearly visible in Fig. 5 b; the inner and outer circumferential supports may be seen in both sections. These two supporting structures are connected by a periodic structure of roughly 260 A spacing. The invaginated cell membrane is closely applied to the inner supports in Fig. 5 a. The number of inner supports is 15 in one and 16 in the other micrograph.
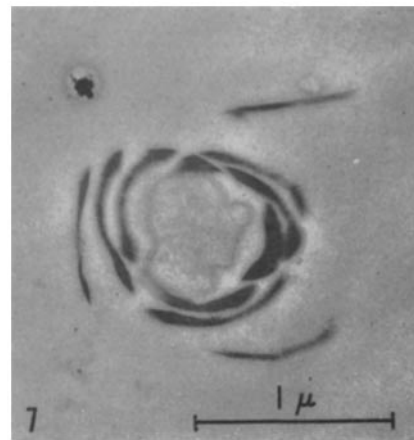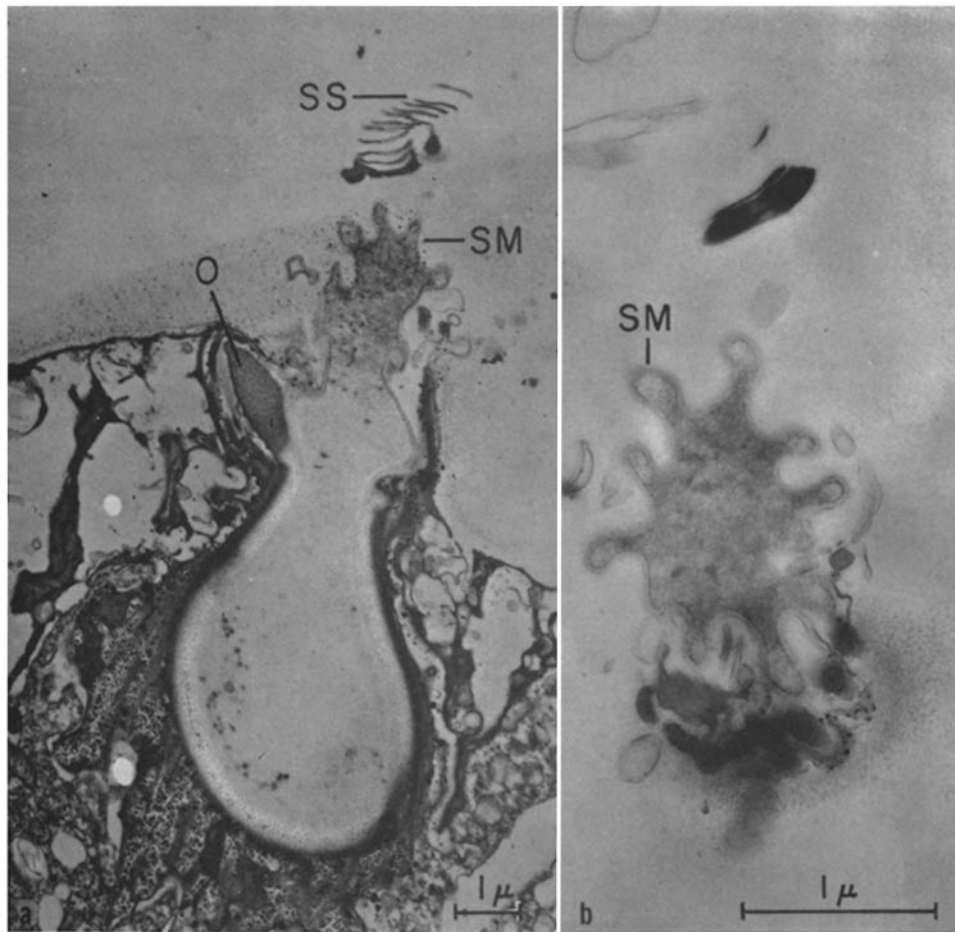
Fig. 5 c is a micrograph of a section of a steno-



FIGURE 7. Cross-section through the tip of an everted (fired) stenotele. Note the small spines, internal membrane, and absence of an external membrane. × 29,500.

FIGURES 8 a and 8 b   Oblique and transverse sections through the everted stenotele membrane showing the pseudo-ninefold symmetry, in the everted state. Note the "sprung" operculum (0). Fig. 8 a, × 10,000; Fig. 8 b, × 29,000.

tele at the level of the stylets. The threefold symmetry is apparent and the stenotele membrane shows a pseudo-ninefold symmetry which is also apparent in micrographs of Slautterback (1961) and Chapman (1961). The absence of matrix material in this and in an adjacent nematocyst (not shown) may result from the stage of maturity or may represent an artefact.

In Fig. 5 d the section is at the level of the spines, again showing a threefold symmetry. Por tions of the tubule and part of the head region of the tubule are seen. The continuation of the tubule with the head has been clearly demonstrated by Chapman (1961).

The appearance of fired stenoteles may be seen

in Figs. 6 to 9. In Fig. 6 a, the complete eversion of the stenotele membrane is visible. That part that formerly (Fig. 1) enveloped the stylets now forms the thick tubule connecting the everted stenotele contents to the empty capsule, as shown by Chapman and Tilney (1959 b). The exterior portion of the stenotele membranes to which the spines were connected in Fig. 1 is now interior. Fig. 6 b shows another section of the same fired stenotele. Unfortunately the grid membrane is wrinkled in this area. Nonetheless, the stenotele membrane, now internal to the spines, can be followed into the tip of the fired, everted stenotele and in all probability connects to the nearly cross-sectioned fine tubule just at the point of this
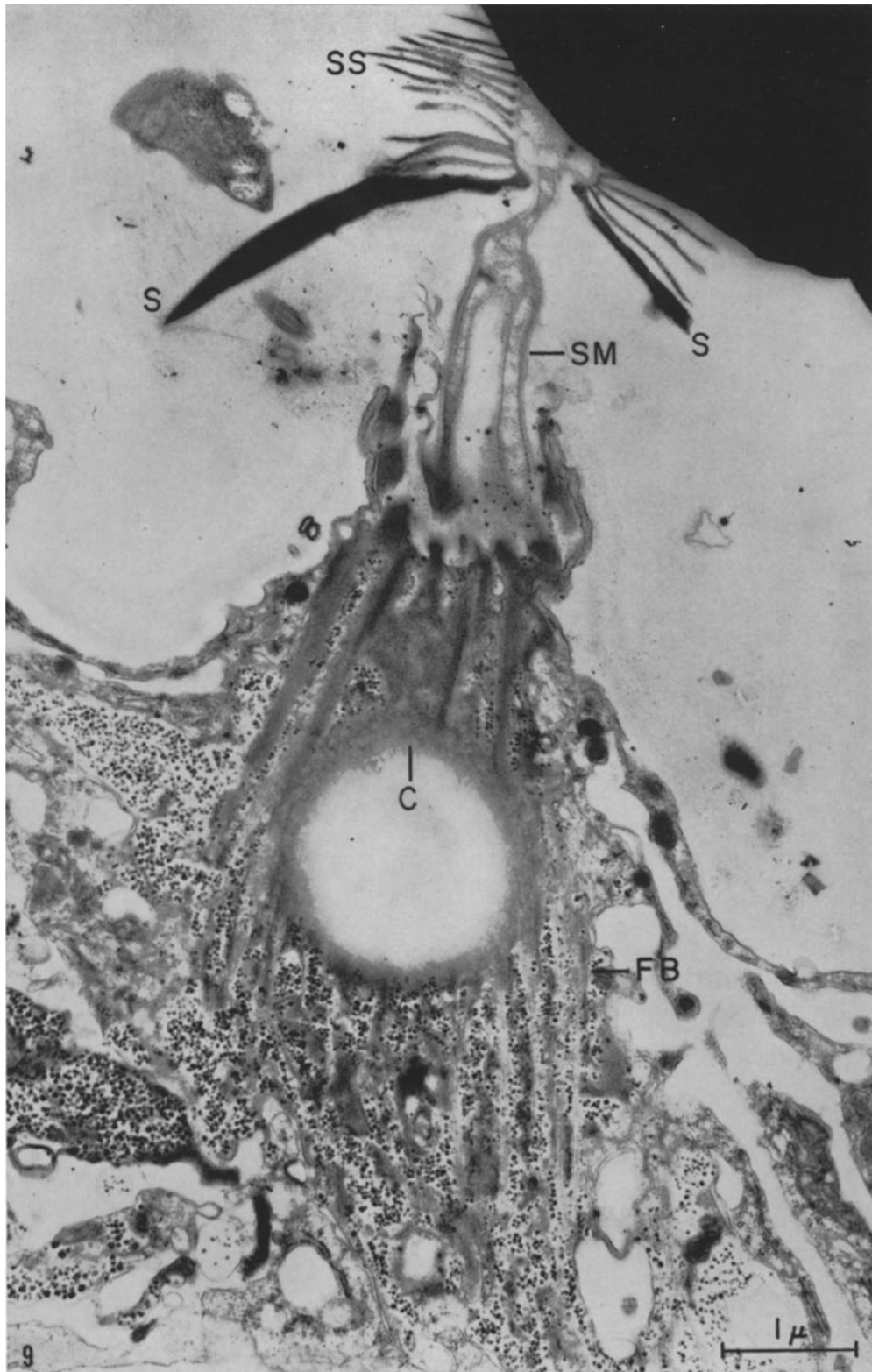
FIGURE 9   A fired nematocyst showing a complex fibrillar (or tubular) system (*FB*) coursing circum-
ferentially about the nematocyst capsule (*C*) and extending nearly to the base of the cell. × 23,000.

FIGURE 10  A cross-section through the large spine region of a stentotele, showing a 146 A periodicity in the stenotele membrane. ✕ 99,000.
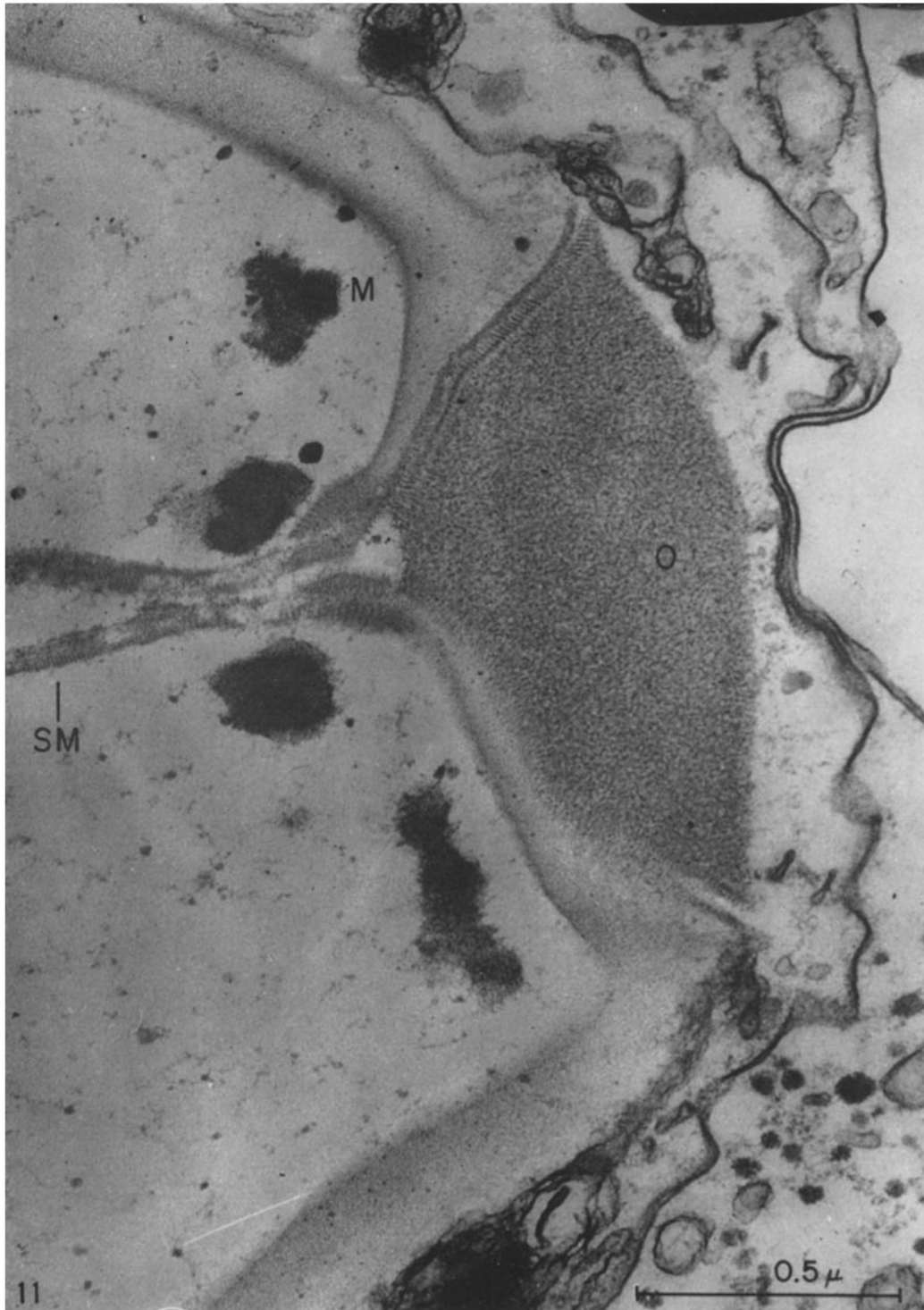
FIGURE 11  A longitudinal section through the operculum ($O$), showing the opercular reflection of the stenotele membrane ($SM$) with mean periodicities varying from 86 to 103 A. $M$, dense matrix. $\times$ 88,500.

FIGURE 12 *a* and *b*   Different sections of the same cnidoblasts containing immature holotrichous isorhizas (*HI*), showing nucleus (*N*), mitochondria (*MI*), endoplasmic reticulum (*ER*), and the paired centrioles (*CT*), one in *a*, the other in *b*. $\times$ 29,000.

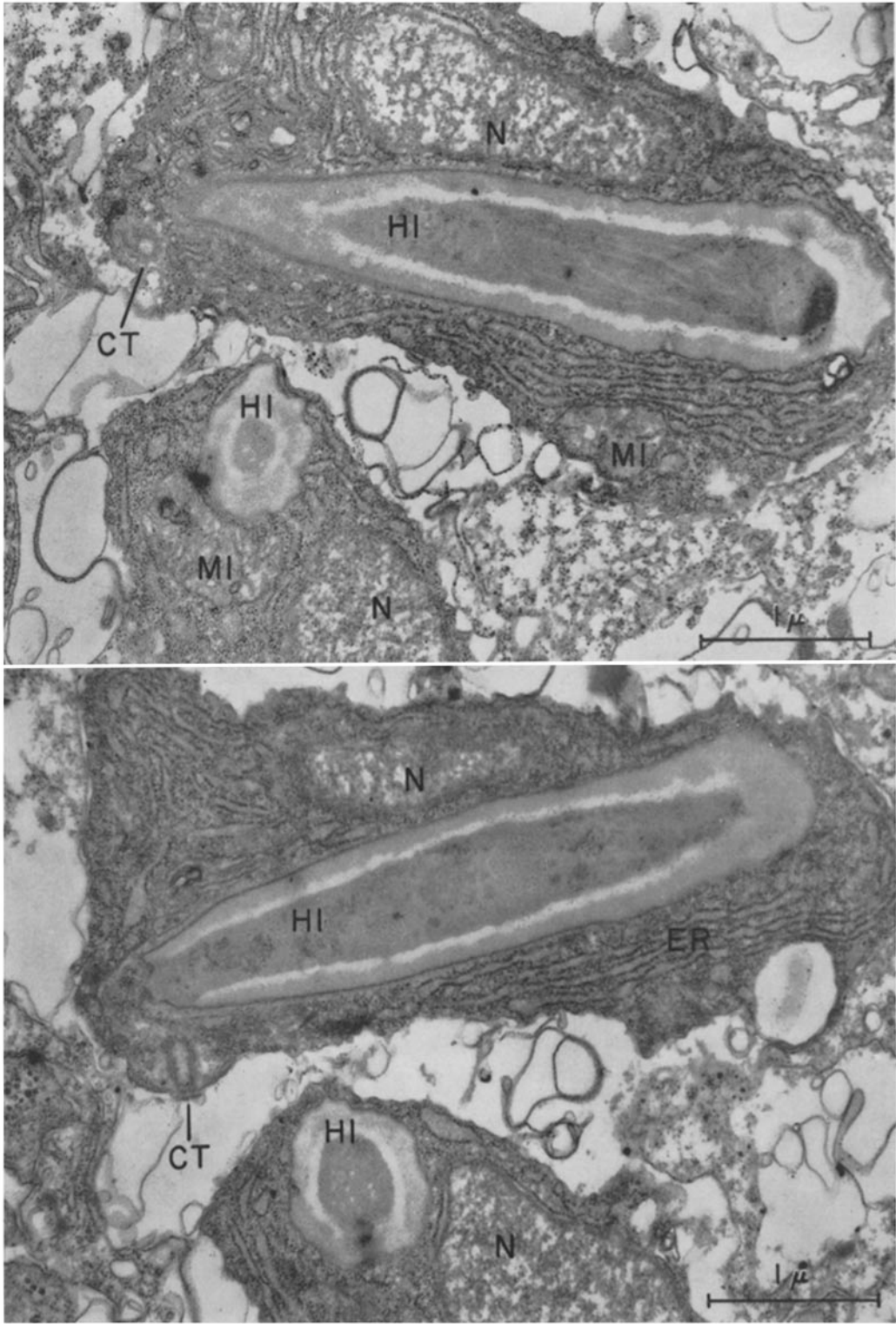organelle (also seen in Fig. 6 *a*), since light micros-copy clearly reveals a very long filament about 0.5 $\mu$ in diameter continuous with the point of the fired stenotele.

Fig. 7 shows a cross-section of the spine region of a fired stenotele. The beginning of the fine tubule or the end of the everted stenotele mem-brane is seen surrounded by spines.

In Fig. 8 *a*, a portion of the spine region of the stenotele is seen as well as the anchor membrane attached to the empty capsule. Several ridges are seen protruding from the anchor membrane. These projecting ridges are seen in Fig. 8 *b* where the pseudo-ninefold symmetry of the stenotele membrane (Fig. 5 *c*) is again visible; here, how-ever, the membrane is completely everted.

In Fig. 9, a fired stenotele is partially obscured by a grid bar. However, portions of two stylets and many spines are still visible. The appearance of the anchoring stenotele membrane is due to the longitudinal sectioning through ridges of this membrane. The section courses just below the tangent to the nematocyst capsule and shows an extensive fibrillar network which is either a con-tinuation of the supporting structures or inde-pendent fibrillar connection between the cnidocil region and a region near the base of the cell. A somewhat similar perimeter of hollow tubules 180 A in diameter was reported by Slautterback (1961, 1963).

Slautterback (1961) and Chapman (1961) have reported, respectively, a 150 to 160 A periodic structure in the "invaginated capsular wall," herein called the stenotele membrane. Such a periodicity of 146 A is seen in Fig. 10 over the entire membrane.

Another periodic structure, varying from 86 to 103 A spacing, is seen in Fig. 11 in the reflection of that part of the stenotele membrane which separates the base of the operculum from the nematocyst capsule. The very dense matrix ma-terial is also seen to have a periodic structure.

In Fig. 12 may be seen sections of two immature nematocysts of another type, the holotrichous isorhiza. Centrioles are seen in different sections of the same cells just lateral to the developing tip of the nematocyst.

## DISCUSSION

These observations bear on several unsettled questions: (*a*) the mode of discharge of the long,

fine tubule of the stenotele; (*b*) the source of the energy for discharge of the nematocyst; (*c*) the nature and purpose of the periodic structure of the stenotele membrane; and (*d*) the origin of the nematocyst. The discussion of these topics will be largely speculative since the electron microscope observations can, at best, only suggest the follow-ing interpretations.

(*a*) The continuity of the membranes observed in Figs. 6 *a* and 6 *b*, as well as the evidence for complete eversion of the spinous region of the stenotele from light microscopy, leaves little doubt doubt that the long, thin tubule of the stenotele nematocyst everts *in toto* as does that of the holo-trichous nematocyst of the jewel anemone (Picken, 1953; Robson, 1953). The tubule must, in fact, be attached near the small spine region and it must evert in order for it to become "exterior" to the stenotele membrane. This eversion would provide an excellent means of getting the tubule head contents, presumably containing a toxin (Welsh and Moorhead, 1960; Chapman, 1961), into the hydra's prey. This is probably effected through the penetration of the cuticle of the prey by the stylet and spines which, after everting, act as an anchored harpoon.

(*b*) As to the source of energy for firing the nematocyst, electron micrographs present little evidence. However, the fine fibrils in Fig. 9 and fine tubules shown by Slautterback (1963) pro-vide a possible clue to the mechanism. These fine structures are very similar to the 150 to 180 A tubular complexes associated with cilia, stereocilia, flagella, centrioles, contractile vacuoles, striated rootlets, and mitotic spindles (Sleigh, 1962; Pitelka, 1963; Grimstone and Cleveland, 1965). A role of these tubules in contraction seems quite certain. A contractile function in their position surrounding the nematocyst could provide an increased pressure within the cyst, which, if suf-ficient to displace the operculum, could result in firing. Compatible with this view is the triggering of the process by the cnidocil, another variant of the cilium-flagellum-complex all related by nine-fold symmetry of microtubules (Sleigh, 1962). The most disturbing feature of this hypothesis is the difficulty in readily demonstrating the fine tubules.

An alternative explanation is that the nemato-cyst develops an increasing internal hydrostatic pressure during differentiation, premature firing being prevented by the cage-like external supports

which converge to a narrow opening. These in turn may be secured by the dense, inner supports to which they are connected by a material having a periodic structure resembling that of ciliary rootlets, common to many metazoa (Sleigh, 1962, pp. 67–69). One might speculate further that, upon impulse from the triggering cnidocil, the two supports are disconnected, and the large supports bend outward permitting the operculum to break loose. The capsular contents would then be free to emerge through the relatively large ring of internal supports and the opened ring of external supports.

(c) It is of interest to note that those membrane regions which likely are subject to the greatest stresses consist largely of highly ordered material of about 85 to 260 A periodicity. These regions are the opercular base, presumably restraining the stenotele until the moment of firing, the stenotele membrane which when everted must hold the harpooned prey, the desmosome region which likely prevents the cnidoblast from being ripped from the organism by the prey, and the periodic matrix into which the inner supports project and which appears to connect the inner and outer supports.

(d) Slautterback and Fawcett (1959) and Slautterback (1961) have described in detail the association of the Golgi complex and the endo-

plasmic reticulum with the developing nematocyst. It seems highly probable that their interpretation that these organelles are involved in active synthesis of materials required by the developing nematocyst is correct.

However, we feel that the site of initiation and source of information for the synthesis of this organelle may lie elsewhere, possibly in the kinetosome. This is supported by the symmetry of the stenotele proper, as well as the previously described sensory-cilium structure of the cnidocil or trigger. The stenotele exhibits a threefold symmetry from the tip of the large spines through the fine tubule, except possibly for the head region of the tubule. In the region of the stenotele membrane, there is also seen a pseudo-ninefold symmetry which in the everted, fired membrane becomes essentially a ninefold symmetry.

This view is consistent with the previous discussion of the possible role of contractile tubules in the firing of the nematocyst. The derivation of nematocysts from kinetosomes provides a reasonable explanation for both the origin and mechanism of discharge of this unusual organelle without unduly taxing the potential of the kinetosome, already implicated in an amazing array of functions.

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