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NEMATOCYSTS OF THE SEA ANEMONE *METRIDIUM*

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SYNOPSIS. Six types of nematocysts and their nematocytes in tentacles and acontia of the sea anemone *Metridium senile fimbriatum* were studied by electron microscopy.

Microbasic b-mastigophores, microbasic amastigophores, and basitrichs have one fundamental feature in common: a straight, complexly-folded shaft with dense spines pointing apically. An additional resemblance between a b-mastigophore and a basitrich is the possession of a long, narrow, coiled thread bearing spines. An amastigophore is characterized by a short, looped, unspined thread and a cup-shaped granular matrix.

Atrich and holotrich nematocysts have a coiled, spined tube of uniform diameter which lies in an evenly granular matrix filling the entire capsule.

The above five nematocysts have three flaps at the apex of the capsule which open upon discharge, and each nematocyte possesses a flagellum with which is associated one or two centrioles and a striated rootlet. The long rootlet of the b-mastigophore-bearing nematocyte passes through a circular band of fibrils surrounding the neck region of the capsule, and the short rootlet of the atrich lies in a dense fibrous sheath surrounding all but the apex of the capsule.

The spirocyst differs from the other nematocysts in having a thin, ridged, single-walled capsule; an inverted tube containing bundles of tubules; an apical disk covered only by a thin layer of granular material and the nematocyst membrane; and the absence of a flagellum in its nematocyte.

Theories of excitation and mechanism of discharge of nematocysts and the function of spirocysts are discussed in the light of this and other recent studies of the fine structure of nematocysts. Special attention is drawn to the probable role of the folds in the walls of shaft and thread in increasing the length of the tube upon discharge.

Nematocysts or stinging capsules which are characteristic of the phylum Cnidaria have been of interest to biologists for several centuries. Although early biologists, Aristotle for example, mentioned the sting of the nettle animals, Trembley was apparently the first to call attention to nematocysts. To the many studies of their unique structure by light microscopy have been added in recent years the findings of electron microscopy. These and other investigations have sought also to elucidate the function of the various kinds of nematocysts.

Two of the most intriguing problems in the physiology of nematocysts are their excitation and the mechanism of discharge. In particular, the question of whether they or the cells which bear them, called nematocytes, are independent effectors, or under the control of the nervous system, has long been controversial and still remains unsolved. Much work has been done on the nematocysts of hydra, but little attention has been given to those of sea anemones. Electron microscopy has shown that a hydra nematocyst possesses a cnidocil (or trigger hair), an apical operculum, and a basket of supporting rod-like structures around the capsule (Chapman and Tilney, 1959a). The cnidocil, although non-motile, exhibits a dense core surrounded by nine elements originating from a centriole-like body, features which strongly suggest that a cnidocil is a modified flagellum (Fawcett, 1961). A cnidocil, operculum, and supporting rods have not been observed in sea anemones (e.g. Pantin, 1942; Hand, 1961).

In electron microscopic studies of anemone nematocytes and nematocysts, on the other hand, I have found a flagellum instead of a cnidocil (Westfall, 1963), an apical apparatus of three flaps instead of an operculum (Westfall and Hand, 1962), and fibrous bands or sheaths about the capsule instead of a basket of supporting rods (Westfall, 1963 and present paper). Considering the similarity of a sea anem-

one's flagellum, apical flaps, and capsular sheaths to hydra's cnidocil, operculum, and supporting capsular basket respectively, one may postulate a similar mechanism of stimulus and release for nematocysts of both hydra and sea anemones. All nematocysts have in common an inverted, folded tube which everts first in the apical region of the capsule, with which it is continuous. The tube progressively turns inside out, dilates, and lengthens until it lies completely outside the capsule. The tube may be of uniform diameter (isorhiza) or divided into a thick basal shaft and a narrow distal thread (heteroneme), and it may be spined or unspined.

The present paper will endeavor to elucidate further the fine structure of nematocysts of the sea anemone *Metridium* and to contribute to an understanding of the process of excitation and mechanism of discharge of stinging capsules.

MATERIALS AND METHODS

Specimens of the Pacific Coast sea anemone, *Metridium senile fimbriatum* (Verrill), used in this investigation were collected mainly from yacht harbor pilings in Sausalito, California. Many methods of fixation have been employed, including glutaraldehyde, but the best general fixative proved to be 2% OsO_4 and 1% $K_2Cr_2O_7$ in 78% sea water at pH 7.2. Mature double-walled nematocysts do not fix well owing to their extreme impermeability. Pieces of tentacles and acontia were excised with iridectomy scissors, placed in cold fixative for approximately two hours, dehydrated quickly by passage through 50, 70, 90, and 100% ethanol, and flat embedded in Epon. Sections were cut on a Porter-Blum ultramicrotome using a diamond knife with a Westfall-Healy section mounter (Westfall and Healy, 1962). Most sections were doubly stained by one half hour in aqueous uranyl acetate, followed by one half hour in lead citrate (Reynolds, 1963). Most micrographs were taken with an RCA EMU 3G electron microscope. Exceptions to the general methods described above are noted in the Explanation of Figures.

OBSERVATIONS

Three types of nematocysts are found in the thread-like acontia from the gastrovascular cavity of *Metridium*: microbasic b-mastigophores, microbasic amastigophores, and basitrichs (Hand, 1955). In the ordinary tentacles one observes an additional and unusual form of nematocyst called a spirocyst (Bedot, 1890). In addition to the many ordinary tentacles in *Metridium*, some specimens may have one or more thickened inner tentacles capable of great extension. These are called catch tentacles (Carlgren, 1929; Hand, 1955), and when present they add atrichous and holotrichous isorhizas to the cnidom of *Metridium*. The catch tentacles also have microbasic amastigophores and spirocysts, although these are fewer in number than the atrichs and holotrichs. These nematocysts are grouped by Weill (1934) and Hyman (1940) into isorhizas, i.e. with a tube of uniform diameter (basitrichs, atrichs, holotrichs), and heteronemes, i.e. with a distinct but (microbasic mastigophores and microbasic amastigophores). They consider the spirocyst as distinct from nematocysts proper.

Microbasic b-mastigophore. In acontia this type of nematocyst is abundant and large, measuring 63 by 4.5 μ. This and all subsequent measurements of undischarged nematocysts were made on a few selected representatives (see Hand, 1955, for ranges of size). In the ordinary tentacles, b-mastigophores are less numerous and smaller in size (18 by 3 μ). These nematocysts are easily discharged, and consist of a heavily spined shaft continuous with a longer, lightly barbed thread. The inverted shaft is straight, having a complexly folded wall within which the dense spines point apically (Figs. 1 and 2). The narrow thread is coiled about the shaft, and bears folds which appear three-armed in cross section (Fig. 3), the spines being centrally located. In acontia the undischarged b-mastigophore lies in a nematocyte which is typically flared at its distal surface, and from which project a long flagellum (12.5 μ as seen in the light microscope) and numerous microvilli (Fig. 1). A long striated

rootlet (Figs. 1 and 4) extends from the centrioles at the base of the flagellum deep into the cell. This rootlet passes through a circular band of thin fibrils surrounding the neck of the nematocyst (Figs. 1, 2, 5, 6). The apex of the undischarged nematocyst consists of three flaps with dense margins which appear to be cemented together (Fig. 7). The apical flaps are folded back against the circular band of fibers after discharge of the b-mastigophore (see Fig. 4, Westfall and Hand, 1962). The cytoplasm in the distal end of the nematocyte, enclosing the apex of the nematocyst, is markedly less dense (see Figs. 2, 5, and 7) in contrast to that of the surrounding five or six narrow, densely fibrous cells, each with a prominent rootlet (Figs. 5 and 7). The rootlet of the nematocyte is difficult to discern in cross sections of the cell. In longitudinal sections it appears to be composed of long, thin, closely apposed fibrillae. The regions of different density along the fibrillae are aligned to give the appearance of cross-banding as a collagen (Fig. 4). The periodicity of the striations is about 800 A, which is somewhat greater than the 640 A periodicity of collagen and the 500-700 A banding in other ciliary rootlets (Fawcett and Porter, 1954). It is about double the banding of the flagellar rootlet described by Batham (1960) in the sea anemone *Mimetridium cryptum* Hand, 1961 (= *Metridium canum*). Moreover the striations in the rootlet of this nematocyte appear complex. The dark bands consist of approximately six stripes, the top one being more dense and slightly separated from the others, giving polarity to the rootlet. The light bands appear to have three groups of two stripes each. Mitochondria and vesicles lie near the rootlet and between the capsule and cell membrane; the nucleus and Golgi apparatus are situated near the base of the nematocyte.

Basitrich. This type of nematocyst is much smaller (13.5 by 1.3 μ) than the b-mastigophore just described, and is difficult to observe with the light microscope. It occurs in the lower part of the tentacles and in the acontia of *Metridium* (Hand, 1955). Basitrichous isorhizas have been described as having a tube that is spiny at the base only (Weill, 1934; Hyman, 1940). Carlgren (1940) suggests that basitrichs are derived from microbasic b-mastigophores. The similarity of the latter two nematocysts is clearly seen in the electron microscope. They appear to differ only in size. Figure 8 shows a cross section of a basitrich from the tentacle of *Metridium*. A large central shaft is surrounded by a narrow thread which has a three-armed appearance, with central spines as in the b-mastigophore. The capsule is double-walled, the inner component being continuous with the wall of the tube as in other nematocysts, and it appears to have three apical flaps. The basitrich lies in a flagellated cell which is wide at its distal surface. A circular band of fibers has not been observed. An elongate nucleus is situated alongside the basal part of the capsule (Fig. 8).

Microbasic amastigophore. Like the microbasic b-mastigophore, this nematocyst is large (63 by 5.4 μ) and abundant in the acontia of small specimens. It is much less frequently observed in the acontia of older animals (Hand, 1955). It is present but not numerous in the tentacles of anemones of all sizes. In ordinary tentacles it measures about 17 by 2.7 μ, and in catch tentacles 36 by 4.5 μ. The amastigophore differs from other nematocysts in having a cup-shaped mass of uniformly granular material surrounding most of the shaft (Fig. 9). A thin, looped thread may be seen at the end of the straight, heavily-spined shaft in the undischarged cnida. Accordingly Cutress (1955) classes this nematocyst with the microbasic p-mastogophore of Carlgren (1940). In a previous report on the fine structure of the amastigophore (Westfall and Hand, 1962) it was stated that the thread of the amastigophore had not been seen in the electron microscope. Since that time, however, I have observed it as a typical three-armed structure, as shown in most cross sections of the thread, but without spines. It can be seen to pass through the cone-shaped indentation of the base of the inverted shaft (Figs. 11 and 12), and to lie alongside the inner capsular wall; but its exact connections to shaft and capsule have

not been observed. Like the b-mastigophore, the amastigophore consists of a double-walled capsule, the inner layer of which is continuous with the wall of the inverted shaft. The nematocyte bearing the amastigophore possesses a flagellum, centrioles, and rootlet and, as in other nematocytes, its distal end is flared (see Fig. 9). Occasionally stacks of closely-spaced membranes are found adjacent to the nematocyte membrane (Figs. 9 and 10). Mitochondria, vesicles, and granules (glycogen?) occur between capsule and lateral cell membrane; the nucleus and Golgi body are situated basally.

Spirocyst. The spirocyst (Fig. 13) measures about 16 by 2.5 μ and has a very thin single-walled capsule. Within the capsule is a coiled tube of uniform diameter which in cross section shows a three-armed arrangement of its wall, owing to folding, as in all nematocysts examined with the electron microscope. Within the body of the tube are bundles of rodlets or tubules (Westfall, 1964). They measure about 300-400 A in diameter and run parallel to the long axis of the tube (Fig. 15). In cross sections of the thread approximately 30-40 tubules appear in hexagonal arrangement, seemingly on one side of the tube (Fig. 16). The other side of the thread is filled with an electron-dense granular material. Occasionally one sees amorphous bodies of similar material (ma_2, Fig. 13) outside the tube, usually in the middle of the capsule. Sections tangential to the capsular wall (Fig. 17) show it to be beautifully patterned with transversely or obliquely oriented parallel ridges having a spacing of about 440-480 A. Each ridge is delicately striated with a periodicity of approximately 100-115 A. In longitudinal sections of the spirocyst the inner surface of the capsular wall appears serrated (Fig. 13). The capsule is closely surrounded by a nematocyst membrane.

The apical area of the capsular wall, a disk of about 1.5 μ in diameter, differs from the general wall just described in that it is apparently free of ridges, judging from the absence of serrations on its inner surface. Note in Figure 14 that between the two x's the lining of the apical disk is smooth. Moreover, the disk is much thicker than the wall of the rest of the spirocyst because of a dense layer of granular material immediately outside the inner lining. This mantle perceptibly narrows at the margins of the apical disk (x-x, Fig. 14) and soon becomes a very thin coat over the remainder of the spirocyst which is ridged as noted above. As the lining of the wall is continuous with the wall of the tube the opening of the latter is covered by only the granular matrix of the wall plus the nematocyst membrane.

The spirocyst lies within an elongate nematocyte containing a nucleus and Golgi apparatus near the base of the cell. Mitochondria and small vesicles are situated between the capsule and cell membrane. Near the apex of the spirocyst, the nematocyte is joined to neighboring cells by junctions of the septate desmosome type. The spirocyst-bearing cell is flared at its surface (not shown in Fig. 13) so that the apex of the capsule projects into a broad, less dense cytoplasmic region. No flagellum or fibrillar apparatus has yet been seen in a nematocyte containing a spirocyst.

Atrich. This nematocyst (36 by 11 μ) has the greatest diameter of any in *Metridium* and is very numerous in the epidermis of catch tentacles. It has a double-walled oval capsule that is uniformly filled with a granular matrix in which a long narrow thread is embedded (Fig. 18). Atrichous isorhizas have been described (Weill, 1930) as having a tube devoid of spines. Electron microscopy, however, shows that the thin, evenly coiled, three-armed thread of uniform bore does contain spines (Figs. 18 and 19), contrary to its name. Atrichs of catch tentacles are characterized by a dense membranous or fibrous sheath which surrounds the nematocyst, and encloses in addition the nucleus of the cell, mitochondria, and vesicles. The nematocyte possesses at its apex a very long flagellum, axial centriole (kinetosome), and a short striated rootlet that extends into the capsular sheath just mentioned. Atrichs and their flagella are generally found deeper in the catch tentacle than holotrichs. The top of the

atrich has the characteristic apical flaps found in the b-mastigophore, amastigophore, and basitrich.

Holotrich. This nematocyst occurs in catch tentacles in approximately the same abundance as atrichs (Hand, 1955). It is smaller in size (18 by 4.5 μ) and less ovoid in shape than the atrich. As suggested by its name, the thread, which is of uniform diameter, is spined along its entire length. The inverted tube is much shorter than that of the atrichous isorhiza and does not coil all the way to the base of the capsule. The thread lies in a dense matrix of unknown nature (Fig. 20). Cross sections of the undischarged tube show centrally placed spines and the same three-fold appearance observed in other nematocysts. The capsule is double-layered, having its inner wall continuous with the tube, and there are three apical flaps like those noted earlier. Some holotrichs are enclosed in a dense sheath, similar to that about atrichs, which closely adheres to the nematocyst membrane and which contains mitochondria and vesicles. In other holotrich-bearing nematocytes, however, this sheath is not present. Such cells possess more cytoplasm, mitochondria, vesicles, and many granules (glycogen?, Fig. 20). Although this nematocyte is broad at its distal surface, as in other types, the apical cytoplasm appears densely fibrous, but less so than that of neighboring cells. The fibrils run both horizontally and vertically, the latter extending into long thick processes which surround the flagellum. These processes, seen in cross sectional view in Figure 21, bear a resemblance to the stereocilia of sensory cells in the vertebrate utricle and saccule (Spoendlin, 1964; Flock, 1964). As seen in the light microscope, these processes together with similar ones from neighboring cells form a nonmotile pyramid measuring 4.5 μ high. At the base of the flagellum lie a kinetosome and a short rootlet.

DISCUSSION

Comparative morphology. Of the six types of nematocysts from the tentacles and acontia of the sea anemone *Metridium* described above, all except spirocysts share the following features: a smooth double-walled capsule, the inner layer of which is continuous with the wall of the internal tube; three apical capsular flaps which fold outward at discharge; a narrow inverted thread which is coiled within the capsule and folded so that it appears three-armed in cross sectional view; nematocyte flared at its distal surface; and the presence of a flagellum associated with one or two centrioles and a striated rootlet. In three of the types, namely the amastigophore, b-mastigophore, and basitrich, the tube is subdivided into a basal thick shaft and a distal narrow thread. The shaft is uncoiled, but its wall is complexly folded and it bears heavy spines that are apically and medially directed. All threads except that of the amastigophore bear small spines.

Spirocysts differ in so many respects from the nematocysts considered above that they merit special attention. They are single instead of double-walled, and the capsule is finely sculptured with striated ridges. They are readily permeable to aqueous solutions and as a consequence they fix well, whereas other nematocysts are relatively impermeable and poorly preserved. No apical capsular flaps have been seen, and the inverted tube is very thinly covered above its opening. The thread of spirocysts is wider but thinner-walled than that of other nematocysts; the thread is filled with long rod-like tubules and granules instead of spines. No flagellum, centrioles, or rootlet have been found in spirocyst-bearing nematocytes. Spirocysts resemble other nematocysts, however, in having a tube that is folded and continuous with the capsule.

Mechanism of discharge. Hyman (1940) considers the immediate causative factor of discharge of a nematocyst to be increased pressure within the capsule. This theory is supported by certain experiments and by some recent electron microscopic findings. That internal capsular pressure everts and extends both shaft and thread is suggested by the observation that in discharging capsules, previously stained with methylene blue, the blue fluid advances with the everting tube. When the thread has completely

unrolled a small blue droplet may form at its tip. The electron microscopic evidence consists of the demonstration of supporting rods in hydra (Chapman and Tilney, 1959a) and fibrous bands and sheaths about some nematocysts in sea anemones. If these rods and fibrous structures were contractile, they could exert pressure on the capsular wall upon excitation. Although as yet I have no evidence that the fibrous collars and tunics are contractile, similar systems of fibers in other cells have been thought to exert tension, namely the cytoplasmic fibers in slime molds and amebae (see Wohlfarth-Bottermann, 1964; Komnick and Wohlfarth-Bottermann, 1964) and the fibrous layer in the bottle cells of the amphibian blastophore (Baker, 1965).

A question related to the mechanism of eversion of the tube is: how is the tube increased in length? Picken (1953) and Robson (1953) proposed an hypothesis, based upon an earlier one by Will (1909, 1914), that "intrinsic swelling" of the thread wall by "imbibition" of water leads to the dilatation and extension of the thread. Hand (1961), however, suggested from observations of electron micrographs of heteronemes by Westfall (unpublished at that time), by Yanagita and Wada (1959), and by Chapman and Tilney (1959b), that the basal shaft, folded like an accordion, may unfold as it everts and thus account for its increased length. Since the thread in heteronemes (see Figs. 1 and 3) and the entire tube of isorhizous nematocysts, including spirocysts, is also folded, and since no folding is observed after eversion, it seems probable that unfolding of the arms would account for the full extension of the thread as well as that of the shaft. Robson (1953) stated that isolated fragments of uneverted thread "lengthen by swelling" when exposed to water. Neither she nor Picken (1953), however, appear to have been aware of the folded condition of the inverted thread. Although water uptake may be involved, it seems to me that the principal factor in the elongation of the tube is the unfolding of its wall.

Excitation of discharge. Pantin (1942) concluded after a careful study of excitation of nematocysts in anemones that: "The stimulus to the cnidoblast which causes discharge of the cnida is primarily mechanical contact. But normally this is only effective if certain chemical substances are present which lower the threshold of the cnidoblast to mechanical contact." I have conducted some simple tests similar to those of Pantin, such as touching the tentacles and acontia of *Metridium* with a thread before and after dipping it in methylene blue or saliva. The results support this hypothesis. In *Metridium* the flagella of nematocytes, the most distal elements in these cells, may function as both mechanoreceptors and chemoreceptors, as suggested for the cnidocil by Chapman and Tilney (1959a).

How might a mechanical stimulus applied to the flagellum trigger the discharge of a nematocyst? Although striated rootlets of flagella and cilia have usually been assigned a supportive function, it is tempting to speculate that in some nematocytes a disturbance of the flagellum may be transmitted mechanically down the fibrillar apparatus to the kinetosome and thence via the striated rootlet to the circular band of fibers investing the neck of a nematocyst (b-mastigophore), or to the fibrous sheath surrounding the greater part of the capsule (atrich and perhaps holotrich). As a result of the mechanical excitation a contraction of the fibers might increase the pressure within the capsule and force open the three apical flaps in the manner discussed above.

Speculation on how a chemical stimulus facilitates the action of a mechanical one is more difficult. Presumably the plasma membrane of the flagellum, or perhaps that of the microvilli, or even the distal surface of the nematocyte represents the chemoreceptor and also the avenue of transmission of the resulting excitation. Pantin (1942) suggests that "normally sensitization is due to some surface active lipoid directly transferred to the cnidoblast by contact." Since presumably a chemical substance would first come into contact with the flagellum, and since there is no evidence that the microvilli and the surface of the nematocyte proper are involved, I think that the

flagellum itself is the chemoreceptor, bearing in mind its role in chemoreception in other animals (e.g., insects, see Slifer and Sekhon, 1964).

The above discussion of the flagellum as a mechanoreceptor and chemoreceptor is in accord with the independent effector hypothesis of nematocyte excitation advanced by several workers (see Lentz and Barrnett, 1962).

The spirocyst, however, has no flagellum or fibrillar apparatus associated with it. This suggests the need for a chemical stimulus, without perhaps an accompanying mechanical stimulus, to evoke discharge. Spirocysts are abundant on the tentacles, and Weill (1961) has found that these, together with other nematocysts, release a dense sheath when a piece of tentacle is given an electric shock. This sheath no doubt contains the rod-like tubules discharged by the everted spirocyst tubes. Perhaps the tubules become adhesive when expelled into a watery medium. This might account for the ability of the anemone *Calliactis parasitica* to adhere firmly by its tentacles to the shell of the hermit crab *Eupagurus bernhardus* during transfer (Ross, 1960, 1965). Davenport, Ross, and Sutton (1961), in their observations of *Calliactis*, concluded that "the nematocysts concerned in attachment can no longer be regarded as independent effectors." They further stated that they are not the same as the nematocysts involved in feeding, owing to the specificity of response to certain shells. One might postulate that the spirocyst-bearing cell is controlled to some extent by the nervous system, which somehow lowers its threshold to the chemical stimulus that elicits discharge of the spirocyst. Thus these nematocytes may be dependent effectors. This would give support to a second theory of excitation held by other workers (see Lentz and Barrnett, 1962) that the nervous system plays a role in controlling discharge of nematocysts.

If neurons were seen next to nematocytes, it would strengthen the idea of nervous control. On the other hand, if no nervous elements were found in contact with nematocytes, it would support the concept of independent effectors. Neural elements have not been identified with certainty in *Metridium*, but in its tentacles I have observed structures resembling the ganglion, sensory, and neurosecretory cells described for hydra (Lentz, 1963; Lentz and Barrnett, 1965). Batham (1965), however, in her report based on light microscopic studies stated that in the sea anemone *Mimetridium* the cell bodies of tentacular neurones lie in the oral disk. Accordingly, it seems best to await further research before drawing any conclusion regarding neural supply to nematocytes.

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EXPLANATION OF FIGURES

Specimens figured below were treated as described in the Materials and Methods section unless otherwise stated.

PLATE 1

FIG. 1. Diagram of longitudinal section of upper half of a nematocyte containing a microbasic b-mastigophore, showing folded wall of both shaft and thread, the latter in both cross sectional and longitudinal view.

PLATE 2

FIG. 2. Apical region of nematocyte (*nc*) and upper fourth of enclosed b-mastigophore (*ns*). Note circular fibers (*cf*) about neck region of nematocyst and a fragment of the flagellar rootlet (*r*) embedded in the fibrous band. The nematocyst membrane (*nm*) is closely adherent to the capsule on one side,

but separated from it by a space on the opposite side. Observe folded wall (*tw*) of shaft (*s*) with spines (*sp*) pointing apically, double nature of capsular wall (*cw*) and an apical flap (*af*). Several oblique fibrils (*of*), a mitochondrion (*m*) and vesicles (*v*) are also seen. Fixed in 2% $KMnO_4$ in 72% sea water; post-fixed in 2% OsO_4-1% $K_2Cr_2O_7$ in 78% sea water at pH 7.2; dehydrated in isopropyl alcohol; sections stained in lead hydroxide. × 23,000.

FIG. 3. A lower level of same nematocyst, same section, showing thread (*t*) with arms (*a*) and spines (*sp*) surrounding central shaft (*s*). A striated rootlet (*r*) lies in neighboring cell. × 23,000.

PLATE 3

FIG. 4. High magnification of segment of flagellar rootlet, showing light (*lb*) and dark (*db*) bands, membrane of an adjacent mitochondrion (*mm*), and vesicle (*v*). Fixed in 2% OsO_4-1% $K_2Cr_2O_7$ in 50% sea water at pH 6.0; sections stained with lead hydroxide. × 130,000.

FIG. 5. Oblique superficial section of several cells in an acontium. Note the flagellum (*f*) surrounded by microvilli (*mv*), flared lip (*lp*) of a nematocyte (*nc*) containing a b-mastigophore (*ns*) which is ringed with circular fibers (*cf*). Rootlets (*r*) are seen in adjacent fibrous cells. Fixed in 2% $KMnO_4$ in sea water; post-fixed in 2% OsO_4-1% $K_2Cr_2O_7$ in 78% sea water at pH 7.6; treated with propylene oxide before embedding; stained with lead hydroxide. × 18,900.

FIG. 6. High magnification of circular fibers (*cf*) surrounding capsular wall (*cw*) of b-mastigophore. Fixed in 2% OsO_4-4% $K_2Cr_2O_7$ at pH 7.6; lead citrate stain. × 32,300.

PLATE 4

FIG. 7. Cross section of apex of b-mastigophore showing three flaps (*af*). Compare less dense region of nematocyte (*nc*) with the neighboring cells each bearing a prominent rootlet (*r*) and transverse fibrils (*tf*). Method same as that for Fig. 6. × 24,500.

FIG. 8. Cross section below middle of basitrich showing central spined shaft (*s*) surrounded by thread (*t*) also bearing spines (*sp*), and nucleus (*n*) of nematocyte (*nc*). × 25,700.

PLATE 5

FIG. 9. Longitudinal section of a typical nematocyte (*nc*) showing flared lip (*lp*), microvilli (*mv*), flagellum (*f*), and a segment of rootlet (*r*). Nematocyst a microbasic amastigophore with double walled capsule (*cw*), apical flap (*af*), and folded armed shaft (*s*) cupped by granular matrix (*ma*). Mitochondria (*m*) and nucleus (*n*) seen in adjacent cell. Method same as that for Fig. 5. × 13,700.

FIG. 10. Stack of tightly packed membranes (*mb*) often seen just inside cell membrane (*cm*) of amastigophore-bearing nematocyte. Fixation same as that for Fig. 6; lead hydroxide stain. × 25,500.

FIG. 11. Cross section through cone-shaped indentation (*co*) at lower end of inverted shaft (*s*) of amastigophore showing unspined thread (*t*). Fixation same as that for Fig. 5; lead citrate stain. × 33,900.

FIG. 12. Same as in Fig. 11 but at lower level to show 3-armed structure of thread (*t*). × 32,200.

PLATE 6

FIG. 13. Longitudinal section of spirocyst in nematocyte (*nc*) with nucleus (*n*), mitochondria (*m*) and vesicles (*v*) near cell membrane (*cm*). Nematocyst membrane (*nm*) shows various sized blebs (*b*) into which capsular wall material projects. Serrated inner lining (*sl*) of wall straightens in apical region before turning inward to form thin wall of tube (see Fig. 14). Thread (*t*) filled with rodlike tubules (*tt*) and granular matrix (ma_1). Occasionally balls of matrix (ma_2) seen outside tube. × 19,500.

FIG. 14. Higher magnification of apical region of spirocyst in longitudinal section showing a smoothing out of serrated capsule lining (*sl*) at points x before forming wall of tube (*tw*). × 31,500.

FIG. 15. Longitudinal view of tubules (*tt*) within folded tube. × 24,200.

FIG. 16. Cross sectional view of tubules (*tt*) within 3-armed (*a*) tube which also contains granular matrix (ma_1) on one side. × 36,300.

FIG. 17. Tangential section of capsule showing striated ridges (*cr*). × 68,800.

PLATE 7

FIG. 18. Longitudinal section of apical region of atrich showing fibrous sheath (*fs*) into which centriole (*c*) and rootlet (*r*) of flagellum (*f*) extend. The capsule (*cw*) bears apical flaps (*af*). Within capsule is granular matrix (*ma*) surrounding folded thread (*t*) bearing spines (*sp*). × 33,900.

FIG. 19. Cross section of 3-armed (*a*) thread at higher magnification to show central spines (*sp*) heretofore undescribed in this nematocyst. × 60,500.

PLATE 8

FIG. 20. Cross section of holotrich showing matrix (*ma*), uniform thread (*t*) bearing spines (*sp*), and capsule wall (*cw*) closely surrounded by nematocyst membrane (*nm*). Glycogen (?) granules (*g*) and vesicles (*v*) seen in nematocyte (*nc*) which in this instance does not contain a fibrous sheath around the nematocyst. Capsule of atrich seen on right. × 29,000.

FIG. 21. Cross section through pyramid of processes (*p*) surrounding flagellum (*f*) from holotrich-bearing nematocyte. × 50,900.

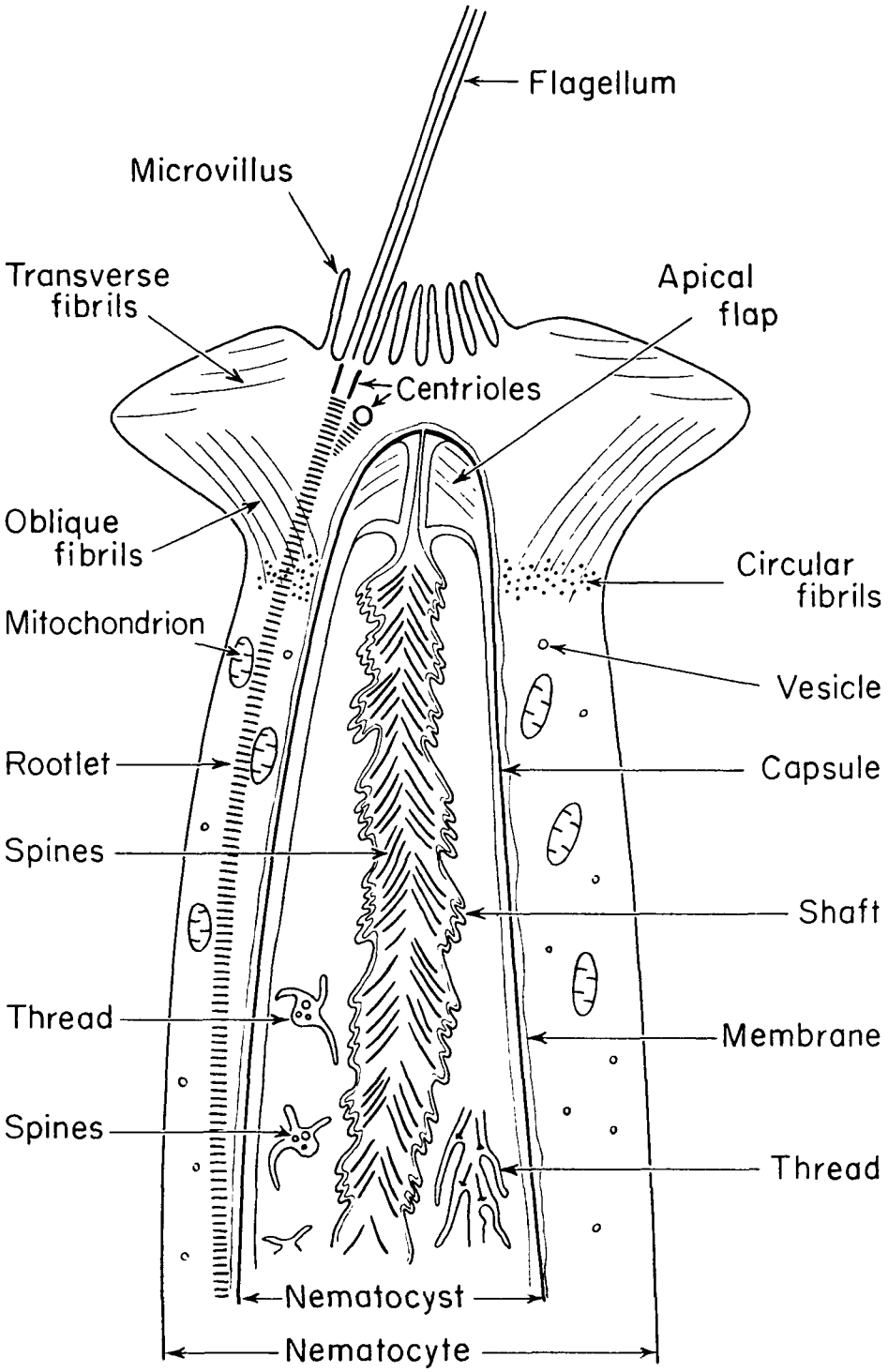
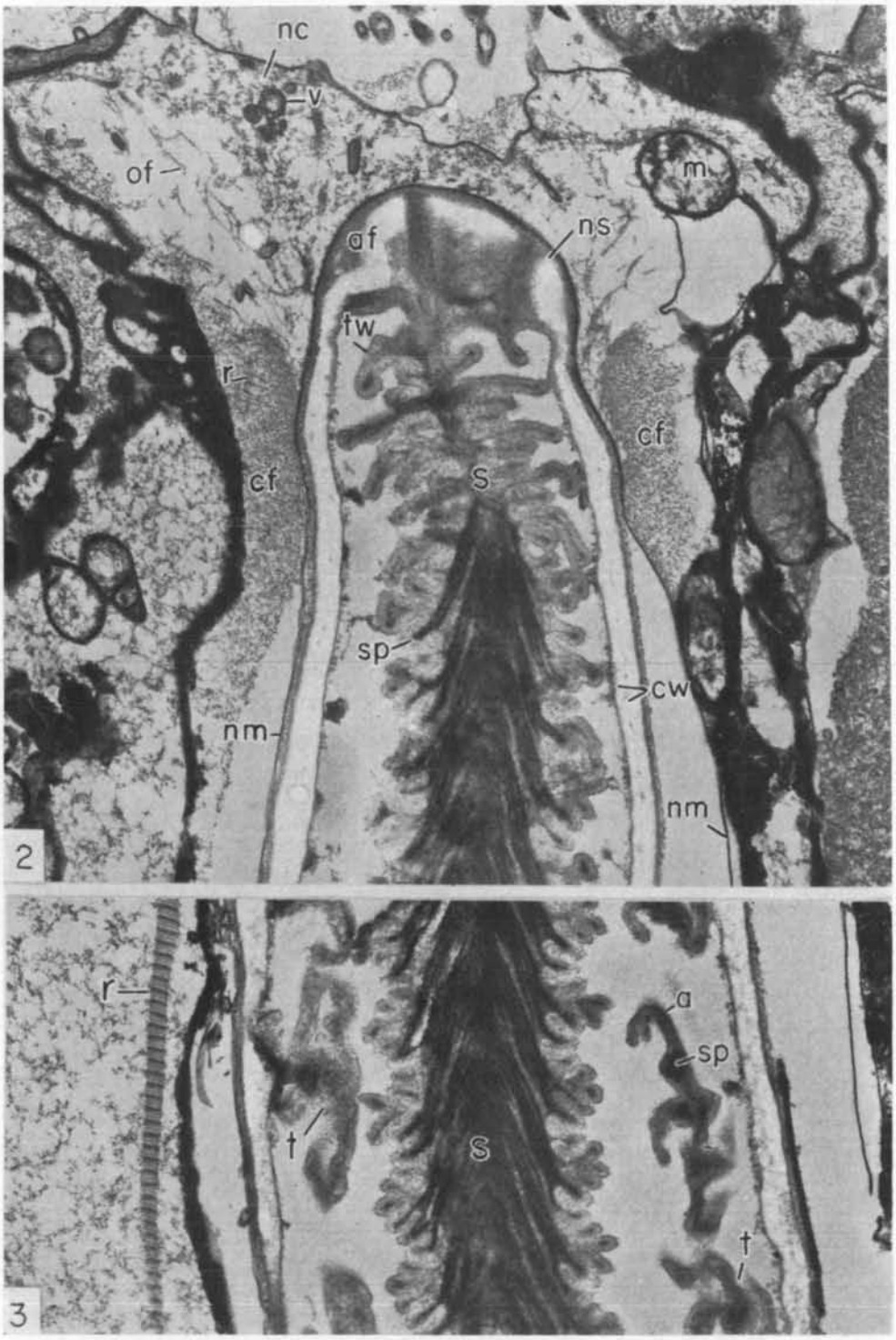


PLATE 1



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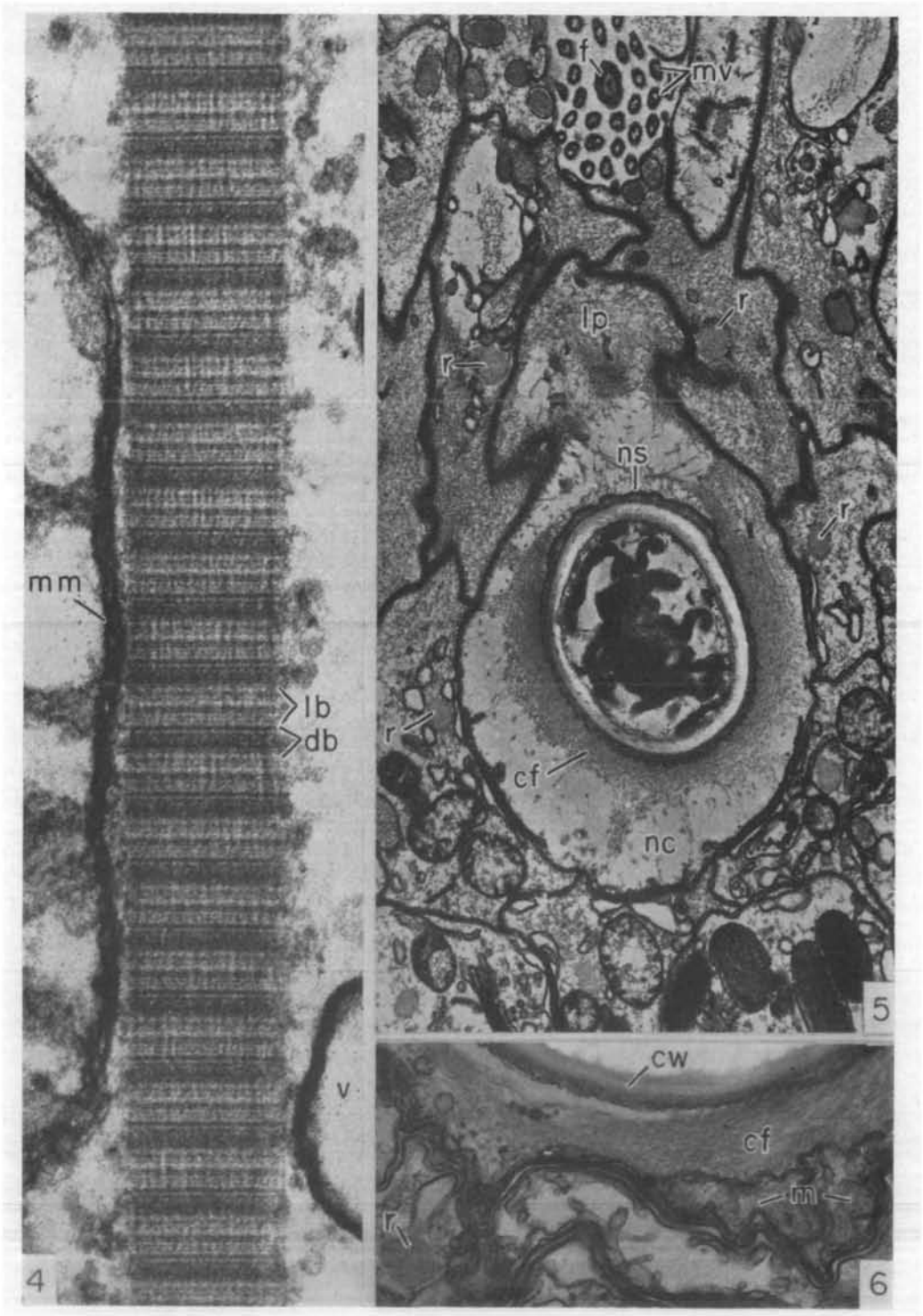
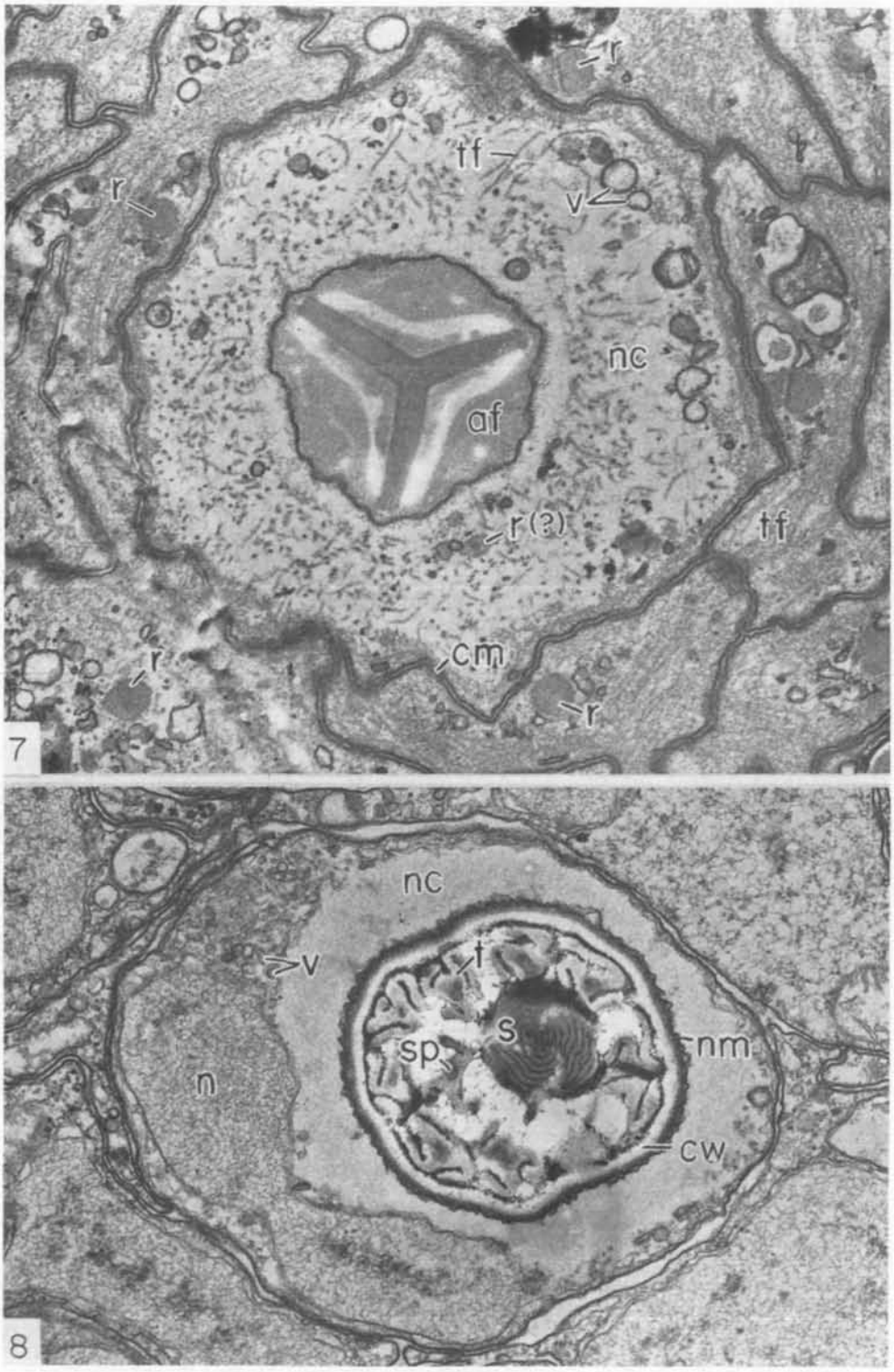


PLATE 3



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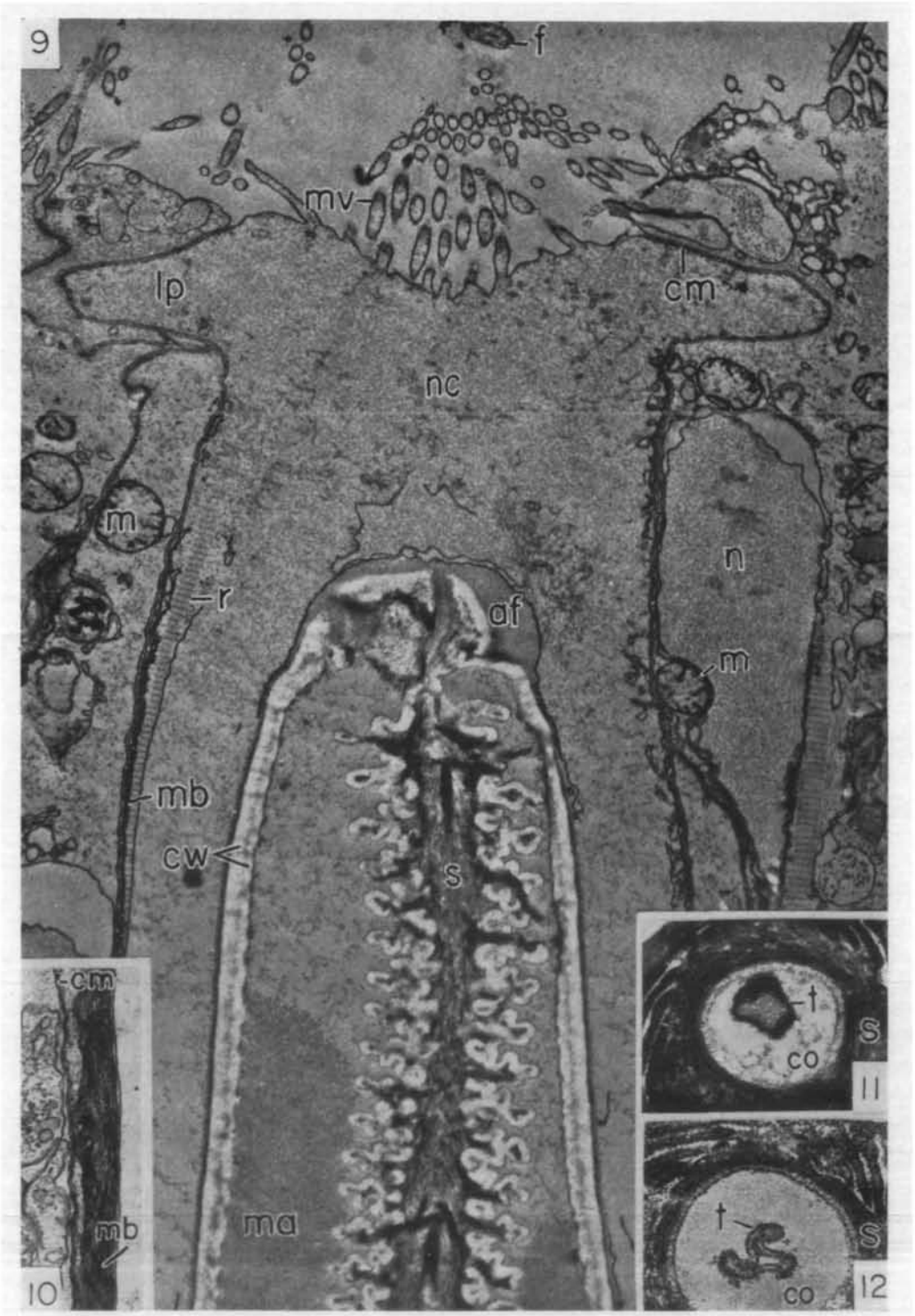

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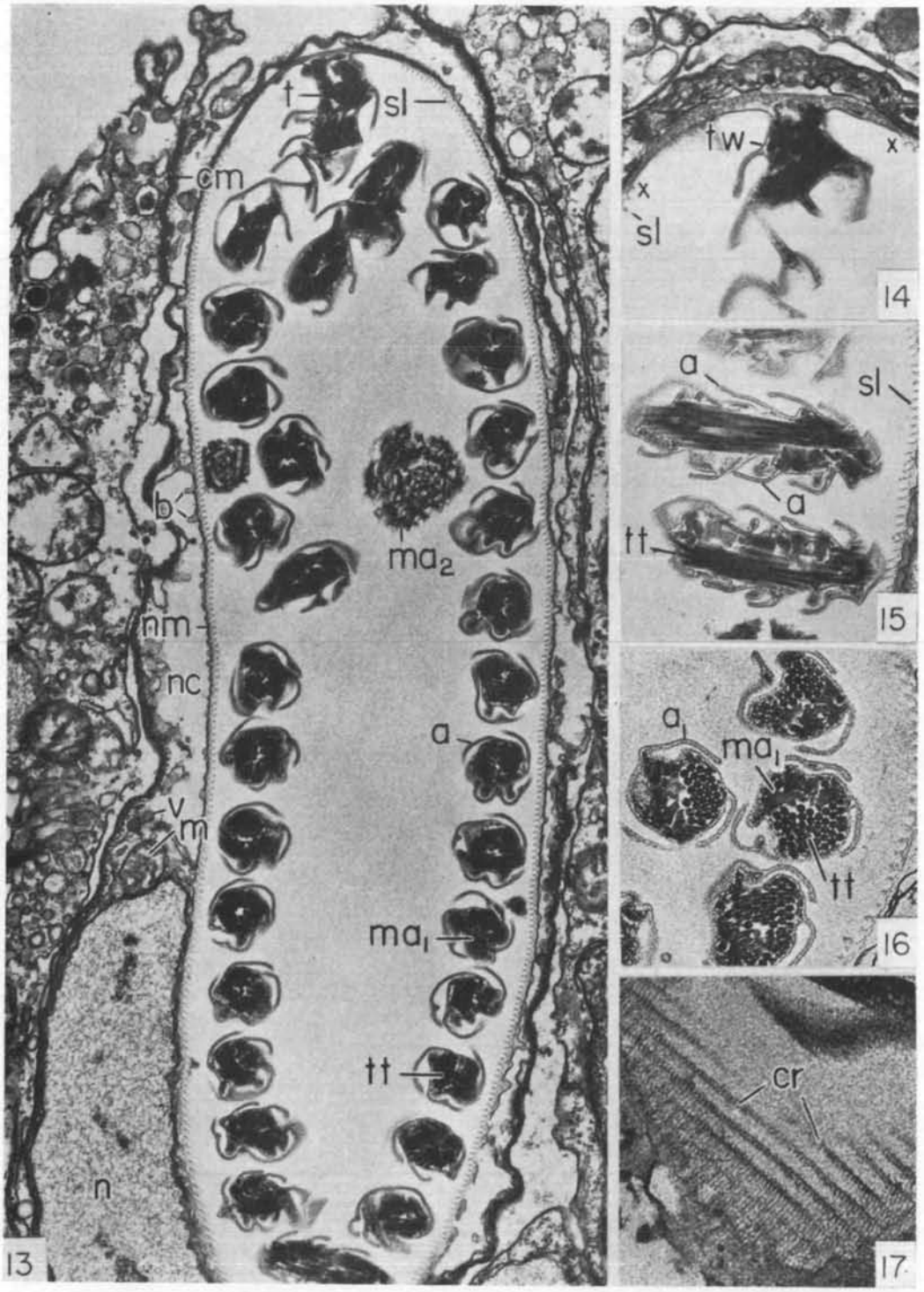

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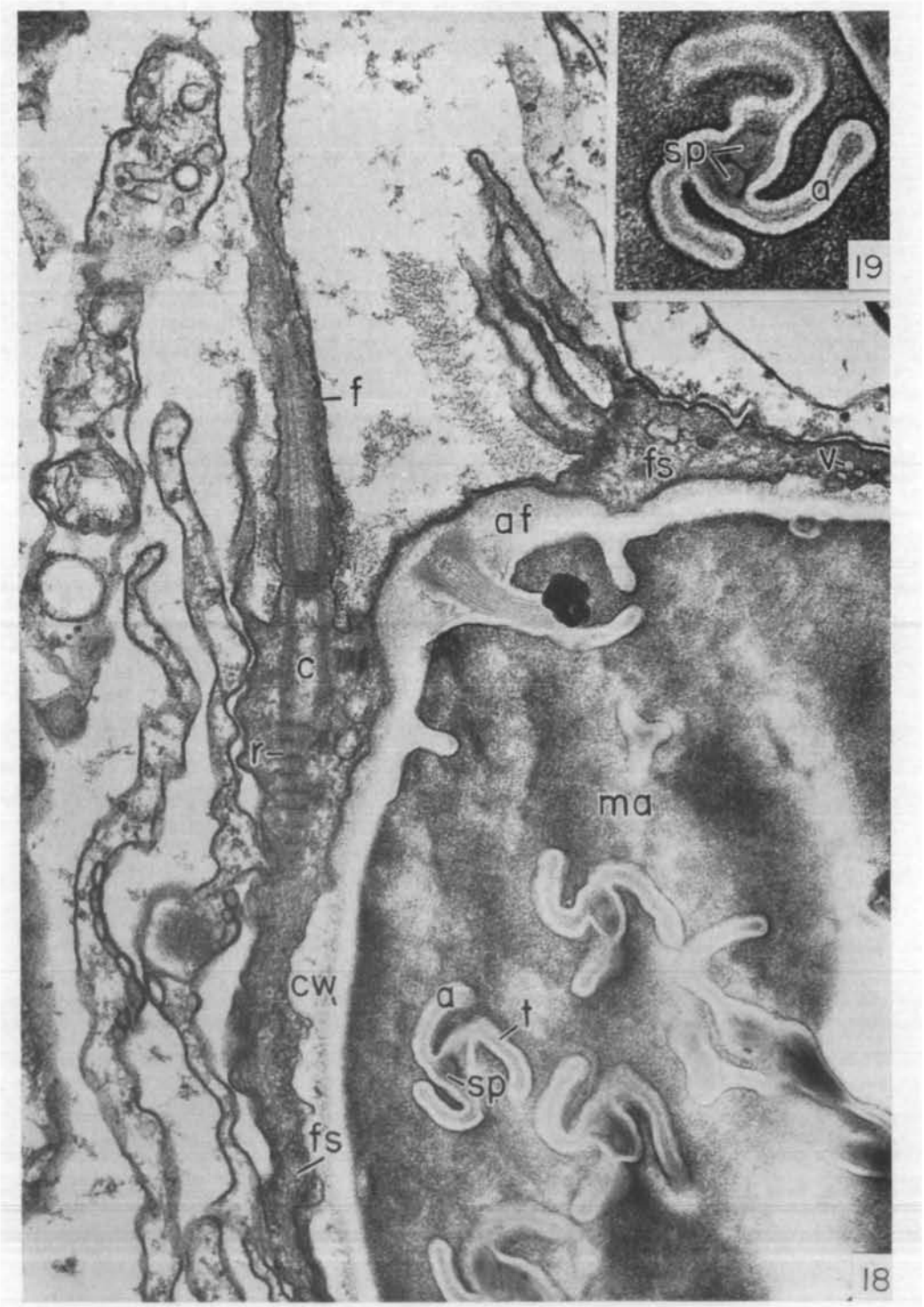

PLATE 7

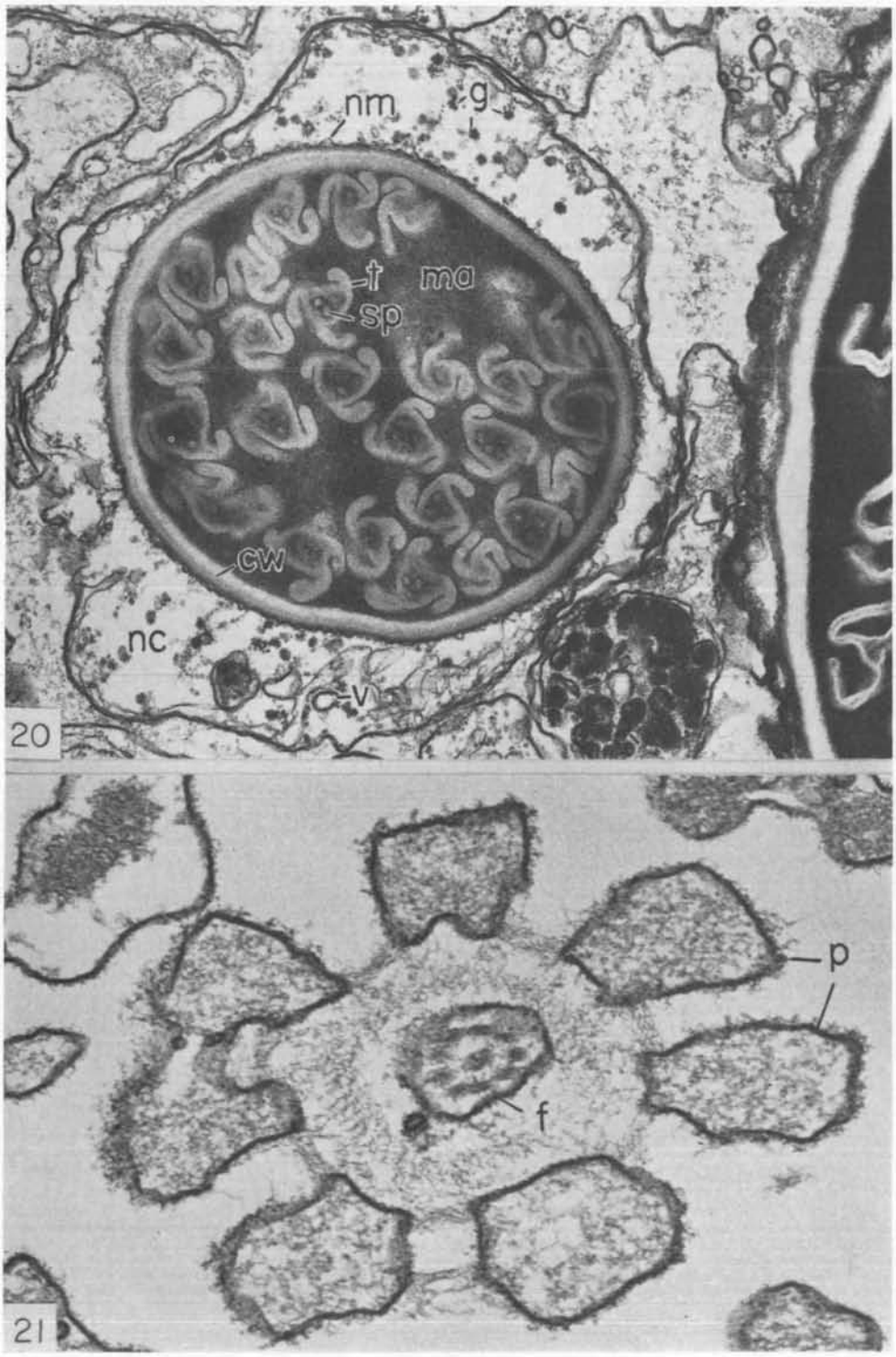

PLATE 8