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ULTRASTRUCTURE OF THE SMALL CNIDOCYTE OF THE PORTUGUESE MAN-OF-WAR (Physalia physalis) TENTACLE

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SUMMARY

The nematocysts of the Portuguese Man-of-war (Physalia physalis) occur in two sizes, large and small. This paper describes the extraordinarily complicated ultrastructure of the Man-of war cnidocyte, with particular emphasis on the small cnidocyte. The nematocyst is located immediately below the apical region of the cnidocyte and is enclosed within a prominent cytoskeletal structure referred to as the fibrillar basket. The base of the fibrillar basket is anchored to the underlying mesoglea via hemidesmosomes. Capping the basal half of the nematocyst and in close association with the fibrillar basket are numerous elongate vesicles of unknown function. The apical region of the cnidocyte, all but the tip of the cnidocil apparatus which contacts the ambient medium, is enclosed by an adjacent epithelial "neighbor" cell. The cnidocil apparatus of the cnidocyte consists of a radially symmetrical ring of 15 to 21 stereocilia surrounding a modified cilium called the cnidocil. At the bases of the stereocilia, the cnidocyte invaginates to form a cavity. The cavity is continuous with the ambient medium and is divided into two interconnected chambers, one on top of the other. These chambers are separated from each other by a narrowing of the cavity wall formed by vertical septate-like processes that project medially from the base of each stereocilium. A spatulate, longitudinally oriented, striated rod originates within each of these vertical processes and terminates basally on the fibrillar basket. The apical ends of the rods are interconnected by a circular, filamentous band that is

situated in a plane perpendicular to the long axis of the cell. The assembly of interconnected striated rods is referred to as the fibrillar collar and is believed to support the cnidocil apparatus. A circular bundle of horizontally-oriented thin filaments called the annular ring surrounds the fibrillar collar. Evidence indicates that extracellular bridges form between the surface membranes of the stereocilia and cnidocil upon chemosensitization. We suggest that this occurs by medial movement of the stereocilia into contact with the cnidocil caused by a contraction of the annular ring against the supportive fibrillar collar.

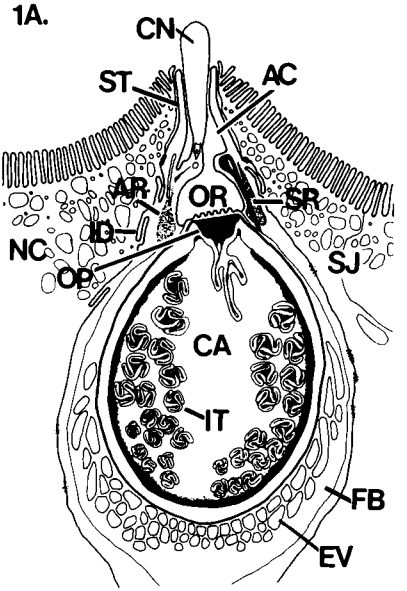
I. INTRODUCTION

The Portuguese Man-of-war is a free-floating, colonial hydrozoan having separate polyps adapted for the capture of prey, digestion of food, and sexual reproduction (Mackie, 1960).

Long fishing tentacles hang from a gas-filled float and drift passively in the ocean's currents. The tentacles consist of thin tubes of contractile tissue which can reach up to 30 meters in length when fully extended (Lane, 1960). They can contract more than twenty-fold (Parker, 1932) and draw captured prey to the digestive gastrozooids located immediately below the float. There, stimulated by reduced glutathione released from the wounded prey, the gastrozooids envelop the prey (Lenhoff and Schneiderman, 1959), which are then digested.

A. Morphology of the fishing tentacle

The "tentacular" canal, which is continuous with the common gastrovascular cavity of the colony, extends the length of the tentacle. Bulbous evaginations of the tentacle called batteries measure approximately 1.5 mm by 0.7 mm and enclose individual fluid-filled chambers connected by narrow channels to the tentacular canal. When the tentacle is fully extended adjacent batteries occur at up to 5 mm intervals along the entire length. When the tentacle is contracted, however, adjacent batteries are in contact with each other. An endoderm lines the chamber of the battery and is separated from the ectoderm by a basement membrane called the mesoglea. The ectoderm is in direct contact with the ambient seawater and is composed of at least three cell types: gland cells, neighbor cells, and cnidocytes (Hyman, 1940). Gland cells



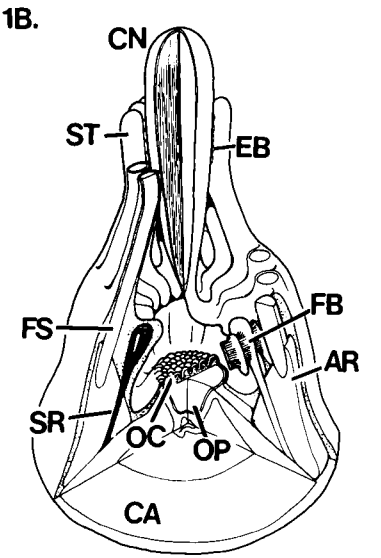


Fig. 1A. Diagram of an idealized longitudinal section of small cnidocyte.

Fig. 1B. Three-dimensional diagrammatic representation of the apical region depicting the relationship between various structural elements. Abbreviations used in these and/or in other figures: AC, apical chamber; AR, annular ring; BB, basal body; BP, basal plate; BR, barb; CA, capsule; CN, cnidocil; CR, ciliary rootlet; CV, crescent-shaped vesicle; DT, double microtubule; EB, extracellular bridge; EV, elongate vesicle; FB, fibrous band or fibrillar basket; FS, filament bundle of the stereocilia; ID, interdigitations; IT, inverted tubule; IV, invaginations; MI, mitochondrion; MT, microtubules; NC, neighbor cell; OC, opercular cap; OP, operculum; OR, opercular chamber; SJ, septate junctions; SR, striated rod; ST, stereocilia.

are columnar and contain large, electron-dense, polymorphic vesicles presumably filled with mucus which these cells secrete to the external surface. The other cells, the neighbor cell and the cnidocytes, are described in detail below.

B. Neighbor cells

The neighbor cells contain pigment granules which impart to the tentacle its characteristic blue color. These cells

possess at least three morphologically distinct vesicles (Fig. 2) but it is not known in which of these, if any, the pigment granules are contained. One type of vesicle averages 0.4 μm in diameter and appears electron-lucent. The second vesicle type is 0.3 μm in diameter and contains a moderately electron-dense material. The third vesicle type measures 0.2 μm in diameter and encloses an electron-dense granular material. The large, electron-lucent vesicles are located near the external surface of the neighbor cell, while the remaining two, electron-dense types of vesicle appear to be distributed more evenly throughout the cell. These three types of vesicles may represent distinct functional compartments or developmental stages of the same type of vesicle.

The external surface of the neighbor cell is covered with numerous microvilli measuring approximately 1.0 μm in length and 80 nm in diameter. A mucous layer extends above the tips of the microvilli and covers the external surface of the battery.

Just below the external surface of the battery, the neighbor cell forms septate junctions with the adjacent cnidocyte (Figs. 1, 4). Beginning below the region of the septate junctions extensive, flap-like interdigitations occur between the neighbor cell and the cnidocyte (Fig. 5). These interdigitations appear in cross section as lamellar processes involving both cells and occur at many locations where these cells are in contact. Such interdigitations occur in tissues subject to mechanical stress. This is consistent with the observation that Man-of-War captures relatively large, swimming prey (Purcell, 1984). Such capture may produce significant local stress on involved cnidocytes.

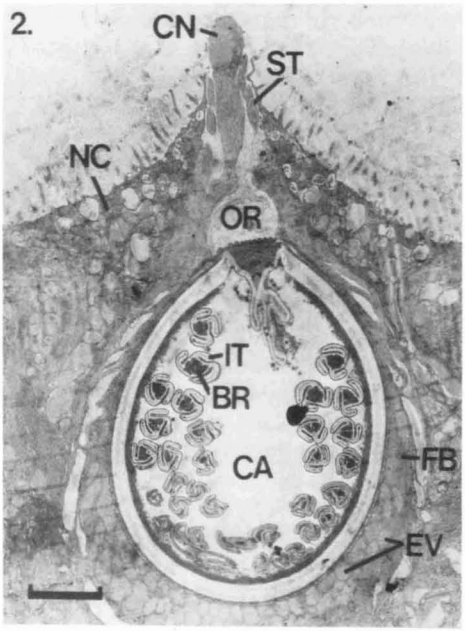
Neighbor cells are perforated by the cone-shaped, apical

Fig. 2. Longitudinal section of small cnidocyte and adjacent neighbor cell (NC). Scale bar = 2.0 μm.

Fig. 3. Cross section of cnidocil apparatus. Extracellular bridges (EB) occur between the cnidocil (CN) and each stereocilium (ST). Scale bar = 0.2 μm.

Fig. 4. Longitudinal section of cnidocil apparatus (Fig. 2). Cnidocil (CN) attaches to cnidocyte below basal body (BB). Septate junctions (SJ) occur between the cnidocyte and neighbor cell (NC). Scale bar = 0.5 μm.

Fig. 5. Cross section of apical region of cnidocyte at level of opercular cap (OC). Striated rods (SR) occur between horizontal fine filaments of annular ring (AR). Scale bar = 0.6 μm.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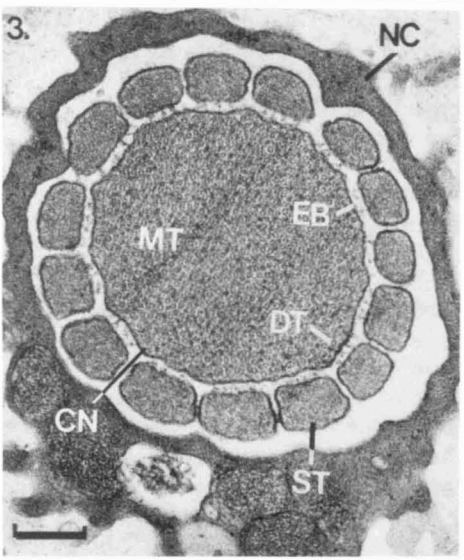
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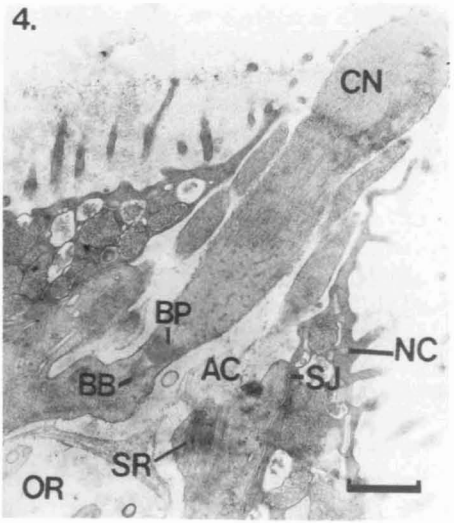
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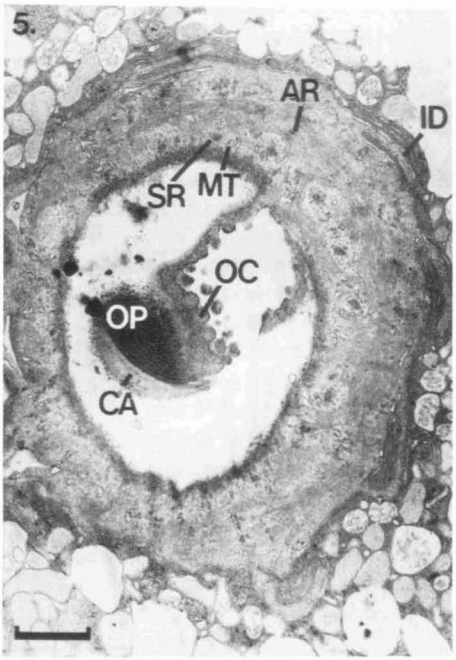
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region of the cnidocyte such that all cnidocytes are surrounded by and extend through an adjacent neighbor cell to make contact with the ambient seawater (Figs. 2-4). The apical margins of the "perforated" neighbor cell almost completely cover the tips of the stereocilia that originate from the cnidocyte (Figs. 1a, 2). This association is quite similar to the relationship between an accessory cell and the mechanoreceptor cell of filiform tentacles of Coryne (Tardent and Schmid, 1972).

Three types of associations between cnidocytes and adjacent accessory cells have been reported. First, in hydra several kinds of cnidocyte occur together within a single battery cell such that the only contact the cnidocytes have with the tentacle is via the battery cell, which in turn is attached to the mesoglea (Wood and Novak, 1982). The battery cell also participates in relatively complicated cellular interactions with elements of the nervous system (Hufnagel and Kass-Simon, 1988). Second, in sea anemone tentacles each cnidocyte is surrounded by two or more supporting cells (G. Watson, unpublished). The supporting cells provide the stereocilia to the sensory cone of the cnidocyte (Westfall, 1965) and carry at least one class of chemoreceptor involved in sensitizing cnidocytes to mechanical stimuli that trigger nematocysts to discharge (Watson and Hessinger, 1988). Third, the cnidocyte in the Man-of-War is anchored directly to the mesoglea (Cormier and Hessinger, 1980a) and is only surrounded at its apical region by a single neighbor cell.

Because of its apparent interaction with many types of cells including more than one type of cnidocyte it appears that the battery cell association in hydra is structurally the most complex of the three types of accessory cells, thus prompting the suggestion that it may also represent the more complicated functional association with cnidocytes. At the other extreme of structural complexity is the situation in the Man-of-War in which the neighbor cell only associates with the apical region of the cnidcyte while the cnidocyte possesses both an independent attachment to the mesoglea (Cormier and Hessinger, 1980a) and provides the full complement of the stereocilia to the cnidocil apparatus (Cormier and Hessinger, 1980b). Thus, the Man-of-War cnidocyte may perform some of the functions provided by accessory cells in the other types of accessory cell associations.

II. THE CNIDOCYTE

The cnidocyte of the Man-of-war is an extraordinarily complicated cell (Cormier and Hessinger, 1980a, b). Cnidocytes from tentacles fixed with glutaraldehyde and osmium fixed are not easily studied by transmission electron microscopy (TEM) because of difficulty in attaining complete infiltration of the nematocyst capsule by embedding media. We have found that post-fixation of samples of tentacles for three hours in ice-cold 1% acrolein in 0.1 M PIPES buffer, pH 7.3 (Baur and Stacey, 1977) prior to preparation for TEM improves infiltration of Spurr's resin into the nematocyst capsule and provides suitable material to study the ultrastructure of the small cnidocyte. The large nematocyst, however, does not adequately infiltrate under these conditions. Indeed, these differences in infiltration may be explained by the fact that capsules of the two size classes of nematocyst have very different permeabilities. For instance, we have found that isolated large nematocysts are impermeable to solutes of molecular weights of at least 300 daltons, whereas the small nematocysts are permeable to solutes of greater than 960 daltons.

In this section we report two novel observations: elongate vesicles cap the basal half of both large and small nematocysts; and the internal structure of the nematocyst barbs is hollow.

A. The base of the cnidocyte

The small cnidocyte of the Man-of-war possesses specialized cytoskeletal structures located both at the base and at the apex of the cell (Figs. 1, 2). At the base of the cnidocyte filaments of the fibrillar basket, which surround the nematocyst (Schultze, 1922; Mackie, 1960;), form a thickened stalk that terminates in hemidesmosomal junctions on the mesoglea (Cormier and Hessinger, 1980a). These junctions, together with the fibrillar basket anchor the nematocyst to the tentacle so that captured prey cannot escape (Cormier and Hessinger, 1980a).

B. Elongate vesicles

Numerous elongate vesicles occur at the basal half of the nematocyst (Figs. 1, 2) where they are densely packed into a layer two to three vesicles across and interspersed in parallel fashion with elements of the fibrillar basket (Fig. 6). The contents of these vesicles in the small cnidocyte

are moderately electron-dense, whereas in the large cnidocyte they appear electron-lucent. Because the vesicles are quite long and conform to the curvature of the nematocyst capsule, we have not been able to measure the full length of the vesicles from thin-sections. Nevertheless, in .the large cnidocytes we can measure them for at least 5.6 μm (Fig. 7). In both the small and large cnidocytes the vesicles measure approximately 0.25 μm in diameter.

These vesicles, not previously described in any cnidocyte, are never seen in preparations of isolated nematocysts although the fibrillar baskets still commonly surround these nematocysts (Cormier and Hessinger 1980a). Either these vesicles are fragile and rupture upon disruption of the cell or else they are easily displaced from the fibrillar basket. The presence of mitochondria in association with the basket has been cited as evidence that the fibrillar elements of the basket may be contractile and used by the cnidocyte to initiate discharge (Cormier and Hessinger, 1980a). If true, these vesicles may function in a way similar to that of the sarcoplasmic reticulum in vertebrate striated muscle by storing and releasing divalent cations. This suggestion, however, is not supported by our failure to demonstrate the presence of divalent cations in these vesicles using pyroantimonate (G. Watson, unpublished). Alternatively, these vesicles may contain water and hydrated monovalent ions which may be released directly to the nematocyst capsule to facilitate or to initiate discharge. In addition, these vesicles may also contain proteolytic enzymes which function to digest the fibrillar basket after the nematocyst has been discharged. Such a situation would

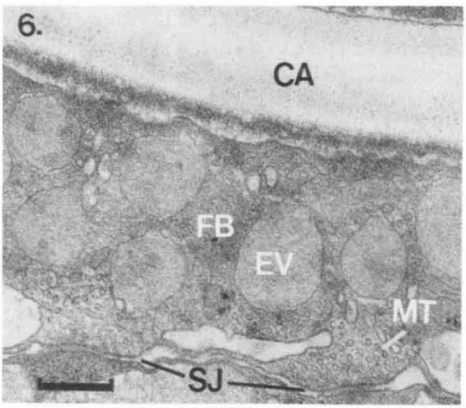
Fig. 6. Cross section of elongate vesicles (EV). Microtubules (MT) and septate junctions (SJ) occur at outer periphery of fibrillar basket (FB). Scale bar = 0.25 μm.

Fig. 7. Longitudinal section of elongate vesicles (EV) and elements of fibrillar basket (FB). Scale bar = 0.5 μm.

Fig. 8. Cross section of cnidocil (CN) above stereocilia. Only singlet microtubules (MT) occur at this level. Scale bar = 0.2 μm.

Fig. 9. Cross section of cnidocil apparatus near bases of stereocilia. Doublet microtubules (DT) persist in cnidocil and fine filaments (FS) fill stereocilia (ST). Scale bar = 0.2 μm.

Fig. 10. Cross section of apical region of cnidocyte at basal plate (BP) of cnidocil. Electron-dense striated rods (SR) occur within medial projections of stereocilia bases. Scale bar = 0.4 μm.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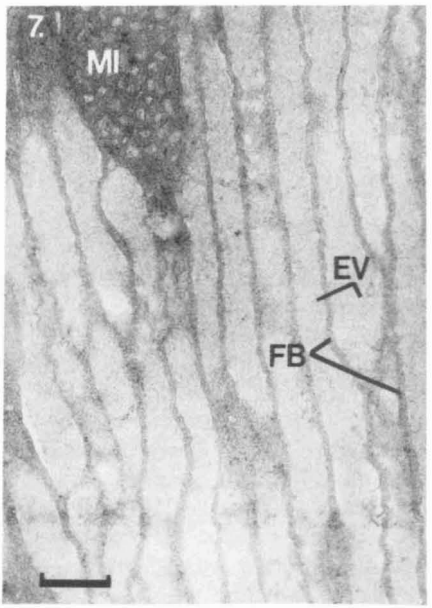
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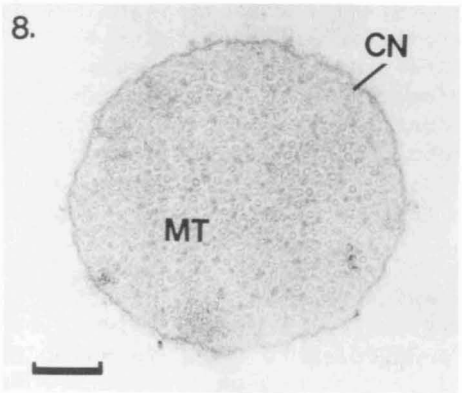
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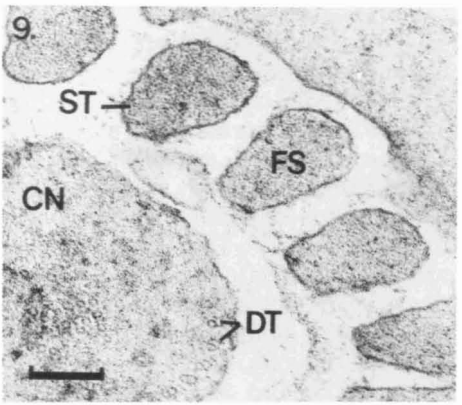
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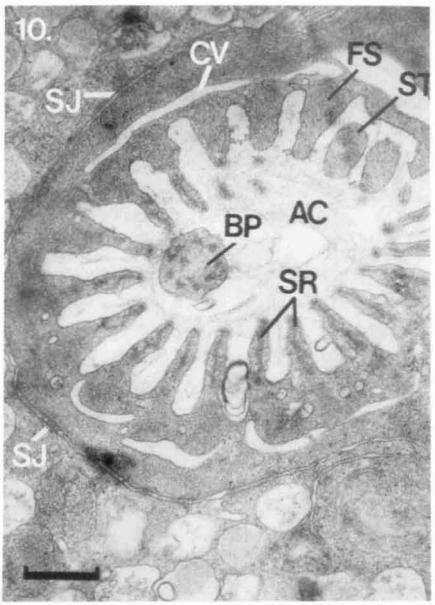
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allow the tentacles to release captured prey and the attached nematocysts to the digestive gastrozooids. The presence of such a stored source of proteases could explain why the ectoderm of the Man-of-War tentacles autolyses so easily (Lane, 1958) and why many laboratories working with Man-of-War venom from isolated nematocysts report both significant protease activity and low toxicities for the nematocyst venom (Hessinger, 1988).

C. The nematocyst

Man-of-War nematocysts are capable of injecting a potent venom (Tamkum and Hessinger, 1981) into prey. Tentacle nematocysts have been classified both as atrichous isorhizas (Weill, 1930) and as holotrichous isorhizas (Cormier and Hessinger, 1980a) and occur in two sizes, large (20-28 μm dia) and small (6-8 μm).

A plug-like operculum (Figs. 2, 13), which appears to be composed of a granular material, is situated in the apical opening of the capsule wall at the junction of the capsule with the inverted tubule. The operculum seals the capsule and hollow tubule from the external environment (Figs. 1, 2, 13). At the point where the tubule is in contact with the operculum, the tubule is cylindrical in cross section. Extending beyond the operculum region, the inverted tubule becomes triply-pleated (Figs. 2, 15). In the small nematocyst the tubule gradually tapers from a diameter of 1.3 μm at its widest aspect at the apical opening of the capsule to 0.45 μm near its tip (Fig. 2) while executing 16-18 concentric coils in planes perpendicular to the polar axis in the small nematocyst.

The proximal two-thirds of the inverted tubule (representing approximately 10 coils) contains electron-dense barbs packed in a staggered, tripartite array within the tubule lumen (Fig. 15).

The distal one-third of the tubule, representing approximately 7 smaller coils, contains no detectable barbs. Such a tubular structure is difficult to classify according to conventional classification schemes (Mariscal, 1974) but the nematocyst is probably best referred to as a holotrichous isorhiza.

At high magnification, the barbs appear to be hollow, with a honeycomb-like internal structure containing crypts that parallel the base-to-tip axis of the barb (Fig. 16). The matrix of the hollow crypts are occupied by electron-dense granules. We suggest this substructure of the spination of the nematocyst tubule may be of general occurrence. In the Man-of-war this may represent a

modification of the barb to minimize its total mass and/or to contain venom proteins, thereby making each barb a tiny hypodermic syringe for delivery of the nematocyst toxins.

III. APICAL REGION OF THE SMALL CNIDOCYTE

The small cnidocyte is approximately 40 μm long and 9.8 μm in diameter at its widest aspect. The apical region of the cnidocyte is cone-shaped and houses the cnidocil apparatus and the fibrillar collar (Cormier and Hessinger, 1980b) which are situated immediately above the operculum of the nematocyst (Figs. 1, 2). The apical region of the cell is believed to be receptive to chemical and mechanical stimuli (Parker and van Alstyne, 1932). We describe below (i) the cytoskeleton of the apical region of the small cnidocyte, (ii) the reversible formation of extracellular bridges between the plasma membranes of the stereocilia and cnidocil under conditions of chemosensitization, (iii) a centrally located, double-chambered cavity in apex of the cell, and (iv) the morphodynamic nature of the apical region of the small cnidocyte.

A. Cnidocil apparatus

The cnidocil apparatus includes the club-shaped cnidocil and a single circlet of stereocilia (Figs. 1, 3). The cnidocil is about 5 μm long, extending into the ambient medium about 1 μm beyond the tips of the microvilli at the surface of the neighbor cell. The tip of the cnidocil contains tightly packed singlet microtubules (Fig. 8; Cormier and Hessinger, 1980b). Directly below the tip of the cnidocil doublet microtubules first appear (Fig. 3). The doublets are restricted, however, to a single peripheral row just within the cnidocil membrane (Cormier and Hessinger, 1980b) where they continue to the base. The cnidocil narrows from 0.9 μm diameter at its widest aspect to 0.3 μm at its base (Fig. 1, 4). Near the base of the cnidocil only a few singlet microtubules and an incomplete ring of doublets remain (Fig.9). At the base, a typical ciliary basal plate and basal body occur (Fig. 4, 10, 11; Cormier and Hessinger, 1980b).

The cnidocil is surrounded by 15 to 21 stereocilia, each measuring about 2.8 μm in length and 0.2 μm in diameter (Figs. 1, 3, 4). Each stereocilium contains a bundle of approximately 125 fine filaments running lengthwise. The

diameter of the stereocilia increase from 0.18 μm at the tips to 0.28 μm at the bases.

In non-treated tentacles the extracellular space between the stereocilia and cnidocil is approximately 0.1 μm (Cormier and Hessinger, 1980b). In tentacles treated with mucin from the surface of prey fish the space between each stereocilium and cnidocil decreases and it appears that each stereocilium becomes interconnected with the cnidocil membrane by moderately electron-dense, extracellular "bridges" (Fig. 3) measuring 34 nm in diameter. In cross-section an average of three bridges occur in the space between the plasma membranes of the cnidocil and the stereocilia. Bridges are observed only in mucin-exposed tentacles in regions where doublet microtubules are present. Bridges are not observed between adjacent stereocilia nor between the stereocilia and the neighbor cell.

The mechanoreceptors of vertebrate hair cells exhibit extracellular bridges between individual stereocilia (Comis et al., 1985; Neugebauer and Thurm, 1985). The cnidocil apparatus is purported to be the mechanoreceptor of the cnidocyte (Parker and van Alstyne, 1932; Pantin, 1942; Cormier and Hessinger, 1980b). We believe such extracellular bridges in Man-of-War cnidocytes (Fig. 3) reversibly "couple" the membrane of the cnidocil with the membranes of the stereocilia under conditions of prey-derived chemosensitization to facilitate sensory transduction of mechanical stimuli in triggering the discharge of nematocysts.

B. Apical Chambers

Beginning at the bases of the stereocilia a deep, central cavity extends nearly to the operculum of the nematocyst (Figs. 1, 2). The apical margin of this cavity appears as a narrow rim on which each stereocilium sits (Fig. 10). Narrow vertical processes, or "flutes," project medially from the bases of the stereocilia into the central cavity (Fig. 10). Basally these processes broaden and fuse (Fig. 11) to form a circular ledge which divides the cavity into two interconnected chambers (Figs. 1, 2). The base of the cnidocil, including the basal body, originates from one side of this ledge (Figs. 1, 2, 4, 11).

Of the chambers formed by the circular ledge, the outer chamber is defined basally by the ledge and laterally by the surrounding neighbor cell (Figs. 1a, 2, 4). It is open to the ambient medium through the interstices between the cnidocil, stereocilia, and the perforation of the neighbor cell.

The narrow opening in the circular ledge connects the outer chamber with the lower, so-called "opercular" chamber (Figs. 1a, 2, 4). The opercular chamber is defined apically by the ledge, laterally by the region of the fibrillar collar (see section III. C., below), and basally by the opercular cap of the nematocyst.

The opercular cap is situated on top of the operculum of the nematocyst (Figs. 1b, 13, 14). It consists of an electron-dense layer 90 nm thick sandwiched between the plasma and nematocyst membranes and is characterized by a total of 45-50 raised, electron-dense bumps, each measuring about 0.13 μm in diameter and 90-95 nm high. At its lateral edge, the electron-dense material of the cap thins to 35 nm and appears to fuse with the electron-dense fibrillar basket (Figs. 5, 13).

Our description of two chambers formed by the apical invagination of the cnidocyte represents a unique finding. The outer chamber is similar to the heart-shaped chamber described in the perioral sensory cells of hydra (Kinnamon and Westfall, 1984), but the lower, opercular chamber of the Man-of-War cnidocyte, with the opercular cap, has not been described for other cnidarian cells. Functionally the opercular chamber likely reflects the capacity of the apical region to undergo morphodynamic changes in response to appropriate chemical and mechanical stimuli.

C. Fibrillar collar

The fibrillar collar is composed of a ring of 15 to 21 vertically-oriented striated rods that surround the opercular chamber. In longitudinal section, each rod is alternately electron-dense and electron-lucent with a periodicity of 19 nm (Fig. 13). Each rod originates within a flute just above the ledge and extends basally for about 3 μm to the fibrillar basket on the "shoulder" of the nematocyst (Fig. 1, 13 insert).

In cross section, each striated rod appears as an electron-dense bar above the circular ledge (Fig. 10, 11) and as a disk of similar density below the ledge (Fig. 14). The diameter of the rod decreases from 0.25 μm at the ledge to less than 80 nm at the fibrillar basket, indicating that the rod tapers along its length.

Within the circular ledge, oblate-shaped, fibrous plates occur equidistant between each rod (Figs. 1b, 11, 12). The plates are 0.15 μm wide, 25 nm thick, and at least 0.32 μm long. Each plate is connected to the adjacent rods by fine filaments. This arrangement of striated rod-filaments-plate-filaments-striated rod, etc. forms a continuous, circular

band within the ledge.

Near the circular band, each striated rod appears hollow. The hollow "space" within each rod is at least 0.32 μm long. In cross section, the hollow space appears elliptical, measuring approximately 0.21 μm wide by 27 nm thick at its largest aspect.

Cormier and Hessinger (1980b) described the striated rods of the fibrillar collar as "striated rootlets, one for each stereocilium." The periodicity of the striations seen in the ciliary rootlet associated with the cnidocil in Figure 8 of that report measures 75 nm, as do the striations of the cnidocyte ciliary rootlets in Aiptasia (G. Watson, unpublished). However, the periodicity of the rod striations seen in Figure 9 of Cormier and Hessinger's report and in Figures 13 and 14 of the present report measure 19 nm. Westfall (1970) observed a periodicity of 20 nm in electron-dense, structural "stiff rods" of the Gonionemus cnidocyte. Furthermore, the striated rods in Physalia appear to have no attachment to or insertion into any of the ciliary structures of the cnidocyte. From these comparisons, it appears that the striated rods in the apex of the Man-of-war cnidocyte are not ciliary rootlets but rather are more similar in structure to the "stiff rods" of Gonionemus.

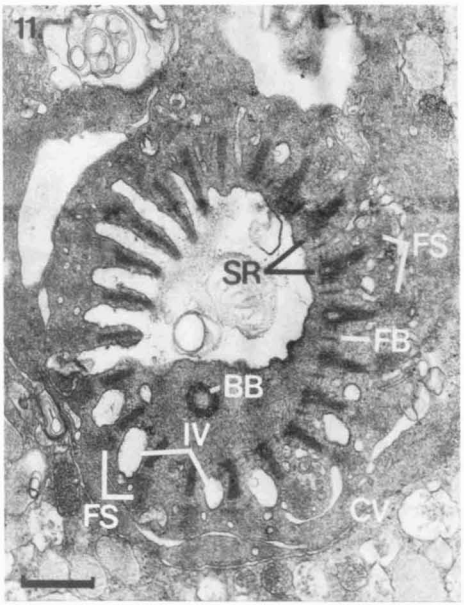
Furthermore, no difference in the periodicity of rod striations occurs between those contained within untreated and mucin-treated Man-of-war cnidocytes, as described above (section III. A.), or between those contained within untreated cnidocytes and cnidocytes electrically stimulated to discharge (G. Watson, unpublished). These observations suggest that the striated rods are primarily supportive

Fig. 11. Cross section of apical region of cnidocyte at supporting ledge. Circular fibrous band (FB) links striated rods (SR) which, at this level, appear hollow. Scale bar = 0.5 μm.

Fig. 12. Cross section of apical region of cnidocyte immediately below supporting ledge. Filament bundles of stereocilia (FS) and a ciliary rootlet (CR) occur next to striated rods (SR). Horizontally oriented fine filaments of annular ring (AR) first appear at this level. Scale bar = 0.25 μm.

Fig. 13. Longitudinal section of apical region of cnidocyte. Scale bar = 1.0 μm. Insert: striated rod (SR) terminating on fibrillar basket (FB). Scale bar = 0.5 μm.

Fig. 14. Cross section of apical region of cnidocyte at opercular cap (OC). Each striated rod (SR) is surrounded by an array of microtubules (MT). Scale bar = 0.3 μm.


11.
SR
FS
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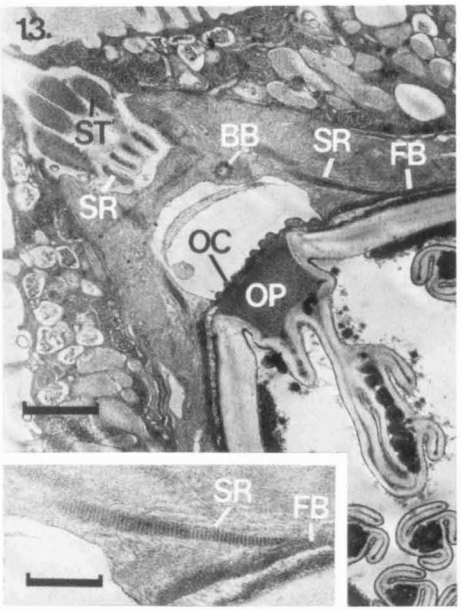
13.
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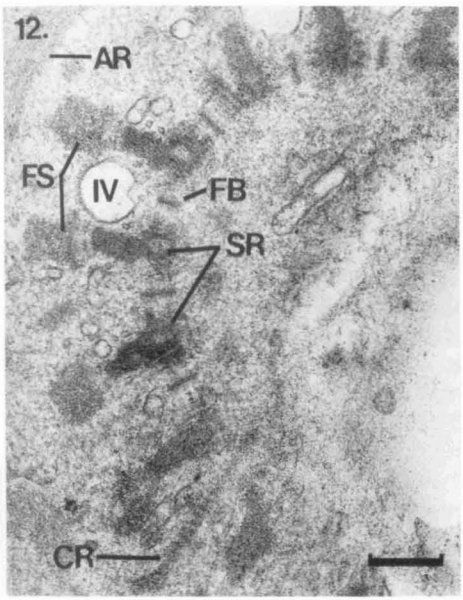
12.
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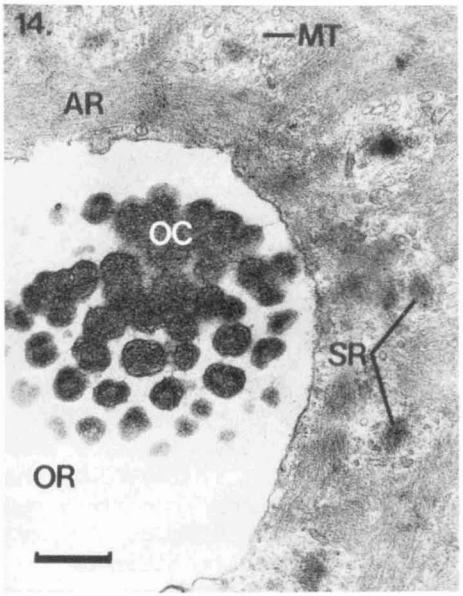
14.
MT
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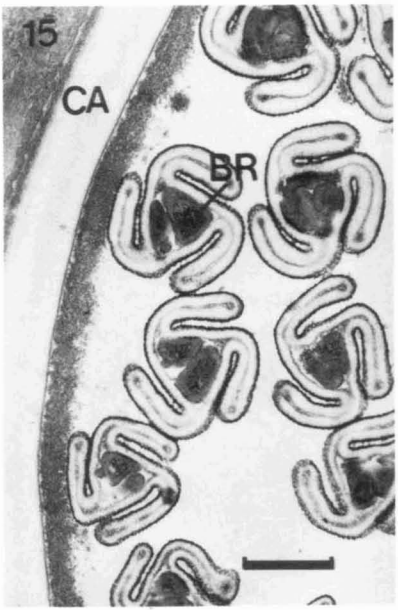

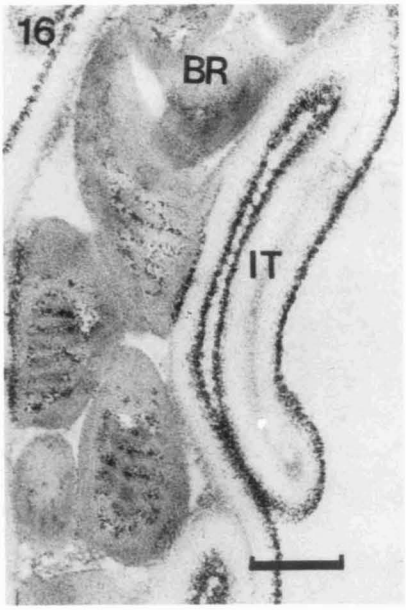

Fig. 15. Cross section of inverted tubules (IT) containing electron-dense barbs (BR). Scale bar = 0.5 μm.

Fig. 16. Longitudinal section of inverted tubule (IT) revealing honeycombed interior of barbs (BR). Bar = 0.2 μm.

structures rather than contractile. In this capacity, we believe the striated rods function to support the cnidocil apparatus of the cnidocyte.

Below the circular band of the fibrillar collar, each striated rod is encircled by a parallel array of five to ten microtubules (Figs. 5, 14) that follow the rod until the rod terminates on the fibrillar basket. The microtubules, however, continue basally beyond this point and separate laterally to encircle the nematocyst at the periphery of the cell (Fig. 6).

Also occurring in the vicinity of the microtubles are numerous small vesicles measuring approximately 40 nm in diameter (Fig. 5, 14). The small vesicles continue alongside the microtubules into the region of the fibrillar basket (Fig. 6). The site of origin of these tiny vesicles is not known. If the location of these vesicles along the microtubules represents a pathway for translocation of vesicles, then it is attractive to suggest that they originate from the deep invaginations that occur in the ledge between the bases of each pair of adjacent stereocilia (Figs.

4, 11, 12). These invaginations of approximately 0.17 μm in diameter penetrate to a depth of at least 0.5 μm. Whether the tiny vesicles are endosomal derivatives of endocytosed chemoreceptor-ligand complexes as is found for chemosensitized sea anemone tentacles chemosensitized by mucin (Watson and Hessinger, 1987; 1988) or pinocytotic vesicles transporting water to the elongate vesicles surrounding the basal half of the nematocyst will have to await further experimentation.

D. Annular ring and associated structures

Beginning at the level of the ledge, a thick, circular bundle of horizontally-oriented fine filaments forms a ring peripheral to much of the fibrillar collar (Fig. 12). This bundle, which we call the annular ring, extends from the basal region of the circular ledge to the shoulder of the nematocyst (Fig. 5). The ring thickens basally and encircles the opercular chamber (Figs. 1a-b). The striated rods along with associated microtubules and tiny vesicles penetrate the medial aspect of the annular ring at the level of the opercular cap and exit its base (Figs. 5, 14).

Broad, electron-lucent vesicles which appear crescent-shaped in cross sections of the cnidocyte apex occur between the rim of the outer chamber and the ledge (Figs.10, 11). These vesicles are located in the cytoplasm between the vertically-oriented fibrillar bundles from the base of each stereocilium and the plasma membrane adjacent to the neighbor cell.

IV. FUNCTIONAL SIGNIFICANCE AND CONCLUSIONS

As already noted in part, several morphological features distinguish the apical region of the small cnidocyte from non-sensitized tentacles (Cormier and Hessinger,1980b) from those of mucin-sensitized tentacles. In chemosensitized Man-of-war tentacles the gap between the stereocilia and cnidocil is decreased and replaced by extracellular bridges that connect the cnidocil to the stereocilia (Fig. 3). In addition, the opercular cap appears thicker and the bumps are more pronounced (Fig. 13) and the opercular chamber is narrower and taller (Fig. 4).

Bigger (1982) reported that the apical region of the cnidocyte thinned in the acrorhagi of certain anemones in response to stimuli from non-clonal tissues. Watson and Hessinger (in preparation) find that the stereocilia of

cnidocyte sensory cones in the sea anemone, Haliplanella luciae, elongate upon mucin-sensitization in correlation with a change in the sensitivity of the mechanoreceptor (submitted). Wood and Novak (1982) using NBD-phallicidin localized actin filaments in the apical region of hydra cnidocytes. If the annular ring in the Man-of-war cnidocyte contains actin it may be contractile and thus capable of inducing morphodynamic changes observed in the present study.

We propose that the morphodynamic changes observed in the Man-of-War cnidocyte upon chemosensitization are due to shortening of the presumed contractile elements of the annular ring. Such a contraction of the annular ring against the supportive fibrillar collar would biomechanically produce the above-mentioned changes including bringing the stereocilia close enough to the cnidocil so that extracellular bridging can occur between them.

We also describe a new type of cnidocyte accessory cell, the neighbor cell, which surrounds only the apical region of the cnidocyte while the cnidocyte has its own direct attachment to the mesoglea and provides all elements of the purported cnidocyte mechanoreceptor, the cnidocil apparatus. On the basis of ultrastructural evidence, it seems that the association between the neighbor cell and the Man-of-War cnidocyte is the least intimate of any reported cnidocyte/accessory cell association. Whether the neighbor cell modulates cnidocyte responsiveness in a manner similar to the supporting cells in sea anemone tentacles (Watson and Hessinger, 1987; 1988) is not yet known. We would predict, however, from the ultrastructural evidence presented here that the Man-of-War cnidocytes have much more autonomy from the accessory cells than tentacle cnidocytes of either hydra or sea anemones.

It is our opinion that as more is learned about the sites and mechanisms of sensory functions controlling cnida discharge in different species some cnidocytes will be found possessing neither chemo- nor mechano-receptor systems of their own, such as we suspect exist on the sea anemone fishing tentacle, and will serve merely as "dependent" or "passive effectors" to adjacent sensory accessory cells. On the other extreme, however, there will also be found cnidocytes, which will function as classical "independent effectors" with no sensory and little or no modulatory influence from accessory cells or the nervous system, such as we suspect exists on the Man-of-War fishing tentacle,

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