

## Cnidocil Apparatus: Sensory Receptor of *Physalia* Nematocytes

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The cnidocil apparatus, a cluster of subcellular structures at the external surface of the nematocytes of *Physalia physalis*, was examined by scanning and transmission electron microscopy. The cnidocil apparatus consists of a modified cilium or cnidocil surrounded by 15 to 21 stereocilia. The stereocilia contain closely packed, longitudinally arranged microfilaments. The cnidocil contains numerous singlet and doublet microtubules that lack the classical 9 + 2 microtubule pattern characteristic of most cilia and flagella. The basal body of the cnidocil does, however, maintain the usual circle of nine triplets of microtubules. It is proposed that the cnidocil of the nematocyte is truly the sensory receptor for mechanical and chemical stimuli that elicit nematocyst discharge.

Nematocysts are intracellular, ovoid organelles that discharge a long tubule to capture prey (1). Both mechanical and chemical stimuli are required to elicit nematocyst discharge (2, 3). It is generally accepted that the sensory receptor for nematocyst discharge is the cnidocil, a cilium- or flagellum-like structure situated on the epithelial surface of the tentacle (3-7). No direct evidence, however, has been presented to support this role of the cnidocil. The nematocyst was originally proposed by Pantin in 1942 (2) to be an independent effector; however, the current view suggests that the nematocyte housing the nematocyst and the surrounding cells interact to control nematocyst discharge (3, 8). Yet the inhibitory or excitatory role of the adjacent epitheliomuscular and nerve cells has not been directly determined (9, 10).

*Physalia physalis* (Portuguese man-of-war) is commonly considered a colonial hydrozoan (phylum Cnidaria) in which individual polyps are specialized for flotation, reproduction, and the capture and digestion of prey (11). The tentacles of *Physalia* are used solely for the capture of prey. It was probable that, like the tentacles, the cells involved in the capture of prey, namely, the nematocytes, would also reflect this high

degree of specialization and organization clearly connecting the cnidocil with nematocyst discharge. An ultrastructural investigation of the *Physalia* cnidocil apparatus was undertaken in order to elucidate the cnidocil structure and its relationship with other nematocyte components as well as providing insights into the function of the cnidocil apparatus.

### MATERIALS AND METHODS

*Physalia physalis* L. (Portuguese men-of-war) were collected in February of 1978 from the surf at Crandon Beach, Key Biscayne, Florida. The fishing tentacles were severed from the animals and immediately fixed on the beach for electron microscopy in ice cold 2.5% glutaraldehyde in filtered sea water. After 8 to 10 hr the specimens were rinsed in sea water and postfixed in 1.0% OsO<sub>4</sub> buffered with 0.1 M collidine buffer for 15 to 30 min. Specimens were dehydrated through a graded series of ethanol terminating with two 1-hr rinses in absolute ethanol. The infiltration of Spurr's embedding medium was facilitated by using acetone as the vehicle and by gradually increasing the concentration of resin by infinite dilution. Thin sections were cut on a Sorvall MT-2 ultramicrotome using a Dupont diamond knife. Silver sections were stained with Reynold's lead citrate for 1 min, followed by staining with a saturated solution of uranyl acetate in ethanol for 5 min. Electron micrographs were taken in both Philips 200 and 301 transmission electron microscopes at 60 kV.

Samples for scanning electron microscopy were dehydrated in ethanol directly following glutaraldehyde

fixation. The postfixation with OsO<sub>4</sub> was routinely omitted to permit removal of the mucus masking the surface during subsequent washings. Specimens were critical-point dried using CO<sub>2</sub> in a Samdri critical-point dryer and were coated three times with gold for 0.5 min at 10 µA using a Commonwealth Scientific mini-coater. Electron micrographs were taken on 4 × 5 Kodak plate film in a Zeiss Novascan electron microscope at 15 kV.

#### OBSERVATIONS

The nematocysts in the fishing tentacles of *Physalia* are grouped into bulbous clusters called batteries. The surface of each battery has a bumpy appearance and is covered with mucus. Each of the surface bumps marks the site of a nematocyst hidden below the external nematocyte membrane. The cnidocil apparatus is generally seen extending from the apex of the raised bump on the free surface of the battery (Fig. 1).

Abrasion of the battery surface with a wooden applicator stick exposes the underlying nematocysts harbored within the nematocyte (Fig. 2). Each nematocyte contains one nematocyst with a single cnidocil apparatus situated distal to the nematocyst. The cnidocil apparatus is composed of a modified ciliary structure 2.5 nm in length surrounded by 15 to 21 shorter stereocilia of equal height.

The cnidocil contains numerous closely packed microtubules arranged parallel to one another along the long axis of the cnidocil. These microtubules converge on a basal plate (Fig. 3). The shorter stereocilia surrounding the cnidocil contain numerous microfilaments also arranged in a parallel and longitudinal fashion. The cnidocil lacks

the classical 9 + 2 microtubule arrangement found in most ciliary and flagellar structures (Fig. 4). Instead, 27 microtubule doublets are positioned around the periphery of the cnidocil. The numerous singlet microtubules are generally centrally situated in the cnidocil; however, some are found near the periphery. Both doublet and singlet microtubules in the *Physalia* cnidocil have a diameter of approximately 13.8 nm, a wall thickness of 3.4 nm, and a central electron translucent region of about 6.9 nm. Dynein arms are not found associated with either the singlet or doublet microtubules. An amorphous electron-dense material of an unknown nature is observed between the microtubules.

At the basal plate, the microtubules form the usual peripheral circle of nine doublets (Fig. 5). The microtubules below the basal plate are arranged in the classical circular pattern of nine triplets to form the basal body (Figs. 6 and 7). Pericentriolar processes radiate out from the innermost microtubules of the triplets. A striated rootlet extends from the basal body and becomes associated with other elements of the fibrillar system situated along the wall of the nematocyst capsule (Fig. 8). A centriole is found near the basal body lateral to the striated rootlet.

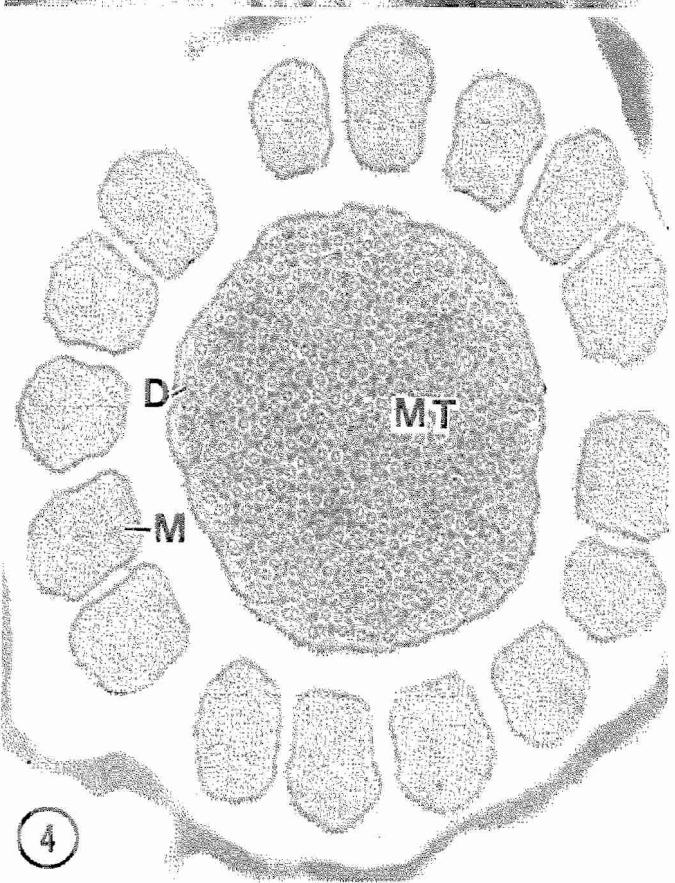
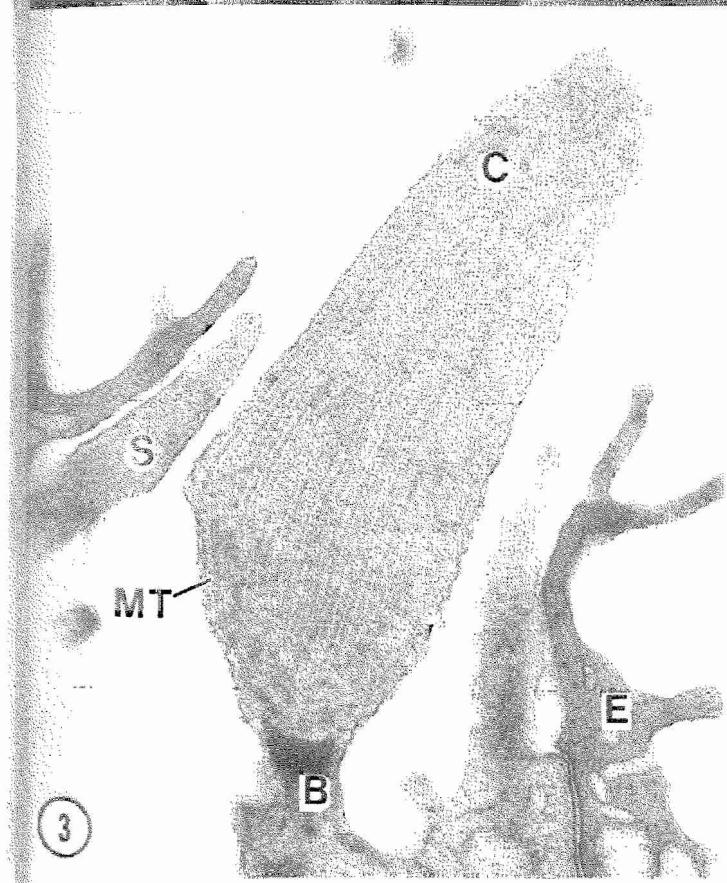
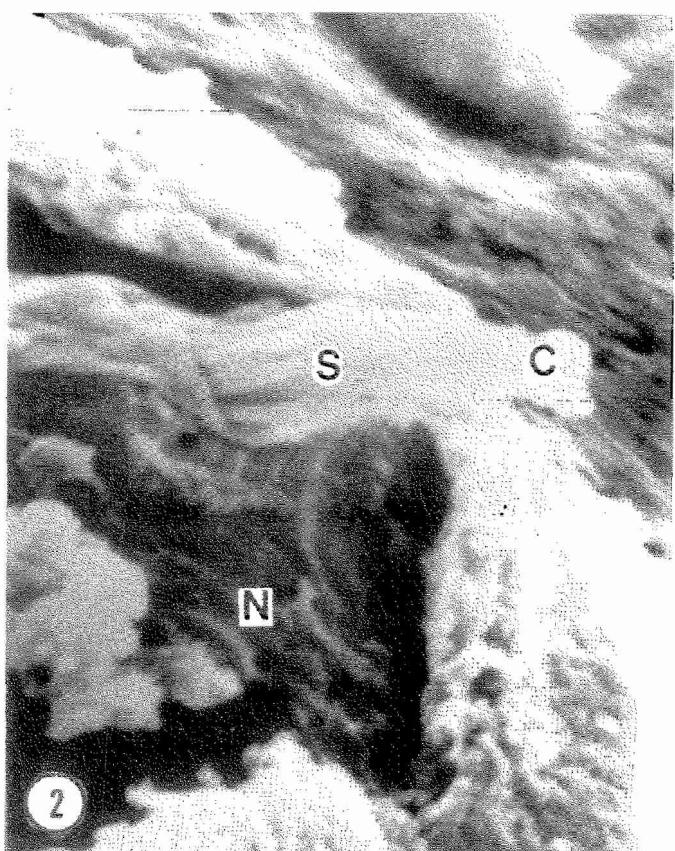
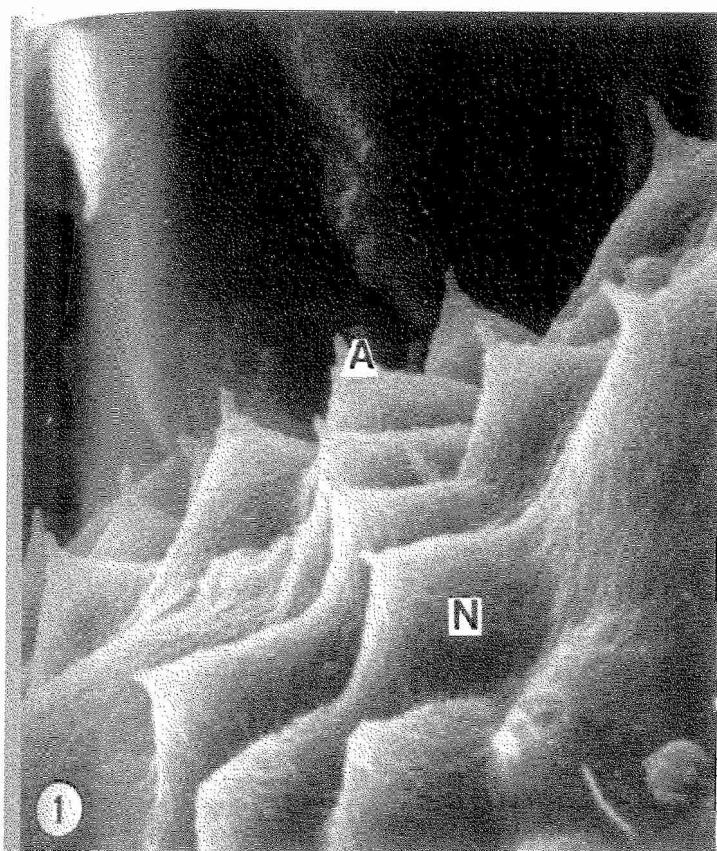
The stereocilia are associated with the fibrillar collar at the apical region of the fibrillar system. The fibrillar collar consists of striated rootlets, one for each stereocilium, that form an internal concentric ring below the crown of stereocilia (Fig. 7). A single fibril lies parallel to and equidistant

FIG. 1. Raised areas on the *Physalia* battery surface owing to underlying nematocysts (N). Cnidocil apparatuses (A) project from the free surface at the apex of each bump.  $\times 1\ 660$ .

FIG. 2. Abraded battery surface revealing large nematocyst (N). Associated cnidocil apparatus is composed of a single modified cilium or cnidocil (C) surrounded by shorter stereocilia (S).  $\times 5\ 000$ .

FIG. 3. The cnidocil (C) contains numerous longitudinally arranged, parallel microtubules (MT) that converge on a basal plate and then form a basal body (B). Processes of neighboring epithelial cells (E) surround ring of shorter stereocilia (S) that contain numerous fine microfilaments also arranged longitudinally.  $\times 33\ 420$ .

FIG. 4. Cross section through distal end of cnidocil apparatus showing central cnidocil containing both singlet (MT) and peripheral, doublet (D) microtubules and peripheral ring of stereocilia with numerous microfilaments (M).  $\times 58\ 800$ .



between adjacent rootlets. The individual rootlets and fibrils are connected by microfilaments perpendicular to the long axes of the rootlets forming a network resembling skeletal muscle (Figs. 7 and 9). Near the level of the nematocyst operculum, the fibrillar collar is connected to the unstriated rods of the fibrillar system that surround the nematocyst in a cage-like structure termed the fibrillar basket. The cnidocil is in close proximity to the fibrillar collar, although no direct association with the fibrillar collar was observed.

#### DISCUSSION

*In vivo* nematocyst discharge is a rapid and dramatic event that requires both mechanical and chemical stimulation (2-4). This suggests that more than one sensory receptor is required for the reception, transduction, integration, and transmission of sensory information. It is believed that the ciliary-cone complex in anthozoans, the flagellum-stereociliary complex in scyphozoans, and the cnidocil apparatus in hydrozoans are the homologous sensory receptors for the initiation of nematocyst discharge in the three classes of Cnidarians (7).

This study has shown on the one hand that the nematocytes of *Physalia physalis* have a cnidocil apparatus that is characteristic of hydrozoans. It is composed of a modified cilium surrounded by stereocilia situated at the free surface of each nematocyte (Figs. 1, 2). On the other hand, the

cnidocil apparatus of *Physalia* differs from the hydrozoans previously studied in that the 15 to 21 stereocilia are of equal height and are evenly distributed around the central cnidocil (Figs. 3, 4). In *Gonionemus*, 27 or 28 short stereocilia surround the opercular region of the nematocyst and 9 longer stereocilia surround the cnidocil (6). In *Hydra*, there are 18 or 20 short and 2 to 5 longer stereocilia (5). In addition, whereas the cnidocils of *Gonionemus* and *Hydra* have a central, dense core of microtubules surrounded by the classical, peripheral array of nine doublets, the cnidocil of *Physalia* has numerous longitudinally arranged doublet and singlet microtubules. The only features of the *Physalia* cnidocil in the region distal to the basal plate reminiscent of the classical 9 + 2 microtubule array are that: (1) the singlet microtubules predominate in the interior of the cnidocil, (2) the doublets are arranged exclusively at the periphery of the cnidocil, and (3) the number of doublets is evenly divisible by 9 suggesting a relationship with the nine doublets found in most cilia. The electron-dense material between the microtubules could be associated with motility or sensory functions or represent degenerate dynein arms.

The unusual microtubule pattern coupled with the apparent absence of dynein arms suggests that the mechanism generally accepted for ciliary motility is not possessed by the cnidocil (12, 13). It is likely,

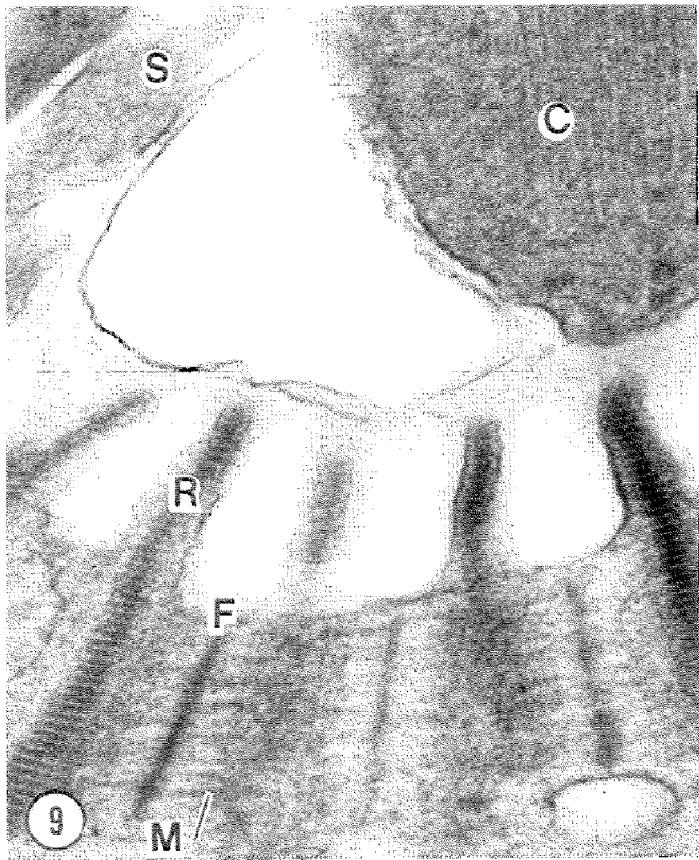
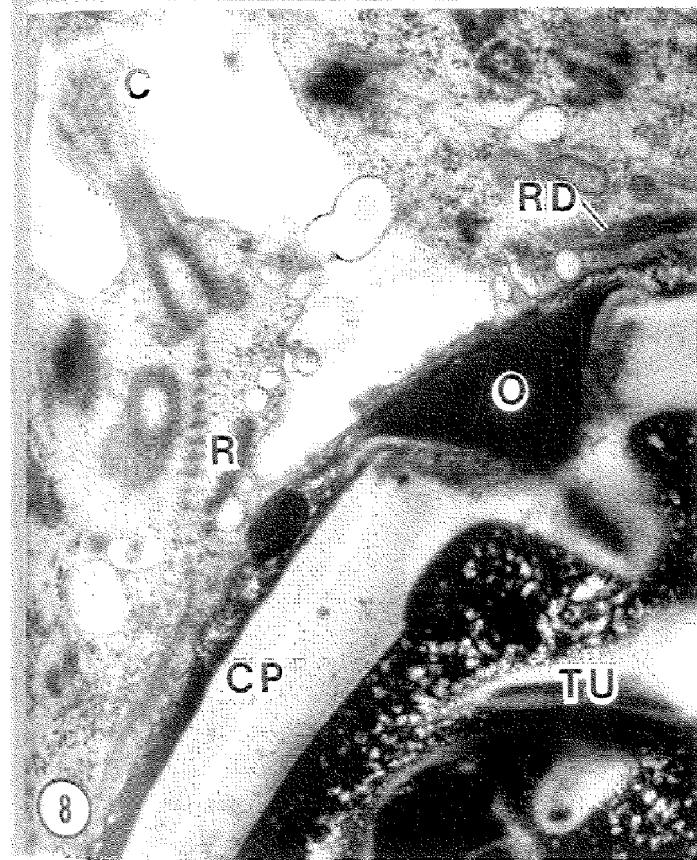
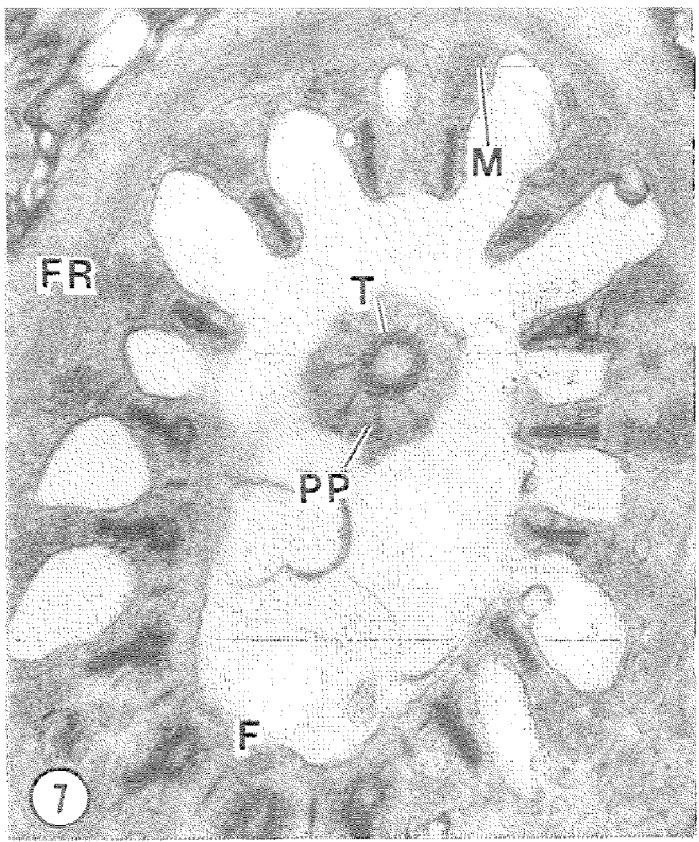
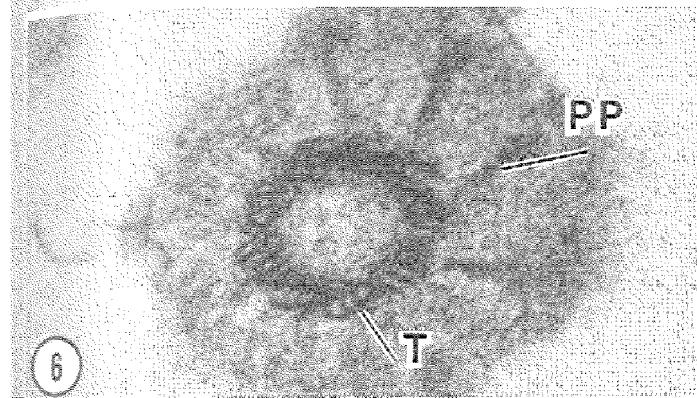
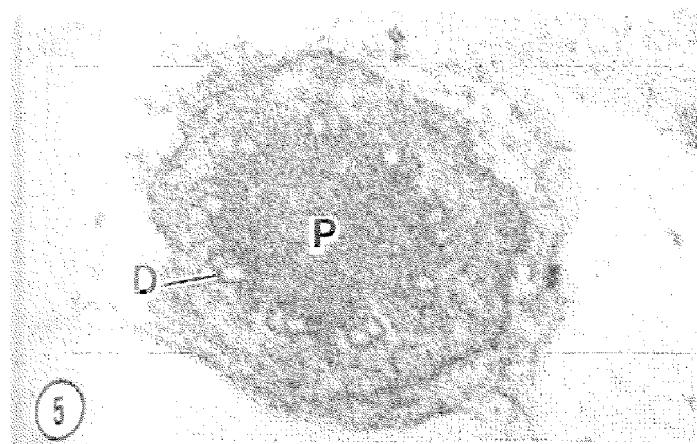
FIG. 5. Electron-opaque basal plate (P) of cnidocil with nine peripheral microtubule doublets (D).  $\times 133$  825.

FIG. 6. Microtubules in basal body of cnidocil arranged in a peripheral ring of nine triplets (T). Pericentriolar processes (PP) radiate from innermost microtubule of each triplet.  $\times 107$  970.

FIG. 7. Cross section of fibrillar collar and cnidocil basal body with microtubule triplets (T) and pericentriolar processes (PP). Microfilaments (M) of stereocilia extend basally into cell. Outer ring of microfilaments (FR) surrounds microfilaments of stereocilia in a plane perpendicular to stereociliary microfilaments. Fibrillar collar forms inner ring of rootlets and fibrils (F) connected by microfilaments.  $\times 40$  260.

FIG. 8. Striated rootlet (R) of cnidocil (C) extending from basal body to level of operculum (O). Rootlet loses its striations and becomes fibrous and indistinguishable from rods (RD) which surround nematocyst. All nematocysts observed in a *Physalia* battery are holotrichous isorhizas since there are numerous barbs ornamenting inverted tubule (TU) coiled within spherical capsule (CP).  $\times 26$  560.

FIG. 9. Fibrillar collar below cnidocil (C) and stereocilia (S) composed of a ring of striated rootlets (R). A thick fibril (F) lies equidistant and parallel to rootlets. Many fine microfilaments (M) connect fibrils and rootlets.  $\times 63$  450.



though, that the cnidocil apparatus is the sensory receptor for the chemical and mechanical stimuli responsible for nematocyst discharge. Like the chemosensory receptors in the antennae of the cockroach *Periplaneta americana*, the cnidocil contains an abundant number of doublet and singlet microtubules lacking the 9 + 2 arrangement (14). The arrangement of stereocilia around the central ciliary structure strongly resembles the mechanoreceptor found at the base of the hydroid, *Coryne pintneri* (15). In addition, the hydroid receptor, like the cnidocil apparatus in *Physalia*, is associated with a fibrillar collar and fibrillar system. In both *Physalia* and *Coryne*, the association of the receptor with the fibrillar system may be related to the transduction and transmission of mechanosensory data. Because of these structural similarities with the chemoreceptor of *Periplaneta* and the mechanoreceptor of *Coryne* and because of its proximity to the nematocyst, it seems likely that the cnidocil apparatus of *Physalia* functions as a sensory receptor-transducer in the initiation of nematocyst discharge.

Further evidence supporting the cnidocil apparatus as the sole mechano- and chemo-receptor for nematocyst discharge lies in the unique histology of the epithelial layer of the *Physalia* battery and tentacle (16, 17). Unlike *Hydra* (5) and *Gonionemus* (6), there are neither epitheliomuscular cells nor nerve cells in the epithelial layer of the *Physalia* tentacle and battery. Rather, the epithelial layer in *Physalia* consists solely of nematocytes, mucus-secreting cells, and epithelial cells attached directly to an acellular mesoglea (16). The absence of nerve conduction in the *Physalia* battery was reported by Parker and Van Alstyne (4). They showed that electrical stimulation can only cause nematocyst discharge in the immediate vicinity of the stimulation. Thus, the histological and physiological evidence suggests that in *Physalia* the nematocyte alone receives and transduces

sensory stimuli while also effecting nematocyst discharge.

Three general theories have been proposed for nematocyst discharge: the constant pressure hypothesis, the osmotic hypothesis, and the contractile hypothesis (8). The constant pressure hypothesis proposes that the contents of the nematocyst exert a continuous internal pressure that is released during nematocyst discharge when the opercular plug or flaps are loosened (18). The last two hypotheses posit that the internal pressure within the nematocyst capsule increases immediately prior to or at the moment of discharge. According to proponents of the osmotic hypothesis, the increased intracapsular pressure is due to an influx of water or hydrated ions (5, 19). In the contraction hypothesis, increased intracapsular pressure occurs as a result of a contractile apparatus or contractile mechanism in or around the nematocyst (2, 4). Hyman suggested that the rods of fibrillar system contract, increasing the pressure on the capsule and thereby eliciting nematocyst discharge (3). Although the whole fibrillar system was originally implicated, the fibrillar collar alone with its muscle-like network of connecting microfilaments may contract to induce discharge.

The cnidocil apparatus that projects from the free surface of the nematocyte is ideally situated for the reception of mechanical and chemical sensory stimuli. Furthermore, if nematocyst discharge occurs by a contractile mechanism, the cnidocil apparatus would provide a short, direct route to conduct transduced external stimuli through the fibrillar collar to other elements of the fibrillar system.

Direct confirmation of the sensory function of the cnidocil is within the reach of current neurophysiological techniques (20). Study of the possible contractile nature of the fibrillar system may also be approached experimentally. Present electron microscopic techniques are available to measure microfilaments in relaxed and contracted

*Physalia* tentacle systems (21, 22). Cellular fractionation (23) and biochemical characterization offer additional means for identifying the possible contractile nature of the fibrillar system. Such studies would help to provide evidence to support or refute the sensory function of the cnidocil and the contractile nature of the fibrillar system in Cnidarians including *Physalia*.

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