

## Chapter 4

### ULTRASTRUCTURE OF PHYSALIA NEMATOCYSTS

William H. Hulet\*

Division of Undersea Medicine  
School of Medicine, University of Miami  
Miami, Florida

J. L. Belleme and G. Musil  
Electron Microscopy Laboratory  
Veterans Administration Hospital, Miami, Florida

Charles E. Lane  
Rosenstiel School of Marine and Atmospheric Science  
University of Miami, Miami, Florida

The siphonophore Physalia physalis or Portuguese-man-of-war is a floating colonial hydrozoan that is particularly well known to bathers along the western Atlantic coast. Inadvertent contact with the venomous fishing tentacles produces a severe and painful sting that may last for several days. For many organisms, blundering into the tentacles of Physalia is a fatal mistake since the trailing retractable tentacles serve as an effective means of food capture. The length of each fishing tentacle is studded with "batteries" of nematocyst-containing cells. These cells, often referred to as cnidoblasts, are located in the epidermis (Lane and Dodge 1958). It is the nematocyst, a cell organelle, that contains the toxin.

Nematocysts are characteristic of all coelenterates, and Weill (1934) has described more than a dozen taxonomically useful categories. Physalia physalis has only one type of nematocyst. Basically it consists of a barbed thread coiled within a spherical capsule. Hyman (1940) states that all nematocysts discharge by eversion of the thread, but as recently as 1961, Chapman and other investigators (1961) could not accept the idea that the heavily armed thread in the stenotelic nematocyst of Hydra leaves the capsule by turning inside out. In a review

\*Present address: Marine Medicine Division, Marine Biomedical Institute, University of Texas, Galveston, Texas

of research on nematocysts, Picken and Skaer (1965) present data on the geometry of discharge in nematocysts of the sea anemone Corynactis viridis. Their electron micrographs clearly show that the thread everts on discharge.

In continuing our work on Physalia and its toxin, we planned to examine the nematocyst with the electron microscope and, as a first step, to clarify the mechanics of discharge. To our great dismay, countless attempts to section a nematocyst failed. The contents of the capsule shattered and were lost from the section. We had no difficulty in preparing sea anemones and corals for electron microscopy, but Physalia eluded our efforts for many months. In the remainder of this chapter we will show how we solved the problem of tissue preparation and demonstrate the pertinent features of nematocyst discharge in Physalia.

## I. MATERIAL

For preparation of undamaged tentacles, we collected live Physalia from the Straits of Florida while on board the R/V Gerda. Free undischarged nematocysts were obtained from fishing tentacles allowed to autolyze in the cold for 24 to 48 hr. Lane and Dodge (1958) previously described the details of this procedure.

## II. METHODS

### A. Light Microscopy

The addition of a few drops of Chlorox to a suspension of nematocysts is a simple method to induce discharge. For preparation of stained permanent mounts we used two procedures. In the first, intact and discharged nematocysts were fixed in 4% formaldehyde for 1 hr and then stained with 1% aqueous eosin solution for 30 min at 100°C. After cooling, the nematocysts were washed in 95% ethanol for 5 min and resuspended in absolute ethanol. The alcohol was replaced with xylene and the preparation mounted on a glass slide. In the second method, we fixed the nematocysts in 2.5% phosphate-buffered glutaraldehyde for 1 hr. After washing with two changes of 0.1 M neutral phosphate buffer, we "stained" with 1.33% buffered osmium tetroxide solution. We dehydrated with ethanol and suspended the nematocysts in xylene prior to mounting.

### B. Electron Microscopy

Immediately after collection, we immersed small pieces of Physalia tentacles in a fixing solution composed of glutaraldehyde, acrolein, paraformaldehyde, and s-collidine buffer. According to the directions given by Hayat (1970), the tissue was postfixed with 2% osmium tetroxide. We used acetone for dehydration prior to embedding the tissue in plastic. Isolated nematocysts were fixed in 2.5% phosphate-buffered glutaraldehyde for 8 hr. We maintained the osmolality of all fixatives and phosphate buffer washes at 2000 milliosmols per liter by the addition of sodium chloride. All aqueous solutions contained 0.1 M phosphate buffer at pH 7.2. The entire procedure was carried out in the cold ( $4^{\circ}\text{C}$ ). Osmium tetroxide, 1.33%, was used for postfixation (1 hr). We dehydrated the fixed nematocysts with ethanol and gradually substituted propylene oxide for optimum miscibility with the embedding plastic. Osmium penetrated the capsule of undischarged nematocysts only when the sample was pretreated with an aldehyde fixative, either glutaraldehyde or formaldehyde.

We believe the crucial factor that enabled us to cut thin sections of Physalia nematocysts was the use of a low-viscosity embedding medium. The Spurr epoxy embedding method is based on vinyl cyclohexene dioxide, which readily penetrated hard tissues and even mineral rocks (Spurr, 1969). Thin sections were cut with a diamond knife on a MT-2 ultramicrotome, stained with uranyl acetate and lead citrate, and examined in a Phillips EM 300 transmission electron microscope.

### C. Scanning Microscopy

For scanning electron microscopy (Cambridge SEM 4), osmium-fixed nematocysts were washed with distilled water, immersed in liquid nitrogen, freeze dried for 12 hr at  $-60^{\circ}\text{C}$ , and gold coated in a vacuum evaporator.

We recently had the opportunity to do a sample run on the Quantimet 720 Image Analysing Computer. In this experiment we measured the diameter of several thousand free nematocysts of P. physalis.

## III. RESULTS AND DISCUSSION

The Physalia nematocyst is a coiled thread coiled within a thick spherical capsule. Isolated nematocysts as well as those observed in sections of fishing tentacles are of one

structural type and two sizes (Fig.1). From a size analysis with the Image Analysing Computer we obtained a bimodal distribution. With a sample size of 4670 nematocysts, two groups

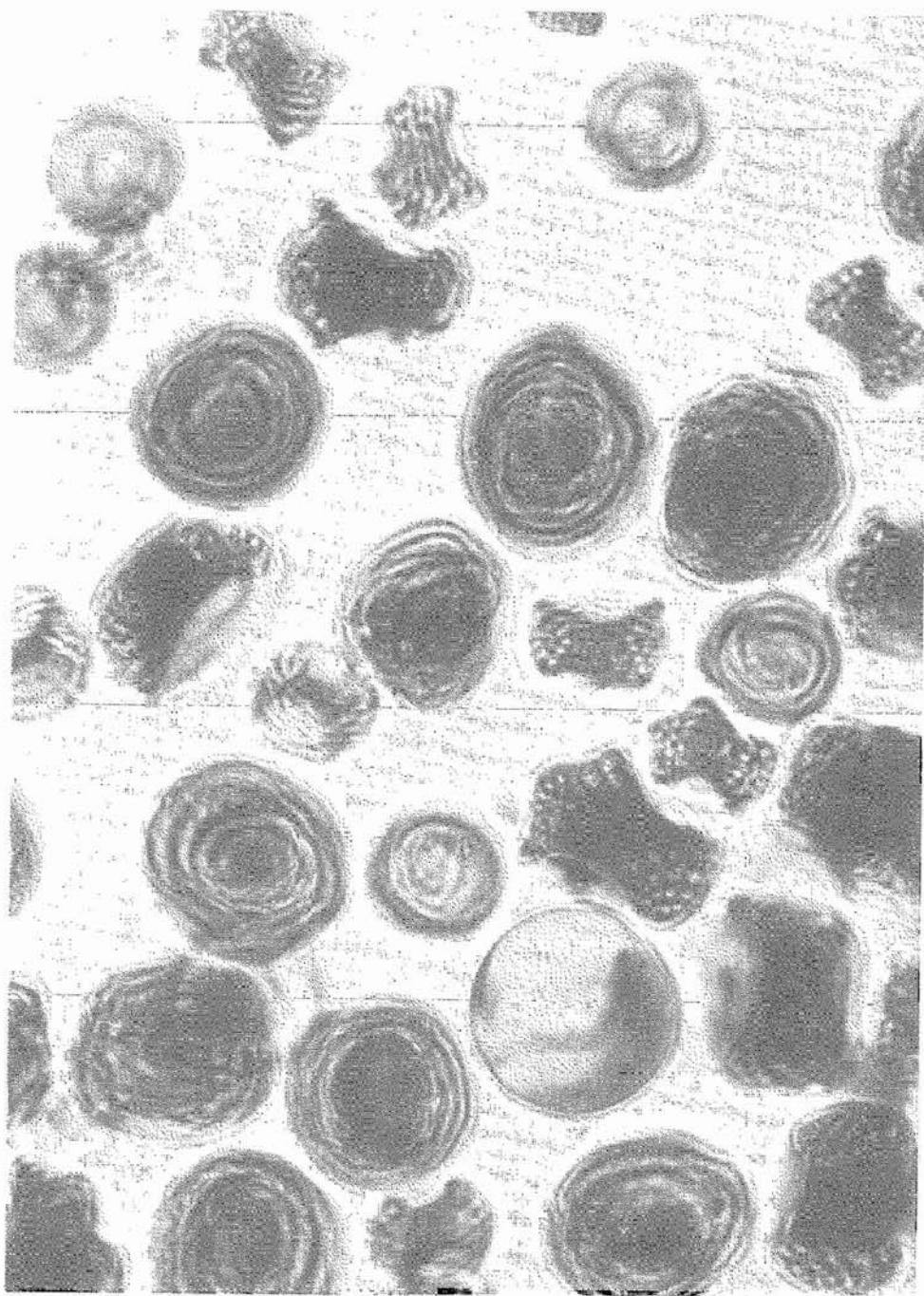


Fig.1. Light micrograph of isolated nematocysts of Physalia physalis; fixation with glutaraldehyde and osmium tetroxide. (X865.)

accounted for 78% of the measurements. Mean diameters were 11.3  $\mu\text{m}$  for the smaller nematocysts (19%) and 25.8  $\mu\text{m}$  for the larger (59%). These data agree closely with the earlier report of Lane and Dodge (1958). Six percent of the nematocysts in our measurements were over 40  $\mu\text{m}$  in diameter. This group probably represents discharged nematocysts in the sample at the time of scanning measurements. Although coelenterate nematocysts are cellular organelles that develop in the cytoplasm, we did not find early stages or what could be called immature nematocysts in histological sections of Physalia tentacles. Siautterback (1961) described the differentiation of nematocysts in Hydra, and we have seen different stages of development in tentacles of the sea anemone Condylactis. We did find in our image analysis study that 435 out of 4670 measurements gave a diameter less than 6.2  $\mu\text{m}$ . This size group may have been immature nematocysts that we did not detect microscopically.

A simple but conceptually valid way to visualize the mechanics of a discharging nematocyst is the process of turning a rubber surgical glove inside out. After stuffing the parts covering the palm and fingers through the opening at the wrist end, air can be trapped inside and the glove held closed. As the partially inflated glove is gently compressed, the fingers begin to evert. It is important to note that eversion begins at the base of the inverted fingers. With additional pressure the everted-inverted junction travels distally until the entire finger is everted. Except for some complexities of rotational symmetry, which we will discuss later, this is the way the nematocyst thread gets out of the capsule.

In our discussion we use the term "mechanics of nematocyst discharge" to describe a sequence of anatomical events. The precise mechanism that incites a living Physalia to discharge its nematocysts remains unknown. Sensory hairs (cnidocils) and a nerve network have been described for the cnidoblasts of other coelenterates (Westfall, 1970). If cnidocils are present in Physalia, they are not a prominent feature since we have yet to find one directly associated with a cnidoblast. In isolated nematocysts of Corynactis, Picken and Skaer (1966) found the intracapsular fluid to have a high osmolality, about 3000 mOsm/liter. They suggest that some mechanism increases the permeability of the capsular wall to water and the rapid expansion of intracapsular fluid exerts a strong driving force for expulsion of the thread.

Returning to Physalia, the photomicrographs of Fig. 2 demonstrate several pertinent structural details. As it so often happens, after we reviewed a number of electron micrographs we were able to recognize anatomical details by light microscopy.

which previously went unnoticed. Beneath the operculum (Fig. 2a) the coiled thread is anchored to the internal wall of the capsule. An end-on view of several coils is shown in Fig. 2b.

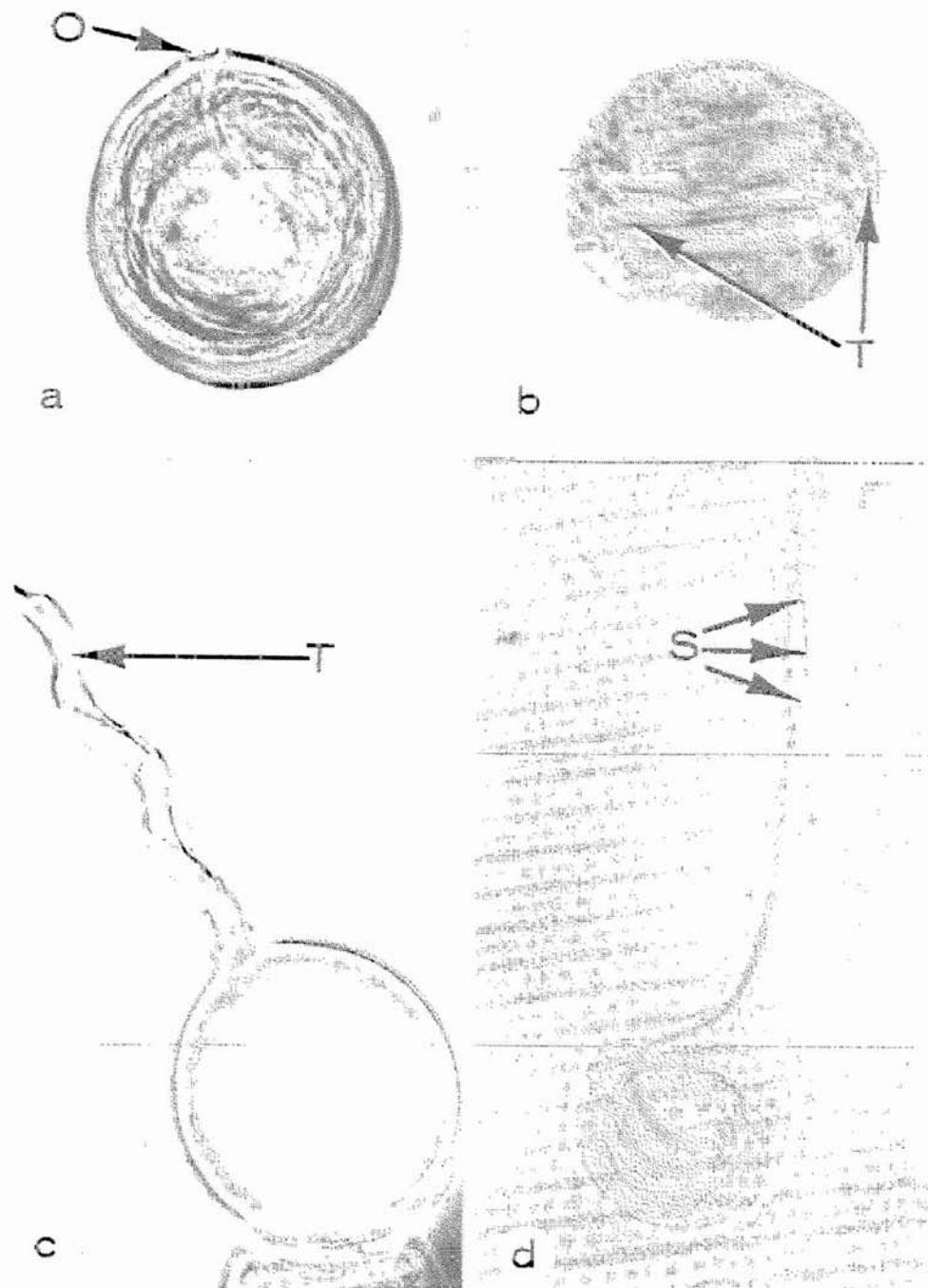


FIG. 2. (a) Undischarged nematocyst showing opercular opening (O) and coiling of the thread; (b) dark area indicates spines inside the thread (T); (c) a discharged nematocyst; (d) partially discharged nematocyst showing helical row of spines (S); eosin stained. (X1200.)

Closely packed spines appear to occlude the lumen of the thread. After ejection of the operculum, the thread begins the eversion process through the opening in the capsule. The thread is attached all around the rim of the opercular opening. As the thread turns inside out, the spine-bearing luminal surface on the inverted thread now becomes the external surface and the spines project to the outside. Figure 2d shows a partially discharged nematocyst. That part of the thread already everted bears rows of spines and serves as a sheath for the advancing remainder of the thread, which has yet to evert. The thread of a completely discharged nematocyst (Fig. 2c) spirals away from the capsule in a clockwise rotation. Spines run the length of the thread and since the latter is of constant diameter the Physalia nematocyst can be classified as a holotrichous isorhiza (Hand, 1961).

The discharged thread of Physalia has three longitudinal pleats and three rows of spines that spiral to the right going away from the capsule (Figs. 3 and 4). The scanning electron micrograph in Fig. 4 shows how the spatulate spines overlap and point backward toward the proximal end of the thread. The nematocyst in this picture was arrested in discharge, and in the upper portion one can see a spine coming over the ridge that is the moving junction between the everted and inverted segments of the thread. The structure of the thread is remarkably suited for rapid eversion with minimal frictional resistance. Since the everted thread has right-handed helical pleats, the unevolved thread, as it lies in the capsule or is sliding outward through the sheath formed by the everted portion, must have the shape of a left-handed screw. This engineering feat of Physalia and other coelenterates is demonstrated in the transmission electron micrographs of Figs. 5 and 6. The unfired thread has the shape of the triquetrum or triskelion, a three-legged ancient magic symbol that runs counterclockwise. This symbol represents one of the simplest forms of rotational symmetry (Weyl, 1952). It should be noted that in sections cut through an intact nematocyst half the coils will rotate to the left and the other half to the right. The spines of the three helical rows form the center of the undischarged thread (Fig. 7). They are triangular in shape and of equal size. The reason for the apparent size difference in Fig. 7 is because the single plane of the section cuts through different relative levels of each helix of spines. The relative position of the spines can be better understood from a longitudinal section of the thread (Fig. 8). Figure 9 shows the cross-sectional appearance of two partly discharged threads. The internal thread is propelled

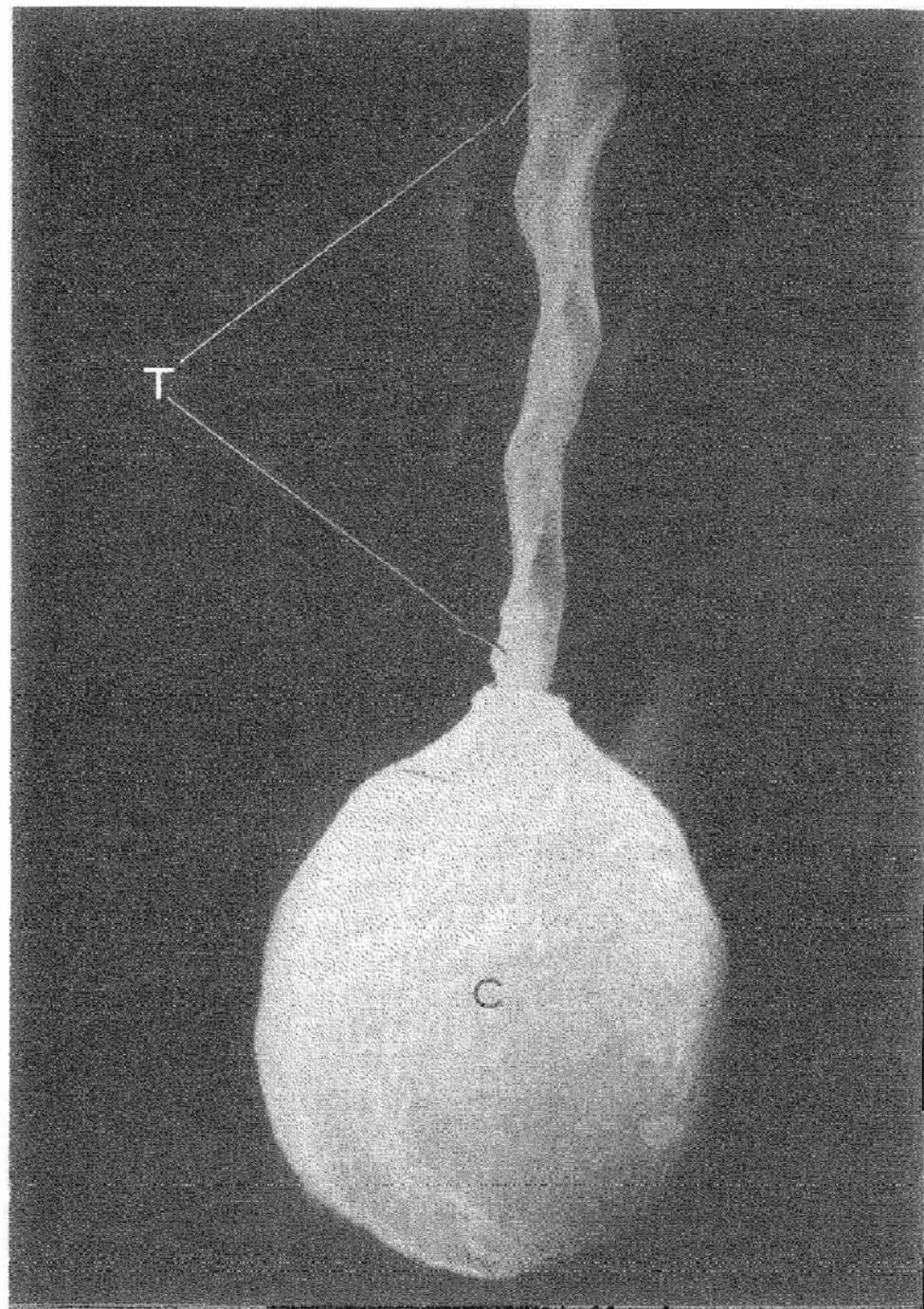


FIG. 3. Scanning electron micrograph showing capsule (C) and thread (T) of a discharged nematocyst. (X3800.)

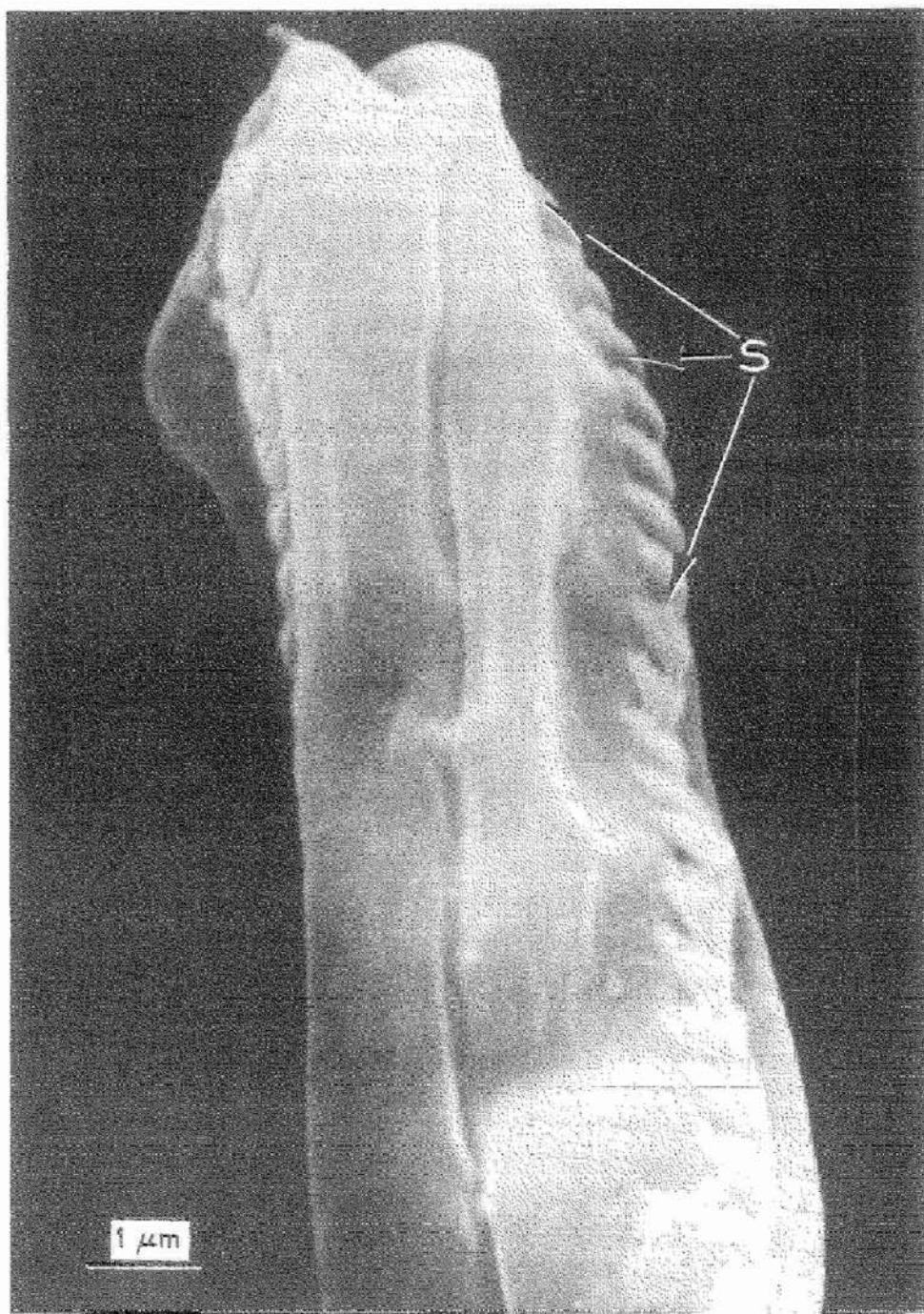


FIG. 4. Scanning electron micrograph demonstrating the spatulate spines (S) of a nematocyst arrested in discharge. (X14,400.)

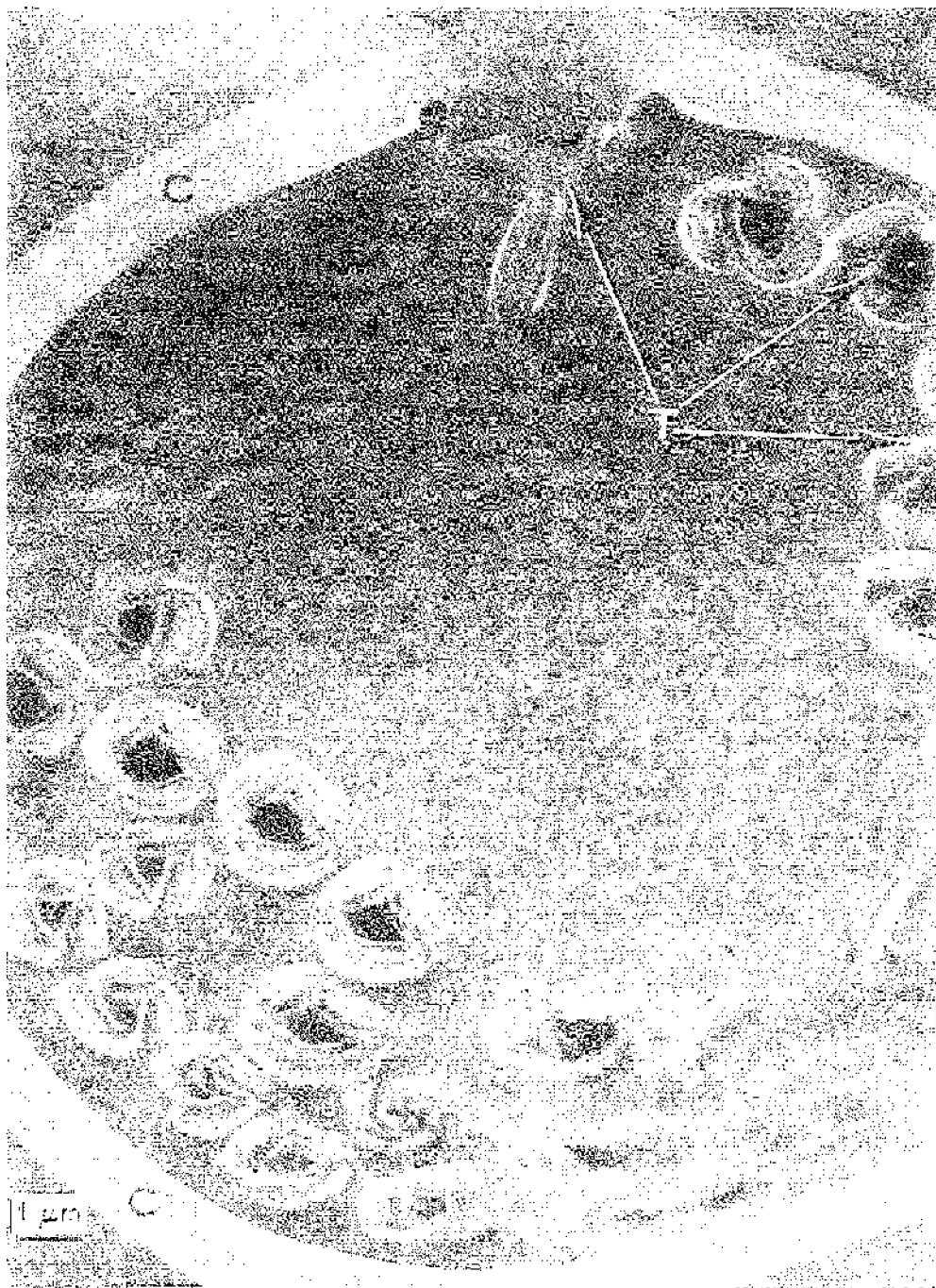


FIG. 5. Transmission electron micrograph of a nematocyst in situ in a fishing tentacle. Note the layering of the capsule (C) and the triquetrum-shaped thread (T). (X9300.)

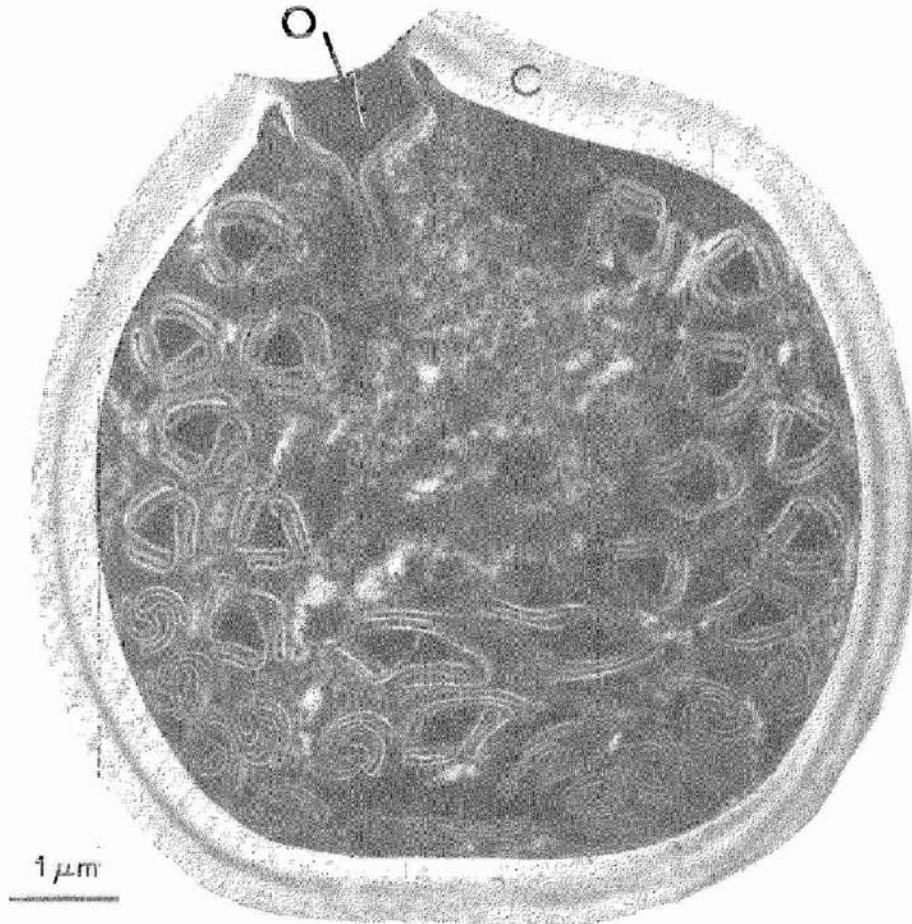


FIG. 6. The first part of the thread to evert is located beneath the operculum (O). (X14,400.)

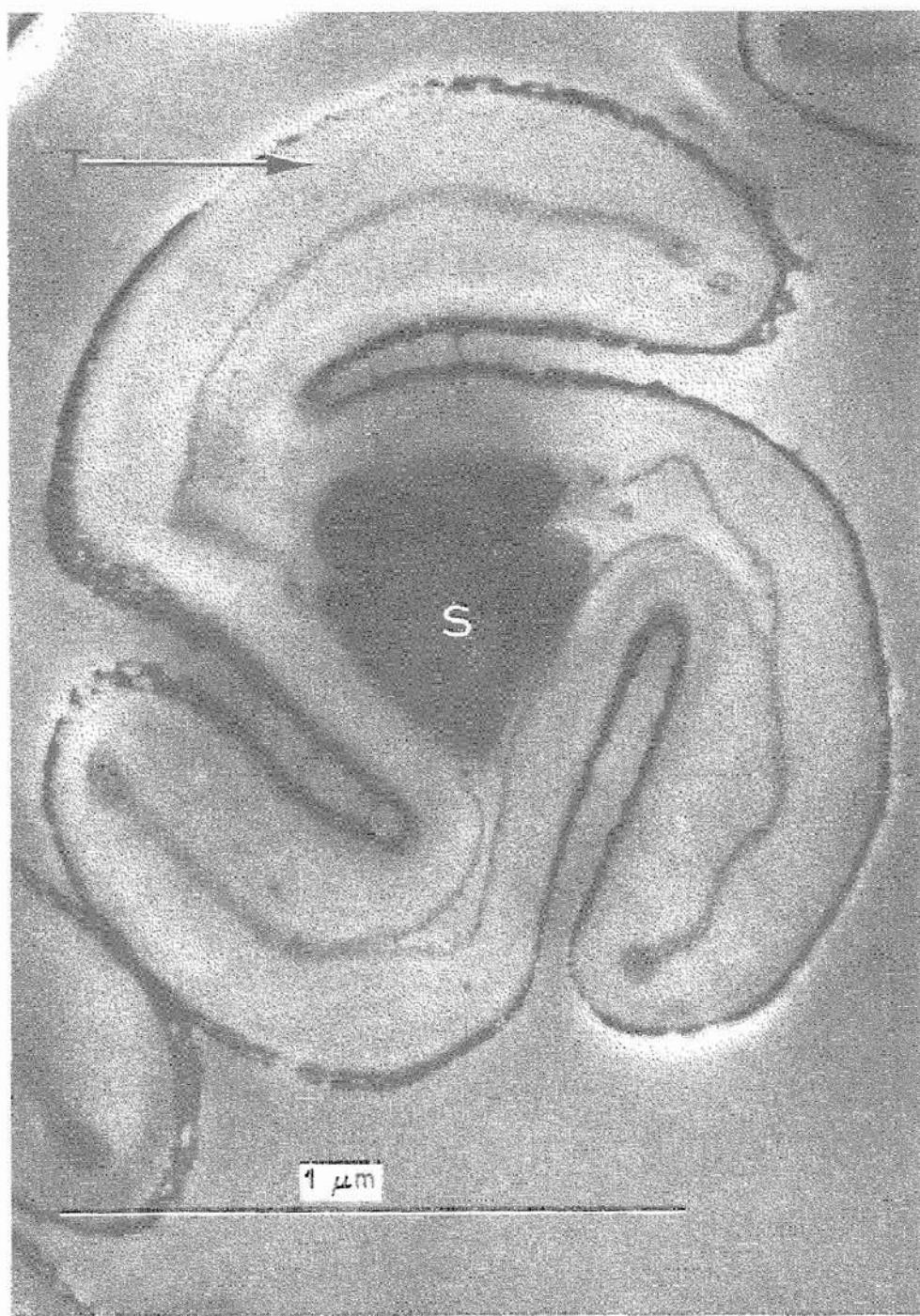


FIG. 7. Cross section of an undischarged thread. Portions of three spines (S) are present on the internal surface. (X84,300.)

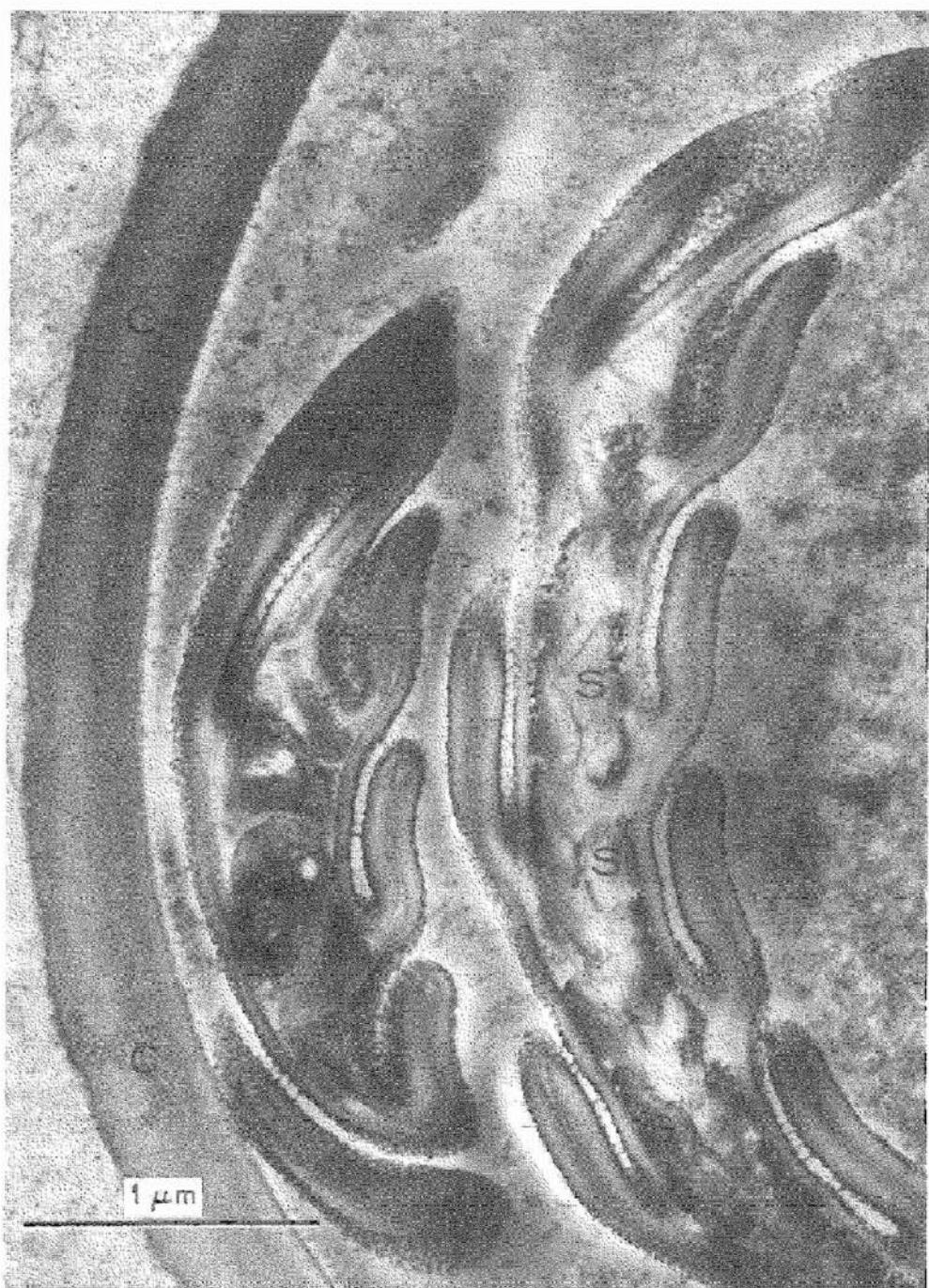


FIG. 8. Tangential section of two thread coils. When the thread is discharged the spines (S) will form three external helical rows. (X41,500.)

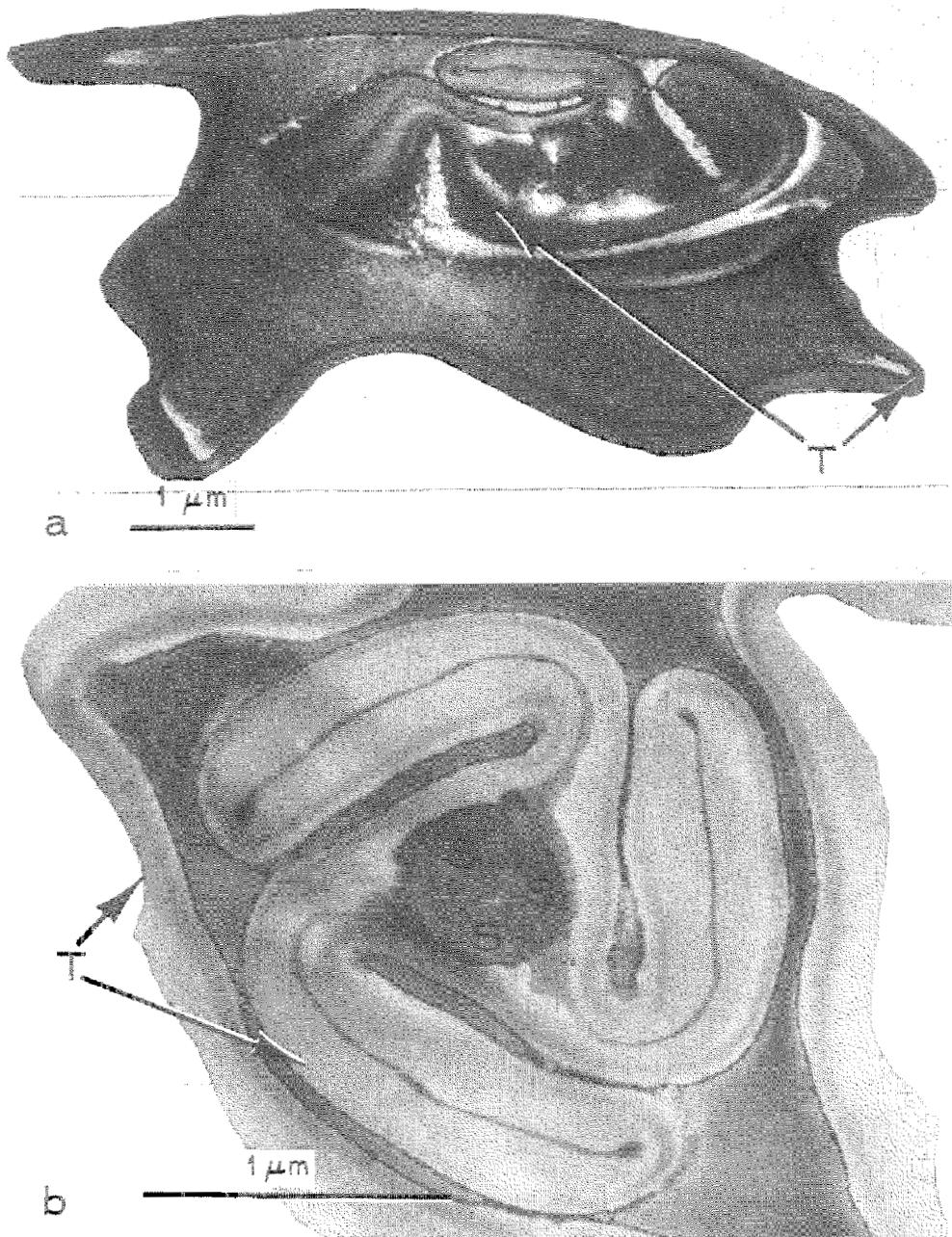


FIG. 9. Section outside the capsule through two partially discharged threads. The unevolved segment is sheathed by the external everted portion of the thread. [(a) X17,600; (b) X41,000.]

through the external everted portion until eversion is complete and all the spines are outside.

We have made one rather obvious conclusion from this study on mechanics of discharge. The barbed thread is in a geometrical position to penetrate and affix itself to the victim's tissue immediately after eversion begins. Total discharge of the nematocyst is not necessary, at least not necessary to produce mechanical injury. The location of the toxin is another question of some importance. We already know that ground up Physalia nematocysts yield a highly poisonous fluid, which is not a constituent of the capsule wall. Some of the toxic protein complex may be deposited on both sides of the thread membrane, and, if so, contact with any portion of an ejected thread would be poisonous.

#### ACKNOWLEDGMENTS

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