

Phosphatic spicules in the nematocyst batteries of *Nanomia cara* (Hydrozoa, Siphonophora)

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Summary. The complex, erupting nematocyst batteries of *Nanomia cara* are described. In addition to the cnidoband, the battery has a central axis containing longitudinal muscles and nerves that run right through to the terminal filament. In addition, an elastic strand lies coiled within the battery. After eruption of the battery, this strand keeps the prey attached to the tentacle. The strand bears hook-like spicules, equipped with barbs that project beyond the surface. Electron microscopy shows that the elastic strand is a mesogloal structure tunnelled through and through with cellular processes deriving from both ectoderm and endoderm and that the spicules lie in cellular pockets in the interior of the elastic strand. There is nothing in the structure of the spicules or their cellular sheaths to suggest an origin from nematocysts. Energy dispersive X-ray microanalysis shows strong peaks for calcium and for phosphorus in the spicules, indicating that the mineral present in them is an apatite. An organic matrix is also found in the form of fine filaments and a granular axial structure. The spicules are arranged in a linear series along the elastic strand showing progressive increase in size and structural elaboration.

A. Introduction

The nematocyst batteries of siphonophores are highly complicated erupting structures containing several sorts of nematocysts mounted in orderly arrays on a coiled or curled structure, the cnidoband. On contact with prey, the battery erupts, the cnidoband is flung out and sticks to the prey and, at the same time, the nematocysts discharge. Some of the nematocysts are adhesive, and others penetrate the prey. A long supposedly elastic strand, the *Angelband* of German writers, secures the prey to the tentacle after the battery has discharged. The elastic strand is known to bear hook-like structures. Purcell (1984) has summarized information on the different types of nematocysts found in the batteries of various siphonophore species, but the organization of the battery itself has not been studied this century. Given the lack of any modern study, we include below (see Discussion) a brief review of the principal findings of nineteenth-century workers on the elastic strand and its hooks.

During a visit to the University of Washington's Friday Harbor Laboratories in 1986, G.O. Mackie, accompanied

by C.E. Mills (University of Washington, USA) and R.J. Skaer (Cambridge University, England), looked at the nematocyst batteries of *Nanomia cara* and ascertained that the hooked structures, unlike nematocysts, dissolve in dilute hydrochloric acid and, therefore, are presumably calcareous spicules. This result was interesting, because the only members of the class Hydrozoa previously known to form calcareous structures are the Milleporidae and Stylasteridae. Subsequently, the present authors took up the project in more detail and examined the batteries by electron microscopy and electron probe microanalysis, the results of which are given here.

Our primary concern in this paper is with the spicules (a term we will now use in place of hooks) but in order to put our account of these structures in a proper morphological context we will start with a brief description of the structure of the battery based on optical and electron microscopy.

B. Material and methods

Specimens of *Nanomia cara* (Agassiz, 1862) were caught off the dock at the University of Washington Laboratories at Friday Harbor, Washington, USA. Living tentillar batteries were observed using bright field, phase contrast and Nomarski differential interference contrast microscopy. Specimens were fixed for routine electron microscopy in 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer at pH 7.2 and postfixed for 1 h in 1% osmium tetroxide in the same buffer. They were stained overnight in a 2% aqueous solution of uranyl acetate at 60°C, dehydrated in ethanol and propylene oxide, embedded in Spurr's resin (Spurr 1969) and examined with a Philips 300 electron microscope. Decalcification was carried out according to the method of Dietrich and Fontaine (1975) by treatment with 2% ascorbic acid in 0.3 M NaCl for 12 h.

Material for scanning electron microscopy was fixed in 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer, dehydrated in ethanol, critical point dried, coated with gold, and examined with a J.E.O.L. JSM 35 scanning electron microscope.

Elemental analysis of the spicules was carried out on 0.5 µm thick sections of material fixed in buffered 2.5% glutaraldehyde without osmium post-fixation and omitting the uranyl acetate treatment. A Tracor Northern 5500 energy dispersive X-ray microanalyser was used in conjunction with a J.E.O.L. 1200 scanning transmission electron microscope.

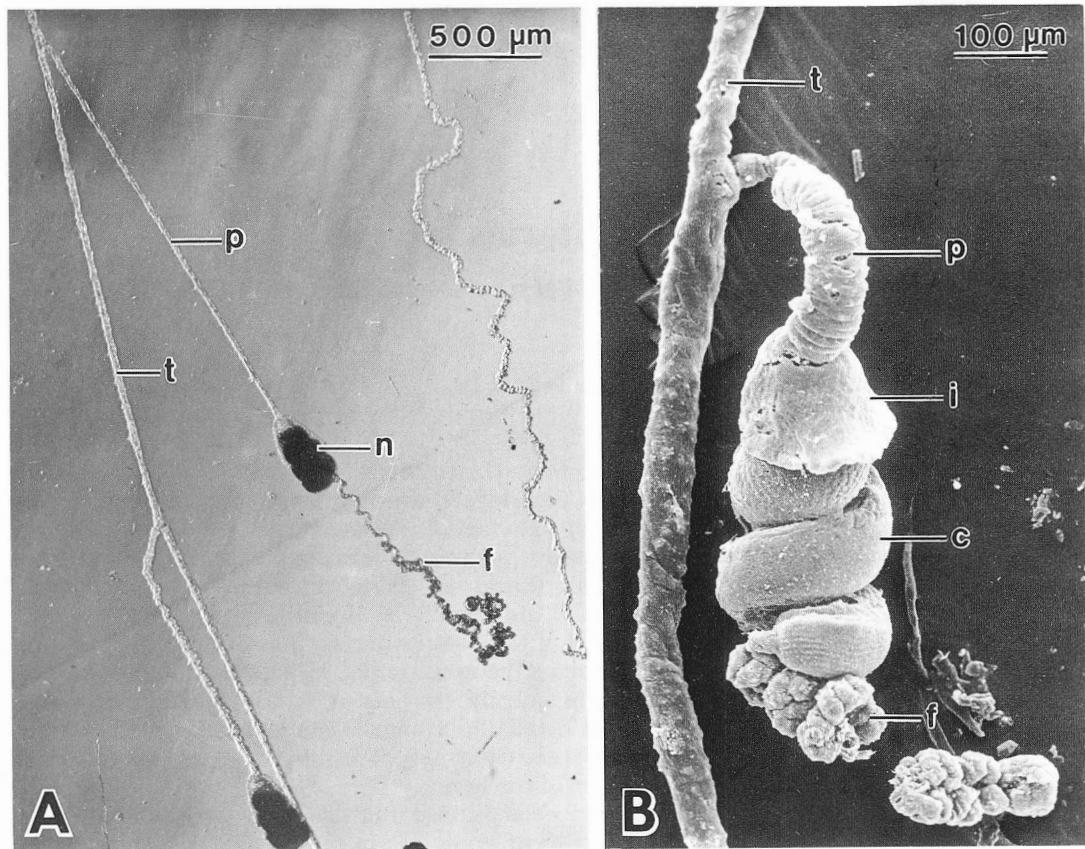


Fig. 1A, B. Nematocyst batteries of *Nanomia cara*. **A** Photomicrograph and **B** scanning electronmicrograph showing; *c* cnidoband; *i* involucrum; *f* terminal filament; *n* nematocyst battery; *p* pedicel; *t* tentacle

C. Results

1. General organization of the nematocyst battery

Each mature gastrozooid tentacle of *Nanomia* has about 12 side branches, or tentilla (Mackie and Boag 1963), consisting of a proximal pedicel, an involucrum (a bell-shaped outfolding of the ectoderm), a nematocyst battery, and a terminal filament (Figs. 1, 2; terminology according to Totton 1965). The internal structure of the battery is quite complicated. There is a central strand of tissue containing an endoderm canal and equipped with longitudinal ectodermal muscle fibres, forming the axis of the battery (Figs. 2, 3). Around this strand the cnidoband, which bears the nematocysts, forms a wide spiral. The elastic strand (*Angelband* used by earlier authors) consists of a descending arm, which is coiled around the central axis, and an ascending arm, which follows the spiral of the cnidoband, adhering to its inner surface.

Seen in cross-section under the microscope (Fig. 3), the ascending and descending arms of the elastic strand are predominantly mesogloal structures and they are connected with the mesogloea surrounding the endodermal canal and supporting the cnidoband. The ectodermal longitudinal muscle is formed from epithelio-muscular cells that lie to one side of the axis on a thickened supporting mass of mesogloea. Loose "spongy" ectoderm fills in the area between the axis and the cnidoband.

Eruption of the nematocyst battery is a violent explosive event that takes place with lightning rapidity on an all-or-

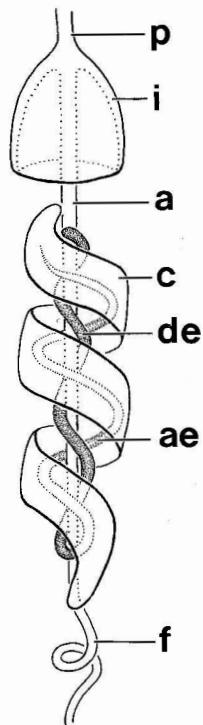


Fig. 2. Battery stretched out slightly to show inner structures: *a* axis; *ae* ascending portion of elastic strand; *c* cnidoband; *de* descending portion of elastic strand; *f* terminal filament; *i* involucrum; *p* pedicel

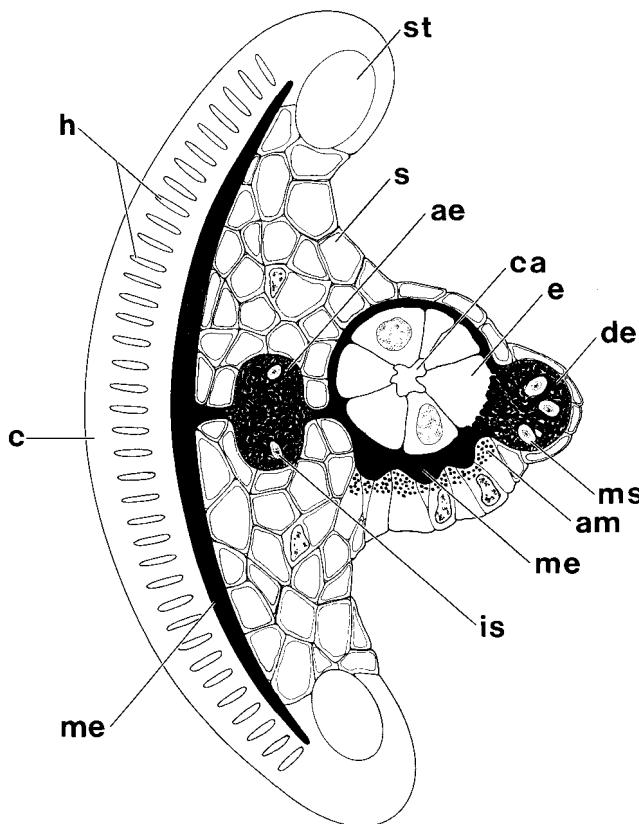


Fig. 3. Diagrammatic cross-section through an undischarged battery, based on electron microscopy; *ae* ascending portion of elastic strand; *am* axial muscle; *c* cnidoband; *ca* endoderm canal; *de* descending portion of elastic strand; *e* endoderm; *h* haploneme nematocyst of cnidoband; *is* immature spicule; *me* mesogloea; *ms* mature spicule; *s* spongy ectoderm; *st* stenotela nematocyst of cnidoband

nothing basis and results in the virtual destruction of the cnidoband as all the nematocysts in it explode, along with the tearing apart of the tissues that hold the battery together in its coiled state. Comparison of batteries before and after eruption (Fig. 4A, B) shows that when the battery erupts, the thin mesogloea lamella holding the ascending elastic strand to the axis tears, along with the spongy ectoderm, allowing the cnidoband to detach, uncoil and apply itself to the prey by means of discharged nematocysts. It may remain attached to the axis at its lower end (as shown in Fig. 4B), but this connection too may sever, leaving the cnidoband held to the axis solely by means of the elastic strand. Towards its lower end the ascending elastic strand also tears away from the cnidoband, but it always remains firmly attached to the cnidoband at its upper end. The descending elastic strand is firmly attached to the axial mesogloea all along its length, and never separates from it.

With reference to the eruption mechanism, manipulation and dissection of living batteries does not suggest that the coiled battery is under tension or that eruption represents release of tension in a springlike system. Eruption is actually hard to provoke. Pulling the terminal filament is not in itself sufficient to provoke it. Even when the cnidoband is cut at its upper end, it remains coiled up. The presence of a complete endodermal canal running through the axis (a point not appreciated by earlier writers) raises the possibility that hydraulic expansion of the axial canal,

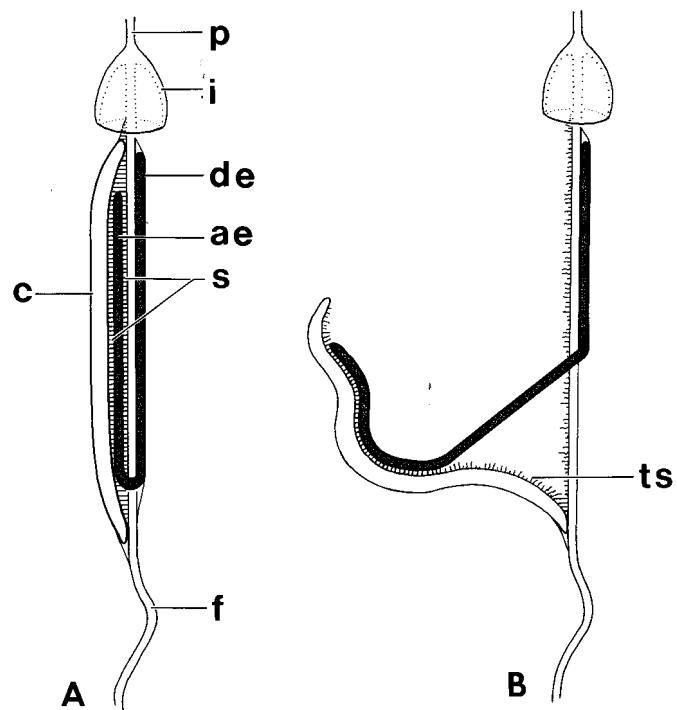


Fig. 4A, B. Diagram to show what happens during discharge. **A** A battery is shown extended (uncoiled) but still undischarged, with all connections still intact. **B** The battery is shown discharged. *ae* Ascending portion of elastic strand; *c* cnidoband; *de* descending portion of elastic strand; *f* terminal filament; *i* involucrum; *p* pedicel; *s* spongy ectoderm; *ts* torn spongy ectoderm

caused by contraction of muscles higher up the tentillum or in the tentacle, may play some part in eruption.

Contrary to earlier reports, the axis contains nerves. These form a bundle closely associated with the axial muscles (Fig. 6E). Electrophysiological evidence (Mackie, unpublished) shows that electrical signals can be propagated right through the tentillum, from pedicel to terminal filament. In the tentacles, two physiological conduction systems, represented by two nerve tracts, are present, one pathway conducting faster than the other (Mackie 1973; Grimmelikhuijen et al. 1986). Although two separate nerve tracts have not been distinguished histologically in the tentillum itself, the physiological evidence shows that two conduction pathways are present. It seems likely, therefore, that the axial muscles of the battery are controlled in the same way and contract at the same time as the ectodermal muscles elsewhere in the tentacle and tentillum. No evidence has come to light for the existence of other more specialized neuromuscular mechanisms that might be implicated in eruption.

2. Distribution and morphology of spicules

Spicules are distributed all the way along the elastic strand. In the ascending arm, they are small and rod shaped, about 15 µm long and arranged in two rows, one on either side of the strand. No barbs are present on these seemingly

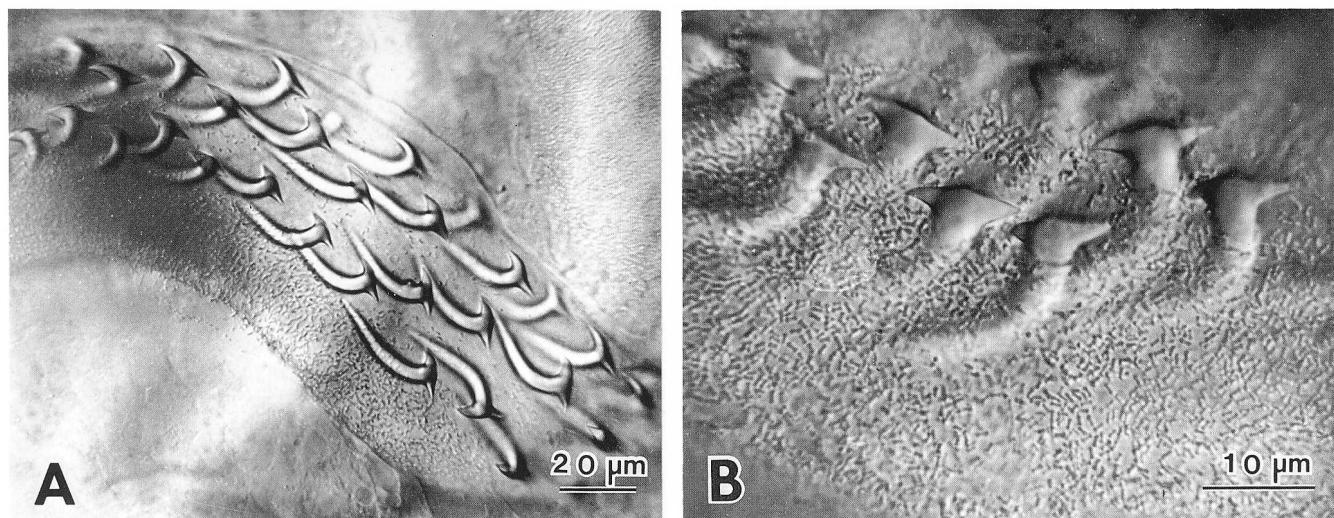


Fig. 5A, B. Spicules in situ, seen in a whole mount by Nomarski microscopy. **A** Concentrations of mature spicules in descending portion of elastic strand. **B** Detail of the same. Note that barbed ends of spicules project freely

immature spicules. Near the point where the terminal filament joins the muscular axis of the battery and where the descending arm of the elastic strand loops around to become the ascending arm, an abrupt transition in spicule form and distribution is apparent. Within a distance of 0.5 mm the spicules change from 15 µm long, unbarbed rodlets to 25 µm long spicules with small protuberances representing nascent barbs at one end. In place of the two rows, we now find a single row containing four to six spicules packed closely together side by side. Towards the top of the descending arm of the elastic strand, the spicules are fully developed, 35 µm long, curved, and with prominent barbs (Fig. 5A). The barbed ends project through the surface of the ectoderm covering the elastic strand, and would thereby presumably be capable of acting as attachment hooks (Fig. 5B).

In electron micrographs of the elastic strand the mesogloea, which constitutes the bulk of the structure, is seen to be tunnelled through and through by fine cellular processes. The spicules, both in their immature rodlet form and in their mature barbed form, are enveloped within cellular sheaths (Fig. 6A, B) that are continuous with these processes. The spicule itself lies within an intracellular pocket. The cytoplasm immediately adjacent to the spicule is in the form of a shell of dense granular material. This granular shell is bounded on both sides by unit membranes, but it does not appear to represent a distinct cell type as it lacks typical cellular organelles; instead, it probably represents a specialized cytoplasmic sac belonging to the same cells that form the rest of the sheath. The cytoplasm of the sheathing cells contains numerous mitochondria.

The cytoplasmic processes penetrating the mesogloea appear to be derived both from the endoderm (Fig. 6C) and from the ectoderm (Fig. 6D). Cellular processes of this type are found only in the mesogloea of the elastic strand. Other mesogloal structures, including the thick pad supporting the axial muscle band, lack them completely. It seems very likely that the processes are responsible for spicule production, and serve for transport of materials required for spicule synthesis. Around the edges of the elastic strand, in regions bounded by ectoderm, there is an orderly

array of cytoplasmic pockets (Fig. 6A, C, D). Some of these are vacuolated, with traces of fibrous material in the vacuole. We have no evidence concerning their function.

With regard to the organic matrix of the spicule, all that can be seen in decalcified material is a cortical mass of fine filaments separated by a space (possibly a shrinkage artefact) from a central mass of similar fibrous material surrounding an axis of dense granular material (Fig. 6B). In sections of spicules that have not been decalcified, the mineral mass appears homogeneous, and we have not been able to make out any microcrystalline substructure.

3. Elemental analysis

Energy dispersive X-ray microanalysis shows strong peaks for calcium and phosphorus in sections of spicules (Fig. 7). Control scans in other parts of the elastic strand showed no such peaks. After decalcification, the spicules no longer showed the peaks. We conclude that the predominant mineral present is a form of calcium phosphate (apatite).

D. Discussion

1. Historical background

The hooked structures, here termed spicules, have been found in several Physonectae but in no members of the Calycophora. They were first described by Sars (1846, p. 36, footnote) as "cartilaginous crescents". Kölliker (1853) was the first to draw a clear distinction between them and nematocysts. Leuckart (1853) described them as rodlets (*Stäbchen*) resembling the setae of tubicolous polychates, but later he concluded that they were merely folds produced by strong contraction of the elastic strand (*Angelband*). Vogt (1854), however, saw the rodlets as true solid structures that shone "like crystals". Huxley (1859) provided the first accurate drawings and description of the spicules, which he described in these terms: "... elongated, slightly curved, and pointed at one end, while the other end is obtuse, and presents three or four elevations." These elevations we term barbs. Claus (1860) noted that the barbed

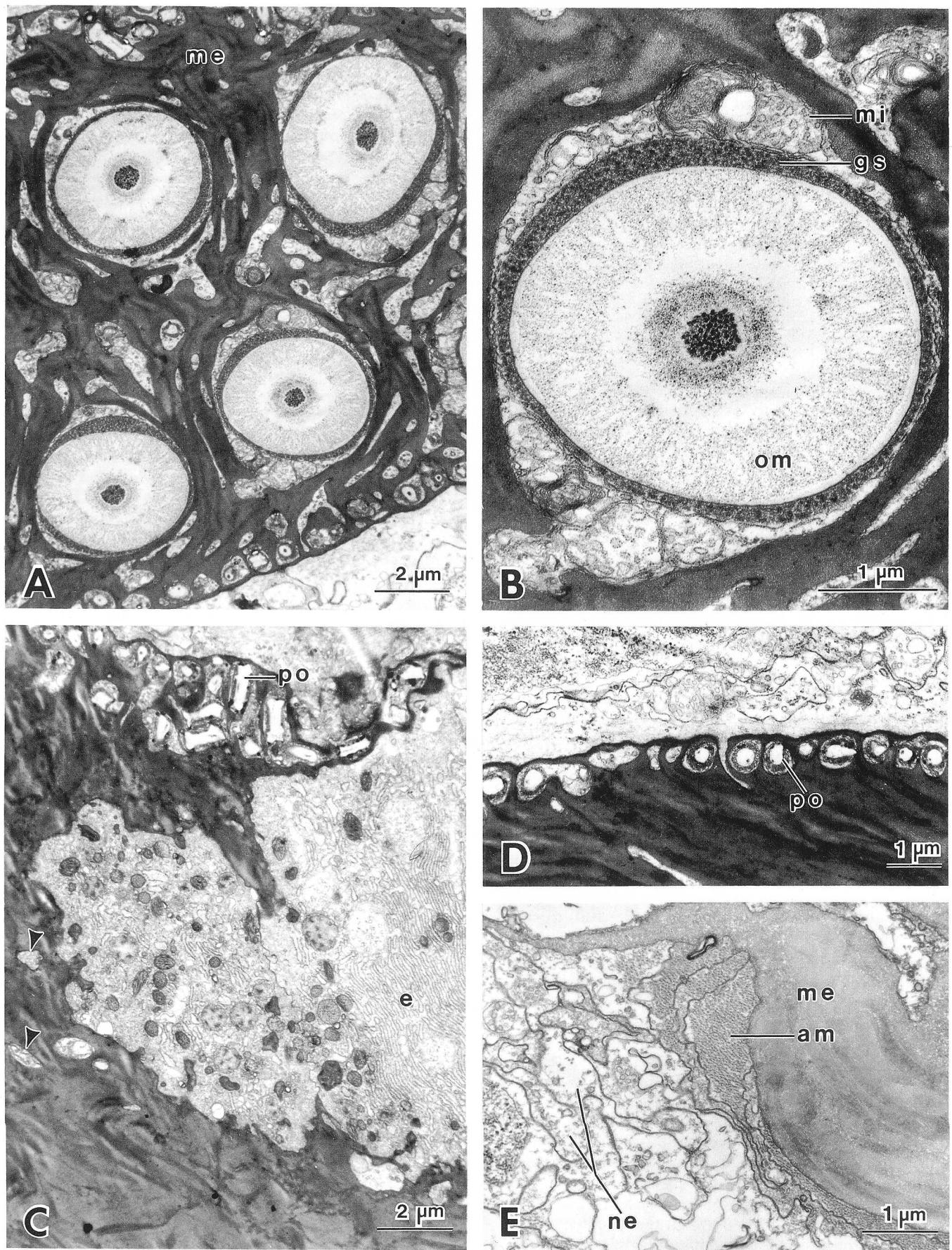


Fig. 6A–E. Electron micrographs of sectioned battery components. **A** Group of four spicules embedded in elastic strand. **B** Spicule enlarged. **C** Interface between descending elastic strand (*on left*) and endoderm (*right*), showing cellular processes entering the strand (*arrowheads*). **D** Interface between ectoderm (*above*) and elastic strand (*below*). **E** Longitudinal muscle of axis and adjacent mesogloea. *am* Axial muscle; *e* endoderm; *gs* granular cytoplasm of cellular sheath around spicule; *me* mesogloea; *mi* mitochondrion; *ne* neurites; *om* organic matrix of spicule; *po* pocket of vacuolated cytoplasm at periphery of elastic strand

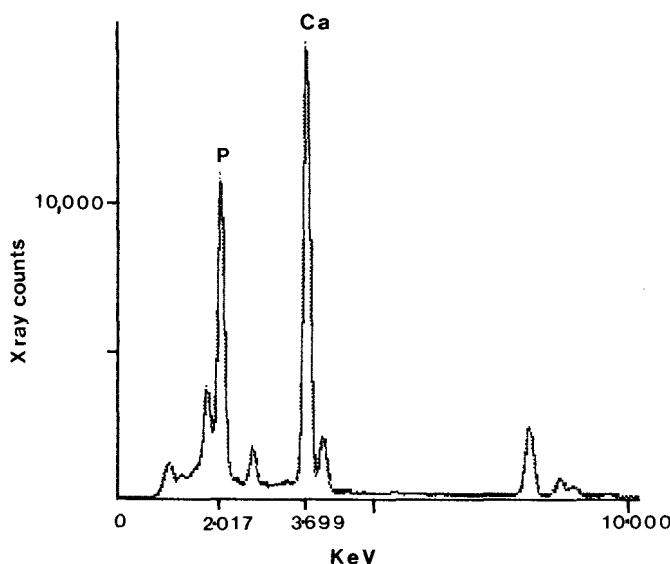


Fig. 7. X-ray energy spectrum in kiloelectronvolts (KeV) of spicule interior, showing phosphorus and calcium peaks

ends of the spicules project freely beyond the surface of the elastic strand. Bedot (1888) saw a central cavity in the spicules, sometimes containing what might be construed as a thread, but he was not convinced that there was any true resemblance to nematocyst structure. His central "thread" may correspond to the central mass of fine filaments seen in our electron micrographs. The only workers to have reported chemical tests on the spicules are Keferstein and Ehlers (1861), who reported that they are alkali-soluble, and Korotneff (1884), who found that they dissolve in weak acids yet (inexplicably) concluded that they contain no calcium salts.

Kölliker (1853) and Leuckart (1854) regarded the elastic strand as a muscular structure, but later workers were unable to verify the presence of muscle tissue. Keferstein and Ehlers (1861) provided accurate drawings showing the arrangement of the spicules in the strand, which they regarded as an elastic structure. Later observers, notably Chun (1891) for Calyphora and Schneider (1899) for Physonectae, agree on the strand's elastic non-muscular nature, and this is in accordance with our own findings. We have therefore adopted the term elastic strand in preference to Totton's (1965) "angleband", which suggests a misunderstanding of the meaning of the original German word. (*Angel* refers to fishing gear, and an *Angelband* would be the "leader" by which the hook is attached to the main part of a fishing line.)

The last major study of the elastic strand and its spicules is that of Schneider (1899). Schneider realized that the elastic strand was a mesogloal structure. Like Claus (1860), who later withdrew this opinion, however (Claus 1878), Schneider regarded the strand as an endodermal derivative. Furthermore, he believed that the endoderm was entirely used up in formation of the strand, which we find to be an error. He correctly understood that the nematocyst battery has a central axis containing longitudinal muscles, and that this structure is distinct from the elastic strand, but he states that the muscles are not innervated, which we find to be incorrect, and his account (not surprisingly in view of the technical limitations of his day) contains a

number of other misinterpretations and questionable points of detail. He observed the spicules, which he likened to boars' teeth (*Eberzähne*). Apart from a brief report of "hook-like attachment structures" by Mackie and Boag (1963), the hooks appear to have received no further mention in the primary literature.

2. Structure, function and chemistry of spicules

Our investigation of the spicules confirms the findings of nineteenth-century workers in several important respects. It is impossible to consider them as nematocyst-derivatives. They do not lie in the ectoderm, but in the mesogloea. They are contained within cellular processes that show none of the characteristic features of cnidoblasts. The spicules possess no true internal filament, cannot discharge and are heavily mineralized. They are evidently neomorphic structures, evolved as part of the complex apparatus of the nematocyst battery, in all probability serving as hooks to secure captured prey.

We have not attempted to explain the mechanism by which the nematocyst battery erupts, and this has never been satisfactorily analysed. It seems possible that hydraulic expansion of the axial canal, accompanied by contraction of the axial muscles (which are arranged asymmetrically), might generate torsional stresses sufficient to rupture the membranes holding the battery together but, even if this is so, it is still unclear why the cnidoband uncoils with such speed and violence.

It is possible to draw certain conclusions regarding the function of the elastic strand and its spicules. There is no reason to suppose that the strand plays an active role in the discharge process, but after discharge, when the cnidoband has adhered to the prey, the elastic strand certainly serves to attach the prey to the tentillum. Composed of collagen, it is a tough resilient structure, which would not readily tear during the struggles of the prey in its attempts to escape, and it probably has sufficient elasticity to provide for a certain amount of "give" in the system. The accounts of earlier authors and our own observations show that the struggling prey may become entangled in the elastic strand. In Physonectae the barbed spicules probably catch in the bristles and other appendages of crustacean prey, strengthening the attachment. The barbs project beyond the surface and would tend to grip such structures. Therefore, even if the prey managed to free itself from the cnidoband by shedding appendages, it would still have to tear itself loose from this long, strong, spiny filament in order to escape.

The elemental analysis carried out in this study indicates that the principal inorganic component of the spicules is some form of calcium phosphate. Calcium phosphate in the form of hydroxyapatite is the major mineral of bone, and similar compounds (apatites) are also present in the shells of certain inarticulate Brachiopoda (Watabe and Pan 1984) and in a few other invertebrates (Pautard 1961), although calcium carbonate is far more commonly found in invertebrates, including Coelenterata. Apatites are harder and more durable than the common calcium carbonate minerals calcite and aragonite (Kemp 1984) and the presence of apatite in the spicules of *Nanomia* presumably endows these structures with the mechanical strength necessary for them to function as attachment hooks. It is clear from the electron micrographs that the spicules contain an organic

matrix, but its nature is at present unknown. Mineralization of vertebrate hard tissues is believed to proceed by the deposition and alignment of hydroxyapatite crystals on collagen fibrils (references in Kemp 1984) and it would not be surprising, therefore, if the matrix protein of the spicules proved to be collagen. An organic matrix is only rarely found in calcium carbonate skeletons (Watabe and Dunkelberger 1979) so the presence of a matrix in these spicules is consistent with other evidence of phosphatic mineralization. The disintegration of the spicules in alkali reported by Keferstein and Ehlers (1861) would presumably be caused by dissolution of the organic component and resulting dispersion of the mineral.

Our observations and those of earlier workers make it clear that the spicules are arranged in a linear developmental sequence along the elastic strand with new ones at one end and mature ones at the other. Functionally, these structures could not act as hooks until they have reached the stage of maturity seen in the descending arm of the elastic strand, with barbs which project beyond the surface. It is hard to account for the seeming overproduction of spicules: there is a long double row of immature "reserve" spicules in the ascending arm, but, as it is inconceivable that the battery could be used more than once, it is not at all clear what purpose is served by maintaining these.

Regardless of the functional significance of the reserve spicules, the series of spicular growth stages spread out along the strand would seem to offer an attractive preparation for study of phosphatic mineralization. Phosphorus is a trace element in seawater, and the animal must somehow be able to concentrate phosphate as well as calcium, and transport it into the mesogloal pockets where the spicules are formed. We have not looked closely for ultrastructural differences in the cytoplasm of the cellular pockets and canals at points along the elastic strand, but such a study might throw an interesting light on how the mineral is deposited in the growing spicules.

Acknowledgements. This study was assisted by operating and equipment grants to G.O. Mackie from the Natural Sciences and Engineering Research Council of Canada. We thank the Canada Council for the award of a Killam Research Fellowship to G.O. Mackie and the University of Victoria for the award of a British Columbia JobTrac Supplementary Scholarship to R.M. Marx. We also wish to thank the director and staff of the Friday Harbor Laboratories, University of Washington, USA, for providing facilities for the collection and study of specimens. We gratefully acknowledge the important contributions made by C.E. Mills and R.J. Skaer at the start of this project. P.R. Pugh kindly sent photocopies of pages from Sars (1846) and Bedot (1888). Finally, we thank H.F. Dietrich, A.R. Fontaine and C.L. Singla for help with the X-ray microanalysis.

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Received November 24, 1987