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Structure and function of the prehensile tentilla of *Euplokamis* (Ctenophora, Cydippida)

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Summary. *Euplokamis* has coiled tentilla on its tentacles, which can be discharged, flicking out at high velocity, when triggered by contact with prey. The tentillum adheres to prey by means of numerous colloblasts. Discharge, which takes 40–60 ms, is accomplished by contraction of striated muscles, found only in this genus among the Ctenophora. Restoration of the coiled state is attributable to passive, elastic components of the mesogloea. Rows of "boxes" (fluid-filled compartments) along the sides of the tentillum appear to stiffen the structure so that it does not collapse, kink or buckle during discharge. Smooth muscle fibres present in the tentillum may help pull the tentillum tight after prey have been captured.

In addition to the rapid discharge response, the tentillum can perform slower, spontaneous, rhythmic movements which, it is suggested, resemble the wriggling of a planktonic worm, enabling the tentillum to function as a lure. These movements appear to be executed by contraction of two sets of myofilament-packed cells which differ in several important respects from conventional smooth muscle. They belong to a novel and distinct cytological subset ("inner-ring cells"), other members of which are packed with microtubules and seem to be involved in secondary structuring of the collagenous component of the mesogloea.

Study of tentilla in different stages of development shows that the striated muscle fibres, originally nucleated, become enucleate as they differentiate and that the colloblasts form in association with accessory cells, as proposed by K.C. Schneider and G. Benwitz. The refractive granules which adhere to the outside of all mature colloblasts derive from these accessory cells. The colloblast nucleus undergoes changes during development suggestive of progressive loss of its role in transcription and protein synthesis, but it remains intact, contrary to statements in the literature.

The tentillum of *Euplokamis* can be regarded as a true food-capturing organ and it is probably the most highly developed organ in the phylum.

A. Introduction

A small cydippid ctenophore of the genus *Euplokamis* (Chun, 1880; Fig. 1A) is found occasionally in surface waters at Friday Harbor, Washington, USA, but is abundant in deeper water below 100 m throughout the Strait of Georgia and in inlets up the coast of British Columbia, where Mackie and Mills (1983) and Mackie (1985), using a submersible, observed it to be the commonest of all the midwater ctenophores, reaching densities of up to 10 per cubic metre. Although it was originally supposed to be a species of *Pleurobrachia,* further study shows that it belongs to Chun's little-known genus *Euplokamis*. A taxonomic description by C.E. Mills establishing it as a new species is now in press. *Euplokamis* is characterized by unique, tightly coiled tentilla, containing striated muscle fibres. As these structures have not been studied since Chun (1880) first described them, and as they are remarkable for their complex histology, we have undertaken a new investigation of them, the results from which are presented here.

B. Material and methods

Specimens of *Euplokamis* were scooped from the surface at the dock at the Friday Harbor Laboratories using small bowls, taking care to minimize disturbance. These ctenophores are extremely fragile and readily disintegrate if subjected to rough treatment, which may help to explain why they have so rarely been recorded in plankton reports based on net sampling. Once captured, however, *Euplokamis* were found to survive in good condition for a week or more if kept in cool (10° C) fresh sea-water in the laboratory. Very few specimens were captured, but they provided sufficient material for preliminary observations on behaviour, for light microscopy of fresh and fixed tissues, and for electron microscopy.

A Gyyr time-lapse video cassette recorder, Model TLC 2051, in conjunction with a Dage 65 monochrome video camera, was used to record discharge of tentilla in isolated tentacles. Fresh tissues were observed by Zeiss-Nomarski differential interference contrast and dark field microscopy. Material for electron microscopy was fixed in 3% glutaraldehyde in 0.4 *M* Millonig's phosphate buffer for 1 h at 20° C, rinsed in the same buffer and osmicated in 1% osmium tetroxide in the same buffer at 4° C for 1 h. After embedding in Epon 812, sections were stained with uranyl acetate and lead citrate.

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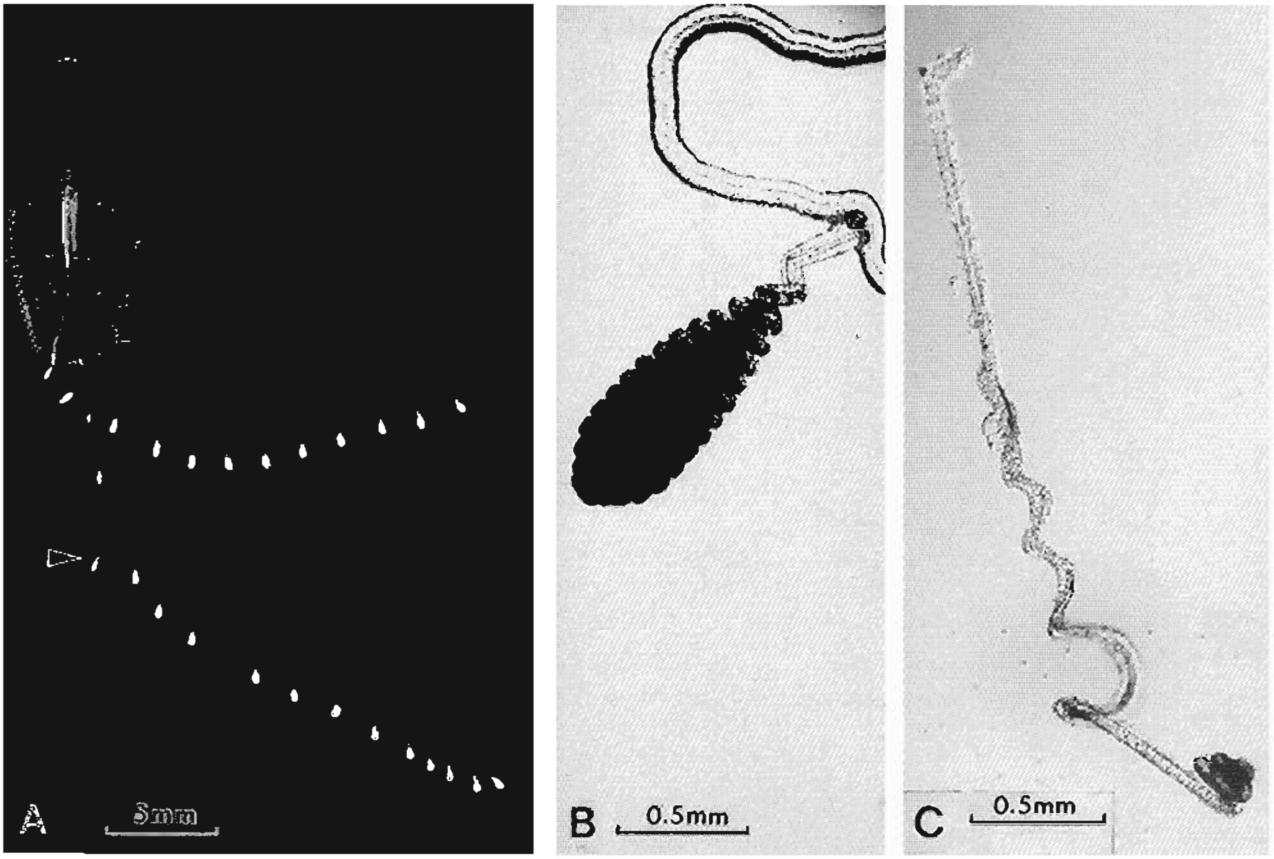

Fig. 1 A–C. Living *Euplokamis*. **A** Whole animal, tentacles extended. *Arrowhead* shows a tentillum which is undergoing slow, spontaneous uncoiling and recoiling. **B** Coiled tentillum attached to tentacle. **C** Detached tentillum partially discharged, adhering to a glass surface

C. Results

I Tentillar activities observed in living specimens

The tentilla are normally kept tightly coiled (Fig. 1A, B) but are capable of actively uncoiling (Fig. 1C). Two types of activity have been observed: slow spontaneous changes in length; and rapid extensions evoked by prey contact or by mechanical or electrical stimulation.

1. Slow spontaneous movements

In freshly caught specimens, the tentilla often perform slow writhing movements, uncoiling and then recoiling (Fig. 1A, *arrowhead*). Not all tentilla perform these movements at the same time and the movements are not synchronized. The same movements can be seen in isolated tentilla under the microscope. Uncoiling and recoiling each take about 2 s and may be performed cyclically with a periodicity of about 60 s. The movements are not blocked by addition of isotonic magnesium chloride to the sea-water to a final concentration of 150 m*M* Mg^{2+}.

2. Rapid extensions

Rapid tentillar extension ("discharge") has been observed in fresh specimens kept in laboratory tanks with prey organisms, and it is clearly effective in food capture. When a tentacle is contacted by a copepod, tentilla at the site of contact are rapidly shot out and adhere to the prey by their colloblasts (Fig. 2). In the case of small copepods (e.g. *Pseudocalanus* sp., 1–2 mm long), the response may be localized to a single tentillum, but larger copepods (e.g. *Neocalanus plumchrus*, 4–5 mm) may discharge several tentilla. Tentilla not touched do not discharge. Captured prey are hauled up toward the mouth by contraction of the whole tentacle. The prey continue to struggle after entrapment, and there is no reason to suspect the involvement of toxic secretions.

Rapid extension can be evoked in isolated tentacles by electrical stimulation (Fig. 1C). Fine coaxial metal electrodes were used to deliver short-duration (2 ms) shocks at just supra-threshold voltage, in the range 5–10 V. In 15 out of 21 trials, when stimuli were applied to the outer surface of the tentillum directly over the longitudinal nerve tract (see Fig. 3) the tentillum responded extremely rapidly, uncoiling and straightening out partially or completely. Stimulating the tentacle near the tentillar stalk may also evoke discharge. Frame-by-frame replay of 14 recordings of rapid discharges made with a video camera showed that the tentillum can extend to its full length of 6 mm from its coiled length of 1 mm in 40–60 ms. If the tentillum has not adhered to some object during discharge, recoiling follows immediately and is completed within about 350 ms. When a tentillum has been discharged electrically there may be some contraction of the tentacle near the tentillar attachment point, but this is usually strictly localized, and contractions do not spread along the tentacle to other tentilla.

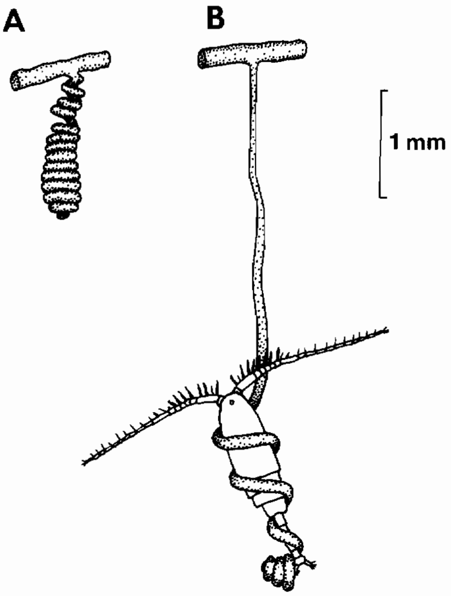

Fig. 2A, B. Drawings of living tentillum **A** at rest and **B** after discharge, adhering to a copepod

In a few cases, discharged tentilla which had adhered only weakly to prey were seen to detach and recoil. It seems likely, therefore, that tentilla can be used more than once, although mechanical damage would probably prevent reuse in cases where a tentillum had adhered strongly to the prey. Repeated use would lead to loss of colloblasts and, as it is doubtful if the latter can be replaced in the mature tentillum, the tentillum would gradually lose its effectiveness. New tentilla, however, are formed as the tentacle grows from its base.

Unlike the slow coiling movements described above, the fast extension response is blocked completely, but reversibly, in Mg^{2+} levels in the range 105–150 mM. Addition of a drop of 0.3 M KCl to a piece of tentacle lying on a glass slide in about 1 ml of sea-water results in the immediate discharge of all tentilla, presumably due to chemical depolarization of the excitatory nerves or striated muscles, or both. This treatment, however, causes obvious epithelial damage, and a tentillum so discharged cannot be recoiled.

All our observations and experiments on the living material support the view that the coiled state of the tentillum is the resting state, and that extension requires activation of powerful, rapidly responding muscles. The existence of slow spontaneous length changes suggests the involvement of a second set of contractile elements. The histological evidence, to be considered next, is in general agreement with this picture. The magnesium results suggest that the fast response is mediated by nerves acting through chemical, neuromuscular synapses, but that the slow response does not necessarily involve such junctions.

II. Histology and ultrastructure

Most specimens of *Euplokamis* have at least ten tentilla per tentacle, of which the one or two nearest the root of the tentacle are immature, as indicated by their smaller size (Fig. 1A), lack of pigmentation and incompletely differentiated cells.

The tentacles themselves, which are about 100 µm in diameter, show structural features similar to those of other cydippids (Hernandez-Nicaise 1973b) and need not be described here, but the prehensile tentilla are unique to *Euplokamis*.

A mature tentillum consists of a narrow (50 µm diameter) proximal portion ("stalk"), which is about 1 mm in length when extended and lacks a covering of colloblasts. The stalk grades into the thicker (75 µm diameter) portion ("shaft"), which is about 5 mm in length, and is covered with colloblasts over its entire surface. The shaft contains striated muscle and is the part which extends actively during prey capture. The main emphasis in this account will be on the structure of the mature tentillum, but some information will also be provided on immature tentilla, as these show important stages in cellular differentiation relevant to an understanding of the mature condition of the tissues.

1. Shaft of mature tentillum

a) General description. In a coiled tentillum seen in cross section (Fig. 3), the core consists of a mass of mesogloea, with collagen built up into fibre bundles of various thicknesses and orientations, most conspicuously into a transverse row of thick, longitudinally oriented bundles. This is the "ligamentous band" observed by Chun (1880), which will be referred to here as the "transverse collagen lamella". On one side of this lamella (the outward-facing side of the coiled structure) lie two symmetrically arranged bundles of longitudinally oriented striated muscle fibres, and on the opposite (inward-lying) side lie two symmetrical bundles of closely packed smooth muscle fibres which together form a sort of "keel" to the whole structure. Both these muscle systems are figured and described by Chun (1880). Internally to the striated and smooth muscles lie another group of cells to be termed "inner-ring cells" (numbered 1–18 in Fig. 3). Unlike the muscle cells they are not grouped into compact bundles, but lie well apart from one another, separated by mesogloea, and form a continuous chain or ring around the periphery of the mesogloea. Some of these are associated with peculiar mesogloeal structures to be termed "boxes". Chun (1880) interpreted the boxes as the outer edges of the collagen lamella, but this is incorrect. The boxes are extracellular compartments formed within the mesogloea and have no connection with the transverse collagen lamella.

All the cells described above (smooth and striated muscles, inner-ring cells) are enclosed within the mesogloea and can therefore be classified as mesogloeal cells. The cells forming the outer covering of the tentillum can be termed epidermal, as they lie outside the mesogloea. The epidermal cells lie upon a thin basal matrix (Fig. 3), which merges with the mesogloea. Colloblasts are the most conspicuous feature of the epidermis. Where they lie over muscles their roots insert between the muscle fibres, as noted by Chun (1880). Epidermal supporting cells fill in the spaces between the colloblasts. At least two sorts of gland cells and two sorts of neurosensory cells are also present in the epidermis. The tentillum is richly innervated, with many neurites running in the epidermis and a large nerve bundle running longitudinally in the groove formed between the striated muscle bundles. A smaller bundle, or sometimes a single axon, lies at the opposite pole, beneath the smooth muscle keel. Nerves do not penetrate the mesogloea.

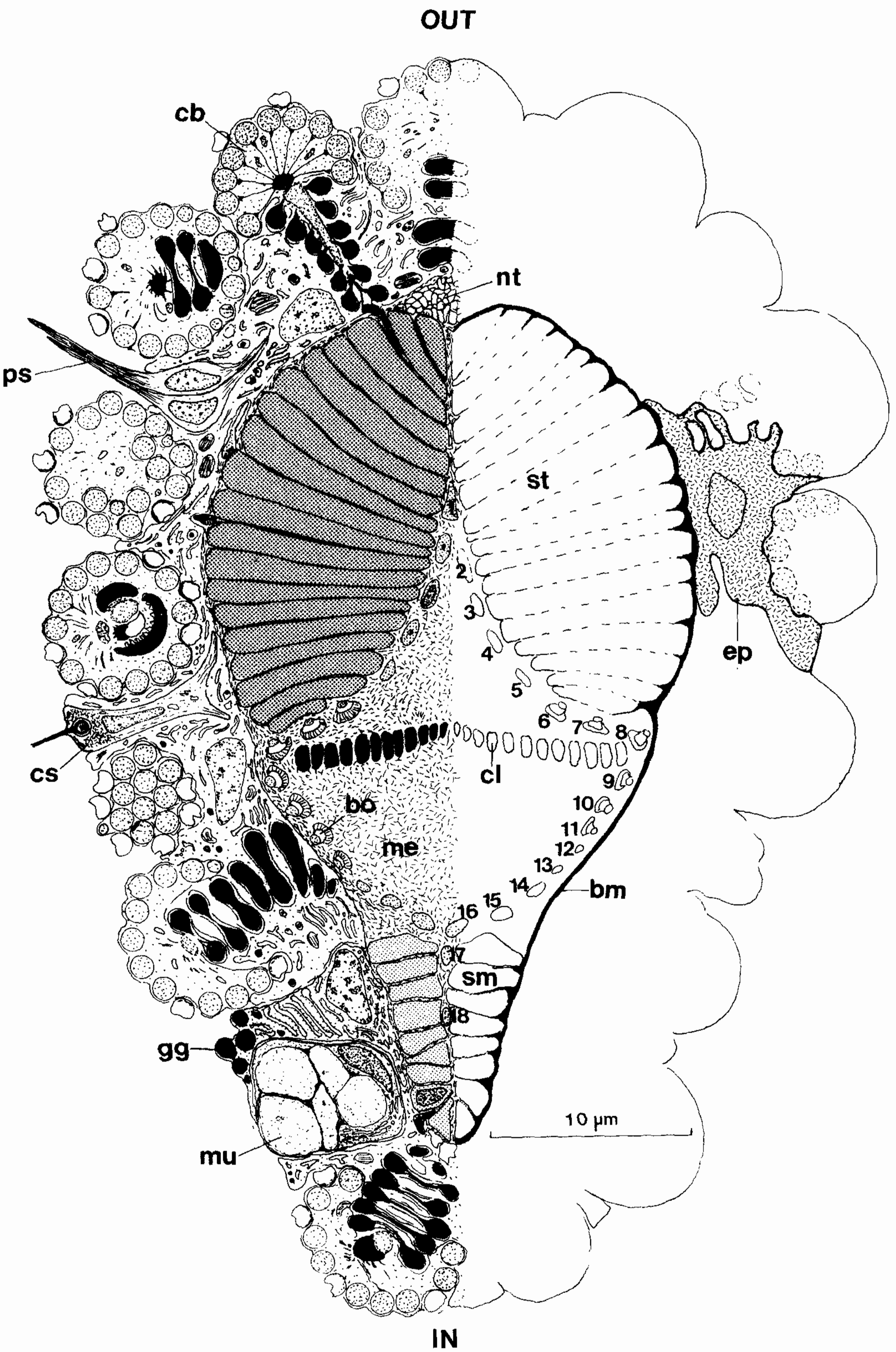

Fig. 3. Cross-section through a tentillum fixed in relaxed (coiled) state. *OUT* refers to the outside of the coil, *IN* to the inside. *bm* basal matrix; *bo* a mesogloeal box; *cb* a colloblast; *cl* a collagen bundle in the transverse collagen lamella; *cs* a ciliated sensory cell whose cilium has been cut short; *ep* an epidermal supporting cell; *gg* a granular gland cell; *me* the mesogloea; *mu* a mucus cell; *nt* neurites composing longitudinal nerve tract; *ps* a peg sensory cell; *sm* a smooth muscle fibre in the keel; *st* a striated muscle fibre (*st*). The outlines of the epidermal supporting cells are omitted in the *left half* of the picture to avoid complicating the drawing, but a single epidermal cell is shown in outline on the *right*. Basal matrix demarcating epidermal-mesogloeal boundary is shown diagrammatically on the right as a *solid black line*. Numbers *1–18* show inner-ring cells, of which *1–4* would typically be myofilament cells, *5* a transitional cell, *6–11* microtubule cells associated with boxes, *12–15* microtubule cells not associated with boxes, *16* a transitional cell, and *17–18* myofilament cells

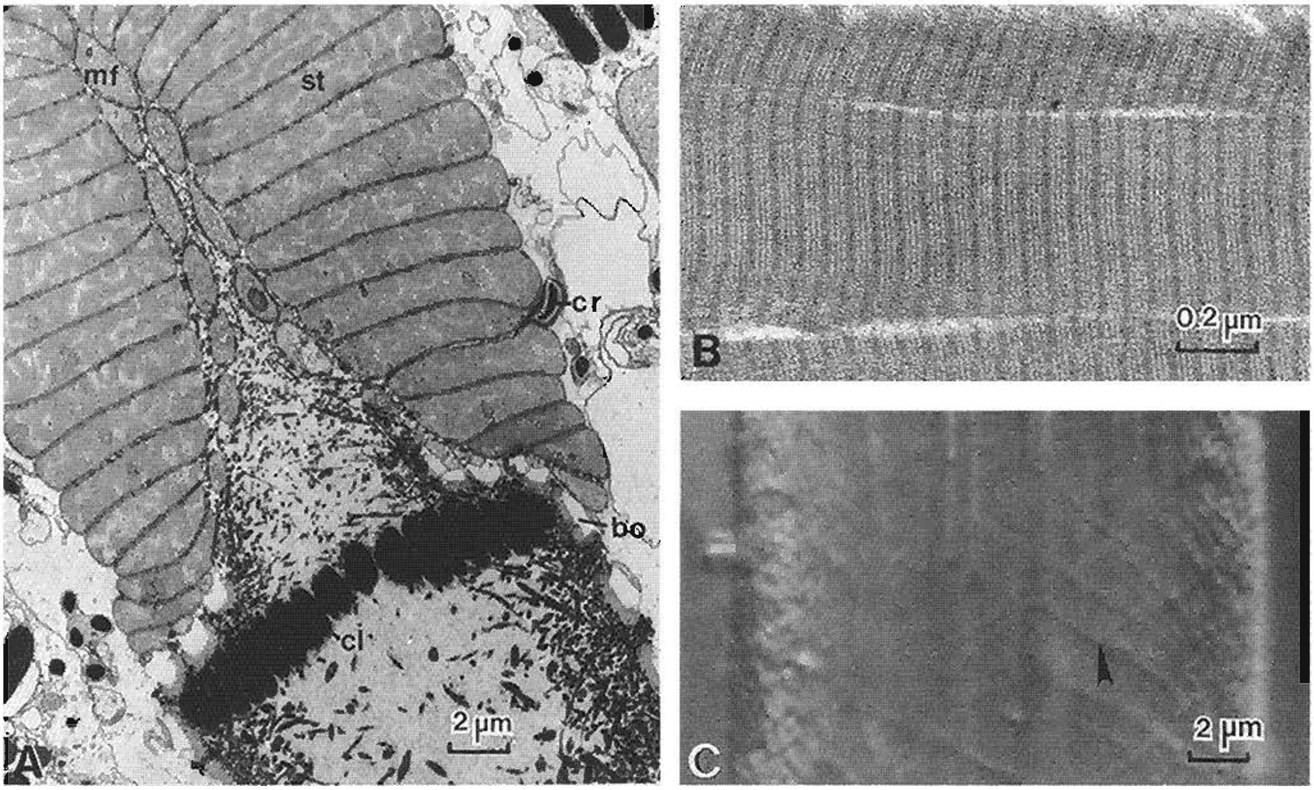

Fig. 4 A–C. Mesogloeal formations. A Cross section as in Fig. 3, showing boxes (*bo*), thick, longitudinally oriented collagen bundles of transverse lamella (*cl*) and smaller collagen bundles running in other orientations in the mesogloea. Other labels show a colloblast rootlet (*cr*), a myofilament cell (*mf*), and a striated muscle fibre (*st*). **B** Longitudinal section of collagen bundle from transverse lamella. **C** Macerated tentillum, after dissolution of all cells, showing criss-cross collagen fibres (*arrow* shows an intersection in the system) and in the background, running vertically, thick collagen bundles of transverse lamella

b) Mesogloea. The most striking component of the mesogloea is the set of about 20 enormous (<2.0 µm diameter) collagen bundles forming the transverse collagen lamella, which runs down the whole length of the tentillar shaft, separating the striated muscle pole from the inner (keel) pole (Fig. 4A, C). Smaller, but still substantial, collagen fibres run elsewhere in the mesogloea, especially immediately beneath the striated muscles and adjacent to the lateral epidermis. These fibres grade down into a fine fibrous meshwork, which forms the base of the main mesogloeal mass and of the basal matrix. Most of the collagen fibres appear to have a random orientation when seen in sectioned material, but light micrographs of intact tentilla macerated in distilled water to remove cells show a system of obliquely arranged fibres forming a criss-cross mesh near the mesogloeal surface (Fig. 4C).

All the larger fibres show a clear and regular 52-nm banding pattern (Figs. 4B, 5A). It is most unusual to see striations in native ctenophore collagen, although Franc (1985) has succeeded in demonstrating the typical striation pattern in reconstituted collagen obtained from extracts of *Beroe* (Fig. 5B). Franc's study shows that proteoglycans are also present in ctenophore mesogloea, and it can be assumed that these are present in *Euplokamis*. With regard to biosynthesis, Franc's labelling studies show that the epidermis is the main site of synthesis of mesogloeal macromolecules. The epidermis of *Euplokamis* contains supporting cells having a rich rough endoplasmic reticulum (Figs. 6D, 13B). These cells abut upon the mesogloea and probably synthesize the collagen. As with other ctenophores there is no separate class of fibroblast-like cells in *Euplokamis* and the muscle fibres and inner-ring cells contain very little rough endoplasmic reticulum and are unlikely to be responsible for collagen secretion.

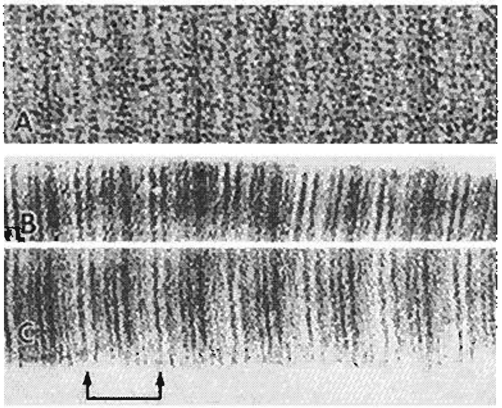

Fig. 5 A–C. Collagen striations. **A** Electron micrograph of a thin section of epon-embedded native collagen from the mesogloea of *Euplokamis*. **B, C** Extracted, purified and reconsitituted collagen fibres from another ctenophore (*Beroe*) and calf skin respectively (from Franc et al. 1976; also Franc 1985) for comparison with **A**. *Scale* showing fundamental periodicity represents 52 nm, 53 nm and 64 nm in **A, B** and **C** respectively

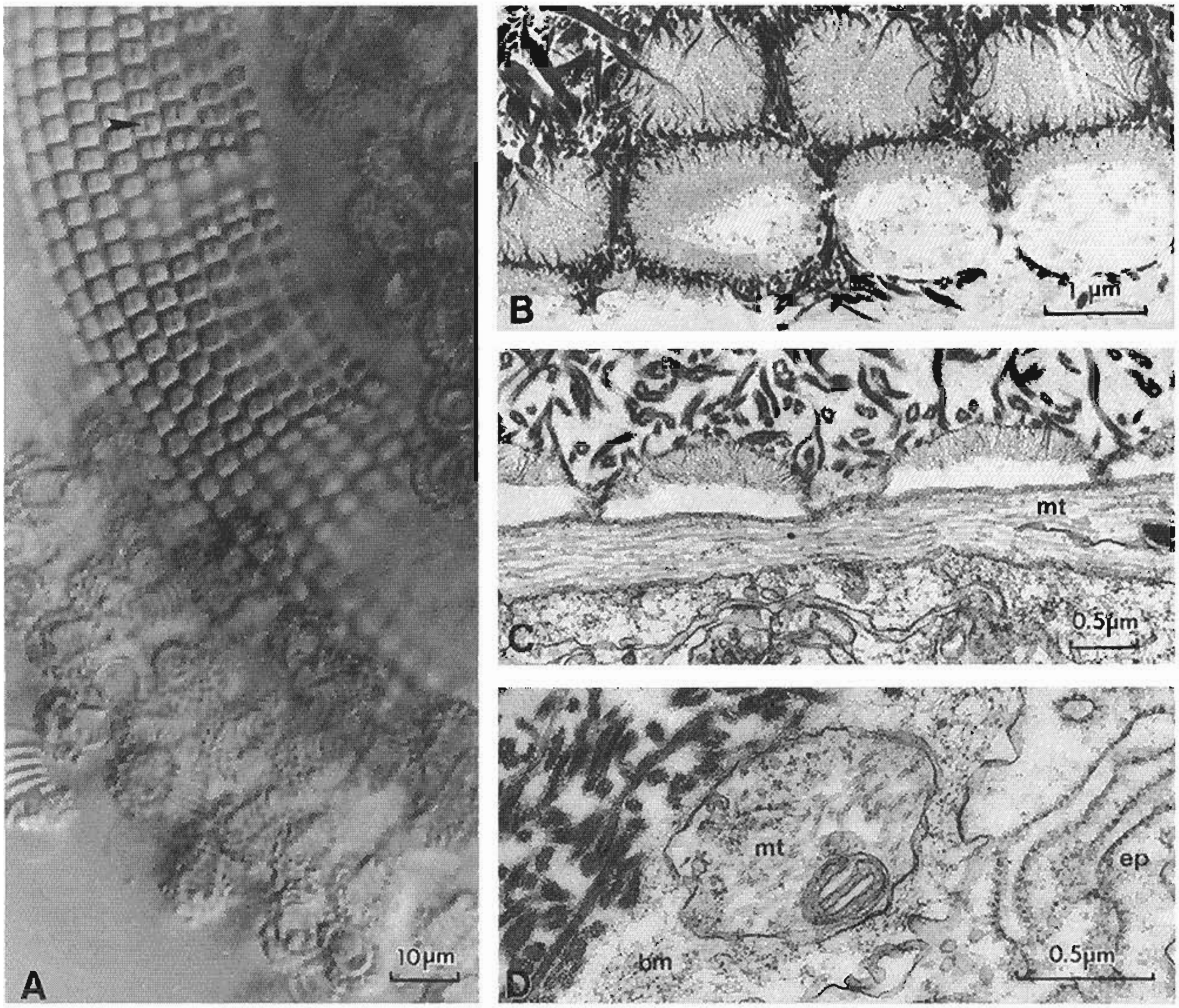

Fig. 6A–D. Boxes and microtubule cells. **A** Light micrograph of whole squashed tentillum showing box rows and colloblasts. *Arrowhead* shows a microtubule cell. **B** Electron micrograph of section cut tangentially to surface, showing boxes. **C** Longitudinal section through a box row, showing microtubule cell (*mt*) running under boxes, as in location 10 (Fig. 3). **D** Cross section of a boxless microtubule cell (*mt*) in location 13 (Fig. 3). Note rough endoplasmic reticulum and vesicles in cytoplasm of epidermal cell (*ep*) on right, and epithelial basement membrane (*bm*)

A unique and striking component of the mesogloea are the lateral rows of "boxes". These structures are readily visible in intact living tentilla as rows of compartments lying along the sides of the tentillum (Fig. 6A, B, C). In the thicker part of the shaft there may be as many as nine rows of boxes on each side, but the number decreases toward the tip as some of the rows peter out.

Seen in cross section, about three box rows lie in the outer half of the tentillum under the striated muscle. The remainder lie around the sides. Each box is a roughly rectangular space (Fig. 6A, B). Under the electron microscope it is seen to be a completely extracellular structure formed within the mesogloea. Its inner wall is composed of fine collagen fibres projecting in towards the box cavity (Fig. 6C). Thicker collagen bundles run under and between the boxes. The fine fibres of the inner wall of the box are formed from the ramifying terminations of some of these thicker fibres. The central cavity of the box usually appears as empty space, but sometimes flocculent material may be seen in it. In life the cavity is presumably filled with fluid.

Boxes are invariably associated with microtubule (*mt*) cells (see below, p. 327). A long mt cell runs along the outer face of each box row (Fig. 6A, C). Its membrane does not extend into or around the box. There are no ultrastructural indications of secretion or transfer of materials from mt cells to boxes.

c) Striated muscle fibres. There are about 20 fibres on each side of the tentillum, each probably a single very long cell; some of them may run the full length of the shaft of the tentillum. The striation pattern is visible under the light microscope (Fig. 7B). Nuclei are absent in the mature tissue, though present in immature stages (see below p. 330, Fig. 15A). Dense masses of material enclosed in membranes are occasionally seen in the mature fibres in the places where nuclei "should" occur, and these probably represent

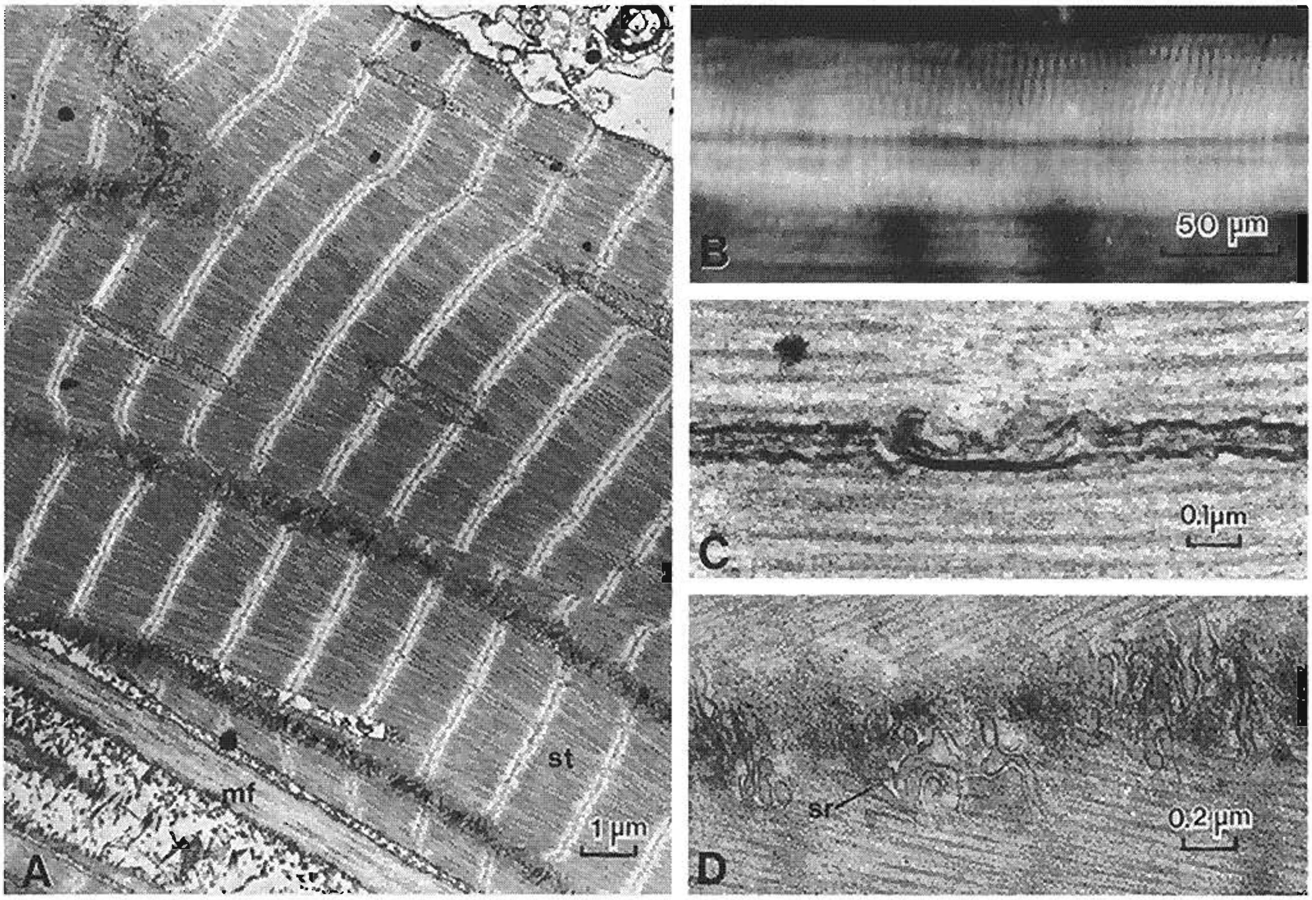

Fig. 7A–D. Striated muscle: general. **A** Longitudinal section of striated muscle fibres (*st*), also showing a myofilament cell (*mf*). **B** Light micrograph of living muscle (dark-field microscopy). **C** Gap junction between striated myocytes. **D** Section cut tangentially to cell surface showing sub-sarcolemmal smooth endoplasmic reticulum (*sr*)

pyknotic relics of nuclei. Each fibre is separated from its neighbour by a thin partition of material similar to that forming the basal matrix. Colloblast roots may extend into this material, but processes of other cells do not. Neuromuscular junctions are observed only at the outer ends of the muscle cells. Gap junctions have been observed between adjacent muscle fibres (Fig. 7C), but these are so rare that they cannot be assumed to play a significant role in cell-to-cell impulse propagation. Each muscle fibre is probably individually innervated. Apart from a few long narrow mitochondria (Fig. 7A), a few microtubules (Fig. 8A), some sarcoplasmic reticulum (Fig. 8A) and some granular material located in central pockets, the interior of the fibre consists entirely of myofilaments. A second tubular system, consisting of many fine ramifying anastamosing tubules lies just underneath the sarcolemma (Fig. 8D). This peripheral portion of the sarcoplasmic reticulum does not appear to be connected directly to the sarcolemma. It is not clear if the peripheral and central sarcotubular networks are at any point interconnected. There is no sign of a transverse tubular system, and the vacuolar systems seen show no regular organization in relation to the striations.

Striations are typically aligned in register across many fibres in both relaxed and contracted muscles, indicating that the fibres normally contract together as a unit. All the fibres in the bundle have similar sarcomere lengths and other cytoplasmic features. The ultrastructural appearance of the sarcomeres and measurements of sarcomere lengths in coiled and extended tentilla show that the muscle is relaxed when the tentillum is coiled, and that straightening (extension) involves active contraction of the muscles. Sarcomere lengths are greater in the coiled than in the straightened state (Fig. 8B, C), but the evidence here is not as tidy as might be wished, and calls for special comment.

Sarcomere length as seen in living tentilla in the relaxed (coiled) state is 1.4 µm (Fig. 7B). In muscle fixed in this state the sarcomeres would be expected to show the same separation, and should display distinct A, I and H bands. However, sarcomere length in the material fixed while relaxed (Fig. 8B) never exceeds 1.1 µm, and no H bands are visible. Thus, the appearance is one of partial contraction. In muscle fixed immediately after treatment with KCl, which causes strong muscle contraction and consequent straightening of the tentillum, sarcomere length is typically about 0.6 µm and the thick filaments come right up to the Z lines, eliminating the I band. They may even interdigitate across the Z line (Fig. 8C), a condition observed in some crustacean and insect striated muscles during strong contractions (Hoyle 1983)

In evaluating measurements made on fixed material, we have to consider (a) that the material shrinks during processing for electron microscopy and (b) some active contraction may occur as a result of the excitation caused by the shock of fixation. If we assume 15% shrinkage during pro-

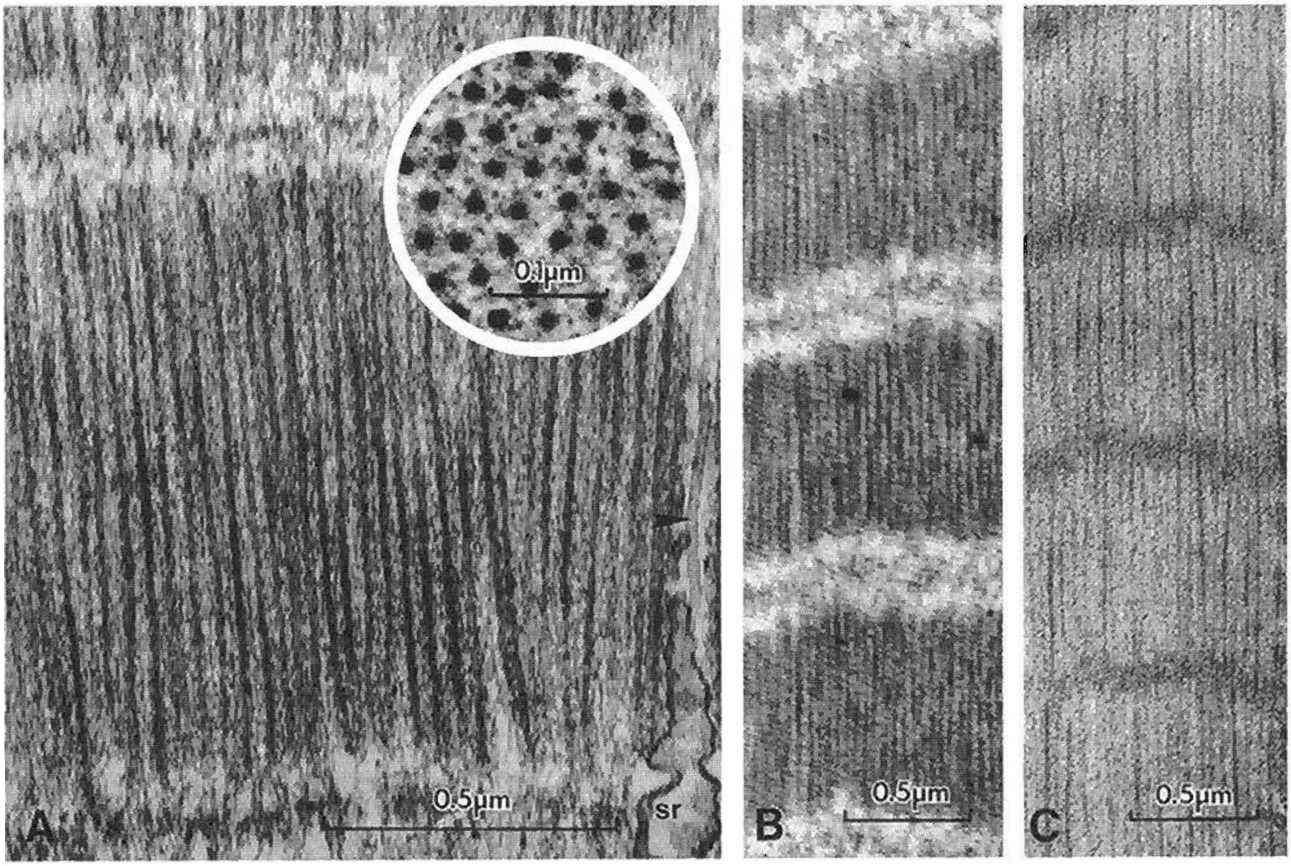

Fig. 8A–C. Striated muscle: details of myofilaments. **A** Longitudinal section showing thick and thin myofilaments, also a microtubule (*arrowhead*) and axial smooth endoplasmic reticulum (*sr*). *Inset* shows cross-section of myofilament array. **B, C** Relaxed and contracted muscle fibres, shown for comparison

cessing (which seems reasonable judged by observations on colloblasts), the sarcomere length in full contraction but without shrinkage would be about 0.7 µm, which would be equivalent to 50% of the relaxed length measured in vivo, a value typical of many sorts of striated muscle (Hoyle 1983). Application of this 15% shrinkage factor to the extended sarcomere length of 1.4 µm measured in living tissue gives 1.2 µm, close to the 1.1 µm actually observed in the fixed tissue. The slight difference (0.1 µm) would presumably represent active contraction caused by the shock of fixation, which would explain the lack of an H band. The fibres would have shortened actively by only 7%, not enough to cause the tentillum to uncoil appreciably.

In cross sections of striated muscle fibres, both thick and thin filaments can be readily observed. There is only one class of thick filament. These units are ca 15 nm in diameter, like myosin filaments in other animals where paramyosin is absent (Hoyle 1983). They are arranged in a regular array about 40 nm apart, each thick filament being surrounded by about six thin filaments, as nearly as can be judged (Fig. 8A, insert). The thin-to-thick packing ratio would therefore be about 2:1, a ratio characteristic of many invertebrate and all vertebrate striated muscles (Hoyle 1983).

We have, then, a picture of a very simple, functionally unitary muscle, enucleate in the mature state and with none of the ultrastructural correlates of intense metabolic activity. The shortness of the sarcomere length suggests that contraction velocity is high. Presumably these muscles are used rarely, possibly only once, and do not have to contract repetitively or in a sustained way, but when they do contract, they do so very rapidly.

d) Smooth muscle. As noted above, two lateral blocks of smooth muscle together form a kind of "keel" along the inner side of the tentillum (Fig. 3). The two blocks are closely apposed along the midline. Each block consists of about light eight fibres arranged in a column. The cells show the same ultrastructural features as smooth muscle fibres in the tentacles of this and other cydippid species (Hernandez-Nicaise 1973b) and in *Beroe* (Hernandez-Nicaise, Bilbaut, Malaval and Nicaise 1982). They contain both thick (ca. 15 nm) and thin (ca. 5 nm) filaments, with the thick filaments 80–100 nm apart, though not arrayed as regularly as in the striated muscle (Fig. 9A, B). Neuromuscular junctions have been observed on some of the smooth muscle cells of the keel (Fig. 13C), and the whole system is probably innervated, though rather sparsely. Gap junctions are absent.

e) Inner-ring cells. These are the deepest lying cells in the tentillum and form a continuous series, lying under the muscles and around the sides between the two poles (Fig. 3). Like the striated and smooth muscle fibres and nerve tracts, they are elongated parallel to the long axis of the tentillum. Those inner-ring cells lying close to the primary muscles show ultrastructural specializations of smooth muscle, in contrast to those arranged around the sides, which are filled

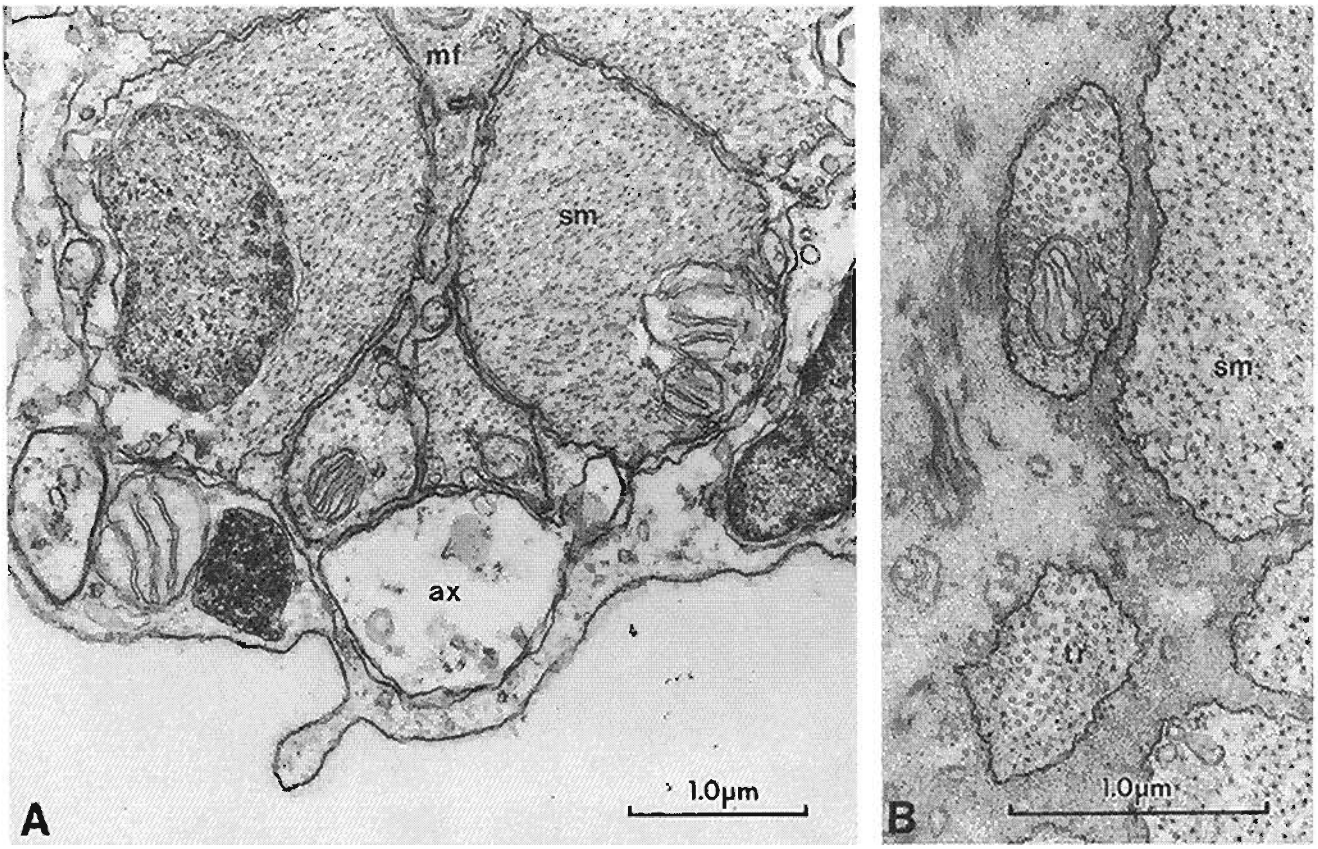

Fig. 9A, B. Smooth muscle of keel and associated inner-ring cells. **A** Smooth muscle fibres (*sm*) and myofilamentous inner-ring cell (*mf*) from bottom of keel, also showing an axon (*ax*). **B** Inner-ring cells showing transitional structure (*tr*) from location 16 (Fig. 3). Note mixture of microtubules and myofilaments in the cytoplasm

with microtubules. The two types will be referred to as myofibrillar (*mf*) and microtubular (*mt*) inner-ring cells respectively. In a typical cross section through the shaft of the tentillum, there would be about 18 inner-ring cells on each side of which about six would be myofibrillar, ten microtubular and two transitional between the two types (Fig. 3).

Mf cells are long tubular cells containing a mass of fine myofilaments and some thick filaments. They are recognizable as a distinct population from the keel smooth muscle cells, by virtue of their smaller size, isolation from one another, more rounded contours and paucity of thick myofilaments. They contain only about half as many thick filaments as the keel muscle cells, and these filaments are not arranged in an orderly array. They are found at the two muscle poles, where they lie closely beside or sandwiched between smooth (Fig. 9A) or striated (Fig. 10A) muscle bundles.

Mt cells (Figs. 6D, 10C) resemble mf cells in their elongated form and in some cases they show a partitioning of the cytoplasm into a central core containing the nucleus, mitochondria, endoplasmic reticulum granules, etc., surrounded by a mass of microtubules (Fig 10C) rather like the mass of myofilaments that surrounds the cytoplasmic core in mf cells. In the regions where mesogloeal boxes are formed, mt cells run along the outer sides of the box rows as noted earlier (Fig. 6C). Of the total mt cell population, 6–16 would be associated with box rows while the others would lie freely in the mesogloea (Fig. 6D).

Inner-ring cells transitional between mt cells and mf cells are found close to the keel smooth muscle cells (Fig. 9B) and under the striated muscle bundles near the point where the muscle lies closest to the collagen lamella (Fig. 10B). These cells show a mixed population of myofilaments and microtubules.

Though they lack an abundant rough endoplasmic reticulum, Golgi component and other features expected of a collagen-secreting cell, it seem likely that mt cells are in some way concerned with organizing the extracellular matrix. Their association with boxes is strongly suggestive of such a role. Also, where they lie freely in the mesogloea they are often encircled by what appear to be developing collagen bundles (Fig. 14C).

f) Nerve cells. Neurite profiles are seen in EM sections throughout the epidermis, but nerves do not enter the mesogloea of the tentillum, contrary to the situation in the tentacles (Hernandez-Nicaise 1968, 1973a, b). They do form synapses of the typical ctenophore type (Hernandez-Nicaise 1973c) with striated myocytes (Fig. 11B), with the smooth muscle cells of the keel (Fig. 13C), with epidermal gland cells and with each other, but not with the inner-ring cells. In the main portion of the tentillum they aggregate to form a substantial longitudinal nerve bundle running along on the outer exposed side of the tentillar coil, next to the striated muscle mass (Fig. 11A). Here as many as 50 neurite profiles may be seen. The larger of these units have diameters in the range 2.0–3.0 µm, but most are about

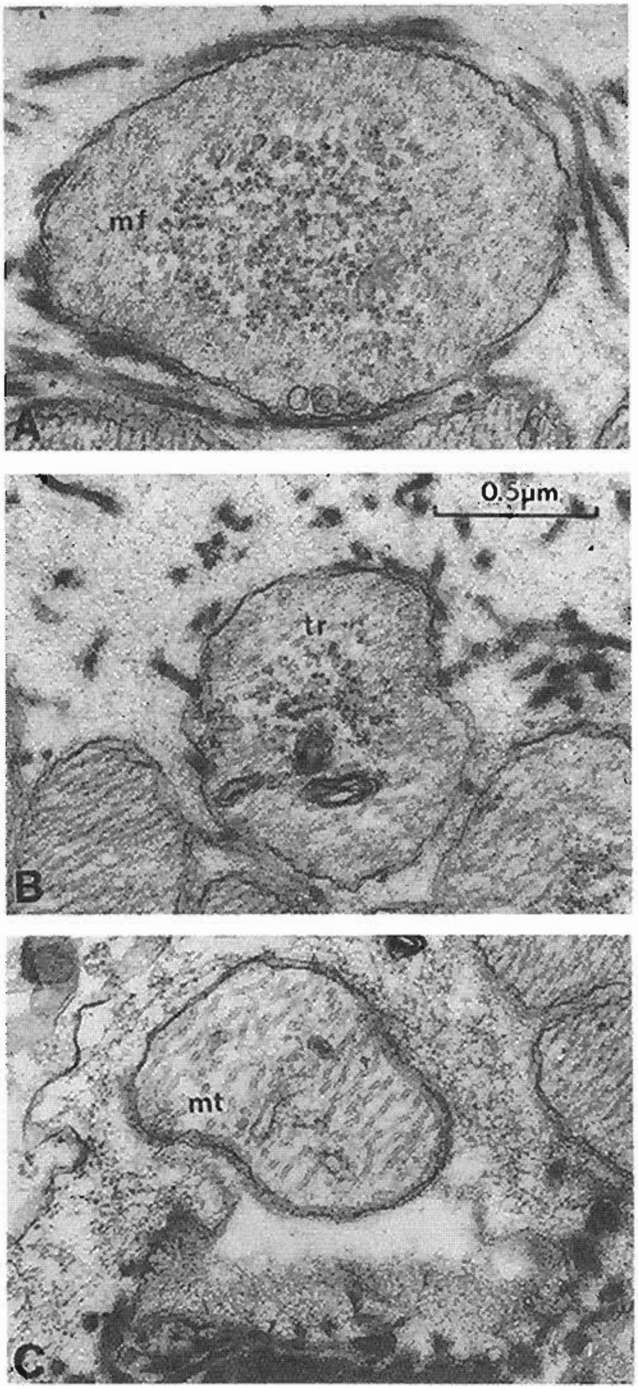

Fig. 10A–C. Inner-ring cells of three types from the same section. **A** Myofilamentous cell (*mf*) from location 3 (Fig. 3). **B** Transitional cell (*tr*), location 5. **C** Box-associated microtubule cell (*mt*), location 7. In **A** and **B** the myofilaments are concentrated in the periphery of the cell, with granular cytoplasm filling the core

1.0 µm or less. It seems likely that the larger neurites (like giant axons in other groups) are responsible for rapid conduction of excitatory signals coordinating the fast discharge response. They run all the way along the tentillum, and make synapses with smaller processes which presumably distribute excitation to the striated muscles and other effectors. Visible in most sections is what appears to be a smaller nerve bundle, sometimes represented by a single axon (Fig. 9A), running under the smooth muscle keel

g) Sensory cells. Sensory cells of two types well known in ctenophores (reviewed by Tamm 1982) are found in the epidermis. These are the ciliated sensory cells and the fibrillar peg cells. Horridge (1965) retrieved and summarized the essential information provided by nineteenth-century workers on these structures, and put it into context with his own findings made with optical and electron microscopy.

The ciliated sensory cells (Fig. 11C, D), which are probably vibroreceptors (Horridge 1965), have processes 300 nm thick and 50 µm long and show a conventional 9 + 2 pattern of microtubules in cross section. Observed in fresh material at 40 × magnification, they were never seen to beat actively. Remarkable structural specializations (onion-like spheroidal basal body, ring of apical dense bodies and vacuoles, smooth endoplasmic reticular complex, etc.) occur at the base of the cilium, as described for other species (Horridge 1965; Hernandez-Nicaise 1974a, b). The basal bodies of *Euplokamis* are exceptionally large, <0.9 µm diameter, as against 0.5 µm for three species studied by Hernandez-Nicaise (1974b), and the striations of the core structure show a very bold 60-nm periodicity compared with the rather faint 20-nm periodicity seen in other cases.

The peg cells in *Euplokamis* are small compared with similar structures in some species (Hernandez-Nicaise 1974b), and have a short stiff process, probably never exceeding 20 µm in length, about 600 nm in diameter at the base, tapering to a narrow tip and packed with microfilaments (Fig. 11E). Numerous microtubules are also present, particularly near the base of the process, but they show no orderly pattern of the type seen in conventional ciliated cells. The cells were termed "hoplocysts" by von Lendenfeld (1885), who thought they might prick and wound prey, a suggestion echoed by several later workers, including Hernandez-Nicaise (1974a, b), who, however, argues for a primary sensory function.

Both sorts of sensory cell may occur either singly or in clusters. They are joined by tight junctions to neighbouring cells, including other sensory cells where present. They have basal neurites which mingle with subepithelial nervous components, making synapses with them. They also make synapses with granular gland cells in the epithelium, as noted by Hernandez-Nicaise (1974a, b) in other species.

h) Colloblasts. Colloblasts dominate the epidermis. Strictly speaking they are not purely epidermal, as their roots run through the basal matrix and penetrate deeply in between the muscle cells. They resemble the colloblasts of other ctenophores in most essential respects although their spiral filaments show up to 11 coils, and unusually large number. There are still several uncertainties and contradictions in the literature on colloblast structure and development, some of which we can resolve by comparing the colloblasts in mature and immature tentilla, and this evidence will be presented below (Sect. 3).

i) Epidermal supporting cells. These fill in the spaces between colloblasts and other epidermal cells, but they do not form a continuous covering on the outside, except in immature tentilla. As "packing" elements (Schneider 1902: "Füllzellen") they have very irregular shapes (Fig. 3). In most places they have numerous large mitochondria and an abundant rough endoplasmic reticulum (Figs. 6D, 13B). Scattered secretory granules and phagosomes are often

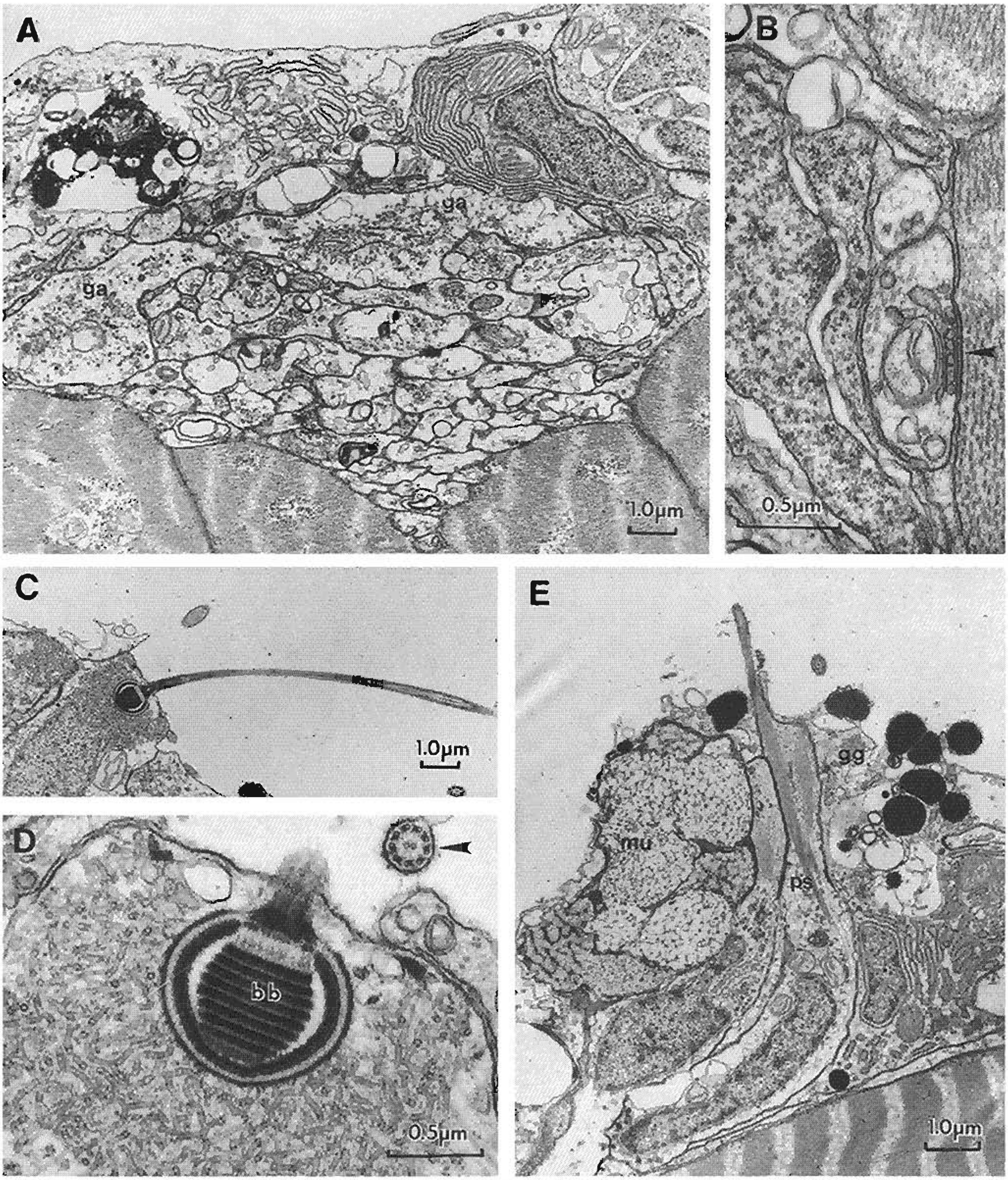

Fig. 11 A–E. Nerves and sensory cells. **A** Cross-section of outer longitudinal nerve tract showing many small axons and a few larger "giant" axons (*ga*). **B** Neuromuscular synapse. *Arrowhead* shows synaptic cleft. **C** Ciliated sensory cell. **D** Basal body (*bb*) of ciliated sensory cell, and cross-section through a sensory cilium (*arrowhead*). **E** A pair of peg sensory cells (*ps*), their shafts partially enveloped by processes of a granular gland cell (*gg*). Also shown is a mucus cell (*mu*)

present. Without an investigation using autoradiography to trace the destination of the secretion product it would be hard to be sure, but there is a strong possibility that these cells secrete the collagen of the mesogloea. They surround the mesogloea on all sides, and membrane-enveloped vesicles are often seen along with concentrations of rough endoplasmic reticulum close to the mesogloea.

j) Granular gland cells. These cells correspond to the "cellules glandulaires sphéruleuses" of French writers, and

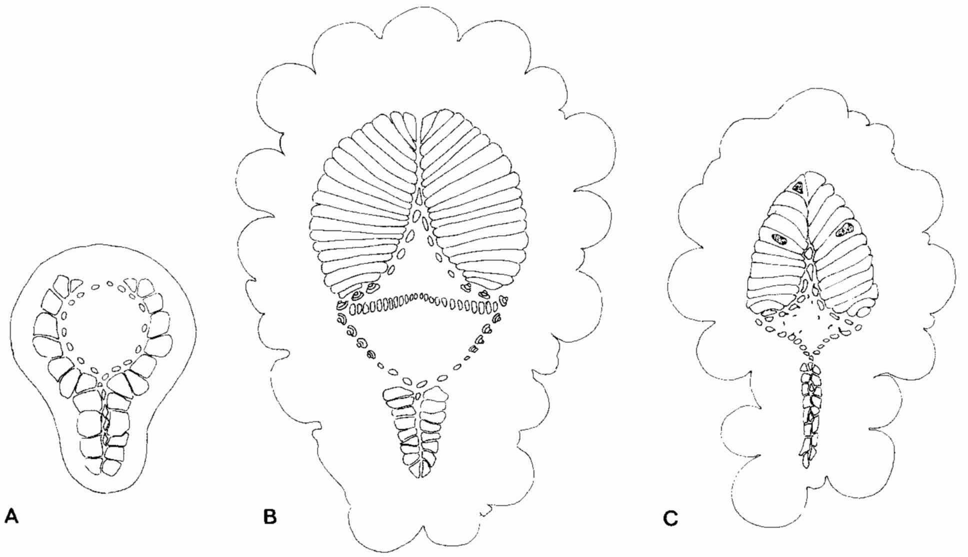

Fig. 12 A–C. Outline drawings to the same scale of cross sections through **A** the stalk and **B** the shaft of a mature tentillum, and **C** the shaft of an immature tentillum, to illustrate differences discussed in the text

should probably not be referred to as "mucus cells" (as, for example, in Hernandez-Nicaise 1974a; Tamm 1982), a term better reserved for the spumous type of gland cell (see below, *k*). They resemble epidermal supporting cells in their irregular outlines and indeed appear to fulfil the same sheathing or packing functions but with a special relationship to sensory cells. Both sorts of sensory cell are typically associated with these granular gland cells, which often form sheaths around the shafts of the sensory processes, particularly in the case of peg cells (Fig. 11 E). They may be innervated by processes of sensory cells or by other neurites. The granules are clustered at the free end of the cell, while RER and Golgi components occupy the basal portions. The granules are strongly electron dense and homogeneous and measure 0.8–1.2 µm in diameter. Hernandez-Ni-caise (1974a, b) proposes that the secretion product is a venom used to kill or paralyse prey after the latter have been pierced by peg-cell processes, which act like stings.

k) Mucus cells (Figs. 11 E, 13 A). These cells ("Schleimzellen, cellules glandulaires spumeuses") figure in numerous previous accounts of ctenophore histology (e.g. Fig. 16 B, lower right). Dispersed throughout the epidermis they have a compact form, which distinguishes them from the granular gland cells, and they contain so much secretory material that their nuclei are usually squeezed over to one side. The secretory granules are much larger (1.5–5.0 µm) than those found in the glandular supporting cells, are less electron-dense and have a rather loose fibrillar composition much like their counterparts in Leucothea (see, Horridge 1965) and *Beroe* (see, Hernandez-Nicaise 1973b). The larger granules evidently arise by fusion of smaller granules, as fragmentary partitions are often seen within and between them.

2. Stalk of mature tentillum

Figure 12 compares the layout of the major histological components of the tentillar stalk with the mature and immature shaft. There is no striated muscle in the stalk, but a smooth muscle "keel" is present, as in the shaft. Smooth muscle cells also extend around the sides in two arms which almost meet at the top (Fig. 13 A). Neurites present in the epidermis form synapses with some of the smooth muscle cells (Fig. 13 C).

Inner-ring cells lie in the narrow spaces between the two sides of the muscle keel and continue up and around the main mass of mesogloea to form a complete circle of up to 20 cells. Most of these cells are of the microtubular type (Fig. 13 B), though some, particularly in the keel region, show transitional structure. There are no mesogloeal boxes in the stalk except close to the junction with the shaft, where the box rows first appear. The mesogloeal collagen is not organized into a transverse lamella as in the shaft; instead, thick collagen bundles are intertwined in seemingly random orientations in the centre of the mesogloea, with smaller bundles around the periphery. The epidermis includes all the cell types seen in the shaft with the exception of colloblasts.

The stalk of the tentillum is basically an outgrowth from the tentacle, and smooth muscle fibres and nerves run directly between the two regions. Mt cells can be traced back into the tentacle for a certain distance, but they are not a feature of tentacle structure except in these regions.

3. Shaft of immature tentilla, and colloblast development

Tentilla near the base of the tentacle are unpigmented and smaller than those lower down. Their shafts show very interesting differences from the mature shaft, which may be summarized as follows:

a) The striated muscle cells are nucleated (Figs. 14 B, 15 A). Often several nuclear profiles are visible in the same cell in cross section, which probably means that at this stage the cells are multinucleate. Signs of degeneration of nuclei have been seen in some cells, and mature striated muscle fibres sometimes contain membrane-enclosed masses of material resembling condensed nucleoplasm, which are probably pyknotic nuclei. Thus, the striated muscle fibres are originally nucleated, probably with many nuclei, but become enucleate as they mature.

b) Nerves and neuro-sensory cells are scarcer than in the mature tissue, and the nerve bundle in the outer "notch" of the striated muscle block is only rudimentarily developed.

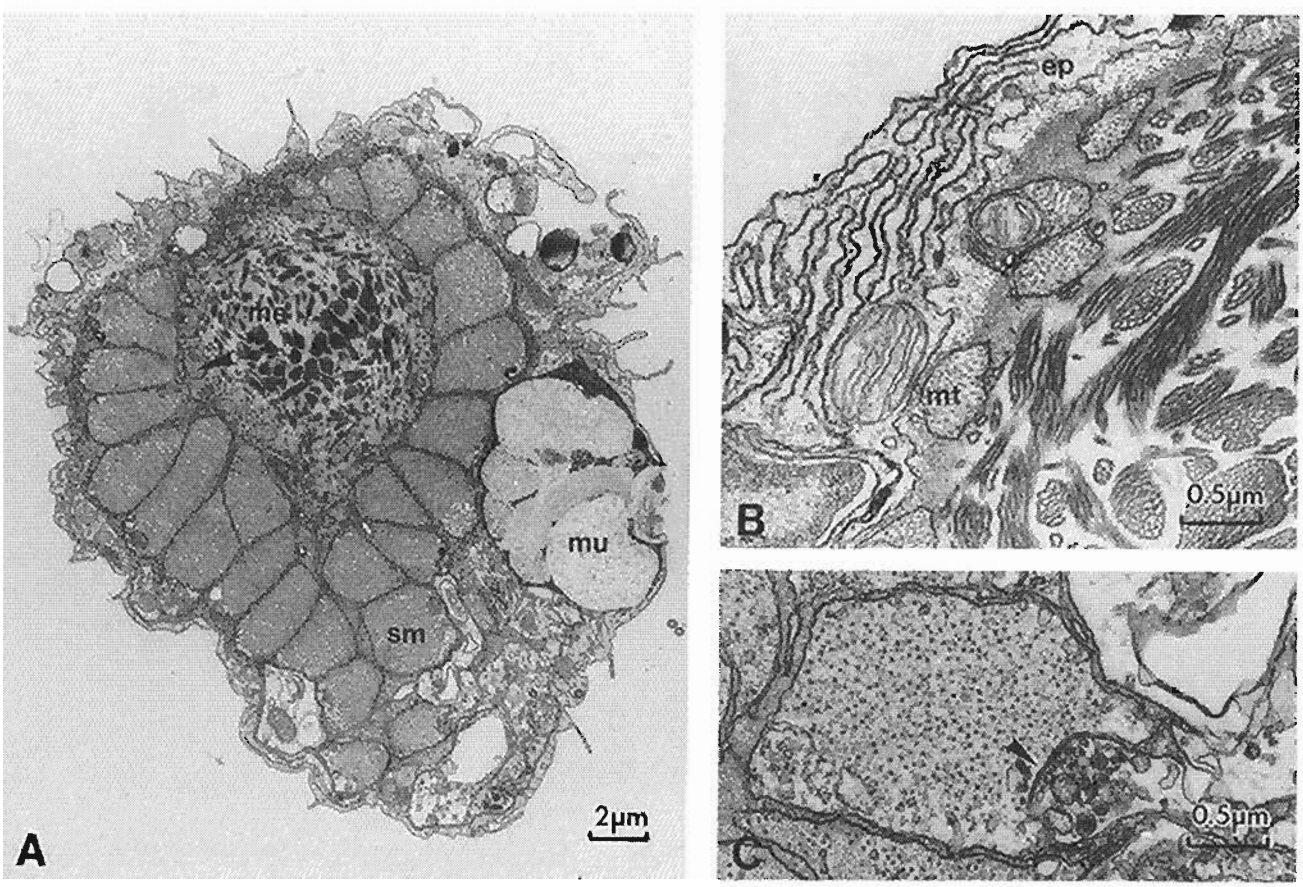

Fig. 13A–C. Stalk of mature tentillum. A cross-section of whole stalk showing mesogloea (*me*), with randomly oriented collagen bundles, a mucus cell (*mu*) and smooth muscle fibres (*sm*). *Arrowhead* shows one of the circle of inner-ring cells. B Details of epidermis (*ep*), inner-ring cell of microtubule type (*mt*) and collagen bundles. C Neuromuscular junction (*arrowhead*) in keel smooth muscle

c) Inner-ring cells of the mt cell or transitional types are present. They have fine processes which run out for long distances into the mesogloea (Fig. 14A). Such processes are never seen in the mature tissue.

d) In the youngest stages observed (Fig. 14A), there is no collagen lamella or other thick collagen bundles, or if distinguishable, they are visible only as vague shadowy forms. The mesogloea generally appears uniformly electron dense, except for the processes of mt cells, and there are no mesogloeal boxes. In a slightly later stage (Fig. 14B), the collagen lamella has appeared, but the background mesogloea is still relatively featureless. Processes of mt cells still run through it, but boxes have not yet begun to specialize. We have not yet come across a stage showing the beginnings of box differentiation. In the mature tentillum, *mt* cells appear to be associated with organization of the collagen into bundles (Fig. 14C), and it may be that they play a similar role in the formation of the bundles composing the transverse collagen lamella, but this remains unclear.

e) The epidermis is dominated by immature colloblasts and by secretory cells ("accessory cells") filled with granules (Fig. 15A, B). Both the colloblasts and the accessory cells are entirely subepithelial at this stage, being covered by processes of epidermal supporting cells (Fig. 15A), whereas later, in mature tentilla, the covering has regressed and the colloblasts along with the various sensory and glandular cells are exposed at their outer ends (Fig. 15C).

With regard to the formation of colloblasts, all the colloblasts in any given tentillum are in the same stage of development, so by examining tentilla of different ages it is possible to see how the cells differentiate. The picture emerging fits well with earlier findings by Schneider (1902) on colloblast maturation. Schneider showed that the colloblasts develop in groups of about half a dozen underneath an umbrella-like covering cell (our accessory cell), the whole complex lying beneath an thin outer epithelial layer. The accessory cell is filled with what Schneider suggested were adhesive granules ("Klebkörner"). As the complex matures, the accessory cell is reduced to a thin "mantle" containing the adhesive granules, which remains attached to the outside of the colloblast. Later workers have termed Schneider's adhesive granules "brilliant" or "refractive", from their appearance in vivo, while the granules lying within the head of the colloblast are termed "eosinophilic", from their appearance in stained paraffin sections.

There has been much uncertainty about the role of the accessory cells. Komai (1922) and Weill (1935) make no mention of accessory cells. Hovasse and de Puytorac (1962) noted the presence of accessory cells but saw no role for them in the production of refractive granules. According to Bargmann et al. (1972) and Storch and Lehnert-Moritz (1974), the refractive granules are produced from within the colloblast itself, as transformation products of the eosin-

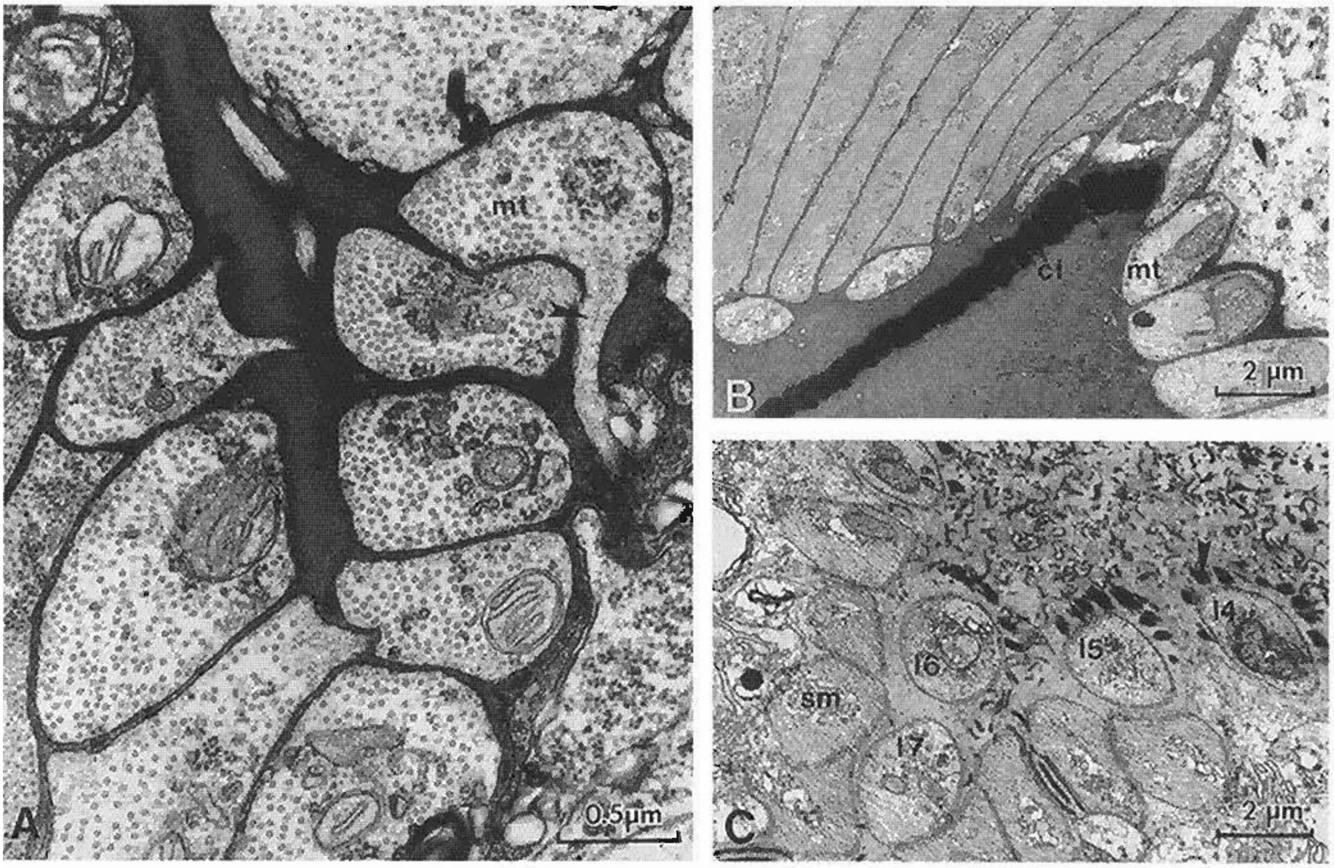

Fig. 14A–C. Immature tentillar shaft showing inner-ring cells of microtubule type seemingly implicated in organization of mesogloea. **A** Microtubule cells (*mt*) at very young stage with processes (*arrowhead*) running in mesogloea. **B** Later stage, with nucleated microtubule cells (still with processes) clustering round edge of forming collagen lamella (*cl*). **C** Later-stage microtubule cells, numbered as in Fig. 3, some with collagen bundles (*arrowhead*) forming around them, also showing smooth muscle (*sm*) of keel

ophilic granules. Franc (1978), however, states that the refractive vesicles are the remains of an accessory cell which disappears during differentiation and this conclusion is documented convincingly by Benwitz (1978) in *Pleurobrachia*. Our findings are in agreement with those of Benwitz, but because of the diversity of opinions expressed on this topic, we will briefly describe the situation in *Euplokamis*.

In very immature tentilla of *Euplokamis*, the subepithelial space is dominated by accessory cells, processes from which extend around the top and sides of the developing colloblasts (Fig. 15A, B). The accessory cells possess nuclei, and it can be seen that a single cell envelops several colloblasts, as noted by Schneider (1902) and Benwitz (1978). In a slightly later stage the accessory cells have regressed somewhat, but still form membrane-bounded caps over the tops of the colloblasts; these are quivalent to the mantles shown by Storch and Lehnert-Moritz (1974). In mature tentilla there is no trace of accessory cells, except for the refractive granules adhering to the surfaces of the colloblasts. The findings of Schneider (1902) and Benwitz (1979) are thus in agreement with our findings on *Euplokamis* as summarized in Fig. 16, and probably apply to all ctenophores possessing colloblasts.

In most other respects, the accounts of colloblast development given by Storch and Lehnert-Moritz (1974) and by Benwitz (1978) for *Pleurobrachia* apply with little modification to *Euplokamis*. These workers show how the spiral filament, formed originally within the spacious basal cytoplasm of the colloblast, elongates and forms an outer spiral around the axial core of cytoplasm, connected to it by a thin fold of cell membrane. Innervation of colloblasts, reported by Franc (1978), has not been observed in our material.

With regard to the vexed question of the colloblast nucleus, Schneider (1902) and Komai (1922) believed that the nucleus of the young colloblast persisted within the axial filament of the mature cell, but according to Weill (1935) the nucleus and cytoplasm of the axial filament degenerate and eventually disappear during normal development. On the basis of electron microscopy, Bargmann et al. (1972) describe a tapering structure lying in the axial cytoplasm of mature colloblasts, directly below their "star-shaped body" here termed "spheroidal body" following Franc 1978), which they refer to as a nucleus, but state that it lacks a membrane over a large part of its surface. The presence of a similar structure was confirmed by Storch and Lehnert-Moritz (1974), who describe it was a degenerating nucleus, which later disappears. Benwitz (1978) identifies the structures as a persistent nucleus, as does Franc (1978), who draws attention, however, to peculiarities in its contents, namely the absence of a nucleolus and the peculiar chromatin. The only histochemical evidence bearing on the

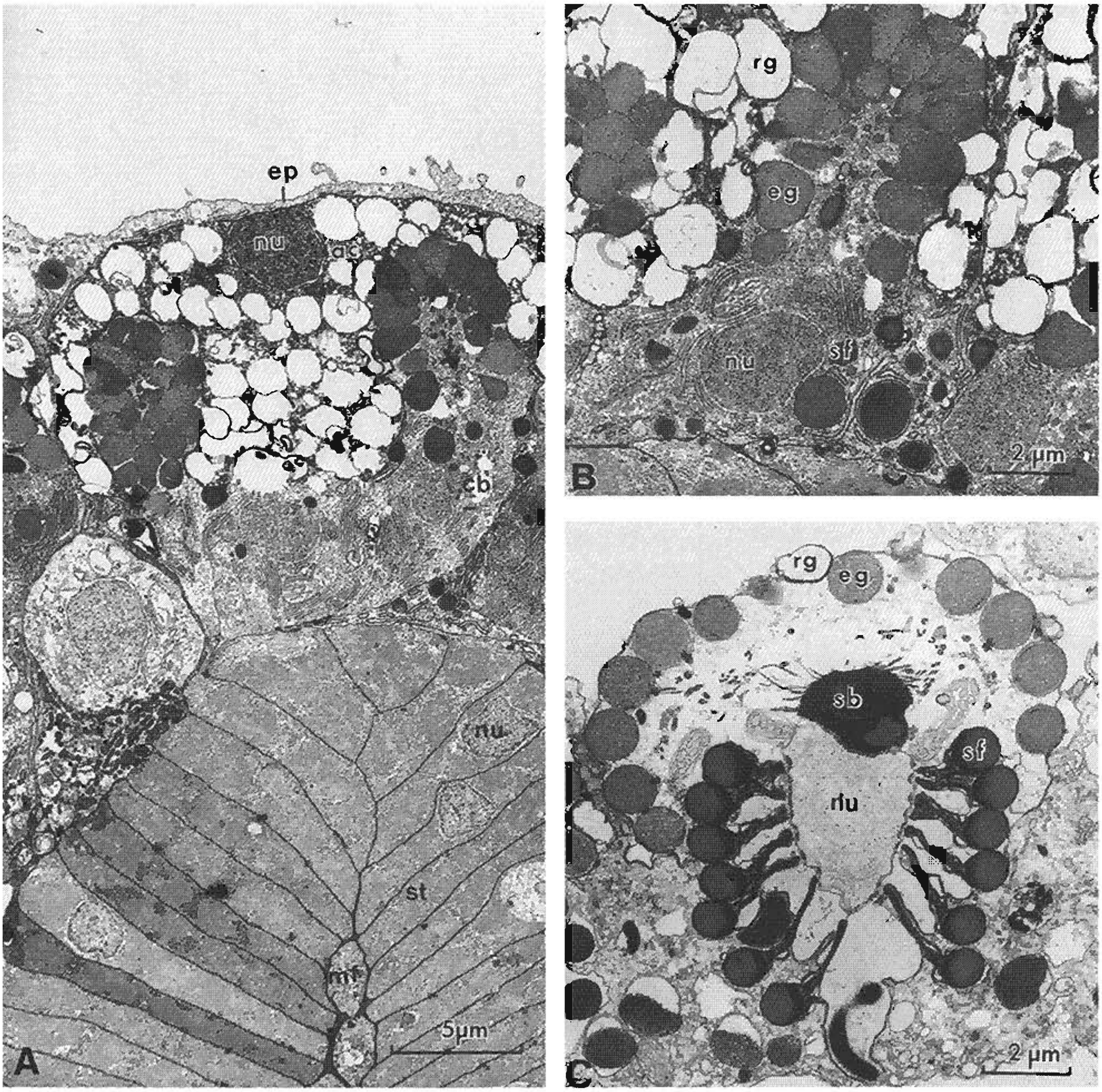

Fig. 15A–C. Colloblast differentiation. **A** Section of tentillar wall in striated muscle zone showing accessory cell processes (*ac*) enfolding immature colloblasts (*cb*), with epidermis (*ep*) forming a continuous outer-covering layer. **B** Enlargement, same stage as A, showing a nucleated young colloblast for comparison with **C** mature colloblast. Also labelled are eosinophilic granules of colloblasts (*eg*), a myofilamentous inner-ring cell (*mf*), nuclei (*nu*) of various cells, refractive granules originating in accessory cells (*rg*), spheroidal body (*sb*) of colloblast, the colloblast spiral filament (*sf*), and a striated muscle fibre (*st*)

question is Hovasse and de Puytorac's (1962) finding that the structure in question is Feulgen-positive. If so, it must almost certainly be a nucleus or nuclear derivative.

In *Euplokamis* the nucleus of young colloblasts is spherical, with granular electron-dense contents like a normal nucleus (Fig. 17A). There is no single prominent nucleolus, so presumably nucleolar organizer sites occur at scattered locations. At about the stage shown in Fig. 16B, as the cytoplasm becomes restricted to form the narrow axial strand the nucleus also elongates and develops patches of fibrous nucleoplasm, presumably chromatin, surrounded by finely granular material, presumably ribonucleoprotein (Figs. 15B, 17B). The fibrous electron-lucent areas are more prominent in later stages, and the nucleus finally becomes filled with the thin fibrous component, sometimes retaining a few scattered patches of denser material (Figs. 15C, 17C). There is no sign of a nucleolus. Despite the changes in the nucleoplasm, the nucleus retains a complete nuclear envelope, and is present in all colloblasts examined. We conclude that the structure is a true nucleus, that it is not lost, does not degenerate, has a complete nuclear membrane at all stages, but undergoes changes indicative of a decrease in its metabolic activity, e.g. cessation or drastic reduction of RNA and protein synthesis, so that the major remaining component is chromatin itself.

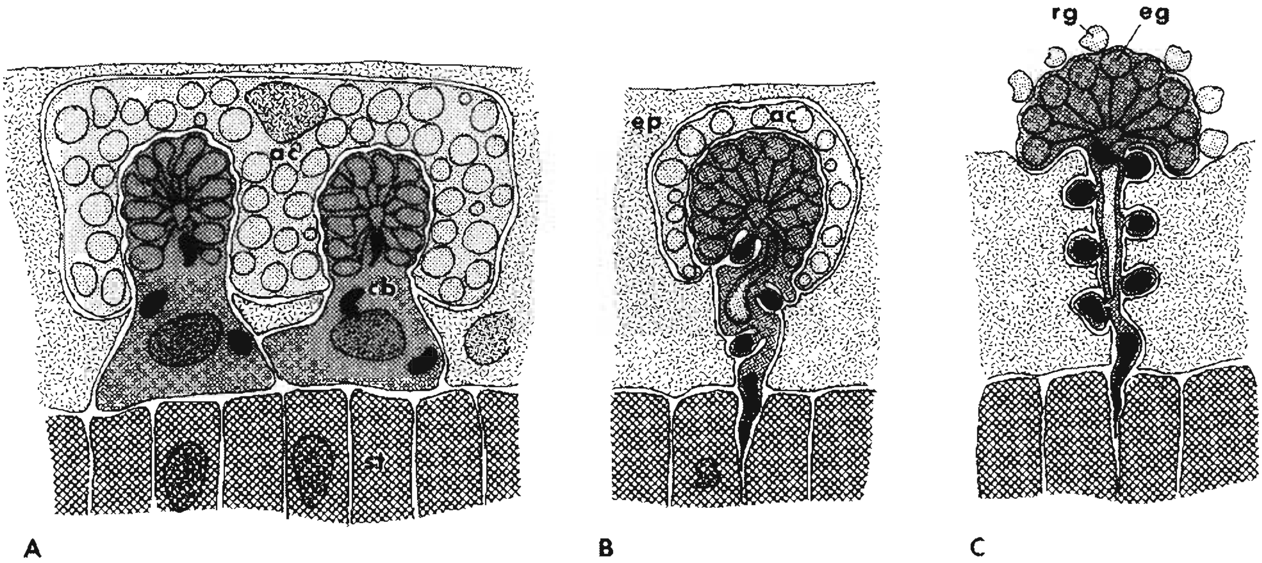

Fig. 16A–C. Changes observed during maturation of the tentillum. A Accessory cells (*ac*) envelop clusters of developing colloblasts (*cb*), providing refractive granules (*rg*) which adhere to the colloblast surface membrane outside the layer of eosinophil granules (*eg*). Retraction of the covering epithelium (*ep*) exposes the mature colloblast. *Also shown*: Striated muscle fibres (*st*), whose nuclei become pyknotic (**B**) and disappear (**C**) at the same time as the colloblast nuclei lose their granular component

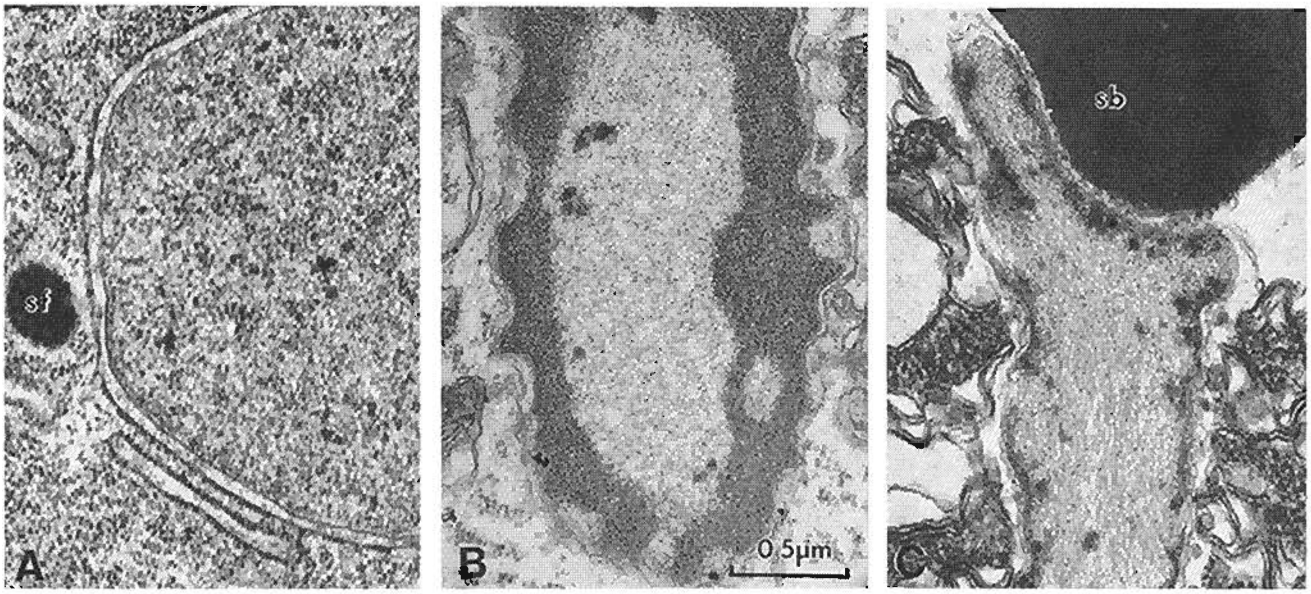

Fig. 17A–C. Nuclei of A very immature, B later and C mature colloblasts, showing transformation of the nucleoplasm from densely granular to thinly fibrous consistency All to same scale. *Also shown*: A section through developing spiral filament (*sf*), C spheroidal body (*sb*)

4. Evidence regarding the mechanisms of fast discharge and of slow length changes

Sarcomere length in the striated muscles is greatest when the tentacle is coiled which, along with other evidence, shows that in the coiled tentillum these muscles are relaxed and that discharge (rapid straightening of the coil) is somehow achieved by contraction of the striated muscles. This seemingly paradoxical conclusion makes sense when we consider that the striated muscles lie on the outside of the coil, so their shortening will tend to straighten the whole structure, assuming that it is fairly resistant to buckling or kinking. We suggest that the rows of fluid-filled boxes along the sides of the tentillum resist compression and prevent buckling of the walls during contraction, acting like flexible box-girders. These and the other structural components implicated in discharge and recoiling are shown diagrammatically in Fig. 18.

Following discharge, the tentillum recoils repidly. This might be due in part to contraction of the smooth muscles of the keel, but it is more likely that passive forces are chiefly responsible. Tentilla were placed in distilled water

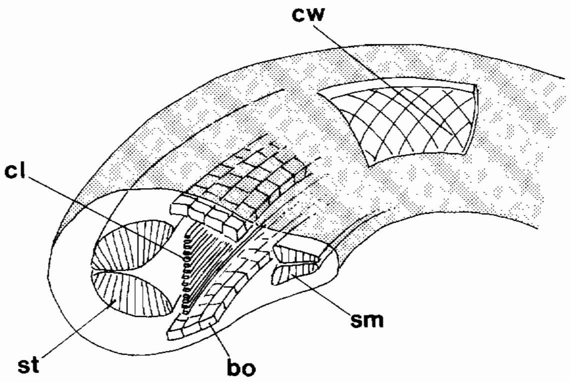

Fig. 18. Simplified model of a coiled (relaxed) tentillum. The rows of boxes (*bo*) would resist compression and prevent the tentillum from collapsing when the striated muscles (*st*) contract, making it possible for the tentillum to straighten out (fast discharge). The transverse collagen lamella (*cl*) and criss-cross collagen web (*cw*) would restore the tentillum to its coiled shape by their elasticity. The smooth muscles (*sm*) on the inner curve of the coil might help retain or haul the prey in after capture

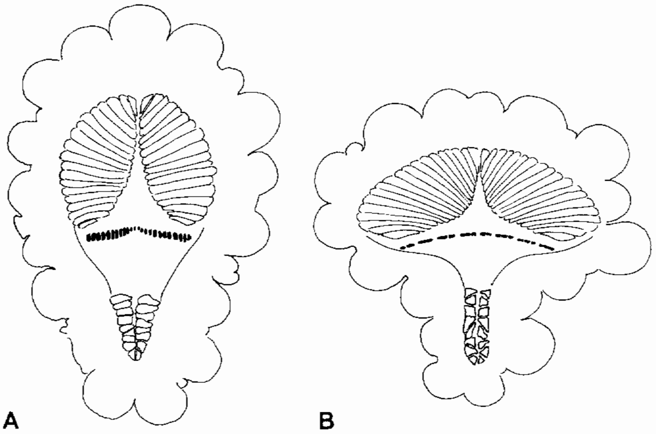

Fig. 19 A, B. Cross sections through the **A** relaxed and **B** actively extended tentillar shaft

for several days to destroy the cellular components. The collagenous components were not destroyed by this treatment, and the core of the tentillum retained its shape and coiled configuration, indicating that coiling is inherent in the mesogloea. It seems likely that the collagen lamella functions as a sort of coiled leaf-spring, returning the tentillum to the coiled state following contractions. Anaesthetized tentilla always show this configuration. We therefore suggest that contraction of the striated muscles straightens out this "spring" and that strain energy stored in the extended mesogloea provides a major part of the restorative force.

Though the boxes must resist compression well enough to keep the wall of the tentillum from buckling, there are signs that the tentillum does shorten to some extent during discharge, although since it straightens out it looks as if it is getting longer. The evidence for this comes from observations on tentilla fixed in the discharged and coiled states. Tentilla were treated with KCl to discharge them, and were then fixed immediately in the (still actively) extended condition. Cross-sectional profiles of tentilla fixed in the coiled and extended states are shown in Fig. 19. Extension is accompanied by flattening of the whole structure, suggesting that when the striated muscles contract they swell, and that the extra bulk is accommodated by bulging at the sides. The collagen lamella is stretched out sideways, large gaps appear between the bundles, and the remaining tissues conform to the new shape.

The mesogloeal skeleton is obviously very complex and includes additional components which may serve important mechanical functions. For instance, the outer wall of the mesogloea is composed of fairly thick collagen bundles, which appear to be arranged in the form of left- and right-handed helices wrapped around the mesogloeal core (Fig. 4C). Such crossed fibre arrays in the nematode cuticle and elsewhere can antagonize the contraction of longitudinal muscles by their elasticity (Wainwright et al. 1976) and may do so here.

The slow spontaneous movements described earlier would presumably be brought about by smooth muscles. If we are correct in our basic assumptions about the mesogloeal skeleton, there is only one set of muscles which can cause slow extension of the tentillum, and that is the small group of myofibrillar inner-ring cells located close to the striated muscles. Recoiling would involve passive elastic forces, possibly aided or modulated by contractile elements (again, most probably the myofibrillar inner-rings cells) located at the opposite pole. It will be recalled that the slow movements are not affected by elevated magnesium concentrations, which would block neuromuscular junctions, so the units responsible need not be innervated. The myofibrillar cells are not innervated, and appear all the more likely candidates for this reason. The keel smooth muscles by contrast are innervated, and would be expected to cease functioning in elevated magnesium, so they are less likely to be involved. Possibly they function in holding the captured prey tightly against the tentacle during transfer to the mouth.

D. Discussion

Hyman (1940), who defines an organ as "a combination of two or more kinds of tissues into a functioning whole", draws a distinction between animals at the tissue level of construction (cnidarians, ctenophores) and those at the organ level (flatworms and above). This distinction is useful in a general way, but organs in the strict sense do occur here and there in both cnidarians and ctenophores; for example, the ocelli and statocysts of medusae, the complex tentillar nematocyst batteries of siphonophores and the apical organ of ctenophores. The tentillum of *Euplokamis* provides another example. As a multi-tissue complex serving for food capture, it is possibly the most elaborate and specialized organ to be found anywhere in these "tissue-level" groups. In support of this contention we may cite (a) ability to perform two distinct types of movement; (b) possession of striated muscles, unique among the Ctenophora; (c) possession of a system of conventional smooth muscles; (d) possession of neuronal components coordinating the activities of these muscles, including what by ctenophore standards may be considered as "giant axons"; (e) presence of the most elaborate mesogloeal specializations to be found in any ctenophore, including elaborate arrays of thick collagen bundles with clearly visible periodic striations and of fluid-filled box-like compartments probably serving a skeletal function; and (f) presence of a unique class of cells, the inner-ring cells, some of which are specialized as a type of smooth muscle (differing, however, from conventional smooth muscle) and others as microtubule-filled cells, some of which appear to be involved in restructuring the collagen skeleton so as to form boxes, and in other ways. These are the components which together characterize the tentillum as an organ. In addition to these components, the tentillum has a normal covering epithelium containing the usual epidermal cell types (colloblasts, secretory cells, sensory cells, etc.).

Regarding the functional aspects, the whole complex serves most obviously as a dischargeable food-capturing device, prehensile in the sense of "adapted for seizing or grasping, especially by wrapping around" (Webster). This is not true of other tentacular structures in cnidarians and ctenophores, which rely upon gravity or drag forces for their extension. The tentillum is actively shot out like the tongue of an insectivorous frog.

In addition to its fast discharge response, however, we have seen that the tentillum performs slow, spontaneous, rhythmical, writhing movements. It is possible that these might simulate the movement of small planktonic worms, enabling the tentillum to function as a lure in attracting fish larvae, etc., into the vicinity of the tentacle, where they can be captured. The bright red pigmentation may also be significant in this context. The pigment lies along the inside of the coil and would be displayed to good effect by uncoiling of the tentillum. A very similar argument has been advanced in the case of the tentilla of certain physonectid siphonophores by Purcell (1980), who terms the phenomenon "aggressive mimicry".

In observing prey capture by *Euplokamis* we found no reason to suspect the involvement of toxins. The prey continue to struggle while being conveyed to the mouth. Regarding the proposed function of the peg cells as stings (von Lendenfeld 1885; Hernandez-Nicaise 1974a, b), it seems unlikely that these structures have the stiffness needed to penetrate a crustacean exoskeleton.

Our studies of the structural components have revealed a number of interesting cellular specializations, of which the striated muscles come first to mind. The striated muscle fibres show a typical myofilament array with what we interpret as a 1:2 thick-to-thin filament packing ratio and an apparent ability to shorten to about 50% of the relaxed length. The shortness of the sarcomere length (1.4 µm relaxed) suggests that contraction velocity is high. The fibres are thin in cross section (<1.0 µm), so the absence of a T-system is not surprising. Depolarizations presumably spread in the sarcolemma from synaptic sites, possibly triggering release of calcium ions from the richly developed sarcoplasmic reticulum, which lies just below the sarcolemma. It is unlikely that the striated muscle fibres are electrically closely coupled, as very few gap junctions are seen. Each fibre is probably innervated. The absence of a nucleus and the paucity of glycogen and mitochondria fit with the picture of a system maintained at low metabolic cost for occasional brief use.

When the striated muscles contract, the tentillum extends, presumably because the muscles are on the outside of the coil, so their shortening will cause uncoiling. We have suggested that the mesogloeal boxes along the sides of the tentillum are compression-resistant structures which prevent the tentillum from crumpling or collapsing when the muscles contract. Restoration of the coiled state is probably due to passive spring-like properties of the mesogloea, which contains a massive transverse collagen lamella and is enveloped in helically wrapped collagen strands.

The smooth muscles of the keel show no particularly novel specializations, but the inner-ring cells, some of which are differentiated as "myofibrillar cells" and others as "microtubule cells", deserve comment. There seems to be no counterpart to these cells in other ctenophores. In young stages of the shaft, and in the stalk, the inner-ring cells are all of the microtubular variety, but in the mature shaft some of them develop thick and thin myofilaments. Our observations suggest that the latter function as contractile units in the slow rhythmic coiling and recoiling movements. The microtubular inner-ring cells, by contrast, seem to be involved in organizing the structure of the mesogloea. It is unlikely that they actually secrete collagen, but in young tentilla they send processes out into the extracellular matrix at a time when the collagen bundles are in process of formation. Later they may still be seen surrounded by bundles of collagen and, in addition, they are clearly implicated in the formation and/or maintenance of the mesogloeal boxes. It is also noteworthy that the microtubule cells extend the whole length of the tentillum from tip to base and pass right into the tentacle at the attachment point. As microtubules are frequently implicated in transport processes (e.g. Gilbert et al. 1985), these cells might serve some role in movement of metabolites down the tentillum.

It would seem that in the inner-ring cells genetic expression can be given primarily to microtubules or primarily to myofilaments; however, cells showing a type of cytoplasm intermediate between the two are also found in specific locations and are indeed a regular feature of the mature tentillum. Simply by looking at cross-sections of fixed cells it is hard to say whether these "transitional cells" are a categorically distinct type of cell, cells in the process of changing their specification over time, or merely long cells intercepted at a point along their length where their cytoplasm is grading from one type to the other spatially. Our attempts to study these cells in longitudinal sections and in different stages of development have so far failed to answer these questions, and more work is needed.

An unexpected bonus of this study was the opportunity to observe stages in the differentiation of colloblasts by looking at tentilla in various stages of development. Our findings confirm observations by Schneider (1902) and Benwitz (1978) that groups of colloblasts form in association with specialized accessory cells. The accessory cells degenerate, but leave behind their secretory vesicles as the "refractive granules" seen attached to the outside of all mature colloblasts. We have also been able to clarify the fate of the nucleus in developing colloblasts, a subject of much disagreement in the past. We find that the nucleus persists and does not degenerate, but undergoes a transformation of the nucleoplasm indicative of reduced metabolic activity. By contrast, in developing striated muscle fibres, the nuclei become pycnotic and degenerate completely as the tentillum matures. The series of developing tentilla would provide excellent material for further studies on differentiation of various cellular and mesogloeal components.

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