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Microanatomy of the cubopolyp, *Tripedalia cystophora* (Class Cubozoa)

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ABSTRACT. The microanatomy of a cubopolyp (polypoid stage of Cubomedusae) is described for the first time. The 0.5–1.0 mm long polyp of *Tripedalia cystophora* has an oral cone with special lip cells at the mouth. Next is a baggy calyx occasionally followed by a slender stalk. The basal region is surrounded by a thin periderm. A single row of tentacles is at the oral cone/calyx junction. The mesoglea is thin and non-cellular. The muscular system of the ectoderm is composed of smooth longitudinal epitheliomuscular cells in the oral cone, tentacle, stalk and calyx. The calyx ectoderm also sends longitudinal muscle fibers into the mesoglea. The mesogleal muscle fibers seem to contain paramyosin and perhaps are doubly innervated: one set of neurites for contraction and one for relaxation. A circular endodermal system of filaments, probably actin, is found in all regions. The tentacles have a solid core of a single row of endodermal cells capable of phagocytosis. The ectodermal tip is swollen with longitudinally aligned nematocysts. The distal part of the tentacle contains striated ectodermal myofibers. The nervous system is unique in having an endodermal/ectodermal nerve ring pair at the calyx/oral cone junction. Ganglion cells are not apparent. Presumed sense cells have complicated microvilli and no flagellar rootlet. A cell fitting the description of a neurosecretory neurone is especially prominent in the oral cone's endoderm. It has a major process reaching the coelenteron. Round macrogranular cells corresponding to the amoebocytes of the Scyphozoa and Anthozoa are found. There are no interstitial cells. The oral cone's flagellated endoderm is made up of mucous cells, cells with small dark granules, cells with large granules and rodlets in the cytoplasm, and a few absorptive cells. The calyx endoderm is very thick (120 μm) and is made up of flagellated absorptive, mucous and granular cells. Ingested food is transformed into basal droplets. 4 size and shape types of the microbasic eurytele category make up the cnidome. The largest nematocyst types are found at the tentacle's tip. Like a hydropolyp, the cubopolyp lacks gastral septa and is in other features radially symmetrical. Like a scyphopolyp, the cubopolyp has mesogleal muscles and no interstitial cells. Unique histological features are the nerve rings and tentacular striated muscles.

INTRODUCTION

Cnidarians come in various forms such as planulae, polyps, ephyrae, actinulae and medusae. The polypoid form occurs in all the cnidarian classes: Scyphozoa, Hydrozoa and Anthozoa. Even within the Class Scyphozoa the different orders usually have distinct polypoid forms. The Stauromedusae are a mosaic of rather medusoid features at the oral end and polypoid features basally. The Coronatae have polyps which are obviously different from those of the Semaeostomeae and

Rhizostomeae. The latter two orders have similar polyps which are called scyphistomae. Missing from this series is the cubomedusan polyp, the last major, cnidarian polyp to be described microanatomically.

Okada (1927) and earlier, Conant (1898) reared cubopolyps to the young four tentacle stage but the polyps succumbed. Conant died before he could put together all his material and it was left to Professor W. K. Brooks to edit Conant's notes. Brooks inserted an editorial note saying that the polyps had four taenioles (septa). He did this because scyphistomae have taenioles and he thought cubopolyps being scyphopolyps would possess them as well. No trace of these formations can, however, be found as will be explained below.

It was only recently that the complete life cycle of the cubomedusa *Tripedalia cystophora* Conant, 1898 could be cleared up by successful rearing experiments (Werner et al., 1971). On the basis of this knowledge other polyps obtained in 1970 and later by diving off Puerto Rico could be identified as cubopolyps. The assignment of polyp to medusa has now been determined as a consequence of the elucidation of the life cycles of *Carybdea marsupialis* (L.) (Cutress & Studebaker, 1973) and *C. alata* Reynaud (Arneson & Cutress, 1976). Werner (1975) has described the morphology and life history of the polyp of *Tripedalia* in detail and has included many drawings of living specimens.

The theoretical interest in this study relates to the taxonomic and phylogenetic position of the Cubomedusae, lately raised to the rank of Class Cubozoa by Werner (1973, 1975). With its shape, velarium and nerve ring, the cubomedusa is unlike a scyphomedusa. The cubopolyp metamorphoses in its entirety into a small medusa without an ephyra stage (Werner et al., 1971). Therefore, this life cycle is unlike either the Hydrozoa or Scyphozoa. It was considered important to study the microanatomy of *Tripedalia cystophora* with a view to ascertaining the cubopolyp's hydrozoan, scyphozoan and peculiar features in greater detail. In a short note Werner et al. (1976) have already communicated some important results on the cubopolyp's muscular and nervous systems.

MATERIALS AND METHODS

The polyps of *Tripedalia cystophora* were reared at 22° C in Dr. Werner's laboratory at the Biologische Anstalt Helgoland in Hamburg. Every two days the polyps were fed on four day old *Artemia* nauplii. Living polyps were sent via air mail to me in thermos flasks.

The polyps were anesthetized in magnesium – sea water (Pantin, 1960) and fixed for electron microscopy in Dorey's (1965) chrome-osmic fluid, embedded in Spurr's (1969) resin and stained in uranium acetate (Kay, 1965; p. 262 after Gibbons & Grimstone) and lead citrate (Venable & Coggeshall, 1965). One half micrometer sections were stained in Richardson et al.'s (1960) solution.

Besides ordinary bright-field microscopy, polarizing optics using a quarter wave retardation plate was employed.

Whole mounts were prepared by extracting specimens from their plastic embedd-

ment using an iodine-acetone soak followed by treatment in 1 % sodium hydroxide in absolute ethanol (Yensen, 1968). The specimens were washed in ethanol and either cleared and mounted in Canada balsam or else hydrated and mounted in glycerin. The whole mounts were examined with Nomarski interference-contrast (Zeiss) optics.

A variety of histochemical techniques was performed, not just to learn something about the chemical nature of the various cells but mainly to determine the number of different endodermal cell types and to be alerted to morphological features not obvious by other microscopic techniques.

The polyps used for histochemistry were starved for a few days then were anesthetized in magnesium – sea water before fixation in 4 % formaldehyde in sea water. Except for the frozen sections, they were dehydrated in alcohol, stained in fast green FCF, cleared in benzene then embedded in Tissumat (Fisher) before sectioning at 5 or 8 μm.

The protein tests were dinitrofluorobenzene (Pearse, 1968; p. 614, after Danielli & Burstone), mercuric-bromphenol blue (Chapman, 1975) and eriochrome cyanin (Chapman, 1968). Certain aspects of carbohydrate chemistry were demonstrated by the periodic acid – Schiff reagent (Pearse, 1968; p. 660), alcian blue at pH 2.5 (Pearse, 1968), astra blue (Barka & Anderson, 1965) and toluidine blue (Pearse, 1968; p. 665, after Kramer & Windrum). For lipid, Sudan black/frozen sections (Pantin, 1960) were used and, roughly for phospholipid, copper phthalocyanin (Pearse, 1968). Fontana's solution (Pearse, 1972) or silver methenamine (Lillie, 1965) were used for the argentaffin reaction.

Silvering techniques were attempted in order to demonstrate the nervous system. Schofield's method (see Drury & Wallington, 1967) was used on paraffin sections of polyps fixed in 10 % buffered formalin. Korn's (1966) modification of Fraser Rowell's method was used on paraffin sections of polyps fixed in buffered and unbuffered 10 % formalin. Polyps fixed in unbuffered 10 % formalin were silvered as whole mounts in Bone's (1972) modification of Winkelmann's technique. Bone's (1972) other modification of Palmgren's method was tried on paraffin sections of polyps fixed in unbuffered 10 % formalin.

RESULTS

Description of polyp

There is some variability in shape especially if the polyp is budding or feeding. Roughly, the polyp looks like a tentaculate baggy olive, set occasionally on a narrow short stalk (Figs. 1, 2). The basal region is usually surrounded by a thin peridermal cup. The mouth is at the apex of the pointed oral cone (Fig. 3). Special lip cells ring the mouth inside and out. The calyx provides the bulging bulk of the body and is found between the oral cone and the stalk. The single row of capitate solid tentacles springs from the calyx at the junction with the oral cone. Large nematocysts are found in the swollen tentacle tips. The tentacular endoderm is composed of a single row of mainly vacuolated cells. The coelenteron's endoderm is very thick (Fig. 4),

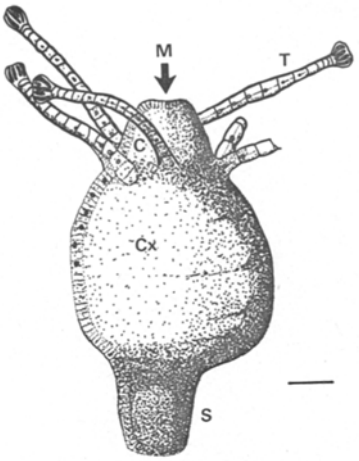


Fig. 1: Drawing of the cubopolyp, *Tripedalia cystophora*, as seen in plastic after fixation and embedding. Some tentacles have broken off. Intact tentacles show the large nematocysts in the swollen tentacle tips. Bar = 100 µm. C, oral cone; Cx, calyx; M, mouth; S, stalk; T, tentacle

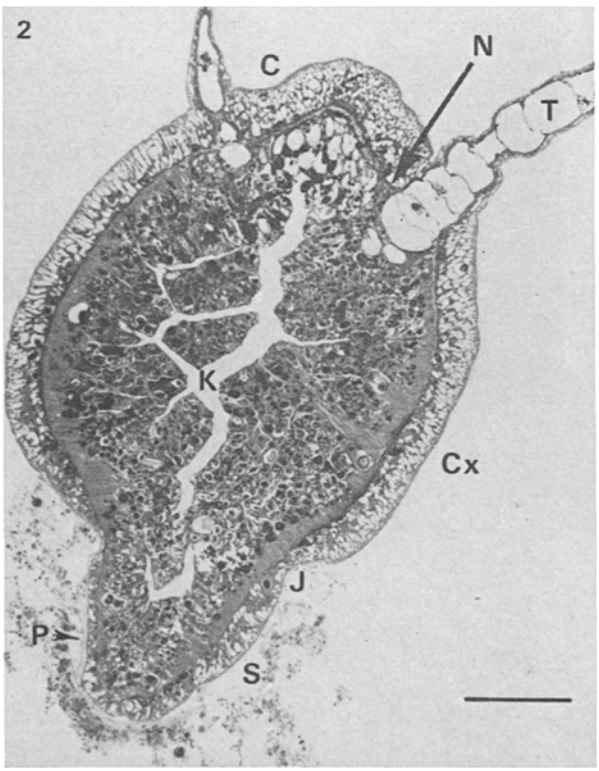


Fig. 2: Light photomicrograph of a longitudinal section to one side of the mouth. Note the thick endoderm. The arrow (N) points to the approximate location of the nerve ring pair. Bar = 100 µm. C, oral cone; Cx, calyx; J, junction between calyx and stalk; K, coelenteron; P, periderm; S, stalk; T, tentacle

leaving scant room for the coelenteron. The 0.5–1.0 mm long body is defined as comprising the oral cone, calyx and stalk.

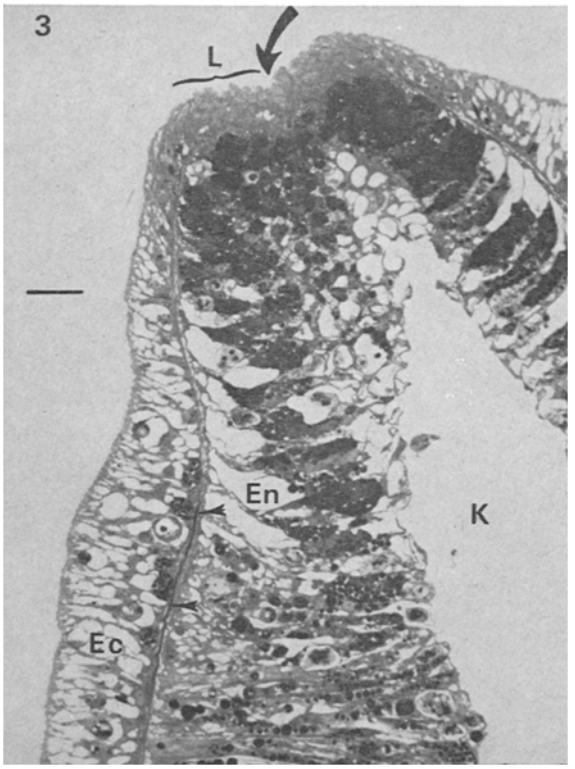


Fig. 3: As in Figure 2 of the oral cone. The broad arrow points in the direction of the mouth. The two fine arrow heads point to muscle strands in the mesogela. Bar = 20 μm. Ec, ectoderm; En, endoderm, K, coelenteron; L, lip cells

The periderm

A periderm surrounds the basal region of the polyp and is continuous with the material separating the basal ectoderm from the substratum (Fig. 5). The flared portion of rived-shaped desmocytes (Chapman, 1969) are embedded in the basal periderm. Where the periderm is opposed to the stalk ectoderm, the apical ectodermal granules (see below) are fewer in number as if they played a role in periderm formation by giving up their contents to the periderm. The periderm is PAS and mercuric bromphenol blue positive hence carbohydrate and protein are present.

Two layers of periderm, each 5 μm thick, are shown in Figure 6. The inner layer is in tight contact with the ectoderm.

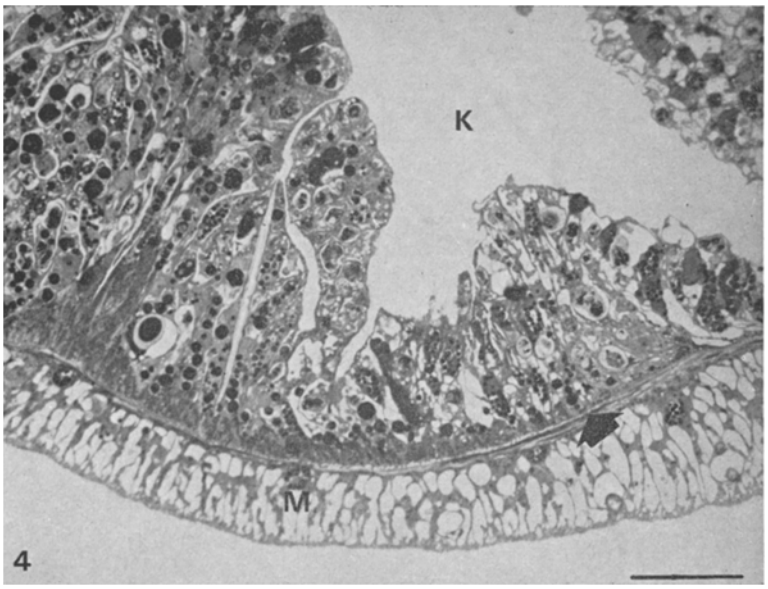


Fig. 4: Longitudinal section of the calyx. The thick but variable endoderm, lines the coelenteron (K). The arrow points to muscle within the mesoglea. Light microscopy. Bar = 40 μm. M, macrogranular cell

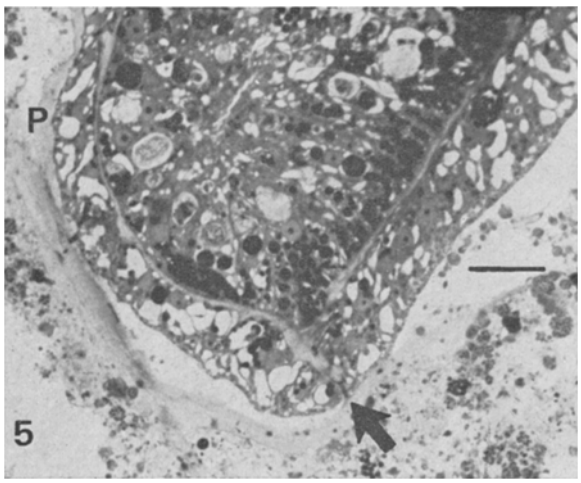


Fig. 5: Longitudinal section of the stalk and pedal disk. At the arrow is a rivet-shaped desmocyte anchoring mesoglea to periderm (P). Light microscopy. Bar = 20 μm

Electron microscopy reveals that the periderm is present as a loose feltwork of irregular minute fibrils. Various microorganisms invade the substance of the periderm.

The mesoglea

This is the layer between the ectoderm and endoderm. In the cubopolyp the mesoglea is thin when compared to scyphistomae. It is acellular if we exclude the invading muscle fibers, accompanying nerve fibers and a few rare macrogranular cells (see below). The mesoglea is fibrous with usually denser accumulations of fibrous

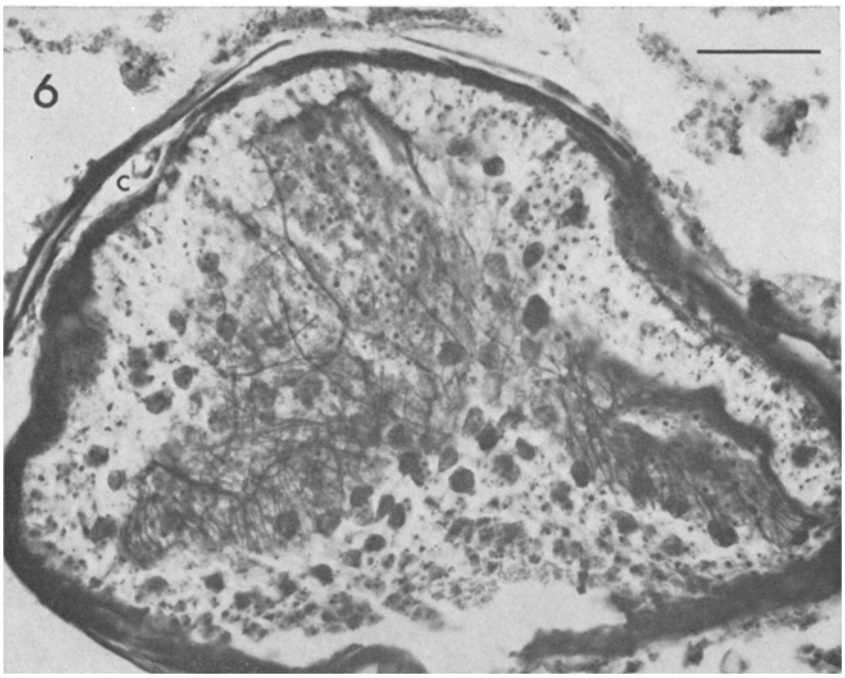


Fig. 6: Transverse section through the ectoderm of the basal disk showing the vaguely radial muscle fibers near the center. On the periphery is the cuticle (C) split in two layers at "C". Korn's silver modification. Bar = 40 μm

material next to the epithelia and invading muscle fibers (Fig. 9). Deeper regions may show bands or patches of this feltlike fibrous material. No crossbanding of the 6 nm wide fibrils could be detected at high magnification. Indistinct material can be seen here and there between the fibrils. The mesoglea is PAS positive.

The muscular system

The body ectoderm. As is usual for this phylum, the contractile apparatus is part of an epitheliomuscular cell. Since the muscle fiber part usually contains just one myofibril, the terms myofiber and myofibril often mean the same thing. Some

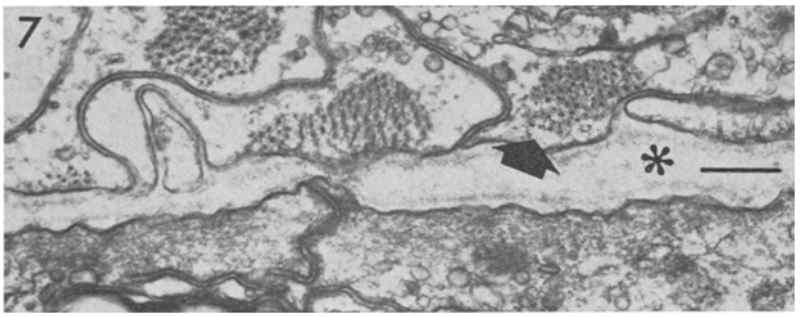


Fig. 7: Transverse section of oral cone showing ectodermal myofibrils (e.g. at arrow). Below are two joined endodermal cells containing a basal circular system of microfilaments. The oral end of the calyx and the whole stalk also resemble this condition. TEM. Bar = 0.5 μm. *, mesoglea

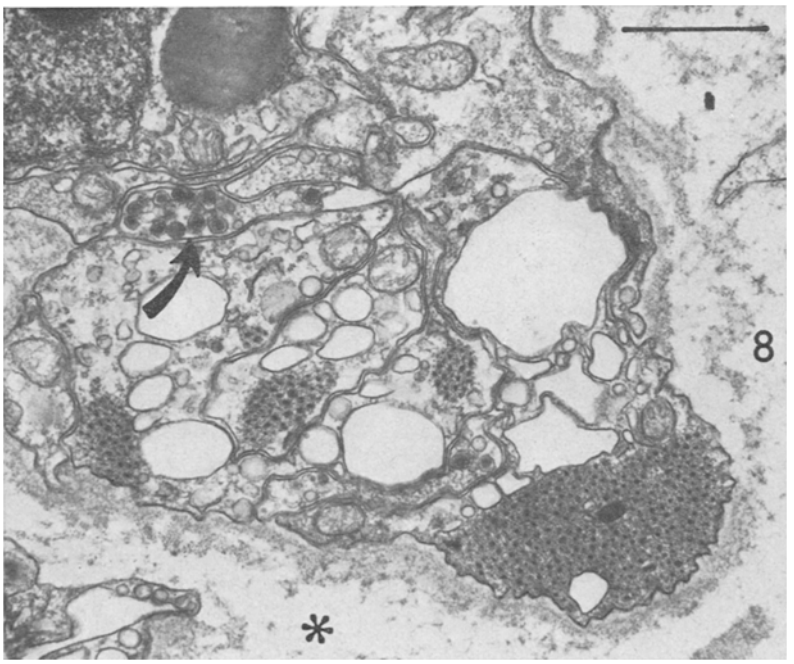


Fig. 8: Transverse section near the oral end of the calyx. Some of the myofibers are bulging together into the mesoglea. Arrow points to neurite. TEM. Bar = 1 μm. *, mesoglea

of the epitheliomuscular cells, however, were noted to have two cytoplasmic processes each connecting separate myofibrils.

The oral cone conforms to this picture with the myofibers running longitudinally along the cone. The same is true for the stalk when it exists. As the myofibrils approach

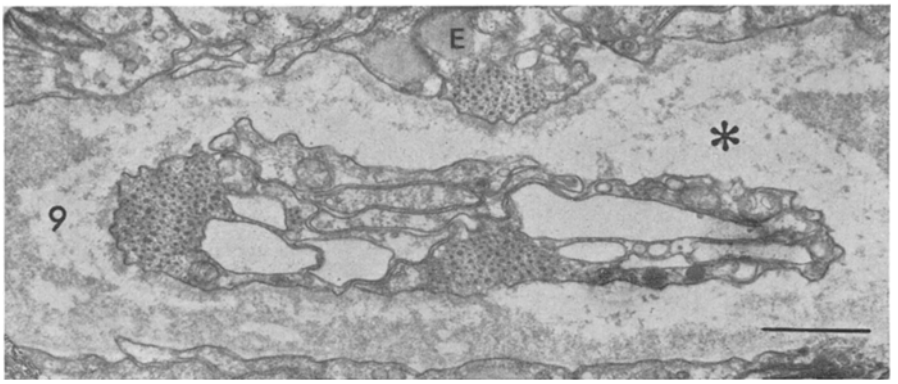


Fig 9: Transverse section of the mid-region of calyx. Some myofibers have separated from the ectoderm (E) and lie in mesoglea (*). Some myofibers remain in ectoderm TEM. Bar = 1 μm

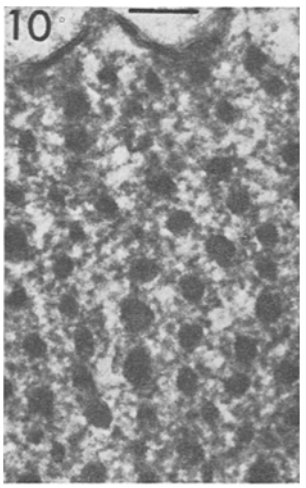


Fig. 10: High power view of a mesogleal myofibril showing the range in size of the myofilaments. TEM. Bar = 100 nm

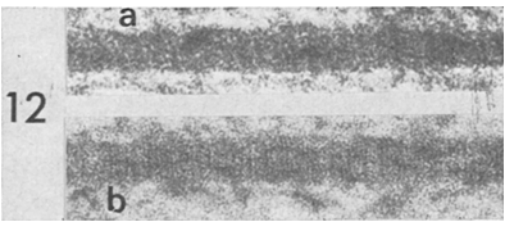


Fig. 11 a, b: (a) Vague transverse periodicity of a thick myofilament from a myofibril undergoing digestion in a tentacular core cell. TEM. (b) Sharper 5 nm periodicity from a mesogleal myofilament from the cubopolyp, *Carybdea marsupialis*. Stained with uranium only. TEM

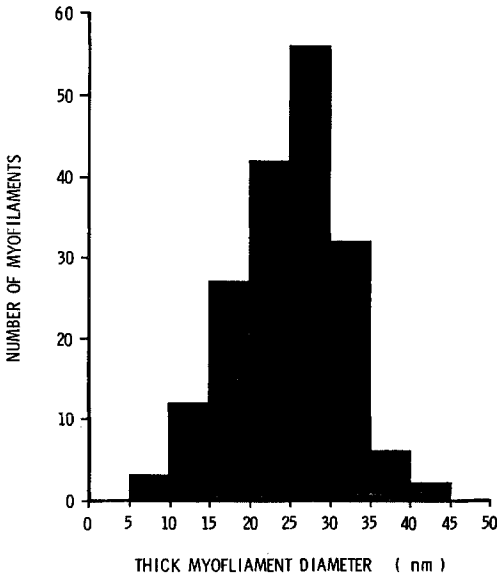


Fig. 12: Histogram of frequency distribution of myofilament diameters in a mesogleal myofibril

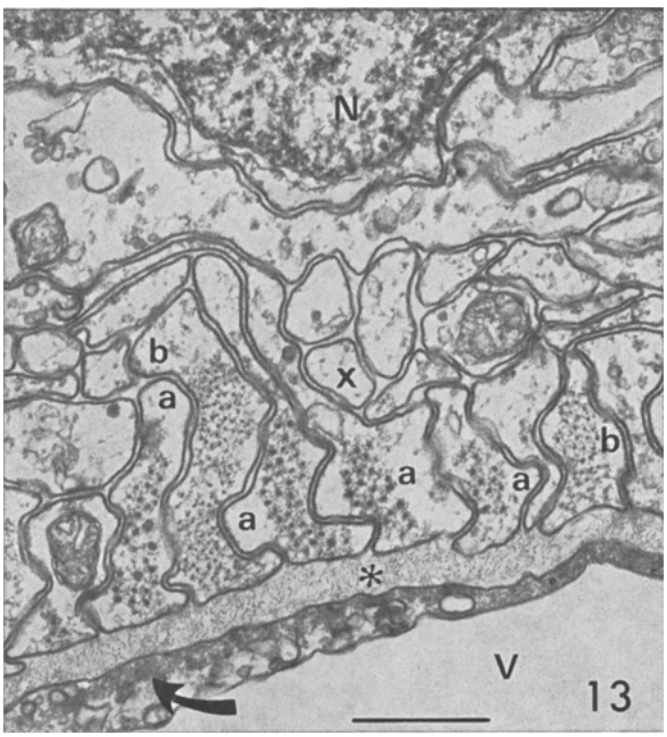


Fig. 13: Transverse section of the tentacle near its tip. The myofibrils are smooth (a) or striated (b). The arrow points to a microfilament layer at the periphery of the endodermal core cell. TEM. Bar = 0.5 µm. a, smooth myofibril; b, striated myofibril; N, nucleus; V, intracellular vacuole; X, neurite; *, mesoglea

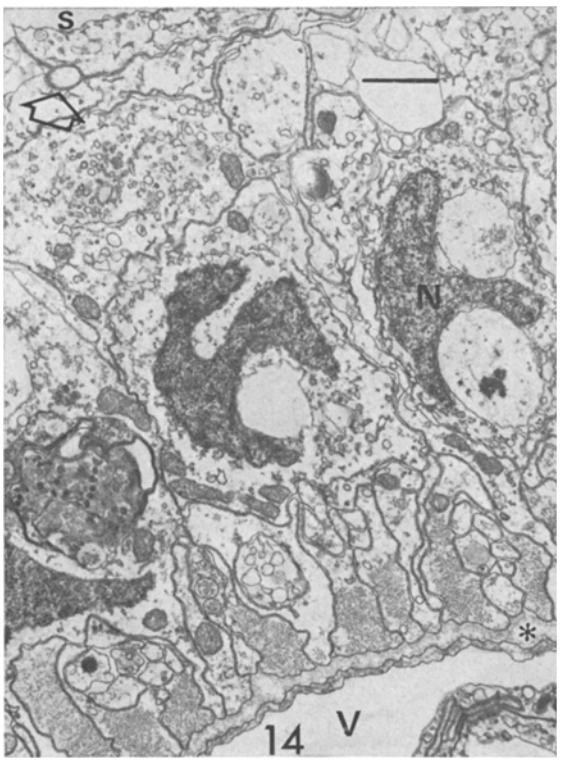


Fig. 14: Transverse section closer to the tentacle's tip. Here the myofibrils are all striated. The arrow points to a plugged apical canaliculus. TEM. Bar = 1 μm, N, nucleus; S, sea water; V, intracellular vacuole; *, mesoglea

the basal disk, they turn through 90° and show a vaguely radial disposition in the basal disk ectoderm (Fig. 6).

The calyx is more complicated for although some myofibers remain in the ectoderm (Fig. 7) others group together and bulge into the mesoglea (Fig. 8). Others, singly or in groups along with neurites, invade the mesoglea and are surrounded by it (Fig. 9). Perhaps these types are continuous but appear different at different levels. The mesogleal muscles stop near the calyx-stalk junction.

Some, at least, of the mesogleal myofibers seem to be pure myocytes, that is to say, muscle cells lacking an epithelial part and having the nucleus in the myofiber, peripheral to or surrounded by myofibrils. These myofibrils are usually larger than those in the ectoderm. End-to-end myofiber junctions were not seen and perhaps the fibers are long enough to span the length of the calyx and end in the mesoglea anchored by mesogleal fibrils.

Just as the myofibers are larger in the mesoglea so too are their thick myofilaments (Fig. 10). The myofilaments measure from 8 to 53 μm in diameter. An earlier hasty observation (Chapman, 1974, p. 3, 5) prompted me to think erroneously that there were two sorts of thick myofilaments; however, the histogram (Fig. 12) indicates there is one variable sort. The diameter of any single filament is quite uniform over

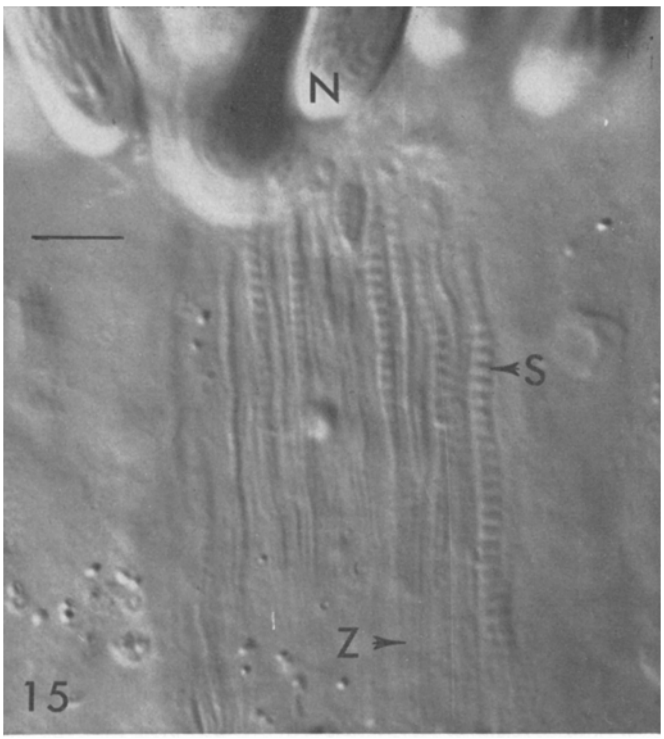


Fig. 15: View of the striated muscle fibers just proximal to the row of nematocysts at the tentacle's tip. Mounted in glycerin. Nomarski optics. Bar = 10 μm. N, nematocyst; S, striated muscle fiber; Z, smooth muscle fiber

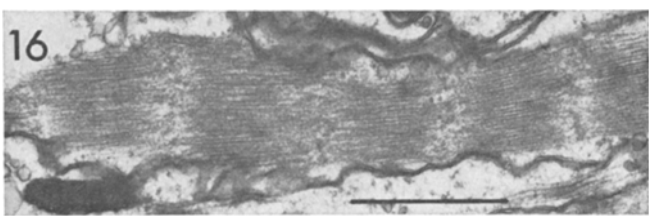


Fig. 16: Striated myofibril from a longitudinal section of the distal region of a tentacle. TEM. Bar = 1 μm

a long distance so that any terminal tapering would account for only a small amount of the narrow diameters measured.

An faint cross-striation with a 5 nm periodicity can be detected (Fig. 11) in this thick mesogleal myofilament.

Another myofilament type exists but is hard to make out consistently with confidence; it measures about 5 nm in diameter and presumably is actin. Because the wide myofilament type is so variable its smaller members can be confused with the other's constant value. If the large type had a sharp geometrical array then the smaller type would be easier to detect; however, the array is rather irregular.

The tentacle ectoderm. Most of the tentacle's musculature resembles that of the oral cone. In both regions the large myofilament type averages 20 nm wide though some are 33 nm. A startling change is noted near the tip of the tentacle: this is the occurrence of striated myofibrils among the smooth ones (Fig. 13); further distally (Fig. 14, 15) all the myofibrils are striated. The purely striated region is about 80 μm long. The sarcomere length is 0.9 μm (Fig. 16).

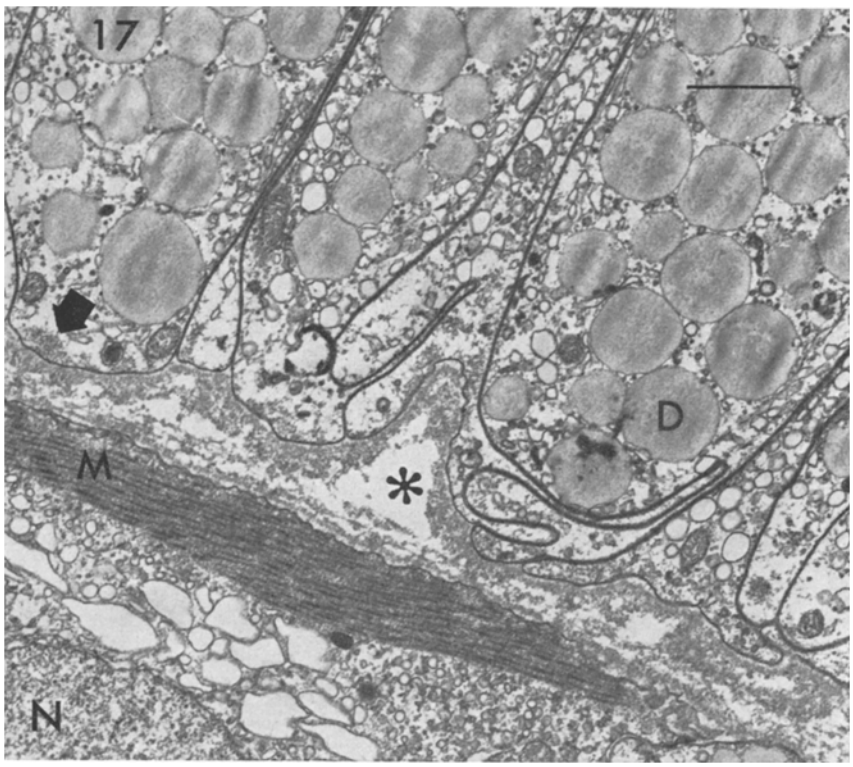


Fig. 17: Longitudinal (slightly oblique) view of a calyx ectodermal myofibril. The arrow points to a transverse view of the circular endodermal microfilaments. TEM. Bar = 1 μm. D, droplets deep in the endoderm; M, myofibril; N, nucleus; *, mesoglea

The endoderm. Each cell of this epithelium has a short basal myofiber-like process oriented in a circular manner at right angles to the ectodermal system of myofibers (Figs. 7, 17). In the body these processes are about 200 nm thick and the contained filaments measure about 5 nm in diameter. In the tentacles the endodermal core cells have a peripheral layer of similar circular filaments.

A detailed search was made along the endodermal and ectodermal surface of the oral cone's mesoglea for a spincter but none was found.

Polarizing microscopy of the calyx showed a circular system of filaments no doubt corresponding to this system of fine endodermal filaments.

The nervous system

Naked neurites with neurotubules and mitochondria are found almost anywhere in the deeper regions of the ectoderm and endoderm. Neurites either contain a few agranular vesicles or else contain many granular vesicles. In muscular regions, neurites are usually just superficial to the muscular layer and in the tentacles they are oriented parallel to the myofibers (Fig. 13). No neuromuscular endings were seen.

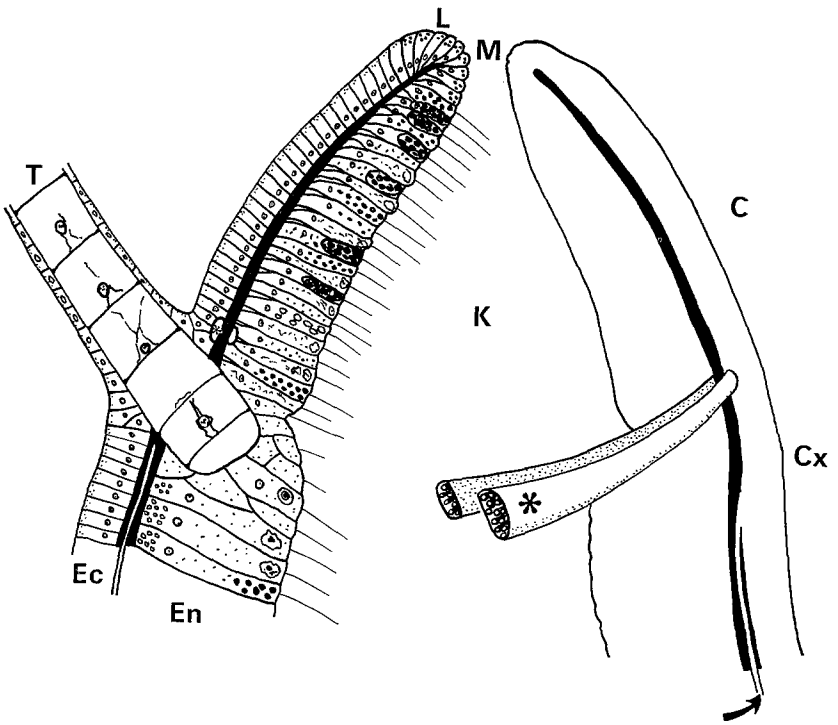


Fig. 18: Longitudinal view of the cubopolyp at the oral end to show the location of the minute nerve rings. The tentacle-bearing side is detailed and the other side, plain. The plain side accentuates the ectodermal (*) and endodermal nerve rings in stereo view. The sheath around the nerve rings' neurites does not really exist. The nerve rings on the detailed side face each other across the black mesoglea near the oral side of the tentacle. The arrow points to a mesogleal muscle fiber which is derived from the ectoderm. C, cone; Cx, calyx; Ec, ectoderm; En, endoderm; K, coelenteron; L, lip cells; M, mouth; T, tentacle

An important discovery is the ectodermal-endodermal nerve ring pair located at the junction between the oral cone and ring of tentacles (Fig. 2, 18). The endodermal nerve ring is embedded in calyx endoderm near the junction with the oral cone endoderm. The example in Figure 19 has about 32 neurites in the ectodermal ring and 9 in the endodermal one. No connectives bridging the mesoglea were noted. Synapses were not observed.

Except for one equivocal example, ganglion cells were not seen. This example consisted of a "perikaryon" next to an ectodermal nerve ring. The absence of cell processes and neurotubules in the plane of section were lacking in order to make a confident diagnosis. Early cnidoblasts can resemble ganglion cells with respect to their basal location and cell processes.

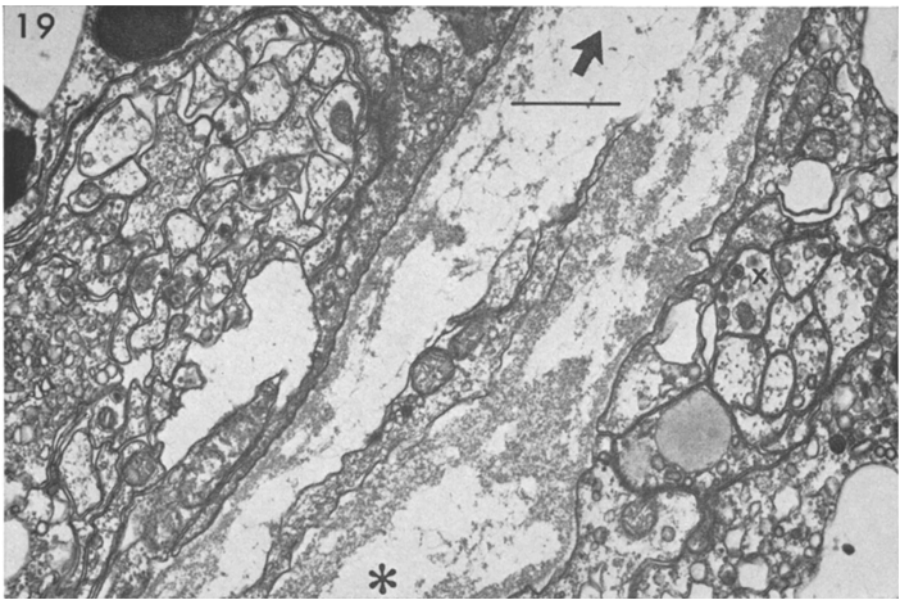


Fig. 19: Longitudinal section of the base of the oral cone showing the nerve ring pair, the larger ectodermal ring on the left and the endodermal one on the right. The arrow points towards the mouth. The neurite marked "X" contains granular vesicles and neurotubules; * mesoglea. TEM. Bar = 1 µm

Neurites are seen around the bases of the cnidoblasts at the tentacle tip but no neurocnidoblast junctions were detected.

Sense cells are seen on the tentacle especially at the tip. This cell is described in the next section.

A presumed neurosecretory cell is described below. The various silver techniques used were unsuccessful in demonstrating any of the elements of the nervous system.

Flagellated cells and flagellar derivatives

There are three ectodermal types. Werner (1975) found that the polyp of *Tripedalia* does not show any flagellar currents on the body surface and that squash preparations only show occasional flagellar movement.

The first type lacks microvilli around the usually static flagellum's base but there is a rootlet to the flagellum (Fig. 20) in the typical cnidarian relationship with the

basal bodies (Chapman, 1974; p. 10). This type is sparsely scattered over the ectoderm. This might be the type showing occasional undulatory movement of the flagellum.

The second type, presumably sensory, is (by electron microscopy) only seen on the distal region of the tentacles where they are common. This type has a field of microvilli at the cell surface (Figs. 21, 22). The microvilli are joined to their neighbors by lateral membranes which have a distinct glycocalyx. Supporting rodlets within

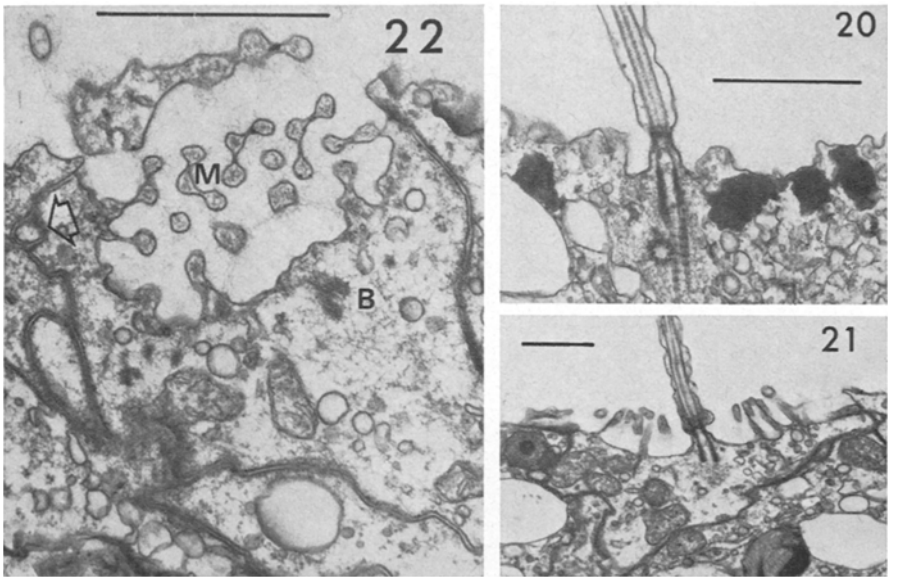


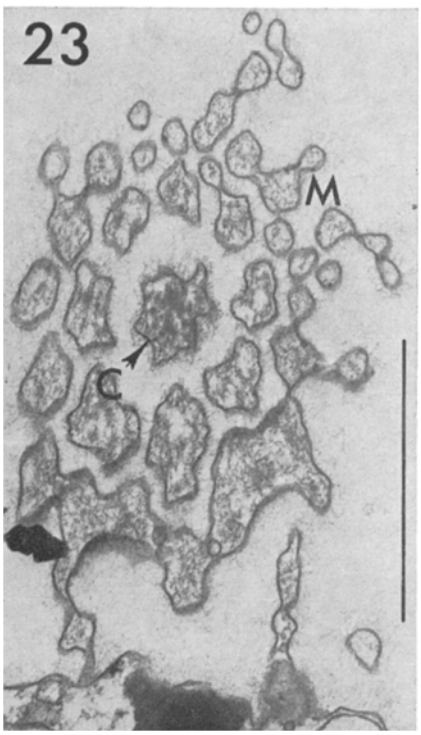
Fig. 20: Flagellated cell at the very end of the tentacle. Note the rootlet, the right angled orientation of the basal bodies and the absence of microvilli. TEM. Bar 1 μm
Fig. 21: Probable sense cell from tentacle. Note absence of rootlet. The oblique proximal basal body is not included in this section. TEM. Bar 1 μm
Fig. 22: Oblique section of a probable sense cell from the tentacle tip. Only one basal body is shown (B). The microvilli (M) are joined laterally and are coated with the glycocalyx. The arrow points to a plugged apical canaliculus. B, basal body; M, microvilli. TEM. Bar 1 μm

the microvilli continue on into the cytoplasm. The distal basal body is attached and in line with the usually static flagellum but the proximal basal body lies obliquely to one side of the distal one and no rootlet is present. The cytoplasm contains many small clear vesicles. At the sides of the tentacle's tip, these cells each have a basal process which curves proximally and deeply for a long distance. Microtubules were not seen in these basal processes. On the rest of the tentacles these cells are rather stubby and are directed radially. These might be the cells whose flagella Dr. Werner sees occasionally beating stiffly from side to side.

The third type, the cnidocil (Fig. 23), also has the usual 9 + 2 construction of microtubules but associated material surrounding the microtubules makes the picture somewhat indistinct. The vague double ring of long glycocalyx-covered microvilli

slant towards the eccentrically based cnidocil. Rodlets also support these microvilli. No flagellar rootlet was seen in the few examples studied.

Examples of intercellular flagella are seen in the tentacular ectoderm and even between two endodermal cells of the tentacle (see Chapman & James, 1973, for a discussion of this phenomenon).



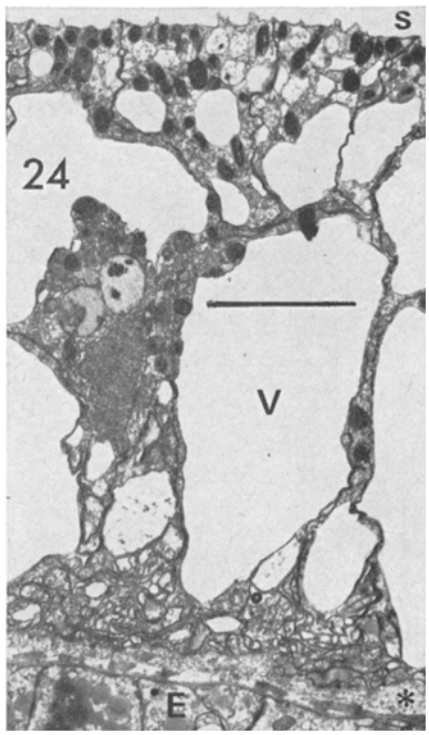


Fig. 23: TEM of a nearly transverse section of the cnidocil and associated microvilli of a cnidoblast. Bar = 1 μm. C, cnidocil; M, microvilli

Fig. 24: Transverse section through the calyx ectoderm. The intracellular vacuoles are large. TEM. Bar = 5 μm. E, endoderm; S, sea water; V, intracellular vacuole, *, mesoglea

The endodermal cells of the coelenteron have basal rootlets and may have basal collars of microvilli (Figs. 35, 41). The oral cone's endoderm has more flagella per unit area than the endoderm of the calyx and stalk. The endodermal flagella exhibit the usual beating movements.

Ectoderm of the calyx

Perhaps the uniform epithelial part of the ectoderm is always part of epitheliomuscular cells but this could not be proved. The columnar epithelial part is about 20–30 μm high by about 5 μm wide and contains a few moderately large intracellular

vacuoles (Figs. 4, 24). Like the rest of the polyp the ectoderm is covered by a delicate glycocalyx. There are a few microvilli and sometimes a flagellum is seen. The round to highly indented nucleus is seen about half way along the cell. This nuclear indentation seems to be characteristic of all ectodermal regions. Near the nucleus is a Golgi apparatus engaged in forming special granules which travel to the cell apex.

Ectodermal granules

Just deep to the surface of the ectodermal cell surface is a fairly even distribution of membrane-enclosed 0.5 μm wide granules having a dense core (Figs. 25, 48). Some

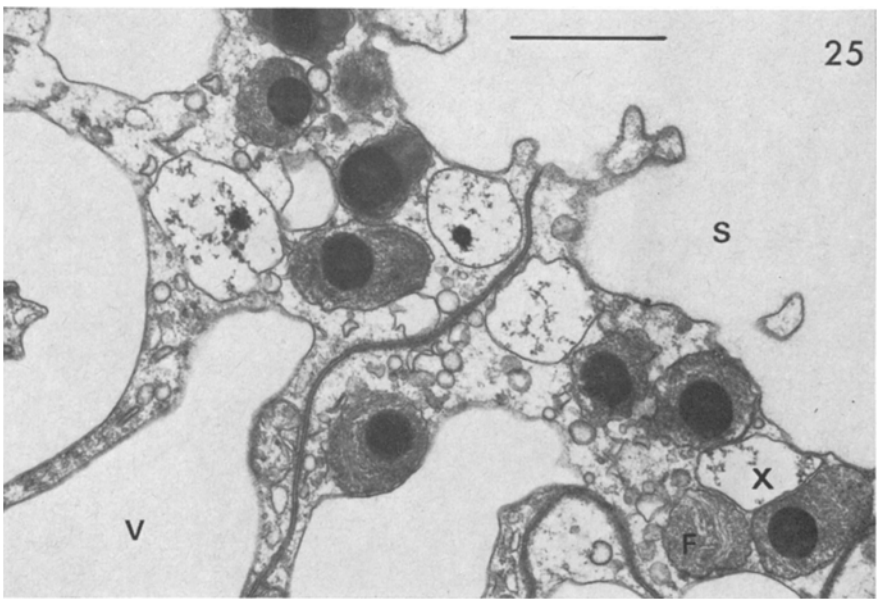


Fig. 25: Ectodermal surface of calyx showing the dense-core granules. The moderately dense material tends to become fibrillar (F). Some vesicles seem to have emptied their contents (X). A faint glycocalyx covers the surface. TEM. Bar = 1 μm. S, sea water; V intracellular vacuole

granules can be seen deeper in the cell where they seem to be formed by the Golgi apparatus. This organelle buds off small granular and agranular vesicles which seem to come together as multivesicular bodies which darken and take on a denser core as they approach the surface. The moderately dense peripheral material can become fibrous (Fig. 25). Some granules form parallel straight microtubules. These granules when found at the tentacle's tip tend to be crenellated. Other granular vesicles look empty; however, a communication to the outside has not been noted.

These granules stain red with the iron acid eriochrome technique and give positive reactions for the mercuric-bromphenol blue and DNFB techniques; hence the granules have at least a basic protein component. They are also PAS, luxol and

argentaffin positive thus indicating respectively carbohydrate, phospolipid and reducing groups.

The stalk

The stalk ectoderm (Fig. 5) is like that of the calyx but may be thinner and normally covered by the periderm. The pedal disk ectoderm shows no degeneration as it often does in *Aurelia* (Chapman, 1968). A few rivet-shaped desmocytes (Fig. 5) (Chapman, 1969) anchor the mesoglea to the basal periderm. The endoderm is like that of the calyx but there are fewer basal droplets.

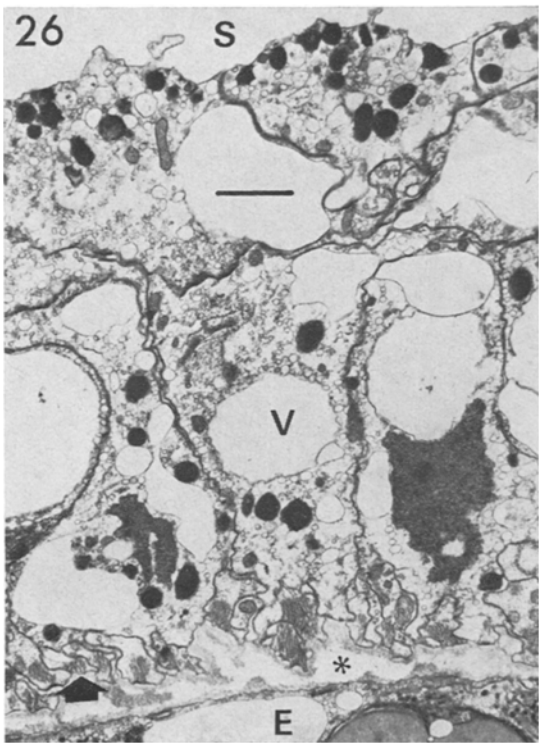


Fig. 26: Transverse section through the oral cone ectoderm. The intracellular vacuoles are smaller than those of the calyx. The arrow points to a myofibril. TEM. Bar = 1 μm. E, endoderm; S, sea water; V, intracellular vacuole; *, mesoglea

Ectoderm of the oral cone

The ectoderm of the oral cone (excluding the lip cells) is like the calyx except that the intracellular vacuoles are smaller but they increase in size from the lip towards the tentacles (Figs. 3, 26).

Ectodermal cell junctions

Sometimes there are no special junctions; however, septate junctions do occur near the surface. These may even encircle a process seen between cells near the cells' apices. In some cnidarians a circumapical canaliculus is present and this can be blocked by a cell process (Chapman, 1974). In the cubopolyp the canaliculus is always plugged (Figs. 14, 22) and does not fully encircle. Tangential sections of the surface show the full longitudinal view of such a plugging process. Faint striations representing the septate junction's septa run lengthwise along the plug. This picture is especially noticeable around sense cells.

Lip

Distinctive cells cover the distal extremity of the mesoglea (Figs. 3, 27) thus forming a lip for the mouth. The apex of a lip cell has a flagellum and a few scattered microvilli on its bulged surface; the basal part curves proximally as it approaches the

Fig. 27: Drawing based on electron photomicrographs of a radial section just to one side of the mouth indicated by the arrow. The black layer at the * is the mesoglea. Around the oral termination of the mesoglea, special lip cells (L) are wrapped. Farther into the coelenteron (K) the lip cells give way to endoderm lining the rest of the oral cone. Bar = 10 μm

mesoglea. The nucleus resides in the basal part. In the cytoplasm are many small clear vesicles as well as membrane-bound granules 0.5 μm wide and having a denser core (Fig. 28). Histochemically lip granules are like the ectodermal surface granules.

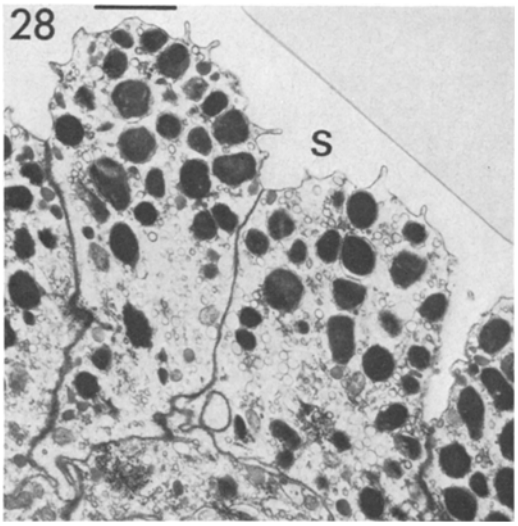
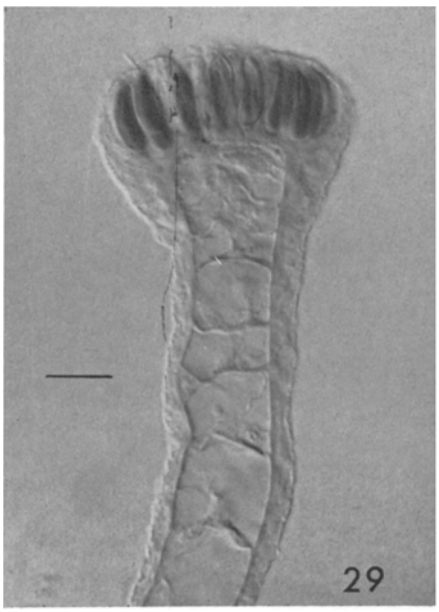


Fig. 28: Radial section of oral cone showing lip cells near the mouth. TEM. Bar = 1 µm. S, sea-water area



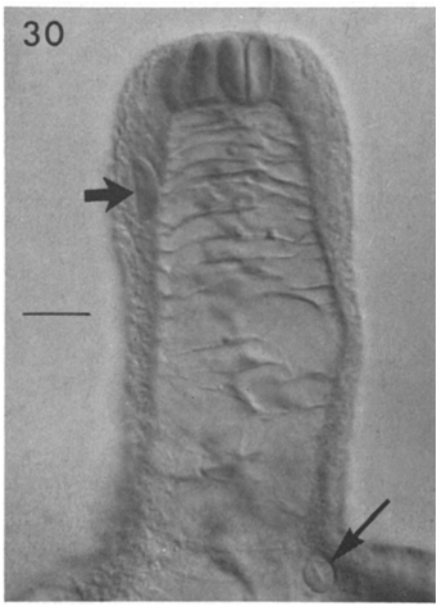


Fig. 29: Fully formed tentacle. The tip is swollen but the rest is more slender than the bud's tentacle. Optical sections of whole mounts mounted in Canada balsam. Nomaraki optics. Bar = 25 μm

Fig. 30: Tentacle of a bud. Note the rectilinear shape with apical packing of nematocysts. A migrating nematocyst is at the broad arrow. At the thin arrow is another smaller nematocyst type which does not enter the tentacle. The endodermal core cells are like thin disks distally. Optical sections of whole mounts mounted in Canada balsam. Nomaraki optics. Bar = 25μm

The tentacles

Briefly, the single row of tentacles (Figs. 1, 2, 18, 29, 30) are capitate, solid, covered on their sides mainly by epitheliomuscular cells and they are part of the calyx as one can see when their endodermal roots are examined because here the endodermal core of the tentacles is covered by endoderm characteristic of the calyx, there often being an identation separating calyx endoderm from the different endoderm of the oral cone.

Tentacle core cells. The core is made up of a single row of large highly vacuolated cylindrical endodermal cells about 40 μm long by 37 μm wide (Fig. 29). The usually single vacuole in mature cells approaches to within 0.13 μm of the plasma membrane (Fig. 13, 14). This thin rim of cytoplasm contains a circular system of filaments as mentioned in the section on the muscle system. The nucleus with its thin rim of cytoplasm lies usually proximally along the central axis of the cell. A few cytoplasmic struts join the perinuclear cytoplasm to the peripheral cytoplasmic rim. Where neighboring core cells make contact, sometimes there is a complicated peripheral interdigitation of the plasma membranes as described in *Aurelia* (Chapman, 1970). Near the nucleus is a Golgi body making granular vesicles. A rather surprising vesicle

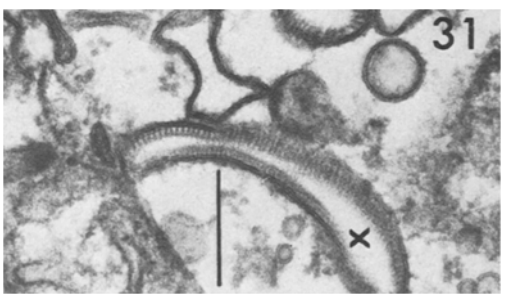


Fig. 31: TEM of a discoid coated vesicle (X) from a tentacle's endodermal cell. Bar = 200 nm

type in the form of discoid coated vesicles is encountered here (Fig. 31). Such vesicles are characteristic of absorptive cells lining the coelenteron (Slautterback, 1967). Structures resembling phagosomes are frequently observed and two even contained a large myofibril. At the tip of the tentacular core, the core cells are short and contain small vacuoles (Fig. 33). The core cells are encased in mesoglea 0.2 μm in thickness.

The tentacle's ectoderm. As mentioned in the section on the muscle system the distal 80 μm stretch of epitheliomuscular cells has striated myofibrils. Next is a neighboring mixed zone of striated and smooth myofibrils. The rest is just smooth myofibrils in the proximal part of the tentacle.

A large montage was made of a transverse section of a tentacle in the distal region. There are only a few smooth myofibers here and altogether the number of both types of myofibers is 144. Neurites can be confused with thin epithelial processes. With this in mind the approximate number of neurites is 156. The outermost layer of the ectoderm is usually a thin non-nucleated cytoplasmic sheet; next is the

nuclear zone of the epitheliomuscular cells which rarely reach the surface. Often the epitheliomuscular cell can be traced to the myofiber and, oddly, the myofibril is rather small here. The neurite layer is superficial to the muscle layer which rests on the mesoglea.

The tip. At the distal end of the tentacle, it flares out a short distance giving a tip resembling an Erlenmeyer flask (Fig. 29). The cells at the tip's sides sweep

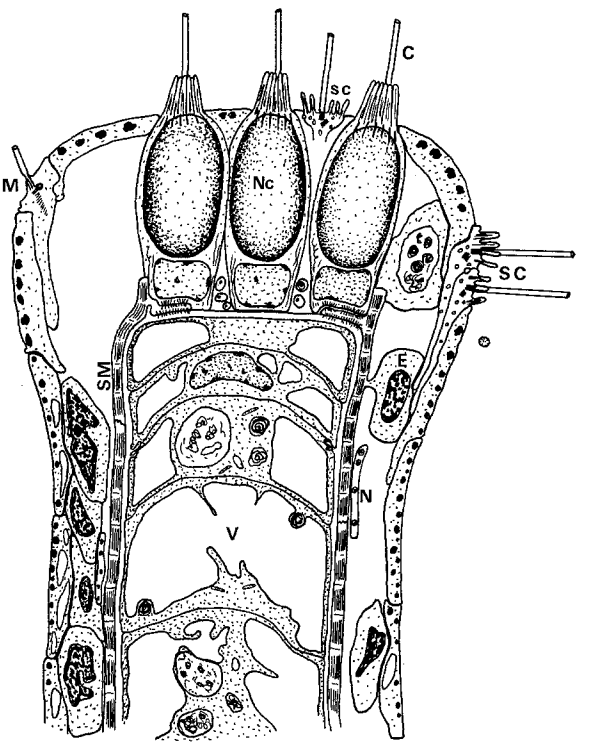


Fig. 32: Longitudinal section of the tentacle tip as constructed from electron photomicrographs. The tip is swollen due to the nematocyst-bearing cnidoblasts. The peripheral cnidoblasts rest their bases on a medially directed pedestal process from the striated muscle fiber. Desmosomes are a feature of the pedestal processes. The nature of the distal end of the muscle fiber was not ascertained. Neurites are found at the cnidoblast base. The mesoglea is just deep to the muscle fiber layer. Bar = 10 μm. C, cnidocil; E, epitheliomusclar cell; M, motor (?) cell; N, neurite; Nc, nematocyst; SC, sense (?) cell; SM, striated muscle fiber; V, vacuolated endodermal cell

proximally. The tip's side and end have the 3 types of flagelled cells mentioned above (Fig. 32).

In with the numerous sausage-shaped nematocysts is a single large ovoid nematocyst; there are rarely more. This often single nematocyst can be located cen-

trally, peripherally or anywhere inbetween. This same picture is seen in the tentacle tips of buds.

About 20–40 columnar cnidoblasts pack the tip and account for its swollen nature. The slightly curved large sausage-shaped nematocysts are packed so that the convex side faces laterally. The nucleus is basal with the cell's base resting on the mesoglea which covers the end of the most distal endodermal core cell.

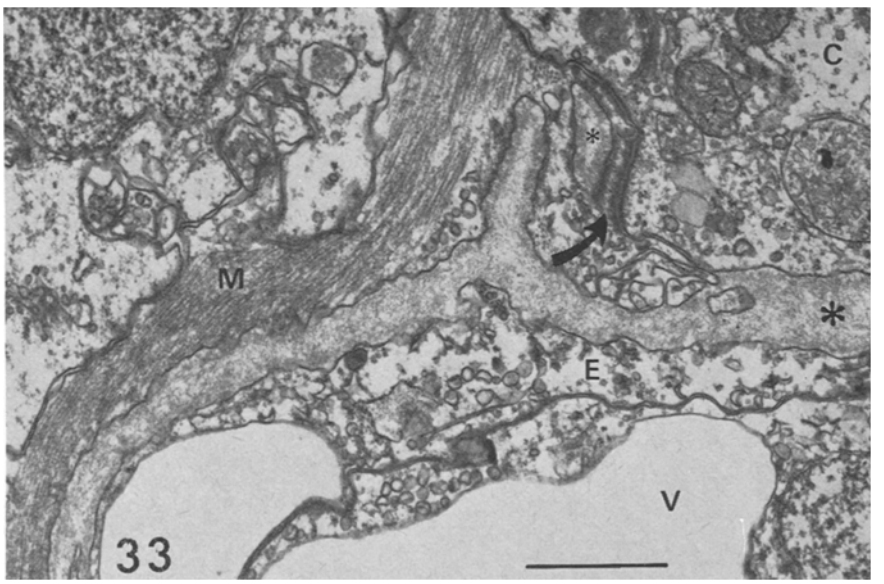


Fig. 33: Longitudinal section of the tentacle at the distal extent of the mesoglea (large *). A pedestal process of the striated muscle fiber extends somewhat medially, providing a base for a cnidoblast. The arrow points to the pedestal process with its desmosomes. This pedestal is split and contains mesoglea (small *). C, cnidoblast; E, endoderm; M, striated myofibril (somewhat oblique); V, intracellular vacuole; *, mesoglea

Polarizing microscopy showed that the striated myofibers continue on to the level of the nematocyst bases. At the level of the cnidoblasts' bases the striated myofibers send out a medially directed process to form a pedestal for a part or the whole of just the peripheral cnidoblasts (Figs. 32, 33). This process stands out because of its one or more desmosomes between cnidoblast and pedestal process and because of the hemidesmosome between the pedestal process and a corresponding thickening in the mesogleal fibril matwork.

Macrogranular cells

Scattered throughout the polyp's tissues are 9 μm wide cells referred to here as macrogranular cells because of the huge cytoplasmic granules (Figs. 4, 34). The cell

shape is round to oval with a peripheral nucleus mottled with much chromatin and a prominent nucleolus. The main feature of the cell is the numerous, large, dark, somewhat oval granules about 2 µm long. Some granules are not so dark but both types are bounded by a membrane. There are many small clear vesicles as well as a few large ones. A small round Golgi apparatus is found in what little cytoplasm is not taken up by granules and vesicles.

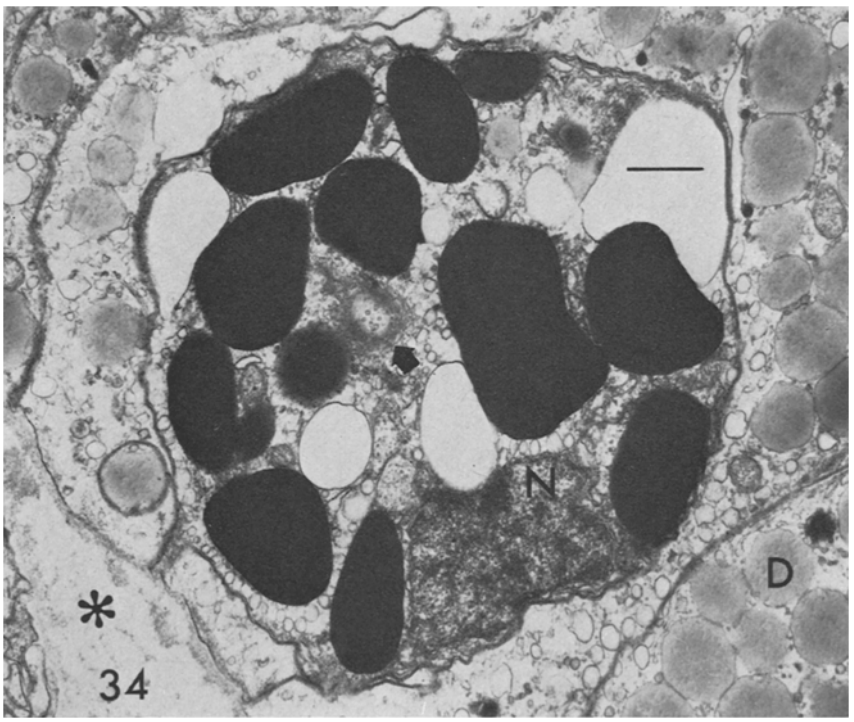


Fig. 34: Macrogranular cell in the endoderm. The huge granules and dark mottled nucleus are characteristic. Arrow points to the Golgi apparatus. TEM. Bar = 1 µm. D, droplets deep in the endoderm; N, nucleus; *, mesoglea

The macrogranules are positive for the DNFB and mercuric-bromphenol blue protein tests and also for the PAS reaction. There was no cytoplasmic basophilia as shown by a negative result using toluidine blue.

In a longitudinal section of a whole polyp, seven of these cells were seen in the ectoderm and five in the endoderm of the calyx and stalk; three were in the oral cone ectoderm – all evenly scattered. A few were noted in the tentacular ectoderm and are even rarer in the mesoglea.

Examples of these cells were found in an elongated condition in the ectoderm with a diminished granular content. The characteristic dark mottled nucleus retains its features in this probable transformations series.

Buds

Buds were not studied in detail. Light microscopy of plastic sections stained according to Richardson's method showed greenish droplets deep in the tentacle's ectoderm and in the cytoplasmic part of the tentacular endoderm. Such a feature was not noted elsewhere in the mature polyp which has these droplets mainly confined to the basal end of the endodermal cells of the calyx and stalk.

The bud's tentacles are wider and more rectilinear than those of the fully developed polyp (Fig. 30).

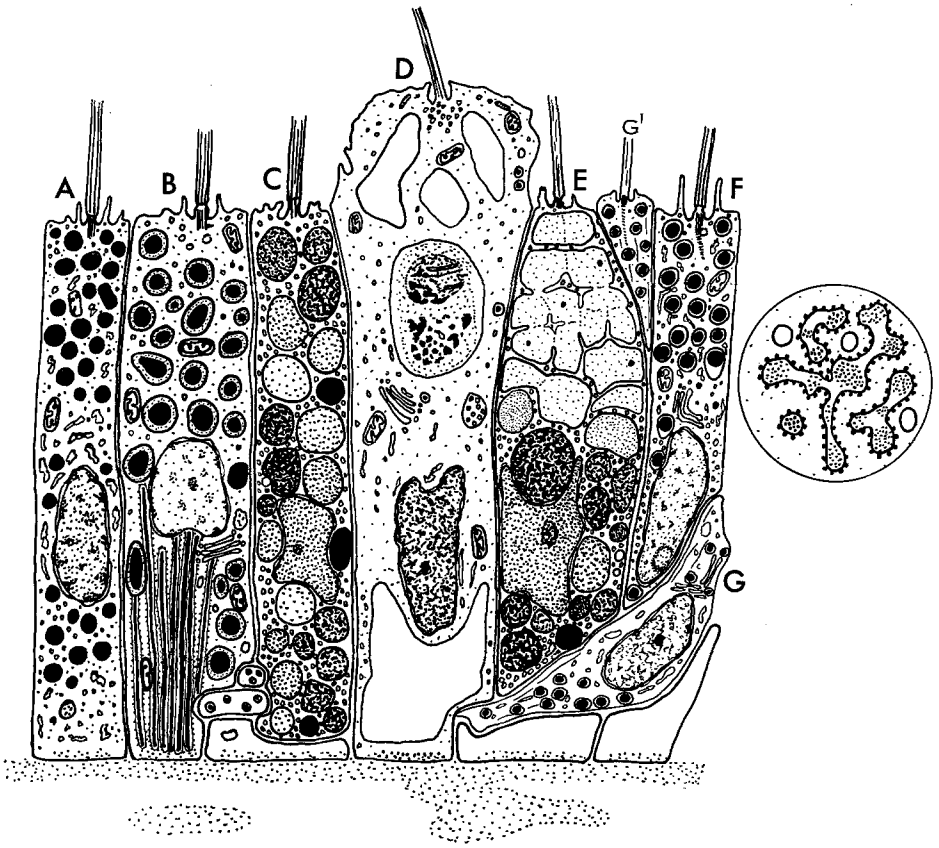


Fig. 35: Drawing of the cell types in the oral cone endoderm. A, Dark-granule cell. F, Dark-granule cell with areolar vesicles. B, Rodlet cell. E, Spumous cell. C, Spumous cell with non-hydrated granules. D, Absorptive cell. G, Basal cell, presumably neurosecretory. G', Apical process of a basal cell. Inset. High power view of a cross section of the rodlet cell's rodlets. Rodlets are enclosed by the rough endoplasmic reticulum

Endoderm of the oral cone, calyx and stalk

Apart from the lip cells which just enter the oral cone, the cone is lined by a special epithelium differing from that of the calyx and stalk which are the same.

Much difficulty was experienced in sorting out the number of different cell types on the basis of morphology because the same cell type in different phases of differentiation or secretion may look different. Histochemistry was attempted in order to give chemical distinctions but the techniques chosen (see Materials and Methods) were not discriminating. Some of the granule types were positive for combinations of the protein tests, the PAS reaction and for acidic mucopolysaccharides (alcian blue and astra blue).

Oral cone

Each of the cells in this simple 50 μm thick epithelium has a single flagellum and perhaps each has basal filaments oriented transversely to the polyp; there are approximately equal numbers of each of the following cell types (Figs. 3, 35). An exception to some of these points is a basal cell resembling a neurosecretory neuron; it is less common and lacks basal filaments.

A b s o r p t i v e c e l l (Fig. 36). This is the most voluminous cell and its apex is bulged beyond the other sorts. Small watery vesicles are found throughout the

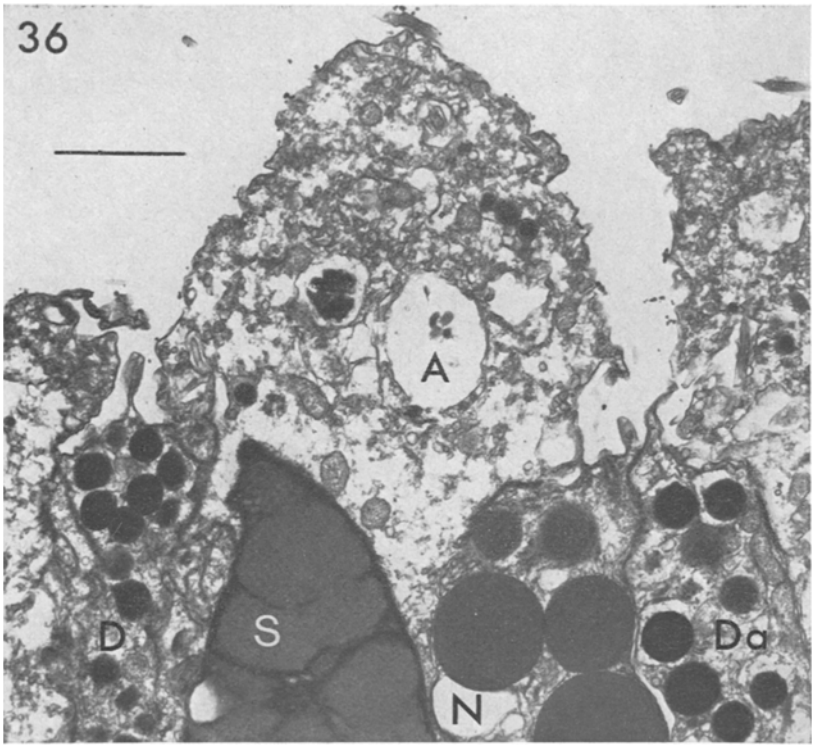


Fig. 36: Lining of oral cone. Surface showing apices of several cell types. TEM. Bar = 2 μm. A, absorptive cell; D, dark-granule cell; Da, dark-granule (areolar form) cell; N, spumous cell (non-hydrated form); S, spumous cell (hydrated form)

cytoplasm especially around the flagellum's basal bodies. A few large watery vacuoles are found, mainly apically; some vacuoles (phagosomes) contain digesting matter. Associated with the presumed absorptive and digestive functions of this cell are some apical discoidal coated vesicles (Slautterback, 1967) and some more numerous scattered granules which are likely lysosomes. The chromatin of the somewhat dark nucleus is uniformly distributed.

S p u m o u s c e l l (Fig. 36). The cell apex is swollen rather like a goblet cell because of the often confluent swollen mucous granules. More basally are the presumably less hydrated membrane-bound, 1–2 µm wide granules whose electron density varies from slight to great. The large nucleolus is rather obscured by the dense uniform scattering of chromatin. The cell labelled "C" is probably a less hydrated form of "E".

R o d l e t c e l l (Fig. 37). This cell has characteristic large 0.5–1.5 µm wide areolar granules and rodlets aligned parallel to the cell's long axis. The bundle of rodlets is either apical or basal to the nucleus but not in both locations. The rodlets measure 30–100 nm in diameter and are in turn made up of filaments 7 nm wide separated by a 6 nm space. The filaments in the widest examples can be seen to wind about themselves in a loose helix thus creating a moiré pattern in thick sections. Rodlets are enclosed by a rough endoplasmic reticulum. In transverse section several

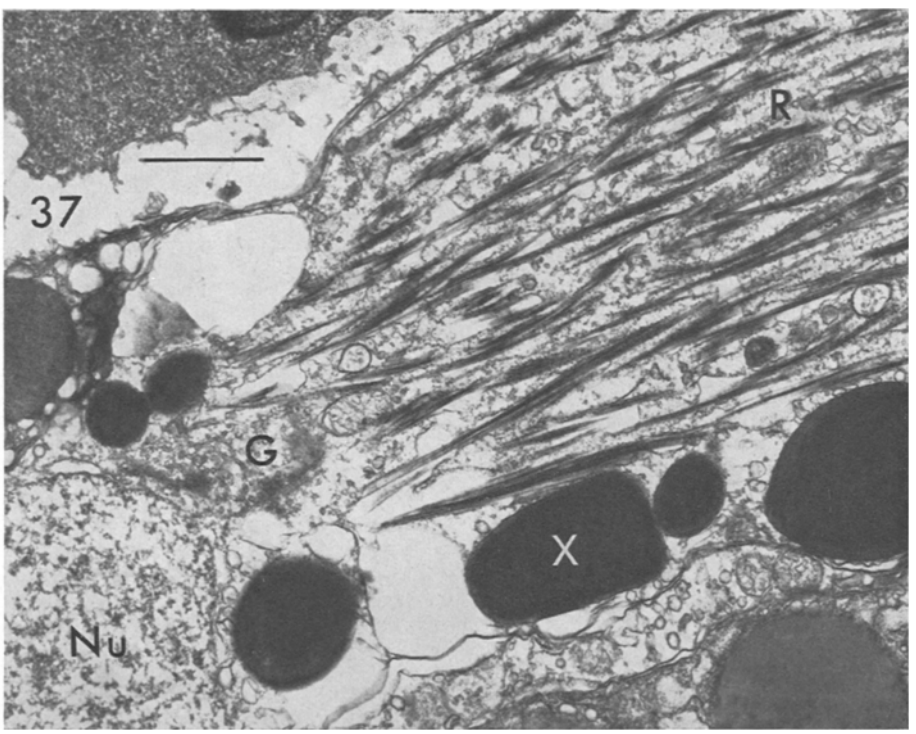


Fig. 37: Lining of oral cone. Rodlet cell showing its characteristic granules and rodlets. TEM. Bar = 1 µm. G, Golgi body; Nu, nucleus; R, rodlets; X, granule of rodlet cell

neighboring rodlets may be seen in the same cisterna which does not necessarily fully surround each rodlet before moving off to partially surround a neighboring rodlet. In this way several rodlets may be irregularly enclosed in the same labyrinthian cisterna (Fig. 35). Sometimes the rodlet indents the nucleus but usually the end nearest the nucleus stops near the Golgi body. Perhaps rodlet material is sent to the Golgi to be used in the formation of the areolar granules. The nucleus has fine granular chromatin.

Dark-granule cells (Fig. 36). These are narrow cells having many electron dense membrane-bound granules about 0.6 μm in diameter. The chromatin is distributed as small, irregular dark patches with intervening smaller granules.

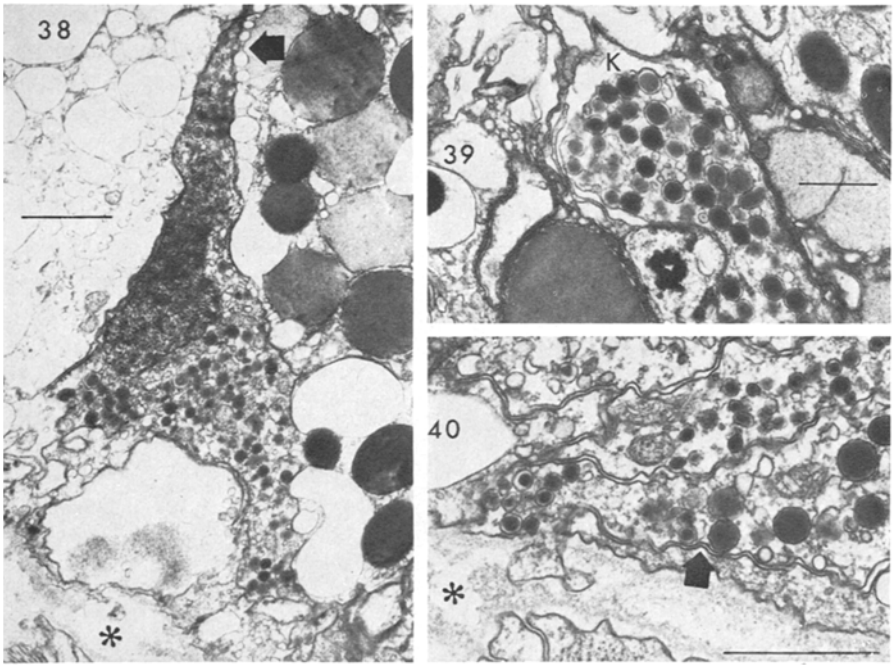


Fig. 38: TEM of basal cell from oral cone endoderm. A tripolar example with a process (at arrow) extending to surface. The nucleus is dark and the cytoplasm contains many areolar vesicles. Bar = 2 μm

Fig. 39: TEM of basal cell from oral cone endoderm. Basal cell process reaching the coelenteron at "K". Bar = 1 μm

Fig. 40: TEM of basal cell from oral cone endoderm. At the arrow, the large vesicles all of a sudden become smaller as they progress distally along a cell process. Bar = 1 μm. K, coelenteron; *, mesoglea

Another similar cell differs in that its granules are areolar (Fig. 36). These two cells might be different secretory phases of the same cell type.

Basal cell. This less common cell is found at or near the mesoglea (Fig. 38). The shape is multipolar, a bipolar spindle or even pear-shaped. This seems to be the cell stained by silver methenamine. The Golgi complex forms 300 nm wide areolar

vesicles with grey to black cores. Along a cell process the vesicles narrow to 100 nm (Fig. 40). Occasional hints of microtubules are encountered in the cytoplasm. These features suggest that basal cells are neurosecretory neurons. The nucleus resembles that of the dark-granule cell.

If basal cells are indeed neurosecretory then they are remarkable in that they are sometimes observed to send a wide process to the surface (Fig. 39) where it ends with a flagellum (Fig. 35). The granules at this location are wide unlike the smaller type in the other cell processes.

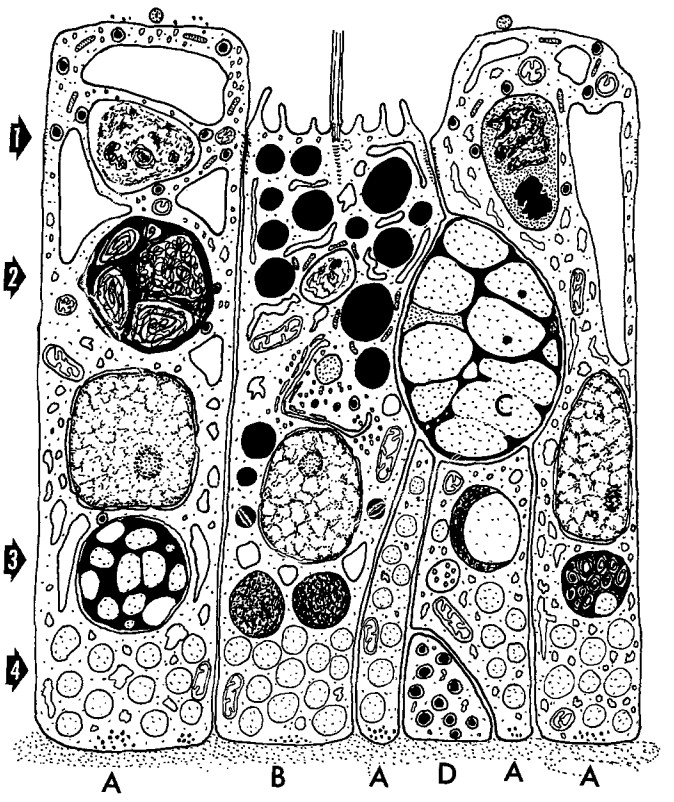


Fig. 41: Drawing of the cell types in the calyx and stalk endoderm. A, Absorptive cell. B, Spumous (non-hydrated form) cell. C, Spumous (hydrated form) cell. D, Process from a basal cell.
Absorptive cell level: #1, Early phase of digestion. #2, Further digestion. #3, Phagosome developing droplets. #4, Basal droplets are now free

Calyx and stalk

This epithelium in the slightly starved polyp can be more than 120 μm thick (Fig. 41), a high value for a simple columnar epithelium (Fig. 4). The thickness is not

uniform from region to region so that peaks and crevasses are present. Recently fed polyps seem to shed the supranuclear cytoplasm but this was only examined with paraffin sections so that detailed information is unavailable.

Absorptive cell. These form the majority (about 80%) of the cells of these regions. These are much like the absorptive cells of the oral cone except that there tends to be more discoidal coated vesicles in the calyx and stalk and that special electron lucent droplets are found basally. Figures 41, 42, 43 and 44 show how ingested material is progressively transformed into free basal droplets (Fig. 17). An irregular osmiophilic layer surrounds the droplets which stain bright green in the stained plastic sections. Now and then similar droplets are found across the mesoglea in the ectoderm. The droplets take up the Sudan black in frozen sections hence they contain lipid.

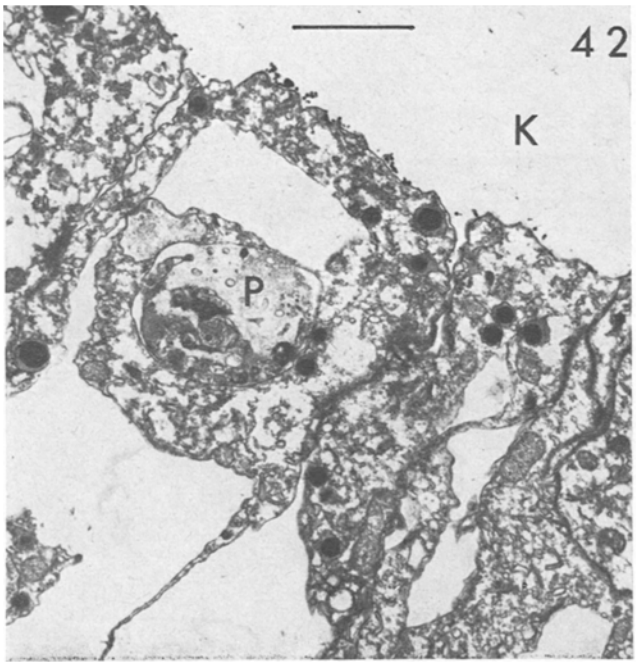


Fig. 42: TEM of the surface of an absorptive cell from the calyx endoderm. Shown are large vacuoles, a phagosome (P) and presumed areolar lysosomes. Bar = 2 µm. K, coelenteron

Spumous cell. The hydrated form of this cell is uncommon but the granular non-hydrated type resembling its counterpart in the oral cone is often encountered (Figs. 41, 45, 46). The difference between the cone and calyx forms relates to the small amount of phagocytosis observed in only the calyx form as if the cell's secretory and absorptive functions were not sharply distinct. Associated with the phagocytic vacuoles are the companion discoid coated vesicles, presumed lysosomes and basal droplets.

It was hoped that nuclear chromatin patterns might aid in sorting out the endodermal cell types. Some pycnosis of naturally moribund cells complicates the picture. The nuclei of the absorptive and spumous cells of the calyx were similar but they differ from their counterparts in the oral cone. In other words the cytoplasmic data do not correspond to the nuclear data.

Basal cells. Wide processes attributible to this cell type are encountered but the nuclear region was not seen (Fig. 41).

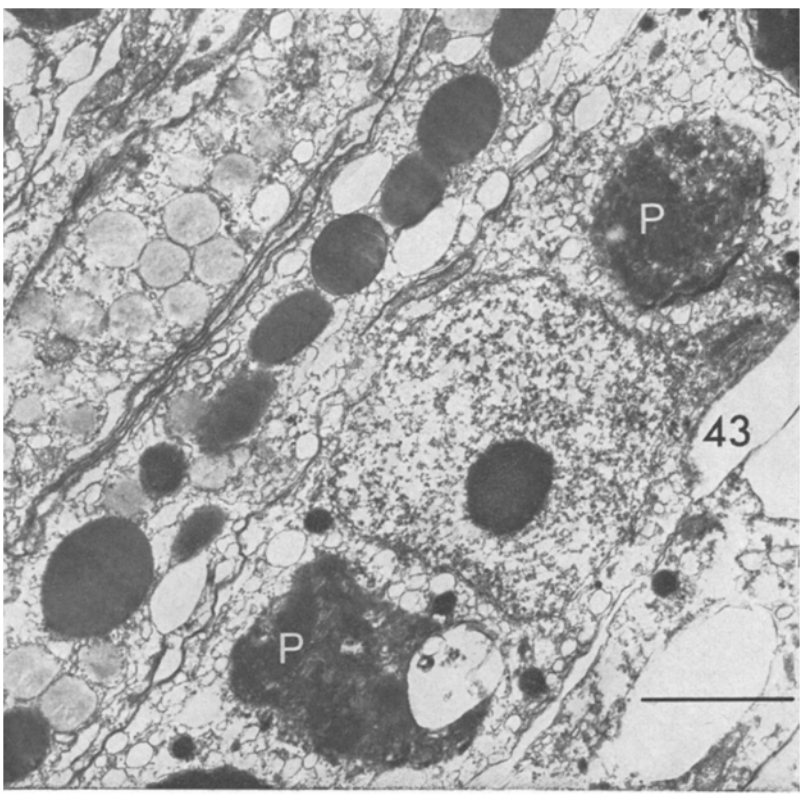


Fig. 43: TEM of the mid-region of an absorptive cell from the calyx endoderm. Shown are more advanced phagosomes (P) on either side of the nucleus. Bar = 2 μm

Macrogranular cell. These cells are found basally but no transition forms linking them to its neighbors in the endoderm are seen.

The coelenteron. In starved or fed polyps the mesoglea is circular as seen in transverse section; there are no centrally directed mesogleal partitions to form the basis of a set of septa. It is only in a recently starved polyp that about seven longitudinal folds of endoderm project into the coelenteron and hence resemble septa (Werner, 1975; his Fig. 11). Because these folds are variable in number, lack permanence and do not contain a mesogleal core, they are not septa.

The cnidome

Werner (1975) has dealt with this topic; however, some details may be mentioned here. But first, in summary, all the polyp's nematocysts belong to the heterotrichous, microbasic, eurytele category. This category is further subdivided into one sausage-shaped subtype, one large ovoid subtype and two small globular subtypes. The two small globular subtypes are confined mainly to the ectoderm of the calyx, stalk and

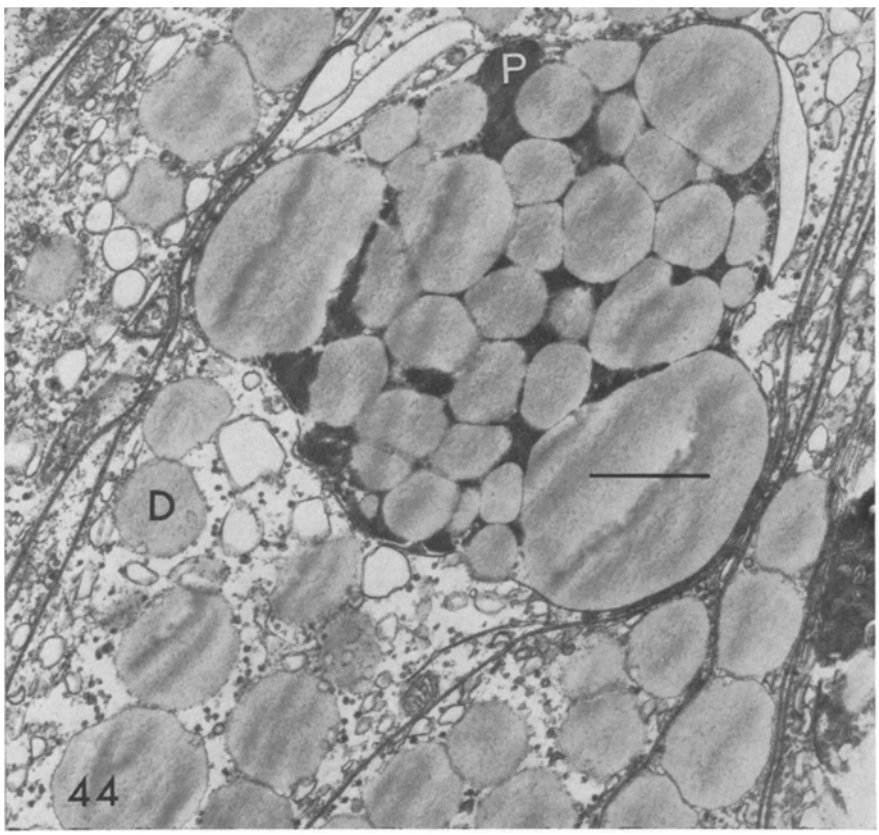
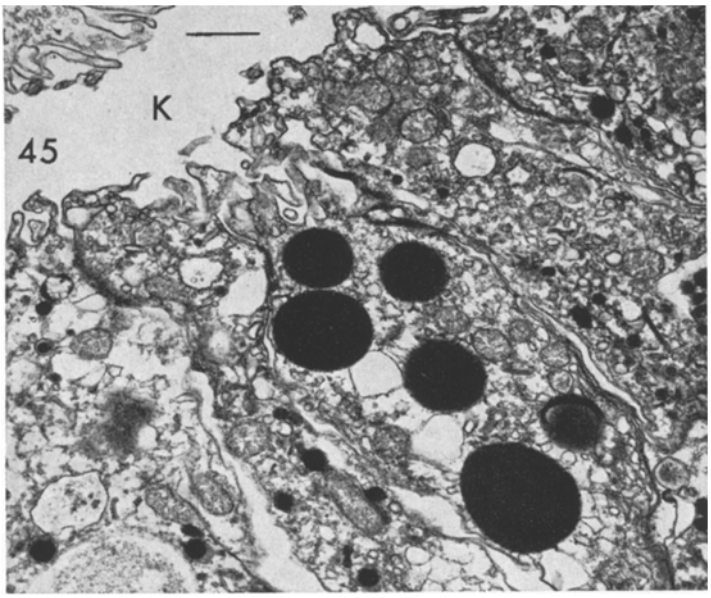


Fig. 44: Basal region of an absorptive cell from the calyx endoderm. Shown is the most advanced stage of the phagosome containing droplets. Basal to this are free droplets. Bar = 1 μm. D, droplet; P, phagosome

base of the oral cone. The tip of the tentacle, as mentioned above, contains the sausage-shaped nematocysts as well as usually just one of the ovoid subtype.

All types develop deep in the ectoderm of the calyx and stalk especially in the calyx near its junction with the stalk. A few developing ones have been noted in the endoderm of this region and others here appeared partly digested.

Cytologic signs of nematocyst loss can be seen here and there. Replacement at the



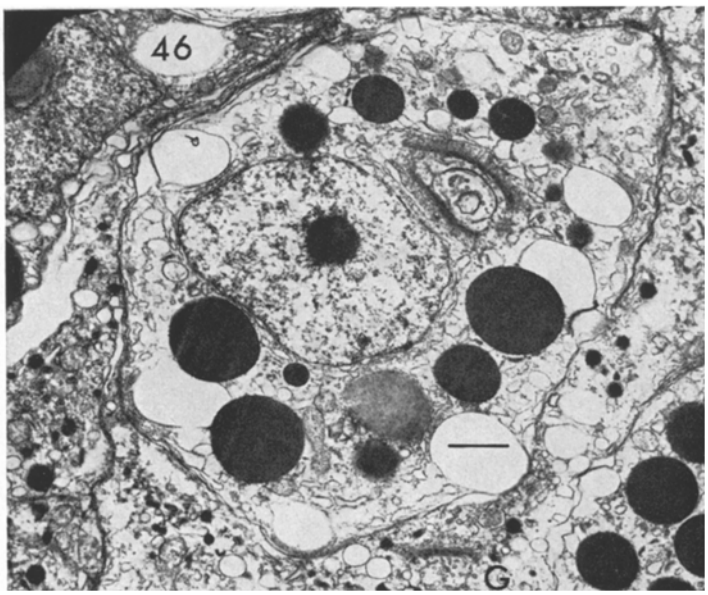


Fig. 45: Calyx endoderm. Longitudinal section of the apical end of the epithelial cells showing a central spumous (non-hydrated) cell surrounded by absorptive cells. TEM. Bar = 1 µm. K, coelenteron

Fig. 46: Calyx endoderm. Transverse section of a spumous (non-hydrated) cell containing a well developed Golgi body. Near the bottom of the photo is a Golgi body in an absorptive cell forming presumed lysosomes. TEM. Bar = 1 µm. G, Golgi body

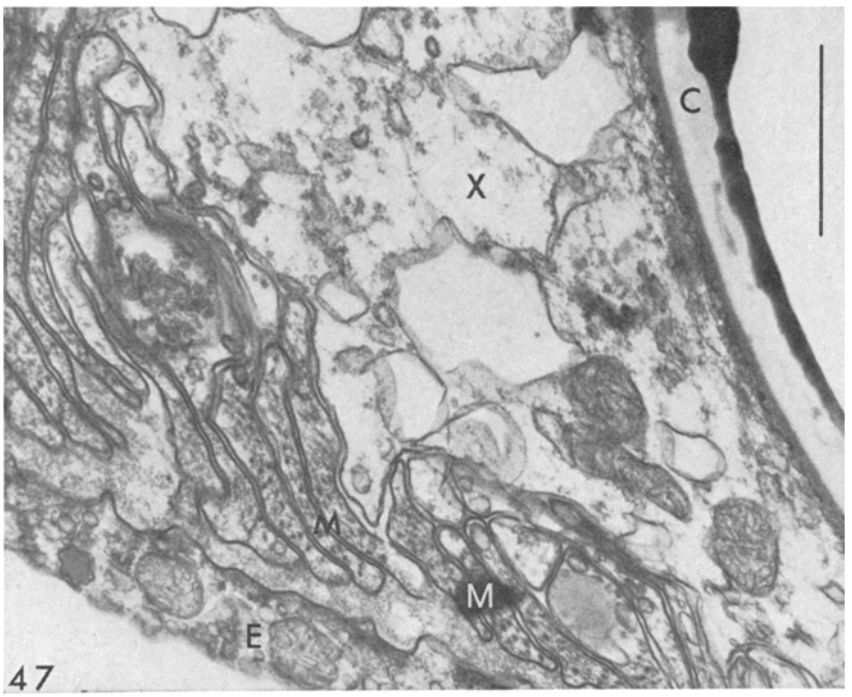


Fig. 47: Transverse section mid-way along a tentacle showing a migrating cnidoblast (X) containing a nematocyst of which only the capsule (C) is preserved. TEM. Bar = 1 µm. E, endoderm; M, muscle fibers

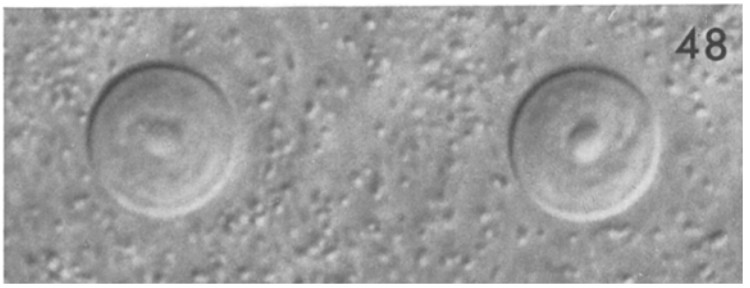


Fig. 48: Optical section just below the ectodermal surface of the calyx showing the surface granules and the apices of nematocysts each with a central operculum. Nomarski optics of a glycerin mount. Nematocyst is about 10 µm wide

tentacle's tip seems to be effected by cnidoblasts with mature nematocysts (Fig. 47) migrating intercellularly in the tentacular ectoderm with the long axis of the nematocyst directed parallel to the tentacle's long axis (Fig. 30). About half of the tentacles have one or seldom two of these migrating cnidoblasts.

Sections give the wrong impression of the number and disposition of nematocysts. It is far better to focus on and below the ectoderm's surface with a light microscope. Fig. 48 shows such a view below the surface.

DISCUSSION

Cytological considerations

The function of the large and numerous ectodermal granules is not apparent. Their argentaffin nature suggests phenolic compounds which might serve as a chemical defense against microorganisms (Monné, 1960). There is also slight evidence that they may participate in the formation of the peridermal cup as in *Aurelia* (Chapman, 1966) because they are depleted opposite the periderm. Their form resembles certain granules in the neurons of *Aplysia* (Henkart, 1975). Henkart finds that the denser central region of the granule is due to the presence of calcium which leaves the granule after light illumination with the result that myelin figures develop.

A proper study of the digestive and absorptive functions of the endoderm requires more work using a series of polyps sacrificed after various durations following feeding. Food markers and enzyme histochemistry would contribute towards sorting out the many cell types found lining the coelenteron. This study does at least prove that the cubopolyp has more endodermal cell types than the hydropolyp (Bouillon, 1966).

Bouillon also showed that a few hydropolyps had a special zone of cells at the lip region remarkably like the cubopolyp with respect to form and histochemistry.

Neurites are often encountered in the deeper regions of the ectoderm and endoderm. The problem is finding their source because ganglion cells were not seen except for one doubtful example lying against a nerve ring. Perhaps further work will disclose more ganglion cells associated with the nerve rings which would be an important source of neurites distributing to other regions. Sense cells and the basal cells (presumed neurosecretory cells) provide other sources; nevertheless, in hydropolyps and anthopolyps it is usual for there to be scattered ganglion cells as well.

As Werner (1975) has pointed out, there is a strange difference between the cubopolyps of *Tripedalia* and *Carybdea: Carybdea* has strong flagellar currents on its ectoderm but *Tripedalia* has none. It is suggested here that the flagellated cell with the rootlet and no microvilli has a "paralyzed" flagellum in *Tripedalia* but a motile flagellum in *Carybdea*. Furthermore, it is suggested that the other ectodermal cell with a static flagellum, no rootlet but with well-formed microvilli is a sense cell.

The basal cells of the oral cone's endoderm look very much like neurosecretory neurons (see Chapman, 1974) because of the microtubules, cell shape, location and nature of the anatomical evidence that some, at least, of these cells are in position to release granules to the coelenteron which is a digestive cavity. Neurosecretory cells in other animals are best known for releasing neurosecretion to the blood or intercellular spaces but it is only lately that it is becoming clear that hormone-containing

cells are located even in the mammalian gut epithelium, and may in some cases secrete into the gut lumen (Forssmann et al., 1969).

One intestinal cell type is the argentaffin cell which contains 5-hydroxytryptamine and may even have basal processes (Falck & Owman, 1968). Histochemical tests for 5-hydroxytryptamine (of which silver methenanine is one test) show up cells in the cnidarian coelenteron (Wood & Lentz, 1964, and *Tripedalia*). Wood & Lentz shrewdly thought that these cnidarian cells might "be analogous to the argentaffin cells of the mammalian intestine". Perhaps in the cubopolyp, neurosecretion plays a role in digestion and absorption.

Cross-striated myofilaments all probably contain paramyosin and usually are wider than those lacking striations (Ebashi & Nonomura, 1973). The periodicity of these striations can vary and its significance is not known (Morrison & Odense, 1974). When an oblique chequered banding is also present then such myofilaments are part of a "catch" fiber in the pelecypods studied by Morrison & Odense. "Catch" is the phenomenon whereby slow but great muscular tension due to little stimulation may be maintained for long periods with scant energy expenditure; there is an associated high rigidity when muscle is in this state (see Prosser, 1973). It is still possible for there to be a degree of catch even when the chequered oblique banding is not present as Swanson (1971 a, b) discovered in the Nematomorpha.

The mesogleal myofibers of both cubopolyps studied have wide cross-striated myofilaments; the other smooth myofibers in the cubopolyps have thinner and vaguely cross-striated myofilaments resembling the four longitudinal muscles in the polyp of *Aurelia* (unpublished observations). Paramyosin is, therefore, likely present. It is not known from the cubopolyp's behavior or physiology whether catch is shown and it is not readily imagined what use catch could serve. Perhaps it is not the catch but the associated rigidity that is of more value to the polyp in providing a skeleton of variable stiffness to the otherwise soft polyp. For the pelecypod this rigidity is presumably a useless concomitant phenomenon.

5-hydroxytryptamine is likely the natural relaxer for the catch mechanism in pelecypods (Twarog, 1954). Fig. 10 shows neurosecretory neurites among the mesogleal myofibers. Since there is reason for believing that basal cells (see below) contain 5-hydroxytryptamine and that they are actually neurosecretory, it may well be that catch is released by the secretions of the neurosecretory neurites. Another neurite type lacking granular vesicles is envisaged as giving contraction and finally catch.

Another example of a paramyosin-like myofilament has been described by Perkins et al. (1971) for *Chrysaora*'s fishing tentacle's ectodermal musculature. This myofilament was only observed if the tentacle had first been highly shortened then allowed to re-extend from 7.2 to 20 times its contracted length. This example is incomprehensible in itself and sheds no light on the cubopolyp's condition.

A muscle cell type containing just fine myofilaments has been recognized for a long time (Chapman, 1965). A good histochemical test for actin filaments is now available (Goldman, 1975) and should be applied to the presumed endodermal musculature of the cubopolyp. It may be, therefore, wrong to think of the hydropolyp and anthopolyp as the only cnidarian polyps with a circular endodermal muscle field.

The cubopolyp shows a gradation series from typical epitheliomuscular cells

with distinctive epithelial and muscular regions to cells in the mesoglea where the nucleus is peripheral to the muscular part to the final stage (also in the mesoglea) where the nucleus is surrounded by the contractile portion thus producing a pure myocyte (Chapman, 1974; p. 3). From this it may be concluded that epitheliomuscular cells and pure myocytes do not represent two basically different muscle cell types in the Cnidaria.

Striated myofibers in the Cnidaria are almost always a feature confined to the pulsating musculature of the medusoid bell. It was a surprise to find striated myofibers near the tips of the tentacles on the polyp of *Tripedalia*. It is difficult to imagine the significance of this.

Sometimes cnidarian myofibrils have a radial component which is in continuity with the main longitudinal component (see Chapman, 1974; p. 4). Certain hydropolyps (e. g. see Bouillon, 1968; his Figs. 4, 5) and the scyphopolyp of *Aurelia* (Chapman, 1970) possess these radial components but the cubopolyp does not.

The endodermal core cells of the scyphistoma's tentacle provide a hydrostatic skeleton for the tentacle (Chapman, 1970). The cubopolyp's tentacular core is similar. Since each seemingly metabolically inert cell is for the most part a huge watery vacuole in the scyphistoma, it comes as a surprise in the cubopolyp that the perinuclear cytoplasm has discoidal coated vesicles and sometimes large phagosomes containing digesting muscle fibers – all indicating more than a little metabolic activity. Dr. Werner suggests that these hints of phagocytosis presage the more intense phagocytic rôle these cells play during metamorphosis when the distal parts of the tentacles are resorbed.

The nematocysts in the tip of the tentacles would seem to pose an interesting packing problem. By analogy, the problem is the same as finding the best way to pack two dozen bananas and one large lemon into a pail. The answer is to have all fruit upright with the lemon central and the bananas in concentric rings concave side in. Although the banana or sausage-shaped nematocysts are oriented with their concavities centrally directed, the location of the ovoid (lemon) nematocyst is only randomly central.

One might wonder about the loss of the ovoid nematocyst from the tip and whether there is a mechanism to recruit selectively a replacement of the same type which would migrate along the tentacle to the tip.

Interrelation among cnidarian polyps

As a cnidarian polyp, the cubopolyp is diploblastic and possesses a mesoglea. At the cytological level there are the cnidarian features of nematocysts, rivet-shaped desmocytes (Chapman, 1969) and the spatial relationship among the flagellum's basal bodies and rootlet complex (Chapman, 1974; p. 10).

At the Class level the cubopolyp may be compared to the scypho-, hydro- and anthopolyps.

The cubopolyp's coelenteron lacks septation and thus resembles a hydropolyp. The pedestal process and its desmosomes resemble the similar condition in the

Hydrozoa (see Chapman, 1974; p. 34). Some hydropolyps and the known cubopolyps have capitate tentacles. Closer examination, however, discloses that they are not exactly the same because in the hydroid the nematocysts project radially in all directions but in the cubopolyp the nematocysts all point distally.

Scyphopolyps differ from both hydro- and anthopolyps in that there are longitudinal muscle fibers free in the scyphopolyp's mesoglea (except where they originate from the oral disk ectoderm). This musculature is present as muscle tubes made up of a ring of muscle fibers. The cubopolyp also has mesogleal muscles in the calyx but here the muscle fibers may be single or a few may be clumped together in a non-tubular array. In this feature the cubopolyp is more like the scyphopolyp than the other two types.

It has been argued that there are functional and morphological similarities between hydrozoan interstitial cells on one hand and the amoebocytes of the Scyphozoa and Anthozoa on the other (Chapman, 1974; p. 24). Since the cubopolyp does not have interstitial cells, nor any small basophilic type of cell, it is suggested that the cubopolyp's macrogranular cell type belongs to the amoebocyte category hence this feature puts the cubopolyp closer to the Scyphozoa than Hydrozoa.

Carybdea spec. has strong ectodermal flagellar currents so that it is odd that *Tripedalia*, another cubopolyp, has none (Werner, 1975). One cannot say what the primitive condition is. The scyphozoan condition is to have ectodermal flagellar currents in the polyp (Chapman, 1973) whereas the hydropolyp's ectoderm is seldom flagellated according to Hyman (1940; p. 411).

An ectodermal-endodermal nerve ring pair seems to be a feature peculiar to cubopolyps. This nerve ring seems to be carried over to the medusa phase. Although hydromedusae have a nerve ring pair, it is confined to the medusa and the components are both ectodermal. The scyphopolyp of *Aurelia aurita* has a number of ectodermal nerve rings in the oral cone (Chapman, unpublished).

Another unique cubopolyp characteristic is the ectodermal striated musculature in the tentacles.

The cubopolyp shares features with the hydro- and scyphopolyps as well as having its own peculiar characteristics. This is sufficient reason, therefore, that these animals be placed in their own category – the Class Cubozoa.

Acknowledgement. I owe thanks to Dr. B. Werner for allowing me to be the first to satisfy the curiosity concerning the structure of the cubopolyp.

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