

Fine Structure and Behaviour of the So-called Cuvierian Organs in the Holothuroid Genus *Actinopyga* (Echinodermata)

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Abstract

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Actinopygid Cuvierian tubules are few in number. They are made of a basal trunk from which arises 2–3 branches. The trunk is smooth and hollow (proximally) or slightly swollen and solid (distally) and the branches consist of a central rachis to which attach many peripheral spherules. The fine structure of the tubules is similar in the three investigated species of *Actinopyga* but differs considerably from that of non-actinopygid tubules. Basic behavioural differences occur also as actinopygid Cuvierian tubules cannot elongate nor become sticky, and are not expelled by the individuals. It is concluded that actinopygid Cuvierian tubules do not fulfil a defensive function.

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Introduction

Some species of aspidochirote holothuroids belonging to the genera *Actinopyga*, *Bohadschia*, *Holothuria* and *Pearsonothuria* have particular intracoelomic organs, namely the Cuvierian organs, that attach to the basal part of a respiratory tree. They consist of several to many blind tubules that are either smooth (most genera) or rugged (*Actinopyga*) (Ludwig & Barthels 1892; Hyman 1955). They have been studied mostly in *Holothuria* spp. where they function as defensive organs. When stimulated, *Holothuria* expel some of the tubules through the anus; expelled tubules lengthen, become sticky, and immobilize the organism with which they come into contact (Jourdan 1883; Mines 1912; VandenSpiegel & Jangoux 1987). The fine structure and the mechanism of discharge of the Cuvierian tubules of *Holothuria forskali* (Delle Chiaje, 1823) were studied by VandenSpiegel & Jangoux (1987). They reported that tubule elongation results from both the mechanical stimulation of the tubule sphincter by hydrostatic pressure and the entry of water into the tubule.

Cuvierian tubules of *Actinopyga* spp. are claimed to be toxic (Nigrelli & Jakowska 1960; Chanley & Rossi 1969a,b). Morphological descriptions by previous authors showed that they are histologically different from those of non-actinopygid genera (Semper 1868; Ludwig & Barthels 1892; Hyman 1955; Mosher 1956). Whether or not they can be expelled through the anus and function like the Cuvierian tubules of *Holothuria* spp. is not known.

The aim of the present paper is to describe the behaviour, investigate the fine structure, and discuss the possible function of the Cuvierian organs in several Indo-Pacific species of *Actinopyga*.

Materials and Methods

Individuals of *Actinopyga echinata* (Jaeger, 1830), *Actinopyga mauritiana* (Quoy et Gaimard, 1833) and *Actinopyga miliaris* (Quoy et Gaimard, 1833) were collected on the reef flat of Laing Island (Hansa Bay, Papua New Guinea) in July and August 1988. *In vivo* observations were made in the King Leopold III Marine Biological Laboratory of Laing Island using individuals kept in an open-circuit marine aquarium.

For histological investigations, Cuvierian tubules were removed from individuals previously anaesthetized for 1 h at 0°C in a 1% marine solution of propylene phenoxetol. Tubules were fixed in Bouin's fluid, embedded in paraplast and cut into 7 µm thick sections. Bouin-fixed sections were used for routine histology (Masson's Trichrome) and histochemistry (the Periodic Acid Schiff and the Alcian Blue pH 2.6 techniques) according to the procedures of Ganter & Jollès (1969–70).

TEM and SEM investigations were performed only on *A. echinata* and *A. mauritiana*. For TEM study, tubules were fixed at 4°C in a 3% solution of glutaraldehyde in cacodylate buffer (0.1 M, pH 7.3). They were washed in buffer, post-fixed for 1 h with 0.1% osmium tetroxide in 0.1 M cacodylate buffer, and washed again in buffer. Tubules were dehydrated in graded ethanol, embedded in Spurr medium and sectioned using a LKB V ultramicrotome. Semi-thin sections were stained according to the method of Humphrey & Pittman (1974). Ultra-thin sections were contrasted with uranyl acetate and lead citrate, and observed with a Zeiss EM 10 transmission electron microscope. For SEM study, tubules were either fixed as above or fixed in Bouin's fluid. They were dehydrated in ethanol before being dried by the critical-point method using CO₂ as the transition fluid. Tubules were mounted on aluminium stubs, coated with gold in a sputter coater and observed with a JEOL JSM 6100 scanning electron microscope.

Results

External morphology and behaviour

When observed *in vivo*, the Cuvierian organs of the three investigated species of *Actinopyga* consist of a reddish tuft of branched tubules (no more than 10 tubules per

individual). Tubules attach independently to the basal part of the left respiratory tree near the cloaca (Fig. 1A). Each tubule is made up of a basal trunk from which arises two or three primary branches which in turn give rise to two elongated secondary branches (Figs 1A, 2). The maximal length of a tubule (measured from the basal part of the trunk to the distal extremity of the longest secondary branch) is c. 4 cm, with the trunk itself accounting for c. 1 cm. In 50 individuals of *A. echinates* and *A. mauritiana*, there was no tubule in the process of regeneration.

For the three species studied, prolonged mechanical stimulation of the body wall results in the overall contraction of the individual. Whatever the intensity of the stimulation, it never results in the expulsion of the Cuvierian tubules. The tubules are passive, immobile organs in the body cavity. They never elongate nor become sticky whether one touches them or pulls their distal end. The water taken in or expelled from the respiratory tree never enters the tubules.

Fine structure

Three parts can be distinguished along an actinopygid Cuvierian tubule: (1) the proximal half of the trunk that is smooth, (2) the distal half of the trunk that is slightly rugged, and (3) the primary and secondary branches that

are highly rugged (Figs 1A, 2, 3A). SEM observation of the latter shows that they are covered by small densely packed spherules measuring from 60 to 90 μm in diameter (Figs 1B, 3A). Each spherule attaches to the tubule rachis by means of a narrow peduncle c. 30 μm in length (Fig. 1B). Together they give the tubule branches the appearance of a bunch of currants. All tubule regions are covered externally by a layer of monociliated cells with scattered microvilli (Fig. 3B).

Despite the change in the tubule outer morphology (namely, from a smooth proximal trunk to highly rugged branches), its gross tissue stratification remains constant all along its length. It consists of an outer mesothelium and an inner epithelium encompassing a connective tissue layer in which muscle fibres and nerve processes occur. The types of cells also remain the same although they organize together in different ways in the trunk and in the branches. At the point of attachment of the tubule to the respiratory tree, the aforesaid tissue layers are continuous with their equivalents in the wall of the respiratory tree.

The tubule trunk. The proximal trunk is a smooth tube with a narrow central cavity (Figs 2, 4, 19A, B). Its outermost part consist of a pseudostratified mesothelium made of an upper layer of peritoneal cells and a basal layer of vesicular cells (Fig. 6). Peritoneal cells line the body cavity. They are T-shaped in section having a flattened apical cell body (Fig. 7A) and an elongated and narrow basal process. The latter crosses the vesicular cell layer and contacts the mesothelial basal lamina through hemidesmosome-like structures. Peritoneal cells attach to each other through apical septate junctions. Each bears a single cilium surrounded by c. 10 much shorter microvilli (Fig. 7B). The cell body houses an ovoid nucleus, a Golgi apparatus and a few scattered mitochondria. Vesicular cells are overlaid everywhere by peritoneal cells. They are large, irregular to ovoid-shaped, closely appressed and have a small ovoid nucleus. Their cytoplasm is filled by conspicuous vesicles (c. 2 μm in diameter) containing mucosubstances (Alcian blue and PAS-positive) (Fig. 6). The vesicular cells rest upon the mesothelial basal lamina without developing any particular attachment structure.

The connective tissue layer contains scattered collagen fibres. The latter have no particular arrangement, and are sometimes associated with small ovoid cells. The outermost part of the connective layer encloses thin bundles of smooth muscle fibres that consist of a continuous sheet of longitudinal fibres and of regularly spaced circular fibres (Figs 8, 9). Both longitudinal and circular fibres are associated with nerve processes. The muscle fibres and nerve processes are surrounded by a common basal lamina. (Nerve processes presumably arise in the hyponeurial nerve plexus which is conspicuously developed where the trunk attaches to the respiratory tree; Fig. 10.)

The inner epithelium is monostratified and includes two cell types, namely, ciliated cells and mucous cells (Fig. 11). Ciliated cells are by far the most numerous. They are monociliated and bear short apical microvilli (Fig. 12). They attach to each other and to the mucous cells by apical septate junctions (Fig. 13). Their nuclei are spherical, centrally located and surrounded by a finely

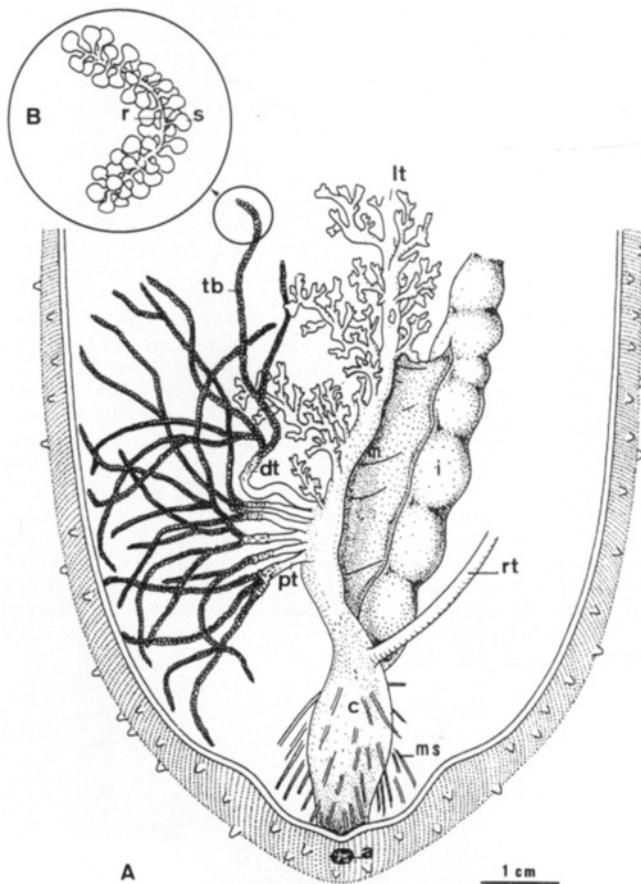
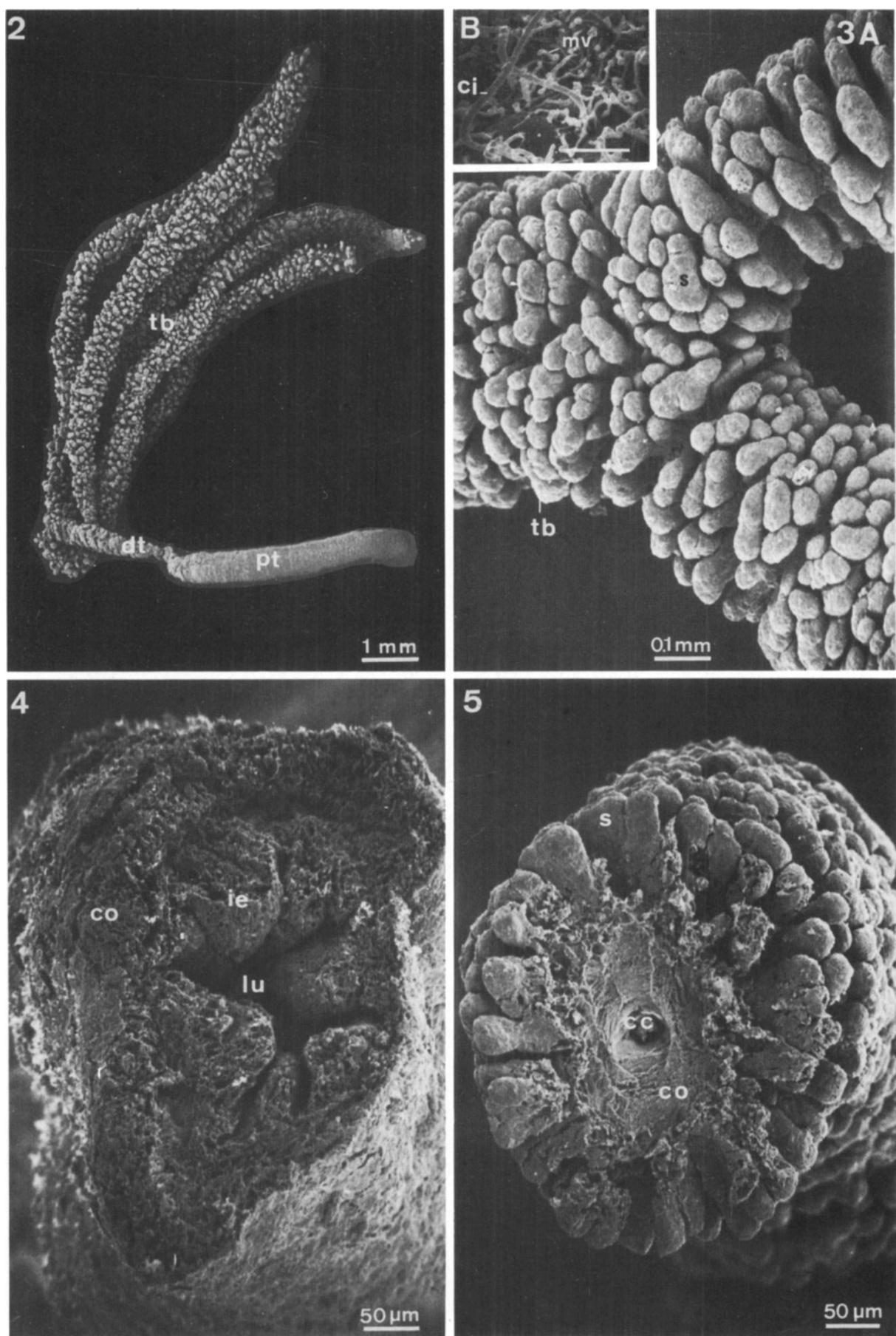
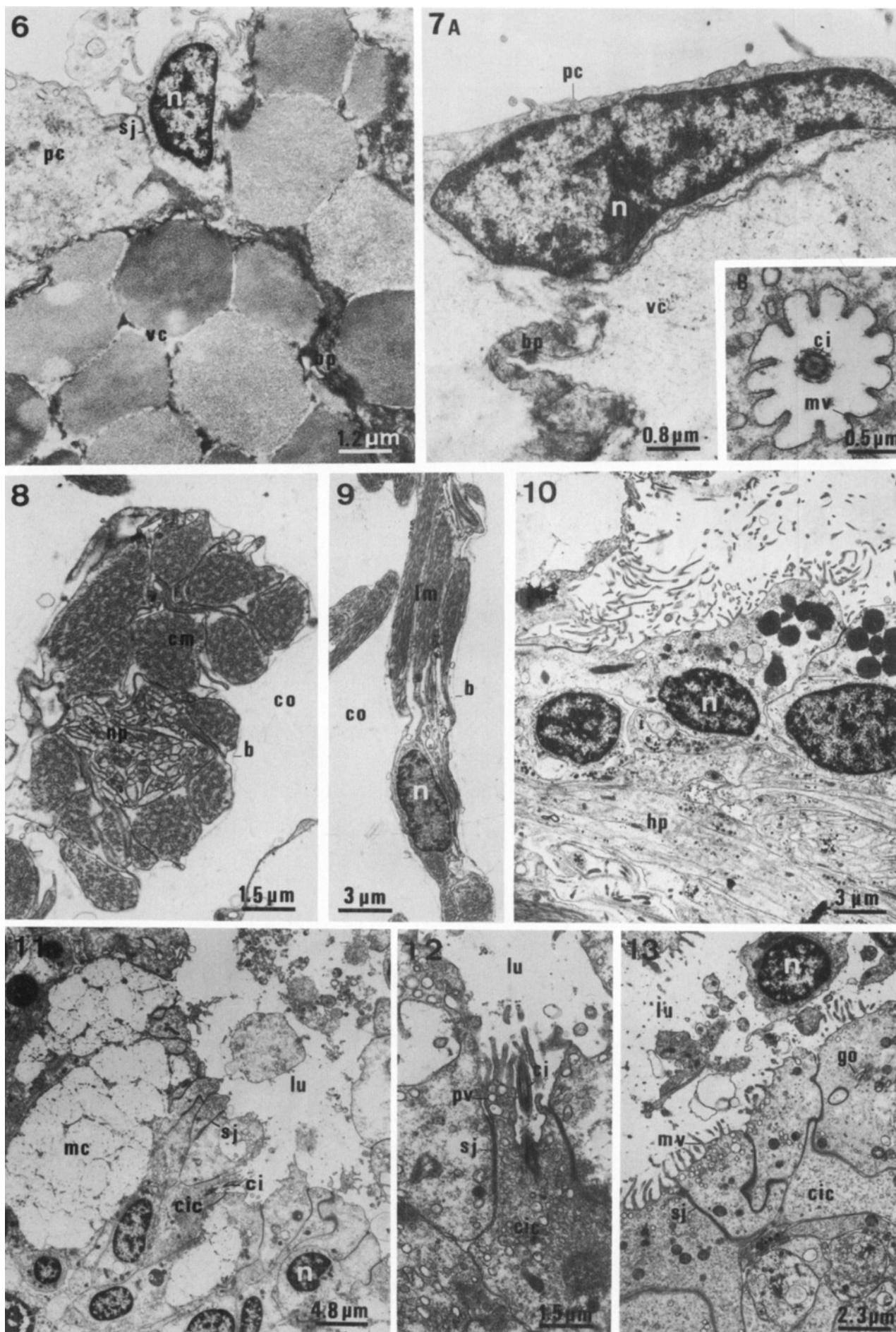


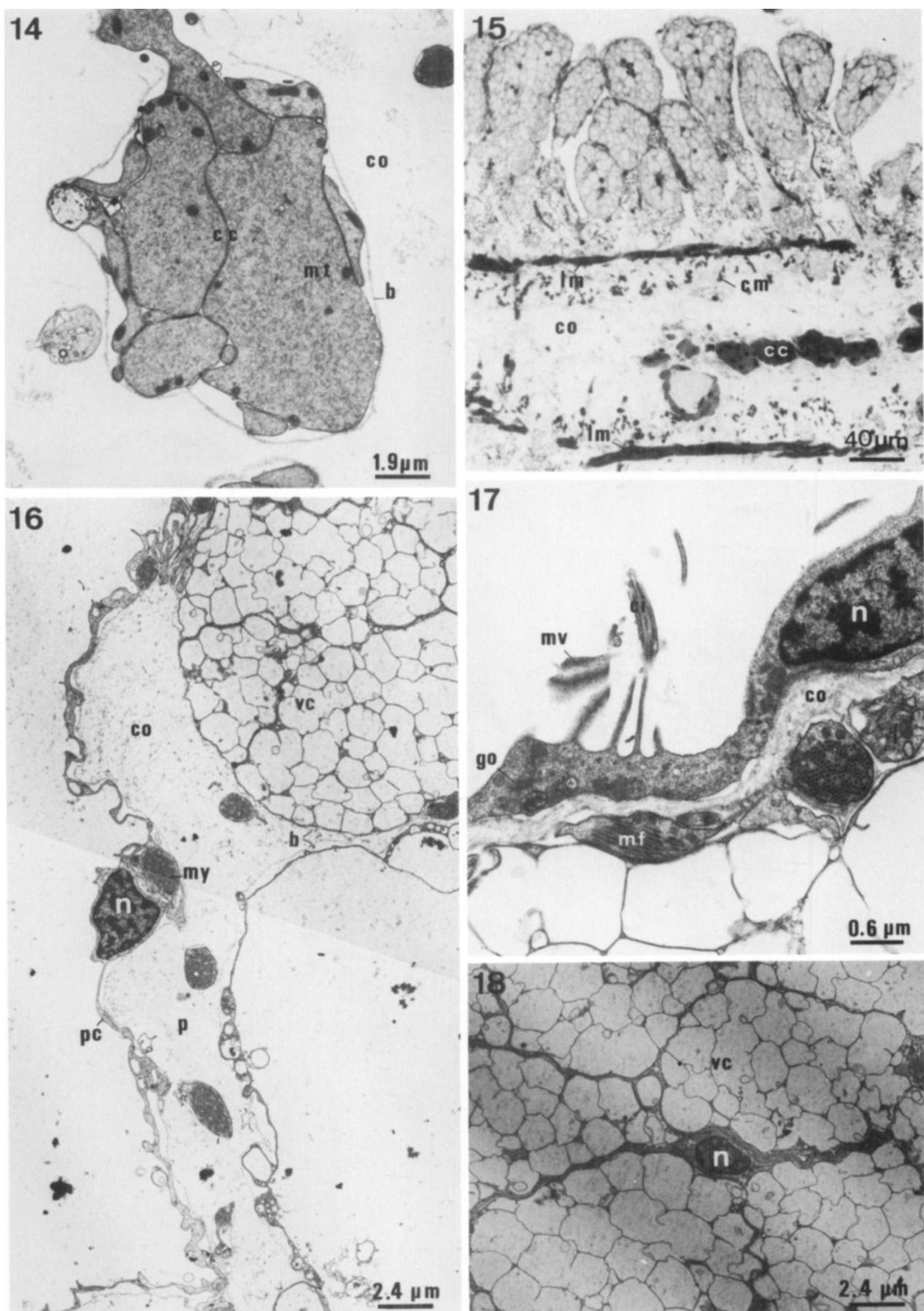
Fig. 1.—A. Anatomical drawing of the Cuvierian organs of *Actinopyga echinates*.—B. Detail of a tubule branch. a anus; c cloaca; dt distal part of the trunk; i intestine; lt left respiratory tree; m mesentery; ms mesenteric strand; pt proximal part of the trunk; r rachis; rt right respiratory tree; s spherule; tb tubule branch.



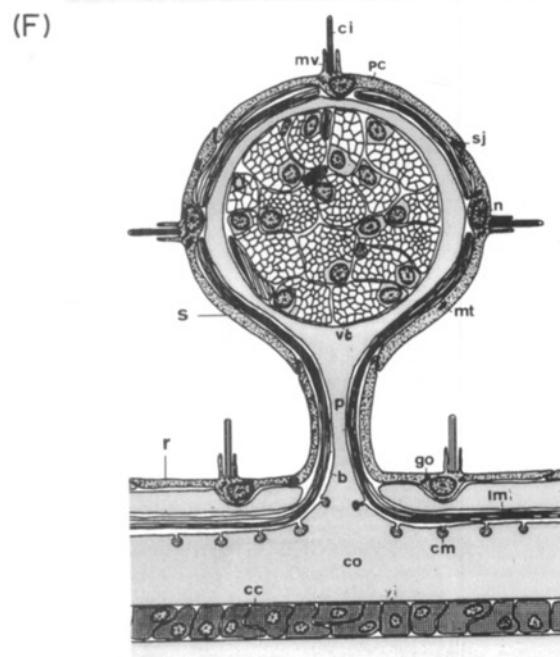
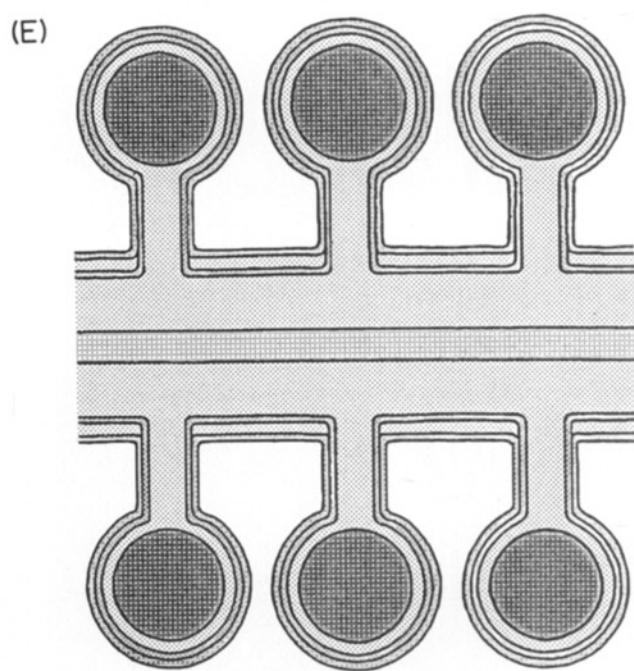
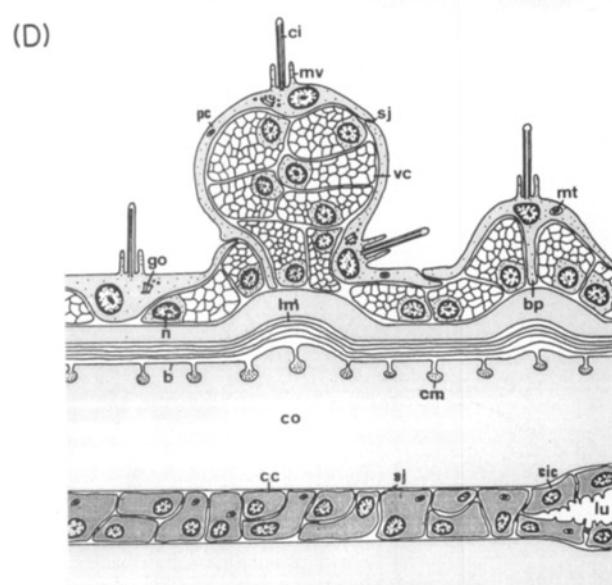
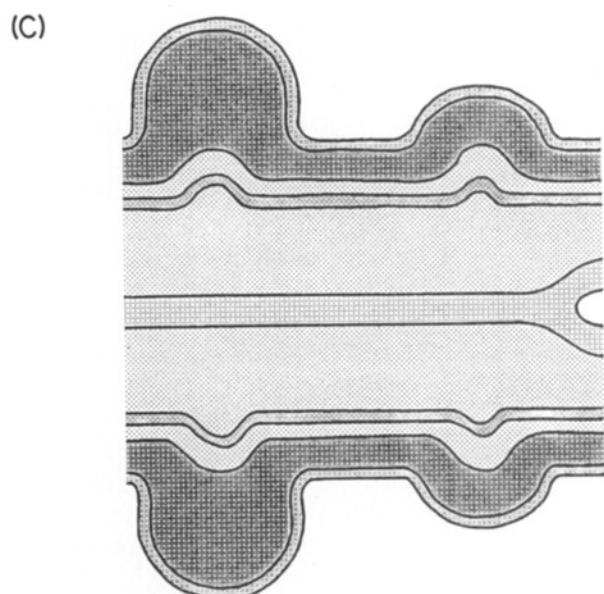
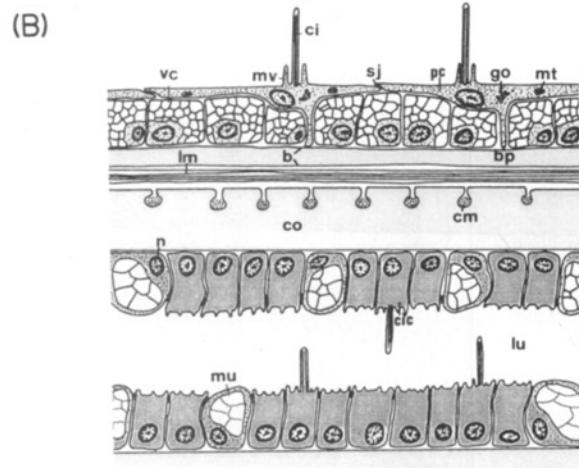
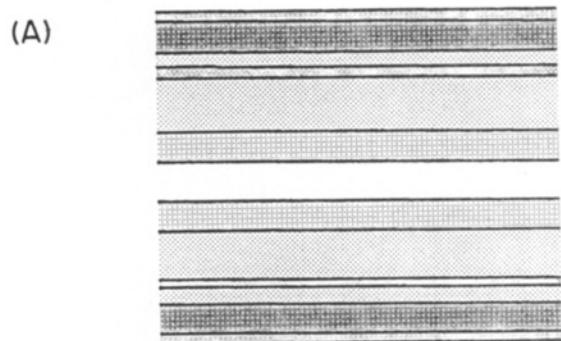
Figs 2-5.—*Actinopyga mauritiana*. Fine structure of the Cuvierian organs (SEM).—Fig. 2. General aspect of a Cuvierian tubule.—Fig. 3.(A) Aspect of a tubule branch. (B) Outer surface of a spherule.—Fig. 4. Cross-section through the proximal part of the trunk of a tubule.—Fig. 5. Cross-section through a tubule branch. *ci* cilium; *cc* central cord; *ct* connective tissue; *dt* distal part of the trunk; *lu* central lumen; *mv* microvilli; *pt* proximal part of the trunk; *s* spherule; *tb* tubule branch.



Figs 6-13. *Actinopyga mauritiana*. Fine structure of the trunk of a Cuvierian tubule (TEM).—Fig. 6. Peritoneal and vesicular cells of the mesothelium.—Fig. 7. (A) Peritoneal cell of the mesothelium. (B) Cross-section through a cilium surrounded by a ring of microvilli.—Figs 8-9. Circular (Fig. 8) and longitudinal (Fig. 9) muscle cells.—Fig. 10. Hyponeurial nerve plexus (basal area of the proximal part of the trunk).—Figs 11-13. Aspects of the mucous cells (Fig. 11) and the ciliated cells (Figs 11-13) of the inner epithelium (proximal part of the trunk). *b* basal lamina; *bp* basal process of a peritoneal cell; *ci* cilium; *cm* circular muscle; *co* connective tissue; *cic* ciliated cells; *hp* hyponeurial nerve plexus; *ie* inner epithelium; *lu* lumen; *lm* longitudinal muscle; *mc* mucous cell; *mt* mitochondria; *mv* microvilli; *n* nucleus; *np* nerve process; *pc* peritoneal cell; *pv* pinocytic vesicle; *sj* septate junctions.



Figs 14–18. *Actinopyga mauritiana*. Fine structure of the branches of a Cuvierian tubule (TEM except Fig. 15).—Fig. 14. Cross-section through the central cell cord of the rachis.—Fig. 15. Longitudinal section through a tubule branch (semi-thin section).—Fig. 16. The spherule peduncle and spherule core.—Fig. 17. Peritoneal cell of the spherule mesothelium.—Fig. 18. Central core of the spherule. *b* basal lamina; *ci* cilium; *cc* central cell cord; *cm* circular muscle; *co* connective tissue; *cic* ciliated cells; *g* golgi; *lm* longitudinal muscle; *mf* myofibrillae; *mt* mitochondrion; *mv* microvilli; *n* nucleus; *p* peduncle; *pc* peritoneal cell; *s* spherule; *sj* septate junction; *vc* vesicular cell.



adluminal cells
 vesicular cells
 muscle layer

connective tissue
 inner epithelium

granular cytoplasm that is Alcian Blue and PAS-positive. The cytoplasm contains almost no other organelles than scattered mitochondria and a few pinocytotic vesicles. The mucous cells have no microvilli nor a cilium, and their cytoplasm consists only of large mucus-containing vacuoles (PAS and Alcian Blue-positive; Fig. 11). Both ciliated cells and mucous cells rest upon a basal lamina without differentiating particular attachment structures.

Within the distal trunk the central part of the tubule consists only of a cell cord (Figs 5, 19C, D). The cell cord is made from closely appressed cells that present the same characteristics as the ciliated cells of the proximal trunk except that, for obvious reasons, there are neither cilia nor microvilli (Fig. 14). As for the mesothelium, its structure is identical to that occurring in the proximal trunk. However, from place to place, the vesicular layer thickens owing to local accumulation of vesicular cells. As a consequence the outer surface of the distal trunk is slightly rugged (Figs 2, 19C, D).

The tubule branches. Each branch consists of a central rachis and many peripheral spherules. Each spherule is attached to the rachis by a short narrow peduncle (Figs 1B, 3A, 5). There is no morphological difference between primary and secondary branches.

The structure of the rachis resembles that of the tubule trunk. There is a central cell cord continuous with the cord of the distal trunk. The connective tissue layer is also similar, and encloses bundles of muscle fibres and nerve processes in its outermost part (Figs 15, 19E, F). The mesothelium is, however, different in that it is monolayered and made only from peritoneal cells.

The peduncle of the spherule consists of a connective tissue stem surrounded by a flattened pseudostratified mesothelium consisting of an external layer of peritoneal cells and an inner layer of myoepithelial cells (Fig. 16). The latter are continuous with the longitudinal muscle fibres of the rachis (Figs 19E, F).

The spherule itself is made from an enlarged spherical central core surrounded by a connective tissue sheet which in turn is surrounded by a mesothelium similar to that of the peduncle (Figs 16, 17). The central core consists chiefly of packed vesicular cells (Figs 16, 18, 19E, F). Muscle fibres occur in between the vesicular cells. The central core is everywhere surrounded by a basal lamina that lines the inner surface of the connective tissue sheet.

Trunk–branch comparison. Figures 19B, D, F summarize the morphological changes occurring along the length of a tubule. There is no particular change in the connective tissue layer which shows the same structure all along the tubule. The inner epithelium consists also of similar cells that either organize around a discrete central lumen

(proximal trunk) or form a single solid cord that occupies the centre of the tubule (distal trunk and branches). The main changes concern the mesothelium which, depending on the part, consists of peritoneal and vesicular cells (trunk), of peritoneal cells only (branch rachis), or of peritoneal and myoepithelial cells (spherules and spherule peduncles). Serial sections of tubules showed that the muscle fibres located in the connective layer of the rachis are continuous with those located in the mesothelium of both the spherule peduncles. This continuity also concerns the basal lamina (BL); that is, the BL surrounding the rachis muscles clearly arises from the BL lining the mesothelium of the spherule peduncles. This suggests that all muscle cells of the tubule actually originate from the mesothelium. As for the vesicular cells, they show a marked tendency to aggregate in more distal regions of the tubule. They form a continuous subperitoneal cell layer in the trunk that is either single (proximal trunk; Figs 19A, B) or locally pluristratified (distal trunk; Figs 19C, D). In both the primary and secondary branches, the vesicular cells detach from the mesothelium and form the central core of the spherules (Figs 19E, F).

Discussion

Cuvierian organs of *Actinopyga* species have few features in common with those of other holothuroid genera except their anatomical relationships and overall tissue organization (both attach to the basal part of the left respiratory tree; both are made of similar tissue layers that are continuous with their equivalents in the wall of the respiratory tree). Actinopygid Cuvierian tubules are not numerous (no more than 10 tubules per individual); they are branched, have a rugged outer surface and are mostly solid (no central lumen except in the proximal part of the trunk; see also Mosher 1956); they are never expelled, and cannot elongate or become sticky. Holothuriid Cuvierian tubules, by contrast, are numerous (several hundred tubules per individual); they are unbranched, have a smooth outer surface and are hollow throughout; when expelled, they elongate and become sticky (Jourdan 1883; Mines 1912; VandenSpiegel & Jangoux 1987).

Elongation of the Cuvierian tubules in holothuriid holothuroids results ultimately from the forceful entry of water into the tubule lumen (VandenSpiegel & Jangoux 1987) and is made possible by the particular organization of the tubules: they have a central cavity that can be distended by hydrostatic pressure; they have a surrounding thick connective tissue layer rich in collagen fibres arranged in helices parallel to the tubule long axis (these helices elongate and flatten owing to the hydrostatic pressure); they also have a heavily folded mesothelium which becomes unfolded when the tubule elongates (see VandenSpiegel & Jangoux 1987 for a detailed explanation of the process of tubule expulsion and elongation). A similar organization is not seen in actinopygid tubules. The latter are solid, without a central cavity for most of their length. The collagen fibres are few in number and do not arrange helically and the mesothelium is not folded (Mosher 1956; present work). As a consequence it is

Fig. 19. *Actinopyga mauritiana*. Histological maps (A, C, E) and drawings (B, D, F) of selected areas of Cuvierian tubule. (A, B) Proximal part of the trunk. (C, D) Distal part of the trunk. (E, F) Tubule branch. b basal lamina; bp basal process; ci cilium; cc central cell cord; cm circular muscle; co connective tissue; cic ciliated cells; go Golgi apparatus; lu lumen; lm longitudinal muscle; mu mucous cell; mt mitochondria; mv microvilli; n nucleus; p peduncle; pc peritoneal cell; r rachis; s spherule; vc vesicular cell.

structurally not possible for an actinopygid Cuvierian tubule to elongate.

Although actinopygid Cuvierian organs do not fulfil a defensive function in the same way as those of other holothuroids, they are nevertheless considered to be defensive because of their alleged toxicity. Nigrelli (1952) first reported that the body wall and internal organs—including the Cuvierian organs—of *A. agassizi* contain toxic substances able to kill fish and various invertebrates. (These substances are actually saponins of the holothurin group; see Kalayani *et al.* 1988.) This led Hyman (1955, p. 163) to suggest that *A. agassizi* Cuvierian organs ‘accomplish the function of defense in another way’. However, Mosher (1956) found that saponins were produced in the body wall epidermis (where they are mixed with mucus) and that the internal organs, although containing the toxins, do not secrete them. This might explain the common occurrence of endosymbiotic organisms within the cavity of the left respiratory tree of *Actinopyga* spp., such as carapid fish or pinnotherid crabs (Smith *et al.* 1981; VandenSpiegel & Jangoux 1989). Consequently, the suggestion of Hyman (1955) seems no longer to be likely.

The only other proposed function for actinopygid Cuvierian organs was that of Mosher (1956) who speculated that they might have a protective role in preventing poisoning of the holothuroid by its own toxins. He did not specify, however, how the Cuvierian organs would act to fulfil such a function.

From the observations we made it appears almost certain that actinopygid Cuvierian organs have no defensive role. Yet they are well-developed organs that occur in all species of *Actinopyga* investigated so far. Moreover, from the way they attach and their overall histology, they could be homologous to those of other holothuroids. Their most remarkable feature is the occurrence in the mesothelium of numerous large vesicular cells, a cell type that does not exist in holothuriid Cuvierian organs. Additional information is required concerning the structure of these cells and on their changes—for example during growth and/or during the annual cycle of the species—before a possible function can be ascribed to these peculiar organs.

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