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# Functional morphology of the locomotory podia of *Holothuria forskali* (Echinodermata, Holothuroida)

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**Summary.** The ventral surface of *Holothuria forskali* (Holothuroida, Aspidochirotida) is almost completely covered by small-sized podia that are locomotory. Each podium consists of a stem that allows the podium to lengthen, to flex, and to retract, and this is topped by a disc that allows the podium to adhere to the substratum during locomotion. Podia of *H. forskali* do not end in a sucker and their adhesion to the substratum thus relies entirely on the disc epidermal secretions. The disc epidermis is made of five cell types: non-ciliated secretory cells of two different types that contain granules whose content is either mucopolysaccharidic (*NCS1* cells) or mucopolysaccharidic and proteinic in nature (*NCS2* cells), ciliated secretory cells containing small granules of unknown nature (*CS* cells), cilitated non-secretory cells (*CNS* cells), and support cells. The cilia of *CS* cells are subcuticular whereas those of *CNS* cells, although also short and rigid, traverse the cuticle and protrude in the outer medium. During locomotion, epidermal cells of the podial disc are presumably involved in an adhesive/de-adhesive process functioning as a duo-gland adhesive system. Adhesive secretions would be produced by *NCS1* and *NCS2* cells and de-adhesive secretion by *CS* cells. All these secretions would be controlled by stimulations of the two types of ciliated cells (receptor cells) which presumably interact with the secretory cells by way of the nerve plexus. The lack of suckers and the coexistence of two adhesive cell types in the disc epidermis give the locomotory podia of *H. forskali* a "compromise" structure which would perhaps explain their ability to move as efficiently along soft and hard substrata.

## A. Introduction

The function of ambulacral appendages, or podia, in echinoderm biology has been reviewed by several authors (Smith 1937; Nichols 1966; Lawrence 1987). Many of these appendages are used in relation to the substratum, allowing the echinoderm either to move on it (locomotory podia) or to handle it (handling podia). Locomotory podia often end in a sucker (e.g., podia of forcipulatid asteroids; Nichols 1966) while handling podia are digitate or penicillate (e.g., podia of spatangoid echinoid; Flammang et al. 1990). In both cases, the relationship between the podia and the substratum is achieved at least partly by secretions of the podial apical epidermis. According to Hermans (1983), some of these secretions are adhesive and others de-adhesive together forming a duo-gland adhesive system.

Aspidochirote holothuroids have both handling podia (buccal tentacles) and locomotory podia. Whereas the buccal tentacles have been the subject of a number of investigations (Bouland et al. 1982; Smith 1983; Cameron and Fankboner 1984; McKenzie 1987, 1988a), the available data about locomotory podia are scarce. The latter are often called "suckered podia" although the operation of the sucker has never been adequately explained and their epidermis appears to be heavily charged with secretory cells (Nichols 1966; Harrison 1968).

The aim of the present work is to describe the ultrastructure of the locomotory podia of the species *Holothuria forskali*, with a particular emphasis on the podial disc, and to consider how these podia adhere to and become detached from the substratum during locomotion.

## B. Materials and Methods

Specimens of *H. forskali* (Delle Chiaje, 1823) were collected by SCUBA diving in the Bay of Morlaix (Brittany, France) in October 1989. They were transported to the marine biology laboratory of the Mons University and kept alive in a closed circuit marine aquarium (13° C, 33‰ salinity) until required.

For light microscopy, podia were cut off from individuals previously anaesthetized with propylene phenoxetol (1‰ in sea water), fixed in Bouin's fluid, embedded in paraplast, and cut into 7 μm thick sections. Sections were stained with Masson's trichrome and Mayer's hemalum coupled with phloxine and light green. Histo-

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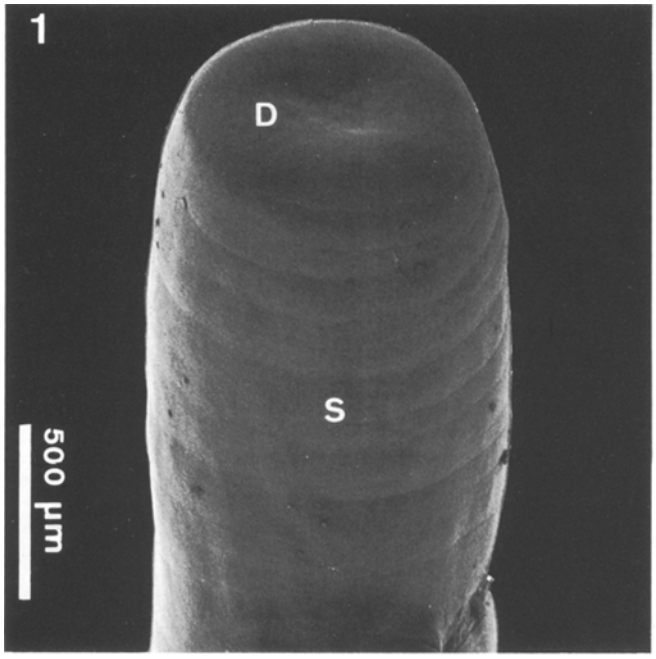
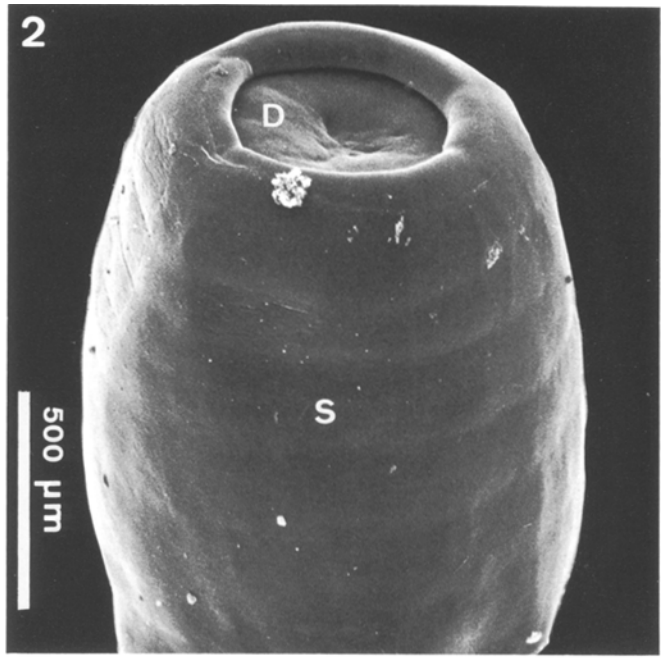
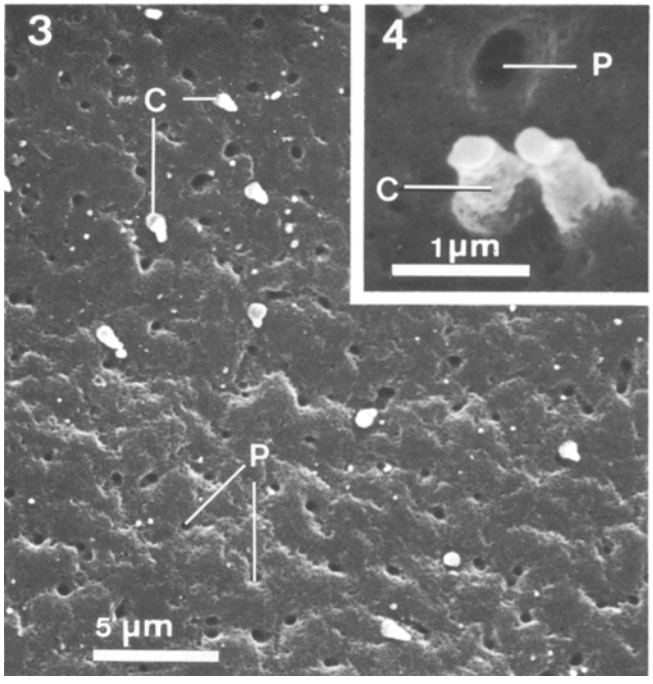
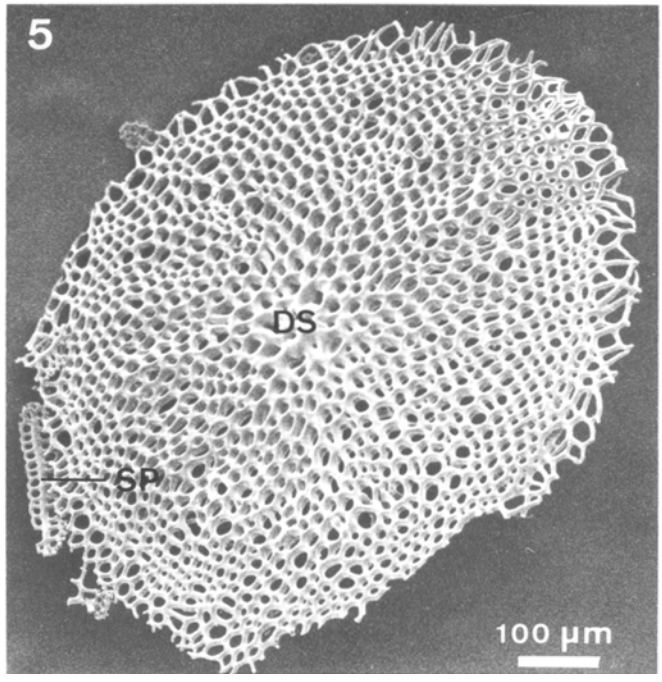









**Figs. 1–5.** Outer aspect of a podium and its spicules in *Holothuria forskali*. *C*, cilium; *D*, disc; *DS*, disc-supporting ossicle; *P*, pore; *S*, stem; *SP*, spicule

**Fig. 1.** Relaxed podium

**Fig. 2.** Retracted podium

**Fig. 3.** Disc surface

**Fig. 4.** Detailed view of cilia and pores

**Fig. 5.** Disc supporting ossicle and one of its associated spicules

chemical observations were performed using alcian blue pH 2.6 and the periodic acid-Schiff (PAS) techniques for the detection of mucopolysaccharides, and the Danielli's technique for the detection of proteins (Ganter and Jollès 1969–70). Footprints left by holothuroids were obtained by allowing individuals to walk across clean glass microscope slides. These were stained with 0.2% toluidine blue in Walpole buffer pH 4.2 (for the detection of mucopolysaccharides; Ganter and Jollès 1969–70) and with 0.06% brilliant blue G in 0.6 N HCl (for the detection of proteins; Sedmak and Grossberg 1977).

For transmission electron microscopy (TEM), two sets of podia were investigated: attached podia (those adhering to the aquarium wall and which were cut off from the holothuroid body using small scissors) and unattached podia (those not adhering to any substratum). Podia were fixed by immersion in 3% glutaraldehyde in cacodylate buffer (0.1 M, pH 7.8) for 3 h at 4° C. They were rinsed in cacodylate buffer and then postfixed for 1 h in 1% osmium tetroxide in the same buffer. After a final buffer wash, they were decalcified according to the method of Dietrich and Fontaine (1975), dehydrated in graded ethanol, and embedded in Spurr. Sec-

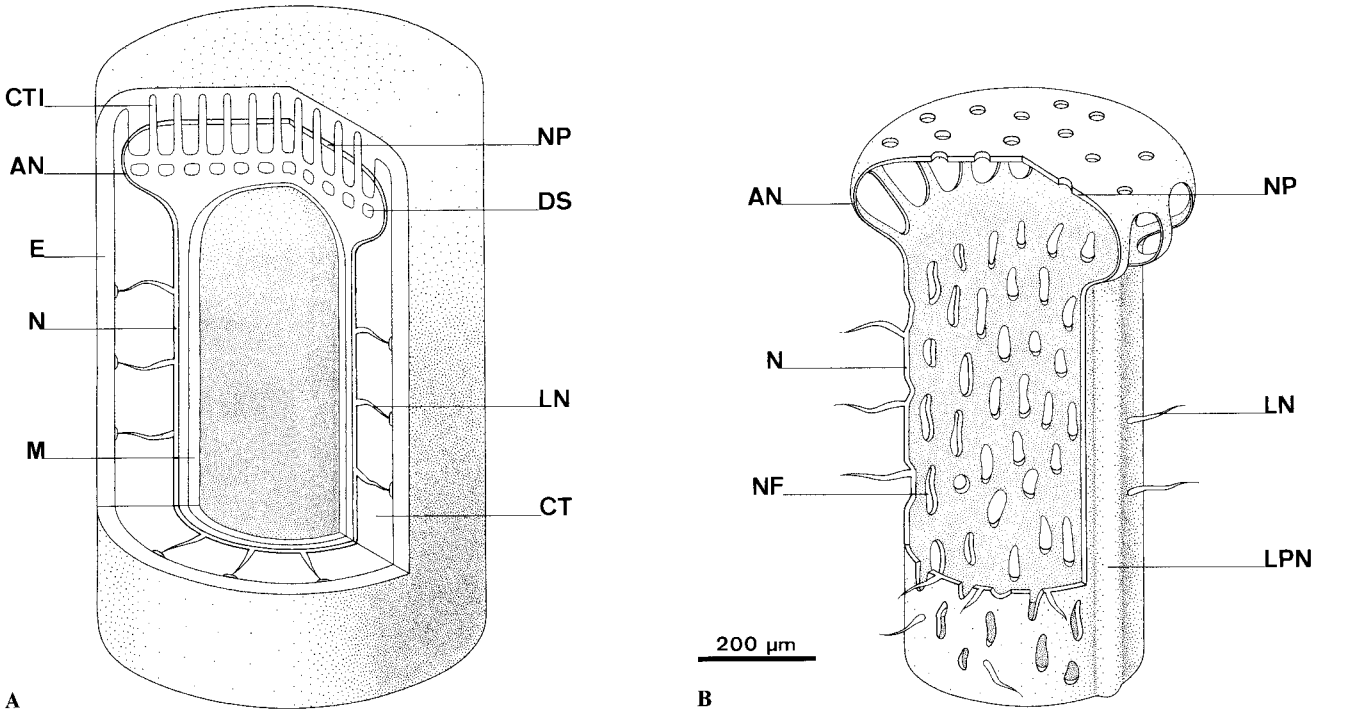



**Fig. 6A, B.** Schematic representation of some aspects of a locomotory podium; **A** distal portion with part of podial stem; **B** podial nerve system. *AN*, apical nerve; *CT*, connective tissue layer; *CTI*, connective tissue indentation; *DS*, disc-supporting ossicle; *E*, epidermis; *LN*, lateral nerve; *LPN*, longitudinal podial nerve; *M*, mesothelium; *N*, nerve plexus; *NP*, nerve plate; *NF*, nerve plexus fenestration

tions were cut on an LKB V ultramicrotome, contrasted with uranyl acetate and lead citrate, and observed in a Philips EM 300 transmission electron microscope.

For scanning electron microscopy (SEM), podia were fixed in Bouin's fluid for 24 h (Lahaye and Jangoux 1985). They were dehydrated in graded ethanol, dried by the critical point method (using $CO_2$ as transition fluid), mounted on aluminium stubs, coated with gold in a sputter coater and observed with a Philips 515 scanning electron microscope. Podial spicules were cleaned off their associated soft tissues in 10% (v/v) common bleach. They were mounted on aluminium stubs and processed as before.

## C. Results

### *I. External morphology of the podia*

Locomotory podia are arranged in four longitudinal rows on the ventral surface (trivium) of the holothuroids. Within each row the podia organize quite irregularly with the result that they almost completely cover the trivium. Each podium consists of a cyclindrical stem (about 5 mm long and 1 mm in diameter in relaxed podia, Figs. 1, 6A) topped by a flat disc. In contracted podia, the disc remains flat, does not change in diameter, and sinks slightly into the stem (Fig. 2).

The disc surface harbors uniformly distributed cilia and pores (Fig. 3). The cilia (about 1.5 µm long) are partly covered by the cuticle and only their tips protrude into the outer medium (Fig. 4). Cilia may occur either singly or in pairs. There are about three cilia per 100 µm$^2$ and approximately four times as many pores. These latter are about 300 nm in diameter (Fig. 4).

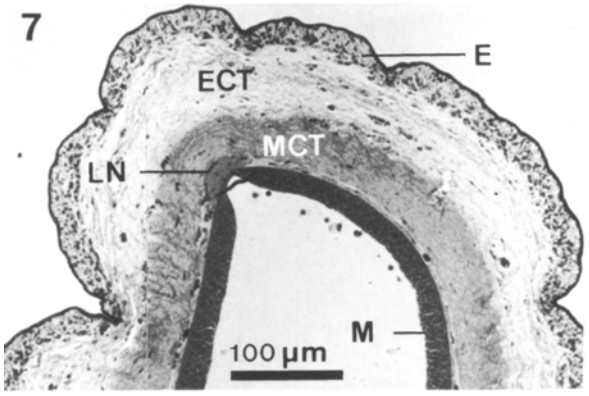
The podial disc is supported by a large circular ossicle (about 900 µm in diameter) located in the connective tissue layer and made of perforate stereom. This ossicle is surrounded by a ring of small elongated spicules about 200 µm long (Figs. 5, 6A, 15).

### *II. Histology and cytology of the podia*

Both the stem and the disc consist of four tissue layers: an inner mesothelium, a connective tissue layer, a nerve plexus, and an outer epidermis covered by a well-marked cuticle. However, these layers are made of different cell types and/or are differently organized according to whether they belong to the stem or the disc (Figs. 6A, 7, 15).

#### 1. Fine structure of the stem

*a) Mesothelium.* The mesothelium (i.e., the most internal layer of the podial wall) surrounds the lumen and is about 25 µm thick except in front of the longitudinal podial nerve where it measures only 5 µm thick (Figs. 6A, 7). The mesothelium is a pseudostratified epithelium made of two cell types, viz. the adluminal cells and the myoepithelial cells, that both contact the underlying basal lamina (Fig. 8). Adluminal cells form the lining of the ambulacral cavity. These are T-shaped monociliated cells harboring a long vibratile cilium surrounded by a ring of about ten microvilli. They are bound together by junctional complexes consisting of an apical zonula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ECT
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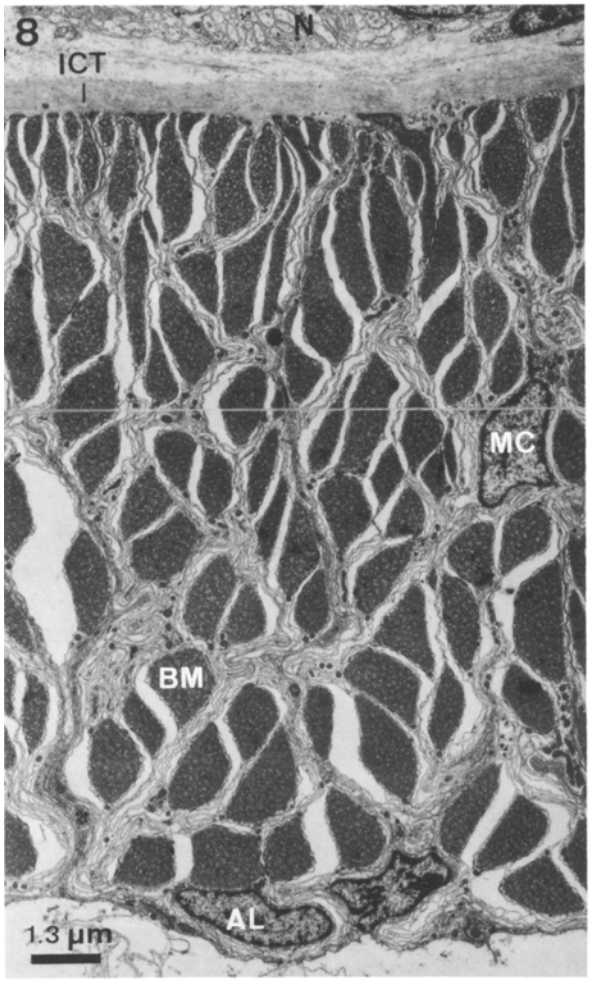
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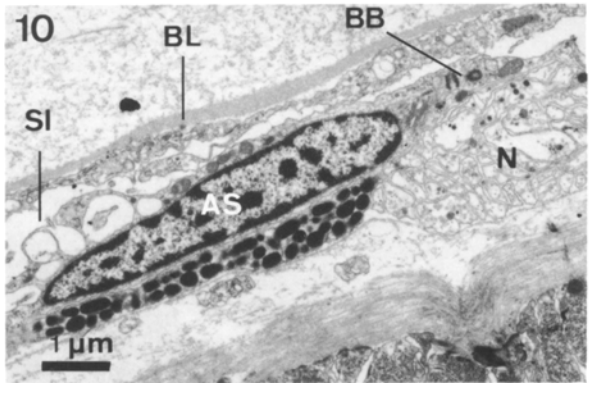
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AS
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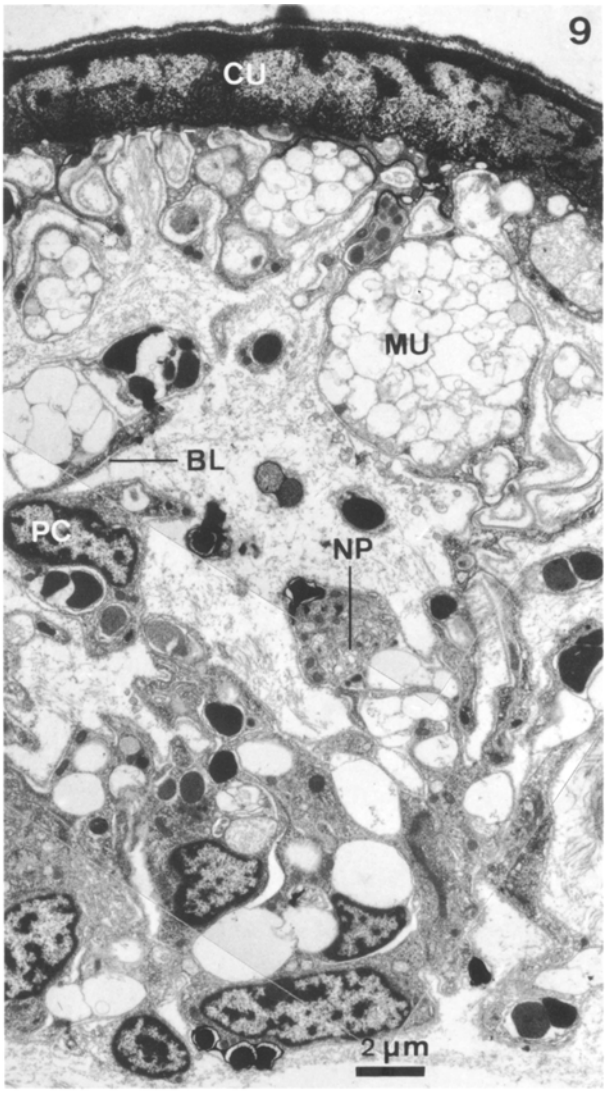
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BL
PC
NP
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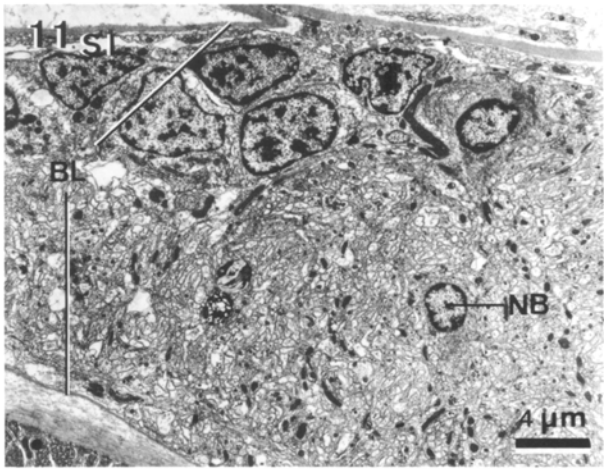
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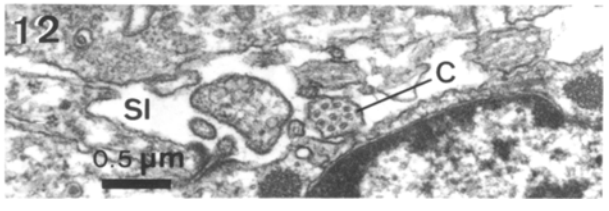

12
C
SI
0.5 µm

adhaerens and a subapical septate desmosome. Adluminal cells send basal processes that pass between myoepithelial cells before contacting the basal lamina. Myoepithelial cells are located below the cell bodies of the adluminal cells. They contain a bundle of myofibrils associated with numerous mitochondria. Myofibrils are oriented longitudinally; they form together an extensive longitudinal muscle layer (viz. the retractor muscle of the podium). Myoepithelial cells are bound together by regularly spaced desmosome-like structures. Intermingled with myoepithelial cells are slender cell processes (cell bodies have never been observed) whose cytoplasm is filled with electron-dense, membrane-bound granules (about 100 nm in diameter). These cells, which also occur in the connective tissue, are similar to the granulocytes described by Wood and Cavey (1981) in the podial mesothelium of the asteroid *Stylasterias forreri*.

*b) Connective tissue layer.* The connective tissue within the stem is divided into three layers: a very dense internal, a dense middle, and a loose external layer, which measure, respectively, ca. 1.3, 50, and 75 µm thick (Figs. 7, 8). In each of these layers, the connective tissue fibers are arranged in a circular to spiral pattern. The connective tissue contains spherulous coelomocytes as well as other cells that may represent fibrocytes or phagocytic coelomocytes.

*c) Nerve plexus.* The nerve plexus is located in the basal part of the middle connective tissue layer. It looks like a cylindrical fenestrated sheath of nervous tissue, asymmetrically thickened to form the longitudinal podial nerve (Figs. 6A, B, 10, 11). All along the stem, this nervous sheath sends small lateral nerves to innervate the epidermis. A single continuous basal lamina lines the epidermis, the small lateral nerves, and both faces of the nerve plexus. Neurone bodies occur only in the longitudinal podial nerve (Fig. 11), the rest of the plexus consisting of a criss-cross of nerve processes. These latter contain mitochondria, microtubules, and clear and/or dense-core vesicles. On the outer face of the nerve plexus is a narrow cavity or sinus (Figs. 10, 12, epineural sinus) which is lined by adsinusal cells that look like the adluminal cells of the podial mesothelium. The outer adsinusal cells are highly flattened (1.5 µm thick at the most) and they rest upon the outer basal lamina of the nerve plexus. The inner adsinusal cells rest upon the nerve plexus itself. They send out basal filament-containing processes that traverse the nerve plexus before contacting its inner basal lamina. Adsinusal cells bear a single cilium that extends into the nerve sinus (Fig. 12).

*d) Epidermis and cuticle.* The stem epidermis (about 30 µm thick) consists of T-shaped cells of two types, viz. mucocytes and pigment cells (Fig. 9). Both cell types have a thin but extended apical area, a neck, and an enlarged nucleus-containing body. They are attached together by apical junctional complexes consisting of a zonula adhaerens and a septate desmosome, and separated elsewhere by connective tissue indentations. The apical processes of these two cell types contain filaments that link the connective tissue fibers and the cuticle together by way of hemidesmosomes. The cytoplasm of mucocytes is filled with densely packed vesicles, about 1 µm in diameter, containing loose amorphous electron-lucent material. The cytoplasm of pigment cells contains numerous granules, about 2 µm in diameter, sometimes enclosing very electron-dense material.

The cuticle (about 700 nm thick) is made of four layers: an electron-dense filamentous layer (the most external), an electron-lucent layer containing scattered granular material, an electron-dense layer consisting of densely packed filaments, and a very electron-dense granular layer (the most internal) (Fig. 9). The cuticle is separated from the epidermis by a subcuticular space (about 1.3 µm thick) that is crossed by the microvilli of the epidermal cells.

## 2. Fine structure of the disc

*a) Mesothelium.* The mesothelium is only made of adluminal cells that are identical to those of the stem and form together with the latter, a continuous lumenal lining (Fig. 15). Myoepithelial cells do not extend into the disc mesothelium. They terminate at the distal end of the stem where they anchor to the connective tissue.

*b) Connective tissue layer.* The disc connective tissue consists of two layers: a very dense internal layer (about 1.3 µm thick) and a dense external layer (about 50 µm thick), that are continuous with the internal and the middle connective tissue layers of the stem. (The external connective tissue layer of the stem terminates at the distal end of the stem). The dense external layer encloses the large circular supporting ossicle (Figs. 6A, 15) together with its associated spicules. It also contains fibrocyte-like cells and spherulous coelomocytes. This layer sends upwards bundles of connective tissue fibers (Figs. 6A, 13, 15, 17) that insinuate themselves between the epidermal cells and attach apically to the support cells of the epidermis by way of hemidesmosomes (see below).

**Figs. 7–12.** Fine structure of the stem of locomotory podia of *H. forskali*. *AL*, adluminal cell; *AS*, adsinusal cell; *BB*, basal body; *BL*, basal lamina; *BM*, bundle of myofilaments; *C*, cilium; *CU*, cuticle; *E*, epidermis; *ECT*, external connective tissue layer; *ICT*, internal connective tissue layer; *LN*, longitudinal podial nerve; *M*, mesothelium; *MC*, myoepithelial cell; *MCT*, middle connective tissue layer; *MU*, mucocyte; *N*, nerve plexus; *NB*, neurone body; *NP*, nerve processes; *PC*, pigment cell; *SI*, sinus

**Fig. 7.** Transverse section through the stem (semithin section)

**Fig. 8.** Transverse section through the mesothelium

**Fig. 9.** Epidermis

**Fig. 10.** Nerve plexus and epineural sinus

**Fig. 11.** Transverse section through the longitudinal podial nerve

**Fig. 12.** Epineural sinus (detailed view of Fig. 11)

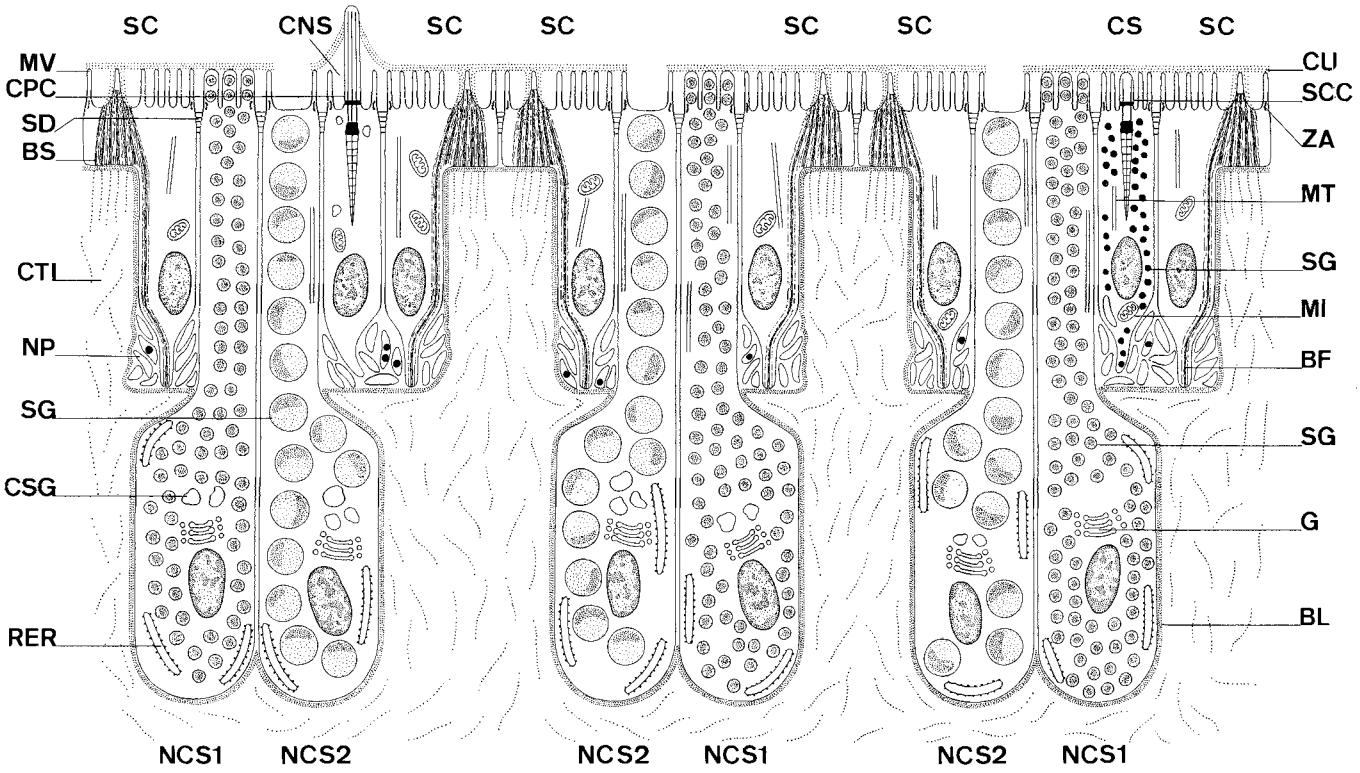



**Fig. 13.** Reconstruction of a longitudinal section through the disc epidermis (not to scale). *BF*, bundle of filaments; *BL*, basal lamina; *BS*, cuticle-connective tissue binding structure; *C*, cilium; *CNS*, ciliated non-secretory cell; *CPC*, cuticle-protruding cilium; *CS*, ciliated secretory cell; *CSG*, condensing secretory granule; *CTI*, connective tissue indentation; *CU*, cuticle; *G*, Golgi zone; *MI*, mitochondrion; *MT*, microtubule; *MV*, microvillus; *NCS1*, type 1 non-ciliated secretory cell; *NCS2*, type 2 non-ciliated secretory cell; *NP*, nerve plate; *RER*, rough endoplasmic reticulum cisternae; *SC*, support cell; *SCC*, subcuticular cilium; *SD*, septate desmosome; *SG*, secretory granule; *ZA*, zonula adhaerens

*c) Nerve plexus.* The nerve plexus of the disc consists mainly of a well-developed plate of nervous tissue in the base of the epidermis (about 10 μm thick) located about 70 μm under the apical surface of the disc (Figs. 6A, B, 13, 15, 23). This "nerve plate" is linked up with the stem nerve plexus by way of thin apical nerves that traverse the outermost part of the dense external connective tissue layer of the disc (Figs. 6A, B). The nerve plate is fenestrated, allowing both the connective tissue indentations and the basal processes of some epidermal cells to traverse it (Figs. 6B, 13, 15). As in the stem, a single continuous basal lamina lines the apical nerves, the nerve plate, and the epidermis. The nerves and the nerve plate enclose neurone bodies as well as numerous nerve processes. In the nerve plate, these processes run mainly in a plane parallel to the apical surface of the podium. They contain mitochondria, microtubules, and clear and/or dense-core vesicles. At the level of the nerve plate, there is no longer any trace of the sinus which presumably ends blindly at the apex of the stem.

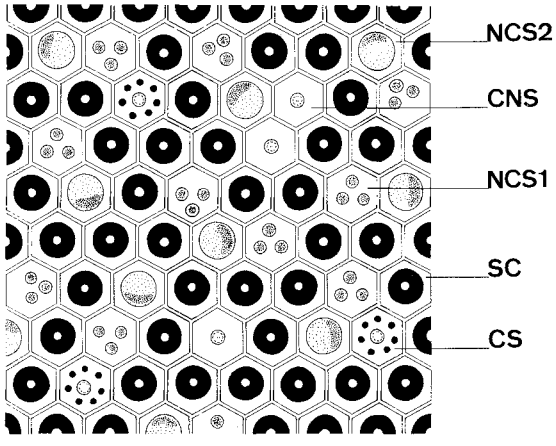


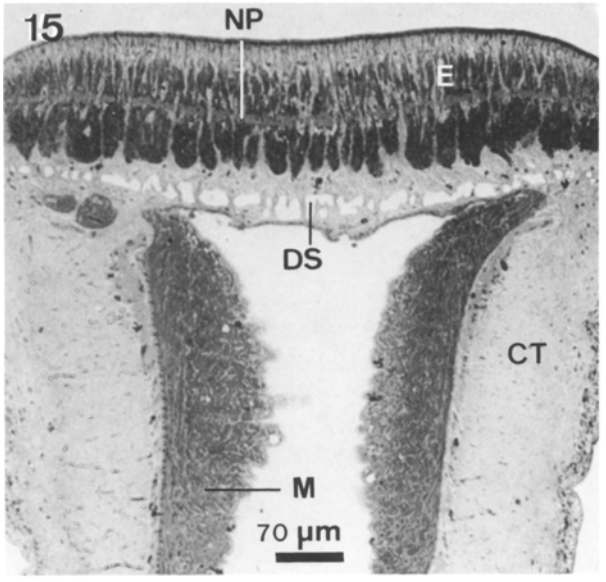

**Fig. 14.** Diagrammatic representation of a transverse section through the apex of the disc epidermis (not to scale). *CNS*, ciliated non-secretory cell; *CS*, ciliated secretory cell; *NCS1*, type 1 non-ciliated secretory cell; *NCS2*, type 2 non-ciliated secretory cell; *SC*, support cell

**Figs. 15–20.** Fine structure of the disc of locomotory podia of *H. forskali*. *BL*, basal lamina; *CNS*, ciliated non-secretory cell; *CS*, ciliated secretory cell; *CT*, connective tissue; *CTI*, connective tissue indentation; CU, cuticle; *DS*, disc-supporting ossicle; *E*, epidermis; *M*, mesothelium; *MV*, microvilli; *NCS1*, type 1 non-ciliated secretory cell; *NCS2*, type 2 non-ciliated secretory cell; *NP*, nerve plate; *P*, pore; *SC*, support cell; *SG*, secretory granule

**Fig. 15.** Longitudinal section through the disc (semithin section)

**Figs. 16–18.** Transverse sections through the epidermis (apex, above the nerve plate, and beneath the nerve plate)

**Figs. 19, 20.** Longitudinal sections through the apex of the epidermis (unattached and attached podia)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CT
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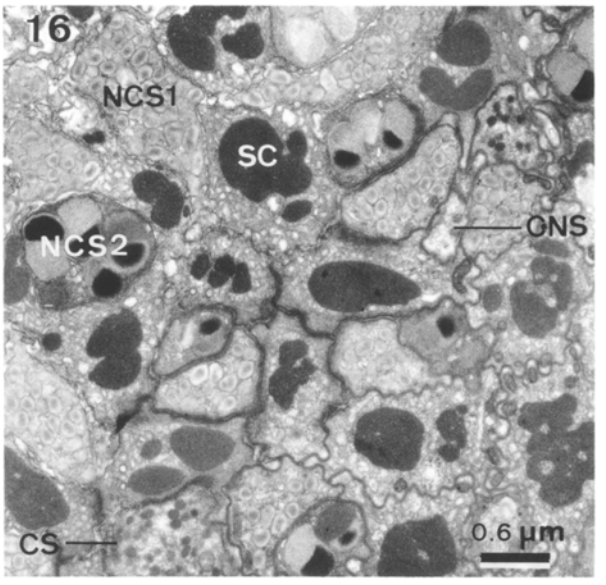
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NCS2
CS
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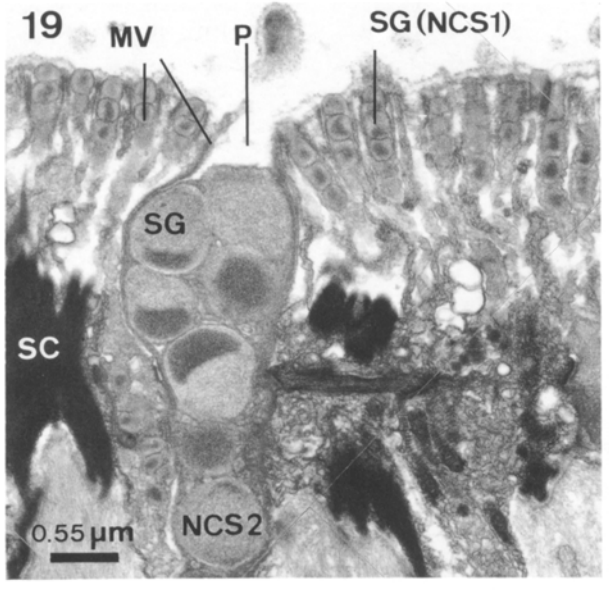
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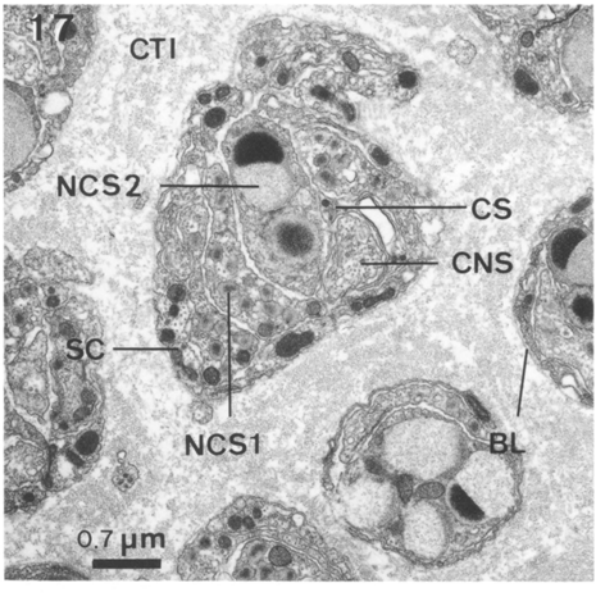
P
SG (NCS1)
SG
SC
0.55 µm
NCS2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CS
CNS
SC
NCS1
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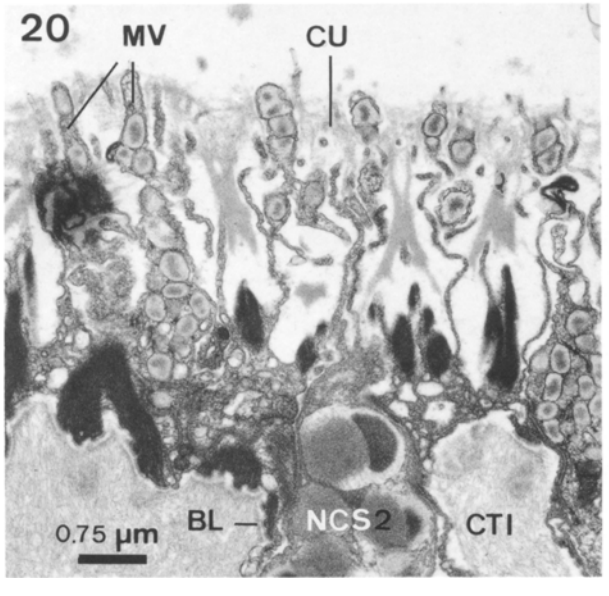
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MV
CU
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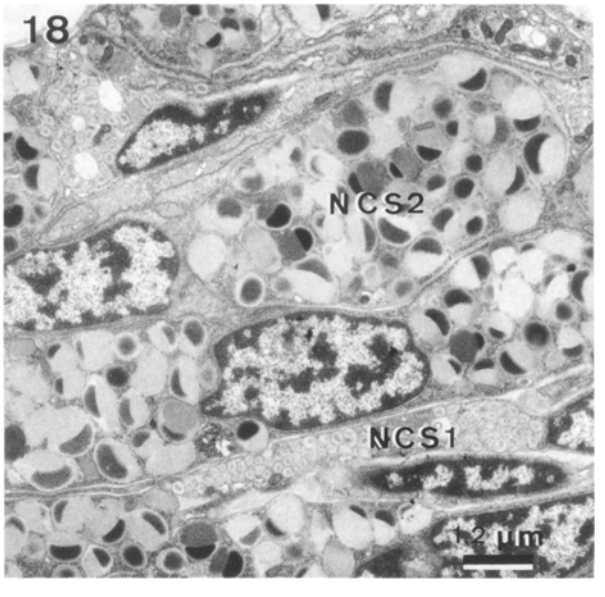
NCS2
CTI


18
NCS2
NCS1
1.2 µm

*d) Epidermis and Cuticle.* The disc epidermis is made of five cell types: non-ciliated secretory cells (*NCS* cells) of two different types (*NCS1* and *NCS2*), ciliated secretory cells (*CS* cells), ciliated non-secretory cells (*CNS* cells), and support cells. All these cells are joined apically by junctional complexes made up of a distal zonula adhaerens and a proximal septate desmosome. Some of these cells terminate at the level of the nerve plate (*CS* cells, *CNS* cells, and support cells) while others traverse the plate and sink into the underlying connective tissue (*NCS1* and *NCS2* cells) (Figs. 13, 15, 23). Epidermal cells are grouped together within islets separated by connective tissue indentations. When observed just above the nerve plate islets are built up by the different cell types, support cells being always located at the islet periphery (Figs. 13, 17). Islets ramify towards the apex of the disc and their upper branches only enclose a few cells. At the apex, support cells enlarge and epidermal cells join one another to form a continuous cellular layer. This layer consists of a support cell meshwork, the meshes of which are filled with the other epidermal cell types: *NCS1* and *NCS2* cells are generally coupled and can be associated with one (*CS* or *CNS* cell), or two (*CS* and *CNS*, or *CNS* and *CNS* cells) ciliated cells (Figs. 14, 16). (Ciliated cells are about four times less numerous than *NCS* cells.)

Non-ciliated secretory cells (*NCS1* and *NCS2* cells) are about 125 µm long and flask-shaped (Fig. 13). They have an enlarged nucleus-containing cell body that is basal and located under the nerve plate (Fig. 18). Each cell body sends a long upper process that traverses the nerve plate (where it is closely associated with axons) and reaches the apex of the podium. The cytoplasm of both the cell body and the upper process is filled with densely packed membrane-bound secretory granules (Figs. 18, 21, 22). The cytoplasm of the cell body also contains a well-developed Golgi apparatus, mitochondria, and cisternae of rough endoplasmic reticulum (RER) sometimes distended and filled with amorphous material. Developing secretory granules are closely associated with Golgi membranes and RER cisternae suggesting that these organelles are involved in the synthesis of the granule content (Figs. 21, 22). Besides secretory granules, the cytoplasm of the apical process encloses longitudinally arranged microtubules. These latter occupy all the cytoplasm of the apical process of *NCS1* cells and are located only peripherally within the apical process of *NCS2* cells.

The secretory granules of *NCS1* cells measure about 250 nm in diameter (Fig. 21). They contain material of low electron density surrounding an electron-dense central core. These materials stain with alcian blue pH 2.6 (Danielli's technique for the detection of proteins has failed). The apex of *NCS1* cells bears "microvillar-like" cell processes filled with secretory granules (Fig. 19). The material enclosed in the granules is extruded at the apical ends of the "microvillar-like" cell processes. *NCS1* cells are similar to those occurring in the terminal buds of the buccal tentacles of *H. forskali* (they correspond to the GV cells of Bouland et al. 1982). The secretory granules of *NCS2* cells are about 1 µm in diameter. These granules contain electron-lucent filamentous material covered, on one side, by a cap of electron-dense granular material (Fig. 22). At least one of these materials stains with alcian blue pH 2.6. At the end of the apical processes, the granules are extruded in the center of a ring of microvilli which terminates in a cuticular pore (Fig. 19). These pores correspond to those observed on SEM pictures of the disc surface.

In attached podia (Fig. 20), the "microvillar-like" cell processes of *NCS1* cells are nearly empty and one can see secretory granules in the process of exocytosis at the tip of some of these processes. *NCS2* cells also appear to have extruded some of their apical secretory granules.

The three other cell types of the epidermis are shorter than *NCS* cells. Their nuclei are located just above the nerve plate (Fig. 13). Ciliated secretory cells (*CS* cells) have cytoplasm filled with small membrane-bound secretory granules, 150 nm in diameter, that enclose electron-dense material (Fig. 23). The cytoplasm also contains numerous mitochondria, a Golgi apparatus, RER cisternae, and longitudinally arranged microtubules. The apex of *CS* cells bears a short subcuticular cilium (about 2.5 µm long) which has a striated rootlet (Fig. 24). This cilium has an irregular arrangement of microtubules and is surrounded by several rings of densely packed microvilli (Fig. 25). The basal part of the cell terminates within the nerve plate where granules similar in both shape and size to those occurring in *CS* cells can be seen in some nerve processes.

Ciliated non-secretory cells (*CNS* cells) are narrow. Their cytoplasm includes mitochondria, a few small clear vesicles of various shapes and sizes, and longitudinally arranged microtubules. Their characteristic features is a single short cilium (about 4 µm long) partly hidden by the cuticle, and whose apex protrudes into the outer medium (Fig. 26). The cilium has a regular 9+2 arrangement of microtubules and possesses a striated rootlet. It is surrounded by a ring of nine microvilli (Fig. 25). These cilia are those visible on SEM pictures of the disc

**Figs. 21–28.** Fine structure of the disc epidermis of locomotory podia of *H. forskali*. *BL*, basal lamina; *BF*, bundle of filaments; *C*, cilium; *CCS*, cilium of ciliated secretory cell; *CCNS*, cilium of ciliated non-secretory cell; *CMV*, central microvillus; *CNS*, ciliated non-secretory cell; *CS*, ciliated secretory cell; *CSG*, condensing secretory granule; *CTI*, connective tissue indentation; *CU*, cuticle; *CUS*, cuticular sheath; *G*, Golgi zone; *MV*, microvilli; *RER*, rough endoplasmic reticulum cisternae; *SD*, septate desmosome; *SG*, secretory granule; *SR*, striated rootlet

**Figs. 21, 22.** Aspect and formation of secretory granules in non-ciliated secretory cells (*NCS1* and *NCS2* cells)

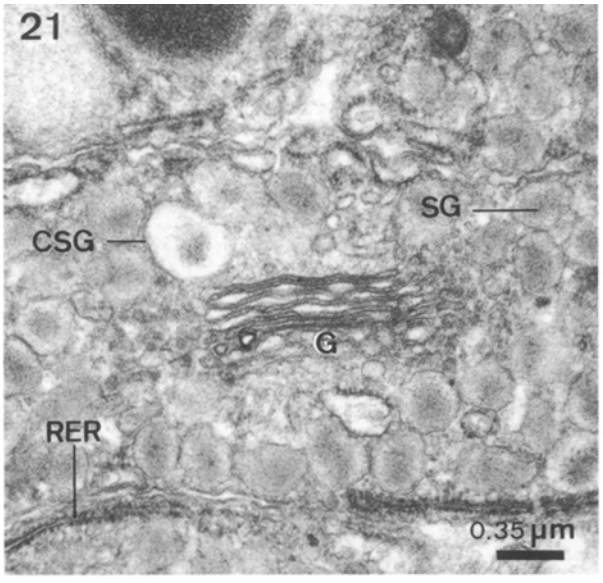
**Figs. 23, 24.** Ciliated secretory cell (cell body and apex)

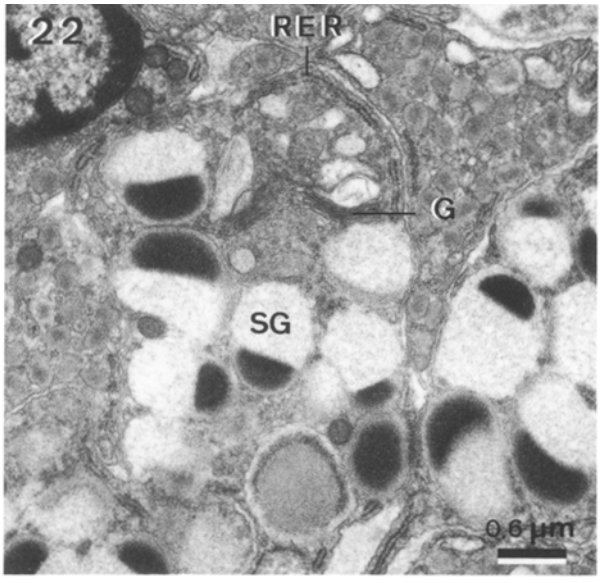
**Fig. 25.** Transverse section through the cilia of a ciliated secretory and a ciliated non-secretory cell

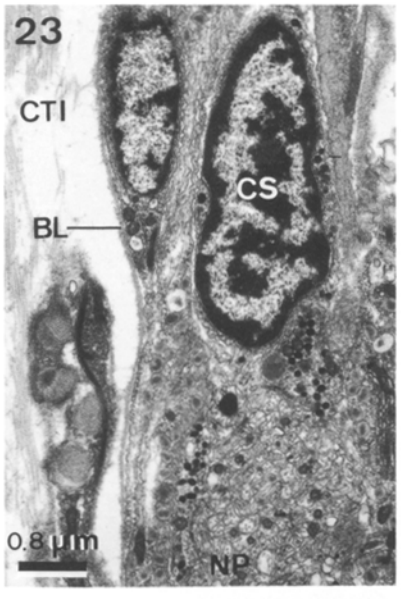
**Fig. 26.** Ciliated non-secretory cell

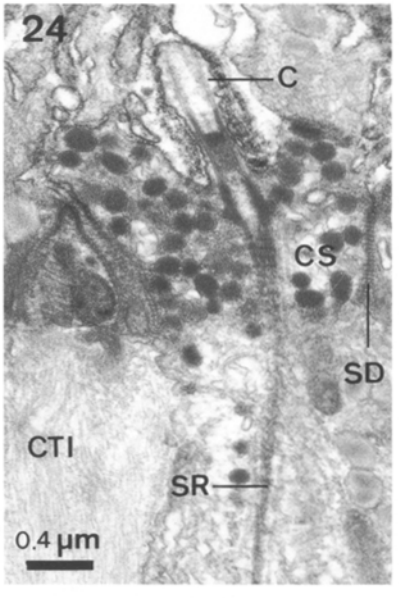
**Fig. 27.** Support cell

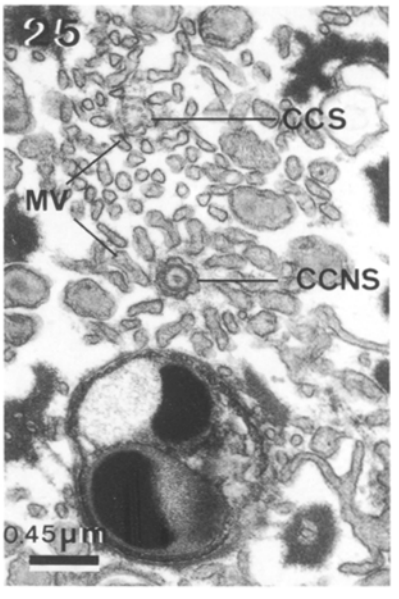
**Fig. 28.** Transverse section through two cuticle-connective tissue binding structures

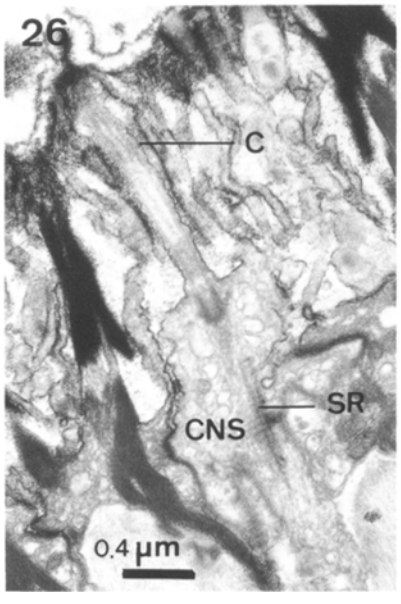

21
SG
CSG
G
RER
0.35 µm

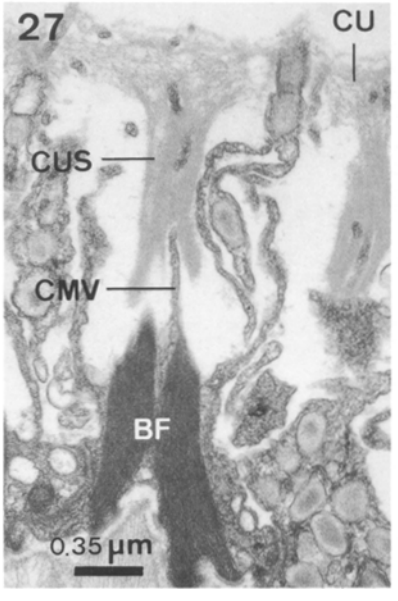

22
RER
G
SG
0.6 µm

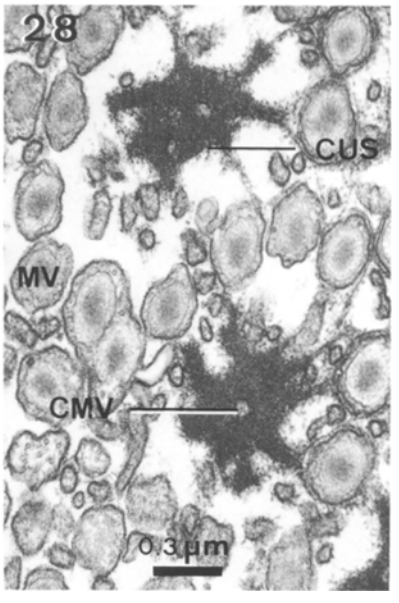

23
CTI
CS
BL
0.8 µm
NP


24
C
CS
SD
CTI
SR
0.4 µm


25
CCS
MV
CCNS
0.45 µm


26
C
SR
CNS
0.4 µm


27
CU
CUS
CMV
BF
0.35 µm


28
CUS
MV
CMV
0.3 µm

surface. *CNS* cells, like *CS* cells, appear to terminate within the nerve plate.

Support cells are the most numerous cells of the disc epidermis. They form a supportive meshwork into which the other cell types fit (Figs. 14, 16). Their cytoplasm contains mitochondria, some clear vacuoles, and a few microtubules. Their apical surface bears numerous microvilli and their basal part presumably contacts the basal lamina at the level of the nerve plate. One or several longitudinal bundles of filaments (about 300 nm in diameter) traverse the cell and join its apical and basal membranes. In the enlarged apical processes of the cell, they develop into a particular structure binding the cuticle to the connective tissue indentations (Figs. 13, 27, 28). This structure consists of a large bundle of intracellular filaments that basally binds the connective tissue fibers by way of hemidesmosomes. This bundle of filaments is topped by a sheath of cuticular material that surrounds a central microvillus and also contacts the neighboring microvilli. These structures probably strengthen the whole disc during adhesion.

A three-layered cuticle (Figs. 19, 20, 27) covers the disc epidermal cells. The more external granular layer (about 50 μm thick) covers the microvilli of all epidermal cells. It is underlain by a thin (about 50 nm thick) electron-lucent layer which in turn is underlain by an internal electron-dense granular layer (about 700 nm thick). The last two layers are penetrated by the microvilli apices. The cuticle is separated from the epidermis by a subcuticular space (about 1.3 μm thick) crossed by the microvilli. The cuticle is only traversed by the tips of *CNS* cells cilia and is regularly interrupted by the secretory pores of *NCS2* cells. It is linked up with the epidermis and underlying connective tissue by way of the attachment structures described above.

### *III. Characterization of the foot prints*

When a holothuroid is placed on microscope glass slides, it not only walks on them, it also sweeps the slides with its buccal tentacles. There are two types of prints on the slides: long filamentous threads up to several mm long left by the sweeping tentacles and circular prints about 800 μm in diameter left by the walking podia. The former stain only with toluidine blue while the latter stain strongly with toluidine blue and lightly with brilliant blue G. This together with the fact that only the podia enclose *NCS2* cells while both the podia and the tentacles enclose *NCS1* cells suggest that the secretory granules of *NCS2* cells are partly proteinic in nature. The secretory granules of *NCS1* cells are rather mucopolysaccharidic in nature.

## D. Discussion

### *I. Gross anatomy of the podia*

Locomotory podia of *H. forskali* consist of a stem topped by a flat disc. The stem and the disc together form a functional unit. The stem allows the podium to lengthen, to flex, and to retract (antagonistic action of the hydrostatic pressure of the ambulacral fluid and the retractor muscles of the podium); the disc allows the podium to adhere to the substratum during locomotion.

The stem has the classic tissue stratification of echinoderm podia, that is, from the inside to the outside: a mesothelium, a connective tissue layer, a nerve plexus, and an epidermis (Kawaguti 1964; Florey and Cahill 1977; Wood and Cavey 1981). The only thing that marks the difference between holothuroid and non-holothuroid podia is the fact that the nerve plexus in holothuroid, instead of lying just beneath the epidermis, is located deep in the connective tissue layer of the stem and connected to the epidermis by way of thin bundles of nerve processes. This peculiar arrangement also occurs in holothuroid buccal tentacles (Bouland et al. 1982; McKenzie 1987, 1988a). The nerve plexus, which may be considered as ectoneural, is associated, at the stem level, with a discrete nerve sinus that is lined by flattened ciliated adsinusal (mesothelial-like) cells. Such a sinus has already been described in holothuroid podia (Hyman 1955) and was reported to arise from the ectoneural sinus lining the radial nerve. The meaning of such a peculiar arrangement of the nervous tissue and the possible function of the sinus are still unknown.

Early authors described locomotory podia of holothuroids as suckered podia although the operation of the sucker has never been adequately explained (see Nichols 1966 for review). Our results show that in *H. forskali* the locomotory podia do not end in a sucker, for the disc morphology (the occurrence of a large circular ossicle underlying the whole disc and the peripheral insertion of retractor muscles) does not allow a mechanical sucker-like operation as in podia of regular echinoids and asteroids. The adhesion of the locomotory podia of *H. forskali* to the substratum thus relies entirely on the disc epidermal secretions.

### *II. Comparison with other echinoderms*

The disc epidermis consists of five cell types: two types of non-ciliated secretory cells containing granules whose content is mucopolysaccharidic (*NCS1* cells) or mucopolysaccharidic and proteinic in nature (*NCS2* cells), ciliated secretory cells containing small granules of unknown nature (*CS* cells), ciliated non-secretory cells (*CNS* cells), and support cells. The cilia of *CS* cells are subcuticular whereas those of *CNS* cells, although also short and rigid, traverse the cuticle and protrude into the outer medium. All these cells are closely associated with a well-developed nerve plexus, the nerve plate.

Cells morphologically similar to all these cell types occur in the podial adhesive areas of most echinoderm species. *NCS1* cells are mainly characterized by an apical tuft of "microvillar-like" cell processes containing secretory granules (McKenzie 1988b, named them "apical tuft" cells). *NCS1* cells of the podial epidermis of *H. forskali* are morphologically similar to the "apical tuft" cells of the buccal tentacles of holothuroids (Smith 1983;

Cameron and Fankboner 1984; McKenzie 1987, 1988a) and of the podia of regular and irregular echinoids (Coleman 1969; Flammang et al. 1991, respectively). The secretion of the "apical tuft" cells of holothuroid buccal tentacles and echinoid podia is adhesive.

*NCS2* cells enclose secretory granules that are conspicuously larger than those of *NCS1* cells. They are extruded through a microvillar duct which opens as a pore onto the secretory surface. McKenzie (1988b) has named these cells goblet cells but we prefer to qualify them "apical duct" cells as opposed to "apical tuft" cells. The secretory granules of the "apical duct" cells of the podial epidermis of *H. forskali* closely resemble those described in the podia of other holothuroid species, in that all are made of two distinct areas, a filamentous area (mucopolysaccharidic in nature) and a granular area (proteinic in nature) (Harrison 1968, Menton and Eisen 1970). They also resemble the granules of their equivalent in ophiuroid (Ball and Jangoux 1990) and asteroid (Harrison and Philpott 1966; Souza Santos and Silva Sasso 1968) podia although they lack the highly organized core present in some of these latter. The secretion of the "apical duct" cells of ophiuroid and asteroid podia is adhesive.

The two types of ciliated cells were observed, viz. secretory (*CS* cells) and non-secretory (*CNS* cells), also occur in the adhesive areas of the podia of most echinoderm species. Whatever the considered species, *CNS* cells are always structurally identical and *CS* cells share many common features. The presence of a short, rigid cilium and the fact that both these cells terminate within the nerve plexus are indicative of their sensory (receptor) function (*CS* cells thus may be considered neurosecretory-like).

*NCS*, *CS*, and *CNS* cells are generally closely associated in the podial adhesive areas but they are differently arranged according to whether the podia are handling or locomotory. In the epidermis of handling podia, these cells join together to form sensory-secretory complexes (wherein the three cell types are present). Such complexes occur, for example, in ophiuroid podia (Ball and Jangoux 1990), in spatangoid podia (Flammang et al. 1991), or in holothuroid buccal tentacles (Cameron and Fankboner 1984; McKenzie 1987). Conversely, in the epidermis of locomotory podia, these three cell types from a homogeneous cellular layer together with support cells (Coleman 1969; present work).

### *III. A model for the functioning of the podial disc*

During locomotion, epidermal cells of the podial disc of *H. forskali* are presumably involved in an adhesive/de-adhesive process functioning as a duo-gland adhesive system as proposed by Hermans (1983). In the functional model we propose adhesive and de-adhesive secretions are produced by *NCS* and *CS* cells, respectively. We consider "apical tuft" (*NCS1*) and "apical duct" (*NCS2*) cells to be adhesive as both of them have extruded some of their secretory granules in attached podia. It should be noticed also that these cells were said to be adhesive in every podia so far studied where they always occur independently (till now their co-occurrence was observed only in the disc epidermis of *H. forskali*). Their secretions would make up a bridge of adhesive material between the podium and the substratum, this bridge being the footprint left by the podium after it has become detached from the substratum. The de-adhesion would be due to the material enclosed in the granules of *CS* cells. This material could either be secreted to the outside and act as a de-adhesive or, as proposed by Ball and Jangoux (1990), could act as a neurosecretion controlling or terminating the release of the adhesive secretions. Whatever the mechanism actually involved, the *CS* cells action would allow the podium to become easily detached from the substratum and to always remain clear of any particle. All these secretions would be controlled by stimulations of the two types of ciliated cells (receptor cells) which presumably interact with the secretory cells by way of the nerve plexus.

The meaning of the occurrence in the disc epidermis of locomotory podia of *H. forskali* of two types of adhesive cells remains enigmatic. Maybe is it necessary to offset the lack of suckers? Such suckers moreover would be poorly efficient on soft substrata where *H. forskali* sometimes moves along. So, owing to the coexistence of two adhesive cell types in their disc epidermis, the locomotory podia of *H. forskali* have a "compromise" structure which perhaps would explain their ability to move as efficiently along soft and hard substrata.

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