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The Role of Podial Secretions in Adhesion in Two Species of Sea Stars (Echinodermata)

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Abstract. Individuals of *Asterias rubens* and *Marthasterias glacialis* use their podia in locomotion, anchorage, and feeding. Each podium consists of a stem with a disk at its tip. The stem allows the podium to lengthen, flex, and retract, and the disk allows the podium to adhere to the substratum. Adhesion of sea star podia seems to rely on the epidermal secretions of the disk and not on a mechanical sucker-like operation. The disk epidermis is made up of five cell types: nonciliated secretory cells (NCS cells) of two different types (NCS1 and NCS2), both containing granules that are at least partly mucopolysaccharidic in composition; ciliated secretory cells (CS cells) containing small granules of unknown content; nonsecretory ciliated cells (NCS cells); and support cells. The epidermal cells of the podial disk are presumably functioning as a duo-gland adhesive system that is involved in an adhesive/de-adhesive process. The following model is presented. Adhesive secretions are produced by NCS1 and NCS2 cells (both of them have extruded some of their secretory granules in attached podia). These secretions constitute a layer of adhesive material between the podium and the substratum, this layer being the footprint left by the podium after it has become detached from the substratum. De-adhesion, on the other hand, would be due to CS cell secretions. All these secretions would be controlled by stimuli perceived by the two types of ciliated cells (receptor cells), which presumably interact with the secretory cells *via* the nerve plexus.

Introduction

Marine organisms have developed a wide range of mechanisms allowing them to attach to a substrate or to handle it (Nachtigall, 1974). Among these mechanisms, one can distinguish between mechanical attachments (with, for example, hooks or suckers) and chemical attachments (with adhesive substances). The way the former operate is generally obvious, whereas the functioning of the latter remains enigmatic.

Asteroid podia are traditionally viewed as suckered organs in which adhesion is partly due to suction (mechanical attachment) and partly to adhesives (chemical attachment) (Smith, 1937; Nichols, 1966). According to Paine (1926), about half of the adhesive force of the podia comes from suction and half from adhesive secretions. However, there is no agreement on this ratio. Some authors argue that the part of the percentage allotted to adhesive secretions has been underestimated (Thomas and Hermans, 1985); others believe that suction has been overlooked (Smith, 1991).

As far as the chemical mechanism is concerned, Hermans (1983) proposed that all echinoderm podia possess duo-gland adhesive systems enclosing two types of secretory cells (*viz.*, cells releasing an adhesive secretion and cells releasing a de-adhesive secretion) that are involved in the attachment to the substratum. But McKenzie (1988) concluded that morphological observations supporting such adhesive systems are rare and that more ultrastructural studies of echinoderm podia are needed.

This work, continuing an extended comparative ultrastructural study of echinoderm podia, describes the locomotory podial disk in two species of asteriid asteroids, *Asterias rubens* and *Marthasterias glacialis*. The study deals with the attachment mechanism of the podia on the substrate (mechanical *versus* chemical). It focuses especially on the epidermal secretory cells and their possible participation in a duo-glandular model of adhesion.

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Materials and Methods

Individuals of *Asterias rubens* Linné, 1758, were collected intertidally in Audresselles (Pas-de-Calais, France) in November 1991; individuals of *Marthasterias glacialis* (Linné, 1758) were collected by scuba diving in Brest harbor (Brittany, France) in November 1990. Individuals of both species were transported to the marine biology laboratory of the University of Mons where they were kept in a marine aquarium with closed circulation (13°C, 33‰ salinity) and fed with mussels (*Mytilus edulis* Linné, 1758).

Light microscopy

Podia were cut off individuals that had previously been anesthetized with propylene phenoxetol (0.1% in seawater). The podia were fixed in Bouin's fluid, embedded in paraplast, and cut into 7 μm thick sections. The sections were stained with Masson's trichrome and Mayer's hemalum coupled with phloxine and light green. Alcian blue (pH 2.6) and the periodic acid-Schiff (PAS) techniques were used in the detection of mucopolysaccharides, and mercuric bromophenol blue and Danielli's technique in the detection of proteins (Ganter and Jollès, 1969–1970).

Scanning electron microscopy (SEM)

Podia were fixed in Bouin's fluid for 24 h. They were dehydrated in graded ethanol, dried by the critical point method (with CO_2 as transition fluid), mounted on aluminum stubs, coated with gold in a sputter coater, and observed with a JEOL JSM-6100 scanning electron microscope.

Footprints were prepared as follows. Podia were allowed to adhere firmly to clean glass coverslips. After the podia had detached themselves, the coverslips were soaked in Bouin's fluid and prepared for SEM by critical-point drying.

Transmission electron microscopy (TEM)

Two sets of podia were investigated: unattached podia that did not adhere to any substratum and attached podia that adhered to hardened Spurr blocks. Because podia do not adhere to clean Spurr blocks, the blocks had been placed in seawater for 4–5 days to allow the formation of a primary film. (The primary film is a moderately negative layer, consisting of adsorbed macromolecules and bacteria, that coats all marine surfaces; Characklis, 1981.) The unattached podia were fixed in 3% glutaraldehyde in cacodylate buffer (0.1 *M*, pH 7.8) for 3 h at 4°C, rinsed in cacodylate buffer, and postfixed for 1 h in 1% osmium tetroxide in the same buffer. The attached podia were fixed according to the same protocol as the unattached podia but with the addition of 0.05% ruthenium red to both the primary fixative and the postfixative (Luft, 1971). This technique allows better preservation of extracellular acid mucopolysaccharides as well as their labeling. After a final wash in buffer, both sets of podia were dehydrated in graded ethanol and embedded in Spurr. Ultrathin sections (40–70 nm) were cut with an LKB III ultramicrotome equipped with a diamond knife. They were stained with uranyl acetate and lead citrate and observed with a Zeiss EM 10 transmission electron microscope.

Results

External morphology of the podia

The locomotory podia of both *Asterias rubens* and *Marthasterias glacialis* are arranged in four longitudinal rows on the oral surface of the arms. In a specimen of *A. rubens* with arms about 5 cm long, a podium at the middle of the arm is about 1 mm in diameter and 10 mm in length when protracted. These measurements, in a specimen of *M. glacialis* of about 10-cm arm length, are 1.5 mm and 20 mm, respectively. Each podium consists of an extensible cylindrical stem topped by a somewhat wider flat disk (Figs. 1, 2).

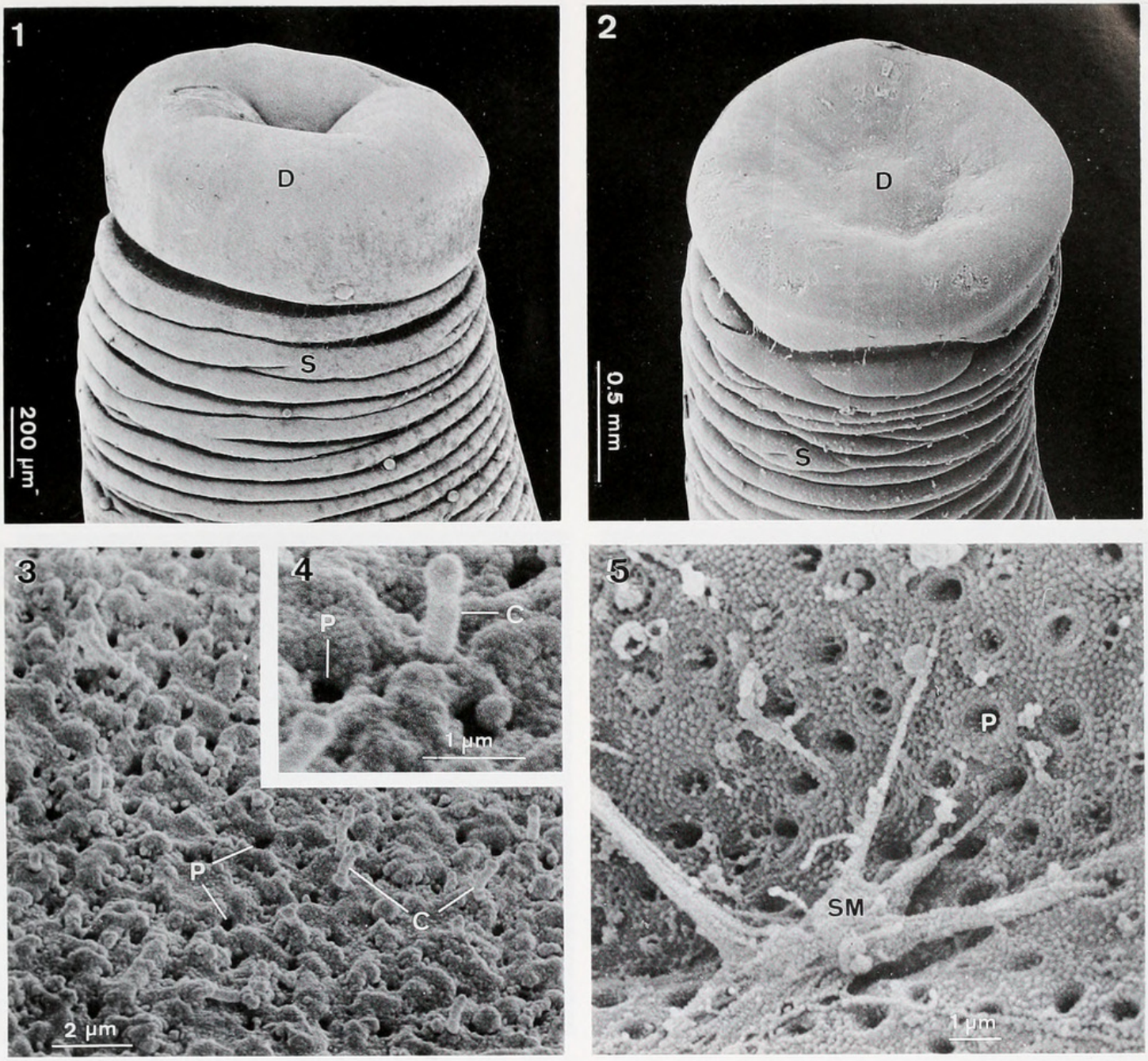
The surface of the disk is sprinkled with uniformly distributed cilia and pores (Fig. 3). In both species, there are about 10 cilia/100 μm^2 and about 4 times as many pores. The cilia are about 1 μm long (Fig. 4). The pores measure about 600 nm in diameter in *A. rubens* (Fig. 5), but only about 450 nm in *M. glacialis* (Fig. 4). Secretory material extruded through the pores was clearly visible on some podia (Fig. 5).

Histology and cytology of the podial disk

The disk consists of four tissue layers that are, from the inside to the outside, a mesothelium, a connective tissue layer, a nerve plexus, and an epidermis covered by a cuticle (Figs. 6, 7).

Disk inner tissues. The inner tissues of the disk in the two species are very similar structurally, and the following description applies to both of them.

The mesothelium surrounds the ambulacral lumen. It is a pseudostratified epithelium comprising three cell types: adluminal cells, glandular cells, and myoepithelial cells; all of them contact the underlying basal lamina. Adluminal cells line the ambulacral cavity. These are T-shaped monociliated cells with a long vibratile cilium (Fig. 8) surrounded by a ring of about 10 microvilli. Glandular cells occur singly among the adluminal cells. Their cytoplasm is filled with membrane-bound, electron-dense secretory granules whose diameter varies between 0.7 and 1.5 μm (Fig. 9). Adluminal cells are bound together and to glandular cells by junctional complexes consisting of an apical zonula adher-



Figures 1–5. Outer aspect of the locomotory podia in *Asterias rubens* and *Marthasterias glacialis*. C, cilium; D, disk; P, pore; S, stem; SM, secretory material.

Figure 1. Podium (*A. rubens*).

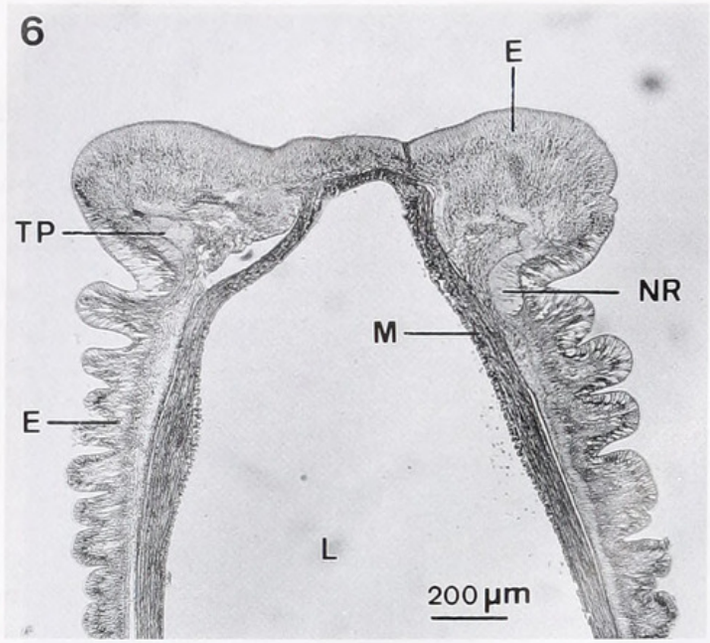
Figure 2. Podium (*M. glacialis*).

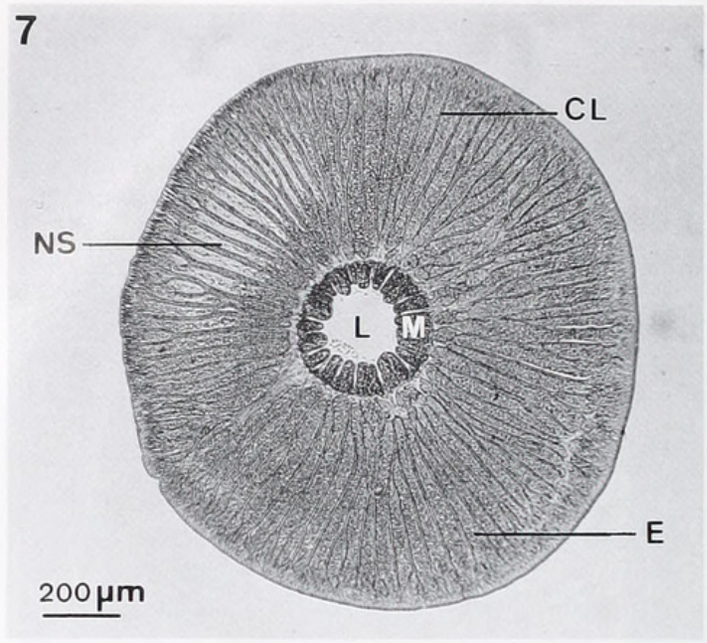
Figure 3. Disk surface (*M. glacialis*).

Figure 4. Detailed view of cilia and pores (*M. glacialis*).

Figure 5. Disk surface with secretory material (*A. rubens*).

ens and a subapical septate desmosome. Both types of 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 and glandular cells. They contain a bundle of myofilaments (Fig. 8) associated with numerous mitochondria and are bound together by regularly spaced desmosome-like junctions. The myoepithelial cells are arranged to form the muscle systems of the podia (that is, the retractor, the levator, and the radial systems; see Smith, 1947, for a detailed description of these systems in *A. rubens*). Intermingled with myoepithelial cells are slender cell processes (cell bodies have never been observed in the mesothelium) whose cytoplasm is filled with electron-dense membrane-bound granules (about 200 nm in diameter). These cells are similar to the


6
E
TP
NR
M
E
L
200 µm


7
CL
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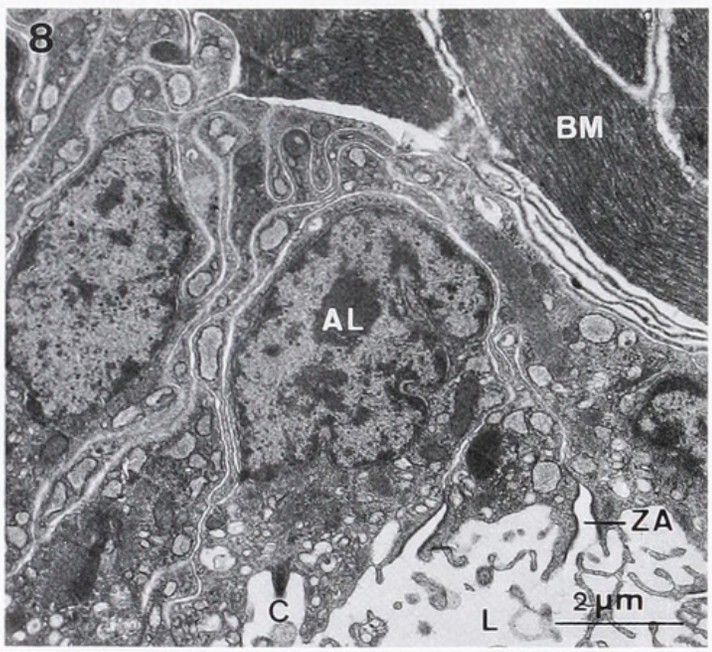
200 µm


8
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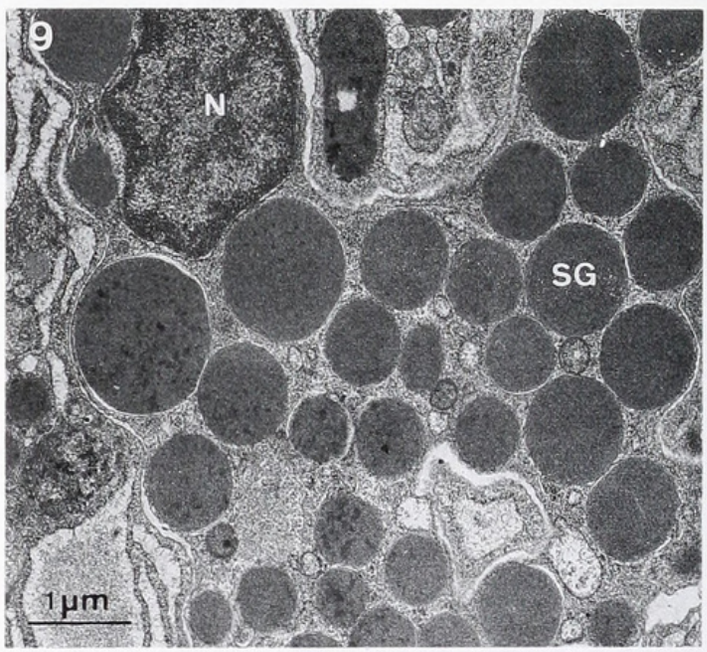
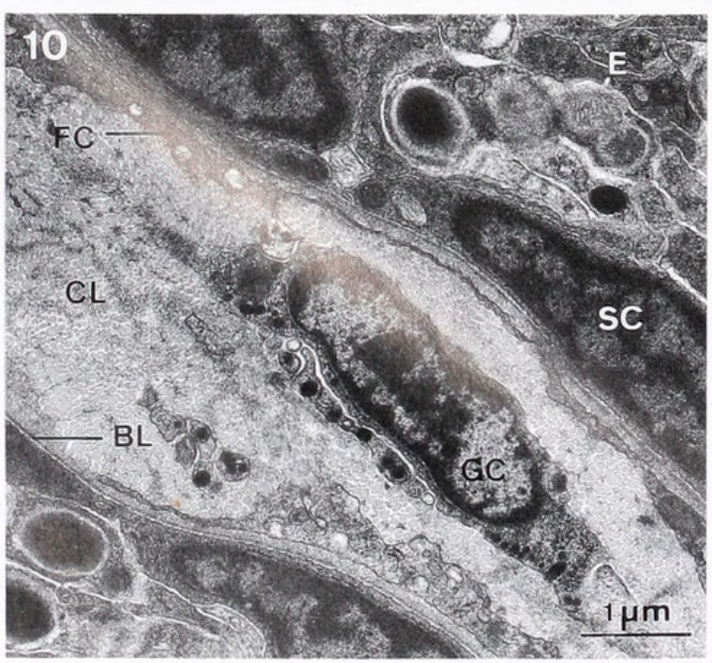
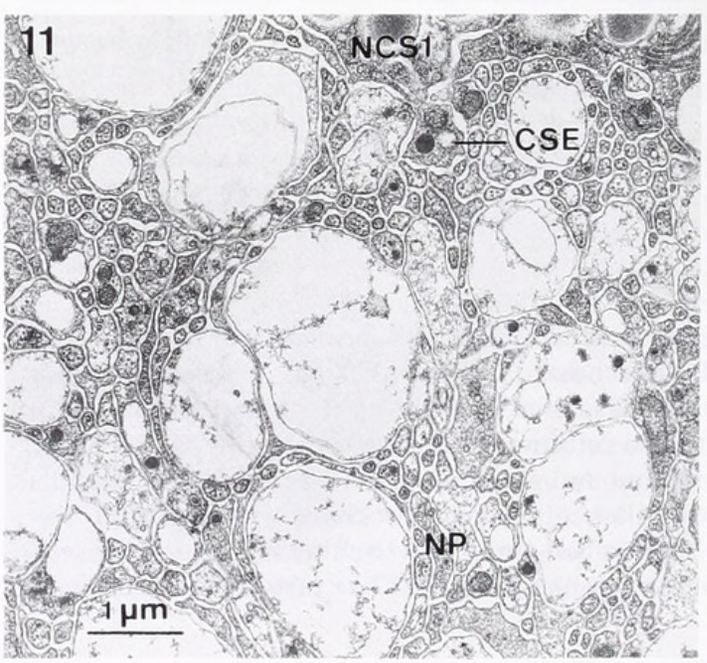
L
2 µm


9
N
SG
1 µm


10
E
FC
CL
SC
BL
GC
1 µm


11
NCS1
CSE
NP
1 µm

granulocytes described by Wood and Cavey (1981) in the podial mesothelium of the asteroid *Stylasterias forreri*.

The connective tissue layer, which occupies most of the volume of the disk, is made of an amorphous ground substance that encloses collagen fibers and elongated cells with an electron-dense cytoplasm, which may be fibrocytes. Granulocytes resembling those in the mesothelium are also present (Fig. 10); they contain many electron-dense, spherical to ellipsoidal granules, 100 to 250 nm in diameter. The connective tissue layer consists mainly of a circular structure—the terminal plate—that underlies and supports the whole disk (Fig. 6). The center of the plate is much thinner than its margin, which forms a thickened rim continuous, proximally, with the cylindrical connective tissue sheath of the stem. Distally, the surface of the terminal plate is drawn out into a series of radial laminae that thrust into the epidermis of the disk (Fig. 7). Within these laminae, collagen fibers are arranged longitudinally (Fig. 10). A few micrometers before reaching the apical surface of the podium, the distal edge of each lamina divides into numerous collagen-rich sheets that insinuate between the epidermal cells and attach apically to the support cells of the epidermis.

The nervous tissue of the disk consists chiefly of two components. First, at the base of the disk, the nerve plexus of the stem is thickened to form the nerve ring (Fig. 6). It comprises nerve cell bodies as well as numerous neurites that contain mitochondria, microtubules, and clear or dense-core vesicles. Second, the nerve ring gives off radial branches that extend over the proximal surface of the terminal plate, where they run between the radial connective laminae. The radial nerve strands are made up of neurites that run mainly in a plane parallel to the apical surface of the podium (Fig. 11). Small bundles of neurites may also be observed between the basal parts of the epidermal secretory cells.

Disk epidermis. In both *A. rubens* and *M. glacialis,* the disk epidermis is made up of five cell types: nonciliated secretory cells (NCS cells) of two different types (NCS1 and NCS2); ciliated secretory cells (CS cells); nonsecretory ciliated cells (NSC cells); and support cells (Figs. 12–15).

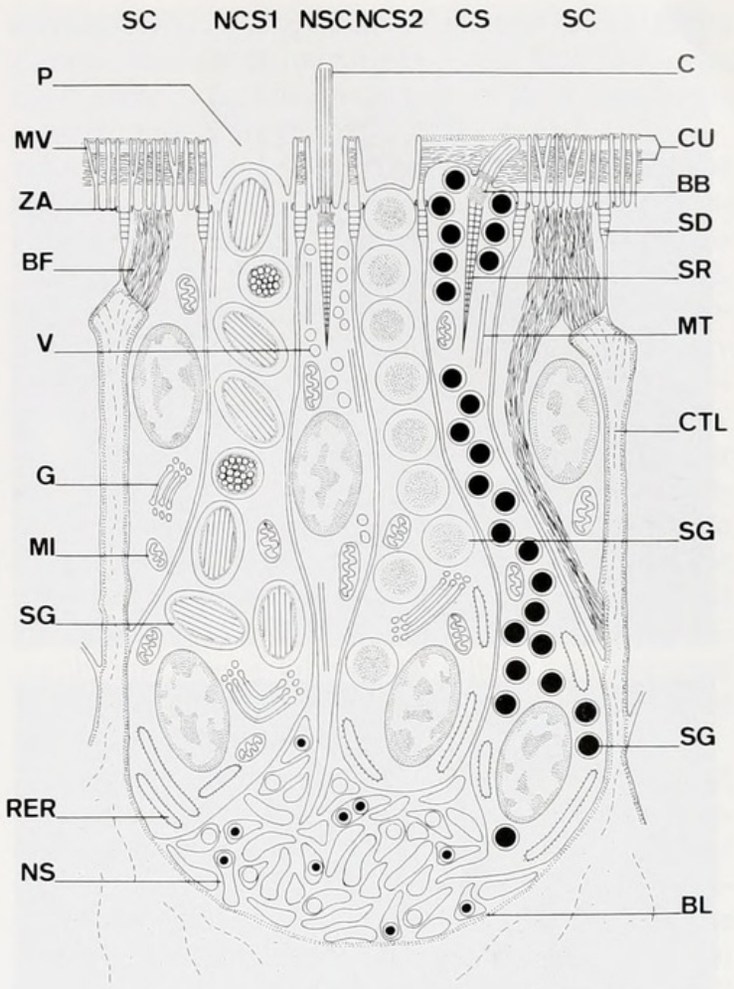
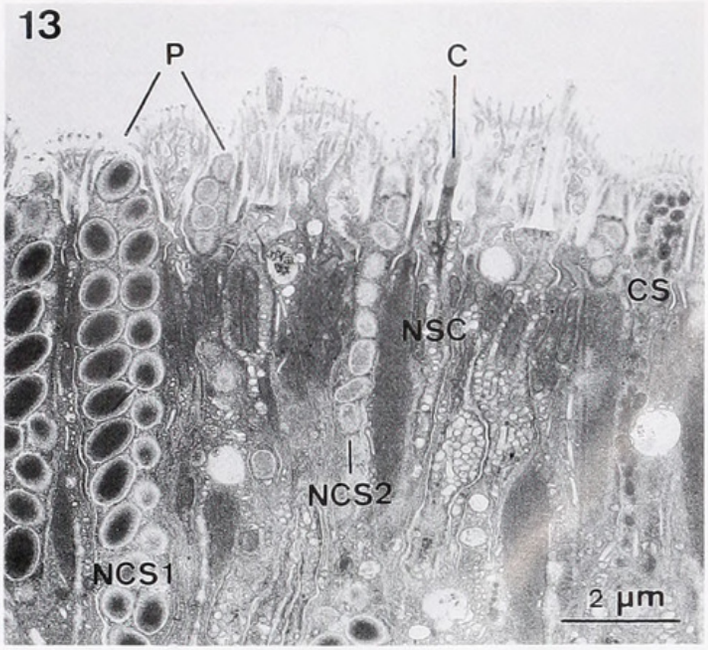


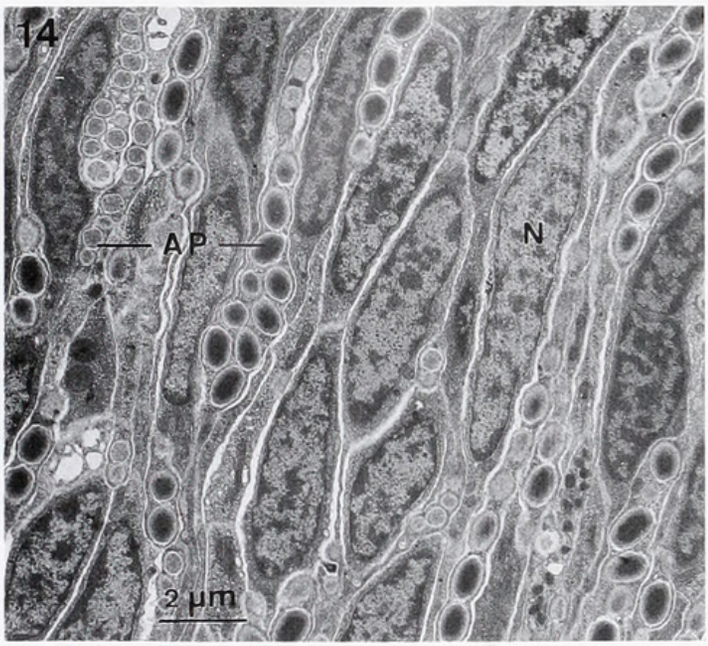
Figure 12. Reconstruction of a transverse section through a radial epidermal strip located between two adjacent connective tissue laminae (not to scale). BB, basal body; BF, bundle of filaments; BL, basal lamina; C, cilium; CS, ciliated secretory cell; CTL, connective tissue lamina; CU, cuticle; G, Golgi zone; MI, mitochondrion; MT, microtubule; MV, microvillus; NCS1, type 1 nonciliated secretory cell; NCS2, type 2 nonciliated secretory cell; NS, nerve strand; NSC, nonsecretory ciliated cell; P, pore; RER, rough endoplasmic reticulum cisternae; SC, support cell; SD, septate desmosome; SG, secretory granule; SR, striated rootlet; V, vesicle; ZA, zonula adherens.

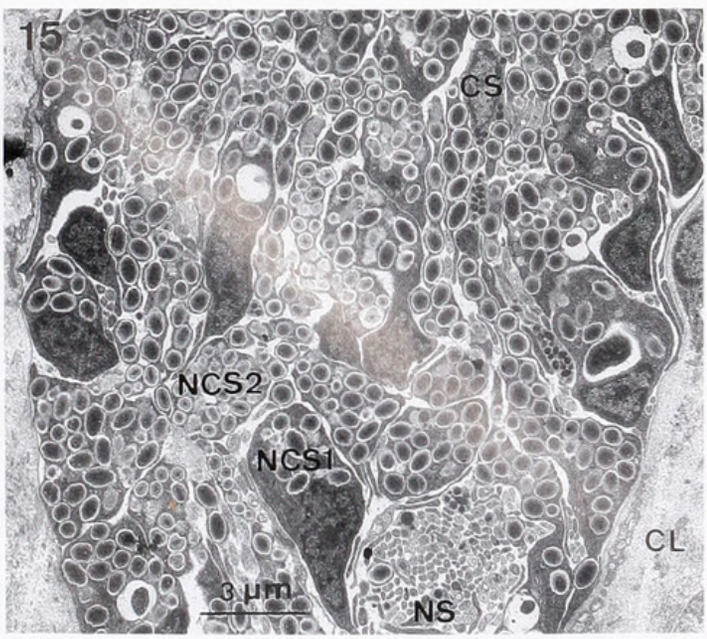
All of these cells are connected apically by junctional complexes made up of a distal zonula adherens and a proximal septate desmosome. In the epidermis, support cells are the most numerous and form a supportive mesh-

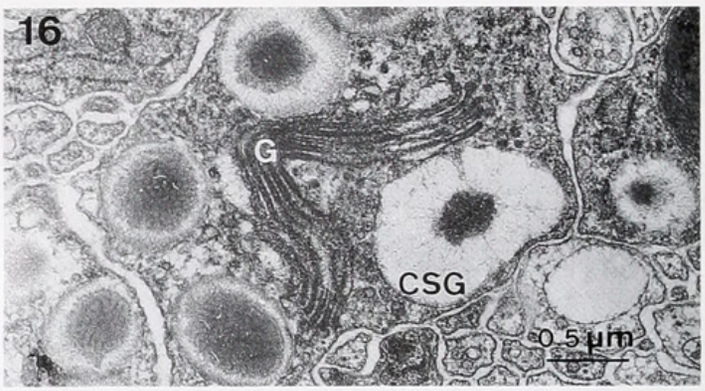
Figures 6–11. Fine structure of the disk of locomotory podia of *Asterias rubens* and *Marthasterias glacialis.* AL, adluminal cell; BL, basal lamina; BM, bundle of myofilaments; C, cilium; CL, connective tissue lamina; CSE, ciliated secretory cell tapered basal end; E, epidermis; FC, fibrocyte-like cell; GC, granulocyte; L, ambulacral lumen; M, mesothelium; N, nucleus; NCS1, type 1 nonciliated secretory cell; NP, nerve processes; NR, nerve ring; NS, nerve strand; SC, support cell; SG, secretory granule; TP, terminal plate; ZA, zonula adherens.

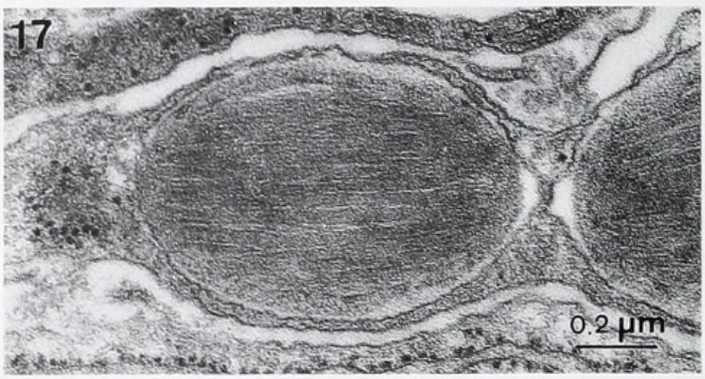
Figure 6. Longitudinal section through the disk (*M. glacialis*).
Figure 7. Transverse section through the disk (*M. glacialis*).
Figure 8. Longitudinal section through the mesothelium (*M. glacialis*).
Figure 9. Glandular cell of the mesothelium (*A. rubens*).
Figure 10. Transverse section through a connective tissue lamina (*M. glacialis*).
Figure 11. Disk nerve strand (*M. glacialis*).

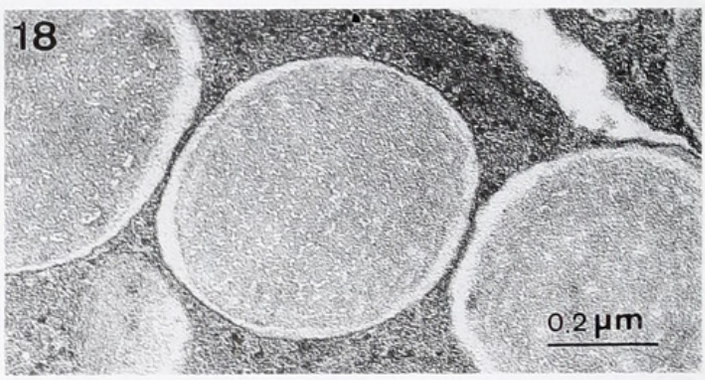

13
P
C
CS
NSC
NCS2
NCS1
2 µm

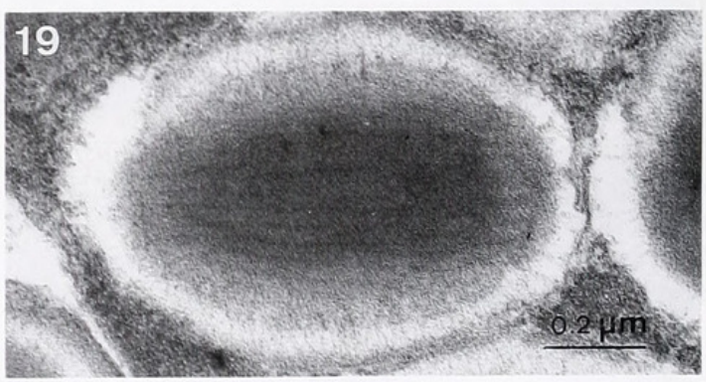

14
AP
N
2 µm

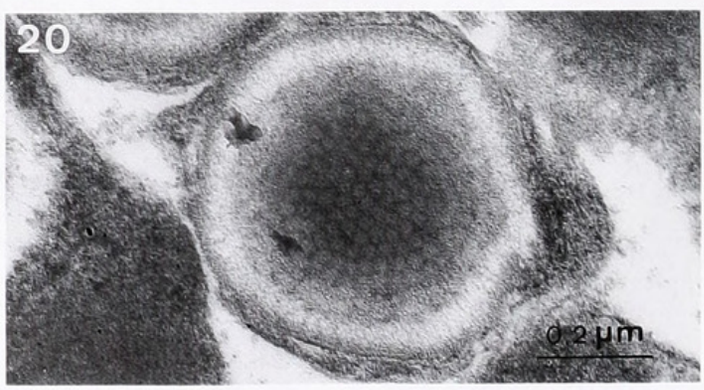

15
CS
NCS2
NCS1
CL
3 µm
NS


16
G
CSG
0.5 µm


17
0.2 µm


18
0.2 µm


19
0.2 µm


20
0.2 µm

work in which the other cell types are homogeneously distributed; the relative proportion of NCS1, NCS2, CS, and NSC cells is, respectively, 4:4:1:2.

Nonciliated secretory cells (NCS1 and NCS2 cells) are about 250 μm long and flask-shaped. Their enlarged cell bodies are located basally, at the bottom of the grooves made up by adjacent connective laminae and close to the radial nerve strands (Figs. 12, 15). Each cell body sends out a long apical process that reaches the apex of the podium (Figs. 12–14). The cytoplasm of both the cell body and the apical process is filled with densely packed membrane-bound secretory granules. In both NCS1 and NCS2 cells, the material enclosed in these secretory granules stains with alcian blue pH 2.6. The cytoplasm of the cell body also contains a well-developed Golgi apparatus, mitochondria, and cisternae of rough endoplasmic reticulum (RER) that are sometimes distended and filled with amorphous material (Fig. 16, 21). Developing secretory granules are closely associated with Golgi membranes and RER cisternae, suggesting that these organelles are involved in the synthesis of the contents of the granules. Besides secretory granules, the cytoplasm of the apical process contains longitudinally arranged peripheral microtubules. At the end of the apical processes, the granules are extruded through a duct delimited by a ring of microvilli and opening onto the disk surface as a cuticular pore (Figs. 12, 13). These pores correspond to those observed on SEM pictures of the disk surface (Figs. 3, 5).

The secretory granules of NCS1 cells are ellipsoids about 1 μm long and 0.6 μm in diameter (Figs. 17–20). They have a complex ultrastructure. Most of their volume is occupied by a bundle of parallel rods about 30 nm in diameter. These rods are somewhat more numerous in the granules of *A. rubens* (about 180, Fig. 18) than in those of *M. glacialis* (about 120, Fig. 20). In the latter, the rods appear to be embedded in material that is more electron-dense. Each bundle of rods is surrounded by a ring of the same density as the rods and separated from the granule membrane by a clear belt. The secretory granules of NCS2 cells are spherical in *A. rubens* (about 550 nm in diameter, Fig. 21) and slightly ellipsoidal in *M. glacialis* (about 500 nm in length and 300 nm in diameter, Fig. 22). They contain electron-lucent, finely granular material surrounded by a clear belt. In *M. glacialis,* some of these granules have an inner core that is more electron-dense (Fig. 22).

Ciliated secretory cells (CS cells) have the same shape and size as NCS cells. They possess an enlarged nucleus-containing cell body and a long and narrow apical process that ends with a bulge just beneath the cuticle (Figs. 12, 13, 23). The entire cytoplasm of the cell (basal part, process, and bulge) is filled with membrane-bound secretory granules. These granules are spherical in *A. rubens,* 300–450 nm in diameter (Fig. 24). They enclose an electron-dense homogeneous material surrounded by a thin, clear belt. In *M. glacialis,* the granules are spherical to ellipsoidal, 250–300 nm in diameter (Fig. 23). They contain an electron-dense fibrillar core surrounded by a less dense ring. The cytoplasm contains many mitochondria and RER cisternae. The apex of the CS cells is devoid of microvilli but bears a subcuticular cilium with a striated rootlet (Fig. 23). The very basal part of the cell is tapered and thrusts into the radial nerve strand (Figs. 11, 12).

Nonsecretory ciliated cells (NSC cells) are narrow and have a centrally located nucleus (Figs. 12, 13). Their cytoplasm includes mitochondria, small, clear vesicles of various shapes and sizes, and longitudinally arranged microtubules (Fig. 25). Their characteristic feature is a single short cilium (about 3 μm long) whose apex protrudes into the outer medium. The cilium has a regular $9 \times 2 + 2$ arrangement of microtubules and possesses a striated rootlet. It is surrounded by a ring of nine microvilli. These cilia are visible on SEM pictures of the disk surface (Fig. 3). NSC cells, like CS cells, terminate within the radial nerve strands.

Support cells have a centrally located nucleus (Figs. 12, 14). Their cytoplasm contains mitochondria and some clear vacuoles. Their apical surface bears numerous microvilli, and their base contacts the basal lamina of the epidermis either on the bottom of the grooves formed by adjacent connective laminae (thus traversing the radial nerve strands) or on the laminae themselves. One longitudinal bundle of intermediate filaments traverses the cell and joins its apical and basal membranes (Figs. 12, 25).

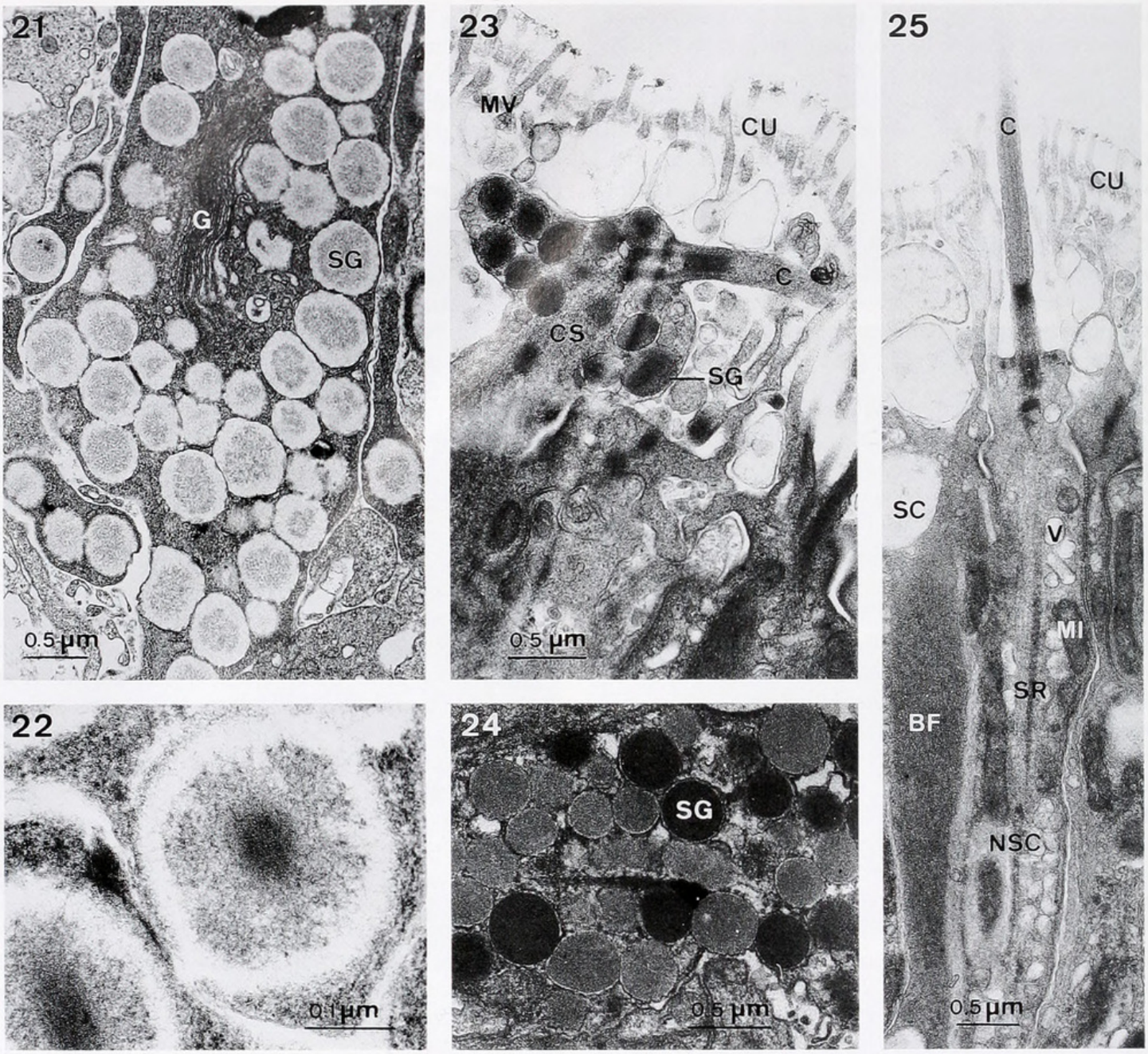
Figures 13–20. Fine structure of the disk epidermis of locomotory podia of *Asterias rubens* and *Marthasterias glacialis.* AP, NCS cell upper process; C, cilium; CS, ciliated secretory cell; CL, connective tissue lamina; CSG, condensing secretory granule; G, Golgi zone; N, nucleus; NCS1, type 1 nonciliated secretory cell; NCS2, type 2 nonciliated secretory cell; N, nucleus; NS, nerve strand; NSC, nonsecretory ciliated cell; P, pore.

Figures 13–15. Longitudinal sections through the disk epidermis (apex, mid part, and basal part; respectively) (*M. glacialis*).

Figure 16. Nucleus-containing basal part of a type 1 nonciliated secretory cell (NCS1 cell) (*M. glacialis*).

Figures 17, 18. NCS1 cell secretory granules in *A. rubens* (longitudinal and transverse section, respectively).

Figures 19, 20. NCS1 cell secretory granules in *M. glacialis* (longitudinal and transverse section, respectively).



Figures 21–25. Fine structure of the disk epidermis of locomotory podia of *Asterias rubens* and *Marthasterias glacialis*. BF, bundle of filaments; C, cilium; CS, ciliated secretory cell; CU, cuticle; G, Golgi zone; MI, mitochondrion; MV, microvilli; NSC, nonsecretory ciliated cell; SC, support cell; SG, secretory granule; SR, striated rootlet; V, vesicle.

Figure 21. Nucleus-containing basal part of a type 2 nonciliated secretory cell (NCS 2 cell) (*A. rubens*).

Figure 22. NCS2 cell secretory granule (*M. glacialis*).

Figure 23. Apical bulge of a ciliated secretory cell (CS cell) (*M. glacialis*).

Figure 24. CS cell secretory granules (*A. rubens*).

Figure 25. Apex of a nonsecretory ciliated cell (*M. glacialis*).

A few micrometers before reaching the disk surface, support cells enlarge, forming an attachment area for the collagen fibers of the connective tissue sheets.

A two-layered cuticle covers the epidermal cells of the disk (Figs. 12, 23, 30). The cuticle is traversed by the epidermal cell microvilli and by the tips of the cilia of NSC cells, and is regularly interrupted by the secretory pores of NCS cells. It consists of an internal filamentous layer (about 600 nm thick in *A. rubens* and 350 nm in *M. glacialis*) and an external granular layer (about 200 nm thick in *A. rubens* and 100 nm in *M. glacialis*). The external layer is strongly labeled with ruthenium red, showing that it bears many negatively charged sites (Luft, 1971). The cuticle is separated from

the epidermis by a subcuticular space that is wider in *M. glacialis* than in *A. rubens*.

Footprints and attached podia

The footprints left by the podia of both *A. rubens* and *M. glacialis* have the same shape and the same diameter as the podial disks (Fig. 26). Almost the whole surface of the print consists of adhesive material, except for some small areas that lack it (*i.e.*, one central circular zone and a few radial or concentric narrow stripes). Other footprints, less numerous, are completely full of adhesive material. Three different aspects of the adhesive material can be recognized; these may occur together in the same footprint and probably represent three successive stages in the accumulation of adhesive during the secretion process. In the first stage, the adhesive material has the shape of a network with a mesh of about 3 μm (Fig. 27). The second stage is characterized by the addition of secretory material that gives the footprint a felt-like appearance (Fig. 28). In the third stage, the adhesive material becomes still thicker, forming a homogeneous layer that completely covers the glass substratum (Fig. 29).

Smaller prints (Fig. 30) observed on the edges of the coverslips correspond to podia that adhered by the margin of their disk. The adhesive strength produced by these podia was equivalent to that of podia attached by their entire disk.

Electron micrographs of attached podia of *A. rubens* and *M. glacialis* reveal secretory material forming an adhesive layer between the podium and the substratum (Fig. 31)—more exactly, between the cuticle and the thin primary film covering the Spurr block. (The film stains heavily with ruthenium red, demonstrating that it bears many negatively charged sites; Luft, 1971). The material of the adhesive layer is a compact fibrillar matrix (Fig. 31). It binds ruthenium red, though the labeling is weaker than on both the primary film and the external layer of the cuticle.

The adhesive material clearly arises from the two types of nonciliated secretory cells (NCS1 and NCS2 cells; Figs. 30–33). The material enclosed in their most apical secretory granules (the outermost few, ranging from 1 up to 10 in some NCS1 cells) appears to swell, losing its usual aspect (Fig. 32). The apical cell membrane and the membranes of the secretory granules vanish, and the granule content is extruded through the cuticular pores (Fig. 33) in the space between the podium and the substratum, forming the adhesive layer.

As for the CS cells, they have the same morphology in attached and unattached podia (Fig. 34); *i.e.*, they do not appear to have extruded any of their secretory granules.

Discussion

Individuals of *Asterias rubens* and *Marthasterias glacialis* use their podia in locomotion and anchorage, and to open the bivalve mollusks on which they feed (Lawrence, 1987). These podia consist of a cylindrical stem topped by a specialized disk. The stem and the disk together form a functional unit: the stem allows the podium to lengthen, flex, and retract—through the action of the podial retractor muscle on the ambulacral fluid—and the disk allows it to adhere to the substratum.

Podial adhesion: mechanical versus chemical attachment

The disk has the classical 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).

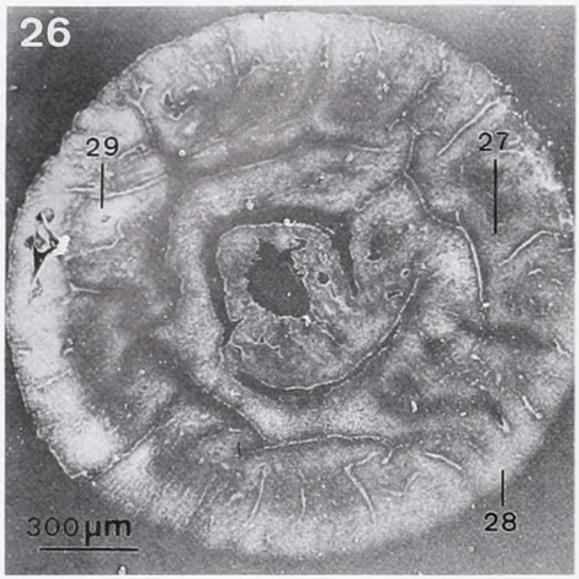
In the mesothelium, myoepithelial cells arrange to form three muscle systems: the retractor, the levator, and the radial systems. The levator and the radial muscle systems have been implicated in a sucker-like mechanical operation of the disk; the former providing the suction and the latter releasing it (Smith, 1947).

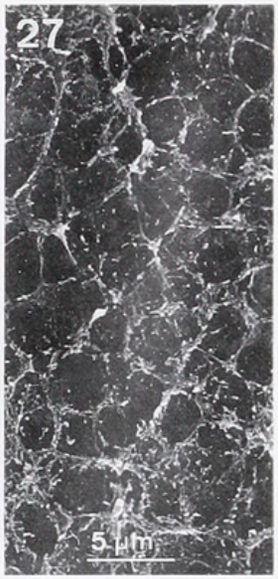
Suction has often been regarded as the primary means of podial adhesion in seastars. Paine (1926), who studied the podia of *Asterias vulgaris*, concluded that 56% of podial attachment would be contributed by suction and the rest by adhesive secretions. Recently, Smith (1991) reevaluated the adhesive strength of suckers in water and suggested that suction may have been underestimated in the adhesive organs of several invertebrates, including sea stars. On the other hand, Thomas and Hermans (1985) found that podia of the sea star *Leptasterias hexactis* adhered very strongly to a fine-meshed, stainless steel plankton screen that precluded the podia from using suction. These workers concluded that, although sea star podia may use suction, it is a secondary adjunct to the adhesion established by secretions.

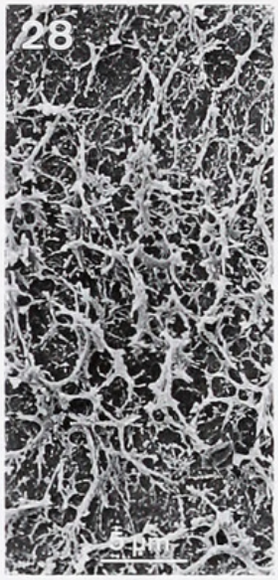
Data presented in this report support this second hypothesis: first, many epidermal secretory cells cover the entire apical surface of the disk; second, the footprints are completely (or almost completely) full of adhesive material; and third, podia may adhere strongly with only the margin of their disk (leaving crescent-shaped footprints). These complementary features argue for an adhesive process principally mediated by secretion. In this event, the musculature of the disk would act to modify the apical surface of the disk, molding it exactly to that of the substratum. However, suction cannot be ruled out altogether; *i.e.*, the muscular equipment in the podial disk can certainly develop suction sufficient to reinforce adhesion (Smith, 1991).

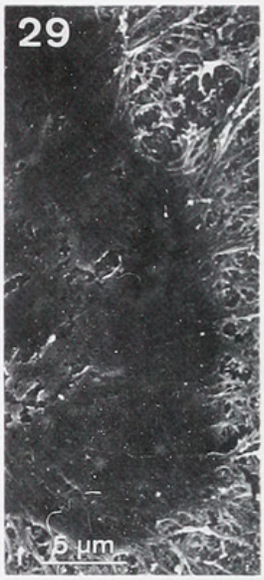
Comparison with other echinoderms

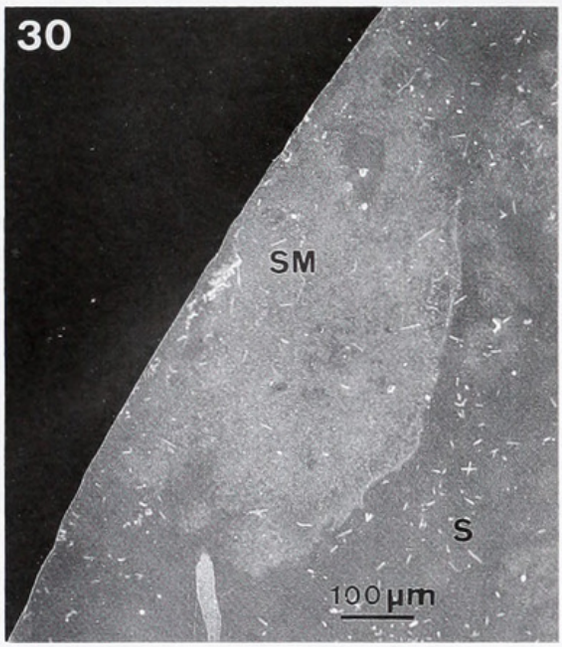
The disk epidermis consists of five cell types: two types of nonciliated secretory cells containing granules whose

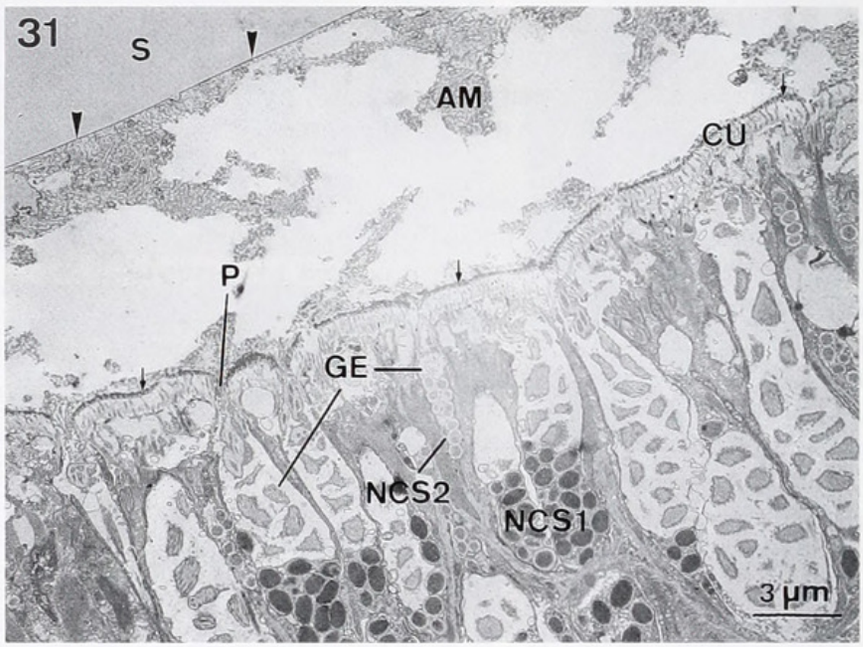

26
29
27
28
300 µm

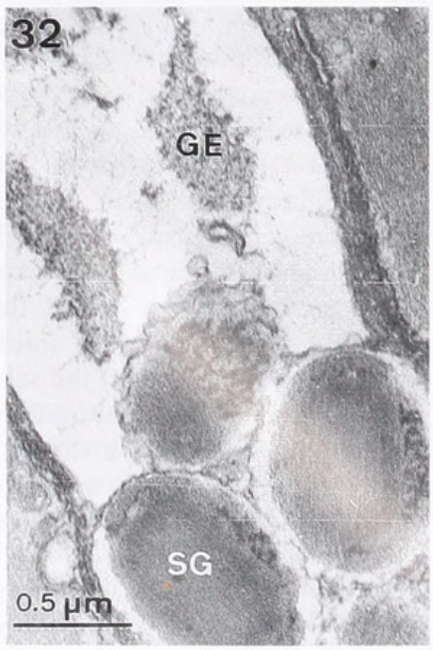

27
5 µm

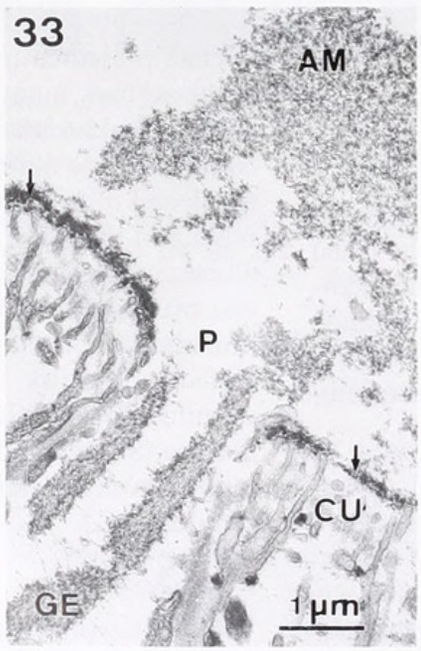

28
5 µm

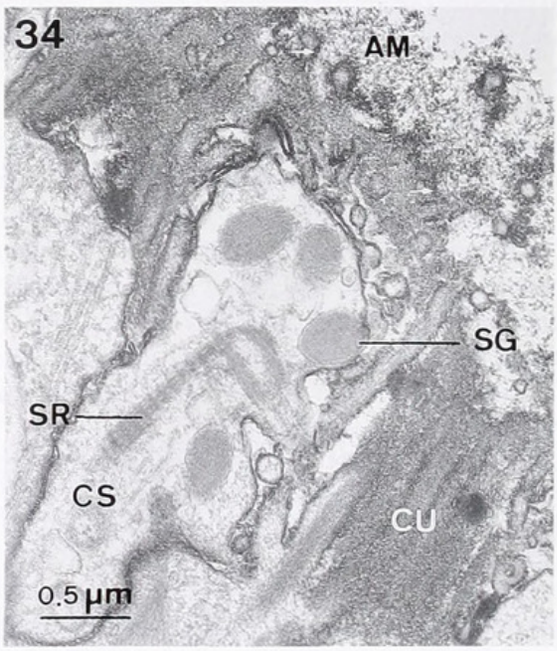

29
5 µm


30
SM
S
100 µm


31
S
AM
CU
P
GE
NCS2
NCS1
3 µm


32
GE
SG
0.5 µm


33
AM
P
CU
GE
1 µm


34
AM
SG
SR
CS
CU
0.5 µm

content is at least partly mucopolysaccharidic (NCS1 and NCS2 cells); ciliated secretory cells containing granules of unknown content (CS cells); nonsecretory ciliated cells (NSC cells); and support cells. The cilia of CS cells are subcuticular, whereas those of the NSC cells, although also short and rigid, traverse the cuticle and protrude into the outer medium. All of these cells are closely associated with well-developed nerve strands.

In both species, the two types of NCS cells occurring in the disk epidermis resemble each other morphologically; moreover, the tinctorial affinities of their secretory granules are also similar. This resemblance probably misled earlier students of asteroid podia, for they described only the NCS1 cells and failed to recognize the NCS2 cells (Harrison and Philpott, 1966; Souza Santos and Silva Sasso, 1968; McKenzie, 1988); only Chaet (1965) described the two types of NCS cells in the disk epidermis of *Asterias forbesi*. The NCS cells of *A. rubens* and *M. glacialis* are typical "apical duct" cells (according to the terminology of Flammang and Jangoux, 1992); *i.e.*, their secretory granules are extruded through a duct that is walled by microvilli and opens as a pore onto the secretory surface. Such cells have already been observed in the podia of paxillosid asteroids, which end not with a disk, but with a conical tip (Engster and Brown, 1972); in ophiuroid podia (Ball and Jangoux, 1990); and in the locomotory podia of several holothuroid species (Harrison, 1968; Flammang and Jangoux, 1992). In the podia of all these species, NCS cells are thought to be adhesive in function.

Both ciliated secretory cells (CS cells) and nonsecretory ciliated cells (NSC cells) terminate within the nerve strands. This, together with the fact that they both bear a short and rigid cilium, is indicative of their nervous origin and sensory function. In addition, CS cells are filled with secretory granules and, therefore, may be considered neurosecretory-like. Ciliated cells morphologically similar to these two cell types occur in the podial adhesive areas of most echinoderm species.

CS cells are remarkably similar in all echinoderm species whose podia have been examined. In all cases they contain small electron-dense granules and often present a subcuticular cilium (Engster and Brown, 1972; McKenzie, 1987; Ball and Jangoux, 1990; Flammang *et al.*, 1991; Flammang and Jangoux, 1992, 1993). CS cells of *A. rubens* and *M. glacialis* are, however, peculiar because they possess a large apical bulge instead of the more common granule-filled, microvillar-like cell processes (Flammang *et al.*, 1991; Flammang and Jangoux, 1993); and also because of the large size of their granules. Some workers have considered CS cells to be de-adhesive, although the way in which they fulfill this function remains obscure. Ball and Jangoux (1990) proposed that CS cell secretions could act as neurotransmitters controlling or terminating the release of the adhesive secretion. On the other hand, Flammang and Jangoux (1993) suggested that their granule content would be released into the outer medium, acting directly as a de-adhesive.

NSC cells are monociliated; their cilium traverses the cuticle and protrudes into the outer medium. These cells, like CS cells, are almost identical in the podia of all echinoderm species studied so far (Engster and Brown, 1972; Burke, 1980; McKenzie, 1987; Ball and Jangoux, 1990; Flammang *et al.*, 1991; Flammang and Jangoux, 1992, 1993).

Comparison with other taxa

Many marine benthic organisms are equipped with particular secretory organs that allow them to adhere to the substratum. Three forms of adhesion may be distinguished: (1) loose adhesion involving mucus and permitting simultaneous adhesion and movement along the substratum (*e.g.*, the foot secretions of some mollusks); (2) permanent adhesion involving the secretion of a cement (*e.g.*, the attachment of barnacles on rocks); and (3) temporary adhesion allowing an organism to attach strongly but momentarily to the substratum (Tyler, 1988).

Among the adhesive organs of marine invertebrates, duo-gland organs, enclosing two types of secretory cells (*viz.*, cells releasing an adhesive secretion and cells releasing a de-adhesive secretion) and involved in temporary attachment, are morphologically the closest to echinoderm podial adhesive systems. These duo-gland adhesive organs are very frequently found in small invertebrates inhabiting

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Figures 26–34. Footprints and attached podia of *Asterias rubens* and *Marthasterias glacialis*. Arrowheads indicate the primary film; arrows indicate the external layer of the cuticle. AM, adhesive material; CS, ciliated secretory cell; CU, cuticle; GE, granules in the process of extrusion; NCS1, type 1 nonciliated secretory cell; NCS2, type 2 nonciliated secretory cell; P, pore; S, substratum; SG, secretory granule; SM, secretory material; SR, striated rootlet.

Figure 26. Footprint of *M. glacialis*.

Figures 27–29. The three aspects of the secretory material (*M. glacialis*).

Figure 30. Crescent-shaped footprint left by the margin of a podium of *A. rubens* on the edge of a coverslip.

Figure 31. Attached podium of *A. rubens*.

Figures 32, 33. Apical part and secretory pore, respectively, of a NCS1 cell (*A. rubens*).

Figure 34. Ciliated secretory cell (*M. glacialis*).

the interstitial environment—for example, in Turbellaria (Tyler, 1976); in Gastrotricha (Tyler and Rieger, 1980); in Nematoda (Adams and Tyler, 1980); and in Polychaeta (Gelder and Tyler, 1986). In every species studied, the adhesive organs contain two types of closely associated secretory cells. Cells of the first type are specialized epidermal cells similar to echinoderm NCS cells, and they release an adhesive material. Cells of the second type are derived from sensory nerve cells and resemble echinoderm CS cells; they are de-adhesive in function. They release their secretory granules through a pore adjoining the secretory pore of the adhesive cell.

A duo-gland adhesive system has also been described, recently, in the captacula (*i.e.*, the food-collecting tentacles) of scaphopod mollusks (Byrum and Ruppert, 1994), still widening the distribution range of this adhesive system in marine invertebrates. In the species examined, *Graptacme calamus,* the secretory cells look strikingly similar to echinoderm NCS and CS cells.

A model for the adhesive mechanism of sea star podia

Epidermal cells of the podial disk of *A. rubens* and *M. glacialis* are presumably involved in adhesion and de-adhesion and function 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 the two types of NCS cells to be adhesive because they are the only secretory cells having extruded some of their secretory granules in attached podia. Moreover, these cells have been suggested to be adhesive in every podium so far studied. Their secretions form the adhesive layer joining the podium to the substratum; this layer remains on the substratum as a footprint after the podium has become detached.

The de-adhesion would be due to the material enclosed in the granules of CS cells. Indeed, there must be a detachment mechanism that is under control of the animal and allows the podia to easily become detached from the substratum. The CS cells are the best candidates for this function. In *A. rubens* and *M. glacialis,* the way their secretions are exocytosed is enigmatic; but, by analogy with echinoid CS cells, we believe that their secretory granules are extruded and act at the surface of the disk. This de-adhesive material would also prevent particles from accumulating on the adhesive surface of the disk.

All of these secretions are presumably controlled by stimulation of the two types of ciliated cells (receptor cells), which then interact with the secretory cells by means of the nerve strands. Because the cuticle-protruding cilia of NSC cells are the first to contact the substratum, it is likely that they trigger NCS cell secretion. On the other hand, the release of secretory granules by the CS cells would be induced by a stimulation of their subcuticular cilia.

The significance of two types of adhesive cells in the podial disk epidermis of both *A. rubens* and *M. glacialis* is obscure. Furthermore, asteroid podia are not exceptions: ophiuroid podia also have two types of adhesive cells (Ball and Jangoux, 1990), as do holothuroid locomotory podia (Flammang and Jangoux, 1992); and in non-echinoderm invertebrates, the co-occurrence of two types of adhesive cells has been reported in a species of turbellarians (Ehlers, 1989). Maybe the two secretions combine to form a special type of adhesive secretion. Another possibility is that one type of adhesive cell is used only in locomotion on horizontal substrata, whereas both types are necessary during locomotion on vertical substrata or for anchorage, when a stronger adhesive bond is required.

Tyler (1988) suggested that most adhesives in marine organisms involve an association of protein and glycans. In both *A. rubens* and *M. glacialis,* the adhesive secretion is probably a polysaccharide-protein complex, with the main component being an acid mucopolysaccharide (positive reaction of NCS cells with alcian blue pH 2.6, ruthenium red labeling of the adhesive layer between podia and substratum). We failed to detect proteins, but Perpeet and Jangoux (1973) found a protein component in NCS cell secretory granules of *A. rubens.* This adhesive material could link the negatively charged cuticle, through divalent cations, to the negatively charged primary film. Such a mode of adhesion has already been described in bacteria (Corpe, 1974; Marshall, 1974) and diatoms (Cooksey, 1981), and has been proposed in several turbellarians and archiannelids (Martin, 1978). This notion is, however, contrary to the model that Thomas and Hermans (1985) proposed for asteroid podia, in which acid mucopolysaccharides are considered to be de-adhesive in function. In the two species studied in this work, the composition of the de-adhesive secretion remains unknown, as it does in all marine invertebrates (Tyler, 1988).

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Literature Cited

Adams, P. J. M., and S. Tyler. 1980. Hopping locomotion in a nematode: functional anatomy of the caudal gland apparatus of *Theristus caudasaliens* sp. n. *J. Morphol.* **164:** 265–285.

Ball, B., and M. Jangoux. 1990. Ultrastructure of the tube foot sensory-secretory complex in *Ophiocomina nigra* (Echinodermata, Ophiuroidea). *Zoomorphology* **109:** 201–209.

Burke, R. D. 1980. Podial sensory receptors and the induction of metamorphosis in echinoids. *J. Exp. Mar. Biol. Ecol.* **47:** 223–234.

Byrum, C. A., and E. E. Ruppert. 1994. The ultrastructure and functional morphology of a captaculum in *Graptacme calamus* (Mollusca, Scaphopoda). *Acta Zool. (Stockh.)* **75:** 37–46.

Chaet, A. B. 1965. Invertebrate adhering surfaces: secretions of the starfish, *Asterias forbesi,* and the coelenterate, *Hydra pirardi. Ann. N.Y. Acad. Sci.* **118:** 921–929.

Characklis, W. G. 1981. Fouling biofilm development: a process analysis. *Biotechnol. Bioeng.* **23:** 1923–1960.

Cooksey, K. E. 1981. Requirement for calcium in adhesion of a fouling diatom to glass. *Appl. Environ. Microbiol.* **41:** 1378–1382.

Corpe, W. A. 1974. Microfouling: the role of primary film forming marine bacteria. Pp 598–609 in *Fouling Biology: Proc. 3rd. Int. Cong. on Marine Corrosion and Fouling,* R. F. Acker, B. F. Brown, J. R. DePalma, and W. P. Iverson, eds. Northwestern University Press, Evanston, IL.

Ehlers, U. 1989. Duo-gland adhesive systems of *Schizochilus caecus* L'Hardy (Platyhelminthes, Kalyptorhynchia). *Microfauna Mar.* **5:** 243–260.

Engster, M. S., and S. C. Brown. 1972. Histology and ultrastructure of the tube foot epithelium in the phanerozonian starfish, *Astropecten. Tissue Cell* **4:** 503–518.

Flammang, P., and M. Jangoux. 1992. Functional morphology of the locomotory podia of *Holothuria forskali* (Echinodermata, Holothuroida). *Zoomorphology* **111:** 167–178.

Flammang, P., and M. Jangoux. 1993. Functional morphology of coronal and peristomeal podia in *Sphaerechinus granularis* (Echinodermata, Echinoida). *Zoomorphology* **113:** 47–60.

Flammang, P., C. De Ridder, and M. Jangoux. 1991. Ultrastructure of the penicillate podia of the spatangoid echinoid *Echinocardium cordatum* (Echinodermata) with special emphasis on the epidermal sensory-secretory complexes. *Acta Zool. (Stockh.)* **72:** 151–158.

Florey, E., and M. A. Cahill. 1977. Ultrastructure of sea urchin tube feet. Evidence for connective tissue involvement in motor control. *Cell Tissue Res.* **177:** 195–214.

Ganter, P., and G. Jollès. 1969–1970. *Histochimie normale et pathologique,* Vols. 1, 2. Gauthiers-Villars, Paris.

Gelder, S. R., and S. Tyler. 1986. Anatomical and cytochemical studies on the adhesive organs of the ectosymbiont *Histriobdella homari* (Annelida: Polychaeta). *Trans. Am. Microsc. Soc.* **105:** 348–356.

Harrison, G. 1968. Subcellular particles in echinoderm tube feet. II. Class Holothuroidea. *J. Ultrastruct. Res.* **23:** 124–133.

Harrison, G., and D. Philpott. 1966. Subcellular particles in echinoderm tube feet. I. Class Asteroidea. *J. Ultrastruct. Res.* **16:** 537–547.

Hermans, C. O. 1983. The duo-gland adhesive system. *Oceanogr. Mar. Biol. Ann. Rev.* **21:** 281–339.

Kawaguti, S. 1964. Electron microscopic structure of the podial wall of an echinoid with special references to the nerve plexus and the muscle. *Biol. J. Okayama Univ.* **10:** 1–12.

Lawrence, J. M. 1987. *A Functional Biology of Echinoderms.* Croom Helm, London.

Luft, J. H. 1971. Ruthenium red and violet. II. Fine structural localization in animal tissues. *Anat. Rec.* **171:** 369–416.

Marshall, K. C. 1974. Mechanism of adhesion of marine bacteria to surfaces. Pp 625–632 in *Fouling Biology: Proc. 3rd. Int. Cong. on Marine Corrosion and Fouling,* R. F. Acker, B. F. Brown, J. R. DePalma, and W. P. Iverson, eds. Northwestern University Press, Evanston, IL.

Martin, G. G. 1978. Ciliary gliding in lower invertebrates. *Zoomorphologie* **91:** 249–261.

McKenzie, J. D. 1987. The ultrastructure of the tentacles of eleven species of dendrochirote holothurians studied with special reference to surface coats and papillae. *Cell Tissue Res.* **248:** 187–199.

McKenzie, J. D. 1988. The ultrastructure of tube foot epidermal cells and secretions: their relationship to the duo-glandular hypothesis and the phylogeny of the echinoderm classes. Pp 287–298 in *Echinoderm Phylogeny and Evolutionary Biology,* C. R. C. Paul and A. B. Smith, eds. Clarendon Press, Oxford.

Nachtigall, V. 1974. *Biological Mechanisms of Attachment. The Comparative Morphology and Bioengineering of Organs for Linkage, Suction, and Adhesion.* Springer-Verlag, Berlin.

Nichols, D. 1966. Functional morphology of the water-vascular system. Pp 219–240 in *Physiology of Echinodermata,* R. A. Boolootian, ed. Interscience Publishers, New York.

Paine, V. L. 1926. Adhesion of the tube feet in starfishes. *J. Exp. Zool.* **45:** 361–366.

Perpeet, C., and M. Jangoux. 1973. Contribution à l'étude des pieds et des ampoules ambulacraires d'*Asterias rubens* (Echinodermata, Asteroidea). *Forma Functio* **6:** 191–209.

Smith, A. M. 1991. Negative pressure generated by octopus suckers: a study of the tensile strength of water in nature. *J. Exp. Biol.* **157:** 257–271.

Smith, J. E. 1937. The structure and function of the tube feet in certain echinoderms. *J. Mar. Biol. Ass. U.K.* **22:** 345–357.

Smith, J. E. 1947. The activities of the tube feet of *Asterias rubens* L. I. The mechanics of movement and of posture. *Quart. J. Microsc. Sci.* **88:** 1–14.

Souza Santos, H., and W. Silva Sasso. 1968. Morphological and histochemical studies on the secretory glands of starfish tube feet. *Acta Anat.* **69:** 41–51.

Thomas, L. A., and C. O. Hermans. 1985. Adhesive interactions between the tube feet of a starfish, *Leptasterias hexactis,* and substrata. *Biol. Bull.* **169:** 675–688.

Tyler, S. 1976. Comparative ultrastructure of adhesive systems in the Turbellaria. *Zoomorphologie* **84:** 1–76.

Tyler, S. 1988. The role of function in determination of homology and convergence—examples from invertebrate adhesive organs. *Fortschr. Zool.* **36:** 331–347.

Tyler, S., and G. E. Rieger. 1980. Adhesive organs of the Gastrotricha. I. Duo-gland organs. *Zoomorphologie* **95:** 1–15.

Wood, R. L., and M. J. Cavey. 1981. Ultrastructure of the coelomic lining in the podium of the starfish *Stylasterias forreri. Cell Tissue Res.* **218:** 449–473.