Zoomorphology (1987) 107:261–272

Zoomorphology

Fine structure of the dorsal arm plate of *Ophiocoma wendti*: Evidence for a photoreceptor system (Echinodermata, Ophiuroidea)

Gordon Hendler[1] **and Maria Byrne**[2]

[1] Life Sciences, Natural History Museum of Los Angeles County, 900 Expositon Boulevard, Los Angeles, CA 90007, USA
[2] Zoology Department, University College Galway, Galway, Ireland

Summary. Three structures in the dermis of the dorsal arm plate (DAP) of the brittlestar, *Ophiocoma wendti,* appear to comprise a photoreceptor system. The upper surface of the DAP bears transparent, knob-like, microscopic structures which are expanded peripheral trabeculae (EPT) of the calcite stereom. The EPT are part of the photoreceptor system and can facilitate light transmission through the DAP by decreasing light refraction, reflection and absorption that occur at stereom/stroma interfaces. Bundles of nerve fibres located below the EPT are a second component of the system, and may function as primary photoreceptors. The intensity of light impinging on the putative sensory tissue is regulated by the diurnal activity cycle of chromatophores, the third element of the system. During the day the chromatophores cover the EPT and thereby shade the nerve fibres. At night they retract into inter-trabecular channels, uncovering the EPT and thereby exposing the nerve fibres to transmitted light. Thus, the transparent stereom may play a role in photoreception, in addition to its generally recognized skeletal function. Although ciliated cells that may be sensory are present in the epidermis of *Ophiocoma wendti,* they do not appear to be photoreceptors. Functional analogues of the brittlestar photoreceptor system in other echinoderms are discussed, emphasizing the relationship between photosensitivity and the transparency of the stereom in several classes of Echinodermata.

A. Introduction

Many echinoderms respond to light, although specialized "eyes" are restricted to one species of holothuroid and members of Asteroidea (Eakin and Brandenburger 1979; Takasu and Yoshida 1983; Yoshida et al. 1984). For most echinoderms, photosensitive behaviour is attributed to a "diffuse dermal light sense", a poorly understood extraocular type of photoreception (reviewed by Millott 1975; Weber 1983; Yoshida et al. 1984). A variety of structures have been suggested to be photoreceptors, and the general consensus is that the primary receptors are unspecialized (Cobb and Stubbs 1982; Pentreath and Cobb 1982; Moore and Cobb 1985; Cobb and Moore 1986).

Offprint requests to: G. Hendler

In this report, we describe for the first time what appears to be a photoreceptor system in the body wall of a brittlestar, for *Ophiocoma wendti* Müller and Troschel, 1842. The species changes colour dramatically, from homogeneous dark brown during the day to gray and black banding at night (Hendler 1984a). Our present study was prompted by the recent demonstration that the diurnal colour change in *Ophiocoma wendti* is associated with fluctuations in the brittlestar's sensitivity to light (Hendler 1984a).

Photosensitivity is an ecologically important behavioral attribute, particularly for escape reactions, in brittlestars such as *Ophiocoma wendti* and other echinoderms (Hendler 1984a, b). In the shallow water habitats occupied by *Ophiocoma wendti,* light levels range over 8 decades of magnitude from sunlight to moonlight (McFarland 1986). Even under these markedly variable light regimes *Ophiocoma wendti* exhibits rapid escape responses, photoreception appearing to play a role in the detection of shadows and in escape from predators into darkened crevices (Hendler 1984a).

It was hypothesized (Hendler 1984a) that the transparent, microscopic "lenses" on the arm plates of *Ophiocoma wendti* conduct light to (and perhaps focus it on) photosensitive tissue within the plate. It was further suggested that the chromatophores, responsible for colour-change of the brittlestar and associated with these calcite "lenses", regulate the intensity of light reaching the photosensitive tissue.

Previous ultrastructural examinations of the epidermis of several ophiuroids revealed the presence of ciliated cells that were suggested to be sensory (reviewed by Holland 1984; Märkel and Röser 1985). Cobb and Moore (1986) described a ciliated cell in the epidermis of *Ophiura ophiura* (Linné, 1758) that may be a photoreceptor. Therefore, we examined the epidermis of *Ophiocoma wendti* for potential ciliary receptors.

In the present study, evaluation of the presumed photoreceptor system is based on examination of the fine structure of the dorsal arm plate (DAP), augmented by observations on the other arm plates of this species. In addition, the DAPs of other ophiocomid brittlestar species are considered, to evaluate the relationship between stereom structure and the previously determined interspecific differences in the behavioral response of ophiocomids to illumination (Hendler 1984a). The analysis is supported by recent information on the neurophysiology of brittlestars (Stubbs 1982; Moore and Cobb 1985).

Our findings put the nebulous concept of "diffuse dermal light reception" in brittlestars on a firmer morphological footing and are consistent with previous suggestions of photoreceptor structures in sea urchins (Millott 1975) and starfish (Döderlein 1898). In addition to serving a skeletal function, the calcite of the dorsal surface of *Ophiocoma wendti* is an integral part of a photoreceptor system. We suggest that photoreception in several echinoderm classes may depend on analogous modifications of the skeleton, integument and nervous system.

B. Materials and methods

Specimens of *Ophiocoma wendti* and the other ophiuroid species examined, *Ophiocoma echinata* (Lamarck, 1816) *Ophiocoma paucigranulata* Devaney, 1974, *Ophiocoma pumila* Lütken, 1859, *Ophiopsila riisei* Lütken, 1859, and *Ophiopsila polysticta* H.L. Clark, 1915, were collected from Southwater and Carrie Bow Cays, on the Belize Barrier Reef, the Florida Keys, USA, and Galeta, Panama.

Brittlestars for scanning electron microscope (SEM) preparations and gross anatomical observations were relaxed by adding magnesium sulfate crystals to the seawater in which they were held, and then preserved in ethanol. Plates for the SEM were dissociated and cleaned by soaking in sodium hypochlorite solution, then in sodium peroxide, followed by washing in ethanol. They were dried and mounted on stubs using toluene-soluble Bakelite glue. Samples were coated with carbon, gold-palladium sputter coated and examined with a Cambridge Stereoscan 100 microscope.

Specimens for light and for transmission electron microscope (TEM) were placed in a recirculating seawater system and maintained in a natural diurnal light/dark regime. DAPs were dissected from the same individuals at 10:00-15:00 h (day samples) and 20:00–23:00 h (night samples) for comparison of the tissue changes associated with the day and night colour phases (Hendler 1984a). For the TEM, portions of arms were dissected from unanaesthetized specimens and placed directly in fixative.

Two methods of fixation were used. In the first, the arms were dissected in 3% glutaraldehyde in 0.2 *M* cacodylate buffer (pH 7.4). The dissected DAPs were then placed in fresh fixative for 1 h at room temperature, rinsed in the same buffer and postfixed in 1% OsO_4 in cacodylate for 1 h at 4° C. In the second method, the arms were placed in 2.5% glutaraldehyde in 0.45 µm Millepore-filtered seawater for 1 h at room temperature. The specimens were washed in 2.5% sodium bicarbonate and post-fixed in 2% OsO_4 for 1 h at room temperature. Both fixation methods yielded good results, but the second method produced the best preservation of the cuticle.

Following fixation, the specimens were rinsed in distilled water and decalcified for 12–24 h in a 1:1 mixture of 2% ascorbic acid and 0.36 *M* NaCl (Dietrich and Fontaine 1975). Following decalcification, the tissues were dehydrated in increasing concentrations of ethanol, transferred through two changes of propylene oxide and embedded in Epon 812. Sections for the TEM were cut with a diamond knife and stained with 2.0% uranyl acetate for 30 min and with 2.0% lead citrate for 10 min. The thin sections were viewed with a Zeiss EM9-S2 electron microscope.

For light microscopy, portions of arms were dissected from specimens anesthetized in a 1:1 mixture of 7% $MgCl_2$ to sea water for 10–20 min. The arms were fixed for up to 1 month in Bouin's fixative. This fixative decalcified the tissue, although some specimens required additional decalcification by the ascorbic acid method. Following decalcification, the tissues were dehydrated with ethanol, cleared in xylene, embedded in paraffin and sectioned at 6–7 µm. The sections were stained with haematoxylin and eosin.

C. Results

As in all brittlestars, the arm of *Ophiocoma wendti* consists of a repeated series of skeletal structures. The major skeletal structures are the dorsal, ventral and paired lateral arm plates which enclose each ambulacral (vertebral) ossicle. The plates consist of two components: the stereom, a three dimensional array of high-magnesium calcite trabeculae, and the stroma, the tissue filling the interstices of the stereom.

I. Scanning microscopy of the dorsal arm plate

The dorsal surface of the DAP bears hundreds of microscopic knobs (Figs. 1–3, 9–12). These knobs are enlarged peripheral trabeculae (EPT), and when viewed with a ster-

Fig. 1. Dorsal arm plate (*DAP*) of *Ophiocoma wendti*. Expanded peripheral trabeculae (*EPT*) of stereom form transparent, microscopic knobs on dorsal surface of plate

Fig. 2. Cross-section of fractured *Ophiocoma wendti* DAP. EPT of dorsal surface easily distinguished from deeper labyrinthine stereom (*L*). Pores (*arrows*) around EPT open into channels (*CH*) in labyrinthine stereom. – Dorsal arm plates of strongly photonegative species with prominent EPT (**Figs. 3–5, 7**) and of species not sensitive to light with EPT poorly developed or lacking (**Figs. 6, 8**)

Fig. 3. Dorsal surface of *Ophiocoma wendti* DAP (detail of **Fig. 1**), showing EPT and pores (*arrow*)

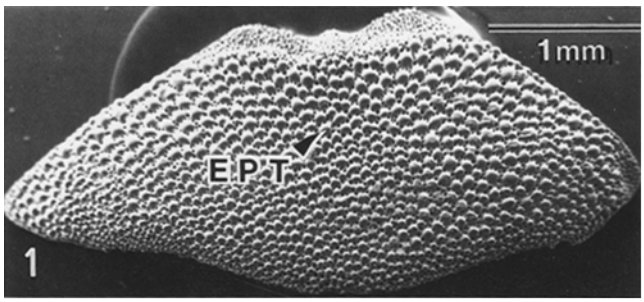
Fig. 4. Dorsal surface of *Ophiocoma echinata* DAP, showing EPT and pores (*arrow*)

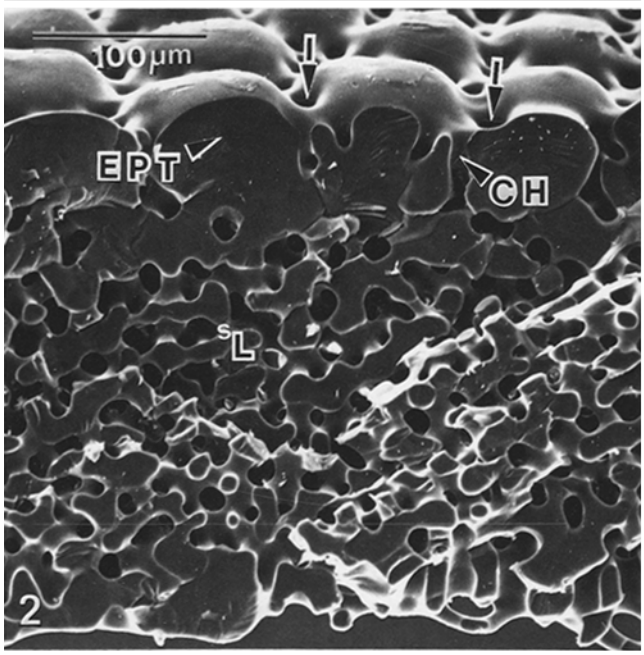
Fig. 5. Dorsal surface of *Ophiocoma paucigranulata* DAP, showing EPT and pores (*arrow*)

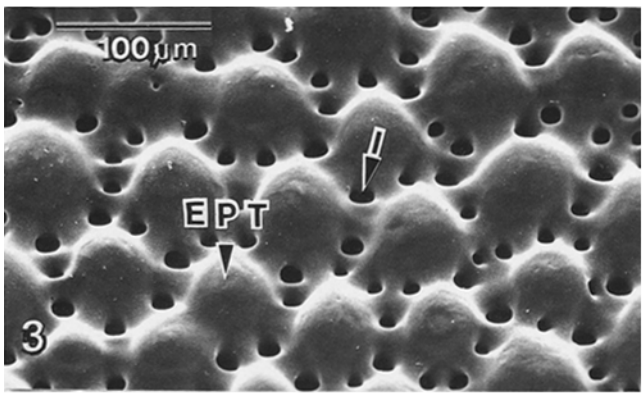
Fig. 6. Dorsal surface of *Ophiocoma pumila* DAP. Stereom appears labyrinthine as EPT are poorly defined and pores (*arrow*) between trabeculae are relatively large

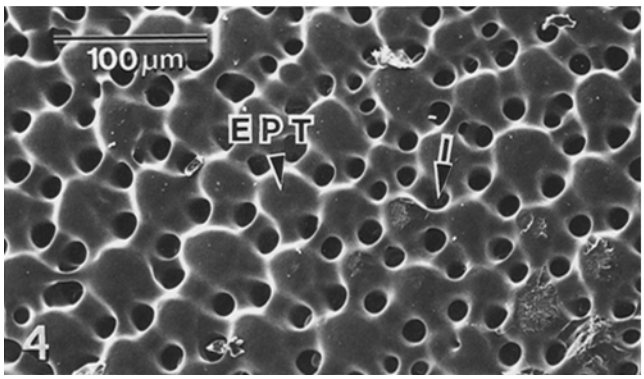
Fig. 7. Dorsal surface of *Ophiopsila riisei* DAP, showing EPT and pores (*arrow*)

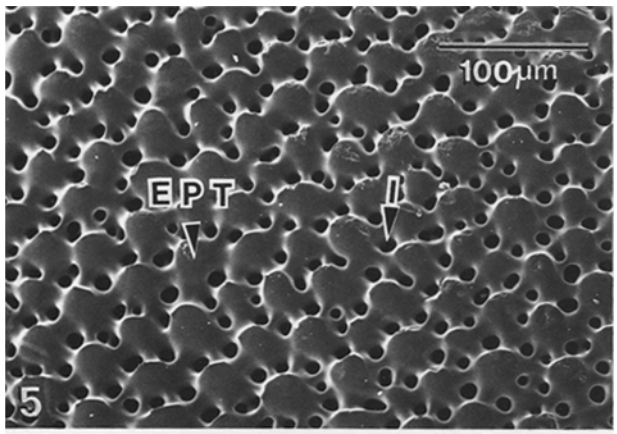
Fig. 8. Dorsal surface of *Ophiopsila polysticta* DAP. EPT absent and pores (*arrow*) between the trabeculae are large

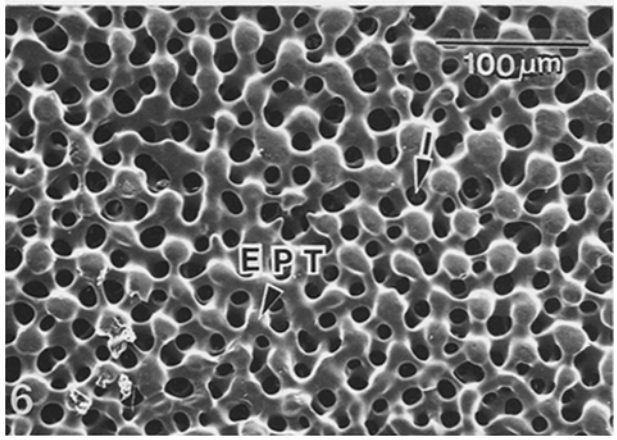

1mm
EPT
1

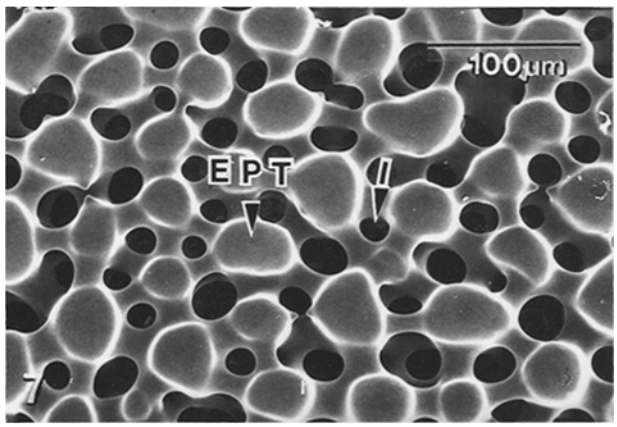

100µm
EPT
CH
SL
2

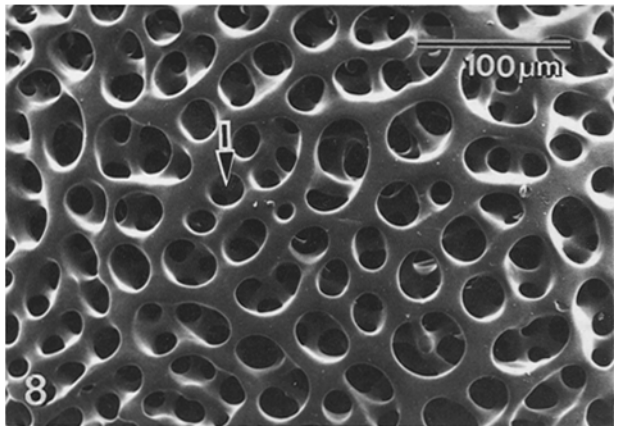

100µm
EPT
3

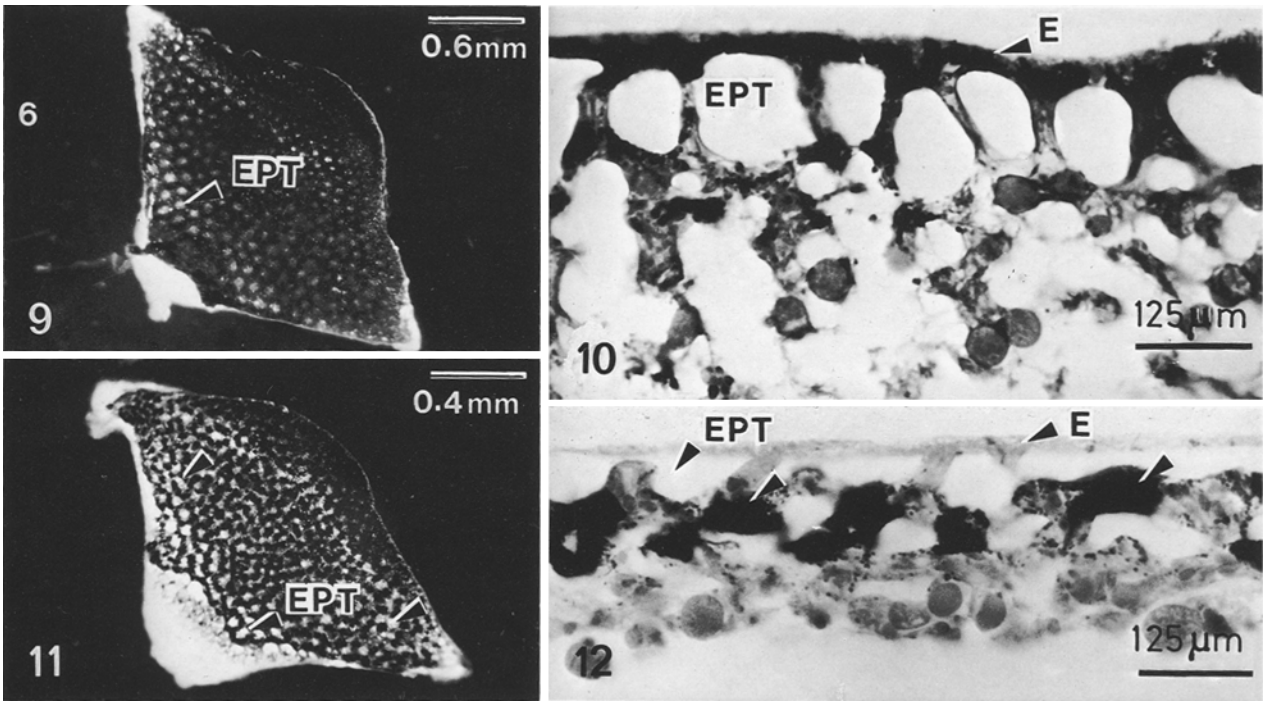

100µm
EPT
4


100µm
EPT
5


100µm
EPT
6


100µm
EPT
7


100µm
8



Figs. 9–12. *Ophiocoma wendti* dorsal arm plates (DAPs): entire (**Figs. 9, 11**) and in cross-section (**Figs. 10, 12**)

Fig. 9. DAP dissected during the day. Chromatophore pigment is dispersed

Fig. 10. Cross-section of DAP dissected during the day. Chromatophore pigment (*P*) dispersed beneath epidermis (*E*) and over EPT. Paraffin section

Fig. 11. DAP dissected at night. Chromatophores aggregated and appear as black dots (*arrowheads*); EPT are uncovered

Fig. 12. Cross-section of DAP dissected at night. Chromatophore pigment aggregated in inter-trabecular channels (*arrowheads*); EPT are uncovered. *E* epidermis. Paraffin section

eomicroscope they appear transparent (Figs. 9, 11). Reconnaissance of the plate with the SEM at low magnification reveals that the EPT are largest near the centre of the plate and smaller at the distal edge (Fig. 1). The EPT are smooth, rounded and somewhat irregular and range from 55 to 73 µm in diameter. They are solid calcite and occupy the upper 53–68 µm of the plate (Fig. 2). Each knob is surrounded by 7–11 pores, measuring approximately 7 to 19 µm in diameter, leading into channels located along the lateral edge of the EPT (Figs. 2, 3). These channels are continuous with the interstices within the stereom. The interior ventral surface of the DAP is composed of labyrinthic opaque calcite (Fig. 2). EPT are absent on the ventral arm plates, but they occur on the outer surface of the lateral arm plates.

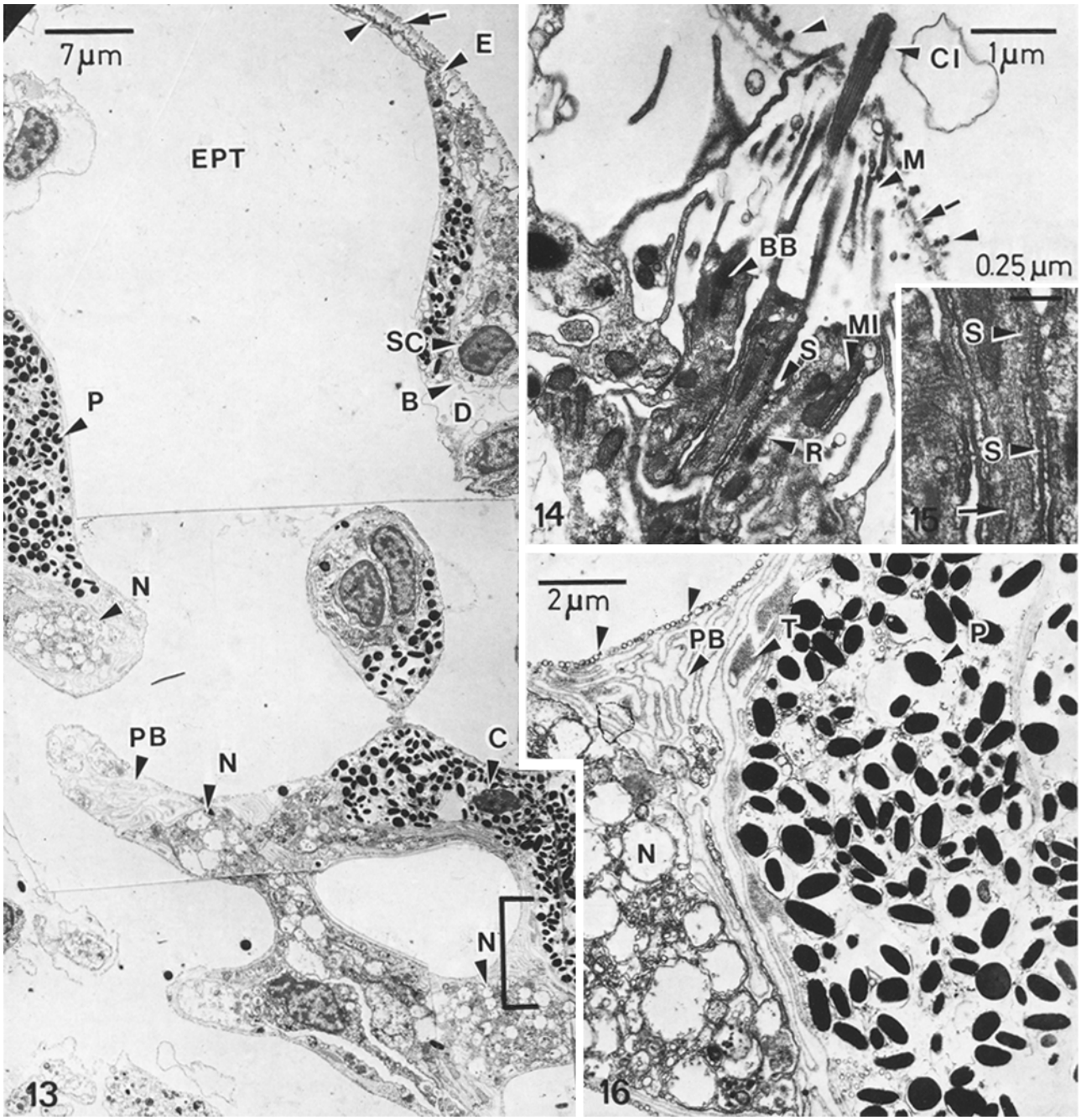
EPT occur on the dorsal and lateral arm plates of the light sensitive species, *Ophiocoma echinata* (Fig. 4) and *Ophiocoma paucigranulata* (Fig. 5) (Hendler 1984a). However, EPT are poorly defined on the arm plates of *Ophiocoma pumila* (Fig. 6), a species that is relatively insensitive to illumination (Hendler 1984a). Another sympatric Caribbean ophiocomid, *Ophiopsila riisei,* which has large EPT (Fig. 7), hides in rock crevices during the day and is active nocturnally. It is extremely sensitive to illumination and markedly photonegative. Its congener, *O. polysticta,* lacks light-transmitting EPT (Fig. 8). It is active diurnally and concealed in sediment at night (Hendler unpublished observation) indicating that it is less photonegative than *Ophiopsila riisei.*

II. Diurnal activity of the chromatophores

Migration of the chromatophores is apparent in light microscopic sections of DAPs prepared from night and day samples (Figs. 10, 12). During the day, chromatophore pigment is located underneath the epidermis and covers the EPT (Fig. 10), whereas at night the pigment is concentrated in the lateral channels (Figs. 12). The chromatophores appear to move their pigment-filled cell processes by an amoeboid type of locomotion (see below, III.2). At dusk (1600–1800 h, they retract their pigment-filled processes through the inter-trabecular pores and aggregate the pigment in the lateral channels. The processes remain retracted through the night and the chromatophores appear as black dots, forming a meshwork of pigment in the DAPs (Fig. 11) and giving the brittlestar a banded colouration. At dawn (0600–0800 h), cell processes are again extended, thereby dispersing the pigment under the epidermis (Fig. 9), giving individuals a homogeneous dark colour.

III. Transmission microscopy of the dorsal arm plate

1. Epidermis. The DAP of *Ophiocoma wendti* is covered by a 0.3–0.6 µm thick bilayered cuticle (Figs. 13, 17). A



Figs. 13–16. Dorsal arm plates (DAPs) of *Ophiocoma wendti*

Fig. 13. Low power cross-section of DAP showing three elements of photoreceptor system: expanded peripheral trabecula (*EPT*), chromatophores (*C*) and nerve fibres (*N*) positioned underneath EPT. *Bracket* marks area enlarged in **Fig. 16.** *B* basal lamina; *E* epidermis; *D* dermis; *P* pigment granules; *PB* plicated basal lamina; *SC* support cell; *arrowhead* support cell process; *arrow* cuticle

Fig. 14. Cross-section of apices of three epidermal ciliated cells with cilium (*Cl*) and associated microvilli (*M*) of one cell penetrating cuticle (*arrow*). Microvillar tips (*arrows*) dot cuticle surface. Septate junctions (*S*) join cells (see **Fig. 15**). *BB* basal body; *MI* mitochondrion; *R* ciliary rootlet

Fig. 15. Detail of septate junction (*S*) between ciliated cells shown in **Fig. 14.** *Arrow* microtubules

Fig. 16. Detail of area bracketed in **Fig. 13.** Nerve fibres (*N*) are separated from chromatophore by plicate basal lamina (*PB*) which is thickened in places (*T*). Processes, presumably from sclerocytes, form discontinuous covering over skeleton (*arrowheads*). *P* pigment granule

distinct basal lamina is situated along the dorsal part of the DAP of *Ophiocoma wendti* (Figs. 13, 17, 20). Using the convention adopted by Märkel and Röser (1985) for *Ophioderma longicaudum* (Retzius, 1805) this basal lamina is taken to delimit the juxtaposition of epidermal and dermal tissue layers. We use the term "dermis" for comparative purposes and to facilitate description of the tissue, although Holland (1984) deems the term inappropriate for descriptive echinoderm anatomy.

The epidermis is largely a single layer of squamous cells covering the skeleton (Figs. 13, 20). Where epidermis extends into the inter-trabecular pores, two or three layers of nuclei are present. There are several types of cells in the epidermis, the most numerous of which are the support cells (Figs. 13, 17, 20). Apico-lateral processes of the support cells cover the trabeculae, and the epidermis is thinnest where these attenuated processes cover the EPT (Fig. 13). Microvilli from the support cells penetrate the cuticle and their tips dot the cuticular surface (Fig. 14). The support cells and their processes are joined laterally to each other by septate junctions (Fig. 17). Support cell secretory activity was evidenced by the release of a flocculent material in the sub-cuticular space (Fig. 17). Narrow, columnar ciliated cells, occurring singly or in groups of several cells (Figs. 14, 18), are joined apico-laterally by septate junctions to each other and to support cells (Fig. 19).

The ciliated cells (Figs. 14, 18) possess a short cilium measuring 3–4 μm in length, of which 0.8–0.9 μm projects beyond the cuticle. The ciliary shaft has the 9+2 axonemal arrangement, but this pattern appears to be lost at the slightly clavate tip. A striated rootlet projects from the basal body of the cilium. The ciliated cells are occasionally found adjacent to secretory cells. There are three types of secretory cells in the epidermis that release their synthetic product into the external environment through cuticular pores (Fig. 22). These include cells containing a homogeneous secretory product, cells filled with a mucus-like material and fibrillar glands (Figs. 18, 21, 22). The cuticle is linked to the dermis by means of double-ended hemidesmosomes, with one end on the apical membrane and the other on the basal membrane of the support cell process (Fig. 19). Anchoring filaments attach to the basal lamina at the junction.

2. Dermis. The dermis includes the skeleton and the cells occupying the stromal space (Fig. 13). Chromatophores are the dominant feature of this tissue. They are situated in channels in the stereom between the EPT and occupy much of the stroma (Figs. 13, 16, 20). During the day, the chromatophore processes are positioned just below the epidermis, rarely, however, some pigment granules are encountered above the subepidermal basal lamina suggesting that pigment cells occasionally send processes across the basal lamina. In low magnification cross-sections, the EPT appear transparent and are the most prominent calcite deposits in the DAP (Fig. 13).

The chromatophores are large cells (at least 20–40 μm in diameter) with a complex architecture (Figs. 20, 23). The cell processes invariably are densely filled with membrane-bounded pigment granules, suggesting that pigment aggregation involves retraction of the entire cell process (Figs. 13, 20). The extent to which the pigment granules aggregate in the stroma at night is apparent in Fig. 24.

The pigment granules are very electron-dense, ellipsoidal in shape and range in length from 0.3 to 1.7 μm (Figs. 16, 23, 25). In addition to pigment granules, the chromatophores contain an abundance of electron-lucent vesicles, microtubules and an occasional Golgi complex (Figs. 23, 25).

The dermis also contains large nerve bundles (12–40 μm in breadth) surrounded by basal lamina (Figs. 13, 26, 27). The bundles are composed of several hundred nerve fibres

Figs. 17–25. Dorsal arm plates (DAPs) of *Ophiocoma wendti*

Fig. 17. Epidermal support cell (*SC*) and underlying basal lamina (*B*). Cell is releasing amorphous material into sub-cuticular space (*arrows*). Cuticle (*C*) is composed of outer homogeneous layer (*HL*) and inner fibrillar layer (*FL*). *M* microvilli; *S* septate junction

Fig. 18. Epidermal secretory cell and adjacent ciliated cell. *BB* basal body; *C* cuticle; *M* microvilli; *MI* mitochondrion; *SV* secretory vesicle; *R* ciliary rootlet

Fig. 19. The cuticle-epidermis-junction is a double-ended hemidesmosome, with one hemidesmosome (*H*) on the apical membrane and one on the basal membrane of the support cell process (*SP*). Extracellular anchoring filaments (*AF*) attach to basal lamina (*B*) and project into dermis (*D*). *C* cuticle; *F* cuticular fibrils

Fig. 20. Cross-section of DAP from a specimen dissected at night. Epidermis (*E*) and dermis (*D*) are separated by basal lamina (*B*) and chromatophore pigment granules (*P*) are aggregated beside the EPT. *C* cuticle; *FB* fibrillar gland; *SC* epidermal support cell

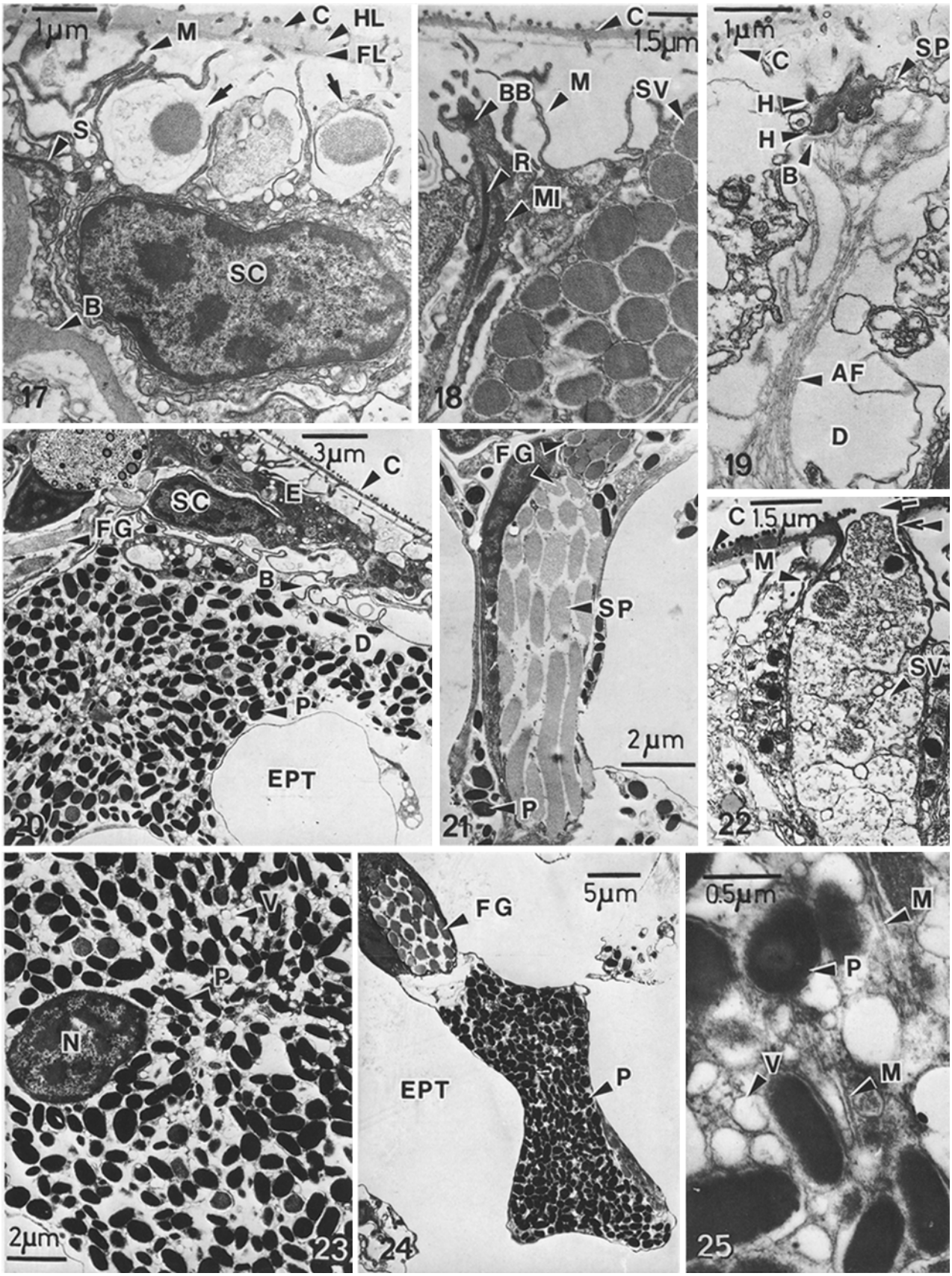
Fig. 21. Fibrillar glands (*FG*) between two EPT. The secretory product (*SP*) is fusiform and appears to be contained within one large vesicle. *P* pigment granules

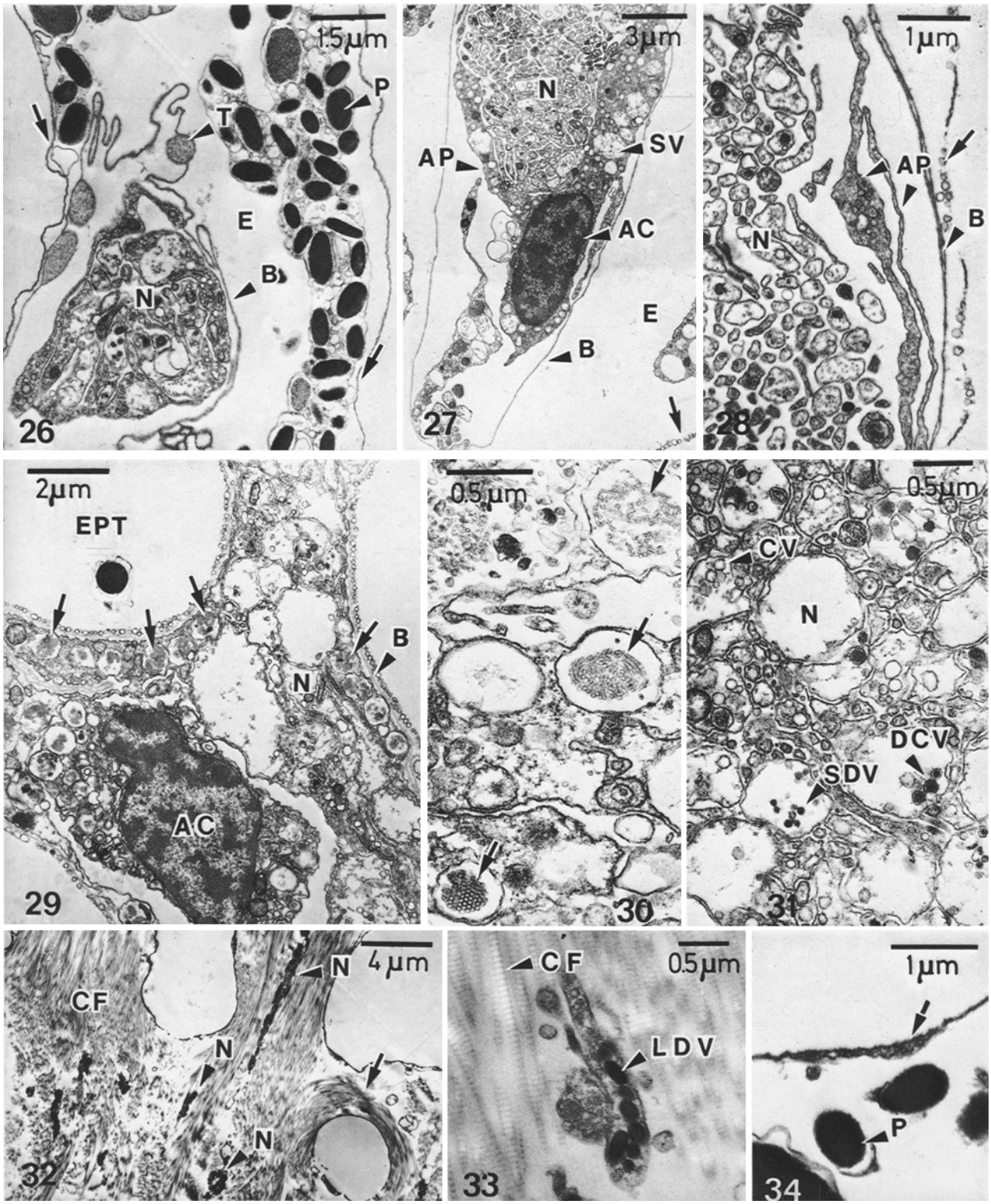
Fig. 22. Epidermal secretory cell filled with mucus-like material which is extruded through a pore (*arrow*) into the cuticle (*C*). Apical portion of cell passes through cuticle-lined channel (*double arrowhead*). Microvilli (*M*) are adjacent to channel. Secretory vesicles (*SV*) have coalesced

Fig. 23. Cell body of chromatophore in inter-trabecular channel, at night, containing aggregated pigment granules (*P*) and vesicles (*V*). *N* nucleus

Fig. 24. Aggregated pigment granules (*P*) between two EPT from specimen at night. *FG* fibrillar gland

Fig. 25. Detail of chromatophore containing microtubules (*M*), pigment granules (*P*) and vesicles (*V*)


1μm
M
C
HL
FL
S
SC
B
17
C
1.5μm
BB
M
SV
R
MI
18
1μm
C
SP
H
H
B
AF
D
19
3μm
C
SC
E
FG
B
D
P
EPT
20
FG
SP
2μm
P
21
C
1.5μm
M
SV
22
V
P
N
2μm
23
5μm
FG
EPT
P
24
0.5μm
M
P
V
M
25



Figs. 26–34. Dorsal arm plates (DAPs) of *Ophiocoma wendti*

Fig. 26. Nerve bundle (*N*) in channel between two EPT, surrounded by basal lamina (*B*) which has occasional thickenings (*T*). Adjacent chromatophore process not ensheathed in basal lamina. *E* extracellular matrix of stroma; *P* pigment granule; *arrows* skeletal covering

Fig. 27. Nerve bundle (*N*) below the EPT layer, surrounded by processes (*AP*) from accessory cell (*AC*) and by basal lamina (*B*). *E* extracellular matrix of the stroma; *SV* secretory vesicle; *arrow* skeletal covering

Fig. 28. Detail of nerve bundle seen in **Fig. 27** showing accessory cell process (*AP*), basal lamina (*B*) and skeletal covering (*arrow*)

ranging in diameter from 0.07–1.3 μm (Figs. 28, 29, 31). They are positioned in the stroma immediately below the level of the EPT, approximately 50 μm below the cuticle (Figs. 13, 29). The nerve fibres contain neurotubules, occasional mitochondria and three types of vesicles (Fig. 31): (1) small dense vesicles (0.05–0.07 μm in diameter); (2) dense cored vesicles (0.05–0.1 μm in diameter) and (3) clear vesicles (0.07 μm in diameter).

Accessory cells are usually found near the nerve fibres and extend narrow processes around the nerve bundles (Figs. 27, 29). The accessory cells contain RER, mitochondria and vesicles containing a flocculent material (Fig. 27). In addition, these cells usually possess vesicles containing stacked tubular structures (Figs. 29, 30). The vesicles are often aligned along the bottom of a trabecula and the tubules appear to be in different stages of organization (Fig. 30). These structures are common, but their functional significance is not known.

There is a complex, plicate system of basal lamina in the dermis below the EPT (Figs. 13, 16). Although the origin of this basal lamina is not clear, it appears to be associated with the nerve bundles and may be elaborated by the accessory cells. This basal lamina possesses occasional thickenings (Figs. 16, 26) which may be areas where the lamina was sectioned transversely. Chromatophores are often adjacent to nerve bundles, but are invariably separated from the nerve fibres by the plicate basal lamina (Figs. 16, 26). The plicate basal lamina was not encountered near the subepidermal basal lamina and the two laminae do not appear to connect, however our evidence is equivocal.

The dermis contains extracellular matrix comprising collagen fibrils, unstriated fibrils and ground substance (Figs. 26, 27, 32, 33). In the vicinity of the chromatophores, the extracellular matrix is largely ground substance (Fig. 26), presumably allowing unimpeded motility of the chromatophore processes. The ventral portion of the dermis is composed of collagen fibrils which attach the DAP to adjacent skeletal plates (Fig. 32). There are small bundles of nerve fibres in the ventral connective tissue (Figs. 32, 33) that contain vesicles similar to those described above and, in addition, possess large electron-dense vesicles (0.14–0.38 μm in diameter).

Sclerocytes (the cells which secrete the stereom) were not encountered in the dermis, and the cytoplasmic processes that cover the skeleton may be derived from inactive sclerocytes (Figs. 16, 34). Viewed in longitudinal section, the processes form a continuous covering over the skeleton and the close apposition of their membranes gives the sheath and extracellular appearance (Fig. 34). In cross section, the processes appear as a chain of minute circles forming a discontinuous skeletal covering (Fig. 16).

D. Discussion

Our general conclusion is that *Ophiocoma wendti* has a photoreceptor system composed of three main components: transparent EPT, chromatophores which exhibit a diurnal migration cycle and inter-trabecular bundles of nerve fibres below the EPT. The form, function and interactions of these components is considered below.

I. Enlarged peripheral trabeculae (EPT)

The arm plates of *Ophiocoma wendti* are typical of echinoderm skeleton, being composed of a calcite stereom with stroma-filled interstices. The plates have two major indices of refraction and other crystallographic properties characteristic of single calcite crystals (Raup 1966). Consequently, the transmission of light through the plate is determined in part by the direction of incident light with respect to the orientation of its optical axis (Raup 1960, 1966).

The major optical axis of the dorsal arm plates of *Ophiocoma wendti* and other brittlestars is dorso-ventral (Raup 1966; Donnay and Pawson 1969; Hendler unpublished observation). This crystallographic orientation tends to reduce light penetrating the dorsal surface of the plate. However, based on Raup's (1960) experiments it is clear that the chief impediment to light transmission through the echinoderm skeleton is the refraction, reflection and absorption of light at the stereom/stroma interface owing to the different optical axes of the calcite and soft tissues.

With this in mind, we suggest that transparent enlarged peripheral trabeculae (EPT) are an adaptation which maximizes the transmission of light to tissues within the DAP. EPT appear transparent because light passes through them relatively readily. In contrast, the labyrinthic calcite near the DAP ventral surface is opaque because of reflection, refraction and absorption of light at a multitude of stereom/stroma interfaces in that region of the plate. In the terminology suggested by Smith (1980), the numerous EPT comprise a layer of compact stereom overlaying the deeper labyrinthic stereom of the arm plate.

Indirect evidence that the EPT are adaptations for light conduction in *Ophiocoma wendti* is their occurrence in other ophiocomid brittlestars that are light sensitive. The EPT are well developed in *Ophiocoma echinata, Ophiocoma pau-*

Fig. 29. Nerve fibers (*N*) and accessory cell (*AC*) immediately below EPT. *Arrows* vesicles containing tubular structures (see **Fig. 30**); *B* basal lamina

Fig. 30. Vesicles containing tubular structures (*arrows*) which may be in different stages of organization

Fig. 31. Sub-trabecular nerve bundle with nerve fibres (*N*) containing small-dense vesicles (*SDV*), dense-cored vesicles (*DCV*) and clear vesicles (*CV*)

Fig. 32. Collagen fibrils (*CF*) attach DAP to adjacent skeletal plates and encircle stereom trabeculae. *N* neurons

Fig. 33. Nerve in connective tissue containing large-dense vesicles (*LDV*). *CF* collagen fibrils

Fig. 34. In longitudinal section, sclerocyte-like processes form continuous covering over skeleton. Close apposition of membranes (*arrow*) gives sheath an extracellular appearance. *P* pigment granules

cigranulata and *Ophiopsila riisei* which respond readily to light, but are absent in *Ophiocoma pumila* and *Ophiopsila polysticta* which are relatively light insensitive species. Both *Ophiopsila* species are luminescent (Hendler unpublished observation), however, differences in their stereom structure are likely a function of their contrasting diurnal behaviour patterns, rather than adaptations for bioluminescence. In the light sensitive species thus far examined, the EPT are surrounded by small pores that open into stroma-filled channels within the plate. In *Ophiocoma wendti,* and probably in other species, chromatophores are associated with the pores.

II. Chromatophores

The chromatophores of *Ophiocoma wendti* are strikingly similar in fine structure to those of diadematid urchins (Weber and Gras 1980). They are also similar to the chromatophores of the ophiuroid *Ophioderma longicaudum* described by Märkel and Röser (1985), but unlike the pigment cells of *Ophioderma longicaudum,* the chromatophores of *Ophiocoma wendti* are not attached to the skeleton. The chromatophores of *Ophiocoma wendti,* like those of diadematid urchins, appear to move freely in the extracellular matrix. Echinoderm chromatophores are mesodermally derived and the chromatophores of *Ophiocoma wendti* and *Ophioderma longicaudum* are located in the adult dermis (Märkel and Röser 1985). In contrast, sea urchin chromatophores migrate to the ectoderm early in development and are thus located in the adult epidermis (Ryberg and Lundgren 1979; Gibson and Burke 1985).

This investigation confirms that the chromatophores of *Ophiocoma wendti* have a diurnal activity cycle (Hendler 1984a). The cycle alternately brings the chromatophore pigment to a position over the EPT or to a lateral position beside the EPT. The response of *Ophiocoma wendti* chromatophores to varying levels of illumination appears to involve migration of the pigment-filled processes resulting in a change of cell shape. The extension and retraction of the processes suggest an amoeboid-type of locomotion, as documented for isolated sea urchin chromatophores (Weber and Dambach 1974; Gras and Weber 1983). Isolated sea urchin chromatophores retract their filopodia in the dark, aggregating their pigment granules; they spread their filopodia in the light, dispersing their pigment granules. This behavioural pattern is similar to that of *Ophiocoma wendti* chromatophores.

Experimental manipulation of isolated diadematid chromatophores provides convincing evidence that the cells detect light and effect movements independently of nervous or humoral cues (Gras and Weber 1983). *Ophiocoma wendti* chromatophores appear to respond to light independently of associated cells, suggesting that they too are independent receptors and effectors. Nerve fibres are adjacent to the chromatophores, but they are separated from the chromatophores by basal lamina and do not appear to innervate the cells. Independence of the chromatophores from the central nervous system is supported by the observation that they alter their shape in isolated DAPs in response to light, as they do in the whole organism (Hendler 1984a).

A microtubule/microfilament mechanism of motility has been suggested for sea urchin chromatophores based on the presence of a microtubule system, the abundance of electron-lucent vesicles and the influence of cytochalasin B on the cell's motility (Dambach and Weber 1975; Weber and Gras 1980). The abundance of microtubules and electron-lucent vesicles in the chromatophores of *Ophiocoma wendti* suggests that a similar system may occur in brittlestars.

III. Nerve bundles

The nerve fibres in the DAPs of *Ophiocoma wendti* are similar to those described for other brittlestars (Brehm 1977; Martínez 1977a; Wilkie 1979; Märkel and Röser 1985; Cobb and Moore 1986). As is characteristic of the echinoderm nervous system, these nerve processes are small and contain vesicles indicative of cholinergic (clear vesicles) and aminergic (dense cored vesicles) transmission (Pentreath and Cobb 1972).

In contrast to the epidermal nerve plexus described in the skeletal plates of *Ophioderma longicaudum* by Märkel and Röser (1985), the nerve plexus of *Ophiocoma wendti* is located in the dermis. In both species, the nerve fibres are ensheathed in basal lamina, but in *Ophioderma longicaudum,* the lamina is derived from epidermal cells whereas that of *Ophiocoma wendti* appears to be elaborated by the accessory cells. The nerve bundles are usually surrounded by processes from accessory cells, suggesting a glia-like function for these cells. Accessory cells have not generally been described for other echinoderms (Pentreath and Cobb 1972), but are associated with nerve bundles in the holothuroid body wall (Byrne 1983). We did not trace the origin of the inter-trabecular nerve fibres but they presumably connect with branches of the ophiuroid ectoneural system that innervate the arm plates (Cobb and Stubbs 1982). The nerve fibres in the ventral connective tissue resemble the juxtaligamental cells of *Ophiocomina nigra* Abildgaard, 1789, that are involved with the variable tensility of the connective tissue (Wilkie 1979).

The nerve fibres situated below the EPT and motile chromatophores, in the path of transmitted light, may transduce the light striking the arm into neurally transmitted signals (Hendler 1984a). In the terminology suggested by Yoshida (1979), we propose that the "diffuse sensitivity" of the chromatophores acts in tandem with "neuronal sensitivity" of the nerve bundles to control the behavioural response of *Ophiocoma wendti* to illumination.

Recent work on the neurophysiology of brittlestars provides evidence for the presence of peripheral photoreceptors. Photic stimulation of the arms of *Ophiura ophiura* elicits electrical activity in the radial nerve cord that presumably can produce complex locomotory behavior (Stubbs 1982; Cobb 1985). This electrical activity is not elicited if the stimulus is presented to portions of arms with the ventral and lateral arm plates removed. Based on the results of the present investigation, we suggest that this lack of response may be due to the severing of the connection between the radial nerve and the primary photoreceptor system located in the arm plates.

Early studies of sea urchin neurobiology provide evidence that the radial nerve is photosensitive (reviewed in Yoshida 1966; Millott 1975). A recent investigation on neurons present in the spine of a diadematid urchin indicates that the neurons may be distal sensory processes and it was suggested that they transmit mechanical, chemical or thermal information (Berrios et al. 1985). Considering the dermal photosensitivity of these urchins, it is equally likely

that neurons in the diadematid spines transmit photic stimuli.

IV. Comparison with other ophiuroids

The structure of the skeletal plates of *Ophiocoma wendti* is similar to that recently described for *Ophioderma longicaudum* (Märkel and Röser 1984). However, there are important differences in the greater size of the peripheral trabeculae, the prominence of the chromatophores and the extensive nerve plexus in the DAP of *Ophiocoma wendti*. A comparison of Fig. 13 with a similar section of *Ophioderma longicaudum* (Märkel and Röser 1984, Fig. 3), attests these differences. Unfortunately, there are no published observations on the photosensitivity and diurnal activity of *Ophioderma longicaudum* that could be used for comparison with *Ophiocoma wendti*.

Ultrastructural surveys for ophiuroid photoreceptors (Stubbs 1982; Moore and Cobb 1985; Cobb and Moore 1986) have failed to locate receptor organs and have concluded that photosensitivity is a function of unspecialized receptors. Cobb and Moore (1986) recently described a ciliated cell in *Ophiura ophiura* that may be a general photic receptor. This putative photoreceptor has a modified cilium which does not project beyond the cuticle and lacks a long ciliary rootlet (Cobb and Moore 1986). In both of these features, the ciliary photoreceptor of *Ophiura ophiura* differs from the epidermal ciliated cells of *Ophiocoma wendti*. Potential ciliary photoreceptors were not encountered in the epidermis of *Ophiocoma wendti*, and were not reported in *Ophioderma longicaudum* by Märkel and Röser (1985). The ciliated cells of *Ophiocoma wendti* possess short cilia and may be sensory, as suggested for similar ciliated cells seen in other ophiuroids (Martínez 1977b; Whitfield and Emson 1983; Märkel and Röser 1985). Their association with the secretory cells suggests that the ciliated cells may be involved with chemo-or thigmo-reception. However, the ciliated cells were not observed to give rise to sensory axons.

V. Implications for echinoderm photosensitivity

A better understanding of the "diffuse dermal light sense" exhibited by echinoderms may be at hand. As suggested for diadematid sea urchins (reviewed by Millott 1975; Yoshida 1979), the chromatophores of *Ophiocoma wendti* may control the transmission of light to neuronal photoreceptors. Based on their location and on corroborative work on brittlestar and urchin neurobiology (Stubbs 1982; Yoshida et al. 1984; Berrios et al. 1985; Cobb 1985; Moore and Cobb 1985), we suggest that the inter-trabecular neurons may function as primary photoreceptors, but this conjecture requires neurophysiological verification.

Photosensitivity is known to exist in molluscan neurons (Robles et al. 1986). The proposal that the sub-trabecular neurons are primary photoreceptors differs from the suggestion, for several echinoderms, that receptors are peripheral ciliated cells (reviewed by Holland 1984; Cobb and Moore 1986). However, Moore (1985) has suggested that there is an interoreceptor deep within the arm spines of *Ophiura ophiura* which detects water movement relative to the position of the animal.

Several of the findings of this investigation support the contention that the EPT, the chromatophores and the inter-trabecular neurons comprise a photoreceptor system in *Ophiocoma wendti*. First, is the relative prominence and spatial relationship of these features in the DAP of *Ophiocoma wendti* in contrast with the body wall morphologies of other brittlestars (unfortunately a small sampling) that have been investigated. Second, is the occurrence of EPT in the plates of other light sensitive species and the absence of these structures in closely related species that are relatively insensitive to light. Moreover, the prevalence of EPT on dorsal plates and absence on ventral plates is as expected for photoreceptor structures. Third, is the correlation between diurnal colour changes and the photosensitivity of *Ophiocoma wendti* (Hendler 1984a). Fourth, is the corroborative results of recent neurophysiological investigations of *Ophiura ophiura* (Stubbs 1982; Cobb 1985; Moore and Cobb 1985).

In diadematid urchins there does not appear to be a modification of the skeleton corresponding to the EPT of *Ophiocoma wendti*. However, sea urchin chromatophores and skeletal nerve plexus are located in the epidermis and the relatively superficial position of the plexus may obviate the need for specialization of the stereom (Millott and Coleman 1969; Weber and Grosmann 1977). For a number of urchins, Smith (1980) describes compact stereom, skeletal "granules" and glassy tubercles, and suggests they may be adaptations to protect the basiepithelial nerves or increase the surface area available for respiratory epithelium. As suggested for the EPT of *Ophiocoma wendti*, these transparent skeletal structures may function in photoreception. Transparent regions of compact stereom are also reported for seastars. They were first described as "Krystallkörper" by Döderlein (1898), who noted that the crystal bodies are covered by pigmented epidermis and was convinced that they were somehow involved in the transmission of light. However, Döderlein concluded that the absence of pigment inside the crystal bodies ruled out the possibility of their being photoreceptors ("Sehorgane"). In light of our findings, the striking similarity in the association of pigmented epithelium and transparent skeletal structures in brittlestars and starfish suggests that Döderlein's initial hypothesis may have been correct. We suggest that in addition to its role for support and protection, the echinoderm stereom (and associated tissue) is specialized for photoreception. In fact, morphological features that enhance the transparency of the stereom may play an important role in the photosensitivity of many echinoderm taxa.

Acknowledgements. Field work was carried out in Belize with Scholarly Studies and Caribbean Coral Reef Ecosystem funding from the Smithsonian Institution. SEM facilities and assistance were provided by the National Museum of Natural History. TEM-facilities were provided by Harbor Branch Foundation, Inc. and the Smithsonian Marine Station-Link Port. Mr. William Lee prepared histological paraffin sections. Dr. Kenneth Towe was our Virgil in the realms of calcite crystallography. Ms Ailsa Clark and Ms Maureen Downey alerted us to the prescient observations by Döderlein, which were ably translated by Ms Regina Wetzer. We thank Drs. Nicholas Holland, John Costelloe and David McGrath for reading and commenting on drafts of this manuscript. This publication is, respectively, contribution number 225, 597, 211 and 296 of the Smithsonian Institution Caribbean Coral Reef Ecosystem/Investigations of Marine Shallow Water Ecosystems, Harbor Branch Oceanographic Institution, Smithsonian Marine Station – Link Port and University College Galway.

References

Berrios A, Brink D, del Castillo J, Smith DS (1985) Some properties of the action potentials conducted in the spines of the sea urchin *Diadema antillarum*. Comp Biochem Physiol [A] 81:15–23

Brehm P (1977) Electrophysiology and luminescence of an ophiuroid radial nerve. J Exp Biol 71:213–227

Byrne M (1983) Evisceration and autotomy in the holothurian, *Eupentacta quinquesemita* (Selenka), Ph. D. Thesis. University of Victoria

Cobb JLS (1985) The neurobiology of the ectoneural/hyponeural synaptic connection in an echinoderm. Biol Bull 168:432–446

Cobb JLS, Moore A (1986) Comparative studies on the receptor structure in the brittlestar *Ophiura ophiura*. J Neurocytol 15:97–108

Cobb JLS, Stubbs TR (1982) The giant neurone system in ophiuroids: III. The detailed connections of the circumoral nerve ring. Cell Tissue Res 226:675–687

Dambach M, Weber W (1975) Inhibition of pigment movement by cytochalasin B in the chromatophores of the sea urchin *Centrostephanus longispinus*. Comp Biochem Physiol [C] 50:49–52

Dietrich HF, Fontaine AR (1975) A decalcification method for ultrastructure of echinoderm tissues. Stain Technol 50:351–354

Döderlein L (1898) Ueber "Krystallkörper" bei Seesternen. Denkschr Med Nat Ges Jena 8:491–494

Donnay G, Pawson DL (1969) X-ray diffraction studies of echinoderm plates. Science 166:1147–1150

Eakin R, Brandenberger JL (1979) Effects of light on ocelli of seastars. Zoomorphologie 92:191–200

Gibson AW, Burke RD (1985) The origin of pigment cells in embryos of the sea urchin *Strongylocentrotus purpuratus*. Dev Biol 107:414–419

Gras H, Weber W (1983) Light-induced alterations in cell shape and pigment displacement in chromatophores of the sea urchin *Centrostephanus longispinus*. Cell Tissue Res 182:165–176

Hendler G (1984a) Brittlestar color-change and phototaxis (Echinodermata: Ophiuroidea: Ophiocomidae). PSZNI Mar Ecol 5:379–401

Hendler G (1984b) The association of *Ophiothrix lineata* and *Callyspongia vaginalis*: A brittlestar-sponge cleaning symbiosis? PSZNI Mar Ecol 5:9–27

Holland ND (1984) Echinodermata: epidermal cells. In: Bereiter-Hahn J (ed) Biology of the integument, vol 1. Invertebrates. Springer, Berlin, pp 756–774

Märkel K, Röser U (1985) Comparative morphology of echinoderm calcified tissues: histology and ultrastructure of ophiuroid scales (Echinodermata, Ophiuroidea). Zoomorphology 105:197–207

Martínez JL (1977a) Ultrasestructura del tejido nervioso podial de *Ophiothrix fragilis*. Bol R Soc Esp Hist Nat Sec Biol 75:315–333

Martínez JL (1977b) Estructura y ultraestructura del epithelio de los podios de *Ophiothrix fragilis* (Echinodermata, Ophiuroidea). Bol R Soc Esp Hist Nat Sec Biol 75:275–301

McFarland WN (1986) Light in the sea – correlations with behaviors of fishes and invertebrates. Am Zool 26:389–401

Millott N (1975) The photosensitivity of echinoids. Adv Mar Biol 13:1–52

Millott N, Coleman R (1969) The podial pit – a new structure in the echinoid *Diadema antillarum* Philippi. Z Zellforsch 95:187–197

Moore A (1985) Neurophysiological studies on the perception of environmental stimuli in *Ophiura ophiura* (L.) (Echinodermata, Ophiuroidea). In: Keegan BF, O'Connor BDS (eds) Echinodermata: proceedings of the 5th International Echinoderm Conference, Galway. AA Balkema, Rotterdam, pp 627–631

Moore A, Cobb JLS (1985) Neurophysiological studies on photic responses in *Ophiura ophiura*. Comp Biochem Physiol [A] 80:11–16

Pentreath VW, Cobb JLS (1972) Neurobiology of Echinodermata. Biol Rev 47:363–392

Pentreath VW, Cobb JLS (1982) Echinodermata. In: Shelton GAB (ed) Electrical conduction and behaviour in 'simple' invertebrates. Clarendon, Oxford, pp 440–472

Raup DM (1960) Ontogenetic variation in the crystallography of echinoid calcite. J Paleontology 34:1041–1050

Raup DM (1966) The endoskeleton. In: Boolootian RA (ed) Physiology of Echinodermata. Interscience, New York, pp 379–395

Robles LJ, Breneman JW, Anderson EO, Nottoli VA, Kegler LR (1986) Immunocytochemical localization of a rhodopsin-like protein in the lipochondria in photosensitive neurons of *Aplysia californica*. Cell Tissue Res 244:115–120

Ryberg E, Lundgren B (1979) Some aspects on pigment cell distribution and function in the developing echinopluteus of *Psammechinus miliaris*. Dev Growth Differ 21:129–140

Smith AB (1980) Stereom microstructure of the echinoid test: special papers in paleontology No 25. Paleont Assoc, London, pp 1–81

Stubbs TR (1982) The neurophysiology of photosensitivity in ophiuroids. In: Lawrence JM (ed) Proceedings, International Echinoderms Conference, Tampa. AA Balkema, Rotterdam, pp 403–408

Takasu N, Yoshida M (1983) Photic effects on photosensory microvilli in the seastar *Asterias amurensis* (Echinodermata: Asteroidea). Zoomorphology 103:135–148

Weber W (1983) Photosensitivity of chromatophores. Am Zool 23:495–506

Weber W, Dambach M (1974) Light-sensitivity of isolated pigment cells of the sea urchin *Centrostephanus longispinus*. Cell Tissue Res 148:437–440

Weber W, Gras H (1980) Ultrastructural observations on changes in cell shape in chromatophores of the sea urchin *Centrostephanus longispinus*. Cell Tissue Res 206:21–33

Weber W, Grosmann M (1977) Ultrastructure of the basiepithelial nerve plexus of the sea urchin, *Centrostephanus longispinus*. Cell Tissue Res 175:551–562

Whitfield PJ, Emson RH (1983) Presumptive ciliated receptors associated with the fibrillar glands of the spines of the echinoderm *Amphipholis squamata*. Cell Tissue Res 232:609–624

Wilkie IC (1979) The juxtaligamental cells of *Ophiocomina nigra* (Abildgaard) (Echinodermata: Ophiuroidea) and their possible role in mechano-effector function of collagenous tissue. Cell Tissue Res 197:515–530

Yoshida M (1966) Photosensitivity. In: Boolootian RA (ed) Physiology of Echinodermata. Interscience, New York, pp 379–395

Yoshida M (1979) Extraocular photoreception. In: Autrum H (ed) Handbook of sensory physiology, vol VII/6A. Springer, Berlin, pp 581–640

Yoshida M, Takasu N, Tomatsu S (1984) Photoreception in echinoderms. In: Ali MA (ed) Photoreception and vision in invertebrates. Plenum, New York, pp 743–771

Received January 28, 1987