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THE ULTRASTRUCTURE OF THE OCELLUS IN THE LARVA OF CLAVELINA LEPADIFORMIS (MÜLLER) (TUNICATA: ASCIDIACEA)

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RESUM

Ultrastructura de l'ocel·le de la larva de Clavelina lepadiformis **(Müller)** (Tunicata: Ascidiacea)

S'estudia la ultrastructura de l'ocel·le larvari d'una espècie d'ascidi: *Clavelina lepadiformis*. Aquest fotoreceptor és format per tres parts: una gran cèl·lula pigmentària en forma de copa, tres cèl·lules que formen un sistema de lents i 15-18 cèl·lules fotoreceptores. Aquesta disposició sembla ésser la més comuna al grup dels ascidis.

La llum incideix damunt el sistema de lents, que probablement actua concentrant-la sobre les membranes fotoreceptores d'origen ciliar situades a l'interior de la copa que forma la cèl·lula pigmentària. Es comenta també l'estructura dels fotoreceptors als grups més propers.

INTRODUCTION

Several studies on the anatomy of the larvae of some ascidian species (GRAVE, 1921; SCOTT, 1946; BERRILL, 1948a, 1948b; TRASON, 1957) have described the structure of the larval ocellus located in the postero-dorsal wall of the sensory vesicle. These studies agree that this photoreceptor is a multicellular organ consisting of a pigmented cup-shaped mass, a lens-system and some photoreceptor cells. Recently, GORMAN *et al.* (1971) have clearly demonstrated its photoreceptor function by registering hyperpolarizing responses to light-exposure in the receptor cells of *Aplidium (Amaroucium) constellatum*. Thus, the ocellus is a true photoreceptor engaged in the phototactic reactions of ascidian larvae (GRAVE, 1921; GRAVE & WOODBRIDGE, 1924; DILLY, 1964; MILLAR, 1971; KAJIWARA & YOSHIDA, 1985).

Other species, however, differ from this general structural pattern in their photoreceptor organs. Some members of the Styelidae family, such as *Botryllus schlosseri* (GRAVE & WOODBRIDGE, 1924; GRAVE & RILEY, 1935), *Stolonica socialis* and *Distomus variolosus* (BERRILL, 1949) possess a combined light-perceiving and equilibrium sense organ, the photolith, consisting of a cup-shaped statocyte related to some photoreceptor cells. *Styela partita* has a reduced ocellus formed by a single cell with a pigment-containing vesicle (GRAVE, 1926; WHITTAKER, 1966). Other examples, such as the Molgulidae family or the genus *Dendrodoa*, completely lack the oce-

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llus (GRAVE, 1926; BERRILL, 1950; BONE & MACKIE, 1982).

In contrast to these studies using light microscopy, fine structure studies (required in order to know the exact nature of these photoreceptors), are far from numerous. The major literature on this topic consists of the work of DILLY (1961, 1964); EAKIN & KUDA (1971); BARNES (1969, 1971, 1974) and KAJIWARA & YOSHIDA (1985), which give information about four species: *Aplidium (Amaroucium) constellatum*, *Ciona intestinalis*, *Ciona savignyi* and *Distaplia occidentalis*. VORONTSOVA & MALAKHOV (1982, 1984), on the other hand, describe the fine structure of the photolith of the styelid species *Cnemidocarpa finmarkiensis*.

In this work, we present data on the ultrastructure of the ocellus of a polycitorid species: *Clavelina lepadiformis*.

MATERIAL AND METHODS

The larvae were obtained during the natural reproduction period of this species. Mature colonies with larvae being incubated in the atrial cavity of zooids were collected by SCUBA diving in the locality of Tossa de Mar (Girona, NE of Spain) in July.

By dissection of tunic and mantle, embryos were released from the atrial cavity and placed in Petri dishes. Within a few minutes, the already developed larvae broke out from their egg-membranes and began to swim actively. They were then gently pipetted out and transferred to the fixative liquid.

The fixation of the specimens was done in glutaraldehyde 2.5 % in 0.2 M phosphate buffer at pH 7.2 for two hours, followed by several washes in buffer and postfixation for one hour in osmium tetroxide (1 %) in the same buffer. Dehydration was carried out using a graded series of acetone solutions. The material was then embedded in Araldite (Durcupan ACM).

Thick serial sections (1 micron) were cut with a glass knife and stained with 1 % methylene blue solution untill the localization of the desired area was achieved with the light microscope. Thin sections were obtained with a Reichert Om-U2 microtome with a diamond knife. These were stained with uranyl acetate and lead citrate (REYNOLDS, 1963). Some of the grids, however, were stained following Thiéry's procedure (THIÉRY, 1967) in order to test the polysaccharid nature of some elements which will be discussed later.

FIG. 1. Lateral view of the trunk region of the larva of *Clavelina lepadiformis*. The two black spots are the eyespot (E) and the statocyte (S) within the sensory vesicle. x 130. ▶
Vista lateral de la regió del tronc de la larva de *Clavelina lepadiformis*. Les dues taques negres corresponen a l'ocel·le (E) i a l'estatocist (S) dins de la vesícula sensitiva. x 130.

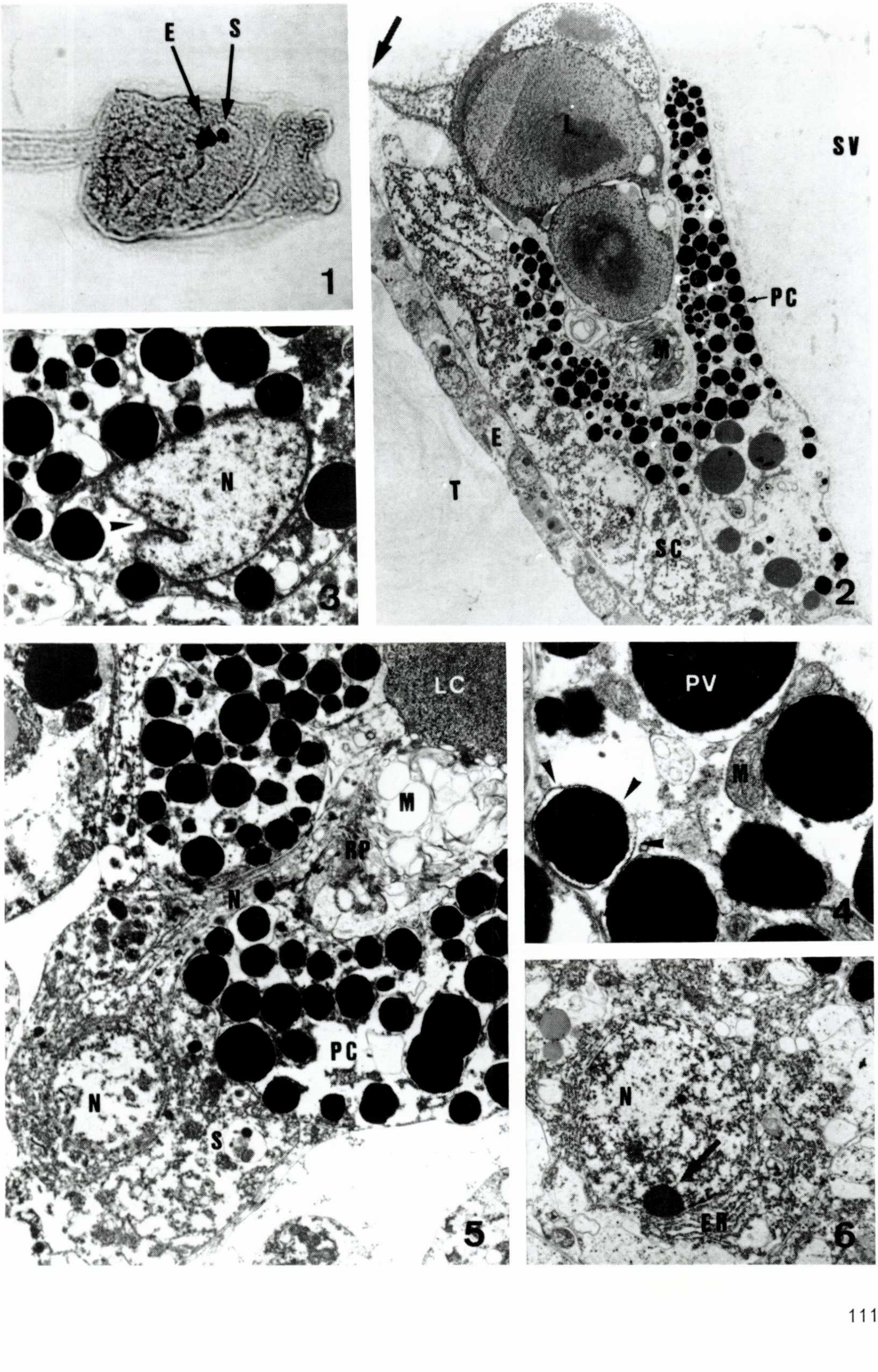
FIG. 2. Section of the ocellus showing its general structure. E: epidermis; L: lens cell; M: membranous processes; PC: pigment cell; SC: sensory cell; SV: sensory vesicle; T: tunic. Arrow marks the wall of the sensory vesicle. x 2200.
Secció de l'ocel·le mostrant la seva estructura general. E: epidermis; L: cèl·lula de la lent;; M: processos membranosos; PC: cèl·lula pigmentària; SC: cèl·lula sensitiva; SV: vesícula sensitiva; T: túnica. La fletxa indica la paret de la vesícula sensitiva. x 2200.

FIG. 3. Nucleus (N) of the pigment cell. Note the deep invagination of its membrane (arrowhead). x 6200.
Nucli (N) de la cèl·lula pigmentària. Observeu la profunda invaginació a la membrana (punta de fletxa). x 6200.

FIG. 4. Pigment vesicles (PV) of the pigment cell. Arrowheads indicate the limiting membrane. M: mitochondrion. x 22140.
Vesícules de pigment (PV) de la cèl·lula pigmentària. Les puntes de fletxa marquen la membrana limitant. M: mitocondri. x 22140.

FIG. 5. Sensory cell showing its soma (S), neck region (N) and receptor processes (RP). LC: lens cell; M: receptor membranes; N: nucleus; PC: pigment cell. x 6190.
Cèl·lula sensitiva mostrant el seu soma (S), la regió del coll (N) i els processos receptors (RP). LC: cèl·lula de la lent; M: membranes receptores; N: nucli; PC: cèl·lula pigmentària. x 6190.

FIG. 6. Basal region of a sensory cell with its nucleus (N), nucleolus (arrowhead) and endoplasmic reticulum (ER). x 6200.
Part basal d'una cèl·lula sensitiva amb el seu nucli (N), nuclèol (punta de fletxa) i reticle endoplasmàtic (ER). x 6200.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The sections were examined under a Philips EM 301 electron microscope in the Microscopy Service of the University of Barcelona.

RESULTS

The ocellus lies anchored to the right postero-dorsal wall of the cavity which forms the sensory vesicle (fig. 1). It is made up of three parts (fig. 2): a pigmented cup-shaped cell (U-shaped in section), whose aperture points anteriorly and slightly ventrally, a lens system consisting of three lenses placed in front of the concavity of the «U», and a group of sensory cells, the cell bodies of which lie outside the dorsal arm of the pigmented cup. Within the cup cavity there are many piled membranous structures that are part of the sensory cells.

The pigment cup is made up of a single cell which is the largest of the cells in the ocellus. Its section displays a cellular soma and two arms that give it its characteristic shape (fig. 2). The length of the cell is about 45-50 μm, its largest diameter being 21 μm. The «U» aperture reaches 14 μm in diameter.

The nucleus is at the base of the cell. It is irregular in shape and a deep invaginatinon of the nuclear membrane is often observed (fig. 3).

The most conspicuous feature of this cell is the large number of electron dense pigment granules which are uniformly distributed in the cytoplasm, except in the basal zone. These granules (fig. 4) are membrane-bounded and their size varies between 0.5 and 1.8 μm in diameter. Numerous vesicles and mitochondria are to be seen in the cytoplasm between the granules.

The position of these pigment vesicles prevents light from entering the cavity of the eyecup except from the direction which passes through the lens system.

MINGANTI (1951) demonstrated that the substance filling the granules is a melanin. The melanogenesis process in ascidian embryos was accurately studied by WHITTAKER (1966, 1976, 1979).

The basal region of the cell is a part of the wall of the larva's sensory vesicle (fig. 2). It is quite free of pigment granules and features some yolk vesicles with associated rough endoplasmic reticulum.

The sensory cells lie dorsally to the pigment cup cell. Peripheral to them is the larval epidermis and, outside this, the tunic.

As many as eight sensory cells have been found in one section, and their estimated number is between 15 and 18, similar to that found in other species (EAKIN & KUDA, 1971; BARNES, 1974). These cells have four parts: a basal axon, a cellular soma, a narrow section or neck traversing the dorsal arm of the eyecup and a laminar process which develops in the lumen of the cup (fig. 5).

The soma is conical or pear-shaped. It contains the rounded nucleus, with a conspicuous nucleolus. The cytoplasm is filled with vesicles, mitochondria, endoplasmic reticulum and yolk droplets (fig. 6).

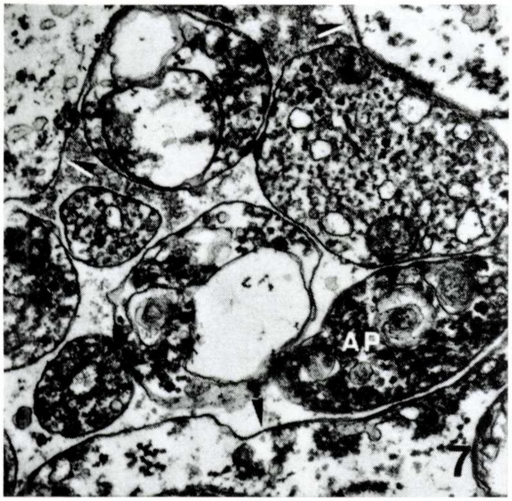
Each cell has a tapering basal process

FIG. 7. Bundle of basal axonal processes (AP) surrounded by the membranes of the sensory cells (arrowheads). x 30000.
Grup d'àxons basals (AP), envoltats per les membranes de les cèl·lules sensitives (puntes de fletxa). x 30000.

FIG. 8. Receptor process of a sensory cell showing its proximal (PS) and distal (DS) segments. BB: basal body; LC: lens cell; PC: pigment cell. Double arrowheads mark a desmosome-like intercellular junction. Single arrowheads indicate a hemi-desmosome-like junction. x 13110.
Procés receptor d'una cèl·lula sensitiva mostrant el segment proximal (PS) i distal (DS). BB: corpuscle basal; LC: cèl·lula de la lent: PC: cèl·lula pigmentària. Les puntes de fletxa dobles marquen una unió cel·lular de tipus desmosoma; les puntes de fletxa senzilles n'indiquen una de tipus hemidesmosoma. x 13110.

FIG. 9. Arrays of lamellae (AL) derived from three receptor processes (asterisks) inside the cavity of the pigment cell (PC). LC: lens cell. x 10025.
Formacions lamel·lars (AL) derivades de tres processos receptors (asteriscs) dins la cavitat de la cèl·lula pigmentària (PC). LC: cèl·lula lenticular. x 10025.

FIG. 10 to 12. Three sections of the same photoreceptor process. BB: basal body; C: accessory centriole; L: lamellae; PC: pigment cell. Arrowheads mark the narrow circumciliary space. x 25700.
Tres seccions del mateix procés fotoreceptor. BB: corpuscle basal; C: centriol; L: lamel·les; PC: cèl·lula pigmentària. Els caps de fletxa marquen l'estret espai circumciliar. x 25700.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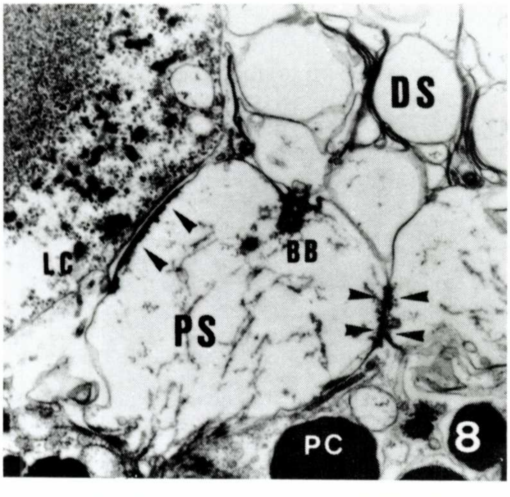
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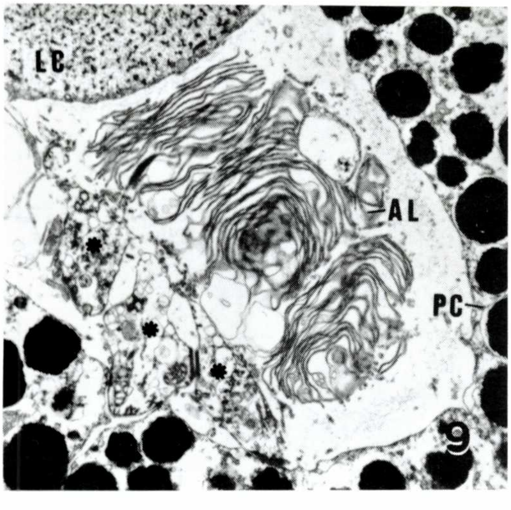
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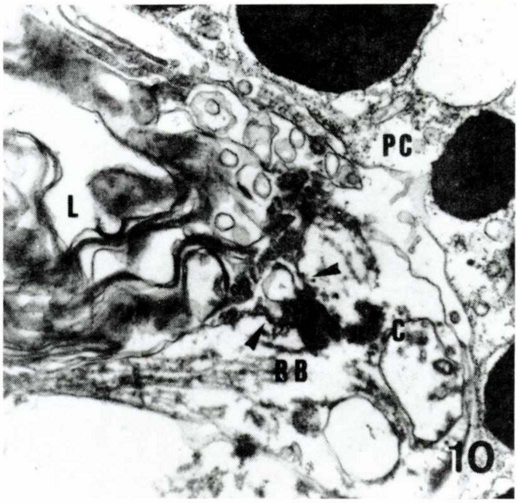
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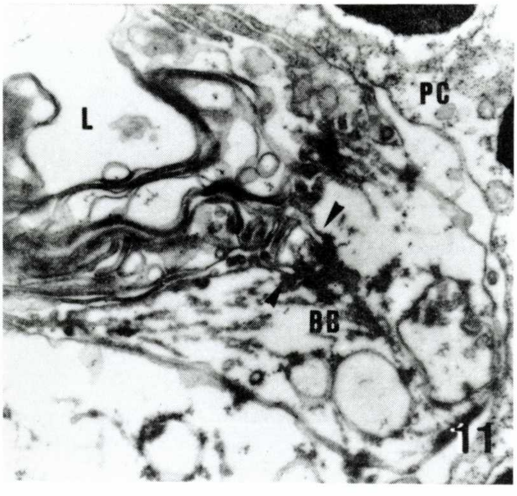
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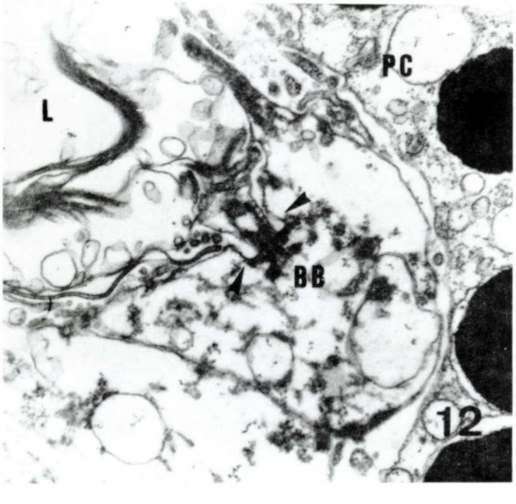
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or axon, with some microtubules and vesicles. Transversally cut groups of these processes can be seen in each section. They run across the basal part of the sensory cells, surrounded by their membranes (fig. 7).

The neckt of cytoplasm has a diameter of about 0.5 μm. It is surrounded by infoldings of the membrane of the pigmented cell, through which it passes. Inside, vesicles and numerous microtubules can be seen. Near the «exit» point of this shaft to the lumen of the eyecup there are intercellular desmosome-like junctions attaching the neck to the adjacent pigmented cell.

The receptor processes of the retinal cells lie within the cavity of the eyecup. They have a proximal and a distal segment. The proximal one is formed by the outer part of the shaft coming from the sensory cell. Here some microtubules and vesicles can be observed. Adjacent segments from different retinal cells are firmly anchored to each other by desmosome-like junctions, whereas the innermost lens cell and the sensory process adjacent to it are attached by a hemi-desmosome-like structure (fig. 8).

The distal segment of the receptor process consists of piled membranes, which form arrays of flattened discs oriented perpendicularly to the long axis of the eyecup (which is the direction of the incoming light passing through the lens cells). In fig. 9 three arrays of discs can be seen, although as many as seven have been found in the same section.

In some well-oriented sections these piled structures are seen to emerge from the membrane of a cilium (fig. 8). Figs. 10 to 12 show three sections of the same photoreceptor process, in which a basal body can be seen at the tip of the proximal segment (an accessory centriole is also to be observed in one of them). In favourable sections, a 9 + 0 axonematic structure, which is characteristic of sensory rather than motile cilia, has been observed. Higher magnification of this region (fig. 13) shows that the arrays of lamellae originate from the ciliary membrane. A circumciliary space appears surrounding the short neck or connecting piece between the basal body and the membranous expansions. Microvilli, longitudinally and transversally (circular profiles) cut, are also to be observed. They emerge from the proximal segment of the photoreceptor process, near the basal body, and interdigitate with the flattened piles of membranes from the modified cilium. Filamentous diverticula that intermingle with the receptoral processes also arise from the pigmented cell and the innermost lens cell (figs. 14, 15).

FIG. 13. High magnification of the ciliary region. Some microvilli emerge from the proximal segment and intermingle with the receptor membranes. They appear longitudinally (arrowheads) and transversally (arrows) cut. BB: basal body; CS: circumciliary space. x 43690. ▶
Visió augmentada de la regió ciliar. Alguns microvil·lis que surten del segment proximal es barregen amb les membranes receptores. Es troben tallats longitudinalment (puntes de fletxa) i transversalment (fletxes). BB: corpuscle basal; CS: espai circumciliar. x 43690.

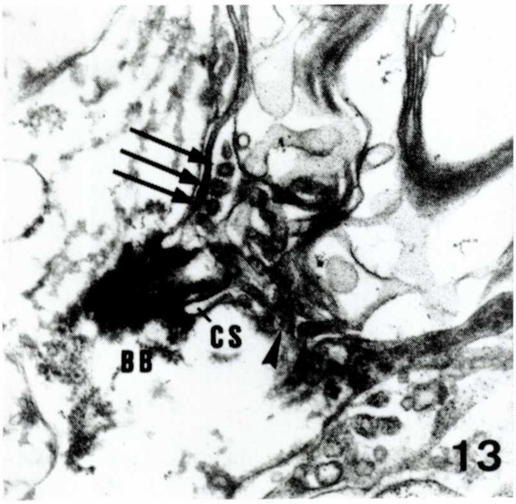
FIG. 14. Two microvilli emerging from the pigment cell (PC) and entering deep into receptor processes (RP). Note the thickening of the membranes (arrowheads). x 43690.
Dos microvil·lis, procedents de la cèl·lula pigmentària (PC), penetren profundament en els processos receptors (RP). Observeu l'engruiximent de les membranes (caps de fletxa). x 43690.

FIG. 15. Array of lamellae (L) from a receptor process. x 131140.
Grup de lamel·les (L) derivades d'un procés receptor. x 131140.

FIG. 16. Margin of the innermost lens cell showing a filamentous outgrowth (arrowheads) protruding into a receptor process. CB: central body of the lens cell; L: lamellae; M: mitochondria surrounding the central body. x 33410.
Marge de la cèl·lula lenticular més interna mostrant una formació filamentosa (puntes de fletxa) que entren en un procés receptor. CB: cos central de la cèl·lula lenticular; L: lamel·les; M: mitocondris envoltant el cos central. x 33410.

FIG. 17. Central body of a lens cell. Note the glycogen? granules and a slight net between them (stained following Thiéry's procedure). x 60000.
Cos central d'una cèl·lula lenticular. Noteu els grànuls de glucogen? i el lleuger reticle entre ells (tenyit amb la tècnica de Thiéry). x 60000.

FIG. 18 Lens cell. C: cytoplasm; CB: central body; M: mitochondrion; N: nucleus with nucleolus (arrowhead). x 6410.
Cèl·lula lenticular. C: citoplasma ;CB: cos central; N: nucli amb nuclèol (punta de fletxa). x 6410.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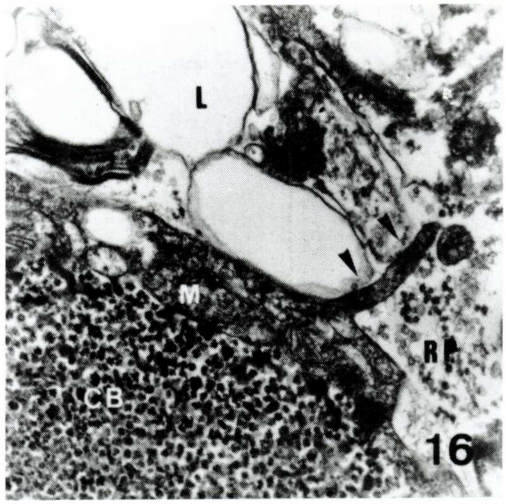
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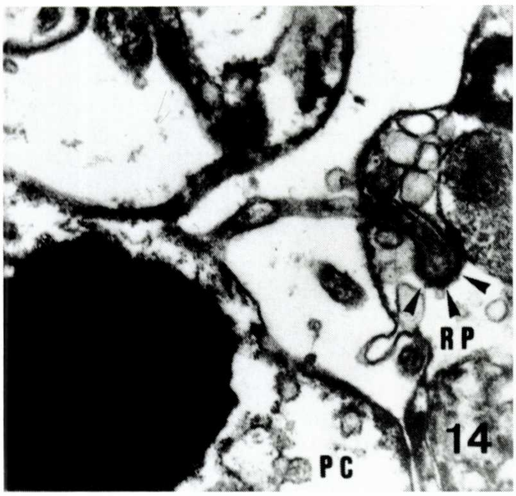
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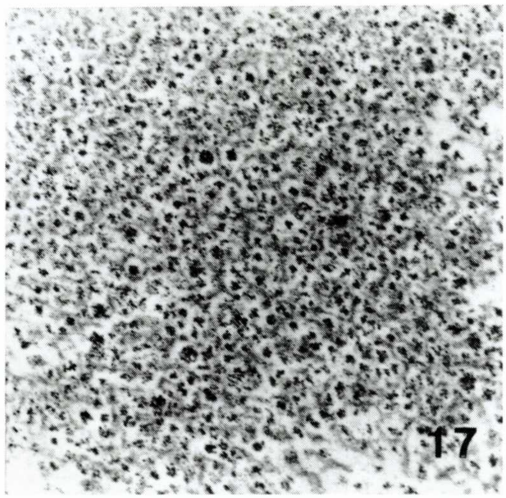
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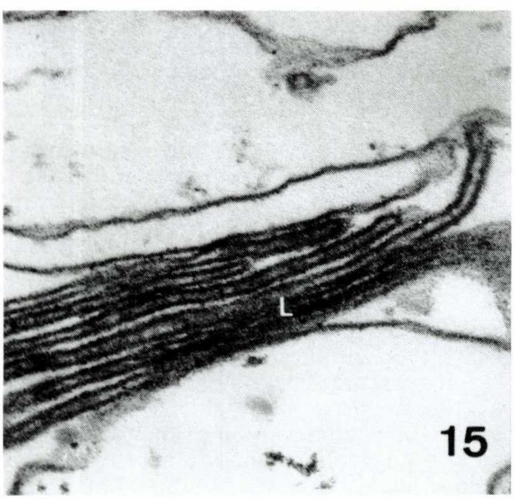
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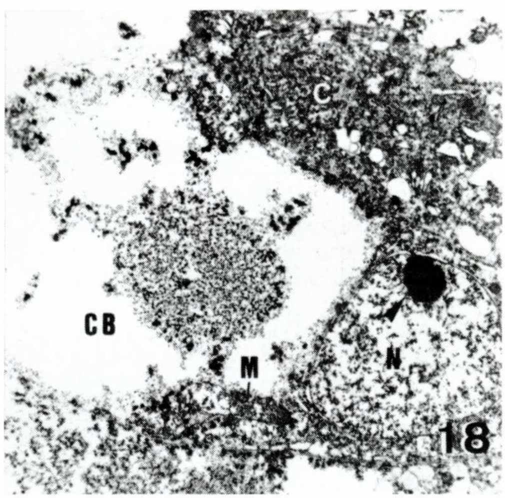
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However, no continuity has been found between these profiles and the discs themselves. The width of the discs is about 200 Å (fig. 16).

There are three lens cells, which are arranged in a row in the anterior part of the eyecup (fig. 2). They are in a line with the direction of light passing into the photoreceptors through the open end of the pigment cup.

The lens cells are oval in shape, roughly 28 μm long and 14 μm wide. Most of the cytoplasm in each cell is occupied by a massive aggregate of electron dense granules (average 300-450 Å in diameter) of irregular shape; between them there is a slight reticular net (fig. 17). There is a higher concentration of these elements at the centre than on the periphery of the central body. This aggregate is limited by a single layer of mitochondria, whose long axis is usually parallel to the granulous mass. Some tiny gaps in the line of mitochondria allow contact between the substance of the central mass and the rest of the cytoplasm. The distally-placed nucleus (fig. 18) is round-shaped and displays a conspicuous nucleolus. As for the rest of the cytoplasm, it is pushed away to the periphery of the cell. It is electron dense and features numerous mitochondria, ribosomes and endoplasmic reticulum.

DISCUSSION

The ultrastructure of the ocellus in *Clavelina lepadiformis* does not differ in any major way from that found in *Aplidium (Amaroucium) constellatum, Ciona intestinalis* and *Distaplia occidentalis* (DILLY, 1961, 1964; EAKIN & KUDA, 1971 and BARNES, 1969, 1971, 1974).

The working of the eye, as inferred from its morphology, is a simple one: the configuration of the pigment cup shields the receptor membranes from all light except that which comes through the lens system. Thus, the eye gives the larva directional information which is important for explaining the photic orientation featured in the behaviour of ascidian larvae (GRAVE, 1921, 1944; GRAVE & WOODBRIDGE, 1924; DILLY, 1964; KAJIWARA & YOSHIDA, 1985).

In addition, a «shadow reaction» has been demonstrated (GRAVE, 1921; GRAVE & WOODBRIDGE, 1924; DILLY, 1964): when light falling on the pigment cup is interrupted, the larva begins to swim immediately. Consequently, during the rotating movement made by ascidian larvae when they swim actively, the receptors are illuminated (i.e. when the axis of the eyecup points towards the surface of water) and immediately shaded once in each rotation, thereby stimulating further locomotive activity.

The gradient in density from the centre to the periphery of the central bodies of the lens cells probably enables them to concentrate light on the receptoral membranes. EAKIN & KUDA (1972) indicated after cytochemical studies that the granules in the «lenses» are particles of glycogen. The positive reaction of these granules found here to Thiéry's staining method (fig. 17) supports this conclusion.

The flattened membranous discs oriented perpendicularly to the path of the incoming light are presumably the site of the photopigment. Numerous microvilli and cellular junctions (figs. 8, 13, 14) give rigidity to these membranous units. EAKIN & KUDA (1971) found microvilli derived from the pigmented cell, whereas BARNES (1971, 1974) described microvilli as arising from the sensory cell, near the basal bodies. Both types exist in *Clavelina lepadiformis* (figs. 13, 14). Their exact function is unknown. BARNES (1974) indicates the possibility of them having a photoreceptor funcion. However, their poor development compared to the other receptor membranes suggests a supporting, and perhaps also a nutritive function (EAKIN & KUDA, 1971). The deep interdigitation between the microvilli and the receptor processes as well as the thickening or union observed between their membranes (fig. 14) supports this latter interpretation.

The transmission of the impulse is, most probably, via the neck of the sensory cell and then through its basal axon. The bundles of axons presumably run backwards and end at the larval cerebral ganglion (BARNES, 1971). No synapses have been observed between the axons and adjacent cells.

Two types of photoreceptors exist among the invertebrates: one of these derives the sensitive organelles from modifications of a ciliary membrane. In the second type, called rhabdomeric, the receptor organelles derive from microvilli (EAKIN, 1965) .The ocellus of the ascidians is of the ciliary type. Its possible homology with the vertebrate photoreceptors was already suggested by SALENSKY (1893). Electron microscopists (DILLY, 1964; EAKIN & KUDA, 1971; BARNES, 1971) also pointed out that the fine structure of the sensitive segment bears a remarkable likeness to the structure of vertebrate receptors (MOODY, 1964; COLLIN, 1969).

As for the related groups, the hemichordate tornarian larva has two eyespots with both cilia and microvilli. It remains unclear which of the structures, if not both, acts as a photoreceptor (BRANDENBURGER *et al.*, 1973).

In *Branchiostoma* (Cephalochordata) two types of photoreceptors have so far been described: the Hesse cells (EAKIN & WESTFALL, 1962) and the Joseph cells (WELSCH, 1968; ANADON, 1976), both related to the rhabdomeric type. However, near the Joseph cells, in the dorsal wall of the cerebral vesicle, there are other cells with membranous appendages derived from cilia which are strikingly similar to the outer segment of the ascidian sensory cells and which may also act as photoreceptors (EAKIN & WESTFALL, 1962; MEVES, 1972).

Other groups of tunicates, such as appendicularia and doliolids, do not have an ocellus (BONE & MACKIE, 1982; HOLMBERG, 1984). Salps possess a microvillous receptor (BARNES *et al.*, 1970; GORMAN *et al.*, 1971). Finally, some adult ascidians like *Ciona intestinalis* have rhabdomeric ocelli at the border of the siphons (DILLY & WOLKEN, 1973).

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