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Ultrastructure and Potential Significance of Cerebral Light-Refracting Bodies of *Stenostomum virginianum* (Turbellaria, Catenulida)*

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Summary. Light-refracting bodies, possibly photoreceptors, occurring in the posterior lobes of the brain are considered characteristic for most species of catenulid turbellarians of the freshwater genus *Stenostomum.* In *S. virginianum,* each of two light-refracting bodies consists of a single cup-shaped granule (3–4 µm) situated in the perikaryon of a single nerve cell (the 'clear vesicle' of earlier papers). TEM reveals each granule as an enlarged and folded mitochondrion with a dense matrix inclusion of undetermined composition. Cristae are well-developed and there is a dense granular extramitochondrial layer of uniform thickness (100 nm) along the posteromedial surface of the mitochondrion. The perikaryon is packed with ribosomes, *β*-glycogen granules and 60–100 nm dense-cored vesicles. A neurite extends from the perikaryon into the neuropile of the brain. Experimental data indicate an absence of phototaxis and photokinesis and an absence of ultrastructural modifications with light- and dark-adaptation. An ultrastructural comparison is made of the light-refracting bodies of *S. virginianum* with those of a second species. Hypotheses regarding the role of light-refracting bodies as photoperiodic receptors and/or specialized neurosecretory cells are advanced.

A. Introduction

Turbellarians of the order Catenulida are common freshwater animals, and stenotopic but not rare marine flatworms (Borkott 1970; Sterrer and Rieger 1974; Faubel 1976; Doe and Rieger 1977; Pennak 1978). Catenulids are considered unique among tubellarians for possessing a single, mid-dorsal protonephridium; a mid-dorsal testis with its gonopore located dorsally, immediately behind

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the head; and a fluid-filled body cavity (in some species) referred to as a "pseudocoel" by classical investigators (Nuttycombe and Waters 1938). Additionally, the Catenulida is one of two orders of Turbellaria in which individuals may divide asexually by paratomy to form chains of zooids, and, as a group, shows degenerate forms of sexuality (Borkott 1970; Doe and Rieger 1977; Rieger 1978).

The complex sensory organs of the Catenulida may be limited to a gravity receptor or statocyst situated within the brain of some but not all species, and a pair of putative chemosensory organs called cephalic pits situated on the lateral margins of the rostrum. Photoreceptors are not reported from catenulid turbellarians although epidermal and cerebral eyes of ciliary, rhabdomeric, and mixed ciliary and rhabdomeric organizations are reported from other orders of Turbellaria (Karling 1974; MacRae 1964, 1966; Ehlers and Ehlers 1977; Ruppert 1978). Most freshwater catenulids of the genus *Stenostomum* possess a pair of colorless, cerebral light-refracting bodies in the posterior lobes of the brain. These light-refracting bodies may resemble eyes when viewed with a light microscope (see Fig. 3, Nuttycombe and Waters (1938), and Fig. 2, this paper) and they are species specific. Nuttycombe and Waters (1938), in their monograph of the genus, recognized 3 types of refractile bodies: (1) those consisting of a clear vesicle with more than ten refractile granules forming a dish to one side of the vesicle, (2) those with 1–5 refractile granules associated with a clear vesicle and (3), those with a vesicle and a single refractile granule, as in *S. virginianum*. Nuttycombe and Waters do not advance any ideas regarding the function of these refractile bodies. Sonneborn (1930) and Borkott (1970), among others, suggested that the light-refracting bodies are photosensory in function whereas Hyman (1951) after reviewing the available literature rejected a potential role in photosensation for these 'bowl-shaped bodies'.

Our objectives were to test *S. virginianum*, a freshwater species, for phototactic or photokinetic responses; to test the light-refracting bodies directly for photosensitivity; and, to correlate light and electron microscopic data for these potential photoreceptors.

B. Materials and Methods

Specimens of *S. virginianum* (Nuttycombe 1931) were obtained alive from Carolina Biological Supply Company and were maintained in the laboratory in wheat grain infusions. *Stenostomum* sp., possibly *S. tenuicaudatum* (Nuttycombe and Waters 1938), was obtained alive from laboratory aquaria courtesy of Drs. J.E. Schindler and J.B. Waide of Clemson University. Living animals were relaxed partially using chloroform vapors prior to examination of live specimens or fixation for light and electron microscopy. Chloretone, menthol crystals, a 0.1% solution of $MgCl_2$ and a 1.0% solution of cocaine hydrochloride in distilled water were uniformly unsatisfactory relaxants. Specimens were fixed in 1.4% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.2 for 45 min to 1 h at room temperature. Postfixation was in 2.0% OsO_4 in 1.25% $NaHCO_3$ buffer, pH 7.2, for 30 min at room temperature. After ethanol dehydration, specimens were embedded in Epon. Serial thick sections (0.5 μm) were obtained in ribbons using diluted contact cement applied to the block prior to sectioning with a glass knife (3 specimens of *S. virginianum*). Serial thin sections were cut with a diamond knife on a Sorvall MT-2B ultramicrotome and collected on Formvar-filmed slot grids (2 specimens of *S. virginianum*). Representative sections of specimens of *S. virginianum* and one light-adapted specimen of *S.* sp. were collected on 200-mesh copper grids for observation with a Phillips 300 TEM. The dark-adapted specimens of *S. virginianum* were maintained in total darkness for 7 days and then fixed in total darkness. The overall quality of fixed animals was variable, even among those that were fixed simultaneously, ranging from excellent to poor tissue and cell preservation.

C. Results

1. Observations of Living Animals

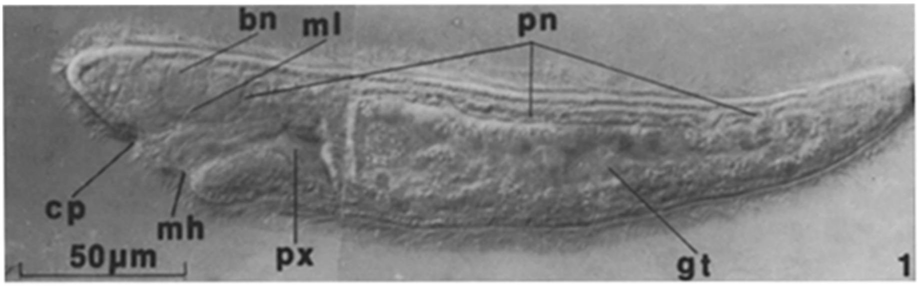
Stenostomum virginianum is fusiform in body outline and circular in transverse section. Anteriorly, the body is short and conical, forming the so-called rostrum. The diameter of the body is greatest just behind the rostrum, then it tapers gradually to a bluntly pointed tail. The surface of the body is completely ciliated.

When the animals are demersal, they glide on cilia at various speeds. This pattern of locomotion is distinctive because *S. virginianum* glides with its oral surface uppermost (also see Borkott 1970). The mouth and pharynx are situated behind the highly mobile rostrum and are directed anteriorly. A recurring behavior of these animals while gliding on a substrate is a periodic reversal of at least the aboral locomotory cilia. This action carries them rearward a distance of approximately $^1/_8$ to one full body length before they again resume forward locomotion, usually in a new direction.

S. virginianum swims actively in pond water in our culture dishes. Swimming activity appears to be directly related to disturbance and inversely related to food availability, but we have not performed any rigorous tests of these hypotheses. Swimming animals spiral through the water while slowly rotating clockwise about their long axes when viewed from the anterior end.

The light-refracting bodies are visible under transmitted light with a dissecting microscope. They appear as nearly transparent, spherical droplets in the brain, one on each side of the mouth (Fig. 1, ml). When viewed with a compound microscope, the light-refracting bodies of *S. virginianum* are visible in the posterior lobes of the brain (Figs. 2, 5). Each body consists of a refractile granule (Fig. 4, in) and a clear vesicle (Fig. 4, cy), as recognized by Nuttycombe and Waters (1938). When the specimen is flattened by withdrawing water from below the coverslip, each refractile body (Fig. 3) is resolved into three distinct structures (Fig. 4): 1) the refractile granule per se (Fig. 4, in), 2) a region of somewhat lower refractive index on the lateral side of the refractile granule (Fig. 4, mt), and 3), the cytoplasm and nucleus of the cell containing the refractile granule (Fig. 4, cy, nu). The 'clear vesicle' of Nuttycombe and Waters is the cytoplasm of the inclusion-bearing cell.

S. virginianum is not phototactic and appears to show no behavioral changes under various light intensities. We tested phototaxis in *S. virginianum* by applying directed light on the animals under various intensities in a totally darkened room. When a microscope illuminator (6 V 15 W) was placed to one side of the culture dish, we did not observe any behavioral changes. Similarly, we directed a beam of light onto a single specimen using a 0.5 mm diameter optical fiber illuminated at several intensities and again observed no responses in *S. virginianum*.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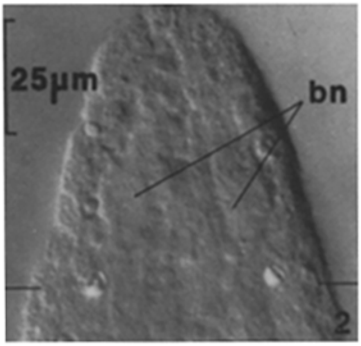
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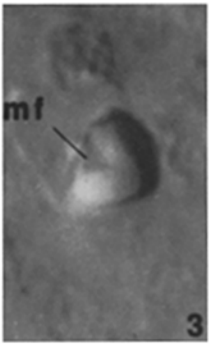
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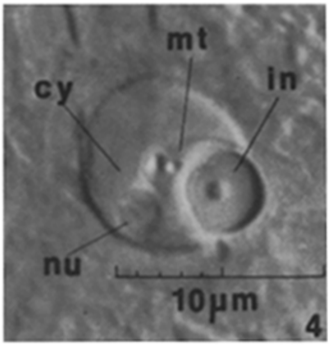
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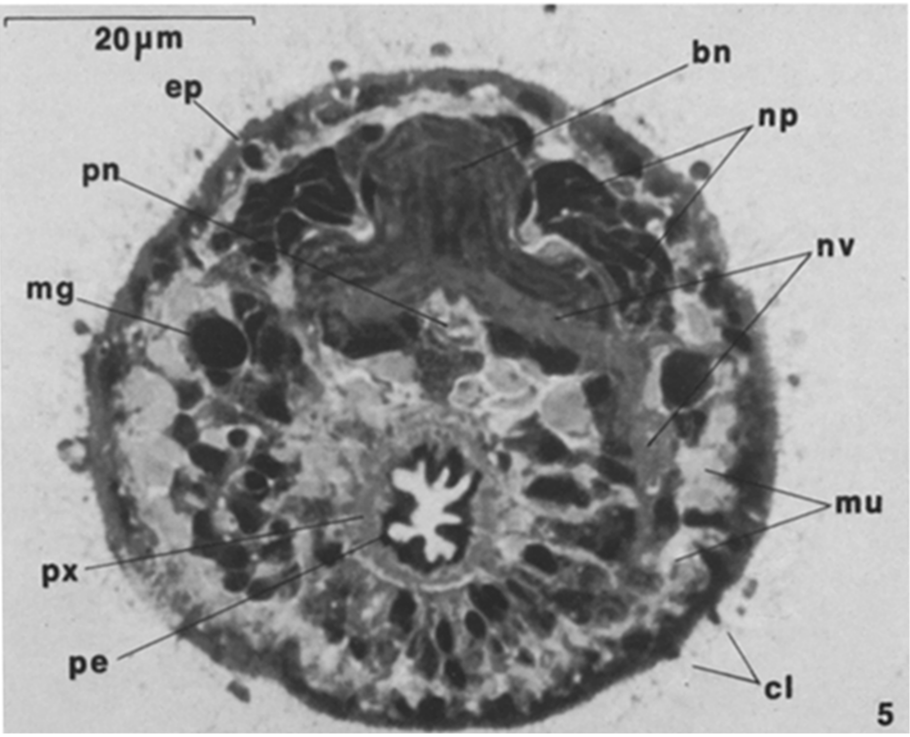
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2. Observations of Fixed Animals

The cerebral ganglion, or brain, of *S. virginianum* is a large, subepidermal mass of neuropile situated aborally and bordered bilaterally by nerve cell perikarya (Figs. 1, 2, 5). Each refractile body (5–8 µm) is situated laterally, immediately internal to the body wall musculature. The refractile granule is situated in the perikaryon of a nerve cell that gives rise to a neurite extending medially into the neuropile of the cerebral ganglion. The nerve cell perikaryon containing the refractile granule is bordered medially by perikarya of several neurons that also contribute neurites to the tract leading to the neuropile. Topographically, each of the two granule-containing perikarya appears to rest in a cup formed of nerve cell perikarya, and each of the two cups is joined to the main mass of the brain by a tract of neurites (Figs. 5 and 6).

When viewed with a transmission electron microscope, the refractile granule was observed to be a single, large mitochondrion (3–4 µm diam.) that contained material of high electron density in much of its matrix (Figs. 6–8). We believe that it is this matrix material that results in the refractile properties of this organelle observed in living animals with the light microscope. Posteriorly, the dense matrix material occupies most of the volume of the organelle (Fig. 8).

Each mitochondrion, one per cell, infolds on its anterolateral face (Fig. 3, mf). This folding is apparent at the level of light observations as irregularities along the anterolateral face of the refractile granule (Figs. 3 and 4). Septate cristae are numerous, well-developed and are often swollen (Figs. 6 and 7). A uniformly dense, finely granular material forms a layer 100–125 nm thick immediately peripheral to the outer membrane of the mitochondrion along its posteromedial surface (Fig. 8, gl).

The cytoplasm of the cells containing these mitochondria is dense with β-glycogen, ribosomes and Golgi-derived dense-cored vesicles, 60–100 nm in diameter (Fig. 9). Small myelin figures were consistent features of these cells (Figs. 7 and 8, my).

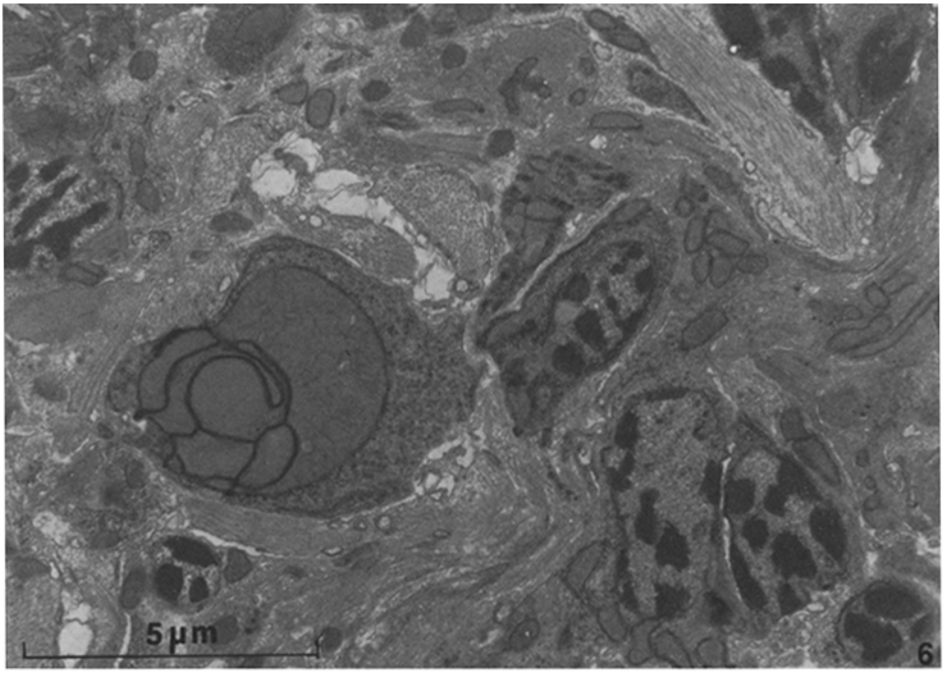
Fig. 1. Living *S. virginianum* in optical sagittal section, specimen slightly contracted longitudinally. Brain (*bn*), location of refractile body (*ml*), protonephridium (*pn*), cephalic pit (*cp*), mouth (*mh*), pharynx (*px*), gut (*gt*)

Fig. 2. Aboral view of the anterior end of *S. virginianum* showing light-refracting bodies and brain. Specimen dorsoventrally compressed. Brain (*bn*)

Fig. 3. Enlargement of the left light-refracting body from the specimen shown in Fig. 2. Mitochondrial folds (*mf*)

Fig. 4. Highly compressed view of a light-refracting body shown in Figs. 2 and 3. Cytoplasm (*cy*), mitochondrion (*mt*), mitochondrial inclusions (*in*), nucleus (*nu*)

Fig. 5. Transverse section (1 µm) through the body of *S. virginianum* at the level indicated by the white dashes in Fig. 2. Epidermis (*ep*), brain neuropile (*bn*), nerve cell perikarya (*np*), neurites (*nv*), body wall muscles (*mu*), locomotory cilia (*cl*), pharynx epithelium (*pe*), pharynx musculature (*px*), refractile granule (*mg*), protonephridium (*pn*)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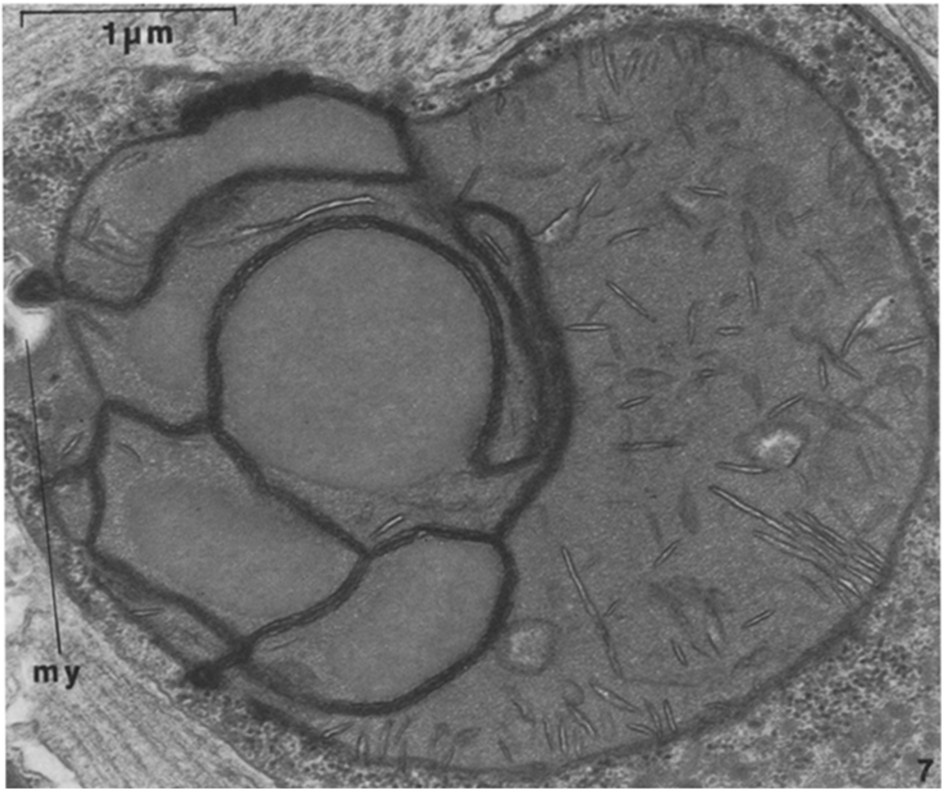
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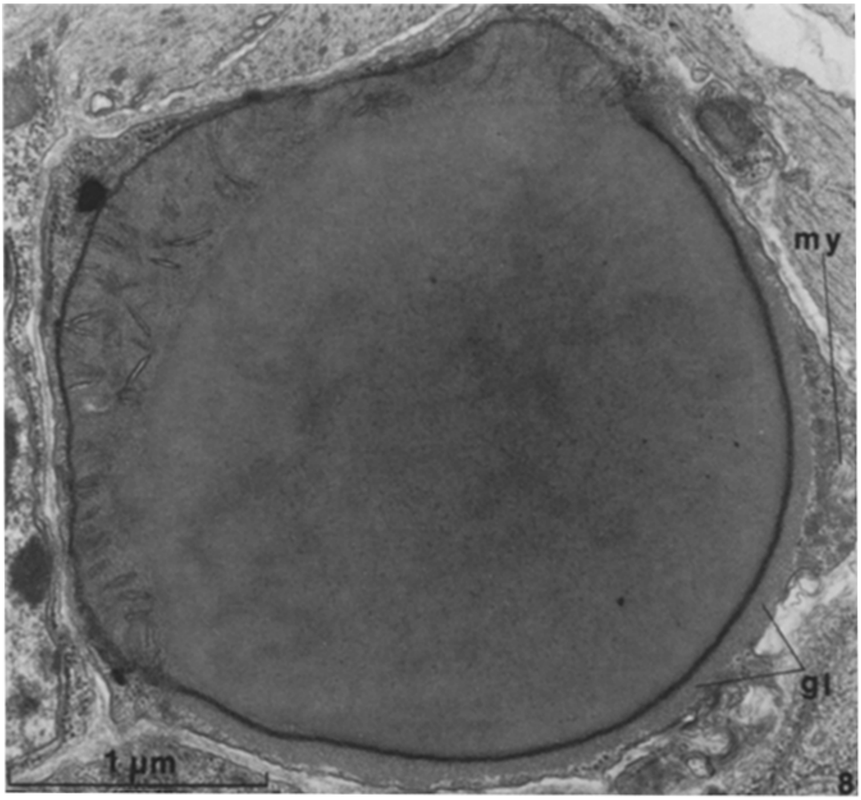

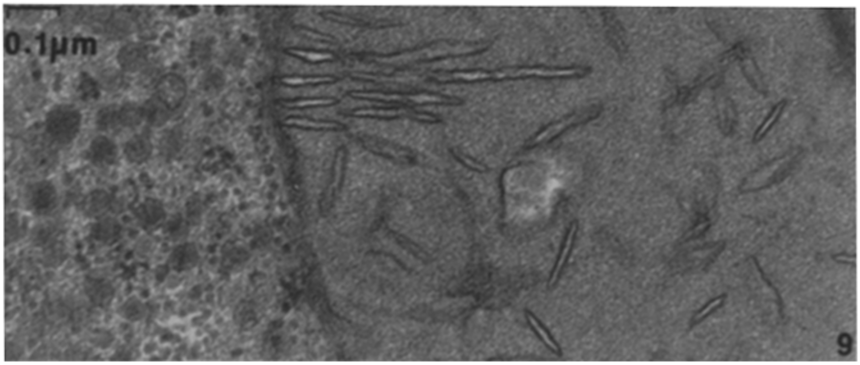

Fig. 8. TEM transverse section through *S. virginianum*, at the level of middle of left giant mitochondrion = refractile granule (oriented as indicated by white dashes in Fig. 2). Intramitochondrial inclusion (*center*), extramitochondrial granular layer (*gl*), myelin figure (*my*)

Fig. 9. TEM section of a part of giant mitochondrion showing cytoplasmic dense-cored vesicles

Fig. 6. TEM transverse section through *S. virginianum* showing left light-refracting granule = giant mitochondrion. The lateral body wall is to the left of the figure, the brain neuropile is to the upper right and the mouth is to the lower right

Fig. 7. Enlargement of the mitochondrion shown in Fig. 6 showing its inclusion and infolding. Myelin figure (*my*)


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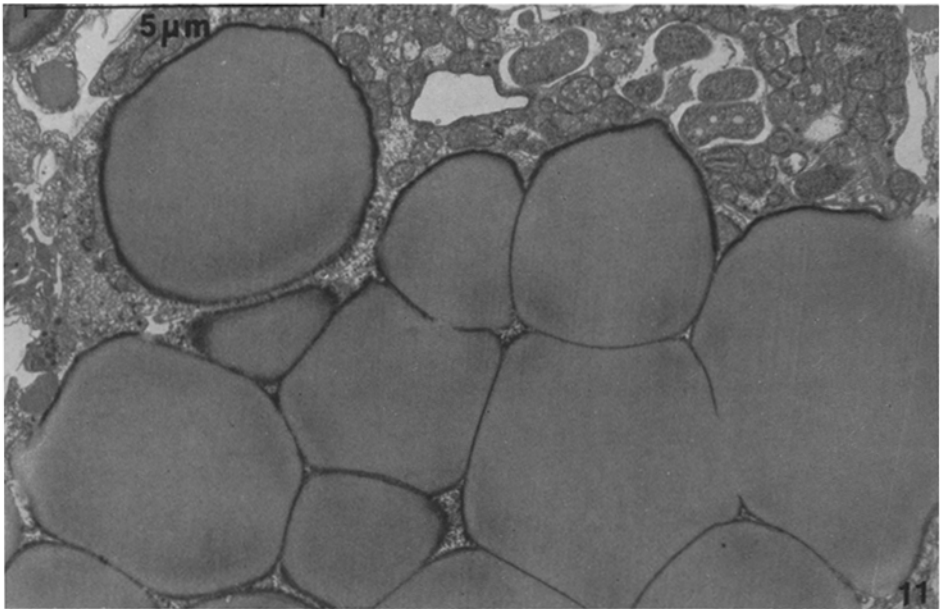
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3. TEM Observations of Dark-Adapted Specimens

The refractile mitochondria of specimens dark-adapted for 7 days showed no measurable changes in the quantity or density of matrix material, the number or appearance of cristae, the density or thickness of the granular extramitochondrial layer, or the degree of infolding at the anterolateral face of the mitochondrion (Fig. 10). Additionally, there was no observable variation in the number or proportion of cytoplasmic components peripheral to the mitochondrion. The sole difference that we observed in each of the two dark-adapted specimens was the appearance of pronounced myelin figures in the cytoplasm of the refractile granule-containing cells. We are reluctant to attribute functional significance to these myelin figures because of their known occurrence in tissues marginally well-fixed. We conclude that an ultrastructural comparison of light-and dark-adapted refractile granules of *S. virginianum* indicates no morphological differences.

4. TEM Observation of the Light-Refracting Bodies of Stenostomum sp.

Stenostomum sp. possesses light-refracting bodies resembling closely those illustrated in Nuttycombe and Waters (1938, Fig. 1A, 'type 1', see introduction to this paper) for *S.tenuicaudatum.* In *S.* sp., the refractile body appears as a 'clear vesicle', in the light microscope, with a cluster of several small, spherical refractile granules on one side of the vesicle. These refractile bodies are found in the posterior lobes of the brain, as in *S. virginianum.*

TEM observation of the refractile bodies of *S.* sp. indicates that the refractile granules may be lipid spheres situated within what we believe to be a single cell. The presumed lipid spheres are numerous and have a maximum diameter of 5 µm. They are in close association with many mitochondria of normal (0.5–1.0 µm) dimensions. The cytoplasm of the cell, or cells, contains dense-cored vesicles (Fig. 11).

D. Discussion

We undertook this project to compare the ultrastructure of catenulid photoreceptors with those known from other platyhelminths (see introduction) and to evaluate the relationship of this singular order to other flatworms. We have demonstrated within the confines of our experiments and observations that *S. virginianum* is not phototactic and that dark-adaptation produces no conformational alterations in the presumed photoreceptors. Such conformational changes would be expected after comparing light and dark-adapted photoreceptoral membranes if the membranes were indeed photoreceptive (Carpenter et al.

Fig. 10. TEM transverse section of a 7-day dark-adapted giant mitochondrion of *S. virginianum*, near anterior end of mitochondrion

Fig. 11. TEM transverse section through a part of the left light-refracting body of *Stenostomum* sp. indicating lipid-like droplets with closely associated mitochondria and cytoplasmic dense-cored vesicles

1974; Bedini et al. 1977; Yamamoto and Yoshida 1978; Röhlich 1968; Eakin and Brandenburger 1979). Consequently, we are unable to draw any conclusions from a comparison of platyhelminth photoreceptors with the refractile bodies of *Stenostomum* except to reaffirm the distinctiveness of the order Catenulida among flatworms (Sterrer and Rieger, 1974; Rieger 1978). We must ask the residual question; if the light-refracting bodies of *S. virginianum* are not photoreceptive, what is their function? We recognize two possibilities.

The light-refracting bodies may be photoreceptive despite the absence of behavioral and experimental-morphological data to support this assumption. The position of the bodies in the brain and their eye-like light microscopic appearance are compelling facts that are not easily disregarded. We have considered that the light-refracting bodies function as photoperiodic receptors, perhaps triggering the onset of sexual reproduction in *Stenostomum*, a genus where asexual division by paratomy is commonplace and where periodic sexual reproduction might be expected (Borkott 1970; Lender 1974; Kaplońska 1967). We have preliminary data for *S. virginianum* cultured in the laboratory on a six hour daylength regime and we have observed eggs in these individuals. The data are not unequivocal, however, because of improper controls on the experiment. Nevertheless, if the refractile bodies are involved in photoperiodic responses, then because of their similarity in position to cerebral eyes in other flatworms and their general eye-like appearance, we would expect them to have evolved from more typical photoreceptors, i.e. ciliary or rhabdomeric photoreceptors (Eakin 1968) that have lost their phototactic or photokinetic function. A comparative investigation of cerebral light-refracting bodies of *Stenostomum* could be made to test this hypothesis.

The light-refracting bodies may be morphologically distinct neurosecretory cells unrelated to photoreceptors. We base this hypothesis on the observation of 60–100 nm dense-cored vesicles in the refractive bodies of *S. virginianum* that agree in size and density with those identified as neurosecretory granules in the brains of *Stenostomum* sp. and *Catenula lemnae* by Moraczewski et al. (1977). We are aware, however, that ultrastructural identification of granules is often inconclusive and we make this comparison cautiously. We are willing to consider that the lipoidal material of the light-refracting bodies of *Stenostomum* sp. (this study, Fig. 11) and the intramitochondrial inclusions of *S. virginianum* could act as substrates for mitochondrial activity indirectly related to the synthesis of neurosecretory vesicles. Large mitochondria have been described associated with neurosecretory cells in invertebrates (Hagadorn 1962) and a close association of mitochondria with their substrates, often lipid, has been recognized previously (Palade 1959).

The function of the light-refracting bodies of *Stenostomum* remains enigmatic despite our elucidation of their detailed structure and after our experimental manipulations. We anticipate additional investigations of these unique structures to further clarify their function.

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