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THE ACTION OF SOME MICROTUBULAR POISONS ON THE RETINOMOTOR RESPONSE OF THE EYES IN *DALYELLIA VIRIDIS (TURBELLARIA RHABDOCOELA)*

Abstract — The effect of some microtubular poisons (colchicine, vinblastine, vincristine) on the retinomotor response of the eyes in the rhabdocoel turbellarian *Dalyellia viridis* was studied. The retinomotor response is characterized, during the dark period, by the displacement of the photosensible segments towards the pigment cup opening; this leaves most of the cavity empty. During light exposure, the photosensible segments again fill all the cavity of the pigment cup. We found that the reinomotor response, in spite of the microtubular damage produced by the poisons, is not altered.

Riassunto — *L'azione di alcuni veleni microtubulari sulla risposta retinomotoria degli occhi di* Dalyellia viridis (Turbellaria Rhabdocoela). È stato studiato l'effetto di alcuni veleni microtubulari (colchicina, vinblastina, vincristina) sulla risposta retinomotoria degli occhi del turbellario rabdocelo *Dalyellia viridis*. Essa è caratterizzata, durante il periodo di buio, da spostamento dei segmenti fotosensibili verso l'apertura della coppa pigmentata, in modo da lasciare vuota buona parte della cavità. Durante il periodo di luce i segmenti fotosensibili riempiono di nuovo la totalità della cavità della coppa pigmentata. È risultato che la risposta retinomotoria, nonostante il danno microtubulare causato dai veleni, non subisce alterazioni.

Key words — Ultrastructure / turbellarian eyes / retinomotor response / microtbular poisons.

INTRODUCTION

In fish, the retinomotor response includes not only pigment migration, but also elongation and contraction of the photoreceptors (AREY, 1915; WALLS, 1942; ALI, 1971, 1975). In the dark, the rod myoids con-

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tract, drawing their outer segment in contact with the outer limiting membrane, while the cone myoids lengthen and move their outer segments sclerally near the melanin granules of the pigmented epithelium. During light adaptation, these processes are reversed. A retinomotor response apparently similar to that of fish rods is present also in the turbellarian rhabdocoel *Dalyellia viridis*. In the dark, the outer segment seems to contract, while following light exposure the outer segment seems to grow in length and to place itself in the cavity of the pigment cup (BEDINI et al., 1977). Over the past few years, the mechanism underlying the retinomotor response in fish has been studied with renewed interest (ALI & CROUZY, 1968; ALI, 1975; Burnside, 1976; WARREN & BURNSIDE, 1968; FERRERO et al., 1979; ANCTIL et al., 1980; KLINE & ALI, 1980; BURNSIDE et al., 1983).

The finding of a retinomotor response similar to that of fish in a turbellarian raises the issue of what is the structural model of non-muscle motility in these lower invertebrates, and what is the mechanism(s) governing such response. Because cell motility is the expression of the properties and interactions of microfilaments, microtubules and ground cytoplasm, we hypothesized that the retinomotor response in *Dalyellia* might result from the interaction between the contraction mediated by myofilaments and the lengthening mediated by microtubules. In the photoreceptors of this turbellarian, both microtubules and microfilaments are present. Our approach was to find out whether microtubular poisons would indice any changes in the retinomotor response. The putative damage caused by microtubular poisons would be particularly evident during light exposure because the ability of the photoreceptor to lengthen would be impaired. It is well known that microtubular poisons prevent the formation of microtubules by binding to the tubuline dimers present in the cytoplasm. During the dark period, on the other hand, microtubular depolimerization would not be altered by the poison effect.

MATERIALS AND METHODS

All *Dalyellia viridis* were adapted to light-dark cicle. Specimens of the turbellarian were maintained in dishes containing 0.01% colchicine or 0.01% vinblastine or 0.01% vincristine in 5 ml of water. Animals were kept in the dishes for 12 hours, during which time they were dark adapted and then exposed to light for 3 hours. Control animals were

maintained in dishes containing water alone. Animals were sacrificed before light exposure or after 3 hours of light. Fixation was performed in 1% osmium tetroxide in 0.13 M phosphate buffer, pH 7.4. Following dehydration, the specimens were included in Epon 812-Araldite mixture according to standard procedures. Ultrathin sections, stained with both solutions of uranyl acetate and lead cytrate, were examined using a Siemens Elmiskop 1A electron miscroscope.

Results

The eyes of *Dalyellia viridis* are made up of a monocellular, bilobed pigment cup in which the photosensible segments of two photoreceptor cells are present. During light exposure, the photosensible segments totally fill the pigment cup cavity. In the dark, they shift toward the cup opening, leaving almost all the cavity empty. The transition from dark to light causes, over a 10 min time period, the re-entry of photosensible segments into the pigment cup, while the reverse transition does not provoke a quick shift back to the cup opening. The latter event does occur but requires a longer time (6 - 9 hours).

The control specimens of *Dalyellia viridis* fixed during the dark period showed partially empty pigment cups (Fig. 1); the photosensible segments were shifted toward the cup opening, which appeared to be collapsed. The controls fixed following light exposure showed the inside of the cup filled up with photosensible segments of the lens process, without empty areas (Fig. 2). The cytoplasm of the phtoreceptors fixed in the light showed numerous microtubules in longitudinal array (Fig. 3). In the treated animals, neither colchicine nor vinblastine nor vincristine were capable of disrupting the retinomotor response. Thus, after 12 hours of dark, the microvilli of the photosensible processes were located near the pigment cup opening (Fig. 4), while after 3 hours of light they had returned into the cup cavity (Fig. 5). This is the opposite of what would be expected were the microtubules responsible for the elongation of the photosensible segments. In fact, the microtubules were never present in the cytoplasm of the photoreceptor (Fig. 6). The action of the microtubular poisons on the microtubular structures was also visible in the other cells of the animals. Of note were that the cytoplasm of the photoreceptor cells of treated animals presented clearly abnormal structures such as large vacuoles and glycogen clusters (Fig. 7).

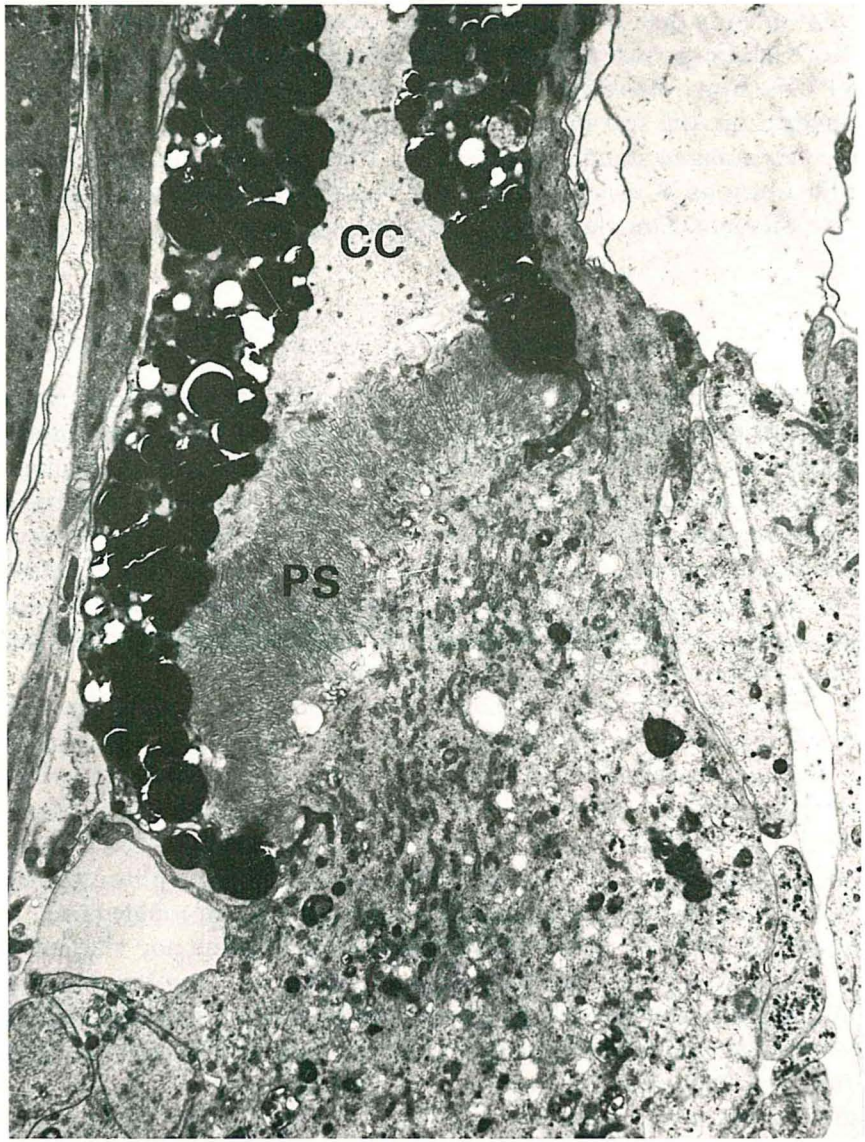

Fig. 1 - Control specimen. Fixation during the dark period. Note the photosensible segment (PS), shifted towards the cup opening, the cavity of which (CC) appears empty and collapsed. × 3000.

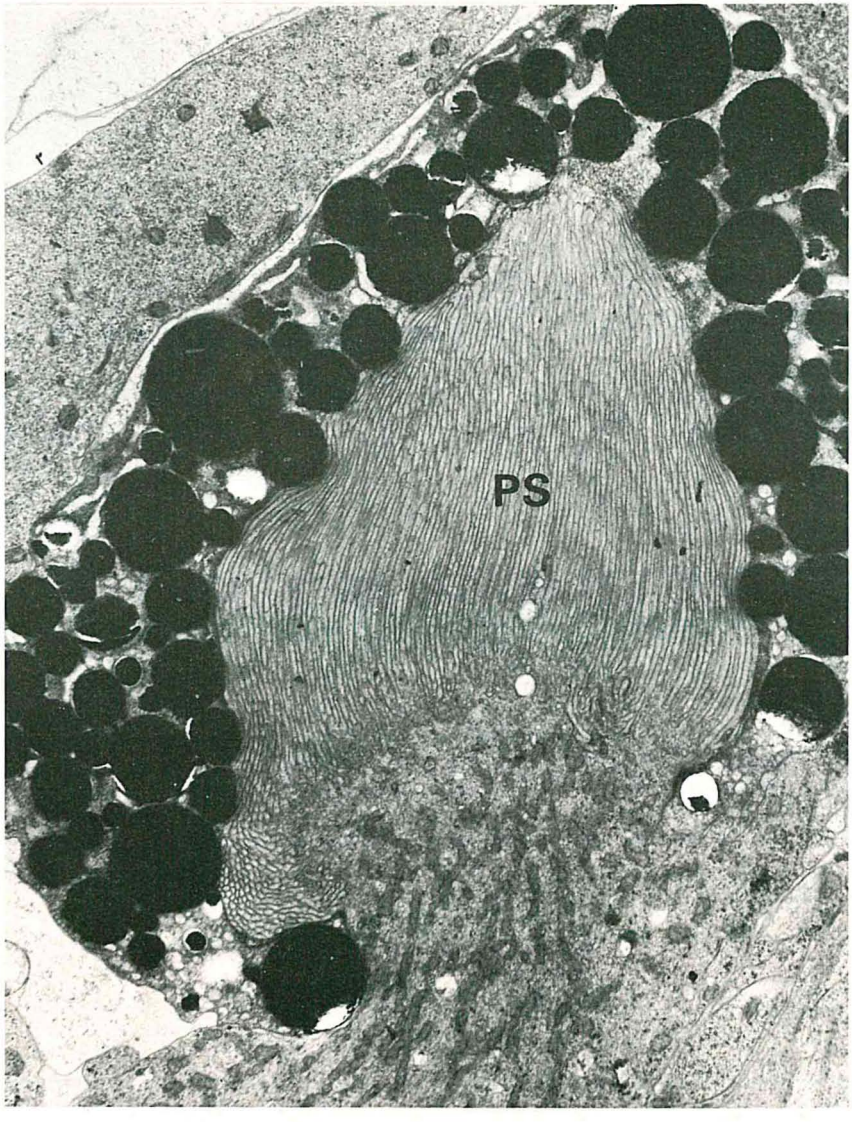

Fig. 2 - Control specimen. Fixation after light exposure. Note the photosensible segment (PS) filling up the cup cavity. × 5000.

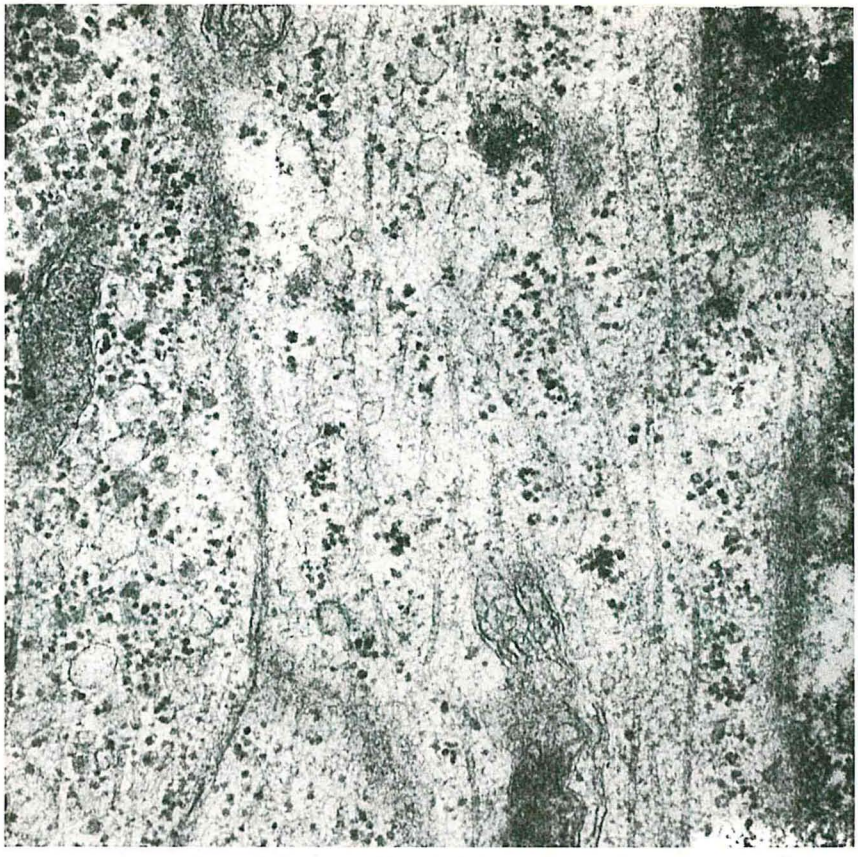

Fig. 3 - Control specimen. Fixation after light exposure. Cytoplasm underlying the microvillar border with numerous microtubules. × 44000.

Discussion

The lack of effect of microtubular poisons on the retinomotor response of light-adapted photoreceptors supports the conclusion that this response is not mediated by the photoreceptor or pigmented cell microtubules, since polimerization of the tubuline dimers is abolished by this class of poisons. Recently, however, Kline & Ali (1980), have re-examined the distribution of microtubules in the pigmented epithelial cells, and the movements of the retinomotor response in the retina of dark/light adapted trouts that had been treated with colchicine or vinblastine. These Authors have shown that this poisons partially

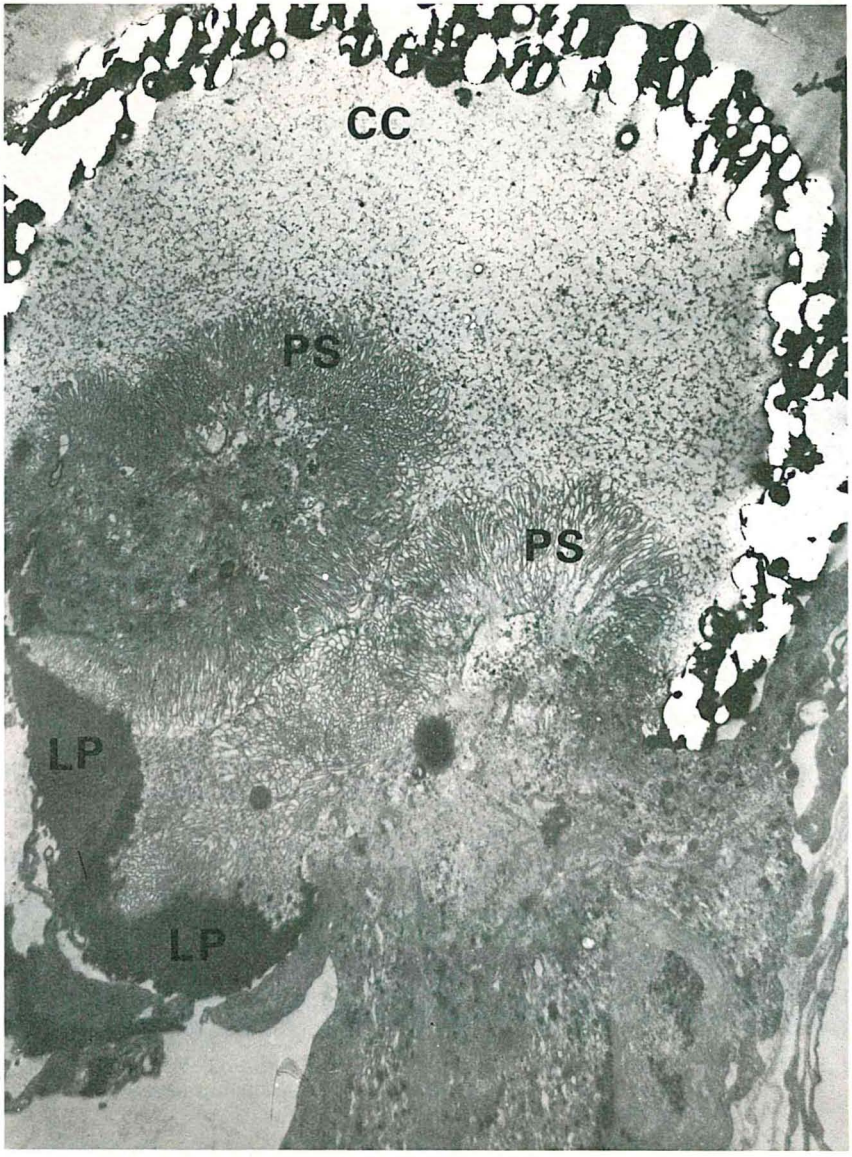

Fig. 4 - Treated specimen. Fixation during the dark period. Photosensible segments (PS) shifted towards the opening of the pigment cup, the cavity of which (CC) appears empty. Note the lens process (LP) located outside the cup. × 2000.

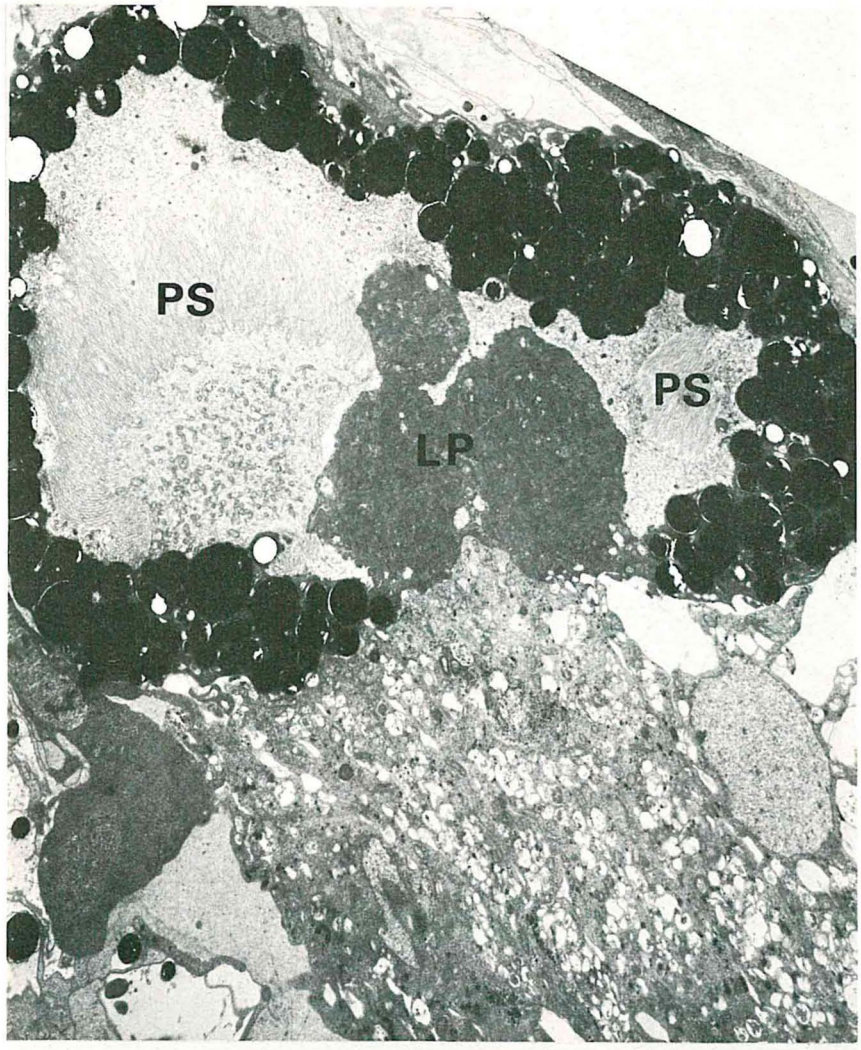

Fig. 5 - Treated specimen. Fixation after light exposure. Photosensible segments (PS) and lens process (LP) located inside the pigment cup. Note the numerous vacuoles present in the cytoplasm. × 2000.

disrupt the photomechanical response. The microtubules appear disassembled and/or at different stages of depolimerization both in the cones an in the rods. KLINE & ALI have therefore proposed that the lack of effect of microtubular poisons is due to the fact that, during contraction, the cones squeeze the rods sclerally; this results in a struc-

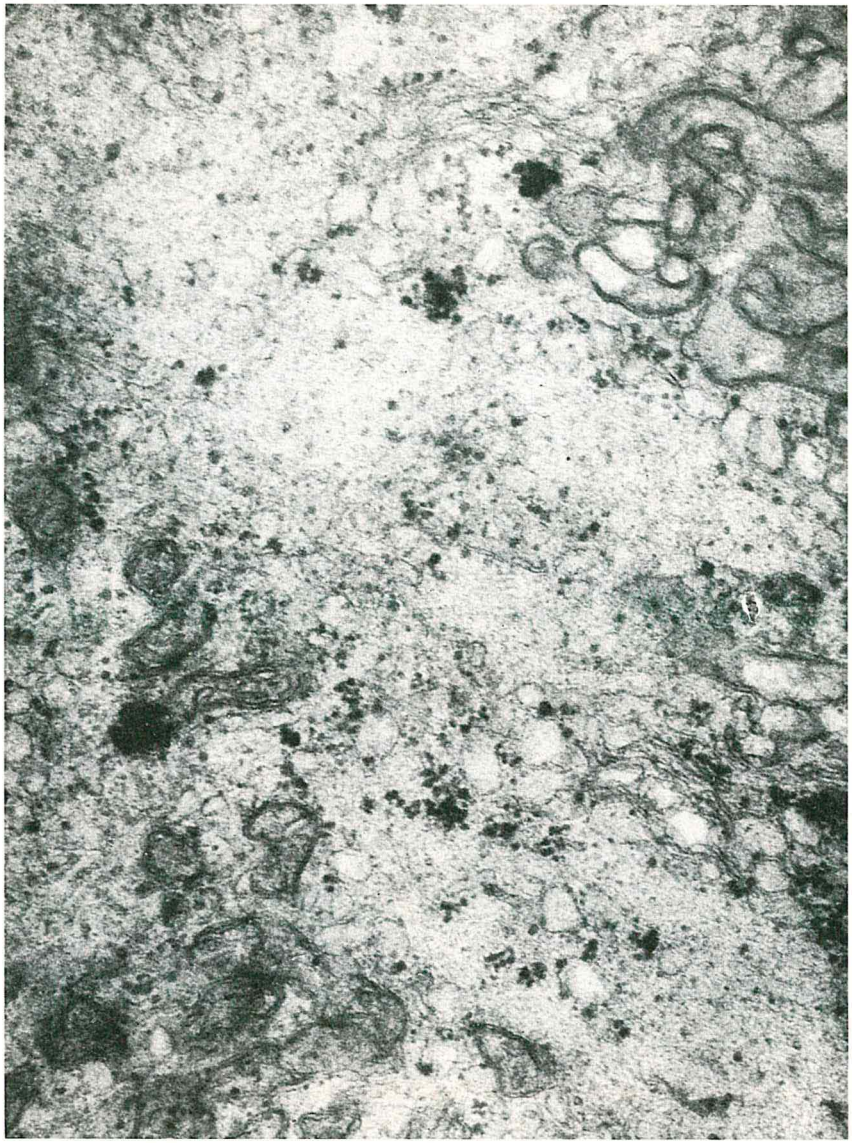

Fig. 6 - Treated specimen. Fixation after light exposure. Note the lack of microtubules in the cytoplasm underlying the photosensible segment. × 44000.

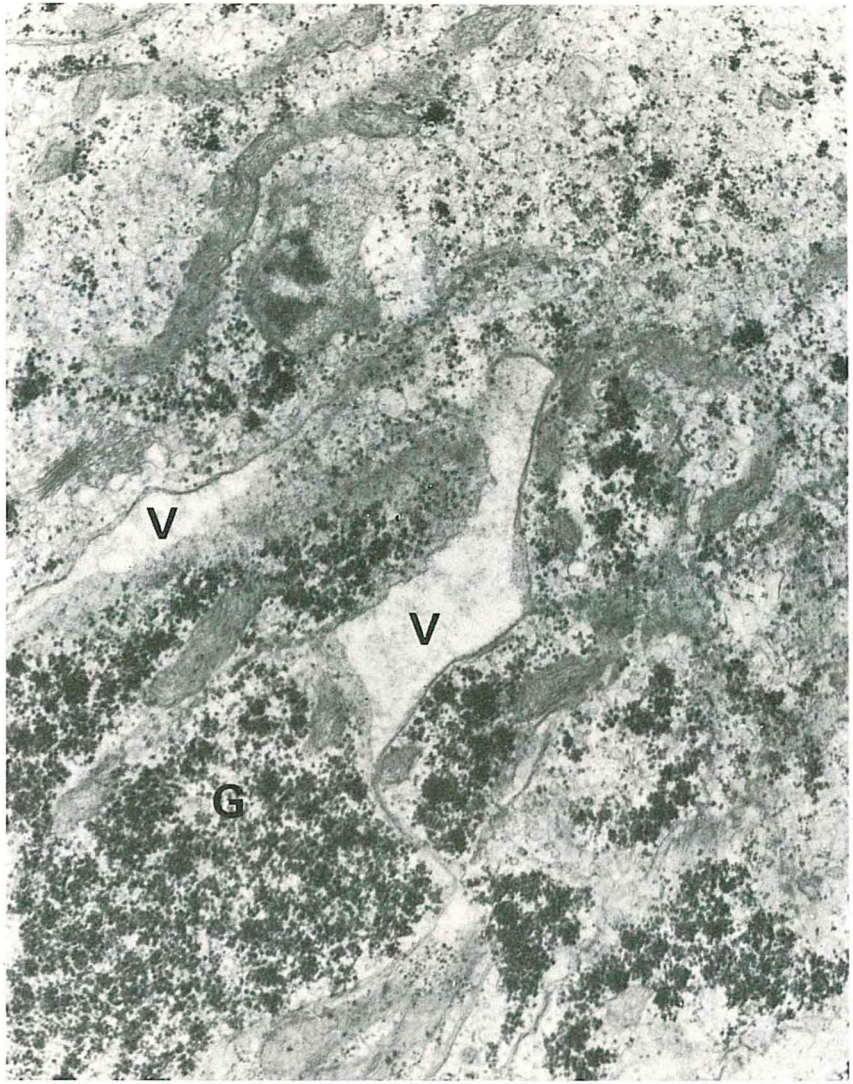

Fig. 7 - Treated specimen. Fixation after light exposure. Cytoplasm of photoreceptor showing glycogen clusters (G) and large vacuoles (V). × 36000.

ture which retains some resemblance to the light adapted retina. Such mechanism is not possible in the eye of Dalyelliidae because of their simpler morphology. Since, in these turbellarians, the retinomotor response to light is very quick whereas the response to dark is much

slower, it is possible that light exposure acts through the release of some substance. This putative chemical mediator could be secreted by the neurosecretory cell, which is in contact with the lens process. The neurosecretory cell could trigger the retinomotor response to light by quickly delivering pre-formed mediator. Upon returning to dark, the retinomotor response is slowly turned off as the mediator is metabolized. Which cellular elements, whether the microfilaments or other structures in the photoreceptor and/or in the pigment cell, would be the target of such neurosecretory mediator remains to be established.

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