Atti Soc. Tosc. Sci. Nat., Mem., Serie B, **91** (1984)
pagg. 161-171, figg. 6, tab. 1.

A. LANFRANCHI (*), C. BEDINI (*), E. FERRERO (**)

TRITIATED LEUCINE INCORPORATION INTO THE EYES OF *DALYELLIA VIRIDIS* (TURBELLARIA RHABDOCOELA)

Abstract — The mechanism of shedding and renewal of the photosensible membranes in the eyes of the rhabdocoel turbellarian *Dalyellia viridis* was studied with the use of tritiated leucine. Nine hr after administration, the label is first seen in the photoreceptor cytoplasm, while later on it migrates to the microvilli. In the microvilli, the amount of labeling increases steadily over 48 hr; in contrast, in the cytoplasm after an initial rise it declines significantly. The labeling of the pigmented cup reaches a maximal value, and then remains constant with time. An apparently continuous turnover of the photosensible membranes, in which the pigmented cup could also participate, has been evidentiated.

Riassunto — *Incorporazione di leucina tritiata negli occhi di* Dalyellia viridis *(Turbellaria Rhabdocoela).* È stato studiato il meccanismo di distruzione e ricostituzione delle membrane fotosensibili negli occhi del turbellario rabdocelo *Dalyellia viridis*, dopo somministrazione di leucina tritiata. È risultato che la marcatura compare per la prima volta nel citoplasma del fotorecettore dopo 9 ore dalla somministrazione e si sposta successivamente sui microvilli. Mentre sui microvilli la percentuale di marcatura aumenta, nel citoplasma, dopo una crescita iniziale, subisce una flessione, ma non viene mai meno. La marcatura della coppa pigmentata, raggiunto un valore massimo, rimane costante nel tempo. È stato evidenziato un turnover apparentemente continuo delle membrane fotosensibili, alla cui ricostituzione potrebbe partecipare anche la coppa pigmentata.

Key words — Ultrastructure / turbellarian eye / photosensible membrane labeling.

INTRODUCTION

Research on vertebrate eyes with the use of autoradiographic techniques have clarified the shedding and renewal process of the rod

(*) Istituto di Biologia Generale dell'Università di Pisa, Via A. Volta, 6 - 56100 Pisa.
(**) Dipartimento di Biologia dell'Università di Trieste, Via A. Valerio, 32/34 - 34127 Trieste.

and cone outer segment (YOUNG, 1974, 1976). In invertebrates, studies on microvillar membrane turnover are based on the assumption that all photoreceptors turn over their photosensible membranes. The mechanisms of membrane shedding are inferred from the examination of the ultrastructural variations of the rhabdomeres and cellular organelles during the circadian cycle. However, this kind of observations cannot provide detailed, quantitative information on membrane turnover. In fact, data on the renewal of the photosensible processes obtained after tracer administration are very few (PERRELET, 1972; KRAUHS et al., 1976).

In turbellarians, morphological studies on the planarian eyes have shown that smooth endoplasmic reticulum components are necessary for the renewal and organization of the microvillar membranes, and that the mobilization of such components is light-dependent (RHÖLICH & TAR, 1968). In previous studies in rhabdocoels (BEDINI et al., 1977), we have analyzed the morphological variations of the photoreceptive segment during the light-dark cycle. Modifications occurring at the base of the microvilli have been observed after light exposure and have been imputed to a resorption process. The maximal microvillar disarray appears 12 hr after light exposure, and is maintained throughout the subsequent dark period. Quick microvillar re-organization occurs within a few minutes of light exposure. This suggests that the cell uses pre-formed material. These observations also are compatible with the existence of a daily rhythm of photoreceptive membrane shedding and renewal in turbellarians. To directly test this possibility, we have used a labeled substrate to follow the extent and time-course of photoreceptive membrane shedding and renewal.

MATERIALS AND METHODS

Specimens of *Dalyellia viridis* were maintained under normal light-dark cycle conditions. For the experiment, dishes each containing six animals were used. The dishes were provided with 1 ml of fresh water, obtained from the animal living area, to which 40 μCi of tritiated leucine were added. Tritiated leucine, purchased from the Radiochemical Centre, Amersham, England, had a specific activity of 57 Ci/mM. The animals were sacrificed 2, 6, 9, 12, 24, 36, or 48 hr after their immersion into the dishes containing the label. They were fixed 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, deposited on 150 - mesh cop-

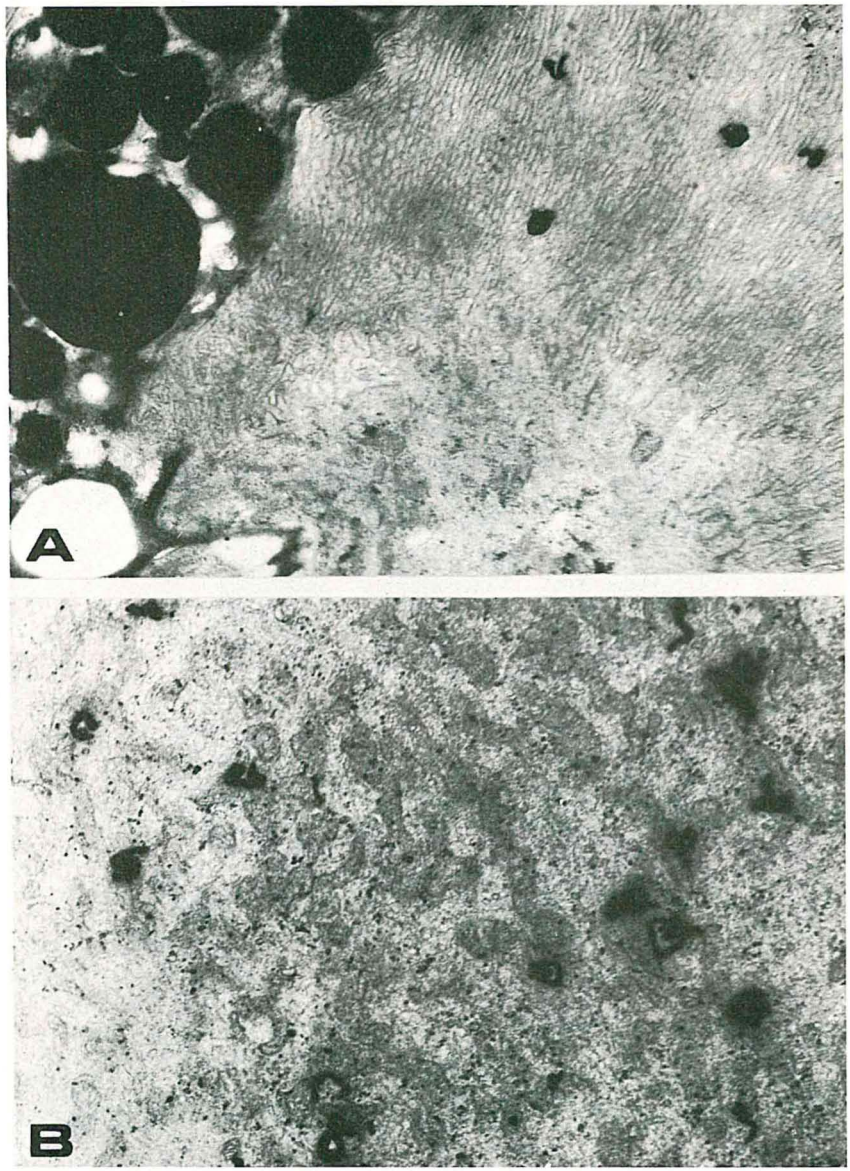

Fig. 1 - Autoradiography of *Dalyellia* eyes 12 hr after treatment with tritiated leucine. A. Few grains are present over the microvilli. × 10,000; B. Numerous grains are associated with the peripheral cytoplasm. × 14,000.

per grids and covered with a film of Ilford L4 emulsion with the loop technique, were exposed for 4-8 wk in the dark. The emulsion was developed in Kodak Microdol X and fixed in Kodak Unifix. The grids were stained with lead citrate and examined under a Siemens Elmiskop 1A electron microscope. Photographic prints, at 10,000 magnifications, were used for counting the autoradiographic grains associated with the different cell components. The radioactivity was expressed as grains per unit area. Data are given as the mean ± standard error.

Results

The autoradiographs taken at different times after the administration of tritiated leucine showed that radioactivity in the photorecep-

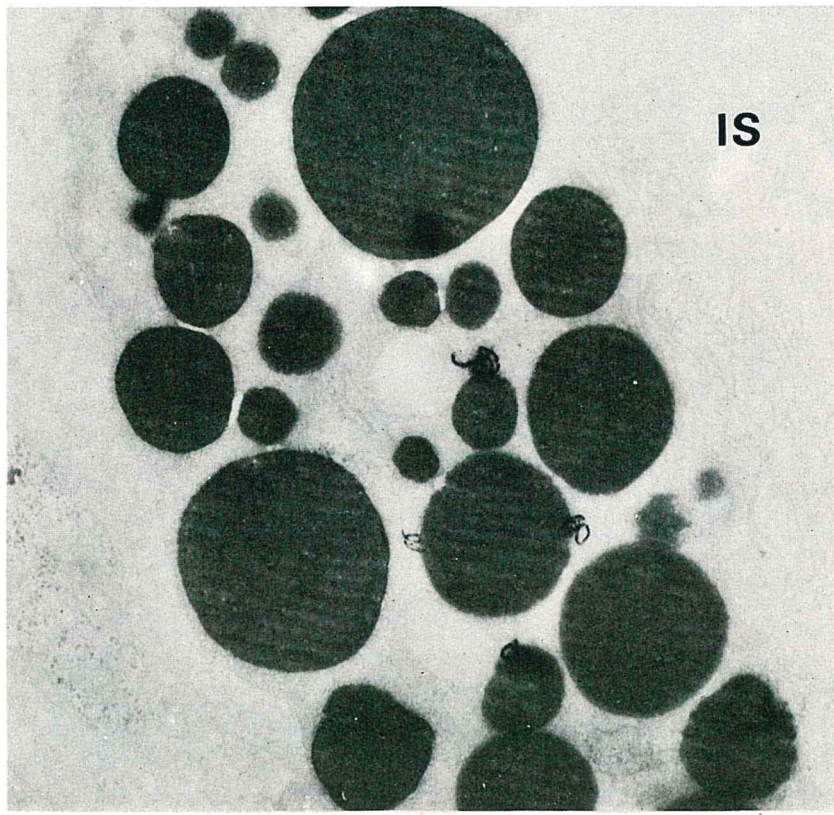

Fig. 2 - Eye 12 hr after tritiated leucine treatment. The grains can be seen over the pigmented cup while the space interposed (IS) between photoreceptor cells and pigmented cup is free of labeling. × 10,000.

tors was still undetectable after 2-6 hr, but appeared in the cytoplasm of the visual cells after 9 hr. The autoradiographic grains appeared to be scattered all over the cytoplasm, while the microvilli were entirely free of radioactivity. Also the pigmented cup and the cells surrounding the eye showed only rare grains of labeling.

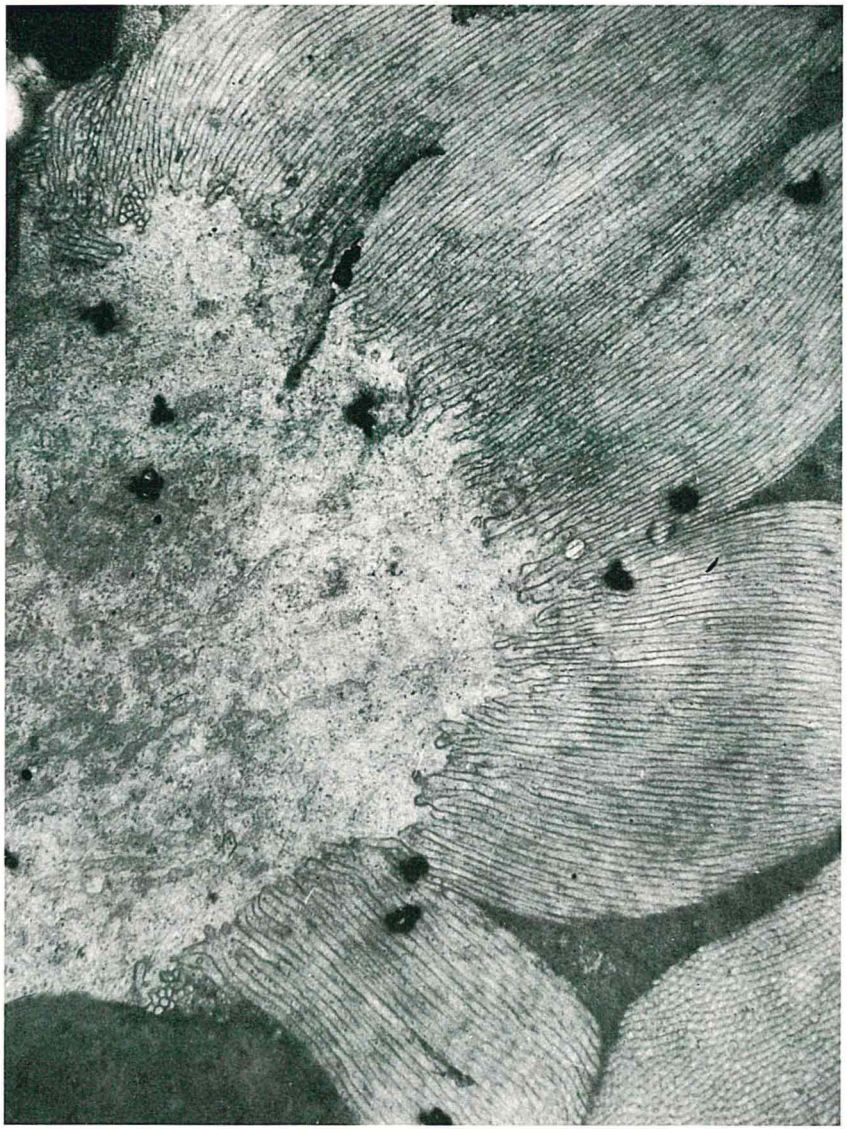

Fig. 3 - Eye 24 hr after tritiated leucine treatment. Note a weak increase in labeling on the microvilli. × 10,000.

Twelve hr after tracer addition, labeling was noted in the cytoplasm and in the microvillar regions (Fig. 1 A, B). The space between the photoreceptors and the pigmented cup presented no labeling, while the cup itself bore labeled areas both over granules and in the cytoplasm (Fig. 2).

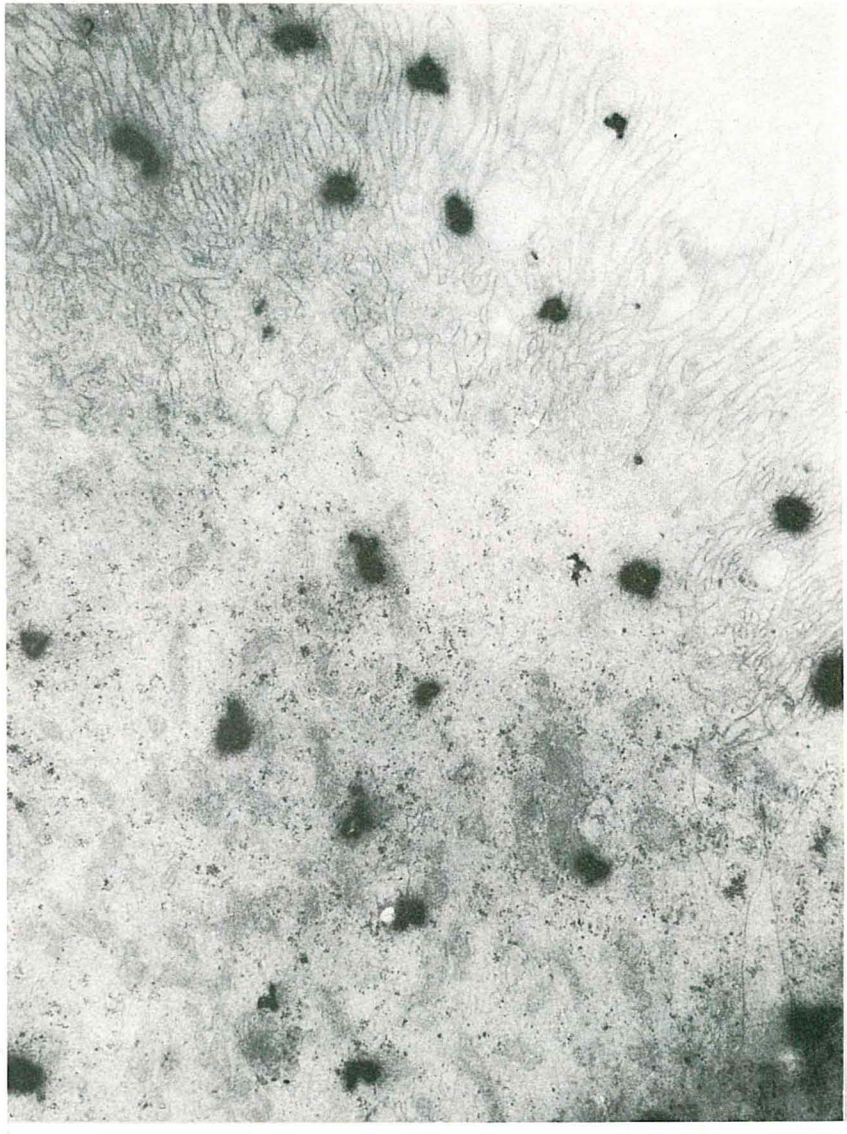

Fig. 4 - Eye 36 hr after tritiated leucine treatment. Note increased radioactivity on the microvilli and the pararhabdomeric cytoplasm. × 10,000.

Twenty-four hr after tritiated leucine treatment, the photoreceptors localized in the pigment cup presented labeling both over the cytoplasm and in the microvillar regions (Fig. 3). In these latter, silver grains were significantly more numerous than at 12 hr. Over the pigmented cup, radioactivity reached the value of 35% of total tracer concentration, and remained constant in time.

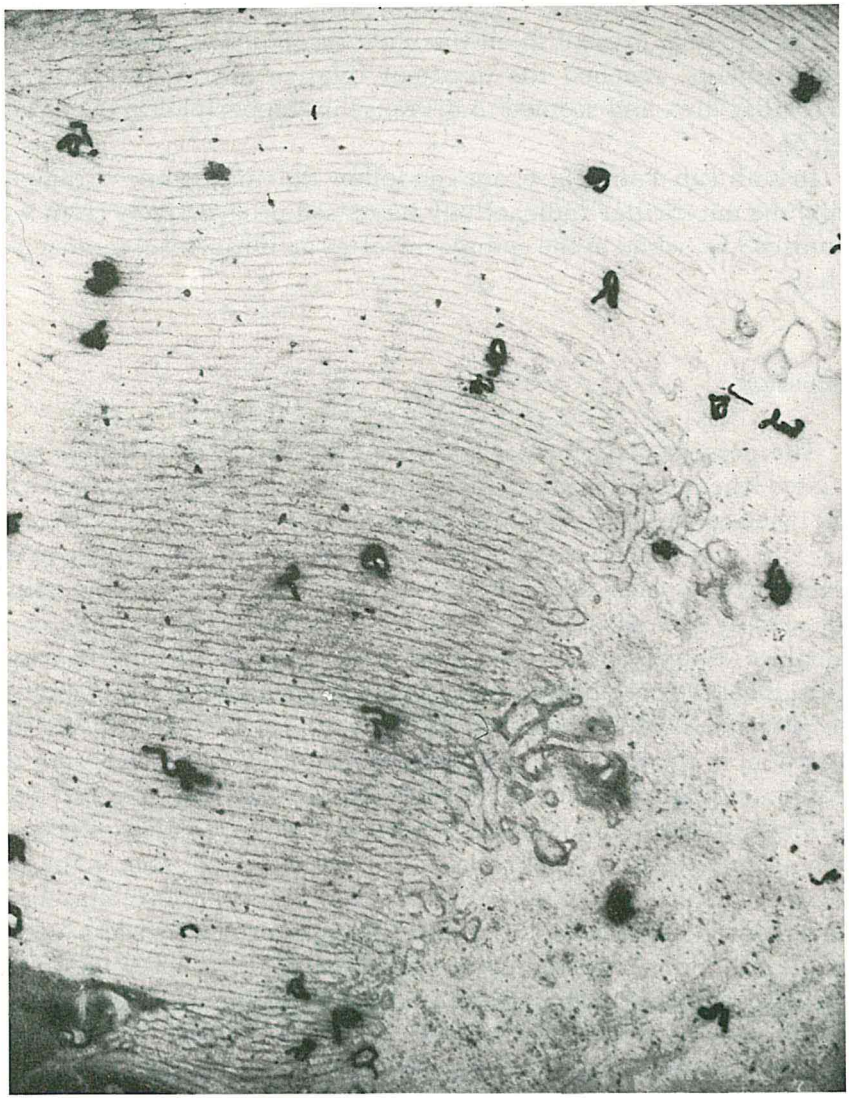

Fig. 5 - Eye 48 hr after tritiated leucine treatment. The labeling is still increased on the microvillar areas. × 10,000.

Thirty-six hr after treatment, the animals (who had been exposed to 12 hr dark + 12 hr light + 12 hr dark) presented eyes with the photosensible segments displaced towards the cup opening, which was characterized by disorganized microvilli. The labeling increased both over the microvilli and in the pararhabdomeric cytoplasm (Fig. 4).

Forty-eight hr after the immersion in tritiated leucine (12 hr dark + 12 hr light + 12 hr dark + 12 hr light), the general picture had changed. The labeling, present in all the photoreceptor and pigmented cup compartments, reached the maximal relative concentration in the microvillar area and seemed to decrease in the peripheral cytoplasm (Fig. 5).

In both Tab. I and Fig. 6, one can follow the time-course of radioactivity: the microvillar radioactivity increased progressively from 9-12 hr until 48 hr, while in the cytoplasm, after an initial rise, radioactivity fell.

Discussion

The present experiments show that 2-6 hr after treatment with tritiated leucine neither the cytoplasm nor the photoreceptor microvilli have taken up any label. The first appearance of radioactivity is detected after 9 hr, and is predominantly localized, with only few grains, in the peripheral cytoplasm of the photoreceptors. Over the following hours, radioactivity increases and also appears on the microvilli. It is therefore logical to think that radioactivity first reaches the endoplasmic reticulum of the peripheral cytoplasm, and then moves to the microvilli. The persistence of radioactivity in the cytoplasm, which masks this migration, is clearly due to the continuous uptake of tracer from the medium. A single front of new synthesis, moving from one cell compartment to another (such as is shown in vertebrate photoreceptors after a single pulse of tritiated leucine), is therefore not seen in the present experiments. Moreover, proteins other than the microvillar membranes incorporate the label. In fact, the pigment cell presents a constant, high level of radioactivity. An increase in the labeling on the microvilli is visible from 24 to 48 hr (Tab. I and Fig. 6). This is accompanied by a simultaneous decrease in cytoplasm radioactivity, although here silver grains remain abundant because of the continuous apposition of new label. Our results are similar to those of Perrelet (1972) on visual receptors of the honeybee drone, and those of Young and Droz

TABLE I

Distribution of autoradiographic grains over eye cell components

	% of autoradiografhic grains SEM			
Time after administration	12 hr	24 hr	36 hr	48 hr
Microvilli	16.94 ± 2.90	16.23 ± 1.11	18.53 ± 2.45	25.97 ± 4.42
Peripheral cytoplasm	33.56 ± 4.01	42.27 ± 1.59	36.73 ± 3.72	29.63 ± 5.49
Pararhabdomeric cytoplasm	10.92 ± 2.97	5.97 ± 1.25	7.60 ± 1.47	9.94 ± 1.87
Pigmented cup	34.60 ± 2.20	35.53 ± 0.11	31.10 ± 1.25	34.46 ± 6.47
Interposed space	3.96 ± 1.78		7.04 ± 1.25	
No. of grains	590	1260	2065	2707

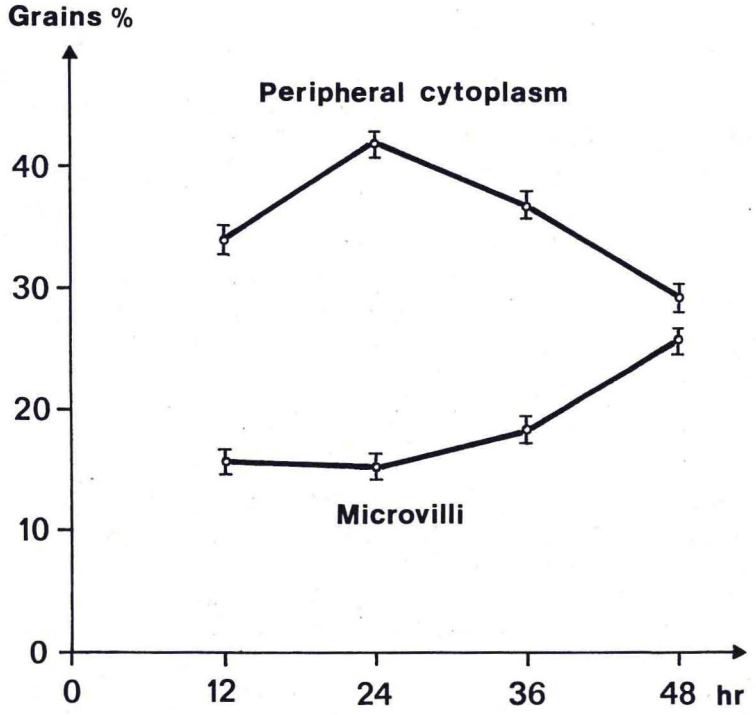

Fig. 6 - Graph showing the time-course of radioactivity on the peripheral cytoplasm and in the microvilli on E.M. autoradiographs.

(1968) in the frog retina. In vertebrates, the labeling pattern of the outer segment of the cones is different from that of the rods (YOUNG, 1969). In the former, the labeling is diffuse whereas in the latter it is in the form of a band which moves along the length of the outer segment. The labeling of the microvilli in *Dalyellia viridis* resembles the cone pattern. Nevertheless, unlike rods and cones (YOUNG, 1978), in *Dalyellia viridis* a daily cycle does not seem to be present. Instead, a continuous turnover of the membranes appears to take place. In *Dalyellia,* the photosensible segment probably reabsorbs its own membranes at the microvillar border by means of pinocytotic vesicles, which penetrate into the inner cytoplasm of the photoreceptor. This sequence is in keeping with the presence of constant and equal radioactivity levels both in the light and in the dark, thereby masking the daily cycle. In addition, it is likely that the pigmented cup participates in the breakdown and renewal of microvillar membranes. Thus, we have observed that, in the dark, the space left free from the photoreceptor microvilli is occupied by flocculent and fibrous material, probably the product of the breakdown of microvillar membranes. This material could be subsequently reabsorbed by the pigment cell. In this case, the radioactivity found in the pigment cell would originate in part from the transfer of material from the photoreceptors to the cup, and in part from the incorporation of labeled precursors of the pigment granules. Morphological observations on the eye of other invertebrates have indicated that microvillar membrane phagocytosis from surrounding pigment cell is possible (BRANDENBURGER and EAKIN, 1980). The material taken up by pigment cells would be metabolized and released, by a process of exocytosis, to the photoreceptor, which would then recycle it for the *de novo* synthesis of membranes.

REFERENCES

BEDINI C., FERRERO E., LANFRANCHI A. (1977) - Fine structural changes induced by circadian light-dark cycle in photoreceptors of Dalyelliidae (Turbellaria: Rhabdocoela). *J. Ultr. Res.*, **58**, 66-77.

BRANDENBURGER J.L., EAKIN R.M. (1980) - Cytochemical localization of phosphatase in ocelli of the seastar *Patiria miniata* during recycling of photoreceptoral membranes. *J. Exp. Zool.*, **214**, 127-140.

KRAUHS J.M., MAHLER H.R., MINKLER G., MOORE W.J. (1976) - Synthesis and degradation of protein of visual receptor membranes in lateral eyes of *Limulus. J. Neurochem.*, **26**, 281-283.

PERRELET A. (1972) - Protein synthesis in the visual cells of the honeybee drone as studied with electron microscope radioautography. *J. Cell Biol.*, **55**, 595-605.

RÖHLICH P., TAR E. (1968) - The effect of prolonged light-deprivation on the fine structure of planarian photoreceptors. *Z. Zellforsch. Mikrosk. Anat.*, **90**, 507-518.

YOUNG R.W. (1974) - Biogenesis and renewal of visual cell outer segment membranes. *Exp. Eye Res.*, **18**, 215-223.

YOUNG R.W. (1976) - Visual cells and the concept of renewal. *Invest. Ophtalmol.*, **15**, 700-725.

YOUNG R.W. (1978) - Visual cell, daily rhythms and vision research. *Vision Res.*, **18**, 573-578.

YOUNG R.W., DROZ B. (1968) - The renewal of protein in retinal rods and cones. *J. Cell Biol.*, **39**, 169-184.

(ms. pres. l'11 ottobre 1984; ult. bozze il 14 dicembre 1984)