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RETINAL DAMAGE AND SENSITIVITY LOSS OF A LIGHT-SENSITIVE CRUSTACEAN COMPOUND EYE (*CIROLANA BOREALIS*): ELECTRON MICROSCOPY AND ELECTROPHYSIOLOGY

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SUMMARY

1. The compound eye of the deep-water-living crustacean *Cirolana borealis* has been exposed to measured amounts of white light, and the effects have been analysed by electron microscopy and electrophysiology (ERG).

2. The threshold for damage of the retinula cells lies between 117 lx ($0{\cdot}47\ W\ m^{-2}$) and 1250 lx ($4{\cdot}9\ W\ m^{-2}$). With daylight exposures of more than $70\ W\ m^{-2}$, there is severe structural derangement and the amplitude of the electroretinogram (ERG) is abolished.

3. No recovery of the retinula cell organization or of the ERG occurs after daylight exposure and a dark period of up to 5 days.

4. A novel type of photoreceptor membrane shedding is described for both dark-adapted and light-exposed eyes.

5. Hence, morphologically and functionally, the *Cirolana* eye is strictly adapted to a dim-light environment and is destroyed by too intense illumination.

INTRODUCTION

The compound eyes of some animals are adapted to dim light. These scotopic eyes have lenses with large acceptance angles (Land, 1981; Nilsson & Nilsson, 1981; Stavenga, 1979) and may contain reflecting layers (tapeta) in crustaceans (for review, see Hallberg, 1978) and other invertebrates (Land, 1972, 1981; Miller, 1979). A tapetum is considered to be an adaptation to ensure an efficient photon capture (Snyder, 1977; Snyder, Laughlin & Stavenga, 1977). In some deep-sea crustaceans the area of the photoreceptor membranes is enlarged, and thus the rhabdoms appear hypertrophied (Elofsson & Hallberg, 1977; Hallberg, Nilsson & Elofsson, 1980; Meyer-Rochow, 1981; Nilsson, 1982, 1983). These morphological adaptations also include an eye sensitivity that can cope with the dim light (Donner, 1971; Lindström & Nilsson, 1983).

Key words: Compound eye, Crustacea, retinal damage.

The deep-sea-adapted eyes have been shown to be vulnerable to excess light. Even moderate amounts of natural daylight or artificial light cause the photoreceptor membranes to break down (Loew, 1976; Meyer-Rochow, 1981; Nilsson, 1982; Tuurala & Lehtinen, 1971). A similar photo-induced destruction has also been observed in *Limulus* (Behrens & Krebs, 1976), in visual mutants of *Drosophila* (Cosens & Perry, 1972; Harris & Stark, 1977), and *Procambarus* (Kong & Goldsmith, 1977), and in a number of vertebrates (for references, see Williams & Baker, 1980).

A correlation between the morphology of the retina and the sensitivity of the eye has been established in the vertebrate eye (Kuwabara, 1970; Lawwill, 1973*a*,*b*; Noell, Walker, Kang & Berman, 1966). The present investigation was undertaken to determine the effects of measured light exposures upon morphology and sensitivity of the eye of the deep-water-living crustacean *Cirolana borealis*. The results show that there is a correlation between these effects and that normal eye function is abolished by too strong illumination.

MATERIALS AND METHODS

Cirolana borealis Lilljeborg (Crustacea: Isopoda) were obtained at night in baited traps from approximately 100-m depth in Raunefjord, south of Bergen, Norway, and sorted in less than 10 min under red safety light. They were shipped by air to the Tvärminne Zoological Station, Finland, where the experiments were performed. The well-fed animals (by the bait) were transferred to 500 ml glass beakers (Jena), filled with approximately 3 cm of water from the sampling station, and with two or three animals in each beaker. These were kept in a 3 °C cooling chamber in a wide light-tight box in a coordinate system, allowing for easy identification of the beakers in the dark. The animals were maintained without food. All handling of the animals took place in the dark using infrared (i.r.) light and i.r. image converters (Find-R-Scope, FJW Industries). The animals were dark-adapted 1–7 days prior to the experiments, and during the experiments the animals were exposed to the light intensities presented in Table 1.

Table 1. *Light exposures of various intensities and durations of white light to the compound eyes of* Cirolana borealis

	Light exposure (LE)				Experiment no. 6 (expt 6)		
Experiment no.	LE Intensity lx ($W m^{-2}$)	Exposure time (min)	Number of animals n_A	Number of animals n_E	Recovery in darkness (h)	n_A	n_E
1	Dark-adapted	–	6	19*	–	–	–
2	117 (0·47)	10, 60	7	3	1, 12	2	2
3	1250 (4·9)	10, 60	–	–	6, 12, 24	4	7
4	2500 (9·8)	10, 60	2	1	2, 6, 12, 24	12	11
5	Daylight (70–136) (≥ 7500 lx)	10–240	13	4	24 h; 2, 3 and 5 days	4	6

In order to study recovery, some animals from experiments nos 2–5 were left to dark-adapt after the light exposures and prior to the histological fixation and the electroretinogram (ERG) measurements (expt 6).
n_A = number of animals fixed for histology, n_E = number of animals sacrificed for ERG measurements.
* See also Lindström & Nilsson (1983).

After light exposure one batch of animals ($N = 50$) was used for histological fixation of the eyes, for light and electron microscopy; and another batch ($N = 53$), for electrophysiological measurements (electroretinogram, ERG).

The following experiments were performed

Expt 1

No light exposure.

Expt 2

($0{\cdot}47\ W\ m^{-2}$). The beaker containing the animals was exposed to ordinary room light (illuminance 117 lx). The light was calibrated ($\mu W\ cm^{-2}$) by using interference filters as for the ERG measurements (see below) and a UVM-8LX Luxmeter calibrated in absolute units by Airam laboratories for a wavelength of 564 nm (Donner & Lindström, 1980). Knowing the filter parameters, an integration of the flux between 393 and 673 nm and in steps of about 20 nm could be done (Fig. 1). Exposures took place at 10 °C.

Expts 3, 4

($4{\cdot}9$ and $9{\cdot}8\ W\ m^{-2}$). The beaker containing the animals stood on a piece of white Styrox and was centred under a vertically mounted Osram 6 V 15 W (79152) microscope lamp with housing. The diameter of the white light patch was adjusted

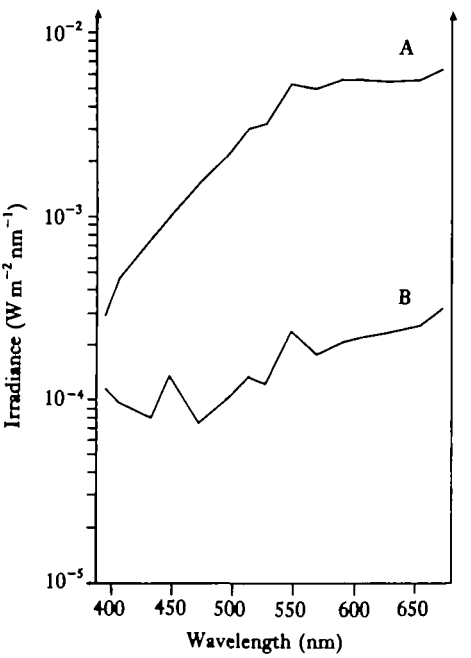

Fig. 1. Calibrated spectral irradiance, in absolute units ($W\ m^{-2}\ nm^{-1}$), for the broad spectrum white light that was used in the light-exposure experiments of the *Cirolana borealis* compound eye. The calculated irradiances are measured over the range 390–690 nm. A, illuminance 2500 lx (1250 lx has the same spectral distribution); B, illuminance 117 lx.

to give uniform light over the bottom of the beaker and with illuminances of 1250 lx (expt 3) and 2500 lx (expt 4) at the level of the *Cirolana* eyes, measured with standard Luxmeter (Dr Bruno Lange, Berlin). The exposure light was calibrated as in expt 2 (Fig. 1). These exposures took place in a cooling chamber at 3 °C.

Expt 5

Three daylight exposures with different intensities were performed: (1) Sunny day with clouds, for 4 h, with an energy content of $1{\cdot}4 \times 10^6$ J m^{-2} (97 W m^{-2}). (2) Sunny day without clouds, for 10 min, with an energy content of $4{\cdot}2 \times 10^4$ J m^{-2} (70 W m^{-2}). (3) Sunny day without clouds, for 2 h with an energy content of $9{\cdot}8 \times 10^5$ J m^{-2} (136 W m^{-2}).

The beaker containing the animals was kept at less than 10 °C in an ice bath outdoors. There was no shading of the beaker. The energy content of the exposures was measured with a solarimeter (Kipp & Zonen Solarimeter integrator CC1) positioned next to the beakers. The irradiance values are calculated averages from the exposure times and the energy contents.

Histological treatment

Dark-adapted eyes were dissected in the fixative (i.r. light) and light-exposed eyes

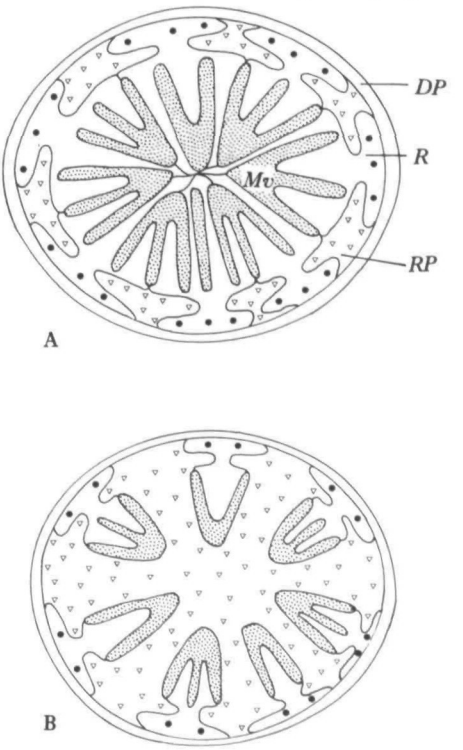

Fig. 2. Schematic drawings (simplified), of cross sections, of the fused distal (A) and proximal open (B) rhabdom portions of the compound eye of *Cirolana borealis*. The rhabdom comprises seven rhabdomeres. *DP*, distal pigment cell sheet; *Mv*, microvillar lamellae of a rhabdomere; *R*, retinula cell; *RP*, reflecting pigment cell.

in red safety light (expts 2–5, Table 1). Fixation for light and electron microscopy was performed in a mixture of paraformaldehyde/glutaraldehyde according to Karnovsky (1965) with Ca^{2+} omitted. Fixation time was 3 h. Post-fixation was carried out in 2 % OsO_4 for 2 h at 4 °C, and 0·2 M-cacodylate buffer, pH 7·2, was used throughout. Further details of the histological procedure are given by Nilsson (1982) and Richardson, Jarret & Finke (1960).

Electroretinogram (ERG) measurements

Following light exposure, animals were handled in the dark using i.r. image converters (Lindström & Nilsson, 1983). Each excised *Cirolana* head was pinned to a piece of cotton, immersed in sea water from the sampling station and placed in the experimental chamber. The preparations were cooled to about 10 °C and the temperature was continuously recorded with a thermocouple.

Flashes of monochromatic light (duration 600 ms; $HW_{max} \sim 10$ nm), focused on the eye, evoked eye potentials, which were recorded through a glass micropipette (tip diameter 10 μm and filled with 1 M-NaCl) on a dual-beam oscilloscope, using a.c. coupling. The interval between the flashes was 2 min. A readily detectable and repeatable on-response of 5 μV at 495 nm was used as criterion response in the sensitivity threshold experiments (see also Lindström & Nilsson, 1983). The intensity of the stimulus was adjusted using neutral density filters and a neutral density wedge. The stimulus and recording system is described in detail by Donner (1971) and by M. Lindström (1983, in preparation). The preparations were allowed to accommodate for approximately 30 min before the first stimulus light was presented.

RESULTS

Morphology

Dark-adapted eye (DA)

The following description of the anatomy of the dark-adapted (DA) compound eye of *Cirolana borealis* concentrates on some details of the structure of the dark-adapted retinula cells (nuclear region) which have a bearing on the results of different light exposures to the eye. A more detailed description of the morphology of the *Cirolana* eye is given by Nilsson (1982, 1983).

The rhabdom was formed by the rhabdomeres of seven retinula cells, and was fused distally, and open proximally (Fig. 2). Each rhabdomere consisted of several cytoplasmic folds bearing microvilli (microvillar lamellae). These folds originated from the retinula cells and projected into the rhabdom space. The lamellae were often multiforked (Fig. 2). The retinula cell cytoplasm outside the rhabdom contained electron-dense pigment granules (Fig. 3).

The orderly arranged microvilli of the rhabdom were aligned parallel to each other and were 2·5–3 μm long and 60–100 nm wide. Each villus originated separately from a short and thin neck (length and diameter about 25 nm) (Fig. 4). This arrangement could be observed as closed microvillar bases, due to a lack in perfect alignment of the section along the necks of the microvilli. A close packing along the circumference of the microvilli appeared as an almost 'tight junction'-like structure (Fig. 5), which

restricted the extracellular space to thin channels (less than 4–6 nm wide) in between the corners of the 'hexagonally' packed microvilli (Fig. 6). An electron-dense extra cellular deposit was present at each microvillar tip (Fig. 3, inset).

Large (150–250 nm diameter) and small (25–60 nm diameter) vesicles occurred in small numbers in the cytoplasm just beneath the microvilli. Depending on the plane of section, these could be seen connected to the microvilli. These vesicles had a double membrane and their abundance and formation will be considered further in the description of the light-exposed eyes (Fig. 4). Occasionally, small (approximately 20–30 nm) coated vesicles were found (Fig. 4).

The Golgi complexes (Fig. 7, inset) were mostly found in the soma region and were fairly frequent. Small fragments of rough endoplasmic reticulum (*RER*) were dispersed in the retinula cell cytoplasm. The smooth endoplasmic reticulum (*SER*) was thin and consisted of a large number of membrane-delimited sacs and tubules (Fig. 7). Only a few free ribosomes were present in the cytoplasm. No lysosome-like bodies were found. A number of irregularly shaped vesicles were present in the cytoplasm and these are interpreted as distended cisternae of the smooth endoplasmic reticulum (Fig. 7). The mitochondria were well preserved.

Light-exposed eye (LE)

Eyes were fixed immediately after exposures to different intensities of white light (Table 1). The anatomy of the retinula cells was altered compared with the totally dark-adapted eye (DA). The changes mostly involved the microvilli, and some of the organelles, such as the Golgi complex, endoplasmic reticulum, mitochondria and pigment granules, and are summarized in Table 2. Unless otherwise stated, the described differences are the same for different exposure times at the same light

Fig. 3. Portion of a rhabdomere from a dark-adapted compound eye of *Cirolana borealis*. The well-organized microvilli (*Mv*) project, at right angles, from a retinula cell (*RC*) fold (*asterisk*), which projects into the rhabdom space. *Large black arrow*, a thin sheet of retinula cell cytoplasm that surrounds the rhabdom; *small black arrows*, pigment granules; *white arrow*, the region where opposing microvilli meet. Scale bar, 2 μm, 5000×. Inset. Electron-dense deposits between the tips of opposing microvilli. Scale bar, 0·1 μm, 34 490×.

Fig. 4. Microvillar bases of a dark-adapted eye. Note membrane vesicles, which are present and connected to a microvillus (*asterisks*) and also the thin necks (*arrowheads*) from which each microvillus originates. *Mv*, microvilli; *S*, single-membraned vesicle; *black arrows*, small extracellular spaces between the microvillar bases; *open arrow*, closed microvillar bases caused by an oblique section; *white arrow*, coated vesicle. Scale bar, 0·1 μm, 59 400×. Inset. Closed microvillar bases (*open arrow*) and the origin of a microvillus through a thin neck (*arrowhead*). Scale bar, 0·1 μm, 19 510×.

Fig. 5. Five-layered appearance ('tight junction'-like) of adjoining microvilli (*arrow*). Scale bar, 0·1 μm, 126 250×.

Fig. 6. Cross-sectioned microvilli (dark-adapted eye), which are hexagonally packed. *Small arrows*, thin extracellular channels between adjoining microvilli; *large arrow*, axial filament, which seems to be connected to the cell membrane. Scale bar, 0·1 μm, 77 550×.

Fig. 7. The retinula cell cytoplasm of a dark-adapted eye. *P*, pigment granulae; *arrows*, smooth endoplasmic reticulum (*ER*); *asterisks*, distended *ER*. Scale bar, 0·2 μm, 47 000×. Inset. Golgi apparatus. Scale bar, 0·1 μm, 61 100×.

Fig. 8. Part of a rhabdomere of an eye exposed to white light, 117 lx (0·47 W m^{-2}) for 60 min. Note swollen and irregularly shaped microvilli (*Mv*), vesicle formation at the microvillar bases and retinula cell pigment granules in the rhabdom space. (Compare with Fig. 3.) Scale bar, 2 μm. 94 000×.

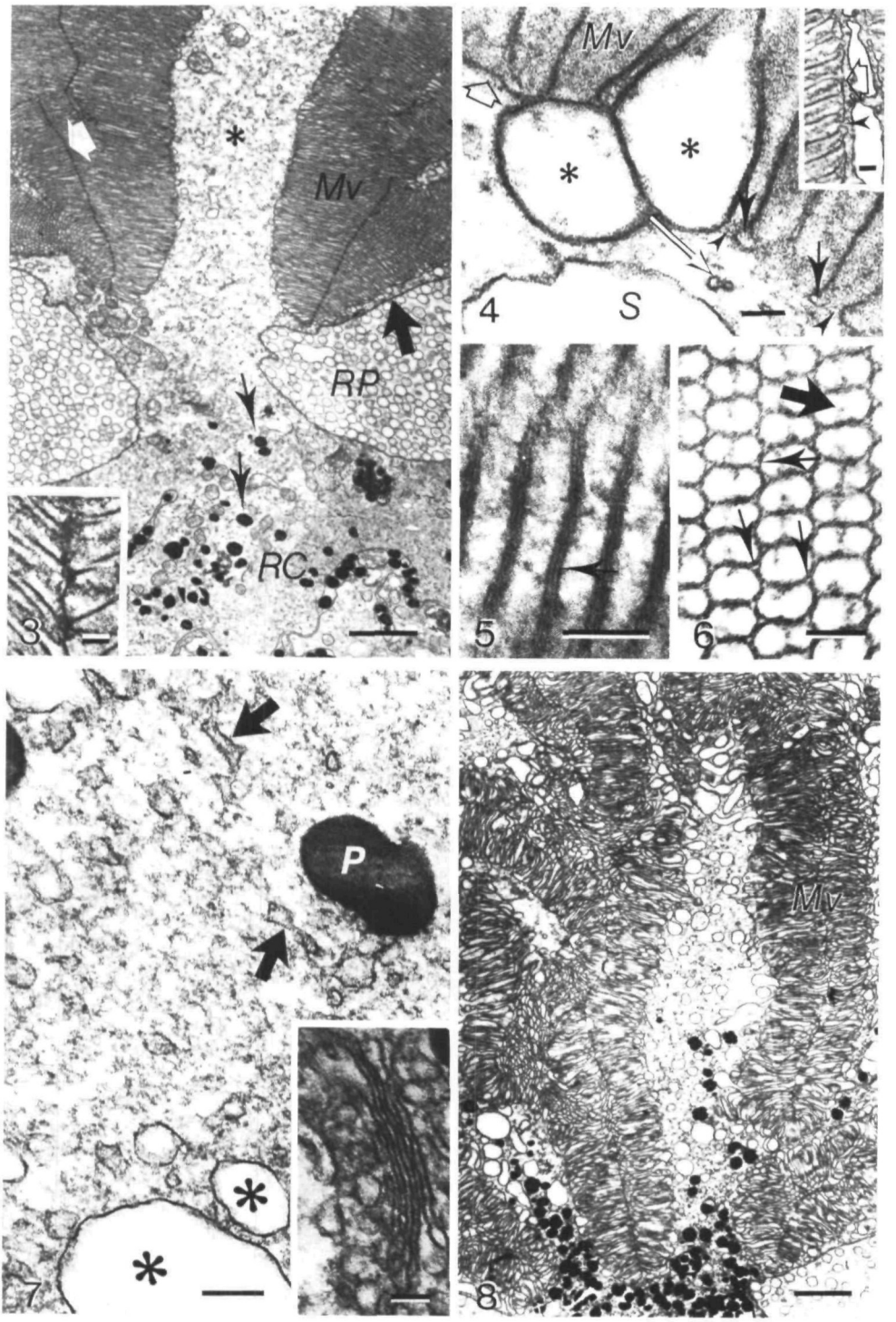
Mv
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Table 2. *The morphological preservation of the retinula cells of the* Cirolana borealis *compound eye, at various conditions of light and darkness*

Retinula cell (*RC*)	DA	Light exposed							
		117 lx		1250 lx	2500 lx		Daylight		
		IF	12 h PF	12 h PF	IF	12 h PF	IF	2–5 days PF	Scale (score)
Microvilli	5	4	4·5	2	4	2	2	1	(5) (1) good → very poor
No. of vesicles at the microvillar base	5	2	5	1	1	1	1	Distorted cytoplasm	(5) (1) low → high
No. of vesicles in the *RC* cytoplasm	5	3	5	1	3	1	1	Distorted cytoplasm	(5) (1) low → high
Golgi complex	5	4	5	2	4	2	2	1	(5) (1) good → very poor
SER	5	5	5	4	5	4	4	1	(5) (1) good → very poor
Mitochondria	5	5	5	4	5	4	4	1	(5) (1) good → very poor
Location of retinula cell pigment granules	5	3	5	1	3	1	1	1	(5) (1) outside → inside the rhabdom
Mean score	5	3·7	4·9	2·1	3·6	2·1	2·1	1·0	mean score

Preservation, or the number (No.) of organelles, is given on a relative scale: 5 – 1.
Comparison and reference are made to the morphology of the eyes, wich have never been exposed to any artificial light or daylight (DA; scale = 5).
IF fixation was performed immediately after the light exposure.
PF prior to fixation the light-exposed eyes were dark adapted for 12 h or 2–5 days.

intensities. Rarely, lysosome-like bodies were observed. Further observations are presented below.

At 117 lx (expt 2) the microvilli maintained a close packing and an orderly pattern, although they were uneven in thickness (20–200 nm) and shorter (1–2 μm) than those of the DA eye (Figs 3, 8).

In contrast to the DA eye, a large number of various sized double-membraned vesicles were present at the microvillar bases. Some of these were continuous with the microvilli and some were free in the cytoplasm (Fig. 8). They were also characterized by a fuzzy coat on both sides of the membrane (Fig. 10).

The Golgi complexes were increased both in size and number. Changes in structure included fragmentation and hypertrophy (Fig. 12). A number of retinula cell pigment granules migrated into the rhabdom area and spread into the cytoplasmic folds (Fig. 8).

As stimulus intensity was increased, there was increased disorder of the microvillar lamellae and the microvilli (Figs 9, 13). Daylight exposures caused the microvilli to disrupt (Fig. 15) and frequently, membrane whorls were seen at the microvillar bases (Fig. 11). Pigment granules were found throughout the rhabdom (Fig. 15).

Recovery experiments (expt 6)

Some animals that were exposed to light according to Table 1 (expts 2–5) were post dark-adapted to determine if the morphology could be returned to the DA condition, and results are summarized in Table 2. Additional comments are given below.

After 117 lx exposure followed by 12 h of dark adaptation, the structural organization was similar to that in the DA eyes (Fig. 16).

After 1250 and 2500 lx light exposures, dark adaptation resulted in further damage. Damage was more extensive with longer dark adaptation (Figs 14, 17). This also includes an increased number of membrane whorls and disruptions. Two and five days of dark adaptation after 4 h of daylight exposure resulted in a retinula cell anatomy that was severely deranged (Figs 18, 19).

Fig. 9. Part of a rhabdomere of an eye exposed to white light, 2500 lx (9·5 W m^{-2}) for 60 min. Note the large number of various sized vesicles. Some are seen connected to the microvilli (*arrow*) and others are free in the cytoplasm of the retinular cell fold. Scale bar, 1 μm, 9460×.

Fig. 10. A double-membraned vesicle still connected to the base of a microvillus. The eye was exposed to 117 lx (0·47 W m^{-2}) for 60 min. Note the five-layered appearance of adjoining membranes and the presence of a fuzzy coat on both sides of the membrane (*arrows*). Scale bar, 0·1 μm, 67 725×.

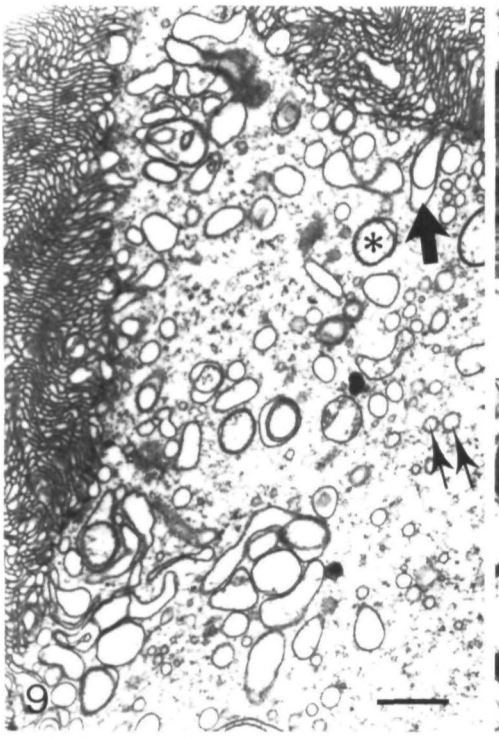
Fig. 11. Membrane whorls of receptor membranes present at the microvillar bases (from an eye exposed to 2 h of unquantified daylight). Scale bar, 1 μm. 11 375×.

Fig. 12. Golgi complexes (*G*) in the retinula cell cytoplasm of an eye exposed to white light for 60 min (117 lx, 0·47 W m^{-2}), *asterisks*, large vesicles associated with the Golgi complex; *arrows*, Golgi cisternae and associated small vesicles (fragmented?). Scale bar, 1 μm, 12 000×.

Fig. 13. Light microscopical cross section of the medial portion of the rhabdom (slightly oblique) of a daylight exposed eye (4 h). Note disordered microvillar lamellae and pigment granules in the rhabdom (*arrow*). Compare with Fig. 2A. Scale bar, 15 μm, 248×.

Fig. 14. Cross-sectioned microvilli of an eye exposed to 1250 lx (4·9 W m^{-2}) followed by dark adaptation for 24 h prior to fixation. Note disrupted and dilated microvilli, but also microvilli that are adjoined. Scale bar, 0·1 μm, 29 575×.

Fig. 15. Cross section of the rhabdom of an eye exposed to daylight for 4 h. Note disrupted microvilli (*Mv*) and pigment granules in the cytoplasmic folds of the rhabdoms (*arrow*). Scale bar, 1 μm, 5000×.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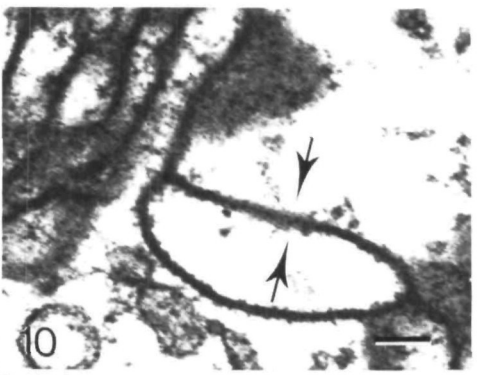
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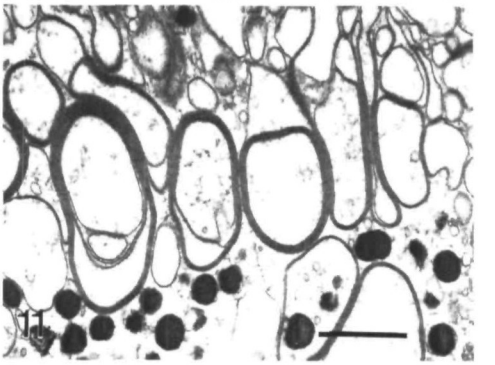
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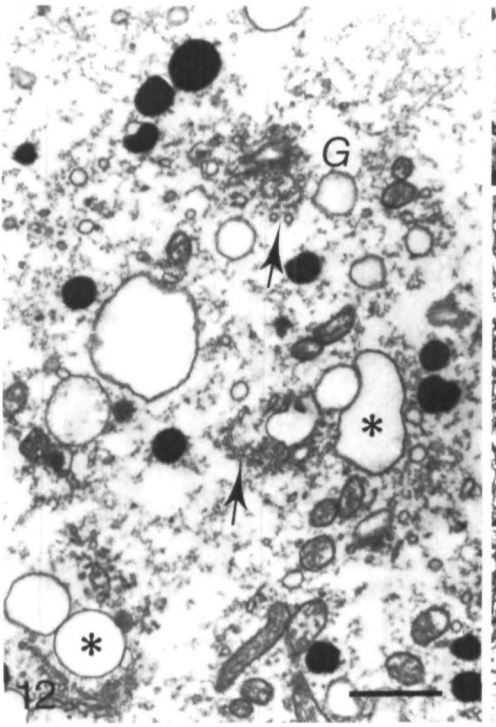
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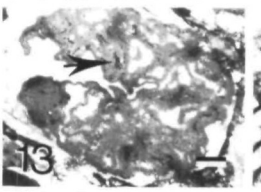
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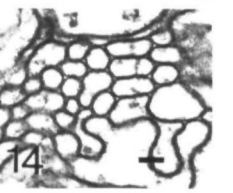
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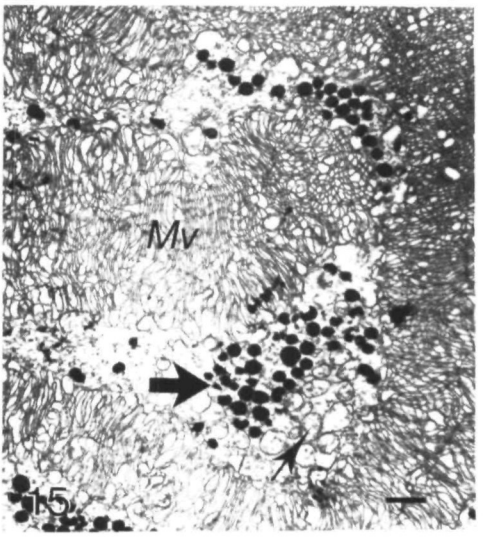
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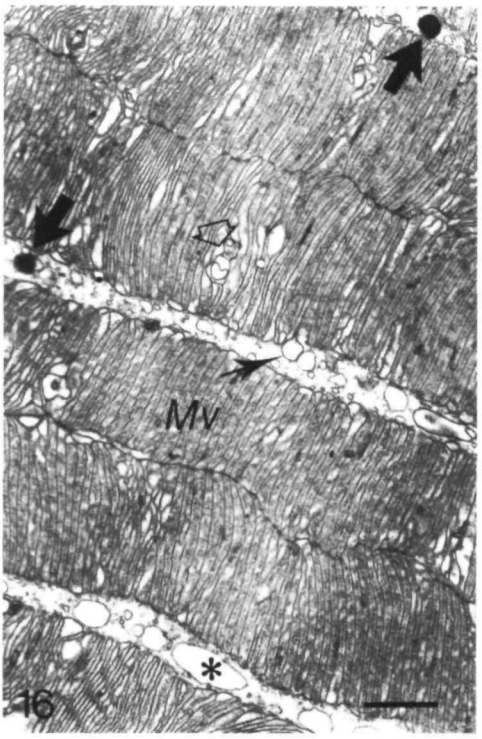
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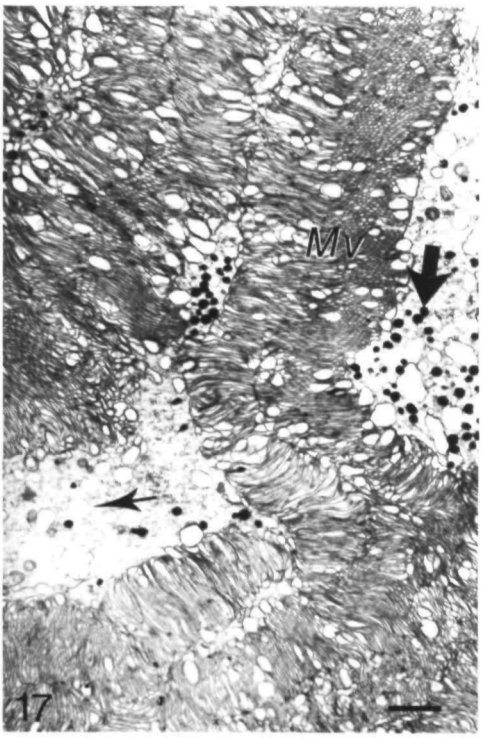
Mv
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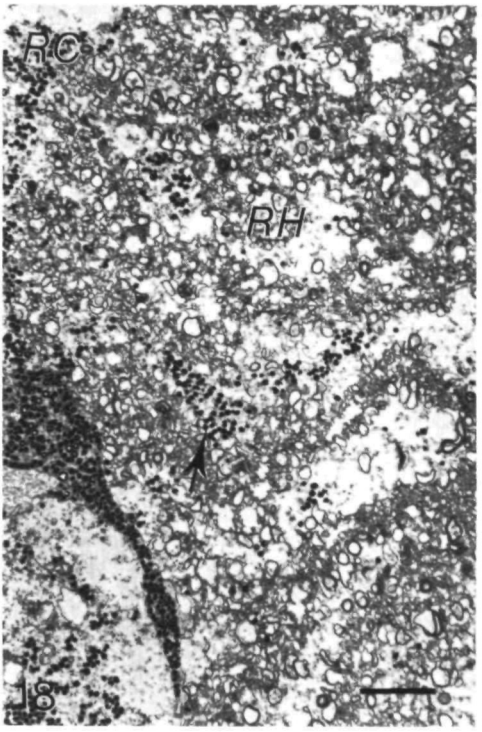
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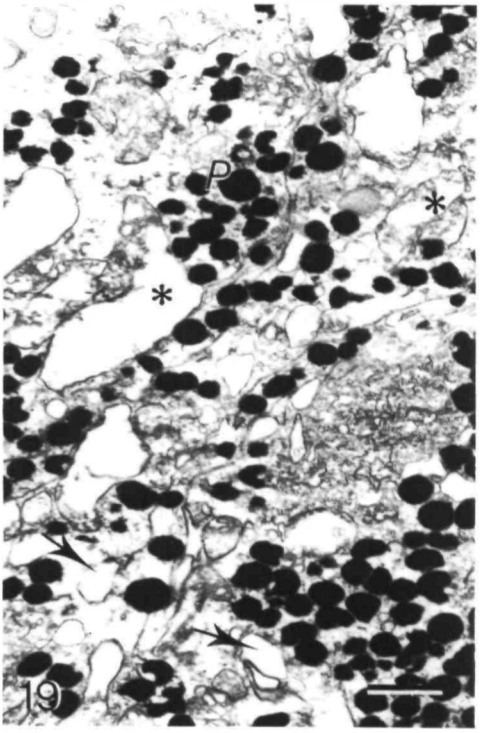
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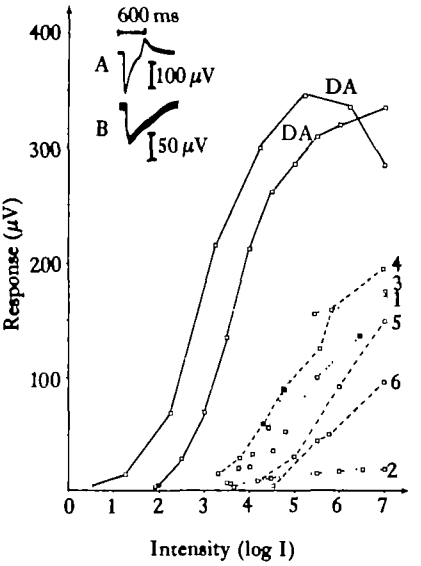

Fig. 20. Response-intensity functions (μV/logI) measured by the ERG for the compound eye of *Cirolana borealis*. The measurements were either performed on truly dark-adapted eyes (*solid lines*, DA) or on eyes exposed to white light followed by various dark adaptation times prior to the recordings (1–6). (Each function represents measurements from one animal.) Note the depression of the peak amplitudes of the light-exposed eyes; *stippled curves*, white-light exposures with an illumination of 1250 lx (4·9 W m^{-2}) for 10 min followed by various dark adaptation periods prior to the measurements. (1) DA 6 h (2) DA 12 h (3) DA 24 h. *Broken curves*, white-light exposures of 2500 lx (9·8 W m^{-2}) for 10 min followed by dark adaptation for 12 h (4 and 5) and light exposure for 60 min followed by DA 12 h prior to the measurements (6). Inset. Typical electroretinogram curves for the dark-adapted eye (A) and the light-exposed eye (B). The recordings were made immediately after adaptation and exposure, respectively.

Electrophysiology – the electroretinogram (ERG)

Dark-adapted eye (DA)

The ERG of the DA eye was similar to that observed previously (Lindström & Nilsson, 1983). On exposure to a light stimulus, there was a cornea negative on-response with peak amplitudes between 300 and 400 μV at the highest stimulus intensities (Fig. 20, inset A). The μV/logI-function had a sigmoid shape (Fig. 20); and the visual threshold sensitivity (= criterion response), defined as the number of photons

Fig. 16. Microvillar lamellae of the *Cirolana borealis* rhabdom. The eye was exposed to 117 lx (0·47 W m^{-2}) for 10 min and then dark adapted for 12 h before fixation. Note well-organized microvilli with but few disruptions (*open arrow*) and a decreased vesicle formation at the microvillar bases (*small arrow*). *Asterisk*, single-membraned vesicle; *large arrows*, pigment granules. Scale bar, 1 μm, 9460×.

Fig. 17. Disrupted microvilli (*Mv*) of the *Cirolana borealis* rhabdom after white-light exposure of 1250 lx (4·9 W m^{-2}) for 10 min followed by dark adaptation for 12 h prior to fixation; *large arrow*, retinula cell pigment granules in the rhabdom; *small arrow*, distended smooth *ER*. Scale bar, 2 μm, 3550×.

Fig. 18. The rhabdom of the *Cirolana borealis* eye after 4 h of daylight exposure followed by dark adaptation for 5 days prior to fixation. Note totally disrupted rhabdom (*RH*). *RC*, retinula cell; *arrow*, retinula cell pigment granules. Scale bar, 5 μm, 1880×.

Fig. 19. The retinula cell cytoplasm of the *Cirolana borealis* eye after 4 h of daylight exposure followed by 5 days of dark adaptation prior to fixation. Note disrupted mitochondria (*asterisks*) and irregularly shaped vesicles (*arrows*). *P*, pigment granules. Scale bar, 1 μm, 11 100×.

required to evoke a 5 μV response at peak wavelength ($\lambda = 495$ nm), was $2{\cdot}1 \times 10^8$ quanta $cm^{-2} s^{-1}$ (Fig. 21).

Light-exposed eye (LE)

Electroretinograms were immediately recorded after light exposure according to Table 1 and yielded weak responses in all cases except in the 117 lx light exposure. These preparations were also typically hypersensitive to strong stimulus light (10^{13} quanta $cm^{-2} s^{-1}$) and a high stimulus irradiance suppressed the sensitivity and it could no longer be recorded. The results are summarized in Table 3.

Recovery experiments (expt 6)

Following light exposure, some animals were dark-adapted (DA) for various periods of time prior to ERG recording (Table 1, expt 6). The irradiance necessary to evoke a 5 μV response at peak wavelength, $\lambda = 495$ nm, was compared with that for the DA eye, as is shown in Fig. 21 and Table 3. Additional comments are found below.

After a 10-min exposure to 117 lx, followed by 12 h of DA, the sensitivity of the ERG visual threshold was similar to that for the DA eye (Fig. 21). With longer exposure, recovery was incomplete.

Dark adaptations for different times (see Table 1, expt 6) after both 1250 and 2500 lx light exposures resulted in threshold sensitivities that could be correlated with the DA times (Fig. 20), and the results were dispersed around 2 and 2·5 log units, respectively (Fig. 21).

All preparations were characterized by a hypersensitivity to strong light stimuli, which radically depressed sensitivity and it could no longer be recorded during the experiment. No responses of the daylight-exposed eyes could be recorded even after 5 days of dark adaptation.

Table 3. *The ERG amplitude and sensitivity ($\lambda = 495$ nm, criterion response = 5μV) of the* Cirolana borealis *compound eye, at various conditions of light and darkness*

		Light exposed							
		117 lx		1250 lx	2500 lx		Daylight		
	DA	IM	PM	PM	IM	PM	IM	PM	Formula: normalized to unity (u) = 5≥u≥0
Amplitude	5·0	1·8	3·8	2·1	0·1	2·5	0·1	0	$\frac{A_I}{A_{max}} \times 5$
Sensitivity	5·0	3·5	5·0	3·6	3·4	3·3	3·2	0	$\frac{\log S_{max}}{\log S_I} \times 5$
Mean score	5·0	2·7	4·4	2·4	1·8	2·9	1·7	0	

Scale = 5 (high) – 0 (none). Values are normalized to unity (5) according to the formula given in the table.
IM Measurements were performed immediately after the light exposure.
PM prior to the measurements the light-exposed eyes were dark adapted for 6–24 h (all measurements included in the group).
A_I maximum amplitude of the light-exposed eye.
A_{max} maximum amplitude typical of the non-exposed eyes (DA) = 400 μV.
S_I sensitivity of the light exposed eye.
S_{max} sensitivity of the DA-eye = $2{\cdot}1 \times 10^8$ quanta $cm^{-2} s^{-1}$.

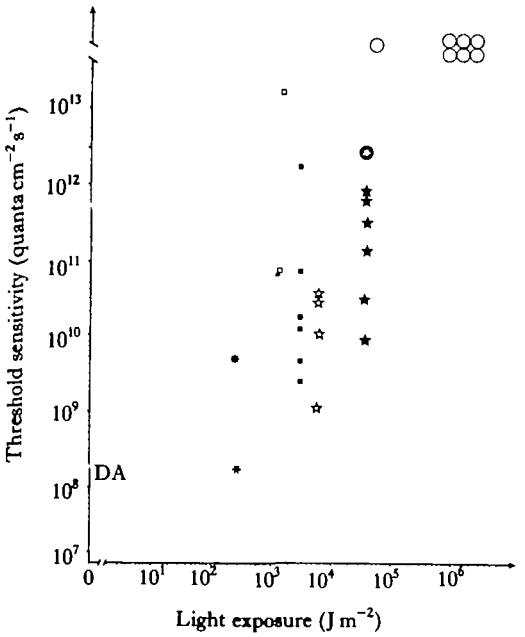

Fig. 21. Electroretinogram (ERG) measurements of visual threshold sensitivities for different white-light exposures, which were followed by a dark recovery time prior to the measurements for the compound eye of *Cirolana borealis*. (Each point represents measurements from one animal.) Abscissa; energy content of the white-light exposures (J m^{-2}). Ordinate; irradiance (quanta cm^{-2} s^{-1}) necessary to evoke a criterion response (5 μV at peak amplitude λ = 495 nm). DA = mean sensitivity of the deeply dark-adapted eye (N = 19). • 117 lx, 10 min DA 0 h, * 117 lx, 10 min DA 12 h, □ 117 lx 60 min DA 0 h, ▲ 117 lx 60 min DA 1 h, ■ 1250 lx 10 min DA 6–24 h, ☆ 2500 lx 10 min DA 12 h, ★ 2500 lx 60 min DA 2–24 h, ● 2500 lx 60 min DA 0 h, ○ daylight – no responses could be detected.

DISCUSSION

The compound eye of *Cirolana borealis* has recently been characterized functionally by the use of the electroretinogram, and this showed that the eyes were well adapted to their dim light environment (Lindström & Nilsson, 1983). It has also been shown that daylight exposures to the *Cirolana* eye alter the morphology of the photoreceptor membranes (Nilsson, 1982). The present results extend these investigations by correlating the function and the morphological appearance of the eye after defined light exposures and periods of recovery.

Exposure to light produced changes in retinula cell morphology. The cytoplasmic folds bearing the microvilli became distended, while the microvilli were shortened or sometimes disrupted. Double-membraned vesicles were present near the microvilli, especially after illumination at 117 lx. There was distension of the smooth endoplasmic reticulum and an increase in the number of Golgi complexes. Pigment moved from a position outside the rhabdom into the rhabdom space. Lysosome-like bodies were rarely seen, and are unlikely to be involved in membrane renewal or breakdown processes as is the case in a number of arthropods (Blest, 1980; Eguchi & Waterman, 1967, 1976; Hafner, Hammond-Soltis & Tokarski, 1980; Nemanic, 1975; White, Gifford & Michaud, 1980). At daylight intensities membrane whorls were seen at the microvillar bases.

These morphological changes are interpreted as a retinal dystrophy, for they are reflected in the electroretinogram recordings as a loss and, except after brief exposure to 117 lx, the changes could not be restored to the condition in the dark-adapted eye. The responses saturated at low amplitudes or were totally depressed. The morphological appearance of the retina has also been correlated with function in the light-exposed eye of rat (Dowling & Wald, 1960; Noell *et al.* 1966), rabbit (Lawwill, 1973*a*; Kuwabara, 1970) and monkey (Lawwill, 1973*b*); and also in crayfishes kept in prolonged darkness (Eguchi, 1965).

Disruption of photoreceptor membranes and whorl formation, caused by excess light, is a well-described phenomenon in both arthropods (Behrens & Krebs, 1976; Blest & Day, 1978; Loew, 1976; Meyer-Rochow, 1981; Nilsson, 1982; Tuurala & Lehtinen, 1971) and vertebrates (Kuwabara, 1970; Lawwill, Crockett & Currier, 1980). Similar effects are also caused by exposure of the eyes to prolonged darkness (Bloom & Atwood, 1981; Edwards, 1969; Eguchi & Waterman, 1966; 1979; Roach & Wiersma, 1974; Welsch, 1977), by a vitamin A-deficient diet (Carlson, Gemne & Robbins, 1969; Dowling & Gibbons, 1961; Yang, Hollenberg & Wyse, 1978), or by temperature (Meyer-Rochow & Tiang, 1979). Membrane changes can be caused by inadequate fixation procedures, as previously observed (Kabuta, Tominaga & Kuwabara, 1968; Williams, 1980; White & Michaud, 1981) but the technique employed in the present study has been found to be satisfactory (Nilsson, 1982, 1983).

Cell damage was indicated by the distended *ER* (David, 1978; Trump, Jesudason & Jones, 1978). This was found only in light-exposed eyes and was located in the nuclear region of the cell, so cannot be regarded as subrhabdomeric cisternae (insects: reviewed, by Autrum, 1981; crustaceans: Eguchi & Waterman, 1967; Nässel & Waterman, 1979; *Limulus*: Behrens & Krebs, 1976).

At 117 lx, the increased number of Golgi complexes, and the associated large vesicles, may indicate a normal role in the membrane turnover process. At higher illumination, these features probably reflect disordered function (Ontell, 1975).

The degeneration produced by exposure to light above the damage threshold continued in the dark. A similar situation is also found in other crustacean eyes (Loew, 1976; Meyer-Rochow, 1981; Meyer-Rochow & Tiang, 1981).

Within the range of illumination in which the eye is not injured we found that even very short exposure times (10 min) needed 12 h of recovery to reach the values of the dark-adapted eye. This type of eye apparently has a very slow metabolism. This conclusion is supported by the finding that the regeneration rate of visual pigment is very slow in the deep-water-frequenting crustacean *Nephrops* (Loew, 1976). Further, the presence of light is not necessarily important (at least within a rather long period of time) to maintain normal function of the eye. This is evidenced by the fact that even after 2 months in total darkness the eye responds electrophysiologically quite normally when compared with dark-adapted eyes (Lindström & Nilsson, 1983).

A characteristic type of double-membraned vesicles was found to occur in association with, or close to, the microvilli. They were infrequent in the dark-adapted eyes, but increased appreciably in eyes exposed to light, both below and above the damage threshold levels. A thorough analysis of the appearance of the vesicles, their morphology and connections with the microvilli revealed a new way, previously undescribed

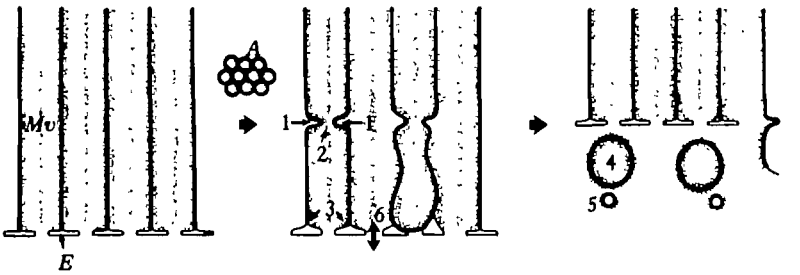

Fig. 22. Schematic drawing illustrating how microvilli of light-exposed eyes in *Cirolana borealis* are thought to be shortened. Double-membraned- (4) and single-membraned-coated (5) vesicles are formed at the microvillar bases. (For further explanation, see the text.) *Mv*, microvillus; *A*, hexagonally packed microvilli seen in a cross section; *E*, extracellular space.

by which retinula cells disintegrate microvilli. The process is thought to occur in several steps, which are illustrated in Fig. 22.

The first step involves a bulging in of the membranes of two or more contiguous microvilli (1), and a new neck of the shortened microvillus is formed (2). A similar invagination of the microvillar membrane occurs basally (3). The next step involves, hypothetically, a closure of the membranes involved (this closure seems to occur basally first) and the double-membraned residual vesicle is formed (4). The original basal extracellular space is released as a coated single-membraned vesicle (5). The cytoplasm of the original microvilli ends joins that of the retinula cell (6). Various intermediate steps in the process are seen in Figs 4, 9, 10 and 14.

This mechanism could occur also in other species. Double-membraned vesicles seem to be present in the *Limulus* eye (Behrens & Krebs, 1976). The coated vesicles, which are normally associated with membrane turnover and breakdown (Blest, 1980; Blest, Kao & Powell, 1978; Eguchi & Waterman, 1976; Stowe, 1980; White, 1967, 1968), seem to play a minor role, at least in the breakdown process, in the *Cirolana* eye, although these vesicles are still present.

Nothing is known at present of the future fate of the degraded microvillar membranes or how the synthesis of new photoreceptor membranes are accomplished.

In conclusion, our investigation has shown that the *Cirolana* eye is functionally adapted to low light intensities. This adaptation has evolved so far that exposure to moderate to high illumination is fatal to the eye and presumably also to the animal. The effects of a harmful initial exposure are continued in the dark and recovery is extremely slow. The described mechanism for microvillar membrane destruction is new among arthropods.

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