STRUCTURE, LIGHT & TEMPERATURE INDUCED CHANGES IN THE COMPOUND EYES OF ANTARCTIC CRUSTACEANS

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ABSTRACT

The structure of the photoreceptors of three different Antarctic crustaceans was investigated by light and electron microscopy. Special attention was paid to light and temperature induced changes of the normal, i.e. dark-adapted eye.

The apposition eye of Orchomene plebs shows structural modifications which are interpreted as adaptations to maximise photon capture in an environment of low ambient light intensities. Dark-light adaptational changes affect the position of the screening pigment granules, the volume of the rhabdom and the composition and density of the organelles in both retinula and interstitial cells. Exposure to a temperature of +10°C for seven hours affects the structural integrity of the rhabdoms and mimicks light-adaptation in animals that are kept in the dark. Rhabdoms regenerate as long as the animals are returned to water of 0°C.

The ommatidia of the dorsal eye of Glyptonotus antarcticus possess very large diameters and are of the apposition type. Dark-light adaptational changes, which are confined to the dark eye if one eye is painted black and the other is left untouched, involve radial migration of screening pigment granules in the retinula cells surrounding the rhabdom. An elevation of the temperature also affects the position of the screening pigments, but the rhabdom ultrastructure is far less affected than that of Orchomene plebs.

The compound eyes of the Ross Ice Shelf amphipod Orchomene grandis show the highest degree of structural adaptation to a dimly-lit environment. Following exposure to sunlight or dark-

ness for one week at a temperature of approximately +1°C, the extraordinarily massive rhabdoms exhibit almost total disintegration. The density of screening pigment granules is so low that migrations upon dark-light adaptation are insignificant. The eye of Orchomene grandis shows the smallest capacity of all three species studied to adapt to different ambient light levels.

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LIST OF ABBREVIATIONS

AX	Axons
BM	Basement membrane
C	Cornea
CC	Crystalline cone
Cor C	Corneagenous cell
CP	Cone cell process
DA	Dark-adapted
DP	Distal pigment
LA	Light-adapted
M	Mitochondria
MVB	Multivesicular bodies
MLB	Multilamellar bodies
N	Nucleus
PP	Proximal pigment
RH	Rhabdom
Ret C	Retinula cell
Ret CN	Retinula cell nucleus
RISP	Ross Ice Shelf Project
S	Link between cone and rhabdom
V	Vacuoles

PREFACE

A. Photic environment

Crustaceans, like most living forms, exhibit the phenomenon of being sensitive to that region of the electronmagnetic radiation commonly referred to as 'light' (wavelengths approximately 350-750nm long). The mechanism associated with photosensitivity is usually mediated by photolabile pigments and specialized membranes. Photoreception especially vision, plays a major role among the many sensory modalities utilized by various animal forms (including most crustaceans) to provide information on the nature of the environment. Interpretation of the photic environment through eyes rather than light-sensitive spots scattered over the entire body is a great advantage because an organized visual system is able to provide the richest source of sensory information in terms of quality and detail.

However, diversity in the photic environment and the different modes of life of animal species have given rise to a spectacular variety of visual adaptation in the animal world. Nocturnal animals or animals living in a dimly lit environment usually exhibit highly specialized visual systems, sometimes combined with bioluminescence. Some animals like the cave-dwelling bat or fish Astyanax have gone to the other extreme: complete regression and degeneration of photoreceptive elements and development of alternative sensory systems like hearing (echo-location) or electro-reception, respectively.

Environments of low ambient light intensity could be instrumental in the development of numerous and sometimes

converging mechanisms directed towards an improvement of photon capture and, thus, sensitivity. Eyes of fishes, insects and crustaceans living in dimly lit environments share certain features, demonstrating that similar visual systems evolve as a response to similar pressures. The eyes of many deep-sea and nocturnal fishes often have pure-rod retinas with photoreceptor outer segments arranged in superimposed layers (Munk 1966, Ali & Anctil 1976, Meyer-Rochow & Tiang 1978). Optical guides in the form of the rod myoid (Locket 1970, Miller & Snyder 1977) which contain microfilaments direct light from one receptor layer to the next (Eakin 1972, Burnside 1976). Similarly, the rhabdomeres of the firefly (Photuris, Horridge 1968) and scarabaeid beetle (Sericesthis, Meyer-Rochow 1977) are arranged in two superimposed layers with a smaller distal and a larger proximal layer. Extending from the corneal lens are four elongated crystalline cone cell processes which contain microfilaments too, and presumably have wave-guide properties directing light onto the photosensitive rhabdoms (Horridge 1968).

Likewise the compound eyes of many crustaceans have undergone structural and functional modifiaations as a result of the environment in which these animals live - especially if the mesopelagial (400-1000m) and the deep-sea (>1000m) are concerned. Environmental conditions in the deep-sea are definitely very different from those at the surface, some of the most significant differences being the general absence or great diminution of sunlight, a relatively constant low temperature and high pressure. However, despite these conditions, crustaceans provide the maximum marine biomass in

sea-water between 1000m and 3000m (Omori 1974). In the eyes of Streetsia (a mesopelogic crustacean, Meyer-Rochow 1978), the unusually large rhabdom and crystalline cone are often interpreted as adaptations to increase absolute sensitivity. The relatively large and long crystalline cones are thought to act as light guides comparable to those found in many nocturnal insects, e.g. scarabaeid beetles (Meyer-Rochow 1978b).

Some of the adaptive modifications occurring in the compound eyes of the deep-sea crustaceans still living in a depth where vision is useful include:

1. Enlargement of eye and ommatidia or even fusion of the eyes (e.g. Streetsia: Meyer-Rochow 1978).
2. Elongation of ommatidia in specialized areas of the eye (e.g. Phronima, Ball 1977) or even splitting of the eye into two functionally different parts (e.g. Boreomysis scyphops, Elofsson & Hallberg 1977).
3. Enlargement of rhabdom and microvillus orientations in more than just 2 perpendicular directions, in other words, increase of photosensitive membrane area at the expense of polarization sensitivity (Meyer-Rochow 1975).
4. Development of bilobed eyes (e.g. deep-sea euphausiid, Kampa 1963).
5. Modifications affecting spectral sensitivity and retinal pigments (e.g. Procambarus, Goldsmith 1978).
6. Possibility of electrical coupling of axons to 'neural superposition eye' (not yet shown to exist in crustaceans).

Crustaceans inhabiting the depths of the oceans (beyond 1500m) do not normally show any of these adaptations but display various degrees of regression and degeneration in their photoreceptive organization.

The marine environment of Antarctica ($77^{o}52'S$) is exceptionally unique even when compared with that of the Arctic (Hedgpeth 1971) because of constantly low temperature, low ambient light levels and stable salinity conditions (Jacobs et. al. 1970). The annual mean sea temperature in the McMurdo Sound is $-1.81^{o}C$ (with a standard deviation of 0.08) and vertical thermostratification is virtually non-existent (Littlepage 1965). Light transmission is influenced by a variety of factors including sun-angle, snow cover, ice condition and epontic phytoplankton (mostly diatoms on the underside of the sea-ice, Burt 1963). However, even during the Antarctic summer with continuous day-light for several months, light intensities measured under 3 meters of solid sea-ice covered by 5 cm of snow give only a noon average light transmission of 0.25% (based on data from the McMurdo Sound obtained during four summer months by Littlepage (1965)). Measurements of light transmission in the sun-free winter months are not available but light levels would undoubtedly reach immeasurably small values a few meters below the ice. It is reasonably safe to assert that benthic organisms of the McMurdo Sound live in total darkness during the sun-free season.

The frozen continent of Antarctica is commonly described as the most inhospitable, white, barren piece of waste land existing on earth. Below the sea-ice, however, one is

surprised to encounter an abundance of marine life forms, some of which survive even under 420m of ice at a latitude 82°22'S and 450km from the ice front in the Ross Sea. Eyes are a prominent feature of almost all free-living Antarctic crustaceans, which, surprisingly enough, includes even those that live under the 420m thick Ross Ice Shelf itself. It is believed that the Antarctic crustaceans may be closely related to certain deep-sea forms (Kussakin 1973). To what extent the compound eyes of Antarctic crustaceans have become modified and adapted to their unique environment and what effects light and temperature may have on the eye ultrastructure have been questions which initiated this investigation.

B. General structure, organization and function in crustacean compound eyes

The structures of the crustacean compound eyes have been reviewed by Waterman (1961), and Bullock & Horridge (1965). The compound eye is constructed of typical anatomical units which are defined in some detail by Eguchi & Waterman (1966). Like all compound eyes, the crustacean eye is made up of ommatidia, each ommatidium being the morphological unit which generally consists of thirteen or fourteen cells. The distal part of the ommatidium is capped by a cuticular covering (the corneal lens), which may attain the form of a convex/concave or biconvex lens. A pair of modified epidermal cells per ommatidium (the so-called corneagenous cells) just below the cornea is responsible for secreting it. Next proximally is a further group of modified epidermal cells which are responsible for the formation of the crystal-

line cone and the stalk if present. Beneath these are the retinula cells and rhabdoms. Each ommatidium possesses a fixed number of retinula cells which may vary from species to species but not within a species. In decapods there are usually seven plus one distal eccentric cell. In amphipods there are five. Although these cells may be arranged in a number of ways, generally the retinula cells are grouped around a central elongated refractile structure, the rhabdom. In crustaceans (as in insects), there are two main forms of eyes. Based on the position of the rhabdom, 'apposition' and 'superposition' eyes are distinguished. In the apposition type, the distal end of the elongated fused rhabdom touches the inner end of the crystalline cone, while proximally it approaches the basement membrane. The superposition type usually exhibits a fusiform, shorter and 'fatter' rhabdom, remote in position from the crystalline cone, separated from it by a 'clear' pigmentless zone (e.g. Palaemon, Astacus, Procambrus, Waterman 1961). The rhabdom is a composite structure derived from centrally projecting membraneous retinula cell specializations called rhabdomeres. Each individual rhabdomere is the product of a single retinula cell. Within the rhabdomere the visual membranes appear like stacked tubes or rods, they are generally known as microvilli and have diameters which range from 40-120nm. The microvilli are oriented obliquely or at right angles to the axis of the rhabdom and contain the visual molecules in their membranes. Below the retinula cell lies the basement membrane which consists of an intricate network of cells and connective tissue fibres pierced at regular intervals by the axons of retinula cells.

Depending on the species one studies, retinula cell nuclei may be found above or below this basement membrane.

The crustacean eye may contain up to three sets of pigments in and around the retinal region varying in their position, number and screening properties. The distal pigment is contained in two or more cells which form a sleeve around the outer part of the ommatidium. Distal pigment migration in crustaceans is now known to be controlled by one or more neurosecretory hormones of the sinus gland which in turn is directly influenced by the immediate photic environment (Kleinholz 1966, 1976). The proximal pigment is located within the retinula-cells and its entire control mechanism has thus far not been elucidated. Recent experiments have not shown that hormonal control does not exist, but have confirmed that migration of retinula cell primary pigment can occur without hormonal intervention, simply as a direct response of the retinula cells to light (Olivo & Larsen 1978). The task of the screening pigments in the distal pigment cells is to absorb light rays which may be refracted or scattered out of the axial dioptric system, for light that is detrimental to the visual image causes a blurred visual image or even damage to unprotected nervous tissue. The screening pigments within the retinula cells, restrict the light that enters to the axial region of the rhabdom. Thus, the function of the screening pigments is to prevent stray light within the eye from interfering with the formation of the image or the information transfer process. Therefore the distal and proximal pigments which may be coupled to the corneal lens and the crystalline cone function as optical stops or a kind of aperture, restricting

the light rays that reach the photosensitive region of the retinula under varying external brightness conditions.

Pigment migration to the dark adapted state, i.e. wide open aperture, is likely to increase sensitivity at the cost of acuity. However, according to de Bruin and Crisp (1957), the movement of the distal pigment alone had little effect on acuity or sensitivity, but when the dark proximal pigment migrated over the reflecting layer around the basement membrane, visual acuity did increase and light sensitivity decreased.

Reflecting pigments are found in many crustaceans. They are located primarily near the basement membrane or in the interstitial cell around the retinula cells. Their main function is to increase light utilization during dark-adaptation by reflecting unabsorbed light back through the photosensitive region. This optical process can give rise to a phenomenon known as eye-glow. The chemical substance responsible for the reflections are guanines and pteridines (Zyznar & Nicol 1971). Reflecting organelles lining the crystalline cones are involved in the formation of a superposition image in the compound eyes of deep-sea crustaceans (e.g. Gigantocypris (Land 1976, 1978)).

There is ample anatomical as well as electrophysiological and biochemical evidence that the site of the occurrence of photoreception is in the rhabdomeres (for review, see Dartnall ed. 1972, Fuortes ed. 1972, Autrum ed. 1979). Crustaceans as well as the majority of other seeing invertebrates and vertebrates have photosensitive pigments located in the

membranes of the microvilli. The rhabdom itself has properties of a di-electric wave-guide (Snyder 1973) so that light preferentially travels within the light-sensitive tissue of one photoreceptor and does not cross into neighbouring ommatidia. Here, it is absorbed by the photosensitive pigments which causes a chain of events which finally leads to a receptor potential. The process is called 'transduction'.

Almost all crustaceans possess a fused rhabdom which indicates that the rhabdomeres function in combination with their neighbours. Considering the accurate aligment of pigment molecules, the arrangement of densely packed parallel molecules within a rhabdomere and the symmetrical orientation of microvilli within the fused rhabdom, Snyder et. al. (1973), who investigated the structure and function of the fused rhabdom in insects, concluded that this type of compound eye "cannot be considered as a loose collection of photoreceptors sharing the same dioptric apparatus but must be viewed as an integrated unit". The fused rhabdom shows a significant advance in arthropod visual system design over the "open rhabdom", characteristic of animals living in bright sunlight like the fly, by allowing high absolute sensitivity to be combined with colour vision and acuity.

The compound eyes of crustacean like those of several other major invertebrate animal groups, particularly insects, exhibit a great variation in their structural organizations. The number of photoreceptive cells present, the number of corneal lenses and the degree to which the cone is developed, all of these are known to vary greatly amongst different species, different stages in the life cycle, and sometimes

between sexes. Usually there are 5-8 retinula cells present per ommatidium in most crustacea, but some Cumaceae have only 3 retinula cells while Oniscus, an isopod, has 17 retinula cells (Debaisieux 1944). The number of ommatidia may be as great as 3000 (Squila mantis, Dethier 1953), or 14.000 (Homarus, Balss 1944), while in some forms (e.g. Acrothoracica), the number of ommatidia may be reduced to five (Waterman 1961). Rarely has this diversity in structural variation in the compound eyes of crustaceans been related to function. There is a traditional assumption that the number of ommatidia and photoreceptive cells is proportional to visual capacity. However, species with only a few photoreceptors are known which are capable of reacting to changes in their photic environment in a more rapid and accurate way than species with hundreds of well developed ommatidia (e.g. the copepod Copilia sp. with only one single ommatidial lens: Gregory et. al. 1964). This demonstrates how little we really know at this stage in our quest to understand the effects of environmental factors on photoreceptor adaptation and evolution.

CHAPTER I

THE COMPOUND EYE OF THE ANTARCTIC AMPHIPOD (*ORCHOMENE PLEBS* : AMPHIPODA)

A. SUMMARY

The anatomy of the compound eye of Orchomene plebs, structural changes in dark-light adaptation and temperature induced changes have been studied by light and electron-microscopy. The spectral sensitivity was investigated by electroretinogram (ERG) recordings from retinula cells and direct observations on the spectral absorption.

1. The gross structural organization of the eye of Orchomene plebs is of the apposition type. The ommatidia (approximately 260) basically consist of a corneal cuticle, a crystalline cone of 'eucone' type, five retinula cells, which make up the centrally fused rhabdom (Plate 1-3).

2. In the dark-adapted state the crystalline cones have a larger diameter than those of the light-adapted eye.(Fig. 1). The 'plug' that connects the crystalline cone to the rhabdom is short and wide. Mean rhabdom widths and lengths are 21.5um and 60um, respectively. Screening pigment occupies a distal position between the cones. This has functional significance in that it allows more light to enter the eye (aperture enlargement) and to be transmitted along the rhabdom columm. The interstitial cells contain peculiar 'echinosome' organelles of 0.3um diameter.

3. Light-adaptation is characterised by screening pigment granules surrounding the whole length of the rhabdom. The crystalline cones taper proximally and the mean rhabdom width is 18.7um. The breakdown of rhabdom microvilli is indicated by formation of multivesicular bodies and multilamellar bodies. Hollow spherical vesicles of 0.3um

diameter are found in the interstitial cells (Plate 4-14).

4. The structural integrity of the rhabdom is profoundly altered by heat-stress (10°C). The rhabdoms appear to be in a state of 'melting' and individual microvilli are not discernible. The position of the screening pigment approaches that of the light-adapted state (Plate 1, 15).

5. The heat-stressed rhabdoms when returned to 0°C sea-water show a remarkable recovery of their ultrastructure. The microvilli membranes are distinguishable again. The position of the screening pigment granules approaches that of the dark-adapted state (Plate 16).

6. The eye of Orchomene plebs most efficiently absorbs light consisting wavelengths of 513-474nm (Plate 17). It is under these conditions that the eyes appear darkest. They are lightest in light of 634nm wavelength i.e. red light. Densitometer readings (Fig. 2) also show strongest absorption for green light and lowest for red light.

7. Electrophysiological recordings show a single sensitivity peak indicating that the maximum sensitivity is near 497nm i.e. blue-green light (Fig. 3, 4).

B. INTRODUCTION

The marine environment of the Antarctic is unique because of constantly low temperatures, low ambient light intensities under the sea-ice and a complex system of currents and counter-currents. It provides an ideal habitat for some rich and diverse marine life. The annual mean sea temperature in the McMurdo Sound is -1.81°C, seasonal variation is about 0.08° and vertical thermostratification is virtually non-existent (Littlepage 1965).

Of all animals living in the southern Antarctic sea including those in the McMurdo Sound, amphipods represent one of the commonest groups. Their taxonomy (Hurley 1965; Bellan-Santini 1972; Lowrey & Bullock 1976), general biology (Bone 1972; Ruakusa-Suszczewski & Klekowski 1973), and ecology (Opalinski 1974; McWhinnie et. al. 1975; De Broyer 1977; Wells 1979) have been studied by a number of investigators, but almost nothing is known about the structure and function of the sense organs of these animals. As the eyes are a prominent feature of almost all free-living Antarctic amphipods, it seemed a worthwhile exercise to begin a sensory physiological programme with an in-depth study of the photoreceptors. The constantly low temperature and ambient light level offer an excellent opportunity for investigations of the effects of environmental temperature and light on the eyes of these animals. Another advantage is that in Antarctic waters it is possible to study alive, species that are thought to be closely related to deep-sea forms (Kussakin 1973) which are hard to come by and usually die before the experimenter can carry out his research.

C. MATERIAL AND METHODS

All amphipods used in this study were approximately 20mm long and identified as Orchomene plebs. This species was first described by Hurley (1965) as Orchomenella plebs, but its generic disignation changed when Barnard (1967) combined the four genera Orchomene, Orchomenella, Orchomenopsis and Allogaussia to the single genus Orchomene.

The specimens were caught off Scott Base in the McMurdo Sound at a depth of 140m (77^{o} 52' S; 166^{o} 41' E) in cages made of chicken wire, which were baited with frozen seal meat. The traps were lowered through a 1.5m wide hole in the 3-4m thick sea-ice and left on the bottom for 24-36 hours. The 'yield' per haul varied from literally buckets full to none, indicating that this amphipod has a patchy distribution.

The animals were maintained in transparent plastic aquaria (37 x 27 x 15 ccm) or 25 l light-proof black plastic drums. The temperature of the sea-water was kept at $0^{o}C \pm 1$. Dark-adapted animals were taken from the black drum after the required time of adaptation and dissected under dim red light. For light-adaptation an aquarium with 20-30 amphipods was placed on snow exposed to the sun for varying periods (see details under Results). The temperature remained at $0^{o} \pm 1$ during the dark-light-adaptation experiments. Temperature control during heat-stress experiments, which were carried out in a dark-room, was achieved through the use of a Grant Instruments liquid expansion thermostat to an accuracy of $\pm 0.5^{o}$.

Methods for histological investigations were identical in all eyes studied, irrespective of the light conditions or temperatures that the animals had been exposed to. A 2.5% glutaraldhyde 2% formaldehyde mixture in Millonig's phosphate buffer, adjusted with d-glucose to a 0.6 Mol solution of a pH of 7.4., served as a prefixative. The specimens stayed in this solution for 12 hours before they were washed in buffer and postfixed for 2 hours in 2% phosphate-buffered solution of OsO_4. Dehydration in a graded series of acetone was followed by infiltration with Epon 812 and hardening for two days at 65°C.

At least 8 eyes of each light regime tested and 5 eyes of temperature-stress experiments were successfully sectioned and examined in both light and transmission electron microscope. For light microscopy 1 um transverse and longitudinal sections were stained with toluidine blue for a few seconds on a hot plate. Electron microscope material consisted of golden sections, which were picked up with uncoated 200 mesh copper grids and double-stained with uranyl acetate and lead citrate for 8 and 2 minutes, respectively. Where statistical analyses were necessary, t-test and a significance level of 5% were used.

D. RESULTS

1. Basic anatomy of the eye

(a) General features

The compound eyes of Orchomene plebs and related Antarctic species such as Orchomene rossi and Orchomene grandis are pear-shaped and brown-black in colour (Plate 1A). The two eyes have their narrower ends nearly touching at the apex of the head. The broader part of the eye lies in the lateral position where it is protected by a transparent cuticle extension of the first thoracal epimere. In an average individual of about 20mm, the eye measures 1.2mm in a dorso-ventral extension and consists of approximately 260 ommatidia. The total functional surface of the eye (cornea) averages 2.7mm^2, while the average ommatidial surface area is 0.031mm^2.

The cornea is unsculptured and smooth with no external facets observable. The transparency of the cornea enables the underlying arrangement of separate ommatidia 40-50um diameter to be seen quite clearly. The curvature of the eye-surface and the interommatidial angle vary depending on the region of the eye in which these parameters are studied. An interommatidial angle of 4° is found in the broad ventral part of the eye where the radius of curvature is 0.6mm. The narrower eye region which occupies the dorsal part of the eye also has an interommatidial of 4° and a radius of curvature of 0.6mm even though here the ommatidia are arranged almost perpendicularly to those of the ventral region (Plate 2B). Based on these data, an eye parameter, defined as $p=D^2/R=D\Delta\phi$ (where D is facet diameter, R-eye radius and ϕ interommatidial angle), of 3.3um is calculated. This is

in agreement with the theory that eyes that are to function optimally under low light level have p values in excess of 3 (for further details and derivation of the formula, see Horridge 1977). A pseudopupil, the size of which fluctuates with the state of adaptation which in turn is controlled by the ambient light intensity, can be seen to extend over an area of at least seven ommatidia in living material.

Each ommatidium (Plate 3A, B) which is the individual unit of the photoreceptive element is approximately 140um long in the broad ventral region while towards the narrower dorsal region, it is approximately 120um long. It consists of the dioptric apparatus i.e. cornea and crystalline cone and the photoreceptive components i.e. retinula cells and their rhabdomeres. The length of the dioptric apparatus is 50 ± 5um (based on the average of 10 measurements of 1um thick longitudinal sections of 10 eyes). Nearly two-thirds of the crystalline cone are surrounded by the five retinula cells containing screening pigment granules of 0.5um diameter. The five retinula cells extend from this distal level down to the basement membrane and completely surround the five rhabdomeres within them. This gives the photoreceptive elements a longitudinal extension of about 90 ± 5um. The interstitial cells, which contain organelles of 0.3-0.4um diameter which, depending on the state of adaptation assume a peculiar sea-urchin like shape (dark-adapted) or are hollow sphericles (light-adapted), extend from the base of the cornea to the basement membrane. They, therefore, surround the retinula cells and provide some kind of optical isolation between neighbouring ommatidia. The retinula cell processes or

axons penetrate the basement membrane which separates the retinula from the sub-retinula space and the lamina ganglionaris. The rhabdom forms the core or central part of the ommatidium and measures approximately 65um in length and 22um in width (in the dark adapted state). A collection of relevant data is given in Table 1.

Table 1. The eye of Orchomene plebs

Morphological Data of one representative individual

Length of Orchomene plebs	20 mm
Size and shape of eye	1.2 mm, pear-shaped
No. of facets	approx. 260
Diameter of facets	18 um
Length of Ommatidium	130 um
Interommatidial angle (Dorsal region)	4^{o}
Interommatidial angle (ventral region)	4^{o}

Anatomical Data

Thickness of cornea	5 um
Length of crystalline cone	42 um
Length of crystalline cone process	8 um
No. of retinula cells	5
Length of rhabdom	60 um
Diameter of microvillus	0.1 um
Diameter of pigment granules	0.5-1.0 um
Diameter of 'grey' granules	0.5-1.2 um
No. of axons in one bundle	5

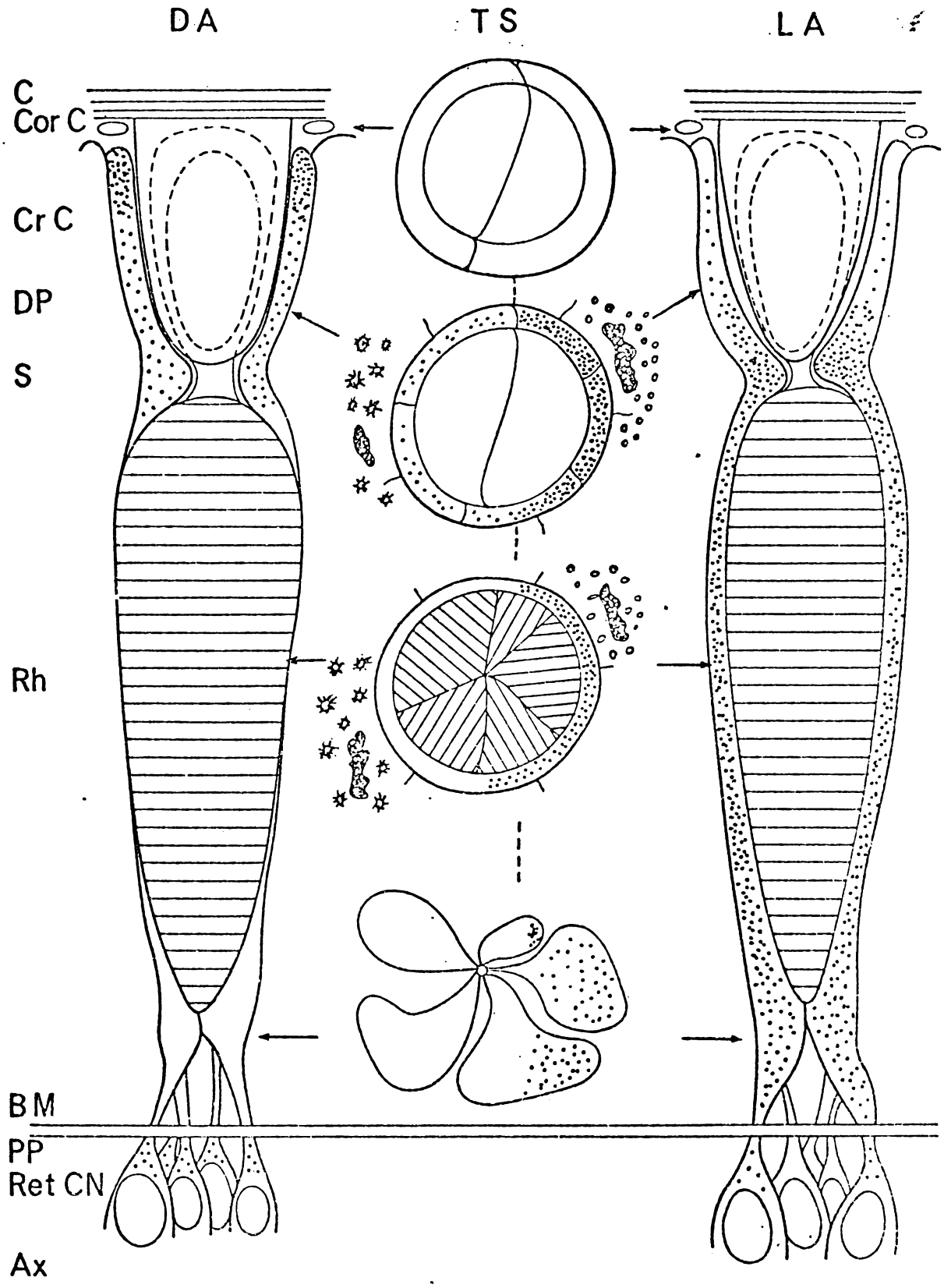
Figure 1

Semi-schematic drawing of dark and light adapted ommatidia in longitudinal and transverse (TS) sections.

When compared with its dark-adapted counterpart, the light-adapted ommatidium has a more strongly pointed proximal end of the cone, an equally long but considerably thinner rhabdom and screening pigment granules that completely envelope the rhabdom. The cone consists of 2 cone cells which are surrounded by distal processes of the 5 retinula cells per ommatidium. The rhabdom is made up of 4 rhabdomeres of more or less equal size plus a very much smaller one belonging to retinula cell number 5. Ultrastructural differences between the two states of adaptation are most obvious in the cytoplasm of the interstitial cells which occupy the spaces between individual ommatidial groups and the nuclei of which, in contrast to those of the retinula cells, are found above and not below the basement membrane.

Abbreviations: C=cornea, Cor C=corneagenous cells, Cr C=crystalline cone, DP=distal pigment, distal processes, S=link between cone cells and rhabdom, RH=rhabdom, EM=basement membrane, PP=proximal pigment, Ret CN=retinula cell nuclei, Ax=axons.

FIGURE 1



(b) Dioptric Apparatus

The dioptric apparatus of the Orchomene plebs eye comprises the smooth cuticle and crystalline cone. When viewed from the outer surface under the light microscope, the corneal cuticle appears to be smooth and featureless (Plate 1A). The surface of the cornea, however, is slightly convex and possesses a radius of curvature of 560 um in the dorsal region and 440um in the broader ventral region. The inner surface of the corneal cuticle is also slightly convex and is separated from the distal end of the crystalline cone by a 4-5um wide space. This space is occupied by the two corneagenous cells whose mottled nuclei and reticulated cytoplasm are easily distinguishable (Plate 1F), particularly towards the edge of the eye from where, after each moult, new ommatidia are recruited.

Semi-thin transverse sections of 1um thickness, stained with toluidine blue, reveal that the corneal cuticle consists of a laminated fine structure. Electron-microscope investigation show that the cornea appears to be composed of three alternately dark and light bands approximately 9-10um thick (Plate 1B). This pattern is characteristic of cuticular chitin (Locke 1964), comparable to that found in insects and other crustaceans. The alternating dark and light lamallae are formed by layers of consistently aligned micro-filaments (Waterman 1961, Bouligand 1965, Fahrenbach 1968, and Meyer-Rochow 1975).

As typical of an 'amphipodean' eye, (Gerstaecker 1884, Debaisieux 1944, Ball 1977 and Meyer-Rochow 1978a), there are just two cone cells per ommatidium. In contrast, the

insects and other crustaceans, usually have four cone cells (e.g. the beetle Creophilus: Meyer-Rochow 1972 and the rock lobster Panulirus: Meyer-Rochow 1975). The two cone cells of the amphipod eye have nuclei located just above the crystalline cone. The two cone cells unite to form a bipartite structure, more or less of a cylindroconical shape with its apex pointing inward. In an average eye, the crystalline cone measures approximately 50um in length. In transverse section, it has a circular outline of approximately 22.8um in its middle plane (Plate 1E). Generally the cone cells are oriented in the same direction with minimal variations and the interface between the two halves of the crystalline cone is regular. The crystalline cone is of the 'eucone-type' which by definition possess a dense cone-shaped central core, secreted intracellularly by the cone regions pointing toward the axis during development. The crystalline cone is found to 'link' the cornea to the rhabdom directly, with no 'clear-zone' in between. This is characteristic of the apposition type of compound eye (Plate 3A, B). The shape and length of the crystalline cone may vary according to the state of adaptation. In the light-adapted state, the cone is found to be more conical in shape and shorter. In transverse and longitudinal semi-thin sections for light microscopy, the crystalline cone exhibits a differential affinity for toluidine blue with a distinctly darker stained central core area. Uranyl acetate and lead citrate stains for electron microscopy also show a similar result with the intracellular differentiation of the cone cells. High magnification electronmicrographs (Plate 1C, D, Plate 4A) show a very electron-dense core surrounded by two layers of lighter

cytoplasm. The electron-dense core is made up of structures approximately 30-40nm in diameter. These minute particles appear to form a very uniform lattice with a spacing constant of 50nm. However, towards the peripheral regions of the cone, the well-ordered arrangement of small core particles breaks up into or is replaced by smaller fragments of approximately 10nm in diameter (Plate 1D). A few mitochondria are also present in this cone. The electron dense particles of the core-centre exhibit similarities with the beta particles of glycogen first described by Drochmans (1962), and later confirmed by Revel 1964, Wolken 1969, Eakin & Kuda 1971, and Elofsson & Odselius 1974. Detailed studies on the biochemical composition of cones have not been carried out but according to Perrelet (1970), who studied the cone composition of the honey bee, beta-glycogen particles present in the cones have no metabolic but an optical function. He postulates that the glycogen particles present in the cones may contribute to the refractive index of the cone and, thus, could be involved in the optics of the compound eye. As glycogen particles are known to be stained by toluidine and lead, the differential staining by these two chemicals may reflect a difference in the amount and distribution of this metabolically and optically important carbohydrate in the cone of Orchomene plebs (Plate 4A).

(c) Photoreceptor Cells

In the eye of Orchomene plebs the photosensory region e.g. the retinula, consists of 5 retinula cells and their centrally fused rhabdomeres. The retinula cells reach distally to form more or less a sleeve round the proximal region

of the crystalline cone (Plate 1E). The retinula cells extend over the entire length of the retinula. Proximally, they taper and separate into individual strands. There is no differentiation into distal retinula cells or proximal retinula cells which are found to occur in other crustaceans and insects. All five retinula cells contribute their centrally pointing rhabdomeres to the fused, spindle-shaped rhabdom in the same position at all levels of the ommatidium (Plate 6A). Interommatidial spaces are occupied by the interstitial cells which number 5-7 per ommatidium. Unlike those of other amphipods such as the deep-sea species Streetsia and the Arctic Pontoporeia, the interstitial cells of the eye Orchomene are devoid of pigment grains, but they do contain epon-resistant granules whose function is yet to be fully explained.

The fused rhabdom, in transverse section, resembles an orange made up of five segments, transversely cut (Plate 6A). With the exception of the fifth cell, the rhabdomeres are more or less of the same size. The segment of the fifth rhabdomere apart from being smaller also exhibits a more distinct reaction to toluidine-blue stain than the rest but microvilli dimensions were found not to be significantly different. The peculiar staining characteristic also helped to establish that the fifth retinula cell bordered by the two cone cell roots (Plate 6B), consistently occupied the same relative position in each ommatidium. The Arctic amphipod, Pontoporeia appears to exhibit this smaller segment of the fifth rhabdomere too, but as in Orchomene plebs the significance of the smaller size of this rhabdomere is not clear.

The size of the rhabdom varies slightly depending on the state of adaptation. In the light-adapted eye the diameter of the rhabdom is approximately 20um, but in the dark-adapted specimens, it is approximately 24um. Individual rhabdomeres possess microvilli which measure 85-100nm in cross section (Plate 11A). These microvilli run in a more or less parallel direction towards the centre of the rhabdom. The well-known pattern in which the microvilli are arranged in alternating layers with their longitudinal axes regularly aligned in a given direction and perpendicular to those of the adjacent layer (commonly found in decapod crustacean, Eguchi & Waterman 1966, and some insects, Meyer-Rochow 1971), thought to aid in the perception of polarized light, is not apparent in Orchomene plebs.

In an average 20mm specimen, the length of the rhabdom is approximately 65um. The rhabdom is connected to the crystalline cone via a peculiar cylindrico-conical buffer zone 8-10um in length and 6-8um in diameter. Apparently this peculiar structure is not present in the eyes of other amphipods (e.g. Gammarus roeselii: Szczawinska 1891, Echinogammarus berilloni: Debaisieux 1944, G. Oceanicus: Ali & Steel 1961, Pontoporeia affinis: Donner 1971, Streetsia challengeri: Meyer-Rochow 1978a). In the eyes of Orchomene this structure consists of densely packed particles which look very similar to the ones found in the peripheral region of the crystalline cone. In cross-section, it shows a bipartite composite structure similar to the crystalline cone structure but lacks the distinctive core-structures (Plate 4B). The position of this structure may be reminiscent of that of the retinula rhabdom lenses in the western rock lobster (Meyer-Rochow 1975)

and the deep-sea euphausiid Thysanopoda tricuspidate (Meyer-Rochow & Walsh 1978), but in view of its composition, there is little doubt that here it is of cone cell origin and not a part of the retinula cells. Orchomene grandis, the related Antarctic amphipod species collected from under 420m of ice i.e. the Ross Ice Shelf, does not possess this peculiar or unusual structure (see Chapter 3).

The rhabdom becomes tapered and phases out at approximately 30um above the basement membrane. The retinula cells at this level branch off into five narrow retinula cell axons being separated from each other by the cytoplasm of the interstitial cells. The retinula cell axons project proximally from each ommatidial group to the basement membrane where they increase in diameter from 5-10um approximately (Plate 3A, B). The nuclei of the retinula cells are found below the 0.5um thick basement membrane which separates the retinula and the sub-retinula space. The retinula cell nucleus is ellipsoid in shape, approximately 11um in length and 6um in width (Plate 3C). At this level most retinula cells still contain grains of screening pigment. Distinct bundles of axons are no longer discernible in the sub-retinula space and connections with second order neurons were not traced.

(d) Screening pigment granules

Generally speaking, arthropod eyes have available a number of mechanisms by which both light acceptance and spectral content of light flux down the photoreceptor can be controlled. Two factors, thought to improve vision in a specific photic environment, are involved i.e. selective absorption and reflection of a specific wavelength of light. Apart from

the dioptric structures, screening pigment granules are known to affect the quality (= colour) and quantity (= the intensity of light) reaching photosensitive cells in the compound eye of arthropods.

Unlike many other crustaceans and insects, the eye of Orchomene does not possess primary or principle and secondary or accessory pigment cells. Instead the screening pigment granules measuring 0.4um in diameter are found within the retinula cells only. The retinula cells have distal processes that stretch between the cones. These retinula processes usually contain numerous pigment granules which account for much of the cytoplasmic inclusions, particularly in the dark-adapted state. Under such condition most of the screening pigment is located either distally around the crystalline cones or proximally near the basement membrane, out of the way of the light-path. Hence, we can distinguish distal screening pigment and proximal screening pigment (Fig. 1). On light adaptation, the screening pigment migrates to its respective position around the rhabdom indicating its screening and absorbing properties. The pigments involved are assumed to be ommochromes (after Becker 1941). The distribution of the screening pigment granules is particularly dense around the rhabdom after light adaptation.

(e) Retinula cell organelles and inclusions

The retinula cells exhibit a wealth of cytoplasmic organelles other than pigment grains, especially in the distal region. However, towards the proximal end with its rhabdomeres, only a few scattered mitochondria and lipid droplets are observed. The presence of these cytoplasmic

organelles suggests that metabolic and synthetic activities are taking place. In the retinula cell cytoplasm endoplasmic reticulum, screening pigment granules, multivesicular bodies, multilamellar bodies, mitochondria and vacuoles of variable shapes and dimensions can be found.

Generally, the mitochondria are found to be arranged along the periphery of the retinula cell membranes. The endoplasmic reticulum is scattered randomly in the cytoplasm but when compared with mitochondria is less abundant here. The distribution of mitochondria within the visual cells of Orchomene plebs is such that mitochondria seem confined to the distal and proximal regions of the retinula cell cytoplasm. A few mitochondria of spherical shape are found at the periphery of the crystalline cone and in the distal interstitial cells (Plate 4A & B, Plate 5A, D, G and H). Structural transformation of the mitochondria may explain the large variety of forms and shapes of these organelles. There appear to be stages of mitochondria transitional between electron dense spherical structures and parallel arrays of crista-like electron-lucent structure (Plate 5H, (Munn 1974)). However, the majority of mitochondria are usually spherical in shape with a few elongated or grotesquely curved ones present amongst them (Plate 5A, G). Near the basal-membrane, the mitochondria appear to congregate around the retinula cell nuclei (Plate 3C). Their size range varies from 1um to 7um in length.

Numerous multivesicular bodies (mvb) are also found in the retinula cells. Most of these are found to be closely associated with the microvillar edge of the rhabdom. Tran-

sitional stages between the multivesicular bodies and multilamellar bodies (mlb), also found in Libinia (Eguchi & Waterman 1967), Squilla (Schiffi & Gervasio 1969) and Palaemonètes (Itaya 1976), were observed in the proximal region of the retinula cells. These transitional stages, involving the mvb and mlb formation, are thought to be associated with membrane turnover, a phenomenon following light adaptation (Itaya 1976, Brammer et. al. 1978). The mvb contain vesicles within them and vary in size from approximately 1.3um to 2.5um. Some of these mvb are found with their membrane ruptured and the vesicles scattered out (Plate 5B, E, H). The multilamellar bodies appear to be concentric ellipsoid structures with sizes ranging from 2.0um to 7um (Plate 5A, C, F).

Vacuoles are a common feature of the cytoplasm. They occur randomly throughout the cytoplasm and also around the edge of the crystalline cone or around the rhabdom as perirhabdomal vacuoles. The perirhabdomal vacuoles observed are not as prominent or obvious as the ones described by Eguchi & Waterman (1967) or the agranular cisternae of Fahrenbach, (1966), the 'palisade' of Horridge & Barnard (1965) or the 'Schaltzone' of Hesse, (1901). These perirhabdomal vacuoles are found to be less abundant and very much reduced in number and size during the process of light adaptation, turning from an elongated convoluted shape into a more or less round shape.

Lipid droplets or granules are found throughout the length of the retinula cytoplasm together with some dense bodies which according to Fahrenbach (1969) are very likely of a lysosomal nature (Plate 4B). It is not surprising that

lipid droplets are a common feature in the Orchomene eye, for it has been established that Antarctic crustaceans are very rich in lipid compounds (McWhinnie et. al. 1975, Meyer-Rochow & Pyle 1979). The lipid organelles of the cytoplasm vary in size from approximately 0.5um to 1.2um and may assume spherical or irregular shapes. They are easily recognizable granules of consistent shape and size and distinguishable from pigment grains by being lighter stained than the latter.

2. Dark-light adaptational changes

(a) The eyes of Orchomene plebs are affected by the presence or absence of light. The changes involve the anatomical integrity of the photoreceptor cells. As with many other arthropods eyes the retino-motoric phenomena and other retinula adaptation mechanisms are designed to control the light flux towards the photosensitive components. These mechanisms are activated when the animal is exposed to light. This causes the protective pigments and sometimes even the nuclei and the rhabdoms to migrate from one position to another (Mazokhin-Porshnyakov 1969, Walcott 1975). Such movements are called retinomotor or photomechanic phenomena and are one of the well-known but not completely understood mechanisms regulating the photosensitivity of the eye. According to Walcott (1975), other mechanism may operate to alter the effective range of the photosensitive components at several levels e.g.

(1) Non-sensory elements inside the eye, such as crystalline cones, as well as sensory structures such as retinula cells may be anatomically affected by ambient light levels.

(2) The receptor can be biochemically affected by bleaching of its photo-pigment and by membrane changes associated with the transduction process.

(3) There may be neuronal adaptations in the integration process in the optic neuropiles.

In this study of Orchomene plebs I have concentrated on anatomical adaptive mechanisms which would affect the light flux within dioptric apparatus and receptors before

the transduction process occurred. Like most apposition eyes, the eyes of Orchomene plebs exhibit pigment granule migrations in the retinula cells in response to dark-light adaptation. A radial pigment migration is known to bring about a change in refractive index within the cytoplasm surrounding the cone and rhabdom in insects (Meyer-Rochow 1974), thereby altering the amount of light capable of passing within and down the rhabdomeres. The same mechanism may operate in Orchomene.

An anatomical and optical study on the firefly, Photuris, led Horridge (1969) to hypothesise that in the light adapted state all the light reaching a receptor comes from its own facet and that it travels by wave-guide modes down the crystalline tract of that same facet. However, the eye of Orchomene does not possess a crystalline tract. It is assumed that in Orchomene the elongated crystalline cone process is to serve the same purpose as a crystalline tract light-guide. Light rays on or near the optical axis of a facet are preferentially accepted. In view of the fact that screening pigment granules enshroud the crystalline cones, light entering the facet at a greater angle of incidence is likely to be absorbed before it has the chance to reach the rhabdom and excite a visual molecule. In the dark adapted situation, additional paths are possible and more light is accepted: because of a sleeve of lower refractive index and fewer pigment granules around cones and rhabdom, light rays striking the facet at a larger angle of incidence, can now be accepted and will travel to the receptor of one, or perhaps even neighbouring ommatidia. Because of the lack of interfering and absorbing screening

pigment granules and because of a wider proximal cone aperture, light may reach the receptor from many facets. This, effectively, increases sensitivity, but unfortunately at the expense of acuity as the image is not necessarily focussed and the acceptance angles of individual ommatidia are large and overlapping (see Parker 1899, Kirschfeld & Franceshini 1969, Horridge 1969, Snyder & Horridge 1972, Kolb & Autrum 1972, 1974, Stavenga 1975, 1977, Ribi 1978). Figure 1 illustrates the anatomical states of dark and light adapted conditions in longitudinal and transverse section. The different specific adaptational states were followed by histological examination involving both light and electron microscopy.

In this investigation of different adaptational states, experiments were carried out to determine the degree of pigment and cell migrations on exposure to varying periods of light and darkness. Most of the more prominent changes can be studied very effectively with the light microscope alone, for instance, the position of the screening pigment. It is found that pigment migrations occur independently of a circadian rhythm. Orchomene plebs can be dark-adapted or light-adapted at any time of the day. Electron microscope investigations were carried out to determine the ultrastructure of the photoreceptors under different experimental conditions and to supplement results obtained by light microscopy.

(b) Light microscopy

With regard to the position of the cells and organelles, the different states of adaptation are best observed in longitudinal sections through the eye of Orchomene plebs (Plate 3A,

B). A typical light-adapted ommatidium is indicated by the characteristic position of the screening pigment granules which are distributed throughout the entire length of the ommatidium (Fig. 1). Light adaptation in the eye of Orchomene plebs appears to be a rapid process inspite of the low environmental temperatures that prevail in the Antarctic seas. Exposures to bright sunlight of 100 000 lux for 40 minutes, 1 hour, 2 hours and 3 hours respectively were carried out while the temperature was carefully maintained at $0\pm1^{\circ}C$.

Complete light adaptation is achieved in less than 40 minutes, which is the shortest time being followed up by histological investigations, for after this time the screening pigment granules had already the characteristic light-adapted position. The distribution of screening pigment granules appeard to play a very important role with regard to the possible light flux down the ommatidia in both light and dark adapted eyes (see Introduction). Clustering of the distal screening pigment granules particular around the narrow 'neck' of the elongated crystalline cone process will change the optical aperture of the ommatidium. This adaptational phenomenon is not restricted to Orchomene plebs but widespread among other crustacean and insect eyes (Walcott 1969, Meyer-Rochow 1975, Nemanic 1975). In the light-adapted lateral eye of Limulus, this very same aperture phenomenon reduces the total amount of light rays reaching the rhabdom to approximately half of that of the situation in the dark-adapted eye (Behrens 1974). In the eye of Orchomene plebs the anatomical effect is clearly observable but it is less pronounced than that of the Limulus lateral eye. The optical

aperture was approximately 7.8um in diameter for the light-adapted and 11.2um in diameter for the dark-adapted state (based on measurements of ten best sections of light/dark eyes). Extensive changes in shape and movement of the cone cells have not been observed but light-adapted crystalline cones are more conical in shape towards the proximal end and measure approximately 45um in length, whereas dark-adapted cones exhibit a broader proximal region and measure approximately 52um in length. These effects are likely to be adaptational changes related to the widening of the optical aperture mentioned earlier. Longitudinal migration of both the distal and proximal screening pigment granules results in the formation of an envelope around the rhabdom in the light-adapted state. Therefore, the distribution of the screening pigment effectively controls the amount as well as the path of the light reaching the receptor cells.

The position of the screening pigment granules is also believed to be involved in the phenomenon of eye-shine (see chapter 4). Eye-shine is caused through illumination by a parallel beam of light which is reflected back out of the eyes by a mirror-like tapetum possibly of guanine or lipid origin. This is in accordance with the principle of the reversibility of light. In light-adapted eyes of Orchomene plebs, eye-shine disappearance may be related to the position of the screening pigment granules. This is supported by the observation of the diminution of the eye-shine area of dark-adapted Orchomene plebs to a minimum size within five minutes after exposure to a bright light (Meyer-Rochow personal communication). It is evident from these observations that much more rapid adaptational phenomena than the ones

observed in histological studies (40 minutes) can occur even at subzero temperature.

Dark-adaptation in the eyes of Orchomene plebs was found to be an almost equally rapid process to that of the light adapted eyes when based on the position of the screening pigment alone. However, in order to ensure that only completely dark-adapted animals were used for this study, they had to stay in total darkness for 7, 24 and 72 hours before being fixed. Temperature once again was maintained at $0\pm1^{o}C$. No significant difference between the three periods of adaptation were observed. In all three cases the results show that the retinula cells and their rhabdoms are generally devoid of screening pigment granules (Fig. 1). The distal screening pigment granules were withdrawn from the rhabdomal region and tended to cluster densely between the distal ends of the crystalline cones and to a lesser extent near the cone-rhabdom junction. The proximal screening pigment granules increase in abundance and become densely distributed in the retinula cells at a level approximately 20um above the basement membrame.

The rhabdom of the dark-adapted eye is more voluminous compared to the light-adapted eye. However, only the diameter and not its length responded significantly to the absence or presence of light (Fig. 1). Results from eight eyes of each light regime studied reveal that the ratio of rhabdom length to width was 2.8 in dark-adapted and 3.2 in light-adapted animals.

(c) Electron microscopy

At the ultrastructural level, the differences in size between light and dark adapted rhabdoms were seen to be negatively correlated with the cytoplasmic area adjacent to the rhabdomeres (Plate 6A, 9A). Such correlation was not observed in the crystalline cone structure, but the width of a space which developed between the crystalline cone and the distal retinula cells was highly variable and affected by dark-light adaptation. In terms of staining properties of the rhabdom as a whole, there was no difference detected with regard to the state of adaptation. Dimensional differences were observed only with regard to the rhabdom as a whole but not in its constituent microvilli. However, the most pronounced changes appear to occur in the retinula and interstitial cytoplasm.

In the light-adapted state, the distal region of the retinula cytoplasm appears to form 'bridges' connecting the peripheral edge of the crystalline cone with the retinula cell plasma (see Plate 3A, 10B). The formation of such 'bridges' gives rise to a number of sub-crystalline cone spaces which are quite different from the perirhabdomal vacuoles occurring near the periphery of the rhabdomes (see chapter 1). The 'bridges' occurred at regular intervals around the periphery of the crystalline cone and a maximum width of 0.7um and a length of approximately 0.5um. The maximum width of the space separating the retinula cytoplasm and the crystalline cone edge is 2.0um. Dark adaptation brought about a reduction in the number of such bridges from about 20 (light-adapted state) to less than five.

Furthermore, the 'bridges' became much narrower in width (about 0.1um). The space on the other hand is very much wider and reaches a maximum of 4um. The formation of such 'bridges' may have functional significance in view of the presence of screening pigment granules (density of 45 granules per $10um^2$ in light-adapted and about 4 granules per $10um^2$ in dark-adapted eye). The narrowness of the space separating the retinula cytoplasm and the cone-edge together with the broader 'bridges' during light-adaptation may enhance the absorbing effect of the screening pigment granules.

The rhabdom in the light-adapted state exhibits a smaller diameter but at the same time a more substantial cytoplasmic envelope (Plate 6A). Screening pigment granules, some of which are in actual physical contact with the microvilli were found within the cytoplasm. The distribution of the screening pigment granules is characteristically dense in the light-adapted state throughout the whole length of the rhabdom (approximately 15 granules per $4um^2$). These screening pigment granules are more or less the same size measuring 0.7um in diameter. On dark adaptation, most of these screening pigment granules are found to be localized in the distal and proximal region of the retinula cytoplasm, hence the density decreased around the rhabdom to as low as 2 granules per $4um^2$. Their functional aspect was discussed earlier. In many rhabdoms of light-adapted eyes, the rhabdomeres show a looser organization with random pock-mark occurring in them (Plate 6A). After exposure to light, the microvilli also bear evidence of membrane disruption. The microvillar organization studied in electron-

micrographs of both cross-section and the longitudinal sections of the rhabdom reveals irregularities (Plate 10B, 11C). In the dark-adapted eyes, the microvilli show a uniformly smooth organization both in cross-section and longitudinal section (Plate 11C). However, the diameter of the microvilli is not affected by the state of adaptation, being a regular 0.1um. The edge of the rhabdom in the dark-adapted state is more or less smooth in contrast to the uneven rhabdom edge of the light-adapted state where pinocytosis seems to be a frequently occuring event (Plate 13A, B).

Pinocytotic phenomena in which round vesicles of approximately 100nm in diameter appear to bud off from the open ends of the microvilli and drift into the cytoplasm were observed only in the light adapted eye (Plate 6A, 13B). It is believed that light absorption is closely coupled to the degree of pinocytosis occurring at the rhabdom edge (Eguchi & Waterman 1967). Vesicles formed during pinocytosis are readily recognized near the base of the rhabdom microvilli. Vesicles of similar dimension were found not only near the site of pinocytosis but further away within the cytoplasm as well (Plate 8A, 13B). Such vesicles may be membrane-bound organelles in the process of forming multivesicular-bodies (mvb) (Plate 8A, 13A). Orchomene plebs definitely differs from the Norway lobster or Orchomene grandis where the photoreceptors are readily and irreversibly damaged by moderate irradiation (Leow 1976, see also chapter 3). Orchomene plebs exhibits close similarities to crustaceans where the rhabdoms show light-induced membrane turnover (Itaya 1976, Eguchi & Waterman 1976, Nassel & Waterman 1979).

Rhabdomal membrane material lost into the cytoplasm was also reported in mosquito rhabdoms, where it is mediated through coated vesicles and multivesicular body formations (Brammer et. al. 1978).

Multivesicular bodies (mvb) which commonly occur in the distal region of the retinula cells appear to increase both in size and number with prolonged illumination. Proximal retinula cell regions contain a much reduced number of mvb even in the dark-adapted eyes. Light-adapted multivesicular bodies when compared to the more tightly packed ones of the dark-adapted eye exhibit a much looser organization (Plate 8A, 13A) within them. Multilamellar bodies (mlb) are generally confined to the proximal parts of the retinula cells in the light-adapted eye. However, there is little and certainly no statistical evidence that mlb are significantly correlated to the state of adaptation in the eye of Orchomene plebs.

Vacuoles in the retinula cells of light-adapted eyes assume a much rounder configuration. Vacuoles appear to be present throughout the whole length of the retinula cells, but near the rhabdom edge they are called perirhabdomal vacuoles (PV), while in the cytoplasm they are referred to as cytoplasmic vacuoles (CV). Perirhabdomal vacuoles which increase in size and number from 0.8um to 1.3um on exposure to light may be of lysosomal nature and related to membrane breakdown. In the dark-adapted state, the vacuoles assume a convoluted shape. Perirhabdomal vacuoles in dark-adapted eyes were rare compared to light-adapted eyes.

Along the length of the retinula cell, the distribution of mitochondria is rather scanty. Most of the mitochondria observed appear to congregate near the distal end of the photoreceptors and in the basal regions where the axons originate. In the light-adapted state, the mitochondria in the distal retinula cell region show an increase in the number of those that are of an elongated shape (density of approximately 5 per $5um^2$). Dark-adaptation causes an increase in the number of spherical mitochondria of smaller dimensions (maximum 1um compared to the length of the longish mitochondria shape of 7um). The density of the mitochondria in the distal region of the rhabdom, is similar to that in the crab Libinia (Hays & Goldsmith 1969), where 70 per cent of the incident light is apparently absorbed, and it is believed that the mitochondria are associated with the active metabolic state of the distal part of the retinula cells. However, in the distal region of the eye of Orchomene plebs, any correlation between dark-light adaptation and the number and size of mitochondria present still lack statistical evidence. Towards the basement membrane, light-adaptation brought about an increase in density, size and shape of the mitochondria around the retinula cell nucleus (see Plate 14A, B). The density may reach a figure as high as 15 per $5um^2$ in the light-adapted state while in the dark-adapted state it is only about 5 per $5um^2$. The mitochondria in the light-adapted eye may well be involved in certain metabolic processess such as membrane recycling.

Lighter stained 'grey' cytoplasmic inclusions, thought to represent lipid granules were found to be richly distributed,

particularly in the distal part of the retinula cytoplasm. These lipid organelles which were clearly more abundant in the light adapted state, clustered near the crystalline cone and the rhabdom edge. The function of any possible lipid structures is not known but because of their characteristic position and their abundance, one may postulate a screening or reflecting function. They may also be involved in membrane turnover, since membranes are largely composed of lipids.

One very significant and important ultrastructure difference between light-adapted and dark-adapted eyes of Orchomene plebs concerns the intracellular organization of the interstitial cells. In the fully light-adapted state, the interstitial cells are densely filled with hollow spherical vesicles (not penetrated very well by Epon) of a rather uniform diameter of 0.3-0.4um (Plate 6B, 9A, 13B), with regard to their size, their fixing and staining characteristics and their abundance (approximately 50 per $10um^2$), these vesicles resemble those reported from pigment cells in the eyes of Astacus (Krebs 1972), Grapsus (Eguchi & Waterman 1973), Procambrus (Fernandez & Nickel 1976) and other crustaceans (E. Eguchi personal communication). They are generally thought to contain reflecting material (Struwe et. al. 1975, Itaya 1976).

Absent from material, light-adapted for 3 hours, but occasionally present among hollow vesicles of the interstitial cells of eyes of creatures, light-adapted for 2 hours and 1 hour are organelles which resemble a sea-urchin. In fully dark-adapted eyes these structures (Plate 9C, 13A) seem

completely to replace the vesicles of the light-adapted cells. Because of their resemblance with a sea-urchin (Plate 9C), the organelles were termed 'echinosomes'. Each echinosome consists of a central hollow area, 300nm in diameter and a dense coat with needle-like 'spines' 80nm long and 8-10nm thick (Plate 9B, C). Cross-sections through the echinosomes reveal that each organelle is surrounded by a membrane approximately 10nm thick. While in dark-adapted material from water of 0°C this membrane surrounds the spiny layer peripherally, it does not do so in animals subjected to heat-stress (see chapter 3). Here the membrane is either lacking or it is found in traces well inside the 'spines' of the echinosomes (Plate 9B).

Temperature induced changes

(a) Apart from light, temperature is one of the most important physical environmental factors that effect the lives of all animals. The poikilothermic animals whose body temperature varies with environmental temperature are particularly affected in terms of their development, growth, metabolism and other complex activities including photosensitivity. The process of photoreception is initiated by photon absorption through the visual pigment molecules and involved photochemical events that lead to the formation of Vitamin A and opsin as end products (Dartnall 1972). Like all chemical reactions this process is temperature-dependent. The chemical reaction is followed by electrical, mechanical and behavioral responses which are also affected by temperature. Electrophysiological recordings in fish and insects have shown changes in potentials such as electronretinogram (ERG), S-potential and spike-potentials (Ali & Kobayashi 1967, Adolph 1973, Ali 1975). Although little studied a photomechanical response of the retinal elements to temperature seems widespread among vertebrates (e.g. fish: Arey 1916, Ali 1964, 1975) and invertebrates (e.g. insects: Day 1941, crustaceans: Parker 1899, Bennit 1924, Eguchi & Waterman 1967, molluscs: Arey 1916). Behavioral responses such as light reflexes, phototaxis and pupillary responses are also affected by temperature (Studnitz 1952). The initiation of flight in some insects e.g. the beetle Acanthoscelides is influenced by both temperature and light intensity (Perttunen and Hayrinen 1969).

According to the law of Van't Hoff the velocity of a chemical reaction is doubled by a rise in temperature of 10°C.

Bearing in mind that the electrical, mechanical and behavioral responses are primarily the result of chemical reations, one could imagine that the role of temperature in photoreception, especially that of poikilotherm animals, would be of immense importance and complexity. In the study of temperature-related photoreceptive adaptations, one has to consider several factors. Some of these factors which are thought to be particularly important in the vision of poikilotherms include: whether or not there is a difference of the visual structure during winter and summer, of animals acclimatized to higher or lower temperatures and of diurnal animals entering shaded conditions and of nocturnal animals entering a bright environment. The marine environments of the tropic and temperate regions are known to fluctuate in temperature often ranging from freezing point to over 30°C in relation to depth, the time of day and the seasons. Most of the marine poikilotherms are able to extend their lethal temperature limits after a period of thermal acclimatization at temperatures near these limits (see review by Newell 1976). In contrast, the Antarctic marine amphipods do not show any improved survival at higher temperature (11°C) when acclimated to the sublethal temperature of ±5°C (Wells 1978).

The Antarctic marine environment with its constant temperature of -1.9°C, where seasonal variations are negligible, provides an ideal natural laboratory for the investigation of the effect of temperature and several species of Antarctic marine amphipods have been studied (e.g. Orchomene chilensis, Armitage 1962, Paramoera walkeri, Rakusa-Suzczewski & Klekowski 1973). However, until now the effect of temperature on photoreceptors of these marine poikilotherms is unknown. In this

investigation the temperature effect on the position of cells and the organelles and the ultrastructure of the eye of Orchomene plebs was studied.

The animals were kept in 10°C warm sea water in a dark-aquarium for a period of 7 hours. After 7 hours at this elevated temperature half of the total number of animals were transferred back to 0°C for another 7 hours before being fixed while the temperature stressed animals were fixed immediately after their 7 hours at 10°C. The temperature induced changes and the possible recovery of any damaged structures could, thus, be studied.

(b) Light microscopy

Animals kept in the dark for 7 hours in sea water of the unnaturally high temeprature of 10°C exhibit grotesquely deformed rhabdoms when compared with light or dark-adapted material at 0°C (Plate 7A, B, C). The rhabdom no longer possess a round profile in transverse section, but have a 'squarish' appearance with concave edges. Longitudinally, some of the rhabdoms appear crooked and no longer attain the usual relatively straight spindle shape. There is some evidence of rupturing of the rhabdom structure observable in some sections. A certain degree of radial expansion of the rhabdom is also seen: maximum diameters reach 30.2um compared to those of light-adapted 0°C material (26.2um), dark-adapted 0°C material (29.2um) and material recovered from heat stress at 0°C (25.4um). Each figure represents the average of 5 measurements taken from five different ommatidia of each experimental state. Furthermore, the position of the

screening pigment granules of heat-stressed dark-adapted animals suggests that the animals had been exposed to light. However, the animals had been kept in the dark and the unescapable conclusion is that an increase in temperature causes the pigment granules to move into a position indicative of the light-adapted state. The screening pigment granules form a denser rim around the edge of the distal rhabdom region which incidentally is also the part that exhibits the greatest degree of distortion. In terms of their staining properties, shape and size, no significant change is observed in the structures of the dioptric apparatus.

When returned to sea water at 0°C and kept in the dark for another 7 hours, many of the heat-stressed amphipods recover. The survival of these amphipods at high temperatures depends on the time of exposure and the rate of temperature increase. According to Wells (1978) at 9.5°C, the median resistance time (LD_{50}) for Orchomene plebs was 12 hours. The survival rate after 7 hours of exposure to heat-stress was approximately 90 per cent. The rhabdom of these recovered animals begin to regain their normal shape and show a decrease in the diameter which indicates contraction of the rhabdom to a certain extent. On the other hand, the cytoplasmic area increases when compared to the heat-stressed eyes (Plate 7C, D). The position of the screening pigment granules approaches that which is characteristic of the typically dark-adapted eye.

(c) Electron microscopy

Under the electron microscope, transverse sections through heat-stressed rhabdoms reveal that the microvilli have become totally disrupted and that holes, irregular folds and vesicles

are scattered between them (Plate 12A, 15A, C). Individual microvillus membranes are no longer discernible and one could get the impression that the entire rhabdom was in a state of 'melting'. In spite of the position of the screening pigment granules which, as in the light-adapted eye, form a sleeve around the whole length of the rhabdom, the ratio of rhabdomere to retinula cell plasma, and the presence of echinosomes in the interstitial cells, clearly show that certain features of the dark-adapted state have been retained.

The retinula cytoplasm of the heat-stressed eyes show a dark stained dense profile when compared to the normal dark-adapted $0^{o}C$ specimens. Even though the rhabdom membranes are in a state of disintegration and 'melting' most peripheral retinula cell boundaries are clearly distinguishable. The screening pigment granules are in extreme proximity of the rhabdom edge, in fact some pigment granules are actually in direct contact with the rhabdom edge (Plate 15C). Other organelles present include electron-lucent bodies which intermingle with the screening pigment granules within the cytoplasm. Their dimensions vary from 0.1um to 0.3um. Multivesicular bodies, multilamellar bodies, vacuoles or mitochondria are scarcely observed. In the specimens that were returned to $0^{o}C$ from $10^{o}C$ heat-stress, the retinula cytoplasm appears to be of an even denser nature. The screening pigment granules begin to move away from the proximity of the rhabdom edge. The electron-lucent bodies show an increase in abundance (approx. 30 per um^2), but other retinula cytoplasmic organelles are not observed. This could be due to the dense nature of the cytoplasm itself.

Because of the severe temperature-induced damage to the

rhabdom membranes, no measurements of the diameters of microvilli in eyes of animals kept at 10°C could be made (Plate 12A, 15A, C). However, rhabdoms of amphipods that were returned to 0°C showed a surprisingly successful recovery. While their rhabdoms usually had a smaller diameter than those of both normal and heat-stressed dark-adapted animals, the rhabdom edge is clearly discernible in contrast to the undefinable rhabdom edge of 10°C heat-stressed. Their microvilli differed hardly at all from those of light or dark-adapted eyes of the normal individuals (Plate 12B, C, D). The only differences observed were that the microvilli and other membrane structures in specimens that had recovered from the heat-stress, stained less strongly and the retinula cytoplasm lacked organelles which were earlier interpreted as liposomes (see chapter 2).

The heat-stress effect is not confined to the retinula region, but extends proximally towards the basement membrane where the retinula cell nuclei are. With regard to staining properties, size and shape, the retinula cell nuclei of heat-stressed eyes differ markedly from those returned to 0°C or the normal light and dark-adapted eyes. The nuclei appear more ellipsoid in shape and have a maximum length of 4um and width of 2.3um. Individuals returned from heat-stress had nuclei which measured 3.7um in length and 2.5um in width. The corresponding values for normal eyes are 4.3um (length) and 3.3um (width) (Plate 16A, B, C). The nuclei are also lighter stained with the nucleus boundary not very clearly marked out at all. The cytoplasm around the nucleus appears to be devoid of the usual mitochondria and lipid granules except for the normal screening pigment granules present. The retinula cell nuclei of those animals that were returned

to $0^{o}C$ approach that of the normal ones. However, the nuclei are darker stained when compared to the heat-stressed animals and the cytoplasm surrounding the nuclei shows the presence of mitochondria. When compared to normal ones, most of them seem to be of elongated shapes and much smaller too.

Significant changes also occur within the interstitial cells of the heat-stressed material. There are fewer echinosomes than in normally dark-adapted eyes, and the ones that are present lack the clear membrane around the 'apines'. The density of these echinosomes is about 10 per $2um^2$ compared to about 30 per $2um^2$ in the material of $0^{o}C$ (data based on 10 measurements taken from material of different adaptational states). This gives the cytoplasm of the interstitial cell a more empty appearance compared to the compact arrangement of the $0^{o}C$ material. Finally, the cytoplasm of the interstitial cells exhibits extraordinary electron transparency and is almost totally devoid of subcellular components other than the echinosomes.

4.

(a) Spectral Sensitivity and Eye Colour

It is an established fact that the ability of an organism to utilize the light quanta incident upon it depends on the spectral sensitivity of its photoreceptors. The term 'spectral sensitivity' refers to the physical concept of the relationship between the sensitivity of a photoreacting system to different wavelengths of the visible spectrum and also intensity. Hence spectral sensitivity is generally used to describe an animal's visual reactivity to light. Colour vision has been intensively and widely studied among vertebrates, especially in humans. However, the phenomenon of colour vision is not an exclusive property of primates and birds. Among the invertebrates, the arthropods (particularly insects and crustaceans) are well known to be able to discriminate wavelengths. Wavelength discrimination in crustacean compound eyes was first demonstrated by Von Frisch & Kupelwieser (1913) in slightly light-adapted Daphnia using behavioural methods. Depending on the species, more than one type of visual pigment may be present in the compound eyes of crustacea. The receptor cells may exhibit absorption maxima, and hence greatest sensitivity, in the violet, yellow and yellow-violet as in the crayfish Procambrus (Eguchi, Waterman & Akiyama 1973). If cells with different spectral sensitivities exist in one eye, colours and colour patterns may be distinguished.

In the determination of the eye's spectral sensitivity or colour vision, usually four general methods can be employed:

1. Behavioural experiments - by conditioning the animal to respond to different colours. This method involves the demonstration of the animal's ability to distinguish

colours seen either successively or simultaneously solely on the basis of the wavelength differences of the test colours and not by the different brightness of the colours.

2. Chemical process - chemical extract of the visual pigment of the eye and study of the transmission of the visual pigment solution with a micro-spectrophotometer.
3. Direct observation both optically and in vivo of the photoreceptors and their absorption characteristics using different spectral lights and
4. Electrophysiology - measuring the response of the eye or the visual cell to brief flashes of light of different colour but equal intensity.

In this investigation, spectral response characteristics of the amphipod Orchomene plebs were obtained using the methods (3) and (4) described above.

(b) Material and Methods

The amphipod Orchomene plebs was obtained as described in chapter (1). Specimens were maintained in aerated sea-water of $0^{o} \pm 1^{o}C$ in a dark room for up to 10 days. Only healthy individuals of at least 15mm total body length were selected for experiments. The experimental set-up consisted of the standard recording equipment (cathode follower, oscilloscope) and the stimulating apparatus (75w Xenon arc lamp, electronically-operated shutter, neutral density filters and Schott interference filters). Photographs of the eyes were taken with a Nikon F2 camera through the optics of a binocular microscope, and electroretinogram recordings were

carried out with KCl -filled glass-electrodes. A densitometer with a mercury vapour lamp (model Vitatron TLO 100) was used to measure absorbance (I_e-log) across the amphipod eye on photographs taken under different spectral lights. More details on filters etc., are described under results section.

(c) Results

The eyes of Orchomene and other Antarctic amphipods show an interesting eye-glow, which is caused by reflecting or fluorescing substances present in the cells surrounding the rhabdom (Meyer-Rochow & Tiang (1979)). As a result of this arrangement the wavelengths which are maximally absorbed by the rhabdoms give the eye a dark appearance. However those wavelengths that pass through the rhabdom without being weakened by absorption are reflected and consequently give the eye a bright appearance or eye-glow. Using this phenomenon, the experiments were carried out to determine the degree of light absorption in Orchomene plebs eyes at 0°C. This is done by exposing them to monochromatic light of the following wavelengths 634, 614, 593, 574, 552, 533, 513, 494, 474, 452, 438, 413, 393, 373, 353 (all in nm and white light).

A series of photographs with exposure times of 5, 10, 20, 40 and 80 seconds for each filter were taken under white light and light of 634, 513, 474 and 393 wavelengths. To illustrate what the human observer sees when he examines the Orchomene eye, pictures most closely resembling the real situation were selected. The results (Plate 17) showed that the eye of Orchomene plebs most efficiently absorbs light consisting of wavelengths of 513-474 nm (Plate 17B, C). It was within this range that the eye appeared darkest. Under

red light (634nm) and also wavelengths shorter than 474 nm the eye was much lighter and most of the light seemed to be reflected out of the eye again (Plate 17A) producing eye-glow. It was assumed that reflecting vesicles in the eye behaved like a 'mirror' and that their own spectral absorbance characteristics were negligible in comparison to those of the rhabdoms.

Quantifying the results obtained further, photographs of eyes illuminated by red, green and blue lights were scanned by a densitometer (Fig. 2). The densitometer reading confirmed that under red light the eye is lightest (i.e. absorbs least light) and under green light the eye is darkest (i.e. absorbs most light). These results and the direct observations of the amphipod eye during exposure to 15 different spectral lights (see filters above) indicated the existence of a single sensitivity peak near the 513 nm mark. Electrophysiological ERG (electroretinogram) technique was used to confirm that there was only a single peaked, sensitivity maximum in the eye of Orchomene plebs.

A glass microelectrode, filled with KCl solution was carefully driven with a micro-manipulator through the cuticle of the eye into the retina of a living, but tethered animal. To calibrate the eye brief flashes of light of one wavelength but varying intensities were delivered to the eye, and the response height in mv of the latter was recorded (Fig. 3). This calibration run was followed by a spectral run in which the responses to all 15 different wavelengths were recorded and mathematically converted into sensitivity equivalents (Fig. 4). Although not many test animals survived the entire run (after the spectral test another calibration series was

carried out to see whether the eye was still healthy and in the same state of adaptation as before the test), results of those animals that did survive supported the theory of a single sensitivity peak. With more filters than were used in the direct observation experiments, the electrophysiologically obtained results allow one to predict that Orchomene plebs possesses eyes with an optimal sensitivity near 497 nm (blue-green light).

E. DISCUSSION

1. Basic anatomy of the eye

There are only minor differences between the gross structural organization of the eye of Orchomene plebs (fam. Lysianassidae) and that of other species, representing different amphipod families (Fam. Gammaridae (Szczawinska 1891; Debaisieux 1944; Ali & Steele 1961)); fam. Scinidae (Hanstrom 1933), fam. Haustoriidae (Donner 1971); fam. Phronimidae (Ball 1977); fam. Oxycephalidae (Meyer-Rochow 1978). The fifth retinula cell and its rhabdomere are considerably smaller in Orchomene plebs than in the other species investigated, and the 'plug' connecting the crystalline cone and the rhabdom seems a unique feature in this eye.

In the Antarctic Orchomene plebs, the eyes are very much larger and the ommatidia more numerous than in the eye of the Arctic Pontoporeia affinis (Donner 1971). A further dissimilarity concerns the crystalline cone which is lens shaped in P. affinis but elongated in Orchomene plebs. While the small, whitish eyes of Pontoporeia appear degenerate and inefficient, there is no doubt that Orchomene individuals use their eyes and can distinguish different light intensities; they aggregate in the darkest corner of the aquarium. In nature, both Antarctic (Orchomene plebs) and Arctic (P. affinis, Donner 1971) species inhabit water from just below the surface to several hundred meters depth, and why there should be a difference in eye development between the Arctic

and Antarctic species is not known at this stage.

Compared with Orchomene plebs anatomical differences were also observed in the eyes of Orchomene grandis (another Antarctic amphipod, living under the Ross Ice Shelf, see chapter III). In a typical ommatidium of Orchomene grandis, the distal rhabdom region is in close contact with the crystalline cone surrounding the proximal tip of the latter. The rhabdoms in O. grandis are very much larger and screening pigments granules are only seen in minute quantities. The 'plug' found in Orchomene plebs is absent. Anatomical differences observed could be due to environmental adaptation, for Orchomene grandis inhabits a virtually completely dark environment under 420 meters of ice. In fact the very existence of a functional eye in Orchomene grandis has been a biological 'puzzle'. However, for O. plebs we can assume that a certain amount of light reaches its habitat and that this light is enough for the animal to 'see' in.

2. Structural changes on Dark-Light-Adaptation

Retinomotoric responses in which the screening pigment migrates inside the retinula cells, resemble those observed in other amphipods (Szczawinska 1891; Parker 1899; Bennit 1924; Debaisieux 1944; Ali & Steele 1961), although Orchomene plebs may not possess quite as many pigment granules as the other species investigated. As in many insects, where similar pigment migrations occur, the position of the screening pigment is thought to influence the light flux down the rhabdom and thus the sensitivity of the eye (Bernhard & Ottoson 1960). With this mechanism alone, sensitivity

improvements of around 3 orders of magnitude should be possible when the eye is dark-adapted (Walcott 1971 recordings from Lethocerus compound eye, which has pigment migrations similar to those reported from Orchomene plebs). The Antarctic environment is so stable that it is not really surprising to find that Orchomene plebs lacks the circadian rhythm which controls the state of adaptation in many decapod crustaceans (e.g. Procambrus (Arechiga & Wiersma 1969)) and terrestrial arthropods (e.g. Tenebrio (Wada & Schneider 1968)). However, even in amphipods from temperate climates these rhythms seem little involved (Szczawinska 1891; Debaisieux 1944; Ali & Steele 1961). A circadian rhythm is also absent in Glyptonotus antarcticus (the Antarctic giant marine isopod) and in Orchomene grandis (RISP amphipod).

Anatomically, the two extreme states of adaptation in the eye of the Orchomene plebs agree with observation made in other amphipods at the end of the last century (Szczwinska 1891; Parker 1899). However, one feature missed by these and later investigators (Bennitt 1924; Debaisieux 1944; Ali Steele 1961) is the diminution of the rhabdom as a response to light. A further significant difference not visible in the light microscope and therefore not known to earlier researchers of the amphipod eye, is the change of hollow vesicles in the light-adapted eye into the 'echinosome' organelles of the dark-adapted interstitial cell. Clearly, the abscence or presence of light not only exerts an effect on light perceiving structures (rhabdom and retinula cells) but also on the contents and the cytoplasmic consistency of the interstitial cells. According to W. Fahrenbach (personal communication) the electron microscopical appearance of the

echinosomes suggest that they contain fluorescent substances, but whether such components which have recently been extracted from a variety of crustacean eyes (Lima-Zanghi & Bouly 1969), are involved in the visual process remains speculation at this stage (Meyer-Rochow 1978c). The eyes of Orchomene plebs can thus be said to follow a normal pattern of dark-light adaptation. However, in contrast, the eyes of Orchomene grandis (RISP amphipod), exhibit strong light-induced damage after only two hours of exposure to sunlight (see chapter III).

3. Temperature induced changes

The most striking result is no doubt the effect of temperature on the structural integrity of the eye of Orchomene plebs. While functional aspects in relation to different temperatures have been studied in a number of compound eyes (Calliphora: Hamdorf & Keller 1962); Eupagurus: Stieve 1963; Apis: Seibt 1967; Limulus: Srebro & Behbehani 1972; Oncopeltus: Dudek 1975; Calliphora: French & Jarvilehto 1978, no research apart from some older light microscopical studies by Congdon (1907), Bennitt (1924) and Ali & Steele (1961) have been carried out to investigate the anatomical and ultrastructural changes induced by temperature.

In this investigation it was found that heat stress causes the retinal pigment in dark-adapted eyes of Orchomene plebs to migrate into a position characteristic of light-adapted eyes. However, investigations of the effect of a raised temperature on the position of the retinal pigment in decapod crustaceans (Congdon 1907), freshwater amphipod and decapod of temperate zones (Bennitt 1924) and an insect (Ephestia: Day 1941) show a contradictary result: increase in temeprature

causes the retinal pigment to migrate towards the dark-adapted state. My result obtained from Antarctic amphipods agree with that of Ali & Steele (1961) who reported a similar phenomenon for the Arctic Gammarus oceanicus when the temperature reached at least 27°C. Temperature-stress investigations on another Antarctic crustacean, Glyptonotus antarcticus, also show that in increase in ambient temperature causes the retinal pigment to migrate towards the light-adapted state (see chapter II D3). Biologically a response of this kind would, of course, makes sense as the warmest conditions would usually be encountered together with the brightest light (for example in a shallow water interstitial melt pool). There exists a casual observation, worth following up, that even the electrophysiologically recorded resting potential of the retinula cell drifts to a level characteristic of the light-adapted state when the temperature is raised (S.B. Laughlin, unpublished observation on Glyptonotus antarcticus).

The mechanism of this heat-generated light adaptation is not clear. As a matter of fact, one ought to consider perhaps the possibility of a light-generated heat effect if it is taken into consideration that in the light the dark screening pigment granules absorb radiation and warm up. Nobody appears to have measured or calculated this localized temperature gain. Whatever the mechanism, the results show that light adaptation in heat-stressed animals kept in a dark environment was not complete; echinosomes, found only in dark-adapted individuals, are still present and the rhabdom has still its large diameter typical of the dark-adapted condition.

Ultrastructurally, the damage to the architecture of the normally well ordered microvilli is the most significant feature. It would be difficult to see how under these conditions the eye could possibly function, particularly if, as Srebro (1966) believes on the basis of electrical responses recorded from the lateral eye of Limulus, the temperature directly affects the visual pigment. However, while one can only speculate on the functional consequences of the destroyed microvilli in Orchomene plebs, one does know from recent gaschromatographical analyses by Meyer-Rochow & Pyle (1979) that the eyes of Antarctic amphipods and fishes are extremely rich in long-chain unsaturated fatty acids that posses characteristically low melting points. Lipids of this sort may be necessary in a very cold environment, but it is conceivable that a sudden temperature increase from 0 to 10°C is enough to cause dramatic alterations of the lipid component of the membranes (as was shown in the muscle lipid composition of the freshwater cryfish Austropotamobius by Cossins 1976). This might explain the disrupted, swollen, fused and twisted microvillus membranes in the heat-stressed materials. However, in the eyes of Glyptonotus antarcticus, heat-stress did not result in a disruption of microvilli comparable in its severity to that observed in Orchomene plebs. There is a possibility of a difference in lipid components of the rhabdom structures for it was shown that Glyptonotus is more resistant to freezing and thawing than O. plebs (even to -6.5°C McWhinnie et. al. 1975). Orchomene plebs was unable to survive even the briefest period of freezing (Wells 1979).

Another remarkable fact of the Orchomene plebs photoreceptor is that within 7 hours, the visual membranes reorganize,

microvillus patterns reform and the rhabdom structure fully regenerates, provided the animal is returned to its normal environmental temperature of $0^{\circ}C$ or below. Whether, however, parallel to the structural recovery, the function of the eye is equally restored, cannot be answered at this stage.

In conclusion, one may note that light directly affects the visual molecules embedded in the membranes of the rhabdom microvilli (the ordinary physico-chemical process of visual perception). Temperature, on the other hand, seems to alter the basic properties of the membranes themselves. How the two stimuli influence the visual properties of the eye, and how they interact are problems that remain to be solved. What has become evident from this investigation is that light and temperature cause separate but intricately related effects that must not be studied in isolation from each other.

4. Spetral Sensitivity and Eye Colour

Considerable research has been carried out to investigate whether animals are able to perceive and distinguish colours in the same way as humans do. The elaborate and sophisticated behavioural experiments of V. Frisch and Daumer (1956) have demonstrated conclusively that the honey bee possesses colour vision. However, the fundamentally trichromatic colour vision system of arthropods differs in one important respect from that of human colour vision: ultra-violet, not normally seen by vertebrates is a colour of great significance in insects and crustaceans (e.g. honey bee von Helversen 1972 and Daphnia Heberdy, 1949). In humans the filtering action of the cornea and lens cut off the short wavelength, i.e. ultra-violet light, and the visual pigment itself shows maximum absorbance in the

green. It is now known that many species of insects, fishes and birds are able to see colours. It became apparent that under dim light conditions (at night, in the deep-sea or in caves) the sensitivity to one region of the spectrum was often improved at the expense of the ability to distinguish individual colours.

Deep-sea organisms in particular exhibit sensitivity peaks which coincide with the type of light present in their environment. The latter could originate from luminescence or sunlight filtered through hundreds of meters of water (Denton & Warren 1957, Boden et. al. 1961, O'Day & Fernandez 1974).

In Antarctica the light not only has to penetrate water, but also has to pass through several meters of ice. The transmission of (white) sunlight through 'in situ' sea ice has been measured by Littlepage (1965), but the spectral composition of the light filtering effect of sea-ice, a one meter round hole of 10cm diameter was melted into the sea ice. It appears blue to the human observer (Plate 17D), and considering that the epontic green and brown diatoms present in and under the sea-ice (Bradford 1978) further affect the spectral composition of the light, this indicates that the light reaching the deep-water of McMurdo Sound is predominantly blue-green in colour.

If this indeed is the case, it is not surprising to find that amphipod eyes possess only one sensitivity peak which lies in exactly this part of the spectrum. Whether the sensitivity peak of the eye of Orchomene plebs changes with the seasons and the sun angle (as in Procambarus crayfish: Nosaki 1969), and whether and to what extent the eye is sensitive to polarized light are problems that remain to be answered.

PLATE 1

The eye of Orchomene and its dioptric structures

A Close-up photograph showing head and darkly pigmented eye of Orchomene.

B The cornea consists of 3 major layers which are separated from each other by narrow dark bands.

C This longitudinal section through the crystalline cone shows that in the border region between cone core (left) and cone margin (right) granules, probably consisting of glycogen, form string-like arrangements (arrowheads).

D Transverse section through the cone reveal that most of the central part of the cone consists of a regular lattice of granules measuring approximately 30 nm in diameter. The surrounding peripherally-located cytoplasm is rich in tiny particles, 10 nm in diameter, which probably represent ribosomes.

E Transverse sections through the cones reveal that the latter are composed of 2 cells orientated in the same way, and that the inner cores of the cones show a stronger affinity to the stain than the peripheral regions do. The screening pigment visible between the cones is contained in distal processes of the retinula cells.

F Longitudinal section through an area of the eye where new ommatidia develop. In this region the corneagenous cells (Cor C) and their nuclei are large. The crystalline cone and its darker core, retinula cells and their pigment grains (Ret C), and interstitial cells filled with spherical vesicles (V), are clearly discernible.

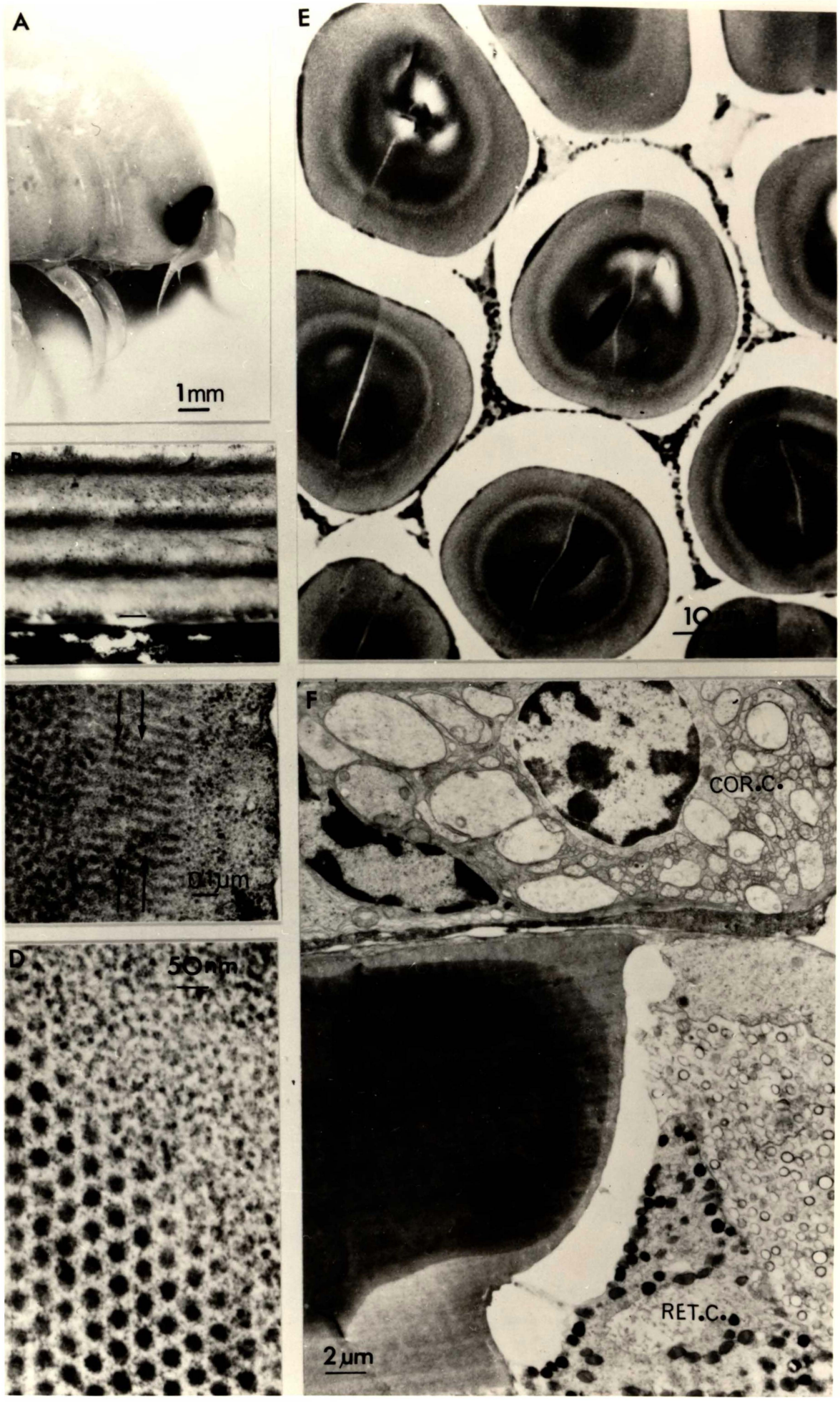
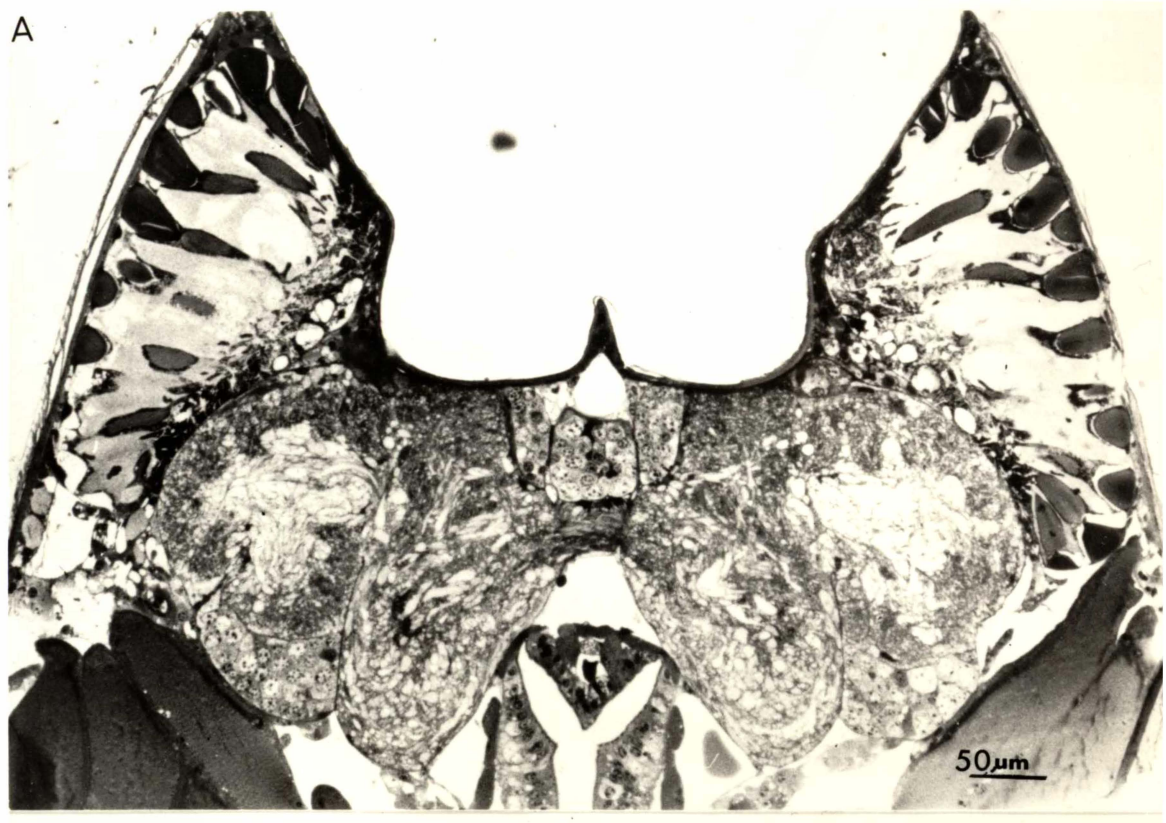

A
1mm
E
10
F
COR.C.
RET.C.
2 µm
0.1µm
D
50nm

PLATE 2

The compound eye of *Orchomene plebs*

A Oblique section through the ventral part of the head. Note the large crystalline cones and rhabdoms of the compound eyes.

B Vertical section through the head of *Orchomene plebs* showing variation in the size and angular arrangement of Ommatidia. Dorsally located ommatidia are oriented towards perceiving light from the dorsal region while the ventral regions have ommatidia arranged towards vision to the sides and below. Interommatidial angles of the extreme dorsal and ventral regions differ from the 4^{o} of the more centrally located ones. The two eyes are separated at the apex by about 400 um.


A
50µm

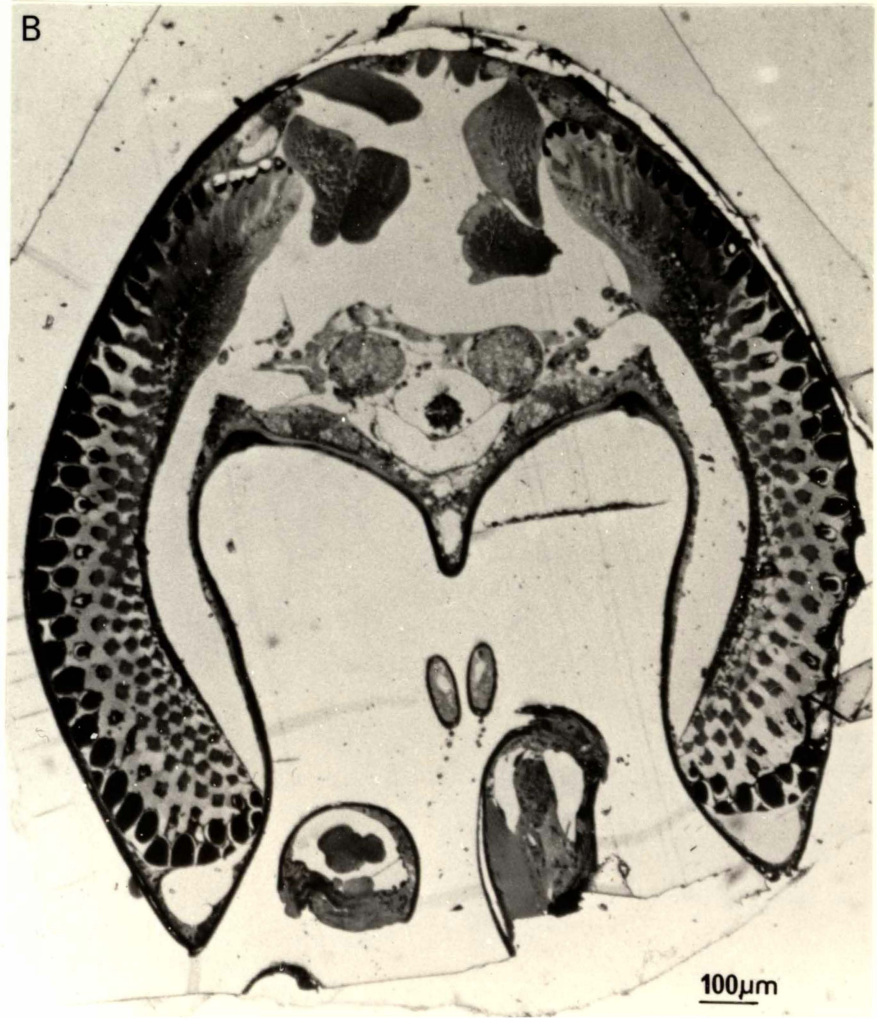

B
100µm

PLATE 3

Ommatidial organization and inter-ommatidial space

A In the light-adapted eye the screening pigment granules of the retinula cells surround the entire rhabdom (RH), and the rhabdom length to width ratio is 3.2.

B In the dark-adapted eye screening pigment is mostly found above and below the rhabdom, which is connected to the dioptric structures via specialized regions of the two cone cells. The ratio of rhabdom length to width is 2.8 in the dark-adapted ommatidium.

C Transverse section through the axons of the photoreceptors at the level where the retinula cell nuclei are located. At this basal level, numerous mitochondria and screening pigment granules are observed.

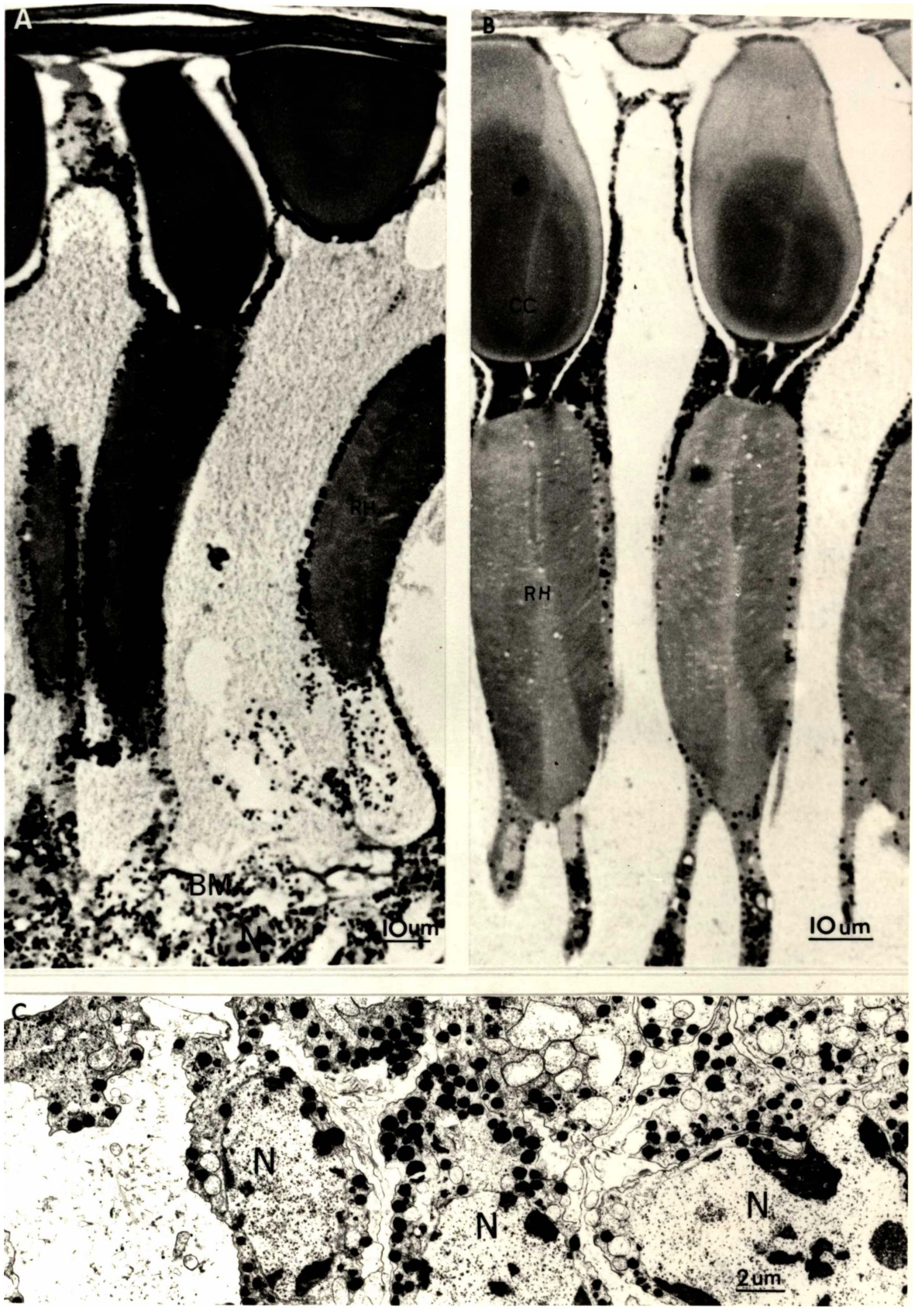
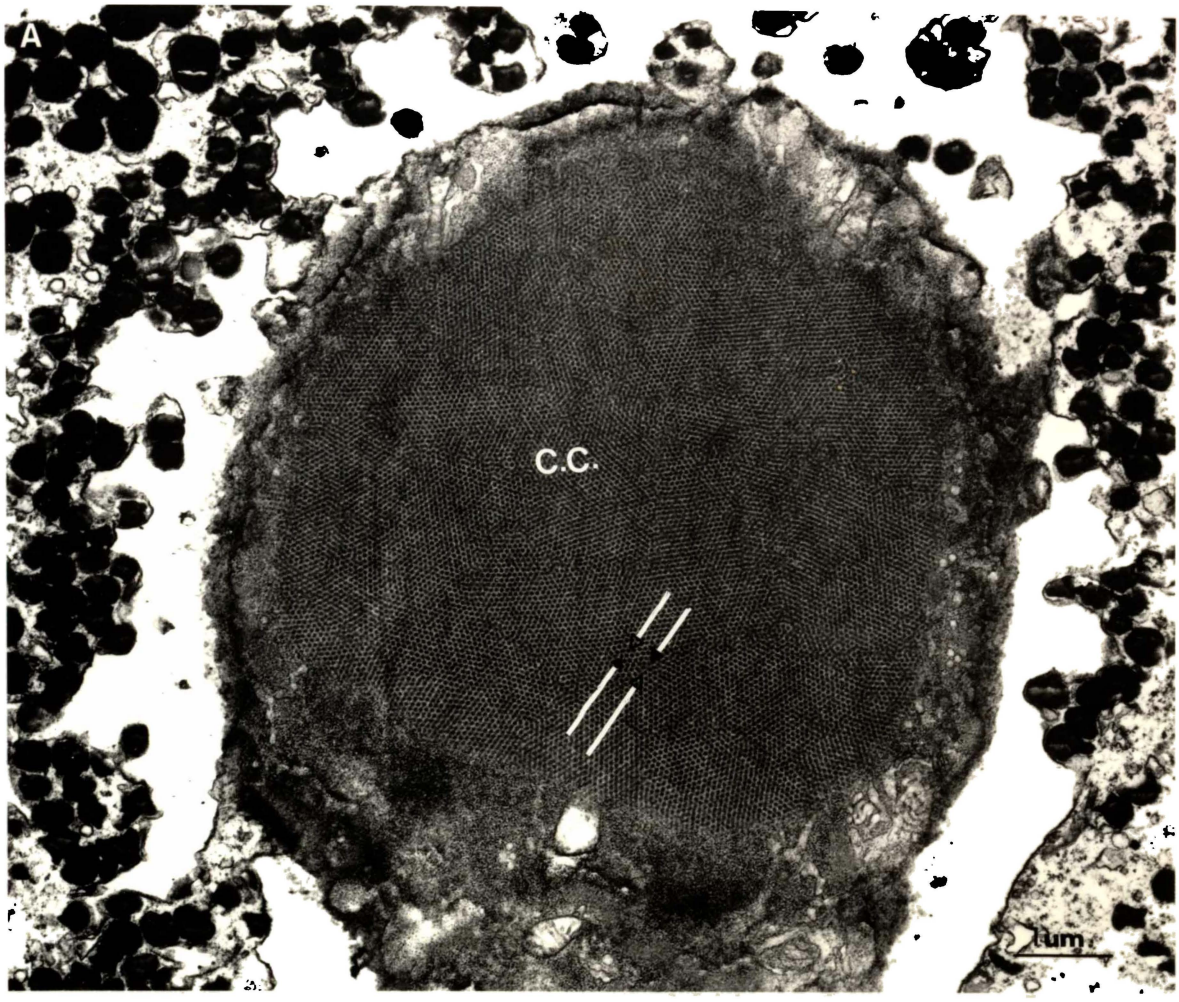

A
RH
BM
N
10um
B
CC
RH
10um
C
N
N
N
2um

PLATE 4

Ultrastructure of dioptric apparatus

A Oblique section through the light-adapted eye of the crystalline cone which shows the central part of the cone to consist of mainly a regular lattice of tiny granules. Towards the periphery even smaller particles and mitochondria are observed. The retinula cytoplasm forms regular 'bridges' joining the periphery of the crystalline cone. Pigment granules are also noticed to increase in abundance around the cone edge.

B Transverse section through the dark-adapted eye of the crystalline cone to the rhabdom. It still shows the continuing bipartite structure of the crystalline cone, surrounded by five retinula cell but it lacks the regular lattice of granules of the central core of the crystalline cone. The so-called 'bridges', with which the retinula cells are connected to the periphery of the cone, have diminished and the space separating the cone and surrounding tissue has increased. The black screening pigment granules around the edge of the cytoplasm have more or less been replaced by the grey granules, which may contain lipids.


A
C.C.
2 µm

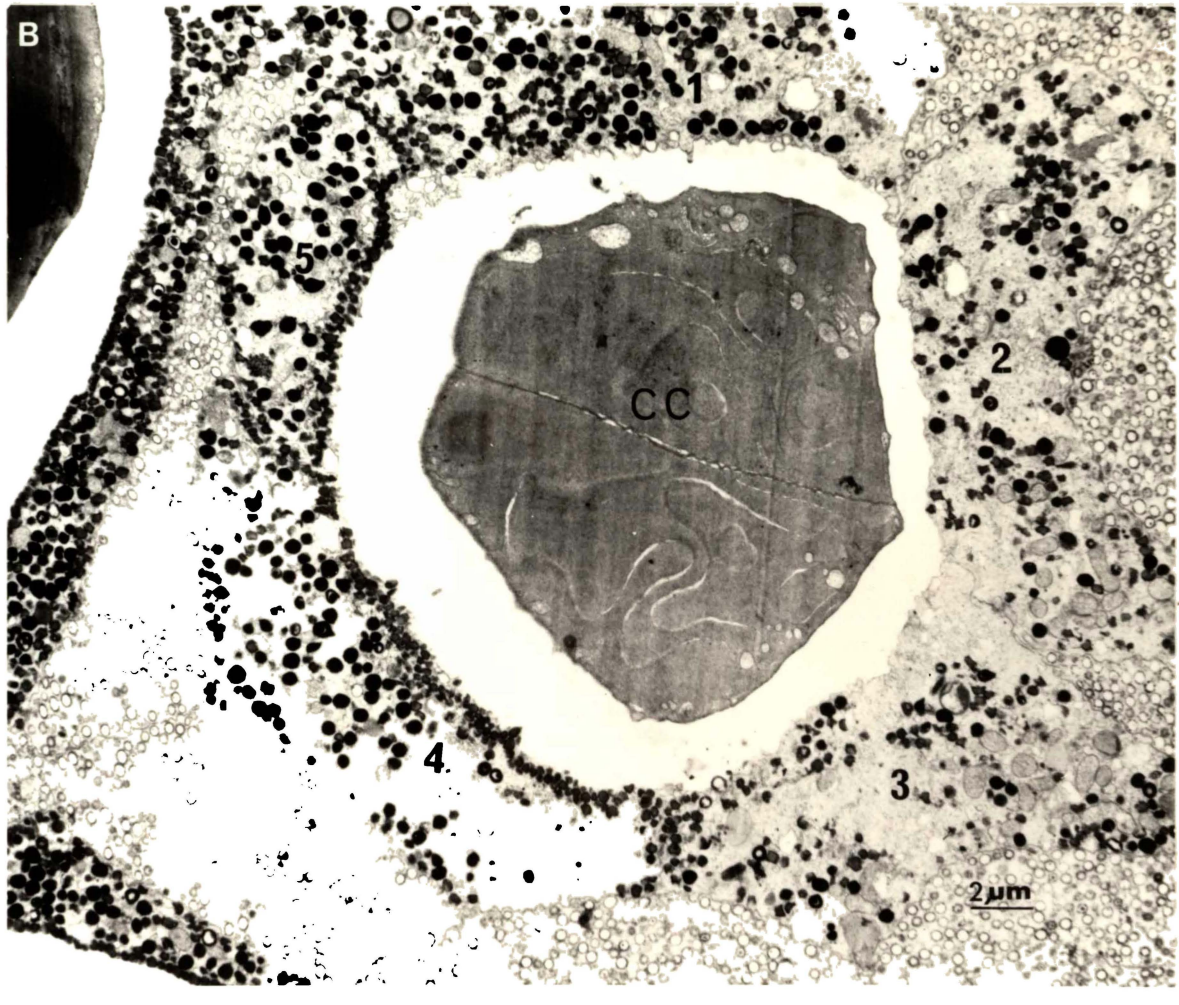

B
1
2
3
4
5
CC
2µm

PLATE 5

Retinula cell organelles from both light and dark-adapted material

A Longitudinally and transversely cut mitochondria of various sizes and shapes of the distal retinula cell region.

B Multivesicular body (mvb) with vesicles closely packed within it.

C Multilamellar body ('onion body') (mlb) with darker stained membranes.

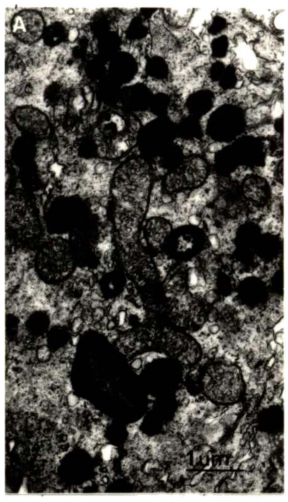
D Screening pigment granules near rhabdom edge with some 'grey' granules in the cytoplasm. Three peculiar mitochondria of the interstitial cells arranged in a row are surrounded by hollow vesicles typical of the light-adapted state.

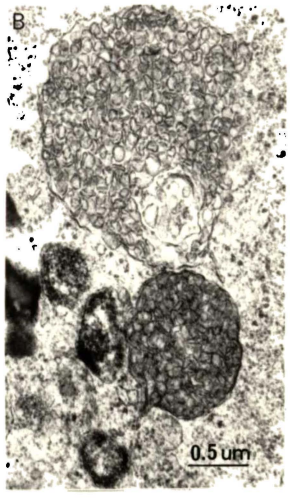
E The complex cytoplasmic bodies from retinula cells include mvb and mlb. It appears that a transitional process is taking place from mvb to mlb.

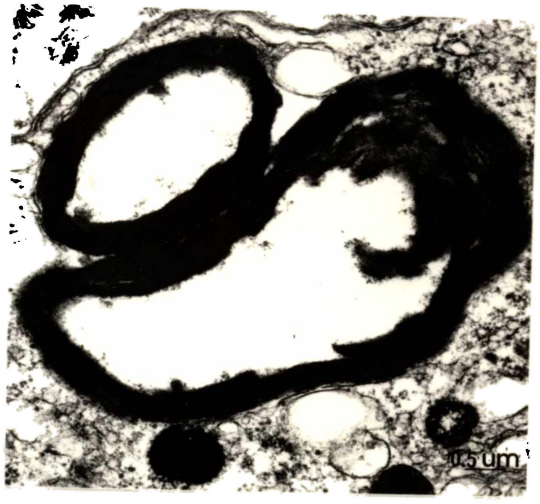
F Multilamellar body surrounded by screening pigment granules. A peculiar membrane bound organelle lies at the lower edge (arrow).

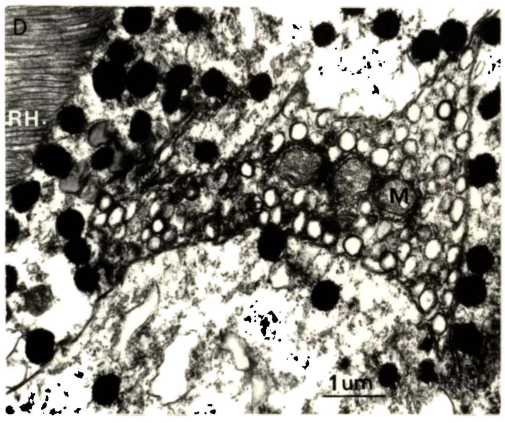
G Concoluted worm-like mitochondrion of the proximal region of the retinula cell.

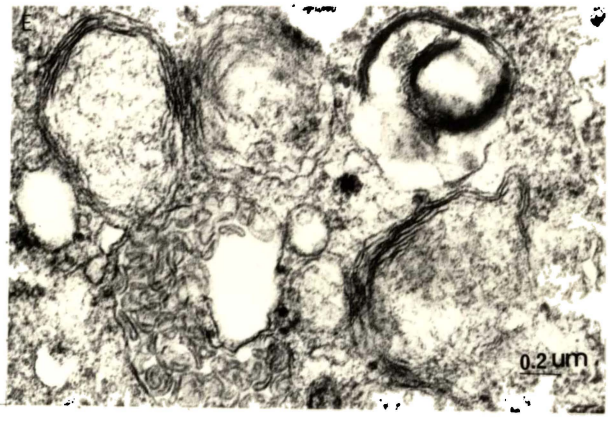
H Multivesicular bodies, mitochondria and unidentified dense bodies. Some of the mvb have ruptured, spilling the membrane-bound vesicles into the cytoplasm. A peculiar membrane-bound organelle with rod like parallel membrane formations inside it is also found (arrows).

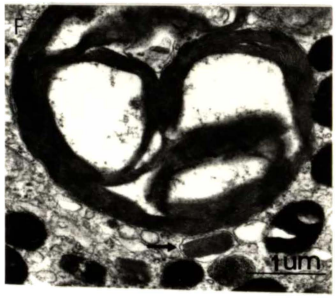

A
1 um

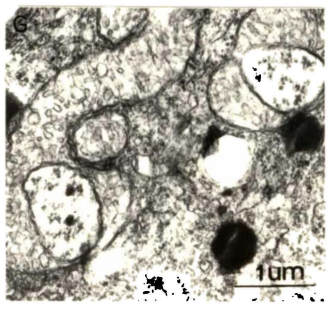

B
0.5 um


0.5 um


D
RH.
M
1 um


E
0.2 um


F
1 um


G
1 um

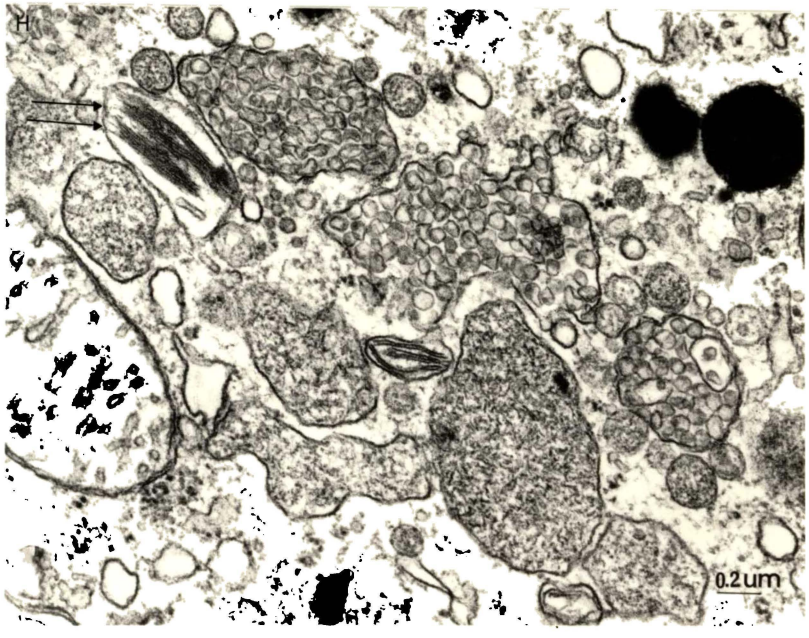

H
0.2 um

PLATE 6

Retinula cells and rhabdom ultrastructure

A This electron micrograph of a transverse section through an ommatidial group of 5 retinula cells at mid-rhabdom level shows how the rhabdomeres of the five cells give rise to the centrally fused rhabdom. Pigment granules surrounding the rhabdom, vesicles budding off the inner margin of the rhabdomeres (inset) and hollow vesicles in the interstitial cells are indications of the light-adapted state.

B The small retinula cell number 5, bordered by two cone cell processes (arrows), has only a tiny rhabdomere, the microvilli of which do not differ from those of the larger rhabdomeres. All retinula cells contain black screening pigment granules of 0.4 um diameter, and grey structures of rather similar dimensions but more irregular outlines. The latter are thought to contain lipids.

C This section through a dark-adapted eye shows a typical retinula cell mitochondrion, which is of the tubular crista type (arrow), and "echinosome" organelles in the cytoplasm of an interstitial cell.

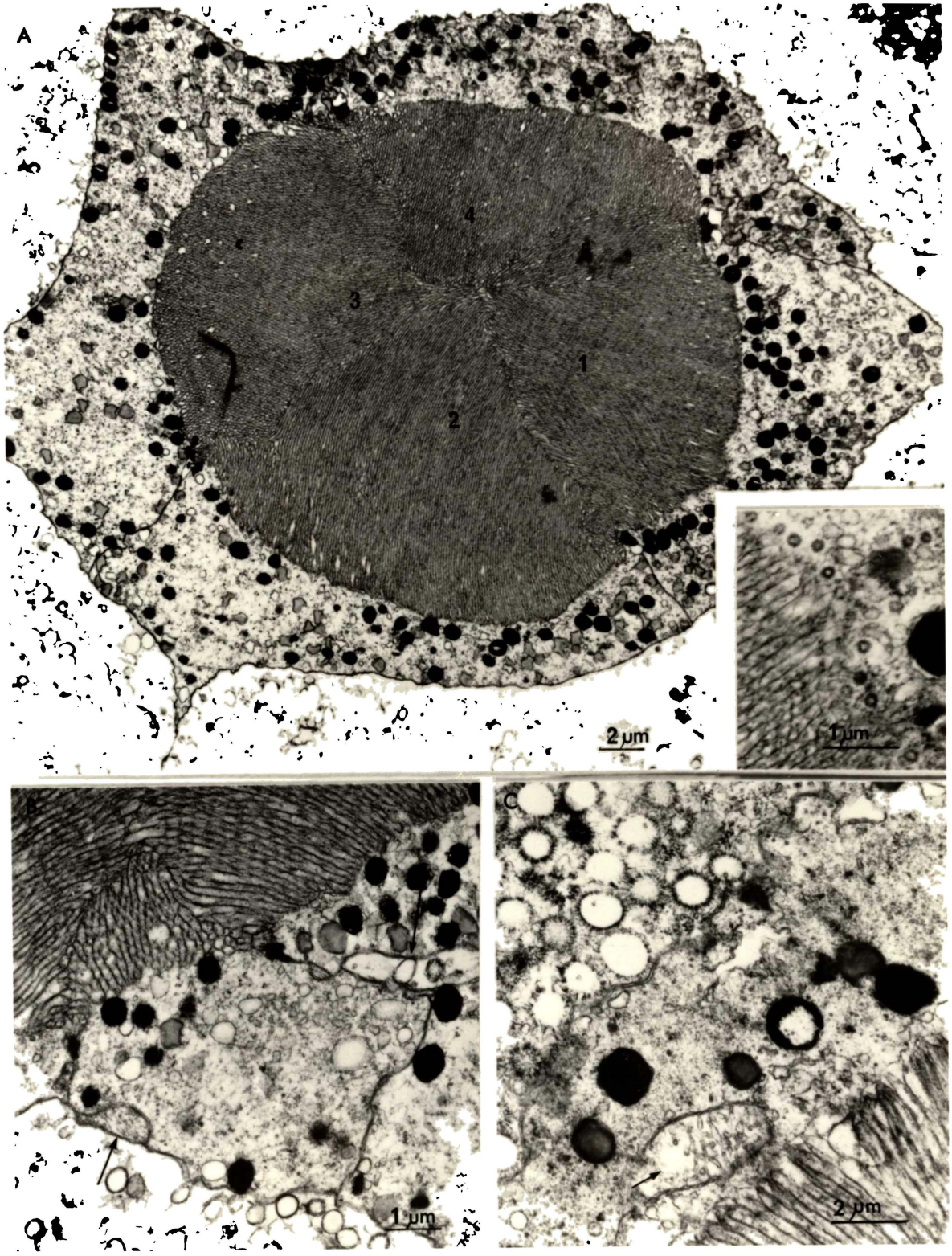
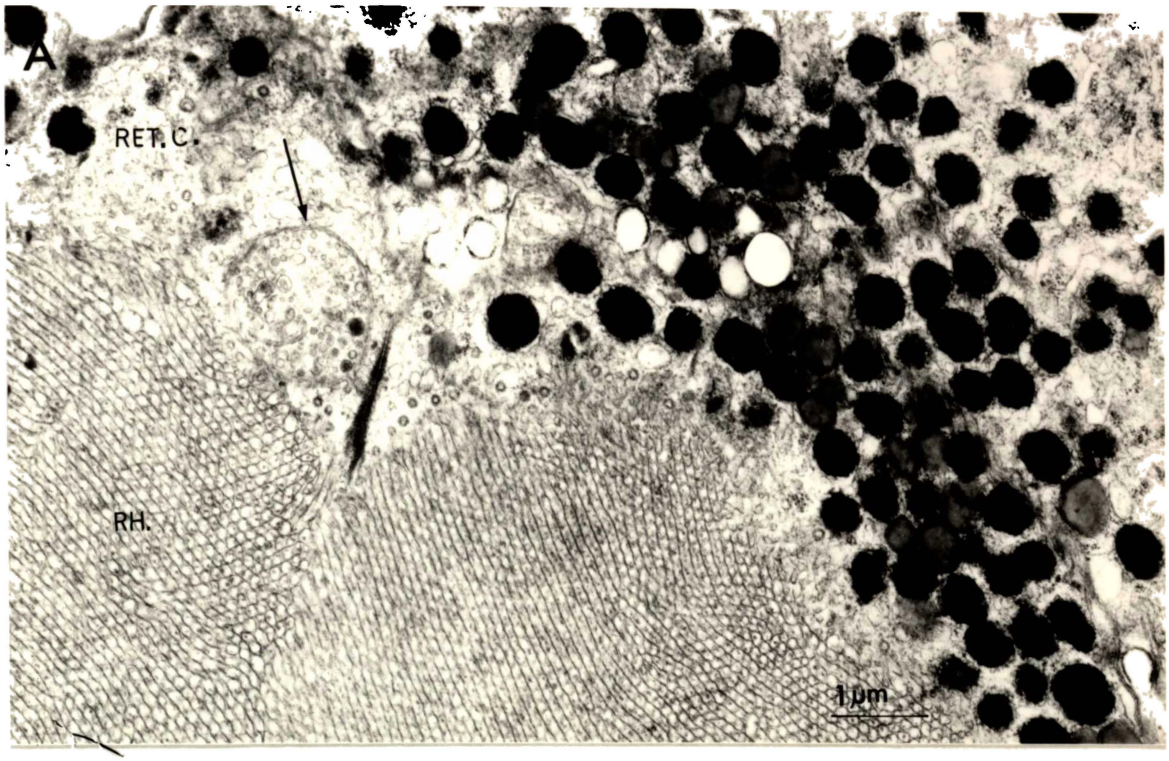

A
4
3
1
2
2 µm
1 µm
B
1 µm
C
2 µm

PLATE 7

Light-adapted retinula cytoplasm and proximal rhabdom

A A large number of dark screening pigment granules and microvillus vesiculation (pinocytosis) are characteristic of the light-adapted state. A multivesicular body located near the rhabdom edge is thought to be involved in the process of membrane turnover (arrow). The dark screening pigment granules are in close proximity around the rhabdom edge.

B Oblique section through the proximal rhabdoms, showing that the fifth retinula cell consistantly occupies the same position and seems to contain fewer pigment granules than the other cells, too. The lower proximal rhabdom ends show a darker stained region which does not contain microvilli. At this level the five retinula cells branch into five independent axons which penetrate the basal membrane.


A
RET. C.
RH.
1 µm

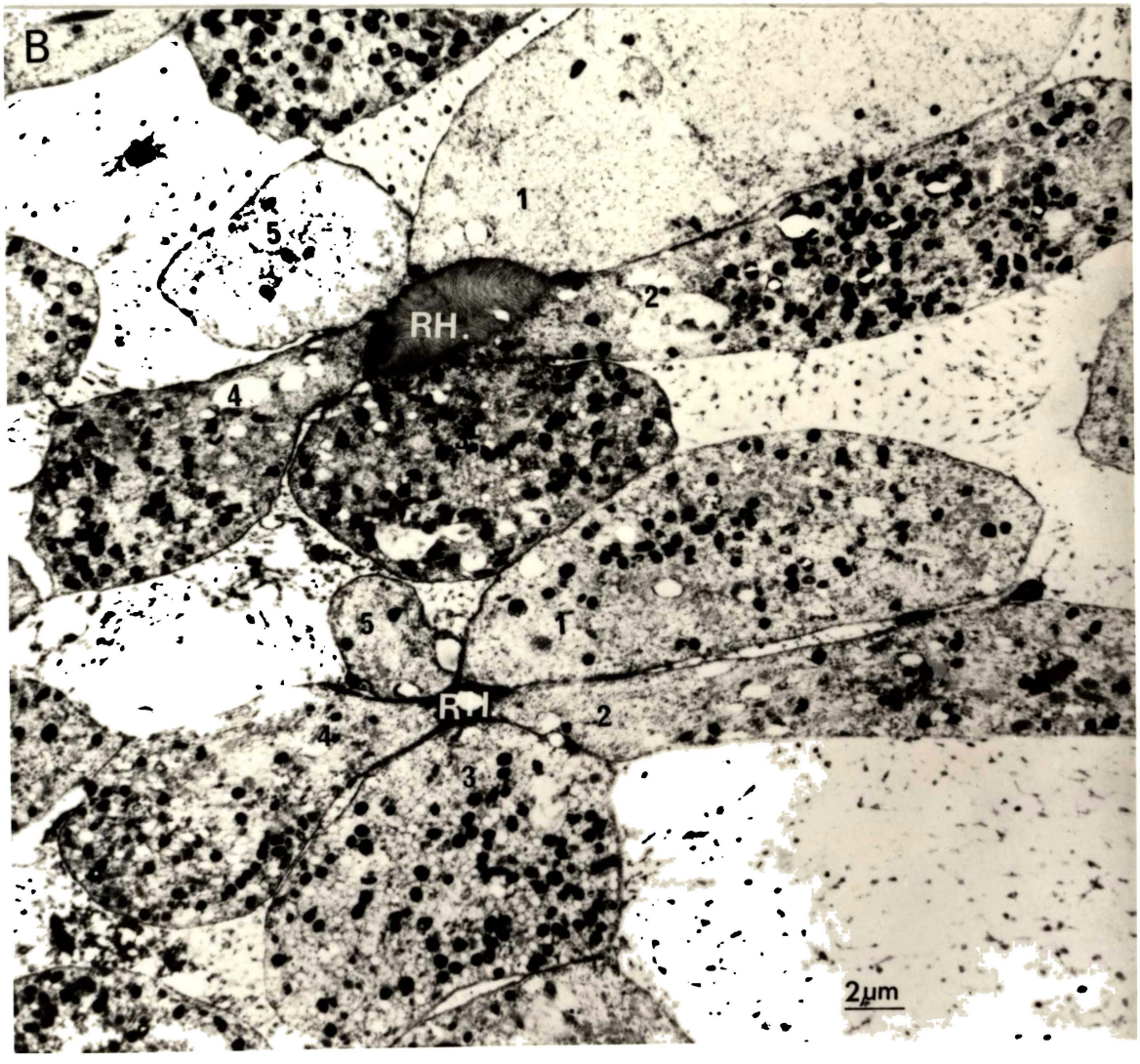
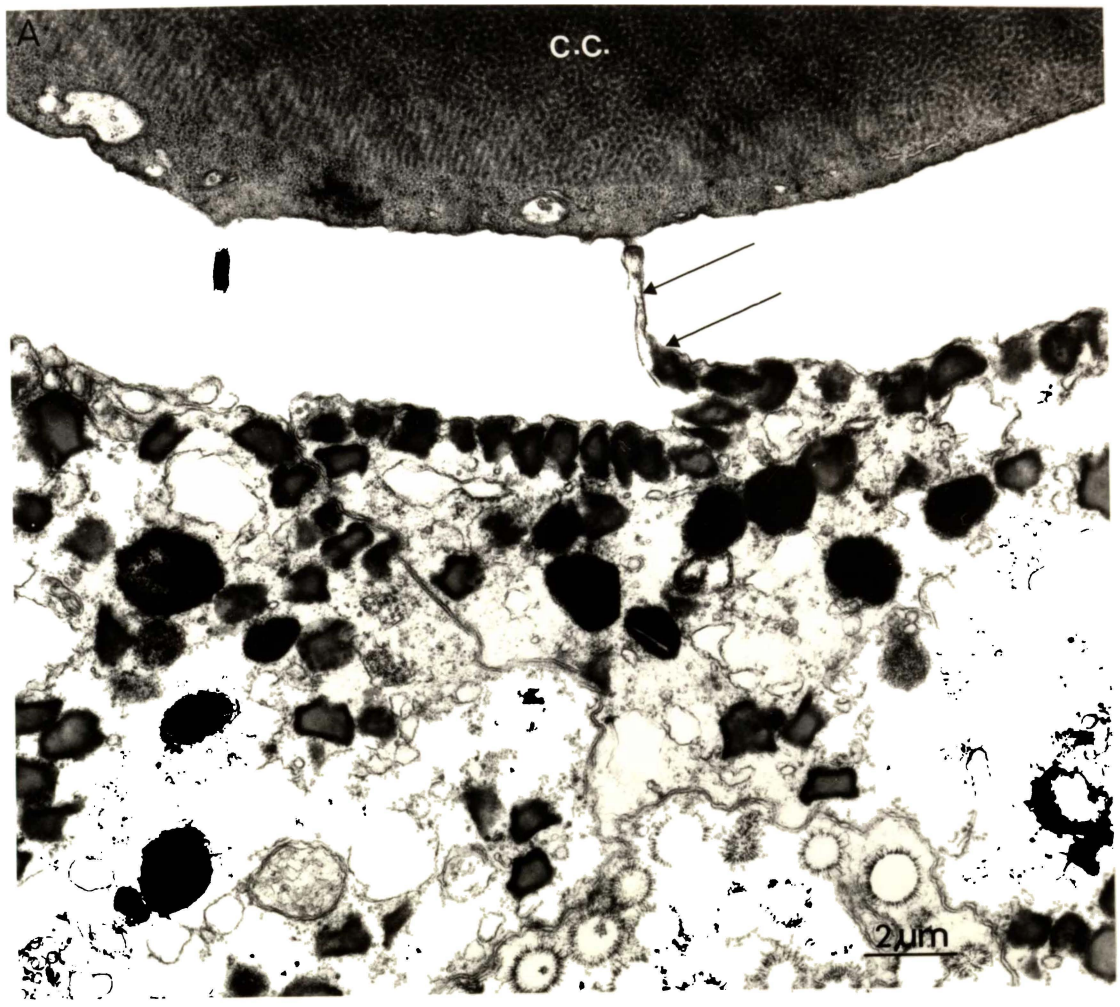

B
1
5
RH.
2
4
5
1
RH.
2
4
3
2µm

PLATE 8

Dark-light adaptation of the crystalline cone edge

A In the dark-adapted eye, narrow 'bridges' (arrows) connect the retinula cytoplasm to the periphery of the crystalline cone. Note the abundance of 'grey' granules near the edge of the cytoplasm and the small number of screening pigment granules present. The space between the crystalline cone and the retinula cytoplasm is much wider when compared to that of the light-adapted state (B).

B In the light-adapted state, cytoplasmic 'bridges' (arrows) occur at regular intervals around the periphery of the crystalline cone. The space between the retinula cytoplasm and the crystalline cone is narrower. The abundance of pigment granules in the retinula cytoplasm has increased.


A
C.C.
2µm

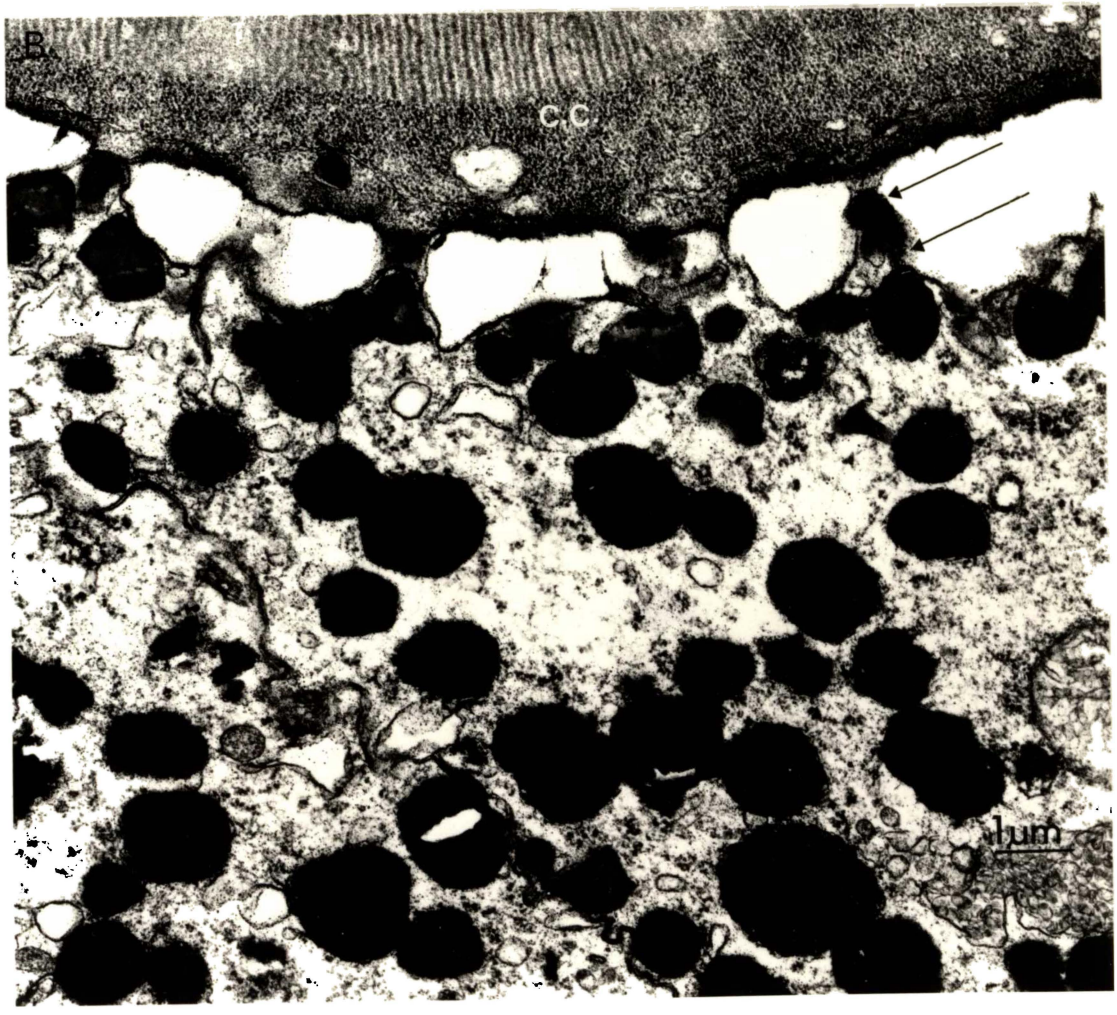

B
C.C.
1µm

PLATE 9

Electron micrographs of intracellular organelles from the interstitial cells of (a) light-adapted, (b) heat-stressed dark-adapted, and (c) normal, dark-adapted eyes. In the light-adapted material the organelles are hollow, spherical, vesicles of 0.3 um diameter. In heat-stressed material they resemble those of the dark-adapted eye, but lack the surrounding membrane (arrows) that is characteristic of these organelles in the dark-adapted state. (d) Between the ommatidial groups of 5 retinula cells and their rhabdoms (RH) interstitial cells and their bizarrely shaped nuclei (N) are found. The cytoplasm of these cells does not contain pigment grains but organelles that change their size and shape according to the state of adaptation.

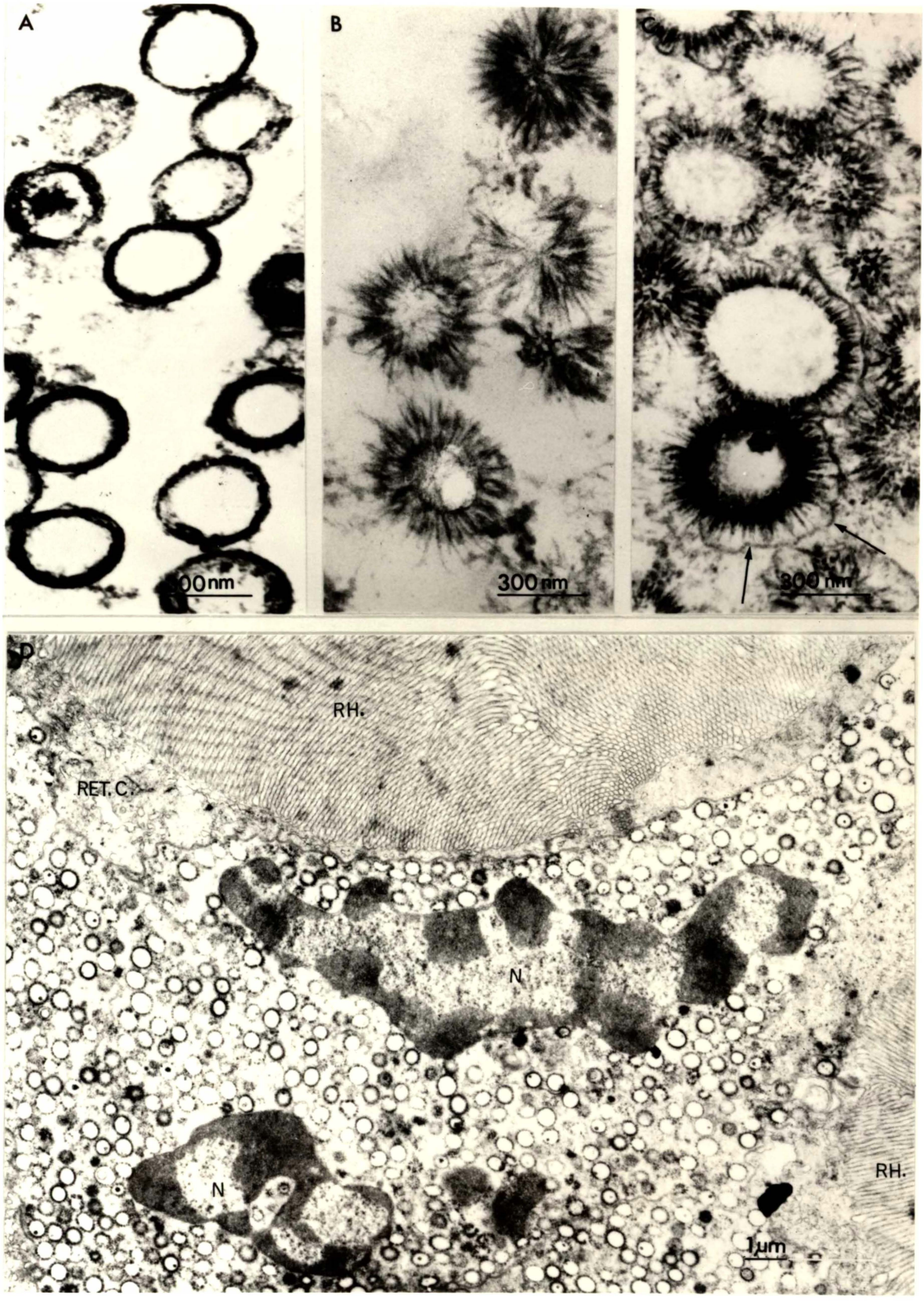

A
00nm
B
300 nm
300 nm
D
RH.
RET. C.
N
N
RH.
1μm

PLATE 10

Light micrographs of transverse sections through (a) normal light-adapted, (b) normal dark-adapted, (c) heat-stressed dark-adapted, and (d) heat stress recovered dark-adapted material. The position of the screening pigment as well as the shape and sixe of the rhabdoms are affected by the adaptations. Grotesquely deformed rhabdoms occur in heat-stressed eyes and the pigments in these eyes, in spite of the dark conditions throughout the experiment, are in a position which resembles that of the normal light-adapted state (a). During the recovery phase (d) rhabdom shapes and pigment positions begin to approach condition (b), that of the normal dark-adapted eye, again.

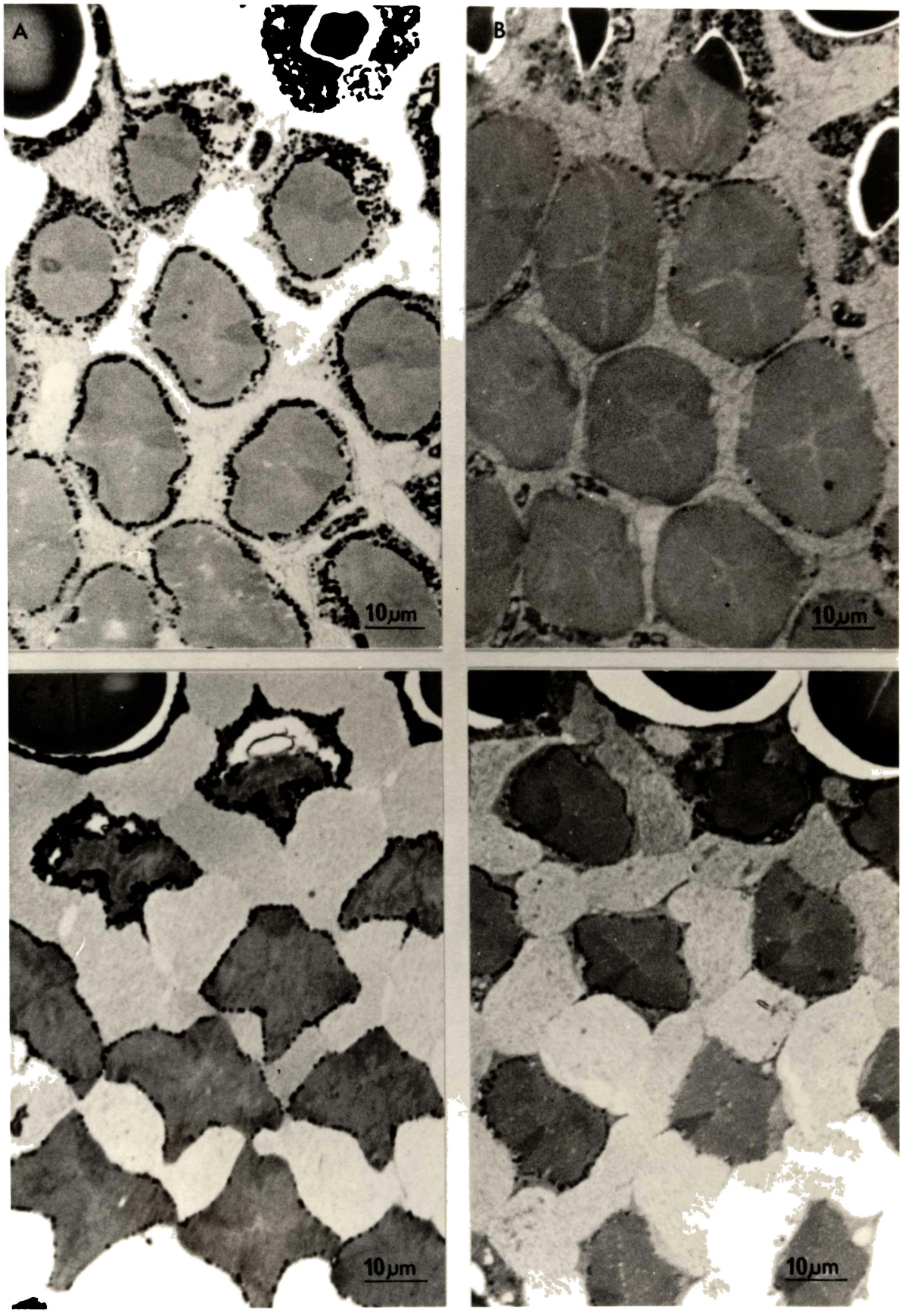

A
10µm
B
10µm
10µm
10µm

PLATE 11

Microvilli arrangement

A High magnifications of microvilli in cross-section of dark-adapted rhabdom. The microvilli membranes and their dark staining contents are readily seen. The microvilli are more regular and uniform in their arrangement.

B After light-adaptation, the microvilli appear less regular in their arrangement and shape. Also the membranes seem not to show up as clearly as in the dark-adapted eyes.

C Longitudinally and transversely cut microvilli, note the uniform and parallel arrangement of the microvilli. The longitudinally sectioned microvilli appear to expand at the edge (arrows).

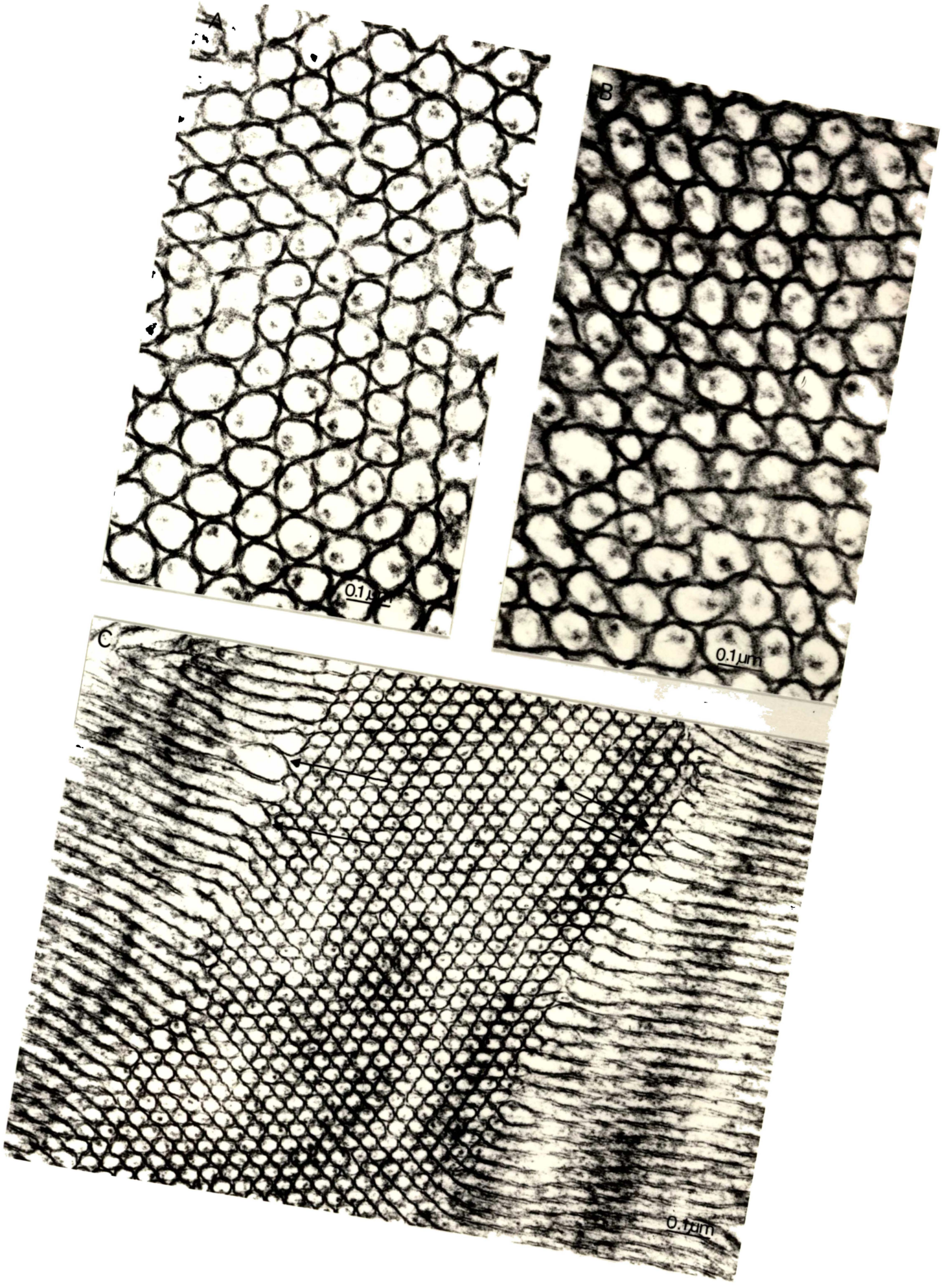

A
0.1μm
B
0.1μm
C
0.1μm

PLATE 12

Ultra-structure of rhabdom and microvilli in different states of adaptation.

A Electron micrograph of a heat-stressed dark-adapted rhabdom in transverse section. There is little retinula cell cytoplasm and the rhabdom is large, but its microvilli are in a state of total disintegration. Microvillus membranes are no longer discernible and the cytoplasm of the interstitial cells seems to contain little else but modified echinosomes, pictured in plate 4 b.

(B,C,D) A comparison between longitudinally sectioned rhabdom microvilli of eyes recovered from heat-stress within 7 hours (b), fully-light-adapted in bright sunlight for 3 hours at 0°C (c), and fully dark-adapted for 3 days at 0°C in a light-proof container (d). Clearly, the most orderly microvillus arrangement is displayed by the dark-adapted eye, but with regard to microvillus diameter, inter-villus space or membrane thickness, no statistically significant changes were detected between the three types of adaptation.

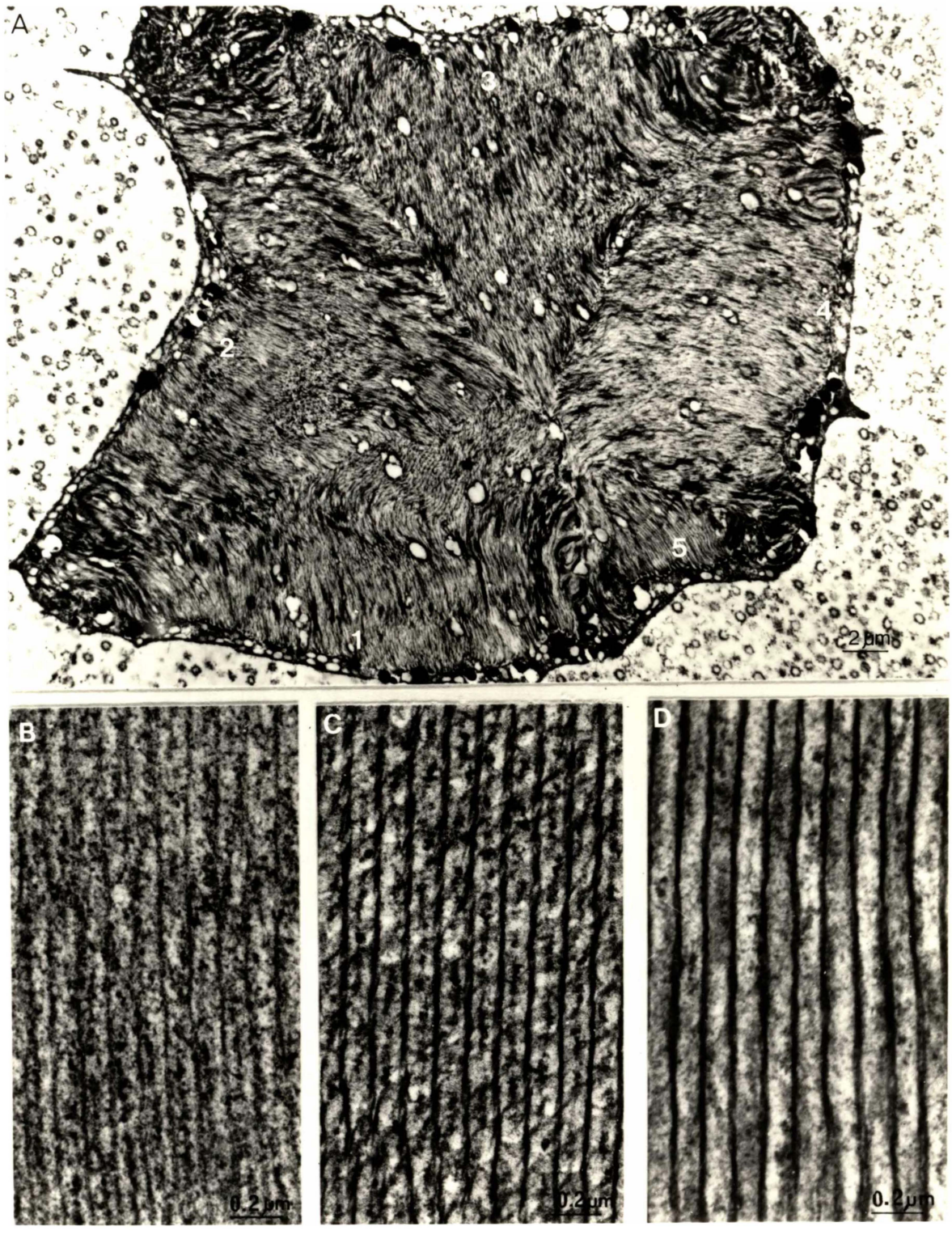
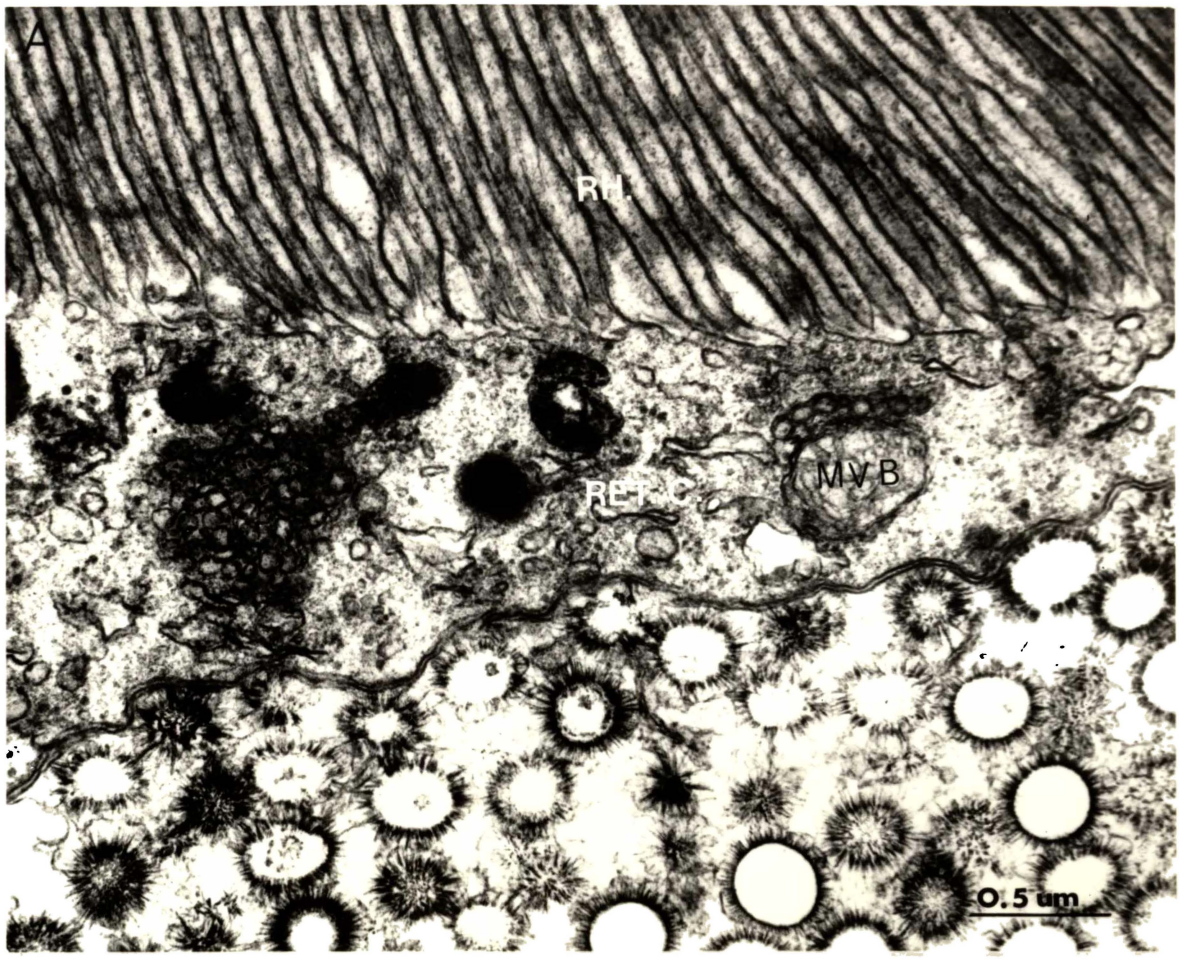

A
1
2
3
4
5
2 μm
B
0.2 μm
C
0.2 μm
D
0.2 μm

PLATE 13

A In this section through the dark-adapted eye of Orchomene plebs, the rhabdom and its microvilli, the surrounding retinula cell plasma and its organization, and the interstitial cells with their characteristics 'echinosomes' are clearly visible. A typical echinosome resembles a miniature sea-urchi and consists of a hollow centre surrounded by a membrane-bound coat of needle-like microstructures.

B In the light-adapted eye, the rhabdom and its microvilli show a looser organization. Several vesicles are seen 'budding' off from the microvilli (rhabdom edge). The screening pigment granules have increased in abundance around the rhabdom. Also note that the interstitial cell plasma contains only hollow spherical vesicles in contrast to the 'echinosomes' of the dark-adapted state.


A
RH
RET. C
MVB
0.5 um

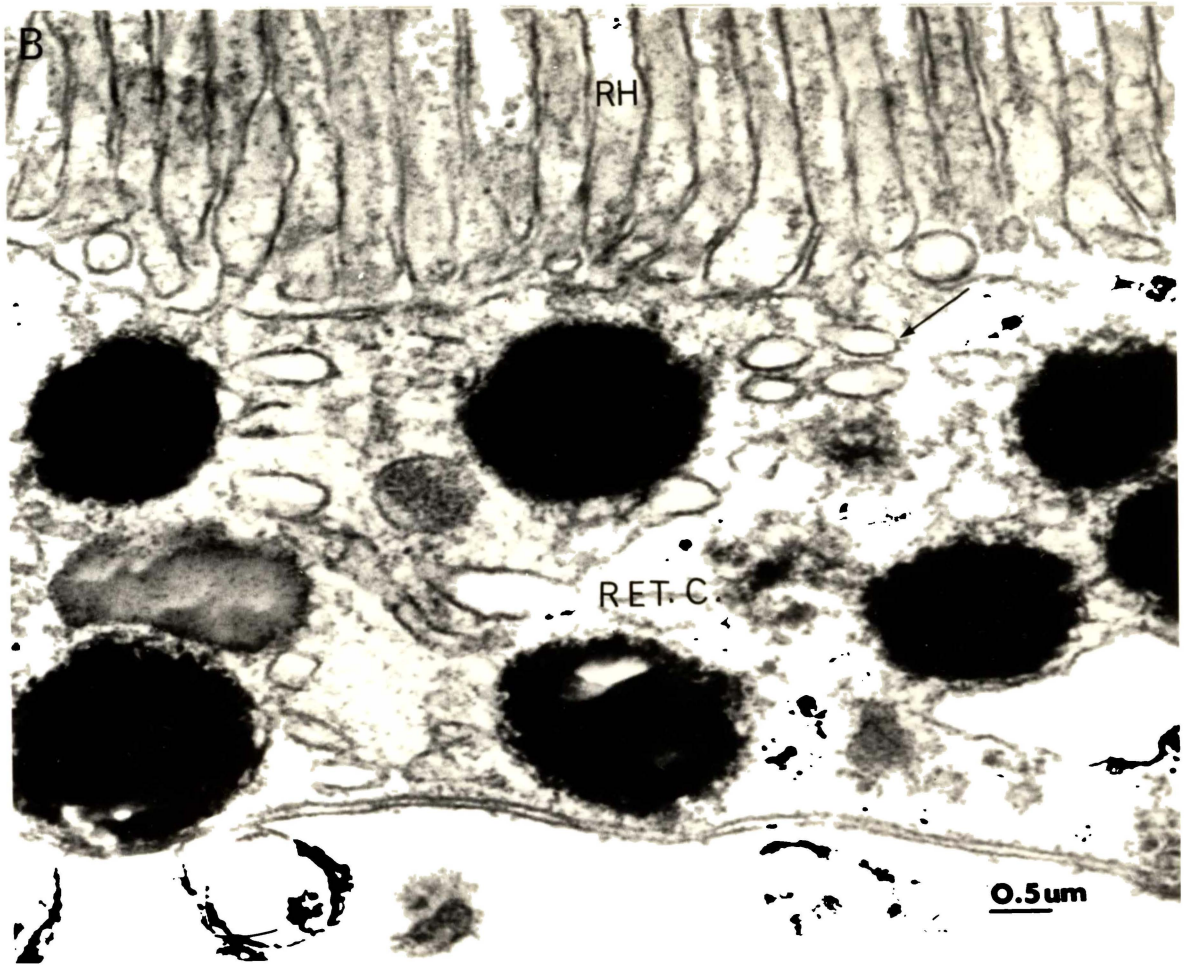
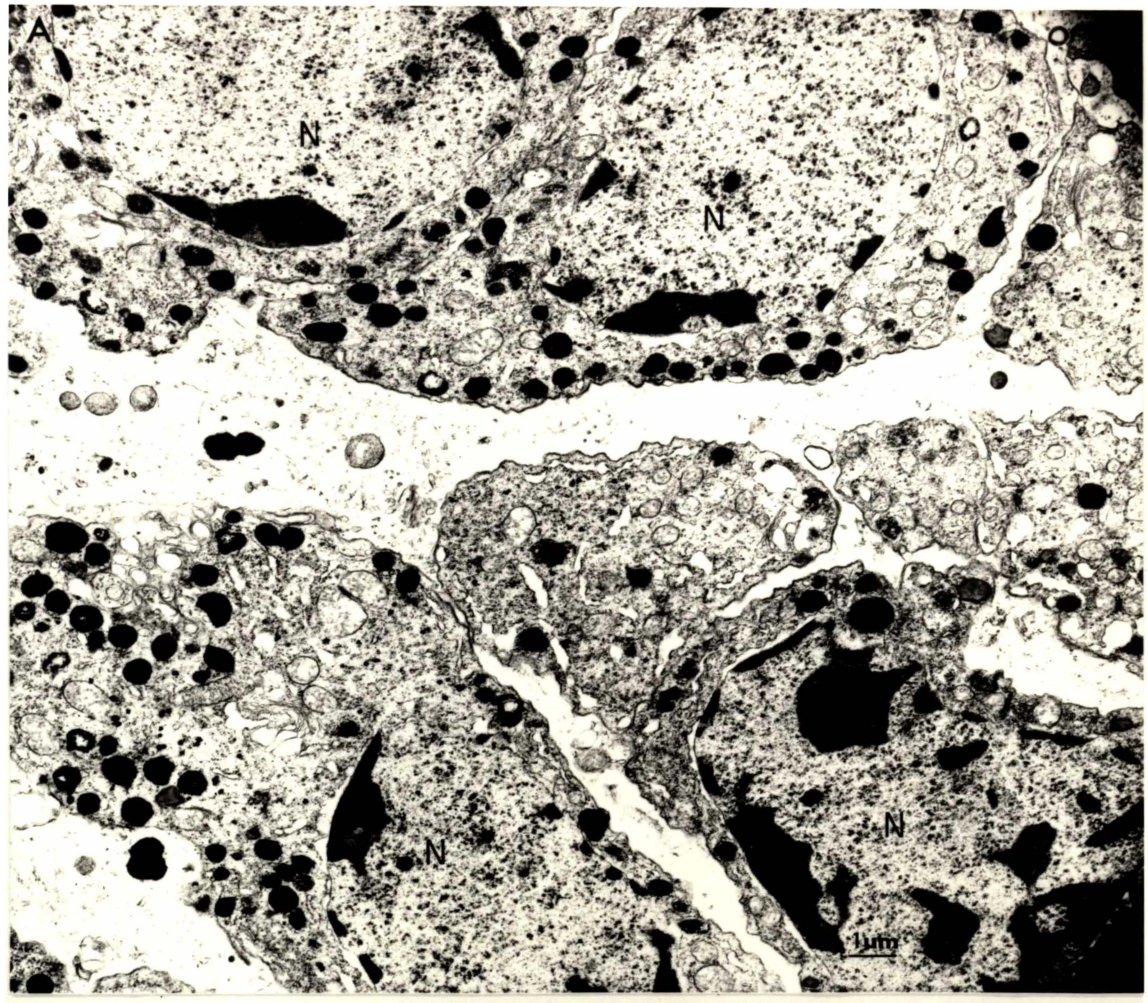

B
RH
RET. C
0.5 um

PLATE 14

Dark-light ultrastructural changes of the proximal retinula cell regions

A Transverse section of the dark-adapted eye through the axonal region. Screening pigments are still present at this basal level around the nuclei, but mitochondria are less abundant when compared with light-adapted material.

B In the light-adapted state, there is an increase in the abundance of the mitochondria, particularly towards the distal region of the retinula cells nuclei. The number of screening pigment granules also increases within the cytoplasm at this level.


A
N
N
N
N
1µm

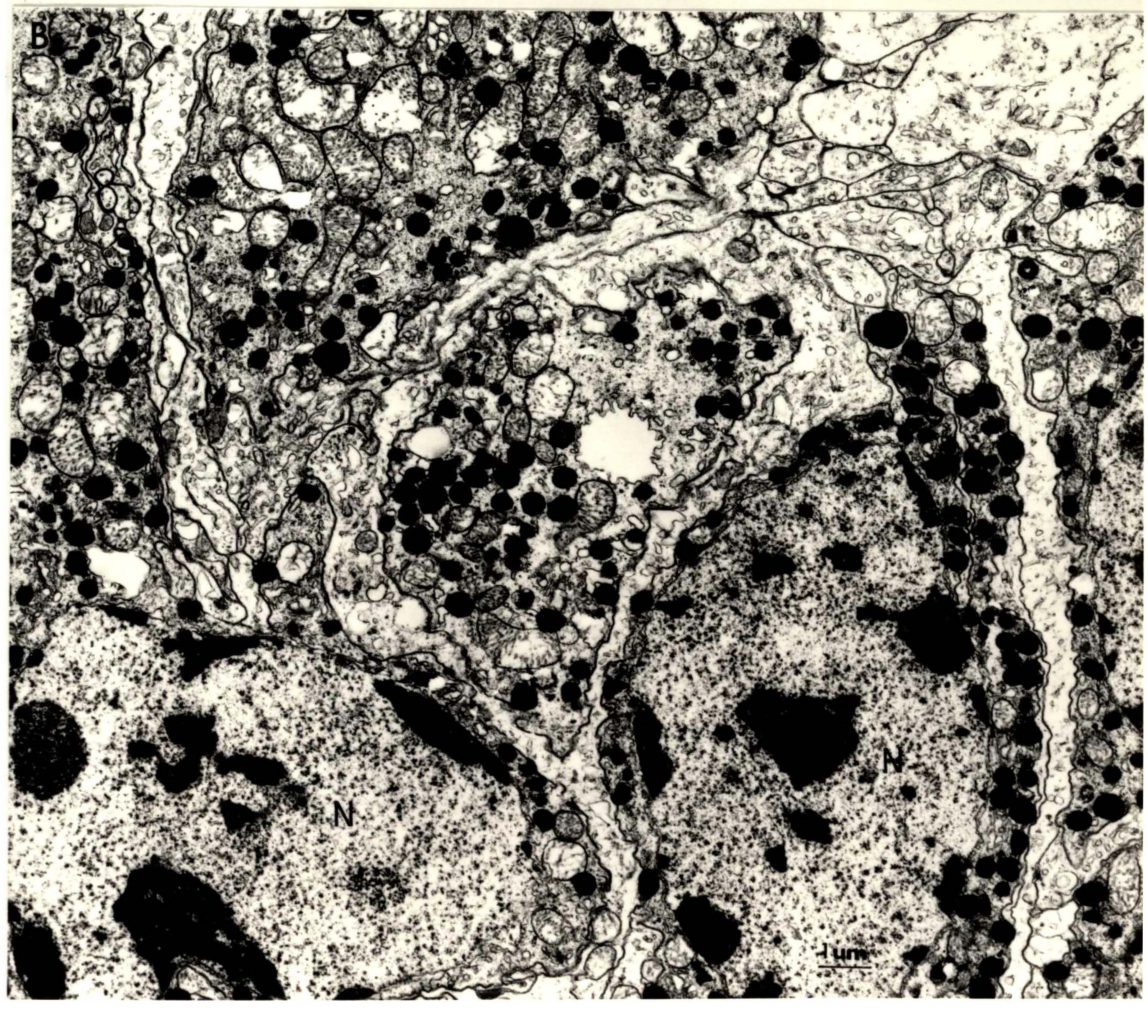

B
N
N
1µm

PLATE 15

Temperature-stressed and recovered rhabdom

A This section shows the cross-section of the temperature-stressed proximal rhabdom. Note that the whole rhabdom is in a sort of 'melting' state where individual microvilli are hardly discernible. The retinula cell plasma exhibits an increase in density compared to normal 0°C material.

B The proximal rhabdom at about the same level of the heat-stressed recovered eyes shows the rhabdom approaching normal integrity. Individual microvilli can be seen clearly. The retinula cell plasma has an even denser appearance (note the dark staining characteristics).

C High magnification of the rhabdom edge of heat-stressed material. The microvilli appear as a stack of contorted membranes (arrow). The pigment granules are in extreme proximity to the rhabdom edge and some are actually in direct contact with the rhabdom - a situation which is characteristic of light-adaptation. Note also the electron-lucent vesicles in the retinula cell plasma.

D The rhabdom of the heat-stress recovered material shows a remarkable recovery of the rhabdomal integrity. Individual microvilli are clearly distinguishable (arrows). The retinula cell plasma has increased in area and some screening pigment granules have moved away from the rhabdom edge.

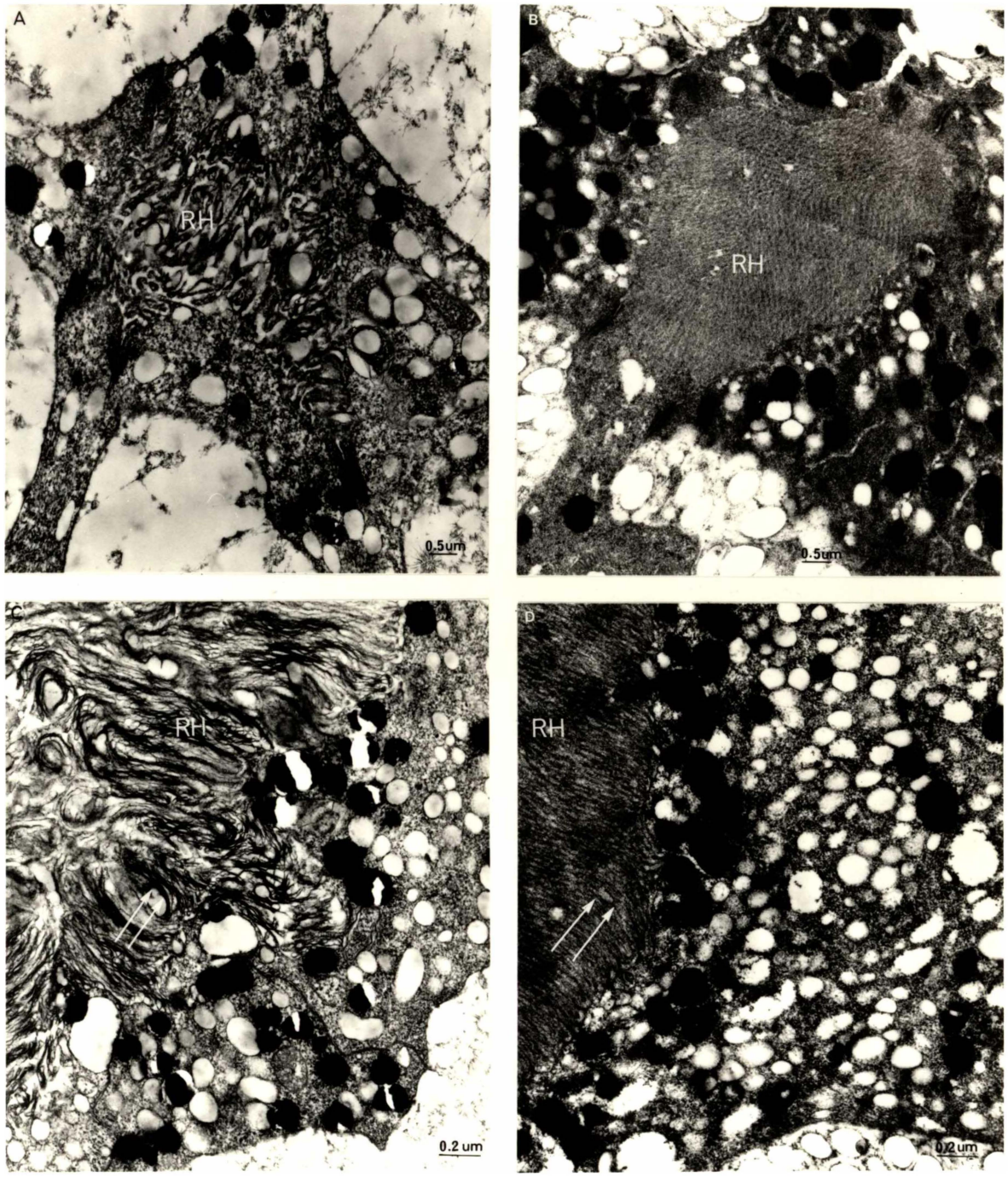

A
RH
0.5um
B
RH
0.5um
C
RH
0.2 um
D
RH
0.2 um

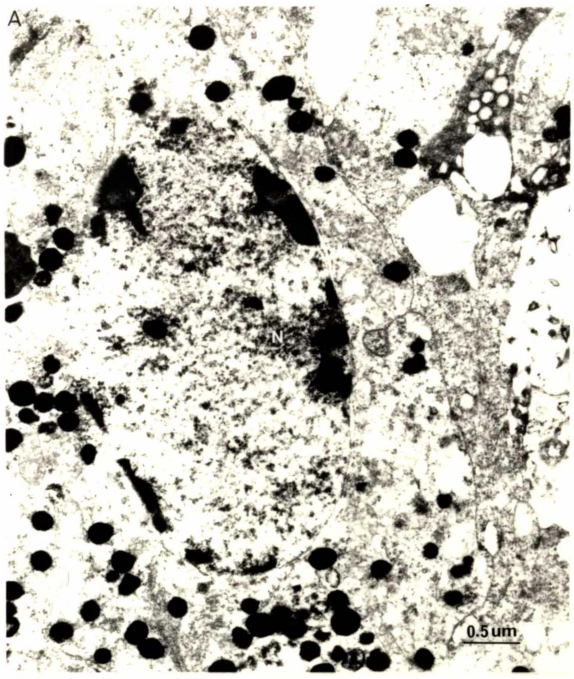
PLATE 16

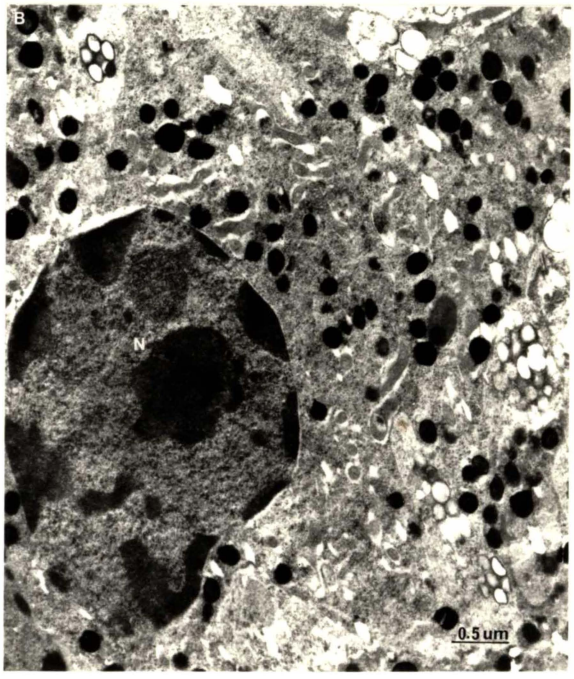
Temperature induced changes in the proximal retinula cell processes and nuclei

A In heat-stressed material, the sahpe of the nucleus changes into a more ellipsoidal form. The retinula cell plasma around the nucleus is devoid of organelles except for the screening pigment granules. The cytoplasm is less dense compared to normal 0°C and recovered materials.

B From heat-stress recovered material shows a denser cytoplasm. The mitochondria are also found randomly scattered in the cytoplasm together with the screening pigment granules. The nucleus and the cytoplasm are darker stained.

C The normal nucleus shows a distinct lamellar boundary. The cytoplasmic organelles show an increase, especially in the abundance of mitochondria. Screening pigment granules are also found close around the nucleus.


A
N
0.5 um


B
N
0.5 um

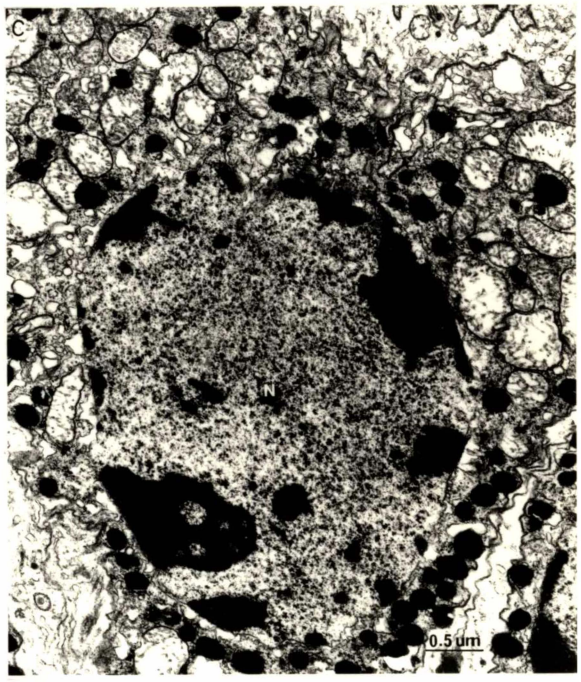

C
N
0.5 um

PLATE 17

(A-C) Close-up photographs of the eye of *Orchomene plebs* under light of 634 nm (red), 513 nm (green) and 474 nm (blue). Under red light most of the light is reflected out of the eye, but in green and blue lights the eye appears dark because of increased absorption. The appearance of the eye was studied under light of 15 different wavelenghts.

(D) This is what a human observer sees when he looks at an oblique 1m long hole of 10cm diameter in the sea-ice. The blue-filter effect of sea-ice is obvious.

FIGURE 2

Densitometer readings of vertical scans across the broad (outer curves) and narrow regions (inner curves) of the eyes depicted in Plate 17A, B, C. The apparatus was adjusted in such a way that a direct comparison between the three eyes was possible. Very clearly, absorbance of the eye is highest under light of 513nm.

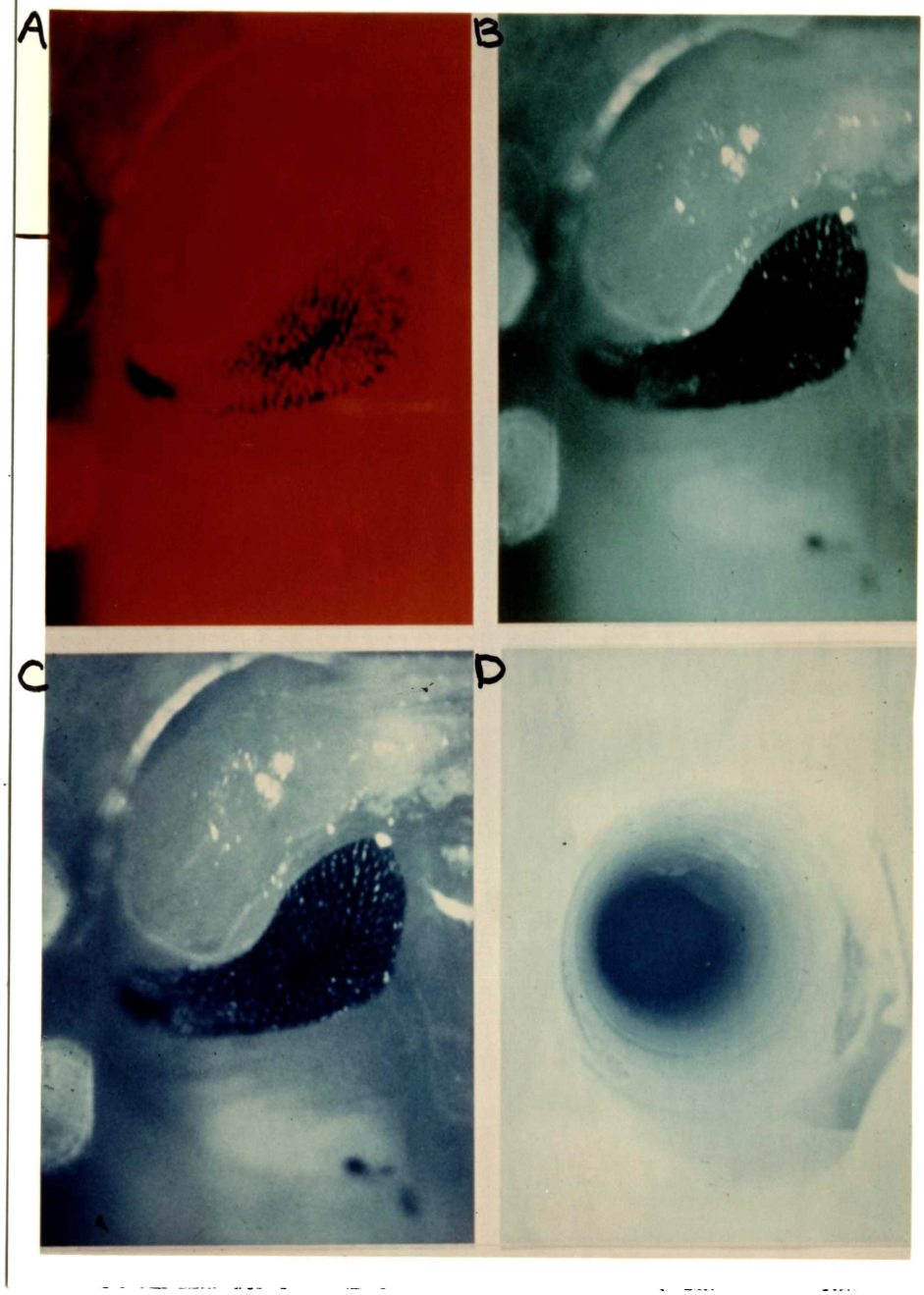


Plate 17

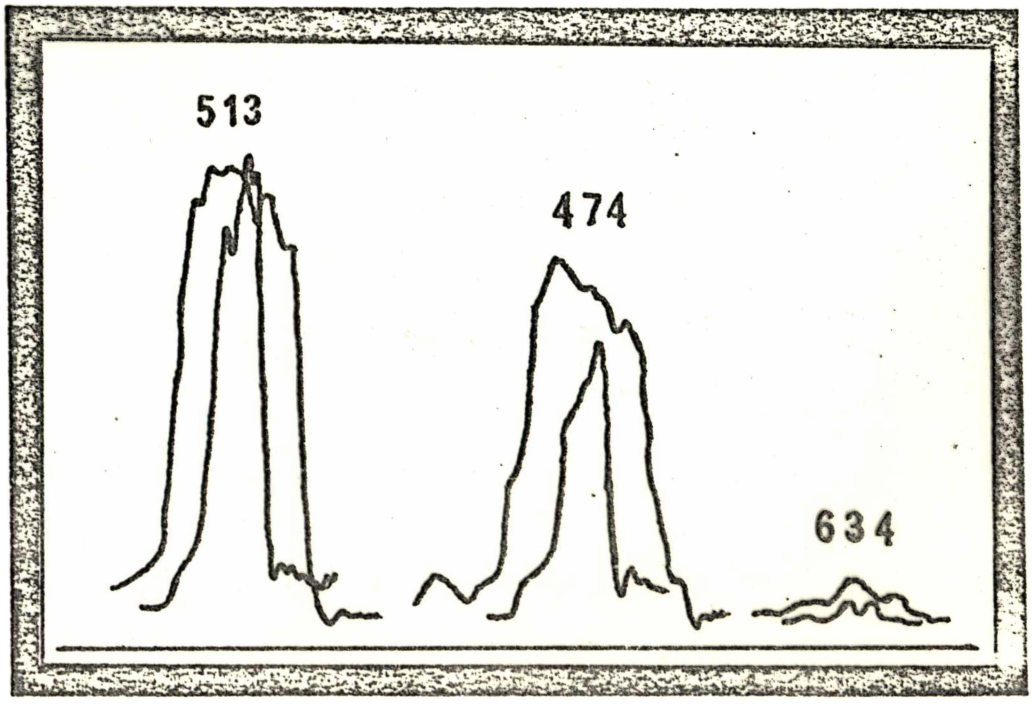


Fig. 2

Figure 3

A typical V/log I calibration curve, with ERG response height plotted on the ordinate and light intensity on the abcissa. The duration of each flash was 200 ms, and the dark interval between flashes was 5 seconds. The curve is almost linear over a range of 3 log units.

Figure 4

Spectral sensitivity of the amphipod, Orchomene plebs, shows a maximum response at about 490 nm. The single maximum response peak confirmed the results obtained from direct spectral absorption and densitometer readings.

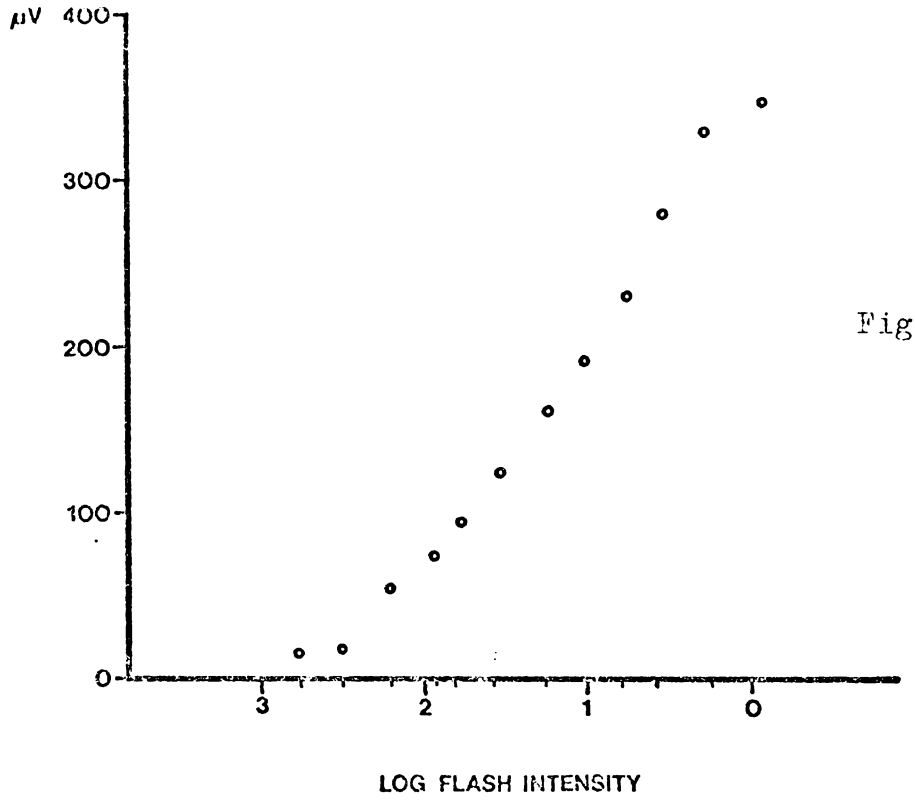

μV 400
300
200
100
0
3
2
1
0
LOG FLASH INTENSITY

Fig. 3

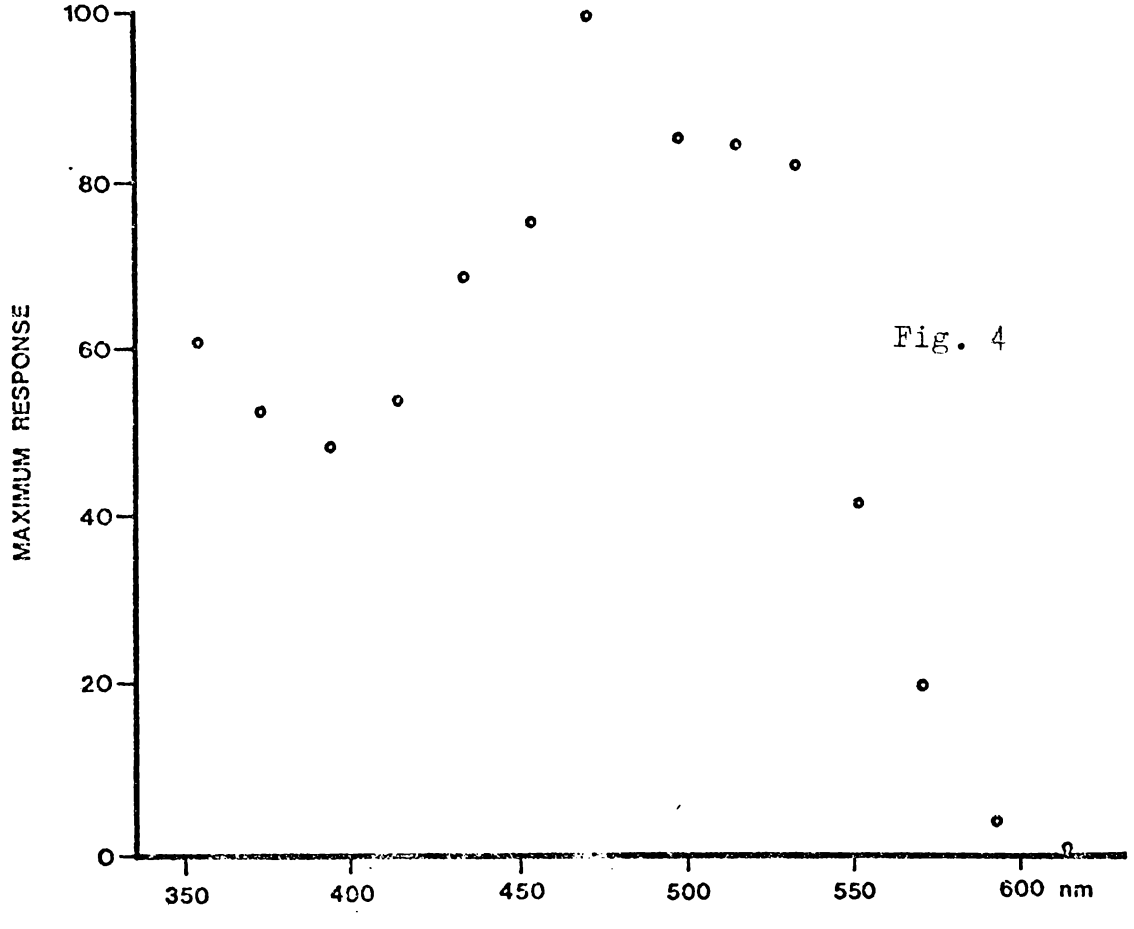

100
80
60
40
20
0
MAXIMUM RESPONSE
350
400
450
500
550
600 nm

Fig. 4

CHAPTER II

THE COMPOUND EYE OF THE ANTARCTIC MARINE ISOPOD (*GLYPTONOTUS ANTARCTICUS*: ISOPODA)

A. SUMMARY

The Antarctic marine isopod Glyptonotus antarcticus has four eyes: two large dorsal eyes and a very much smaller ventral pair, both are of the apposition type. Only the dorsal eyes are being investigated by light and electron microscopy. The research centered on the anatomy, structural changes in dark-light adaptation and temperature-induced changes.

1. The dorsal eye has no externally visible facets and represents an example of a typical apposition type of compound eye. It consists of corneal lens, 'eucone' crystalline cones and the retinula cells with their centrally fused rhabdoms, (Plate 18-22).

2. Dark-adaptation is characterized by the absence of the screening pigment granules from the retinula cytoplasm adjacent to the rhabdomeres. Furthermore, the volume of the retinula cell cytoplasm is increased when compared with that of the light-adapted eye (Plate 23, 24).

3. Light-adaptation is characterized by screening pigment granules having migrated towards the vicinity of the rhabdom. The formation of pinocytotic vesicles and multilamellar bodies along the rhabdom edge is also indicative of the light-adapted state. The cellular volume of the retinula cell is reduced (Plate 25-27).

4. The structural integrity of the rhabdom is intact after being kept at the elevated temeprature of 10°C, but individual microvilli increase in length and diameter. The

position of the screening pigment granules is that of the strongly light-adapted state (Plate 28, 29). The cellular volume of the interstitial cells increases drastically with a corresponding reduction of the volume of the retinula cell. The situation resembles that of light adaptation.

B. INTRODUCTION

Even though there is a wealth of research information on the structure and function of the crustacean compound eye very few investigations have dealt with the compound eyes of isopods, let alone Antarctic isopods. The early work on the isopod visual system was restricted to light microscopy e.g. Porcellio (Grenacher 1879); and Oniscus (Debaisieux 1944) and provides an excellent background to the understanding of the structural organization of the eyes of this group of crustaceans. Apart from the ultrastructural dark-light adaptational studies on Oniscus (Tuurala & Lehtinen 1966, 1967, 1971) and Porcellio (Nemanic 1975) a detailed knowledge of the ultrastructural organization of the eye of marine isopods is still lacking. Furthermore, a comparative ultrastructural study may help to understand the adaptational modifications of the visual systems of crustaceans to various environmental conditions.

The unique Antarctic marine environment off Scott Base in the McMurdo Sound with its constantly low temperatures and low ambient light levels is now known to provide an ideal habitat for a surprisingly rich and diverse fauna of both pelagic and benthic crustaceans including Glyptonotus antarcticus. In the fully grown adult, this giant Antarctic marine isopod measures about 117mm in length (Plate 18). There are two large compound eyes present, one on each dorso-anterior side of the head. The animal has another pair of very much smaller eyes which are located on the ventral side of the head. G. antarcticus is benthic and has a circumpolar distribution from relatively shallow depths down to 300m deep water. The

presence of well-developed functional eyes may be important (a) in providing visual cues for the animal's scavenging necrophagic behaviour and (b) to evade dangers, such as hungry seals or large fish. Like Orchomene plebs (and other Antarctic crustaceans), the ultrastructural organization of the photoreceptors of this isopod has not been studied before. This investigation describes the ultrastructure of the dorsal compound eye of Glyptonotus and the effects of light and temperature on the structural integrity of the retina. It also represents part of an attempt to understand the structure and function of the photoreceptors of Glyptonotus and to support or supplement the results already obtained from Orchomene plebs.

C. MATERIAL AND METHODS

All isopods used in this study were about 80-110mm long and positively identified as Glyptonotus antarcticus var. acutus (Tattersall 1921). This species was first described by James Eights (1852) as Glyptonotus antarctica, but the specific name was changed to antarcticus by subsequent workers (Pfeffer 1887; Richardson 1906; Collige 1918; Tattersall 1921; Nordenstan 1930; Sheppard 1957). Even though the genus Glyptonotus is often regarded as 'monotypic' (Kussakin 1973) two sub-species or varieties have been described. Glyptonotus antarcticus var. acutus (Richardson 1906) and newly discovered Glyptonotus antarcticus var. obtusus (Meyer-Rochow 1979) can be distinguished by the different shapes of their long and pointed pleotelsons.

The specimens were caught off Scott Base in the McMurdo Sound at the same site from where Orchomene plebs were obtained. Chicken wire cages baited with frozen seal meat were used as traps. They were lowered to the bottom through a 1.5m hole in the 3-4m thick sea ice and the traps were hauled up, emptied and rebaited once every two days.

The animals were maintained in transparent plastic aquaria (37 x 27 x 15 ccm) or 25l light-proof black plastic drums. The temperature of the sea-water was kept at $0^{\circ}C \pm 1$. Dark-adapted animals were taken from the black drum after the required time of adaptation and dissected under dim red light. For light adaptation an aquarium with 1-5 isopods was placed on snow and exposed to the sun for 1 hour. The temperature remained at $0^{\circ}C \pm 1$ during the dark-light adaptation experiments.

Temperature control during heat-stress experiments, which were carried out in a darkroom, was achieved through the use of Grant Instruments liquid expansion thermostat to an accuracy of $\pm 0.5^{o}$. In one set of experiments the left eye of 3 individuals was covered with black opaque nail-varnish, while the other eye remained unpainted. Animals treated in this way were kept in the laboratory for one week at $0^{o} \pm 1^{o}C$ and an ambient light intensity if approximately 50 lux.

Methods for histological investigations were identical in all eyes studied, irrespective of the light conditions or temperatures that the animals had been exposed to A 2.5% glutaraldehyde - 2% formaldehyde mixture in Millonig's phosphate buffer, adjusted with d-glucose to a 0.6 Mol solution of a pH of 7.4, served as a prefixative. The specimens stayed in this solution for 12 hours before they were washed in buffer and post fixed for 2 hours in a 2% phosphate-buffered solution of Os 04. Dehydration in a graded series of acetone was followed by infiltration with Epon 812 and hardening for two days at $65^{o}C$.

At least 5 eyes of each dark and light regime and 5 eyes of temperature-stress experiments were successfully sectioned and examined in both light and transmission electron microscope. For light microscopy 1um transverse and longitudinal sections were stained with toluidine blue for 10-15 seconds on a hot plate. Electron microscope material consisted of golden sections, which were picked up with uncoated 200 mesh copper grids and double-stained with uranyl acetate and lead citrate for 8 and 2 minutes, respectively. Formvar coated copper grids were used for some sections, especially at lower magnification when 100 mesh grids were used. Where statistical analyses were necessary t-test and a significance level of 5% were used.

D. RESULTS

1. Basic anatomy

(a) General features

Glyptonotus antarcticus possesses two pairs of compound eyes, one large dorsal and a very much smaller ventral pair. All the experiments were carried out on the larger dorsal compound eye. The dorsal eye is heavily pigmented, oval or kidney-shaped and has a maximum diameter of 3.2mm in an individual of 100mm total body length. Across its narrower central region, the diameter is 2.0um. The dorsal eye is oriented for vision towards the front, above and the side. It consists of approximately 220 ommatidia, each having a diameter of about 70um. A difference between eyes of males and females was not detected.

Externally, the cornea is completely smooth with no external facets discernible. Corneal nipples, interfacetal hairs or other innervated structures like those present in nocturnal Lepidoptera (Bernhard et. al. 1970) or trichoid sensilla reported in Porcellio (Nemanic 1975) are absent here. Scanning electronmicrographs (Plate 19B) show that the smooth external surface of the eye is covered by a hard chitinous epi-cuticle which presumably offers some form of physical protection to the compound eye. This hard epi-cuticle is a hazard to the glass-knives during sectioning for it causes the cutting edge to become blunt very rapidly. After a partial removal of the external tough layer, the inner side of the cornea reveals slightly convex 'bumps' on its surface which indicate the facets of individual ommatidia (Plate 19C). The

curvature of the corneal surface and the interommatidial angle do not vary significantly from one region of the eye to another. Only towards the extreme periphery some slight variations may occur. An interommatidial angle of 3^{o} - 4^{o} is found for ommatidia in the central region of the eye, but in view of the regularity of the general corneal surface it is assumed that the peripheral ommatidia do not differ significantly from this value.

The ommatidium (Plate 19D) which is the morphological unit of the photoreceptor is approximately 300um long and 65-70um wide. It consists of the dioptric apparatus i.e. cornea and cone and the photoreceptive elements i.e., retinula cells and their rhabdomeres. A clear-zone commonly found in arthropods living in a dimly-lit environment is not developed in the Glyptonotus eye. However, the crystalline cone is separated from the rhabdom by a narrow space of 2-4um width. A summary of the relevant data is given in Table 2 below.

Table 2: The eye of Glyptonotus antarcticus.

Morphological Data (based on one 'average' individual)

Length of Glyptonotus antarcticus	100 mm
Size and shape of eye	3.2 mm oval or kidney-shaped
No. of facets	approx. 220
Diameter of single facet	65-70 um
Length of ommatidium	approx. 300 um
Interommatidial angle	3^{o} - 4^{o}

Anatomical Data

Thickness of corneal lens	76 um

Length of crystalline cone	84 um
No. of retinula cells	5 or 6
Length of rhabdom	100 um
Diameter of microvillus	0.06 - 0.1 um
Diameter of pigment granules (Primary)	approx. 0.04-0.08um
Diameter of pigment granules (Secondary i.e. in retinula cell)	0.1 - 0.4 um
No. of axons in one bundle	5 or 6

(b) Dioptric apparatus

The dioptric apparatus of the Glyptonotus eye consists of a convex-concave corneal lens and the nearly spherical crystalline cone. The unspecialized region of the corneal lens i.e. the cuticle, is not compartmentalized into individual facets, but is assumed to be of chitinous material of about 2um thickness. Chitin is one of the major components of the crustaceans cuticle. The exocuticle which is slightly thicker than the external epi-cuticle, measuring 10um, consists of heavily calcified material which may be responsible for the tough and rigid nature of the cornea (Nemanic 1975). This calcified exocuticle covers the inner multilamellated structure of the endocuticle (i.e. the corneal lens). Longitudinal semi-thin sections through the cornea (Plate 19D) reveal the differential staining characteristics of the outer thin epicuticle layer, the darker stained calcified thick layer of the exocuticle and the multi-lamellated inner corneal lens. Electron-micrographs through the corneal cuticle further confirm the multilamellated nature of the corneal lens (Plate 20A). A transverse section shows concentric rings of rotating layers

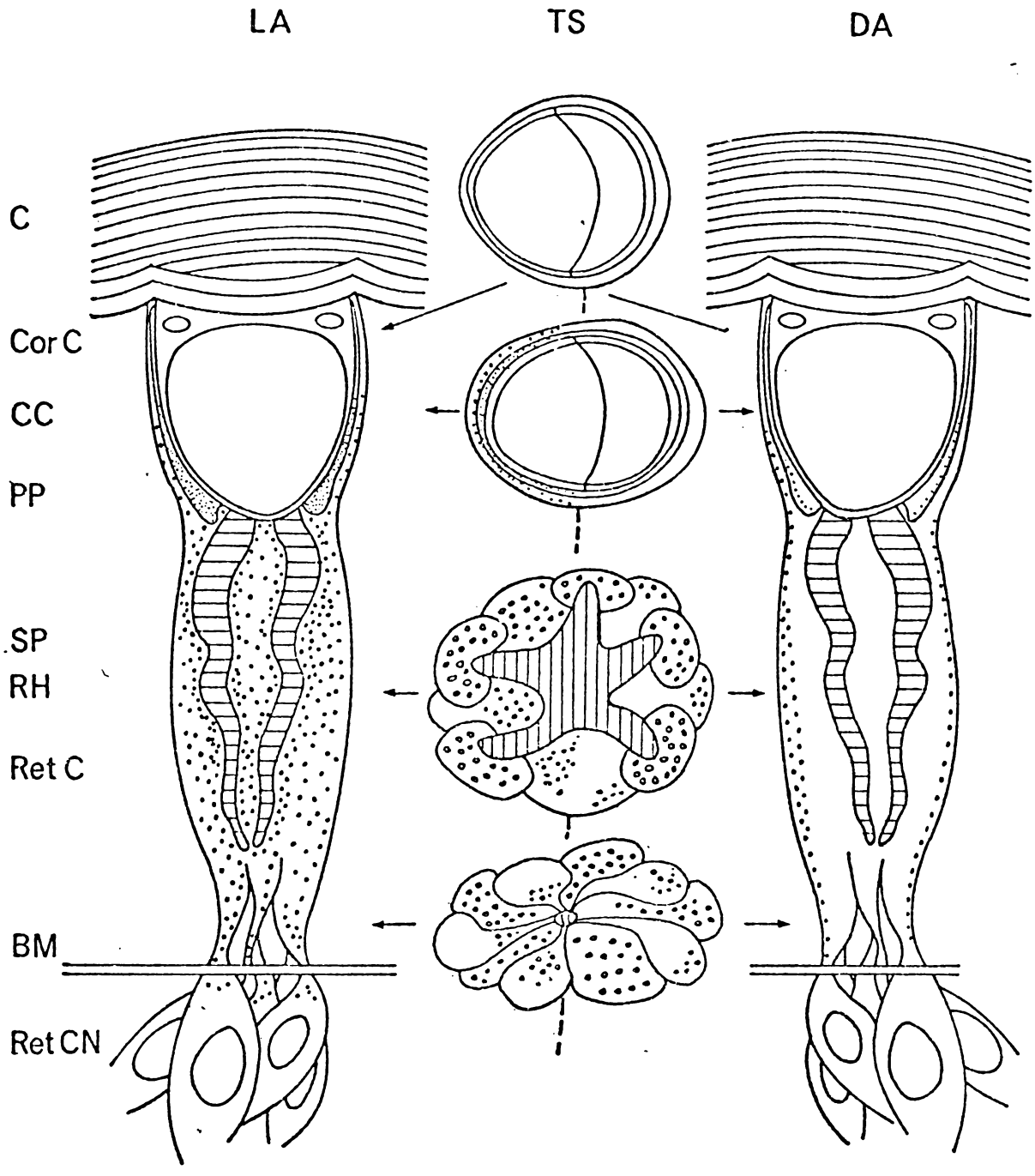
FIGURE 5

Semi-schematic drawing of dark and light ommatidia in longitudinal and transverse (TS) sections.

Dark-light adaptational changes involve primarily the primary and secondary screening pigment granules, which migrates towards the proximal crystalline cone region and the rhabdom edge. The crystalline cone is made up of 2 cone cells. The rhabdom consists of 5 rhabdomeres of more or less equal size.

Abbreviations: C=cornea, Cor C-corneagenous cells, CC=crystalline cone, PP=primary pigment cell, SP=secondary pigment cell, RH=rhabdom, BM=basement membrane, Ret CN=retinula cell nuclei.

FIGURE 5



of chitin/protein fibrils, thought to be responsible for the multilamellated appearance. Similar structural arrangements which are characteristic of cuticular chitin (Locke 1964) are known from insects (Meyer-Rochow 1975a) and other arthropods (e.g. Limulus, Fahrenbach 1968, Porcellio, Nemanic 1975). The convex-concave corneal lens exhibits an optically homogenous structure (Plate 20C) which is comparable to that found in certain insect eyes (Meyer-Rochow 1972) and other crustaceans (Carricaburo 1968). The two modified epidermal cells, i.e. the corneagenous cells lying below the corneal lens, are presumed to be responsible for secreting the lens.

The crystalline cone which lies directly below the two corneagenous cells consists of a bipartite nearly spherical structure. The cone or 'Semper' cells which clearly surround the crystalline cone, are responsible for secreting it (Plate 20C). The central core of the cone is darkly stained and dense but structurally homogenous under the light and electron-microscope (Plate 20B, C). Towards the periphery the dense structure of the core, however, gives way to a lighter stained region which often contains mitochondria and endoplasmic reticulum.

The detailed biochemical composition of the dense central core of the crystalline cone was not studied, but glycogen has been found to be a major component of the crystalline cones of the bee (Perrelet 1970). Nemanic (1975) in her study on the eye of the terrestrial isopod, Porcellio, discovered that a protein-carbohydrate complex was present in the crystalline cones. The same dual component complex has also been reported to occur in Pieris (Kim, 1964) and Anoplog-

nathus cones (Meyer-Rochow & Horridge 1975). It is believed that the ultrastructural demonstration of small particulate matter together with the presence of endoplasmic reticulum and mitochondria, in the peripheral regions of the cone of Glyptonotus, point towards the same two chemical components, namely carbohydrate (= glycogen) and protein.

(c) Photoreceptor cells

The amphipods are known for their consistent arrangement of five retinula cells secreting the centrally fused rhabdom (e.g. Orchomene plebs, Pontoporeia affinis, Gammarus oceanicus). In contrast, the isopods exhibit variations in their structural organization of the photoreceptor cells: e.g. Oniscus has about the highest number (approx. 17) of retinula cells known amongst crustacea (Debaisieux 1944), Porcellio has 7 retinula cells plus one eccentric cell (Nemanic 1975), Ligia has also 7 retinula cells but an unusual open-type of rhabdom, and in the Antarctic marine isopod, Glyptonotus antarcticus, there are 5 (90%) or 6 (10%) retinula cells contributing to the centrally fused rhabdom.

The retinula cells have distal processes which extend to the inner surface of the corneal cuticle (Plate 20A), Proximally, the retinula cells project towards the fenestrated basement membrane where they turn into axons. Glyptonotus differs from Porcellio, many other crustaceans and insects in that it lacks a separation into distal and proximal specializations of the retinula cells. Each of the individual retinula cells contributes equally to the centrally fused rhabdom. The interstitial cells which number 6-7 per ommatidium occupy the inter-retinula cell spaces and provide for the inter ommatidial isolation.

In transverse section, depending on the number of retinula cells involved, the rhabdoms are seen as a five-pointed or a six-pointed star (Plate 21, 22). The pentagonal rhabdom arrangement outnumbers the six-pointed rhabdom by some 90 per cent (based on cross-sectional counts of 10 eyes). Any special significance related to the differences of retinula cells within the eye is not known. The rhabdoms show a profound affinity for both toluidine and lead whereby they are distinctly stained and easily distinguishable from the rest of the retinula components including the dark screening pigment grains. The size of the rhabdoms does not vary significantly during dark-light adaptation. A maximum diameter of 49um and a length of 100um (based on the average of 10 ommatidia of 5 eyes each of dark-light adapted state) were recorded. The microvilli which form the rhabdomere sub-structure, are projected in a convex arc-like arrangement towards the centrally fused rhabdom. Thus, each 'arm' of the star-shaped rhabdom comprises two opposing rows of microvilli from 2 separate rhabdomeres which meet along the middle ridges. This arrangement differs from that of Oniscus (Tuurala & Lehtinen 1964, 1966) in which the 'arm' of the stella-shaped rhabdom comes from a single rhabdomere. The diameter of a single microvillus ranges from 0.08um to 0.12um and the length of the individual microvillus varies from 0.8um at the tip of the 'arm' to 2.4um in the centre of the rhabdom.

The length of the rhabdom is about 100um in an average 100mm adult specimen. Distally, the rhabdom is separated from the crystalline cone by a narrow 2-4um wide space. As in other isopods that have been investigated before, the crystalline stalk or tract and a clear-zone commonly found

in crustaceans living in dimly-lit environments are absent in Glyptonotus. The star-shaped rhabdom extends proximally until it decreases in size and phases out at approximately 35um above the fenestrated basement membrane. The retinula cells at this level branch off into five or six narrow processes being isolated from each other by voluminous interstitial cytoplasm. Before penetrating the basement membrane, the retinula cell processes increase in diameter again. The retinula cell processes or axons do not exhibit specialization of encapsulating material reported from the axonal groups of Ligia oceanica by Edwards (1969), but demonstrate a certain similarity to those of Porcellio scaber (Nemanic 1975) in which the axons exit ' en masse' with glial cells wrapped around them. The retinula cell nuclei lie 20-50 um below the basement membrane and are of spherical shape. The screening pigment granules which migrate radially to and from the rhabdom edge, depending on the state of adaptation, are completely absent in the retinula cytoplasm below the basement membrane. However, unidentified grey vesicles of approximately 0.25um diameter cluster around the nuclei (Plate 19D).

(d) Screening pigment granules.

Crustacean eyes in general have available to them a host of different types of screening pigment granules. These screening pigment granules are generally localized in special pigment cells and/or retinula cells. Their primary function is to act as shield between individual ommatidia and to regulate the light flux in the rhabdomere (Walcott 1975). The pigment screen has been demonstrated to exhibit selective absorption and reflection, both necessary to improve the

spectral sensitivity of the photoreceptors (Struwe et. al. 1975). The number of different pigment granules present varies from species to species e.g. in Squila mantis (Schonenberger 1977) found five kinds of pigment cells with different pigment grains, while in Gennadas (Meyer-Rochow & Walsh 1977) no pigment cells whatsoever were observed.

Glyptonotus, unlike Orchomene, possesses distal primary pigment cells as well as secondary pigment granules within the retinula cell. The primary pigment cells extend from the proximal region of the crystalline cones to the distal edge, near the inner surface of the cornea. The exact number of these pigment cells per ommatidium is difficult to determine as their cellular boundaries are not clear. However, a conservative estimate would be that there are about 2-4 cells per ommatidium. The pigment grains are extremely small (approximately 0.04-0.08 um in diameter) but they have a tendency to form clusters measuring up to 0.8um in diameter. They migrate proximally and radially towards the crystalline cones when irradiated (see Fig. 5). The distribution of the screening pigment grains within the retinula cells reaches distally towards the inner surface of the corneal lens. The retinula cell pigment grains are much larger and measure approximately 0.1-0.4um diameter. They are distributed throughout the whole length of the retinula cell and are affected by an increase in ambient light in such a way that migrate radially towards the rhabdom edge.

(e) Retinula cell organelles and inclusions

Being the primary visual neuron of the ommatidium, the retinula cells exhibit a host of cytoplasmic organelles and

inclusions. The retinula cytoplasm shows an abundance of endoplasmic reticulum often occurring as a concentric array of cisternae (Fahrenbach 1969) or whorled structures (Plate 20, 27). Free ribosomes are widely distributed in the cytoplasm together with mitochondria. The presence of these organelles indicates that active metabolic and synthetic processes are taking place within the retinula cells mitochondria are particularly abundant in the distal and proximal region rather like in Orchomene. Mitochondria range from 0.8um to 2.8um. Most of the mitochondria are of spherical or longitudinal shape and have a crista-like ultrastructure. Within the retinula cytoplasm the density and position of the mitochondria is affected by the state of adaptation, dark-adaptation bringing about an increase and light-adaptation causing a reduction in the total number.

Multivesicular bodies (MVB) occur frequently in the retinula cells. These organelles vary in size from 0.6um to 1.28um and consist of vesicles of 0.04-0.08um in diameter packed within a membranous sheath. Most of the MVBs exhibit tightly packed vesicles but some have a looser organization or may even show signs of lysis of the vesicles within them (Plate 27B). Some of the MVBs are not membrane bound while others show rupturing of their membranes with their contents spilled out into the cytoplasm. Multivesicular bodies have been found to undergo a series of transformations which via multilamellar bodies (MLB) leads to the formation of large residual bodies (Eguchi & Waterman 1976; Itaya 1976; Blest et. al. 1978).

Multilamellar bodies (MLB) or 'onion bodies' are structures which assume the shape of a concentric whorl. They

are especially common around the proximity of the rhabdom (Plate 25A). Some of these multilamellar bodies may enclose within them vesicles or even some pigment granules. The abundance of this organelle is positively affected by dark-light adaptation. In the light-adapted state, more multilamellar bodies are formed near the rhabdom edge than when the eye is dark-adapted. Multilamellar bodies are known from crustacean (e.g. Crayfish: Eguchi & Waterman 1976) and insect-eyes (e.g. spider: Blest et. al. 1978) and are thought to be involved in the photoreceptor's membrane recycling process (Itaya 1976, Blest et. al. 1978).

The retinula cell plasm possesses numerous cytoplasmic vacuoles, scattered randomly together with other organelles throughout the cell. However, vacuoles that are associated with the rhabdom edge and found in several arthropod eyes under different names such as perirhabdomal vacuoles, palisade, 'Schaltzone' etc. are absent here. Those vacuoles with diameters ranging from 0.2um to 1.2um are affected by dark-light adaptation. During light adaptation the number of these cytoplasmic vacuoles is drastically reduced. In the dark-adapted state practically the whole of the retinula cytoplasm is filled with these vacuoles (Plate 25A, B).

2. Dark-light adaptational changes

(a) The compound eyes of crustaceans, like those of other animals are able to adapt and function over a wide range of environmental light intensities (sometimes up to 10 log units of light intensities are covered). In order to meet such a demand, the photoreceptors have the ability to adjust to the visual conditions at any given time by a two-step process. Through the involvement of dioptric elements (lenses, reflectors, light guides) and catoptric elements (screening, filtering and scattering pigments) the light entering the eye is influcenced. Through biochemical processes at the molecular or 'primary' level an equilibrium state between bleached and unbleached pigments is established. According to Nassel and Waterman (1979) the steady state of the visual membranes at the primary level is maintained by the continual membrane synthesis and breakdown which must be superimposed on and partly coincident with the secondary regulatory changes that established the state of adaptation. Therefore the position of individual cells as well as the organization of the intracellular components of the photoreceptors like pigment granules, nuclei, rhabdomeres, are not static but in a dynamic state of movement, regulated by light and other factors like temperature (Ali 1975; Meyer-Rochow & Tiang 1979) and colchicine (Miller 1975). Light-induced cell movements or the so-called photomechanical phenomena are well established in many compound eyes and have been studied intensely ever since Exner's pioneer work on the eyes of beetles (1891), but the detailed mechanism of this light-induced phenomenon is still not clear. In this investigation

of the dark-light adaptational changes in the Glyptonotus eye, the aim is to follow the changes at the ultrastructural level to compare them with those of other arthropods in particular those that live in less cold and less stable environments.

(b) Light microscopy

The anatomical changes following dark-light adaptation in the eye of Glyptonotus affect (a) the position of the screening pigment granules in the primary pigment cells and in the retinula cells and (b) the volume of the retinula cells. Fig. 5 illustrates the anatomical organizations of dark and light-adapted ommatidia in longitudinal and transverse sections. The compound eyes of Glyptonotus, like those of Orchomene plebs, do not exhibit circadian rhythm in the control of pigment migration. Thus, Glyptonotus can be dark-adapted or light-adapted at any time of the day, as long as one is aware of the fact that dark-adaptation in Glyptonotus requires several days.

Light adaptation in the eye of Glyptonotus is affected by the intensity of the light and the duration of exposure to it. The 1 hour exposure to bright sunlight of 100 000 lux was found to have an effect similar to that of an animal kept for 1 week in dim room-light of about 200 lux. The temperature was carefully maintained at $0 \pm 1^{\circ}C$ for both sets of experiments. In both experiments, the position of the secondary pigment granules within the retinula cells was that of the light-adapted state i.e. a radial migration of the pigment granules towards the proximity of the rhabdom (see Plate 23, Plate 24A, B). Light adaptation just like in Orchomene

plebs must be a rapid process in Glyptonotus too for histological investigations that followed 1 hour exposure to light of 100 000 lux, reveal that complete light adaptation had occurred.

Maybe because of the exposure to light during the hauling up process, maybe because all observations were carried out in the continuous light of the Antarctic summer, or maybe simply because the cellular and subcellular adjustments necessary to turn the light-adapted into a dark-adapted eye take longer in Glyptonotus than in other crustacean eyes, to obtain fully dark-adapted eyes is more difficult. It was found that several hours in the dark, immediately after the animals had been caught, was not long enough to get complete dark adaptation and I almost concluded that Glyptonotus eyes cannot be dark-adapted. However, finally fully dark-adapted eyes were observed, but only in animals which had been kept in the aquarium for 1 week with their left eyes pointed with black nailvarnish. The right eye remained unpainted as a control. Dark-adaptational features, but to a lesser extent, were also seen in 3 days dark-adapted material, and once recognized and identified as signs of proper dark-adaptation, they were even seen to a minor extent in 7 hours dark-adapted individuals. Dark adaptation was indicated by the absence of screening pigment granules from the proximity of the rhabdom edge (Plate 23B, 24B).

The primary pigment cells which contain minute pigment granules, form a dense sleeve around the crystalline cone during light adaptation. This is evident from both 1 hour exposure to bright sunlight and 1 week exposure to dim light

of unpainted eye of the animal (Plate 23A, Fig. 5). Such behaviour of the primary pigment may act as a pseudopupil mechanism that effects the selective reduction of radiant flux on the photosensitive rhabdoms. This anatomical effect is observable in Orchomene plebs (see Chapter I, D2), Limulus (Behrens, 1974) and insects e.g. dragonfly (Stavenga 1979). In the dark-adapted state, the minute pigment granules tend to cluster together and retract to the distal periphery, thereby effectively increasing the aperture.

The size and shape of the rhabdom is not noticeably affected by dark-light adaptation. However, the cellular areas of the retinular cells and the interstitial cells are much affected by the presence or absence of light. In light-adapted eyes the area occupied by retinula cells is small in comparison to that of the interstitial cells, but the reverse holds true for the dark-adapted eye. The ratio of the area of the retinula cell to that of the interstitial cell was found to be 0.54 in the dark-adapted eye and 0.19 in light-adapted material (based on 10 ommatidia of 5 eyes of each adaptational state). This effect is very strong in the temperature stress experiment (see next section).

(c) Electron microscopy

Differences in the rhabdom and the retinula cell organizations at the ultrastructural level were detected in both light and dark-adapted animals when studied with the electron microscope. The rhabdom size and shape do not change significantly, but the microvilli arrangement of the individual rhabdomere is affected by the state of adaptation. Light absorption and the effect of adaptation are thought to be

closely coupled to microvilli disruption of the rhabdom (Eguchi & Waterman 1967; Tuurala & Lehtinen 1971; Brammer et. al. 1978). However, in the eyes of Glyptonotus, there appear to be variations in the degree of microvillar disruption following light adaptation. Some rhabdomeres exhibit intense disruption of the orderly array of the rhabdomeric microvilli causing the villar membranes to form extensive whorled structures, multilamellar bodies (MLBs) or 'onion bodies' (Plate 25A). In others, pinocytotic vesiculations of villar membrane material are seen but the microvilli still maintain an orderly pattern which is similar to that of the dark-adapted state. The microvilli of the dark-adapted state are uniformly arranged along the rhabdomere. Most of the microvilli have slim extensions, up to 1um long, and positioned at regular intervals along the rhabdom edge into the retinula cytoplasm. The length and diameter of the individual mecrovillus is not significantly affected by the state of adaptation. However, more profound ultra-structural changes in relation to the different states of adaptation do not occur within thr rhabdom but in the retinula cytoplasm.

Pinocytic vesiculations of the microvillus membrane during light adaptation are more prominent in the eyes of Glyptonotus than in Orchomene plebs. The vesicles formed during pinocytosis occur regularly at the rhabdom edge. Their sizes range from 0.56um to 2.32um. Pinocytosis is found to occur in both the normal 1 hour light-adapted eyes and the 1 week unpainted eyes. Glyptonotus antarcticus differs from the Norway lobster, Nephrops norvegicus in the extent to which prolonged exposure to low intensity light produced photoreceptor degeneration, (Leow 1976). It was demonstrated that after an exposure of 1 week to light of low intensity,

the photoreceptors show similarities to those exposed to bright sunlight for 1 hour. Thus, Glyptonotus appears to react to light in the same way that Orchomene plebs and other crustaceans do: light induces or triggers membrane turnover of the rhabdom (Tuurala & Lehtinen 1971; Eguchi & Waterman 1976; Nassel & Waterman 1979). Radioisotope tracing of tritiated leucine in the photoreceptors of Oniscus (Tuurala & Lehtinen 1974) also showed that light promotes the breakdown of the microvillus material which is related to membrane turnover. This process of membrane turnover is thought to be mediated by multivesicular bodies and multilamellar bodies (Itaya 1976, Brammer et. al. 1978).

Multivesicular bodies (MVB) occur within the retinula cytoplasm adjacent to the rhabdomere. In the light-adapted state, the number of this complex structure appears to decrease in number. However, during dark adaptation, the multivesicular bodies increase in number and size, a situation that was also found in the terrestrial isopod, Porcellio scaber (Nemanic 1975). Some of these multivesicular bodies have loosely packed vesicles while others show signs of lysis of the vesicles contained within them. On the other hand, the closely related structure, (MLB) appears to increase both in size and number with light adaptation. Most of the compound and complex microstructures are of various shapes and dimensions and are localized near the vicinity of the rhabdom edge. Some have vesicles enclosed within them and their dimensions range from 0.5um to 5um in diameter. In the dark-adapted state, the number and sizes of these multivesicular bodies, are reduced drastically and are no longer localized near the

rhabdom but distributed randomly in the retinula cytoplasm.

Scattered throughout the retinula cytoplasm are the so-called cytoplasmic vacuoles. They are a prominent feature of the cytoplasm of the dark-adapted eye. Both number and size of these vacuoles are much reduced upon light adaptation. Their density may reach approximately 50 per $5um^2$ and the dimensions of an individual vacuole range from 0.2um to 1.2um in diameter during dark adaptation. In the light-adapted state, the abundance decrease to about 10 per $5um^2$ and the maximum diameter is reduced to 0.5um (values calculated from 10 sections of 5 eyes representing different states of adaptation). Some of these vacuoles may be of lysosomal nature, but the actual part these vacuoles play in the cytoplasm particularly in the dark-adapted state following previous exposure to light is uncertain.

Mitochondria are distributed throughout the length of the retinula cytoplasm. Their number but not their size is apparently affected by dark-light adaptation. In the light adapted state, an average section may show 3 mitochondria per retinula cell, while in the dark-adapted state, the density may have increased so that now 10 per retinula cell can be counted in a section. During light adaptation, most of these mitochondria appear to migrate towards the periphery of the retinula cell and also towards the distal region of the cytoplasm (Plate 27A, B).

Another ultrastructural difference of significance related to dark-light adaptation in the eye of Glyptonotus, involves the interstitial cytoplasm. The hollow spherical vesicles of the interstitial cells exhibit darker-staining

characteristic in the light adapted state and have a maximum diameter of 0.28um. In the dark-adapted state, these hollow spherical vesicles increase in size, now reaching maximum diameters of 0.44um. These hollow vesicles are not identical to the 'echinosomes' of the interstitial cells of Orchomene plebs, but they, nevertheless, appear to exhibit some resemblance, for they may be covered by a rather irregular membrane on the outside (Plate 27B).

3. Temperature induced changes

(a) The extremely low and constant temperature of the Antarctic marine environement has recently stimulated a strong interest in research on the physiological effects of environmental temperature in marine poikilothermic animals. Metabolic activities, energy balances and lethal temperatures have been studied in a number of Antarctic marine crustaceans (e.g. amphipods, Paramoera Walkeri; Opalinski 1974 and Orchomene plebs, Wells 1978; decapods McWhinnie et. al. 1975) and fishes (MacDonald & Wells 1978).

Apart from the casual observation by Wells (1978) on the lethal temperature of Glyptonotus no systematic investigation has been carried out with regard to the effect of temperature on the eyes of Glyptonotus. The aim of this investigation is to attempt to elucidate the effect of temperature on the position of cells and organelles and the ultrastructure of the photoreceptors of Glyptonotus, and to compare them with Orchomene plebs. The animals were kept in 10°C warm sea-water in a dark-aquarium but exposed to a dim background light of about 50 lux or less at the animal's eye level. After 7 hours at this elevated temperature, the eyes were carefully dissected out and fixed immediately.

(b) Light microscopy

The size and shapes of the rhabdom were found to be affected in the eyes of the animals exposed to the unnaturally high temperatures of 10°C (Plate 24C). When compared with material of the light or dark-adapted state at 0°C (Plate 24A, B), the rhabdoms still possessed their star-shaped profile,

but their overall sizes were very much reduced (to about 30.7 um in diameter from 40um of the normal 0°C material). The rhabdom's 'arms' were also found to be much broader but less long (about 11um in length, compared with 18um of the light- or dark-adapted eyes at 0°C). (The figures were obtained as an averages of 10 ommatidial measurements from 5 eyes each). Despite rather dark conditions throughout the experiment (ambient light level of 50 lux or less), the screening pigment granules clearly occupy a position which is characteristic of totally light adapted material (Plate 24A, C). The screening pigment granules in the retinula cytoplasm adjacent to the rhabdomere, represents a more darkly-stained mass than in the lighter stained light-adapted material. Another profound structural change affected the interstitial cells. The size of these cells increased significantly which may have been caused by the corresponding reduction of size of the retinula cells. The ratio of the size of the retinula cell to that of the interstitial cell was found to be 0.11, compared to 0.19 of the light-adapted eye and 0.54 of the dark-adapted eye (based on the average of 10 ommatidia of 5 eyes from each state of adaptation). The recovery experiment of returning the animals to sea-water of 0°C was not carried out. However, more than 90 per cent of the animals survive the 7 hours of exposure to 10°C warm water (Wells 1978).

(c) Electron-microscopy

The electron-micrograph of a transverse section through the 10°C heat-stressed dark-adapted rhabdom shows that the ultrastructural integrity is still retained. The retinula cytoplasm has decreased in area and the 'arms' of the rhabdomeres have also become reduced in length (Plate 28).

However, relative to the total size of the rhabdom, the microvilli appear to increase in length giving the rhabdomeres a short and stout appearance. The microvillar arrangement still remains more or less undisturbed with villi being uniformly aligned except in some rhabdomeres where membrane disruptions have already begun. This is in contrast to the heat-stressed retinas showed that individual microvillus-membranes were no longer discernible and that the entire rhabdom was in a state of 'melting'.

The dimensions of the microvillar structures are apparently affected by heat-stress. The length of the individual microvillus varies from 0.5um at the tip of an 'arm' to 3.5 um in the centre of the star-shaped rhabdom (compared with 0.08um and 2.4um, respectively for light and dark-adapted eyes at $0^{o}C$). The diameters of the microvilli have also increased ranging from 0.12um to 0.32um compared with 0.08um to 0.12um of the material kept at $0^{o}C$. The microvillar edge along the rhabdomere is rather smooth in contrast to the uneven slim projections of the dark-adapted or pinocytic vesiculations of the light-adapted eye. The inner-villus space has increased and microvilli are lighter stained in contrast to the closely packed and darkly stained microvillar membranes of the light and dark-adapted eye at $0^{o}C$ (Plate 29A, B, C).

The retinula cytoplasm of the heat-stressed eyes stains considerably, although unlike the situation found in the light and dark-adapted eye kept at $0^{o}C$, there are very few cytoplasmic organelles present. Minute particles of unknown nature, presumably the remnants of some thermo-labile organelles, appear to increase in abundance within the cytoplasm. The

distribution of multivesicular bodies along the edge of the rhabdomere increase too, with sizes ranging from 0.15um to 4.0um in diameter. Mitochondria and cytoplasmic vacuoles which were abundantly distributed within the dark-adapted retinula cytoplasm and to a lesser extend in the light-adapted eye, seem to be lacking in the heat-stressed material. The dark screening pigment granules appear to form the major cytoplasmic inclusions. From the position of these screening pigment granules, one gets the impression that the animal has been exposed to bright sunlight, but the animals had really been under nearly dark conditions. The pigment granules have migrated closer to the edge of the rhabdom when compared with sections of the light-adapted material. Electron-lucent structures which were abundantly found within the retinula cytoplasm of heat-stressed material of the eye of Orchomene plebs are completely absent in Glyptonotus.

Alterations in the structural components of the interstitial cells also occur in the heat-stressed material. The vesicles within the interstitial cells, judging from their appearance as empty holes, do not seem to be very well penetrated by Epon after exposure to 'heat'. The cytoplasm also appears more transparent with fewer vesicles present, when compared with dark and light-adapted eyes of animals kept at $0^{o}C$.

E. DISCUSSION

1. Basic anatomy of the eye

The general organization of the Glyptonotus compound eye and its constituent ommatidia is different from that of other isopods as well as crustaceans of other groups. Isopods are known to have a wide-ranging geographical distribution and the geologically more recent isopod fauna of the deep-sea is thought to have evolved from the Antarctic fauna (Kussakin 1973). However, the diversity of their photoreceptor organization is wide ranging too. It ranges from the eyeless isopod Paragnathia (Monod 1926) to Oniscus (Debaisieux 1944) which possesses the largest number of retinula cells known for any crustacean eye. Ligia (Ruck & John 1954, Edwards 1969) has an unusual open type of rhabdom and Glyptonotus further differs from other isopods and other crustaceans in having two sets of retinula cells (5 or 6) per ommatidium. Most crustaceans are known to have a more or less consistent number of retinula cells within each ommatidium e.g. amphipods have five (see Chapter I D 1), decapods have seven plus one ecentric cell (Waterman 1961; Meyer-Rochow 1975; Meyer-Rochow & Tiang 1979).

The gross structural arrangement of the eye of Glyptonotus is of the apposition type first described by Exner (1891) and shows a certain similarity to the eye of Orchomene plebs. The distal ends of the photoreceptors are capped by the unfaceted outer corneal cuticle different from the faceted biconvex lenses of the isopod Porcellio (Nemanic 1975) or the convex corneal cuticle of Ligia (Edwards 1969). A multilamellated regular structure of the corneal lens was suggested to be

less likely to cause image distortion than one with more irregularly organized epi-cuticle (Nemanic 1975). The concentric rotating fibers in the corneal lenses of Limulus polyphemus have been reported to act as light depolarizers (Fahrenbach 1968). Polarization sensitivity in Glyptonotus has not yet been demonstracted. A definite optical significance for these rotating fibers has not been found, but apart from their effect on the plane of polarization they may be able to 'bend' rays that strike them at an angle, and thus they may create optical path differences indistinguishable from those caused by a non-homogenous medium with changes in the refractive index (Meyer-Rochow 1975a). Glyptonotus appears to possess a larger crystalline cone than other crustaceans living in dimly-lit environments (e.g. Panulirus: Meyer-Rochow 1975a; Gennadas: Meyer-Rochow 1977; Squilla: Schonenberger 1977; Phronima: Ball 1977; Thysanopoda: Meyer-Rochow & Walsh 1978; Orchomene plebs: Meyer-Rochow & Tiang 1979). The homogenous lens and the large crystalline cones may together contribute in providing a more efficient window for the ommatidium, thereby maximising photon capture.

The retinula of Glyptonotus eye consists of 5 or 6 retinula cells radially arranged around the ommatidial axis. There is probably no functional significance attached to this dual number of retinula cells within the eye, as no pattern for the distributing of the two types of ommatidia (with 5 and 6 retinula cells) was apparent. Specialization of the retinula cells at the proximal region, commonly found in other isopods and marine arthropods (e.g. isopod Ligia (Edwards 1969); isopod Porcellio (Nemanic 1975); stomatopod Squila (Schonenberger 1977); xiphosuran Limulus (Fahrenbach 1969) where a

basal or eccentric cell is developed) are absent in Glyptonotus.

The centrally fused star-shaped rhabdom is formed by the equal contribution of the retinula cells, unlike the situation found in Orchomene plebs where an unusually small fifth cell contributed a small rhabdomere. A differentiation of the rhabdom into distal and proximal parts (found in Streetsia: Meyer-Rochow 1978) and Sericesthis (Meyer-Rochow 1977) and thought to improve absolute sensitivity) has not taken place in Glyptonotus. Instead, a uniform layer of rhabdoms stretching from the inner surface of the cone to the proximity of the basement membrane is present. Rhabdomeres with their microvilli at right angles to each other arranged in alternating superimposed layers (a situation found in the lobster: Rutherford & Horridge (1965), Daphnia: Waterman & Eguchi (1966) and Gennadas: Meyer-Rochow & Walsh (1977) and thought to be related to polarization sensitivity) are not developed in Glyptonotus. However, the microvilli have about the same range of diameters found for those of other crustacea (0.06um - 0.1um). The nuclei of the retinula cells are located below the basement membrane, a feature shared with Orchomene plebs and other amphipods but different from isopods like Ligia (Edwards 1969) and Porcellio (Nemanic 1975) in which the nuclei are located well above the basement membrane.

2. Dark-light adaptational changes

Dark-light adaptational changes affecting the position of retinula cells and pigment granules, size and shape of rhabdom and microvilli, and the width of the clear-zone have been

reported in several insects and crustaceans (see review by Walcott 1975). Usually light adaptation brings about significant movements of retinula cells, rhabdom, crystalline tract and primary pigment cells. The crustacean, Squila mantis (Schönenberger 1977) can serve as an excellent example, for it exhibits all these changes and movements upon dark-light adaptation. In the eye of Glyptonotus, apart from the increase in cellular volume of the interstitial cells and the migration of pigment granules, no significant movements occurred within the structural components of the ommatidia during dark-light adaptation. On light adaptation the cellular volume of the interstitial cells increased which brought about a corresponding decrease in the cellular volume of the retinula cells. The screening pigment granules which in the dark-adapted state were located in the outer peripheral region migrated into the inner region near the rhabdom, presumably to control the photon flux (see Chapter I D3). The exact function of the volume change of the interstitial cells is uncertain but it may facilitate the radial migrations of screening pigment granules during different states of adaptation.

The rhabdom being the site of photo-transduction process in the ommatidium has been the centre of interest for a long time (see compound eye research by Langer 1966, Eguchi & Waterman 1966, 1967, Hamdorf 1979). The effects of prolonged absence and presence of light on rhabdom organization are well documented and it is known that the structural integrity of the rhabdom is altered in both cases (Eguchi & Waterman 1966, 1979; Tuurala & Lehtinen 1971, Behrens & Wolf 1976, Brammer et. al. 1978). Before structural change manifests itself in the

rhabdom relatively long exposure to light up to 2 hours may be necessary (Loew 1976). The major changes observed in such a situation have been a significant reduction in the volume of the rhabdom (Behrens & Wolf 1976; Brammer & Clarin 1976) and a 'loosening' of the microvilli architecture (Loew 1976). The visual pigments which form the biochemical units of the photosensitive membranes are freed during the destruction of the membranes, they are thought to be recycled through stages which involve transformation into coated vesicles and multivesicular bodies (Brammer & Clarin 1976). In the eye of Glyptonotus, coated vesicles (through pinocytosis) and multilamellar bodies appear to increase following light adaptation. However, the number of multivesicular bodies appears to increase during dark-adaptation a feature shared with the isopod Porcellio (Nemanic 1975) but not with Oniscus (Tuurala & Lehtinen 1971) and the crab Libinia (Eguchi & Waterman 1967), in which the converse is found. However, in view of the difficulty in getting a fully dark-adapted state at the initial stage, exposure to light during the hauling-up of Glyptonotus from under the sea-ice may have had a long lasting effect. This suggests that the increase in MVB in the dark-adapted eyes could be the result of the aftereffect of initial light exposure. The actual function of MVBs is still controversial. They have been suggested to either transport photopigments to the rhabdom (Brammer & White 1969) or carry catabolites away (Rutherford & Horridge 1965). The presence of light is directly correlated to membrane breakdown via the formation of multilamellar bodies in the eye of Glyptonotus. The variations from massive multilamellar bodies formation to pinocytic vesiculations in some eyes could be due to differences in age, size

or perhaps even the diet and state of nutrition of the animals. Since none of these factors have so far been studied in any crustacean, a comparative analysis would not have been possible here. The renewal of the photosensitive membranes in Glyptonotus is probably brought about by the increase in the number of multivesicular bodies. Turnover of photosensitive membrane structures in Limulus shows a very similar formation of multilamellar bodies on either light adaptation or mimicking the effect of light by efferent optic nerve stimulation (Chamberlain & Barlow 1979). The renewal of the membranes is associated with an increase in multivesicular bodies (Brammer et. al. 1978).

3. Temperature induced changes

The effect of temperature on the structural integrity of the eye of Glyptonotus has revealed some interesting results. Heat-stress (10°C) causes the retinal pigment in dark-adapted eyes of Glyptonotus to migrate into a position characteristic of the light-adapted state. Influences of temperature on the state of adaptation of the retinal screening pigments have been found not only in the invertebrate but also in the vertebrate eye. Early light microscopical work on crustacean eyes by Congdon (1907) and Bennitt (1924) showed that an increase in temperature caused a movement of the pigment in a direction opposite to that produced by light, a situation contradictory to Glyptonotus or Orchomene. However, in the frog (Fujita 1911), heat-stress (34° - 37°C) in darkness caused an expansion of the retinula epithelial pigment and a strong contraction of the cones. Therefore, an elevated temperature in the dark has the same effect as light-adaptation in the frog. This agrees with results obtained from the Arctic crustacean Gammarus

oceanicus (Ali & Steele 1961), the Antarctic Orchomene plebs and Glyptonotus, and even the mouse in which Hollyfield & Bersharse (1978) have shown that both light and temperature increase the rate of membrane turnover. Electrophysiological recordings on the eye of Glyptonotus also show that the resting potential of the retinula cell drifts to a level characteristic of the light-adapted state, when the temperature is raised (Laughlin 1979, unpublished observation).

The mechanism of this heat-generated light adaptation or perhaps a light-generated heat effect is not clear. The dark screening pigment granules may be involved by absorbing radiation as suggested for Orchomene plebs. The reason why light adaptation in heat-stressed Glyptonotus kept in a dark environment was more complete than that observed in Orchomene plebs may lie in the fact that Glyptonotus was not kept in complete darkness (approx. 50 lux). Under these conditions the screening pigment granules are more dense and the cellular volume of the interstitial cells have increased more significantly, drastically reducing the cellular volume of the retinula cells, than in the heat-stressed eye of Orchomene plebs.

Ultrastructurally, the microvilli arrangement is more or less intact in Glyptonotus and does not exhibit any signs of 'melting' like in the eye of Orchomene plebs where individual micorvilli were not discernible. The microvilli in heat-stressed Glyptonotus rhabdomeres appear to be unusually long, which could be related to the general shortening of the rhabdomeric 'arm'. The microvillus diameter as well as the inter-villus space have increased. The performance of microvilli in this state is still speculation, but one can perhaps

state that the lipid components of the Glyptonotus microvilli are likely to be different from Orchomene plebs.

Thus, one can draw the same conclusion as with Orchomene and say that in Glyptonotus, too, temperature and light both cause separate but closely related effects which must not be studied in isolation from each other.

PLATE 18

Antarctic isopod : Glyptonotus antarcticus

Side view of Glyptonotus antarcticus. When fully grown this giant Antarctic marine isopod measures about 117.5mm in length. In comparison, the common garden slater (arrow) only reaches about 12.5mm. Note also the dorsal eye (arrows) which is one of the two compound eyes present on the dorsal side of the head. Glyptonotus has another pair of very much smaller eyes which are located on the ventral side of the head. This animal is benthic and has a circum-polar distribution from relatively shallow down to 300m deep water. The eyes may be important in providing visual cues for the animals in its scavenging feeding behavior.


5mm

PLATE 19

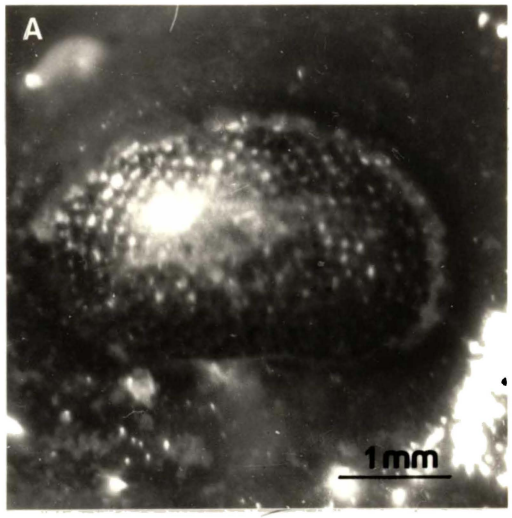
The compound eye of Glyptonotus antarcticus

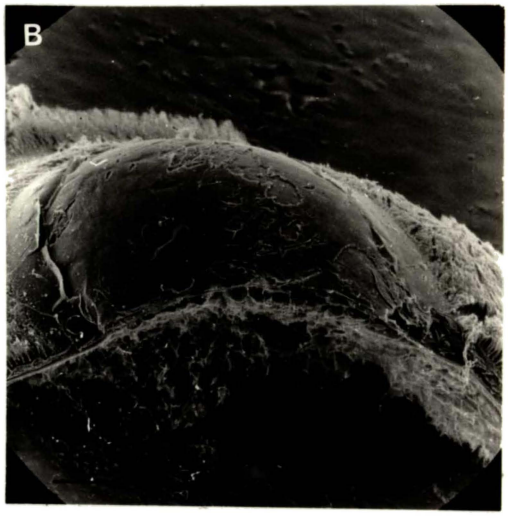
A Close-up photograph showing the whole dorsal compound eye of Glyptonotus antarcticus. The eye appears heavily pigmented and has a dark appearance.

B Scanning electron-micrograph of the external appearance of the compound eye. Note the smooth cuticle; individual facet are not discernible. The outer cuticle forms a hard and tough protective covering for the compound eye.

C Scanning electron-micrographs of the inner side of the corneal cuticle after partial removal of the external tough layer. Note the slightly convex 'bumps' on the inner surface of the cornea indicating the facets of individual ommatidia.

D Composite of two light micrographs showing obliquely sectioned ommatidium. The cornea (C) is dense and clearly lamellated. Note the darker stained epi-cuticle. Below the cornea are two corneagenous cells. The crystalline cones (C.C.) lie just below the corneagenous cells. The rhabdom (RH) is separated from the crystalline cone by a narrow space of 2-4um width. The retinula cells (RET.C.) can be distinguished by the lighter stained cytoplasm. The massive interstitial cell help to optically isolate the ommatidia as well as individual retinula cells. They extend from the proximal region of the crystalline cone to basement membrane (BM). Below the basement membrane are the retinula cell nuclei.


A
1mm


B


C
0.1mm

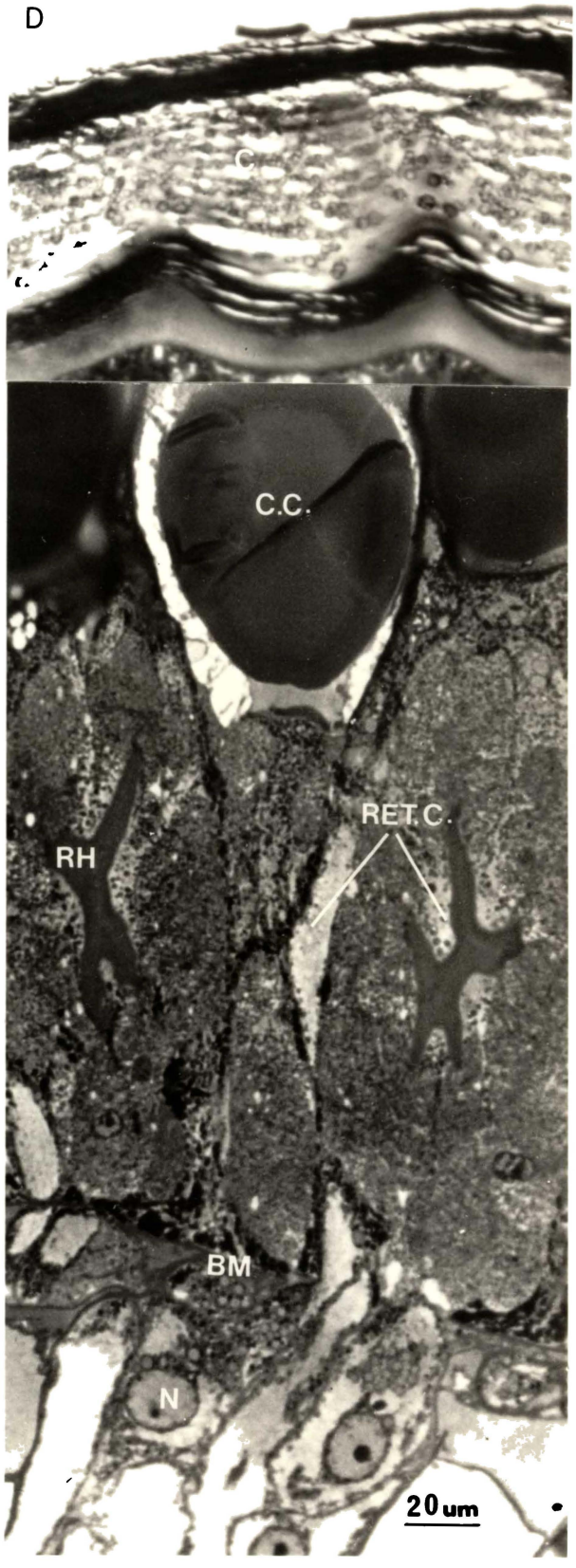

D
C
C.C.
RET.C.
RH
BM
N
20um

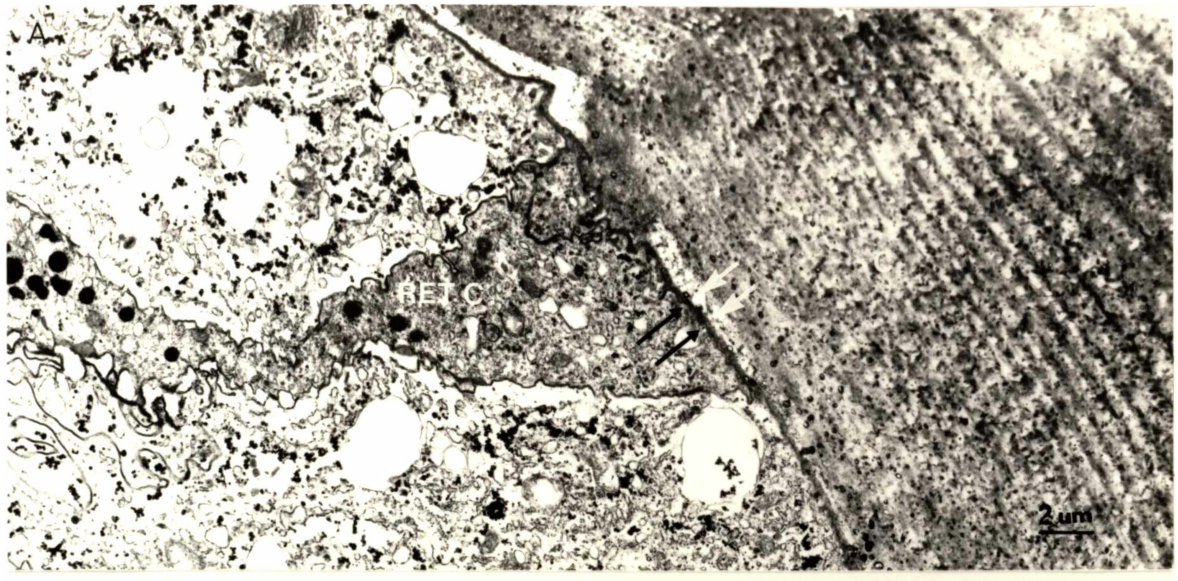
PLATE 20

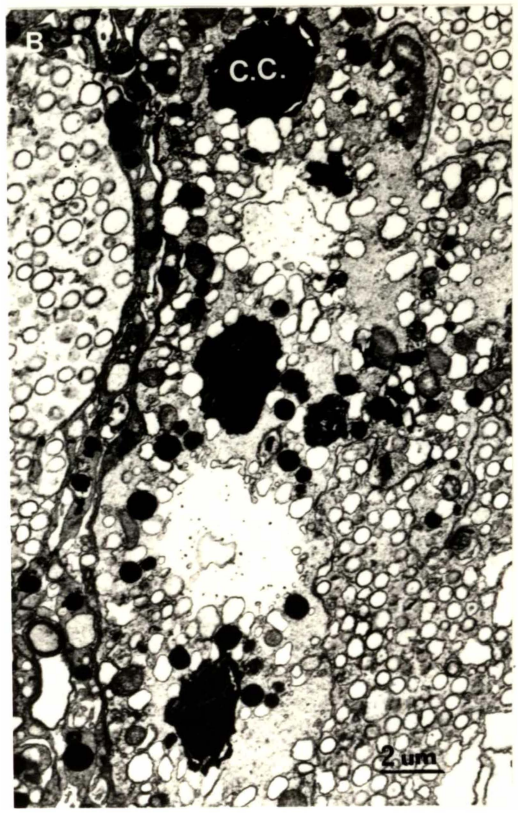
The Dioptric apparatus

A Electron-micrographs of a longitudinal section through the cornea (C) and the distal retinula cell (RET.C.) region. Note the cell boundary where the distal retinula cell region meets the cornea (arrows). The cornea is seen here as a multi-lamellated structure. The retinula cell is distinguished by the cytoplasmic inclusions, the dark-screening pigment granules. The smaller particles, often occuring in cluster, presumably belong to the primary pigment cells adjacent to the retinula cell.

B Electron-micrograph of the proximal region of the crystalline cones. Note the arrangement of the cones in a more or less straight line. The cell boundaries of cone cells were not traceable at this level, but it is clear that the cones are optically isolated from each other.

C Light micrograph showing transverse section through the crystalline cones. This section reveals that the cones are composed of two cells, often oriented more or less in the same direction. The peripheral region appears to be stained darker when compare with the inner cone. The cones are clearly surrounded by the cone or 'Semper' cells, which secreted it. The dark screening pigment particles seen between the cones presumably belong to the primary pigment cells which surround the crystalline cone cells. Inset shows the inner surface of the facet with the hard corneal cuticle peeled away.


A
RER
2 um


B
C.C.
2 um

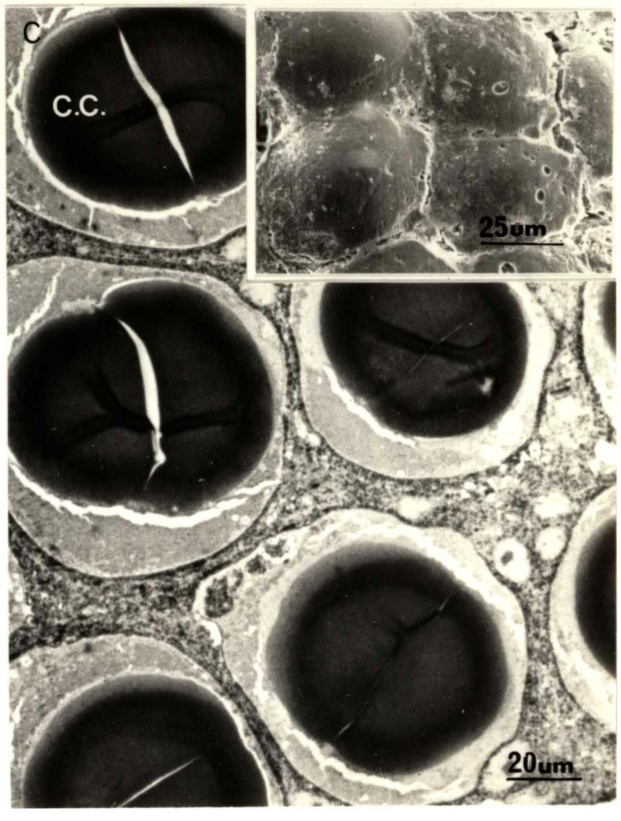

C
C.C.
25um
20um

PLATE 21

Dark-adapted retinula cells and rhabdom ultra-structural organization

This transmission electron-micrograph of a transverse section through an ommatidial group of five retinula cells (numbered 1-5) shows the organization of the rhabdomeres and the five retinula cells which form the centrally fused rhabdom. The retinula cytoplasm is particularly rich in various cytoplasmic organelles including multivesicular bodies, multilamellar bodies, cytoplasmic vacuoles and mitochondria of various shapes and sizes. Note the general absence of screening pigment granules within the retinula cell plasm adjacent to the rhabdom edge, which is characteristic of the dark-adapted state. The whole retinula cells and rhabdom structure are surrounded by six or seven interstitial cells which contain spherical hollow vesicles.

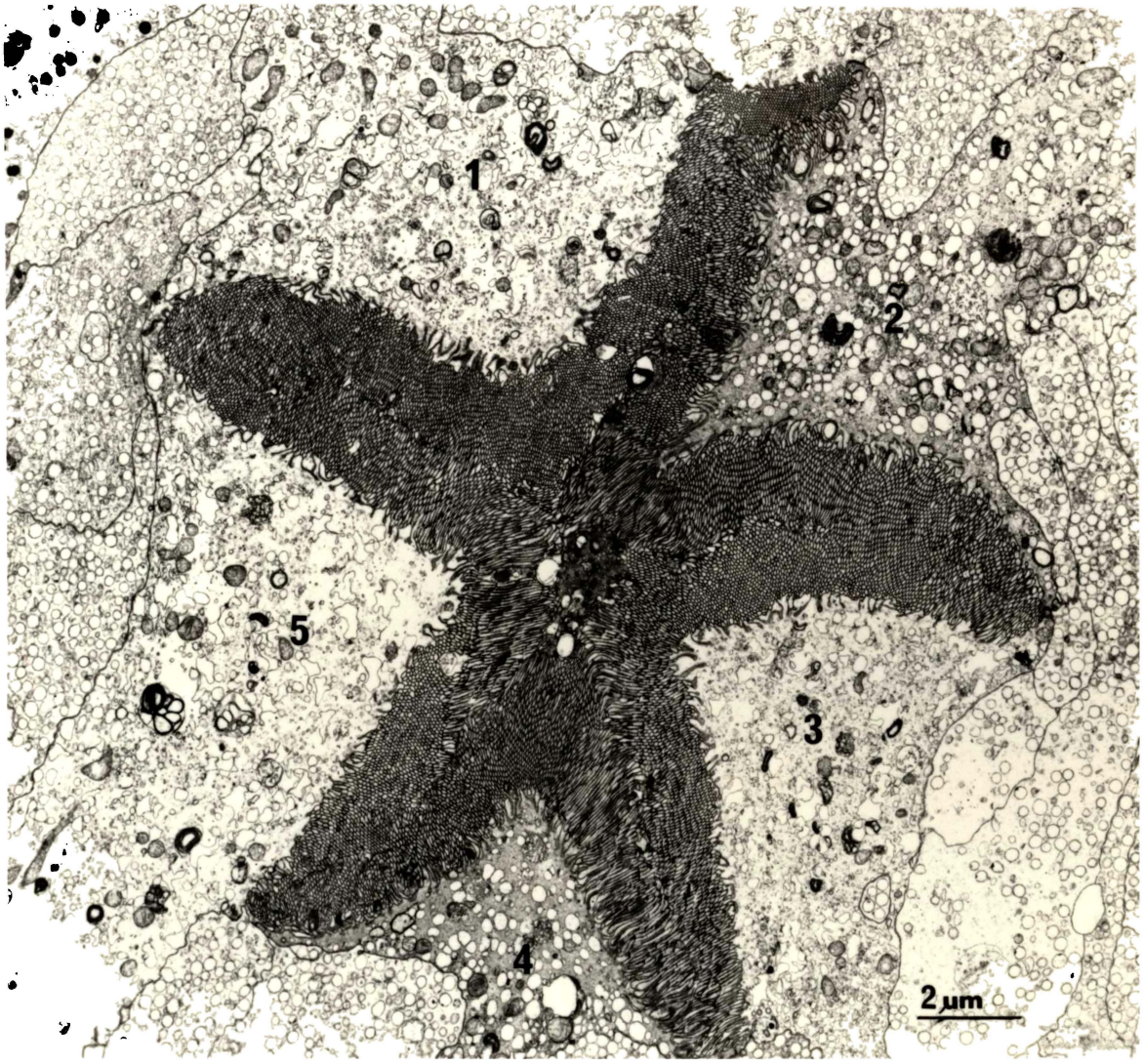

1
2
3
4
5
2 µm

PLATE 22

Light-adapted retinula cell and rhabdom organization

This transverse section through the ommatidium shows that the rhabdom consists of rhabdomeres belonging to six retinula cells (number 1-6). Each rhabdomere consists of microvilli i.e. finger-like projections of the retinula cells which contribute to the six pointed, star-shaped, centrally fused rhabdom. The rhabdom edge shows signs of membrane break-down through the process of pinocytosis whereby vesicles 'bud-off' from the microvillar edge to aggregate into multilamellar bodies. The screening pigment granules have migrated inwardly towards the edge of the rhabdomal region of the retinula cells plasm. This is quite characteristic of the light-adapted state.

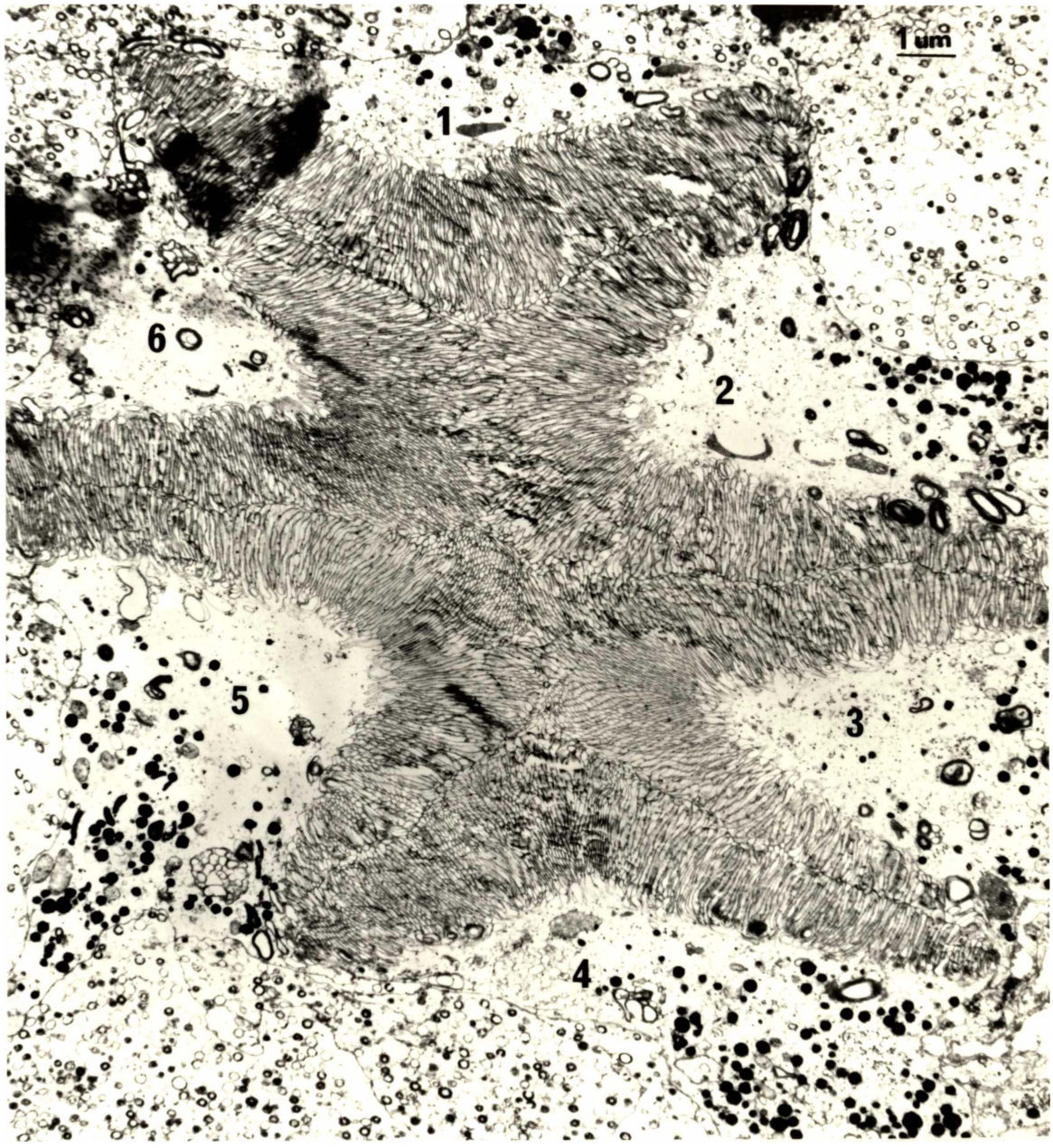

1 um
1
2
3
4
5
6

PLATE 23

Ommatidial organization of painted and unpainted eyes

A This light-micrograph represents an oblique section through the unpainted eye. Note the position of the screening pigment granules in the retinula cells (RET.C.) which is typical of the light-adapted eye. The rhabdom (RH) with its shape of a five pointed star is of normal configuration and size. The pigment grains of the primary pigment cells around the periphery of the crystalline cones (C.C.) show increased abundance when compared with the dark-adapted eye.

B In this oblique-section through the painted eye, the ommatidial organization is characteristic of that of a dark-adapted eye. The screening pigment granules in the retinula cells (RET.C.) have migrated away from the proximity of the rhabdom (RH) edge and concentrate in the outer periphery of the retinula cell plasm. The primary pigment cells around the crystalline cones (C.C.) also reveal a decrease in other pigment grain density indicated by their lighter staining characteristic.

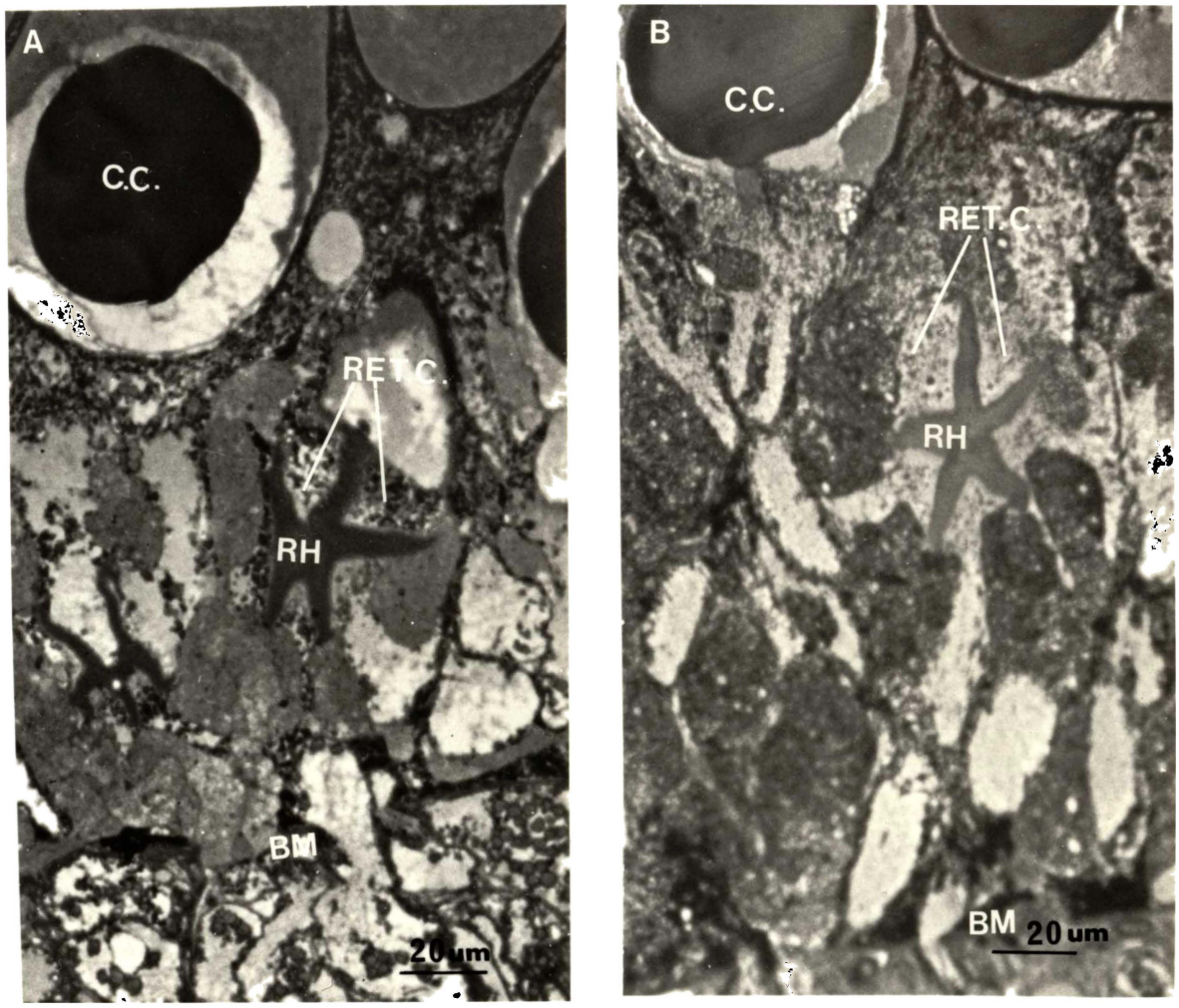

A
C.C.
RET.C.
RH
BM
20 um
B
C.C.
RET.C.
RH
BM
20 um

PLATE 24

Light micrographs of transverse-sections through (A) normal light-adapted, (B) normal dark-adapted and (C) heat-stressed dark-adapted eyes. The position of the screening pigment granules as well as the shape and size of the rhabdoms are affected by the adaptations. In the normal light-adapted state (A), the pigment granules within the retinula cells migrates towards the proximity of the rhabdom edge. The interstitial cells which isolate the individual retinula cell increase in size and appear to reduce the size of the retinula cell plasm. Dark-adaptation (B) brings about the migration of the screening pigment granules away from the rhabdom edge so that a dense concentration of pigment in the outer periphery of the retinula cells is formed. In the heat-stressed eyes (C), the rhabdom 'arms' are found to be much shortened but broader in size compared with the dark or light-adapted eyes at $0^{o}C$. Despite rather dark conditions throughout the experiment (ambient light level 50lux or less), the screening pigment granules occupy the position which is characteristic of the light-adapted state or even to a greater degree. The interstitial cells have increased in size and correspondingly reduce the size of the retinula cells.

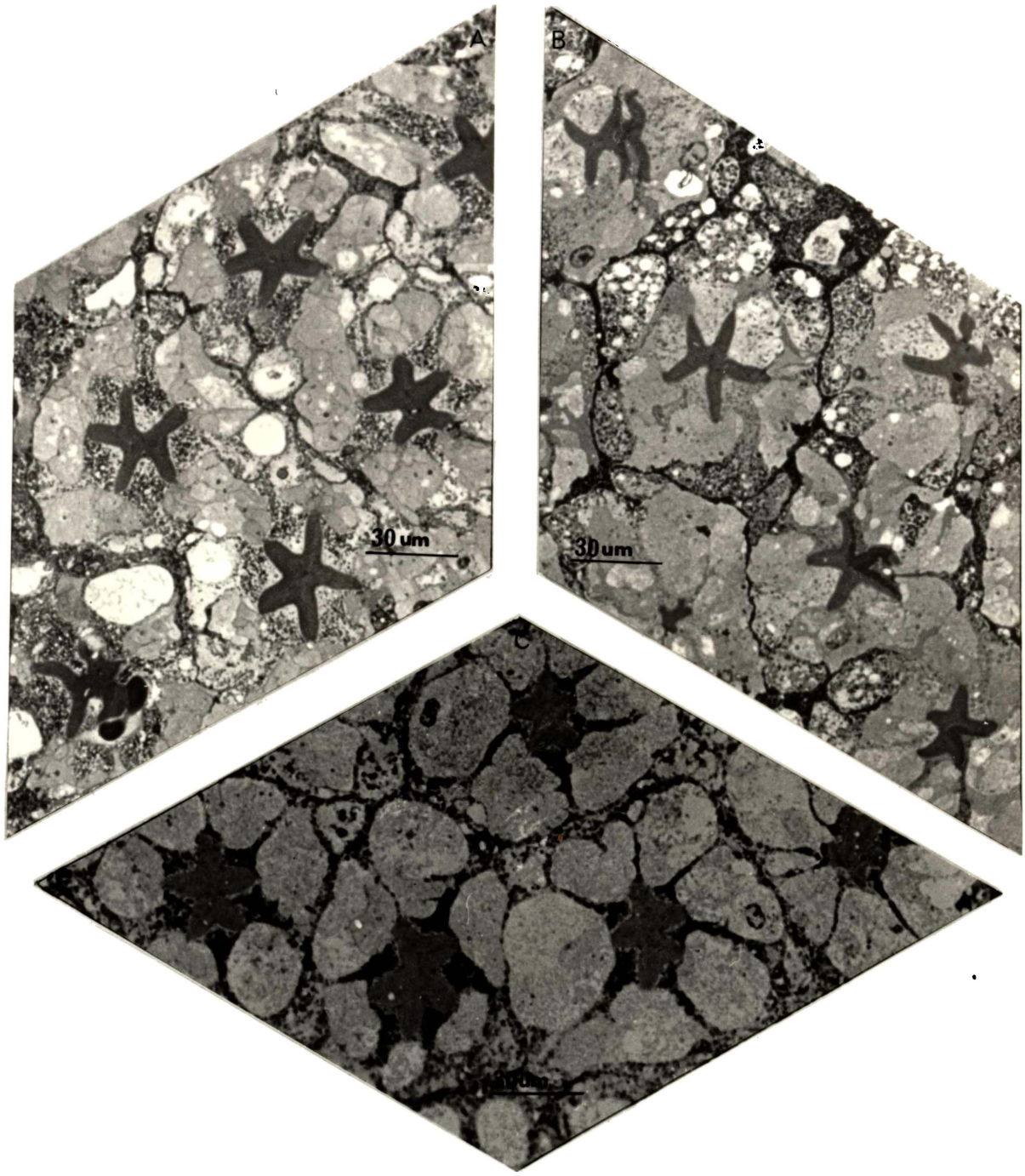

A
30 um
B
30 um

PLATE 25

Dark-light ultrastructural organization of the rhabdom edge

A This electron-micrograph of the light-adapted eye shows massive microvilli disruption at the rhabdom edge. The alignment of the microvilli along the rhabdomere is also greatly distorted. Large numbers of multilamellar bodies (MLB) form around the vicinity of the rhabdom edge indicating microvillar membrane break-down. The screening pigments have migrated into the cytoplasm adjacent to the rhabdom. This is typical of the light-adapted state.

B This electron-micrograph shows the uniformly aligned microvilli of the dark-adapted rhabdom. The multilamellar bodies (MLB) which were common in the light-adapted eye are absent here. Instead, multivesicular bodies (MVB) and cytoplasmic vacuoles increase in abundance. The absence of screening pigment granules in the cytoplasm adjacent to the rhabdom is characteristic of dark-adaptation.

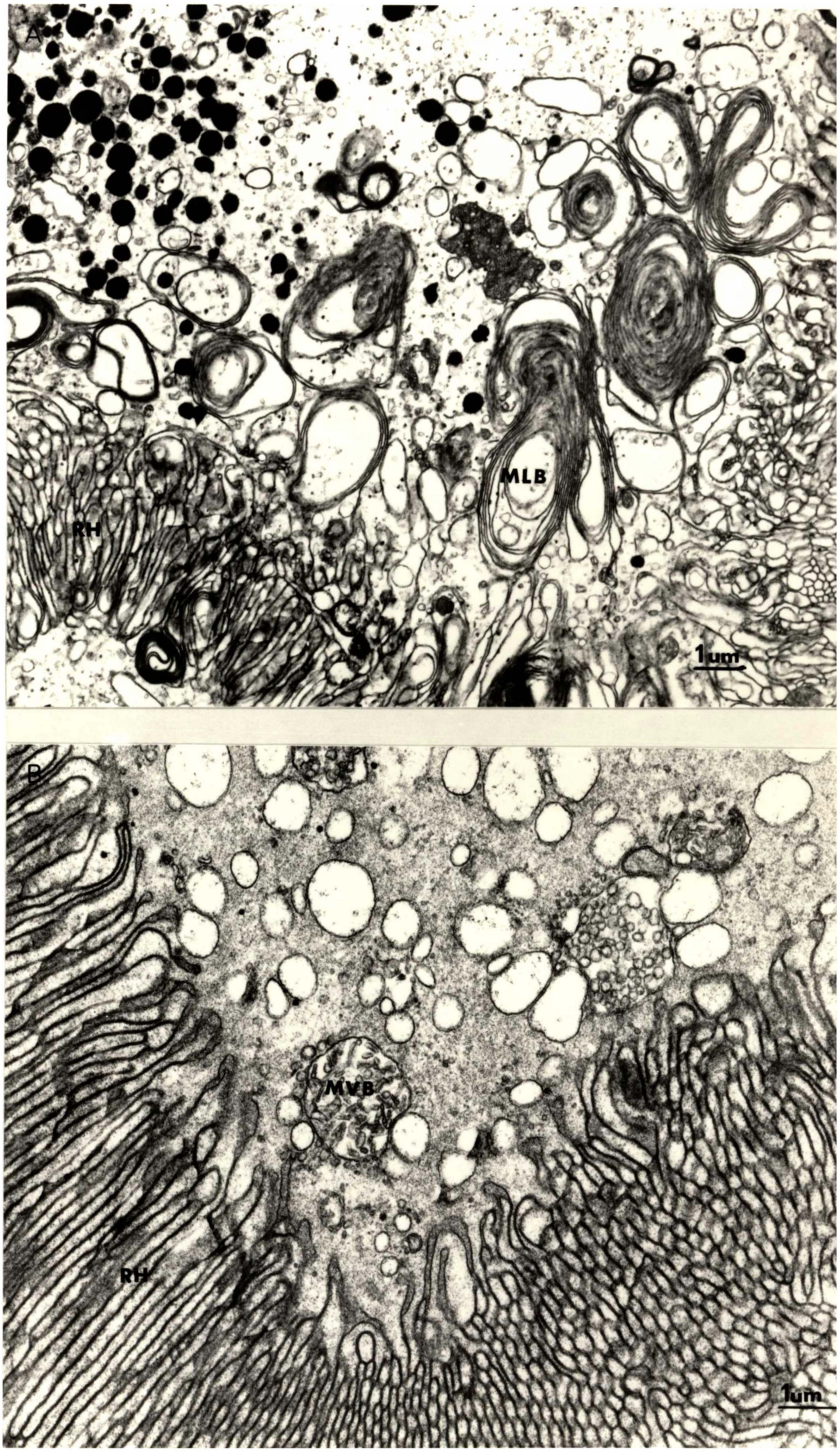

A
MLB
RH
1um
B
MVB
RH
1um

PLATE 26

High-magnification of the ultrastructure of the rhabdom edge of painted and unpainted eye

A This electron-micrograph through the unpainted eye shows that the rhabdom ultrastructure resembles that of the light-adapted state. Vesicles of various sizes were found in the cytoplasm. Some vesicles were found to be 'budding off' at the microvillar edge of the rhabdom. The screening pigment granules restricted to the outer periphery of the retinula cell plasm in dark-adapted eye, have migrated towards the proximity of the rhabdom edge. A typical retinula cell mitochondrion (M) of the tubular crista type is also found in the cytoplasm.

B The painted and, therefore, dark-adapted eye shows a structural organization which is characteristic of the dark-adapted state. Microvillar disruption through 'budding-off' of vesicles from the rhabdom edge are less common than in light-adapted material. Screening pigment granules are usually absent from the vicinity the rhabdom edge.

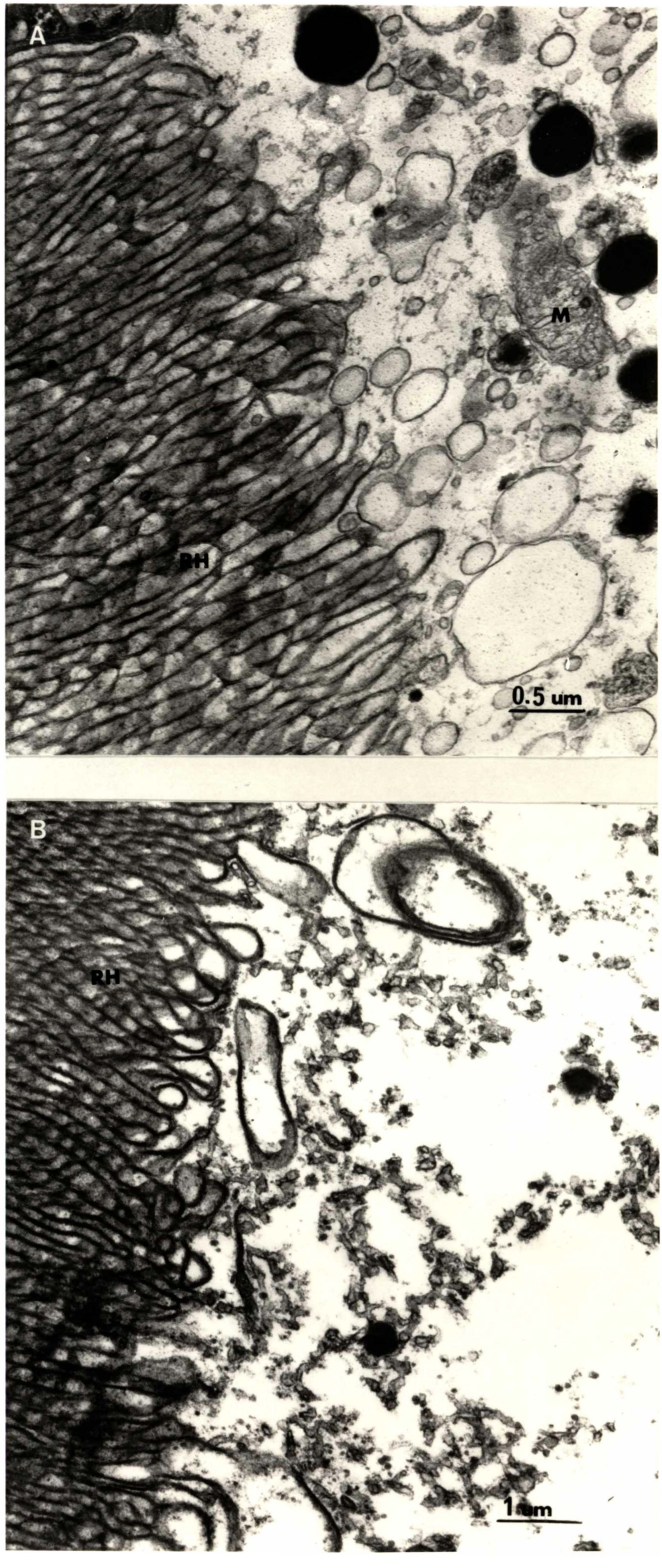

A
M
RH
0.5 um
B
RH
1 um

PLATE 27

Retinula cell of Dark-light adapted state

A This section through the light-adapted eye shows the typical location of the screening pigment granules within the retinula cell plasma. Note the general poverty of intracellular organelles except for some mitochondria, multilamellar bodies (MLB) and some vesicles. The peripheral region of the retinula cell appears to decrease in area forming a 'narrow channel' through which the screening pigment granules migrate towards the rhabdom. The interstitial cells appear to increase in size, when compared with some of the dark-adapted eye.

B In this transverse section through the dark-adapted eye, the retinula cell shows a wealth of intracellular organelles but lacks the screening pigment granules so characteristic of light-adaptation. The number of mitochondria has not only increased but also the variety of sizes and shapes. Cytoplasmic vacuoles have also increased many fold encompassing most of the cytoplasm. Some multivesicular and multilamellar bodies are also present. The retinula cell cytoplasm has increased in area (especially in the peripheral region) probably at the expense of interstitial cell volume.

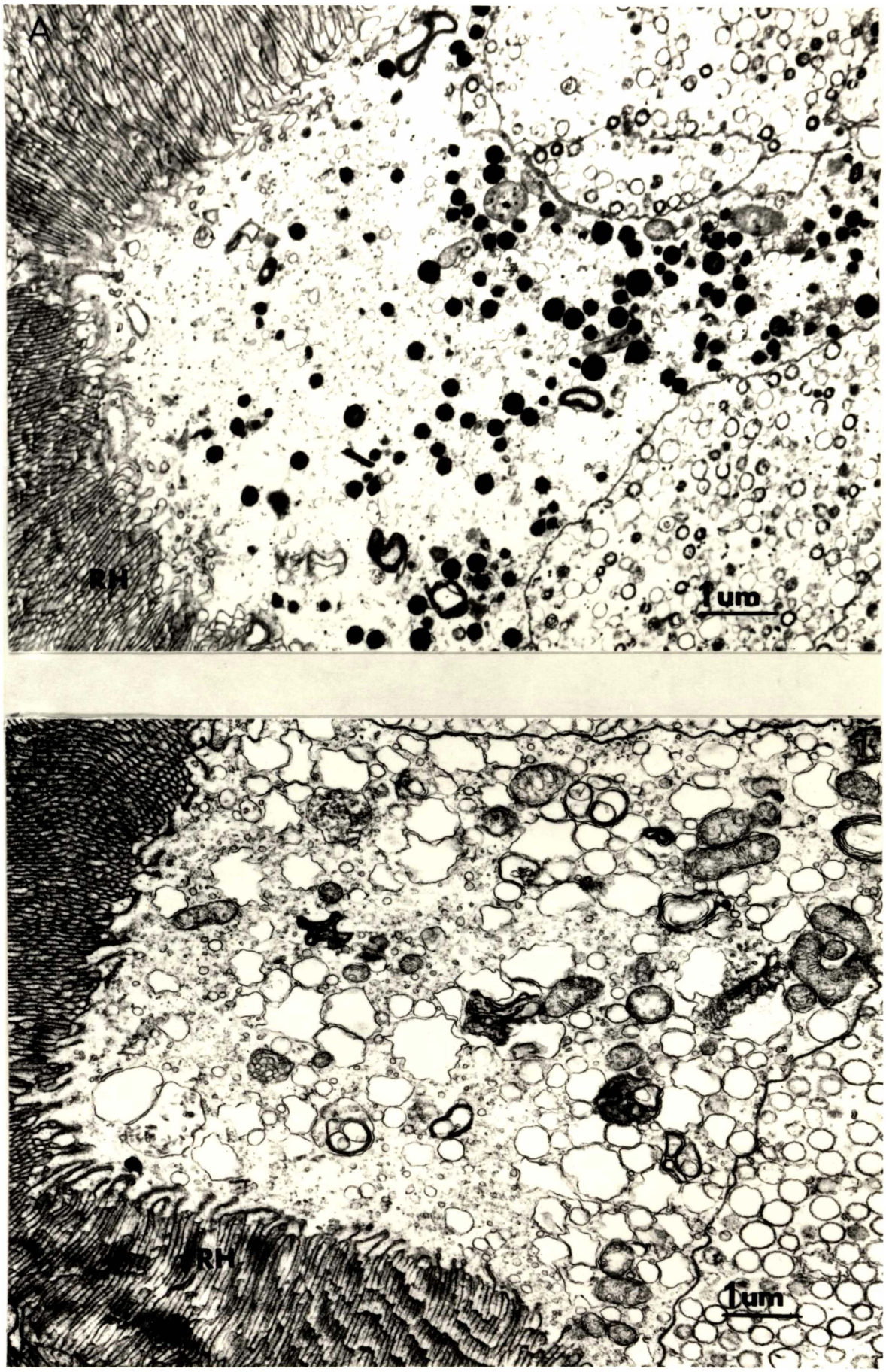

A
RH
1um
RH
1um

PLATE 28

This electron-micrograph, a transverse section through the 10°C heat-stressed dark-adapted rhabdom, shows that the retinula cell plasm has decreased in area and that the 'arms' of the rhabdomeres have also reduced in length. However, relatively to the total size of the rhabdom the microvilli appear to increase in length giving the rhabdomeres a short and stout appearance. The microvillar arrangement still remain more or less undisturbed with villi being uniformly aligned except in some rhabdom area where membrane distruptions have already begun. The retinula cell plasm is quite empty with regard to intracellular organelles except for a few multivesicular bodies (mvb). One significant change occurring within the retinula cell plasm is the migration of screening pigment granules towards the proximity of the rhabdom edge. This is similar to that found in the light-adapted state. The vesicles in the interstitial cells, judging from their appearance as empty holes, do not seem to be very well penetrated by Epon.

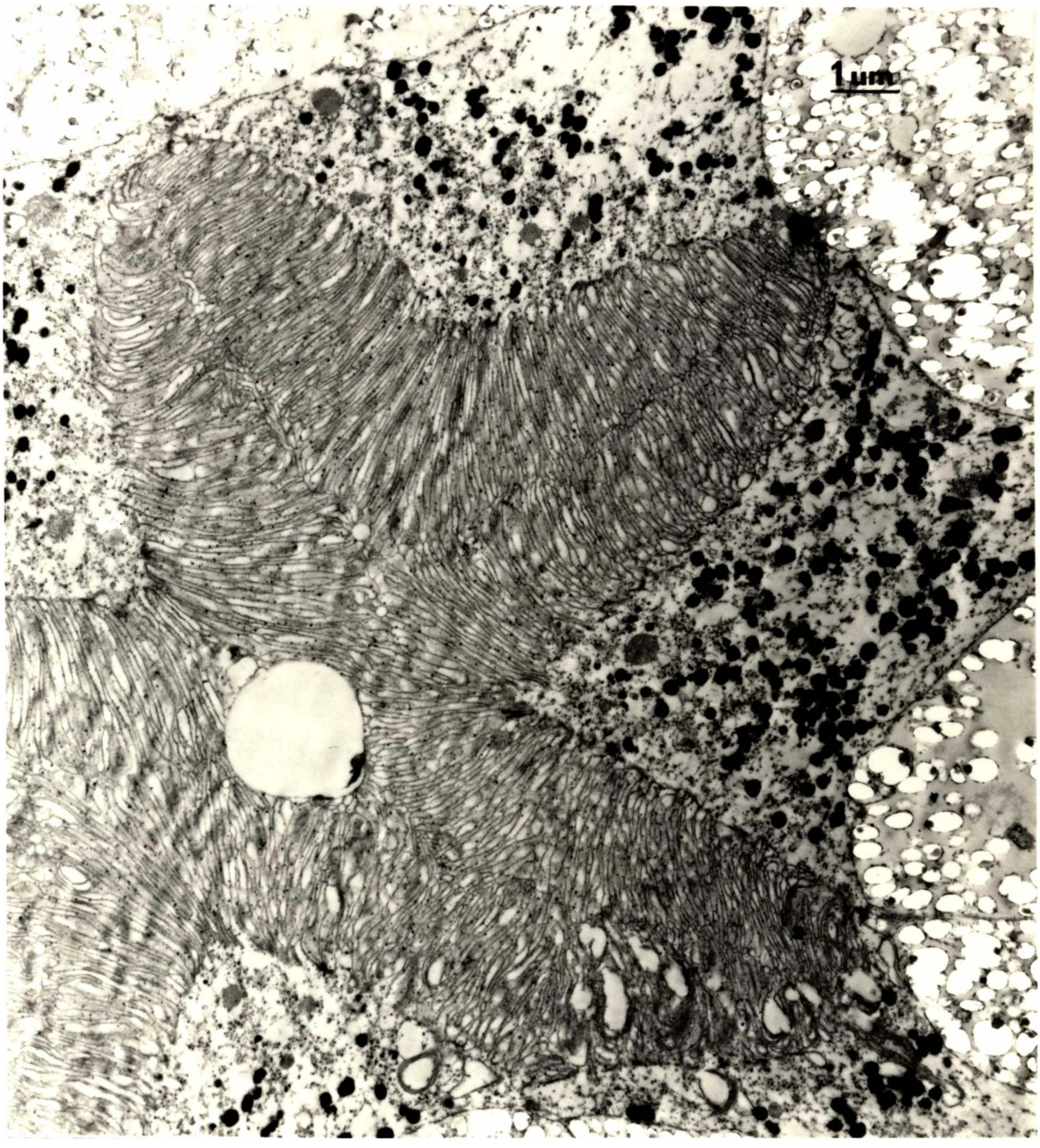
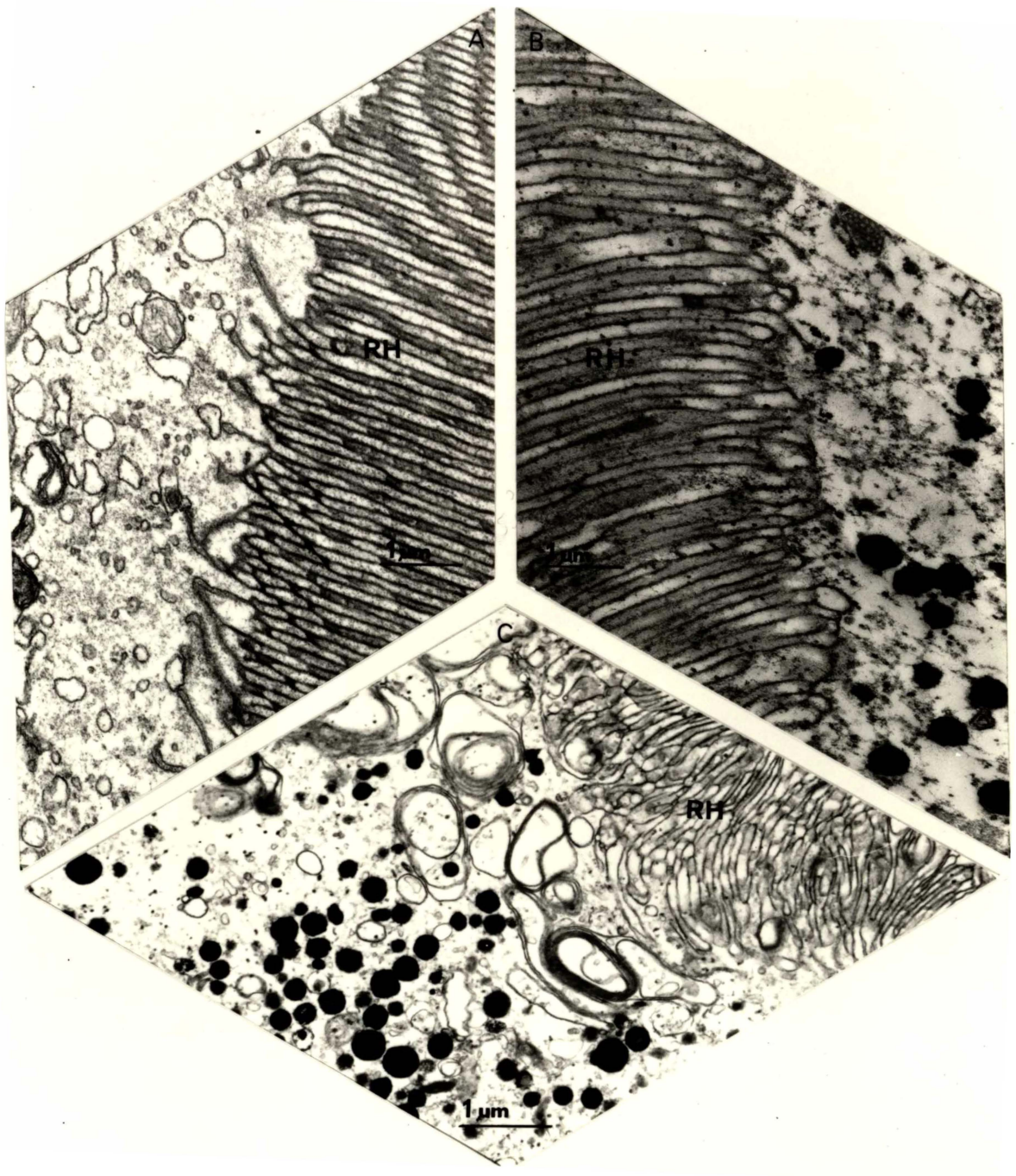

1 µm

PLATE 29

Ultrastructure of the rhabdom edge in different states of adaptation

A comparisen between longitudinally sectioned rhabdoms of eyes of: (A) a fully dark-adapted animal, kept at $0^{o}C$ in a light proof container, (B) a heat-stressed animal, kept at $10^{o}C$ for 7 hours under ambient light level of 50lux or below, and (C) a fully light-adapted individual, exposed to bright sunlight at $0^{o}C$ for an hour.

From the electron-micrographs, the most orderly microvillus arrangement is displayed by (A) the dark-adapted eye at $0^{o}C$. With regard to microvillus diameter, no statistically significant changes were detected between dark and light-adapted state, but in the temperature stressed eyes, the microvillus exhibits a small increase in diameter. The inter-villus space, too, is increased and microvilli are lighter stained when compared with those of normal dark and light-adapted animals kept at $0^{o}C$. Screening pigment granules have migrated to the proximity of the rhabdom edge in both the light-adapted state and temperature stressed material. Microvilli disruption at the rhabdom edge are observed primarily in the light-adapted eye.


A
RH
1 µm
B
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C
RH
1 µm

CHAPTER III

THE COMPOUND EYE OF *ORCHOMENE GRANDIS*, AN AMPHIPOD FROM UNDER THE ROSS ICE SHELF

A. SUMMARY

The structural organization of the compound eye of Orchomene grandis and the effects of light and darkness have been investigated by light and electron microscopy.

1. Superficially, the anatomy of the compound eye of Orchomene grandis resemble that of the related species Orchomene plebs and other amphipods: a smooth non-faceted cornea, two cone cells giving rise to a 'eucone' type of crystalline cone, and five retinula cells each contributing its rhabdomere to the centrally-fused rhabdom (Plates 30-40).

2. Dark-adapted eyes with non-disrupted, normal ultrastructure were not found. The rhabdoms either showed that a degenerative process had begun (2 days dark-adapted eyes) or exhibited a complete breakdown of visual membranes and other fine structures (1 week dark-adapted eye, Plate 42, 43).

3. Light-adaptation does not show the same characheristics as in O. plebs. The 1 hour light-adapted eye had the most 'normal' photoreceptor organization, but animals exposed to light for 2 hours exhibited widespread disintegration of the photoreceptor (rhabdom).

4. The high light intensity of the Antarctic summer sun has disruptive effect on the structural integrity of the eye of O. grandis.

B. INTRODUCTION

Whether or not any life could exist at all far from the open ocean beneath the permanent Ross Ice Shelf of Antarctica was only a matter of speculation until a year ago. Today, a few species are known to inhabit this poorest or harshest aquatic environment on earth. In terms of nutrient supply, this habitat is even more impoverished than that found in very deep sea trenches. The bacterial population is sparse and infaunal detridal feeders representing parts of the food chain are missing (Lipps et.al. 1978).

The Ross Ice Shelf, which is covered by a layer of about 500m thick solid ice, is about the size of Spain. It covers a triangular-shaped ocean area bordered by 160°E; 15°W and 78°S. The average thickness of the ice-shelf is 400m. The depth of water below the ice-shelf is 200m. However, in a few spots, the ice-shelf actually touches the bottom of the sea. Like all glaciers, the Ross Ice Shelf moves but with what speed is inadequately known. Where the shelf ice reaches the northern boundary, it abuts on the sea-ice. Although only 5m thick, the sea-ice in winter covers an enormous area, but unlike the Ross Ice Shelf, sea-ice breaks up in summer. Parts of the Ross Ice Shelf may also break off at the edge through a process called 'glacier calving' giving rise to floating table mountains. The floating table mountains slowly drift north together with sea-ice floes. Cracks often develop when glacier currents clash. These cracks may occur some 40km from the ice-front of the Ross Ice Shelf, providing a natural 'fishing' hole for sampling of the benthic fauna

(Littlepage & Pearse 1962; Lipps et. al. 1977). The composition of animals found here resembles that of the typical benthic communities off McMurdo Sound (Heywood & Light 1975; Lipps et. al. 1977). The presence of these animals was interpreted as direct evidence that a marine biome occurs under the entire Ross Ice Shelf.

A hole was drilled through the Ross Ice Shelf at 82°22'S, 168°37'W, 500km from the open Ross Sea and 600km from the South Pole. A camera and traps both baited with seal meat were lowered through the 30cm wide and 400m deep hole. The presence of a fish Trematomous spp. (identified by Meyer-Rochow) occurred twice in camera observation but none was ever caught. Crustaceans, however, were trapped and identified as amphipods, (Orchomene spp.) and one isopod (Serolis trilobitoides). Surprisingly all amphipods possessed large eyes. A preliminary simple test revealed light sensitivity of the eyes of these animals when a plastic aquarium, half of it covered by a black plastic sheet, is placed in the sun, after 30 min. all amphipods congregate in the shaded area. When the position is reversed, the animals migrate to the shaded part again (Meyer-Rochow, unpublished observation in the field).

The presence and existence of these animals under the Ross Ice Shelf 500km away from the edge of the ice-front still lacks a proper explaination. Furthermore, the presence of fully developed functional eyes in these animals living in virtual darkness is a 'biological puzzle'. This investigation is focused on the structural organization of the eyes of 'Orchomene grandis' (the preliminary but as yet unpublished name given to the Ross Ice Shelf amphipod). An attempt is made to

explain its possible function and any adaptive modifications. The effects of light on the ultrastructure of the photoreceptors are also considered and compared with results already obtained from Orchomene plebs and Glyptonotus antarcticus.

C. MATERIALS AND METHODS

All amphipods used in this study were approximately 30 mm long and identified as Orchomene grandis (identified by Slattery 1978). This species has never been described before and there is some controvery as to whether it could be a sub-species of Orchomene rossi, because of its close resemblance to the latter or not. However, most scientists appear to favour the concept that it is a new species.

This specimens were caught under the Ross Ice Shelf at the J-9 drill hole ($82^{o}22'S$, $168^{o}37'W$), 500km from the ice-front at depth of 597m below sea-level. The trap was made of 1.3cm mesh steel screen around a cylindrical steel frame 20cm in diameter and 60cm in length (Lipps et. al. 1979). The baited traps was lowered through the 30cm wide hole in the 400m thick ice-shelf and left near the bottom. Yields per haul were variable indicating sparse distribution.

The animals were maintained in the fridge in transparent aquarium or light-proof containers. The temperature of the sea-water was kept at $0\pm1^{o}C$ for the first 2-4 hours after capture. The dark-adapted animals were kept in a refrigerator and taken from the light-proof container after a required time of adaptation and dissected under dim red light. For light adaptation the animals kept in the transparent aquarium were

placed on snow and exposed to the sun for the required periods. The temperature was -1 - -2°C for light adaptation and +1 - +3°C for dark adaptation.

Methods for histological investigations were identical in all eyes studied, irrespective of the light conditions that the animals had been exposed to A 2.5% glutaraldehyde -2% formaldehyde mixture in Millonig's phosphate buffer, adjusted with D-glucose to a 0.6M solution with a pH of 7.4, served as a prefixative. The specimens stayed in this solution for 12 hours before they were washed in buffer and post-fixed for 2 hours in a 2% phosphate buffered solution of Os O4. Dehydration in a graded series of acetone was followed by infiltration with Epon 812 and hardening for two days at 65°C.

At least three eyes of each light regime were successfully sectioned and examined in both light and transmission electron microscope. For light microscopy, 1um transverse and longitudinal sections were stained with toluidine blue for a few seconds (-10 -15 seconds) on a hot plate. Electron microscope material consisted of 'golden' sections, which were picked up with 200 mesh copper grids and double-stained with uranyl acetate and lead citrate for eight and two minutes respectively.

D. RESULTS

1. Basic anatomy of the eye

(a) General features

The two pear-shaped compound eyes of Orchomene grandis are orange in colour (Plate 30A). Like other Orchomene species,

the two eyes have its narrower ends nearly touching at the apex of the head, while the large parts lie in a latero-ventral position protected by a transparent cuticle extension of the first thoracal epimere. In an average adult of about 30cm length, the eye measures 2.25um in dorso-ventral extension, and the broad ventral region is 1.36mm wide. The number of ommatidia present is approximately 360. On the basis of external morphology there is no clear differentiation of the eye in the dorsal or ventral regions. No structural differences were observed in the ommatidia from both the narrow dorsal region and the larger ventral region of the eye. All ommatidia appear to be oriented primarily for vision towards the side.

Externally the eye is covered by a smooth and unsculptured non-faceted cornea. The extreme transparency of the cornea allows the underlying arrangement of ommatidia, 50-60um in diameter to be observed. The cornea appears to extend 0.2mm around the periphery of the whole eye (Plate 30A). The curvature of the corneal cuticle and the ommatidial angle do not vary significantly from one region of the eye to another, with the exception of the extreme periphery where some slight variations may occur. An interommatidial angle of 4-5^{o} is found for ommatidia in the dorsal and ventral regions of the eye. Variations of the ommatidial angle at the extreme periphery are common in most crustacean eyes (Schonenberger 1977). However, in the eye of Orchomene grandis, it is suggested that the peripheral ommatidia do not differ significantly from the value of 4-5^{o} measured from the central region.

As in other crustaceans, one ommatidium, which form the morphological unit of the eye, consists of two distinct subunits: the dioptric apparatus and the retinula (Plate 33A,

Fig. 6). The dioptric apparatus is made up of the corneal cuticle and the cylindrical crystalline cone. The retinula represents the morphological basis for photosensitivity, and consists of five retinula cells and the centrally-placed, compact, spindle-shaped rhabdom. A oblique section of the eye is shown in Plates 31, 32A depicting the arrangement of the ommatidia. The length of the dioptric apparatus varies indiscriminately throughout the whole eye ranging from 60um to 90um. The variations involves primarily the crystalline cones whose lengths vary from 35um to 65um. The retinula cell plasm has distal extensions which form a narrow cytoplasmic sleeve around the crystalline cones (Fig. 6). The slender interstitial cells reach distally to the inner surface of the cornea and extend proximally towards the basement membrane. They probably contribute to the optical isolation of the individual ommatidia but to a lesser extent than those of the eye of Orchomene plebs for here in O. grandis the isolation of the individual ommatidia is not complete. Some distal rhabdom ends appear to be in close apposition or may even to be fused together. The total length of the photoreceptive elements stretching longitudinally is approximately 190um. The retinula cells continue as axons from the level at which the rhabdom has phased out. They penetrate the basement membrane at regular intervals. A summary of the relevant data is given in Table 3.

Table 3 : The eye of Orchomene grandis (RISP amphipod).

Morphological Data of one representative individual.

Length of O. grandis	30 mm
Size and shape of eye	3.0 mm, pear-shaped
No. of ommatidia	Approx. 360
Diameter of single ommatidium	56 um
Length of ommatidium	260 um
Inter ommatidial angle	4^{o} - 5^{o}

Anatomical Data

Thickness of corneal cuticle	34 um
Length of crystalline cone	56 - 65 um
No. of retinula cells	5
Diameter of rhabdom	30 - 70 um
Length of rhabdom	150 - 170 um
Diameter of microvillus	0.04 - 0.19 um
Diameter of dark pigment granules	0.5 - 1.0 um
Diameter of grey pigment granules	0.5 - 0.9 um
No. of axons in one bundle	5

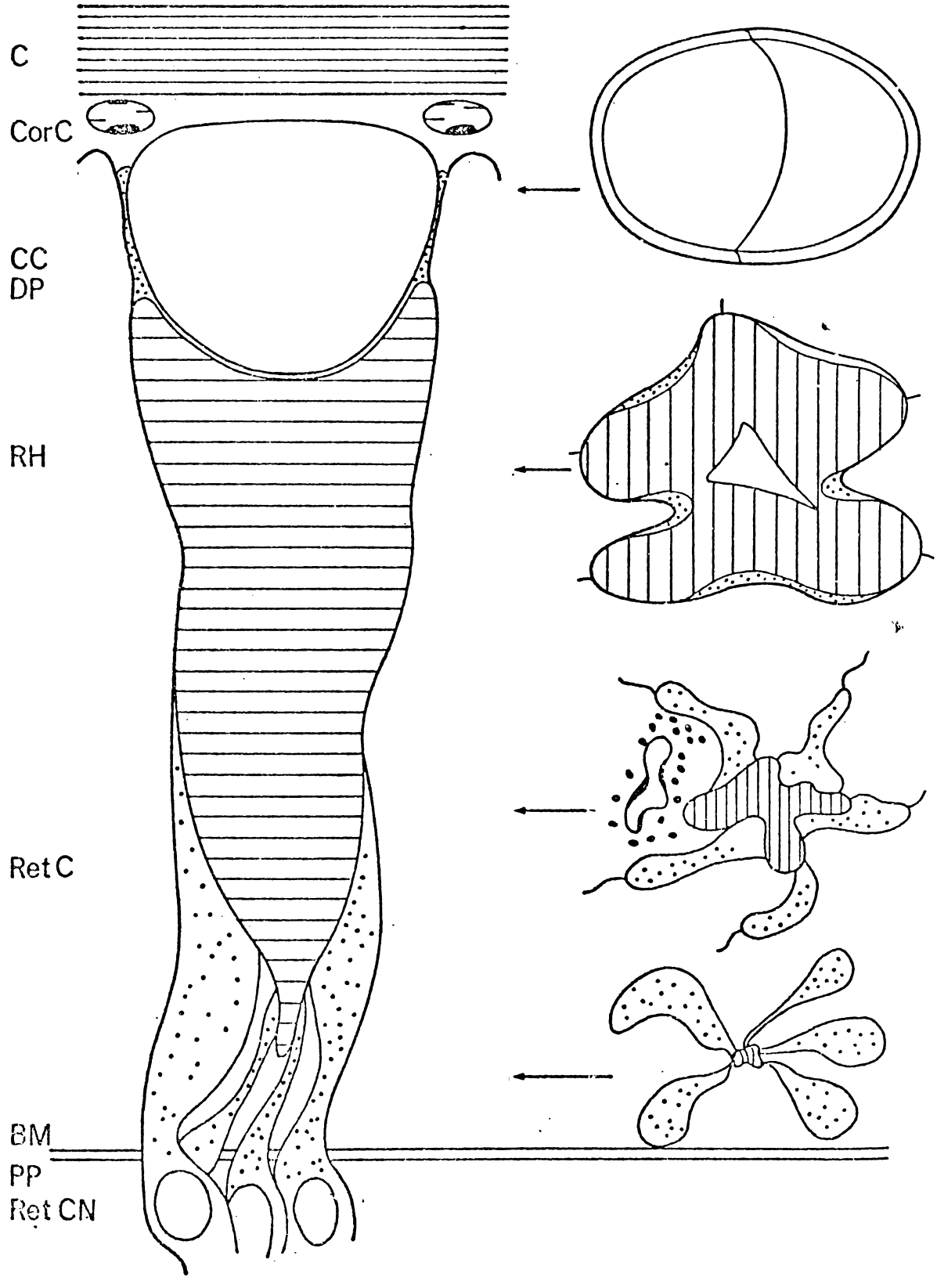
FIGURE 6

Semi-schematic drawing of an ommatidium in longitudinal and transverse sections.

The density of screening pigment granules is very much reduced when compared to Orchomene plebs and Glyptonotus. The crystalline cone is made up of 2 cone or 'Semper' cells, typical of all amphipod eye. The rhabdom consists of 5 rhabdomeres of approximately equal size.

Abbreviation: C=cornea, Cor C=corneagenous cells, CC=crystalline cone, DP=distal pigment, distal processes, RH=rhabdom, Ret C=retinula cell, BM=basement membrane, PP=proximal pigment, Ret CN=retinula nucleus.

FIGURE 6



(b) Dioptric apparatus

The corneal cuticle and the crystalline cones make up the dioptric apparatus of Orchomene grandis. The thick cornea as in Sicyonia brevirostris, is thought to be supportive in function allowing the eye to maintain its turgor (Zyznar 1970). The outer surface of the cornea is smooth and not sculptured. A longitudinal section through the cornea reveals its multi-lamellar structure (Plate 30C). Three horizontal regions exist clearly in the cornea which is not compartmentalized into individual facets. The outer layer consists of 7 thin lamellae (each 0.8um thick), the middle layer of 4 thick lamellae (each 2.6um thick) and the inner most layer of 5 lamellae (each 1.8um thick). This gives the total thickness of the cornea a value of about 25um. Both the inner and outer surfaces of the cornea are slightly convex. The cornea can be considered as the direct continuation of the body cuticle without the innermost layer (Locke 1966). During the development of the corneal cuticle and the body cuticle of arthropods similar events appear to take place (Delachambre 1971). The corneal cuticle is separated from the crystalline cone by a space 10-15um wide which is occupied by modified epidermal cells or so-called 'corneagenous cells'. The corneagenous cells are responsible for the secretion of the corneal cuticle (Waterman 1961) and are readily distinguished by their nuclei and reticulated cytoplasm (Plate 30B).

The crystalline cones which lie directly below the corneagenous cells, are circular when sectioned transversely, and are made up of two cells. It is probably typical of all amphipod eyes that only two cone cells are found for each omma-

tidium (Ball 1977; Meyer-Rochow 1978a; Meyer-Rochow & Tiang 1979). The two cone cells or 'semper cells' secrete intracellularly the relatively solid cone structure. The arrangement of the crystalline cones can be clearly observed from the surface through the transparent cornea. Structurally, the two cone cells are responsible for the bipartite configuration and the cylindrical shape of the crystalline cones. Unlike other crustaceans (e.g. Panulirus: Meyer-Rochow 1975a; Orchomene plebs and Glyptonotus antarcticus, see Chapter I & II) where the sizes of the crystalline cones vary from the dorsal, ventral and central region, Orchomene grandis exhibits a random variation throughout the whole eye. The length of the crystalline cones may vary from 40um to 65um, while the diameter measures from 25um to 40um in width. The crystalline cone appears to taper inwardly where it makes direct contact with the distal region of the rhabdom. Like in Orchomene plebs, the crystalline cone, according to Grenacher (1879), is defined as of 'eucone' type, in which the dense cone-shape core is secreted intracellularly towards the axis during development. However, semi-thin transverse section for light microscopy show that the crystalline cone consists of material which appear to be completely featureless, readily stained by toluidine blue. High magnification electron micrographs (Plate 30B, D) show that the cores of the crystalline cone consist of electron-dense particles of approximately 0.15 - 0.20um in diameter (resembling glycogen granules) which break up into minute particles towards the periphery. The association of glycogen with the 'eucone' type of crystalline cone has been reported in a number of insects and crustacean eyes (Drochmans 1962); Revel (1964); Wolken (1969); Perrelet (1970); Barra (1971); Elofson & Odselius (1974); Nemanic (1975). These glycogen granules may

be of optical significance in the perception of polarized light (Skrzipek 1971). According to Meyer-Rochow (1975b), light absorption is thought to vary with the amount of glycogen granules present in the cone cells. Since the glycogen serve no metabolic function in the cone (Perrelet 1970), an optical function seems most appropriate, but its exact significance remains speculation at this stage.

One interesting feature by which one can easily differentiate between the dioptric apparatus of Orchomene plebs is the absence of the 'plug' or crystalline stalk from the former. The 'plug' which connects the crystalline cone to the distal end of the rhabdom in Orchomene plebs was assumed to serve as a light guide, but in Orchomene grandis the crystalline cone is directly connected to the rhabdom. This could be an adaptational modification to enhance photosensitivity.

(c) Photoreceptor cells

The photoreceptive layer of the eye of Orchomene grandis consists of 5 retinula cells and their centrally fused rhabdomeres. Towards the distal region, the retinula cells appear to consist of nothing else but microvilli with little cytoplasm. There are flimsy extensions towards the proximal ends of the crystalline cones. Demarcation of the retinula cell border at this distal level is not very clear because of the enormous development and irregular shapes of the rhabdoms, some of which actually in direct contact with each other. A rudimentary retinula cell or eccentric cell commonly occurring amongst crustaceans (and insects) (e.g. Astacus: Krebs 1972; Formica: Menzel 1972; Panulirus: Meyer-Rochow 1975a; Squila:

Schonenberger 1977) is lacking in Orchomene grandis. The interstitial cells provide the intercellular as well as interommatidial isolation in the mere proximal region of the retinula cells. The number of interstitial cells is about 6-9 per ommatidium and appears to be related to the number of the retinula cells within the rhabdom. The retinula cell boundaries are not very distinct making it difficult to assess whether the retinula cells contribute equally to the centrally fused rhabdom. The small fifth cell which contributed the smallest segment to the rhabdom of Orchomene plebs (Chapter I D1) and the Arctic amphipod, Pontoporeia (Donner 1971) is lacking. The cytoplasmic area of the retinula cells is much reduced compared to that of Orchomene plebs. The retinula cell is seen to occasionally shed some of its cell plasm which may include screening pigment granules, into the interstitial cell (see Plate 36). The retinula cell is relatively poor in intracellular organelles when compared with that of Orchomene plebs or Glyptonotus. Narrow radially-projecting retinula cell process connecting with the retinula cells of adjacent ommatidium are observed towards the proximal region (Plate 38). These processes also serve to isolate the individual interstitial cell around the rhabdom. There appear to be some intercellular relationship between the retinula cells and the interstitial cell. In some rhabdoms the cytoplasms of both cells appear to be involved in the cycle of rhabdomeric microvillar break-down and regeneration. Membranes are observed to form vesicles of round shapes which seem to be transferred into the retinula cytoplasm and later excluded into the interstitial cytoplasm (see Plate 37).

The rhabdom which is spindle-shaped abuts directly on

the flat proximal end of the crystalline cone. In some ommatidia, the rhabdoms forms a 'hemi-spherical' profile at the inner side of the cone (Fig. 6). Semi-thin transverse sections for light-microscopy (Plate 34A, C) invariably show remnants of proximal cone material still attached to the central region of the rhabdom. Unlike Orchomene plebs, where the transverse section through the rhabdom reveals round profiles, in Orchomene grandis, the rhabdoms assume all sorts of irregular twisted shapes with a maximum diameter of 70um, and a minimum of 30um, such diverse variation in shape and size of the rhabdoms also differs from the situation in the deep-sea amphipods (e.g. Phronima: Ball (1977); Streetsia: Meyer-Rochow (1978)) and Arctic amphipods (e.g. Gammarus: Ali & Steele (1961); Pontoporeia: Donner (1971)), where more uniform rhabdom shapes are encountered. Apart from the normal variation in size and shape of rhabdoms within the eye, the usual dark-light adaptational changes involving rhabdom dimension are not observed, instead irreversible disintegration of the rhabdom occurs under prolonged light (2 hours) as well as darkness (1 week).

Electron micrographs of a transverse section through the rhabdom show the multidirectional arrangement of the microvilli (Plate 35) in contrast to the unidirectional uniformly arranged pattern of the Orchomene plebs rhabdomere. The alternating tiered arrangement of the microvilli along the longitudinal axis of the rhabdom presumed to enhance polarization sensitivity (e.g. Daphnia: Eguchi & Waterman 1966; Streetsia: Meyer-Rochow 1978) is lacking in Orchomene grandis. The microvilli arrangement appear to resemble that of the deep-sea mysid Boreomysis scyphops (Elofsson & Hallberg 1977)

which also exhibits a multidirectional microvilli pattern.

In longitudinal sections, the rhabdom does not always assume the uniformly cylindrical shape typical of the O. plebs eye. Variations in diameter along the length of the rhabdom occur frequently at various levels. The rhabdom directly is connected to the crystalline cone at its distal end (the buffer zone found in Orchomene plebs is lost here) via a broad face. Towards the proximal end, the rhabdom usually tapers and phases out at approximately 20-30um above the basement membrane. However, some rhabdoms actually have their proximal tips touching the basement membrane (Plate 32A). At this proximal level, the retinula cells are clearly separated by the cytoplasm of the interstitial cells. The retinula cell processes increase in width (from 4um to 10um) before penetrating the basement membrane at regular interval. The basement membrane separates the retinula and the sub-retinula space with the retinula cell processes providing the necessary communication between the different optic elements. The retinula cell nuclei are located approximately 20-30um below the basement membrane. The retinula cell nuclei are ovoid in shape and measures approximately 12um in diameter (long axis). The sub-retinula space has a rich inclusion of organelles e.g. pigment granules, mitochondria and vacuoles. The retinula cell processes or axons at this level lie closely to one another and possess numerous screening pigment granules and mitochondria (Plate 33B, C). Distinct bundles or groups of axons are not discernible and connections with the lamina ganglionaris have not been traced.

(d) Screening pigment granules

The physical and morphological properties of the pigment screen in the eyes of crustaceans (and insects) play an important role, not only in terms of light sensitivity but also of spectral sensitivity (Strume et. al. 1975). The dark screening pigment granules have been demonstrated to migrate in the cone region acting as a pseudopupil during light adaptation in the eyes of Orchomene plebs. Unlike other arthropods in which the eyes possess specialized pigment cells which varying in number and pigment densities, Orchomene grandis has pigment granules located within the retinula cells. However, there are two different types of pigment grains present, a darker electron-dense type of granule and a lighter greyish sort. The total number or abundance of these pigment granules is very much reduced in O. grandis when compared with O. plebs. The size of the screening pigment granules varies from 0.5-1.0um in diameter. The change of distribution of the screening pigment granules which were closely related to the state of dark-light adaptation in the eye of O. plebs, is lacking in O. grandis i.e. absence or presence of light has no significant effect on the position of the pigment grains.

However, the screening pigment granules show a dense distribution immediately below the basement membrane (Plate 32B) and around the distal part adjacent to the retinula cell nuclei (approximately 30 grains per $5um^2$, count) based on the average of 5 eyes. Since all the pigment granules have electron-dense staining characteristics, it is assumed that as in other arthropods, they are present to serve as a protective, light absorbing screen. However their mere presence

in the eye of O. grandis remains a puzzle as we can sefely assume that no light would penetrate 400m of ice plus several meters of loose surface snow. In other words, there would not be any light to see in let alone to be absorbed or screened out.

(e) Retinula cell organelles and inclusions

Compared with the retinula cytoplasm of Orchomene plebs and Glyptonotus, the retinula cells of Orchomene grandis are most deficient in cytoplasmic organelles and inclusions. The distal part of the rhabdom, where the retinula cells persist as a flimsy cytoplasmic area surrounding the enormous rhabdom, are quite empty and lack any kind of cytoplasmic organelle. Towards the mid-rhabdom level, where the retinula cytoplasm is slightly more extensive, some screening pigment granules, mitochondria multivesicular body, multilamellar body and vacuoles can be seen (Plate 35).

Mitochondria are more abundant within the proximal retinula cytoplasm especially below the basement membrane. Towards the distal region, mitochondria are randomly distributed along the length of the ommatidium. The density and position of the mitochondria are not affected by the state of adaptation. Most of the mitochondria observed are spherical-shaped with diameters ranging from 0.4um to 1.8um.

Irrespective of the state of adaptation, multivesicular bodies (MVB) are very rare in the retinula cytoplasm even though special attention was paid to their occurrence or absence. Less than five MVB were accounted from about 20 sections screened under the electron microscope. With a maximum diameter of approximately 3um (Plate 36) the MVB occurring in Orchomene grandis are larger than those observed in O. plebs

and Glyptonotus. Changes of number and size of these MVBs lack any statistical evidence in relation to dark-light adaptation in O. grandis.

Multilamellar bodies (MLB) or 'onion bodies' are particularly common around the vicinity of the rhabdom (Plate 37) or in the rhabdom itself when it undergoes structural disintegration (see Plate 42B, D). The formation of these whorled concentric structures appears to immediately follow the initial stage of irradiation as no fully dark-adapted eyes could be observed. It is believed that the process of hauling the animals up from beneath the Ross Ice Shelf during which they were exposed to sunlight triggers off an irreversible process of rhabdom disintegration through the formation of these MLBs. Although, the abundance of MLBs present in the retinula cytoplasm has been positively associated with light adaptation in the eye of Glyptonotus (see Chapter II D2). Vacuoles also is another common feature in the retinula cytoplasm, but as with almost all organelles their abundance is also much reduced when compared with corresponding features in the eyes of O. plebs and Glyptonotus. These vacuoles vary extensively in size and shape, ranging from 0.4um and spherical shape to elongated regions of 3.4um length. These vacuoles may be of lysosomal nature as suggested for Glyptonotus (see Chapter II D1).

The structural organization of the ommatidium in O. grandis points towards an adaptation for vision under very low ambient light levels. The extreme transparency of the cornea and the large crystalline cone could provide an efficient window for the under lying photosensitive rhabdom. Moreover the rhabdom which has established an intimate and

extensive contact zone with the flat proximal cone end is voluminious and with its multidirectional microvilli seems geared towards maximising photon capture. There is a general lack of distal screening pigment migration on light adaptation and the concentration of screening pigment seems inadequate to effectively shield the rhabdom against light damage received by exposure. These adaptations must be regarded as reflections on the dimly-lit environment in which O. grandis lives, for it is possible that O. grandis normally inhabits deep-water of a depth to which sunlight might just reach, but sometimes is carried under the Ross Ice Shelf. Here it may survive, exist and multiply in total darkness, but not being genetically isolated population, eyes and screening pigment granules persist (see also Discussion).

2. Dark-light adaptation

(a) Both light and darkness appear to have some profound effect either directly or indirectly in photoreceptor membrane regulation. The photoreceptor membranes of many vertebrates and invertebrates (e.g. Besharse et. al. 1977; Chamberlain & Barlow 1979) have been demonstrated to regularly shed and renew the parts of the cells that transduce the visual stimuli. On the onset of light, the regenerative mechanisms operate in providing turnover and replacement of the functional photosensitive membrane adaptive mechanisms involving the adjustment to ambient light levels as well as incorporating endogenous circadian (diurnal) rhythms further enhance visual adaptation (Eguchi & Waterman 1979; Nassel & Waterman 1979). Therefore membrane loss and regeneration due to dark-light condition

have to be critically balanced. Excess of either darkness or light will invariably lead to temperal or even irreversible disruption of the visual receptor organization. Light induced structural changes in the photoreceptors have been well-documented in several animals (e.g. Noell et. al. 1966; Eguchi & Waterman 1967; Brammar & Clarin 1976; Bruno et. al 1977; Nassal & Waterman 1979). In Limulus, light triggers the daily rapid Synchronous disassembly and buildup of the rhabdom in each photoreceptor (Chamberlain & Barlow 1979). In contrast, the spider Dinopis exhibits a complete destruction of the rhabdom at the first light of dawns, Blest et. al. (1978).

Prolonged light exposure has a variable effect on different animals; e.g. in shrimp (Itaya 1976) and crayfish (Eguchi & Waterman 1976), temperal membrane turnover involving multilamellar and multivesicular bodies occurs, while in rats (Noell et. al. 1966) and lobster (Loew 1976) irreversible membrane breakdown has been reported.

Darkness on the other hand, has also been found to affect a number of visual elements. In the eye of the spider Dinopis (Blest 1978) and in crab (Nassal & Waterman 1979), membrane synthesis occurs rapidly in the dark. Disruptive effects of darkness on the photoreceptor membrane and microvillus pattern organization occur in a number of animals including (e.g. isopod: (Edwards 1969; Nemanic 1975) and crayfish (Eguchi & Waterman 1979)).

In this investigation, attention is focussed on the effects light and darkness have on the structural organization of the photoreceptor in general and the fine structure of rhabdomeric membranes in particular. Orchomene grandis with fully developed

eyes which lives in an environment virtually devoid of any light could be expected to react sensitively to light induced anatomical changes and thus may be an excellent subject for this investigation.

(b) Light microscopy

The structural organization of the ommatidia in the different states of adaptation are best observed in longitudinal sections through the eye (Plate 33). In the typical ommatidium, the well defined crystalline cones and rhabdom structures are shown (Plate 33A). Unlike the situation in O. plebs or Glyptonotus, where light exposure led to cell movements, it appears that O. grandis, exposure to light results solely in a disruptive effect on the photoreceptors. Exposures to bright sunlight of 100 000 lux for 1 hour and 2 hours were carried out immediately after the amphipods had been hauled up from beneath the Ross Ice Shelf (a process that took about 25 minutes). The water temperature, although not checked, must have been between -1 and -2°C in the experimental aquarium.

Structural integrity of the photoreceptor in maintained in animals exposed to light for 1 hour. The large crystalline cones and rhabdom show virtually no disruptions or any other damage (Plate 33A, 34A). In view of its scarce distribution the position of the screening pigment granules is insignificant. This is in contrast to O. plebs and Glyptonotus where screening pigment granule migrations played a very important role in light adaptation (see Chapter I D2, II D2). Rhabdom size and shape appear to be 'normal' : the rhabdom outlines

are distinct and show close contacts with adjacent rhabdoms (Plate 34A). In animals that were exposed to light for 2 hours the photoreceptors appear to be a process of degeneration. Both the crystalline cones and the rhabdoms seem to be affected. In longitudinal sections through the eye (Plate 33B), the crystalline cone appears to be smaller than after only 1 hour light exposure. The rhabdoms have retracted from the proximity of the crystalline cones leaving a space 12um to 30um wide. Moreover, the rhabdoms have lost their structural integrity. There are many holes along the entire length of the rhabdom in contrast to the 'solid' cylindrical rhabdoms of the 1 hour light adapted eye. In the distal region especially, the cylindrical outline of the rhabdom shape gives way to grotesque distortions. In transverse sections through the ommatidia reveal a total disintegration of the rhabdoms into a mess of tissues. Individual rhabdoms are often completely indistinguishabel, especially when the remenants of the rhabdoms have become fused together, surrounded or interspread by empty space and interstitial cell (Plate 34B).

However, in the dark-adaptational experiment, the animals kept in a light-proof containers and placed inside a refridgerator for two days and 1 week respectively. Temperature inside the refridgerator fluctuated between 1^{o} and 3^{o}, but as the containers with the amphipods floated in brine/ice solution, one can assume that the temperature never rose above $+1^{o}C$. The 2 days dark-adapted animals appear to exhibit more lightly stained rhabdom (Plate 33B, 34C) when compared with those of the 1 hour light-adapted animals. In both longitudinal and transverse sections, the structural integrity of the rhabdom

is still largely retained. Some screening pigment granules are distinguishable in the retinula cytoplasm, especially in the proximal region. Towards the distal region, these screening pigment granules are also found randomly distributed around the narrow retinula cytoplasm surrounding the rhabdoms. In contrast, the screening pigment granules are located primarily below the basement membrane in the 1 hour light-adapted eyes.

The rhabdoms in the 2 days dark-adapted eye exhibit signs of damage with holes in them, when compared with the 1 hour light adapted rhabdoms. In the one week dark-adapted eyes, damage to the rhabdoms is more extensive. The rhabdoms have completely detached from the proximity of the crystalline cones. A wide space of approximately 80um has developed between the cones and the distal part of the rhabdoms. However, the cellular boundaries of the retinula cells are still intact indicating the withdrawal of the rhabdoms from the cone edge. The distal part of the rhabdoms appears to be completely fused (Plate 33D). Only towards the proximal region are individual rhabdom distinguishable. Transverse sections through the ommatidia reveal a total disruption and disintegration of the rhabdom structure, comparable to that which has been described for the 2 hours light-adapted animals (Plate 34D).

(c) Electron microscopy

At the ultrastructural level, further and more profound changes occurred within the rhabdoms in the different states of adaptation. Animals exposed to light for 1 hour exhibited

by far the most 'normal' and 'natural' rhabdoms. Transverse sections through the ommatidium reveal a clear multidirectional arrangement of the rhabdomeric microvilli (Plate 35). The retinula cytoplasm is unusually narrow with very few intracellular organelles present. The rhabdom edge shows the regular arrangement of the microvilli. With light or magnification, it was possible to determine inter-villus space and microvillus diameter (Plate 39A, 41A), both of which were regular and constant.

However, in the two days dark-adapted eyes, the rhabdom ultrastructure revealed regular 'pock-mark' holes. Ultrastructural changes also occurred along the rhabdom edge, where the microvilli arrangement exhibited signs of the beginning of membrane disruptions. Membranous, whorled structures or 'onion bodies' are commonly found along the rhabdom edge of this adaptational state. Some of these structures are attached to the rhabdom edge. The membranous structures often enclose cytoplasmic organelles including mitochondria, pigment granules and vesicles. These membrane bound structures with organelles in them have been observed to be liberated into the interstitial cytoplasm (Plate 41B). Irregularities in the microvillus arrangement are observed together with variations of microvilli diameter and intervillus space. Diameter in contrast to the regular 0.14um diameter of the 1 hour light-adapted state (based on the average of 10 ommatidia of the different state of adaptation range from 0.04um to 0.19um. Screening pigment granules which were relatively rare in the narrow retinula cytoplasm of the 1 hour light-adapted appears to have increased in abundance in 2 days dark-adapted eye. Both the 'dark' and 'grey' pigment grains are found near the rhabdom edge. However,

its absorbing capacity is questionable in view of their generally low concentration. Unlike the situation in Orchomene plebs where light adaptation brought about a rapid migration of screening pigment granules along the length of the rhabdom, in O. grandis, pigment migration in the dark may be due to the after effect of the initial light exposure during 'hauling up' process.

In both the 2 hours light adapted and the one week dark adapted eyes, the rhabdom ultrastructure exhibited massive disruptions visual membranes. The whole rhabdom appears as a mess of twisted and convoluted whorls of membranous structures. Individual microvilli are no larger discernible (Plate 42B, D, 43B, D). This disruptions of microvillus membranes appears to be even more severe in the 2 hours light adapted eye, whereas in the 1 week dark-adapted eye, remnants of the retinula cytoplasm are still visible. One interesting feature in the 1 week dark adapted eye concerns the interstitial cytoplasm. Unlike Orchomene plebs where fully-dark-adapted eyes possess 'echinosome' organelles in the interstitial cells, O. grandis exhibits spherical hollow vesicles in the interstitial cells. This is in contrast to the flake-like organelles which occur in hour, 2 hours light-adapted and 2 days dark-adapted eye. The functional significance of these hollow spherical vesicles is not known with certainty, but they could be reflecting or fluorescing elements as suggested for the 'echinosome' organelle of Orchomene plebs (Meyer-Rochow 1978).

E. DISCUSSION

1. Structural organization of the eye

In the Antarctic summer, during the existence of continuous daylight for several moths, light intensities measured under 3m of solid sea-ice gave only a noon average light transmission of 0.25% (Littlepage 1965). Therefore, under the 400m thick Ross Ice Shelf, it is reasonably safe to assume that light is virtually non-existent. As a result, animals that live in complete dark environments like in caves or great ocean depths are usually blind or possess degerate eyes (Hallberg 1977; Elofsson & Hallberg 1977). Surprisingly enough, Orchomene grandis possesses fully developed functional compound eyes. The very existence of the eyes in animals under the Ross Ice Shelf has been a 'biological puzzle' because it is not efficient for an animal to maintain such a highly organized, energy-requiring organ in a completely dark environment, unless there is something to see. However, bioluminescence, thought to be correlated with the existence of eyes of crustaceans at great depths in the ocean (Welsh & Chace 1937), is lacking in Orchomene grandis.

There is probability that O. grandis is not endemic to the Ross Ice Shelf but originates from animals that live at greater depth further north to the Ross Ice Shelf and occasionably drift under the Ross Ice Shelf with the ocean current (Meyer Rochow, personal communication). As a result, a gradual displacement and mixing of the populations of O. grandis stretching from the area north of the Ross Ice Shelf to the very southern most tip of the Ross Ice Shelf, may occur. Therefore, one would not be able to talk

about a genetically isolated population under the Ross Ice Shelf. Here vision would no longer be important and the animals could live without eyes, but in order for this to happen the animals would have to be completely isolated from the original gene-pool where eyes are part of the normal animal.

The gross structural organization of the eye of Orchomene grandis can be compared with that of Orchomene plebs (see Chapter I) and other amphipods (Grenecher 1879; Debaisieux 1974; Ali & Steele 1961; Donner 1971; Ball 1977; Meyer-Rochow 1978). The cornea is not compartmentalized into facets; two cone cells are contributing to the bipartite structure of the crystalline cone and the five retinula cells give rise to a centrally-fused rhabdom (see Table 4).

In the eyes of O. grandis, the photoreceptors differ from those of the related species O. plebs in four points: (1) the cornea is much thicker and more transparent, (2) the crystalline cone has a larger diameter and length, (3) the crystalline cone is connected directly to the underlying large rhabdom and (4) there exists a complete lack of screening pigment granules between the cones. These morphological differences appear minor but are in fact important in terms of functional performance.

The unsculptured and highly transparent cornea in the eye of O. grandis appears to function as an efficient window for the ommatidium, which stands in strong contrast to the extremely thick and calcified layer of the epicuticle of Glyptonotus. The crystalline cones which are relatively long and wide for an amphipod eye may probably act as a light

TABLE 4

QUATITATIVE COMPARISONS OF OMMATIDIAL STRUCTURE

SPECIES NAME	OMMATIDIUM		CONE CELLS	RETINULA CELLS		RHABDOM		MICROVILLI
	Length (um)	Dia. (um)	No.	Length (um)	No.	Length (um)	Dia. (um)	Diameter (um)
PONTOPOREIA AFFINIS[1]	68	32	2	52	5	32	20	0.1
STREETSIA CHALLENGERI[2]	1900	32	2	170	5	150	18	0.05
PHRONIMA SP.[3] (MEDIAL EYE)	4600	120	2	450	5	350	30	0.1
ORCHOMENE PLEBS	130	18	2	75	5	60	24	0.1
ORCHOMENE GRANDIS	260	56	2	170	5	160	35	0.1

Note: 1 From K.O. Donner (1971)

2 From V.B. Meyer-Rochow (1978)

3 From E.E. Ball (1977)

guide. The closely knit arrangement of the cones which are separated by narrow interstitial cytoplasm from each other, undoutedly increases the functional, light-gathering surface area and must be regarded as an adaptational modification to conditions of low environmental brightness. The enormous rhabdom, which distally terminates in a 'bowl-shaped' profile surrounding the inner region of the crystalline cones, and proximally ends near the basement membrane, is typical of an apposition eye adapted to seeing in a dimly-lit environment (Meyer-Rochow & Walsh 1977). The relatively large size and wide diameters of both rhabdom and crystalline cone are often interpreted as adaptations to increase absolute sensitivity (Horridge 1975; Meyer-Rochow 1978a). Similar features have been found in many nocturnal insects where the large rhabdom and crystalline cone modifications have been demonstrated to improve sensitivity at the expense of acuity (e.g. Meyer-Rochow & Horridge 1975; Meyer-Rochow 1977b). Light sensitivity in the marine environment is associated with primary production of phytoplankton. Cracks occurring in the Ross Ice Shelf may allow enough light to penetrate through the crevices, enabling algae to grow and start off a food chain. Under such conditions it would be an advantage for O. grandis to possess vision and home in towards lighter regions. Whether or not it could utilize the phytoplankton directly is unimportant in this context as there would always be something to scavenge near such an 'oasis'. Like most benthic animals, amphipods like O. grandis are opportunists in their feeding behaviour and under the Ross Ice Shelf where food is so scarce, a functional eye would enable the animal to notice and migrate towards any crevices which may create an 'oasis' environment at a long distance. Fish are known to exist under the Ross Ice

Shelf and to be able to 'see' one's predator could be a further advantage, especially if it is a bioluminescent species (there are some bioluminescent squids and ctenophores in the Antarctic Seas).

The presence of some screening pigment granules in the retinula cells (which are less abundant than in O. plebs and Glyptonotus) may be compared with some deep-sea crustaceans where pigment granules are totally lacking (e.g. Gennadas: Meyer-Rochow & Walsh 1977; Boreomysis: Elofsson & Hallberg 1977). The lack of screening pigments is regarded as a special adaptation to a dark environment and not as a sign of degeneration (cave organisms frequently possess screening pigment, but no dioptric apparatus or visual membranes in their eyes).

2. Effect of light and darkness

The compound eye of O. grandis does not exhibit the typical anatomical changes in response to light and darkness commonly found in insects and crustaceans (e.g. Walcott 1975; Schonenberger 1977; McKean & Horridge 1977). However, the structural disruption of the rhabdoms appear to be similar to that found in Nephrops norvegicus (Leow 1976) and in photoreceptors of cave salamander (Besharse & Hollyfield 1977) on irradiation. Since O. grandis live in an environment where virtually no light exists, it is not surprising that light has such a profound effect on the structural integrity of the photoreceptors, namely the rhabdoms. It is believed that the bleaching process of the visual pigment affects the structural stability of the rhabdoms which results in their degeneration (Leow 1976). Degeneration of the photoreceptor

appears to be related to a time-intensity threshold as demonstrated in rats (Noell et. al. 1966; O'Steen 1970) whereby prolonged low-intensity light results in photoreceptors degeneration, while short term exposure of a higher intensity does not normally produce any apparent damage (Bauman 1972). Similarly, it has been shown in the Norway lobster that the degeneration process of the photoreceptors in initiated after two hours of light exposure. As has been found in O. grandis, the 1 hour light exposure exhibited the most 'normal' rhabdom organization. This may be interpreted in such a way that the degenerative process has either not yet started or it has just begun without any visible signs yet. The 2 hours light exposure experiment provide a strong contrast to the results obtained from the 1 hour light exposure experiment. Only in the former did a total disintegration of the rhabdoms occur, which agrees with result obtained in Nephrods norvegicus Leow (1976). Ultrastructurally, the disrupted microvillar membranes of the rhabdoms of O. grandis are different from those of the heat-stressed rhabdoms of O. plebs (see Chapter I D3) but they do closely resemble those of Nephrods norvegicus Leow (1976). Both O. grandis and Nephrods norvegicus show irreversible damage of the rhabdom structure, but the heat-stressed rhabdoms of O. plebs demonstrated an ability to recover and regenerate rhabdom structure when returned to normal temperature. There is a strong posibility that individuals vary with regard to their screening pigments, for some animals appeared to have slightly darker eyes than others. Because of the small number of individuals available, this aspect was not investigated further.

In the dark-adaptation experiments, a fully dark-adapted eye, or what was thought would be 'fully dark-adapted', was neither observed in after a period of 2 days nor 1 week confinement to a dark container. The rhabdoms of the 2 days dark-adapted eyes show signs of membrane disruption, e.g. multilamellar bodies form around the edge of the rhabdom. There are holes within the rhabdom, too, which are similar to those seen in the light-adapted state of the eye of O. plebs. Another interesting fact of the 2 days dark-adapted eyes is the apparent increase in the abundance of screening pigment granules around the narrow retinula cytoplasm. Screening pigment migrations towards the rhabdom edge are closely associated with light adaptation and elevated temperature in the eyes of O. plebs and Glyptonotus. Why the pigment granules apparently started to migrate in the 2 days dark-adapted eyes in O. grandis remains specualtion at this stage. It is probable that it represents one of the long-lasting after-effects of exposure to light of the photoreceptors during the 'hauling up' procedure of the animals through 400m of ice. It took about 25 minutes to get the trap from the bottom of the Ross Ice Shelf to the surface and under the Antarctic summer, initial light exposure is unavoidable. Temperature stress may have contributed to the pigment granule migration, for under the Ross Ice Shelf an extremely stable temperature of $-2.1^{o}C$ prevails, whereas an elevation of temperature to refridgerates level of $+1^{o}C$ may have had an adverse effect on the animals as a whole or the eyes in particular. Moreover, the entire process of obtaining the animals involved some very rough and harsh handling, whereby the animals were lifted from their $-2.1^{o}C$ environment and briefly exposed to the Antarctic summer air,

which at that time of capture was -20°C (measured inside the drilled-hole). The air temperature outside the drill hole was -12°C. It is estimated that most amphipods would have been exposed to light for at least 15 minutes, before any handling could commence, and some individuals would be put into the fridge. The 1 week dark-adapted eyes show a complete disintegration of the rhabdom structure, similar to that of the 2 hours light adapted eye both of which positively differ from the long term dark-adapted eye of Crayfish (Eguchi & Waterman 1979).

Finally, the structural organization of the eyes of O. grandis is evidently equipped for vision a low light intensity environment. How O. grandis makes use of its eyes under the specific conditions of the virtually lightless environment beneath the Ross Ice Shelf is not known and could have to be studied by behavioural means. A high sensitivity to light is deduced from the observation of irreversible degeneration of the rhabdoms in both the 2 hours light adapted and 1 week dark-adapted eyes. Exposure to light of high intensity could possibly triggers off a degenerative process which is retarded, but nonetheless continues, during dark-adaptation. Therefore light can be both beneficial or disruptive depending on the intensity and exposure and the conditions of the photic enviroments to which the animals is adapted. In O. grandis light exceeding a certain brightness or duration is definitely disruptive and the eye of O. grandis is more sensitive than that of the adaptable O. plebs and Glyptonotus.

PLATE 30

The compound eye of Orchomene grandis and its dioptric structures

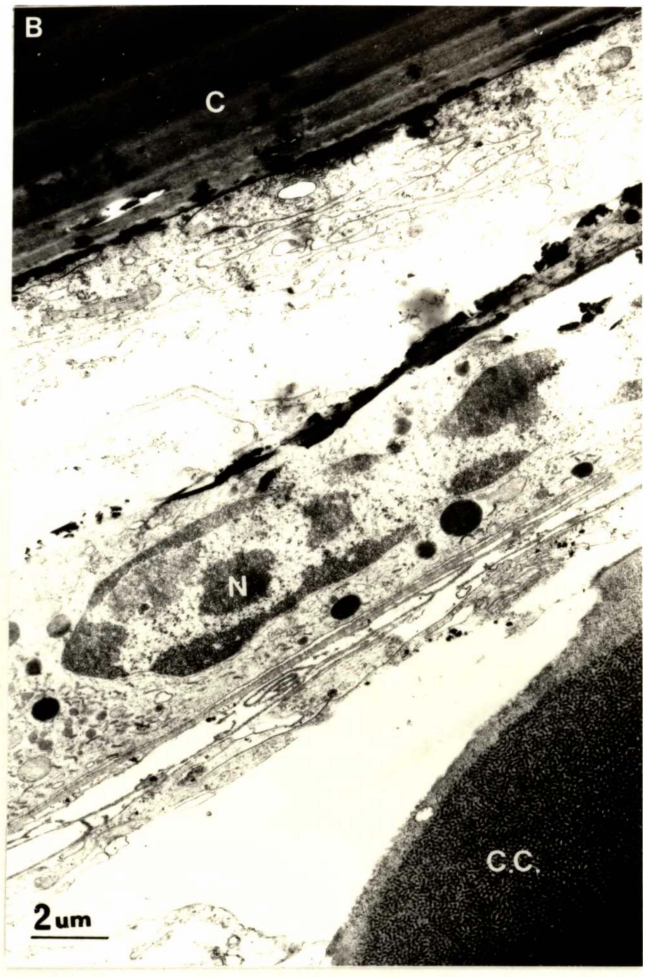
A Close-up photograph showing head and the orange pigmented compound eye.

B An oblique section through the distal region of the eye showing the cornea (C), corneagenous cell nucleus (N) and the crystalline cone (C.C.). The cornea is separated from the crystalline cone by a space of about 12um which is occupied by the corneagenous cells and their large nuclei (N). The crystalline cones lie just below the corneagenous cells.

C A longitudinal section through the cornea reveals the multilamellar structure of the latter. The cornea is unusually thick for an amphipod eye: approximately 20um. There exist clearly 3 horizontal regions in the cornea. The outer consists of 7 thin lamellae, the middle of 4 thick lamellar layers and the inner of 5 thin lamellar layer (arrows).

D High magnification transmission electronmicrograph of the crystalline cone revealing that most of the cone's ultrastructure consists of electron-dense granules of approximately .15 - .20um.


A


B
C
N
C.C.
2um


C
2um

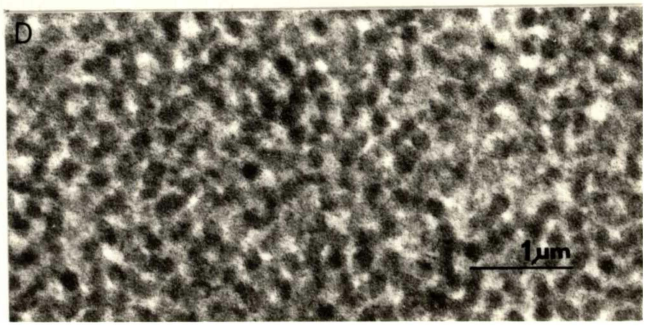

D
1um

PLATE 31

The structure of compound eye

This is a light-micrograph of an oblique section through the right eye of Orchomene grandis. The arrangement of the ommatidia suggests that the eye is internally divided into two separate region i.e. the large ventral region and the smaller dorsal region (arrows). However, external differentiation of the eye is not observed. Externally the ommatidia are covered by a thick and smooth cornea (C). The large crystalline cones (C.C.) lie below the corneagenous cells which are found next to the cornea. The large crystalline cones are stained distinctly darker than the rest of the photoreceptive tissues. Next below, the crystalline cones are the equally large rhabdoms (RH) which make direct contact with proximal region of the cones. Through the different staining characteristics it is possible to distinguish the lighter stained retinula region from the darker stained epi-retinula region.

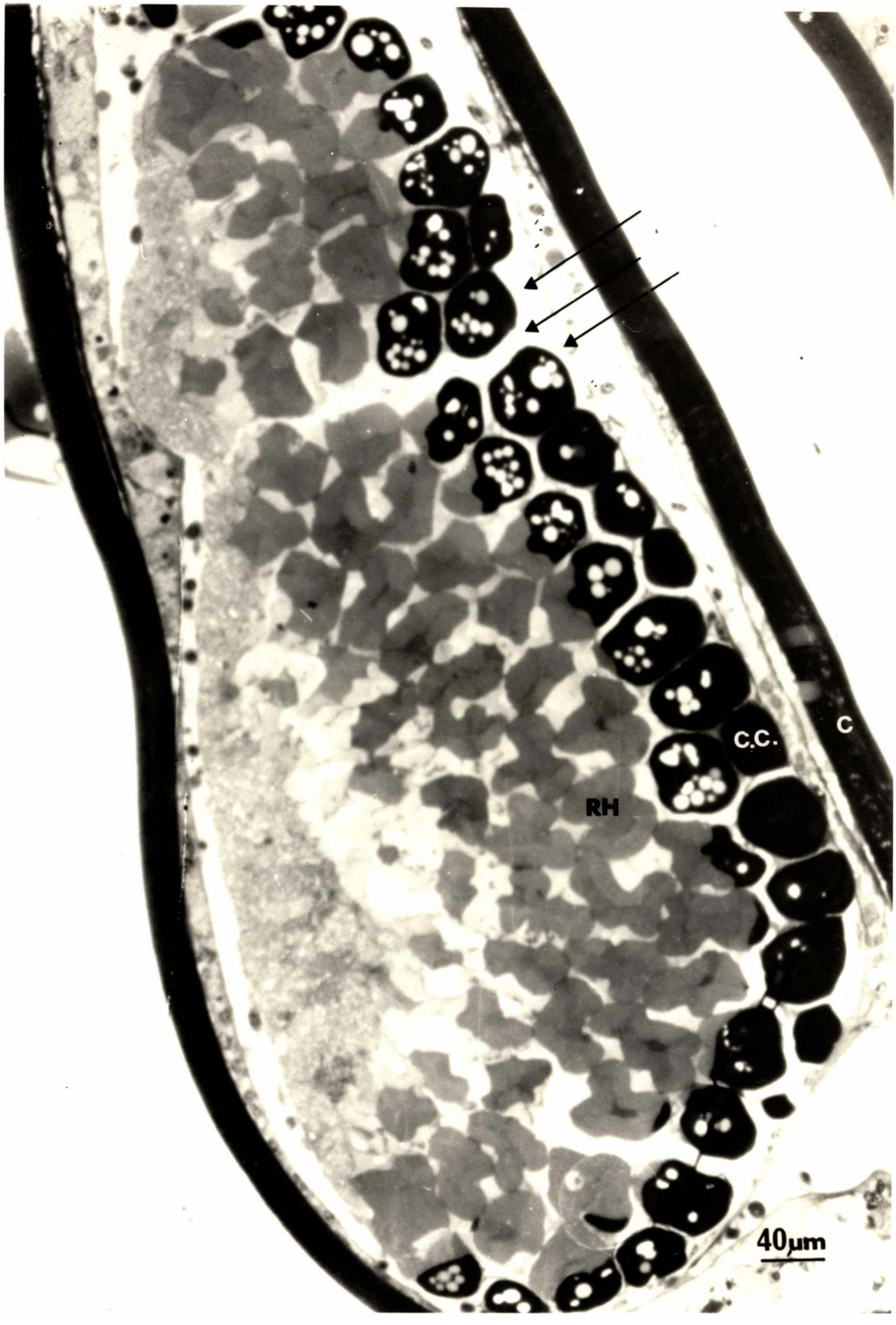

C.C.
C
RH
40µm

PLATE 32

Proximal region of the Ommatidia

A In this light-micrograph, the longitudinal section through the proximal region of one ommatidium reveals the proximity of the rhabdoms (RH) to the basement membrane (BM). In fact three rhabdoms appear to make direct contact with the basement membrane. The retinula cell processes (AX) narrow proximaly but increase in width before they penetrate the basement membrane. The nuclei (N) of the retinula cells are located 25um below the basement membrane, and measure approximately 8um in diameter.

B Longitudinal sections through the basement membrane reveal the ultrastructure of the retinula and the sub-retinular space. Note the large number of inclusions made up of pigment granules, mitochondria and vacuoles in the distal region of the sub-retinular space. The inset shows a single axons with the retinula cell nucleus (N) surrounded by some screening pigment granules.

C This electron-micrograph of an oblique section through the axonal region of the ommatidium at the level where the retinula cell nuclei are located, shows the scanty distribution of screening pigment granules within the cytoplasm. The cell boundaries are clearly distinguishable.

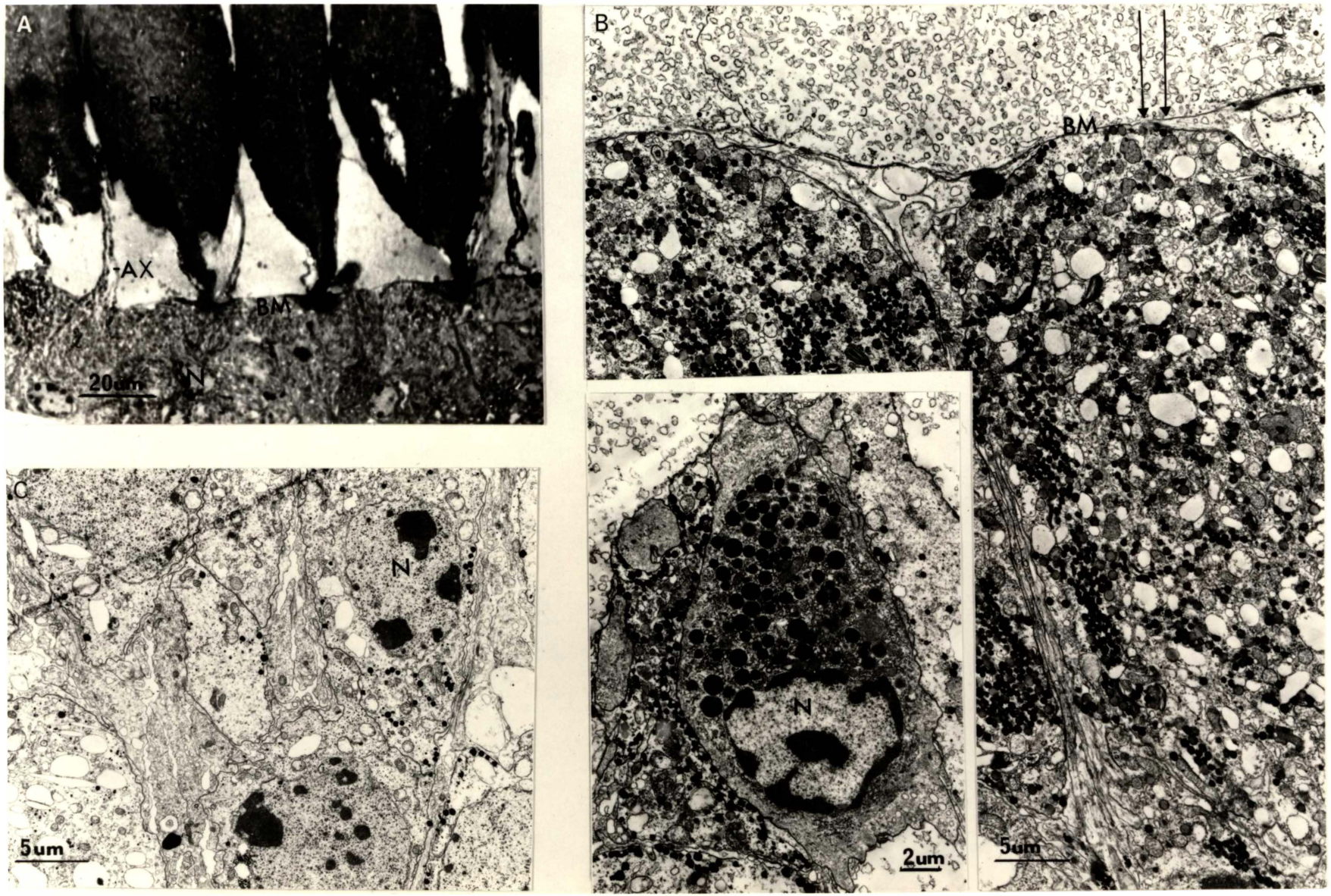

A
AX
BM
20um
N
B
BM
N
2um
5um
C
N
5um

PLATE 33

Comparison between the ommatidial organization of the different states of adaptation

A This light-micrograph of a longitudinal section through the 1 hour light-adapted eye shows the typical ommatidial organization of Orchomene grandis. Note the very large area with which the crystalline cones (C.C.) whose corneagenous cells can just be seen above them, abut on the distal ends of the large rhabdoms (RH). The rhabdoms and the crystalline cones appear to be in direct contact. Proximally the elongated spindle-shaped rhabdom tapers off and disappears 20um above the basement membrane (BM). The retinula cells have processes which penetrate the basement membrane and turn into axons (AX). The axons are separated by the cytoplasm of the interstitial cell.

B This somewhat oblique section through the 2 days dark-adapted edge reveals the lighter stained rhabdoms. The rhabdoms still retain their structural integrity and the direct contact with the crystalline cones is intact.

C In the 2 hours light-adapted state, the longitudinal section reveals disruptions of the rhabdom structure especially in the distal region. The intimate connection between the rhabdom and the crystalline cone is lost, instead a space of 20um developed in its place.

D One week dark-adapted eyes show an even greater degree of disorganization of the rhabdom. The distal part of the rhabdom has receded from the crystalline cone leaving an empty space of 70um between them. This space is not regarded as an artefact, for it was observed in several eyes.

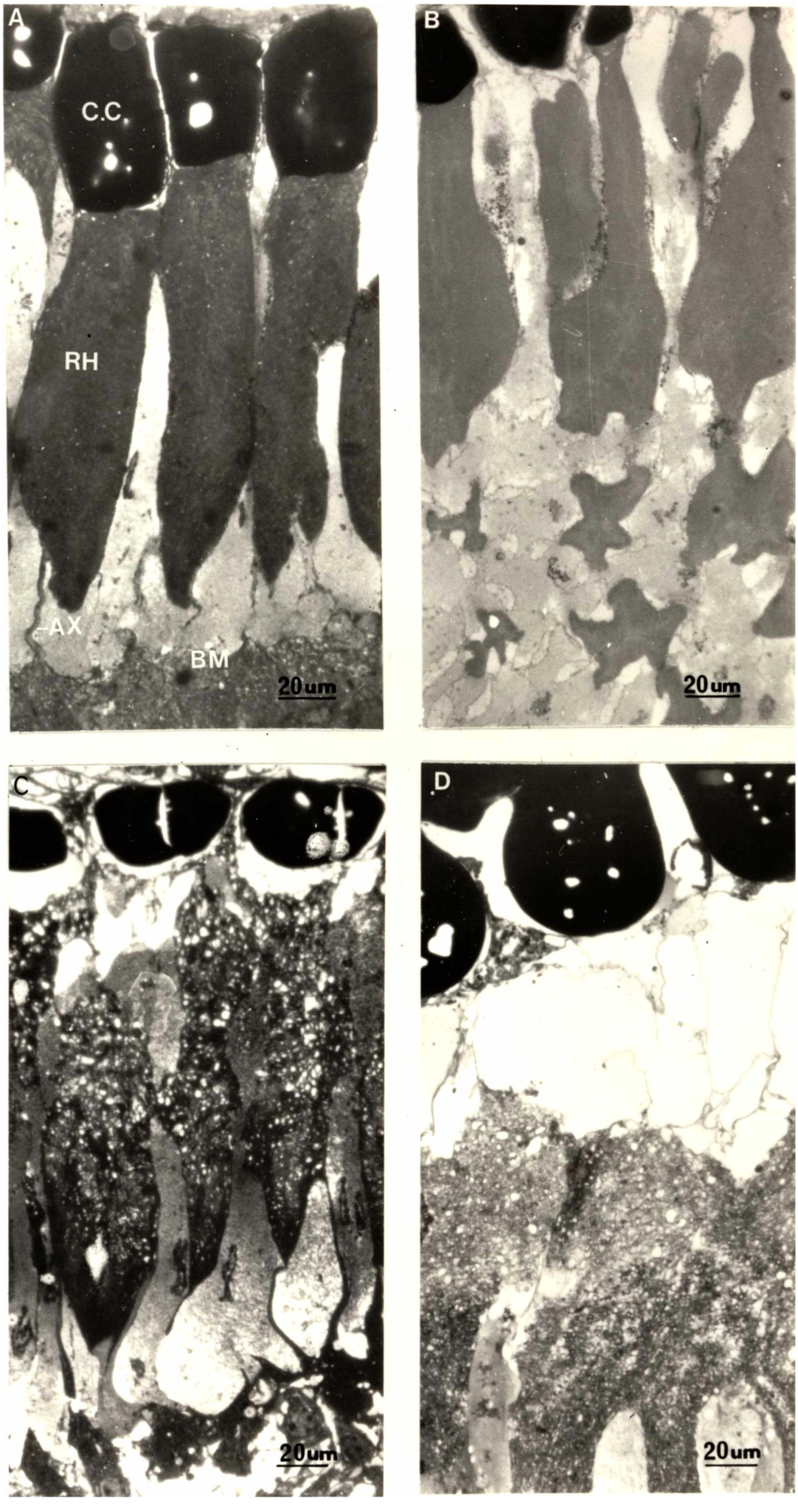

A
C.C.
RH
AX
BM
20um
B
20um
C
20um
D
20um

PLATE 34

Light micrographs of transverse sections through (A) 1 hour light-adapted, (B) 2 hours light-adapted (C) 2 days dark-adapted, and (D) 1 week dark-adapted eyes. Shape, size and structural integrity of the rhabdom are all affected by the adaptations. The most 'normal' rhabdoms are found in the 1 hour light-adapted animal (A), here rhabdoms of variable shape but uniform optical density are found to be in close proximity with each other. In the two hours light-adapted material (B) individual rhabdom structures are hardly distinguishable and appear to have disintegrated. The two days dark-adapted eyes show minor signs of rhabdom damage e.g. most of the rhabdoms have holes in them. They also stain less strongly than (A) material. One week dark-adapted rhabdoms resemble those of the two hour light-adapted material. Individual rhabdoms are hardly discernible and the structural integrity in them seems lost.

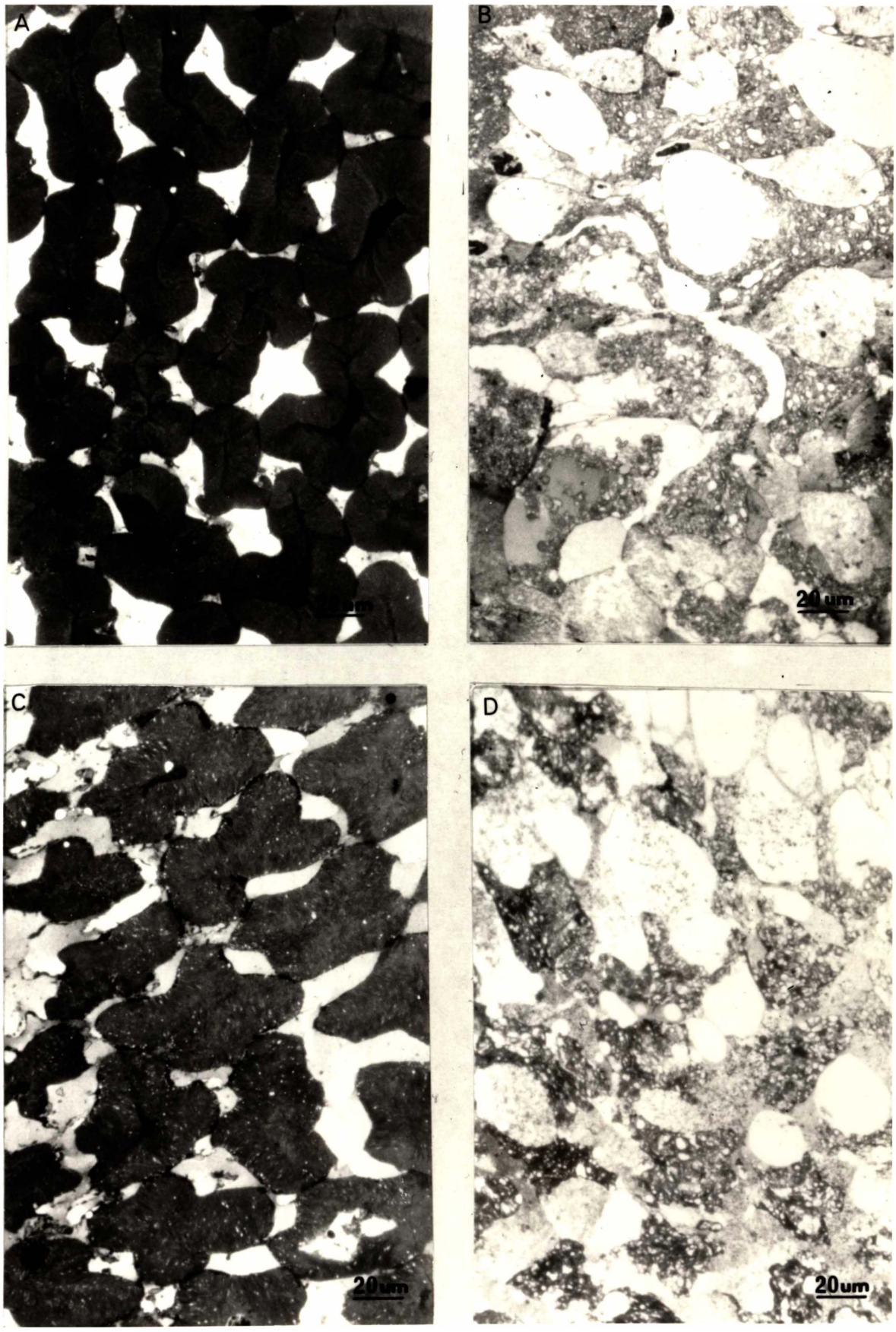

A
B
20 um
C
20um
D
20um

PLATE 35

Retinula cells and rhabdom ultrastructure

This is a transmission electron-micrograph of a transverse section through an ommatidial group of five retinula cells (numbered 1-5) at about mid-rhabdom level. It shows how the rhabdomeres, whose borders are not discernible, of the five retinula cells give rise to the centrally fused rhabdom. The retinula cytoplasm is unusually small with very few intracellular organelles present. A few mitochondria (M) and a few screening pigment granules are found scattered randomly in the narrow layer of cytoplasm. The retinula cell plasm possesses thin processes that extent radially and make contact with the retinula cells of the adjacent ommatidial group. Individual rhabdomeres are hard to distinguish and the microvilli arrangement is irregular, in contrast to the uniformly arranged microvilli of Orchomene plebs.

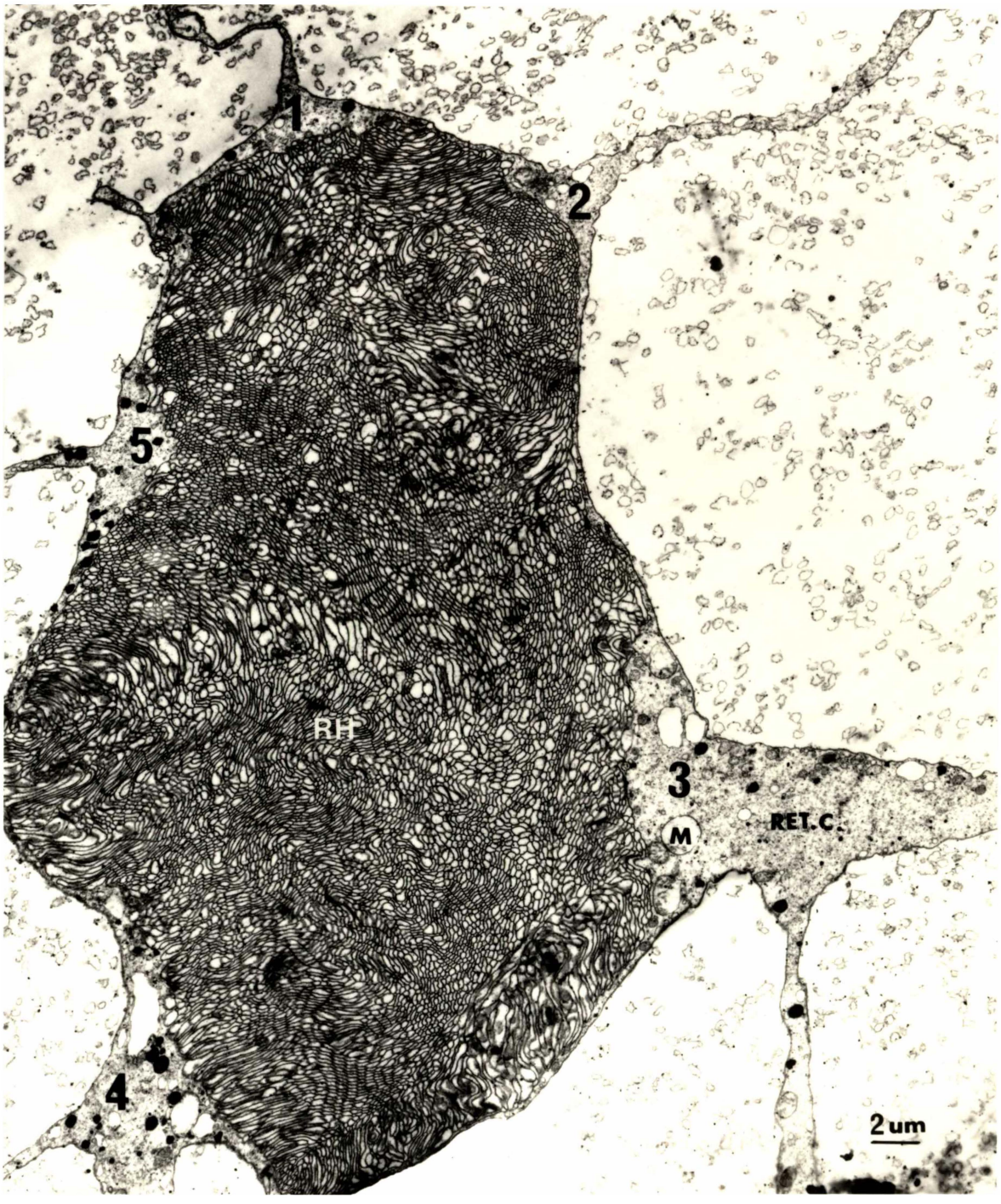

1
2
3
4
5
RH
M
RET.C.
2 um

PLATE 36

The rhabdom, retinula cell and interstitial cell

This transverse section through the one hour light-adapted material shows that the microvilli are arranged irregularly and vary in size and directions, thus giving the rhabdom its peculiar shape. The cytoplasmic area of the retinula cells is much reduced compared to that of Orchomene plebs. The distribution of the screening pigment granules is scanty and in this particular section, the ones that are present appear to be localized in one particular region. The intracellular organelles present include multivesicular body (MVB), mitochondria and cytoplasmic processes which extend to the other retinula cells of the adjacent rhabdom and sometimes may contain within them mitochondria and cone process (CP). The retinula cell is seen to occasionally shed some of its cell plasma which may include screening pigment granules, into the interstitial cell (see arrows).

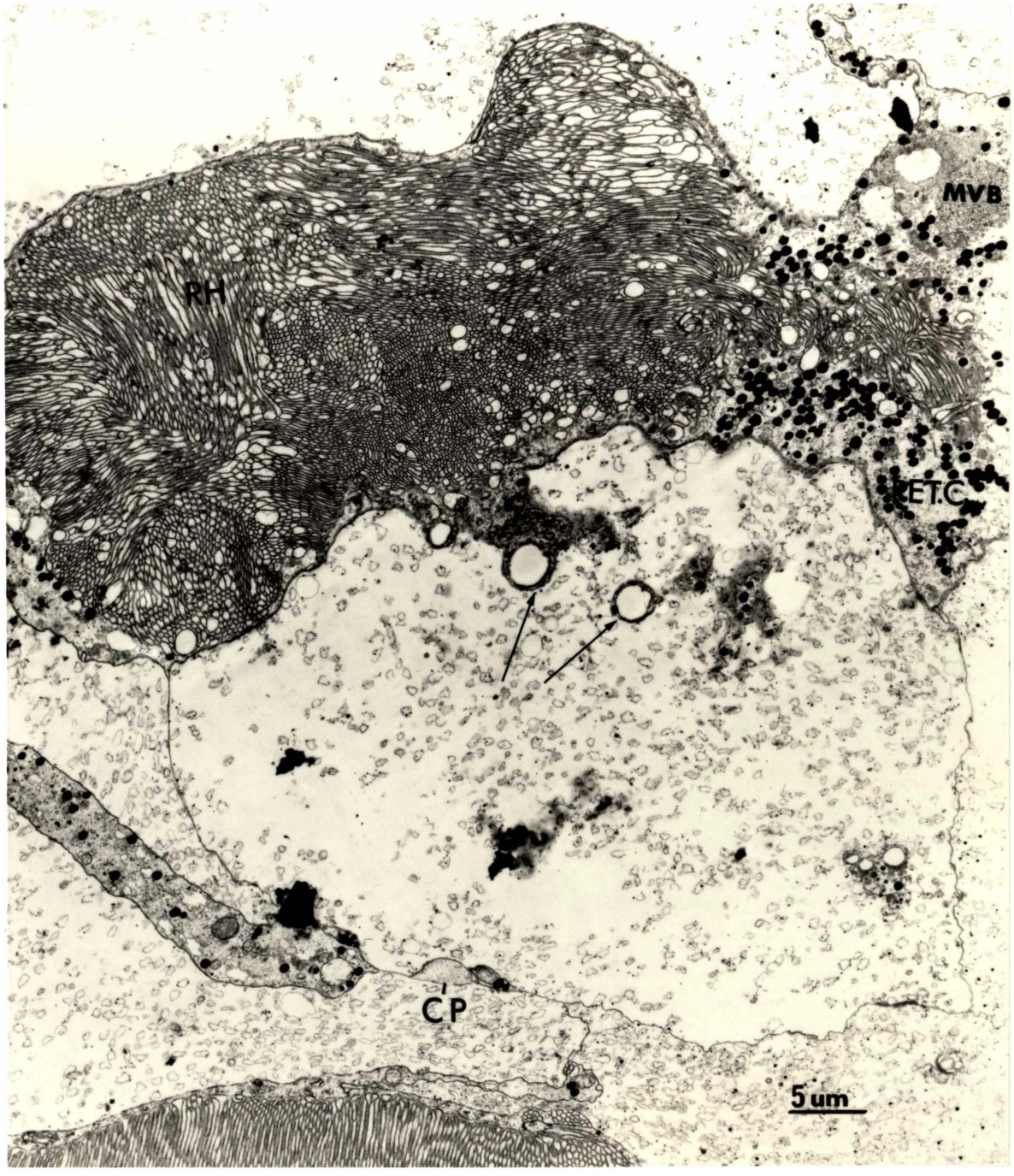

MVB
RH
E.T.C.
CP
5 um

PLATE 37

Rhabdom edge and interstitial cytoplasm

This oblique section reveals the relationship between the retinula cytoplasm and the interstitial cytoplasm. Along the rhabdom edge the cytoplasms of both cells appear to be involved in the cycle of microvillar breakdown and regeneration. Membranes are observed which form vesicles of round shapes which seem to be transferred into the retinula cytoplasm and later excluded into the interstitial cytoplasm (lower arrows). Membranous structures near the rhabdom edge are found to enclose cytoplasmic vesicles and even pigment (see arrows). Mitochondria (M) are more abundant in the interstitial cytoplasm when compared with retinula cell plasma.

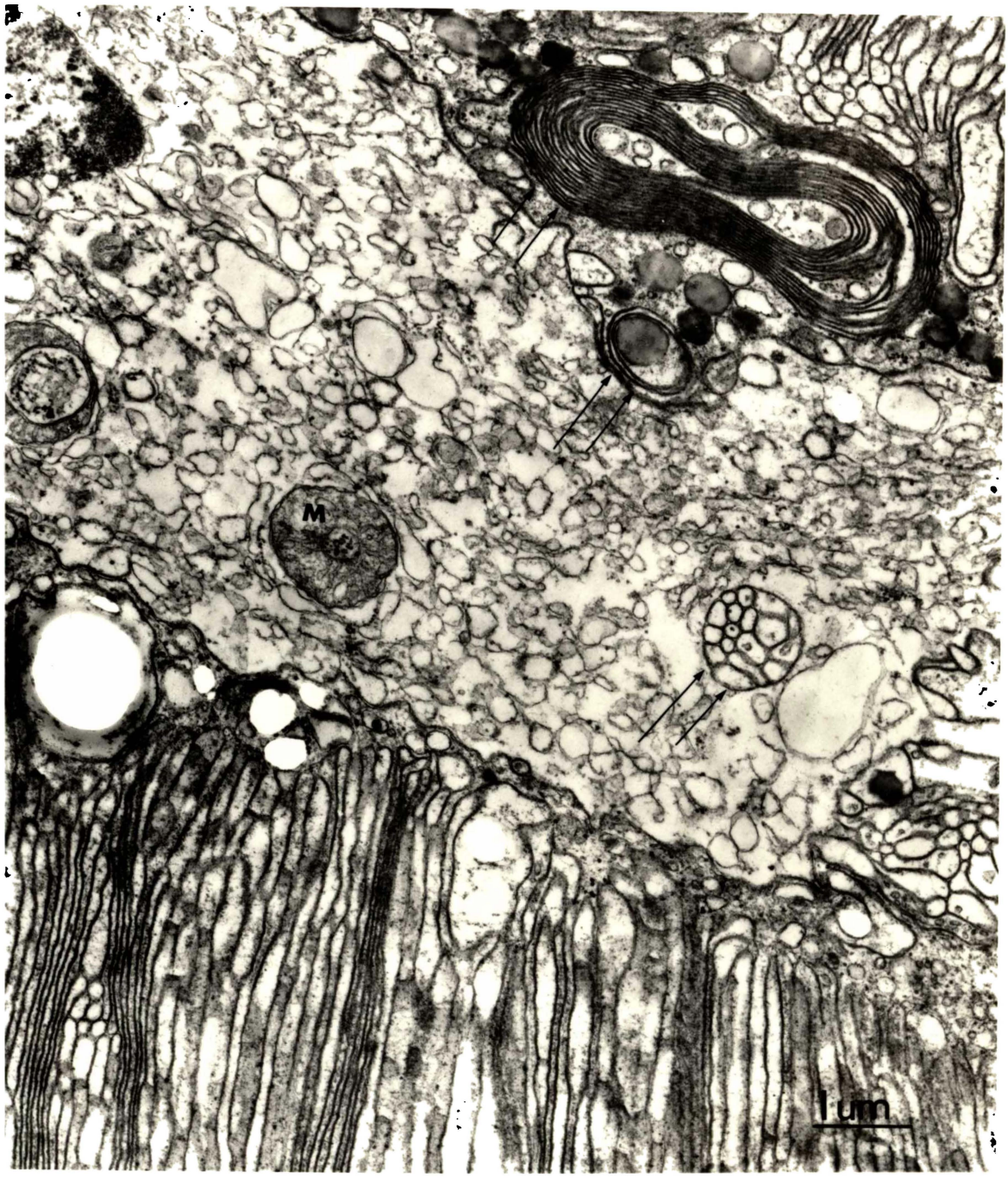

M
1 um

PLATE 38

Retinula cell ultrastructure

A The electron-micrograph shows retinula cell plasm (RET.C.) and microvilli organization along the rhabdom (RH) edge. Note that apart from a few mitochondria (M) and vesicles the retinula cytoplasm does not contain many intracellular organelles. One of the interstitial cells which surround the retinula cell displays its characteristically shaped nucleus (N).

B This section shows the narrow, radially-projecting retinula cell process connecting with the retinula cells of the adjacent ommatidium (see arrows). These processes also serve to isolate the individual interstitial cell around the rhabdom.

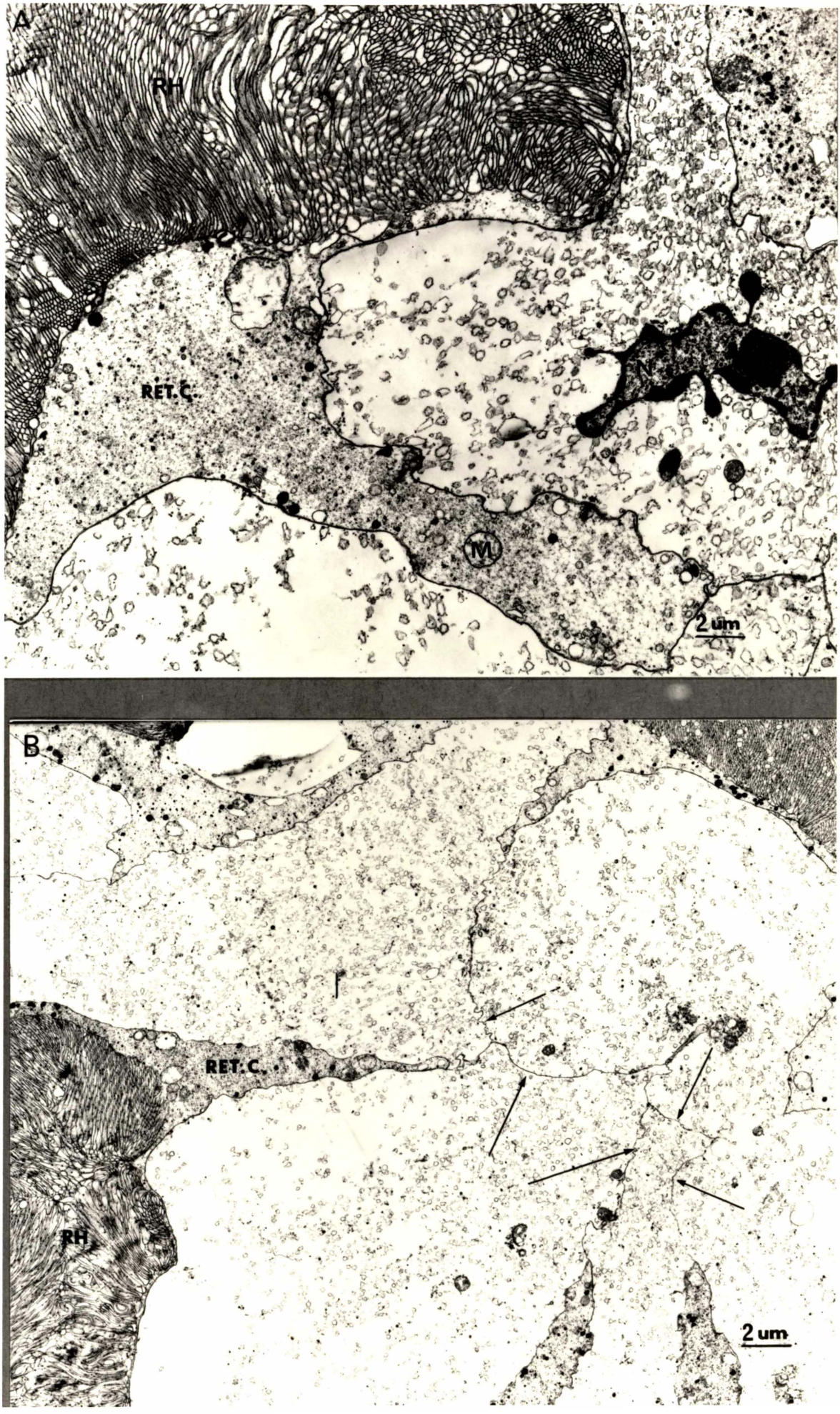

A
RH
RET.C.
N
M
2 um
B
RET.C.
RH
2 um

PLATE 39

Ultrastructure of microvilli

A High magnification electron-micrograph of longtudinally sectioned rhabdom showing microvilli in the 1 hour light-adapted eyes. Note the uniformly arranged microvilli with an almost regular intervillus space and microvillus diameter.

B This section through the two days dark-adapted eyes shows irregularities in the microvillus arrangement. The microvillar membrane appears to be darker stained. Variations in the diameter of the microvilli are also observed.

C Cross-section through the microvilli reveal variations of the microvillus dimension. The diameters of the microvilli may range from 80nm to 0.6um in two days dark-adapted eyes.

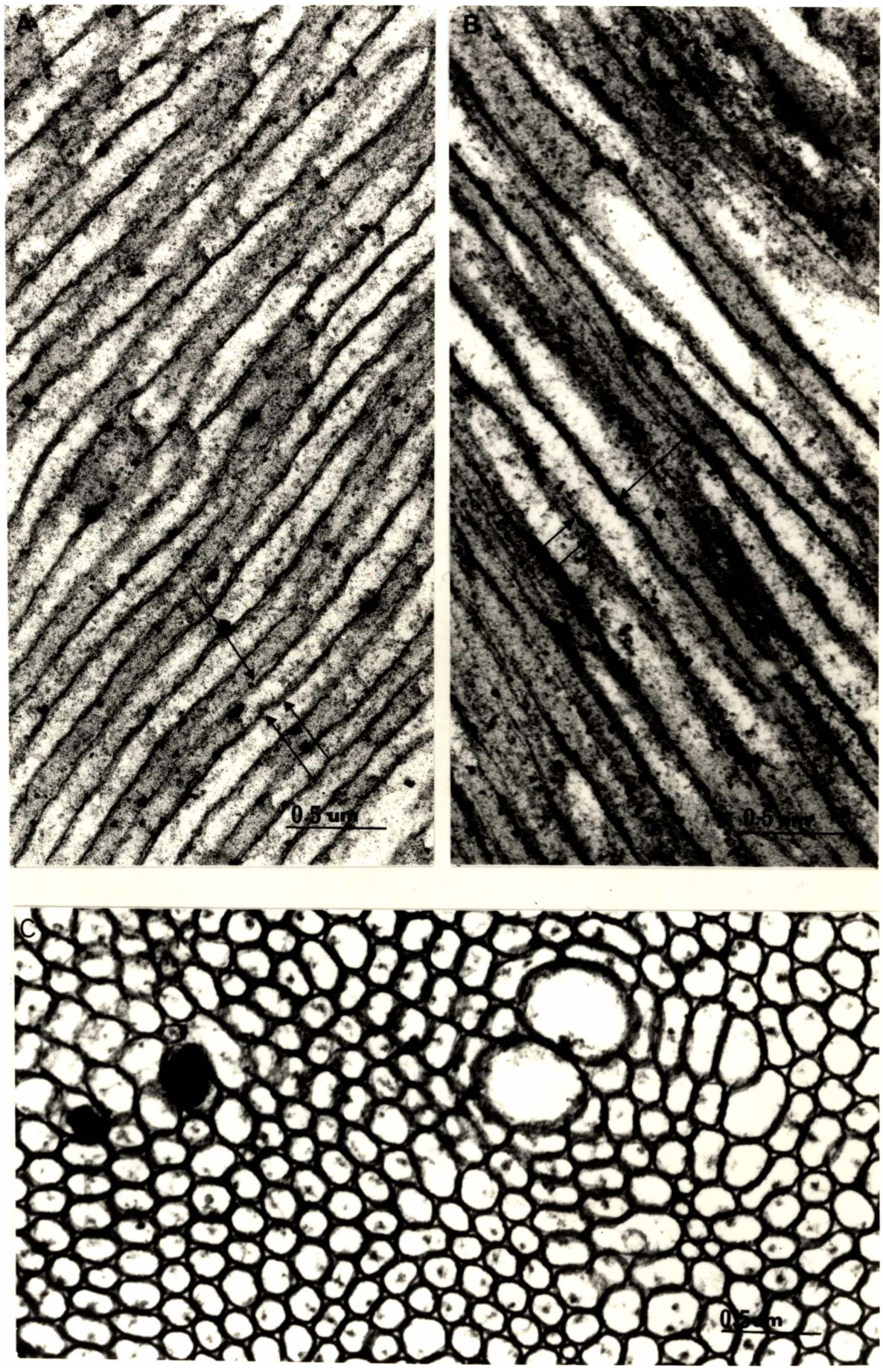

A
0.5 um
B
C

PLATE 40

Variations in the ultrastructure of microvilli within one rhabdom

A This electron-micrograph of 1 hour light-adapted eye shows massive microvillar disruption along the rhabdomeral edge. The microvilli form concentric whorls and other multimembranous structures (arrow) which may enclose vesicles within them. Some of these structures are lost to the retinula cytoplasm. Mitochondria and screening pigment granules appear to increase in abundance.

B This section shows another part of the rhabdomeral edge but within the same rhabdom as in (A). Note the rather well arranged microvilli arrangement which is in strong contrast to that found in (A). However, the retinula cytoplasm is quite empty with regard to the intracellular organelles.

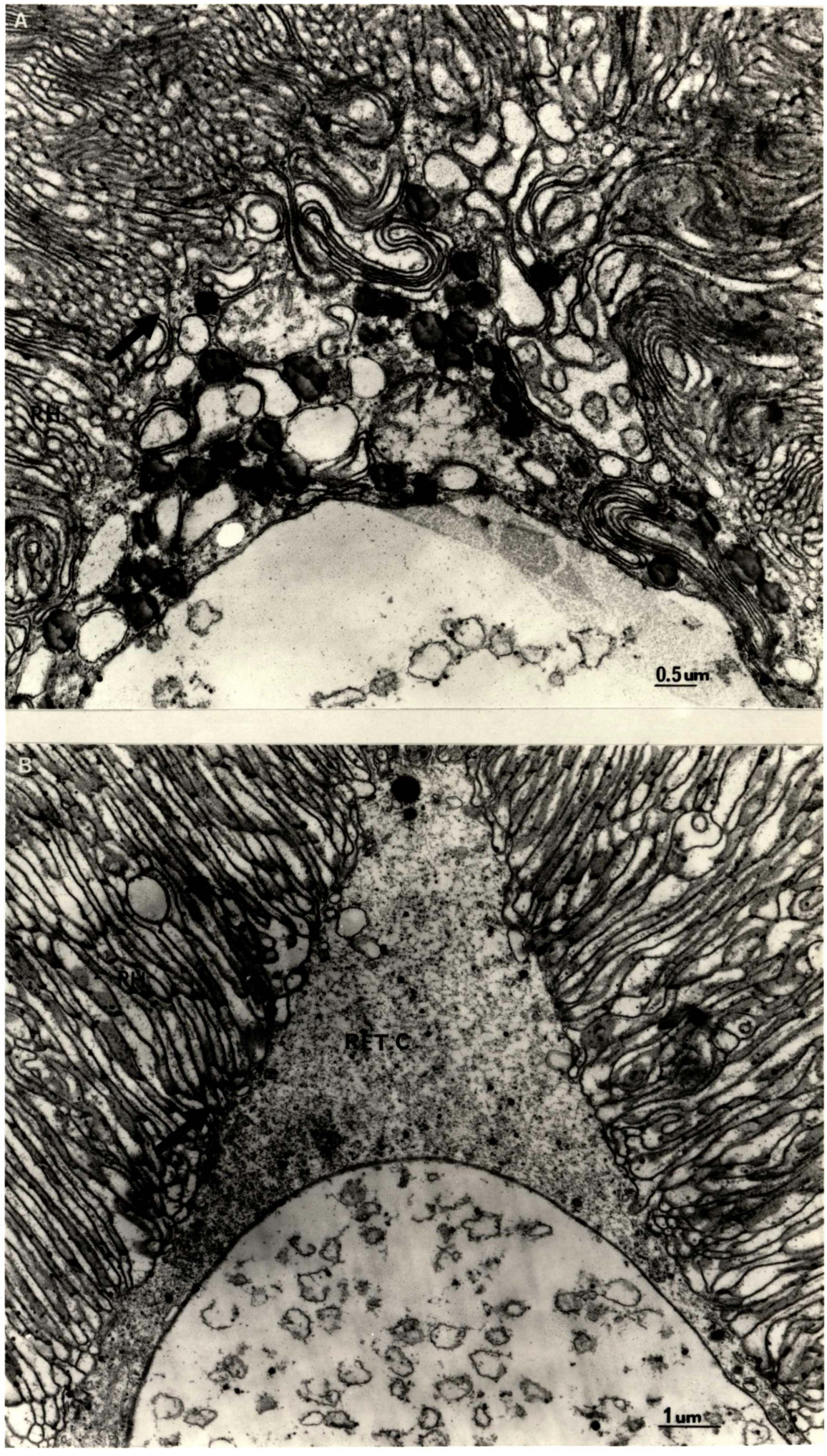
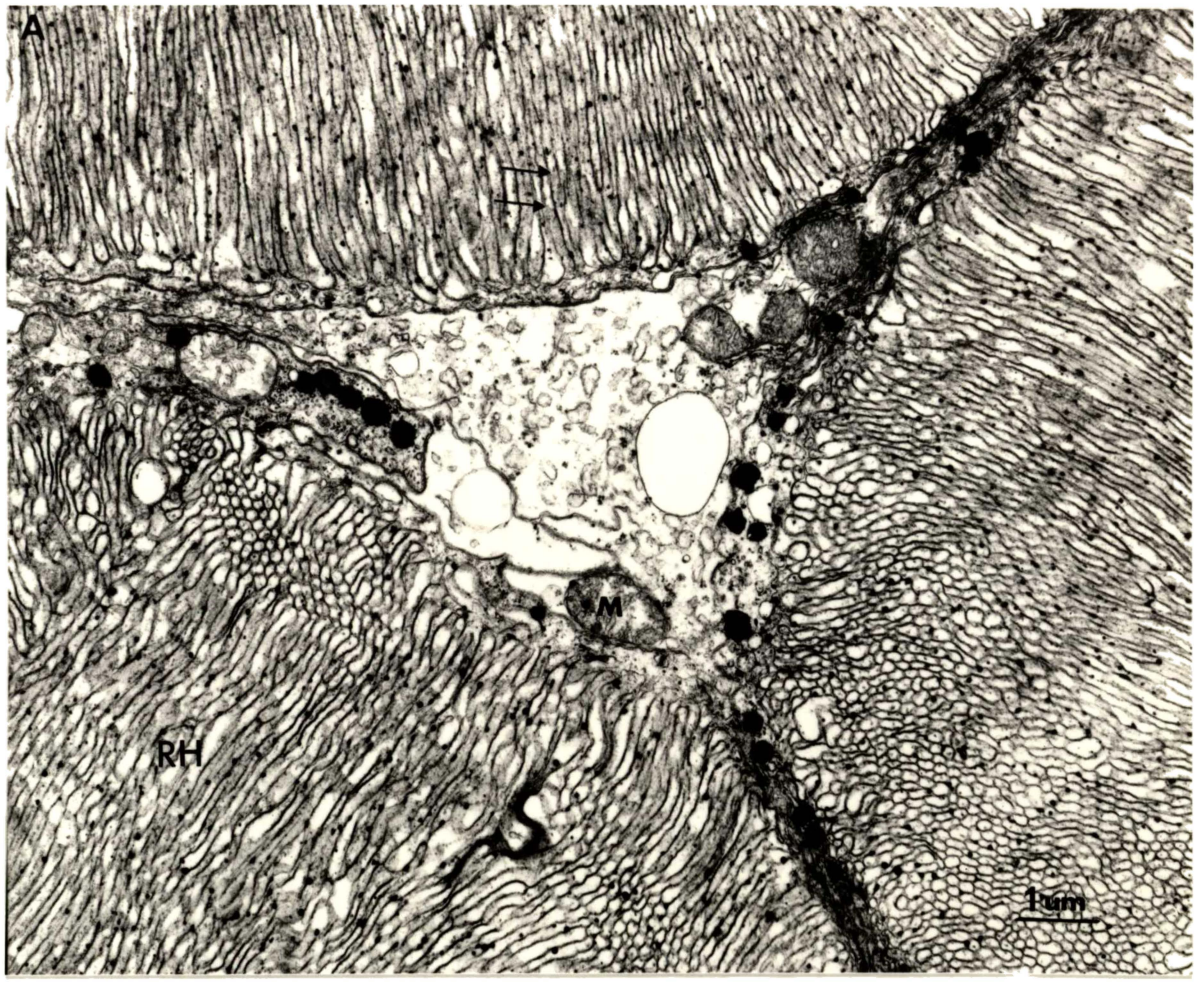

A
RH
0.5um
B
RH
RETC
1um

PLATE 41

Ultrastructure of the distal ends of the rhabdoms

A This transverse section through the distal rhabdoms of the 1 hour light-adapted eye shows the microvillar organization. Note the proximity of one rhabdom to the adjacent one. The retinula cytoplasm is very much reduced with a few mitochondria and pigment granules present. The microvilli are quite uniformly arranged, running almost parallel to one another (arrows) for any given rhabdom area.

B This transverse section through the two days dark-adapted eyes shows a much a looser rhabdom organization. A number of 'holes' occur within the rhabdom and near the rhabdom edge. Signs of membrane breakdown microvilli are found along the rhabdomeral edge where the membranes extend into the retinula cytoplasm enclosing the organelles within it. Membrane bound structures with organelles like mitochondria and pigment granules in them are observed to be liberated into the interstitial cytoplasm. The number of dark and grey granules increase in abundance.


A
M
RH
1um

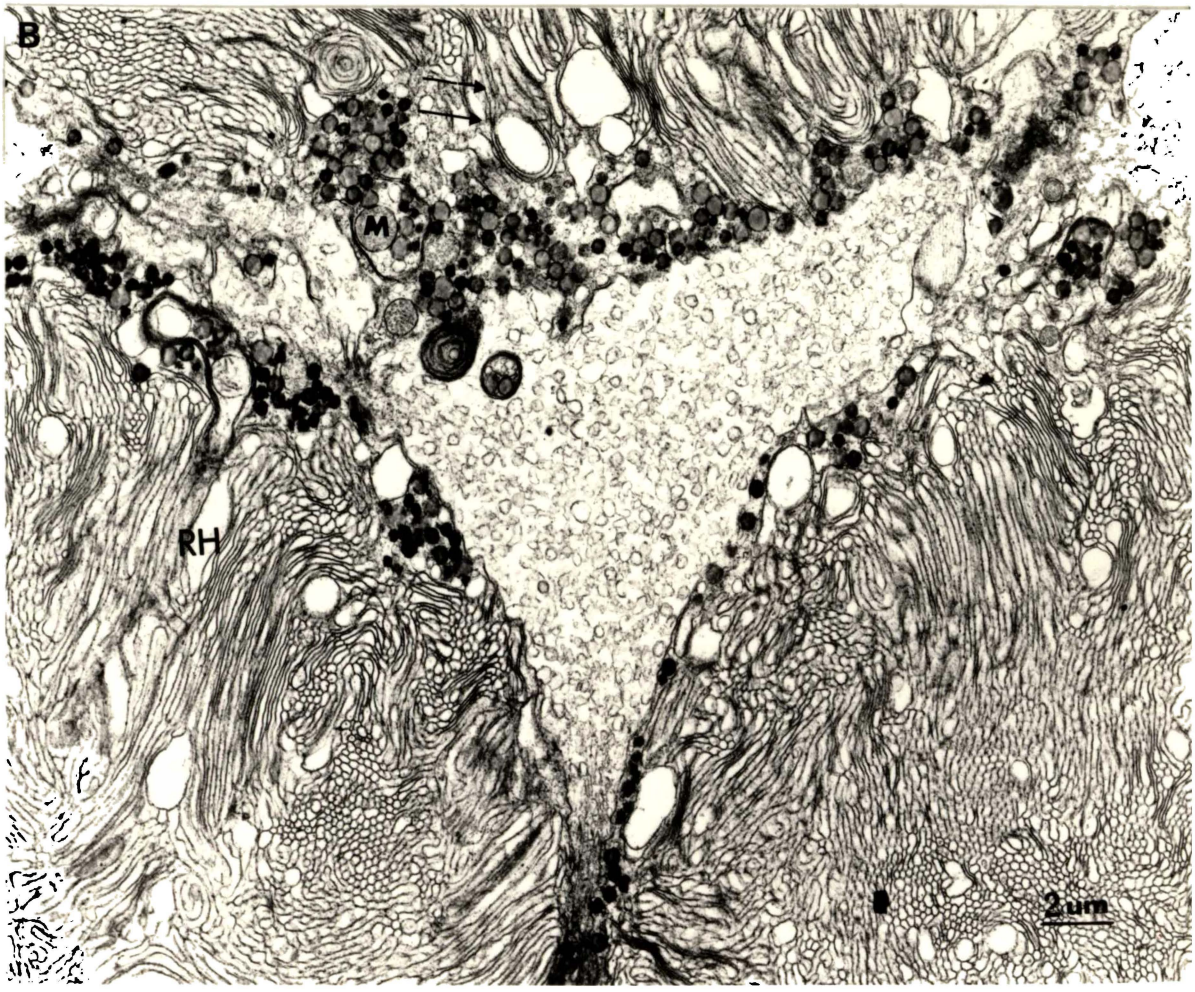
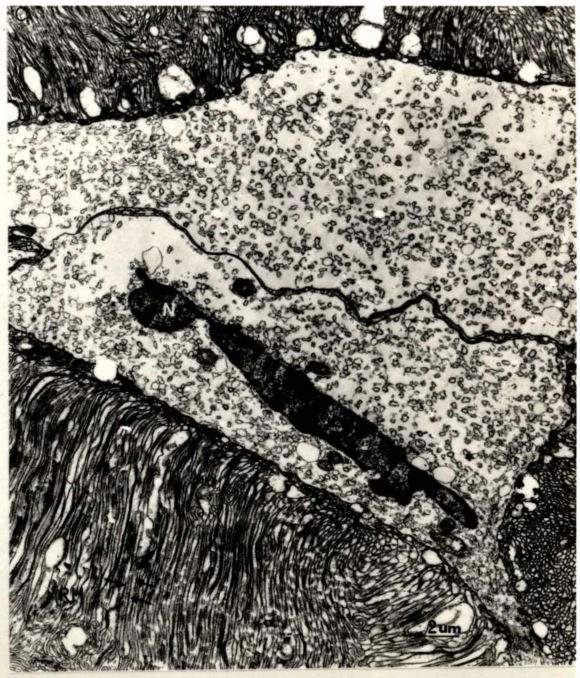

B
M
RH
2um

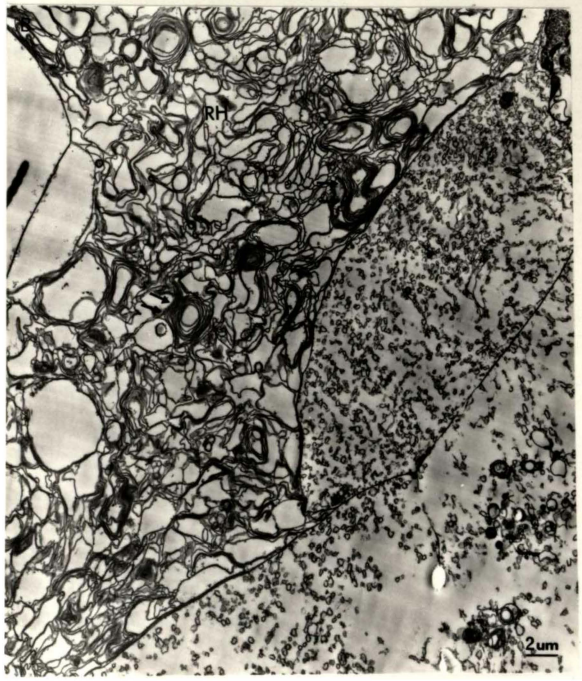
PLATE 42

Ultrastructure of the rhabdom in different states of adaptation

A comparison between transversely sectioned rhabdoms of eyes from (A) 1 hour light-adapted, (B) 2 hours light-adapted, (C) 2 days dark-adapted, and (D) 1 week dark-adapted. The electron-micrographs reveal that the most orderly and presumably normal rhabdom arrangement is displayed by (A), the 1 hour light-adapted eye, and to a lesser extent in the 2 days dark-adapted eye (C). Both, the 2 hours light adapted (B) and 1 week dark-adapted (D) material exhibit disintegration of the rhabdom structure. The microvillus arrangement in both (A) and (C) shows a regular pattern of organization (arrows). However in (B) and (D), the microvilli form whorls of concentric membranous structure or 'onion bodies' (arrows). Screening pigment granules which occur randomly in the narrow layer of retinula cytoplasm of 1 hour light-adapted and 2 days dark-adapted eye, are absent from the disintegrated rhabdoms of (B) and (D).


RH
CP
2um


N
RH
2um


RH
2um

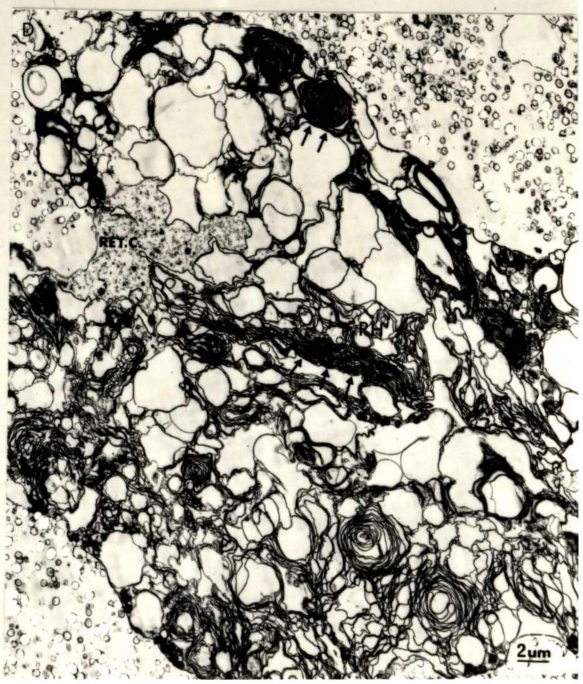

RET.C.
RH
2um

PLATE 43

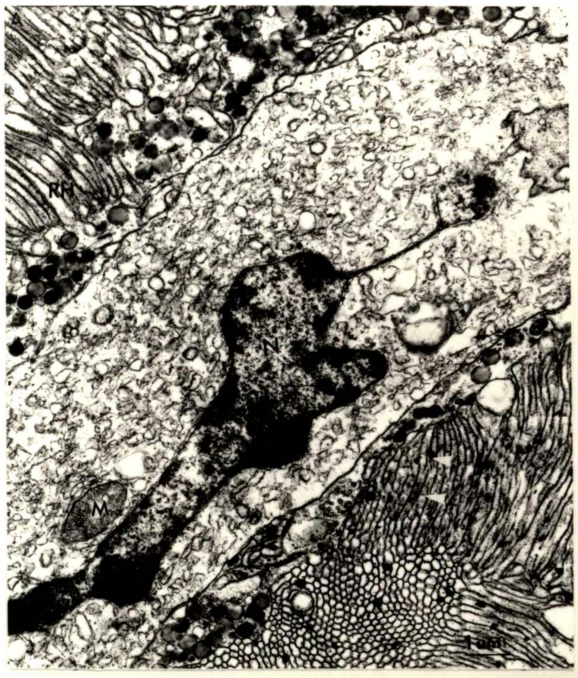
Ultrastructure changes in microvilli organization upon dark-light adaptation.

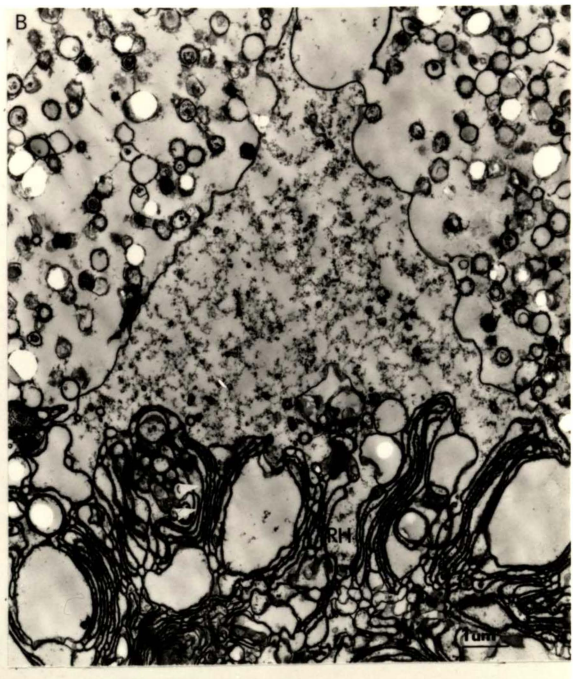
A This high magnification electron-micrograph shows regular, well organised microvilli along the rhabdomeral edge. The bizarrely shaped nucleus (N) is typical of the interstitial cell. M denotes a typical mitochondrion.

B This section through the one week dark-adapted eye shows severely disrupted microvilli along the rhabdomeral edge. The distortion of the microvillar structures has reached such an extent that individual microvilli are hardly distinguishable. Note that the interstitial cytoplasm contains hollow vesicles which are all of the same spherical shape in contrast to those of 1 hour light-adapted, 2 hours light-adapted and 2 days dark-adapted eyes, where these vesicles were of irregular shapes.

C In the 2 days dark-adapted state, high magnification electron-micrograph show that the microvilli are still well ordered and of an arrangement which is typical of that of the normal eye. The slim convoluted retinula cell process that extent radially is characteristic of the photoreceptor structure in this species.

D The 2 hours light-adapted eye shows complete disintegration of rhabdomal microvilli. The microvillar membranes have totally been transformed into a mess of contorted membranes.


RH
N
M
1 um


B
RH
1 um


RH
1 um


RH
1 um

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