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The Photoreceptors Of The "four-eyed Fish," Anableps Anableps (l)

Bessie Borwein

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THE PHOTORECEPTORS OF THE "FOUR-EYED FISH"

ANABLEPS ANABLEPS L.

by

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ABSTRACT

The photoreceptors of the adult *Anableps anableps* were investigated by light microscopy, scanning and transmission electron microscopy. The fish is a surface swimmer and the eye is divided by the water meniscus. In general, the photoreceptors (rods, single cones, and double equal cones) resemble those of other vertebrates, but there are several unusual features: (1) The outer segment discs of the double cones differ in the two members. (2) All cones have a prominent accessory outer segment derived from the single connecting cilium, and there is no second centriole. (3) The exterior of the inner segments is ridged and grooved longitudinally, most markedly so in the cones. (4) A membrane-bounded oil droplet is present in the distal cone inner segment, formed from mitochondria which enlarge, fuse and transform in a vitreal-scleral gradient. (5) There are knob-like invaginations of rod cytoplasm into the cones immediately scleral to the external limiting membrane. (6) Subsurface cisterns underlie apposed plasma membranes of double cone inner segments and direct rod-cone inner segment contacts. (7) Fine "fins" on the cones interdigitate, with Müller cell cytoplasm between, just scleral to the external limiting membrane.

(8) In the rod spherule there is a greater density of vesicles and the cytoplasm is darker than in the cone pedicle.

The well-defined cone mosaic has a linear pattern peripherally and a square pattern centrally. The photoreceptors undergo photomechanical movements. Photoreceptor ultrastructure is alike in both dorsal and ventral parts of the retina, but the ventral retina contains more cells and is thicker than the dorsal retina.

The adjustments necessary for simultaneous air and water vision are to be found in the ocular anatomy, mainly in the lens shape, corneal thickness and curvature, and the greater number and dense packing of the cells in the ventral retina.

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My husband and children endured this investigation cheerfully and sustained me with that domestic co-operation and support that freed time and energy for laboratory and library. Their help, encouragement and delight in learning with me made this work possible.

to

DAVID

and

Jonathan, Peter & Sarah

and

my mother

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Notes:

1. Figure 113 summarises the observations made on the structure of the rods and cones of Anableps anableps.

2. Throughout this thesis the terms 'inner' or 'vitreal' and 'outer' or 'scleral' are used in reference to orientation within the retina. 'Inner' or 'vitreal' refer to areas lying closer to the inside or vitreal chamber of the eye; 'outer' or 'scleral' refer to those structures positioned nearer to the sclera or outside of the eye.

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Chapter 1

INTRODUCTION

General Review of Anableps

Anableps anableps L. (Gr. upward-looking), commonly called the four-eyed fish, is a teleost of the order Cyprinodontiformes (the top-minnows or toothed-carps), family Anablepidae. The genus contains three species, *A. anableps*, *A. microlepis*, and *A. dowei*, distinguished from each other by the number and colour of narrow longitudinal bands along the flank and the number of scales on the lateral line.

Anableps was named by Linnaeus (1758) who based his work on that of Artedi (1738). In different editions of Linnaeus' *Systema Naturae* the fish was given different names, *A. anonymus* in the second edition (1744), *A. anableps* in the ninth edition (1758), and *Cobitis anableps* in the tenth edition (1760). Bloch (1794) named it *A. tetrophthalmus*, and Walbaum (1792) named it *A. gronovii*. By these various names *A. anableps* can be identified in the literature.

This fish is a surface dweller of the rivers

and tidal estuaries of central and northern South America and the Caribbean. One of the earliest descriptions of the fish is that of Harcourt (1756) (quoted in Klee, '68) who sailed the Amazon and saw a "rare fish called Cassoorwa, which hath in each eye two sights, and as it swimmeth it beareth the lower sights within the water and the other above.....it is somewhat bigger than a smelt but far exceeding it for daintie meat."

The adult shows marked sexual dimorphism. The male has a complex gonopodial organ, developed ontogenetically from the anal fin through which sperm bundles are transferred to the female genitalium for internal fertilisation. There are sexual rights and lefts, with a preponderance of dextral males and sinistral females. Male rights, in which the gonopodial organ curves markedly to the right, can mate only with female lefts, in which the large foricula scale which covers the genital aperture is not attached on its left side. Male lefts can mate only with female rights. This is a striking peculiarity not found in any other fish group. (Garman, 1895a, 1895b; Breder and Rosen, '66).

There is an extremely well-developed viviparity, including a pseudoplacenta of new and temporary structures developed in the ovarian follicle. The survival rate from egg to birth is very high and the one - five young are 3 1/4 - 5 3/4 cms. long at birth. At 15 cms. sexual

maturity is reached and adults range up to 30 cms. in length. (Wyman, 1854; Garman, 1895a; Turner, '38,'39,'40a,'40b,'50).

The Eyes of Anableps

Anableps is a very active fish, feeding by vision, and its most prominent feature is the presence of two large,protuberant spherical eyes, partially protected by bony ocular shields (fig. 1). The fish normally swims just below the water level, often in muddy waters, and thus occupies a very restricted part of the aquatic environment. The lidless, bulging eye is bisected by the water meniscus with the lower half of the eye exposed to the water and the upper half to the air (figs. 2,3,4). It dips its head two to four times every minute. This keeps the upper cornea from drying.

A horizontal corneal pigment band at the water level divides the thicker upper cornea from the thinner lower counterpart. The thicker upper cornea has a greater curvature than is usual among fish where, in general, the cornea is not important in refraction. Two movable horizontal iris flaps also divide the eye at the water level (fig. 3). In the new-born and in the dark-adapted state there is one hour-glass-shaped pupil with a larger upper aperture. In the light-adapted state there are two separated pupillary apertures and the anterior horizontal iris flap then overlaps the posterior one (fig. 2). The

dorsal iris has a wide range of movement while the ventral aperture changes little. (Schneider, 1803; Klinckowström 1895; Schneider-V. Orelli, '07; Arruga, 41; Walls, '42; Schwassmann & Kruger, '65).

The burrowing serpent eel of New Zealand has an hourglass-shaped pupil and an egg-shaped lens (Prince, '56). The only other fish known to have a divided eye is the small marine blenny, *Dialommus fuscus*, which positions itself vertically in the water with part of its head and half of its eye in the air. The iris is single, and there are two clear areas in an otherwise pigmented cornea. (Breder and Gresser, '39). Seals see either in air or in water. In air, the pupil closes to a pinhole to provide universal focus, like a pinhole camera (Walls, '42).

Anableps is almost unique in its ability to see simultaneously in two different refractive media, air and water. The dorsal part of the single retina receives images from the water, and the larger ventral part of the retina from the aerial field. The very large lens is eggshaped, unlike the spherical lenses of most teleosts. The long axis of the lens is directed into the water and the short axis into the air. "The long axis acts like the spherical lens of other fish, and the short axis gives a reasonable imitation of the ovoid lens of terrestrial animals" (Prince, '56). There is a sharper curvature and greater diameter of the lens in the water visual axis, and a flatter curvature and shorter diameter in the air visual axis.

These different curvatures of the lens surface in the two different visual axes provide the two different refractive powers, assisted by the different curvatures and thicknesses of the cornea (fig. 4). The horizontal iris flaps appear to prevent double-image formation due to refraction of light at the water surface and they reduce the glare reflected at the water surface which would otherwise interfere with visual acuity (Schwassmann & Kruger, '65).

The lens capsule is thickened at the attachment of the dorsal suspensory ligament and there is a conspicuous small bulge on the ventral portion of the lens, in the region of the underwater aperture (Schneider, 1803; Klinckowström, 1895; Garman, 1895a; Schneider-v. Orelli '07). Contraction of the retractor lentis muscle, unique to teleosts (Walls, '42) pulls the lens towards the lower pupillary aperture, i.e. backward and temporal. (Arruga, '41; Walls, '42; Rochon-Duvigneaud, '43; Duke-Elder, '58).

The ciliary zone in fish is narrow, without folds or processes, and the choroid appears to pass directly into the iris. *Anableps* is an exception, along with a few other amphibious fish, in having a few ciliary folds. (Duke-Elder, '58; Walls, '42). In teleosts there is an elaborate annular ligament, continuous with the corneal endothelium, between the cornea and the outer rim of the iris and it is peculiar to teleosts (Walls, '42; Duke-Elder, '58; Lagler, Bardach and Miller, '62).

but this has not been specifically described for *Anableps*.

In *Anableps*, a large crescent-shaped choroidal gland lies alongside the ventral retina from the head of the optic nerve to the ciliary region. The optic nerve enters the globe through the lower part of the posterior pole and follows an intraocular path upwards before forming the papilla (Arruga, '41).

Schwassmann and Kruger ('65) in an electrophysiological study, found that, unlike other freshwater fish, *Anableps* has an area specialised for visual acuity, a horizontal band above the water level in the aerial field which has a non-linear, greatly magnified projection on the optic tectum. This area is correlated with a ventral retinal region (with a greater ganglion cell density) of enhanced visual acuity above the water line. In other teleosts, as in other nonmammalian vertebrates, the majority of optic nerve fibres terminate in the optic tectum with a uniformly linear representation.

Optic nerve fibres cross completely at the optic chiasma (Schwassmann, '68). There are three pairs of ocular motor muscles for each eye (Lagler, *et al*, '62).

"The eye of the Cuatro ojos is one of the most remarkable of vertebrate eyes" (Walls, '42). It was first

described by Artedi (1758). Lacépède (1797) noted that the popular name of "four-eyes" was incorrect as he recognised the eye as one organ, but mistakenly wrote of a double iris and a double cornea. He recognised that the dorsal and ventral parts of the cornea differ in curvature and thickness. Schneider (1803) provided a detailed and remarkably accurate account of the *Anableps* eye, but without drawings and without reference to the retina. Klinckowström (1895), also without reference to the retina, illustrated and described the eye of the newborn. Wyman (1854) reported that *Anableps* is born with the horizontal iris flaps; so did Schneider-v. Orelli ('07), whose extensive (but little-illustrated paper) is much-quoted. In this latter paper the retina is described in only one paragraph which notes that the histology is like that of other fish with clearly demarcated layers and with upper and lower retinas alike. Arruga ('41) described and fully illustrated the general ocular anatomy but the only reference to the retina points out its general resemblance to that of higher animals; that there are few cones in proportion to the rods, and many, closely-packed ganglion cells. Schwassman and Kruger ('65) described, by light microscopy, some histological features of the retina in the course of a study which mapped the projection of the aerial and aquatic fields on the optic tectum of *Anableps microlepis*. They found that there are twice as many smaller cones in the ventral retina

as in the dorsal; that the ventral internal nuclear layer is thicker with many more bipolars; and that there are more ganglion cells in the ventral retina.

Many early travellers, naturalists, and ichthyologists commented on *Anableps* and its strange eyes. (Soemmerring, 1818; Meckel , 1818; Clarke and Mortimer, 1839; Jenyns, 1842; Cuvier and Valenciennes, 1846; Smith, 1850; Wyman, 1854; Dow, 1861; Gill, 1861; Agassiz, 1868; Jordan, 1885, '05,'63; Evermann & Goldsborough, '02; Klingelhoffer, '10; Noble, '35; Coates, '36; Walls, '39; Norman, '47; Grassé, '58; Rosen and Bailey, '63). There are some surprising omissions (Polyak, '57) and a few strange ambiguities ('there are two retinas in each eye') (Young, '62). A description of the eye can be found in Rochon-Duvigneaud ('43).

Ultrastructural Studies of Teleost Eyes

Teleost fish represent a vast and varied group occupying a very wide range of habitats from muddy flats to deep ocean waters. Yet the ultrastructure of their retinas has been scantily investigated.Of the few studies done, most have been concerned entirely or principally with synaptic relationships and neuronal wiring; and the relationships of the special horizontal cells, and the question of whether these are neuronal, or neuroglial components (Villegas, '60, '61; Villegas and

Villegas, '63; Stell, '64, '65a, '65c, '67; Yamada and Ishikawa, '65 ; Borovyagin, '66; Goodland, '66; Witkowsky and Dowling, '69). Kuwabara ('65) included goldfish retina in a study of microtubules, especially abundant in the inner segment of the photoreceptor, but without description of the goldfish photoreceptor structure in general.

The literature on photoreceptor ultrastructure is very extensive, but work on teleost photoreceptors is limited to eight investigators. Sjostrand ('53a, '53c, '59) used the perch for his now classic studies on photoreceptor structure, especially the membranes of the outer segments, and also their synaptic terminals. Villegas ('60, '61) briefly described the photoreceptors of *Centropomida,* the main interest centering on synaptic and neuroglial relationships. Borovyagin ('66) described some aspects of photoreceptor structure in pike, perch and orfe, and Stell ('65b), in a brief unillustrated abstract, described the goldfish rods and cones. Engstrom ('63a) studied extensively the ultrastructure of the visual cells of the Labridae and reported the accessory outer segment, the only feature of which he examined by electron microscopy in the Cyprinids (Engstrom, '60). Berger ('63, '64, '65, '66, '67) examined the visual cells of guppies in detail and described the mitochondrial origin of their oil-droplets, and the subsurface cisterns of the apposed membranes of their double cones. Blaxter and

Jones ('67) include some ultrastructural details of visual cells in their study of the larval herring. Locket ('69, '70a,'70b,'70d,'71a,'71b) investigated the retinas of deep sea fish, and mitosis in the African lungfish retina ('70c). Ali ('71),in an elaborate study of photomechanical movements of visual cells, shows some electron photomicrographs of dark and light adapted myoids of goldfish rods and cones. Pedler ('65) in the methods section of his paper says he investigated three teleosts, saithe, cod and dragonet but neither in text nor in illustration is there any specific reference to their photoreceptors.

There is at one and the same time a fundamental constancy in basic ultrastructure of photoreceptors and a remarkable diversity among them, often in relation to particular habits and habitats. Cohen ('63a) warned that certain organisms, by virtue of size or availability or other convenience factors, tend to be more frequently investigated and it may well be that the sampling of the chordate kingdom has been restricted to too small a number of species. This may interfere with efforts to generalize about photoreceptor structures and to understand their evolutionary and functional significance. Until very recently it was held that teleost cones do not have oil droplets (Walls, '42; Wolken, '63; Meyer *et al.*,'65). Now they have been demonstrated in *Lebistes* (Berger, '65,'66) and this study reports oil droplets in *Anableps*.

Scope of This Study

No ultrastructural studies on *Anableps* eye have been reported. The aim of this investigation was to study, by light microscopy, scanning electron microscopy, and mainly by transmission electron microscopy, the morphology of the photoreceptors of a uniquely specialized vertebrate eye; and also to ascertain whether the receptors of the dorsal and ventral portions of the retina show any structural differences associated with the permanent bifocal vision in air and water, i.e. with true amphibious vision. Several unusual features are reported and this study adds to the body of knowledge of photoreceptor structure in general and teleost visual cells in particular.

Chapter 2

MATERIALS AND METHODS

Sources of Materials, and Care of the Fish

Specimens of *Anableps anableps L.* were obtained from dealers in California, Toronto and London, Ontario. It is difficult to get fish in a healthy condition. Those that were vigorous on arrival generally proved relatively easy to keep, despite their reputation for poor survival in captivity. The fish were kept in 15 gallon covered tanks with added Instant Ocean (pH 7-8; temperature 23-25°C). (Instant ocean is supplied by Aquarium Systems, Inc. 1450 E. 289th St., Wickliffe, Ohio.) They were fed on live food (meal worms, *Daphnia*, brine shrimp, baby guppies, wingless *Drosophila*, mosquito larvae and freshly drowned mosquitos), supplemented with small amounts of tetramin and bran. The most frequent loss was associated with a nematode infestation of the liver; and secondarily to Ichthyophthiriasis.

Dark Adaptation

For dark adaptation the fish were transferred to a small tank, covered with aluminum foil and kept in a dark room for about 3 hours. All procedures were then

carried out under red light. The fish were decapitated, the eyes quickly dissected free of the orbit, punctured anteriorly, and plunged into fixative. Then, in a drop of fixative each eye was cut into dorsal and ventral halves using the corneal horizontal pigment band as a guide. The lens and vitreous were removed. The dissected retinas were left in the dark in fixative for a further half-hour before being exposed to the light. When whole eyes were required, the anterior parts were dissected away in the fixative and the lens and vitreous removed.

Light Microscopy

(a) The eyes were fixed in Bouin's fixative (6-10 hrs.); processed through 100% dioxane (three changes of 2 hrs. each and a further change overnight); and into parawax mixture (1 parawax:1 Tissuemat). Sections were cut at 5-8μ, stained with Gomori's Trichome, Hartwig's method ('67) or Hematoxylin-Eosin. Some sections were bleached with H_2O_2-NH_4OH (Curd, '68) or permanganate and H_2SO_4 (Chesterman and Leach, '58). (b) Epon-or vestopal-embedded retinas, fixed in glutaraldehyde and post-fixed in osmium tetroxide (as for transmission electron microscopy) were sectioned at 0.5μ on a Reichert OmU2 ultramicrotome, mounted on glass slides and stained with 0.5% cresyl fast violet or 10% methylene blue at 60°C for 5 minutes. (c) Retinas were fixed in 10% aq. acrolein (0°, 12-24 hrs.) (Feder and O'Brien, '68), processed through alcohols at 0°C and embedded as in (a).

Micrographs were taken with a Wild M20 photomicroscope on 35 mm Panatomic X Kodak Film, and developed for 9 minutes in Kodak Microdol-x at 20°C.

Scanning Electron Microscopy

Dark and light adapted eyes were separated into ventral and dorsal portions and fixed in cold 3-4% glutaraldehyde in 0.3M Sorenson's phosphate buffer at pH 7.4, for 2-3 hours.

The retinas were cut up with a razor blade and the pigment epithelium was separated as far as possible from the retina by flushing with a gentle stream of fixative from a flattened syringe. The separation of pigment epithelium and retina was particularly difficult in the light-adapted retina. After fixation, the tissue was washed with 0.3M Sorenson's phosphate-buffered 5% sucrose and dehydrated in a graded ethyl alcohol series to absolute ethanol, then transferred to propylene oxide (2 changes of 15 minutes each) and slowly air-dried. The tissue samples were then oriented and glued onto specimen stubs with Du Pont Duco cement; rotary coated *in vacuo* with a 40:60 mixture of gold-palladium and examined in a Cambridge Stereoscan Mark II scanning electron microscope.

Transmission Electron Microscopy

All retinas were light adapted. Half eyes were plunged in cold fixative, dissected in fixative, and the retina was cut with a sharp razor blade to separate either peripheral from central retina or dorsal from ventral retina. Later, in 70% alcohol, the retina was further cut up.

Satisfactory fixation of fish retinas by currently acceptable criteria is difficult and a variety of fixation methods were used. Those methods relevant to the illustrations in this thesis are described below:-

(1) Cold 4% glutaraldehyde, with Sorensen's phosphate buffer (0.2 M, pH 7.4) for 5 hours; Sorensen's buffered 5% sucrose wash overnight; postfixed in cold 2% OsO_4 in 5% Sorensen's buffered sucrose for 2 hours; through graded ethanols to acetone and then styrene and embedded in Vestopal W.

(2) Cold 2.5% glutaraldehyde, buffered with sodium cacodylate, (0.1 M, pH 7.33) for 1 hour; 5% cacodylate buffered sucrose wash, 1/2 hour; post-fixed in cold 2% OsO_4 in the above buffer for 1 hour, through graded ethanols to 1 absolute alcohol: 1 acetone; stained in block with uranyl nitrate and lead acetate for 2 hours (Kushida and Fujita, '66); taken through acetone and embedded in Epon 812.

(3) Cold 3% glutaraldehyde with 0.1 M sodium cacodylate

buffer and 0.0015 M $CaCl_2$, pH 7.35, for 3 hours; washed in 6.9% sucrose with the above buffer, overnight; post-fixed in cold 0.1 M phosphate buffered 1% OsO_4 for 2 hours; through graded ethanols to acetone, then styrene and embedded in Vestopal W. This was generally the most satisfactory method used.

(4) Cold 5% glutaraldehyde in 1/4 strength Instant Ocean, with Sorensen's phosphate buffer (0.2 M, pH 7.5) for 1 1/4 hrs.; 5% sucrose, with Sorensen's buffer, overnight; postfixed in cold 2% OsO_4 in buffered sucrose, 1 hr.; through graded ethanols to acetone and embedded in Epon 812.

(5) 2% OsO_4 in 1% sucrose with Zetterquist buffer for 2 hours and taken through graded ethanols and acetone and styrene to Vestopal W.

Silver-grey (500 - 600 Å) sections were cut on a Porter-Blum MT-1 or a Reichert Om U_2 ultramicrotome, placed on formvar-coated copper grids and double stained with uranyl acetate (sat. aq.) for 45 mins., and lead citrate (Reynolds, '63) for 45 secs. Sections were examined in a Phillips 100c electron microscope operated at 60 kv.

For photography, Kodak Fine Grain Positive 35 mm film was used, and it was developed in Kodak D-19 for 6 mins. at 20°C.

The Mosaic Diagrams

The mosaic diagrams were constructed from tracings from light and electron photomicrographs of transverse sections, approximately 0.5 μ and 600 Å, respectively.

Chapter 3

OBSERVATIONS

Visual Behaviour

In our tanks the fish normally feed at the surface, on floating food, but they dive down after actively wriggling mosquito larvae. Staying down for long seems to be an effort and they surface periodically when feeding on below-surface food. They leap out of the water to catch, with great accuracy, meal worms, dangled loosely, from forceps, up to about an inch above the water surface. Peripheral vision is poor and they fail to grasp stationary food when it is not in the direct line of vision.

There is a marked response to movement across considerable distances. All the fish swim up to the front of the tank as soon as the laboratory is entered and feeding preparations are made, and they line up like a herd of cows to watch the feeding of the neighbouring tank. They have remarkable sensitivity to any movement above, and in response to this, they scatter vigorously and wildly by darting and leaping out of the water with much splashing, and they appear to be very alarmed. They then swim down

to near the tank bottom and stay under water in an agitated state. Movement at the sides of the tank does not disturb them although, when 'domesticated', they respond by swimming towards it.

Light Microscopy

The large choroidal gland occupies a considerable area of the ventral half of the eye, and the ventral retina is in close association with it (fig. 5). There is a very well developed pigment epithelium (figs. 5,7,11). The sclera is cartilagenous (fig. 5). There is no fovea

Each member of the double cone is essentially very similar in appearance to the single cone (figs. 6,10). The cone nuclei are more scleral in position than the rod nuclei and frequently project beyond the external limiting membrane (figs. 6,9,10).

The elliptical oil droplets stain very darkly, and so do the membranes between the inner segments of the apposed members of the double cone (figs. 6,10). The outer segments of the double cone stain differently, the one staining more darkly (fig. 6).

The vitreal ends of the rod outer segments can be seen at the level of cone outer segments (fig. 6), but the former are much longer and extend well beyond the cones and into the thick pigment epithelial layer (fig.9). Rod myoids are

very long and slender and are more easily seen in electron microscopic than in light microscopic preparations. The inner pigment epithelial cell processes, laden with pigment granules, extend alongside the cone ellipsoids (figs. 6,9,10).

The ventral and dorsal retinas differ (figs. 7,8,11, 12). The ventral retina (figs. 7,12), which receives the light from the aerial visual field, is about twice as thick as the dorsal retina (figs. 8, 11). It has far more cells in the ganglion cell layer very closely packed together. The ventral inner plexiform layer is much wider and seems to be divided into two sublayers (figs. 7,12), and the ventral inner nuclear layer has 10 to 12 strata of nuclei (figs. 7,12) compared to only 3 to 5 in the dorsal retina (figs. 8,11). There are also more nuclei in the ventral outer nuclear layer, indicating that there are more photoreceptor cells.

Rarely, mitosis is observed in a cone nucleus (fig. 10). There are hyaloid blood vessels on the vitreal surface of the retina, on both the dorsal (fig. 11) and ventral (fig. 12) portions.

Scanning Electron Microscopy of Light- and Dark-adapted Retinas

The surface topography of the receptors is shown in figures 13-22. Dark adapted photoreceptors are shown in figures 8 and 10.

The cones, both single and double, are much wider than the rods in the light-adapted state (figs. 5,9,10). They appear rather squat with stumpy outer segments, especially so in the light-adapted state, in comparison to the rods. Their oil droplets produce very pronounced bulges at the distal ends of the inner segment (figs. 13,17-22). The junction of inner segment and outer segment is clearly demarcated (figs. 17,19-22). The entire inner segment bears longitudinal striations of ridges and grooves in both the light-adapted and dark-adapted states and in both single and double cones (figs. 17,18,21,22).

The calycal processes arise at the inner-outer segment junction in both rods and cones and they appear to be continuous with the longitudinal surface striations of the inner segment (figs. 17-22). The calycal processes form a palisade surrounding the cone outer segment which is short, blunt and slightly conical (figs. 17-22). These calycal processes are more massive than those of the rod, and extend over most of the length of the cone outer segment, some of them even to the apex (fig. 20). In the dark-adapted state,the inner-outer segment junction is not so marked and the entire cone is more elongated (fig. 18) than in the light-adapted state. Figures 13,21 & 22 show entire double cones from synaptic pedicle at the base of the micrograph to the outer segments above the bulging oil droplets; and also the surface folds and the longitudinal "fins" can be seen at the vitreal

ends of the inner segments.

The rods in the light-adapted state are very elongated with very attenuated myoids. In the dark-adapted state the rods are much shorter. There is a widening in the rod inner segment at about the level of the cone oil droplet and outer segment (figs. 13,14,19,21,22), and the entire rod is a thin and very elongated cell (figs. 14-16). The rod outer segments are so long that their most scleral portions break off readily (fig. 14,16). The rod inner segment is also ridged and grooved longitudinally but in a less pronounced way than the cone (figs. 17-19,21,22). The widest part of the rod is the outer segment which is uniformly cylindrical and ends abruptly (fig. 17,18).

The abundant pigment epithelium occupies a large area (fig. 14) and is densely packed with pigment granules. The pigment granules are cylindrical with convex ends and some of these can be seen lying free in figures 21 & 22. It is very difficult to separate the pigment epithelium from the photoreceptors in the light-adapted state in tissue preparation. It is essential to do so to get an unobstructed view of the long rod outer segments which are wholly enveloped by the pigment epithelial cell processes. Since the processes extend towards and almost reach to the external limiting membrane,the cone outer segments are also enclosed, but less intimately so.

The entire retina is thicker when light-adapted as the rods are much longer (fig. 15) than in the dark-adapted retina (fig. 16).

Conventional transmission electron microscopy

Light-adapted photo-receptors

The following descriptions of the rods and cones apply to those in both dorsal and ventral parts of the retina since they are of the same appearance throughout the retina.

Rods

The rod outer segment has one incisure, opposite the cilium, which penetrates about one-third of the rod diameter and often ends in a small bifurcation (figs. 23, 25-29). These incisures are aligned to form a groove (fig. 23,28). The rod outer segment is wider than that of the cone and they can readily be distinguished from each other in cross sections (figs. 26,28,29). The distal part of the rod inner segment and the outer segment have much the same diameters (figs. 23,25,27-29). The rod discs appear to be uniformly electron dense except at their edges and they measure 210 - 280 Å in total thickness (figs. 23,25,38). The eccentrically placed connecting cilium (figs. 27-29) is long. It lacks the central pair of microtubules (fig. 29), is surrounded by extracellular space and is deeply inserted in the inner segment (figs. 23,25). The niche in the inner segment within

which the cilium is inserted often has six or more lobules (figs. 28,29) and the cilium outline undulates in a similar but less marked way. There is a distinct basal body, but ciliary rootlets and a second centriole were not observed. The microtubules of the rod cilium do not splay out very much (figs. 23,25,29a).

The ellipsoid is packed with mitochondria (figs. 29,31,45,54), which have distinct cristae and intramitochondrial granules (figs. 25,29). These mitochondria (6-9) are arranged around the cilium like the wedge-shaped slices of a pie (figs. 27-29). The rod mitochondria always look the same, in all the material, and they are not altered in appearance by the various fixation methods used in this study. They do not show the transformations seen in the cone inner segments.

The long rod myoid is much narrower (figs. 24,47, 53,54,59,62) than the ellipsoid (figs. 28,31). It tapers vitreally and it contains many cisternae. Longitudinally oriented microtubules are especially abundant in the narrowest portions (figs. 31,61,65). Apart from the outer segments, in all other regions the rod has a denser cytoplasm than the cone (figs. 61,62,66,74,75,97,102,103,104).

Up to fourteen fine calycal processes surround the rod outer segment (fig. 27). They extend only a short part of the outer segment length leaving most of the scleral part without calycal processes (figs. 26,28-30). The

pigment epithelial processes are in apposition to the calycal processes, and in the more scleral parts there is direct contact between the outer segment and the pigment epithelial processes (figs. 26,28,29,30).

There are many pigment epithelial cell processes, laden with pigment granules and sometimes containing lamellated bodies (figs. 26,28,30). These processes do not separate all the rod outer segments from each other (fig. 30); or even all the rod and cone outer segments (figs. 26,28); or inner segments from outer (figs. 28,29). Up to eighteen rods were seen in contact with each other, without intervening pigment epithelium cell processes. However, each rod outer segment abuts on a pigment epithelial cell process on some part of its circumference (fig. 30).

The rod incisures are not oriented in an orderly way, nor are the rods grouped in specific patterns (figs. 26,28,30). The oval rod nucleus lies vitreally in the outer nuclear layer and it is smaller and darker staining than that of the cone (figs. 32,33). The rod spherule is described with the cone terminal receptor, below.

Cones

(a) Double Cones

The outer segments

The terms "twin cone" and "double cone" have been used synonymously and without clear definition. The

apposed cones pairs seen in *Anableps* are referred to as double "equal" cones. However, they are not quite "equal" cones, since they show structural differences in their outer segments. The two outer segments differ from each other, markedly and consistently, and the difference is apparent in almost all photomicrographs, both in longitudinal sections (figs. 36,37,40) and in cross sections (figs. 26,28,41,43,45). The difference in the cross-sectional appearance of the two outer segments of the double-cone pair is so consistent that it is very unlikely to be due to angle of sectioning or any artifact.

The single cone discs are uniformly dense and measure 115 - 170 Å (figs. 34,39). The double cone discs are of two kinds: in one of the members of the pair, the discs are like the single cone discs (140 - 170 Å); in the other, the discs have the overall dimensions of the rod discs (210 - 280 Å) but they contain a clear intradisc space (115° thick) not seen in the rod discs. There appear to be irregular globular masses closely juxtaposed to form the dark bounding membranes of these discs, so that at lower magnifications these discs appear simply to have a wide clear intradisc space (figs. 36,37,40). The disc measurements for each photoreceptor type are remarkably uniform, but the interdisc spaces vary considerably.

The outer segments of the double cone members are never separated by pigment epithelial cell processes,

or by their accessory outer segments, or by calycal processes (figs. 36,37,40,41,43,45,46).

The outer segment is surrounded by a palisade of calycal processes, there being twenty in figure 35. Twenty-five was the maximum number counted. The calycal processes do not extend entirely to the scleral ends of the outer segments. They are more massive than those of the rods and extend over most of the cone outer segments almost to the apex (figs. 31,41-43,45,46).

The longitudinally oriented microfilaments of the calycal processes are continuous with those that are seen in groups at the peripheral zone of the ellipsoid (figs. 34,36,51,52) and the myoid (figs. 60,61,63).

The pigment epithelial processes lie immediately adjacent to the accessory outer segments, and where there are calycal processes the pigment epithelial processes do not abut on the rod and cone saccule regions directly, except perhaps at rare intervals (fig. 35). However, their contact with the inner segments is direct (figs. 27, 28,42-44,47,54).

The accessory outer segment

The accessory outer segment of the cone is apparent in all cross sections (figs. 27,28,31,35,41-46,53,54) and is quite frequently seen in longitudinal sections,

(figs. 47-50). The accessory outer segment has a ciliary origin, vitreal to the inner-outer segment junction (figs. 43,44,48-50). It seems devoid of any obvious organelles and shows only a marked (figs. 35,46) or a vague (figs. 41-44,47-50) granularity.

The ciliary microtubules (there is no central pair) quickly splay out in the accessory outer segment and become single, and are found in the region of the plasma membrane (figs. 41,43,53). This may be seen even at the level of the oil droplet, before the start of the outer segment discs (fig. 53). The basal body and cilium are very short compared to those of the rod. The microtubules extend for only a short distance within the accessory outer segment (figs. 49,50). The accessory outer segment may be of the same diameter as the outer segment, or even larger (figs. 31,41,42,49,50,54). However, it tapers more rapidly than the cone itself to become very small at the scleral end (figs. 45,47,48). A second cone cilium was never seen. There is only one cilium per cone, and it is associated with both the accessory outer segment and the discs of the outer segment. Many hundreds of cones were examined and a second centriole was never observed, nor were ciliary rootlets seen. The accessory outer segments are always on the surfaces away from, and often opposite to, the apposed membranes of the double cone, with calycal processes intervening between the outer segment and the accessory outer segment (figs. 31,41,42,45,46,54).

Reconstructions from cross sections show that the plasma membrane of the accessory outer segment is always continuous with that of the outer segment by a series of fine processes (figs. 41,42,45,46,48-50,54). Since these protoplasmic continuities are seen at all levels, in cross sections, it appears that there are web-like cytoplasmic connections between the outer segment and its accessory outer segment. Figure 111 summarises features of the accessory outer segment.

The Inner Segments

The cone members of the double cones are flattened on the apposed contiguous surfaces of their inner segments (figs. 43,53,55-58).

In cross sections it is seen that the cone inner segment does not have a regular smooth outline. It shows irregularities, ridges and undulations of its surface (figs. 42,53,55,58,59,75,90), except on the apposed surfaces of the double cone members (figs. 73, 74).

The Mitochondria

The vitreal part of the inner segment of the cone is divided for descriptive purposes into three intergrading regions. Most vitreally, i.e. supra-nuclearly, in the large myoid, there is an area of cytoplasm in which there may be only a few mitochondria, or, most often, none (figs. 60,61,62,66). This region is characterized by the presence of free ribosomes, and many

irregularly arranged cisternae, large and small, smooth and rough-surfaced. The area appears very "vesicular" or "vacuolated" and the cisternae gradually become more elongated and more longitudinally oriented towards the more scleral parts of the inner segment (fig. 63).

The next region, moving sclerally, contains 'empty' or 'vague' mitochondria with very few cristae (figs. 60,61,62, 65). Some of these show luminal continuities between smooth or rough-surfaced cisternae of the endoplasmic reticulum and the outer mitochondrial membrane, and others show closely associated cisternae (figs. 62,64). Some 'vague' mitochondria appear to have one membrane only which may be due to the angle of sectioning. However, such single-membraned mitochondria were never observed in rods, nor in the ellipsoid area with 'typical' mitochondria. In the 'vague' mitochondria the outer membrane is often at a very irregular distance from the inner membrane (figs. 60,62,65) and again this is not seen in the typical cone mitochondria of the next region. The inner membranes are also vesicular, typical cristae are sparse and the matrix is pale and clear (figs.60-62,65,66). The mitochondria are surrounded by much cytoplasm, containing free ribosomes, and the many large and small cisternae produce a generally vacuolated appearance of the cytoplasm (figs. 60-62,65). The appearance of distended cisternae was, at first, assumed to be due to poor fixation. However, in the same sections, apart from the

most proximal (vitreal) parts of the cone inner segments, fixation appeared satisfactory. The 'vacuolation' is constant and is probably only in part due to fixation artefact.

The Golgi apparatus (there may be several stacks) is found in this area (fig. 64), or a little more sclerally and alongside more typical mitochondria (fig. 63). The Golgi is not immediately supra-nuclear in position.

Progressing sclerally, the next region contains typical mitochondria, very closely packed together and gradually increasing in size (figs. 63,79,83,90) and density of staining (figs. 65-67). The enlarged mitochondria appear to fuse by first becoming enclosed in one outer mitochondrial membrane (figs. 67-73).

There is a distinct vitreal-scleral gradient in the appearance of the mitochondrial transformations (figs. 47,48,67,77,83). These involve enlargement (figs. 65-67, 70,73-75); a darkening of the matrix and the loss of cristae (figs. 44,47,51,58,65,67,73-80); and fusions (figs. 44,55,57,67-75,78). The enlargment of the mitochondria, their fusions and other aspects of their transformation, may proceed independently of each other.

The two members of the double cone inner segments often stain differently, one being darker than the other (figs. 75,79,80,99,101,107,108). This appears to

be a result of differences between the two cones of the pair in the extent of their mitochondrial transformations. This is seen also by light-microscopy (figs. 105,106). The gradient is not uniformly strictly developed and it is not necessarily synchronous in neighbouring cones (figs. 47,74,75,77,78), or even in the two members of the double cone (figs. 79,80). In some cases there is an approximately equivalent development of the mitochondrial changes in nearby cones (figs. 65,66,74,80,83), and within the double-cone pairs (figs. 74,80).

The oil-droplet

There are, then, various patterns of mitochondrial fusions and transformations (figs. 44,47,48,51,67, 74-78,83). No matter what the pattern, there is most often formed, in the most distal (scleral) part of the inner segment, a membrane-bounded, very dark-staining, oval, cylindrical or slightly pear-shaped structure, the oil-droplet (figs. 31,36,37,42,43,47,50,53-56,58-60,74,75, 79,83). In rare cases, the oil-droplet is seen to be nearly round (fig. 50).

Individual transformed mitochondria can be seen in close apposition to the oil droplet and appear to be contributing to it or fusing with it (figs. 36,51,53,56-59, 74,75,77,79-81). No mitochondria are seen on the distal side of the oil-droplet. Very rarely, a mitochondrion is seen towards the scleral end, apparently about to fuse,

in that position, into the oil-droplet (figs. 36,81).

On the proximal (vitreal) side of the oil-droplet, the mitochondria vary in their appearance, but only rarely do they appear to be 'normal' (or 'typical') (figs. 66,79,86). More often, they are altered to some degree; enlarged, and with varying amounts of dense matrix (figs. 47,51,79,80,83-85,97).

Around the oil-droplets, there may be mitochondria which appear to contain substances of the same electron density as the oil-droplet does (figs. 53,54,56-59,75,85).

Not every cone contains a fully formed oil-droplet (figs. 34,37,44,48,49,66,67,76,77). Where oil-droplets are absent, there are enlarged and variously transformed mitochondria. Typical mitochondria are never seen in the oil droplet position. In the position occupied by the electron-dense oil-droplet there may be found one or more very enlarged mitochondria, showing cristae-like remains and containing granular and fibrous material, with little electron density (figs. 34,37,48,49,66,85,86). Figure 34 shows such a single mitochondrion, enlarged to fill almost the entire diameter of the cone, fusing with a similar one below it. Figure 86 shows a double-cone in which the oil-droplet of the one member and the very enlarged mitochondrion of the other member are approximately the same size.

It is difficult to know when exactly to affix the appelation "oil-droplet" to the structure that forms

in the most scleral end of the cone inner segment, as there are intergrading but varied patterns of formation. A moderate degree of electron density in a single, more-or-less oval structure, occupying the entire 'oil-droplet position', has been taken as the definition. Thus, in figures 44,67 & 78, the structures are not considered oil-droplets whereas that in figure 51 is so accepted. Figure 84 shows an anomalous rare situation where there are two large and three small oil-droplets in the one cone.

Many oil-droplets show channel-like markings throughout (figs. 51,53,55,56,80,81). Many have uniformly electron-dense centres surrounded by a 'channelised' periphery. The proportion of these two zones varies (figs. 36,37,57-59,75,80-82). Some enlarged and electron-dense mitochondria show similar 'channelisation', especially at the peripheral areas (fig. 73). In figure 67 the very enlarged mitochondria are 'channelised' throughout.

There seems to be a gradient also in the 'densification' of the oil-droplet with the outermost (i.e. peripheral) region remaining less dense and retaining visible cristae-like channels for longer than the centre,which earlier becomes homogeneously dense and dark and shows no internal structure (figs. 36,37,50,53,57-59, 75,80,85,86). Distal portions of the oil-droplet may remain more 'mitochondrial' in appearance than the

proximal part which is electron-dense (fig. 82).

The foregoing description of the oil-droplets applies to retinas fixed in glutaraldehyde and postfixed in osmium. When retinas were fixed in osmium only, then large areas of the oil-droplets appear clear, and lying within the clear area is an ill-defined fibrillar network (figs. 87,88,89). Some areas of the oil-droplet appear to be homogeneously electron dense, especially the peripheral areas.

Oil-droplets, with electron dense centers, were measured in twenty micrographs, with 1 - 5 oil droplets per micrograph. The range of the length is 1.2μ - 8.4μ, with the great majority (80%) between 1.8μ - 6.1μ; 10% are smaller and 10% are larger than this range. All the oil droplets are 1.4 - 2.9μ wide. The length varies much more than the width, as is expected, since the mitochondria seem to continue to transform and add to the length of the oil droplet (figs. 51,58,79,80). In cases where transformed mitochondria occupy the oil droplet position they are in the size range 0.9 - 4μ long and 0.8 - 2.8μ wide. In figure 34, there is a very enlarged mitochondrion, 3.1μ × 2.3μ, showing distintegrating cristae and granular-fibrillar matrix.

The mean dimensions of the oil droplets are: 4.4μ long ± 3.6μ x 2.03μ wide ± 1.2μ. Statistically

then, 95% of the oil droplets are expected to fall between 0.8 μ - 7.9 μ long x 0.8 μ - 3.2 μ wide and in this sample the range actually is 1.6 μ - 7.2 μ long x 1.4 μ - 2.8 μ wide. (see table 1)

The apposed plasma membranes

The double cone members have apposed membranes with a low undulating interdigitation with each other and these extend along almost the entire lengths of their inner segments, from the inner-outer segment junction, (figs. 85,86) to a point just scleral to the external limiting membrane (figs. 63,91). Along this continuous surface the membrane-complex (figs. 19,20,23-28,35) is resolvable at higher magnifications into 6 membranes (figs. 55,63,91,92,93). At low magnifications this appears, in cross sections, at all levels, as a very dark thick line separating the double cone member inner segments (figs. 53,55-59,62,65,74,75,97). This makes the double cones very easy to identify.

The subsurface cisterns

The apposed plasma membranes are closely paralleled by subsurface cisterns, and this association is responsible for the image of six membranes (described above). There appear to be many cisterns, individual ones of which may be quite long. This was seen in both

cross sections (figs. 90,112) and longitudinal sections (figs. 63,66,79,91). The cisterns often bear ribosomes on their cytoplasmic aspects (figs. 60,61,86) and mitochondria are often closely associated (figs. 60,61,63,65, 74,75,80,90).

Subsurface cisterns are also present where rods abut directly on cones without intervening Müller cells (figs. 62,96,97) and where rod processes invaginate into the cone cell, just scleral to the external limiting membrane (figs. 94,96,97).

The rod invaginations

The rod invaginates into the cone by knob-like projections (figs. 94-97) and one section of a cone may show up to four of these. Over these rod-knob-invaginations there is always a membrane-complex similar to that found between the apposed inner segments of the double cone members, but more studded with ribosomes, and the subsurface cisterns are more distended.

Cytoplasmic fins

At the level immediately scleral to the external limiting membrane, and only there, the cones have fine longitudinal cytoplasmic protrusions, microplicae or 'fins', at right angles to the long axis of the cell. These are clearly seen in cross sections. They form gear-like

interdigitations with those of neighbouring cones, with pale Müller cell processes filling the spaces between them (figs. 98,99,100,101,107,108,112).

Müller Cells

Fine Müller cell processes are abundant, just scleral to the external limiting membrane, but, nonetheless, rods abut on each other and on cones, and cones are in direct contact with other cones vitreal to the external limiting membrane (figs. 62,65,74,96,97). The Müller cells have very fine processes which are arranged in whorls around the terminals (fig. 102) but especially around the cone pedicles (figs. 102-104). The Müller cells contain granules which are probably glycogen (figs. 102,104).

Cone Nuclei

Cone nuclei are elongated ovals, irregular in outline, and sometimes deeply indented. They are paler and larger than the rod nuclei (figs. 32,33). They often protrude through the external limiting membrane (figs. 60, 61,62).

The Receptor Terminals

The cone receptor terminals occur at one level, but the rod spherules may be at the cone levels, or more

scleral. Each cone has a long fibre connecting the nucleus to the pedicle (fig. 33). The rod nucleus, more vitreal in position than the cone nucleus, has a short connecting fibre (fig. 33). There is a distinct difference in appearance between the two receptor terminals (figs. 102,103,104). The rod spherule is of a smaller diameter and it contains a greater density of synaptic vesicles and denser, darker cytoplasm than the cone pedicle. The synaptic vesicles were measured on micrographs which showed both rod and cone synaptic terminals. Four such micrographs were analyzed and the results are set out in table 2.

In each case the absolute value of the difference of the means is less than 1.96 times the appropriate standard deviation, i.e. $|\bar{x}_1 - \bar{x}_2| < 1.965$. This shows that one cannot infer at the 95% confidence level that there is a difference in size between the rod and cone synaptic vesicles (Wonnacott and Wonnacott, '69).

The rod spherule contains a synaptic ribbon (figs. 102,103,104) with an associated arciform density, and the synaptic vesicles from an orderly border parallel to the synaptic ribbon, with intervening hazy fibrous material. There appear to be two large synaptic vesicles at the synaptic ribbon at the arciform density end which are in contact with the presynaptic membrane (fig. 103). Processes of what are probably horizontal cells invaginate on either side of the synaptic ribbon and a bipolar cell dendritic invagination occupies a central position between them. Membrane

thickenings are clear, both at the invaginated zones (fig. 103) and superficially (fig. 104). The cone pedicle is larger and bulbous and it contains many triads (synaptic units around a synaptic ribbon). In the cones, expansions of invaginated processes show thickenings on their postsynaptic membranes which appear, in cross sections, as rings with discontinuous dense masses (fig. 102). No mitochondria are seen in the receptor terminals.

(b) Single Cones

The shape and structure of the single cone is essentially like that of a member of the double cone pair but without the one flattened surface of the double cone member. The single cone shows the same ridges and grooves on the surface. Very infrequently, a very short cone is seen whose outer segment, with its accessory outer segment, appears in cross sections among the more vitreal parts of the inner segments of the great majority of the other cones (figs. 100,101,107). In some low magnification crosssectional micrographs none of these are seen; in others, one, two or three are found among 35-55 cones.

In analyzing cross sectional micrographs for the mosaic arrangement of the cones it became clear that in the cone ellipsoid regions and the vitreal regions of the cone outer segments there are equal numbers of double and single cones. However, in the most scleral regions, single cones become rare, and it is therefore apparent

that the single cones are somewhat shorter than the double cones.

The Mosaic

The mosaic patterns were analyzed by light microscopy from 0.5 μ thick sections and from low magnification electron micrographs made from 600 Å sections. The presence of the double cone membrane complex makes the mosaic pattern readily apparent.

There is one pattern common to the periphery and another to the center, both in the dorsal and ventral retina.

There is a square mosaic pattern in the center, made up of repeating square units of three alternating rows each of three alternating single and double cones. In these successive rows the double cone membrane complexes are also usually at right angles to each other (fig. 105,106,107,110).

The periphery has a linear pattern of alternating rows of single and double cones, and the double cones themselves alternate so that in any one row of double cones the membrane complexes are more or less at right angles to those of the next double cone rows (fig. 108,109). When the plane of the section is right, then the regular alternation of single cones and double cones can also be seen in longitudinal

sections, by both light and electron microscopy.

There are zones in which the pattern, while distinct, is not complete. The orientation of the double cones with regard to the right-angled arrangement of the membrane complexes in successive double-cone rows may be somewhat irregular (fig. 108).

The linear mosaic pattern, in its fully developed form, is illustrated in figure 109; the square pattern in figure 110.

TABLE 1

OIL DROPLETS

MEAN:	4.4μ	- ± 3.6μ		
	2.03μ	- ± 1.2μ		
95% EXPECTED TO BE:	0.8μ	-	7.9μ	LONG
	0.8μ	-	3.2μ	WIDE
95% IN THIS SAMPLE ARE:	1.6μ	-	7.2μ	LONG
	1.4μ	-	2.8μ	WIDE
ENTIRE RANGE OF SIZE:	1.2μ	-	8.4μ	LONG
	1.4μ	-	2.9μ	WIDE
80% ARE IN RANGE:	1.8μ	-	6.1μ	LENGTH
	10% are smaller			
	10% are larger			

TABLE 2

		I	II	III	IV
Cone synaptic vesicles					
	$\bar{X}_1$	460 Å	457 Å	384 Å	407 Å
	S_1	91	91	76	71
	n_1	40	40	42	42
Rod synaptic vesicles					
	$\bar{X}_2$	454 Å	463 Å	402 Å	434 Å
	S_2	86	85	83	81
	n_2	40	40	44	42
$\bar{X}_1 - \bar{X}_2$		6	6	18	27
$S = \sqrt{\frac{S_1^2}{n_1} + \frac{S_2^2}{n_2}}$	-	20	20	17	17

$\bar{X}_1$, $\bar{X}_2$ = mean diameters

S, S_1, S_2 = standard deviations

n_1, n_2 = no. in the sample

CHAPTER 4

DISCUSSION

General Morphology of Chordate Photoreceptors

The morphology of the retinal photoreceptors in all classes of vertebrates has been studied by electron microscopy and the literature is too extensive to be generally reviewed here. Photoreceptor structure in the Vertebrates is summarised in several reviews (Cohen, '63a, '69; Dartnall and Tansley, '63; Moody, '64; Pedler, '65, '69; Dowling, '66,'70; Young, '69a). Of special interest, however, is the fact that some *Urochorda* have photoreceptors which degenerate on metamorphosis. In this sub-phylum of the *Chordata*,only the freeliving larva has gill-slits, a notochord and a dorsal nerve cord in the tail; the adult is attached, has no notochord or dorsal nerve cord and only the gill-slits suggest it is a Chordate. Yet the larval photoreceptor cell has outer segments of a 9 + 0 ciliary origin and membranous lamellae formed from infoldings of the ciliary membrane (Eakin and Kuda, '71; Barnes, '71; Gorman *et al.*, '71).

The eye of these chordate larvae is homologous with the vertebrate median eye, and it has been found that the pineal organs of fish (Oksche and Kirschstein '71

Rudeberg, '69,'71), amphibians (Bunt and Kelly, '71; Eakin, '61; Eakin and Westfall, '61; Kelly and Smith, '64), reptiles (Eakin and Westfall, '59,'60; Steyn, '59,'60; Petit, '68) birds (Oksche, '68,'69; Oksche *et al.*, '69) and albino rat (Wolfe, '65) have receptor cells developed from modified ciliary cells with characteristic outer segments, and synaptic terminals with ribbons. Bunt and Kelly ('71) found in frog pineal photoreceptors that there was a continual renewal of outer segment discs, although the receptors were cone-like in that their discs were in continuity with the plasma membrane. Macrophages acted in ingesting shed discs, as the pigment epithelium does in the lateral eye (Young, '71c; Young & Bok, '69).

The jawless *Agnatha* (lampreys and hagfish) are the only vertebrate parasites known and they have eyes which are degenerate and functionless in varying degrees. Nonetheless, these eyes have photoreceptor cells (Holmberg, '70,'71). The outer segments may have membranes in whorls, as in *Myxine* (Holmberg, '70), or as discs in parallel array, perpendicular to the long axis of the cell *(Polistotrema,* Holmberg, '71), which suggest a functional state. Even inclusions in the pigment epithelium suggest phagocytosis of the outer segment discs (Young, '67,'71a, '71c; Young and Droz, '68; Young & Bok, '69).

The Choroidal Gland

The eyes of almost all bony fish contain a choroidal gland and it is especially well developed in predators. It is absent only in small-eyed fish living in muddy waters, and in night-feeders. In *Anableps*, the gland is very prominent in the entire ventral hemisphere (figs. 4,5). Teleosts do not have retinal blood vessels, eels being the only known exception. *Anableps* has hyaloid blood vessels on the vitreal retinal surface (figs. 11,12). These are quite different from mammalian hyaloids which nourish the fetal lens. Hyaloids are never present in those fish which have a falciform process (embryonically derived extension of the choroid) projecting into the vitreous (Walls, '42).

The choroid gland was first shown by Albers (1806) to be a rete mirabile, and neither a gland nor a muscle, as had been supposed. Its thickness is not affected by vasodilators or vasoconstrictors, or by squeezing between plates (Barnett, '51). In it there is a very regular alternating pattern of arterial and venous streams. The presence of the choroidal gland is associated absolutely with the pseudobranch (a vestigial hyoid gill) from which the ophthalmic artery brings aerated blood to the choroidal gland and from there to the choriocapillaris. Absorption spectra of choroidal gland blood and pseudobranch blood

(Dartnall., reported in Barnett, '51) show absorption bands of haemoglobin only in the latter while the former contains substances resembling cytochrome C. Barnett ('51) concluded that the 'gland' was an organ of biochemical interchange preventing loss of cytochrome C from retinal cells into the general circulation.

Wittenberg *et al.*, ('62) found high oxygen tensions in the eyes of marine teleosts. They suggest the choroidal gland is concerned with oxygen transport and that it creates a large oxygen tension behind the retina, a pressure head for diffusion to supply the vigorous oxygen demands of a relatively avascular retina. Walls ('42) suggested that the 'gland' smoothed out arterial pulsations which would otherwise disturb the retina's accurate focussing in an eye of short focal length. If it does so, it affects only the last traces of pulsations, since these have already been reduced by passage through the capillaries of the gills and pseudobranch (Barnett '51). Arruga('41) suggests the choroidal gland adjusts the oxygen tension in adaptation to different pressures. "One of the most unique physiological features of the teleost eye is its ability to generate superatmospheric oxygen tensions" (Fairbanks *et al.*, '69).

The choroidal gland is the oxygen-concentrating organ of the teleost eye and oxygen tensions are high in teleost ocular fluids. The afferent blood supply to the

the pseudobranch from the first gill arch is maximally saturated with oxygen; and blood from the pseudobranch is the sole source of oxygen for the choroid gland.

Visual Behaviour

Dr. Herald ('61) says that there is no record that *Anableps* can catch insects above the water-line. Jordan ('05) and Coates ('36) never saw the fish jump out of the water after food, and Klinckowstrom (1895) never saw them swim down. Dr. Herald ('67) described pond-kept *Anableps* in California that came out of the water to take food from the human hand In their natural habitat they have been seen sunning on muddy banks (Agassiz, 1868; Beebe and Beebe, '10). During the course of this investigation *Anableps anableps* were readily trained to catch food above the water-line; they swam down regularly after food, or when frightened by overhead motion.

The Thicker Ventral Portion of the Retina

Many teleosts have a thicker ventral than dorsal retina, associated with greater cone density andgreater thickness of all layers (figs. 7,8) but especially the inner layers (Vilter, '50; Baburina, '55; Ali, '59; Engstrom '63a,'63b; Blaxter and Jones, '67). This is connected with greater acuity in the corresponding visual field

(Duke-Elder, '58) and it is in no way peculiar to the bifocal vision of *Anableps*.

Anableps, a photopic, surface fish with acute vision in the air axis shares this feature of greater thickness of the ventral retina with *Ericymba*, a minnow, which is a bottom dwelling fish (Moore, *et al.* '50). In both, the outer plexiform layer of the ventral part of the retina is nearly twice as thick as that of the dorsal retina. The rudd, on the other hand, is a shallow water fish living in dim light and it has a great density of cones and even greater density of rods in the dorsal half of the retina which is specialised for greater sensitivity and not acuity (Muntz and Northmore, '71). Deprivation of light from birth causes a decrease in the width of the inner plexiform layer in rats (Sosula and Glow, '70). The far greater thickness of the ventral part of the inner nuclear and plexiform layers of *Anableps* retina may be associated with its exposure to the brighter light of the air axis, and to its acute vision in the air axis.

The inner plexiform layer of the ventral retina appears to be layered in *Anableps* (figs. 7,12). Locket ('70a) saw this layering in the deepsea fish, *Sternoptyx*, which he says is common in shallow water teleosts. This layering in fish retinas is illustrated, but not commented on by Ali and Hanyu ('63) and Anctil ('69). Schwassmann ('68)reported two layers of the inner plexiform layer in

bass. Dowling ('70) says that the amacrines may extend laterally for some considerable distances and may form a discrete stratum in the inner plexiform layer. The amacrines are known to synapse with the bipolars and the ganglion cells. It may be that the layering demarcates a distinct zone in an area of the retina where these cell processes are more abundant and closely packed.

Schwassman and Kruger ('65) reported, without illustrations, that in *Anableps microlepis* the dorsal and ventral portions of the retina are of identical thickness but that the ventral retina has a thicker internal nuclear layer with more bipolars, and far more ganglion cells, more closely packed. If the internal nuclear layer is much thicker and the retinas are of equal width then another layer(s) must be thinner for both to achieve equal thickness. I find that the ventral retina of *Anableps* is always thicker (and the choroidal gland serves as an excellent marker) and the greater thickness of its inner nuclear and inner plexiform layers is very pronounced, as is also the density and number of ganglion cells (figs. 7,8,11,12). The density of the ganglion cells in the *Anableps* retina was commented on by Arruga ('41) but not specifically for the ventral region.

Schwassmann and Kruger ('65) in an electrophysiological study found in *A. microlepis* that the size threshold of moving stimuli is twice as high in the

aquatic visual field as in the aerial, and it is usually assumed that cone density determines retinal resolution and limits visual acuity. They mapped the projection of the aerial and aquatic fields on the optic tectum. They found that, unlike other freshwater fish, *Anableps microlepis* has an area specialized for acute vision, a horizontal band in the aerial field, just above the water level, which has a greatly magnified projection on the tectum. They found no difference of brightness thresholds in the two fields.

This specialized retino-tectal area is correlated with that part of the retina that has a flatter density of photoreceptor cells, bipolars, and ganglion cells and that part of the eye with a larger pupil, greater curvature and thickness of cornea, and greater curvature of the lens (figs. 3,4). These specializations account for the amphibious vision of *Anableps*, and its marked sensitivity to movement overhead.

Hans Hvass ('65) found no special reason for the peculiar eye development of *Anableps* as it can see to catch food at the surface. Breder and Gresser ('39) doubt if *Anableps* has "better" vision than other surface fish, (e.g. Poecilids) but find that the fish are alert to conditions above the surface. From observations of the author and others quoted here, it is abundantly clear

that *Anableps* has its most acute vision just above the water line; that it can see well out of water; that its marked sensitivity to overhead motion is probably a significant adaptation to its environment, perhaps a protection against birds. It probably has little need of acute vision below water as it swims in muddy waters in its natural habitat.

Photomechanical Movements

Arey ('15,'16) described, in some detail, the photomechanical movements in teleost fish. The cone myoids elongate when dark-adapted, and shorten in the light, while the rod myoids lengthen in the light and shorten in the dark. Equal double cones generally move together in retinomotor migrations. The evidence for the widespread occurrence of photomechanical movements is well documented (Walls, '42; Müller, '54; Brett and Ali, '58; Duke-Elder, '58; Ali, '59,'61,'64a,'64b,'71; Engstrom, '61,'63a,'63b; Engstrom and Ahlbert, '63; Engstrom and Rosstorp, '63; O'Connell, '63; John, *et al.* '67).

It has long been known that it is easier to separate the retina from the eye if it is dark adapted (Kuhne, 1879) and this was found to be the case when it was necessary to separate the pigment epithelium from the visual cells of *Anableps* for scanning electron

microscopy. Teleosts generally do not have pupillary control of light entrance to the eye (*Anableps* is an exception), and the photomechanical changes provide protective shields for the rods in conditions where the cones are exposed to bright light (O'Connell, '63). No detailed investigation of retinomotor movements was made in *Anableps* beyond establishing the existence of the phenomenon.

The Double Cones

Multiple cones, mainly unequal double cones, are reported in all vertebrate groups below that of placental mammals. Paired equal cones are a teleost monopoly, and the two components are approximately the same size and shape (Walls, '42; Müller, '51; Lyall, '57a,'57b;Duke-Elder, '58; Engstrom, '60,'61,'63a,'63b; O'Connell, '63).

The widely used terms "twin" and "double" cones, synonymous with equal and unequal cones, have been employed interchangeably and vaguely, e.g. Ali ('59) refers to unequal twin cones in *Oncorhynchus*. Lyall ('57a) and Engstrom ('63b) comment on and list the confused usage and they chose to use "equal double cones" and "unequal double cones". Cohen ('69) has suggested the terms "similar" and "dissimilar" double cones which avoids the semantic and logical awkwardness

of terms like "mainly equal" or "slightly unequal" double cones (Engstrom, '63a).

Staining differences in the equal double cones have been reported (Schultze, 1867; Butcher, '38; Walls, '42; Müller, '51,'54; Lyall, '57a; Engstrom, '61; Engstrom and Ahlbert, '63).

In *Anableps* the equal double cones show staining differences in the ellipsoids of some pairs, seen in cross sections especially (figs. 75,99,101,107,108) but this difference is related to the variations in the mitochondrial transformations into oil droplets. Enoch ('63,'66) says staining differences in the ellipsoids are associated with the state of activity of the given photoreceptors.

The *Anableps* double cone outer segments display a different and more significant difference in that the constituent discs differ in the two members (figs. 36-40). This difference between the two outer segments can be seen regularly in cross sections also (figs. 26, 41,45) and by light microscopy (fig. 6). This has not been reported before. The disc differences seen in the two outer segments of the double cone is a constant feature, irrespective of fixation. There is a tendency for the large saccules to ripple (Cohen, '69) so that it is difficult to measure the inter-disc space, but in

Anableps the measurements of the discs themselves are remarkably constant in each of the four kinds of outer segments.

Villegas ('60,'61) found no differences in the discs of the double cones she studied, including those of the fish, *Centropoma*. Sjostrand and Elfvin ('56) found the discs differed in the unequal double cone outer segments of the toad, but disc sizes of the double cones are reported to be the same in Amphibians by De Robertis and Lasansky ('61) and Moody and Robertson ('60). Nilsson ('65) provides a table of the reported disc measurements. There is some agreement that the interdisc space distance is different in rods and cones. Brown *et al.*, ('63) found the spacing of the discs in the double cones of *Necturus* to be the same. Discs are very susceptible to change with hypotonicity and fixation and other experimental procedures (Sjostrand, '53a; Cohen, '61,'64; Nilsson, '65; Sjostrand and Nilsson, '65). Nilsson ('65) discusses the many sources of error in measurements at the ultrastructural level, and Sjostrand ('70) and Sjostrand and Barajas ('68) have shown in mitochondria that the thickness of membranes measured to date is too small, as standard electron microscopic procedures cause extensive denaturation of the unit membrane. With their new methods they found membrane thicknesses to be 200-300 Å.

Individual receptors contain only one pigment (Marks, '65; Marks *et al*, '64). The photopigment protein, made in the inner segment, is in the outer segment discs (Dowling and Gibbons, '61; Sjostrand, '61; Liebman, '62; Droz, '63; Hall *et al.*, '68,'69; Matsubara *et al.*, '68; Bargoot *et al.*, '69; Wald, '69; Bok and Young '72). Marks ('65) showed, by microspectometry, that there are three kinds of cones in goldfish, each with its own visual pigment. Tomita *et al.* ('67) found three kinds of cones in the carp. Rods, on the other hand, are spectrally homogeneous (Liebman, '62). The basis for the functional differences between components of the cone pairs is the presence or absence of specific visual pigments in each cone (Hibbard, '71). There is an unusual diversity of visual pigments in the cones of fish (Dartnall, '62). The structural differences between the outer segment discs of each component of the double cones of *Anableps* may be related to properties associated with different visual pigments. Wolbarscht (discussion, following Pedler, '65) comments that double cones may contain different pigments and this would account for the observed staining differences referred to by Crescitelli in the same discussion. However, he points out that double cones do not necessarily connect to the same ganglion cell and therefore functionally there does not seem to be any advantage in having two different pigments in the double cones rather than in two single cones.

The functions of double cones are not established. They are most frequent in surface fish and are related to diurnality and to bright light vision, but are also present in deep sea fish (Walls, '42; Duke-Elder, '58; Ali and Hanyu, '63; Engstrom, '63b; O'Connell, '63). Cohen ('69) points out that light moves through the double cone members as if they were one receptor; the two cells sample identical parts of the visual field yet the information is handled separately by each member. Their sensitivity was found to be intermediate between that of single cones and of rods (Willmer, '53; Engstrom, '63b; Anctil, '69). Kalberer and Pedler ('63) think the double cones provide a metabolic and packing economy, and, as their outer segments are closer together than those of other cells, there may be an associated greater acuity. However, equal double cones are absent from the foveas of those few fish which have foveas. The compact packing arrangement and the orderly arrangements of photoreceptors in mosaics, especially well developed in fish that feed by vision, may be important in movement perception (Lyall, '57a; Engstrom, '63b). Many invertebrates can distinguish the plane of polarized light (Waterman and Forward, '70). Underwater space-light is polarized as a result of the scattering of sunlight and Lythgoe and Hemmings ('67) suggest that the plane of polarized light is used as a navigational aid by some underwater animals. They point out that divers use

polaroid glasses to see further. Locke (cited in Underwood, '68) has suggested that the double cones serve as polarized light analysers. Waterman & Forward ('70) attribute this ability of fish retinas to perceive polarized light to the mosaic of the double cones.

The Very Short Single Cones

The rare, very short single cone seen in *Anableps* (figs. 100,107) has also been reported in other fish as an infrequent type (Müller, '51; Lyall, '57b; Engstrom, '60, '63a; Engstrom and Rosstorp, '63; Anctil, '69). Moore *et al.* ('50) report a very small cone near the external limiting membrane in *Ericymba* and suggest it has a high threshold and reacts slowly to light, where the large double cones have a lower threshold and react rapidly in photomechanical movements. In *Anableps*, the short single cone is a very rare cell and it is difficult to see then what advantage there would be in a slow reaction to light. However, the double cones of *Anableps* are somewhat longer than the single cones, and here such a real and useful difference may exist. Engstrom ('63b) found that the single cones elongate in a higher light value than do the double cones. Double cones were also found to be longer than single cones by Lyall ('57b) in trout. There are also fish which have shorter and longer single cones in definite positions in the mosaic (Engstrom, '63b).

The Mosaic

A regular arrangement of double cones and single cones is common in teleost retinas (Eigenmann and Shafer, 1900; Walls, '42; Müller, '52; Lyall, '57a,'57b; Duke-Elder, '58; Engstrom, '60,'61,'63a; Engstrom and Ahlbert, '63; O'Connell,'63; Goodland, '66; Anctil, '69; Dathe, '69). These patterns, or mosaics, were first described by Hannover (1840) who saw a regular alternation of single and double cones in fresh unfixed retinas. The definite arrangement of the axes of the double cones was first described by Ryder (1895).

The cone mosaic is formed at the ora serrata in rows directed towards the centre of the eye. The basic pattern most commonly found at the periphery of the retina in teleosts that have a mosaic is one of parallel rows, either of double cones alone, or double cones and single cones in alternating rows. In some retinas this pattern is found throughout the retina. In others, in the centre there is a quadrangular unit pattern, derived from the parallel rows of single cones at the ora (Shafer, 1900; Müller, '52; Lyall, '57a,'57b; Engstrom, '63b). Engstrom ('63b) considers that the pattern alters from parallel rows to square units by a zig-zaging change in positions of the cells.

The basic mosaic pattern most commonly found

in teleosts is that which is found in *Anableps*: parallel alternating rows of single cones and double cones peripherally, and centrally there is a quadrangular unit of 9 visual cells. This occurs also in *Oncorhynchus* (Ali, '59), *Salmo salar* and *S. trutta* (Ryder, 1895; Eigenmann and Shafer, 1900; Engstrom, '63b; Lyall, '57b), *Lebistes* and *Molliensia* (Müller, '52). However, in *Salmo salar*, Lyall ('57a and '57b) and Engstrom('63b) found that there is only one single cone in the adult unit, where there are 5 in the young.

Mosaics can best be seen in cross sections at the inner segment level where the visual cells are intimately bound together by the Müller cells and their processes. At the outer segment level the short cones may no longer be apparent and the pattern may appear less regular. In *Anableps* the densely staining complex of apposed plasma membranes and subsurface cisterns greatly facilitates recognition of the double cones at their inner segment levels, in sections (figs. 74, 101, 107, 108).

Mosaic patterns may vary in different regions of the retina, and also according to the age of the retina. The pattern is constant for a given species and is related to phylogeny (Eigenmann and Shafer, 1900; Engstrom, '63b) but the degree of "accomplishment" or perfection of the mosaic is influenced by environment and habit. The patterns are most regular in fish with large eyes, in the

temporal (posterior) area of the retina or in those areas of the eye adapted for more acute vision, and in fish which rely on vision for food capture, especially in those which feed on fast-moving prey (Lyall, '57b; Engstrom, '63b). The mosaic pattern disintegrates in bottom-dwellers and in fish with nocturnal habits. There may be no mosaic at all in fish which do not rely on vision for food capture (e.g., catfish, sole, *Catastomus* (a sucker) and *Liparis liparis*, a sedentary fish (Engstrom, '63b)).

No functional significance has been ascribed with certainty to the mosaic. If the different cone types have different functions then their patterned arrangement is important in providing uniform distribution of these differing cone types. Most teleosts feed by sight (Bateson, 1889) and it has been suggested that the mosaic aids movement perception, which is more important than acuity in catching prey (Lyall, '57a). However, the presence of the most regular patterns temporally has led Engstrom ('63b) to correlate this with need for high visual acuity. The double cones are said to be intermediate in spectral sensitivity between single cones and rods (Willmer, '53; Engstrom, '63b; Anctil, '69) and the cone types have also been associated with different regions of the visible spectrum (MacNichol, '64; Anctil, '69).

Teleost retinas are highly differentiated, with very regular layers. Fish have colour vision. Svaetichin ('53, '56) found three types of spectral response curves associated with three types of cones. The mosaics, he thinks, achieve a regular distribution of receptors with different colour sensitivities. He believes that the double cone is responsible for colour vision and the single cone for general light sensitivity. In the square pattern the two opposite double cones represent the same complementary colour.

Waterman and Forward ('70) have suggested that the ordered arrangement of the twin cones might be implicated in the ability of fish retinas to perceive polarized light, a function that has also been ascribed to the double cone itself.

It is said that the regular visual cell mosaic facilitates integration of information at the visual cell level, and the repeating units are linked in an ordered way to inner layers of the retina which also show ordered arrangement. Thus, physiological units are formed (Engstrom, '63b; Dunn, '66b; Goodland, '66; Hibbard, '71). Each square group or unit of the cone layer has its own horizontal cell situated centrally in the mosaic. The horizontal cells form regular patterns. The inner plexiform layer has a pattern corresponding to that of the cones, at the same distances apart as the cone rows (Engstrom,

'63b). Goodland ('66), in *Cottus bubalis*, found a visual cell square mosaic, a squared lattice of the synaptic pedicles of the outer plexiform layer, a regularity of horizontal cell arrangements and a single-layered regular grid in the inner plexiform layer. There are several layers of grid in the inner plexiform layer of *Callionymus lyra*, a marine fish, and these are made up of axons of cone bipolars and associated fibres from amacrine and ganglion cells (Vrabec, '66, quoted in Goodland, '66; Hibbard, '71). Hibbard ('71) in the freshwater fish, *Astronotus ocellatus*, found fibrous grid patterns in the outer and inner plexiform layers with the same orientation and dimensions as the squares of the mosaic formed by the double cones; and there were horizontal and amacrine cell processes intimately associated with these plexiform grids.

In the mudpuppy, *Necturus*, the amacrine cells appear to be involved in motion detection. Cells with laterally extending processes (amacrines and horizontals) seem to be intimately associated with the grid patterns in the plexiform layers (Werblin and Dowling, '69; Kaneko, '70). Hibbard ('71) suggests that the grids serve to channel cell processes in specific directions and increase convergence of input from first and second order neurons onto the ganglion dendrites. It seems that the grid patterns form the structural basis which underlies at least

some of the analysis of the image and the processing of visual information within the retina itself (Goodland, '66; Hibbard, '71).

There is then, a consistent continuity of the visual cell pattern so that regular ordered arrangements of patterns are found throughout the retina. This suggests fixed relationships between retinal components. A fish that hunts prey for food, such as *Anableps*, needs to know the size, shape and appearance of the prey and its direction and speed of movement. A regular visual cell and rectilinear mosaic could be part of the basis of this information.

Subsurface Cisterns

Subsurface cisterns, closely parallel to the contiguous membranes of the inner segments of the double cone, have been reported in several fish. Engstrom ('63a) in the "more equal cones" and "extremely unequal cones" of Labrid fishes, found that the two components of the double cone are joined together by pressbutton formations in the contacting cell membranes of their inner segments. Underlying these plasma membranes along the entire contact surfaces are subsurface cisterns. Stell ('65b), in goldfish, found them in inner segments, in rod spherules opposite invaginated processes; and also alongside the membranes where cones contact rod myoids

near the external limiting membrane, as they appear in *Anableps* (figs. 61-63,90,91,93-97). He found these subsurface cisterns unchanged after four days dark adaptation. Borovyagin ('66) saw them in pike but not in perch or orfe, and referred to them as 'membrane sacs'. Berger ('67) studied the subsurface cisterns from serial sections of guppy cones. He reconstructed them as rectangular cisterns, co-extensive with and closely parallel to the apposing plasma membranes of the double cone inner segments, lacking in fenestrations and not continuous with the plasma membrane.

In *Anableps* the apposing membranes of the double cone inner segment have a low undulating interdigitation with each other (figs. 63,90) but no "pressbutton" formation. The subsurface cisterns appear as a series of discrete cisterns. These may be part of a tubular flattened anastomosing system (Ericsson, '64) or they may be discrete cisterns (Pappas and Purpura, '61). The latter seems more likely as both cross sectional and longitudinal sections produce similar images, without evidence of tubular structure and anastomosomes.

Rosenbluth ('62) named subsurface cisterns and he defined them as large, flattened, single-membrane-limited vesicles which are very closely apposed to the inner aspect of the plasma membrane but distinct from it. They were first described in hair cells of the organ of Corti

(Engstrom, '58) and are most frequently described in nervous tissue. They have synaptic locations (Spoendlin, '60; Smith and Sjostrand, '61; Charlton and Gray, '66) and they have been seen also in hepatocytes (Trump *et al.*, '62);in muscle cells (Richardson, '62; Kumegawa *et al.*, '68); in various sites in teleosts (Rosenbluth and Palay, '61; Stefanelli and Caravita, '64; Hama, '65,'69); and in the auditory nerve of chincilla (Smith and Rasmussen, '65). They are considered a specialized part of the endoplasmic reticulum and may be in direct continuity with elements of it. Berger ('67) described their appearance in the developing retina of the neonate guppy, in the primitive inner segments, in which two cisterns contact and appose, and the zone of contact lengthens as they grow.

Their inner (i.e. cytoplasmic aspect) membranes may bear ribosomes, and they are not infrequently closely associated with mitochondria, often longitudinally aligned ones (figs. 63,90,91,96) (Herndon, '63; Borovyagin, '66; Siegesmund, '68).

The functions ascribed to the subsurface cisterns are many. The close association of mitochondria, in particular,(Herndon, '63) has suggested a metabolic role (Engstrom, '63a; Takahashi and Wood, '70) such as transfer of metabolites (Siegesmund, '68; Bucek, '69). An

electrophysiological role has been postulated (Takahashi and Wood, '70), such as transmission of neuronal impulses (Rosenbluth, '62) since they are seen so frequently in cells which generate or conduct electrical potential charges. In muscle cells they may play a role in excitation-contraction coupling and their frequent association with rough endoplasmic reticulum suggests a cellular role in protein synthesis (Kumegawa *et al.*, '68). Berger ('67) thinks that the alignment of six membranes in *Lebistes* double cones may act as an insulator, as myelin does, or that the entire area may be a specialized cell-to-cell contact zone. Rosenbluth ('62) points out that the membranes are so close that electrical and chemical forces can operate and he suggests ion-binding or flux as one role. They may co-ordinate excitation and intercellular metabolic activity (Brzin *et al.*, '66).

Hartmann ('66), commenting on the absence of any considerable extracellular space in brain tissue, suggests that subsurface cisterns may form intercellular fluid compartments for transport of water and ions, as the extracellular fluid does. In photoreceptors, subsurface cisterns have been reported in inner segments along the abutting surfaces of the equal double cones of teleosts where there is no extracellular material. Unequal cones do not move together in photomechanical responses and their area of contact is less (myoids only).

It would be enlightening to know if subsurface cisterns occur in conjunction with unequal double-cone pair contiguous surfaces. Structures are often not noticed or reported if the main direction of the study leads to concentration elsewhere. In this study, it is interesting that subsurface cisterns appear where cone abuts on cone, or rod on cone, but not where there is Müller cell material or extracellular space. Contact adhesion and the co-ordination of impulses may thus be particularly important in the double equal cone which move as a unit in photomechanical responses. It is possible, of course, that subsurface cisterns have multiple roles in the photoreceptor, as they may have elsewhere, e.g. in the co-ordination of impulses, in the transference of water and ion metabolites, and in contact adhesion.

The Müller Cell Processes

The Müller cell, or radial fibre, described by Müller (1851,1853,1856) is the glial cell of the retina. It branches and ramifies throughout the retina between the inner and outer limiting membranes, and many microvillous processes project beyond the outer limiting membrane around the bases of the inner segments (figs. 62, 65,67). The Müller cells form the limiting "membranes" of the retina; the outer limiting membrane being formed by the tight junctions between visual cell inner segments and the Müller cells (figs. 60,61,99). The Müller cell

cytoplasm is less dense than that of the visual cells (figs. 99,100). This is also reported by Hollenberg and Bernstein ('66).

There is very little extracellular space in the retina between the limiting membranes and the Müller cells fill all the spaces between the cells of the retina. Pedler ('63) referred to the Müller cells as optical filling material and Sjostrand ('58) considered that they represent extracellular space. Where there are blood vessels within the retina, they run through glial tunnels lined by basement membrane, that is, they are still "outside" of the ectodermally derived tissue (Cohen, '63a). Although the Müller cells fill spaces between the neuronal elements they do not do so completely, and there are many areas of contact, known as interreceptor contacts, between the visual cells (figs. 62,65,96,97).

The retina uses more oxygen than any other tissue and the Müller cells are very rich in glycogen, especially so in avascular retinas. Synthesis of glycogen is a prominent Müller cell activity and the glycogen concentration rises inwardly with the distance from the choroidal blood vessels and is highest in the middle zone of the retina. Anaerobic glycolysis occurs here. The abundant glycogen of this nutritive cell is visualized at the ultrastructure level as particulate matter especially around the synaptic bodies (figs. 102,

104) and nuclei of the photoreceptor cells (Yamada, '60a; Cohen, '61; Kuwabara and Cogan, '61; Eichner and Themann, '62; Lasansky, '65; Young, '69a; Magalhaes and Coimbra '70).

The lamellar nature of the Müller cell thin processes in whorls around the receptor terminals has been noted in fish by Locket ('70a,'71a,b); in gecko by Pedler ('63) and in frog by Evans ('66). They were traced unequivocally to the radial fibres. Thin processes of glial cells around the receptor terminals were also seen in guinea pig (Sjostrand, '58) and in human (Villegas, '61) retinas. Such lamellae also separate bipolar and amacrine cells. The whorls of Müller cell processes are a prominent feature of the outer plexiform layer of *Anableps*, especially around the cone pedicles (figs. 102,104).

The interdigitation of Müller cells with inner segment fins at the vitreal end of the ellipsoid is discussed later. It is assumed that there is a nutrient flow here and a transfer of metabolites. The Müller cells contain most of the retinal glycogen and the enzymes for glycolysis and this region of the inner segment in *Anableps* is distant from both the abundant choroidal vascular supply and the sparser hyaloid vessels. There are no Müller cell processes between the double cones (figs. 62,97).

The Müller cells have an important metabolic interaction with neurons, and the presence of pinocytotic vesicles suggests an active transport role. They have been considered as insulators of the receptors to prevent signal interaction, controllers of the ionic environment, and spacers to position the receptors in a precise geometry (Cohen, '61). An optical function was ascribed them by Locket ('71b), and Dunn ('66a) regarded them as the circulators of metabolites in the retina. Marchesi *et al.* ('64) found nucleoside phosphatase, which is implicated in transport at other sites, at the interfaces of glia and neurons, and ascribed a transport function to the Müller cells. Magalhaes and Coimbra ('70) considered the Müller cells to be specialized for storage and delivery of carbohydrates, and for support. Svaetichin (quoted in Cohen, '61) found that they undergo potential shifts during illumination of fish retinas and he thought they could modify neuronal responses. Svaetichin *et al.* ('61) proposed that, through potential shifts of their membranes, Müller cells modify or regulate the excitability of adjacent neuronal elements. A participation in neuronal function has also been suggested by Galambos ('61) and Villegas and Villegas ('63). No synaptic contacts occur between Müller cells and photoreceptor cells (Dowling, '70).

Rod and Cone Differences

Structural differences between rods and cones have been widely reported ever since Schultze (1866, 1867,

1872,1873) first clearly formulated the Duplicity Theory. He distinguished scotopic rods from photopic cones on the basis of outer segment shape (elongate cylinders in rods and short cones in the cones); nuclear position; and size and shape of the foot piece (receptor terminal). Many exceptions and anomalies have been reported and these are reviewed and discussed by Pedler ('69). However, ultrastructural studies have in general emphasized and reinforced the duplicity theory, as most visual cells studied fall into the two classes remarkably well (Cohen, '63a,'69; Dartnall and Tansley, '63; Pedler, '69; Young, '69a).

In the *Anableps* retina, the rods differ from the cones in (1) outer segment size, disc structure and the presence of an incisure; (2) the inner segment size, shape and surface ridging; (3) the details of the connecting cilium; (4) the calycal processes; (5) the mitochondria of the inner segment; (6) that the cones have an oil-droplet in the distal inner segment; (7) behavior in photomechanical movements; (8) the presence of marked fins on the cones; (9) the appearance and position of the nuclei; (10) the synaptic terminals and the Müller cells around these; (11) the absence of an accessory outer segment; (12) the denser and darker cytoplasm of the rod inner segment and rod synaptic terminal. The photoreceptor cells do not

differ in structure in ventral and dorsal retinas and the binocular vision of *Anableps* can be accounted for by its general ocular anatomy. These points, inter alia, are elaborated in the discussion which follows.

The Outer Segment Discs

The discs were first reported by Schultze (1867) but disputed until the advent of electron microscopy (Sjostrand, '49,'53a; DeRobertis, '56; Porter, '57). The most consistent difference found to date between rods and cones is the difference between their outer segment discs. These form in the same way in both, by repeated infolding of the base of the plasma membrane of the growing outer segment, one disc at a time. Cone discs retain the continuity between disc and plasma membrane, except perhaps at their scleral ends, and hence the cone outer segment disc is open to the extracellular space. Rod discs become pinched off from the plasma membrane to form independent, free-floating discs (SJostrand, '59, '61; Missotten, '60; Moody and Robertson, '60; Yamada, '60b; Cohen, '61,'63a,63b; De Robertis and Lasansky, '61; Brown Gibbons and Wald, '63; Nilsson, '64a,'64c; Hollenberg and Bernstein, '66; Young, '69b).

By autoradiography it has been established that rod discs are continually renewed one at a time, and shed intermittently in groups in a balanced way

at the apex, to be phagocytosed in the pigment epithelium (Young, '71a,'71b). In the cones, labelled protein is incorporated diffusely into the existing discs which are not shed. This indicates that subunits of discs are renewed in cones, whereas whole discs are renewed in rods (Bairati and Orzalesci, '63; Droz, '63; Nilsson, '64b; Young, '67,'68,'69a,'71a,'71b; Hall *et al.*, '68,'69; Matsubara, *et al.*, '68; Young and Droz, '68; Young and Bok, '69,'70; Spitznas and Hogan, '70). Young ('71b) accounts for the cylindrical shape of the rod outer segment by the shedding of their first formed smaller discs. In the cones, the disc formation process ceases early in development and the smaller first-formed discs are retained and thus produce the conical shape.

Membranous lamellae formed from infoldings of the membrane of a cell of ciliary origin have been seen in photoreceptor cells of *Urochordata* larvae (Barnes, '71; Eakin and Kuda, '71; Gorman *et al.*, '71); in *Cyclostomata* (Holmberg, '70,'71); in the pineal organs of fish (Oksche and Kirschstein, '71; Rudeberg, '71); of amphibians (Bunt and Kelly, '71); of reptiles (Eakin and Westfall, '59,'60; Steyn, '60; Petit, '68); birds (Oksche, '68,'69; Oksche *et al.*, '69) and of mammals (Wolfe, '65). Bunt and Kelly ('71) found that frog pineal photoreceptors constantly renewed their outer segment discs, and these were phagocytosed by macrophages.

Anableps rods and cones are very clearly differentiated and in every respect are exemplary models of the Duplicity Theory model as proposed by Schultze (1872). Many differences, in addition, between these two cell types are reported in this study.

The *Anableps* rods are numerous, very elongated cells with cylindrical outer segments and free floating discs (figs. 17,19,24,38), while the cones are shorter stockier cells with conical outer segments (figs. 17,20, 21,22,66) and infolded discs (fig. 40).

The Rod Incisure

The *Anableps* rod outer segment discs show a single incisure (figs. 23,25,26,29). Incisures of the outer segment discs have been found only in rods (Cohen, '69) where they are aligned, for considerable distances (figs. 23,25).

A single incisure was reported by Cohen ('63a) as unique to rodents (a sampling error!). Locket reports a single incisure in Platytroctes ('71a) and in Poromitra ('69) but none in Sternoptyx ('70a) and Scopelarchid fish ('71b), all deep-sea fish. Catfish rods have many deep incisures (Cohen, '63a) and Labrids have two or three deep incisions (Engstrom, '63a).

Sjostrand ('53a) was the first to report the

incisure, seen as a single incision reaching towards the centre, in perch rods. He equated this groove with the fibre seen by Kolmer ('04). Cohen ('63a) thought that they might be the vertical striae seen by light microscopists, but these are more likely to be the calycal processes.

There are many deep incisions in Amphibian rods (Porter, '57; Sjostrand and Elfvin, '56; Yamada, '57; Wald, '58; Brown *et al.*, '63; Cohen, '63a; Nilsson, '65; Dickson and Hollenberg, '71). The gecko has one incision (Dunn, '66a). Pigeons (Cohen, '63b), the American gray squirrel (Cohen, '64), monkeys (Cohen, '61) and man (Cohen, '65; Hogan *et al.* '71) have scalloped rods with many shallow incisions.

Why are there fissures in rods but not in cones? How are these related to their modes of origin and to their functions? Wald ('58) and Brown *et al.*, ('63) suggest incisures are related to the need for exchange of metabolites, as rod discs, but not cone discs, are separated from the extracellular space. The fissures increase the surface area of the discs. What is the significance of the number and depth of incisions? The sample is too small to generalize about their form within phylogenetic groupings, if indeed any such association exists, or their relation to habit and environment. Whatever the special significance in the number and depth

of rod incisions, many forms exist among the teleosts.

The Connecting Cilium

Sjostrand ('49,'53a,'53b) was the first to report that the outer segment and inner segment are connected by a stalk with nine fibril bundles. Willmer ('53) recognised the similarity of the stalk to the cilium and De Robertis ('56,'60) described the ciliary structure in mouse rods (nine pairs of fibrils with no central pair; the basal body; the matrix material and the surface membrane). He recognised that the entire outer segment results from the differentiation of a primitive cilium arising from a centriole in the distal inner segment.

The association of the outer segment with a cilium and its basal body was seen by light microscopists. Krause (1892,1893,1894) regarded the outer segments as derived from modified cilia of neural canal ependyma. Held ('06) recognised a filamentous start to the human visual cell. Fürst ('04) saw, in Salmon embryo, a cilial origin to photoreceptor cells and that the cilium was connected to one of the pair of centrioles. This structure became known as Fürst's fibre. Retzius ('05) noted the cilium in Selachian rods. Kolmer ('04,'14) claimed that the visual cell cilium was connected to one member of the diplosome and that another fibre, traversing the

inner segment longitudinally, was attached to the other member of the diplosome. Leboucq ('09) recognised the ciliary origin of outer segments and he described axial filaments in rods and cones which ended in one member of the diplosome.

In embryogenesis, a presumptive cilium, arising from a centriole, influences the distal plasma membrane to infold and form the discs of the outer segment (De Robertis, '56; Sjostrand, '59,'61; Tokuyasu and Yamada, '59; De Robertis and Lasansky, '61; Dowling and Gibbons, '61; Ueno, '61; Missotten, '65; Nilsson, '65; Olney, '68).

The cilia of visual cells resemble non-motile cilia of the C.N.S. (Cohen, '63a) and all non-motile cilia with sensory conducting function (Barnes, '61). This includes those cilia seen rarely in bipolar and amacrine cells and in Landolt's clubs (Hendrickson, '66; Locket, '71b). Such cilia have a 9+ 0 structure. Barnes ('61) says that in cilia or flagella that have only one centriole associated, the centriole forms the basal body of the cilium. Motile cilia (9 + 2) generally have one centriole only. All two-centrioled cilia show a 9 + 0 structure but not all 9 + 0 cilia have two centrioles. Each outer segment arises from one cilium and Cohen ('69) claims that all visual

cells contain a pair of centrioles but there are reports of those with only one centriole. (De Robertis and Lasansky, '58, rabbit; Stell, '65b, goldfish; Locket, '69, '70a,'71a,'71b, deepsea fish). Ueno ('61) reported that one centriole was lost during development in the chick, but Morris and Shorey ('67) saw a pair of centrioles in the chick cones. No second centriole was seen in either rods or cones of *Anableps*. In gecko cones, Pedler and Tansley ('63) did not find any clearly defined ciliary structures, and abnormal lamellae were often seen.

There is no connection between the discs and the filaments of the connecting structure, but in frogs (Cohen, '69) the ciliary region is so short that the outer and inner segments abut, as they do in the cones of *Anableps* (figs. 17,19,20,47-49,85).

Pedler ('65) reports that, in general, the rod cilium appears more complete. In the rabbit, De Robertis and Lasansky ('58) report a long rod cilium with a basal body while the cone cilium lacks a basal body and the cilium is very short, and, as a result, more difficult to find. In *Anableps* the rod cilium is well-developed, prominent and compactly organised (figs. 23,25,29). The cone cilium, however, is very short and much less frequently seen. It is associated also with what Stell ('65b) calls an "elaborately evaginated structure, the accessory outer segment of Engstrom ('63a) (figs. 48-50,85). However, Engstrom ('63a) says

the accessory outer segment arises from a second cilium. In *Anableps* cones there is always only one cilium associated with both the outer segment discs and the accessory outer segment, as Stell ('65b) reports for the goldfish. Brown *et al.* ('63) suggest that the main role of the cilium is probably in embryogenesis and regeneration of outer segments. This could account for the far better developed cilium in the rods, in which outer segments discs are constantly renewed.

The ciliary microtubules end as singles within the vitreal one-third of the cone outer segment, in general (Brown *et al.*, '62; Cohen, '65; Hogan *et al.* '71), but in *Anableps* they end very close to the vitreal end of the cone outer segment after splaying out widely, over a short distance, and becoming single (figs. 41,43,44). The rod ciliary microtubules do not extend very far into the outer segment, but they do not splay out very much (fig. 29a).

In *Scopelarchus*, a deepsea fish, Locket ('71b) found all the rod cilia oriented in the same way with an unexpected and uniquely regular pattern. 'In *Anableps* the rod cilia appear randomly oriented but there is a suggestion of an oriented arrangement of the cone and its cilium, associated with the position of the accessory outer segment.

In *Anableps*, the niche in which the rod cilium is inserted in the inner segment often has six lobules

or more (figs. 28,29,29a), a condition also reported in the deepsea fish, *Platytroctes* (Locket, '71a).

There are often ciliary rootlets, arising from or near the second centriole and running a good part of the length of the inner segments. These may be cross-striated, and have nearby vacuoles. (Sjostrand, '59, guinea pig; Tokuyasu and Yamada, '59 kitten; Cohen, '60 mouse, '61 monkey; Missotten, '60, human; Locket, '70b, Landolt's clubs of lungfish). In several deepsea fish, Locket ('60,'70a,71a) found only vague traces of ciliary rootlets and in scopelarchid fish (Locket, '71b), goldfish (Stell, '65b) and hagfish (Holmberg, '71) there are none. None is seen in *Anableps*.

The cilium is the sole morphological connection between the inner and outer segments (De Robertis, '56; Sjostrand, '59; Cohen, '69; Nilsson, '64a; Dunn, '66a; Young, '69a,'71b) and its core looks empty (Cohen, '65a). The ciliary connecting stalk(as the sole connection between the site of light absorption and the rest of the cell where proteins are manufactured) must, therefore, be involved in both impulse conduction and metabolite transfer, and also in growth, maintenance and regeneration of the outer segment. Labelled proteins made in the ergastoplasm of the myoid were seen to travel slowly through the Golgi apparatus and the mitochondria of the ellipsoid, be detained momentarily at the base of the

cilium and then to travel quickly through the cilium and accumulate prominently in the discs. (Droz, '63; Young, '68). There is no protein synthesis in the cilium itself, which seems to act as a pipeline for the transport of newly formed protein to the outer segment. Microtubules elsewhere in the central nervous system are associated with transport and Young ('68) speculates that the absence of the two central microtubules may also be related to the pipeline transport function.

There is a most remarkable uniformity of ciliary structure in the animal kingdom (Fawcett, '66). The normal ciliary function is in the movement of fluids. In the visual cells cilia are associated with embryogenesis (an inductive-like role), with regeneration of the outer segment which is possible only as long as the inner segment and cilium are intact (Dowling and Gibbons, '61), with transport of proteins and, presumably, the conveyance of nervous impulses.

Richardson ('69) has questioned the now accepted view that the connecting cilium is the only pathway between inner and outer segments. In mammals (rhesus monkeys, albino rats, hooded and albino guinea pigs and the ground squirrel, all light and dark adapted) he found that the plasma membrane of the connecting cilium was thicker than that of inner or outer segment and he confirmed that there is a distinct inner core separated by a thin membrane from the radially arranged tubules.

Cohen ('69) reported an empty looking core with an interface where the core abuts on the circle of ciliary tubules.

Richardson ('69) found in all 4 species he studied that, in addition to the connecting cilium, there is a cytoplasmic bridge, separated completely by extracellular space, joining inner and outer segments. The bridges are seen in longitudinal sections and are missed where the bridge is very narrow.

He found two constant features: the connecting cilium is always separated from the surrounding cytoplasm by a continuous extracellular channel, and there is always some area of contact between the cytoplasm of the inner segment and the lowest discs of the outer segment, an area where no membrane intervenes between inner and outer segments. He suggests that materials from the inner segment are more likely to pass through the cytoplasmic bridge. Such connections seen before (Pedler and Tilly, '65; Tokuyasu and Yamada, '60) were thought to be abnormal. He found ground substance from the inner segment running up beside the rod outer segment discs, in the monkey. If this is so, one wonders what the associations may be with the accessory outer segment.

The accessory outer segment of the cones forms a connecting channel between inner and outer segments. Is Richardson correct and has the connecting cytoplasmic

bridge been missed because 'man zeht nur was man weiss' (Liebig)? The weight of evidence and opinion to date is against Richardson's view.

Calycal Processes

Sjostrand and Elfvin ('56) saw 'fibrils' around the Bufo rod outer segment. This was the first electronmicroscopic reference to what have since become known as calycal processes. Schultze (1873) saw these and called them 'striae'. Hoffman (1876, cited in: S.E.G. Nilsson, '65) called them 'hairs' and Arey ('32) referred to them as 'fine peripheral fibres'. Howard (1908) saw 20-30 longitudinal peripheral fibres projecting around the outer segment surface in the rod of *Necturus*, and Franz ('10, cited in: L.B. Arey, '32) saw them in birds. Engstrom ('63a) described 'plasma strains' as extensions of the cytoplasm of the ellipsoid around the proximal part of the outer segment, and Blaxter and Jones ('67) saw them closely applied to the cones in the very early stages of development of larval herrings. Cohen ('61,'63b) saw 'tongue-like' extensions of the inner segments around the proximal parts of the outer segments and likened them to the flower calyx calling them 'calycal processes', and this term has gained general acceptance.

Early reviews of ultrastructure of photoreceptors do not mention these processes (Dartnall and Tansley, '63; Moody, '64).

Brown *et al.* ('63) described these processes in *Necturus* and, for the first time, gave importance to them. They saw a circlet or palisade of 27-30 fibrillar microvillous processes surrounding the base of the outer segment and extending more than half-way up it. In the rod these processes were shorter than those of the cones and there was one in each fissure or groove of the lobulated rod saccules. These structures they called 'dendrites' but as this term already has another clearly defined meaning, Cohen's ('63b) term "calycal processes' is preferred.

Once Brown *et al.* ('63) had emphasised their presence, structure and possible roles, references in the literature to the calycal processes increased. They seem to be present in all visual cells since investigated, with the exception of the albino rat (Dowling and Gibbons, '61), which suggests an association with the condition of albinism and more specifically with the absence of pigment granules in the pigment epithelium.

The functions of the calycal processes are not known. Cohen ('63a) points out that Müller cell microvilli, pigment epithelial processes and calycal processes (and, one may add, the inner segment fins) may all be paths for metabolites and also serve as receptor cell spacing and positioning devices. Brown *et al.* ('63) think there might be a significant interchange of nutrients between

the outer segment and the pigment epithelium involving the calycal processes. Dunn ('66a) suggests that the calycal processes take up nutrients from the extracellular space at the outer segment level and carry them to the ellipsoid region of the visual cell. Nilsson ('65) considers these processes to be supportive and nutritional and also involved in excitation conduction. Hollenberg and Bernstein ('66) refer to calycal processes as an array of external supports.

Locket ('71a) found that the deep sea fish, *Platytroctes*, has calycal processes which form a continuous low wall with an irregular scleral border, and this wall arises vitreal to the apex of the inner segment. On the other hand, *Poromitra*, another deep-sea fish, has the usual discrete processes, albeit rather short ones (Locket, '69). The 9 - 12 human calycal processes are said by Cohen ('65a) not to be closely applied, whereas Missotten ('65) says that they are, and Hogan *et al.* ('71) show 16 closely applied calycal processes extending one-third of the way of the rod outer segment length, and they report that these are similar in the cone. The human calycal processes seem to be shorter than those reported, for example, in *Necturus* (Brown *et al.*, '63).

In *Anableps*, the calycal processes extend over almost the entire length of the outer segment of the

cone (figs. 20-22). Proportionally, they are much shorter in the rod (figs. 17,19,21,22). In the more vitreal regions of the rod outer segment, near the junction with the inner segment, where the rod outer segment discs are being formed, the pigment epithelium is not in direct contact with the outer segments. Here the calycal processes intervene (figs. 27,35). In the scleral regions of the rod outer segments (figs. 26,28,29) at the apical zones where the discs are being shed and engulfed by the pigment epithelial processes, there are no calycal processes (fig. 30). There the outer segment disc zone is in direct contact with the pigment epithelial processes. The rod outer segment then, is directly surrounded by processes, either calycal or pigment epithelial. The calycal processes seem to have a support function, strongly suggested by the longitudinal orientation of their fibrillar contents. It is also possible that the calycal processes protect the discs from the 'engulfing' properties of the pigment epithelium processes, and such a protective role could also account for the more massive and proportionally much longer cone calycal processes, as cone discs are not shed.

The Accessory Outer Segment

In light microscopic studies Engstrom ('60,'61) first described the 'accessory elements' of varying lengths and widths lying alongside the outer segments of

teleost visual cells. Ultrastructural details followed (Engstrom, '63a) and the structure was renamed the accessory outer segment. Engstrom ('63a) described it as originating in the distal ellipsoid outer edge, close to the base of a cilium, extending alongside the outer segment, and inserting in a pigment epithelial cell. He reported that it arose from a cell with two cilia but the illustrations show only one cilium. He showed the accessory outer segment with certainty in the cones only; in the rods they showed only with Bodian's silver impregnation as very thin threads.

Munk and Anderson ('62) reported that, by light microscopy, they had seen the accessory outer segment in Bodian impregnanted retinas of deep sea fish and humans, but later decided that what they had seen were cilia (Munk, '66). Dathe ('69) found accessory outer segments in the European fresh-water fish he studied by light microscopy. He found, as Engstrom ('63a) had reported, that these structures had homogeneous-looking cytoplasm devoid of obvious organelles and were associated with a second cilium. The rod accessory outer segment was more readily seen in the dark-adapted state, and in bleached sections. Locket ('69,'70a) could not find these structures in his electron microscopic studies of deep sea fish. Stell ('65b), in an electron microscopic study (without illustration) of goldfish photoreceptors, reported that the cilium formed a separate "elaborately

evaginated structure" alongside the outer segment. He never found a co-existant ordinary ciliary connective.

In *Anableps* only one cilium per cone was seen and always in association with both the accessory outer segment and outer segment (figs. 47-50,85). The accessory outer segment is connected to the outer segment by short cytoplasmic bridges which are found at all levels indicating that there is a web-like connection (figs. 41,42,45,46,54, 75). This Engstrom ('63a) also found in labrid fishes.

Transverse sections are necessary to see the accessory outer segment which in longitudinal sections may be confused with an enlarged ciliary backbone. The latter is not separated by membranes from the disc zone. Light microscopic identification alone is hazardous. Attempts have been made to equate earlier descriptions with the later identified accessory outer segment. Fürst's ('04) and Kolmer's ('04) fibres have been interpreted both as the connecting cilium (Sjostrand, '53a) and also as the accessory outer segment (Engstrom, '63a). Early reports of these various fine and coarse fibres are summarized by Arey ('32). There seems little to be gained by establishing the pedigree of historical antecedents. Knowledge of the morphology, function and phylogenetic distribution of the accessory outer segment needs present-day study by all available techniques.

Engstrom ('63a) generalized from his own findings, from Munk's since retracted support ('66) and from the early findings of fibres associated with the outer segment (Kolmer, '04; Fürst, '04; Retzius, '05; Held, '04) that the accessory outer segments are widespread organelles and regular components of the vertebrate visual cells. So far, however, they have been identified with ultrastructural certainty only in some shallow water fish. It was not possible to identify any accessory outer segment in any other group from photomicrographs in the literature. In *Anableps* they were seen in cones only.

Engstrom ('63a) suggested that they have a mechanical contact function between visual cells and pigment epithelium, and in the supply of metabolites, such as oxygen or vitamin A. Why this should be so only in retinas of shallow water fish is not obvious. Perhaps they represent a residual outer segment, a double cone on its way to becoming single or a triple cone becoming double.

The accessory outer segment seems devoid of microtubules or filaments, pinocytotic vesicles or desmosomes, indeed any organelle associated with contact adhesion or cell transport. However, this does not entirely rule out such roles. If the structure is indeed found to be present only in shallow water fish and

only in cones, then this peculiarity will be of additional interest. It may be that they play a role in light capture (Synder & Hamer, '72) as the photoreceptor has a light capture area larger than its geometric cross-section. It is hoped that embryological studies of *Anableps* retina may shed some light on the development, at least, of the accessory outer segment.

The Rod Inner Segment

In *Anableps* the mitochondria of the rod ellipsoid are arranged radially around the base of the eccentric cilium, in a very uniform way to form a hilum, and this is widely reported (Yamada, '57,'60b; Engstrom, '63a; Nilsson, '64c; Locket, '69,'71a). There are seven to eight (Villegas, '60) mitochondria or five-seven (Villegas, '61) in the rods of the fish, *Centropomida*. *Anableps* has six-nine. Engstrom ('63a) reports a hilum in cones also, but in *Anableps* cones there is no hilum as the distal mitochondria are replaced by an oil-droplet and the cilium has a very short insertion. Villegas ('60,'61) found no hilum in the cones of the fish *Centropomida*.

Proteins and phospholipids manufactured in the myoids are known to pass through the mitochondria en route to the outer segment across the connecting cilium (Young, '69b). The convergence of the rod mitochondria suggests they form a directional pathway towards the cilium; or they may be so arranged because of the

directional flow (Young, '68).

In *Anableps* there is staining difference between the rods and cones in the proximal parts of their inner segments, the rod cytoplasm always staining more darkly (figs. 60-62,65,66,96,97). The rod myoid is especially narrow (figs. 31,53)whereas the cone myoid is about the same size as the cone ellipsoid (fig. 66).

Fins and Inner Segment Ridges

Carasso ('56,'57,'58) first described the fins as "cytoplasmic prolongations", gearlike, and interdigitating about the level of the external limiting membrane in the visual cells of frog, larval frog and lizard. These longitudinal folds of the inner segment surface also interdigitate with Müller cells. Transverse sections are needed to recognise them clearly as in longitudinal sections the inner segment may appear smoothly cylindrical. They have been variously described as toothed, wheel-like projections (Yasuzumi *et al.*, '58-weaver finch), pleats (Yamada and Ishikawa, '67-lamprey), ridges which appeared gear-like in cross section (Cohen, '63b-pigeon), and fins (Pedler and Tansley, '63-lizard and grey squirrel). Among lower vertebrates they have been very widely

reported (Yamada, '60a,'60b-turtle; Kalberer and Pedler, '63-alligator; Pedler, '63-reptiles; Nilsson, '64c-frog (illustrated only); Borovyagin, '66-pike, orfe, frog, tortoise; Dunn, '66a-gecko; Locket, '71b-Scopelarchid fish).

Pedler and Tansley ('63) reported fins in the grey squirrel, *Sciurus carolensis*, and this is the only report of their presence in mammals, but Cohen ('64) failed to observe any in the same species. Sjostrand (cited in Dunn, '66a) found that there are none in the guinea pig. Morris and Shorey ('67) found none in the chick but Yasuzumi *et al.* ('58) saw them in the weaver-finch and Cohen ('63b) identified them in the pigeon. Fins have been found so far in avascular retinas only and they are assumed to function in water and metabolite transport (Yamada, '60b; Dartnall and Tansley, '63; Pedler and Tansley, '63; Dunn, '66a).

Pedler ('63) suggested the presence of fins is related to interchange in retinas lacking intra-retinal vessels. Dunn ('66a) speculated that lateral fins are present in retinae having a pecten or a conus papillaris, physiologically equivalent structures. He thought it was difficult to see a functional relationship between the presence of lateral fins and the absence of

retinal vascularization. Since Dunn's ('66a) work the sample range has been extended and fins have been reported also in fish and lampreys, which do not have a conus or pecten. Young ('69a) reports fins, and their interdigitations with Müller cells, to be present in lower vertebrates and he associates this with active transmembrane exchange, pointing out that in higher vertebrates Müller cells at this region are rather watery and devoid of organelles.

Fins increase the surface area for transport and they have been ascribed a role in active water and metabolite (including glucose) transfer (Yamada, '60a,'60b; Dartnall and Tansley, '63; Pedler and Tansley, '63; Dunn, '66a). Yamada ('60b) found that the narrow fins of the Müller cells at this region showed no glycogen reaction.

Fins are most clearly seen in transverse sections of *Anableps* cones, but these are not so long as those reported in reptiles by Pedler and Tansley ('63) and they are found only immediately scleral to the external limiting membrane. As reported for all double cones, there are no fins separating the double cones; they surround the entire double cone as a unit (figs. 99,101,108). The fins become shallower and appear only as small ridges in the more scleral regions of the inner segment (figs. 101,108) (Yasuzumi *et al.*,

'58). The cone inner segment in *Anableps* is not a smooth cylinder but is clearly ridged and grooved (figs. 13,17,18,20,21,22,37,44,74,75,90), and so is the rod, but to a lesser extent and then mainly in the myoid region (figs. 17,19,21,22). Longitudinal ridging has been reported previously (Schultze, 1873; Howard, '08; Cohen, '61,'63a; Pedler and Tilly, '64; Pedler and Tansley, '63; Blaxter and Jones, '67). Engstrom ('63a) comments that the 'plasma strains' (calycal processes) do not really begin at the outer tip of the ellipsoid but run like mouldings along the surface of the entire ellipsoid.

This can be seen in figures 20,21,22 and it seems that the ridges or mouldings may contain bundles of microtubules, longitudinally oriented. *Anableps* inner segment has a peripheral lining of microtubules or filaments (figs. 34,36,51,52,61,63,82,83,86) as in chicks (Morris and Shorey, '67) and these are prominent also in the myoid, and they continue into the calycal processes (figs. 34,47,61,65).

These external ridges of the inner segment were seen by light microscopists. Howard ('08) saw twenty-thirty fibres along the entire length of the visual cells which produced a longitudinal ribbing, and Schultze (1873) saw them on the inner segment and illustrated them clearly continuing onto the outer segment.

Microtubules are direction markers. They maintain cell shape, symmetries and polarities, guide the movement of materials and direct cell shape changes. They are abundant in nerve cells, where vesicles move relative to stationary microtubules. In some protozoans, microtubules disassemble in retraction of axopodia and assemble in their expansion. The arrangement of microtubules in the visual cells suggests structural and transport functions (Roberts, '72) and a role in photomechanical movements.

The Oil Droplets and Their Formation

The oil-droplets were so named by Hannover (1840). He inferred that they were oily because they floated, were immiscible in aqueous media, and were soluble in ether. Sidman and Wislocki ('54), by histochemical means, found that they contained unsaturated lipids and were rich in phospholipids. Wald and Zussman ('38) isolated three distinct stable carotenoids from chick retina and observed absorption spectra similar to those of the oil-droplets. The colours of the avian oil droplets are attributable to these carotenoid pigments. Short single cones bear red droplets, the principal cones of the double cones have yellow droplets and, contrary to reports that there is no oil-droplet in the accessory cones, Meyer *et al.*, ('65) found there a very small yellow green oil-droplet. In the retinal thickness

there are three layers of oil-droplets formed, the proximal layer is red, the intermediate is yellow-green and the distal layer is green.

Yamada ('60b) illustrates an oil-droplet in the accessory cone of the turtle but Nilsson ('64a) did not locate any in that cell of *Rana pipiens*. Meyer and Cooper ('66) say that Engstrom ('58) by electron-microscopy saw an oil-droplet in the accessory cone of the great tit. Engstrom's was a light microscopic study on the mosaic, and the oil-droplet is not mentioned. The line drawing in that paper is of a double cone, derived from Walls ('42), with full acknowledgements.

The function(s) of oil-droplets, generally restricted to the cones, is still conjectural (Pedler, '69). Light must pass through the oil-droplet to reach the outer segment so that cone sensitivity depends on both the visual pigments in the outer segments and the transmission of light through the inner segment (Kingsmith, '69). Coloured oil-droplets, most common in diurnal Sauropsideans (Walls, '42; Duke-Elder, '58) do not provide the bases of colour perception but they modify the perception of colour by altering the wavelength of light reaching the outer segments (Walls and Judd, '33). Coloured oil-droplets act as colour filters and they remove the low wavelengths (violet and ultraviolet) for which the eye has considerable chromatic aberration, thus reducing glare and improving contrast and acuity

(Walls, '42; Duke-Elder, '58; Strother *et al.*, '60; Pedler and Boyle, '69). 80% of the incoming light is removed by the oil-droplets irrespective of colour (Strother, '63). Sjostrand ('59), Meyer *et al.*, ('65) and Pedler('69) suggest that the ellipsoid mitochondrial mass may act as a focussing device. Cohen ('63a) points out that any structure of a distinct refractive index and shape can modify the passage of light through it. The clusters of modified mitochondria may act as oil-droplets do to condense light onto the outer segments (Pedler, '69). They act as bandpass filters,both absorbing and condensing light (Meyer *et al.*, '65).

The guppy and *Anableps* are both shallow water fish, but *Anableps* is a surface swimmer and it has far more oil-droplets than the guppy (Berger, '65,'66) and this may well be associated with the problem of glare at the water surface.

Oil-droplets of the adult visual cells are permanent inclusions. Young and Bok ('70) found no turnover in the cone oil-droplets of *Rana pipiens*, whereas the oil-droplets of the pigment epithelium, which concentrate Vitamin A derived from the blood, show a rapid turnover of Vitamin A and galactose. Craig *et al.* ('63) found that neither the oil droplets of *Rana pipiens* nor their adjacent mitochondria were affected by the nutritional states of the animal, and they concluded that the oil-droplet does not serve as a source of energy in

starvation. The presence of oil-droplets in cones of *Rana pipiens* made no difference to radioactive labelling of the outer segment (Bok & Young, '72). It is interesting that the paraboloid also is not affected by starvation (Gourévitch, '54).

Meyer ('71) and Meyer *et al.* ('71) fed Japanese quail, which have coloured oil-droplets, for eleven months with a carotenoid-free, Vitamin A-supplemented diet. The parents retained their coloured oil-droplets, but their offspring had colourless oil-droplets in cells that were normal in all other respects, and these offspring had normal vision. The carotenoids of the oil-droplets are obviously derived from the egg and do not contribute to visual pigments. Adult colours of the coloured oil-droplets are present at birth (Cooper and Meyer ('68).

Oil-droplets are generally reported to be membrane-bounded, but there are reports of continuities between the adjacent mitochondria and the oil-droplets (Craig *et al.*, '63; Pedler and Tansley, '63; Ishikawa and Yamada, '69). There is most often only one large oil-droplet in the distal inner segment of each cell, but multiple small droplets (Pedler and Boyle, '69) or small granular structures occur (Cohen, '63b-pigeon). These may be oil-droplet precursors.

There are reviews available about oil-droplets at the light microscopic level (Walls, '42; Prince, '56; Duke-Elder, '58; Meyer and Cooper, '65). There are no reviews which deal with the ultrastructural complexity of oil droplet morphology, their associated mitochondria and the transformation or transmutation of mitochonrida into oil-droplets.

In the guppy (Berger, '66) the single oil-droplet is double membrane bounded, as in *Anableps*, and almost all the mitochondria are situated vitreally to the oil-droplets. Mitochondria on the scleral side of the oil-droplet are seen in amphibia (Craig *et al.*, '63; Nilsson, '63,'64c) in reptiles (Pedler and Tansley, '63; Pedler and Tilly, '64) and in birds (Cohen, '63b; Morris and Shorey, '67). Some reptiles (Dunn,'66a) and some frogs (Nilsson, '64a) do not have any oil droplets.

Until Berger ('65,'66) reported the presence of oil-droplets (colourless)in guppy (*Lebistes*) cones, it was repeatedly said that teleosts do not have oil-droplets. This emphasizes again the ever-present danger of generalizing from too restricted a sample. In some principal cones of *Lebistes*, Müller ('52) saw, by light microscopy, a light homogeneous mass, whereas in all other cones the entire ellipsoid appeared granular. The granular material may represent the mitochondria and the light homogeneous mass the oil-droplet, as is the

'globule' Butcher, ('38) saw in the *Fundulus* distal single cone inner segment. In the principal member of the double cone he saw a dark granular mass which may be transformed mitochondria; he thought they were not oil-droplets.

Ishikawa and Yamada ('69) studied by electron microscopy, in seven species, the transformations of inner segment mitochondria. They described large electron-dense, homogeneous bodies in the carp ellipsoid, like lipid droplets, each one double-membrane bounded with cristae-remains. A variety of intergrading forms were seen with varying densities of mitochondrial matrix and varying amounts of cristae, and a gradual appearance of normal mitochondria at the ellipsoid periphery. They thought that the original mitochondrial components were replaced by a specialized substance in very modified enlarged mitochondria that resemble lipid droplets.

In *Lebistes*, Berger ('65,'66,'64) found oil-droplets in some of the principal cones of the double unequal cones and in some single cones, these two cone types being morphologically equivalent. Those cones that lacked oil-droplets had normal mitochondria. In *Anableps*, most cones have oil-droplets and where fully-formed recognizable oil-droplets are not seen the scleral-end mitochondria are either very enlarged and transformed to some degree, or a single very large mitochondrion, with fibrillar contents and disorganized cristae, occupies

the oil-droplet position (figs. 34,37,49,67,85,86). Berger ('64) illustrates such very large mitochondria in this position in the neonate guppy.

Ishikawa and Yamada ('69) felt there were difficulties in referring to the lipoidal enlarged mitochondria of the carp ellipsoid apex as oil-droplets since they possess rudimentary cristae and bordering double membranes and are clearly intermediate between typical and modified mitochondria. In *Anableps*, hundreds of cones were examined and all intergrading stages were seen from incipient transformation of mitochondria to homogeneous electron-dense oil-droplets. There is little doubt that the oil-droplet forms from mitochondria that enlarge, alter (transform) and fuse as they mature in a vitreal to scleral gradient, with progressive increase in density of matrix, and apparent disorganization of cristae. Fusions were often seen (figs. 36,44, 49,68-75,78,92). It is most improbable that fission occurs in this situation, the only other explanation possible, since that would involve the unlikely postulate that the mitochondria bud off from the oil-droplet.

Pedler and Tansley ('63) thought that the inner segment mitochondria synthesise an oily product which accumulates to form an oil-droplet at the ellipsoid apex. Pedler and Tilly ('64) found transmuted mitochondria in nocturnal geckos, in a variety of forms,

sometimes resembling the oil-droplets in the diurnal species. Berger ('66) believed the oil-droplet to be of mitochondrial origin, the result of fusion of mitochondria which modify in a vitreal-scleral gradient. In *Anableps* this gradient, while general, is not altogether strict, and some small mitochondria may be seen at the scleral end in close contact with the oil-droplet (figs. 36,50,66,85,86). Transforming mitochondria with some very electron-dense contents may be vitreal to the oil-droplet (fig. 16) or at the same level (figs. 36,56,57,58, 92); or several highly transformed mitochondria may occupy a good portion of the distal inner segment and it would seem that they will fuse more or less simultaneously to form an oil-droplet, (figs. 47,75,78,84).

There are,then,different patterns in the 'densification' of the oil-droplet and the apparent obliteration of the cristae, but the periphery seems the last zone to become homogeneous in appearance (figs. 36,37,50,57-59,75,80,82,85,86). Pedler and Tansley ('63) found that the oil-droplet in the gecko sometimes looked homogeneous but in the thinnest sections cristae could be clearly seen. However, in *Anableps*,variations were seen within one section and within a pair of cones and within one oil-droplet so that the variations are unlikely to be due to section thickness or to maturity differences. It was thought that a very dense substance

might obscure the cristae which remain and perhaps even continue to function in some way. But when some of the lipoidal contents are dissolved out in osmium fixation no framework of cristae remains (figs. 87-89). The rod mitochondria of *Anableps* are always of the same appearance, irrespective of fixative used and thus serve in a way as a control.

In the chick, the principal members of the double cones have a pale-staining oil-droplet and the single cones have either a dark (often irregularly outlined) or a palestaining oil-droplet. The accessory cone of the double cones has no oil-droplet, but a small granular vesicle instead (Morris & Shorey, '67). In the pigeon, in the position of a typical oil-droplet Cohen ('63b) sometimes found a granular body of the same size (up to 2μ). Other smaller granular bodies were sometimes seen near the oil-droplet and there were also minute oil-droplets among the mitochondria.

Berger ('65,'66) reported two kinds of oil-droplets in the guppy, but also found many intergrading forms, and some structures that seemed to have only partial oil-droplet appearance. The *Anableps* oil-droplets are either round or ovals of varying lengths or slightly pear-shaped ovals and I suspect that those that appear round or short ovals are sectioned nearer the oil droplet periphery. There may (figs. 37,57) or may

not (figs. 36,53,55,56,58,59,74,75,79,85,86) be synchrony in the transformations seen within one double cone. The fibrous, granular, cristae-containing structures (figs. 34,37,49,85,86) are postulated to be early stages in the process of oil-droplet formation.

Berger ('66) reported the guppy oil-droplets to be in the range of 1.7±0.5 μ. *Anableps* oil-droplets have a far greater size variation and it seems that the oil-droplets continue to grow in length, postnatally, for an extended period, being added to at their vitreal ends (figs. 51,77,79,80,83).

Hudson *et al.*, ('71) were able to isolate chick oil-droplets and by chromatographic analyses they found at least nine lipid components, with cholesterol representing 15% of the mixture. Detailed histochemistry on oil-droplets is difficult because reagents may not penetrate the surface layers of the droplet.

As in *Anableps*, unusually dense mitochondria are seen in association with oil-droplets in other retinae (Craig *et al.*, '63; Pedler and Tansley, '63) but very dense, or otherwise altered mitochondria are also reported in the cone ellipsoids of retinas that do not possess an oil-droplet. Ishikawa and Yamada ('69) in a study of 7 species report such modifications in scleral-end mitochondria, some of which seem unique. There are two kinds, those with changes within single mitochondria,

in the toad (with glycogen particles), lampreys and giant salamanders; and those with areas of specialized mitochondria, in the gecko, carp and snake. Dickson and Hollenberg ('71) saw enlarged central mitochondria in the cones of *Triturus*, often with curved cristae, and both in rods and cones there were intramitochondrial granules. These were identified as glucose by enzyme digestion in the rat (Ishikawa and Pei, '65) but not in the newt (Dickson and Hollenberg, '71) where they persisted after alpha amylase digestion, and they were considered by Dickson and Hollenberg ('71) as sites of ion accumulation (Peachey, '64). In *Anableps*, such granules are especially common in the rod mitochondria. In the chick (Morris & Shorey, '67) the two kinds of oil droplets (pale-staining and dark-staining) in the single cones are associated with different kinds of mitochondria, the pale one with mitochondria with few cristae. Peculiar configurations of mitochondria are shown by Ishikawa and Yamada ('69) in the ellipsoids they studied. In the snake ellipsoids there are large, very dense-staining cores in the principal and single cones. The central mitochondria contain osmiophilic granules arranged regularly along the mitochondrial inner membrane. In *Gekko japonicus*, in the class B doubles (as named by Underwood, '51), and in the carp, central areas of the ellipsoid have 'bodies' with a homogeneous lipid-like substance, each surrounded by a double membrane with rudimentary cristae, and there

are transitional forms. They conclude that these bodies are extremely modified mitochondria in which a special substance has replaced the original components. Engstrom ('63a)in Labrids, saw ellipsoid mitochondria filled with a dense, seemingly granular material. Locket ('71a)reports that *Platytroctes*, which has an all rod retina, has large tightly packed mitochondria "markedly different from those found elsewhere in the retina", but he does not give any details. Pedler and Tilly ('64) found three kinds of mitochondria in the gecko, one small with few cristae, one larger with dense cristae in only part of it, and one with many well aligned cristae, this latter kind occurring only most distally. Some cells have very elongated mitochondria, others have large dense structures like oil-droplets which are transmuted mitochondria. Aligned, transmuted and conventional mitochondria were sometimes found in one cell.

In some cases where there is a well defined oil-droplet, the neighbouring mitochondria are often larger but do not appear to be denser. This is seen in turtle (Ishikawa and Yamada, '69), pigeon (Cohen, '63b; Pedler and Boyle, '69) diurnal gecko (Pedler and Tansley, '63), chick (Morris and Shorey, '67) and frog (Craig *et al.*, '63). In *Anableps*, mitochondria neighbouring the oil-droplets were never 'normal' in appearance and this seems , from the illustrations, to be the case in the guppy also (Berger,'66).

In chicks, Ueno ('61) saw irregular dense oil-

droplets in 8 day old embryo which developed after the inner segment mitochondria had increased in number. Meyer *et al.* ('65) and Cooper and Meyer ('68), in light microscopic studies, reported that in chick embryo oil-droplets develop at the same time as the mitochondrial mass develops in the inner segment and the discs form in the outer segment. Also in chicks, Coulombre ('55) saw oil-droplets appear at the distal end of the inner segment on the 13-14th day of incubation and by the 15th day they were coloured. The oil-droplets formed in the midst of mitochondria that would form the ellipsoid, and each droplet concentrated only one species of carotenoid. Witkovsky ('63) saw oil-droplets in the 15 day old chick when first the electroretinogram became recordable. Meyer *et al.* ('65) found in birds that the first oil-droplets to appear are colourless. The last to form are the red droplets (Cooper and Meyer'68).

In the very young visual cells of larval Amphibia, Carasso ('58) saw sparse mitochondria around lipid droplets which coalesced to produce the spherical oil-droplets of older cells, and the mitochondria re-arranged themselves around this large oil-droplet. Saxen ('54) observed oil-droplets in the developing visual cells of Amphibian eyes, including *Triturus* and *Xenopus*, which droplets disappeared at maturity, and last of all in the principal cone of the double cone.

Prince ('56) also reports that oil-droplets in some frog tadpole rods disappear at or before metamorphosis. Dickson and Hollenberg ('71) found no oil-droplets in the adult *Triturus*. Nilsson ('64b), in the *Rana pipiens* tadpole, saw many dense bodies in the oil-droplet position by the 6th day of development. All but one disappeared and this one persisted as the oil-droplet. In *Lacerta*, Tiemann ('70) saw oil-droplets develop eight weeks after hatching. Berger ('66) found no oil-droplets in the twin cones of the neonate guppy, but oil-droplets are present in some of the principal cones of the adult fish.

It appears that there are different times and different ways in which oil-droplets form, and there are also different forms of oil-droplets. In order to follow the development of the oil-droplet in *Anableps* cones it would be necessary to study the cones of the prenatal, neo-natal, small and large fish, and also the peripheral-central gradient since fish eyes are reputed to grow continuously from the periphery of the retina.

One kind of oil-droplet seems to be derived from a secretion of the mitochondria (Pedler and Tansley ('63) and other kind, as in *Anableps*, seems to arise from mitochondria which themselves transform. (Berger, '66; Pedler and Tilly, '64). Some may develop very early ontogenetically (Saxen, '54,'56; Coulombre, '55; Carasso, '58; Ueno, '61; Nilsson, '64b); others may be adult

structures only (Berger, '66; Tiemann, '70). Greater knowledge about the ultrastructure of the prenatal, neonatal and adult oil-droplets and their associated mitochondria is necessary. However, it seems clear that there is a mitochondrial participation in the formation of the oil-droplet and in some cases the mitochondria themselves transmute into an oil-droplet.

The Cone Mitochondria and Mitochondriogenesis

Mitochondria are multifunctional organelles of considerable diversity and versatility and their known functions include lipid synthesis and catabolism. There are many recent reviews which discuss these aspects and which evaluate the theories of mitochondrial origin. (Criddle and Schatz, '69; Roodyn and Wilkie, '68; Rabinowitz and Swift, '70; Racker, '70; Schatz, '70; Boardman *et al.*, '71; Flavell, '71).

A de novo origin has been postulated by Linnane *et al.* ('62) in yeasts cells, by Berger ('64,'65,'66) in guppy cones, by Threadgold *et al.*, ('67) in larval sardine and by Stangvos ('70) in Annelid spermatids. The weight of evidence is against acceptance of a de novo origin from molecular components but favours the promitochondrial concept. Promitochondria, somewhat analagous to proplastids, are small, membrane-bounded, cristae-less

empty-looking vesicles which grow by the addition of subunits into visually and functionally recognisable mitochondria. In other words, the mitochondria form from the assembly of structurally organised sub-unit precursors. (Tzagaloff *et al.*, '67; Penniston *et al.*, '68; Roodyn and Wilkie, '68; Wallace *et al.*, '68; Kellerman *et al.*, '69; Racker, '70; Lenaz, *et al.*, '71). Baxter ('71) points out that this concept is akin to a de novo synthesis of mitochondria from molecular components but conceives of larger, more complex and constantly present subunits as the building blocks, instead of molecular size units.

Other structures assemble from subunits, namely, microtubules (Tilney, '71) centrioles (Fulton, '71) and flagella and viruses (Roodyn and Wilkie, '68).

The failure to visualise mitochondria in anaerobic yeasts and their appearance on the return to aerobiosis has been taken as evidence of their de novo origin (Wallace and Linnane, '64); or of evidence of their formation from non-mitochondrial membranes (Linnane *et al.*, '62). However, Damsky *et al.*, ('69) showed that mitochondria-like bodies are present in the anaerobic yeast but poorly visualised because they lack sterols and unsaturated fatty acids. Subunits are added to these bodies for the respiratory state. Criddle and Schatz ('69) found that biochemically viable

yeasts always contain mitochondrial inner membrane. They isolated promitochondria (0.2-0.6 μ diameter) which looked like featureless vesicles, which, on oxygenation, differentiated into mitochondria. By blocking with cycloheximide in label transfer experiments with 3H-leucine, Plattner *et al.*, ('70,'71) showed that nonrespiring, incomplete promitochondria of anaerobically grown yeast cells are the structural precursors of functional mitochondria. The promitochondria gradually become mitochondria-like and larger, and they develop cristae and their lipid complement changes (Paltauf and Schatz, '69).

Descriptions of yeast promitochondria in anaerobiosis and their development into mitochondria in aerobic conditions can be found in Linnane *et al.*, ('62), Wallace and Linnane ('64) Criddle and Schatz ('69) and Plattner *et al.*, ('69,'70,'71).

These vesicular featureless promitochondria (and their manner of growth into mitochondria) are very similar to the structures seen in the vitreal end of the inner segment of *Anableps* and in the guppy, in which Berger ('66) said mitochondria arose *de novo*. By serial sections he saw structures (he called them I-bodies) in the very vesicular vitreal-end ergastoplasm. He interpreted them as invaginations of vesicular membranous sacs which eventually became independent and matured into typical

mitochondria. Cytoplasmic intrusions into the proplastids reminiscent of Berger's I-bodies were described by Schiff in Euglena ('71).

In *Anableps*, the vitreal end of the ellipsoid is very vesicular and vacuolated and the few mitochondria that are present are very small with very small, sparse cristae, the matrix is pale and the outer membrane is irregular and 'loosely-fitting'. Some of these "vesicular" structures have only one membrane. They are often closely apposed to or in direct continuity with cisternae of the endoplasmic reticulum (figs. 60,61,62,64,96). This vacuolated vesicular look has been seen in the vitreal ends of guppy cone inner segments (Berger, '66) and in the vitreal ellipsoids of other fish cones (Engstrom, '63a).

Similar 'vague' mitochondria that are seen in *Anableps* have also been described where there are young or newly formed mitochondria and where rapid growth is occurring (Rabinowitz *et al.*, '70), for example, in oocytes (Adams *et al.*, '64); in neonatal liver (Stempak, '67) in regenerating liver (Claude, '65); in brown adipose tissue development (Suter, '69); regenerating limb of larval *Amblyostoma* (Hay, '58); in yeasts, in the change from anaerobic to aerobic conditions (Linnane *et al.*, '62; Wallace *et al.*, '64,'68) and in the integumentary cells of the larval sardine

(Threadgold *et al.*, '67).

Oxygen acts as a mitochondrial inducer, and glucose and low oxygen tension act as repressors (Tustanoff and Bartley, '64; Wallace *et al.*, '68). Addition of glucose to aerobic yeasts returns the mitochondria to the promitochondrial condition just as anaerobiosis does. (Chapman and Bartley, '68; Lenaz *et al.*, '68,'71). In such conditions, mitochondria dedifferentiate or fail to differentiate (Criddle and Schatz , '69). Aschenbrenner *et al.*, ('69) found in rat heart that there is a destruction of mitochondria in low oxygenation and a rapid resynthesis of mitochondria in the return to normal oxygen tensions.

In *Anableps* the vitreal end of the cone ellipsoid and the myoid are rich in glycogen and this area has fins closely associated with glycogen-rich Müller cell processes.

In cones in general, the scleral-end mitochondria may exist in a richer oxygen milieu than do the vitreal end ones. The former are nearer the choroidal gland and the blood vessels of the choroid. The glucose concentration is lower than in the more vitreal zones, the'fin' zone. These differentials in the glucose and oxygen concentrations might be associated with the assembly of mitochondria in the vitreal inner segment

of the cone of *Anableps* and with the gradient of their development as they age.

Scarpelli *et al.*, ('70) point out that initiation of mitochondriogenesis depends on integration of synthetic events in both the mitochondria and the endoplasmic reticulum. The close association between these two organelles and its importance in mitochondrial growth was noted by Fawcett ('55). This association, and even luminal continuities between the inner-outer-membrane mitochondrial space and the cisternae, is well documented (Claude, '65; Bracker and Grove, '71; Franke and Kartenbeck '71; Ruby *et al.*, '71; Morré *et al.*, '71). These continuities may be transfer channels. Proteins synthesised on the endoplasmic reticulum are transferred to the mitochondria (Kadenbach, '66; Gonzalez-Cadavid, and Campbell '67) and so are phospholipids (McMurray and Dawson, '69; Jungalwala and Dawson, '70). The close association of lipid droplets and mitochondria is well-known (Palade, '52,'59; Napolitano and Fawcett, '58; Novikoff, '61; Lindberg, '70).

There is a functional relationship between endoplasmic reticulum and mitochondria in adrenal hormone synthesis where steroid hormones, or their precursors, are made within the mitochondria (Lever, '55; Kadioglu and Harrison, '71). Marek *et al.*, ('70) describe a lipoidal transformation of mitochondria in rat adrenals in which the membranous components dissolve. In sheep adrenal

zona glomerulosa cells the aldosterone is produced within the mitochondria (Luthman, '71). Carasso and Favard ('58) and Ward ('62,'64) saw yolk platelets form within oocyte mitochondria. Aging Tetrahymena accumulate lipids in their mitochondria (Elliott and Bak, '64) which degenerate and transform. Lung surfactants,which contain a unique lecithin,are produced by alveolar cell mitochondria which transform (Tombropoulos, '71).

Elaborate fusions of mitochondria occur in spermatogenesis (Grassé *et al.*, '56a; André, '59; and are also seen in *Euglena* (Calvayrac *et al.*, '71).

It has been suggested from embryonic studies that inner mitochondrial membranes with different enzymatic-specific activities may be synthesised at different stages of development (Rabinowitz and Swift '70) and there may be different populations of mitochondria in different tissues and even within one cell (Flavell, '71).

The Mitochondrial Gradients

In the ellipsoids of many non-mammalian vertebrates there is commonly a linear or circular gradient of mitochondrial size. In the forms with a linear gradient, the smallest mitochondria with fewest cristae are at the vitreal end (Yamada, frog '57; Engstrom, Labrid fish, '63a;

Pedler and Tansley, diurnal gecko, '63; Berger, guppy, '64, '65,'66; Pedler and Tilly, nocturnal gecko, '64; Morris and Shorey, chick, '67; Ishikawa and Yamada, lamprey, '69). In forms with a central gradient, the smallest mitochondria with the fewest cristae are peripheral and the largest are central (Villegas, *Centropoma*, a fish, '60; Pedler and Tilly, *Gecko gecko*, '64; Borovyagin, pike, '66; Dunn, *Coleonyx*, a gecko, '66a; Dickson and Hollenberg, *Triturus*, '71).

In *Anableps* the mitochondria are organised vitreally and there is a distinctly linear vitreal-scleral gradient; but, in addition, in the middle regions of the ellipsoid the smaller mitochondria tend to be on the periphery (figs. 65,74,78). These mitochondrial arrangements may well help to condense light onto the outer segments.

The Nuclei

In *Anableps*, the cone nuclei lie more sclerally (at the level of the external limiting membrane) and are larger; they tend to be more elongated and more irregular, often lobed; paler, with more dispersed, "tigroid" chromatin; and they have a longer fibre (figs. 32,33) than the rod nuclei. This is a very common finding but there are exceptions e.g., in the frog the

cone nuclei are closer to the outer plexiform layer than are the rod nuclei (Nilsson, '64c).

Inter-receptor Contacts and Rod-Knob Invaginations into Cone Inner Segments

Areas of contact between adjacent neuronal elements are found in the CNS generally. Direct contacts in *Anableps* photoreceptors are seen between inner segments of double cones (figs. 36,37,61-63,65,74,75) along their apposed membranes. Direct contacts are also seen between cone and rod synaptic terminals (fig. 104) and between rod and cone inner segments (fig. 62). There are often membrane densities in the two latter cases but there are also contact zones without any apparent membrane alterations.

Subsurface cisterns are found where there are contact zones in the double cones (figs. 90,91); where there are apposed rod and cone membranes (figs. 62,97); and they are especially prominent where rod cell 'knobs' actually invaginate into the cones, slightly scleral to the external limiting membrane (figs. 94-96).

This rod invagination of the inner segment is a novel finding and suggests particularly intimate interaction between the two cell types. Processes of cone cells were seen, albeit rarely, invaginated into rod

spherules by Missotten ('60), and also by Borovyagin ('66) who found direct contacts at the synaptic level in the pike, including 'finger-like outgrowths' at the synaptic level.

Inter-receptor contacts were first reported by Sjostrand ('58). They occur with or without membrane densities and are very widely reported at the synaptic level either by lateral expansions several microns long (Cohen, '63b; Borovyagin, '66) or between directly abutting synaptic terminals (Cohen, '60,'61,'63a,'63b,'65b; Fine and Zimmerman, '63; Kalberer and Pedler, '63; Nilsson, '63,'64a; Missotten, '65; Borovyagin, '66; Evans, '66; Hollenberg and Bernstein, '66; Morris and Shorey, '67; Uga *et al.*, '70; Hogan, *et al.*, '71). Inter-receptor contacts are also reported at the inner segment level, often involving membrane thickenings and densities (Carasso, '57; Pedler and Tansley, '63; Borovyagin, '66; Morris and Shorey, '67; Pedler and Tilly, '69; Uga *et al.*, '70). A synaptic function has been suggested by Cohen ('64), but no certain synaptic function has been demonstrated.

Inter-receptor contacts may simply be strengthening and attachment devices. The retina is an epithelial derivative and it may have the same sort of functional associations as epithelia elsewhere.

Desmosomal contacts of synaptic terminals are reported by De Robertis and Bennett ('55); Palay, ('56) and Gray ('59). Locket ('70a)saw tight junctions in the more scleral regions of the inner segment of fish. Uga *et al.*, ('70) in human retina reported a reduced intercellular space associated with apposing membrane areas that show increased thickness and electron-density, as in tight junctions. Similar junctional associations were seen in photoreceptor cells by Sjostrand ('58),Cohen ('61) and Dowling and Gibbons ('62).

There is also the suggestion that lateral inter-receptor contacts facilitate the integration of information by lateral inhibition which acts as a contrast-enhancing mechanism (Elenius and Heck, '57; Sjostrand, '58, Cohen, '63a).

There are known to be permeability barriers involving junctional complexes in the pigment epithelium (Bernstein and Hollenberg, '65a,'65b; Hollenberg and Ghosh (unpublished); Moyer '69) and at the external limiting membrane. Large molecular substances injected into the vitreous readily find their way into the retina but only as far as the outer limiting membrane (Peyman, *et al.*, '71). Between the two limiting membranes there is considerable diffusion and this is the region in which inter-receptor contacts are seen. Perhaps the inter-receptor contacts vitreal to the external limiting

membrane are important in diffusion processes.

The Synaptic Terminals

Synapses of the photoreceptor cells of vertebrate retinas were first described by Sjostrand ('53b, '53c, '58, '59), De Robertis and Franchi ('56), De Robertis ('58), and Ladman ('58). Subsequent studies have shown that their basic morphology is remarkably uniform in all classes of vertebrates, from fishes to primates, including cyclostomes with degenerate eyes (Holmberg, '71).

There are many reviews of photoreceptor synaptic structures (De Robertis and Lasansky, '61; Cohen, '63a, '69; Dartnall and Tansely, '63; Dowling and Boycott, '65, '66; Evans, '66; Dowling, '70; Kolb, '70). Fish horizontal cells and their connections, and the neuronal or glial nature of these cells, have been studied by Sjostrand ('53c, '58, '59, '61), Villegas ('60, '61), Engstrom ('63a), Villegas and Villegas ('63), Stell ('64, '67), Yamada and Ishikawa ('65), Borovyagin ('66), Pedler and Tilly ('66), Witkowsky and Dowling ('69).

The synaptic terminals of the photoreceptors have distinctive presynaptic organelles, the synaptic ribbons or semicircular lamellae in synaptic grooves (Gray & Pease, '71), associated with deeply inserted bipolar cell dendrites and horizontal cell processes (probably both axonal and dendritic). The synaptic ribbons were

first seen by Sjostrand ('53b) who called them 'rodlets'. There are also superficial conventional synaptic associations with the expected membrane densities, vesicle accumulations, and widened synaptic clefts.

Frequently, direct presynaptic inter-receptor contacts occur. Dowling and Boycott ('65) have suggested a presynaptic interaction between receptors. No definite synaptic function has been clearly demonstrated for these contacts.

The invaginations are very orderly. Each invaginated synaptic unit (Stell, '67) or triad (Missotten, '65) of a cone pedicle consists of a central bipolar dendrite and on either side of it are more deeply inserted processes of horizontal cells, probably from two different cells (Kolb, '70). These processes are presumed to synapse at the region of the base of the synaptic lamella and its arciform density (Ladman, '58). More superficially invaginated into this area are other processes from other bipolar cells which make conventional synapses (Kolb, '70). Opposite the edge of the synaptic ribbon are typical membrane thickenings. Each cone pedicle contains many triads, and some triads may even overlap to share elements, whereas the rod spherule has only one triad, or rarely, a few (Cohen, '60; Missotten, '65), with the invaginated dendrites from only one bipolar cell. The rod synaptic ribbon tends to be larger than

that of the cone. The anatomy of the synaptic unit suggests that bipolar dendrites and horizontal cell processes are activated together at the ribbon synapses along the bases of the receptors (Sjostrand, '53b,'58; De Robertis and Franchi,'56; Carasso, '57; Ladman, '58; Yamada *et al.*, '58; Cohen, '60; Okuda, '60; Stell, '65a,'67; Dowling and Boycott, '65,'66; Missotten, '65; Dowling, '66,'70; Hollenberg and Bernstein, '66; Dowling and Werblin, '69; Kolb, '70). Rarely, a lateral process from a cone may be enclosed in the invagination of the rod spherule (Missotten, '60). Cohen ('63a) suggests that the pit in the receptor base might in part act to shield the terminating processes from the activity of the neighbouring receptor bases.

Ladman ('58) described the synaptic lamella and its associated arcuate or arciform density continuous with the plasma membrane between the synaptic ribbon and the synaptic groove. He ascribed to it an important role in impulse transmission. Sjostrand ('58) introduced the term 'synaptic ribbon' which more accurately describes its shape. The ribbon has a pentalaminate structure of three dense lines and two pale ones (Lanzavecchio, '60; Cohen, '63b). The arciform density is a 3-pronged fork, with the prongs directed towards the synaptic ribbon. Matsusoka ('67) found that uranyl acetate staining obscures its fine structure. Opposite the synaptic groove, the postsynaptic membranes are

thickened and the gap widens to 200-400 Å (Peters *et al.*, '70).

Synaptic ribbons are found in spherules and pedicles and, in addition, they have been seen in the bipolar retinal cells (Kidd, '62; Fine and Zimmerman, '63; Dowling and Boycott, '65; Goodland, '66), in cochlear organ of Corti hair cells (Smith and Sjostrand, '61), in lateral-line receptors (Trujillo-Cenoz, '61; Barets and Szabo, '62; Hama, '65); in invertebrate eye neuropil (Trujillo-Cenoz, '65; Cohen, '69); in pineal receptors of Amphibians (Kelly and Smith, '64); in ampullary electric receptors in *Amiurus* (Mullinger, '64) and in the hair of the vestibular sensory cells (Wersall *et al*, '65).

Mitochondria are reported present in some visual cell synaptic terminals (in mammals: Ladman,'58; Cohen, '60,'61; Villegas, '60; Missotten, '65; Evans, '66; Hogan *et al.*, '71; and in the pedicles of scopelarchid fish; Locket, '71b), but they are not a common feature of the presynaptic terminal (Peters *et al.*, '70) and apart from the deep sea scopelarchids, they have not been found in fish receptor terminals. In *Anableps*, mitochondria are found only in a perinuclear (figs. 62,97) or in supranuclear position, in the myoid, and concentrated in the ellipsoid. In figure 97 a very large and

unusual mitochondrion is seen at the nuclear level. This was seen only twice.

Sjostrand ('53c) describes 'granules' in the photoreceptor synaptic terminals and Palade ('59) saw similar organelles in the neuromuscular junction. These 'granules' were identified and named synaptic vesicles (De Robertis and Bennett, '54, '55; De Robertis and Franchi, '56) and it was proposed that a specific transmitter substance is stored in these vesicles and released synchronously on the presynaptic membrane on arrival of nerve impulses (De Robertis and Bennett, '54, '55; Del Castillo and Katz, '56; De Robertis *et al.*, '70). Each synaptic vesicle represents a quantal unit of transmitter (De Robertis *et al.*, '70, De Robertis '71).

Synaptic vesicles are seen distributed throughout all visual cell synaptic terminals, and are often the only organelles present apart from the ribbon structure. Continuities between vesicles and the pre-synaptic membrane are reported (Westrum, '65). They are organized around the synaptic ribbon in a very orderly way,

halolike, parallel to it but distant from it (Cohen, '63a, -300-500 Å; Stell, '67, -100-200 Å; Hogan *et al.*, '71-250 Å; Cohen, '69, -200 Å) and the intervening space contains fibrillar material which seems to attach to both the vesicles and the ribbon (Gray and Pease, '71; Hogan *et al.*, '71).

A difference between the rod and cone terminals was reported by Evans ('66) in *Rana*, chick and tortoise. She found that the synaptic vesicles of the two terminals differ in size (300-500 Å in the cone, 500-600 Å in the rod), shape, electron density, and concentration per unit area. In all rod retinas she found only one class of synaptic vesicles. Morris and Shorey ('67) found more vesicles in the rod terminals of chicks than in the cones, and in addition, the cone vesicles varied more in size, density and distribution. In *Rana*, Nilsson ('64c) and Dowling ('68) did not find any differences between rod and cone vesicles, in osmium fixed material. Dickson and Hollenberg ('71) reported that in the newt the cytoplasm of the rod spherule is darker and denser than that of the cone and they suggested this was shown up by glutaraldehyde fixation and obscured in osmium fixation. They found that the rod synaptic vesicles were approximately 490 Å, while the cone vesicles were 330 Å.

In *Anableps*, the rod and cone synaptic terminals

always look different and can also be easily identified by their association with differing nuclei, at different levels. The cone terminal is larger and appears much paler staining with a relatively sparse population of synaptic vesicles, while the rod spherule is smaller, and contains a denser concentration of synaptic vesicles. There is no evidence that their vesicles are not of the same size. In general, in *Anableps* the rod cytoplasm of the inner segment appears darker than that of the cones and the difference in appearance extends to the terminals (figs. 32,33,102,103,104).

Villegas ('61) reported that in Primates the rod synaptic terminal has a greater concentration (190-250/μ_2) of vesicles and looks darker than that of the cones (170/μ_2), but that in the fish, *Centropomida*, the vesicles were alike in size (500 Å) and concentration (200-220/μ_2), in both spherules and pedicles. But Villegas ('60) reports the cone terminal in the fish *Centropoma* as darker than the rod, presumably due to cytoplasmic density. Evans ('66) suggested that one transmitter might be involved in cones and another in rods, on the basis of the consistent differences she found. However, there is not much agreement in the reported sizes of the synaptic vesicles. Synaptic vesicles of the CNS generally are of the order of 400-500 Å (De Robertis *et al.*, '70; Peters *et al.*, '70). The synaptic vesicles of the visual cells usually fall

within this range (Table 3).

Palay ('56) considered that the synaptic vesicles arise from the endoplasmic reticulum and that the Golgi and neurotubules have continuities.

Another view is that the synaptic vesicles originate from the plasma membrane of the presynaptic bag. Pinocytotic vesicles occur there and coated vesicles arise. The smooth vesicles emerge from the coated vesicles, leaving the empty shells near the sites of origin, and are guided to the dense projections of the presynaptic membrane or to the synaptic lamella of the photoreceptor terminal. The filamentous material effects the contacts between the vesicles and the ribbon. Just as dense projections guide the vesicles to the specific sites on the presynaptic membrane, so the synaptic ribbon guides the vesicles in an orderly progression to the synaptic zone (Gray and Pease, '71). The vesicles arrive at the innermost end of the synaptic ribbon (Bunt, '71) and discharge at the outermost, at the synaptic cleft. Furthermore, Gray and Pease ('71) suggest that the arciform density serves to anchor the ribbon to the presynaptic membrane at the synaptic gutter and that the ribbon has two slopes to the rim of the trough which guide the vesicles of the ribbon on to the presynaptic membrane. Vesicles at the base of the receptor are seen close to or in contact with the presynaptic membrane in their illustrations, in our figures

102,103 and in figure 23 of Cohen ('63b).

The ribbon, it is postulated, directs two streams of vesicles at the same time, one towards each of the postsynaptic elements, and the transmitter diffuses down the gap to the bipolar processes, only 0.3μ away (rat, guinea pig). The shape of the cleft is such that the transmitter is conserved and the area of formation of complex vesicles is close to that of synaptic vesicle discharge, so recycling is possible.

This hypothesis of Gray and Pease ('71) is a very attractive one, giving function to the ribbon and suggesting an orderly way of contacting several postsynaptic elements, but as yet, the transmitter substance is not known with certainty and there is no physiological evidence for chemical transmission at this synapse.

Dickson et al ('71) found acetylcholinesterase at the synaptic contact sites of the rod and cone terminals but not within the photoreceptor cells themselves.

In the perch, Borovyagin ('66) reported club-shaped expansions of the horizontal cells with postsynaptic thickenings, which sometimes appeared as discontinuous osmiophilic globules. Such structures are seen in the cone pedicles of *Anableps* but the cell type to which

they belong has not been identified (fig. 102). Pedler and Tilly ('66) saw, in serial section reconstruction of pigeon retina, that the horizontal cells make contact with a terminal ring containing protrusions, a claw-shaped array with end bulbs, of lone long invaginated horizontal cell process with many processes off it.

TABLE 3

Rod Vesicle Size Å	Cone Vesicle Size Å	One size only reported Å	Animal	Author	Fixation	Other Comments
490	330		Newt	Dickson *et al.* ('71)	Glutaraldehyde	cytoplasm denser darker in rods
		400-600	Goldfish	Stell ('67)	osmium	granules 200-300 Å in cone spherules
	300		Centropoma(Telost)	Villegas ('60)	osmium	
		500	Centropomidae	Villegas ('61)	osmium	same conc. rods and cones 200-220/u_2
	250-300		Labrid fish	Engstrom (63a)	osmium	
		300-500	review-article	Cohen (63a)		
		300-500	pigeon	Cohen (63b)	osmium, permanganate	
	300		American Gray Squirrel	Cohen (64)	osmium	all-cone retina
500-600 larger vesicles more crowded	300-500 smaller vesicles less crowded. Some cone vesicles:		frog, chick, tortoise	Evans ('66) Evans ('66) Evans ('66)	osmium osmium osmium	rod vesicles more uniform in shape and all are pale-centred; some cone vesicles are dense - centred
		800-850				
looks darker; vesicles more concentrated		300-400 300-400	monkey Man	Villegas ('61)	osmium: potassium dichromate	Cone: 170/u_2 Rod:Monkey:190/u_2 Rod:Man:250/u_2

TABLE 3 - Cont'd

Rod Vesicle Size Å	Cone Vesicle Size Å	One size only reported Å	Animal	Author	Fixation	Other Comments
400-600	450-600			Missotten ('65)	many	
av.-386	av.-338		albino rabbit	De Robertis and Franchi ('56)	osmium	
spread -200 650	spread -150 550					
		400-500	hagfish	Holmberg ('71)	formaldehyde glutaraldehyde osmium	
		500-600	mammals	Lovas ('71)		
		450-600	lamprey	Yamada *et al.* ('67)	glutaraldehyde	

SUMMARY

The fish are remarkably sensitive to movement overhead.

There is a prominent horseshoeshaped choroidal gland in the ventral half of the eye, and hyaloid vessels on the vitreal surface of the retina.

The ventral half of the retina is thicker, and its inner nuclear layer is especially thick and crowded with nuclei and the very large inner plexiform layer has two strata. There is also a greater number and density of ganglion cells in the ventral half of the retina.

There are photomechanical movements, the cones elongating in the dark, and rods in the light.

There is a distinct mosaic, a linear arrangement of alternating rows of rods and cones in the periphery of the retina, and a square pattern (of four double cones and five single cones) in the central retina.

The photoreceptors are very clearly divided into two types, (a) very elongated cylindrical rods with very long and narrow myoids and very long outer segments

(b) shorter, stockier, squatter, fatter cones with short conical outer segments. There are double equal cones and slightly shorter single cones and, rarely, very short single cones. The outer segment discs differ in the two classes of photoreceptors and in the two members of the double cone pair. There is one deep incisure in the rod outer segment discs.

The pigment-laden pigment epithelial cell processes surround the outer segments and much of the inner segments, scleral to the external limiting membrane. Contact with the inner segments is direct. Long calycal processes and the accessory outer segment separate the cone outer segment from the pigment epithelial processes except perhaps at the very apex. Rod calycal processes are relatively short. Over much of their lengths the rod outer segments may be in direct contact with pigment epithelial processes. Many rod outer segments abut on each other in groups without intervening pigment epithelial processes; and rod inner segments, rod outer segments and cone outer segments may abut on each other directly.

The calycal processes contain longitudinal fibrillar material continuous with that of the inner segment microfibrillar border, especially marked in the ridges. The inner segments are not smooth cylinders but are grooved and ridged on their surfaces, most markedly so in the cones. The fins and the calycal processes appear to be continuous with these ridges.

The cytoplasm of the rod inner segment and of the

terminal is denser and darker than that of the cones.

There is only one basal body and one cilium in each photoreceptor cell and ciliary stalks are absent. The rod cilium is longer, more prominent, much more deeply inserted and the niche within which it lies is lobulated. The single cone cilium is very short and associated also with the accessory outer segment.

The rod ellipsoid mitochondria are arranged very regularly to form a hilum around the cilium. The rod mitochondria are constant in appearance. The cone ellipsoid mitochondria transform in a vitreal to scleral gradient, into the distal oil-droplet. The transformation involves hypertrophy, fusions, increase in matrix density, and disorganization of the cristae. Where no recognizable oil-droplet is present, its position in the cone is occupied by very enlarged and transformed mitochondria.

The vitreal end of the cone ellipsoid is very vesicular in appearance and is the 'nursery' where it seems that mitochondria are assembled.

The Golgi apparatus of the cones is not immediately supranuclear in position but is near the ellipsoid mitochondria.

Subsurface cisterns are found closely parallel

to the apposed membranes of the double cones, and where rod and cone inner segments and where rod and cone synaptic terminals abut on each other. Small knob-like portions of the rod cells invaginate into the cones immediately scleral to the external limiting membrane and their subsurface cisterns are particularly prominent.

There are cytoplasmic fins extending from the most vitreal end only of the cone inner segments immediately scleral to the external limiting membrane and these interdigitate with each other and with Müller cell processes.

The accessory outer segment is a constant and prominent organelle of all the cones. It is associated with the cone cilium and is attached to the outer segment by cytoplasmic bridges.

The larger cone nuclei are scleral, often protruding through the external limiting membrane. They are more elongated and often lobed, paler staining with more dispersed "tigroid" chromatin than the rod nuclei. The cone fibre connecting to the synaptic terminal is longer than that of the rod.

There are inter-receptor contacts between rod and cone inner segments and rod and cone synaptic terminals; cones abut on cones and on rods; and rods abut on rods at their inner segments.

There are whorls of thin Müller cell processes around the synaptic terminals, especially prominent around the cone pedicles.

The synaptic terminals of the cones are much larger than those of the rods and they contain many triads, whereas the rod spherule has one triad. The rod spherule is very dark compared to the cone pedicle. The synaptic vesicles of the two terminals are of the same size, but they are densely concentrated in the rod spherule and are relatively sparse in the cone pedicles. The rod cytoplasm is denser. Synaptic vesicles are seen in contact with the base of the synaptic ribbon at the synaptic groove.

The rods then differ from the cones in

(1) outer segment size, shape, disc structure and the presence of an incisure;

(2) the inner segment size, shape and surface ridging;

(3) the details of the connecting cilium;

(4) the calycal processes;

(5) the appearance of the mitochondria of the inner segment;

(6) behaviour in photomechanical movements;

(7) the presence of the oil droplet; (in the cone only)

(8) the appearance and position of the nuclei;

(9) the synaptic terminals and the Müller cells around these;

(10) the absence of an accessory outer segment in the rods;

(11) the denser & darker cytoplasm of the inner segment and

synaptic terminal of the rod compared to that in the cone.

The photoreceptor cells do not differ in structure in ventral and dorsal retinas and the binocular vision of *Anableps* can be accounted for by its general ocular anatomy.

Figure 113 summarises the salient features of the rod, single cone and double cone of Anableps anableps.

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Abbreviations

a..... accessory outer segment

b..... bipolar cell process

c..... cone

C..... corneal pigment band

ce..... cone ellipsoid

cg..... choroidal gland

ci..... cilium

cis..... cone inner segment

cm..... cone myoid

cn..... cone nucleus

co..... cone outer segment

cp..... calycal process

ct..... cone pedicle

dc..... double cone

e..... external limiting membrane

f..... fins

g..... ganglion cell layer

G..... Golgi apparatus

h..... horizontal cell process

hy..... hyaloid blood vessels

i..... rod invagination

I..... horizontal iris flaps

in..... inner nuclear layer

ip..... inner plexiform layer

is..... inner segment

L..... lamellated bodies

m..... mitochondrion

M..... Müller cell

mc..... membrane complex

n..... nucleus

o..... oil-droplet

op..... outer plexiform layer

os..... outer segment

p..... pigment epithelium

P..... pigment granule

pn..... photoreceptor nuclei

r..... rod

R..... invaginated processes

re..... rod ellipsoid

ri..... rod inner segment

rm..... rod myoid

rn..... rod nucleus

ro..... rod outer segment

rt..... rod spherule

s..... sclera

sc..... single cone

sr..... synaptic lamella or ribbon

su..... subsurface cistern

t..... receptor terminal

Figure 1. Photograph of *Anableps anableps*, swimming in a tank, showing the protruberant eyes and the larger dorsal (aerial) pupil.

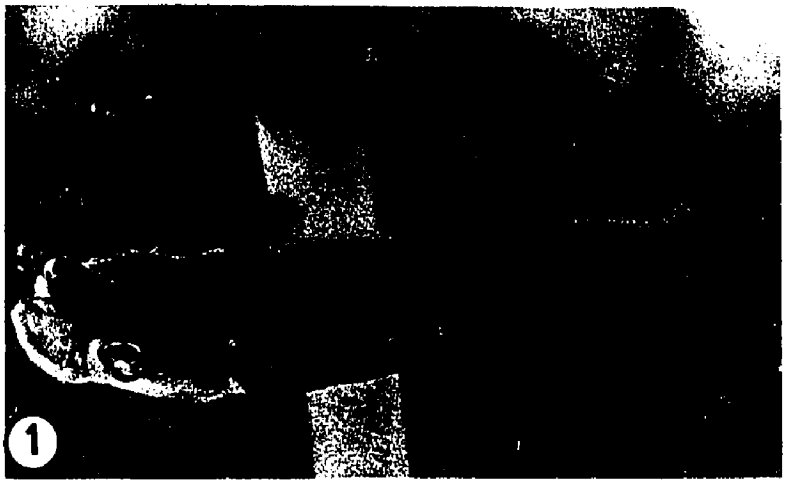
X 2

Figure 2. Photograph of *Anableps anableps*, at the water-surface, showing the smaller ventral (water) pupil. The fish is reflected at the water surface (arrows).

X 4

Figure 3. Photograph of part of the head of *Anableps* to show a side view of the eye, with its horizontal corneal pigment band (C), the horizontal iris flaps (I) and the two pupils. The line represents the water level.

X 8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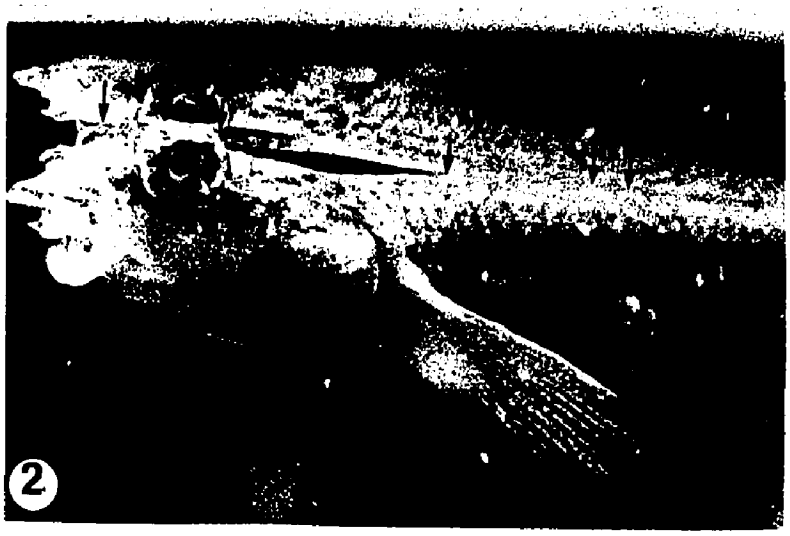

2


3


1


2

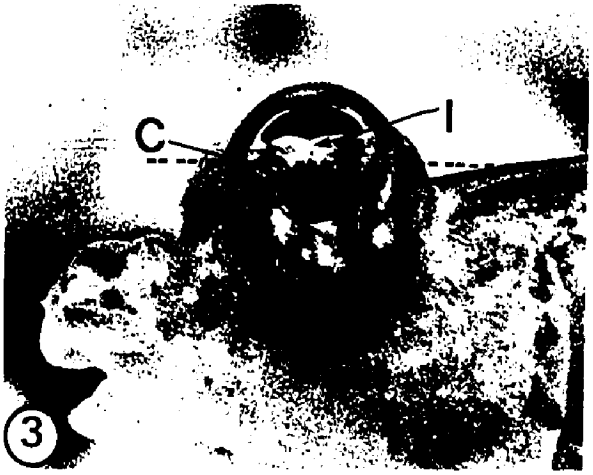
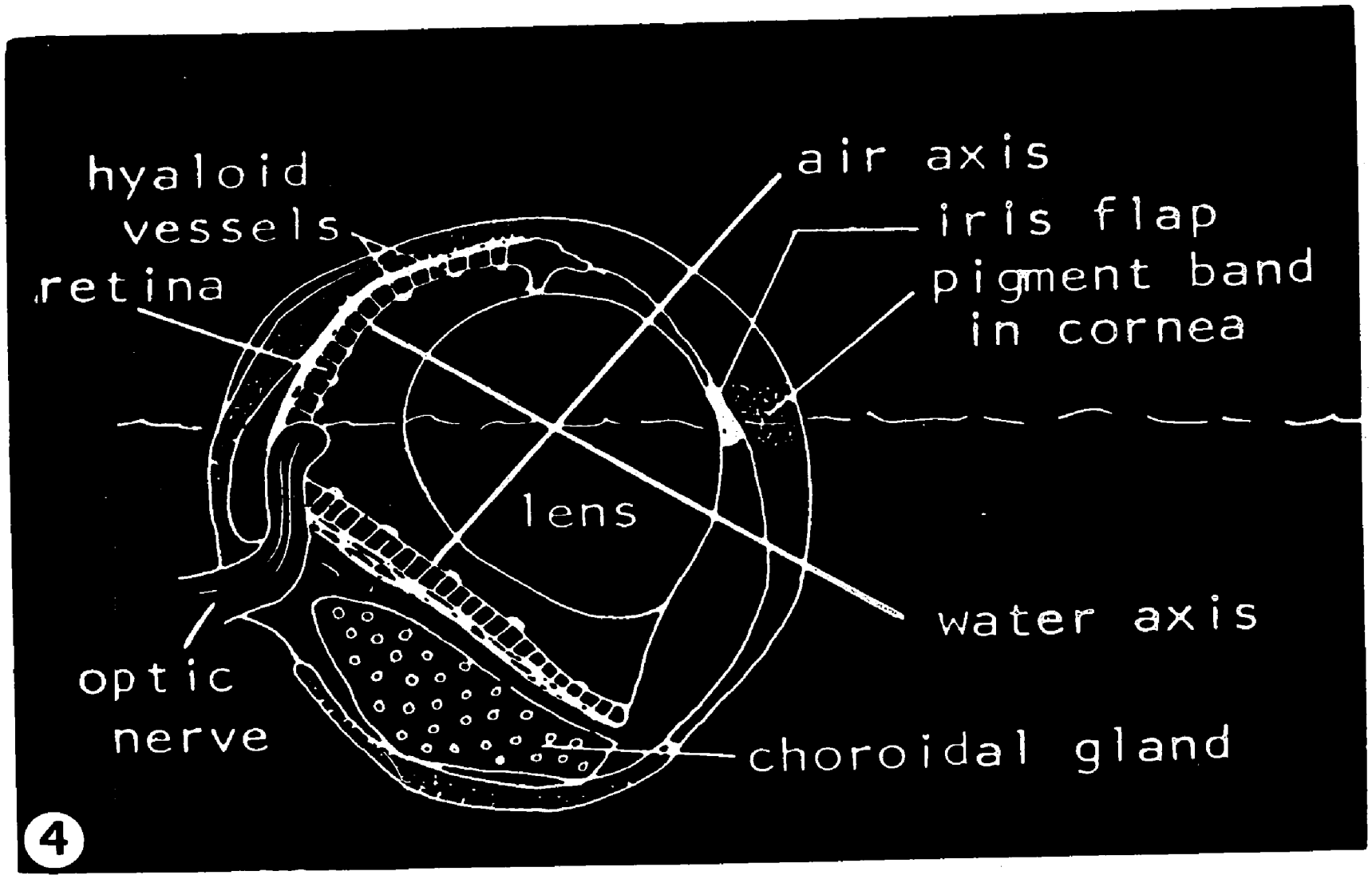
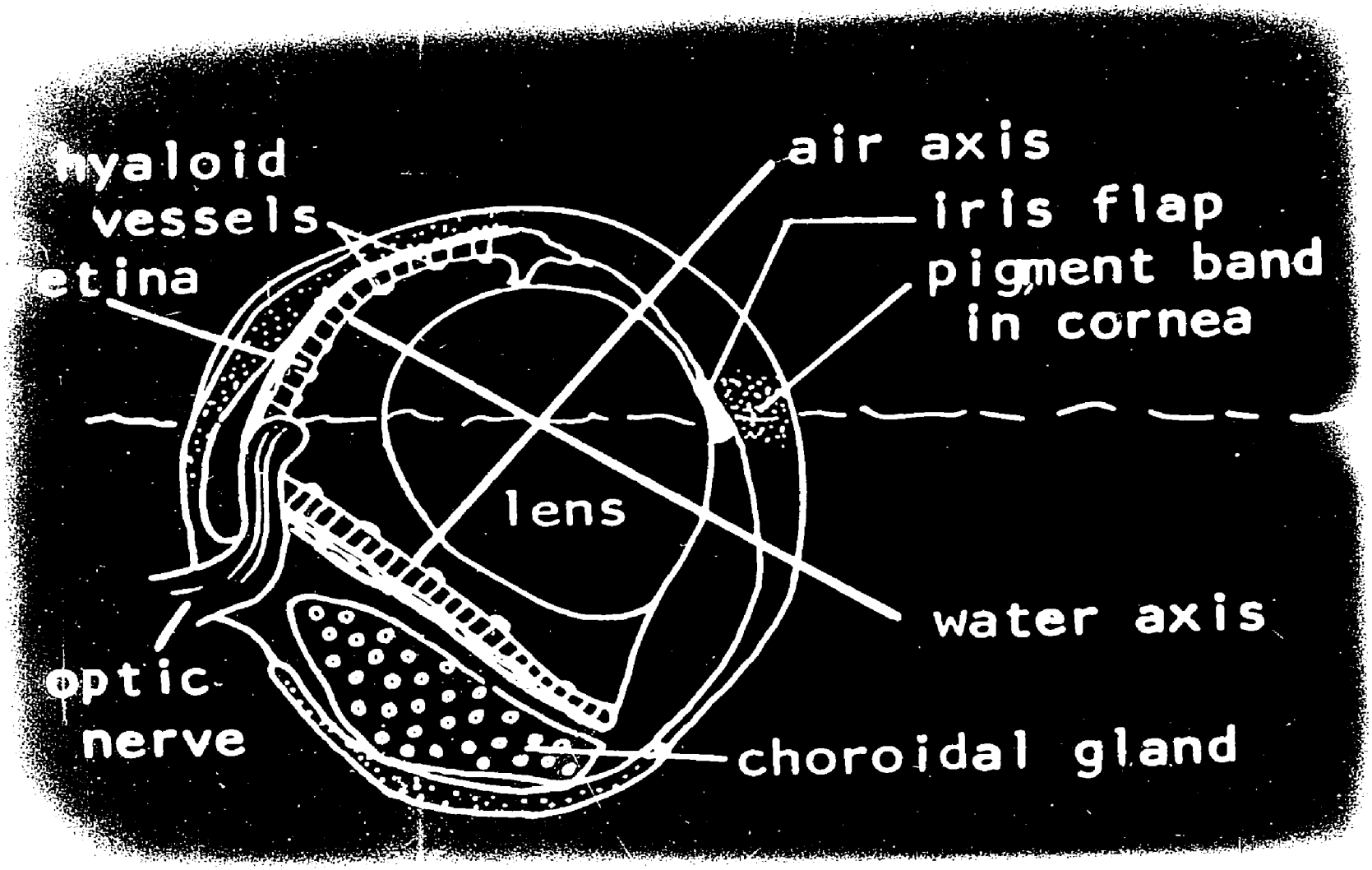

C
I
3

Figure 4. Diagram of a vertical section through the eye showing the greater thickness and curvature of the dorsal cornea, and its smaller anterior compartment. The line represents the water level.


hyaloid
vessels
retina
air axis
iris flap
pigment band
in cornea
lens
water axis
optic
nerve
choroidal gland
4


hyaloid
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iris flap
pigment band
in cornea
lens
water axis
optic
nerve
choroidal gland

Light micrographs of light-adapted retinas

Figure 5. The ventral retina is shown, in horizontal section, to the left, and the large choroidal gland (cg) to the right. The cartilagenous sclera (s) is visible on the extreme right.

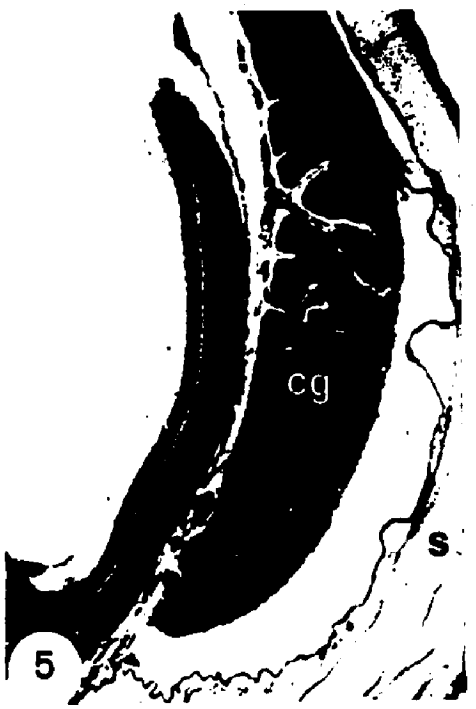
X 144

Figure 6. This half micron section of epon-embedded retina shows several double cones (dc) and a single cone (sc). Note that in the double cone (dc) which is labelled, the two outer segments stain differently. Note also the dense oil droplets (o) of the cones, and the position of the rod outer segment (ro). Photoreceptor nuclei (pn) and receptor synaptic terminals (t) can be identified. Pigment epithelial cell processes (p) are prominent and surround the cone ellipsoids. Note the dark line (the apposed membranes) demarcating the two members of the double cone (dc), and the cone nucleus (n) protruding through the external limiting membrane.

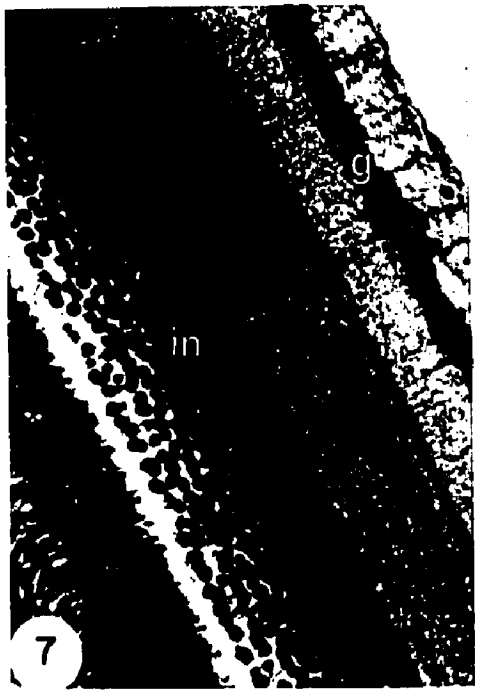
X 2,200

Figures 7,8. The ventral (7) and dorsal (8) portions of the retina of a single eye are shown here at the same magnification. Note the greater overall thickness of the ventral retina, which has a wider inner nuclear layer (in) with many more nuclei, a greater thickness of the inner plexiform layer (ip), and a greater density of ganglion cells (g). The inner plexiform layer of the ventral retina has two strata. Fixed in Bouins.

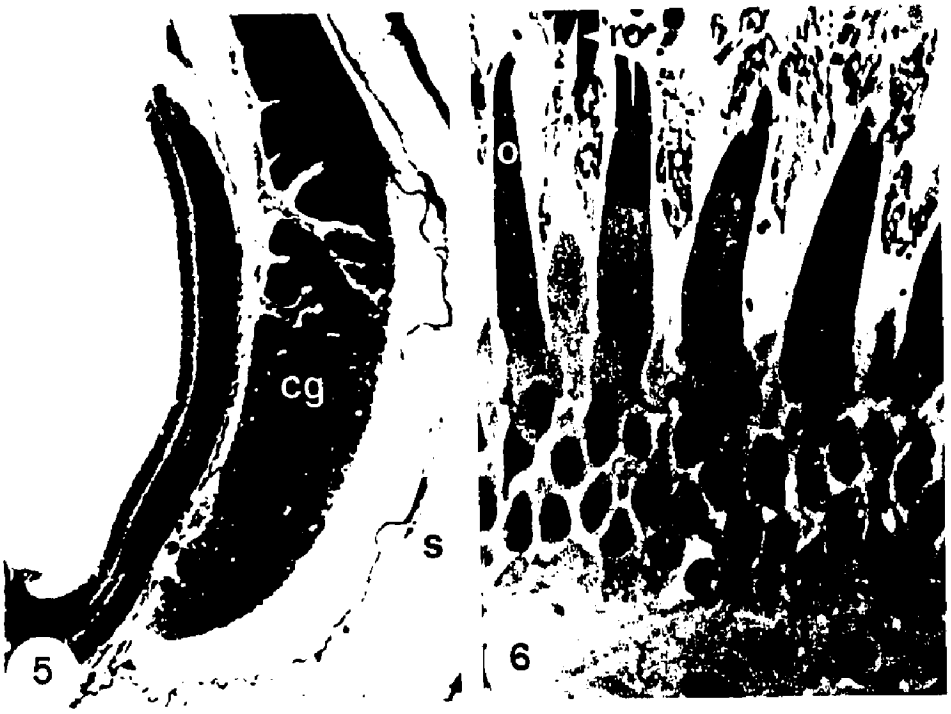
X 510

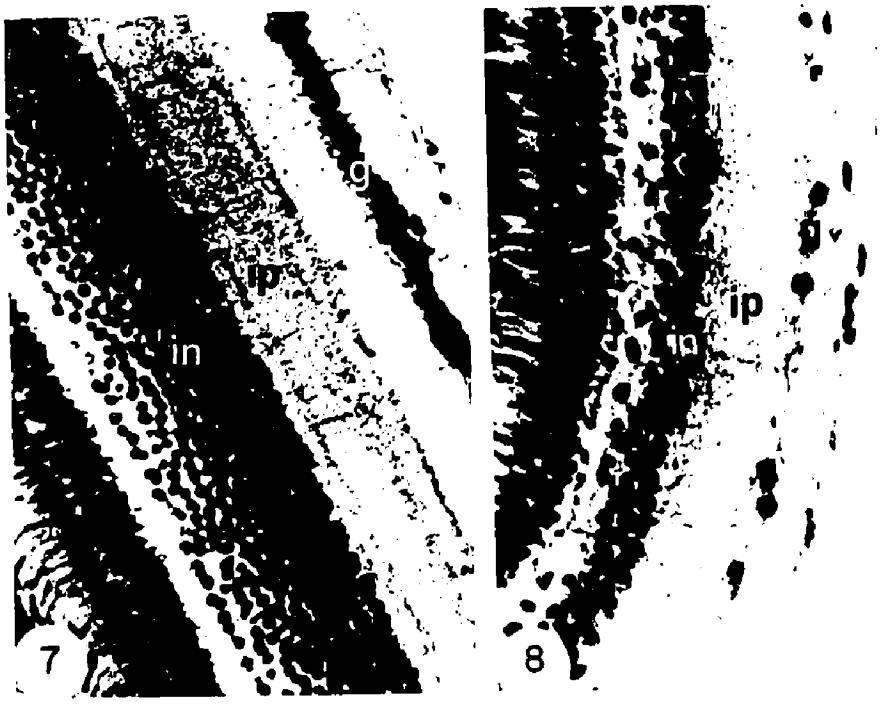

cg
s
5


o
6


g
in
7


in
8


cg
s
5
o
6


g
ip
in
7
ip
8

Light micrographs of light-adapted retinas

Figure 9. A half-micron section of epon-embedded retina showing cones (c), rods with their outer segments (ro) extending far beyond those of the cones. Pigment epithelial cell processes (p) laden with pigment granules can be seen between the cones. Cone nuclei extend through the external limiting membrane (e).

X 1,400

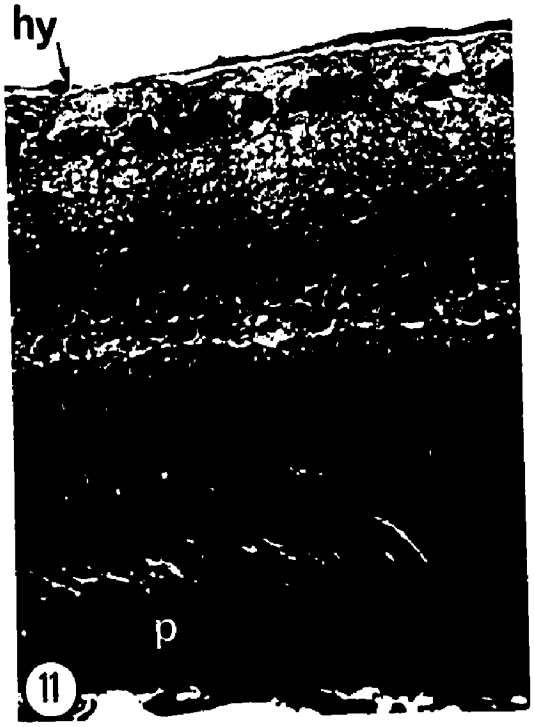
Figure 10. A half-micron section of epon-embedded retina showing cones (c) with their nuclei (n) extending through the external limiting membrane. There are abundant pigment granules in the pigments epithelium (p). One cone nucleus is undergoing mitosis (arrow).

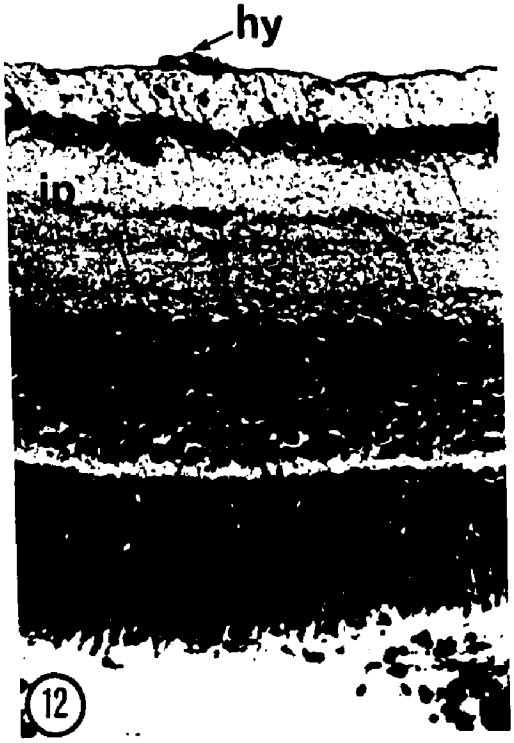
X 3,500

Figures 11,12. The ventral (12) and dorsal (11) portions of retina showing the hyaloid blood vessels (hy) on the vitreal retinal surface. Note the abundant pigment epithelium in 11. Note the differences described in figures 7 and 8. Fixed in Bouins.


9

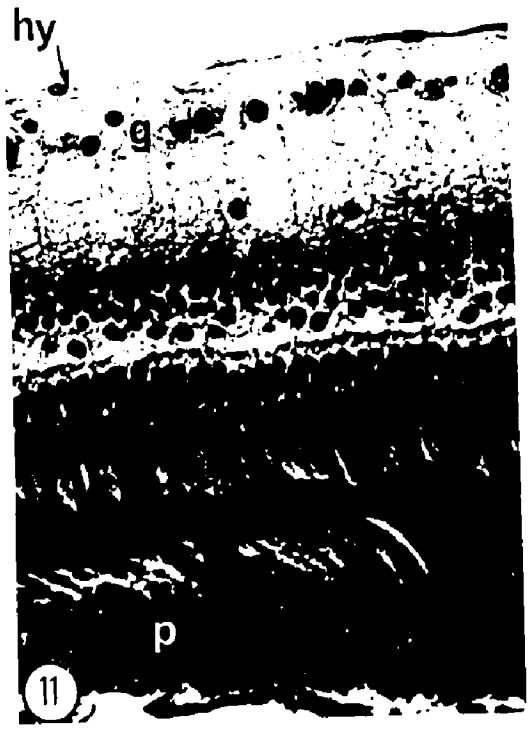

c
n
10


hy
p
11


hy
ip
12

9

10



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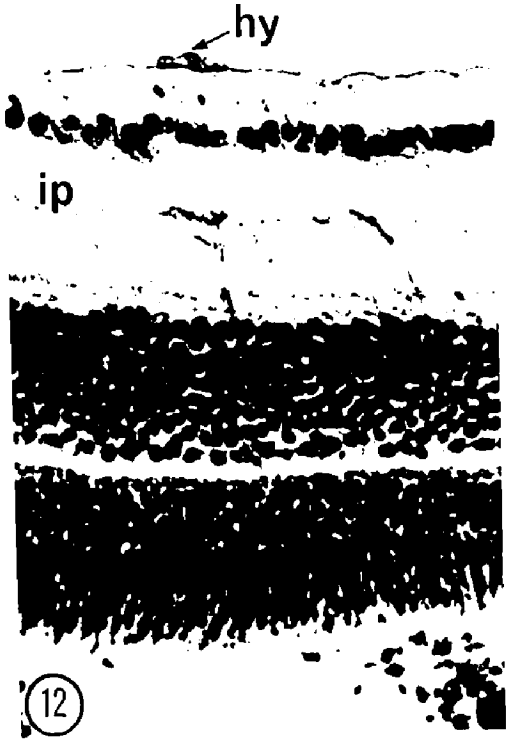


12

Scanning electron micrographs

Figure 13. The dorsal retina in the light-adapted state. Several cone photoreceptor cells and one double cone (dc) can be seen throughout almost their entire lengths from synaptic pedicles (below) to outer segments (above). (See figures 21 and 22). A displaced rod (r) lies across some of the cone inner segments. Note the bulge (arrow) produced by the oil droplet in the double cone inner segment (dc); and the longitudinal surface folds of the inner segment, and the fins (f).

X 8,000

Figure 14. A more scleral portion of the same specimen shown in figure 5. The retinal pigment epithelium (p) occupies more than half of the upper part of the micrograph. The lower half of the micrograph shows rod outer segments (ro) lengthened in the light-adapted state, and several cones (c).

X 4,200

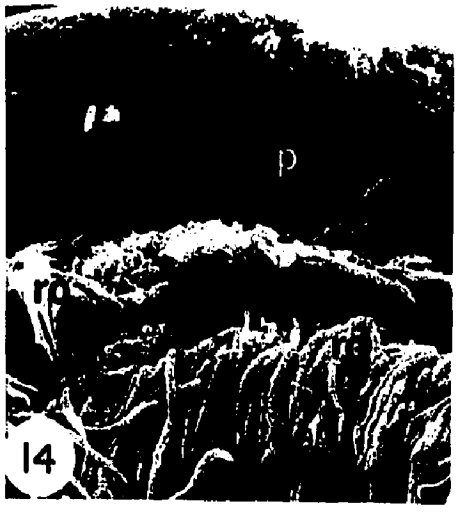
Figure 15. The ventral retina, light-adapted. Note the great length of the rod outer segments (ro).

X 4,000

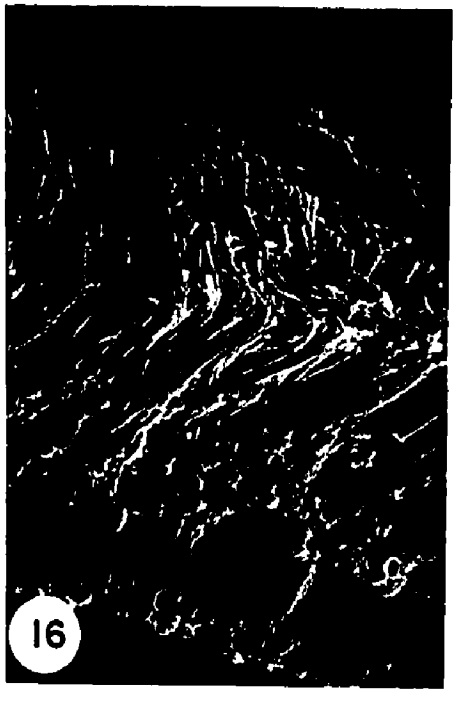
Figure 16. The ventral retina, dark-adapted. The rod outer segments (ro) are somewhat shorter and squatter than those shown in figure 15.

X 4,000

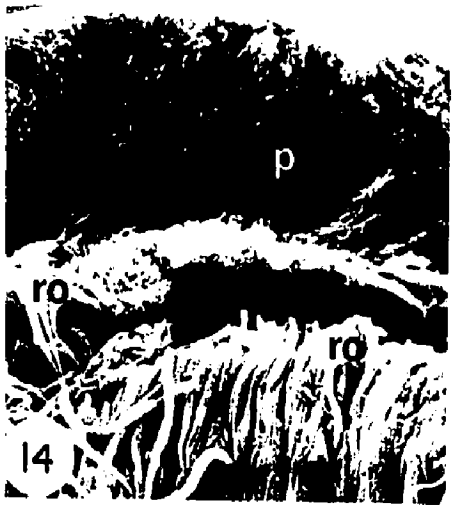

13


p
14


15


16


r
r
r
13


p
ro
ro
14


15


16

Scanning electron micrographs

Figure 17. Rod inner and outer segments (ri, ro) and cone inner and outer segments (co, cis) from a specimen of light-adapted dorsal retina are shown. The rod inner segments (ri) appear as long, thin, tapering cylindrical structures. The cones (c) are larger, with plump inner segments of more uniform diameter (cis) and short outer segments (co). The calycal processes (cp) of the cone form a palisade around the outer segment. Note the longitudinal ridges on the surface of the cone and rod inner segments.

X 21,700

Figure 18. Rods (r) and cones (c) from a dark-adapted ventral retina. The rod outer segment (ro) appears as a thin cylinder of uniform diameter. The cone inner segment (cis) and cone outer segment (co) are more elongated than in the light-adapted state. Note the double cone (dc) in the center of the micrograph.

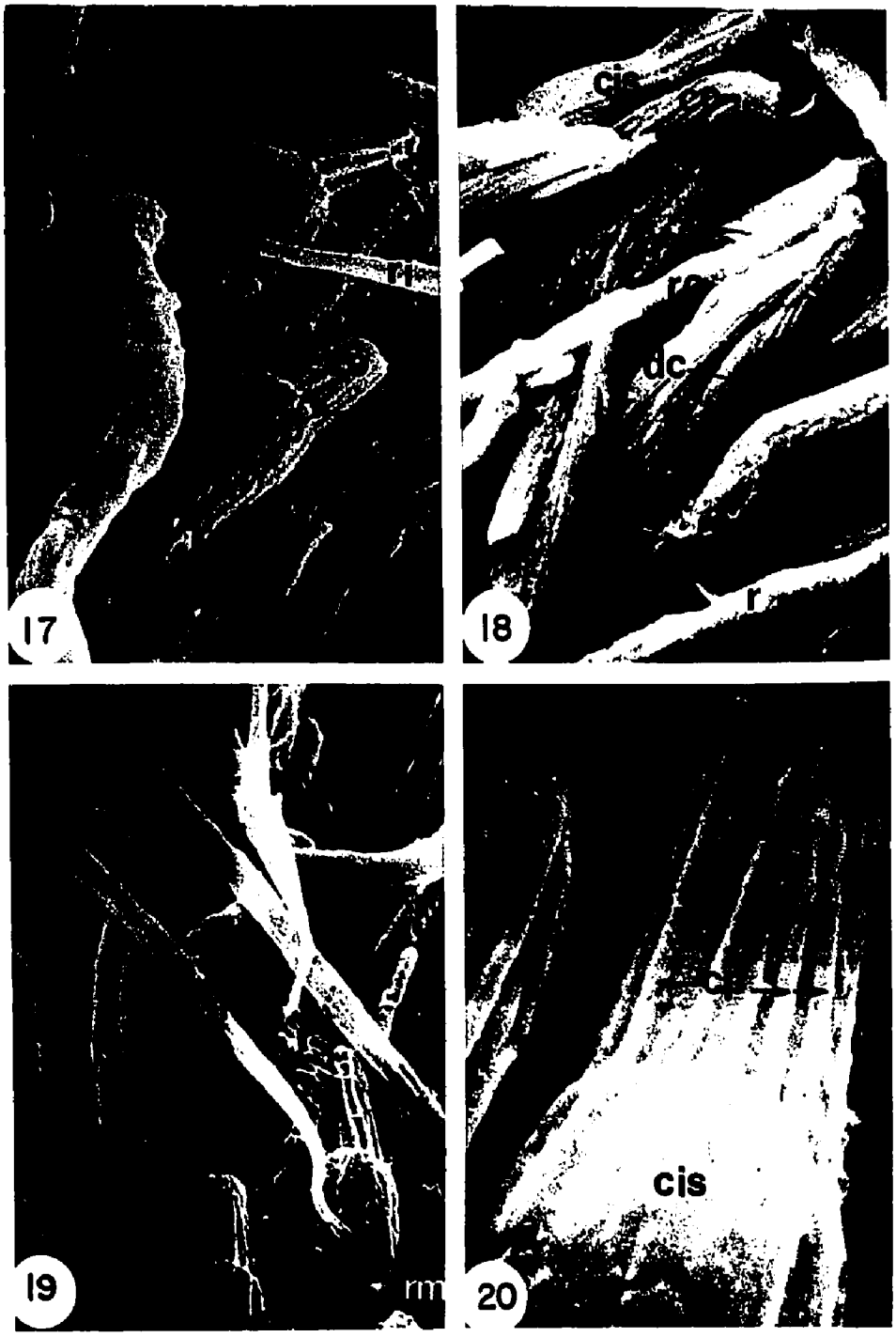
X 21,300

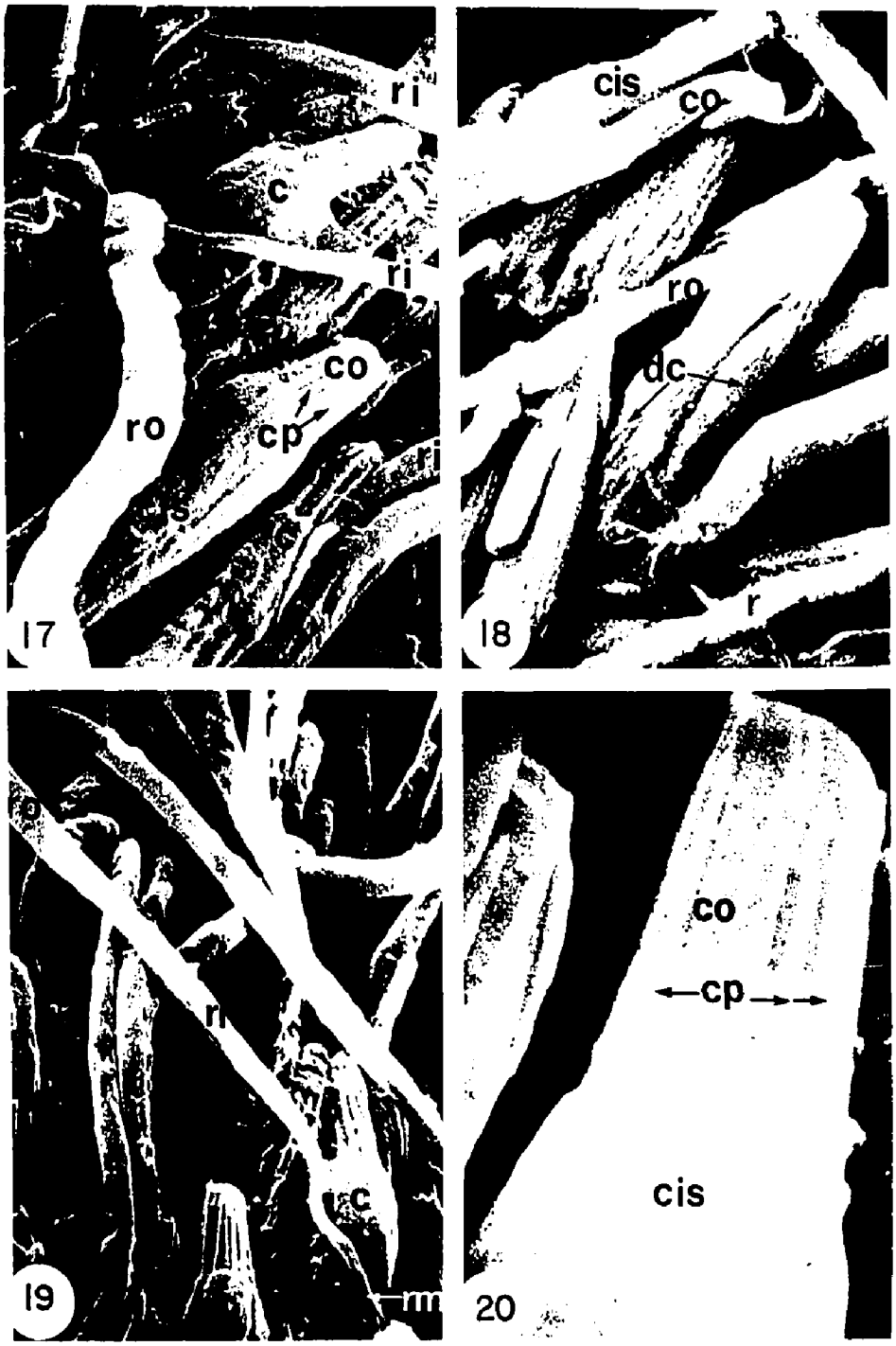
Figure 19. Rods (r) and cones (c) from the light-adapted dorsal retina. The rods lengthen in the light-adapted state and extend much further sclerally (above) than the cones. Note the tapering, narrow rod myoid (rm), the rod inner segment (ri) and the wide rod outer segment (ro).

X 19,500

Figure 20. Enlargement of figure 19 showing the cone outer segment (co) surrounded by calycal processes (cp) originating from the cone inner segment (cis) at the outer-inner segment junction. Note the longitudinal ridges on the surface of the cone inner segment, which appear to be continuous with the calycal processes.

X 83,000


17
18
19
20
cis


ri
c
ri
co
ro
cp
ri
17
cis
co
ro
dc
r
18
ri
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m
19
co
cp
cis
20

Scanning electron micrographs

Figures 21,22. Cones are shown from their outer segments (co) to their nuclei (n) and synaptic terminals (t). Note the tapering inner segment (myoid) of the rod (r); the bulge of the oil-droplet of the cone (o); and the cylindrical pigment granules (p). The longitudinal ridges (arrows) of the cones are much more prominent than those of the rods generally. Sometimes, the rod inner segment ridges are distinct also (ri). Broken off parts of the broad rod outer segment (ro) can be seen.

X 30,000


ro
21
o
p
ro
22


ro
r
r
r
r
co
o
p
21
r
r
r
r
r
o
p
dc
c
ro
22

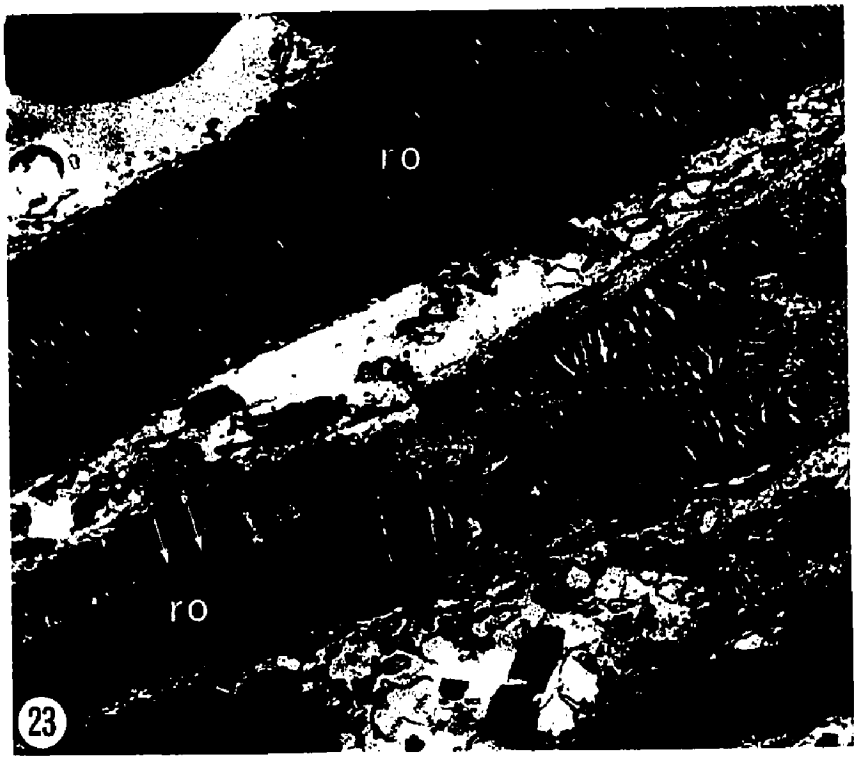
Transmission electron micrographs

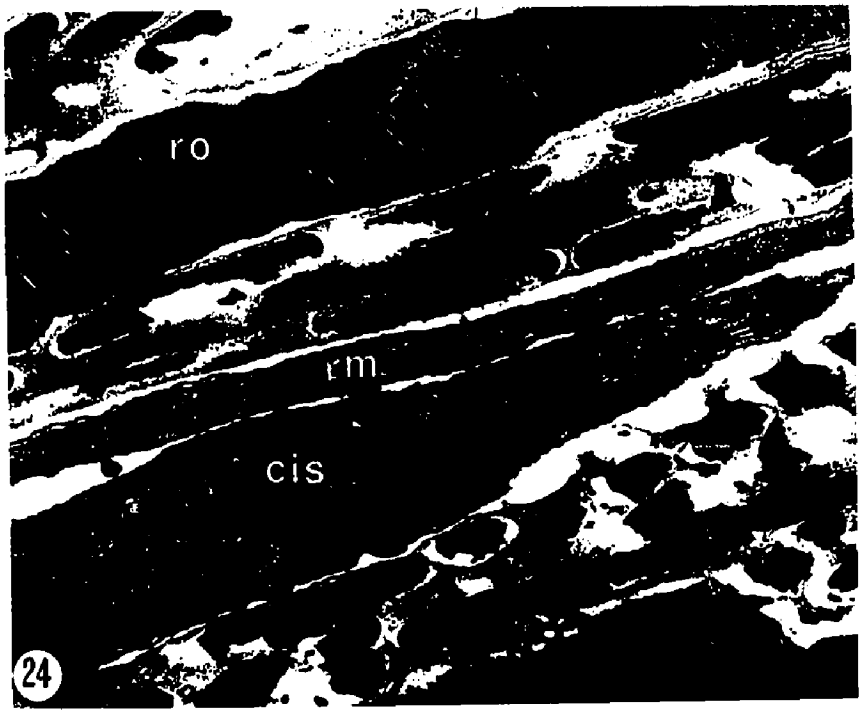
Figure 23. Longitudinal section of rods from ventral retina. Note that the rod inner segment (ri) is about the same width as the rod outer segment (ro). The single incisure is apparent (arrows). Note the cilium, and the mitochondria of the ellipsoid.

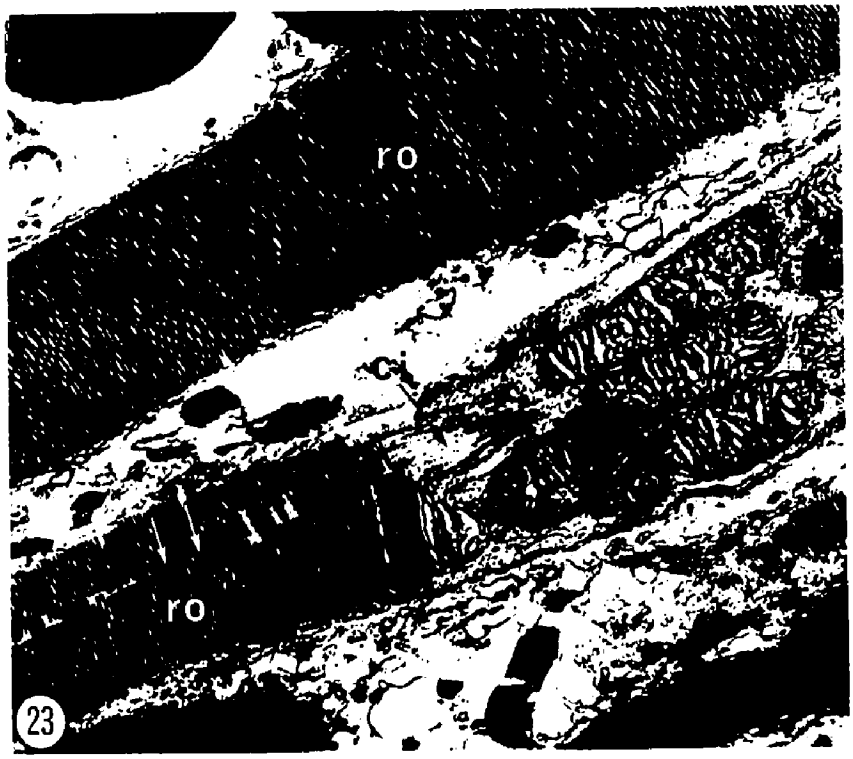
X 17,300

Figure 24. Longitudinal section of rods from the dorsal retina showing rod outer segment (ro), and the very narrow rod myoid (rm). Note that the myoid has a central area with cisternae and a peripheral border with microfibrils. The cone ellipsoid (cis) shows an increasing size gradient (from right to left) of the mitochondria.

X 12,200


ro
ro
23


ro
rm
cis
24


ro
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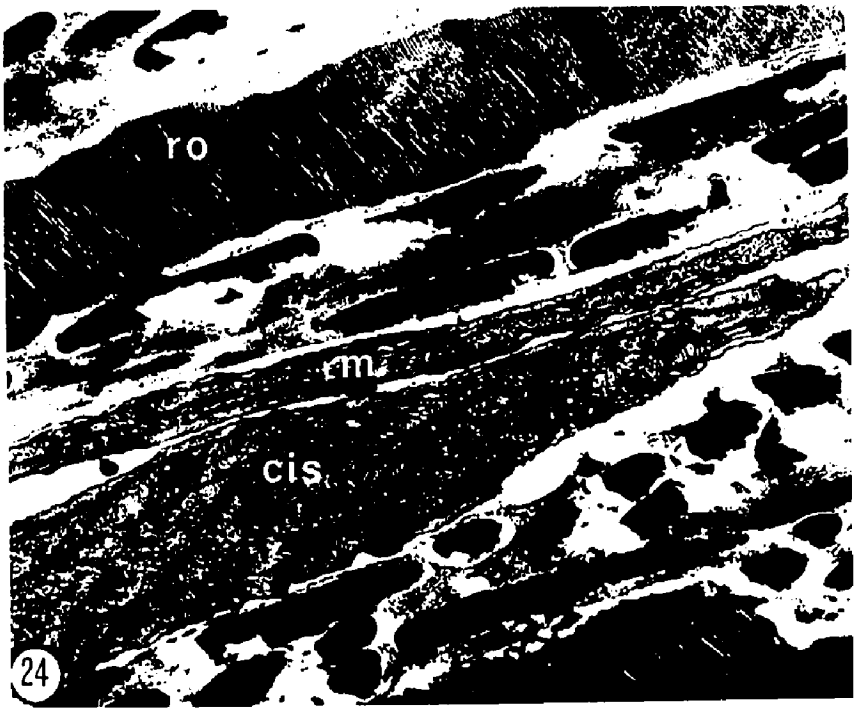
23


ro
rm
cis
24

Transmission electron micrographs

Figure 25. A portion of a rod, from the ventral retina, shown in longitudinal section. The rod outer segment has a single incisure (arrow). Note the cilium (ci) connecting the inner and outer segment. The ellipsoid is packed with mitochondria (m) which display distinct cristae and intra-mitochondrial granules.

X 15,900

Figure 26. Pigment epithelial processes and rod outer segments from the dorsal retina are shown in cross section. Each rod outer segment (ro) has a single incisure (arrow) which penetrates about a third of the diameter of the rod and often ends in a small bifurcation. Towards their scleral ends the rod outer segments lack the surrounding calycal processes. Note that the two outer segments of a double cone (co) differ from each other in appearance. The pigment epithelial processes (p) contain abundant pigment granules and lamellated bodies (L).

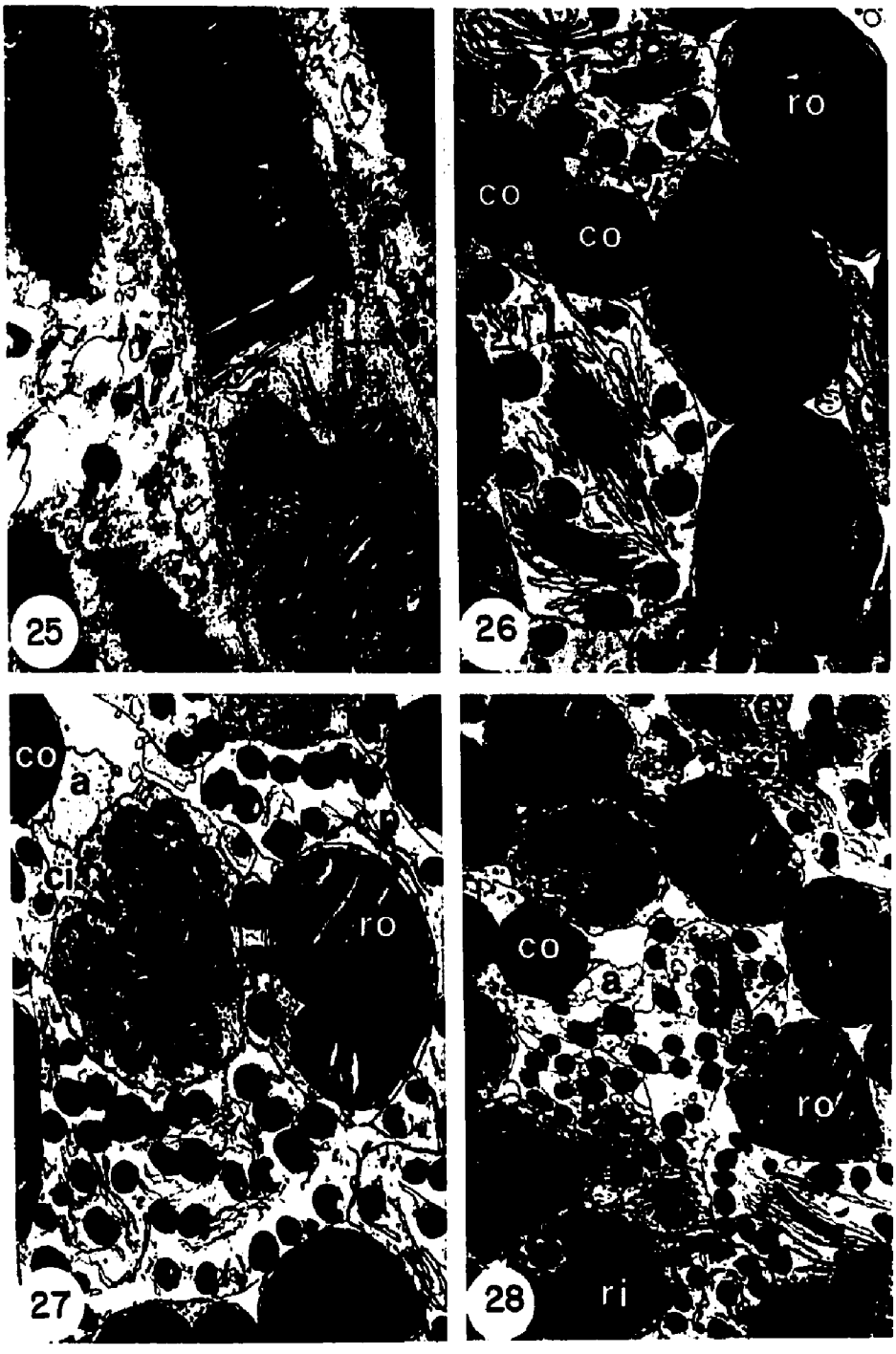
X 13,800

Figure 27. Rod inner (ri) and rod outer segments (ro) (from the dorsal retina) among pigment-laden processes from the retinal pigment epithelium are shown in cross section. This section is slightly more vitreal than that shown in figure 26. Thirteen calycal processes (cp) surround the rod outer segment shown here. The rod inner segment displays mitochondria, characteristically arranged like the wedge-shaped portions of a pie around the cilium (ci). Note the cone outer segment (co) with its accessory outer segment (a).

X 11,900

Figure 28. Survey micrograph of a portion of the dorsal retina in cross section. It shows rod outer segments (ro) and their incisures (arrow) and three of these show the connecting cilium (ci). Note that the niche through which the cilium is inserted is scalloped into lobules. There are pigment epithelial processes (p), and a cone outer segment (co) with its accessory outer segment (a).

X 8,600


25
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26
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27
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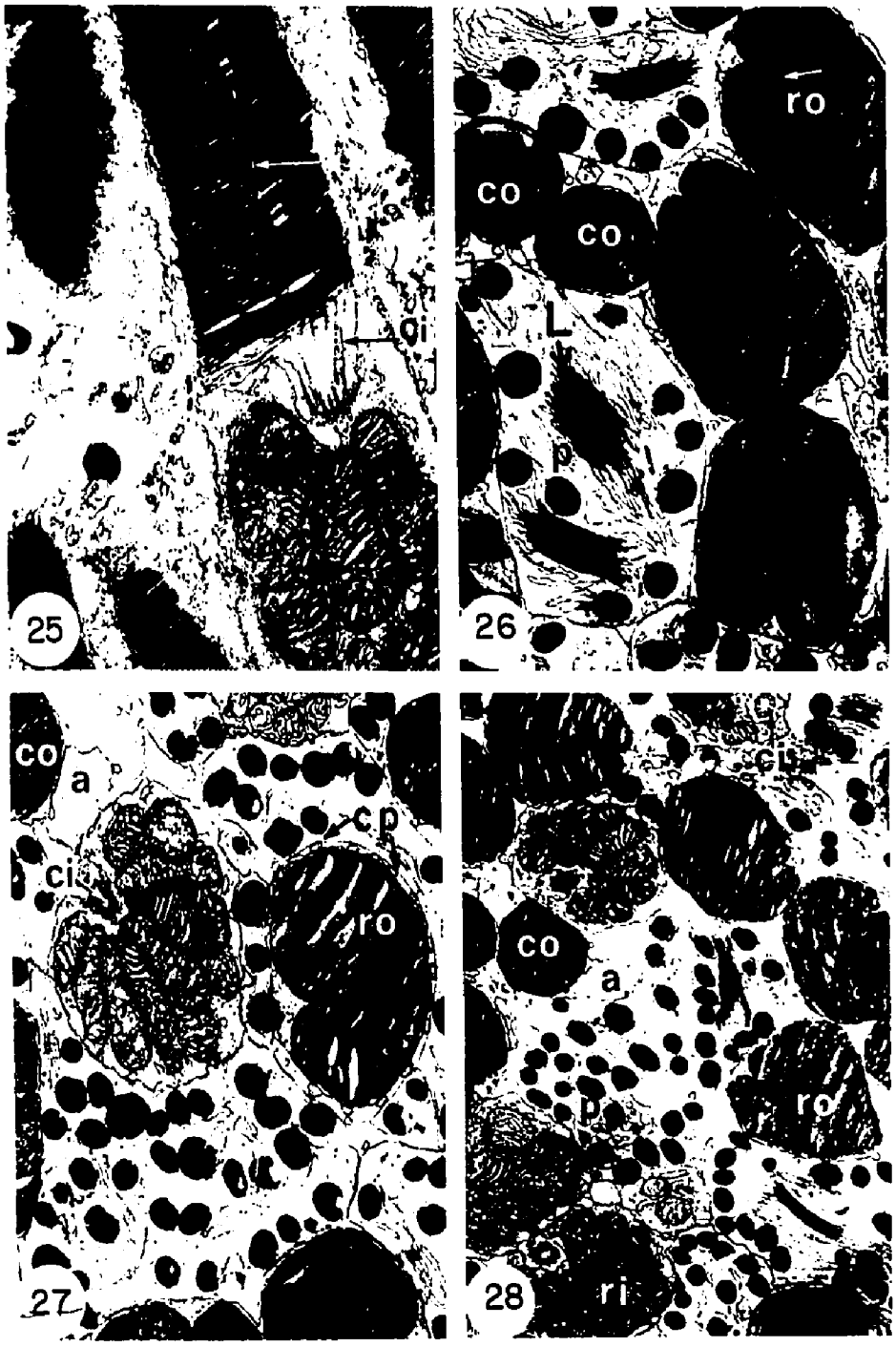
28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28

Transmission electron micrograph

Figure 29. Cross sections of rods and cones from the dorsal retina showing the position of the rod cilium (ci) in its lobulated niche, the single rod incisures (arrows), and the difference in size between the outer segments of the rod (ro) and the cone (co). The cone has an accessory outer segment (a). Note that the rod outer segment (ro), the rod inner segment (ri) and the cone outer segment (co) are not separated from each other by pigment epithelial cell processes.

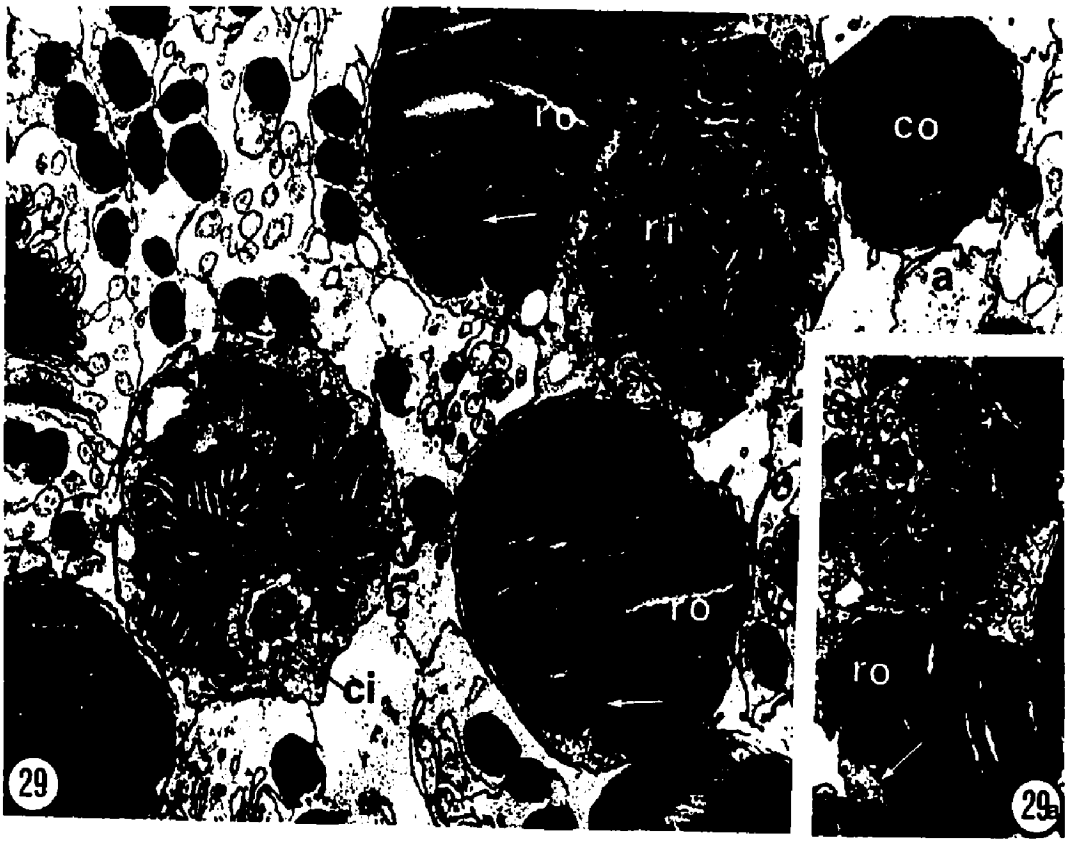
X 17,600

Figure 29a. Cross sections of rods from the dorsal part of the retina showing the rod cilium (ci) in its lobulated niche in the inner segment, and the 9 ciliary microtubules (arrow) in the outer segment (ro).

X 13,700

Figure 30. Cross section through the dorsal retina showing groups of rod outer segments in direct contact with each other, without intervening processes of pigment epithelial cells. However, each rod over some part of its circumference, is in contact with such a process. Note the apparently random orientation of the rod incisures. The two cone outer segments (c) are labelled. One has an accessory outer segment (a).

X 4,600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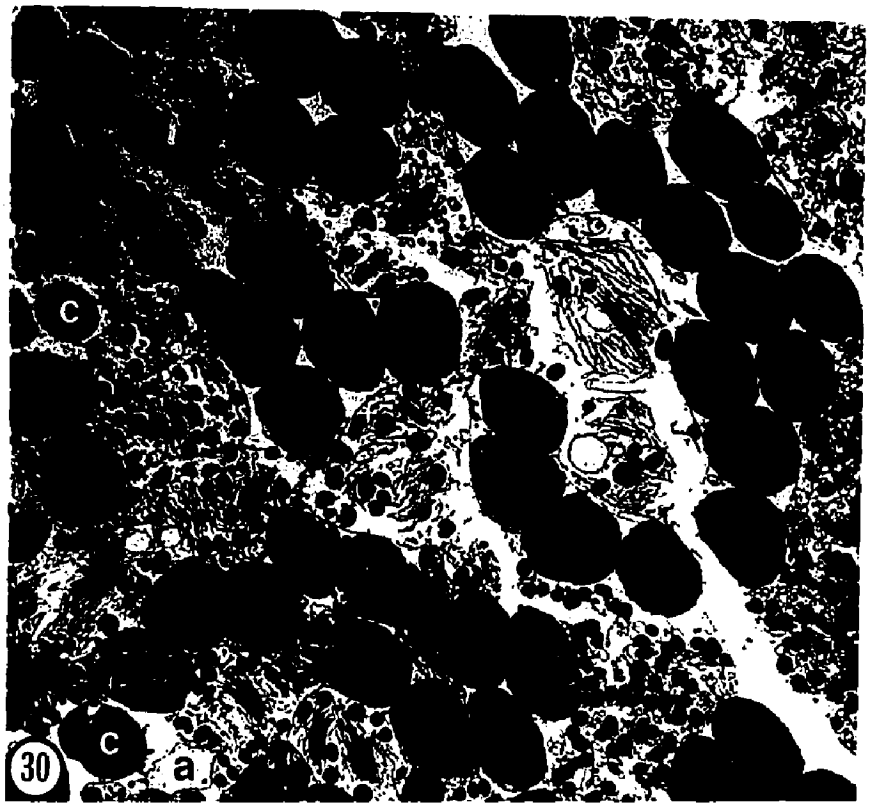
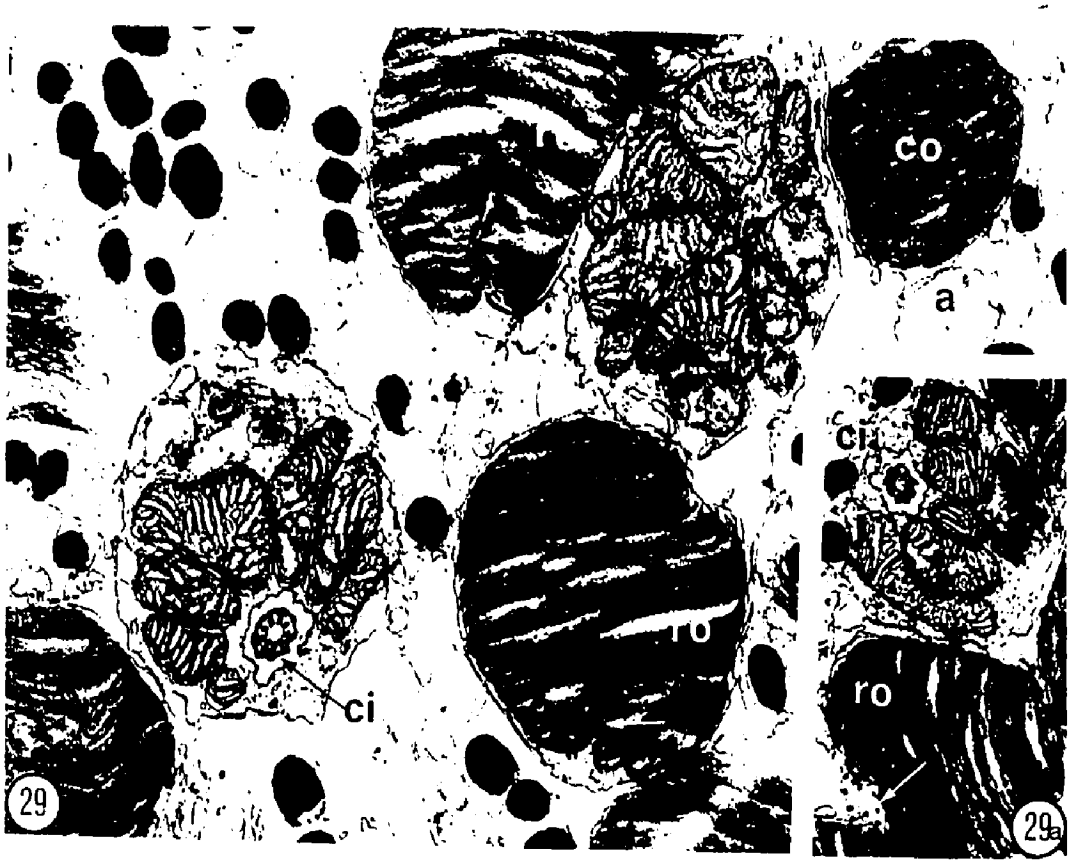
29
ro
29a


c
c
a
30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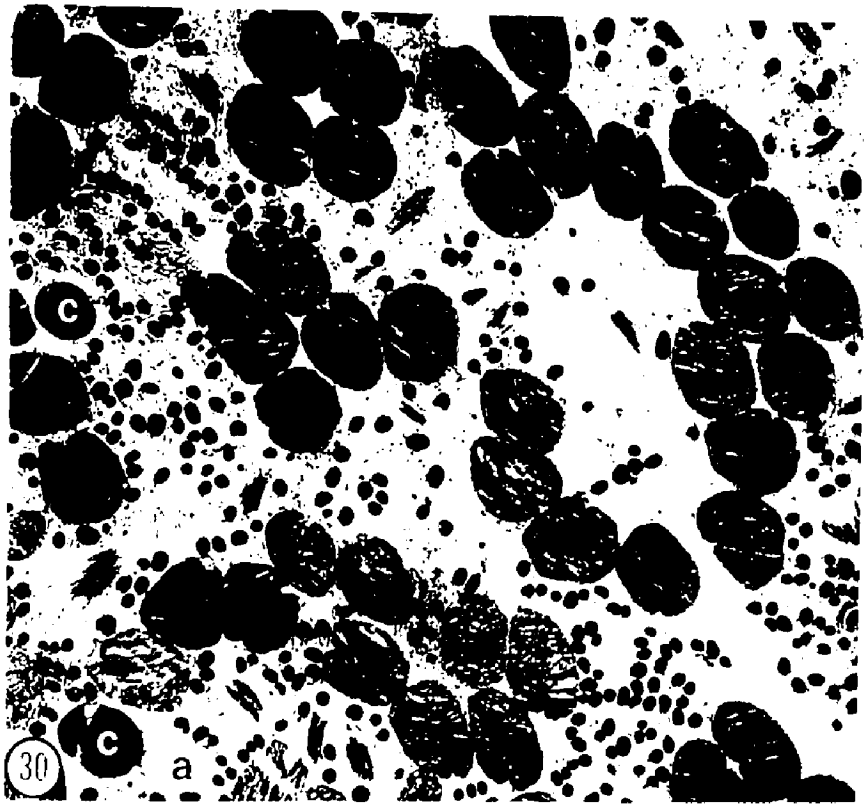
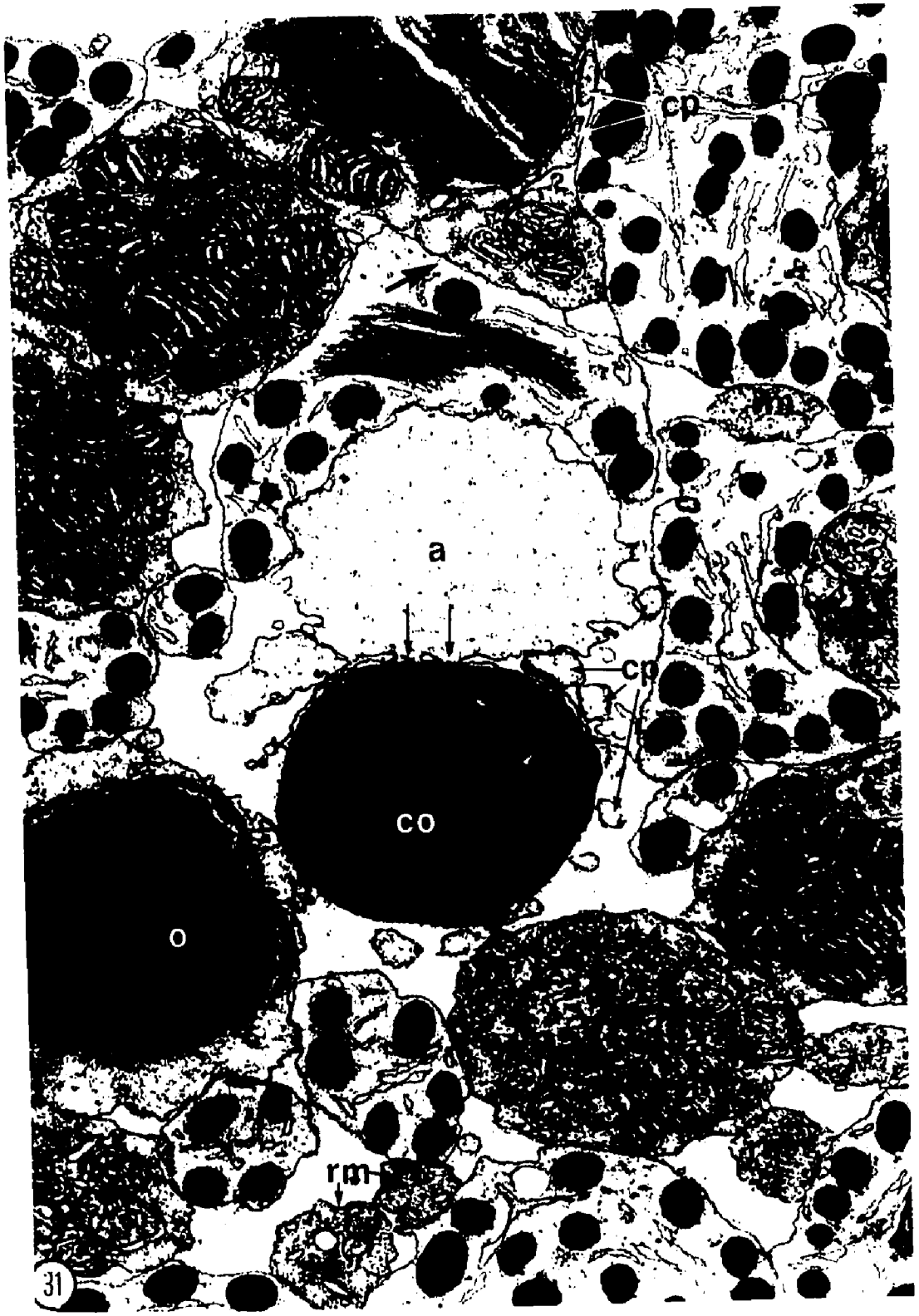

c
c
a
30

Transmission electron micrographs

Figure 31. A cross section from the dorsal retina showing rod ellipsoids (re) packed with normal mitochondria. The circumference of the rod ellipsoid is only very slightly ridged. The rod myoid (rm) is much narrower than the ellipsoid. Note the tapering area of the rod ellipsoid (big arrow), and the direct contact between some of the rod ellipsoids and myoids. An oil droplet (o) can be seen. There is a cone outer segment (co) with surrounding calycal processes (cp). The accessory outer segment (a) is in continuity with the outer segment at two places (arrows).

X 17,300


a
CO
O
31

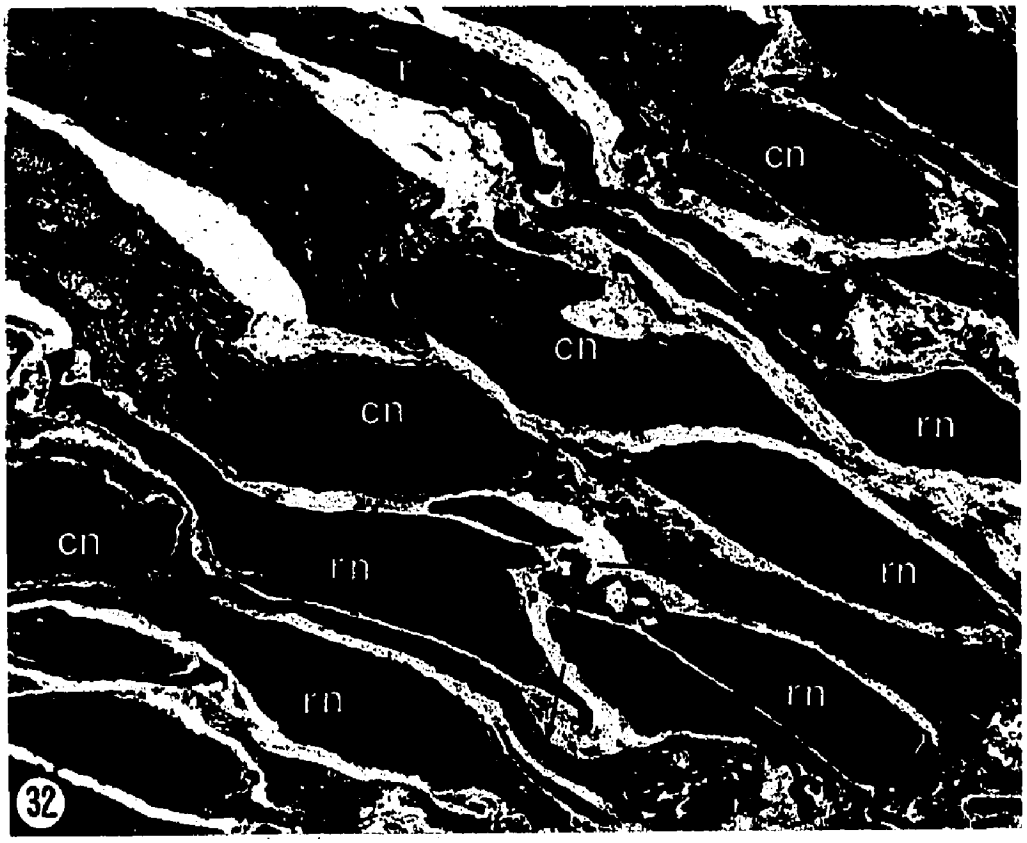

cp
a
cp
co
o
rm
31

Transmission electron micrographs

Figures 32,33. Longitudinal sections showing: the rods (r) and cones (c), with the deeply indented cone nuclei (cn); the darker and denser rod nuclei (rn); the shorter rod fibres and the longer cone fibres (arrows); the dark staining rod spherule (rt) and the lighter staining and larger cone pedicle (ct). The Müller cell cytoplasm between these structures is barely stained at all. Note that the rod stains more intensely than the cone at all regions shown here. The rod nuclei are more vitreal in position than the cone nuclei.

Fig. 32 (central area of retina) X 6,300

Fig. 33 (dorsal area of retina) X 6,000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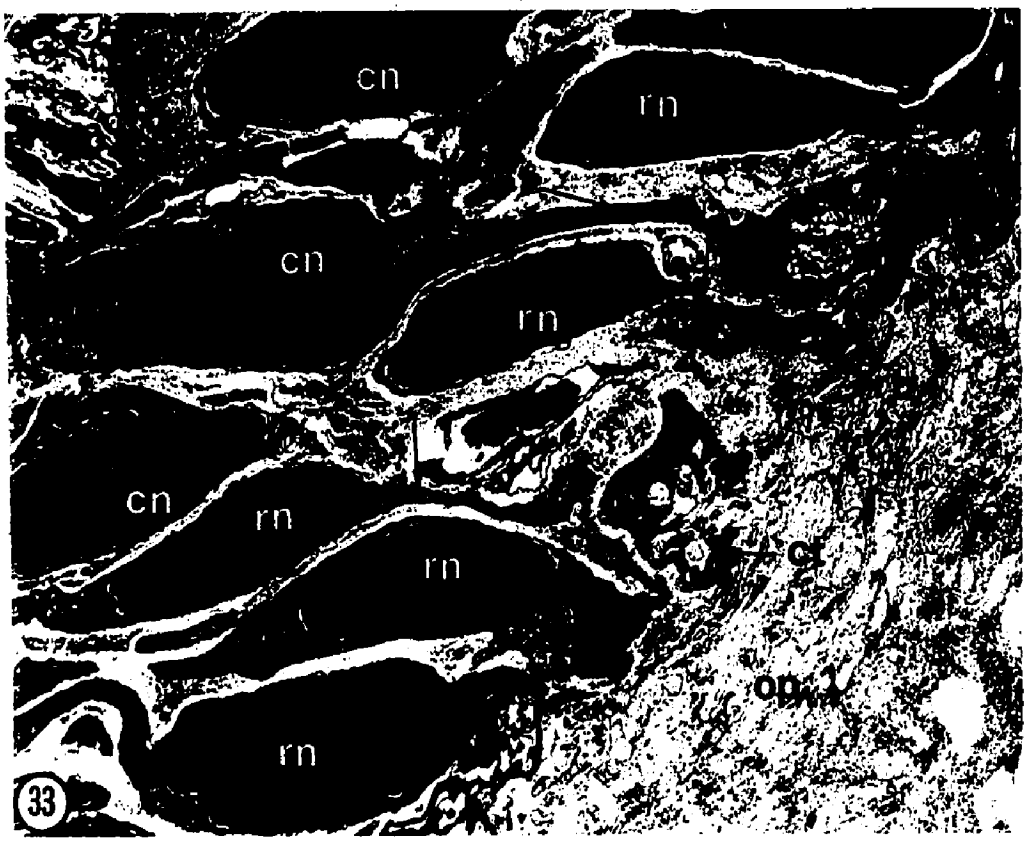
32


cn
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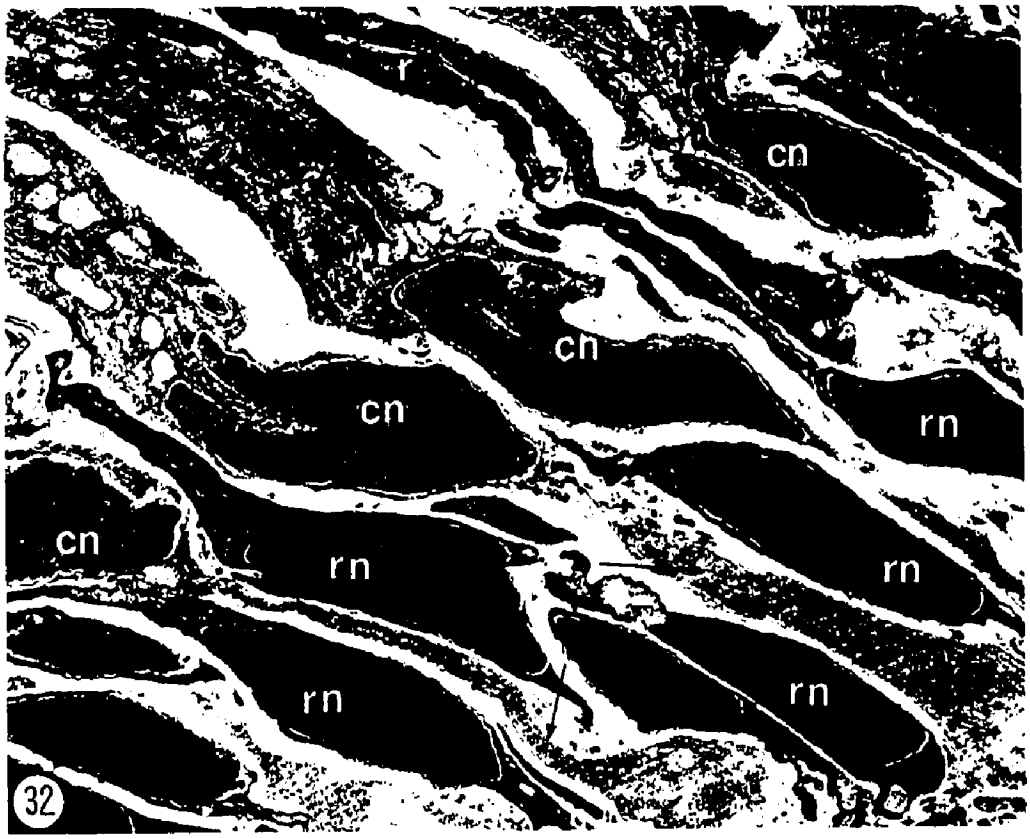
op
rn
33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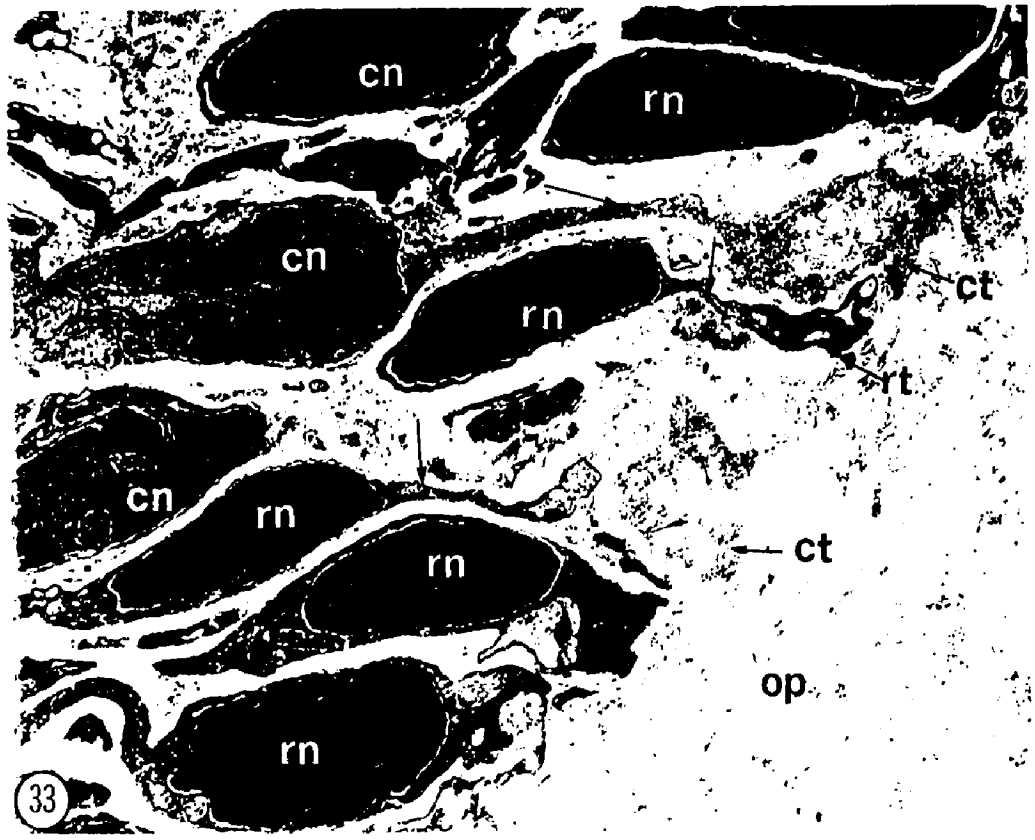
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Transmission electron micrographs

Figure 34. Longitudinal section showing a single cone at the inner-outer segment junction. The regularly arranged flattened saccules or discs forming the cone outer segment are clearly visible. The calycal process (cp), arising from the inner segment, contains longitudinal filaments. The large membrane-bounded structure in the inner segment represents a forming oil droplet (o). In its scleral portion especially, structures resembling mitochondrial cristae are clearly visible.

X 18,400

Figure 35. Slightly oblique cross section of a single cone outer segment surrounded by twenty-one calycal processes (cp) and showing the separate accessory outer segment (a).

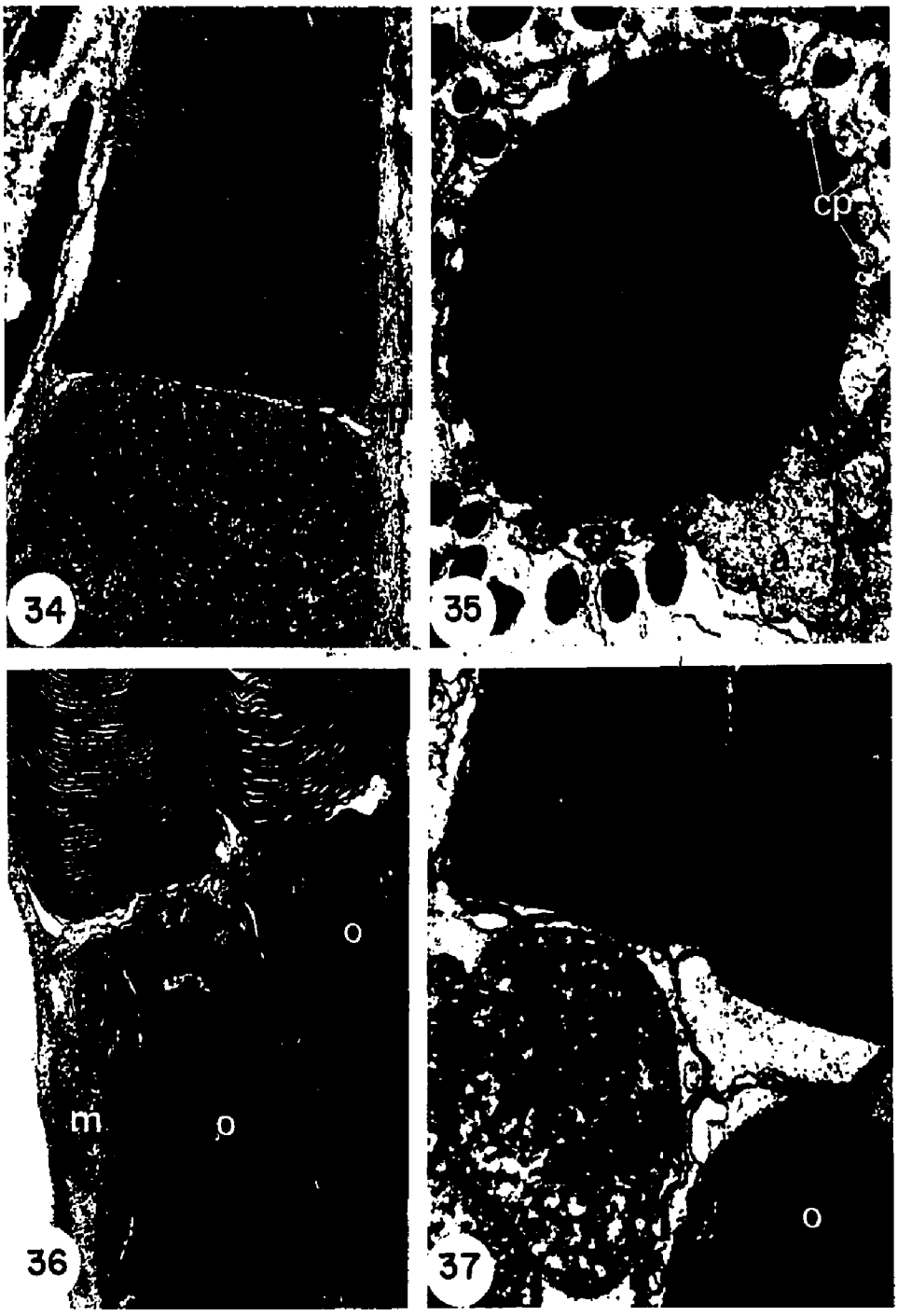
X 17,300

Figure 36. Longitudinal section of a double cone from the ventral retina. There is a marked and constant structural difference between the discs of the two outer segments of a double cone. In the outer segment to the right, the space between the membranes forming each of the flattened saccules (the intralamellar space) is wider. The inner segments show stages in the formation of the oil droplet (o) and display remnants of mitochondrial cristae. Note the mitochondrion (m) adjacent to the forming oil droplet.

X 21,500

Figure 37. Longitudinal section of a double cone from the dorsal retina. Note the difference in the appearance of the outer segments, as in figure 49. In the inner segment to the left, mitochondria (very enlarged) can still be recognized but in the inner segment to the right, the oil droplet (o) is formed, although channels resembling mitochondrial cristae are still visible at its periphery.

X 22,900


34
cp
35
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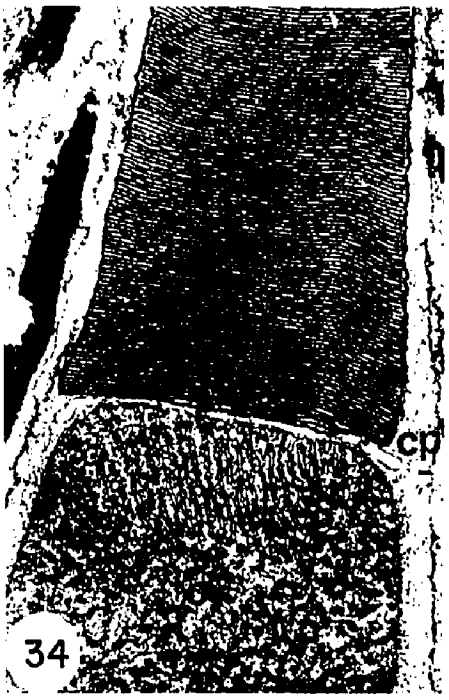

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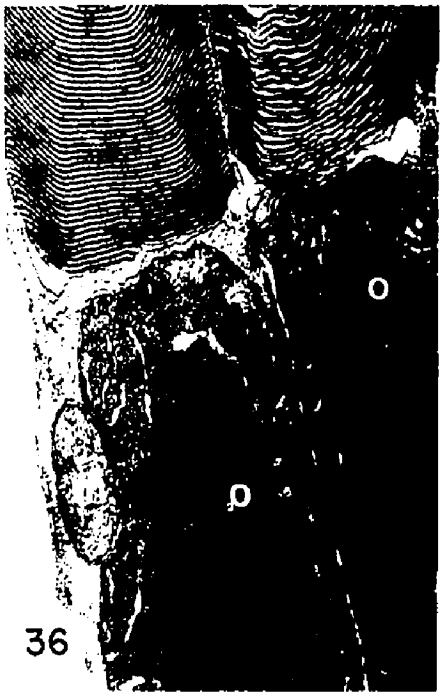

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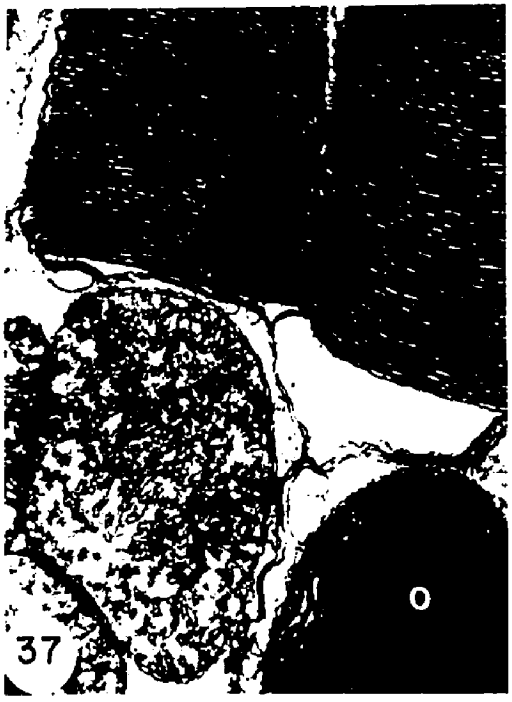


37

Transmission electron micrographs

Longitudinal sections

Figure 38. Part of the outer segment of a rod from the ventral retina showing the discs, which are independent of each other except for those at the base of the outer segment which are infolded.

X 33,300

Figure 39. Part of the outer segment of single cone showing the discs. In the lower right hand portion the flattened saccules appear to be continuous with each other.

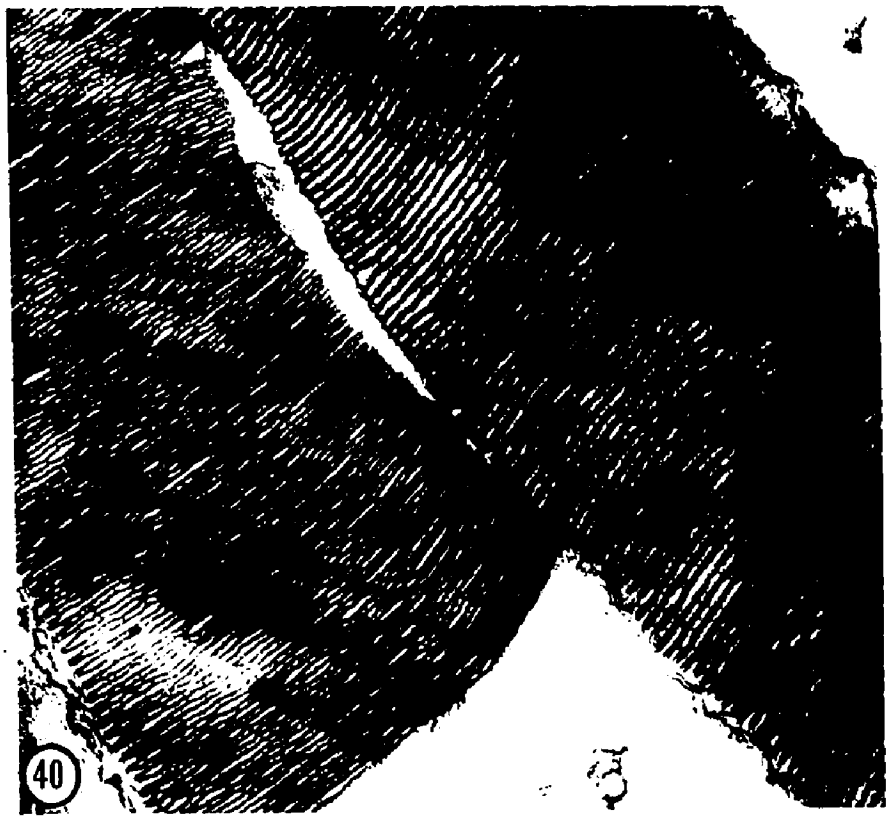
X 34,600

Figure 40. Part of the outer segments of a double cone from the ventral retina showing the differences between the discs of the two members of the cone pair.

X 34,600


38


39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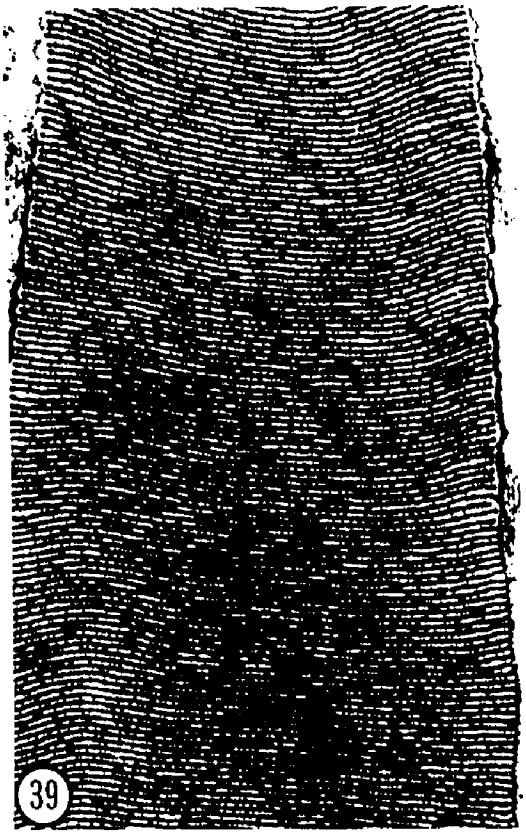
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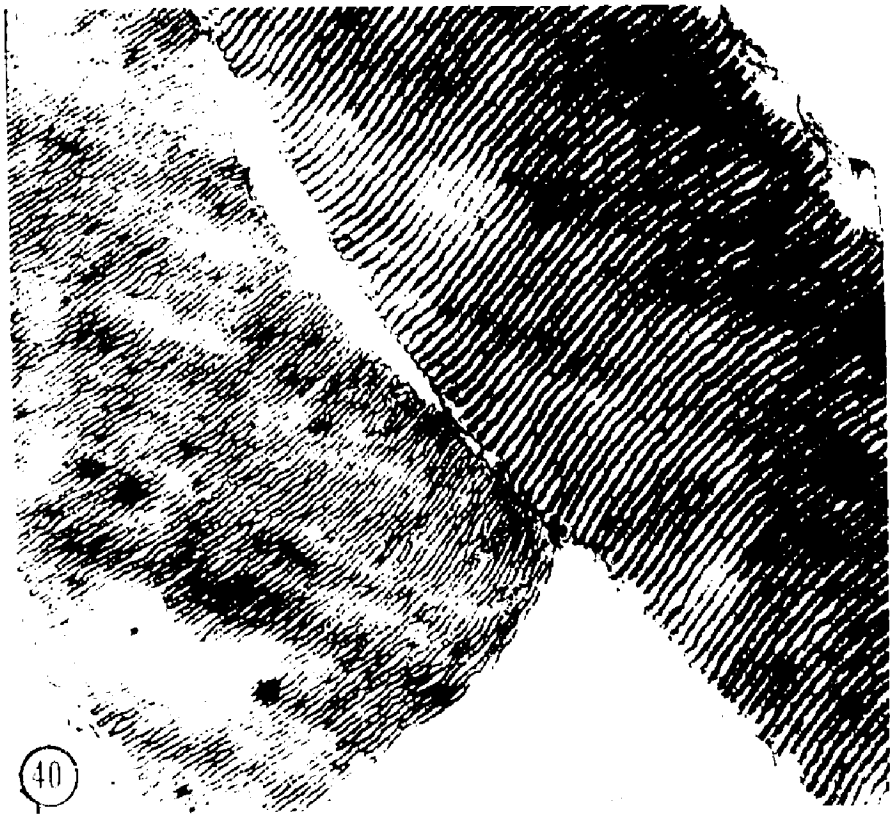
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Transmission electron micrographs

Figure 41. A double cone shows the difference between the two outer segments. Each member of the double cone has an associated accessory outer segment (a) connected at intervals to the outer segment (arrows).

X 12,500

Figure 42. A double cone sectioned through the oil droplet (o) of one member, and the outer segment of the other member. The accessory outer segment (a) shows places of attachment to the outer segment (arrows) and a calycal process (cp) intervenes between them. Note the relative sizes of the rod ellipsoid (ri) and the two rod myoids below it; and also the ridging of the outline of the cone ellipsoid containing the oil droplet (o). The rod mitochondria appear 'typical' or 'normal'.

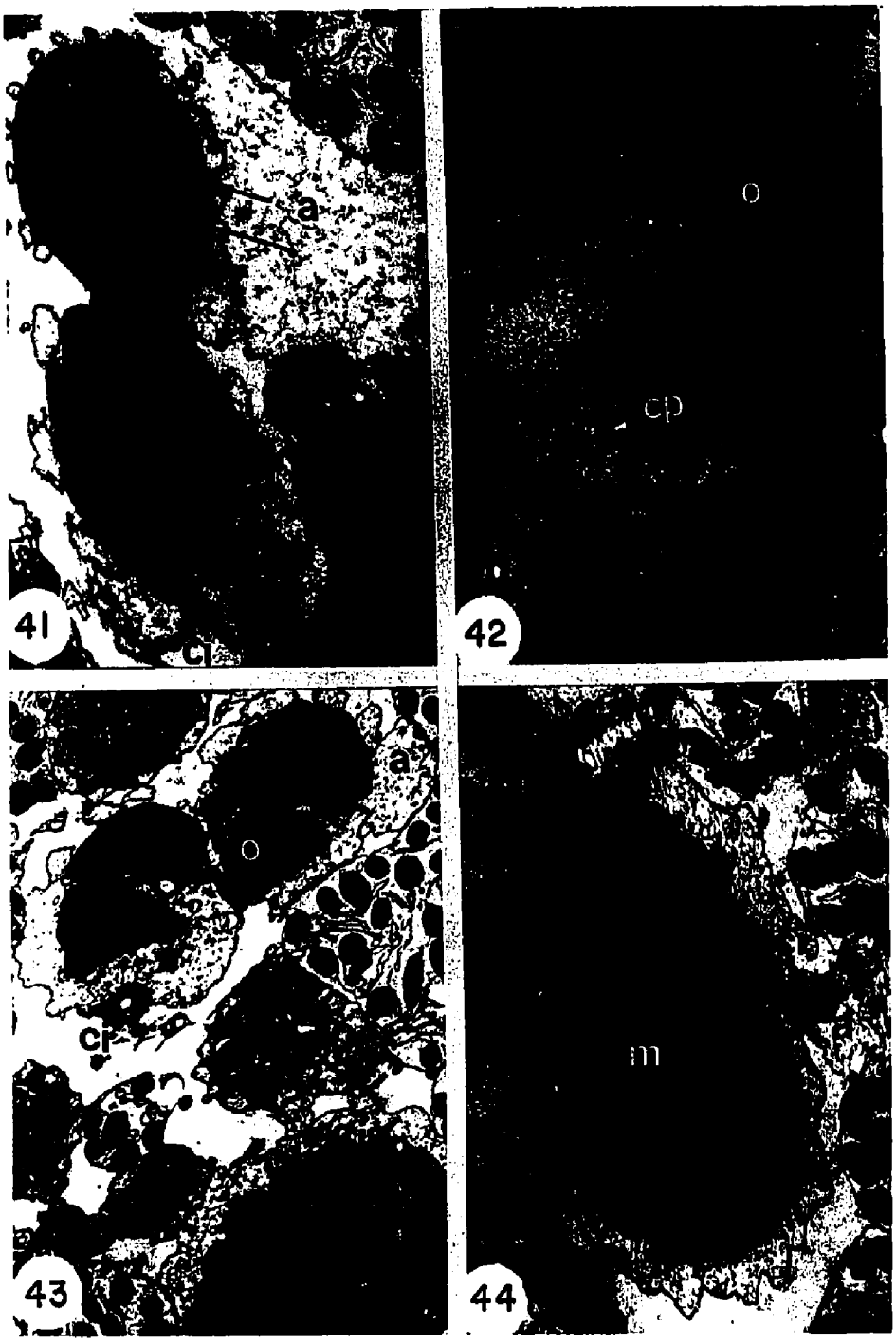
X 8,300

Figure 43. The double cone has been cut obliquely. In one member, part of the outer segment with its accessory outer segment (a) and part of the oil droplet (o) of the inner segment can be seen. To the lower left of it is the other member of the pair, part with the outer segment and part with inner segment and cilium (ci). On the outer segment portions there are calycal processes. In the accessory outer segment there are 9 microtubules of the cilium splayed out. Note the mitochondria of the cone inner segment (cis) in various stages of transformation, whereas the rod mitochondria are normal.

X 9,000

Figure 44. The ellipsoid of a single cone, oblique, showing the irregular (ridged) outline of the cone, the cilium (ci), the accessory outer segment (a) and the membrane-bounded groups of fusing and transformed mitochondria (m) of the scleral end of the ellipsoid.

X 12,000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a
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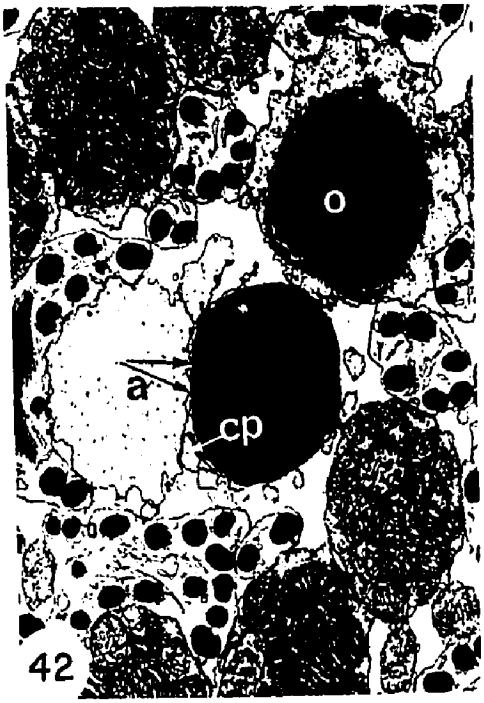
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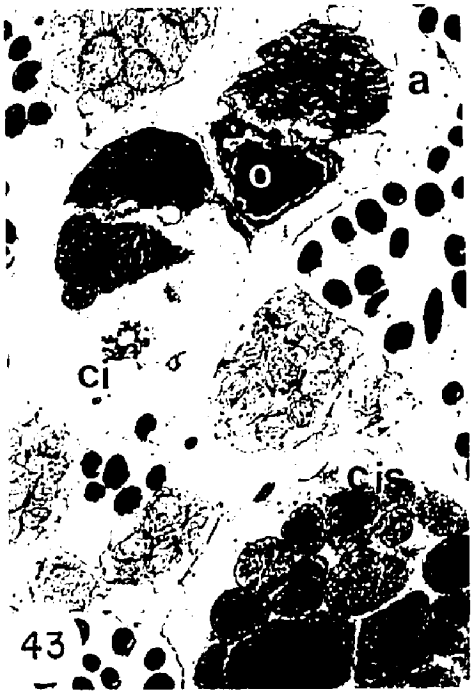
42


a
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43


ci
a
m
44

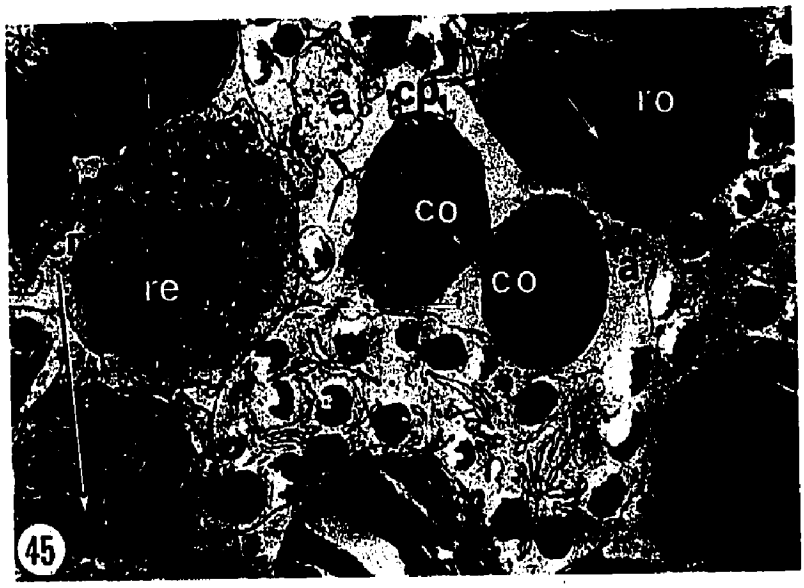
Transmission electron micrographs

Figure 45. A cross section through the dorsal part of the retina showing the outer segments (co) of a double cone, each with an accessory outer segment (a). There are some calycal processes (cp). Note the thin attachment (black arrow) of one of the accessory outer segments to the outer segment. The other accessory outer segment is very small at its most scleral end. The rod ellipsoids (re) show cilia (ci); the rod outer segment (ro) shows the incisure (arrow).

X 14,500

Figure 46. A cross section of a double cone from the dorsal part of the retina, with the accessory outer segments (a) showing a distinct granularity, as compared to the vague granularity of that shown in figure 45.

X 13,400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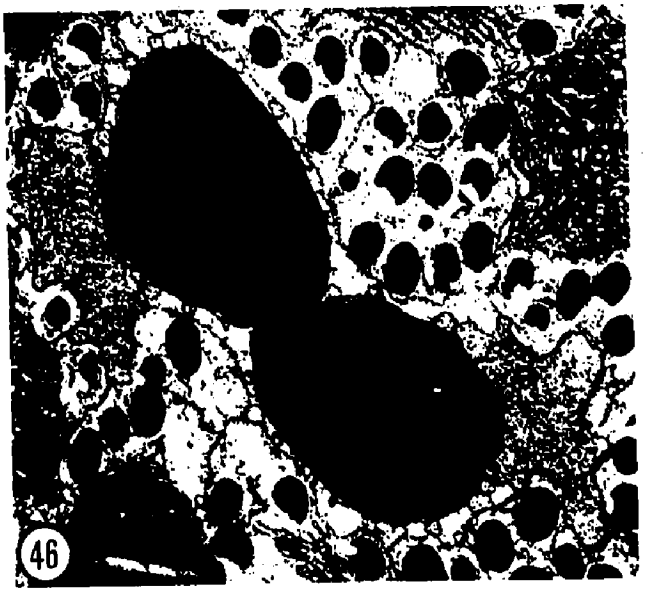
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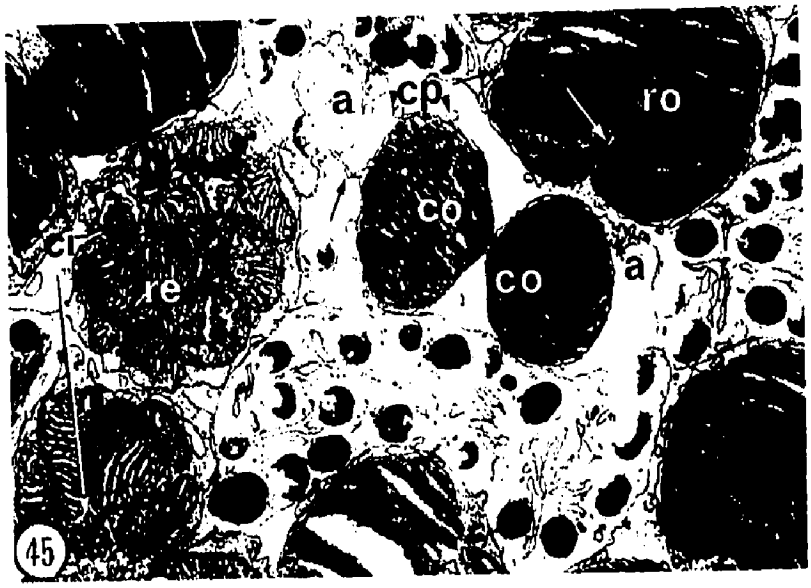
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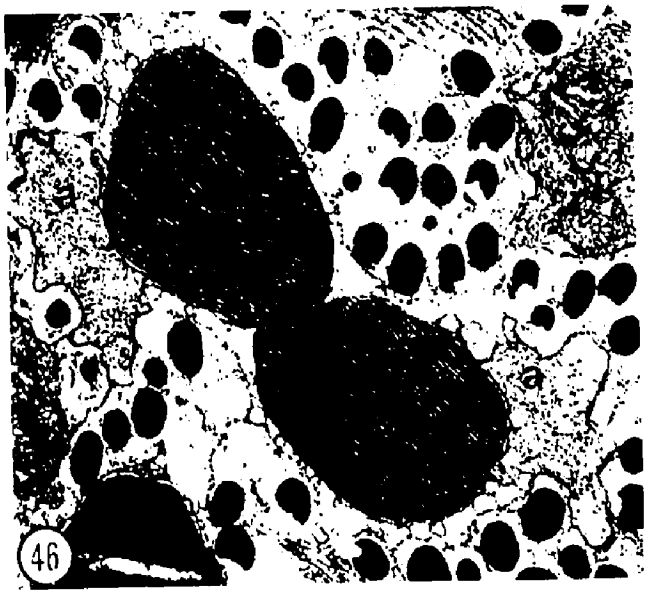

46

Transmission electron micrographs

Figure 47. Survey micrograph of the dorsal retina in longitudinal section. Note the gradual increase in size and density of the mitochondria of the ellipsoids of the cones (c) in a vitreal-scleral gradient. A rod (r) shows unaltered mitochondria, in the ellipsoid, and the narrow myoid. There is a double cone with a forming oil droplet in one member (dc). Note the accessory outer segment (a).

X 3,200

Figure 48. Micrograph of cones from the ventral retina in longitudinal section. Note the tapering accessory outer segments (a), the one showing the cilial origin in the inner segment (arrow). The mitochondria nearest the outer segment are very enlarged and show the beginnings of transformation. Note the increasing size of the mitochondria in a vitreal-scleral gradient.

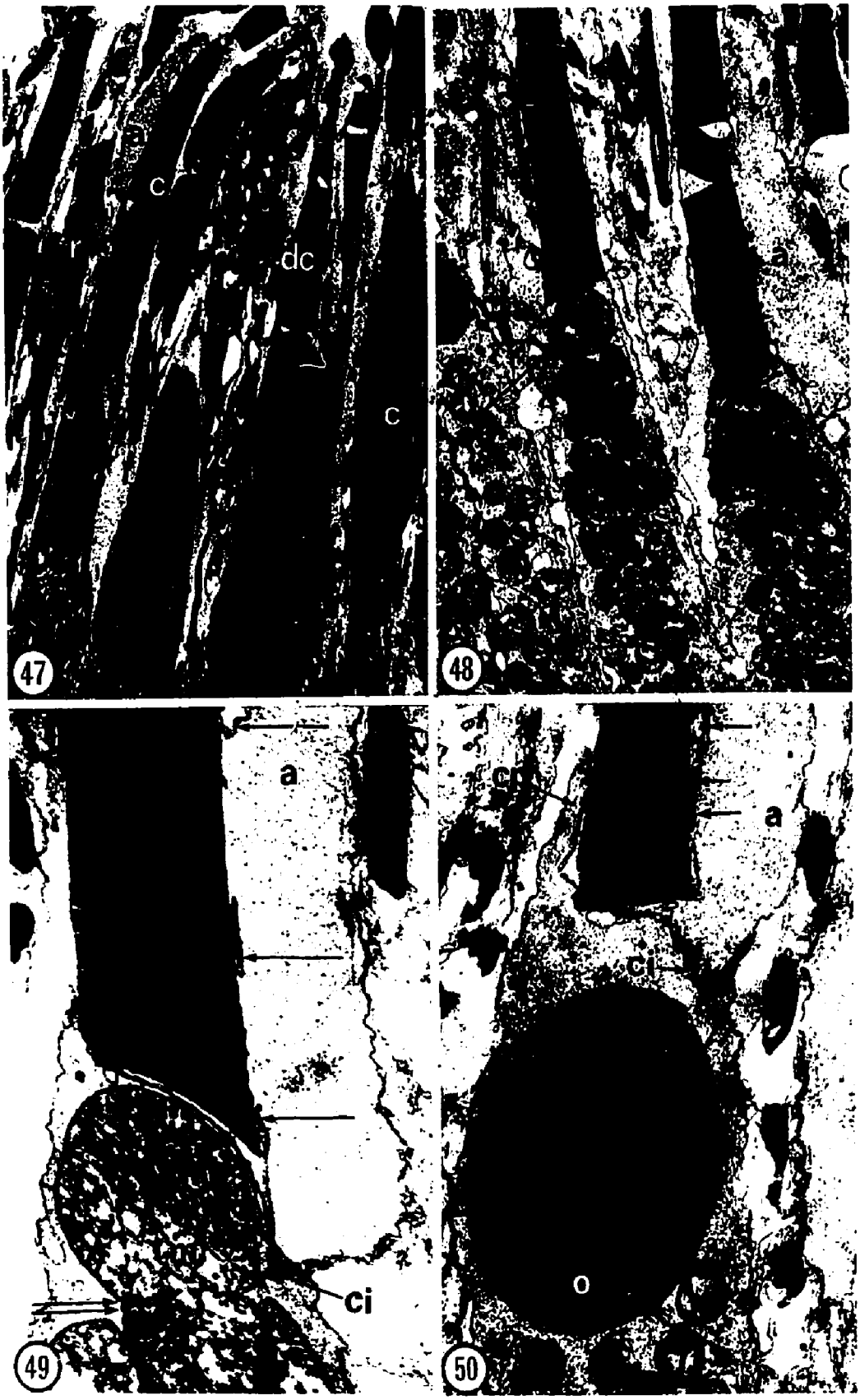
X 6,500

Figure 49. Longitudinal section of a single cone from the ventral retina. Note the accessory outer segment (a) and its ciliary origin (ci) in the inner segment. Over part of its length, the accessory outer segment is continuous with the outer segment, but there are areas showing separating plasma membrane (arrows). Note the very large and unusual mitochondrion (m) in the inner segment which appears to be fusing (double arrow) with one vitreal to it.

X 19,400

Figure 50. Longitudinal section of one member of a double cone from the ventral retina, showing the accessory outer segment (a), its ciliary origin (ci), and a calycal process (cp). The accessory outer segment is partly separated from the outer segment by plasma membrane (arrows). There is a well-formed membrane-bounded oil droplet (o) with a few membranous infoldings, reminiscent of cristae. Note that the lamellae of the outer segment differ from those of the single cone shown in figure 49.

X 18,700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a
48
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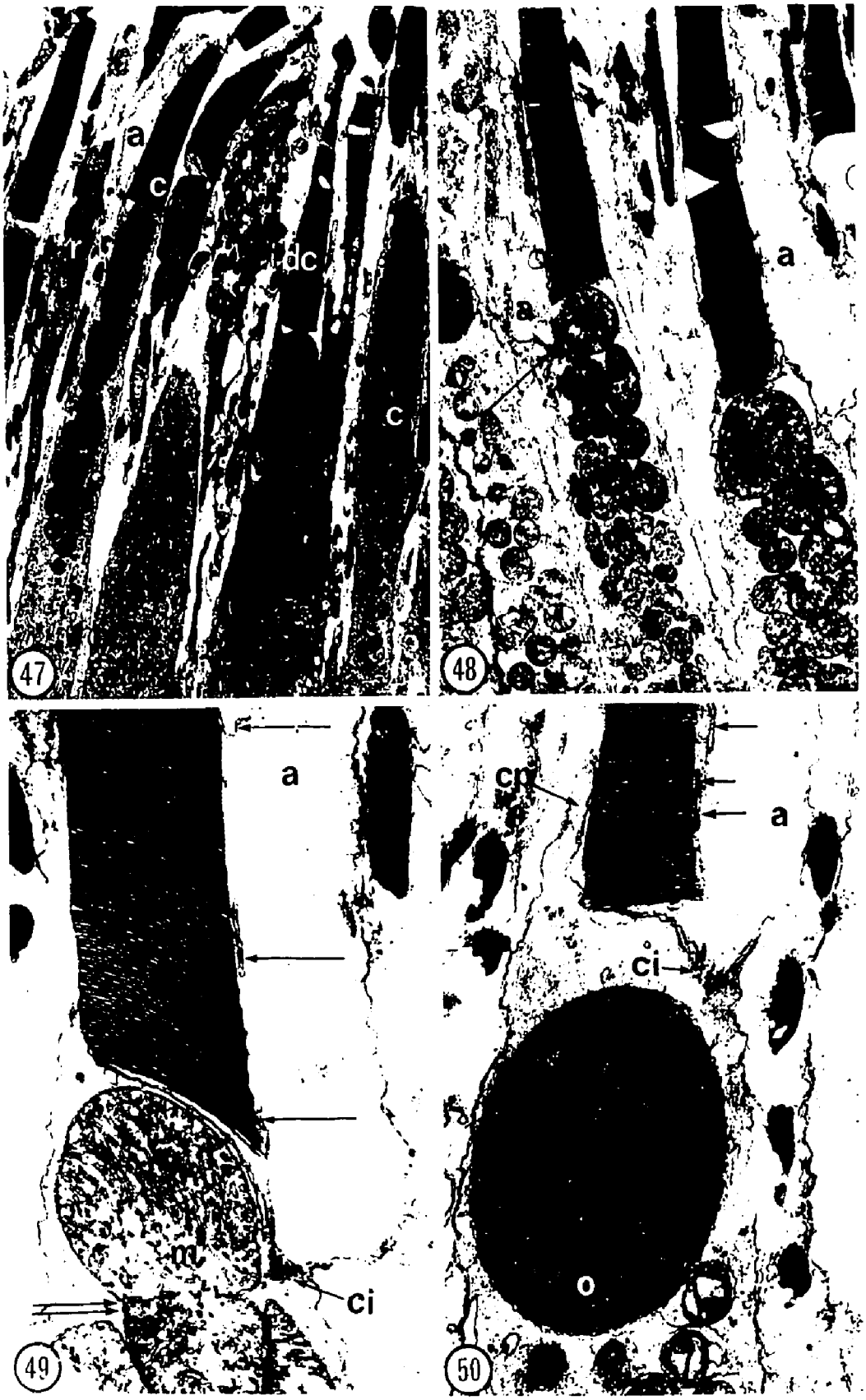
o
50


a
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47
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50

Transmission electron micrograph

Figure 51. A longitudinal section from the dorsal part of the retina. The oil droplet (o) shows channel-like markings throughout and on the left of it a transformed mitochondrion of similar appearance seems to be fusing into the oil droplet. Note the transformed mitochondria (m) with their dense matrices. Longitudinally oriented microfibrils are abundant (arrows) in the inner segment.

X 19,000


o
m
m
51


o
m
m
51

Transmission electron micrograph

Figure 52. A longitudinal section through part of a cone showing the longitudinal microfilaments (arrows) of the calycal processes (cp) continuous with those of the inner segment. Note the cylindrical pigment granule (p), part of an oil droplet (o), and part of the ciliary apparatus (ci) and accessory outer segment (a).

X 34,600


P
P
O
52


p
o
52

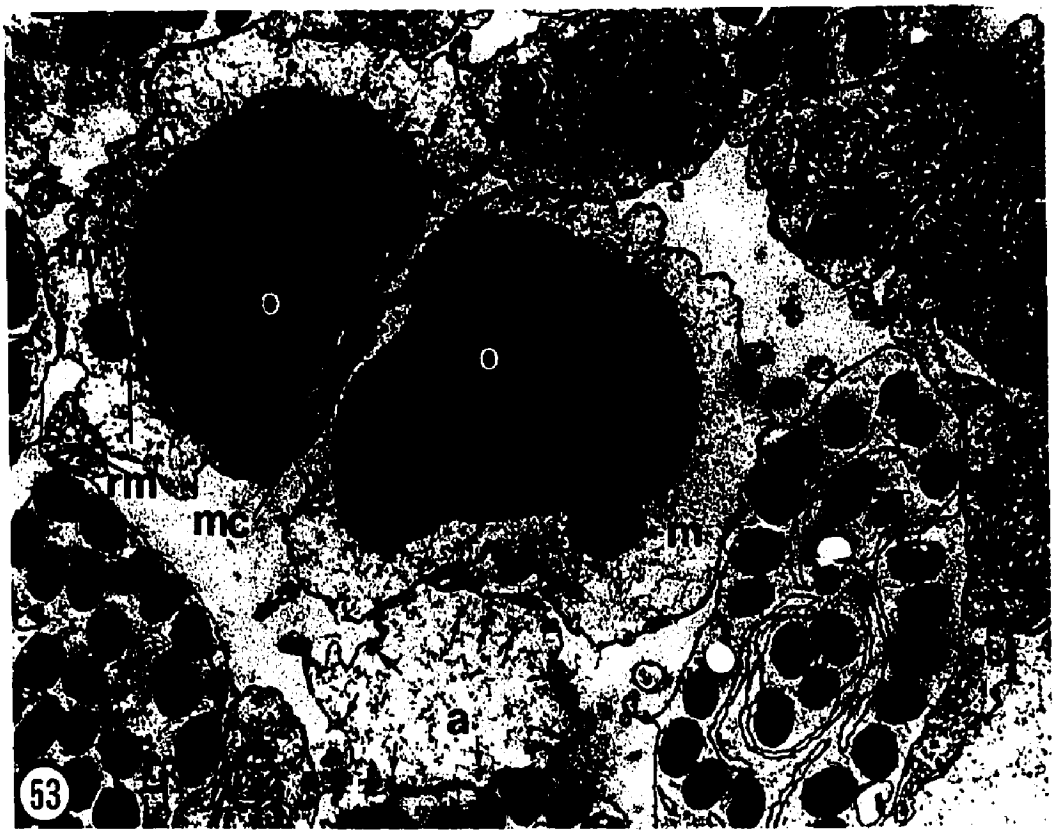
Transmission electron micrographs

Figure 53. Cross section through the dorsal part of the retina showing a double cone with the membrane complex (mc) between the two members. Each cone has an oil-droplet (o) in different stages of formation. The right-hand one is channelised, whereas the left-hand one is uniformly dense, except at the periphery. The mitochondrion (m) on the left is enclosed within the membrane bounding the oil droplet (arrows). The accessory outer segment (a) shows nine microtubules of the cilium, around its periphery (short arrows). Note the rod myoid (rm) and rod ellipsoids (re).

X 19,000

Figure 54. Cross section through the dorsal part of the retina showing the rod ellipsoids (re), rod myoids (rm), abundant pigment granules, and accessory outer segments (a) of single cones (sc) and double cones (dc). One cone shows an oil droplet (o) and the ridged and grooved circumferential surface.

X 8,200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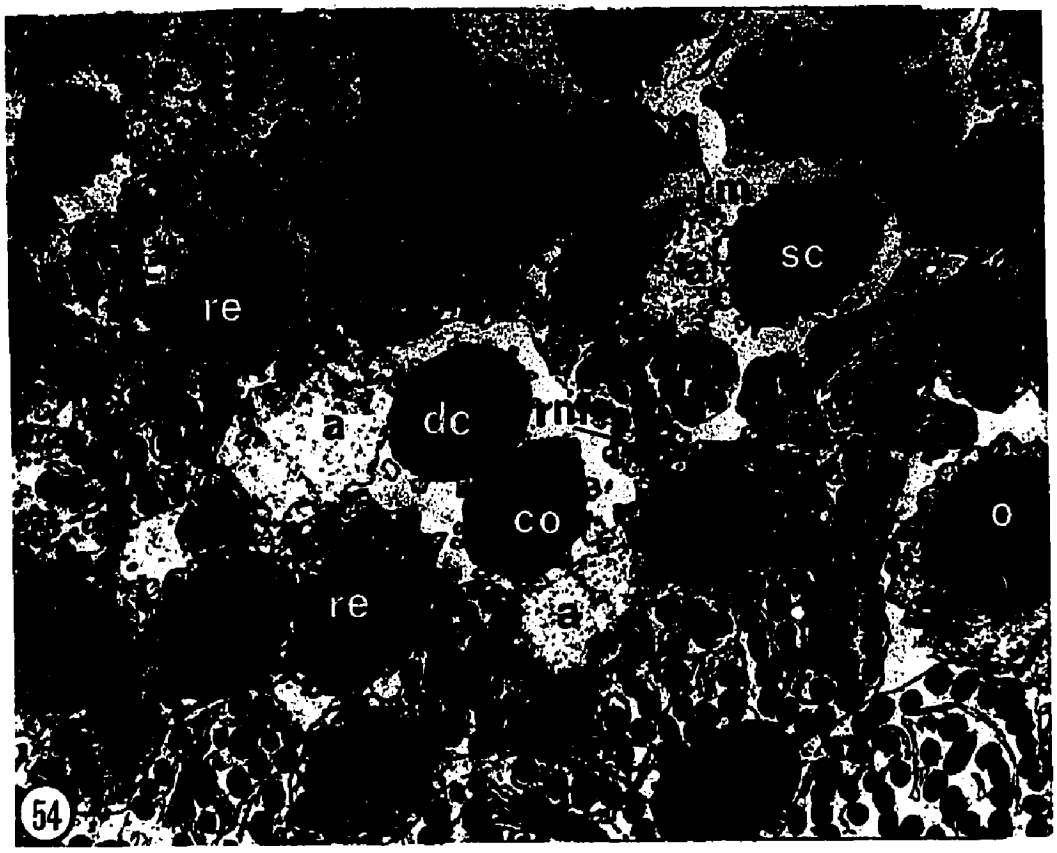
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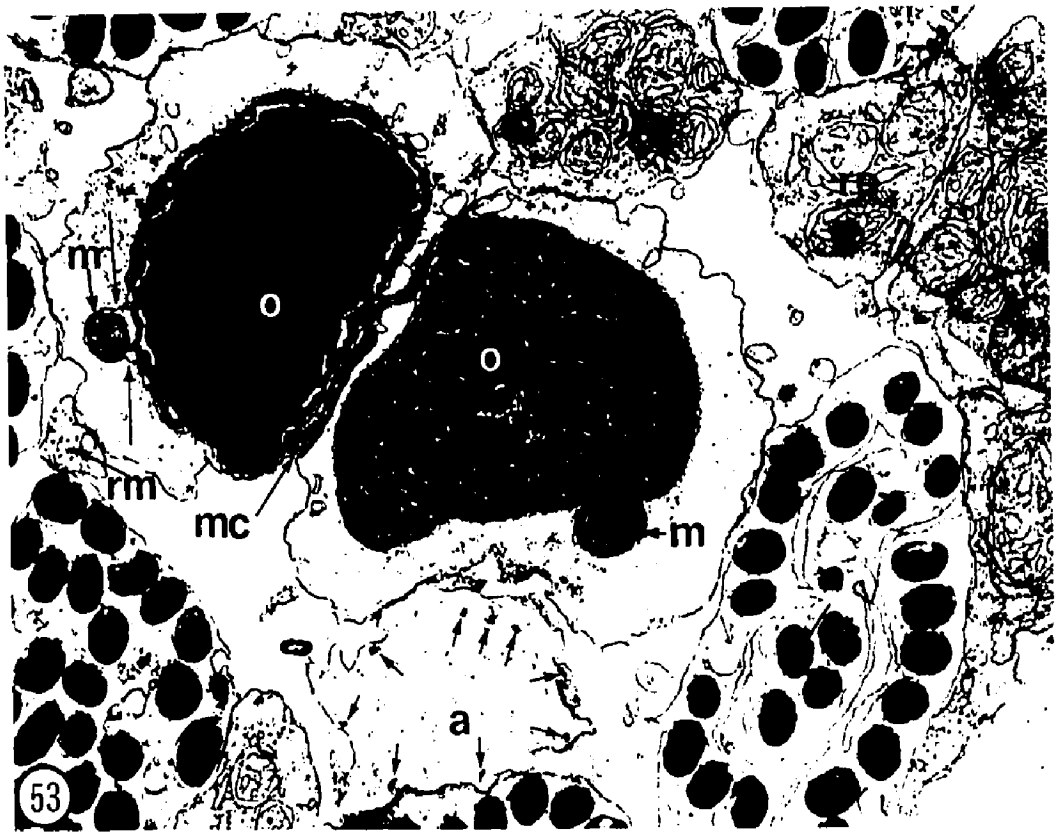
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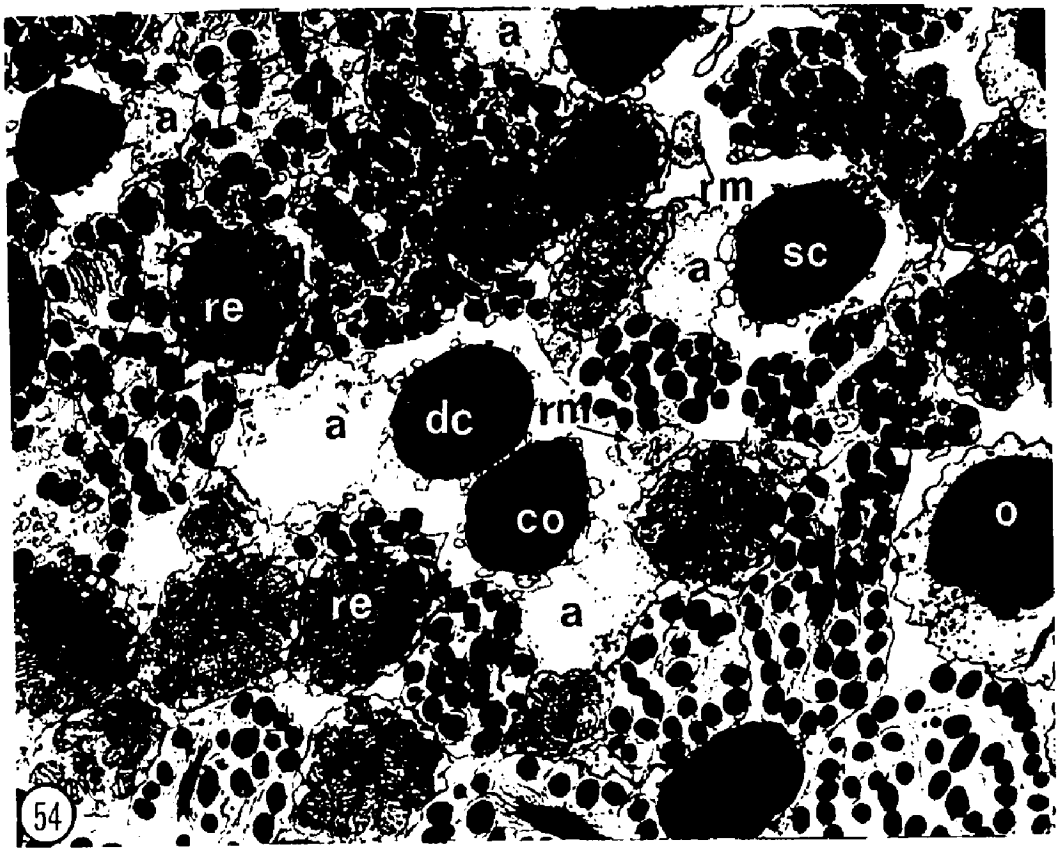
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Transmission electron micrographs from the dorsal part of the retina

Figures 55-58. Cross-sections through double-cone inner segments at the level of the oil droplet. The oil droplets are in various stages of formation. The membrane complexes of the apposed surfaces of the double cone pairs are obvious.

Figure 55. One oil droplet is dense and homogeneous (o). The other contains numerous channels and two transformed mitochondria which are separated from the main body of the oil droplet by membranes (arrows). Note that the rod ellipsoid (re) has normal mitochondria.

X 13,800

Figure 56. One member of the pair has a fully formed oil droplet (o); the other has a droplet with channels throughout, and a neighbouring mitochondrion of similar appearance (arrow).

X 12,500

Figure 57. One oil droplet (o) has a dense centre and channels around the periphery, and one protruberance. The other shows a moderately dark centre and more channels. There are also more protruberances on the circumference, formed by fusing mitochondria. Note the small neighbouring mitochondrion (arrow).

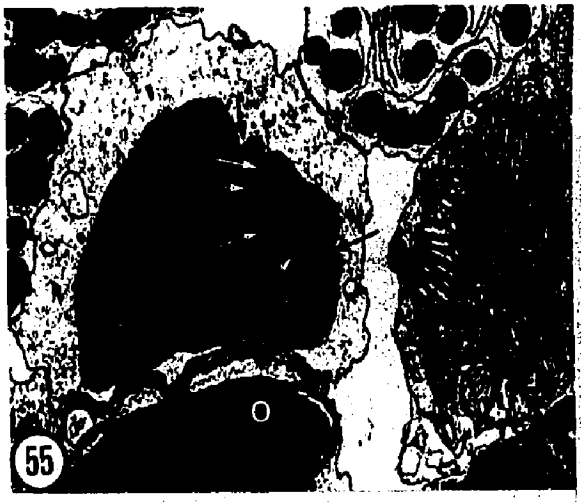
X 15,100

Figure 58. There is a homogeneously dense oil droplet (o); alongside it are transformed mitochondria (m) with matrix that is as dark and dense as that of the oil droplet. The other oil droplet has a moderately dark centre with channels around the periphery.

X 13,600

Figure 59. One oil droplet (o) is uniformly dark and dense, except for a peripheral area. The other oil droplet has a small centre that is very dark, but there are many channels elsewhere, and there is a neighbouring density that is part of a transformed mitochondrion (arrow). Note the narrow rod myoid (rm).

X 12,900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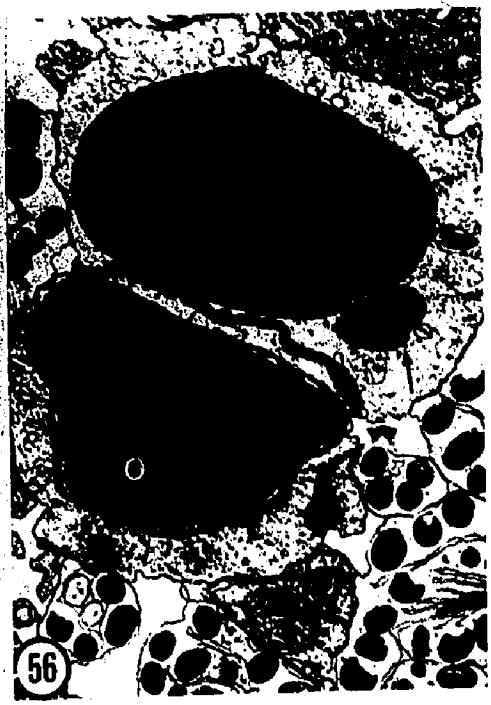
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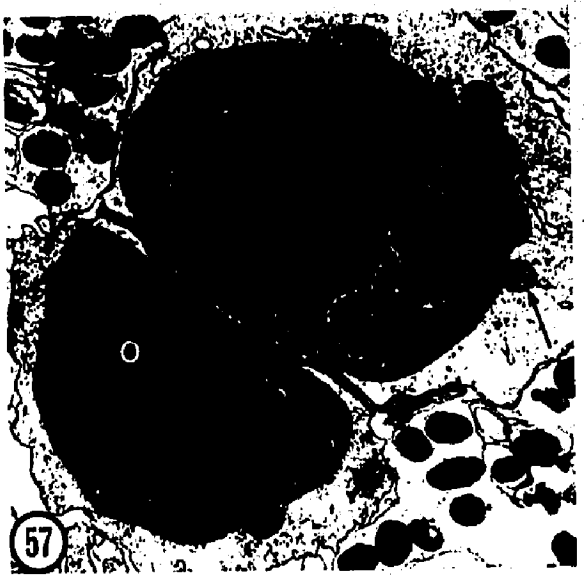
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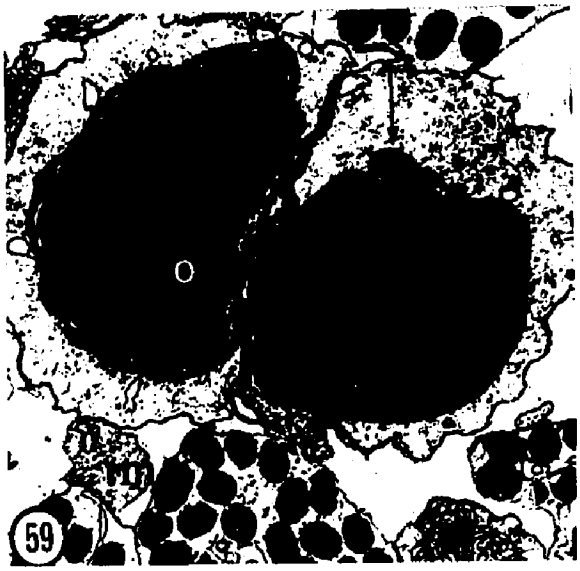
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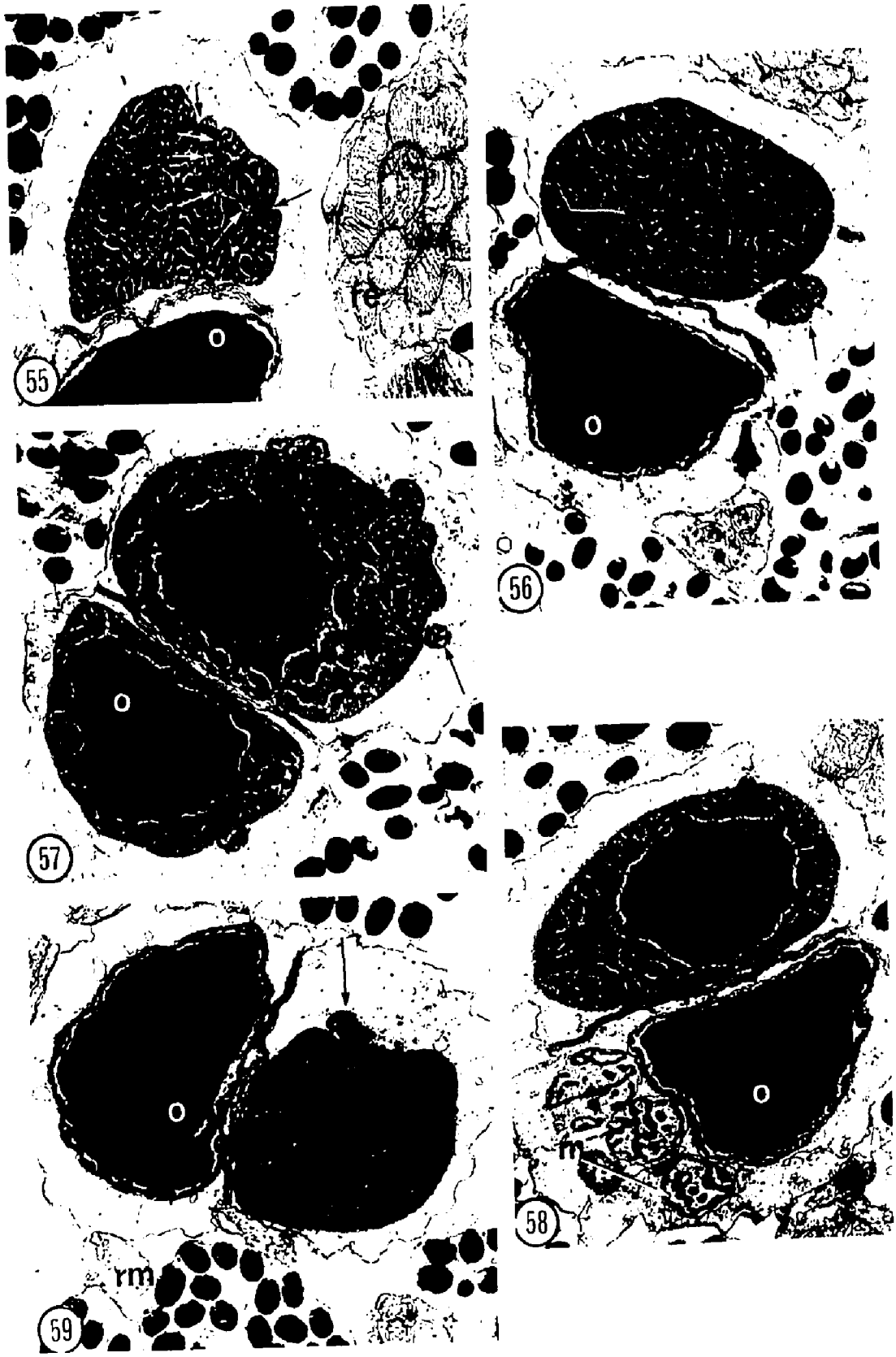
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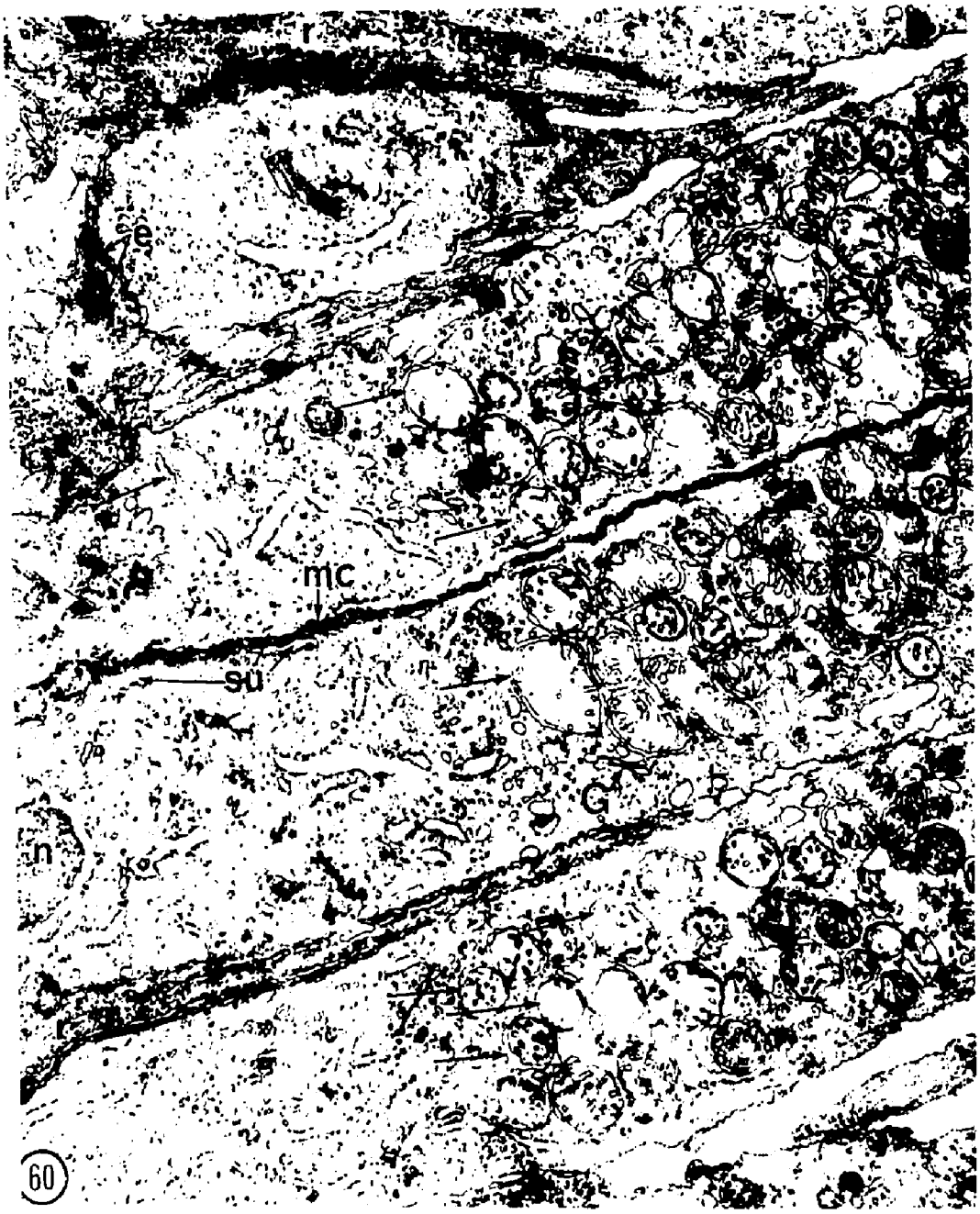
rm

Transmission electron micrographs

Figure 60. Longitudinal section through the ventral part of the retina showing the vitreal ends of the cone inner segments; the external limiting membrane (e), portion of a cone nucleus (n) and rods (r). Note the membrane complex (mc) of the double cone and the associated subsurface cisterns (su). The myoid is rich in ribosomes and polysomes, and cisterns of smooth and rough endoplasmic reticulum. Part of a Golgi stack (G) can be seen near the mitochondria. The most vitreal mitochondria (arrows) have few cristae, loose-fitting outer membranes, and pale matrix. Note the cisternae among the mitochondria.

X 11,700


60


r
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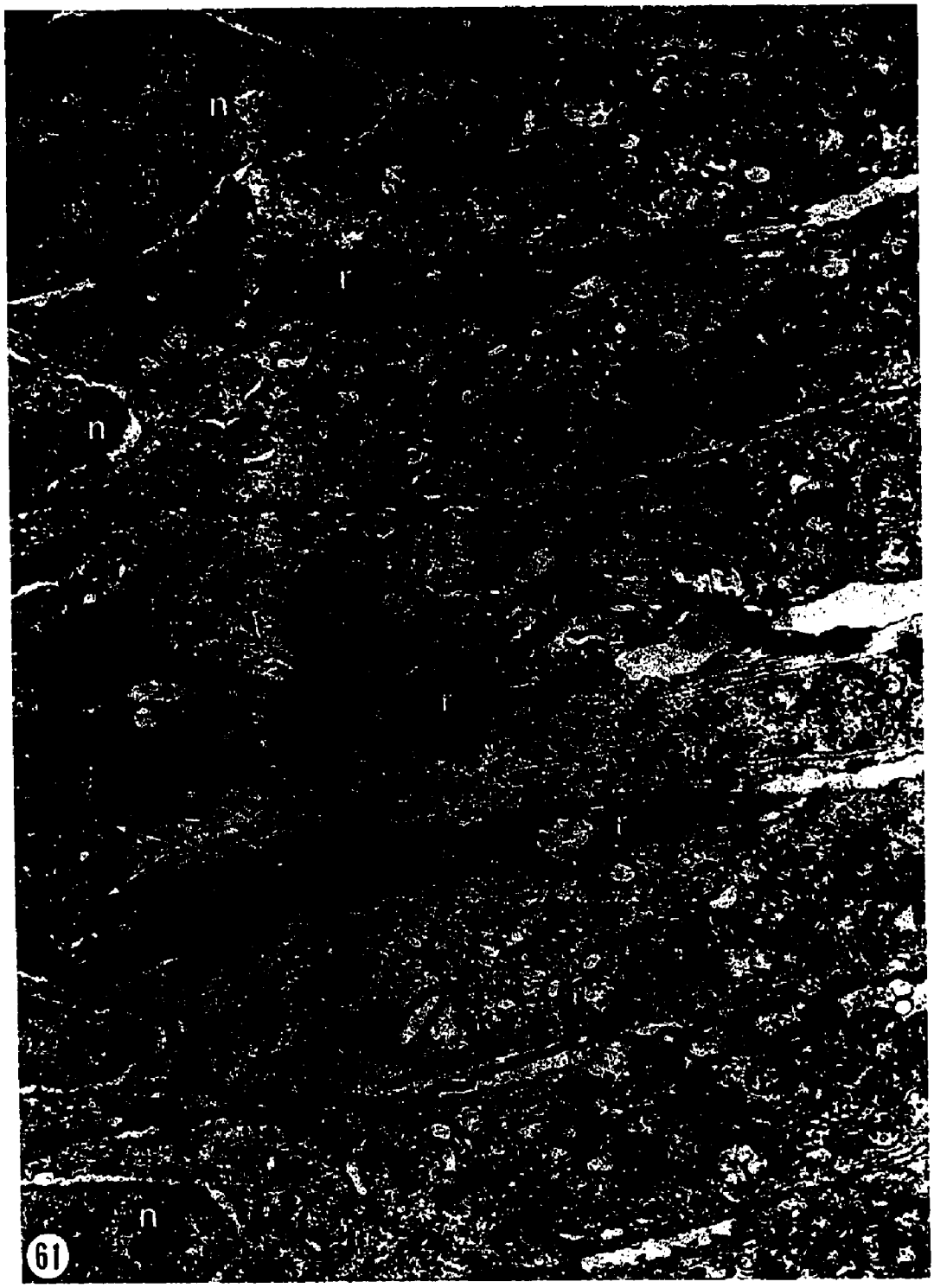
mc
su
n
60

Transmission electron micrographs

Figure 61. Longitudinal section through the ventral part of the retina, showing the external limiting membrane (e); portions of cone nuclei (n); the cone myoids (cm) and the beginning of the ellipsoids; and rods (r). The cone myoids contain longitudinally oriented microfibrils and cisternae (smooth and rough) of endoplasmic reticulum. "Vague" mitochondria (arrows) with very few cristae and pale matrix are seen. More sclerally (righthand side) the mitochondria contain more cristae.

X 10,300

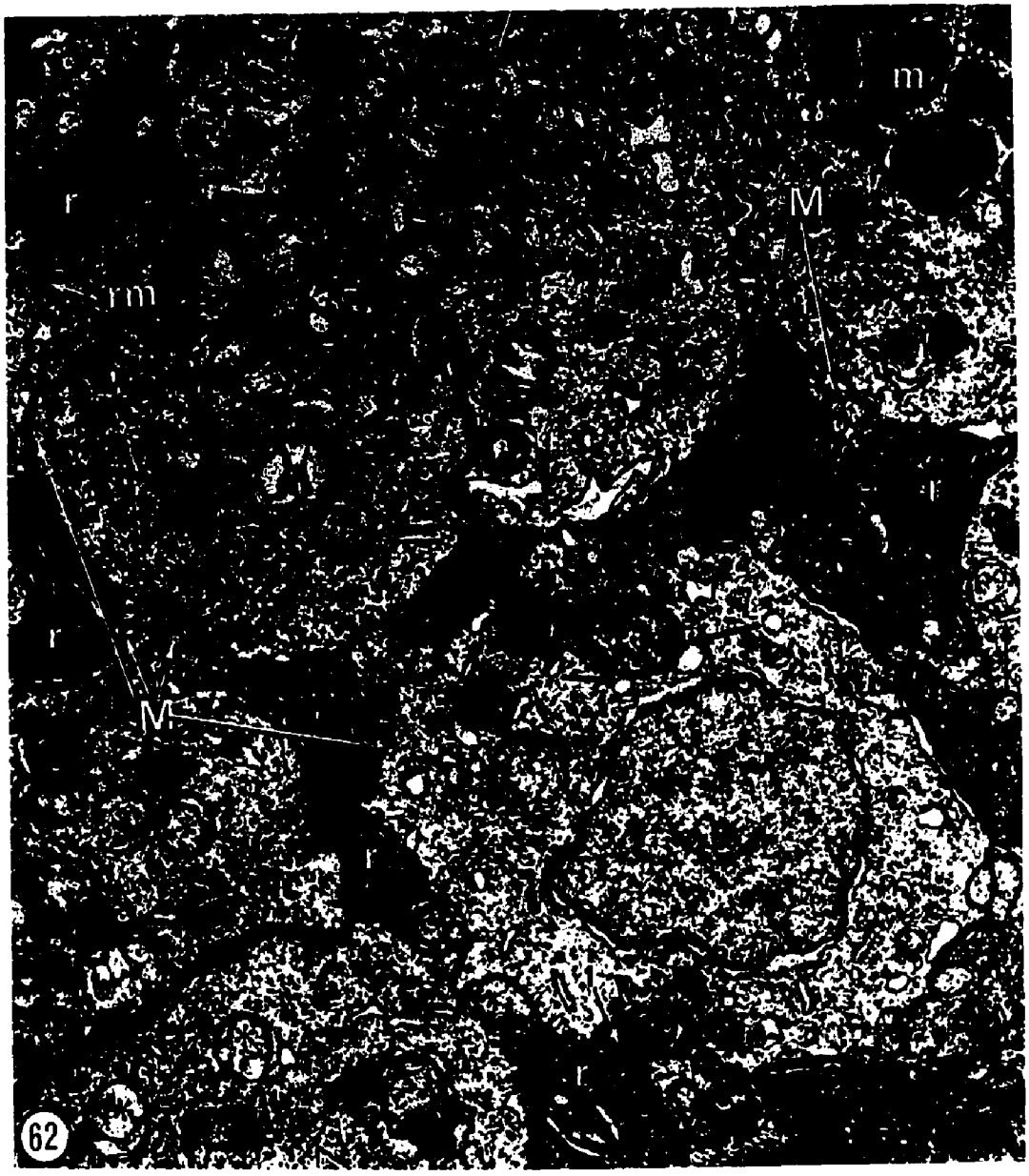

n
r
n
r
r
n
61


n
n
r
e
cm
61

Transmission electron micrograph

Figure 62. Cross section of rods and cones from the dorsal retina, just scleral to the outer limiting membrane. The thick dark lines are the membrane complexes (mc) between two cones of the double cone inner segments. The rods (r) stain more deeply than the cones (c). Note the rod invagination (i), and also the close association of cisternae of the endoplasmic reticulum with mitochondria (arrows). Müller cell processes (M) are abundant and some rods and cones contact each other directly without Müller cell intervention. Subsurface cisterns (su) can be seen where rods (r) abut on cones (c). Note the transforming mitochondria (m) with dense matrix in the upper right-hand corner, and the 'vague' mitochondria (*) with very few cristae and pale matrix (in the lower left hand corner) Note the rod myoid (rm).

X 15,300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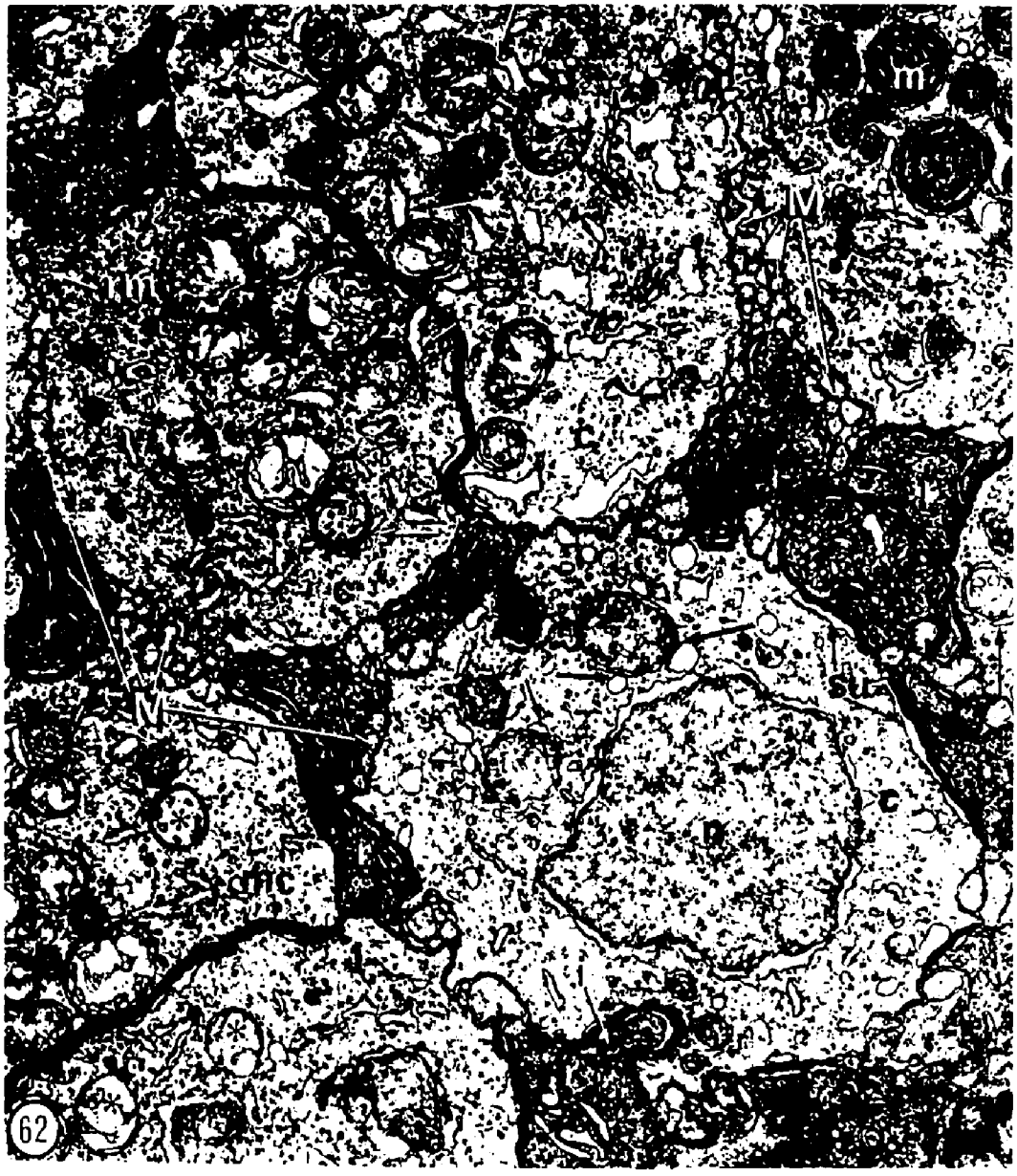
r
62

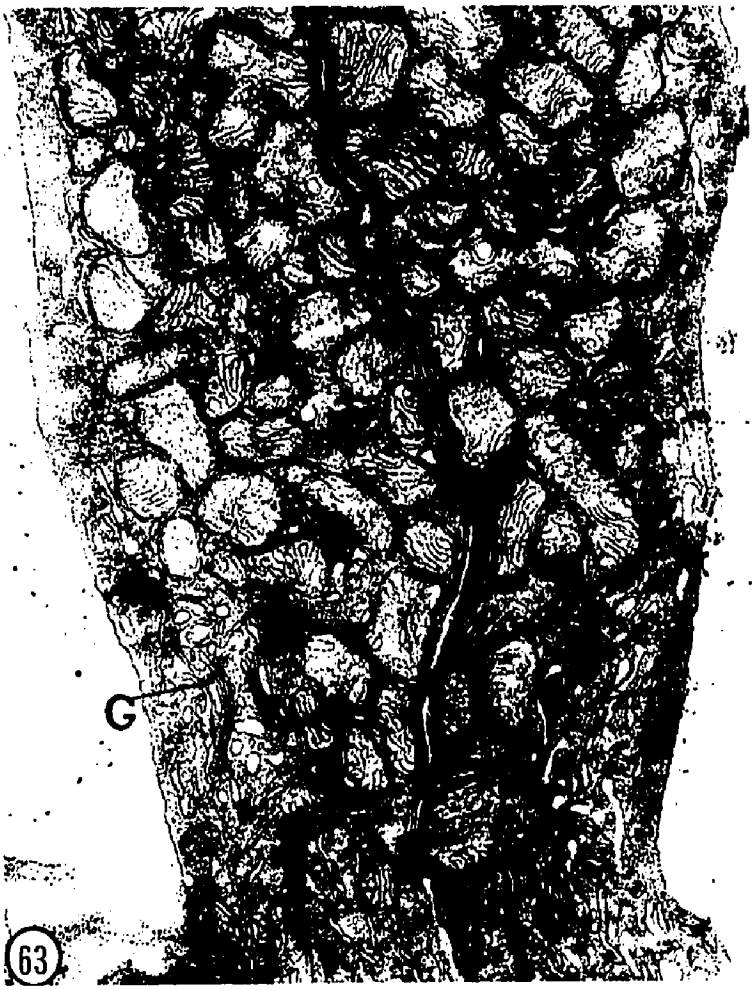

m
M
M
r
r
str
c
c
chc
62

Transmission electron micrograph

Figure 63. Part of the vitreal portions of the ellipsoids of a double cone from the periphery of the dorsal retina just scleral to the external limiting membrane (scleral surface upper, and long axis horizontal). Note the Golgi apparatus (arrow), the numerous crowded mitochondria (m), and the membrane-complex (mc) with subsurface cisterns separating the apposed ellipsoids. There are many cisternae of endoplasmic reticulum in the lower part of the ellipsoids. (Longitudinal section).

X 24,900


G
63


G
63

Transmission electron microscopy

Figure 64. Longitudinal section through cones from the dorsal part of the retina showing the Golgi apparatus (G) in the myoid. The cone myoid is rich in ribosomes & cisternae of the endoplasmic reticulum. Note the association of the cisternae and the mitochondria (arrows).

X 23,800


64

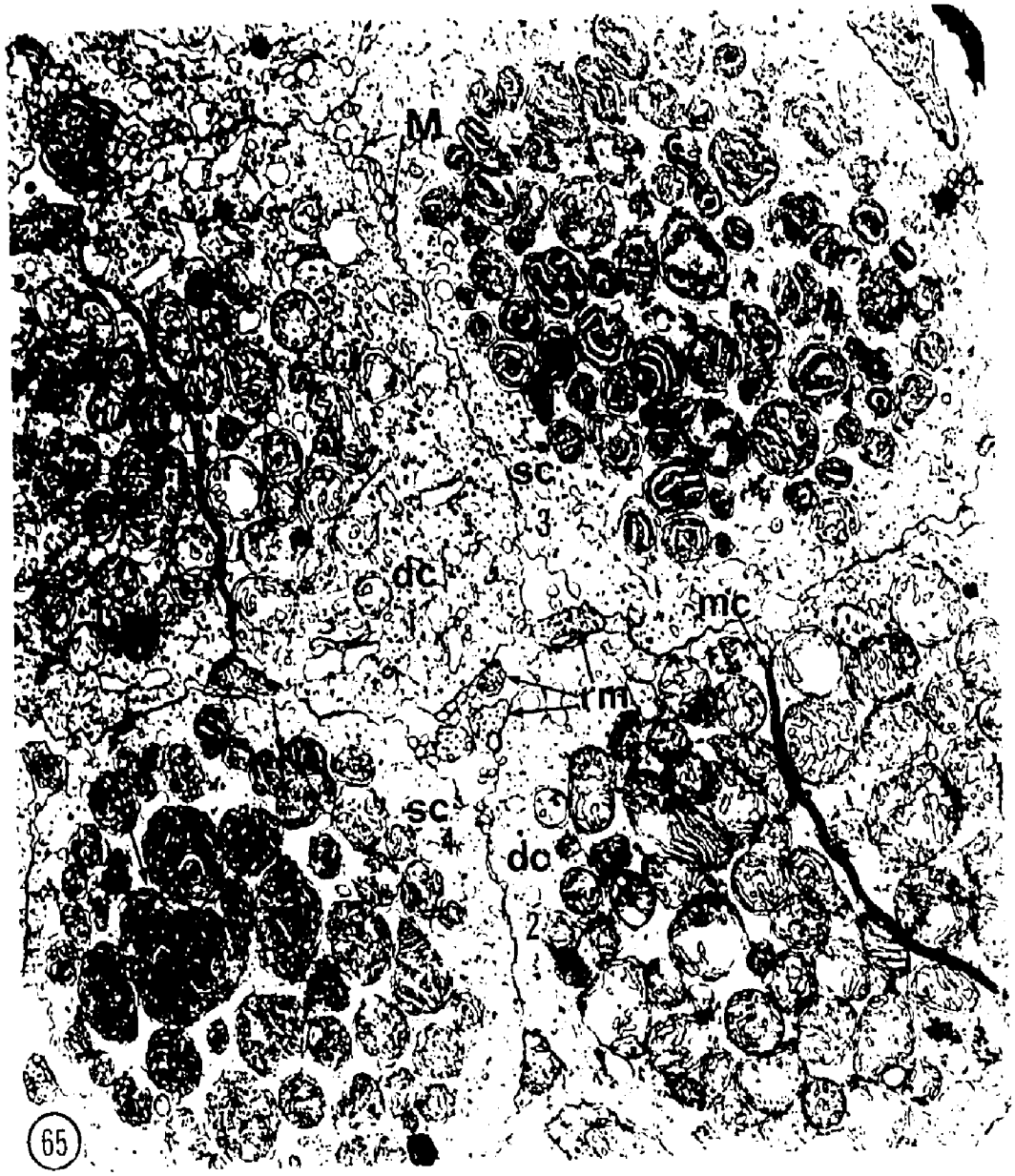

64

Transmission electron micrograph

Figure 65. Cross section through the region of the cone ellipsoids from the dorsal part of the retina, showing single cones (sc) and double cones (dc). The membrane-complex (mc) between the double cones appears as a thick, dark, wavy line. Müller cell (M) microvillous processes and rod myoids (rm) can be seen between the cones. Note the differences in the structure of the mitochondria in the different cones. In cone I there are many cisternae and many 'vague' mitochondria, with few cristae. Some of these may be fusing (arrows). In cone 2, there are more and larger mitochondria, with more cristae. The mitochondria are crowded together and there are fewer cisternae. In cone 3, the mitochondria have dense dark matrix with distinct cristae. In cone 4, the mitochondria are enlarged, some have fused (arrows) and the dark matrix has increased in amount. The cristae are disappearing. Note the cisternae among the dark mitochondria.

X 11,900


65


M
sc
3
dc
mc
rm
sc
4
dc
2
65

Transmission electron micrograph

Figure 66. Longitudinal section of cones, from the ventral part of the retina, extending from their nuclei (n) and the external limiting membrane (e) to their outer segments (co). The cone myoids (cm) have few or no mitochondria. There are pale, small & 'vague' mitochondria at the vitreal ends of the ellipsoids and they increase in size and staining in a vitreal-scleral direction (thick arrow). Three cones show oil droplets (o), and two show enlarged and transformed mitochondria (m) in the oil droplet position. Note the accessory outer segments (a), and also the long cylindrical pigment granules (P). The membrane complexes and the associated subsurface cisterns (su) show clearly. The rod (r) cytoplasm stains more darkly than that of the cones.

X 4,700


co
m
co
o
o
o
r
66


co
m
P
m
a
o
o
o
su
su
cm
cm
66

Transmission electron micrographs

Figure 67. Longitudinal section of part of a cone showing the ellipsoid. The vitreal-scleral gradient (dark arrow) of mitochondrial size change is shown and also the increase in the amount and density of the matrix, and the loss of cristae. At the scleral end of the inner segment, the structures seen are very like early-formed oil-droplets (o). There is a distinct but intergrading transition from mitochondria (m) to oil droplet (o). Note the separating membranes (arrows).

X 18,700


O
O
67


67

Transmission electron micrographs

Figures 68-73. Cross sections showing fusing mitochondria from cone ellipsoids. The mitochondria are in various stages of their developmental transformations into oil droplets, from those with few cristae (figs. 68,69); to 'typical' mitochondria (figs. 71,72); to very enlarged mitochondria with dense, fibrillar-granular matrix and few cristae (fig. 70); to very dense & dark mitochondria, much like oil droplets (fig. 73). Arrows indicate fusing mitochondria.

Figure 68	X 27,600
Figure 69	X 27,600
Figure 70	X 34,000
Figure 71	X 19,000
Figure 72	X 27,600
Figure 73	X 20,700


68


69


70


71


72


73


68


69


70


71


72


73

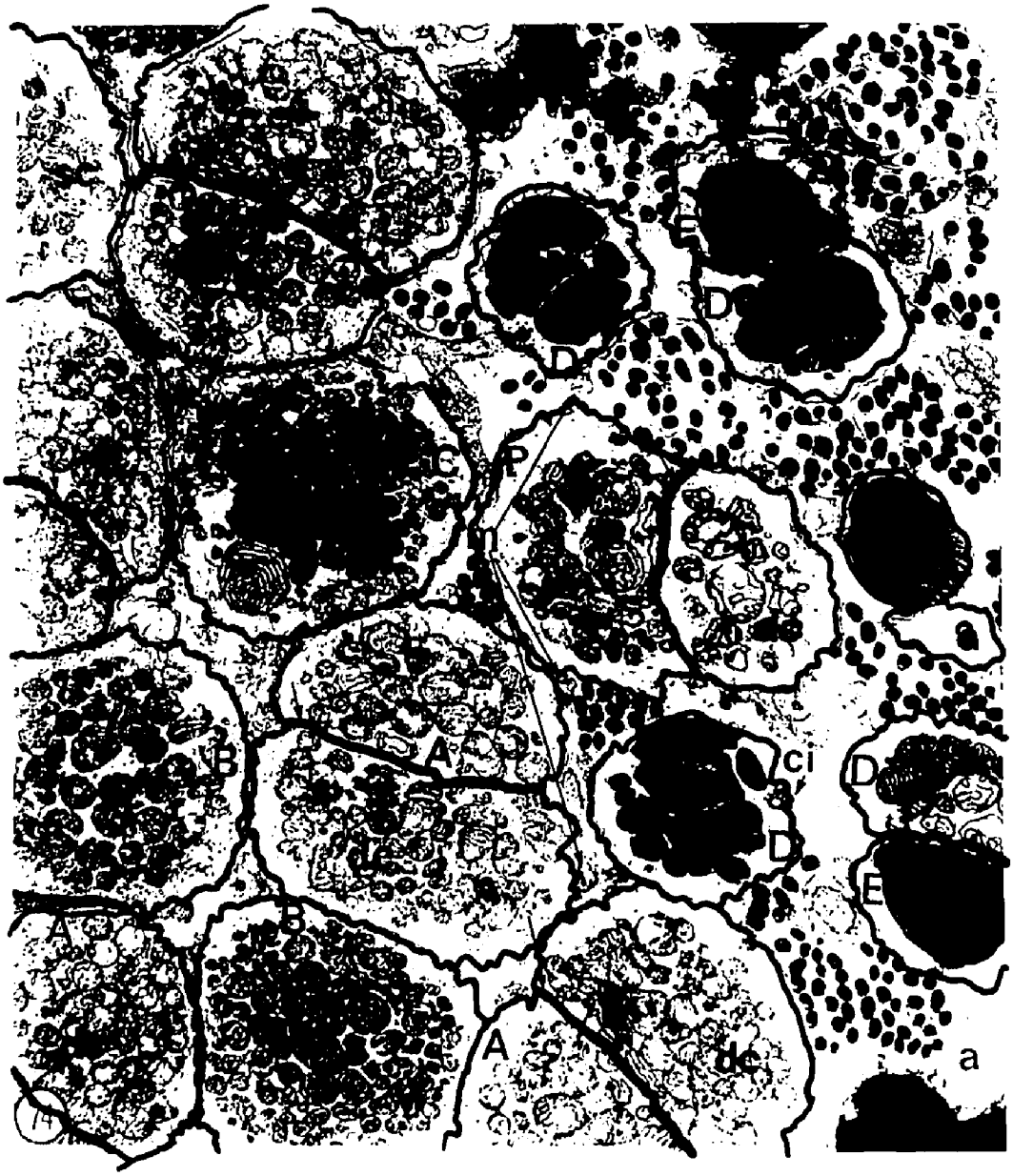
Transmission electron micrograph

Figure 74. Cross section through the dorsal part of the retina, showing double-cones with their separating dark membrane-complexes (arrows). The mitochondria show a range of the changes they undergo from small and pale (A); to small and dark (B); to enlarged with dark matrix (C); to enlarged, very dark, & fused together (D);to the oil-droplets (E).

Note the cone cilium (ci), the accessory outer segment (a) and the small rod myoids between the cones (rm). The mosaic pattern of the single & double cones is drawn on the covering transparency.

X 7,560

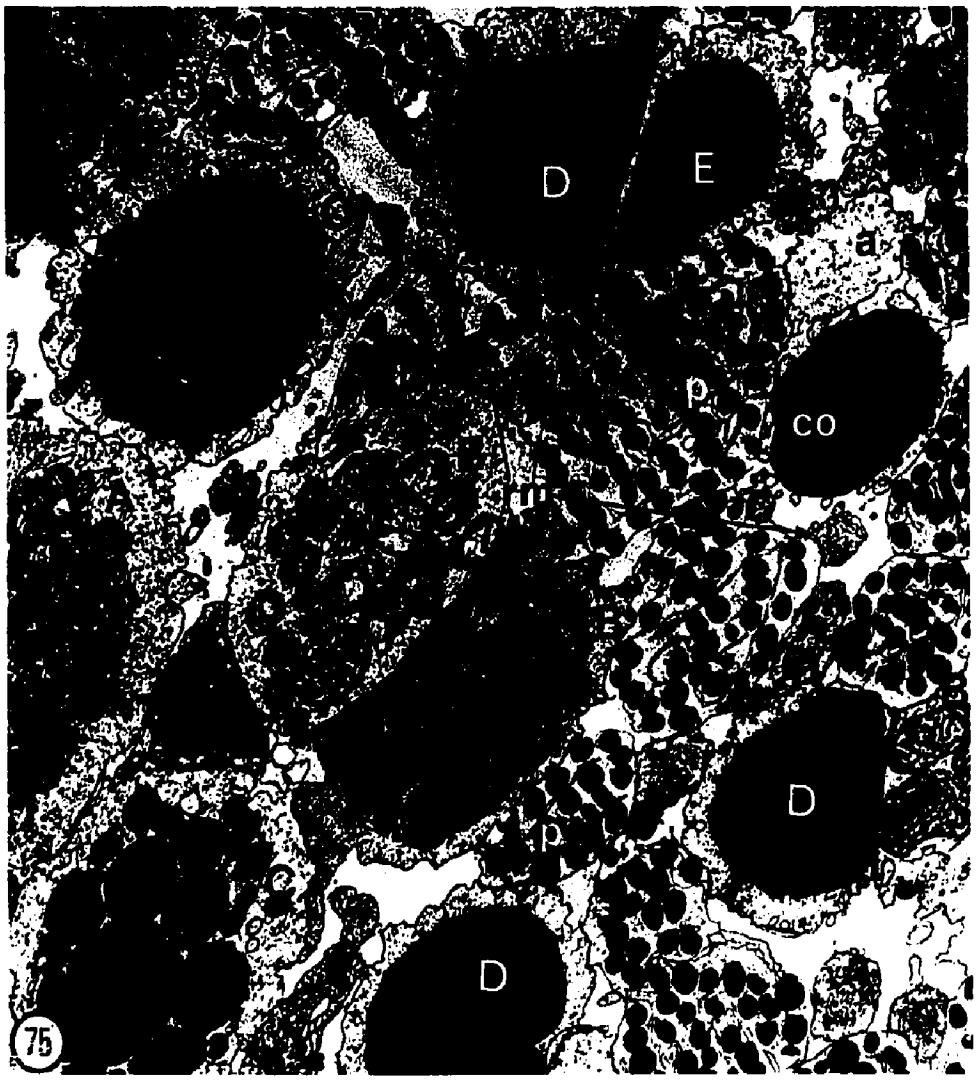
a
b

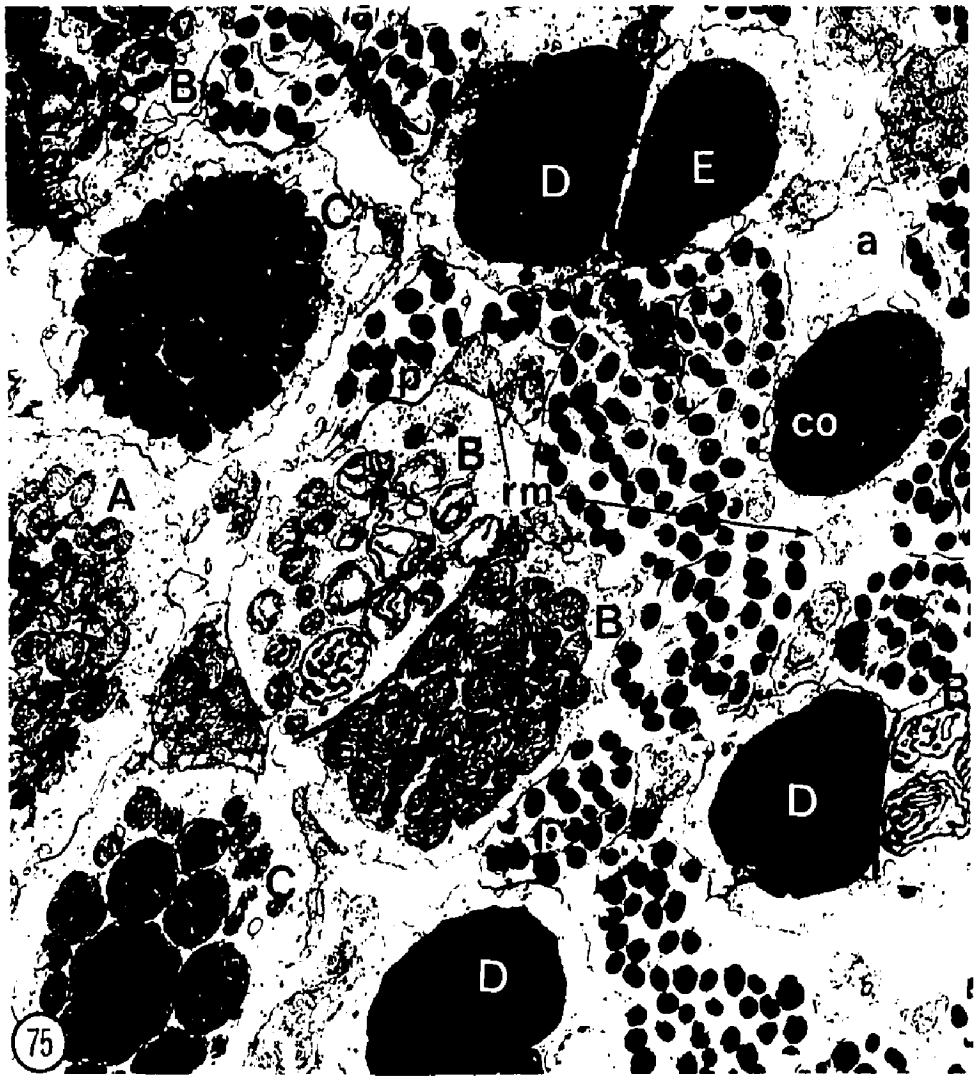

E
D
D
C
P
A
B
ci
D
D
E
B
A
A
de
a

Transmission electron micrograph

Figure 75. Cross section from the dorsal part of the retina showing cones with a range of mitochondrial transformations from normal (A), to the beginnings of the accumulation of dense matrix (B), to very dense, enlarged and fused mitochondria (C) to oil droplets that are still channelised (D) and finally to uniformly dense and homogeneous oil droplets (E). Note the accessory outer segment (a) of the cone outer segment (co), the rod myoids (rm), and the pigment-epithelial cell processes (p) laden with pigment granules.

X 21,600


D
E
a
p
co
D
p
D
75


B
D
E
C
a
p
co
B
rm
A
B
B
D
p
C
D
75

Transmission electron micrographs

Figure 76. Longitudinal section of a cone from the dorsal peripheral part of the retina. Note the very enlarged and 'channelised' mitochondria separated from each other by membranes (arrows).

X 15,600

Figure 77. Longitudinal section of two cones from the dorsal peripheral part of the retina. A very large and transformed mitochondrion (m) occupies the entire width of the distal inner segment. Note the oil-droplet-like mitochondria separated from each other by membranes (arrows).

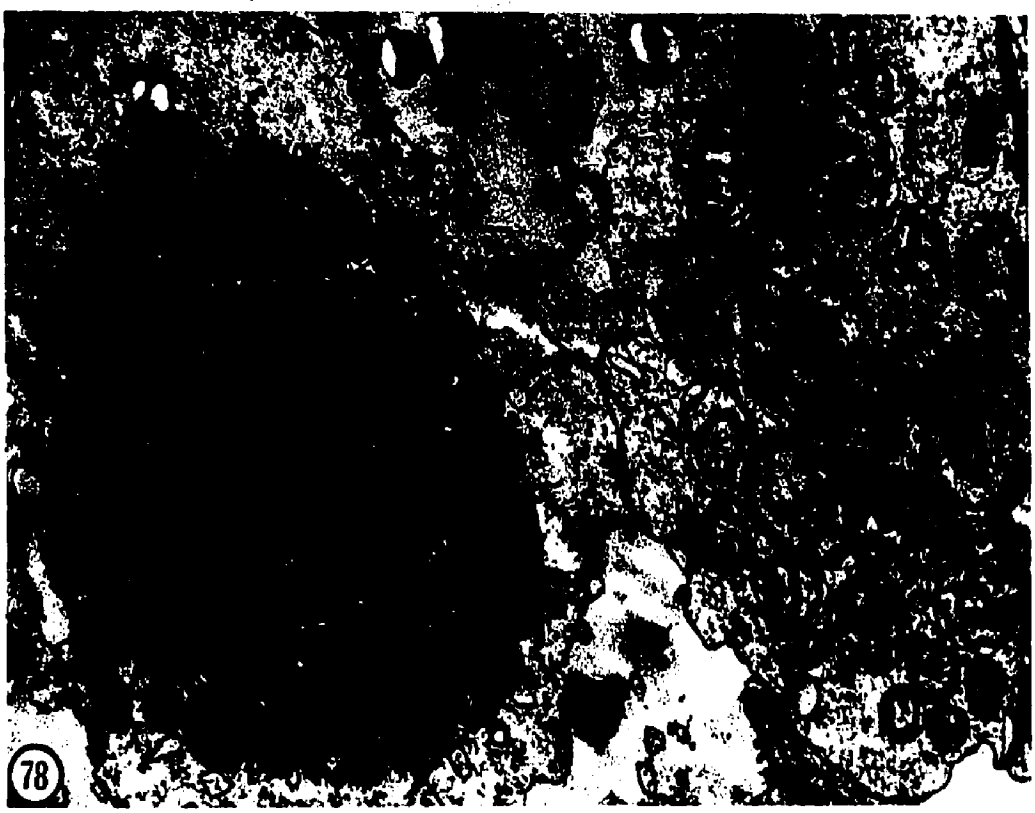
X 11,200

Figure 78. Cross section through the dorsal central part of the retina showing two cones. The right hand cone shows mitochondria in which the dense matrix is beginning to become apparent. The left-hand cone shows enlarged, fused, dark, dense and 'channelised' mitochondria. Smaller mitochondria are on the periphery; larger ones are central.

X 20,400

76

77



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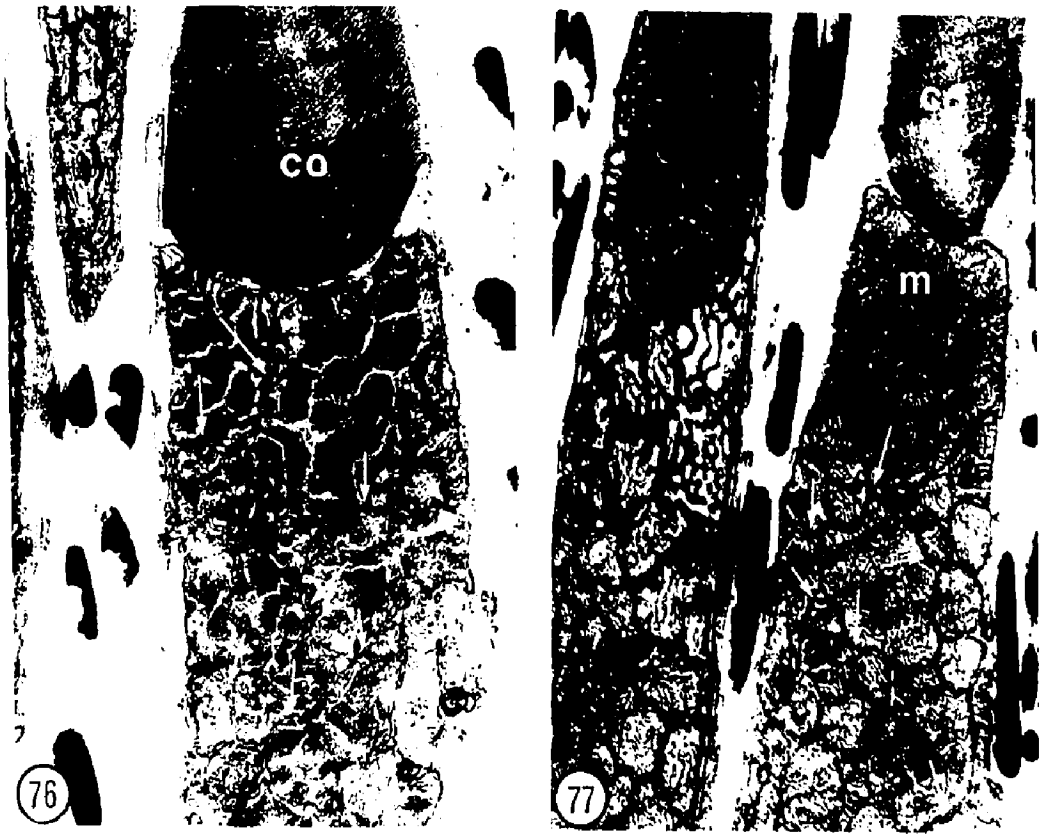
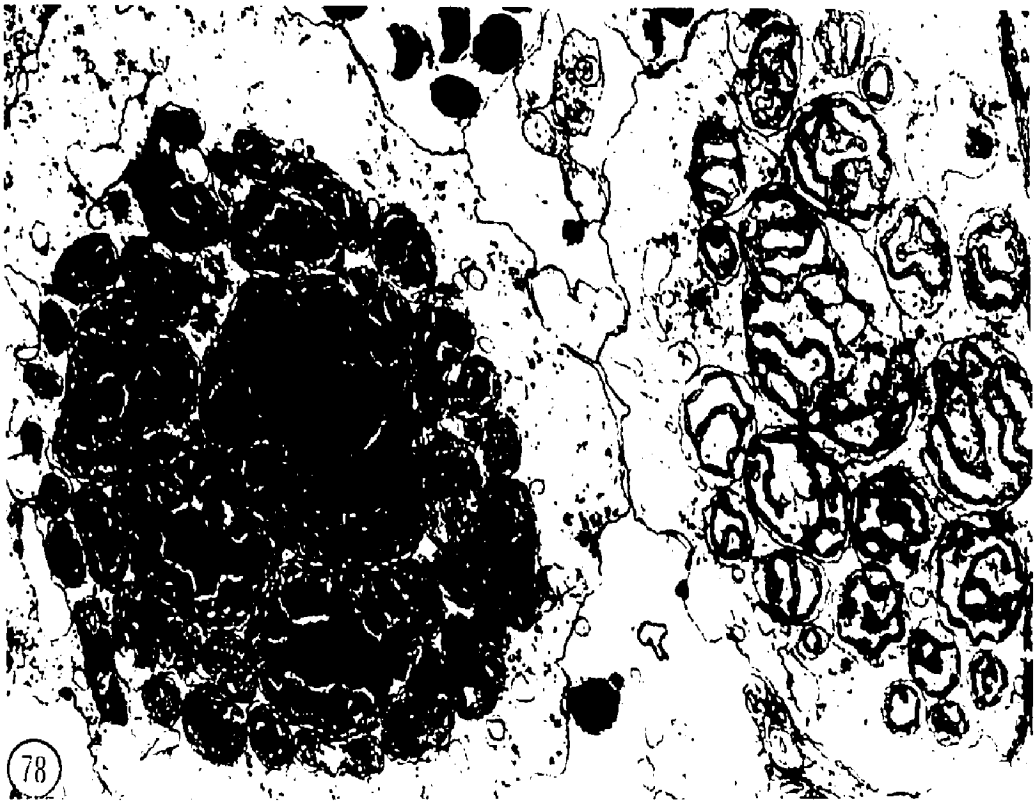

co
m
76
77


78

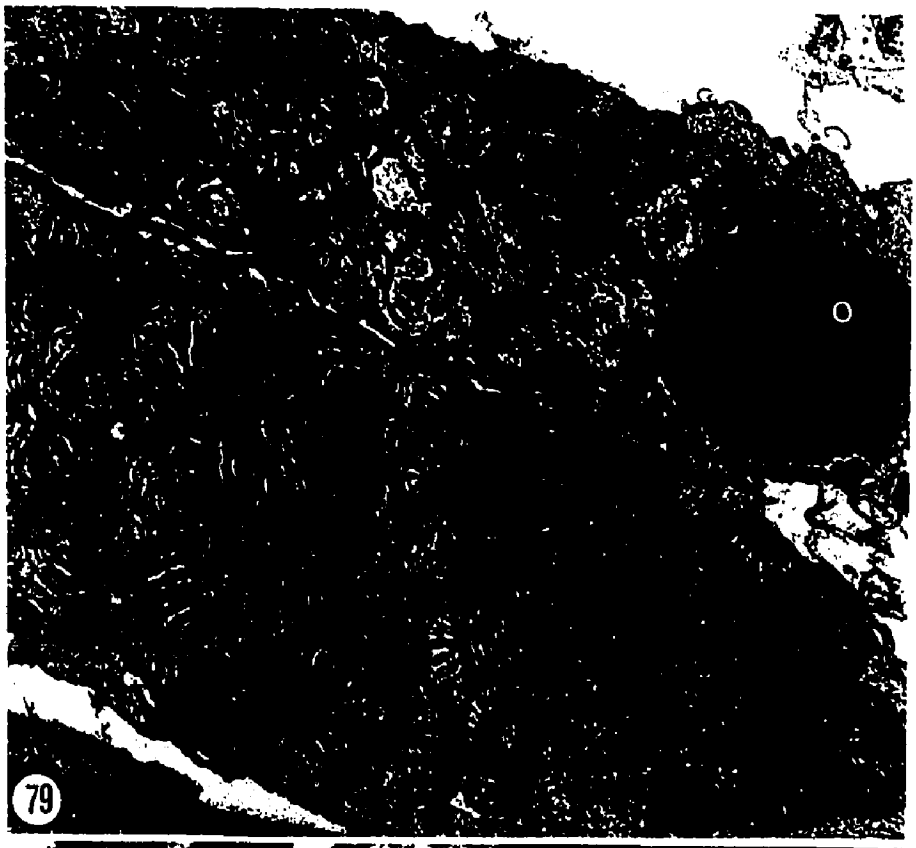
Transmission electron micrographs

Figure 79. Longitudinal section through the ventral part of the retina showing part of a double cone inner segment with the membrane complex and its underlying subsurface cisterns (su). One cone shows mitochondria that are only a little altered at the scleral end with matrix that is fibrillar-granular, and there are few cristae. At the vitreal end, the mitochondria (m) are 'normal' in appearance. The other cone of the pair has an oil droplet (o) with neighbouring mitochondria that contain dark dense matrix. More vitreally, the mitochondria (m) are normal.

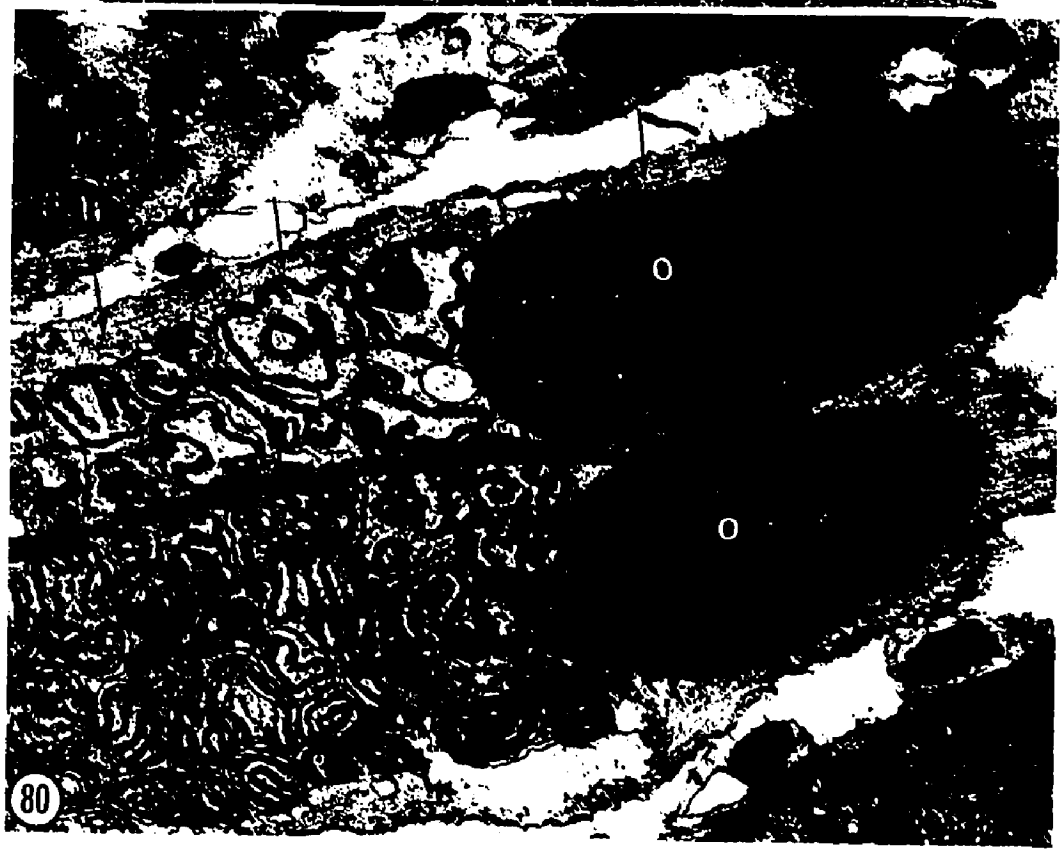
X 15,600

Figure 80. Longitudinal section through the dorsal part of the retina showing a double cone with each member of the pair containing an oil-droplet (o). The immediately neighbouring mitochondria contain areas of very dense matrix. Note the membrane-complex (mc) and the microfibrillar border (arrow).

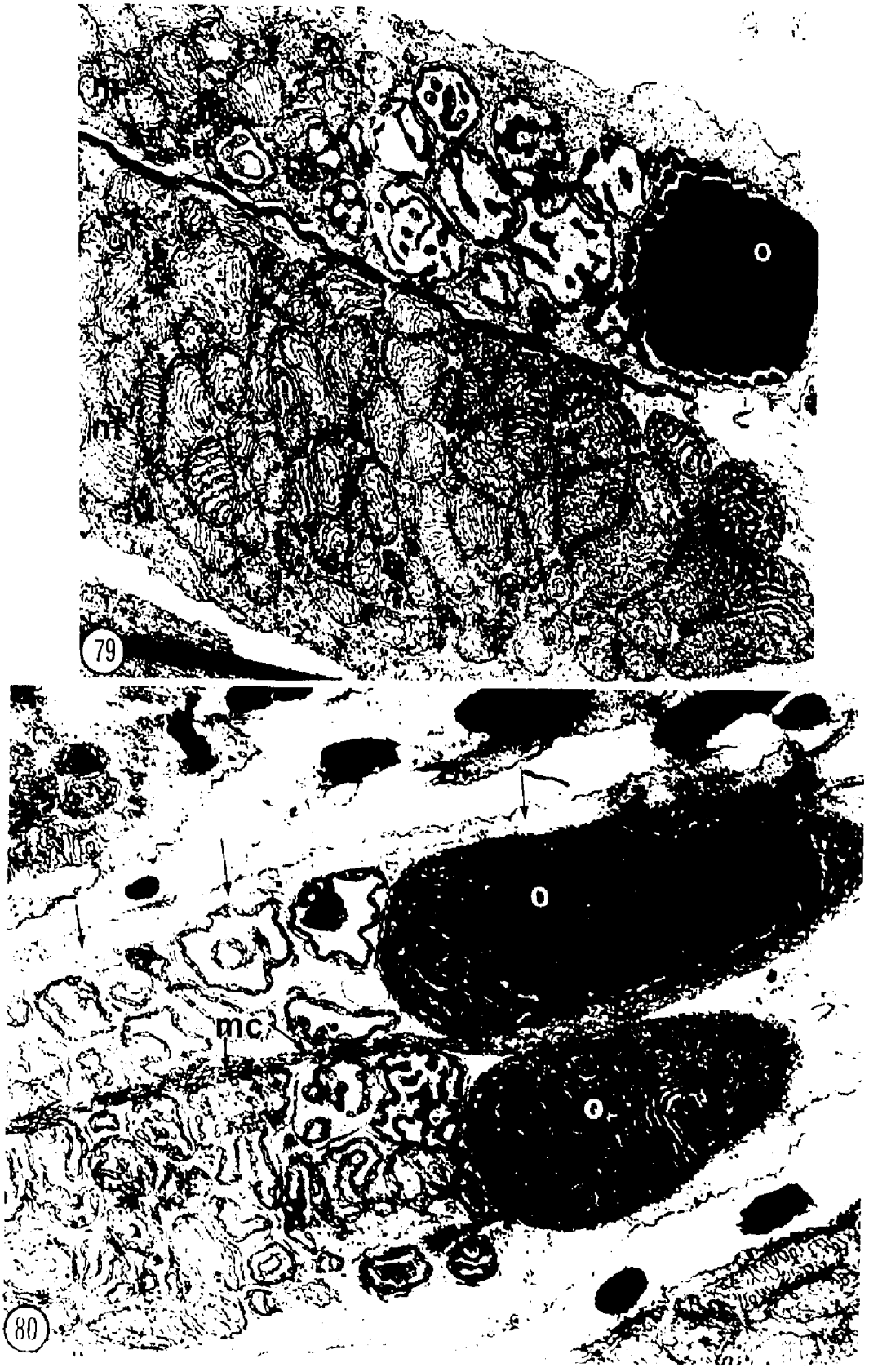
X 20,800



79



80


nf
nf
O
79
O
O
mc
80

Transmission electron micrographs

Figure 81. Longitudinal section through a cone from the dorsal part of the retina. The oil-droplet (o) is dense but has irregular light areas and towards its scleral end there is a mitochondrion (m) partly inserted into the oil-droplet. Note the rod (r).

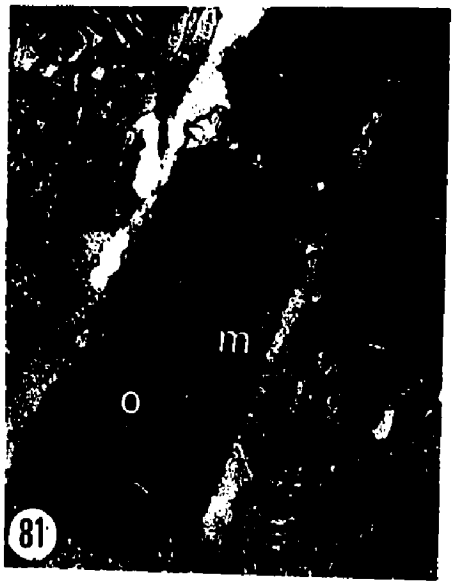
X 14,300

Figure 82. Longitudinal section of a cone from the same retina as figure 81. The oil-droplet (o) is very dark and dense, except for a narrow peripheral area (white arrow) and a larger area distally which bears cristae-like structures (black arrow). Longitudinal microfibrils can be seen on either side of the oil-droplet.

X 19,000

Figure 83. Portions of two cones from the central part of the retina, in longitudinal section. The mitochondria (m) immediately vitreal to the oil-droplets (o) are more transformed than those further from the oil droplet. However, their appearance differs in these two neighbouring cones. In the left-hand cone the cristae have disappeared. In the right-hand one the dense matrix does not fill the entire mitochondrion. Note the increasing size of the mitochondria from the base of the micrograph towards the oil droplet.

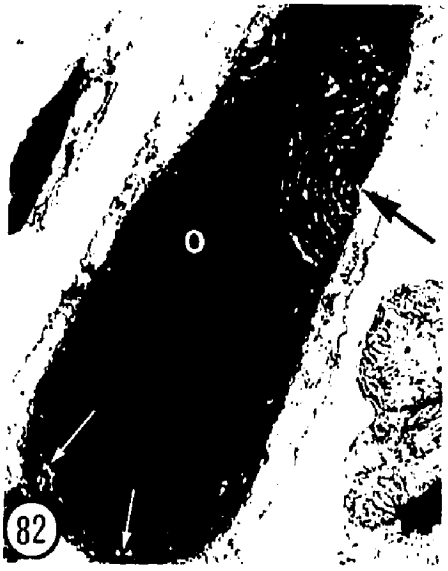
X 11,900


m
o
81


o
82


o
o
m
83


m
o
81


o
82


o
o
m
83

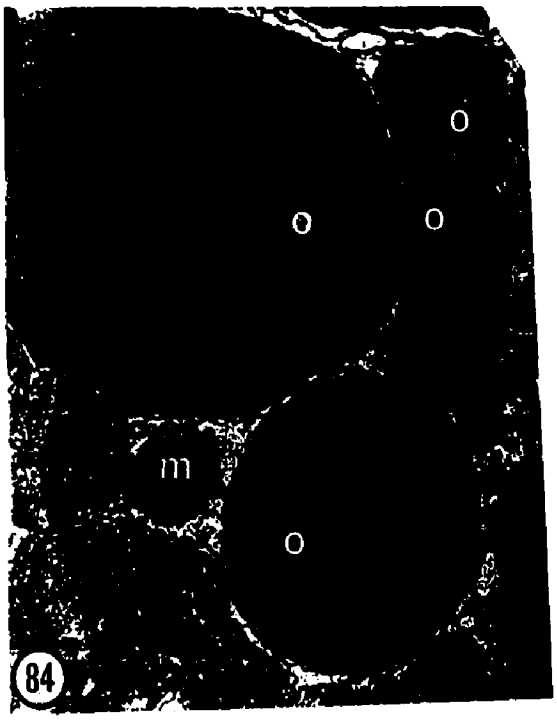
Transmission electron micrograph

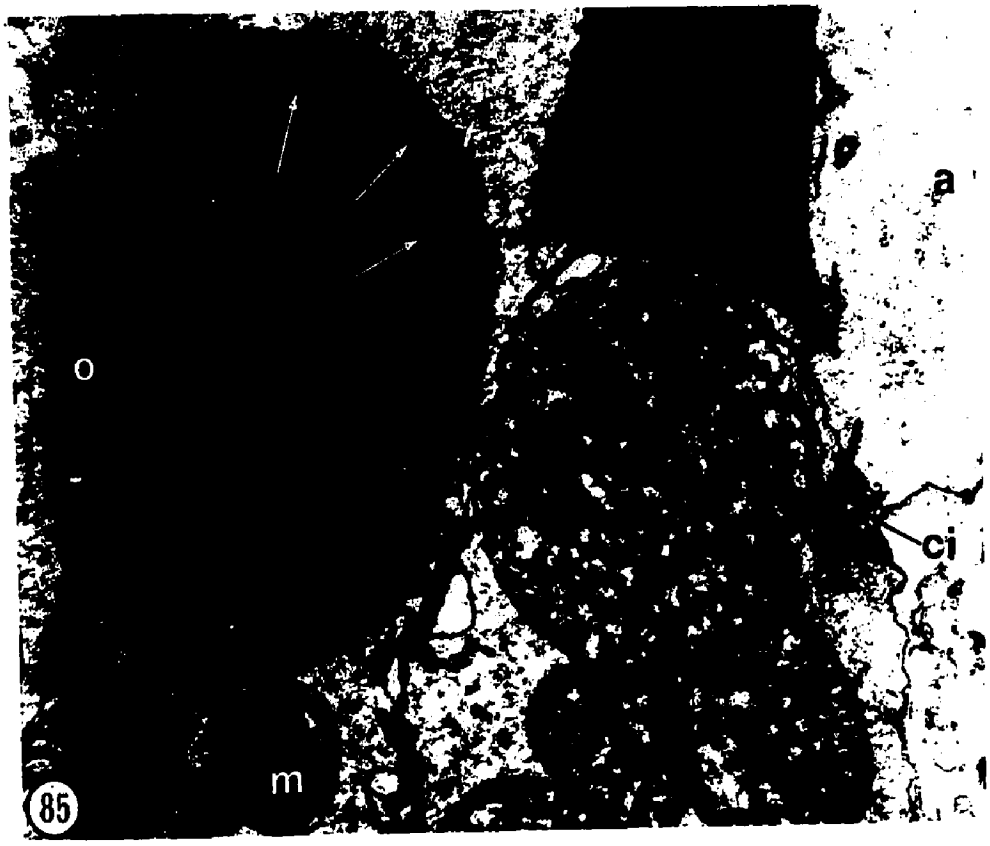
Figure 84. Longitudinal section of a portion of a cone ellipsoid from the ventral part of the retina. There are two large and two or three small oil-droplets (o), and the neighbouring mitochondria show loss of cristae and they are filled with a granular matrix. One small mitochondrion (m) has darker and denser matrix, similar to that of the oil droplets.

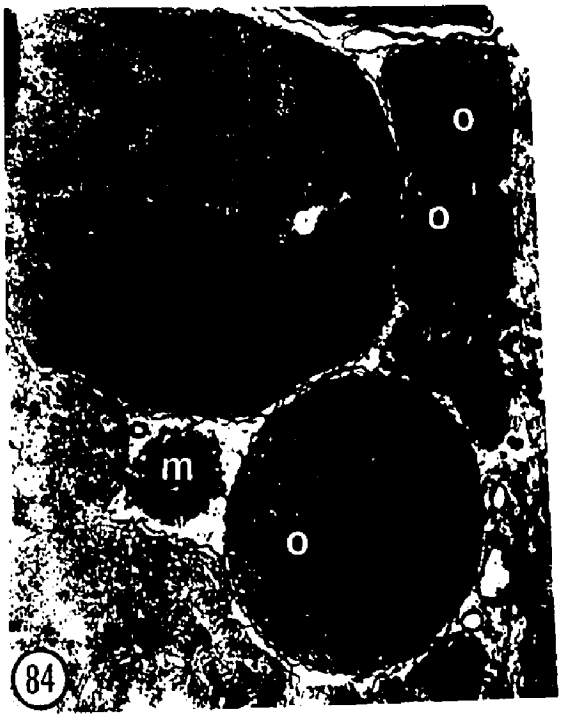
X 19,000

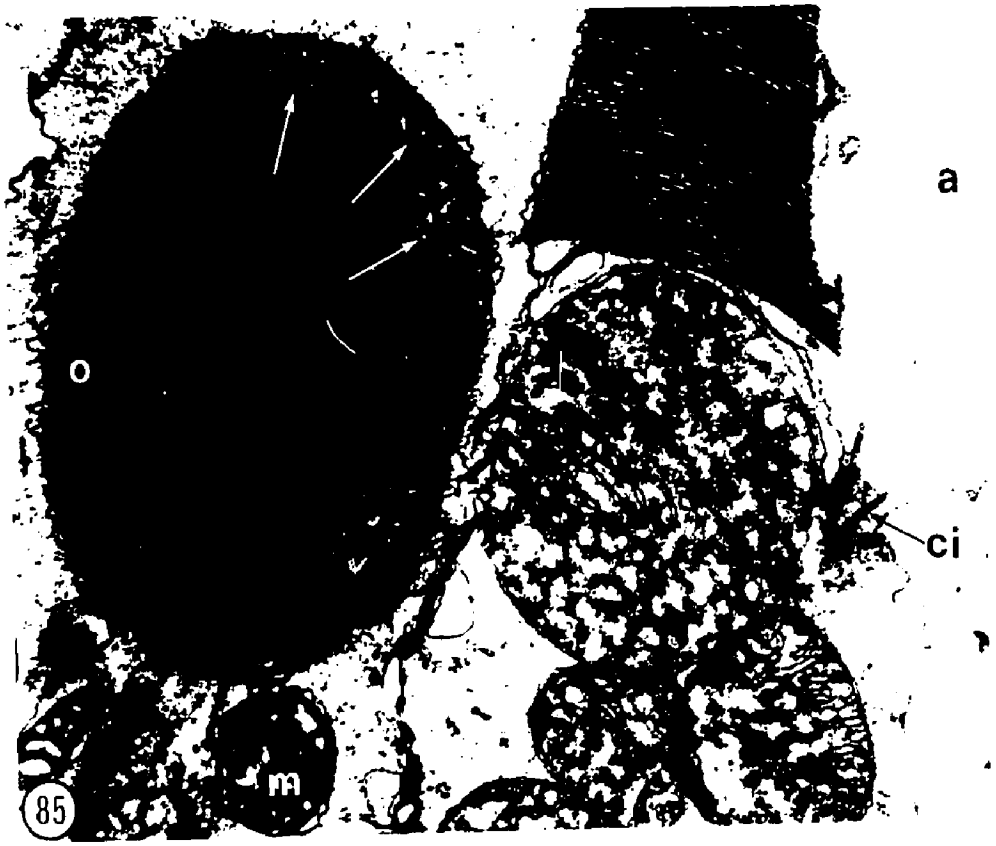
Figure 85. Longitudinal section of the ventral part of the same retina as that shown in figure 84. In one of the members of the double cone there is a well-formed oil droplet (o), with more cristae-like remnants (arrows) on its scleral side than on its ventral aspect. The mitochondria (m) neighbouring the oil-droplet contain a very dark-staining substance. The other member of the pair contains very enlarged mitochondria with disintegrating cristae, and fibrillar-granular contents. This cone shows an accessory outer segment (a) and the associated cilium.

X 25,900


o
o
o
m
o
84


a
o
ci
m
85

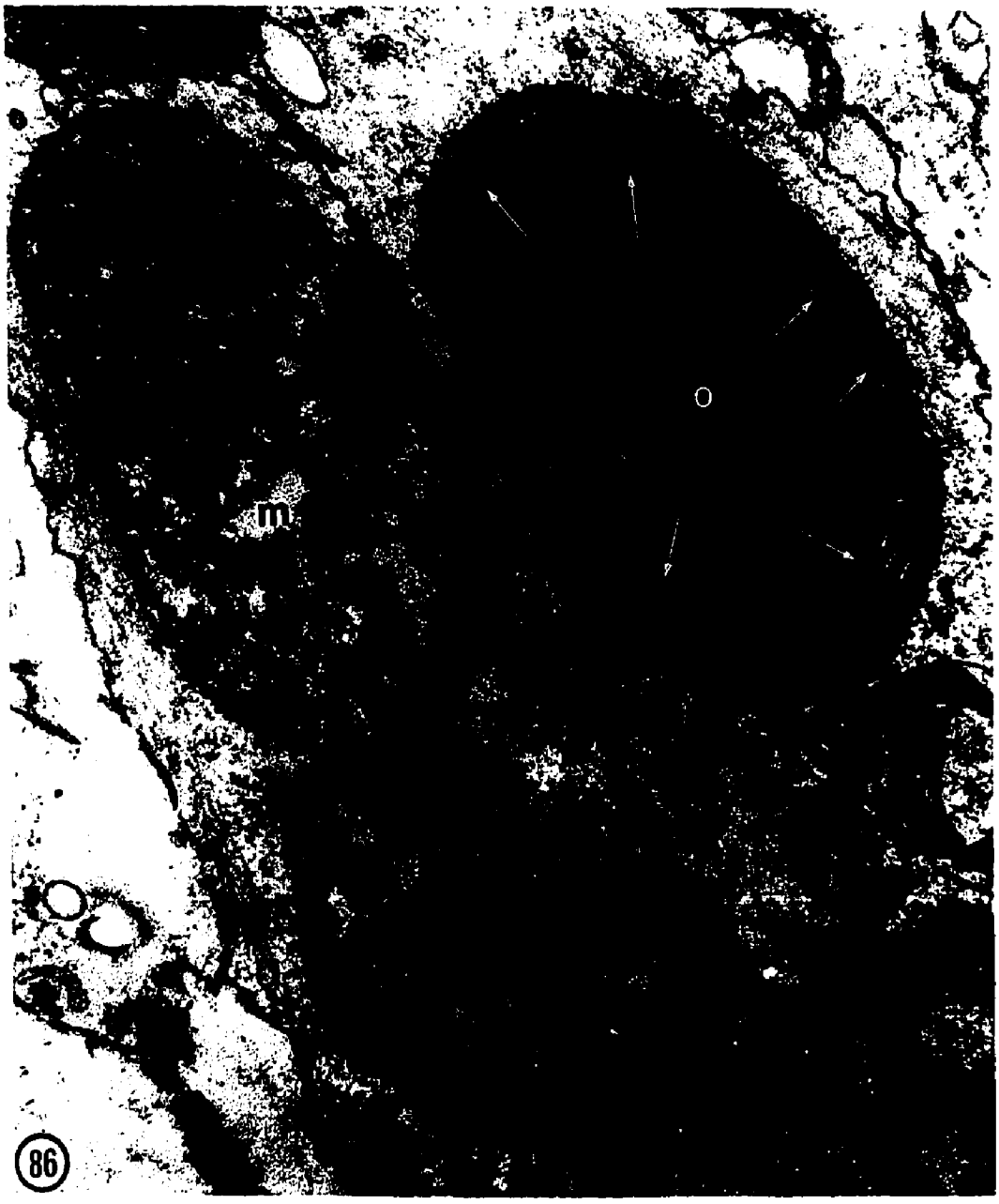

o
o
m
o
84


a
o
ci
m
85

Transmission electron micrograph

Figure 86. A double cone from the ventral retina shown in longitudinal section. The very enlarged mitochondrion (m) in the one member of the cone pair is almost the same size as the oil droplet (o) in the other member. Note that in both cones, the neighbouring mitochondria present a very similar appearance. There are cristae-like remains in the oil droplet (arrows).

X 27,600


O
m
86

86

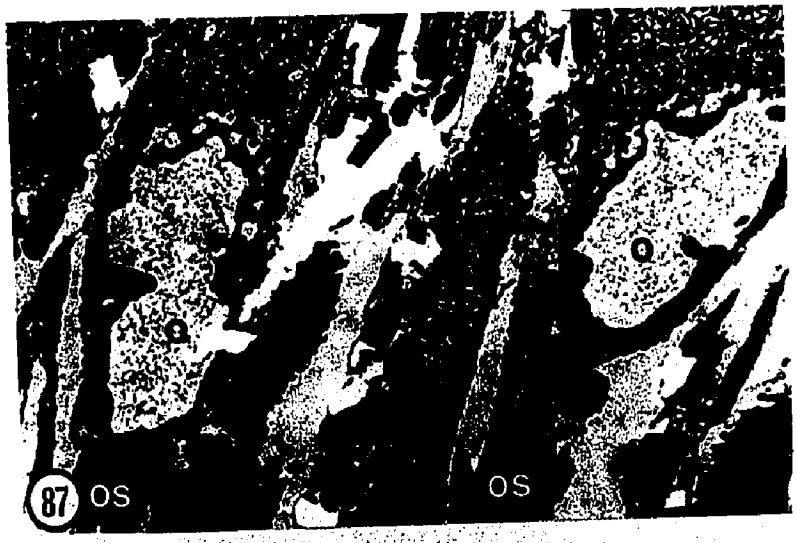
Transmission electron micrographs

Figures 87,88,89. Sections from the dorsal part of the retina fixed in osmium only, showing oil-droplets (o). Large areas have been dissolved away, leaving dispersed fibrillar remains. In figure 88 large areas of the oil-droplet have remained dense and dark staining (arrows). Note also the mitochondrial gradient (m).

Figure 87, X 10,500

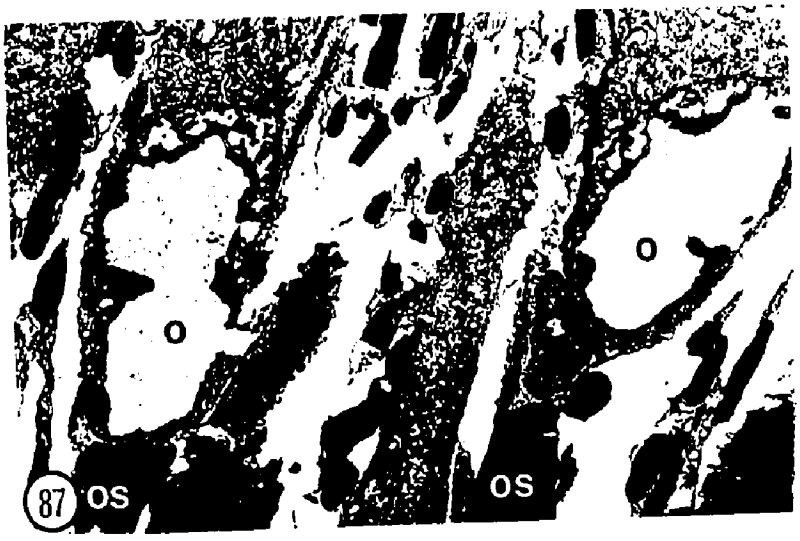
Figure 88, X 10,500

Figure 89, X 19,439

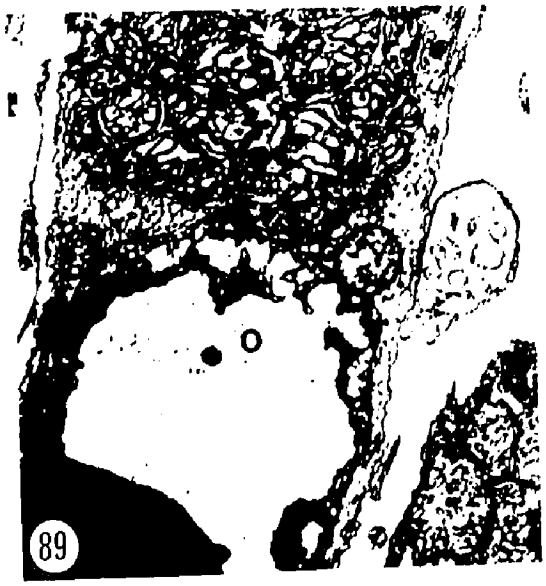

87
OS
OS


88


89


87
OS
OS


88


89

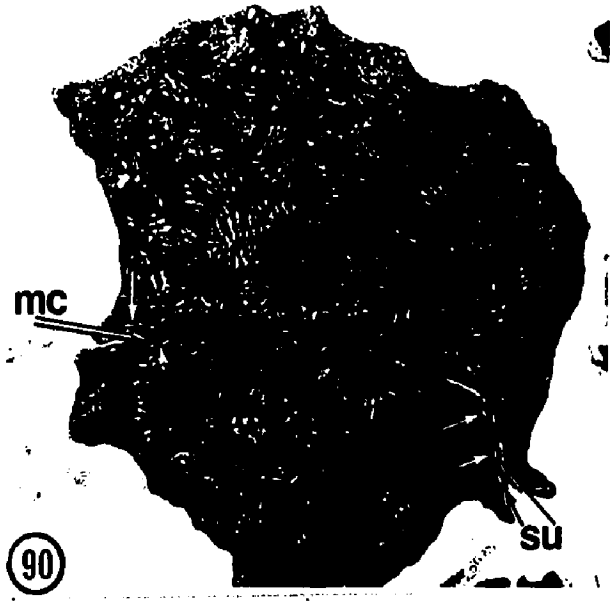
Transmission electron micrographs

Figure 90. Cross section through the ellipsoid region of a double cone from the ventral part of the retina. The membrane-complex (mc) between the two cones of the pair has associated subsurface cisterns (su and arrows).

X 22,400

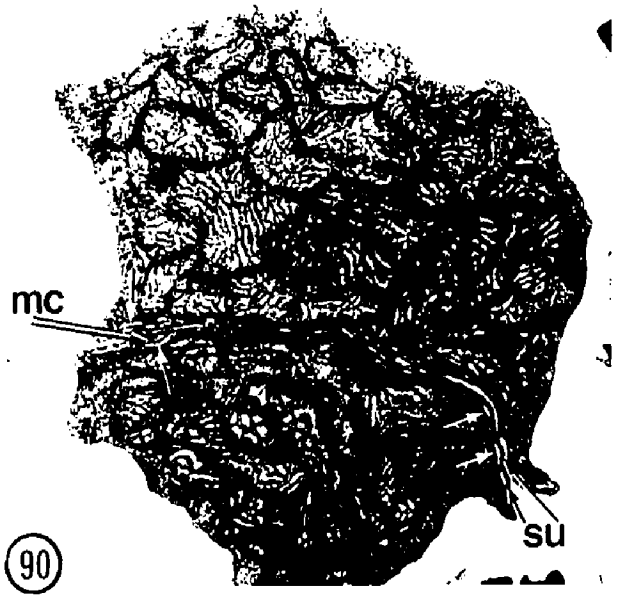
Figure 91. Longitudinal section of a double cone from the dorsal part of the retina, showing the vitreal-end origin (arrow) of the separating membrane-complex (mc) of the apposed surfaces of the double cone. Subsurface cisterns can be seen in association with this membrane-complex (arrows). The cone nucleus (n), in this case, projects well beyond the external limiting membrane. There are many cisternae of the endoplasmic reticulum present, alongside and between the mitochondria (m).

X 43,200

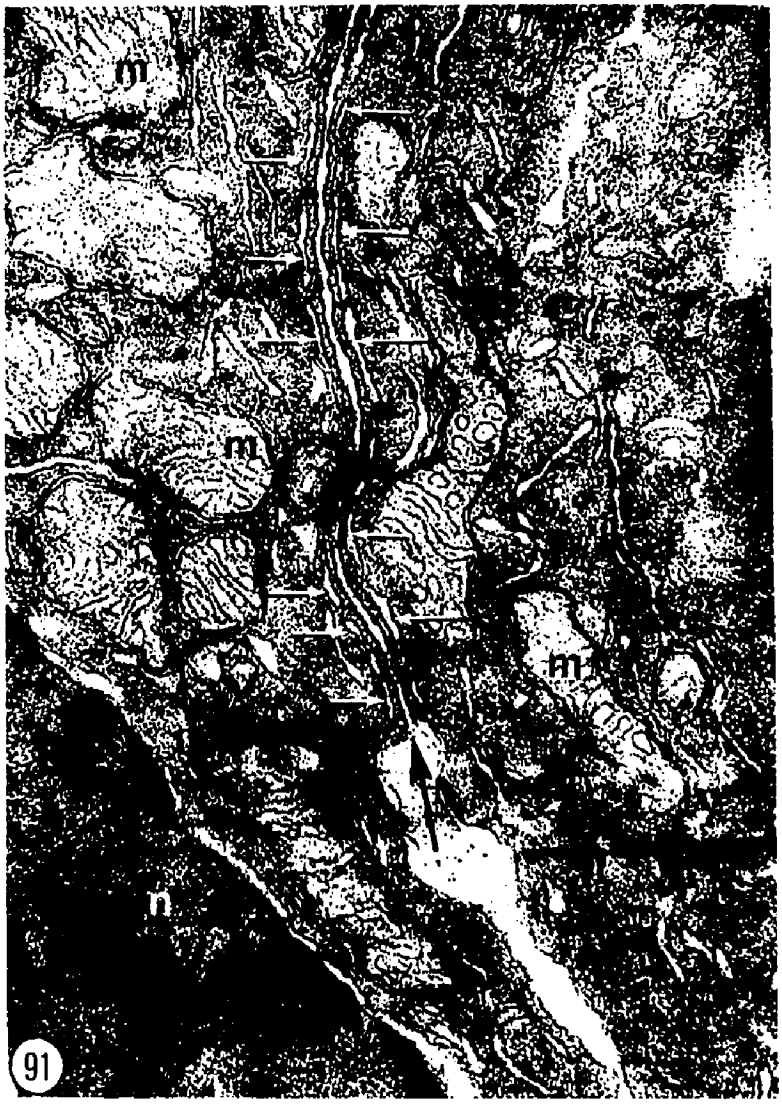


90

91



90



91

Transmission electron micrographs

Figure 92. Cross section through the dorsal part of the retina showing a double cone with its membrane complex (mc). Note the oil droplet (o) and the mitochondria (m) near it containing a very dense and dark staining substance. In the other member of the cone pair, the mitochondria show a range of transformation.

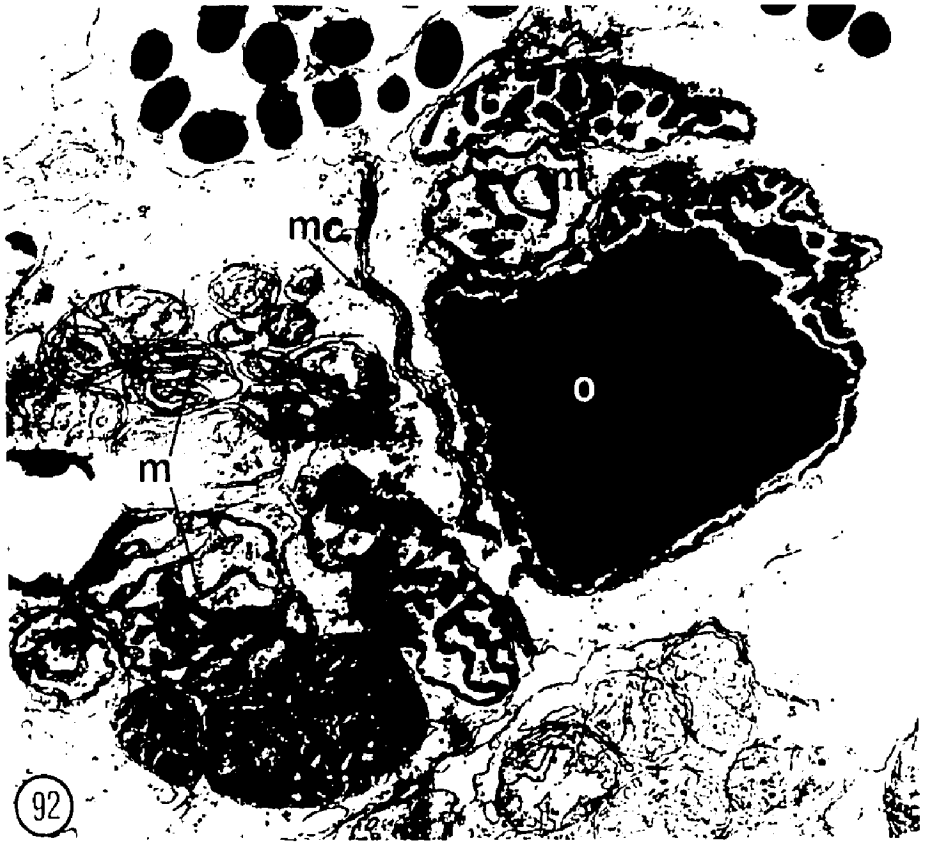
X 20,400

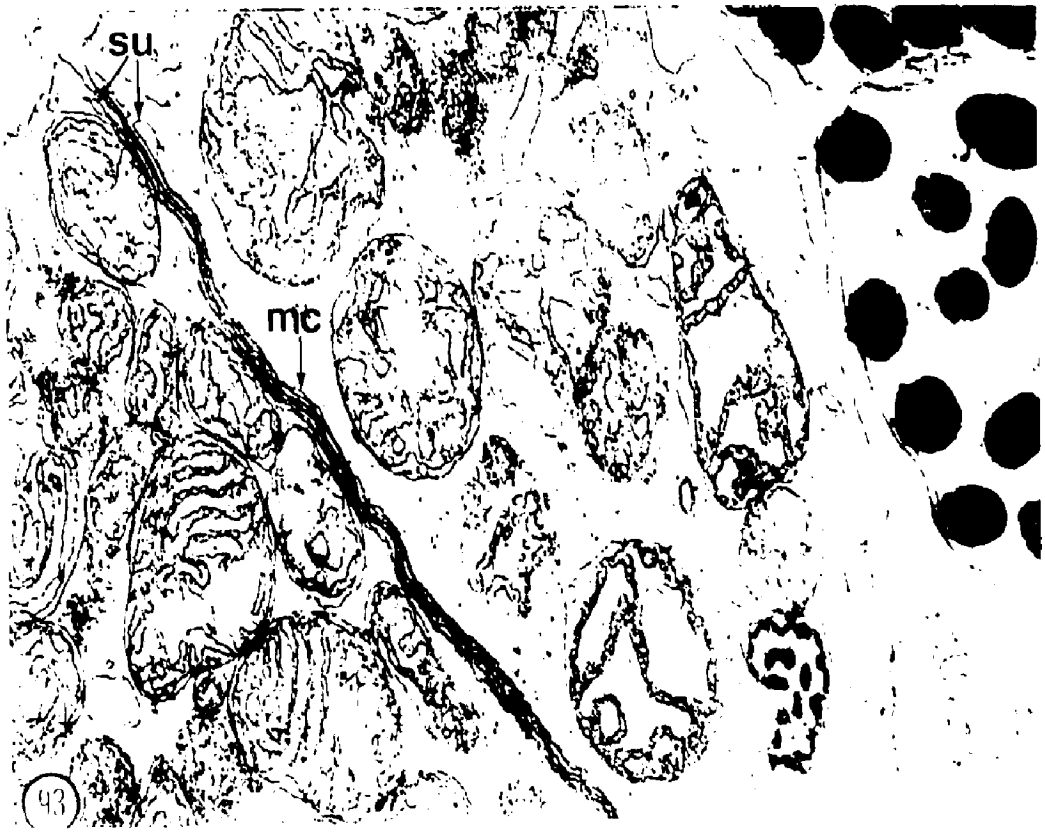
Figure 93. Cross section through a double cone from the dorsal part of the retina showing the six membranes of the membrane-complex (mc), and subsurface cisterns (su).

X 27,600


92


93


mc
o
m
92


su
mc
93

Transmission electron micrographs

Figures 94,95. Portions of cones (c) and rods (r) showing the rod knob-like invagination (i) into the cone (c). Over these invaginations there is a membrane-complex (mc), and associated subsurface cisterns (su).

Figure 94. Cross section, dorsal part of retina.

X 22,800

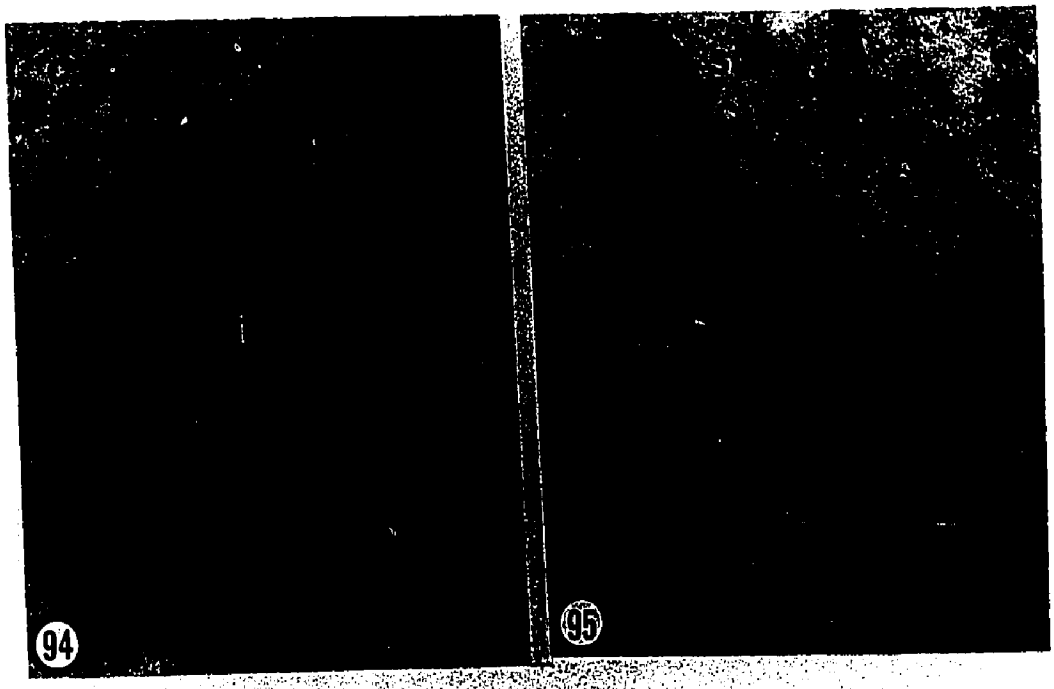
Figure 95. Longitudinal section, ventral part of retina.

X 13,900

Figure 96. Cross section through a cone and neighbouring rods, from the dorsal part of the retina

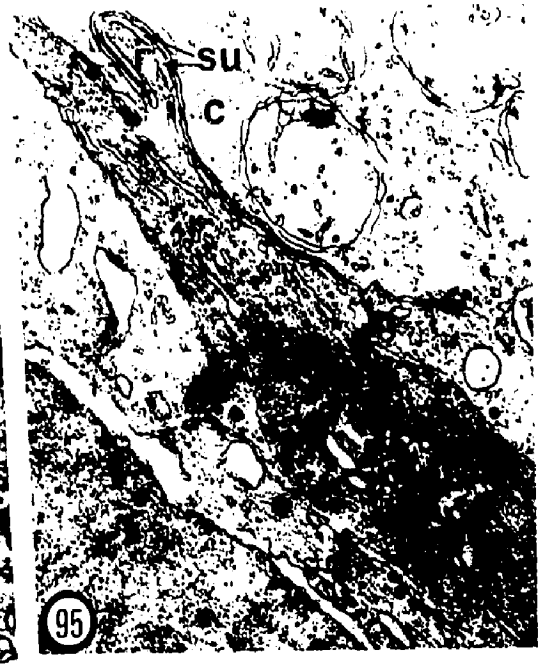
At this level, the cone is rich in ribosomes and cisternae of the endoplasmic reticulum. The mitochondria (m) are sparse, and contain few cristae and pale matrix. There are three rod invaginations (i), each characterized by a complex of four membranes and associated subsurface cisterns (su) on both the rod and cone aspects. Note subsurface cisterns also occur where rod and rod abut (arrows). There is a close association of cisternae of endoplasmic reticulum and mitochondria. The tight junctions of the external limiting membrane (e) can be seen in the upper right-hand corner.

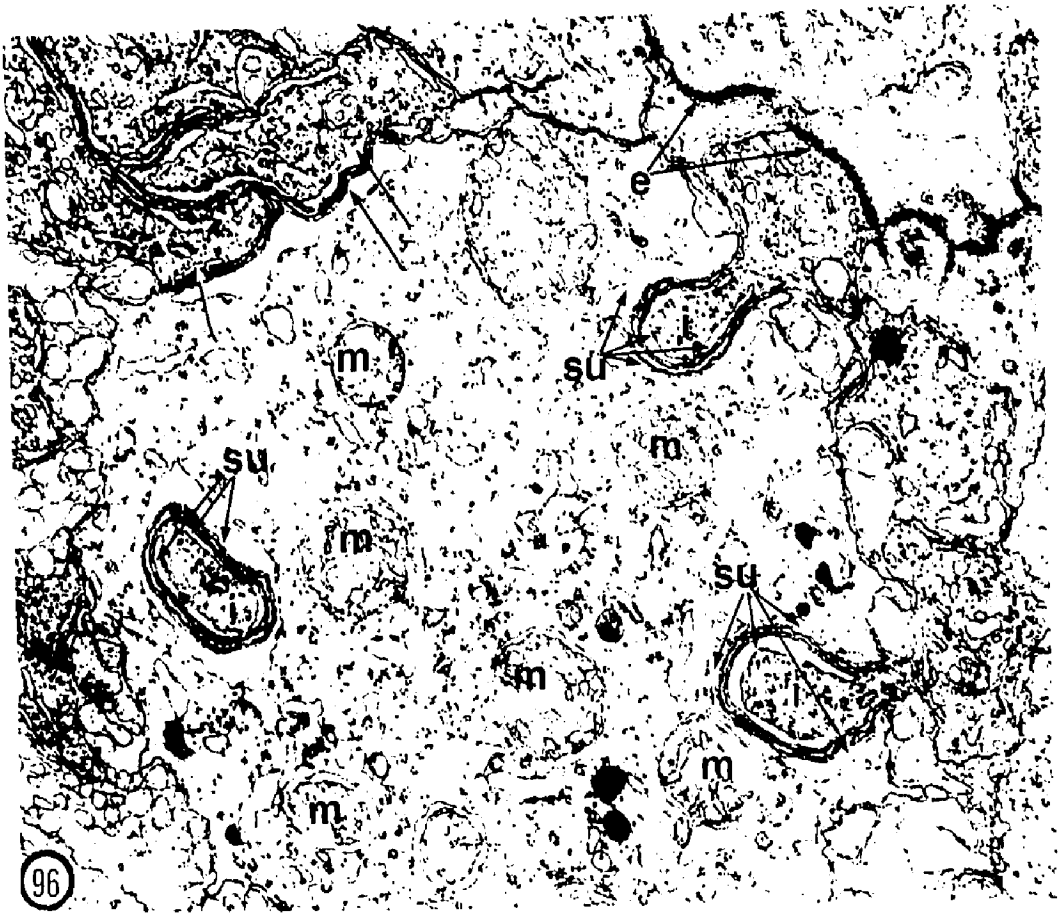
X 19,000


94
95


96


c
mc
94

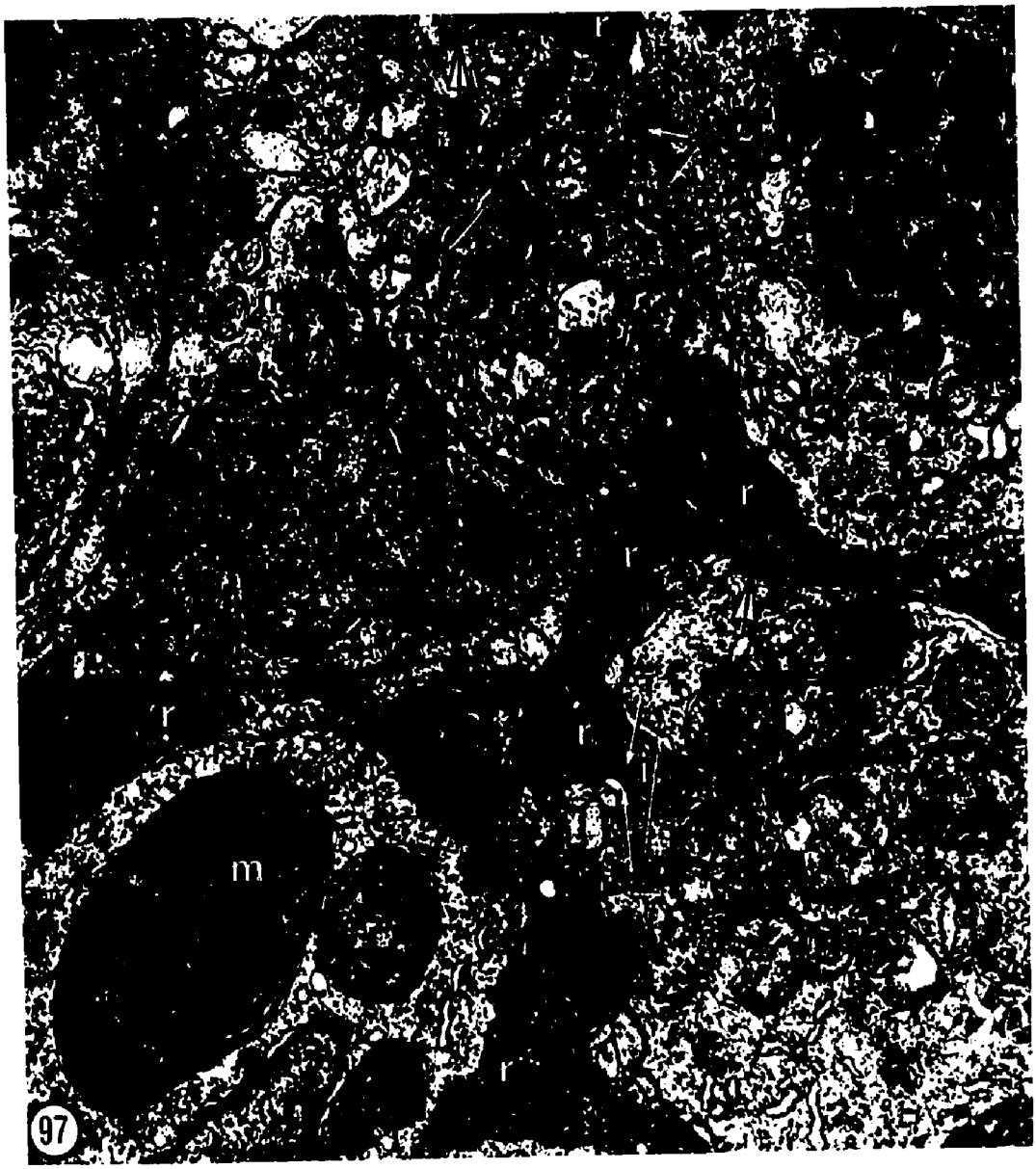

su
c
95

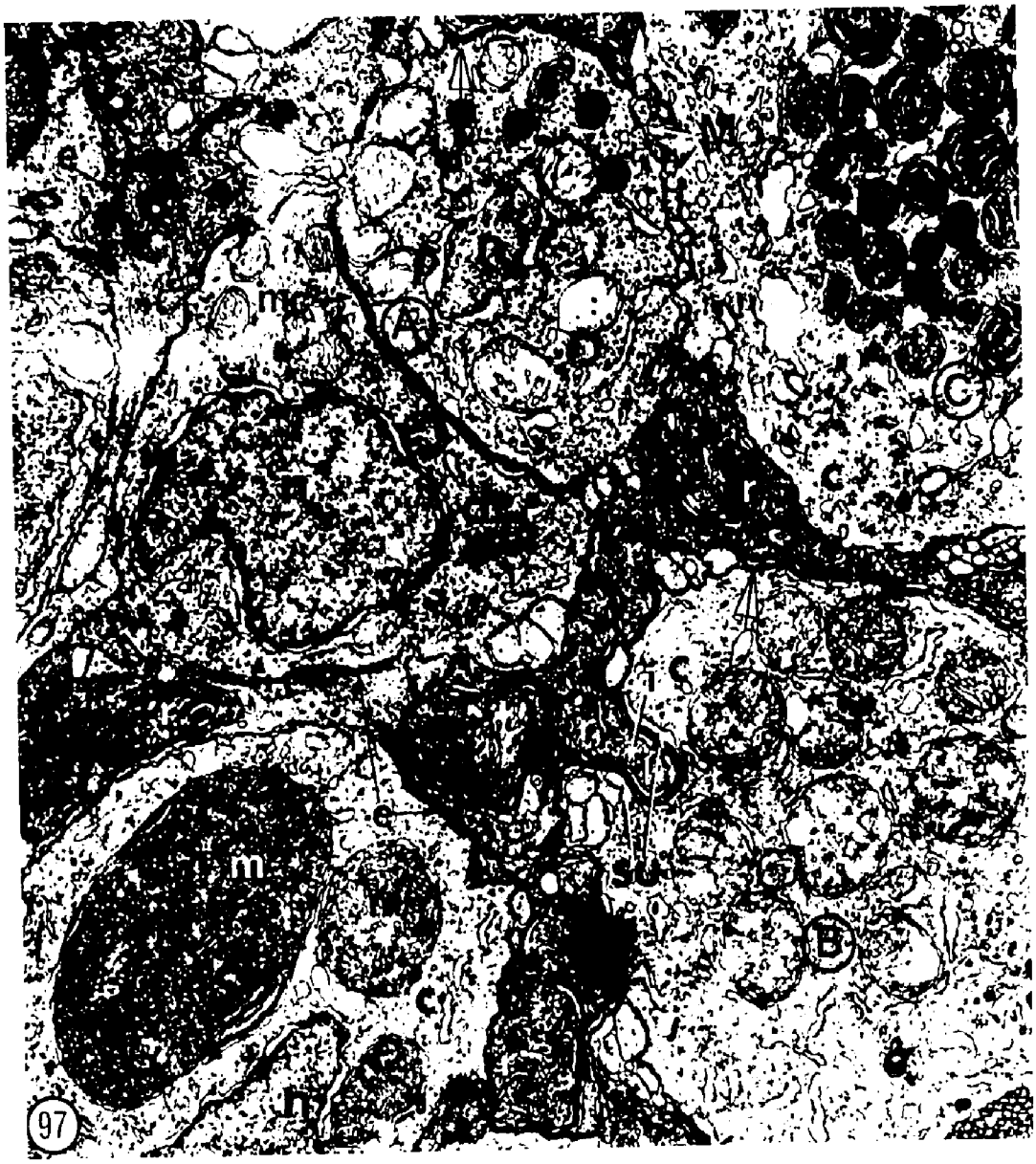

e
su
m
su
m
su
m
m
m
96

Transmission electron micrographs

Figure 97. Cross section of the dorsal retina approximately at the level of the external limiting membrane (e). The rod (r) cytoplasm is darker than that of the cones (c). There is a rod invagination (i) with its membrane-complex and subsurface cisterns (su). The double cone membrane-complex (mc) shows as a thick dark line, as also does the membrane-complex between the rods and cones (* and arrowheads). Müller cell (M) microvillous processes are present. In one cone, near the nucleus, there is a very enlarged and transformed mitochondrion (m). It is very unusual to find transformed mitochondria so vitreal in position in the ellipsoid. In the other cones, mitochondria (m) can be seen in varying stages: A-small, pale with few cristae; B-enlarged, with matrix that is somewhat granular; C-with very dark matrix. The cytoplasm is rich in ribosomes and cisternae of endoplasmic reticulum.

X 13,000


r
r
r
r
r
m
r
97


97

Transmission electron micrographs

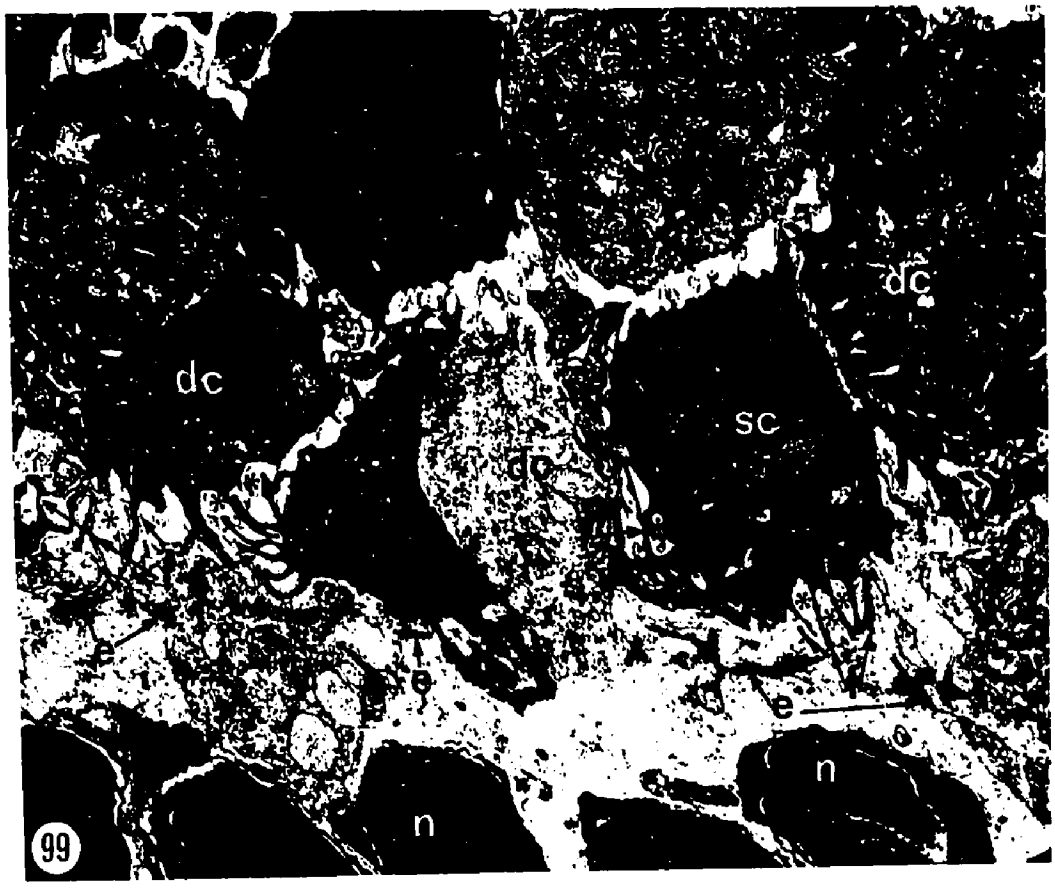
Figure 98. Cross section of the dorsal retina at the level of the external limiting membrane (e). The cones have fine cytoplasmic protrusions or 'fins' (f) and between these interdigitating fins are pale Müller cell processes (*).

X 12,900

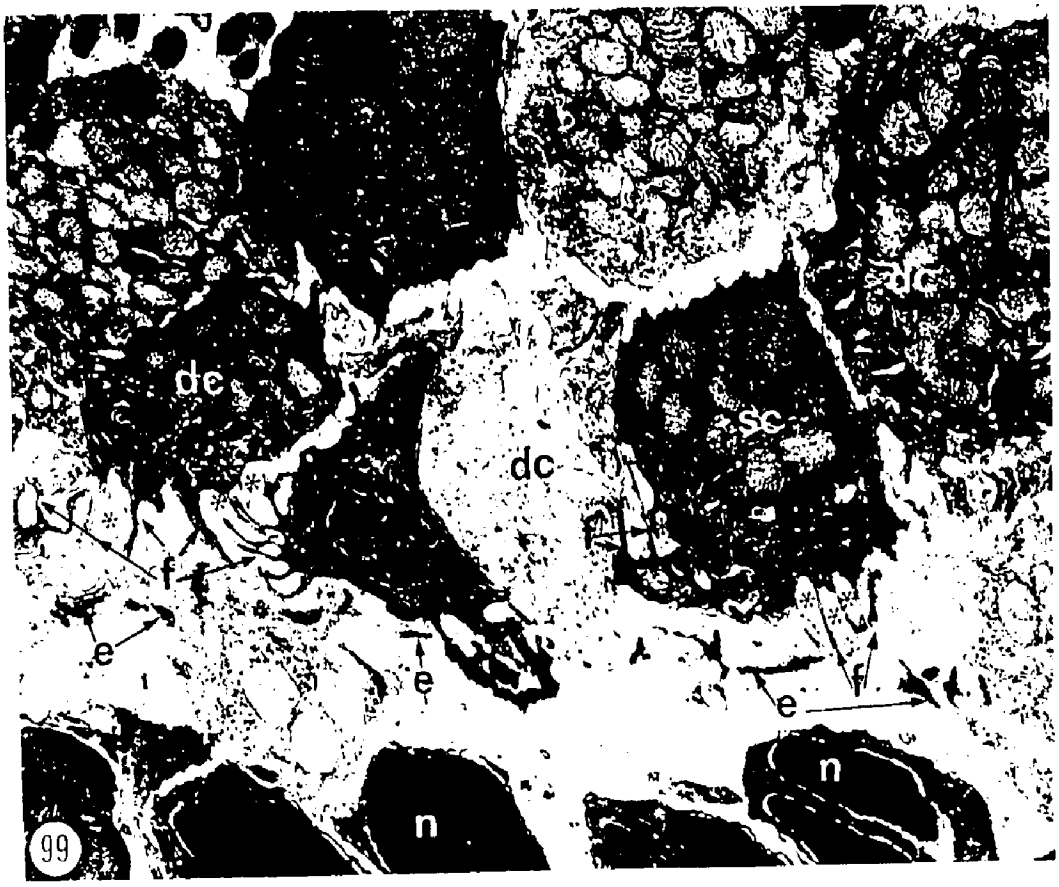
Figure 99. Cross section of the dorsal retina at the level of the external limiting membrane (e), showing the cytoplasmic fins (f) of the single (sc) and double cones (dc). Note the nuclei (n). The Müller cell cytoplasm, very pale indeed, can be seen between the fins (*).

X 8,300


f
f
f
f
98


dc
dc
sc
e
e
e
e
n
n
99


f
f
f
e
98


dc
dc
sc
dc
f
e
e
e
f
n
n
99

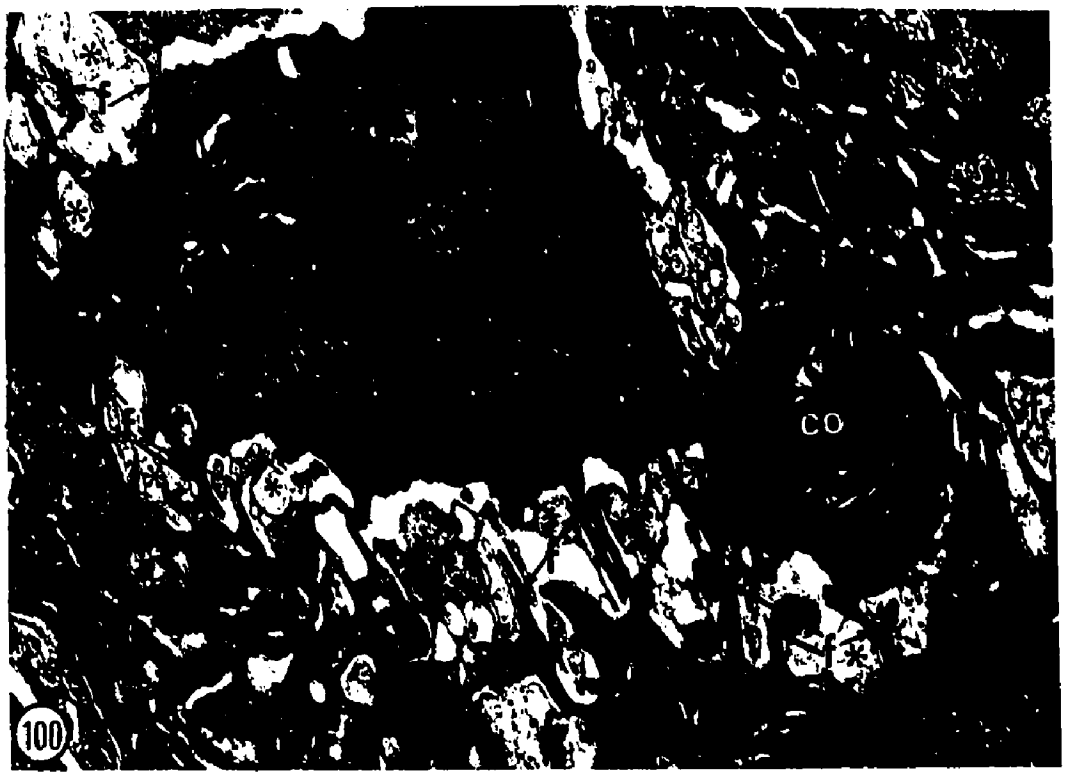
Transmission electron micrographs

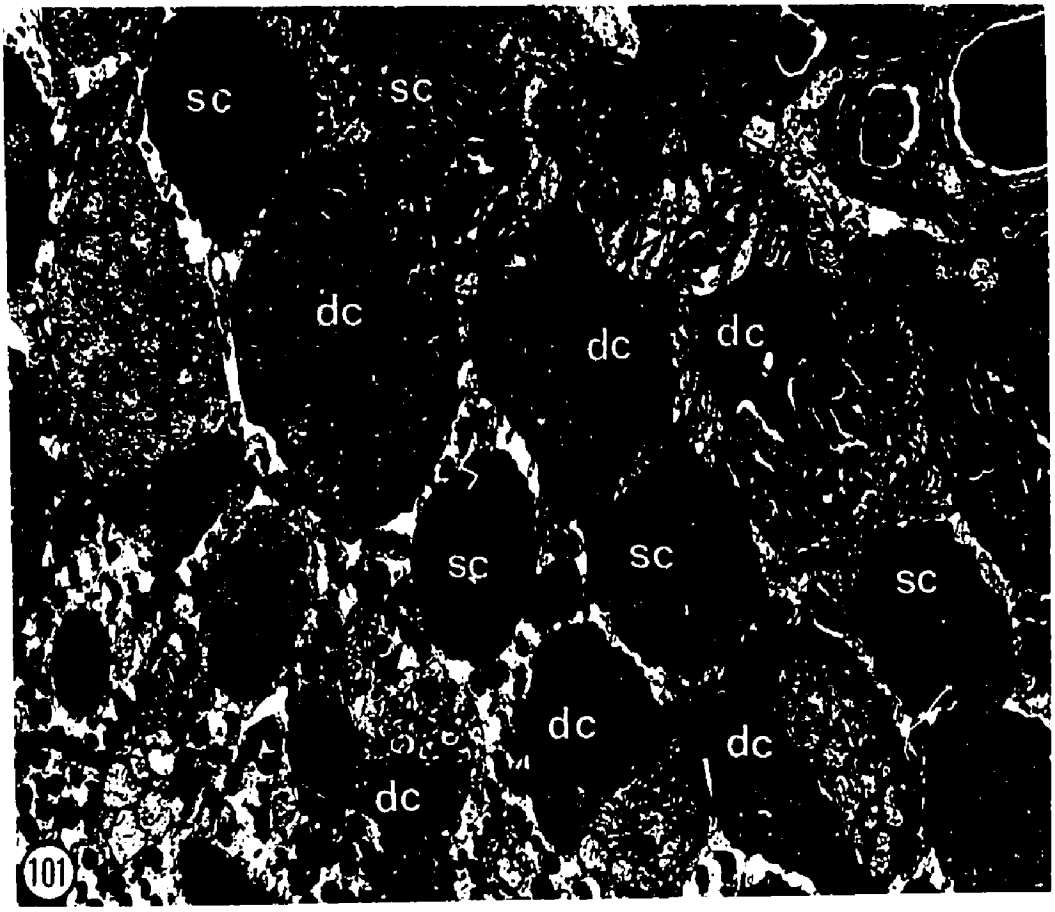
Figure 100. Cross section through the dorsal peripheral part of the retina showing the fins (f) of the cones, with very pale Müller cell processes (*) between the fins. This section is immediately scleral to the external limiting membrane. A cone outer segment (co) of a very short cone is present among the myoids and the vitreal parts of the ellipsoids of the other cones.

X 19,000

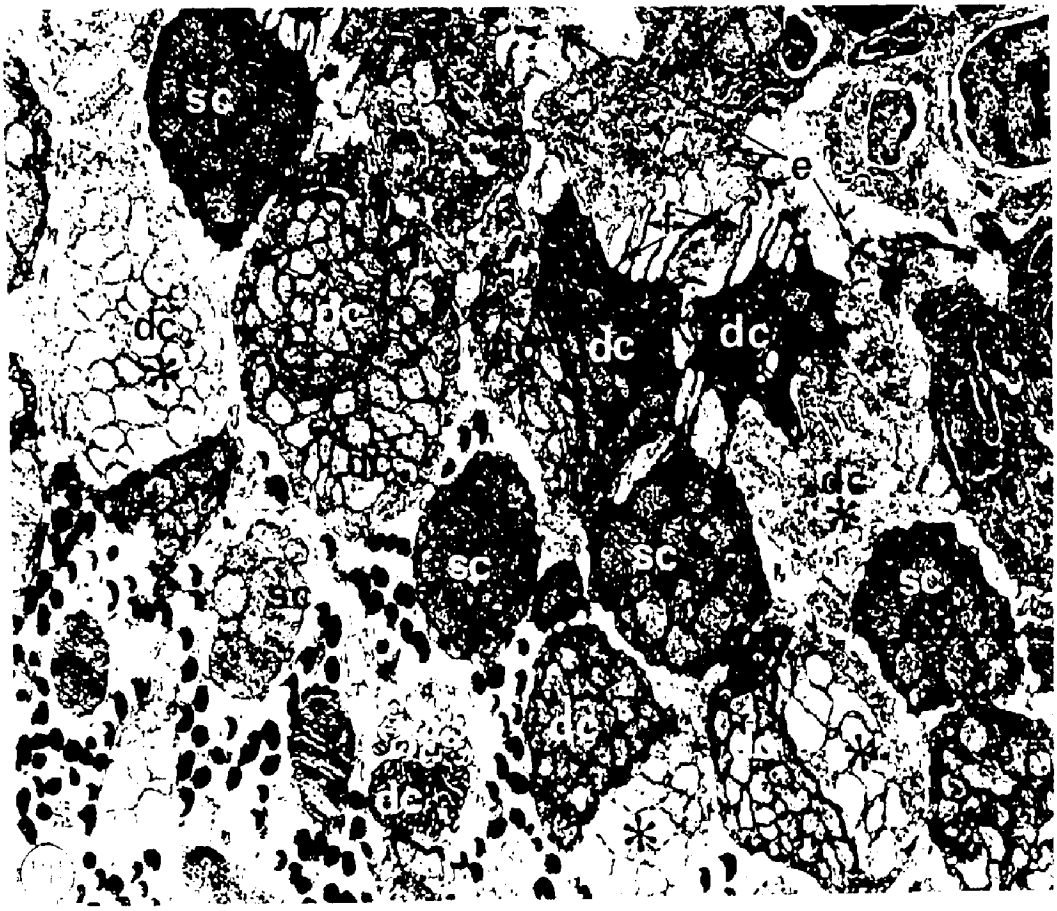
Figure 101. Cross section through the dorsal peripheral part of the retina showing the linear pattern of the mosaic arrangement of alternating rows of single cones (sc) and double cones (dc). Fins (f) are present on the cones near the external limiting membrane (e). Many of the double cones (dc) show differences in staining between the two members of the pair (*).

X 5,700


co
100


sc
sc
dc
dc
dc
sc
sc
sc
dc
dc
dc
101

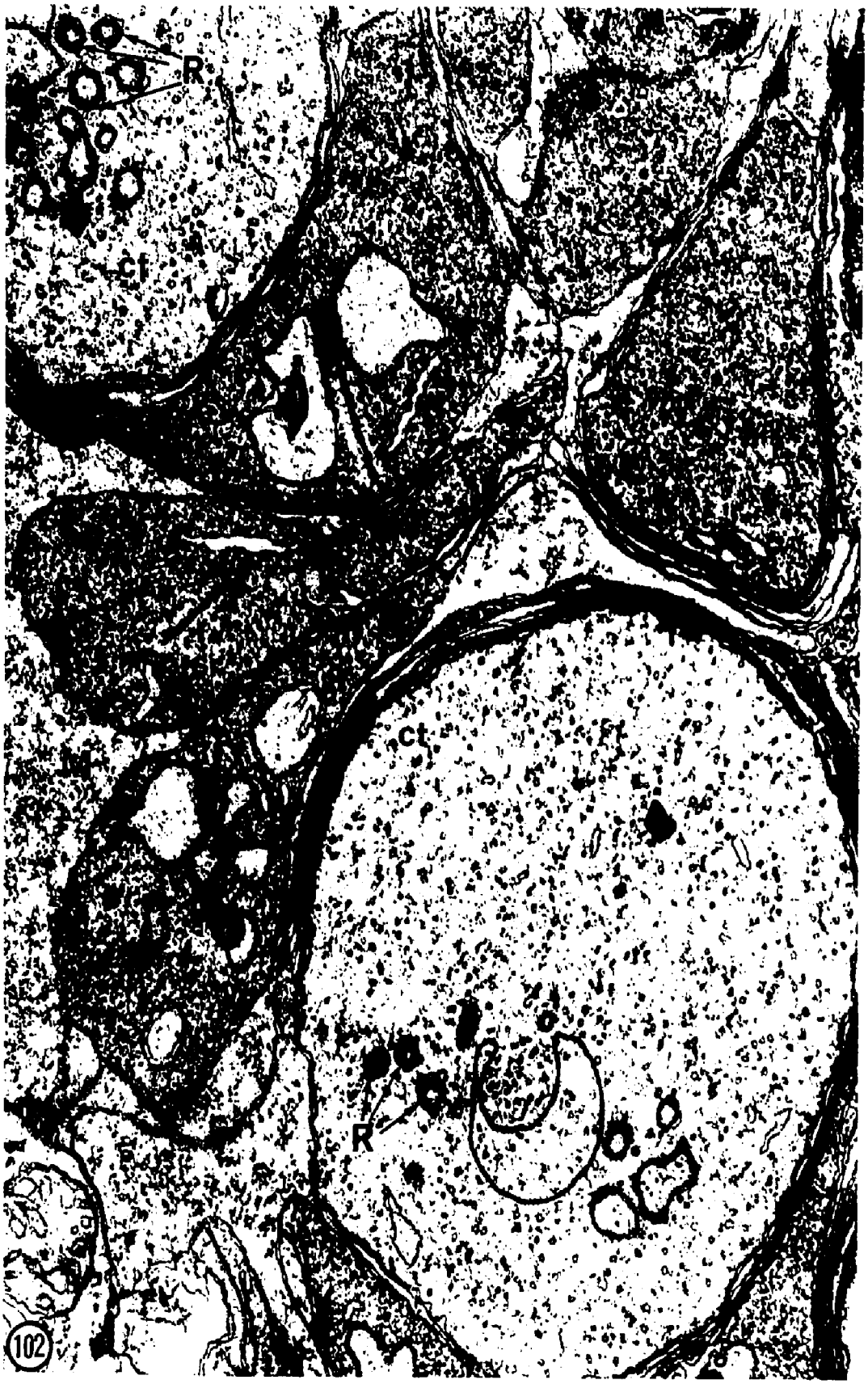

f
f
f
f
f
CD
100


sc
dc
dc
dc
dc
e
sc
sc
sc
sc
dc
dc
dc

Transmission electron micrographs

Figure 102. Cross section of the outer plexiform layer showing the two types of receptor terminals; the dark rod spherule (rt) and the larger and lighter cone pedicle (ct). Note that the rod spherule has a far greater density of synaptic vesicles. In the rod spherule (rt) synaptic ribbons (sr) can be seen, associated with invaginations of horizontal cell and bipolar cell processes. In the cone pedicle (ct) invaginated processes (R) in cross sections, show thickenings on their postsynaptic membranes, and appear as rings with discontinuous thickenings. Müller cells (M), rich in glycogen, have fine processes which surround the cones in whorl-like arrangements.

X 29,400



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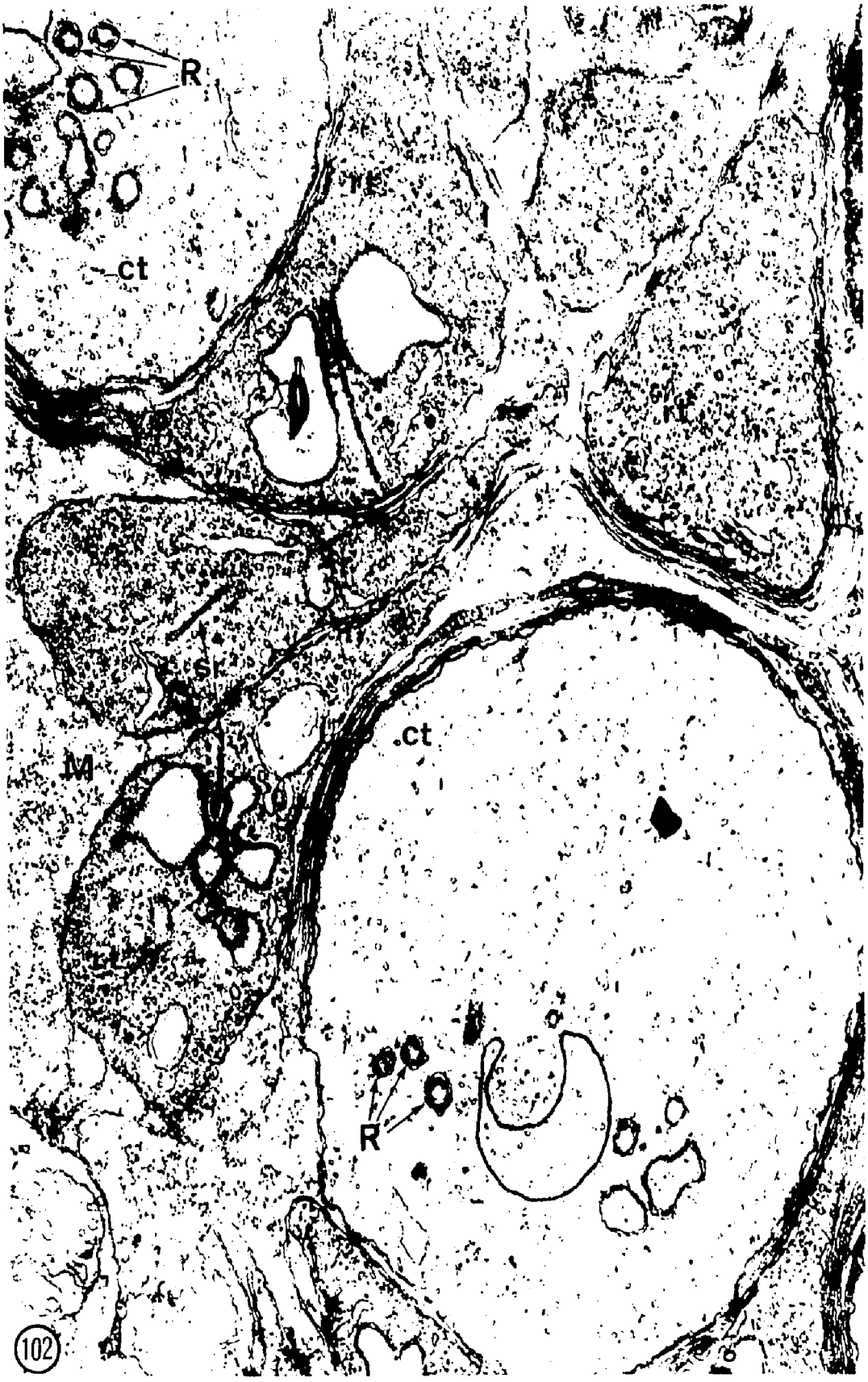


102

Transmission electron micrographs

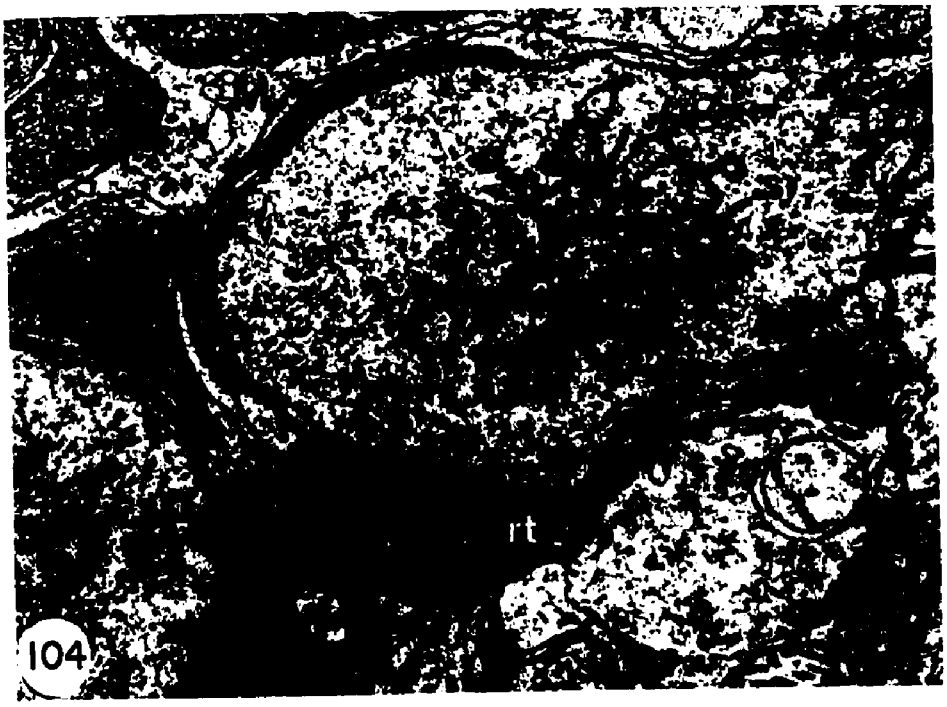
Figure 103. Longitudinal section of the outer plexiform layer of the ventral retina showing two types of receptor terminals. The rod spherule (rt) appears darker than the cone pedicle (ct) because it contains a greater concentration of synaptic vesicles. Note the synaptic ribbon (sr), lateral horizontal cell processes (h) and the central dendritic process of the bipolar cell (b). The synaptic vesicles of the rod spherule (rt) form orderly borders parallel to the ribbon leaving an intervening area clear of vesicles but containing filamentous material. There are marked postsynaptic membrane densities of the horizontal cell processes in the area around the arciform density. There is one pair of large synaptic vesicles (arrows), one on each side of the margin of the ribbon at its arciform density end. These vesicles appear to be in contact with a presynaptic membrane at the level of horizontal cell postsynaptic densities.

X 35,000

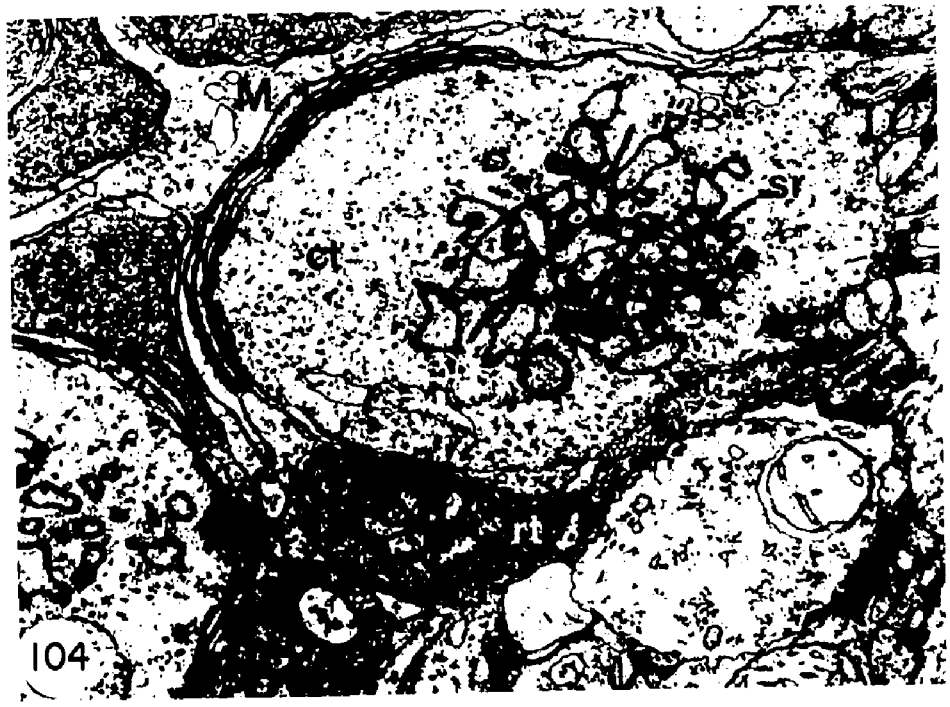
Figure 104. Cross section of the photoreceptor terminals from the dorsal retina showing the dark rod spherules (rt), and the lighter cone pedicle (ct) with its more complex neuronal invaginations and the multiple synaptic units around synaptic ribbons. Müller cells (M) are present containing glycogen particles and with many fine processes extending around the receptor terminals. Arrows indicate membrane densities.

X 17,600


rt
n
b
n
103


rt
104

103



104

Light micrographs

Figure 105. Thick (epon-embedded) section from the central area of the retina, showing the square mosaic pattern. The single and double cones are shown in outline on the accompanying transparency.

X 1,400

Figure 106. Thick section of epon-embedded material from the central area of the retina showing the square pattern of the mosaic. The single and double cones are shown in outline on the accompanying transparency.

X 5,000

Light micrographs

Figure 105. Thick (epon-embedded) section from the central area of the retina, showing the square mosaic pattern. The single and double cones are shown in outline on the accompanying transparency.

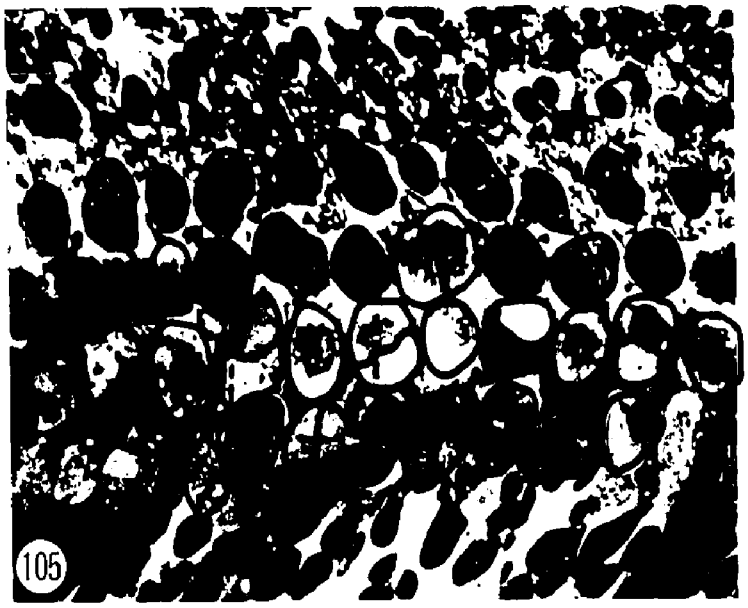
X 1,400

Figure 106. Thick section of epon-embedded material from the central area of the retina showing the square pattern of the mosaic. The single and double cones are shown in outline on the accompanying transparency.

X 5,000


105


106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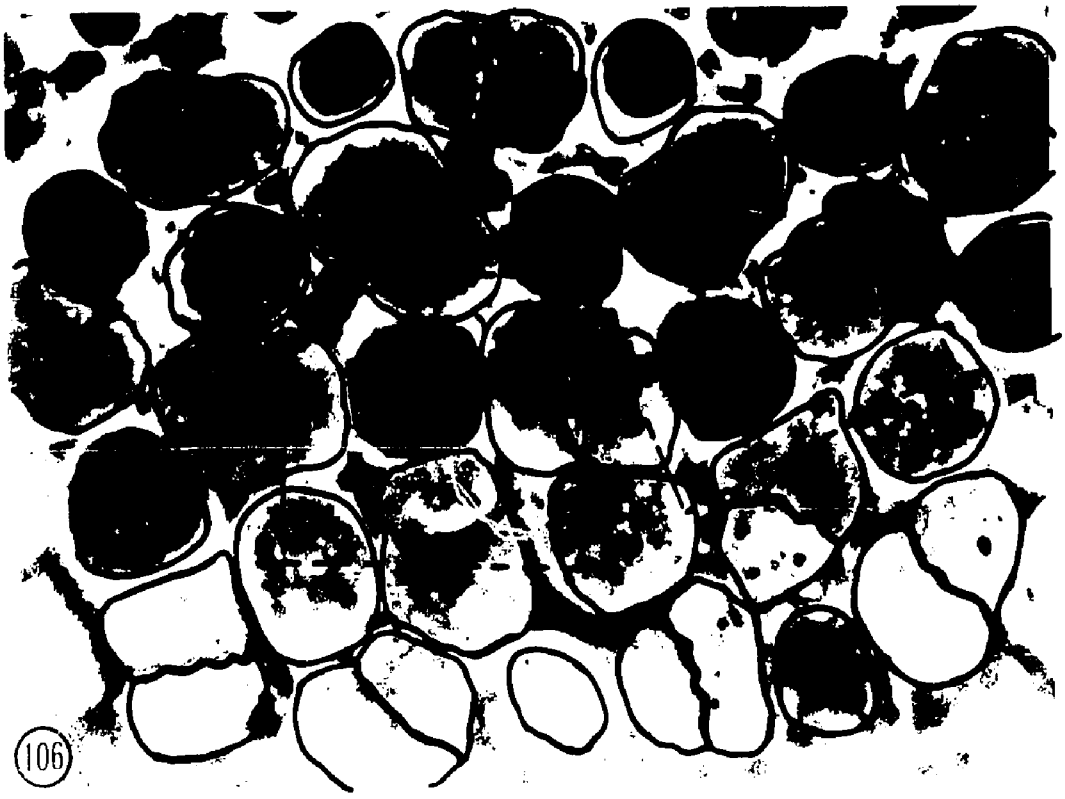

106

Transmission electron micrographs

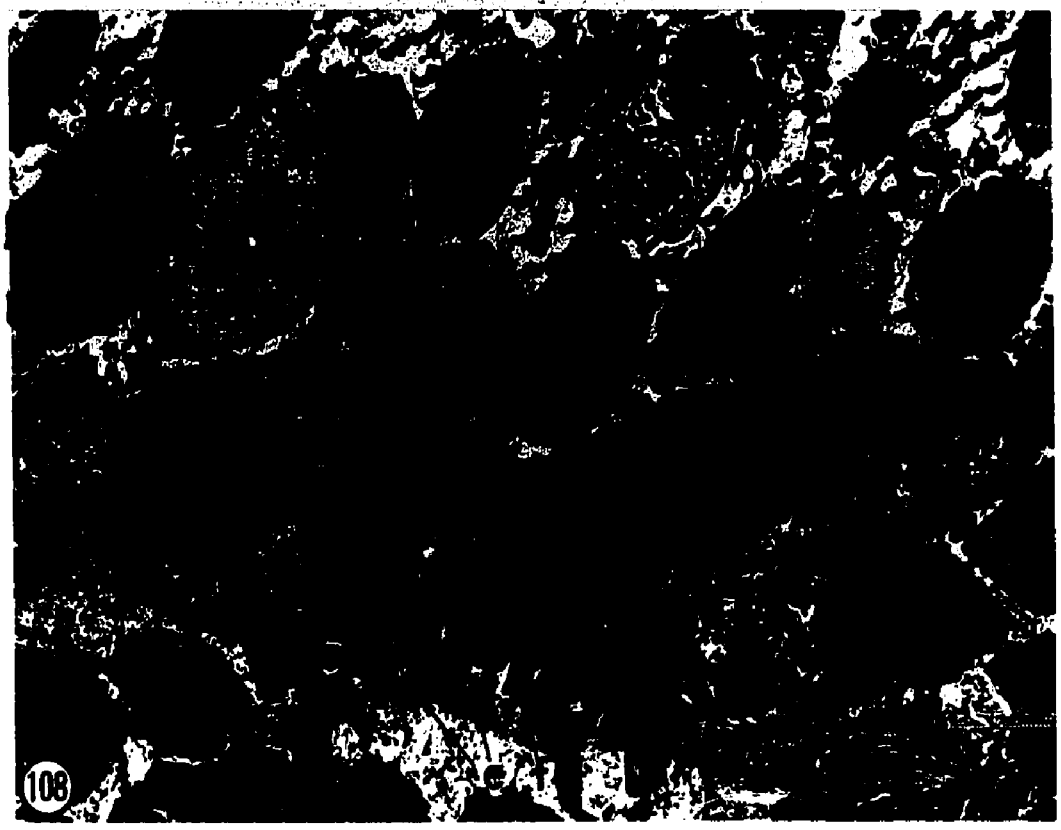
Figure 107. Cross section through the ventral peripheral part of the retina, showing the linear mosaic pattern. Note the fins (f) and the very short single cone outer segment (co). Staining differences between the two members are apparent in some of the double cones (*). The transparency delineates the single and double cones.

X 5,400

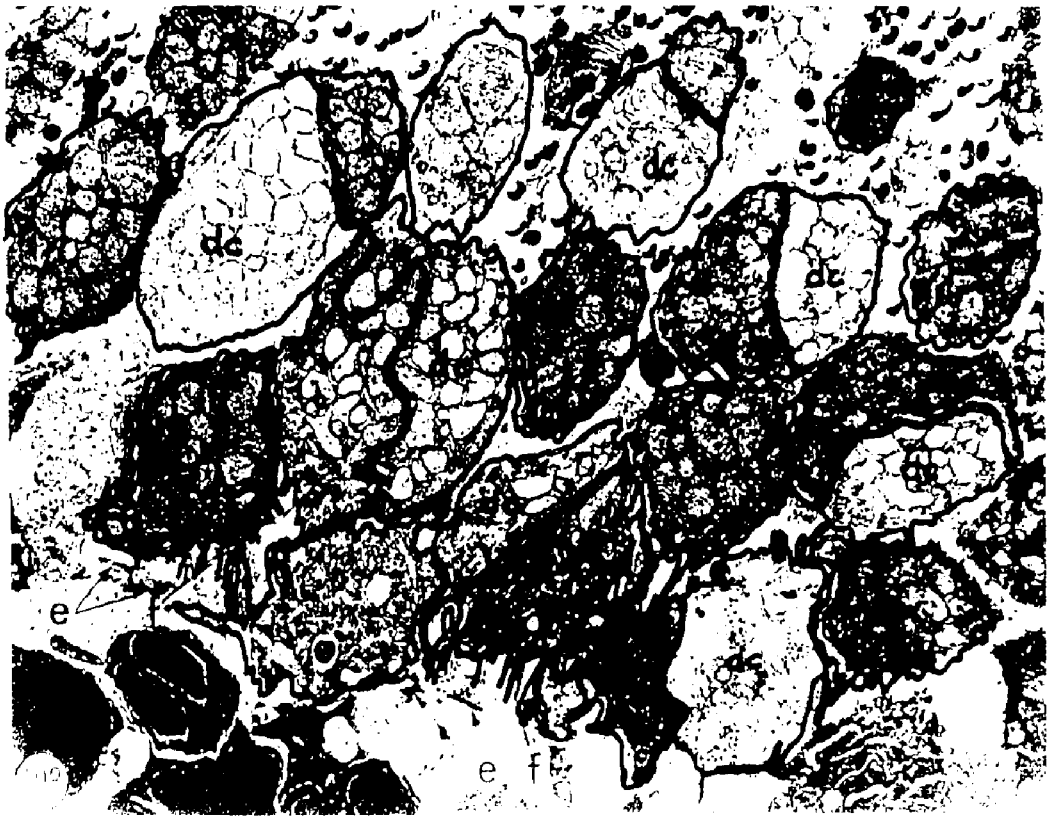
Figure 108. Cross section through the dorsal area of the retina, showing a linear pattern of the mosaic. There is a suggestion that the zig-zag change of position is occurring from linear to square pattern. Outlines of single and double cones are shown on the accompanying transparency. Fins (f) are present close to the tight junctions of the external limiting membrane (e).

X 5,400


107


108


dc
dc
dc
co
107


dc
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e f

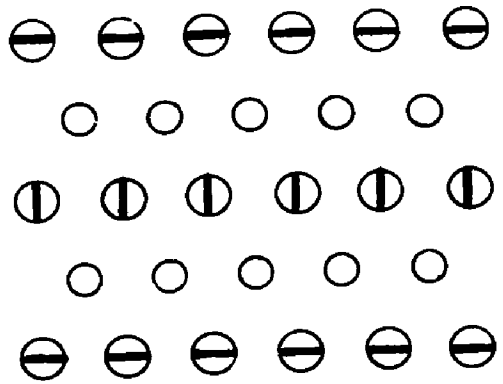
Line Drawings

Figure 109. The linear mosaic pattern is set out in its fully developed, or most perfected form. The small circle represents the single cone and the larger circle with the dark mid-line, represents the double cone.

Figure 110. The square mosaic pattern in its most perfected form.

Figure 111. A single cone and two double cones showing the attachment of the accessory outer segment (a) to the cone outer segment (co), and the relationship to the calycal processes (cp). Note that the accessory outer segment (a,1) may be larger than its associated outer segment. At the scleral end of the cone outer segment, the accessory outer segment (a,2) tapers and becomes very small.

Figure 112. Diagram of a double cone at the vitreal end of the ellipsoids showing the fins, the pale Müller cell processes, and the position of the subsurface cisterns.



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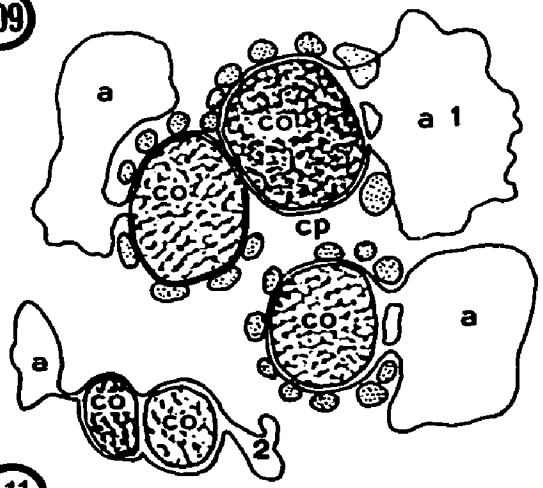

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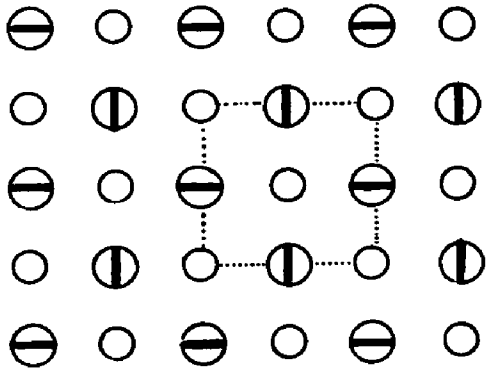

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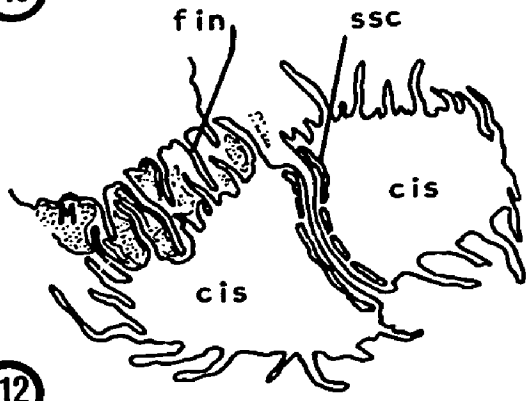
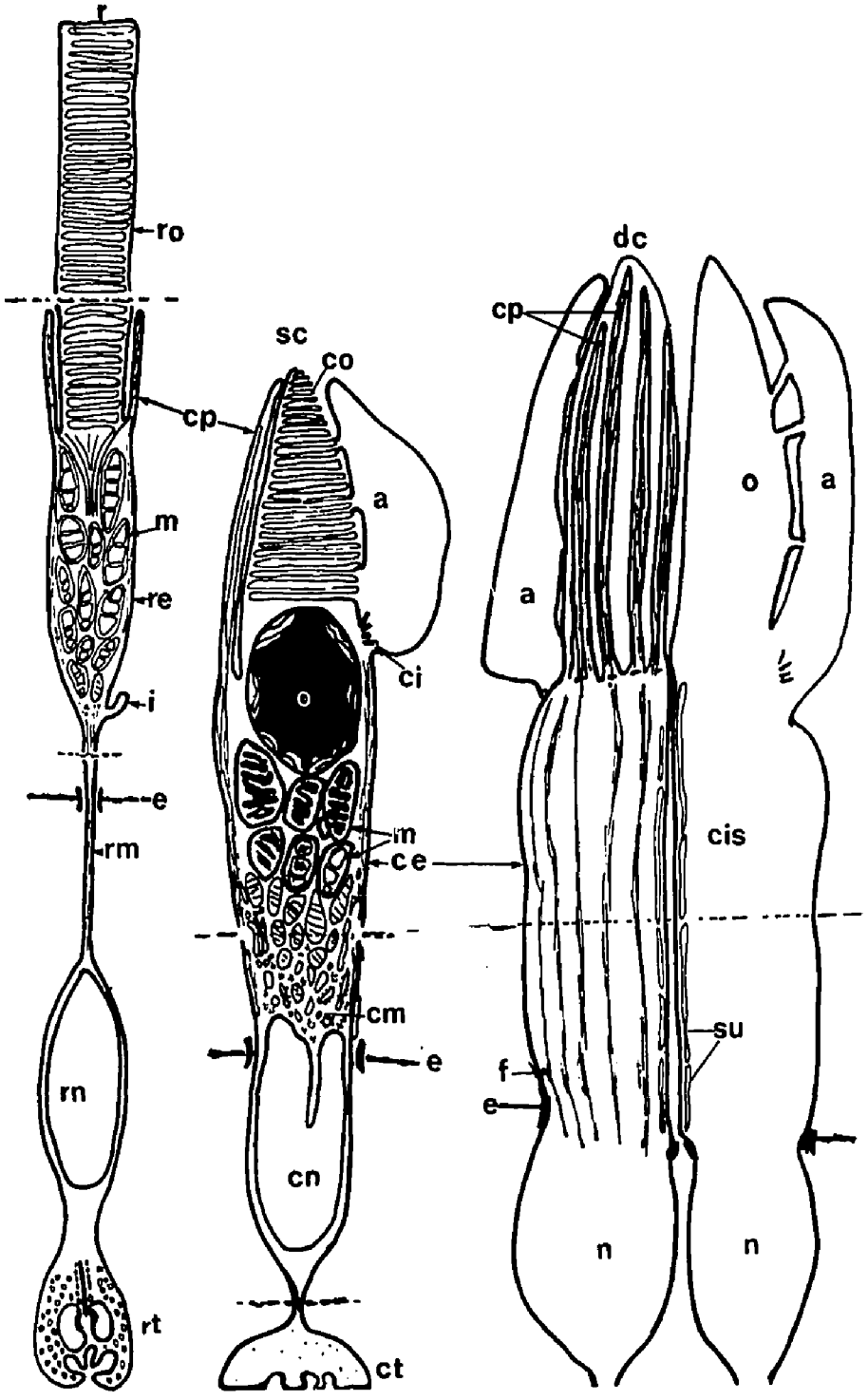


112

Figure 133. Line diagram to illustrate the salient features of the rod, single-cone and double-cone of Anableps anableps.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