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Fine structure of the retinal epithelium of the tiger salamander *(Ambystoma tigrinum)*

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Summary. The retinal pigment epithelium (RPE) of the tiger salamander *(Ambystoma tigrinum)* consists of a single layer of large squamous-shaped cells. These RPE cells are but minimally infolded basally (sclerally) but show numerous apical (vitreal) processes that interdigitate with the photoreceptor cells. A series of apically-located tight junctions joins the RPE cells to form part of the blood-occular barrier. Internally the RPE nucleus is large, vesicular and flattened. Smooth endoplasmic reticulum predominates in these cells with only occasional small profiles of rough endoplasmic reticulum present. Polysomes and basally-located mitochondria are plentiful. Golgi zones, lipid droplets, lysosome-like bodies and phagosomes of outer segment material are also present. Myeloid bodies which may show ribosomes on their surface are also numerous. The round to oval melanosomes undergo retinomotor movements and in the light-adapted state reach almost to the external limiting membrane. Bruch's membrane or complexus basalis shows a pentalaminate structure with the central elastic lamina poorly represented. The choriocapillaris is a single layer of large anastomosing capillaries which are but minimally fenestrated.

Key words: Retinal epithelium (RPE), Electron microscopy, Amphibian, Tiger salamander, *Ambystoma tigrinum*

Introduction

The retinal epithelium (RPE) is a normally pigmented single layer of cells which forms the outermost (scleral) layer of the vertebrate retina. It is intimately involved in several processes vital to the proper functioning of the photoreceptors and hence to the visual process itself. Included in the roles of the RPE are: 1) the structural support and proper orientation of the elongate and light-sensitive photoreceptor outer segments (Bernstein, 1961; Enoch, 1979), 2) the RPE apical processes are involved in intraretinal adhesion of the RPE and photoreceptor cells to prevent a retinal detachment (Zinn and Benjamin-Henkind, 1979), 3) the transport of materials between the choriocapillaris and photoreceptors (Steinberg and Miller, 1973), 4) the storage and modification of vitamin A precursors of the visual pigments (Young and Bok, 1970), 5) the phagocytosis and subsequent degradation of shed outer segment disks (Bok and Young, 1979) and 6) the absoprtion of light that has passed through the photoreceptor layer to prevent backscatter and subsequent reduction of visual acuity (Moyer, 1969; Zinn and Benjamin-Henkind, 1979).

As a consequence of its involvement in a variety of functions, the RPE and closely associated Bruch's membrane (complexus basalis) and choriocapillaris have been investigated in a number of vertebrates with a variety of different techniques. Morphological studies have shown a remarkable interspecies similarity to this region of the eye with any structural modifications apparently dictated by visual requirements (Nguyen-Legros, 1978; Kuwabara, 1979; Braekevelt, 1986, 1988, 1990).

While numerous studies of the morphology of the retinal epithelial region are available, relatively few reports deal with amphibian species (Porter and Yamada, 1960; Steinberg, 1973) and fewer still on the urodeles or tailed amphibians (Dickson and Hollenberg, 1971; Keefe, 1971). This report describes the fine structure of the RPE, Bruch's membrane (complexus basalis) and choriocapillaris of the tiger salamander *(Ambrystoma tigrinum)* and compares and contrasts these observations with other vertebrate species.

Materials and methods

For this study, the eyes from six adult tiger salamanders *(Ambystoma tigrinum)* were examined by light and electron microscopy. The specimens were maintained on a 12 hr light-dark cycle with the lights

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coming on at 6 a.m. and going off at 6.p.m. Three animals were sampled at noon (light-adapted) and midnight (dark-adapted) when the specimens were decapitated and the eyes quickly removed. The eyeballs were opened at the equator and immersion fixed for 5 h at 4 °C in 5% glutaraldehyde buffered to pH 7.3 with 0.1M Sorensen's phosphate buffer. The posterior halves of the eyeballs were then removed, washed in 5% sucrose in 0.1M Sorensen's buffer (pH 7.3) and cut into pieces less than 1 mm^2. This tissue was then postfixed for 2 h in 1% osmium tetroxide in the same phosphate buffer, dehydrated up through graded ethanols to methanol and then to propylene oxide and embedded in Araldite.

Pieces of plastic-embedded tissue were subsequently reoriented to desired angles by means of a wax mount and thick sections (0.5 μm) were cut, stained with toluidine blue and examined by light microscopy. Thin sections (60-70 nm) were then cut of selected areas and collected on copper grids. These sections were stained with aqueous urnayl acetate and lead citrate and examined and photographed in a Philips EM201 tranmission electron microscope.

Results

The RPE of the tiger salamander *(Ambystoma tigrinum)* consists of a single layer of large squamous-shaped cells which measure about 10 μm in height (Figs. 1, 2, 5) and up to 30 μm in width. Basally (sclerally) these cells are but moderately infolded (Figs. 2, 5, 7) while apically (vitreally) they display numerous microvillar processes which interdigitate with photoreceptor outer segments (Figs. 1, 3, 4). Wandering phagocytes were occasionally noted at this retinal epithelial-photoreceptor interface (Fig. 6). Laterally these epithelial cells are joined by a series of tight junctions located in the apical region of the cells (Figs. 1, 5).

Internally the RPE nucleus is large and vesicular and flattened with the long axis of the cell (Figs. 5-7). The mitochondria of these epithelial cells are small, round to oval in shape and in both light- and dark-adaptation are predominantly basally located (Figs. 2, 8, 9).

Smooth endoplasmic reticulum (SER) is the most obvious and abundant cell organelle and is located throughout the RPE cell body but not usually within the apical processes (Figs. 2, 4, 5). Rough endoplasmic reticulum (RER) is scarce while polysomes are scattered throughout the cell body (Figs. 2, 5, 9). Occasional Golgi zones (Figs. 2, 8), lysosome-like bodies (Figs. 5, 7, 8) and lipid droplets (Fig. 8) are also noted within these epithelial cells.

Myeloid bodies displayed much the same appearance in both light- and dark-adaptation in that they are small lentiform structures composed of smooth membranes and widely scattered throughout the cell body (compare Figs. 1 and 2, and 7 and 9). While myeloid bodies were noted to be in continuity with the SER membranes, they also displayed ribosomes on their outer surface (Figs. 2, 7-9). Phagosomes of outer segment material while not abundant within the RPE cells were noted in both light- and dark-adapted specimens (Fig. 2).

The melanosomes of the RPE cells are small and spherical to oblong in shape (Figs. 1-5). These melanosomes are extremely electron dense and are presumably quite brittle as they often shatter during sectioning (Figs. 2, 6). No premelanosomes were noted. In the light-adapted state the melanosomes are located within the apical region of the cell body and within the long apical processes when they reach to within 3-4 μm of the extermal limiting membrane and ensheath both outer and inner segments of the photoreceptors (Fig. 3). In dark-adaptation, the melanosomes within the apical processes retreat to be located in the apical region of the cell body and within the basal region of the apical processes (Fig. 4).

Bruch's membrane or complexus basalis in this urodele is a pentalaminate structure consisting of 1) the basal lamina of the retinal epithelium 2) the basal lamina of the choriocapillaris endothelium and 3) a central discontinuous elastic layer (lamina densa) separating an 4) inner and 5) and outer collagenous layer (Figs. 1, 5, 9). In the tiger salamander, Bruch's membrane measure about 0.7 μm in thickness and the central elastic layer is poorly represented as in the basal lamina of the choriocapillaris endothelium (Figs. 5, 7-9).

The choriocapillaris in this species form a single layer of large-caliber anastomosing capillaries immediately adjacent to the choroidal aspect of Bruch's membrane (Figs. 1, 2, 5). The endothelium facing Bruch's membrane while usually very thin is but minimally fenestrated (Figs. 1, 5, 9).

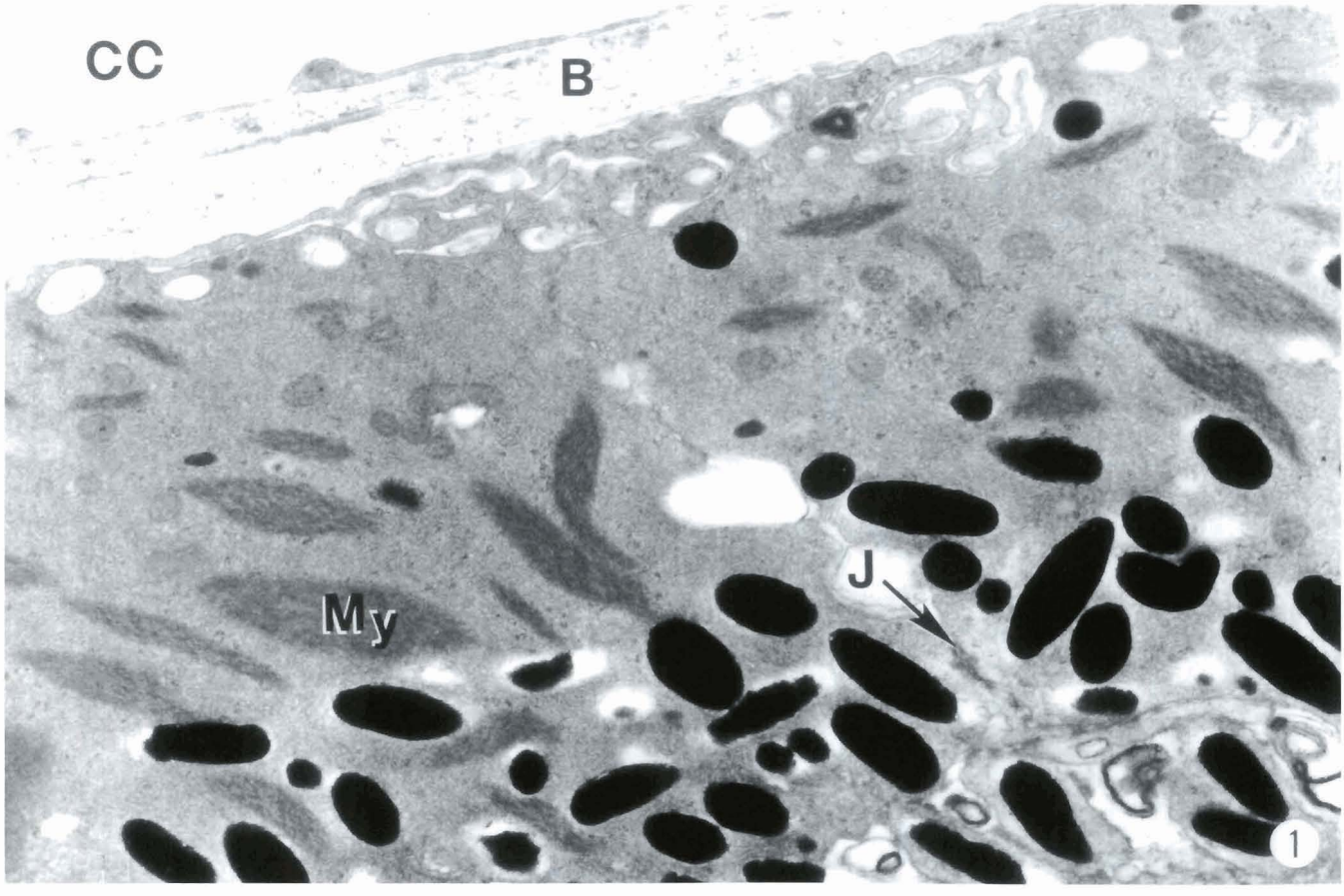
Discussion

The morphology of the RPE of the tiger salamander *(Ambystoma tigrinum)* is comparable to that described in other vertebrate species with characteristics that appear to be representative of amphibians (Nguyen-Legros, 1978; Kuwabara, 1979; Braekevelt, 1980, 1982, 1984, 1986, 1988, 1990).

As in all species investigated to date the RPE of the tiger salamander consists of a single layer of cells. In teleosts the RPE cells tend to be columnar in shape while in mammals, reptiles and birds they are mostly cuboidal

Fig. 1. Electron micrograph of the retinal pigment epithelium of a light-adapted tiger salamander. The choriocapillaris (CC), Bruch's membrane (B), a cell junction (J) and myeloid bodies (My) are all indicated. x 12,800

Fig. 2. Electron micrograph of the retinal pigment epithelium of a dark-adapted tiger salamander. Bruch's membrane (B), a Golgi zone (G), a phagosome (Ph) and myeloid bodies (My) are indicated. x 12,800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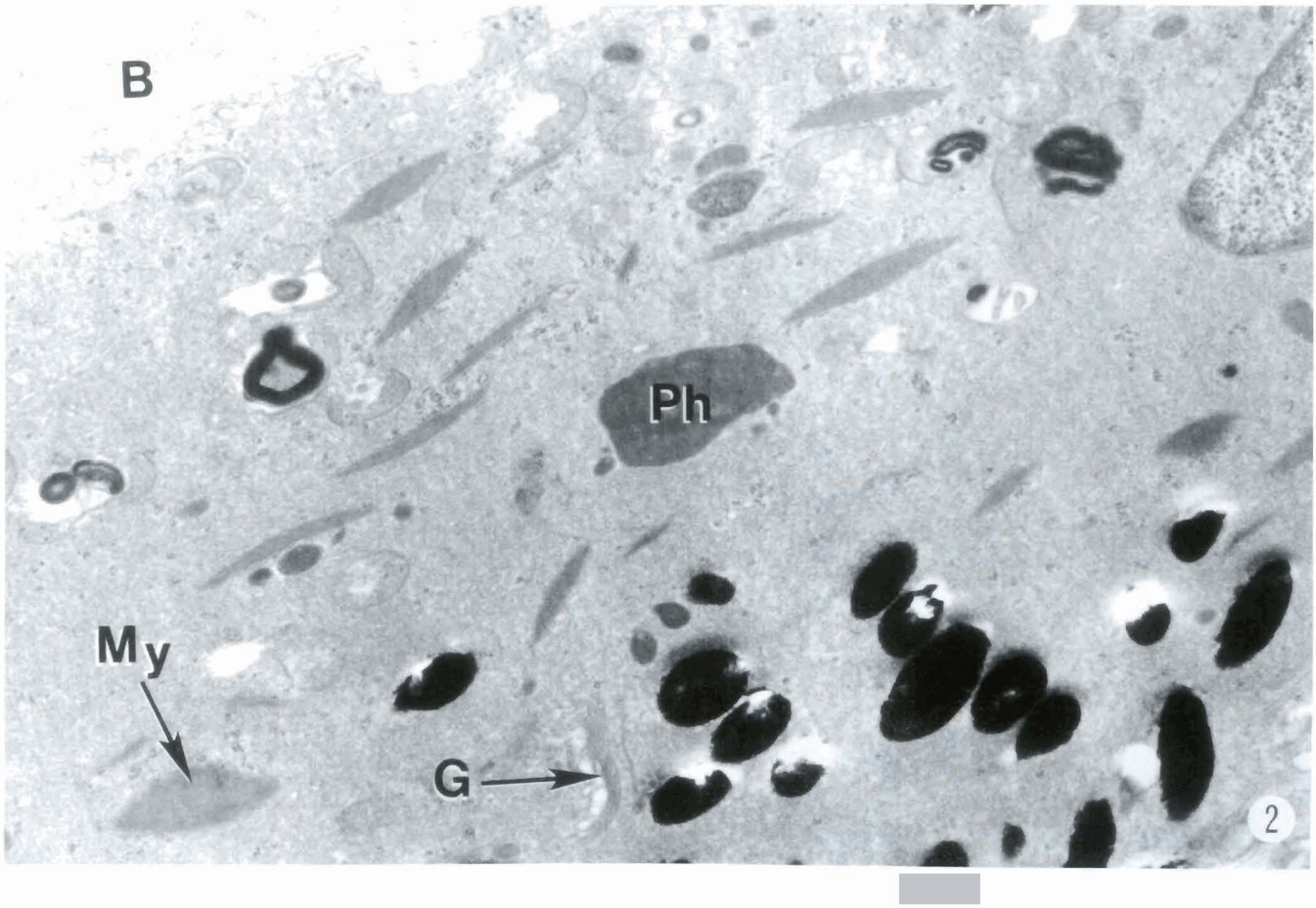
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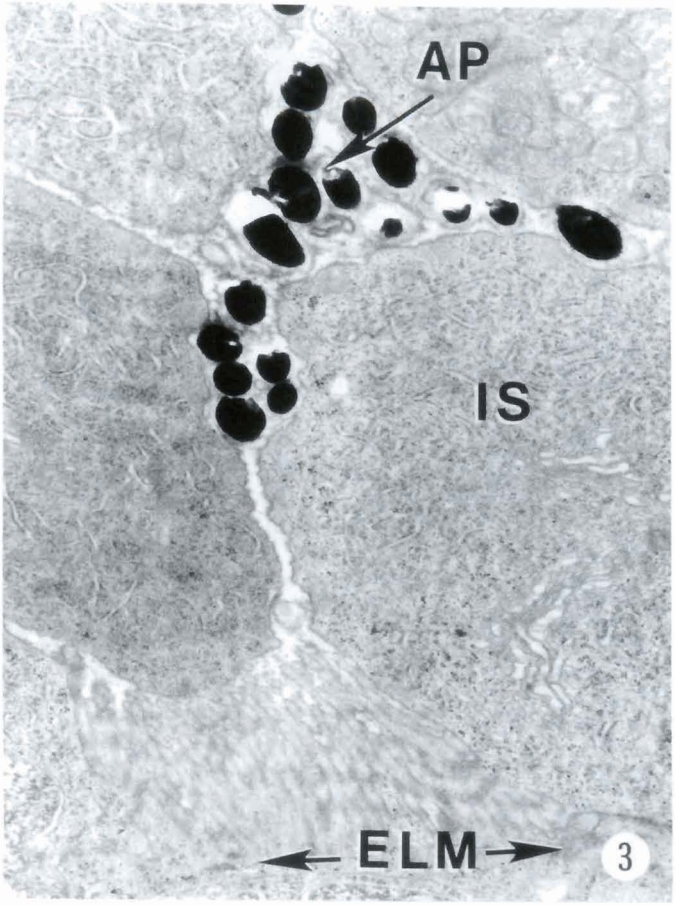
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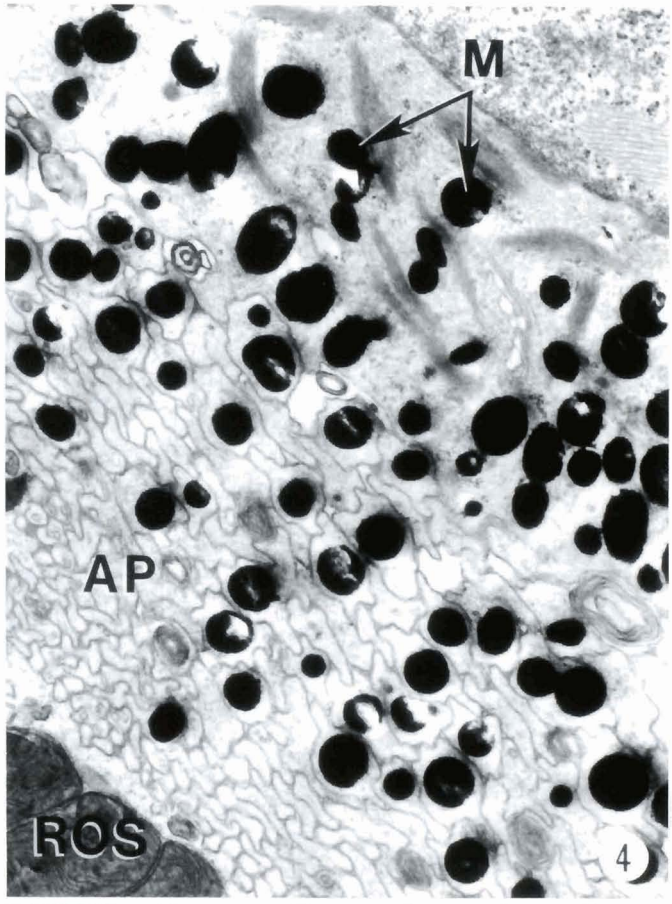
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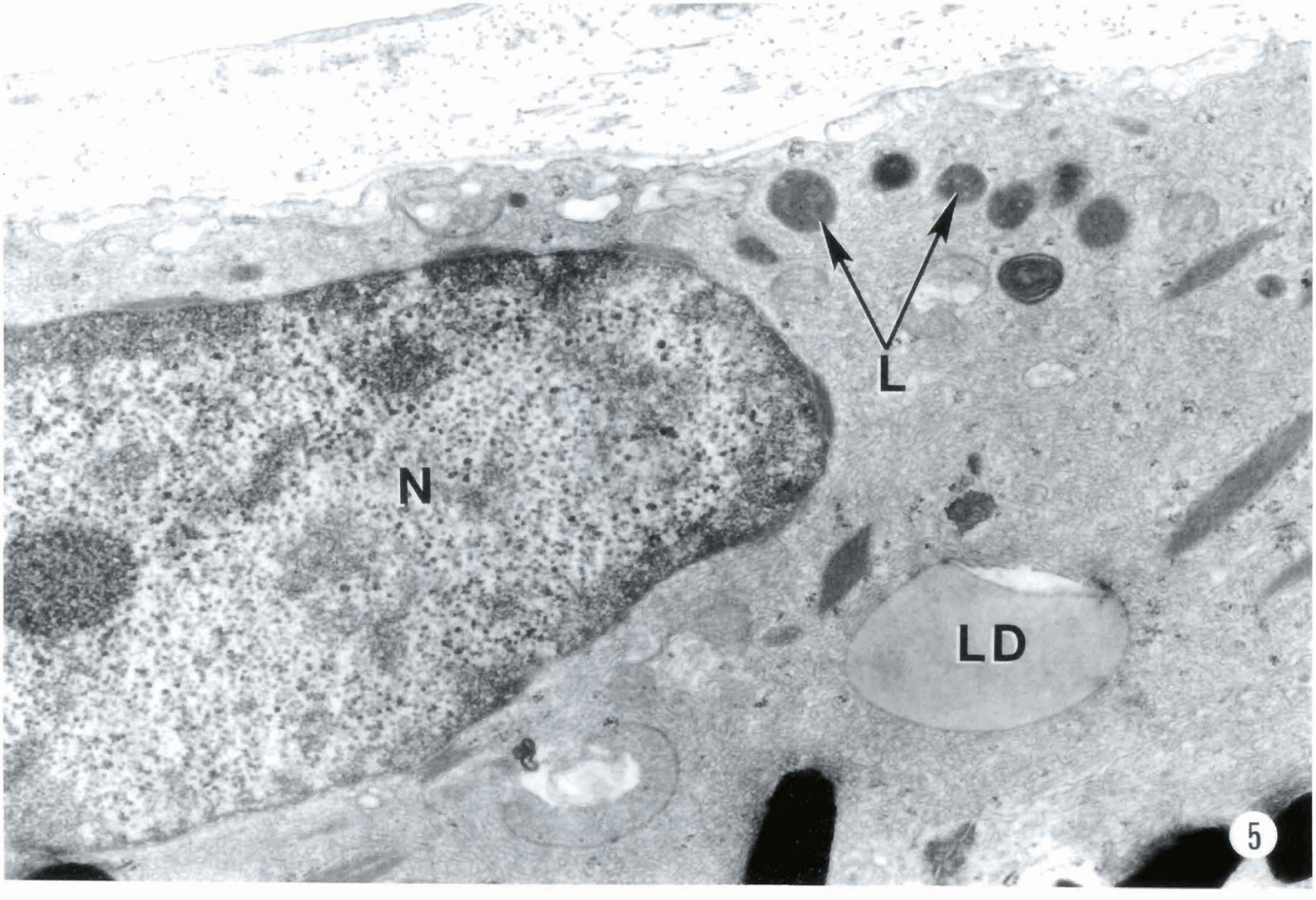
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5

Fig. 3. Electron micrograph taken from a light-adapted specimen. Pigment laden apical processes (AP) of the RPE cells reach almost to the external limiting membrane (ELM). Photoreceptor inner segments (IS) are also indicated. x 13,000

Fig. 4. Electron micrograph of the retinal epithelium in a dark-adapted specimen. The melanosomes (M) are clustered in the apex of the cell body and in the bases of the apical processes (AP). A rod outer segment (ROS) is also indicated. x 13,000

Fig. 5. Electron micrograph of the RPE (dark-adapted) to illustrate an epithelial nucleus (N), a lipid droplet (LD) and several lysosome-like bodies (L). x 18,700

(Kuwabara, 1979; Braekevelt, 1984, 1990). In marsupials and amphibians the cells are much lower and tend to be squamous in shape (Braekevelt, 1973, 1991; Young and Braekevelt, 1992).

The basal (scleral) region of the RPE cells in most species is extensively infolded which is felt to be indicative of an increased rate of transport by these cells (Dowling and Gibbons, 1962; Bernstein and Hollenberg, 1965). Teleosts are an exception to this observation and show but minimal infolding of the basal membrane of the RPE cells (Okuda, 1962; Braekevelt, 1982, 1985). This reduction of basal infoldings is felt to be due to the presence of a choroid gland which maintains a high oxygen pressure and presumably lowers transport requirements by the RPE in teleost species (Wittenberg and Wittenberg, 1974). In the tiger salamander there is also only minimal infolding of the basal region of the RPE cells but in this case may be due to the large size of both the RPE cells and the photoreceptors which is a characteristic of amphibian species (Walls, 1942).

The apical (vitreal) processes of the RPE cells are a constant finding in all vertebrate species and are an important feature in the structural support of the elongate photoreceptor outer segments (Bernstein, 1961) and in their proper orientation to incoming light (Enoch, 1979). In addition these processes are involved in the intraretinal adhesion required between the RPE and the photoreceptor cells (Zinn and Benjamin-Henkind, 1979) as well as in the phagocytosis of shed outer segment discs (Bok and Young, 1979). In this salamander, only one type of microvillar process is noted and it appears to contact both rods and cones.

The series of cell junctions at the lateral cell borders of the RPE cells constitute an effective barrier to the intercellular movement of materials and therefore form part of the blood-ocular barrier (Zinn and Benjamin-Henkind, 1979). These tight junctions are normally apically located in mammalian species and more mid to basally located in lower vertebrates (Kuwabara, 1979; Braekevelt, 1982, 1988, 1990). The tiger salamander is somewhat unusual in this respect in that the lateral cell junctions are predominantly located at the apical end of the RPE cell bodies.

Internally the RPE cells display a large, flattened and vesicular nucleus and a wealth of cell organelles indicative of highly active cells (Alberts et al., 1989). SER is the most abundant organelle within these cells. An abundance of SER is common to cells heavily involved in lipid biosynthesis (Enders, 1962) and it is well established that the RPE is crucial in the storage, transport and esterification of vitamin A compounds (Zinn and Benjamin-Henkind, 1979). RER is not abundant within these epithelial cells indicating that little protein is produced for export by these cells in the adult condition. The widespread appearance of polysomes on the outer hand would indicate that protein is being produced for internal use. The mitochondria in this species are small and less numerous than is normally observed for RPE cells and this may reflect the presumed lower amount of transport by the RPE in this species.

The occurrence of lysosome-like bodies within the RPE cells is to be expected as one of the important roles of the RPE is the lysosomal degradation of outer segment material (Young and Bok, 1969; Young, 1978; Bok and Young, 1979). The relative scarcity of phagosomes within the RPE cells of the tiger salamander is probably due to the sampling times employed. It is known that rods shed outer segment discs soon after the lights come on (6 a.m.) and cones shed soon after lights off (6 p.m.) (Young, 1978). By sampling at noon and midnight, approximately six hours have elapsed since shedding occurred and most phagosomes of outer segment material will have digested.

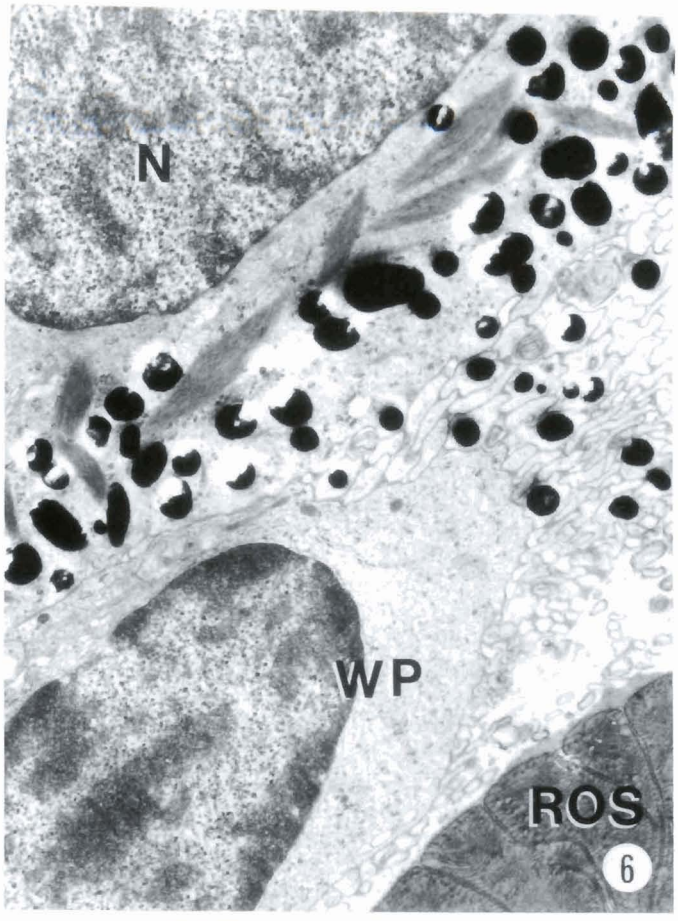
The melanosomes within the RPE cells of the tiger salamander undergo retinomotor or photomechanical movements in response to environmental lighting. In light-adaptation the melanosomes are located in the

Fig. 6. Electron micrograph of the RPE-photoreceptor interface. An epithelial nucleus (N), a rod outer segment (ROS) and a wandering phagocyte (WP) are all indicated. x 9.000

Fig. 7. Electron micrograph to illustrate the abundance of smooth endoplasmic reticulum (SER) within RPE cells. An RPE nucleus (N) and Bruch's membrane (B) are also labelled. x 13,000

Fig. 8. Electron micrograph to illustrate a few basal infoldings (BI), the minimally fenestrated choriocapillaris (CC) and numerous scattered polysomes. x 13,800

Fig. 9. Electron micrograph to indicate a phagosome (Ph) and myeloid bodies (My) some of which display ribosomes on their outer surface. Bruch's membrane (B) is also indicated. x 19,500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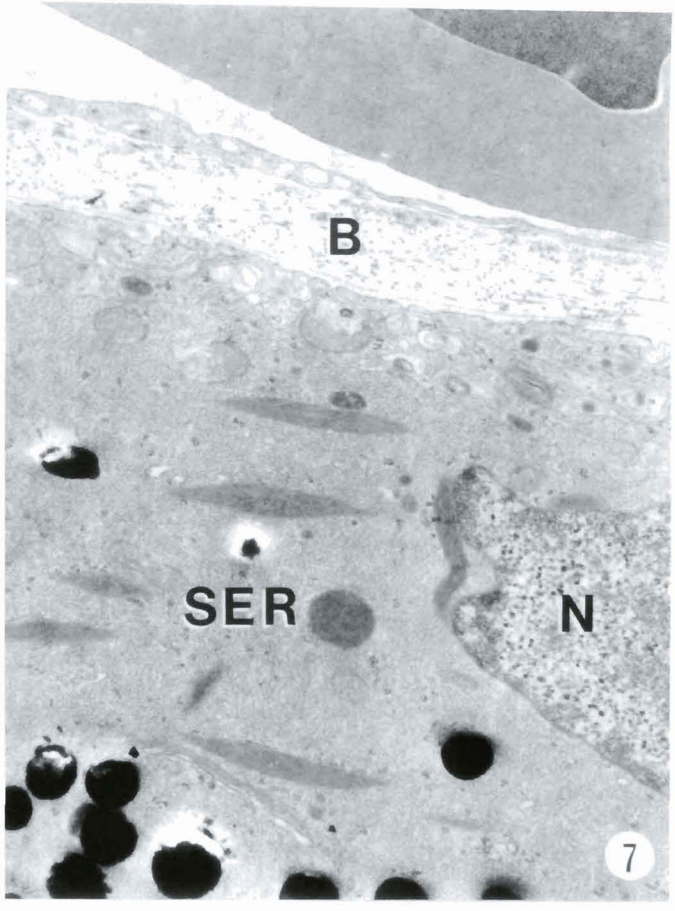
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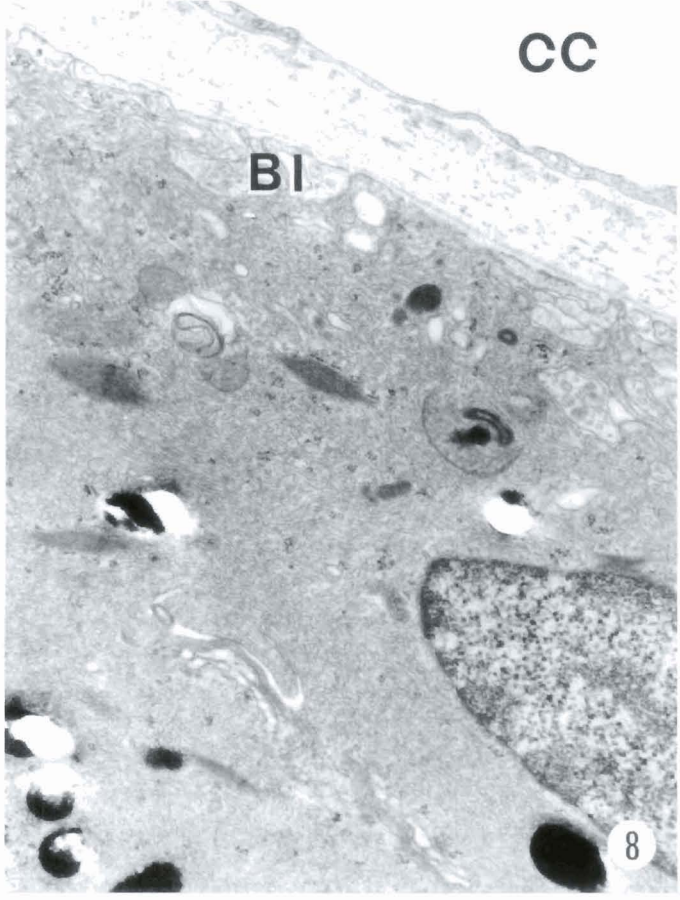
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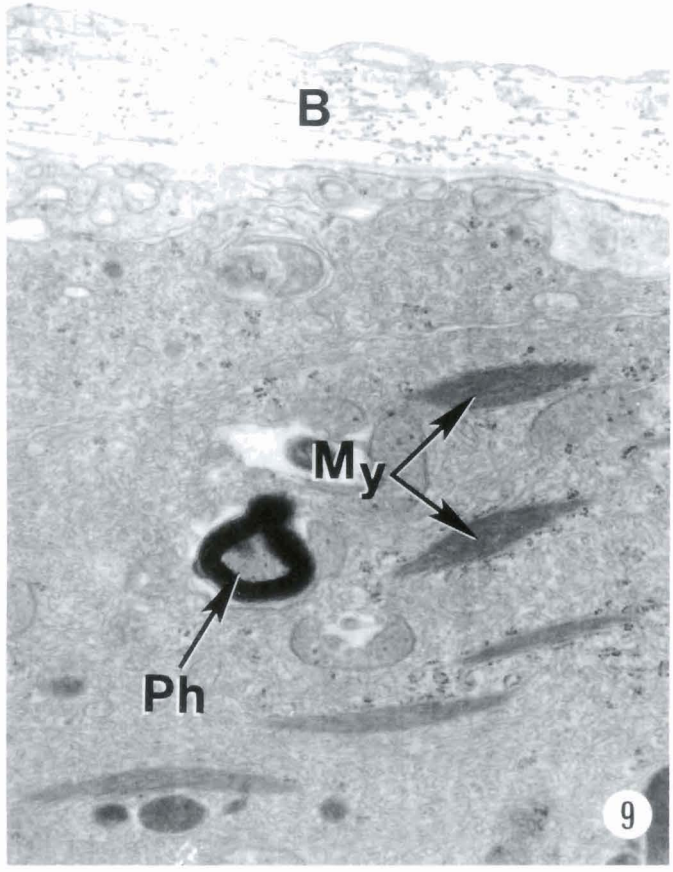
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apical region of the cell body and basal region of the apical processes. In this location the photoreceptor outer segments are not separated by melanosomes. In dark-adaptation while some melanosomes are located within the apical region of the cell body they are for the most part located within the apical processes and reach very close to the external limiting membrane. In this location they shield the outer segments of rod photoreceptors from overstimulation and by isolating individual photoreceptors probably also increase acuity.

Myeloid bodies which are organelles composed of a stacked array of membranes continuous with the endoplasmic reticulum are a common feature within the RPE cells of most non-mammalian species (Braekevelt, 1977, 1980, 1982; Kuwabara, 1979). They are most often noted in species which show photomechanical movements of the melanosomes (Porter and Yamada, 1960; Braekevelt, 1982, 1985) and while this may indicate a connection with retinomotor responses, they are also conjectured to be involved in the nutritional metabolism of the cell (Kuwabara, 1979) or to act as temporary storage sites for the large quantity of lipids entering the RPE during phagocytosis of outer segment discs (Yorke and Dickson, 1984, 1985). The myeloid bodies of the tiger salamander often display ribosomes on their outer surface, an observation which has only previously been noted in avian species (Braekevelt, 1988, 1990, 1991).

Bruch's membrane or complexus basalis in the tiger salamander shows the typical pentalaminate structure noted for all vertebrates except teleosts (Nakaizumi, 1964; Braekevelt, 1982, 1985). Teleosts characteristically only show a trilaminate complexus basalis with the central elastic layer (lamina densa) being absent (Braekevelt, 1982, 1985). In most avian species the central lamina densa while present is often very poorly represented (Braekevelt, 1984, 1989) and that is also the case in this species.

The choriocapillaris in all described species is composed of a single layer of large-caliber anastomosing capillaries (Rodieck, 1973; Kuwabara, 1979). Typically the endothelium facing Bruch's membrane is very thin and highly fenestrated indicative of the movement of large quantities of material from these capillaries (Bernstein and Hollenberg, 1965). Teleosts normally show very few choriocapillary fenestrations and this is felt to be due to the presence of the choroid gland (Braekevelt, 1985). In the tiger salamander the choriocapillaris endothelium facing Bruch's membrane shows very few fenestrations but is felt to be due tin this case to the reduced number of large photoreceptors which is characteristic of the amphibian retina (Walls, 1942; Dowling and Werblin, 1969).

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