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Retinal pigment epithelial fine structure in the red-backed salamander *(Plethodon cinereus)*

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Summary. The retinal pigment epithelium (RPE) of the red-backed salamander *(Plethodon cinerus)* consists of a single layer of large squamous shaped cells. The RPE cells are but minimally infolded basally (sclerally) but show many large apical (vitreal) processes interdigitating with the rod outer segments. These epithelial cells are joined laterally by prominent tight junctions located in the mid region of the cells. Internally smooth endoplasmic reticulum is very plentiful while rough endoplasmic reticulum is not. Polysomes, small dense mitochondria and small round to oval melanosomes are plentiful. Golgi zones and lysosome-like bodies are also present as are phagosomes of outer segment material and myeloid bodies. The RPE cell nucleus is large and vesicular. It is felt that the melanosomes undergo retinomotor movements but as only light-adapted specimens were examined it is not known how extensive are these movements. Bruch's membrane or complexus basalis shows the typical pentalaminate structure noted for most vertebrates. The choriocapillaris is a single layer of large anastomosing capillaries which are minimally fenestrated facing Bruch's membrane.

Key words: Retinal pigment epithelium (RPE), Electron microscopy, Amphibian, Red-backed salamander, *Plethodon cinereus*

Introduction

The retinal pigment epithelium forms the outermost (scleral) layer of the vertebrate retina and is intimately involved in several processes vital to the proper functioning of the visual system. Amongst the best understood roles of the RPE are: 1) the structural support and proper orientation of the photoreceptor outer segments (Bernstein, 1961; Enoch, 1979) 2) the storage and modification of vitamin A precursors of the visual pigments (Young and Bok, 1970) 3) the transport of materials to and from the photoreceptor cells (Steinberg and Miller, 1973) 4) the phagocytosis and subsequent degradation of shed outer segment discs (Bok and Young, 1979) and 5) internal adhesion of the neurosensory retina (Zinn and Benjamin-Henkind, 1979).

As the RPE is involved in a variety of functions, this area of the vertebrate retina has been investigated in a variety of vertebrates and with a number of different techniques. Morphological studies in particular have shown a remarkable interspecies similarity to this region with modifications apparently dictated by visual requirements (Nguyen-Legros, 1978; Kuwabara, 1979; Braekevelt, 1983, 1985, 1986, 1988, 1990a).

While numerous reports of the morphology of the RPE are available, relatively few reports deal with amphibian species (Porter and Yamada, 1960; Nilsson, 1964; Steinberg, 1973) and fewer still with the urodeles or tailed amphibians (Dickson and Hollenberg, 1971; Keefe, 1971). This report describes the fine structure of the retinal pigment epithelium (RPE), Bruch's membrane (complexus basalis) and choriocapillaris of the red-backed salamander *(Plethodon cinereus)*.

Materials and methods

For this study, the eyes from five adult, light-adapted red-backed salamanders *(Plethodon cinerus)* were examined by light and electron microscopy. The animals were decapitated and the eyes were 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 0.1 M Sorenson's phosphate buffer. The posterior half of the eyeball was then removed, washed in 5% sucrose in 0.1 M Sorenson's buffer (pH 7.3) and cut into pieces less than 1 mm^2. This tissue was then post-fixed for 2 h in 1% OsO4 in the same phosphate buffer (pH 7.3) dehydrated through graded ethanols to methanol and then to propylene oxide and embedded in Araldite.

Pieces of plastic-embedded tissue were subsequently

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reoriented to desired angles by means of a wax amount 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 uranyl acetate and lead citrate and examined and photographed in a Philips EM 201 transmission electron microscope.

Results

The retinal pigment epithelium (RPE) of the red-backed salamander *(Plethodon cinerus)* consists of a single layer of large squamous-shaped cells which measure about 10 µm in height and 20 - 30 µm in width (Figs. 1, 2, 4). Basally (sclerally) these cells are but minimally infolded (Figs. 2, 5) but apically (vitreally) they display large microvillus processes that interdigitate with the rod outer segments (Figs. 1, 8). Laterally the cell borders are relatively smooth and are joined by a series of tight junctions located at the midregion of these cells (Figs. 2, 4, 5).

Internally the RPE nucleus is large, vesicular, flattened with the long axis of the cell (Figs. 1, 3) and is located close to the basal membrane of the RPE cell (Figs. 1 - 3). The mitochondria in this species are small (0.5 µm) and spherical, display an electron dense matrix (Figs. 2, 4, 5) and are also predominantly basally located (Figs. 1 - 3).

Smooth endoplsmic reticulum (SER) is the most obvious and abundant cell organelle and is located throughout the RPE cells (Figs. 2, 4, 6) often almost to the exclusion of all other organelles (Fig. 7). Rough endoplasmic reticulum (RER) is extremely scarce while polysomes are scattered throught the RPE cells (Figs. 3, 4, 6, 7). Occasional Golgi zones (Fig. 6) and lysosome-like bodies (Figs. 2, 3, 6) are also noted within these epithelial cells.

Myeloid bodies in these light-adapted specimens were small and flattened and widely scattered (Figs. 2, 4, 5, 6). Phagosomes of outer segment material while not abundant were also present (Figs. 2, 4, 6). The melanosomes of the RPE cells are small and spherical to oblong in shape (Figs. 1, 3, 5). These melanosomes are extremely electron dense and no premelanosomes were noted. In the light-adapted state the melanosomes are predominantly located in the apical region of the RPE cells and within the numerous apical processes between the rod outer segments (Figs. 2, 5, 8).

Bruch's membrane or complexus basalis in this urodelean species 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, a 4) inner, and 5) an outer collagenous layer (Figs. 2, 3, 7). In the red-backed salamander, Bruch's membrane measures about 0.7 µm in thickness and the central elastic layer is very well developed and only discontinuous in a few locations (Figs. 2, 4, 5).

The choriocapillaris in this species forms a single layer of large caliber capillaries immediately adjacent to Bruch's membrane (Figs. 1 - 7). The endothelium facing the complexus basalis while usually very thin is but sparsely fenestrated (Figs. 2, 3, 6, 7). The nuclear region of these endothelial cells is normally on the choroidal aspect of these capillaries where the endothelium is totally without fenestrations (Figs. 2 - 4).

Discussion

The morphology of the retinal pigment epithelial region of the red-backed salamander *(Plethodon cinereus)* is similar to that described in other vertebrates with modifications that seem to be peculiar to amphibian species (Nguyen-Legros, 1978; Kuwabara, 1979; Braekevelt, 1980, 1982, 1984, 1986, 1988, 1990a).

As in all described species the RPE of this urodele is composed 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 most usually cuboidal (Kuwabara, 1979; Braekevelt, 1977, 1984, 1990a). In marsupials and amphibians on the other hand the retinal epithelium is normally much lower and the cells are squamous in shape (Braekevelt, 1973, 1991; Young and Braekevelt, 1992).

The basal (scleral) region of the RPE in most species is extensively infolded which is felt to indicate an increased rate of transport by these cells (Dowling and Gibbons, 1962; Bernstein and Hollenberg, 1965). Teleosts are an exception to this observation, showing but minimal infolding of the basal membrane of the

Fig. 1. Electron micrograph of the retinal epithelium of the red-backed salamander. The choriocapillaris (CC), Bruch's membrane (B), an epithelial cell nucleus (N) and rod outer segments (ROS) are all indicated. x 8,600

Fig. 2. Electron micrograph of the retinal epithelium to indicate a cell junction (J), a phagosome (Ph) and numerous melanosomes (M). The choriocapillaris (CC) is also indicated. x 8,600

Fig. 3. Electron micrograph to illustrate the pentalaminate Bruch's membrane (B) an epithelial cell nucleus (N) and several mitochondria (Mi). x 12,800

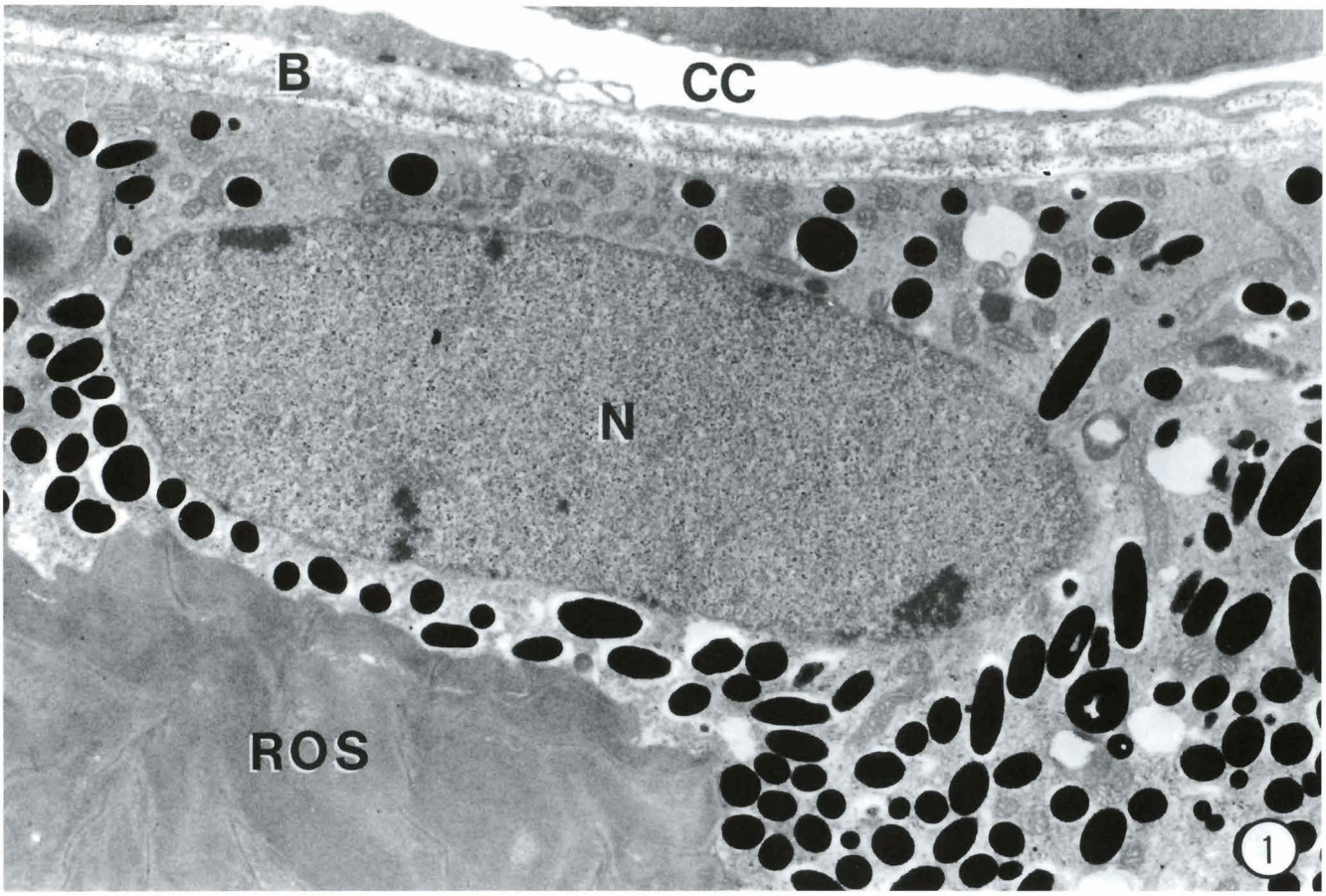
Fig. 4. Electron micrograph to indicate the abundance of smooth endoplasmic reticulum (SER), a phagosome (Ph), myeloid bodies (My) and a lateral cell junction (J). x 12,800

Fig. 5. Electron micrograph to illustrate a myeloid body (My), numerous melanosomes (M), a cell junction (J) and a lysosome-like body (L). x 8,900

Fig. 6. Electron micrograph to indicate a Golgi zone (G), phagosomes (Ph) and Bruch's membrane (B). x 8,900

Fig. 7. Electron micrograph to indicate the abundance of smooth endoplasmic reticulum (SER). Mitochondria (Mi), melanosomes (M) and the poorly fenestrated chroriocapillaris (CC) are also indicated. x 12,900

Fig. 8. Electron micrograph to indicate the pigment-laden apical processes (AP) of the RPE. Rod outer segments (ROS) are also indicated. x 16,700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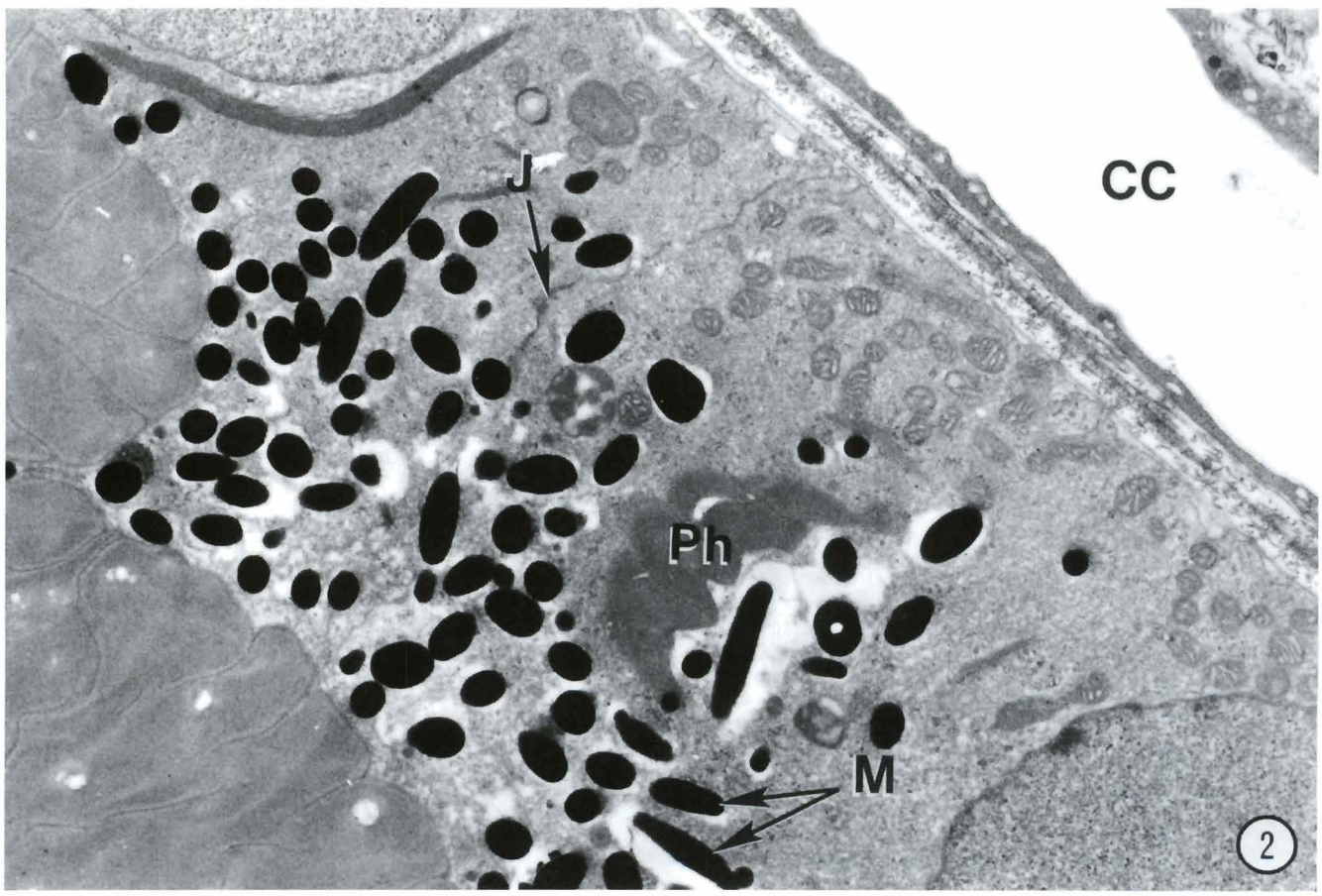
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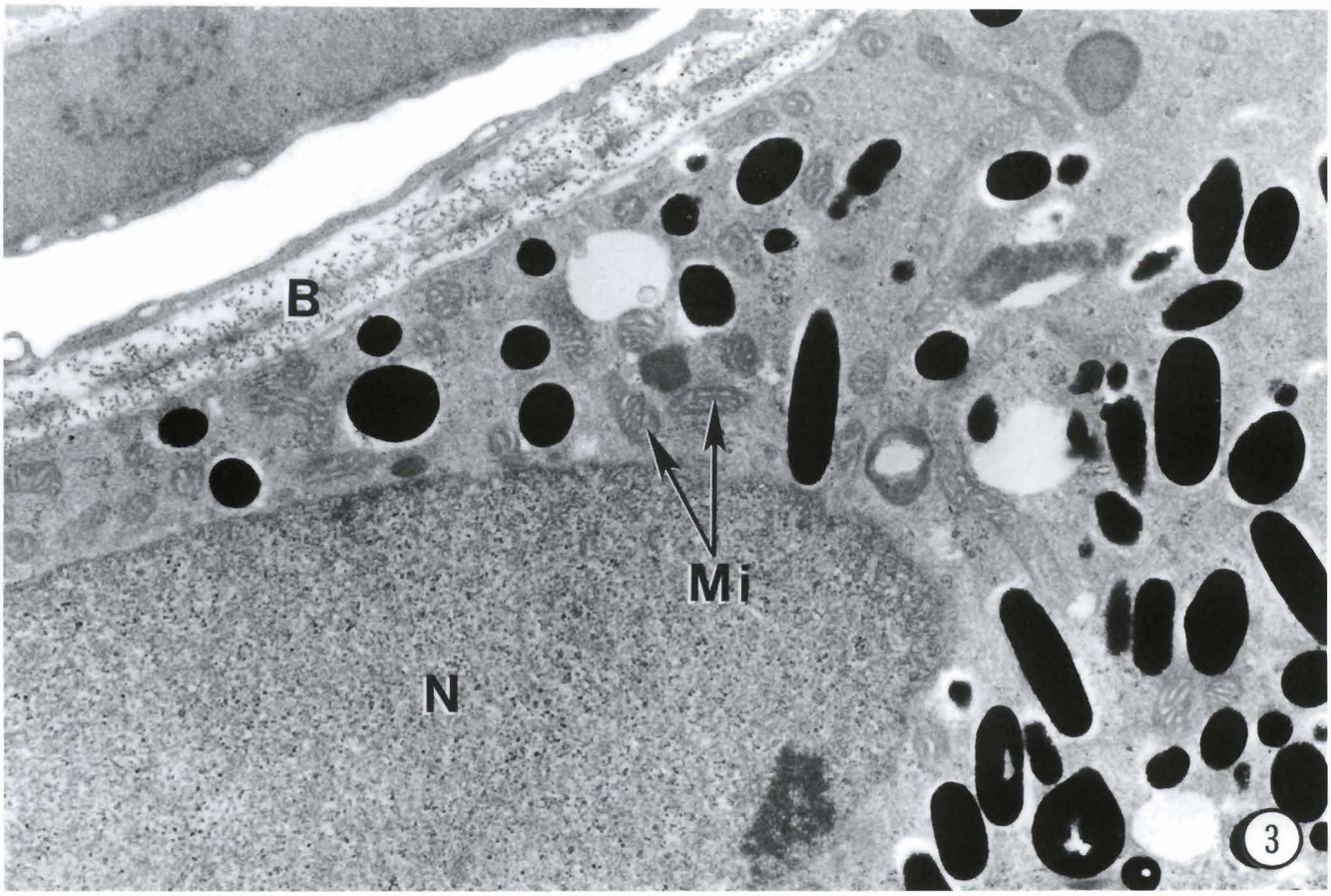
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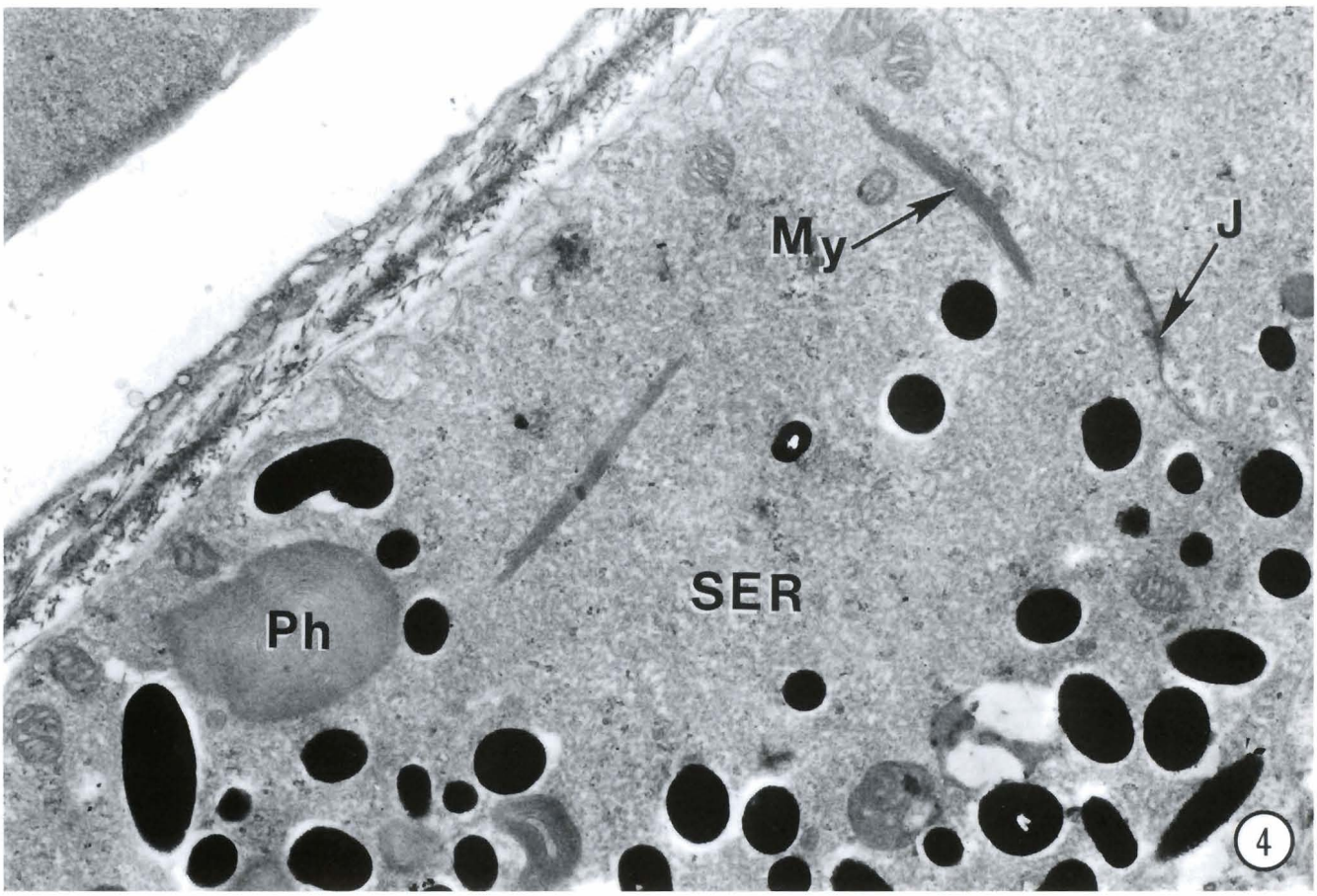
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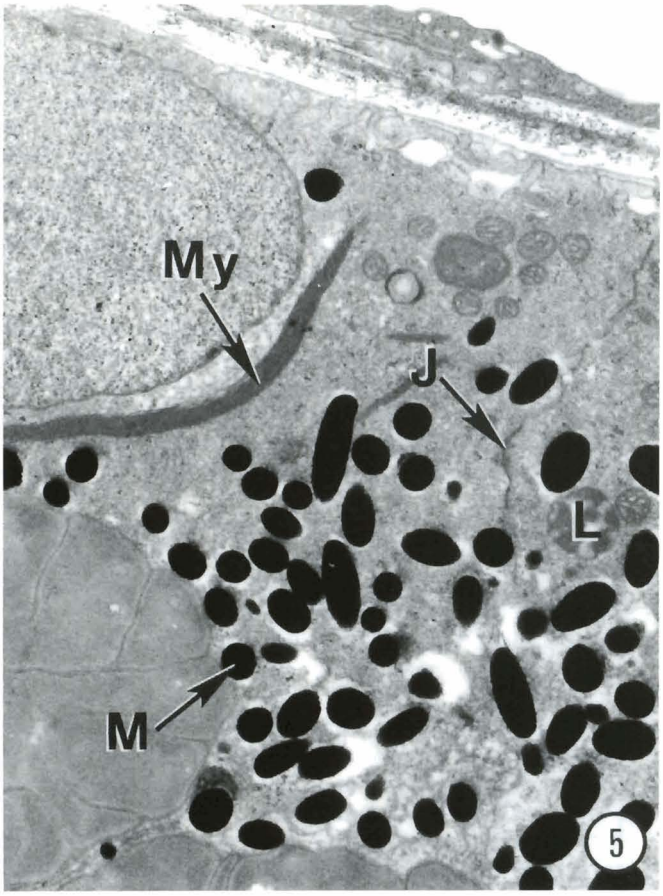
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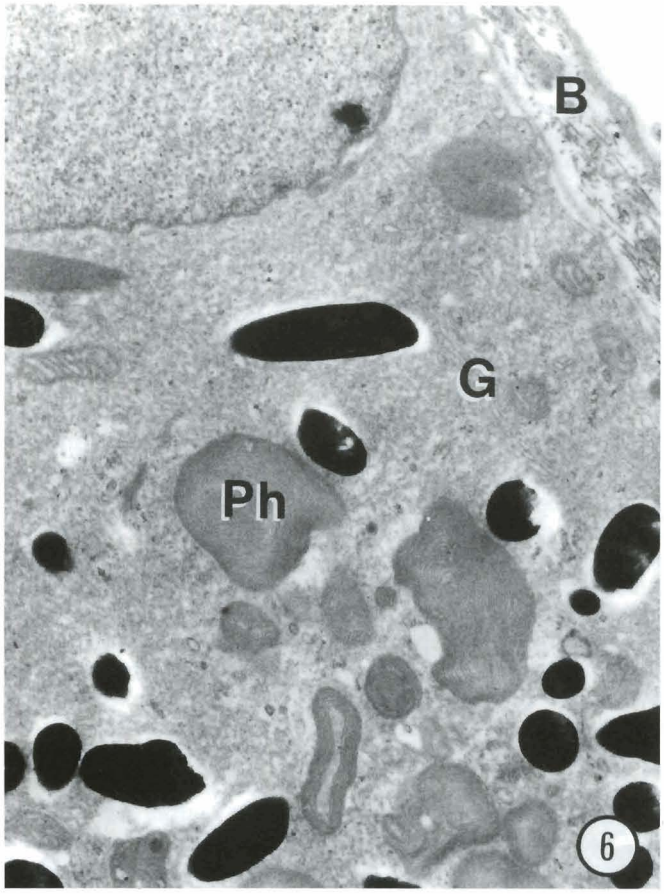
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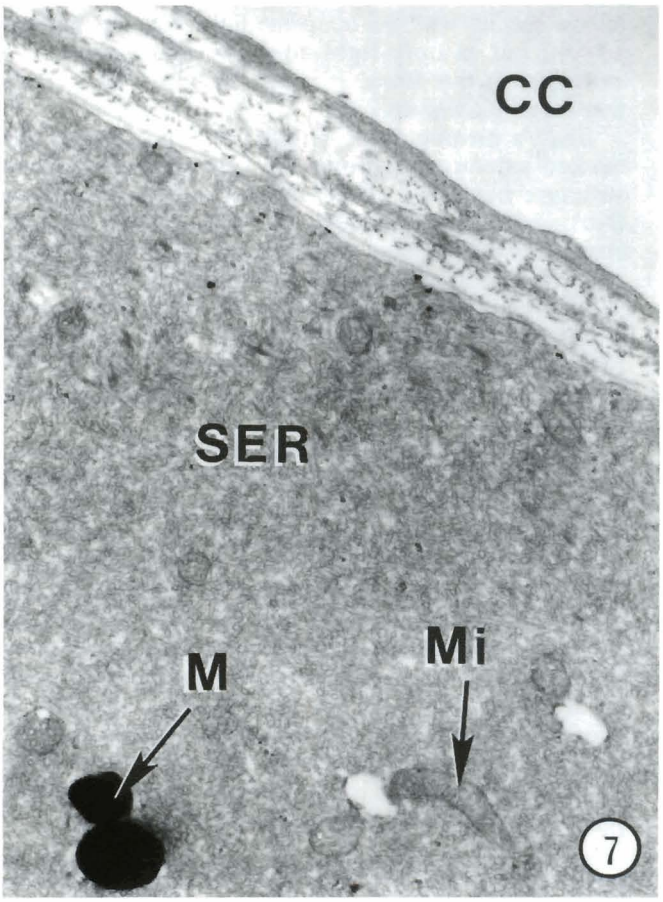
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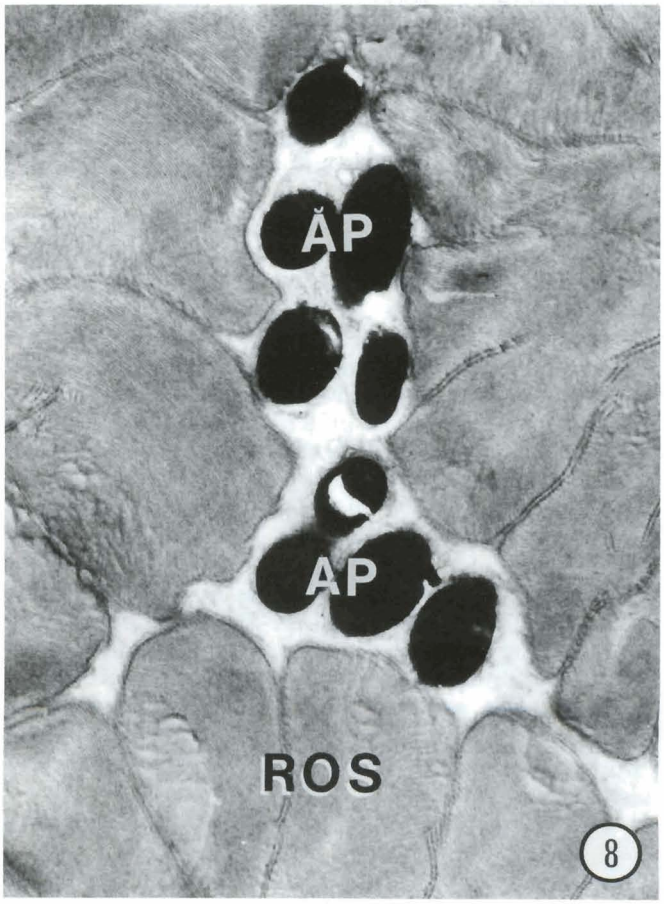
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RPE (Okuda, 1962; Braekevelt, 1982, 1985). This lack of basal infoldings is felt to be due to the presence of a choroid gland which maintains a high oxygen presure and presumably lowers transport requirements in teleosts species (Wittenberg and Wittenberg, 1974). In the red-backed salamander there is also but 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. The large size of the photoreceptors results in fewer cells to be serviced from the choriocapillaris and hence lessens transport requirements by the RPE.

The apical processes of the retinal epithelial cells are important in the structural support of the elongate photoreceptor outer segments (Bernstein, 1961) and in their proper orientation (Enoch, 1979) as well as in the internal adhesion required within the neural retina (Zinn and Benjamin-Henkind, 1979). These apical processes are also involved in the phagocytosis of shed outer segment discs (Bok and Young, 1979) and in non-albino species normally contain melanin granules which shield and isolate the photoreceptor outer segments and enhance acuity (Moyer, 1961; Braekevelt, 1982). In the red-backed salamander only one type of large finger-like apical process is noted and appears to contact both rods and cones.

The series of cell junctions at the lateral cell borders of the RPE cells are a constant feature of all vertebrates studied (Nguyen-Legros, 1978; Kuwabara, 1979). They constitute an effective barrier to the intercellular movement of materials and hence from part of the blood-ocular barrier (Zinn and Benhamin-Henkind, 1979). In mammalian species these junctions are normally located apically (vitreally) between the RPE cell while in lower vertebrates they are located in the mid to basal (scleral) region of the cells (Kuwabara, 1979; Braekevelt, 1982, 1988, 1990a).

The large vesicular nucleus found in RPE cells coupled with the abundance of mitochondria and other cell organelles are all indicative of highly active cells (Alberts et al., 1989). As noted in most vertebrates, smooth endoplasmic reticulum is abundant within RPE cells while rough endoplasmic reticulum is not (Nguyen-Legros, 1978; Kuwabara, 1979). The presence of only small amounts of RER would indicate that little protein is produced for export by these cells in the adult condition. The widespread appearance of polysomes on the other hand would indicate that protein is being produced for internal uses. An abundance of SER is common to cells heavily involved in lipid biosynthesis (Enders, 1962) and it is well established that the RPE is important in the storage, transport and esterification of vitamin A (Zinn and Benjamin-Henkind, 1979). The mitochondria in this species are small and somewhat less numerous than is normally observed for RPE cells and this may reflect the presume lowered amount of transport by these cells in the red-backed salamander.

The occurrence of lysosome-like bodies in this epithelial layer is to be expercted as one of the most important roles of the RPE is the phagocytosis and 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 this species in the light-adapted state is probably due to the sampling time as it is known that rods shed soon after lights on and cones shed soon after lights off (Young, 1978).

In the light-adapted specimens examined in this study the small melanosomes are predominantly located in the apical (vitreal) region of the cell body and within the apical processes. This would indicate that the melanin granules undergo retinomotor movements but gives no indication as to the extent or rapidity of these movements.

Myeloid bodies which are organelles composed of a stacked array of membranes continuous with the endoplasmic reticulum have been reported in most non-mammalian species (Braekevelt, 1976, 1977, 1985, 1990b; Kuwabara, 1979). They are most often noted in species with retinomotor movements (Porter and Yamada, 1960; Braekevelt, 1984, 1985) and while this may indicate a connection with photomechanical 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 lipids entering the RPE during phagocytosis of outer segment discs (Yorke and Dickson, 1984, 1985). Previous studies have shown that the morphology of these myeloid bodies changes in teleost species with the circadian light cycle (Braekevelt, 1990b) but as only light-adapted salamanders were examined in this study, it is not known if this is also true for amphibian species.

Bruch's membrane or complexus basalis in the red-backed salamander shows the typical pentalaminate structure noted for all vertebrates except for teleosts (Nukaizumi, 1964; Braekevelt, 1983, 1986). Teleosts characteristically have a trilaminate complexus basalis with the central elastic layer (lamina densa) being absent (Braekevelt, 1982, 1985). In avian species the central lamina densa while present is very often only poorly represented (Braekevelt, 1984, 1989) while in this urodele the central lamina densa is exceptionally well represented.

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 red-backed salamander the choriocapillaris is also but minimally fenestrated but is felt to be due in this case to the reduced number of large photoreceptors which is characteristic of the amphibian retina (Walls, 1942; Dowling and Werbling, 1969).

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