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Retinal pigment epithelial fine structure in the bobtail goanna *(Tiliqua rugosa)*

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Summary. The retinal pigment epithelium (RPE), the choriocapillaris and Bruch's membrane (complexus basalis) have been studied by light and electron microscopy in the bobtail goanna *(Tiliqua rugosa)* an Australian diurnal lizard. The RPE consists of a single layer of cuboidal cells which display very deep and tortuous basal (choroidal) infoldings as well as numerous apical (vitreal) processes which interdigitate with the photoreceptor cells. The lateral cell borders are relatively smooth and joined by basally located tight junctions. Internally smooth endoplasmic reticulum is abundant while rough endoplasmic reticulum is not. The RPE cell nucleus is large and vesicular and basally located in the light-adapted state. Polysomes, mitochondria and myeloid bodies are present and widely distributed. Melanosomes are plentiful in the apical region of the epithelial cells in light-adaptation. Bruch's membrane is pentalaminate with the basal lamina of the choriocapillaris being exceptionally thick. The choriocapillaris is a single layer of large-caliber capillaries with thin but only moderately fenestrated endothelium. Numerous dense granules are always present within these endothelial cells.

Key words: Retinal pigment epithelium, Bruch's membrane, Choriocapillaris, Electron microscopy, Lizard

Introduction

The retinal pigment epithelium (RPE) forms the outermost layer of the vertebrate retina and is intimately involved in several processes essential to the normal physiology of the photoreceptors and eventually to vision itself. Amongst the best understood roles of the RPE are 1) the architectural stabilization and proper orientation of the photoreceptor outer segments (Bernstein, 1961; Enoch, 1979) 2) internal adhesion of the neurosensory retina (Zinn and Benjamin-Henkind, 1979) 3) the storage of vitamin A precursors of the visual pigments (Young and Bok, 1970) 4) the selective transport of materials between the choriocapillaris and photoreceptors (Kroll and Machemer, 1968; Steinberg and Miller, 1973) and 5) the phagocytosis and lysosomal breakdown of photoreceptor outer segment discs (Young, 1978a; Bok and Young, 1979).

In view of these several vital functions, the RPE region of the vertebrate retina has been studied in a variety of animals. While this region is essentially similar in design in all vertebrates, species differences in morphology are often observed (Braekevelt, 1977, 1980, 1983, 1985, 1986, 1988; Nguyen-Legros, 1978; Kuwabara, 1979).

While numerous reports of the fine structure of the RPE are available, relatively few deal with reptilian species (Yamada, 1961; Braekevelt, 1977; Kuwabara, 1979). Consequently as part of a comparative morphological study of this region in vertebrates, the fine structure of the RPE, Bruch's membrane and choriocapillaris in the bobtail goanna *(Tiliqua rugosa)* is reported in this study.

Materials and methods

For this study the eyes from ten healthy adult bobtail goannas *(Tiliqua rugosa)* were examined by light and electron microscopy. Specimens were 3-4 years old, of both sexes, weighed 420-550 gm and were light-adapted when sampled.

With the animals under deep anesthesia, the eyeballs were quickly removed, opened at the equator and fixed for 5h in 5% glutaraldehyde buffered to pH 7.3 which 0.1M Sorensen's phosphate buffer at 4°C. The posterior half of the eyeball was then removed, washed in 5% sucrose in 0.1M Sorensen's buffer (pH 7.3) and cut into

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pieces less than 1mm^2. The tissue was then post-fixed for 2h in 1% osmium tetroxide in the same phosphate buffer, dehydrated through graded ethanols to methanol and propylene oxide and embedded in Araldite.

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

Results

The retinal pigment epithelium (RPE) in the bobtail goanna consists of a single layer of cuboidal to columnar cells (Fig. 1). These cells display extremely deep and often very tortuous basal (choroidal) infoldings (Figs. 1, 2, 4). Apically (vitreally) the RPE cells show numerous processes which interdigitate with the inner and outer segments of the photoreceptor cells (Figs. 1, 7). The lateral cell borders of these cells are relatively smooth and are joined together by a series of prominet basally-located tight junctions (Figs. 1, 4).

Internally the RPE cell nucleus is large, extremely vesicular and normally basally located in the light-adapted state (Figs. 3, 4). A single nucleolus is usually obvious (Fig. 3).

Smooth endoplasmic reticulum (SER) is the most abundant cell organelle and except for the basal infoldings is present throughout the cell (Figs. 4, 5, 7). Polysomes are also numerous and widespread while profiles of rough endoplasmic reticulum (RER) are scarce (Figs. 3, 4). Mitochondria are small and numerous and also scattered throughout the cell cytoplasm with a slight preponderance in the basal region (Figs. 1, 4, 7).

Myeloid bodies are present and in the light-adapted state are large and compact (Figs. 3, 5). Melanosomes are small and numerous and in light-adaptation are almost exclusively located in the apical region of the cell body and within the apical processes (Figs. 1, 5, 7). Lysosome-like bodies and phagosomes of outer segment material while present were not abundant (Figs. 2, 6, 7).

Bruch's membrane or complexus basalis in this species is pentalaminate, consisting of 1) the basal lamina of the RPE layer 2) the basal lamina of the choriocapillaris 3) a central discontinuous elastic layer (lamina dense) separating 4) an inner and 5) an outer collagenous layer. The basal lamina of the choriocapillaris in the bobtail goanna is unusually thick and in many locations extends from the choriocapillaris to the central lamina densa, covering the entire outer collagenous layer (Figs. 2, 4, 8). The basal lamina of the RPE layer is also more prominent than usual but does not spread into the inner collagenous layer (Figs. 3, 4, 8).

The choriocapillaris consists of a single layer of large-caliber capillaries located immediately adjacent to Bruch's membrane (Figs. 1, 4, 8). The endothelium facing Bruch's membrane is thin and normally moderately fenestrated (Figs. 2, 4) although areas without fenestrations are also common (Fig. 8). The endothelium facing the choroid is thicker and contains the nuclear region and most of the cell's other organelles (Figs. 1, 8). In addition, numerous spherical granules of varying electron density are a constant feature within these endothelial cells' cytoplasm (Figs. 1, 8).

Discussion

The retinal epithelial region of the bobtail goanna *(Tiliqua rugosa)* displays a morphology essentially similar to that described for other vertebrate species, although some unique variations are noted (Nguyen-Legros, 1978; Kuwabara, 1979; Braekevelt, 1980, 1984, 1988).

As is invariably the case, the retinal pigment epithelium (RPE) in this species consists of a single layer of cells (Nguyen-Legros, 1978). The height of the RPE cells varies widely amongst species but is most usually cuboidal as is the case in this lizard. The RPE cells normally display an extensive array of basal infoldings which are felt to be microfolds and indicative of a heavy involvement in the transport of materials from the choriocapillaris to the photoreceptors (Steinberg and Miller, 1973). The basal infoldings noted in this species are deeper and more extensive than is normally observed in other species.

The numerous apical processes of the RPE cells are necessary for the structural support and proper orientation of the elongate photoreceptor cells, particularly the outer segments (Bernstein, 1961; Enoch, 1979) as well as in the internal adhesion required within

Fig. 1. Low power electron micrograph of the retinal epithelial layer (RPE) in the bobtail goanna. The chororiocapillaris (CC) and photoreceptors (Ph) are indicated. × 3,900

Fig. 2. Electron micrograph of the basal region of the RPE to indicate the extensive basal infoldings (BI) and Bruch's membrane (B) × 13,000

Fig. 3. Electron micrograph of the RPE to indicate an epithelial nucleus (N), myeloid bodies (My) and numerous mitochondria (Mi). × 13,000

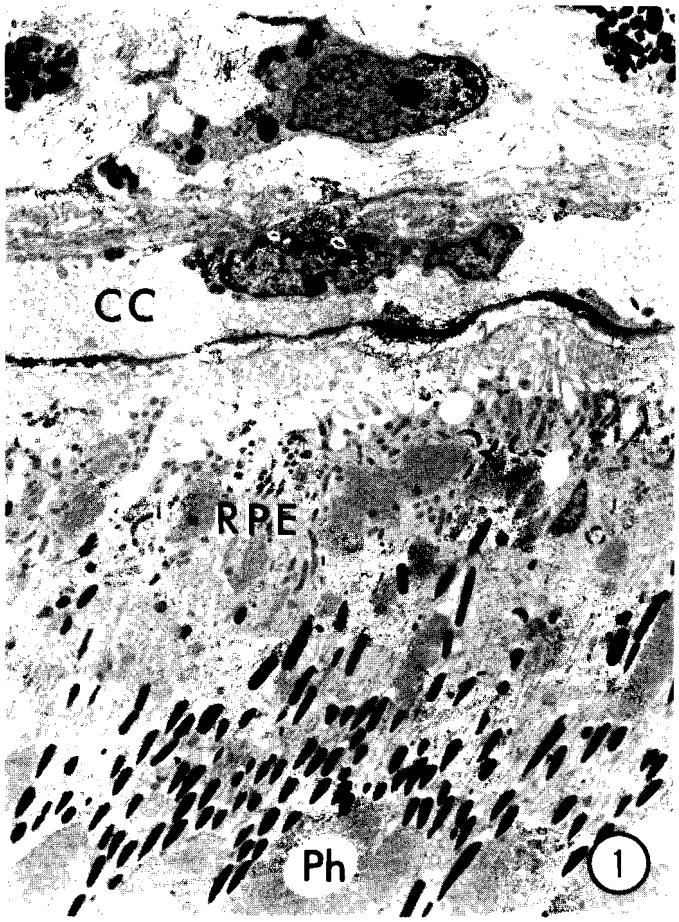
Fig. 4. Electron micrograph of the basal region of the RPE to indicate a cell junction (J), basal infoldings (BI) and Bruch's membrane (B). × 13,500

Fig. 5. Electron micrograph to indicate the abundance of smooth endoplasmic reticulum (SER). Myeloid bodies (My) and a photoreceptor outer segment (OS) are also indicated. × 13,800

Fig. 6. Electron micrograph of the apical region of the RPE to indicate much SER, apical processes (AP), melanosomes (M) and lysosome-like bodies (L). × 14,400

Fig. 7. Electron micrograph of the apical region of the RPE to indicate the length of the apical processes (AP) and a photoreceptor outer segment. (OS). × 9,500

Fig. 8. Electron micrograph of the choriocapillaris (CC) to indicate numerous dense endothelial granules (G). Bruch's membrane (B) is also indicated. Note the thickened basal lamina of the choriocapillaris and the absence of fenestration in the endothelium. × 13,500


CC
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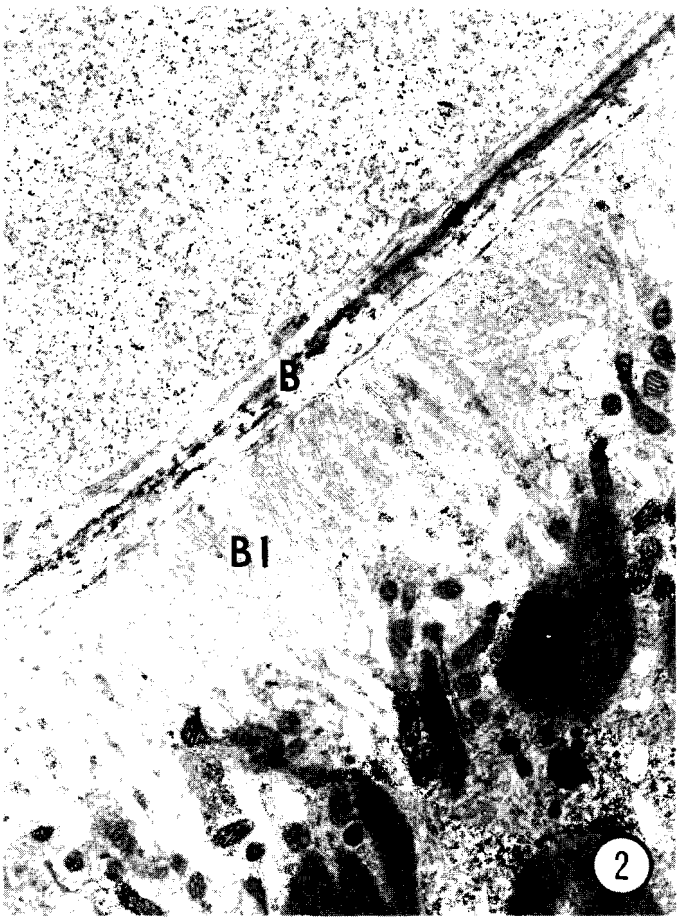
1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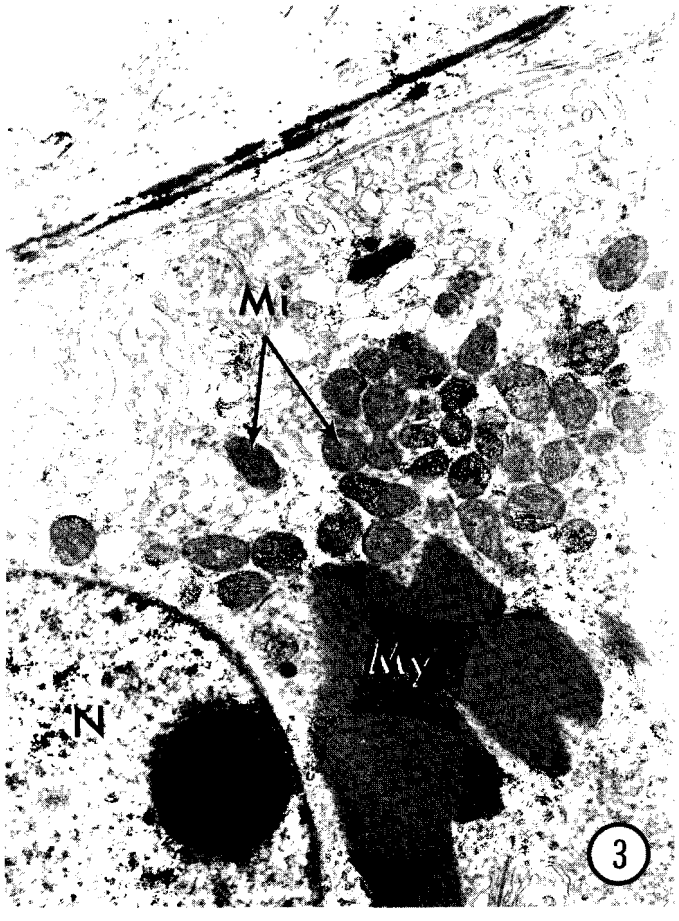
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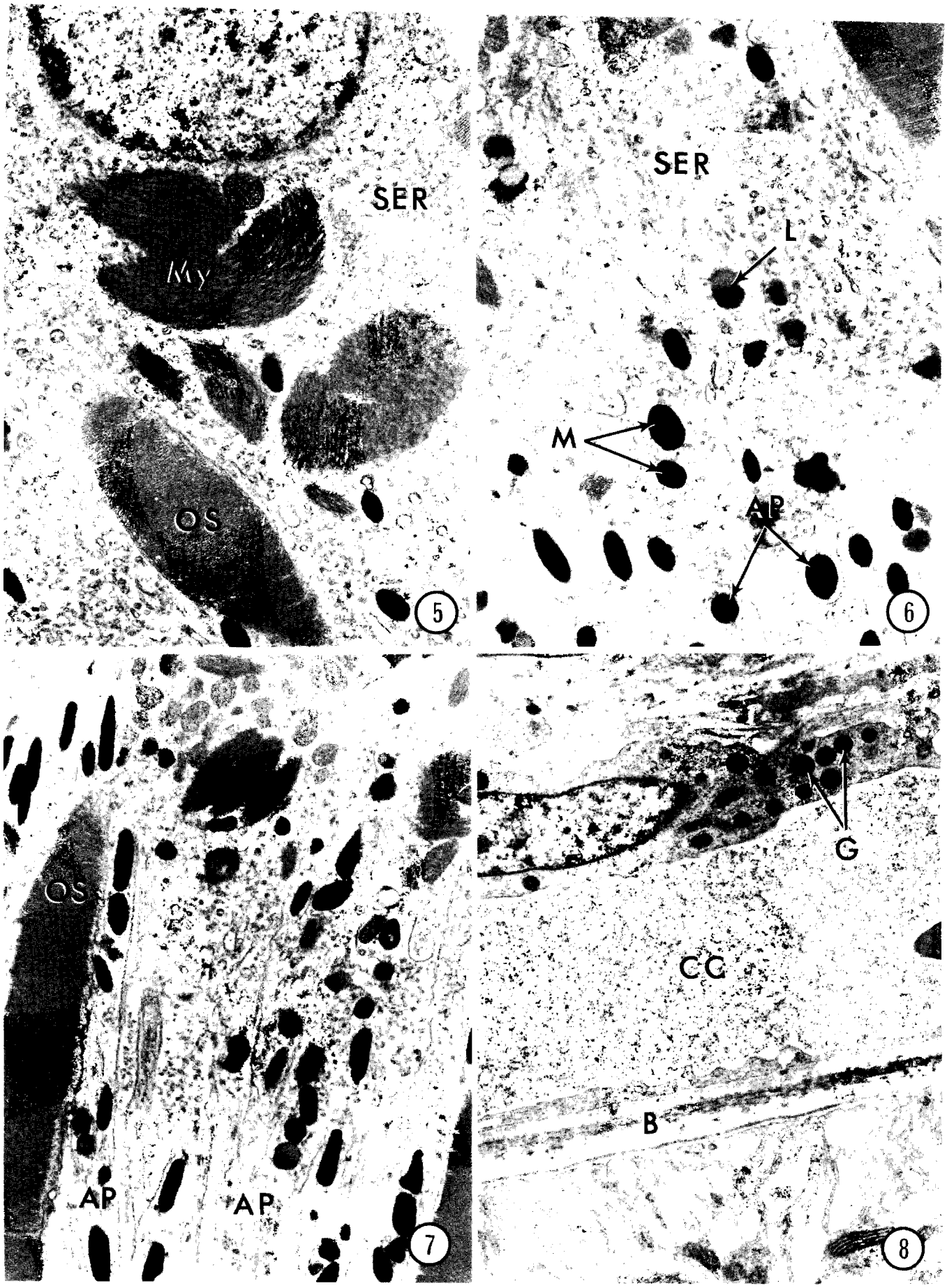
SER
My
OS
5
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the neural retina (Zinn and Benjamin-Henkind, 1979). These apical processes are also important in the phagocytosis of shed outer segment discs (Bok and Young, 1979). While in some species two or more morphologically distinct types of apical process are reported (Steinberg and Wood, 1974; Braekevelt, 1982), this species has only one type of apical process that contacts both rods and cones.

The cell junctions located at the lateral cell border of the RPE cells are a constant feature in all vertebrates studied. They are felt to constitute an effective barrier to the intercellular movement of materials and hence form part of the blood-ocular barrier (Zinn and Benjamin-Henkind, 1979).

The large vesicular nucleus and abundance of cell organelles is a common finding within the RPE cells of most species and is characteristic of metabolically very active cells (Nguyen-Legros, 1978; Kuwabara, 1979; Young and Bok, 1979; Braekevelt, 1984, 1986, 1988). The wealth of SER reflects the heavy involvement of these cells in the storage, transport and esterification of lipid photopigment precursors (Zinn and Benjamin-Henkind, 1979). The paucity of RER noted in the RPE cells would indicate that little protein is being produced for export by these cells in the adult condition. The abundance of polysomes however indicates that these cells still require proteins for their internal structures and functions. In line with the abundance of other organelles, the number of mitochondria is high to supply the energy requirements of these cells.

The melanosomes noted in the retinal epithelial cells of this lizard are in the light-adapted state grouped in the apical region of the cell body and within the apical processes separating photoreceptors. While only light-adapted specimens were examined in this study, judging by the location of the melanosomes, retinomotor or photomechanical responses of the pigment are felt to occur in this species as is reported for other diurnal reptilian species (Walls, 1942). The almost total lack of phagosomes of outer segment discs within the RPE cells in the light-adapted state is presumably due to the high preponderance of cones over rods in this species, as it is known that cones shed soon after the offset of light (Young, 1977, 1978b).

Myeloid bodies are a common feature within the RPE cells of a variety of lower vertebrates (Nguyen-Legros, 1978; Kuwabara, 1979; Braekevelt, 1982, 1984). While they have been implicated as the organelle that triggers photomechanical movements (Porter and Yamada, 1960; Braekevelt, 1982) and also as the sites of storage of lipids prior to esterification (Yorke and Dickson, 1984, 1985), their function remains uncertain. In most species, including this one, myeloid bodies are seen to be continuous with the membranes of the SER, although in avian species they often have ribosomes on their outer surface indicating a possible continuity with the RER (Braekevelt, 1984).

Bruch's membrane (complexus basalis) in mammalian species is invariably pentalaminate with the five layers as described in the results portion of this report being quite distinct and obvious (Nakaizumi, 1964; Braekevelt, 1986, 1988). Teleosts characteristically only have a trilaminate Bruch's membrane with the central elastic layer (lamina densa) being absent (Braekevelt, 1980, 1982, 1985). In this lizard while Bruch's is pentalaminate, the basal lamina of the choriocapillaris is unusual in being very thick and often covering the entire outer collagenous layer.

The choriocapillaris in all vertebrate species is composed of a single layer of large-caliber capillaries (Rodieck, 1973; Kuwabara, 1979). With the exception of teleosts the choriocapillaris endothelium facing Bruch's membrane is normally very thin and highly fenestrated, indicative of the movement of large quantities of material (Bernstein and Hollenberg, 1965). In teleosts the presence of a choroid gland which is important in the maintenance of a high oxygen pressure is felt to be the reason for the reduction in the number of fenestrations in the choriocapillaris endothelium (Braekevelt, 1985). The choriocapillary endothelium in the bobtail goanna is thin but only moderately fenestrated facing Bruch's membrane. It is also unusual in displaying a large number of electron dense granules of unknown material and function within the endothelial cells of the choriocapillaris.

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