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# Fine structure of the retinal pigment epithelium of the great horned owl *(Bubo virginianus)*

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**Summary.** The fine structure of the retinal epithelium (RPE), choriocapillaries and Bruch's membrane (complexus basalis) has been studied by light and electron microscopy in the great horned owl *(Bubo virginianus)*. The RPE consists of a single layer of cuboidal cells joined laterally in the mid to basal region by a series of tight junctions forming part of the blood-ocular barrier. Basally (sclerally) the epithelial cells show numerous deep infoldings while apically (vitreally) a wealth of microvillar processes interdigitate with the photoreceptor cells. Internally the RPE cells display a large vesicular nucleus, plentiful smooth endoplasmic reticulum (SER) and polysomes with only small scattered profiles of rough endoplasmic reticulum (RER). Numerous pleomorphic mitochondria are basally located. In the light-adapted state the melanosomes are located almost exclusively within the apical processes indicating retinomotor movements. Myeloid bodies are numerous and often show ribosomes on their outer surface. Bruch's membrane is typical of avian species in that it is pentalaminate and the lamina densa is displaced towards the choriocapillaris. The choriocapillaris itself is but minimally fenestrated facing Bruch's membrane. Most fenestrations present show a single layered diaphragm while others display a double-layered diaphragm.

**Key words:** Retinal pigment epithelium (RPE), Electron microscopy, Great horned owl, *Bubo virginianus*

## Introduction

The retinal pigment epithelium (RPE) derives from the outer wall of the optic cup to become the outermost (scleral) layer of the neural retina. In this location it is intimately involved in several processes essential to the proper functioning of the photoreceptor cells and hence to vision itself. Amongst the best understood roles of the RPE are 1) the architectural stabilization and proper orientation of the photoreceptor outer sements (Bernstein, 1961; Enoch, 1979), 2) the storage and modification of vitamin A precursors of the visual pigments (Young and Bok, 1970, 1979), 3) the selective transport of materials to the photoreceptors (Kroll and Machemer, 1968; Steinberg and Miller, 1973), 4) internal adhesion within the neurosensory retina to prevent a retinal detachment (Zinn and Benjamin-Henkind, 1979), and 5) the phagocytosis and degradation of shed photoreceptor outer segment debris (Young, 1978; Bok and Young, 1979).

As one consequence of the RPE involvement in several vital processes, this region of the vertebrate retina has been investigated in a variety of animals with a range of techniques. Morphological studies in particular have shown a remarkable structural similarity throughout vertebrate species but with generic differences usually present (Nguyen-Legros, 1978; Kuwabara, 1979; Braekevelt, 1980, 1982, 1984, 1986, 1988, 1990a).

While numerous reports of the fine structure of the retinal epithelium are available, avian species have been relatively neglected (Nishida, 1964; Matsusaka, 1966; Meyer et al., 1973; Dieterich, 1975; Braekevelt, 1984, 1989, 1990b). Consequently as part of an ongoing comparative study of the RPE region, this report deals with the fine structure of the retinal pigment epithelium, choriocapillaris and Bruch's membrane (complexus basalis) in the great horned owl *(Bubo virginianus)*.

## Materials and methods

For this study both eyes from an adult, light-adapted great horned owl *(Bubo virginianus)* were examined by light and electron microscopy. With the bird under deep anaesthesia, the eyeballs were quickly enucleated, sliced open 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 half of the eyeball was then removed and washed in 5% sucrose in

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0.1M Sorensen's buffer (pH 7.3) and cut into pieces less than 1 $mm^2$, taking care not to detach the retina. The tissue was then postfixed for 2 h in 1% osmium tetroxide in the same phosphate buffer (pH 7.3), dehydrated up through graded ethanols to methanol and then propylene oxide and embedded in Araldite.

Pieces of plastic-embedded tissue were subsequently reoriented to desired angles using a wax mount and both thick (0.5 μm) and thin (50-60 nm) sections were cut on an LKB ultramicrotome. Thick sections were stained with toluidine blue and examined by light microscopy. Thin sections of selected areas were stained in aqueous uranyl acetate and lead citrate and examined and photographed in a Philips EM201 transmission electron microscope.

## Results

As in all vertebrates investigated, the retinal pigment epithelium (RPE) of the great horned owl *(Bubo virginianus)* consists of a single layer of cells joined laterally by a series of tight junctions (Figs. 1, 4). In this species the cells are cuboidal measuring about 12 μm in width and 14 μm in height with the lateral cell junctions located in the mid to basal region (Figs. 1, 4). Basally (sclerally) these epithelial cells show numerous deep branching microfolds (Figs. 1, 2, 4, 6, 7). These basal infoldings average about 1.0 μm in depth and are normally quite uniform in depth within each cell (Figs. 2, 4, 6). Apically (vitreally) the epithelial cells display numerous processes that extend from the cell body and interdigitate with the photoreceptor outer segments (Figs. 3, 5). Only one type of microvillar process is noted in this species and it appears to contact both rods and cones (Figs. 3, 5). Laterally the retinal epithelial cell borders are comparatively smooth and joined by tight junctions (Figs. 1, 4).

Within the RPE cells the nucleus is large, vesicular and fairly centrally located (Figs. 1, 2). The mitochondria in this species are extremely pleomorphic and many of them show long thin projections which do not appear to contain cristae (Figs. 1, 2, 4, 6). In addition, ring-shaped mitochondria as reported in other avian species are also noted (Figs. 2, 6). In the light-adapted state these mitochondria are predominantly located basally within the RPE cells (Figs. 1, 2, 6).

The most abundant cell organelle is smooth endoplasmic reticulum (SER) and with the exception of the basal infoldings is found throughout the cytoplasm including the apical processes (Figs. 2, 3, 5, 6). Rough endoplasmic reticulum (RER) is not abundant but is present as short profiles (Fig. 5). Polysomes are however abundant and widespread (Figs. 2, 4, 5). Lysosome-like bodies and lipid droplets are also a regular feature of these cells (Figs. 1, 2, 4). Phagosomes of outer segment material while not abundant in this light-adapted specimen are present in various stages of degradation (Figs. 1, 2, 4).

Myeloid bodies which are seen as compact arrays of membranes are also noted within the RPE cells of the great horned owl (Figs. 1, 2, 3, 5). Normally the membranes of these myeloid bodies are seen to be continuous with the SER membranes (Figs. 2, 4) but as has been noted in other avian species the myeloid bodies in this species often display ribosomes on their outer surface and may indicate a continuity with RER membranes as well (Fig. 5).

The melanosomes of the RPE cells are in the light-adapted state located almost exclusively within the apical processes where they serve to surround and isolate photoreceptor outer segments from one another (Figs. 3, 5). This leaves the RPE cell body essentially devoid of melanosomes in light-adaptation (Figs. 2, 4). In the great horned owl, melanosomes are small (about 0.5 μm) and predominantly round to oval in shape (Figs. 3, 5).

Bruch's membrane or complexus basalis in this avian species is a pentalaminate structure. It consists of 1) the basal lamina of the RPE and 2) the basal lamina of the choriocapillaris enclosing 3) a discontinuous elastic layer (lamina densa) which separates 4) an inner and 5) an outer collagenous layer (Figs. 1, 4, 6, 7). Also as noted in other birds, the lamina densa is located much closer to the choriocapillaris and hence the two collagenous layers are unequal in thickness (Fig. 4).

The choriocapillaris forms a single layer of large-caliber capillaries immediately adjacent to the choroidal aspect of Bruch's membrane (Figs. 1, 4, 6, 7). The endothelium facing Bruch's membrane is normally quite thin but only minimally fenestrated (Figs. 1, 2, 4, 7). While the majority of the fenestrations present display a single-layered diaphragm, some of them show a double-layered diaphragm (Figs. 4, 6, 7).

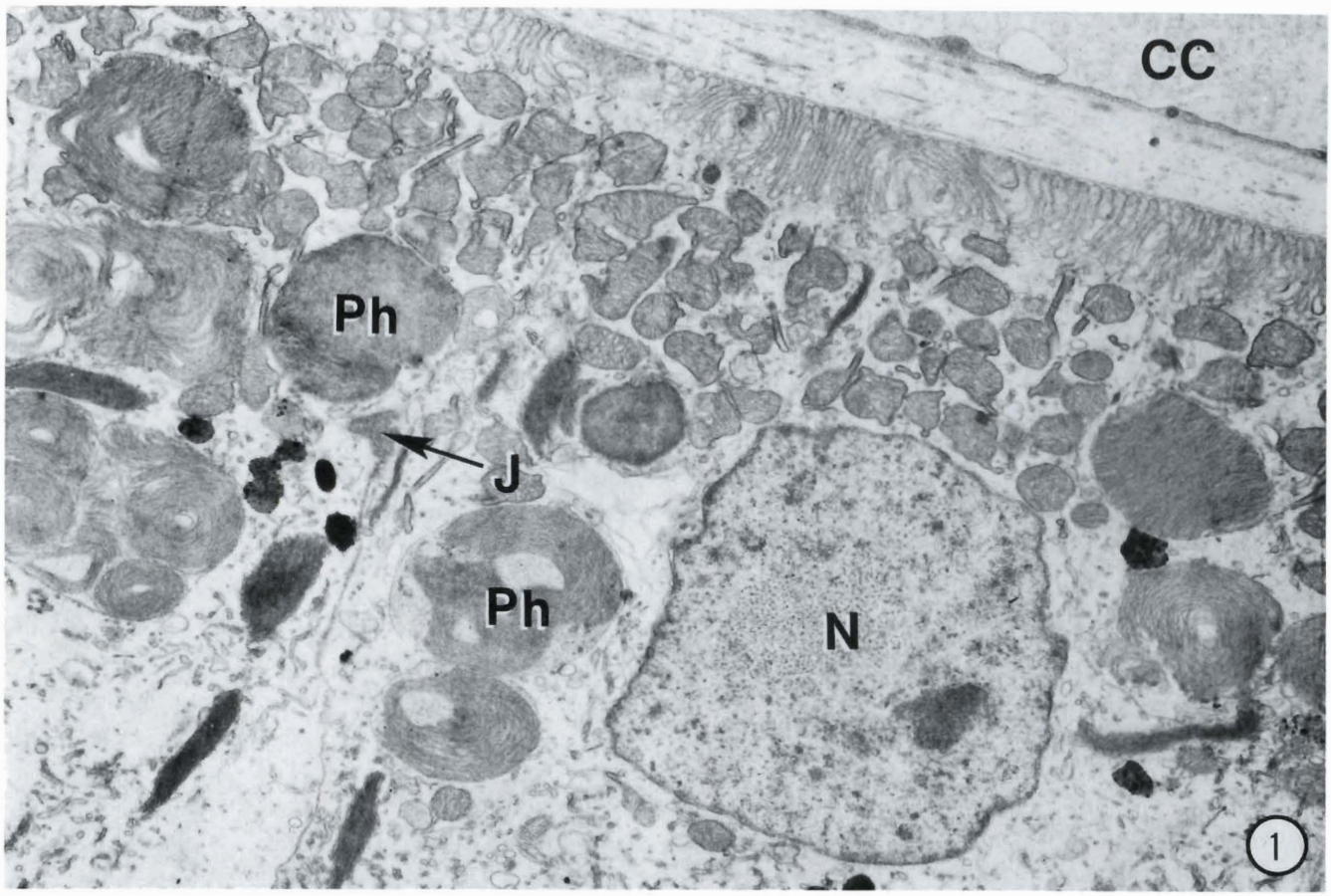
## Discussion

The retinal pigment epithelium (RPE) of the great horned owl *(Bubo virginianus)* is fundamentally similar to that described for all vertebrates with modifications or refinements that seem to be specific to avian species (Rodieck, 1973; Nguyen-Legros, 1978; Kuwabara, 1979;

**Fig. 1.** Electron micrograph of the retinal pigment epithelium in the great horned owl. The choriocapillaris (CC), an RPE nucleus (N), phagosomes (Ph) and a cell junction (J) are all indicated. x 9,000

**Fig. 2.** Electron micrograph of the basal region of an RPE cell to illustrate the basal infoldings (BI), the abundance of smooth endoplasmic reticulum (SER) and numerous pleomorphic mitochondria (Mi). Bruch's membrane (B) is also indicated. x 9,500

**Fig. 3.** Electron micrograph of the apical region of an RPE cell to illustrate the apical processes (AP) and melanosomes (M). Rod outer segments (ROS) and a myeloid body (My) are also indicated. x 10,300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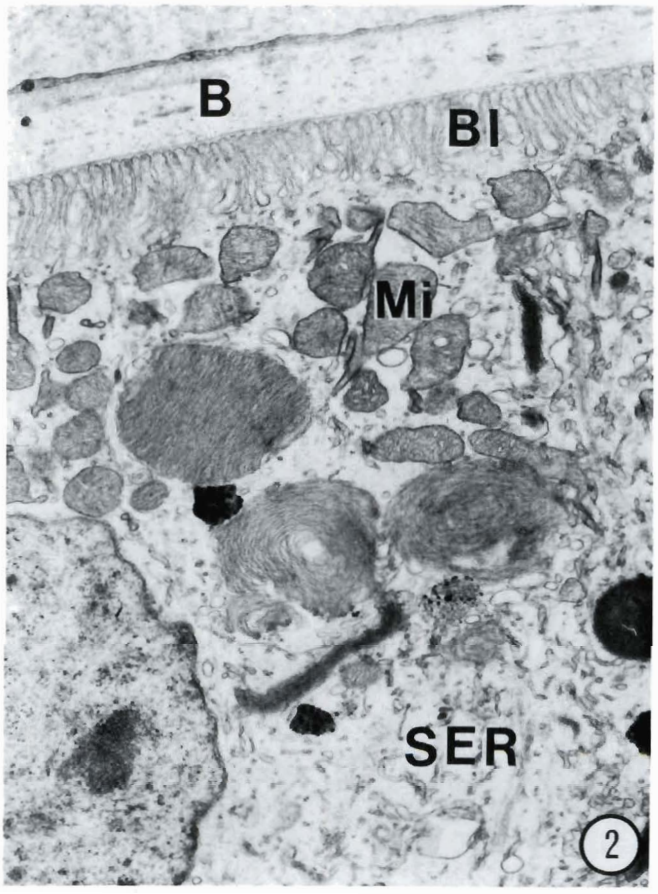
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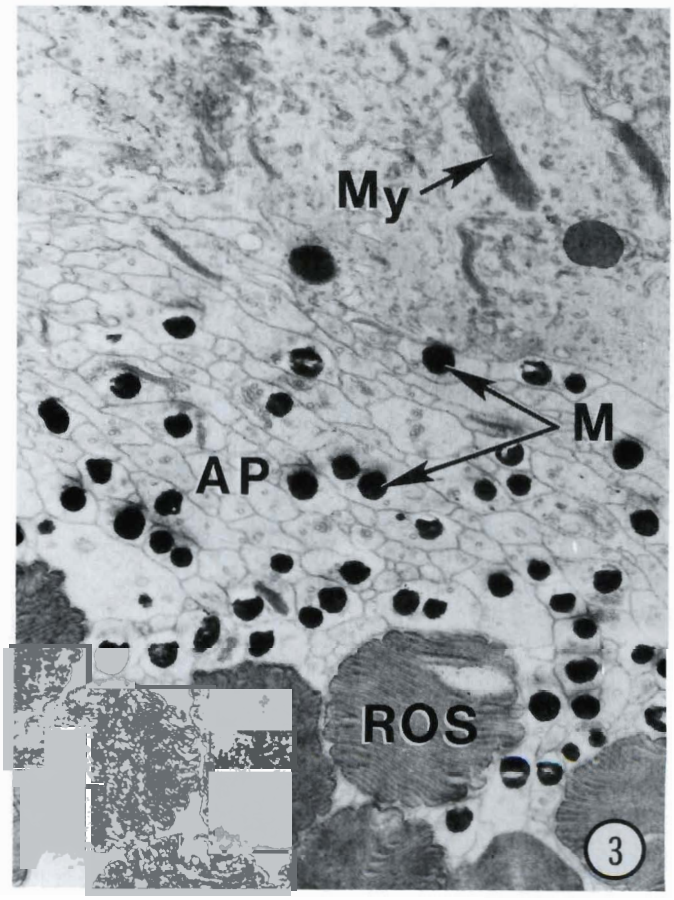
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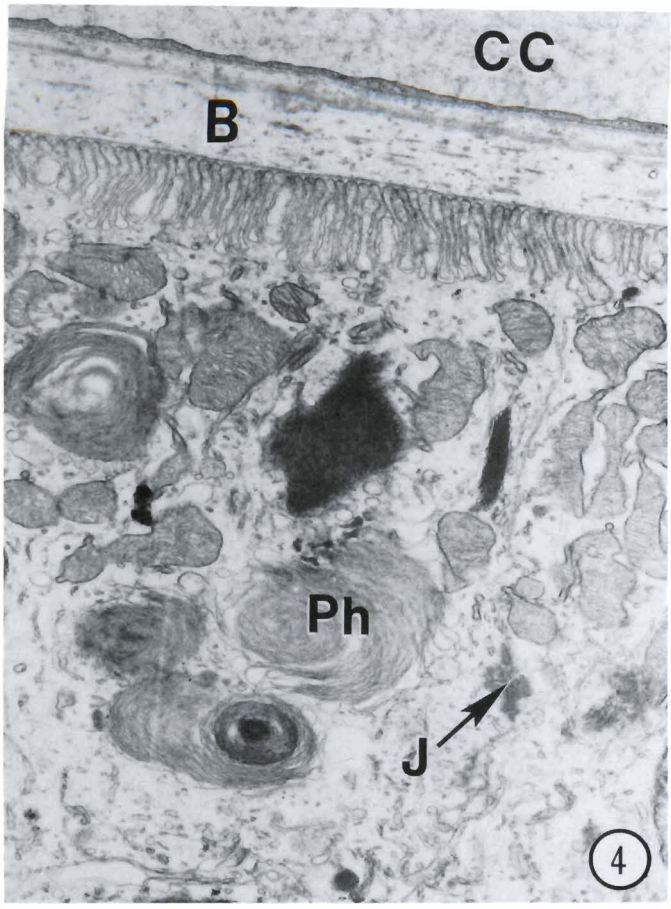
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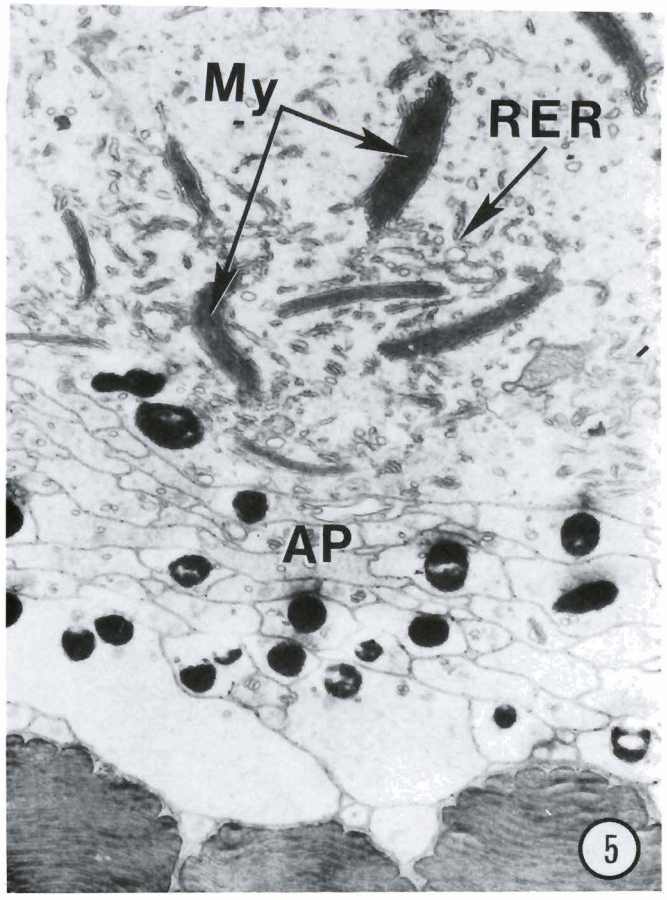
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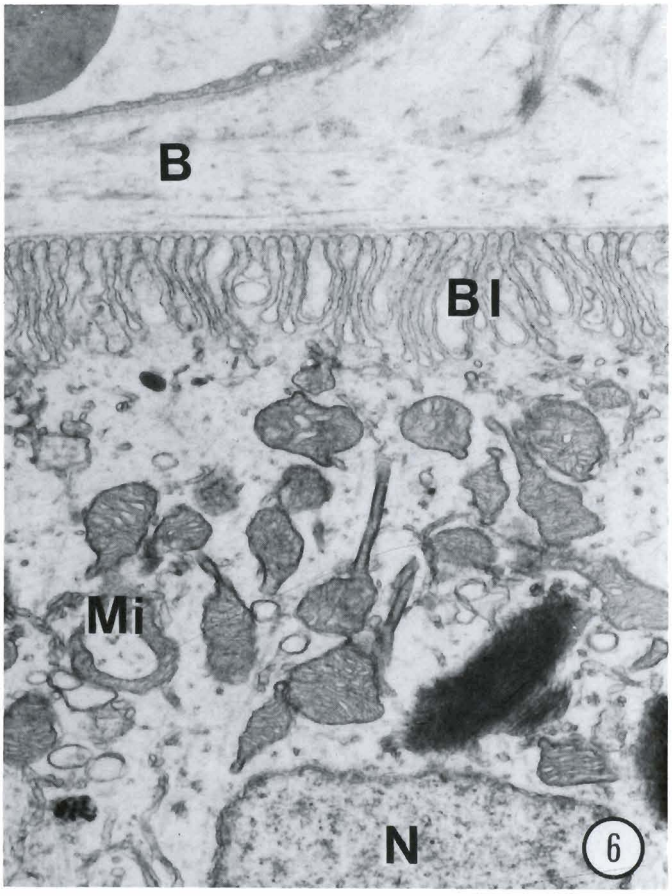
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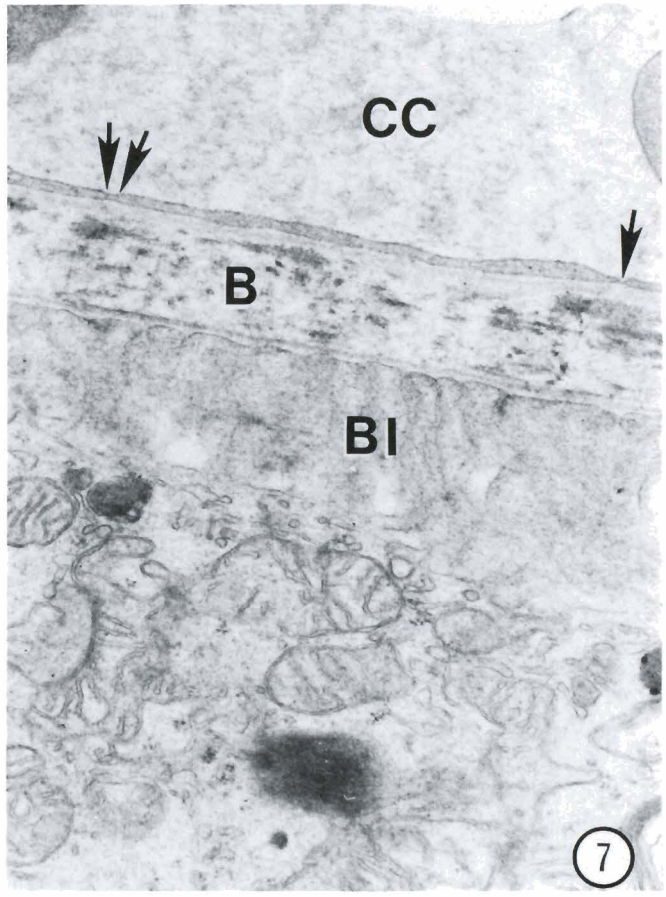
CC
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7

Fig. 4. Electron micrograph to indicate the poorly fenestrated choriocapillaris (CC) and the pentalaminate Bruch's membrane (B). A phagosome (Ph) and a lateral cell junction (J) are also indicated. x 10,300

Fig. 5. Electron micrograph to illustrate several myeloid bodies (My) some of which have ribosomes on their outer surface. RPE apical processes (AP) are indicated as are small profiles of rough endoplasmic reticulum (RER). x 13,800

Fig. 6. Electron micrograph to indicate a ring mitochondrion (Mi), numerous basal infoldings (BI), an RPE nucleus (N) and Bruch's membrane (B). x 14,400

Fig. 7. Electron micrograph to illustrate fenestrations with a single diaphragm (arrow) and others with a double diaphragm (double arrow). The choriocapillaris (CC). Bruch's membrane (B) and tangentially sectioned basal infoldings (BI) are also labelled. x 26,000

Braekevelt, 1980, 1982, 1984, 1986, 1988, 1990b).

As in all vertebrate species investigated to date, the RPE of the great horned owl is composed of a single layer of cells with extensive basal infoldings and numerous apical processes. The presence of highly infolded basal membranes and plentiful mitochondria is felt to be indicative of a heavy involvement in transport and this function is well established for the retinal epithelium (Steinberg and Miller, 1973). In this species as in other birds studied these basal infoldings are deeper than is normally reported for mammals and may be necessary to compensate for the less fenestrated choriocapillary endothelium noted in birds (Braekevelt, 1989, 1990b, 1992).

The numerous apical processes of the RPE cells are important in the structural support of the elongated photoreceptor outer segments (Bernstein, 1961) as well as their proper orientation to incoming light (Enoch, 1979). These apical processes are also involved in the internal adhesion required within the neural retina for it is between the RPE and the photoreceptors that a retinal detachment can occur (Zinn and Benjamin-Henkind, 1979). A fourth important function of these apical processes is in the phagocytosis of shed outer segment discs so as not to interfere with the transport of materials between the RPE and the photoreceptors (Bok and Young, 1979). In some mammalian species, two different types of apical process are reported associated with either rods or cones (Steinberg and Wood, 1974; Braekevelt, 1982, 1990a). In birds however only one type of microvillar process is noted and it appears to contact both rods and cones.

The cell junctions at the lateral cell borders of the retinal epithelial cells are a constant feature in all vertebrate species. They constitute an effective barrier to intercellular movement of materials and hence form part of the blood-ocular barrier (Zinn and Benjamin-Henkind, 1979). As noted for non-mammalian species, these cell junctions in the great horned owl are located in the mid to basal region of the epithelial cells (Kuwabara, 1979; Braekevelt, 1984, 1989).

As previously noted in most other species, smooth endoplasmic reticulum is abundant within RPE cells while rough endoplasmic reticulum is not (Nguyen-Legros, 1978; Kuwabara, 1979; Braekevelt, 1983, 1986, 1988, 1990a). The abundance of SER is due to the heavy involvement of this epithelium in the storage, transport and esterification of the lipid precursors of the visual pigments (Zinn and Benjamin-Henkind, 1979). The lipid droplets normally found within these cells are also presumably associated with this function. The small amounts of RER present would indicate that little protein is being produced for export by these cells in the adult condition. The abundance of polysomes on the other hand is felt to reflect an ongoing need of protein for internal requirements by these cells (Alberts et al., 1989). A large vesicular nucleus coupled with an abundance of cell organelles are all indicative of metabolically very active cells.

The wealth of mitochondria within RPE cells has been noted in most other species but the ring-shaped mitochondria noted in the great horned owl appear to be unique to the avian retina (Lauber, 1983a; Braekevelt, 1984, 1989, 1990b, 1992). Lauber (1983a,b) has shown that this shape effectively doubles the surface area of the mitochondrion. The numerous pleomorphic mitochondria noted in this species may also be an attempt to increase surface area although why the great horned owl possesses such unique mitochondria is unknown. Lauber (1983a,b) has also shown a variation in the number of ring-shaped mitochondria associated with the photoperiod with a peak in the early dark period. This may explain their relative scarcity in the great horned owl as only a light-adapted specimen was studied. In like manner the phagosomes of outer segment material noted within the RPE cells of this light-adapted great horned owl are presumably the remains of the burst of rod outer segment shedding which is known to occur soon after the onset of light (Young, 1978; Young and Bock, 1979).

Myeloid bodies which normally present as a stack of smooth membranes in continuity with the SER are a common feature within the RPE cells of a variety of non-mammalian species (Kuwabara, 1979; Braekevelt, 1982, 1984, 1988, 1990b). While they have been implicated as sites of storage of lipids prior to esterification (Yorke and Dickson, 1984, 1985) and as the organelle that triggers photomechanical movements (Porter and Yamada, 1960) their function remains uncertain. Only in avian species however have ribosomes been noted on the surface of myeloid bodies and this may indicate another or secondary function for this organelle (Meyer et al., 1973; Braekevelt, 1989, 1990b, 1992). Also the number and size of myeloid

bodies have been noted to change dependent upon the photoperiod in a variety of species but as only a light-adapted specimen was examined in this study it is uncertain as to whether the myeloid bodies of avian species undergo such changes.

The small melanosomes of the retinal epithelial cells of the great horned owl are felt to undergo retinomotor movements in response to environmental lighting. Judging from their almost exclusive location within the apical processes of the RPE in light-adaptation where they would shield rod photoreceptor outer segments from excess light it is presumed that they have undergone photomechanical movements. While Meyer (1977) states that photomechanical movement of pigment within the RPE cells of all birds is rapid and extensive, examination of a dark-adapted great horned owl would be required to confirm this.

Bruch's membrane or complexus basalis in mammalian species is invariably a pentalaminate structure with the five layers as described in the results portion of this report being quite obvious (Nakaizumi, 1984; Braekevelt, 1986, 1988, 1990a). Teleosts characteristically display a trilaminate Bruch's membrane with the central elastic layer (lamina densa) being absent (Braekevelt, 1982, 1985). In avian species the central lamina densa may occasionally be poorly represented but is always shifted towards the choriocapillary side of Bruch's membrane (Braekevelt, 1984, 1989, 1990b). The significante (if any) of these variations in the structure of the complexus basalis is obscure.

The choriocapillaris in all vertebrate species is composed of a single layer of large-caliber anastomosing capillaries (Rodieck, 1973; Kuwabara, 1979). The endothelium facing Bruch's membrane is typically very thin and in mammals highly fenestrated indicative of the movement of large volumes of material across these capillaries to the RPE (Berstein and Hollenberg, 1965). Teleost species normally show only a minimally fenestrated choriocapillaris and this is felt to be due to the presence of a choroid gland which is responsible for the maintenance of a high oxygen pressure due to its counter current arrangement (Wittenberg and Wittenberg, 1974). Avian species including the great horned owl also normally show a reduction in the number of fenestrations in the choriocapillary endothelium which in this case may be due to the presence of a pecten oculi (Braekevelt, 1989, 1990). Finally a number of the fenestrations that are present in the choriocapillary endothelium of birds show a double-layered diaphragm rather than the more conventional single-layered diaphragm (Braekevelt, 1989, 1990b, 1992). Such double diaphragm fenestrations are also seen in the great horned owl but their significance is unknown.

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