Fine structure of the retinal pigment epithelium of the barred owl (*Strix varia*)

C.R. Braekevelt[1], B.J. Smith[2] and S.A. Smith[2]
[1]Department of Anatomy, The University of Manitoba, Winnipeg, Manitoba, Canada and [2]Department of Biomedical
Sciences and Pathobiology, Virginia-Maryland Regional College of Veterinary Medicine, Blacksburg, Virgnia, United States

Summary. The retinal pigment epithelium (RPE) as well as the choriocapillaris and Bruch's membrane (complexus basalis) have been studied by light and electron microscopy in the barred owl (*Strix varia*). The RPE consists of a single layer of cuboidal cells joined laterally by a series of tight junctions that forms part of the blood-ocular barrier. Basally (sclerally) the retinal epithelial cells display numerous deep infoldings while apically (vitreally) microvillar processes interdigitate with the photoreceptor outer segments. Internally the RPE cells show a large vesicular nucleus, plentiful smooth endoplasmic reticulum (SER) and polysomes but very little rough endoplasmic reticulum (RER). Numerous pleomorphic (including ring-shaped) mitochondria are basally located. In the light-adapted state the small melanosomes are almost exclusively located within the apical process indicating that retinomotor movements probably occur. Phagosomes and lysosome-like bodies are present as are myeloid bodies which may show ribosomes on their outer surface. Bruch's membrane is typical of avian species in that it is pentalaminate and the central lamina densa is displaced towards the choroid. The choriocapillaris endothelium is thin but only minimally fenestrated facing Bruch's membrane. Most fenestrations present show a single-layered diaphragm while others display a double-layered diaphragm as noted in other avian species.

Key words: Retinal pigment epithelium (RPE), Fine structure, Aves, Barred owl, *Strix varia*

Introduction

The retinal pigment epithelium (RPE) is the outermost (scleral) layer of the neural retina and in association with Bruch's membrane (complexus basalis)

and the choriocapillaris is involved in several processes crucial to the proper functioning of the retina.

Amongst the best understood roles of the RPE are: 1) phagocytosis and removal of shed photoreceptor outer segment debris (Young, 1978; Bok and Young, 1979); 2) the storage and modification of vitamin A precursors of the visual pigments (Young and Bok, 1970); 3) selective transport of materials to the photoreceptors (Kroll and Machemer, 1968; Steinberg and Miller, 1973); 4) internal adhesion within the neurosensory retina to prevent retinal detachment (Zinn and Benjamin-Henkind, 1979); 5) architectural stabilization and proper orientation of the photoreceptor outer segments; and 6) the normal presence of pigment that enhances visual acuity by absorbing reflected light (Moyer, 1969).

As a result of these several important functions, this region of the vertebrate retina has been investigated in a variety of animals using a variety of techniques. Morphological studies in particular have shown a remarkable similarity throughout vertebrate species, though generic differences are usually present (Nguyen-Legros, 1978; Kuwabara, 1979; Braekevelt, 1980, 1982, 1984, 1986, 1988, 1990, 1992, 1994).

While numerous reports of the fine structure of the retinal epithelial region are available, relatively few deal with avian species (Nishida, 1964; Matsusaka, 1966; Meyer et al., 1973; Dieterich, 1974; Braekevelt, 1984, 1989, 1992; Braekevelt and Thorlakson, 1993). As part of an ongoing comparative study of the RPE region in general and in avian species in particular, this report describes the fine structure of the RPE, choriocapillaris and Bruch's membrane in the barred owl (*Strix varia*).

Materials and methods

For this study the eyes from two adult and one juvenile light-adapted barred owls (*Strix varia*) were examined by light and electron microscopy. With the specimens under deep anesthesia the eyes were quickly enucleated. The eyeballs were slit 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, washed in 5% sucrose in 0.1M Sorensen's buffer (pH 7.3) and cut into pieces less than 1 mm². This tissue was then postfixed for 2 h in 1% OsO_4 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 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 uranyl acetate and lead citrate and examined and photographed in a Philips EM201 transmission electron microscope.

Results

The RPE in the barred owl (*Strix varia*) consists of a single layer of cuboidal cells which average about 12 µm in height and width (Fig. 1). Basally (sclerally) the retinal epithelial cells display numerous infoldings which penetrate to a depth of about 1.5 µm (Fig. 2). Apically (vitreally) the RPE cells show numerous finger-like processes which enclose photoreceptor outer segments (Figs. 1, 3). Laterally the cell borders of the epithelial cells are relatively smooth and are joined by a series of basally-located tight junctions (Figs. 1, 5).

Internally the retinal epithelial cells display a single vesicular nucleus located in the mid-region of the cell in the light-adapted condition (Figs. 1-3). Mitochondria are numerous and for the most part basally located (Figs. 2, 4). These mitochondria are extremely pleomorphic and ring-shaped mitochondria are noted as are reported in other avian species (Fig. 5).

As in all other vertebrate species described to date, 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. 1-3). Rough endoplasmic reticulum (RER) is not abundant but is noted as isolated profiles (Fig. 4). Polysomes are however widespread and abundant (Figs. 2-4).

Phagosomes of outer segment material are surprisingly large and numerous in these light-adapted specimens and are seen in various stages of degradation (Figs. 1-3). Lysosome-like bodies were often noted and usually in a heterogeneous condition indicating a secondary lysosome (Figs. 2, 3).

Myeloid bodies which are organelles usually noted within the RPE cells of non-mammalian vertebrates are also present within the retinal epithelial cells of the barred owl (Figs. 1, 2, 4). Normally they are composed of compact arrays of membranes which are continuous with the SER (Fig. 4) but as has been noted in other avian species the myeloid bodies in this owl often display ribosomes on their outer surface which may indicate a continuity with the RER membranes as well (Fig. 2).

The melanosomes within the RPE cells are in the light-adapted condition mostly located within the apical processes with some melanosomes also located within the apical region of the epithelial cell body (Figs. 1, 3). Judging by their location in the light-adapted condition, it is felt that the melanosomes in this species undergo retinomotor movements. Melanosomes within the apical processes supposedly serve to surround and isolate the photoreceptor outer segments from one another (Fig. 3). The melanosomes in this species are small (about 0.5 µm) and round to oval in shape (Figs. 1, 3). The retinal epithelial cells in the barred owl are not heavily pigmented and it is doubtful that the melanosomes when dispersed within the apical processes are indeed effective in isolating individual photoreceptors (Fig. 3).

Bruch's membrane or complexus basalis in this avian species is a pentalaminate structure and consists of: 1) the basal lamina of the RPE; 2) the basal lamina of the choriocapillaris enclosing; 3) a discontinuous elastic layer (lamina densa) which separates; 4) an outer; and 5) inner collagenous layer (Figs. 2-6). Often as is noted in other avian species the lamina densa is located much closer to the choriocapillaris and hence the two collagenous layers are often of unequal thickness (Fig. 5). Bruch's membrane averages about 1.0 µm in thickness in this owl (Figs. 2, 5, 6).

The choriocapillaris forms a single layer of capillaries immediately adjacent to the choroidal aspect of Bruch's membrane (Figs. 4-6). The endothelium facing the complexus basalis is normally quite thin but only minimally fenestrated (Figs. 4, 6). While the majority of these fenestrations display a single-layered diaphragm, as in other avian species some of them show a double-layered diaphragm (Figs. 4, 6).

Discussion

The (RPE) of the barred owl (*Strix varia*) is essentially similar to that described for other vertebrates with modifications or refinements that seem to be specific to avian species (Rodieck, 1973; Nguyen-Legros, 1978; Kuwabara, 1979; Braekevelt, 1980, 1982, 1984, 1988, 1990, 1992, 1994).

As in all vertebrate species investigated to date, the RPE of the barred owl is composed of a single layer of cells with extensive basal infoldings and numerous apical processes which interdigitate with photoreceptor outer segments. The occurrence of highly infolded basal

Fig. 1. Low power electron micrograph of the retinal epithelium. For orientation the choroid (Ch) and rod outer segments (ROS) are indicated. An RPE nucleus (N), phagosomes (Ph) and apically located melanosomes (M) are also indicated. x 5,600

Fig. 2. Electron micrograph to illustrate a cell junction (J), myeloid bodies (My) and plentiful basal infoldings (BI). The choriocapillaris (CC) is also indicated. x 12,800
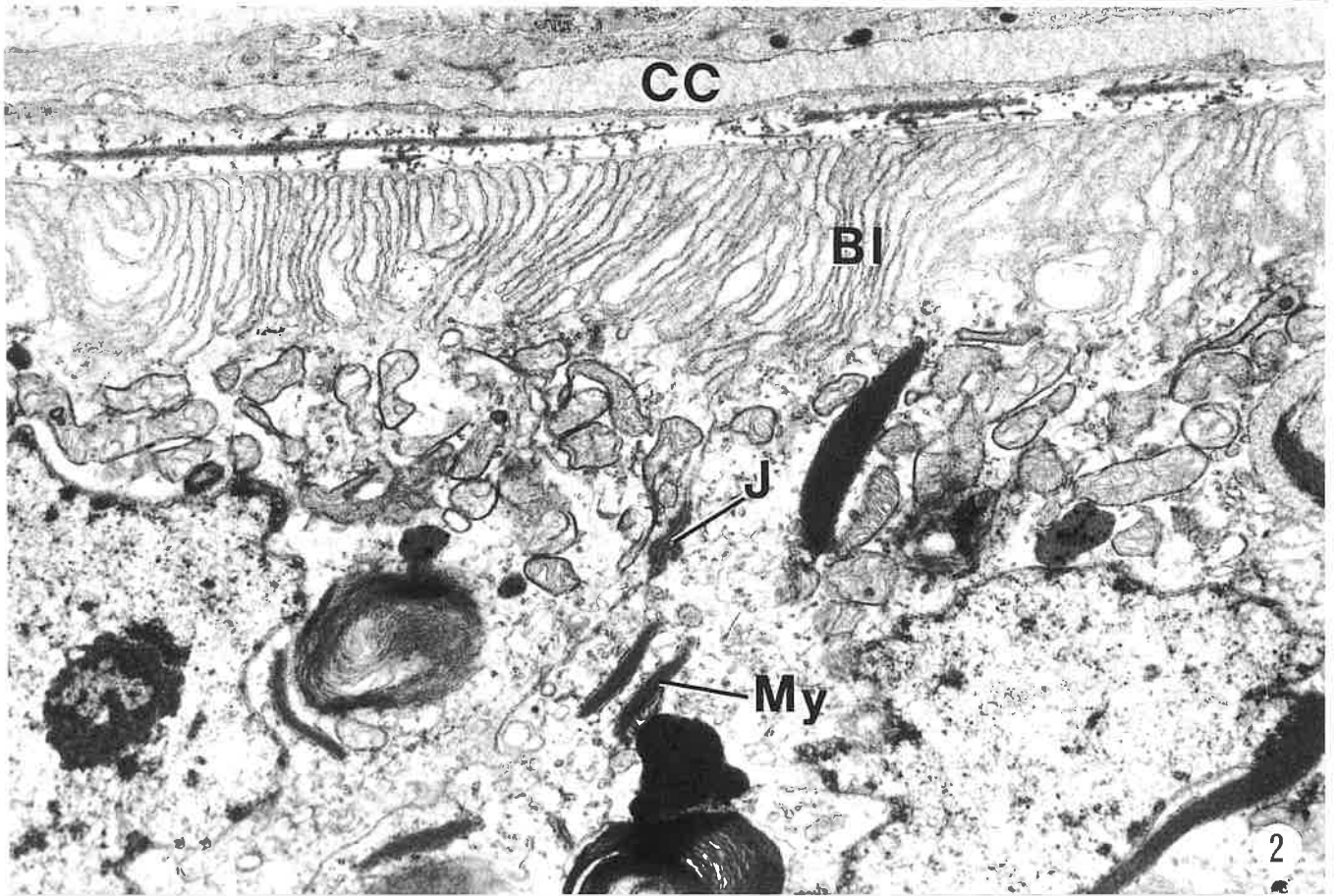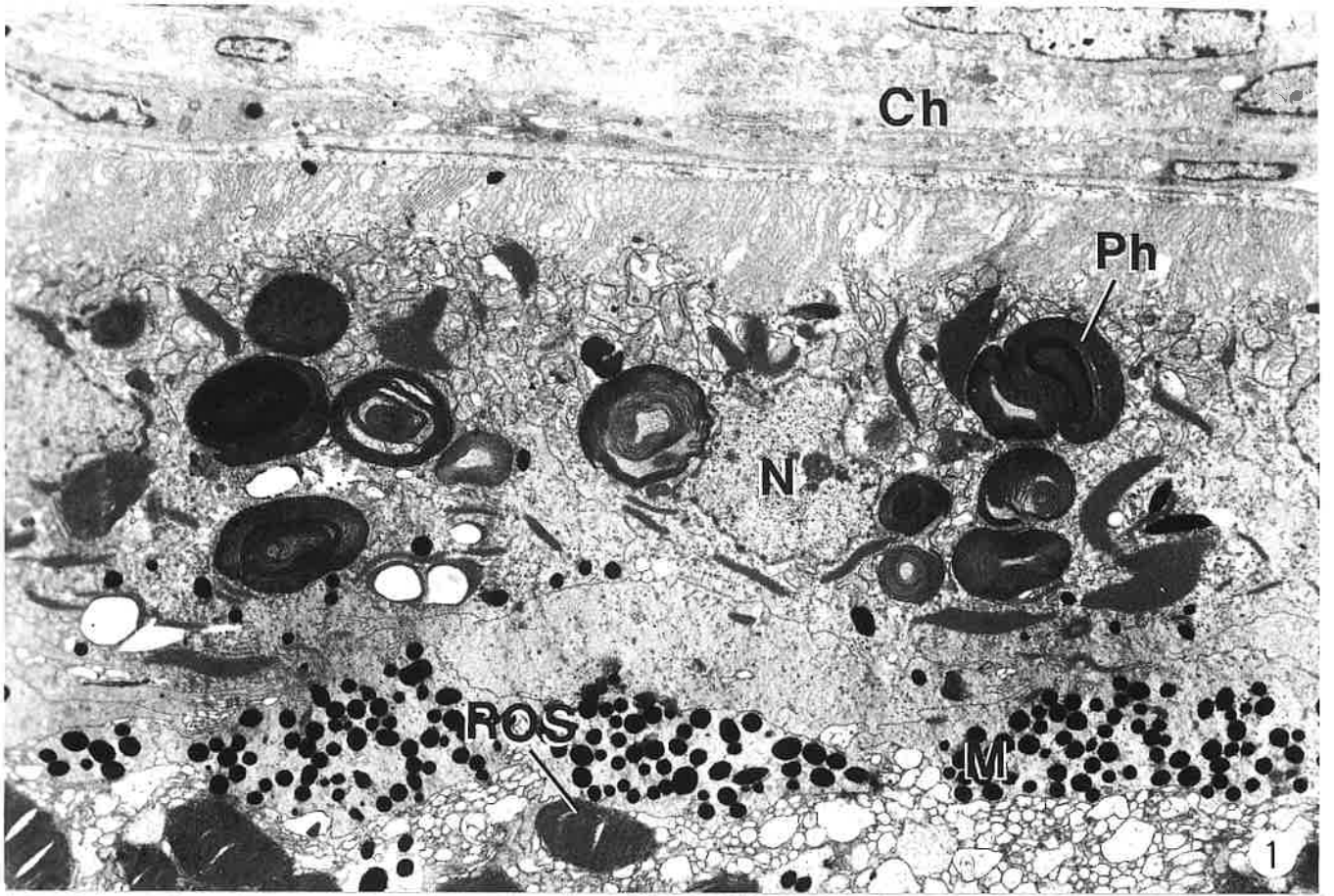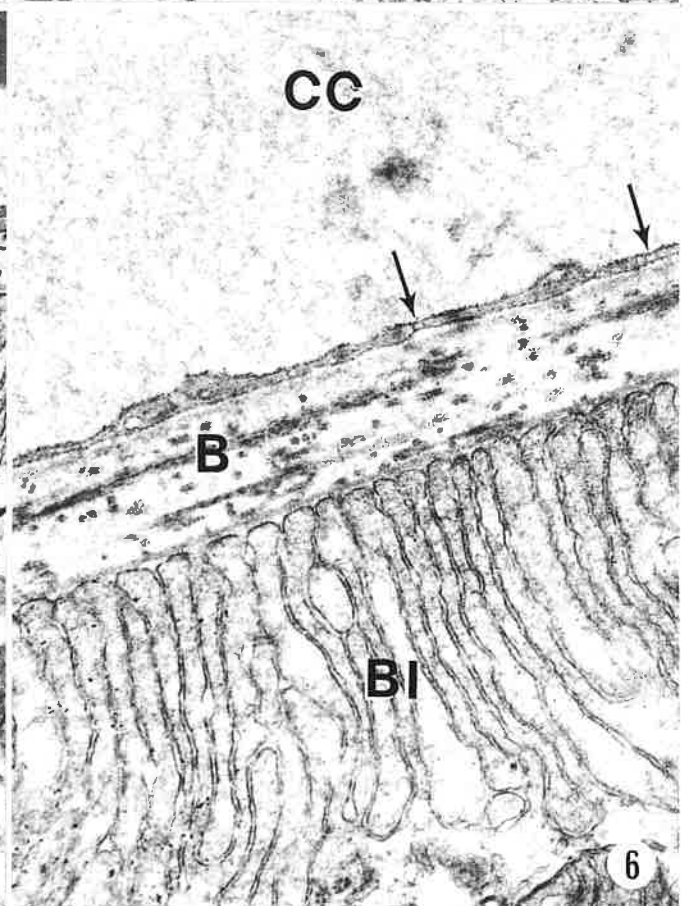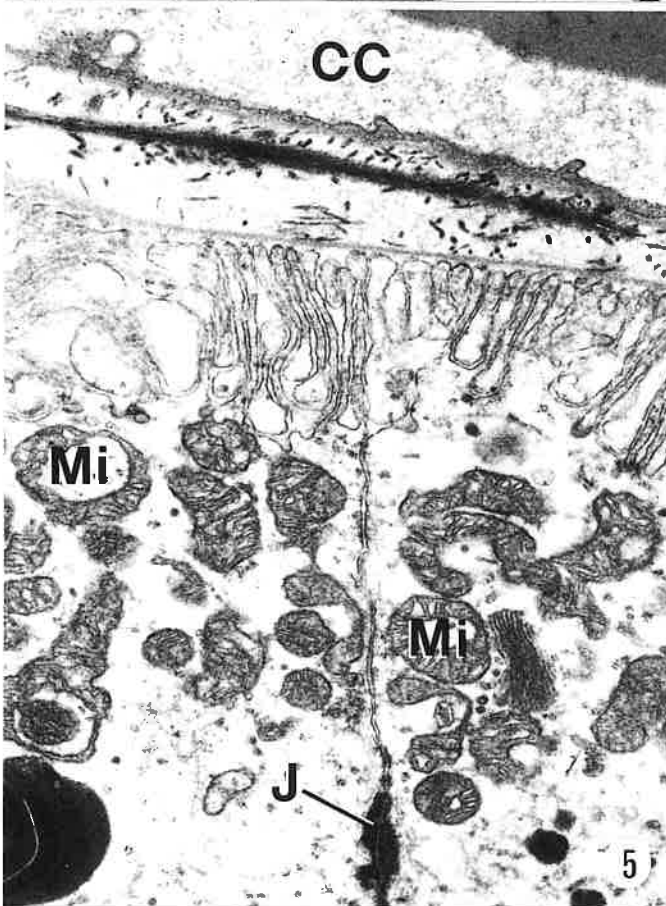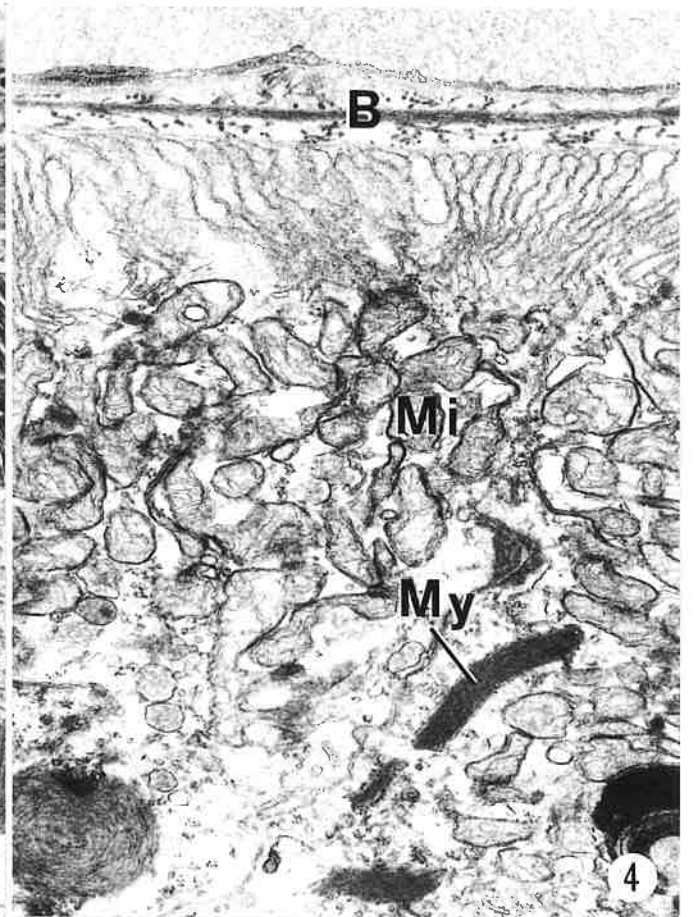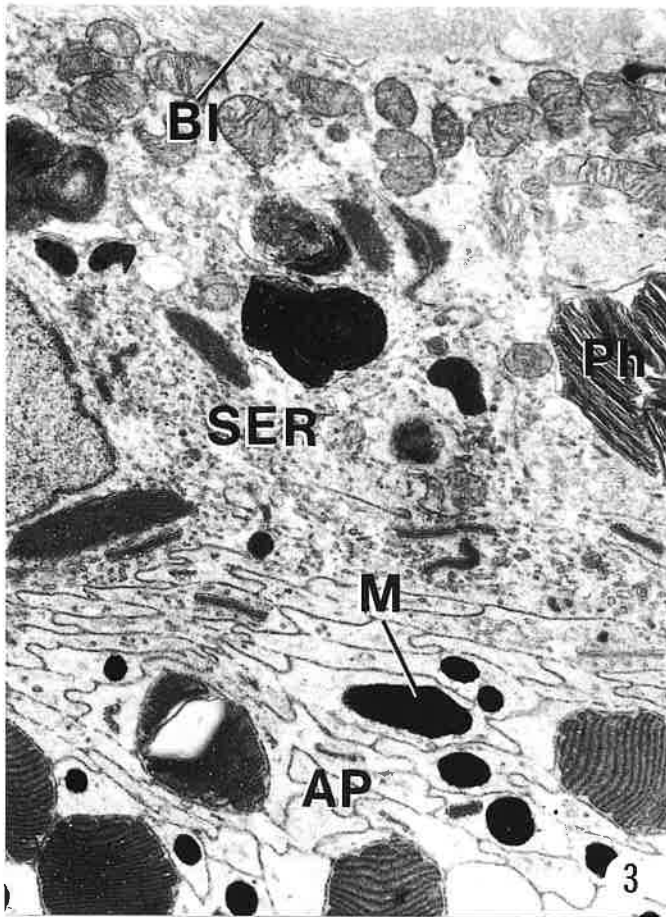
Fig. 3. Electron micrograph to illustrate plentiful smooth endoplasmic reticulum (SER), melanosomes (M), phagosomes (Ph), basal infoldings (BI) and apical processes (AP) of the retinal epithelial cells. x 10,600

Fig. 4. Electron micrograph to illustrate the extremely pleomorphic mitochondria (Mi) found in this species. A myeloid body (My) and Bruch's membrane (B) is also indicated. x 15,800

Fig. 5. Electron micrograph to illustrate ring mitochondria (Mi) and a cell junction (J). The choriocapillaris (CC) is also indicated. x 15,800

Fig. 6. Electron micrograph to indicate fenestrations with a double diaphragm (arrows) in the choriocapillaris (CC). Bruch's membrane (B) and the deep basal infoldings (BI) are also labelled. x 22,300

membranes in epithelial cells (coupled with the presence of 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 the basal infoldings are deeper than is normally reported for mammals and may therefore indicate an increased transport role for this epithelium in avian species (Braekevelt, 1989, 1990, 1992; Braekevelt and Thorlakson, 1993).

The numerous apical processes of the RPE cells are felt to be important for a number of reasons. They are necessary for the structural support of the elongated photoreceptor outer segments (Bernstein, 1961) as well as in their proper orientation to the 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). Another important function of these apical processes is in the phagocytosis and removal of shed outer segment material so as not to interfere with the transport of materials between the RPE and the photoreceptors (Bok and Young, 1979).

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

As has been reported in most species, SER is abundant within RPE cells while RER is not (Nguyen-Legros, 1978; Kuwabara, 1979; Braekevelt, 1983, 1986, 1988, 1990, 1992, 1994). This abundance of SER is probably due to the heavy involvement of this epithelium in the production, storage, transport and esterification of the lipid precursors of the visual pigments (Zinn and Benjamin-Henkind, 1979). The smaller 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 the ongoing need for protein for the internal requirements of these cells (Alberts et al., 1989). The large vesicular nucleus coupled with an abundance of cell organelles are all indicative of metabolically very active cells.

The wealth of basally-located mitochondria within RPE cells has been noted in most other species but the ring-shaped mitochondria noted in the barred owl appear to be unique to avian RPE cells (Lauber, 1983a; Braekevelt, 1984, 1989, 1990; Braekevelt and Thorlakson, 1993). Lauber (1983a,b) has shown that this ring shape effectively doubles the surface area of the mitochondrion. The numerous pleomorphic mitochondria noted in this species has also been reported in the great horned owl (Braekevelt and Thorlakson, 1993) and may represent a mitochondrial morphology unique to this order of birds. 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 barred owl as only light-adapted specimens were available for study. Perhaps the pleomorphic shapes of the mitochondria will also vary with the photoperiod. In like manner the phagosomes of outer segment material noted within the RPE cells of the light-adapted barred owl are presumably the remains of the burst of rod outer segment shedding which is known to occur soon after the onset of light and their numbers will vary with the photoperiod (Young, 1978; Young and Bok, 1979).

The small melanosomes of the retinal epithelial cells of the barred owl are felt to undergo retinomotor movements in response to environmental lighting judging from their almost exclusive location within the apical processes of these epithelial cells in light-adaptation. While this agrees with Meyer (1977) who states that photomechanical movement of pigment within the RPE cells of birds is rapid and extensive, examination of the RPE of a fully dark-adapted barred owl would be required to confirm the movement of pigment in this species. The light pigmentation of the retinal epithelial cells in the barred owl present relatively few melanosomes available for dispersal into the apical processes. Thus, it is doubtful that the melanosomes when dispersed within the apical processes are indeed effective in isolating individual photoreceptors. Such a masking function for the RPE melanosomes may not be as crucial in a nocturnal animal as in a diurnal one.

Myeloid bodies which normally present as a stack of smooth membranes are a common feature within the retinal epithelial cells of a variety of non-mammalian species (Kuwabara, 1979; Braekevelt, 1982, 1984, 1988; Braekevelt and Thorlakson, 1993). While they have been implicated as sites of storage prior to esterification (Yorke and Dickson, 1984, 1985) and as the organelles

that trigger photomechanical movements (Porter and Yamada, 1960; Braekevelt, 1982) their function remains uncertain. Only in avian species however have ribosomes been noted on the surface of myeloid bodies which may indicate another or secondary function for these organelles (Meyer et al., 1973; Braekevelt, 1989, 1990). The number and size of myeloid bodies have also been noted to change dependent upon the circadian cycle in a variety of species. Since only light-adapted specimens were examined in this study, it is uncertain as to whether the myeloid bodies of avian species will undergo such changes (Braekevelt, 1982, 1990).

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, 1964; Braekevelt, 1986, 1988, 1990). Teleost fish characteristically display a trilaminate Bruch's membrane with the central elastic lamina layer (lamina densa) being absent (Braekevelt, 1982, 1985). In avian species, Bruch's membrane is pentalaminate, and while the central lamina densa may be poorly represented, it is always shifted towards the choriocapillary side of Bruch's membrane (Braekevelt, 1984, 1989, 1990). The significance (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 highly fenestrated indicative of the movement of large quantities of material across these capillaries to the RPE (Bernstein and Hollenberg, 1965). Teleost fish normally show only a minimally fenestrated chorio-capillaris which is felt to be due to the presence of a choroid gland important in the maintenance of a high oxygen pressure due to its counter current arrangement (Wittenberg and Wittenberg, 1974). Avian species including the barred 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, 1990, 1992). Such double diaphragm fenestrations are also seen in the barred owl but again their significance is unknown.

References

Alberts B., Bray D., Lewis J., Raff M., Roberts K. and Watson J.D. (1989). Molecular biology of the cell. Garland. New York. Second edition.

Bernstein M.H. (1961). Functional architecture of the retinal epithelium. In: The structure of the eye. G.K. Smelser (ed). Academic Press. New York. pp 139-150.

Bernstein M.H. and Hollenberg M.J. (1965). Movement of electron-opaque tracers through the capillaries of the retina. In: The structure of the eye. II. Rohen J.J. (ed). Schgattauer-Verlag. Stuttgart. pp 129-138.

Braekevelt C.R. (1980). Fine structure of the retinal epithelium and tapetum lucidum in the giant danio (*Danio malabaricus*) (Teleost). Anat. Embryol. 158, 317-328.

Braekevelt C.R. (1982). Fine structure of the retinal epithelium, Bruch's membrane (*complexus basalis*) and choriocapillaris in the domestic ferret. Acta Anat. 113, 117-127.

Braekevelt C.R. (1983). Fine structure of the choriocapillaris, Bruch's membrane and retinal epithelium in the sheep. Anat. Embryol. 166, 415-425.

Braekevelt C.R. (1984). Retinal pigment epithelium fine structure in the nighthawk *Chordeiles minor*. Ophthalmologica 188, 222-231.

Braekevelt C.R. (1985). Fine structure of the retinal pigment epithelial region of the archerfish (*Toxotes jaculatrix*). Opthalmic. Res. 17, 221-229.

Braekevelt C.R. (1986). Fine structure of the choriocapillaris, Bruch's membrane and retinal epithelium of the cow. Anat. Histol. Embryol. 15, 205-214.

Braekevelt C.R. (1988). Retinal epithelial fine structure in the vervet monkey (*Cercopithecus aethiops*). Histol. Histopathol. 3, 33-38.

Braekevelt C.R. (1989). Fine structure of the retinal epithelium, Bruch's membrane and choriocapillaris of the pigeon (*Columba livia*). Can. J. Zool. 67, 795-800.

Braekevelt C.R. (1990). Fine structure of the retinal pigment epithelium of the mallard duck (*Anas platyrhynchos*). Histol. Histopathol. 5, 133-138.

Braekevelt C.R. (1992). Retinal pigment epithelial fine structure in the red-tailed hawk (*Buteo jamaicensis*). Anat. Histol. Embryol 21, 48-56.

Braekevelt C.R. (1994). Retinal pigment epithelium fine structure in the short-tailed stingray (*Heterodontus phillipi*). Histol. Histopathol. 9, 501-506.

Braekevelt C.R. and Thorlakson I.J. (1993). Fine structure of the retinal pigment epithelium of the great horned owl (*Bubo virginianus*). Histol. Histopathol. 8, 17-23.

Bok D. and Young R.W. (1979). Phagocytic properties of the retinal pigment epithelium. In: The retinal pigment epithelium. Zinn K.M. and Marmor M.F. (eds). Harvard Univ Press. Cambridge. pp 148-174.

Dieterich C.E. (1975). On the retinal pigment epithelium of the barn owl (*Tyto alba*). Graefe's Arch. Opthalmol. 196, 247-254.

Enoch J.M. (1979). Vertebrate receptor optics and orientation. Doc. Opthalmol. 48, 373-388.

Kroll A.J. and Machemer R. (1968). Experimental retinal detachment in the owl monkey III. Electron microscopy of retina and pigment epithelium. Am. J. Opthalmol. 66, 410-427.

Kuwabara T. (1979). Species differences in the retinal pigment epithelium. In: The retinal pigment epithelium. Zinn K.M. and

Marmon M.F. (eds). Harvard Univ. Press. Cambridge. pp 58-82.

Lauber J.K. (1983a). Retinal pigment epithelium: ring mitochondria and lesions induced by continuous light. Curr. Eye Res. 2, 855-862.

Lauber J.K. (1983b). Diurnal mitochondrial changes in avian retinal pigment epithelium: a search for correlation with thyroid state. Curr. Eye Res. 2, 863-868.

Matsusaka T. (1966). Electron microscopic observations on the basal zone of the pigment epithelial cell of the chick embryo. Folia Opthalmol. Jpn. 17, 320-322.

Meyer D.B. (1977). The avian eye and its adaptations. In: Handbook of sensory physiology. Vol VIII/5. The visual system in vertebrates. Crescitelli F. (ed). Springer-Verlag. Berlin. pp 549-612.

Meyer D.B., Hazlett L.D. and Susan S.R. (1973). The fine structure of the retina in the Japanese quail (*Coturnix coturnix japonica*). 1. Pigment epithelium and its vascular barrier. Tissue Cell 5, 489-500.

Moyer F.H. (1969). Development, structure and function of the retinal pigment epithelium. In: The retina. Straatsma B.R. et al. (ed). Univ. of Calif. Press. Los Angeles. pp 1-30.

Nakaizumi Y. (1964). The ultrastructure of Bruch's membrane. I. Human, monkey, rabbit, guinea pig and rat eyes. Arch. Opthalmol. 72, 380-387.

Nguyen-Legros J. (1978). Fine structure of the pigment epithelium in the vertebrate retina. Int. Rev. Cytol. Suppl. 7, 287-328.

Nishida S. (1964). Electron microscopic study of chicken retina. The ultrastructure of the retinal pigment epithelium of light adapted chicken. Acta. Soc. Ophthalmol. Jpn. 68, 1431-1433.

Porter K.R. and Yamada E. (1960). Studies on the endoplasmic reticulum V. Its form and differentiation in pigment epithelial cells of the frog retina. J. Biophys. Biochem. Cytol. 8, 181-205.

Rodieck R.W. (1973). The vertebrate retina. Principles of structure and function. W.H. Freeman. San Francisco.

Steinberg R.H. and Miller S. (1973). Aspects of electrolyte transport in frog pigment epithelium. Exp. Eye Res. 16, 365-372.

Wittenberg J.B. and Wittenberg B.A. (1974). The choroid rete mirabile of the fish eye. I. Oxygen secretion and structure: comparison with the swim bladder rete mirabile. Biol. Bull. 146, 116-136.

Yorke M.A. and Dickson D.H. (1984). Diurnal variations in myeloid bodies of the newt retinal pigment epithelium. Cell Tissue Res. 235, 177-186.

Yorke M.A. and Dickson D.H. (1985). A cytochemical study of myeloid bodies in the retinal pigment epithelium of the newt *Notophthalmus viridescens*. 240, 641-648.

Young R.W. (1978). Visual cells, daily rhythms and vision research. Vision Res. 18, 573-578.

Young R.W. and Bok D. (1970). Autoradiographic studies on the metabolism of the retinal pigment epithelium. Invest. Opthalmol. 9, 524-536.

Young R.W. and Bok D. (1979). Metabolism of the retinal pigment epithelium. In: The retinal pigment epithelium. Zinn K.M. and Marmor M.F. (eds). Harvard Univ. Press. Cambridge. pp 103-123.

Zinn K.M. and Benjamin-Henkind J.V. (1979). Anatomy of the human retinal pigment epithelium. In: The retinal pigment epithelium. Zinn K.M. and Marmor M.F. (eds). Harvard Univ. Press. Cambridge. pp 3-31.