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Fine structure of the retinal pigment epithelium of the mallard duck *(Anas platyrhynchos)*

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Summary. As part of a comparative morphological study, the fine structure of the retinal pigment epithelium (RPE), the choriocapillaris and Bruch's membrane (complexus basalis) has been investigated by light and electron microscopy in the mallard *(Anas platyrhynchos)*. In this species the RPE consists of a single layer of cuboidal cells which display numerous very deep basal (scleral) infoldings and extensive apical (vitreal) processes which enclose photoreceptor outer segments. The RPE cells are joined laterally by prominent basally-located tight junctions. Internally smooth endoplasmic reticulum is the most abundant cell organelle with only small amounts of rough endoplasmic reticulum present. Polysomes are abundant as are basally-located mitochondria which often displayed a ring-shaped profile. The cell nucleus is large and vesicular. Melanosomes are plentiful only within the apical processes of the RPE cells in the light-adapted state. Myeloid bodies are large and numerous and very often have ribosomes on their outer surface. Bruch's membrane (complexus basalis) shows a pentalaminate structure but with only a poorly represented central elastic lamina. Profiles of the choriocapillaris are relatively small and the endothelium of these capillaries while extremely thin facing the retinal epithelium is but minimally fenestrated.

Key words: Retinal pigment epithelium, Bruch's membrane, Choriocapillaris, Electron microscopy, Mallard duck, *Anas platyrhynchos*

Introduction

The retinal epithelium (RPE) forms the outermost layer of the vertebrate retina and is intimately involved in several processes essential to the proper functioning of the photoreceptors and eventually to vision itself. Amongst the best understood roles of the RPE are: 1) the selective transport of materials to and from the photoreceptors (Kroll and Machemer, 1968; Steinberg and Miller, 1973) 2) the storage and modification of vitamin A precursors of the visual pigments (Young and Bok, 1970) 3) the architectural stabilization and efficient orientation of the photoreceptor outer segments (Bernstein, 1961; Enoch, 1974) 4) the internal adhesion of the neurosensory retina (Zinn and Benjamin-Henkind, 1979) and 5) the phagocytosis and lysosomal degradation of photoreceptor outer segment discs (Young, 1978; Bok and Young, 1979).

As a consequence of these vital functions, the RPE region of the inverted vertebrate retina has been investigated by a variety of workers employing a wide range of techniques designed to determine various parameters of this epithelial layer. From a morphological standpoint while this region is similar in all vertebrates, generic differences are usually present (Nguyen-Legros, 1978; Kuwabara, 1979; Braekevelt, 1980, 1983, 1985, 1986, 1988).

Although numerous reports of the fine structure of the RPE are available, with the possible exception of the chicken, this layer has not been extensively studied in the avian retina (Nishida, 1964; Matsusaka, 1966; Fite et al., 1985) with only a few reports on other species (Meyer et al., 1973; Dieterich, 1975; Braekevelt, 1984, 1989). Consequently as part of a comparative morphological study of this region in vertebrates, this study deals with the fine stucture of the RPE and closely related choriocapillaris and Bruch's membrane (complexus basalis) in the mallard duck *(Anas platyrhynchos)*.

Materials and methods

For this study the eyes from six healthy adult, light-adapted mallards *(Anas platyrhynchos)* of both sexes were examined by light and electron microscopy. With the

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birds under deep anesthesia, the eyeballs were enucleated, opened at the equator and 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 1mm^2. The tissue was then postfixed for 2h in 1% osmium tetroxide in the same phosphate buffer, dehydrated through graded ethanols to methanol and propylene oxide and finally embedded in Araldite.

Pieces of plastic-embedded tissue were reorientated 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) of selected areas 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

As in many other described vertebrates the retinal pigment epithelium (RPE) of the mallard duck *(Anas platyrhynchos)* consists of a single layer of cuboidal cells joined laterally by a series of tight junctions (Figs. 1, 3, 5). In this species the epithelial cells measure about 15-20 μm in width (Fig. 1) with the lateral cell junctions located near the basal region of the cells (Figs. 3, 5). Basally (sclerally) these cells show numerous deep and often branching microfolds. These folds are 1.0 to 1.5 μm in depth within each cell (Figs. 2, 3). Apically (vitreally) numerous processes extend from the epithelial cell body to enclose and more or less isolate the photoreceptor outer segments from one another (Fig. 1). Laterally the epithelial cell borders are relatively smooth (Figs. 3, 5).

Internally the RPE cells are uninucleated with the nucleus being large and vesicular and basally located in the light-adapted condition (Figs. 1, 2, 4). A single prominent nucleolus is often present (Fig. 4). Mitochondria are small and numerous and in the light-adapted specimens examined were predominantly basally located (Figs. 1, 2, 6). While most mitochondria appeared to be round to oval in shape (Figs. 3, 4) ring-shaped mitochondria were also noted (Fig. 7).

Smooth endoplasmic reticulum (SER) is the most abundant cell organelle and except for the basal infoldings is distributed throughout the entire epithelial cell cytoplasm (Figs. 3, 4, 6, 7). Rough endoplasmic reticulum (RER) while not abundant is also present usually as very short profiles (Figs. 3, 4, 7). Polysomes are however plentiful and widely dispersed (Figs. 2, 3, 5). Phagosomes of outer segment material while not abundant in these light-adapted specimes were also present in various stages of degradation (Figs. 4, 5, 7). Lysosome-like bodies are also a regular feature of these cells (Figs. 1, 3, 6). Lipid droplets are common and usually occur as a series of small, spherical bodies (0.8-1.0 μm) rather than a single large droplet (Figs. 1, 6).

Myeloid bodies are also a common feature of the RPE cells of the mallard. These appear as compact oblong or lentiform-shaped structures composed of tightly-packed membranes (Figs. 1, 3, 4, 7). These membranous bodies are often seen to be in continuity with the membranes of the SER (Figs. 4, 5). As has been reported in other avian species however, ribosomes are frequently noted on the outer surface of these myeloid bodies which may also indicate an association with the RER membranes (Figs. 4, 5, 7).

In the light-adapted condition, the melanosomes of the RPE cells of the mallard are almost entirely located within the apical processes of these cells, leaving the cell body essentially devoid of pigment granules (Figs. 1, 4, 5). These melanosomes are small and predominantly spindle-shaped and when located within the apical processes are arranged parallel to the long axes of these processes (Fig. 1).

Bruch's membrane or complexus basalis in this avian species is pentalaminate, consisting of: 1) the basal lamina of the retinal epithelial cells 2) the basal lamina of the choriocapillaris 3) a central discontinuous elastic lamina (lamina densa) separating 4) an inner and 5) an outer collagenous layer (Figs. 2, 4, 5). The central elastic layer (lamina densa) is very poorly represented in this species (Figs. 3, 6). Also the lamina densa, when present, is usually located much closer to the choriocapillary side of the complexus basalis producing two collagenous layers of unequal thickness (Figs. 2, 7). In various locations, cell processes are also noted within Bruch's membrane (Fig. 6).

The choriocapillaris forms a single layer of capillaries on the choroidal aspect of Bruch's membrane (Figs. 1, 2). The nuclear region of the endothelial cells is normally located at the periphery or on the choroidal aspect of

Fig. 1. Low power electron micrograph of the retinal pigment epithelial layer (RPE) in the light-adapted mallard. The choriocapillaris (CC), rod outer segments (ROS) and two cell junctions (J) are indicated. Melanosomes (M) are predominantly located in the apical processes of the RPE cells. × 8,600

Fig. 2. Electron micrograph of the basal region of the RPE to indicate the extensive and uniform basal infoldings (BI). The choriocapillaris (CC), Bruch's membrane (B) and an RPE cell nucleus (N) are also indicated. × 13,000

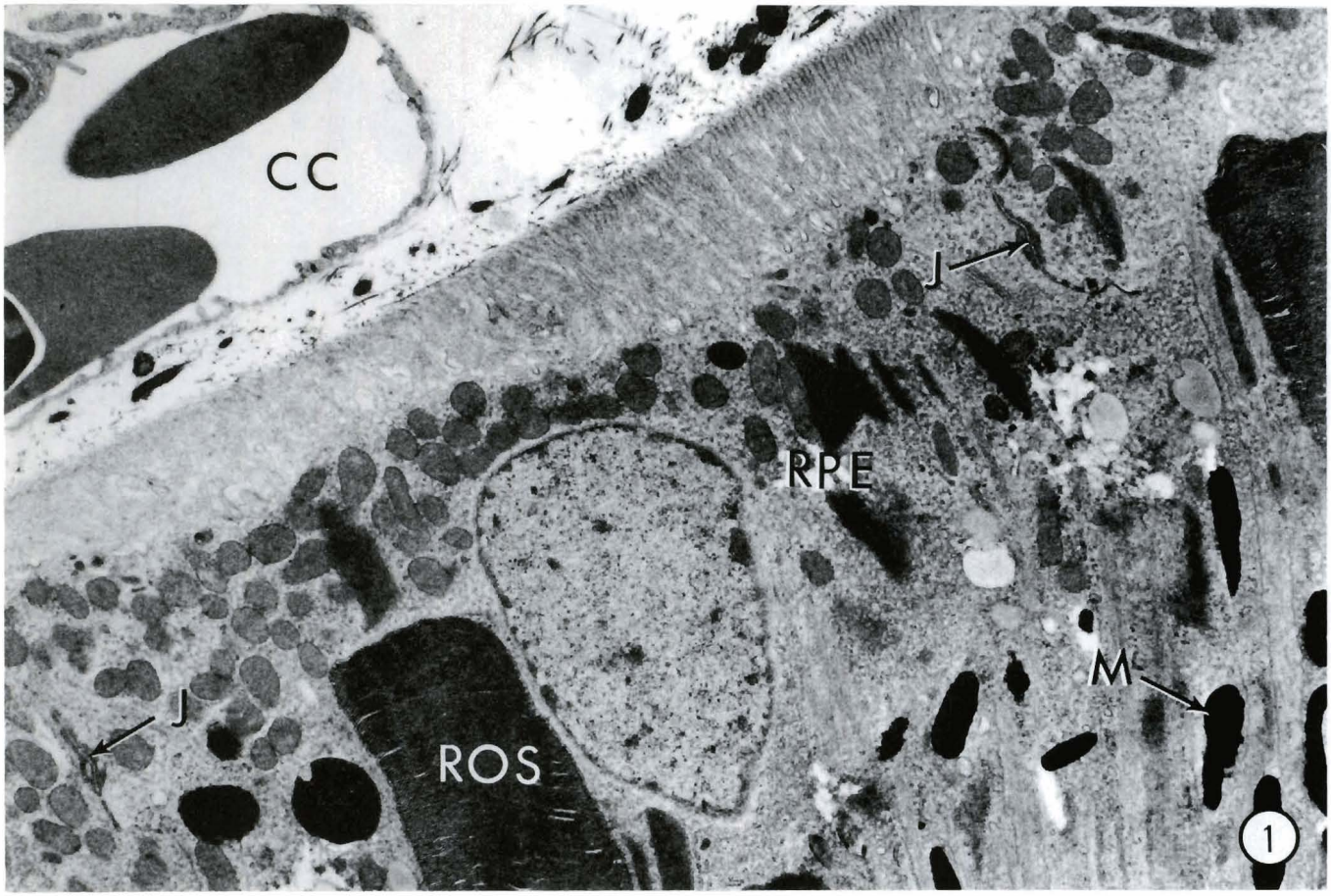
Fig. 3. Electron micrograph of the mallard RPE to indicate numerous basally-located mitochondria (Mi), myeloid bodies (My) and a cell junction (J). × 13,000

Fig. 4. Electron micrograph to indicate myeloid bodies (My) and the abundance of smooth endoplasmic reticulum (SER). The slightly fenestrated choriocapillaris (CC) is also indicated as is an RPE cell nucleus (N). × 13,500

Fig. 5. Electron micrograph to indicate phagosomes (Ph) and myeloid bodies with ribosomes on their surface (My). Bruch's membrane (B) is also indicated. × 13,200

Fig. 6. Electron micrograph to indicate several lipid droplets (L) and a cell junction (J). Polysomes are widespread and obvious. Cell processes are noted within Bruch's membrane (B). × 13,500

Fig. 7. Electron micrograph to indicate a phagosome (Ph), myeloid bodies (My) and a ring-shaped mitochondrion (Mi). The choriocapillary (CC) endothelium is non-fenestrated in this location. × 18,700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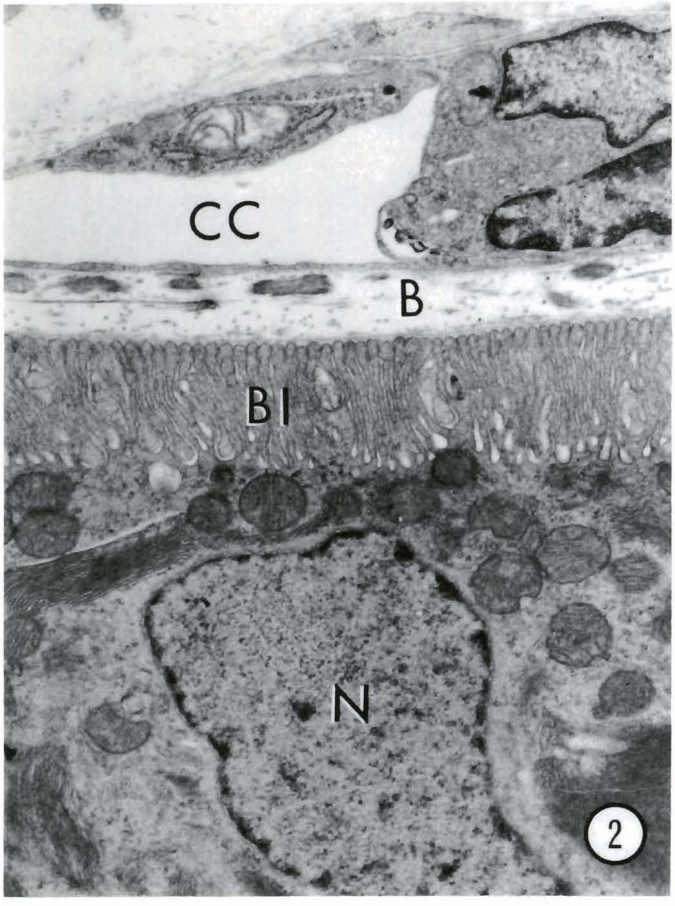
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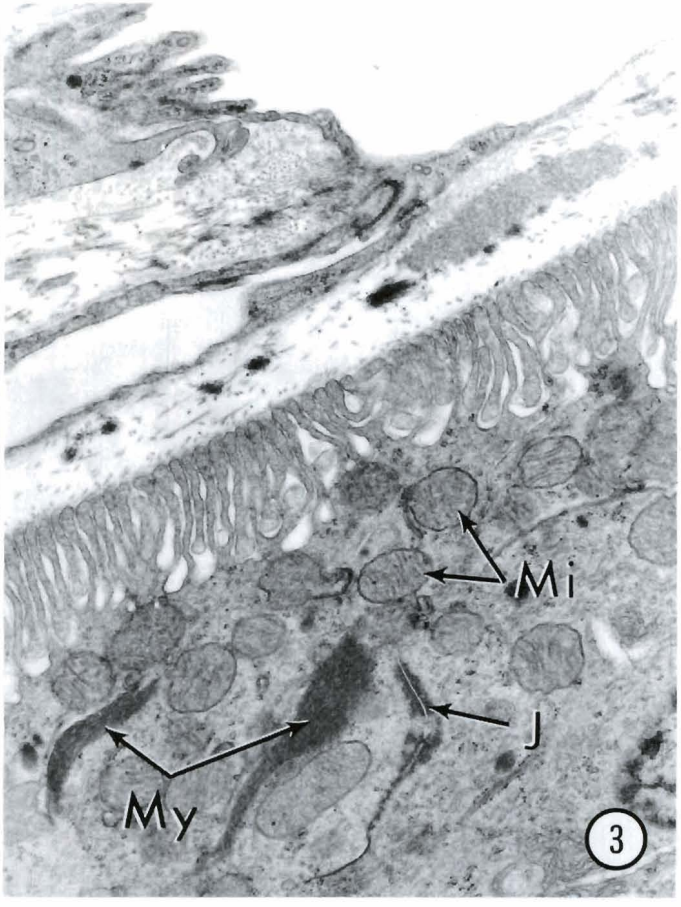
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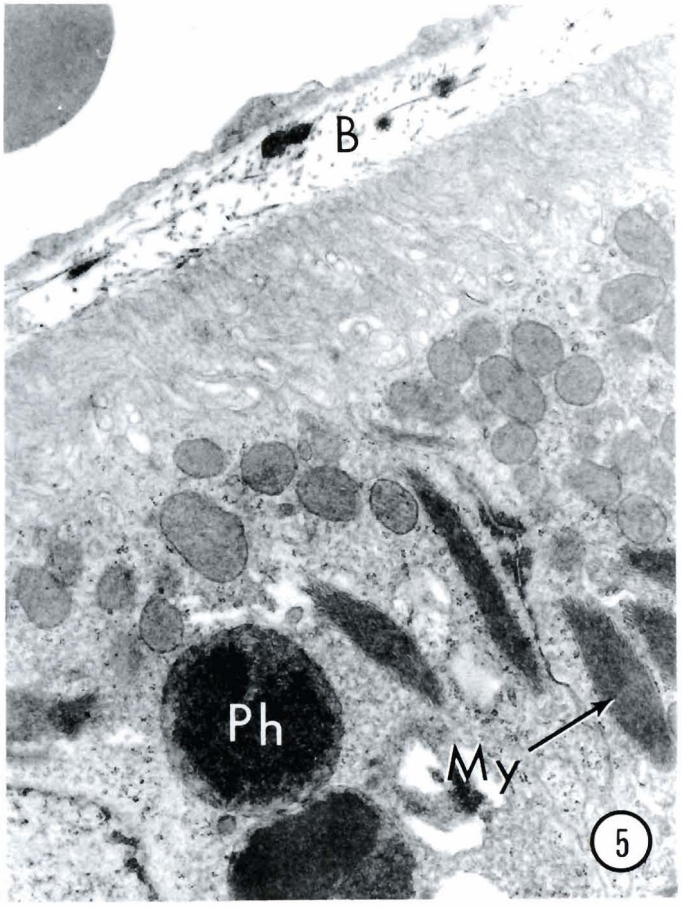
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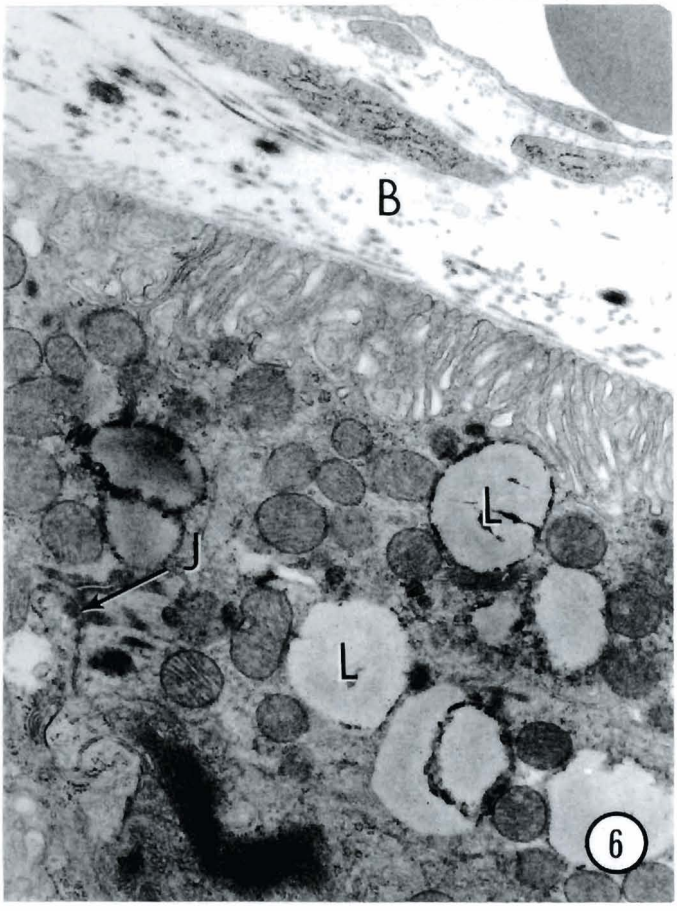
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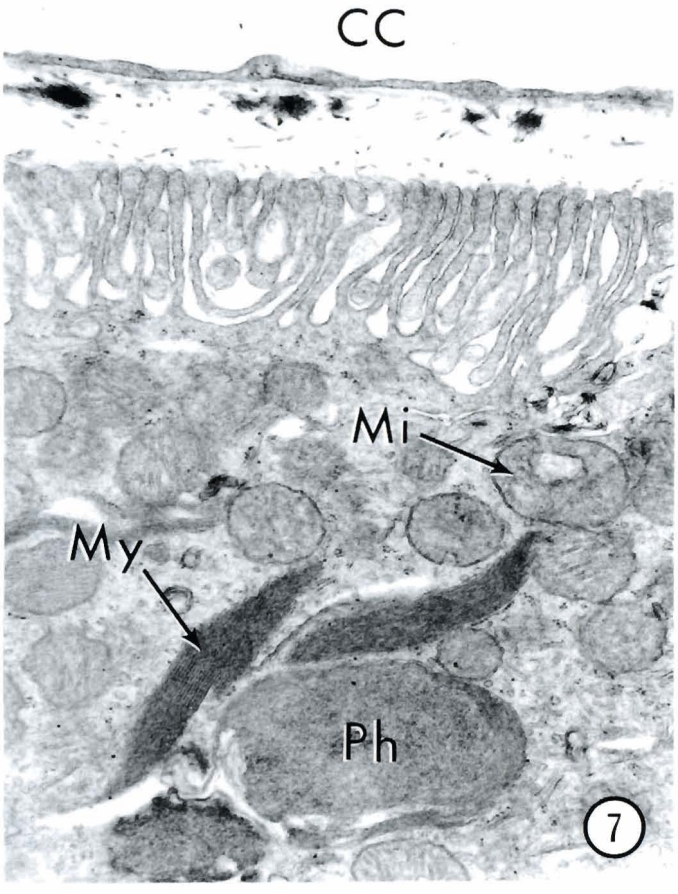
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the chorioscapillaris (Figs. 1, 2). The profiles of the the choriocapillaris are not overly abundant and are often widely separated (Fig. 1). The endothelium facing Bruch's membrane while normally extremely thin is but poorly fenestrated (Figs. 3-5).

Discussion

The retinal pigment epithelium (RPE) of the mallard *(Anas platyrhynchos)* is essentialy similar to that described for all vertebrate phyla but with modifications present that seem to be specific to avian species (Rodieck, 1973; Nguyen-Legros, 1978; Kuwabara, 1979; Braekevelt, 1980, 1982, 1983, 1984, 1988, 1989).

As in all vertebrate species described to date, the RPE of the mallard is composed of a single layer of cells with extensive basal infoldings and numerous apical processes. The presence of highly infolded basal epithelial membranes 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). These basal infoldings are deeper in avian species than is normally reported for mammalian species and may be necessary to compensate for the less-well-fenestrated choriocapillary endothelium noted in birds (Braekevelt, 1984, 1989).

The numerous apical processes are important in the structural support of the elongate photoreceptor outer segments (Berstein, 1961) as well as their proper orientation to incoming light (Enoch, 1979) and also in the internal adhesion required within the neural retina (Zinn and Benjamin-Henkind, 1979). Another important function of these apical processes is in the phagocytosis of shed outer segment debris (Bok and Young, 1979). In a variety of species, two different types of apical process are noted, associated with either rods or cones (Steinberg and Wood, 1974; Braekevelt, 1982b). In the mallard however only one type of finger-like process is noted and appears to contact both rods and cones.

The cell junctions at the lateral cell borders of the RPE cells are a constant feature of all vertebrates and are believed to 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 other avian species, these junctions in the mallard are located near the base of the epithelial cells (Kuwabara, 1979; Braekevelt, 1984, 1989).

The large vesicular nucleus and abundance of cell organelles are all indicative of metabolically very active cells. As is noted in most other species, SER is abundant within the RPE cells while RER is not (Kuwabara, 1979; Braekevelt, 1983, 1986, 1988). The abundance of SER is presumably due to the heavy involvement of the RPE in the storage, transport and esterification of the lipid precursors of the visual pigments (Zinn and Benjamin-Henkind, 1979). The smaller amounts of RER present probably 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 cells internal requirements for protein.

The wealth of mitochondria within RPE cells has been noted in most other species but the ring-shaped mitochondria found in the mallard appear to be unique to the avian species (Lauber, 1983a; Braekevelt, 1984, 1989). Lauber (1983a,b) has shown that this shape effectively doubles the surface area of the mitochondrion and has also noted 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 abundance in the mallard as only light-adapted species were investigated. In like manner, the few phagosomes of outer segment material noted within the RPE cells of the light-adapted mallard are presumably the remains of the burst of rod outer segment sheding which is known to occur soon after the onset of light (Young, 1978; Young and Bok, 1979).

The melanosomes of the retinal epithelial cells of the mallard are in the light-adapted state, located almost exclusively within the apical processes of these cells. While only light-adapted specimes were examined in this study, judging by the location of the melanosomes, retinomotor responses or photomechanical movements are felt to occur in the mallard as is reported for other birds (Walls, 1942). The location of melanosomes only within the apical processes of the RPE cells in light-adaptation may be explained by Meyer's (1977) observation that the photomechanical changes in birds are quite rapid and extensive.

Myeloid bodies are a common feature within the RPE cells of a variety of lower vertebrates (Kuwabara, 1979; Braekevelt, 1982a, 1984). While they have been implicated as sites of storage of lipid prior to esterification (Yorke and Dickson, 1984, 1985) and as the organelle that triggers photomechanical movements (Porter and Yamada, 1960; Braekevelt, 1982a) their function remains uncertain. Only in avian species however have ribosomes been noted on the surface of myeloid bodies (Meyer et al., 1973; Braekevelt, 1984, 1989) and this may indicate a further or secondary function of these compact arrays of membranes.

Bruch's membrane (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, 1983, 1986). Teleosts characterstically display a trilaminate Bruch's membrane, with the central elastic layer (lamina densa) being absent (Braekevelt, 1980, 1982a, 1985). In avian species the central lamina densa while usually present is but poorly represented (Braekevelt, 1984, 1989). Also the inner collagenous layer (closest to the RPE) is usually much wider than the outer collagenous layer (Braekevelt, 1984, 1989).

The choriocapillaris in all described species is composed of a single anastomosing layer of large-caliber capillaries (Rodieck, 1973; Kuwabara, 1979). With the exception of teleosts, the endothelium facing Bruch's membrane is normally highly fenestrated, indicative of the movement of large quantities of material across the wall of these capillaries (Bernstein and Hollenberg, 1965). In teleosts the presence of a choroid gland which

is important in the maintenance of a high oxygen pressure due to its counter current arrangement is conjectured to be the reason for the reduction in the number of fenestrations in the choriocapillaris endothelium (Braekevelt, 1985). A similar reduction of fenestrations is noted in the mallard choriocapillaris endothelium and may in this case be due to a lessened volume of transport required because of the presence of a large pecten oculi. A similar reduction of choriocapillary fenestrations has also been noted in the nighthawk and pigeon (Braekevelt, 1984, 1989).

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