

Retinal epithelial fine structure in the vervet monkey (*Cercopithecus aethiops*)

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Summary. The morphology of the retinal pigment epithelium (RPE) and closely associated Bruch's membrane (complexus basalis) and choriocapillaris have been investigated by electron microscopy in the vervet monkey (*Cercopithecus aethiops*). The RPE is composed of a single layer of cuboidal cells joined laterally by apically-located junctional complexes. Basally (sclerally) these cells display numerous infoldings while apically (vitreally) abundant processes enclose and interdigitate with rod outer segments. Internally the large vesicular nucleus is centrally located and smooth endoplasmic reticulum, mitochondria and lysosome-like bodies, are plentiful. Rough endoplasmic reticulum, polysomes and melanosomes while present are not abundant. Phagosomes of outer segment discs are noted in various stages of uptake and degradation. The choriocapillaris is highly fenestrated over large areas. Bruch's membrane shows the typical pentalaminar structure noted in other mammalian species without a tapetum lucidum.

Key words: Retinal pigment epithelium - Electron microscopy - Vervet monkey

Introduction

The retinal pigment epithelium (RPE) is heavily involved in a number of processes vital to the maintenance of the photoreceptor cells and ultimately to the proper functioning of the retina. Amongst the best known duties of the RPE are: 1) the transport of materials to and from the photoreceptors (Kroll and Machemer, 1968; Steinberg and Miller, 1973), 2) the architectural stabilization of photoreceptor outer segments (Bernstein, 1961), 3) adhesion of the neurosensory retina to the choroid (Zinn and Benjamin-Henkind, 1979), 4) the storage of vitamin A precursors

of the visual pigments (Young and Bok, 1970) and 5) the phagocytosis and lysosomal degradation of photoreceptor outer segment discs (Young, 1974, 1978; Bok and Young, 1979).

As a consequence of these important functions, the retinal epithelial region has been investigated in a variety of animals and while this region is essentially similar in vertebrates, species differences are usually noted (Braekevelt, 1973, 1976, 1977, 1980, 1983, 1985, 1986; Nguyen-Legros, 1978; Kuwabara, 1979).

Despite numerous studies of retinal fine structure, the RPE of the monkey has been described in only very few species (Kroll and Machemer, 1968; Young 1971; Sakuragawa and Kuwabara, 1976; Nilsson, 1978; Kuwabara, 1979). This report deals with the fine structure of the RPE and closely associated choriocapillaris and Bruch's membrane (complexus basalis) in the vervet monkey (*Cercopithecus aethiops*).

Materials and methods

For this study the eyes of four healthy adult vervet monkeys (*Cercopithecus aethiops*) were examined by light and electron microscopy. With the animal under deep surgical anesthesia, the eyeballs were quickly removed, opened at the equator and fixed for 5 h in 5% glutaraldehyde buffered to pH 7.3 with 0.1 M 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 pieces less than 1mm², taking care not to detach the retina. The tissue was then post-fixed for 2 h in 1% osmium tetroxide in the same phosphate buffer, dehydrated through graded ethanols to propylene oxide and embedded in Araldite.

Pieces of plastic-embedded tissue were reorientated to desired angles by means of a wax mount. Thick sections (0.5 µm) were cut, stained with toluidine blue and examined by light microscopy. Thin sections (600-700 Å) 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 EM 201 transmission electron microscope.

Results

As in other species described, the retinal pigment epithelium (RPE) of the vervet monkey comprises the outermost (scleral) layer of the neural retina. It consists of a single layer of low cuboidal cells that vary in height depending upon their location within the retina. Nearer the optic nerve head the RPE cells measure up to 9 μm in height (Fig. 1) while near the equatorial region they are approximately 5 μm in height (Fig. 2). At the extreme periphery, the retinal epithelial cells are even lower and can become squamous in appearance.

In all locations, the epithelial cells display numerous basal (scleral) infoldings and apical (vitreal) processes which enclose the tips of photoreceptor outer segments (Figs. 1,2,4,5). In the vervet monkey, these apical processes are of different shapes with the most common form being numerous villous-like processes which interdigitate with the rod outer segments (Figs. 2,4). The other group of apical processes form short sheath-like wrappings around the rod photoreceptor outer segment tips (Fig. 5). The lateral borders of the RPE cells are relatively smooth and are joined by typical tight-junctional complexes located near the apices of the cells (Figs. 3, 4).

The nuclei of the epithelial cells are large, moderately vesicular and located in the central region of the cell body (Figs. 2, 4). Smooth endoplasmic reticulum (SER) is the most abundant cell organelle within these cells. (Figs. 3, 4,6). Rough endoplasmic reticulum (RER) while much less abundant is also present (Fig. 4). Scattered polysomes and basally located mitochondria are also plentiful (Figs. 3, 4, 6). Melanosomes are large, predominantly spindle-shaped and are located both within the epithelial cell body as well as within the apical processes (Figs. 2,3,5). Lysosomes are found within all RPE cells but are occasionally extremely abundant in a particular cell (Fig. 3). Melanosome-lysosome complexes are also noted (Figs. 3, 4). Phagosomes of outer segment material while not abundant are observed in various stages of engulfment and degradation (Figs. 5, 6, 7).

Bruch's membrane (complexus basalis) in the vervet monkey displays a pentalaminar substructure (Figs. 4, 6, 7). The five layers are 1) the basal lamina of the retinal pigment epithelium 2) the basal lamina of the choriocapillaris 3) an inner and 4) an outer collagenous layer and 5) a central discontinuous elastic layer (Figs. 6, 7, 8). Although Bruch's membrane is slightly thicker in the posterior fundus, its structure remains the same in all retinal locations.

The choriocapillaris consists of a single layer of large-caliber, anastomosing capillaries located immediately scleral to Bruch's membrane (Figs. 1, 2). The endothelium bordering on Bruch's membrane is very thin and very heavily fenestrated (Figs. 6, 7). These fenestrations continue within the endothelium facing

other capillary profiles (Fig. 8) but are not normally found within the endothelium facing the choroid. The nuclei of the choriocapillary endothelial cells are normally located at this choroidal edge of the choriocapillaris (Fig. 2).

Fig. 1. Electron micrograph of the retinal epithelium (RPE) near the optic nerve head. Rod outer segments (ROS) and the choriocapillaris (CC) are indicated. $\times 4,800$

Fig. 2. Electron micrograph of the retinal epithelium (RPE) in the equatorial region. Bruch's membrane (B), an RPE nucleus (N) and rod outer segments (ROS) are indicated. $\times 4,500$

Fig. 3. Electron micrograph of the RPE near the optic nerve head. Mitochondria (M), melanosomes (Me) and lysosomes (L), are indicated. $\times 8,900$

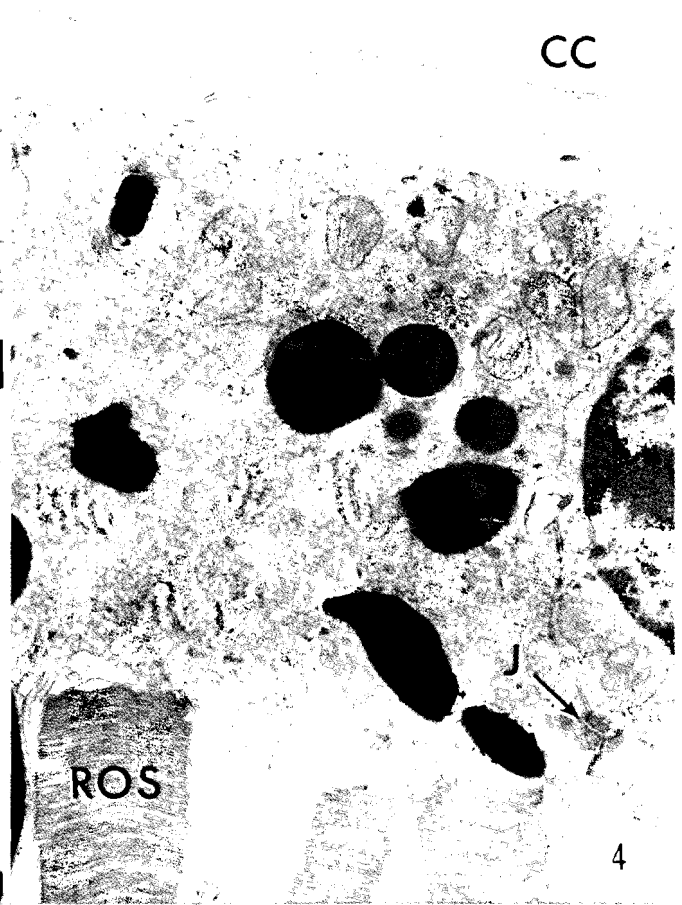
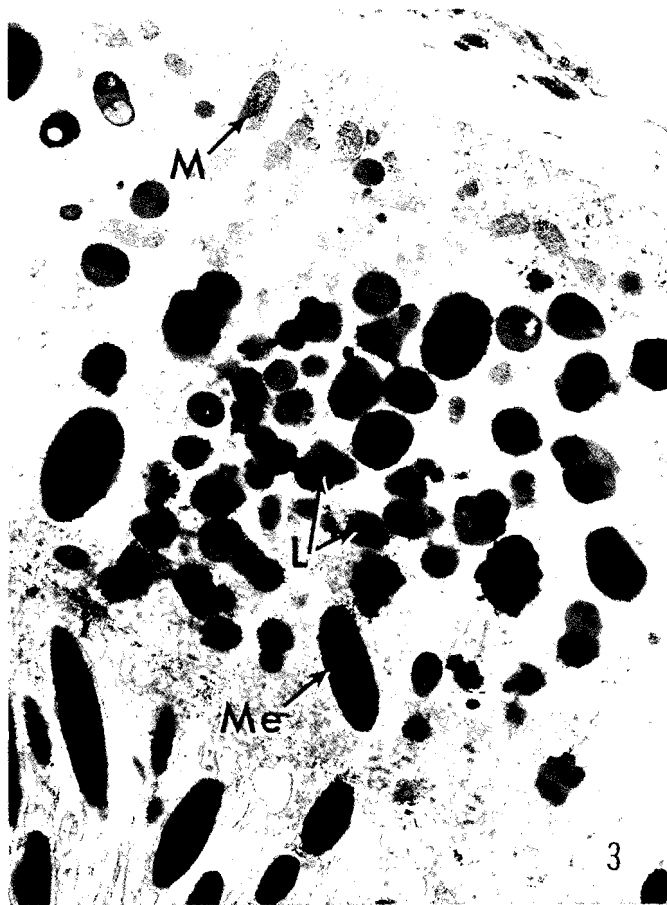
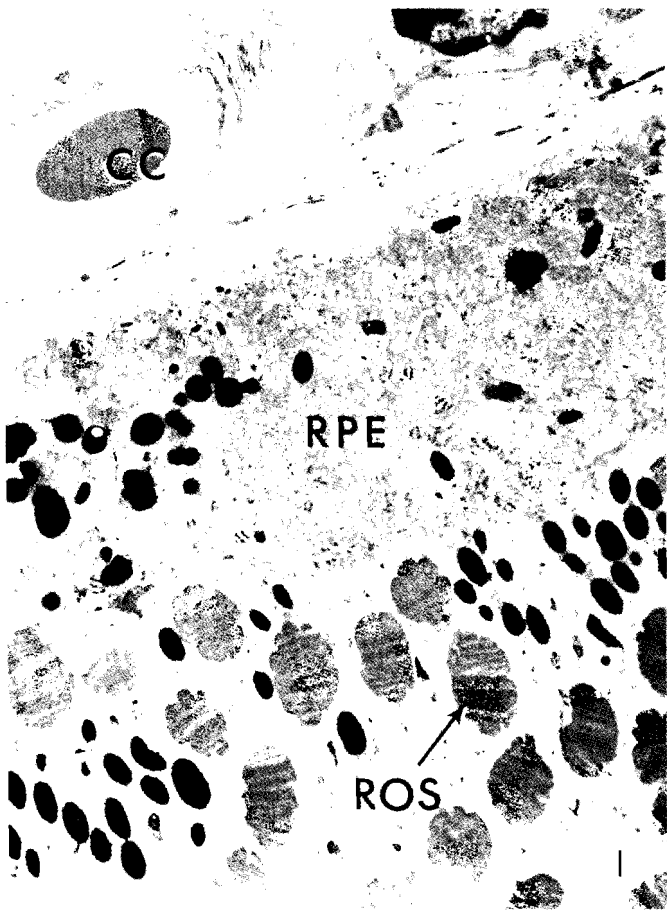
Fig. 4. Electron micrograph of the RPE in the equatorial region. A cell junction (J), rod outer segments (ROS) and the choriocapillaris (CC) are indicated. $\times 12,900$

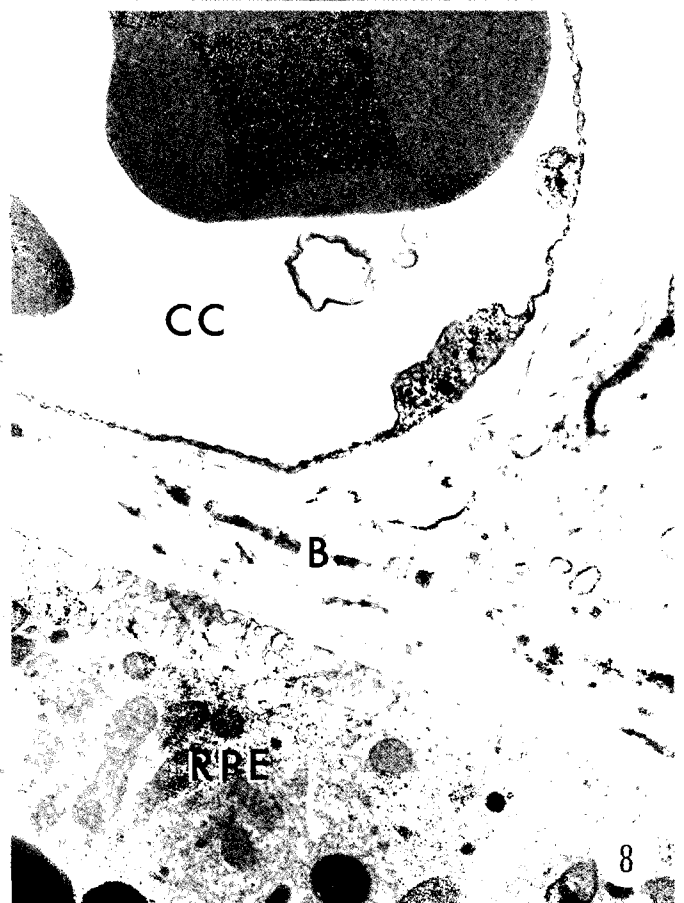
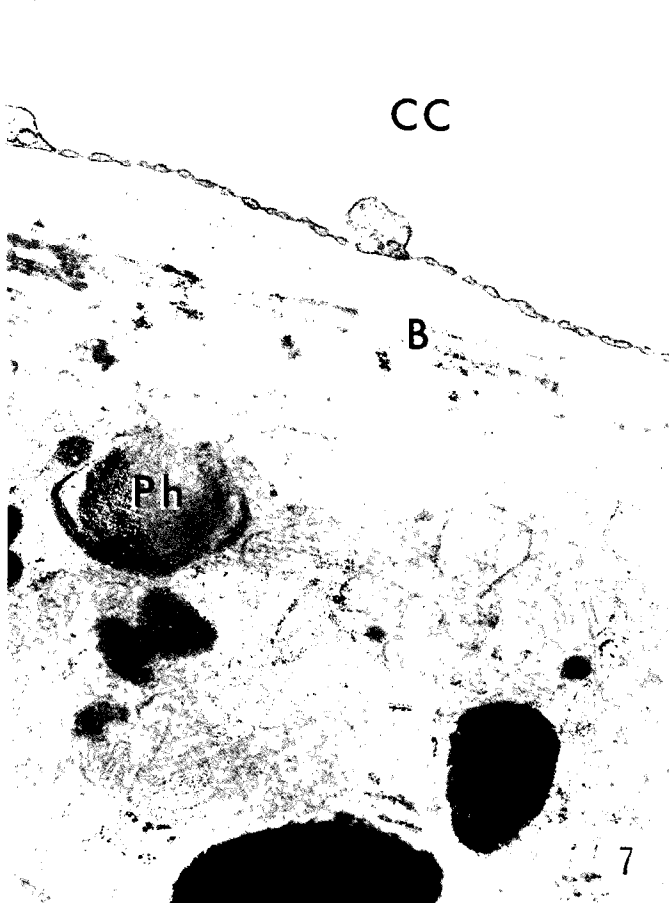
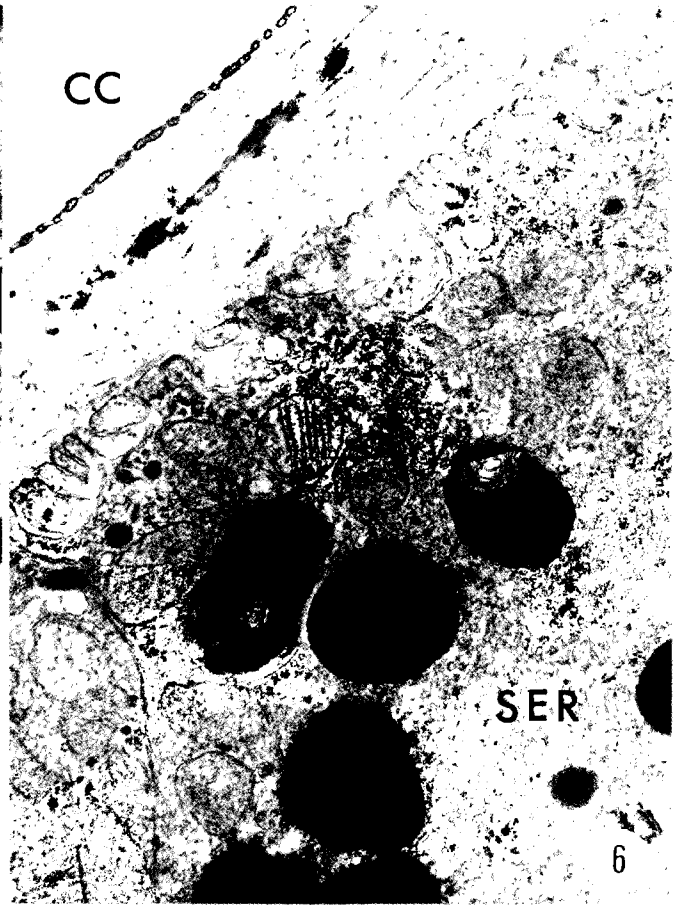
Fig. 5. Electron micrograph of the apical region of the RPE. A rod outer segment (ROS) is shedding some discs as a phagosome (Ph). $\times 13,800$

Fig. 6. Electron micrograph of the RPE to indicate the abundance of smooth endoplasmic reticulum (SER). The choriocapillaris (CC) is also indicated. $\times 19,500$

Fig. 7. Electron micrograph of the RPE to indicate a phagosome (Ph). Bruch's membrane (B) and the highly fenestrated choriocapillaris (CC). $\times 20,300$

Fig. 8. Electron micrograph to indicate the fenestrated choriocapillaris (CC), Bruch's membrane (B) and the infolded basal region of the RPE. $\times 15,000$





Discussion

The morphology of the retinal pigment epithelium (RPE) of the vervet monkey (*Cercopithecus aethiops*) is essentially similar to that described for most non-albino vertebrates (Braekevelt, 1973, 1982, 1983, 1986; Spitznas 1974; Nilsson, 1978; Kuwabara, 1979)

The observation that RPE cells are taller in the posterior retina than in the peripheral regions has also been noted in other species (Tso and Friedman, 1967; Hogan et al., 1971; Braekevelt, 1983, 1985). This is presumed to indicate a higher state of activity for the posteriorly located epithelial cells as these cells are probably in contact with more photoreceptors.

The presence of numerous basal infoldings in RPE cells is a constant feature of most retinal epithelia described (Nilsson, 1978; Kuwabara, 1979; Braekevelt, 1982, 1984). These infoldings are felt to be indicative of an active role in transport by these cells (Dowling and Gibbons, 1962). A number of teleost species have been described with relatively few basal infoldings (Braekevelt, 1980b, c, d, 1985). The usual presence of a choroid gland in the teleost eye however probably makes transport by these epithelial cells somewhat less important (Wittenberg and Wittenberg, 1974).

Apical (vitreal) processes of the retinal epithelium which interdigitate with photoreceptor outer segments and which are important in both the phagocytosis of outer segment material (Young, 1976, 1978) and the architectural stabilization and orientation of the outer segments (Bernstein, 1961; Enoch, 1979) have been reported in all vertebrates to date. In some species two strikingly different apical processes are described. One type is the long finger-like process associated with the rod outer segments (Kuwabara, 1979; Braekevelt, 1983). Some of these rod-associated processes are sheath-like in appearance as they wrap around rod outer segment tips (Burnside and Laties, 1979). The villous-like processes are considered to be most important in outer segment orientation (Enoch, 1979) and adherence of the neurosensory retina (Zinn and Benjamin-Henkind, 1979) while the sheath-like processes are felt to be significant in the phagocytosis of outer segment discs (Burnside and Laties, 1979). The second type of apical process is much larger and extends to the normally shorter cone cells (Steinberg and Wood, 1974; Braekevelt, 1982). Only the apical processes associated with rod photoreceptors are noted in the vervet monkey.

The lateral borders of the RPE cells are normally relatively smooth when compared with the apical and basal borders of these cells (Braekevelt, 1983, 1984, 1986). The retinal epithelial cells are joined laterally by a series of typical tight junctions (Kuwabara, 1979) which form an effective barrier to the intercellular movement of materials and forms part of the blood-ocular barrier (Cohen, 1968; Shiose, 1970).

The relatively large and vesicular nucleus found in the retinal epithelial cell coupled with the abundance of mitochondria and other cell organelles is indicative of a highly active cell. As has been noted in most other

species, smooth endoplasmic reticulum (SER) is the most obvious cell organelle within RPE cells (Nguyen-Legros, 1978; Kuwabara, 1979; Braekevelt, 1982, 1984, 1986). This abundance of SER is common to cells heavily involved in lipid biosynthesis (Enders, 1962) and it is certainly well established that the RPE is crucial in the storage, transport and esterification of vitamin A (Zinn and Benjamin-Henkind, 1979). While profiles of rough endoplasmic reticulum (RER) are noted within the RPE of most species, they are not abundant and would indicate that little protein is being produced for export by these cells.

Melanosomes are a constant feature of the RPE in non-albino species and are felt to be important in the absorption of light which has not struck a photoreceptor to prevent light scattering and the subsequent reduction of visual acuity (Moyer, 1969; Zinn and Benjamin-Henkind, 1979). The presence of numerous lysosomes (both primary and secondary) and phagosomes of photoreceptor outer segment debris are also a prominent feature of the retinal epithelium in all species investigated (Braekevelt, 1982, 1984, 1986). One of the most important functions of this epithelial layer is of course the phagocytosis and subsequent lysosomal degradation of shed outer segment material (Young and Bok, 1979).

In mammalian species, Bruch's membrane (complexus basalis) is invariably reported as the pentalaminate structure which is noted in the vervet monkey (Nakaizumi, 1964a; Kuwabara, 1979; Braekevelt, 1983, 1986). In species with a chroidally situated tapetum lucidum, Bruch's membrane is reduced to only the two basal laminae of the choriocapillaris and the retinal epithelium (Nakaizumi, 1964b; Braekevelt, 1980a, 1986). In teleosts on the other hand, Bruch's membrane is normally trilaminate with the central elastic layer being absent (Braekevelt, 1980b, c, d).

The choriocapillaris in all mammalian species described is heavily fenestrated on the border facing the RPE and the important role of the nourishment of the outer retina by the choriocapillaris is well established (Zinn and Benjamin-Henkind, 1979). The choriocapillaris in the vervet monkey is highly fenestrated not only on the border facing the RPE but also on the sides facing other capillary profiles. In species with a choroidally located tapetum, all aspects of the choriocapillaris are normally fenestrated and this is felt to indicate an involvement of the choriocapillaris in the nourishment of the relatively poorly vascularized tapetal region (Braekevelt, 1983, 1986; Lesiuk and Braekevelt, 1983). As the fenestrations are felt to indicate areas of heavy transport across the endothelium, this may indicate an involvement of the choriocapillaris in this species in the metabolic support of the choroid.

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