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Retinal epithelial fine structure in the southern fiddler ray *(Trygonorhina fasciata)*

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Summary. The morphology of the retinal epithelium (RPE), choriocapillaris and Bruch's membrane (complexus basalis) has been investigated by light and electron microscopy in an elasmobranch, the southern fiddler ray or guitarfish *(Trygonorhina fasciata)* . The RPE consists of a single layer of cuboidal cells which display basal (scleral) infoldings as well as numerous apical (vitreal) finger-like processes which interdigitate with the photoreceptor outer segments. The lateral cell borders are relatively smooth and are joined in the mid-region by a series of tight junctions. Internally the RPE nucleus is large, vesicular and centrally located. Smooth endoplasmic reticulum (SER) is abundant while rough endoplasmic reticulum (RER) is scarce. Polysomes are however widespread and mitochondria are plentiful. Two unusual organelles are also noted. One consists of a membrane bound array of tubules while the other is a membrane bound structure consisting of a granular matrix with again an internal tubular array. This species possesses a choroidally located tapetum lucidum in the superior fundus and over this tapetal area, melanosomes are absent from the RPE cells. In non-tapetal locations a few melanosomes are present that do not appear to undergo photomechanical movements. Bruch's membrane is a pentalaminate structure with an almost continuous central elastic layer (lamina densa). The choriocapillaris forms a single layer of capillaries with a thin but only minimally fenestrated endothelium facing Bruch's membrane.

Key words: Retinal Pigment Epithelium (RPE), Electron microscopy, Elasmobranch, *(Trygonorhina fasciata)*

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Introduction

The retinal pigment epithelium (RPE) is an essential layer of the vertebrate retina and along with the choriocapillaris and Bruch's membrane (complexus basalis) is intimately involved in several processes vital to the proper functioning of the photoreceptor cells and hence to vision itself. Amongst the best known roles of the RPE are: 1) the selective transport of materials to and from the photoreceptors (Kroll and Machemer, 1968; Steinberg and Miller, 1973) probably mediated by the interphotoreceptor matrix (IPM) (Uehara et al., 1990); 2) the storage and modification of vitamin A precursors of the visual pigments (Young and Bok, 1970); 3) the architectural support and proper orientation of the photoreceptor outer segments (Bernstein, 1961; Enoch, 1979); 4) the adhesion of the photoreceptors to the RPE (Zinn and Benjamin-Henkind, 1979); and 5) the phagocytosis and lysosomal degradation of shed photoreceptor outer segment discs (Young, 1978; Bok and Young, 1979).

As a consequence of these important functions, the RPE region of the vertebrate retina has been investigated in a variety of animals and while the essential design of the RPE is remarkably similar in all vertebrates described to date, generic differences in morphology are almost always observed (Nguyen-Legros, 1978; Kuwabara, 1979; Braekevelt, 1980, 1983, 1985, 1986, 1988, 1990).

While numerous reports of the fine structure of the retinal epithelium (RPE) are available, relatively few deal with elasmobranches (sharks and rays) (Nicol, 1989). Consequently as part of the comparative morphological study of the RPE region in vertebrates, the fine structure of the RPE, Bruch' membrane (complexus basalis) and choriocapillaris in the southern fiddler ray or guitarfish *(Trygonorhina fasciata)* is reported in this study.

Materials and methods

For this study the eyes of four adult southern fiddler rays (or guitarfish) *(Trygonorhina fasciata)* were investigated by light and electron microscopy. Eyes from both sexes of both light- and dark-adapted specimens were examined.

The animals were killed by severing the spinal cord and 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 Sorenson's phosphate buffer at 4° C. The posterior half of the eyeball was then removed, washed in 5% sucrose in 0.1 M Sorenson's buffer (pH 7.3) divided into tapetal and non-tapetal portions and cut into pieces less than 1 mm^2. The tissue was then post-fixed for 2 h. in 1% osmium tetroxide in the same phosphate buffer, dehydrated through graded ethanols to methanol and then propylene oxide and embedded in Araldite.

Pieces of plastic-embedded tissue were subsequently 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 (50 - 60 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 EM 201 transmission electron microscope.

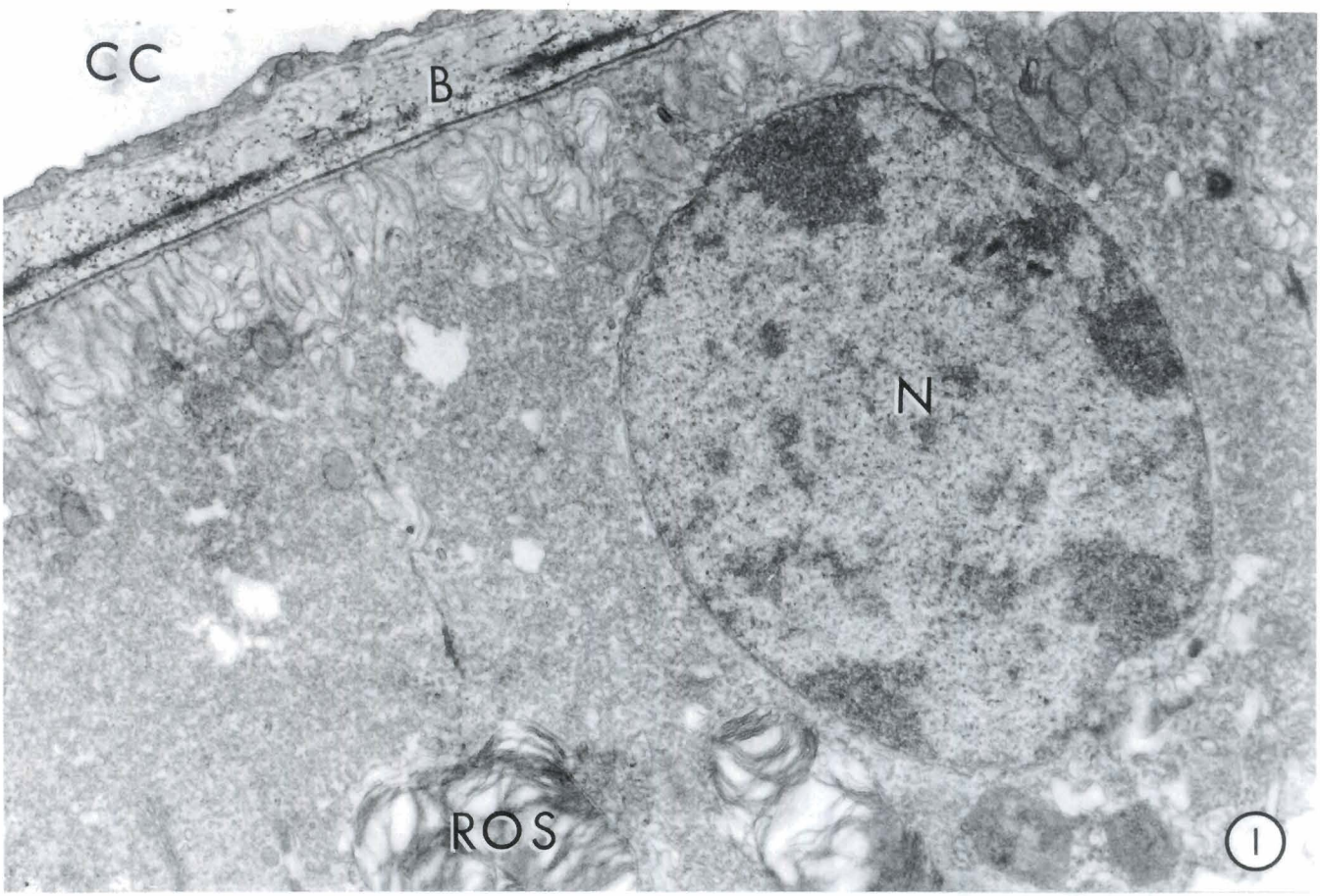
Results

In the southern fiddler ray *(Trygonorhina fasciata)* the retinal epithelium (RPE) consists of a single layer of cuboidal cells which average between 6 and 9 μm in height throughout the retina (Figs. 1, 2). The southern fiddler ray or guitarfish however possesses an occlusible tapetum lucidum located in the choroid of the superior fundus (Braekevelt, 1991) and the morphology of certain aspects of the RPE varies somewhat depending upon retinal location. In all regions, the RPE cells display numerous basal (scleral) infoldings which penetrate to much the same depth (1.2 - 1.5 μm) in both tapetal and non-tapetal locations (Figs. 1, 2). Apically (vitreally) the RPE cells in all locations show numerous finger-like processes which enclose rod photoreceptor outer segment tips (Figs. 2, 8). The lateral cell borders of the retinal epithelial cells are relatively smooth and are joined in their mid-regions by a series of tight junctions (the so-called Verhoeff's membrane) (Figs. 1 - 4). Internally the RPE cells display large, vesicular and roughly spherical nuclei with a fairly dispersed chromatin (Figs. 1, 4, 7, 8) and only uninuclear RPE cells were encountered (Figs. 1, 2). Smooth endoplasmic reticulum (SER) is the most abundant cell organelle and dominates the cytoplasm (Figs. 1 - 8). Profiles of rough endoplasmic reticulum (RER) are extremely rare while polysosomes are scattered amongst the abundant SER (Figs. 3, 4). Mitochondria are abundant in the basal region of the RPE cells, either clustered at the bottom of the basal infoldings or in some cases located against the basal cell membrane when the infoldings are absent (Figs. 2, 3, 7). These mitochondria are round, oval or oblong in shape (Figs. 3, 4, 7). Phagosomes of shed outer segment discs were noted in both light- and dark-adapted specimes but were never abundant (Figs. 2, 3, 7). Lysosome-like bodies were also frequent within the RPE cells with only occasional Golgi zones being noted (Figs. 1 - 3). Myeloid bodies were not observed within the RPE cells of the southern fiddler ray but a couple of unusual organelles were frequently present. One of these consisted of a small (1.0 - 2.0 μm) membrane-bound electron dense body with an internal array of tubular profiles apparently embedded in a granular matrix (Figs. 3 - 6). The other unusual organelle consisted of an array of 10 - 12 closely packed tubular profiles also surrounded by a membrane (Figs. 7, 8). Both these inclusions were present in both light- and dark-adaptation and in both tapetal and non-tapetal locations. As both these organelles displayed tubules as part of their morphology they may be inter-related.

Over the choroidally located tapetum, the retinal epithelial cells are totally devoid of melanosomes (Figs. 1, 3, 8). In non-tapetal locations, melanosomes are present within the RPE cells but are never very abundant (Figs. 2, 4, 7). When melanosomes are present they are normally small (< 1.0 μm) and located in the apical (vitreal) region of the cell body (Figs. 2, 8). No appreciable movement of melanosomes was observed in light- and dark-adaptation and it is unlikely that they undergo retinomotor responses. Also because of the limited number of melanosomes present it is unlikely that they can form an effective barrier to incoming light. The choroid in non-tapetal locations is however more heavily pigmented than are the RPE cells and probably acts as the light-absorbing layer in extra-tapetal locations (Fig. 5).

Bruch's membrane in the guitarfish is in all locations a pentalaminate structure (Figs. 1, 3, 6). It consists of 1) the basal lamina of the RPE layer; 2) the basal lamina of the choriocapillaris; 3) an inner and 4) an outer collagen layer with 5) a central layer of elastic tissue (Figs. 2, 4). While this central elastic layer is discontinuous in some locations it is for the most part a distinct and continuous layer (lamina densa). Bruch's membrane is marginally thicker in the posterior fundus in both tapetal and non-tapetal locations (at about 1.0 μm) than it is in peripheral locations (Figs. 2, 3).

The choriocapillaris consists of a single layer of large-calibre capillaries, immediately adjacent to Bruch's membrane (Figs. 1, 2). The endothelium of these capillaries facing Bruch's membrane is typically very thin but minimally fenestrated (Figs. 1, 3, 6).



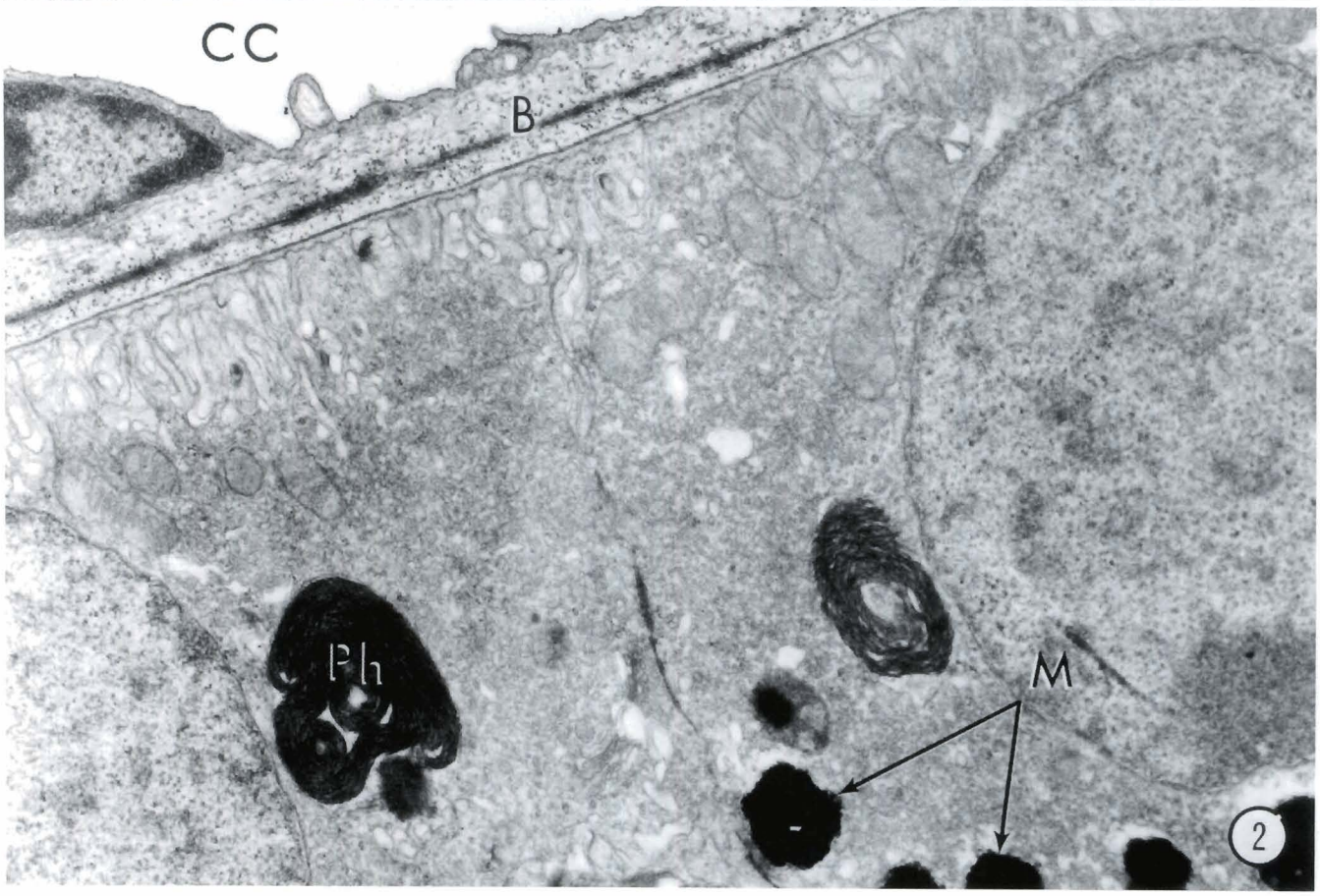
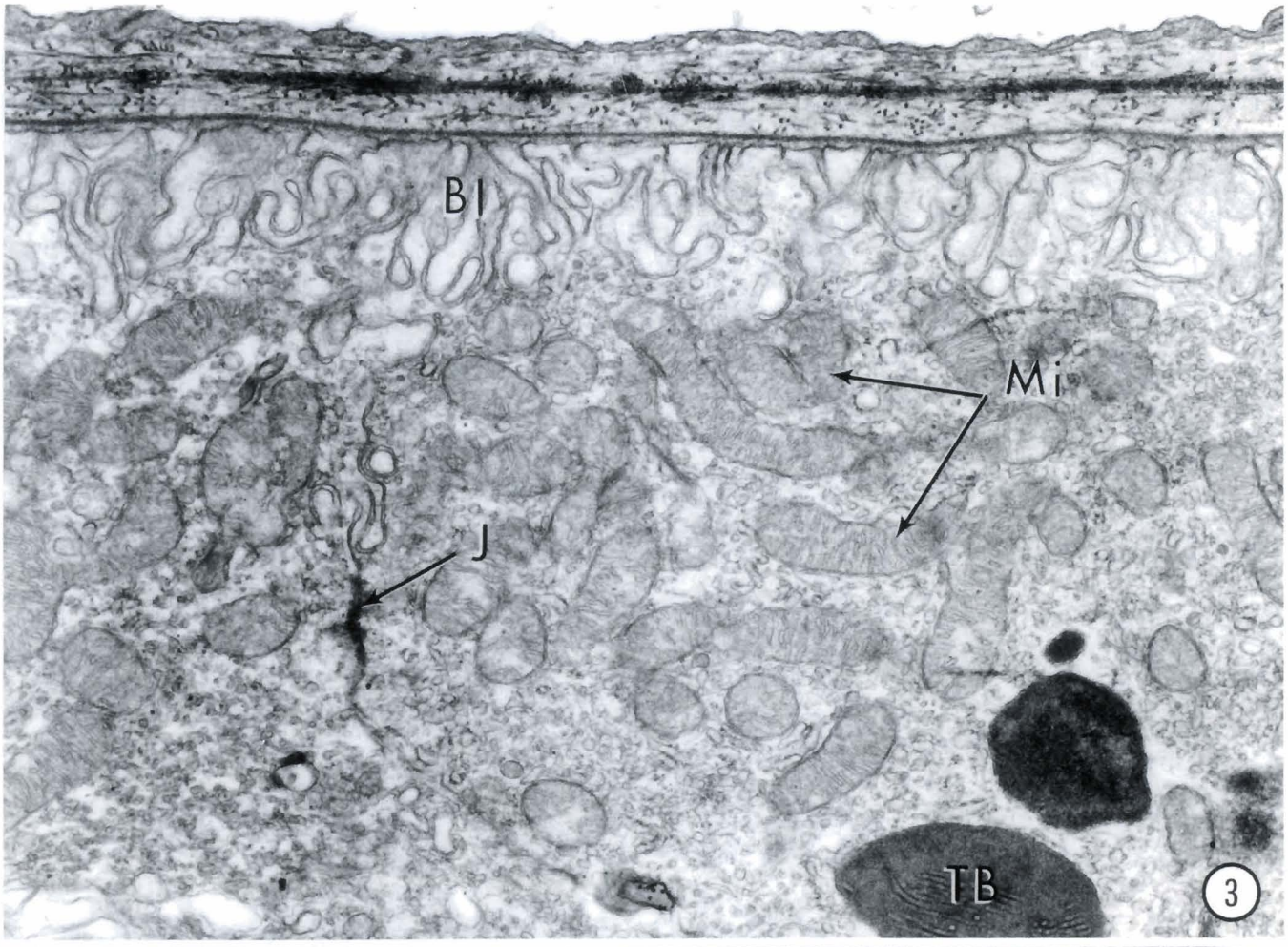


Fig. 1. Electron micrograph of the retinal epithelium overlying the tapetum. The choriocapillaris (CC), Bruch's membrane (B) and a rod outer segment (ROS) are labelled as is an RPE nucleus (N) × 12,800

Fig. 2. Electron micrograph of the RPE from a non-tapetal location. The choriocapillaris (CC), Bruch's membrane (B), a phagosome (Ph) and melanosomes (M) are indicated. × 12,600



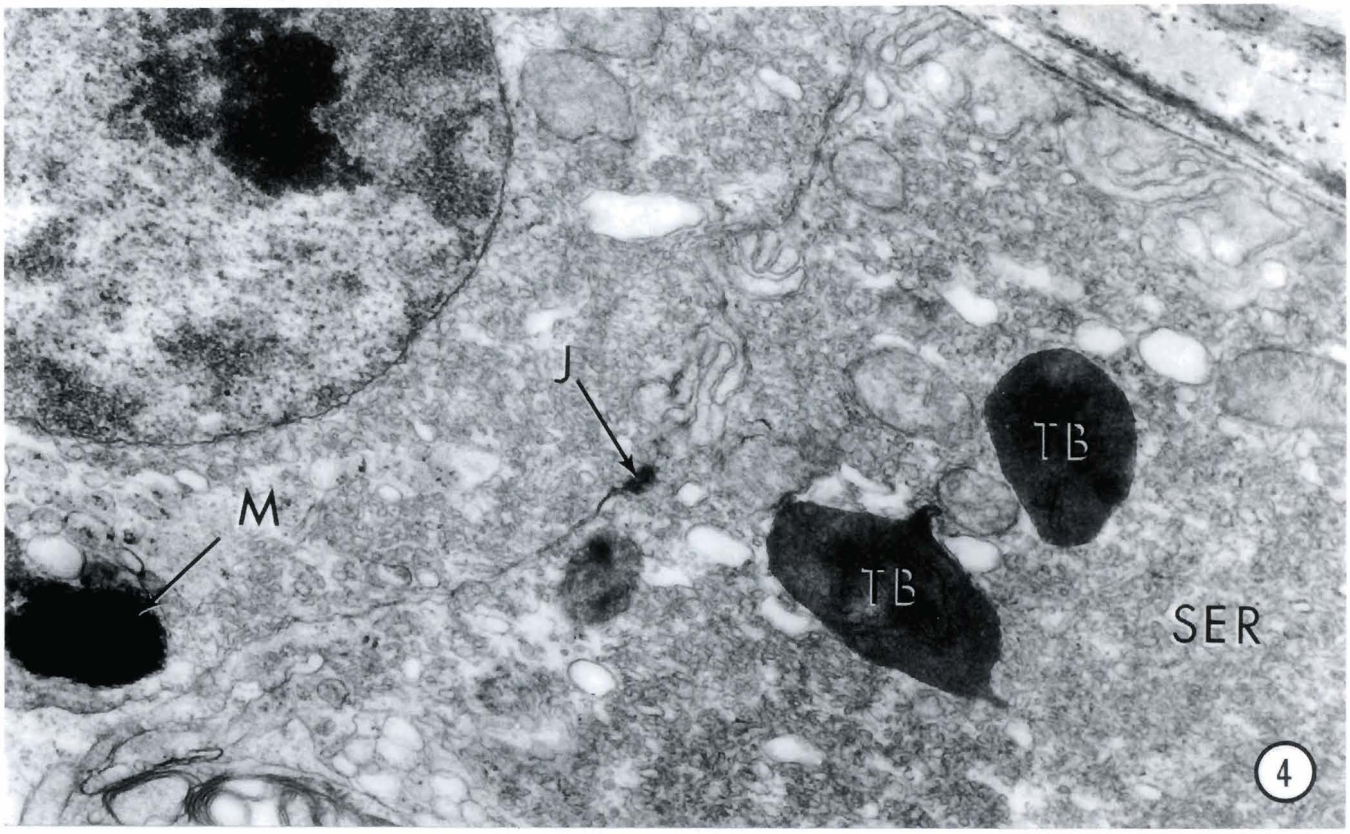


Fig. 3. Electron micrograph of the RPE from a tapetal region. Basal infoldings (BI), mitochondria (Mi) a cell junction (J) and a dense tubular body (TB) are all labelled. × 18,400

Fig. 4. Electron micrograph of the RPE from a non-tapetal location. A cell junction (J), smooth endoplasmic reticulum (SER), melanosomes (M) and two dense tubular bodies (TB) are indicated. × 18,700

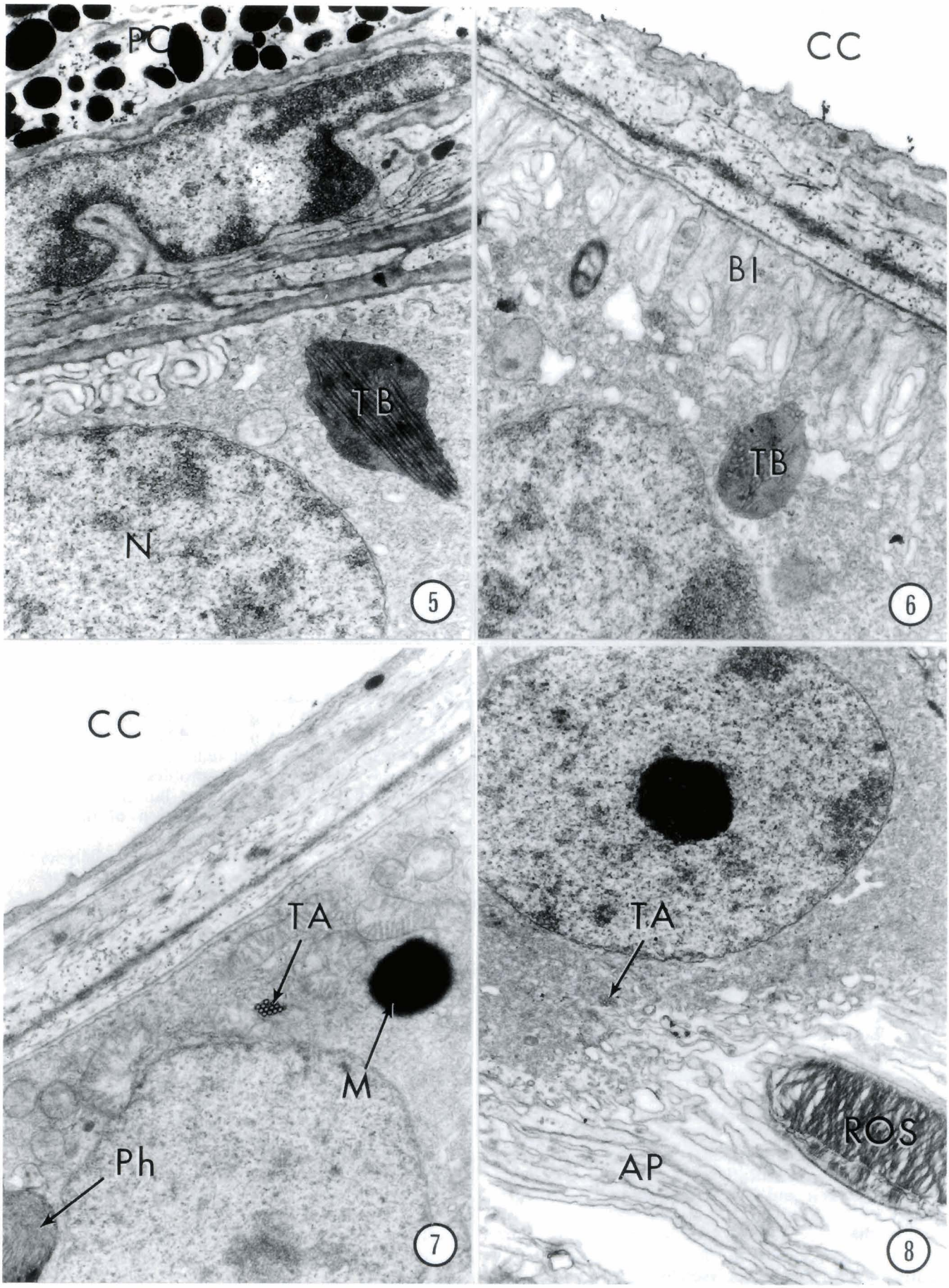


Fig. 5. Electron micrograph from a non-tapetal area to illustrate the heavily pimented cells (PC) of the choroid. A dense tubular body (TB) and the RPE nucleus (N) are also indicated. × 13,000

Fig. 6. Electron micrograph from a tapetal location to indicate a few fenestrations in the choriocapillaris (CC). Basal infoldings (BI) and a dense tubular body (TB) are also indicated. × 18,700

Fig. 7. Electron micrograph from a non-tapetal region to illustrate an array of tubules (TA), a melanosome (M), a phagosome (Ph) and the choriocapillaris (CC). × 13,200

Fig. 8. Electron micrograph of the RPE from a non-tapetal location to indicate the numerous apical processes (AP) a rod outer segment (ROS) and a small array of tubules (TA). × 10,000

Discussion

The retinal epithelial region of the southern fiddler ray or guitarfish *(Trygonorhina fasciata)* is morphological very similar to that described for other vertebrate species with some variations (Nguyen-Legros, 1978; Kuwabara, 1979; Braekevelt, 1980, 1984, 1988, 1989a, 1990).

As in all reported cases, the retinal epithelium (RPE) in this species consist of a single layer of cells (Nguyen-Legros, 1978). The height of RPE cells varies widely amongst species from the tall columnar cells reported for many teleosts (Braekevelt, 1982, 1985) to the squamous type noted in marsupials (Braekevelt, 1973; Young and Braekevelt, 1991). In most species however the RPE consists of cuboidal cells that are slightly taller in the posterior fundus (Kuwabara, 1979) and that is the case in this elasmobranch.

Retinal epithelial cells normally display an extensive array of basal infoldings which are felt to be microfolds and indicative of a heavy involvement by this epithelial layer in the transport of materials from the choriocapillaris to the photoreceptors (Steinberg and Miller, 1973). The abundant apical processes of the RPE cells are necessary for the structural support and proper orientation of the elongate photoreceptor cells (Bernstein, 1961; Enoch, 1979) as well as in the adhesion required between the photoreceptors and retinal epithelium (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 morphological distinct types of apical process are reported (Steinberg and Wood, 1974; Braekevelt, 1982, 1989b) in the guitarfish only one type of finger-like process is noted.

The cell junctions located at the lateral cell border of the RPE cells are a constant feature in all vertebrate retinas (Nguyen-Legros, 1978). They are felt to constitute an effective barrier to the intercellular movement of materials and hence form part of the blood-occular 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, 1970; Braekevelt, 1986, 1988, 1990). The preponderance of SER reflects the heavy involvement of this epithelial layer in the storage, transport and esterification of lipid photopigment precursors (Zinn and Benjamin-Henkind, 1979). The paucity of RER noted within the RPE cells would indicate that very little protein is being produced for export by these cells in the adult condition. The relative abundance of polysomes however indicates that these cells still require proteins for internal structures and functions. The abundance of mitochondria within RPE cells is indicative of the high energy requirements of these cells as they fulfill their various functions. The presence of phagosomes of outer segment material and lysosome-like bodies within the RPE cells of the guitarfish is also very typical as the phagocytosis and subsequent degradation of outer segment discs in one of the main functions of the RPE layer in all vertebrate species (Nguyen-Legros, 1978; Young, 1978).

Myeloid bodies which are arrays of stacked membranes usually formed by the SER and which are a common feature in most lower (non-mammalian) vertebrates are not present within the RPE cells of the southern fiddler ray. The function of myeloid bodies is uncertain although they have been implicated as the organelle that triggers photomechanical movements (Porter and Yamada, 1960; Braekevelt, 1982) as well as being storage sites of lipids prior to esterification (Yorke and Dickson, 1984, 1985). As it is felt that the melanosomes of RPE in this species show no appreciable movements, the absence of myeloid bodies may strengthen the hypothesis that they act as the organelle that generates retinomotor responses. The function (if any) of the two unusual organelles noted with the RPE cells of this species is unknown. One of these structures, the small array of tubular profiles is similar to a structure noted in bats (Yamada, 1958; Kuwabara, 1979). Kuwabara (1979) reported that these tubular profiles became large and conspicuous during hibernation but its function (if it is a comparable structure) in the fiddler ray is unknown. The function of the other unusual structure which is large and contains tubular profiles embedded in a granular matrix is also obscure but it may perhaps represent the lysosomal degradation of the tubular arrays.

As in other species with a choroidally-located tapetum lucidum, the RPE cells overlying the tapetum are totally devoid of melanosomes (Walls, 1942; Rodieck, 1973; Braekevelt, 1986, 1989b). This allows for the passage of light to and from the tapetum and in this region, the RPE layer is similar to that noted in albino species (Braekevelt and Hollenberg, 1970). In non-tapetal locations, the RPE is typically heavily pigmented (Braekevelt, 1986, 1990). In this species however, the extratapetal RPE is characterized by only a few melanosomes which do not change position during light- and/or dark-adaptation and which probably do not seriously block the passage of light. The choroid overlying such areas is however more heavily pigmented and would act as an efficient light-absorbing layer.

Bruch's membrane (complexus basalis) in mammalian species is invariably reported as a pentalaminate structure with the five layers as described in the results portion of this report being quite distinct and obvious (Nakaizumi, 1964; Braekevelt, 1986, 1988, 1990). Teleosts characteristically only show a trilaminate Bruch's membrane with the central elastic layer (lamina densa) being absent (Braekevelt, 1980, 1982, 1985). In this elasmobranch, while Bruch's membrane is

pentalaminate it is somewhat unusual in that the basal lamina of the choriocapillaris is often apparently absent and the lamina densa shows very few discontinuities.

The choriocapillaris in all vertebrate species described is composed of a single layer of large-calibre capillaries (Rodieck, 1973; Kuwabara, 1979). With the exception of teleosts the choriocapillary endothelium facing Bruch's membrane is very thin and highly fenestrated, indicative of the movement of large quantities of material across this endothelium (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 (Braekelvet, 1985). The choriocapillary endothelium in the southern fiddler ray is thin but only minimally fenestrated facing Bruch's membrane and yet elasmobranchs do not possess a choroid gland.

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