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Retinal pigment epithelial fine structure in the short-tailed stingray *(Dasyatis brevicaudata)*

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Summary. The retinal pigment epithelium (RPE), choriocapillaris and Bruch's membrane (complexus basalis) have been studied by light and electron microscopy in the short-tailed stingray *(Dasyatis brevicaudata)*. The RPE consists of a single layer of cuboidal cells which display numerous basal (choroidal) infoldings as well as many apical (vitreal) processes which interdigitate with photoreceptor outer segments. The lateral cell borders are relatively smooth and joined by a series of tight junctions. Within these epithelial cells, smooth endoplasmic reticulum is the dominant organelle with only scattered profiles of rough endoplasmic reticulum. Polysosomes, mitochondria and phagosomes are abundant. Melanosomes are totally absent over the tapetum and are scarce in non-tapetal locations. The RPE nucleus is large, vesicular and centrally located. Bruch's membrane is a pentalaminate structure. The choriocapillaris is a single layer of large capillaries. The endothelium of these capillaries is typically thin but only minimally fenestrated.

Key words: Retinal Pigment Epithelium (RPE), Fine structure, Elasmobranch, Short-tailed Stingray, *Dasyatis brevicaudata*

Introduction

The retinal epithelium (RPE) forms the outermost (scleral) layer of the vertebrate retina and along with the choriocapillaris and Bruch's membrane (complexus basalis) is intimately involved in several processes essential to the proper functioning of the photoreceptors and ultimately 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) mediated by the interphotoreceptor matrix (IPM) (Uehara et al., 1990); 2) the storage and esterification 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 internal adhesion of the sensory retina (Zinn and Benjamin-Henkind, 1979) and 5) the phagocytosis and subsequent digestion of shed outer segment discs (Young, 1978; Bok and Young, 1979).

Because of these several important functions, the RPE region of the vertebrate retina has been investigated in a variety of animals and with a variety of techniques. From a morphological standpoint the essential design of the RPE is remarkably similar in all vertebrates described to date but with generic differences usually present (Nguyen-Legros, 1978; Kuwabara, 1979; Braekevelt, 1983, 1985, 1986, 1988, 1990, 1992a, 1993).

While numerous reports of the fine structure of the RPE are available, relatively few deal with elasmobranchs (sharks and rays) (Nicol, 1989; Braekevelt, 1992b). Consequently as part of the comparative morphological study of this region in vertebrates, the fine structure of the RPE, Bruch's membrane (complexus basalis) and choriocapillaris in the short-tailed stingray *(Dasyatis brevicaudata)* is reported in this study.

Materials and methods

For this study, the eyes from two adult light-adapted short-tailed stingrays *(Dasyatis brevicaudata)* were examined by light and electron microscopy. The specimens were killed by severing the spinal cord and the eyes quickly removed. 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 mm^2. This tissue was then postfixed for 2 h in 1% osmium tetroxide in the same phosphate buffer, dehydrated through graded ethanols to methanol and then to propylene oxide and embedded in Araldite.

Pieces of plastic-embedded tissue were reoriented to

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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 short-tailed stingray *(Dasyatis brevicaudata)* consists of a single layer of cuboidal cells which average about 10 μm in height (Fig. 1). This species possesses a large tapetum lucidum located in the choroid of the superior fundus but the RPE layer does not display marked differences in morphology to reflect this. In all locations the retinal epithelial cells show numerous basal (scleral) infoldings which penetrate to much the same depth (about 1.2 μm) in both tapetal and non-tapetal locations (Figs. 1, 3, 6). Apically (vitreally) the RPE cells display numerous finger-like processes which enclose rod photoreceptor outer segments (Figs. 1, 3). Laterally the cell borders of these epithelial cells are joined in their mid to apical region by a series of tight junctions (Figs. 1-3).

Internally the RPE cells display a single large vesicular nucleus which is normally spherical and quite centrally located (Figs. 1, 4). Smooth endoplasmic reticulum (SER) is the most abundant cell organelle and dominates the cytoplasm (Figs. 1, 4). Profiles of rough endoplasmic reticulum (RER) are extremely scarce while polysomes are scattered throughout the cytoplasm (Figs. 3, 4). Small rounded mitochondria are abundant in the basal region of the RPE cells where they are predominantly clustered at the bottom of the basal infoldings (Figs. 1, 2, 4). Phagosomes of shed outer segmental material are noted in these light-adapted specimens, usually in an advanced state of lysosomal digestion (Figs. 1, 3, 4). Lysosome-like bodies were also noted within these RPE cells while Golgi zones were scarce (Figs. 3, 4). Myeloid bodies were not observed within the RPE cells of these light-adapted specimens.

Over the choroidally located tapetum lucidum the retinal epithelial cells are totally devoid of melanosomes (Figs. 1, 2). In non-tapetal locations, melanosomes were occasionally noted but never in sufficient numbers that they could form an effective barrier to incoming light. The choroid is more heavily pigmented than the RPE layer in all locations (both tapetal and non-tapetal) and probably acts as the light absorbing layer in this species (Figs. 1, 2, 5).

Bruch's membrane or complexus basalis in the short-tailed stingray is in all locations a pentalaminate structure (Figs. 1, 4, 5). It consists of 1) the basal lamina of the RPE layer, 2) the basal lamina of the choriocapillaris, 3) a central layer of elastic tissue, and 4) an inner and 5) an outer collagenous layer (Figs. 1, 4). In some locations the central elastic layer is but poorly represented but in most locations is a distinct and continuous layer (lamina densa) (Figs. 2, 4, 6). Bruch's membrane averages about 1.0 μm in thickness in all locations (Figs. 3-5).

The choriocapillaris consist of a single layer of large-calibre capillaries immediately adjacent to Bruch's membrane (Figs. 1, 2, 4, 5). Except for their nuclear region the endothelial cells of these capillaries are quite thin facing both Bruch's membrane and the choroid (Figs. 1, 2, 5). In all locations the endothelium facing Bruch's membrane displays a moderate number of fenestrations (Figs. 2, 4). In tapetal locations, the endothelium facing the choroid also shows a few fenestrations (Fig. 5).

Discussion

The retinal epithelial region of the short-tailed stingray *(Dasyatis brevicaudata)* is morphologically very similar to that described for other vertebrate species (Nguyen-Legros, 1978; Kuwabara, 1979; Braekevelt, 1988, 1989, 1990, 1993) including elasmobranchs (Braekevelt, 1992b).

As in all report species, the RPE in this stingray consists of a single layer of cells. The height of RPE cells varies widely amongst vertebrates from the tall columnar cells reported for many teleosts (Braekevelt, 1985, 1990) to the squamous type noted in marsupials (Braekevelt, 1993; Young and Braekevelt, 1993). In most species however the RPE consists of cuboidal cells (Kuwabara, 1979) and that is the case in this elasmobranch.

Retinal epithelial cells normally display an extensive array of basal infoldings which are truly microfolds (rather than microvilli) and are felt to be indicative of a heavy involvement by these cells in the transport of materials from the choriocapillaris to the photoreceptors (Steinberg and Miller, 1973). The abundant apical processes typical of 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 morphologically distinct types of apical

Fig. 1. Low power electron mcirograph to illustrate the retinal epithelium (RPE), photoreceptor outer segments (OS) and a portion of the tapetum lucidum (TL). x 5,900

Fig. 2. Electron micrograph to indicate the abundance of smooth endoplasmic reticulum (SER), the extensive basal infoldings (BI) and a lateral cell junction (J). The choriocapillaris (CC) and the tapetum lucidum (TL) are also indicated. x 9,800

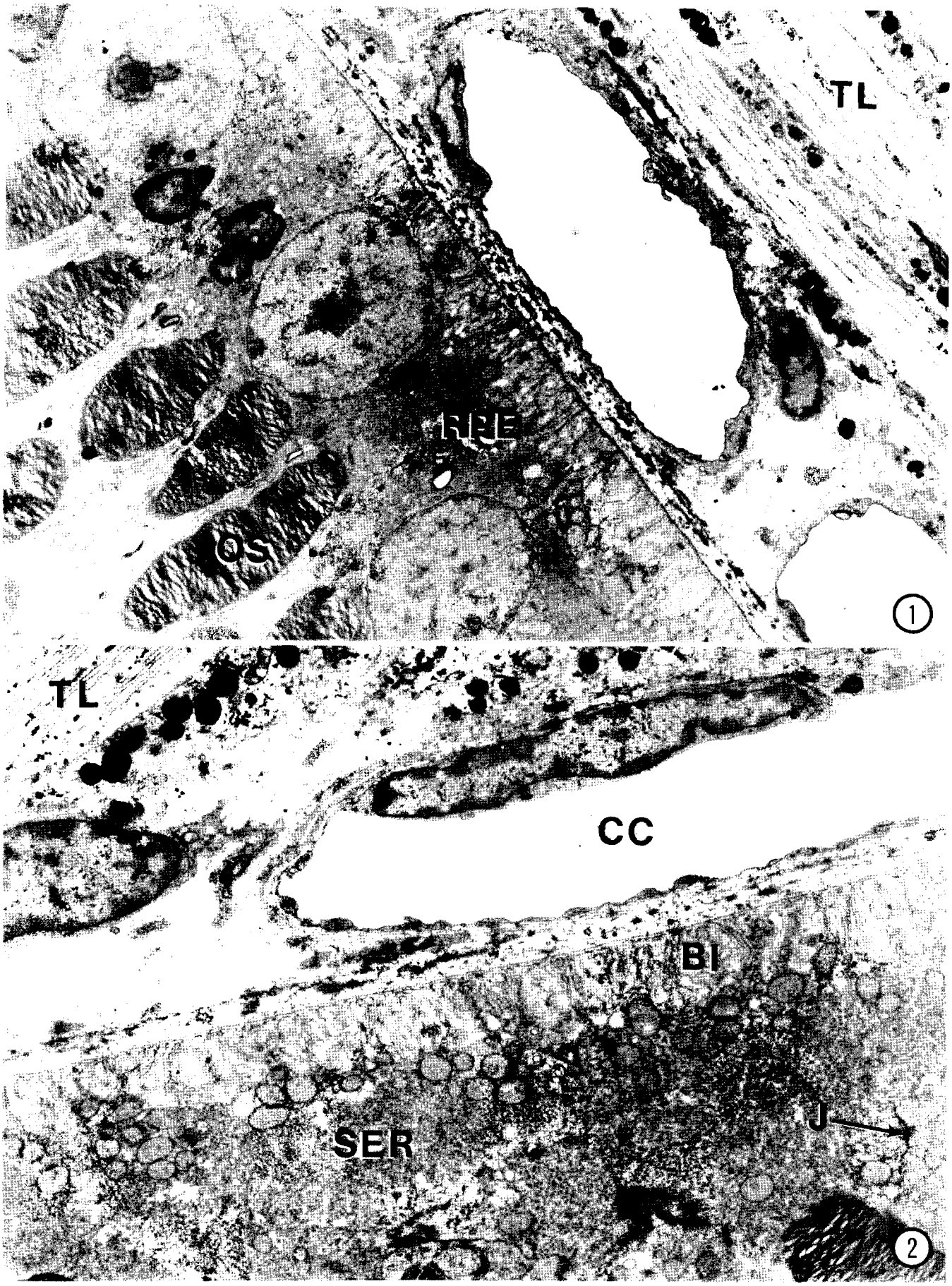
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Fig. 3. Electron micrograph of the RPE in a non-tapetal location. Phagosomes (Ph) of outer segment material, abundant smooth endoplasmic reticulum (SER) and photoreceptor outer segments (OS) are all labelled. Note the absence of melanosomes. x 6,200

Fig. 4. Electron micrograph to illustrate the thin fenestrated choriocapillary (CC) endothelium facing Bruch's membrane (B). An RPE nucleus (N) is also indicated. x 9,500

Fig. 5. Electron micrograph from a tapetal location. The tapetum (TL) is indicated as is Bruch's membrane (B). Note the fenestrations in the choriocapillaris (CC) endothelium facing both the tapetum lucidum and Bruch's membrane. x 9,500

Fig. 6. Electron micrograph of a tangential section through Bruch's membrane (B) and the basal infoldings (BI) of the RPE to illustrate the extent of the microfolds. x 14,000

process are reported (Steinberg and Wood, 1974; Braekevelt, 1982, 1989) in the short-tailed stingray only one type of finger-like process is noted.

The series of tight junctions located at the lateral cell borders of RPE cells is a constant feature in all vertebrate species (Nguyen-Legros, 1978). Collectively these form Verhoeff's membrane and constitute an effective barrier to the intercellular movement of materials to form part of the blood-ocular 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 (Kuwabara, 1979; Braekevelt, 1986, 1988, 1990, 1992a). The preponderance of SER reflects the heavy involvement of this epithelial layer in the transport, esterification and storage of lipid photopigment precursors (Zinn and Benjamin-Henkind, 1979). The lack of RER noted within 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 are still providing protein for their internal requirements.

The abundance of mitochondria within RPE cells is a common feature seen in all species and is indicative of the high energy requirements of these cells as they fulfil their various functions. The presence of phagosomes of outer segment material and lysosome-like bodies within the RPE cells of the short-tailed stingray is also very typical as the phagocytosis and subsequent degradation of outer segment discs is one of the main functions of this epithelial layer in all vertebrate species (Nguyen-Legros, 1978; Young, 1978).

Myeloid bodies which are stacked arrays of membranes usually formed by the SER and which are a common feature in most lower (non-mammalian) vertebrates are not present within the light-adapted RPE cells of the short-tailed stingray, nor were they noted in another elasmobranch, the guitar fish *(Trygonorhina fasciata)* (Braekevelt, 1992b). The function of myeloid bodies is uncertain although they have been implicated as the organelle that triggers photomechanical movements (Porter and Yamada, 1960) as well as being storage sites for lipids prior to esterification (Yorke and Dickson, 1984, 1985).

As is the case 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, 1989). This allows for the free passage of light to and from the tapetum. In non-tapetal locations, the RPE is normally pigmented (Braekevelt, 1986, 1990). In this species as in the guitar fish (Braekevelt, 1992b) the extra tapetal RPE is characterized by only a few melanosomes 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 characeristically only show a trilaminate Bruch's membrane with the central elastic layer (lamina densa) being absent (Braekevelt, 1980, 1985). In this elasmobranch and in the guitar fish (Braekevelt, 1992b), Bruch's membrane is pentalaminate.

The choriocapillaris in all vertebrate species 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 choriocapillary endothelium (Braekevelt, 1985). The choriocapillary endothelium in the guitar fish (Braekevelt, 1992b) and in this species is thin but only minimally fenestrated facing Bruch's membrane and yet elasmobranchs do not have a choroid gland. In the short-tailed stingray the endothelium facing the tapetum lucidum also displays some fenestrations which may indicate that the choriocapillaris is also supplying the tapetum lucidum to some extent.

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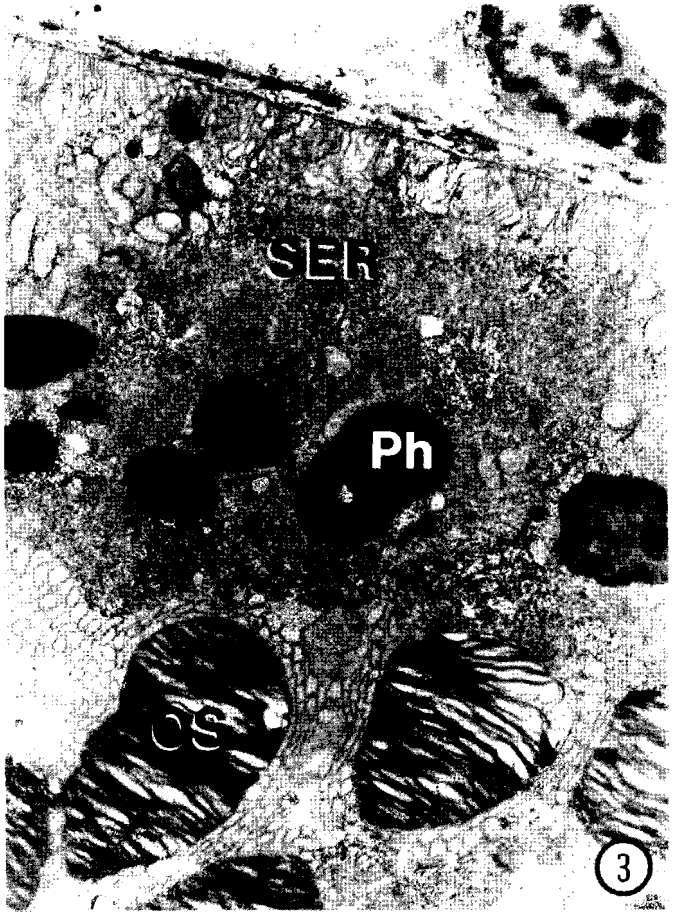
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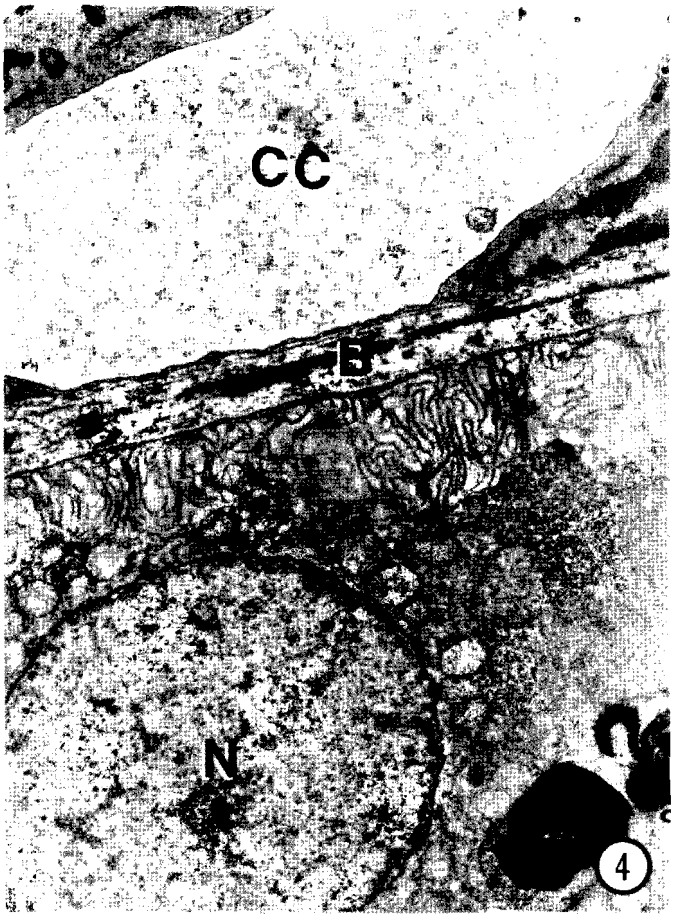
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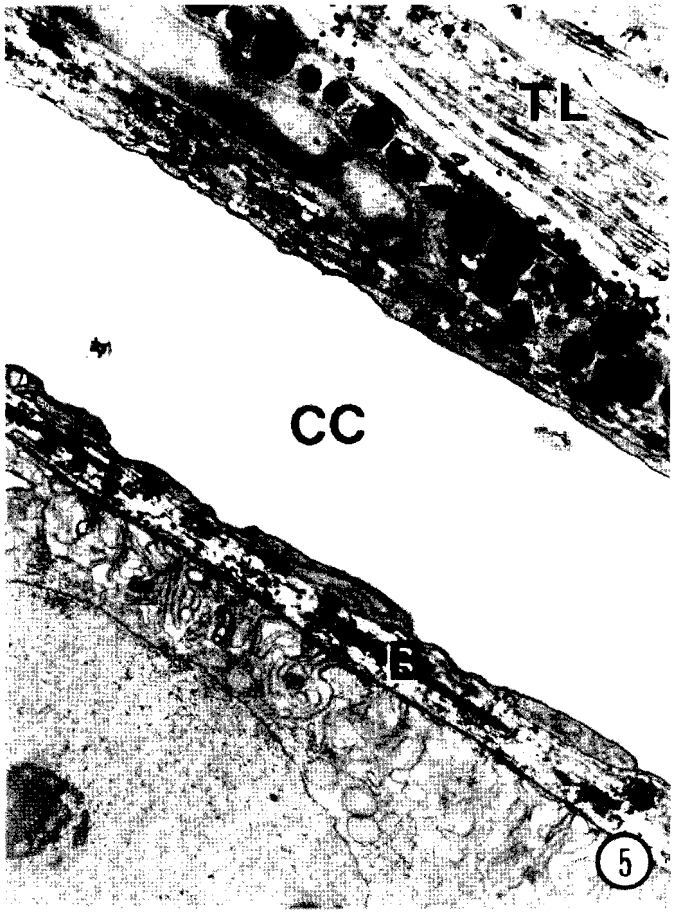
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