

# The retinal pigment epithelium of the teleost *Notopterus notopterus* (Pallas): Appearance of basal infoldings during prolonged dark-adaptation

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**Summary.** In teleosts, the basal part of the retinal pigment epithelium (RPE) is relatively smooth, i. e., it is free of basal membrane infoldings. In the featherback, *Notopterus notopterus*, whereas this is the situation in light adaptation, during dark-adaptation, especially when kept for prolonged periods (6-9 hour), numerous infoldings appear at the basal region, as found uniquely by transmission electron microscopy. In this teleost, during retinomotor movements, the rods move vitreally during dark-adaptation, while the cones do not elongate, and remain stationary in both light- and dark- adaptation. The significance of the appearance of basal infoldings in dark-adapted RPE is explained in terms of the pattern of retinomotor responses and the features of RPE and photoreceptors in this species. It is suggested that (1) the thick, impervious tapetal layer present in the RPE, (2) the unusual position of the photoreceptors in the visual cell layer of dark-adapted retina, and (3) the presumably high demand for glucose and O<sub>2</sub> of the outer retina during dark-adaptation might contribute to cause this phenomenon in this species. The available evidence tend to associate this phenomenon with the involvement of the RPE in nutrient and O<sub>2</sub> delivery to the photoreceptors via the basal infoldings of the RPE in dark-adapted state in this species. This has not been reported for any other teleosts to date.

**Key words:** Retinal pigment epithelium, Basal infoldings, Teleost, Dark-adaptation, Retinomotor response

## Introduction

The retinal pigment epithelium (RPE) is the outermost (scleral) layer of the vertebrate retina. Typically, it consists of a single layer of cuboidal to low

columnar epithelial cells, and is identified by its characteristic possession of the brownish melanin pigments. In certain mammals and teleosts, these cells are reported to be exceptionally tall and broad (Steinberg and Wood, 1974; Lockett, 1977; Wagner and Ali, 1978; Nag, 1993). The individual cells appear to taper and at the apex, they possess numerous fine, cytoplasmic processes that interdigitate with photoreceptor outer segments (Bairati and Orzalesi, 1963; Steinberg and Wood, 1974; Pfeffer and Fisher, 1981; Nag, 1994). Basally, the RPE is highly infolded, especially in mammals (Bernstein, 1961; Spitznas, 1974; Braekevelt, 1983, 1988), whereas in teleosts, this region is reported to be relatively smooth (Braekevelt, 1982a; Braekevelt et al., 1998; Collin et al., 1996).

The choroidal circulation is responsible for supplying nutrients to the outer retina, especially to the RPE and photoreceptors, whereas the retinal circulation provides with nutrition to the inner retinal layers (Rodieck, 1973; Delaey and Van de Voorde, 2000). In teleosts, the retina is avascular and therefore, it depends on the choroidal circulation for its nutrition and O<sub>2</sub> supply (Walls, 1942). Experimentally, it has been found that the retinal demand for O<sub>2</sub> and glucose varies over a circadian light-dark cycle: the photoreceptors, e.g., consume less O<sub>2</sub> during light adaptation, whereas the consumption increases during dark-adaptation (Bill and Sperber, 1990; Wang et al., 1997). The retina of teleosts shows vigorous movements of photoreceptors and melanin pigments in response to changing ambient illumination. This phenomenon, which is known as retinomotor responses, allows the eyes to adapt to photopic or scotopic vision (Walls, 1942; Ali, 1975). Although this phenomenon is common to the majority of the teleosts studied thus far, some species variations are there; for example, in the mormyrids, and in goldeyes and mooneyes, the cones do not migrate during dark-adaptation (McEwan 1938; Wagner and Ali, 1978; Braekevelt, 1982b; Nag and Bhattacharjee, 2002).

In an experiment on light- and dark-adaptive retinomotor responses in the teleost *Notopterus*

*notopterus*, it was found that the retinal rods contracted vitreally during dark-adaptation, while the cones did not elongate, their outer segments remained stationary near the apex of the photoreceptor bundles, as in light adaptation. Additionally, there was an associated anatomical change at the basal border of the RPE during dark-adaptation: the smooth basal border of the RPE, as seen in light adaptation, changed into an infolded structure during prolonged dark-adaptation. The present paper reports on this novel finding and explains its possible significance in this teleost.

## Material and methods

### Specimens

Specimens of *N. notopterus* (order Osteoglossiformes), a shallow water-dwelling species, were procured from dealers at Kolkata, eastern India. Their standard length ranged from 13.3 to 22.7 cm, n=13). They were reared in glass aquaria for 7-10 days at 12 h light: 12 h dark photoperiod regime. The light-adapted specimens (n=3) were sacrificed during midday (12 noon). The retinal samples (embedded in Epon 812) of three light-adapted specimens from an earlier study (Nag, 1993) were pooled and reexamined. Dark-adaptation was carried out by keeping the specimens (n=10) in well-aerated, large earthen pitchers (water temperature: 22-23 °C) and the mouth sealed with aluminium foils. The light was switched off in the evening at 6 p.m. and the dark-adaptation continued for 9 h. The specimens were sacrificed by decapitation at 3 h intervals under a dim, red lamp and the eyeballs enucleated.

In a similar way, healthy specimens of another teleost, *Labeo rohita* (Cyprinidae; n= 3, length: 11-15.7 cm) were reared in separate pitchers in dark condition, as mentioned above. The specimens were sacrificed, and their eyeballs enucleated, trimmed off cornea and lens, fixed and processed for transmission electron microscopy (TEM). Earlier, by light microscopy, the species was found to exhibit prominent retinomotor rhythms involving rod contraction and cone elongation during dark-adaptation (unpublished). The features of the RPE were not examined by TEM.

### TEM

The eyeballs (from both light-and dark-adaptation) were trimmed off cornea and lens, and fixed in 2.5% glutaraldehyde and 1% paraformaldehyde in 0.1 M phosphate buffer (pH 7.3) for 3 h at 4 °C. The retina was separated from the globe, cut region wise (dorsal, ventral and centre of fundus), washed, and postfixed in 1% OsO<sub>4</sub> for 2 h at 4 °C. The materials were dehydrated, infiltrated and embedded in araldite CY212. Thick sections (1 μm) were cut, stained with toluidine blue and observed under a light microscope. Thin sections (60-70 nm) were contrasted with uranyl acetate and lead citrate

and viewed under a Philips CM10 transmission electron microscope at an operative voltage of 60 kv.

## Results

The retina of *N. notopterus* was peculiar in that the photoreceptors were arranged in bundles (Fig.1). The duplex nature of its retina was earlier described by Ali and Anctil (1976). The photoreceptor layer was exceptionally thick, and measured about 240 μm at the centre of the fundus (specimen length: 20.0 cm). Rods and cones occurred separately in individual bundles, the rod outer segments were located at the scleral half, whereas the cone outer segments occupied the vitreal half of the photoreceptor layer in light-adapted retina (Fig. 1A). The rods were approximately 150 μm long (from the external limiting membrane to the tip of their outer segments) in light adaptation. The cones possessed exceptionally long inner segments (ca 50 μm) and due to this morphological peculiarity, they were not contracted near the external limiting membrane, as seen in most teleosts (Ali and Anctil, 1976). Thus, the cone outer segments were located (at the apex of the photoreceptor bundles) at approximately 75-80 μm sclerad from the external limiting membrane to the tip of their outer segments. Many features of the photoreceptors of *N. notopterus* resembled with those described for the goldeye, *Hiodon alosoides* (Wagner and Ali, 1978; Braekvelt, 1982b) and therefore, are not mentioned here.

As was reported earlier (Nag, 1993), the retinal pigment epithelial cells were extremely tall in this species, each measured about 180 μm (in a 20.0 cm long specimen). There was a paucity of melanosomes and the pigment epithelial cells were studded with reflective crystals that collectively formed the retinal tapetum lucidum (Figs. 1, 2A).

During dark-adaptation, the melanosomes migrated towards the basal part (Fig. 2A) and apices of the RPE (Fig. 2B). The tapetal crystals concentrated near the centre of the RPE (Fig. 2A). The rod inner segments contracted to the vitread-half of the photoreceptor layer (Fig. 1B). The cone inner segments did not elongate at all even after 9 h of dark adaptation (Fig. 1B). Thus, the outer segments of both rods and cones were aligned at the same level (vitread-half), and within the same photoreceptor bundles in dark-adapted retinas of all 10 specimens.

In light-adapted retinas (n=6), the basal part of the RPE was smooth (Fig. 3) in all retinal regions examined. In dark-adapted retina, numerous infoldings appeared at its basal surface (Figs. 4-6). In the initial three hours of dark-adaptation, however, the RPE did not show any presence of infoldings, other than the basal movement of the melanin granules and nucleus. The infoldings appeared during prolonged period of dark-adaptation only (>6 h). Furthermore, observations have suggested that the basal infoldings elongated with time, they were taller and more prominent in specimens that were dark-

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adapted for nine hours (Fig. 6A, B, D) than in those kept for six hours (Figs. 4, 5). The presence of basal infoldings was consistently found in seven specimens that were reared for 6-9 h under dark condition. No inter-individual variations in the appearance or morphology of these infoldings, was observed.

Bruch's membrane was pentalaminate, as described earlier (Nag, 1993) and appeared normal in all specimens. The fenestrae of the endothelium of the choriocapillaris were present (Fig. 6C) in all materials examined.

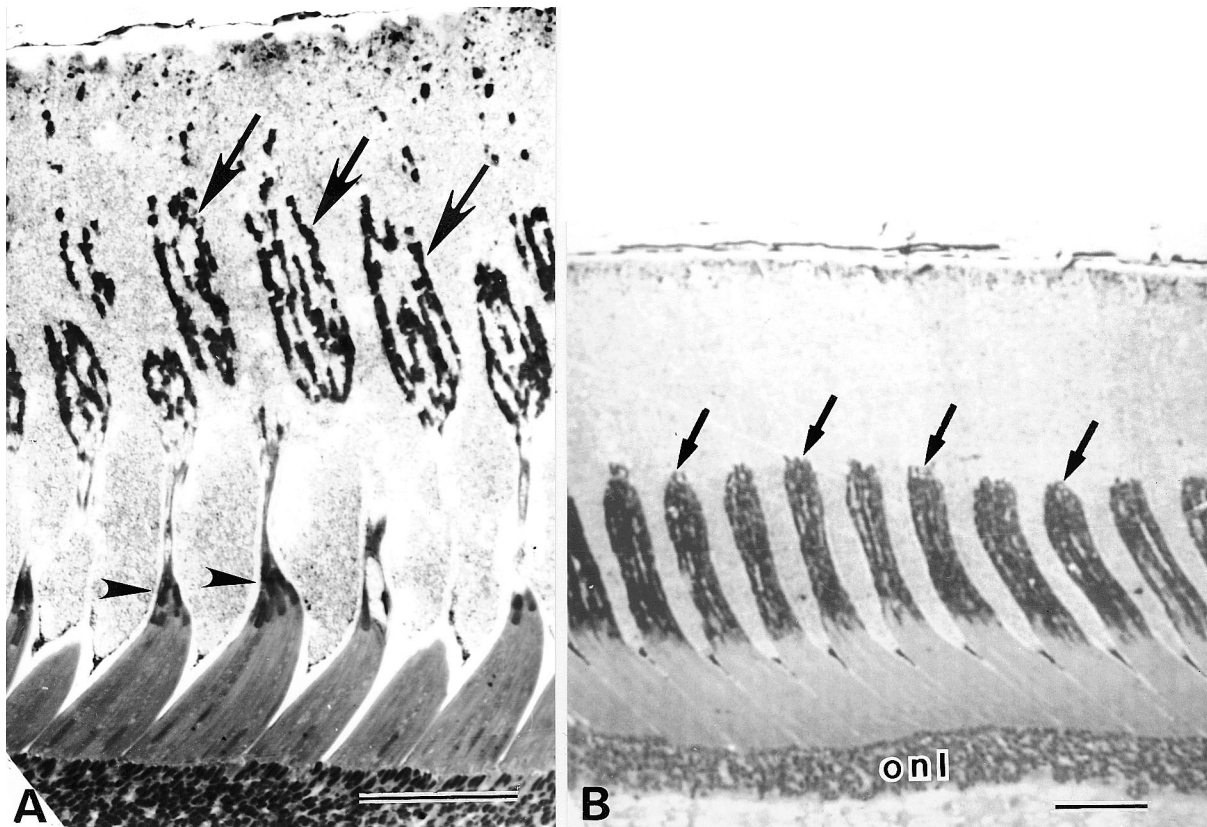
There were no basal infoldings in dark-adapted RPE of *Labeo rohita* (Fig. 7). The RPE appeared normal in its appearance; organelles like mitochondria, smooth endoplasmic reticulum and phagosomes were plentiful. Lateral cell junctions between the pigment epithelial cells were present. The melanosomes were abundant and appeared to have concentrated towards the basal aspect of the RPE in dark-adapted samples.

### Discussion

Under certain physical conditions, such as retinal exposure to light, there is an increase of the choroidal

blood flow velocity (Parver et al., 1982). During dark-adaptation, on the other hand, there is an associated decrease of the choroidal blood flow, as has been found in human (Havelius et al., 1999). Experimental studies with animals have shown increased retinal metabolic demands during dark-adaptation, while consumption of glucose and O<sub>2</sub> increases significantly (Bill and Sperber, 1990; Wang et al., 1997). It is suggested that, irrespective of light and dark conditions, the inner retina is in a state of maintaining its oxidative metabolism (Havelius et al., 1999). Based upon these facts, it is logical to presume that the demand for O<sub>2</sub> and nutrients of the outer retina, especially the photoreceptors, should be higher in dark-adapted state than compared with that in light adaptation.

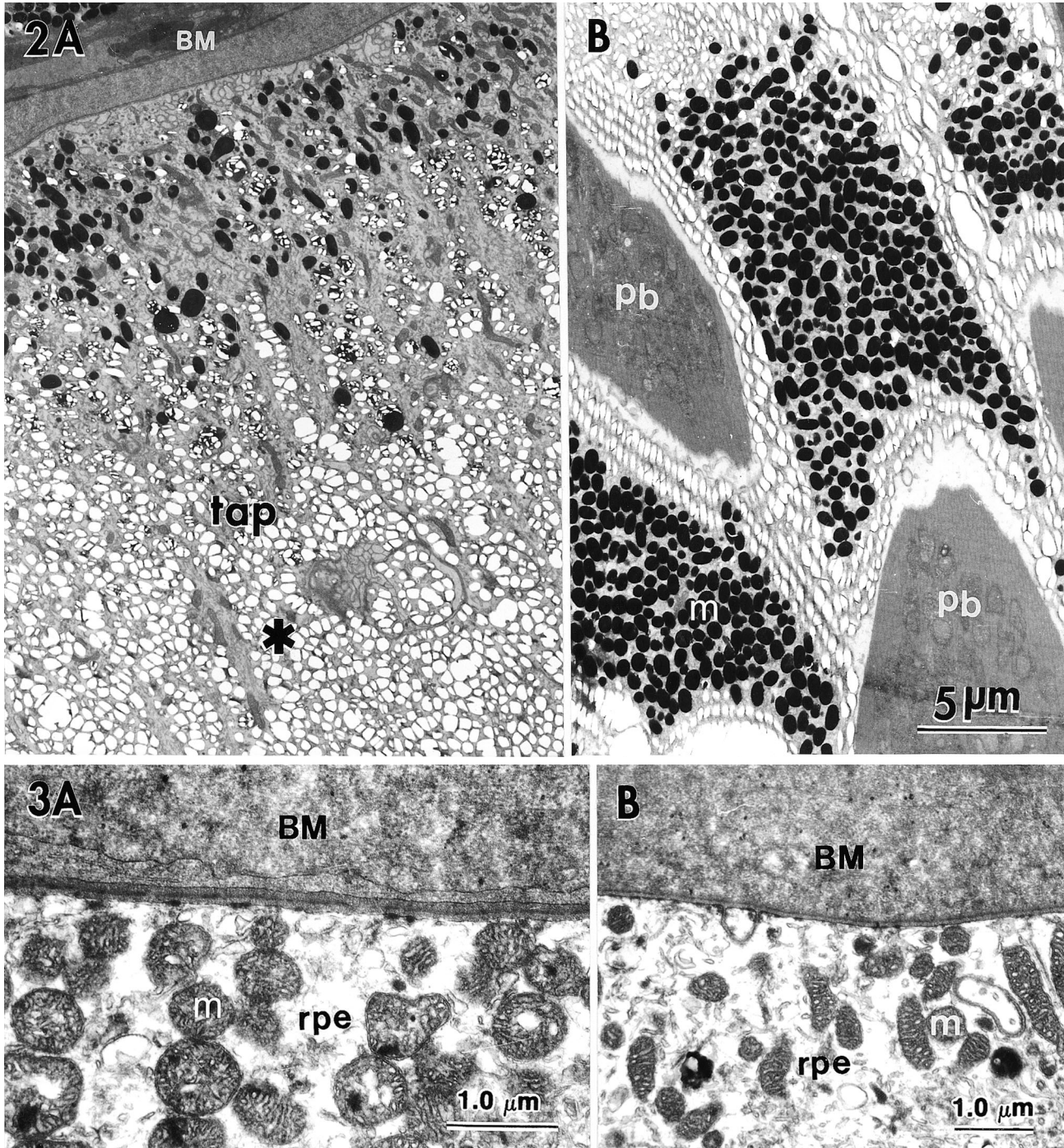
In mammals, the basal part of the RPE is highly infolded. This anatomical arrangement is held to assist in active fluid transport by the RPE into the photoreceptor layer. As the outer retina is avascular, it is believed that O<sub>2</sub> and nutrient requirements to the photoreceptors are met by the active fluid transport via the basal infoldings of the RPE (Bernstein, 1961; Spitznas, 1974). Whereas basal infoldings are reported in some elasmobranchs (e. g., stingrays, Braekevelt, 1994), on the contrary, in



**Fig. 1.** Light micrographs of the retina of *N. notopterus*. In light-adapted retina (A), rods (arrows) and cones (arrowheads) occupy the scleral and vitreal half, respectively, of the photoreceptor layer. In dark-adapted retina (B), both rods and cones lie within the same photoreceptor bundles (arrows). There is no cone elongation. onl, outer nuclear layer (in B). Scale bars: 50  $\mu$ m.



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**Fig. 2.** Electron micrographs of dark-adapted (6 h) retinal pigment epithelium. Melanosomes are located basally, near Bruch's membrane (BM, **A**) and at the apex (**B**, m) of pigment epithelial processes ensheathing the photoreceptor bundles (pb). Note the tapetum (tap) in **A**, the tapetal crystals are concentrated towards the middle (O) of the pigment epithelium. Scale bar: 5 μm in both micrographs.

**Fig. 3.** Electron micrographs of the retinal pigment epithelium (rpe) in light adaptation. Note that there are no basal infoldings. BM: Bruch's membrane; m: mitochondria.

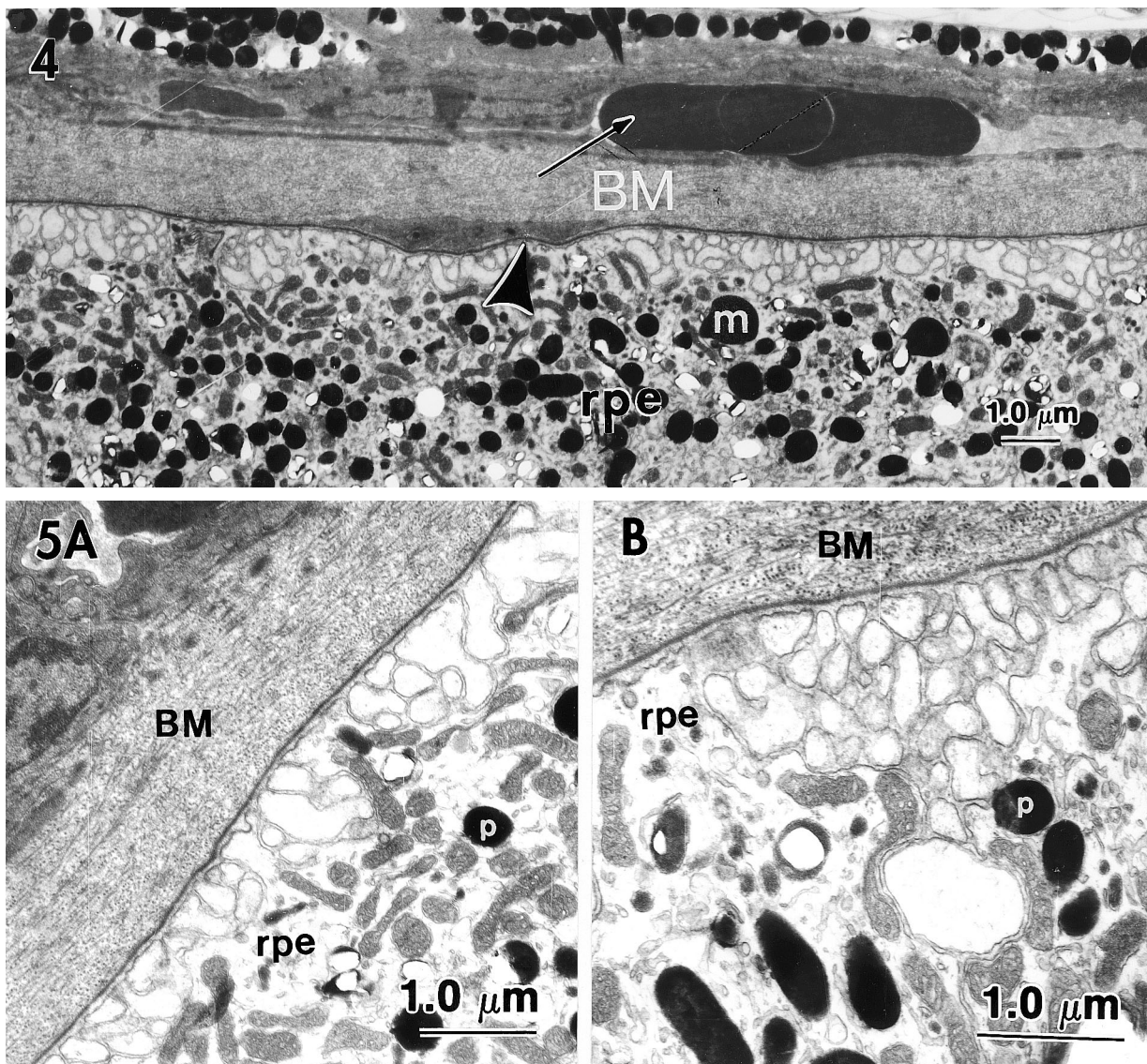


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teleosts, the basal part of the RPE is seen to be smooth (Braekevelt, 1982a; Braekevelt et al., 1998; Nag, 1993; Collin et al., 1996). Although the outer retina of the teleosts is also avascular, their choroid has a high  $O_2$  tension owing to the presence of a peculiar rete called the choroid gland (Barnett, 1951). The latter consists of many, small closely packed arterial and venous capillaries, arranged to act like a counter-current system. According to Wittenberg and Wittenberg (1962), the choroid gland is responsible for gas secretion into the teleost eye. It is, therefore, possible that the choroid of

teleosts delivers a large flow of  $O_2$  to the outer retina, thereby minimising the need for fluid transport by the RPE in lower vertebrates like teleosts (Braekevelt, 1982a).

One might argue then, why does the basal part of the RPE become infolded during dark-adaptation in *N. notopterus* retina? The present author is of the idea that this could be a sign of novel adaptive response of the RPE during dark-adaptation to increased nutritional demand of the photoreceptors in this species. According to the concept of Michaelson (1954), the choriocapillaris



**Fig. 4.** Part of the dark-adapted retinal pigment epithelium (rpe, 6 h) from the centre of fundus, showing basal infoldings. The arrowhead indicates the basal membrane of rpe. M: melanosomes. One nucleated RBC is indicated (arrow) inside the choriocapillaris. BM: Bruch's membrane.

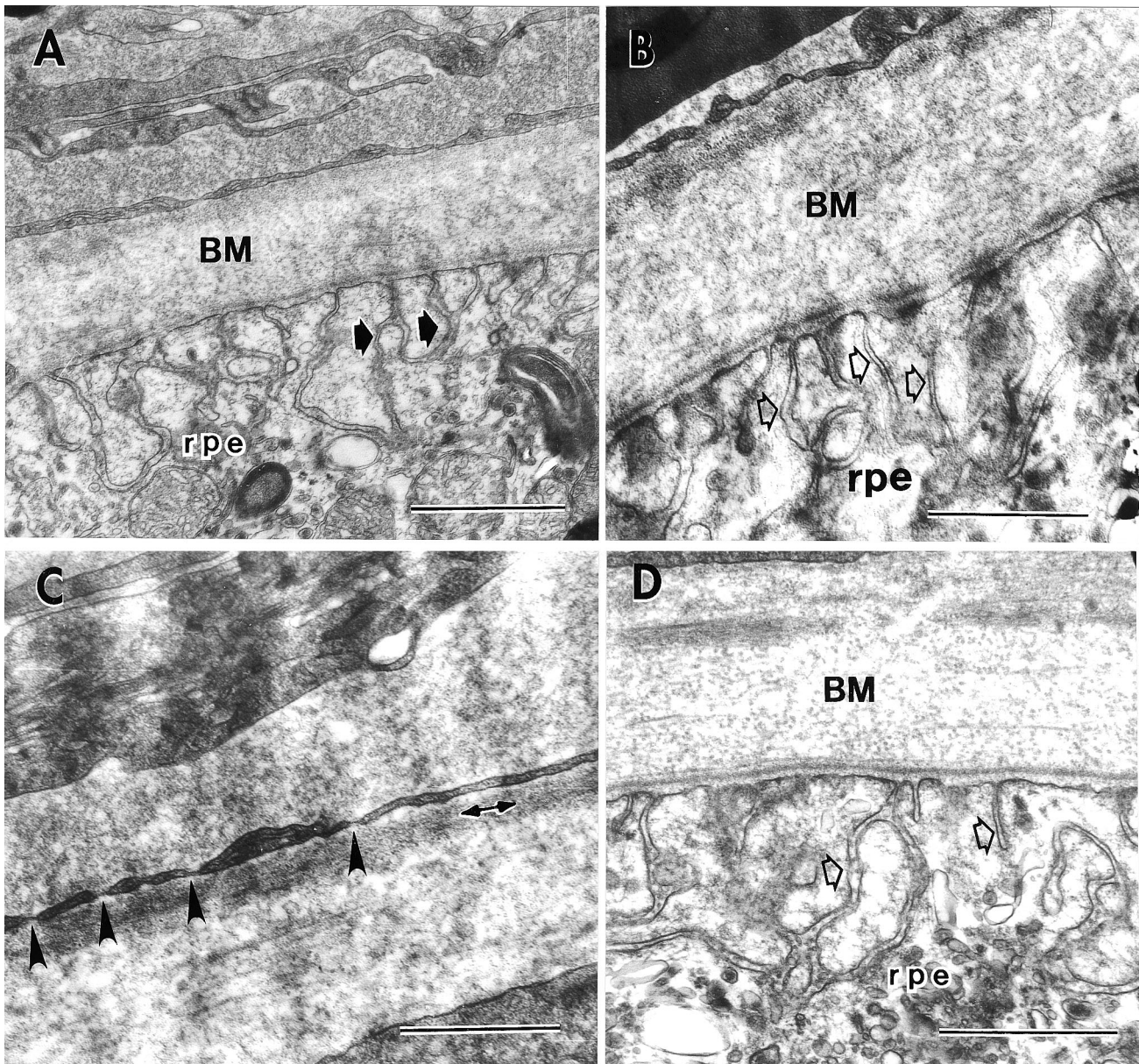
**Fig. 5.** Basal infoldings of retinal pigment epithelium (rpe) from dorsal (A) and ventral retina (B), after 6 h of dark-adaptation. BM: Bruch's membrane; p: melanin pigment.



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can supply with nutrients and  $O_2$  to the outer retina for a distance of  $130\ \mu\text{m}$  only. In *N. notopterus* retina, as already mentioned, the photoreceptor layer is exceptionally thick. In light adaptation, the elongated rods occupy the scleral half of the photoreceptor layer (within a distance of  $80\text{-}120\ \mu\text{m}$  vitread from the basal lamina of the RPE) and therefore, are closer to the source of the choroidal circulation. The cone outer segments, on the other hand, are located towards the middle of the photoreceptor layer. Like many teleosts, this species must also possess a high choroidal  $O_2$

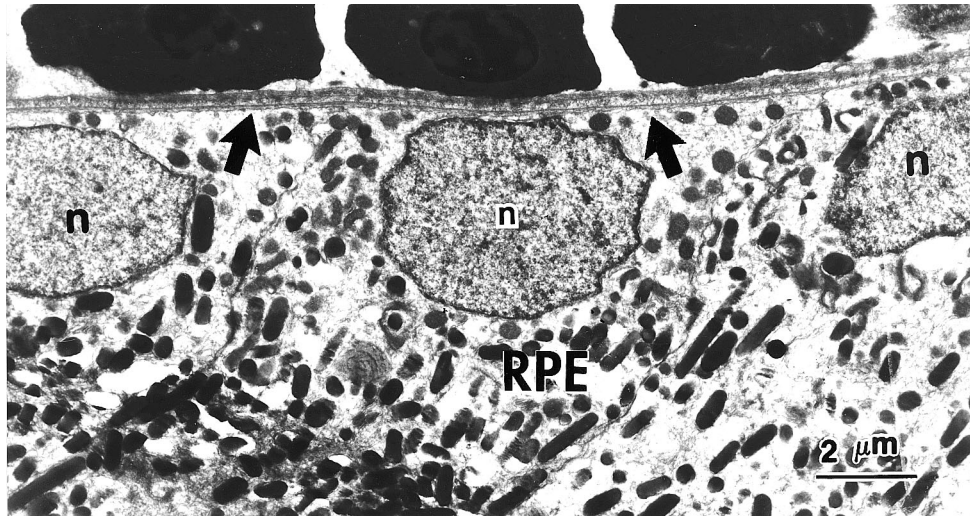
tension. This is evident, indirectly, from the existence of a large horse-shoe shaped choroidal gland in this species.  $O_2$  and nutrient supply, therefore, can easily be met to the photoreceptors from the choriocapillaris circulation without the aid of active fluid transport by the RPE in light adaptation. Perhaps this is the reason why the basal part of the RPE remains smooth in light adaptation in this species (and also in all other teleosts reported to date). During dark-adaptation, on the other hand, the rod inner segments contract towards the vitreal half of the photoreceptor layer (distance: approximately  $150\text{-}170$



**Fig. 6. A, B, D.** Electron micrographs showing tall, prominent basal infoldings (arrows) of retinal pigment epithelium (rpe) after 9 h of dark-adaptation (respectively from three different specimens). BM: Bruch's membrane. **C.** showing endothelial fenestrae (arrowheads). The basal lamina of the endothelium of choriocapillaris is indicated by arrow (bothway).



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**Fig. 7.** Part of the dark-adapted retinal pigment epithelium (RPE) of *Labeo rohita*. The nuclei of RPE, now become spherical, have moved basally, as did the melanosomes (features of dark adaptation). Note that there are no basal infoldings, the basal membrane of RPE (arrows) is smooth.

$\mu\text{m}$  from the basal lamina of the RPE). Since they are now actively engaged in scotopic vision, their energy demand must have been increased significantly. As choroidal blood flow has diminished in dark-adaptation, the best possible way to provide with nutrients and  $\text{O}_2$  to the rods (now lying far away from the source of choriocapillaris circulation) is by making basal infoldings to facilitate active fluid transport via them.

The significance of the abnormal migration of melanosomes towards the apices of the retinal pigment epithelial cells during dark-adaptation is not clearly understood. To some extent, this arrangement may help in shielding the cones located vitreally, in order to protect them from sudden exposure to bright light.

Let us critically judge if other factors could be involved somehow in the phenomenon of appearance of basal infoldings in dark-adaptation. Locket (1977, p 153), while describing the adaptive features of the tapeta lucida in bony fishes, pointed out that the retinal tapetum behaves as an impermeable layer and therefore might hinder  $\text{O}_2$  supply to the outer retina from the choroidal circulation. As mentioned earlier, there is a retinal tapetum in *N. notopterus*. In light adaptation, the tapetal bodies are distributed along the whole length of the retinal pigment epithelial cells. During dark-adaptation, these reflective bodies tend to aggregate in the mid region of the epithelial cells, a fact that was also observed in goldeyes and mooneyes having retinal tapeta lucida (Braekevelt, 1982a). As the photoreceptor outer segments are aligned close to the apex of the photoreceptor bundles in dark-adapted retina, the tapetal crystals, by way of their location in the middle of the pigment epithelial cells, might interfere with  $\text{O}_2$  diffusion from the choriocapillaris to the photoreceptors.

The appearance of basal infoldings during dark-adaptation may require some sort of structural support and in this respect, the author wonders if the elastic lamina of Bruch's membrane, a feature to the eye of *N.*

*notopterus* (Nag, 1993) plays a supportive role in it.

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## References

- Ali M.A. (1975). Retinomotor responses. In: Vision in fishes. Ali M.A. (ed.). Plenum Press. New York. pp 313-355.
- Ali M.A. and Ancil M. (1976). Retinas of fishes. Springer-Verlag. Berlin.
- Bairati A. and Orzalesi N. (1963). The ultrastructure of the pigment epithelium and of the photoreceptor-pigment epithelium junction in the human retina. *J. Ultrastruct. Res.* 9, 484-496.
- Barnett C.H. (1951). The structure and function of the choroidal gland of teleostean fish. *J. Anat.* 85, 113-119.
- Bernstein M.H. (1961). Functional architecture of the retinal epithelium. In: The structure of the eye. Smelser G.K. (ed.). Academic Press. London. pp 139-150.
- Bill A. and Sperber G.O. (1990). Aspects of oxygen and glucose consumption in the retina: effects of high intraocular pressure and light. *Graefes Arch. Clin. Exp. Ophthalmol.* 228, 124-127.
- Braekevelt C.R. (1982a). Fine structure of the retinal epithelium and retinal tapetum lucidum of the goldeye (*Hiodon alosoides*). *Anat. Embryol.* 164, 287-302.
- Braekevelt C.R. (1982b). Photoreceptor fine structure in the goldeye (*Hiodon alosoides*) (Teleost). *Anat. Embryol.* 165, 177-192.
- 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. (1988). Retinal epithelial fine structure in the vervet monkey (*Cercopithecus aethiops*). *Histol. Histopathol.* 3, 33-38.
- Braekevelt C.R. (1994). Retinal pigment epithelial fine structure in the short-tailed stingray (*Dasyatis brevicaudata*). *Histol. Histopathol.* 9,

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- 501-506.
- Braekevelt C.R., Smith S.A. and Smith B.J. (1998). Fine structure of the retinal pigment epithelium of *Oreochromis niloticus* L. (*Cichlidae: Teleostei*) in light- and dark-adaptation. *Anat. Rec.* 252, 444-452.
- Collin S.P., Collin H.B., Ali M.A. (1996). Ultrastructure and organization of the retina and pigment epithelium in the cutlips minnow, *Exoglossum maxillingua* (Cyprinidae, Teleostei). *Histol. Histopathol.* 11, 55-69.
- Delaey C. and Van de Voorde J. (2000). Regulatory mechanisms in the retinal and choroidal circulation. *Ophthalmic Res.* 32, 249-256.
- Havelius U., Hansen F., Hindfelt B. and Krakau T. (1999). Human ocular vasodynamic changes in light and darkness. *Invest. Ophthalmol. Vis. Sci.* 40, 1850-1855.
- Locket N.A. (1977). Adaptations to the deep-sea environment. In: *Handbook of sensory physiology*. Crescetelli F. (ed). vol. VII/5. The visual system in vertebrates. Springer-Verlag. Berlin. pp. 67-192.
- McEwan M.R. (1938). A comparison of the retina of the mormyrids with that of various other teleosts. *Acta Zool.* 19, 427-465.
- Michaelson I.C. (1954). *Retinal circulation in man and animals*. C.C. Thomas. Springfield.
- Nag T.C. (1993). Bruch's membrane with an elastic lamina in the eye of a teleost. *J. Anat.* 182, 113-115.
- Nag T.C. (1994). Retinal pigment epithelium-calycal processes association in the catfish *Clarius batrachus* (Linnaeus, 1758). *Acta Zool. (Stockholm)* 75, 209-211.
- Nag T.C. and Bhattacharjee J. (2002). Retinal cytoarchitecture in some mountain-stream teleosts of India. *Environ. Biol. Fish.* 63, 435-449.
- Parver L.M., Auken C.R., Carpenter D.O. and Doyle T. (1982). Choroidal blood flow. II. Reflexive control in the monkey. *Arch. Ophthalmol.* 100, 1327-1330.
- Pfeffer B.A. and Fisher S.K. (1981). Development of retinal pigment epithelial surface structures ensheathing cone outer segments in the cat. *J. Ultrastruct. Res.* 76, 158-172.
- Rodieck R.W. (1973). *The vertebrate retina. Principles of structure and function*. W.H. Freeman. San Francisco.
- Spitznas M. (1974). The fine structure of the chorioretinal border tissues of the adult human eye. *Adv. Ophthalmol.* 28, 78-174.
- Steinberg R.H. and Wood I. (1974). Pigment epithelial cell ensheathment of cone outer segments in the retina of the domestic cat. *Proc. Roy. Soc. B* 187, 461-478.
- Wagner H.-J. and Ali M. A. (1978). Retinal organisation in goldeye and mooneye (Teleostei: Hiodontidae). *Rev. Can. Biol.* 37, 65-84.
- Walls G.L. (1942). *The vertebrate eye and its adaptive radiation*. Bulletin No. 19, Cranbrook institute of Science, Cranbrook Press. Bloomfield Hills, Michigan.
- Wang L., Kondo M. and Bill A. (1997). Glucose metabolism in the cat outer retina: Effects of light and hyperoxia. *Invest. Ophthalmol. Vis. Sci.* 38, 48-55.
- Wittenberg J.B. and Wittenberg B.A. (1962). Active secretion of oxygen into the eye of fish. *Nature* 194, 106-107.

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