MATTERS ARISING

Porphyropsin in retina of four-eyed fish, *Anableps anableps*

A RECENT investigation[1] reports the same visual pigments in the dorsal and ventral regions of *Anableps* retina. I present here some previously unpublished studies that demonstrate the existence of porphyropsin as well as rhodopsin in this species. *Anableps* is therefore similar to two related poeciliids, *Belonesox belizanus* and *Mollienesia latipinna*[2]. The *Anableps* used in the present work were examined only 3 days after collection from their native waters in Guiana, whereas the fish used by Avery and Bowmaker[1] originated from a fish supplier in London. Mixed rhodopsin–porphyropsin retinas are highly variable in composition; this variability probably affects the cone system also[3] and often depends on environmental factors[4]. For example, it has been shown that *Belonesox* may lose much of its porphyropsin if kept in unnaturally bright surroundings[5] and that its rhodopsin–porphyropsin balance shifts markedly with season and local habitat[6]. Other species are known to behave similarly[4]. Therefore, Avery and Bowmaker's results do not exclude the possibility that in its natural habitat *Anableps* may have appreciable proportions of porphyropsin that could be segregated in its dorsal retina.

Specimens of *Anableps* were flown directly from Guiana to Miami. A total of 3 days elapsed between the time of netting and delivery (August 1963) to my laboratory in the Bascom Palmer Eye Institute, University of Miami, Florida. After overnight dark-adaptation at 80°F, 11 fish ranging in standard length from 7 to 10 cm were killed by decapitation. The retinas were then dissected out and the photoreceptor outer segments prepared by sucrose flotation[2]. Further procedures used have been described previously[2]. The visual pigment was extracted in three successive 0.5-ml volumes of 2% digitonin (Merck), and each extract was buffered to *p*H 8.8 with sodium borate.

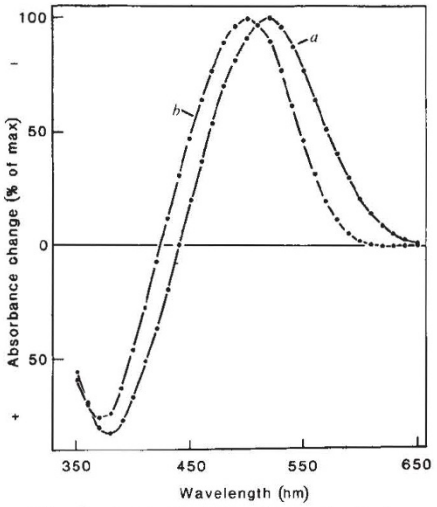

Fig. 1 Analysis of *Anableps* visual pigments. Curve *a* represents the difference spectrum obtained by bleaching a digitonin extract with light of wavelength 660 nm in the equipment described by Bridges[2]: the maximum absorbance loss was 0.077 at 519 nm. After several subsequent exposures to red light (660 and 620 nm), the visual pigment was completely bleached by exposure to yellow light (>440 nm). Curve *b* represents the difference spectrum for this final irradiation: the maximum absorbance loss was 0.0944 at 500 nm (see text for details).

The data presented here are for the first extract from the above procedure, which had a peak absorbance of 0.57 at 507 nm. The extract had more than one visual pigment—this was demonstrated by exposing it to a series of irradiations at wavelengths ranging from 660 to 620 nm, then finally to non-isomerizing yellow light of wavelengths >440 nm. Figure 1 curve (*a*) shows the difference spectrum for an initial exposure to 660 nm light. The amount bleached represents 14% of the total visual pigment, and the difference spectrum has a λ_{max} at 519 nm, close to that of porphyropsin. The difference spectrum for the final exposure to yellow light (representing 16% of the total pigment) had a λ_{max} at 500 nm, typical of rhodopsin. As Fig. 1 shows, the λ_{max} of the bleaching product also shifted to shorter wavelengths. A single fish from this group gave similar results: the digitonin extract had a peak absorbance of 0.12 at 506 nm. As the cone pigments of fishes do not survive digitonin extraction, it seems that the *Anableps* used here contained a mixture of rhodopsin and porphyropsin in their retinas.

The λ_{max} of *Anableps* rhodopsin was calculated[4] to be identical to that reported for *Belonesox*[2] that is, 498 nm, and the proportion of porphyropsin was 25–30%. The presence of porphyropsin may have accounted for the elevated λ_{max} of 506 nm previously assigned to the rhodopsin of this species[7]. The apparent agreement with Avery and Bowmaker's mean rod λ_{max} may be fortuitous, because *in situ* values are often displaced to longer wavelengths[8,9].

Thus, *Anableps* appears to be similar to several of its close relatives in having a mixture of rhodopsin and porphyropsin. Extensive studies of other species have established that such mixtures may vary widely in composition, and that ambient illumination is an important controlling factor[4]. Therefore, further studies of *Anableps* in its natural habitat or in a variety of controlled lighting conditions are needed before a final conclusion on its dorso-ventral distribution of visual pigments can be reached.

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AVERY AND BOWMAKER REPLY—Bridges presents data from partial bleaching experiments to show that the rod pigment of *Anableps* (λ_{max} = 506–507 nm) is actually a mixture of rhodopsin (λ_{max} = 498 nm) and a porphyropsin (λ_{max} = 519 nm), and is not a pure rhodopsin as reported by Schwanzara[1]. In our recent investigation of the dorso-ventral distribution of visual pigments in *Anableps*[2], we suggested from the shape of the absorbance spectra of the rods, obtained by microspectrophotometry, that the pigments were probably rhodopsins, but we are not surprised to find that they are rhodopsin–porphyropsin mixtures.

Bridges suggests that we found no difference between the visual pigments of the dorsal and ventral parts of the retina possibly due to a change in the rhodopsin–porphyropsin ratio caused by keeping the fish in artificial light. Our value of the mean λ_{max} of the rods is, however, within 1 nm of the value he and Schwanzara[1] obtained for extracts of the rod pigment, inferring that the rhodopsin–porphyropsin ratio in the rods we measured in both regions of the retina was the same as that of their extracts. If *Anableps* does have higher proportions of porphyropsin in the dorsal retina in its natural habitat, we would have expected the λ_{max} of Bridges' extract to have been longer than our rod mean λ_{max}. Unfortunately, extracts normally contain only rod pigments and give no information about the distribution of pigments across the retina or the nature of the cone pigments. Thus, further microspectrophotometric studies are required.

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