lence and the ability of certain bacterial strains to elaborate sulfatides. Thus while lysosyme may digest the carbohydrate part of the bacterial envelope, arylsulfatase A may act upon bacterial membrane sulfolipids.

Analysis of saliva and tears for hexosaminidase A was proposed for diagnostic purposes in early detection of Tay-Sachs syndrome[24]. Similarly, analysis of these excretions could be adapted into a convenient clinical tool for identifying metachromatic leukodystrophy, a syndrome associated with arylsulfatase A deficiency.

[24] J. D. SINGER, Lancet *2*, 1116 (1973).

On partial and differential bleaching experiments with the visual pigments in a fresh water euryhaline teleost (Etroplus suratensis)

T. RAMAKRISHNA*

Department of Biophysics, National Institute of Mental Health and Neurosciences, Bangalore–560 027 (India),
4 February 1976

Summary. Visual pigment was extracted from a fresh water toleost, *Etroplus suratensis* and the optical density measured over a range of 300–650 nm. The absorption spectrum indicates the λ_{max} at 550 nm and a small hump at 530 nm. Through partial bleaching at 630 nm, it was confirmed that the fish possesses a mixture of 2 visual pigments: the one with the λ_{max} at 550 nm predominating over the other.

Fishes of widely seperated habitats ranging from fresh water to sea water have received much attention in the analysis of visual pigments[1–6]. As a result, the influence of diverse factors, such as the habitat salinity of the natural environs and the artificial photoperiod in the laboratory, on the nature of their visual pigments has come to be understood only partially. Hence the discussion continues on the nature of the visual pigments in fishes[7,8]. The teleost, *Etroplus suratensis* is known to inhabit fresh water as well as brackish waters along the coasts of Ceylon and India[9], and thus offers a great potential for investigations into the nature of the visual pigments as related to the habitat[10]. As a first step in this direction, the present study was undertaken in *E. suratensis*, obtained from fresh water.

Material and methods. The fish procured from a local dealer were stocked in glass aquaria and fed on cooked rice everyday in the laboratory. Mostly they were used for the experiments within a week of their procurement from their natural habitat.

Extraction of the pigments. The method of CRESCITELLI[11,12] was followed for the extraction and analysis of the retinal pigment. The fish were dark-adapted overnight, before extracting the pigment. The eyes were dissected out and kept in 4% potassium alum solution for 1–2 h after corneal puncture. After repeated washing with distilled water and borate-KCl buffer at pH 8.3, retinal pigment was extracted into 2% digitonin in alkaline borate-KCl buffer. The homogenate was centrifuged at 4000 rpm for about 30 min. The optical density of the unbleached extract was measured at 300–650 nm in a Hilger-Watt UVISPEK spectrophotometer, using a microcuvette. In all, the optical density of 12 extracts

* Present address: Fulbright Hays Senior Scholar, North Carolina Department of Mental Health, Division of Research, Box 7532, Raleigh, N. C. 27611 (USA).

[1] G. WALD, in *Comparative Biochemistry* (Eds. M. FLORKIN and H. S. MASON; Academic Press, New York 1960), vol. 1, p. 311.

[2] H. J. A. DARTNALL, in *The Eye* (Ed. H. Davson, Academic Press, New York 1962), vol. 2, p. 323.

[3] F. W. MUNZ and D. D. BEATTY, Vision Res. *5*, 1 (1965).

[4] D. D. BEATTY, Can. J. Zool. *44*, 429 (1966).

[5] S. A. SCHWANZARA, Life Sci. *6*, 157 (1967).

[6] V. VIRABHADRACHARI, R. V. KRISHNAMOORTHY and V. PARVATHESWARA RAO, J. exp. Biol. *47*, 307 (1967).

[7] M. A. ALI and W. R. HEUMANN, Vision Res. *10*, 1307 (1970).

[8] F. CRESCITELLI, in *Handbook of Sensory Physiology* (Ed. H. J. A. DARTNALL; Springer Verlag, Berlin 1972), vol. 7/1, p. 245.

[9] F. DAY, *The Fishes of India, being the natural history of the Fishes Known to Inhabit the Seas and Fresh Water of India, Burma and Ceylon* (Today and Tomorrow's Book Agency, New Delhi 1967), vol. 1, p. 414.

[10] T. RAMAKRISHNA, Indian J. Physiol., Pharmac. *16*, 277 (1972).

[11] F. CRESCITELLI, J. gen. Physiol. *39*, 423 (1956).

[12] F. CRESCITELLI, J. gen. Physiol. *40*, 217 (1956).

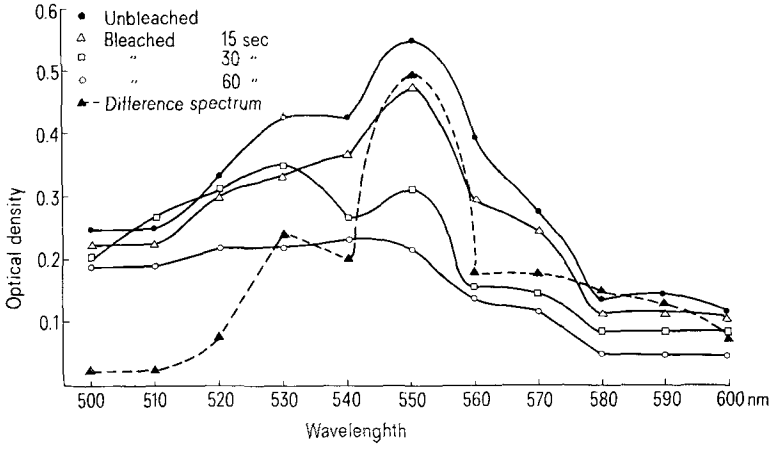

Fig. 1. Absorption spectrum of retinal extract of 24 eyes obtained from 12 animals. ●, Unbleached extract; △, partially bleached at 630 nm for 15 sec; □, – 30 sec; 0,60 sec; ◇, difference spectrum, obtained as a result of total bleaching with white light.

were measured. All operations were carried out in a dark room under a dim red light, at the temperature 22 ± 1 °C.

Partial bleaching. Partial bleaching of the extract was carried out by exposing the microcuvette with the extract to a source of white light against a corning interference filter of long wavelength (630 nm) for 15, 30 and 60 sec to ensure slow and progressive bleaching of the visual pigment.

Difference Spectrum. The difference spectrum was obtained after total bleaching of the extract to a white light of 100 watt source for 10 min.

Results. Figure 1 shows the optical density measurements of an extract pooled from 24 retinae shown against a wavelength range of 500–600 nm. The absorption spectrum of the unbleached extract showed a λ_{max} at 550 nm. However, a small hump around 530 nm could also be seen. The optical density of the extracts, partially bleached, at a long wavelength (630 nm), over increasing periods of 15, 30 and 60 sec showed a gradual decrement in optical density. While the shape of the curve for the extract bleached only for 15 sec more or less conforms to that of the unbleached extract, the same, when bleached for 1 min, does not show any peak. The most noteworthy feature of the results of partial bleaching is that the absorption spectrum of the extract, bleached for 30 sec, indicates a predominant hump at 530 nm, in contrast to that of the unbleached extract.

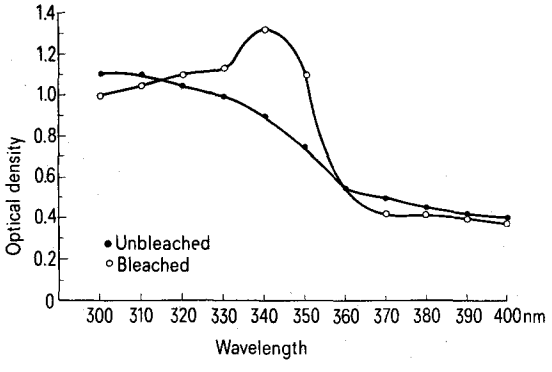

Fig. 2. Optical density of the pooled retinal extract (same as in Figure 1) at the wavelength range of 300–400 nm in unbleached (●) and totally bleached (0) conditions.

The difference spectrum, obtained between the unbleached and totally bleached extracts, shows a peak around 550 nm and a small hump around 530 nm, indicating the maximum density loss on bleaching, at the λ_{max} of the unbleached retinal extract.

Figure 2 indicates the optical density measurements of the unbleached and totally bleached extracts shown over a wavelength range of 300–400 nm. The bleached retinal extract showed a peak around 349 nm, indicating the absorption spectrum of the photoproducts.

Discussion. The results seem to indicate that *E. suratensis*, obtained from fresh water possesses 2 visual pigments, one maximally sensitive at 550 nm and the other at 530 nm, the former being predominant over the latter. The partial and differential bleaching with a long wavelength towards the red and white light has served as a reliable method for testing the presence of more than one visual pigment[2,8,13]. It is clear from the present partial bleaching experiments that the break down of the visual pigment maximally sensitive to a longer wavelength is more complete when exposed to a source of 630 nm for 30 sec. However, the approximate similarity of the shape in the curves of the absorption spectrum between the unbleached extract and the same exposed to a source of 630 nm for 15 sec seems to indicate the incomplete and inadequate bleaching of both the pigments.

The peak obtained around 340 nm in the absorption spectrum of the bleached extract confirms the photosensitive pigment, extracted, as it represents the nature of the photoproducts as a result of bleaching.

The visual pigments extracted in *E. suratensis* are comparable to those obtained from the other fishes like bleak (533, 550 nm) and carp, (523, 550 nm)[13,14]. It is of interest to note that a closely related species, *E. maculatus* apparently has a mixture of 2 pigments i.e., porphyropsin and rhodopsin[6], although their λ_{max} are far to the left (515 and 495 nm, respectively) of the same for the pigments obtained in *E. suratensis*. It should be pointed out, however, that many of the fresh water fishes possess visual pigments, whose peak sensitivity ranges from 520–550 nm[15].

[13] H. J. A. DARTNALL, *The Visual Pigments* (John Wiley & Sons, New York 1957), p. 216.
[14] H. J. A. DARTNALL, J. Physiol., Lond. *128*, 131 (1955).
[15] F. A. BROWN JR. and C. L. PROSSER, in *Comparative Animal Physiology*, 2nd edn. (Eds. C. L. PROSSER and F. A. BROWN; W. B. Saunders Company, London 1961), p. 357.

Histochemical demonstration of adrenergic nerve fibres and serotonin-containing mast cells of the knee joint synovial membrane in rats

SEISUKE TANAKA, NORIO OHNISHI and YOSHINORI TOMINAGA[1]

Department of Orthopaedic Surgery, Faculty of Medicine, Kyoto University, Kyoto 606 (Japan), 6 July 1976

Summary. Distribution of noradrenergic nerves and serotonin-containing mast cells in the synovial membrane of rat knee joints was demonstrated using the histochemical fluorescence technique of Falck-Hillarp.

Using conventional histochemical techniques, many investigators have demonstrated that the joint capsule is supplied with both myelinated and unmyelinated nerves, which are afferent and autonomic, associated with blood vessels[2], and that mast cells lie around the blood vessels and in the loose connective tissue[3,4]. Cellular localization of monoamines in various organs and tissues has been demonstrated using the histofluorescence technique of FALCK-HILLARP[5]; but demonstration in the joint capsule has not been reported.

We investigated the distribution of noradrenergic nerves and serotonin-containing mast cells in the rat, using the Falck-Hillarp method applied in such a way that the joint capsular tissue could be visualized.