

scribed as the respiratory current (1), shows that when the book gills are active, the lateral slits entrain dye ventrally and dorsally. In both cases, dye passes the flabella. The flabella are therefore situated in the respiratory current where they can monitor chemical stimuli drawn in from a distance. However, when the last pair of legs, known as the "pusher" legs, are active and the book gills are inactive, these slits can serve as expulsion sites. The book gills were also able to entrain dye placed within 2 cm lateral to the opisthosoma. *Limulus* spends time with its prosoma burrowed in the sand (4); as a result, we hypothesize that the flabella allow the animal to sense chemical signals in its environment while partially buried. Both currents described above are expelled posteriorly under the telson when the book gills are active. Currents can be reversed and dye forced out

the flare, however, when an animal flaps its opisthosoma and telson forward.

Although the specific sensory functions of the flabella remain unclear, the two currents we describe target two different (chemo)receptor organs, the gnathobases and the flabella. Our findings indicate that *Limulus* may be more capable of long-range chemoreception than previously thought.

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UV Radiation Blocking Compounds in the Eye of the Cuttlefish *Sepia officinalis*

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UV-absorbing compounds are well documented in the light-passing components (cornea and lens) of vertebrate eyes, including numerous fish species (reviewed in [1, 2]). In fishes, three main classes of such compounds have been found: aromatic amino acids of proteins, such as tryptophan, tyrosine, and phenylalanine; mycosporine-like amino acids (MAAs); and derivatives of tryptophan such as kynurenine and 3-hydroxykynurenine (2, 3). Whereas aromatic amino acids may be contained in structural proteins of cornea and lens, the role of the MAAs and the kynurenine type molecules is believed to be primarily the filtering of UV radiation. Of the latter two classes, MAAs, which are of algal or bacterial origin, are obtained from the animals' diet, whereas kynurenine and 3-hydroxykynurenine are synthesized within the lens.

Cuttlefish, *S. officinalis*, live in shallow waters along the east coast of the Atlantic ocean and in the Mediterranean sea. These waters are classified (4) as types I, IA, IB, II (open waters) and I and 3 (coastal waters). In these waters, UV radiation penetrates to considerable depths (4) including a large portion of the shallow waters that *S. officinalis* inhabits. In this study, we examined the light transmission properties of the lens and cornea of *S. officinalis*, identified compounds that modify these properties, and compared lens and corneal transmission with the sensitivity of the animal's photoreceptors.

High performance liquid chromatography (HPLC)

Cornea and lenses were frozen (–70°C) until further analysis. Samples were extracted overnight in 100% HPLC-grade metha-

nol at 4°C. Individual pigments were separated by reverse-phase, isocratic HPLC on a Brownlee RP-8 column (Spheri-5, 4.6 mm ID × 250 mm) that was protected with an RP-8 guard column (Spheri-5, 4.6 mm ID × 30 mm). The mobile phase consisted of 25% methanol (v:v) and 0.1% glacial acetic acid (v:v) in water with a flow rate of 0.7 ml · min⁻¹. Detection of the peaks was carried out using a diode array, UV absorbance detector (Beckman Gold System). Standards (originally isolated by W. Dunlap) were available for seven MAAs (mycosporine-glycine, shinorine, porphyra-334, palythine, asterina-330, palythinol, and palythene); and 3-hydroxykynurenine was identified following Truscott *et al.* (3). Identities of the peaks were confirmed by co-chromatography with standards and by on-line diode array spectroscopy.

Three MAAs, 3-hydroxykynurenine, and two unknown compounds were found in the lens and cornea of cuttlefish (Fig. 1B). The two unknown compounds and one of the MAAs (palythinol) were found in both lens and cornea, whereas 3-hydroxykynurenine and palythene were limited to the lens, and shinorine was identified only in the cornea.

Bon *et al.* (5) noted a non-protein compound in the lens of *Octopus vulgaris* with maximal absorption at 334 nm. It is likely that octopus, like cuttlefish, also possess UV-absorbing compounds such as MAAs in their lenses.

Microspectrophotometry (MSP)

Samples of cornea and lens, used whole or in 40 μm slices (lenses), were mounted between quartz cover slips and examined through a microspectrophotometer capable of measuring light fluxes passing through microscopic samples (6). By this technique we were able to measure light absorbance through a biologically significant optical path in the sample and visually

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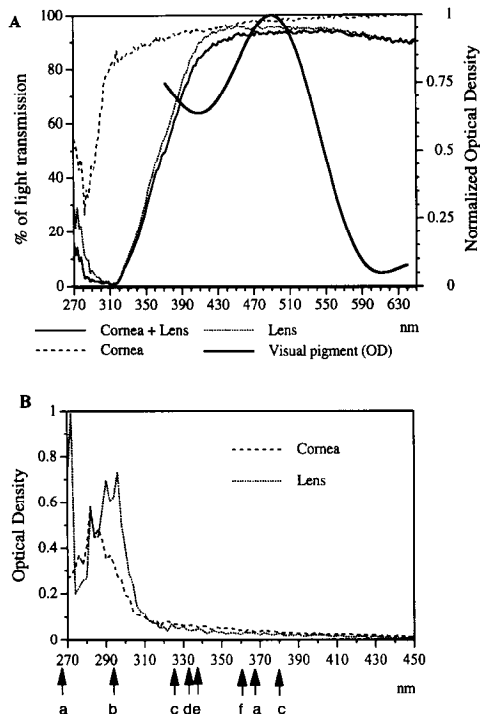


Figure 1. (A) Transmission spectra (left scale) through a lens (5 mm in diameter, animal age is 5 months) and a cornea of a cuttlefish, each measured intact, and the combined transmission through cornea and lens; and normalized optical density (right scale) of the cuttlefish visual pigment having a λ_{max} at 490 nm (light-scatter by screening pigments and possibly by visual products interfered with the signal at short wavelengths and hence the spectra is plotted only at the 370–640 nm range). Transmission spectra show that the lens acts as a UV-A blocking filter with 50% transmission at about 365 nm, while the cornea's 50% cut-off is at about 295 nm. (B) Optical density of a cornea and a 40 μ m thick section of a lens. Low levels of scattering and higher absorption are revealed at wavelengths shorter than 390 nm. Arrows indicate λ_{max} of compounds identified in the lens and cornea (a, 3-hydroxykynurenine 267 and 368 nm; b, unknown #1–295 nm; c, unknown #2–324 and 380 nm; d, palythanol–332 nm; e, shinorine–334 nm; f, palythene–360 nm). Values demonstrate that the combined effect of these compounds is responsible for attenuating UV radiation.

ensure the quality of the part of the sample examined. Fresh corneas and lenses were rapidly frozen, cut frozen, and thawed only on the measuring slide. Sivak (7) and Thorpe *et al.* (2) found that this method does not distort the transmittance of light through the tissue. Additionally, a fresh lens, 5 mm in diameter, was examined within a specially prepared water well, using 10 \times Ultrafluor lenses as objective and condenser. Each sample

was measured at 5 different locations. Each recording consisted of 16 scans in which the light transmittance of the tissue was measured over the range of 270–650 nm, in 2 nm intervals. These measurements were compared to a 16-scan reference measurement of a clear portion of the sample, and the optical density of the tissue was computed. Repetitive measurements of each sample type showed very little variation and were averaged together. No differences were found between the anterior and posterior parts of the lens and the measurements were averaged as well.

MSP recordings were further performed on photoreceptors of a dark-adapted, 4-month-old cuttlefish. The technique followed Hárosi (6), but recordings were taken from groups of photoreceptors. In 22 measurements, a single photopigment was found having maximal absorption at 490 nm (Fig. 1A).

Both lens and cornea showed high transmittance in the 470–550 nm range, to which cuttlefish are most sensitive (Fig. 1A). The lens was found to be somewhat less transparent in the long wavelengths (>590 nm), and both lens and cornea absorbed short-wavelength radiation, acting together as a long pass filter with 50% transmission at about 370 nm. Close examination of the optical density of cornea and lens revealed that most absorption can be correlated with that of proteins and 3-hydroxykynurenine, with a smaller impact by MAAs. However, since MAAs are derived from the diet, the concentrations of MAAs in the eye may be food-dependent.

UV-absorbing compounds in lenses were suggested to function both in preventing damage to the retina and in absorbing short-wavelengths that have a greater tendency to cause blurring (2). As cuttlefish are likely to have a single visual pigment (8) with maximal absorption near 490 nm (Fig. 1A), such short-wavelength blocking compounds would be expected to have a small impact on the cuttlefish visual sensitivity.

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