Euphausiid Visual Pigments

The Rhodopsins of Euphausia superba and Meganyctiphanes norvegica (Crustacea, Euphausiacea)

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ABSTRACT The rhabdoms of *Euphausia superba* contain one digitonin-extractable rhodopsin, λ_{max} 485 nm. The rhodopsin undergoes unusual pH-dependent spectral changes: above neutrality, the absorbance decreases progressively at 485 nm and rises near 370 nm. This change is reversible and appears to reflect an equilibrium between a protonated and an unprotonated form of the rhodopsin Schiff-base linkage. Near neutral pH and at 10°C, the rhodopsin is partially converted by 420-nm light to a stable 493-nm metarhodopsin. The metarhodopsin is partially photoconverted to rhodopsin by long-wavelength light in the absence of NH_2OH; in the presence of NH_2OH, it is slowly converted to retinal oxime and opsin. The rhodopsin of *Meganyctiphanes norvegica* measured in fresh rhabdoms by microspectrophotometry has properties very similar to those of the extracted rhodopsin of *E. superba*. Its λ_{max} is 488 nm and it is partially photoconverted by short wavelength irradiation to a stable photoconvertible metarhodopsin similar to that of *E. superba*. In the presence of light and NH_2OH, the *M. norvegica* metarhodopsin is converted to retinal oxime and opsin. Our results indicate that previous determinations of euphausiid rhodopsin absorbance spectra were incorrect because of accessory pigment contamination.

INTRODUCTION

The Antarctic krill, *Euphausia superba*, and the north Atlantic *Meganyctiphanes norvegica* are 2 of the 85 species of pelagic euphausiids that constitute a large part of the marine zooplankton (Mauchline and Fisher, 1969). Like other euphausiids, they are blue-green bioluminescent and share behavioral features that may be mediated by the visual system, such as swarming and diel vertical migration (Mauchline and Fisher, 1969).

Besides studies of eye structure, published data on euphausiid vision are confined to a few studies of spectral sensitivity and extracted visual pigments.

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The reported absorbance maxima of their visual pigments range from 462 to 480 nm (Table I). Based on the relationship of the rhodopsins of marine fishes to the prevailing wavelength of light reaching their habitat (Denton and Warren, 1956, 1957; Munz, 1957; Wald et al., 1957), one could expect that the rhodopsins of euphausiids found at greater depths would absorb at shorter wavelengths than the rhodopsins of those from shallower water. Among euphausiids, the relationship between depth and visual pigment appears to be

TABLE I
DEPTH OF DISTRIBUTION AND VISUAL PIGMENT λ_{max} OF SELECTED EUPHAUSIID SPECIES

Species	Rhodopsin λ_{max}	Day depth distribution*
	nm	*m*
Meganyctiphanes norvegica	460–465‡ 462§	100–500
Euphausia pacifica	462 ‖	<300
Thysanoessa raschii	460–465¶	<200
Nematoscelis megalops	465¶	>300
Stylocheiron maximum	470¶	>500
Thysanopoda acutifrons	480¶	to 4,000

* Mauchline and Fisher, 1969.
‡ Fisher and Goldie, 1959.
§ Fisher, 1967.
‖ Kampa, 1955.
¶ Fisher and Goldie, 1961.

the reverse of that expected: species found at greater depths reportedly have rhodopsins with longer wavelength absorbance maxima (Table I).

Data on euphausiid vision are anomalous in other respects. Unlike other crustacean rhodopsins that have stable metarhodopsins (Bruno and Goldsmith, 1974; Bruno et al., 1973; Goldsmith et al., 1968; Hays and Goldsmith, 1969; Kong and Goldsmith, 1977; Wald and Hubbard, 1957), euphausiid rhodopsins reportedly bleach directly to retinal and opsin (Fisher, 1967; Fisher and Goldie, 1959, 1961; Kampa, 1955).

There is also a marked discrepancy between spectral sensitivity and the λ_{max} of extracted visual pigments. For the north Atlantic species *M. norvegica*, Kay (1965) determined that the flash-stimulated luminescent response to light of various wavelengths peaked at ~480 nm, whereas spectral sensitivity

measured by electroretinogram (ERG) (Boden et al., 1961) showed this species to be equally sensitive to wavelengths near 460 and 515 nm, with a third subsidiary peak near 490 nm. The visual pigment, however, was reported to peak at ~462 nm (Fisher, 1967; Fisher and Goldie, 1959). There is a similar discrepancy between ERG spectral sensitivity (Boden et al., 1961) and visual pigment absorbance (Kampa, 1955) for *Euphausia pacifica.*

Other evidence indicated that the characteristics of euphausiid visual pigments should be re-examined. Although Fisher (1967) reported the λ_{max} of *M. norvegica* rhodopsin to be 462 nm, the description of his results of a homogeneity test suggested that there were two photosensitive pigments present in his extracts rather than one. One pigment bleached maximally at ~485 nm, and a second bleached maximally at ~452 nm. It is now apparent that the 452-nm pigment resembles a light-sensitive ommochrome that was first described in the ocelli of an anthomedusan (Yoshida, 1963; Yoshida and Ohtsuki, 1966; Yoshida et al., 1967). Such a photosensitive xanthommatin-like pigment is also present in the eyes of *E. superba* and presumably other euphausiids as a component of the screening pigment granules (Denys, 1982). The methods of preparation and extraction of visual pigment used by previous investigators would not have removed this xanthommatin pigment, which would seriously interfere with rhodopsin characterizations.

To investigate this problem, rhodopsin was extracted from the isolated rhabdoms of *E. superba* with particular attention to eliminating the ommochrome pigment. The results indicate that the digitonin-extracted rhodopsin of *E. superba* has a 485-nm λ_{max} and that it forms a stable 493-nm metarhodopsin. Similar results were obtained for the rhodopsin of *M. norvegica* measured in the rhabdom membrane by microspectrophotometry: the rhodopsin λ_{max} is 488 nm, and a stable metarhodopsin is formed at ~495 nm. Unlike most other rhodopsins, however, the absorbance of *E. superba* rhodopsin is pH sensitive. With increasing pH, there is a progressive absorbance decrease at 485 nm and a concomitant increase near 370 nm. This pH-dependent absorbance change is reversible.

MATERIALS AND METHODS

Animals

All *E. superba* used for these studies were caught with a 1-m Isaacs-Kidd Mid-Water Trawl from aboard the National Science Foundation research vessel *Hero.* Fishing was done in the vicinity of 64°S and 65°W, near Palmer Station on Anvers Island, west of the Antarctic peninsula. On board ship, the animals were kept in dark flow-through seawater live boxes. They were transferred to large (2′ × 2′ × 11′) flow-through seawater aquaria at Palmer Station until used. The animals were kept in dim white tungsten light at a photoperiod of 16L:8D, to approximate prevailing Antarctic light conditions during the period of this study (February–March). Water temperature varied between 0 and 2°C.

Live *M. norvegica* were obtained from the Department of Oceanography of Dalhousie University (Halifax, Nova Scotia). They were kept at Harvard University in the dark in a seawater aquarium with an aerator at 6°C. All of the animals were used within 6 d of being received.

Extraction of Rhodopsin from the Eyes of E. superba

Animals were dark-adapted for a minimum of 12 h. The eyes were removed by pinching the eyestalk with fine forceps and pulling them gently free of the head. The removal of the eyes and all subsequent preparatory procedures were performed in dim red light (Kodak deep red safelight filter No. 2; Eastman Kodak Co., Rochester, NY) (T <0.1% at λ <640 nm) at 2–4°C. 300–700 eyes were used for each preparation. Extraction procedures were always started immediately after the eyes were removed from the animals.

The eyes were gently homogenized in cold natural seawater, and the mixture was centrifuged for 1 min at 2,000 rpm in an IEC model B-20A refrigerated centrifuge (International Equipment Co., Needham Heights, MA) at 4°C. The supernatant resulting from this gentle centrifugation was a deep red suspension. It was decanted, fresh seawater was added, the precipitate was resuspended by gentle vortexing, and the mixture was centrifuged again. The eye material was washed a total of four times with seawater. This washing procedure removed a great deal of accessory pigment and facilitated further purification. At the end of these washes, the rhabdoms in the pellet were relatively intact, as determined by examination in the light microscope during methodological trials.

The precipitate was then resuspended in cold 50% (wt/vol) sucrose in seawater, layered over with cold seawater, and centrifuged for 5 min at 6,000 rpm. Both the interface and the 50% sucrose were saved, and the seawater layer was discarded. The precipitate was resuspended and treated as before. The resulting interface was pooled with the previously saved fractions, diluted threefold with seawater, and centrifuged for 5 min at 10,000 rpm.

This precipitate was washed repeatedly with M/15 phosphate buffer, pH 8.0, to remove contaminating ommochrome pigments. Approximately 10 successive washes were required. After the phosphate buffer washes, the precipitate was lyophilized and washed with petroleum ether (bp 20–40°C) until the washes contained no measurable astaxanthin. The petroleum ether was carefully evaporated with compressed air. The residue was again washed with phosphate buffer until free of measurable ommochrome.

The precipitate was suspended in 1.0 ml of 2% digitonin (J. T. Baker Chemical Co., Phillipsburg, NJ) in M/15 phosphate buffer, pH 6.5, and allowed to extract in the refrigerator at ~4°C for a minimum of 2 h. The extract was cleared by centrifugation for 15 min at 15,000 rpm and the supernatant was transferred to a 1-cm light path semimicro quartz cuvette. A second digitonin extract of the same precipitate contained very little rhodopsin.

The pH of a visual pigment extract was adjusted as follows: the extract was transferred from the spectrophotometer cuvette to a test tube in an ice bath and the desired pH value was obtained by adding either solid Na_2CO_3 or 1 N HCl. The pH was measured with an Orion model 601A pH/mV meter (Orion Research Inc., Cambridge, MA) with a Fisher Scientific Microprobe combination electrode (Fisher Scientific Co., Pittsburgh, PA). After centrifuging for 10 min at 15,000 rpm, the supernatant was transferred back to the cuvette for spectral measurements.

The spectrophotometer used for scanning extracts at Palmer Station, Antarctica, was a Hitachi model 100-60 equipped with an Hitachi X-Y recorder (NSA Hitachi, Mountain View, CA). In those procedures where temperature was controlled, the extract was held at 8–10°C by circulating local seawater through the cell holders. Extracts were irradiated at specific wavelengths with a holographic monochromator, (model H10V; ISA Instruments SA, Inc., Metuchen, NJ) with a 1-mm slit. The

monochromator was equipped with a 24-V 100-W halogen (Osram Sales Corp., Newburg, NY) light source.

Measurement of Rhodopsin in the Rhabdoms of M. norvegica

M. norvegica were dark-adapted for a minimum of 48 h before measuring the rhodopsin in the rhabdom membrane. Single eyes were removed in dim red light as described earlier for *E. superba*. The eye was held by the stalk with forceps over a piece of dry ice and frozen slices were cut by hand, under a dissecting microscope, with a razor blade (Gillette Platinum Plus; Gillette Safety Razor Co., Andover, MA). The slices were mounted between two 22-mm-diam thin Pyrex cover slips (microcell) in either cold seawater or in cold 0.1 M hydroxylamine in seawater. The microcell was transferred to a cold circular brass holder and then to the stage of the microspectrophotometer (MSP) microscope (Carl Zeiss, Inc., New York) for spectral measurements. In the MSP, the preparation was kept at ~0°C with a constant temperature stage refrigerated by circulating cold ethylene glycol. The MSP was otherwise the same as described by Brown (1961) and Brown and White (1972). Absorbance spectra were scanned from 700 to 300 nm at 2.5 nm/s.

Within the eye slices, rhabdom areas appropriate for measurement were chosen by viewing the preparation in the MSP microscope with a focused light source (Osram quartz iodine 12-V 50-W lamp, plus two Schott KG3 heat filters [Schott Optical Glass, Inc., Duryea, PA]) and a Schott RG-8 red filter. This same light source, plus appropriate filters, was used to irradiate the retina during experimental procedures. Table II contains a list of the filters used in these experiments.

TABLE II

IDENTIFICATION OF FILTERS USED IN MSP MEASUREMENTS OF THE RHODOPSIN OF *M. norvegica*

Color	Type and number	Transmission characteristics
Red	RG-8, Schott	Percent T <1% at λ <670 nm
Green	IF-554, Baird	229 Å half-bandwidth
Yellow	C-3385, Corning	Percent T <1% at λ <470 nm
Yellow	GG-3, Schott	Percent T <1% at λ <435 nm
Blue	IF-418, Baird	96 Å half-bandwidth
Heat filter	KG-1, Schott	
Heat filter	KG-3, Schott	

RESULTS

E. superba Rhodopsin Spectrum

The direct spectrum of a digitonin extract of *E. superba* rhodopsin at pH 6.5 is shown in Fig. 1, curve 1. The λ_{max} of the α band is at 485 nm. Initially, photobleaching was attempted at room temperature (~25°C) and spectra were measured without cooling the spectrophotometer sample chamber (~30°C). A 15-min irradiation at 485 nm at room temperature caused a substantial decrease in absorbance in the visible region, and a comparable increase in the near ultraviolet (UV) region (Fig. 1, curve 2), which indicates the absence of a stable metarhodopsin at that temperature. The spectrum

continued to change during a 40-min interval in the dark at 30°C, which indicates that the pigment was also thermally bleaching (Fig. 1, curve 3). The addition of hydroxylamine (NH_2OH) to the extract increased the absorbance in the near UV and shifted the peak from 380 to ~365 nm (Fig. 1, curve 4), the absorbance characteristic of all-*trans* retinal oxime (Hubbard et al., 1971). Thus, the chromophore was retinal$_1$ and the photosensitive pigment in the extract was rhodopsin.

Because of the thermal lability of the solubilized *E. superba* rhodopsin, all subsequent experiments were carried out at 10°C, unless a thermal bleach

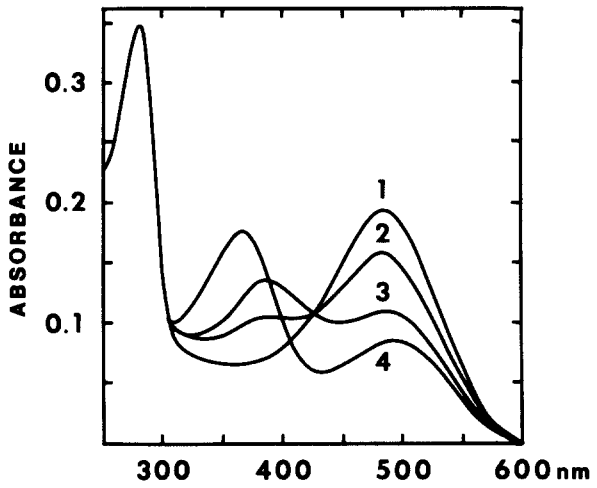

FIGURE 1. Bleaching of a digitonin extract of *E. superba* rhodopsin at pH 6.5. 1, initial dark spectrum measured after the extract had reached room temperature; 2, after a 15-min irradiation at 485 nm, at room temperature; 3, after 40 min in the dark at 30°C; 4, after addition of NH_2OH to a final concentration of 0.04 M. The pigment responsible for the residual absorbance is probably ommin.

was specifically intended. At 10°C, the rhodopsin is stable in darkness in either the absence or presence of NH_2OH.

Metarhodopsin

Although rhodopsin bleaches at room temperature, irradiation at 10°C produces a stable photoequilibrium mixture of metarhodopsin and rhodopsin. Irradiation at 410–420 nm for 20 min caused a shift of λ_{max} from 485 (Fig. 2, curve 1) to 490 nm and an increase in absorbance (Fig. 2, curve 2). The absence of a change in the absorbance spectrum below ~430 nm indicates that the absorbance of rhodopsin and metarhodopsin at shorter wavelengths is the same, and that the metarhodopsin is stable at 10°C and pH 6.5, i.e., it is not hydrolyzed to retinal and opsin.

The photoreversibility of the rhodopsin-to-metarhodopsin transition was investigated by irradiating the rhodopsin-metarhodopsin mixture at 550 nm. The resulting spectrum (Fig. 2, curve 3) is intermediate between the initial dark spectrum and that measured after short-wavelength (410–420 nm) irradiation. Thus, some metarhodopsin was photoconverted back to rhodopsin.

Although stable in the absence of NH_2OH at 10°C, the metarhodopsin is very slowly converted to opsin and retinal oxime in the presence of 0.04 M NH_2OH. For example, continued irradiation of an extract containing NH_2OH results in the total bleaching of rhodopsin and the formation of retinal oxime. The total rhodopsin difference spectrum in the presence of NH_2OH has a

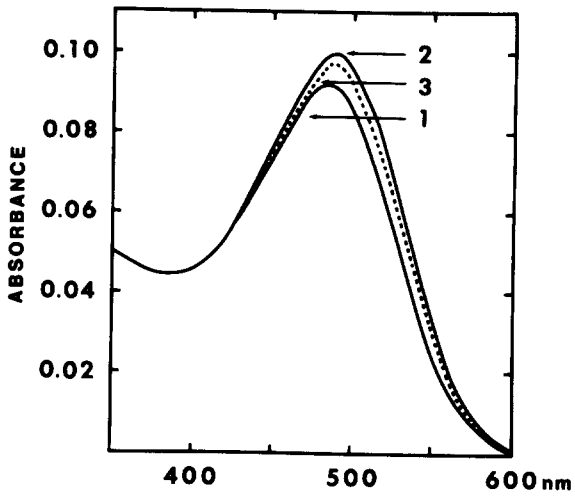

FIGURE 2. Formation of metarhodopsin and its photoconversion back to rhodopsin in digitonin at pH 6.5 and 10°C. 1, initial dark spectrum of rhodopsin; 2, mixture of rhodopsin and metarhodopsin after irradiation at 410–420 nm for 20 min; 3, partial photoregeneration of rhodopsin after a 10-min irradiation at 550 nm.

485-nm λ_{max}, as does the original direct spectrum. In addition, the normalized difference spectrum is nearly identical to a 485-nm difference spectrum nomogram based on cattle rhodopsin bleached in the presence of NH_2OH (P. K. Brown, unpublished results) (Fig. 3). We conclude that *E. superba* rhabdoms contain a single rhodopsin and that there were no significant photosensitive contaminants in the digitonin extract.

pH Sensitivity of Euphausiid Rhodopsin

The rhodopsin of *E. superba* forms a stable metarhodopsin and is therefore similar to the rhodopsins of several other crustaceans (Goldsmith et al., 1968; Kong and Goldsmith, 1977; Wald and Hubbard, 1957). However, *E. superba* rhodopsin is unusual in having a spectrum that varies with the pH of the extract. When the pH of three separate extracts was raised from 6.5 to 8.5,

9.3, or 10.9, respectively, the absorbance band at 485 nm progressively decreased with the appearance of one at ~370 nm (Fig. 4). In all cases, the λ_{max} of the α band remained at 485 nm, as did the λ_{max} of the difference spectrum after thermal or photobleaching. Acidifying extracts also caused the spectrum to change, though less markedly. At pH 6.0, there was a small decrease in absorbance at 485 nm. At pH 5.0, the λ_{max} shifted to 480 nm.

pH Sensitivity and Absorbance Spectrum of Metarhodopsin

The metarhodopsin of *E. superba* is also pH sensitive. It was stable at pH 6.0 and 6.5, but not at 5.3 and below or 8.5 and above. Irradiation at pH 5.3 (Fig.

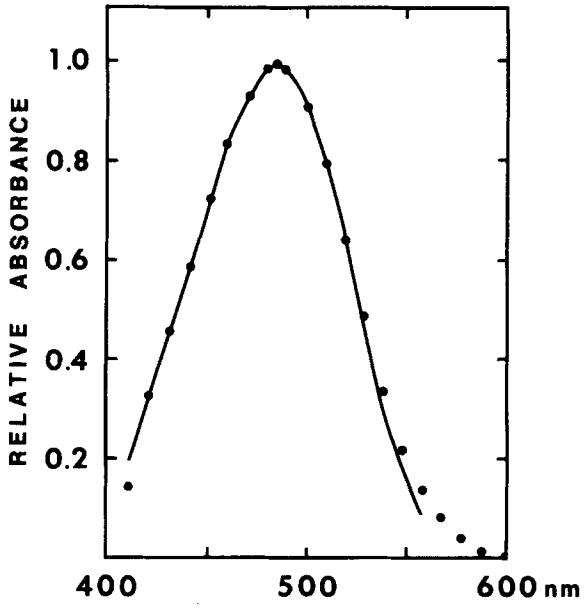

FIGURE 3. Normalized difference spectrum for *E. superba* rhodopsin bleached at 10°C, pH 6.5, in the presence of 0.04 M NH_2OH (solid circles), compared with a 485-nm difference spectrum nomogram derived from the bleaching of cattle rhodopsin in the presence of NH_2OH (solid line).

5) resulted in a progressive decrease in the α band that continued in the dark. The dark changes ended in a stable spectrum with a peak at ~470 nm and a second shallow peak at ~380 nm (Fig. 5, curve 2). Further irradiation produced no significant change in spectrum 2. The extract was then allowed to warm to 30°C in the dark. After 60 min the absorbance had decreased further in the visible and increased at 380 nm (Fig. 5, curve 3). The addition of NH_2OH to the extract shifted the UV absorbance maximum to 365 nm (Fig. 5, curve 4), typical of retinal oxime. The λ_{max} of the difference spectrum (curve 1 minus curve 4) was at 485 nm.

Irradiation of an extract at pH 5.0 (Fig. 6, curve 1) resulted in a stable

spectrum with a 450-nm λ_{max} (Fig. 6, curve 2). The 450-nm peak is presumed to represent the formation of the acidified Schiff base of retinal because adjusting the pH of a separate rhodopsin extract from 6.5 to 2.8 resulted in the same spectrum.

At alkaline pH, the metarhodopsin (Fig. 7, curve 1) is also unstable. Irradiation of an extract at pH 8.5 for 5 min at 485 nm and for 5 min more at 420 nm caused three changes in the spectrum (Fig. 7, curve 2): (*a*) an increase in absorbance in the visible range; (*b*) a shift in the visible λ_{max} to longer wavelengths; and (*c*) an increase in absorbance in the near UV. The extract was then stored at 5°C for 5 h in the dark, frozen for 2 d, thawed at

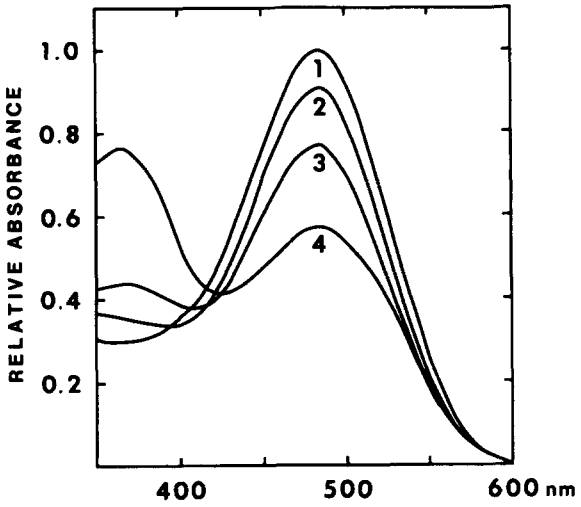

FIGURE 4. Progressive change in the spectrum of the rhodopsin of *E. superba* with increasingly alkaline pH, at 10°C. 1, pH 6.5; 2, pH 8.5; 3, pH 9.3; 4, pH 10.9. The absence of an isosbestic point may be due to the use of several extracts that were not of identical purity. All initial spectra at pH 6.5 were normalized to 1.0 at 485 nm to facilitate comparison.

8°C, and centrifuged at 4°C, and the spectrum was remeasured at 8°C (Fig. 7, curve 3). The changes that took place represent the transformation of virtually all of the metarhodopsin to a UV-absorbing molecule with a 370-nm λ_{max}. Thus, although metarhodopsin is formed at pH 8.5, it is unstable in darkness even at low temperature at this pH. The instability of the metarhodopsin at pH 8.5 provided the opportunity to determine its λ_{max}. The difference spectrum between curves 2 and 3 of Fig. 7 has a 493-nm λ_{max} (Fig. 8).

To determine whether the peak at 370 nm represented a metarhodopsin like the alkaline metarhodopsin found in cephalopods (Hubbard and St. George, 1958; Brown and Brown, 1958), the extract was irradiated for 9.5 h with 370-nm light from the spectrophotometer. Spectra measured several

times during this interval showed no evidence of rhodopsin photoregeneration. The pigment absorbing maximally at 370 nm is therefore probably not a metarhodopsin but rather alkaline *N*-retinylidene opsin (NRO), i.e., the all-*trans* retinal chromophore attached to its initial site on the opsin molecule (Collins, 1953).

Reversibility of the pH-induced Spectral Change of E. superba Rhodopsin

The unusual spectral change caused by alkalinization of rhodopsin extracts is reversible. A nonirradiated extract was adjusted from pH 6.4 to 10.0 (Fig. 9, curves 1 and 2), whereupon the absorbance decreased at 485 nm and increased

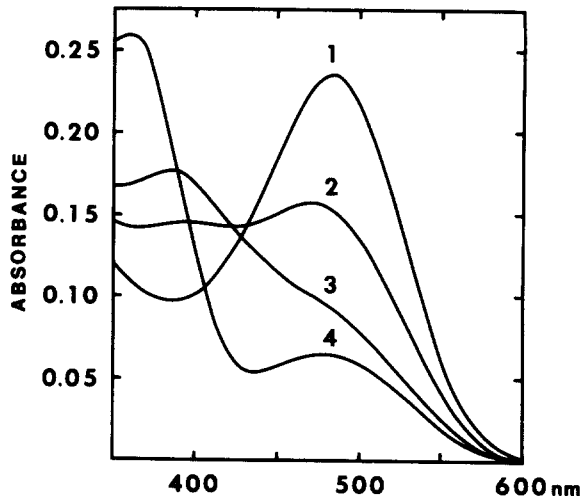

FIGURE 5. Bleaching of a digitonin extract of *E. superba* rhodopsin at 10°C and pH 5.3. 1, initial dark spectrum at pH 5.3; 2, after irradiation for 30 min and 6.5 h in the dark; 3, after 60 min warming to 30°C; 4, 60 min after the addition of NH_2OH.

at 370 nm. It was demonstrated above that increasing the pH of the solubilized rhodopsin results in a progressive decrease in absorbance at 485 nm and a progressive increase in absorbance at 370 nm. The absorbance at 485 nm at pH 10.0 is therefore "acid rhodopsin" that has not converted to the alkaline form. A 5-min bleach with 480-nm light then caused a 0.011 decrease in absorbance at 485 nm and an increase in the near UV (Fig. 9, curve 3). When the pH was readjusted to pH 6.4 in darkness, the absorbance decreased in the UV and increased again in the visible (Fig. 9, curve 4). The difference in absorbance at λ_{max} between curves 1 and 4 was 0.011, the same as the decrease caused by the 5-min bleach. Returning the extract to pH 10.0 shifted the spectrum back to curve 3.

These spectral shifts indicate that the change in the spectrum of *E. superba* rhodopsin with pH is reversible, whereas the decrease in absorbance after

irradiation is not. The second point confirms our earlier conclusion that no stable alkaline metarhodopsin is formed. If alkaline metarhodopsin had been present, then the 493-nm metarhodopsin should have reappeared when the extract was adjusted back to pH 6.4.

M. norvegica Rhodopsin and Metarhodopsin

The rhodopsin and metarhodopsin of *M. norvegica* were measured in the rhabdom membranes at 0°C by microspectrophotometry. The initial spectrum had a 485-nm λ_{max} (Fig. 10, curve 1). After a 2-min irradiation with 418-nm light (IF-418), the absorbance rose and the maximum shifted toward 490 nm (Fig. 10, curve 2), which indicated the formation of metarhodopsin. Continued

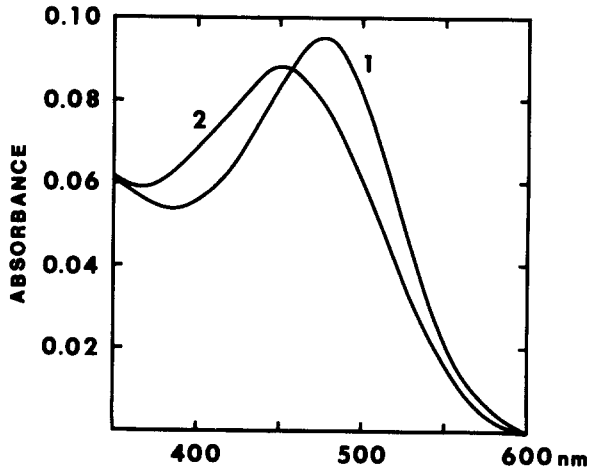

FIGURE 6. Bleaching of a digitonin extract of *E. superba* rhodopsin at 10°C and pH 5.0. 1, initial dark spectrum at pH 5.0; 2, after 30 min irradiation. Curve 2 is the spectrum of the presumed acidified Schiff base produced from this rhodopsin.

irradiation caused no further change, which showed that a photoequilibrium had been reached. As in the case of *E. superba* rhodopsin in solution, *M. norvegica* rhodopsin in the rhabdom membrane can be partially photoregenerated by 550-nm irradiation of the rhodopsin-metarhodopsin mixture (Fig. 10, curve 3). At 0°C, the metarhodopsin is stable: there was no evidence of bleaching during an 85-min exposure to yellow light (Corning 470-nm cutoff filter; Corning Glass Works, Corning, NY).

Light bleaching of metarhodopsin in invertebrate rhabdoms is usually possible after fixation with glutaraldehyde. This method was not successful for the eyes of *M. norvegica* because the glutaraldehyde caused the slow solubilization of the accessory pigments, whose diffusion into the rhabdom area made it impossible to obtain stable spectra. In addition, the photosensitive xanthommatin-like pigment would have interfered with rhodopsin measurements.

Suspending fresh eye slices in 0.1 M NH_2OH in seawater at ~0°C was an acceptable alternative to glutaraldehyde fixation because it allowed the rhodopsin and metarhodopsin to be bleached with light and did not solubilize the accessory pigments. Slices were cut parallel to the long axis of the rhabdoms. Sequential absorbance spectra were measured with nonpolarized light after placing the preparation in the microspectrophotometer. They showed increases in absorbance, which indicates that the amount of measurable rhodopsin was increasing. Re-examination of the slice in red light showed that the structure of the rhabdoms in the measuring beam had disappeared. It is probable that the observed increase in rhodopsin absorbance resulted

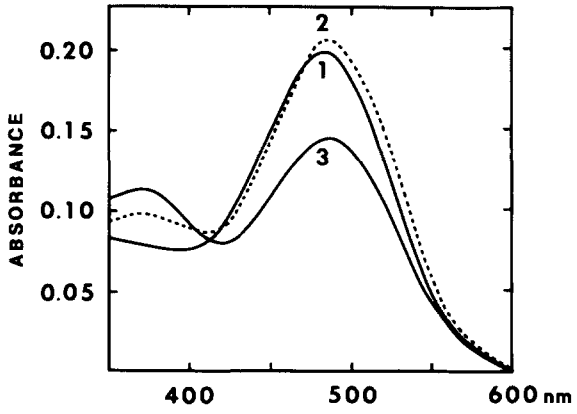

FIGURE 7. Instability of *E. superba* metarhodopsin at pH 8.5 and 10°C. 1, initial dark spectrum of rhodopsin at pH 8.5; 2, after irradiation at 485 and 420 nm, each for 5 min; 3, after 5 h at 5°C, plus 2 d at −10°C in the dark. The increase in absorbance and the shift of λ_{max} to longer wavelengths in spectrum 2 relative to spectrum 1 indicates the formation of some metarhodopsin. The rise in absorbance in the near UV in spectrum 2 is evidence for the instability of the metarhodopsin. The change between spectra 2 and 3 represents the loss of metarhodopsin.

from the disruption of the microvilli into vesicles so that the rhodopsin molecules became more randomly oriented.

After the spectrum had stabilized (Fig. 11, curve 1), the rhodopsin was completely bleached with yellow light (GG-3) in 60 min (Fig. 11, curve 2). An average of the normalized difference spectra of two rhabdom preparations was best fit by a 488-nm difference spectrum nomogram. The λ_{max} of *M. norvegica* rhodopsin is therefore at 488 nm, differing only slightly from the rhodopsin of *E. superba* (λ_{max}, 485 nm) in digitonin (Fig. 12).

DISCUSSION

Thermal Stability of Rhodopsin and Metarhodopsin

The 485-nm rhodopsin of *E. superba*, solubilized in 2% digitonin at pH 6.5, is unstable at 30°C, and its 493-nm metarhodopsin is unstable at 25°C. This is

unlike the metarhodopsins of other invertebrate species, which are usually stable in digitonin extracts at room temperature (Brown and Brown, 1958; Hamdorf et al., 1971; Hubbard and St. George, 1958; Wald and Hubbard, 1957). The thermal instability of the rhodopsin and metarhodopsin is not surprising, however, since these animals are stenothermal poikilotherms and inhabit waters where the temperature rarely exceeds 4°C. Near physiological temperature, at 10°C, both the rhodopsin and metarhodopsin of *E. superba* are stable.

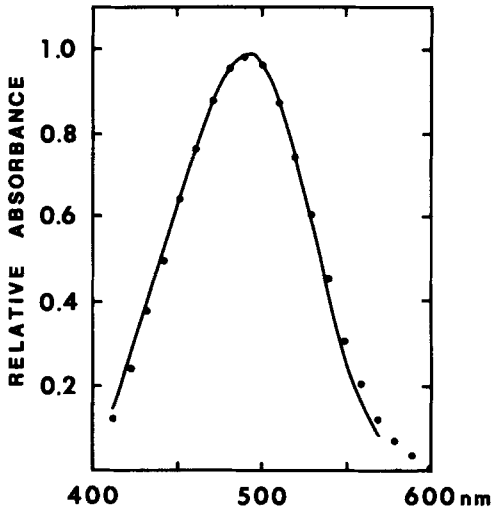

FIGURE 8. Normalized difference spectrum for *E. superba* metarhodopsin bleached in the dark at 10°C, pH 8.5 (solid circles) compared with a 493-nm difference spectrum nomogram derived from the bleaching of cattle rhodopsin in the presence of NH_2OH (solid line). The slight narrowing of the metarhodopsin spectrum at wavelengths shorter than 450 nm is attributed to the 370-nm λ_{max} of the bleaching product.

Metarhodopsin

At pH 6.5 and 10°C, short wavelength irradiation of *E. superba* rhodopsin produces a photoequilibrium mixture of the 485-nm rhodopsin and its 493-nm metarhodopsin. In *M. norvegica* the metarhodopsin λ_{max} is also shifted to slightly longer wavelengths than the rhodopsin 488-nm λ_{max}. In both cases, the molar extinction of the metarhodopsin is greater than that of the corresponding rhodopsin. It is the higher molar extinctions of the metarhodopsins relative to their rhodopsins, and their longer-wavelength absorbance maxima, that make it possible to partially photoregenerate rhodopsin from metarhodopsin in both *E. superba* and *M. norvegica*. Total photoregeneration is possible only if metarhodopsin absorbs at a much longer wavelength than rhodopsin (Hamdorf et al., 1971; Höglund et al., 1973).

If photoconversion were the only pathway of regeneration available to *E.*

superba and *M. norvegica*, one would expect to find that extracts and rhabdoms from animals previously exposed to light would contain some metarhodopsin. This was not the case. In all extracts to which NH_2OH was added before irradiation, there was no evidence of the formation of retinal oxime during 30-min initial dark scans of digitonin extracts. Rhabdoms suspended in NH_2OH in seawater also showed no evidence of retinal oxime formation before irradiation. The evident ability of these animals for dark regeneration may be related to the high concentration of 11-*cis* retinol found in the eyes of both *E. superba* (Denys, 1981) and *M. norvegica* (Wald and Brown, 1957).

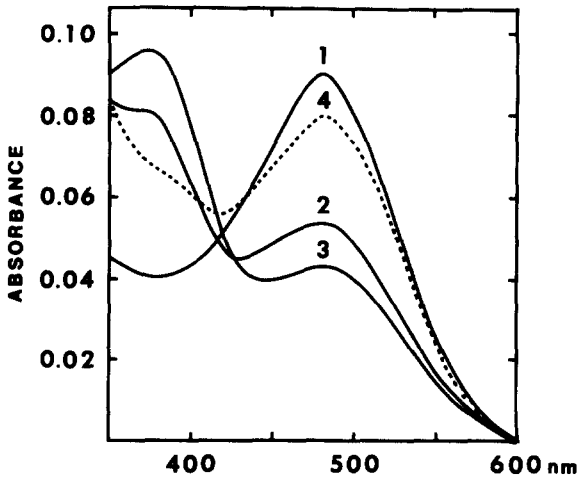

FIGURE 9. Reversibility of the pH-dependent spectral changes of *E. superba* rhodopsin at 10°C. 1, initial dark spectrum at pH 6.4; 2, after adjusting the pH to 10.0 in the dark; 3, after a 5-min irradiation at 480 nm at pH 10.0; 4, after adjusting the pH of the irradiated extract back to pH 6.4 in the dark. The difference in absorbance at 485 nm between spectra 1 and 4, and spectra 2 and 3, is 0.011. The failure of spectrum 4 to decrease in absorbance in the UV to the extent expected may have been caused by the use of HCl to adjust the pH. The addition of HCl to rhodopsin extracts always caused an increase in absorbance in the UV, which is tentatively attributed to protein denaturation. This had little effect on the difference spectrum in the visible, as determined by the fit of the total difference spectrum with a 485-nm difference spectrum nomogram.

pH Stability of Metarhodopsin

The metarhodopsin of *E. superba* is stable only within a relatively narrow pH range. At pH 6.0, the results of short wavelength irradiation are the same as they are at pH 6.5, i.e., a stable mixture of rhodopsin and metarhodopsin is formed. At pH 5.3, the metarhodopsin is not stable. At this pH, the final spectrum after irradiation has two maxima, one at ~380 nm and a second at ~470 nm. The 470-nm pigment is reminiscent of other unexplained bleaching intermediates with similar absorbance maxima in other animals. Bruno (1971)

detected a 475-nm intermediate while bleaching *Homarus* rhodopsin in single rhabdoms suspended in 5% glutaraldehyde in seawater. She reported that this intermediate was less photolabile than *Homarus* rhodopsin or metarhodopsin. A similar 475-nm pigment appears as an intermediate in the bleaching of frog retinas fixed in 5% glutaraldehyde (P. K. Brown, unpublished data); it, too, is less photolabile than the rhodopsin. In squid, a 470-nm pigment is formed when retinochrome in 2% digitonin is bleached at pH 5.3 (Hara and Hara, 1973). The entire UV-visible spectrum of bleached retinochrome is in fact very similar to the spectrum of *E. superba* rhodopsin bleached at pH 5.3. The

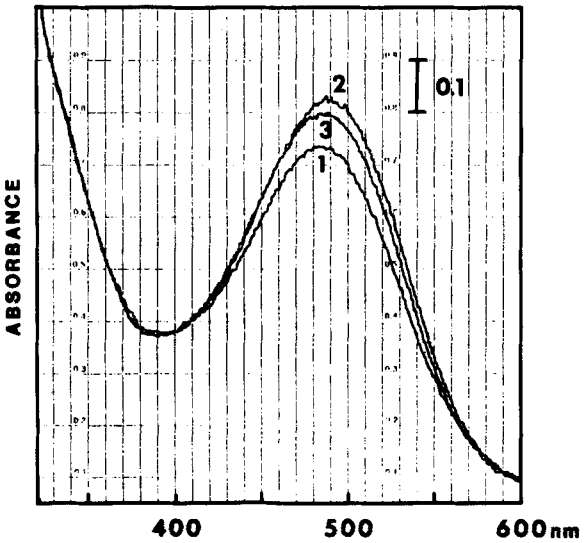

FIGURE 10. Microspectrophotometric measurements of rhabdoms in an eye slice from a dark-adapted *M. norvegica*. The slice was suspended in natural seawater at 0°C. Curve 1 is the initial dark spectrum. Curve 2 was recorded after a 2-min exposure to 418-nm light (IF-418). An additional 5-min irradiation with 418-nm light caused no further change in spectrum. Curve 3 was recorded after a 5-min irradiation with 554-nm light (IF-554). The measuring beam was 50 × 238 μm at the specimen and included only rhabdoms. Scanning rate was 2.5 nm s^{-1}.

identity of the molecule(s) absorbing at ~470–475 nm is unknown. Their appearance under only very limited circumstances would suggest that they are not normal bleaching intermediates.

Neither the 470-nm pigment nor metarhodopsin appeared when *E. superba* rhodopsin was bleached at pH 5.0. At this pH, the λ_{max} shifts directly from 485 to 450 nm, the absorbance maximum characteristic of the acidified Schiff base of this rhodopsin. At pH 8.5 and above, the 493-nm metarhodopsin is transformed to alkaline NRO (λ_{max} = 370 nm). The 493-nm metarhodopsin of *E. superba* does not appear to be in equilibrium with a stable alkaline

metarhodopsin, as has been reported for the cephalopods (Brown and Brown, 1958; Hagins and McGaughy, 1967; Hubbard and St. George, 1958; Matthews et al., 1963), and the insect *Ascalaphus macaronius* (Hamdorf et al., 1971).

pH Dependence of the Rhodopsin Spectrum of E. superba

The most surprising finding of this investigation was the pH dependence of the spectrum of the digitonin-solubilized rhodopsin of *E. superba*. A progressive change in pH to more alkaline values resulted in a progressive decrease in absorbance at 485 nm and a concomitant absorbance increase at ~370 nm. The spectral change is reversible, as demonstrated by successively adjusting the pH of an extract from 6.4 to 10.0 and then back to 6.4 (Fig. 9).

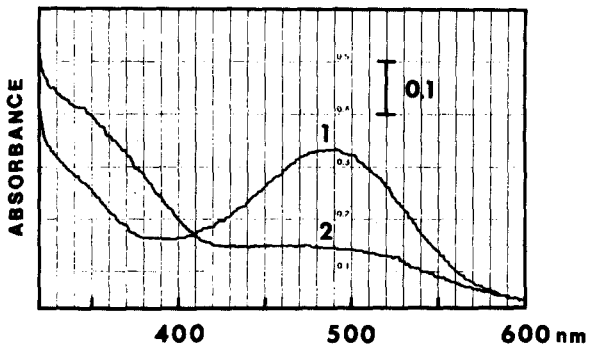

FIGURE 11. The bleaching of *M. norvegica* rhodopsin in the rhabdom membranes in an eye slice suspended in 0.1 M NH_2OH in seawater at 0°C. Curve 1 is the initial dark spectrum. Curve 2 was recorded after a 60-min bleach with yellow light (GG-3). Continued irradiation caused no further change in spectrum. The increase in absorbance in the UV reflects the formation of retinal oxime. The measuring beam was 17 × 217 μm at the specimen and included only rhabdoms. Scanning rate was 2.5 nm s^{-1}.

The appearance of a short-wavelength band at higher pH parallels the shift in λ_{max} of Schiff bases of retinal with amino compounds from ~440 nm in acid solution to ~365 nm in alkaline solution (Ball et al., 1949). This reaction has been attributed to the deprotonation of the Schiff-base linkage at alkaline pH (Morton and Pitt, 1955). Such a protonation-deprotonation system has been invoked by Matthews et al. (1963) as the mechanism underlying the reversible spectral changes between acid and alkaline metarhodopsin demonstrated by Brown and Brown (1958) in the cephalopods. If the Schiff-base linkage in the rhodopsin of *E. superba* is assumed to be protonated, as is generally assumed for other rhodopsins (reviewed by Ebrey and Honig, 1975), then the reversible pH-dependent spectral changes may represent a simple equilibrium between a protonated and an unprotonated form of *E. superba* rhodopsin. The implication is that the binding site of the chromophore is unusually accessible in this rhodopsin, as it is in the metarhodopsins of

cephalopods and the insect *Ascalaphus* (Hamdorf et al., 1971). Whether such spectral shifts would occur with the *E. superba* rhodopsin in the rhabdom membrane or in digitonin at lower temperature has not been determined. In either of these situations, the rhodopsin molecule might be more tightly folded and the chromophore-opsin linkage less accessible to changes in the hydrogen ion concentration of the surrounding medium.

The situation is very different from that described for the reversible pH-dependent spectral changes of crayfish rhodopsin in the rhabdom membrane. In the crayfish, the λ_{max} of both the direct and difference spectra of the rhodopsin move gradually toward shorter wavelengths with decreasing pH,

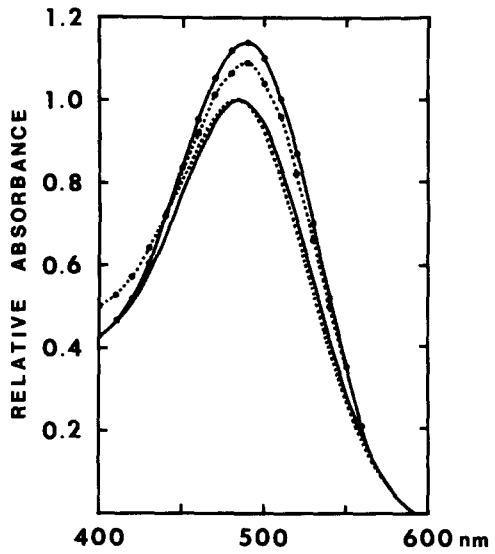

FIGURE 12. The formation of metarhodopsin in the rhabdoms of *M. norvegica* and in a digitonin extract of *E. superba* rhodopsin by 420 nm irradiation. The absorbance of the initial rhodopsin spectra at λ_{max} have been normalized to 1.0. *M. norvegica*: membrane rhodopsin (—); membrane rhodopsin plus metarhodopsin (—•—). *E. superba*: extract rhodopsin (- - -); extract rhodopsin plus metarhodopsin (-••-).

and there appear to be a number of stable intermediate forms of rhodopsin with absorbance maxima between the extremes of 535 nm (at pH 7.8) and 460 nm (at pH 2.0) (Goldsmith, 1978). The reversibility of the acid effect on the crayfish rhodopsin spectrum is thought to reflect a reversible denaturation of the opsin, perhaps made possible by the stabilization of the opsin by other membrane constituents (Goldsmith, 1978). In contrast, the reversible pH-dependent spectral changes of *E. superba* rhodopsin occur at alkaline pH, and in extracts, where stabilization by membrane components would be minimal. Also, there appear to be only two interchangeable forms of *E. superba* rhodopsin.

The retinal-protein pigment whose pH-dependent properties most closely resemble those of the rhodopsin of *E. superba* is the retinochrome of cephalopods, which is not a visual pigment. Adjusting the pH of a retinochrome extract from *Ommastrephes* to more alkaline values causes the absorbance to decrease near its λ_{max} (495 nm) and to increase near 370 nm. Although the λ_{max} of the α band shifts to slightly longer wavelengths with increasing pH, the λ_{max} of the difference spectrum always remains the same, regardless of the pH at which the retinochrome is bleached. The alkaline pH-induced spectral changes of unirradiated retinochrome are also entirely reversible (Hara and Hara, 1965).

Despite the similarities between retinochrome and the pigment we have extracted from the rhabdoms of *E. superba*, the latter is clearly a rhodopsin rather than a retinochrome for several reasons. Near neutrality, irradiation converts the *E. superba* pigment to a stable mixture of rhodopsin and metarhodopsin, whereas retinochrome is bleached directly to retinal and protein (Hara et al., 1967). At 10°C, the *E. superba* pigment is stable to NH_2OH, whereas retinochrome is converted directly to retinal oxime and protein in the dark when NH_2OH is added (Hara and Hara, 1967). The oxime formed upon irradiation of the *E. superba* pigment has the 365-nm λ_{max} characteristic of all-*trans* retinal oxime, whereas the oxime formed when NH_2OH is added to bleached retinochrome has the 355-nm λ_{max} characteristic of 11-*cis* retinal oxime (Hara and Hara, 1967). Finally, the 488-nm pigment was the only photosensitive pigment detected in fresh rhabdoms of *M. norvegica* and therefore must be the rhodopsin of this euphausiid. Its similarity to the *E. superba* pigment supports the identification of the latter as a rhodopsin. The resemblance between the spectra of *E. superba* rhodopsin and cephalopod retinochrome bleached at pH 5.3, and the pH sensitivity of the dark spectra of both pigments stem undoubtedly from similarities in chromophore-protein interaction; otherwise, the two pigments are very different.

Verification of Rhodopsin Characteristics in the Membrane

A question remains as to whether the digitonin-solubilized rhodopsin of *E. superba* has the same λ_{max} as it does in the rhabdom membrane. Bruno and Goldsmith (1974) reported a 23-nm discrepancy between the absorbance maxima of the *Callinectes sapidus* rhodopsin in digitonin (λ_{max} = 477 nm) and the rhodopsin in the membrane (λ_{max} = 500 nm). Absorbance measurements of digitonin-solubilized rhodopsin preparations alone have therefore been considered inadequate until confirmed by microspectrophotometric studies of the rhodopsin in the photoreceptor membrane.

We originally intended to verify the characterization of the *E. superba* rhodopsin in digitonin extracts by returning glutaraldehyde-fixed eyes to the United States for microspectrophotometric measurements of the visual pigment in the rhabdom membrane of *E. superba* retinas. However, the glutaraldehyde fixation had solubilized the photosensitive xanthommatin-like accessory pigment, allowing it to spread throughout the eye. Consequently, the results could not be confirmed directly by microspectrophotometry of *E.*

superba rhabdoms. However, Fisher's (1967) homogeneity test on digitonin-solubilized pigments from the eyes of the euphausiid *M. norvegica* had revealed the existence of a 485-nm rhodopsin in these eyes, although he did not recognize it as such at the time. Microspectrophotometric measurements of the spectral changes occurring upon irradiation of the rhabdoms in fresh eye slices from *M. norvegica* showed that the rhodopsin λ_{max} in the membrane is shifted to slightly longer wavelengths and has a λ_{max} at ~488 nm. Such bathochromic shifts have been observed before for some vertebrate rhodopsins measured in the disk membranes and in extracts (Wald and Brown, 1958; Wald et al., 1962). The 488-nm rhodopsin is the only visual pigment we have detected in this species. The very close agreement with the digitonin extract data for this species makes it reasonable to assume that the same is true in *E. superba*, i.e., the rhodopsin in the membrane has approximately the same λ_{max} as it does in digitonin extracts.

Moreover, the changes in the rhodopsin spectrum that occur upon irradiating fresh *M. norvegica* rhabdoms with 420-nm light are nearly identical to the changes upon irradiating *E. superba* rhodopsin in digitonin with the same light (Fig. 12). Thus, the metarhodopsins are essentially the same. These two species therefore appear to have nearly identical rhodopsins and metarhodopsins.

Such similar rhodopsins may reflect an adaptation to similar photic environments, since the two species have similar depth distributions. It is possible that other factors are involved, however. With the exception of the benthic species *Bentheuphausia amblyops*, all euphausiids possess photophores (Mauchline and Fisher, 1969) and so are presumably bioluminescent. What few measurements exist in the literature indicate that the peak emission wavelengths of the photophores center around 470 nm, regardless of the species (Boden and Kampa, 1959; Herring and Locket, 1978; Kampa and Boden, 1956; Swift et al., 1977). If these animals use their bioluminescence as a mode of communication (to maintain swarms, for example), then a conservative evolutionary strategy may have maintained a single rhodopsin, regardless of the depth of habitat. The determination of which of these two possibilities is the correct interpretation must await studies of the rhodopsins of other euphausiid species with different depth distributions.

Evaluation of Previous Euphausiid Vision Studies

The 488-nm absorbance maximum of the rhodopsin of *M. norvegica* measured by MSP is ~25 nm to longer wavelengths than the λ_{max} reported in previous studies of digitonin extracts of *M. norvegica* rhodopsin. The 485-nm absorbance maximum of *E. superba* rhodopsin in digitonin is at longer wavelengths than the absorbance maxima of any other extracted euphausiid rhodopsin (Table I). We attribute the differences between our rhodopsin data and those from previous investigators to the presence of a photolabile xanthommatin-like ommochrome screening pigment in their extracts of euphausiid eyes. In digitonin, this xanthommatin pigment bleaches maximally at 440 nm (Denys, 1982). The rhodopsin preparation methods used by previous investigators would have included large amounts of photosensitive pigment, thereby pro-

ducing an artificially short wavelength λ_{max}. This interpretation is supported by the observation that bleaching unpurified extracts of *E. superba* eyes yields a difference spectrum λ_{max} of ~462 nm (Denys, 1982) instead of the 485-nm λ_{max} of purified rhodopsin. Since the photolabile xanthommatin pigment is presumably present in the eyes of other euphausiid species as well, the absorbance characteristics of their rhodopsins will need to be re-investigated.

In this context, one can re-examine the anomalous relationship between the λ_{max} of euphausiid visual pigments and depth of habitat (Table I). If ommochromes are reduced in the eyes of crustaceans that live at very low light levels (Green, 1972), then it is likely that the eyes of shallow-living euphausiid species will have more xanthommatin-like pigment than deeper-living species. Consequently, unpurified digitonin extracts of the eyes of the shallower species would contain more xanthommatin contamination, and yield shorter wavelength absorbance maxima upon bleaching. Artifacts caused by such quantitative differences in screening pigment could account for the anomalous relationship between rhodopsin absorbance maxima and depth of habitat.

With the present results, we still cannot explain the difference between the spectral sensitivity of *M. norvegica* measured by ERG and the λ_{max} of its rhodopsin. There is, however, close agreement between the 480-nm spectral sensitivity peak measured by flash-stimulated luminescent response (Kay, 1965) and the 488-nm rhodopsin absorbance maximum. It can only be concluded that more detailed investigations of spectral sensitivity will be required to resolve these discrepancies.

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REFERENCES

BALL, S., F. D. COLLINS, P. D. DALVI, and R. A. MORTON. 1949. Studies in vitamin A. 11. Reactions of retinene$_1$ with amino compounds. *Biochem. J.* **45**:304–307.

BODEN, B. P., and E. M. KAMPA. 1959. Spectral composition of the luminescence of the euphausiid *Thysanoessa raschii*. *Nature* (*Lond.*). **184**:1321–1322.

BODEN, B. P., E. M. KAMPA, and B. C. ABBOTT. 1961. Photoreception of a planktonic crustacean in relation to light penetration in the sea. *In* Progress in Photobiology. B. Christensen and B. Buchmann, editors. Elsevier North-Holland, Inc., New York. 189–196.

BROWN, P. K. 1961. A system for microspectrophotometry employing a commercial recording spectrophotometer. *J. Opt. Soc. Am.* **51**:1000–1008.

BROWN, P. K., and P. S. BROWN. 1958. Visual pigments of the octopus and cuttlefish. *Nature* (*Lond.*). **182**:1288–1290.

BROWN, P. K., and R. H. WHITE. 1972. Rhodopsin of the larval mosquito. *J. Gen. Physiol.* **59**:401–414.

BRUNO, M. S. 1971. Microspectrophotometry of isolated crustacean rhabdoms. Ph.D. Thesis. Harvard University, Cambridge, MA.

BRUNO, M. S., and T. H. GOLDSMITH. 1974. Rhodopsin of the blue crab *Callinectes*: evidence for absorption differences *in vitro* and *in vivo*. *Vision Res.* **14**:653–658.

BRUNO, M. S., M. I. MOTE, and T. H. GOLDSMITH. 1973. Spectral absorption and sensitivity measurements in single ommatidia of the green crab, *Carcinus*. *J. Comp. Physiol.* **82**:151–163.

COLLINS, F. D. 1953. Rhodopsin and indicator yellow. *Nature* (*Lond.*). **171**:469–471.

DENTON, E. J., and F. J. WARREN. 1956. Visual pigments of deep-sea fish. *Nature* (*Lond.*). **178**:1059.

DENTON, E. J., and F. J. WARREN. 1957. The photosensitive pigments in the retinae of deep-sea fish. *J. Mar. Biol. Assoc. U. K.* **36**:651–662.

DENYS, C. J. 1981. The visual pigment and photoreception of the Antarctic krill, *Euphausia superba* (Crustacea, Euphausiacea). Ph.D. Dissertation; De Paul University, Chicago, IL.

DENYS, C. J. 1982. Ommochrome pigments in the eyes of *Euphausia superba* (Crustacea, Euphausiacea). *Polar Biol.* In press.

EBREY, T. G., and B. HONIG. 1975. Molecular aspects of photoreceptor function. *Q. Rev. Biophys.* **8**:129–184.

FISHER, L. R. 1967. Pigments of euphausiid eyes. *J. Mar. Biol. Assoc. India.* 1074–1080.

FISHER, L. R., and E. H. GOLDIE. 1959. The eye pigments of a euphausiid crustacean, *Meganyctiphanes norvegica* (M. Sars). Proc. XV Intern. Congr. Zool., London. 533–535.

FISHER, L. R., and E. H. GOLDIE. 1961. Pigments of compound eyes. *In* Progress in Photobiology. B. Christensen and B. Buchmann, editors. Elsevier North-Holland, Inc., New York. 153–154.

GOLDSMITH, T. H. 1978. The spectral absorption of crayfish rhabdoms: pigment, photoproduct and pH sensitivity. *Vision Res.* **18**:463–473.

GOLDSMITH, T. H., A. E. DIZON, and H. R. FERNANDEZ. 1968. Microspectrophotometry of photoreceptor organelles from eyes of the prawn *Palaemonetes*. *Science* (*Wash. D. C.*). **161**:468–470.

GREEN, J. 1972. Pigmentation of the eyes of *Nebalia bipes* (Leptostraca). *Crustaceana* (*Leiden*). **22**:206–207.

HAGINS, W. A., and R. E. MCGAUGHY. 1967. Molecular and thermal origins of fast photoelectric effects in the squid retina. *Science* (*Wash. D. C.*). **157**:813–816.

HAMDORF, K., J. SCHWEMER, and M. GOGALA. 1971. Insect visual pigment sensitive to ultraviolet light. *Nature* (*Lond.*). **231**:458–459.

HARA, T., and R. HARA. 1965. New photosensitive pigment found in the retina of the squid *Ommastrephes*. *Nature* (*Lond.*). **206**:1331–1334.

HARA, T., and R. HARA. 1967. Rhodopsin and retinochrome in the squid retina. *Nature* (*Lond.*). **214**:573–575.

HARA, T., and R. HARA. 1973. Biochemical properties of retinochrome. *In* Biochemistry and Physiology of Visual Pigments. H. Langer, editor. Springer-Verlag, New York. 181–191.

HARA, T., R. HARA, and J. TAKEUCHI. 1967. Vision in octopus and squid. *Nature* (*Lond.*). **214**:572–573.

HAYS, D., and T. H. GOLDSMITH. 1969. Microspectrophotometry of the visual pigment of the spider crab, *Libinia emarginata*. *Z. Vgl. Physiol.* **65**:218–232.

HERRING, P. J., and N. A. LOCKET. 1978. The luminescence and photophores of euphausiid crustaceans. *J. Zool.* (*Lond.*). **186**:431–462.

HÖGLUND, G., K. HAMDORF, and G. ROSNER. 1973. Trichromatic visual system in an insect and its sensitivity control by blue light. *J. Comp. Physiol.* **86**:265–279.

HUBBARD, R., P. K. BROWN, and D. BOWNDS. 1971. Methodology of vitamin A and visual pigments. *In* Methods in Enzymology. D. B. McCormick and L. D. Wright, editors. Academic Press, Inc., New York. **18**:615–653.

HUBBARD, R., and R. C. C. ST. GEORGE. 1958. The rhodopsin system of the squid. *J. Gen. Physiol.* **41**:501–528.

KAMPA, E. M. 1955. Euphausiopsin, a new photosensitive pigment from the eyes of euphausiid crustaceans. *Nature (Lond.)*. **175**:996–998.

KAMPA, E. M., and B. P. BODEN. 1956. Light generation in a sonic-scattering layer. *Deep-Sea Res.* **4**:73–92.

KAY, R. H. 1965. Light-stimulated and light-inhibited bioluminescence of the euphausiid *Meganyctiphanes norvegica* (G. O. Sars). *Proc. R. Soc. Lond. B Biol. Sci.* **162**:365–386.

KONG, K.-L., and T. H. GOLDSMITH. 1977. Photosensitivity of retinular cells in white-eyed crayfish (*Procambarus clarkii*). *J. Comp. Physiol.* **122**:273–288.

MATTHEWS, R. G., R. HUBBARD, P. K. BROWN, and G. WALD. 1963. Tautomeric forms of metarhodopsin. *J. Gen. Physiol.* **47**:215–240.

MAUCHLINE, J., and L. R. FISHER. 1969. The Biology of Euphausiids. F. S. Russell and M. Yonge, editors. Academic Press, Inc., London and New York.

MORTON, R. A., and G. A. J. PITT. 1955. Studies on rhodopsin. 9. pH and the hydrolysis of indicator yellow. *Biochem. J.* **59**:128–134.

MUNZ, F. W. 1957. Photosensitive pigments from retinas of deep-sea fishes. *Science (Wash. D. C.)*. **125**:1142–1143.

SWIFT, E., W. H. BIGGLEY, and T. A. NAPORA. 1977. The bioluminescence emission spectra of *Pyrosoma atlanticum*, *P. spinosum* (Tunicata), *Euphausia tenera* (Crustacea) and *Gonostoma* sp. (Pisces). *J. Mar. Biol. Assoc. U. K.* **57**:817–823.

WALD, G., and P. K. BROWN. 1957. The vitamin A of a euphausiid crustacean. *J. Gen. Physiol.* **40**:627–634.

WALD, G., and P. K. BROWN. 1958. Human rhodopsin. *Science (Wash. D. C.)*. **127**:222–226.

WALD, G., P. K. BROWN, and P. S. BROWN. 1957. Visual pigments and depths of habitat of marine fishes. *Nature (Lond.)*. **180**:969–971.

WALD, G., P. K. BROWN, and I. R. GIBBONS. 1962. Visual excitation: a chemoanatomical study. *In* Symposia of the Society for Experimental Biology. Number XVI, Biological Receptor Mechanisms. 32–37.

WALD, G., and R. HUBBARD. 1957. Visual pigment of a decapod crustacean: the lobster. *Nature (Lond.)*. **180**:278–280.

YOSHIDA, M. 1963. A photolabile pigment from the ocelli of *Spirocodon*, an anthomedusa. *Photochem. Photobiol.* **2**:39–47.

YOSHIDA, M., and H. OHTSUKI. 1966. The effect of light on the ocellar pigment of *Spirocodon*, an anthomedusa. *Photochem. Photobiol.* **5**:99–111.

YOSHIDA, M., H. OHTSUKI, and S. SUGURI. 1967. Ommochrome from anthomedusan ocelli and its photoreduction. *Photochem. Photobiol.* **6**:875–884.