The Spectral Sensitivity of Crayfish and Lobster Vision

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ABSTRACT (1) The spectral sensitivity function for the compound eye of the crayfish has been determined by recording the retinal action potentials elicited by monochromatic stimuli. Its peak lies at approximately 570 mμ. (2) Similar measurements made on lobster eyes yield functions with maxima in the region of 520 to 525 mμ, which agree well with the absorption spectrum of lobster rhodopsin if minor allowances are made for distortion by known screening pigments. (3) The crayfish sensitivity function, since it is unaffected by selective monochromatic light adaptation, must be determined by a single photosensitive pigment. The absorption maximum of this pigment may be inferred with reasonable accuracy from the sensitivity data. (4) The visual pigment of the crayfish thus has its maximum absorption displaced by 50 to 60 mμ towards the red end of the spectrum from that of the lobster and other marine crustacea. This shift parallels that found in both rod and cone pigments between fresh water and marine vertebrates. In the crayfish, however, an altered protein is responsible for the shift and not a new carotenoid chromophore as in the vertebrates. (5) The existence of this situation in a new group of animals (with photoreceptors which have been evolved independently from those of vertebrates) strengthens the view that there may be strong selection for long wavelength visual sensitivity in fresh water.

The comparative biochemistry of vertebrate visual pigments has progressed to the point of giving us a molecular natural history of almost unrivaled completeness. Their occurrence and distribution have revealed interesting correlations with ecology and with phylogeny (Denton and Warren, 1957; Crescitelli, 1958; Wald, 1958). Unfortunately, the extension of the search to invertebrates has only begun.

The crustacea, a large-eyed group with forms occupying a number of different environments, present particularly tempting opportunities for compara-

tive study. Kampa (1955) has briefly reported the extraction of a photosensitive pigment ("euphausiopsin," λ_{max} 460 mμ) from euphausiids, and Wald and Hubbard (1957) have extracted and characterized lobster rhodopsin (λ_{max} 515 mμ). Neither pigment has been compared with the *in vivo* spectral sensitivity of its owner; more important, no fresh water representatives of the group have been examined. The latter would be of special interest in view of the fact that fresh water vertebrates possess both rod and cone pigments which absorb at longer wavelengths than those of their marine relatives, a state of affairs achieved through the substitution of retinene$_2$ for retinene$_1$ as the carotenoid chromophore. It is difficult to know whether this phenomenon is correlated with an as yet unknown adaptive value associated with the employment of pigments absorbing at longer wavelengths for vision in fresh water; or whether, as recently proposed by Wald (1957), "the genetic characters which decide the environment seem also to determine the choice of visual system as a gratuitous by-product." The latter possibility would clearly turn upon examination of a parallel situation in an independently evolved set of photoreceptors from some invertebrate group.

Such comparative studies are often difficult to make by the techniques of biochemical analysis developed for vertebrate retinal photopigments. These methods often encounter special difficulties in invertebrate eyes (Wald and Hubbard, 1957); and frequently the eyes themselves are too small to provide good yields. The electrophysiological measurement of spectral sensitivity, however, provides a way of asking at least the initial question about absorption maximum, and can make possible predictions about some other properties of the photosensitive pigment involved. Such methods have been applied with success to vertebrates (Granit, 1947; Kennedy, 1957) and to some invertebrates (Graham and Hartline, 1935); and they have recently been effectively utilized in an analysis of the visual systems of insects (Goldsmith, 1960).

The experiments reported here are the first spectral sensitivity measurements of this kind on crustacea; they have been used to verify the spectral sensitivity of the lobster's compound eye which Wald and Hubbard's (1957) absorption spectrum predicted. More significantly, however, the same methods reveal a very large shift towards longer wavelengths in the sensitivity maximum of fresh water crayfish—a shift which parallels in direction that exhibited by the fresh water vertebrates. Some properties of the crayfish visual pigment can be predicted from the electrophysiological data; and the question of the adaptation of visual pigments to the environment will be discussed below. A preliminary account of the results has appeared elsewhere (Kennedy and Bruno, 1960).

METHODS

1. *Animals*

Crayfish (*Procambarus clarkii*) were obtained from Louisiana and maintained in aerated aquaria in the laboratory. Lobsters (*Homarus americanus*) were purchased as needed from the pound of a local supermarket. The animals were immobilized on their sides for recording in paraffin-bottomed preparation boxes with appropriate fluid media: van Harreveld's (1936) solution for crayfish and sea water for lobsters. Under these conditions at room temperature (24–27°C.), crayfish would survive a 4 to 8 hour experiment, during which visual thresholds remained approximately constant, with no ill effects. Lobsters would not; and all experiments with them were terminated after 1½ to 3 hours when thresholds began to rise.

2. *Recording*

The compound eye was immobilized with plastacene and contact made with a cotton-wick electrode for the purpose of recording the retinal response to illumination. The wick was placed laterally on the corneal surface so that it did not interfere with the stimulating light beam, and was connected *via* an Ag–AgCl electrode to one input grid of a preamplifier (coupling time constant 1 sec.). Recording was differential, with the second electrode shielded from light and placed in the bathing fluid. Responses were displayed on a cathode ray oscilloscope and recorded photographically. Trial repeats of all the basic observations were also made using a direct coupled recording system; the slight attenuation of long duration potentials produced by the capacitance coupling affects none of the measurements or observations reported.

3. *Optical System*

The system for monochromatic stimulation used has been employed on other preparations in this laboratory, and has been described previously (Kennedy, 1960). Briefly, it consisted of a zirconium arc source, a grating monochromator with linear dispersion of 1.6 mμ/mm operated at slits of 3 mm or less, spectrally calibrated "neutral" density filters and an annular wedge, and a photographic shutter. A part of the beam was diverted to produce signals for a photocell used in monitoring the stimulus duration during recording.

The stimulus patch provided was a square of approximately 3 mm side which completely covered the compound eye. The optical system had previously been tested for reliability by using it (with slight modifications) to determine the human scotopic visibility function by psychophysical methods; the curve so obtained was in good agreement with the standard (Stiles and Smith, 1944).

4. *Experimental Procedures*

Three methods were used in locating the region of maximum sensitivity and/or determining the shape of the spectral sensitivity function. In all of them, the index of sensitivity used was the amplitude of the retinal action potential. This potential undoubtedly contains contributions from structures other than receptor cells, as does that from insect eyes (Bernhard, 1942; Goldsmith, 1960); such a conclusion is suggested by the polyphasic responses obtained from crayfish compound eyes with extracellular microelectrodes (Naka and Kuwabara, 1959). These doubts about the

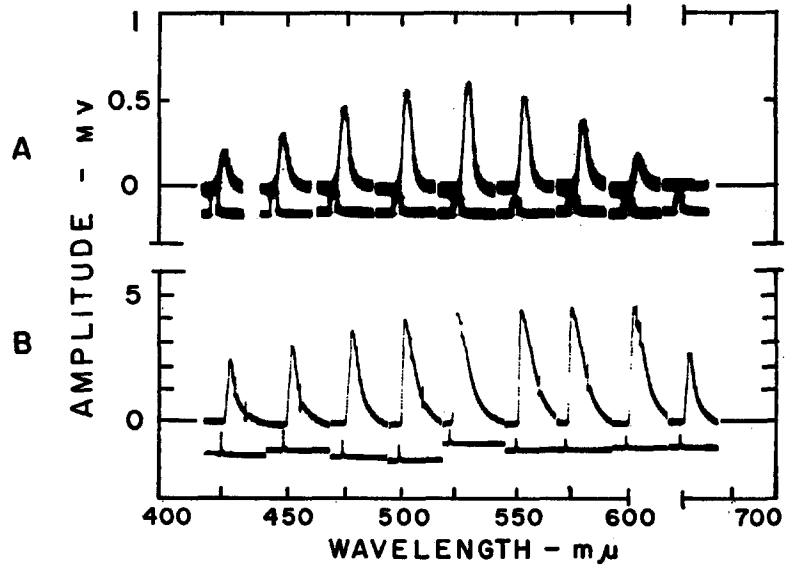

FIGURE 1. Records of responses from compound eyes of lobster (*A*) and crayfish (*B*) to stimulation with equal energies of monochromatic light. The duration of the stimuli is 0.2 second in *A*, 0.01 second in *B*; energy of all stimuli approximately 4×10^{-3} watts/cm^2. Gain in *A* ten times that in *B*.

origin of the ERG, however, do not vitiate its usefulness as an index of the sensitivity of the visual system.

In method 1, wedge adjustments were calculated which enabled the presentation of a series of monochromatic flashes having equal energy content. This method yielded the relationship between wavelength and response amplitude; but since this relationship is not a sensitivity function it was useful only for locating the region of maximum response. In method 2, stimuli of a number of intensities were presented at each of a series of wavelengths and the responses photographed. For each wavelength, a relationship was thus established between stimulus energy and response amplitude. The members of this family of curves should be parallel if a single pigment is responsible for the photoreceptor act, and their distribution along the energy axis at any chosen amplitude is a measure of the spectral sensitivity.

In method 3, a "criterion amplitude" of retinal potential was chosen which haP been previously determined to lie in a region of large amplitude change for small intensity increment, *i.e.*, on the steep part of the response amplitude *vs.* intensity function. At each wavelength, repeated short duration test flashes were given and the optical wedge adjusted until a response of the criterion amplitude was obtained. Sensitivity at a particular wavelength was rechecked during the run to make sure that over-all sensitivity was not changing. Usually, the spectral sensitivity of each

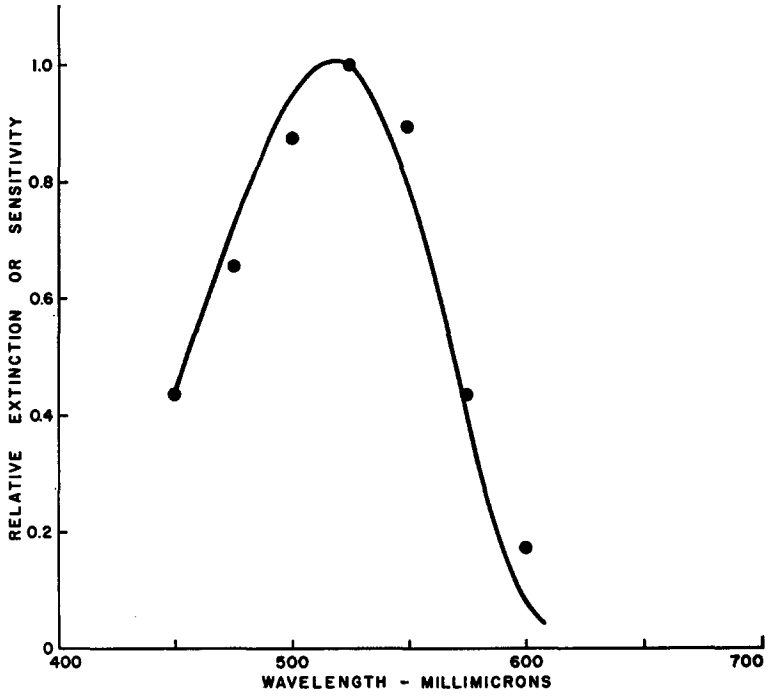

FIGURE 2. Solid line, difference spectrum of lobster rhodopsin, redrawn from Wald and Hubbard (1957). Points, average determinations of lobster spectral sensitivity from ten runs on five preparations. The average value at 525 mμ has been arbitrarily made equal to 1.

preparation was measured twice in this way, once going from short to long wavelengths and once in the reverse direction. Reciprocals of the energy values at each wavelength were then plotted against wavelength to give the spectral sensitivity function. All stimulus durations were kept below 50 msec. and the preparation was dark-adapted for 1 minute between wavelength presentations.

RESULTS

A demonstration of the striking difference between the position of peak sensitivity for lobsters and for crayfish is given by Fig. 1. The responses are to equal energy stimuli; those from the lobster eye are largest in the region of 525 mμ,

while those from the crayfish eye are largest between 550 and 600 mμ. The region of maximum response in the crayfish is quite broad in the records shown because the energies used were high; in the region 500 to 600 mμ, in other words, there was some "saturation."

Sensitivity measurements on the compound eyes of lobsters (made using method 3 above) confirm the position of maximum sensitivity suggested by the equal energy data. In Fig. 2, the points are averaged measurements from ten runs on five different preparations. These experiments were actually per-

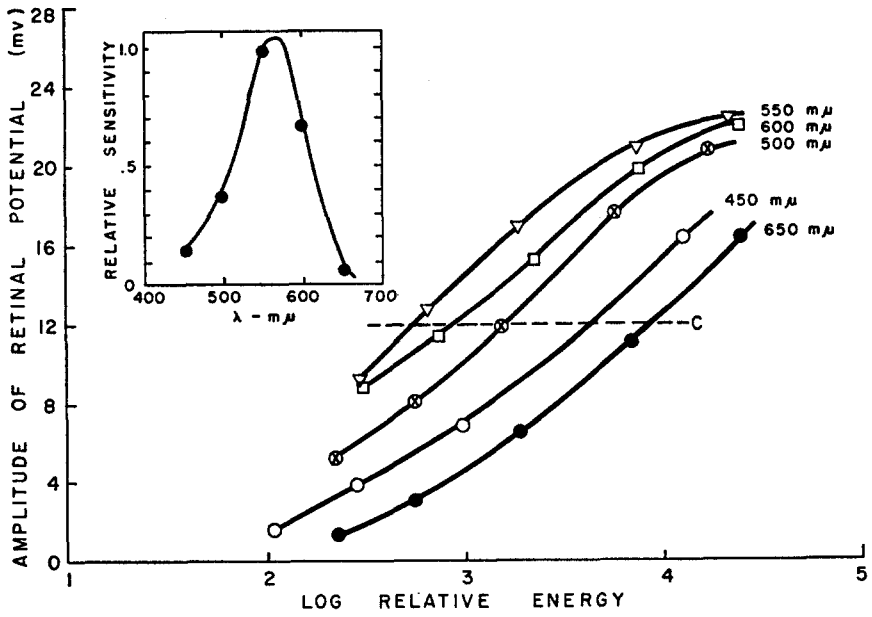

FIGURE 3. Curves relating retinal potential amplitude to log stimulus energy for five different wavelengths. Dark-adapted crayfish eye. A stimulus energy of approximately 6×10^{-4} watts/cm^2 is equivalent to a log relative energy value of 3. Inset, spectral sensitivity function constructed by plotting the reciprocals of energies required to elicit a criterion response of 12 mv (line marked "C" on graph).

formed after the sensitivity data on crayfish had been obtained; we felt that the wide disparity between the sensitivity function for the crayfish and the absorption spectrum of visual pigment from its close marine relative, the lobster, required some careful verification. It was, in other words, necessary to show that in the lobster the absorption spectrum of the visual pigment really does agree with the *in vivo* spectral sensitivity. In Fig. 2 the quantized sensitivity points are compared with the absorption spectrum of lobster rhodopsin. Agreement is quite good. There is a displacement in the sensitivity maximum of 5 to 10 mμ towards the red end of the spectrum, which can perhaps be accounted for by the high concentration of astaxanthin as a screening pigment in lobster

eyes (Wald and Burg, 1957). A filter of free or protein-bound astaxanthin (λ_{max} approximately 460 mμ) would have the effect of selectively reducing sensitivity in the blue and pushing the sensitivity maximum towards longer wavelengths.

In Fig. 3, method 2 has been employed to derive the spectral sensitivity function for the crayfish compound eye. The curves relating retinal potential

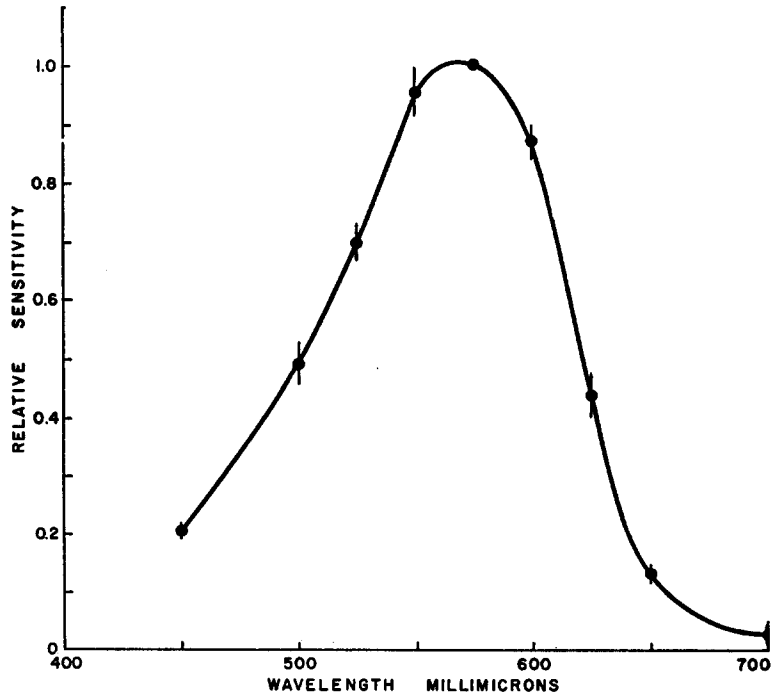

FIGURE 4. Spectral sensitivity function for the crayfish compound eye. Each point represents the average of twelve determinations made on six different preparations. Sensitivity at 575 mμ has been arbitrarily made equal to 1. Vertical lines indicate standard error.

amplitude to log stimulus energy are approximately parallel for all wavelengths, so that the choice of a criterion amplitude to use as a sensitivity index does not materially affect the resulting function. The inset shows the spectral sensitivity curve derived from the reciprocals of the energies needed to produce responses of the arbitrary amplitude labeled C.

As would be expected, method 3 yields an averaged spectral sensitivity function agreeing closely with that obtained by method 2. The averages of twelve runs made on six different preparations by experimental threshold determinations are plotted in Fig. 4 after quantizing.

To assure ourselves that a single pigment was being dealt with in these ex-

periments, and to determine the approximate rate of dark adaptation, we performed the experiment shown in Fig. 5. Test flashes at 600 and 500 mμ were matched in energy so as to elicit approximately equal amplitudes of retinal potential from the dark-adapted eye. Then a monochromatic adapting light of 600 mμ (left-hand curve) or 500 mμ (right-hand curve) was turned on for 30 seconds. Following the adaptation, the recovery of response amplitude in the dark was tested at both wavelengths. The 600 mμ adapting light was about ten times as bright as the 600 mμ test flash in the first experiment; in the sec-

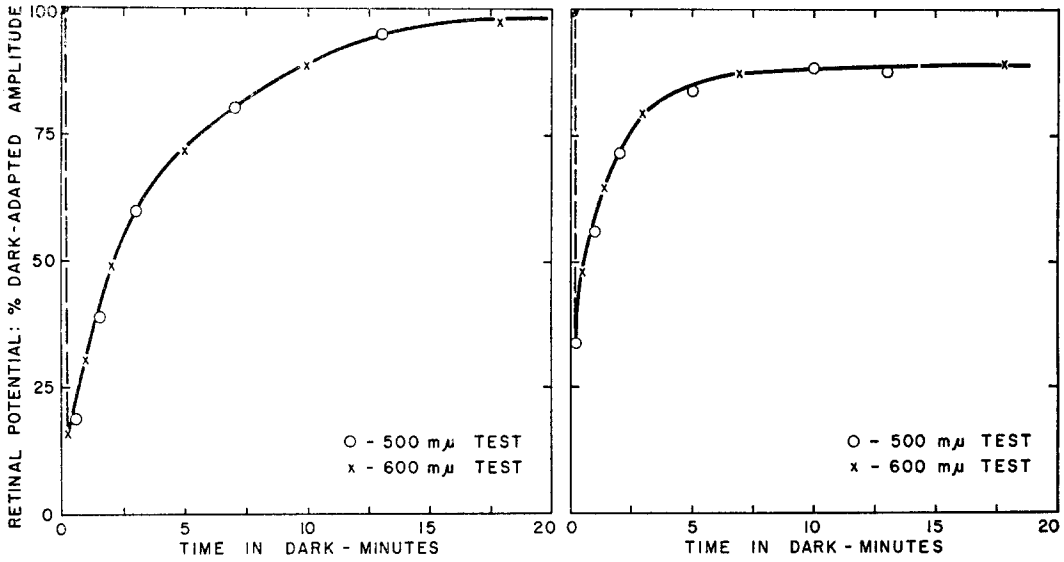

FIGURE 5. Selective adaptation of the crayfish compound eye. Left, light adaptation at 600 mμ, 1.7×10^{-2} watts/cm^2; right, light adaptation at 500 mμ, 1.2×10^{-2} watts/cm^2. Recovery in both cases is tested with alternating 500 mμ and 600 mμ test flashes previously equated to elicit equal retinal potentials from the dark-adapted eye. Energy of 500 mμ test flashes (○) = 6.2×10^{-3} watts/cm^2; of 600 mμ test flashes (×), 1.6×10^{-3} watts/cm^2.

ond, the 500 mμ adapting light was only three times as bright as the 500 mμ test flash because of limitations in the source output. Thus recovery is quicker in the second case because the adapting light used is (*a*) less bright and (*b*) not as close to the spectral region of greatest sensitivity. In both experiments, however, the responses to the two colors of test flash recover along the same curve. If more than one visual pigment were participating in the photoreceptor process, monochromatic adaptation would have selectively reduced the responses to test flashes of that wavelength, and slowed their recovery. The time course of dark adaptation, despite the fact that the adapting lights were of rather low intensity, is fairly slow, and probably comparable to adaptation rates in most vertebrate rods.

DISCUSSION

1. *Properties of Crayfish Visual Pigment*

The demonstration in these experiments that the spectral sensitivity maximum in the crayfish is displaced by 50 to 60 mμ from that of the lobster is unqualified; there also seems very little doubt that this sensitivity function is determined by a single receptor system utilizing one visual pigment. The latter point may seem overemphasized; but such assurances are necessary with arthropod eyes, especially since the demonstration (*e.g.*, Goldsmith, 1960) of more than one receptor system in the compound eyes of some insects.

The important question to be asked about the data concerns the precision with which the absorption spectrum of the visual pigment may be inferred from the spectral sensitivity function. Though agreement between the two is normally assumed, and has been obtained in a great variety of visual systems, some possible sources of error should be considered. First, it might have been assumed that for some reason the technique used gives spuriously high sensitivity readings in the red. This objection is, of course, removed by the close agreement obtained between the sensitivity function for lobster vision (determined by the same methods) and the absorption spectrum of lobster rhodopsin.

The very small magnitude of the discrepancy between these two functions in the lobster also makes it highly unlikely that filtering by screening pigments could account for the position of the 570 mμ peak in crayfish. The major colored accessory pigment present is astaxanthin; this is present in the lobster eye in very large amounts. In fact, these eyes contain three times as much astaxanthin as vitamin A, and only a small fraction of the latter substance is tied up (as retinene$_1$) in visual pigment. So the amount of screening pigment, which is at least partially contained in the ommatidial sleeves and is therefore potentially capable of acting as a filter, is huge with respect to the amount of visual pigment. Yet in the lobster eye, it has only a very small effect on the position of peak sensitivity. Such an effect would be even harder to achieve with a visual pigment absorbing at longer wavelengths; a photosensitive pigment having $\lambda_{max} = 570$ mμ, screened with a solution of astaxanthin having a density of 1.0 at its absorption maximum, would show a displacement of less than 10 mμ in the sensitivity maximum. It is thus almost inconceivable that alterations of spectral sensitivity by the presence of such a filtering pigment could constitute a major error in the prediction of the absorption maximum of the photosensitive pigment. It would seem conservative to state, then, that the sensitivity data indicate the presence in crayfish eyes of a single visual pigment, λ_{max} 570 ± 15 mμ.

Since the visual pigments of vertebrates, cephalopod molluscs, and all

arthropods so far examined are built according to the same basic plan, there seems no reason to doubt that like these others, that of the crayfish consists of a protein ("opsin") and a carotenoid chromophore (retinene). Adjustments in the position of the absorption maximum are achieved by substitution of retinene$_2$ for retinene$_1$ as the chromophore (as in fresh water fish, etc.); or by utilization of different opsins. It is the latter sort of manipulation which is responsible for the difference in sensitivity maximum ("Purkinje shift") between rods and cones, both in the retinene$_1$ system (rhodopsin, λ_{max} 480 to 524 mμ to iodopsin, λ_{max} 562 mμ) and in the retinene$_2$ system (porphyropsin, λ_{max} 522 mμ to cyanopsin, λ_{max} 620 mμ). The only difference between these pairs of pigments is in their protein moieties. Altered proteins are also responsible for the large spread in the absorption maxima of different marine fish rhodopsins (for a summary, see Crescitelli, 1958).

Since the eyes of the crayfish have been shown by Wald (1941) to contain vitamin A$_1$, the chromophore of the crayfish visual pigment must be retinene$_1$; thus the displaced absorption maximum must be due to the possession of an opsin different from that of the lobster. Crayfish opsin clearly shares some properties with that from vertebrate *cones*: the λ_{max} of crayfish visual pigment is close to that of the retinene$_1$ cone pigment iodopsin (562 mμ). Yet in other properties they diverge. For example, structural properties of the opsin determine its affinity for the chromophore and thereby, presumably, the rate of regeneration of the pigment. Iodopsin has an extremely fast regeneration rate, reaching half-completion in about 15 seconds at 10°C; yet the dark adaptation data in Fig. 5, which should approximately parallel the pigment regeneration rate (*cf.* Wald, Brown, and Kennedy, 1957), show that crayfish visual pigment regenerates rather slowly.

2. *Nomenclature and Invertebrate Visual Pigments*

These properties raise a peripheral question: What should the pigment be called? Though this should perhaps be answered after its extraction and biochemical characterization, it is worth mentioning that the occurrence of an arthropod visual pigment with λ_{max} 570 mμ is bound to do violence to the now established habit of calling invertebrate photopigments "rhodopsins." This practice was sensibly adopted by the Wald group (see Wald and Hubbard, 1957), to block the chaos emerging from the practice of applying proprietary names to essentially similar molecules (*e.g.*, "euphausiopsin," "cephalopsin"). Since the crayfish visual pigment is going to be well out of the spectral range of rhodopsins—and since there really is no reason for thinking that arthropod retinula cells should have rod rather than cone pigments anyway—it should certainly not be called a rhodopsin; and it hardly seems justified to call it an iodopsin. It is likely too, that the invertebrates will pro-

duce other surprises; and so it would seem wise at the moment simply to call all these new pigments carotenoid proteins, further specifying them by their absorption maxima and chromophores. This procedure might be a slight nuisance; but it at least avoids the polar alternatives of false homology and terminological jungle.

3. *Visual Pigments and Environment*

It is worth looking briefly at the present picture of distribution of visual pigments in aquatic invertebrates as compared to the rather complete one available for vertebrates. Among molluscs, the visual pigments of three cephalopods have been examined: *Sepia* and *Loligo* have rhodopsins with λ_{max} 493 mμ, but that of *Octopus* has λ_{max} 475 mμ (Brown and Brown, 1958; Hubbard and St. George, 1958). Among arthropods, lobster rhodopsin has λ_{max} at 515 mμ (Wald and Hubbard, 1957), that of *Limulus* at 520 mμ (Hubbard and Wald, 1960). The latter absorption peak agrees with the spectral sensitivity maximum found almost 25 years earlier by Graham and Hartline (1935). This inventory does not include the "euphausiopsin" (λ_{max} 460 mμ) extracted by Kampa (1955); this pigment was extracted without preliminary tissue purification, and no retinene was identified in the bleached extracts. Since the absorption maximum is (*a*) close to that of protein-bound astaxanthin, which is the main potential impurity and (*b*) has not been found to be in good agreement with the spectral sensitivity function (Kampa, Boden, and Abbott, 1959), inclusion of this pigment in such a list should await additional information.

The distribution is a reasonable replica of that found among the vertebrates. Absorption maxima of rhodopsins from these marine invertebrates are in the neighborhood of 500 mμ; the octopus is an exception, but the exception in itself has its vertebrate parallel in the rhodopsins of deep water fish, which in general absorb in the region of 480 mμ (Denton and Warren, 1957). The rules of visual pigment distribution in vertebrates, of course, are not absolute. It is, however, remarkably general that fresh water forms show the shift to the retinene$_2$–porphyropsin system, with its concomitant adjustment of the sensitivity maximum to longer wavelengths. This alteration in sensitivity involves not merely the scotopic shift from the 500 mμ region to 522 mμ; more significantly, it moves the maximum for cone vision from 560 mμ to 620 mμ, the peak absorption for the retinene$_2$ cone pigment cyanopsin (Wald, Brown, and Smith, 1953). So ubiquitously does this exchange of retinene chromophores accompany entry into the new environment among vertebrates that it is seen even during the life cycles of anadromous and catadromous fish, and in amphibian metamorphosis (Wald, 1958).

It would obviously be premature to assume a similar ecological correlation among crustacea on the basis of having examined a single fresh water representative. Yet the wide separation of the crayfish maximum from those of all marine invertebrates, and the similarity of the latter group to their vertebrate analogues, encourage at least the suspicion that this correlation will be upheld. Even from such a single reinforcing instance, a strong argument can be made for the view that there is, in fact, a positive adaptive coefficient in fresh water for visual sensitivity at long wavelengths. What the advantage might be is problematical. Theoretical assumptions about thermal bleaching and its contributions to retinal "noise" have been made (Barlow, 1957); these rest upon a false assumption of equivalence between thermal and photic bleaching of visual pigments (Hubbard, 1958), and at any rate lead one to the conclusion that short (not long) wavelength absorption maxima are an advantage. One can, however, take some comfort in the old measurements of light penetration into lakes. These show (James and Birge, 1938) that fresh waters generally contain dissolved or suspended materials which selectively reduce the transmission of short wavelength light. While it is difficult to see how this could be a selective factor of overriding importance in extremely shallow waters, it could well be significant in certain situations. For example, in some lakes (cited by Hutchinson, 1957, p. 396) the spectrum at a depth of 1 meter is already altered so that 78 per cent of the radiation present is of wavelength longer than 600 mμ; and even in quite transparent lakes, the spectral band at 10 meters is narrow and centers around 550 mμ. Such conditions, if they prevailed at the time of the evolutionary transition to fresh water, would surely exert powerful influences upon the selection of visual pigments. Whatever the selective challenge, it has apparently been met in the crayfish by altering the protein structure of the visual pigment instead of by the vertebrate method of employing a new carotenoid.

Note Added in Proof Since this manuscript was submitted, a report has appeared by Stieve (1960) on the spectral sensitivity of the marine crab *Eupagurus*. His extremely careful measurements, made with an electrophysiological technique similar to that reported in this paper, show that the peak sensitivity for *Eupagurus* lies very close to 500 mμ. This result provides a pleasing expansion of the generality that the visual pigments of marine crustacea, like those of most marine vertebrates, cluster in the region of 500 mμ. *Reference:* Stieve, H., Die spektrale Empfindlichkeitskurve des Auges von *Eupagurus bernhardus* L., *Z. vergleich. Physiol.*, 1960, **43**, 518.

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