Single and Multiple Visual Systems in Arthropods

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ABSTRACT Extraction of two visual pigments from crayfish eyes prompted an electrophysiological examination of the role of visual pigments in the compound eyes of six arthropods. The intact animals were used; in crayfishes isolated eyestalks also. Thresholds were measured in terms of the absolute or relative numbers of photons per flash at various wavelengths needed to evoke a constant amplitude of electroretinogram, usually 50 μv. Two species of crayfish, as well as the green crab, possess blue- and red-sensitive receptors apparently arranged for color discrimination. In the northern crayfish, Orconectes virilis, the spectral sensitivity of the dark-adapted eye is maximal at about 550 μm, and on adaptation to bright red or blue lights breaks into two functions with λ_{max} respectively at about 435 and 565 μm, apparently emanating from different receptors. The swamp crayfish, Procambarus clarkii, displays a maximum sensitivity when dark-adapted at about 570 μm, that breaks on color adaptation into blue- and red-sensitive functions with λ_{max} about 490 and 575 μm, again involving different receptors. Similarly the green crab, Carcinides maenas, presents a dark-adapted sensitivity maximal at about 510 μm, that divides on color adaptation into sensitivity curves maximal near 425 and 565 μm. Each of these organisms thus possesses an apparatus adequate for at least two-color vision, resembling that of human green-blinds (deuteranopes). The visual pigments of the red-sensitive systems have been extracted from the crayfish eyes. The horseshoe crab, Limulus, and the lobster each possesses a single visual system, with λ_{max} respectively at 520 and 525 μm. Each of these is invariant with color adaptation. In each case the visual pigment had already been identified in extracts. The spider crab, Libinia emarginata, presents another variation. It possesses two visual systems apparently differentiated, not for color discrimination but for use in dim and bright light, like vertebrate rods and cones. The spectral sensitivity of the dark-adapted eye is maximal at about 490 μm, and on light adaptation, whether to blue, red, or white light, is displaced toward shorter wavelengths in what is essentially a reverse Purkinje shift. In all these animals dark adaptation appears to involve two phases: a rapid, hyperbolic fall of log threshold associated probably with visual pigment regeneration, followed by a slow, almost linear fall of log threshold that may be associated with pigment migration.
A few years ago Kennedy and Bruno (1960–1961) reported measuring by means of the electroretinogram (ERG) the spectral sensitivities of the compound eyes of the swamp crayfish (Procambarus clarkii) and the common lobster (Homarus americanus). In the lobster they found the spectral sensitivity to be maximal at 520–525 mμ, in fair agreement with the absorption spectrum of the visual pigment (lobster rhodopsin; $\lambda_{max}$ about 515 mμ) extracted earlier by Wald and Hubbard (1957). In the crayfish, however, the spectral sensitivity was found to be maximal near 570 mμ, far to the red of any known vertebrate or invertebrate rhodopsin, and close to the $\lambda_{max}$ of the vertebrate cone pigment iodopsin (562 mμ).

It seemed interesting therefore to attempt to extract the visual pigment from the crayfish eye. This was tried first with the common northern crayfish, Orconectes virilis, with the unexpected result that two photosensitive pigments were found, with absorption maxima at about 510 and 562 mμ, close therefore to vertebrate rhodopsin and iodopsin. The examination of similar extracts from eyes of the swamp crayfish also yielded evidence of more than one visual pigment (Wald, 1962, 1967).

These observations raised the question, what multiple visual pigments might be doing in such eyes. The fact that so far as is known they contain only one anatomical type of receptor is irrelevant, since that is equally true of human cones, which though anatomically identical contain three different visual pigments (Brown and Wald, 1963, 1964; Marks, Dobelle, and MacNichol, 1964). Kennedy and Bruno had tested for the possibility that their measurements on the crayfish eye might involve more than one visual pigment, and concluded that they did not. The identification of multiple pigments in the eye extracts, however, reopened this issue, and led to the experiments to be described.

The upshot of these experiments was that both species of crayfish, as well as the green crab, Carcinides maenas, have visual systems composed of at least two types of receptor, grossly differing in spectral sensitivity, and presumably serving the function of color discrimination. The compound eyes of the horseshoe crab, Limulus, as well as the lobster, each appears to possess a single visual system, based upon a visual pigment that has already been extracted (Wald and Hubbard, 1957; Hubbard and Wald, 1960). The spider crab, Libinia emarginata, introduces another variation; it possesses two visual systems differentiated, not for color vision, but for use in dim and bright light, as are vertebrate rods and cones.

**PROCEDURE**

Light from a 100 w zirconium arc lamp was projected with glass lenses through a grating monochromator (Bausch and Lomb, 250 mm focal length, grating 1200 lines/mm). With its slits set at 1.0 mm, this instrument transmitted a wave band 3.3 mμ
wide throughout the spectrum. A photographic shutter at the exit slit regulated the 
exposure, and a lens projected the image of the exit slit onto a pair of circular neutral 
wedges (Eastman, dyed gelatin), rotating in opposite directions so as to compensate 
each other. The wedges yielded a range of densities of about 3.5, which could be 
extended with Wratten neutral filters.

The energy distribution of this optical system was calibrated with a Moll thermo-
pile (Kipp and Zonen, Delft, Holland) of 80 elements, which generated a mean emf 
of 0.083 μV/μW/cm². The voltage was measured with a Keithley 150 A electronic 
microvolt-ammeter, reading down to 0.1 μV. The wedge was calibrated with a Welch 
Densichron (Welch Scientific Co., Skokie, Ill.) in a series of overlapping ranges, at 
each 20 μ from 380 to 700 μ. Since its attenuation relative to the open position 
varied somewhat across the spectrum, a number of calibration curves were needed to 
represent the various wave bands. The transmission spectra of each of the neutral 
filters were measured with a Beckman DU spectrophotometer.

Electroretinograms were measured with a Tektronix 502 dual beam oscilloscope 
and a Grass P5 AC preamplifier with a half-amplitude frequency of 0.1 cps, run from a 
Grass regulated power supply. Electroretinograms were photographed with a Polaroid 
camera (Dumont) mounted on the oscilloscope. For long exposures of the eye to 
light, a Grass P6 DC preamplifier was used.

Contact with the eyes was through cotton wick electrodes, held in glass tubes 
pulled out at one end to capillary tips, and filled either with frog Ringer's solution 
for the crayfishes, or with seawater for the marine arthropods. Contact with the re-
cording system was through nonpolarizable silver-silver chloride electrodes. Most of 
the work was done with whole live animals that were not harmed by the experiments, 
individuals being used repeatedly, sometimes over periods of weeks.

All the measurements involved determining the relative—or sometimes the abso-
lute—numbers of photons per flash at various wavelengths, needed to evoke a con-
stant peak amplitude in the ERG, usually 50 μV. The reciprocal of these values is the 
sensitivity.

NORTHERN CRAYFISH

The first experiments were performed with the common northern crayfish, 
Orconectes (formerly Cambarus) virilis. Some years ago it was shown that pe-
troleum ether extracts of the dark-adapted eyes of this species contain vitamin 
A₁ (retinol). Reextraction with petroleum ether in bright light yields only 
a little more vitamin A, apparently left over from the dark extraction. Ex-
traction with chloroform, however, denatures the visual pigments liberating 
retinal (formerly retinene). Apparently the visual pigments of this freshwater 
arthropod possess retinal as chromophore; but—as in a number of other in-
vertebrates—this remains attached to the visual proteins or opsins in the 

As already said, digitonin extracts of rhabdomere preparations from these 
eyes contained two photopigments, both having retinal as chromophore: 
a red-sensitive pigment with λ_max about 562 μ, and a second pigment with
$\lambda_{\text{max}}$ about 510 m$\mu$. These displayed altogether different kinetics of bleaching. On exposure to light the 562 m$\mu$ pigment is converted to labile intermediates which require over an hour in the dark (23° C, pH 7.0) to finish bleaching. The final product absorbs maximally near 390 m$\mu$, representing retinal probably still attached to opsin. The 510 m$\mu$ pigment, however, bleached so rapidly under the same conditions that no intermediates were apparent (Wald, 1967 b). The discovery of these two pigments was the particular incentive for the present investigation; but the electrophysiological experiments find place as yet only for the 562 m$\mu$ pigment.

Crayfish were immobilized by wrapping in wet cheesecloth, leaving only the rostrum and eye stalks exposed. The eye to be examined was fixed in place with a plug of wet absorbent cotton stuffed into the recess behind the eyestalk, and the indifferent electrode made contact with this plug. The active electrode lightly touched the corneal surface near its distal pole. The animal was frequently rewetted with tap water during a run, which usually took several hours. Room temperatures tended to be about 24–29°C. The animals were altogether unhurt by the experiment, and often were used again and again.

In this first approach to the problem, several possible roles were considered for the two visual pigments that had been found: (a) They might represent separate systems for vision in dim and bright light, like vertebrate rods and cones. (b) There might be a topographic segregation of pigments in the eye, in that case most likely a dorsoventral differentiation. (c) The pigments might be oriented in the rhabdomeres so as to respond selectively to one or another orientation of plane-polarized light. (d) The animal might possess color vision.

The first possibility was tested by measuring dark adaptation at two widely separated wavelengths. The point was to determine whether the spectral sensitivity changes in the course of dark adaptation, as evidence that different pigments govern vision in the light- and dark-adapted states. So long as one spectral sensitivity function applies, the thresholds ($I$) measured at any two wavelengths maintain a constant ratio to each other, i.e. the log thresholds maintain a constant separation ($I_{\lambda_1}/I_{\lambda_2} = \text{constant}$; log $I_{\lambda_1}$ - log $I_{\lambda_2} = \text{constant}$). If the spectral sensitivity changes, the separation of log thresholds at the two wavelengths must also change. In human dark adaptation, for example, such a simple test indicates precisely the shift from cone to rod vision (Wald, 1960).

Fig. 1 shows the result of a typical experiment. The eye had been exposed to the concentrated white light of a microscope lamp for 5 min. Then this was put out, and with the animal in the dark, repeated determinations were made of the absolute intensities (photons/cm$^2$/flash) at 480 and 570 m$\mu$ needed to evoke a 50 $\mu$V amplitude of ERG. At 480 m$\mu$ not enough energy...
was available to produce this response until the animal had dark-adapted for 8.5 min. Thereafter, however, the log thresholds at these two wavelengths pursued a parallel course. A number of such experiments yielded the same type of result, whether the test light fell upon dorsal or ventral areas of the eye. These experiments show that the spectral sensitivity does not change over the ranges of adaptation investigated. Admittedly the ranges were not very wide; and it is possible that higher states of light adaptation would have yielded another result.

These dark adaptation curves have a special property that, as will appear shortly, was encountered also in some of the other animals. Vertebrate dark adaptation curves, whether rod or cone, tend to have a hyperbolic form when plotted as log threshold against time in the dark. Log threshold falls rapidly, then more slowly, approaching a final level at which it remains constant.

The crayfish dark adaptation curves begin in much the same way; but at the end of the hyperbolic phase, the log threshold, instead of remaining constant, continues to fall linearly with time. Thus in Fig. 1, after log threshold had fallen hyperbolically for about an hour, it continued to fall linearly for about 3 hr longer. It seemed still to be falling perceptibly during the 5th hr of the experiment.

It is tempting to think that, as in vertebrates, the hyperbolic phase of
dark adaptation represents the regeneration of visual pigment bleached during light adaptation. The linear phase looks like something else; and here the migrations of screening pigments, so prominent in these crustacean eyes, may by exposing the receptors more fully to the incident light, continue to lower the threshold (compare Bernhard and Ottoson, 1960–61; Post and Goldsmith, 1965).

Fig. 2 shows a test of the second possibility, already begun with the dark adaptation experiments: that different visual pigments function in the dorsal and ventral portions of the eye. The spectral sensitivity of the dark-adapted eye was measured with the test flashes directed onto either a dorsal or a ventral area. In both regions of the eye essentially the same type of spectral sensitivity was found, maximal at about 550 mμ. Small and unspecific variations were encountered, owing probably to minor differences in the transmission and reflection of light by the eye structures under the particular conditions of the experiment. No experiments of this type, however, offered any clear indication of a dorsoventral differentiation of the eye with regard to spectral sensitivity.

The third possibility, that the spectral sensitivity might vary with the plane of polarization of the light, is intrinsically rather unlikely. Nevertheless this was tested by setting a polaroid plate at various angles in the
incident test flash at a few selected wavelengths. This had negligible effects on the threshold.

We are left with the fourth possibility, that Orconectes may have color vision; and since that has appeared to be the case, most of the remaining experiments are devoted to clarifying this issue.

**Figure 3.** Red- and blue-sensitive mechanisms of the eye of the northern crayfish. The dark-adapted eye displays a broad, simple spectral sensitivity curve, maximal at about 550 μm. Adaptation to bright red light (Jena filter RG 1), left on throughout the experiment, depresses the sensitivity of the red receptor (λ_max about 570 μm), so that the thresholds are mainly those of the blue receptor (λ_max 435 μm). Conversely, continuous exposure to bright blue light (Wratten filter 47) depresses the blue receptor sensitivity, exposing the red receptor. 76 msec flashes; 50 μv responses.

Fig. 3 shows the results of a typical experiment. The spectral sensitivity of the dark-adapted eye takes the usual form already shown in Fig. 2, with λ_max about 550 μm. 5 days later the same animal was used in the color adaptation experiments shown in the remainder of the figure.

The area of the eye on which the test flashes impinged was bathed in the concentrated, bright blue light of a tungsten filament projection lamp, passing through the blue Wratten filter 47, which transmits a band of wavelengths from about 375 to 520...
maximal at 447 mμ. With this light on the eye continuously, the threshold to evoke a 50 μv response was measured throughout the spectrum, with 76 msec flashes superimposed on the blue background. The curve shown in Fig. 3 was obtained, with λmax about 570 mμ. In other experiments this maximum was found to lie between 560 and 570 mμ.

Conversely, irradiating the eye continuously with bright red light (Jena filter RG 1, which passes all wavelengths longer than 610 mμ), and repeating these measurements just as before, yielded the curve at the lower left in Fig. 3, with λmax about 435 mμ (430–440 mμ in other experiments). The former maximum near 570 mμ is now reduced to a minor inflection.

In this experiment the bright blue adapting light had so greatly lowered the sensitivity of a blue-sensitive mechanism that the measurements involved primarily, or perhaps entirely, a red-sensitive mechanism. Conversely the red adapting light had so lowered the sensitivity of the red mechanism that the measurements were dominated by the blue mechanism. The presence of two such visual systems, widely different in spectral sensitivity, yet poised at about the same level of thresholds, suggests that they serve the function of color discrimination.

The spectral sensitivities maximal at about 435 and 565 mμ appear to reflect the presence of a blue-sensitive and a red-sensitive pigment of crayfish vision. The latter of these agrees reasonably well with the pigment extracted from Orconectes eyes, with λmax about 562 mμ; but no evidence has yet been obtained for the presence in such extracts of a visual pigment that might account for the blue sensitivity.

This method of differential color adaptation tends to single out photopigments rather than receptor types. If in this instance the two photopigments were present in one receptor cell, the same result might have been obtained. That is, Fig. 3 presents evidence for the operation of two visual pigments, but conveys no assurance that they occur in different receptors.

Fig. 4, however, makes that seem highly probable. This experiment was performed with dc amplification to avoid any distortion of the ERG. The intensities of the flashes were so adjusted as to yield the same amplitude of response at 430 and 650 mμ (about 320 μv). The ERG has a different shape at each of these wavelengths. At 430 mμ it takes a simple form; whereas at 650 mμ there are sharp cusps on both the rising and falling phases. These characteristics appear regularly, and seem to represent genuine differences in the emf-generating properties of the red- and blue-sensitive systems. They are strong evidence that these two systems involve different receptors, as also differences in whatever afferent pathways contribute to the ERG.

1 Goldsmith (1959–60) in our laboratory used such differential color adaptations in analyzing the color vision system of the bee.
A number of experiments were undertaken with the isolated eyestalks of these animals. The rostrum with both eyestalks was cut away, and laid on a bolster of absorbent cotton soaked in Van Harreveld's crayfish Ringer's solution. In several instances the spectral sensitivity of the dark-adapted eye was measured in such preparations, as also dark adaptation following intense light adaptation. With fresh preparations the results of such procedures were much like those with intact animals.

What had led me to experiment with such preparations was a difficulty experienced with whole animals, in which the regular sweep of the gill baler (scaphognathite) across the gills introduced a large periodic fluctuation of potential that prevented any work with the ERG until it had died down. This sometimes meant waiting for several hours, and sometimes blocked an experiment entirely. The fresh, isolated preparation was, of course, altogether steady in this regard, and sometimes yielded results that were not only qualitatively like those with whole animals, but approached about the same levels of sensitivity.

Once, after finishing with such a preparation, I put it away under an

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2 I think now that this difficulty might have been avoided by immersing the animal, for preparations immersed in water such as those of the lobster and crabs were quiet. Presumably in the latter preparations the seawater shunted out the beat of the gill balers. Freshwater might not have done as well by the crayfish; but it probably could have tolerated at least dilute salinities.

3 Stieve (1960) has worked extensively with the ERG of isolated eyestalk preparations in the hermit crab, Eupagurus bernhardus. He found the sensitivity of the dark-adapted eye to be maximal at about 500 m\(\mu\).
inverted beaker in the refrigerator, at about 6°C. To my surprise, on taking it out next day, it still responded and yielded a creditable spectral sensitivity curve, though at a considerably depressed level of sensitivity. It also became dark-adapted after light adaptation. In one instance I tested such a prepara-

![Image](image_url)

**Figure 5.** Blue- and red-sensitive systems in the eye of the swamp crayfish. Isolated eyestalk preparation. The dark-adapted eye displays a broad sensitivity function, maximal at about 560 mμ, with a broad inflection in the blue. On steady adaptation to bright red light (Coming filter 2403), the sensitivity function is dominated by the blue receptor, with \( \lambda_{\text{max}} \) about 450 mμ. Conversely, adaptation to bright blue light (Wratten filter 47) exposes the red-sensitive mechanism, with \( \lambda_{\text{max}} \) about 570 mμ.

I worked with it for about an hour each day at temperatures of 24–30°C, and kept it otherwise at 6°C in the refrigerator. On the 1st day its log threshold at 550 mμ was 7.95, on the 2nd day, 10.75, on the 3rd day, 11.25, and on the 4th day, 11.6. Once I obtained a small response from such a preparation after 7 days. It should be added that in the isolated preparation kept working for 4 days, on the last 2 days the spectral sensitivity curves of the dark-adapted eye displayed a distinct separate
maximum at about 430-450 m\(\mu\), due apparently to the blue receptor, but appearing more prominently than in the fresh preparation or in whole animals.

SWAMP CRAYFISH

As already said, Kennedy and Bruno's work with this organism (*Procambarus clarkii*) provided the first incentive for these experiments. The preparations were just like those of the northern crayfish, but involved much greater use of the isolated rostral preparation. 4

Fig. 5 shows typical results, obtained in this instance with such an isolated rostrum. The spectral sensitivity of the dark-adapted eye, whether measured in such preparations or in whole animals, is a broad function, maximal at 565-570 m\(\mu\), with a broad inflection near 450 m\(\mu\) that appears to be due to the blue receptor. When the eye is adapted continuously to bright red light (Corning filter 2403; wavelengths longer than 635 m\(\mu\)), the spectral sensitivity function is reduced almost entirely to a band maximal at about 450 m\(\mu\) (440-450 m\(\mu\) in various experiments). This seems to represent an almost complete isolation of the blue-sensitive system, complicated only by a small inflection at about 580 m\(\mu\) due to a residue of the red mechanism.

Conversely, if the eye is adapted continuously to bright blue light (Wratten

4 Naka and Kuwabara (1959) have carried out penetration experiments with microelectrodes in isolated eyes of *Procambarus clarkii*. 
filter 47), the spectral sensitivity curve is reduced to a single band maximal at about 575 m\(\mu\) (572–585 m\(\mu\)), which apparently represents the red-sensitive mechanism.

Here again, as in *Orconectes*, an attempt was made to decide whether the two visual pigments responsible for the spectral sensitivity curves exist in separate receptors. In Fig. 6 this point is tested by exposing the eye to flashes of light at 420 and at 690 m\(\mu\), with their intensities adjusted so as to evoke the same amplitude of ERG (2.1 mV). In this case, unlike *Orconectes*, the response in the red is simple in form whereas that in the violet displays cusps on both the rising and falling phases, as well as a longer latency and a slightly delayed peak.

Fig. 7 shows such recordings made at intervals across the spectrum. The response retains some complexity up to 570 m\(\mu\), but is simple at 630 m\(\mu\) and beyond. As Fig. 5 shows, up to 570 m\(\mu\) the blue mechanism may still be stimulated, whereas at the longer wave lengths it has dropped out.

In this crayfish also, therefore, there is clear evidence of the operation of two types of receptor, blue-sensitive and red-sensitive, poised at about the same level of thresholds, so that they could well serve the function of color differentiation. A visual pigment has also been extracted from this eye, with \(\lambda_{\text{max}}\)
about 556 mμ, that may be the pigment of the red-sensitive receptors, though displaced considerably from the spectral sensitivity maximum at about 575 mμ, perhaps owing to distortions produced by the screening pigments which are unusually prominent in this eye (Wald, 1967 b). Here, as in Orconectes, no evidence of the blue-sensitive pigment has been found in extracts.

HORSESHOE CRAB

One might ask whether such color adaptations as used with the crayfish in themselves distort the shapes of spectral sensitivity functions. They should not do so when a single visual pigment is involved; yet the point is so crucial to these experiments that there is some reassurance in demonstrating it directly. The horseshoe crab, Limulus polyphemus, offers an opportunity to do so.

Figure 8. Dark adaptation of the lateral eye of Limulus, measured at two wavelengths. Over this range the curves are parallel, showing that the spectral sensitivity does not change; i.e., that one receptor mechanism governs the threshold. Log threshold falls hyperbolically for about 1 hr, and thereafter continues to fall almost linearly for another 1-2 hr.

Young animals, 7-8 cm in width, were used in these experiments. A small hole was bored through the shell behind the eye, and a plug of absorbent cotton stuffed into it. This provided the contact for the indifferent electrode. The surface of the cornea of the eye to be examined was scraped gently with a razor blade, to provide better contact for the active electrode. The animal was laid on a cushion of absorbent cotton soaked in seawater, and held with pins through the borders of the shell.

Fig. 8 shows the dark adaptation, measured at 450 and 520 mμ. Readings could not be begun at 450 mμ until the animal had been 12 min in the dark. Thereafter, as the figure shows, adaptation followed a parallel course at both wavelengths, indicating that over this, admittedly small range of adaptation the spectral sensitivity did not change. As in the crayfish, log threshold, having fallen hyperbolically for the 1st hr, continues to fall almost
linearly for about 2 hr longer (24.5–25.5°C). It may be that here again dark adaptation can be divided into a hyperbolic phase that goes with the regeneration of visual pigments, and a second phase that may involve pigment migrations in the eye (cf. Miller, 1958).

Hartline (1930) has measured dark adaptation in Limulus through the ERG; Hartline and McDonald (1947) have made a detailed analysis of the

![Graph showing spectral sensitivity of Limulus eye](image)

**Figure 9.** Evidence that the lateral eye of Limulus possesses a single type of receptor system. Spectral sensitivity measured in one animal (19–24 July 1962) in the dark-adapted condition, or adapted to bright red or to bright blue light. All these curves, brought to the same height to facilitate comparison, prove to be identical, with \( \lambda_{\text{max}} \) about 520 nm. They agree also with the absorption spectrum of the visual pigment of Limulus, extracted by Hubbard and Wald (1960).

kinetics; and Hartline, Milne, and Wagman (1947), the quantum relationships in light and dark adaptation in single photoreceptor elements. Benolken (1962) has made a careful study of the effects of light and dark adaptation on the generator potential of single elements in the Limulus eye. All these measurements involve primarily the first, hyperbolic phase of dark adaptation.

Measurements of the spectral sensitivity of the Limulus eye are shown in Fig. 9. The spectral sensitivities of single receptor cells were measured many years ago by Graham and Hartline (1934–35). They found the average
sensitivity to be maximal at about 520 mμ, varying somewhat from cell to cell.

Fig. 9 shows that the spectral sensitivity curve remains unchanged, whether measured in the dark-adapted eye, under bright red light, or under bright blue light. To show this, the curves measured under all three conditions are arbitrarily brought to the same height in Fig. 9. Under all circumstances λ_max lies at 520–525 mμ. The absorption spectrum of the visual pigment extracted from this eye by Hubbard and Wald (1960) is plotted in the same figure. It also has λ_max about 520 mμ, and agrees well in shape with the spectral sensitivity measurements.

In Fig. 10 the same test is performed that in the crayfishes yielded evidence of the operation of different receptors at the two ends of the spectrum. In Limulus the ERG’s obtained at 400 and 650 mμ appear to be identical in every regard.

These measurements indicate that in the lateral eye of Limulus only one visual pigment functions in a single type of receptor, and that this accounts precisely for the spectral sensitivity. As expected, adaptation to colored lights, though it changes the level of sensitivity, has no effect on the shape of the spectral sensitivity function.

Since these measurements were performed, a more complete spectral sensitivity function for the lateral eye that continues to 320 mμ has been measured by Wald and Krainin (1963.) This shows that the curve flattens out between about 400 and 350 mμ, then descends rapidly into the shorter ultraviolet. The same workers found that the median ocellus in
this animal is an ultraviolet receptor organ, in which the spectral sensitivity is dominated by a narrow band maximal at about 360 m\(\mu\). In the ocellus there is also a broad secondary band of sensitivity maximal at 530–535 m\(\mu\), close therefore to the main peak in the compound eye. These observations suggested that a more careful search might reveal the presence of ultraviolet receptors in the lateral eye; but all our attempts in this direction have so far proved fruitless (unpublished experiments with E. B. Seldin). The lateral eye of *Limulus* appears to possess only one visual system.

**LOBSTER**

The lobster, *Homarus americanus*, is such an impressive and colorful animal that it is hard to believe that if other crustacea have color vision, it does not. The experiments to be described, however, seem to show this to be the case.

I should like to emphasize one feature of the lobster preparations that may be useful to other workers. As everyone knows, it is hard to keep lobsters alive, not only while experimenting with them, but in the much more familiar situations preliminary to cooking them. Kennedy and Bruno (1960–61) remark that though their crayfish survived experiments lasting 4 to 6 hr with no ill effects, lobsters would not, and that their experiments had to be terminated within 1–1\(\frac{1}{2}\) hr when the thresholds began to rise.

Since I had had similar experiences with squid years ago, which proved to be due mainly to asphyxiation, I was concerned to keep my lobsters well supplied with oxygen. For a time I thought it might be necessary to circulate oxygenated water about them; but a lobster does its own circulating, its gill balers keeping a constant stream of water entering at the back of the thorax and ejecting it at the front. I worried also about the temperature, which during these experiments sometimes rose to 29°C.

It turned out that all that mattered was to bubble oxygen continuously through the small bath of seawater that held a lobster with all but its rostrum submerged. Under these conditions I regularly worked 5–8 hr a day with single individuals, day after day. One such lobster participated in eight sessions spread over a period of 3 wk, after which in gratitude I tossed him back into the ocean.

The lobster was held by tying the chelae together with string, wrapping the thorax and abdomen in wet cheesecloth, and holding the thorax in a condenser clamp against the bottom of the dish, so that one eye was well exposed over the rim. This eye was immobilized with a plug of absorbent cotton stuffed into the recess behind it, which offered contact also for the indifferent electrode.

Fig. 11 shows dark adaptation measured at 450 and 530 m\(\mu\). Log threshold descends in parallel at these two wavelengths, evidence that the spectral sensitivity does not change over this range of adaptation. The log threshold
falls hyperbolically for the first 20 min, then linearly for at least an hour longer.

The electroretinogram of the lobster has a remarkable feature, something of which is shown in Fig. 15. Near the ERG threshold of the dark-adapted eye, the response consists of a small wave, with about 60 msec latency, and peaking at about 100-120 msec. As the intensity of stimulus is raised, an earlier wave appears, peaking at 30-40 msec. With further rise in intensity this earlier wave grows in amplitude much faster than the second wave, overtaking it and then far exceeding it in height. So in Fig. 15, the first wave is about twice as high as the second, and at such intensities one sees also a third and fourth wave. At still higher intensities the response takes the form of a highly damped oscillation of potential, in which as many as six or seven waves can be detected. Also on turning off a light that has been on for several seconds, there is an oscillating off-response that after a complex initial phase included as many as six regular oscillations. These phenomena emerge clearly only with dc recording, or long time constant—0.1 cps—dc amplification.

The dark adaptation curves in Fig. 11 were measured to a constant height—50 µv—of the first wave. Light adaptation tends to depress the later waves in the ERG relative to the first, dark adaptation to accentuate them. These and other characteristics of the lobster ERG are discussed in a paper later in this issue (Wald, 1967-68).

Fig. 12 shows measurements of the spectral sensitivity of the lobster eye, with the criterion response in all cases an amplitude of 50 µv in the first wave of the ERG. The sensitivity is maximal at 520–525 µm, as Kennedy and Bruno found. It maintains the same form whether measured in the 5

5 Apparently the common European lobster, Homarus vulgaris, displays very nearly the same spectral sensitivity function, with λmax 516–531 µm (Kampa, Abbott, and Boden, 1963). These authors worked with the excised eye, cooled to 9°C, and penetrated with microelectrodes.
dark-adapted eye, or under adaptation to bright red or bright blue light. It would seem from this that one visual pigment governs these measurements.

Fig. 13 compares the spectral sensitivity curve with the absorption spectrum of the visual pigment extracted by Wald and Hubbard (1957). Lobster rhodopsin has \( \lambda_{\text{max}} \) about 515 m\( \mu \), slightly shorter than the sensitivity maximum, as is usual. The lobster eye is heavily pigmented with astaxanthin and other screening pigments, which could well account for some small distortions of sensitivity that are encountered.

Fig. 14 shows that the second wave in the ERG has the same spectral sensitivity as the first wave. The experiment was done in two ways: either to a constant height—25 \( \mu \)v—of second wave; or the intensity at each wavelength was adjusted to yield equal amplitudes of first and second waves (in achieving that condition the height of the first wave varied between 35 and 50 \( \mu \)v). With either method about the same result was obtained as in Fig. 12. It can be concluded that the same receptors and visual pigment are responsible for the second wave in the ERG as for the first wave.
Fig. 15 shows a further test of the thesis that a single type of receptor and visual pigment governs lobster vision. Flashes at 425 and 630 μm were so adjusted in intensity as to yield the same height of first wave in the ERG (360 μν). With this as the sole condition, the ERG’s at both ends of the spectrum are virtually identical throughout. Each takes the form of a highly damped oscillation of potential, in which three to four waves are evident.

Since these first measurements were made, the spectral sensitivity of the dark-adapted eye has been pursued to 340 μm (unpublished experiments with J. M. Krainin and E. B. Seldin). The complete curve displays a plateau of sensitivity between about 420 and 370 μm, falling precipitately at shorter wavelengths. A more searching test to see whether the sharp shoulder at about 370 μm involves the presence of an ultraviolet receptor yielded no evidence of this. The rapid drop of sensitivity below this wavelength is probably associated with low transmission by the dioptric structures of the ommatidia—cornea and crystalline—in this region of the spectrum.
It seems clear that the lobster eye, like the lateral eye of Limulus, possesses one type of receptor and visual pigment. This accounts satisfactorily for its spectral sensitivity under all conditions of adaptation. The oscillatory ERG may represent electrical activity at successive levels in the chain of neurons that conducts the visual excitation centrally, or a reverberatory feedback circuit, or some other neural arrangement, in any case contingent upon the excitation of one receptor mechanism.

**Green Crab**

The green crab, *Carcinus maenas*, was mounted for experiment much like the lobster. The legs and chelae were held close to the body by wrapping the animal in wet cheesecloth, leaving one eye exposed. This was immobilized as in the other animals with a plug of cotton, and the animal was clamped so as to be immersed in seawater except for its rostrum. Oxygen was bubbled through the water continuously.

Spectral sensitivity measurements on one of these animals are shown in Fig. 16. The spectral sensitivity of the dark-adapted eye presents a broad,
somewhat skewed curve, maximal at 500–520 m\(\mu\). From one animal to another it displayed differences of form that, though they did not involve clear additional maxima, seemed to reflect some complexity of composition.

Adaptation of this eye to bright red light (Corning filter 2403; wavelengths longer than 625 m\(\mu\)) skewed the spectral sensitivity function markedly toward the blue, so that \(\lambda_{\text{max}}\) now lay at 420–430 m\(\mu\). Conversely, adaptation to bright blue light (Wratten filters 35 + 47; transmission 400–452 m\(\mu\), peaking at 428 m\(\mu\)) skews the spectral sensitivity toward the red, so that it is maximal at 560–570 m\(\mu\).

These observations seem clearly to indicate the presence of at least two visual mechanisms, maximally sensitive near about 425 and 560 m\(\mu\). Yet the curves have a very odd appearance compared with other known spectral sensitivity functions. Each of them maintains a high level of sensitivity throughout the entire middle range of wavelengths, as though each curve were a composite of both, with perhaps a third receptor filling in the middle. An effort was made to isolate such a third mechanism by adapting simultaneously with wave bands in the violet and red, i.e. with purple light (Wratten filter 36; transmission bands at 350–450 m\(\mu\), and wavelengths longer than 665 m\(\mu\)). This yielded only broad spectral sensitivity curves, simple in form and maximal at 560–570 m\(\mu\), resembling those obtained on blue adaptation. I have not succeeded in finding in this animal such narrow,
more or less symmetrical curves as with the other forms, nor any reliable evidence of a third visual mechanism.

**SPIDER CRAB**

This animal, *Libinia emarginata*, was handled much like the lobster and green crab. It presented a situation quite different from that encountered in the other animals.

![Graph showing spectral sensitivity of the eye of the green crab.](image)

**Figure 16.** Spectral sensitivity of the eye of the green crab. In the dark-adapted eye this presents a broad, somewhat skewed curve, maximal at 500-520 mμ. On continuous exposure to bright red light (Corning filter 2403) the spectral sensitivity is skewed toward the blue (λ<sub>max</sub> about 430 mμ); and on adaptation to bright blue light (Wratten filters 35 + 47) it is skewed toward the red (λ<sub>max</sub> about 560 mμ). These curves are decidedly different in shape from the absorption spectra of all known visual pigments, in a way that suggests that each represents the mixed effects of both the blue- and red-sensitive pigments and something in between; but no reliable indications of the latter have been observed.

Fig. 17 shows measurements of spectral sensitivity in this animal. The dark-adapted eye yields a broad, relatively symmetrical spectral sensitivity curve, maximal at about 490 mμ. In this instance adapting the eye with bright blue, or red, or white light yields much the same result. All these ways of light adaptation tilt the spectral sensitivity function toward short wavelengths, so that it hardly changes between about 500 and 390 mμ.
It seems that in this case the brightness of the adapting light is more important than its color. That is, at least two visual systems seem to be involved, but apparently differentiated, not primarily for color vision, but for use in dim and bright light, as are vertebrate rods and cones. The dark-adapted condition seems to involve a single type of receptor and visual pigment, the latter probably absorbing maximally near 490 mμ, like a vertebrate rhodopsin. Light adaptation displaces the sensitivity toward the violet and ultraviolet, where some other visual pigment that absorbs primarily at shorter wavelengths takes over. As one might expect, adaptation to red light pushes the curve a little farther in this direction than do the other radiations, but otherwise has about the same effect.

In Fig. 17 this change has begun, but is far from complete. The states of light adaptation achieved bring the animal into what would be called in human vision the "mesopic" range, intermediate between the dark-adapted ("scotopic") and light-adapted ("photopic") states. Unfortunately, my
equipment was not sensitive enough to permit the measurement of higher levels of light adaptation. I would suppose from the way these measurements go, however, that at higher states of light adaptation this transition would be completed, with the visual sensitivity shifted to a new maximum in the violet or near ultraviolet.

The changes shown in Fig. 17, though incomplete, are analogous to the Purkinje phenomenon in vertebrate eyes; yet with the striking difference that whereas in vertebrates light adaptation shifts the spectral sensitivity toward the red, in the spider crab it shifts the spectral sensitivity toward the violet. This is in a sense a reverse Purkinje phenomenon.

Fig. 18 shows another manifestation of this condition. Measurements of dark adaptation made at two wavelengths, such as yielded parallel curves with all the animals discussed above, here yield an entirely different result. In the light-adapted condition, i.e. at the beginning of dark adaptation, the threshold at 420 m\(\mu\) is lower than at 520 m\(\mu\); but after a few minutes of dark adaptation the curves cross, and thereafter the threshold at 520 m\(\mu\) is the lower. Also after about 8 min of dark adaptation the curves are parallel,
and have the spacing that goes with the spectral sensitivity of the dark-adapted eye. Comparable changes accompany the transfer from cone to rod thresholds in human dark adaptation, measured by a similar procedure (Wald, 1960), yet with the striking difference that the shift of sensitivity is in the opposite direction.

As in the other arthropods, dark adaptation in the spider crab seems to proceed in two phases: a rapid hyperbolic fall of log threshold for the first 15–20 min, followed by a long linear phase that persists for another hour. Thereafter one can observe a very slow further fall of log threshold, that possibly represents the tail end of the hyperbolic phase.

### Table I

<table>
<thead>
<tr>
<th>Animal</th>
<th>Shape of ommatidia</th>
<th>Diameter</th>
<th>Area</th>
<th>Log threshold</th>
<th>Threshold</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Limulus</em></td>
<td>Circular</td>
<td>101</td>
<td>7,850</td>
<td>8.20</td>
<td>12,500</td>
</tr>
<tr>
<td>Lobster</td>
<td>Square</td>
<td>61</td>
<td>3,600</td>
<td>9.05</td>
<td>40,300</td>
</tr>
<tr>
<td><em>Orconectes</em></td>
<td>Square</td>
<td>61.5</td>
<td>3,780</td>
<td>8.62</td>
<td>16,000</td>
</tr>
<tr>
<td><em>Procambarus</em></td>
<td>Square</td>
<td>55</td>
<td>3,000</td>
<td>8.50</td>
<td>9,600</td>
</tr>
<tr>
<td>Green crab</td>
<td>Hexagonal</td>
<td>35*</td>
<td>1,060</td>
<td>10.00</td>
<td>106,000</td>
</tr>
<tr>
<td>Spider crab</td>
<td>Hexagonal</td>
<td>35*</td>
<td>1,060</td>
<td>9.25</td>
<td>19,000</td>
</tr>
</tbody>
</table>

* The diameter is that of the inscribed circle \(D\). \(D = 1.732 \times \text{length of one side of the hexagon}\). The area, \(A = 2.60 \times \text{length of one side}^2\).

### Discussion

Table I brings together a number of anatomical observations on these eyes, and estimates of the absolute amounts of light needed to evoke the criterion responses. The facets of the ommatidia display extraordinary variations in shape. Those of *Limulus* are circular in outline, those of the lobster and crayfishes square, and those of the green and spider crabs hexagonal. *Limulus* ommatidia are very large, each about 100 \(\mu\) in diameter, whereas those of green and spider crabs are only about one-third as wide.

The visual thresholds cited in Table I are for the dark-adapted eye, at the wavelength of maximum sensitivity. In terms of intensities incident on the eye—photons/cm\(^2\)/flash—to evoke the criterion response, *Limulus* is most sensitive, the green and spider crabs least. Computation of the number of photons that enter each ommatidial facet per flash yields a somewhat different order: *Procambarus* now leads, and the green crab comes off by far the worst.

* Further data of this kind have recently been published by Eguchi and Waterman (1966), including measurements on *Procambarus* and *Libinia*. 
These numbers of photons are too large to be interesting; but one should remember that the criterion responses lie considerably above the thresholds for just perceptible ERG's, and that the latter lie far above the absolute thresholds of vision in these animals.

As was said in the introduction to this paper, the original incentive for these experiments was finding two visual pigments in extracts of crayfish eyes (Wald, 1967b). The experiments have so far found functions for only one of these pigments—the red-sensitive pigment—in each crayfish. That of _Orconectes_ has $\lambda_{\text{max}}$ about 562 m$\mu$ in solution, as compared with the sensitivity maximum of the red receptor at about 565 m$\mu$. That of _Procambarus_ has $\lambda_{\text{max}}$ about 556 m$\mu$, compared with the maximum sensitivity of its red receptor at about 575 m$\mu$. With due allowance for possible distortions introduced by screening pigments, which are prominent in _Orconectes_ and much more so in _Procambarus_, it seems probable that the red-sensitive visual pigment in each of these animals accounts for the red-sensitive component in what appears to be its color discriminating system.

These color vision systems involve also a blue-sensitive mechanism, peaking at about 435 m$\mu$ in _Orconectes_ and 450 m$\mu$ in _Procambarus_. No corresponding visual pigments have yet been found in extracts of crayfish eyes. Some years ago, however, Goldsmith (1958) extracted from honeybee eyes a visual pigment with $\lambda_{\text{max}}$ 440 m$\mu$; and measurements of the difference spectra of single human cones have revealed a blue-sensitive pigment at about the same wavelength (Marks, Dobelle, and MacNichol, 1964; Brown and Wald, 1964). Both the latter pigments appear to have retinal (vitamin A aldehyde) as chromophore. It is not surprising that the blue-sensitive pigments of crayfish eyes cannot be found in extracts, for judging from the spectral sensitivity functions of the dark-adapted eyes, these must be minor components in their vision, just as the analogous pigment is in human vision (cf. Wald, 1964).

Each of the crayfish eye extracts contained also a minor pigment, in _Orconectes_ well-defined and with $\lambda_{\text{max}}$ about 510 m$\mu$, in _Procambarus_ not as well-characterized, and with $\lambda_{\text{max}}$ apparently near 525 m$\mu$ (Wald, 1967b). In their absorption spectra, this and the red-sensitive pigment have much the same relationship as the visual pigments of the vertebrate rods and cones, rhodopsin and iodopsin. The comparison is apt in a number of other ways also. Just as crayfish "iodopsin" is the red-sensitive pigment of its color vision system, so vertebrate iodopsin is the red-sensitive pigment of human color vision (Brown and Wald, 1963); and, one may add, cyanopsin, the dominant retinal$_2$ pigment of freshwater vertebrate cones (Wald, Brown, and Smith, 1953), is the red-sensitive pigment of color vision in the goldfish (Marks, 1965) and the carp (Tomita et al., 1967).

No function can yet be assigned to crayfish "rhodopsins", the extracted
pigments with \( \lambda_{\text{max}} \) 510 and 525 m\( \mu \). They have such very different kinetics of bleaching from the red-sensitive pigments as to make it very unlikely that they cooperate with the latter in color vision. It was pointed out some years ago that one of the basic conditions that must be fulfilled for a color vision system to function reasonably is a “principle of parallel kinetics.” To maintain some degree of color constancy under a wide variety of states of adaptation and other conditions, the color vision pigments must possess approximately parallel kinetics of bleaching and regeneration so as to keep in step with one another in light and dark adaptation (Wald, 1944, 1949). This consideration alone makes it improbable that the crayfish pigments at 510 and 525 m\( \mu \) can function together with the 558–562 m\( \mu \) pigments in color discrimination.

It seems possible that the crayfish rhodopsins play a quite different role, functioning perhaps at higher levels of adaptation than were achieved in the present experiments.\(^7\) If that were so, the crayfishes would exhibit a reverse Purkinje shift as does the spider crab, their spectral sensitivities moving from 550 or 570 m\( \mu \) in the dark-adapted eye to 510 or 525 m\( \mu \) on high light adaptation. Obviously all of this awaits further examination. It is interesting also that Bruno and Kennedy (1962) found the caudal photoreceptor of *Procambarus* to be maximally sensitive at about 500 m\( \mu \).

The reverse Purkinje shift already observed in the spider crab raises the question why an arthropod may differ so strikingly in this regard from vertebrates. We are at present engaged in a further analysis of this condition in the spider crab, and so should be in better position to discuss it shortly (Wald and Seldin, unpublished observations). One aspect of this situation, however, may be pointed out at once.

A lens eye such as vertebrates possess suffers all the defects of chromatic aberration. Unlike even the cheapest cameras, no vertebrate eye has a color-corrected lens. In all such eyes the lens system brings blue or violet light to a shorter focus than red, so that the eye is never in focus for more than one color at a time. The chromatic aberration of the human eye, for example, rises gradually from the red to about 500 m\( \mu \), then more and more steeply into the blue, violet, and ultraviolet. Chromatic aberration makes difficulties primarily for pattern vision; and in those circumstances in which pattern resolution is most at stake the vertebrate eye—particularly the primate eye—introduces a number of special devices for withdrawing vision from the short wavelengths of the spectrum in which the chromatic aberration is

\(^7\) It is also possible that, as in vertebrates, the crayfish rhodopsins function at lower levels of illumination than those required to evoke the criterion ERG in the dark-adapted eye. The ERG is at best a coarse, high-level response; and the rhodopsins may also be relatively ineffective in contributing to it. For both reasons their contribution may have been missed.
greatest. One of the most effective of such devices is the Purkinje shift from rod to cone vision, with its transposition of visual sensitivity toward the red (cf. Wald, 1967 a).

It will perhaps suffice here to recognize that these considerations have no force in the compound eyes of arthropods, where each ommatidium probably contributes only a patch to a mosaic image, and focusing by lens systems plays little part except to concentrate light upon the rhabdomeres. Along with obvious disabilities, a compound eye has certain virtues, among them the lack of chromatic aberration. It can at least be noted therefore that a reverse Purkinje shift does not embarrass a compound eye as it would that of a vertebrate.

When, as in the two crayfishes and the green crab, I have found evidence of the presence of at least two visual systems differing widely in spectral sensitivity and poised at about the same levels of threshold, I have thought of them as color vision mechanisms. Properly speaking, they represent only the potentiality for color vision. The demonstration that these organisms discriminate colors—that they differentiate aspects of the visual environment on the basis of wavelength—must depend ultimately on behavioral experiments. On the other hand, there are few tasks in all science as difficult as to demonstrate behaviorally that an organism possesses or lacks color vision. One thinks of the enormous effort and patience that went into von Frisch's demonstration that bees possess color vision; and conversely the skepticism with which many of us regard the evidence that all our common domestic animals lack it.

In view of these circumstances I think that the demonstration that an animal possesses an apparatus capable of color differentiation creates a strong presumption that it uses it. Indeed if behavioral experiments failed to substantiate a capacity for color vision in such animals, I would rather suspect the adequacy of those experiments than surrender the likelihood that the animal exploits its potentialities in this regard.

The present status of Johannes Müller's mosaic theory is assessed in a number of papers in The Functional Organization of the Compound Eye (C. G. Bernhard, editor). Oxford, Pergamon Press. 1966. The theory continues to find strong support, particularly as regards the ommatidia acting as units in pattern vision. Whatever doubts may exist in this regard have more force in those insect eyes in which the rhabdomeres are well separated from one another (cf. Fernández-Morán, 1958) than in those insects (e.g. the silk moth; Eguchi, Naka, and Kuwabara, 1962-63) and Crustacea (Eguchi and Waterman, 1966) in which they fuse to form a rhabdome, or in Limulus, in which the dendrite of the eccentric cell picks up the responses of the whole ommatidium (Hartline, Wagner, and Mac-Nichol, 1952).

It is interesting that in the lens eye of the scallop, Cronly-Dillon (1966) has found some evidence of a Purkinje shift, this time in the usual direction. The spectral sensitivity in bright light exhibits two maxima, at 480 and 540 mμ; and lowering the intensity seems to favor the 480 mμ maximum.

Compare also Mazokhin-Porshnakov (1959) on the discrimination of green, yellow, and orange lights by bees; and the same author (1960) on the discrimination of violet and red in the fly Calliphora.
In the present instance there is an added consideration. When I had performed these experiments in the summers of 1962 and 1963, I should, of course, have done more than publish them in abstract. By that time, however, I was so taken with the idea that similar experiments might isolate color vision mechanisms in man that I began such trials at once, and have been so deeply immersed in them ever since that the more complete publication of the arthropod experiments has waited until now. That involves some advantages, however; for now that I have carried through the same pattern of experiments in human subjects with much the same kind of result (Wald, 1964, 1966), one has at least the reassurance of a striking analogy, to grant color vision to these arthropods, though in default of behavioral evidence.

Normal human color vision is trichromatic; it involves three types of cone and three visual pigments, blue-, green-, and red-sensitive, with maximal sensitivities and absorptions at about 435, 540, and 565 m. The two species of crayfish examined seem to possess analogous blue- and red-sensitive mechanisms. I have not been able to find evidence in them of a third mechanism. So far as the present experiments go, they have only dichromatic vision; indeed their responses are strikingly similar to those of human green-blind subjects (deuteranopes) (Wald, 1966).

In the compound eye of arthropods, each ommatidium contains a number of receptor cells, the retinulae, each with its rhabdomere containing visual pigment, and each ending in an optic fiber that runs centrally to the optic ganglion. In those animals that possess multiple visual systems, whether differentiated for color vision or for use in dim and bright light, one must ask whether this differentiation is on the basis of whole ommatidia or the individual retinulae. Do the crayfishes, for example, have blue- and red-sensitive ommatidia, or does each ommatidium contain a distribution of blue- and red-sensitive retinular cells?

This kind of question has begun to be answered for the eye of an insect, the fly Calliphora. Autrum and Burkhardt (1961) measured spectral sensitivities in this eye with microelectrodes fine enough to pick up the responses from single retinular cells. Three different types of spectral sensitivity function were observed, with $\lambda_{\text{max}}$ about 468, 491, and 524 m, each coupled with a minor band in the ultraviolet at about 345 m. Their frequency of occurrence suggested that some distribution of all three types occurs in each ommatidium. In similar experiments with drone bees, Autrum and von Zwehl (1962) found two receptor types, with $\lambda_{\text{max}}$ about 340 and 447 m (compare Goldsmith's observation of a spectral sensitivity maximum in

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11 I have taken qualitative differences in the shape of the ERG at different wavelengths to be presumptive evidence that different receptors are responding. It is of some interest that such differences in the form of the ERG with wavelength, particularly involving the off-response, have been observed in the compound eyes of the worker bee, an animal generally conceded to possess color vision (Goldsmith, 1959–60).
drones at about 440 m\(\mu\), and his extraction of a corresponding visual pigment (1958, 1959–60)).

Recently Langer and Thorell (1966) have succeeded in measuring the absorption spectra of pigments in single rhabdomeres of a white-eyed mutant ("chalky") of Calliphora. They found a major type of spectrum that seemed to be shared by six of the seven rhabdomeres, with a main \(\lambda_{\text{max}}\) at about 510 m\(\mu\) and a minor absorption in the near ultraviolet; and some indication that the seventh rhabdomere in each ommatidium may possess another pigment with \(\lambda_{\text{max}}\) at about 470 m\(\mu\). There was no report in either instance of changes induced by light; yet these bands come close to two of the three sensitivity maxima observed by Autrum and Burkhardt, and presumably were associated with visual pigments. No similar investigations have yet been carried out on the eyes of Crustacea.

This investigation was supported in part with funds from the National Science Foundation and the Office of Naval Research. The experiments were carried out in the summers of 1962 and 1963. Since 31 January 1966, I have had no support from the Office of Naval Research. Preliminary abstracts on these experiments have appeared earlier (Wald, 1962, 1963).

Received for publication 17 August 1967.

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