The Spectral Sensitivities of Single Receptor Cells in the Lateral, Median, and Ventral Eyes of Normal and White-Eyed *Limulus*

JOHN NOLTE and JOEL E. BROWN

From the Department of Biology and Research Laboratory of Electronics, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139

ABSTRACT Spectral sensitivity curves can be distorted by screening pigments. We have determined whether this is true for *Limulus polyphemus* by determining, from receptor potentials recorded using intracellular microelectrodes, spectral sensitivity curves for normal animals and for white-eyed animals (which lack screening pigment). Our results show: (*a*) In median ocelli, the curve for UV-sensitive receptor cells peaks at 360 nm and does not depend on the presence of screening pigment. (*b*) The curve for ventral eye photoreceptors is identical to that for retinular cells from the lateral eyes of white-eyed animals and peaks at 520–525 nm. (*c*) In normal lateral eyes, when the stimulating light passes through screening pigment, the curve indicates relatively more sensitivity in the red region of the spectrum than does the curve for white-eyed animals. Therefore, the screening pigment is probably red-transmitting. (*d*) In median ocelli, the curve for visible-sensitive cells peaks at 525 nm and is approximately the same whether the ocelli are from normal or white-eyed animals. However, the curve is significantly broader than that for ventral eyes and for lateral eyes from white-eyed animals.

INTRODUCTION

A number of studies on *Limulus polyphemus* have indicated that the compound lateral eyes, the median ocelli, and the ventral rudimentary eyes may differ in spectral sensitivity. All the studies on lateral eye (Adolph, 1968; Chapman and Lall, 1967; Graham and Hartline, 1935; Srebro, 1966; Wald and Krainin, 1963; Wasserman, 1969) agree that there is one major population of photoreceptors in this eye, whose spectral sensitivity curve peaks at 520–525 nm. Moreover, the absorption spectrum of the only photopigment that has been extracted from the lateral eye peaks at 520 nm (Hubbard and Wald, 1960). The possibility has been raised that there is a small second population

of receptors in this eye with a broad band of sensitivity in the blue and green regions of the spectrum, whose spectral sensitivity curve nevertheless peaks near 525 nm (Wasserman, 1969). In the median ocellus, it was inferred from ERG studies (Chapman and Lall, 1967; Wald and Krainin, 1963) that two populations of receptors are present; this was confirmed by intracellular recordings from single receptors (Nolte, Brown, and Smith, 1968; Nolte and Brown, 1969 *a*). One population was found to be maximally sensitive in the ultraviolet at 360 nm, and the other in the visible at 530–535 nm. Ventral rudimentary eye spectral sensitivity was determined from intracellular recordings from single receptor cells, and was found to be maximal in the visible at 535–545 nm (Millecchia et al., 1966). A difference spectrum peaking at 530 nm has been found by microspectrophotometry of ventral rudimentary eye receptors (Murray, 1966).

Comparisons of the data from these various studies indicate that there may be small differences among those receptors whose sensitivity maxima lie in the visible region of the spectrum. For example, reported sensitivity maxima range from 520 nm (lateral eye, Wald and Krainin, 1963) to 535–545 nm (ventral eye, Millecchia et al., 1966). In addition, the shapes of spectral sensitivity curves are sometimes reported to be different for (*a*) different eyes (e.g., median ocellus vs. lateral eye; cf. Wald and Krainin, 1963), and (*b*) different studies on the same eye (e.g., Fig. 2, A and B). However, any conclusions which might be drawn from such comparisons are uncertain for two reasons. First, the data come from several laboratories, so it is unclear whether the relatively small differences observed represent differences in the receptors or in the equipment and methodology. Second, the lateral eye, at least, is known to contain screening pigment. Thus, in some cases the spectral sensitivity curves may not be solely a function of the photopigment involved.

In the present work, we have remeasured the spectral sensitivity of lateral eye, median ocellus, and ventral rudimentary eye. Since the equipment and methodology were uniform throughout, the curves may be reliably compared with one another. Also, we have discovered what appear to be screening pigment-free mutants of *Limulus*. These animals made it possible to assess the contribution of the screening pigment to previously reported spectral sensitivity curves. Therefore, we have been able to make unambiguous comparisons of the photopigment systems in the three types of receptor cell. Some of these results have already been published in an abstract (Nolte and Brown, 1969 *b*).

MATERIALS AND METHODS

The optical and recording systems, and the methods of intracellular recording from median ocelli, have been described previously (Nolte et al., 1968; Nolte and Brown,

1969 *a*). Ventral rudimentary eye recordings were made from receptors in the isolated, desheathed nerve (Millecchia et al., 1966). Lateral eye recordings were made from receptors in ommatidia exposed in a slice of lateral eye or in a bisected eye (Fuortes, 1958; Tomita, 1956). In order to project the stimulus onto the side of an ommatidium, the light beam was adjusted to be nearly perpendicular to the optical axis of the ommatidium. The beam was focused to a 100 μ spot which was centered on the ommatidium. When the stimulus was projected through the lens, the light beam was adjusted to be perpendicular to the cornea, and the focused spot was restricted to the intact lens of a single ommatidium. The basic method of determining spectral sensitivity was the same as that described previously (Nolte and Brown, 1969 *a*). Briefly, response magnitude was plotted against the log of the stimulus intensity for a series of different wavelengths. From the resulting family of curves, the relative number of photons needed to elicit a response of criterion amplitude (usually 15 mv) could be found for each wavelength of the series. The reciprocal of this number of photons is defined as the spectral sensitivity. The peak of each curve was arbitrarily set at 5.0. The only exceptions to this technique were the following. In one instance a UV-sensitive cell which generated large (>30 mv) spikes was found in a median ocellus; both the number of spikes and the steady-state depolarization elicited by 5 sec stimuli were determined and used as response parameters in two separate spectral sensitivity measurements. Also, in several lateral and ventral eye receptors, the steady-state depolarization elicited by a long flash was used as the response parameter. These were cells in which the large size of the "bumps" (20 mv or more) made measurements of the size of the responses to brief flashes near threshold impossible. The different choices of response parameter produced identical results, which is expected when a single photopigment is involved in the generation of a response. This follows from the Principle of Univariance discussed by Naka and Rushton (1966), which states that a system involving only one photopigment can signal only the rate at which it absorbs effective quanta, and not the wavelengths of these quanta. One response parameter should serve as well as another for indicating the rate of quantum absorption. The assumption that only one photopigment is involved in the generation of the response seems valid, since chromatic adaptation experiments under similar conditions failed to produce evidence of the activity of more than one pigment in single receptors of the median ocellus (except in the case of "UV-visible cells," which are not considered in this report [Nolte and Brown, 1969 *a*]). Also, chromatic adaptation failed to alter the spectral sensitivity curve of the lateral eye, as determined from ERG measurements (Chapman and Lall, 1967; Wald and Krainin, 1963).

White-Eyed Limulus

We have discovered that, extremely infrequently, specimens of white-eyed *Limulus* can be collected on the west coast of Florida. In spite of the long history of *Limulus* as an experimental animal in visual physiology, such animals appear not to have been described before. These animals have the same general appearance as a normal *Limulus* but can be recognized by the complete absence of screening pigment around the lateral eye ommatidia (Fig. 6), the absence of the faint reddish coloration sometimes seen in median ocelli, and the absence of the pigment layer normally seen between the

inner face of the carapace and the underlying tissue. We assume that these animals are mutants incapable of producing screening pigment, but that their visual systems are otherwise comparable to those of normal animals.

RESULTS

Median Ocellus

1. UV CELLS The averaged data from 10 spectral sensitivity determinations on UV cells from the median ocelli of white-eyed animals are shown in Fig. 1 A. Also shown are the results of an earlier determination (Nolte and Brown, 1969 *a*) on the same type cells from normally pigmented animals. The two curves are quite similar and peak at about 360 nm. As shown in Fig 1 C, the curve for UV cells from white-eyed animals is slightly broader than a nomogram curve (Dartnall, 1953) which peaks at 360 nm.

2. VISIBLE CELLS The averaged data from spectral sensitivity determinations on eight visible cells from the median ocelli of white-eyed animals are shown in Fig. 1 B. Also shown are the results of an earlier determination (Nolte and Brown, 1969 *a*) on visible cells from the ocelli of normal animals. The two curves fit each other best if the curve for normal animals is lowered 0.15 log unit along the sensitivity axis, and this has been done in Fig. 1 B, although a difference of 0.15 log unit is near the limit of resolution of our technique and may not be significant. The curve from normal animals then indicates too little sensitivity in the green and perhaps also in the blue, compared to the curve from white-eyed animals. Both curves peak around 525 nm and have a distinct shoulder in the UV around 350–375 nm. As shown in Fig. 1 C, the curve obtained from white-eyed animals agrees very well with the curve predicted from Dartnall's nomogram for a visual pigment with its peak absorption at 525 nm.

Lateral Eye and Ventral Rudimentary Eye

The averaged data for spectral sensitivity determinations on five retinular cells from the lateral eyes of normally pigmented animals, in which the stimulating light was projected onto the sides of the ommatidia, are shown in Fig. 2 A. Also shown are data redrawn from Wasserman (1969) for the same type receptors (alpha cells, in his terminology), obtained by stimulating in the same way. Both curves peak near 525 nm and the agreement is good throughout the spectrum, except around 350 nm. However, when these same lateral eye data are compared to those obtained by Wald and Krainin (1963) from ERG measurements, as in Fig. 2 B, large discrepancies at both ends of the spectrum are evident; although both curves peak near 525 nm, the ERG curve falls off much more rapidly at both ends of the spectrum than does the single receptor curve.

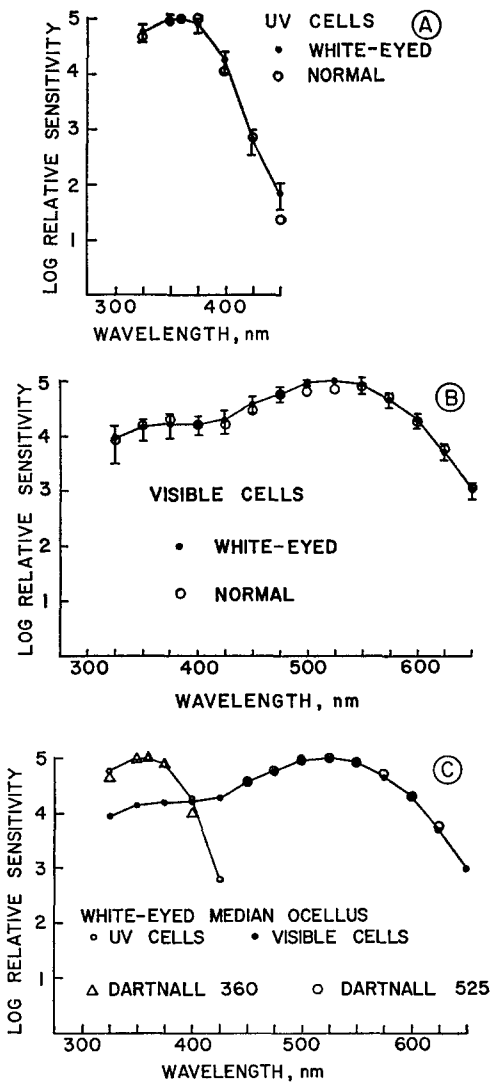


FIGURE 1. A. Spectral sensitivity curve for UV cells in the median ocelli of white-eyed animals. Filled circles are the means of 10 determinations on 9 cells. Error bars are ±1 SD. Open circles are the values found in an earlier determination (Nolte and Brown, 1969 *a*) on similar receptor cells in normally pigmented animals. B. Spectral sensitivity curve for visible cells in the median ocelli of white-eyed animals. Filled circles are the means of determinations on eight cells. Error bars are ±1 SD. Open circles are the values found in an earlier determination (Nolte and Brown, 1969 *a*) on similar receptor cells in normally pigmented animals. The points from normal animals have all been lowered 0.15 log unit in order to obtain the best fit (see text). C. Comparison between experimental spectral sensitivity values (from Fig. 1, A and B) for receptor cells in the median ocelli of white-eyed animals and those predicted by Dartnall's nomogram. Small open circles are experimental values for UV cells; triangles are values predicted by Dartnall's nomogram for a pigment with λ_{max} at 360 nm. Filled circles are experimental values for visible cells; large open circles are values predicted by Dartnall's nomogram for a pigment with λ_{max} at 525 nm.

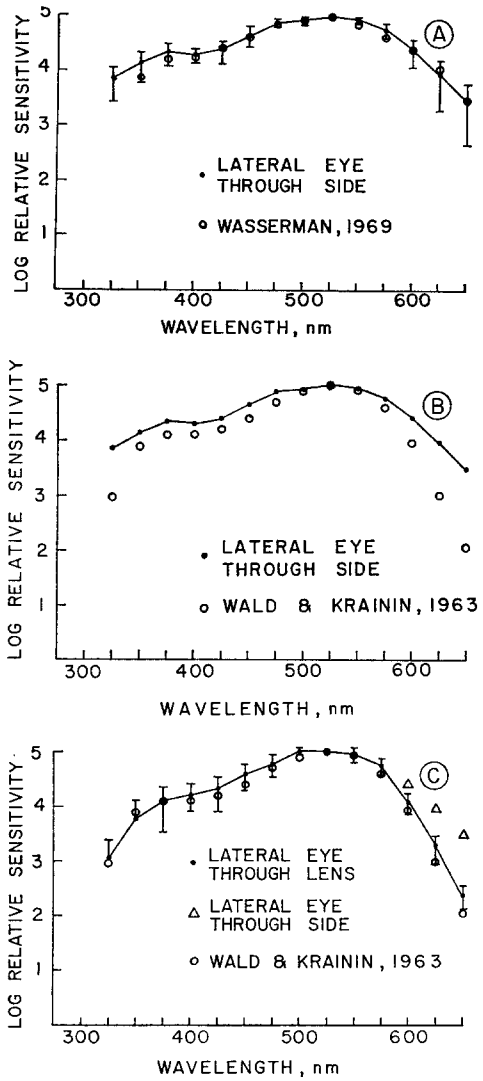


FIGURE 2. A. Spectral sensitivity curve for retinular cells from the lateral eyes of normally pigmented animals, when the stimulus is projected onto the side of the ommatidium. Filled circles are the means of determinations on five cells. Error bars are ±1 SD. Open circles are data redrawn from Wasserman (1969) for one of the two receptor types (called alpha cells) he found by similar techniques. B. Spectral sensitivity curve for retinular cells from the lateral eyes of normally pigmented animals, when stimulus is projected onto the side of the ommatidium. Filled circles are the means of determinations on five cells (same data as Fig. 2 A). Open circles are data from Wald and Krainin (1963), and were obtained from ERG measurements on lateral eyes. C. Spectral sensitivity curve for retinular cells from the lateral eyes of normally pigmented animals, when stimulus is projected through the lens. Filled circles are the means of determinations on six cells. Error bars are ±1 SD. Open circles are data redrawn from Wald and Krainin (1963), as in Fig. 2 B. For comparison, the triangles are points from the data of Fig. 2 A, for lateral eye retinular cells when the stimulus is projected onto the side of the ommatidium.

We used an alternate stimulus path for six lateral eye retinular cells; the light was projected through the lens, rather than onto the sides of the ommatidia. The averaged data are shown in Fig. 2 C; in this case, the curve agrees fairly well with that found by Wald and Krainin, at both ends of the spectrum.

We also examined the spectral sensitivity of ventral eye photoreceptors. The averaged data for determinations on nine such cells are shown in Fig. 3 A. This curve also peaks around 525 nm, and agrees well with the lateral eye ERG data of Wald and Krainin, except at the short wavelength end of the spectrum.

Finally, we examined the spectral sensitivity of receptors from the lateral eyes of white-eyed animals; in all these cases the stimulus was projected onto the side of the ommatidium. The averaged data for 10 determinations on eight receptor cells are shown in Fig. 3 B. The curve peaks near 525 nm, and agrees very well with the ventral eye curve of Fig. 3 A.

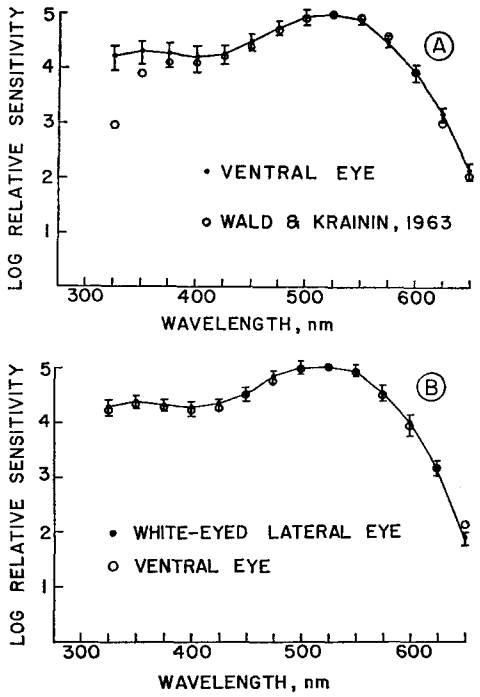


FIGURE 3. A. Spectral sensitivity curve for photoreceptor cells from the ventral rudimentary eye. Filled circles are the means of determinations on nine cells. Error bars are ±1 SD. Open circles are data redrawn from Wald and Krainin (1963), as in Fig. 2, B and C. B. Comparison of spectral sensitivity curves for retinular cells from the lateral eyes of white-eyed animals, when the stimulus is projected onto the side of the ommatidium, with the spectral sensitivity curve for ventral eyes. Filled circles are the averages of 10 determinations on 8 cells. Error bars are ±1 SD. Open circles are the ventral eye data of Fig. 3 A.

Fig. 4 A shows a comparison between the spectral sensitivity curve for ventral eye receptors and the curve predicted by Dartnall's nomogram for a pigment with maximal absorption at 520 nm. This is the closest fit we can obtain, but the ventral eye sensitivity curve is too narrow to fit the nomogram curve satisfactorily.

Fig. 4 B shows the spectral sensitivity curves obtained for ventral eye receptors and for visible cells from the median ocelli of white-eyed animals. The agreement is surprisingly poor: the ventral eye curve falls off more rapidly at long wavelengths, and has a higher UV shoulder.

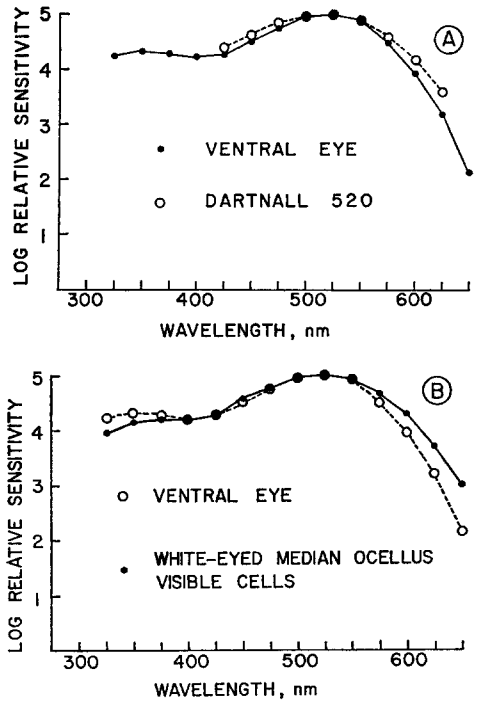


FIGURE 4. A. Comparison between experimental spectral sensitivity values (from Fig. 3 A) for receptor cells in the ventral rudimentary eye and those predicted by Dartnall's nomogram. Filled circles are the experimental values. Open circles are values predicted by Dartnall's nomogram for the pigment with λ_{max} at 520 nm. B. Comparison between the spectral sensitivity curve for ventral rudimentary eye photoreceptors and that for visible cells in the median ocelli of white-eyed animals. Open circles are the data for ventral eye receptors shown in Fig. 3 A. Filled circles are the data for visible cells in the median ocelli of white-eyed animals shown in Fig. 1 B.

DISCUSSION

Median Ocellus

1. UV CELLS An earlier study of the spectral sensitivity of median ocellus UV cells (Nolte and Brown, 1969 *a*) could not eliminate the possi-

bility that the shape of the spectral sensitivity curve was in some measure due to the presence of a screening pigment. That is, if a visible-blocking screening pigment were present, then either (*a*) the β-band absorption of a photopigment with λ_{max} in the visible, or (*b*) the UV-stimulated fluorescence of some cell component, could explain the UV sensitivity of these receptors. However, since the spectral sensitivity curves for UV cells from the median ocelli of white-eyed and normal animals agree, the curve previously found for normal animals cannot be due to the presence of screening pigment. This lends further credibility to the idea that a property of the receptors themselves is reflected in the UV sensitivity of the numerous functional UV receptors reported by others (e.g., Autrum and von Zwehl, 1962, 1964; Bennett, 1967; Bruckmoser, 1968; Gogala, 1967; Goldsmith and Fernandez, 1968; Hasselmann, 1962; Wald and Seldin, 1968; Walther and Dodt, 1959). The curve obtained from white-eyed animals raises some doubt as to whether UV cell spectral sensitivity can be described by a Dartnall nomogram, as we had previously thought. It now seems that the true spectral sensitivity curve may be slightly broader than the nomogram curve (Fig. 1 C). Autrum and von Zwehl (1964) found a similar discrepancy for UV receptor cells of the bee *Apis*.

2. VISIBLE CELLS The spectral sensitivity curves obtained for visible-sensitive receptor cells from the median ocelli of normal and white-eyed animals agree reasonably well. The comparison shown in Fig. 1 B suggests that perhaps in normal animals the sensitivity of these cells to red light is slightly enhanced relative to the sensitivity in the green and blue; in agreement with this, we sometimes see a slight reddish pigmentation in median ocelli (see below for a further discussion of this pigment), which could produce such an effect in averaged data. However, the observed differences of about 0.15 log unit are near the limits of resolution of our technique, and may not be real.

The lack of agreement between the spectral sensitivity curves for median ocellus visible cells, and for lateral and ventral eye photoreceptors (Fig. 4 B), is somewhat disturbing. The difference at the long wavelength end of the spectrum was noticed by Wald and Krainin (1963) when comparing curves, obtained from ERG measurements, for the median ocellus and the lateral eye. It seems clear that the differences are real, but they are difficult to interpret unequivocally. One possible explanation is that two different photopigments, both with nearly the same λ_{max}, exist in the photoreceptors of different eyes of *Limulus*. On the other hand, unless the median ocellus pigment is extracted and its absorption properties in dilute solution analyzed, one cannot exclude the possibility of self-screening. As Goldstein and Williams (1966) have indicated, any receptor which has a relatively high optical density (due to its photopigment) along the path of the measuring light beam will appear to

have an absorption spectrum (or spectral sensitivity curve) broader than the true absorption curve measured in dilute solution. That this kind of self-screening operates in median ocellus photoreceptors seems unlikely to us, since the total length of rhabdomere along the axis of a median ocellus photoreceptor is not greater than that along a lateral eye retinular cell; in order for self-screening to be operative in the former but not the latter, one would have to postulate that the pigment content of the rhabdomeric membranes is different in the two cell types. This kind of morphological difference seems less likely than the existence of two different pigments.

Lateral Eyes and Ventral Rudimentary Eyes

Spectral sensitivity curves determined from intracellular recordings in single *Limulus* photoreceptors can be distorted, if the stimulating light passes through a colored screening pigment. An earlier report (Wasserman, 1967) indicated that the density of *Limulus* lateral eye screening pigment is constant from 375 to 675 nm (i.e., the pigment is black). We feel that the evidence presented here shows that the pigment screen is not black, but rather may be red-transmitting. Thus, for wavelengths longer than 400 nm, two kinds of spectral sensitivity curve can be found for lateral eye photoreceptors. The first type is found when the stimulating light passes through screening pigment (Fig. 2 A) and is characterized by a relatively slow decline of sensitivity with increasing wavelength. The second type is found when the screening pigment is bypassed, by projecting the stimulus through the lens (Fig. 2, B and C), or by using lateral eyes from white-eyed animals (Fig. 3 B). This type is characterized by a relatively rapid decline in sensitivity with increasing wavelength. Since sensitivity in the red is enhanced (relative to sensitivity in the green) when the stimulus passes through the screening pigment, we conclude that the screening pigment is more transparent to red light than to green. These results are similar to those found for the fly *Musca*, in which spectral sensitivity curves can be distorted by a red-transmitting screening pigment which surrounds the ommatidia (Goldsmith, 1965; Strother, 1966).

Subtracting the spectral sensitivity curve for normal lateral eye retinular cells, when the stimulus passes through screening pigment, from that obtained from retinular cells from white-eyed mutants should yield an average absorption spectrum for the screening pigment (Fig. 5). ("Screening pigment" in this context means the conglomerate of all light-absorbing substances between the rhabdom and the exterior of the ommatidium.) The results indicate that the absorption of the screening pigment is constant from 375 to 550 nm, decreases for wavelengths longer than 550 nm, and increases slightly for wavelengths shorter than 375 nm. The increased absorption in the UV should be functionally insignificant, since the absorption of the lateral eye lens increases much more rapidly in the UV than does that of the screening

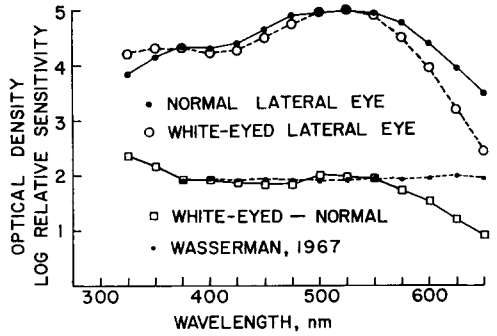
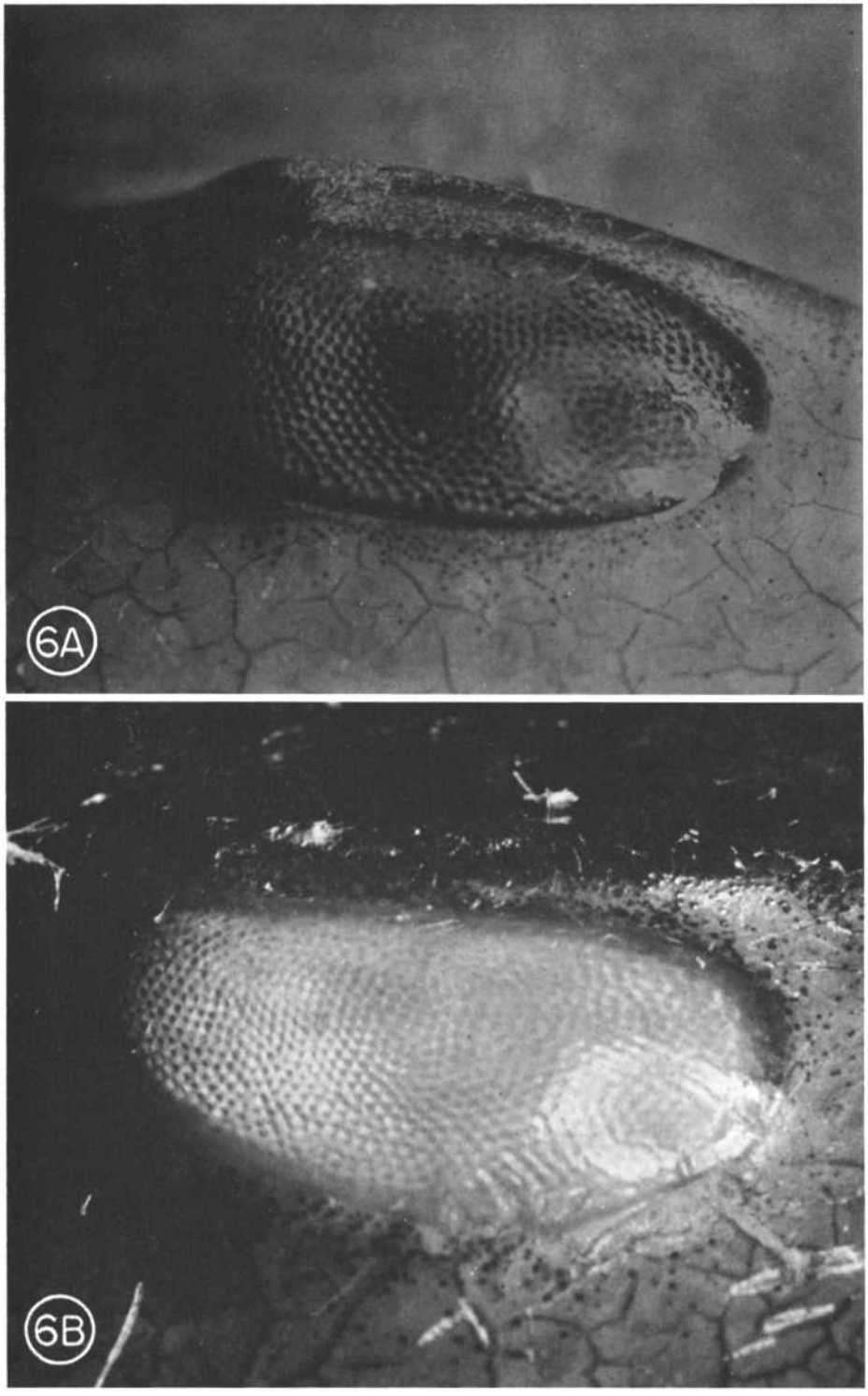


FIGURE 5. Properties of the pigment screen surrounding lateral eye ommatidia in normally pigmented animals. Large filled circles are the data from Fig. 2 A for lateral eye retinular cells from normally pigmented animals, when the stimulus is projected on the side of the ommatidium. Open circles are the data from Fig. 3 B for lateral eye retinular cells from white-eyed animals. Squares show the difference between these log sensitivity values. This should be a measure of the optical density of the pigment screen in normal lateral eyes. For comparison, small filled circles are the neutral density spectrum reported by Wasserman (1967) for lateral eye screening pigment. The ordinate at 525 nm for both density spectra is arbitrary, so the curves indicate only relative density across the spectrum.

pigment. This can be seen from a comparison of the spectral sensitivity curves obtained when the stimulus is projected through the lens (Fig. 2, B and C) with those obtained when both lens and screening pigment are bypassed, as in lateral eyes from white-eyed animals (illuminated from the side, Fig. 3 B). Presumably the presence or absence of a lens in the light path is the only difference between the two situations; with the lens present, a precipitous drop in sensitivity at wavelengths shorter than 375 nm is seen. We conclude that the absorption of the lens is nearly constant from 650 to 375 nm and increases rapidly for wavelengths shorter than 375 nm.

There is an alternative explanation for the distortions of spectral sensitivity found when the stimulating light passes through screening pigment. This is that the screening pigment particles are relatively opaque, but have a non-neutral reflection spectrum, i.e. the pigmented particles selectively reflect long wavelength light. Then the fraction of the incident light which passes through the pigment screen, through the rhabdom, and is reflected by the pigment screen on the opposite side of the ommatidium would contain relatively more long wavelength energy than would the incident light. The net effect would be to make long wavelength light a relatively more effective stimulus than would be predicted from the absorption spectrum of the photopigment. Such a mechanism would be consistent with Wasserman's (1967) observation that the screening pigment appears black by transmission spectroscopy. Since we have no information on the chemical nature of the screening pigment, an unequivocal decision between the alternatives is not possible



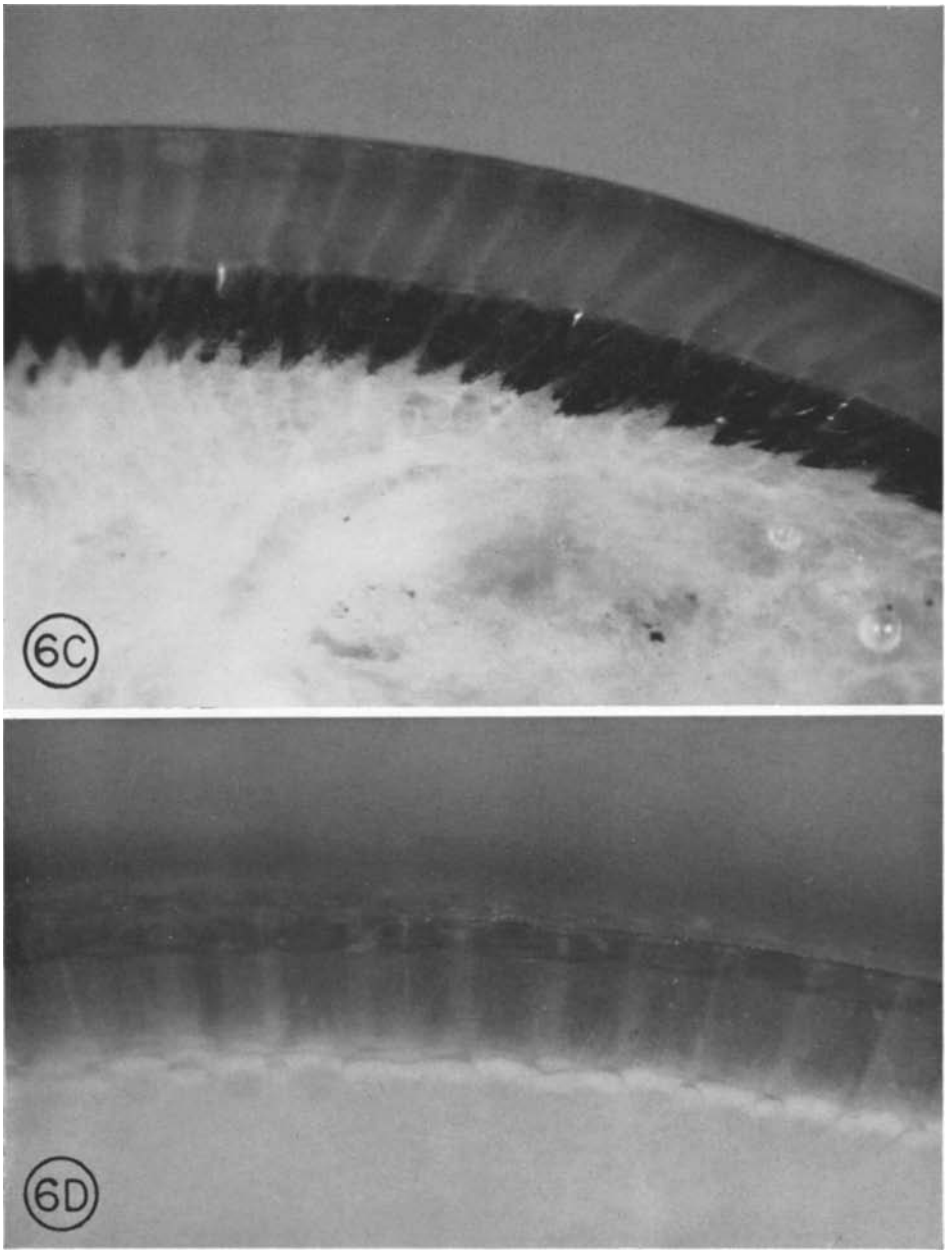


FIGURE 6. Photographic comparison of the lateral eyes of (A) a normally pigmented and (B) a white-eyed *Limulus*. Notice that the dark screening pigment is especially apparent in the "pseudopupil" of A, but is absent in the pseudopupil of B. The white streaks and annulus are reflections of the light source. Photographic comparison of lateral eyes fixed in buffered glutaraldehyde and sectioned parallel to the ommatidial axis of (C) a normally pigmented and (D) a white-eyed *Limulus*. Notice that an elongated ommatidium can be seen lying behind each lenslet in the normal eye. In (D), the ommatidia cannot be distinguished, due to the lack of pigment. Figs. 6 A and 6 B, × 9; Figs. 6 C and 6 D, × 33.

at present. However, a mechanism based on the preferential reflection of red light (and absorption of the shorter wavelength light) would also require multiple internal reflections. The maximum possible effect of one reflection is to double the path length (through the photopigment) for red light relative to green light whereas the sensitivity in the red is increased (relative to the sensitivity in the green) by a factor of 10 (Fig. 5). We feel that such a mechanism, which depends on high internal reflection of long wavelength light, is unlikely.

A further conclusion from these comparisons is that lateral and ventral eye photoreceptors contain the same photopigment, with peak absorption at about 520 nm. This is indicated by the very close agreement between the spectral sensitivity curves for lateral eye cells from white-eyed animals and that for ventral eye receptor cells (Fig. 3 B). The ventral eye curve also agrees well with the curve obtained by projecting the stimulus through the lens of lateral eyes from normal animals, for wavelengths longer than the lens cutoff (Fig. 3 A).

The peak of 520 nm which we find for ventral eye photoreceptors lies at a slightly shorter wavelength than the 535–545 nm peak reported by Millecchia et al. (1966).

We have not found any receptors in either lateral, median, or ventral eyes with spectral sensitivity curves similar to those of the beta cells described by Wasserman (1969).

Hubbard and Wald (1960) have reported extracting a photopigment with λ_{max} at 520 nm from *Limulus* lateral eyes. The difference spectrum for this pigment was matched by Dartnall's nomogram. Murray (1966) recorded the absorption spectrum of a photopigment in *Limulus* ventral eye photoreceptors by difference microspectrophotometry. This pigment had λ_{max} at 529 nm and was also matched by Dartnall's nomogram. In comparison to these two studies, we find that lateral and ventral eye photoreceptors yield the same spectral sensitivity curve, with λ_{max} at 520 nm, which is too narrow to be fit by Dartnall's nomogram (Fig. 4 A).

This work was supported by the National Eye Institute (Grants 5 R01 EY00377-03 and 5 R01 EY00312-05).

The authors express their appreciation to Mr. Jack Rudloe of the Gulf Specimen Company for collecting specimens of white-eyed *Limulus* and to Dr. Charles Holt and Kenneth Muller for reading the manuscript.

Received for publication 29 December 1969.

REFERENCES

ADOLPH, A. R. 1968. Thermal and spectral sensitivities of discrete slow potentials in *Limulus* eye. *J. Gen. Physiol.* **52**:584.

AUTRUM, H., and V. VON ZWEHL. 1962. Zur spektralen Empfindlichkeit einzelner Sehzellen der Drohne (*Apis mellifica* ♂). *Z. vergl. Physiol.* **46**:8.

AUTRUM, H., and V. VON ZWEHL. 1964. Die spektrale Empfindlichkeit einzelner Sehzellen des Bienenauges. *Z. vergl. Physiol.* **48**:357.

BENNETT, R. R. 1967. Spectral sensitivity studies on the whirligig beetle, *Dineutes ciliatus*. *J. Insect Physiol.* **13**:621.

BRUCKMOSER, P. 1968. Die spektrale Empfindlichkeit einzelner Sehzellen des Rückenschwimmers *Notonecta glauca* L. (Heteroptera). *Z. vergl. Physiol.* **59**:187.

CHAPMAN, R. M., and A. B. LALL. 1967. Electroretinogram characteristics and the spectral mechanisms of the median ocellus and the lateral eye in *Limulus polyphemus*. *J. Gen. Physiol.* **50**:2267.

DARTNALL, H. J. A 1953. The interpretation of spectral sensitivity curves *Brit. Med. Bull.* **9**:24.

FUORTES, M. G. F. 1958. Electric activity of cells in the eye of *Limulus*. *Amer. J. Ophthalmol.* **46**:210.

GOGALA, M. 1967. Die spektrale Empfindlichkeit der Doppelaugen von *Ascalaphus macaronius* Scop. (Neuroptera, Ascalaphidae). *Z. vergl. Physiol.* **57**:232.

GOLDSMITH, T. H. 1965. Do flies have a red receptor? *J. Gen. Physiol.* **49**:265.

GOLDSMITH, T. H., and H. R. FERNANDEZ. 1968. Comparative studies of crustacean spectral sensitivity. *Z. vergl. Physiol.* **60**:156.

GOLDSTEIN, E. B., and T. P. WILLIAMS. 1966. Calculated effects of "screening pigments." *Vision Res.* **6**:39.

GRAHAM, C. H , and H. K HARTLINE. 1935. The response of single visual sense cells to lights of different wavelengths. *J. Gen. Physiol.* **18**:917.

HASSELMANN, E-M. 1962. Über die relative spektrale Empfindlichkeit von Käfer- und Schmetterlingsaugen bei verschiedenen Helligkeiten. *Jahrb. Abt. Allg. Zool. Physiol. Tiere.* **69**:537.

HUBBARD, R., and G. WALD. 1960. Visual pigment of the horseshoe crab, *Limulus polyphemus*. *Nature* (*London*). **186**:212.

MILLECCHIA, R., J. BRADBURY, and A. MAURO. 1966. Simple photoreceptors in *Limulus polyphemus*. *Science* (*Washington*). **154**:1199.

MURRAY, G. 1966. Intracellular absorption difference spectrum of *Limulus* extra-ocular photolabile pigment. *Science* (*Washington*). **154**:1182.

NAKA, K-I., and W. A. H. RUSHTON. 1966. S-potentials from colour units in the retina of fish (Cyprinidae). *J. Physiol.* (*London*). **185**:536.

NOLTE, J., and J. E. BROWN. 1969 *a*. Spectral sensitivities of single photoreceptors in the median ocellus of *Limulus*. *J. Gen. Physiol.* **54**:636.

NOLTE, J., and J. E. BROWN 1969 *b*. Spectral sensitivity of single photoreceptors in the lateral and ventral eyes of *Limulus*. *Biol. Bull.* (*Woods Hole*). **137**:410.

NOLTE, J., J. E. BROWN, and T. G. SMITH, JR. 1968. A hyperpolarizing component of the receptor potential in the median ocellus of *Limulus*. *Science* (*Washington*). **162**:677.

SREBRO, R. 1966. A thermal component of excitation in the lateral eye of *Limulus*. *J. Physiol.* (*London*). **187**:417.

STROTHER, G. K. 1966. Absorption of *Musca domestica* screening pigment. *J. Gen. Physiol.* **49**:1087.

TOMITA, T. 1956. The nature of action potentials in the lateral eye of horseshoe crab as revealed by simultaneous intra- and extracellular recording. *Jap. J. Physiol.* **6**:327.

WALD, G., and J. M. KRAININ. 1963. The median eye of *Limulus*: an ultraviolet receptor. *Proc. Nat. Acad. Sci. U.S.A.* **50**:1011.

WALD, G., and E. SELDIN. 1968. Spectral sensitivity of the common prawn, *Palaemonetes vulgaris*. *J. Gen. Physiol.* **51**:694.

WALTHER, J. B., and E. DODT. 1959. Die Spektralsensitivität von Insekten-Komplexaugen im Ultraviolett bis 290 mμ. *Z. Naturforsch.* **14b**:273.

WASSERMAN, G. S. 1967. Density spectrum of *Limulus* screening pigment. *J. Gen. Physiol.* **50**: 1075.

WASSERMAN, G. S. 1969. *Limulus* receptor action spectra. *Vision Res.* **9**:611.