Electroretinogram Characteristics and the Spectral Mechanisms of the Median Ocellus and the Lateral Eye in *Limulus polyphemus*

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ABSTRACT Electrical responses (ERG) to light flashes of various wavelengths and energies were obtained from the dorsal median ocellus and lateral compound eye of *Limulus* under dark and chromatic light adaptation. Spectral mechanisms were studied by analyzing (*a*) response waveforms, e.g. response area, rise, and fall times as functions of amplitude, (*b*) slopes of amplitude-energy functions, and (*c*) spectral sensitivity functions obtained by the criterion amplitude method. The data for a single spectral mechanism in the lateral eye are (*a*) response waveforms independent of wavelength, (*b*) same slope for response-energy functions at all wavelengths, (*c*) a spectral sensitivity function with a single maximum near 520 mμ, and (*d*) spectral sensitivity invariance in chromatic adaptation experiments. The data for two spectral mechanisms in the median ocellus are (*a*) two waveform characteristics depending on wavelength, (*b*) slopes of response-energy functions steeper for short than for long wavelengths, (*c*) two spectral sensitivity peaks (360 and 530–535 mμ) when dark-adapted, and (*d*) selective depression of either spectral sensitivity peak by appropriate chromatic adaptation. The ocellus is 200–320 times more sensitive to UV than to visible light. Both UV and green spectral sensitivity curves agree with Dartnall's nomogram. The hypothesis is favored that the ocellus contains two visual pigments each in a different type of receptor, rather than (*a*) various absorption bands of a single visual pigment, (*b*) single visual pigment and a chromatic mask, or (*c*) fluorescence. With long duration light stimuli a steady-state level followed the transient peak in the ERG from both types of eyes.

The horseshoe crab (*Limulus polyphemus*) possesses compound lateral eyes and simple median ocelli. The structural and functional properties of these eyes are similar in some respects and differ in others. The structure of the lateral eyes of *Limulus polyphemus* is well-known (Miller, 1957). The median ocelli

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are less than 2 mm in diameter and lie at the base of the median frontal spine. Histological examination (Demoll, 1914) shows a cuticular cornea fused with an almost spherical lens beneath which lies a vitreous humor region and the retina. Limiting membranes composed of epidermal cells line the inner and outer border of the retina. Some of the epidermal cells penetrate the retina as perpendicular strings which divide the retina into cell groups. Sometimes a rhabdomere type of structure, presumed to contain the visual pigment in the lateral eye, is visible along the border of larger retinula cells in the ocellus. Demoll reported that each retinula cell leaves the retina by an optic nerve fiber without synapse. In the whole ocellus, Wulff and Pandazi (1951) counted 50 to 80 sense cells.

Earlier studies indicate that the lateral eyes have a single spectral mechanism[1] (Graham and Hartline, 1935; Wald and Krainin, 1963; Lall and Chapman, 1964) and the median ocelli have two spectral mechanisms (Lall, 1962; Wald and Krainin, 1963; Lall and Chapman, 1964). In the present experiments electrical responses were obtained from both kinds of eyes in order to (*a*) describe the response waveforms and their relation to different spectral mechanisms, (*b*) study the relation of response-energy functions to spectral mechanisms, (*c*) determine the spectral sensitivity curves for the various spectral mechanisms, (*d*) isolate each spectral mechanism by chromatic light adaptation, and (*e*) relate the data to various hypotheses involving visual pigments, screening pigments, and fluorescent pigments (see Discussion). The spectral analyses of (*a*) response waveform, (*b*) slopes of amplitude-energy functions, and (*c*) spectral sensitivity by the criterion amplitude method are independent in that they involve independent aspects of the data and different conclusions might be reached. For example, the spectral sensitivity analysis could indicate two spectral mechanisms although all responses might show the same waveform and all amplitude-energy functions might show the same slope.

Several questions involve the time course or waveform of the ERG. Is there a steady-state component which lasts as long as the stimulus light is maintained? Wald and Krainin (1963) were unable to find one for the ocellus. The ocellar waveform differences with wavelength obtained by Wald and Krainin (1963) are not the same as those obtained in this laboratory. Stray light is discussed as the source of the discrepancy. Since the ERG waveforms vary with light energy, how may the ERG changes with light energy and wavelength be systematized to reveal wavelength effects? It is shown here that the ocellar waveform differences are related to different spectral mechanisms. Waveform differences in responses attributed primarily to visual re-

[1] By "spectral mechanism" is meant a part of the visual system which functions as a unit with respect to light wavelength and whose only dependence on wavelength is relative sensitivity. Thus, a spectral mechanism may involve, but not necessarily be limited to, one kind of receptor (e.g. Stiles, 1953).

ceptors themselves support the possibility of temporal coding of wavelength information, on the one hand, and may relate to the mechanisms of visual excitation, on the other.

Only white light has previously been used to study the relation between the amplitude of the ocellar response and light energy (Wulff and Pandazi, 1951). In the present experiments the light wavelength is varied and the slopes of the response-energy curves are shown to differ for different spectral mechanisms.

The use of dark and chromatic (violet and orange) adaptation helps to separate and identify the different spectral mechanisms.

As the *Limulus* lateral eye has been useful in studying lateral interaction (for review, Ratliff, 1965), the *Limulus* ocellus may be useful in studying spectral mechanisms. Some ocellar features which appear helpful include the structural simplicity, the presence of two spectral mechanisms located well apart in the spectrum, and the simplicity of the ERG waveforms.

METHODS

Optical System

The optical system used a 100 w tungsten ribbon filament light source (operated at 17.5 amp with a regulated power supply) and a Bausch and Lomb grating monochromator (10 mμ half-band width). The stimulus durations were controlled by various shutters, photographic, electromagnetic, or sectored disc. Spectrally calibrated Wratten neutral density filters in the light path controlled the energy of the stimuli. The infrared radiation was absorbed either by Corning heat filters or by a $CuCl_2$ quartz cell. The relative energies of the monochromatic lights were measured with a Farrand thermocouple placed in the eye position. The thermocouple output was averaged on a computer and the relative energies determined by the criterion amplitude method (see below).

Chromatic adaptation using either long or short wavelengths was obtained by a 6 v microscope illuminator with Corning glass filters (2-61, 2-73, 3-67, 5-57, 5-59, 7-51, and 7-59). During the adaptation session the maintained light fell on the eye at about a 45° angle to the stimulus light beam.

Animal Preparation and Recording

Limulus polyphemus (carapace width 8–14 cm for young animals and 25–40 cm for adult animals) were obtained from the Marine Biological Laboratory in Woods Hole and kept in aerated seawater aquaria. Most of the data were obtained from the intact animal at room temperature (23–25°C).

An image of the grating of the monochromator was focused on the lateral eye. The active electrode was a cotton wick bathed in seawater. This electrode, shielded from the light beam, was placed laterally on the scraped corneal surface. The slit image of the monochromator was focused on the ocellus in order to deliver the maximum light flux to this small eye. The active electrode was either a seawater-filled

glass pipette (25–75 μ tip diameter) placed in a hole made in the ocellar lens or a cotton wick placed on the cornea. In all experiments an Ag-AgCl electrode made contact between the seawater and the input of the amplifier. The reference electrode was embedded underneath the carapace through an opening made near the eye while the ground electrode was placed on the scraped surface of the carapace in all intact preparations.

In some experiments records were obtained from excised ocelli using the method of Wulff and Pandazi (1951). The ocellus was dissected free of carapace and cornea, carefully removed with a section of ocellar nerve, and introduced into a small capillary tube by suction. The ocellar preparation lodged against the constriction in one end of the tube. The tube was then sealed into a small hole in the partition of the electrode chamber. The chambers on each side of the partition contained seawater and Ag-AgCl recording electrodes.

A Philbrick P-2 DC operational amplifier used differentially with an input impedance of 1 meg or a Tektronix Type 122 AC-coupled amplifier (0.2 cps) was the first stage of amplification. Further amplification was by DC amplifiers with the high frequency noise reduced by low pass filtering (50 cps). The responses were displayed on an inkwriter and a CRO. In some experiments responses were converted into 256 amplitude measurements spaced 1, 2, or 4 msec apart starting with the light onset. These digital values were handled by a general purpose computer (Packard Bell 250) which was programmed to plot the waveform on an X-Y plotter and punch the values on paper tape. In some cases average waveforms were computed. Various waveform characteristics were measured by the computer from the punched tape.

PROCEDURE Light flashes of 0.1 sec duration of selected wavelengths (320–680 mμ) were given over 3 log units of light energy. For the lateral eye 3–5 min between successive flashes permitted recovery of sensitivity, while 1 to 2 min were sufficient for the *in situ* ocellus. During the collection of dark-adapted data the preparation was kept in complete darkness for at least 30 min prior to experimentation. A "standard" flash was periodically given to check the reliability.

The use of intact animals permitted successful recording sessions lasting up to 28 hr and involving up to 339 responses.

SPECTRAL SENSITIVITY FUNCTIONS The amplitude of the ERG was plotted as a function of log energy of the test flash at each test wavelength. From such a family of curves a spectral sensitivity function was obtained by choosing a criterion amplitude and determining the relative energy at each wavelength required to elicit that criterion amplitude. The reciprocal of this energy, expressed in relative number of quanta, as a function of wavelength gave a spectral sensitivity function.

RESULTS

General Characteristics of Lateral Eye and Median Ocellar Responses

When the *Limulus* median ocellus or lateral eye was illuminated, the corneal surface became negative with respect to a reference elsewhere. These electrical responses (ERG's) showed a transient peak followed by a steady-state

level maintained for the duration of the stimulus (Fig. 1). Both transient and steady-state parts of the ERG were recorded from both the median ocellus and lateral eye. However, for the ocellus the steady-state amplitude was a smaller fraction of the transient amplitude than it was for the lateral eye. This probably accounts for Wald and Krainin's (1963) failure to obtain the steady-state amplitude from the ocellus. The transient time course appeared to be faster in the ocellus than in the lateral eye. In keeping with the pre-

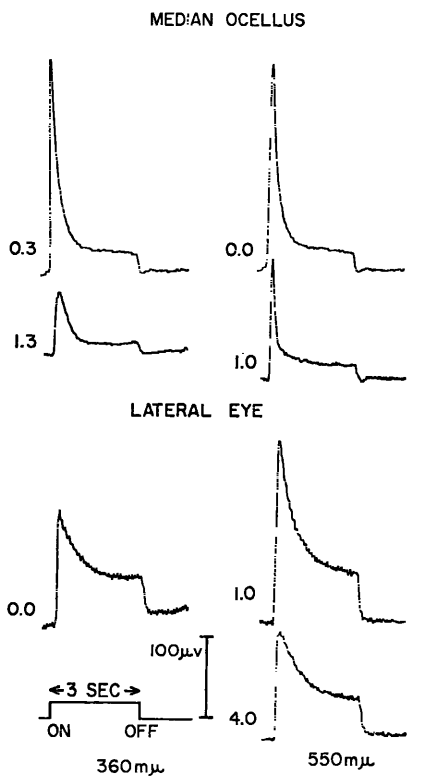

FIGURE 1. Electroretinograms illustrating the phasic and steady components of the responses to long duration stimuli (3 sec) at 360 and 550 mμ from median ocellus and lateral eye in *Limulus*. Numbers to left of ERG's refer to neutral density filter in light path. Forty responses are averaged for median ocellus and 24 for lateral eye. DC recording. Data from a single preparation.

dominantly transient, fast nature of the ocellar ERG, the sensitivity recovery after a light flash was faster for the ocellus than for the lateral eye.

Both transient and steady-state parts of the ERG were elicited by a wide range of wavelengths and energies (Fig. 1). Both ERG parts increased in amplitude with higher light energies. Light wavelength did not appear to affect the relative amplitude of the transient and steady-state features for each kind of eye. This relation was not studied quantitatively.

Quantitative analyses were based on responses to relatively short duration light flashes (approximately 0.1 sec) which elicited relatively simple waveforms (Fig. 2) to a wide range of wavelengths and energies.

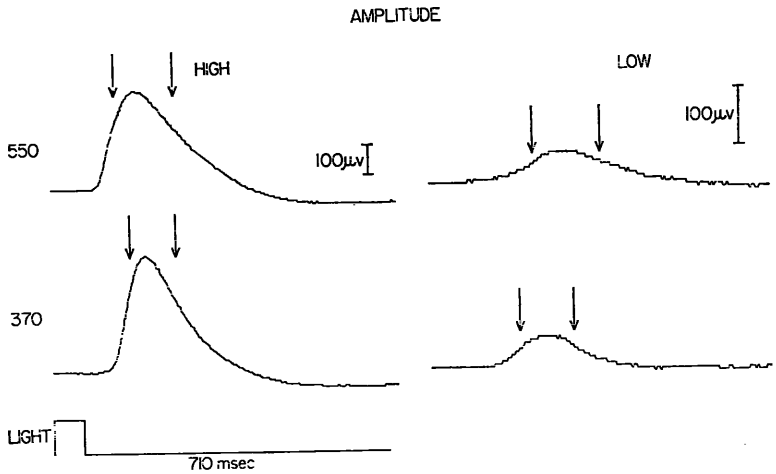

FIGURE 2. High and low amplitude ERG's from *Limulus* median ocellus for high and low energy lights at 370 and 550 mμ. Arrows mark the rise and fall times. Flash duration 0.1 sec. AC recording. Data from one experiment.

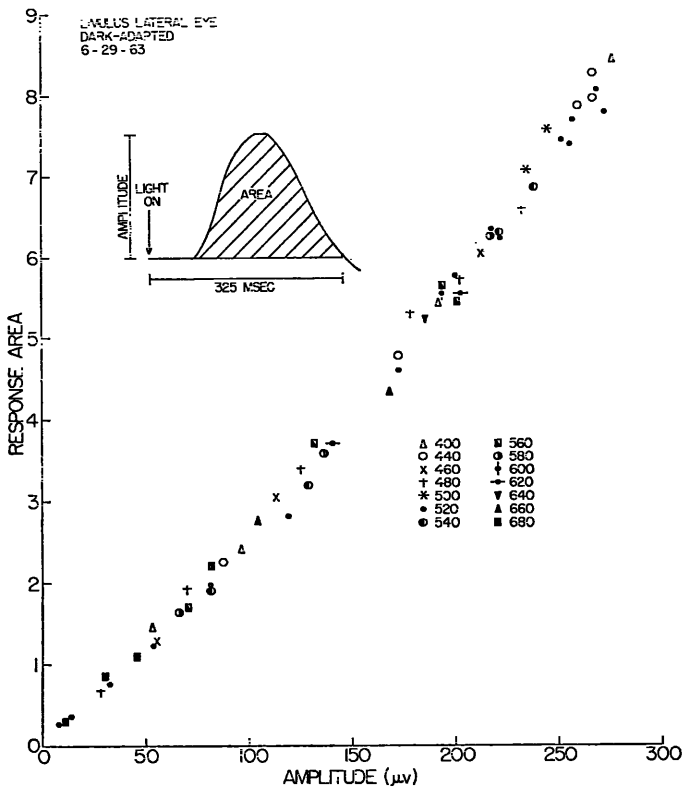

FIGURE 3. Response area (arbitrary units) as a function of amplitude of the ERG in dark-adapted *Limulus* lateral eye for various wavelengths of light. Inset shows the method of measurement. Data from one preparation. Stimulus duration 0.1 sec.

Although the response waveforms appear relatively simple we have not found a simple analytical expression to describe them. Instead certain features of the waveform were measured, namely the area under the response, rise and fall times, and response width, and these were related to response amplitude.

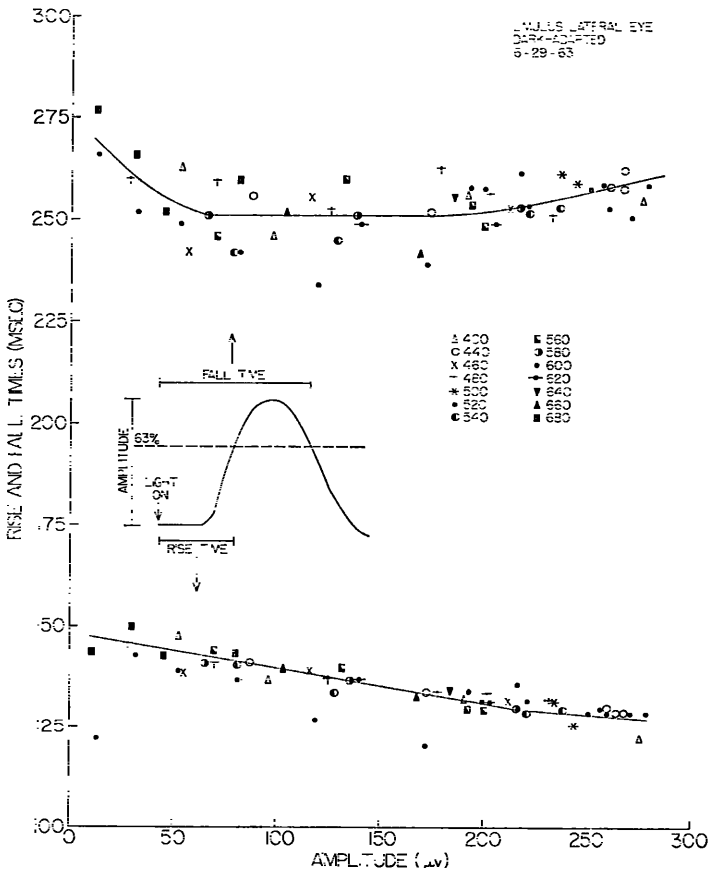

FIGURE 4. Rise and fall times as a function of the ERG amplitude for various wavelengths from a dark-adapted lateral eye. Inset explains method for measuring rise and fall times. Stimulus duration 0.1 sec.

RESPONSE AREA The area under the response was studied since it condenses information from the entire response to a single term. Response area was defined as the area enclosed by the extended base line and the transient negative (upward) potential (Fig. 3, inset). Response area was measured by a computer program which summed the digitized amplitudes at regular intervals for 325 msec from the light onset.

The relation between response area and amplitude for lateral eye responses is shown in Fig. 3. Without adjustment the data for various wavelengths

tended to fall along the same curve, as would be expected if a single spectral mechanism were operating. The response area increased at a slightly faster rate than the response amplitude (curve positively accelerated). A linear relation is expected if the ERG is the output of a system with a constant waveform.

Response area data for the ocellus are not presented, since the dependence of waveform on wavelength is clearly established below with rise and fall time measures.

RISE AND FALL TIMES Latencies to the relatively steeply rising and falling parts of the ERG were measured to assess waveform changes. A computer program was designed to measure the rise and fall times which were defined as the times from the stimulus onset to the points where the ERG was 63% of the peak amplitude on the rising and falling sides of the peak, respectively (inset Fig. 4).

Rise and fall times for lateral eye data are plotted in Fig. 4 as a function of ERG amplitude. The data for various wavelengths appeared to superimpose within the error of measurement. Wavelength had no discernible systematic relation to the rise and fall times. Curves were drawn by inspection through the data in Fig. 4. If the waveforms differed only by a gain factor the rise and fall times should be invariant with response amplitude (horizontal line). The rise times decreased slightly with increasing amplitude. The fall times changed very little with amplitude, showing a slight tendency to increase at the higher amplitudes. These measures are less reliable at the lowest amplitudes where the signal-to-noise ratio is less favorable. The distance between the rise and fall times may be considered as a measure of response width. The response widths of the lateral eye ERG's tended to increase with higher amplitudes.

The rise and fall times of the ocellar ERG showed quite different characteristics (Fig. 5), the dependence on light wavelength being especially noteworthy. The data appeared to be grouped into two main categories, short wavelengths (380–425 mμ) and long wavelengths (500–675 mμ) with the transition wavelengths (especially 450 mμ) different from the rest. The rise and fall times for long wavelengths (500–675 mμ) decreased markedly, and approximately linearly, with increasing amplitude. The fall times decreased with amplitude more than rise times, indicating that the larger amplitude responses move forward in time and are narrower. The rise and fall times for short wavelengths (near UV) also decreased with increasing amplitude. However, the relation was curvilinear and covered a smaller range. The data for the short and long wavelengths crossed showing that at low amplitudes the responses occurred sooner for the short wavelengths, although at high amplitudes the responses occurred sooner for the long wavelengths. These characteristics are illustrated in Fig. 2 for another dark-adapted ocellus

by average high and low amplitude responses to 550 and 370 mμ light flashes. Compared to the short and long wavelength patterns (Fig. 5), the transition wavelengths (especially 450 mμ) gave relatively long rise and fall times at high amplitudes. These characteristics are attributed to the dual action of UV and green spectral mechanisms.

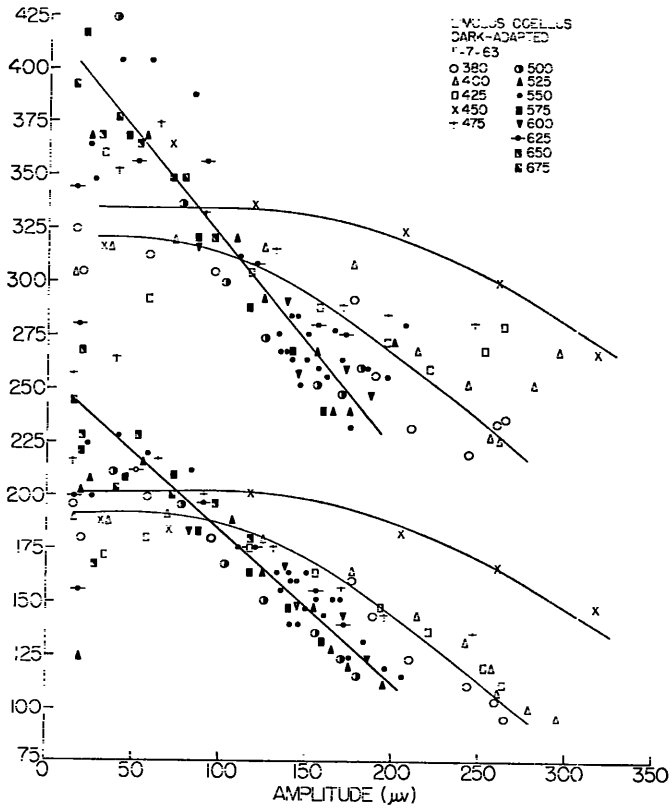

FIGURE 5. Rise and fall times as a function of ERG amplitude from a dark-adapted median ocellus. By inspection, straight lines were drawn through long wavelength data (500–675 mμ), curves through short wavelength data (380–425 mμ) and transition wavelength data (450 mμ).

Chromatic adaptation provided a further test of the waveform characteristics of the two presumed spectral mechanisms of the *Limulus* ocellus. The rise and fall times for short wavelengths when the ocellus was adapted by orange light and for long wavelengths when adapted by violet light are shown in Fig. 6. The data agree with the trends described for the short and long wavelengths in the dark-adapted ocellus (Fig. 5). These findings, together with the relative reduction of response amplitude at transitional wavelengths, support the dual spectral mechanism interpretation.

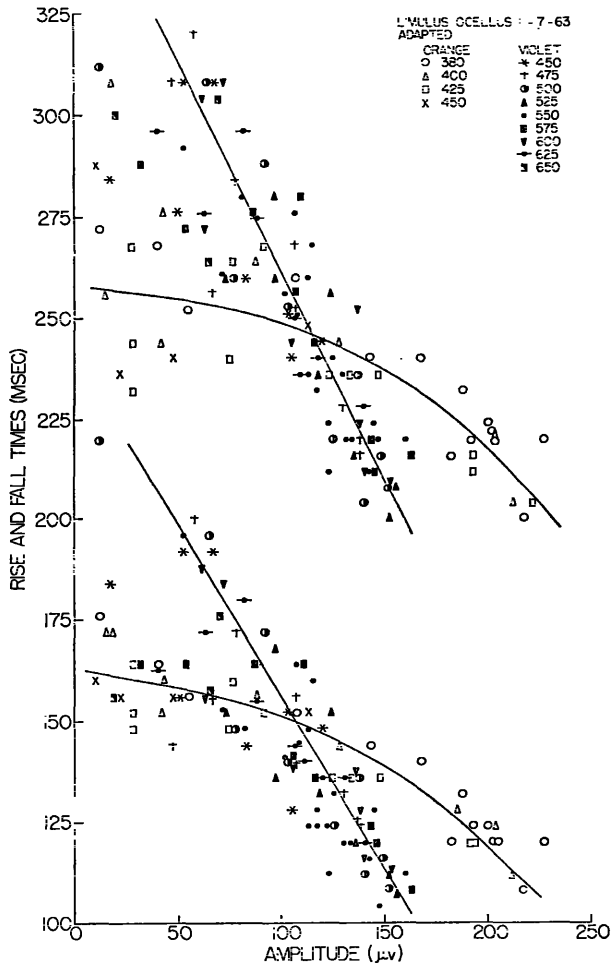

FIGURE 6. Rise and fall times as a function of ERG amplitude in a chromatically adapted median ocellus. By inspection, straight lines were drawn through the violet-adapted (Corning Cs7-51, 5-59, 5-60) data and curves through the orange-adapted (Corning Cs3-67) data.

Amplitude-Energy Functions

LATERAL EYE The amplitude of the lateral eye ERG was plotted as a function of log relative energy for various wavelengths of the stimulating light. Data from one preparation are given in Fig. 7. The data for each wavelength were fitted reasonably well by straight lines which tended to have the same slopes.

MEDIAN OCELLUS The amplitude of the ocellar ERG, measured from the base line to the negative peak, is plotted as a function of log relative energy

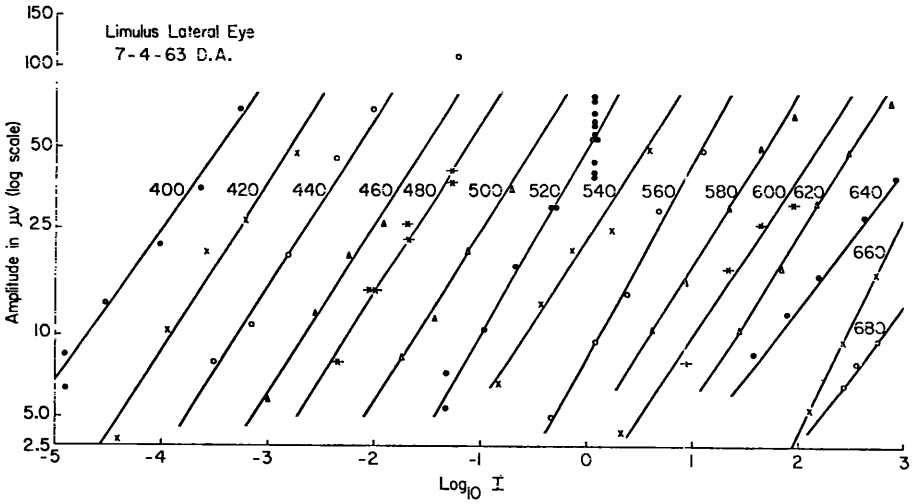

FIGURE 7. Amplitude of ERG (log scale) as a function of log relative energy for several wavelengths in dark-adapted *Limulus* lateral eye. The curves were arbitrarily displaced along the abscissa. Lines are least-square fits to data points. Data from a single preparation.

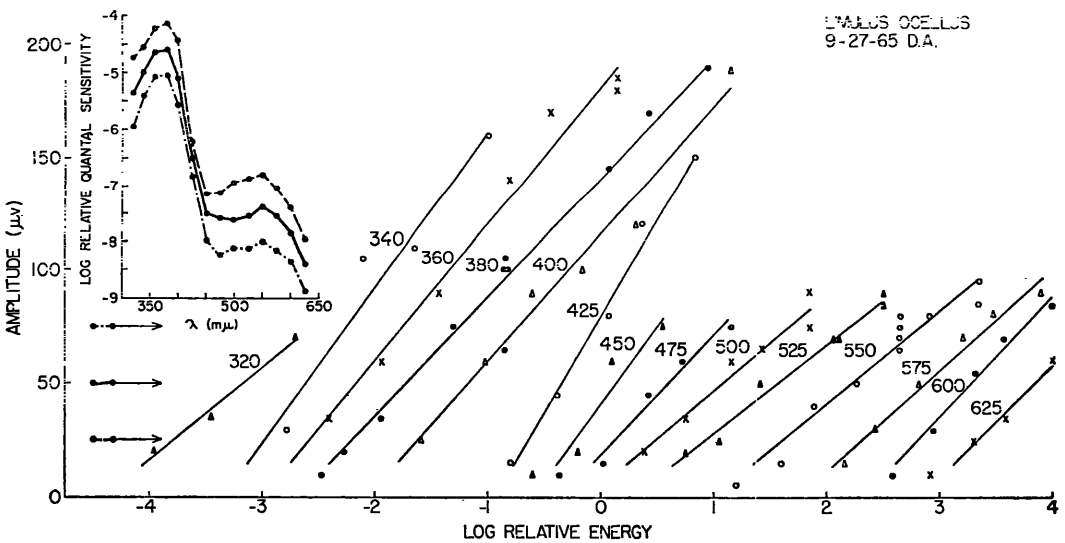

FIGURE 8. The amplitude of ERG as a function of log relative energy for several wavelengths in dark-adapted *Limulus* median ocellus. The curves for each wavelength were arbitrarily displaced along the abscissa. The straight lines were fitted to the data points by the least-square method at each wavelength. Data from a single preparation. Stimulus duration 0.1 sec. Inset, relative spectral quantal sensitivity obtained by three different criterion levels (horizontal arrows), 25, 50, 75 μv.

for various wavelengths in Fig. 8. The slopes of the functions were systematically related to the wavelength, with shallowest slopes at wavelengths longer than 500 mμ, slightly steeper slopes at wavelengths shorter than 425 mμ, and steepest slopes at transition wavelengths in the region near 450 mμ. The slopes at the transition wavelengths lost their steepness when the ocellus was chromatically adapted with light from either long or short wavelengths.

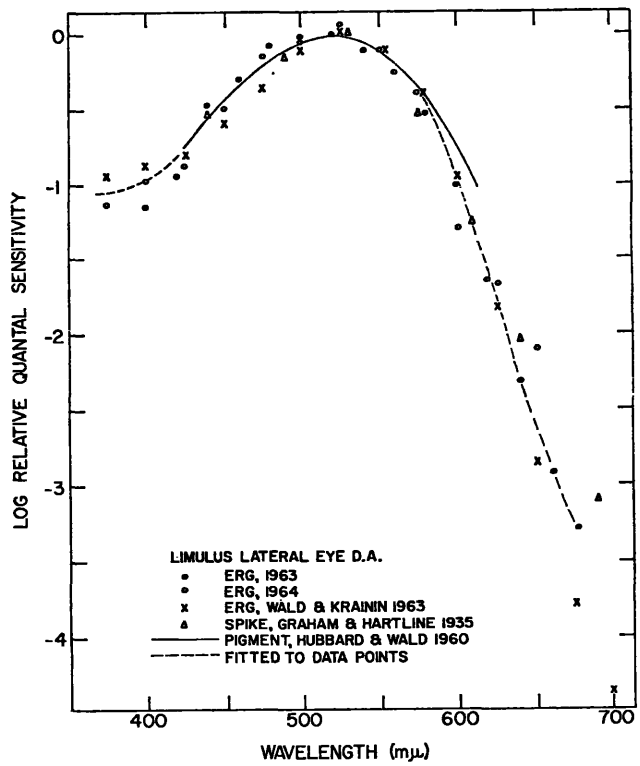

FIGURE 9. Spectral sensitivity of dark-adapted *Limulus* lateral eye. Filled circles, the mean curve from seven large animals (1963); open circles, the mean of seven large animals (1964); open triangles, points from the average curve of Graham and Hartline (1935). Solid line represents the photopigment difference spectrum curve of Hubbard and Wald (1960); dashed line, fitted to the data points by inspection. Sensitivity values are quantized.

Spectral Sensitivity Curves

LATERAL EYE The constancy of the slopes of the amplitude-energy functions at various wavelengths (Fig. 7) means that the relative spectral sensitivity function is independent of the criterion amplitude chosen. The criterion amplitude varied among preparations (10–140 μv) and was selected to correspond to the most reliable parts of the amplitude-energy functions for most wavelengths.

For the dark-adapted preparation, the maximum sensitivity was near 520 mμ with a fair amount of agreement between various sets of experiments (Fig. 9). The mean data shown are based on individual curves which were adjusted vertically by inspection to achieve minimum dispersion prior to averaging. The standard errors of the mean for the points shown lay between 0.03 and 0.21 log unit with a median of 0.06 log unit. The data on mature animals (1963 and 1964, glass optics) were similar to the data obtained on young animals (1965, quartz optics).

The lateral eye was light-adapted with violet (>36% transmission at 400–440 mμ) and orange (>45% transmission at 620 mμ and above) light and spectral sensitivities determined. The spectral sensitivity curves (Fig. 10) obtained with either chromatic adaptation were similar in shape to the dark-adapted curve. The adapting lights were of sufficient energy to change over-all sensitivity (1.06 and 1.28 log units).

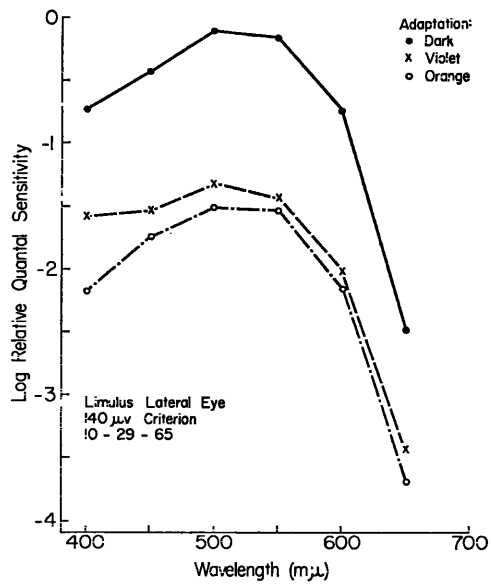

FIGURE 10. The effect of violet and orange chromatic adaptation on the dark-adapted *Limulus* lateral eye. The adaptation was accomplished with Corning glass filters; Cs5-59 and 5-60 for violet and Cs2-61 and 2-73 for orange adaptation. Data from one preparation. Order, dark, violet, and orange adaptations.

MEDIAN OCELLUS The heterogeneity of slopes of the amplitude-energy curves from the ocellus causes variation in the relative spectral sensitivity depending on the criterion selected (Fig. 8). The inset shows that the UV peak was relatively higher than the green wavelength peak with higher response criteria (lower sensitivity curves). The criteria used for different preparations varied from 25–200 μv depending on the response amplitudes obtained.

The average dark-adapted curve for *Limulus* ocellus (Fig. 11) showed two sensitivity peaks, with maxima at about 530–535 mμ and at about 360 mμ.

The relative sensitivity varied among animals and was about 200–320 times greater to light at 360 mμ than at 525–550 mμ. The data obtained from excised preparations were in good agreement with the data from intact preparations. The standard error of the mean for each data point from intact ocellus (same procedure as for Fig. 9) lay between 0.03 and 0.20 log unit, with a median of 0.11 log unit.

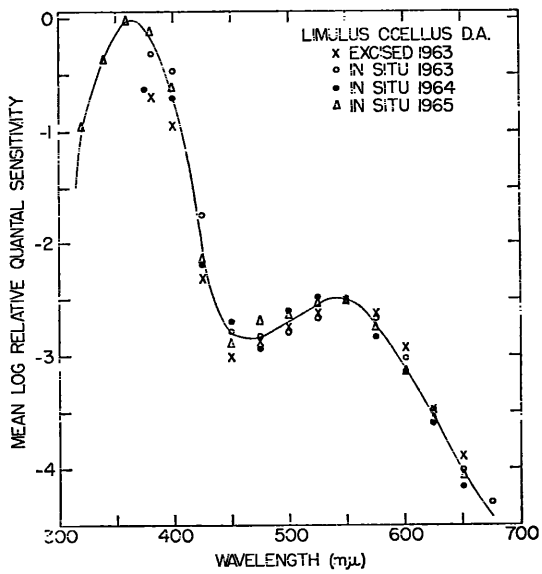

FIGURE 11. Average spectral sensitivity of dark-adapted *Limulus* median ocellus. Open circles, mean of data from eight runs on eight different large animals (1963). Filled circles, mean of data of five runs on four different animals (1964). Open triangles, mean of data of eight runs on eight different small animals (1965). Small x, data from one excised ocellar preparation. The sensitivity values are quantized.

If the two sensitivity maxima are due to separate spectral mechanisms, then an orange-adapting light will be absorbed more by the green receptor than by the UV receptor system, while the effect of a violet-adapting light will be the opposite. Adaptation with orange light (>50% transmission at 560 mμ and above) almost eliminated the response in the visible region of the spectrum but left the near ultraviolet region relatively unaffected (Fig. 12). Adaptation with violet light (>47% transmission 320–390 mμ), reduced the ocellar sensitivity in the blue-violet region of the spectral curve more than in the green-yellow region. All the chromatic adaptation experiments conducted on the ocellus demonstrated spectrally selective effects.

DISCUSSION

Limulus Lateral Eye—A Single Spectral Mechanism

The evidence for a single spectral mechanism is based upon (*a*) the presence of a single maximum (Fig. 9), (*b*) spectral sensitivity invariance in chromatic

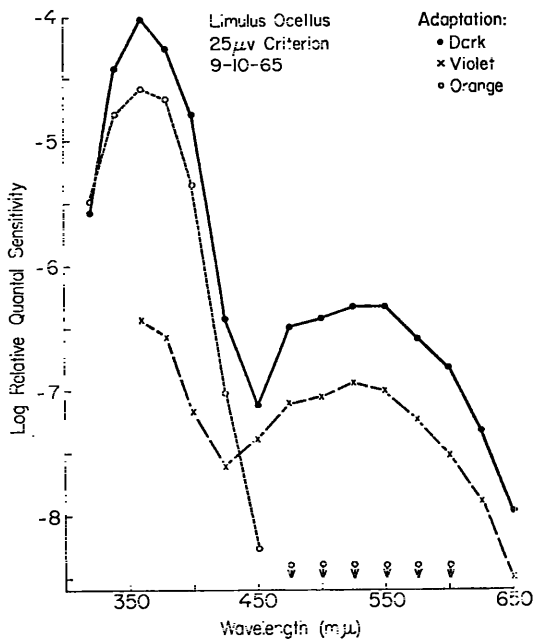

FIGURE 12. Chromatic adaptation compared with dark adaptation in *Limulus* median ocellus. The adaptation was accomplished with Corning glass filters, Cs7-51 for violet (small x) and Cs3-67 for orange adaptation (open circles). The violet-adapted curve has been shifted up 0.7 log unit. Data from one preparation. Order, dark, orange, violet adaptation.

adaptation experiments (Fig. 10), (*c*) homogeneity in the slopes of the response-energy curves (Fig. 7), (*d*) similarity in response waveform measures at different wavelengths (Figs. 3 and 4), and (*e*) essentially the same visual pigment difference spectrum with white and orange bleaching lights (Hubbard and Wald, 1960). The data in Fig. 9 include single fiber spike recording from optic nerve and ERG recordings from the corneal surface and they are essentially in agreement. Compared to Fig. 9 data, our curve from an earlier, more limited series (Lall and Chapman, 1964) was shifted unexplainably toward longer wavelengths.

The spectral sensitivity data (Fig. 9) and the difference spectrum for the photopigment extracted from the *Limulus* lateral eye (Hubbard and Wald, 1960) showed good correspondence with no correction for ocular media. The *Limulus* rhodopsin appears to be the basis for the spectral sensitivity in the lateral eye. Srebro (1966) showed that the long wavelength part of the curve depends on temperature.

Limulus Median Ocellus—Dual Spectral Mechanisms

The evidence for the presence of two spectral mechanisms in the ocellus is based upon (*a*) the presence of two different waveform characteristics, one for the UV and the other for the green mechanism (Figs. 5 and 6), (*b*) the response-energy functions show steep slopes for the UV mechanism and shallow slopes for the green mechanism (Fig. 8), (*c*) the presence of two spectral sensitivity peaks (360 mμ and 530–535 mμ) in the dark-adapted data (Fig. 11), and (*d*) selective depression of either spectral sensitivity peak by appropriate chromatic adaptation (Fig. 12). The dark-adapted spectral sensitivity data (Fig. 11) are in essential agreement with those of Wald and Krainin (1963).

The *Limulus* ocellus is more than 200 times more sensitive to UV light than to green light. Such high relative sensitivity to UV light has not been found in electrophysiological studies of other invertebrates for whom the UV peak was not more than three times as sensitive as the visible peaks (Burkhardt, 1962, 1964; Ruck, 1965; Goldsmith, 1960, 1964; Goldsmith and Ruck, 1958; Walther, 1958; Walther and Dodt, 1959). The pineal body in vertebrates is the structure analogous to the median ocellus (Walls, 1942). The spectral sensitivity data on the frog's pineal body (Dodt and Heerd, 1962) showed close similarity with our data from *Limulus* median ocellus.

Analysis of Response Waveform

Responses from a single spectral mechanism to all wavelengths would be alike provided the light energy were adjusted appropriately for the spectral sensitivity (Graham and Hartline, 1935). However, if several spectral mechanisms with different time courses contribute to the response, then no adjustment of the light energy will make the responses to all wavelengths alike. Data showing that responses to all wavelengths cannot be matched are evidence for the presence of several spectral mechanisms possessing different time courses. The approach taken here was the graphical equivalent of matching responses on the basis of peak amplitude and then comparing other characteristics of the waveform, e.g. response area, rise and fall times, and response width. Matching the responses for peak amplitude employs the typical amplitude criterion for adjusting the light energy to compensate for spectral sensitivity differences.

This general procedure of waveform analysis may be useful for other sense

modalities, e.g. taste, where not even the relevant physical dimensions, analogous to light wavelength, are known. Since the graphs plot a response waveform measure against response amplitude, the relevant stimulus dimensions need not be known and, in fact, may be identified by the grouping of the data.

These plots not only systematize the dependence of waveform on wavelength but also summarize how the waveform of presumably a single spectral mechanism changes with response amplitude (Figs. 3, 4, and 6). The curvilinear relation between response area and amplitude in the lateral eye data shows that the responses differ by more than a simple gain factor. The rise and fall times of the lateral eye responses changed relatively little (under 25 msec) over a good range of amplitude (250 μv). However, the rise and fall times of both UV and green mechanisms of the ocellus decreased appreciably as the amplitude of the responses increased (more than 50 msec and 100 msec, respectively). The responses of the green mechanism were not always faster, but at low amplitudes were actually slower than those of the UV mechanism (Figs. 2, 5, and 6).

The long rise and fall times of the high amplitude responses to the transition wavelengths around 450 mμ (Fig. 5) may be interpreted as the long rise and fall times of lower amplitude responses of the UV and green mechanisms summated together. The sum of two similar responses increases the amplitude without changing the rise and fall times.

STRAY LIGHT AND OCELLAR WAVEFORM Because of the high UV sensitivity of the ocellus (Figs. 1, 11, and 12), stray UV light may have a relatively large effect. Such an effect may be the basis of the ocellar waveform differences reported by Wald and Krainin (1963). Their 550 mμ response declined over a broad hump. Stray UV light accompanying high-energy light at 550 mμ could especially contribute to the late part of the response, since a low amplitude response of the UV mechanism has a later rise and fall time than a high amplitude response of the green mechanism (Fig. 6). The action of such stray UV light may be detected by its elimination with a cutoff filter (e.g. Corning 3-71) and by noting the faster fall in the ERG waveform. A further test was to change the falling part of the waveform by adding UV light from a second source to that obtained from a filtered stimulator delivering 550 mμ. The results reported here for the long wavelengths have negligible contamination from stray UV light.

WAVEFORMS IN OTHER EYES The variations in response waveform which were related to light wavelength in the *Limulus* ocellus were all based on relatively simple, negative, monophasic waveforms. This is to be contrasted with wavelength effects from some other invertebrate eyes which show more complex polyphasic responses (e.g. Goldsmith, 1960, 1965; Ruck, 1965). Since the work of Hartline (1938) and Bernhard (1942), the simple negative

potential has often been considered to be the response of the retinular cells and the waveform complications attributed to postsynaptic activity or neural interaction. Consequently, the data reported here may most reasonably be attributed primarily to receptor processes.

In the only other study known to relate the simple monophasic waveform to spectral mechanisms (Walther, 1958), the response of the upper edge of the cockroach compound eye had a longer decay to half-time for violet (407 mμ) than for red light (605 mμ). The decay to half-time (similar to fall time with a 50% amplitude criteria) increased at higher light energies (larger ERG amplitudes) at both violet and red wavelengths. The direction and small amount (about 30 msec increase for an increase of about 3 log units of light energy) of change were similar to those found for the *Limulus* compound eye and opposite to those found for the *Limulus* ocellus. However, like the *Limulus* ocellus at matched large amplitudes the short wavelength mechanism had a longer fall time than the long wavelength mechanism.

Photochemical Basis for Ocellus Spectral Sensitivity

The photochemical substances responsible for the UV and the green sensitivity peaks in the ocellus should be considered. Various possibilities are related to two general hypotheses: (*a*) the presence of one visual pigment alone or in conjunction with another light-absorbing substance, and (*b*) the presence of two visual pigments.

UV SENSITIVITY AND β-BAND It has been postulated for the *Calliphora* eye that a single visual pigment causes both UV and green sensitivity due to the α- and β-bands of the rhodopsin molecule (Burkhardt, 1962). In such a system the UV and the green peaks should be affected equally by any kind of light adaptation. The selective effects of chromatic adaptation in the *Limulus* ocellus (Fig. 12) are at variance with the hypothesis.

SCREENING PIGMENT AND ONE VISUAL PIGMENT If a screening pigment lay in the light path to some receptors, but all receptors had the same visual pigment, many characteristics of a two pigment system could be obtained (Autrum, 1955; Burkhardt, 1964; Goldsmith, 1965). A simple model of such a single visual pigment system is at variance with the ocellar data. The model assumes (*a*) a chromatic mask in the light path to the masked receptors is the only difference between masked and naked receptors, which all have the same visual pigment, average response-energy function, etc.; (*b*) the responses of each receptor are independent and add with fixed coefficients at the recording electrode; (*c*) the response-energy functions (response amplitude plotted vs. log energy) of each receptor are not concave down over the lower part of their curves (Fig. 8); and (*d*) the density of the chromatic mask is intermediate at wavelengths which are between those to which the masked and naked re-

ceptors are most sensitive. The last proposition is necessary since the mask must be relatively opaque to wavelengths at the absorption maximum of the visual pigment in order to shift the sensitivity maximum of the masked receptors. With these assumptions it can be shown that the slopes of the recorded response-energy functions over their lower range should not be steepest at the intermediate wavelengths. However, the slopes at these wavelengths were found to be the steepest (e.g. 425 mμ, Fig. 8). Additional evidence against a chromatic mask interpretation is the selective suppression of *either* spectral sensitivity peak by corresponding chromatic adaptation (Fig. 12). Adaptation with wavelengths which are attenuated by a chromatic mask and absorbed by a visual pigment relatively spares the masked receptors while adapting the naked receptors. However, such a model is not symmetrical, since wavelengths which pass the chromatic mask adapt both masked and naked receptors when only one visual pigment is involved.

FLUORESCENT PIGMENT AND ONE VISUAL PIGMENT The UV sensitivity in the ocellus has been attributed to a fluorescent substance (Chance, 1964). A fluorescent substance would have to have an improbably high quantum efficiency in order to account for the UV sensitivity being so much greater than the green sensitivity. Such a difference in sensitivity could only be explained by fluorescence if the long wavelength light reaching the receptors were reduced for external illumination but not for the fluorescent illumination. Selective chromatic adaptation effects cannot be explained by a fluorescent system, since chromatic adaptation of the green mechanism leaves a relatively high UV sensitivity (Fig. 12).

TWO VISUAL PIGMENTS AND SEPARATE RECEPTORS Since evidence has been presented against various ways in which one visual pigment could account for UV and green peaks, an alternative hypothesis of two visual pigments seems likely. Two visual pigments could be formed if two different opsins conjugated with the chromophore retinene$_1$. In *Musca*, UV sensitivity depended on vitamin A (Goldsmith et al., 1964).

The purest spectral sensitivity curves for the two presumed visual pigments in the ocellus were those obtained by chromatic adaptation (Fig. 12). With the use of these data, log relative quantal sensitivity was plotted as a function of frequency (wave number). With appropriate shifting of the curves on these axes, the UV and the green sensitivity curves were superimposable with each other and with Dartnall's nomogram (Dartnall, 1953). This extends the applicability of Dartnall's nomogram into the ultraviolet region of the spectrum. It suggests that UV sensitivity may be dependent on a visual pigment belonging to the same class as other known visual pigments. It seems unlikely that the two spectral mechanisms are based on two visual pigments mixed at random in each receptor, since the waveforms of the two spectral mechanisms

exhibited different characteristics. Thus, the data favor the hypothesis that the UV and green spectral mechanisms are based on two visual pigments housed in different receptors.

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