

## SPECTRAL SENSITIVITY OF SCORPION EYES AND THE POSSIBLE ROLE OF SHIELDING PIGMENT EFFECT

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### INTRODUCTION

The structure of the median and lateral eyes of the scorpion and the nature of the electroretinogram (ERG) have been described elsewhere, as has been the basic arrangement of apparatus used in the electrophysiological aspects of this study (Machan, 1966, 1967).

The position of the shielding pigment in scorpion eyes has been shown to play a role in the general sensitivity of the eye (Machan, 1968). For this reason an attempt has been made in this study to determine to what extent, if any, shielding pigment plays a part in the spectral sensitivity.

### MATERIALS AND METHODS

Fifteen Balzers narrow-band interference filters were mounted in a metal, circular filter holder, 50 cm. in diameter. This was placed in the optical system in a collimated part of the beam. These filters, ranging in wavelength from 371 to 640 m $\mu$  as well as the Wratten neutral density filters had been previously calibrated on a Beckman D-2 spectrophotometer. Quanta determinations for the interference filters in the optical system were made by means of a model 151 Keithley null-detector microvoltmeter and an R.C.A. 935 photo tube of known spectral sensitivity.

Eighteen animals, including three different species were used: *Centruroides sculpturatus* (Ewing), *Vejois spinigerus* (Wood), and *Opisthacanthus validus fulvipes* (Pocock). Stainless-steel electrodes were used. A control run with glass pipette electrode showed that responses with either type of electrode had the same waveform, amplitude, and spectral sensitivity. This was also the case if the remaining eyes were left exposed to scattering light or covered.

Eyes were dark-adapted from 45 min. to 1 hr. Readings were at threshold level (ca. 80  $\mu$ V.), with test flashes of  $\frac{1}{10}$  sec. duration, allowing sufficient time for recovery between stimulations. This was tested for each animal, and did not vary with wavelength. Test-flash intensity was increased from subthreshold levels until threshold was reached. Two or three readings were taken at each wavelength and a whole run was repeated for each curve determined. Since the neutral density filters were spaced in 0.2 units, points in between had to be interpolated. Estimates were within 0.1 unit of the true value. Data are graphed as the reciprocal of the number of quanta required to evoke a threshold response ( $1/E$ ) as a function of wavelength.

The same procedure was followed for the light-adapted eye, the eye being allowed to recover before repeating adaptation with light of another colour. With each selective adaptation the intensity of the adapting light was controlled by adjusting the adapting light so that the same response of the eye to a stimulus at a control wavelength was obtained with all adapting lights used for an experiment.

The source of the adapting light was a 100 W. microscope lamp, mounted in line with a series of lenses so that a spot of light was focused on one end of a flexible length of fibre optics, the other end of which was positioned so that the adapting light beam struck the eye as closely as possible at the same angle as that of the principal optical system. A heat filter was placed close to the light source, and selective lighting was achieved by use of Corning glass filters. Table 1 shows the transmission range of the

Table 1. *Transmission range of Corning filters used in selective adaptation*

Filter	Percentage transmission at wavelength in $m\mu$		
	80% or more	70%	Less than 10%
2-58	678-750	650	630
2-60	658-750	630	620
2-73	618-750	608	590
3-73	511-750	460	410
7-59	365	405	460

filters used. Absorption determinations on shielding pigment were made on a modified Leitz, microspectrophotometer, a detailed description of which has been published elsewhere (Rasch, Darnell, Kallman & Abramoff, 1965). All readings were taken in the same optical system on areas measuring  $2 \mu$  in diameter. Determinations were made on the eyes of three animals. From two of the animals fresh eye-squash preparations were set up and determinations made on dense and diffuse areas of pigment in each preparation. Additional readings on the lateral eye were taken at shorter wave lengths.

Data were also obtained from sections of eyes fixed in alcoholic Bouin's solution and cut at  $5 \mu$ . Uncorrected curves and curves corrected for fixative are shown for the sections. Readings of optical density were taken for: (1) pigment, (2) a translucent piece of cuticle retaining the yellow coloration of the fixative, and (3) a background area. The uncorrected curve was obtained by the difference in optical density between (1) and (3), and the corrected curve was obtained by subtracting from this uncorrected curve the curve obtained by the difference in the optical density between (2) and (3). Optical density values were converted to the percentage transmission for graphing, since spectral sensitivity curves are not in logarithmic units.

A fixative correction was introduced beyond the one implied in the background correction, since the background correction included only the glass and mounting medium. It was considered of interest to know to what extent the fixative itself might have changed the curve, and the correction showed a difference only at shorter wave-lengths. The principal value of using fixed sections was in providing a method of comparing pigments of median and lateral eyes, since the eyes were subjected to the same treatment and sectioned to the same thickness, the latter a factor not possible to control in the eye squash preparations.

## RESULTS

Figure 1 shows the normalized composite spectral sensitivity curves from the dark-adapted median and lateral eyes of fourteen animals. The standard error of the mean is shown at points where variability was large enough to graph. On the same graph, the normalized curves from the lateral eye of four individual animals are shown.

The lateral eye shows a pronounced rise in sensitivity in the near ultraviolet and a secondary rise in the blue-green region of the spectrum. The curve is probably

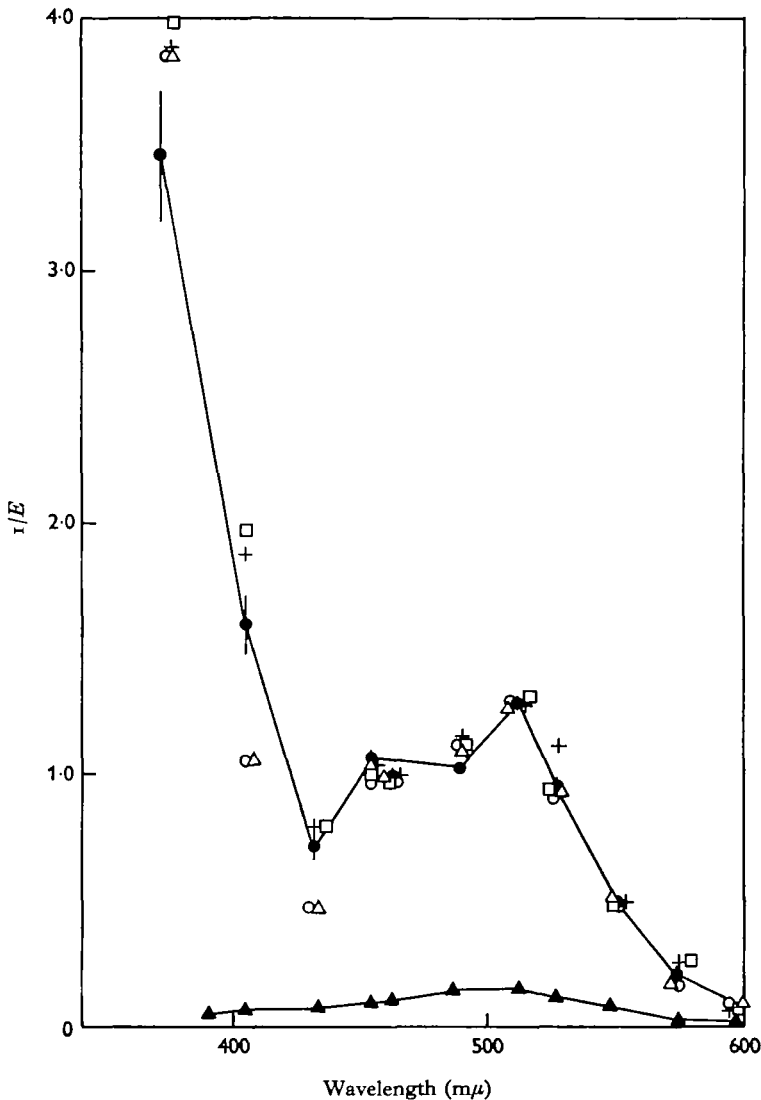


Fig. 1. Normalized spectral sensitivity curves of lateral and median eyes. Mean curve is the average of fourteen animals. Standard error is indicated at points where variability was large enough to graph. Lateral eye curves were normalized by setting point at wavelength 551 mμ equal to 0.505. Median eye curve was normalized by setting point at wavelength 462 mμ equal to 0.112. Lateral eye: ●, mean curve of fourteen animals; ○, animal 20; □, animal 10; +, animal 8; △, animal 5. Median eye: ▲, mean curve of fourteen animals.

somewhat distorted due to the limited number of interference filters. The median eyes show little or no elevation in the near ultraviolet, but the rise in the blue-green is evident. In general, as has been pointed out in previous papers, the median eyes are less responsive than the lateral eyes, unless dark-adapted for prolonged periods (Machan, 1968). Statistical analysis shows for the lateral eye a high variability at the three shortest wavelengths tested. The scatter at these wavelengths is also shown in the graphs of individual animals included in Fig. 1. The curves of these animals follow each other and the mean rather closely down to a wavelength of  $431\text{ m}\mu$ . At wavelength  $371\text{ m}\mu$ , where energy is low, it is possible that some scatter is due to

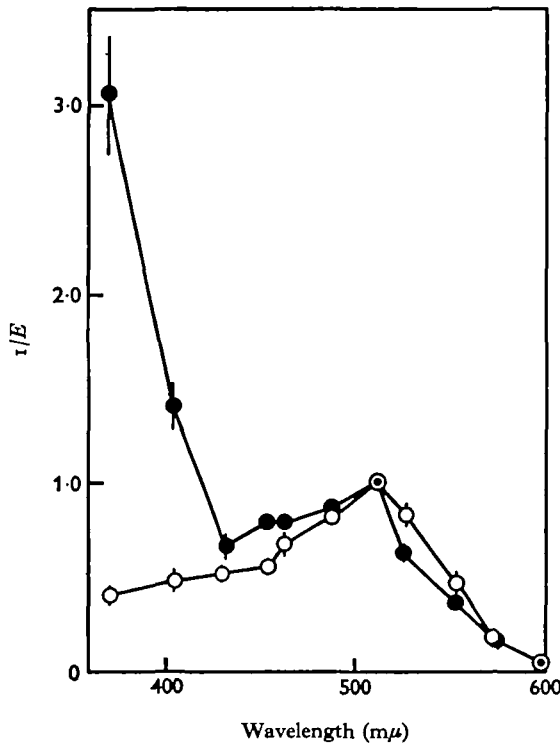


Fig. 2. Spectral sensitivity curves of lateral and median eyes, averaged from fourteen animals, and normalized by setting the point at wavelength  $509\text{ m}\mu$  equal to one. Standard error is indicated at points where the variability was large enough to graph. ●, Lateral eye normalized at  $\lambda_{509} = 1$ , with standard error of mean. ○, Median eye, normalized at  $\lambda_{509} = 1$ , with standard deviation of mean.

inability to interpolate points for less than  $0.1$  unit optical density. At this wavelength a difference of  $0.05$  unit can mean a difference of  $0.3$  sensitivity unit, which statistically can create a significant deviation. However, the energy difference at wavelengths  $404$  and  $431\text{ m}\mu$  is not sufficiently greater than that at  $509$  and  $526\text{ m}\mu$  to account for the selective variability. Since the position of the pigment in dark-adapted states might vary in different animals, there is also the possibility of some deviation in the near ultraviolet and far blue regions resulting from a shielding pigment effect, if the pigment selectively absorbs at the shorter wavelengths. Figure 5 showing the absorption curves on shielding pigment from the lateral eye supports this possibility.

Because of its low relative sensitivity and the ordinate scale chosen, deviations from the mean in the median eye are negligible. In order to obtain a comparison of curve shape and degree of variation, curves from both eyes of fourteen animals were normalized by setting the point at wavelength 509  $m\mu$  equal to one. The results are shown in Fig. 2. As can be seen in the graphed standard error, there is not much difference in scatter as compared with the lateral eye curve shown in Fig. 1. The scatter for the median eye, now apparent, is seen to be more or less equal throughout the curve.

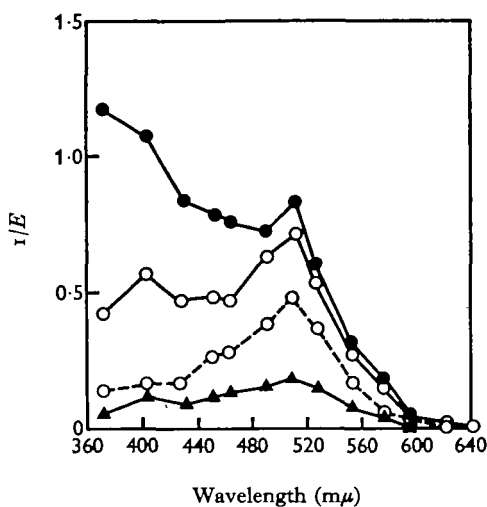


Fig. 3

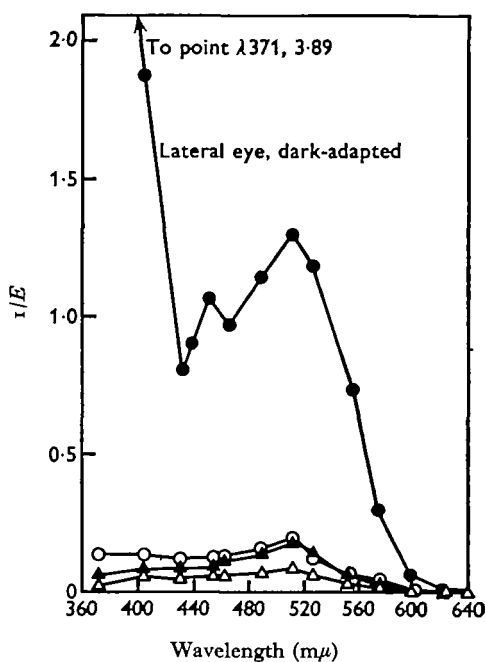


Fig. 4

Fig. 3. Spectral sensitivity of lateral and median eyes, adapted as indicated. ●, Lateral eye, dark-adapted. ○—○, Lateral eye adapted *w/2-73* filter. ○— —○, Lateral eye; intensity of adapting light increased. ▲, Median eye, dark-adapted.

Fig. 4. Spectral sensitivity of lateral and median eyes, adapted as indicated. The median eye did not respond to adaptation with filter 2-58. ○, Lateral eye, adapted with filters 2-58, 2-60, 2-73, 3-73. ▲, Median eye, dark-adapted. △, Median eye, adapted with filters as above listing.

Sensitivity curves on light-adapted and dark-adapted eyes were obtained on ten animals. Figures 3 and 4 show curves from two animals. Data from the other eight animals were similar. Light adaptation of the lateral eye resulted in a drop throughout the spectrum, but most substantially in the near ultraviolet. As seen in Fig. 3 the drop is relative to the degree of light adaptation. Selective adaptation of lateral and median eyes showed no detectable change in the light adapted curve, all adapting lights used producing the same effect. The intensity of the adapting light was controlled so that the same response of the eye was obtained with all adapting lights used for an experiment.

Although it was not possible to obtain more than a few readings without sensitivity change from a median eye after prolonged dark-adaptation, Table 2 shows the change

Table 2. *The effect of prolonged dark-adaptation on the relative sensitivity of the median eye at selected wavelengths*

Upper table, animal 23; lower table, animal 24

Wavelength (m $\mu$ )	Light adapted	1 hr. dark adapted	16 hr. dark adapted	$\frac{16 \text{ hr. dark-adapted}}{\text{light-adapted}}$
404	0.000020	0.000060	0.1958	$\frac{1.958 \times 10^{-1}}{2 \times 10^{-5}} = 0.979 \times 10^4$
509	0.000040	0.000180	0.1296	$\frac{1.296 \times 10^{-1}}{2 \times 10^{-6}} = 0.324 \times 10^4$
597	0.000002	0.000003	0.0080	$\frac{8 \times 10^{-3}}{2 \times 10^{-8}} = 0.400 \times 10^4$
Wavelength (m $\mu$ )	1 hr. dark adapted	42 hr. dark adapted	60 hr. dark adapted	$\frac{60 \text{ hr. dark-adapted}}{1 \text{ hr. dark-adapted}}$
404	0.000060	0.3844	8.030	$\frac{8.03}{6 \times 10^{-5}} = 1.4 \times 10^5$
509	0.000092	0.2176	2.150	$\frac{2.15}{9.2 \times 10^{-4}} = 0.23 \times 10^5$
597	0.000003	0.0080	0.124	$\frac{0.124}{3 \times 10^{-4}} = 0.41 \times 10^5$

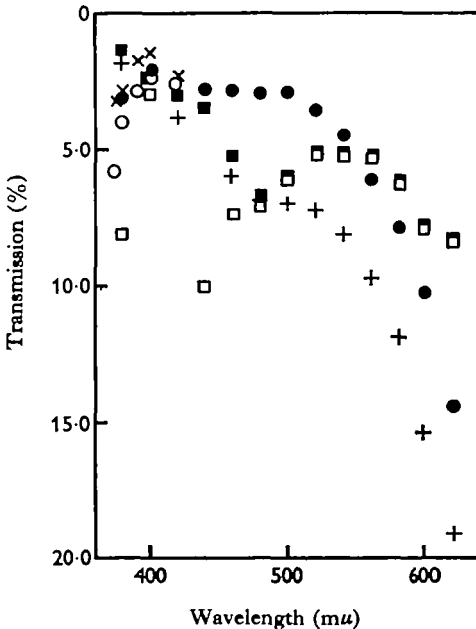


Fig. 5

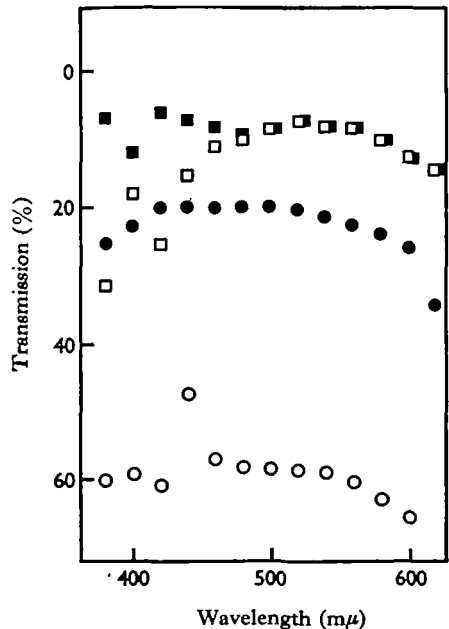


Fig. 6

Fig. 5. Absorption spectrum of shielding pigment of the lateral eye. See text for explanation. Animal 18: +, x, dispersed area; O, ●, dense area. Animal 5: ■, 5  $\mu$  section fixed in Bouin's; □, with fixative correction.

Fig. 6. Absorption spectrum of the shielding pigment of the median eye. See text for explanation. Animal 5: ■, 5  $\mu$  section fixed in Bouin's; □, with fixative correction. Animal 19: ●, dispersed area; O, dense area.

in sensitivity at three wavelengths following different periods of dark-adaptation from two animals. There is a marked increase in sensitivity to all three wavelengths, but the greatest rise is at wavelength  $404\text{ m}\mu$ .

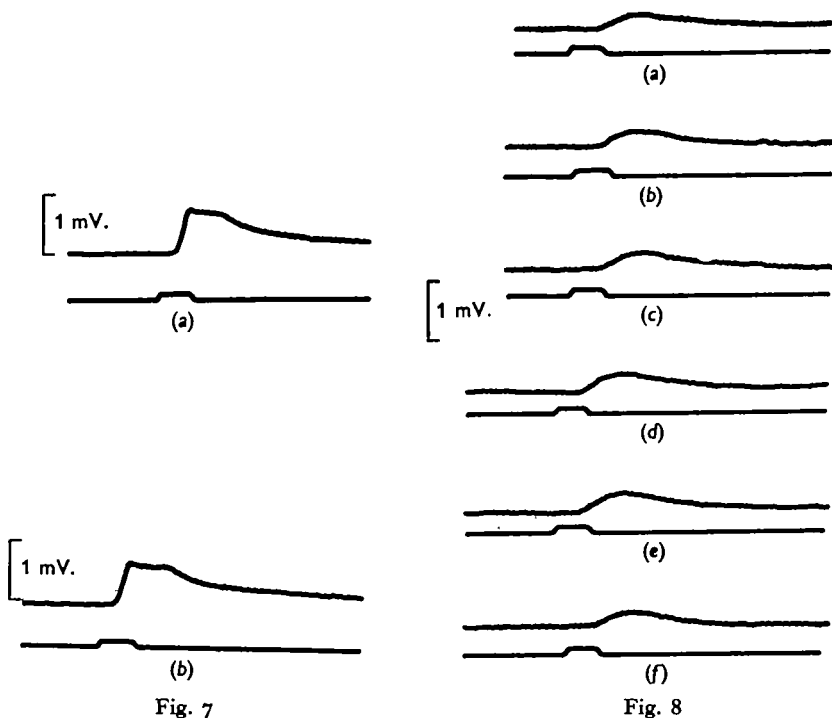


Fig. 7. Responses from lateral eye dark-adapted 1 hr.  $E$  value indicated after wavelength at which response was obtained. (a)  $404\text{ m}\mu$ , 3.1; (b)  $509\text{ m}\mu$ , 4.0.

Fig. 8. Responses from median eye dark-adapted 1 hr.  $E$  value indicated after wavelength at which response was obtained. (a)  $404\text{ m}\mu$ , 3.1; (b)  $431\text{ m}\mu$ , 3.1; (c)  $449\text{ m}\mu$ , 3.1; (d)  $488\text{ m}\mu$ , 3.3; (e)  $509\text{ m}\mu$ , 4.0; (f)  $554\text{ m}\mu$ , 3.5.

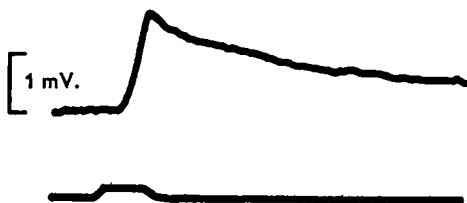


Fig. 9. Response of median eye after 40 hr. dark-adaptation at wavelength  $404\text{ m}\mu$ . Compare with Fig. 8a.

The percentage transmission curves for the shielding pigment are graphed in Figs. 5 and 6. The median eye shows a flatter curve than the lateral eye. Pigment of lateral eyes showed a maximum absorption around wavelength  $400\text{ m}\mu$ .

Figure 7 shows the equal amplitude responses, which were also almost equal energy responses at wavelengths  $404$  and  $509\text{ m}\mu$  from the lateral eye. The waveforms are not significantly different. For the intensity range of light available for these experiments

(maximum intensity *ca.* 10,000 foot candles), amplitudes could be matched with matching waveforms throughout the spectral range 400–600  $m\mu$  and were typical of low intensity responses.

Equal energy responses for the median eye are shown in Fig. 8. Although the value was the same as that used for the lateral eye responses in Fig. 7, the height of the responses here is considerably smaller than those of the lateral eye, and shows very little difference over a wide range of wavelengths. Figure 9 shows the response of the median eye at wavelength 404  $m\mu$  after 40 hr. of dark-adaptation. For purpose of comparison, attempts were made on three animals to obtain photographs of responses at wavelengths 404 and 509  $m\mu$  with the eye in the state of prolonged dark-adaptation, but the same sensitivity level of the eye, indicated by threshold level, could not be obtained.

#### DISCUSSION

The spectral sensitivity curve of the lateral eye of the scorpion like those of a number of arthropod eyes that have been studied, shows two maxima in the visible light range. The steep rise of the curve at wavelength 371  $m\mu$  at which point it may not as yet have reached its maximum height, indicates a sensitivity in the ultraviolet, and a sensitivity which exceeds that found in the blue-green region of the spectrum. A complete characterization of sensitivity in the ultraviolet could not be demonstrated since the optical system did not accommodate an analysis of this region.

As in any spectral sensitivity study which yields a curve having two maxima, the question here arises as to whether the two maxima are the result of one receptor type or two receptor types in the retina. The usual test for qualitative difference in waveform at various wavelengths could not carry much significance. As shown in Fig. 10, some animals show very little change in amplitude for test flashes of a thousandfold difference in intensity. Waveform change is a consequence of intensity change.

The results of selective adaptation show a maximum drop of sensitivity in the near ultraviolet, irrespective of the colour of the adapting light. This result is indicative of some effect other than selective fatigue of an ultraviolet receptor. This could conceivably be due to the effect of a shielding pigment for which the following hypothesis might be postulated:

1. Stimulation by light of any wavelength produces a migration of accessory pigment into the light-adapted position.
2. The accessory pigments of the eye transmit considerably less ultraviolet light than light in the 400–600  $m\mu$  range.

The absorption spectrum of the shielding pigment of the lateral eye does show a maximal absorption at about wavelength 400  $m\mu$ , but not to the extent one would expect if pigment movement alone were responsible for the large drop in sensitivity in the light-adapted eye. This can be seen if one examines the dark-adapted and light-adapted spectral sensitivity curves of animal 18 in Fig. 3 and the shielding pigment absorption curves from the same animal in Fig. 5. If the drop in ultraviolet sensitivity were due to the effect of a shielding pigment, the ratio of sensitivity change between points 400  $m\mu$  and 500  $m\mu$  should be about 0.06 or 6%. But the actual ratio of decreased sensitivity between these points, as seen in the spectral sensitivity curves, is about 3.0. Therefore, a shielding pigment hypothesis is ruled out.



Another possibility for the selective adaptation result might be found in a theory such as that of Gaunt (1964) for human rod function. His mathematical predictions coincide with experimental data from the initial threshold to the saturation level of intensity with various amounts of rhodopsin bleaching.

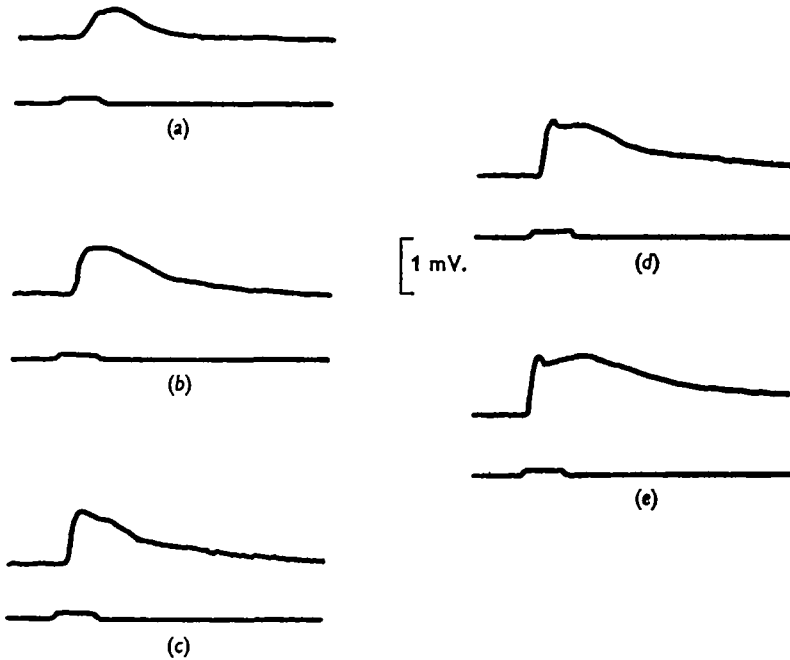


Fig. 10. Animal 23: ERG from lateral eye, D.C. recording, corneal lead, with test flash  $\frac{1}{10}$  sec. at (a)  $\log I = -4$ , (b)  $\log I = -3$ , (c)  $\log I = -2$ , (d)  $\log I = -1$ , (e)  $\log I = 0$ . Each test flash was preceded by 1 hr. dark-adaptation following 2 min. of light-adaptation at  $\log I = 0$ .

The most important aspect of the theory is that when regeneration of the bleached visual pigment occurs it produces a subthreshold response in the receptor, which is inadequate for visual excitation, but which prevents the receptor from transmitting further signals during a refractory period. The absorption of light can produce a visual signal only if a given amount of time has elapsed since the transmission of either a previous visual signal or a regeneration signal. The rate of rhodopsin regeneration is proportional to the number of molecules bleached, so that the regeneration signal would be increased with bleaching. There is evidence that bleached rods do produce a signal which, though not visual, controls pupil size while the eye is dark-adapting.

Applying this theory in a modified form to the scorpion eye, the results of selective adaptation could now be explained by two receptors:

- (1) an ultraviolet receptor,
- (2) a receptor, the visual pigment of which has an  $\alpha$  band in the blue-green, and a  $\beta$  band in the ultraviolet.

If we suppose that the regeneration signal transmitted in one type of receptor not only prevents visual stimulation in that receptor but also exerts a neural inhibitory effect on the other receptor, then the maximal drop in the ultraviolet with a red or orange light

can be explained through the combined effect of bleaching the pigment of the second receptor, which has a  $\beta$  band in the ultraviolet, plus the subsequent effect of inhibition produced in the ultraviolet receptor by the regeneration signal of the second receptor.

The maximal drop in ultraviolet sensitivity with the blue light can be explained by the combined effects of bleaching in both receptor types.

The indirect evidence of selective adaptation appears to support the concept of two receptor types, since it is not easy to find an explanation that would reconcile the results with a single receptor type than other a shielding-pigment hypothesis which does not seem tenable here.

The data for the median eye are not very conclusive. First of all, the low level of sensitivity of the eye in anything but a prolonged dark-adapted state does not allow much distinction of points in the dark-adapted and light-adapted sensitivity curves. In most of the animals tested exposure to light using Corning filters 2-58 and 2-59 was totally without effect. The heavy shielding pigment shows a very flat absorption curve with a small and questionable rise at about wavelengths 420-440  $m\mu$ . An effect suggestive of neutral density action seems to be indicated in the almost identical responses shown in Fig. 8. The absorption curves obtained on the fixed sections of median and lateral eyes indicate, just as do the fresh squash preparations, that the lateral eye pigment absorbs more selectively in the deep blue.

Prolonged dark-adaptation results in a very marked increase in sensitivity as is seen from Table 2 and Figs. 8 and 9. This leads one to believe that in a fully dark-adapted state the spectral sensitivity of this eye would closely resemble that of the lateral eye.

#### SUMMARY

1. The lateral eye shows one maximum in the ultraviolet and another in the blue-green region of the spectrum, probably attributable to two receptor types.
2. Light-adapted lateral eyes show the peculiar effect of a marked drop in ultraviolet sensitivity, irrespective of the colour of the adapting light.
3. Absorption by shielding pigment of the lateral eye is slightly selective in the short wavelengths.
4. The median eye has one maximum in the blue-green, violet sensitivity being apparent only with prolonged dark-adaptation.
5. Absorption by the shielding pigment of the median eye appears to be non-selective.

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