

# Spectral Sensitivity of the Barnacle, *Balanus amphitrite*

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**ABSTRACT** The extracellular ocellar potential was used to evaluate the spectral sensitivity of the ocellus of the barnacle, *Balanus amphitrite*. Maximum relative sensitivity was at 530–540 nm. Studies with chromatic adapting lights suggest that the receptors contain a single photopigment. The spectra were relatively broader in the dark as compared to the light-adapted state. This effect was shown to be due to an increase in the slope of the amplitude-intensity function, caused by light adaptation. Studies of tapetal fluorescence and corneal transmission indicate little effect of the ocellar media on the determination of sensitivity.

## INTRODUCTION

The ocellus of the barnacle offers many attractions to the visual neurophysiologist. It is a simple eye containing three photoreceptor cells, two of which are as large as 100  $\mu$  (Fales, 1928; Fahrenbach, 1965). The photoreceptor axons also are large (10–15  $\mu$ ), as much as 8–10 mm long, and the eye and ocellar nerve can be removed from the animal for in vitro studies. Data are easily acquired from these cells; photoreceptors can be penetrated with one or two microelectrodes under direct visual control (Brown, Hagiwara, Koike, and Meech, 1970) or the summed extracellular current of the three cells can be evaluated by registration of an I-R drop between the receptors and their axons (Gwilliam, 1963, 1965; Stratten and Ogden, 1969).

The latter of these techniques has been used in the present study to evaluate the spectral sensitivity of *Balanus amphitrite*. Studies of the optical properties of the cornea and tapetum are also presented.

## MATERIALS AND METHOD

Specimens of *Balanus amphitrite* were obtained from the Marine Biological Laboratory at Woods Hole. These hardy animals were easily maintained in a saltwater aquarium for months at a time.

*Recording* The ocellar potential (OP) was recorded in vitro. The eye and 4–5

mm of ocellar nerve were carefully cleaned of connective tissue and placed across an air gap in a moist chamber; a 1 mm section of the nerve spanned the gap. Since the potential developed between the ends of the preparation was determined by the extracellular current density and resistance across the gap, it was important to remove as much shunting connective tissue from the specimen as possible. An OP of 5 mv could be recorded from a well-cleaned preparation. The recording electrodes were silver-silver chloride–barnacle Ringer–agar bridges. Data were monitored on a CRO and recorded on a Grass polygraph.

*Light Stimulus* Calibrated spectral lights were obtained from a xenon source (360–450 nm) or a tungsten-halogen source (450–650 nm) and a Bausch & Lomb diffraction grating monochromator operated with a half-intensity bandwidth of  $\pm 5$  nm. Monochromator side bands in the range below 430 nm and above 600 nm were eliminated with appropriate interference filters. Light intensity was controlled with a calibrated Wratten neutral density wedge. Stimulus energy was measured with a Gaertner evacuated thermopile (Gaertner Scientific Corp., Chicago, Ill.) used with an appropriate galvanometric readout of sufficient sensitivity to permit direct registration of a criterion spectral energy at all wavelengths ( $1000 \text{ erg s}^{-1} \text{ cm}^{-2} \text{ nm}^{-1}$ ). The system was adjusted to deliver spectral stimuli of equal quantal content at the specimen. Light for chromatic adaptation was obtained from a low voltage tungsten source, used with an appropriate interference filter (Balzer B-40; HIBW = 20 nm).

Unless otherwise stated, the lines drawn through the data points for the various curves represent a best-fit 5-point parabolic regression curve for the points.

## RESULTS

The analyses presented in this paper are based on measurements of the amplitude, from base line to peak, of the extracellular photoreceptor or ocellar potential. The OP is depicted in Fig. 1 which illustrates the effect of stimulus intensity on response amplitude and form. As in most invertebrates, the photoreceptor has an initial transient phase followed by a sustained plateau phase: both phases are exaggerated at high intensities of stimulation.

The OP was dependent on several stimulus parameters: intensity, duration, repetition rate, background illumination, and history of previous stimuli. If

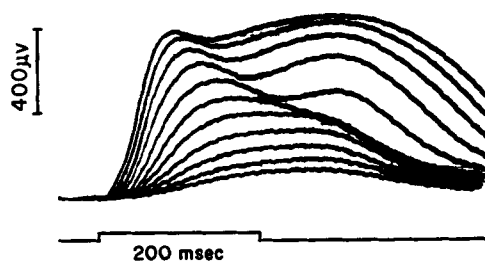


FIGURE 1. Effect of stimulus intensity on the ocellar potential. Each trace shows an OP evoked by an equal log increment of 530 nm light. Stimulus duration indicated on lowest trace.

the preparation was dark-adapted, and experienced infrequent low intensity stimuli, its ability to respond deteriorated gradually. If the ocellus was strongly illuminated for a brief interval, at least once per minute, it would provide stable responses to test stimuli for a period of several hours. Thus best results were obtained when a white conditioning flash (5000 lux/20  $\mu$ sec) was alternated with a test stimulus every 30 sec. This also provided assurance that the preparation was in an equivalent state of adaptation at the instant of test stimulus presentation.

*Equal Quanta Spectral Response* The correlation of spectral response functions was complicated by variations in the state of adaptation among animals in an apparently identical experimental paradigm. This probably resulted from differences in pigmentation of ocelli and from differences in metabolic status of animals kept for varying lengths of time in our saltwater aquarium. Spectral response functions obtained from animals that were light-adapted only slightly were rather flat and showed a relatively increased sensitivity to red and blue light, but particularly to near UV light (Fig. 2, upper curve). Stronger light adaptation considerably narrowed the spectra (Fig. 2, lower curves). Because of the variation in curve form found among the 15 animals from which equal quanta spectral response functions were obtained, simple grouping of the data did not provide a meaningful representation of a "typical" spectral response curve. However, each animal showed its maximum response to 530–540 nm stimulation.

The state of adaptation was clearly a highly individual circumstance and could not be adjusted reliably to an equivalent level among animals. However, it was possible to evaluate the state of adaptation in a given animal from studies of the effect of stimulus intensity on amplitude. As in most other animals, the barnacle photoreceptor voltage ( $V$ )–log intensity ( $\log I$ ) relation was an S-shaped function, the slope of which was determined by the state of

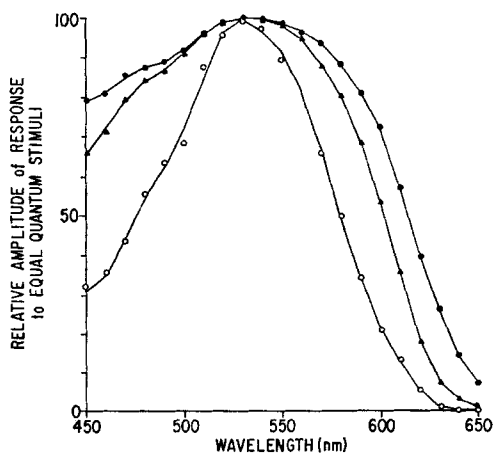


FIGURE 2. Spectral response curves from a single preparation under three conditions of adaptation. Stimuli were spectral lights of equal quantum content. Upper curve dark-adapted. Middle curve moderately light-adapted. Lower curve strongly light-adapted. Response amplitude is shown as % of maximum response under each condition of adaptation.

adaptation. Fig. 3 shows a series of intensity-amplitude studies from a single preparation. Each curve was obtained with the use of a different spectral stimulus light of various intensities. The light line labeled 530 represents a best-fit 5-point parabolic regression curve for the 530 nm points. This curve has been drawn over the other wavelength data points and clearly represents the  $V$ -log  $I$  relation for each wavelength. Now the relationship between normalized response amplitude and the logarithm of stimulus intensity has been found to be closely approximated, in a number of sensory systems, by the log-hyperbolic tangent curve (Naka and Rushton, 1966; Lipetz, 1969). The log tanh curve which would fit best the data points of the 530 nm curve was calculated and visually positioned in Fig. 3 (dark line).

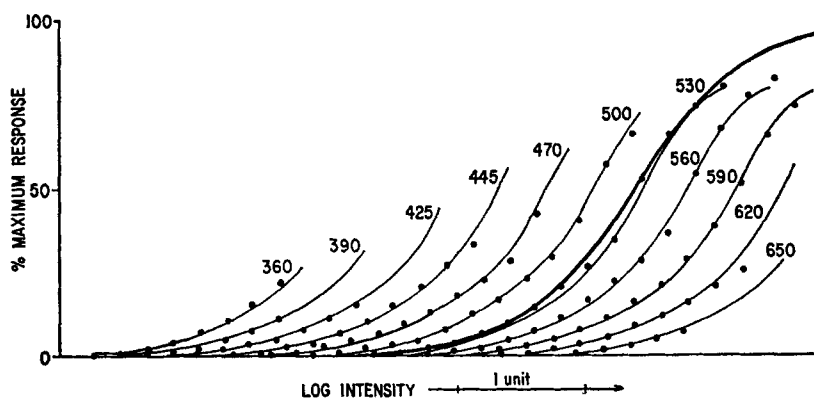


FIGURE 3. Amplitude-intensity curves. Each curve obtained with various intensities of light of the indicated wavelength. Amplitude is represented as percentage of maximum response to 530 nm light. Curves are displaced on abscissa for clarity of presentation. Light lines are best-fit parabolic regression for 530 nm points. Heavy line is best fit tanh for 530 nm points.

Clearly, response amplitude is a function of both stimulus intensity and state of adaptation. Some aspects of this relationship are shown in Fig. 4. The upper graph shows the  $V$ -log  $I$  relations obtained from the same preparation and under the three conditions of adaptation represented by the spectral response functions shown in Fig. 2. The effect of light adaptation was to increase the slope of the  $V$ -log  $I$  curve and the light level required to elicit a given response. The change of slope of the  $V$ -log  $I$  curve was best evaluated by reduction of the data to a linearized plot. This was accomplished, with the assumption that the tanh curve adequately fits the  $V$ -log  $I$  data, by a manipulation of the logistic function used by Naka (1969) to investigate the  $V$ -log  $I$  relationship of S-potentials of the fish:

$$V_o = 1/(1 + de^{-kj}),$$

where  $V_o$  is the ratio of response amplitude to maximum response in units of 0-1;  $J = \log I$ ;  $d = I$ ; the intensity required to evoke  $V_o = 1/2$ ; and  $k$  is an arbitrary constant near unity in magnitude. If we let  $d = e^{-2a}$ , and  $k = 2b$ , then

$$V_o = 1/(1 + e^{-(a+bJ)}),$$

and if we let  $a + bJ = w$ , then

$$\begin{aligned} V_o &= 1/(1 + e^{-2w}) = e^{2w}/(e^{2w}+1) \\ &= 1/2 + 1/2 \frac{e^w - e^{-w}}{e^w + e^{-w}} = 1/2 + 1/2 \tanh w. \end{aligned}$$

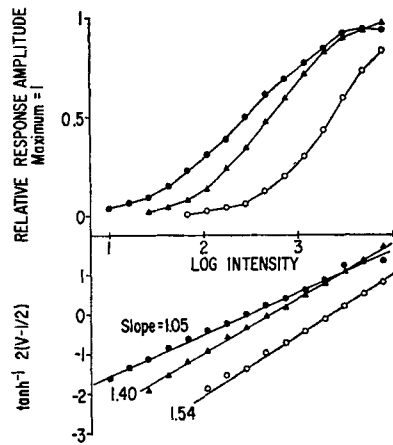


FIGURE 4. Upper graph, effect of light adaptation on the  $V$ -log  $I$  relation. Same preparation and conditions of adaptation as for Fig. 2. Solid circles, dark-adapted; triangles, moderate, open circles, strong light adaptation. Lower graph, linearized plot of data of upper graph to show slope changes caused by light adaptation (see text).

From this

$$V_o = \frac{1}{2} + \frac{1}{2} \tanh (a + bJ).$$

This is equivalent to

$$\tanh^{-1} 2(V - \frac{1}{2}) = a + bJ,$$

the desired linear equation.

In the lower graph of Fig. 4, the inverse hyperbolic tangent,  $\tanh^{-1} 2(V - 1/2)$ , for the above data, is plotted against log  $I$ . The slopes ( $b$ ) are increased and the point of interception of each curve with the ordinate ( $a$ ) is decreased by light adaptation. The effect of the change in slope is to increase the sensitivity of the ocellus to changes in intensity of stimulation. This in turn serves to emphasize responses to stimuli in the midportion of the normalized spectrum. The curves are shifted along the ordinate when the preparation is

light-adapted. This results from an equivalent decrease in sensitivity to all wavelengths of stimulation.

In order to represent accurately the form of the spectral response curves of the barnacle, it was necessary to correlate data from many preparations in varying states of adaptation. The following analysis, which uses the linearized  $V$ -log  $I$  function, was developed to circumvent the effects of adaptation on the form of the curves. If a single pigment is present in a photoreceptor (see below), its response to different wavelengths of light is determined by its ability to catch quanta at each wavelength. In an analogous manner, the response of a photoreceptor to monochromatic light of different intensities is dependent on its ability to catch quanta at each intensity. Since quanta at one wavelength are equivalent to more or less quanta of another wavelength in their ability to cause a response, it was possible to construct an "equivalent quantum" scale which relates the response at each wavelength to quanta of a reference wavelength.

Thus for each animal the following were obtained: (a) Experimental spectral response function using spectral lights of equal quantal content. (b) Experimental amplitude-intensity curve, using various intensities of 530 nm light. (c) Linearized amplitude-intensity plot, using a  $\tanh^{-1}$  program. From the equation  $a + bJ = \tanh^{-1} 2(V - \frac{1}{2})$ , the intensity values ( $J$ ), and the amplitude values ( $\tanh^{-1} 2(V - \frac{1}{2})$ ) were entered into a linear regression program in a small computer. This program calculated the appropriate values of (a) and (b) and a Pearson correlation coefficient ( $r$ ). (d) The spectral response curve was calculated. In these curves each point represents the relative number of quanta of 530 nm light required to elicit a response of an amplitude equal to that evoked by a different spectral light. In this analysis, variations in the slope of the  $V$ -log  $I$  curve, which are independent of wavelength but dependent on adaptation, nullify the effect of adaptation on the calculated spectral response functions.

Fig. 5 shows the spectral response function, in terms of equivalent 530 nm quanta, from 23 curves obtained in 15 preparations. The function is somewhat narrower than the Dartnall (1953) nomogram of a  $\lambda_{535}$  pigment. It is of interest that the Pearson correlation coefficient for the  $\tanh^{-1} 2(V - \frac{1}{2})$  curve was never less than 0.99. This indicates a close fit of the tanh curve to the  $V$ -log  $I$  data for the barnacle photoreceptor and supports the initial assumption on which this analysis was based.

*Spectral Sensitivity* In its normal environment the barnacle may be subjected to high levels of ambient illumination. A sudden decrease in light, i.e. a shadow falling on the animal, initiates closure of the operculum. This shadow reflex is mediated by the ocelli (von Buddenbrock, 1930; Gwilliam, 1963, 1965). Thus it was of interest to evaluate the spectral sensitivity of the ocellus in terms of its response to "flashes" of darkness. For the study shown in Fig. 6,

continuous spectral lights were briefly interrupted for 150 msec at a rate of once per 10 sec. The amplitude of the off-response at each wavelength was set to a criterion level by appropriate adjustments of the spectral light intensity. Fig. 6 shows for two preparations the relative intensity of spectral light required to elicit, when interrupted, an off-response of constant amplitude. The

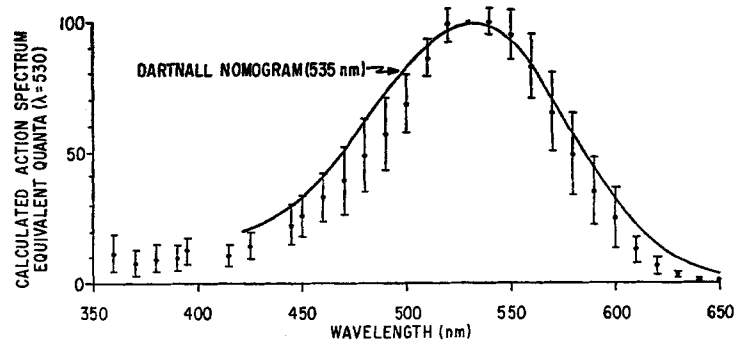


FIGURE 5. Spectral response curve. Each point represents the equivalent quanta of 530 nm light required to evoke a response of the same amplitude evoked by a spectral test stimulus of equal quantum content. Amplitude is represented as a percentage of the maximum response. Pooled results from 23 curves in 15 preparations. Bars indicate  $\pm 1$  SD.

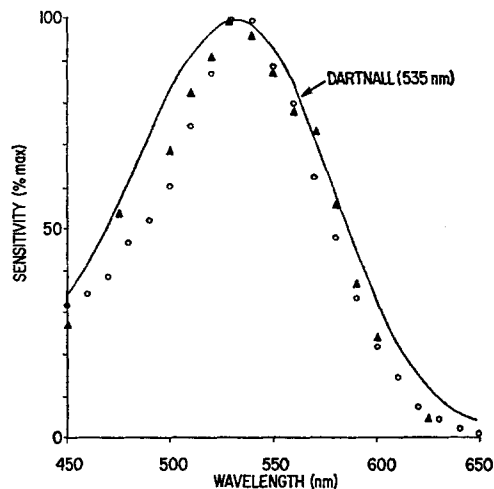


FIGURE 6. Spectral sensitivity. Inverse of energy of background light required to sustain a  $50 \mu\text{v}$  off-response is normalized and plotted against wavelength of background light. Off-responses were evoked by 150 msec flashes of darkness.

spectral sensitivity, as the spectral response function, showed a maximum effectiveness of 530–540 nm light. The incident light used in these experiments was of sufficient intensity to evoke a near maximal on-response from the animal, which was accordingly strongly light-adapted during the study.

*Chromatic Adaptation* It was convenient to use the off-response to evaluate the effects of chromatic adaptation on the spectral response function. For the

studies illustrated in Fig. 7, the preparation was continuously illuminated with different spectral lights of equal quantum content. This light was interrupted for 150 msec every 10 sec to elicit an off-response whose amplitude was proportional to the effectiveness of the incident light (top curve). The addition of spectral bleaching light at 590 or 490 nm did not alter the form of the spectral response curve which still peaked at 535 nm (open circles and triangles). This study supports the hypothesis that a single photopigment mediates the light sensitivity of the barnacle photoreceptor. The light-adapted curves are somewhat narrower than the control curves. This resulted from an increase in the slope of the  $V$ -log  $I$  functions caused by adaptation.

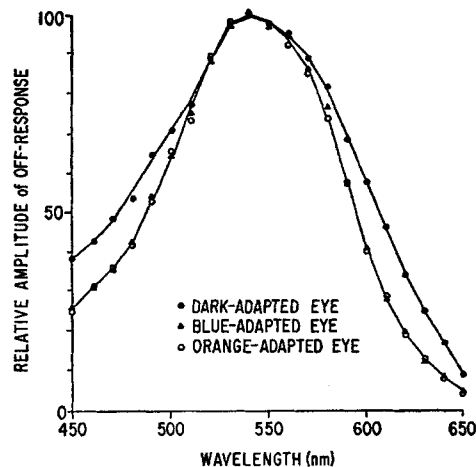


FIGURE 7. Effect of chromatic adaptation on spectral response. Normalized amplitude of off-response is plotted against wavelength of background lights of equal quantum content (solid circles). For curves shown as open circles and solid triangles, an additional adapting light at 490 or 590 nm was turned on. Off-responses were evoked by 150 msec flashes of darkness. The adapting lights were adjusted in intensity to reduce the off-response at 530 nm by 50%.

**Fluorescence** The relatively greater sensitivity of the photoreceptor to short wavelength stimulation was probably related to the decreased slope of the  $V$ -log  $I$  curve found when the animal was dark-adapted. However, there was obvious tapetal fluorescence to incident light in the near UV. It was important to determine whether the intensity of this fluorescence was sufficient to account for some portion of the heightened responsiveness to blue light. A Gamma microspectrophotometer was used in conjunction with a Leitz Ultra-pak incident light microscope to determine the emission spectrum and intensity of tapetal fluorescence excited by the 360 nm stimulus light. The system was calibrated against a standard MgO surface placed in the position of the ocellus.



The emission spectrum, derived from studies of six specimens, is shown in Fig. 8. The greatest flux was at 460 nm. In these studies emission from the entire specimen was evaluated. Approximately 60% of the flux passed through both the cornea and the receptor cells. The remaining 40% passed through the cornea only. Thus the emission spectrum has been altered to some extent by absorption within the ocellus. The data of Fig. 5 were used to convert the emission spectrum into its 530 nm equivalent (Fig. 8, dark line). The area under the 530 nm equivalent curve represents the total fluorescence flux in terms of an equivalent quantum flux of 530 nm light. When this equivalent quantum flux, due to fluorescence, was compared with the incident quantum flux at 360 nm used in the spectral response studies, it was found that fluorescence could account for less than 10% of the observed response.

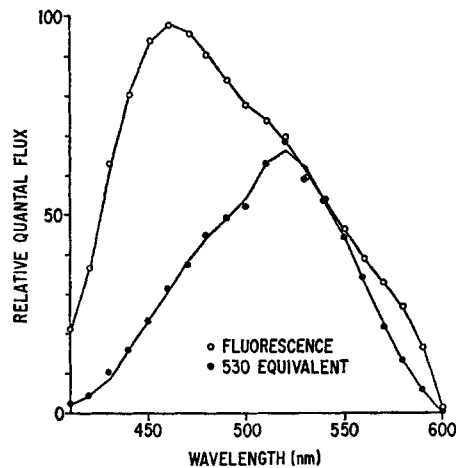


FIGURE 8. Emission spectrum of ocellar fluorescence excited by 360 nm light. Relative flux is presented as a percentage of the maximum. 530 equivalent spectrum calculated from spectral efficiency shown in Fig. 5. The area under the 530 equivalent curve represents the total ocellar fluorescent emission in terms of equivalent 530 nm quanta.

*Optical Considerations* The tapetum is a highly reflecting surface which lines the inside of the ocellar cup (Fales, 1928). Its concave surface, although not perfectly regular, probably serves to concentrate incident light onto the receptor cells, somewhat in the manner of a parabolic mirror. The spectral characteristics of tapetal reflectance were determined for the isolated tapetum with the use of narrow band Balzer interference filters in the path of the incident light. The Gamma microspectrophotometer was used to evaluate the intensity of the reflected spectral light. The relative spectral reflectance of the tapetum was flat, to within about 10%.

The spectral density of the cornea also was determined. For this study, the isolated cornea was placed atop a glass fiber through which spectral lights were conveyed. As in many other arthropods (Carricaburu and Chardenot, 1967) corneal spectral transmission was relatively uniform, with the greatest density to short wavelengths of light. However, transmission of 390 nm light was at least 90% of transmission of 650 nm light. Thus the optical properties

of the cornea and tapetum introduce no significant error into our determination of the wavelength of maximum sensitivity of the barnacle photoreceptor.

#### DISCUSSION

In previous studies of the barnacle (Gwilliam, 1963, 1965; Brown et al., 1970) and in the present study, no evidence of ocellar nerve action potentials was seen. Apparently, central effects of light are mediated through electrotonic spread of the receptor potential to the axon terminals.<sup>1</sup> Thus a study based on the measurement of extracellular potential is highly pertinent to the function of the ocellus; most of the extracellular current responsible for our receptor potentials emerges from the cut end of the ocellar nerve, as much as 5–6 mm from the receptor. In a well-cleaned preparation, the extracellular pathway had an impedance of about 1 megohm, across which receptor potentials of 2–5 mv developed. Thus axial current flow was on the order of 2–5 namp. The photoreceptor axons are on the order of 15, 15, and 5  $\mu$  in diameter (Fahrenbach, 1965) with a total cross-sectional area of about  $3.75 \times 10^{-6}$  cm<sup>2</sup>. Thus the density of current at the cut surface of the axon was on the order of 1.33 ma cm<sup>-2</sup>. This is remarkably comparable to the peak axial extracellular current flow along the mammalian rod, 0.59 ma cm<sup>-2</sup> (Penn and Hagins, 1969).

These spectral sensitivity and chromatic adaptation studies suggest that the ocelli of *Balanus amphitrite* possess a single photopigment with a  $\lambda_{\max}$  of 530–540 nm. The spectral response function of the barnacle, like the spectral sensitivity of the octopus (Hamasaki, 1968), was considerably broadened in the relatively dark-adapted state. However, absorption spectra tend to become broader as pigment concentration is increased. For example, if a solution of some given concentration has an absorption of near 100% at  $\lambda_1$  and 10% at  $\lambda_2$ , an increase in concentration can have little effect at  $\lambda_1$  but will increase absorption at  $\lambda_2$ . It is for this reason that the density curve is used as the basis of the Dartnall nomogram, since this curve represents the limiting case where the concentration of pigment is near zero (Dartnall, 1957). The most obvious effect of light adaptation is to reduce pigment concentration; i.e., to reduce the population of available chromophores. It is under these conditions that a spectral response function is expected to resemble Dartnall's nomogram in form.

It is of interest that the change in slope of the  $V$ -log  $I$  curves caused by light adaptation also can be explained on the basis of a change of population density of the chromophores. Let us assume that the available pigment constitutes a population of chromophores of varying quantum catching capability (sensitivity). In Fig. 9 this population is represented as a frequency distribution  $f(s)$ .

Stimuli of increasing strength may be expected to recruit progressively chromophores of decreasing sensitivity. The number of chromophores which

<sup>1</sup> Shaw, S. 1970. Personal communication.

are activated by a stimulus of a given intensity (i.e. the response to the stimulus) is represented by the accumulated area under the frequency distribution and may be plotted as the integral of  $f(s)$ ;  $F(s) = \int f(s)$ . Then if sensitivity is plotted on a log scale,  $F(s)$  is analogous to our  $V$ -log  $I$  curves. If the distribution of sensitivities is Gaussian, the curve,  $F(s)$ , closely approximates a hyperbolic tangent curve (Pearson correlation coefficient = 0.998).

Light adaptation of a given intensity may be expected to inactivate the high sensitivity chromophores, thus to reduce the variance of the population  $f(s)$ . This in turn causes the slope of the normalized intensity response curve  $F(s)$  to be increased (Fig. 9, dotted lines) (Goldstein, 1964). As noted above, if a

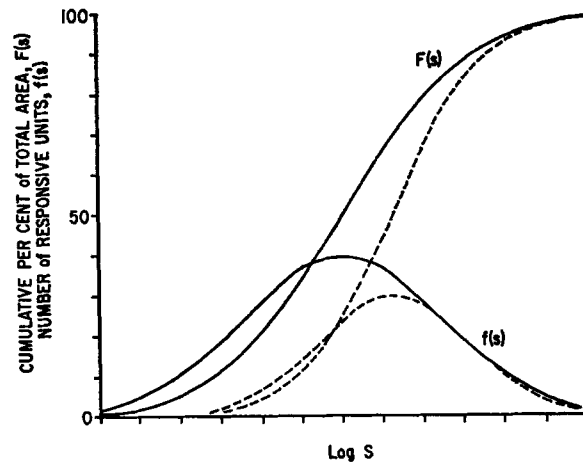


FIGURE 9. Representation of the  $V$ -log  $I$  curve as an accumulated frequency distribution. A population of chromophores is assumed to be variant in its sensitivity to stimulation as shown by the frequency distribution  $f(s)$ . The number of chromophores activated by a stimulus of strength  $b$  is  $\int_0^b f(s) = F(s) |_0^b$  (solid line). If light adaptation reduces the variance of  $f(s)$ , the slope of  $F(s)$  is increased (dotted line).  $F(s)$  is analogous to the  $V$ -log  $I$  curve.

single pigment is present in a photoreceptor, the width of its spectral response curve is determined by the slope of the  $V$ -log  $I$  curve.

The effect of adaptation on the slope of the  $V$ -log  $I$  curve and thus on the spectral response curve also could be based on changes at the level of the photoreceptor membrane. It has been suggested that the  $V$ -log  $I$  relationship found in many receptors has as its basis a self-shunting receptor membrane (Loewenstein, 1961; Naka and Rushton, 1966 *b, c*). In this hypothesis, receptor activation results in the opening of a population of low resistance transmembrane paths. Loewenstein (1961), from an analysis of an electrical analogue, suggests that the relation of the response amplitude ( $V$ ) to the number of opened shunting membrane paths ( $x$ ) should be

$$V = kbx / (1 + bx)$$

where  $k$  and  $b$  are constants. Of course,  $x$  is also a measure of stimulus strength. Naka and Rushton (1966 *a*) have shown that this expression is equivalent to

$$V_o = \frac{1}{2} + \frac{1}{2} \tanh x$$

where  $V_o$  is the ratio of the response,  $V$ , at a given stimulus strength to the maximum response, and  $x$  is the stimulus intensity expressed in appropriate units. The  $\tanh x$  curve, which represents the  $V$ - $\log I$  relation at the membrane, may be considered to be the cumulative frequency distribution of a population of open membrane shunts. If the population of photoreceptor membrane shunts is variant in its sensitivity to light, and if light adaptation reduces this variance, then from the above discussion, it may be expected that the slope of the  $V$ - $\log I$  relation will be increased by light adaptation. Thus the form of the spectral response function, which is dependent on the slope of the  $V$ - $\log I$  curve, could be based on either a pigment or a membrane phenomenon, or both.

Behavioral studies of the spectral sensitivity of barnacles have been reported. These utilize the positive or negative phototactic response of the cyprid or nauplius larvae of various species to evaluate sensitivity (Visscher, 1928; Visscher and Luce, 1928). Of particular interest, the cyprid larvae of *B. amphitrite* showed a maximum phototactic sensitivity to light of 530–545 nm wavelength (Visscher and Luce, 1928). The free-swimming cyprid larvae possess a pair of lateral compound eyes and a median nauplius eye. The compound eyes later degenerate and the adult lateral eyes form by division and migration of the simple median nauplius eye (Fales, 1928). Which of these eyes is responsible for cyprid larval phototaxis is not known. However, the similarity of the maximum spectral sensitivities found in our studies of the adult to those of Visscher and Luce, of cyprids, suggests involvement of the nauplius eye.

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