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Control of Retinal Sensitivity

I. *Light and Dark Adaptation of*
Vertebrate Rods and Cones

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ABSTRACT   Rods and cones in *Necturus* respond with graded hyperpolarization to test flashes spanning about 3.5 log units of intensity. Steady background levels hyperpolarize the rods, and the rod responses become progressively smaller as background level is increased. In cones, higher background levels reduce the effectiveness of test flashes, so higher ranges of test intensities are required to elicit the full range of graded responses. When backgrounds are terminated, cones return rapidly, but rods return slowly to the dark potential level. The effects of backgrounds on both rods and cones can be observed at intensities that cause negligible bleaching as determined by retinal densitometry. During dark adaptation, changes are observed in the rods and cones that are similar to those produced by backgrounds. Receptor sensitivities, derived from these results, show that rods saturate, cones obey Weber's law, and sensitization during dark adaptation follows a two-phase time-course.

INTRODUCTION

In this series of three papers we have studied the effects of background illumination upon the response characteristics of the receptors, bipolars (Werblin, 1973), and ganglion cells (Werblin and Copenhagen, 1973). From these studies, a series of transformations that are concatenated to form the retinal output have been derived. Retinal sensitivity is controlled initially by the state of adaptation of the photoreceptors, then modified by lateral interactions at the outer plexiform layer, and finally influenced by lateral interactions at the inner plexiform layer. These papers deal successively with each of the three sites, then the cumulative effect of each transformation on the form of retinal output is evaluated.

Recent electrophysiological evidence suggests that the desensitizing effects of full field backgrounds, measured at more proximal sites in the visual system, may take place in the photoreceptors themselves. Both Boynton and

Whitten (1970) recording from monkey cones, and Dowling and Ripps (1972) recording from skate rods, have shown that the test flash intensity required to elicit a criterion receptor response was proportional to background intensity over a broad range of background levels, satisfying Weber's law. Furthermore, in the presence of backgrounds, graded responses were elicited by brighter test flashes that had previously saturated the receptors. This was attributed to pigment bleaching in the monkey cones, but it was shown that the same phenomenon was observed in skate rods at backgrounds that bleached negligible quantities of pigment.

The different role of bleaching in the light adaptation of skate rods and monkey cones could be due to differences in the properties of rods and cones, or might be attributable to differences in species. The retina of *Necturus* contains only one rod and one cone type (Liebman, 1972) so a comparison between rod and cone behavior can be made in one animal. We studied the effects of backgrounds and bleaching levels, as determined by retinal densitometry, upon the response characteristics of both rods and cones in *Necturus*, and interpreted our results in terms of receptor sensitivities.

METHODS

Preparation

Eyes were enucleated from adult *Necturus* and the anterior portion and excess vitreous humor were removed under dim red light. The opened eyecup was then placed upon the cone of an air suspension loudspeaker which was used both for advancing the retina into the electrode and for jolting the retina to facilitate cell impalement. This technique was used in conjunction with a conventional hydraulic microelectrode drive system. The eyecup and loudspeaker cone were enclosed in a chamber containing water-saturated wicks which minimized retinal dehydration.

Electrodes

The intracellular micropipette electrodes used in all recordings were made from fiberglass-filled Pyrex no. 7740 capillary tubes (Corning Glass Works, Corning, N.Y.) which were pulled on a Livingston type microelectrode puller (Otto Hebel, Rutledge, Pa.), injection-filled with 5 M potassium acetate, and observed under a microscope for a coarse inspection of the electrode. For these experiments, 350–500-MΩ electrodes were used. The electrodes were coupled to an FET operational amplifier through a chlorided silver wire; the eyecup was grounded with a chlorided silver wire which made contact with moistened filter paper beneath the eyecup. The extracellular electrodes used in this study were of the wick variety and were made from 5-c lengths of the Pyrex no. 7740 capillary tubing which were half-filled with a tight packing of thread. They were then placed in Ringer's solution and filled by vacuum.

Ringer's Solutions

Two types of Ringer's solutions were used in these experiments. The aspartate Ringer's solution was used to isolate photoreceptors from the other retinal neurons

(Cervetto and MacNichol, 1972) and hence allowed the mass receptor response to be recorded (Sillman et al., 1969). It was also used to fill the wick electrodes used in the mass receptor measurements. The composition of each solution is shown in Table I.

Photostimulator

The photostimulator used in this study contained two independent channels of light stimulation, a test channel and a background channel. Only one channel is described as the optics of both were identical. The light from a 15-W Zeiss illuminator (Carl Zeiss, Inc., New York, N.Y.) was first passed through infrared absorbing glass and then through a 12-position neutral density filter wheel (made from calibrated no. 96 Kodak Wratten neutral density filters, Eastman Kodak Co., Rochester, N.Y.) with density steps of 0.5 log units, producing a range of 0.0–5.5 log units of light attenuation. The light was then focused with a 10X microscope ocular onto one end of $\frac{1}{8}$-inch diameter light pipe. Between the ocular and the light pipe was another filter wheel containing a 2.0-log unit neutral density filter, an open space where the emerg-

TABLE I

COMPOSITION OF RINGER'S SOLUTIONS. ALL VALUES IN MILLIMOLAR
CONCENTRATIONS.

	NaCl	KCl	CaCl₂	MgCl₂	HCl	NaHCO₃	Dextrose	Na aspartate
Normal Ringer's	108	4.0	0.9	1.0	to pH 7.7	7.0	10.5	0.0
Aspartate Ringer's	78	4.0	0.9	1.0	to pH 7.7	7.0	10.5	30.0

ing beam was unattenuated, a red interference filter (605 nm), and a blue interference filter (495 nm). A third 2.0-log unit neutral density filter could be interposed between the second filter wheel and the light pipe, providing a total light attenuation range of 0.0–9.5 log units in 0.5-log unit steps. The ends of both the test channel and the background channel light pipes were placed 1 cm away from the eyecup and postioned so as to provide uniform illumination over the entire retina. All test stimuli and background illuminations were, therefore, full field illuminations which were normally incident upon the retina from slightly different angles.

The two light beams were shuttered with a single axial solenoid (Unilex Electromagnetic Actuator, Velmex Inc., Holcomb, N.Y.) with thin, brass-covered, balsa wood blades attached to each end of the solenoid. Depending on the state of activation of the solenoid, the blades occluded either the test channel or the background channel. The distance between the ends of the blades was made equal to the distance between the ends of the light pipes so that no intermediate period of darkness occurred as the solenoid switched from the background channel to the test channel. The time required to switch between channels was less than 5 ms.

Using this shuttering arrangement coupled with the independent test and background channels, test intensities greater or less than any background intensity were produced.

Light Source Calibration

The emission spectrum of the test channel source was determined using interference filters and a light meter that has a flat spectral response curve from 450 to 1,000 nm (United Detector Technology Inc., Santa Monica, Calif., no. 40A). The source produced a total output power from 450 to 700 nm of 2,070 μW/cm². To calibrate this source in terms of incident photon fluxes, the Dartnall nomograms for the rod and cone photopigments, whose peak absorptions are 525 and 575 nm, respectively (Liebman, 1972), were each weighted with the emission spectrum of the source. From the rod and cone average outer segment diameters of 10 and 7 μm, respectively, these fluxes of incident absorbable quanta were calculated to be 1.06 $\times$ 10⁹ quanta (*Necturus* scotopically equivalent to 525 nm) incident at the retina per second per rod, and 1.25 $\times$ 10⁹ quanta (*Necturus* photopically equivalent to 575 nm) incident at the retina per second per cone. These maximum intensities are produced by our "9.0-log unit test flash" so the intensity scale factors in the text and in the figures represent approximately the log of the incident fluxes of equivalent absorbable quanta. The calibration of the source in terms of levels of bleaching produced by steady backgrounds is discussed in Appendix I.

The technique of substitution of test flashes for backgrounds removed all calibration requirements from the background channel since the intensity of each particular background was determined by comparing it with the calibrated test intensities using the receptors as null indicators. Because of this automatic calibration of the backgrounds used in the course of each experiment, no effort was made to balance the light output of the lamps in the test and background channels. This explains why some of the background intensities referred to in the succeeding text are not integral multiples of 0.5 log units.

RESULTS

Identification of Intracellularly Recorded Photoreceptor Responses

Photoreceptor identification was accomplished by eliminating the electrical responses of the retinal neurons proximal to the receptors with sodium aspartate Ringer's solution (Cervetto and MacNichol, 1972). The eyecup was immersed in the 30 mM sodium aspartate Ringer's solution for 30 min, then placed in the experimental chamber, and rod and cone identification was then accomplished using the following spectral test. Liebman (1972) has shown that *Necturus* has only one rod type and one cone type with maximum pigment absorptions near 525 nm and 575 nm, respectively. From Dartnall nomograms constructed around these absorption peaks, we selected two interference filters, 495 nm and 605 nm, which would provide the greatest differential absorption (and hence excitation) of the rods and cones. The ratio of the magnitudes of the receptor responses to test flashes elicited when each filter was interposed in the test beam could be used to distinguish between rods and cones. We equalized the magnitudes of the responses for one class of receptor by adding appropriate neutral density filters to the

interference filters. After equalization the other class of receptors always produced larger responses when the 605-nm filter was interposed in the test beam. The latter receptors were identified as cones; the receptors in which equal responses were elicited when each filter was interposed were identified as rods.

Effects of Background Illumination on the Rod Responses

A series of intracellularly recorded rod responses, elicited by 2-s test flashes of increasing intensity in a dark-adapted retina, pretreated with the aspartate
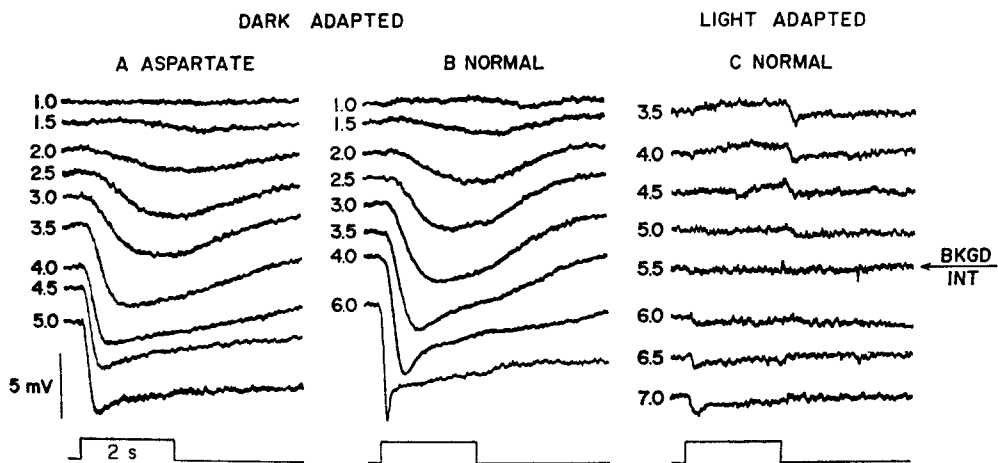
FIGURE 1. Intracellular rod responses. (A) Dark-adapted responses elicited in the aspartate-treated retina to 2-s test flashes of increasing intensities. (B) Dark-adapted responses from a normal, nonaspartate-treated retina. (C) Responses from a normal retina in the presence of a 5.5-log unit background. The intensity of the test flash is indicated at the left of each recording. The test flash was substituted for the background so test intensities greater and less than the background intensity were used. Responses to test flashes brighter than the background are shown below the "bkgd int" and responses to test flashes dimmer than the background are shown above the "bkgd int".

Ringer's solution, is shown in Fig. 1 A. In order to maintain the retina in its dark-adapted state, the period between test flashes was lengthened from 10 s for dim test flashes to 2 min for the brighter test flashes. The minimum test intensity that elicited a rod response distinguishable from noise was typically about 1.0 log unit of intensity, which corresponds to an incident photon flux of 10 quanta (525 nm)/s/rod (*see* Light Source Calibration in Methods). As the test intensity was increased, the amplitude of the rod response increased in a graded manner, the latency decreased, the initial phase of the response showed a faster rise time, and a small initial overshoot appeared. The most characteristic feature of the rod response was the absence of a

rapid return of the rod potential to its dark-adapted value at the termination of the test flash. For test intensities greater than 3.5 log units, the rod remained hyperpolarized and returned very slowly to its dark-adapted potential; for lower test intensities, the potential returned more rapidly, but there was never a clear discontinuity in the rod potential just after the flash was terminated for any test intensity. The maximum response amplitude elicited in the dark-adapted rods was typically about 6 mV.

Rod responses, recorded intracellularly in a dark-adapted retina not pretreated with the aspartate Ringer's solution are shown in Fig. 1 B. A more pronounced "overshoot" of the rod potential was consistently found in the normal retina. A similar effect of aspartate on the turtle receptor response has been reported by Cervetto and MacNichol (1972). These normal rod responses are very similar to those recorded in axolotl rods by Grabowski et al. (1972).

The effect of background illumination on the rod responses elicited by a wide range of test intensities was studied using the following protocol. The retina was first allowed to equilibrate to a given background for 2 min. (The process of intracellular recording necessarily limited this time period.) Then 2-s test flashes were delivered to the retina. Since the test flash was substituted for the background rather than superimposed upon it, we were able to use test intensities that were dimmer as well as brighter than the background intensity. As before, the period between test stimuli was 10 s for test intensities close to the background intensity and about 1 min for test intensities far greater or far less than the background. After the responses were recorded, the background was increased by 1 log unit and this protocol was repeated. A series of rod responses elicited by 2-s test intensities greater and less than a 5.5-log unit background is shown in Fig. 1 C. This background bleaches only minor amounts of pigment as is shown in Appendix I. The rod responses are still graded with increased test intensities, but the magnitude of these responses has been considerably reduced by the presence of the background illumination.

The effects of background illumination on the full range of the receptor responses are shown in the rod operating curves of Fig. 2 A. These curve were generated by plotting the amplitude of the rod response (measured as the difference between the peak of the response and the steady rod potential just before the response) versus the logarithm of the test intensity that elicited each response. The data points below the abscissa represent the magnitudes of the responses elicited by test intensities greater than the background level, while those above the abscissa represent the magnitudes of the responses elicited by test intensities less than the background level. The intersection of each curve with the abscissa represents the value of the background intensity at which the curve was generated; a test flash of the same

intensity as the background elicited no response. For example, the middle two curves of Fig. 2 A were generated from responses elicited around background intensities of 3.5 and 4.5 log units. The operating curves of this figure illustrate that increased background illumination caused a progressive reduction in the maximum response that could be elicited in the rod even by intense test flashes.

The square data points in this figure represent the magnitudes of the rod responses elicited by test intensities greater than various backgrounds in the aspartate-treated rod. Responses to test intensities dimmer than these backgrounds were not studied in the aspartate-treated rods. The aspartate responses were scaled so that the dark-adapted operating curves were approximately the same size. Because the data points recorded under light-adapted conditions are close to the normal rod operating curves, it appears that both the normal and aspartate-treated rod show a similar reduction in the maximum elicitable response with increased backgrounds.

Steady bright background illumination of the retina always caused sustained hyperpolarization of the rods. Fig. 3 A shows the sustained rod hyperpolarization recorded in the aspartate-treated retina in the presence of a 3.5-log unit background intensity. Background illumination had two main effects on the electrical properties of the rods: it produced a sustained hyperpolarization and it dramatically reduced the size of the maximum response that could be elicited in the rods by bright test flashes.

The inability of the rods to generate large responses to bright test intensities under conditions of background illumination might result from the presence of the sustained polarization produced by the background. This would be the case if the rod polarization were limited at some maximum hyperpolarized level. As the sustained polarization increased with increased background levels, the difference between the sustained level and the maximum level would decrease. This would consequently cause a reduction in the maximum elicitable rod response.

The reduction in response amplitude, and the sustained hyperpolarization recorded in the rod under conditions of light adaptation were also observed during the dark adaptation of the rods after a bleach. Upon the termination of our standard 9.0 log unit, 30-s bleaching stimulus (this bleached more than 65% of the rod pigment), the rod exhibited no "off response" but remained hyperpolarized. Unfortunately, because of drift inherent in these electrical measurements, we were unable to follow the slow return of this rod hyperpolarization back to its dark-adapted level, but in all cases, this afterpolarization appeared to persist for many minutes (the length of time that we could have confidence in our DC recordings). This sustained afterpolarization observed after termination of bright backgrounds and bleaches
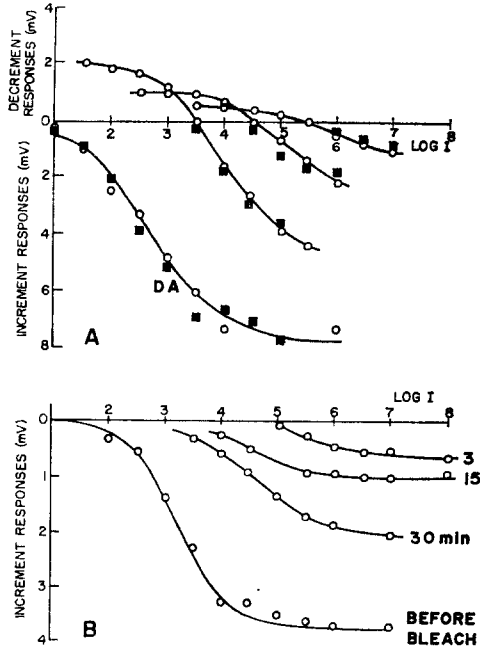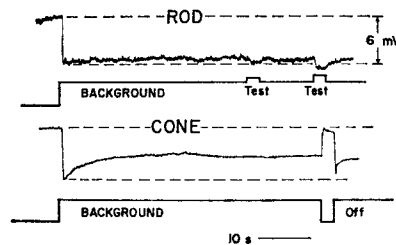
FIGURE 2

FIGURE 3

FIGURE 2. Rod operating curves for backgrounds and after a bleach. These curves are generated from the magnitude of the rod response (measured as the difference between the peak of the response and the steady polarization just before the response). The responses to flashes brighter than the background are shown below the abscissa and the responses to test flashes dimmer than the background are shown above the abscissa. (A) Operating curves for the rod, dark-adapted (DA) and at three background intensities generated from 2-s test flashes. The background intensities can be read as the test intensity that elicits no response so the backgrounds shown were 3.5, 4.5, and 5.5 log units. Circles show responses from single-rod in normal retina and squares show responses from a single rod in a different retina which had been pretreated with aspartate. The rod responses to test intensities dimmer than the background were not recorded in the aspartate-treated retina. (B) Operating curves generated from 100-ms test flashes delivered before a bleaching stimulus and at times 3, 15, and 30 min after the termination of the bleach. The bleaching stimulus bleached more than 65% of the rod photopigment (see Appendix I). The curves labeled DA and "before bleach" were generated from the equation $V_r/V_{r_{max}} = I^n/(I^n + k^n)$, with $n = 0.7$ and 1.0, respectively.

FIGURE 3. Receptor hyperpolarization in the presence of background illumination. Responses were recorded intracellularly in the aspartate-treated retina. (Top) Rod potential before and during a 4.5-log unit background. Responses to test flashes brighter than the background are superimposed upon this DC level. (Bottom) Cone potential before and during a 5.7-log unit background illumination. When background was briefly extinguished (off), the cone potential returned to its value measured before the background was applied. Because the cone potential exhibits a greater decay than the rod, responses to test intensities greater than the background can always be elicited in the cone.

is another example of the inability of the rods to generate large responses to test intensities dimmer than the background.

During the dark adaptation of the rod, the maximum response which could be elicited in the rod was also much smaller than the maximum elicitable dark-adapted response but, as dark adaptation proceeded, the maximum rod response grew in magnitude. This aspect of the dark adaptation of the rods is illustrated in the rod operating curves of Fig. 2 B which were generated from responses measured before the bleach and at times 3, 15, and 30 min after the termination of the bleach.

Effects of Background Illumination on the Cone Response

The set of intracellularly recorded cone responses elicited by a series of 2-s test flashes of increasing intensity in a dark-adapted retina pretreated with the aspartate Ringer's solution is shown in Fig. 4 A. The operating curve generated from these data is shown in Fig. 4 C as the curve labeled *DA*. The minimum test intensity that could elicit a response distinguishable from noise was about 2–2.5 log units which corresponds to an incident photon flux of 100–300 quanta (575 nm)/s/cone (*see* Light Source Calibration in Methods). As the intensity of the test flashes was increased, the magnitude of the cone response increased in a graded manner, the latency of the response decreased, the rise time became faster, an initial overshoot appeared, and, at the termination of the test flash, the potential fell rapidly to the dark-adapted resting potential. The maximum response recorded from the dark-adapted cone was typically about 6 mV, the same as for the rod.

The set of cone responses elicited by substituting test flashes brighter and dimmer than a 5.7-log unit background is shown in Fig. 4 B. The light-adapted cone responded rapidly, reaching an initial peak potential that then decayed to a steady level for test flashes brighter and dimmer than the background. The maximum response to test flashes brighter than this particular background was about 3 mV and the maximum response to test flashes dimmer than the background was also about 3 mV. Thus, the total voltage range of the cone responses was about 6 mV, the same as that of the cone under dark-adapted conditions. Therefore, this background caused little or no diminution of the total potential range over which the cone could respond but it shifted the operating curve of the cone to the right along the log-test intensity axis where it spanned an entirely different, higher range of log-test intensities around the new background level. This is more clearly illustrated in the operating curves generated from these data shown in Fig. 4 C. From the retinal densitometry measurements described in Appendix I, it was determined that this 5.7-log unit background intensity bleached negligible visual pigment.

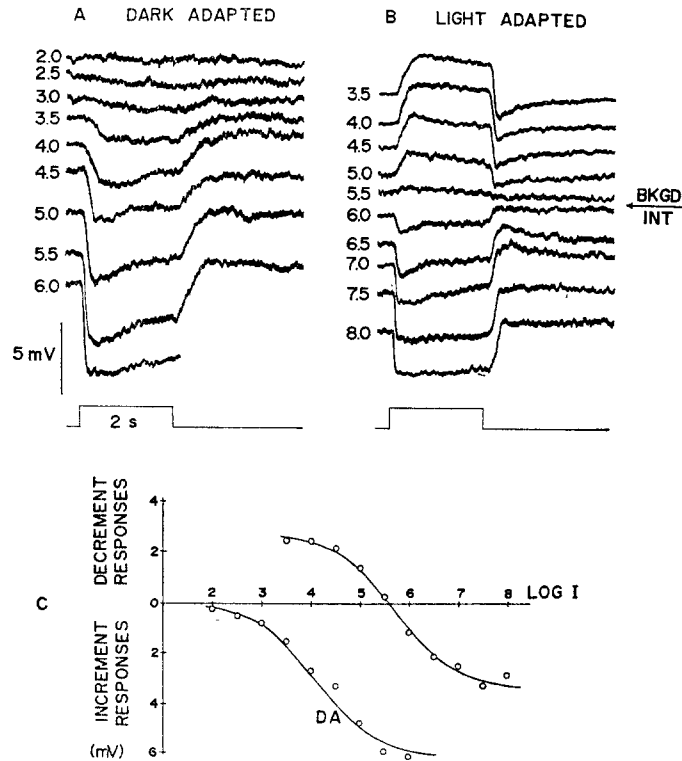The cones in the aspartate-treated retina, like the rods, also exhibited a

Figure 4. Intracellular responses from cones in the aspartate-treated retina. (A) Responses from dark-adapted retina to 2-s test flashes of increasing intensities. Only the initial phase of the response to the 6.0-log unit test flash is shown because the stimulus duration was lengthened for this particular intensity. (B) Responses in the presence of a 5.7-log unit background. The response to the 5.5-log unit test flash is minimal because this test flash is of almost the same intensity as the background. The apparent depolarizations to test flashes less than background result from the cone becoming less hyperpolarized than at background level (Fig. 3). (C) Cone operating curves generated from responses of A and B. Both curves were generated from the same template which was generated from the equation $V_r/V_{r_{max}} = I^n/(I^n + k^n)$, where $n = 0.7$.

sustained hyperpolarization in the presence of backgrounds. Fig. 3 B illustrates the cone potential before and in the presence of a 5.7-log unit background intensity. However, the steady level of cone polarization was always less than was observed in the rod. Comparison of the rod and cone polarization levels of Fig. 3 illustrates this point.

To summarize, background illumination caused two effects in the cone: it produced a sustained hyperpolarization and it shifted the entire operating curve along the log-test intensity axis so that it spanned a new, higher range of intensities, but the total potential response range was maintained.

Comparison of Intracellularly Recorded Rod and Cone Activity

At low test flash intensities, the waveforms of the rod and cone responses were quite similar although the rods were about 1.0–1.5 log units more sensitive. A difference in rod and cone sensitivity of 1.4 log units has been observed by Fain and Dowling (1973) also in *Necturus*. The most striking difference in the rod and cone responses is in the nature of their behavior at the termination of brighter test flashes. The cone response exhibited a fast "off" component; the rod did not generate any off response but returned only gradually to its dark potential level.

Both rods and cones exhibited graded operating curves over a range of test intensities spanning about 3.5 log units when the peak response was plotted versus the log of the stimulus intensity. This graded curve has been observed in turtle cones by Baylor and Fuortes (1970), in the axolotl rod by Grabowski et al. (1972), and in the skate rod by Dowling and Ripps (1972). The functional relationship between the receptor response magnitude and the stimulus intensity can be described by the equation

$$\frac{V_r}{V_{r\max}} = I^n/(I^n + k^n),$$

where V_r is the response amplitude, $V_{r\max}$ is the maximum response amplitude, I is the stimulus intensity, and n and k are constants which best fit their data with values of n between 0.9 and 1.0. Our data for the dark-adapted rods and cones is also approximately described by this equation but a better fit is obtained for values of n of 0.7, the value measured by Boynton and Whitten (1970) for the monkey cones. This difference in the exponent is possibly a result of the longer stimulus periods used in ours and Boynton and Whitten's studies. When 100-ms test flashes were used (as was done in Fig. 2 B), the exponent of $n = 1.0$ in the equation gives a better fit to the data than $n = 0.7$.

Background illumination produced a dramatic difference in the behavior of the operating curves for the rods and cones. The size of the rod operating curve was severely reduced by background intensities of 5.5 log units and greater while the curve for the cone shifted across the log-test intensity axis and did not change in total magnitude as the background level was increased.

Light Adaptation of the Mass Receptor Response

The rods and cones have operating curves that behave differently with increased background illumination. The number of background levels used in the intracellular experiments was necessarily limited because of the

limited time that one is able to make reliable intracellular recordings from each receptor impaled. Extracellular measurements, however, can be made for long periods of time with excellent stability. We used sodium aspartate to isolate the mass receptor response from the electroretinogram (Sillman, et al., 1969). Then we attempted to correlate rod and cone components of the mass receptor response waveforms with the waveforms recorded intracellularly in the individual rods and cones. We could then study light and
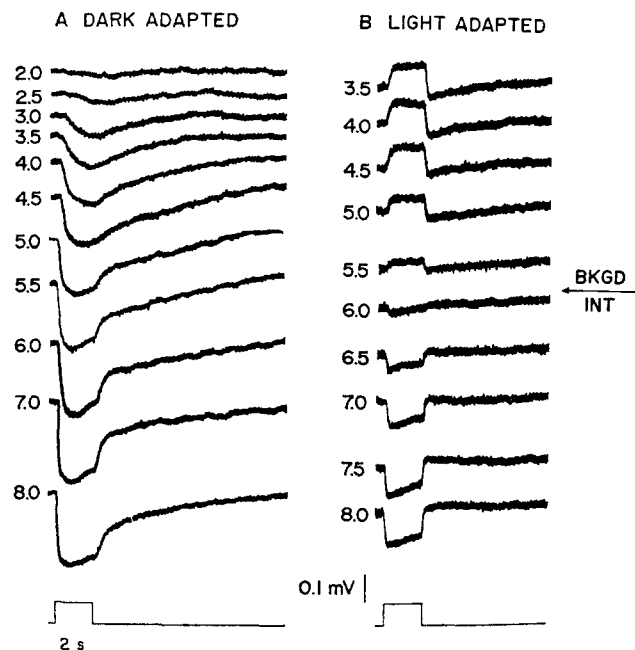

FIGURE 5. Mass receptor response isolated by the aspartate Ringer's solution. (A) Responses to 2-s test flashes of increasing intensities in the dark-adapted retina. (B) Responses in the presence of a 5.7-log unit background. The maximum response was typically about 300 μV. The positive-going responses in the light-adapted recordings are to test intensities less than the background level.

dark adaptation of the receptors in greater detail because we were no longer time limited.

Mass receptor responses, isolated by a 30-min pretreatment of the retina in the aspartate Ringer's solution and elicited by 2-s test flashes in the dark-adapted retina and in the presence of a 5.5-log unit background are shown in Fig. 5 A and 5 B. The experimental protocol used to generate these responses was identical to that described for our intracellular recordings. The waveforms of Fig. 5 A and 5 B illustrate two points. First, these waveforms which do not appear to exhibit a large "Proximal PIII" component (Witkovsky et al., 1973), are quite similar to the intracellularly recorded rod

and cone response waveforms shown in Figs. 1 and 4. This suggests that the mass receptor response is a good index of receptor activity. Second, it appears that the mass receptor responses elicited in the two types of receptors summate. In the dark, the responses of both the rods and the cones seem to contribute to the mass response which shows both a fast off response, characteristic of the cones and a prolonged afterpolarization, characteristic of the rods. These characteristics of the extracellularly recorded rod and cone responses have been demonstrated in monkey by Brown et al. (1965), and more recently by Whitten and Brown (1973).

The operating curves, generated from increment and decrement responses around each of a wide variety of background levels in a single aspartate-treated retina are shown in Fig. 6 A. Background illumination caused a shift in the operating curve along the log-test intensity axis as observed in the intracellular recordings. This figure also illustrates that as the background illumination was increased, the magnitude of the operating curves decreased until background levels of about 5.5 log units were reached. The magnitude of the curve then remained approximately constant for all higher
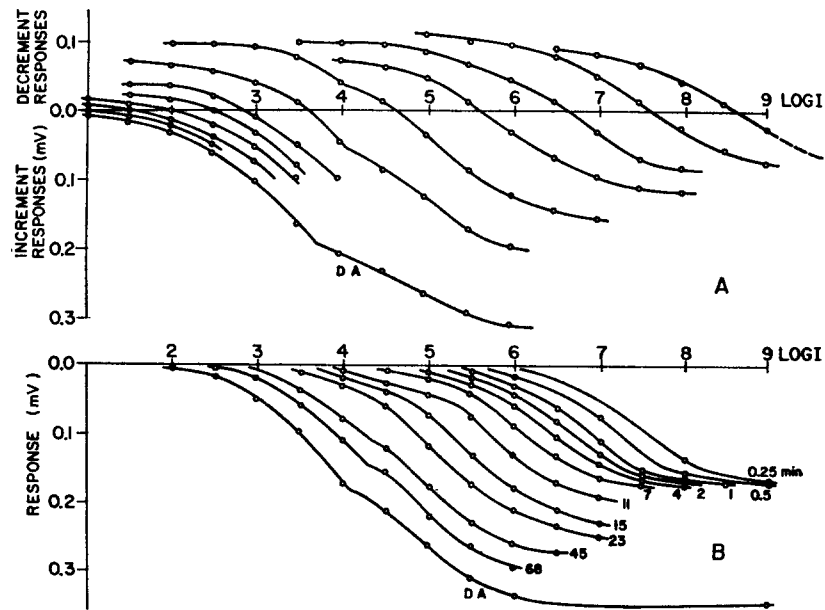

FIGURE 6. Operating curves of the mass receptor response during light and dark adaptation. (A) Light-adapted curves at 11 background levels. Peak of responses to test flashes brighter and dimmer than each background level are plotted below and above the abscissa, respectively. (B) The time-course of dark adaptation after a 30-s exposure to a 9.0-log unit background (bleaches greater than 85% of the cone photopigment) is manifest as a shifting position of the operating curve. All responses are hyperpolarizing here. The two families of curves are taken from different retinae.

backgrounds. This is consistent with the notion that rod and cone responses add to produce the total mass receptor response: for low background levels, both the rods and cones contribute to the magnitude of the operating curve, but for backgrounds greater than 5.5 log units, the rod responses become reduced to such an extent that we measure only the cone contribution to the response. The cone curve is not reduced, but continues to shift to the right along the log-test intensity axis as the background is elevated.

Dark Adaptation of the Mass Receptor Responses

Complete dark adaptation of the retina following a bleaching stimulus takes more than an hour to complete so intracellular studies of the changes in the responses of the rods and the cones during dark adaptation were extremely difficult to perform. We, therefore, measured the mass receptor response during dark adaptation.

The operating curves generated from the mass receptor response at various times after our standard bleach (which bleaches more than 85% of the cone photopigment) are shown in Fig. 6 B. The first two curves on the right side of this figure are drawn through only a few data points. The justification for drawing complete curves through these few data points is established in Appendix II which outlines the experimental protocol we used to study the initial shifting of the operating curve following a decrease in the background intensity. We found that the shifting of the operating curve during the initial phases of dark adapation proceeds in a form similar to that observed during the latter phases.

For the first 7 min of dark adaptation, the operating curve of the mass receptor response did not increase significantly in magnitude but simply shifted to the left along the log-test intensity axis. After about 11 min, the operating curve continued to shift but it increased substantially in magnitude. The curve also exhibited an inflection at the lower test intensities. At this time, an afterpolarization began to appear in the mass receptor response waveforms. These results suggest that for the first few minutes of dark adaptation, the cone responses predominate the mass receptor response and consequently, the operating curve during this early phase reflects mainly cone behavior. As dark adaptation progresses, the rods function more and their responses begin to add to those of the cones. The operating curve, which at this point contains contributions from both rods and cones, increases in magnitude because, as was shown in Fig. 2 B, the magnitude of the rod response becomes larger during further dark adaptation.

The operating curves of Fig. 6 seem to reflect a fundamental property of the photoreceptors; both the reduction in the size of the rod operating curve and the shifting of the cone operating curve are affected in very similar ways by either backgrounds or bleaches.

DISCUSSION

Receptor Operating Curves with Inferred DC Levels

Our results show how backgrounds and bleaches determine the range of log-test intensities that elicit the graded rod and cone responses. However, the level of steady receptor hyperpolarization in the presence of the background (as shown in Fig. 3 A and 3 B) has not yet been considered. The rod and cone operating curves would have greater significance if this DC-level information were included. Because of the drift inherent in intracellular recordings made with high impedance microelectrodes, the steady hyperpolarizations produced by the backgrounds could not be measured with sufficient precision to provide this information directly. Therefore, we have inferred these receptor DC levels from short term, intracellular recordings in the aspartate-treated retina in the presence of background illumination. The effects of drift were minimized in these observations made over brief periods.

For any state of background illumination, the cone polarization always seemed to lie between two fixed limiting potentials: the dark-adapted resting potential, and a "maximum" potential level. Fig. 3 B illustrates the cone potential before and in the presence of a bright background. When this background was briefly extinguished, (off), the cone polarization returned to the same dark-adapted potential level measured before the background was applied. This observation suggests that the potentials reached by cone responses to test intensities less than the background are bounded by the dark-adapted resting potential. We displaced the operating curves of Fig. 6 A vertically (those measured around background intensities greater than 4.5 log units were probably cone dominated) so that the maximum decrement response was aligned with the dark-adapted potential, as illustrated in Fig. 7. Since the curves all start from the same dark-adapted potential level and
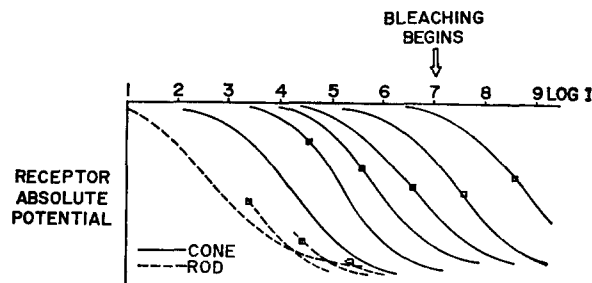

FIGURE 7. Operating curves for rods and cones in the aspartate-treated retina with inferred DC levels. The squares mark the intersection of each operating curve with the background level around which each curve was measured. The DC levels increase more rapidly with backgrounds in rods than in cones. The cone curves begin at higher test intensities than the rods, reflecting their lower sensitivity.

the magnitude of each curve is invariant with background intensity, the curves all appear to be bounded by the same maximum potential level as well.

The degree of sustained cone polarization in the presence of any background can be obtained from this figure by projecting the intersection of the background level on each operating curve onto the ordinate. This figure illustrates that as backgrounds are increased, the sustained polarization of the cones increases, but the increase is small so that even at the highest backgrounds used in this study, the steady polarization is less than two-thirds of the maximum cone potential.

The hyperpolarization that accompanies background illumination increases much more dramatically in the rods than in the cones, particularly in the aspartate-treated retina. Fig. 3 A shows that the rod polarization in the presence of a background falls only slightly from its initial value measured at the presentation of the background; thus, the steady rod polarization rises with increased backgrounds only slightly less than the extent to which the rod response increases with increased test flash intensities. The maximum elicitable rod responses in the aspartate-treated retina appeared to be limited by a maximum potential as were the cone responses. Both of these observations were used in Fig. 7 to vertically displace the rod operating curves measured in the aspartate-treated retina shown in Fig. 2 A (squares) so that the maximum potential levels were aligned. In this way, the steady level of rod hyperpolarization could be inferred.

The differing effects of background illumination on the rod and the cone operating curves is apparent from the vertical displacement of the operating curves. The main effect of background illumination on the cone operating curve is to shift the entire operating curve horizontally to the right along the log-test intensity axis so that graded responses are elicited by a higher range of log-test intensities around each new background level. The shifted cone operating curves of Fig. 7 could also have been generated if, instead of increasing the background illumination, neutral density filters were interposed in the test beam. This suggests that background illumination attenuates the effects of the test beam at an early stage in the cone; somewhere before the initiation of electrical activity. Boynton and Whitten (1970) have indicated that bleaching could account for such an attenuation, based on the reflection densitometry measurements of Rushton and Henry (1968). These measurements have shown that at high bleaching levels, each 10-fold increase in background bleaches 90% of the remaining photopigment. Boynton and Whitten suggest that backgrounds will thus cause a decrease in the ability of the cones to catch quanta which would be manifest as a shift in the cone operating curve along the log-test intensity axis. However, from our retinal densitometry measurements described in Appendix I, we have

shown that the curve shifting in the cones of *Necturus* occurs at backgrounds which bleach negligible photopigment. Therefore, a mechanism proximal to the site of photon absorption must account for the changes in adaptation that we have observed.

The rod responses changed in a quite different way in the presence of background illumination. The rod operating curves shown in Fig. 7 could not be artificially generated by inserting neutral density filters in the test channel. It appears that the rod input signal is not attenuated; the rod hyperpolarization increases rapidly with increasing backgrounds and is apparently "compressed" against some fixed maximum potential, severely reducing the maximum elicitable incremental rod response. However, because the rod operating curves of Fig. 2 A generated at various background intensities cannot be superimposed upon the single, dark-adapted curve by a vertical shift of each curve, this simple "compression" hypothesis is inadequate to completely specify the rod behavior under conditions of background illumination. There is apparently another mechanism that causes a slight shift in the operating curves with increased backgrounds similar to that which is observed in the cones.

Receptor Operating Curves and Receptor Sensitivity

Sensitivity is generally defined as the reciprocal of threshold, but since the receptors produce graded responses, we were unable to measure a unique receptor threshold. Instead, we have used the operational definition that receptor sensitivity is inversely related to the amount of light required to elicit a small, fixed-magnitude increment response. Thus, receptor sensitivity, S, can be defined as being equal to $(c_1) \cdot (dV/dI)$ where, in the limit, dI is the change in intensity required to elicit a criterion response, dV, and c_1 is a constant of proportionality. Because the operating curves generated from our data are displayed on semi-log coordinates, we can represent this sensitivity in terms of the slope and position of the operating curves as follows:

$$S = c_1 \frac{dV}{dI} = c_1 \frac{dV}{d(\log I)} \cdot \frac{d(\log I)}{dI} = c_1 \frac{dV}{d(\log I)} \cdot \frac{\log e}{I}.$$

The sensitivity of the receptors is proportional to the slope of the operating curve, dV/d (log I), and inversely proportional to the background intensity I. If the slope of the operating curves for small increments is constant at each background level, then

$$\frac{dV}{dI} = \frac{c_2}{I},$$

or,

$$\frac{dI}{I} = \frac{dV}{c_2},$$

where c_2 = (slope of the operating curve at the background intensity) · (log e). For a constant criterion response, dV is (by definition) constant and this relation describes Weber's law, dI/I = constant.

The operating curves of Figs. 4 C, 6 A, and 7 show that the condition of constant slope at each background level is approximately met for all backgrounds greater than about 3.5 log units, and therefore, the desensitization produced by increased backgrounds should be described by Weber's law. The "increment-threshold" curve of Fig. 8 A was derived from the curves of Fig. 6 A by calculating the intensity required to elicit a criterion response at each background. The desensitization described by Weber's law is represented on a graph of this type by a linear curve with unity slope. As the background level was increased, the slope of the receptor increment-threshold curve increased from zero to unity where it remained, even for the high background levels used in these experiments. Fig. 2 A shows that the rod responses are dramatically reduced over most of these background levels; therefore, the linear portion of this curve probably mainly reflects the behavior of the cones.

The increment-threshold curve obtained in this manner is similar to that obtained by Boynton and Whitten (1970) for the monkey cone system and that obtained by Dowling and Ripps (1972) for the skate rod system. It is also similar to the increment-threshold curve generated from data measured psychophysically in the human visual system by Koenig and Brodhum (1889), data which span a very wide range of background intensities.

The condition of a constant slope at each background level was not met in the rods as is seen in Fig. 2 A. The rods were more desensitized by increased backgrounds than were the cones because the slope of the operating curve decreased with increased background intensity. The rod increment-threshold curve, generated by applying the constant response criterion to the rod operating curves of Fig. 2 A, is shown in Fig. 8 B. This curve illustrates that the slope of the increment-threshold curve increases monotonically: it is initially zero, approaches a value of one, and finally increases to values considerably greater than one. Thus, Weber's law is obeyed over a broad range in the cones of *Necturus*, but not in the rods.

The effects of background illumination on rod sensitivity cannot be predicted from a simple compression hypothesis, as is illustrated in Fig. 8 B. The dashed curve shows the increment-threshold relationship that would be predicted from a compression hypothesis, assuming that the rod responses under light-adapted conditions are still described by the single dark-adapted operating curve of Fig. 2 A. Here, increases in background would cause the
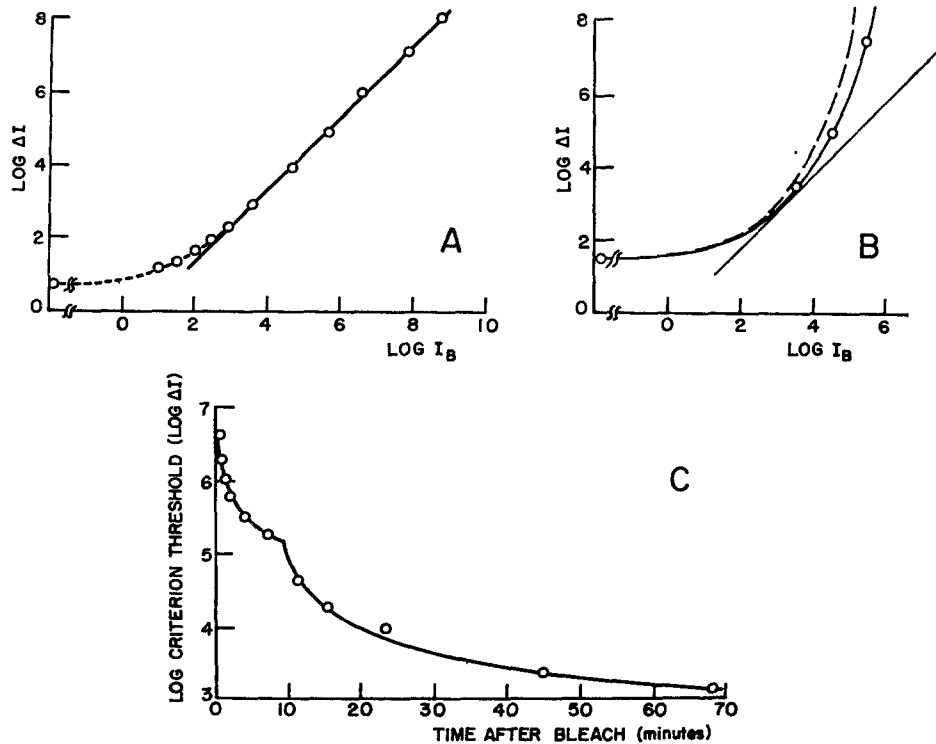
FIGURE 8. Receptor sensitivity for backgrounds and bleaches. (A) The "increment-threshold" curve for the receptors derived from the operating curves of Fig. 6 A, showing straight line Weber's law relation. Because the rod responses are severely reduced over most of this linear region, this curve reflects mainly cone desensitization. (B) Solid line: rod increment-threshold curve derived from the rod operating curves of Fig. 2 A. Weber's law is not obeyed in the rod and a form of "saturation" is observed at moderate backgrounds. Dashed line: "increment-threshold" curve derived from single dark-adapted operating curve of Fig. 2 A. Rod sensitivity was always higher than predicted from "compression" hypothesis. (C) The time-course of sensitivity changes after a bleach. This curve was derived from the operating curve of Fig. 6 B and shows both cone and rod components of resensitization. These curves were all derived on the assumption that receptor sensitivity is proportional to the amount of light required to elicit a constant criterion receptor response.

position on the curve around which responses are generated to be moved to the right along the single curve. Comparison of the curves of Fig. 8 B shows that for the backgrounds used in our experiments, rod sensitivity was always higher than that predicted by the compression hypothesis. Therefore, the slight shifting of the rod operating curve along the log-test intensity axis, which accompanies increased backgrounds, increases rod sensitivity at these background levels.

While the rods of *Necturus* exhibit a severe diminution in the magnitude of

their responses, and a form of "saturation" at high background levels, Dowling and Ripps (1972) have shown that after long periods of bright background illumination, the rods in the all-rod retina of skate do not saturate. Thus, the rods of skate seem to behave more like the cones of *Necturus*. The saturating nature of the *Necturus* rods and the nonsaturating nature of skate rods support the generalization of Daw and Pearlman (1971) that rods in rod-cone retinae exhibit saturation while those in all-rod retinae do not.

Our measurements of the light and dark adaptation of the mass receptor response suggest that there are two distinct phases of receptor resensitization following a bleach: a slow phase where the receptor operating curves shift back along the log-test intensity axis, and an extremely rapid phase that occurs immediately upon the termination of the bleaching stimulus. The rapid phase results from the movement of the operating point of the receptors (the point along the operating curve around which responses are generated) from some mid position along the operating curves in the presence of a background (Fig. 7) to the leftmost end of the operating curve when the background is terminated (Fig. 6 B). This repositioning of the operating point, which occurs so rapidly that the operating curve does not appreciably move back along the log-test intensity axis, can produce a large increase in cone sensitivity within its 100-ms response time.

The slower resensitization of the receptors is described by the curve shown in Fig. 8 C which was generated by applying the constant criterion condition to the dark-adapting mass receptor responses of Fig. 6 B. Since rods have a lower threshold than the cones, the final portion of this curve reflects mainly rod resensitization but, because the rod responses are severely reduced by moderate backgrounds and remain so when these backgrounds are terminated, the initial portion of this curve reflects mainly cone resensitization. Psychophysical measurements, made originally by Kohlrausch (1931) for the resensitization of the visual system following a bleaching stimulus typically exhibit similar biphasic kinetics.

SUMMARY

(*a*) We have observed the effects of full field background illumination and bleaches on the responses elicited in rods and cones by test flashes which were substituted for, rather than superimposed upon, the background intensity. (*b*) Measurable rod responses were elicited by test flashes 10–30 times less intense than those which elicited measurable cone responses. Each receptor type exhibited graded responses over a range of test intensities about 3.5-log units wide (its "operating range") and each produced maximal responses typically of 6 mV. At the termination of brighter test flashes, the rod exhibited little or no off response; the potential only slowly returned to its "dark" level. The cones, however, always exhibited a sharp off response.

(c) Sustained polarizations were always observed in each receptor type in the presence of backgrounds, however, in the aspartate-treated retina, the cones exhibited a larger decay in this sustained level from its initial value than did the rods. (d) As the level of background illumination was increased, the maximum elicitable rod responses to test intensities greater and less than the background were progressively reduced. In the cone, increased backgrounds also reduced the maximum elicitable response to test intensities greater than the background, but the maximum elicitable response to test intensities less than the background was enhanced. Thus, the total range of potentials over which the cone could respond was not reduced by backgrounds. (e) As the background intensity was increased, the 3.5-log unit operating range of the cone shifted along the log-test intensity domain so that it spanned new regions around each new background intensity. After termination of backgrounds that bleached substantial photopigment, the operating range of the cone slowly shifted back down the intensity domain to its dark-adapted contion. (f) Assuming that receptor sensitivity is proportional to the increment in intensity required to elicit a fixed criterion response, we have shown that the desensitization of the cones produced by backgrounds is described by Weber's law. The rods do not obey Weber's law but exhibit a form of saturation at moderate background levels. Following a bleach, the resensitization of the receptors exhibits a two-phase dark adaptation curve. (g) From retinal densitometry measurements, the saturation of the rods and the shifting of the cone operating ranges occurs at backgrounds that do not bleach substantial photopigment. Therefore, it appears that the desensitization of each receptor that accompanies background illumination occurs after the initial quantum events. In the cones, it appears that the site of the desensitization is before the initiation of the electrical events but this may not be the case in the rods.

APPENDIX I

Kinetics of Photopigment Bleaching

To determine the rate of photopigment bleaching produced by each background, the changes in retinal light transmission at 605 nm and 1,000 nm were monitored as a function of the duration of the background intensity. The retina was removed from the eyecup and mounted in a chamber which was filled with normal Ringer's solution. The chamber was then mounted over a photodiode which was located so that the retina was in the identical position used when electrophysiological measurements were made. The intensities of the 605-nm and 1,000-nm monitoring beams were adjusted so they both produced the same photodiode output. A given background intensity was then shown upon the retina for a predetermined length of time, ex-

tinguished, and the increase in transmission at 605 nm relative to the transmission at 1,000 nm was recorded. This protocol was repeated until no further increase in transmission occurred. The kinetics of the change in transmission at 605 nm relative to that at 1,000 nm for a variety of background intensities is shown in Fig. 9 A. This figure demonstrates that bleaching begins to become appreciable at background intensities somewhat greater than 7.0 but is not substantial for backgrounds less than this.
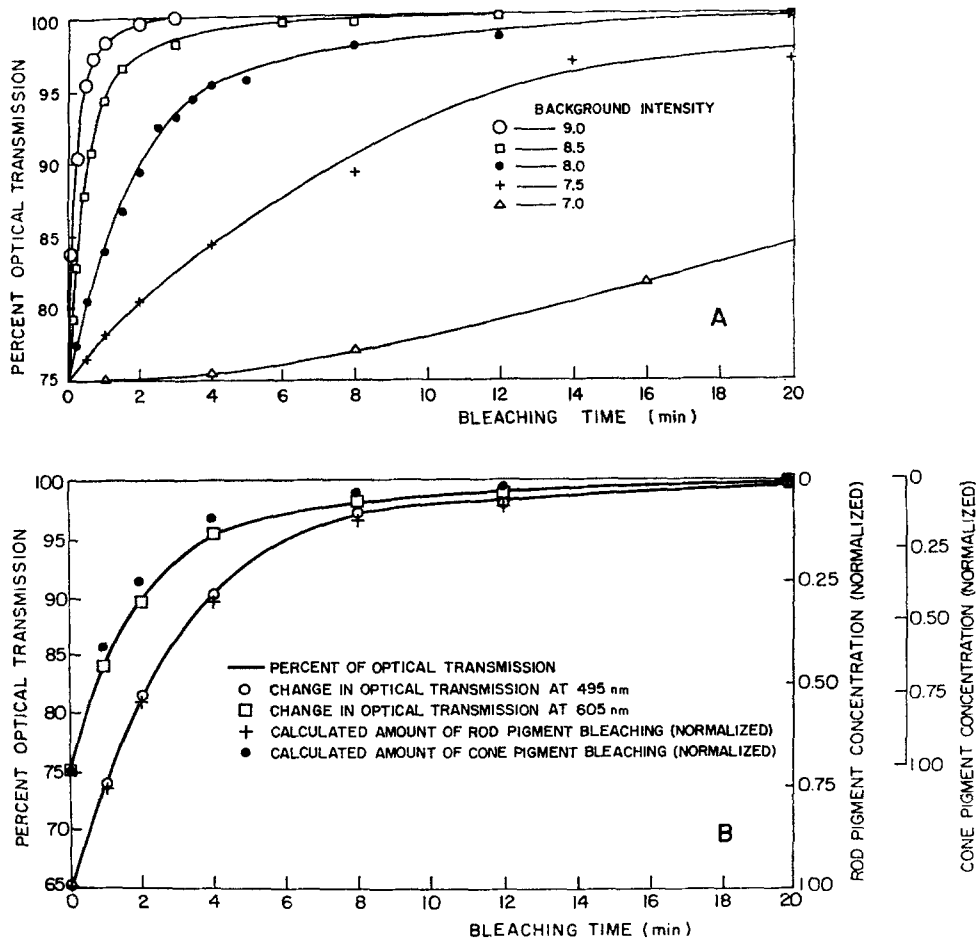

FIGURE 9.   Photochemical bleaching kinetics. (A) Normalized kinetics of retinal absorption changes at 605 nm relative to absorption at 1,000 nm produced by various background intensities. The background was applied for a given time and extinguished. The absorption at 605 nm relative to that at 1,000 nm was recorded and this procedure was repeated until no further change in transmission was observed. (B) Normalized calculated kinetics of rod and cone pigment bleaching produced by 8.0-log unit background intensity and the normalized changes in retinal transmission at 605 nm and 495 nm relative to that at 1,000 nm. Bleaching does not become substantial until backgrounds are increased past 7.0 log units.

Because the peaks of the rod and cone absorption spectra are only separated by 50 nm, the retinal absorption at 605 nm reflects mainly cone, but also some rod pigment. To determine the actual kinetics of rod and cone bleaching produced by an 8.0-log unit background, the time-course of the changes in transmission at 495 nm and 605 nm were both measured relative to the transmission at 1,000 nm and converted to optical density units. The cone and rod bleaching kinetics were calculated from these changes in optical densities by using the following simplified simultaneous equations;

$$D_{495}(t) = w(C(t)) + x(R(t))$$

$$D_{605}(t) = y(C(t)) + z(R(t)),$$

where w and y are the cone pigment absorptions at 495 nm and 605 nm, respectively, as obtained from the Dartnall nomograms for a 575-nm cone pigment absorption maximum and x and z are the rod pigment absorptions at 495 nm and 605 nm, respectively obtained from the Dartnall nomogram for a photopigment absorption maximum at 525 nm. $D_{495}(t)$ and $D_{605}(t)$ are the optical densities of the retina at 495 nm and 605 nm and $C(t)$ and $R(t)$ are the concentrations of cone and rod pigment at each time the optical densities were measured. The rod and cone bleaching kinetics and the changes in retinal transmission at 605 nm and 495 nm are shown in Fig. 9 B.

APPENDIX II

Initial Kinetics of Operating Curve Shifting during Light and Dark Adaptation

After a change in background level, and after the initial rapid phase of resensitization, the operating curve of the cones shifts along the intensity axis to new positions. In order to determine the initial shifting kinetics of the mass receptor response operating curve, produced by a 2-log unit decrease in the background level, the following experiment, illustrated in Fig. 10 A was performed. A 6.0-log unit background intensity was presented to the retina and an operating curve was generated from the responses elicited by test flashes delivered around this background. This operating curve is drawn through the filled circles of Fig. 10 C. After the retina had equilibrated to the 6.0-log unit background, the intensity of the background was then changed routinely to 4.0 log units and held there for 2 s at which time the test flash was substituted for this new 4.0-log unit background. The magnitude of the response to this test flash was recorded as in the example of Fig. 10 B. When this protocol was repeated for a variety of test flash intensities, we plotted a complete operating curve which characterized the operating range of the receptors after a 2-s decrease in the background intensity from 6.0 log units to 4.0 log units. This curve is shown in Fig. 10 C as the second curve from the left (labeled 2). This experimental protocol was then repeated for time intervals of 1 and 4 s at the 4.0-log unit background and the operating curves generated at these times of dark adaptation are shown in Fig. 10 C
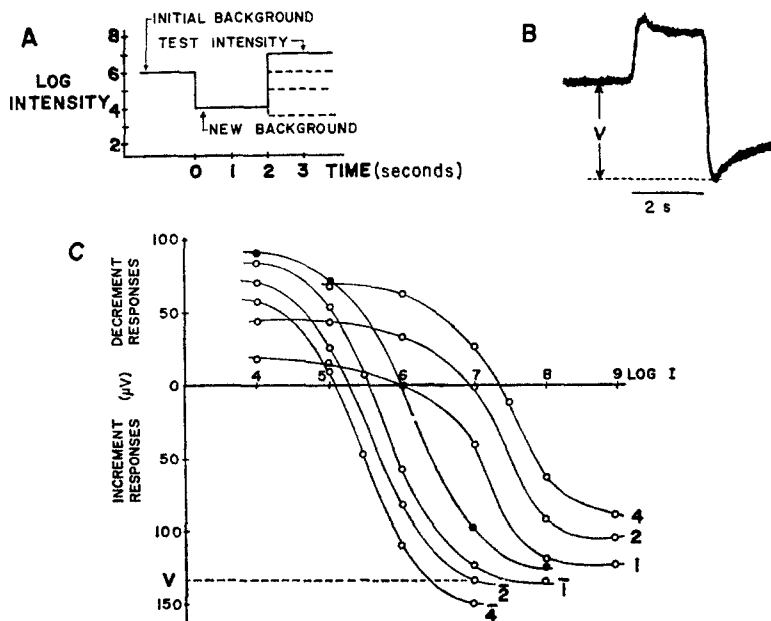
FIGURE 10. Initial kinetics of curve shifting after a 2-log unit increase or decrease in background illumination. (A) Experimental protocol used in measurement showing the initial background (6.0 log units), followed by the presentation of the new background (4.0 log units), and then the presentation of the test flash (7.0 log units) which generated the response shown in B. (B) The behavior of the mass receptor response to the sequence of events in A. V is the magnitude of the response to a 7.0-log unit tes-flash after the change in background from 6.0 to 4.0 log units. (C) The family of operating curves measured at various times after the change in background level. Filled circles show the operating curve at the initial background level. The curves labeled 1, 2, and 4 are the operating curves measured at 1, 2, and 4 s after 2-log unit increase in background; $\bar{1}$, $\bar{2}$, and $\bar{4}$ are the operating curves at 1, 2, and 4 s following a 2-log unit decrease in background level.

as the curves labeled $\bar{1}$ and $\bar{4}$, respectively. A similar protocol was used to determine the initial shifting of the operating curves following a 2-log unit increase in the background intensity and the operating curves generated at times 1, 2, and 4 s after the increase in background level from 6.0 and 8.0 log units are also illustrated in Fig. 10 C.

Fig. 10 C shows that the initial phases of dark adaptation proceeded similarly to the later stages of dark adaptation; the magnitudes of the operating curves did not change but the curves simply shifted along the log-test intensity axis. The kinetics of the initial phase of light adaptation were somewhat faster and the curves behaved slightly differently. Within 1 s of the increase in background, the magnitude of the operating curve was reduced slightly and, as light adaptation proceeded, the curve both grew in magnitude and shifted along the log-test intensity axis. Similar kinetics of curve shifting were obtained from S-potential recordings in turtle by Byzov and Kusnezova (1971).

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