Adaptation in Skate Photoreceptors

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ABSTRACT Receptor potentials were recorded extracellularly from the all-rod retina of the skate after the application of sodium aspartate. This agent suppresses the responses of proximal elements, but leaves relatively unaffected the electrical activity of the photoreceptors (*a*-wave) and pigment epithelium (*c*-wave). Since the latter develops too slowly to interfere with the receptor response, it was possible to isolate receptor potentials and to compare their behavior in light and dark adaptation with earlier observations on the S-potential, *b*-wave, and ganglion cell discharge. The results show that the photoreceptors display the full complement of adaptational changes exhibited by cells proximal to the receptors. Thus, it appears that visual adaptation in the skate is governed primarily by the photoreceptors themselves. Of particular interest was the recovery of sensitivity in the presence of background fields that initially saturate the receptor potential. Analysis of this recovery phase indicates that a gain-control mechanism operates within the receptors, at a distal stage of the visual process.

INTRODUCTION

Although the mechanisms subserving light and dark adaptation in the vertebrate visual system are not yet well understood, their control seems to depend largely upon events occurring at the initial stage of the sensory process. This was clearly implied in the experiments of Dowling (1960, 1963) and Rushton (1961, 1965) who showed that after exposing the eye to very intense (bleaching) lights, the slow return of sensitivity during dark adaptation was quantitatively related to the concentration of photosensitive pigments in the receptors. More recently, Frank (1971) has demonstrated that the isolated P-III response of the frog electroretinogram (ERG) shows the early, rapid phase of dark adaptation (which occurs independent of changes in visual pigment concentration), suggesting that this component of dark adaptation is also a property of the photoreceptors.

The importance of receptor processes in adaptation to background illumination (light adaptation) appears also to be firmly established. For example, Boynton and Whitten (1970) have shown for cone receptor potentials in the monkey retina that the incremental intensity required to generate a threshold

response increases with background intensity (I_B) along a curve that parallels the increment threshold function measured psychophysically in the human fovea. They further demonstrated that the curves describing response amplitude as a function of log intensity (V-log I) are shifted to the right on the scale of intensities as a result of a steady background light; the magnitude of the displacement provides a measure of the threshold change due to light adaptation. They proposed that I_B causes a maintained receptor potential and suggested that this steady voltage accounts for the loss in gain that accompanies exposure to I_B. That is, I_B pushes the receptor towards its maximal (saturating) response, thereby *compressing* the dynamic range over which incremental responses can be generated. The recent intracellular recordings by Werblin (1971) suggest that a similar mechanism may operate in mud puppy cones.

One difficulty with this idea is that it predicts that photoreceptors cease to function when I_B is of saturating intensity, but primate cones continue to function at levels of I_B far above those required to saturate the receptor potential. Since saturation of the cone response requires intense (bleaching) lights, Boynton and Whitten suggested that when I_B bleaches a large fraction of visual pigment, the rate of quantal absorption is reduced. This lowers the amplitude of the potential generated by I_B to below saturation, thereby extending the dynamic range of the receptor response (cf. Alpern et al., 1970). However, such a mechanism would not seem to be applicable to vertebrate rods, for their receptor potentials reach saturation voltage well before significant amounts of pigment are bleached (Hagins et al., 1970). Indeed, it has been shown that the rod system in many rod-cone retinas saturates at background luminances that bleach less than 1% of the available rhodopsin (Aguilar and Stiles, 1954; Daw and Pearlman, 1969). On the other hand, Dowling and Ripps (1970, 1971 *a*) found that the increment thresholds for S-potentials, *b*-wave, and ganglion cell responses in the *all-rod* retina of the skate can be measured upon all background intensitites, up to 7 log units above threshold, suggesting that other mechanisms must exist to prevent saturation of skate photoreceptors.

To study this problem we have recorded trans-retinal potentials after isolating the photoreceptor response with sodium aspartate (Sillman, Ito, and Tomita, 1969). The present paper describes the action of Na aspartate on the ERG and horizontal-cell potentials, the adaptation properties of skate photoreceptors, and the relation between receptor activity and that of more proximal neurons in the skate retina.

MATERIALS AND METHODS

Small pieces of eyecup ($\sim$1 cm square), excised under dim red light from the tapetal region of the dark-adapted skate (either *Raja erinacea* or *R. oscellata*), were prepared and maintained as described previously (Dowling and Ripps, 1970). After most of the

vitreous had been teased away, the tissue was immersed for 3 min in oxygenated elasmobranch Ringer in which 50–100 mM L-Na aspartate was substituted for an equivalent amount of NaCl. Various concentrations (10–200 mM) of aspartate were tested; all gave similar results, except that with the lower concentrations some return of the *b*-wave response was usually observed during the course of an experiment. After removal from the solution, the eyecup was drained of excess fluid, and placed on a moist piece of filter paper in contact with a chlorided silver wire that served as the reference electrode.

The electroretinogram was recorded from the vitreal surface of the retina with a Ringer-filled pipette, drawn by hand and flame-polished to a tip diameter of 50–100 μm. A second chlorided silver wire connected the pipette to the input stage of a differential preamplifier (Princeton Applied Research Corp., Princeton, N. J.) which was used either directly coupled or with a time constant of 5.5 sec. Responses were displayed on an oscilloscope and recorded on a Brush penwriter (Gould, Inc., Cleveland, Ohio). For intracellular recording of S-potentials, glass micropipettes were pulled to give input resistances of 35–75 megohms when filled with 2 M KCl. The pipette, mounted on a hydraulic microdrive, was connected to the input stage of a unity gain, negative capacitance amplifier. The signals were amplified further and displayed on another channel of the Brush recorder.

Photic stimuli were delivered by a dual-beam optical system (Dowling and Ripps, 1971 *a*) that provided independent control of intensity, duration, and spectral composition of test and adapting fields. These were focused in the plane of the retina and the flux density measured with a calibrated thermopile and microammeter. The light sources were 12 v 100 w tungsten-halogen lamps operated at a color temperature of 2750°K. The beams passed through BG38 and KG 1 heat filters which strongly transmitted wavelengths below 560 nm and virtually eliminated wavelengths >700 nm. Both test and adapting fields were slightly larger than the piece of eyecup, and evenly illuminated the retinal surface.

Experiments usually lasted 2–4 hr. Control preparations showed that no significant alterations in sensitivity or amplitude of the receptor potential occurred during this period. Throughout the paper thresholds refer to the intensity required to elicit a just detectable voltage (3–5 μv). Maximum amplitude responses, on the other hand, were evoked with flashes at least 1 log unit more intense than those needed to produce a saturating voltage at any given level of adaptation. The intervals between test flashes varied with the experimental protocol, but were kept sufficiently long so as not to influence subsequent responses.

RESULTS

The Action of Na Aspartate

Fig. 1 shows electroretinographic records from the eyecup and isolated retina of the skate before and after the application of aspartate-Ringer's solution; the responses were elicited by 200 msec flashes of "white" light at two stimulus intensities. In the normal eyecup preparation (Fig. 1, upper traces) there are present the three principal ERG components of the rod-dominated vertebrate

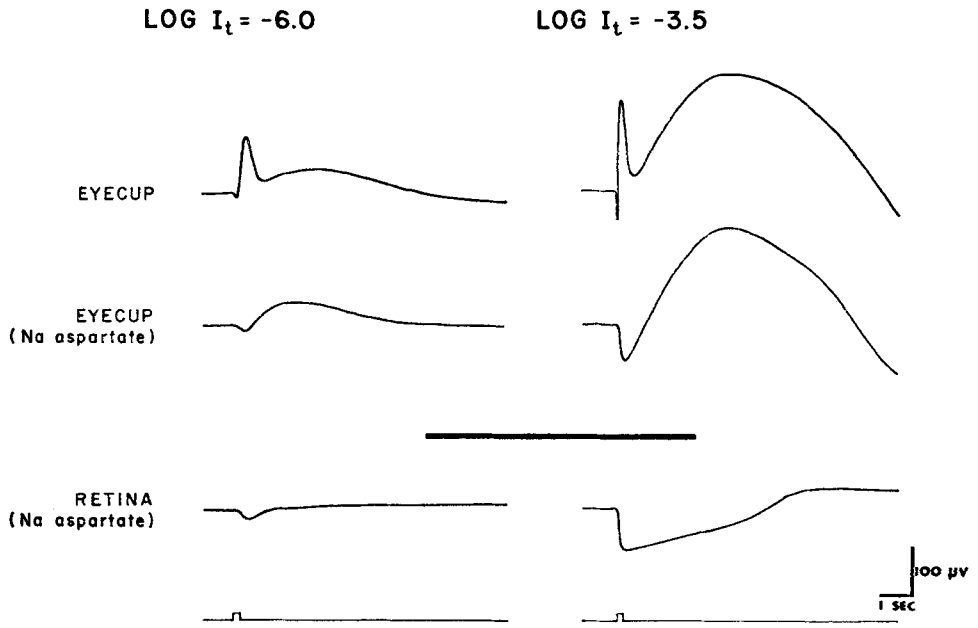

FIGURE 1. The effects of sodium aspartate on electroretinographic responses from the eyecup and isolated retina of the skate. Responses were elicited by 0.2 sec stimuli at two intensities (log I_t = −6.0 and −3.5). The upper traces were recorded from the untreated eyecup preparation, and show the *a*-, *b*- and *c*- waves of the normal ERG. After immersion for 3 min in Ringer's containing 50 mM L-Na aspartate (middle records), the *b*-wave was suppressed, but *a*- and *c*-waves were essentially unaltered. Removing the aspartate-treated retina from the eyecup eliminated the *c*-wave. Unless indicated otherwise, log I_t values give the filter density attenuating the test beam (log $I_t \sim -D$), where log I_t = 0 corresponds to 1.1 mw cm^{-2}.

retina (Granit, 1955): (*a*) an initial negative deflection (*a*-wave) that arises primarily from the photoreceptors (Brown and Watanabe, 1962 *a*; Penn and Hagins, 1969), but possibly containing a contribution from more proximal elements (Murakami and Kaneko, 1966); (*b*) a relatively fast positive transient (*b*-wave) generated probably by the Müller (glial) cells as a consequence of the discharge of neurons in the inner nuclear layer (Faber, 1969; Miller and Dowling, 1970); and (*c*) a slow positive potential (*c*-wave) originating in the pigment epithelial cells (Noell, 1954; Steinberg et al., 1970), although its response properties depend largely on the activity of the photoreceptors (Schmidt and Steinberg, 1971). All components of the ERG varied with intensity, but the *c*-wave continued to increase at intensities above those which evoked maximum amplitude *a*- and *b*-waves (compare *a*- and *c*-waves of Fig. 8). This was observed also by Schmidt and Steinberg (1971) who suggest that the pigment epithelial cells are capable of integrating the prolonged rod afterpotential elicited by brief, intense stimuli (cf. Brown and Watanabe,

1962 *b*). The *c*-wave is prominent also in the electroretinogram of the dogfish, *Mustelus canis* (Ripps and Siegel, unpublished observations), although it has not been detected in recordings from three other species of elasmobranch (Hamasaki et al., 1967).

After immersion of the eyecup in Na aspartate (Fig. 1, middle traces), the *b*-wave was lost, but *a*- and *c*-waves remained relatively intact. However, *a*-wave amplitude was usually larger after aspartate, owing primarily to the absence of the *b*-wave which, when present, interrupted the *a*-wave before it reached peak amplitude. When the aspartate-treated retina was separated from the pigment epithelium and removed from the eyecup (Fig. 1, lower traces), the *c*-wave was eliminated and only the vitreous-negative *a*-wave was registered. There is evidence that the initial portion of this response is derived from the photoreceptors (Sillman et al., 1969; Penn and Hagins, 1969), whereas a slower wave of negativity that follows the receptor potential (see Fig. 5 of Sillman et al., 1969), may be of glial origin (Faber, 1969; Arden and Ernst, 1972; Witkovsky, personal communication). In the eyecup, the *c*-wave obscured this slow negative potential, but it appeared to have no discernible effect on either threshold or amplitude of the initial negative deflection. For example, no *c*-wave is observed with near-threshold stimuli in both light- and dark-adapted retinas (Fig. 8), and careful examination of the responses to long duration test flashes showed that the *c*-wave does not begin until the receptor potential has begun to decay. Furthermore, the voltage-intensity relation for the receptor potential is identical in the eyecup and isolated retina preparation (Compare Figs. 3 and 4). Thus, equivalent receptor potentials were obtained after aspartate treatment in both the eyecup and isolated retina. We used the eyecup preparation in most experiments, since it was subjected to less trauma and remained viable for longer periods than the nonperfused isolated retina.

It should be noted that Na aspartate was not entirely without effect on the receptor potentials. Control experiments, in which the leading edge of the *a*-wave was recorded at fast sweep speeds, showed a small but significant reduction in the slope (i.e. rise-time) of the response as compared with that obtained before aspartate; response latency, on the other hand, appeared unaltered by Na aspartate. Nevertheless, the rapid and complete elimination of the *b*-wave with retention of the receptor potential, suggests that aspartate exerts its effects primarily on cells proximal to the receptors. Electron microscopic examination of the retina after immersion of the eyecup in aspartate Ringer revealed no alterations in fine structure of the receptors, although swelling of processes in the inner plexiform layer was obvious in preparations fixed 30 to 90 min after aspartate treatment (cf. Wald and De Robertis, 1961).

The view that aspartate affects primarily cells postsynaptic to the receptors is supported also by the reaction of the horizontal cells to local application of

Na- aspartate (Dowling and Ripps, 1971 *b*; Cervetto and MacNichol, 1971). In the experiment illustrated in Fig. 2, a horizontal cell was penetrated with a micropipette, and after satisfactory control responses (S-potentials) were obtained, a small amount of aspartate-Ringer's solution was pressure-injected onto the surface of the retina. After a delay of 20–30 sec, due presumably to the diffusion time for aspartate to reach the recording site, the horizontal cell rapidly depolarized. The depolarization was accompanied at first by a significant increase in the amplitude of the light-evoked responses. As the cell depolarized further, the S-potentials gradually diminished, and, after the membrane potential had stabilized, continued to decrease until all light-evoked activity had vanished. When maximally depolarized in the presence of aspartate, the horizontal cell maintained a small resting potential, for upon withdrawing the electrode, there was a positive shift in potential of 5–10 mv.

In a few experiments, S-potentials and the ERG were recorded simul-

FIGURE 2. The effect of sodium aspartate on the intracellularly recorded S-potential of the skate. Stimulus flashes of constant intensity (not indicated) were given at 10- to 20-sec intervals before and after applying a drop of aspartate-Ringer to the retinal surface (arrow). See text for details.

taneously during the application of aspartate. The loss in amplitude of the *b*-wave and the S-potential followed very nearly the same time-course, although a small *b*-wave usually persisted for a short time after the S-potential was no longer detectable. It would clearly be of interest to know how the bipolar cells as well as other retinal neurons are affected by the sodium salt of aspartic acid, and whether this or other short-chain amino acids are of functional significance in the retina (cf. Kishida and Naka, 1967).

Receptor Potentials in the Dark-Adapted Retina

Experiments on a variety of vertebrate species (Naka and Rushton, 1966; Baylor and Fuortes, 1970; Boynton and Whitten, 1970) have consistently shown that the amplitude-intensity relation for photoreceptor and S-unit responses is adequately described by the expression:

$$V/V_{max} = I^a/(I^a + k), \tag{1}$$

where V is the potential elicited by a retinal irradiance I; V_{max} is the saturation

voltage; and a and k determine, respectively, the slope of the function and its position on the abscissa.

Fig. 3 shows results for the isolated receptor potential of the skate, together with the graph of equation 1 plotted on semi-logarithmic coordinates. The data points are the averaged values from nine experimental runs, and best fit the curve of equation 1 for $a = 1$. The V-log I functions obtained by Naka and Rushton (1966) for tench S-potentials and by Baylor and Fuortes (1970) with intracellular recordings from turtle cones are virtually identical in form with those shown here. Skate horizontal cells, on the other hand, respond to a slightly broader range of intensities; i.e. $a = 0.7$ (Dowling and Ripps, 1971 *a*).

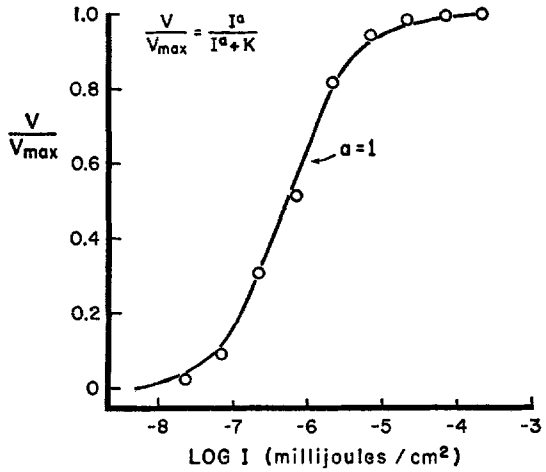

FIGURE 3. The intensity-response relation for the mass receptor potential of the skate eyecup. Stimulus duration = 0.2 sec. Data points are the averaged results of nine experimental runs on as many different preparations.

However, it is noteworthy that skate receptor potentials, as well as S-potentials, saturate at intensities more than 3 log units below those which bleach a significant fraction of the available rhodopsin (compare Fig. 3 of Dowling and Ripps, 1971 *a*).

In order to obtain a more precise estimate of quantal absorption, the voltage-intensity relation was measured with relatively monochromatic stimuli (λ_{max} = 500 nm, half-band width ±3 nm) on the isolated retina. Removing the retina eliminates from consideration the reflectivity of the silvery tapetum, although it should be mentioned that isolated retina and eyecup preparations gave similar results. In Fig. 4, the open symbols plot log voltage as a function of log intensity, and illustrate the linear relation between receptor potentials and stimulus intensity that holds over the first 1.5 log units of effective energies. Beyond this, the curve flattens as saturation is rapidly approached. If we arbitrarily select for absolute threshold a potential of 1

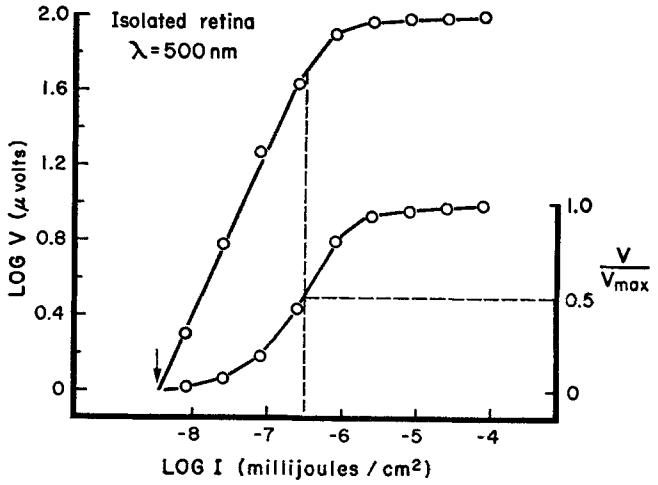

FIGURE 4. The voltage-intensity relation obtained from the isolated retina with monochromatic stimuli. Plotting amplitudes as a fraction of the saturation voltage (right-hand scale of ordinates) yields a function like that shown in Fig. 2. The dashed line is drawn to the curve from $V/V_{max} = 0.5$, and its perpendicular intercepts the abscissa at log $I_t = -6.5$. The log-log plot (left-hand ordinates) has a slope of unity, i.e. $V \sim I$, over the initial portion of the dynamic range. The arrow indicates the energy density at which log $V = 0$. Details in text.

μv; i.e. log $V = 0$, then, by extrapolation, a criterion response is elicited when the energy density E_t delivered to the retina in a 0.2 flash is 3.66×10^{-9} millijoules cm^{-2}.

Since 1 mJ $= 10^4$ erg, $E_t = 3.66 \times 10^{-5}$ erg·cm^{-2}, and the quantum content Q_t at threshold irradiance is obtained from the relation:

$$\epsilon_\lambda = \frac{hc}{\lambda},$$

where ϵ is the energy per quantum of monochromatic light of wavelength λ, c is the speed of light, and h is Planck's universal constant of 6.624×10^{-27} erg·sec. Thus, for $\lambda = 5 \times 10^{-5}$ cm,

$$\epsilon_{500} = 3.97 \times 10^{-12} \text{ erg and } Q_t = 9.2 \times 10^{-2}\, q/\mu^2.$$

The mean diameter of skate rods, taken from light and electron microscopic observations is approximately 3.8 μ; i.e. the cross-sectional area $= 11.3\ \mu^2$. Hence, Q_t corresponds to approximately 1 quantum incident per rod in a threshold flash lasting 0.2 sec. However, transmissivity measurements on the isolated retina, corrected for the number of receptors per unit area (Ripps and Weale, 1965), indicate that the *in situ* density ($\lambda = 500$ nm) of rhodopsin is about 0.18, i.e. only 33% of the incident quanta are absorbed. Thus, assuming

a quantum efficiency of one, a 1 μv response would be elicited when on average one out of every three rods absorbs a single quantum of light.

Also shown in Fig. 4 are the same data fitted to the curve of equation 1 and plotted on V-log I coordinates. Since $a = 1$, the value of k is given by the intensity required to produce a half-saturating voltage. Now $V/V_{max} = 0.5$ when $I = 3.26 \times 10^{-7}$ mJ/cm², and a computation similar to that given above yields a quantal absorption of 30 quanta per rod, which compares favorably with the value of 27 quanta/rod reported by Hagins et al. (1970) to evoke a half-saturating voltage in rat rods.

Receptor Adaptation

Earlier observations on the adaptation process in skate horizontal cells led us to suggest that the photoreceptors probably undergo large changes in sensitivity during light and dark adaptation (Dowling and Ripps, 1971 *a*). The experimental results of the following sections not only confirm our expectations, but indicate that the changes are in each instance similar to those occurring in more proximal elements.

THE INCREMENT THRESHOLD Fig. 5 is a plot of log increment threshold (ΔI), the intensity required to elicit a 3–5 μv potential, against log background intensity (I_B). The relation is linear with a slope of 1, the same as reported previously for incremental threshold measurements of S-potentials, *b*-wave, and ganglion-cell responses in skate (Dowling and Ripps, 1970, 1971 *b*). Although it is not immediately apparent in the graph of Fig. 5, ΔI is a time-dependent variable. After the onset of a bright adapting field, the threshold changed markedly with time, and in some cases 20–30 min elapsed before it reached a stable (minimum) value (cf. Fig. 9). Furthermore, the background itself evokes a receptor potential which, *initially*, is related to I_B according to the dashed-line curve of Fig. 5. But the increment threshold was measurable upon adapting fields that were 3–4 log units brighter than the saturating I_B (arrow); i.e., into the intensity range where large fractions (>90%) of the visual pigment are bleached. Clearly, then, the potential due to I_B must also be time-variant, since incremental responses can not (by definition) be elicited in the presence of a maintained saturating voltage. The temporal course of light adaptation will be considered in the following section; here it is important to note that the increment thresholds plotted in Fig. 5 represent the values obtained after the receptors had adapted fully to I_B.

The increment threshold is also measurable over an extensive range of background intensities in extra-foveal regions of the human retina (Stiles, 1939, 1959) as well as in other vertebrates with mixed retinas (Dodt and Echte, 1961; Daw and Pearlman, 1969). However, in these cases the function is comprised essentially of two segments: a low intensity branch subserved by

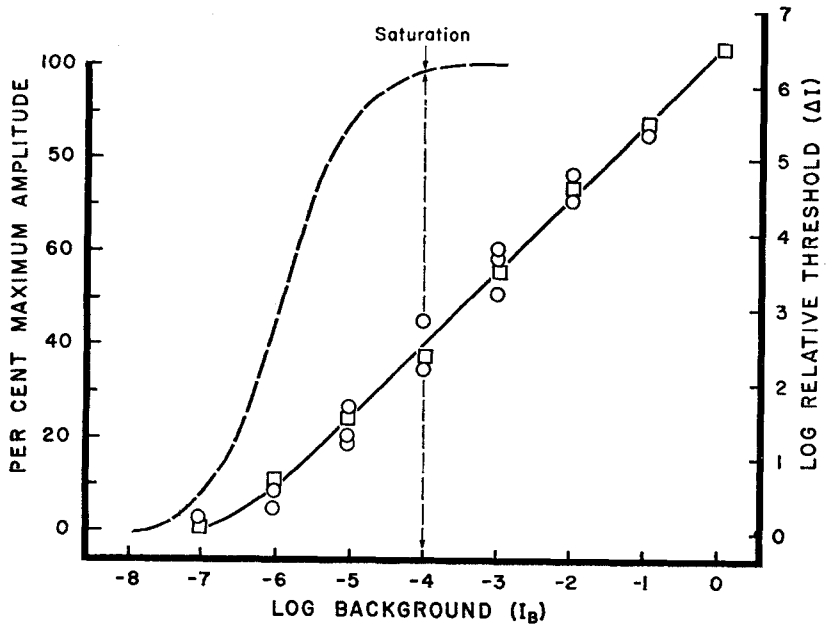

FIGURE 5. The increment threshold function (solid line) for the isolated receptor potential in skate. Thresholds (ΔI) are plotted relative to dark-adapted values. The dashed-line curve and left-hand scale of ordinates give the voltage-intensity relation produced by the onset of I_B. Thus, all increment threshold data for levels of $I_B \geq -4.0$ (arrow) were obtained on backgrounds which initially saturated the receptor potential. Here and in subsequent figures, log $I_B = 0$ corresponds to a retinal irradiance of 1.66 mw/cm^2. Square symbols are the results from a single experimental run in which the background was raised sequentially in 1 log unit steps over an intensity range of 7 log units. Circles are the results from separate experiments in which a dark-adapted retina was exposed to a given background intensity.

rods, and a cone portion that comes into play when the retinal illuminance due to I_B exceeds approximately 300 scotopic trolands, the intensity at which the rod mechanism begins to "saturate" (Aguilar and Stiles, 1954). The transition from scotopic to photopic function is not always marked by a discontinuity in the increment threshold curve, but the different mechanisms can often be identified by a change in spectral sensitivity as between the dark- and light-adapted retina (Dodt and Echte, 1961; Green, 1971).

Earlier findings, both histological and electrophysiological, suggest that skate photoreceptors are of one type, namely rods (Dowling and Ripps, 1970, 1971 *a*), and the V-log I_B curve of Fig. 5 shows that the response saturates at about 3 log units above threshold. Since the increment threshold curve of Fig. 5 continues far above saturating intensity, we have tried in two ways to determine whether there is any sign of a spectral shift when I_B exceeds the saturation level. In the first experiment, spectral sensitivity measurements on

the dark-adapted retina were compared with those obtained in the presence of a background irradiance of 0.01 mw/cm², approximately 1.5 log units above saturation. The results from one preparation (Fig. 6) show that whereas light adaptation reduced sensitivity almost 10,000-fold, the spectral functions were identical, and in good agreement with the density spectrum (λ_{max} = 500 nm) of skate rhodopsin (Dowling and Ripps, 1970).

The results of a second experiment demonstrate even more convincingly that a single mechanism mediates sensitivity in both the dark-adapted state

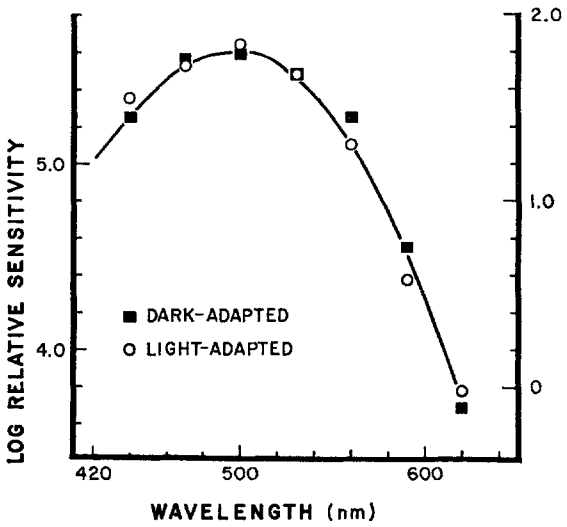

FIGURE 6. Spectral sensitivity of the receptor response in the dark-adapted retina (solid symbols, ordinates on the left) and after light adaptation to log I_B = −2 (open symbols, ordinates on right). Light adaptation decreased sensitivity by almost 4 log units, but produced no lateral displacement of the spectral function (Purkinje shift). The curve is derived from the Dartnall nomogram for a vitamin A_1 pigment λ = 500 nm.

and under conditions of strong light adaptation. The data represented by squares in Fig. 5 were obtained from a particularly hardy preparation in which it was possible to test increment thresholds as the background was raised sequentially in 1 log unit steps over an intensity range spanning 7 log units. When measurements upon the brightest background were completed, the retina had already been subjected to more than 2 hr of continuous light adaptation. I_B was then extinguished, and thresholds were determined during dark adaptation with white light and with two monochromatic test fields (λ = 470 nm and λ = 560 nm). Although dark adaptation was relatively slow (cf. Dowling and Ripps, 1970) after this exhaustive bleaching exposure (at least 99% of the rhodopsin had undergone photolysis), Fig. 7 shows that (*a*) the three dark adaptation curves followed exactly the same time-course, (*b*) during dark adaptation the data for each of the test fields retained the

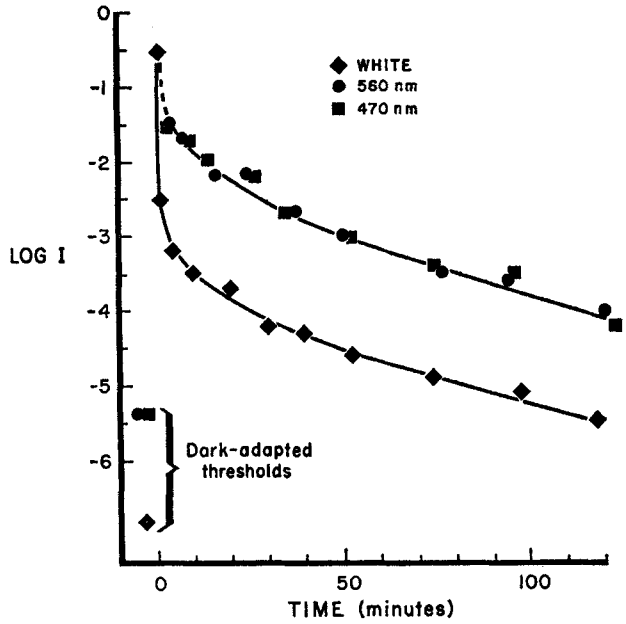

FIGURE 7. The course of dark-adaptation after exhaustive bleaching. Thresholds were monitored with white and monochromatic (λ = 470 nm and 560 nm) test flashes. The three sets of data follow the same time-course and retain throughout the same relative sensitivities as measured initially in the dark-adapted retina.

same relative position on the scale of ordinates as in the dark-adapted preparation, and (*c*) none of the curves showed a cone-rod kink in the course of dark adaptation.

These experimental findings are clearly consistent with the view that the receptor potentials from the light- and dark-adapted skate retina arise from one type of photoreceptor, subserved by a single visual pigment. Moreover, skate rods do not saturate permanently with intense background illumination, but continue to respond even after losing nearly all of their rhodopsin.

THE KINETICS OF LIGHT AND DARK ADAPTATION We mentioned earlier, and Fig. 8 illustrates, that the saturating effect of a bright steady background is not maintained indefinitely. In the upper left corner of the figure are control records from the dark-adapted retina showing the responses to 200 msec test flashes that elicit threshold, nearly maximal, and maximum amplitude receptor potentials (V_{max}). Note that as the receptor potential neared its full excursion (at log I = -5), raising the intensity by 2 log units produced only a slightly larger voltage, while the *c*-wave more than doubled in amplitude. In subsequent recordings, test flashes were delivered at the times indicated after the onset of a background field (log I_B = -3) that saturated the receptors. Thus, after more than 7 min of light adaptation, responses could not be

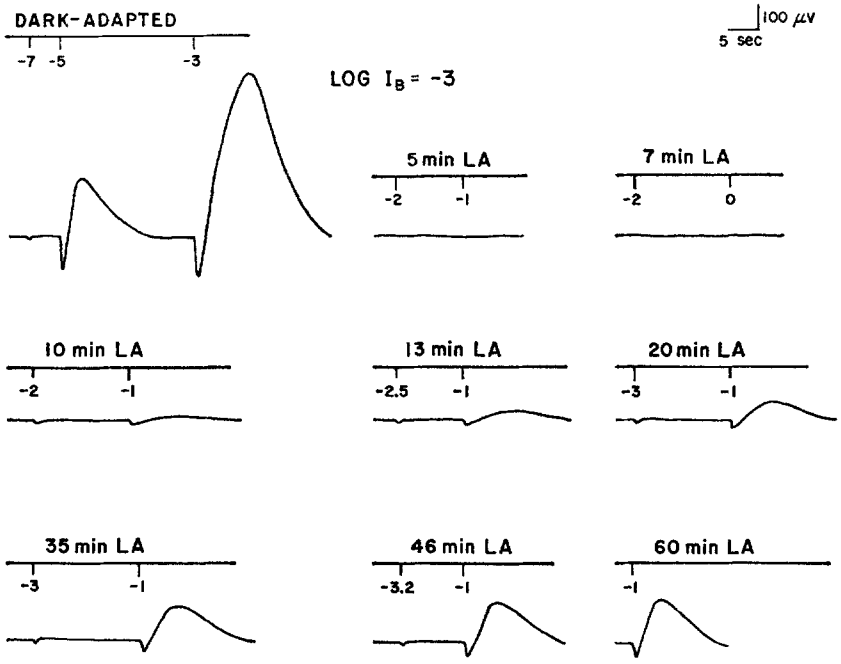

FIGURE 8. The effects of light adaptation (log $I_B = -3$) on threshold and maximum amplitude responses in the aspartate-treated eyecup. Dark-adapted responses to threshold (log $I_t = -7$) and supra-threshold stimuli (log $I_t = -5$ and -3) are shown in the upper left-hand corner; receptor potentials (*a*-waves) are followed by large positive deflections (*c*-waves). Note the "silent period" during the first 7 min of light adaptation. Subsequent records show the decline in threshold and the growth in maximum response amplitude, although the latter appears to continue after threshold has almost stabilized (compare records at 20, 35, and 46 min).

elicited with stimulus intensities 7 log units above dark-adapted threshold. But as exposure to I_B continued, the transient nature of saturation became apparent. After 10 min, for example, receptor potentials were obtained in response to very bright incremental stimuli (threshold ≈ -2), and at the 20 min mark, threshold had fallen to log $I \approx -3$, which was approximately the level it maintained throughout the remainder of the experiment. On the other hand, the maximum receptor potential, which after 10 min of light adaptation barely exceeded threshold amplitude, continued to increase over the next 25–30 min.

Essentially the same procedure was used to determine changes in threshold and V_{max} during adaptation to various levels of I_B, and in the course of dark adaptation after the background was extinguished. Threshold data at three background intensities are shown in Fig. 9. With the weaker fields (log $I_B = -5, -4$), measurements could be made almost immediately after the start of light adaptation, since neither background produced a fully

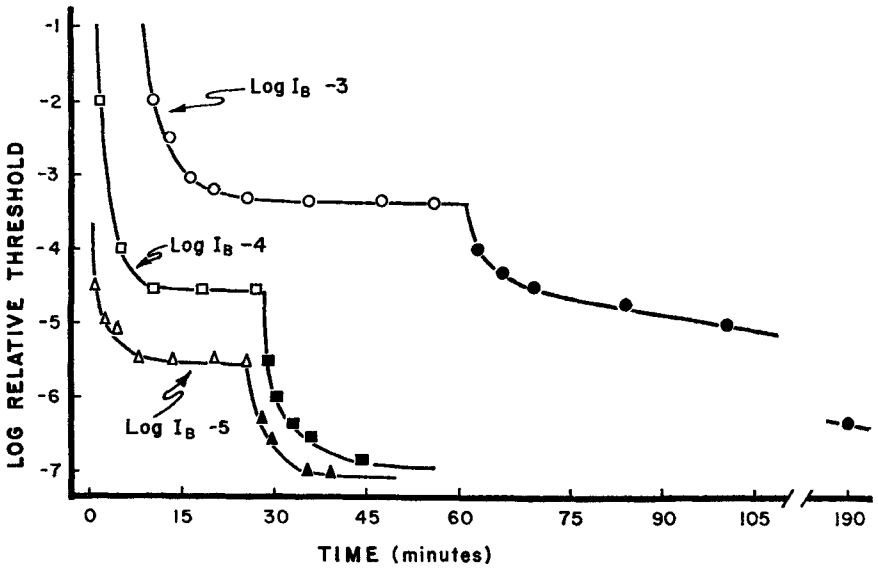

FIGURE 9. Thresholds during light- (open symbols) and dark-adaptation (filled symbols) for three different background intensities. With the dimmer adapting lights (log I_B = −5 and −4), increment thresholds were high initially, but fell rapidly to a steady level, 1.5–2.5 log units above absolute threshold. With the brighter adapting field, there was an initial period during which no responses could be elicited, but when excitability returned, the light-adapted threshold was quickly established. When I_B was extinguished, dark adaptation was rapid if the exposure had been to a dim background, slow after exposure to bright (bleaching) backgrounds.

saturating receptor potential (cf. Fig. 5). Although the instantaneous effect of I_B was to raise receptor thresholds more than 3 log units above the dark-adapted value, thresholds fell sharply within the first 10 min to a plateau that was maintained until I_B was extinguished.

With brighter adapting fields, the background illumination caused the receptor potential to saturate, and consequently, there was always an initial period during which time incremental responses could not be elicited. In the example shown in Fig. 9 (log I_B = −3), the saturating effect continued for 10 min, but still brighter backgrounds completely suppressed responses for almost 15 min (cf. Fig. 11). However, regardless of its intensity, the steady background does not clamp the receptor at V_{max} ; incremental responses eventually appear and thresholds become measurable (cf. Fig. 8). Once this occurs, thresholds again fall rapidly to a stable (but higher) level, where they remain for the duration of light adaptation. When the background lights were extinguished, thresholds returned to the dark-adapted level (Fig. 9, filled symbols). The return was rapid ("neural" adaptation) if the prior exposure bleached only a trivial amount of visual pigment, or slow ("photochemical" adaptation) if a significant fraction of the pigment had been bleached. *It is*

noteworthy that every feature of the light and dark adaptation process described above, was seen previously in the responses of proximal neurons in the skate retina (Dowling and Ripps, 1970, 1971 a).

Concomitant with the changes in increment threshold was a continuous increase in V_{max} during exposure to the ambient illumination. Figs. 10 and 11 show the time-course of this phenomenon at several background intensities. Since a fresh preparation was used for each experiment, the filled symbol to

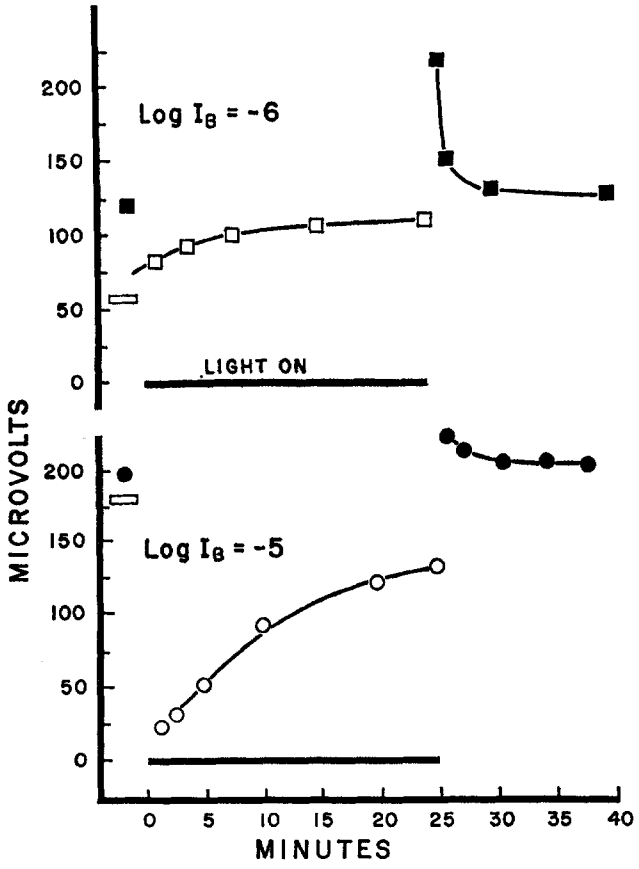

FIGURE 10. Temporal changes in the maximum amplitude of the receptor potentia during and after exposure to relatively weak adapting fields (log I_B = −6 and −5). The background was turned on at t = 0 and remained on for about 25 min during which time intense test flashes were delivered at widely spaced intervals. The filled symbol to the left of time zero is the maximum response obtained in the dark (i.e. the saturation potential), and the clear bar indicates the potential elicited by the onset of I_B. During light adaptation, maximum amplitude increased, approaching asymptotically a plateau (V'_{max}). On extinguishing the light, there was usually a transient period of hyperexcitability, during which time the potentials exceeded the dark-adapted maximum. Within a few minutes, however, the responses were similar to those of the dark-adapted preparation.

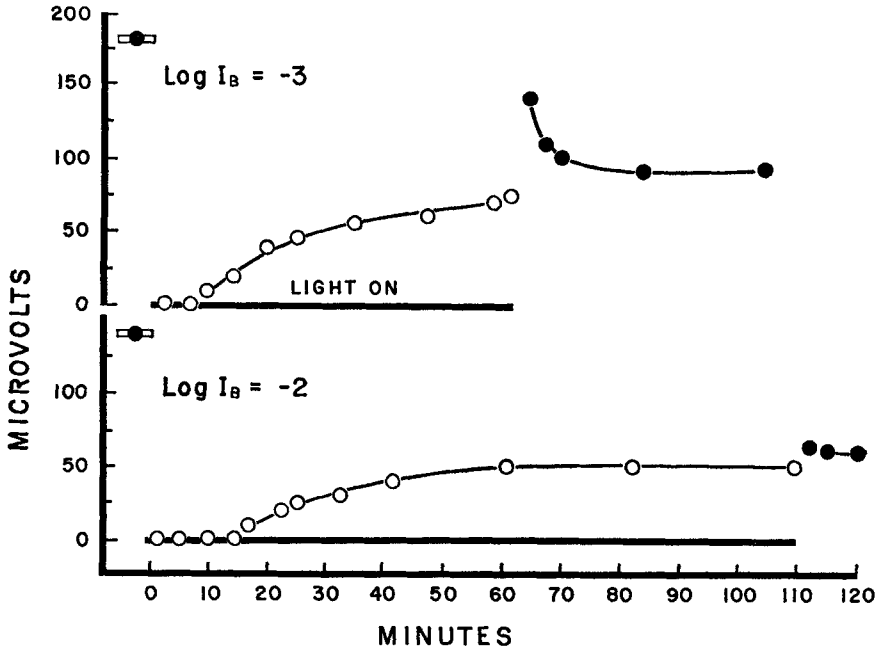

FIGURE 11. The effects of bright (bleaching) backgrounds on the maximum amplitude of the receptor potential. Details same as in Fig. 10. Note that the onset of I_B saturates the receptor potential (rectangle) and induces a silent period lasting 8 min (log I_B = −3) to 15 min (log I_B = −2). However, with further time in the light, excitability returned and the potentials grew in amplitude. At the beginning of dark adaptation, there was again a transient increase in maximum amplitude, but within a few minutes the responses were similar in magnitude to those recorded when the light was on.

the left of zero denotes the maximum response (saturation potential) recorded from the dark-adapted retina. When the background field was turned on, V_{max} was immediately reduced by an amount which clearly depended upon I_B. In fact, the initial response amplitude was simply the algebraic difference between V_{max} of the dark-adapted retina and the response generated by I_B (horizontal rectangle). Thus, if the latter evoked a saturating potential (Fig. 11), the retina was completely unresponsive to incremental stimuli no matter how intense. But in every instance, the receptors *adapted* to I_B, and the amplitude of V_{max} increased, in some cases throughout a 60 min period of light adaptation.

The filled symbols of Figs. 10 and 11 show the changes in V_{max} after extinguishing I_B. If the prior exposure had not significantly affected the concentration of visual pigment (Fig. 10), there was often a brief period during which V_{max} exceeded that of the dark-adapted preparation, but the responses quickly settled down to a constant voltage, approximately the same as at the start of the experiment. However, after a prolonged period of intense light

adaptation, considerably smaller potentials were obtained in the early stages of dark adaptation (Fig. 11).

DISCUSSION

The experimental results show that the isolated receptor potentials in skate display the full complement of adaptational changes that have been observed previously in the responses of more proximal elements of this all-rod retina (Dowling and Ripps, 1970, 1971 *a*). These parallels, which include the increment threshold functions, action spectra, and the temporal changes in sensitivity and response amplitude during light and dark adaptation, strongly support the view that visual adaptation in the skate is governed to a considerable extent by the photoreceptors themselves.

In the earlier papers (Dowling and Ripps, 1970, 1971 *a*), we reported that the ganglion cell discharge, ERG *b*-wave, and S-potential all showed the surprising capacity to respond to incremental stimuli flashed upon bright, steady background fields—even when the intensity of the latter was such as to bleach large fractions of the visual pigment. These findings were particularly difficult to reconcile with the performance of the rod system in vertebrates with mixed retinas, where rod saturation occurs when less than 1% of the available rhodopsin is bleached (Aguilar and Stiles, 1954; Daw and Pearlman, 1969). In the skate, however, we noted that saturation was a transient phenomenon. The initial effect of a bright background is to induce a "silent period" (response saturation) during which time the retina does not respond to stimulus flashes > 7 log units above the absolute threshold. Although this phase of suppression can last for 20 min or longer (with very intense backgrounds), it is followed invariably by a recovery cycle signaled by the appearance of incremental responses and a rapid fall in threshold.

The same sequence of events has now been observed in the responses of the receptors (Fig. 8). Since it is hardly likely that the proximal neurons can discern intensity variations not distinguished by the receptors, rod saturation must result in the complete suppression of retinal sensitivity. Conversely, the recovery of rod activity leads to the return of excitability throughout the retina. It is important to stress that this adaptation process occurred at all levels of I_B. Thus, whether I_B was below saturating intensity or sufficient to bleach more than 90% of the rod photopigment, there followed a decrease in increment threshold to a stable plateau (Fig. 9) and a progressive increase in the maximum potential which the receptors could generate (Figs. 10 and 11). Plotting the final values of ΔI_t as a function of I_B yielded the increment threshold function of Fig. 5, identical in all respects to that obtained from measurements of the S-potential (cf. Fig. 14 of Dowling and Ripps, 1971 *a*).

It will be recalled that Boynton and Whitten (1970), recording the receptor potentials of monkey cones, also found a linear relation between increment

thresholds and I_B. Their study (like ours) did not provide measurements on the maintained receptor potential in effect when the incremental flash was delivered. Using alternative procedures, however, Boynton and Whitten showed that when I_B bleaches only trivial fractions of the cone pigments, the background itself produces a receptor potential V_B in accordance with equation 1. Thus, for n = 1,

$$V_B = \frac{I_B}{I_B + k}. \tag{2}$$

They suggest that this voltage is maintained during exposure to I_B, and compresses the range over which the receptors can respond to incremental flashes. Since the maximum response of the photoreceptors cannot exceed the saturation potential, the total voltage due to the adapting field plus the test flash is given by the expression

$$V_{t+B} = \frac{I_t + I_B}{I_t + I_B + k}, \tag{3}$$

and the response due to the increment test flash is obtained by subtracting equation 2 from equation 3, or

$$V_t = \frac{kI_t}{(I_B + k)(I_B + I_t + k)}. \tag{4}$$

Equation 4 states that V'_{max} (the limit of V_t as $I_t \rightarrow \infty$) will decrease as the steady voltage induced by I_B increases. Thus, the decrement in V'_{max} is obtained directly from equation 2.

Although the mechanism of response compression adequately describes the results obtained by Boynton and Whitten for cone receptor potentials, Fig. 12 shows that it does not predict the incremental response properties of light-adapted skate photoreceptors at any but the lowest value of I_B (i.e. log $I_B = -7$). The V-log I_B curve of Fig. 12 is a semi-log plot of equation 2 for the instantaneous voltage generated by I_B. Since the entire dynamic range of the receptor potential is produced by intensities which do not significantly alter the concentration of rhodopsin, each increment in V_B should, according to the compression hypothesis, produce a corresponding decrement in the maximum voltage (V'_{max}) elicited by a superimposed flash. But this is clearly not the case. The data points show that the decrement in V'_{max} was always appreciably smaller than predicted by equation 4. Thus, the voltage loss imposed by response compression is apparently opposed by an adaptive process which, in effect, *increases* the gain and maximum response of the receptors.

Evidence for a gain-control mechanism at the most distal stage of the visual

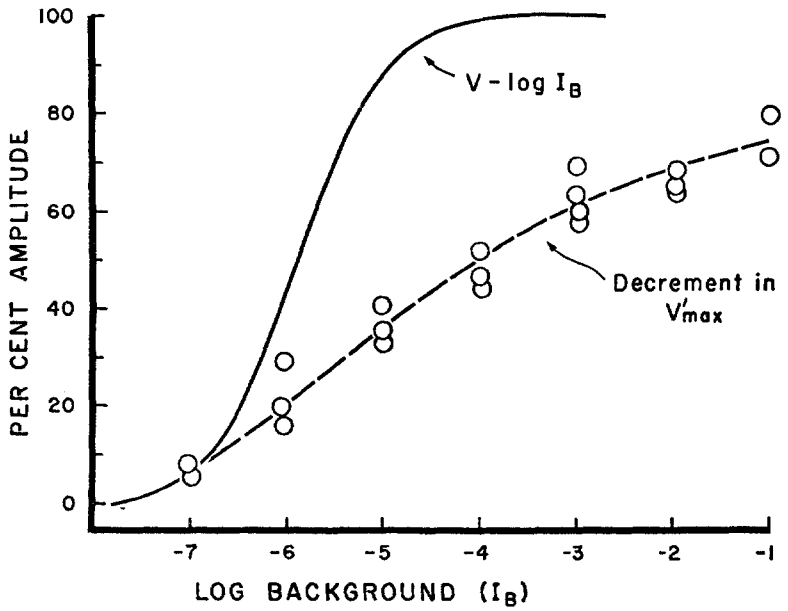

FIGURE 12. Decrement in maximum amplitude of the receptor potential as a function of background intensity. The data points were obtained by subtracting the maximum amplitude response (V'_{max}) elicited in the presence of I_B from the saturation potential of the dark-adapted preparation. The brighter the background, the greater is the decrement in V'_{max}, but nearly all points lie below the values predicted by the V-log I_B function (continuous curve). See text for details.

process can be seen by comparing the responses of dark-adapted receptors with those obtained in the course of light adaptation to a steady, bright background. The voltage-intensity curves plotted on log-log coordinates in Fig. 13 show results obtained before (filled circles) and at the times indicated after turning on a background field of log $I_B = -4$ (open symbols). The onset of I_B initially saturated the receptor potential (Fig. 12, solid curve), and this condition was maintained for nearly 1 min; i.e., responses could not be elicited with our brightest test flashes. After 1 min of light adaptation, however, small amplitude responses could be evoked with intense stimuli, and consequently the voltage-intensity curve was shifted far to the right on the abscissa. Associated with this lateral displacement was a compression of V'_{max}, and a marked loss in sensitivity. With progressively longer periods of light adaptation, V'_{max} grew, and the log V versus log I functions were shifted successively to the *left* on the intensity scale. Between 18 and 25 min, however, light adaptation produced no further change in the position of the curves, although there was a small increase in V'_{max}.

A striking feature of the results shown in Fig. 13 is that the rising portion of each curve has a slope of 1. On log-log coordinates, the unit slope reflects

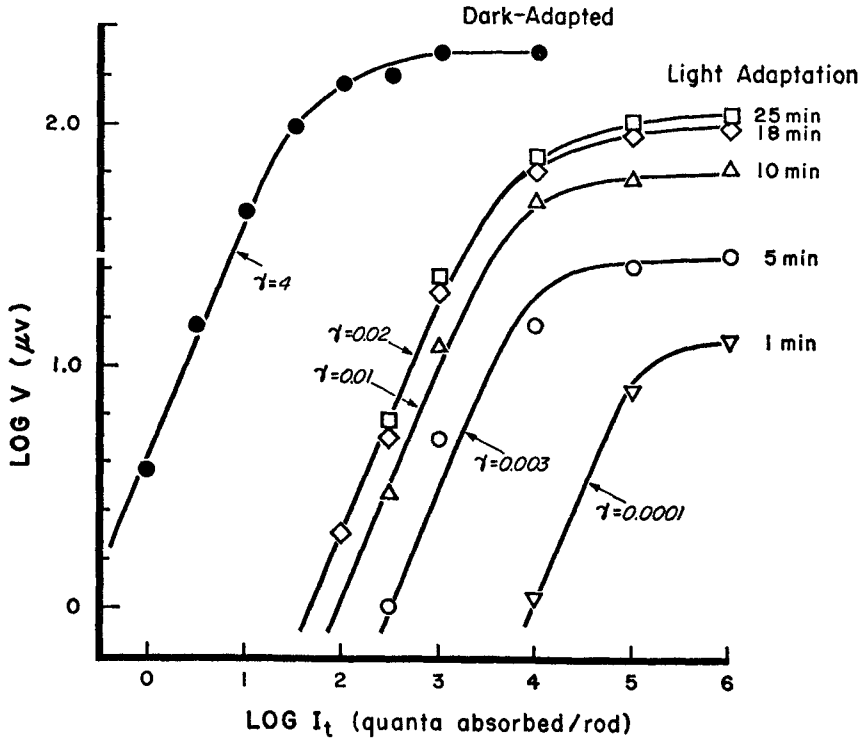

FIGURE 13. Log-log plot of the voltage-intensity functions obtained in the dark-adapted retina (filled circles), and at times indicated after the onset of a steady background (open symbols). The scale on the abscissa was obtained from Fig. 4 where it was shown that a half-saturation potential is elicited in the dark-adapted preparation when 30 quanta are absorbed per rod. Duration of test flash = 0.2 sec. Values of γ for each curve are the slope constants for a linear plot of voltage versus quantal absorption.

a linear relation between voltage and intensity, but a shift in the position of the curve on the scale of abscissae indicates a change in the slope constant of this linear relation as a function of time. Thus, for the initial segment of each curve, $V = \gamma I_t$, where $\gamma = f(t, I_B)$. If we define gain as the voltage increment per absorbed quantum, then gain $= dv/dI = \gamma$. The values of γ associated with each response function of Fig. 13 reveal that during the period from 1 to 18 min of light adaptation, gain increased 200-fold. However, there was no further change with time, so that the gain remained greatly reduced as compared with that of the dark-adapted preparation. This adaptive phenomenon was observed at all background intensities, although the quantitative relations are obviously dependent upon the level of I_B employed.

Lastly, it is important to point out that the maximum amplitude responses recorded during the light adaptation process do not appear to be determined simply by the gain of the system. For example, we have observed that during

light adaptation to different backgrounds, V-log I curves that reach the same V'_{max} (relative to the dark-adapted saturation potential) may be at significantly different positions along the intensity axis. That is, voltage-intensity functions having very different values of γ may have identical amplitude maxima. A similar divergence is found in comparing the temporal changes in log threshold (Fig. 9) and maximum amplitudes (Figs. 10 and 11) during light adaptation. Thresholds fall at a more rapid rate than the growth in V'_{max}. Thus, if the maximum amplitude of the receptor response during light adaptation is determined by level of membrane potential (e.g. by response compression), it appears that the gain control mechanism may be located closer to the initial photochemical event.

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