

# Desensitization of Skate Photoreceptors by Bleaching and Background Light

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**ABSTRACT** Through extracellular measurements of photoreceptor responses to flashed stimuli, we examined how the bleaching of rhodopsin affects increment receptor threshold in the isolated retina of the skate (*Raja ocellata* and *R. erinacea*). Both initially unbleached and previously bleached photoreceptors, when exposed to full-field luminous backgrounds of fixed intensity, attain approximately stable levels of increment threshold that vary with the intensity of the background light. Values of stabilized increment thresholds measured after various extents of bleaching (less than ~50%), when plotted against background intensity in log-log coordinates, tend to converge with increasing intensity of the background; this relationship of the increment threshold functions resembles that which Blakemore and Rushton (1965*b*) found to describe the transient effect of bleaching on psychophysical increment threshold for the human rod mechanism. Our data are consistent with the possibility that related photochemical processes govern the stabilized levels of receptor sensitivity exhibited by the isolated retina (*a*) during steady illumination and (*b*) long after substantial bleaching.

## INTRODUCTION

There is ample evidence that the bleaching of visual pigment in the photoreceptors markedly depresses the sensitivity of the vertebrate visual system during dark adaptation (Dowling, 1960, 1963; Rushton, 1961, 1972; Donner and Reuter, 1968; Dowling and Ripps, 1970; Hollins and Alpern, 1973). The directness of the relationship between bleached pigment and reduced sensitivity and the expression of this relationship at the level of the photoreceptors have been emphasized in numerous recent studies (Frank, 1971; Witkovsky et al., 1973, 1976; Hood et al., 1973; Grabowski and Pak, 1975; Pepperberg et al., 1978; Donner and Hemilä, 1979; Clack et al., 1982). The results of these studies have been interpreted to suggest that bleached visual pigment, that is, opsin in the state ultimately arising on the decay of photoinduced intermediates and the dissociation of the retinal chromophore (Matthews et al., 1963; Brin and Ripps, 1977; Ostroy, 1977), acts to sustain the operation of some process which in darkness depresses the sensitivity of the photoreceptors.

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However, the nature of this postulated activity of the bleached pigment and the relationship of such activity to the overall process of light adaptation remain unclear (for discussions of this question, cf. Rushton, 1965, 1972; Donner and Reuter, 1968; Barlow, 1972, 1977; Pepperberg et al., 1978; Ernst et al., 1978; Bäckström and Hemilä, 1979; Geisler, 1980, 1981; Lamb, 1980, 1981; Ashmore and Falk, 1981).

This paper describes experiments aimed at further understanding how the bleaching of rhodopsin affects the visual responses of vertebrate rod photoreceptors. Our principal approach has been to examine how the photoreceptors, previously brought to a defined state of bleaching, respond to the presence of luminous backgrounds which themselves cumulatively bleach relatively little of the remaining visual pigment. The comparative effects of intense conditioning irradiations (i.e., substantial bleaches) and weak, "nonbleaching" backgrounds have been examined in previous studies, both electrophysiological and psychophysical, as a means of analyzing the mechanisms that underlie visual adaptation (Stiles and Crawford, 1932; Crawford, 1947; Hattwick, 1954; Rushton, 1961; Barlow and Sparrock, 1964; Blakemore and Rushton, 1965 *a, b*; Donner and Reuter, 1968; Baron et al., 1979; Donner and Hemilä, 1979). However, most of these studies have involved the measurement of visual responses arising post-receptorally, and/or have been carried out in the intact eye or eyecup preparation, where the regeneration of rhodopsin in the rods proceeds spontaneously during dark adaptation (Ripps and Weale, 1969; Dowling and Ripps, 1970; Alpern, 1971; Ripps et al., 1981). We reasoned that the measurement of photoreceptor responses in an isolated retina preparation might afford a relatively direct analysis of the effects of bleaching on increment thresholds of the receptors. In the isolated retina, there ordinarily occurs little or no regeneration of the rod visual pigment after a bleaching irradiation; apparently as a direct consequence of the interruption of regeneration, rods of this preparation attain stabilized levels of threshold during dark adaptation that are correlated with the extent of bleaching (for a review, see Pepperberg et al., 1978). Thus, in the isolated retina, losses of sensitivity induced by weak backgrounds may be measured as departures from a relatively well-defined state of bleaching-induced desensitization. The present experiments have involved the extracellular measurement of aspartate-isolated photoreceptor potentials in the isolated, all-rod retina of the skate, *Raja ocellata* and *R. erinacea* (Dowling and Ripps, 1970, 1972; Green and Siegel, 1975; Gruber and Cohen, 1978). In this preparation, the effects of brief, intense irradiation on levels of rhodopsin and on relative photoreceptor thresholds have already been closely examined (Brin, 1975; Pepperberg et al., 1976, 1978; Brin and Ripps, 1977).

#### METHODS

Skates (*Raja ocellata* and *R. erinacea*), typically 30–50 cm long, were obtained from the Marine Biological Laboratory, Woods Hole, MA, and maintained in a 60-gal aquarium containing aerated, artificial seawater (Instant Ocean; Aquarium Systems, Eastlake, OH) at a temperature of ~11°C. All experiments used sections of isolated retina (~3 × 5 mm), obtained from the eyes of animals that had been dark-adapted

for 11–18 h. Procedures used for the isolation of the retina and for the positioning of the retina in the recording dish were identical to those previously described (Pepperberg et al., 1978). The aspartate-containing Ringer's solution used in all experiments was identical to that used previously. The ambient temperature in the room used for the dissections and experiments was 14–16°C; in most of the experiments, the temperature of the preparation was maintained at  $16.0 \pm 0.5^\circ\text{C}$  with the use of a thermoelectric stage (Cambridge Thermionic Corp., Cambridge, MA) and controlling unit. After isolation and positioning in the electrophysiological apparatus, the retina was subjected only to occasional test flashes until a stable condition of maximal sensitivity was attained (typically 30–50 min); this condition will be referred to as the fully dark-adapted state.

Responses of the retina to test flashes, 0.2 s in duration, were recorded transretinally with the use of a Ringer-filled pipette (recording electrode) placed in contact with the upper, photoreceptor surface of the retina, and a loop of chlorided silver wire (reference electrode) positioned beneath the retina. The responses were amplified (model P-16; Grass Instrument Co., Quincy, MA; or model M701; WP Instruments, Inc., New Haven, CT) and displayed on an oscilloscope (model 5112 or 5113; Tektronix, Inc., Beaverton, OR); both AC amplification (band pass of 0.1–300 Hz) and DC amplification (0–300 Hz) were used in the experiments. The amplified signals were recorded on a Brush pen writer (model 2400; Gould, Inc., Cleveland, OH).

Test flashes and adapting irradiations were delivered to the retina with the use of a dual-beam photostimulator. The two beams of this instrument originated from separate tungsten-halogen lamps which were powered by a regulated supply (model SP20-20; Deltron, Inc., North Wales, PA; operating current = 7.5 amp). Exposures to both of the beams were controlled by electronic shutters (Vincent Associates, Rochester, NY) driven by an interval generator (model 830; WP Instruments). Each beam passed through Schott KG-1 and KG-3 filters (Schott Optical Works, Inc., Duryea, PA), and was variably attenuated by passage through Schott neutral density filters. Beam 1, used for the 0.2-s test flashes and the brief bleaching irradiations, was spectrally shaped by passage through a Wratten 58 (green) filter (Eastman Kodak, Inc., Rochester, NY); beam 2, used for background illuminations, passed through a Schott 500-nm interference filter. The two beams were combined by a beamsplitter cube (Ealing Optics Division, Cambridge, MA) and focused onto the plane of the retina, yielding homogeneous incident fields substantially larger than the retina.

The irradiance of each beam was measured after each experiment with the use of a calibrated photodiode (PIN-5DP; United Detector Technology, Inc., Santa Monica, CA) operated in the photovoltaic mode. The physiologically effective quantal irradiance (at 500 nm) of the Wratten 58-filtered light was calculated as previously described (Pepperberg et al., 1978). Throughout this paper, irradiations delivered by both beams are expressed in units of  $N_{\text{eff}}$ , the physiologically effective equivalent in 500-nm quanta incident per  $\mu\text{m}^2$  ( $\text{q}\cdot\mu\text{m}^{-2}$ ) at the plane of the preparation over a defined period. Except where noted, the incident intensities of test flashes (including flash intensity at threshold,  $I_t$ ) and background illuminations ( $I_b$ ) are indicated in logarithmic units of attenuation from a common reference of  $7.0 \times 10^5 \text{ q}\cdot\mu\text{m}^{-2}\cdot\text{s}^{-1}$ .

Throughout this study, the amplitude of the photoreceptor potential was defined as the peak voltage of the response to a 0.2-s test flash, obtained with the use of AC amplification (band pass of 0.1–300 Hz). These conditions of stimulation and recording are similar to those used in earlier studies of the aspartate-treated skate retina (Dowling and Ripps, 1972; Brin and Ripps, 1977; Pepperberg et al., 1978); these studies have discussed the correspondence of the AC-amplified response with the "rapid," receptor component (Sillman et al., 1969) of the DC response. In the

present series of experiments, under conditions of intense stimulation yielding clearly resolvable rapid and slow components of the PIII response (cf. Witkovsky et al., 1975), the peak voltage of the AC-amplified signal usually differed by <25% from the amplitude of the rapid component of the DC signal. In all of our experiments, an amplitude of  $3 \mu\text{V}$  of the AC-amplified response to a 0.2-s test flash was used as the criterion for photoreceptor threshold; receptor sensitivity was defined as the inverse of the measured threshold. Each determination of threshold was carried out by varying the intensity of a near-threshold test flash in steps of 0.1 log unit; the interval between flashes, at least 30 s, was sufficiently large to avoid light adaptation of the retina by the flashes themselves. The fractional extent of bleaching of the rhodopsin in the photoreceptors was calculated from the previously reported bleaching curve describing the isolated skate retina (Fig. 5 of Pepperberg et al., 1978). Crystalline 11-*cis* retinal, generously provided by Paul K. Brown of Harvard University, was stored under nitrogen at  $-20^\circ\text{C}$ . Suspensions containing 11-*cis* retinal were prepared and applied to the retina as previously described (Pepperberg et al., 1976, 1978).

#### ALGEBRAIC EXPRESSION OF THRESHOLD RELATIONSHIPS

The principal aim of this study was to examine how steady background light affects the threshold response of photoreceptors containing a substantial quantity of bleached visual pigment. We began with the knowledge that, in skate rods, as in other vertebrate photoreceptors, the sustained elevation of threshold associated with a given extent of prior bleaching greatly exceeds the elevation predictable solely by the reduction in the efficiency of quantum capture (Brin and Ripps, 1977; Engbretson and Witkovsky, 1978; Pepperberg et al., 1978). This fact seemed consistent with two potentially resolvable possibilities concerning the influence of partial bleaching on the increment threshold properties of the photoreceptors. The first of these possibilities supposes that the pronounced effect of bleaching described in previous studies of dark adaptation is the manifestation (for the case in which background intensity equals zero) of a process that elevates threshold by a fixed factor dependent on the extent of bleaching. A straightforward prediction arising from such a possibility is that, after correction is made for the prevailing efficiency of quantum capture, increment threshold functions for "unbleached" vs. substantially bleached receptors, obtained on the presentation of weak background light, should differ by a *scaling* factor (i.e., by a fixed vertical shift, on a logarithmic scale) which is correlated with the level of bleached pigment. The second of these possibilities supposes that bleaching contributes a fixed threshold elevation that behaves formally (i.e., algebraically) as a residual "dark light" (cf., for example, Barlow, 1972), to which the threshold elevation induced by weak background illumination adds. This latter possibility predicts a *convergence*, with increasing value of background intensity, of logarithmic increment threshold functions exhibited after varying extents of bleaching. Such a relationship was found by Blakemore and Rushton (1965*b*) to describe increment psychophysical threshold for the rod mechanism during the period of slow, "photochemical" dark adaptation after a bleaching irradiation (cf. Dowling, 1963).

The alternative possibilities just considered can be formulated algebraically as follows.

(a) Let  $I_b$ ,  $I_t$ , and  $I_{t_0}$  equal, respectively, the intensity of incident background light, the measured, prevailing value of receptor threshold for a 0.2-s test flash, and the minimal, fully dark-adapted value of receptor threshold.

(b) Throughout this paper, we shall approximate the factor describing the relative efficiency of quantum capture by  $(1 - B)$ , where  $B$  equals the fractional extent of

bleaching (that is, the fractional decrease in transretinal absorbance that occurs upon progressive bleaching of the isolated retina [Pepperberg et al., 1978]). With the use of this factor, we can express the relative effectiveness with which partially bleached receptors absorb incident light; the "reduced," or "effective," intensities of a background field ( $I'_b$ ) and threshold stimulus ( $I'_t$ ), after correction for efficiency of quantum capture, become:

$$I'_b = I_b (1 - B) \quad (1a)$$

$$I'_t = I_t (1 - B). \quad (1b)$$

(Because the fully dark-adapted retina contains no bleached pigment,  $I'_{t0} = I_{t0}$ .)

(c) Let the expression  $[1 + F(I'_b)]$  describe the variation of photoreceptor threshold with  $I'_b$  for the previously studied case of relatively little bleaching (Baylor and Hodgkin, 1974; Green et al., 1975; Bastian and Fain, 1979); that is,

$$\frac{I'_t}{I_{t0}} = 1 + F(I'_b) \quad \text{for } B \ll 1 \quad (2)$$

where  $F(I'_b) = 0$  for  $I'_b = 0$ . Let the expression  $[1 + G(B)]$  describe the previously observed variation of receptor threshold with  $B$  (Brin, 1975; Pepperberg et al., 1978); that is,

$$\frac{I'_t}{I_{t0}} = 1 + G(B) \quad \text{for } I'_b = 0 \quad (3)$$

where  $G(B) = 0$  for  $B = 0$ . Eqs. 2 and 3 can be viewed as special cases ( $B = 0$  or  $I'_b = 0$ , respectively) of the two alternative relationships under consideration here:

$$\frac{I'_t}{I_{t0}} = [1 + F(I'_b)] [1 + G(B)] \quad (\text{"scaling" relationship}). \quad (4a)$$

$$\frac{I'_t}{I_{t0}} = 1 + F(I'_b) + G(B) \quad (\text{"convergence" relationship}). \quad (4b)$$

## RESULTS

### *Increment Photoreceptor Thresholds*

**TEMPORAL COURSE OF THRESHOLD CHANGES** Fig. 1 illustrates representative data used in the determination of increment threshold functions. The data illustrated by filled and open squares were obtained before, during, and after the presentation of two relatively weak backgrounds to a retina that initially was fully dark-adapted. Data illustrated by filled and open circles were obtained from a second retina that, before exposure to the backgrounds, was subjected to a 100-s irradiation that bleached ~87% of the rhodopsin originally present in the photoreceptors. In each of these experiments, stepwise increases (of sufficient magnitude) in the intensity of the background light elevated threshold for the response to a superimposed flash to a new, relatively stable value. Extinction of the background light in each case led to a rapid decrease in threshold to a stable value relatively close to that which had prevailed immediately before the increment threshold run.

It will be noted that the background irradiations used in each experiment

of Fig. 1 cumulatively bleached  $<5\%$  of the total complement of rhodopsin. In other experiments (for which temporal data are not illustrated), the intensity and duration of a background light were adjusted so as to induce a cumulative bleaching of as much as 50%. During all such exposures, increment threshold gradually declined from the (maximally elevated) level exhibited immediately after onset of the background and ultimately attained a relatively stable value.

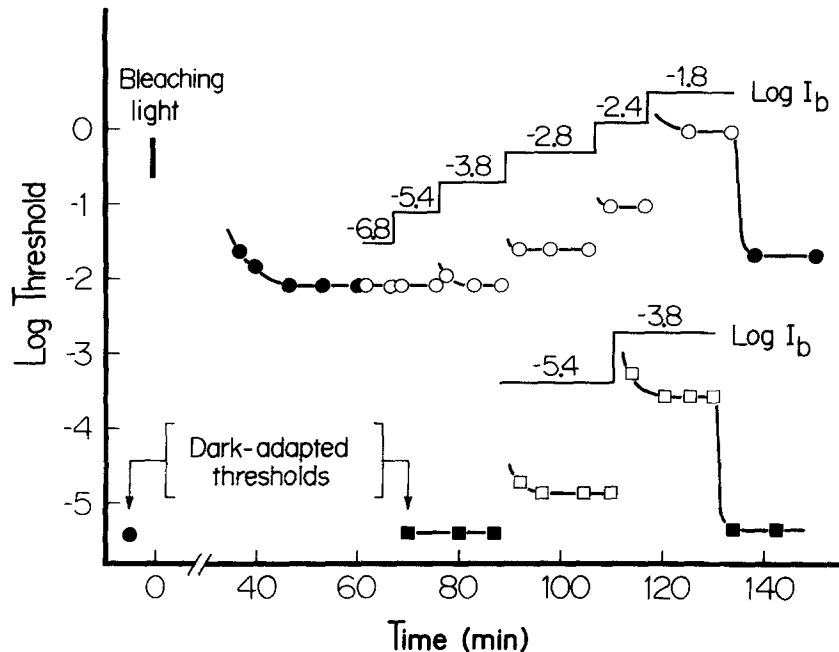


FIGURE 1. Photoreceptor thresholds measured in the presence (open symbols) and absence (filled symbols) of adapting light. Numbers above the increment threshold data identify the logarithmic intensity of the background light; horizontal lines indicate the period of each background irradiation. In experiment 1 (squares), the adapting irradiation ( $1.4 \times 10^5 \text{ q} \cdot \mu\text{m}^{-2}$ ) cumulatively bleached  $\leq 2\%$  of the rhodopsin initially present. In experiment 2 (circles), the retina initially was subjected to brief irradiation ( $6.0 \times 10^7 \text{ q} \cdot \mu\text{m}^{-2}$ ), which bleached  $\sim 87\%$  of the rhodopsin; the backgrounds subsequently presented to this retina delivered an additional  $1.1 \times 10^7 \text{ q} \cdot \mu\text{m}^{-2}$ , an amount expected to have bleached an additional 4% of the rhodopsin.

**THRESHOLD FUNCTIONS** Summarized in Fig. 2 are threshold data obtained during 24 experiments (on as many retinas) of the types described in Fig. 1. Each data point in this figure represents the stabilized value of photoreceptor threshold, relative to the initial, fully dark-adapted value, exhibited by the receptors in the presence of a background light of fixed incident intensity ( $I_b$ ). The open and half-filled symbols show data from 18 experiments that involved the presentation of increasingly intense backgrounds to initially unbleached retinas. The solid curve drawn through these

data was taken as a standard for comparison with data obtained from retinas that were bleached before the increment threshold run (filled symbols; see below). It should be emphasized that data contributing to the upper portion of this standard curve were obtained from preparations that (as a consequence of cumulative background illumination) had undergone substantial bleaching. For example, the data point represented by  $\blacksquare$ , with coordinates of  $\log I_b = -1.6$ , log relative threshold = 4.5, was obtained at a time by which the

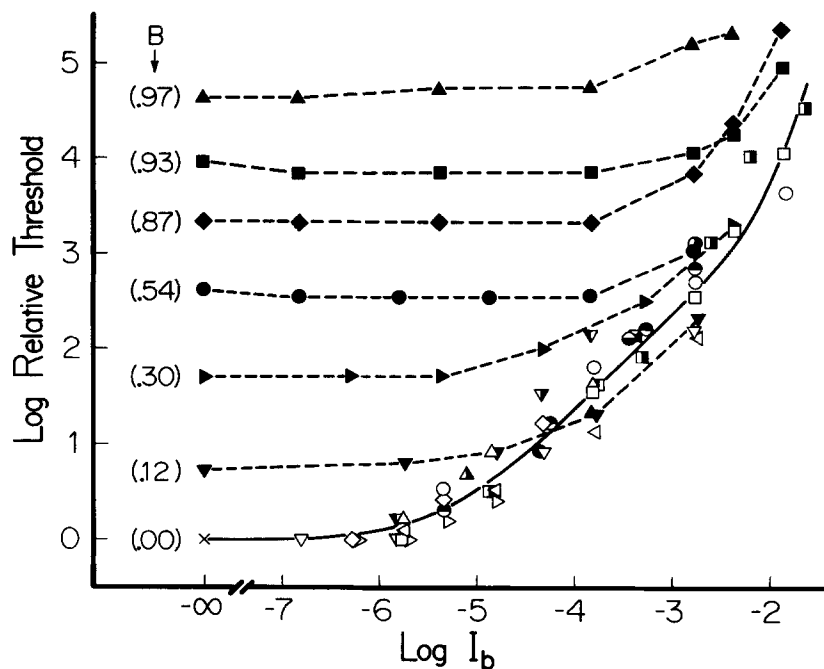


FIGURE 2. Stabilized photoreceptor thresholds exhibited by isolated retinas that were either fully dark-adapted (open and half-filled symbols) or partially bleached (filled symbols) at the time of initiation of increment threshold measurements. Data obtained from the same preparation are shown by identical symbols. The index  $B$  identifies the fractional extent of bleaching that prevailed at the time increment threshold measurements were begun. For  $\log I_b$  less than  $-2.8$ , the solid curve conforms to the relationship:  $(I_t/I_{t0}) = 1 + (I_b/I_0)$ , where  $I_0 = 3.1 \text{ q} \cdot \mu\text{m}^{-2} \cdot \text{s}^{-1}$ .

background light had bleached  $\sim 71\%$  of the rhodopsin in the retina. As we have indicated earlier, exposures to weaker backgrounds, for the periods necessary for stabilization of receptor threshold, induced relatively little cumulative bleaching; for the set of data shown in Fig. 2 by open and half-filled symbols, the prevailing extent of bleaching was  $<4\%$  for all data points falling to the left of  $\log I_b = -3.0$ . Also shown in Fig. 2 (filled symbols) are results obtained from six preparations that received brief, intense irradiation before the measurement of increment thresholds. (For example, the filled diamonds in Fig. 2 represent data obtained from the retina described by

circles in Fig. 1.) These bleaching irradiations (values of  $N_{\text{eff}}$  ranging between  $1.8 \times 10^6$  and  $1.1 \times 10^8 \text{ q} \cdot \mu\text{m}^{-2}$ ) brought the receptors to stabilized states that differed substantially in fractional extent of bleaching (denoted in Fig. 2 by the index  $B$ , to the left of the data) and in dark level of threshold.

To compare more directly the increment threshold behavior of receptors containing substantially different levels of bleached pigment, we subjected all of the data in Fig. 2 to the transformations denoted by Eqs. 1*a* and *b*; these transformations approximately corrected for differences in the efficiency of quantum capture of retinas in different states of bleaching. To accomplish the transformations, it was necessary to calculate the fractional extent of bleaching ( $B$ ) that prevailed at the time of determination of each threshold point shown in Fig. 2. Most of these points were determined during exposure of the retina to background light, i.e., during a gradual increase in the total photic energy delivered to the retina. In all such cases, the "time of determination" of stabilized receptor threshold was taken as the moment immediately preceding any further adjustment in the level of background illumination. From the value of cumulative irradiation calculated in this way, we obtained the value of  $B$ , with the use of the bleaching curve previously reported (Pepperberg et al., 1978).

Figs. 3A and B show data resulting from the transformations just described and compare the relationship of these data with the predictions of the alternative models considered earlier (Eqs. 4*a* and *b*). In each part of this figure,  $\times$ 's represent the data obtained from initially unbleached retinas (open and half-filled symbols in Fig. 2); the dashed curve, fitted visually to these data, was taken to represent the expression  $[1 + F(I'_b)]$ . Also illustrated in each part of the figure are data obtained from the six experiments of Fig. 2, which involved an initial bleaching exposure (filled symbols in Fig. 2). (For clarity in Figs. 3A and B, the symbols used to illustrate some of these data have been changed.) Fig. 3A tests the predictions of Eq. 4*a*; for each of the six sets of data just described, we equated the expression  $[1 + G(B)]$  with value of  $I'_t/I_{t0}$  exhibited at  $I'_b = 0$ , and plotted the function  $[1 + F(I'_b)] [1 + G(B)]$  (solid curves in Fig. 3A). Fig. 3B tests the predictions of Eq. 4*b*; for each set of data, we again equated  $[1 + G(B)]$  with the value of  $I'_t/I_{t0}$  exhibited at  $I'_b = 0$ , and plotted the function  $[1 + F(I'_b) + G(B)]$  (solid curves in Fig. 3B).

The results shown in Figs. 3A and B emphasize a pattern already evident in Fig. 2: namely, that for receptors containing less than  $\sim 54\%$  of their visual pigment in the bleached state, measured values of log increment threshold tend to approach one another with increasing value of log background intensity. This behavior is roughly in accord with the predictions of Eq. 4*b* and is qualitatively similar to that observed by Blakemore and Rushton (1965*b*) in psychophysical experiments. Figs. 3A and B further indicate that for receptors initially subjected to nearly complete bleaching (greater than  $\sim 87\%$ ), data describing the variation of  $\log I'_t/I_{t0}$  with  $\log I'_b$  exhibit a reduced tendency toward convergence with data characterizing less extensively bleached receptors. The relatively sharp rise (i.e., departure from convergence) in the Fig. 3 data shown by  $\diamond$ ,  $\blacksquare$ , and  $\triangle$  appeared not to be due (solely) to a



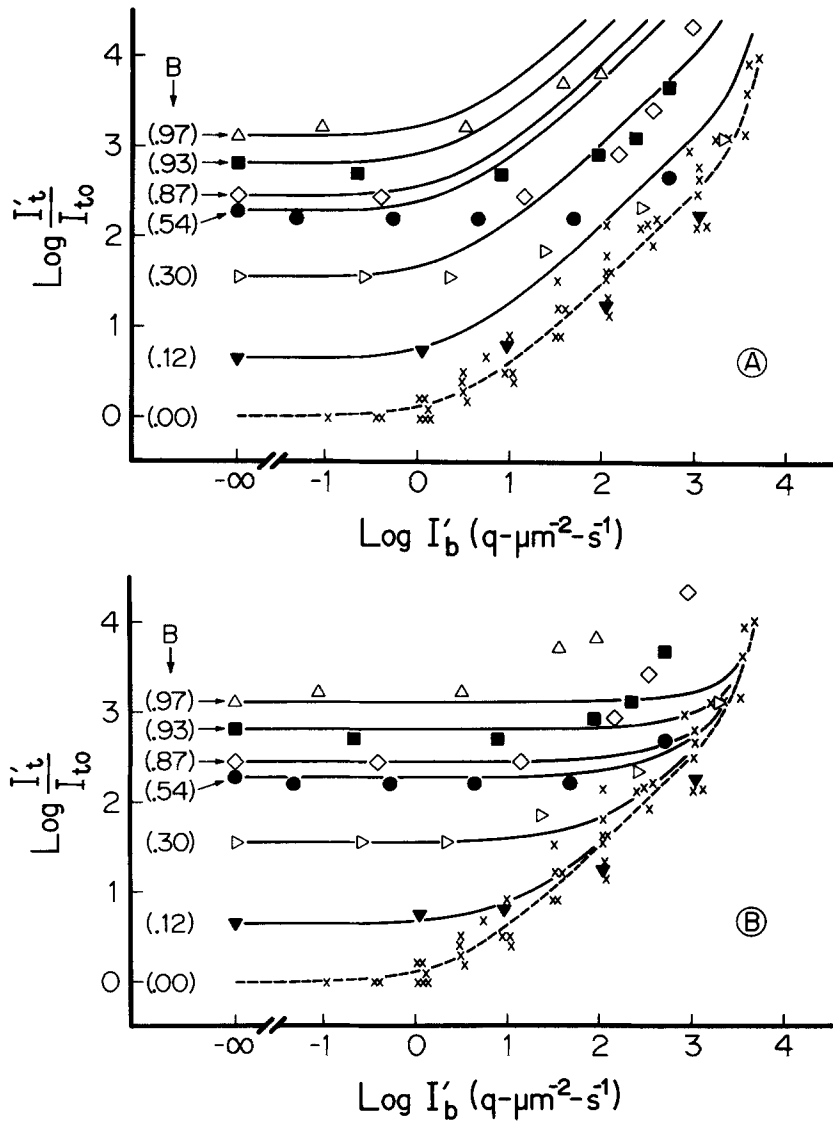


FIGURE 3. Effects of prior bleaching and sustained background light on stabilized photoreceptor thresholds, after correction for the reduction in quantum capture efficiency due to bleaching. Solid curves in panels A and B were obtained, respectively, with the use of Eqs. 4a and b. See text for further details.

deterioration of the retina, for, in such preparations, extinction of the background light led to a substantial recovery in the value of log threshold (cf. Fig. 1). In this domain, the data suggest an increasing dominance of the “scaling” relationship, Eq. 4a, as a description of the increment threshold data.

The data of Figs. 2 and 3 bear also on the functional relationship between  $I_t/I_{t0}$  and  $B$ , a subject addressed in many previous studies (cf. Introduction).

The behavior of values for  $I_t/I_{t_0}$  (at  $I_b = 0$ ) emphasize a frequently noted property of the bleaching vs. threshold relationship for photoreceptors of the isolated retina preparation: namely, that sustained levels of log relative threshold (both before and after correction for efficiency of quantum capture) increase with  $B$  in nonlinear fashion (Fig. 5 of Pepperberg et al., 1978, and accompanying references; also cf. Gordon and Hood, 1976; Bäckström and Hemilä, 1979). This behavior of (sustained) receptor thresholds in the isolated skate retina differs from the (transient) behavior of electroretinographic  $b$  wave and ganglion cell (spike-firing) thresholds in the dark-adapting skate eyecup; in the latter preparation, values of  $\log I_t/I_{t_0}$  for the  $b$  wave and ganglion cells are linearly correlated with values of  $B$  over (at least) the range spanning  $0.0 \leq B \leq 0.7$  (Figs. 12 and 18 of Dowling and Ripps, 1970).

**MASKING OF THE SENSITIZING ACTIVITY OF 11-*cis* RETINAL** The results presented in Figs. 1–3 indicate the importance of background intensity as a determinant of receptor threshold, even in the presence of a considerable quantity of bleached visual pigment. This indication led us to ask whether the regeneration of rhodopsin, if initiated during a period of steady illumination, substantially alters the level of threshold ordinarily maintained during background illumination. Fig. 4 shows the results of an experiment in which the photoreceptors, before their exposure to background light, were subjected to a brief irradiation that bleached ~35% of the rhodopsin initially present. After the stabilization of receptor threshold in darkness, the intensity of an adapting field was adjusted to a value ( $\log I_b = -2.6$ ) that led to a further desensitization of the receptors. (This background, in the absence of any further treatment of the retina, would have bleached an additional 19% of the rhodopsin during the 102-min period that it remained on. The bleaching induced by the  $\log -4.9$  background was negligible.) During the period of background illumination, 11-*cis* retinal was introduced into the fluid surrounding the retina. As a consequence of this treatment, there immediately developed a small (and somewhat transient) elevation of log threshold; however, no major change in threshold occurred during the remainder of the period of illumination. By contrast, on the extinction of the adapting light, there rapidly occurred a large decrease in log threshold to a level that was within 0.6 log unit of the initial, fully dark-adapted value and was 1.5 log units below the value attained earlier in the experiment (after the brief bleaching irradiation). The decrease in threshold observed after the period of illumination confirmed the general responsiveness of this preparation to the action of 11-*cis* retinal (Pepperberg et al., 1976); furthermore, the rapidity with which the sensitization occurred (largely complete within 5 min) strongly suggested that the 11-*cis* retinal had entered the photoreceptors and combined with available opsin before the extinction of the adapting light. The absence of a decrease in threshold *during* the adapting exposure therefore was consistent with the possibility that background illumination of relatively low bleaching efficiency can suppress the sensitivity change associated with the regeneration of rhodopsin during dark adaptation. In a separate series of experiments (not illustrated), weak backgrounds ( $I_b < 3.2 \times 10^3 \text{ q} \cdot \mu\text{m}^{-2} \cdot \text{s}^{-1}$ ) were applied to initially unbleached

retinas in the presence of 11-*cis* retinal. The stabilized elevations of receptor threshold induced by these backgrounds were similar to those induced in the absence of 11-*cis* retinal.

#### *Saturation of the Photoreceptor Response Function*

Conditions of prior bleaching or sustained background illumination which induced a measurable, stable elevation of receptor threshold led also to a decrease in the saturating, or maximal, amplitude ( $V_{\max}$ ) of the response to a test flash (Boynton and Whitten, 1970; Dowling and Ripps, 1972; Brin and Ripps, 1977; Hemilä, 1977; Pepperberg et al., 1978). (Occasionally, increases

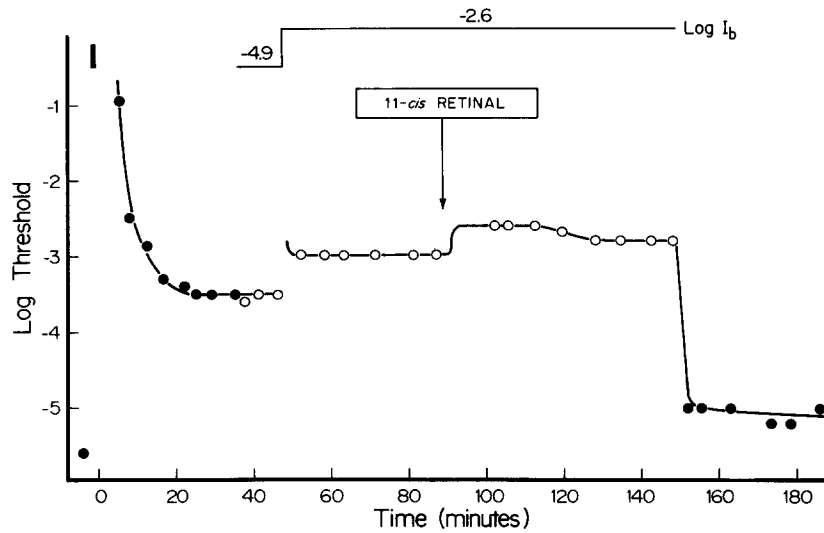


FIGURE 4. Treatment of a partially bleached retina with 11-*cis* retinal during a period of steady illumination. The data point to the left of time zero shows the initial, dark-adapted threshold. The brief, intense irradiation ( $1.1 \times 10^7 \text{ q} \cdot \mu\text{m}^{-2}$ ) delivered early in the experiment (time indicated by the heavy vertical bar) bleached  $\sim 35\%$  of the rhodopsin initially present. At the time shown by the arrow, 0.11 ml of a suspension containing 11-*cis* retinal ( $5.2 \mu\text{mol/ml}$  in ethanol-Ringer's solution, 2:100 by volume) was applied in dropwise fashion (over a period of  $\sim 1$  min) to the upper photoreceptor surface of the retina.

in the stabilized value of threshold were accompanied by an increase in  $V_{\max}$ ; cf. the experiment in Fig. 7 illustrated by (14).) An interesting question arising from these observations was whether the photoreceptors, if brought to equal levels of relative threshold by prior (bleaching) irradiation and sustained background light, exhibit similar decreases in the maximal amplitude of their responses to flashed stimuli. The degree of similarity amounts to a test of the "equivalence" of states of light and dark adaptation in the receptors (cf., for example, Baylor and Hodgkin, 1974; Kleinschmidt and Dowling, 1975; Baron et al., 1979).

Figs. 5 and 6 show results obtained in two experiments, each of which

involved the comparison of response functions exhibited by a single retina under different desensitizing conditions. In each of these experiments, a retina that initially was fully dark-adapted first was exposed to relatively weak background illumination; data illustrated by open circles and open squares in

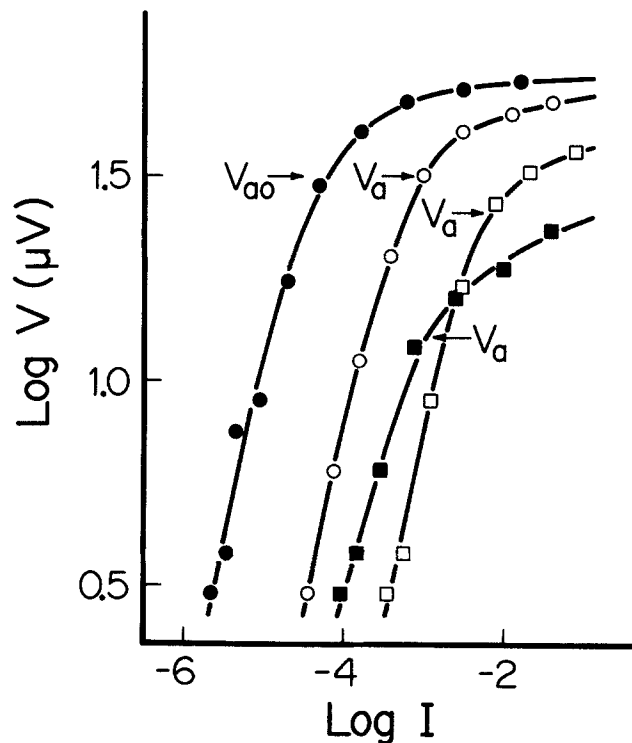


FIGURE 5. Response amplitudes measured at several stages of light adaptation of an isolated retina. After measurements of the response function of the fully dark-adapted preparation (filled circles), the retina was exposed, successively, to background lights of log intensity  $-4.3$  and  $-3.3$ ; data illustrated by open circles and open squares, respectively, were obtained in the presence of these backgrounds. The filled squares describe the response function of the photoreceptors later in the experiment (after further exposure to adapting light), in the absence of background illumination. All data were obtained during periods of stabilized photoreceptor threshold; approximate values of cumulative adapting irradiation delivered to the receptors at the times of recording were  $3.3 \times 10^4 \text{ q} \cdot \mu\text{m}^{-2}$  (open circles),  $5.0 \times 10^5 \text{ q} \cdot \mu\text{m}^{-2}$  (open squares), and  $3.6 \times 10^6 \text{ q} \cdot \mu\text{m}^{-2}$  (filled squares). Values of  $V_{a0}$  (for the fully dark-adapted retina) and  $V_a$  (for the desensitized retina) identify amplitudes at which visually fitted curves through the data points exhibit a slope of  $1/3$  on the  $\log V$  vs.  $\log I$  plot; cf. Fig. 7, below.

Fig. 5, and by open circles in Fig. 6, describe response amplitudes measured during periods of stabilized threshold in the presence of these backgrounds; in each case, the extent of bleaching cumulatively induced by the background light at the time of measurement was  $\leq 4\%$ . After exposure to the back-

ground(s), each retina briefly was exposed to intense light. These adapting irradiations led, cumulatively, to bleaches of 18 and 31%, respectively, in the preparations described by Figs. 5 and 6. In each figure, filled squares illustrate the response function measured after the stabilization of receptor threshold in

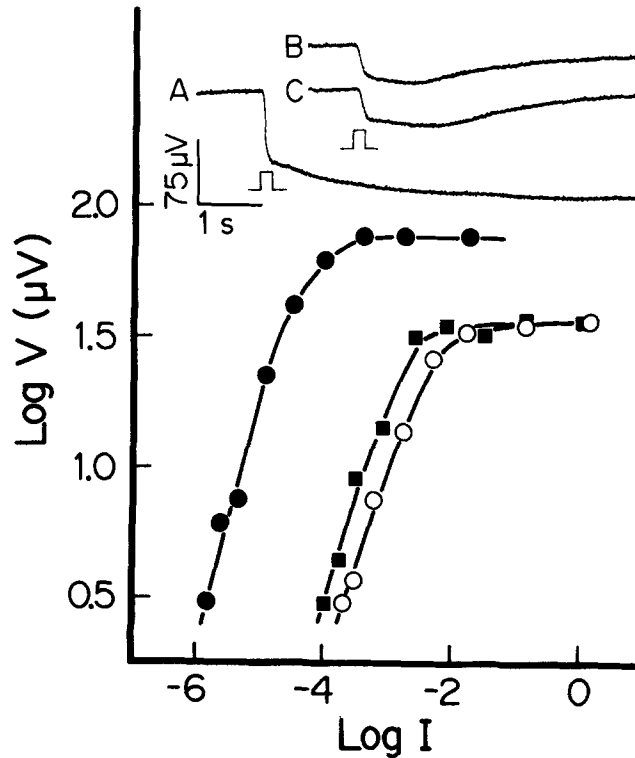


FIGURE 6. Desensitization of an isolated retina by weak background light and a subsequent bleaching irradiation. Voltage-intensity data first were obtained from the fully dark-adapted preparation (filled circles). A background light ( $\log I_b = -3.4$ ) was then turned on ( $t = 0'$ ), and the data shown by open circles were obtained during a brief period ( $t = 28'-33'$ ) after increment threshold had stabilized; the cumulative irradiation (background light and test flashes) delivered to the retina by the conclusion of this second set of measurements was  $6.9 \times 10^5 \text{ q} \cdot \mu\text{m}^{-2}$ . The background light was then extinguished, and the retina was exposed for 9 s to intense light; this exposure increased the value of cumulative irradiation to  $9.3 \times 10^6 \text{ q} \cdot \mu\text{m}^{-2}$ . Data illustrated by the filled squares were obtained over a brief interval ( $t = 79'-84'$ ) after receptor threshold had stabilized in darkness. The DC responses labeled A, B, and C were recorded at approximately the same times as the data shown, respectively, by filled circles, open circles, and filled squares; each of the DC responses was elicited by a 0.2-s test flash of log intensity =  $-1.8$ .

darkness. The results of these two experiments, and results obtained in 34 additional experiments examining saturation of the response function, are summarized in Fig. 7. In this figure, we have used the parameter  $V_a$  (defined in the legend of Fig. 5) as an approximate measure of the relative amplitude

at which the response function tends toward saturation; circles and squares in Fig. 7 describe, respectively, data obtained in the presence and absence of background light. The data of Figs. 5–7 together suggest that, for a given value of stabilized relative threshold, the effects of sustained background light and prior (bleaching) irradiation on  $V_a$  do not differ dramatically. The results give some indication that, for certain levels of relative threshold, the decrease in  $\log V_a$  associated with prior bleaching is slightly more pronounced than that associated with exposure to background light.

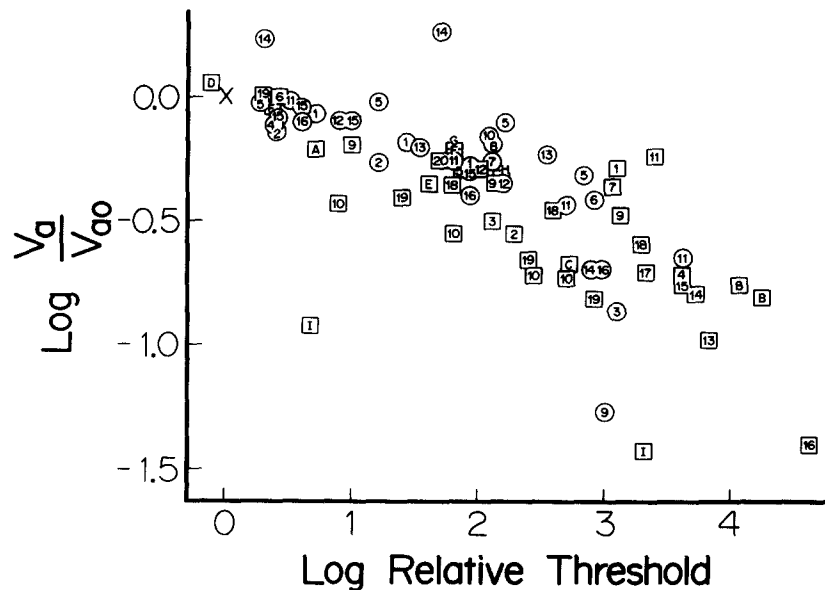


FIGURE 7. Effects of light adaptation on relative threshold and  $V_a$  of the photoreceptor response. All of the data were obtained during periods of stabilized receptor threshold; data illustrated by the same symbol and numerical index were obtained from the same preparation. Circles represent data obtained from 16 retinas which, at the time of analysis, had been subjected only to background illumination. Numbered squares represent data obtained from 20 retinas subjected only to brief bleaching irradiations. Squares indexed by A–I show data obtained during experiments ①–⑨, respectively, in the absence of background light.

#### DISCUSSION

##### *Bleaching and Increment Thresholds*

Our results indicate that partially bleached photoreceptors in the isolated retina of the skate undergo pronounced further decreases in sensitivity on the presentation of relatively weak luminous backgrounds. Under steady illumination, the bleached receptors attain a relatively stable level of increment threshold. As is the case for receptors subjected only to weak illumination, the stabilized levels of increment threshold exhibited after bleaching vary with

the intensity of the sustained illumination. In addition, background intensity appears to be a principal determinant of stabilized level of threshold under conditions that favor the regeneration of rhodopsin (Fig. 4). The expression of these properties in the isolated retina preparation, which ordinarily lacks the capacity to regenerate rhodopsin, emphasizes the importance of receptor mechanisms which, over a wide range of bleaching states, link the sensitivity of the visual response with the prevailing (effective) intensity of incident light, that is, with the rate at which background light photoactivates rhodopsin remaining in the photoreceptors.

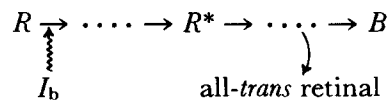
Numerous studies, examining responses arising at several levels within the eyecup preparation and intact visual system, have shown that, in certain respects, intermediate states of dark adaptation exhibited during the regeneration of visual pigment resemble steady states of adaptation attained during exposure to fixed background lights (cf. references cited in the Introduction; also, cf. Cone, 1964; Maffei and Poppele, 1968; Ernst, 1968; Kleinschmidt and Dowling, 1975). The present study of photoreceptor responses in the isolated retina relates directly, we believe, to these previous observations. In this study, we have asked how the persisting threshold change associated with bleaching (Pepperberg et al., 1978) combines with that induced by fixed backgrounds. We have found that the effect of small and moderate bleaches ( $B$  less than  $\sim 0.54$ ) can be viewed roughly as an increase in the limiting, intensity-independent value of the increment threshold function (Eq. 4b). By analogy with interpretations of earlier psychophysical data (Blakemore and Rushton, 1965 *b*; Barlow, 1972), this finding suggests that, in this domain of  $B$ , the sustained, bleaching-induced threshold change behaves formally (i.e., in an algebraic sense), as if induced by a fixed luminous background. Our data comparing relative changes in threshold and  $V_a$  seem consistent with such an interpretation; they suggest that, for  $I_t/I_{t_0} \sim 2$  log units, stabilized elevations of threshold, whether brought about by prior bleaching or a steady background, cause generally similar changes in the tendency toward saturation of the photoreceptor response function. As Figs. 3A and B have indicated, the interpretation of increment threshold data for large fractional bleaches ( $B$  greater than  $\sim 0.87$ ) is less clear. Interestingly, the relationship of increment threshold functions obtained under such conditions resembles that predicted by certain "response compression" models (Williams and Gale, 1978; Dawis, 1978, 1981). Although our data do not establish the involvement of a compression mechanism in the determination of measured threshold, it is apparent that threshold elevations induced by extensive bleaches are accompanied by substantial decreases in  $V_{\max}$  of the photoreceptor response (cf. Fig. 7).

#### *Implications for Photochemical Processes*

The results of this study lead directly to consideration of a general question that has received much attention in previous discussions of the "equivalent background" principle: does the resemblance in the adapting effects of bleaches and backgrounds derive from the expression, under these two conditions, of similar physiological activities of the visual pigment molecule? In

this section, we discuss a notion, considered in somewhat different contexts by previous investigators (Donner and Reuter, 1968; Rushton and Powell, 1972; Brin and Ripps, 1977; Donner and Hemilä, 1979; Gale and Williams, 1980; Lamb, 1981), that suggests a parallel basis for desensitizations induced by sustained background light and by prior bleaching. The discussion below concerns only the description of "stabilized" states of reduced sensitivity exhibited by photoreceptors in the isolated retina. (For example, we do not address the basis of the "neural" process through which receptor thresholds decrease in the early phase of dark adaptation; cf. Frank, 1971; Grabowski et al., 1972; Brin and Ripps, 1977).

The specific notion we wish to consider may be developed as follows. (i) During exposure of the isolated retina to a steady background light ( $I_b = \text{constant}$ ), rhodopsin ( $R$ ) in the rods is gradually bleached (i.e., converted to bleached visual pigment,  $B$ ). The pathway leading to the formation of  $B$  involves passage of the pigment molecule through a transient (as yet unidentified) state, defined here as  $R^*$ , which precedes the dissociation of the retinal chromophore from opsin:



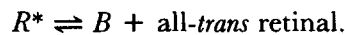
(ii) Under conditions of steady illumination in which the level of  $R$  decreases very slowly (say,  $\leq 1\%$  per minute, a condition prevailing in most of the present experiments employing background light), the effective background intensity,  $I_b$ , is relatively constant, and most of the pigment is either already bleached, or is in its native, photosensitive state. Such a condition leads to the appearance of an approximately steady level of  $R^*$ , the magnitude of which is small by comparison with the total complement of pigment in the receptors. That is, if  $R$ ,  $R^*$ , and  $B$  represent the fractional molar levels of the respective forms of the pigment molecule,  $R + B \cong 1$ , and  $R^* \ll (R + B)$ . (iii) We propose that  $R^*$  and  $B$  function in similar biochemical processes to regulate the stabilized states of photoreceptor adaptation exhibited (a) during exposure to fixed  $I_b$ , and (b) after substantial bleaching. Under this hypothesis, the observed effect of bleaching ( $B$  less than  $\sim 0.5$ ) on increment thresholds (Fig. 3B) derives from the ability of bleached pigment,  $B$ , to mimic the action of the transient species,  $R^*$ . Although a detailed evaluation of this hypothesis is beyond the scope of the present paper, it is helpful to comment here on the following relevant points.

(a) The level of  $R^*$  arising under a particular condition of steady illumination remains unclear. However, consideration of the hypothesis just described immediately suggests that the desensitizing activity associated with a given molar quantity of  $R^*$  is far greater than that associated with the same molar quantity of  $B$ . This follows most directly from the finding that pronounced, sustained elevations of receptor threshold can be induced by backgrounds that cumulatively bleach very little of the rhodopsin. For example, in experiment 1 (squares) of Fig. 1, the background that elevated threshold by 1.8 log units



cumulatively bleached at most 2% of the rhodopsin initially present. Thus, even on the clearly conservative assumption that the lifetime of  $R^*$  is infinite, the maximum (i.e., cumulative) level of  $R^*$  arising during the stable condition of light adaptation would have been  $\cong 0.02$ . A comparable, stable elevation of threshold, to have been induced by prior bleaching, would have required the value of  $B$  to be  $\sim 0.15$  (cf. Pepperberg et al., 1978). It is of interest to note here that Rushton and Powell (1972), in a study of the early stage of dark adaptation in man, hypothesized the existence of a transient species ("X-opsin") whose desensitizing activity was implied to be  $\sim 50$  times as great as that of the final bleaching product (also cf. Donner et al., 1979; Lamb, 1981).

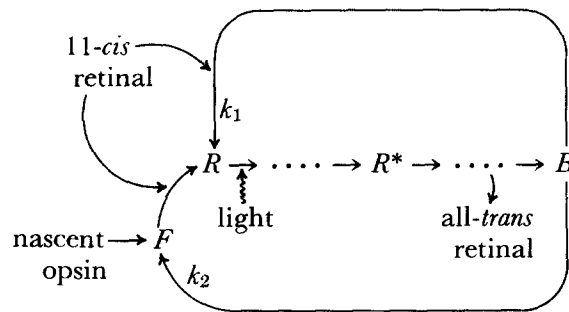
(b) A somewhat different photochemical scheme, in which  $B$  lacks desensitizing activity, also could account for the present findings. This alternative explanation supposes that the persisting desensitization exhibited after bleaching is due to the thermal equilibration of  $R^*$  with its ultimate products,  $B$  and all-*trans* retinal:



That is, a residual level of  $R^*$ , remaining after an equilibration favoring  $B$ , accounts for the desensitization persisting long after substantial bleaching. Such a possibility cannot be ruled out, but a recent observation made by Brin and Ripps (1977) appears to us to argue against it. In their study of photoreceptor sensitivity in the isolated skate retina, Brin and Ripps used treatment with hydroxylamine to accelerate the decay of bleaching intermediates after intense irradiation of the retina. These investigators found that in both hydroxylamine-treated and control preparations (subjected to identical bleaching irradiations), the final level of relative threshold attained by the receptors was the same ( $\sim 3$  log units above the fully dark-adapted level; Fig. 13 of Brin and Ripps [1977]). Because  $R^*$ , as a relatively short-lived intermediate, is likely to contain bound retinal, the equilibrium level of  $R^*$  would be expected to decrease dramatically in the presence of hydroxylamine (because of the reaction of free retinal with the hydroxylamine). The fact that treatment with hydroxylamine did not influence the final level of threshold thus implies that the sustained threshold elevation associated with bleaching is not governed by the level of a species containing retinal, such as  $R^*$ .

(c) A final note, relevant to the postulated activity of  $B$ , regards observations made by Engbretson and Witkovsky (1978) in a recent study of rod sensitivity in *Xenopus* larvae. With the use of dietary deprivation of vitamin A, Engbretson and Witkovsky reduced the level of visual pigment in the rod photoreceptors and examined how this reduction in pigment level influences the sensitivity of (dark-adapted) rods in the eyecup preparation. Their results indicated that the appearance of "free opsin" in the outer segments (that is, visual pigment devoid of its retinal chromophore, symbolized by " $F$ " in the scheme below) is not associated with an intrinsic desensitization of the receptors (i.e., an elevation of threshold exceeding the factor predictable on the basis of quantum capture efficiency). The data of the present study, and the considerations discussed above, do not exclude the existence of such an "inactive" species,  $F$ ;

they suggest only that in the skate retina, the bleaching of rhodopsin does not lead to the appearance of a substantial quantity of  $F$  during the period (on the order of several hours) over which thresholds were measured in our preparations. A simple scheme accounting for both our results and those of Engbretson and Witkovsky is as follows:



where  $k_2^{-1}$  exceeds several hours, and  $[k_1(11\text{-cis retinal})] \gg k_2$ . This scheme (including the indicated relationships for the rate constants) supposes that the formation of  $F$ , although occurring in the vitamin A-deprived eye (i.e., in the absence of 11-*cis* retinal) through the generation of nascent opsin or through some (slow, as yet unidentified) transformation of  $B$ , is suppressed in vivo under normal conditions by the availability of 11-*cis* retinal chromophore, which largely restricts states of the opsin molecule to those involved in the cycle of bleaching and regeneration.

We thank William L. Pak, Gerald S. Wasserman, and William A. Cramer for helpful discussions during the course of this study. Much of this material was submitted by J. W. C. to the Department of Biological Sciences of Purdue University in partial fulfillment of the requirements for the Ph.D. degree.

This research was supported by grants EY-02103, EY-07008, and EY-00198 from the National Institutes of Health, and by a David Ross grant from Purdue University.

Received for publication 17 February 1982 and in revised form 30 July 1982.

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