# Rhodopsin Kinetics in the Cat Retina

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ABSTRACT The bleaching and regeneration of rhodopsin in the living cat retina was studied by means of fundus reflectometry. Bleaching was effected by continuous light exposures of 1 min or 20 min, and the changes in retinal absorbance were measured at 29 wavelengths. For all of the conditions studied (fractional bleaches of from 65 to 100%), the regeneration of rhodopsin to its prebleach levels required >60 min in darkness. After the 1-min exposures, the difference spectra recorded during the first 10 min of dark adaptation were dominated by photoproduct absorption, and rhodopsin regeneration kinetics were obscured by these intermediate processes. Extending the bleaching duration to 20 min gave the products of photolysis an opportunity to dissipate, and it was possible to follow the regenerative process over its full time-course. It was not possible, however, to fit these data with the simple exponential function predicted by first-order reaction kinetics. Other possible mechanisms were considered and are presented in the text. Nevertheless, the kinetics of regeneration compared favorably with the temporal changes in log sensitivity determined electrophysiologically by other investigators. Based on the bleaching curve for cat rhodopsin, the photosensitivity was determined and found to approximate closely the value obtained for human rhodopsin; i.e., the energy $E_c$ required to bleach $1 - e^{-1}$ of the available rhodopsin was 7.09 log scotopic troland-seconds (corrected for the optics of the cat eye), as compared with ~7.0 in man.

## INTRODUCTION

The recovery of visual sensitivity that follows exposure to intense (bleaching) lights has been studied in almost every class of vertebrate. Although the mechanisms subserving the dark-adaptation process are still poorly understood, numerous attempts have been made to link quantitatively the slow fall in visual threshold to the regeneration of the bleached photopigment (Hecht et al., 1937; Dowling and Wald, 1960; Donner and Reuter, 1965 and 1968). Most successful in this regard has been the formulation proposed by Dowling (1960) to account for electroretinographic (*b*-wave) threshold changes during dark adaptation in the rat retina. Dowling found *log* threshold to be a linear function of the fraction of rhodopsin still in the bleached state, an empirical relation that describes, over most of the time-course of dark adaptation, the data obtained in man (Rushton, 1961 *a*) and several other vertebrate species (Dowling and Ripps, 1970; Baumann, 1967). Moreover, the expression he

J. GEN. PHYSIOL. © The Rockefeller University Press · 0022-1295/81/03/0317/18 $1.00 317
Volume 77 March 1981 317-334

derived states unambiguously that bleached photopigment exerts a far greater effect on visual threshold than can be accounted for by the loss in quantal absorption.

It is generally recognized that the log-linear relationship does not predict accurately threshold data obtained over the entire bleaching range (Dowling and Ripps, 1970; Ripps and Weale, 1976), nor can it account for results obtained under all of the various stimulus-response conditions encountered in studies of dark adaptation (cf. Brin [1975], Grabowski and Pak [1975], Matsuura [1975], Witkovsky et al. [1976], and Pepperberg et al. [1978]). Nevertheless, if the fraction of bleached photopigment is the principal determinant of visual sensitivity during the late stages of dark adaptation, then the time at which the pigment has regenerated almost completely should mark the time at which threshold approximates its fully dark-adapted ("absolute") level. However, a very different conclusion was reached by Bonds and Enroth-Cugell (1979) in their detailed study of dark adaptation in the cat. Measuring the sensitivity of the ganglion-cell discharge in the retina of the anesthetized animal, they found that the decline in threshold continued for 70 min or longer after photic exposures that bleached 60% or more of the rhodopsin content of the test area. On the other hand, earlier results obtained by retinal densitometry on the regeneration of cat rhodopsin after similar bleaching conditions indicated that the time required for the resynthesis of the bleached rhodopsin never exceeded 35 min (Bonds and MacLeod, 1974). This large discrepancy prompted Bonds and Enroth-Cugell (1979) to suggest that processes other than those under the influence of photopigment kinetics contribute to the regulation of sensitivity after bleaching. Although there is ample evidence of adaptive mechanisms operating within the neural network of the retina (Dowling and Ripps, 1971; Green et al., 1975), it seems strange that they should continue to function after so long a period of dark adaptation, and long after the bleached pigment had regenerated. Moreover, it will be recalled that in the preceding paper (Ripps et al., 1981) we showed that after a flash bleach the regeneration of rhodopsin in cat continues for >1 h. A cursory comparison with the electrophysiological dark-adaptation data of Bonds and Enroth-Cugell (1979) revealed somewhat similar time-courses, despite differences in the bleaching conditions of the two experiments. Since flash exposures and exposures of longer duration may give rise to different regeneration data (Hollins and Alpern, 1973), we present in this paper the results obtained after bleaches lasting 1 min or longer. In addition, we have measured the photolysis of rhodopsin for a range of exposure conditions, and determined the photosensitivity of the bleaching process in cat.

## METHODS

The preparation of the cat and the fundus reflectometric technique for this study were the same as described in the preceding paper (Ripps et al., 1981). However, flash bleaching was not employed. All bleaching exposures were derived from the heat-filtered, tungsten-halogen lamp; either "white" or orange (Corning Glass Works [Corning, N. Y.] 3482) light was used. Unattenuated, the retinal illuminance (corrected for cat) of the white field was 7.86 log scotopic troland (td); that of the orange was 6.82 log scotopic td.

## RESULTS

### *Difference Spectrum and Photosensitivity of Rhodopsin*

Fig. 1 shows density difference spectra obtained from one cat during a series of experimental runs in which the retina was exposed for 1-min periods to bleaching lights of various intensities (4.66–6.82 log scotopic td). All but one

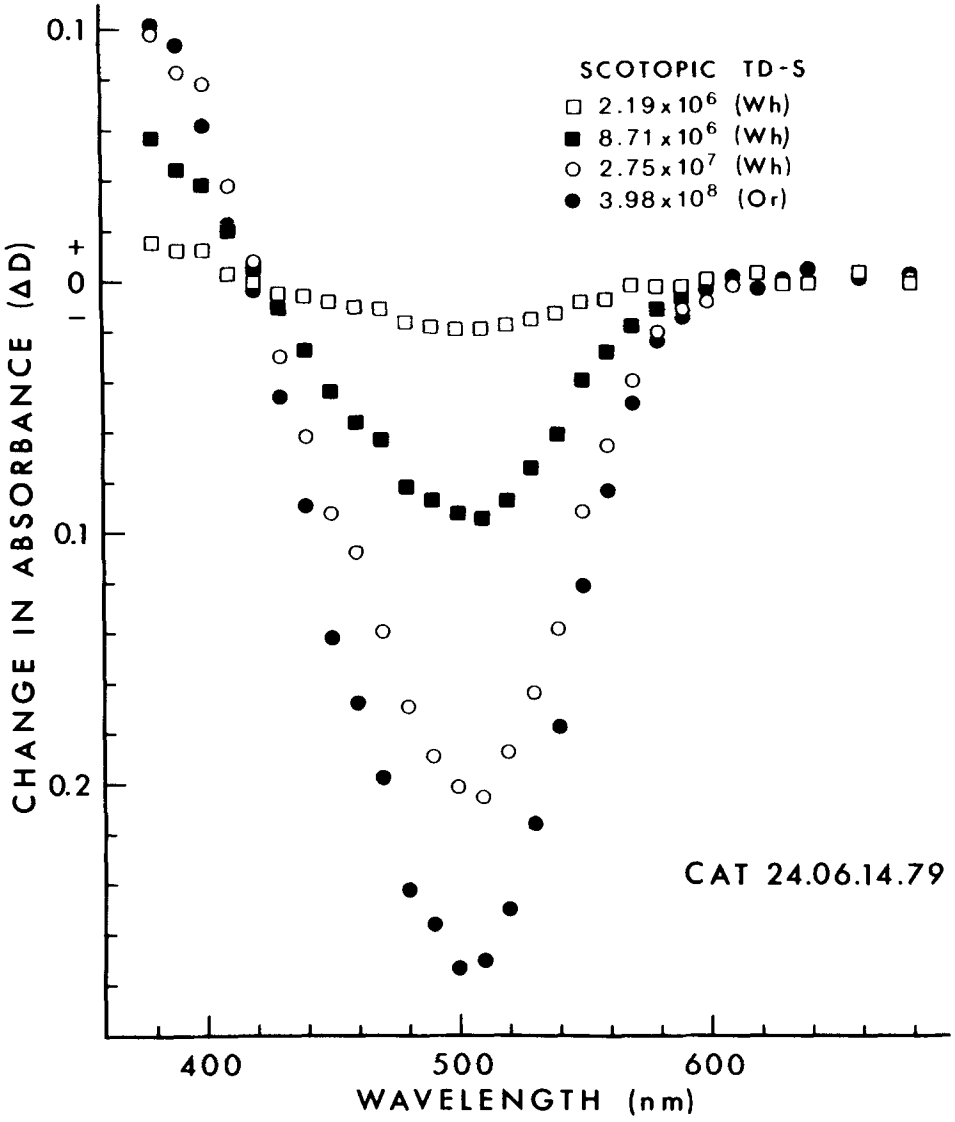


FIGURE 1. Density difference spectra obtained after 1-min exposures to various intensities of the bleaching light. The latter, expressed in scotopic td-s, have been corrected for the optics of the cat eye. Between bleaching exposures the eye was allowed to dark adapt for at least 70 min. Orange (*Or*) light, which minimizes absorption by early photoproducts, was used to bleach completely the available rhodopsin. *Wh*, "white" light.

of the experiments were followed by a period of dark adaptation sufficient for full regeneration of the bleached photopigment. The data for the full bleach are the exception, having been obtained immediately after the final partial bleach, and the difference spectrum (filled circles) thus represents the density changes between absorbance measurements in the dark adapted state and those obtained after two consecutive bleaches: the partial bleach and a 1-min exposure to the unattenuated orange field (Corning 3482; 8.60 log scotopic troland-seconds [td-s]).

The spectral variation of $\Delta D$ shown in Fig. 1 is typical of data obtained from more than 80 experimental runs in which bleaching-difference spectra

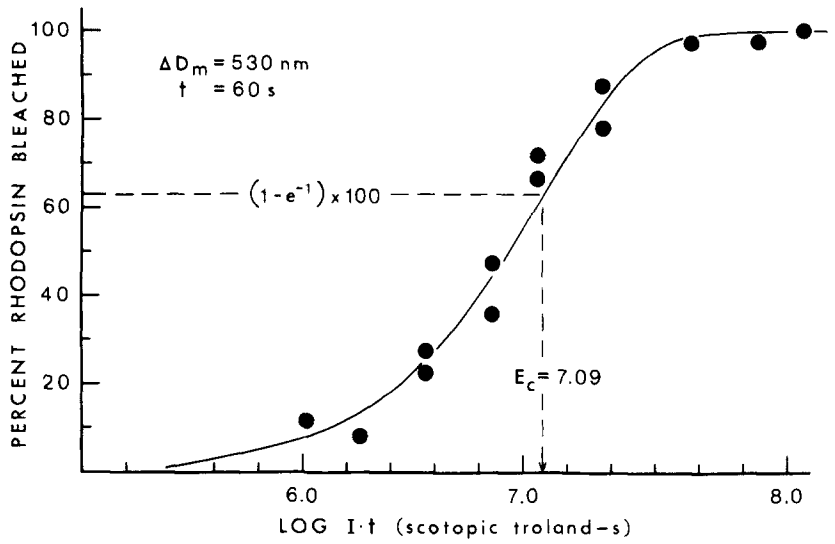


FIGURE 2. The bleaching curve of cat rhodopsin. Data points represent the changes in absorbance ($\Delta D_{530}$) expressed as a percentage of the maximum change recorded at this wavelength. The curve drawn through the data is derived from Eq. 1 and is positioned on the scale of abscissae to best fit the experimental points. The dashed lines show the determination of the photosensitivity constant $E_c$ (see text).

were recorded. The wavelength at which the maximum density change for each spectral curve occurred was approximately $\lambda$ = 502 nm (based on the midpoint of the half-bandwidth). The $\lambda_{max}$ of the absorption spectrum of cat rhodopsin is reported to be at 500 nm (Bridges, 1970), and the shift of the difference spectrum to slightly longer wavelengths is attributable to the formation of a photoproduct absorbing strongly at short wavelengths (i.e., <410 nm). These findings and the location of the isosbestic point at ~420 nm (cf. Dartnall [1957]) provide good evidence that the measurements represent changes in the absorbance of rhodopsin. Moreover, attempts to detect other photosensitive substances were unsuccessful; partial bleaching with progressively longer exposures to long-wavelength light did not produce a shift in the $\lambda_{max}$ of the difference spectrum (Dartnall, 1952).

Difference spectra such as those shown in Fig. 1 were used to generate a function from which the photosensitivity of cat rhodopsin could be estimated. Fig. 2 shows results culled from 18 cats in which $\Delta D$ (measured at 530 nm) was determined as a function of the retinal irradiance of the bleaching light. Since the $[\Delta D_{max}]_{530}$ varied among animals, absorbance changes were expressed as the fraction bleached $F$ of the available rhodopsin. The data points are described well by the exponential equation

$$F = 1 - \exp(-\alpha\gamma It). \quad (1)$$

The terms of this expression are defined in the preceding paper (Ripps et al., 1981). It follows from Eq. 1 that the retinal exposure $E_c$ that bleaches $1 - e^{-1}$ of the available rhodopsin provides a measure of the photosensitivity, i.e., $E_c = \alpha\gamma^{-1}$. Expressed in log td-s, $E_c = 7.09$, a value not significantly different from the figure of $E_c \approx 7.0$ obtained from measurements on the normal human retina (Ripps and Weale, 1969 *a*; Alpern, 1971). The true value of $\alpha\gamma$ is probably slightly lower than our measurements would indicate because, as noted in the Appendix to the preceding paper (Ripps et al., 1981), the effect of stray light is to cause the $\Delta D$ measurements to underestimate the in situ density change; i.e., a shift in the curve of Fig. 2 to the left on the scale of abscissae.

We mentioned above that the maximum density change recorded between dark-adapted and fully bleached conditions varied from one animal to the next, and it is of some interest to note that in the 25 cats tested the values of $[\Delta D]_{500}$ (i.e., the $\Delta D$ for double transit through the retina measured at 500 nm) ranged from 0.206 to 0.289, with a mean and SD of $0.256 \pm 0.022$. The value of the mean is about twice that obtained from measurements on the human retina (Rushton, 1956; Alpern and Pugh, 1974; Ripps et al., 1978). Not included in the statistics are data from one animal for which the $\Delta D$ was 0.422; in this instance the test spot was probably positioned fortuitously over the retinal region containing the highest concentration of rods (Steinberg et al., 1973).

### *Regeneration of Rhodopsin*

Determining the time-course of rhodopsin regeneration after brief (≤2-min) photic exposures proved difficult due to the presence of photoproducts that form and decay slowly after the initial exposure (Weale, 1967; Ripps and Weale, 1969 *b*; Brin and Ripps, 1977). The problem can be anticipated by considering the difference spectra of Fig. 3, recorded at various times after the bleaching light ($It = 7.14$ log scotopic td-s; $F = 65\%$) was extinguished. During the first 3 min in darkness, the $\lambda_{max}$ at ~478 nm suggests that the formation of metarhodopsin III is the dominant factor contributing to the absorbance increase, whereas the concomitant loss in density at wavelengths <420 nm is most probably due to the decay of metarhodopsin II ($\lambda_{max} \approx 380$ nm). Between 7 and 10 min in darkness, the difference spectra broaden, and a second hump at $\lambda \approx 500$ nm forms as rhodopsin makes an appearance. Thereafter, synthesis of the visual pigment predominates; $\Delta D_{500}$ continues to grow while metarhodopsin III is decaying to relatively colorless photoproducts.

The regeneration of rhodopsin is usually determined from a graph of the time-dependent change in density at some wavelength within its absorption spectrum. Fig. 4 shows data obtained from another experimental run in which the eye was exposed for 1 min to a retinal illuminance of 6.06 log scotopic

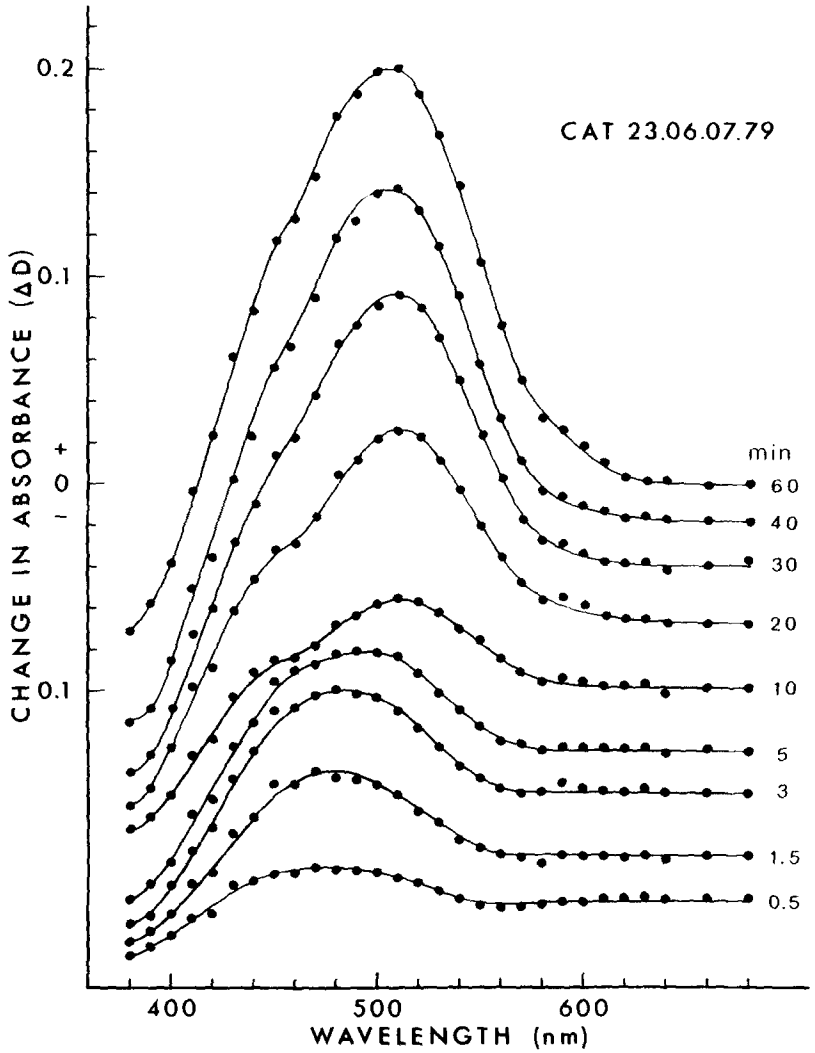


FIGURE 3. Difference spectra obtained at the times indicated after a 1-min exposure that bleached ~65% of the available rhodopsin ($It$ = 7.14 log scotopic td-s). The results at 60 min are placed correctly on the scale of ordinates; all others are displaced downward for clarity. Note the shift in $\lambda_{max}$ during the first 10 min of dark adaptation.

td. Note that the thermal reactions referred to above create "regeneration" curves that vary with the wavelength selected. For example, at wavelengths 475 and 510 nm, both of which lie near the $\lambda_{max}$ of rhodopsin, the formation of metarhodopsin III tends to obscure the early stages of rhodopsin regenera-

tion, whereas its decay distorts the curve representing later stages of the regenerative process. Even at $\lambda = 560$ nm, a wavelength beyond those often regarded as free of contamination by late photoproducts, a discontinuity in the curve at the 10-min mark suggests that absorption by photoproducts may be impressed upon the results. Thus, it would appear that to obtain regeneration data in which the intrusion of thermal intermediates is minimized requires prolonged bleaching exposures that allow ample time for the decay of most of the photoproducts (cf. Bonds and MacLeod [1974]). Nevertheless, reasonable estimates of the time-course of regeneration can be obtained by

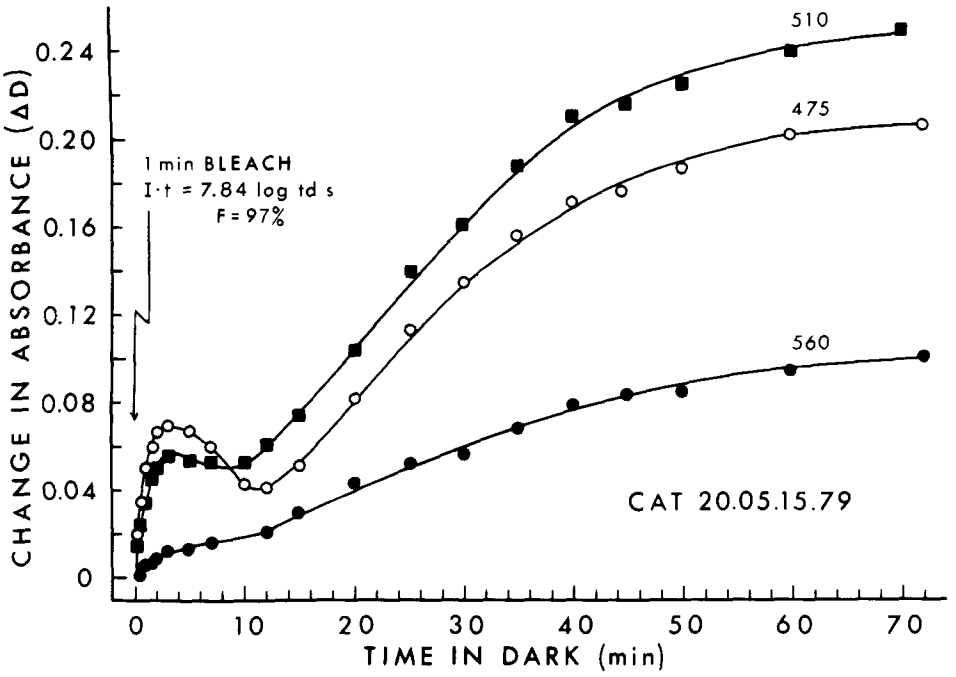


FIGURE 4. Time-course of the changes in absorbance measured at three test wavelengths after a 1-min bleaching exposure. The data were obtained from a family of curves such as that shown in Fig. 3. The curves show the influence at all three wavelengths of the formation and decay of early photoproducts. See text for details.

plotting the density changes recorded at 540 nm (since the small density changes at longer wavelengths provide less reliable data) and considering as representative of rhodopsin regeneration the data collected at times >15 min (i.e., after the early photoproducts have started to decay). Fig. 5 shows the results for $\Delta D_{540}$ obtained from three cats after bleaching between 65 and 98% of the available rhodopsin. The main point to note in Fig. 5 is that in all three experiments rhodopsin continued to regenerate for >60 min. Furthermore, it is apparent that the data do not follow the exponential time-course expected of first-order reaction kinetics (see below).

*Regeneration after Prolonged Bleaches*

As already mentioned, extending the exposure duration of the bleaching field allows time for the intermediates formed initially to decay to relatively colorless photoproducts. Nevertheless, the difference spectra of Fig. 6, recorded during the course of dark adaptation after a 20-min exposure to an intense white light (6.66 log scotopic td), reveal a small but progressive loss of absorbance for $\lambda$ <430 nm, due most likely to the disappearance of retinal ($\lambda_{max} \approx 387$) present at the termination of bleaching. However, the data show far less evidence of the formation and decay of photoproducts than was seen

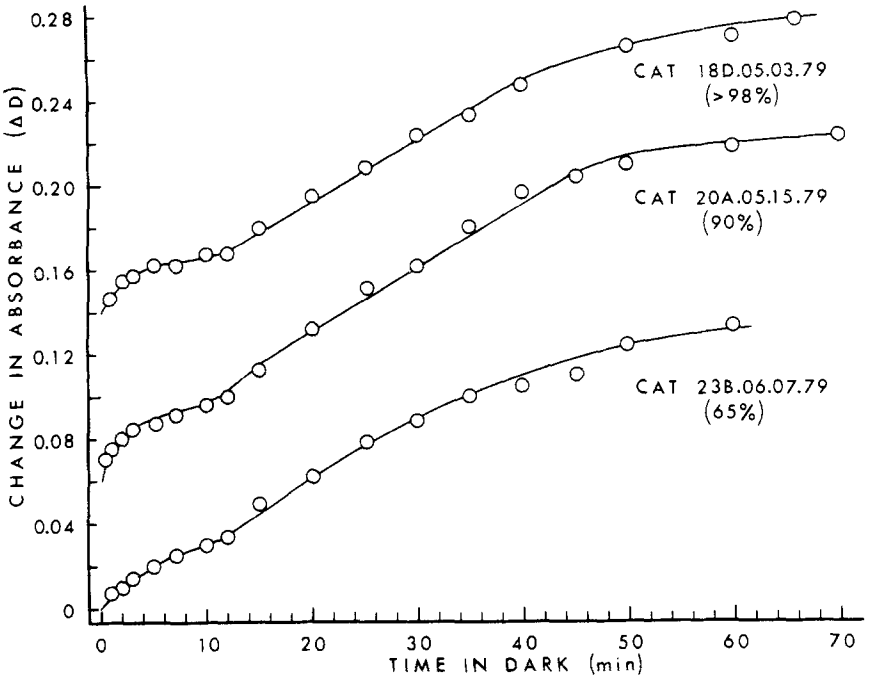


FIGURE 5. Time-course of the absorbance changes ($\Delta D_{530}$) that followed bleaches of various strengths. The data from three cats are shown. The curve for the 65% bleach is correctly positioned on the scale of ordinates; the other two have been displaced upward to avoid overlap.

in the results shown in Fig. 3. Compare, for example, the difference spectra of Figs. 3 and 6 taken after 1.5 and 3 min in darkness. In Fig. 3 the $\lambda_{max}$ of these curves is in the region of 480 nm due to the formation of metarhodopsin III; in Fig. 6 the $\lambda_{max}$ is at 505 nm, and there is no distortion of the curves at shorter wavelengths to indicate that a significant amount of metarhodopsin III is present (cf. the data of Fig. 3 for $t \leq 10$ min). In fact, difference spectra recorded after 20-min bleaching exposures maintained the same spectral form throughout the period of regeneration. Consequently, if the values of $\Delta D_{500}$ are plotted as a function of time (data points of Fig. 7 *A*), the result is a

monotonic increase in absorbance that is probably due almost exclusively to the resynthesis of rhodopsin.

But even under these circumstances the regeneration of rhodopsin does not follow a simple exponential curve. This can be illustrated by considering the

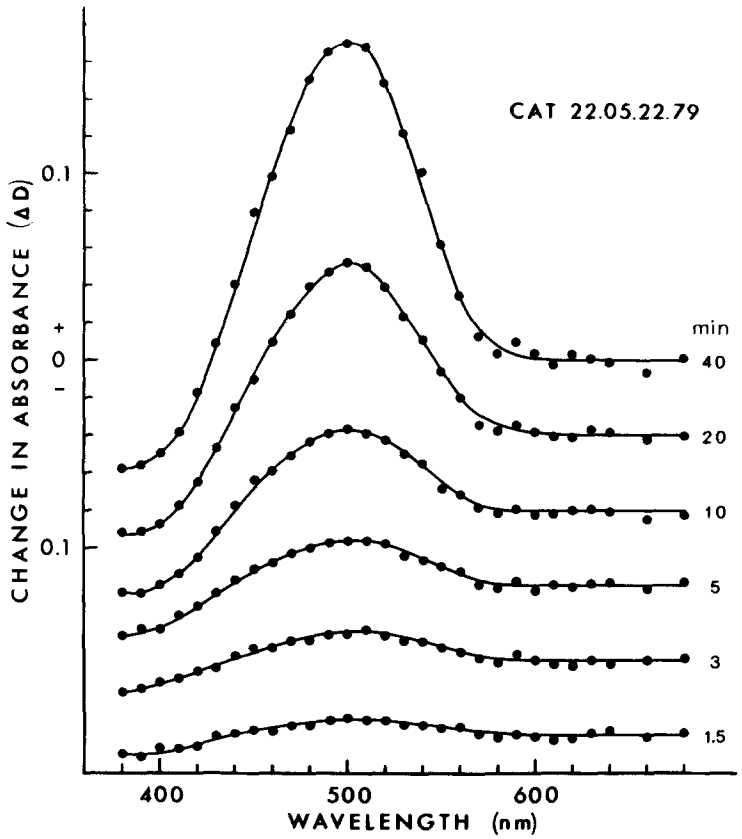


FIGURE 6. Density difference spectra obtained during dark adaptation after a 20-min exposure (9.74 log scotopic td-s) which bleached >99% of the available rhodopsin. The data for $t = 40$ min are correctly positioned on the scale of ordinates; all others are displaced downward for clarity. Note that one consequence of the long-duration exposure is the invariant position of the $\lambda_{max}$ in each set of data.

exponential function that describes the regeneration of rhodopsin in man (Rushton, 1961*b*; Alpern, 1971):

$$C_t = C_0 [1 - \exp(-t/\tau)], \quad (2)$$

where $C_t$ is the rhodopsin regenerated at time $t$ after the bleaching exposure, $C_0$ is the amount bleached by the exposure, and $\tau$ is the time constant of regeneration.

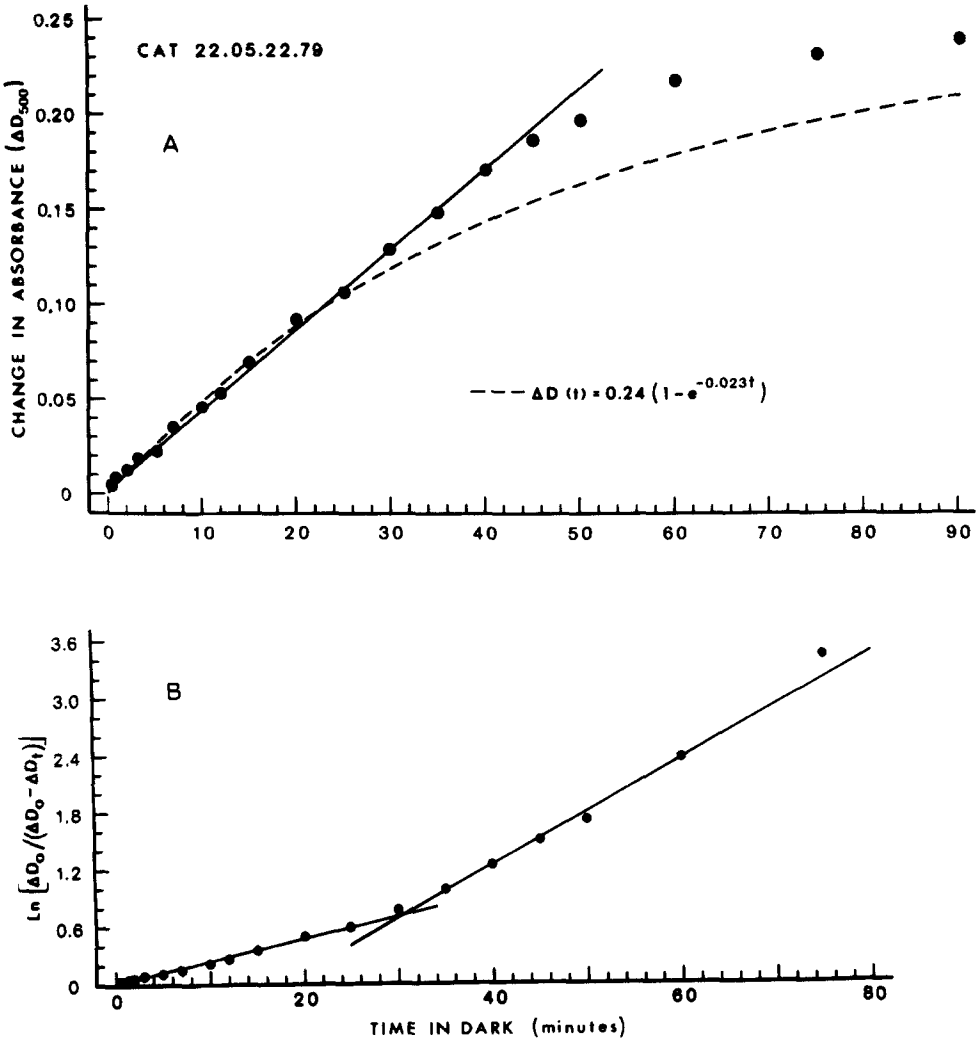


FIGURE 7. (*A*) The resynthesis of rhodopsin after an intense (6.66 log scotopic td), 20-min exposure. Data points represent the changes in absorbance at 500 nm recorded at various times in darkness. The dashed line is a graph of the exponential function shown to the right of the data; the straight line was fit by eye to the data obtained at $t < 30$ min. (*B*) The data of *A* replotted in transformed coordinates. See text for details.

Rearranging terms and eliminating the exponential leads to the expression

$$\ln\left[\frac{C_0}{C_0 - C_t}\right] = t/\tau. \tag{3}$$

The left side of Eq. 3 can be written in terms of the density difference

measurements ($\Delta D$) to give

$$\ln\left[\frac{\Delta D_0}{\Delta D_0 - \Delta D_t}\right]_\lambda = t/\tau, \tag{4}$$

where $\Delta D_0$ refers to the absorbance change at wavelength $\lambda$ produced by exposure to the bleaching field.

Fig. 7 *B* shows the regeneration data of Fig. 7 *A* graphed in the form of Eq. 4. It is clear that the data points are not satisfied by a single exponential function, i.e., a straight line with slope $\tau^{-1}$. Between zero and 30 min, regeneration kinetics are described adequately by an exponential with $\tau =$ 43.5 min; beyond this a second exponential is required, although the fit to the remaining points is less than perfect. Translating the first exponential to the conventional graph of Fig. 7 *A* (dashed curve) illustrates how well the exponential fits the early results. However, for $t > 30$ min the regenerative process proceeds more rapidly than predicted by a first-order reaction, and the data depart significantly from the curve. In fact, a straight line provides a far better fit to the $\Delta D$ values over the first 40 min of dark adaptation, after which the data approach asymptotically the maximum change in absorbance ($\Delta D_{500} \approx 0.24$) reached in ~90 min.

*Regeneration and Dark Adaptation*

The degree to which our data on the regeneration of rhodopsin parallels the recovery of visual sensitivity in cat is shown in Fig. 8. The sensitivity data (small filled symbols) are taken from threshold measurements for the *b*-wave of the electroretinogram (ERG) (Dodt and Elenius, 1960) and for on-center ganglion cells (Bonds and Enroth-Cugell, 1979) and are plotted logarithmically with reference to the left scale of ordinates; results on the resynthesis of rhodopsin (unfilled symbols) refer to the linear (percentage) scale at the right of the figure. Both parameters are graphed as functions of the time in darkness after various bleaching exposures.

Two points should be made in connection with a comparison of the results shown in Fig. 8. First, no attempt was made in the photochemical experiments to match precisely the bleaching conditions of the electrophysiological studies. And, second, the distinctly biphasic character of the recovery of ganglion-cell sensitivity after the more intense bleach (Fig. 8 *B*) is due to the fact that during the early portion ($t < 25$ min) threshold is subserved by the cone mechanism (Bonds and Enroth-Cugell, 1979). Nevertheless, the fractional bleaches in each half of the figure are very nearly the same; and for the rod-mediated portion of the electrophysiological data, there is a measure of agreement between the rates at which log sensitivity and the concentration of rhodopsin return to their prebleach levels. The correspondence between the two sets of data is even more impressive when one considers the fact that the measures of sensitivity are derived from such varied responses as the *b*-wave and the spike discharge of different classes of ganglion cell (Y-cell in *A*; X-cell in *B*).

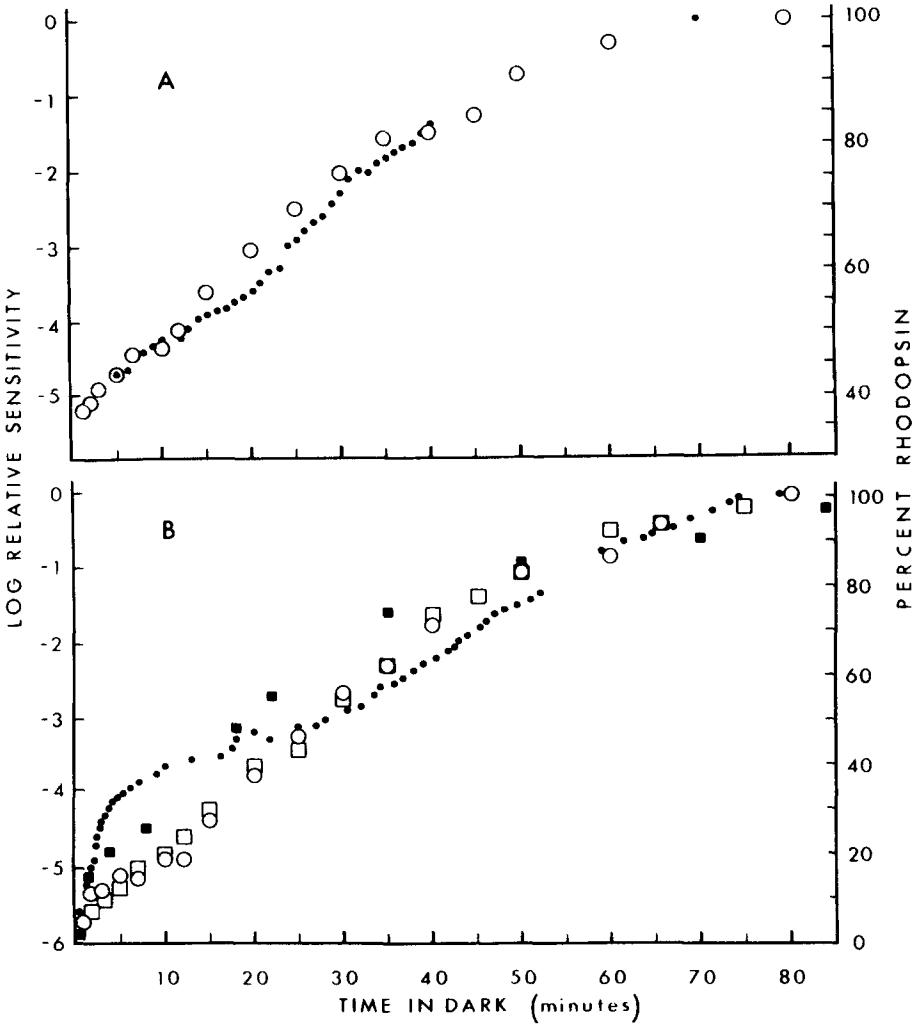


FIGURE 8. Rhodopsin regeneration (large symbols) and the changes in sensitivity measured electrophysiologically (small filled symbols) are plotted as functions of the time in darkness after comparable bleaching exposures. The lengths of the oridinates have been adjusted to equate the full extent of the log scale (*left*) with that of the linear scale (*right*). (*A*) Results obtained after 60–65% bleaches. (●) Ganglion cell. Bonds and Enroth-Cugell (1979). 60% bleach, 5.4 log scotopic td for 1 min. (○) Rhodopsin ($\Delta D_{540}$). 65% bleach, 5.36 log scotopic td for 1 min. (*B*) Data for 90–100% bleaches. (■) ERG. Dodt and Elenius (1960). 100% bleach, 6.02 log scotopic td for 15 min. (●) Ganglion cell. Bonds and Enroth-Cugell (1979). 90% bleach, 6.6 log scotopic td for 1 min. (○) Rhodopsin ($\Delta D_{540}$). 98% bleach, 6.82 log scotopic td for 1 min. (□) Rhodopsin ($\Delta D_{540}$). 100% bleach, 6.66 log scotopic td for 20 min.

### *The Effect of the Test Beams*

The divergence between the regeneration data presented here and the findings of Bonds and MacLeod (1974) is too great to be attributed to the normal variation encountered in biological study. A possible cause of the discrepancy is bleaching by the spectral measuring beams, i.e., it is possible that the monochromatic test beams of the reflectometer bleach in the course of measurement amounts of rhodopsin sufficient to retard regeneration.

It will be recalled that eight spectral scans are required for each recording (time vector) and that up to 22 such recordings were taken during the 60–100-min time period during which the rate of rhodopsin regeneration was determined. Although the eight scans are collected in ~3 s, the test beam is occluded by the opaque interfilter spaces for half of the time. Thus, the spectral lights are exposed for a total of ~1.5 s at each of the preselected times in darkness at which regeneration data are collected. Clearly, one way to answer the question as to the possibility of bleaching by the test lights is to (*a*) determine the retinal illuminance (scotopic troland) of each of the 29 spectral beams, (*b*) multiply each value by the 32-ms exposure that occurs during the collection of a time vector (the 4-ms spectral pulse times eight scans), (*c*) calculate the bleaching efficacy of each from the measured photosensitivity of cat rhodopsin (Fig. 2), and (*d*) then determine the fraction of the available rhodopsin bleached (bearing in mind that the quantity available varies throughout dark adaptation).

However, there is a less tedious and more direct method of demonstrating the innocuous behavior of the test beams—at least in so far as rhodopsin regeneration is concerned. Since 22 time vectors represent the maxium number of recordings taken during an entire dark adaptation run, the *total* exposure to the spectral test scans is $22 \times 1.5 = 33$ s, distributed over a period of more than 1 h. In one experiment, however, we allowed the test beams to be exposed *continuously* for 180 s, to a fully dark-adapted retina (with its full complement of rhodopsin). Reflection measurements were collected in the dark-adapted state and at 30-s intervals throughout the exposure period. Fig. 9 shows several of the resultant difference spectra, each of which represents the difference between the retinal transmissivity of the dark-adapted eye and that of the light-adapted eye after the indicated exposure duration. The data show that it required ~3 min of continuous exposure to effect a $\Delta D_{500}$ of ~0.01 density unit, i.e., to bleach <5% of the available rhodopsin. On the basis of the bleaching curve of Fig. 2, these results indicate that the mean retinal illuminance of the spectral test beams is ~3.34 log scotopic td.

## DISCUSSION

In this study we have shown that after extended bleaching exposures (1 and 20 min), the regeneration of rhodopsin follows the slow time-course expected from the findings reported in the preceding paper (Ripps et al., 1981). For the 1-min bleach (Fig. 1), the results are similar to those obtained after flash photolysis, i.e., the difference spectra are dominated during the early stages of

dark adaptation by the formation and decay of thermal intermediates (primarily metarhodopsin III). Thus, when the temporal changes in $\Delta D$ are plotted for any appropriate test wavelength, biphasic regeneration curves (Fig. 3) result. But it is apparent even in these data (cf. Fig. 5) that, after a large fraction (≥65%) of the available rhodopsin is bleached, the photopigment reforms at a very slow rate, requiring >60 min to reach completion.

A clearer picture of the kinetics of the regenerative process emerges when the absorbance changes obtained after 20-min bleaches are plotted in this way (Fig. 7). It is obvious that these data do not follow the exponential curve of first-order kinetics that describes rhodopsin regeneration in man (Rushton,

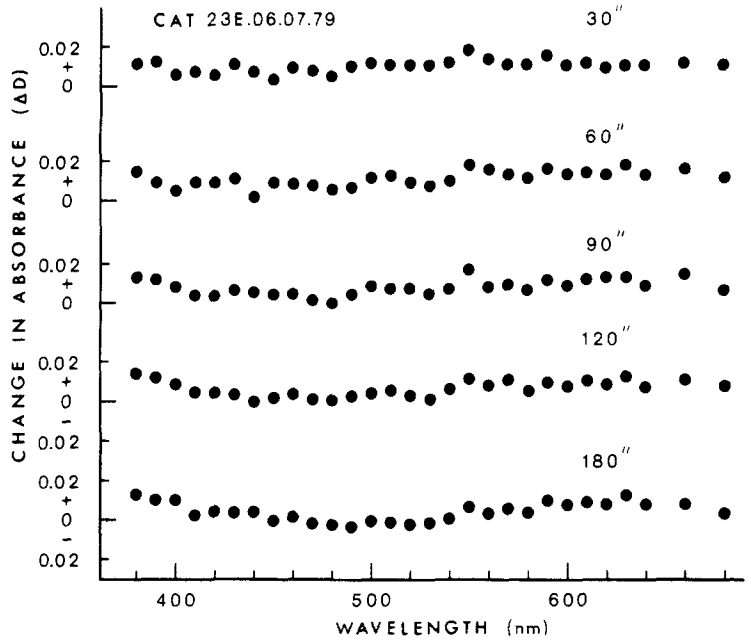


FIGURE 9. Density difference spectra obtained at various times during continuous exposure of the retina to the test beams. Each set of data shows the total change in absorbance that was produced from the start of the exposure (i.e., in the fully dark-adapted eye) up to the time indicated.

1961 *a*; Ripps and Weale, 1969 *b*; Alpern, 1971). Rhodopsin density rises linearly for the first 40–45 min at a rate of ~0.004 density unit/min and then slows abruptly to reach completion in ~90 min. The time-course in cat is, in fact, remarkably similar to that obtained in the albino rabbit by Rushton et al. (1955), who not only point out that the first half of regeneration proceeds at a uniform speed but state also "that in the rabbit regeneration takes some 80 min, and that it is much the same in the cat."

The linear increase in the dark suggests saturation at some stage in the sequence of the regenerative process. Hagins and Rushton (1953) have postulated that the rate-limiting step may be enzymatic; e.g., a high affinity

between enzyme and substrate that leads to occupation of all reaction sites on the enzyme causes the reaction velocity to become independent of changes in substrate concentration. Thus, for the early portion of the regeneration curve, when precursor concentration is presumably above the saturation level, reaction velocity would be constant. Alternatively, the linear rise in absorbance could result from a saturated carrier system (diffusion limited). Although this idea stems from results obtained in vitro with the delivery of retinol congeners to the rod outer segments (ROS) via a liposome carrier (Yoshikami and Nöll, 1978), the logic can be extrapolated to the in vivo system in which a retinol binding protein serves as the carrier of precursors between the pigment epithelium and the ROS (cf. Futterman et al. [1977]).

Zero-order kinetics is not the only means of accounting for the apparent linearity in the early stages of regeneration. The regenerative process may be subserved by parallel pathways in addition to a number of sequential reactions. For example, two parallel pathways, each of which obeys first-order kinetics, may be operating. One pathway begins to produce rhodopsin immediately upon cessation of bleaching, whereas the other enters after a delay; from then on, both contribute to the formation of rhodopsin, although the initial pathway ceases production before regeneration is completed. Such a situation might pertain if the first pathway involved the production of rhodopsin from a limited supply of precursor already present in the retina at the end of the bleach (cf. Cone and Brown [1969] and Baumann [1970]) while the delayed route represented rhodopsin formation from precursor transported to the ROS from the pigment epithelium. Although these notions are obviously speculative, the data of Fig. 7 *A* can be fit satisfactorily by an expression that incorporates two first-order reactions working in parallel to regenerate rhodopsin.

We should mention that neither the slow rate of regeneration nor the linear change in rhodopsin concentration after a bleach are peculiar to the cat and rabbit. These properties have been observed also in the rat (Lewis, 1957), frog (Peskin, 1942), skate (Dowling and Ripps, 1970), and bush baby.[1] Of the species studied thus far, only the regeneration of human rhodopsin appears to exhibit first-order kinetics and a significantly faster time-course, $\tau = 4$ (Ripps and Weale, 1969 *b*) to 6 min (Rushton, 1961 *b*). It is not clear whether this exception to the pattern adhered to by most other animals represents some modification of existing reaction pathways or the addition of an alternative route in response to evolutionary demands. In this connection, it is of interest that in fundus albipunctatus, a human night-vision disorder characterized by extraordinarily slow rates of dark adaptation, rhodopsin regeneration requires >2 h for completion, and for the first 100 min in darkness proceeds at a constant rate (Carr et al., 1974).

Before attempting to deal with the relation between the photochemical data and visual sensitivity, it is important to note the large disparity between our findings on rhodopsin kinetics and those of Bonds and MacLeod (1974). These authors found that complete regeneration after a full bleach was

[1] Ripps, H., and I. M. Siegel. Unpublished observation.

completed in 35 min, about half the time required in the experiments of Figs. 4, 5, and 7. We cannot point directly to the cause of this discrepancy, but the results shown in Fig. 9 demonstrate convincingly that the prolonged time-course of regeneration cannot be attributed to bleaching by the spectral measuring beams of our fundus reflectometer.

*Rhodopsin and Visual Adaptation*

Most investigators who have undertaken a comparison between the regeneration of rhodopsin and some postreceptoral measure of dark adaptation (e.g., *b*-wave, ganglion-cell response, or subjective thresholds) have found that the two are related over most of their time-courses in accordance with the log-linear expression first proposed by Dowling (1960):

$$\log (I_t/I_0) = k(C_0 - C_t)/C_0, \tag{5}$$

where $C_t$ and $I_t$ are, respectively, the rhodopsin concentration and threshold intensity at time $t$ during dark adaptation, $C_0$ and $I_0$ represent these parameters in the fully dark-adapted eye, and $k$ is a constant of proportionality.

The relationship applies to man, where regeneration and log threshold are exponential functions of time (Rushton, 1961 *a*; Alpern, 1971), as well as to the rat (Dowling and Wald, 1960; Dowling, 1963; Perlman, 1978), skate (Dowling and Ripps, 1970), and frog (Baumann, 1967), where the two processes follow initially a linear time-course. The fact that Eq. 5 is limited with regard to the range over which it can apply is well known (cf. Dowling and Ripps [1970], Hollins and Alpern [1973], Ripps and Weale [1976], and Bonds and Enroth-Cugell [1979]). Nevertheless, the results shown in Fig. 8 demonstrate that good agreement with the predictions of Eq. 5 is obtained in cat when bleaching by the preadapting exposure is appreciable (i.e., $C_B \geq 65\%$).

In addition to the sensitivity data shown in Fig. 8, there are other experimental studies of the cat which show the prolonged temporal course over which electrophysiologically determined thresholds dark adapt (Granit et al., 1939; Barlow et al., 1957). On the other hand, the one behavioral study of dark adaptation in cat (La Motte and Brown, 1970) produced a faster time-course, not unlike that obtained for human vision, but the bleaching and test conditions were less than ideal, and the results may not be directly comparable with those of the present study. In any event, the significance of any parallelism between rhodopsin regeneration and the recovery of visual sensitivity in darkness will remain obscure until we better understand the rate-limiting steps in the regenerative process and the mechanisms by which bleached rhodopsin influences visual threshold.

We are grateful to Miss Jane Zakevicius for her assistance during the course of this study and for her help in the preparation of the manuscript.

This work was supported by research grant (EY 00285) and a research center award (EY 01842) from the National Eye Institute, U. S. Public Health Service, by the Retinitis Pigmentosa Foundation, and by an unrestricted award from Research to Prevent Blindness, Inc.

*Received for publication 23 June 1980.*

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