Dark Adaptation and Visual Pigment Regeneration in Human Cones

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ABSTRACT Foveal threshold elevation and red-green cone pigment regeneration have been studied in the dark after a wide range of bleaches in normal man with a view to probing the limits of the application of the Dowling-Rushton relation: i.e., the direct proportionality between log threshold elevation and fraction of unregenerated pigment. Cone pigment regeneration (and threshold recovery) is much faster after short bleaches than expected from the kinetics of a simple monomolecular reaction. Recovery is faster after a fixed (short) duration bleach the weaker it is. Except for the first 30 s after relatively weak bleaches and the entire recovery after a very brief (<0.001 s) saturating bright flash which bleaches a little more than 50%, the results are accurately fit by the Dowling-Rushton relation over the entire range tested with only one arbitrary constant (the proportionality factor). Theory predicts too low threshold in comparison with what is obtained, for both of these exceptions

It is a familiar fact that the recovery of visual sensitivity in the dark after significant bleaching proceeds only very slowly, and it was long presumed that this was because the visual pigment regenerated at a corresponding rate. However, experimental attempts to provide the justification for this presumption for rods went unrewarded until Dowling (1960), working with the electroretinogram of the rat, and Rushton (1961), measuring psychophysical thresholds on a human subject deficient in cones, found a linear relationship between the log threshold and the fraction of unregenerated rhodopsin. Their equation can be written

$$\log (E_t/E_a) = \alpha(1 - p). \qquad (1\ a)$$

E_t is the intensity of a threshold flash at any moment t in the dark after a bleach, E_a is the value of E_t found after full dark adaptation, p is the fraction of pigment present, and α is a constant. This equation has since been verified

for rhodopsin both in normal persons (Alpern et al., 1970; Alpern, 1971; Rushton and Powell, 1972 *a*) and in the skate (Dowling and Ripps, 1970). In man α has a value somewhere between 12 and 37, while it is about 5 in skate.

For human cones the matter is less clear. Rushton (1963, 1965 *a*; Baker and Rushton, 1965) found that Eq. 1 *a* (with α equal to about 3.0) described the recovery after full bleaches, but we are cautioned that it ". . . does not claim to describe other manoeuvres" (Rushton, 1965 *a*, p 42). Though foveal cone pigments follow simple monomolecular regeneration kinetics (Rushton, 1958) over a wide (if not the whole) range of bleaching circumstances (Alpern et al., 1971) it is known that the time constant of the exponential curve which describes the recovery of log foveal sensitivity in the dark varies with the intensity and the duration of the bleach (Mote and Riopelle, 1951). Furthermore, a bright 1 ms flash elevates foveal threshold in the dark for more than 10 min, although virtually all of the cone pigment has regenerated after only 3. This is known as the theta effect (Rushton and Baker, 1963; Rushton, 1964).

Considerations such as these no doubt give rise to Weale's (1972) skepticism as to ". . . the splendid correlation claimed to exist between foveal dark-adaptation and cone pigment regeneration." We have therefore taken the matter up once more, testing the validity of Eq. 1 *a* for cones by measuring both foveal cone pigment regeneration and dark adaptation over a wide range of bleaching conditions on the same subject.

METHODS

Dark Adaptometer

A Maxwellian view optical system was used to bring the light from a xenon arc to a focus in the observer's pupil. There were two channels, which merged at a beam splitter: one for bleaching, the other for testing. Both the 7° adapting field and the 1° test spot were centered on the fovea. Fixation in the former case was maintained with crosshairs, and in the latter by means of four grain-of-wheat lamps mounted in the target plane symmetrically flanking the test spot. The observer kept these fixation lamps as dim as was consistent with clear visibility. The bleaching exposure was regulated either manually (for long bleaches) or with an electronic shutter controlled by a Hunter timer (Hunter Mfg. Co. Inc., Iowa City, Iowa). The test beam was interrupted by a sector disk in such a way that the 83 ms flash appeared twice a second. A red (675 nm) narrow-band interference filter with an appropriate blocking filter was used in this channel throughout the experiments. Test luminance was adjusted with neutral-density filters and a balanced neutral-density wedge under the observer's control. The adapting beam was also attenuated with neutral-density filters, and with an infrared-blocking filter. The arc images of the test and adapting channels were superimposed in the plane of the observer's pupil. This pencil of rays was approximately 2 mm wide as it passed through the center of the pupil, which was always dilated with 0.5% Mydriacyl, Alcon Laboratories, Inc., Ft. Worth, Tex.

Densitometer

This ophthalmoscopic densitometer, similar to the one developed by Hood and Rushton (1971), has been described earlier (Alpern et al., 1971). It involves a measuring light in which two beams, differing in color, are sinusoidally alternated. The deep red beam is mainly composed of infrared light which is only trivially absorbed by visual pigments, and the amount of it reflected back through the pupil is not measurably dependent on the state of the eye; but the amount of green light reflected is a function of the amount of photolabile pigment on the fundus. If the output of the photocell is not to fluctuate sinusoidally as the two beams alternate, the experimenter must make on-line changes in the intensity of the red beam—changes paralleling those which the retina brings about in the green beam. The adjustment of the red beam which the experimenter finds to be necessary constitutes the datum. The measurements are relatively imprecise, but they allow an estimate of the fraction of pigment present under any given set of conditions with an error of just under 10% of the maximum pigment density. A bleaching beam, not given access to the photocell, is also present.

Procedure

The authors served as observers in the psychophysics. M. Hollins alone was the subject in the densitometry.

In the psychophysics, the dark-adapted observer was exposed to a bleaching light for some predetermined length of time. At the offset of this light, the observer began adjusting the test flash to threshold by moving the wedge. When he had achieved an acceptable setting, he signalled the experimenter, who noted both the time and the position of the wedge. During the 5 min or so that threshold continued to drop, about a dozen settings were made.

Measurements in the densitometer were made in a similar way: after the administration of a bleach, the experimenter recorded the acceptable settings of the red wedge, together with the time at which these settings were made.

The use of a very narrow band of radiation centered at 675 nm as a test flash insures that what is principally studied by threshold measurements is the recovery of sensitivity of the red cone mechanism—the π_5 of Stiles (1939, 1959); Du Croz and Rushton (1966). On the other hand, in densitometry the measuring wavelength was 565 nm and no doubt the kinetics of bleaching and regeneration of chlorolabe in the green cones as well as erythrolabe in the red cones were under observation. Rushton (1958, 1963, 1965 *a*) has shown, however, that the photosensitivity and time constant of regeneration of each are so similar to those of the other that with white light bleaching the characteristics of both can be studied as though they were but a single pigment. In this paper, for simplicity, the bleaching and recovery of erythrolabe is referred to as if it alone was examined, although the densitometer, used in this way, in fact always studied both together.

Photometry

The bleaching light was caused to illuminate a white diffusely reflecting illuminometer test plate (reflection coefficient 0.82) placed at a fixed (20 cm) distance from the

aperture stop image normally occupied by the observer's pupil. The luminance of the bright patch of light was then measured with an S.E.I. photometer (Salford Electrical Instruments Times Mill, Heywood, Lancashire, U.K.).

To determine the retinal illuminance of the xenon flash, it was viewed in a Maxwellian view optical system by an observer whose widely dilated pupil (2 drops 1 % Mydriacyl) was slightly larger than the image of the flash tube in the pupil plane. The beam was then attenuated by calibrated Inconel neutral filters and a Wratten #96 wedge (Eastman Kodak, Rochester, N. Y.) and 10 measurements of absolute threshold for the fully dark-adapted retinal rods (15° on the temporal retina from the fovea) were obtained. The xenon flash was then replaced by a 6 V, 18 A incandescent tungsten ribbon filament, and the measurements of dark-adapted rod threshold for 97-ms pulses of light repeated on the same subject with the same (10° in diameter) circular test patch and on the same retinal position. The observer's eye was then replaced by the white illuminometer test plate 20 cm from the tungsten filament image and (after removing all filters) the brightly illuminated plate photometered in the usual way. Assuming the mean rod thresholds obtained in these two successive conditions were identical, the areal density of luminous energy incident on the retina from the full xenon flash could be determined by a straightforward calculation. It was 7.582 log scotopic troland seconds (td-s). To convert from scotopic to photopic units we obtained the spectral distribution of the xenon flash (E_λ) at 15 wavelengths through the visible spectrum by imaging the tube on a calibrated Pin-10 silicon photodiode after attenuating the beam by narrow-band Baird Atomic interference filters (Baird Atomic, Inc., Bedford, Mass.). (We are indebted to Dr. E. N. Pugh, Jr. for these measurements.) The transmission characteristics of each filter was measured with a Beckman Acta II recording spectrophotometer (Beckman Instruments, Inc., Fullerton, Calif.). The conversion factor was then calculated by the formula

$$[2.567 \int V'_\lambda E_\lambda d\lambda / \int V_\lambda E_\lambda d\lambda]^{-1},$$

in which V_λ and V'_λ are the ordinates of the Commission Internationale de l'Eclairage photopic and scotopic luminosity curves, respectively (Wyszecki and Stiles, 1967). The log of the correction factor so computed was −0.278. In bleaching, the eye viewed the flash directly (not in Maxwellian view). This effects the calculation in two ways: It increases the log of the areal density of luminous energy in bleaching by the difference between the logarithm of the pupil area (fully dilated to 8 mm) when bleaching and the logarithm of the area of the flash tube image in the pupil when viewing it in Maxwellian view (about 6.5 mm). 2 log (8/6.5) = 0.180. However, because of the Stiles-Crawford Effect the light going through the edge of the pupil is not as effective in bleaching cones as is light going through the center and the *effective* areal density of luminous energy is less than its actual areal density by a factor (49.5/24.5) = antilog 0.305. Hence, the effective areal density of luminous energy (neglecting any transclera illumination) incident on the retina from the flash tube in bleaching was 7.17 log *effective* td-s.

Curve Fitting

The pigment regeneration curves were fit by single exponential functions by plotting $1 - p$ on a logarithmic scale vs. linear time. That one straight line estimated by eye to give the best fit to the data was drawn through the results. The value of t_o could be directly measured from the slope of this line and the value of p_o determined from the value of $(1 - p)$ where the straight line intersected the ordinate axis ($t = 0$). For threshold log E_t − log E_a was plotted on a logarithmic scale against time in the dark (on a linear scale) and a straight line drawn through the results in a similar way. The value of the slope of this line gave t_o of the dark-adaptation curve. The ordinate intercept of this latter line was divided by $(1 - p_o)$ to obtain the estimate of α.

RESULTS

The kinetics of bleaching and regeneration of the foveal red and green cone pigments have been extensively studied (Rushton, 1958, 1963, 1965 *a*; Rushton and Henry, 1968; Alpern et al., 1971). Suppose the fovea at time $t = 0$ is suddenly exposed to a light of intensity I which may assume any value (including zero) and which then remains constant. Then (over a range of conditions) the proportion of red (or green) cone pigment (p) present at time (t) is given by the equation

$$p(I + I_o) = I_o + [p_o(I + I_o) - I_o]e^{-t(I+I_o)/t_oI_o}, \quad (2)$$

where p_o is the fraction of pigment present when $t = 0$, and I_o and t_o are other constants, discussed below. All bleaching began with the fully dark-adapted fovea ($p_o = 1.0$). Under these conditions Eq. 2 reduces to

$$p(I + I_o) = I_o + Ie^{-t(I+I_o)/t_oI_o}. \quad (2\,a)$$

Eq. 2 *a* can be used to determine the fraction of erythrolabe (and/or chlorolabe) present after a range of bleaching conditions once its two constants I_o and t_o have been determined. I_o is the retinal illuminance which bleaches half the pigment at equilibrium. It has been determined by measuring the amount of pigment present at equilibrium for a variety of different intensities (Rushton, 1958, 1963, 1965 *a*). The results of one such experiment on M. Hollins are shown by the open circles in Fig. 1. According to Eq. 2 *a* when t is infinity,

$$p = I_o/(I + I_o). \quad (3)$$

The smooth curve drawn through the circles in Fig. 1 has the form defined by Eq. 3 where I_o is 3 $(10)^4$ td.

In the dark, $I = 0$, so that Eq. 2 reduces to

$$(1 - p) = (1 - p_o)e^{-t/t_o}, \quad (2\,b)$$

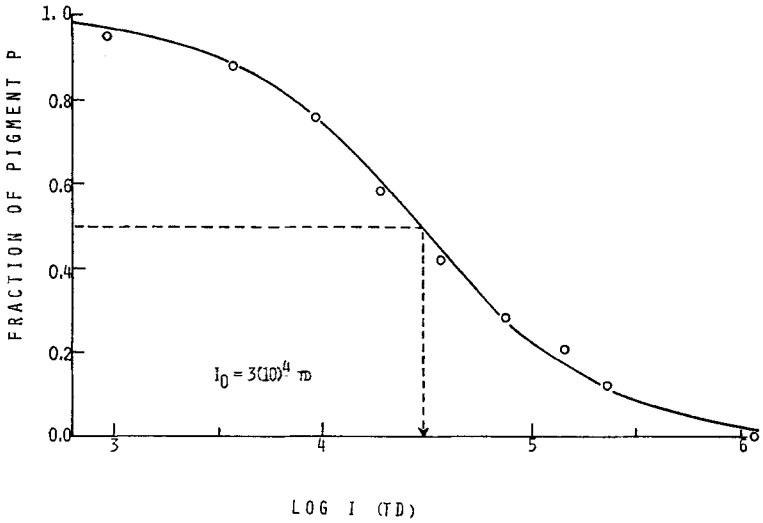

FIGURE 1. Fraction of erythrolabe bleached at equilibrium by lights of different intensities. The experiment repeats for M. Hollins the experiment of Rushton (1958). The dark-adapted retina was exposed for 10 s to a brighter light, after which the appropriate filter (which reduced the retinal illuminance to that required to hold the amount of pigment constant at the level reached by the end of the 10 s initial exposure) was introduced and the measurements were made with the densitometer. I_o, the light level required to bleach 50% of the erythrolabe at equilibrium, is $3.0(10)^4$ td.

and it is evident that t_o is the time constant of the exponential curve which describes the time-course of the recovery of the full dark complement of erythrolabe. The filled symbols in Fig. 2 *a* show the regeneration in the dark of foveal visual pigment after long full and partial bleaching. All of the results showing recovery after equilibrium bleaching (of which the single runs shown in Fig. 2 *a* are only a representative sample) are well described by a single exponential curve with a time constant of 105 s.

These two constants have been substituted in Eq. 2 *a* to estimate the fraction of pigment present at the end of the exposure of a variety of bleaching intensities and durations. Many, but not all, of these calculations have been verified by actual measurement of the fraction of erythrolabe bleached and wherever it has been tested the results confirmed the prediction (within the precision of measurement). Thus, there are no practical restrictions on the use of Eq. 2 to estimate the amount bleached in these experiments, though this equation (and the monomolecular kinetics, on which it is based) has been shown to be limited in its ability to describe regeneration of cone pigments (Rushton and Henry, 1968; Rushton et al., 1969; Alpern et al., 1971).

I. *Recovery after Equilibrium Bleaches*

If the dark-adapted fovea is suddenly exposed to a steady light of intensity I, the pigment bleaches along an exponential curve whose time constant is seen

in Eq. 2 *a* to be $t_o I_o/(I + I_o)$. For small values of I the equilibrium value of p is reached only very slowly. To obviate the long fixation required for such bleaches, we have sometimes resorted to the expedient of bleaching for 10 s with a brighter light which brought p to the desired level at the end of the 10th s; at this moment a filter, so selected that the new intensity (I) held p at this same level indefinitely, was introduced. After 1 min further adaptation to this new level the bleaching light was extinguished and dark adaptation (or pigment regeneration) was studied. It is not evident a priori that these two ways of bleaching will be followed by identical dark adaptation curves, even

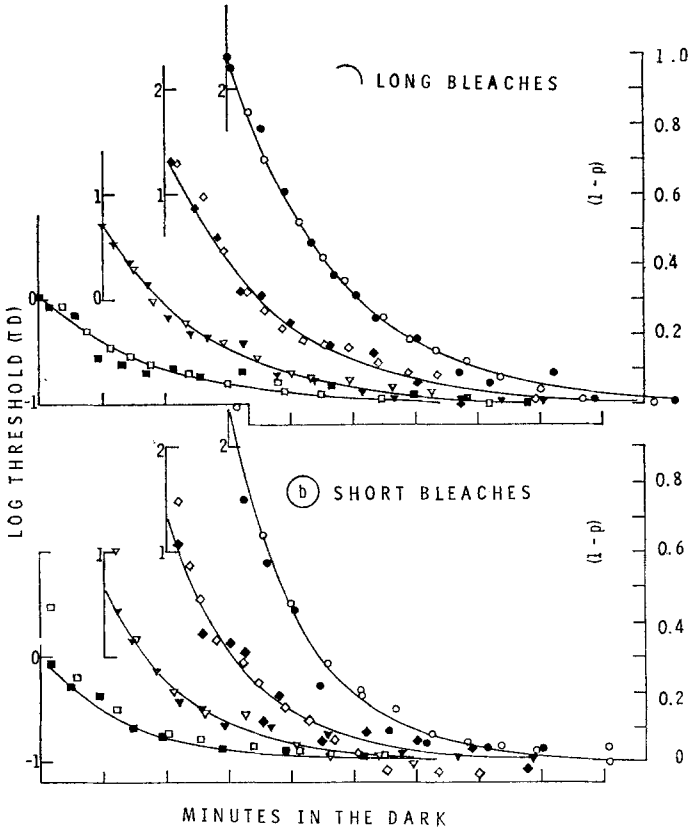

FIGURE 2. Log π_5 threshold elevation (open symbols, ordinate scale to the left) and foveal pigment regeneration (solid symbols, scale to the right) curves after 30% (squares), 50% (triangles), 70% (diamonds), and full (circles) bleaches. Each plotted point is a single measurement. The results for each successively more intense bleach have been shifted 60″ to the right, in succession, in order to obviate confusing overlap of the points near full regeneration. (*a*) Recovery after prolonged (equilibrium) bleaches. (*b*) Recovery after short exposures (9.3 s for the 30% and 50%, 8.7 s for the 70%, and 10 s for the full, bleach). The smooth curve shows prediction of Eq. 1 *a* with $p(t)$ defined by Eq. 2 *b*, with $\alpha = 3.33$ and with $t_o = 105$ s (above) and 73 s (below), respectively. Each abscissa marker corresponds to 1′.

though the fraction of pigment present at the termination of the bleach in the two instances was identical. In fact, however, within the precision of the measurements, the curves so obtained were found to be identical.

What features characterize the time-course of regeneration after equilibrium bleaches of different fractions of the pigment? Alpern et al. (1971) showed that regeneration from prolonged partial bleaches follows an exponential curve with the same time constant (t_o) independent of the value of p_o, as is predicted from Eq. 2 *b*. New data to support the same conclusion are shown by the filled symbols in Fig. 2 *a* which give individual measurements of pigment regeneration after 30% (squares), 50% (triangles), 70% (diamonds), and 100% (circles) long equilibrium bleaches. In this figure, each successive set of curves has been arbitrarily shifted 60″ to the right with respect to its immediate left-hand neighbor to avoid intermingling of the data points near full regeneration. That Eq. 1 *a* also validly describes the relation between threshold and pigment for these partial bleaching conditions is shown by the open symbols in Fig. 2 *a*, which represent results of single sets of threshold measurements (scale to the left) after the identical bleaches. Single

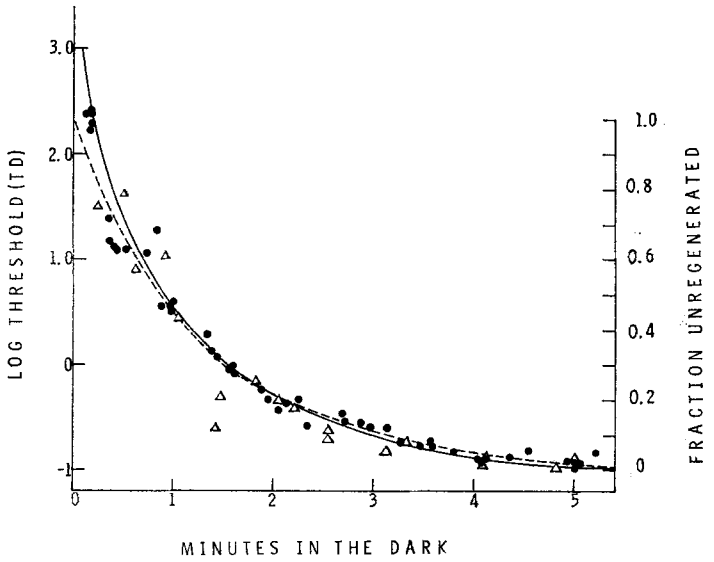

FIGURE 3. π_5 foveal dark adaptation (filled circles, ordinate scale to the left) and foveal erythrolabe regeneration (triangles, ordinate scale to the right) after a prolonged full bleach. The ordinates have been scaled so that the two sets of data should coincide according to Eq. 1 *a*, with E_a = 0.1 td and α = 3.33. The dashed curve describes this same prediction. The solid curve is based on Eq. 1 *b*, with E_a = 0.1 td and α = 2.9 and is intended to be compared with the psychophysical data only. For both curves t_o = 105 s and in each case the time-course of the change in $(1 - p)$ is defined by Eq. 2 *b*. Either curve provides a satisfactory fit to the threshold data. Each plotted point is a single measurement.

exponential curves with a time constant of 105 s describe both dark adaptation and pigment regeneration results. In Fig. 2 each 10% of the pigment bleached is associated with 0.33 log unit threshold elevation.

The agreement between pigment recovery and threshold expected from Eq. 1 *a* should not be pressed too far. According to Eq. 1 *a*, when $p = 0$ the threshold in Fig. 2 would be elevated about 3.33 log units. In fact, when $p = 0$ there will be no pigment left to catch the quanta, and the test flash must be invisible no matter how intense it is made. According to the simplest idea it would be the log of the elevation of the threshold quantum catch rather than log (E_t/E_a) which is proportional to the fraction of unregenerated erythrolabe. That is

$$\log \frac{E_t(1 - e^{-\beta p})}{E_a(1 - e^{-\beta})} = \alpha(1 - p).$$

This equation cannot be solved without knowledge of the in vivo Naperian density (β) of erythrolabe in normal foveal cones. Fortunately, however, the matter is made simpler by testing at 675 nm, a wavelength at which it is known (Alpern and Torii, 1968) that the fraction of incident quanta absorbed by erythrolabe is only 2.7% of the fraction of incident quanta erythrolabe absorbs at its λ_{max}. Even if we assume an improbably high (Miller, 1972) density of erythrolabe at its λ_{max}, say $\beta_{\lambda_{max}} = 3.0$, straightforward calculation shows that β_{675} is only about 0.026, a value so small that one can neglect the higher order terms in the series expansion of $e^{-\beta_{675}}$

Hence, the above equation can be written as

$$\log (E_t/E_a) = \alpha(1 - p) - \log p \qquad (1\ b)$$

without loss of generality for the experiments with test wavelength 675 nm.

Clearly the discrepancy between the expectation of Eq. 1 *a* and that of 1 *b* is greatest after a full bleach. Fig. 3 shows dark adaptation (solid circles) and erythrolabe regeneration (triangles) after identical long full bleaches. The dotted smooth curve drawn through these results has the form of Eq. 1 *a* and the solid curve that of Eq. 1 *b*, where in each case $(1 - p)$ is defined by Eq. 2 *b*. The two curves are remarkably alike and for this condition where the discrepancy is expected to be the largest, one is hard pressed to defend the fit to the psychophysical data of one curve as opposed to the other given the imprecision of the measurements. What makes this paradoxical result possible is that the constant α has no a priori contraints, and we are free to select it so that the measurements are best described by the equation. For the results in Figs. 2 and 3 where Eq. 1 *a* was used, $\alpha = 3.33$; for those in Figs. 3 and 4 where Eq. 1 *b* was used, $\alpha = 2.9$. Hence, the important theoretical distinction between Eqs. 1 *a* and 1 *b* can only assume predictive value in practice if the

value of α has been predetermined by independent experiments. Without such a limitation either provides an equally satisfactory description of the results. Hence, in what follows this empirical relation is referred to as "Eq. 1."

We have not tried to follow pigment regeneration after equilibrium bleaches at levels other than those shown in Fig. 2 *a*. For levels intermediate to those in the figure, the measurements are straightforward, if monotonous, but the matter has already been extensively tested by Alpern et al. (1971), and the close agreement between the present results and those found in that study wherever they have been compared leaves little doubt that Eq. 2 *b* would describe additional results as effectively as it does the results shown by the filled symbols in Fig. 2 *a*. However, for equilibrium bleaches smaller than about 20%, the level of bleaching is too small with respect to the size of the measurement error (nearly 10% of the maximum pigment density) to expect an exact evaluation of the time-course of regeneration with existing techniques of densitometry. Assuming that the validation both here and by Alpern et al. (1971) of Eq. 2 *b* as a description of regeneration from all equilibrium bleaching levels which have been examined justifies its application to regeneration from those equilibrium bleaches where it has not been, and/or cannot be, tested, the study of the validity of Eq. 1 was pursued by dark adaptation alone.

The results of these measurements are shown in Fig. 4, in which log threshold is plotted against time in the dark. Data for different strength bleaches have been slid horizontally for clarity, the moment of bleach offset in each case being indicated at the top of the figure.

The bleaching in this case was always of the two-stage variety (10 s of a bright light followed by a 1 min exposure to a weaker light whose intensity was selected from Eq. 2 *a* to hold the pigment at the desired level). The individual settings from five experimental repetitions are shown in the figure for each condition.

A single smooth curve whose form is given by Eq. 1 *b* with $\alpha = 2.9$ and $\log E_a = -1.0$ log td has been drawn through all of these results. No further adjustment (either vertically or horizontally) of curve to data has been carried out. The agreement is reasonable except in the first 30 s after relatively weak bleaches when thresholds are consistently higher than the theoretical expectation.

II. *Recovery from Short Bleaches*

Dark adaptation after short bleaches proceeds much faster than after long ones, even when the level of pigment is essentially the same at the moment the bleach is terminated. Is this because the value of t_o in Eq. 2 *b* is reduced (Rushton and Henry, 1968; Rushton et al., 1969; Alpern et al., 1971), because Eq. 1 is no longer valid, or for both these reasons? The results in Fig. 2 *b* answer this question in favor of the first possibility. They show both pigment

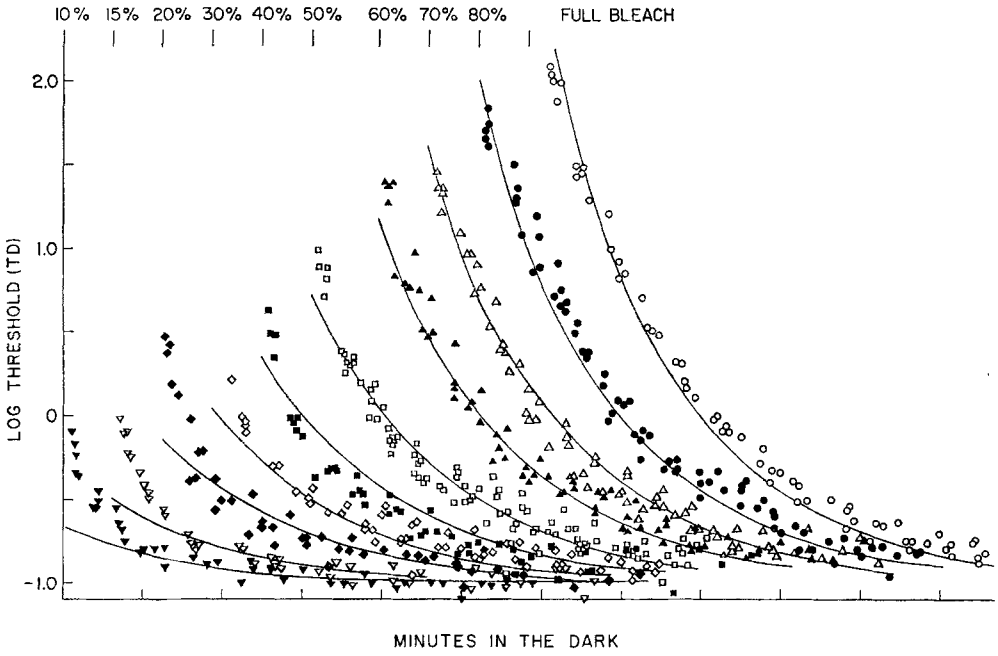

FIGURE 4. Foveal π_5 dark adaptation curves after prolonged exposures (10 s of a brighter light followed by 1 min at the intensity which at equilibrium held the bleaching level at the indicated value: open circles—100%, filled circles—80%, open erect triangles—70%, filled erect triangles—60%, open squares—50%, filled squares—40%, open diamonds—30%, filled diamonds—20%, open inverted triangles—15%, and filled inverted triangles—10%). The smooth curve is defined by Eq. 1 *b* and with E_a = 0.1 td, α = 2.9 and t_o = 105 s. No further vertical or horizontal scaling of curve to data has been performed. For clarity, the results (and curve) after each successively brighter bleach have been shifted progressively to the right with respect to their nearest left hand neighbor 37.5 s for all results except those after 60% bleach which alone have been shifted 50 seconds to the right. To assist in visualizing this shift the vertical lines at the top indicate the 0 abscissa scale for each dark adaptation curve. Each point represents a single measurement. Each abscissa marker below corresponds to 1′.

regeneration (solid symbols) and dark adaptation (open symbols) after short (about 10 s) bleaches which removed 30% (squares), 50% (triangles), 70% (diamonds), or 100% (circles) of the foveal erythrolabe. Each plotted point represents the result of only a single measurement.

Rushton and Henry (1968) and Alpern et al. (1971) have already shown that the regeneration rate after short full bleaches is approximately twice that after long full bleaches, and Rushton et al. (1969) demonstrated that threshold follows Eq. 1 *a* after short full bleaches as faithfully as it does after long full bleaches. The results in Fig. 2 *b* confirm these observations and extend them to short partial bleaches as well.

The time constant of pigment regeneration and the time constant of

the exponential recovery of log sensitivity during dark adaptation were compared for a variety of other (nonequilibrium) bleaching circumstances. The results are plotted in Fig. 5 in which each plotted point is the mean of at least five experimental repetitions of both pigment regeneration and dark adaptation measurements. In this figure, the ordinate shows t_o (in seconds) of the pigment recovery curve, the abscissa that of dark adaptation. With the single exception of the filled circle (c.f. below), the two kinds of measurements are well correlated, falling very close to the perfect correlation line of 45° slope extending between about 65 s (the lower limit for short bleaches) and 105 s (the time constant of recovery after equilibrium bleaches). The solid circle shows the results after a very short (less than 1 ms) bright xenon flash (Strobonar 65 A). We confirm Rushton and Baker (1963) that such brief saturating (7.17 log effective td-s) flashes bleach a little more than ½ the measurable foveal pigment and that the rate of pigment regeneration is then fast (t_o = 64 s) though the recovery of log sensitivity in the dark is drastically slowed. The dark adaptation curves, as Rushton and Baker (1963) showed,

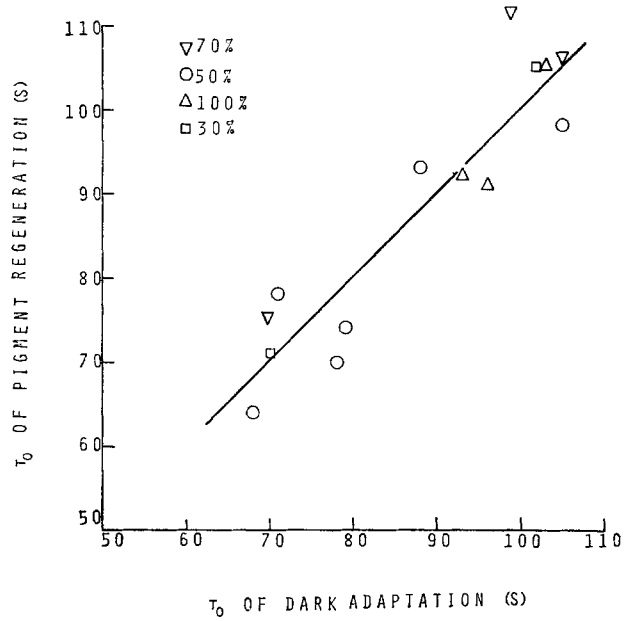

FIGURE 5. Time constant (in seconds) of foveal pigment regeneration is plotted against time constant (also in seconds) of log π_5 dark adaptation. Each point represents a different bleaching condition and is based on about five dark adaptation runs and a comparable number of runs in the densitometer. The different symbols represent different levels of bleaching: 100% (△), 70% (▽), 50% (○), 30% (□). The filled circle is a 50% bleach obtained with an extremely bright (7.17 log effective td-s) and short (<0.001 s) xenon flash. The line shows the expected locus of points if Eq. 1 *a* held exactly and there were no experimental variability.

have a break after several minutes in the dark. (It is the time constant of the first of the two curves, 105 s, that is plotted in Fig. 4.) An interpretation of this result is to be found in Rushton (1964).

III. Intermediate Bleaches

In order to define those conditions in which the value of t_o changes from that found in the recovery from equilibrium bleaches (Figs. 2 *a* and 4) to those

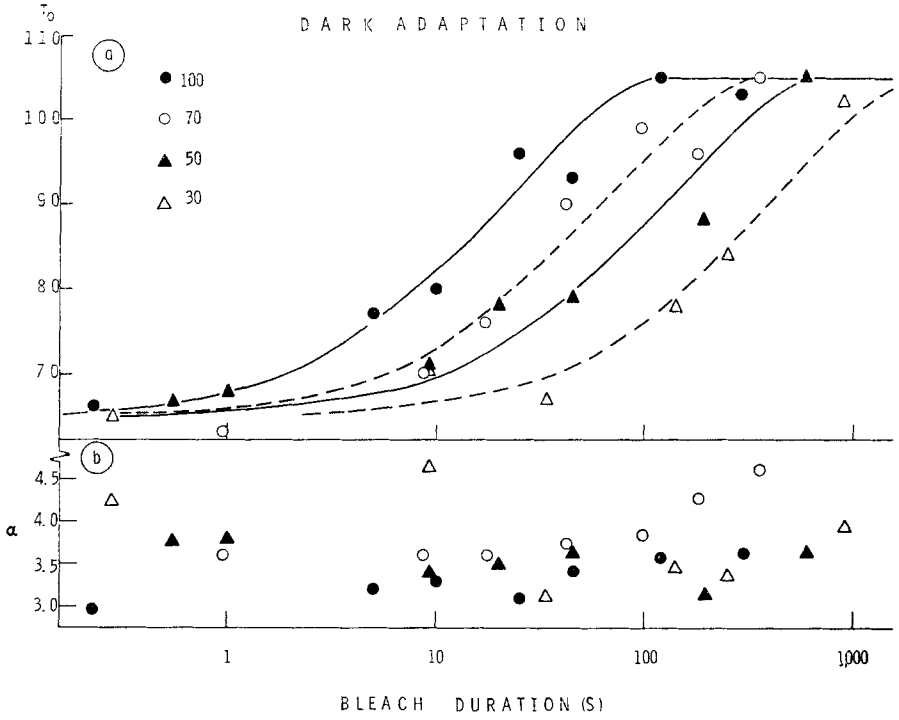

FIGURE 6. (*a*) The time constants (in seconds) of exponential log π_5 dark adaptation curves are shown as a function of bleach duration (logarithmic scale). Bleaches removed either 100% (●), 70% (○), 50% (▲), or 30% (△) of the foveal pigment. Each point summarizes five or more runs. The same curve, drawn by the eye, has been slid horizontally for the best fit with the different sets of points. Fig. 6 *b* shows the value of α in eq. 1 *a* for these same dark adaptation curves.

obtained in the recovery from short bleaches (Fig. 2 *b*), a variety of intermediate bleaches were studied by dark adaptation. The results from those in which pigment regeneration was also followed have been used to plot Fig. 5.

Fig. 6 *a* shows how the time constant of the resulting dark adaptation curves varied with the bleach duration for full (solid circles), 70% (open circles, 50% (solid triangles), or 30% (open triangles) erythrolabe bleaches. A smooth curve has been drawn through each set of symbols to show the time constant as a function of the log of the bleach duration when the fraction

bleached by the adapting light is held fixed. The same curve has been drawn through each set of results but displaced successively to the left for the successively higher bleaching fractions. In general, in order to obtain a given value of t_o a longer bleach duration was required for a weak than for a strong bleach. This trend is not obvious in the individual measurements shown in Fig. 2 *b* because the bleach duration in that figure is near the point where the curves converge.

Although only one value of α (3.33) was used in constructing all of Fig. 2, small differences in α were found from run to run, from observer to observer, and from one bleaching level to the next. These could not be related in any meaningful way to the parameters of bleaching, and the best guess is that they are random variations. Average values of α are shown in Fig. 6 *b* for each of the conditions given in Fig. 6 *a*. The pooled value of α for 39 different bleaching conditions (each the mean of at least five experimental repetitions) yielded $\alpha = 3.8 \pm 0.25$ (mean $\pm$ 1. SEM). (Some of these conditions were the two-stage equilibrium bleaches, not shown in Fig. 6 *a*.) Rushton et al. (1969) also reported variation in α from one condition to another.

IV. *Generality of the Results*

The data reported so far were obtained on subject M. Hollins. The generality of these results was tested psychophysically for a second observer (M. Alpern) and confirmed in all essential features. The value of α was somewhat higher for him than for M. Hollins, but the time constants of his dark adaptation curves showed the same trend with change from long to short bleaches already described for M. Hollins. The dark adaptation time constant after equilibrium bleaches was about 116 s for M. Alpern and 105 s for M. Hollins. The first of these is close to the values commonly reported (Rushton, 1958; Rushton and Henry, 1968; Alpern et al., 1971). The second is one of the smallest yet reported, and demonstrates that there is considerable variability, probably on the order of 20%, from one individual to another.

Finally, the cone part of the dark adaptation curve of the 5° temporal retina of M. Hollins was studied in order to examine the possibility that the kinetics of erythrolabe in the fovea (where they can be measured with the retinal densitometer)may be different from those in the peripheral retina (where—because of the much greater amount of rhodopsin present—they cannot be measured). Dark adaptation curves were obtained after prolonged 30%, 50%, 70%, and 100% erythrolabe bleaches, assuming that the kinetics were, in fact, unchanged. The time constants of these π_5 cone dark adaptation curves (as in the foveal measurements) were found to be about 105 s. Shorter bleaches were explored at the 50% level and confirmed once more the foveal result of much more rapid dark adaptation curves than those found after long bleaches. None of these experiments provided the slightest hint that the

kinetics of erythrolabe recovery in the 5° peripheral retina differ at all from those found in the fovea.

DISCUSSION

The surprising result of these experiments is the wide range of experimental conditions over which Eq. 1 describes the relation between erythrolabe regeneration and log π_5 threshold recovery in the dark. In Fig. 4, for example, recovery after bleaches from 10 to 100% are reasonably described by the same theoretical curve without vertical or horizontal shifting. The relation is approximately valid for any bleach duration over a 3 log unit range whether tested on cones in the foveal or extrafoveal retina.

There are two limitations on the generality of these results. The first is the theta effect, i.e., the prolonged threshold elevation produced by saturating flashes (less than a millisecond in duration) which is associated with a much more rapid regeneration of erythrolabe than Eq. 1 predicts.

The second is the large threshold elevation obtained immediately after relatively weak bleaches and its rapid subsequent fall in the first half minute or so in the dark. This effect has not previously been explicitly identified for cone dark adaptation, but it has long been familiar for rods. Dowling (1963) working on the rat called such recovery "neural" and sharply distinguished between it and the slow or "chemical" phase of threshold recovery. Donner and Reuter (1967, 1968) and Baumann (1967), on the other hand, suggest that for frog this recovery is due to the photoproduct metarhodopsin II, while Frank (1971) attributes it to "passive ionic diffusion down membrane gradients." Rushton and Powell (1972 *b*) have mustered strong evidence for human rods that the early phase is "chemical" though their inference that metarhodopsin II is the agent responsible for the early threshold rise is not compelling. In two quantitative ways the early phase for cones, shown in Fig. 4, differs from the Rushton-Powell results for rods: (*a*) For rods the early deviations appear only after bleaches equal to or smaller than 7%, but in Fig. 4 one finds traces of the effect even with a 60% bleach. (*b*) The "meta II phase" for rods lasts as long as 5 min; for cones it is rarely longer than 30 s and never more than a minute. Whether these differences reflect anything more than differences between the kinetics of the rod and cone visual pigments and their respective photoproducts remains to be established.

No one knows what physiology underlies the deceptively simple empirical principle summarized by Eq. 1. For human rods the concept of the equivalent background of the bleach (Crawford, 1947; Blakemore and Rushton, 1965) unifies results from a variety of different sizes of test, and this is compelling evidence for the idea of threshold elevation determined by an excitation pool of a large number of rods (Rushton, 1965 *b*; Alpern et al., 1972). But intracellular records from axolotl rods (Grabowski et al. 1972) and extracellular

recording from the skate retina treated with Na aspartate (Dowling and Ripps, 1972) provide just as convincing evidence that the threshold of each receptor itself is elevated by bleaching and backgrounds. Very probably both mechanisms are involved. Is the matter just as complicated for the cones studied in the present work? Perhaps not. No one has yet demonstrated the unification of the results of different target areas for a single cone mechanism by the equivalent background principle. The negative results of Rinalducci et al. (1970) in this regard are equivocal, and the matter needs careful study under circumstances in which it can be crisply established that the recovery of threshold of only a single mechanism has been studied. It seems pointless to speculate further about the physiology of the present results until we know how essential the concept of excitation pools is for the understanding of cone threshold elevation in human bleaching adaptation.

SUMMARY

1. Foveal π_5 dark adaptation and erythrolabe regeneration curves obtained on the same observer are compared for a variety of bleaching conditions.

2. All of these results, densitometric and (after 30 s in the dark) psychophysical, are reasonably described by exponential curves.

3. The time constants of the two processes are found to be the same after any given bleach (with the single exception of a very brief flash bleach), but to be dependent on the properties of the bleach.

4. The time constant of regeneration and of dark adaptation varies between 1 and 2 min.

5. Recovery after long (or equilibrium) bleaching is described by curves whose time constant is nearly 2 min independent of the level of the bleach.

6. For shorter (nonequilibrium) bleaches recovery rate from a fixed level is slower the greater the duration of the bleach. For a fixed duration, recovery rate is slower the greater the level of the bleach.

7. Except for the first half minute after weak bleaches and recovery from a brief saturating flash the results of all these experiments are accurately described by the Dowling-Rushton relation (Eq. 1) with only a single arbitrary constant.

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