

# Flash Photolysis of Rhodopsin in the Cat Retina

H. RIPPS, L. MEHAFFEY III, I. M. SIEGEL, W. ERNST,  
and C. M. KEMP

From the Department of Ophthalmology and the Department of Physiology and Biophysics, New York University School of Medicine, New York, New York 10016, and the Institute of Ophthalmology, University of London, London WC1H 9QS, England. L. Mehaffey's present address is Vassar College, Poughkeepsie, New York 12601.

**ABSTRACT** The bleaching of rhodopsin by short-duration flashes of a xenon discharge lamp was studied *in vivo* in the cat retina with the aid of a rapid, spectral-scan fundus reflectometer. Difference spectra recorded over a broad range of intensities showed that the bleaching efficacy of high-intensity flashes was less than that of longer duration, steady lights delivering the same amount of energy. Both the empirical results and those derived from a theoretical analysis of flash photolysis indicate that, under the conditions of these experiments, the upper limit of the flash bleaching of rhodopsin in cat is ~90%. Although the fact that a full bleach could not be attained is attributable to photoreversal, *i.e.*, the photic regeneration of rhodopsin from its light-sensitive intermediates, the 90% limit is considerably higher than the 50% (or lower) value obtained under other experimental circumstances. Thus, it appears that the duration (~1 ms) and spectral composition of the flash, coupled with the kinetic parameters of the thermal and photic reactions in the cat retina, reduce the light-induced regeneration of rhodopsin to ~10%.

## INTRODUCTION

A change in the isomeric form of its chromophore constitutes the initial event in the photolysis of rhodopsin (Hubbard and Kropf, 1958; Goldschmidt *et al.*, 1976; Hurley *et al.*, 1977). The molecule then degrades thermally through a series of spectrally distinct intermediates that lead ultimately to the formation of a colorless ("bleached") photoproduct. However, many of the intermediates are themselves light sensitive and capable of being photically isomerized back to the parent pigment or one of its steric analogues (Hubbard and Kropf, 1958; Bridges, 1961; Baumann, 1970; Ernst and Kemp, 1978). Thus, the efficacy of the bleaching process depends upon a number of factors, among which are the duration and spectral composition of the incident light, the formation and decay rates of the intermediates, and the relative photosensitivities of rhodopsin and its photoproducts. Due to the transient nature of the photoproducts at physiological temperatures (Cone and Cobbs, 1969), a significant measure of "photoreversal" is produced *in situ* only by exposure to brief light flashes that deliver large numbers of quanta during the lifetimes of the absorbing species (Williams, 1964; Ernst and Kemp, 1979). Clearly, if the

conditions required to produce equivalence in the forward and back reactions are satisfied, a bleaching limit of 50% will obtain no matter how intense the photic exposure (Williams, 1964).

There is little doubt that photoreversal can be induced in the mammalian retina *in vivo* (Hagins, 1955; Dowling and Hubbard, 1963), but there is not complete agreement with regard to the degree to which it occurs under well-defined experimental conditions (cf. Pugh [1975 *a*]). The issue is of more than academic interest. Important conclusions concerning the factors that influence human visual sensitivity have been drawn from the results of flash-irradiation studies in which it was stated that the flash exposure could not have bleached more than half of the available rhodopsin (Rushton, 1963; Rushton and Baker, 1963; Alpern, 1971; Pugh, 1975 *b*). This view originated with Hagins's report that in rabbit an upper limit of 50% is obtained when the flash duration is  $\sim 1$  ms and received support from studies on man that utilized comparable densitometric techniques and flash durations of  $\sim 0.6$  (Pugh, 1975 *a*) and 4 ms (Rushton, 1963). These findings are rather surprising in view of the evidence that at mammalian body temperatures the bleaching of rhodopsin in solution reaches 88% with flashes as brief as 2 ms (Williams, 1974). Moreover, Ripps and Weale (1969 *a*) had shown that a single flash from a xenon discharge lamp with characteristics nearly identical to the one used by Pugh (i.e., duration  $< 1$  ms) bleached  $> 75\%$  of the rhodopsin content of the human retina; because they were unable to increase further the flash intensity, the upper limit of photolysis was not determined.

In this report we provide further observations on flash photolysis of rhodopsin in the mammalian eye obtained by fundus reflectometry of the cat retina. In addition, we present the results of a theoretical analysis that predicts remarkably well the intensity variation in the fraction of rhodopsin bleached, as well as the upper limits of flash photolysis in cat.

#### METHODS

The data presented here and in the papers that follow were obtained from the study of 29 adult cats, each weighing between 3.5 and 6 kg. Protocols for preparation of the animals for study and the essential features of the anesthesia were modified from the procedures of Enroth-Cugell and Pinto (1972) and Bonds and MacLeod (1974). After sedation with ketamine (35 mg/kg, intraperitoneal) the animals were given an initial dose of sodium thiamylal (Surital, Parke-Davis, Div. of Warner-Lambert Co., Morris Plains, N. J., 2.5%, intravenous) and maintained under urethane anesthesia by continuous intravenous infusion at a rate of 20–100 mg/kg·h; an intramuscular injection of atropine (0.1–0.2 mg) served to suppress salivation. The animal's head was held immobile by a special clamp that was affixed to a sturdy metal base that could be accurately positioned in three dimensions by geared-motion drives. A slit was cut in the temporal corner of the eyelid, the anterior portion of the nictitating membrane was excised, and a circumcorneal incision was made in the bulbar conjunctiva. To eliminate eye movements, the extraocular muscles were transected near their insertions, and the limbal margin of the globe was sutured to a Flieringa ring (J. L. Storz, Div. of PSG Industries, Perkasi, Pa.) held rigidly by a micromanipulator attached to the metal base. Atropine sulphate (1%) and neosynephrine (10%) were administered topically several times during the preparative procedures to dilate

the pupil. Corneal transparency was maintained by a steady flow (gravity feed) of silicone fluid (Dow Corning Medical Fluid 360 [Dow Corning Corp., Midland, Mich.]; viscosity, 20 centistokes) that formed a protective layer of uniform thickness over the entire cornea.

### *Fundus Reflectometry*

The technique employed and the factors to be considered in the analysis of reflectometric data have been described in detail (Ripps and Weale, 1965 and 1970; Carr and Ripps, 1967). Briefly, collimated light from a 150-W xenon arc lamp (powered by a current-stabilized DC supply) passed sequentially through a series of 29 narrow-band interference filters mounted in spectral order on a wheel rotating at 3 rps. The nearly monochromatic lights (half-bandwidth  $\leq 5$  nm), covering the range from 380 (or 400) to 680 nm in steps of  $\sim 10$  nm, comprised the test (measuring) beams that entered the eye in Maxwellian view. A fraction of the incident light was reflected from the fundus and emerged through the upper half of the dilated pupil (after having traversed the retina twice), where a reflecting prism directed the rays to the cathode of an EMI 9558Q photomultiplier (Varian/EMI, Plainview, N. Y.). The output of the latter was fed through an operational amplifier to the analog-to-digital converter of a PDP8I computer (Digital Equipment Corp., Marlboro, Mass.); the signals consisted of a series of 29 positive deflections, each corresponding to the wavelength of light derived from one of the interference filters in the spinning wheel. The area under each of the deflections was approximated by converting the analog signals to digital values at brief, regular time intervals, and integrating these values using the trapezoidal rule (Ripps and Snapper, 1974); the sums were held in a temporary buffer, each in its appropriate wavelength bin. This process was repeated for eight complete spectral scans, after which averages and variances of the eight areas corresponding to each test wavelength were calculated, and the means were stored as one time vector. Thus, a time vector contains 29 reflection measurements (stored in 29 bins) obtained during the  $\sim 3$  s required to collect eight successive scans. Absorbance (density) differences were calculated from the time vectors according to the equation  $[\Delta D_{1-2}]_{\lambda} = [\log_{10}(A_1/A_2)]_{\lambda}$ , where  $A_1$  and  $A_2$  are the areas at each wavelength  $\lambda$  for time vectors 1 and 2. The absorbance-difference spectrum represents, therefore, the wavelength variation in retinal transmissivity between scans recorded at times  $t_1$  and  $t_2$  (e.g., variation between a dark-adapted retina and the retina immediately after it has been exposed to light). In this formulation,  $[\Delta D]_{\lambda}$  is treated as the density change for double passage through the rod outer segments. However, the measured values are diluted by light reaching the photocell that has not transversed the photoreceptors twice or even once. Thus, the measured density changes do not correspond to the in situ change within the receptors. The effects of stray light on the measurements are considered in the Appendix, where it is shown that the relative changes with which we are concerned are not affected significantly by this factor; in fact, the in situ density change is slightly greater than our measurements would indicate.

### *Bleaching Lights*

A solenoid-controlled mirror rapidly ( $<100$  ms) exchanged the spectral measuring beam for the light from one of two bleaching sources. Brief, intense light flashes were obtained from a xenon flash lamp (Strobonar, model 65D, Honeywell, Inc., Denver, Colo.). Although seen in Maxwellian view, the image of the discharge tube exceeded in area that of the dilated pupil, and, thus, the latter provided the effective entrance pupil for the incident light. The temporal characteristics of the flash are a major

concern in studies of flash bleaching, since only energy emitted during the lifetimes of absorbing photoproducts can be effective in photoregeneration. Fig. 1 is a photograph of the screen of a Tektronix, Inc. (Beaverton, Oreg.) type 454 oscilloscope (150 MHz bandwidth) and shows the output of the Strobunar flash as recorded by an EG & G, Inc. (Salem, Mass.) model HAD 1000A silicon photodiode-operational amplifier unit with rise and fall times of  $<0.4 \mu\text{s}$ . The numbers in brackets give the percentages of the total flash energy emitted at the various time intervals after onset. A comparison of Fig. 1 with Fig. 4 of Pugh (1975 *a*) shows that the emission characteristics of the discharge tubes used in these studies were nearly identical.

The quantity of light incident at the retina (i.e., the retinal illuminance  $\times$  time, in scotopic troland-seconds) was determined by the method of Ripps and Weale (1969 *a*). In short, scotopic thresholds were measured subjectively with both the xenon flash

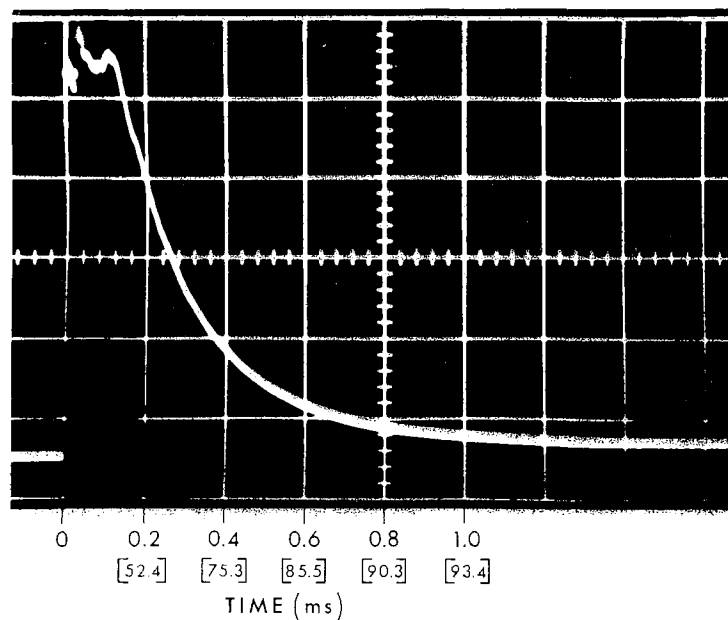


FIGURE 1. Temporal characteristics of the Strobunar flash. The numbers in brackets indicate the percentage of the energy of the flash dissipated during the time elapsed. The scale of ordinates is in arbitrary units.

and 10-ms light pulses from a continuous xenon arc whose retinal illuminance had been calibrated with an SEI (Salford Electrical Instruments Ltd., Essex, England) photometer according to the procedure outlined by Rushton (1956 *a*); the test fields subtended the same visual angle ( $2^\circ$ ) and were located in the nasal field  $15^\circ$  from fixation. Since the reciprocity law ( $I \cdot t = C$ ) applies for  $t \leq 0.1$  s, comparing the neutral density filters used in obtaining the threshold data for the two sources made it possible to determine the luminous energy of the flash in photopic troland-seconds. The photopic value was converted to scotopic troland-seconds (Mainster et al., 1971), assuming a color temperature of  $6,000^\circ\text{K}$  for the Strobunar flash (manufacturer's specifications). It remained then to convert the value obtained from man to its feline equivalent. Corrections were applied first for the large area of the dilated cat pupil ( $144 \text{ mm}^2$  as compared with  $49 \text{ mm}^2$  for our subject), and for the shorter nodal distance of the cat eye ( $12.5 \text{ mm}$  vs.  $17 \text{ mm}$  for the human eye; Vakkur et al., 1963).

To take into account reflection from the cat's tapetum, a correction factor (*CF*) was derived from the expression

$$CF = \frac{\sum_{460}^{650} [E_{\lambda}AV'_{\lambda} + AV'_{\lambda}R_{\lambda}^c E_{\lambda}(1 - AV'_{\lambda})]}{\sum_{460}^{650} [E_{\lambda}AV'_{\lambda} + AV'_{\lambda}R_{\lambda}^h E_{\lambda}(1 - AV'_{\lambda})]}$$

where  $E_{\lambda}$  is the energy incident upon the retina at wavelength  $\lambda$ ,  $A$  is the maximum retinal absorption (at 500 nm),  $V'_{\lambda}$  is the scotopic relative luminous efficiency,  $R_{\lambda}^c$  is the reflectivity of the cat tapetum, and  $R_{\lambda}^h$  reflectivity of the human fundus. In this formulation,  $E_{\lambda}AV'_{\lambda}$  is the energy absorbed during the initial traverse of the retina,  $E_{\lambda}(1 - AV'_{\lambda})$  is the energy transmitted through the retina,  $R_{\lambda}E_{\lambda}(1 - AV'_{\lambda})$  is the amount reflected back from the tapetum or fundus, and  $AV'_{\lambda}R_{\lambda}E_{\lambda}(1 - AV'_{\lambda})$  gives the additional energy captured on the second pass through the retina.  $E_{\lambda}$  was obtained from the xenon flash spectrum (Wyszecki and Stiles, 1967) as modified by transmission through a Schott Optical Glass Inc. (Duryea, Pa.) BG 38 heat filter, the values of  $R_{\lambda}^c$  are from Weale (1953), and those of  $R_{\lambda}^h$  are from Brindley and Willmer (1952); 0.55 for  $A$  is based on the estimated value for the in situ density of human rhodopsin (Alpern and Pugh, 1974). The results of this computation gave 1.24 for the value of *CF*; i.e., due to tapetal reflection, the flash was 1.24 times more effective in the cat eye than in man. With the correction factor applied, the effective luminous energy at the retina from the unattenuated flash was 7.51 log scotopic troland-second for the cat. Correction factors were also calculated for our tungsten source (unfiltered or in combination with Corning glass filters). The values given both in the text and in the figures have been corrected for the optics of the cat eye.

When longer bleaching exposures were required, a collinear beam from a heat-filtered 100-W tungsten-halogen lamp could be substituted for the flash by a second movable mirror. An electronic shutter controlled the exposure duration, whereas neutral density and color filters could be introduced to attenuate and spectrally shape the bleaching beam; all filters were calibrated on a spectrophotometer (Gilford Instrument Laboratories Inc., Oberlin, Ohio). After correction factors for the optical properties of the cat eye (see above) were applied, the unattenuated "white" field of the bleaching beam produced a retinal illuminance of 7.86 log scotopic troland (td); after passage through an orange (Corning 3482) glass filter, the illuminance was 6.82 log scotopic td (cat). The bleaching field (whether derived from the flash source or the tungsten lamp) subtended at the eye a visual angle of 3° and was concentric with the 2° field of the test beams.

**PROCEDURE** The mirror of a reflex housing (E. Leitz, Inc. [Rockleigh, N. J.] Visoflex) could be interposed in the path of the emergent light beams to provide an ophthalmoscopic view of the fundus, which enabled the experimenter to position the eye for study of the desired retinal location. Most measurements were made in a tapetal region 2° in diameter situated in the superior retina ~2 mm temporal to the area centralis. The rod density of this region is ~500,000/mm<sup>2</sup> and exceeds that of the cones by nearly 60-fold (Steinberg et al., 1973).

After alignment in the apparatus, the animal was dark adapted for at least 90 min before the start of an experimental run. Fundus reflection measurements were obtained (a) in the dark-adapted condition, (b) after exposure to bleaching flashes of various energies, (c) at various times after the bleaching light was extinguished, and (d) after a 1-min exposure to the intense orange light (6.82 log scotopic td-s) that bleached at least 98% of the available rhodopsin. Difference spectra computed from

these data were used in determining the density loss due to the flash bleach (*a-b*), the kinetics of regeneration (*b-c*), and the rhodopsin density of the area under study (*a-d*).

## RESULTS

### *Flash Bleaching*

Fig. 2 shows a series of flash-bleaching difference spectra (filled symbols) together with the spectrum resulting from a 1-min exposure to a steady orange field (unfilled circles) that bleached at least 98% of the photopigment in the test area. The results are from one animal, and a period of darkness, sufficient for complete regeneration of the bleached rhodopsin, was interposed between exposures. The flash-bleaching data were obtained for luminous energies ranging from 6.00 to 7.51 log scotopic td-s; the latter was the maximum energy available from the unattenuated flash. In each instance the wavelength at which the maximum density loss occurred (i.e., the  $\lambda_{\max}$  of the absorbance spectrum) was 510 nm, typical of the rhodopsin difference spectrum. At the low-energy end (triangles), the flash caused a small fractional loss of rhodopsin (9.6%), whereas at its maximum energy (filled circles), the Strobolar bleach amounted to 80% of that produced by complete photolysis (unfilled circles) of the available rhodopsin ( $\Delta D_{510} = 0.26$ ). Note also that the absorbance spectra continue to grow with each increment in flash energy, suggesting that the upper limit of bleaching was not reached even at the maximum energy. Although it is not apparent from these data, photolysis may be presumed to have progressed through several intermediate states during the nearly 3 s between the bleaching and measurement. At physiological temperatures, the early photoproducts form and decay so rapidly that they require for their detection instruments capable of nonsecond resolution (Cone, 1972). Thus, the increase in  $\Delta D$  at 400 nm is most likely due to the presence of the photoproduct metarhodopsin II ( $\lambda_{\max} = 380$  nm).

The photolytic effect of the maximum energy flash was tested on six cats and found to be remarkably consistent. A single flash from the Strobolar bleached between 79.1 and 84.1% (mean = 82.8%) of the rhodopsin content of the test area. In one of the experiments, the retina was exposed to two unattenuated flashes, separated by a 70-min period of dark adaptation; as shown in Fig. 3, the difference spectra (half-filled circles) were very nearly the same. When these density changes are compared with the full bleach spectrum produced by the final 1-min exposure to orange light (unfilled circles), the results indicate that the initial exposure bleached ~82% of the rhodopsin, and that after regeneration, the second flash bleached 84% of the photopigment. Thus, there can be little doubt that the xenon discharge lamp, emitting 85.5% of its radiant energy within 600  $\mu$ s, bleached >80% of the exposed rhodopsin in the cat retina.

The effect of flash energy on the fraction *F* of rhodopsin bleached is shown by the data points of Fig. 4. The continuous curve represents the function obtained in man for extended ( $10 \text{ s} \leq t \leq 2 \text{ min}$ ) exposure times (cf. Ripps and Weale, 1969 *b*; Alpern and Pugh, 1974; Pugh, 1975 *a*; Ripps et al., 1978) and satisfies the equation  $F_{\lambda} = 1 - \exp(-\alpha_{\lambda}\gamma I t)$ , where  $F_{\lambda} = [(\Delta D)_{\lambda}] /$

$(\Delta D_{\max})_{\lambda}$  is the fractional change in absorbance at wavelength  $\lambda$  produced by an exposure for time  $t$  to a retinal illuminance  $I$ , and  $\alpha_{\lambda}\gamma$ , the photosensitivity of the light-sensitive substance, is the product of the absorption coefficient  $\alpha_{\lambda}$

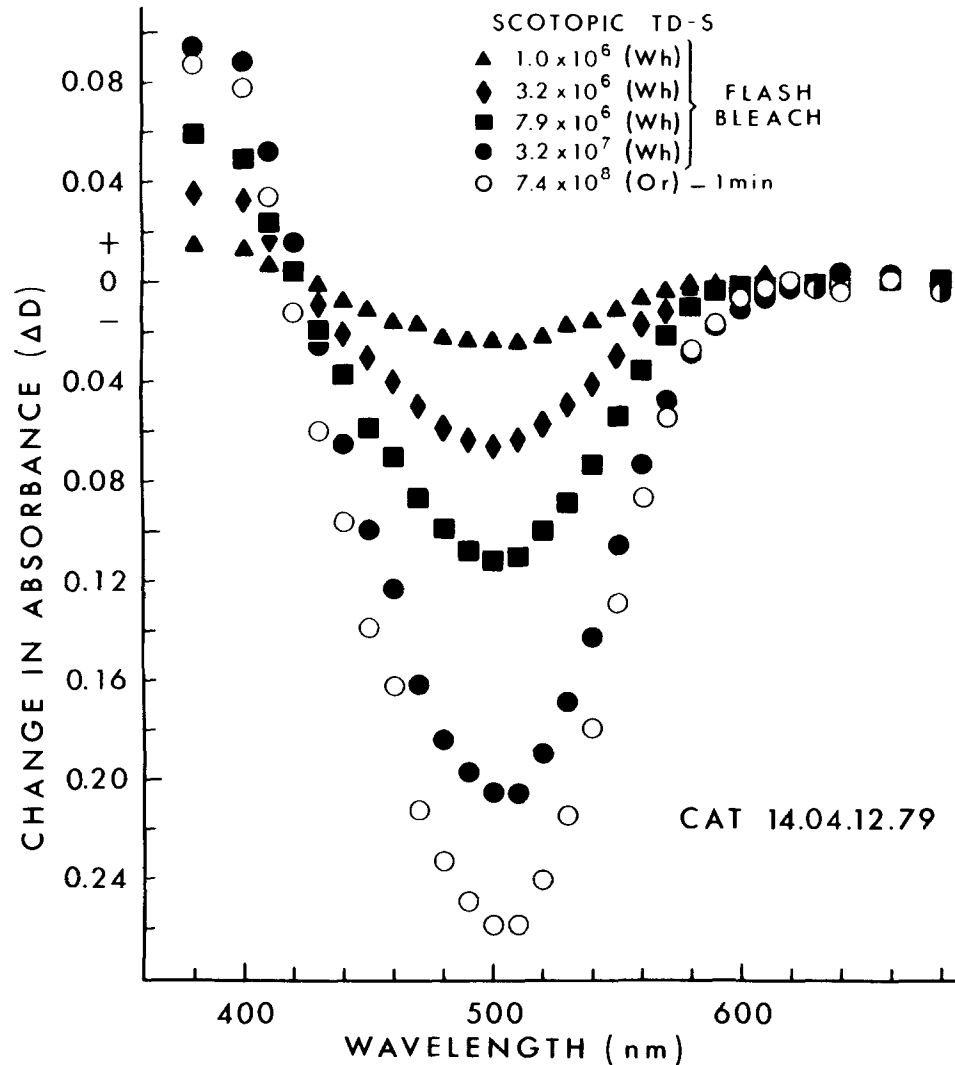


FIGURE 2. Difference spectra resulting from flash-bleaches at various energies (filled symbols) and after a 1-min exposure to an intense orange light (open circles). Each set of results shows the spectral changes in absorbance between the prebleach and postbleach conditions. The data were obtained from the same animal, with 70 min of dark adaptation between each bleaching condition.

the light-sensitive substance, is the product of the absorption coefficient  $\alpha_{\lambda}$  and the quantum efficiency  $\gamma$ . The curve is positioned on the scale of abscissae to fit best the data obtained with the weakest flashes; i.e., at the energies least

likely to induce photoreversal (Williams, 1964). The degree to which the results of prolonged bleaches in cat fit the curve and a determination of the photosensitivity of cat rhodopsin are considered in the following paper (Ripps

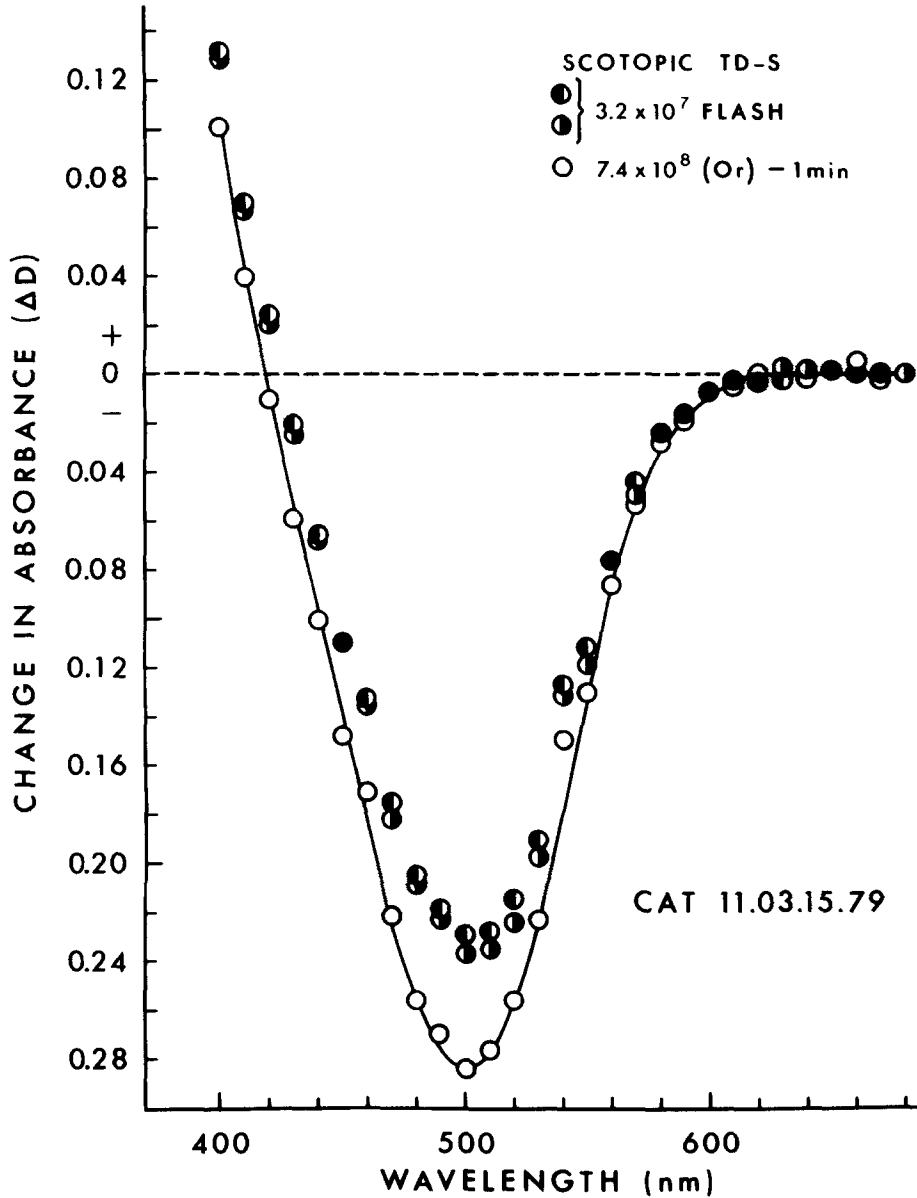


FIGURE 3. Absorbance-difference spectra resulting from two-flash bleaches of the same energy (half-filled circles); a 70-min period of dark adaptation intervened between flashes. Unfilled circles represent the density differences recorded from the same animal after exposure to a steady orange light that bleached fully the available rhodopsin.



et al., 1981). With regard to flash bleaching, the data begin to depart from the continuous curve at energies that produce fractional bleaches of  $\sim 0.5$ , and the two become more divergent as the flash energy is raised further. Although an upper limit to  $F$  is not reached, the data seem to approach asymptotically a bleaching level of  $\sim 90\%$ , and are in splendid agreement with predicted values (dashed-line curve in Fig. 4) derived independently from a theoretical treatment of the conditions of the flash-bleaching experiment (see below).

#### *Regeneration of Rhodopsin*

We mentioned above that interspersed between flash exposures were dark intervals long enough for the bleached rhodopsin to reform. Representative

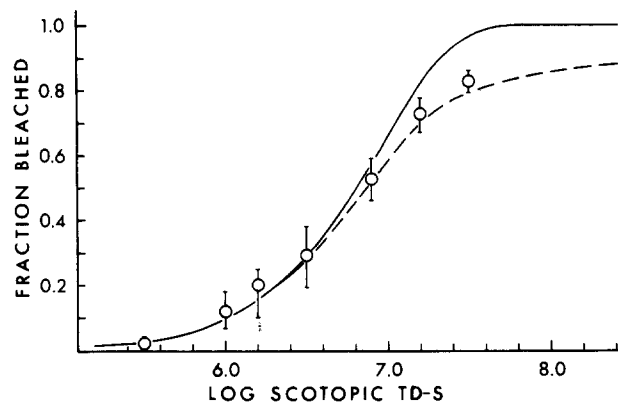


FIGURE 4. The bleaching efficacy of the Strobolar flash. Circles show mean values of  $\Delta D$  ( $\lambda = 510$  nm) taken from difference spectra such as those shown in Figs. 2 and 3; vertical bars indicate the range of values for  $n = 4-6$ . The solid line is the conventional bleaching curve derived from the expression  $F = 1 - e^{-\alpha \gamma I t}$ ; the dashed line was obtained from the model (Eq. 5c) and incorporates photoreversal from metarhodopsin I. Both theoretical curves were aligned with the experimental points by sliding along the scale of abscissae so as to fit best the low-energy data. See text for details.

difference spectra recorded during one such period are shown in Fig. 5. These are, in fact, the results obtained during the 70-min dark adaptation that intervened between the two flash-bleach experiments of Fig. 3. Particularly significant are the shifts in the  $\lambda_{\max}$  of the difference spectra with time in darkness. Almost immediately after the flash (i.e., during the first 15 s in darkness) there was an increase in absorbance, maximal at  $\sim 475$  nm. Over the next 5 min the absorbance values continued to increase and there was little change in  $\lambda_{\max}$ . However, between 7 and 10 min the difference spectrum broadened noticeably toward longer wavelengths, and by 15 min in darkness the  $\lambda_{\max}$  had shifted to 510 nm, where it remained throughout dark adaptation. Other noteworthy features are the continued growth of the difference spectrum up to and including the 60-min record and the density losses that occur for wavelengths below  $\sim 410$  nm.

It is clear from the spectral variation in  $\Delta D$  that the regeneration of rhodopsin is not the only process contributing to the absorbance changes of Fig. 5. The nature of the underlying events is perhaps more apparent in Fig. 6, where data from the complete series of difference spectra have been graphed to show the temporal changes in  $\Delta D$  at five selected wavelengths. Measurements at 400 nm, for example, show at first a rapid fall in optical density that reflects primarily the decay of the metarhodopsin II formed after the flash; later variations in absorbance at this wavelength are probably due to changes in both metarhodopsin II and retinal, which exhibit similar spectral properties. At 475 nm, on the other hand, the rise in absorbance during the first 3 min in darkness and the subsequent decline are due almost entirely to the formation and decay of metarhodopsin III (Matthews et al., 1963; Weale, 1967; Brin and Ripps, 1977). However, the slow decay appears to be interrupted at  $\sim 14$  min, and the curve rises monotonically thereafter until an asymptote is approached between 60 and 70 min in darkness. This rising phase is characteristic of the data at all wavelengths that fall within the absorption spectrum of rhodopsin, and must represent the increase in optical density due to the regeneration of rhodopsin itself.

At the three longer wavelengths, the influence of metarhodopsin III becomes progressively less as the measuring wavelength moves away from the peak of the metarhodopsin III absorption band. However, even at 560 nm, the curve has a bipartite form, indicating a contribution to absorbance from metarhodopsin III. Under the circumstances it is difficult to define precisely the time-course of rhodopsin formation, although extrapolating the late branches of the curves in Fig. 6 back to the baseline suggests that the onset of regeneration is delayed by  $\sim 6$ – $8$  min after the flash bleach. The kinetics of regeneration will be considered in greater detail in the following paper (Ripps et al., 1981).

#### *Analysis of Flash Photolysis*

Pugh (1975 *a*) has shown that the analysis of photoreversal experiments requires a kinetic approach in which the rate constants and quantum efficiencies of the thermal intermediates are essential parameters in determining the bleaching efficacy of a flash exposure. Fig. 7 is the scheme of reactions that have to be considered. However, for the flash intensities and duration used here, almost all of the photoreversal is from metarhodopsin I. This allows the simplification made by Pugh in which photoreversal from bathorhodopsin and lumirhodopsin is ignored. Indeed, computations in which the earlier intermediates were included (kinetic parameters from Ernst and Kemp [1979]) gave nearly identical results.

The appropriate rate equations for the simplified kinetic scheme are

$$\dot{C}_R = -\gamma_{R,MI}J_R + \gamma_{MI,R}J_{MI}, \quad (1)$$

$$\dot{C}_I = -\gamma_{I,MI}J_I + \gamma_{MI,I}J_{MI}, \quad (2)$$

$$\dot{C}_{MI} = \gamma_{R,MI}J_R + \gamma_{I,MI}J_I - J_{MI}(\gamma_{MI,R} + \gamma_{MI,I}) - k_{MI,MII}c_{MI}, \quad (3)$$

$$\dot{C}_{MII} = k_{MI,MII}c_{MI} - J_{MII}(\gamma_{MII,R} + \gamma_{MII,I}), \quad (4)$$

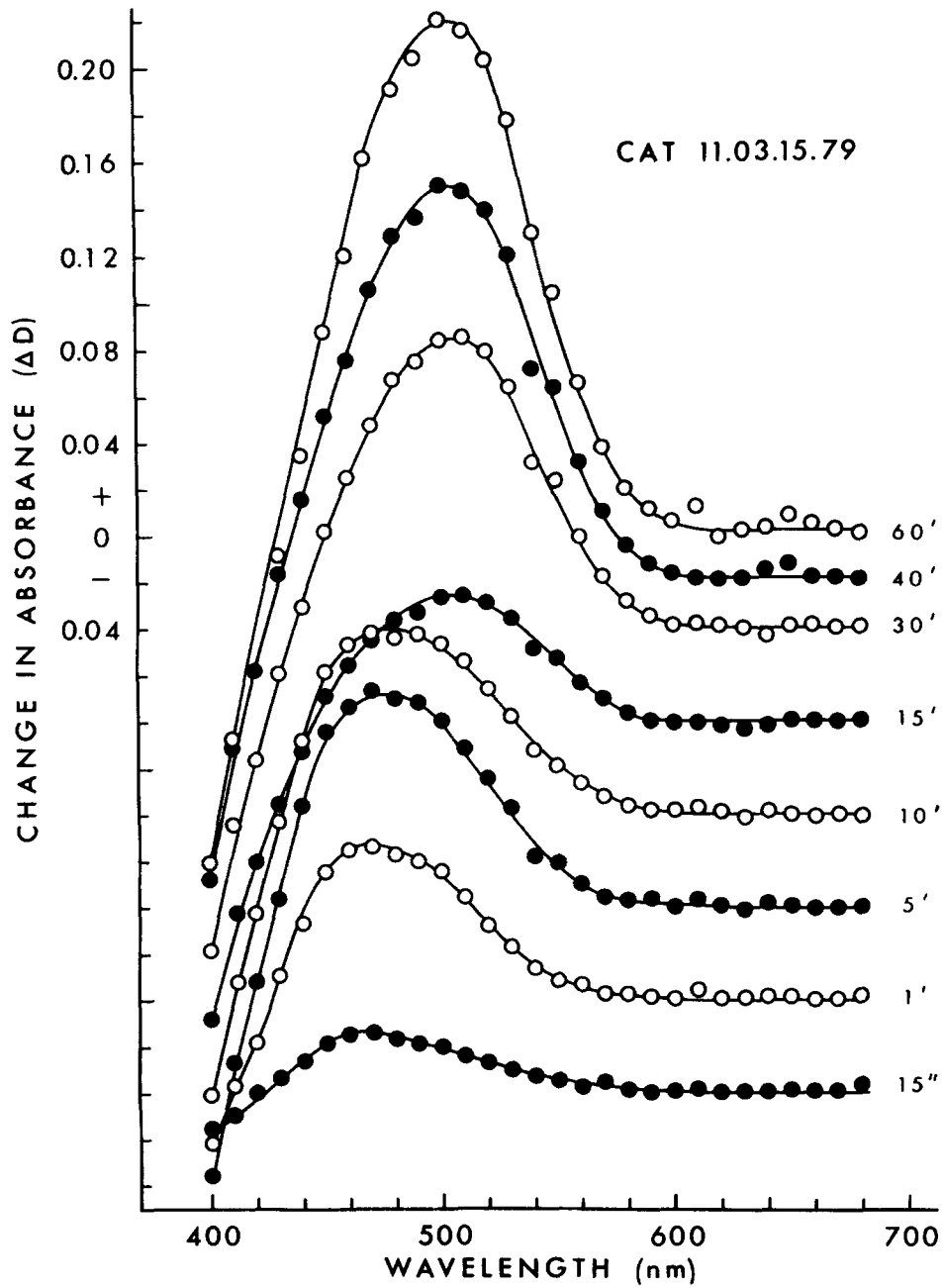


FIGURE 5. Difference spectra obtained at the times indicated during dark adaptation after a flash exposure that bleached 82% of the rhodopsin.  $\Delta D$  values are the absorbance differences between recordings made immediately after the flash and those obtained after each period in darkness. The data for 60' are positioned correctly on the scale of ordinates; all other results are displaced downward for clarity.

where  $c_x$  represents the concentration of X (molecules·cm<sup>-3</sup>) and  $\dot{C}_x$  its differential with respect to time; R, I, MI, and MII stand for rhodopsin, isorhodopsin, metarhodopsin I, and metarhodopsin II, respectively;  $J_x$  are the quanta absorbed by reactant X per centimeter squared per second;  $\gamma_{x,y}$  is the quantum efficiency for the reaction  $X \rightarrow Y$ ; and  $k_{x,y}$  is the rate constant for the reaction  $X \rightarrow Y$ .

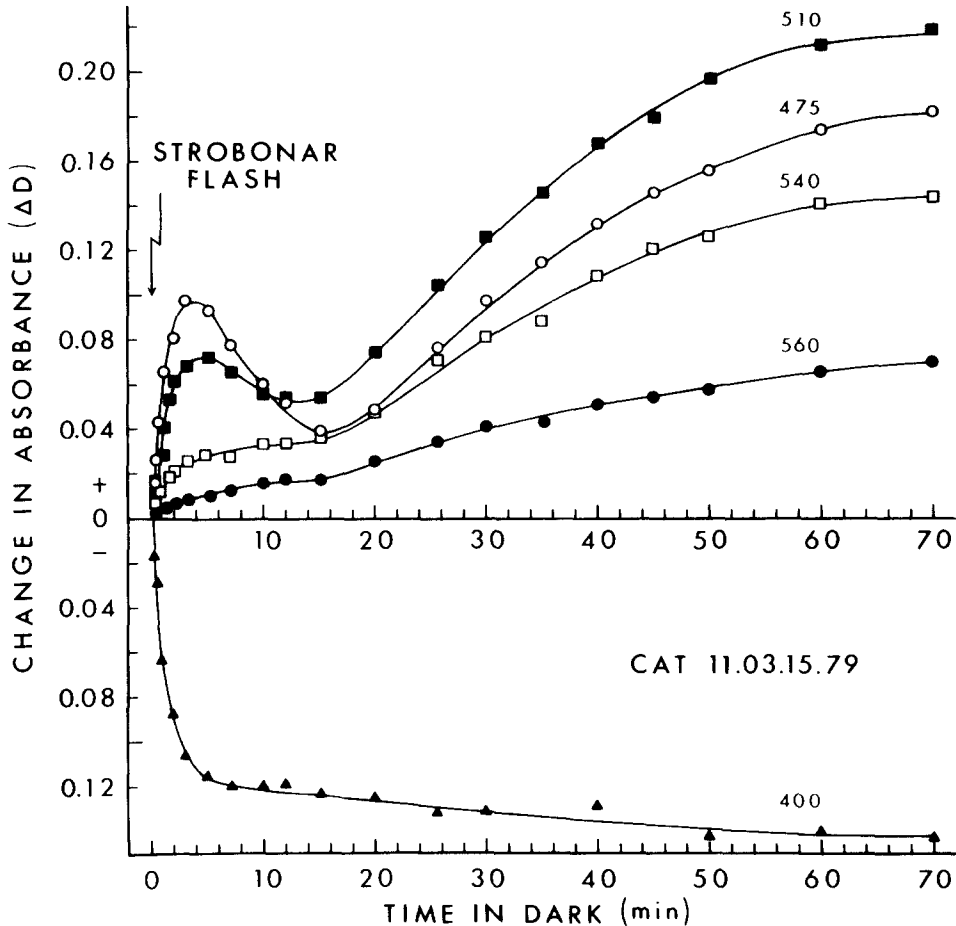


FIGURE 6. The temporal changes in absorbance measured at several wavelengths.  $\Delta D$  values at the wavelengths indicated were obtained from the more extensive series of curves that comprised the experiment shown in Fig. 5 and were plotted as a function of time in darkness after the bleach.

From the Lambert-Beer Law,

$$J_x = I_{\text{ros}}(\lambda, t) \cdot [1 - e^{-\epsilon_x(\lambda) \cdot C_x(t) \cdot l}], \quad (5)$$

where  $I_{\text{ros}}$  is the quantal flux incident at the rod outer segment (quanta·cm<sup>-2</sup>·s<sup>-1</sup>);  $\epsilon_x$  is the extinction coefficient of X (cm<sup>2</sup> chromophore<sup>-1</sup>);  $l$  is the effective

path length (in centimeters) through the rod outer limbs; and parentheses denote that the variables are functions of wavelength ( $\lambda$ ) and/or time ( $t$ ).

Because the exponential term in Eq. 5 is small, the equation reduces to

$$J_x(t) \approx I_{\text{ros}}(\lambda, t) \cdot \epsilon_x(\lambda) \cdot c_x(t) \cdot l. \tag{5a}$$

We have confirmed numerically that eliminating the exponential term in Eq. 5 makes little difference to the solution of Eqs. 1-5, and we therefore rewrite the model in the simplified form

$$I_{\text{ros}}(\lambda, t) = a \cdot Q \cdot i(t) \cdot \int_0^\infty \rho(\lambda) d\lambda,$$

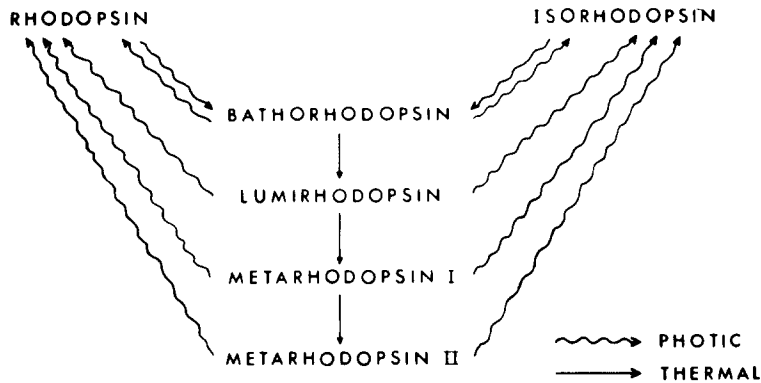


FIGURE 7. Scheme of bleaching and photoreversal reactions during submillisecond flashes. The rate constants for the photic reactions are assumed to be  $>10^{13} \text{ s}^{-1}$ . These reactions, therefore, are treated as instantaneous. The rate constants for the thermal reactions are assumed to have the following order of magnitude: bathorhodopsin  $\rightarrow$  lumirhodopsin,  $10^6$ - $10^7 \text{ s}^{-1}$ ; lumirhodopsin  $\rightarrow$  metarhodopsin I,  $10^4$ - $10^5 \text{ s}^{-1}$  (Ernst and Kemp, 1979); metarhodopsin I  $\rightarrow$  metarhodopsin II,  $10^3$ - $10^4 \text{ s}^{-1}$  (Table I).

where  $Q$  is the estimated luminous energy at the retina in scotopic troland-seconds;  $a$  is a constant that allows the temporal distribution of quantal flux to be determined from  $Q$  and the normalized distribution  $i(t)$ ; and  $\rho(\lambda)$  is a normalized function representing the spectral distribution of flash energy reaching the retina that takes into account the spectral characteristics of the xenon flash and the spectral absorption function of the ocular media.

Eq. 5a can therefore be reorganized:

$$J_x(t) = a \cdot Q \cdot i(t) \cdot c_x(t) \cdot l \cdot e_x, \tag{5b}$$

where  $e_x = \int_0^\infty \rho(\lambda) \cdot \epsilon_x(\lambda) d\lambda$ .

Eqs. 1-5 can be rewritten so that all concentration terms are normalized relative to the initial concentration of rhodopsin, all quantum efficiency terms

are expressed relative to  $\gamma_{R,MI}$  and all  $e_x$  terms are relative to  $e_R$ . This allows Eq. 5 to be expressed as

$$J_x(t) = a' \cdot Q \cdot i(t) \cdot \frac{e_x(t)}{e_R(0)} \cdot \frac{e_x}{e_R}, \quad (5c)$$

where  $a'$  now subsumes the constants  $a$  and  $l$  and those arising from normalization.

Eqs. 1–5 in the normalized form were solved numerically by means of the FACSIMILE program (Chance et al., 1977) for various values of  $Q$ . Table I shows the values assumed for the other parameters of the model and the sources from which these values were obtained. A value of  $a'$  was chosen that aligned the theoretical curve of Fig. 4 with the empirical result for the lowest value of  $Q$ , i.e., for the condition under which the extent of bleaching is a

TABLE I

Parameter	Value (or expression)	Reference
$\gamma_{L,MI}/\gamma_{R,MI}$	0.5	Ernst and Kemp (unpublished data)
$\gamma_{ML,R}/\gamma_{R,MI}$	0.5	Hubbard and Kropf (1958)
$\gamma_{ML,I}/\gamma_{R,MI}$	0.1	
$k_{ML,MLI}$	$3 \times 10^3 \text{ s}^{-1}$	Cone and Cobbs (1969); Pratt et al. (1964)
$e_I/e_R$	0.9*	
$e_{MI}/e_R$	0.8*	
$e_{MII}/e_R$	0*	
$i(t)$	$e^{-xt} - e^{-yt}$ , where $x=3.25 \times 10^3 \text{ s}^{-1}$ and $y=2.23 \times 10^4 \text{ s}^{-1}$	Approximation to flash intensity vs. time function; $x$ and $y$ found by FACSIMILE program (Chance et al., 1977)

\* Calculation based upon the assumption that (a) reactants are nomogram pigments with peak extinction coefficients at 502 nm (rhodopsin), 487 nm (isorhodopsin), 478 nm (metarhodopsin I), and 380 nm (metarhodopsin II); (b) the peak values for the first three stand in the ratio 1:1.13:1.06 (Kropf and Hubbard, 1958); and (c) Pugh's (1975 a) curve for spectral distribution of flash energy corrected for media losses can be applied to cat eye.

linear function of flash energy. Note that there is a good fit between the curve and the remaining points. This is in contrast to the poor fit that would have been obtained if the parameter values suggested by Pugh (1975 a) to account for his human bleaching data had been used. Pugh's curve, which levels off at ~50% for high flash energies, requires a value of  $k_{ML,MLI}$  that is more than 10 times smaller than the one used here (which is based on independent measurements; see Table I) and a ratio of  $\gamma_{ML,R}/\gamma_{R,MI}$  equal to 1 rather than 0.5 (which, again, is based on an independent estimate).

#### DISCUSSION

The results of the present study demonstrate unequivocally that a brief exposure ( $\approx 1$  ms) to an intense white xenon flash can bleach >80% of the rhodopsin contained within the retinal area irradiated by the flash. This finding is clearly at odds with the view that there is a 50% limit to the flash photolysis of rhodopsin when the exposure duration is on the order of 1 ms or

less (Hagins, 1955), and with reports of a comparable ceiling on the bleaching of rhodopsin in the human retina (Rushton, 1963; Pugh, 1975 *a*).

The observation by Hagins (1955) of a 50% upper limit to bleaching with very short flashes prompted Williams (1964 and 1965) to develop a theoretical explanation based upon the photoreversal of bleaching. Williams attempted to quantify his analysis both in terms of reaction rate theory and by application of Poisson statistics, but he warned that the 50% limit on bleaching obtains only if it is assumed that rhodopsin and its photoproducts are equally photosensitive with respect to the incident light. Recently, however, Williams (1974) has stressed that the upper limits to the bleaching of rhodopsin in solution do not depend solely upon the duration of the flash; the temperature of the solution as well as the spectral content of the radiant energy influence the bleaching efficacy of the flash. Thus, at mammalian body temperatures ( $\sim 37^\circ\text{C}$ ), Williams found that a Strobolar flash devoid of ultraviolet light (to minimize photoreversal from metarhodopsin II) is capable of bleaching  $\sim 88\%$  of the rhodopsin. The present results in cat and those obtained in the human retina by Ripps and Weale (1969 *a*) and by Debecker and Zanen (1975) are in line with this observation.

A more general approach to the problem of flash photolysis was taken by Pugh (1975 *a*), who showed that the quantitative treatment of bleaching and photoreversal requires a kinetic analysis of the relevant reactions. Such an approach makes it clear that there is no *a priori* justification for assuming an upper limit of 50%. Given appropriate values for the spectral composition, duration, and intensity of the light reaching the outer segments, and for the photosensitivities and rate constants of rhodopsin and its photoproducts, the predicted bleaching limit may range from well *under* 50% (Ernst and Kemp, 1978 and 1979) to nearly 90%, as seen in the present experiments. Indeed the results of this study are wholly consistent with an analysis based on the scheme shown in Fig. 7. When credible values are used for the parameters, the results predicted by Eq. 5c agree remarkably well with the experimentally derived bleaching data (Fig. 4). The main factors relevant to this outcome are (*a*) that the combined quantum efficiencies of the photoreversal reactions are less than those of the bleaching reactions; (*b*) that the rate constant of the metarhodopsin I  $\rightarrow$  II reaction dictates that significant amounts of metarhodopsin II are formed during the flash; and (*c*) that the spectral transmissivity of the ocular media precludes significant absorption of the flash by metarhodopsin II, and hence no photoreversal occurs from this species. In the case of experiments with a similar flash on isolated retinas, the third factor no longer applies and an upper limit of  $\sim 60\%$  would be expected; this is near the value observed by Baumann (1970) for a xenon flash lasting  $< 500 \mu\text{s}$  applied to isolated frog retina.

The kinetic parameters selected by Pugh (1975 *a*) are difficult to explain. To account for the 50–60% upper limit on bleaching that he observed, he has had to reject plausible values for the rate constant of the metarhodopsin I  $\rightarrow$  II reaction and for the quantum efficiencies of the photoreversal reactions (see Appendix II of Pugh [1975 *a*]). Instead, it was necessary for him to assume a

value for the rate constant that is at least six times less than any value that has been reported (cf. Pugh [1975 *a*], Table 3). In addition, the quantum efficiency for the photoreversal from metarhodopsin I to rhodopsin had to be assigned a greater value than has been found in other studies (Kropf and Hubbard, 1958). On the other hand, inserting in Pugh's equations more reasonable estimates for these parameters results in a bleaching curve that approaches an upper limit of ~90%. However, this still leaves us with no obvious explanation for the discrepancy between Pugh's *experimental* finding of a 50–60% limit to the bleaching of human rhodopsin and the ~80% bleach achieved in the present study in cat and in those of other investigations in man (Ripps and Weale, 1969 *a*; Debecker and Zanen, 1975).

In addition to our findings on flash photolysis, difference spectra recorded at various times during the dark adaptation period between bleaches provided data on the temporal course of rhodopsin regeneration. The results shown in Figs. 5 and 6 demonstrate that for several minutes after the flash exposure the resynthesis of rhodopsin is masked by the presence of thermal intermediates. However, when the photoproducts have decayed sufficiently to reveal the kinetics of regeneration (i.e., after 15 min in darkness), two interesting features emerge. First, rhodopsin regeneration does not appear to follow the simple exponential time-course predicted by first-order reaction kinetics. And, second, regeneration after a large fractional bleach requires 60 min or more of dark adaptation. The latter finding is noteworthy in view of the results of a recent study by Bonds and Enroth-Cugell (1979) showing that the recovery of ganglion cell sensitivity follows a comparable time-course. These topics are dealt with more extensively in the following paper (Ripps et al., 1981).

#### APPENDIX

##### *Stray Light as a Source of Error in Fundus Reflectometry*

Throughout this paper, the measured values of  $\Delta D_\lambda$  computed from the spectral reflections are treated as if representing the absorbance changes for double transit through the receptor outer segments, i.e.,  $\Delta D(2)$ . However, the photocell may receive light other than that which has traversed the retinal receptors twice or even only once (Rushton, 1956 *b*; Ripps and Weale 1965 and 1970). Since some of the light it collects at each test  $\lambda$  has been reflected or scattered from surfaces located in front of the receptors, let the fraction of the incident light  $I_{\text{inc}}$  so described be  $\rho$ . In addition, due to the interstices between the retinal receptors, light may pass through the interreceptor spaces; if the fractional cross section of retina covered by rhodopsin is  $f$ , then that not covered by pigment is  $(1 - f)$ . Let the transmissivity of the dark-adapted retina be  $T_d$  and let  $T_b$  represent the retinal transmissivity after a bleaching exposure. If the reflectivity of the fundus is  $R$  and the overall transmissivity of the ocular media is  $t$ , then it follows that for double transit through the eye the measured intensity  $I$  of the emergent light for each test wavelength is given by

$$I_d = I_{\text{inc}}[f(1 - \rho)t^2T_d^2R + (1 - f)(1 - \rho)t^2R + \rho] \quad (6)$$

and

$$I_b = I_{\text{inc}}[f(1 - \rho)t^2T_b^2R + (1 - f)(1 - \rho)t^2R + \rho]. \quad (7)$$



Dividing Eq. 6 by Eq. 7 we obtain the measured change in transmissivity ( $\tau_m$ ) produced by an exposure that bleaches some fraction of the visual pigment:

$$\frac{I_d}{I_b} = \tau_m = \frac{fT_d^2 + (1-f) + \frac{\rho}{(1-\rho)t^2R}}{fT_b^2 + (1-f) + \frac{\rho}{(1-\rho)t^2R}} \quad (8)$$

Recalling that  $-\log_{10}\tau_m = \Delta D_m$ , and setting  $\frac{\rho}{(1-\rho)t^2R} = \sigma$ , we obtain

$$\begin{aligned} \Delta D_m &= \log_{10} \left[ \frac{I_b}{I_d} \right] = \log_{10} \left[ \frac{f(T_b^2 - 1) + \sigma + 1}{f(T_d^2 - 1) + \sigma + 1} \right] \\ &= \log_{10} \left[ \frac{T_b^2 + \left( \frac{\sigma + 1}{f} - 1 \right)}{T_d^2 + \left( \frac{\sigma + 1}{f} - 1 \right)} \right] \quad (9) \end{aligned}$$

Because the actual double density change at the retinal level is  $\log [T_b^2/T_d^2]$ , the measured density differences are affected by the sources of stray light  $\sigma$  and  $f$ . Minimizing specular reflection from the cornea and the surfaces of intraocular preretinal structures by appropriate subject alignment and masking of reflections reduces superficial stray light to <5% of the signal received from the eye (Rushton, 1956 *b*). Thus,  $\sigma$  is not likely to exert a marked effect on the measurements. On the other hand,  $f$  may not be a trivial factor, and its possible effects on the data and their interpretation should be considered.<sup>1</sup>

Let us assume, for example, that interreceptor spaces are a major source of stray light and that any observed change in retinal transmissivity underestimates the actual change that has taken place due to signal dilution by light that has reached the photoreceptor but has not twice traversed the receptors. An approximate value for  $f$  can be obtained by estimating first the in situ density of rhodopsin in a cat rod and then comparing the value with that measured by the reflectometer. The results of microspectrophotometric studies on the photoreceptors of a variety of vertebrate species indicate that the axial absorbance is  $\sim 0.013$  density units/ $\mu\text{m}$  (Liebman, 1972). Since the length of an adult cat rod outer segment is  $\sim 16 \mu\text{m}$  (Tucker et al., 1979), we arrive at an axial density of 0.208 for single passage and a double density of  $\sim 0.416$ . But our measurements gave a mean value of only 0.256 for the  $\Delta D(2)$ ; it is clear that the reflection signals were diluted by stray light. Eq. 9 can be used to estimate  $f$ , the fractional area of retina covered by rod outer segments. Since the maximum density change  $(\Delta D_m)_{\text{max}}$  is measured when all of the available rhodopsin is bleached away, i.e.,  $T_b^2 \approx 1$ ; and if  $\sigma$  is ignored, Eq. 9 reduces to

$$\Delta D_m(2) = \log_{10} \left[ \frac{1}{(1-f) + f \cdot 10^{-D_d(2)}} \right], \quad (10)$$

<sup>1</sup> In partitioning stray light into its components, light that passes once through the rods and enters or returns outside them is not treated separately. It is considered to produce the same effect as if half this light had twice traversed the rods and half had missed the rods entirely (Rushton, 1956 *b*).

where  $D_d(2)$  is the in situ density for double transit in the dark-adapted retina. And, substituting the aforementioned values for  $\Delta D_m$  and  $D_d$  in Eq. 10, we calculate that  $f$  is 0.72, i.e., ~28% of the light reaching the photocell has not passed through the rod outer segment. Fig. 8 shows how the apparent absorbance will vary with the true absorbance at any value of the latter, and hence the measured change  $\Delta D_m(2)$  as a

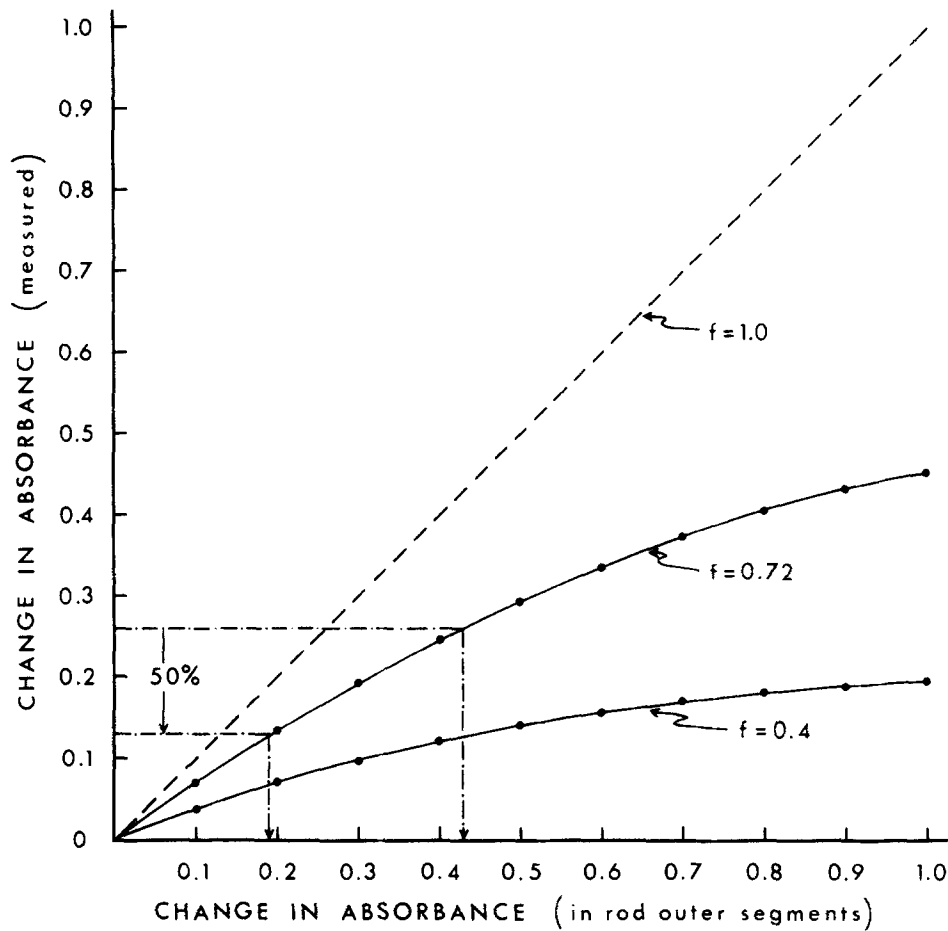


FIGURE 8. The effect of stray light on the absorbance changes measured by fundus reflectometry as a function of the density changes occurring in situ within the rod outer segments.  $f$  gives the fractional area occupied by the rhodopsin-bearing rods;  $(1 - f)$  is the area through which the measuring beams pass without attenuation by visual pigment. Note that a 50% change in the measured values of  $\Delta D$  indicates that a slightly greater density change has taken place in situ.

function of the in situ change in  $\Delta D(2)$ . Thus, if  $f \approx 0.72$ , and we had measured a density change of 50%, i.e., from 0.26 to 0.13, then the in situ density would have been reduced from 0.4 to 0.19, a change slightly >50%. And if we have overestimated the fractional area covered by rods, or underestimated the true in situ density of rhodopsin in cat rods, the measurement indicating a 50% bleach would have under-

estimated the true in situ change by an even greater amount (see curve marked  $f = 0.4$  in Fig. 8). The important point is that as a result of signal dilution by stray light, our measurements tend to *underestimate* the actual (in situ) density change produced by the bleaching exposure.

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#### REFERENCES

- ALPERN, M. 1971. Effect of a bright light flash on dark adaptation of human rods. *Nature (Lond.)* **230**:394-396.
- ALPERN, M., and E. N. PUGH, JR. 1974. The density and photosensitivity of human rhodopsin in the living retina. *J. Physiol. (Lond.)* **237**: 341-371.
- BAUMANN, CH. 1970. Flash photolysis of rhodopsin in the isolated frog retina. *Vision Res.* **10**: 789-798.
- BONDS, A. B., and C. ENROTH-CUGELL. 1979. Recovery of cat retinal ganglion cell sensitivity following pigment bleaching. *J. Physiol. (Lond.)* **295**:47-68.
- BONDS, A. B., and D. I. A. MACLEOD. 1974. The bleaching and regeneration of rhodopsin in the cat. *J. Physiol. (Lond.)* **242**: 237-253.
- BRIDGES, C. D. B. 1961. Studies on the flash photolysis of visual pigments. 2. Production of thermally stable photosensitive pigments in flash-irradiated solutions of frog rhodopsin. *Biochem. J.* **79**:135-143.
- BRIN, K. P., and H. RIPPS. 1977. Rhodopsin photoproducts and rod sensitivity in the skate retina. *J. Gen. Physiol.* **69**:97-120.
- BRINDLEY, G. S., and E. N. WILLMER. 1952. The reflexion of light from the macular and peripheral fundus oculi in man. *J. Physiol. (Lond.)* **116**:350-356.
- CARR, R. E., and H. RIPPS. 1967. Rhodopsin kinetics and rod adaptation in Oguchi's disease. *Invest. Ophthalmol.* **6**:426-436.
- CHANCE, E. M., A. R. CURTIS, I. P. JONES, and C. R. KIRBY. 1977. FACSIMILE: a Computer Program for Flow and Chemistry Simulation, and General Initial Value Programs. H. M. Stationery Office, London.
- CONE, R. A. 1972. Rotational diffusion of rhodopsin in the visual receptor membrane. *Nat. New Biol.* **236**:39-43.
- CONE, R. A., and W. H. COBBS III. 1969. Rhodopsin cycle in the living eye of the rat. *Nature (Lond.)* **221**:820-822.
- DEBECKER, J., and A. ZANEN. 1975. Flash bleaching of visual pigments in man investigated by early receptor recording. *Vision Res.* **15**:113-116.
- DOWLING, J. E., and R. HUBBARD. 1963. Effect of brilliant flashes on light and dark adaptation. *Nature (Lond.)* **199**:972-975.
- ENROTH-CUGELL, C., and L. H. PINTO. 1972. Properties of the surround mechanism of cat retinal ganglion cells and centre-surround interaction. *J. Physiol. (Lond.)* **220**:403-439.
- ERNST, W., and C. M. KEMP. 1978. Limits of bleaching in isolated frog retinas by monochromatic microsecond flashes. *J. Physiol. (Lond.)* **276**:38P. (Abstr.).

- ERNST, W., and C. M. KEMP. 1979. Reversal of photoreceptor bleaching and adaptation by microsecond flashes. *Vision Res.* **19**:363-365.
- GOLDSCHMIDT, CH. R., M. OTTOLENGHI, and T. ROSENFELD. 1976. Primary processes in photochemistry of rhodopsin at room temperature. *Nature (Lond.)*. **263**: 169-171.
- HAGINS, W. A. 1955. The quantum efficiency of bleaching of rhodopsin *in situ*. *J. Physiol. (Lond.)*. **129**:22P-23P. (Abstr.).
- HAGINS, W. A. 1956. Flash photolysis of rhodopsin in the retina. *Nature (Lond.)*. **177**:989-990.
- HUBBARD, R., and A. KROPF. 1958. The action of light on rhodopsin. *Proc. Natl. Acad. Sci. U. S. A.* **44**:130-139.
- HURLEY, J. B., T. G. EBREY, B. HONIG, and M. OTTOLENGHI. 1977. Temperature and wavelength effects on the photochemistry of rhodopsin, isorhodopsin, bacteriorhodopsin and their photoproducts. *Nature (Lond.)*. **270**:540-542.
- KROPF, A., and R. HUBBARD. 1958. The mechanism of bleaching rhodopsin. *Ann. N. Y. Acad. Sci.* **74**:266-280.
- LIEBMAN, P. 1972. Microspectrophotometry of photoreceptors. In *Photochemistry of Vision*. H. J. A. Dartnall, editor. Vol. VII/1 of *The Handbook of Sensory Physiology*. Springer-Verlag, Berlin.
- MAINSTER, M. A., P. W. WILSON, and J. H. TIPS. 1971. Calculation of photopic and scotopic parameters. *Vision Res.* **11**:1011-1014.
- MATTHEWS, R. G., R. HUBBARD, P. K. BROWN, and G. WALD. 1963. Tautomeric forms of metarhodopsin. *J. Gen. Physiol.* **47**:215-240.
- PRATT, D. C., R. LIVINGSTON, and K. H. GRELLMAN. 1964. Flash photolysis of rod particle suspensions. *Photochem. Photobiol.* **3**:121.
- PUGH, E. N., JR. 1975 *a*. Rhodopsin flash photolysis in man. *J. Physiol. (Lond.)*. **248**:393-412.
- PUGH, E. N., JR. 1975 *b*. Rushton's paradox: rod adaptation after flash photolysis. *J. Physiol. (Lond.)*. **248**:413-431.
- RIPPS, H., K. P. BRIN, and R. A. WEALE. 1978. Rhodopsin and visual threshold in retinitis pigmentosa. *Invest. Ophthalmol. Visual Sci.* **17**:735-745.
- RIPPS, H., L. MEHAFFEY III, and I. M. SIEGEL. 1981. Rhodopsin kinetics in the cat retina. *J. Gen. Physiol.* **77**:317-334.
- RIPPS, H., and A. G. SNAPPER. 1974. Computer analysis of photochemical changes in the human retina. *Comput. Biol. Med.* **4**:107-122.
- RIPPS, H., and R. A. WEALE. 1965. Analysis of foveal densitometry. *Nature (Lond.)*. **205**:52-56.
- RIPPS, H., and R. A. WEALE. 1969 *a*. Flash bleaching in rhodopsin in the human retina. *J. Physiol. (Lond.)*. **200**:151-159.
- RIPPS, H., and R. A. WEALE. 1969 *b*. Rhodopsin regeneration in man. *Nature (Lond.)*. **222**:775-777.
- RIPPS, H., and R. A. WEALE. 1970. The photophysiology of vertebrate color vision. In *Photophysiology*, Vol. 5. A. C. Giese, editor. Academic Press, Inc., New York. 127-168.
- RUSHTON, W. A. H. 1956 *a*. The difference spectrum and the photo-sensitivity of the rhodopsin in the living eye. *J. Physiol. (Lond.)*. **134**:11-29.
- RUSHTON, W. A. H. 1956 *b*. The rhodopsin density in the human rods. *J. Physiol. (Lond.)*. **134**: 30-46.
- RUSHTON, W. A. H. 1963. Effect of instantaneous flashes on adaptation of the eye. *Nature (Lond.)*. **199**:971-972.
- RUSHTON, W. A. H., and H. D. BAKER. 1963. Effect of a very bright flash on cone vision and cone pigments in man. *Nature (Lond.)*. **200**:421-423.

- STEINBERG, R. H., M. REID, and P. LACY. 1973. The distribution of rods and cones in the retina of the cat. *J. Comp. Neurol.* **148**:229-248.
- TUCKER, G. S., D. I. HAMASAKI, A. LABBIE, and J. MUROFF. 1979. Anatomic and physiologic development of the photoreceptor of the kitten. *Exp. Brain Res.* **37**:459-474.
- VAKKUR, G. I., P. O. BISHOP, and W. KOZAK. 1963. Visual optics in the cat, including posterior nodal distance and retinal landmarks. *Vision Res.* **3**:289-314.
- WEALE, R. A. 1953. The spectral reflectivity of the cat's tapetum measured *in situ*. *J. Physiol. (Lond.)*. **119**:30-42.
- WEALE, R. A. 1967. On an early stage of rhodopsin regeneration in man. *Vision Res.* **7**:819-827.
- WILLIAMS, T. P. 1964. Photoreversal of rhodopsin bleaching. *J. Gen. Physiol.* **47**:679-689.
- WILLIAMS, T. P. 1965. Rhodopsin bleaching: relative effectiveness of high and low intensity flashes. *Vision Res.* **5**:633-638.
- WILLIAMS, T. P. 1974. Upper limits to the bleaching of rhodopsin by high intensity flashes. *Vision Res.* **14**:603-607.
- WYSZECKI, G., and W. S. STILES. 1967. *Color Science*. John Wiley & Sons, Inc., New York.