THE DENSITY OF HUMAN RHODOPSIN IN THE RODS

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Abstract—The density of human rhodopsin in the rods has been estimated psychophysically by following the change in the relative quantum sensitivity for rod vision at two wavelengths (one near the peak, the other far off on the long wave tail of the action spectrum) in the dark after a full rhodopsin bleach in a region of a typical π_0 monochromat's retina where cones make no contribution to threshold. Metameric matching and dark adaptation curves yield results (about 0·75 ln units at 500 nm) consistent with recent densitometric estimates of Alpern and Pugh (1974) made on the normal retina.

INTRODUCTION

When it first became possible to study the difference spectra of visual pigments in the outer segments of vertebrate photoreceptors under a microscope (Denton and Wyllie, 1955), it was evident that the *in vivo* concentration of these substances was surprisingly high. There is now considerable psychophysical evidence to substantiate the validity of such observations for human cones (Brindley, 1955; Enoch and Stiles, 1961; Walraven, 1962; King-Smith, 1973; Miller, 1973). However, following Hecht, Shlaer and Pirenne (1942) it is usually assumed that the density of rhodopsin in human rods is small (about 0·23 ln units at the wavelength of peak absorbance). This assumption has now been undermined by the recent densitometry analysis of Alpern and Pugh (1974) which suggests that human rhodopsin concentration *in vivo* is about three times higher than this (i.e. more or less ·what is found in cones). Can psychophysical evidence be obtained which validates this estimation?

When a visual pigment is in high concentration, *in vivo*, its action spectrum will depend upon the fraction of the total complement in the unbleached state. Immediately after a full bleach, the fraction of unbleached molecules is very low and a light of wavelength near the tail of the absorption spectrum should require relatively more quanta to produce some constant physiological effect in comparison with a light of wavelength near the peak of the absorption spectrum than will be the case when all the pigment has regenerated. In effect then as rhodopsin regenerates in the dark, rod sensitivity tested at the λ_{max} becomes progressively reduced relative to that at the "tail". This effect is known as "self screening".

Even before the absorption spectrum of human rhodopsin was on firm experimental grounds, Nagel (1911) seems at least partly to have appreciated this fact. In a posthumous unrefereed report in the Appendix to the 3rd Edn. of Helmholtz' *Physiological Optics* (the editorial preparation of which he had begun but had not completed at his untimely death), he describes psychophysical evidence of this kind obtained by Stegmann and himself on the rod vision of both normals and one typical π_0 monochromat. On the other hand, de Vries (1946) failed completely to confirm these observations on normal subjects.

Evaluating this contradiction is complicated by the fact that in the dark after a full bleach almost all rhodopsin in the normal retina has regenerated before rod sensitivity exceeds that of cones and rod vision becomes testable (Rushton, 1961). We have, therefore, analyzed the problem anew in a region of the retina of one typical π_0 monochromat with no measurable cone function. The procedure was to compare rod spectral sensitivity at two wavelengths, one (500 nm) near the peak; the other, (632·8 nm) far off on the low frequency "tail", of the absorption spectrum under different bleaching circumstances (and, therefore, different rhodopsin concentrations).

The subject

The *S* was a 25-yr-old patient referred to this laboratory by Dr. H. F. Falls with the diagnosis of typical π_0 monochromacy. She had been under his care for the last 7 yr and had been studied by him and in this laboratory intermittently during that interval with no change in signs and symptoms. These included: a purely scotopic spectral sensitivity curve irrespective of how it is tested, a central scotoma, 4° dia, a minute pendular nystagmus, visual acuity R.E. 20/200 + 1, L.E. 20/100-1 which could not be improved with the spectacle correction for her small amount of ·simple hyperopic astigmatism. She reads J. O. at nearby holding the card very close to her eyes. The slit lamp examination was free of evidence of any pathological abnormalities and the ophthalmoscopic examination failed to reveal any evidence of macular disease; the discs were normal as were the vascular trees. She is totally color blind. Retina densitometry of the retina area used for central fixation revealed only rhodopsin (Alpern, 1974). Rod saturation at moderately bright backgrounds (equal to or greater than 1000 scotopic td) results in a "photophobia" but exhibits no evidence of the long term (up to 2 hr) "purblindness" described by Walls and Heath (1954) in a somewhat similar subject after such exposures. Nor do we confirm for her the observations of Sakitt (1973) on a so-called rod monochromat that thresholds after bleaching saturate so that however intense the test it remained invisible until about 75% of the rhodopsin had regenerated. Rather, in general, the constraints on visibility early in the dark after a full bleach for this subject appeared to be those imposed by the upper limit of the intensity of the test flash.

Occasional ("rhodopsin") cones can be found in some regions of the retinas of monochromat eyes. Psychophysically they are revealed by a double-branched dark adaptation curve measured after a full bleach and by an increment threshold curve which does not show the characteristic rod saturation. We have deliberately tested a region of this subject's retina which manifests neither of
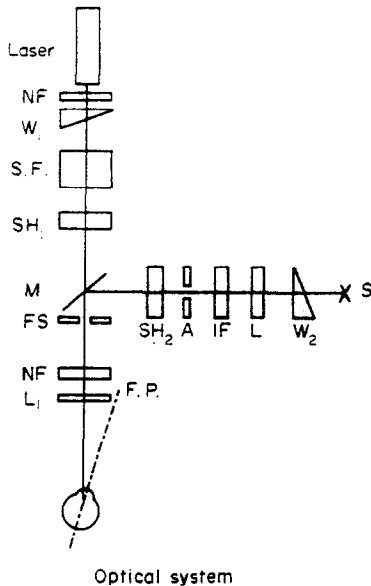
Optical system

Fig. 1. Schematic drawing of the optical system as viewed from above (not to scale). Light from two sources, a Spectra Physics helium–neon laser ($\lambda = 632\cdot8$ nm) and a tungsten filament S are mixed together at M. The field stop, FS, is located at primary focal plane of L_1 (a type I Kodak Ektar f 2·5, 155 mm focal length lens); the aperture stop, A, in Channel 2 and the pinhole of the spatial filter, S.F., (Channel 1) are equidistant from M and both are imaged in the subject's pupil by L_1. The Baird-Atomic interference filter, IF, renders Channel 2 monochromatic at $\lambda = 500$ nm with a half band width of 10 nm. F. P. is the fixation point; SH_1 and SH_2 are Uniblitz (Vincent Associates, Rochester, N.Y.) shutters; W_1, W_2 are "neutral" wedges and NF "neutral" density filters. The lens, L_2, images the tungsten filament on A.

these evidences of cones. In particular, log threshold in the dark after a bright bleach follows the time course of a simple exponential curve and the increment threshold curve saturates at backgrounds of about 10^3 scotopic td (with a semi-saturation constant of 300 hν absorbed rod^{-1} flash^{-1}).

METHOD

Figure 1 is a line drawing of the apparatus. Monochromatic lights from two separate sources (one, a laser providing 632·8 nm, the other, a tungsten filament attenuated by an interference filter transmitting 500 ± 10 nm) were brought together at the mirror M. The intensity of each light beam was reduced by balanced Inconel Wedges (W_1 and W_2) and by "neutral" Inconel filters, all calibrated with a Beckman Acta II spectrophotometer. The test target was viewed by a patch of retina 8° temporal from the fixation point. The circular stop FS regulated the field size; in testing, it was 3° and in bleaching, 8° dia. Bleaching was provided by the tungsten source after the interference filter (IF) was removed from the beam; it then produced 6·5 log td and was exposed for 2 min. This was sufficient to bleach all measurable rhodopsin in the patch of retina illuminated by the focused light.

The subject's head was fixed by biting into a wax dental bite. The image of the laser aperture stop in the plane occupied by her pupil was 0·5 mm; that of the tungsten source was 1·0 mm. These two images were carefully centered on each other. They were positioned in the center of the pupil while she fixated. Widely dilating her pupil with one drop of 1% cyclogyl® obviated eclipsing either beam by the iris due to the small nystagmus

movements characteristic of the fixation of such subjects. The test flashes were exposed for 50 msec once a second by two shutters, one in each beam, controlled by Grass Physiological stimulators. This permitted the experimenter to determine the wavelength under test at any moment in the dark after bleaching. In brightness matching, both channels were simultaneously exposed; in this case, only a single shutter mounted in the common beam was used.

All components of the optical system were mounted on saddles fixed to triangular optical bench rails clamped to a 1·5-in. 8-ply plywood table top. Three different mirrors, each mounted in a separate saddle, served the function in turn of the mirror at M. In bleaching, this was a full silvered mirror reflecting all the light from the tungsten source and transmitting none of the laser light. After the bleach, the saddle containing this mirror was removed and replaced with one or the other of the remaining two. In those experiments in which threshold was measured, its replacement contained a beam mixer which reflected about 30% of the incident tungsten light and transmitted about 30% of the incident laser light. In brightness matching, the third saddle was used. It contained a full surface mirror, the edge of which vertically bisected the field of view into two equal parts: (i) that light reflected at the mirror from the tungsten source and (ii) that part of the laser beam unobstructed by the mirror. Each of these mirrors had been precisely aligned prior to the experiment. Since the location of the proper position of the replacing saddle was easily identified and achieved in the dark, these mirrors were readily interchanged manually during the run without disturbing the optical alignment.

The maximum exposure of the laser beam in these experiments was 2·95 $(10)^{-3}$ W/cm^2 at the retina. This is more than 2·5 log units dimmer than the minimum intensity of a 50 msec flash for damage to any ocular tissue (Peppers and Hammond, 1969).

PART I. METAMERIC MATCHING

Procedure

The test was divided into two half fields: on the left, the monochromatic green light, and the laser monochromatic red light on the right. Both beams were exposed together (for 50 msec) and the observer's task was to adjust the red wedge so that the two fields matched under two different observation conditions (and, therefore, two different presumed rhodopsin concestrations): (1) after 30 min in the dark $[c = \beta(\lambda)/\alpha(\lambda)l]$ and (2) in the dark immediately after a full rhodopsin bleach ($c = 0$). In fact, no measurements were possible immediately after the bleach; it required a full minute at the least before the bipartite field was visible. The comparison was, therefore, between the matches made between 60 and 150 sec after the bleaching and those obtained after complete dark adaptation.

The red and green intensities were first matched in full dark adaptation by placing neutral filters in the common path immediately behind the lens L_1 and then by setting the wedges, W_1 and W_2, somewhere in the middle of their respective ranges. The final match was always established with the red wedge W_1 and only it was adjusted during the experiment before or after bleaching. Following bleaching, both beams were made considerably brighter by removing neutral filters from the common path. Throughout brightness matches were established between lights always approx 1 log unit above threshold.

Analysis

It is assumed that: (i) only absorbed quanta enter into the photochemical events which initiate vision (ii) Beers and Lambert's Law adequately describes quantum absorption in the rods and (iii) equal quanta absorptions cause equal visual effects

independent of wavelength: This last assumption (Naka and Rushtons' (1966) univariance) applies to one photoreceptor cell or to groups of such cells, all of which have the same visual pigment. In the typical π_0 monochromat here under study, we have deliberately sought out that region of her retina where dark adaptation and increment threshold measurements show that the latter is clearly the situation.

When two half fields of wavelengths λ_1 and λ_2 are illuminated with lights of intensity I_1 and I_2 respectively, so adjusted that they appear identical:

$$I_1 T_1[1 - \exp(-\alpha_1 lc)] = I_2 T_2[1 - \exp(-\alpha_2 lc)]. \qquad (1)$$

In this equation T_1 and T_2 represent the respective transmissivities of the ocular media at λ_1 and λ_2.

$\alpha(\lambda) =$ the chromophoric extinction coefficient in cm^2 per chromophore,

$c =$ concentration of rhodopsin in the rods in chromophores cm^{-3},

$l =$ length of the light path in the outer segments of the rods in cm,

$c_d =$ concentration of rhodopsin in full dark adaptation,

$\beta(\lambda) =$ Napierian density of rhodopsin in-the rods in full dark adaptation $[\alpha(\lambda)c_d]$,

$p(t) =$ $c(t)/c_d$ and

$t =$ time in min in the dark after a full bleach.

The wavelengths of the two test lights here under consideration were so selected that according to the C.I.E. scotopic spectral sensitivity curve

$$[1 - \exp(-\beta_1)]/[1 - \exp(-\beta_2)] = 2 \cdot 7 \, (10)^{-3}.$$

Under these conditions, it is reasonable to assume that in the series expansion

$$1 - \exp(-\beta_1) = \beta_1 - \beta_1^2/2! + \beta_1^3/3! - \beta_1^4/4! \ldots .$$

β_1 is so small that the higher order terms can safely be neglected. Accordingly, in full dark adaptation equation (1) reduces to

$$I_1 T_1/I_2 T_2 = [1 - \exp(-\beta_2)]/\beta_1.$$

After a full bleach $c \to 0$, so that the matching intensities at the two wavelengths I_1', I_2' are related by

$$I_1' T_1/I_2' T_2 = \beta_2/\beta_1,$$

so that

$$I_1 I_2'/I_2 I_1' = [1 - \exp(-\beta_2)]/\beta_2. \qquad (2)$$

If k is the density of the "neutral" filters in the common beam in full dark adaptation which were removed for the matches after the bleach and W_1 is the transmissivity of the red wedge (plus any transmissivity differences at the two wavelengths in the "neutral" filters removed from the common

beam), then

$$\log I_2/I_2' = k,$$

and

$$\log(I_1/I_1') = k + \Delta \log W_1.$$

Hence from equation (2)

$$\Delta \log W_1 = \log[1 - \exp(-\beta_2)]/\beta_2. \qquad (3)$$

Results

The results of this experiment (repeated five times) are summarized in Table 1. The first column gives the average (± 1 S.D.) of red wedge density in the interval between 1 min and 2·5 min in the dark after a full bleach. The second column gives the average density (± 1 S.D.) of the same wedge settings after 30 min (corrected for non neutrality of the common filters added). It is seen that in each of the five sets of experiments relatively more red light was required for the match immediately after bleaching than was the case in full dark adaptation. This is the expected result if the rhodopsin in the monochromat's rods was in considerable density. The densities β_2 (i.e. at 500 nm) calculated from these measurements according to equation (3) are given in the third column.

Student's *t*-test was utilized to evaluate the significance of the differences in the settings of the red wedge (the degrees of freedom for each experiment being listed in the fourth column in the table).

These results show considerable variability in the estimation of β_2 from one run to the next. Furthermore, only two of the five estimations in Table 1 differ significantly from zero.

The reasons for these difficulties are not hard to find. Rushton (1961) and Alpern (1971 a,b) have independently shown that after a full bleach rhodopsin regenerates *in vivo* according to equation

$$p(t) = c(t)/c_d = [1 - \exp(-t/\tau)], \qquad (4)$$

in which τ is the time constant of regeneration of rhodopsin (about 9·33 min for the patch of retina under consideration here).

Since, in the above experiments, the first measurements were made between 1 and 2·5 min in the dark, the rhodopsin concentrations at these instances were not zero but between 0·1 and 0·235

Table 1. Brightness matches (red wedge densities) at beginning and end of dark adaptation. Typical π_0 monochromat

60 - 150 sec.	After 30 min.		
$\bar{x} \pm \sigma$	$\bar{x} \pm \sigma$	β_{500}	DF
.841 $\pm$ .246	.888 $\pm$ .108	0.235 n.s.	76
.298 $\pm$ .0787	.378 $\pm$ .0517	0.400 *	17
.953 $\pm$ .217	1.03 $\pm$ .038	0.380 n.s.	54
.859 $\pm$ .133	.920 $\pm$ .070	0.310 n.s.	23
.710 $\pm$ .204	.850 $\pm$ .046	0.700 *	34

* p = 0.01

n.s. = not significantly different from zero

times its full dark concentration. Part of the variability in these results must be due to the fortuitous set of circumstances under which the initial measurements were completed sooner after the bleach in one run than in another. Since no settings could be made near zero concentration, the results in Table 1 can be regarded only as estimates of the lower limit of rhodopsin density.

To obviate these problems, an attempt was made to extrapolate the red wedge setting to the value it would have had at the moment ($t = 0$) the bleaching light was extinguished (when the rhodopsin concentration was known to be very nearly zero). The calculation was strengthened by making brightness matches throughout the 30 min or so in the dark after bleaching while rod sensitivity is changing (rather than only at the very beginning and at the very end of adaptation as was done above). It is seen from equation (3) that

$$\Delta \log W_1(t) = \log \{[1 - \exp(-\beta_2) \\ \times (1 - e^{-t/\tau})]/[\beta_2(1 - e^{-t/\tau})] + a\}. \quad (5a)$$

In this equation, a is the asymptotic value of log W_1 when $t = \infty$.

Experiment

As soon as the test target was visible in the dark after a full bleach, the observer made brightness matches by adjusting the red wedge. The process was repeated over and over until the values of her settings leveled off after 30 min or so. The experimenter recorded the time and the red wedge setting of each adjustment and periodically added a neutral filter to the common beam so that the intensity of the green field always hovered around a level about 1 log unit above its absolute threshold. The experiment was repeated four times on a single day.

The results of each run were analyzed by computer to determine the values of β_2 and a in equation (5a) which minimized the squares of the deviations from the curve described by this equation (given $\tau = 9.33$ min). The best estimates of β_2 (± 1 S.D.) resulting from the individual runs are summarized in the insert in Fig. 2.

The value for a is a function of more or less arbitrary decisions of the experimenter regarding the neutral filters in the common beam and differed from run to run. In pooling the results from the four runs, the individual sets of results were vertically shifted by computer to provide the best overall fit to equation (5a). The individual data points in Fig. 2 are the results from each run plotted as $\Delta \log W$ after this shifting. The smooth curve drawn through these results is the least squares estimate of the best fit to equation (5). It yields an average estimate of $\beta_2 = 0.733 \pm 0.093$ (mean ± 1 S.D.). Although there is considerable scatter in Fig. 2, the ordinate scale of that figure is very enlarged. The density estimate obtained is significantly different from zero and in substantial agreement with the Alpern and Pugh densitometric value, hence considerably higher than that predicted from Hecht, Shlaer and Pirenne (1942). The right hand ordinate scale in Fig. 2 shows how the value of $\beta_2 p(t)$ increases with time in the dark as the rhodopsin concentration
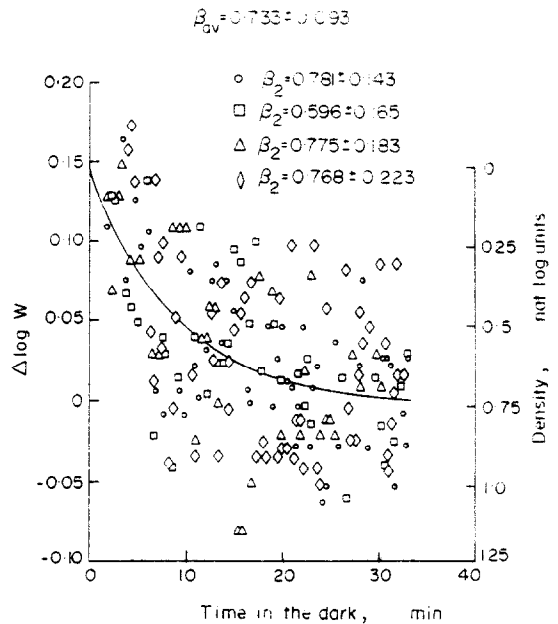

Fig. 2. Changes in the red wedge setting required for brightness matches as a function of time in the dark after a full bleach. The four different symbols represent results from four experimental repetitions completed in a single day. The experimental results from each run were utilized to generate by computer those values β_2 and a which, when substituted in equation (5a), minimized the squares of the deviations from the theoretical curve (given $\tau = 9.333$ min). The points plotted in this figure have been vertically shifted so that all results have a common asymptote. The average estimate of $\beta_2 = 0.733 \pm 0.093$. The table insert gives the estimate of β_2 from each run. The ordinate scale on the right shows how density at 500 nm increased as rhodopsin concentration returned to the full dark value after the bleach.

changes. Comparing the two ordinate scales in this figure emphasizes further the difficulty in obtaining accurate psychophysical estimates of the rhodopsin density from individual expreiments. Given the high density of 0.733, the total range of $\Delta \log W$ is only 0.15 log units. The precision of individual brightness matches in rod vision is typically only 0.05–0.10 log units.

PART II. ABSOLUTE THRESHOLD CHANGES IN THE DARK

For this reason, a second psychophysical estimate of the density of rhodopsin in this monochromat's rods was obtained by measuring threshold changes during dark adaptation.

Procedure

After the bleach, the subject sat in the dark and observed the flashing green light set at its maximum intensity signalling the moment it became visible. The experimenter then switched the shutter so that the red test was flashing and the subject adjusted the red wedge for threshold. The experimenter then switched the shutters so that the green test was flashing and recorded both the red wedge setting and the moments of its adjustment from the stop watch reading; meanwhile, the subject adjusted the green wedge for threshold. The experiment proceeded in this way alternatively testing for green then red throughout some 33 min in the dark after the full bleach. The shaft on which the green wedge rotated was geared to a

potentiometer which was wired to the Y-axis of a Hewlett–Packard 7035B X–Y plotter, the X-axis of which was driven by a 17108A External Time Base reset at zero at the moment the bleaching light was extinguished. In this way, the subject automatically noted the green wedge settings by activating a signal marker; the experimenter recorded both the moment and the value of the red wedge settings while continuously switching the shutters from one beam to the other throughout the run. As before, neutral filters were added to the common beam whenever they were needed.

Analysis

The assumptions made in the derivation of equation (2) are as appropriate for the analysis of dark adaptation measurements as they are for brightness matching. In using this equation in this context, I_λ represents the threshold intensity at wavelength λ_1, λ_2 after infinite time in the dark and I'_λ the threshold intensities immediately after a full rhodopsin bleach.

In practice, I_λ, I'_λ must be estimated by extrapolations. To this end, we take advantage of the fact (Rushton, 1961; Alpern, 1971 a,b) that a single exponential curve describes the time course of the recovery when log threshold is plotted as a function of time in the dark after a full bleach and the test is made of a region of the monochromat's retina in which rods alone contribute to threshold. A computer program (Chandler, 1965) was utilized to fit equations of the form $y = A + B \exp(-t/\tau)$ [in which y is log threshold (in quanta flash^{-1})] to the measured dark adaptation results so as to minimize the square of the deviations from the curves. The values of $A = \log I_\lambda$ and $A + B = \log I'_\lambda$ were then determined directly from these best fitting exponential curves.

Results

The results of four experimental repetitions are shown in the usual way in Fig. 3. In the major part of this figure, each plotted point is a single measurement and the smooth curves drawn through them are the best fitting exponential recovery curves obtained from the computer analysis. The values of I_1, I'_1, I_2 and I'_2 obtained from these curves when substituted into equation (2) yield the estimate of $\beta(500) = 0.787$ in good agreement with that obtained from brightness matching. In order to appreciate how subtle the difference in recovery is which yields this estimate of high density, the two computer generated curves alone (without the data points) are replotted to a smaller scale in the inset with the asymptotic value of the curve for the 632·8-nm test (dashed curve in the figure) arbitrarily equated to that of the 500-nm test (solid curve). The two curves are virtually indistinguishable except for the first 9 min or so in the dark.

A word regarding the curve fitting in Fig. 3 used to obtain I'_λ by extrapolation is in order. This procedure is based upon the observation that simple exponential curves provide the best (albeit purely empirical) description of the experimental results. On theoretical grounds, if $p = 0$ at $t = 0$ there is the difficulty that there would be no rhodopsin molecules available to catch the quanta in the test flash whose intensity must be infinitely large, though the exponential extrapolations in Fig. 3 to $t = 0$ yield log $[I'/I_\infty] = A + B$. Hollins and Alpern (1973) developed a
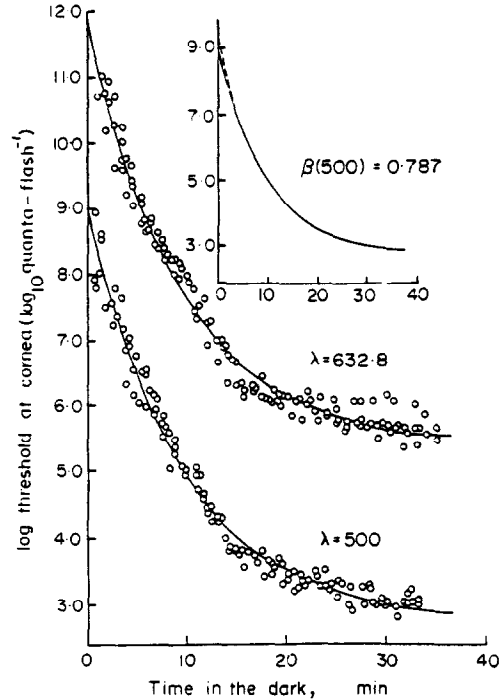

Fig. 3. Simultaneous rod dark adaptation threshold measurements for a red and a green test as a function of time in the dark after a full bleach. The results of four experimental repetitions are presented, and the smooth curves drawn are the best fitting exponential functions obtained by a computer analysis. The equations for the two curves are

$$y_2 = 2{\cdot}803 + 6{\cdot}323 \exp(-t/9{\cdot}54)$$

and

$$y_1 = 5{\cdot}486 + 6{\cdot}486 \exp(-t/9{\cdot}34).$$

The parameters from these equations (i.e. log $I'_1 = 11{\cdot}972$, log $I'_2 = 9{\cdot}126$, log $I_1 = 5{\cdot}486$ and log $I_2 = 2{\cdot}803$) when substituted into equation (2) give an estimate for the density (at 500 nm) equal to 0·787. To appreciate the small difference which yields this value, the two computer generated curves are replotted in the inset with the curve for the red test (dotted curve in figure) arbitrarily shifted until its asymptote was identical to that of the curve for the green test.

more general equation to deal with this difficulty for foveal π_5 cones. Their equation,

$$\log[I(t)/I_\infty] = k_1(1 - e^{-\beta})(1 - p)/(1 - e^{-\beta p}), \qquad (6)$$

(in which k_1 is a constant) did not, however, describe their results more successfully than simple exponential curves, such as the ones drawn in Fig. 3. We have applied equation (6) to the results in Fig. 3, but the best fitting curves of this kind are about 15% poorer fits than those shown in the figure, the root mean square deviation of the measurements from which are relatively small (0·22 and 0·23 respectively). For this reason, and because all known theories which have attempted to relate log rod threshold elevation with fraction of unregenerated pigment after a full rhodopsin bleach are clear oversimplifications (Alpern, 1971a; Duffy, 1971; Pugh, 1972), we have selected the superior empirical description. Conceptually this assumes that p only approaches zero at $t = 0$. It should be appreciated, however, that the application of equation (6) does allow an estimation of I'_λ/I'_λ even though I'_λ approaches infinity at $t = 0$ and that the resulting value of

β_2 obtained from the results in Fig. 3 with this estimation (1·28) is even higher than that obtained from the curves drawn in Fig. 3. Hence, the decision to use the purely empirical equation may prove ultimately to have yielded only a lower limit estimate (unconventionally high as it is) of the density of rhodopsin in this monochromat's rods.

DISCUSSION

The results of the two different methods used here to study rhodopsin density psychophysically in a typical π_0 monochromat's retina are in good agreement with the estimates of rhodopsin density made with retinal densitometry on the normal retina by Alpern and Pugh (1974). These converging lines of evidence for a value much higher than that given by Hecht et al. are summarized in Table 2.

The results in this table are also in good agreement with microspectrophotometric measurements of rhodopsin density in individual rods, the most recent of which (MacNichol, Feinberg and Harosi, 1973) are considerably larger (0·0175 ± 0·0015 common log units/μm) than Liebman's (1962) earlier results (0·013 ± 0·0002). For a rod outer segment 25 μm long, the respective optical densities corresponding (0·4375–0·325) are a good deal higher than the macrospectrophotometric measurements of Bauman and Bender (1973) of 0·1, in part no doubt because the latter estimates are diluted by stray light (Alpern and Pugh, 1974).

In view of the results illustrated in Fig. 3, it is not surprising that de Vries (1946) found no evidence for the effect of bleaching on rod spectral sensitivity of his own (normal) eye. It is now known that in light adaptation rod vision saturates before any significant rhodopsin is bleached (Aguilar and Stiles, 1945) and the dark adaptation curves in Fig. 3 clearly show that after a full bleach the rod dark adaptation curves for a red and green test would have superimposed before rod sensitivity exceeded that of cones in the normal peripheral retina. It is for this reason that these measurements had to be made on a typical π_0 monochromat retina to demonstrate the subtle effect of high density on rod spectral sensitivity.

De Vries' selection of 580 nm for a long wave test (λ_1) was also not judicious for obtaining evidence for high in vivo rhodopsin density. His value of $[1 - \exp(-\beta_1)]/[1 - \exp(-\beta_2)]$ is only 0·121. With the two wavelengths used here, this ratio is 0·0027 but still only very small distinctions in the earliest moments in the dark after a full bleach were found.

Although our conclusion is qualitatively, at least, identical to that of Stegmann and Nagel, we do not know how to deal adequately with the experimental results which led them to it:

"If, for example, after being dark-adapted for a period of 5 to 10 min 'twilight matches' were made

between a spectral orange and blue-green (both of which would, of course, look colourless), and if then this match was considered by the same eye after it had been dark-adapted for a much longer time, it ceased to be valid: the light of longer wavelength (orange) looked the brighter of the two, and it was necessary to reduce its intensity to three-fourths of what it had been."

We have been unable to find any more extensive description of these experiments, but this result appears compatible with those described here only if the kinetics of the rhodopsin in the eyes making these observations were radically different from those of any human eyes studied so far with the retinal densitometer.

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Table 2. Mean estimate of rhodopsin density (λ = 500 nm)

Method:	Density Natural logarithmic units	Density Common logarithmic units
Densitometry (Alpern & Pugh, 1974)	0.788	0.342
Brightness matching	0.733	0.318
Dark adaptation curves	0.787	0.342

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