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PSYCHOPHYSICAL ESTIMATES OF VISUAL PIGMENT DENSITIES IN RED-GREEN DICHROMATS

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SUMMARY

1. The spectral sensitivity of red-green dichromats was determined using heterochromatic flicker photometric matches (25–30 c/s) on the fovea. These matches are upset after a bright bleach and consequently the spectral sensitivity is altered.

2. Preliminary experiments indicate that under the conditions in which these experiments were performed, the blue cone mechanism of deuteranopes and protanopes cannot follow 20 c/s flicker. If dichromats lack one of the normal pigments then the upset of these matches monitors the change in spectral sensitivity of a single mechanism.

3. After a bleach which removes all the cone pigments, the spectral sensitivity recovers with the time course of pigment kinetics as measured by densitometry.

4. An intense background also changes the relative spectral sensitivity of the dichromats. On real equilibrium backgrounds, the changes in spectral sensitivity follow those predicted by the pigment changes measured by densitometry. The predicted changes are obtained by modifying the Rushton equilibrium equation to take into account the density of pigment.

5. The relationship of these changes to the luminance of the background is independent of the colour of the background light.

6. In contradistinction the effect is dependent on the colour of the lights which were flickered. These experiments indicate that a narrowing of the spectral sensitivity curves takes place on both sides of the dichromats' λ_{max}.

7. The change in relative spectral sensitivity as a function of background intensity was also determined by increment threshold measurements. These changes can be expressed in terms of deviations from Weber's law ($\Delta I/I$ = const.) if ΔI and I represent the number of chromophores destroyed by the test and background.

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8. The relative spectral sensitivity of the dichromat was changed by decentering the point of pupil entry. This upset was abolished by bleaching. The size of the upset was correlated with the magnitude of the S–C *I* effect.

9. Given the hypothesis of pigment density (self-screening), the results of expts. (3)–(8) are consistent and allow the calculation of a maximum optical density for those pigments which underlie the dichromats' long-wave mechanism. For the deuteranope a $D_{\lambda max}$ of 0·5–0·6 is calculated and for the protanope a $D_{\lambda max}$ of 0·4–0·5 is obtained.

INTRODUCTION

Normal human foveal colour vision is mediated by three channels whose peak spectral sensitivities lie in the long, middle and short wave regions of the spectrum. If the spectral sensitivities of these three channels do not change shape as a result of adaptation, then metameric matches made at one level of adaptation will hold at all levels (von Kries, 1878). However, Wright (1934, 1936) and de Vries (1948) showed that after adaptation to sufficiently bright light, metamers became distinguishable and therefore the spectral sensitivity of at least one of the three systems is altered by the adaptation. The change in the shape of the spectral sensitivity curve can be explained by assuming that one or more of the underlying photopigments whose peak sensitivity lie in the red-green region of the spectrum is present in high optical density (0·5–1·0) which is very much reduced by bleaching (Brindley, 1953, 1955, 1970; Walraven & Bouman, 1960; Enoch & Stiles, 1961; Tersteige, 1967).

In this paper the question of pigment density (or self-screening) is re-examined by several psychophysical techniques using dichromats rather than normal trichromats. The protanope lacks the normal trichromat's long wave (or red) pigment and the deteranope lacks the normal's middle wave (or green) pigment (Nuberg & Yustova, 1955, 1961; Rushton, 1963*a*, 1965*b*; Mitchell & Rushton, 1971*a*, *b*). The dichromatic system was further simplified by using lights flickering at a rate of 20 c/s or more which are signalled by the blue cone mechanism as steady (Brindley, Du Croz & Rushton, 1966; Green, 1969). The red and green cone mechanisms, however, signal flicker at much higher frequencies and this property was used to monitor the change in action spectrum as the light level was increased. In this way it was possible to test the hypothesis of self-screening on a single receptor mechanism.

METHODS

Screening procedure for dichromats. The dichromats were screened with AO H-R-R pseudoisochromatic plates, Farnsworth Dichotomus test for colour vision and the Nagel anomaloscope. Both the protanopes and deuteranopes were able to match

the yellow for all ratios of R/G with the deuteranopes having very little variation in the amount of yellow needed regardless of R/G ratio. In addition it was found that some subjects who were able to match over the entire range of the anomaloscope could not match a 540 nm light to a 650 nm light (+desaturating light of 460 nm). These subjects were not used since a dichromat by definition should require only two degrees of freedom to match all colours.

The initial flicker experiments were performed on four dichromats (two protanopes and two deuteranopes) and the results were essentially the same. Therefore all the results of this paper will be confined to one protanope (S.N.) and one deuteranope (R.F.). Some control experiments were done on normal and anomalous trichromats.

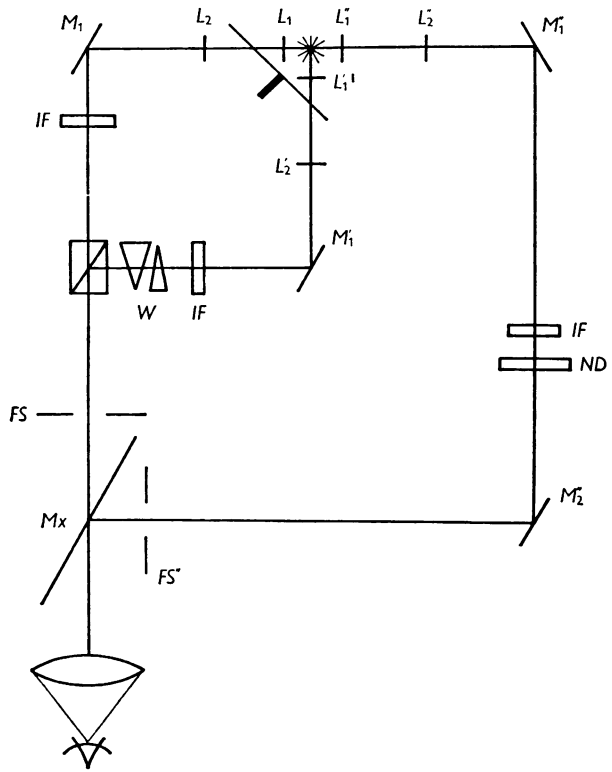

Fig. 1. Optical system. The elements of the system are discussed in the text.

Apparatus and calibration procedure. All of the experiments described below were performed on the apparatus shown schematically in Fig. 1. The source is xenon 150 W high stability w/1 Osram arc lamp with a suitable power supply. The three optical trains shown in Fig. 1 have identical small f number lenses placed close to the source. These lenses then bring the beam down to a small parabola-shaped image in the L_i, L' channels to a somewhat larger one in the background beam, L''_i. In the L_i, L'_i channels, which have been constructed as symmetrically as possible, these images are interrupted by a sector disk run by a variable speed synchronous motor. Mx is a beam mixer (dielectrically coated glass) which allows 30–50 % of the light to be reflected. The final Maxwellian lens is a 155 mm $f/2$ Ektar lens. The circular

wedge W (with balancer) was controlled by the subject with a gear and rod system. This system was geared so that the control knob turned one-half a revolution for about 0·12 log unit change on the wedge.

Some care was required in the calibration of the apparatus since the maximum size effect to be measured was about 0·2 $\log_{10}$ unit. The wedge, a Wratten no. 96, has a density range of approximately 0–3 $\log_{10}$ units and its linearity with angle was determined using a Helium Neon gas laser (632·8 nm). M_1' was removed and replaced by the laser and a calibrated photodiode (Pin-10; United Detector Technology) was placed in the plane of the final image. The beam was chopped at 30 c/s to obviate stray light effects. The output of the photodiode was amplified and displayed on a 502 Tektronic cathode ray oscilloscope.

The first $\log_{10}$ unit was stepped off in 0·1 $\log_{10}$ unit steps and the rest in 0·3 $\log_{10}$ unit steps. This was done by placing a 2×2 N.D. filter 0·1 or 0·3 (both calibrated throughout the visible spectrum on a Carey 14 spectrophotometer) in the beam, obtaining a reading on the scope, then removing the filter and turning W until the signal returned to its former amplitude. The linearity was also calibrated at the several other wave-lengths used during the course of the experiments. In these cases the xenon source itself provided the light but, in addition, the narrow band interference filters used in the experiments were placed in the beam. These filters (Baird Atomic B-1 series) have a band width at half maximum of 5 nm and are blocked to infinity on either side of the pass band. These interference filters are necessary for any calibration of the system using the photodiode since it is sensitive in the infrared and the source provides as much or more energy in this region of the spectrum as in the visible. The interference filters were calibrated on a Zeiss spectrophotometer and outside the pass band they were blocked to 4 $\log_{10}$ units or more. Both the Carey 14 and the Zeiss were checked against an absolute standard provided by Eastman Kodak and against each other. The error in the 1·0 O.D. range is $\pm$ 0·03 $\log_{10}$ unit.

The subject's head was positioned with respect to the light beams by means of a drill press table which had three orthogonal degrees of motion. A metal bar is secured to this table by means of set screws, and mounted in the bar is a dental wax impression of the subject's teeth. In this way the position of entry on the light beams into the pupil could be kept relatively fixed during the course of the experiment. The sessions did not last more than 2 hr. The subject was protected from stray light by a series of light baffles in the various optical channels and by a thick curtain of black cloth which separated the source and M_1', M_2' from the rest of the system. In addition, a soft flexible light tight tube one end of which was fitted to the subject's eye cup extended continuously from the final lens in the system to the eye. The other eye was always covered.

The relative spectral sensitivity of the visual system was required for the wave-lengths of interest. This means that the relative spectral distribution of the optical system (light source, optics, filter transmission) as a whole was determined at these wave-lengths. This was accomplished by using the calibrated Pin-10 diode. The filters used in the experiments were placed in the system and the voltage output was recorded on the oscilloscope. The photometry of the test and background fields was done with a SEI photometer which was calibrated against a Macbeth illuminometer.

The recovery of flicker matches after a bright bleach. In these experiments the observer's visual field consisted of a 1·6° test light superimposed on a 10° background. The test light was flickered (25–30 c/s) between two wave-lengths λ_P and λ_T which fell on the peak and long wave tail of the dichromats' long wave receptor system. For protanopes the wave-lengths used were 535 and 625; for deuteranopes 570 and 650 nm. The observer set a wedge in one of the test beams in order to just detect (or to eliminate) the flicker. This manoeuvre, described in more detail in the results

section, was performed before and at various times after a full bleach (10^6 td for 2 min). After the bleaching light was extinguished, the background light was turned on again. This light was filtered through a 445 nm narrow band (± 5 nm at half maximum) interference filter and its luminance was 10^3 td. This bleaches, at most, 10% of the pigment (Rushton, 1963*a*, 1965*b*). This background was provided in order to further obviate the contribution of the blue cone mechanism to the flicker matches.

Increment thresholds and flicker matches against real backgrounds. The interference filters and size of visual fields used in these experiments are identical to those described above. In the increment threshold experiments two different test flashes (0·2 sec every sec) were used. The wave-length of one test flash was selected to fall at the peak of the dichromats' long wave spectral sensitivity function and the other to fall on its long wave tail. The wave-length of the background always falls on the peak of this curve. Only its luminance is varied. At each background level the subject adapts for 2 min or more and then manipulates a wedge W in the test beam to just detect a test flash whose wave-length may be λ_p or λ_T. To obtain the log relative spectral sensitivity at each background level, the calibrated wedge setting for each test light is subtracted.

The flicker experiments (30 c/s) were also performed against continuous backgrounds. The advantage of this class of experiment over the bleaching experiment is that the subject, in a relaxed manner, can repeat his settings a fairly large number of times at each background level. The background was varied between $10^{2\cdot5}$ td and $10^{5\cdot4}$ td and at each level a 'stopping range' was determined. This is the range of wedge settings over which the observer can detect no flicker. The technique used by both observers was to move in from a position of noticeable flicker on either side of the stopping range to the first stopping point. That the blue cone mechanism has a flicker fusion frequency less than 25 c/s was verified for the subjects in these experiments by using the techniques of Stiles (Stiles, 1959) under conditions designed to isolate the response of these receptors alone (Brindley, Du Croz & Rushton, 1966; Green, 1969).

In addition to the filters mentioned above, several other wave-length pairs were flickered against each other. In these experiments the upsets were measured only on a full bleaching background. However, for some wave-lengths (shown in Table 1) it was not possible to bleach all the pigments and still detect the flickering lights on the background. These cases will be denoted by an asterisk and have the percentage pigment bleached in parentheses next to it. All other cases refer to full bleaches (90% of the pigment or more). Each measurement is given ± 2 S.E. of mean. The subject usually controls the 570 nm (or 535 nm) beam and the upset is in the direction of needing less light in the 570 nm beam relative to 650 nm. This is the upset expected if the relative spectral sensitivity curve narrowed as a result of the bleach. If the subject controlled the 650 nm (or 625 nm) beam (as in some earlier trials), he would require more light in the 650 nm (625 nm) beam. The subject makes about twenty settings per trial. For example, in the case of the protanope, the maximum density inferred from the upset between 535 nm and 625 nm is used along with the measured relative spectral sensitivity between 535, 605; 535, 575; 535, 495; and 535, 440 nm (under conditions where a very small fraction of pigment is bleached) to predict the upset when over 90% of the pigment is bleached. The calculation also required the use of eqns. (2), (3) and the calibration of the optical system at all the above wave-lengths.

Stiles–Crawford measurements. The brightness Stiles–Crawford effect (S–C I) was obtained for these observers by having them determine, as a function of pupil position, the amount of light needed in a test flash (330 msec every 660 msec) to

detect it against a luminous background (which bleaches less than 5% of the pigment). The flicker beam was then brought into the eye at those pupil positions (centre and 3·5 mm nasal) which correspond to the maximum and minimum of this effect.

In these experiments the lights flickered were again at the peak and tail of the long wave receptor system. The size of the test light was 1·6° and the background (kept on continuously) was 10°. The test beam (flickered between λ_T and λ_p at 30 c/s) was moved across the plane of the pupil by means of a variable prism inserted immediately after *FS* in Fig. 1. The observer was required to stop the flicker at the centre and edge of the pupil and this was done on two different backgrounds (filtered by Wratten 44A), one which bleached a very small fraction of the pigment and another which bleached a very large fraction. In order to do this the subject controlled the amount of light in one of the beams with the wedge *W* (570 nm for deuteranope R.F. and 535 nm for protanope S.N.).

Theoretical analysis of the data

The dichromats' data can be analysed in terms of their long wave receptor mechanisms. Let λ_p fall on the peak of the dichromats' long wave pigment, λ_T on its tail. Since the blue cone mechanism is unable to follow the flicker between these wavelengths, its output is equivalent to that produced by a single steady light. That the observer (protanope or deuteranope) can stop the flicker by adjusting the luminance in one of the two beams strongly suggests that the quantum catches at λ_T, λ_p have been equated for that pigment which underlies the long wave-length spectral sensitivity curve. Mathematically this is written as

$$I_{inc}(\lambda_T)(1-\exp[-2{\cdot}3D_{\lambda T}]) = I_{inc}(\lambda_p)(1-\exp[-2{\cdot}3D_{\lambda p}]), \qquad (1a)$$

where the losses in the observer's eye are not included and D_λ is the optical density of that pigment which underlies the long wave mechanism. Coloured bleach products are here assumed to be negligible. If $1-\exp(-2{\cdot}3D_\lambda)$, the relative spectral sensitivity of the pigment is denoted by K_λ, then

$$\log\left[\frac{I_{inc}(\lambda_T)}{I_{inc}(\lambda_p)}\right] = \log\left[\frac{K_{\lambda p}}{K_{\lambda T}}\right]. \qquad (1b)$$

If the pigment is initially present in concentration, the change in wedge setting $\Delta \log W$ as a result of a bleach is given by

$$\Delta \log W = \log\left[\frac{I'_{inc}(\lambda_T)}{I'_{inc}(\lambda_p)}\right] - \log\left[\frac{I_{inc}(\lambda_T)}{I_{inc}(\lambda_p)}\right] = \log\left[\frac{K'_\lambda / K'_{\lambda T}}{K_{\lambda p}/K_{\lambda T}}\right], \qquad (2)$$

where the prime notation indicates the spectral sensitivity of the bleached pigment. $\Delta \log W$ is determined in each experiment; $K_{\lambda p}/K_{\lambda T}$ is known from separate flicker measurement at non-bleaching levels; therefore $K'_{\lambda p}/K'_{\lambda T}$ can be calculated. If α_λ denotes the molecular extinction coefficient of the pigment molecules and c, the number of molecules per unit area of receptor, then $D_\lambda = \alpha_\lambda c$. Therefore at low pigment concentration $K'_{\lambda T}/K'_{\lambda \rho} = D'_{\lambda T}/D'_{\lambda p} = D_{\lambda T}/D_{\lambda p}$ and

$$\frac{K_{\lambda T}}{K_{\lambda p}} = \frac{1-\exp[-2{\cdot}3D_{\lambda T}]}{1-\exp[-2{\cdot}3D_{\lambda p}]} = \frac{1-\exp[(-2{\cdot}3D_{\lambda p})(K'_{\lambda T}/K'_{\lambda p})]}{1-\exp[-2{\cdot}3D_{\lambda p}]}. \qquad (3)$$

$D_{\lambda p}$ is then calculated from eqn. (3) using the experimentally determined values of $K_{\lambda T}/K_{\lambda p}$ and $K'_{\lambda T}/K'_{\lambda p}$.

RESULTS

In Figs. 2 and 3 the abscissa denotes the time after the cessation of the bleaching light and the ordinate denotes a change in relative wedge setting $\Delta \log W$ as a result of the bleach. These results are typical of a large number of trials on four subjects. As soon as the observer detects the light

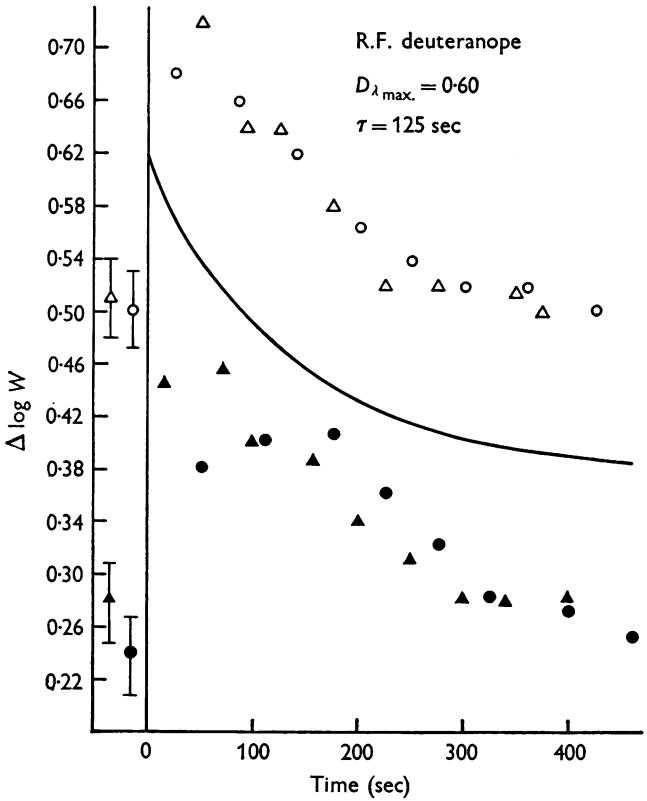

Fig. 2. Change in the end-points of flicker as a function of time after a full bleach (two trials). The test light was flickered between $\lambda = 650$ nm and 570 nm at 30 c/s. The end-points of flicker ± 2 S.E. of mean (at least ten measurements per trial) before the bleach are denoted at the left, and their mid-point provide an asymptote for the smooth curve which has a time constant of 125 sec. The value of this curve at $t = 0$ is $\Delta \log W = 0{\cdot}24$ and this value was used in eqns. (2) and (3) to calculate $D_{\lambda max}$. A variation in $\Delta \log W$ of 0·20–0·26 allows $D_{\lambda max}$ to vary from 0·50 to 0·65.

flickering, a reading was taken (e.g. in Fig. 3 open square nearest to $t = 0$). Then the wedge was turned until the flicker disappeared and again reappeared. This setting and time were also noted (in Fig. 3 filled square at $t = 50$ sec). Repetition of this sequence of reversals in wedge rotation

generates the open and filled symbols shown in these Figures. Settings were also determined for each trial prior to the bleach when the pigment was present in full density. The average value of these end-points ± 2 S.E. of mean (ten settings per trial) is shown for each trial. The mid-point between these averages was used to define the asymptote for the recovery curves shown in Figs. 2 and 3.

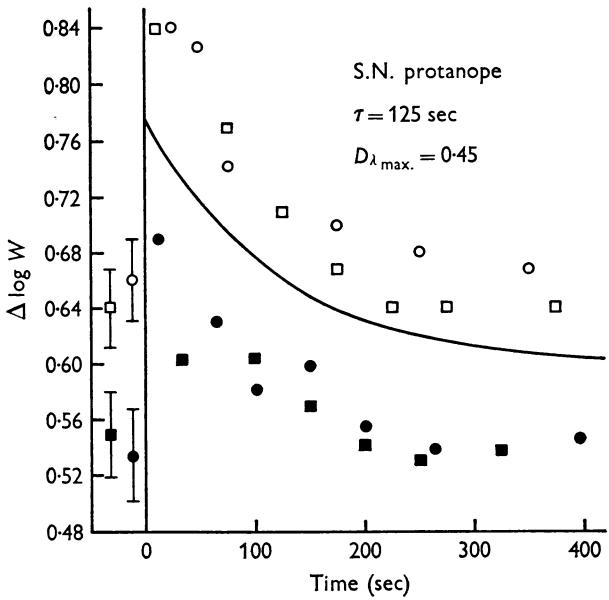

Fig. 3. Change in the end-points of flicker as a function of time after a full bleach (two trials). The test light was flickered between λ = 625 nm and 535 nm at 30 c/s. The end-points of flicker ± 2 S.E. of mean (at least ten measurements per trial) before the bleach are denoted at the left, and their mid-points provide an asymptote for the smooth curve which has a time constant of 125 sec. The value of this curve at t = 0 is $\Delta \log W$ = 0·18 and this value was used in eqns. (2) and (3) to calculate $D_{\lambda max}$. A variation in $\Delta \log W$ of 0·16–0·20 allows $D_{\lambda max}$ to vary from 0·40 to 0·50.

In the region between the flicker end-points (open and filled symbols) the observer sees the flicker lights fused. The recovery of the flicker mid-points specifies the recovery of $\Delta \log W$ with time. The theoretical recovery curves fall nearly midway between the open and closed symbols with a time constant of 125 sec. The time course of the effect follows the pigment kinetics as measured by densitometry (Rushton, 1965*a*, 1963*b*).

The flicker matches do not change very much over a period of time during which threshold sensitivity varies by over 3 log units. One may wonder whether the changes in the subjective brightness of these lights,

during dark adaptation, causes the observed change in $\Delta \log W$ with time. To eliminate this possibility the observer sometimes kept the subjective brightness constant by putting neutral density filters in the test beam. Some of the settings in the figures were made this way. The recovery of the flicker matches were no different where the subjective brightness was fixed or allowed to vary.

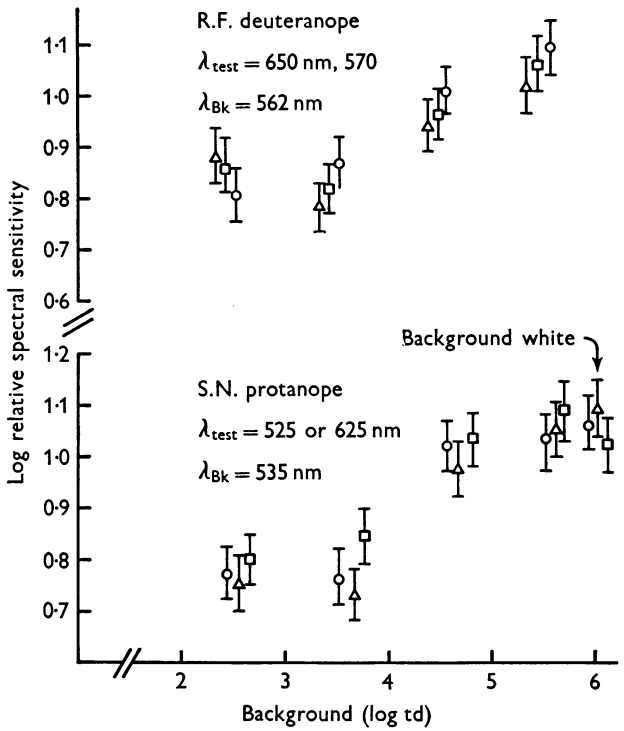

Fig. 4. Change in relative spectral sensitivity as a function of background luminances as measured by increment thresholds. For S.N. the background wave-length was 535 nm and the test wave-length either 525 or 625 nm. The background of largest luminance, 10^6 td, was attained using white light, and the value of $\Delta \log W$ (0·24) at this background is consistent with a $D_{\lambda max}$ of 0·6. A variation in $\Delta \log W$ of 0·20–0·28 allows $D_{\lambda max}$ to vary from 0·5–0·7. For R.F. the background wave-length was 562 nm and the test wave-lengths were 570 and 650 nm. In this subject $D_{\lambda max}$ is 0·5 ($\Delta \log W$ = 0·20) and a variation in $\Delta \log W$ of 0·16–0·24 allows $D_{\lambda max}$ to vary from 0·4 to 0·6.

These results demonstrate the way in which a full bleach alters the flicker match. From the maximum observed effect at t = 0 and eqns. (2) and (3) the calculated optical density for the deuteranope (R.F.) was 0·6 and for the protanope (S.N.) 0·45.

Increment thresholds and flicker matches against real backgrounds. In Fig. 4 the change in log relative sensitivity as determined by thresholds (method of adjustment) is measured as a function of background intensity I (log trolands). On each trial at least five determinations of the threshold are made at every level of background. Three separate trials are shown along with error bars which represent ± 2 S.E. of mean (two of the symbols are displaced from the centre for the sake of clarity but the background levels for each trial were identical).

If we express Weber's law ($\Delta I/I$ = const.) in terms of the number of chromophores destroyed by the test light ΔI and the number of chromophores destroyed by the background light I, then

$$\frac{\Delta I_{\text{inc}}(\lambda_T)(1-\exp[-2{\cdot}3D_{\lambda T}])}{I_{\text{inc}}(\lambda_p)(1-\exp[-2{\cdot}3D_{\lambda p}])} = \text{constant}, \tag{4}$$

where the optical density D_λ is directly proportional to the concentration, c, of photopigment. In the limit as $c \to 0$ we have

$$\frac{\Delta I'_{\text{inc}}(\lambda_T)D'_{\lambda T}}{I_{\text{inc}}(\lambda_p)D'_{\lambda p}} = \text{constant}, \tag{5}$$

where the prime notation indicates that the concentration has been altered. The observed change in wedge setting $\Delta \log W$ is obtained from (4) and (5) with a little algebra,

$$\begin{aligned}\Delta \log W &= \log\left[\frac{\Delta I'_{\text{inc}}(\lambda_T}{\Delta I'_{\text{inc}}(\lambda_p)}\right] - \log\left[\frac{\Delta I_{\text{inc}}(\lambda_T)}{\Delta I_{\text{inc}}(\lambda_p)}\right] \\ &= \log\left[\frac{D'_{\lambda p}/D'_{\lambda T}}{1-\exp[-2{\cdot}3D_{\lambda p}]/1-\exp[2{\cdot}3D_{\lambda T}]}\right].\end{aligned} \tag{6}$$

Each term of the left-hand side of eqn. (6) represents the relative spectral sensitivity between λ_T and λ_p as determined by thresholds. Notice that eqn. (2) and (6) are identical. If the pigment is initially present in density, the right-hand side of (6) represents the maximum observable change in relative spectral sensitivity due to bleaching. If the pigment is not present in density, then this term is zero and no change in relative spectral sensitivity due to pigment concentration changes should occur. $D_{\lambda_{\max}}$ can be calculated using eqn. (3) since the left-hand side of eqn. (6) is observed and

$$\frac{1-\exp[-2{\cdot}3D_{\lambda T}]}{1-\exp[-2{\cdot}3D_{\lambda p}]}$$

is known. A value of about 0·5 was obtained for the deuteranope and 0·6 for the protanope.

Self-screening predicts that the spectral sensitivity curve should show similar changes as a result of bleaching on both sides of the $\lambda_{\max}$. There-

fore the open diamonds of Fig. 5 and the filled triangles of Fig. 6 are of particular interest, since they represent the change monitored on the short wave side of the dichromats' long wave spectral sensitivity function. The effect of the blue cone mechanism was further obviated by using a background (Wratten 44A) which specifically suppressed them relative to the long wave cones. The stopping range for this pair of wave-lengths was as narrow as that which involved long wave-lengths. In both cases the values

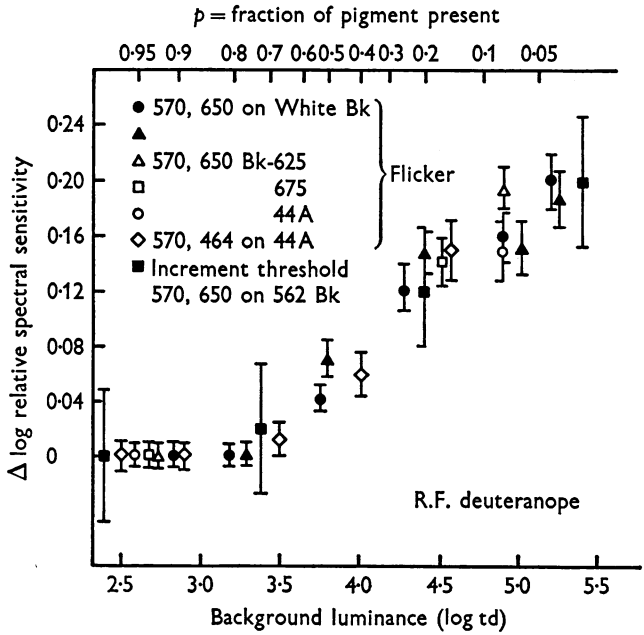

Fig. 5. Change in log relative spectral sensitivity as a function of background luminance (or p) for different coloured backgrounds and for different coloured flickering lights. The increment threshold data of Fig. 5 is replotted here for comparison. Note that the p axis is non-linear. The relative spectral sensitivity between 570, 464 nm and 570, 650 nm are approximately equal. The best choice for $D_{\lambda max}$ is 0·5 and a variation in $\Delta \log W$ between 0·18 and 0·22 allows $D_{\lambda max}$ to vary from 0·45 to 0·55.

of $\Delta \log W$ for relatively small values of p are not obtained due to the limitations of the source. Bleaching backgrounds on both sides of the long-wave peak have been used. These measurements test the photochemical nature of the effects which should not depend on the colour of the bleaching light, but only on the number of quanta absorbed by the pigment. These data suggest a $D_{\lambda max}$ of 0·5 for the deuteranope and 0·44 for the protanope.

The increment threshold data (Fig. 4) are determined on steady-state

backgrounds I. In order to compare these data with the flicker data (Figs. 5 and 6), we first combine the three trials and then calculate

$$\Delta \log W = \log \left[\frac{K'_{\lambda p}/K'_{\lambda T}}{K_{\lambda p}/K_{\lambda T}}\right]$$

where $\log (K_{\lambda p}/K_{\lambda T})$ is the relative spectral sensitivity of the receptor determined on a background which bleaches less than 5% of the pigment and $\log (K'_{\lambda p}/K'_{\lambda T})$ is the relative spectral sensitivity determined on backgrounds which bleach increasing amounts of the pigment. These values of $\Delta \log W$ are then plotted at the appropriate I value in Figs. 5 and 6. The

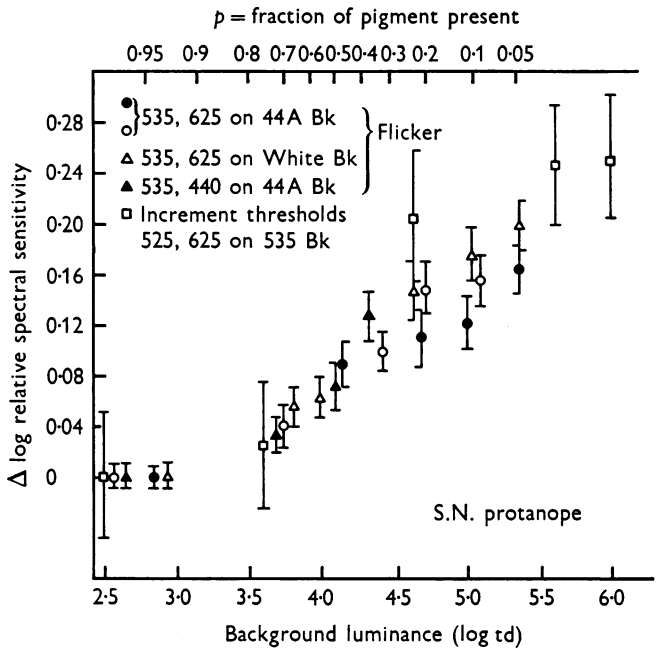

Fig. 6. Change in log relative spectral sensitivity as a function of background luminance (or p) for different coloured backgrounds and for different coloured flickering lights. The increment threshold data of Fig. 5 is replotted here for comparison. Note that the p axis is non-linear. The relative spectral sensitivity between 535, 440 nm and 535, 625 nm are approximately equal. The best choice for $D_{\lambda max}$ is 0·44 and a variation in $\Delta \log W$ between 0·16 and 0·22 allows $D_{\lambda max}$ to vary from 0·40 to 0·54.

error bars in their case are significantly larger than those of the flicker matches. The consistency between flicker and increment thresholds seems to be better in the case of the deuteranope. However, it should be noted that the effect is normalized with respect to the spectral sensitivity determined at low levels of bleaching. Therefore these two sets of data are forced to agree at the origin of co-ordinates for large values of p. There is nothing sacred about this choice and we could as well shift the points at

small p down by 1 S.E. of mean in which case the error bars for the points at all values of p would overlap the flicker data.

Fig. 7 compares the flicker data with what would be predicted on the basis of Rushton's densitometry data. Given the kinetic relations it is possible to calculate $\Delta \log W$ as a function of I for different assumptions

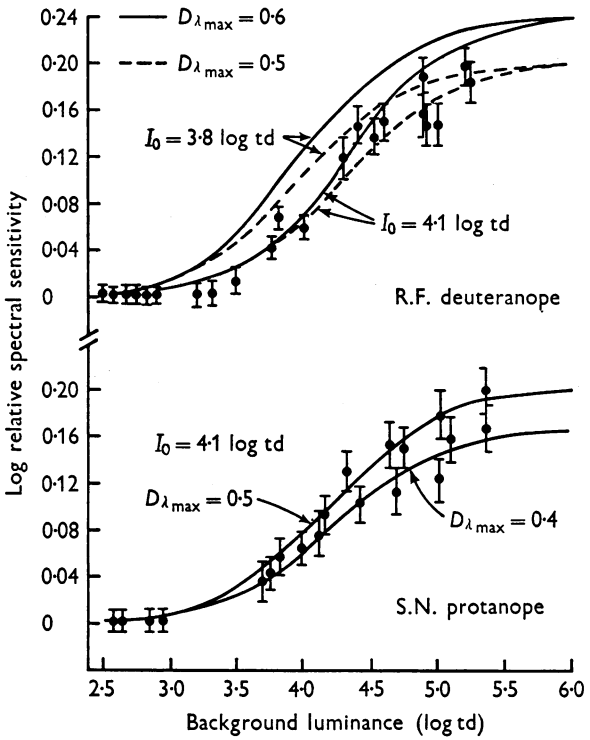

Fig. 7. Comparison of a theoretical prediction with all the flicker data of Figs. 5, 6 treated equally. For R.F. both the dashed and solid curves are obtained using the results of densitometry for different assumed maximum optical densities and different I_0s (eqn. (8)). The same comments apply to S.N. except that only one value of I_0 is used.

about the maximum optical density. In order to obtain the curves of Fig. 7, we first replace the usual pigment equilibrium relation

$$I/I_0 = \frac{1-p}{p} \tag{7}$$

by one which takes into account the pigment density, that is,

$$I/I_0 = \frac{(1-p')\alpha_{\lambda_p} c_0}{1-\exp\left[-\alpha_{\lambda_p} c_0 p'\right]}, \tag{8}$$

where $D_{\lambda_{max}} = \alpha_{\lambda_p} c_0/2{\cdot}3$ and p (or p') is the fraction of unbleached pigment, I the luminance in td, and I_0 that luminance which bleaches 50 % of the pigment at equilibrium. Of course (8) reduces to (7) in the limit of small $D_{\lambda_{max}}$. Next we consider the transition from $c_0 \rightarrow c$ in eqn. (2) which can be written as

$$\Delta \log W = \log \left[\frac{1-\exp(-\alpha_{\lambda_p} c)}{1-\exp(-\alpha_{\lambda_p} c_0)} \cdot \frac{1-\exp(-\alpha_{\lambda_T} c_0)}{1-\exp(-\alpha_{\lambda_T} c)} \right], \tag{9}$$

where $K'_\lambda = 1-\exp(-\alpha_\lambda c)$. In this expression the ratio

$$\frac{1-\exp(-\alpha_{\lambda_T} c_0)}{1-\exp(-\alpha_{\lambda_p} c_0)}$$

is known from the onset. It is determined directly from flicker measurements once the optical system is calibrated at these wave-lengths. There is, however, an adjustable parameter in this expression, $\alpha_{\lambda_p} c_0$, and once this parameter is assigned a value; then $\alpha_{\lambda_T} c_0$ is also determined. For example, for the deuteranope we have

$$\Delta \log W = \log \left[\frac{1-\exp(-\alpha_{\lambda_p} p c_0)}{1-\exp(-\alpha_{\lambda_T} p c_0)} \right] - 0{\cdot}85, \tag{10}$$

where $p = c/c_0$ and 0·85 is the log relative spectral sensitivity between $\lambda = 570$ nm and $\lambda = 650$ nm. Assuming a particular value for $\alpha_{\lambda_p} c_0$, the maximum optical density of the pigment, we use (8) to obtain a p' for each I and (10) to obtain $\Delta \log W$ for each p'. In this way, a family of curves characterized by $\alpha_{\lambda_p} c_0$ and relating $\Delta \log W$ and I may be drawn. The curve which best fits the data gives us the optical density consistent with pigment kinetics.

The densitometry data for deuteranopes (Rushton, 1965*b*) is best fit by choosing $I_0 = 3{\cdot}8$ log td. But the data of Fig. 7 is consistent with $I_0 = 4{\cdot}1$ log td. A part of this difference is due to a shift in the equilibrium curve along the luminance axis (to higher values) which occurs just on the basis of the density assumption (about 0·2 log units when the density goes from $0 \rightarrow 1$). But more importantly, this difference is consistent with the intersubject variation of this parameter. The best estimate for $D_{\lambda_{max}}$ is seen to be between 0·5 and 0·6. The densitometry data for protanopes (Rushton, 1963*a*) is best fit by $I_0 = 4{\cdot}1$ log td as is the data of Fig. 7. In this case the best estimate for $D_{\lambda_{max}}$ lies between 0·4 and 0·5.

The self-consistency of the flicker data can be tested by using the upset ($\Delta \log W$) at one pair of wave-lengths (535, 625 nm or 570, 650 nm) to calculate on the basis of self-screening the upset between other wavelength pairs. The data (± 2 S.E. of mean) of Table 1 demonstrates this consistency. That the size of effect is not symmetrical with respect to the peak can be seen by comparing the upset between 535, 575 and 535, 495 in

Table 1*B* and by comparing 570, 495 and 570, 650 in Table 1*A*. The effect is somewhat smaller on the short wave side and this is what we expect qualitatively of foveal action spectra corrected for media losses (Wald, 1966; Wyszecki & Stiles, 1967). This observation is consistent with the

TABLE 1. Comparison of predicted and observed $\Delta \log W$ for several wave-length pairs

A. Protanope S.N.

Wave-lengths flickered against each other	Observed upset ($\Delta \log W$)	Predicted upset
535 *vs.* 625	$0{\cdot}16 \pm 0{\cdot}04$ (see Fig. 6)	—
535 *vs.* 605	$0{\cdot}15 \pm 0{\cdot}04$	0·15
535 *vs.* 575	$0{\cdot}09 \pm 0{\cdot}04$	0·09
535 *vs.* 495	$0{\cdot}04 \pm 0{\cdot}04$	0·07
535 *vs.* 440*	$0{\cdot}13 \pm 0{\cdot}04$ (75% bleach)	0·11

B. Deuteranope R.F.

Wave-lengths flickered against each other	Observed upset ($\Delta \log W$)	Predicted upset
570 *vs.* 650	$0{\cdot}20 \pm 0{\cdot}04$ (see Fig. 5)	—
570 *vs.* 625	$0{\cdot}09 \pm 0{\cdot}04$; $0{\cdot}11 \pm 0{\cdot}04$	0·13
595 *vs.* 650	$0{\cdot}16 \pm 0{\cdot}04$	0·16
570 *vs.* 464*	$0{\cdot}15 \pm 0{\cdot}04$ (80% bleach)	0·16
570 *vs.* 495	$0{\cdot}07 \pm 0{\cdot}04$; $0{\cdot}05 \pm 0{\cdot}04$	0·11
625 *vs.* 495	$0{\cdot}05 \pm 0{\cdot}03$ (subject controls 625 beam which requires less density after the bleach)	

TABLE 2. Observed $\Delta \log W$'s on decentring test beam in plane of pupil

Subject	Size of S-C effect	Wave-lengths flickered	Pupil position W (log units) Centre	Pupil position W (log units) Edge	Bleaching level	Percent pigment bleached*
R.F.	~ 0·5 log units	570, 650	$0{\cdot}33 \pm 0{\cdot}02$	$0{\cdot}37 \pm 0{\cdot}01$	2·5 log td	4%
	λ_{test} = 570, 650		$0{\cdot}55 \pm 0{\cdot}04$	$0{\cdot}55 \pm 0{\cdot}04$	5 log td	95%
R.F.	~ 0·81 log units	570, 650	$0{\cdot}37 \pm 0{\cdot}02$	$0{\cdot}47 \pm 0{\cdot}05$	1·8 log td	< 4%
	λ_{test} = 570, 650		$0{\cdot}55 \pm 0{\cdot}05$	$0{\cdot}57 \pm 0{\cdot}05$	4·8 log td	90%
S.N.	~ 0·87 log units	535, 625	$0{\cdot}34 \pm 0{\cdot}03$	$0{\cdot}52 \pm 0{\cdot}04$	1·8 log td	< 4%
	λ_{test} = 535, 625		$0{\cdot}47 \pm 0{\cdot}05$	$0{\cdot}50 \pm 0{\cdot}05$	4·4 log td	74%
S.N.	~ 0·80 log units	535, 625	$0{\cdot}33 \pm 0{\cdot}03$	$0{\cdot}45 \pm 0{\cdot}05$	2·4 log td	< 4%
	λ_{test} = 535, 625		$0{\cdot}50 \pm 0{\cdot}05$	$0{\cdot}50 \pm 0{\cdot}05$	4·4 log td	74%
S.N.	0·75 log units	535, 625	$0{\cdot}33 \pm 0{\cdot}01$	$0{\cdot}46 \pm 0{\cdot}05$	1·8 log td	< 4%

* Rushton, 1963*a*, 1965*b*.

absorption characteristics of the visual pigments extracted thus far (Dartnall, 1962).

Stiles–Crawford measurements. The data ($\pm$ 2 S.E. of mean) of Table 2 show that the flicker matches for the deuteranope R.F. and the protanope S.N. are upset after the beam moves from the centre to the edge of the pupil. The second line in each trial shows that the effect is abolished by bleaching. This has also been shown by Brindley (1953) in a qualitative way (the apparent hue shift on decentring was abolished by adaptation) and suggests a connexion between the directional sensitivity of the retina and the concentration of visual pigment. Starting at the top of the Table with subject R.F., we see that for a small S–C I effect (2·2 mm from centre to edge position) the upset, although small, is in the same direction as would be predicted by self-screening. When the flickering beams are moved between two more widely spaced pupil positions (S–C I effect of 0·81 log units), the upset, in moving from centre to edge, was doubled and the effect of bleaching at the edge was reduced which is also consistent with the idea that more peripheral rays pass through more dilute pigment.

Eqns. (2) and (3) allow us to calculate the reduction in the effective peak density $D_{\lambda_{max}}$ which would occur as a result of oblique incidence. For the deuteranope this reduction is 0·17 log units. The data for this calculation is taken from the second trial of R.F. where $\Delta \log W = 0{\cdot}1$ (obtained by subtracting the value of $\log W$ in column 5 from that in column 4). On the protanope, if we take the decentring effect as

$$\Delta \log W = 0{\cdot}12$$

(second or third trial) then this corresponds to a reduction of $D_{\lambda_{max}}$ by 0·25 log units.

Brindley (1953) found that the upset of metamers which occurs as a result of decentring in the plane of the pupil could be explained by the self-screening hypothesis if this decentring resulted in a reduction of $D_{\lambda_{max}}$ by 0·2 log.

DISCUSSION

The above results are explained if it is assumed that the visual pigments which underlie the dichromats' long wave spectral sensitivity mechanisms are present in rather high optical density. However, there exist other explanations which cannot, with certainty, be excluded (Brindley, 1955).

For example, coloured bleach products may be produced preferentially by one receptor system as a result of the bleach and stay in the vicinity of that receptor or they may migrate preferentially to another. We can ask what the absorption characteristics of these coloured bleach products need to be in order to explain the results of this paper. For the protanope

the upsets given in Table 1*A* tell us that this absorption must be decreasing between 625 and 535 nm and then rising between 535 and 440 nm. In the case of the deuteranope (Table 1*B*) the absorption by bleach products must be decreasing between 650 and 570 nm and then rising between 570 and 464 nm. The results between 625 and 495 nm tell us that the distribution must be falling at one rate between 625 and 570 nm and rising at a slower rate (rate = d(effect)/$d\lambda$) between 570 and 495. Figs. 2 and 3 indicate that these coloured bleach products regenerate hand in hand with the visual pigments. These hypothesized sets of circumstances seem unrealistic in view of the fact that all known lasting products of rhodopsin bleaching transmit strongly at long wave-lengths and absorb in the short wave-lengths (Hubbard, Bownds & Yoshizawa, 1965). No counterpart in the iodopsin sequence to metarhodopsin II or pararhodopsin (long lasting products of rhodopsin) has yet been reported (Yoshizawa & Wald, 1967). There seems to be no obvious reason why these bleach products should regenerate with the time course of the pigments themselves. Moreover, it seems unlikely that the predictions in Table 1 could be fortuitous. Consequently there seems no good reason for further entertaining this hypothesis.

Comparison with previous studies. De Vries (1948) has done heterochromatic flicker photometry on dichromats and, although the choices of wave-lengths and flicker rate were not optimal, he obtained results very close to those reported here. He thought this effect was due to an accumulation of coloured bleach products. Brindley (1955), measuring the upset of metameric matches (on normal trichromats) after adaptation to bright lights, has found his data consistent with a density of 0·98 for the long wave pigment and less than 0·1 for the middle wave pigment. However, his data is not inconsistent with both pigments being present in high optical density (Brindley, 1970). The present data can be used to calculate the approximate maximum observable effect of Brindley (1955) by assuming the long wave pigment has density 0·55 and the middle wave 0·45. Tersteige (1967) obtained the colour matching functions for a normal trichromat before and after very bright bleaches and found his results consistent with the self-screening hypothesis (both red and green pigments present in density 0·7–0·9). The Stiles–Crawford results, although different in character from previous studies (Brindley, 1953; Walraven & Bouman, 1960; Enoch & Stiles, 1961) are consistent with them. The objective methods (microspectrophotometry, densitometry) have in the past given no support to the psychophysical results but there the difficult stray light problems would result in an underestimation of the visual pigment density However, the recent refined microspectrophotometry study of Dobelle, Marks & MacNichol (1969) demonstrates a primate cone (peak sensitivity about 535 nm) whose optical density is equal to or greater than 0·3.

Recent densitometry studies especially designed to determine the pigment density also indicate high (0·34) optical densities (King-Smith, 1971) for deuteranopes. These studies were performed on the Florida retinal densitometer (Hood & Rushton, 1971).

In summary, the present study strongly supports the pigment density hypothesis for a single receptor mechanism. The overall best estimates for the optical densities are 0·5–0·6 for the deuteranope and 0·4–0·5 for the protanope. This means, in the case of the protanope, that the flicker data are weighed more heavily than the increment threshold data. In any case, an upper limit for either dichromat is about 0·7 O.D.

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