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THE OPTICAL DENSITY OF ERYTHROLABE DETERMINED BY RETINAL DENSITOMETRY USING THE SELF-SCREENING METHOD

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SUMMARY

1. Retinal densitometry has been used to determine the optical density of erythrolabe in foveal cones.

2. A self-screening method was used, as in Rushton's (1963*b*) measurements on chlorolabe.

3. Psychophysical experiments have demonstrated that self-screening in cones depends on the direction of the light in the same manner as the Stiles–Crawford efficiency. Stiles–Crawford measurements were therefore made on each subject and a method was devised to allow for the corresponding reduction in self-screening in densitometry.

4. Complications arising from coloured bleaching products, the presence of a second visual pigment and the fluorescence of visual pigments are considered.

5. The mean value for optical density at 560 nm was found to be $0{\cdot}40 \pm 0{\cdot}07$ S.E. of mean for the five subjects (two deuteranopes, two deuteranomalous subjects and one normal subject).

6. This result is in good agreement with Rushton's (1963*b*) value of 0·35 for chlorolabe using retinal densitometry.

7. The densitometry results are also in reasonable accord with recent psychophysical and microspectrophotometry measurements. This provides evidence that densitometry measures all the cone pigment in an unbiased manner and not just a superficial sample.

INTRODUCTION

A knowledge of the optical density of visual pigments in human cones is of general importance in the understanding of visual function. For

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example, various aspects of the Stiles–Crawford effect and of adaptation to bright lights are thought to be dependent on cone optical density (Brindley, 1953, 1955; Walraven & Bouman, 1960; Enoch & Stiles, 1961; Miller, 1972). A knowledge of optical density is also necessary for the precise determination of pigment kinetics by retinal densitometry (Rushton, 1963*b*).

Apart from these general considerations, the present investigations have been concerned with two specific points.

(1) Rushton (1963*b*) used retinal densitometry to derive a value of 0·35 for the optical density of the green-sensitive pigment, chlorolabe, in the eye of a protanope. However, he later expressed doubt about this figure (Rushton, 1965*a*). It therefore seemed of interest to perform similar measurements on the red-sensitive pigment erythrolabe to see if similar results would be obtained.

(2) The optical density of cone pigments has been estimated from the psychophysical observations mentioned above. It is of considerable interest to determine whether the optical density derived from densitometry is in agreement with the psychophysical values. For suppose that a small quantity of light is reflected at points *within* the cone outer segments; this light would contribute significantly to the responses of the densitometer photo-cell and would have passed (twice) through only a fraction of the outer segment. Thus, the optical density derived from densitometry would be less than the psychophysical value (Rushton, 1958). As a corollary, if agreement is found between the results of densitometry and psychophysics, this would be evidence that no significant light is reflected within the outer segments, i.e. that densitometry measures all the pigment present without a bias in favour of the superficial layers. A similar argument has been applied to the Stiles–Crawford effect measured psychophysically and by densitometry (Coble & Rushton, 1971).

In this paper, the optical density of erythrolabe is derived from the 'self-screening' method of Rushton (1963*b*); in the next paper (King-Smith, 1973) a new method for the estimation of optical density is described based on a consideration of the stray light which is present in densitometry.

THEORY

Light transmission through the outer segment layer – the single light class model

To deduce the optical density of a cone pigment, an assumption must be made concerning the manner that light passes through the layer of cones, i.e. through the cone outer segments and the interspaces between the outer segments.

In Rushton's (1963*b*) analysis, he assumes that the light which has passed twice through the cone layer may be treated as the *two* classes which are represented schematically in Fig. 1*a*; 'signal' light which has passed twice through the cones and 'stray' light which has passed twice through the interspaces. There are, perhaps, two weak points in this assumption.

(1) Light which travels once through a cone and once (in the opposite direction) through an interspace must be considered as contributing

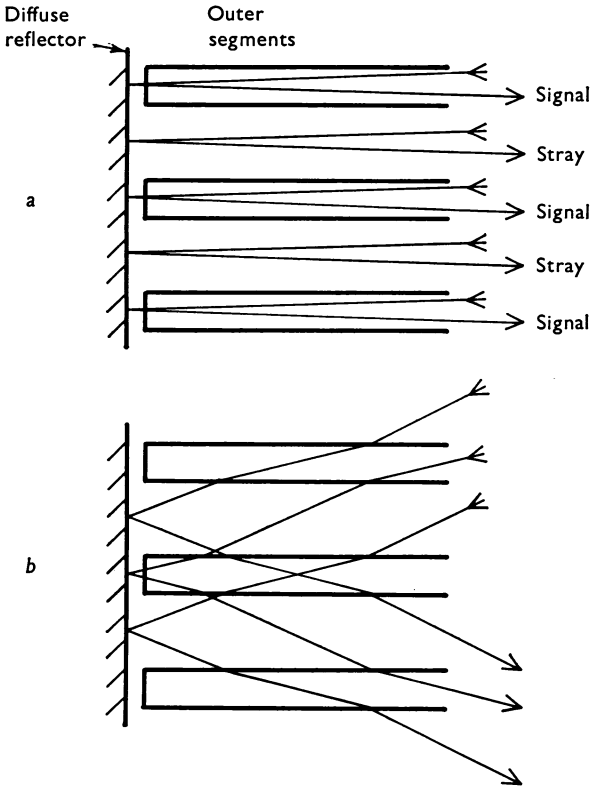

Fig. 1. A schematic illustration of the passage of light through the layer of outer segments. (*a*) *Rushton's* (1963*b*) *model.* The light which has passed twice through the outer segment layer is considered to be of two classes; *signal* light which has passed through the outer segments in both directions and *stray* light which has passed through the interspaces in both directions. (*b*) *The single light class model.* The light which has returned through the outer segment layer is considered to be of only one class; for each direction of passage, a certain fraction (f) of the path occurs within the outer segments and the remainder of the path is within the interspaces. This is the model used in the present analysis.

partly to signal light and partly to stray light; this assumption would be rather inaccurate if the cone optical density is quite large (say 0·4).

(2) The assumption takes no account of the observation that the self-screening in the cones (and hence their apparent optical density) is considerably reduced when light passes through the cone layer at an oblique angle of incidence (i.e. away from the Stiles–Crawford maximum, Stiles, 1939; Brindley, 1953; Walraven & Bouman, 1960; Enoch & Stiles, 1961; Miller, 1972). As the Stiles–Crawford efficiency falls, so does the observed self screening, i.e. the light which is absorbed in the cones has, on average, spent less of its path within the cones and more in the interspaces.

To overcome these objections, it was decided to replace Rushton's two class model with the following *single* light-class model.

(1) Light passing through the layer of cones (either once or twice) may be considered as just a single class whose path is partly within the cones and partly in the interspaces (as in Fig. 1*b*).

(2) The fraction of path length which the light spends within the cones, for a given incidence angle, determines the Stiles–Crawford efficiency at that angle. If this fraction is denoted by f, and D is the optical density for maximum Stiles–Crawford efficiency, the retina will behave, for oblique incidence, like a sheet of uniform material of density fD.

(3) It is assumed that the fundus acts as a diffusing surface rather than as a mirror (Campbell & Gubisch, 1966).

In practice, the f values have been taken to equal the Stiles–Crawford efficiency for the particular path considered, and f values could thus be estimated for the input path (f_I) and the output path (f_O) used in the densitometer. The corresponding transmission factors of the cone layer are thus given by

$$T_I = 10^{-f_I D} \quad \text{and} \quad T_O = 10^{-f_O D}.$$

The intensity of light returning after the double passage is proportional to the product of these two transmission factors i.e. it is proportional to

$$T' = T_I\, T_O = 10^{-f_I D}\ 10^{-f_O D} = 10^{-f_D D} = 10^{-D'}, \qquad (1)$$

where $f_D = f_I + f_O$. T' will be called the two-way transmission factor of the retina, and $D' = f_D D$ will be called the two-way density.

It should be noted that, in this model, there is only *one* class of stray light and this corresponds to the 'superficial' stray light of Rushton (1965*b*), i.e. light reflected from parts of the eye superficial to the layer of cones.

The importance of stray light

Difference spectra derived by retinal densitometry have often been expressed in terms of pigment 'double density'; why may we not calculate

the optical density of a cone pigment simply by halving these 'double density' figures? The answer lies in the quantity of 'stray light' returning to the photocell – light which has not passed through the layer of cones, and whose size is not readily determined. Thus the total intensity of light reflected to the photocell consists of two parts, stray light and 'cone-layer' light, and this may be expressed in the following simplified equations

$$R_B = \rho + s, \tag{2}$$

$$R_D = \rho T'_D + s, \tag{3}$$

where R_B, R_D are the intensity of light reflected to the photocell in fully bleached and fully regenerated states, s is the intensity of stray light, ρ is the intensity of light reflected by the layers behind the cone layer in the fully bleached eye, T' is the two-way transmission factor of the retina (see eqn. (1)).

From eqn. (1), the two-way density, D', may be derived from

$$D' = \log (1/T'_D).$$

Substituting expressions for ρ and T'_D from eqn. (2) and (3), we obtain

$$D' = \log \frac{R_B - s}{R_D - s}. \tag{4}$$

The significance of stray light may now be appreciated by a numerical example. In a deuteranope, complete bleaching of the visual pigments is found to cause an increase in reflexion of about 60% to 560 nm. Thus $R_B = 1{\cdot}6\, R_D$. Assuming no stray light, the two-way density is found to be 0·20 from eqn. (4). But suppose that in fact 90% of the light reflected in the dark-adapted eye is stray light, i.e. $s = 0{\cdot}9 R_D$. D' is now readily shown to be 0·85, and the derived value of optical density is thus greatly dependent on the assumptions made about stray light. Thus optical density cannot be determined directly from the ratio R_B/R_D and more subtle methods must be used such as the self-screening method described below.

The principle of the self-screening method

The self-screening effect has been the basis of most previous estimates of cone optical density, whether by densitometry (Rushton, 1963*b*) or by psychophysical methods (e.g. Brindley, 1955; Walraven & Bouman, 1960; Miller, 1972). The basis of this effect is the fact that light reaching the deeper layers of visual pigment has been attenuated and coloured by absorption in the more superficial layers. This attenuation will be greatest for wavelengths where the pigment extinction coefficient is highest.

The effect of self-screening may be detected by retinal densitometry by using light of two wavelengths, say λ_g (green) where the pigment extinc-

tion is high and λ_r (red) where the extinction is comparatively low. The corresponding two-way transmission factors of the cone-layer in the partially bleached eye will be (cf. eqn. (1))

$$T'_r = 10^{-pD'_r}, \tag{5}$$

$$T'_g = 10^{-pD'_g}, \tag{6}$$

where p is the fraction of pigment still unbleached.

These relations are represented graphically in Fig. 2*a* for assumed values of D'_g and D'_r of 0·5 and 0·15 respectively. (The stronger curvature of the function for green light indicates the greater self-screening for this light.) It is thus possible (from Fig. 2*a*) to determine how T'_r varies as a function of T'_g during bleaching (Fig. 2*b*). What should be noted is that the relation between T'_r and T'_g is curved and that the curvature of this line is, in fact, dependent on the optical density values assumed.

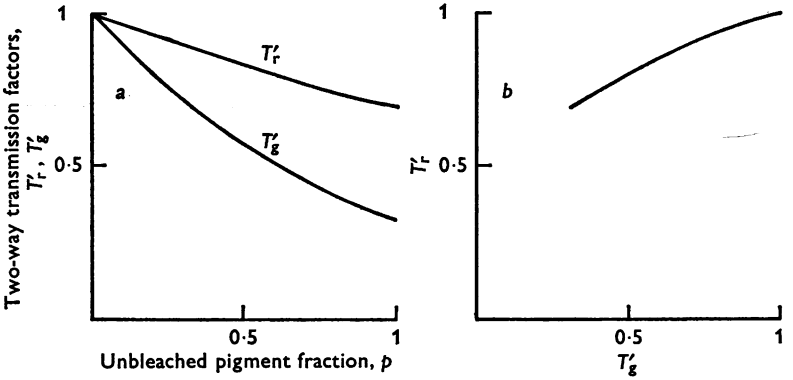

Fig. 2. To illustrate the principle of the self-screening method. (*a*) The variation of the two-way transmission factors, T'_r for red light (low extinction) and T'_g for green light (high extinction) as a function of p, the unbleached pigment fraction. The corresponding two-way optical densities for fully unbleached pigment have been assumed to be 0·15 and 0·50 respectively. (*b*) The two-way transmission for red light as a function of that for green light derived from *a*. The curvature of this line may be used to determine the optical density values.

Now note that, for a given wave-length, the observed reflexion intensity, R, is *linearly* dependent on T' (cf. eqn. (3)). Therefore a plot of R_r as a function of R_g should show a curvature similar to that in Fig. 2*b*. The curvature of this plot of the observed reflexions should again depend on the optical densities at the two wave-lengths, and this curvature can therefore be used to derive a figure for the optical density of the cones (see Discussion). In practice, measurement of R_r and R_g under the three conditions of full regeneration, partial bleach and full bleach is sufficient to determine this curvature and hence the optical density.

METHODS

The densitometer

The densitometer has been fully described by Hood & Rushton (1971). The following two points should be noted.

(1) The intensity of the reflexion from the eye is determined by adjusting the intensity of a deep red 'comparison' light (which enters the eye along the same path as the measuring light) until the photocell responses for the measuring and comparison lights are equal. As the absorption of the comparison light by the visual pigment is negligible, the intensity of this comparison light is directly proportional to the reflexion intensity of the measuring light. In practice, the measuring and calibration lights enter the eye in rapid alternation, and the matching condition corresponds to zero alternating output from the photocell.

(2) It is possible to apply a bleaching light and to measure the eye's reflexion at the same time – a valuable facility for the present work.

The densitometry measurements

Measurements of reflexion from the eye were made at two wave-lengths (a red of 620 nm and a green of 560 nm) for each of three different conditions, full dark adaptation, partial bleach and full bleach.

In practice, the subject, whose pupil had been dilated with Mydriacil, was aligned in the densitometer so that the measuring beam entered the pupil about 1 mm from the centre on the temporal side, and the light returning to the photocell left through the nasal one half of the pupil. After allowing 10 min for the cone pigments to regenerate, measurements were made in the following order.

(1) A set of eight reflexion measurements was made with no bleaching light and using the two measuring lights alternately (i.e. *rgrgrgrg*). The subject was asked to keep his eyes closed between measurements to minimize the effect of bleaching by the measuring lights.

(2) The pigment was then partially bleached by a green (540 nm) bleaching light; the eye was adapted to this light for 2 min and then a further eight measurements were taken in the order *rggrgrgr*. This order was chosen to minimize any difference between the mean value of p (the fraction of pigment still unbleached) for the red measurements and that for the green measurements due to the fact that bleaching equilibrium had not quite been reached at the beginning of the measurements.

(3) The retina was exposed to a very bright bleaching light of 6·4 log trolands which is sufficient to bleach over 99 % of the cone pigment (Rushton, 1965*a*); after an adapting period of 30 sec, reflexion measurements were made alternately with the red and green measuring lights.

Usually, three such blocks of twenty-four reflexion measurements were made in one experimental session and at least 10 min was allowed between each block to allow complete pigment regeneration.

The measuring beam illuminated a 3° circle on the retina which was centrally fixated, but only the central 2° was accepted by the photocell.

Flicker photometry

As will be shown in the discussion section, it was necessary to determine the relative spectral sensitivity of the subjects for the 560 and 620 nm measuring lights. For this determination, the densitometer was used as a flicker photometer; the 'measuring' beam was alternated with the 'comparison' beam (as in densito-

metry – see above – but at the lower frequency of 10 Hz) and the intensity of the comparison beam was adjusted by the subject for a sensation of minimum flicker. The deep red filter of the comparison beam was replaced by a 580 nm filter. The 560 and 620 nm filters used in the densitometry were placed alternately in the measuring beam (which had previously been aligned to pass through the centre of the pupil) and four flicker matches were made for each filter.

The relative energy outputs of the 560 and 620 nm filters were determined at the eye position using a calibrated photocell (Lite-Mike, E.G. & G., Boston, Mass.). A small correction for infra-red radiation was determined by measuring the photocell response with a Schott RG-715 filter interposed. For the flicker photometry, calibrated neutral filters were introduced into the pathways to reduce the retinal illumination to about 30 td (cf. de Vries, 1948).

Stiles–Crawford effect measurements

The present investigation requires a knowledge of how the Stiles–Crawford efficiency for each subject varies as a function of the entry point in the pupil plane (see theory). For this determination, a matching method was used with a bipartite field of 3° diameter. The left semi-circle was derived from the 'measuring' beam and was kept fixed in intensity and point of pupil entry. The right semi-circle was derived from the bleaching beam and could be made to enter the pupil in any of five positions – central and 2·5 mm nasal, temporal, superior, and inferior respectively. One of the semi-circles could be moved horizontally to realign the two vertical edges when the pupil entry position was altered; the intensity of the right semi-circle could then be adjusted to re-establish a match. A 640 nm filter was placed in the combined beam.

RESULTS

The results of a typical session of three 'blocks' of measurements on the deuteranope E.A. are represented in Fig. 3. Each point corresponds to the mean of four reflexion measurements for green light and four for red light. Each set of three points joined by lines (circles, triangles and squares) corresponds to one 'block' of measurements (see Methods). It is seen that, for each block, there is a curvature in the relationship between the reflexion intensities for red and green lights in the manner expected from Fig. 2. However, it can be seen that there is a considerable variation between one block of measurements and the next, and as the curvature to be measured is small, it was necessary to take many blocks of measurements to obtain a reasonably accurate estimate of optical density.

In all, twenty-eight blocks of measurements were made from five subjects. Of these subjects, two were classified as deuteranopes and two as deuteranomalous using an anomaloscope designed by Rushton (Mitchell & Rushton, 1971). The final subject had normal colour vision judged by anomaloscope settings and Ishihara charts; but it was thought that her central retina contained mostly red cones and few green cones for the following reasons.

(1) The difference spectrum found by densitometry for a deep red

bleaching light was found to be indistinguishable from that for a bright white bleach; this deep red light should have bleached nearly all the erythrolabe present but only a small fraction of any chlorolabe.

(2) Her spectral sensitivity to red (620 nm) light compared to green (560 nm) determined by flicker photometry (see Methods) was found to be very close to that for the deuteranopes and indeed agreed to within 1 % with the value found for one of them. This is to be expected if her eye contains little chlorolabe (Rushton & Baker, 1964).

The derivation, from these measurements, of a value for optical density will be considered in the Discussion section.

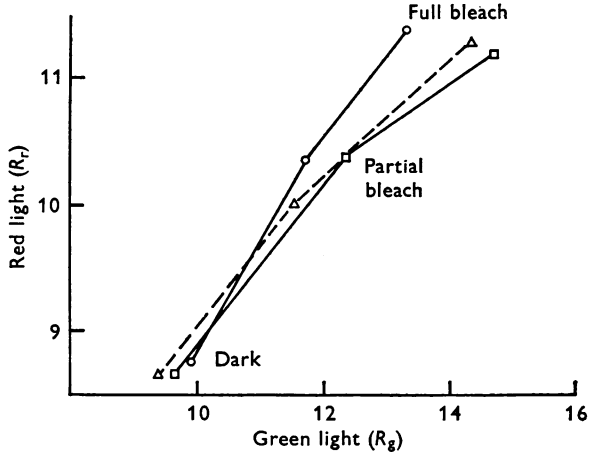

Fig. 3. The effect of partial and full bleaching in a deuteranope on the reflexion intensity for red light (620 nm) ordinate as a function of the reflexion intensity for green light (560 nm) abscissa. Squares, triangles and circles represent the results of three separate 'blocks' of measurements. Note the non-linear relation between the reflexion intensities for red and green light which indicates the greater self-screening for green light (cf. the non-linear relation between two-way transmission factors in Fig. 2*b*).

DISCUSSION

Symbols used in the discussion

For convenience, symbols used in the derivation of optical density are listed here.

f_I, f_O, f_C: the fraction of the light path through the cone layer which is contained within the outer segments (see Theory, the single light class model). The subscripts refer respectively to the input and to the output path used in densitometry, and to the path through the centre of the pupil used in the flicker photometry.

$f_D = f_I + f_O$ (see eqn. (1)).

R_B, R_P, R_D: the intensity of light reflected to the photocell in the fully bleached, partially bleached and fully regenerated states.

ρ: the intensity of light reflected from behind the cone layer in the fully bleached eye.

s: the intensity of stray light reflected to the photocell.

L, (L_r, L_g) = $(R_B - R_P)/(R_B - R_D)$: subscripts r and g refer to red and green measuring lights respectively.

D, (D_r, D_g): the optical density of fully regenerated cone pigment for a single passage in the direction of maximum Stiles–Crawford efficiency (r and g refer to red and green measuring lights).

p: the fraction of unbleached pigment in the condition of partial bleach.

T', (T'_P, T'_D): the 'two-way' transmission factor of the outer segment layer in the conditions of retinal densitometry (see Theory, eqn. (1)). Subscripts P and D refer respectively to partially bleached and fully regenerated pigment.

S_r/S_g: the relative quantum sensitivity of the subject to red and green lights determined by flicker photometry.

t_r, t_g: the transmission factors of the pre-retinal ocular media for the wavelengths of the red and green measuring lights.

The single light class model – calculation of f values

In the single light class model (see Theory), the f value for a given light path corresponds to the fraction of the path through the cone layer which light spends within the outer segments. In practice, the f values are taken to equal the Stiles–Crawford efficiency derived from the measurements (see Methods) at 640 nm where self-screening can be neglected. The f values were calculated from the measurements at the five pupil points by assuming a Gaussian variation in efficiency along both horizontal and vertical axes of the pupil (Stiles, 1937). f values are readily determined for the input path in densitometry (f_I) and for the central pupil position used in the flicker photometry (f_C). For the outgoing path in densitometry light could pass through the whole nasal one half of the pupil; a considerable variation in Stiles–Crawford efficiency occurs within this area and so a mean f value (f_O) was determined by integration of the calculated efficiency over the nasal half of the pupil using a Linc 8 computer. The calculated values of $f_D (= f_I + f_O)$ ranged from 0·92 to 1·53 for the five subjects.

The calculation of optical density

The method used to calculate optical density may now be described and is as follows. First define L_r and L_g in terms of the observed reflexion measurements by two equations (for red and green lights respectively) of the form

$$L = \frac{R_B - R_P}{R_B - R_D} \qquad (7_r, 7_g)$$

where the notation is that for eqns. (2) and (3), and subscript P denotes partial bleaching. Then $(L_g - L_r)$ is an indication of the curvature of the relation between R_g and R_r and so of the optical density. L_r and L_g were calculated for each block of measurements and so was the S.E. in $(L_g - L_r)$ which was derived from the variability of the measurements.

Now R_B, R_D and R_P may be expressed in terms of the retinal two-way transmission factors by eqns. (2) and (3) and the relation

$$R_P = \rho T'_P + s \quad \text{(cf. eqn. (3))}.$$

Substituting these expressions in eqns. (7_r) and (7_g) we obtain two equations of the form

$$L = \frac{1 - T'_P}{1 - T'_D}.$$

Finally we may substitute expressions for T'_P and T'_D (cf. eqn. (1)) yielding

$$L_r = \frac{1 - 10^{-pf_D D_r}}{1 - 10^{-f_D D_r}}, \qquad (8_r)$$

$$L_g = \frac{1 - 10^{-pf_D D_g}}{1 - 10^{-f_D D_g}}. \qquad (8_g)$$

Note that these two equations relate the three unknowns, p, D_r and D_g to the experimentally determined quantities f_D, L_r and L_g. One further relation may be obtained between D_r and D_g, if we note that the quantum sensitivities to red and green light (S_r, S_g) are directly proportional to the fraction of light absorbed in a single passage through the layer of cones; thus

$$\frac{S_r}{S_g} = \frac{t_r(1 - 10^{-f_C D_r})}{t_g(1 - 10^{-f_C D_g})}, \qquad (9)$$

where f_C is the fraction of the light path within the cones for central pupil entry (see above), and t_r and t_g are the transmission factors of the pre-retinal ocular media (t_r/t_g was taken to be 1·05; Boettner & Wolter, 1963). S_r/S_g was determined for each subject by flicker photometry (see Methods), and ranged from 0·37 to 0·44.

We now have sufficient relations to determine the optical density, D_g,

and, in practice, the calculation was performed on a Linc 8 computer, by a method of successive approximation, as follows.

(1) Assume a trial value for D_r (say 0·1).

(2) Calculate p from eqn. (8_r).

(3) Calculate D_g from eqn. (8_g).

(4) Calculate a new value for D_r from eqn. (9).

(5) If this value of D_r does not correspond to the previous value to within 0·001, return to step 2 using the new value of D_r and continue repeating the cycle until two successive estimates of D_r agree to within this limit.

(6) The variance in the estimate of D_g was derived from the calculated error in $(L_g - L_r)$ for that block of measurements. The statistical weight for that block was taken as the reciprocal of this variance.

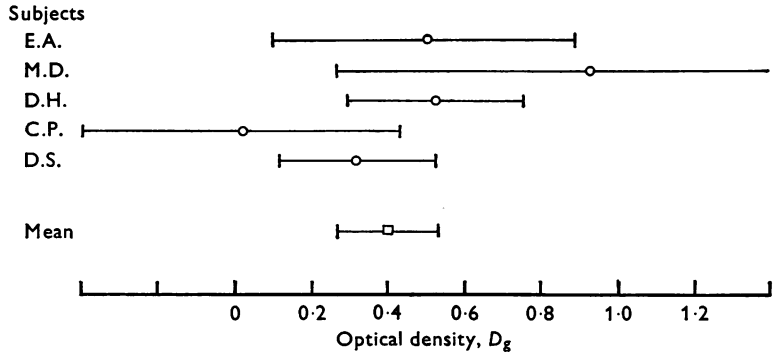

Fig. 4. The circles represent the mean value of unbleached optical density measured at 560 nm for each subject. The weighted mean for the five subjects is represented by the square. The lines represent ± 2 S.E. in each case. E.A. and M.D. were deuteranopes, D.H. and C.P. were deuteranomalous and D.S. had normal colour vision (but see text).

The results of these calculations are represented in Fig. 4 where the circles represent the weighted mean of D_g (± 2 S.E. of mean) for each subject. The square represents the grand mean value for five subjects of $D_g = 0{\cdot}40 \pm 0{\cdot}07$ S.E. of mean. It can be seen that the results for any of the subjects do not differ significantly from the grand mean (within the accuracy attainable).

Some complicating factors

It has been assumed, in the above calculation, that *coloured photoproducts* are not present in the cones in significant quantities; however, if it is assumed that a coloured photoproduct is generated in proportion to the bleached visual pigment, it may be shown that the value of D_g

derived above corresponds to the density *difference* spectrum of the pigment (cf. Rushton, 1963*b*). However, it seems likely that coloured photoproducts are not very significant in cones at the wave-lengths used; for Marks (1965) has shown that the *difference* spectrum of goldfish cones corresponds closely to Dartnall's (1953) nomogram for *extinction*, and the difference spectra for cone pigments, found by densitometry, do not fall at the blue end of the spectrum when compared with the corresponding spectral sensitivity curve (as would be expected if a blue-absorbing photoproduct was formed; Rushton, 1963*a*).

A further complication is the presence of a *second visual pigment* absorbing in the red-green range, at least for the normal and two deuteranomalous subjects. The normal subject must certainly have possessed some chlorolabe, although it was argued above that this could only have been a small fraction (perhaps 5–10 %). The deuteranomalous subjects must also have a second pigment; however, either this second pigment is present in very small amounts, or else its difference spectrum is similar to that of erythrolabe. The justification for this statement is that partial bleaches with blue-green and with deep red lights yield indistinguishable difference spectra (W. A. H. Rushton, personal communication), and this was confirmed with the present subjects.

Note that if the partial bleaching light should cause a greater bleach of the more red-sensitive pigment than for the other pigment, this by itself would give rise to a difference between L_g and L_r of the same sign as the difference due to self-screening. For this reason a partial bleaching light of 540 nm was used which should cause an equal fractional bleach in erythrolabe and chlorolabe (D. Mitchell & W. A. H. Rushton, personal communication).

Following the report of *fluorescence* of rhodopsin (Guzzo & Pool, 1968), it may be wondered whether fluorescence of visual pigments may not contribute significantly to the response of the densitometer photocell. However, no significant fluorescence could be detected in control experiments, either from rhodopsin or from cone pigments; if present at all, it probably contributes less than 1 % of the photocell signal in the present conditions.

Comparison with previous determinations of cone optical density

The mean optical density value of 0·40 for erythrolabe in this determination lends support to the validity of Rushton's (1963*b*) determination of 0·35 for chlorolabe. In fact, the assumptions made in the 'single light-class model' above, would tend to lead to a higher optical density value than that derived with Rushton's assumptions. The present results agree less well with the estimate of 0·22 made by Ripps & Weale (1965)

in their analysis of densitometry. The discrepancy may be related to the treatment of stray light; Ripps & Weale assume that stray light constitutes one third of the light reaching the photocell from a fully bleached eye; in the present study, no *a priori* assumption is made about the value of stray light but the results indicate that stray light is considerably greater than the amount assumed by Ripps & Weale.

The present determination is also in good agreement with microspectrophotometry measurements of Dobelle, Marks & MacNichol (1969), who estimate an optical density of 0·3 for primate foveal cones. The authors point out that this estimate may be too low on account of stray light passing round the cones in their measurements.

Psychophysical estimates of optical density have been based on a number of manifestations of the self-screening effect: the disturbance of a colour match after bleaching adaptation (Brindley, 1953, 1955); the Stiles–Crawford type II (hue shift) effect (Walraven & Bouman, 1960; Enoch & Stiles, 1962), the variation in the size of the Stiles–Crawford effect as a function of wave-length (Walraven & Bouman, 1960), and the disturbance of spectral sensitivity in dichromats caused by bleaching adaptation (Miller, 1972). There is a considerable scatter in these estimates of optical density for chlorolabe and erythrolabe (from negligible up to about 1·2), but perhaps the most direct and reliable determination is that of Miller who finds values of 0·4–0·5 for chlorolabe and 0·5–0·6 for erythrolabe.

If we accept that there may be considerable variations between subjects, and we allow for the uncertainty in the present determination, then the psychophysical and densitometry results are not inconsistent. But we would not expect this agreement if densitometry measures only a superficial sample of cone pigment (see Introduction); it thus seems probable that densitometry measures all the cone pigment in an unbiased way, a view which is supported by the findings of Coble & Rushton (1971).

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