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THE OPTICAL DENSITY OF ERYTHROLABE DETERMINED BY A NEW METHOD

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SUMMARY

1. A new method is described for the determination of optical density by retinal densitometry based on an analysis of stray light.

2. The basic assumption used is that the stray light present in retinal densitometry is independent of wave-length. The justification for this assumption is considered.

3. The stray light and hence the optical density was determined by a method of successive approximations using a Linc 8 computer.

4. The optical density of erythrolabe for the direction of maximum Stiles–Crawford efficiency was determined for five subjects, two deuteranopes, two deuteranomalous and one red-rich normal subject. The mean values for three of the subjects were close to the grand mean value of 0·42, but the other two subjects had considerably higher and lower values respectively.

5. The results are in satisfactory agreement with optical density values determined by the self-screening method using retinal densitometry, and also with determinations using microspectrophotometry and psychophysics.

6. The relevance of dielectric wave-guide modes in the outer segments is considered.

INTRODUCTION

In the previous paper (King-Smith, 1973), the optical density of erythrolabe was determined using the property of self-screening, i.e. the fact that the light absorbed by a pigment is *not* directly proportional to the amount of pigment present, because the light reaching the deeper layers of pigment has been attenuated by absorption in the superficial layers. This attenuation causes a non-linear relation between the fraction of

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light absorbed and the quantity of pigment present and this non-linearity was used to determine the optical density of the visual pigment. The self-screening method has also formed the basis of all attempts to determine optical density psychophysically (see King-Smith, 1973, for references). In the present paper, a new method of determining optical density is described which does not depend on the self-screening effect but is based instead on an analysis of the stray light present in densitometry.

The constant stray light model

Rushton (1963) has pointed out that there is a significant quantity of stray light present in densitometry, i.e. light which has not passed through the visual pigment. This stray light is thought to come from the rear surface of the lens, the internal limiting membrane and the inner layers of the retina. The presence of this unknown quantity of stray light makes it impossible to derive directly a value of optical density from measurements of reflexion in the bleached and unbleached eye. Thus the two-way optical density of pigment is given by

$$D' = \log \left[\frac{R_B - s}{R_D - s}\right] \tag{1}$$

(King-Smith, 1973, eqn. (4)), where R_B and R_D are the measured reflexion fractions (ratio of reflected to incident light) in the bleached and dark-adapted eye respectively and s is the contribution due to stray light. To determine D' from eqn. (1) we must first determine s.

It will be shown in the Discussion that the stray light may be determined by the following method.

(1) Reflexion fraction measurements (for bleached and unbleached retina) are made at two wave-lengths, a red (620 nm) for which stray light is relatively unimportant and a green (560 nm) for which stray light is considerably more important.

(2) The subject's relative spectral sensitivity for the two wave-lengths is determined by flicker photometry.

(3) The basic assumption of the method is that the reflexion fraction (ratio of reflected light to incident light) for stray light is the same for the two wave-lengths.

Using this 'constant stray light model', it is possible to derive a value for the stray light fraction and hence to determine the optical density of cone pigment (eqn. (1)).

METHODS

The densitometer, the densitometry measurements, flicker photometry and Stiles–Crawford effect measurements

The apparatus and measurement techniques are as described in King-Smith (1973). The same five subjects were used, two deuteranopes, two deuteranomalous and one 'red rich' subject with normal colour vision.

Calibration of the reflexion measurements

The present technique requires a knowledge of the relative ocular reflexion fractions (reflected light energy divided by incident light energy) at the two wavelengths used. To calibrate the apparatus, the subject's eye was replaced by an artificial eye consisting of a convex lens and a 'retina' of high-reflectance white paint (Kodak Ltd). The photocell response was then observed for the red and green measuring lights respectively; as these photocell responses correspond to equal reflexion fractions for red and green, it was thus possible to calculate the relative reflexion fractions for red and green for the subject's eye.

RESULTS

Reflexion fraction measurements for the deuteranope E.A. are represented in Fig. 1 for the dark-adapted retina (filled circles) and the fully bleached retina (open circles). The scale for the reflexion fraction is not absolute, but it applies to both the red and green measurements (the absolute size of the reflexion fraction is about 10^{-5} but only the relative sizes are required for the present analysis). The much greater reflexion for red light corresponds to the pink or orange colour of the fundus seen in an ophthalmoscope.

DISCUSSION

The single light-class model

Before an analysis of the data can be made, some assumption must be made about the manner in which light passes through the layer of cones. In the present analysis, the single light class model (King-Smith, 1973) is assumed, namely that light which passes through the cone layer may be considered as a single class whose path is partly within the cones and partly in the interspaces. The Stiles–Crawford efficiency factor is assumed to be a measure of the fraction of the light path within the cones; thus the light path fraction within the cones (f value) may be determined for the conditions of densitometry (see King-Smith, 1973).

The constant stray light assumption

In the present analysis, light passing in the spaces between cones is considered as part of the general 'single light class' discussed above. There is therefore no separate 'fundal' or 'interstitial' stray light as discussed by Rushton (1965). Thus, the stray light in the present model corresponds to the 'superficial' stray light of Rushton, light reflected at points superficial to the cone layer. This stray light probably consists of three components.

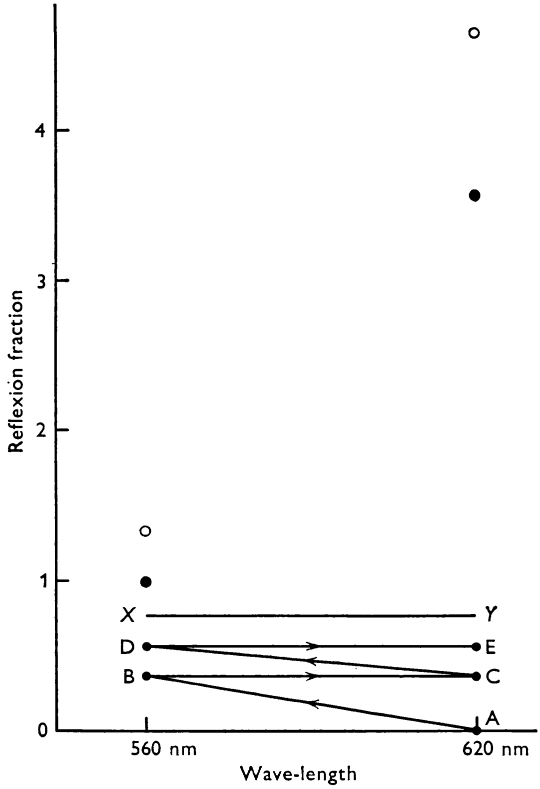

Fig. 1. Reflexion fraction measurements for the deuteranope E.A. for the unbleached retina (filled circles) and the fully bleached retina (open circles). The scale for reflexion fraction is arbitrary, but the red and green reflexion fractions have the correct relative values. The height of the line XY represents the contribution of stray light to the reflexion fraction; this line is horizontal because the stray light contribution at 560 nm is assumed to equal the stray light contribution at 620 nm (the constant stray light assumption). The points ABCDE illustrate stages in the calculation of the optical density (see text).

(1) A reflexion may sometimes be observed from the back of the lens; reflexions from the cornea are always avoided and reflexions from the front of the lens were not observed.

(2) Reflexions may be observed from the internal limiting membrane.

(3) It is probable that there is a significant contribution of stray light

from the superficial (inner) layers of the retina. Any surface where there is a discontinuity of refractive index (e.g. at the boundary of cell nuclei) will reflect a fraction of the incident light.

The basic assumption of the present model is that all three of these components are essentially *white* reflexions, i.e. independent of wave-length. The justification for this assumption is that all three components correspond to reflexions at the boundaries between media of different refractive indices, and such reflexions are characteristically white provided:

(1) The refractive indices of the media do not vary significantly in the wave-length range considered (the refractive index of water in fact increases by only about 0·002 from 620 to 560 nm).

(2) There is no absorption of light by pigments (the macular pigment does not absorb significantly in the range 560–620 nm).

(3) There is no regular structure which may cause interference colours.

(4) The reflecting structures are large compared with the wave-length of light. It is possible that there may be significant reflexion from structures that are smaller than the wave-length of light; for very small structures the Rayleigh scattering law would hold, i.e. scattering is proportional to $1/\lambda^4$. However, reflexion from such structures is likely to be weak (reflexion intensity is proportional to the square of the volume of a very small particle) and Rayleigh scattering has been neglected in the calculations below. If it were assumed that all the stray light obeyed the $1/\lambda^4$ law, the calculated value of optical density would be about 15 % higher.

The principle of the optical density calculation

The use of the constant stray light assumption in determining optical density may be understood by reference to Fig. 1.

Firstly note that the reflexion fraction measured by densitometry has two components, a 'cone-layer' component (i.e. light reflected from behind the cone layer and which has therefore passed twice through the visual pigment) and a stray-light component. Thus the reflexion fraction is given by

$$R = \rho T' + s, \tag{2}$$

where ρ is the fraction of light reflected from behind the cone layer in the fully bleached eye, T' is the two-way transmission factor of the retina and s is the contribution of stray light.

The constant stray light assumption implies that s is independent of wave-length; thus the contribution of stray light may be represented in Fig. 1 by the height of a horizontal line such as XY. If we knew the height of this line, we could determine the 'cone-layer' components for both full bleach and full regeneration (from Fig. 1 or eqn. (2)) and hence determine the optical density of the visual pigment.

The position of XY may be determined by successive approximations as follows. It can be seen that stray light is relatively less important at 620 nm, so, as a first approximation, assume that stray light is zero for this wave-length. Thus, the stray light would be represented by the point A. An approximate value for the optical density at 620 nm may now be calculated. Next, an approximate value for the optical density at 560 nm may be derived from the 620 nm value by using the subject's spectral sensitivity data. A better value for the stray light component, s, may now be calculated from this estimated density value and from the observed reflexion fractions at 560 nm. This estimate of stray light is represented by the height of point B and, using the constant stray light assumption, it is also an estimate of stray light at 620 nm (i.e. point C). A new estimate of optical density at 620 nm may now be made and the whole cycle may be repeated many times yielding points D, E, etc., until no further change occurs in the estimate of stray light. This will be the required value of stray light (corresponding to the line XY) and the optical density at 560 nm may be derived from this value and the observed reflexion fractions at 560 nm (Fig. 1).

The calculation procedure

In the above description, the effect of absorption in the lens has been neglected for simplicity. In fact, the stray light component in densitometry must be of the form

$$s = t^2\sigma, \tag{3}$$

where t is the transmission factor of the lens and σ is the reflexion fraction for stray light which would be observed if there were no lens absorption. It is σ which is assumed to be independent of wave-length.

Thus the basic equation for optical density (eqn. (1)) may be rewritten

$$D' = \log\left[\frac{R_{\mathrm{B}} - t^2\sigma}{R_{\mathrm{D}} - t^2\sigma}\right]. \tag{4}$$

D' is, in fact, the 'two-way' density; if D is the optical density for a single passage in the direction of maximum Stiles–Crawford efficiency, then D may be derived from the 'single light class model' (see above and King-Smith, 1973) by the equation

$$D' = f_{\mathrm{D}}D, \tag{5}$$

where f_{D} is related to the fraction of the light path in the cone layer which is within the outer segments (f_{D} is typically about 1·3; see King-Smith, 1973).

In practice, a Linc 8 computer was used to calculate the optical density as follows.

(1) As a first approximation the stray light was assumed to be zero.

(2) An estimate of the optical density at 620 nm could now be derived from eqns. (4) and (5). (The values of lens transmission t were derived from the measurements of Boettner & Wolter (1963) and are consistent with the measurements of Said & Weale (1959).)

(3) The optical density at 560 nm was now estimated from the subject's spectral sensitivity data using the relation

$$\frac{S_r}{S_g} = \frac{t_r(1-10^{-f_c D_r})}{t_g(1-10^{-f_c D_g})}, \tag{6}$$

where S is the quantum sensitivity of the subject determined by flicker photometry and f_c is the fraction of the light path in the cone layer which is spent within the outer segments for the conditions of the flicker photometry (King-Smith, 1973).

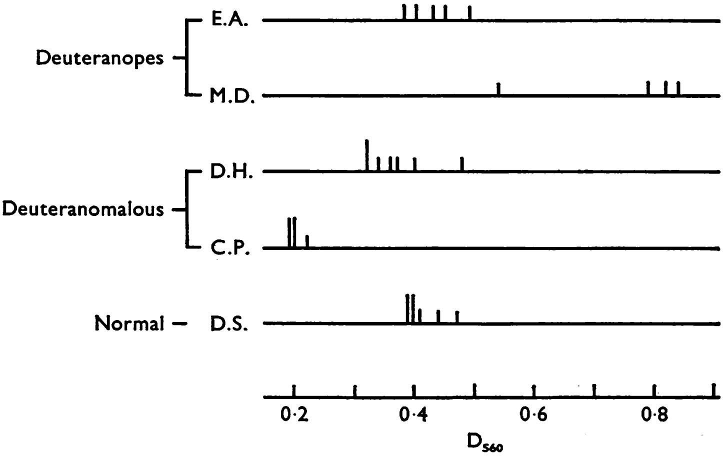

Fig. 2. Results of the optical density calculations for the five subjects. Each vertical bar represents the result from one 'block' of measurements; bars of double height correspond to two results of the same value.

Subscripts r and g in eqn. (6) refer respectively to red (620 nm) and green (560 nm).

(4) A new estimate of the stray light factor σ was determined by applying eqns. (5) and (4) for the green wave-length.

(5) Step 2 was now repeated with this new value for stray light. In practice, steps 2–4 were repeated cyclically until two consecutive values of σ agreed to within one part in 10^4.

The results of these calculations are represented in Fig. 2. Each small vertical line represents the value of optical density of erythrolabe at 560 nm determined from one 'block' of measurements. The following points may be noted:

(1) The determinations for a single subject show less variability than determinations using the self-screening method (Fig. 3, King-Smith, 1973).

(2) The means for three subjects (E.A., D.H. and D.S.) lie in the range 0·38 to 0·43, but M.D. has a significantly higher value (mean 0·75) and C.P.'s mean is only 0·20. These variations are consistent with the determinations using the self-screening method (Fig. 3, King-Smith, 1973).

(3) The mean value of optical density derived from all determinations is 0·42 and so is in good agreement with the value of 0·40 derived by the self-screening method.

Complicating factors

The calculations above determine the optical density of cone pigment only if it is assumed that *coloured photoproducts* are absent during a full bleach. If this is not so, then it may be shown that the above calculation yields the *difference* spectrum value for optical density (see Rushton, 1963).

A *second visual pigment* absorbing in the red/green range must be present in the eyes of the deuteranomalous subjects and the red-rich normal subject. The normal subject must have some cholorolabe in her eye, while the deuteranomalous subjects may similarly have a small amount of a second pigment or else a considerable amount of a pigment very similar to erythrolabe (see King-Smith, 1973). In either case, the observed value of optical density is likely to be slightly less than the optical density for pure erythrolabe.

A comment on wave guide modes

One point about the 'single light class model' requires elucidation. It has been assumed that when light passes through the cone layer in the direction of maximum Stiles–Crawford efficiency, the light is entirely contained within the outer segments (and thus the 'light path fraction' f will be unity). This is what would be predicted by *ray* optics if total internal reflexion was assumed within the inner and outer segments. However, the diameters of outer segments are not much larger than the wave-length of light and, in this situation, wave optics rather than ray optics should be used. In fact, the outer segments may be treated as dielectric wave guides (Toraldo di Francia, 1948; Enoch, 1967). For the present considerations, the important consequence is that some of the light energy in the wave guide modes is transmitted *outside* the outer segment. For the direction of maximum Stiles–Crawford efficiency, most of the light energy is probably transmitted in the basic HE_{11} wave guide mode; in this situation, about 80 % of the light flux is transmitted within the outer segments (derived from Kapany (1967, p. 69) using the following parameters: outer segment diameter 1 μm (Polyak, 1957), free space wave-length 560 nm, refractive index of outer segment 1·385 (Sidman, 1957), refractive index of the interspace 1·334). Correspondingly, the 'effective absorption coefficient' of the visual pigment will be only 80 % of the bulk coefficient (Kapany, 1967).

Thus the single light class model should be more properly stated as follows.

(1) For the direction of maximum Stiles–Crawford efficiency, a certain fraction F_0 of the light flux is contained within the outer segments.

(2) For other directions of incident light the fraction of the light flux within the outer segments is reduced to fF_0.

These alterations make no difference to the calculations; the value of optical density determined is the optical density of the outer segment layer for the direction of maximum Stiles–Crawford efficiency and will be rather less than the optical density of a corresponding lamina of visual pigment of the same thickness and concentration, for the reasons discussed above.

Comparison with previous determinations

The value of 0·42 for the optical density of erythrolabe determined in this investigation is in satisfactory agreement with the value of 0·40 determined by densitometry in the same subjects using the self-screening method (King-Smith, 1973). Comparison with other techniques were considered in that paper; in the summary there is reasonable agreement with the following determinations.

(1) The value of 0·35 for the optical density of chlorolabe determined by retinal densitometry (Rushton, 1963).

(2) The value of 0·30 for primate foveal cones estimated by microspectrophotometry (Dobelle, Marks & MacNichol, 1969).

(3) The values of 0·4 to 0·6 determined psychophysically by Miller (1972).

A notable exception is the psychophysical value of 0·98 for erythrolabe due to Brindley (1955); however, Miller (1972) has shown that Brindley's observations are also consistent with his own values.

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