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STRAY LIGHT AND THE MEASUREMENT OF MIXED PIGMENTS IN THE RETINA

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When light falls upon visual pigments the chemical changes resulting usually lead to an alteration in optical density—'bleaching'. When the pigment is in solution in a uniform trough (as in ordinary densitometry) each ray of light has a passage the same as each other ray, and the density of the solution is simply the difference of the logarithms of incident and transmitted light. By this definition if two solutions in different troughs are placed one behind the other, the combined density is the sum of the two densities measured separately.

It is an experimental fact that if those two solutions were mixed together (and did not interact chemically) the densities would be the same as when placed one behind the other. Thus the principle of the addition of densities in uniform solutions evidently holds down to molecular levels.

But the condition is otherwise when the pigment is not presented in a uniform film but in a mosaic of receptors with interspaces. It is obvious that a change in the density of one pigment will only alter the amount of light through the corresponding receptors and will leave the light contribution from the other pathways unaffected. Thus the change in the logarithm of the *total* light will not be related in any simple manner to the logarithm of the light through the cones that are altering. It is clear that the signal from a change in one kind of cone will be diluted by the unchanging *stray* light, and that the effect of the stray will depend upon the wave-length, the degree of bleaching of the pigment studied and also the degree of bleaching to which all the other pigments have been brought and held. If two pigments change simultaneously the apparent density change is not the sum of the change in density of each nor is it easy to find what the relation is.

On the fovea of protanope and of deuteranope (who have but one measurable visual pigment there) I have made measurements of action spectra and kinetics that are not seriously invalidated by uncertainties about the amount and spectral distribution of stray light (Rushton,

1963*a*, *b*, *c*; 1965*a*, *b*). But before taking up the more difficult question of mixed pigments upon the normal fovea it seemed worth while to demonstrate two things. First, to show theoretically how the experimental results may be plotted so that the various pigments contribute by simple (vector) addition. Secondly, to show experimentally that some 98% of the light reaching the photocell has been reflected from the retina and fundus behind it. This suggests an important simplification.

THEORY

All our densitometry measurements are made by balancing the photocell output in light of wave-length λ against the output in deep red light. The latter is regulated by a wedge calibrated in units of transmission energy, and the signal at λ is measured by the deep red energy that balances it—read at once from the wedge scale. The light incident upon the eye is measured by replacing the subject's eye by an artificial eye with a white retina (essentially a lens with a white sheet in its focal plane). The energy of incident light is taken as the red wedge setting for the balance in this case. For simplicity we shall assume that the pigments in the cones bleach to transparency, though it adds little complication to assume coloured photoproducts.

I_W = light incident upon eye (measured by white artificial retina).
ρ = reflexion fraction at fundus (with which may be multiplied the double transmissivity of the eye media).

Thus

$I_W\rho$ = total light reflected from the fundus in the fully bleached state. It is composed of three parts.
$mI_W\rho$ = light reflected through fully bleached erythrolabe cones.
$nI_W\rho$ = light reflected through fully bleached chlorolabe cones.
$aI_W\rho$ = stray light reflected from fundus (fundal stray).
bI_W = stray light from elsewhere (superficial stray).
D_1, D_2 = density of erythrolabe, chlorolabe (response) in cones for single passage.
ϵ, χ = $(1-10^{-2D_1})$, $(1-10^{-2D_2})$ = fraction of light absorbed on double passage.
T = total light received by photocell (measured in transmission of the balancing red light).
T_o = T in the condition of total bleaching.

Now T, the total light received by the photocell, is the sum of the lights through the two kinds of cone and of the two kinds of stray light

$$T = I_W\rho(m10^{-2D_1}+n10^{-2D_2}+a)+I_Wb.$$

In total bleach

$$T_o = I_W\rho(m+n+a)+I_W b,$$

therefore

$$\frac{T_o-T}{T_o} = \frac{m\epsilon+n\chi}{m+n+a+b/\rho} \quad (1)$$

This is the fundamental formula for retinal densitometry. If all fully bleached cones and their interspaces are supposed to be transparent (or all the same colour) then the Greek letters in expression (1) alone are dependent upon wave-length. The quantities m and n represent the relative number of red and green cones, and a is proportional to the area between them.

From the equation it is seen at once that, if only erythrolabe changes, then only ϵ on the right changes so that $(T_o-T)/T_o$ alters linearly with ϵ. If only chlorolabe changes similarly $(T_o-T)/T_o$ alters linearly with χ. When both ϵ and χ change, the result is the sum of the two separate changes.

If white light falls upon the eye, it is probable that b the superficial stray light will be more or less white. The fundal stray light ρ on the other hand will be reddish (the 'red reflex' of ophthalmology) since fundal reflectivity is less the shorter the wave-length (curve A, Fig. 4). Thus b/ρ, the contribution of superficial stray light in eqn. (1) is much larger for green than for red light.

If in (1) b/ρ is negligible in comparison with $(m+n+a)$ and if only ϵ (or only χ) change, then $(T_o-T)/T_o$ measured at various wave-lengths gives the true difference in absorption of ϵ (or of χ); the Roman symbols simply alter the vertical scaling of the absorption difference spectrum so obtained.

We now proceed to the experimental demonstration that $b/\rho(m+n+a)$ is small.

MEASUREMENT OF SUPERFICIAL STRAY LIGHT

Principle. Figure 1 shows the optics normally used in our densitometer. E is the image of the exit slit of the spectrometer which is focused by L_1 on to the cornea just nasal to the centre. At F in the focal plane of L_1 (for emmetropic eyes) is a stop with cross-hairs which produces a sharp circular patch of $2\frac{1}{2}°$ subtense upon the retina. The reflected light that leaves the temporal half of the pupil falls upon the ophthalmoscopic mirror M and is reflected upon a second lens L_2 to give a real image of the illuminated retina in its focal plane at S. Here an iris stop S could limit the area admitted to the photocell and 2° in the centre of the $2\frac{1}{2}°$ patch was normally received. The adjustment of the stop was generally made by the experimenter substituting his eye for the photocell *P.C.*, viewing the real retinal image at S and adjusting the amount and position of what was accepted.

To measure the superficial stray light, instead of a circular aperture at F there was an annular aperture that formed upon the retina a bright ring R with a dark centre C, Fig. 2. The iris stop S, Fig. 1, was closed until all the bright ring was excluded and also a small margin of the black centre. But, though the bright ring itself was excluded at the iris stop, all the light scattered from it that could reach the central pathway would be accepted

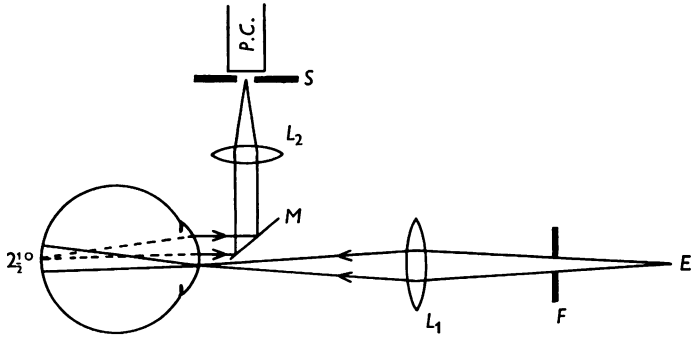

Fig. 1. Optics we use in retinal densitometry (see text).

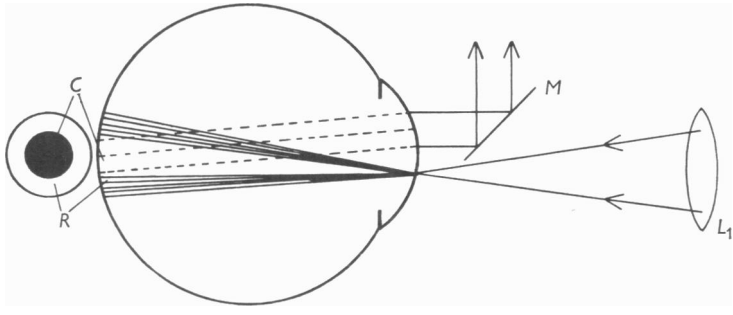

Fig. 2. Optics modified from Fig. 1 to produce a bright ring image round the fovea. The black centre only is accepted by the photocell by stopping down S, Fig. 1. Light from ingoing ring path scattered into out-going central path (dotted) is received by photocell and measured.

by the photocell. As is seen in the diagram of Fig. 2 this pathway starts from the black centre on the retina and passes through the bright cone of rays. It is assumed that the amount of *superficial stray* light received in this arrangement is about the same as the amount of *superficial stray* that would be received if the same energy of incident light had fallen not on the ring but on the centre. In that position the stray would not have been detectable against the direct reflexion; the ring arrangement permits the stray to be detected alone.

The amount of superficial stray light is very small and we need high sensitivity to detect it. The stray light that was scattered to the dark centre was measured by the ordinary densitometry technique of balancing it against deep red light that was arranged to fall upon the centre directly. But in order to do this we have to modify the way in which lights fall upon the eye. In ordinary densitometry the polaroid, rotating at p radians per

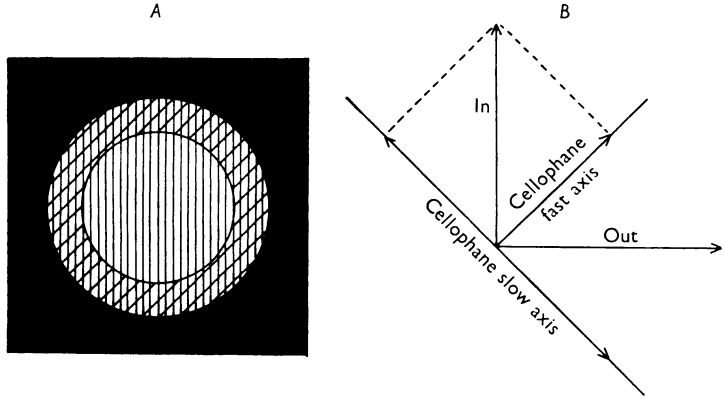

Fig. 3. *A*. The stop used in position *F*, Fig. 1. The aperture is covered by a polaroid with axis vertical, and partly covered by a ring of cellophane (half-wave plate for green) with axis at 45° to the vertical. *B*. Light vector diagram showing phase change on passing through half-wave plate.

sec, causes red light (polarized vertically) to alternate with green light (polarized horizontally). Both fall upon the same retinal region, the first giving a photocell output proportional to $\sin^2 pt$, the second to $\cos^2 pt$; thus, when the wedge is set at balance, the sum of these is unity and there is no a.c. output. In the present case the green light has to fall only upon the ring and alternate with the red light that falls only upon the centre. This optical condition is easily satisfied with the aid of a piece of cellophane by the arrangement of Fig. 3*A*.

In an opaque sheet a hole 6·3 mm in diameter is drilled. Over this is stuck a piece of polaroid with axis vertical. Between the polaroid and the sheet is stuck a piece of cellophane perforated by a hole 4·1 mm in diameter centred upon the other.

The cellophane used happens to be a 'half wave plate' for green light. That is to say it has an axis in the plane of the sheet such that when the light falling upon the sheet is polarized in the plane containing the axis it takes one (or some other odd number) of half vibrations longer to traverse the sheet than when polarized orthogonally. As indicated in Fig. 3*A*, the axis of the polaroid is vertical, that of the cellophane at 45°.

Turn now to the light vector diagram of Fig. 3*B*. If the light incident upon the cellophane is polarized vertically (In), it may be resolved into two equal components, one along the fast axis and one along the slow. But since these are in phase at incidence they will be exactly out of phase upon leaving, so the upward arrow on the fast axis which was synchronous with 'up' on the slow axis when the light went *in* will be synchronous with the downward arrow on the slow axis as light goes *out*. And these two will recombine to form the horizontal arrow (Out). The net result of passage through the cellophane (at 45°) is therefore to turn vertically polarized light into light polarized horizontally. Also by the same argument (or from the principle of ray reversal) horizontally polarized light becomes polarized vertically after its passage.

In application, the stop of Fig. 3*A* is placed in position *F*, Fig. 1, so that light from the spectrometer falls first upon the cellophane ring and then upon the polaroid. The deep red light emerging from the spectrometer is always polarized vertically and in the present case it passes straight through the cellophane hole and through the vertically orientated polaroid beyond. But the red light that falls upon the cellophane ring becomes thereby polarized horizontally and is cut off as it falls upon the vertical polaroid.

Conversely the green light emerging from the spectroscope is always polarized horizontally, and hence is cut off when, after passing through the hole in the cellophane, it falls upon the vertical polaroid. But where this light falls upon the cellophane ring it becomes vertically polarized; hence the ring of light now passes the vertical polaroid. This satisfies all our conditions, for the red and green measuring lights are flickered one against the other as before, but now green falls only upon the ring and red only upon the centre. The outer diameter of the ring subtended 4·3°, the inner diameter 2·8°.

Experimental results. The subject with dilated pupil was aligned in the usual way. All the wave bands from the spectrometer were used in conjunction and a white ring seen upon the subject's retina through the ophthalmoscopic equipment. The iris stop *S* was closed down just to exclude safely all parts of the ring from acceptance by the photocell. Then measurements were made of 'stray light' by balancing against the deep red light, applied to the centre directly, several spectral wave bands together (applied to the ring, of course, and only reaching the centre by scatter). It was then necessary to measure the brightness of the ring itself in order to see what *fraction* escaped to the centre. For this purpose the iris stop *S* was opened to include the whole ring and a new balance against red was made. Since opening the iris also increased slightly the amount of red accepted by the photocell, this increase was measured directly and allowed for.

Figure 4, curve A, shows the proportion of light incident in the ring upon the retina that is reflected back into the photocell when the iris stop S was opened to receive it. The proportion as plotted is multiplied by an arbitrary constant to bring the ordinate at 640 mμ to the value of 100. This curve shows roughly the reflectivity of the (unbleached) fundus and exhibits the well-known fact that red light is much better reflected than is green.

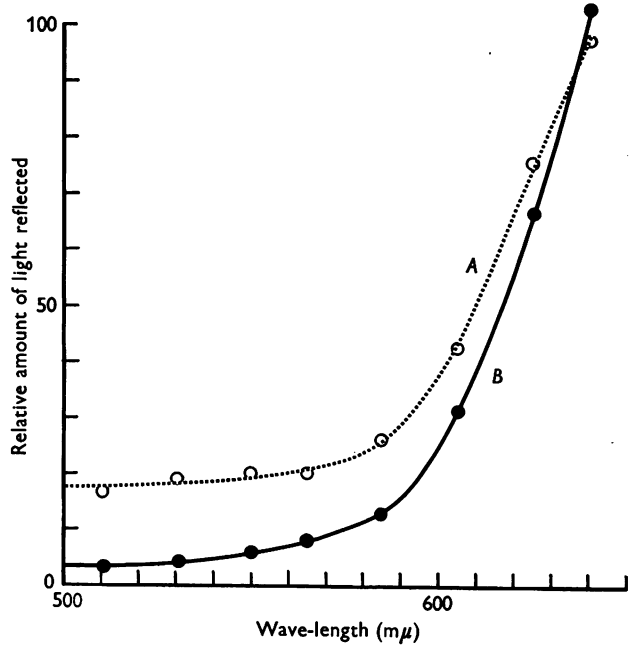

Fig. 4. A. Relative spectral reflectivity of unbleached fundus oculi. B. Proportion of incident light scattered from ring to centre, i.e. to dotted pathway, Fig. 2. Scale of B 0·1 that shown for A; thus at 640 mμ 10 % of reflected light is so scattered.

Curve B shows the proportion of incident light scattered from the ring into the photocell when the iris stop was reduced just to exclude all direct light from the ring. It is plotted at 10 times the magnification of curve A so that at 640 mμ the light is only 10 units. Consequently at this wavelength the light scattered from annulus to centre is 10 % of the total reflected light; in the green it is nearer 2 %. The sum of all the lights together works out at 6 %.

DISCUSSION

The light that is 'scattered' from ring to centre, amounting on average to 6 %, is made up of three parts.

(i) Light not in fact excluded by crossed polaroids. This was less than 1 %, due chiefly to some depolarization in the complicated optical path (to the right of *E*, Fig. 1) between the two polaroids.

(ii) Light scattered in the anterior part of the eye from ingoing to outcoming pathway. Since the two paths are separated in cornea and lens, a double scattering here is needed.

(iii) Light that passed deep through the choroid beneath the ring, then spread centrally through the white translucent sclera and finally returned through the choroid in the dark centre. It was easy to observe in the ophthalmoscopic arrangement that red light falling upon the ring spread in this way and caused the centre to glow. But green light remained confined to the ring with a crisp black border.

Light in category (ii) might be expected to be scattered nearly equally at all wave-lengths or somewhat more at short waves (Rayleigh effect). In category (iii) the scatter would be greatest at long wave-lengths, and the spectral relations should be similar to the curve of fundal reflexion since both depend in much the same way upon the absorption by melanin and haemoglobin.

Now, in Fig. 4, curve *A* is the spectral reflectivity of the fundus and curve *B* the spectral distribution of scatter, so obviously the scattered light falls chiefly in category (iii). Of the 6 % of signal that is scattered to the centre, not more than 1 % is likely to consist of superficial stray that has never reached the retina. If this was all to be said on superficial stray light, we could safely omit the superficial stray term b/ρ from eqn. (1), reducing it to

$$\frac{T_o - T}{T_o} = \frac{m\epsilon + n\chi}{m+n+a}. \tag{2}$$

In this form of the equation none of the coefficients depend upon wave-length, thus $(I_o - I)/I_o$ gives for each wave-length values proportional to the true difference in absorption spectrum.

Unfortunately there remains one source of stray light that I do not know how to measure. It is the light scattered back from the layers of the retina before the outer segments of the cones are reached. The light reflected at the internal limiting membrane of the foveal cup is brought to the focus of this tiny concave mirror about 30 μ in front of the retina. The brightness of the point of light formed varies enormously from subject to subject, but if troublesome it may be excluded by a minute opaque spot placed in the image plane *S*, Fig. 1, and adjusted to coincide with the light point and thus suppress it.

The light from the layers of the retina, on the other hand, cannot by any means be separated from the cone signal passing through them, and, although the retina at the fovea is very thin and very transparent, it remains

a distinct possibility that the light scattered there has an appreciable effect upon pigment measurements.

However, when the superficial stray light is as small as we find in satisfactory subjects with our present technique, we may be justified in taking the transmissivity spectrum $(T_o - T)/T_o$ as giving approximately the absorption difference spectrum. Certainly the curve plotted in this way in the deuteranope (Rushton, 1965*a*) fitted reasonably the deuteranope's threshold spectral sensitivity (Hsia & Graham, 1957); and in the following paper (Baker & Rushton, 1965) a similar fit will be shown between the results of bleaching normal cone pigments so plotted, and the spectral sensitivity of Stiles's colour mechanisms π_4 and π_5.

The superficial stray light has been found to be about 1% of the signal received from the eye. But this in turn is only 10^{-5} to 10^{-6} of the light incident upon the cornea (depending upon wave-length). Thus the superficial stray has now been reduced to less than one part in ten million of the incident light. Only by the completest possible separation of ingoing and out-coming paths has this low figure been attained, aided by the phase-sensitive rectifier that eliminates all stray lights that do not oscillate with the period and phase of the signal.

SUMMARY

1. The distribution of light through a mosaic of red cones, green cones, interspaces and general scatter is worked out theoretically and a formula is derived in which the contributions of the two types of cone combine by simple addition.

2. Stray light is of two kinds. The first, *fundal* stray light, dilutes the signal from the cones equally at all wave-lengths. This therefore will not distort the difference spectrum but only change its amplitude.

3. *Superficial* stray light on the other hand dilutes signals far more in the blue than in the red. It therefore changes the shape of the recorded difference spectrum.

4. In order to measure the proportion of superficial stray light the usual optical arrangement was modified so that the retina was illuminated by a ring of light extending 4·3° round the fixation point with a 2·8° dark centre.

5. The amount of light scattered into the pathway from the dark centre was measured for various wave-lengths with the result that red light was found to be scattered most, and the spectral distribution was very similar to that of reflexion from the fundus.

6. Of all the light returning from the eye into the measuring equipment, 6% on average was scattered to the pathway from the dark centre. This light came partly from superficial scatter and partly from deep

penetration and scatter in the sclera. The similarity of its spectral distribution with that of light reflected deep from the fundus points to the predominance of scleral scatter. Thus of the 6% of light that is scattered, only 1% is likely to be superficial.

7. It is hard to measure or estimate the amount of light scattered in passing through the retinal layers of the fovea. If this were very small the transmissivity spectrum $(T_o - T)/T_o$ should correspond closely to the difference spectrum.

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