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THE RED-SENSITIVE PIGMENT IN NORMAL CONES

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It is ten years since it was demonstrated by retinal densitometry that there were two visual pigments in the red–green range upon the normal fovea, and only one upon the protanope's (Rushton, 1955). Now that this pigment *chlorolabe* has been studied by itself in the protanope (Rushton, 1963*a*, *b*, *c*) and a more red-sensitive pigment *erythrolabe* has been studied by itself in the deuteranope (Rushton, 1965*a*, *b*) we are in a position to begin the study of the mixture on the normal fovea.

The red pigment is the easiest to isolate, so in this paper we shall analyse that and show that it appears to be identical with erythrolabe of the deuteranope by the four kinds of measurements that can be used—the difference spectrum, the action spectrum, the photosensivity and the regeneration rate. It will also be shown to be a visual pigment by the two kinds of measurement that can be used—the correspondence of the action spectrum with the visual spectral response of Stiles' red mechanism π_5 and the correspondence of the time course of pigment regeneration with the dark adaptation curve of π_5.

METHODS

The equipment and procedure was the same as in the other investigations upon foveal pigments and has been already described in some detail (Rushton, 1963*a*). In brief, subjects with the left pupil dilated by homatropine were fixed in exact alignment in the densitometer by a dental impression and brow rest. They fixated upon cross-hairs in the centre of a $2\frac{1}{2}°$ field that constituted the retinal region upon which the bleaching and measuring lights fell. Light entered the eye by Maxwellian view through the nasal half of the pupil and the reflected portion that left through the temporal half encountered a small mirror and was deflected into a photocell. By means of a lens and a stop only light coming from the direction of the central 2° of the fovea was accepted by the photocell. Light from the anterior part of the eye could therefore only be received by a *double* horizontal scatter and the signal was polarized in the sense that made scatter chiefly vertical. A bleaching light could be substituted for the measuring light upon the fovea by interposing a mirror. Two such mirrors mounted like the sails of a windmill were generally used for bleaching. The windmill turned at 5–10 rev/sec and flashes of bleaching light were applied at twice this frequency, shining through $\frac{1}{4}$ of the cycle. Between the bleaching flashes the measuring light fell upon the same retinal region and by means of an electromagnetic shutter only the measuring light reached the photomultiplier cell. In this way it was possible to measure retinal reflectivity at one wave-length while bleaching with light of another wave-length.

In particular, equilibrium states could be investigated and re-checked by this technique and some confidence in some results built up.

Measurements were always made by balancing the reflected light at the wave-length investigated against the reflexion of a deep red light (700 mμ) to which all retinal pigments are transparent. The method (tried earlier) of balancing the signal against a control that did not enter the eye was very much less reliable with the human eye (though not with an artificial eye). This is presumably due to the ceaseless minute eye movements which cause perpetual fluctuations in the quantity of light reflected, but only second-order fluctuations in the ratio green:red light reflected. One further advantage of the steady wedge balance employed is that one can see when the reading is steady and when it is fluctuating unreliably —as happens on occasion with all subjects and perpetually with some.

RESULTS

The transmissivity spectrum

If light passes through the retina whether *in situ* or excised, some light will pass through red cones, some through green cones and some through neither. If this mixed light is used as the basis of density measurements and the change of that density upon bleaching is plotted as a difference spectrum, nothing very secure results. It has been shown (Rushton, 1965*a*) that even in the simpler case where only one visual pigment and some stray light was present a spurious second pigment might appear. When two cone pigments are present the situation is naturally more complex since for each species of cone the (coloured) light through their neighbours acts as additional stray light, and in various stages of bleaching that light is constant neither in quantity nor in relative spectral composition. It was shown, however, that if the change in the energy of the light received by the photocell (not the change in its logarithm) is recorded, the situation becomes less confusing.

In Fig. 1 of the former paper (Rushton, 1965*a*) the change in transmissivity was plotted before and after bleaching with various wave-lengths of light and with various energies, and the expectation verified that the transmissivity difference spectrum so plotted changed only in amplitude but not in shape. This must follow from the physics of the situation if only one pigment is present and its self-screening is negligible. In Fig. 1 of the present paper, results of two bleachings are similarly plotted not with the deuteranope but a normal subject (H.D.B.). Obviously curve B is not just curve A scaled down, hence the normal eye has more than one photosensitive pigment in the red–green range.

In Fig. 1 the amount of light reflected from the eye was measured at eight wave-lengths by retinal densitometry, balancing against a deep red light whose energy T was measured. The *transmissivity* was obtained for each wave-length by dividing T by T_o, the value of T when all the pigment had been bleached away by bright white light. If T_D is the value of T in

the dark-adapted state and T_R in equilibrium under bleaching by strong deep red light, then $(T_R - T_D)/T_o$ is the transmissivity difference spectrum of red bleaching, and this is plotted as the black circles of curve A, Fig. 1. Clearly, a red-sensitive pigment is present. Curve B shows the further transmissivity difference $(T_G - T_R)/T_o$ when the red light is exchanged for a blue-green light that bleaches erythrolabe equally, T_G being the value of T in equilibrium under this condition. When this experiment was performed

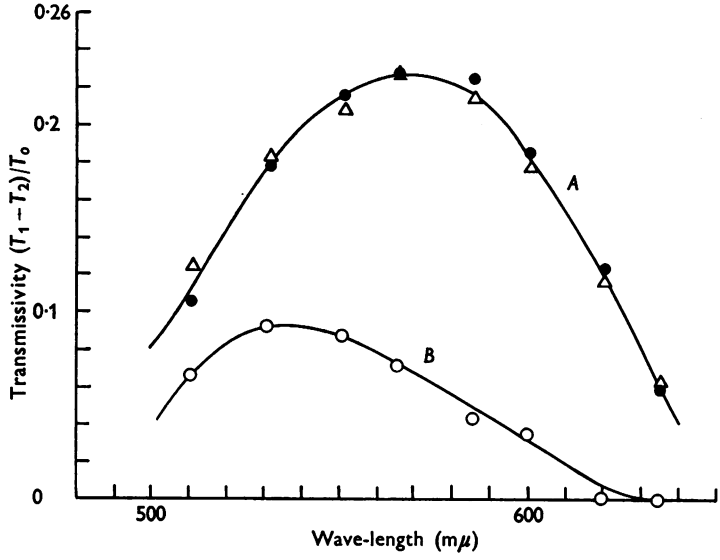

Fig. 1. Black circles show the increase in transmissivity in a normal eye (measured at eight wave-lengths) after bleaching with deep red light. Triangles show the same with a deuteranope (results scaled to coincide at 565 mμ). White circles show in the normal the further increase in transmissivity when the bleaching light is changed from red to a blue-green that was equivalent for the deuteranope.

on the deuteranope, we obtained the difference between the white and black circles in the lower curve of Fig. 1 of the former paper (Rushton 1965*a*). This difference is close to zero everywhere. In the normal, on the other hand, not only is $(T_G - T_R)/T_o$ not zero but, as might be expected, curve B is different in shape from A. Hence the change seen in Fig. 1 must signify the presence of a second pigment more green-sensitive, relatively, than that responsible for curve A.

It is the principal object of this paper to study the properties of the more red-sensitive of these two pigments and to eliminate the other from the measurements. In this we have been helped by noting (Rushton & Baker, 1964) that normal foveas differ widely in the proportions of red and green pigments present. Naturally subjects with red-rich foveas are most

suitable and have been used for our present purpose. One of us (H.D.B.) is the most experienced of them and most of the results actually quoted in this paper are from his left eye.

Measurements at 625 mμ

Photosensitivity. In Fig. 1 it is seen that there appears to be very little absorption of the green pigment at 625 mμ. If then we confine our measurements to that wave-length, there is the chance that the changes observed will be due entirely to the red pigment. If so, the measurements at 625 mμ will be exactly comparable with those in the deuteranope, the green cones simply adding to the interspace area from which stray light returns. We now show that this is the case.

The black circles of Fig. 2 show the results of an experiment upon a deuteranope. Starting dark-adapted, he was bleached to equilibrium with a deep red light that increased in intensity in steps of 0·3 log units. For each step, when equilibrium had been reached, the level of pigment was measured at wave-length 625 mμ by retinal densitometry giving the measured value T. The dark-adapted value T_D was noted and the fully bleached value T_o found using strong white light. It is assumed that at 625 mμ the density of erythrolabe is small enough for self-screening to be negligible. In that case from eqn. (2) Rushton (1965*a*) we may evaluate p the fraction of erythrolabe present

$$p = \epsilon/\epsilon_D = (T_o - T)/(T_o - T_D). \tag{1}$$

In Fig. 2 the points plot p derived from this formula, the various values of I being measured in the appropriate states of adaptation. The continuous curve of Fig. 2 is the theoretical relation from the general kinetic equation (Rushton, 1965*b*, eqn. 5), when dp/dt is put zero for the equilibrium condition under bleaching intensity I. This gives

$$I = k(1-p)/p \tag{2}$$

and when plotted upon a scale of log I we obtain the hyperbolic tangent curve shown. The constant k (which depends upon the light source, coloured filter transmission, etc.) determines the lateral shift of the curve. It has been arbitrarily shifted to fit the filled circles.

If in the deuteranope a blue-green light was used for bleaching instead of a deep red one, naturally the same result would be obtained provided that the two lights appeared to the deuteranope equally bright. It so happens that with our equipment the Ilford filters (206) and (623) exactly fulfil this condition; thus repeating the experiment with nothing changed except the substitution of (623) for (206) gives the white circles (Fig. 2) which coincide almost exactly with the black circles.

This experiment is now repeated except that the subject instead of

being a deuteranope was a normal. He was in fact H.D.B. who is a normal with an unusually large amount of red pigment on the fovea. With the deep red bleach (206) the black triangles of Fig. 2 were obtained. These are plotted with no lateral shifting, and scaled vertically so that the full change in reflexion $(T_o - T_D) = 1{\cdot}0$. As before, T_D is the wedge transmission in dark adaptation, T_o in full *white* bleaching.

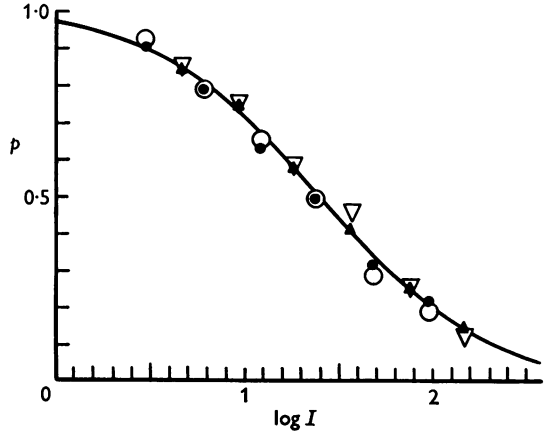

Fig. 2. Ordinates plot p, the fraction of pigment present measured at 625 mμ in equilibrium under lights of log energy shown as abscissae. Black circles, deuteranope bleached by deep red light (Ilford filter 206); black triangles, normal similarly bleached. White circles, deuteranope bleached by blue-green light (623); open triangles, normal similarly bleached.

The fact that the black triangles fall upon the curve is consistent with the idea that the pigment measured in the normal by this technique is erythrolabe and that only. But this conclusion is not inevitable. If two pigments were being bleached by the red light, and if the more green-sensitive were intrinsically far more photosensitive than the other, then it might so happen that both were bleached at equal rates, the diminished absorption by the green pigment just balancing its superior photosensitivity. This possibility is excluded by the open triangles of Fig. 2 which show the effect of bleaching with the blue-green light (623) which matched the deep red light in the bleaching of erythrolabe.

Now a green light which matches the deep red one in bleaching erythrolabe cannot possibly match also our hypothetical green pigment, since both its greater intrinsic photosensitivity and its superior absorption in the green would conspire to cause faster bleaching. This, however, is not found. The open triangles, plotted without any manipulation, coincide with the filled triangles just as white circles coincide with black circles.

There is no sign of that much faster bleaching under green light that must have appeared if a green-sensitive pigment had been involved. These measurements at 625 mμ evidently refer to one pigment only and that pigment has the same photosensitivity as erythrolabe in the deuteranope when measured both by deep red and by blue-green bleaching.

TABLE 1. Action spectrum of erythrolabe and π_5

Peak transmission of interference filters (mμ)	646	615	590	576	552	527	516	White	S.D.
Red pigment (normal)	0·54	0·70	0·70	0·81	0·74	0·52	0·55	2·16	$< \pm 0{\cdot}05$
Erythrolabe (deuteranope)	0·54	0·70	0·70	0·82	0·73	0·53	0·55	2·13	$< \pm 0{\cdot}05$
π_5 (normal)	0·51	0·66	0·77	0·81	0·76	0·51	0·56	2·17	$< \pm 0{\cdot}05$

Action spectrum. Since all measurements at 625 mμ in the normal eye refer only to a single pigment, we may measure the action spectrum of that pigment by the method already used for chlorolabe and erythrolabe in protanopes and deuteranopes respectively. The method consists in attempting to maintain the pigment in an exactly steady state of bleaching (about 50 %) though at the end of each minute the colour (and intensity) of the bleaching light is suddenly changed. The colour of the bleaching light was controlled by one of a set of interference filters and the intensity was adjusted by a neutral wedge. If the wedge was correctly set for each filter, then the bleaching of the single pigment measured would be always equivalent, and hence the pigment would show no change in level when the bleaching light was changed. The actual change that occurred at the end of the minute was noted for each filter and thus a better estimate could be made of the correct wedge setting for that filter from the full kinetic eqn. 5 (Rushton, 1965*b*). In this way a good approximation was obtained in a few runs, and thereafter the measurement consisted in trimming the estimate from the statistics of the results.

Table 1 shows, in the top row, the dominant wave-length of the interference filters used in the bleaching light. The second row gives the relative densities that must be interposed in order to keep the red-sensitive pigment 50 % bleached. It is the average of six runs on H.D.B., and the S.D. of the mean is always less than $\pm 0{\cdot}05$. The third row gives the similar figures for the deuteranope taken from Table 1 of the previous paper (Rushton, 1965*a*).

We worked out these figures for the normal without looking at those for the deuteranope and, though we knew that they were more or less the same, we were astonished to find how identical the results turned out to be. Certainly the accuracy of the work does not justify belief in a fit quite as close as this, nevertheless, there is not much doubt that the red-sensitive pigment in the normal is the same as erythrolabe in the deuteranope both by action spectrum and by absolute bleaching rate—the photosensitivity.

In the deuteranope the pigment was shown to be a visual pigment because the action spectrum corresponded to his spectral sensitivity curve. In the normal eye, on the other hand, it is recognized that both red- and green-sensitive pigments contribute to brightness, thus a different measure is needed to establish the visual function (if any) of erythrolabe in normal vision. We need to measure the spectral sensitivity not of the whole fovea but of the red-sensitive mechanism there. Stiles has shown us how to do this.

Stiles's red mechanism π_5

Increment threshold. The well-known two-colour increment threshold work of Stiles (1949, 1959) has proved that there are colour mechanisms that behave independently one of another in a large range of threshold conditions. In particular the red mechanism π_5 has a spectral sensitivity which is the same whether measured by the energy of test flashes of various wave-lengths that are just detectable when presented superposed upon a fixed background, or measured by the energy of backgrounds of various wave-lengths that raise the threshold of some red test flash to a given extent. Stiles's work has not pursued the question as to the physiological mechanism underlying the π_5 mechanism, but a likely interpretation has always been that it represented the organization of signals from the red-sensitive visual pigment. We are now in a position to test this, for if it were true, background fields of different wave-lengths that were equivalent in raising the π_5 threshold should also be equivalent in bleaching the red pigment.

The experiment was performed by employing as background the very lights that had been used to bleach the pigment in the action spectrum measurements. The subject assumed the position for densitometry measurements and the bleaching light was sent into the eye from the stationary mirror of the 'mirror windmill' giving a $2\frac{1}{2}°$ field centred upon the fovea. Since when running the mirrors lay in the light beam for only a quarter of the cycle, when they were stationary, the light had to be reduced to $\frac{1}{4}$ in order to give the same mean retinal illumination. This light was used as background for an increment threshold determination. The red test flash was superposed by placing a piece of plain glass at 45° obliquity in the background beam and directing upon it a deep red flashing light that after reflexion in the glass entered the eye by Maxwellian view and was seen as a small circular field subtending about 0·5° in the centre of the background. The intensity of the test flash could be controlled by a photometric wedge operated by the subject.

If the background fields were always exactly those which bleached half the pigment and if its action spectrum was the same as that of π_5, then the

subject would have always set the wedge controlling the flash at the same value. As will be seen from Table 1, this turns out to be very nearly the case. In increment threshold measurements it is much more accurate to keep the background fixed and to vary the flash (as was done here) than to keep the flash fixed and vary the background, since that changes the adaptation level. But it is the equivalent background for a fixed flash threshold, not the reverse, that we wish to know. This is found at once,

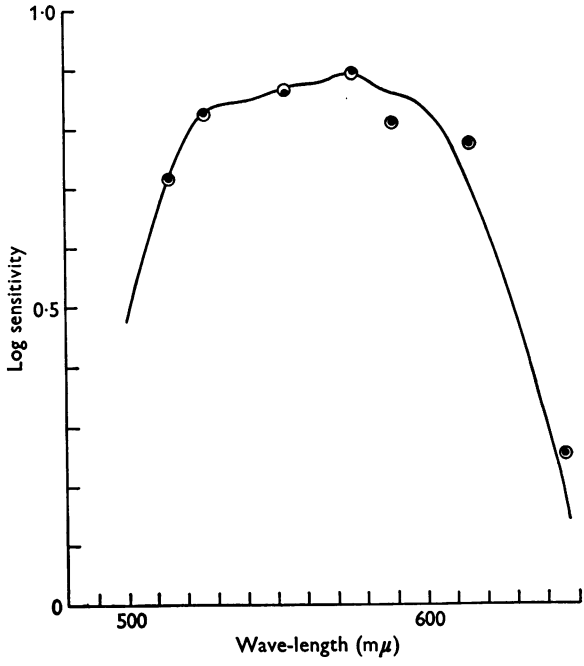

Fig. 3. Curve is log quantum spectral sensitivity of Stiles's red mechanism π_5. Assuming that H.D.B.'s π_5 increment threshold falls exactly upon this curve, his red-sensitive pigment has the action spectrum given by the open circles. The deuteranope's pigment corresponds to the closed circles. These results are taken from Table 1.

however, from the flash threshold readings by confirming the fact that in the conditions of these measurements, the Weber–Fechner relation $\Delta I/I = k$ held exactly. Knowing, then, the small variations in ΔI, it was easy to calculate the corresponding change in background I that would have kept ΔI exactly unchanged.

The results are shown in the fourth row of Table 1. The figures there show the neutral wedge densities required for each interference filter in order that the increment deep red flash (Ilford 206) of fixed intensity should always be exactly at threshold. As with the two previous rows it

is the relative sensitivity alone that is measured, and some fixed density can be added to every member of a row without affecting this. Such fixed densities have been added to make the figures closely comparable. Increment thresholds are never so accurate as perfect matching of fields, and the figures of Table 1 are each the average of 18 determinations in 4 runs made also on H.D.B.

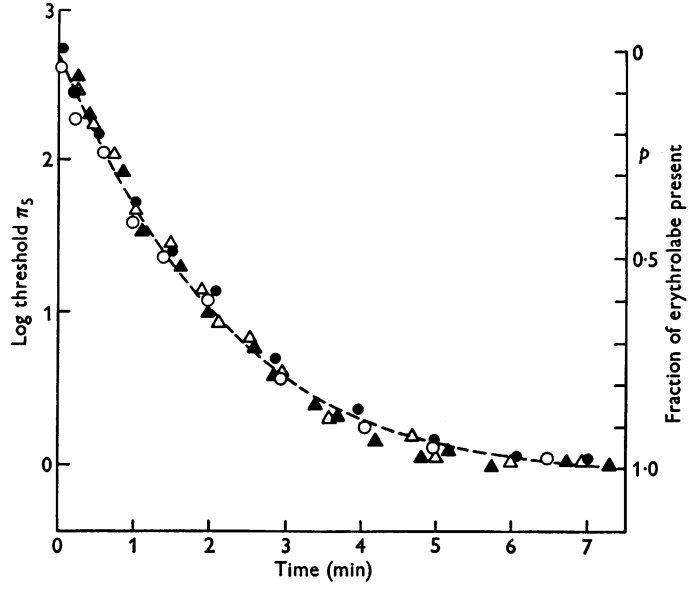

Fig. 4. Recovery after full bleaching. Abscissae time (min). Black and white triangles, dark-adaptation curve plotted as log threshold using a red test flash (scale on left). Black and white circles, regeneration of erythrolabe plotted as fraction of total pigment present (scale on right). The curve is precisely that from the recovery in the deuteranope (Rushton, 1965*b*, Fig. 3) without any scaling.

However, the divergences are small enough to justify us in identifying erythrolabe in the normal eye with the pigment underlying Stiles's π_5 colour mechanism. In Fig. 3 Stiles's π_5 is plotted upon a scale of log quantum sensitivity. If we assume that the π_5 results of Table 1 lie upon this curve, the log action spectra for erythrolabe in the normal and in the deuteranope are given by white and black circles in the figure, since these points diverge from the curve by the amounts that rows 2 and 3 in Table 1 diverge from row 4.

Dark adaptation. The technique of Fig. 2 which permitted the measurements of the red-sensitive pigment alone at all stages of bleaching will also allow the regeneration curve of this pigment to be followed in the dark after full bleaching. The results are shown by the black and white circles

of Fig. 4 which represent two runs alternating with the black and white triangles that represent two dark adaptation curves of π_5. This was obtained by bleaching with a full white light and using a fairly deep red test flash in order to measure the π_5 log threshold. The curve drawn through the points of Fig. 4 is precisely that in Fig. 3 of the deuteranope paper (Rushton, 1965*b*) unscaled and unaltered in any way. It is plain that the time course and absolute rate of regeneration of the red-sensitive normal pigment is the same as that of erythrolabe in the deuteranope. Moreover, just as in the deuteranope the rise of log threshold above the dark value was proportional to the fraction of erythrolabe bleached, so in the normal the same rise of log threshold for π_5 is proportional to the fraction of red pigment bleached. And the constant of proportion is the same in the two cases.

It was stated above that when dark adaptation was investigated with a deep-red test flash, it was the recovery of the π_5 mechanism alone that was being measured. This rests upon an analysis completed, but published only in summary (du Croz & Rushton, 1963). In that work it was shown that Stiles's red, green and blue mechanisms were independently affected by strong bleaching with coloured lights and that each mechanism regenerated independently of the others and could be isolated by the methods Stiles had used in his conditions with steady backgrounds.

Selective bleaching

So far we have been able in normal eyes to measure the red pigment alone by confirming our measuring wave-length to 625 mμ and working with eyes that are sufficiently rich in red pigment to give a good difference in transmissivity there. Obviously if we wish to measure the transmissivity at various wave-lengths we cannot confine our measurements to 625 mμ and thus we must use a different technique to exclude the green pigment.

Figure 2 (black symbols) shows that if we bleach with the deep red light (Ilford filter 206) our full light bleaches about 85% of the red pigment. We now show that this light at the same time bleaches but a negligible amount of the green pigment.

If the change in reflected light is measured not simply at 625 mμ as in Fig. 2 but also at 550 mμ, then at each equilibrium level of bleaching we may plot the light at one wave-length against that at the other as in Fig. 5.

It is obvious that if only one pigment were involved (as in the deuteranope) and if self-screening is negligible, then as the density alters the change in transmission measured at one wave-length will always be proportional to that measured at another. Thus the effect of bleaching the deuteranope to equilibrium under full light or light $\frac{1}{2}$, $\frac{1}{4}$, $\frac{1}{8}$, etc., as bright

will result in reflected lights measured at 620 and 550 mμ which when plotted one against the other as in Fig. 5 must lie upon a straight line. In Fig. 5 the actual deuteranope results are not plotted but the line upon which they fall is. When the same experiment is done with normal eyes (still bleaching with deep red light) the results fall close to the deuteranope's straight line; it must, therefore, have bleached only a single photosensitive pigment—namely erythrolabe. For when the bleaching light is not deep

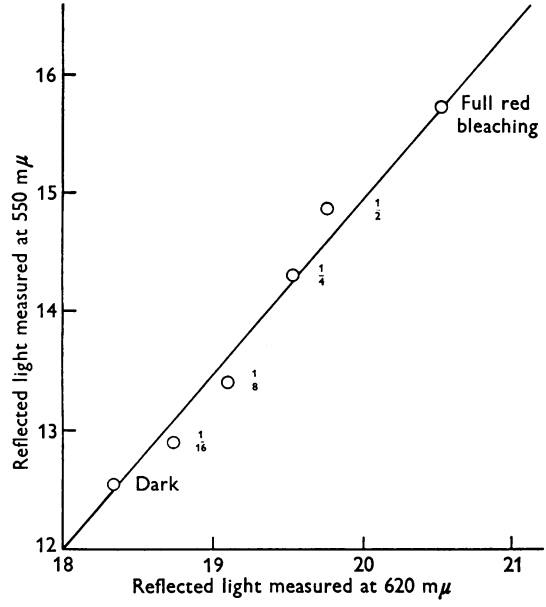

Fig. 5. Transmitted light measured at 550 mμ plotted against that at 620 mμ for equilibrium under deep red bleaching of various intensities. Straight line, the results in the deuteranope; circles, normal subject.

red and some green pigment is also bleached, the points are found to lie above the deuteranope line as may be seen in Fig. 3 of Rushton & Baker (1964)—though there the unsatisfactory 'double-density' was plotted instead of transmitted light.

Now, turning back to Fig. 1, we may interpret the results there more securely. The black circles show the results of bleaching the normal eye with the deep red light that we have just shown bleaches only erythrolabe. This same experiment was performed upon the deuteranope (Rushton, 1965*a*) and the triangles of Fig. 1 are the re-plot of Fig. 1 of that paper, scaled so that triangles and black circles are brought into coincidence at wave-length 565 mμ (black triangle). It is unlikely that the coincidence at all other points would be so good unless the superficial stray light had

been nearly eliminated. And if it has been, we may add similarity of difference spectrum to the three properties by which the red pigment in normal eyes has already been identified with erythrolabe in the deuteranope.

But if this identification is accepted, it must follow that curve *B*, Fig. 1, does not involve erythrolabe, for the method of plotting combines the changes due to each pigment by simple addition, and the change due to erythrolabe was zero in the procedure that resulted in curve *B*. If then there are two visual pigments in the red–green spectral range, curve *A* is due to the red-sensitive pigment alone, curve *B* to the green-sensitive alone.

DISCUSSION

It has been recognized for a hundred years that there is a red- and a green-sensitive mechanism upon the normal fovea, and 10 years ago two different photosensitive pigments were found there by retinal densitometry. In this paper we have examined the properties of the more red-sensitive pigment in conditions where the other was shown to have no detectable effect.

Normal subjects vary considerably in the relative amounts of red and green pigment on the fovea (Rushton & Baker, 1964) and most of those helping in the present experiments had a high proportion of red. With these subjects we were able to show that retinal reflexion measurements at 625 mμ were affected only by the red-sensitive pigment, for Fig. 2 shows that the equilibria under various intensities of either deep-red or blue-green light follow the theoretical curve of a single pigment and indeed coincide both in absolute photosensitivity and in relative red/blue-green sensitivity with erythrolabe of the deuteranope. Seeing that only this one pigment is concerned in measurements at 625 mμ, the whole action spectrum may be determined using as criterion of action the half bleach measured at that wave-length. The action spectrum is the same as that of erythrolabe in the deuteranope (Fig. 3) and corresponds to that of Stiles's red-sensitive colour mechanism π_5. Finally, the rate of regeneration of the pigment (Fig. 4) coincides with that in the deuteranope and with the dark adaptation curve of the π_5 mechanism. We here have wider evidence than is often adduced to help us to identify erythrolabe of the deuteranope with the normal red pigment, for not only is the action spectrum the same but so is the photosensitivity and the rate of regeneration.

The straight line relation of Fig. 5 gives us a second and independent method of isolating the red-sensitive pigment, for it shows that when the normal fovea is bleached with a deep red light (that does not affect chlorolabe in the protanope) it only bleaches erythrolabe in the normal.

This allows us to investigate at various wave-lengths the effects of bleaching erythrolabe alone. If ordinary 'density' measurements are made the results will depend upon the amount and nature of the unbleached green-sensitive pigment present, but this complication is eliminated if not the 'density' but the transmissivity is plotted as in Fig. 1. Since the total transmissivity is the sum of that of the red cones and of the green cones, plotting this quantity combines the contributions from red cones and from green by simple addition.

Transmissivity spectrum. In the previous paper (Rushton, 1965*c*) a useful formula (1) p. 58 was derived

$$\frac{T_o - T}{T_o} = \frac{m\epsilon + n\chi}{m+n+a+b/\rho}. \tag{3}$$

In this ϵ, χ are the fractions of light absorbed in double passage through the red and the green cones respectively. T is the light transmission measured by the wedge setting, and T_o its value in the fully bleached state. Of the light coming from the bleached fundus, proportions m, n and a come from red cones, green cones and from elsewhere, and ρ is the fundal reflectivity. The superficial stray light is b, and in the same paper b/ρ was shown to be exceedingly small in our experiments. Roman letters on the right side of eqn. 3 are likely not to depend upon the wave-length of light.

From eqn. 3 it is easy to obtain an expression for the transmissivity difference spectra of Fig. 1. Curve A is the result of a deep red bleach which Fig. 5 showed to be without effect upon χ. Thus

$$\frac{T_R - T_D}{T_o} = \frac{T_o - T_D}{T_o} - \frac{T_o - T_R}{T_o} = \frac{m(\epsilon_D - \epsilon_R)}{m+n+a+b/\rho} \tag{4}$$

and the shape of curve A is unaffected by the presence of chlorolabe. In the same way since curve B is the result of a bleach that did not change erythrolabe

$$\frac{T_G - T_R}{T_o} = \frac{T_o - T_R}{T_o} - \frac{T_o - T_G}{T_o} = \frac{n(\chi_D - \chi_G)}{m+n+a+b/\rho} \tag{5}$$

and the shape of B is seen to be unaffected by the presence of erythrolabe.

Now in expression (4) if self-screening is small, $(\epsilon_D - \epsilon_R)$ will be proportional to the change in pigment density, and if b/ρ is small the right side of that equation will approximate to the difference spectrum of erythrolabe. But if the curves of Fig. 1 represent the difference spectra of erythrolabe in normal and deuteranope it will be interesting to see how they compare with the spectral sensitivity of those eyes. To display this, the points of Fig. 1 are transferred to the curves of Fig. 6.

The continuous curve A, Fig. 6, is the antilogarithmic plot of Stiles's

π_5 curve. This was given in the logarithmic form in Fig. 3. Dr Stiles kindly supplied us with a table of average field sensitivities for his π_5 and π_4 mechanisms and the computation and plots of the curves were taken from this. The dotted curve *A*, Fig. 6, is taken from Hsia & Graham (1957) who investigated the spectral sensitivity of deuteranopes, protanopes and normals by a threshold method and tabulated their results in log threshold (ergs): in Fig. 6 their results have been converted to quantum energies. It is plain that the transmission difference spectrum of erythrolabe fits these spectral sensitivities of normal and deuteranope red vision reasonably well, and supports the view that in these experiments b/ρ is negligible.

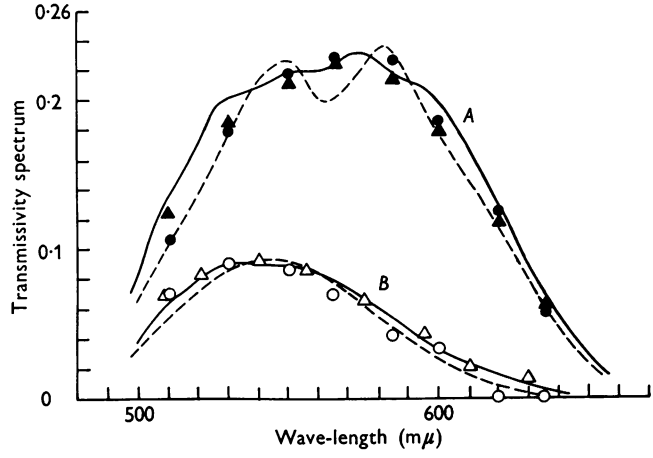

Fig. 6. Transmissivity difference spectrum. Curve *A* circles, the change in normal after bleaching with deep red light; triangles, the same with deuteranope. Continuous curve, spectral sensitivity of Stiles's red mechanism π_5; dotted curve, deuteranope threshold (Hsia & Graham, 1957). Curve *B* circles, the effect in the normal of changing the bleaching light from deep red to blue-green; triangles, transmissivity spectrum in the protanope. Continuous curve, Stiles's green mechanism π_4; dotted curve, protanope threshold.

But if b/ρ is negligible we may conclude from eqn. 5 that the white circles of Fig. 6 which correspond to Fig. 1, curve *B*, represent the absorption difference spectrum of the green-sensitive pigment in the normal eye. The white triangles give the transmissivity spectrum of the protanope, the continuous curve *B* shows Stiles's green mechanism π_4 and the dotted curve *B* is the spectral sensitivity curve of the protanope derived from the results of Hsia & Graham (1957) by the same transformation as in curve *A*.

From the time of König & Dieterici (1886) it has often been held that the normal green mechanism was the same as that in the protanope. In recent times this view has been strengthened by the close fit between the

protanope's spectral sensitivity, Stiles's green mechanism π_4 and also Brindley's (1953) artificial protanopia produced by adapting the eye to a very bright red light. To those subjective measurements we now can add the objective evidence of Fig. 6 which shows in the first place that the transmissivity difference spectrum of the green normal pigment is very similar to that of chlorolabe in the protanope, and in the second that this curve fits well Stiles's π_4 in the normal eye and Hsia & Graham's threshold curve from the protanope.

In this section we set out to compare the transmissivity difference spectrum of the two pigments with the subjective spectral sensitivity. In Fig. 6 we see that the correspondence is good in deuteranopes, protanopes and in normal eyes. This could hardly be the case if the difference spectrum upon bleaching left a photoproduct with substantial absorption in the range studied.

We conclude that the red-sensitive pigment in normal eyes is erythrolabe which is found alone in the red–green range of the deuteranope; the green-sensitive pigment is chlorolabe found alone in this range in the protanope. The action spectrum of these pigments corresponds to Stiles's π_5 and π_4 mechanisms; their difference spectra also correspond, hence the photoproducts must in this range be nearly transparent.

Retinal densitometry in the living eye has nothing to say upon the ancient and important question of whether the visual pigments are segregated each in a different type of cone, or whether all cones are similar and have all three pigments appropriately organized in their structure. That question can only be answered by the immensely difficult technique of micro-spectrophotometry. The answer emerged when Marks (1963) measured the pigments in the outer segments of single isolated cones in fish. Now this work has been extended to monkeys and man in the intact excised retina (Marks, Dobelle & MacNichol, 1964), and Brown & Wald (1964) independently describe how the same thing may be measured by a somewhat different technique. Both groups have demonstrated that there are three types of cone containing red-, green- and blue-sensitive pigments, though (like erythrolabe) the maximum sensitivity of the 'red' pigment is no redder than yellow.

The optical difficulties of obtaining anything like a true difference spectrum by axial passage down the outer segment of a cone—whose aperture at tip is commensurate with the wave-length of light—are so formidable that to get the results that have been obtained is a remarkable achievement. These fine techniques, further perfected, may allow many of the problems of visual chemistry to be studied isolated and at the receptor level. But the relation of pigments to seeing depends of course upon the action spectrum and upon the rate of regeneration in the living eye. The

first is very hard and the second impossible to measure in excised retinas, and so it may well turn out that reflexion densitometry, despite its considerable limitations, will be found helpful in the study of the organization of human vision.

Note. The substance of this paper was presented to The Physiological Society in September 1963 (Baker & Rushton, 1963), and we are indebted to Dr Wald for his comments on that occasion.

SUMMARY

1. In order to study the red-sensitive pigment in the normal eye, subjects specially rich in this pigment but with normal colour vision were used.

2. When reflectivity measurements were made at a wave-length of 625 mμ, only one pigment was involved and that had the same photosensitivity as erythrolabe in the deuteranope when measured both in deep red and also in blue-green light.

3. In fact the photosensitivity was the same at all wave-lengths and the two action spectra coincided.

4. The action spectrum also corresponds to that of Stiles's red mechanism of colour vision π_5.

5. The time course of pigment regeneration after full bleaching coincides with that in the deuteranope and with the normal dark adaptation curve using a deep red test flash.

6. Instead of isolating the red pigment by measuring always at 625 mμ we may bleach with a deep red light that bleaches only the red pigment and measure at any wave-length. The transmissivity spectrum resulting resembles very closely that in the deuteranope and also his visibility curve and the spectral sensitivity of π_5.

7. We may conclude that the erythrolabe of the deuteranope is the same as the red-sensitive pigment of the normal, since the action spectrum, the transmissivity difference spectrum, the photosensitivity and the time course of regeneration are all nearly identical.

8. Consequently a change in equilibrium bleaching from a deep red light to a blue-green light that produces no change in the deuteranope will cause no change in erythrolabe in the normal. The change that does occur in the transmissivity spectrum must therefore be due to the green-sensitive pigment alone.

9. This change fits the transmissivity spectrum of chlorolabe in the protanope and also his visibility curve and the spectral sensitivity of π_4, Stiles's green mechanism in the normal eye.

10. We conclude that deuteranopes and protanopes have one each of

the two normal pigments in the red–green range, and in this range both of these pigments bleach nearly to transparency.

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