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CONE PIGMENT KINETICS IN THE DEUTERANOPE

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In the previous paper (Rushton, 1956) it was shown that the deuteranope contains upon his fovea only one photosensitive pigment in the red–green range, since bleaching either by deep red or by blue-green light results in the same change in spectral reflectivity. Moreover, this pigment *erythrolabe* is the deuteranope's visual pigment since lights of various wavelengths, adjusted by the deuteranope to appear equally bright, produce equal physical bleaching of erythrolabe. Consequently erythrolabe in the deuteranope is precisely analogous to chlorolabe in the protanope.

In a recent paper (Rushton, 1963*c*) the kinetics of bleaching and regeneration of chlorolabe were investigated. In the present paper exactly the same kind of measurement is made on erythrolabe, and almost identical results are obtained. It was pointed out in the former paper that kinetics relate to D the density of the visual pigment, but what is measured is the wedge setting W; thus we need some way of relating these two quantities. If we know σ, the fraction of stray light to signal returning from fully bleached cones, we may obtain the relation between D and W at once from the nomogram (fig. 4, Rushton, 1963*b*). I am now dissatisfied with the value of σ found in the protanope and with the reliability of the difficult method used. In the present paper it will be assumed that in full regeneration the density D at $\lambda = 580$ mμ is 0·2. From this assumption by means of the nomogram the value of σ may be found in any experiment and hence, for each observed value of W, the corresponding D may be read off.

I do not know how to find the density of visual pigments by retinal densitometry. The density cannot well be less than half the 'double-density' measured, for stray light must dilute the signal. If we knew the amount of the stray light for each wave-length it would be easy to make the correction, but I know no method of direct measurement. Indirect measurements of density depend upon self-screening and may be objective or subjective. Essentially they are the matching of the absorption by green and red lights in conditions of bleaching and of full regeneration. I have tried a large number of methods both before and after carrying out the most exact of them, already described for the protanope (Rushton, 1963*b*). But results do not cohere, and subjective red–green matches in the protanope lead to a value lower even than half the double density. The trouble is that self-screening derives its answer from a very small differential change and argues from the assumption that

coloured photoproducts are either absent during steady bleaching–regeneration conditions or have special properties which may well not be true.

It thus seemed better to invert the situation and argue that, if no measurement can be found to distinguish one density value from another, then one density is as good as another in the analysis of all our measurements. For the deuteranope 0·2 peak density is estimated as the geometrical mean of the range over which the above statement holds.

METHODS

Bleaching. This is almost identical with the previous work on the kinetics of chlorolabe (Rushton, 1963*c*). The deuteranope with dilated pupil was aligned in the densitometer, and the image of a semi-circular source was focused upon the pupil so that it nearly filled the nasal half and never fell upon the iris. The temporal half of the pupil was covered by the ophthalmoscopic mirror. The brightness of the white bleaching light was measured by receiving it upon a white screen placed 31·6 ($=10^{\frac{3}{2}}$) cm behind the spot where the pupil had been. The luminance on the screen (I milli-lamberts) was measured with an S.E.I. meter, from which the retinal illumination of $I \times 10^6$ trolands is immediately obtained.

The bleaching light was applied alternating about 10 times per sec with the measuring light by means of the 'mirror windmill' (Rushton, 1963*a*, Methods) and shone during only $\frac{1}{4}$ cycle; it is treated as though steady but $\frac{1}{4}$ the intensity of the actual flash.

Measurement. To measure the amount of pigment bleached by various 10 sec exposures, (as before) the light after 10 sec was not extinguished but reduced about 10 times in intensity to a level that exactly 'froze' the change in density, neither allowing bleaching to go further not yet to be reversed by the process of regeneration. For every intensity I_1 of bleaching light there was an intensity I_2 of maintaining light that kept the level exactly constant. It was the object of the experiment to find for each I_1 the proper I_2 and the subsequent steady pigment level was then easy to measure at leisure.

The regeneration of erythrolabe in the dark after full bleaching occurred at the same rate as the regeneration of chlorolabe (in the protanope) and was slow enough to measure by sample wedge settings taken every 30 sec near the beginning of regeneration, every 60 sec later. The subject closed his eyes except for the 7 sec or so required for the measurement.

Dark adaptation. Foveal dark adaptation in the deuteranope after full bleaching was determined by presenting a red flash, on for $\frac{1}{2}$ sec, off $\frac{1}{2}$ sec. The flashing field subtended 1° and in its centre there was a brighter unflashing point that served for fixation. The subject moved the wedge to bring the flash to threshold, indicated when he was satisfied, and the time and wedge setting were then recorded.

RESULTS

Bleaching

Figure 1 shows the results of one experiment. The ordinate p is the fraction of erythrolabe left unbleached by the 10 sec exposure to an intensity I_1 plotted as log I_1 horizontally. The value of p was found by obtaining the density D from the nomogram having taken σ so that D read 0·2 at full regeneration. Then p is $D/0{\cdot}2$. The white circles show the relation between p and I_1, the intensity of the 10 sec exposure. During that 10 sec, regeneration is negligible; hence we should expect the rate of bleaching to

be proportional to the rate at which quanta are caught by the pigment, that is proportional both to I_1 and to p

$$-\frac{dp}{dt} = pI_1/10I_e \tag{1}$$

where I_e is the value of I_1 which in 10 sec will bleach erythrolabe to 1/e of its initial value. From Fig. 1, I_e is seen to be $10^{5\cdot0}$ trolands.

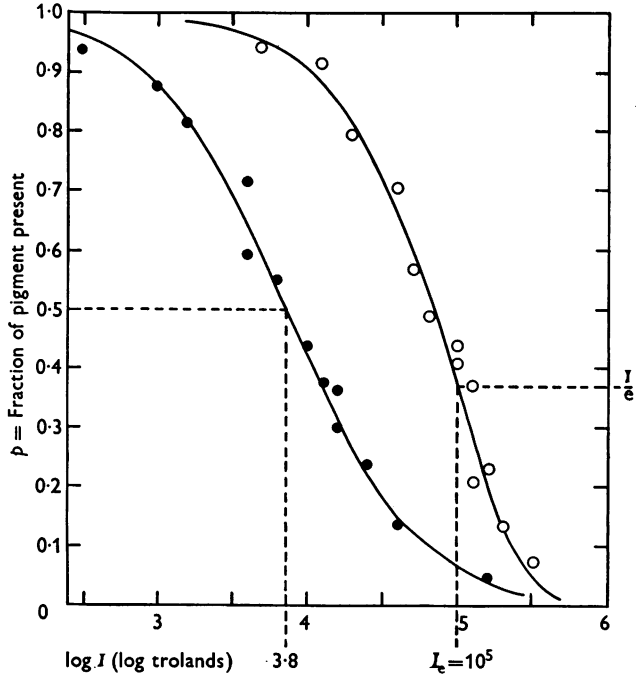

Fig. 1. Ordinates: p, fraction of pigment left unbleached; abscissae: bleaching illumination in log trolands. White circles, amount bleached in a 10 sec exposure to light plotted. Black circles, amount of pigment in equilibrium with steady light of intensity plotted. Curves plot theoretical relations from eqn. (5).

Integrating (1) and taking common logarithms we obtain to a good approximation (as with the protanope)

$$\log\log(1/p) = \log(I_1 t) - 6\cdot4, \tag{2}$$

where $6\cdot4 = \log(10I_e) + 0\cdot4$.

Relation (2) is the theoretical curve drawn through the white circles of Fig. 1. It has one arbitrary constant I_e, variations in which cause the curve to slide sideways. The value $I_e = 10^{5\cdot0}$ gives a good fit.

Regeneration in the light

The black circles of Fig. 1 show the value of the steady illumination I_2 that holds erythrolabe in equilibrium at the level of p indicated upon the ordinate scale.

Now from equation (1) (with I_2 substituted for I_1) we know the rate at which I_2 is bleaching erythrolabe since the black experimental points have co-ordinates that give both p and I_2 and we have found that $I_e = 10^5$. In Fig. 2 the ordinates represent, for each black point in Fig. 1, this rate

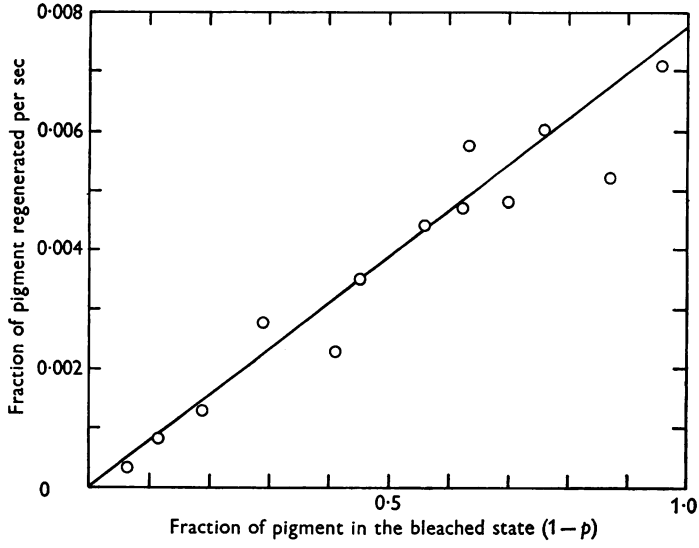

Fig. 2. Abscissae $(1-p)$, the fraction of pigment in the bleached state. Ordinates the rate of regeneration in this state of bleaching under illumination which bleaches at the same rate and hence keeps the pigment level in equilibrium.

of bleaching plotted against the corresponding value of $(1-p)$. Now under the illumination I_2 the pigment level keeps exactly in equilibrium; thus the bleaching rate must be the same as the regeneration rate. Consequently in Fig. 2 is plotted equally the regeneration rate of erythrolabe in varying states of bleaching. The points lie close to the line through 0; hence the regeneration rate is proportional to $(1-p)$, the fraction of pigment bleached, or free opsin; thus

$$\frac{dp}{dt} = \frac{1-p}{130}. \tag{3}$$

Regeneration in the dark

In Fig. 3 the filled symbols plot three determinations of regeneration in the dark after a full white bleach. They were taken in succession at one

sitting and show the kind of reproducibility obtainable with a good subject. The curve of Fig. 3 is that which would be followed if the rate of regeneration at every stage corresponded to the straight line of Fig. 2. Since the experimental points lie closely upon it we may conclude that

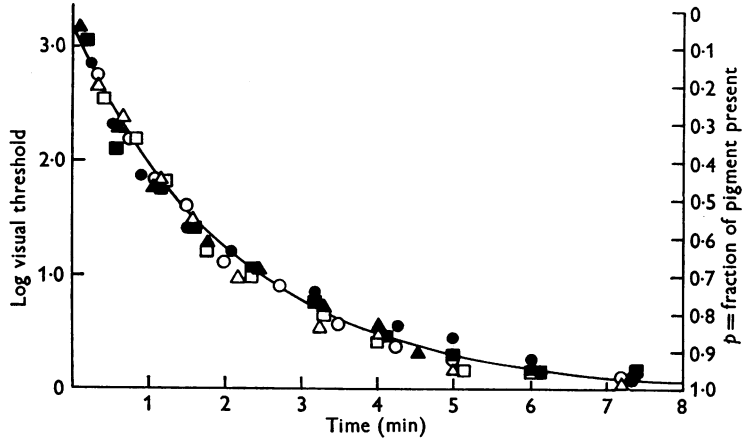

Fig. 3. Filled symbols show the time course of regeneration of erythrolabe in the dark (scale on right). The three runs alternated with the empty symbols that plot three dark adaptation curves (scale on left). In both cases recovery followed full bleaching of erythrolabe. The curve is a re-plot of the straight line of Fig. 2.

regeneration in the dark proceeds at the same rate as in the light, namely that 1/130 of the free opsin combines with retinene every second independent of the amount bleached or the conditions of bleaching here considered. The situation is quite different after intense bleaching with an electronic flash (Baker & Rushton, 1963; Rushton, 1964).

Dark adaptation

In Fig. 3 the open symbols plot three determinations of the dark-adaptation curve, i.e. the return of log threshold after its rise following the full bleaching of erythrolabe.

Each run was taken alternately with the pigment regeneration measurements. The axis on the left, which shows log threshold, is scaled to fit that on the right, which shows the fraction of pigment regenerated. As with the protanope the correspondence is good and satisfies the relation

$$\log(\Delta I/\Delta I_o) = 3(1-p), \tag{4}$$

where ΔI, ΔI_o are the thresholds during and after complete dark adaptation respectively. The relation is obtained for the period in the dark following full bleaching and does not claim to describe other manoeuvres.

The general kinetic equation

Since the rate of regeneration depends upon $(1-p)$ and not upon any concurrent bleaching we may express the general kinetic relation by adding together the two independent equations, namely for bleaching alone (1) and for regeneration alone (3):

$$-\frac{dp}{dt} = pI \times 10^{-6} - (1-p)/130. \quad (5)$$

Solution of this will give the fraction of erythrolabe present at any moment as a result of any manoeuvre involving regeneration and bleaching with I as any function of t. The equation is without arbitrary constant; t is in sec, I in trolands and p is the fraction of the total erythrolabe. It does not describe, however, the results of flash photolysis.

DISCUSSION

The kinetics of erythrolabe are so nearly identical with those of chlorolabe described in a former paper (Rushton, 1963*c*) that only one thing needs discussion here that was not discussed there. It is a conceivable interpretation of why both pigments are so similar.

Only one cone pigment iodopsin has ever been found in the retinas of fowls (Wald, Brown & Smith, 1955), but birds have good colour discrimination and the explanation probably lies in the various coloured oil droplets which Schultze (1866) described in the outer segments of those cones. These could impose a differential colour sensitivity upon the subjacent iodopsin. Human cones do not have oil droplets but many suggestions have from time to time been made as to possible colour-sensitive wave guides, interference filters, etc., of which the latest is Enoch's (1961) wave modes.

Suppose the deuteranope had erythrolabe with no colour-selector mechanism and the protanope also had erythrolabe but with some auxiliary mechanism that prevented it from receiving red light. Then green light would bleach the two systems equally (which they do) and, in the dark, regeneration would proceed identically (which is the case). We know that the rate of combination of retinene with free opsin of the human rods is much slower than with the opsin of the cones; thus if there were two types of cone there would seem no reason to expect that they should regenerate at exactly the same rate (as they do); but that must follow if there is only one cone pigment. Is this possibility the truth?

Brindley and I tried to settle this question and succeeded in making the concept of a single pigment hard to sustain, for we found that monochromatic lights appeared nearly their normal hue when judged by light

that reached the cones by passing through the sclera from the back (Brindley & Rushton, 1959). Light entering the cones from the choroid might be expected to miss the wave-length selectors, or at any rate be differently selected, but that was not the case. Any lingering doubts that cones do contain different photosensitive pigments have now been removed by the beautiful analysis of single cones that has just appeared (Marks, Dobelle & MacNichol, 1964; Brown & Wald, 1964).

It therefore seems safe to conclude that chlorolabe and erythrolabe are two well-matched but distinct cone pigments, that deuteranopes lack the first, and that protanopes lack the second.

SUMMARY

1. Erythrolabe was measured on the fovea of a deuteranope by the method of retinal densitometry and the amount bleached and regenerated in various procedures found.

2. During a 10 sec bleach there is no time for appreciable regeneration and the rate of bleaching was found to be proportional to the rate at which quanta were caught.

3. The rate of regeneration, on the other hand, was proportional to the amount of pigment bleached, the constant being the same for regeneration in the dark or in the light. The two results may thus be combined to give the general equation

$$-\frac{dp}{dt} = pI \times 10^{-6} - \frac{1-p}{130}.$$

4. The shape and time course of pigment regeneration is the same as that of the cone dark-adaptation curve.

5. All these results are precisely similar to those of chlorolabe in the protanope.

6. This suggests that erythrolabe and chlorolabe might be one and the same pigment, operating in conjunction with colour selectors etc. In the Discussion that view is rejected.

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