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MEASUREMENT OF THE SCOTOPIC PIGMENT IN THE LIVING HUMAN EYE

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This paper describes a technique designed to measure rhodopsin in the living human eye. From the experiments quoted one may obtain an idea of the precision of the method and gain some grounds for believing that it is in fact rhodopsin which is measured.

The work of Kühne on the one hand, and the founders of the Duplicity Theory on the other, have made it clear that rhodopsin plays an important role in scotopic vision, and it is now pretty universally held that human rods contain rhodopsin whose photochemical change constitutes the first step in the process of twilight vision. It is outside the immediate scope of this paper to discuss the very considerable amount of work which, following the lead of Hecht and Wald, has attributed to the bleaching and resynthesis of rhodopsin, the changes in visibility during the light and dark adaptation. It will be allowed that since this bleaching and resynthesis has not hitherto been measured in the human eye, a technique which can do this should place such theories upon a surer foundation.

Until recently the only method used to measure rhodopsin from any source has been to excise the eye and to examine the rhodopsin either in the rods or extracted from them. In the living eye something different must be done. Abelsdorff (1895, 1897, 1898) observed but did not measure the rhodopsin (or porphyropsin) in crocodiles and some fish with white tapeta, while they were alive and unanaesthetized. He simply clamped the animal's head and looked at the *fundus oculi* with an ophthalmoscope. The purple colour he saw against the white background could be bleached locally at the image of a bright light, and later the purple was seen to be 'regenerated'. He correctly interpreted his observations and pointed out the difficulty of doing the same sort of thing in man—that the human fundus is lined with black not white, so very little light returns, and one cannot appreciate its colour. We in fact have never been able to *see* any detectable change in the appearance of the human fundus,

even though we had good reason to suppose that a small known area with a crisp margin was completely bleached.

It is surprising that no development has been made in the ophthalmoscopic method of rhodopsin measurement until recently, for the technique of Brindley & Willmer (1952) requires only such equipment as has been available for half a century. They were concerned to measure reflected fundal light chiefly in connexion with *in vivo* estimates of the macula lutea. They matched in a bipartite field the fundal light against a comparison light which could be varied by means of a photometric wedge. When light of wavelength 0·5μ was used with a dark-adapted subject they found that the initial setting very quickly became out of balance. This they attributed to bleaching of the rhodopsin of the retina by the fairly bright light required to make the match.

This partial success encouraged one of us to develop a photocell technique, and experiments were first performed in collaboration with Brindley upon cats (Rushton, 1952). These were soon abandoned when they were found to exhibit quick changes which superficially resembled rapid photo-chemical reactions, but did not have the corresponding action spectrum, and were tentatively attributed to reflex changes in the diffraction patterns upon which the characteristic colour of the tapetum is known to depend. The work was continued with Brindley upon the albino rabbit which has no diffracting tapetum and where none of this difficulty arose (Rushton, 1953*a*, *b*). The technique was improved in conjunction with Hagins (Hagins & Rushton, 1953) and though this work has not yet been published in detail, a recent description of the method and type of result obtainable (Rushton, Campbell, Hagins & Brindley, 1955) shows the power of the technique. There is no doubt that rhodopsin can be measured with considerable accuracy in the anaesthetized albino rabbit. This knowledge has encouraged us to proceed with the problem of the human eye whose smaller aperture and black fundus makes the technique a great deal harder. The principles of the method and summary of some results have already appeared in preliminary notes (Campbell & Rushton, 1954; Rushton & Campbell, 1954), and in an account appearing elsewhere (Rushton *et al.* 1955). Though all the experiments are different from those described here, the conclusions are similar.

METHOD

Principle

The density of retinal rhodopsin is to be measured by 'viewing' the *fundus oculi* with a photocell in green light. Any increase in rhodopsin density will absorb more green light and diminish the signal from the photocell. Since the change expected is very small it is important to eliminate any other factor which could vary the output of the photocell.

Principal factors are: (i) variation in the brightness of the light source; (ii) variation in the amplification of the photocell signal; (iii) small movements of the eye and head; (iv) 'noise'

variations of signal from moment to moment since the signal light is very weak and the amplification great. We have taken ordinary precautions in the first place to make these factors as small as practicable, but the apparatus is designed in addition to reduce the effects to second-order magnitudes.

Using the fact that rhodopsin is practically transparent to orange-red light we have balanced the photocell output in green light against the output in orange light. The balance point is practically unaffected by change (i) above, but it still measures directly the change in rhodopsin density. This device equally eliminates effect of variation (ii). The way that the green light is balanced against the orange is to apply the two lights in rapid alternation along the same path. In this way, though eye movements are always more or less troublesome, the shift applies equally to both lights, and the situation becomes manageable. Random 'noise' can always be reduced in relation to a regularly repeated signal by integrating over sufficient time. In the present case this is limited by the need for making measurements quickly in changing conditions. Our compromise is an integration period of 1 sec or so. In detail the apparatus is as follows:

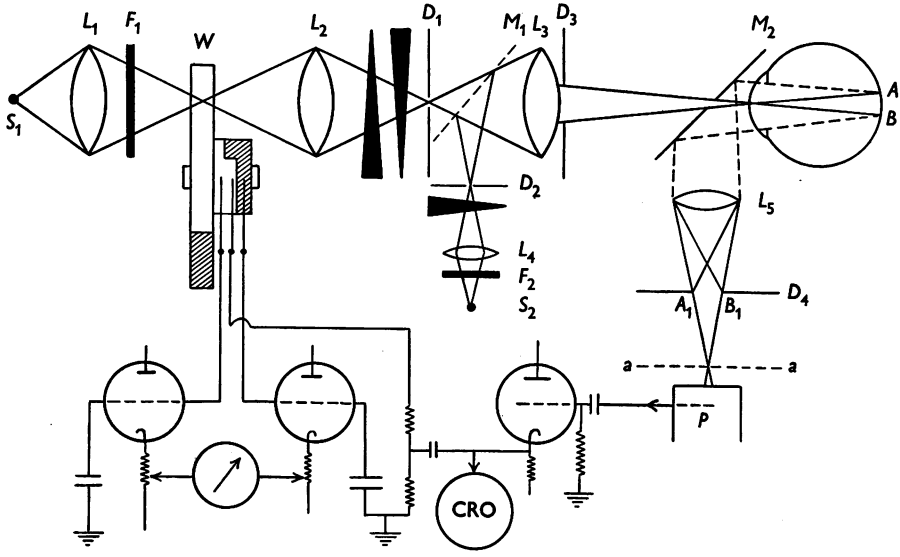

Fig. 1. Diagrammatic representation of the rhodopsinometer. Not to scale. See under Methods for description.

The rhodopsinometer (Fig. 1)

Light was focused on to the subject's pupil by the lens L_3 passing through the variable diaphragm D_3, and an ellipitical hole in the silvering of the mirror M_2, so that a 5° cone resulted and illumined a corresponding circular patch of retina. Light reflected at the choroid passed again through the retina and after reflexion at M_2 fell upon L_5. This was an f2, 50 mm camera lens attached to a Wrayflex camera, which formed an image of the illuminated patch of retina in its focal plane at D_4 (for the emmetropic eye). The variable diaphragm D_4 was diminished so that only about 2° in the centre of the 5° image was allowed to pass. In this way eye movements of 1° might occur without the image being lost.

The aperture D_4 not only selects precisely the desired region of illuminated retina, but it is a powerful excluder of stray light. For any light which is not parallel on entering L_5 will form a diffuse blurred image in the plane D_4 and only a fraction will be admitted through the small hole which, however, accepts *all* the light transmitted by L_5 from the 2° patch of retina.

An image of the cornea is formed at the plane a, a and by mounting L_5 upon a suitable extension tube from the camera, the cornea and pupil could be viewed through the reflex focusing system of the camera. This permitted exact adjustment and centring of the optical system. Considerable care is required to eliminate light reflected at the anterior surface of the cornea, for this is so bright that even after passing through D_4 it could swamp the faint signal from the retina. In most experiments it was excluded by suitably decentring the incident beam on the subject's pupil. On a few occasions it was excluded by placing a small piece of plasticine on a glass plate in the plane a, a adjusted so as to block the real image of the corneal reflex there. In this manner a fairly pure light signal from the illuminated patch of retina fell upon the cathode of a photo-multiplier tube P, placed just behind a, a.

In order to compare the photocell signals in orange and in blue-green light, the beam incident upon the eye flickered between these two colours by the following arrangement. The image of a compact filament lamp (6 V 36 W car head light) was focused by the lens L_1 upon a rotating transparent wheel W, one half of which transmitted orange and the other half blue-green by the incorporation of suitable Ilford filters (nos. 204, 623). The diverging beam is focused again by L_2 upon a small diaphragm D_1 with an aperture of 0·75 mm, and on the way it passes through a cyan colour wedge (Ilford, RA) and a purple colour wedge (Ilford GA). D_1 selects a portion of the filament which is uniformly bright and a small image (2 mm diameter) of aperture D_1 is formed in the plane of subject's pupil by the achromatic lens L_3. Movement of the coloured wedges permits a delicate adjustment of the relative intensities of the orange and blue-green beams, and in this way the alternating photocell outputs can be balanced. If bleaching removes some purple from the retina, the balance may be restored by introducing a corresponding increase of purple in the wedge. In this way the change in retinal rhodopsin is read directly by the change in purple wedge setting required to maintain the photocell balance.

Modern photocell sensitivity is the key to the success of the method. Very little of the light incident upon the retina can reach the photocell, and if the incident light is made too strong, the rhodopsin will be bleached away before it can be measured. In the earlier experiment we used an RCA 931 A, 9-stage photomultiplier tube specially selected for high sensitivity and low noise level. In later work we had an EMI 11-stage tube with improved performance.

The output of the photomultiplier after passing through a cathode follower stage was displayed upon a cathode-ray tube to monitor the level and nature of the performance. But signal-to-noise ratio was much improved by reading the balance after integration. We are indebted to Dr Frankenhaeuser of Stockholm for the following neat arrangement.

The output from the cathode follower was passed (Fig. 1) through a commutator mounted upon the shaft of the colour wheel. The contacts were arranged to connect the signal to one or other of two condensers. The change-over coincided with the change in colour of the light, so that one condenser acted as a reservoir for the output from the orange signal, the other from the blue-green. The time constant was about 1 sec, the rate of alternation about 20 c/s. The condensers were each joined to the grid of one of a pair of cathode followers between whose cathodes a sensitive galvanometer was connected. Equality of photocell output was detected by null deflexion of the galvanometer. Change from zero was immediately restored by a corresponding movement of the purple wedge, and in the experiments a zero setting took 5–7 sec to make.

In some experiments it was necessary to project a bright white or coloured light on to the part of the retina being investigated. This was achieved by means of an auxillary light source S_2 (36 W, 6 V car headlamp) with condensing lens L_4 and diaphragm D_2. This beam could be brought into line with the rest of the optical system by means of a mirror or mixing cube placed at 45° before lens L_3. The intensity or colour could be varied by placing filters and a neutral wedge in the pathway of the beam.

The brightness of the measuring and bleaching lamps was controlled and monitored by conventional means. Luminances were measured with an S.E.I. photometer which was calibrated at frequent intervals against a standard lamp calibrated by the National Physical Laboratory.

The subject's head was steadied by means of a dental wax mouth grip and a firm forehead rest.

The region of retina sampled was controlled by adjusting a red fixation mark at the required angle. Pupil dilatation was ensured by instilling homatropine hydrochloride 2% and Paradrene into the conjunctival sac.

Unless otherwise stated, W.A.H.R. was the subject using his left eye and F.W.C. was the observer.

RESULTS

If the apparatus described is capable of measuring the rhodopsin density in the retina at any moment, it should reveal characteristic changes in the course of light and dark adaptation. We shall first give one or two examples of such changes in order to make clear the sort of results obtained. We shall then assemble the evidence that it is rhodopsin that we measure by a more exact investigation of bleaching as a function of time, of wavelength and of retinal position.

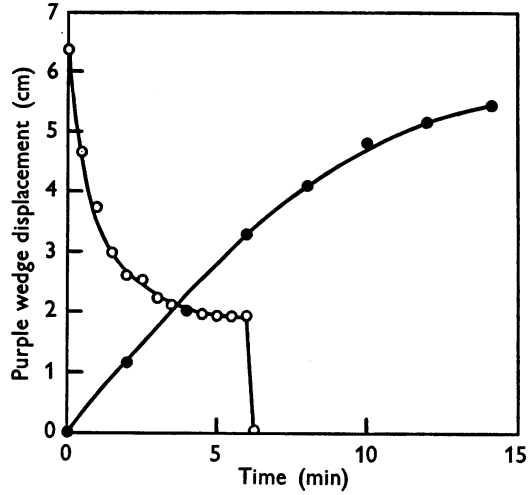

Fig. 2. Time course of bleaching (○) and subsequent dark regeneration (●) of retina. Intensity of bleaching light 50,000 trolands, ending with a very bright flash at 6 min.

The simplest way to measure the time course of bleaching is to use the measuring light so strong that it will itself perform some bleaching. In this way the sensitivity of the measurements is high, and no manipulation has to be made during the course of bleaching except to move the purple wedge so as to keep the null galvanometer in balance. Fig. 2 gives the result of such an experiment.

The ordinates are plotted in cm change in the balance position of the purple wedge: the absolute value is arbitrary. The eye had been dark adapted for about 15 min, and at zero time the bright measuring light fell upon a region of the retina 15° temporal to the fovea. Fixation was maintained throughout the entire bleaching. It is seen (white circles) that the wedge is shifted at first fast

and later more slowly, reaching equilibrium in about 5 min. The direction in which it is shifted introduces more purple into the light path, i.e. it would compensate for removal of purple from the retina.

Now when rhodopsin in a glass cell is bleached by exposure to a constant light, the fall in density is at first rapid and later slower as the solution becomes progressively bleached and hence less capable of absorbing further bleaching light. The curve should be almost exactly an exponential decay. Such a curve describes well enough the bleaching in Fig. 2, but in the living eye there is more than in a glass cell, for regeneration occurs, and equilibrium will be reached, not when all the rhodopsin is bleached away, but when the rate of break-down is equal to the rate of regeneration. If this were the condition at equilibrium in Fig. 2, the amount of rhodopsin still present should be revealed by suddenly applying a very bright flash to the eye. This was done and resulted in a further substantial drop in wedge setting.

The black circles show the dark regeneration curve which followed after a short interval (which included a further bleaching flash). The curve is plotted starting again at zero time for compactness of representation. In this case the subject remained with his eyes closed (but with head still held in position), except for a brief period of about 5 sec every 2 min. At that time he was instructed to open his eyes and fixate, and a measurement was taken with the same strong measuring light which caused the exponential bleaching curve. After 14 min most of the rhodopsin appears to have regenerated, and somewhat more would no doubt have done so but for the brief bleachings every 2 min. It will be recollected that the whole experiment began after 15 min dark adaptation, and so the return towards the initial conditions is satisfactory.

Fig. 3 shows the results of somewhat different conditions of light adaptation. In this case the *measuring* light was 0·1 the brightness of the former case. Bleaching was performed by white light from the side arm, applied by swinging the mirror M_1 (Fig. 1) into the optic axis. The bleaching light was applied for 23 sec and the measuring light for 7 sec in each half minute. Three different bleaching strengths were used, 1, 5 and 100 units of intensity. Each causes a bleaching of exponential type to reach an equilibrium where the rate of bleaching balanced the regeneration rate. The dark regeneration curve is similar to that of Fig. 2, though in Fig. 3 the measuring light was much weaker. Both curves are exponential in form and return half way to full regeneration in 6 min.

The results shown in Figs. 2 and 3 appear consistent with the belief that we are measuring the level of retinal rhodopsin in the various conditions of light and dark adaptation described, and we have interpreted the curves in this way in order to make the facts readily comprehended. But it is the facts of the curves, not our interpretation which we wish to stress at this stage. Our analysis follows.

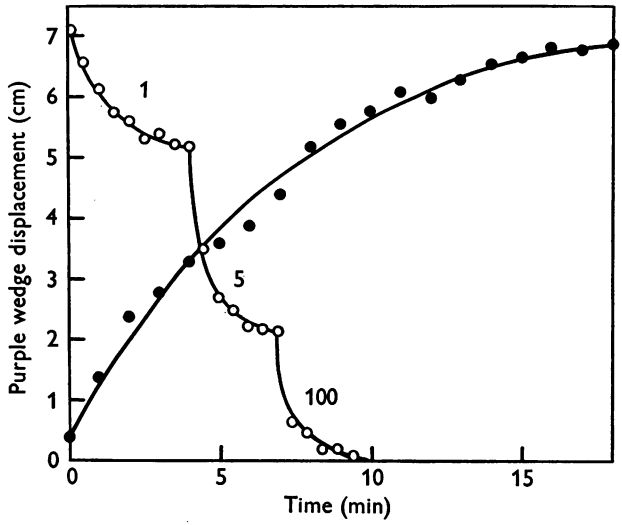

Fig. 3. ○—○, effect of bleaching the dark adapted retina with lights of intensities 1, 5 and 100 units (1 unit = 20,000 trolands). ●—●; dark regeneration time course.

A. *The Bunsen-Roscoe law*

Any process described by the bleaching curves of Figs. 2 and 3 is bound to exhibit some kind of inverse relation between the intensity of the bleaching light and the duration of its application. The present experiments were performed to find how accurate is the relation

$$It = \text{constant}$$

and over what range this holds.

The method was to dark-adapt for 15 min and fixate so that the flash always fell exactly upon the same region of the retina. The wedge balance point was measured a few times initially (using a rather weak measuring light to avoid bleaching), then the mirror (M_1, Fig. 1) was thrown in to cut off the measuring light and permit bleaching from the side arm. The bleaching light was then applied for the required time and finally the wedge balance was again measured. The subject then dark-adapted for 15 min and the procedure was repeated with a change in the value of I and an inverse change in t so that $It = k$.

The bleaching intensity was varied by neutral filters placed in the path of the bleaching light whose steady intensity was monitored by a photocell. The shorter exposure times were controlled by a shutter consisting of slots in a disk on the axis of a synchronous clock mechanism (4 rev/min).

The results of one experiment are set out in Table 1 in which (except for the line at the bottom) the values of It are kept constant throughout at 10^7 troland-sec. The first column gives the exposure time (the intensity varying

inversely). The next two columns give the actual wedge settings as they were taken before and again after the bleaching exposure. The initial measurements show slight bleaching by the measuring light since repeated readings tend to rise. The final readings likewise tend to fall, indicating a little regeneration. We have therefore in general taken for the difference, tabulated in the last column, not the means of the readings but the last reading before bleaching and the first after it. Though the results have been entered in order of increasing exposures, the measurements were obtained in an irregular order over

TABLE 1. Rhodopsin bleaching due to a given total energy of white light delivered in various exposures.

t (sec)	Wedge settings (mm) Initial			Final			Difference
0·3	24	28	28	71	70	70	43
	32	32	33	—	75	—	42
3·0	24	25	27	69	68	66	42
	31	31	31	74	74	—	43
30	30	30	31	73	70	68	42
	29	31	31	74	72	—	43
48	33	33	—	74	73	—	41
90	27	28	29	66	65	—	37
	35	35	—	73	—	—	38
300	35	36	—	66	—	—	30
	42	41	—	73	73	72	31
48	32	33	34	87	—	—	53

The difference column gives the change in setting immediately before and after the exposure.

a large part of the day. So the repeated results represent a stability of measurement over several hours. It will be seen that the difference upon bleaching is more constant than the initial values of wedge setting. Very small movements of the head in its rest produce a few mm fluctuation. This can be controlled when the subject has simply to remain in position for a rather short time, but there will almost inevitably be some change when he returns after 15 min dark adaptation.

The figures in Table 1 show that $It=k$ holds fairly exactly for times up to 48 sec. A deviation is perhaps occurring here, by 90 sec the net bleaching is definitely less, and there is a marked deficit at 300 sec.

If the figure 43 for the change on bleaching represented the entire bleaching of which the retina was capable, then the fact that several of the bleaching exposures gave this value would not prove that they were equally effective but only that they all were effective enough to bleach entirely. This interpretation is excluded by the final entry at the foot of Table 1, where it is seen that a tenfold stronger bleaching exposure $It=10^8$ troland-sec produces a change of 53. So 43 cannot represent total bleaching, and hence equal changes in the table signify equal effectiveness of the bleaching exposure.

B. *The spectral sensitivity*

In the study of the visual pigments, by far the most common and satisfactory way of identifying the pigment involved is to determine its absorption spectrum. Our apparatus with its orange and green filters fixed in the colour wheel, does not permit us very readily to measure the absorption spectrum, but it is possible to investigate *the action spectrum*, that is, the relative efficacy with which lights of different wavelengths produce their effect in conditions similar to those considered in the previous section. The action spectrum is generally far harder than the absorption spectrum to determine, and involves some careful energy calibrations. Moreover, any application to the human eye requires an estimate at each wavelength of the transmission losses through the eye media of the subject in question. Both these difficulties may be neatly circumvented in the present case by the following considerations.

It is generally admitted that the conditions for two scotopic fields to appear equally bright is that they should be equally effective in bleaching the rhodopsin in the retina. Thus we have only to make a scotopic match of various spectral colours to secure at once the condition that they should bleach equally. By removing neutral filters from the path of the matched lights they are increased in brightness to a level where they may cause substantial bleaching. If then rhodopsin is what we measure, the two lights should cause an equal change in our wedge setting. It will be observed that since the scotopic match is made by the subject of the subsequent bleaching experiment, the method is self-compensating for transmission losses through the eye media.

The conditions of bleaching were similar to the experiments in section A, but in the side path of the bleaching light was a neutral wedge and one of the set of Ilford Spectrum filters. After 12 min dark adaptation the subject made a scotopic match of the colour to be used in the next bleaching measurement. This was carried out by standing away from the apparatus so the unobstructed bleaching beam passed across the room in a 5° cone and, much attenuated, fell upon a normal surface of white card. A similar surface at right angles received the light from a weak comparison source, and the subject matched the two by viewing each from 45° and using about 20° of surface as matching field. The operator adjusted the neutral wedge in the path of the spectral bleaching light and determined the position where the subject judged the fields to be equal (correct to density 0·05).

The light as received upon the white card was above the scotopic level and the subject made the match looking through two density 1·0 'neutral' filters held close to the eye. To correct for non-neutrality, separate measurements using the light of each spectral filter were made of the relative density of the 'neutrals', and of the reflectivity of the white card as compared with MgO freshly deposited upon a mirror. The operator made the appropriate correction

for non-neutrality thus measured, when he set the final adjustments of the bleaching lights.

The procedure for bleaching was much the same as in section A. Initial dark-adapted readings of the purple wedge were taken, then the matched intensity of light was exposed for 30 sec and the change in wedge balance determined. Finally, in most cases a further 60 sec of bleaching was given with the same light and the total change of wedge setting found.

Table 2 gives one experiment where conditions were fairly stable and repeatable. The colours were selected in an irregular order to prevent progressive changes appearing as a regular function of wave length. But in fact progressive changes were not noticeable as appears from duplicate determinations which were made at an interval of some hours from one another.

TABLE 2. Rhodopsin bleaching due to two fixed exposures of various monochromatic lights whose relative intensities had been matched to give an equal scotopic brightness.

Filter in bleaching light		Difference after 30 sec	Total
601	Violet	34	44
602	Blue	39	47
603	Blue-green	33	44
604	Green	38	—
		36	45
605	Yellow-green	31	48
		34	45
606	Yellow	38	—
		35	42
607	Orange	31	41
	White	40	48
604	(−0·2 Neutral)	43	—
		42	—
604	(+0·2 Neutral)	27	—

Bleaching with a strong light gives a total change 55

The first column of Table 2 gives the number of the Ilford Spectral filter with its colour. The transmissions of these filters in slightly different conditions of illumination are shown in Fig. 5 of Denton & Pirenne (1954). The actual wedge settings (which are not included in Table 2) exhibited just about the same consistency and scatter as in Table 1. The second column shows the change in wedge setting after 30 sec bleaching, the third column the total change after a further 60 sec bleaching. All the 30 sec bleaching values lie within the extremes of 31 and 40 mm of wedge, and the question is what change in light intensity would be responsible for this variation. At the foot of the table are shown the results of bleaching with green light in which a density of 0·2 was added to or subracted from the standard matched level. It is seen that these values lie well outside the extremes of bleaching with matched light of any colour. Moreover, the variations which do appear among the different colours cannot be held significant, since the extremes of the list, orange, blue and white, gave, as it happened, bleaching values identical with

each other in a comparable experiment 4 days previously. It seems, therefore, reasonable to conclude that the change we measure has the same action spectrum as scotopic visibility to well within 0·2 density unit over the whole spectrum.

C. *The retinal distribution*

It is well known that rhodopsin is found only in the rods, and that the distribution of rods in the human retina is far from uniform; in particular there are none upon the fovea or the optic disk. If then the change we measure represents the bleaching of rhodopsin, it should be absent from those two special regions and elsewhere it might be expected to go somewhat hand-in-hand with the rod distribution.

In Fig. 2 of a preliminary publication (Rushton & Campbell, 1954) we showed the well-defined exponential time course of bleaching which is obtained by a steady light applied to a point 15° temporal to the fovea (similar to Fig. 2 of this paper). We also showed that when the light was applied to the fovea or optic disk and a 1° patch measured in the centre of each of these regions, there was no change in wedge setting—a 1 or 2 mm fluctuation instead of the 50 mm change.

We have used this method systematically in a series of retinal locations along the horizontal meridian. For each point the bleaching curve was obtained and from such curves the retinal content of rhodopsin determined. This was performed upon two subjects and gave more or less the same distribution; the distribution for one of them (W.A.H.R.) is appearing elsewhere (fig. 8, Rushton *et al.* 1955) and is also included in Fig. 4 of the present paper.

There are several difficulties in using the moderate bleaching lights and 3 min exposures necessary for determining the time course of bleaching. For instance, it is not easy to maintain good steady fixation throughout 3 min with eyes deviated 40° from their normal position. The method of short bright exposures in sections A and B, on the other hand, gave such consistent results that we decided to repeat the measurements of retinal distribution of rhodopsin along the horizontal meridian using this technique. The details are as follows.

After a preliminary dark adaptation period of 15 min the subject fixated upon a red light placed at a measured angle from the bleaching-measuring axis. The camera-photocell equipment had now to be brought into adjustment so that it would receive light from the centre of the 4° illuminated retinal patch without contamination from corneal reflex, etc. The adjustment was made in the light of the beam passing through the orange sector of the stationary colour wheel. This light had been shown not to bleach appreciably. The camera was correctly aligned, and the diaphragm in the retinal image plane (D_4, Fig. 1) stopped down to admit 1½° in the centre of the bright 4° retinal image.

After 5 min further dark adaptation the usual sequence of operations for flash bleaching were made. First a few wedge measurements were taken using a fairly weak measuring light, then the very bright 3 sec flash was applied and finally a few more wedge measurements were taken.

The fixation point was then moved by at least 30° and the adjustment to the new position begun at once. Preliminary measurements had established that very intense bleaching at one

point did not affect detectably the wedge setting at a point a few degrees distant, so we did not wait to dark-adapt when passing from one point to another at some distance from it. In this way forty determinations could be made at one session. The bleaching flash was strong enough to produce 95% of the change that could be produced by keeping the light on for much longer than 3 sec.

In Fig. 4 the black circles show the results obtained by this technique, the white circles by the earlier technique. The two sets are in reasonable agreement. Both show that no bleaching occurs at the fovea or optic disk, and in other places the amount depends in a characteristic way upon the distance

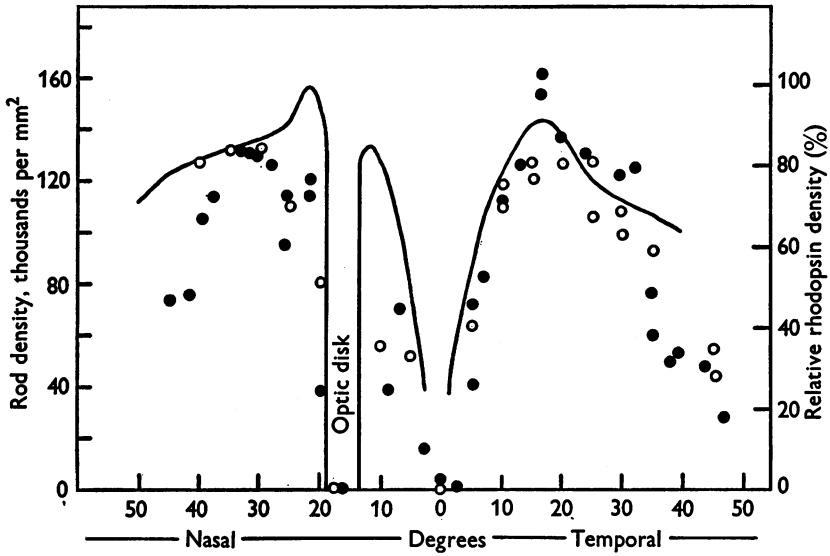

Fig. 4. Relative amounts of bleaching obtained along the horizontal meridian. ○, measurements obtained by steady bleaching; ●, measurements obtained by bright flashes. Continuous line is the rod count per sq.mm along the horizontal meridian of the human eye as published by Østerberg (1935).

from the fovea. It is clearly relevant to ask how does the distribution of *rods* depend upon the distance from the fovea. The authority upon this is Østerberg (1935) and the curve shown in Fig. 4 is taken from that source as replotted by Pirenne (1948) in terms of angular distance from the fovea instead of mm along the retina. Østerberg's material was from a fresh human retina, and the curves record the rod density at various points along the horizontal meridian by actual rod counts of a large number of sample areas.

Contours of equal density were found to be roughly circles with the fovea as centre (the region of the optic disk excepted). This has justified Østerberg in composing his curve out of counts which did not always lie upon the horizontal meridian, but deviated as much as 30°. We too, have deviated for readings beyond 35° temporal where the fixation light could not conveniently be seen in the horizontal plane, and those results lie upon the meridian inclined upwards about 40°.

It is clear that there is some correlation between our results and the rod density distribution of Østerberg. If we had reason to believe that we were measuring rhodopsin density then it would be natural to expect that where the rods were dense we should find great bleaching, where sparse we should bleach little and where absent we should not bleach at all. These conditions are seen to be fulfilled.

DISCUSSION

Section A established that the Bunsen–Roscoe law holds for the change we measure for durations of light exposure up to about 48 sec. The direction of change in wedge setting for balance can be interpreted in three ways. Either some purple pigment is removed, or some blue pigment is generated, or some quite other kind of change occurs.

The first is consistent with what Abelsdorff saw in the eyes of crocodiles, and what many people following Kühne have seen in the dark adapted retinas of all kinds of animals when excised and exposed to light. No one has ever seen a blue pigment develop as a result of 'bleaching', and we do not consider it worth while entertaining this possibility.

The only other likely kind of interpretation which could be given to our results is that they are due to the *movement* of some part of our optical system. An important observation in this connexion is that though a 3° bleaching patch changes the wedge setting as measured in that patch, it has no effect upon measurements made a few degrees outside the patch. This experiment may be performed simply as follows. Two fixation points are set up 6° apart, and preliminary adjustments secure that measurements may be satisfactorily obtained from either position. Then wedge readings are made from each retinal region both initially and after bleaching one region only. It is found that light far stronger than that which 'bleaches' the first entirely, is without appreciable effect upon the second.

It follows that if the wedge change on 'bleaching' is due to something moving, this must be localized upon the part of the retina 'bleached'. Thus movement of head, eyes, iris and lens etc. are each excluded (even if their apparent immobilization is distrusted). We are left with the possible movement of the pigment epithelium or of the receptors themselves which, though thought not to occur in mammals, certainly occurs in some vertebrates. The stimulus for this movement must be some photochemical change which obeys the Bunsen–Roscoe law. What this change is we can learn from the results of section B.

'Bleaching' with lights of different wavelengths showed that lights of equal scotopic visibility produced equal changes in wedge settings. From this we must conclude that it is the absorption of light by rhodopsin which causes the photochemical reaction producing the change we measure.

If this change were the movement of the pigment epithelium, it does not accord with Kühne's (1877) observation in the frog that the movement, so far

from being provoked by rhodopsin absorption, was stimulated by red light even more than by white. Nor is it easy to suppose that movement whether of epithelial or receptor cells could take place so rapidly that in a 0·3 sec flash the whole change has been made and is beginning to return by the time—a few seconds later—that a measurement can be taken. Moreover, it is by no means clear why the effect of such a movement should be equivalent to the removal of a purple pigment.

Up to this point we have not sought the support which can be derived from animal experiments. But the measurements which Brindley, Hagins and Rushton have performed with the albino rabbit, and Weale (1953) with the cat, greatly strengthen the case that the change we measure not only is produced by rhodopsin bleaching, but in fact *is* the rhodopsin bleaching.

The description of one such experiment (Rushton *et al.* 1955) shows the change in the light reflected back from the eye after bleaching. The method allowed measurement to be taken in light of different wavelengths so that a difference spectrum could be obtained, and as Fig. 3 of that paper shows, the change which occurs on bleaching has the difference spectrum of rhodopsin. In the rabbit therefore the change in reflected light certainly represents the bleaching of rhodopsin, and strengthens the case that in the human eye it does so too.

We may now turn to section C and speak with more confidence of Fig. 4 as a plot of the rhodopsin distribution in the retina. But this interpretation immediately raises the question 'Why is the rhodopsin density as measured, then, not proportional to the rod density of Østerberg?' There is no reason why it should be. If there were no stray light and none reflected back except through the rods, the density as measured would be just the density in the rods independent of the number of rods per mm^2. If the light is reflected just as readily between the rods as through them (in the bleached state) then Østerberg's curves and ours should coincide. If from between the rods light is reflected more strongly than through the rods, then our curve should deviate from Østerberg's in the way it does. But so many factors could enter into the interpretation of measurements of this kind that at present it is not worth developing this analysis.

We conclude that we measure light passing through the rods, diluted with 'stray' light that has not passed through the rods. The more concentrated the rods the less is the rhodopsin signal diluted, and thus our distribution is similar to Østerberg's, though not necessarily proportional to it.

There is, however, one region of the retina which deserves special mention. It will be observed that in the neighbourhood of the optic disk our rhodopsin values are exceptionally small. If 10° temporal is compared with 10° nasal, it is seen that both have about the same rod density, but on the nasal side the rhodopsin density appears to be only half the value of the temporal side. Now

we know that from the disk, there is a great outflow of nerve fibres which spread out above the rod population. It would seem inevitable that some of the light falling upon these fibres must be reflected and scattered back, and hence the signal from this region will be diluted with extra stray light. The choroid is very black, but the nerve fibres are rather white so that it does not seem unreasonable to suppose that as much light is reflected from the nerves as from

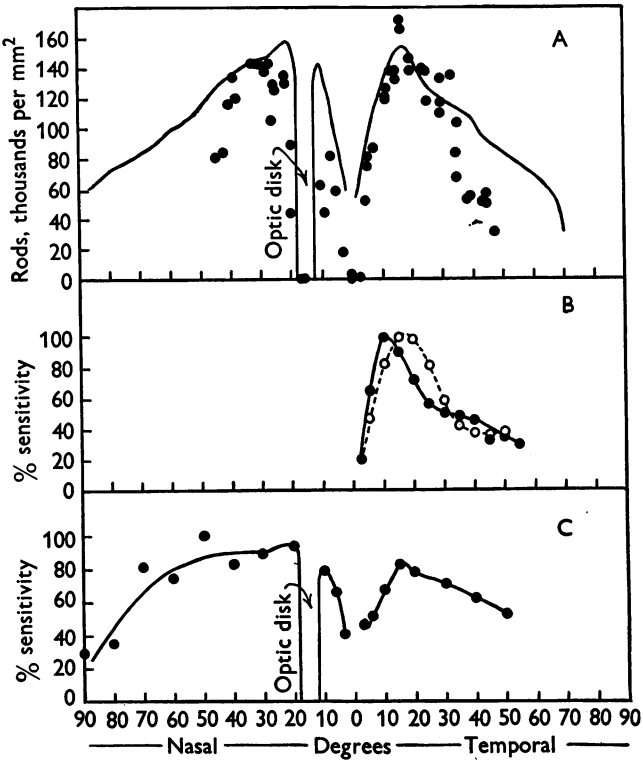

Fig. 5. A. Fig. 4 replotted. B. Relative sensitivity of the dark adapted eye along the horizontal temporal meridian as measured by Stiles & Crawford (1937) on two subjects using a 0·072° test spot flashed for 0·05 sec. C. Relative sensitivity of the scotopic eye as measured by Sloan (1950) using a 1° test spot for 1 sec.

the pigment—less than one per cent of incident light in each case. If this were so, it would account for the half value we find for the density in this neighbourhood.

The alternative interpretation, that rods near the disk have an unusually low rhodopsin content, does not agree well with measurements of absolute rod threshold. Many workers who have measured the absolute threshold of various regions of the retina have considered the relation to rod density. Ten Doesschate (1949) discusses this specifically and relates the threshold observations of

Stiles & Crawford (1937) to the rod density measurements of Østerberg, and Sloan (1950) has carried out an independent set of measurements upon absolute threshold to establish the same relation.

Now if the rods near the disk contain only half the normal amount of rhodopsin, we should expect that the threshold for 10° nasal on the retina should be about twice that of 10° temporal. If, on the other hand, the nasal rods contained the normal amount but were veiled by nerve fibres which allowed some 99% of the light to pass, we should expect the thresholds at the two places to be about the same. And this is what Sloan has found to be the case.

Fig. 5A is a replot of our Fig. 4 and C gives Sloan's results and B Stiles & Crawford's as corrected by ten Doesschate. It is to be remarked that Sloan used a 1° test spot flashed on for 1 sec; Stiles & Crawford used a 0·072° spot flashed for 0·05 sec; thus if the periphery of the retina has greater summation area and summation time than the more central region, Sloan's sensitivity curves would be raised from this cause and despite her closer agreement with the Østerberg counts, Stiles & Crawford should give a closer agreement with the rhodopsin density, as in fact they do with our values. However, when we consider the difficulties, the possible sources of error and the individual variations in all these determinations, it is not the minor divergences which strike us so much as the surprising fact that there is hardly a 2:1 discrepancy in the whole range of these varied observations.

SUMMARY

1. This paper describes a technique devised to enable the density of rhodopsin in the human eye to be measured.

2. It uses the principle that when the fundus oculi is observed in an ophthalmoscope, it is seen by light which has passed twice through the retina. If the viewing light is green it will be attenuated from absorption by retinal rhodopsin, so by measuring the attenuation the amount of rhodopsin may be estimated.

3. Since a measurement may be performed in 7 sec or less, the change in rhodopsin density may be followed throughout the course of light or dark adaptation. In the light the density falls and that more rapidly the stronger the light. It rises again gradually in the dark taking some 6 min for half return.

4. Quantitative measurements on the effects of bleaching show that the Bunsen–Roscoe law ($It = k$) holds for exposures up to 48 sec. If monochromatic bleaching lights are used, the effect of every colour is the same if the lights have previously been adjusted to have the same scotopic brightness.

5. Measurements performed upon different regions of the retina show that

there is no effect of bleaching at the fovea or optic disk, and that the size of the effect in any region depends in a characteristic way upon the distance from the fovea. A very similar dependence has previously been found for the distribution of rod density and for the absolute threshold of vision (see Fig. 5).

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REFERENCES

ABELSDORFF, G. (1895). Ueber die Erkennbarkeit des Sehpurpurs von *Abramis Brama* mit Hülfe des Augenspiegels. *S.B. preuss. Akad. Wiss.* pp. 325–329.

ABELSDORFF, G. (1897). Die ophthalmoskopische Erkennbarkeit des Sehpurpurs. *Z. Psychol. Physiol. Sinnesorg.* **14**, 77–90.

ABELSDORFF, G. (1898). Physiologische Beobachtungen am Auge der Krokodile. *Arch. Anat. Physiol.* (*Physiol. Abt.*), pp. 155–166.

BRINDLEY, G. S. & WILLMER, E. N. (1952). The reflexion of light from the macular and the peripheral fundus oculi in man. *J. Physiol.* **116**, 350–356.

CAMPBELL, F. W. & RUSHTON, W. A. H. (1954). The measurement of rhodopsin in the human eye. *J. Physiol.* **126**, 36–37*P*.

DENTON, E. J. & PIRENNE, M. H. (1954). The absolute sensitivity and functional stability of the human eye. *J. Physiol.* **123**, 417–442.

TEN DOESSCHATE, J. (1949). Extra-foveal scotopic absolute threshold and the distribution of retinal rods. *Ophthalmologica, Basel*, **117**, 110–115.

HAGINS, W. A. & RUSHTON, W. A. H. (1953). The measurement of rhodopsin in the decerebrate albino rabbit. *J. Physiol.* **120**, 61*P*.

KÜHNE, W. (1877). Zur Photochemie der Netzhaut. *Unters. physiol. Inst. Heidelb*, **1**, 1–14.

ØSTERBERG, G. (1935). Topography of the layer of rods and cones in the human retina. *Acta ophthal. Kbh.*, supp. **6**.

PIRENNE, M. H. (1948). *Vision and the eye*, fig. 29, p. 30. London: Chapman and Hall.

RUSHTON, W. A. H. (1952). Apparatus for analysing the light reflected from the eye of the cat. *J. Physiol.* **117**, 47–48*P*.

RUSHTON, W. A. H. (1953*a*). Aspects of retinal physiology. *Publicações do Instituto de Biofísica*, Rio de Janeiro.

RUSHTON, W. A. H. (1953*b*). The measurement of rhodopsin in the living eye. *Acta physiol. scand.* **29**, 16–18.

RUSHTON, W. A. H. & CAMPBELL, F. W. (1954). The measurement of rhodopsin in the living human eye. *Nature, Lond.*, **174**, 1096.

RUSHTON, W. A. H., CAMPBELL, F. W., HAGINS, W. A. & BRINDLEY, G. S. (1955). The bleaching and regeneration of rhodopsin in the living eye of the albino rabbit and of man. *Optica acta*, **1**, 183–190.

SLOAN, L. L. (1950). The threshold gradients of the rods and the cones in the dark-adapted and in the partially light-adapted eye. *Amer. J. Ophthal.* **33**, 1077–1089.

STILES, W. S. & CRAWFORD, B. H. (1937). The effect of a glaring light source on extrafoveal vision. *Proc. Roy. Soc.* B, **122**, 255–280.

WEALE, R. A. (1953). Photochemical reactions in the living cat's retina. *J. Physiol.* **122**, 322–331.