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RHODOPSIN MEASUREMENT AND DARK-ADAPTATION IN A SUBJECT DEFICIENT IN CONE VISION

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This paper is the third in a succession, of which the first (Rushton, 1961) will be referred to as paper I, the second (Fuortes, Gunkel & Rushton, 1961) as paper II. In paper II we described some studies upon a photanope—a subject whose photopic vision was very defective, but whose rod vision appeared to be normal. In particular, the increment threshold, obtained from a retinal region distant 7° from the fovea, coincided with the results of Aguilar & Stiles (1954), obtained from normal eyes when special precautions were taken to exclude the contribution from the cones. Now a subject with normal rods, but practically no cone responses, might be expected to give very valuable information upon the relation between log. sensitivity during the course of dark-adaptation and the regeneration of rhodopsin.

In paper I the course of rhodopsin regeneration was measured in the normal eye, and was found to develop according to a monomolecular reaction with time of half regeneration 4 or 5 min. It was seen, moreover, that after substantial bleaches of various amounts, when rod vision first appeared (at about 2 log. units above the final threshold I_0), rhodopsin was always about 90 % regenerated. It was argued that there was a fixed relation between the threshold I and the fraction of opsin still uncombined with retinene—in fact, that this fraction was proportional to log. (I/I_0).

It was impossible, however, to test this relation experimentally over a range greater than 10 % of bleaching, since this already causes the rod threshold to rise to the level of that for the cones, and with further bleaching all vision appears to be cone vision and rod thresholds ascend upon a curve of conjecture somewhere in the space above the horizontal cone branch.

But a subject without cones would not in this way conceal the ascending rod curve, and we might hope to follow rod dark-adaptation through a much greater range. This hope has been fulfilled, and we have been able to

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plot a rod dark-adaptation curve over a range of nearly 7 log. units and to correlate this with rhodopsin regeneration measured physically upon the same subject.

MEASUREMENT OF VISUAL PIGMENT

Rhodopsin

The apparatus and principles of measurement were those already described in paper I, and the procedure was very similar. The subject, with fully dilated pupil, was aligned in the densitometer and held in position by a dental impression and a moulded forehead rest. After remaining in the dark for 30 min she resumed her position in the densitometer, and wedge settings were taken to give the value corresponding to the fully regenerated condition. Then the subject turned her head and received the bleaching exposure. This was a 20° Maxwellian field of 10^6 td for 30 sec, which should bleach 97 % of the rhodopsin in the retinal area involved (see formula 1, paper I).

The subject tolerated this well, and was seen neither to blink much nor shift her gaze from the fixation point placed just outside the Maxwellian lens. She returned at once to position in the densitometer and fixated upon the small red light (always recognized as 'red') situated 12° from the region measured. This region was a 3° circle near the centre of the 20° area bleached.

The subject remained in position for the next 40 min with eyes closed except for brief periods of about 7 sec during which wedge settings were being taken. At the end of 40 min no further regeneration of pigment occurred, and the initial full dark level had been reached.

Rhodopsin measurements with our photanope were not so self-consistent as with the best of our normal subjects. This may have been due to her difficulty in maintaining accurate fixation, for her central acuity was poor, and there persisted a tiny residue of her earlier nystagmus. The irregularities are greatest within the first few minutes after bleaching, and it is probable that this enormously bright light left a temporary impairment of steady fixation, as indeed for a few seconds it does with normal subjects.

The filled circles of Fig. 1 show one such measurement of rhodopsin regeneration in the photanope; the open circles show it in the normal subject. Both ordinates are expressed as fraction of total rhodopsin present, and the actual amounts of rhodopsin, as measured by change in wedge setting after a full bleach, do not differ by more than 10 % from one to the other.

We may conclude, therefore, that the photanope has about the normal amount of rhodopsin in the retina at 12° temporal to the fovea and that it regenerates at the normal rate.

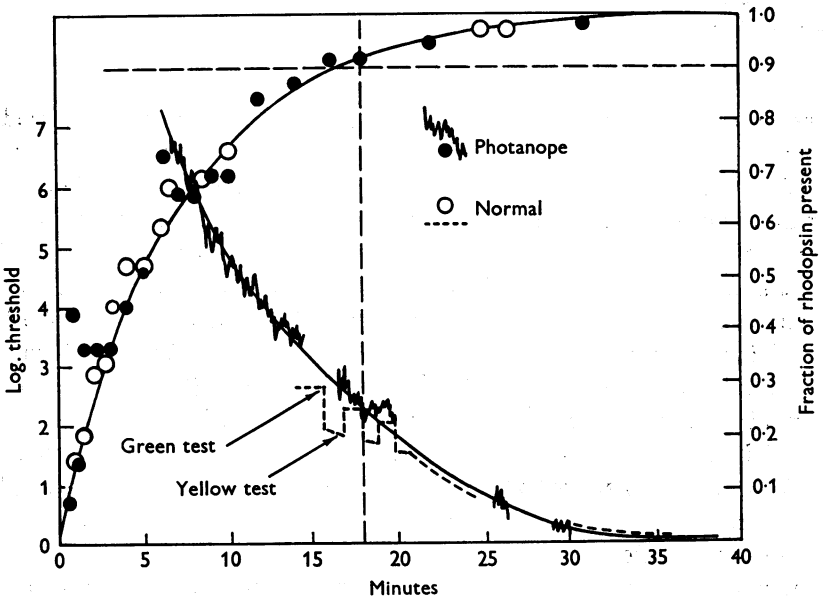

Fig. 1. ○, regeneration of rhodopsin in a normal subject; ●, in the photanope. Irregular curve, dark-adaptation curve of the photanope; interrupted curve, the same experiment with a normal subject.

Foveal pigment

An attempt was made to measure any visual pigment that there might be upon the photanope fovea by means of a 2° measuring field centrally fixated. The bleaching exposure was for 30 sec to a white light of 10^6 td, which should have bleached all the cone pigments present, but measurements with lights of 500, 540 and 580 mμ all failed to detect reliably any change in foveal reflectivity. These central measurements were less steady than the peripheral rhodopsin values, because the macula lutea and foveal reflex emphasize irregularities due to eye movements. Moreover, cone pigments regenerate fast, and thus can only be detected within a minute or two of bleaching, and this is just the period where that intense light leaves irregular fixation.

Despite these uncertainties, an amount of pigment corresponding to 10% of that present upon the normal fovea could perhaps have been detected—20% with some confidence. Neither rhodopsin nor cone pigments were found in this amount, nor indeed was it to be expected, judging by visual function. For our subject had an acuity of 20/80, which might correspond to a coarsening of the mosaic grain of the fovea by $\frac{1}{4}$, linear, or $\frac{1}{16}$ by area. Thus, if all the cones present had normal pigment,

the total pigment change would only be $\frac{1}{16}$ of normal, which in this subject certainly could not have been seen.

DARK-ADAPTATION

The apparatus was Gunkel & Bornschein's (1957) adaptometer, used as described in paper I. The subject traced out her own curve by continually reversing a motor driving a photometric wedge so that the test light alternated between being seen and not seen; thus the tracing appeared as a saw-tooth curve oscillating about the curve of true threshold. In the normal subject the cone branch of the dark-adaptation curve can be distinguished from the rod branch by changing the wave-length of the test flash. This was shown in Fig. 1 of paper I, where the test flash alternated each minute between yellow (580 mμ) and green (520 mμ) of equal scotopic intensity. The rods, therefore, could not distinguish between the two test lights and the rod curve was smooth, but the cones were some three times more sensitive to the yellow than the green. Consequently, the cone threshold jumped half a log. unit at each change of light, and the cone curve presented a castellated appearance.

Now we have seen in paper II that our photanope, D.M., has a cone of spectral sensitivity rather similar to that of normal cones and very different from that of normal rods; thus her dark-adaptation curve traced by the alternating test-colour technique would be expected to show a smooth rod branch and a castellated cone branch. Figure 2 shows that this is the case. The curves are the actual tracings made by the photanope in two experiments from the same sitting, following different bleaching exposures. Each curve exhibits an initial castellated cone branch where the yellow flash is far more effective than the green, and where very little fall in threshold is seen. The second branch *per contra* shows no castellation in any part (though the alternation of flash colour continued throughout the whole curve) and the fall in threshold for a green flash covered about 6 log. units.

If we are correct in interpreting the tracings as cone and rod branches, we should expect the first branch to show a Stiles–Crawford effect, and the second branch to show none. This, in fact, was confirmed in a separate experiment.

The subject was aligned in the apparatus shown in Fig. 1 of paper II, with the background field abolished by an opaque screen at F_1. After full bleaching the fairly constant cone threshold was measured during the first 5 min of dark-adaptation with the point of entry alternately through the centre of the pupil or through the edge, as at A or B (inset) Fig. 2 of paper II. It was found that there was a Stiles–Crawford effect during the first few minutes, though only about 0·2 log. units in magnitude (consistent

with 0·5 for the double value found in paper II). Upon the rod branch, on the other hand, no Stiles–Crawford effect was detectable.

The great range of dark-adaptation shown by the rods in Fig. 2 suggests a possible source of error. If the retinal area exposed to the bleaching light is limited (it was a 20° circle in our case), the rods outside this area will not be bleached, and hence will remain fairly dark-adapted and capable of being excited by stray light if sufficiently intense. Now it will be observed that when rods first appear in Fig. 2 their threshold is raised

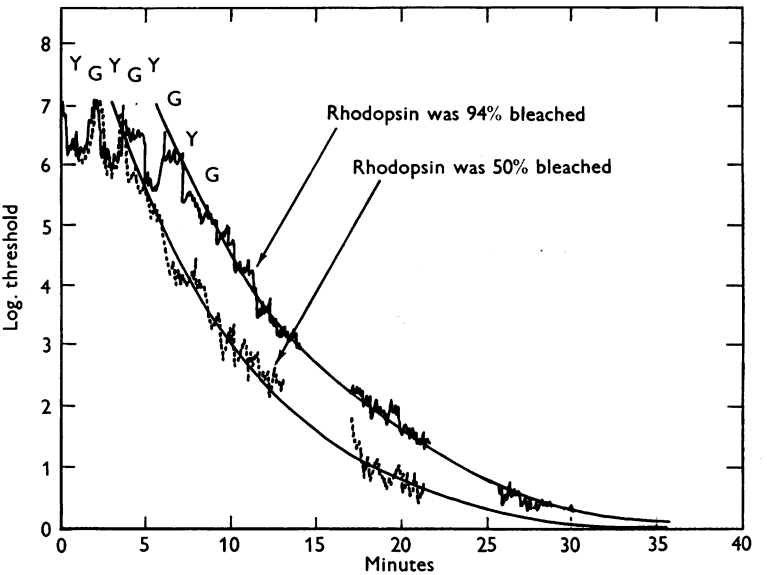

Fig. 2. Dark-adaptation in photanope following 94 and 50 % bleachings. Alternate yellow, Y, and green, G, test flashes throughout the whole curve.

about a million times above the final value, so 1/100,000 part of this test intensity scattered into the periphery would probably suffice to excite the rods there. And indeed attention was drawn to this by our subject who said, 'During the first few minutes, I can see out of the corner of my eye that the light is flashing, but I suppose that you only want me to say I see it when I see it where it really is.'

In a pure cone-free retina an error of this kind would produce an early branch resembling that of Fig. 2, but without castellation, and it might be interpreted as due to receptors which contain rhodopsin but regenerate very fast—'rhodopsin cones' or 'daylight rods'. This effect of scatter cannot account for the early branch of Fig. 2, for the receptors here showed the Stiles–Crawford effect and had a spectral sensitivity very different from that of rhodopsin, and hence could not have been peripheral rods;

moreover, the possibility of error was detected and set aside by the quick intelligence of our subject. I do not wish to suggest that this error has in fact misled any former workers, but when dark-adaptation in the photanope appears to reveal 'daylight rods', the possibility of scatter should clearly be borne in mind and carefully excluded.

One way to do this is to reduce the sensitivity of all the rods in the retina by giving a preliminary bleach from a large milk-glass screen at moderate illumination placed very close to the eye. This is then followed immediately by the strong bleach applied to the limited region to be tested. Some experiments were performed in this way and showed no difference in the resulting dark-adaptation curve of our subject.

The relation to normal dark-adaptation

In normal subjects we can only plot the rod dark-adaptation curve below the level of the cone threshold, and how the curve may run above this level is largely a matter of conjecture. According to Elenius & Heck (1957), the cones inhibit the rods and during dark-adaptation delay the moment when rods are effectively connected to the optic nerve. If this were true, we should expect dark-adaptation in the photanope to be distinguished from normal by the virtual absence of cone inhibition, with the result that the recovery of rod vision would be less delayed.

The observed facts are displayed in Fig. 1, where the interrupted curve represents the dark-adaptation curve of a normal subject (M.G.F.F.) tested by the alternating green-yellow flash technique. The continuous curve is traced by the photanope D.M. (with rest pauses at 15 and 20 min), using a white test flash. The two curves have been reproduced with vertical displacements so that they coincide at full dark-adaptation. Both subjects had a similar bleaching exposure (97 % bleach) and both showed similar rhodopsin regeneration, as indicated in Fig. 1 by the black and white circles. The dark-adaptation curve of the normal subject shows the castellated cone branch for the first 20 min, at which point the photanope curve is met. Thereafter the two curves march together. This does not at all support the idea of rods being inhibited by cones, but it is precisely what should happen if rods and cones are independent mechanisms and if the more sensitive in any situation alone determines the threshold, as Stiles (1949) has established for increment thresholds.

The evidence upon which Elenius & Heck (1957) based their conclusions was derived, not from visual thresholds but from e.r.g. measurements upon photanope and normal subjects. This work we have attempted to repeat and shall discuss elsewhere; here we simply state that we have not confirmed their observations.

It seems reasonable to conclude that in the photanope both rods and

rod vision are normal. For not only is the regeneration of rhodopsin the same in photanope and normal subjects (black and white circles, Fig. 1), but photanope and normal rod thresholds also coincide throughout the whole range where normal rods can be measured. It seems likely, therefore, that the thresholds of normal rods, where they cannot be directly observed (namely, above the level of the cones) are also given by the full sweep of the photanope curves as shown in Figs. 1 and 2. The relation, then, between log. threshold and the fraction of rhodopsin present which, for the photanope, is plotted over a large range in Fig. 1, may be considered as the relation of the normal rods; and we may now measure the curves and find what the relation is.

The relation between rhodopsin and threshold

Suppose that we draw across Fig. 1 a series of vertical lines, the ordinate values of the rhodopsin curve will give the fraction of pigment present, the ordinates of the dark-adaptation curve, the corresponding value of

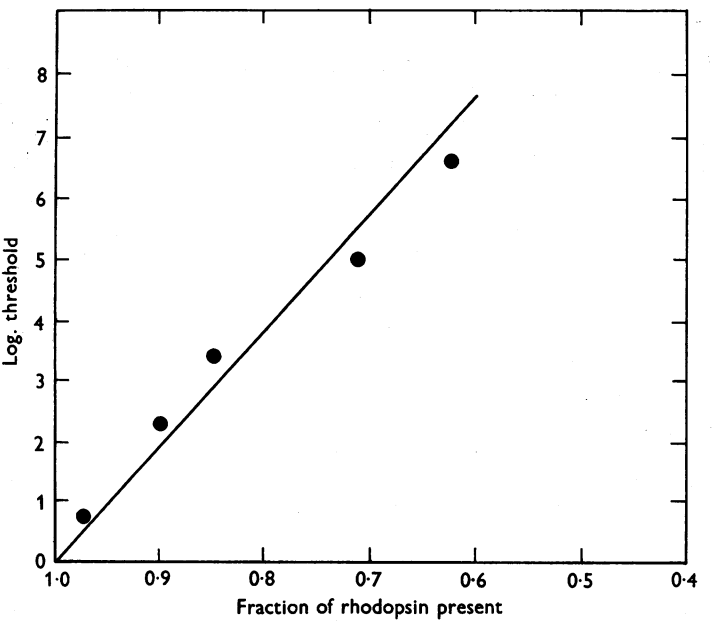

Fig. 3. Relation between log. threshold and fraction of rhodopsin present in photanope.

log. I/I_0—the logarithm of the threshold when this is expressed as a multiple of the fully dark threshold. In Fig. 3 these two values have been plotted one against the other, and a reasonably linear relation is found. This is the relation which in paper I it was argued should occur, and which has been

found by Dowling & Wald (1960) to exist between the rhodopsin content and log. e.r.g. threshold in the rat.

The lateral displacement between the two curves of Fig. 2 is consistent with this relation between rhodopsin and threshold. The bleaching exposures in each case lasted 30 sec and amounted to 6·8 and 7·4 log. td sec, respectively. Now in a previous investigation (Rushton, 1956) it was shown that 6·8 log. td sec leaves $\frac{1}{2}$ the rhodopsin unbleached. Therefore, 7·4 log. td sec, which is 4 times this energy, will leave $(\frac{1}{2})^4$ or about 6 % unbleached. But from Fig. 1 it is seen that rhodopsin takes 4 min to regenerate from the 6 % to the 50 % level. Hence the two curves of Fig. 2 should be laterally separated by an interval of 4 min. In fact, the curves are not quite the same shape, being separated by about 3 min in the upper part and 5 min in the lower part, but the displacement is of the right order of magnitude.

DARK-ADAPTATION AND INCREMENT THRESHOLD

We may obtain some important information about the nature of dark-adaptation by measuring the threshold flash in the period following light-adaptation, not against a black background, but superposed upon fields of various luminances. This experiment has been performed by Crawford (1947) and Hattwick (1954), and in modified form by Sloan (1950), upon normal subjects with the following results. A dim background will raise the final threshold for the rods without greatly affecting the cones; a brighter background will raise somewhat the cone threshold, but elevate that of rods so much that they are hardly detectable and further brightness abolishes the rod branch entirely by raising it above cone threshold.

The high cone threshold of the photanope permits this study to be made upon rods over a much greater range.

The increment threshold equipment shown in Fig. 1 of paper II was used, and a region 7° parafoveal examined. First the increment threshold was determined for the whole range of background fields, with a white flash which could be made bright enough to show some very high threshold cones (black triangles, Fig. 5). Then a total bleach was applied in the usual way and dark-adaptation measurements initiated. The increment light flashed continually and the subject moved the interposed wedge until threshold was obtained. When satisfied she remained in position upon the biting block and snapped her fingers, the time was immediately noted and then the wedge setting was recorded. Filters were changed in flash and background beams between each reading so that five dark-adaptation curves were simultaneously investigated, each with a different background field. Finally, after 45 min, the whole increment-threshold curve was redetermined (dots) and found to agree with the initial values.

Dark adaptation against a fixed background light

The five dark-adaptation curves are plotted in the usual way as a family of curves in Fig. 4. With the exception of the upper curve at a background of 2·4 log. td, which lies close to rod saturation (paper II; Aguilar & Stiles,

1954), the curves show the features already clear from the results of previous workers. The dark-adaptation curves all coincide for the first 14 min. and then, one by one, suddenly stop their increase in sensitivity and rather abruptly level out at a value dependent upon the background intensity.

There has been a tendency to explain this in terms of the bleaching of rhodopsin by the background light, which thereby opposes regeneration and brings the pigment into equilibrium at a level somewhat short of full regeneration and, hence, of full sensitivity. There are strong arguments against this explanation.

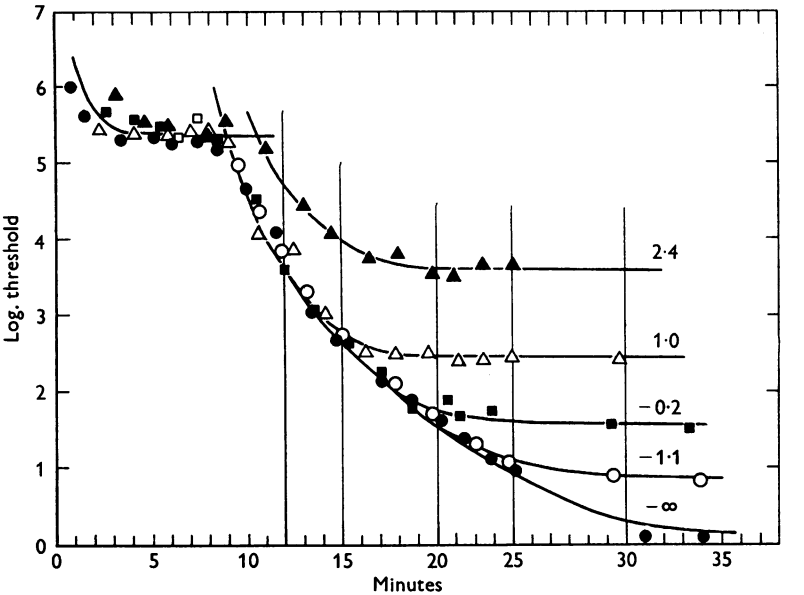

Fig. 4. Dark-adaptation curves of photanope against background fields of luminance shown by number (log. td), against each curve.

From Fig. 4 it appears that a background field of −1 log. td raises the increment threshold by 1 log. unit. If this is due to bleaching, we can see from Fig. 1 that about 4% of rhodopsin must have been bleached, since this raises the dark-adapted threshold 1 unit above the absolute value. But, −1 log. td corresponds to a retinal illumination of about 1 quantum (500 mμ) incident per rod per second, and even in complete absence of regeneration such a light would take 4 months to bleach away the required 4%, accepting the photosensitivity measurements of Dartnall, Goodeve & Lythgoe (1938) for rhodopsin in solution, which have been shown to apply approximately to the living human eye (Rushton, 1956).

But a sudden change of background field causes the new increment

threshold to be established, not in the matter of months nor even of minutes, but within a second or so. It cannot, therefore, depend upon the bleaching and regeneration of rhodopsin.

The dark-adaptation threshold obtained against various backgrounds depends, in fact, upon the background intensity, but not upon the time (greater than a second or two) through which this background has been maintained. In sharp contrast to the 20–30 min delay required to reach full dark-adaptation with any background is the mere 2 sec wait needed when changing back and forth between various fields at every stage of dark-adaptation, as experienced in the determinations of Fig. 4. Increment thresholds, therefore, are not coupled to the rhodopsin level, but constitute a largely independent mechanism.

But if the increment-threshold relation—the Aguilar & Stiles (1954) curve—is not coupled to rhodopsin bleaching, it might be expected not to be affected by light- and dark-adaptation. A re-plot of the results of Fig. 4 shows that this is the case. Ordinates drawn in Fig. 4 at times 12, 14, 20, 24 and 30 min cut all five curves, and hence give at each stage of adaptation the increment threshold against these five given background illuminations. The results re-plotted in this way are shown in Fig. 5.

Increment thresholds during dark adaptation

The initial and final increment threshold curves simply confirm what was found in the previous paper, and the Aguilar & Stiles (1954) curve for normal rods is drawn through them. The rods become saturated at about 2·7 log. td, and very high threshold cones then appear.

At an earlier stage in the dark adaptation process thresholds are raised (as would be expected) but also the horizontal part of the curves (Fig. 5) stretch further to the right before turning up; so these results cannot be described by a vertical shift of the Aguilar & Stiles curve. Nor would the curve be expected to shift vertically. For if bleaching renders the rods less able to generate a signal, this incapacity should affect equally both flash and field. So if the curve must move upwards 2 units because the flash is so ineffective, it must also move to the right 2 units because the field is equally ineffective. The Aguilar & Stiles curve, therefore, should be displaced upwards and to the right along the 45° line. The family of curves drawn in Fig. 5 is, in fact, the Aguilar & Stiles curve so displaced and it is seen to fit the results for all backgrounds below near-saturation at all stages of adaptation.

But the saturation barrier was never crossed by the rods. Specific investigation of this point confirmed the fixity of that vertical saturation line. For instance, the uppermost square (Fig. 5) was compared with the same flash upon a background 2·8 log. td—a point just the other side of

the saturation line. In the dark-adapted state, the square would be seen and the other point would not. Raising the threshold 10,000 times by light-adaptation did not change this result. Though the Aguilar & Stiles curve shifted 4 log. units up and to the right, the saturation line did not shift at all. To the left of the line the flash was seen; to the right, it was not.

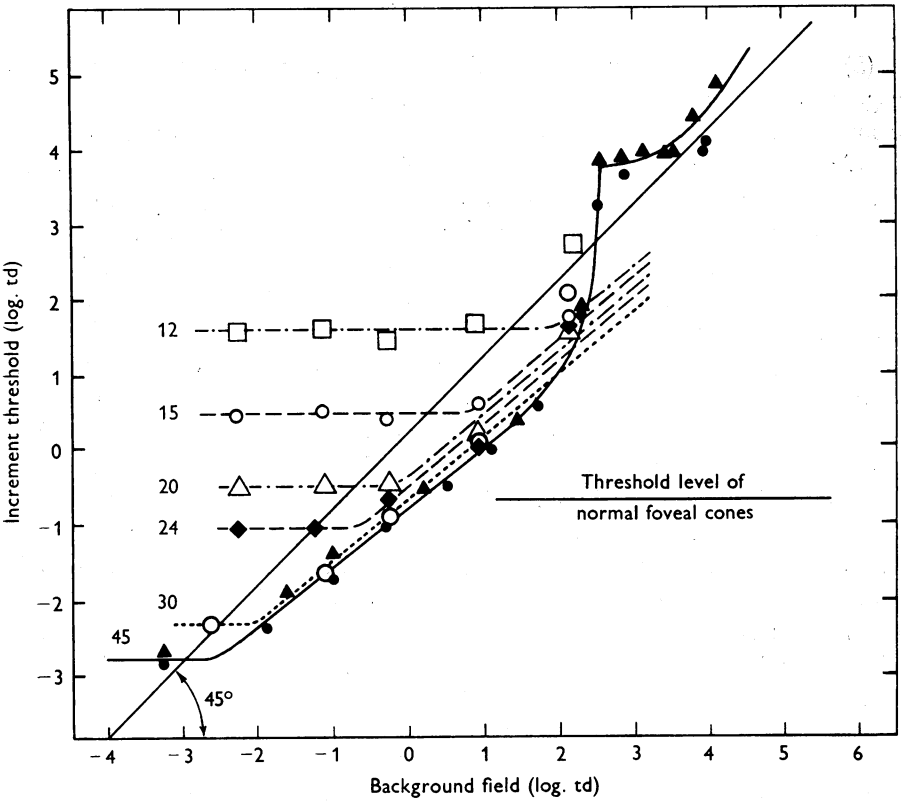

Fig. 5. Increment threshold curves at various stages of dark-adaptation derived from re-plotting the results of Fig. 4. ▲, initial increment threshold relation. Number against each curve shows number of minutes of dark-adaptation.

DISCUSSION

The experiment of Fig. 1 confirms by direct measurement conclusions derived by argument in paper I, and establishes a simple linearity between the amount of rhodopsin still unregenerated and log. threshold for the perception of a test flash. Yet it is important to bear in mind that this simplicity is the result of restricting the range of our investigations, and that in two ways.

(*a*) Though after *strong* bleaches the rod branches of adaptation curves are always the same shape, so that they may be made to coincide by

lateral displacement, this is not the case after weak bleaches. As Winsor & Clark (1936), Hecht, Haig & Chase (1937) and many others have pointed out, rod curves which appear early return quickly. That complication is here avoided by investigating the consequences only of substantial bleaches where rods do not appear early. But this constitutes an important limitation of the conditions over which the pigment–log. threshold relation has been established.

(*b*) Any attempt to relate threshold during dark-adaptation with the regeneration of rhodopsin must face the difficulty that threshold depends upon the size and duration of the test flash, whereas regeneration naturally does not. If changing the parameters of the flash simply shifted the rod branch of the dark-adaptation parallel to the axis of log. threshold, this would be no objection, but in fact the curve changes its shape.

Craik & Vernon (1941), Crawford (1947), Rushton & Cohen (1954), Arden & Weale (1954) and others have found that the extent of dark-adaptation recorded—the total change of log. threshold—depends upon the size of test flash used and perhaps somewhat upon its duration. It is reasonable to suppose that the integrative properties of the human retina, like those of the cat's (Barlow, FitzHugh & Kuffler, 1957) change during dark-adaptation in such a way that space–time precision is exchanged for increased absolute sensitivity. This might easily lead to such dependence of dark-adaptation curves upon the flash parameters as in fact is observed. What, however, is far less obvious is the way in which such neural organization can be uniquely determined by the amount of unregenerated opsin in the rods, and how the simple relation found between log. threshold and pigment is to be reconciled with the complexities of lateral inhibitions and summations in the layers of the retina.

SUMMARY

1. Visual pigments were measured in the eye of one incomplete rod monochromat by retinal densitometry. No visual pigment was detected upon the fovea, but in the periphery rhodopsin was found to be normal both as to amount and the rate of regeneration.

2. The absence of nearly all cones in the periphery permitted the rod branch of the dark-adaptation curve to be followed over 6 log. units of threshold intensities. The curve coincided with that of the normal subject when this appeared below the cone branch (Fig. 1), thus the monochromat's curve probably represents the normal rod threshold where this cannot be measured above the cones.

3. At each stage of dark-adaptation there is a linear relation between log. threshold and the amount of rhodopsin still unregenerated (in the particular conditions investigated).

4. Measurements of increment thresholds upon various background fields at different stages of dark-adaptation show a simple relation. Over most of the range the effect of light-adaptation could be imitated by placing a suitable neutral filter in front of the eye, thus raising the threshold equally for flash and field.

5. But the luminance of background which saturates remains constant at about 1000 td, independent of the state of dark-adaptation.

It is a pleasure to express my great indebtedness to Drs M. G. F. Fuortes and R. D. Gunkel who have modestly refused to allow their names to appear as joint authors of this paper though they co-operated fully in all the experiments. I should like to thank the many authorities at N.I.H. for inviting me to come, and for placing every facility at my disposal. And I am especially grateful for the unfailing support and encouragement of Dr L. von Sallmann in whose department I was privileged to work.

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