J. Physiol. (1965), **178**, *pp.* 141–160
With 5 text-figures
Printed in Great Britain

THE SENSITIVITY OF RODS UNDER ILLUMINATION

BY W. A. H. RUSHTON
From the Physiological Laboratory, University of Cambridge

(*Received* 11 *November* 1964)

Anyone who has noticed that stars are visible at night but not in day time has experienced the fact that the visual mechanism is less sensitive to a fixed test light when presented upon light rather than dark surroundings. The object of this paper is to consider what it is that becomes less sensitive. (*a*) Do rods have to catch more quanta in order to generate a signal, or (*b*) do more signals have to be generated in order to excite a visual response? Or do both these requirements increase together? Following the terminology of a former paper (Rushton & Westheimer, 1962) we shall call (*a*) 'the change in threshold for a rod signal', (*b*) 'the change in threshold of the summation pool', and use 'rod threshold' in the ordinary way without distinguishing between (*a*) and (*b*).

The fact that light can bleach rhodopsin away naturally led to the idea that steady illumination might raise the threshold simply by removing most of the light-catching pigment. This idea which in its quantitative treatment was the climax of Hecht's (1937) photochemical theory of visual performance has long since been abandoned. Almost at once Dartnall, Goodeve & Lythgoe (1936, 1938) showed that rhodopsin is in fact bleached millions of times more slowly than Hecht had to assume, and Lythgoe (1940) in the last paper before his untimely death argues convincingly that the change in sensitivity is due at least in part to the alteration of nerve organization. The focus of attention upon the quantum aspects of vision (Hecht, Slaer & Pirenne, 1942; de Vries, 1943; van der Velden, 1944; Bouman & van der Velden, 1947) has made it plain that over most of rod vision we are dealing not so much with changes in chemical equilibria under the law of mass action but with 'explosive' events triggered by the photo-chemistry of single molecules (Baumgardt, 1949). And now it is generally accepted that in conditions of full dark adaptation, a weak flash against a black background may be seen when rods absorb only one quantum each, and when some small number of rods (more than one) are thus excited together.

Now when the flash is presented not in darkness but super-imposed upon a luminous background, it is known that the background that raises the increment threshold 100 times above the absolute dark value produces a quite negligible bleaching of rhodopsin. Thus the threshold is raised not because a smaller fraction of incident quanta are caught, but because a greater number need to be caught in order to be seen.

One way in which this might happen is that a rod after absorbing a quantum becomes 'refractory'. That is to say it is put out of action for a time, during which all further quanta caught are ineffective. Since the background field is continually exposed to a shower of quanta, a state would soon arise where a proportion of rods would always be ineffective. If, for instance, 90 % were ineffective the threshold would be raised 1 log unit. Pirenne (1958) by a neat experiment has excluded this as a principal factor. Pirenne used a large luminous background 32° in diameter with a black hole 2° at the centre. In the middle of this the test flash was placed, 0·1° in diameter and less than 3 msec in duration. The luminous surround was rather weak but it raised the threshold of the test flash 3 times. If this was done by light that fell directly upon the very rods that were excited by the test flash, it must be by stray light scattered from the surround. Using Le Grand's (1956) estimates Pirenne calculated the magnitude of this scattered light, and then he applied light of this strength directly to the place where the test fell (in the absence of surround). He found that now there was no detectable rise in threshold above the absolute dark value, and concluded that the surround causes a rise in threshold not by light scattered to the centre but by interaction between the nerves from the test and nerves from the surround. From this he drew the important generalization that when background and flash fall upon the same region of the retina, the rise in the increment threshold is still influenced by nerve interaction (inhibition).

In the present paper, Part I confirms Pirenne's conclusions, Part II extends the investigation to higher background levels.

PART I

Principle of the experiment

The idea is to measure in a fully dark adapted eye what is the intensity of background (expressed as quanta absorbed per rod) that raises the absolute threshold about 3 times. The longer the background lasts the more quanta are absorbed; convenient timing to get a low figure is shown in Fig. 1*B*. At zero sec a warning was given, at 1 sec the background was exposed, at 2 sec the test flash was superimposed on it, at 3 sec all lights were extinguished and the subject stated whether or no he had seen the superimposed flash. The threshold value for the flash was found with and without a background and a background brightness was chosen such that it raised the threshold some 3 times. The luminance of this background was measured and converted into quanta absorbed per rod during the 2 sec exposure. The result turns out to be about 1 quantum per 100 rods. It is therefore plain that the threefold threshold rise cannot be the result of 'refractoriness' of quantum-hit rods since this would raise the threshold 1 % not 300 %. If 99 % of the rods had never 'seen' the background their threshold must have been raised by the 1 % that had.

METHOD

Figure 1*A* shows the spatial arrangement—a 2° flash centred upon a 10° surround with fixation point 10° temporal. The lights were projected through a small hole in the wall of a dark room and fell upon a white screen where they subtended the angles shown in Fig. 1*A* at the eye of the observer. The absolute threshold was obtained by presenting (with zero background) a flash either of 1 or 4 units of luminance. The order of their presentation was predetermined from a table of random numbers so arranged that each luminance was presented 50 times. The increment thresholds were determined similarly (except that the luminances used were 4 and 8 units. The order of the runs was 50 with zero background,

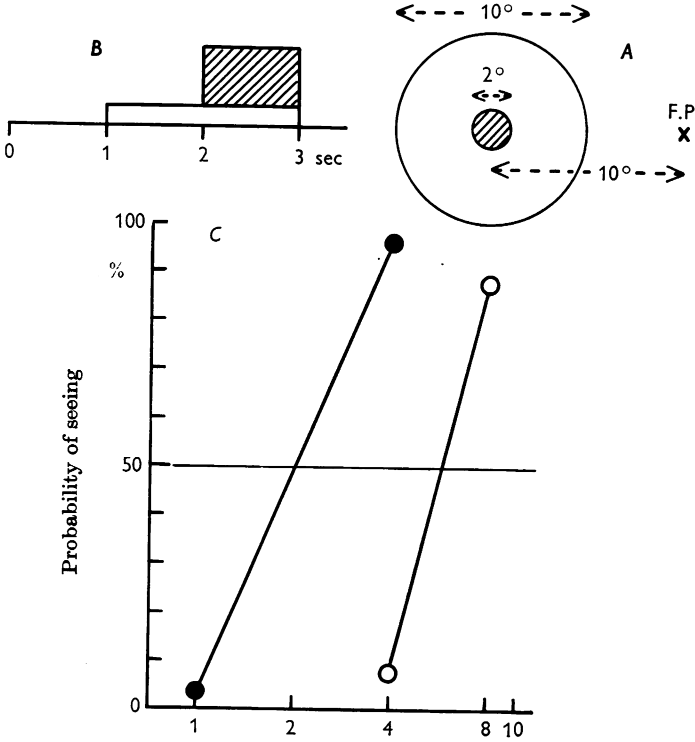

Fig. 1. *A* shows spatial arrangement of flash (shaded) in the centre of background field outside which is fixation point F.P. *B* shows temporal arrangement with flash (shaded) presented 1 sec later than background. *C* Probability of seeing a flash plotted against flash intensity. Black points with black background; white points with white background.

100 with the fixed background, then 50 more with zero background. The time course of presentation was always as shown in Fig. 1*A*. All lights were white.

The luminance of the background was found by measuring with an S.E.I. luminance meter the much brighter field obtained by removing a calibrated 2·0 density from the projected beam, and bringing the screen close to the projector. Since all rays of the projected beam passed through a focal 'point' less than 2 mm in extent, the attenuation with 330 cm of screen distance from that point could legitimately be calculated by the inverse square law. The subject's pupil size in the dark was measured by inspection of the eye illuminated by oblique dim light.

RESULTS

Figure 1*C* shows two frequency-of-seeing curves where the probability of seeing (ordinate) is plotted against the log energy of the flash. Filled circles represent the absolute threshold, open circles the increment threshold. It is seen that with zero background 1 unit of flash intensity was only seen on 4% of presentations, 4 units was only missed on 4% of presentations. The absolute threshold is taken as 2 units, which is where the line joining the filled circles cuts the 50% horizontal.

When the 4-unit flash fell upon the luminous background already exposed for 1 sec, the frequency of detection fell from 96 to 8%. An 8-unit flash, however, was seen on 88% of occasions and the threshold given by crossing the 50% horizon is 5·8 units—nearly three times the absolute threshold (as in Pirenne's experiment quoted above).

Now the luminance of the background directly measured (see Method) was $2{\cdot}5 \times 10^{-5}$ cd/m^2, the pupil area 0·32 cm^2, the transmission through the eye is taken as 0·5, hence from Le Grand's formula (1957) the retinal illumination

$$\begin{aligned} E &= 0{\cdot}36 \times 0{\cdot}32 \times 0{\cdot}5 \times 2{\cdot}5 \times 10^{-5} \\ &= 1{\cdot}4 \times 10^{-6}\ \mathrm{lm/m^2}. \end{aligned} \tag{1}$$

The luminance was actually measured in photopic units. By definition these would also be scotopic units if the colour temperature of the projection light had been 2042° K (the platinum point). Since it was about 1000° higher we convert to scotopic units by adding 0·2 to the log photopic value, and this conversion is given in (1). Now we know that 1 scotopic lumen = $1{\cdot}46 \times 10^{15}$ quanta ($\lambda = 507\ \mathrm{m}\mu$) per sec. Hence the illumination in expression (1) above, that lasted for 2 sec, amounts to a shower of

$$2 \times 1{\cdot}46 \times 10^{15} \times 1{\cdot}4 \times 10^{-12} = 4 \times 10^{3}\ \text{quanta/mm}^2 \tag{2}$$

Denton & Pirenne (1954) have carefully considered what proportion of the area of the peripheral retina is occupied by rods. They conclude that 30% of light incident upon the *retina* misses the rods altogether, and the remaining 70% is distributed among a population of 134,000 rods per mm^2. Thus expression (2) amounts to

$$\begin{aligned} &0{\cdot}7 \times 4 \times 10^{3}/134{,}000\ \text{quanta incident per rod} \\ &= 2{\cdot}1\ \text{quanta incident on 100 rods.} \end{aligned} \tag{3}$$

It is probable (Rushton, 1956) that only 30% of light ($\lambda = 507$) falling on a human rod is absorbed, thus less than 1% of the rods exposed to the background of expression (3) absorbed one quantum from it.

The above calculation was made on the assumption that the background was a light of wave-length 507 mμ. But light of any other wave-length of the same luminance in scotopic units will be absorbed by the rods to exactly the same extent. Thus the above result applied equally to every wave-length in the white light that actually was used, and hence to their sum—the white light itself.

This result helps us to answer our question 'What becomes less sensitive as a result of the luminous background?' For the background that raised the threshold 3 times was only 'seen' by 1% of the rod population; the rest did not catch a single quantum from it. But clearly it is the 99% fully dark rods that determine the threshold, so it is *their* threshold that has been raised 3 times as a result of the activity of the 1% that caught a quantum. There are two conceivable ways in which this could happen (*a*) the active minority might in some way influence the sensitivity of all the other rods so that their threshold for a rod signal was raised; (*b*) the minority might raise the threshold of the summation pool.

Both structure and function are hard to reconcile with any simple interpretation of (*a*). It is not clear through what connecting pathways a quantum caught in any rod could influence the threshold for rod signals of a thousand neighbours. And if all the rods were thus influenced it does not appear that the result would correspond to the *gradual* rise of threshold with the increase of weak backgrounds. For if all rods became unable to respond to one quantum they must await that much rarer event, the near coincidence of two—a step transition never seen in increment threshold curves no matter how small the area of test flash used nor even when observations are made upon single ganglion cell discharges (Donner, 1959).

Alternative (*b*) on the other hand is acceptable. We know that rods from a large cluster converge upon a summation pool. Thus pathways are certainly present to conduct signals to the pool from whichever rods constitute the 1% that happen to catch a quantum. We know from Hecht *et al.* (1942) that about six of these signals in near space-time coincidence can cause a visual sensation, and it is generally accepted that some such coincidence is required to obtain sufficient signal significance, against the noisy background of retinal dark activity (Barlow, 1957). Obviously when the background is luminous, the 'noise' will increase by the addition of random light signals from the whole background, and the flash threshold of the pool will have to be increased also if reliability of detection is to be maintained. Thus the idea that the threshold rises not in the rods but in the pool is anatomically acceptable and functionally necessary.

Higher background levels

In the foregoing experiment the background was very weak; it seemed of interest to extend considerations and observations to higher background levels. The procedure was a simplified version of the previous work.

In order to obtain rod thresholds with bright fields the technique of Aguilar & Stiles (1954) was followed, both flash and background were presented in Maxwellian view, the background being a red light (Ilford filter 205) and the flash green (Ilford 624). The background was exposed for 1 sec (not 2) and for the second half-second the flash was superimposed on it. Exposures were presented every 15 sec and the subject stated whether or no he saw the flash upon the background. Usually some four or five presentations sufficed to determine the threshold correct to ± 0·1 log unit. If further observations were needed they were given after about 2 min rest in the dark.

In Fig. 2 black circles show the log threshold obtained in this way; white circles show the log increment threshold measured (in the more usual manner) with the background continuously exposed. It will be noted that though the rods are in a much more 'light adapted' state under a steady field than under one that lasted but 1 sec in all, yet the threshold is more raised by the 1 sec exposure. This, however, was expected, for it is common experience upon exposure to a high steady background that repeated threshold measurements show improved discrimination and a progressive fall in threshold during the first minute or so.

The background luminance in Fig. 2 is given in log scotopic trolands and also in quanta absorbed per rod during the 1 sec exposure. The scotopic value of the red light was found by equating it with a white background that raised the rod increment threshold to the same extent. The conversion of scotopic trolands to quanta absorbed per rod per sec is calculated as above with the same assumptions. This works out as

$$1\ \text{td} = 4\ \text{quanta absorbed per rod per sec} \tag{4}$$

which is the value stated by Pirenne (1962) and the horizontal scale of Fig. 2 is graduated in a the value of this average quantum catch per rod.

We may easily calculate the threshold to be expected if rods that have not caught a single quantum are unaffected, those that have caught one (or more) are made 'refractory' for at least 1 sec and the threshold of the summation pool remains constant. For from Poisson's formula when the average quantum catch per rod is a, the chance that any given rod shall catch zero is e^{-a}. And since these are the only rods that can respond to the test flash, the threshold will be e^{a} times the absolute value. Thus the $\log_{10}$

threshold should be raised 0·43a above the absolute value. The dotted exponential curve of Fig. 2 shows this expected relation. The calculation actually applies to an instantaneous test flash presented at the end of the 1 sec field exposure; the proper curve lies between that shown and its displacement 0·3 log unit to the right (corresponding to a 0·5 sec field exposure). But the curve is so grossly inappropriate as description of the increment threshold that we need not be concerned with the detail of its true shape.

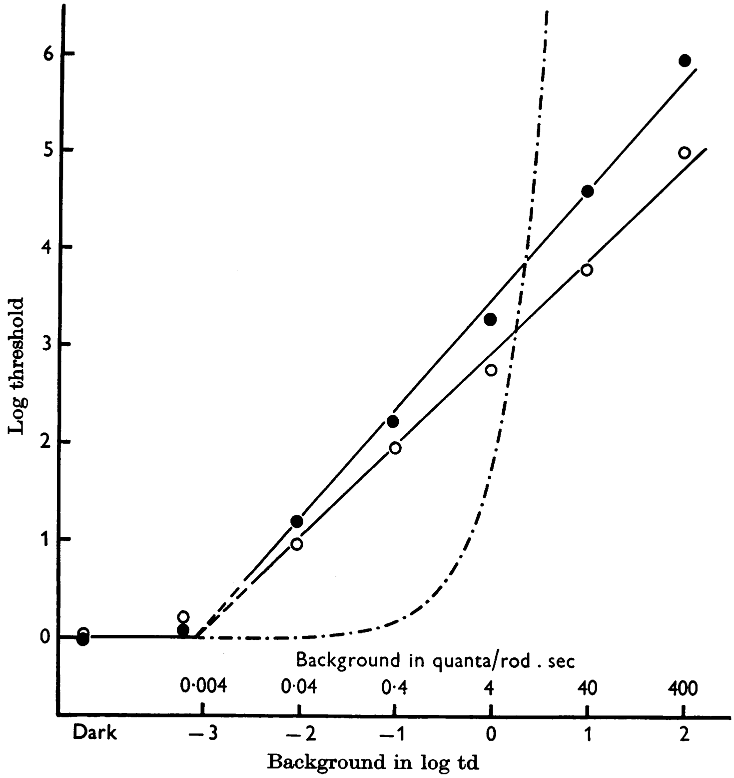

Fig. 2. Increment threshold for a 0·5 sec green flash upon a red background. Abscissa in log trolands and in quanta/sec per rod. White circles with maintained background, black circles with background lasting 1 sec. Dotted curve, the threshold relation expected if catching a quantum inactivated that rod and had no other effect.

Obviously at the lower range of backgrounds even a Medusa-like property that turned to stone every rod that saw the field would not account for the rise in threshold observed; there must be a rise in the signal requirement of the pool. On the other hand at high background levels the computed curve rises so very sharply that it excludes absolutely the idea of a 'refractoriness' of 1 sec—at a background of 2 log td, i.e. 400 quanta per rod per sec for instance the threshold should be raised 172 log units instead of the mere 6 observed. If refractoriness occurs at all it must last only a small fraction of a second and is likely to contribute not to the ordinary increment threshold but to the appearance of 'rod saturation' as Aguilar & Stiles (1954) have already pointed out.

PART II

In Part I of this paper on the effect of luminous backgrounds we have replied to the question 'What becomes less sensitive?' by the answer 'The summation pool: it needs more rod signals'. But we have not been able to answer the next question 'Is the threshold *entirely* dependent upon the pool or is there also a rise in the threshold for rod signals?' A simple way to decide this in principle is as follows. The background instead of being a uniform field is made of bright and dark stripes each subtending 0·25° in width, so that the whole 0·5° period falls within the receptive field of the summation pool, smaller than in the experiment of Rushton & Westheimer (1962). If now the test flash is also a series of bright stripes with the same period, then the threshold may be compared when the bright test stripes fall (*a*) on the dark, (*b*) on the bright stripes of the field. Ideally in (*a*) the out-of-phase position the flash falls on dark-adapted rods and in (*b*) on light-adapted rods but both controlled by the same summation pool. Thus the threshold difference in the two situations will show the rise in threshold for rod signals: if the threshold rise is entirely in the pool the threshold will be the same whether the bright bars of the test fall upon the bright or the dark bars of the background. This is what is found to be the case.

Voluntary fixation: experiments by M.F.C. Crick

A difficulty in performing an increment threshold with a striped background is that eye movements could smudge the background and make it in effect rather uniform. However, if a clear threshold difference had appeared between the in-phase and out-of-phase position of the flash, it would have shown that despite smudging the threshold for the generation of rod signals was affected by the illumination or non-illumination of the background. I am indebted to Mr Crick for undertaking this investigation.

METHOD

The background was a red-black grating projected upon a screen and viewed at such a distance that the width of the red band = width of the black band = 0·25°. The test flash was a set of green lines of width 0·05° spaced 0·5° apart and arranged either (*a*) to fall in the centre of the black bands, or (*b*) the centre of the red bands of the background. Both background and flash were variable in luminance, the background remaining fixed and the flash being brought to threshold value. A small fixation point was situated 10° temporal to the test flash.

For each luminance of background the fixation point was observed steadily and at a satisfactory moment the subject released a photographic shutter and obtained a 0·01 sec exposure of the green test grating. The threshold was found for the in-phase and the out-of-phase positions, and a new background luminance was then tested.

RESULTS

There was never found any significant difference between the threshold in the out-of-phase and in-phase positions. Consequently there was no reason to suppose that the background raised the threshold for rod signals. Whether we think there is reason to suppose that it did not, depends on our view of the stability of fixation. The observer was not aware of eye movements, at the end of a fixation period he had a rather sharp after-image of the grating background, and eye movements less than 0·1° would not smudge significantly. It seems very likely therefore that Crick's results are not invalidated by accidental, unperceived and rather large eye movements just at the time that he released the shutter. In that case they show that the rise in increment threshold with background is due solely to the insensitivity of the summation pool, affecting alike rods exposed to the background and their neighbours in the shade. But in order to test further the soundness of this conclusion it was decided to repeat the experiment using a striped background that was stabilized on the retina.

PART III. STABILIZED IMAGES

The principle

The idea was to place upon the retina the striped image of a grating, and to *stabilize* it there so that it moved exactly with the eye. The flash is a similar stabilized grating adjusted so that its bright bars fall either upon the bright or upon the dark bars of the background. If, despite stabilization the threshold was the same in the two positions it would be strong support for the view that the background raises the threshold by some modification not of the rods but of the summation pool.

The image of the grating was stabilized on the retina by the method of Yarbus (1956, 1957*a*, *b*) in which a device is attached by suction to the anaesthetized cornea. The device I used was designed by Barlow (1963) and used also by Barlow & Sparrock (1964). The special modification that I needed was constructed for me by Mr Sparrock to whom I am grateful also for help in its maintenance and application.

METHOD

The sucker. The appliance is fixed to the anaesthetized cornea as shown in Fig. 3*A*. It consists of a light conical frustrum of thin aluminium whose basal rim is turned outward so that the smooth shoulder makes contact with the cornea at the limbus. The cone is filled with alkaline saline (to avoid misting) reduced in pressure by release of the firm rubber sucker *S*. The lens L_s, 3 mm wide and nearly hemispherical, is sealed into the small end of the cone by black wax so that all light entering the eye must pass through the lens which focuses sharply upon the retina the grating *G* held on a light aluminium girder 2 cm long. The grating is a small piece of photographic film consisting of black and transparent strips of equal width such that the image on the retina has a period of 0·5° subtense. It is clear that if the grating is uniformly illuminated by light that enters the eye, it will be seen as a uniform set of black/bright stripes and that a flash added to the central region of the grating will constitute an in-phase increment test in conditions of stabilized background. What is less clear is how to obtain the out-of-phase arrangement. This, however, is easily managed.

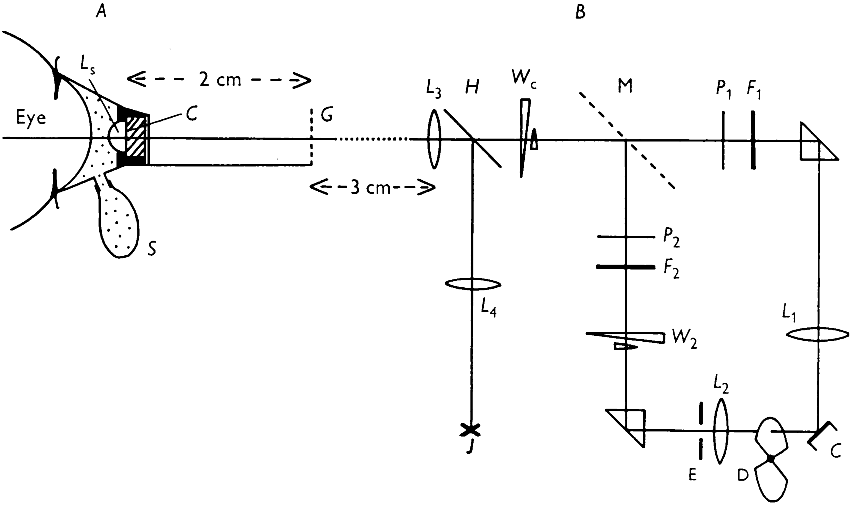

Fig. 3. *A*, eye wearing stabilizing device adhering by action of sucker *S*. Grating *G* focused on to retina by lens L_s operating in conjunction with double-image crystal *C*. *B*, Unstabilized equipment represented at much reduced scale. *J*, fixation point focused on to *G*; *D*, rotating shutter for test flash; L_1 focused by L_3 on to *G* constitutes background field.

The plate *C* is a flake of calcite about 1 mm thick sheared off a crystal in the cleavage plane by a sharp tap applied to a knife. As is well known, calcite is a double-image crystal, the ordinary and extraordinary rays being polarized in orthogonal planes. When the crystal flake was placed upon the grating film from which the fragment *G* was cut, two gratings were seen. If now the crystal is rotated as it lies flat on the film, one image of the grating remains fixed, the other moves. Each point of the second describes a circle around the corresponding fixed point of the first image so the bars of the grating remain parallel but their lateral displacement oscillates about the coincidence position. The amplitude of oscillation is proportional to the thickness of the crystal flake, and with 1 mm thickness the amplitude to right or left is just greater than the thickness of the dark or bright band of the grating. Thus a small rotation of the crystal from position of maximal displacement serves to bring the displacement to exactly the width of a band. In this position the dark bands of one image coincide with the bright bands of the other. This is the orientation of crystal to grating used in setting up the sucker device. The crystal was cemented in this sense upon the plane surface of L_s, and a fragment of cover-slip was cemented on to the opposite crystal face to improve the optics and protect the soft calcite surface.

When the sucker so constructed was worn and the appearance examined at once by foveal vision in good light (e.g. looking at the sky), a nearly uniform field was seen. There were faint very thin parallel lines where the light-dark transitions of one grating were not exactly cancelled by the dark-light transitions of the other. When, however, a polaroid was interposed with direction either vertical or horizontal, a well contrasted bright/black grating was seen, the same with each direction except that the grating seemed to jump half a period; where bright had been, the line now is black. Slow turning of the polaroid shows (as was to be expected) that the bright bands gradually dim and the black bands brighten, the condition of a uniform field being reached after 45° of rotation.

The application to our problem is now clear. The background light is polarized (say) vertically and for each level of intensity two thresholds are measured, one with the test flash also vertically polarized (in-phase presentation) one with it horizontally polarized (out-of-phase).

Unstabilized optics. The optical arrangement needed in conjunction with these sucker measurements presents two requirements. (*a*) Backgrounds and test flashes must be adjustable in intensity, time sequence and polarization plane and must be projected sharply on G, the grating of the sucker. (*b*) The light there has to be not only uniform in spatial distribution, but so directed that the rays fill L_s, the sucker lens that is the effective pupil of the eye. Figure 3B, represented in conjunction with 3A but on quite a different scale, shows diagrammatically how this was done.

The light source C was a horizontal ribbon filament set obliquely so that it would illuminate paths 1 and 2. These are symmetrical and united in the beam-splitter M. Lenses L_1, L_2 focus an enlarged image of the ribbon upon L_3 which fills the 10 mm aperture of that lens. D is a rotating disk with apertures that admit the test flash $\frac{1}{2}$ sec on $\frac{1}{2}$ off. The wedge W_2 varies the flash intensity. P_1, P_2 are polaroids mounted so that they can be set at any required angle; F_1, F_2 are coloured and neutral filter combinations. W_c is a wedge in the common beam. The subject's head was fixed by a dental impression and brow rest and the grating G was held 3 cm away from L_3, which brought the image of L_1 and L_2 (limited by the stop E) in focus upon G. The red fixation point J seen by reflexion in the thin glass cover-slip H was also focused by L_4 to lie in the plane of G. The image of L_1 on G subtends a field of 9° upon the retina, and (retracing rays) every ray from the aperture L_s that passes through any point in G (within the 9° field) will reach the conjugate focus on L_1. Thus the fields at G were uniform since the illumination of L_1, L_2 was uniform, moreover every luminous point in the 9° field of G filled L_s with light.

The background field was red (Ilford 205), the flash green (624). The fixation light was situated about 10°–7° temporal to the flash. The appearance or non-appearance of the flash upon the stabilized background was easy to detect, and reliable increment threshold measurements could be made over about 4 log units of background. The thresholds against zero background were probably some 1 log unit too high since the sucker could not be applied to the eye nor the eye brought into proper adjustment without some light adaptation; and it was judged better not to leave more than 5 min dark adaptation before starting the measurements.

RESULTS

The set of 4 curves marked A in Fig. 4 shows the results of two experimental runs, in the first (open symbols) the background field was polarized vertically (see inset), in the second (filled symbols) horizontally. In each run the test grating was exposed alternately polarized vertically (circles) and horizontally (triangles). With zero background (dark) it might have been expected that all four symbols should coincide. The separation between the open pair and the filled is simply due to different stages of rod dark adaptation, neither complete. The separation between circles and triangles is due to the fact that even when the polaroid P_2 (Fig. 3) was removed, the light of the test flash was partly polarized, so that a photocell receiving the light that normally fell upon the grating *G*, Fig. 3, registered nearly a 2:1 change when P_2 was rotated from vertical to horizontal. Now P_2 was horizontal for triangles; thus these points should stand about 0·3 log unit above the circles from this cause.

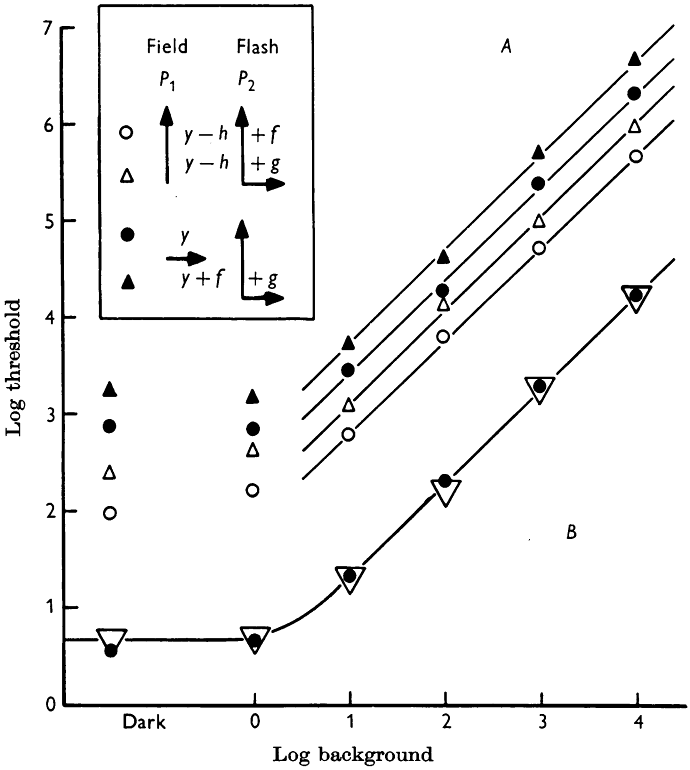

Fig. 4. *A*. Increment thresholds when background (controlled by P_1) is vertically or horizontally polarized and when flash (P_2) is vertically or horizontally polarized. The inset shows what the symbols signify; for instance, white circles show log threshold when P_1 was vertical and P_2 was vertical. *B*, is displaced downwards 2 log units for clearness. Dots plot the average of white circles and black triangles; inverted triangles plot the average of black circles and white triangles. The difference, dots minus triangles, measures the effect of light adaptation of rods in contrast to adaptation of the pool.

Clearly with the dark background this 0·3 separation, triangles above circles, is just what is found in each case. But obviously the same compensation for adventitious polarization must apply to every other background level, and it is seen that the same 0·3 separation approximately occurs throughout. When this is allowed for by lowering the triangles 0·3 log unit, the filled symbols both coincide and the open symbols both coincide (or nearly so) and there is no residual divergence when the flash grating falls in-phase or out-of-phase with the background. It might perhaps be wondered whether this slightly arbitrary displacement of the points might not remove, besides the discrepancy due to polarization, also that due to the grating phase that we wish to measure. This, however, is not so. If in-phase exposure raised the threshold above out-of-phase exposure it would raise white circles and black triangles and thus enlarge the gap between filled symbols and diminish that between empty symbols. It is precisely the equality between these gaps that shows phase to be without effect. We may analyse more accurately.

In order to find the compensation for adventitious polarization, photocell measurements are unsatisfactory for they do not embrace polarization occurring in the optics of the sucker device; and it is unsound to rely (as above) upon the threshold measurement with zero background, since not only does this involve reassessing thresholds where they are accurate (at high levels) in terms of measurements where they are most inaccurate, but since dark adaptation was not complete, the threshold with zero background was slowly falling and accurate comparison hard. The following consideration seemed better.

The thresholds described by the 4 curves of Fig. 4*A* depend upon three factors.

(i) Whether the flash and background are in or out of phase. Let f be the increase in log threshold for the in-phase position; then f is what we seek to measure.

(ii) On account of adventitious polarization in the flash pathway, when P_2 is horizontal the observed log threshold is increased by g.

(iii) On account of adventitious polarization in the background pathway, when P_1 is vertical the background becomes weaker and the log threshold is reduced by h.

Now consider any vertical ordinate cutting all four lines of Fig. 4*A* and having the value of y where it cuts the black circles. The ordinate value for the intersection with each curve may be written down at once, and is easily checked by applying the conditions just defined to the inset interpretation of symbols in Fig. 4. To the value y add g when P_2 is horizontal, $-h$ when P_1 is vertical and f when P_1 is in phase with P_2. This gives

$$\left.\begin{aligned}\text{black circles} &= y\\ \text{white triangles} &= y+g-h\end{aligned}\right\}\ \text{average} = \tfrac{1}{2}\,(2y+g-h), \tag{1}$$

$$\left.\begin{aligned}\text{black triangles} &= y+g+f\\ \text{white circles} &= y-h+f\end{aligned}\right\}\ \text{average} = f+\tfrac{1}{2}\,(2y+g-h). \tag{2}$$

Thus the required value of f, the rise of threshold when the flash grating is in-phase instead of out-of-phase is found by the difference in the two averages.

These are plotted in Fig. 4*B*, displaced down 2 log units below *A* for clearness. Inverted triangles plot average (1) above, dots plot average (2); the quantity f is given by the height of dots above triangles. The near coincidence shows that f is practically zero; the luminous background raises the log threshold 2000 times but the reduced sensitivity lies entirely in the pool and not in the rods since the threshold rise is the same whether the rods tested lie under the bright or the dark bars of the stabilized background.

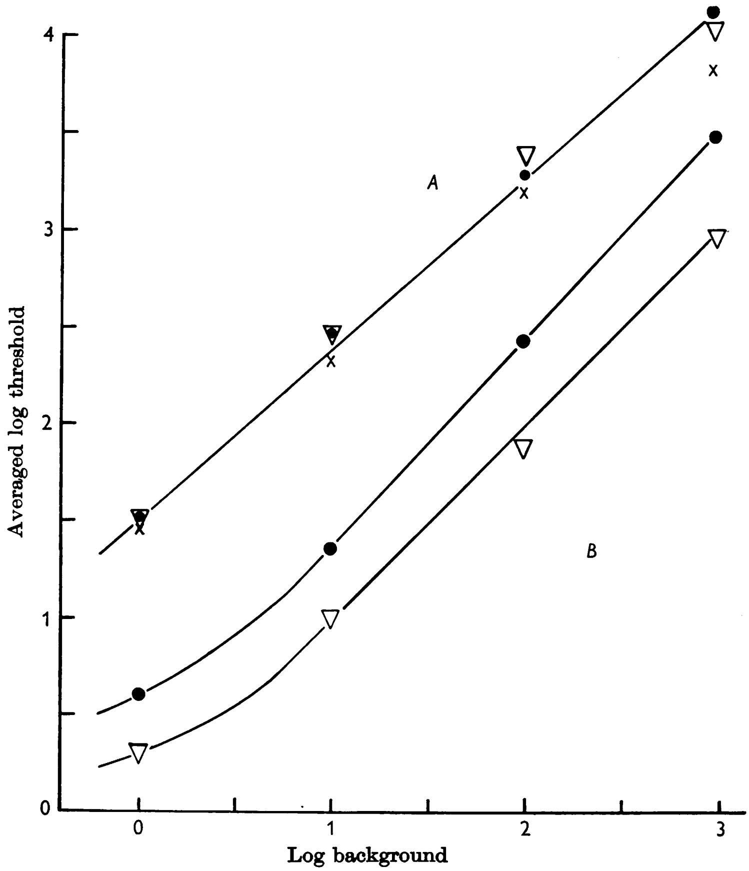

Fig. 5. *A*, dots and triangles as in Fig. 4*B*, but in this experiment it was P_1 instead of P_2 (Fig. 3) that was alternated during the run. Crosses are when P_1 was at 45° and the background was nearly uniform and unstriped. *B*, symbols as in *A*. Flash and background were white and fell on fovea. Light units different from those in *A*. With cones phase is significant.

In Fig. 5*A* are shown the reduced results of an experiment similar to that recorded in Fig. 4 except that in each run the polarization of the test flash was kept fixed and the background changed by rotating the polaroid P_1 into the vertical, the horizontal and the 45° position, this last position giving a uniform (unstripped) background. The symbols in Fig. 5*A* are the same as in Fig. 4*B* and the effect of background upon *f* the threshold for rod signals is measured by the height that the dots stand above triangles—nothing significant.

The crosses show the average threshold for the 45° position of P_1 and indicates that the threshold is nearly the same whether the test grating flash falls upon a uniform background or upon the same total light gathered into stripes either in-phase or out-of-phase. The threshold is raised by a change in the summation pool, and this change depends simply upon the total illumination of its receptive field but not upon the light distribution within it.

DISCUSSION

This paper directs attention to the very familiar fact that a fixed test flash becomes faint and finally invisible when the background field upon which it is projected is made brighter and brighter. To the question 'What becomes less sensitive' I should like to offer this answer.

Whenever a rod absorbs a quantum it generates one signal that travels to the 'summation pool'. These signals have two quite distinct effects. (*a*) If some critical number of signals n arrive at the pool within a short time interval, a message will be relayed to the brain and the light will be seen. (*b*) The influx of rod signals at the moment and in the recent past determine the size of that critical number n.

For simplicity in this paper it has rather been implied that it is the flux of rod signals in the summation pool that determines the value of n. But Pirenne's (1958) experiment quoted at the beginning shows that the value of n may be raised by a luminous surround that falls almost entirely outside the summation pool. And Alpern's recent work (1965) shows that the threshold may be raised by a 5 msec flash that falls not only outside the spatial confines of the pool but is actually presented 50 msec later than the test flash in time. Thus we need the concept of an *adaptation pool,* a centre of organization that controls amongst other things n the critical number of rod signals that must reach the *summation pool* in near-coincidence if a flash is to be seen. It is plain that the full receptive field of the adaptation pool is far larger than that of the summation pool, but the dispositions and interactions of the two kinds of pool and their relation to retinal structure are still obscure. The important distinction between threshold for rod signals and threshold for the pool has been somewhat over-simplified. If, as seems very likely, every quantum caught elicits a signal, it does not necessarily follow that all such signals are equivalent for pool excitation. It might well be that hard-worked rods with fast repeating signals transmit smaller waves and release weaker pool stimulations. Hagins, Zonana & Adams (1962) investigated a slice of squid retina cut parallel to the receptors. If a well-localized light stimulus fell upon a small region of the receptors that had been light adapted it generated a smaller local response than when it fell upon a dark adapted region *in the same receptors*. Thus if in our experiments the in-phase threshold is somewhat higher than out-of-phase, it need not mean any break-down of the 'one quantum, one signal' rule; perhaps the hard-worked in-phase messengers knock more feebly upon the door of the pool. However, that enfeeblement, if indeed it occurs, lies scarcely above the level of detection achieved in this paper.

In Part I it has been shown that a background from which 99% of the rods had not caught a single quantum, nevertheless, raised the visual threshold 3 times. It is plain that the rise was the result of signals sent by the 1% of rods that caught a quantum—sending perhaps 100 signals to the summation pool, and thereby raising the level of 'noise' against which the threshold must be detected. Any detector in order to maintain its reliability must react to this situation by raising the critical coincidence number n for a response. The adaptation pool somehow does this and the familiar rise in rod threshold results from this raised requirement for rod signals, induced in the summation pool. It is easy to prove, as in Part I, that the threshold rise occurs partly in the pool. It is hard to prove that it occurs there entirely and that (below the onset of rod saturation) an absorbed quantum always elicits one rod signal. The experiments of Part II are consistent with this view, but they do not exclude the alternative very firmly.

The method used was to project a striped test flash upon a striped background either in-phase or out. It was found that with 0·25° bright and 0·25° dark bands of grating, the phase never made any difference to rod vision—the threshold was the same whether the bright test bar fell upon the bright (light adapted) rods of the background or upon the dark rods, their neighbours in the shade. This is consistent with the view that the pools are influenced only by the total flux of rod impulses received and not by the place in the receptive field of the summation pool from which the impulses came.

But the sharpness of this argument depends upon the sharpness of the grating on the retina. Though it is rather unlikely that in Crick's experiments involuntary eye movements smudged badly the background over the retina, yet some smudging must have occurred and it was worth repeating the work with a stabilized image. The same absence of phase effect was found but these experiments too may be called in question upon grounds of optical precision. It is certain that the wearing of the sucker device (Fig. 3*A*) will not improve the precision of the grating image on the retina, and it may be asked whether phase was without effect upon threshold because the image was too blurred. To this there are three replies, though not as strong as could be desired.

(i) When the grating was projected upon the fovea and observed by quickly changing the plane of polarization from horizontal to vertical and back, the grating looked sharp and very well contrasted. This is not a good way to measure the percentage modulation of a grating but it counts for something.

(ii) When the experiment of Fig. 5*A* was repeated using cones, not rods, as detectors the reduced results plotted in Fig. 5*B* were obtained and now phase is no longer insignificant. This experiment was conducted as in the case of rods, but the area of test flash was reduced to 1° and fell upon the fovea, both flash and background lights being white. In Fig. 5*B* the arbitrary units of light on each scale are different from those used for curve *A*.

It is plain that in *B* the dots lie consistently above the triangles so that thresholds are higher when bright bands of the flash fall upon the bright rather than the dark bands of the field.

It is natural to regard the summation pools for cones as so small that the majority lie entirely within a bright band or a dark band of the striped field. It would be mistaken, however, to suppose that the adaptation pool was entirely of similar dimension. For Alpern & Rushton (1965) have shown that the threshold for a 1° foveal flash may be greatly raised by a flash presented 50 msec later falling not upon the test area but upon the surrounding region within a radius of 4·5°. It would not be correct, therefore, to regard the dots and triangles of Fig. 5*B* as recording *independent* thresholds under light bars or dark bars. The fact that both bars lie within the same part of the cone adaptation pools must to some extent close the gap between dots and triangles, but it is hard to estimate the magnitude of this effect.

Despite these uncertainties, Fig. 5 permits a tentative limit to be placed upon the change in threshold for rod signals. Let x_1, x_2 be the in-phase, out-of-phase log threshold rise for rods when the actual log luminance on the retina of bright and dark background bars is J_1, J_2. Let the contribution to this log threshold rise by the pool be p, and by the rods be r_1, r_2.

Then $$x_1 = r_1+p, \quad x_2 = r_2+p, \quad \therefore x_1-x_2 = r_1-r_2.$$

Now from Fig. 5 the height of dots above triangles in *A* is $x_1-x_2 = r_1-r_2$, and in *B* it is something less than J_1-J_2, so we may say that (r_1-r_2) is only 10% of $(J_1 - J_2)$ or less. Now with *uniform* backgrounds of J_1, J_2 log luminance, the log threshold X_1, X_2 will be made up of p_1, p from the pools and r_1, r_2 as before from the rods. Thus

$$X_1 - X_2 = (r_1 - r_2) + (p_1 - p_2) = J_1 - J_2$$

from the observed Fechner relation. But $(r_1 - r_2)$ is only 10% of $(J_1 - J_2)$, as was seen in Fig. 5, thus not more than 10% of the rise in log thresholds X_1, X_2 is due to a change in the threshold for rod signals; at least 90% is due to a change in the threshold of the summation pool.

(iii) Similar results have been found with the isolated retina of goldfish by Wagner and Wolbarsht (personal communication and discussion by Wolbarsht, 1965). Already early in 1963 they had made careful measurements upon the threshold for single ganglion discharges essentially with in-phase and out-of-phase increment thresholds and found them the same. First, the receptive field of the ganglion was mapped and two places well separated within it found that had low and equal thresholds for a small well-focused light flash. Now one half of the receptive field was illuminated by a steady light and the other half left dark, the demarcation line falling midway between the two excitable places. Naturally the place that now lay in the light was found to have a raised threshold, but they found that the other place, still in the dark, had its threshold raised equally.

These results greatly strengthen the conclusions of this paper for there could be no smudging by eye movements, the optics were good, and the receptive fields were so large that it is likely that scattered and diffracted light can be nearly excluded. When that fine work is perfected and published it will give precision to the answers which have been attempted in this paper.

Note. Some of the results of this paper have already been quoted (Rushton, 1963).

SUMMARY

1. It is a familiar fact that if a fixed flash is projected upon a background that becomes brighter and brighter, the flash will appear fainter and finally become invisible. This paper investigates for rod vision what it is that becomes insensitive to the fixed flash.

2. Does rhodopsin catch fewer quanta, or is a greater catch needed to generate a rod signal or are more signals required for vision?

3. It was found that a background that raised three-fold the threshold of a superimposed flash was still so weak that not 1% of the rods caught one quantum from it. Obviously this 1% that 'saw' the background must somehow have raised the threshold of the 99% that did not.

4. It is concluded that a rod generates a signal whenever it catches a quantum, and the near coincidence of n signals arriving at the summation

pool is the criterion for seeing. The value of *n*, however, is not fixed but depends upon the flux of signals to the pool.

5. This view was supported by having as background a black–red grating whose bars subtended 0·25°, and as test flash a black–green grating of the same period. The flash could be presented in-phase (bright bar on bright bar) or out-of-phase (bright bar on dark bar). It was found that the threshold was the same in either position, both in experiments with voluntary fixation and in those with a stabilized image.

6. It follows that the rod threshold does not depend upon whether the actual rods tested lie in the light or in the dark. It does depend upon (*a*) the total flux of signals to the pool from the background for that determines the magnitude of *n*, and (*b*) the total flux from the test for that determines whether *n* has been reached. But in all these experiments the pool appears quite indifferent to the provenance of its signals.

My thanks are due as usual to Mr Clive Hood for his assistance in all practical aspects of the work, to Mr Crick for his observations under conditions of voluntary fixation, to Mr Sparrock for constructing the sucker equipment and to Dr Pirenne for reading the manuscript. I am also indebted to U.S. Public Health (N.I.N.D.B.) for Research Grant NB 03014–04.

REFERENCES

ALPERN, M. (1965). Rod-cone independence in the after-flash effects. *J. Physiol.* **176**, 462–471.

ALPERN, M. & RUSHTON W. A. H. (1965). The specificity of the cone interaction in the after-flash effect. *J. Physiol.* **176**, 473–482.

AGUILAR, M. & STILES, W. S. (1954). Saturation of the rod mechanism of the retina at high levels of stimulation. *Optica Acta*, **1**, 59–65.

BARLOW, H. B. (1957). Increment thresholds at low intensities considered as signal-noise discriminations. *J. Physiol.* **136**, 469–488.

BARLOW, H. B. (1963). Slippage of contact lenses and other artefacts in relation to fading and regeneration of supposedly stable retinal images. *Quart. J. exp. Psychol.* **15**, 36–51.

BARLOW, H. B. & SPARROCK, J. M. B. (1964). The role of after-images in dark adaptation. *Science*, **144**, 1309–1314.

BAUMGARDT, E. (1949). Les théories photochimiques classiques et quantiques de la vision et l'inhibition nerveuse en vision liminaire. *Rev. Opt.* **28**, 453–478, 661–690.

BOUMAN, M. A. & VAN DER VELDEN, H. A. (1947). The two-quanta explanation of the dependence of the threshold values and visual acuity on the visual angle and the time of observation. *J. opt. Soc. Amer.* **37**, 908–919.

DARTNALL, H. J. A., GOODEVE, C. F. & LYTHGOE, R. J. (1936). The quantitative analysis of the photochemical bleaching of visual purple solutions in monochromatic light. *Proc. Roy. Soc.* A, **156**, 158–170.

DARTNALL, H. J. A., GOODEVE, C. F. & LYTHGOE, R. J. (1938). The effect of temperature on the photochemical bleaching of visual purple solutions. *Proc. Roy. Soc.* A, **164**, 216–230.

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

DONNER, K. O. (1959). The effect of a coloured adapting field on the spectral sensitivity of frog retinal elements. *J. Physiol.* **149**, 318–326.

HAGINS, W. A., ZONANA, H. V. & ADAMS, R. G. (1962). Local membrane current in the outer segments of squid photoreceptors. *Nature, Lond.*, **194**, 844–847.

HECHT, S. (1937). Rods, cones and the chemical basis of vision. *Physiol. Rev.* **17**, 239–290.

HECHT, S., SCHLAER, S. & PIRENNE, M. H. (1942). Energy, quanta and vision. *J. gen. Physiol.* **25**, 819–840.

LE GRAND, Y. (1956). 3. L'Espace visuel. *Optique Physiologique.* Ed. de la Revue d'Optique. Paris.

LE GRAND, Y. (1957). *Light, Colour and Vision.* London: Chapman and Hall.

LYTHGOE, R. J. (1940). The mechanism of dark adaptation. A critical resume. *Brit. J. Ophthal.* **27**, 21–43.

PIRENNE, M. H. (1958). Some aspects of the sensitivity of the eye. *Ann. N.Y. Acad. Sci.* **74**, 377–384.

PIRENNE, M. H. (1962). Contribution to *The Eye*, ed. H. DAVSON, vol. 2. New York: Academic Press.

RUSHTON, W. A. H. (1956). The rhodopsin density in human rods. *J. Physiol.* **134**, 30–46.

RUSHTON, W. A. H. (1963). Increment threshold and dark adaptation. *J. opt. Soc. Amer.* **53**, 104–109.

RUSHTON, W. A. H. & WESTHEIMER, G. (1962). The effect upon the rod threshold of bleaching neighbouring rods. *J. Physiol.* **164**, 318–329.

VAN DER VELDEN, H. A. (1944). Over het aantal lichtquanta dat nodig is voor een lightprikkel bij het menselijk oog. *Physica,* **11**, 179–189.

DE VRIES, HL. (1943). The quantum character of light and its bearing upon threshold of vision, the differential sensitivity and visual acuity of the eye. *Physica,* **10**, 553–564.

WOLBARSHT, M. L. (1965). Physiology and experimental psychology of colour vision. *Ciba Foundation Symposium.*

YARBUS, A. L. (1956). The perception of a stabilized retinal image. *Biofizika,* **1**, 435–437.

YARBUS, A. L. (1957*a*). A new method of studying the activity of various parts of the retina. *Biophysics,* **2**, 165–167.

YARBUS, A. L. (1957*b*). The perception of an image fixed with respect to the retina. *Biophysics,* **2**, 683–690.