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VISUAL ADAPTATION OF THE RHODOPSIN RODS IN THE FROG'S RETINA

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SUMMARY

1. The threshold of the discharge from single ganglion cells in the excised and opened frog's eye has been measured with on/off stimuli and test parameters that make it possible to activate the rhodopsin rods only. The test stimuli have been restricted to the central part of the receptive field, where no nervous reorganization can be observed with changes in the state of adaptation.

2. When such thresholds and the intensities of the background lights are expressed in terms of the number of quanta absorbed per unit time, it is found that three factors can be correlated with the thresholds measured in various states of light- and dark-adaptation: (i) the intensity of a steady background, (ii) the rate of regeneration of rhodopsin, and (iii) the amount of metarhodopsin II present in the rods.

3. The threshold is found to be proportional both to the intensity of a background and to the rate of regeneration, whereas there is a linear relationship between the logarithm of the threshold and the amount of metarhodopsin II.

4. The presence of metarhodopsin elevates all thresholds, the absolute threshold, increment thresholds and the thresholds elevated by regenerating rhodopsin in the same way.

5. The saturation of the rods at high background intensities is found to be correlated with the accumulation of significant amounts of metarhodopsin in the rods, caused by the bleaching effect of the background.

6. The effect of metarhodopsin on the threshold is independent of the amount of rhodopsin present in the rods.

7. The combined effect of all three factors can be expressed in a general formula, given as eqn. (7) on p. 74.

8. A background not only reduces the signals from the rods illuminated, but also those from neighbouring unilluminated rods. This effect is rapidly decreased with increasing distance from rods covered by the background. This kind of lateral spread in the retina probably occurs also when the

rate of regeneration affects the threshold. The effect of metarhodopsin, on the other hand, appears restricted to those receptors that contain this substance.

INTRODUCTION

The complexity of the structure and the synaptic connexions of the vertebrate retina has histologically been amply demonstrated (cf. e.g. Cajal, 1894; Polyak, 1941; Sjöstrand, 1959; Pedler, 1965). Functionally, the pathways of excitation and inhibition that follow from the structure, give rise to patterns of activity in the fibres of the optic nerve that are specifically dependent on rather complex stimulus parameters, as demonstrated, for instance, in the work on the frog retina by Lettvin, Maturana, Pitts & McCulloch (1961). In a study of the effects of light- and dark-adaptation, where the changes in the physiological state of the receptors are especially investigated, it may then appear less appropriate to use the discharge from the retinal ganglion cells or from their nerve fibres as an index. The same objection naturally applies to the use of the e.r.g. or psychophysical measurements of sensitivity. However, experiments on adaptation, and especially so on dark-adaptation, must utilize methods of recording that give reliable and comparable readings for considerable lengths of time. This condition is so far not easily combined with various methods of intraretinal recording, which otherwise would possibly give a more direct approach to the problem.

By the use of certain kinds of stimuli it is, however, possible to reduce the complexity of the input–output relations in the pathway from receptors to ganglion cells. In this way an attempt can be made to give a fairly precise interpretation of the origin of the sensitivity changes observed in light- and dark-adaptation.

In the frog the receptive fields of the on-, on/off- and off-units (classes I, III and IV in the terminology of Lettvin *et al.* 1961) are known to have a practically circular central area, where the product of stimulus area and intensity at threshold is constant with stationary stimuli flashed on and off (Hartline, 1940; Barlow, 1953). According to Hartline (1940) and Barlow (1953) this area has a diameter of about 0·2 mm. In our experience, however, the product of area and intensity remains constant to diameters of 0·4–0·5 mm in many cases. Because the area–intensity relationship remains unchanged within this central area during light- and dark-adaptation, it appears that the connexions between the receptors and the ganglion cell are not altered during the sensitivity changes involved (Donner & Reuter, 1965). It also follows that here the receptors of a certain type all have the same chance of activating the ganglion cell when they are excited by stationary stimuli flashed on and off.

Within the central area of the receptive field both rods and cones contribute to the discharge, the type of receptor activated being dependent on the state of adaptation and the wave-length and intensity of the stimulus (Donner & Rushton, 1959*a*, *b*; Donner, 1959). However, the use of test lights of wave-lengths close to 500 nm and, in light-adaptation, of backgrounds of long wave-lengths makes it possible to activate the rhodopsin rods only in threshold measurements during most phases of both light- (Donner, 1959) and dark-adaptation (Donner & Reuter, 1965).

Usually, thresholds of this kind are expressed in terms of the intensity of the stimulating light incident on the retina. In the frog eye, especially so during dark-adaptation, there are two factors that from an analytical point of view make this less satisfactory: (i) Photomechanical movements occur (cf. Walls, 1942; Detwiler, 1943; Duke-Elder, 1958) which affect the fraction of the incident light reaching the receptors so that more light is admitted to the rods when the retina is in the dark-adapted state. The quantitative effect of this migration of melanin pigment from the pigment epithelium has been experimentally measured by Bäck, Donner & Reuter (1965). (ii) During dark-adaptation from a light-adapted state where practically all the rhodopsin in the rods has been bleached, there is a continuous increase in the density of rhodopsin that thus directly causes an increase in the fraction of the incident light absorbed by the rods.

Consequently in order to get comparable values for the actual receptor sensitivities, thresholds should be expressed in terms of quanta absorbed per unit time (Donner & Reuter, 1965).

In micro-electrode recordings from single ganglion cells or nerve fibres of excised and opened frog's eyes and with test parameters as defined above, the following quantitative relations have been found to hold between the threshold, backgrounds of light of various intensities and the different states of bleaching and rhodopsin regeneration in the rods (Donner, 1959; Donner & Reuter, 1965, 1967).

1. *The effect of a background.* In this case the same relation holds between threshold and background as in the human eye, as originally formulated by Fechner (1860), and can thus be expressed in the same way as for human vision (cf. Rushton, 1965*a*)

$$\Delta J = \Delta J_0(1 + J/J_D), \tag{1}$$

where ΔJ is the threshold intensity, J the background intensity, J_D the intensity of the 'dark light' (i.e. the visual effect of some intrinsic activity or state that sets a lower limit to the increment threshold) and ΔJ_0 the absolute threshold. All these units are expressed here, in terms of quanta absorbed per rod per second. This does not, however, make any difference when compared to the conventional way of expressing the intensities as

long as the background is weak enough not to reduce significantly the amount of rhodopsin in the rods. In the frog (Donner, 1959) this relation has been found to hold for the rods over a range of background intensities of at least 4 log units.

2. *Dark-adaptation.* In this case we have been able to distinguish between two effects (Donner & Reuter, 1965, 1967):

(i) The effect of bleaching, where the presence of metarhodopsin (most likely metarhodopsin II) in the rods is paralleled by a quantitatively corresponding increase of the logarithm of the threshold of the ganglion cell discharge (the metarhodopsin effect). This relation can be written (Donner & Reuter, 1967) as

$$\Delta J = \Delta J_0 . 10^{aM_{II}}, \tag{2}$$

where the symbols are the same as in eqn. (1) and where M_{II} denotes the amount of metarhodopsin II present in the rods and a is a constant. Metarhodopsins I and II are the first more stable photoproducts of rhodopsin and exist in a tautomeric equilibrium, which depends, among other factors, on temperature and pH (Matthews, Hubbard, Brown & Wald, 1963). Metarhodopsin shows an exponential decay in the retina, the time constant being about 6·5 min at $+15°$ in the rods of Finnish *Rana temporaria* (Donner & Reuter, 1967).

(ii) The effect of the synthesis of rhodopsin, where the threshold is increased in proportion to the rate of regeneration (the regeneration effect). We may therefore write, by analogy with eqn. (1)

$$\Delta J = \Delta J_0 (1 + J_R/J_D), \tag{3}$$

where J_R is the 'equivalent background' produced by the rate of synthesis of rhodopsin. It is, however, very much weaker in its effect than a background of real light if the comparison is based on the effects produced by the synthesis of a rhodopsin molecule and by the absorption of a quantum of light (Donner & Reuter, 1965).

Strictly, the equations (1)–(3) are, however, valid only as descriptions of the sensitivity changes occurring under the conditions of the relevant experiments. Thus eqns. (1) and (2) refer to experiments on initially fully dark-adapted eyes; and eqn. (3) applies to dark-adaptation of an initially fully bleached eye during the later stages of adaptation when the more rapid metarhodopsin effect is absent. The question of the general validity of these expressions is then open and can only be solved in experiments where the sensitivity-reducing effects occur together in a quantitatively known manner. The present paper is an attempt to test experimentally various combinations of these effects with the aim of making it possible to describe the adaptation process of the rhodopsin rods in a general formula.

It will be evident to the reader that this work is highly indebted to Rushton's outstanding work on adaptation in the human eye, with its precise statement of the quantitative aspects of the problem (cf. Rushton, 1965*a*, *b*).

METHODS

Preparation and recording. The excised and opened eyes of common frogs (*Rana temporaria* from S. Finland) were dissected and treated according to our standard procedure (for a detailed description see Donner & Reuter, 1962). Recording from single ganglion cells or nerve fibres on the retinal surface was carried out with glass micropipettes filled with 3 M-NaCl, with the eye resting in a chamber, which made it possible to keep the temperature

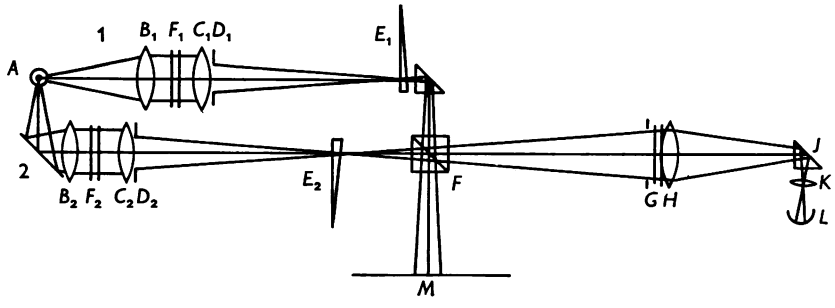

Fig. 1. The optical system. The test stimuli were given through channel 1, the bleaching exposures and the backgrounds were provided by channel 2. *A*, xenon arc, which provides light for both channels that are united at the beam-splitting cube *F*. The lens *K* images the lens *H* on the retina in the opened eye *L*. The interference filters were inserted at F_1 and F_2; the intensity of the light was controlled by wedges E_1 and E_2, and by neutral filters at F_1 and F_2 and at *G*. The size and shape of the fields on the retina could be controlled by stops at D_1 and D_2. These could be moved both horizontally and vertically and thus their position on the retina could be controlled. The test flashes were given by a shutter at E_1 operated by hand. An image of both fields was obtained on the screen at *M*.

constant and to bring about controlled temperature changes (Donner & Reuter, 1967). All the experiments reported here were carried out at a temperature of 13–14° C. When needed, rhodopsin measurements were carried out by extraction of the retina with digitonin (see Reuter, 1966).

Optics. The optical system used for stimulation, for the production of backgrounds of various intensities and for bleaching is shown in Fig. 1. The light source (*A*) was a high pressure xenon arc (Osram XBO, 900 W). The light passed along two pathways (1 and 2), each with arrangements for inserting interference and neutral filters (at F_1 and F_2 between lenses B_1 and C_1 and B_2 and C_2) and with neutral wedges E_1 and E_2 for intensity adjustments. Double heat filters were used at B_1 and B_2. At the beam-splitter *F* the pathways were united, passing through lenses *H* and *K* to the eye at *L*. On the retina an image of lenses C_1 and C_2 was formed; just in front of these there were holders (D_1 and D_2) for the insertion of stops to restrict the size of the field to that desired. These were adjustable, both vertically and horizontally, with micrometer screws. Part of the light passed through the beam-splitter to a screen at *M*, where a magnified image of the field projected on the retina was obtained. This made it possible to map the receptive fields and to record their position on the retina. In the case of dark-adapted eyes, when the sensitivity is extremely high, threshold measurements required an insertion of neutral filters at *G* attenuating the light by 6–8 log units.

Double interference filters (Schott & Gen., DIL) with half-bandwidths of 8–10 nm were used which had a much greater spectral purity of the light transmitted than ordinary interference filters (see e.g. Dodt, 1957). The density of the neutral filters and the density gradient of the neutral wedges were calibrated with a spectrophotometer at the wave-lengths corresponding to the interference filters used. This was especially important in measurements of the increment threshold curve.

The intensities of the lights were calibrated in terms of quanta with the aid of a vacuum thermocouple of known sensitivity and a sensitive galvanometer. The bleaching lights used were further empirically calibrated by measurements of their actual bleaching power on the rhodopsin in eye-bottoms with the retina *in situ*, as described by Bäck *et al.* (1965). This procedure also made it possible to calculate the quantum efficiency of the bleaching of rhodopsin in the rods (see below).

RESULTS

The number of molecules of rhodopsin and the quantum efficiency of bleaching in frog rods. Because much of the quantitative aspects of the results and arguments to be presented depends on the value assumed for the actual number of rhodopsin molecules in the rods, as well as on the quantum efficiency of rhodopsin when bleached *in situ* a brief mention will here be made of the evidence available and of the data that will be used in the subsequent analysis of our results.

We have calculated the number of rhodopsin molecules in a rod assuming that the molar extinction of rhodopsin is 40,600 at 500 nm, which is the value given by Wald & Brown (1953) for cattle rhodopsin. This value is probably valid for frog rhodopsin also, because in our measurements frog rhodopsin in a solution containing hydroxylamine bleaches in the same way as cattle rhodopsin, so that the density loss at 500 nm is the same as the density increase at 370 nm within 2 %. Previously we have found (Donner & Reuter, 1965) that digitonin extracts, after double extractions, contain amounts of rhodopsin corresponding to an average density of 0·2 when spread over a surface equal to that occupied by the retina. Over an area of 1000 cm^2 there are thus 0·2/40,600 moles of rhodopsin, and hence in 1 mm^2 $0{\cdot}2 \times 10^{-5}/40{,}600$ (see Brown, Gibbons & Wald, 1963). Our measurements on fresh, isolated retinae show that in Finnish *Rana temporaria* there are about 20,000 rhodopsin rods/mm^2. It then appears that each rod contains $0{\cdot}2 \times 10^{-5}/40{,}600 \times 2 \times 10^4$ moles. When multiplied by Avogadro's number this gives $1{\cdot}5 \times 10^9$ molecules of rhodopsin. Hubbard (1954) made a calculation based on the data given by Broda, Goodeve & Lythgoe (1940) and obtained the value $2{\cdot}1 \times 10^9$ molecules, a result that should apply to English *R. temporaria*. Now the outer segment of Finnish frogs measures about 40–45 μ in length (Donner & Reuter, 1965), compared to 55 μ in English frogs (Denton, 1959). This difference in length fully explains our lower value, if a constant amount of rhodopsin per unit length of rod outer segment is assumed. We shall use the value $1{\cdot}5 \times 10^9$ in the subsequent presentation, being a value only half of that

previously used by us (Donner & Reuter, 1965, 1967), which was based on the data given by Wolken (1961).

The quantum efficiency, defined as the ratio between the number of molecules bleached and the number of quanta absorbed can be calculated from the data obtained in connexion with the calibration of the bleaching lights used. When bleaching the rhodopsin *in situ* in the retina we find values for the quantum efficiency around 0·6. This is assuming that 20 %

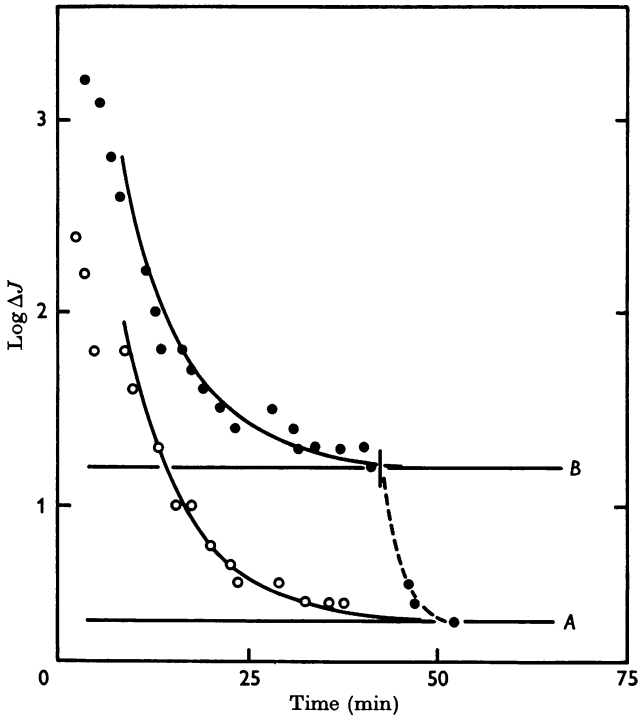

Fig. 2. On-thresholds of an on/off-unit in an initially fully dark-adapted eye, measured with a 0·35 mm circular test field of 513 nm, after bleaching 2 % of the rhodopsin. Open circles: without a background, the absolute threshold is shown by the line *A*. Filled circles: in the presence of a 639 nm background that elevated the threshold before the bleach to *B*. Continuous curves: exponentials with time constant 8·7 min.

of the incident light is reflected from the retinal surface or scattered in the tissues in front of the receptors. In solution we have obtained a value of 0·56. The results are in good agreement with earlier data, thus Wald & Brown (1953) give the value 0·58 and Kropf (1967) 0·66 for cattle rhodopsin in solution. It thus appears that the quantum efficiency for rhodopsin *in situ* is approximately the same as in solution.

The metarhodopsin effect in the presence of a background. If the effect

of bleaching a small percentage of the rhodopsin in the rods is measured in the same retinal unit, first in a condition of full dark-adaptation and then in the presence of a background, it is possible to compare the results in order to see how the sensitivity-reducing effects of background and metarhodopsin combine. Such an experiment is illustrated in Fig. 2. Here the lower set of points (open circles) represents the measured recovery in full dark-adaptation of the on-effect of an on/off-unit after bleaching 2% of the rhodopsin with a light of wave-length 553 nm. The absolute threshold is indicated by the line A. On the other hand, recovery of the same unit after an identical 2% bleach in the presence of a weak red (639 nm) background, which raised the threshold to line B, is shown by the filled circles. When the threshold has returned to its initial level the background was removed (indicated by short vertical bar at 42 min) and there was a rapid recovery to the former dark-adapted level A.

With the exception of the first 7–8 min after the bleach when the thresholds are determined by the cones (as can be shown by spectral sensitivity measurements at this stage) both adaptation curves can be described by the same exponential (Fig. 2, continuous curves) on a linear scale of ordinate with a half-return time of 6 min (time-constant 8·7 min), and thus by a curve that describes the decay of metarhodopsin at the temperature of this experiment (13–14° C) (Donner & Reuter, 1967). But in the case of the results with the background the curve has been slid vertically upwards by 0·8 log units, i.e. by the same amount as the background elevated the threshold with no metarhodopsin present. Consequently, it appears that eqn. (2) holds in this case not only, as would be expected, in full dark-adaptation, but also in the presence of a background, provided that the absolute threshold (ΔJ_0) in eqn. (2) is replaced by the threshold measured in the presence of the background light. But the value of this is given by eqn. (1). Thus we may write

$$\Delta J = \Delta J_0(1 + J/J_D).10^{aM_{II}} \qquad (4)$$

which, in the absence of a background J, is reduced to eqn. (2) and thus describes the metarhodopsin effect both without and in the presence of a background. On the other hand, this expression, in the absence of any metarhodopsin produced by a preceding bleach, is reduced to eqn. (1), and thus adequately describes the effects of backgrounds of various intensities.

Here the sensitivity-reducing effect of a background has been described without reference to the obvious fact that it also bleaches some of the rhodopsin in the rods and that this continuous process produces metarhodopsin that decomposes at a fairly slow rate. Some metarhodopsin will thus always be present in the rods with any background. A calculation of

the actual amounts can easily be done if it is assumed that the rhodopsin density in the rods is unchanged by the background, which is true so long as only weak or moderate intensities are used. In that case the number of quanta caught per rod per second multiplied by the quantum efficiency (γ) gives directly the rate of formation of metarhodopsin. At equilibrium this and the rate of decomposition should be equal. The decay of metarhodopsin proceeds exponentially (Donner & Reuter, 1967) and we may write

$$M = M_0 e^{-t/k},$$

where M is the concentration of metarhodopsin (expressed as molecules per rod) at time t, M_0 the initial amount and k the time constant, which at 13° C is about 8·7 min. The rate of decomposition is hence given by

$$-dM/dt = 1/kM_0 e^{-t/k} = M/k.$$

If J denotes the number of quanta caught per rod per second, then at equilibrium

$$J.\gamma = M/k. \tag{5}$$

Here J is directly obtained in each experiment from the intensity and wave-length of the background used, $k = 8{\cdot}7$ min and $\gamma = 0{\cdot}6$ and thus M can be calculated. In Fig. 2 the background has elevated the threshold by 0·8 log units. This effect in our experiments corresponds to an absorption of approximately 10 quanta/rod/sec. Hence the value of M in this case is about $3{\cdot}1 \times 10^3$ molecules of metarhodopsin per rod. At the temperature of this experiment, 13–14° C, approximately 45 % of this amount is in the metarhodopsin II state (Donner & Reuter, 1967). We have found (see below, p. 74) that the constant a in eqns. (2) and (4) is of the order of 10^{-7}, which means that amounts of metarhodopsin II less than 10^6 molecules per rod give an elevation of threshold smaller than 0·1 log unit, and thus an effect that cannot be detected with the accuracy of the threshold measurements used here. In the case of Fig. 2 we can then safely conclude that the metarhodopsin effect is not involved in the reduction of sensitivity caused by the background. We can further conclude that the rapid, but not instantaneous, dark-adaptation phase after the background has been removed, cannot be explained as an effect of metarhodopsin. This kind of rapid sensitivity changes is not included in our present description of the dark-adaptation process.

It is, however, obvious that at high background intensities sufficiently high metarhodopsin concentrations are reached for their effect to become significant. This will, according to eqn. (4), result in a situation where the rod increment thresholds at high background intensities become higher than expected on the basis of the normal linear relation between threshold

and background. It would thus constitute an effect similar to the saturation of the rods in the human retina as described by Aguilar & Stiles (1954). Figure 3 shows the result of an experiment where the increment thresholds of the on-effect of an on/off-unit have been measured in the presence of a 615 nm background covering the whole retina. The test light used was a 0·35 mm spot of wave-length 513 nm centred on the receptive field. With these wave-lengths of the test spot and the background the increment

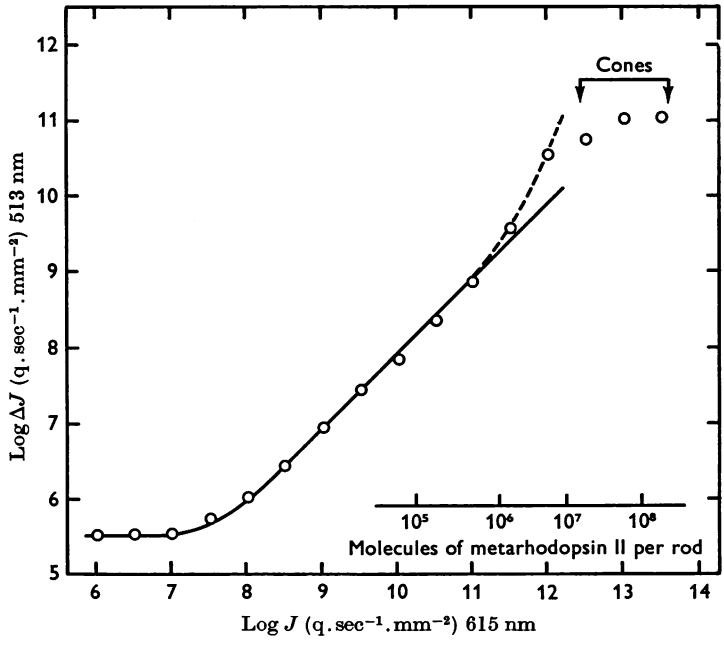

Fig. 3. Increment thresholds for the on-effect of an on/off-unit of an initially fully dark-adapted eye (open circles). Test stimulus: 0·35 mm circular field, 513 nm. Background: 615 nm, covering the whole retina. Second scale on the abscissa gives the number of molecules of metarhodopsin II per rod (see the text). Interrupted line: expected course of the increment threshold curve when the effect of metarhodopsin is accounted for. The intensities are given in terms of quanta incident on the retina.

thresholds are determined by the rhodopsin rods over a large range of background intensities (cf. Donner, 1959). In Fig. 3 the thresholds over a range of 4–5 log units are seen to follow the relation to be expected from eqn. (4) as given by the 45° straight line drawn, if there is no effect of accumulated metarhodopsin. But at the highest intensity levels there is a deviation from this so that the thresholds become higher than would be expected. This deviation cannot be explained on the assumption that the cones begin to determine the threshold; in that case the deviation from the straight line would be in the opposite direction. It is, however, true that

at the very highest background intensities used the spectral sensitivity changed and was apparently determined by the cones, as indicated in Fig. 3.

A calculation of the metarhodopsin equilibria reached at the different background intensities used in Fig. 3 has been carried out. The result is given as the metarhodopsin II-scale on the abscissa. It is based on direct measurements of the actual bleaching power of the 615 nm background at its maximum intensity with the rhodopsin content of opened eyes as an index. This gives a reliable value for $J.\gamma$ in eqn. (5) and makes it possible to calculate the amount of metarhodopsin present. The metarhodopsin II-scale has then been used to calculate the expected deviation from the Fechner line with the aid of eqn. (4) using the value 10^{-7} for the constant a. It is found that the later part of the rod increment threshold curve can be expected to follow the course of the interrupted line in the experiment of Fig. 3. This gives a quantitative explanation to the observed deviation from Weber's law up to the point where we know that the cones begin to determine the thresholds.

The metarhodopsin effect during the regeneration of rhodopsin. The observed equivalence in the effect on the threshold between a background and J_R, the rate of regeneration of rhodopsin (Donner & Reuter, 1965) suggests that the validity of eqn. (4) can be extended to the case in which effects of metarhodopsin and regeneration occur simultaneously. As such this seems likely, but there is at least one important difference between the two cases, which makes it necessary to test the validity of this inference experimentally. In the previous experiments (Figs. 2 and 3) and in our earlier work (Donner & Reuter, 1967) the effect of metarhodopsin has been evaluated for fully dark-adapted rods only, containing the full amount of rhodopsin except for the small fraction bleached. It is hence possible that the efficiency of metarhodopsin as a sensitivity-reducing agent varies with the rhodopsin content of the rods in some way. More specifically it can be stated that, if the effects of background and regeneration are equivalent as stated above, we can also write by analogy with eqn. (4)

$$\Delta J = \Delta J_0(1 + J_R/J_D)10^{aM_{II}}. \qquad (6)$$

This should describe the course of rod dark-adaptation, including effects both of metarhodopsin and regeneration.

The kind of experiment carried out is illustrated in Fig. 4. The eye was initially fully light-adapted so that only about 2 % of the full amount of rhodopsin was present in the rods (Reuter, 1966). This was the situation at 0 min. The eye was then left to dark-adapt and the thresholds of the unit under study were measured. Under our standardized conditions the eye is known to regenerate rhodopsin according to Reuter's (1964, 1966) curve.

At 42–43 min a 60 sec 553 nm exposure was given, calibrated to bleach 6 % of the *full* amount of rhodopsin. This exposure must for two reasons be given with a higher intensity than in the case of a fully dark-adapted eye. First, at 42 min the rods contained only 45 % of the full amount of rhodopsin. It was thus necessary to bleach 13 % of this quantity in order to remove 6 % of the full amount. Secondly, at 42 min the rods were still screened by the melanin pigment of the pigment epithelium, which made it necessary to increase the light intensity a further 3 times (Bäck *et al.* 1965).

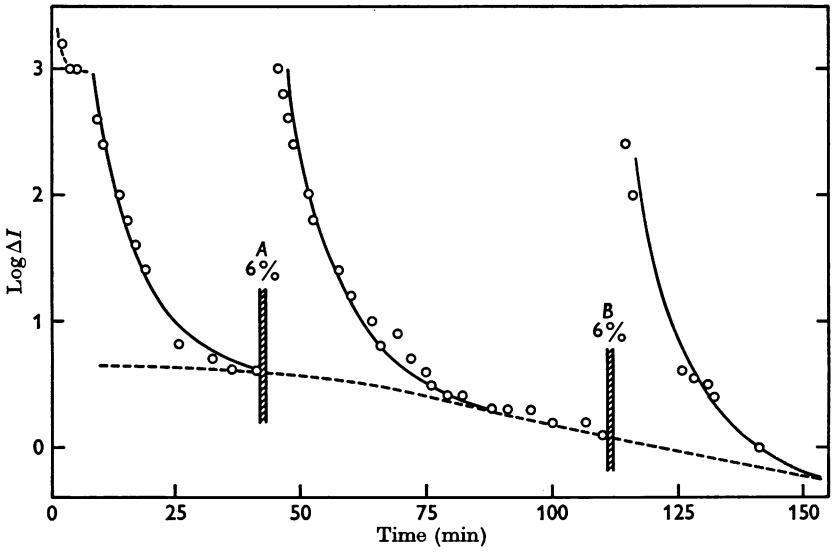

Fig. 4. Off-thresholds of an off-unit in an initially fully light-adapted eye during subsequent dark-adaptation. 0·35 mm circular field, 513 nm. At *A* and *B* 6 % of the full amount of rhodopsin has been bleached. Interrupted line indicates the assumed course of the dark-adaptation curve without the bleaches. Continuous curves, exponentials with time-constant 8·7 min with the interrupted line as the base line.

After the 6 % bleach at 42–43 min the threshold of the unit in Fig. 4 showed a rapid recovery and reached a sensitivity level expected without the bleach at about 85 min. At 111–112 min a new bleach was given, again calibrated to bleach 6 % of the full amount of rhodopsin. Now a much weaker light was used to bleach the same number of rhodopsin molecules, because the density was higher and screening by the melanin pigment was absent. The recovery after this was followed to a point when it could be assumed that most of the metarhodopsin effect had disappeared. At 141 min contact with the cell was lost.

It appears that in this experiment there were three periods of meta-rhodopsin decomposition: (i) after removal of the initial light-adapting

light at 0 min, (ii) after the first bleach at 42–43 min, and (iii) after the second bleach at 111–112 min. In the first case the rods at 0 min contain about 10 % metarhodopsin (10 % of the full amount of rhodopsin) as can be estimated from Reuter's (1966) data on the bleaching conditions during our standard light-adaptation procedure. In the two latter cases the short bleaches were calibrated to give 6 % metarhodopsin at the end of the bleach as stated above.

During all these three periods of metarhodopsin decay and the corresponding periods of recovery of rod sensitivity, there was a continuous synthesis of rhodopsin and hence a gradual increase in the rhodopsin content of the rods. The average rate of regeneration and the rhodopsin content is given by Reuter (1966) as a function of time in the dark. Because, however, short and moderate bleaches of the kind used in Fig. 4 might in some way affect the rate of synthesis, it was necessary to examine this possibility before proceeding further with the analysis of the results. For this purpose rhodopsin measurements were carried out with the following procedure.

Both eyes of the same frog were excised, opened and light-adapted according to our standard procedure. After 40 min of subsequent dark-adaptation one of the eyes was given a 558 nm exposure for 48 sec, calibrated to bleach about 8 % of the full amount of rhodopsin. Then both eyes were either immediately extracted for measurement of the rhodopsin, or given a further 20 min of dark-adaptation before the extraction. Because both eyes of the same frog have been found to contain very nearly the same amount of rhodopsin at the same stage of dark-adaptation (Zewi, 1939; Reuter, 1966) a comparison of the amounts of rhodopsin in each pair gives the amount removed by the bleaching light. Provided the rate of regeneration remains constant in both eyes, which during a normal uninterrupted dark-adaptation is true between 30 and 70 min, the absolute difference in the rhodopsin content between the two eyes in a pair is the same both when the extraction has been carried out immediately after the bleach and when the eyes have been allowed a further 20 min of dark-adaptation. The following values were obtained:

Difference between unbleached and bleached eyes expressed as % of the full rhodopsin value

Extraction immediately after the bleach (%)	Extraction after 20 min of additional dark-adaptation (%)
	6·3
	7·5
9·6	14·2
4·1	4·2
10·6	9·4
Average 8·1	Average 8·3

Considering the great variability of the results, much significance cannot be attached to the good agreement between the average obtained. A substantial effect of the bleach on the rate of regeneration seems, however, to be excluded. The validity of this conclusion is restricted to the period between 30 and 70 min of dark-adaptation (at 13–14° C) when the

rate of regeneration is constant and apparently independent of the amount of free opsin in the rods (Reuter, 1966). This linear phase of rhodopsin regeneration, which normally ends at 70 min, may possibly be extended by a short bleach that removes some of the rhodopsin. In Fig. 4, for instance, 6 % had been bleached at 42–43 min. Thus with a rate of synthesis of 0·93 %/min (Reuter, 1966) the linear phase may last 6–7 min longer than normally.

Considering this—the general effect of the rate of regeneration (Donner & Reuter, 1965) and the increasing rhodopsin density in the rods during dark-adaptation as well as the screening effect of the melanin pigment of the pigment epithelium—it may be assumed that in the absence of any effect of metarhodopsin the unit of Fig. 4 would have dark-adapted along the curve given by the interrupted line. By analogy with Fig. 2 and with reference to eqn. (6), this line can be used as a base line for the construction of the curves that would be expected to describe the recovery of sensitivity in the three cases of metarhodopsin decomposition. The curves drawn in Fig. 4 (continuous lines) are thus exponentials drawn with the same scale of linear ordinates, but with the interrupted line as the 0-level at each instant. The time constant of these curves is 8·7 min, which is the time constant of metarhodopsin decomposition at this temperature. The thresholds after the bleaches at 42–43 min and 111–112 min represent recoveries after bleaching the same number of rhodopsin molecules and the curves drawn are the same. On the other hand, the curve drawn to describe the initial phase of rod adaptation (after 0 min) has, compared to these, been shifted horizontally in the figure about 3 min to the right to fit the points. This would suggest an initial metarhodopsin concentration of 8·5 % which is not far from the estimated 10 %.

The agreement between the curves and the experimental points shows that eqn. (6) can indeed be applied to describe dark-adaptation when both metarhodopsin and regeneration affect the threshold. And this can apparently be done using the same value of the constant a, irrespective of the rhodopsin concentration in the rods. It is clear that if there were a dependence between a and the amount of rhodopsin it would not even be possible to describe the recovery after a single bleach such as that between 42 and 43 min by a simple exponential of this kind, considering that at 50 min there is about 46 % of the full amount of rhodopsin (52 % minus the 6 % bleached), whereas at 75 min about 70 % is present.

The quantitative relation between metarhodopsin and the elevation of threshold from the experiment of Fig. 4 is shown in Fig. 5, where the increase in log threshold is plotted against the amount of metarhodopsin present. The squares refer to the period at the beginning of dark-adaptation, the open circles to the recovery after the bleach at 42–43 min and the filled circles to the recovery after the second bleach at 111–112 min. According to eqn. (6) the points would be expected to fall along the straight line A, which corresponds to the exponentials of Fig. 4. A consistent

deviation from this line is observed during the early phases of recovery, in a direction that suggests a sensitivity of the rods greater than that predicted. This may be caused by any of the following alternatives, or a combination of them. (i) The theoretical formulation is only an approximation that can be applied for small (< 2–3 %) amounts of metarhodopsin only. (ii) Some contributions from the cones increase the sensitivity at this stage. This is known to occur in the mesopic state especially with relatively

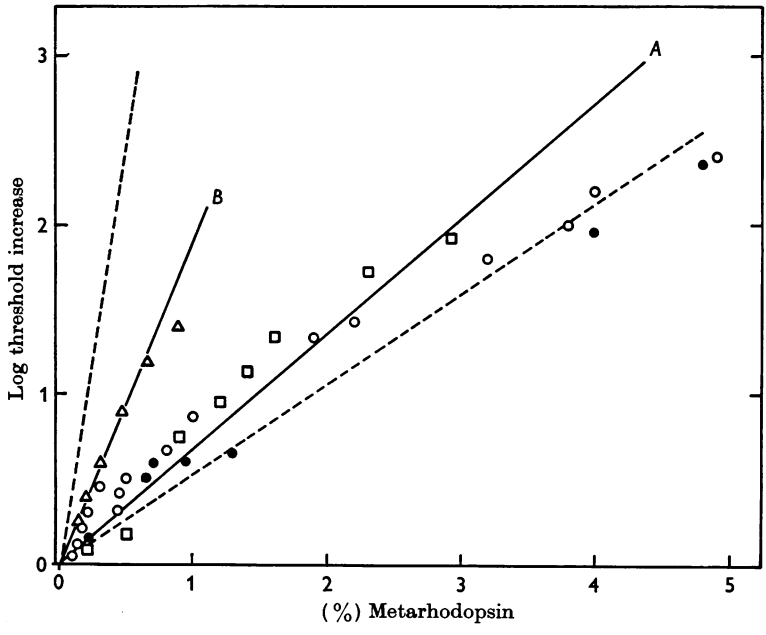

Fig. 5. The relation between log threshold increase and the amount of metarhodopsin in the rods. Line *A* corresponds to the exponentials drawn in Fig. 4, □ = the recovery after the end of light-adaptation, ○ = after the 6% bleach at 42–43 min, ● = after the 6% bleach at 111–112 min in Fig. 4. △ = thresholds from Fig. 2. Interrupted lines give the approximate limits for the relation between log threshold and metarhodopsin.

long wave-lengths of the stimulating light (Donner & Rushton, 1959*b*). (iii) Regeneration after small bleaches of this kind is somehow slightly changed and distorts the results. (iv) This rate of metarhodopsin decomposition may be slightly lower in the living eye than that observed in the isolated retina, which may become slightly acidic under the conditions used for the measurements (Donner & Reuter, 1967 and unpublished observation).

In Fig. 5 the second line (*B*) and the triangles give the corresponding data for the experiment of Fig. 2. Lines *A* and *B* are both seen to fall between the two interrupted lines in the Figure that represent the extremes

of the relation between metarhodopsin and log threshold increase, as found in a large number of experiments both during initially complete dark-adaptation, with backgrounds and during regeneration. In general it can thus be said that the rod threshold is raised 1 log unit by the presence of 0·35–1·9 % metarhodopsin (of the full amount of rhodopsin), in the majority of cases about 1 %, or in other words, by $0{\cdot}5–2{\cdot}8 \times 10^7$ molecules in each rod. Of this amount about 45 % will be in the metarhodopsin II state at 13–14° C (Donner & Reuter, 1967) and hence the value of the constant a in eqn. (6) will assume values between $4{\cdot}5 \times 10^{-7}$ and $0{\cdot}8 \times 10^{-7}$.

The summation of effects of background and regeneration. The fact that the rate of regeneration appears to affect the threshold in the same way as the presence of a background, as is evident from eqns. (4) and (6), makes it likely that there is a direct summation of these two effects when they occur together, in the same way as two superimposed backgrounds of real light add their effects on the threshold.

This question can be examined in dark-adaptation experiments, with the eye initially fully bleached. In such a case there is no metarhodopsin effect after 40–50 min of dark-adaptation (Donner & Reuter, 1965, 1967) and hence only the rate of regeneration determines the threshold. Furthermore, between 30 and about 70 min of dark-adaptation the rate of regeneration is constant. Application of real backgrounds of light and measurements of the increment thresholds during this phase of dark-adaptation gives results like that shown in Fig. 6 (circles). The experiment refers to the on-threshold of an on/off unit between 55 and 75 min of dark-adaptation. In this experiment there is a constant 'background' of regeneration to which is added a background of real light. If these quantities are directly additive the same increment threshold curve as in Fig. 3 should result, but displaced 45° up and to the right (cf. Rushton, 1965*a*, p. 30). This is indeed the case. Figure 6 gives the increment threshold curve of Fig. 3 correctly placed in relation to the intensity scales (interrupted line), the continuous curve being the same curve displaced 45° up and to the right to give the best possible fit to the points.

The curves of Figs. 6 and 3 are not, however, fully comparable because in the experiment of Fig. 3 the rods contained the full amount of rhodopsin, while in that of Fig. 6 only 60–70 % was present. Thus in this case a constant background of light should give a slightly smaller number of absorbed quanta per rod per unit time.

The general formula. The present evidence suggests that all sensitivity-reducing effects treated here can be united in a single formula that can be obtained by the combination of eqns. (4) and (6)

$$\Delta J = \Delta J_0 \left(\frac{1 + J + bJ_R}{J_D} \right) 10^{aM_{II}}, \tag{7}$$

where the symbols and units are the same as those used before. The rate of regeneration (J_R) is here multiplied by a constant b, which converts it to the same units as those used for the intensity of a real background. The value of this constant can be estimated on the basis of our previous finding (Donner & Reuter, 1965, fig. 7) that during the linear process of the regeneration process the threshold is elevated, on average 1·3 log units. The average rate of regeneration being 0·93 % per minute at this stage (Reuter, 1966), it can be calculated that this equals $0{\cdot}23 \times 10^6$ molecules per rod

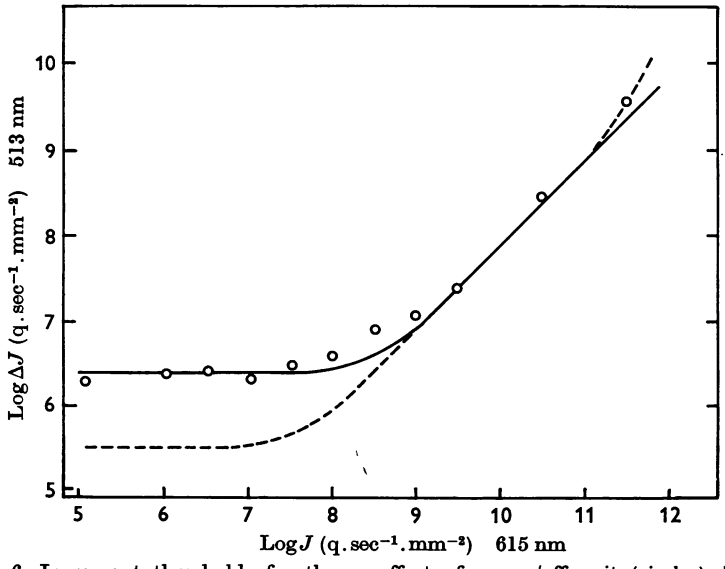

Fig. 6. Increment thresholds for the on-effect of an on/off unit (circles). Test stimulus: 0·35 mm circular field, 513 nm. The eye was initially fully light-adapted and the thresholds were measured during the period 55–75 min after the beginning of dark-adaptation. Background: 615 nm, covering the whole retina. Interrupted line: increment threshold curve from Fig. 3. Continuous line: same but slid 45° up and to the right to fit the points. The intensities are given in terms of quanta incident on the retina.

per second. From Figs. 3 and 6 it can be seen that a threshold elevation of 1·3 log units is produced by the absorption of 50–100 molecules per rod per second, which gives a value around 3×10^{-4} for the constant b in eqn. (7). This means that identical effects would be produced by the bleaching of a single molecule of rhodopsin and the synthesis of approximately 3000 molecules per unit time.

The lateral spread in the retina of the effects of adaptation. In the experiments on adaptation presented above and in most of our earlier work (Donner & Reuter, 1965, 1967) we have tried to eliminate the complication introduced by the possible occurrence of lateral spread of the effects of adaptation in the retina in two ways. First, we have kept all the rods over

a large retinal area in the same state of adaptation; and, secondly, we have used as an index of sensitivity the threshold of the ganglion cell discharge when stimulating only the central part of the receptive field, where all the rods have the same chance to contribute to the signal. The results have led us to differentiate between two kinds of sensitivity-reducing factors, on the one hand the effects of the rate of regeneration and of a background of real light and on the other hand the effect of metarhodopsin.

From the work of Lipetz (1961) on frog ganglion cells and Rushton & Westheimer (1962) on the human eye we know that lateral spread of the effect of a steady background occurs in the sense that the illumination of some rods will attenuate the signals also from neighbouring, dark-adapted rods. This raises the question whether such lateral spread is demonstrable in our experiments also and if so whether there is a difference in this respect between the two kinds of adaptation effects found. If that is the case it will clearly give further support to the idea of the different nature of the two processes.

The first object has been to see whether there is a lateral spread of the effect of a background and, if so, to compare the extent of this spread with the size of the central part of the ganglion cell receptive field. Such a comparison may show if the same cellular structures can be thought to mediate both of them. In Fig. 7*A*, as illustrated in the inset, a circular test light *T* of 0·11 mm diameter was centred on the receptive field. Thresholds were measured in the presence of a constant 553 nm background that covered almost the whole retina, but was excluded from the area covered by *T* by a black circular stop of radius *r*, concentric with *T*. Figure 7*A* gives the thresholds measured in relation to the radius of the stop. The threshold without the stop is given at 0 mm. It is seen that as soon as the black stop excludes the background light from the area covered by the test light, the threshold is lower and gradually sinks until the radius of the stop exceeds 0·3 mm. As will be argued below, it is unlikely that this result is due to stray light but is a result of a physiological spread of activity from the illuminated rods. This effect seems to be rapidly attenuated outside the illuminated area, which is in apparent contrast to the unattenuated summation of signals from all the rods within the central part of the receptive field of the ganglion cell.

A similar result is shown in Fig. 7*B*, where the threshold has first been measured along a line through the centre of the receptive field, showing a plateau of high sensitivity of about 0·3 mm diameter (open circles). This is in full agreement with similar results obtained by Hartline (1940) and Barlow (1953) and again shows that all the rods within this area have the same chance to contribute to the threshold signal. Then the test light (diameter 0·08 mm) has been placed in the centre of the receptive field, in

the position indicated by the arrow, and the threshold has been measured in the presence of a 0·11 mm illuminated spot of constant intensity placed in the positions before occupied by the test flash along the line. The corresponding thresholds (dots) have been plotted according to the position of the illuminated spot constituting the background. The threshold in the

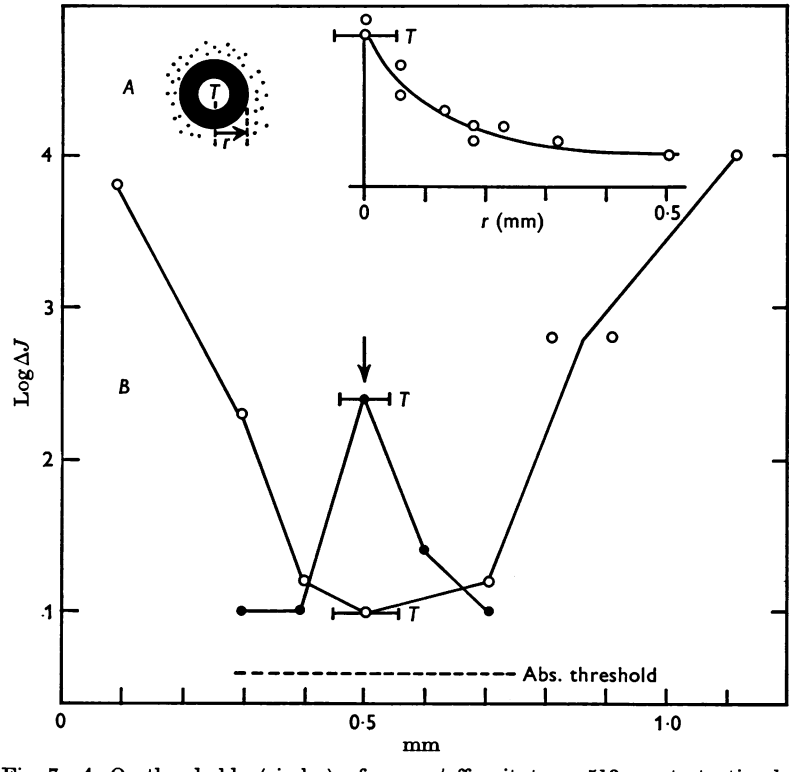

Fig. 7. *A*: On-thresholds (circles) of an on/off-unit to a 513 nm test stimulus (*T*) 0·11 mm in diameter in the presence of a 553 nm background covering the whole retina but excluded from the test area by a stop of radius *r* (see inset). The thresholds are given for different values of *r*.

B: Off-thresholds of an on/off-unit of an initially fully dark-adapted eye. Open circles: thresholds to 513 nm stimulus 0·11 mm in diameter in various positions (abscissa) along a line across the centre of the receptive field. Filled circles: thresholds to a 0·08 mm 513 nm test stimulus in the centre of the field (arrow) with a 553 nm 0·11 mm field as a background in different positions on the same line through the centre of the receptive field.

absence of the spot is shown by the line marked 'abs. threshold' in the Figure. The effect of the background on the threshold is very clear when the background spot and the test flash coincide, but the effect of the background spot again decreases very rapidly when moved away from this position.

It is evident that the background light does not affect equally the whole central part of the receptive field. The agreement between the size of the dendritic tree of the ganglion cells in the frog retina and the size of the central part of the receptive field (Lettvin *et al.* 1961; T. Reuter, unpubl. observations) suggests that the final summation of excitation from this central region occurs at the ganglion cell level. If the effects of adaptation reach this level in the retina one would be inclined to think that they would lower the sensitivity of the whole receptive field, as apparently is the case with effects from the inhibitory surround, which is activated when the illumination is *changed* in the area surrounding the central part of the receptive field (Barlow, 1953). We have previously described the effect of the inhibitory surround in dark-adaptation (Donner & Reuter, 1965) but this effect is excluded in the present experiments. It is thus likely that the lateral spread observed here is mediated by structures at a more peripheral level in the retina. In this context it is of interest to note that Brindley (1956) found a lateral spread of about 0·2–0·3 mm of the intraretinal electrical activity on illumination in the frog retina. In the tench retina Naka & Rushton (1967) reported that the S-potentials can spread laterally across the retina within a thin layer at about the horizontal cell level. The potentials suffer an exponential attenuation with distance; at a distance of 0·5 mm they are reduced to about 1/5.

The sensitivity-reducing effect of metarhodopsin, on the other hand, seems to have quite different properties regarding lateral spread in the retina as shown by the experiment in Fig. 8. In the left-hand part of Fig. 8 are given the absolute threshold (*A*) of the fully dark-adapted eye, the threshold of this unit (*C*) in the presence of a 553 nm background and the threshold (*B*) obtained with the same background but with a stop of 0·36 mm diameter that excluded the background from the area covered by the circular test field, 0·35 mm in diameter. The correct positioning of the stop and the test field was microscopically controlled after the experiment. After measurement of the thresholds *A*, *B* and *C*, the 553 nm background without the stop but now considerably increased in strength, was used to bleach 12 % of the rhodopsin in 60 sec over the whole retina and thus also over the area tested for sensitivity, and the recovery was followed (open circles). After full recovery, the same bleach was given again, but now excluding the light with the 0·36 mm stop from the area tested. This bleach was given twice, the second one after full recovery after the first (filled circles and squares).

The dark-adaptation after the bleach without the stop in the bleaching light (open circles), shows a clear division into a cone and a rod branch. The rod branch can be described by an exponential (continuous line) with a time constant of 8·7 min, in agreement with our previous findings and

with the time constant of the fading of metarhodopsin. The same curve, shifted 27·5 min to the left can be used roughly to describe the recovery after the two later bleaches with the bleaching light excluded from the test area. In both cases (filled circles and squares) and also to some extent in the dark-adaptation curve after the bleach without the stop, there is a deviation of the thresholds from the theoretical curve. This is most likely caused by the regeneration of the pigment bleached (Donner & Reuter, 1967). According to Reuter's (1966) model for the regeneration process in

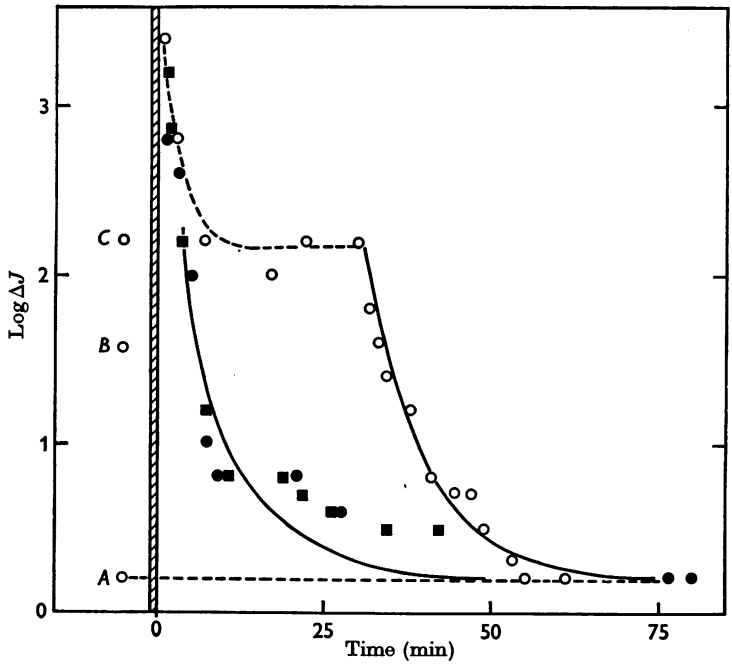

Fig. 8. Off-thresholds of an on/off-unit of an initially fully dark-adapted eye. Test stimulus: 513 nm, diameter 0·35 mm. Open circles: recovery after bleaching in 60 sec 12 % of the rhodopsin. Filled circles and squares: recovery after the same bleach but with the bleaching light excluded by a 0·36 mm stop from the area illuminated by the test stimulus. A = the absolute threshold, C = the threshold with 553 nm background covering the whole retina, B = the threshold with the same background but with the light excluded from the test area by a 0·36 mm stop, concentric with the test stimulus. Continuous curves: identical exponentials with a time constant of 8·7 min.

these eyes, the rate of regeneration is proportional to the amount of free opsin when more than 70 % of the rhodopsin is present. In the experiment of Fig. 8, 12 % has been rapidly bleached. At the end of the bleach most of the rhodopsin bleached will be in the metarhodopsin form, and because the decay of metarhodopsin is a more rapid process than regeneration from free opsin (time constants 8·7 and 32 min respectively) there will be

gradually increasing amounts of free opsin in the rods during the beginning of the dark-adaptation process.

The much faster recovery of the rods in the experiment of Fig. 8, when the bleaching light has been excluded from the test area, shows that the metarhodopsin effect in this case is very much weaker. If the two exponentials are taken to describe the recovery of rod sensitivity in both cases, it can be calculated from (2) that the effect with the bleaching light excluded from the test area corresponds to an amount of metarhodopsin only 4% of that produced when the bleach was not excluded from the test area. This amount is approximately what would be expected if the metarhodopsin effect did not show any physiological spread to neighbouring rods at all. This is because the occurrence of a certain amount of stray light illuminating the test area cannot be avoided, in spite of the fact that the bleaching exposure is prevented from illuminating it directly. Inspection of the image of the black stop on the retina with the aid of a dissection microscope and small neutral density filters inserted into the eyepiece gave about 5% as an approximate figure for the amount of stray light present.

It is illustrative here to compare this strong reduction of the metarhodopsin effect, brought about by the stop that excludes the bleaching light from the test area, with the preceding measurements of the increment thresholds on the same unit and exactly the same positions of the test field and the stop, as shown in the left-hand part of Fig. 8. Here the thresholds C and B differ by 0·65 log units. Considering the linear relation between the increment threshold and the background, this means that the threshold with the stop in the background is affected in a way corresponding to a background of uniform light 0·65 log units lower, in other words as if the background were reduced to about 22% of its former value. In the absence of any physiological lateral spread of the background effect it would thus be necessary to assume that 22% of the background intensity would occur as stray light on the test area, a value that completely disagrees with our estimates. A lateral spread of the sensitivity-reducing effect of the background must then have occurred. This is probably true regarding the effect or regeneration too, considering its equivalence with the effect of a background. The argument is strengthened by the fact, clearly seen in Fig. 8, that the effect ascribed by us to regeneration in the left hand curve was rather strong, although the bleaching of the receptors tested was very small, as stated above.

The metarhodopsin effect then seems to be restricted to those receptors only that have been exposed to light. Whatever the nature of this fast phase of recovery, it certainly appears on these grounds less 'neural' in origin than the other effects of adaptation.

DISCUSSION

The adaptation formula. The experimental combination in pairs of the three sensitivity-reducing effects described by eqns (1), (2) and (3) has enabled us to construct the general formula

$$\Delta J = \Delta J_0 \left(\frac{1 + J + bJ_R}{J_D} \right) 10^{aM_{II}} \tag{7}$$

with the symbols as given in the Introduction. The numerical value of the constant a has been found to vary in different experiments between $0{\cdot}8 \times 10^{-7}$ and $4{\cdot}5 \times 10^{-7}$, but we have been unable to correlate this variation with the amount of rhodopsin in the rods or with changes in the rate of regeneration or in the background. The value of the constant b has been found to be approximately 3×10^{-4}.

It appears that in this formulation the amount of rhodopsin in the rods does not enter as a factor of significance for adaptation. However, it should be observed that the thresholds are expressed here in terms of quanta absorbed by the receptors, which eliminates the purely physical effect of changes in the density of rhodopsin in the rods that changes their quantum catching power.

Although eqn. (7) has been found to describe the thresholds of the red rods during greatly varying states of light- and dark-adaptation, there are at least two aspects of the adaptation problem that are not included. (i) After removing a weak background light that could not have produced any significant amount of metarhodopsin, we have observed a rapid but not instantaneous dark-adaptation phase (Fig. 2 and its explanation in text) down to the absolute threshold. This sensitivity change, or more precisely the elevated threshold during this change, cannot be explained in the terms of our general formula. (ii) The formula does not describe the lateral spread of the sensitivity-reducing effects. It refers to experiments in which the three parameters J (background intensity), J_R (rate of regeneration) and M_{II} (amount of metarhodopsin II) in a given moment have the same value over the whole retina.

The different kinds of lateral effects observed may be summarized as follows:

1. The sensitivity-reducing effect ascribed to metarhodopsin II spreads very little or not at all outside the bleached rods.

2. The sensitivity-reducing effect of a steady background of real light begins to sink immediately outside the area illuminated and is gradually attenuated with increasing distance from the exposed rods so that only a small fraction of this effect remains when this distance is 0·3 mm. The same probably applies to the sensitivity-reducing effect ascribed to the regeneration of rhodopsin.

3. Within the central area (diameter in many cases 0·4–0·5 mm) of the receptive field of a ganglion cell there is full summation at threshold of the signals from separate rods elicited by a test flash (Hartline, 1940; Barlow, 1953; Donner & Reuter, 1965).

4. When both the central area of the receptive field and the adjacent retinal areas outside are simultaneously stimulated it is often observed that the central area is surrounded by an inhibitory zone of great extension. This inhibitory surround is not activated by a steady background, but when the illumination here is changed the simultaneously measured threshold of the ganglion cell rises (Barlow, 1953; Donner & Reuter, 1965). This effect has been avoided in all the experiments described in this paper.

Regarding the effect of light absorbed in the rods the general formula (7) establishes that it acts in two ways: (i) it leads to an activation of the ganglion cell when the illumination is changed, and (ii) it reduces the sensitivity of the rods under conditions of steady illumination. Because we know that the latter effect is spread laterally to neighbouring rods, we must assume that there are two kinds of signals, the activating signal and the background signal, that leave the rods stimulated. The most remarkable difference between these signals is that, according to eqn. (7), the former but not the latter is attenuated by the presence of metarhodopsin II in the rods. Figure 2 shows this very clearly. Here, with the logarithmic scale used, the desensitization caused by decomposing metarhodopsin II simply adds to a constant background level.

The slow phase of dark-adaptation and the e.r.g. threshold. In the rat eye it has been convincingly demonstrated that there is a linear relation between the logarithm of the e.r.g. threshold and the concentration of rhodopsin in the eye. The relation has been found to hold both in vitamin A deficiency (Dowling & Wald, 1958) and dark-adaptation (Dowling, 1960, 1963) and recently also in the isolated rat retina (Weinstein, Hobson & Dowling, 1967) where there is no regeneration of rhodopsin and this factor thus is excluded. This difference in relation to our results does not depend on differences between the mammalian and the amphibian retina, as shown by Baumann's (1966, 1967*a*, *b*, *c*) e.r.g. measurements on the isolated frog (*Rana esculenta*) retina that show a similar linear relation between log threshold and rhodopsin content as Dowling's measurements on the rat.

On the other hand, our results (Donner & Reuter, 1965, 1967) where the threshold of the ganglion cell discharge has been used as an index of sensitivity, are very difficult to reconcile with any simple relation between log threshold and rhodopsin content. The existence of a real difference between the e.r.g. and the ganglion cell threshold has been established by Baumann (1967*d* and personal communication) for the isolated frog

retina. He finds a different and non-linear relation between the logarithm of the ganglion cell threshold and rhodopsin compared to that obtained by the e.r.g. method on the same preparation. With rhodopsin contents between 80 and 100% his results can be accounted for by the simple physical effect of the reduced density of the photopigment, a result in full agreement with our analysis.

It can further be noted that in measurements of the increment threshold with psychophysical methods on the human eye (Aguilar & Stiles, 1954) and with the ganglion cell discharge as an index as in the present work (Fig. 3, p. 68) saturation of the rods is observed at high background intensities, i.e. the increment threshold begins to rise very rapidly with increasing background and appears to approach infinity. This has not been observed in e.r.g. measurements even at background intensities that bleach the rhodopsin almost completely (Dodt & Echte, 1961; Dowling, 1963). In view of our finding that the saturation effect can be explained by an accumulation of metarhodopsin in the rods, an effect that does not show any lateral spread in the retina, it is likely that the e.r.g. is dominated by potentials associated with the lateral spread of adaptive effects in the retina. This is supported by the results of recent attempts to correlate the slow S-potentials of the retina with the ganglion cell discharge and with adaptive processes (Naka & Kishida, 1967; Naka & Rushton, 1967, 1968; Witkowsky, 1967) that suggest that these potentials do not take part in the generation of spikes from the ganglion cells but are more likely involved in the adaptation to background fields.

Quite generally it then appears that the e.r.g. and the ganglion cell thresholds are not comparable criteria of sensitivity but reflect different aspects of the retinal processes.

Dark-adaptation in the human eye. In many aspects there is a close resemblance between rod adaptation in the frog and in the human eye. This is also borne out by the fact that the description of the adaptation process in the frog as given by eqn. (7) includes in a modified form the elements contained in Rushton's (1965*a*, *b*) analysis of the adaptation process in the rods of the human eye.

After bleaching a substantial fraction of the rhodopsin Rushton has shown that there is a linear relationship between the log threshold and the amount of rhodopsin during the whole course of the dark-adaptation curve. This relation thus appears to be the same as that established in the e.r.g. work on the rat and the frog (cf. above). However, all of the e.r.g. work refers to the threshold during the later, slow phase of dark-adaptation only or to the final threshold reached in the absence of regeneration, as is the case in the isolated retina. Thus the first, rapid phase of adaptation that is included in Rushton's analysis, cannot be accounted for in the

e.r.g. measurements as being caused by changes in the amount of rhodopsin in the rods, as already shown by Granit, Holmberg & Zewi (1938).

If, as an alternative, dark-adaptation in the human rods is assumed to follow the rules laid down in eqn. (7) it is evident that it will depend on two factors: (i) the amount of metarhodopsin present at the beginning of dark-adaptation, and (ii) the rate of regeneration.

The regeneration of rhodopsin in man follows a first-order reaction with a time constant of about 6 min (Rushton, 1961). The rate of metarhodopsin decomposition has not been directly measured *in situ* and cannot be obtained from the data on frog metarhodopsin, which according to Hubbard, Brown & Kropf (1959) hydrolyses more readily at the same temperature than mammalian metarhodopsin. Hagins (1957) has, however, observed in the rabbit's eye after a strong bleach a slow fading reaction, which when measured at 400 nm at a temperature of 33°, reaches half-completion after about 6 min. The Q_{10} of this reaction was found to be 3·5, which gives a half-decay time of 3·6 min at 37° C. Although this fading reaction probably does not refer to a single substance, metarhodopsin II is likely to dominate the measurements at 400 nm.

In terms of eqn. (7) it is obvious that with short and small bleaches the metarhodopsin produced and its decay will dominate the adaptation process, thus giving a fast recovery of the type reported by Rushton & Cohen (1954) (see also Haig, 1941). Rushton & Cohen found a roughly exponential recovery of sensitivity with a half-return time of about 3 min, which agrees well with the estimate given above for the rate of decay of metarhodopsin. On the other hand, when a substantial fraction of the rhodopsin is bleached the situation is different. The dark-adaptation process can in that case be described by a curve constructed in the same way as the curves in Fig. 4. That is, the effect of the rate of regeneration is given by a straight line with a negative slope of 0·3 log units in 4 min, which is the half-completion time for regeneration, on top of which are added the ordinates of an exponential of about 3 min half-return time, representing the metarhodopsin effect of the type observed by Rushton & Cohen. With short and strong bleaches this latter component will be more prominent. The result is that the shapes of the dark-adaptation curves will be influenced by the time and intensity of the preceding light-adaptation in the way described by a number of earlier workers (Wald & Clark, 1937; Haig, 1941; Crawford, 1946; Mote & Forbes, 1957). This is also a property observed in the dark-adaptation of a single *Limulus ommatidium* (Hartline & McDonald, 1947).

It further appears that the saturation of the rods as described by Aguilar & Stiles (1954) can be explained as being caused by the accumulation of metarhodopsin. According to the data given by them, the

bleaching effect of the background when saturation occurs is such as to produce amounts of metarhodopsin comparable to those in Fig. 3 at the saturation point.

This qualitative agreement between predictions based on eqn. (7) and actual experiments regarding various aspects of the adaptation process thus seems to support a description of the rod adaptation process in the human eye in these terms. It is, however, conceivable that there are other alternatives as, for instance, that in a mammalian retina the free opsin molecules may have an effect similar to that of metarhodopsin, so that various combinations of rod desensitization caused by metarhodopsin and opsin may occur.

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