J. Physiol. (1974), **242**, *pp.* 729–758
With 15 text-figures
Printed in Great Britain

CHANGES IN TIME SCALE AND SENSITIVITY IN TURTLE PHOTORECEPTORS

BY D. A. BAYLOR* AND A. L. HODGKIN

From the Physiological Laboratory, University of Cambridge, Cambridge, and the Department of Physiology, University of Colorado Medical Center, Denver, Colorado, U.S.A.

(*Received 2 April 1974*)

SUMMARY

1. In turtle cones the steady-state relation between the internal potential and log light intensity was much flatter in the steady state than it was at 30 msec after the beginning of a step of light; this is attributed to a desensitization which develops with a delay of 50–100 msec.

2. When a weak flash was superposed on a steady background light which hyperpolarized the cone by 3–6 mV the amplitude of the linear response to a flash was reduced to 1/e and the time to maximum was shortened from about 110 to 70 msec; the response also became diphasic. With stronger background lights the flash sensitivity continued to fall, but the time to maximum did not become shorter than 40–50 msec and lengthened again with very strong lights.

3. In cones the flash sensitivity S_F was reduced to half its dark value S_F^D by a light intensity of $1/S_F^D\zeta$ where ζ is about 20 sec/V.

4. At low levels of background light, about two-thirds of the change in sensitivity was time-dependent and one-third was attributable to the 'instantaneous non-linearity' described in the previous paper.

5. The reduction in time to peak and the decrease in sensitivity produced by a background light which hyperpolarized by about 3 mV was little affected by changing the diameter of the area illuminated from 12 to 800 μm.

6. An experiment with a rod showed that a very weak light which hyperpolarized by only 0·5 mV decreased the linear response to 1/e and shortened the time to maximum from 300 to 180 msec.

7. With weak or moderate flashes the time-dependent desensitization lagged behind the potential by 50–100 msec.

8. The desensitization and shortening of time scale which persisted after

* Present address: Department of Physiology, Stanford University, Stanford, California 94305, U.S.A.

a flash or step were associated with an after-hyperpolarization. The relaxation of potential, sensitivity and time scale became slower as the preceding illumination was increased from 10^3 to 10^{10} photons μm^{-2}; the increase seemed to occur in steps involving components which relaxed with time constants of the order of 0·1, 1, 10 and 100 sec. A rebound phenomenon was observed after steps longer than 30 sec and with equivalent intensities greater than 10^5 photons μm^{-2} sec^{-1}.

9. Several of the observations are explained by a hypothesis in which the central assumption is that the particles which block the ionic channels are degraded or removed by an autocatalytic reaction.

INTRODUCTION

In the preceding paper (Baylor, Hodgkin & Lamb, 1974*a*) we showed that the early stages of the electrical response of turtle cones to flashes or steps of light can be explained by assuming that absorbed quanta start a linear chain of reactions leading to the production of particles which block ionic channels in the outer segment. During the first 50–100 msec, lack of proportionality between response and stimulus and saturation can be explained by the combined effect of the cell's equivalent circuit and the competition between blocking particles for ionic channels. At times longer than 50–100 msec the simple analysis fails – the most obvious defect being that the peak of the response to a flash moves earlier as the flash becomes brighter. This is attributed to a desensitization which develops with a time lag and which may be brought about by an increase in the rate at which the blocking particles are removed or inactivated. In studying this phenomenon, the general strategy was to compare the sensitivity and time course of small linear responses in the dark with those in the light (cf. Fuortes & Hodgkin, 1964). In such experiments it is important that the intensity of the test flashes should be adjusted so that the electrical responses are small and linear in the dark as well as in the light. Unless this is done the response in the dark will be too large and the control flash will itself have introduced some of the desensitization and shortening of time scale that are being investigated.

In addition to studying the action of steady background lights on time scale and sensitivity, this paper is also concerned with the rather similar effects seen after the eye has been exposed to lights of increasing intensity.

METHODS

The experimental methods were the same as those described by Baylor & Hodgkin (1973).

As in the preceding paper (Baylor *et al.* 1974*a*, p. 687) light intensities are sometimes expressed as equivalent to a certain rate of photoisomerization per cone. For

red-sensitive cones, where the intensity of the unattenuated light was equivalent to 67×10^6 photons μm^{-2} sec^{-1} at 644 nm, the rate is taken as $67\sigma \times 10^6$ photoisomerizations sec^{-1} $cone^{-1}$ where σ, the effective collecting area of the cone in μm^2, is obtained by dividing the observed flash sensitivity in μV $photon^{-1}$ μm^2 by 25 μV/photoisomerization (see Baylor & Hodgkin, 1973).

RESULTS

The relation between light intensity and hyperpolarization in the steady state

In the present context the term steady state means the approximately steady level of potential established at 0·5–1·0 sec after the beginning of a step of light. With strong lights this level is not maintained indefinitely but drifts towards the resting potential with a time constant of the order of 30 sec (see Fig. 14). However, this drift is not detectable with steps of duration 1 sec and the hyperpolarization at 0·6–1·2 sec will be taken as the steady-state value.

In Fig. 1 the hollow circles give the relation between the potential at

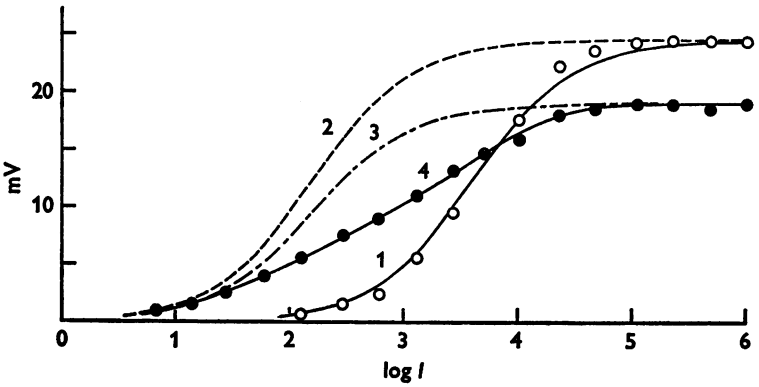


Fig. 1. Comparison of relation between potential at 800 msec after beginning of step ●, with potential at 50 msec after beginning of step ○. The ordinate is the hyperpolarization and the abscissa is the logarithm of the light intensity expressed in units such that the unattenuated light was 10^6 relative units. In this red-sensitive cone 1 relative unit is equivalent to 67 photons μm^{-2} sec^{-1} at the optimum wave-length of 644 nm.

Curves 1, 2, 3 are Michaelis relations, i.e.

$$\frac{U}{U_L} = \frac{I}{I+I_{\frac{1}{2}}}$$

with U_L = 24·5, 24·5 and 19 mV and $I_{\frac{1}{2}}$ = 4000, 160, 160 relative units for curves 1, 2 and 3 respectively.

Curve 4, which fits the steady-state points, is drawn from eqns. (1) and (2) in the text with U_L = 19 mV, P = 0·0075, Q = 0·001 and R = 0·0125.

Resting potential −41 mV; 75 μm diameter white spot; 20·9° C; flash sensitivity 24 μV $photon^{-1}$ μm^2.

50 msec after the beginning of a step and the logarithm of the light intensity; the continuous curve near these points is a rectangular hyperbola. The filled circles give the potential at 0·8 sec after the beginning of the step. In the absence of any time-dependent desensitizing mechanism one would expect the steady-state relation to be close to the interrupted curve (2) which is parallel to curve 1, but shifted to the left by the amount required to fit the potential at low light intensity. As can be seen from the Figure the steady-state points lie on a much flatter curve with a maximum of 19 instead of 25 mV. The change in maximum, which may depend on a change in a voltage-sensitive conductance (Baylor *et al.* 1974*a*), is considered to be a separate phenomenon from the flattening which is attributed to progressive desensitization as the light intensity increases. Reducing the maximum of the rectangular hyperbola from 25 to 19 mV gives curve 3 which is still much steeper than the steady-state relation.

Curve 4 which fits the steady-state values was calculated from

$$\frac{U}{U_L} = \frac{f}{1+f} \tag{1}$$

with

$$f = \frac{PI_S[1+QI_S]}{1+RI_S}, \tag{2}$$

where U is the steady hyperpolarization produced by a steady light of intensity I_S, U_L is the maximum steady hyperpolarization and P, Q and R are constants with the values given in the Figure legend. A theoretical basis for this equation is described on p. 756.

Effect of steady light on response to flashes

Fig. 2 shows a series of Biomac records obtained by averaging sixteen linear responses to flashes. The records on the left give the effect of a standard flash in darkness recorded at various times during the experiment. Those on the right give the effect of the flash superposed on a background light of increasing intensity; the numbers give the displacement of the potential from its steady value which is shown for each record. The test flash was kept constant for the first three backgrounds and was then increased in about the same proportion as the background. The experiment shows that the effect of steady light is (1) to reduce sensitivity progressively to 1/50 in record 11, where there is a hyperpolarization of 5·5 mV, and (2) to shorten the time scale of the response and eventually to make it diphasic.

In order to examine the effects of stronger background lights the 10 msec test flash was applied at 1·1 sec after the beginning of a conditioning step lasting 1·7 sec. The effect of the test flash was examined on an expanded time base and the result of four to five separate trials of step and flash

were averaged. Sufficient time was left between trials to allow full recovery, and the effect of control flashes in darkness was examined at frequent intervals. The average flash responses expressed as hyperpolarization/light intensity are given in Fig. 3. Up to a hyperpolarization of 10 mV the effect of the conditioning light was again to shorten the time to peak of the flash response as well as to reduce sensitivity. With stronger conditioning steps the cone continued to become less sensitive but the time to peak ceased to decrease and eventually increased again. In another experiment of a similar kind the time to maximum was 135 msec in darkness, shortened to 68 msec at $2{\cdot}4 \times 10^5$ photoisomerizations sec^{-1}

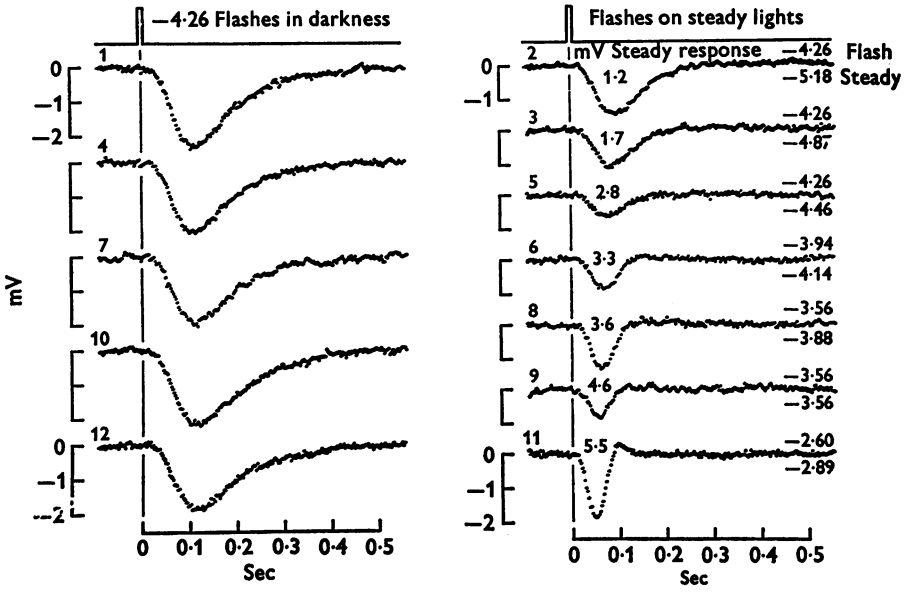


Fig. 2. Effect of steady light on sensitivity of red-sensitive cone to 10 msec flashes. The $\log_{10}$ intensity of the flash, the $\log_{10}$ intensity of the steady light and the hyperpolarization in mV are shown against each record in the right-hand column. The left-hand column gives the responses to test flashes in the dark. The records were carried out in the order shown by the numbers on the left of each record. The light intensity I is expressed in units such that the unattenuated light had intensity 1, which was equivalent to 67×10^6 photons μm^{-2} sec^{-1} at 644 nm. The flash sensitivity of this red sensitive cone was 55 μV $photon^{-1}$ μm^2. To convert relative light intensities shown here into photoisomerizations per sec as in Fig. 3 multiply by $10^{8{\cdot}16}$.

Sixteen responses, spaced at 3 sec intervals were averaged for each trace. Simultaneous inkwriter records showed that there was little drift in the size of the flash response but that with the stronger steady lights there was some drift of the membrane potential towards its original value; the values shown here are approximately average values.

Temperature 21·5° C; resting potential −41 mV; peak response 17·5 mV; 150 μm diameter white spots for both flashes and steady lights.

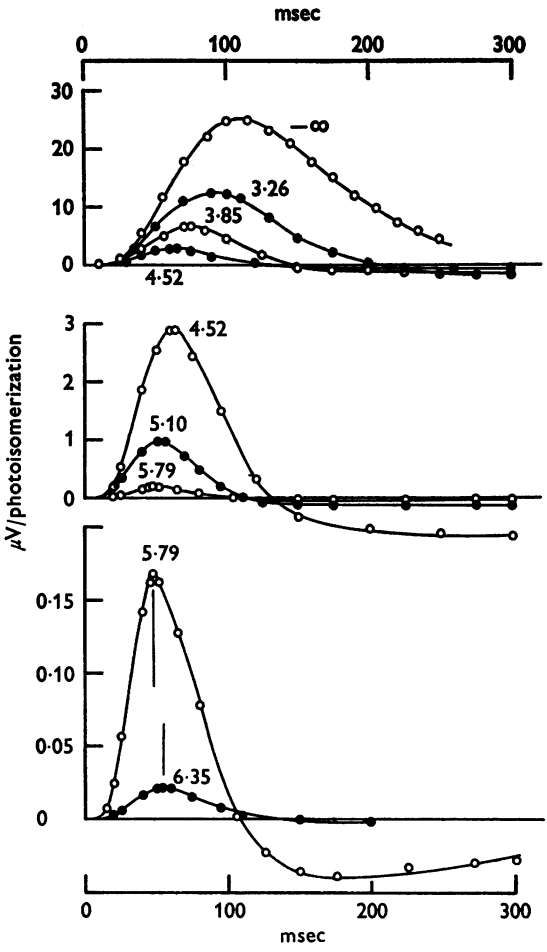


Fig. 3. Effect of increasing intensity of conditioning step on response to 11 msec flash applied 1·1 sec after beginning of step lasting 1·7 sec. The abscissa is the time after the middle of the flash and the ordinate is $U(t)/I^{\phi}\Delta t$ where $U(t)$ is the hyperpolarization, Δt is the pulse duration and I^{ϕ} is proportional to light intensity and gives the calculated rate of photoisomerization per cone (see p. 730). The numbers against the curves give the logarithm of the conditioning light expressed in photoisomerizations cone^{-1} sec^{-1}. Four frames of amplitude 1–2 mV were averaged except for the control flash in the dark when 31 frames were averaged.

The flash sensitivity in the dark was 19·5 photon^{-1} μm^2 and the effective collecting area was taken as 0·78 μm^2. The hyperpolarizations corresponding to the conditioning steps were $-\infty$, 0 mV; 3·26, 2·5 mV; 3·85, 4·3 mV; 4·52, 7·2 mV; 5·10, 9·6 mV; 5·79, 12·8 mV; 6·35, 14·5 mV.

Red-sensitive cone with maximum hyperpolarization of 20 mV; 150 μm diameter white light spot; temperature 21° C; resting potential −39 mV.

cone^{-1} and lengthened to 95 msec at 6×10^6 photoisomerizations sec^{-1} cone^{-1}. This lengthening of the time to peak is rather surprising; a possible explanation of the effect will be considered further in a later paper (Baylor *et al.* 1974*b*).

In Fig. 4 four quantities which can be obtained fairly directly from the records are plotted against the background light intensity. Curve 1 is the steady hyperpolarization plotted downwards and linearly, curve 2 is the

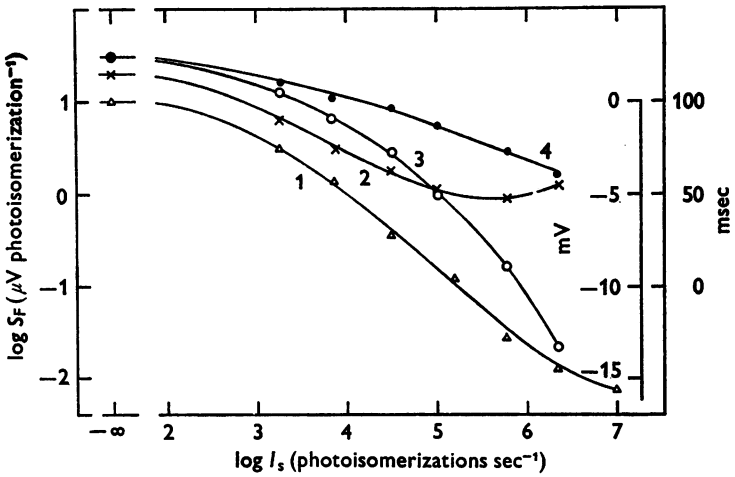


Fig. 4. Effect of steady light on membrane potential and on response to test flashes (from Fig. 3). Abscissa: log of steady light intensity in same units as in Fig. 3. Ordinate: curve 1 steady potential at 1·1 sec (inner right-hand scale); curve 2, time to peak of linear response from middle of test flash (right-hand scale); curve 3, log sensitivity to test flash (left-hand scale); curve 4, log sensitivity to test flash corrected for instantaneous non-linearity by the method given in the text (left-hand scale).

time to maximum of the response to flashes, plotted linearly, and curve 3 is the logarithm of the flash sensitivity. Curve 4 was derived from curve 3 by correcting for the instantaneous non-linearity introduced by the approximately hyperbolic relation which holds before the onset of any additional desensitization. To make the correction it is assumed that the hyperpolarization U is related to the concentration y of a blocking substance by the instantaneous relation

$$\frac{U}{U_L} = \frac{y}{y+b}, \tag{3}$$

where U_L is the maximum hyperpolarization and b is a constant (see Baylor *et al.* 1974*a*). A flash of strength ΔQ produces a peak concentration given by

$$\Delta y = c\Delta Q, \tag{4}$$

where c is a proportionality factor which decreases with a time lag to a lower value when the cone is exposed to light. The flash sensitivity is given by

$$S_F = \frac{dU}{dy}\frac{dy}{dQ} \tag{5}$$

$$= \frac{U_L bc}{(y+b)^2} \tag{6}$$

$$= \frac{cU_L}{b}\left(\frac{U_L - U}{U_L}\right)^2. \tag{7}$$

Hence the flash sensitivity at a steady potential U is related to that in darkness by

$$\frac{S_F}{S_F^D} = \frac{c}{c_D}\left[\frac{U_L - U}{U_L}\right]^2. \tag{8}$$

In Fig. 4 curve 4 gives the flash sensitivity multiplied by $[U_L/(U_L - U)]^2$; these values should be proportional to c and provide an estimate of the time-dependent component of the sensitivity. The analysis shows that changes in c account for about $\frac{2}{3}$ of the desensitization at low light intensities but are less important with strong light.

The procedure outlined in the previous paragraph may underestimate the change in the time-dependent component of the sensitivity and over-estimate the effect of instantaneous non-linearity. Baylor *et al.* (1974*a*) found that the relation between potential and light intensity during the rising phase was usually steeper than a rectangular hyperbola which means that dU/dy would be larger than the value calculated on the basis of a hyperbola. Another difficulty with the analysis is that the saturating level which U approaches after a strong flash declines from a maximum of, say, 20 mV when the eye is in darkness to one of about 16 mV when illuminated with a strong light. To deal with this, we assumed that U_L declined linearly with the steady level U_S between the limits of 20 mV when $U_S = 0$ and 16 mV when $U_S = 16$ mV. Thus if the steady level were 8 mV we took U_L as 18 mV.

Quantitative characterization of effects of background light

Although the desensitization of photoreceptors by background lights may be complicated in origin, in that it involves both instantaneous and time-dependent non-linearities, it is nevertheless desirable to look for some empirical parameter which quantifies the ease with which a receptor is desensitized by background light. One method is to determine the hyperpolarization associated with a given decrease in sensitivity. When the logarithm of the flash-sensitivity was plotted against hyperpolarization a curve of the same general shape as that in Fig. 5 was usually obtained. Table 1 gives values of the exponential parameter U_e defined by

$$\frac{d \ln S_F}{dU} = -\frac{1}{U_e}. \tag{9}$$

For lights which hyperpolarized between 1 and 7 mV, U_e was between 2 and 4 mV with a mean value of 2·9 mV. For weak lights hyperpolarizing less than 1 mV, U_e was between 4 and 10 mV, but the exact values given in Table 1 are uncertain because they depend on the way in which the curve is drawn near the origin.

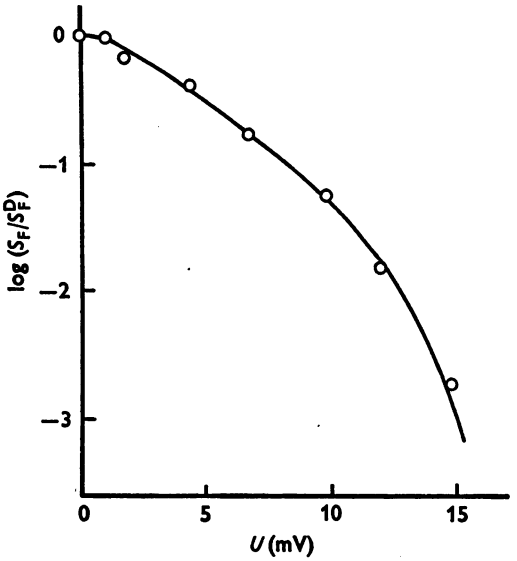


Fig. 5. Relation between hyperpolarization and logarithm of flash sensitivity. Abscissa, U, hyperpolarization in mV at time of test flash, 1·1 sec after beginning of conditioning step of light. Ordinate, logarithm of ratio of flash sensitivity in presence of background light to flash sensitivity in the dark. From the same experiment as Fig. 3 but from the first run.

An alternative and probably more useful way of characterizing the effect of a background light, which is illustrated in Fig. 6 is to plot the reciprocal of the flash sensitivity against the background light I_S. In most cases this method, which is equivalent to plotting ΔI against I in a psychophysical experiment, gave a straight line over a considerable range. Such behaviour is described by the equation

$$\frac{1}{S_F} = \frac{1}{S_F^D} + \zeta I_S, \qquad (10)$$

where S_F is the flash sensitivity associated with a steady light intensity I_S and S_F^D is the flash sensitivity in the dark. ζ is a parameter with the dimensions of sec/V; in cones its average value at 20° C was about 20 sec/V (see Table 1).

An alternative form of eqn. (10) is

$$\frac{1}{S_F^R} = 1 + I_S/\bar{I}, \tag{11}$$

where S_F^R is the relative flash sensitivity, i.e. S_F/S_F^D and $\bar{I} = (S_F^D\zeta)^{-1}$ is the light intensity which reduces the flash sensitivity to half its dark value. If S_F^D is taken as 25 μV/photoisomerization and ζ is 20 sec/V then $\bar{I}$ is 2000 photoisomerizations per second. Although the meaning of the constant $\bar{I}$ is easy to appreciate we prefer to employ ζ because its value is independent of the units in which light intensity is expressed.

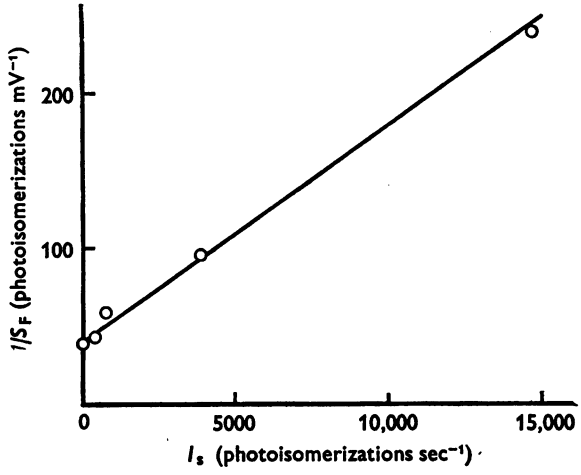


Fig. 6. Relation between the conditioning light intensity I_s and the reciprocal of flash sensitivity with both coordinates linear. The light intensity which is expressed in photoisomerizations/sec was calculated on the assumption that the flash sensitivity in the dark is 25 μV/photoisomerization. From the same set of data as Fig. 5.

To test eqn. (10) over a wider range log $(1/S_F - 1/S_F^D)$ was plotted against log I_S, as in Fig. 7. The straight line on the Figure was drawn with a slope of unity and at a position corresponding to the value of ζ of 16 sec/V. As can be seen the agreement with eqn. (10) is not perfect, although probably as good as in most psychophysical experiments. The deviations from the equation are of the kind expected if ζ declined to about half its dark value with increasing light intensity and then increased again as the cell approached saturation.

Relation between ζ and other parameters

Since the dimensions of the parameter ζ are in sec/V it is natural to inquire how it is related to the exponential voltage constant U_e and the

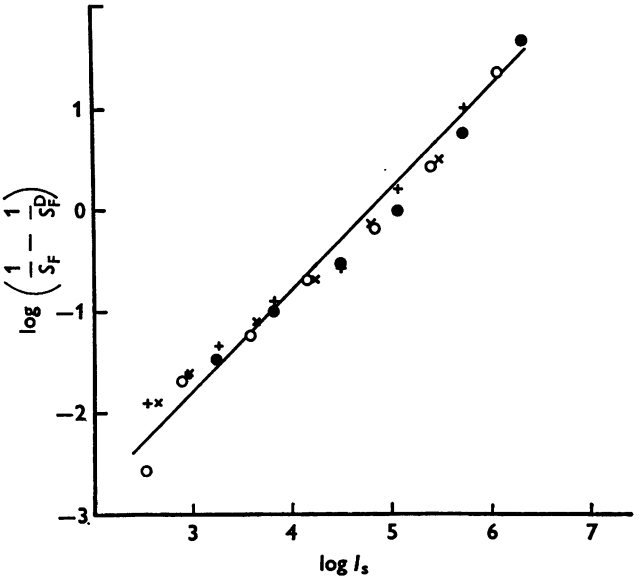


Fig. 7. Effect of background light on sensitivity. Abscissa, log I_s where I_s is background light intensity in photoisomerizations sec^{-1}. Ordinate, log $(1/S_F - 1/S_F^D)$ where S_F is the flash sensitivity in μV photoisomerization^{-1} and S_F^D is the flash sensitivity in the dark. The straight line is drawn from eqn. (10) with $\zeta = 15{\cdot}8$ sec/V. ○, First run on cone R_5 (Table 1). ●, Second run on cone R_5. +, cone R_3. ×, cone R_4.

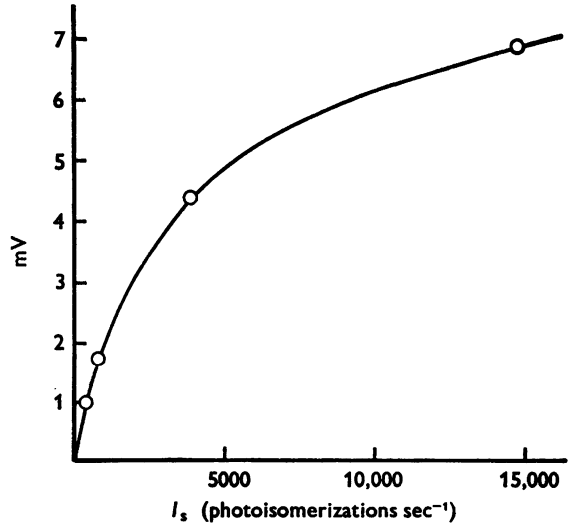


Fig. 8. Relation between conditioning light intensity I_s (abscissa) and the steady hyperpolarization at 1·1 sec after beginning of conditioning step. From the same set of data as Figs. 5 and 6.

integration time t_1. Rather surprisingly it turns out that even though ζ, U_e and t_1 all vary with background light, I_S, these three parameters should nevertheless be connected by the simple relation

$$\zeta = t_1/U_e. \tag{12}$$

To make the proof clear we need first to consider a third type of experimental plot, namely steady hyperpolarization U as a function of steady light intensity I_S. An example from the same experiment as Figs. 5 and 6 is given in Fig. 8, and we shall take the slope of this curve as defining the step sensitivity S_S; i.e.

$$S_S = \frac{dU}{dI_S}. \tag{13}$$

From eqn. (10) we have

$$\frac{d}{dI_S}\left(\frac{1}{S_F}\right) = \zeta, \tag{14}$$

or

$$-\frac{1}{S_F^2}\frac{dS_F}{dI_S} = \zeta, \tag{15}$$

so

$$-\frac{d \ln S_F}{dI_S} = \zeta S_F. \tag{16}$$

From eqn. (9)

$$-\frac{d \ln S_F}{dI_S}\frac{dI_S}{dU} = \frac{1}{U_e} \tag{17}$$

which in conjunction with (13) and (16) leads to

$$\zeta = \frac{S_S}{S_F U_e}. \tag{18}$$

In a previous paper (Baylor & Hodgkin, 1973) it was shown both theoretically and experimentally that flash and step sensitivities were related by

$$S_S = S_F t_1, \tag{19}$$

in which t_1 the integration time is defined by $\int_0^\infty f(t)\,dt$, where $f(t)$ is the response to a flash normalized to unity at the peak. In the paper quoted we were concerned with experiments in which small flashes or steps of light were applied to the eye in darkness. However, the equation should be equally applicable to the more general case in which the sensitivity is determined by superposing a small flash or step on a background.

On combining (18) and (19) we obtain

$$\zeta = \frac{t_1}{U_e}. \tag{20}$$

In Table 1 measurements of the parameter ζ are compared with values calculated by eqns. (18) and (20). In the first experiment where there is a

discrepancy between observed and calculated values of the integration time there are similar discrepancies between observed values of ζ and values calculated by eqn. (20). This discrepancy is explained by an error in the factor taken for the ratio of the intensities of the lights used for the conditioning step and test flash. If present, such an error would have no effect on the value of ζ calculated by eqn. (20) but would introduce similar proportional errors both in the observed value of ζ and in the value calculated by eqn. (18).

The general conclusion from Table 1 is that in these experiments the value of ζ was 15–40 sec/V and that this value is reasonably consistent with the relation $\zeta = t_1/U_e$.

Effect of area illuminated on desensitization by steady light

In all the experiments described so far, both testing and conditioning spots of light were coincident circles of diameter 50–150 μm. Since spots of this size produce only small responses in horizontal cells it is likely that the desensitization and shortening of the response produced by background light depend on local events in the cones and are not mediated by feed-back from the horizontal cells. Further evidence for this conclusion was provided by an experiment in which the test spot was always a 14 μm diameter circle, and the conditioning spot, which was concentric with the test spot was either 12·4, 206 or 803 μm in diameter. The results of this test showed that for equal hyperpolarizations small spots are at least as effective as large ones in desensitizing and shortening time-scale. Thus the values obtained for U_e, the voltage to give an e-fold decrease in sensitivity, were 2·6 mV for 12·4 μm, 3·1 mV for 206 μm and 3·0 mV for 830 μm diameter. The reduction in the time to peak of the flash response was also independent of the area illuminated.

Relation between sensitivity and time to peak of response

The decrease in sensitivity associated with background lights, or with previous exposure to bright flashes, was accompanied by a reduction in the time to peak of the response to a test flash. This is illustrated by Fig. 9 in which flash sensitivity is plotted against time to peak with both scales logarithmic; the open symbols are for experiments in which flashes were superposed on a steady background and the filled symbols are for experiments in which the sensitivity was reduced by previous exposure to bright lights. With weak conditioning lights the relation between sensitivity S_F and time to maximum is approximately described by

$$S_F \propto (t_{max})^n, \tag{21}$$

where n = 3–5; this is quantitatively different from the *Limulus* ommatidium where Fuortes & Hodgkin (1964) found $n \doteqdot 7$.

TABLE 1. Effect of steady light on potential, sensitivity and related quantities

(1) Cone	(2) I_s ϕ sec^{-1}	(3) U mV	(4) S_F μV ϕ^{-1}	(5) S_S μV ϕ^{-1} sec	(6) t_1 (obs) sec	(7) t_1 (calc) sec	(8) U_c mV	(9) ζ (obs)	(10) ζ (calc)$_1$ sec/V	(11) ζ (calc)$_2$
R_1	0	0	25	2·1	0·17	0·084	5·8	19	15	29
	875	1·2	16	0·75	0·12	0·046	2·5	19	18	48
	1800	1·7	13	0·4	0·086	0·031	2·3	19	13	37
	4640	2·8	8	0·2	0·07	0·026	1·9	16	14	36
	9633	3·3	5	0·13	0·06	0·025	1·5	15	17	42
Mean								18	16	38
R_2	0	0	25	5·3	0·19	0·21	5·8	22	36	31
	1520	2·6	14	0·8	0·11	0·06	4·1	22	15	26
	3060	4·0	9	0·5	0·09	0·06	3·3	22	18	27
Mean								22	23	28
R_3	0	0	25	3·5	0·16	0·14	4·2	23	33	38
	450	0·9	19	2·3	—	0·12	4·2	23	28	—
	920	1·9	16	1·2	—	0·07	4·2	21	17	—
	4580	4·2	8·6	0·5	—	0·058	4·2	15	14	—
	17550	6·8	4·2	0·1	—	0·23	3·4	10	7	—
Mean								18	17	—
R_4	0	0	25	3·0	0·15	0·12	6·3	19	19	23
	860	1·8	17	1·7	—	0·10	4·1	19	25	—
	1760	3·0	12	0·7	—	0·06	3·5	19	17	—
	6740	5·4	6	0·23	—	0·04	2·4	19	15	—
Mean								19	19	—

TABLE 1 (*cont.*)

R_5	0	0	25	4·0	0·15	0·16	10	14	16	15
	780	1·8	17	2·5	0·08	0·15	5	14	30	16
	3860	4·4	10	0·4	0·06	0·04	32	14	12	19
	14780	6·7	4	0·11	—	0·026	29	14	9	—
Mean								14	17	17
G_1	0	0	25	5·4	0·22	0·22	9	18	24	24
	985	2·1	18	0·8	—	0·044	2·1	18	21	—
	2000	2·6	12	0·45	—	0·04	1·9	18	21	—
Mean								18	22	—

Column (1) R and G indicate red and green sensitive cones respectively. (2) Background light intensity, in photoisomerizations sec^{-1}, assuming $S_F = 25\ \mu V \phi^{-1}$ in dark. (3) Steady hyperpolarization. (4) Flash sensitivity scaled to $25\ \mu V\ \phi^{-1}$ in dark. (5) Step-sensitivity $(dU/dI)_{t=\infty}$. (6) Observed integration time. (7) Integration time calculated from S_S/S_F. (8) $U_e = -[d(\ln S_F)/dU]^{-1}$. (9) Observed value of $\zeta = d(S_F^{-1})/dI$. (10) ζ calculated as $S_S/S_F\ U_e$. (11) ζ calculated from $\zeta = t_1\,(\text{obs})/U_e$.

The symbol ϕ stands for photoisomerizations.

In the first two experiments the background light was on continuously; in the other four experiments, in which all the cones were from one turtle, the light was applied for about 1 sec before the flash. Absolute sensitivities at the optimum wave-length were calculated as 55, 54, 24, 9, 20, 21 μV photon^{-1} μm^2 (from above downwards). The ratio of intensities of the two beams which affects columns 5, 7, 9, 10 is more liable to error in the first two experiments, temperature 21–22° C.

Equation (21) holds only for weak backgrounds; with strong backgrounds the response ceases to shorten and may lengthen again with a sufficiently strong background.

A phenomenon which must be closely related to the shortening of time scale produced by weak lights is the movement to shorter times of the peak response to flashes of increasing intensity. Fig. 10 illustrates the

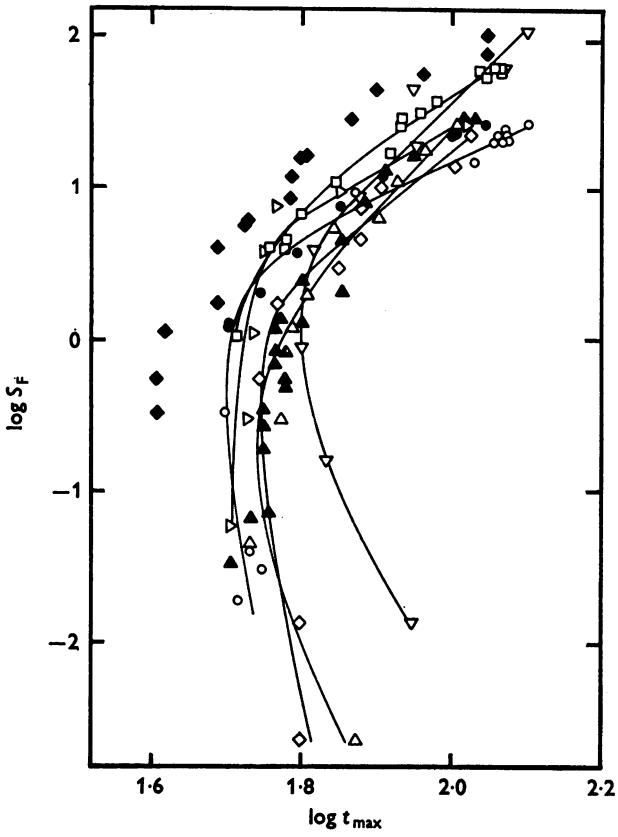


Fig. 9. Relation between sensitivity and time to peak for linear responses to 10 msec flashes during 'steady' background lights (open symbols) or after exposure to bright light (filled symbols). Collected results from eight red-sensitive cones. The abscissa is the $\log_{10}$ of the time to peak of the flash response corrected to 21° C assuming a Q_{10} of 1·8. The ordinate is the $\log_{10}$ of the flash sensitivity S_F in μV photon^{-1} μm^2 which was measured by dividing the peak of the response by the photon density at 644 nm to which the quantity of light was equivalent. Curves have been drawn by eye through the sets of points obtained with steady lights; △ and ▲ were from the same cell. Both test and conditioning lights were coincident white spots of the same diameter, between 50 and 150 μm; temperatures were between 19·8 and 21·5° C.

relation in eleven red-sensitive cones, including an 'isolated cone' (●) which showed no sign of coupling to its neighbours (see Baylor & Hodgkin, 1973).

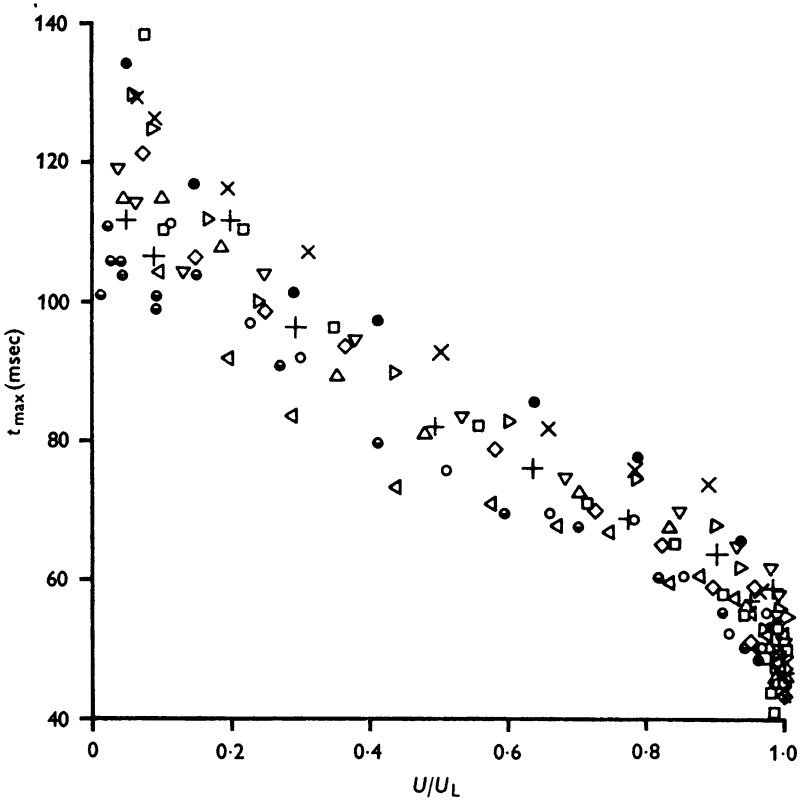


Fig. 10. Relation between time to peak and amplitude of responses to 10 m-sec flashes in eleven red-sensitive cones. The ordinate is the time to peak from the middle of a 10 msec flash corrected to 21° C with a Q_{10} of 1·8 from a temperature range of 20·6–21·8° C. The abscissa is the amplitude of the response divided by the maximum amplitude with strong flashes which varied between 18 and 25 mV. The filled circles are from an isolated cone which showed no sign of coupling to its neighbours (Baylor & Hodgkin, 1973). Stimuli were circular spots 50–150 μm in diameter.

RODS

Fig. 11 shows the effect of a very weak background light on the time scale and flash sensitivity of a rod. From these records it was found that a hyperpolarization of 0·6 mV reduced the flash sensitivity to 1/e of its dark value and shortened the time to peak from 0·53 sec to 0·29 sec; a stronger light which hyperpolarized by 1·2 mV reduced the flash sensitivity to 0·1 and shortened the time to peak from 0·51 to 0·19 sec. From these

figures we conclude that U_e, the hyperpolarization needed to reduce the sensitivity to 1/e, is about 0·5 mV in rods as compared with 2–4 mV in cones.

The weak background lights used in this experiment were from an uncalibrated source but from the flash sensitivity and integration time we estimated that the intensity of a steady light which hyperpolarized by 1 mV was of the order of 1 photon μm^{-2} sec^{-1} at the optimum wave-length. If the collecting area of this rod is taken as 10 μm^2, this result implies that absorption of only a few photons per sec in one rod lowers the sensitivity to 1/e.

One other experiment with a rod that was very much less sensitive gave a value of U_e = 1·1 mV.

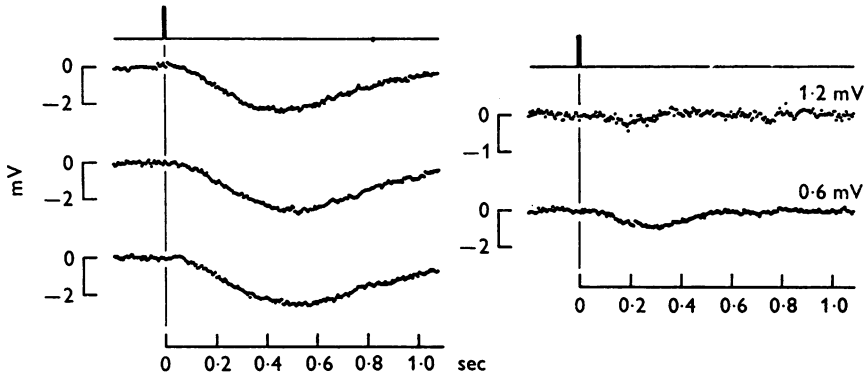


Fig. 11. Changes in sensitivity and time scale of a rod with background lights. Biomac records averaging responses to sixteen stimuli. Records on left obtained with no background light and flashes delivering 2·7 photons μm^{-2} at 519 nm. Records on the right, obtained between runs shown at left, show responses to the same flashes delivered on diffuse white background lights which hyperpolarized by 1·2 and 0·6 mV respectively. Membrane potential in darkness −47 mV, U_L = 15 mV. 22·5° C; flash sensitivity in dark about 1000 μV $photon^{-1}$ μm^2.

The time course of desensitization

Flashes of moderate intensity

The experiment of Fig. 12, which was designed to provide information about the time course of the desensitization mechanism, consisted in applying a test flash F_2 at various times before and after a conditioning flash F_1. F_1 and F_2 were approximately equal in strength. When the two responses overlap, the combined effect of the two flashes is less than the sum of the individual responses. The reduction is due partly to an instantaneous non-linearity and partly to an additional desensitization which

develops with a time delay. To distinguish between the two effects we assumed that the instantaneous relation between hyperpolarization $U(t)$ and a controlling linear variable $y(t)$ was a Michaelis relation. On this assumption $y(t)$ was calculated from the equation

$$y(t) = \frac{U(t)}{1 - U(t)/U_L}, \tag{22}$$

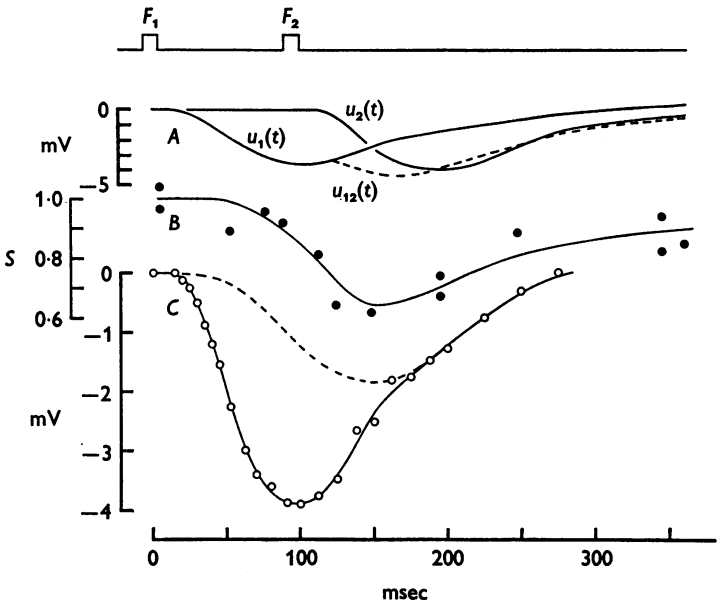


Fig. 12. Time course of delayed reduction in sensitivity produced by conditioning flash in red-sensitive cone.

A, tracings of records obtained (1) with conditioning pulse F_1 applied alone at $t = 0$, labelled $U_1(t)$; (2) with test pulse F_2 applied alone at $t = 95$ msec, note maximum occurs 100 msec after F_2, i.e. at $t = 195$ msec, tracing labelled $U_2(t)$; (3) with both pulses applied together, F_1 at $t = 0$ and F_2 at $t = 95$ msec, labelled $U_{12}(t)$.

B, time-dependent component of sensitivity expressed as a fraction of its normal value and measured at the time after the first pulse shown on the abscissa. Since measurements were always made at the maximum of the unconditioned test response, which occurred 100 msec after the flash, the abscissa is the time interval between flashes plus 100 msec. The method of estimating the ordinate is given in the text.

C, continuous curve and circles; time course of conditioning response. Interrupted curve, possible time course of second component (see text). The quantity of light in the conditioning flash was equivalent to 83 photons μm^{-2} at 644 nm. The quantity in the test flash was about 10% larger; both pulses were 10 msec in duration.

Temperature 20·4° C; maximum hyperpolarization 17·5 mV; resting potential −33 mV; flash sensitivity 53 μV photon^{-1} μm^2; coincident 50 μm diameter spots of white light for both flashes.

where U_L is the maximum hyperpolarization and the scaling factor is unity so that $y = U$ as $U \to 0$.

For the case illustrated in the upper records of Fig. 12 we have three experimental curves $U_1(t)$, $U_2(t)$ and $U_{12}(t)$, where $U_1(t)$ is the hyperpolarizing response to the conditioning flash alone, $U_2(t)$ is the response to the test flash alone and $U_{12}(t)$ is the response to both. In all three cases t means time from the centre of the conditioning pulse F_1. In the particular case illustrated the time interval between the two flashes was 95 msec. The electrical response to the test pulse alone was maximal 100 msec after the pulse, i.e. at $t = 195$ msec so the interaction is measured at that time. From the maximum hyperpolarization of 17·5 mV and the values of U_1 (195), U_2 (195) and U_{12} (195) of 1·38, 4·0 and 3·88 mV respectively we obtain values of y_1 (195) = 1·49 mV, y_2 (195) = 5·19 mV and y_{12} (195) = 4·99 mV. The time-dependent component of the sensitivity at $t = 195$ msec is then calculated as proportional to

$$\frac{y_{12}(195) - y_1(195)}{y_2(195)} = 0{\cdot}67.$$

Repeating this procedure at different time intervals gave the middle curve in Fig. 12 and showed that the sensitivity was lowest at $t = 155$ msec, which is 55 msec after the peak of the conditioning response. If the only desensitizing mechanisms present were some kind of instantaneous non-linearity, one would expect the time of maximum interaction to coincide with the peak of the conditioning response. A different way of stating this result is that for instantaneous non-linearity the condition for maximum interaction is that two equal pulses should be synchronous whereas for delayed desensitization the test response will be most reduced when the test stimulus is applied after the conditioning stimulus.

The time constant with which the sensitivity recovered is not well defined in Fig. 12 but experiments described in the next section show that it is of the order of 0·1 sec.

In a previous paper (Baylor *et al.* 1974*a*) it was shown that the response to flashes of moderate intensity could often be resolved into two components called C_1 and C_2 where C_2 reached a peak at 50–100 msec after C_1 and subsequently declined to zero with a time constant of 60–80 msec. The experiment of Fig. 12 suggests that the time course of the desensitizing mechanism may be similar to that of the second component C_2, which might have the time course shown by the interrupted curve in the lower part of Fig. 12.

The recovery of sensitivity

Fig. 13 illustrates the recovery of potential and sensitivity after applying different quantities of light. In the top curve, *A*, where the equivalent of $1{\cdot}8 \times 10^3$ photons μm^{-2} (at λ_{max}) were delivered in 10 msec, potential and sensitivity recovered fully in about 0·3 sec; the exponential time constant of recovery was about 0·1 sec. In the middle curve, *B*, where the equivalent of $0{\cdot}7 \times 10^6$ photons μm^{-2} were delivered in 10 msec, full recovery of potential and sensitivity took about 3 sec and the time constant of recovery was about 1 sec. In *C*, where the equivalent of 2×10^8 photons μm^{-2} were delivered in 3 sec, full recovery was not complete until more than 30 sec after the end of the pulse; the time constant of recovery was about 10 sec. It will be shown later that if the duration of the illumination is increased to 100 sec and the total equivalent quantity of light to $> 10^9$ photons μm^{-2} the final recovery of potential, sensitivity and time to peak is delayed by yet another order of magnitude, the final time constant of recovery being then 100–200 sec. There is a suggestion of this final slow phase in Fig. 13*C*, where sensitivity is still incomplete at 30 sec and recovers very slowly thereafter. The experiments with long-lasting illuminations, which bring in some new phenomena, will be considered further in the next section.

In all the three cases illustrated in Fig. 13 the depression of sensitivity was accompanied by a shortening of the time to peak and a speeding up of the falling phase of the response which were similar to those seen when the sensitivity was decreased by exposure to steady light.

Although the after-hyperpolarization produced by strong flashes was accompanied by a decrease in sensitivity it should be made clear that hyperpolarization itself is not a cause of the decrease in sensitivity. Thus Baylor & Fuortes (1970) showed that when a cell is hyperpolarized by inward current the response to flashes increases in size by an amount consistent with the increase in the membrane resistance during the response.

The parallel time course of the after-hyperpolarization and the depression of sensitivity can be explained by supposing that light produces a 'substance' which has some hyperpolarizing effect as well as depressing the sensitivity and accelerating the falling phase of the response. This is supported by the finding that the potential associated with an e-fold reduction in sensitivity was about 2 mV for each of the three cases illustrated in Fig. 13 (see Table 2).

Effect of prolonged illumination (ca. 100 *sec*)

At low levels of light intensity, the effects of prolonged illumination are in satisfactory agreement with the results obtained with relatively brief

rectangular pulses. Long steps of bright light on the other hand bring in some rather complicated effects which are illustrated in Fig. 14 and Fig. 15.

In the upper record of Fig. 14, a light delivering the equivalent of 9×10^3 photons μm^{-2} sec^{-1} at the optimum wave-length of 644 nm was applied for 120 sec. After a rapid initial decline, the hyperpolarization

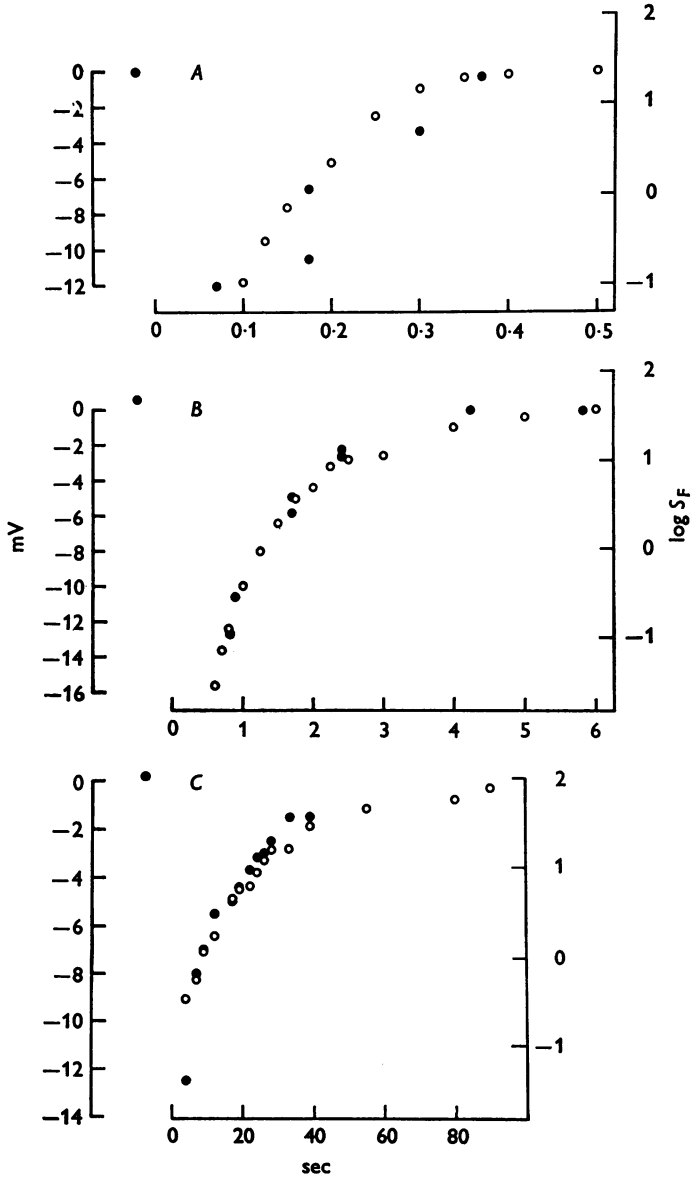


Fig. 13. For legend see facing page.

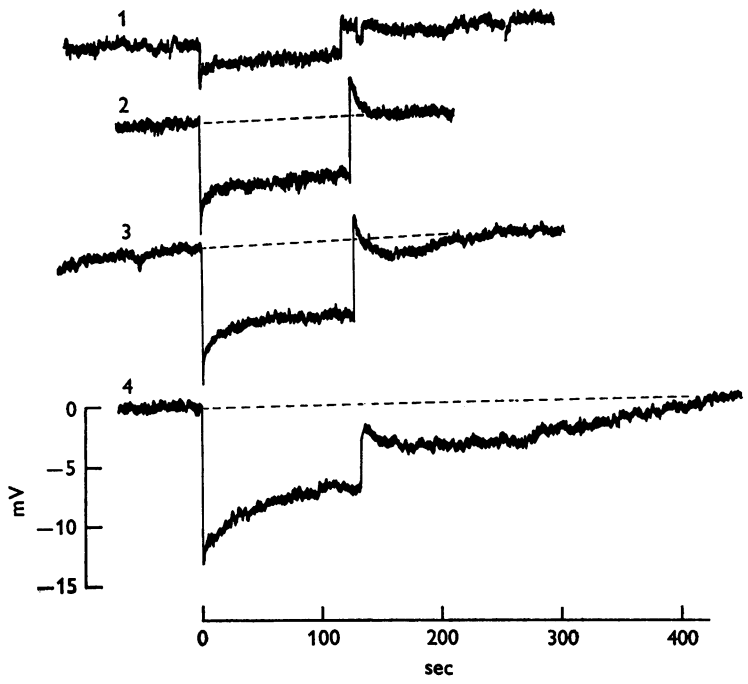


Fig. 14. Responses of a red-sensitive cone to long steps of light. Tracings from inkwriter records. Equivalent strengths of the stimuli, in photons μm^{-2} sec^{-1} at the optimum wave-length of 644 nm were: $9{\cdot}3 \times 10^3$ in record 1, $1{\cdot}6 \times 10^5$ in record 2, $2{\cdot}9 \times 10^6$ in record 3 and $5{\cdot}2 \times 10^7$ in record 4. For clarity, tracings have not been made of small responses to flashes used in testing sensitivity. Stimulating spot 75 μm in diameter, white light. Temperature 21·8° C.

Fig. 13. After effects of increasing quantities of light on potential and sensitivity in three red-sensitive cones. Abscissa, time after flash or step. Ordinate, ○, after-hyperpolarization; ●, log (flash sensitivity in μV $photon^{-1}$ μm^2) with the initial value shown by the left-hand point.

In *A* the conditioning flash was the equivalent of $1{\cdot}8 \times 10^3$ photons μm^{-2} at 644 nm delivered in 10 msec; in *B* the equivalent of $0{\cdot}7 \times 10^6$ photons μm^{-2} were delivered in 10 msec; in *C* the equivalent of 2×10^8 photons μm^{-2} were delivered in 3 sec. Note increase of time scale of recovery as quantity of light increases.

Experimental details for *A*, *B*, *C* respectively: temperature 21·2, 21·2, 22·1° C; resting potential −40, −39, −38 mV; maximum hyperpolarization 18, 19·5, 18 mV; flash sensitivities 24, 47, 105 μV $photon^{-1}$ μm^2.

All three experiments were carried out with coincident 150 μm diameter white spots in both testing and conditioning stimuli.

from the steady light was well maintained and disappeared rapidly when the light was switched off. The sensitivity of the cell, which was tested with brief flashes, dropped to about one third within less than 2 sec of switching on the light and recovered within a few seconds of switching it off. These effects and the decline from an initial peak to a plateau are consistent with the properties of the time-dependent desensitization described in the previous parts of this paper. However, brighter lights delivering the equivalent of $1{\cdot}6 \times 10^5$ photons μm^{-2} sec^{-1} in record 2 and $2{\cdot}9 \times 10^6$ photons μm^{-2} sec^{-1} in record 3 gave a slowly declining hyperpolarization after switching on the light and a rebound which depolarized the cell by a few millivolts after switching off the light. This component of the response which resembles the response of a condenser-coupled amplifier to a rectangular input will be called the differentiated component. In record 3 the depolarization resulting from the rebound at the end of the step is followed by a small hyperpolarization which declined with a time constant of 50–70 sec. The after-hyperpolarization is considerably larger in record 4 in which the light delivered the equivalent of $5{\cdot}2 \times 10^7$ photons μm^{-2} sec^{-1}. In this case the differentiated component did not make the internal potential more positive than in the resting condition but it prevented the after-hyperpolarization from reaching a maximum until about 40 sec after the end of the light step. A delayed maximum of this sort was present consistently in red-sensitive cones illuminated for more than 30 sec with lights of equivalent strength greater than 5×10^6 photons μm^{-2} sec^{-1}.

The slow decline in hyperpolarization during the period of illumination could be attributed either to a decrease in the saturation level or to a reduction in sensitivity, in which we include loss of pigment. The first type of explanation can be excluded as the sole cause of this effect because superposed bright flashes did cause some additional hyperpolarization although not to the original saturating level.

Fig. 15 compares the recovery of potential, log sensitivity and time to peak after applying a light equivalent to 67×10^6 photons μm^{-2} sec^{-1} for 130 sec to a red-sensitive cone. In the later stages of the response all three variables recover with a similar time course. The scaling constant U_e required to bring log sensitivity and potential into coincidence is 1·8 mV for an e-fold change in sensitivity; this is similar to values found for after-hyperpolarization in other cases (Table 2). The final slow phase of recovery can thus be explained by the disappearance of a substance which hyperpolarizes as well as decreasing sensitivity and time scale. However, this is clearly not the explanation of the whole recovery curve, because at times less than 30 sec the sensitivity increased rapidly at a time when the hyperpolarization was increasing and it is impossible to bring the two curves into coincidence by a single scaling constant. It is therefore necessary to

postulate some additional factor which decreases sensitivity without hyperpolarizing and which declines with a time constant of the order of 20 sec. If this factor depolarizes the cell it would explain the presence of a differentiated component in Figs. 14 and 15.

In the experiment of Fig. 15 the light delivered the equivalent of about 10^{10} λ_{max} photons μm^{-2} over a period of 130 sec. Since this should have bleached nearly all the visual pigment it is highly probable that regeneration of visual pigment makes some direct contribution to the recovery of

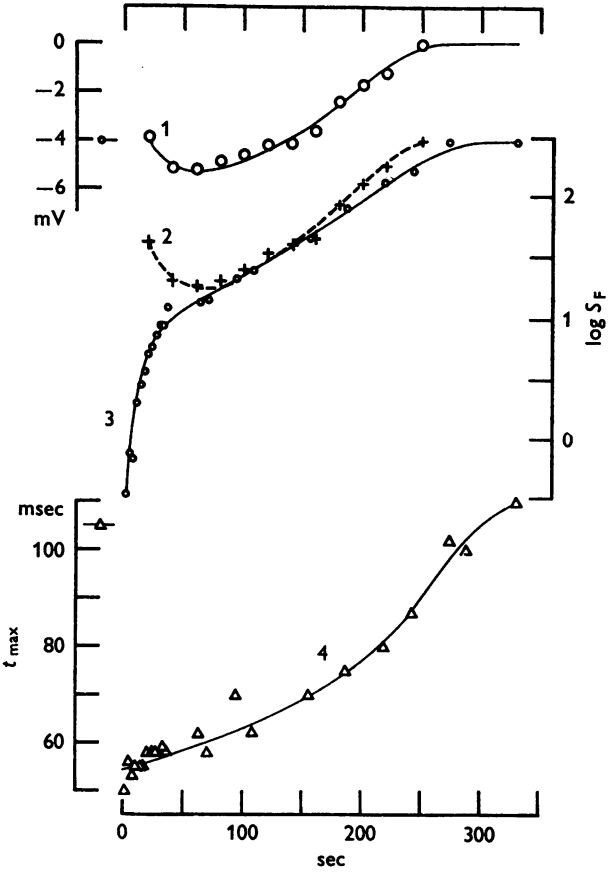


Fig. 15. Comparison of recovery of potential, log sensitivity and time to peak of flash response after illuminating red-sensitive cone for 130 sec with light of intensity equivalent to 67×10^6 photons μm^{-2} sec^{-1}. The abscissa is time after the end of the 130 sec step. Curve 1 is the potential; curve 3 is the $\log_{10}$ of the flash sensitivity in μV $photon^{-1}$ μm^2; curve 4 is the time to peak of the flash response and curve 2 is calculated from the after-hyperpolarization (curve 1) on the assumption that 1·8 mV of hyperpolarization is associated with an e-fold decrease of sensitivity. Temperature 21·3° C; resting potential −43 mV; maximum hyperpolarization 21 mV; flash sensitivity in dark 28·4 μV $photon^{-1}$ μm^2; 75 μm diameter coincident white spots.

sensitivity. However our measurements give no indication whether pigment regeneration occurs with a time constant of 20 sec which would fit the early recovery or with one of 150 sec which would fit the late stage or in two phases, which would fit both.

Comparison of U_e determined by different methods

The exponential parameter U_e which relates potential and change in log sensitivity (eqn. (9)) can be measured either in the presence of background light or from the reduction of sensitivity associated with an after-hyperpolarization. In the former case U_e was measured from the voltage at which the flash sensitivity was reduced to one tenth and in the latter from the scaling factor needed to bring potential and log sensitivity curves into coincidence. Table 2 summarizes the two sets of measurements which give

TABLE 2. Values of U_e obtained by different methods in red-sensitive cones

	U_e (mV)	U_L (mV)
I. After-effect of light		
A. 0·1 sec component	2·2	17
	3·0	17
B. 1 sec component	2·2	19
C. 10 sec component	1·6	22
D. 100 sec component	1·8*	20
	1·2*	
	1·5	18
Mean	1·9	
II. 0·7–1·1 sec after beginning of conditioning light		
	3·2	23
	1·7	16
	2·5*	20
	2·1	16
	3·5	19
	3·1	17
	3·5	20
III. During steady light		
	1·8	18
	2·8	26
Mean of II and III	2·7	

In section I, U_e was obtained from the scaling factor used to bring potential and log sensitivity into coincidence; see Fig. 13 for *A*, *B*, and *C* and Fig. 15 for *D*. In sections II and III, U_e was taken as $0{\cdot}434U_{10}$, where U_{10} is the steady potential at which the flash sensitivity was reduced to one-tenth. U_L is the peak hyperpolarization. Values marked with an asterisk were obtained on the same cone. The last five experiments are those analysed in Table 1.

a mean of $U_e = 2{\cdot}8$ mV in the presence of light and $U_e = 1{\cdot}9$ mV for after-hyperpolarizations. Tests on the same cone (marked with an asterisk in Table 2) support the conclusion that U_e is larger when measured in the presence of light.

DISCUSSION

The main conclusion from the experiments described here is that background lights decrease the amplitude and time scale of the response of turtle rods and cones to flashes of light. This helps to explain the familiar observation that the time resolution of vision improves as the light intensity increases. The result is also interesting because there has been considerable discussion (see Rushton, 1965*b*) as to whether the changes in sensitivity associated with bleaching or with the presence of background light occur in receptors or at some later stage of the visual pathway. Our results show that there is a large change at the receptor level, but do not exclude neuronal changes later on. Nor do they rule out participation of horizontal cell processes in some kind of localized feed-back affecting the receptors. What we do regard as established is that applications of spots of light with a diameter as small as 12 μm produces striking changes in the sensitivity and time scale in the cone on which the circle of light is centred. Since spots with diameter less than 100 μm give virtually no change in the potential of the horizontal cell layer, it seems reasonable to conclude that light adaptation is at least partly dependent on a mechanism which is localized in the receptor and its immediate surroundings.

Nature of the mechanism responsible for decreasing sensitivity and time scale

The aim of this section is to describe a hypothesis which accounts for the main features of the electrical response of turtle cones to flashes and steps of light. It is assumed that a flash of light starts a chain of events which leads to the appearance of a blocking substance Z_1 near the light-sensitive channels. The concentration z_1 of the blocking substance is reduced by the reaction

$$Z_1 \underset{\kappa_{21}}{\overset{\kappa_{12}}{\rightleftharpoons}} Z_2 \xrightarrow{\kappa_{23}} Z_3, \qquad \text{(Scheme 1)}$$

where the κ's are rate constants. When considering the effect of strong flashes or steps it is necessary to introduce a back reaction κ_{32} and further components Z_4 and Z_5. For weak steps or moderate flashes Scheme 1 is all that is required, and its implications will be considered before discussing the full model. As in the previous paper it is assumed that only Z_1 blocks and that Z_2 exerts its influence on potential through the back reaction κ_{21}. In order to explain the desensitization and shortening of time scale it is assumed that the conversion of Z_1 to Z_2 is autocatalytic. Hence as

Z_2 accumulates Z_1 reaches a smaller maximum earlier and declines more rapidly than it does in the absence of Z_2. The simplest quantitative assumption that can be made is

$$\kappa_{12} = \bar{\kappa}_{12} + \nu z_2, \tag{23}$$

where $\bar{\kappa}_{12}$ is the value of κ_{12} in the dark and ν is a constant characterizing the efficacy of the autocatalytic system. As in all cases of genuine catalysis the catalyst increases both forward and back reaction velocities without affecting the equilibrium constant so

$$\kappa_{21} = \bar{\kappa}_{21} + \frac{\nu \bar{\kappa}_{21}}{\bar{\kappa}_{12}} z_2. \tag{24}$$

In a later paper (Baylor *et al.* 1974*b*) it will be shown that this assumption accounts fairly well for the shortening of time scale as the cone is desensitized. Here we consider only the relatively simple problem of calculating the steady-state relation between light intensity and hyperpolarization. To do this we make the additional assumption that the steady rate (M) of producing blocking molecules is proportional to the steady light intensity I_S. In the steady state we then have the following relations

$$M = \kappa_{12} z_1 - \kappa_{21} z_2, \tag{25}$$

$$M = \kappa_{23} z_2. \tag{26}$$

On eliminating the variable quantities, z_2, κ_{12} and κ_{21} from eqns. (23–26) we obtain the relation between z_1 and M as

$$z_1 = \frac{M(\kappa_{23} + \bar{\kappa}_{21}) + M^2 (\bar{\kappa}_{21} \nu / \bar{\kappa}_{12} \kappa_{23})}{\bar{\kappa}_{12} \kappa_{23} + \nu M}. \tag{27}$$

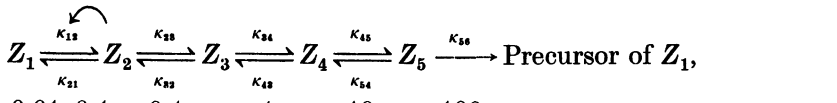
Since M is proportional to I_S and z_1 is proportional to f this equation is of the same form as eqn. (2) which in conjunction with (1) describes the steady-state relation between potential and light intensity. In a subsequent paper we shall use eqn. (27) with other relations to estimate values of the constants $\bar{\kappa}_{12}$, $\bar{\kappa}_{21}$ and ν. We can then make a thorough test of the hypothesis by seeing whether it accounts for the changes in time course and sensitivity produced by a background light.

The basis of eqn. (2) is more general than might appear from the simple derivation given above. In a subsequent paper (Baylor *et al.* 1974*b*) it will be shown that eqn. (2) also applies in the more complicated situations in which κ_{12} reaches a limiting value and eqn. 23 is replaced by the rectangular hyperbola

$$\kappa_{12} - \bar{\kappa}_{12} = \frac{(\kappa_{12m} - \bar{\kappa}_{12}) \nu z_2}{(\kappa_{12m} - \bar{\kappa}_{12}) + \nu z_2}, \tag{28}$$

where κ_{12m} is the upper limit to κ_{12}. Changing from the simple chain in Scheme 1 (page 755) to the more complex one in Scheme 2 (page 757) also does not alter eqn. (2), although both this alteration and the change from eqn. (23) to eqn. (28) naturally affect the relations between the constants P, Q, R and the underlying rate constants.

The model can be extended to cover the wide variations in the time taken for sensitivity and potential to return to their resting values after illumination with increasing numbers of photons. As has been shown in this and the preceding paper the final time constant observed experimentally varies between about 0·1 sec with moderate flashes (10^3 photons μm^{-2}) to 100 sec with long, strong rectangular pulses delivering the equivalent of 10^9 photons μm^{-2}. There also seem to be separate components with time constants of the order of 0·1, 1, 10 and 100 sec, in addition to the first component of potential with a variable time constant. However in all cases (except for the complication mentioned on p. 752) it was found that the exponential constant U_e relating potential to log threshold was about 2 mV. In order to accommodate this complex set of observations we adopt the scheme proposed on p. 719 of Baylor *et al.* (1974*a*) with the addition of an extra rate constant κ_{56} and an autocatalytic link between Z_2 and κ_{12}. The full scheme is then



(Scheme 2)

where the numbers below each stage give the order of magnitude of the time constant in seconds. Back-reactions such as κ_{32} are all small compared to forward reactions such as κ_{23}.

On this scheme the concentration of Z_2 after a strong flash should be given approximately by

$$I\Delta t\,[A\,e^{-10t} + B\,e^{-t} + C\,e^{-0\cdot1t} + D\,e^{-0\cdot01t}],$$

where t is time in seconds and A to D are constants of decreasing order of magnitude, i.e. $A \gg B \gg C \gg D$. When the concentration of Z_2 is high the reaction between Z_1 and Z_2 should be nearly in equilibrium, so a similar expression should describe the decline of Z_1. With moderate flashes only the first term has any detectable effect in hyperpolarizing or reducing sensitivity but the later terms are brought in sequentially as the number of absorbed quanta increases. Hence the time constant for recovery of sensitivity and potential varies between 0·1 sec for weak flashes and 100 sec for strong steps lasting 10–100 sec. Since all components hyperpolarize through Z_1 and reduce sensitivity through Z_2 the desensitization associated with a given hyperpolarization should be the same for all components.

A possible objection to the hypothesis outlined here is that it does not leave room for a connexion between the fraction of pigment bleached and the decrease of log sensitivity after exposure to strong light, for which there is evidence from Dowling (1960) in the rat retina and from Rushton (1961,

1963, 1965*a*) in human rods and cones (see also Donner, 1973). We should be in a better position to discuss this question if there were any information about the rate at which visual pigments are regenerated in the retina of *Pseudemys*. In the absence of such information we shall assume that the cone pigments of *Pseudemys* are regenerated with a time constant of about 100 sec at 20° C and that we have to explain why κ_{56} in Scheme 2 should be the same as the rate constant for regeneration of cone pigment. This might be achieved in the following way. Suppose that calcium ions are the blocking particles released by light and that in unbleached cones one or more calcium ions are locked away behind the chromophore. On such a scheme Z_1 represents free calcium near the sodium channel in the outer segment; Z_2, Z_3 and Z_4 are unspecified forms of bound calcium and Z_5 is calcium bound in the original position behind the chromophore but with the latter bleached instead of unbleached. Since restoration of the initial condition, with calcium firmly bound but releasable by light, depends on the rate of regeneration of unbleached pigment, we should expect there to be a close correlation between the concentration of Z_5 which decreases sensitivity and the fraction of unbleached pigment present at any particular time.

Part of this work was supported by USPHS Grant 1 R01 EY00904 and special Fellowship 2 F11 NS02246 to D.A.B.

REFERENCES

BAYLOR, D. A. & FUORTES, M. G. F. (1970). Electrical responses of single cones in the retina of the turtle. *J. Physiol.* **207**, 77–92.

BAYLOR, D. A. & HODGKIN, A. L. (1973). Detection and resolution of visual stimuli by turtle photoreceptors. *J. Physiol.* **234**, 163–198.

BAYLOR, D. A., HODGKIN, A. L. & LAMB, T. D. (1974*a*). The electrical response of turtle cones to flashes and steps of light. *J. Physiol.* **242**, 685–727.

BAYLOR, D. A., HODGKIN, A. L. & LAMB, T. D. (1974*b*). Reconstruction of the electrical responses of turtle cones to flashes and steps of light. *J. Physiol.* **242**, 759–791.

DONNER, K. O. (1973). Rod dark adaptation and visual pigment photoproducts in *Biochemistry and Physiology of Visual Pigments*, ed. LANGER, H., pp. 205–209. Berlin: Springer-Verlag.

DOWLING, J. E. (1960). Chemistry of visual adaptation in the rat. *Nature, Lond.* **188**, 114–118.

FUORTES, M. G. F. & HODGKIN, A. L. (1964). Changes in time scale and sensitivity in the ommatidia of *Limulus*. *J. Physiol.* **172**, 239–263.

RUSHTON, W. A. H. (1961). Rhodopsin measurement and dark-adaptation in a subject deficient in cone vision. *J. Physiol.* **156**, 193–205.

RUSHTON, W. A. H. (1963). Cone pigment kinetics in the protanope. *J. Physiol.* **168**, 374–388.

RUSHTON, W. A. H. (1965*a*). Cone pigment kinetics in the deuteranope. *J. Physiol.* **176**, 38–45.

RUSHTON, W. A. H. (1965*b*). The Ferrier Lecture 1962. Visual adaptation. *Proc. R. Soc.* B **162**, 20–46.