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THE ELECTRICAL RESPONSE OF TURTLE CONES TO FLASHES AND STEPS OF LIGHT

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SUMMARY

1. The linear response of turtle cones to weak flashes or steps of light was usually well fitted by equations based on a chain of six or seven reactions with time constants varying over about a 6-fold range.

2. The temperature coefficient (Q_{10}) of the reciprocal of the time to peak of the response to a flash was 1·8 (15–25° C), corresponding to an activation energy of 10 kcal/mole.

3. Electrical measurements with one internal electrode and a balancing circuit gave the following results on red-sensitive cones of high resistance: resistance across cell surface in dark 50–170 MΩ; time constant in dark 4–6·5 msec. The effect of a bright light was to increase the resistance and time constant by 10–30 %.

4. If the cell time constant, resting potential and maximum hyperpolarization are known, the fraction of ionic channels blocked by light at any instant can be calculated from the hyperpolarization and its rate of change. At times less than 50 msec the shape of this relation is consistent with the idea that the concentration of a blocking molecule which varies linearly with light intensity is in equilibrium with the fraction of ionic channels blocked.

5. The rising phase of the response to flashes and steps of light covering a 10^5-fold range of intensities is well fitted by a theory in which the essential assumptions are that (i) light starts a linear chain of reactions leading to the production of a substance which blocks ionic channels in the outer segment, (ii) an equilibrium between the blocking molecules and unblocked channels is established rapidly, and (iii) the electrical properties of the cell can be represented by a simple circuit with a time constant in the dark of about 6 msec.

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6. Deviations from the simple theory which occur after 50 msec are attributed partly to a time-dependent desensitization mechanism and partly to a change in saturation potential resulting from a voltage-dependent change in conductance.

7. The existence of several components in the relaxation of the potential to its resting level can be explained by supposing that the 'substance' which blocks light sensitive ionic channels is inactivated in a series of steps.

INTRODUCTION

There is now general agreement that light hyperpolarizes many vertebrate photoreceptors (see Tomita, 1970; Hagins, 1972), and in some cases there is evidence that the hyperpolarization is generated by a decrease in the conductance of ionic channels in the outer segment (Toyoda, Nosaki & Tomita, 1969; Baylor & Fuortes, 1970; Hagins, Penn & Yoshikami, 1970; Korenbrot & Cone, 1972). To explain the dependence of the conductance change on light, Baylor & Fuortes (1970) suggested that absorption of photons leads to the production of an intermediary substance which decreases membrane conductance by interacting with ionic channels in the outer segment. The aim of the present paper is to explore the hypothesis proposed by Baylor & Fuortes and if possible to put it on a quantitative basis. The first move was to look for a reasonably simple way of describing the linear response of turtle cones to flashes or steps of light. We then attempted to extend the analysis to larger signals by assuming that the concentration of the intermediary blocking molecules varied linearly with the light intensity and that non-linearities arose partly from the nature of the equivalent circuit of the cone and partly from competition between blocking molecules for the photo-sensitive channels. This proved successful up to 50 msec after the beginning of a flash or step, but at longer times it is necessary to assume that the behaviour of the blocking molecule is also non-linear. A description of the nature of this non-linearity is deferred to the next paper of this series (Baylor & Hodgkin, 1974).

The second half of the paper deals with the relaxation of the potential to its resting level and provides qualitative evidence for the existence of several components of widely different time constant.

The theoretical section (pp. 688–698) summarizes the equations used in analysing or reconstructing the first 50 msec of the response to flashes or steps covering a 10^5-fold range of intensities. In a later, and more speculative paper (Baylor, Hodgkin & Lamb, 1974) we shall attempt to extend the reconstruction to times of the order of 2 sec.

METHODS

The apparatus and method were as described in the paper by Baylor & Hodgkin (1973). The resistance and capacity of certain cones were measured with a single electrode, using the bridge-circuit principle (Fein, 1966). In order to reconstruct the rising phase, equation (5) was integrated numerically using the Taylor series method (Norman, 1973).

NOMENCLATURE

Equivalent light intensities

When monochromatic light is used the intensity at the retina can be given directly in photons (or quanta) μm^{-2} sec^{-1}. With white light the total photon density is not a relevant quantity because much of the spectrum is not absorbed by the receptor. However, for any given source and receptor a calibration factor can be determined which allows one to give the equivalent effect of the light at the optimum wave-length (see Baylor & Hodgkin, 1973). In the present series of experiments the unattenuated white light was equivalent in its effect on red-sensitive cones to that of a monochromatic light of wave-length 644 nm and intensity 67×10^6 photons μm^{-2} sec^{-1}. The corresponding figure for green-sensitive cones at 559 nm was 36×10^6 photons μm^{-2} sec^{-1} and for rods at 519 nm was 30×10^6 photons μm^{-2} sec^{-1}. The initial rate at which chromophores are isomerized in an unbleached cone can be estimated by multiplying the light intensity by the effective collecting area which was probably about 1 μm^2 in most of the experiments in this paper, but may be 10 μm^2 in the most sensitive cones (see Baylor & Hodgkin, 1973).

Sensitivities

The flash sensitivity, S_F, is defined as the peak hyperpolarization produced by a weak flash divided by the density of applied photons at the optimum wave-length (μV $photon^{-1}$ μm^2).

The step sensitivity S_S (or simply S) is the steady hyperpolarization due to a very weak steady light divided by the light intensity at the optimum wave-length (μV $photon^{-1}$ sec μm^2) (see Baylor & Hodgkin, 1973).

Integration time

If the linear response to a weak flash is $f(t)$ where $f(t) = 1$ at the peak of the response, the integration time, t_1, is defined as $\int_0^\infty f(t)\,dt$. From this definition it follows that the step sensitivity is equal to the flash sensitivity multiplied by the integration time (see Baylor & Hodgkin, 1973).

Electrical effect of one photoisomerization; effective collecting area

Baylor & Hodgkin (1973) obtained evidence that much of the variation in sensitivity between cones arises from the differences in effective collecting area that result from variation in the angle of incidence of the light relative to the axis of the cones. They suggest that the maximum flash sensitivity of 250 μV photon^{-1} μm^2 corresponds to axial entry of light and that the effective collecting area is then 10 μm^2. On this basis a cone with a sensitivity of 25 μV photon^{-1} μm^2 is taken as having an effective collecting area of 1 μm^2 and both the sensitive and insensitive cone then have the same absolute sensitivity of 25 μV per photoisomerization. The symbol S_F^ϕ is used to denote the peak voltage per photoisomerization.

Rising and falling phase of response

By rising phase of the response we mean the initial hyperpolarizing phase and by falling phase we mean the later phase of repolarization.

Michaelis equation

We shall follow Dixon & Webb (1964) in describing the rectangular hyperbola

$$\frac{U}{U_{\max}} = \frac{x}{k+x}$$

as a Michaelis equation. Other names associated with this relation are Michaelis' colleague Menten (1913), Langmuir (1916) and Briggs & Haldane (1925).

If $U/U_{\max}$ is known and we wish to calculate a variable proportional to x this can be done by the formula

$$\frac{x}{k} = \frac{U}{U_{\max} - U},$$

which will be called an inverse Michaelis relation.

THEORETICAL SECTION

Equivalent circuit theory: the relation between membrane potential and the fraction of light-sensitive channels blocked

The object of this section is to derive equations which enable one to calculate the extent to which the light sensitive channels are blocked during the response to a flash or step of light. For this purpose the network proposed by Baylor & Fuortes (1970) will be used, with the addition of a parallel capacitance C (Fig. 1). In this circuit $\bar{g}$ is the constant conductance in series with the voltage E. g_1 is the conductance of the channels affected

by light and has the value g_D in darkness; for simplicity the equilibrium potential of the light-sensitive channels is taken as zero, as was found to be approximately the case by Baylor & Fuortes (1970). The assumption is not critical since the theory is primarily concerned with displacements of the potential from its dark value.

V is the internal potential, V_D is its value in darkness and V_L ($= E$) is the limiting value of V with a light of saturating intensity.

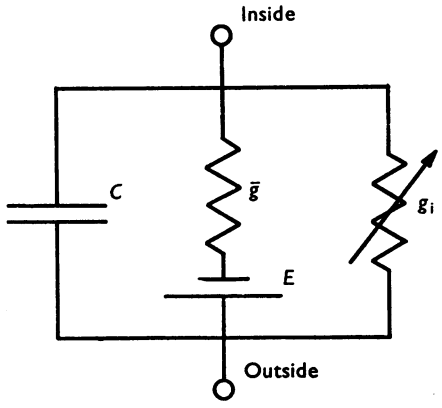

Fig. 1. Equivalent circuit of cone after Baylor & Fuortes (1970). C is the membrane capacity, $\bar{g}$ is the fixed conductance in series with the battery E which maintains the resting potential and g_i is the variable conductance which is reduced by light.

$\tau_D = C/(g_D + \bar{g})$ is the time constant in the dark and $\tau_L = C/\bar{g}$ is the time constant when g_i is reduced to zero under the influence of a bright light.

$$a = \frac{g_D + \bar{g}}{\bar{g}} \tag{1}$$

is a ratio which enters into a number of relations such as

$$a = \frac{V_L}{V_D}, \tag{2}$$

$$a = \frac{\tau_L}{\tau_D}, \tag{3}$$

$$a = \frac{R_L}{R_D}, \tag{4}$$

where R is the d.c. resistance between the inside and outside of the cell.

The differential equation for the circuit in Fig. 1 is

$$C\frac{dV}{dt} + V(g_i + \bar{g}) = E\bar{g}. \tag{5}$$

The light-sensitive conductance g_l is regarded as the sum of the conductances of the channels remaining open, and a variable B defined by

$$B = \frac{g_D - g_l}{g_D} \tag{6}$$

gives the fraction of light-sensitive channels blocked at any particular time. If B is known as a function of time, V can be computed by solving

$$\tau_L \frac{dV}{dt} + V[a-(a-1)B] = E, \tag{7}$$

which follows from eqn. (5) and the definitions of τ_L, a and B. An alternative form of eqn. (7) using the hyperpolarization U $(=V_D - V)$ is

$$\tau_L \frac{dU}{dt} + U[a-(a-1)B] = BU_L, \tag{8}$$

where U_L is the maximum hyperpolarization.

A useful relation which follows at once from eqn. (8) is

$$B = \frac{aU + \tau_L dU/dt}{U_L + (a-1)U}. \tag{9}$$

In the steady state this reduces to

$$B = \frac{aU}{U_L + (a-1)U}, \tag{10}$$

or

$$\frac{U}{U_L} = \frac{B}{a - B(a-1)}. \tag{11}$$

Baylor & Hodgkin (1973) estimated that in turtle cones one photoisomerization gave a peak hyperpolarization ΔU of about 25 μV. From eqn. (9) it follows that at the peak of the response the fraction of conductance channels blocked by one photoisomerization is given by

$$\Delta B = \frac{a\Delta U}{U_L}.$$

If $a = 1{\cdot}6$ and $U_L = 25$ mV, $\Delta B = 1/630$; this estimate is of the same order of magnitude as that obtained by Cone (1973).

Eqns. (8) to (11) are more general than might appear from the derivation given here. Thus they apply to systems in which complete activation involves a change from a dark state in which the light-sensitive pathway is a conductance g_D in series with a battery E_D to a light state in which the pathway is a conductance g_L in series with a battery E_L. For these systems eqns. (8)–(11) apply if B remains the fraction of channels activated, τ_L is the time constant in strong light, $C/(\bar{g} + g_L)$, and a is the ratio of total conductances in dark and light, i.e. $(\bar{g} + g_D)/(\bar{g} + g_L)$.

Possible relations between concentration of activating substance and fraction of ionic channels blocked

This paper provides evidence that light produces a state of activation whose intensity at any fixed time up to 50 msec after a flash varies linearly with the light intensity over several log units. In the model used for analysis the state of activation is characterized by a variable y which represents the number of blocking particles per receptor. Combination of a single particle with a channel is assumed to reduce the conductance of the channel to zero. We shall make the provisional assumption that there is an instantaneous relation, for example, a Michaelis relation, between B, the fractional closure of the ionic channels and the linear variable y. The purpose of this section is to provide a physical basis for alternative forms of the function relating B to y.

It is possible that the division of a receptor outer segments into sacs and disks provides a series of 'compartments' within which any blocking particles released by light would be to some extent confined. In the general case of a compartment having n channels and r particles the probability f that a channel is blocked under equilibrium conditions can be obtained from

$$[r-(n-1)f]\,[1-f] = kf, \tag{12}$$

where k is the dissociation constant. The basis of this equation is that a single channel is uncombined for a fraction $(1-f)$ of the time and during that time there are $[r-(n-1)f]$ free particles available to combine with it. Hence the mean rate of association is proportional to the left-hand side of eqn. (12) and the mean rate of dissociation to the right-hand side. At equilibrium these two rates must be equal.

Two extreme cases will be considered – the first being that all channels and activating particles are confined in a single large compartment which can be identified with the receptor, and the second that there are a large number of compartments each containing a single channel. In the first macroscopic case, arguments based on the Law of Mass Action can be used but in the second it is necessary to consider statistical variation in the number of particles per compartment as well as the fraction of time for which each particle is combined. In both cases the relation between B and y reduce to a Michaelis equation when the affinity of the activating molecule for the channel is low, and both lead to the same relation for $\mathrm{d}B/\mathrm{d}y$ as y approaches zero.

One large compartment; equations based on Law of Mass Action. If N_c is the total number (both blocked and unblocked) of light-sensitive channels per cone and y is the total number of activating particles per cone (both free and bound) then BN_c is the number (or concentration) of blocked

channels, $(1-B)N_c$ is the concentration of unblocked channels and $y-BN_c$ is the concentration of free y. Application of the Law of Mass Action on the assumption that an equilibrium has been established gives

$$(y-BN_c)\,(1-B)N_c = BN_c k. \tag{13}$$

This is equivalent to (12) above, because for a single macroscopic compartment

$$r \equiv y, \quad f \equiv B \quad \text{and} \quad n \equiv N_c \gg 1.$$

Equation (13) has two solutions for B, one of which is greater than unity and therefore not physically meaningful. The solution of interest is

$$B = \frac{1}{2N_c}\{y+N_c+k-\sqrt{[(y+N_c+k)^2-4N_c y]}\}. \tag{14}$$

If $k \gg N_c$, which implies that free y always greatly exceeds bound y, this approximates to the rectangular hyperbola

$$B = \frac{y}{y+k+N_c} \doteq \frac{y}{y+k}. \tag{15}$$

If $k \ll N_c$ which implies that free y is negligible until all the channels are blocked eqn. (14) reduces to

$$B = y/N_c \quad \text{for} \quad y < N_c,$$

$$B = 1 \quad \text{for} \quad y > N_c.$$

For comparison with experiments it is convenient to eliminate N_c from eqn. (14) by using the variables $Y = y/N_c$ and $K = k/N_c$. Y is then the mean number of blocking molecules per ionic channel and K is a dissociation constant expressed in the same units as Y. In terms of these variables eqn. (14) becomes

$$B = \tfrac{1}{2}\{Y+1+K-\sqrt{[(Y+1+K)^2-4Y]}\}. \tag{16}$$

Fig. 2 illustrates the relation between B and $\log\,[Y/(1+K)]$ calculated from eqn. (16) with different values of K. The choice of horizontal scales is made in order that the curves coincide for small Y.

The inverse equation which allows Y to be calculated from B is

$$Y = B + \frac{BK}{1-B}. \tag{17}$$

Single channel compartments. On the assumption that the N_c channels are distributed one per compartment there will be N_c compartments. If it is also assumed that each of the N_ϕ photoisomerizations leads to a blocking particle with probability $P(t)$ at time t after the flash then the mean number of blocking particles per compartment, Y, is

$$Y = \frac{N_\phi}{N_c} P(t). \tag{18}$$

On putting $n = 1$ in eqn. (12) we obtain

$$r(1-f) = Kf,$$

where r is the number of particles, f is the fraction of time for which the channel is closed and K is the dissociation constant. Hence

$$f = \frac{r}{r+K}. \tag{19}$$

As the particles are assumed to be distributed randomly among compartments the probability of a compartment containing r particles is

$$P_r = \frac{Y^r e^{-Y}}{r!} \tag{20}$$

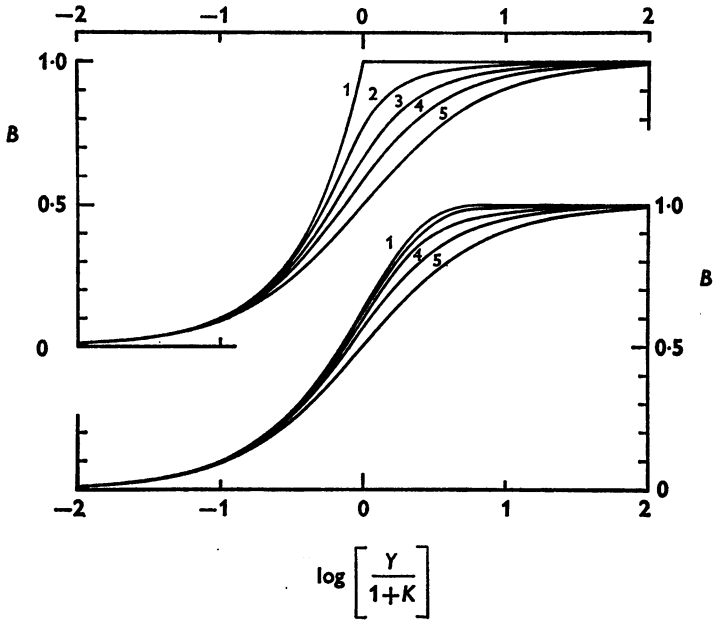

Fig. 2. Theoretical curves giving instantaneous relation between B, fraction of ionic channels blocked, and the log of the linear variable $Y/(1+K)$ which is proportional to light intensity. In terms of the underlying theory Y is the total number of blocking particles divided by the total number of channels and K is the dissociation constant in the same units as Y (see text).

In the upper curves the cone is regarded as a single compartment and the curves were calculated from eqn. (16). In the lower curves it is assumed that there is one light-sensitive channel per compartment and the curves were calculated from eqn. (22).

The values of K were as follows $K \to 0$ (curves 1), $K = \frac{1}{10}$ (curves 2), $K = \frac{1}{3}$ (curves 3), $K = 1$ (curves 4), $K \to \infty$ (curves 5) in which case both curves are Michaelis relations.

and the probability of a channel being blocked in a compartment containing r particles is

$$P_r f(r) = \frac{Y^r e^{-Y}}{r!}\left(\frac{r}{r+K}\right). \tag{21}$$

Hence the fraction of channels blocked is

$$B = e^{-Y} \sum_{r=1}^{\infty} \frac{Y^r}{r!}\left(\frac{r}{r+K}\right). \tag{22}$$

Eqn. (22) can be simplified in certain cases. If the affinity of the particles for the channels is very high $K \to 0$ and

$$B = 1 - e^{-Y}, \tag{23}$$

which is the fraction of compartments containing one or more particles. If $K = 1$,

$$B = 1 - (1 - e^{-Y})/Y, \tag{24}$$

and if $K \gg 1$,

$$B \doteqdot Y/(Y+K). \tag{25}$$

Consequences of taking $B(Y)$ as a Michaelis function. The assumption that the instantaneous relation between the fraction of channels blocked, B, and the concentration of blocking particles Y is a Michaelis relation (eqn. (25)) has a number of interesting implications. If B is eliminated between eqn. (25) and (11) we obtain for conditions in which $dU/dt = 0$,

$$\frac{U}{U_L} = \frac{Y}{Y+aK}. \tag{26}$$

Thus if the relation between B and Y is a rectangular hyperbola the relations between U and Y will be similar but the dissociation constant will be aK instead of K. Since Y is assumed to be proportional to light intensity it follows that if a light of intensity I' blocks half the sodium channels, a light of intensity aI' will give half maximal hyperpolarization. Another interesting point about this assumption is that the increment in the resistance of the light-sensitive channels should be directly proportional to light intensity.

If the Michaelis relation (25) applies, the inverse relation for calculating the linear variable Y is

$$\frac{Y}{K} = \frac{B}{1-B}, \tag{27}$$

which we assume applies at any instant or

$$\frac{Y}{aK} = \frac{U}{U_L - U}, \tag{28}$$

which is valid only under steady-state conditions.

In applying the Michaelis relation to reconstruct the rising phase it is

convenient to eliminate constants by using the step-sensitivity which is defined as

$$\left(\frac{\mathrm{d}U}{\mathrm{d}I}\right)_{\substack{I\to 0\\ t=\infty}} = S. \tag{29}$$

Thus suppose that for a step of light of intensity I

$$Y = c'I\phi(t), \tag{30}$$

where c' is a constant and $\phi(t) = 1$ for $t = \infty$,

$$S = \left(\frac{\mathrm{d}U}{\mathrm{d}I}\right)_{\substack{I\to 0\\ t=\infty}} = \left[\frac{\mathrm{d}U}{\mathrm{d}Y}\frac{\mathrm{d}Y}{\mathrm{d}I}\right]_{\substack{I\to 0\\ t=\infty}}. \tag{31}$$

From eqns. (28) and (30)

$$S = \frac{c'U_{\mathrm{L}}}{aK} \tag{32}$$

and

$$B = \frac{SI\phi(t)}{SI\phi(t)+a^{-1}U_{\mathrm{L}}}. \tag{33}$$

If $\phi(t)$ is known this equation can be used in conjunction with eqn. (8) to reconstruct the rising phase (see page 711).

Kinetics of the activation substance in the linear region

The aim of this section is to derive equations which describe the linear response of the cell to small flashes or steps of light. The distortion introduced by the Michaelis equation, or more complicated relations such as eqns. (16) or (22) will have negligible effects if the signal is less than a millivolt. Apart from the slight lag introduced by the cell time constant, which is about 5 msec in the dark, records of small signals should therefore give accurate information about the time course of the closure of ionic channels. As the total delay between a flash and the peak of the linear response is of the order of 100 msec we shall neglect the effect of the cell's electrical time constant in a preliminary description of the response.

Fuortes & Hodgkin (1964) found that the linear responses of *Limulus* ommatidia could be fitted by mathematical equations in which the effects of light pass through a chain of low-pass filters of equal time constant. The linear responses of turtle cones and rods to flashes are more asymmetrical than those of *Limulus* ommatidia but can be fitted by a similar model in which the time constants are not all equal (see Penn & Hagins, 1972). This model will be described in terms of a chain of chemical reactions rather than an electrical network as this gives a possible meaning to the variation of rate constants along the chain.

Suppose that the absorption of a photon by a chromophore uncovers or releases a particle A on which six sites are combined with a ligand L

(e.g. a proton) which is lost with a rate constant α after the photon has been absorbed. Activation by light then starts the following chain of reactions

$$\begin{array}{ccccccccccccc} \mathrm{AL_6} & \xrightarrow{\alpha_1} & \mathrm{AL_5} & \xrightarrow{\alpha_2} & \mathrm{AL_4} & \xrightarrow{\alpha_3} & \mathrm{AL_3} & \xrightarrow{\alpha_4} & \mathrm{AL_2} & \xrightarrow{\alpha_5} & \mathrm{AL_1} & \xrightarrow{\alpha_6} & \mathrm{A} \\ y_1 & \longrightarrow & y_2 & \longrightarrow & y_3 & \longrightarrow & y_4 & \longrightarrow & y_5 & \longrightarrow & y_6 & \longrightarrow & y_7 \end{array}$$

where the α's are rate constants and the y's are concentrations. Of these seven compounds we assume that only $\mathrm{AL_1}$ can block the 'sodium' channels in the outer segment. The concentration of $\mathrm{AL_1}$, which is denoted by y_6 at a time t after the beginning of a light stimulus $I(t)$ can be obtained by solving the following set of equations:

$$\left.\begin{aligned} \frac{\mathrm{d}y_1}{\mathrm{d}t} + \alpha_1 y_1 &= cI(t), \\ \frac{\mathrm{d}y_2}{\mathrm{d}t} + \alpha_2 y_2 &= \alpha_1 y_1, \\ &\cdots\cdots\cdots\cdots \\ \frac{\mathrm{d}y_6}{\mathrm{d}t} + \alpha_6 y_6 &= \alpha_5 y_5, \end{aligned}\right\} \tag{34}$$

where c is a proportionality constant. An identical set of equations would hold for a ligand becoming attached to empty sites or for a series of intramolecular rearrangements. The property which all these systems must have in common is that the rate constants for the back reactions should be negligible compared to the forward rate constants. In the following sections we consider three possible ways in which the time constants might vary along the chain.

Independent activation. The simplest assumption to make about such a chain of reactions is that they proceed independently. In that case the relation between the α's is as follows

$$\alpha_1 = 6\alpha, \quad \alpha_2 = 5\alpha, \ldots \alpha_6 = \alpha.$$

Thus α_1 is six times α_6 because $\mathrm{AL_6}$ has 6 times more L than $\mathrm{AL_1}$.

On solving eqn. (34) with this restriction for the case where $I(t)$ is an instantaneous flash $I\Delta t$, we obtain

$$y_6 = 6cI\Delta t\, \mathrm{e}^{-\alpha t}\,(1-\mathrm{e}^{-\alpha t})^5. \tag{35}$$

This relation can also be obtained in a simpler manner. The probability that a given site on a particle released by one photon is occupied by an L is $\mathrm{e}^{-\alpha t}$ and the probability that the site is vacant is $1-\mathrm{e}^{-\alpha t}$. For a particle with six sites, the probability of the state in which five sites are vacant and one site is occupied is

$$P(t) = 6\,\mathrm{e}^{-\alpha t}\,(1-\mathrm{e}^{-\alpha t})^5. \tag{36}$$

For a particle with n sites, the probability is

$$P(t) = n\,\mathrm{e}^{-\alpha t}\,(1-\mathrm{e}^{-\alpha t})^{n-1}. \tag{37}$$

In terms of y_n the response to a flash is

$$y_n = ncI\Delta t\, e^{-\alpha t}(1-e^{-\alpha t})^{n-1} \tag{38}$$

and to a step is

$$y_n = \frac{cI}{\alpha}(1-e^{-\alpha t})^n. \tag{39}$$

If we assume that for small signals the change in membrane potential U is linearly related to y_n by $U = k'y_n$ and define a step-sensitivity constant S by $S = ck'/\alpha$, then

$$U = IS(1-e^{-\alpha t})^n \tag{40}$$

gives the response to a step,

$$U = I\Delta tSn\alpha\, e^{-\alpha t}(1-e^{-\alpha t})^{n-1} \tag{41}$$

the response to a flash and

$$U = IS\left\{F\left(t+\frac{\Delta t}{2}\right)-F\left(t-\frac{\Delta t}{2}\right)\right\}, \tag{42}$$

where $$F(t) = (1-e^{-\alpha t})^n \ldots t > 0$$

gives the response to a rectangular pulse lasting from $-\frac{1}{2}\Delta t$ to $\frac{1}{2}\Delta t$.

Rate constants in arithmetic progression. Although most of our results with flashes were fitted by eqn. (41) some were more symmetrical, although less symmetrical than the Poisson curves given by the original *Limulus* model. A useful equation, which can be fitted to all the results and which reduces to eqn. (41) as one special case and to the Poisson curve as another, can be obtained by assuming that the rate constants are in arithmetical progression. If $\alpha_1-\alpha_2 = \alpha_2-\alpha_3\ldots$etc., then the response to a flash is

$$y_n = \frac{I\Delta tc\,(\alpha_1\alpha_2\ldots\alpha_{n-1})}{(n-1)!}\left\{\frac{e^{-\alpha_n t/(n-1)}-e^{-\alpha_1 t/(n-1)}}{\alpha_1-\alpha_2}\right\}^{n-1}. \tag{43}$$

The model in which the ligand is lost independently is a special case of arithmetic progression in which $\alpha_1 = n\alpha$, $\alpha_2 = (n-1)\alpha\ldots\alpha_n = \alpha$, and as would be expected eqn. (43) then reduces to eqn. (38). On the other hand if $\alpha_1 = \alpha_2\ldots = \alpha_n = \alpha$, eqn. (43) reduces to the Poisson curve considered by Fuortes & Hodgkin (1964), i.e.

$$y_n = \frac{I\Delta tc\,(\alpha t)^{n-1}\, e^{-\alpha t}}{(n-1)!}. \tag{44}$$

Final rate constant different. The third model considered is a system in which all the rate constants except the last are equal, i.e.

$$\alpha_1 = \alpha_2 = \ldots\alpha_{n-1} = \alpha,$$

$$\alpha_n = \beta\alpha.$$

In this case the solution for an instantaneous flash is

$$y_n = \frac{I\Delta tc}{(1-\beta)^{n-1}}\left\{e^{-\beta T} - e^{-T}\sum_{r=0}^{n-2}\frac{(1-\beta)^r T^r}{r!}\right\}, \tag{45}$$

where $T = \alpha t$.

Although this model does not give such a neat solution as the other two it has certain advantages when we come to describe the effect of a background in shortening and reducing the response to a test flash. For it turns out that this effect and others related to it can be described fairly well by assuming that the final rate constant α_n increases with the concentration of the inactive end-product y_{n+1} and that the rest of the chain stays constant.

Another advantage of this class of model is that it seems quite likely that the final rate constant might be very different from those in the rest of the chain. Thus it might take five or six steps to release a calcium ion which would be available to block channels until combined with some chelating compound. It is also not hard to visualize a system with equal rate constants, rather than with rate constants varying according to the independence relation. What is required is some kind of bottle-neck or rate-limiting step which is the same for all reactions.

RESULTS

The linear response to flashes and steps of light

The average response of a red-sensitive cone to flashes of light has the characteristic shape shown by the circles in Fig. 3. The continuous curve which is clearly a good fit to the points was calculated on the independent activation formula derived on p. 697, i.e.

$$U = I\Delta tSn\alpha\, e^{-\alpha t}(1-e^{-\alpha t})^{n-1}, \tag{41}$$

where U is the hyperpolarization, I is the light intensity, Δt is the pulse duration (11 msec), S is the step-sensitivity, α is the rate constant ($16{\cdot}7\ \text{sec}^{-1}$), t is the time from the midpoint of the flash and n is the number of 'reactions' which was taken as six.

Fig. 4, in which $\log U/I$ is plotted against $\log t$, extends the treatment to stronger flashes and shorter times. If the system were strictly linear all the points would fall on a common curve. This is plainly not the case but the left-hand parts of all the curves do appear to converge on a common curve and appreciable non-linearities do not appear until the hyperpolarization exceeds 1–2 mV.

The continuous curve which is a fairly good fit to the left-hand parts of all curves was again drawn from eqn. (41) with exactly the same parameters. However, as the flash had a finite duration of 11 msec the

comparison is not fair at times less than 20 msec where eqn. (41) should strictly be replaced by the finite difference equation

$$U = IS\left\{F\left(t+\frac{\Delta t}{2}\right)-F\left(t-\frac{\Delta t}{2}\right)\right\}, \tag{42}$$

where

$$F(t) = (1-e^{-\alpha t})^n, \quad (t > 0),$$
$$= 0, \quad (t < 0).$$

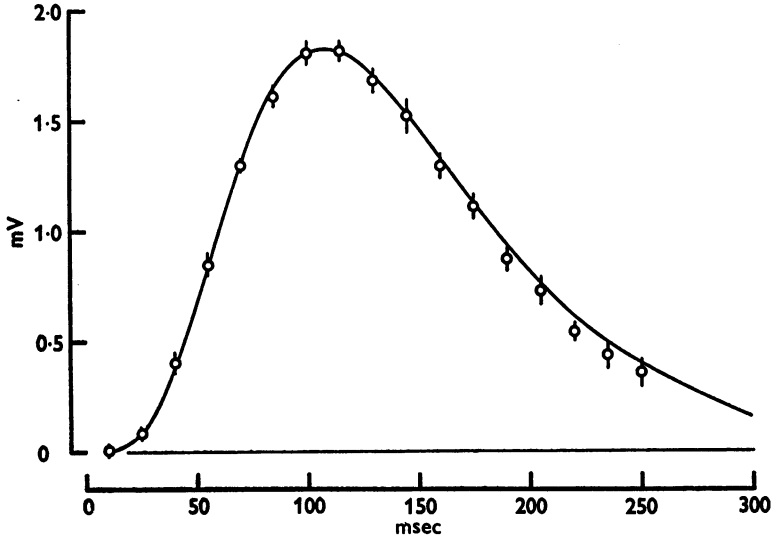

Fig. 3. Mean linear response of red-sensitive cone to weak flashes of white light of duration 11 msec; 31 frames were averaged and the vertical lines through the points are ± 1 S.E. of mean; hyperpolarization is plotted upwards. The continuous curve was calculated from the independent activation formula eqn. (41) (pp. 697, 698) with $n = 6$, $\alpha = 16{\cdot}7$ sec^{-1} and $S = 2{\cdot}92\,\mu$V photon^{-1} μm^2 sec; Δt was 11 msec and $I\Delta t$ was equivalent to 93·6 photon μm^{-2} at λ_{max}. Maximum hyperpolarization 20 mV; flash sensitivity 19·5 μV photon^{-1} μm^2; 150 μm diameter light spot; temperature 21° C; resting potential −39 mV. The abscissa is the time in msec from the middle of the 11 msec flash.

With strong flashes and at times less than 10 msec the experimental points lie to the right of this relation which is shown by the interrupted curve in Fig. 4. These points require an n approaching 7, but the fit to the linear response is then rather less good. This type of discrepancy which was observed in most experiments could be explained if one of the delays in the model were composite. Thus if the shortest time constant of $(6\alpha)^{-1} = 10$ msec were replaced by ten 1 msec delays (which would be a fair representation of the delay introduced by the diffusion of a small molecule over a distance of 2 or 3 μm) it would have little effect over most of the curve but would reduce the theoretical values at small times.

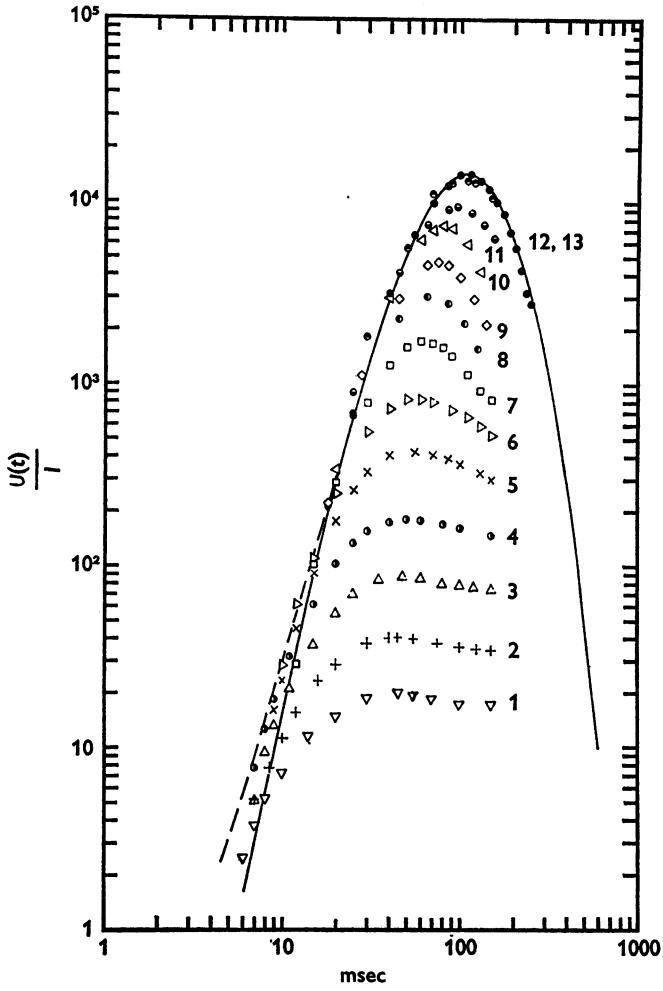

Fig. 4. Responses of red-sensitive cone of Fig. 3 to 11 msec flashes of light. The abscissa is time, t, on a log scale from the middle of an 11 msec flash. The ordinate which is also plotted on a log scale is the hyperpolarization divided by the intensity of the light (I) which is given relative to its unattenuated value. In these units log I was as follows: curve 1, 0; 2, $-0{\cdot}31$; 3, $-0{\cdot}65$; 4, $-0{\cdot}97$; 5, $-1{\cdot}33$; 6, $-1{\cdot}65$; 7, $-2{\cdot}00$; 8, $-2{\cdot}30$; 9, $-2{\cdot}57$; 10, $-2{\cdot}88$; 11, $-3{\cdot}22$; 12, $-3{\cdot}53$; 13, $-3{\cdot}90$. The continuous curve was calculated from eqn. (41) with the same constants as in Fig. 3, i.e. $n = 6$, $\alpha = 16{\cdot}7\ \text{sec}^{-1}$ and $S = 2{\cdot}92\ \mu\text{V photon}^{-1}\ \mu\text{m}^2\ \text{sec}$ if I is expressed in equivalent photon flux. In the Figure, where I is given relative to its unattenuated value of 67×10^6 photon $\mu\text{m}^{-2}\ \text{sec}^{-1}$ at 644 nm, $S = 1{\cdot}96 \times 10^5$ mV per unit of unattenuated light intensity. The interrupted curve which allows for the width of the pulse was calculated from the finite difference eqn. (42) with the same constants. Experimental details as in Fig. 3.

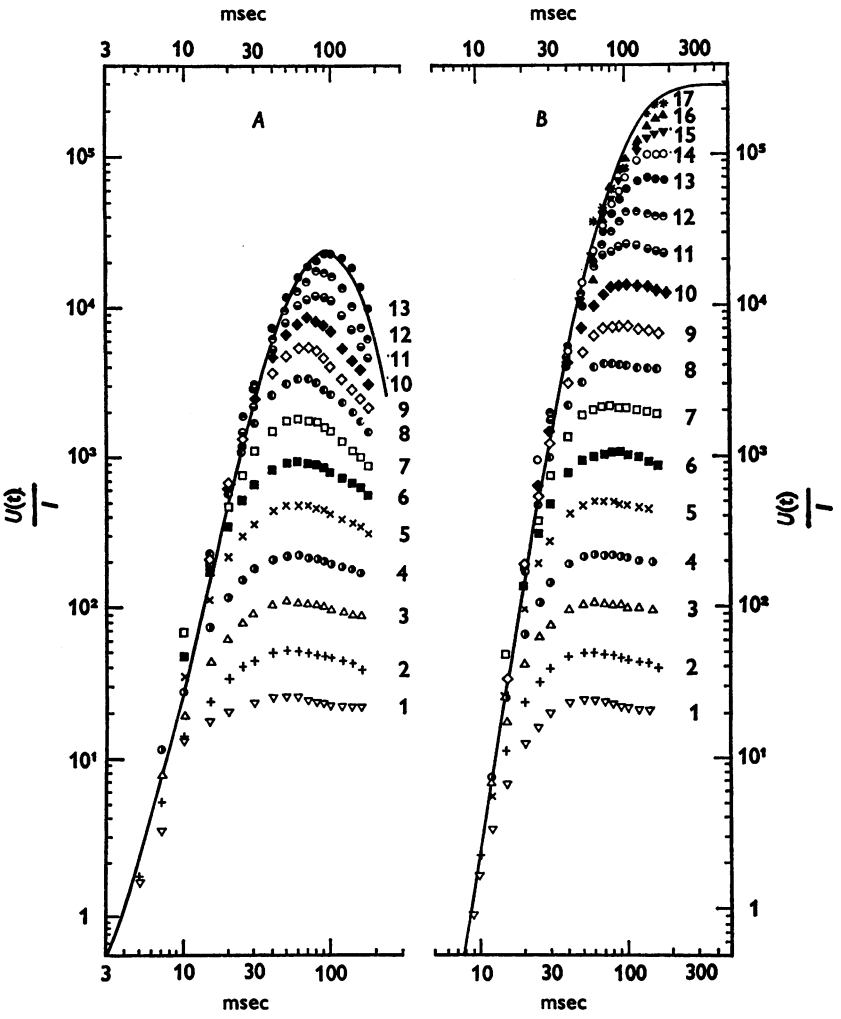

Fig. 5. Response of red-sensitive cone to 9·6 msec flashes, left (*A*) and steps, right (*B*) plotted on log × log scale as in Fig. 4. The logarithms of the light intensity *I* (which is given relative to its unattenuated value) were: curve 1, 0; 2, −0·31; 3, −0·65; 4, −0·97; 5, −1·33; 6, −1·65; 7, −2·00; 8, −2·30; 9, −2·57; 10, −2·88; 11, −3·22; 12, −3·53; 13, (*B*) −3·90; 14, −4·21; 15, −4·55; 16, −4·86; 17, −5·17. In *A* curve 13 is the mean of eight responses obtained with a log mean *I* of −4·14. For the steps (*B*) the smooth curve was calculated from eqn. (40) with $n = 7$, $\alpha = 20{\cdot}8\ \text{sec}^{-1}$ and $S = 2{\cdot}88 \times 10^5$ mV per unit of unattenuated light. For the flashes the finite difference eqn. (42) was used with the same constants and $\Delta t = 0{\cdot}0096$ sec. One relative unit is equivalent to 67×10^6 photons $\mu\text{m}^{-2}\ \text{sec}^{-1}$ at 644 nm. In those units the step sensitivity was 4·3 μV photon^{-1} μm^2 and the flash sensitivity was 35 μV photon^{-1} μm^2. White light; diameter of spot 50 μm; resting potential −39 mV; peak hyperpolarization 25·5 mV; temperature 21·8° C.

Fig. 5 which is from another red-sensitive cone extends the treatment from flashes (left) to steps (right). The continuous curves were drawn with $n = 7$ and $\alpha = 20{\cdot}8$ sec^{-1} using eqn. (42), above, for flashes and eqn. (40) for steps, i.e.

$$U = IS\,(1-e^{-\alpha t})^n. \tag{40}$$

Although the agreement with the independent chain model is impressive other models work equally well and in some cases better. The first point is illustrated by Fig. 6 in which the mean response of nine red cones is compared with two theoretical formulae. The continuous curve is the

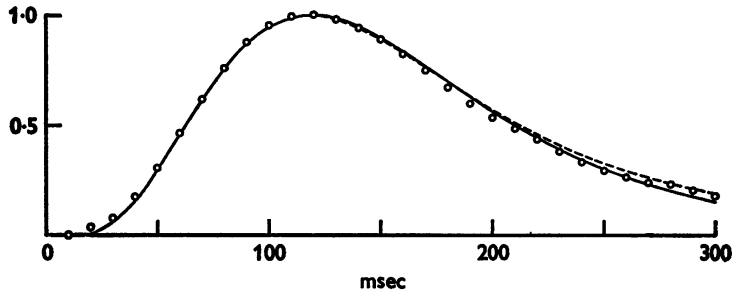

Fig. 6. ○, Average linear response of nine red-sensitive cones to weak flashes, corrected to 20° C from Baylor & Hodgkin (1973). Before averaging, the mean response in each experiment was scaled to give a peak of unity. The continuous curve was calculated from the independent activation eqn. (41), with $n = 6$, $\alpha = 15{\cdot}15$ sec^{-1} and the appropriate scaling factor to give a peak close to unity. The interrupted curve was calculated from a model with five stages of time constant 17·2 msec and one of 86 msec, i.e. eqn. (45) with $n = 6$, $T = t/17{\cdot}2$ (with t in msec), $\beta = \frac{1}{5}$ and an appropriate scaling factor to give a peak close to unity.

independence model with $n = 6$ and $\alpha^{-1} = 66$ msec and the interrupted curve which is an equally good fit was calculated from a model in which five of the reaction time constants were equal to 17·2 msec and the sixth was 86 msec. Our tentative conclusion is that any model with $n = 6$–7 can be fitted to the results provided that we allow sufficient dispersion of time constants. It should also be emphasized that in the linear systems considered here we can change the order of the reactions without affecting the results.

Fig. 7 illustrates the response of a red-sensitive cone which does not agree with the independence formula but which can be fitted by either model 2 (rate constants in arithmetic progression) or model 3 (five time constants of 17 msec and one of 51 msec).

Table 1 summarizes the parameters obtained by fitting the three models to red- or green-sensitive cones and to rods.

TABLE 1. Constants used in fitting linear responses to flashes with three different formulae

Cell	Method	Temperature (°C)	t_{max} (msec)	n	A	B		C	
					τ (msec)	τ_1 (msec)	τ_n (msec)	τ_1 (msec)	τ_n (msec)
				Red-sensitive cones					
1	F	19·6	138	6	78	13	78	20	100
2	F	21·8	95	7	48	7	49	12	70
3	F	21·0	108	6	—	15	30	17	51
4	F	21·5	112	6	60	10	60	16	80
5	F	21·3	108	6	60	10	60	15	75
6	F	21·0	125	7	61	8·7	61	15	75
7	L	22·2	108	6	62	10	60	16	80
8–16	L	20·0	118	6	66	11	66	17·2	86
				Green-sensitive cones					
1	F	19·6	163	6	—	13	87	20	140
2	F	21·3	150	6	80	13	80	21	105
3–10	L	20·0	145	6	80	13	80	21	105
				Rods					
1	L	20·3	475	6	240	—	—	—	—
2	F	20·4	350	6	—	67	67	67	67
3	F	22·0	300	6	160	—	—	—	—

In the first column 8–16 and 3–10 mean that linear responses from nine and eight cones respectively were averaged.

Under Method, L indicates that only the linear response to weak flashes was fitted, as in Fig. 3, and F indicates that the fit was extended to shorter times by using the family of curves, as in Fig. 4.

t_{max} is the time to maximum of the linear response.

Under A, B, C time constants (which are the reciprocal of the rate constants in the text) are given for: A, fitting with eqn. (41) or (42) (independent activation), B, fitting with eqn. (43) (rate constants in arithmetic progression), C, fitting with eqn. (45) (all except last rate constant equal).

n was the same for all three fits.

With cones the diameter of the spot was 50–150 μm, and with rods it was 150–300 μm.

The goodness of fit achieved with rods was lower than that found with cones.

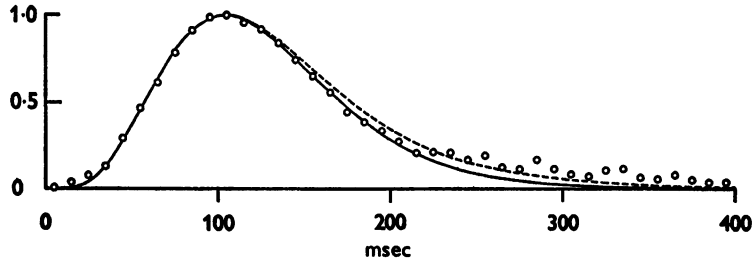

Fig. 7. Average linear response of a red-sensitive cone to weak 10·5 msec flashes. 160 frames were averaged giving a mean peak response of 0·7 mV to an average quantity equivalent to 7·3 photon μm^{-2} at λ_{max}; flash sensitivity, 94 μV photon^{-1} μm^2. In this cone the response was more symmetrical than usual. The continuous curve was drawn from eqn. (43) with $n = 6$, $\alpha_1 = 66{\cdot}7$ sec^{-1} and $\alpha_6 = 33$ sec^{-1}. The interrupted curve was drawn from eqn. (45) with $n = 6$, $T = t/17$ (with t in msec) and $\beta = \frac{1}{3}$. The peaks of both experimental and theoretical curves have been scaled to unity. Temperature 21° C; spot diameter 75 μm; white light in this and all subsequent Figures.

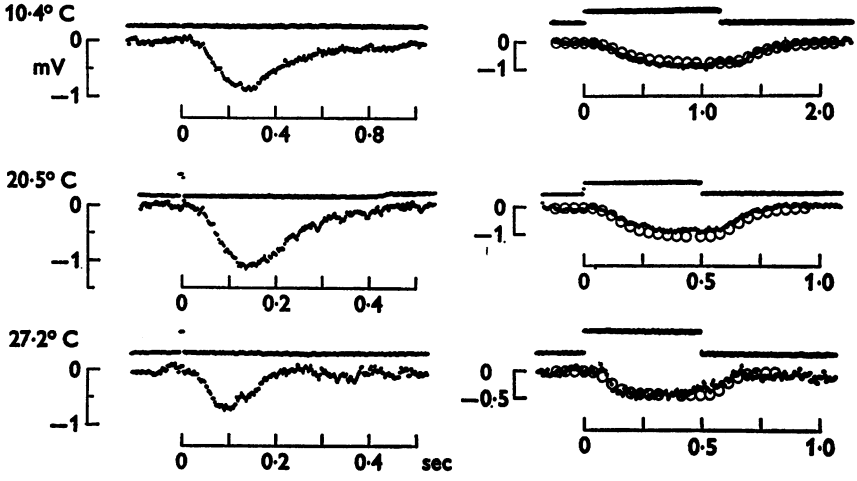

Fig. 8. Response of red-sensitive cone to weak 9 msec flashes (left) and steps (right) of white light, at three different temperatures. Each trace is the average of 16 frames. The intensity of the flashes was 22 times that of the steps. The circles in the right-hand column were obtained by integrating the flash responses (see Baylor & Hodgkin, 1973). The maximum hyperpolarization in this cell was 12 mV at 10·4 and 20·5° C and 10 mV at 27·2° C. The diameter of the light spot was 140 μm.

Effect of temperature on the linear response

The effect of a change of temperature from 10·4 to 27·2° C on the response to flashes and steps of light is shown in Fig. 8. The intensity and duration of the flashes was constant and these records were taken at the

same gain. The intensity of the step at 10·4° C was half that at the other two temperatures and the gain at 27° for the step record was twice that in the records at 10·4 and 20·5° C. The records were taken in the linear region of the response and the form and amplitude of the step response agree with the circles calculated by superposition from the flash response (see Baylor & Hodgkin, 1973). In this experiment the flash sensitivity was approximately constant but the step sensitivity decreased as the temperature was raised – as would be expected from the decrease in area and approximate constancy of the peak amplitude of the flash responses.

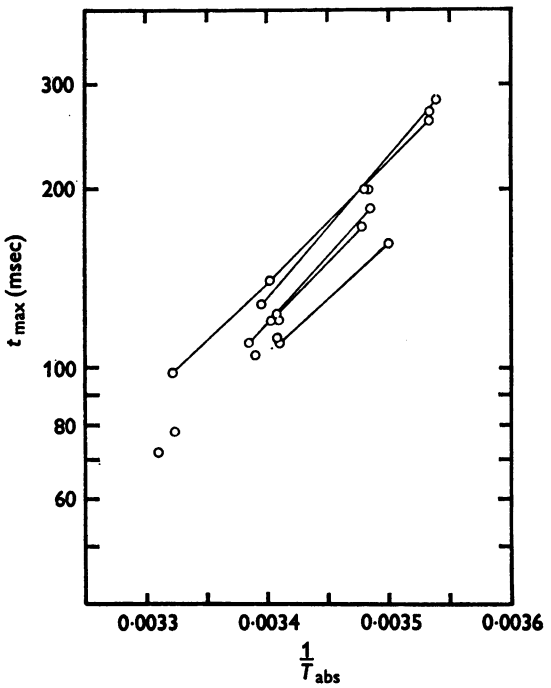

Fig. 9. Effect of temperature on the kinetics of responses to flashes. Pooled results from fourteen red cones. The time to peak in msec of linear responses to brief flashes is plotted logarithmically against the reciprocal of the absolute temperature. The slopes of the lines through the points correspond to an activation energy of about 10 kcal/mole.

Borsellino, Fuortes & Smith (1965) found that *Limulus* ommatidia did not behave in this way since the flash sensitivity increased with temperature and the step sensitivity remained approximately constant. However, before concluding that there is a real difference it should be said that our result is uncertain because we were never able to hold an electrode in a cell over a downward change in temperature, and the approximate constancy

of the flash responses in Fig. 8 could be attributed to a progressive decline in sensitivity. The point that is clear from this and other experiments is that increasing the temperature accelerates the response. From the Arrhenius plot in Fig. 9 we conclude that the apparent activation energy of the rate processes underlying the response is 9·84 kcal/mole and that the temperature coefficient (Q_{10}) of the reciprocal of the time to peak is about 1·8 between 15 and 25° C. These are somewhat smaller than the values of 19·56 kcal/mole and 3·2 for the Q_{10} obtained for *Limulus* ommatidia by Borsellino *et al.* (1965) but still sufficiently high to eliminate straightforward aqueous diffusion as a main cause of the delay.

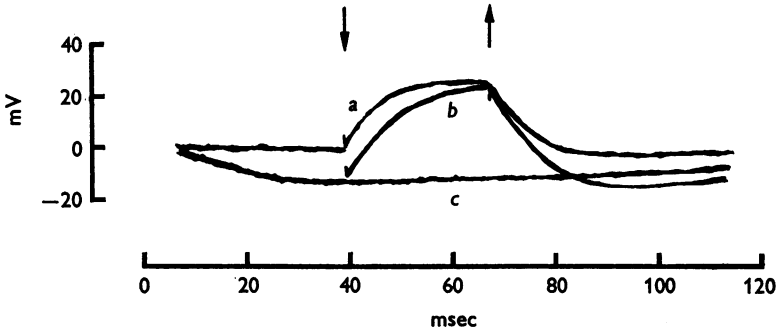

Fig. 10. Changes in potential produced by current in darkness (*a*), and during the response to light (*b*), superimposed tracings. Between the arrows, a rectangular pulse of depolarizing current (strength $1{\cdot}5 \times 10^{-10}$ A) was passed through the micro-electrode. *c* is the response to light without current. Red-sensitive cone 2 in Table 2; 20·8° C. Spots of white light, diameter 75 μm were flashed for 10 msec just before the beginning of the trace; the intensity was equivalent to $1{\cdot}5 \times 10^6$ photons μm^{-2} sec^{-1} at λ_{max}.

In terms of the independent activation model outlined on page 696 our result means that the activation energy for loss of a single particle is 9·84 kcal/mole and that α has a Q_{10} of 1·8.

So far as we can tell a Q_{10} of about 1·8 applies to the non-linear responses produced by strong flashes as well as to the linear responses.

A curious feature of the experiments at 27° C was that a strong flash often dislodged the electrode from the cone – as if strong lights caused some movement or rapid volume change in the cone.

Measurement of the cone's electrical resistance and capacity

In spite of making many trials we were unable to insert two microelectrodes into the same cone. However, in cones with a high resistance it was possible to obtain satisfactory records by balancing out the voltage across the electrode resistance with the equivalent of a bridge-circuit (Baylor & Fuortes, 1970). Fig. 10 illustrates one of these experiments and

TABLE 2. Electrical characteristics of four red-sensitive cones

Cone	V_D (mV)	V_L (mV)	R_D (MΩ)	R_L (MΩ)	τ_D (msec)	τ_L (msec)	V_L/V_D	R_L/R_D	τ_L/τ_D
1	−32	−43	69	86	4·0	5·7	1·34	1·25	1·42
2	−37	−48	173	226	6·5	8·4	1·30	1·31	1·29
3	−41	−63	57·4	63	5·5	6·3	1·54	1·10	1·15
4	—	—	54	64	3·8	4·6	—	1·18	1·21

In cone 4 the resting potential was not recorded; the peak hyperpolarization was 15 mV. V is the internal potential, R the resistance across the cell membrane and τ the time constant in the dark (subscript D) or after a bright flash (subscript L). The temperature was 19–21° C.

shows that the beginning of the charging curve is well defined. As can be seen from the figure and from the data against cone 2 in Table 2, a strong flash of light increased the time constant and resistance by about 30%, as would be expected from eqns. 2–4 since $V_L/V_D = 1{\cdot}3$. The agreement is poor in the case of cone 3 which had a larger response and a lower resistance, but we do not know whether the discrepancy should be attributed to a genuine divergence from the simple network or to the difficulty in choosing the right setting for the bridge balance. An interesting feature of the records is that when the current was superposed on the response to a flash of light, the extra voltage produced by the current swung past zero when the current was switched off; this will be discussed further on page 717.

The values in Table 2 for the resistance R_D across the cell membrane in the dark are much higher than that of 25 MΩ reported by Baylor & Fuortes (1970). This may be because cone resistances vary greatly, perhaps as a result of variations in the amount of coupling, and that the cones in Table 2 are from the upper end of the distribution. On this basis it seems possible that cone 2 with 170 MΩ in the dark is an isolated cone (Baylor & Hodgkin, 1973). From the time constant and resistance the capacity is found to be 37·6 pF in the dark and 37·2 pF in the light. This does not seem an unreasonable value for a single cone. If the inner segment is regarded as a cylinder 8 μm in diameter and 40 μm long its surface area would be 10^{-5} cm^2. If there are 800 sacs in an outer segment with a mean diameter of 2 μm the effective area might be 5×10^{-5} cm^2 so that the capacity per unit area would be

$$\frac{37 \times 10^{-12}}{6 \times 10^{-5}} = 0{\cdot}6\ \mu\text{F cm}^{-2}.$$

Analysis of the rising phase

Relation between channel closure and light intensity at fixed time. The results described in the previous sections are consistent with the hypothesis that flashes and steps of light produce a blocking substance whose 'concentration' is proportional to light intensity in the early stages of the response. Linearity between voltage and light intensity breaks down if the response exceeds a few millivolts but it is not clear whether this is caused by an instantaneous non-linearity of the type considered in the theoretical section or by some more complex kind of desensitization. In an attempt to answer this question we calculated the fractional closure of the light-sensitive channels from the hyperpolarization U and its rate of change $\dot{U}$ by eqn. (9):

$$B = \frac{aU + \tau_L \dot{U}}{U_L + (a-1)U}. \tag{9}$$

In the experiment illustrated in Fig. 11 the resting potential was -39 mV, the maximum hyperpolarization U_L was 20·3 mV, hence

$$a = \frac{59{\cdot}3}{39} = 1{\cdot}52.$$

With strong flashes the variable conductance g_i should be reduced to a very small fraction of the fixed conductance $\bar{g}$ and the potential should approach its maximum exponentially with the light time constant $\tau_L = C/\bar{g}$. In the experiment considered, a plot of $\ln(U_L - U)$ for the strongest flash response gave $\tau_L = 8{\cdot}3$ msec which is similar to that found directly

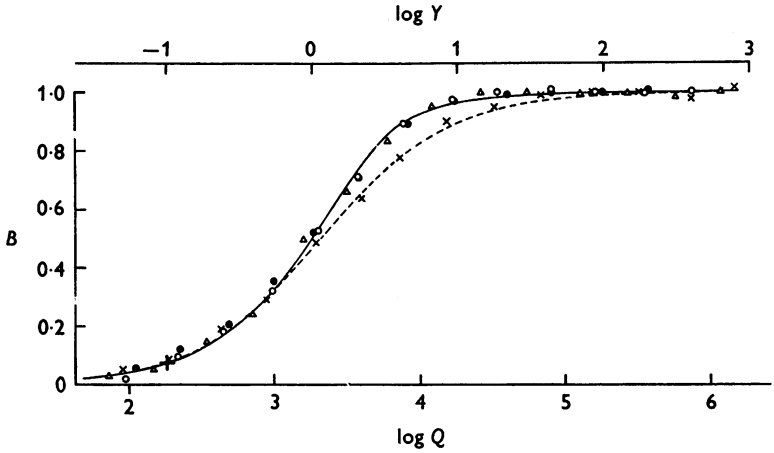

Fig. 11. Relation between fraction of channels blocked (B) and log quantity (Q) of light at various times after a flash. ○, 40 msec after flash; ●, 30 msec after flash, curve shifted 0·3 log units to left; △, 50 msec after flash, shifted 0·2 log units to right, + single point at maximum (110 msec after flash) of linear responses, shifted 0·6 log units to right. The ordinate of these experimental points was calculated by eqn. (9) with $a = 1{\cdot}52, \tau_L = 8{\cdot}4$ msec and $U_L = 20{\cdot}3$ mV. The abscissa of the experimental curves (lower scale) gives the logarithm of the equivalent photon density of the flash in photons μm^{-2} at the optimum wave-length of 630 nm. The continuous curve, for which the upper scale gives log Y was calculated by eqn. (22) with $K = \frac{1}{3}$. ×, the relation between the peak voltage expressed as a fraction of the maximum hyperpolarization and the intensity of the flash; the interrupted curve which fits these points is the Michaelis curve $Q/(Q + Q_i)$, where Q_i is the equivalent photon density at which the peak voltage is half maximal. For further experimental details see Fig. 3, which was obtained on the same red-sensitive cone.

in other cones (Table 2). It can be seen from Fig. 11 that values of B calculated at times of 30, 40 and 50 msec all fall on a common curve when shifted by appropriate amounts along the horizontal axis of log light intensity. This common curve deviates from a Michaelis relation in the

direction expected if the affinity of blocking molecules for channels is high. As reported by Baylor & Fuortes (1970) a Michaelis curve usually fits the relation between peak voltage and light intensity as it did in this experiment. However, since the time to peak changes from about 110 msec with weak flashes to 50 msec with strong flashes, it is clear that the potential at a fixed time cannot also be fitted by a rectangular hyperbola. Such points deviate from a Michaelis curve in the same way as those in Fig. 11 although the discrepancy is not as great as it is for the variable B.

The continuous curve in Fig. 11 which is a good fit to the experimental points was calculated by eqn. (22) (p. 694)

$$B = e^{-Y} \sum_{r=1}^{\infty} \frac{Y^r}{r!} \left(\frac{r}{r+K}\right), \tag{22}$$

with $K = \frac{1}{3}$. By making certain assumptions we can use the agreement of this theoretical expression with the experimental results to estimate the number of light sensitive channels in the cone. In eqn. (22) Y the mean number of blocking particles per channel is given by

$$Y = \frac{N_\phi}{N_c} P(t),$$

where N_ϕ is the number of photons successfully absorbed by the cone, N_c is the number of channels and $P(t)$ is the probability of a blocking particle being present in a compartment containing one channel which has absorbed a photon. The cone in this experiment had an absolute flash sensitivity of 22 μV photon^{-1} μm^2 which is about one-tenth of that seen in the most sensitive cones for which Baylor & Hodgkin (1973) considered an effective collecting area of 10 μm^2 to be appropriate. We shall therefore take the collecting area of this cone to be 1 μm^2. If we make the further assumption that each photoisomerization starts a conservative chain of reactions we can calculate $P(t)$ by eqn. (37), i.e.

$$P(t) = 5\, e^{-\alpha t}(1 - e^{-\alpha t})^4,$$

which was found to fit the variation of B with t in this receptor with $\alpha = 14$ sec^{-1}. By a conservative chain we mean that each photoisomerization liberates one y_1 particle and that the reactions from y_1 to y_5 proceed without loss or amplification. On that basis $P(t) = 0{\cdot}4$ at the maximum which occurred with $t = 110$ msec; at 50, 40 and 30 msec $P(t)$ is respectively 0·16, 0·1 and 0·05. From these values and the observed ratios of N_ϕ to y it is found that the number of channels is 178–187 per cone. In terms of the particular theory used this number is also the number of compartments. However it should be made clear that even if the estimate of the number of channels is approximately correct the data in Fig. 11 provide no real evidence for the existence of compartments. By using a slightly larger value of K we were able to fit the data in Fig. 11 reasonably well by eqn. (16) which was derived without assuming any compartmentalization. If each photoisomerization liberates R particles the argument used above gives the number of channels as about $180R$.

The results in Fig. 11 are typical of experiments in which the diameter of the light spot was 100–150 μm. In experiments with 50 μm spots both the curves for B and peak voltage were usually somewhat flatter and B was then well fitted by a Michaelis curve (Fig. 12). It can be argued either

(1) that the steeper curves in Fig. 11 represent the behaviour of an

isolated cone and that the flatter curves in Fig. 12 occur because the impaled cone is coupled to others which receive less light (see Baylor & Hodgkin, 1973), or

(2) that the Michaelis relation for B in Fig. 12 is 'correct' and that the steeper curves in Fig. 11 arise from some extraneous effect such as feedback from the horizontal cell.

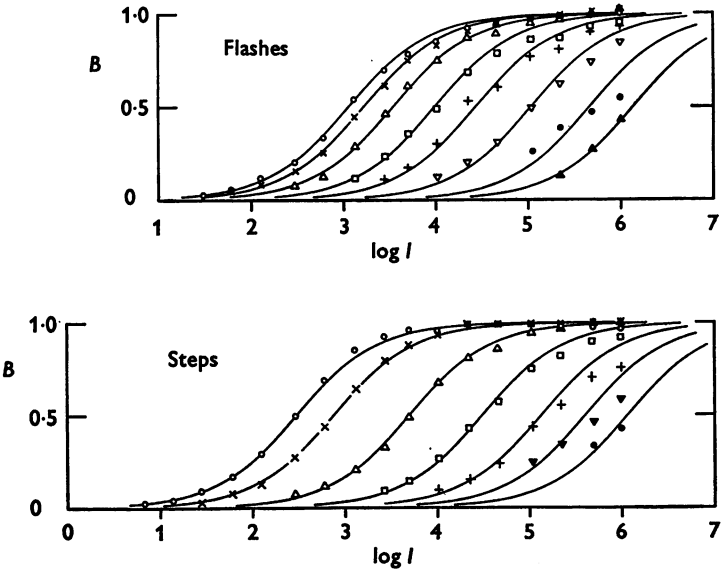

Fig. 12. Relation between fractional closure of ionic channels, B, and log light intensity at fixed times during the rising phase of responses to flashes and steps of light. Each set of points was calculated at a particular time after the stimulus by eqn. (9) with $a = 1{\cdot}64$, $U_L = 25$ mV and $\tau_L = 10$ msec. For the flash run (with a 9·6 msec flash) times in msec from the middle of the flash are: ○, 50; ×, 40; △, 30; □, 20; +, 15; ▽, 10; ●, 7; ▲, 5 and for the step run times in msec from the beginning are ○, 70; ×, 50; △, 30; □, 20; +, 15; ▼, 12; ●, 10. Continuous curves are Michaelis curves displaced along the abscissa. The light intensity is given in relative units such that the unattenuated light had an intensity of 10^6; 1 relative unit ≡ 67 photons μm^{-2} sec^{-1} at 644 nm. For further details of this red-sensitive cone, which was illuminated with a 50 μm diameter spot see Fig. 5.

The first alternative is supported by the fact that the isolated red-sensitive cone described by Baylor & Hodgkin gave steep curves which were almost identical with those in Fig. 11.

Reconstruction of rising phase of the response to flashes and steps of light. The electrical responses of the cone which gave the most consistent and noise-free records are illustrated in Fig. 13. In this experiment the light spot was 50 μm in diameter and the instantaneous relation between B

and light intensity was a Michaelis equation, as can be seen from Fig. 12 which is from the same cell.

In order to reconstruct the rising phase we need to solve two equations derived in the theoretical section, i.e.

$$\tau_L \frac{dU}{dt} + U[a-(a-1)B] = BU_L \tag{8}$$

and

$$B = \frac{SI\phi(t)}{SI\phi(t)+a^{-1}U_L}. \tag{33}$$

In the cone of Fig. 13, τ_L was estimated as 10 msec, a as 1·64 and the step sensitivity S as 3×10^5 mV per unit of unattenuated light; from the maximum response, U_L was taken as 25 mV for the flash run and 24·25 mV for the step run. From the horizontal shifts in Fig. 12 it is a simple matter to obtain experimental estimates of $\phi(t)$ and these were found to be well fitted by the following expressions with t in msec.

Steps
$$\phi(t) = [1-\exp(-t/48)]^6. \tag{46}$$

Flashes of duration 9·6 msec

$$\phi(t) = \{1-\exp(-t/48)\}^6 - \{1-\exp[(9{\cdot}6-t)/48]\}^6 \tag{47}$$

with the second term zero for $t < 9{\cdot}6$ msec.

The expressions for $\phi(t)$ are consistent with those used in Fig. 5 to fit the small changes in potential in this cell, i.e.

$$F(t) = [1-\exp(-t/48)]^7 \tag{48}$$

for steps and the corresponding finite difference formula for flashes. In the linear analysis of Fig. 5 we neglected the cell time constant and used a chain of seven steps of time constant

6·86, 8, 9·6, 12, 16, 24, 48 msec.

In the non-linear analysis with which we are now concerned we have a variable time constant of 6–10 msec and six steps of time constant

8, 9·6, 12, 16, 24, 48 msec,

so the two treatments are nearly equivalent. The two values of S are also internally consistent since the difference between $2{\cdot}875\times10^5$ and 3×10^5 allows for the slight non-linearity of the small signals measured in Fig. 5.

Having established the form of $\phi(t)$ and obtained values for all the constants we can check the analysis by comparing the experimental results with numerical solutions of eqns. (8) and (33). As can be seen from Fig. 13 there is good agreement up to about 50 msec for flashes and steps over a 10^5-fold range of light intensities. At longer times signals of more than about 2 mV deviate in the manner expected from a mechanism which desensitizes with a time delay.

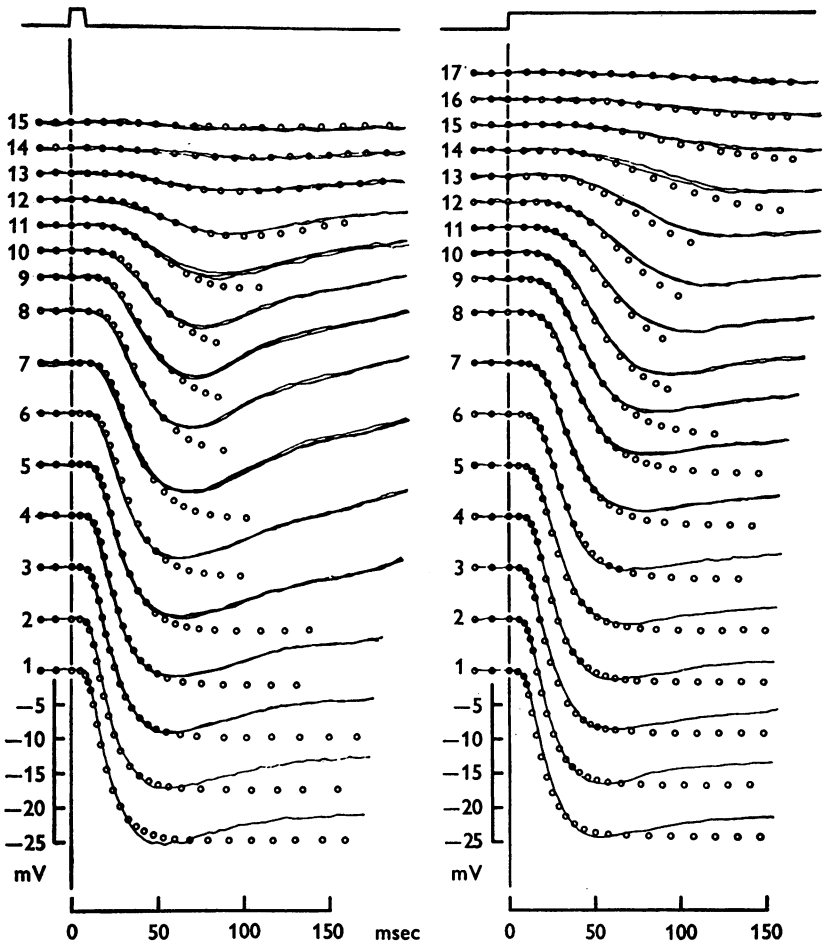

Fig. 13. Reconstruction of the 'rising phase' of the responses of a red-sensitive cone to flashes and steps of light. The continuous lines are tracings of oscilloscope records with hyperpolarization downwards and the circles were calculated from eqns. (8), (33) and (46) (steps) or (47) (flashes). The light intensity I varied approximately in steps of 2·11 between 7×10^{-6} (curve 17) and 1 (curve 1); the 'exact' value of log I for each experimental curve is given in Fig. 5. The unattenuated white light was equivalent to 67×10^6 photons μm^{-2} sec^{-1} at 644 nm. 'Exact' rather than nominal values of I were used in calculating the theoretical curves. For further details of this red-sensitive cone see Fig. 5 and Fig. 12.

Experiments with gaps of darkness

In Fig. 14 the eye was exposed to a light equivalent to $3{\cdot}7 \times 10^4$ photon μm^{-2} sec^{-1} for 1 sec. After 1 sec which is redefined as zero in Fig. 14 the light intensity was either doubled or reduced to zero for 40 msec. The

experiment therefore allows one to compare the effect of adding or subtracting the same light intensity from a steady background. As can be seen from the figure the depolarization resulting from the gap of darkness is much larger than the hyperpolarization resulting from the added light.

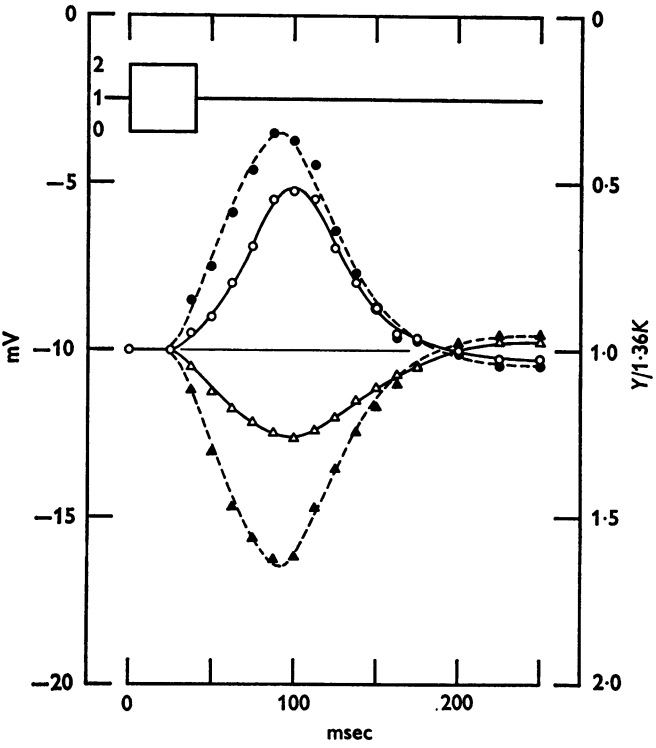

Fig. 14. Effect of interrupting or brightening a steady light on membrane potential and calculated concentration of blocking substance. The cell was stimulated with a steady light of intensity equivalent to $3{\cdot}74 \times 10^4$ photons μm^{-2} sec^{-1} at the optimum wave-length of 644 nm. The steady light was turned off for 40 msec or the intensity was doubled for 40 msec; the resulting changes in membrane potential were averaged in a few frames and plotted (continuous lines, open symbols). The solid symbols show the hypothetical concentrations of activator, calculated from eqn. (49) with $\tau_L = 11$ msec, $a = 1{\cdot}63$, $U_L = 22$ mV. Values of Y/K thus obtained were normalized by dividing by 1·36, the steady level of Y/K. Dashed curves through these points are symmetrical and were fitted by eye. 50 μm white stimulating spot. Membrane potential in darkness −35 mV, 19·8° C.

This asymmetry disappeared when a linear variable proportional to the concentration of hypothetical blocking particle was calculated by the following procedure. The variable B, representing the fraction of ionic channels blocked, can be obtained from eqn. (9) and the linear variable Y,

which is proportional to the concentration of the hypothetical blocking particles, can be then calculated from B by the inverse Michaelis relation (27). Elimination of B between eqns. (19) and (27) gives

$$\frac{Y}{K} = \frac{aU + \tau_L \dot{U}}{U_L - U - \tau_L \dot{U}}. \tag{49}$$

This procedure was justified by the fact that in this experiment, which was carried out with a 50 μm diameter spot the $B(I)$ curve at a fixed time was close to a Michaelis relation. As can be seen from the Figure, the values of Y/K are almost perfectly symmetrical, for the curve through both sets of points were drawn from a common template. The experiment supports the conclusion that there is a rapidly established equilibrium between the concentration of a blocking particle which varies linearly with light intensity and the fractional closure of the ionic channels. It also shows that the response to a pulse of light added on to a background (or to a gap of darkness) is diphasic; this point will be considered further in later papers.

Analysis of the falling phase

Initial peak and plateau in cone responses. A characteristic feature of the response of turtle cones is that after a strong flash the potential sags from a peak of 15–25 mV to a plateau of 12–20 mV. This effect could be attributed either to a desensitization of the transduction mechanism which leads to an increase in the variable conductance g_i or to the development of an additional leak across the battery and conductance which determine the potential when g_i is zero. Experiments of the kind illustrated in Fig. 15 support the second alternative. Here the records labelled A and B show the separate effects of two strong flashes and C gives the effect of combining them at an interval of 200 msec. If the decline from the peak were due to desensitization one would expect the second flash to give a second peak when superposed on the plateau. Fig. 15 shows that this does not happen and that the only effect of the second flash is to prolong the plateau produced by the first. This is consistent with the idea that the light-sensitive conductance remains at zero throughout the whole of the plateau and that the sag from the initial peak depends on a decrease in the potential at which the cell saturates.

Fig. 16 shows a modified circuit which accounts for the initial spike and plateau. Here it is assumed that there is a second variable conductance g_f in parallel with the light sensitive conductance g_l. This hypothetical conductance is not directly affected by light but increases with a delay of about 60 msec when the cell is hyperpolarized. In the dark, with $V \doteqdot -40$ mV, g_f is supposed to be negligible but it increases to a limiting value ($\bar{g}_f$) of about $0{\cdot}2\bar{g}$ when the cell is hyperpolarized to more than

about -55 mV; this causes the potential to sag from its initial value to a level of -60 mV which is maintained as long as there is enough blocking substance to keep g_1 zero. If the light-sensitive conductance became zero instantaneously and there were no capacity, a very strong flash would cause the potential to rise suddenly to -70 mV and then decline to -60 mV. Calculations described in a later paper (Baylor *et al.* 1974) show that with a cell time constant (τ_L) of 10 msec, a sag time constant of

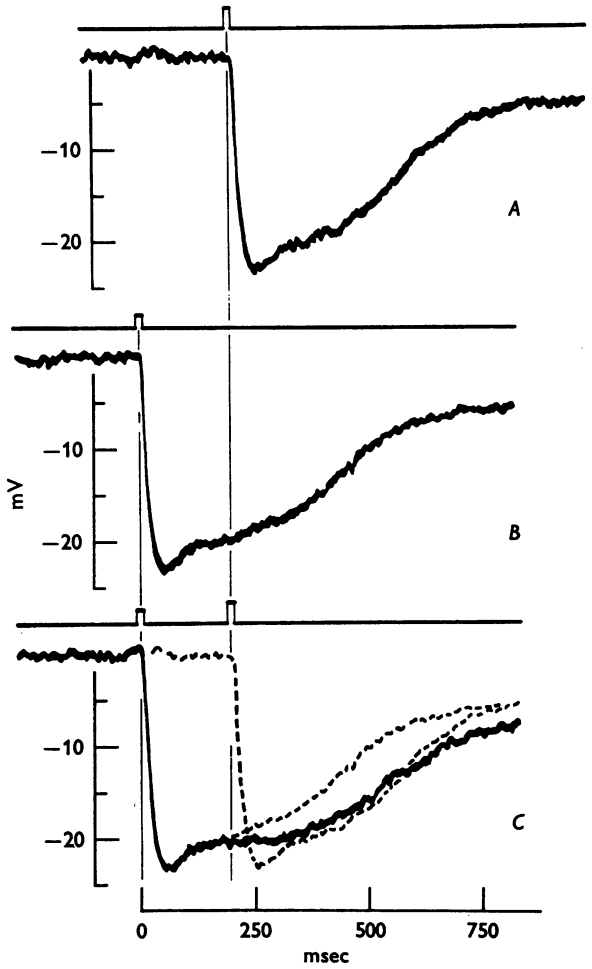

Fig. 15. Effect of a bright conditioning flash on the response to a subsequent bright test flash. Tracings from oscilloscope recordings. *A*: response to test flash alone. *B*: response to conditioning flash alone. *C*: response to both flashes, with the upper two responses dotted in. Red cone, coincident 75 μm white stimulating spots. Flashes of strength equivalent to $6{\cdot}7 \times 10^5$ photon μm^{-2} at the optimum wave-length of 644 nm. 20·2° C.

67 msec and $\bar{g}_t = 0{\cdot}2\bar{g}$ the initial peak would be about 5 mV beyond the plateau.

Fig. 10 on page 706 provides some evidence for the presence of a conductance varying with potential in the manner assumed in the previous paragraph. In that experiment and in others of a similar kind it was found that when a depolarizing current was applied to a cone hyperpolarized by a strong flash the extra voltage produced by the current swung past zero when the current was switched off; no such effect was seen when the same current was applied in the dark. This type of rebound is expected from a mechanism which increases a parallel conductance with a time delay when the cell is hyperpolarized.

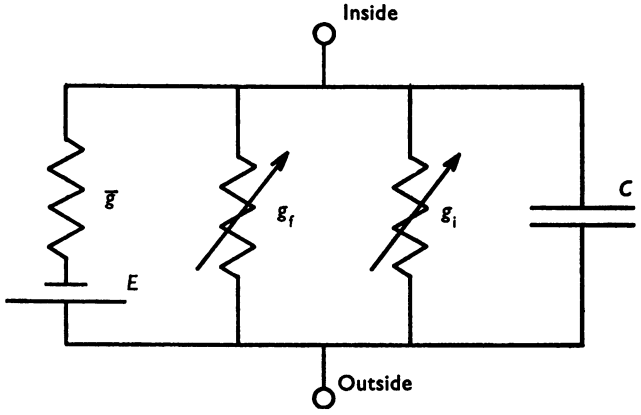

Fig. 16. Equivalent circuit which accounts for the initial spike and plateau. The variable conductance g_f increases reversibly and with a delay when the cell is hyperpolarized; g_i is the light-sensitive conductance.

When the diameter of the illuminated area was increased from 100 μm to 1·5 mm the drop in hyperpolarization after the peak merged into the larger decrease associated with feed-back from the horizontal cells (Baylor, Fuortes & O'Bryan, 1971). This raises the possibility that the sag seen with spots of 100 μm or less might also be due to horizontal cell feed-back. Flashes illuminating less than 100 μm give very little change in the potential recorded from the horizontal cell layer but there might none-the-less be localized electrical changes, with corresponding variations of transmitter release, in the fine horizontal cell processes which make contact with the cones.

Evidence supporting the idea that horizontal cell feed-back could account for the spike and plateau phenomenon has been obtained in the rods of *Gekko gekko* by Kleinschmidt (1973). These photoreceptors normally show an initial spike and plateau not unlike that in turtle cones. However, after

the preparation has been treated with 50 mM aspartate, which depolarizes horizontal cells and renders them insensitive to light, the initial spike of the rods disappears and the records then have a well-defined flat maximum.

Components in the falling phase of the response of cones to flashes and steps of light. When the response to a flash is between 30 and 90% maximal a distinct hump can often be seen on the falling phase. The general appearance of records such as those in the left-hand column of Fig. 17 suggests

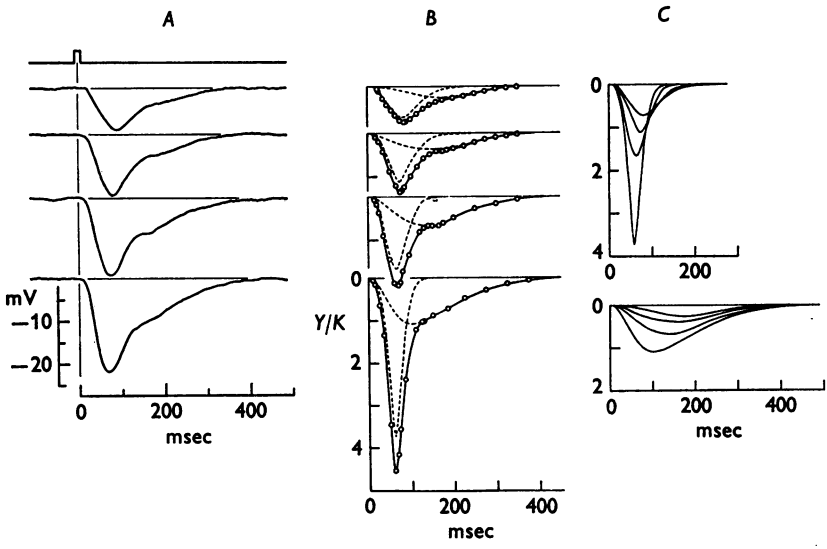

Fig. 17. Division of response to flashes into two components. *A*, tracings of responses of a red-sensitive cone to flashes equivalent in strength to $8{\cdot}75 \times 10^2$, $1{\cdot}34 \times 10^3$, $3{\cdot}37 \times 10^3$ and $6{\cdot}93 \times 10^3$ photons μm^{-2} at 644 nm. *B*, open circles, hypothetical concentrations of blocking particles calculated from eqns. (9) and (17) with $a = 1{\cdot}6$, $U_L = 25{\cdot}5$ mV, $\tau_L = 6{\cdot}3$ msec and $K = \frac{1}{3}$. The interrupted curves depict one way in which the 'composite' response can be split into two components C_1 and C_2, which are shown as separate families in the right-hand column. Resting potential −42 mV; 75 μm diameter white spot; 20·9° C.

that it may be legitimate to regard such responses, and others in which the division is less obvious, as made up of two components which will be called C_1 and C_2. Before attempting to divide the response into two components it is desirable to transform it into a linear form by calculating the variable Y representing the concentration of blocking molecules. The fraction (B) of channels blocked was first obtained by eqn. (9) and Y was then calculated by eqn. (17) i.e.

$$\frac{Y}{K} = \frac{B}{1-B} + \frac{B}{K}. \qquad (17)$$

A value of $K = \frac{1}{3}$ was chosen as this gave a good fit to the experimental points relating B to light intensity at fixed times of 30 and 50 msec. The results of the analysis are shown in the middle column where the dotted lines show plausible but admittedly arbitrary divisions of the response into two components; these are shown as separate families in the right-hand column.

According to this analysis the first component C_1 reaches an earlier peak and the rate constant with which it relaxes increases dramatically as the flash increases. On the other hand the rate constant with which the second component declines remains more nearly constant and the movement of the peak towards earlier times can be explained by supposing that the second component is generated by the first.

With stronger flashes the second component becomes of saturating intensity and may hold the potential on the plateau for 300 msec (Fig. 18*A*). After C_2 has relaxed, a third component C_3 which disappears with a time constant of about 1 sec can be seen at the end of the record. If the duration of the flash is increased to 1 sec, C_3 itself can become of saturating intensity and with maximal light intensity holds the potential at the plateau for 3 sec after the light has been switched off. In that case as can be seen from the lower sets of records the third component is followed by a fourth component C_4 which relaxes with a time constant of 10–20 sec. A still slower component can be identified after steps lasting more than 30 sec but consideration of these results is deferred to the next paper (Baylor & Hodgkin, 1974).

The existence of several components in the relaxation of the potential to its resting level can be explained by supposing that the 'substance' which blocks the light-sensitive channel is inactivated in a series of steps. In the model of the rising phase it was assumed that an absorbed photon started a chain of five or six consecutive reactions leading to the blocking molecule y_n. We now suppose that something rather similar may happen on the falling phase and that y_n is degraded sequentially in a series of steps $(y_n)_1 \rightarrow (y_n)_2$, etc. To simplify nomenclature we shall use the variable z instead of y_n when considering the degradation of the blocking particle, thus z_1 and y_n are equivalent. The scheme adopted in order to explain the present results as well as those in a later paper (Baylor *et al.* 1974) is

$$Z_1 \underset{\kappa_{21}}{\overset{\kappa_{12}}{\rightleftharpoons}} Z_2 \underset{\kappa_{32}}{\overset{\kappa_{23}}{\rightleftharpoons}} Z_3 \underset{\kappa_{43}}{\overset{\kappa_{34}}{\rightleftharpoons}} Z_4 \xrightarrow{\kappa_{45}} Z_5. \tag{50}$$

It is assumed that only Z_1 blocks and that Z_2, Z_3, etc. exert their influence through the back reactions κ_{21}, κ_{32} etc. which maintain Z_1 and lead to its disappearing with a series of distinct time constants. One alternative to the above scheme is to neglect back-reactions and assume that Z_2, Z_3 and

Z_4 are also able to block the light sensitive ionic channels. Another is to assume that Z_1 is degraded in a series of parallel reactions with different time constants. Thus if one adopts the popular idea that calcium ions are the agents which block the light-sensitive ionic channels, then one might suppose that the concentration of these ions is reduced by combination with several types of molecule having widely different affinities and reaction velocities.

Methods of estimating the rate constants in 'equation' (50) will be given in a later paper (Baylor *et al.* 1974). Here we shall be concerned with the

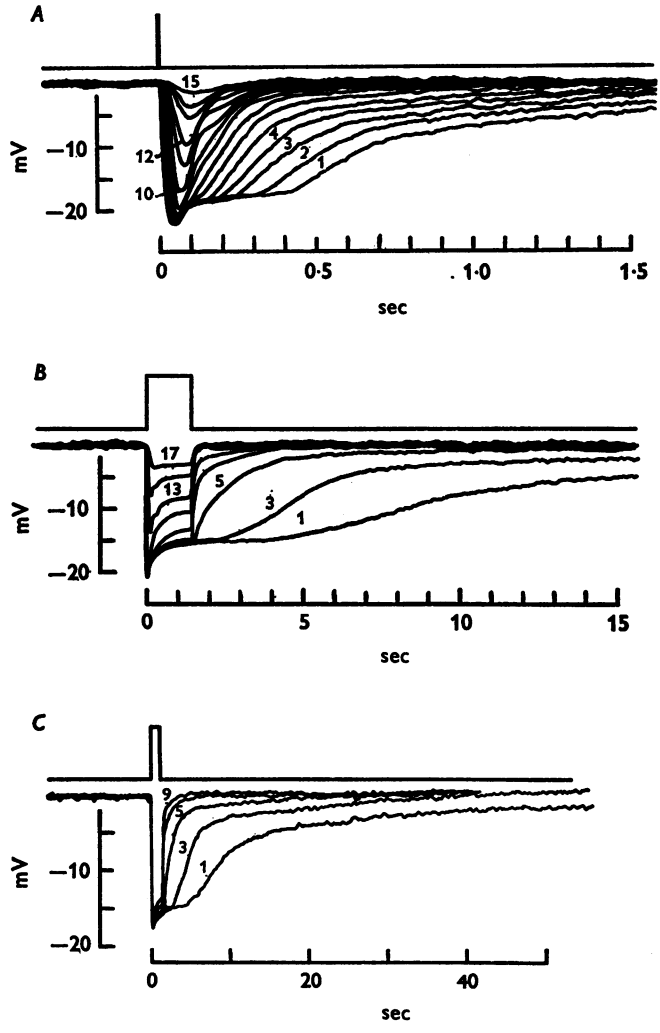

Fig. 18. For legend see facing page.

measurement of the time constants for the disappearance of the various components.

For small signals in darkness τ_1, the time constant for conversion of Z_1 to Z_2 is about 100 msec if one adopts model 3 on page 698. For large signals or for small signals on a background τ_1 is much less and from the analysis of Fig. 17 may decrease to 10 msec or less. τ_2 the time constant with which the second component disappeared was determined by plotting the logarithm of the intensity of the light stimulus against the time taken for the potential to fall from its peak to some fixed value in a typical flash run such as the family in Fig. 19. It can be seen from those records that over a considerable range the falling phases of responses to flashes increasing in geometric progression have approximately the same shape but are shifted along the time axis by fixed amounts. This result is explained if the concentration z_1 of the blocking substance at any fixed time is proportional to light intensity and if its concentration falls exponentially. If

$$z_1 \propto I\,\mathrm{e}^{-t'/\tau_2}, \tag{51}$$

then for z_1 constant

$$t' = \tau_2 \ln I + \text{constant} \tag{52}$$

so that for I increasing in steps of 2·1, t' shifts by constant increments of 0·7 τ_2. This method of calculating τ_2 was the one commonly employed but a more elaborate procedure was employed in plotting the lower part of Fig. 19 in order to test the assumptions that during the plateau the concentration of blocking particles at a fixed time is proportional to light intensity and declines exponentially. For this purpose we calculated a

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Fig. 18. Records illustrating presence of components which relax at different rates after applying intense flashes or steps of light to red-sensitive cone.

A, response of red-sensitive cone to 10 msec flashes recorded on medium time base. The light intensity varied in steps of about 2·11 between the equivalent at 644 nm of $7{\cdot}6 \times 10^3$ photon $\mu m^{-2}\,sec^{-1}$ in record 15 and $2{\cdot}68 \times 10^8$ in record 1.

B, response to 1·5 sec steps of light recorded on slow time base. The light intensity varied in steps of about 4·45 between $1{\cdot}8 \times 10^3$ photon $\mu m^{-2}\,sec^{-1}$ in record 17 and $2{\cdot}68 \times 10^8$ in record 1.

C, same responses as 1, 3, 5, 7, 9 in *B* but recorded on a still slower time scale on an inkwriter.

Four components can be tentatively identified, e.g. C_1, C_2 in record *A* 10; C_2, C_3 in record *A* 1; C_3, C_4 in records *B* 1, or *C* 1. The unattenuated light in this experiment was made 4 times its normal value by using only one light channel and removing the combining prism.

The flash sensitivity of this cone was about 30 μV photon^{-1} μm^2; resting potential -43 mV; white light, spot diameter 103 μm; 21° C.

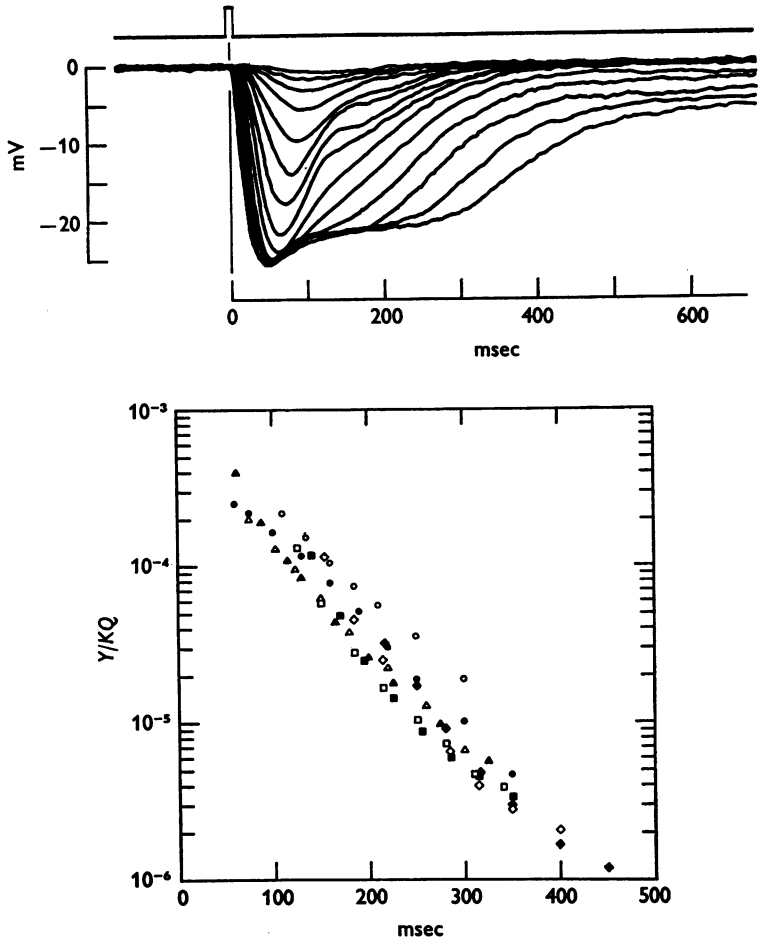

Fig. 19. Relaxation of second component of response. Above, family of fourteen responses to 10 msec flashes increasing in steps averaging 2·11 from a quantity equivalent to 41 photons μm^{-2} to $6{\cdot}7 \times 10^5$ photons μm^{-2} (at 644 nm). Below semi-logarithmic plot of Y/KQ where Y/K is proportional to the concentration of blocking particles calculated by eqns. (9) and (27) with $\tau_L = 6{\cdot}3$ msec, $U_L = 21{\cdot}5$ mV and $a = 1{\cdot}54$. $Q\ (= I\Delta t)$ is the equivalent quantity of light which was: ○, $3{\cdot}4 \times 10^3$ photons μm^{-2}; ●, $6{\cdot}9 \times 10^3$; △, $1{\cdot}5 \times 10^4$; ▲, $3{\cdot}1 \times 10^4$; □, $7{\cdot}3 \times 10^4$; ■, $1{\cdot}5 \times 10^5$; ◇, $3{\cdot}3 \times 10^5$; ◆, $6{\cdot}7 \times 10^5$. The abscissa is the time after the initial peak. The Figure indicates that during the second component of the response the concentration of hypothetical blocking particles is proportional to the quantity of light and declines exponentially with a time constant of 60 msec. Resting potential −41 mV; 75 μm diameter white spot; 20·9° C; red-sensitive cone; flash sensitivity 24 μV photon^{-1} μm^2.

variable Y/K, which is proportional to the concentration of blocking particles, by the simple inverse Michaelis relation

$$\frac{Y}{K} = \frac{B}{1-B}, \tag{27}$$

where B is the fraction blocked which was calculated in the usual way using eqn. (9). It may seem illogical to use the simple relation rather than eqn. (17) which was considered applicable at earlier times, but the second rather than the first component is now being considered and the effective value of K in eqn. (17) may be different. In a later paper (Baylor *et al.* 1974) it will be shown that κ_{12}/κ_{21} in eqn. (50), is of the order of 10 which would make the effective value of K 3·3 rather than $\frac{1}{3}$; a simple inverse Michaelis relation is then a reasonable approximation. More generally one might say that there is no reason why the unknown factors which make $B(Y)$ deviate from a Michaelis relation at 50 msec should also operate at 100–300 msec. At all events the procedure works reasonably well since the points in Fig. 19 which are proportional to Y/Q all fall fairly close to the same straight line when plotted semilogarithmically in spite of the fact that the quantity of light Q varied over a 200-fold range.

TABLE 3. Relaxation time constants in seconds in five red-sensitive cones (R) and one green-sensitive cone (G)

	τ_2 (sec)			τ_3 (sec)			τ_4 (sec)
Cone	(1)	(2)	(3)	(4)	(5)	(6)	(7)
R1	0·062	0·065	0·075	—	—	—	—
R2	0·058	—	—	—	—	—	—
R3	0·052	—	—	0·9	2	—	10–20
R4	0·061	—	—	1	2	0·76	12–20
R5	0·067	—	—	—	—	1·4	10
G1	0·061	—	—	1·6	—	—	10–20

Notes on method

τ_2 was measured from standard 10 msec flash run using the following method: (1) eqn. (51) and time shift corresponding to e-fold increase in light intensity, (2) method illustrated in Fig. 19, (3) from curves in Fig. 17.

τ_3 from after effect of strong 10 msec flashes or 1 sec rectangular pulses using (4) moderately intense and (5) intense 1 sec steps or (6) intense 10 msec flashes; moderate implies a light equivalent to 10^6 to 10^7 photons μm^{-2} sec^{-1} and intense one of *ca.* 10^8 photons μm^{-2} sec^{-1}.

τ_4 was estimated from the final tail seen in Fig. 18C. (7). Temperatures 20–22° C.

Table 3 summarizes the measurements of the time constants underlying the relaxation of potential from the plateau. τ_3 and τ_4 were obtained from the rate of return of potential to its resting level after strong 10 msec flashes (τ_3) or strong 1 sec steps (τ_4).

Behaviour of different components during long pulse. The experiments described in the previous sections suggest that the hyperpolarizing effect of light can be attributed to several components which relax at different rates when the light is switched off. By changing the length of a rectangular pulse and studying the relaxation of potential at the end of the pulse it is possible to obtain tentative information about the way in which the different components vary during a long step. For example, with an unattenuated light of 67×10^6 photons μm^{-2} sec^{-1} it was found in one red-sensitive cone that the component which relaxed with a 1–2 sec time constant was larger after a 1 sec step than after a 10 sec step. In another experiment with a cell of similar sensitivity a 3 sec step equivalent to 67×10^6 photons μm^{-2} sec^{-1} had about the same effect as a 1·5 sec step of the same intensity indicating a maximum near 2·2 sec. From the flash sensitivity of 36·3 mV photon^{-1} μm^2 we estimate the effective collecting area as 1·45 μm^2 so the light should produce about 10^8 photoisomerizations per second. As there are about 10^8 chromophores per cone the time constant for loss of photopigment should be roughly 1 sec at this level of light intensity. On this basis a component which relaxes with a 1 sec time constant should have the time course $t\,e^{-t}$ which has a maximum at $t = 1$ sec. The discrepancy between this and the experimental estimate of 2 sec could arise from an error in estimating the effective collecting area of the cone, or from rapid regeneration of pigment or saturation in the rate of destruction of the 1 sec component.

In preliminary experiments with weaker lights we obtained some evidence that the component which relaxed with a time constant of *c.* 0·1 sec might reach a maximum between 0·1 and 0·5 sec and then decline to about $\frac{1}{4}$ with a time constant of roughly 1 sec. This effect which was observed on one occasion with a light equivalent to $0{\cdot}7 \times 10^6$ photon μm^{-2} sec^{-1} in a cone with an effective collecting area of 1·5 μm^2 cannot be attributed to loss of chromophores since the time constant with which these disappear should be of the order of 100 sec at the level of illumination mentioned above.

DISCUSSION

Like other photoreceptors the electrical response of turtle rods and cones has an S-shaped delay in the response to flashes or steps of light. Previous authors (Baylor *et al.* 1971; Schwartz, 1973) have found that the early part of the rising phase and the linear response to weak flashes were reasonably well fitted by an equation of the type proposed by Fuortes & Hodgkin (1964), i.e.

$$U = AQT^6 e^{-T}, \tag{53}$$

where U is the hyperpolarization, Q is proportional to the quantity of light, T is proportional to time and A is a constant. This corresponds to seven stages of exponential delay in the Fuortes–Hodgkin model. In these earlier studies no attempt was made to find the exact form of the linear response by averaging, so eqn. (53) was not subjected to any very critical test. Our experiments show that if linear responses are determined accurately by averaging many responses to weak flashes they are much more asymmetrical than eqn. (53). To obtain a good fit we retained the idea of six or seven stages of exponential delay but abandoned the assumption that all time constants are equal, as in the Fuortes–Hodgkin model, and

assumed instead that they vary over a 5- to 7-fold range (cf. Penn & Hagins, 1972). In the particular case described as independent activation, the theoretical treatment leads to the following relatively simple expressions for the responses to steps (54) and flashes of light (55)

$$U = AI\,(1-\mathrm{e}^{-T})^n, \tag{54}$$

$$U = A'Q\,\mathrm{e}^{-T}\,(1-\mathrm{e}^{-T})^{n-1}, \tag{55}$$

where A and A' are constants, I is the light intensity and $n = 6$ or 7. These relatively simple expressions give a surprisingly good fit to the linear responses of red- and green-sensitive cones.

In seeking to explain the delay, three general types of hypothesis might be considered. In the first place it seems possible that the model might be literally correct in the sense that five or six events have to take place before a chromophore which has absorbed a photon can release the blocking particle or particles which close the ionic channels.

Another possibility which at first seems plausible is that each absorbed photon might start an autocatalytic reaction so that the concentration of blocking particles would rise initially as $y = \mathrm{e}^T$. However, this idea can be ruled out because if such a curve is plotted on double logarithmic paper its shape remains unchanged. Thus if $Z = \ln y$ and $W = \ln T$, $y = \mathrm{e}^T$ becomes $Z = \mathrm{e}^W$. Such a curve is of a completely different shape from the double logarithmic plots in Figs. 4 and 5 which appear to have straight lines as their asymptotes at short times. This would seem to exclude any simple kind of autocatalytic model as the explanation of the S-shaped delay. In a later paper we shall consider evidence that the blocking particle may be destroyed or degraded by an autocatalytic reaction, but that in no way affects the conclusion that the blocking particles are not produced by a regenerative reaction.

Several authors (e.g. Ives, 1922; Cone, 1964; Rushton, 1965) have considered the possibility that a particle released by light might have to diffuse a certain distance before reacting with an ionic channel. If one dimensional diffusion from an instantaneous point source to a sink at a distance l is considered, one obtains a series in which the dominant term at short times is

$$y \propto T^{-\frac{1}{2}}\,\mathrm{e}^{-L^2/4T}, \tag{56}$$

where L and T are in units to make the diffusion coefficient unity. If this expression is transformed by the substitutions $Z = \ln y$, $W = \ln T$ one obtains

$$Z = \text{const} - \tfrac{1}{2}W - (L^2/4)\,\mathrm{e}^{-W}. \tag{57}$$

This again is very different from the asymptotic straight line of slope 6–7 seen in Figs. 4 and 5. The diffusion theory might be rescued by

assuming some variation in the diffusion distance L but there are two other difficulties which should be mentioned. For the diffusion theory to work with a small ion or molecule there must be a minimum distance of the order of 10 μm in order to give a delay of 100 msec in the time to peak hyperpolarization. By assuming a large particle or some sort of restricted diffusion one could reduce this distance to 1 μm and propose that a particle must diffuse from the periphery of the sac to a channel on the axis of the cone. But photons can be absorbed anywhere in the sac and those absorbed close to the channel will act with very little delay. For these reasons diffusion would not be expected to give more than one stage of delay and if the molecule involved were small the delay might not exceed a few msec. Another difficulty with the diffusion theory is the high activation energy of the initial rate of rise, about 10 kcal/mole in turtle cones and 20 kcal/mole in *Limulus* ommatidia (Borsellino *et al.* 1965), which rules out simple aqueous diffusion as a cause of the delay.

Although diffusion may perhaps be excluded as the main cause of delay it almost certainly makes some contribution as the diffusion time from the edge to the centre of a cone of radius 1 μm is of the order of 1 msec for a small molecule.

It is important to notice that the S-shaped rise in potential soon after applying a step or flash of light cannot be explained by assuming that six particles are required to block a single channel unless one makes the additional and somewhat unlikely assumption that all six particles originate from one and only one photoisomerization. If six particles are required and come from more than one photon then superposition would not apply and the effect of two photons would be more than twice that of one.

Another possibility which can be rejected is that there is one ionic channel per chromophore and that one photoisomerization blocks one channel. Since there are some 10^8 chromophores per cone, the effect of one photoisomerization would on this hypothesis reduce the variable conductance g_1 by 1 part in 10^8. As complete suppression of g_1 hyperpolarizes the cell by only 25 mV, one photoisomerization would therefore hyperpolarize the cell less than 25×10^{-5} μV instead of 25 μV as estimated by Baylor & Hodgkin (1973). In order to account for the observed flash sensitivity it seems necessary to suppose that the number of light-sensitive channels is very much less than the number of chromophores and that the average effect of one photoisomerization is to block about 1/600 of the total number of channels (see p. 690).

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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., FUORTES, M. G. F. & O'BRYAN, P. M. (1971). Receptive fields of cones in the retina of the turtle. *J. Physiol.* **214**, 265–294.

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. (1974). Changes in time scale and sensitivity in turtle photoreceptors. *J. Physiol.* **242**, 729–758.

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

BORSELLINO, A., FUORTES, M. G. F. & SMITH, T. G. (1965). Visual responses in *Limulus*. *Cold Spring Harb. Symp. quant. Biol.* **30**, 429–443.

BRIGGS, G. E. & HALDANE, J. B. S. (1925). A note on the kinetics of enzyme action. *Biochem. J.* **19**, 338–339.

CONE, R. A. (1964). The rat electroretinogram. II. Bloch's law and the latency mechanism of the b-wave. *J. gen. Physiol.* **47**, 1107–1116.

CONE, R. A. (1973). The internal transmitter model for visual excitation: some quantitative implications. In *Biochemistry and Physiology of Visual Pigments*, ed. LANGER, H., pp. 275–282. Berlin: Springer-Verlag.

DIXON, M. & WEBB, E. C. (1964). *Enzymes*, 2nd edn. New York: Academic Press.

FEIN, H. (1966). Passing current through recording glass micro-pipette electrodes. *IEEE Trans. bio-med. Engng* **13**, 211–212.

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

HAGINS, W. A. (1972). The visual process: excitatory mechanisms in the primary receptor cells. *A. Rev. Biophys. Bioeng.* **1**, 131–158.

HAGINS, W. A., PENN, R. D. & YOSHIKAMI, S. (1970). Dark current and photocurrent in retinal rods. *Biophys. J.* **10**, 380–412.

IVES, H. E. (1922). A theory of intermittent vision. *J. opt. Soc. Am.* **6**, 343–361.

KORENBROT, J. T. & CONE, R. A. (1972). Dark ionic flux and the effects of light in isolated rod outer segments. *J. gen. Physiol.* **60**, 20–45.

KLEINSCHMIDT, J. (1973). Adaptation properties of intracellularly recorded Gekko photoreceptor potentials. In *Biochemistry and Physiology of Visual Pigments*, pp. 219–224. Berlin: Springer-Verlag.

LANGMUIR, I. (1916). The constitution and fundamental properties of solids and liquids. *J. Am. chem. Soc.* **38**, 2221–2295.

MICHAELIS, L. & MENTEN, M. L. (1913). Die Kinetik der invertinwirkung. *Biochem. Z.* **49**, 333–369.

NORMAN, A. C. (1973). *Taylor User's Manual.* University of Cambridge Computer Laboratory.

PENN, R. D. & HAGINS, W. A. (1972). Kinetics of the photocurrent in retinal rods. *Biophys. J.* **12**, 1073–1094.

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

SCHWARTZ, E. A. (1973). Responses of single rods in the retina of the turtle. *J. Physiol.* **232**, 503–514.

TOMITA, T. (1970). Electrical activity of vertebrate photoreceptors. *Q. Rev. Biophys.* **3**, 179–222.

TOYODA, J., NOSAKI, H. & TOMITA, T. (1969). Light-induced resistance changes in single photoreceptors of *Necturus* and *Gekko*. *Vision Res.* **9**, 453–463.