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INTERNAL RECORDING OF THE EARLY RECEPTOR POTENTIAL IN TURTLE CONES

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SUMMARY

1. Early receptor potentials (E.R.P.s) were recorded with internal electrodes in turtle cones by applying brief flashes from a xenon tube with a maximum photon density equivalent to $2–3 \times 10^8$ photons μm^{-2} at the optimum wave-length.

2. The E.R.P. was separated from the late receptor potential (L.R.P.) by superposing the flash on a step of light which was strong enough to saturate the L.R.P.

3. In red-sensitive cones the E.R.P. consisted of a brief depolarizing phase (R_1) followed by a hyperpolarizing phase (R_2) of maximum amplitude 10 mV and duration 30–40 msec. R_1 was small or absent in green-sensitive cones.

4. With flashes of increasing intensity the E.R.P. approached its maximum exponentially with an exponential constant $\bar{Q}$ of about 10^8 photons μm^{-2} which is of the same order as the reciprocal of the photosensitivity of porphyropsin; the implication of this result, which is considered in the theoretical section, is that the E.R.P. is proportional to the number of photoisomerizations.

5. When tested with a constant xenon flash at varying times after the beginning of a bleaching light the E.R.P. declined exponentially with a similar value of $\bar{Q}$.

6. After prolonged bleaches the E.R.P. recovered with a time constant of about 100 sec but much quicker recoveries were observed after relatively brief bleaches.

7. The form and size of the E.R.P. are consistent with the accepted view that it arises from a redistribution of charge in the cone pigment molecule.

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8. The effect of a single photoisomerization in an isolated cone was estimated as about 10^{-10} V or one electronic charge through about 10% of the membrane.

INTRODUCTION

There is general agreement that the early receptor potentials first described by Brown & Murakami (1964) are closely related to the initial photochemical changes in the photolysis of rhodopsin (Cone & Pak, 1971; Brindley & Gardner-Medwin, 1966; Hagins & McGaughy, 1967). It is therefore of interest to obtain early and late receptor potentials with internal electrodes on the same cell. This was first done by Murakami & Pak (1970) who described the early receptor potentials (E.R.P.s) which they recorded with internal electrodes from the photoreceptors of *Gekko*, *Necturus* and axolotl (*Amblystoma mexicanum*). These internally recorded E.R.P.s were longer in duration and about 20 times larger than those recorded with external electrodes. The form of the external E.R.P. was approximately equal to the first derivative of the internal E.R.P., a result which is to be expected if the external E.R.P. is proportional to membrane current and the membrane time constant is long compared to the duration of the early receptor current.

The aim of the present work was to extend Murakami & Pak's observations using the cones of the swamp turtle, *Pseudemys*, in which late receptor potentials have been intensively studied with internal electrodes. The general method was similar to that used previously on *Pseudemys* photoreceptors except that the intensity of the xenon flashes employed was about 1000 times greater than that of the tungsten iodide lamp used in earlier work (see Baylor, Fuortes & O'Bryan, 1971; Baylor & Hodgkin, 1973).

The general conclusion is that in *Pseudemys* cones the size of the E.R.P. seems to be proportional to the number of molecules bleached by the flash and that the maximum E.R.P. which can be elicited may be proportional to the number of unbleached molecules. If this is correct studies of the E.R.P. may provide useful information about the rate at which pigment is isomerized by light, or regenerated in the dark. The change in potential produced by one photoisomerization is about 10^{-10} V and a strong flash which isomerizes most of the molecules gives a saturating response of about 10^{-2} V.

METHODS

Material

The experiments were carried out on isolated eyecups from the red-eared swamp turtle, *Pseudemys scripta elegans*. Preparation of the eyecup and recording methods were similar to those described by Baylor & Hodgkin (1973) except that maximum

capacity compensation was employed in order not to attenuate the E.R.P. Tests showed that slightly reducing the amount of compensation did not materially affect the E.R.P.; the electrode resistances were 130–300 MΩ. The xenon flash unit was screened and except in preliminary experiments electrical artifacts from the flash were either absent or so brief as not to interfere with the E.R.P., as was proved by tests made with the electrode outside the cone (see Fig. 1, records *E* and *F*).

Light stimuli

The optical stimulator was the same as that used by Baylor & Hodgkin (1973) with the following exceptions. In one half of the stimulator the tungsten light source and shutter were replaced by a xenon flash unit (Mecablitz 202, Metz, Fürth, Germany); this delivered a flash which rose to a peak in about 100 μsec and then declined exponentially with a time constant of 1·5 msec. The light from the xenon source was collected by a field lens of focal length 21·6 cm, passed through a series of neutral density filters and combined with the light from the tungsten source with a beam combining plate (Balzer: Transflex-TF-MJ-45). Both the xenon flash and the conditioning light from the tungsten source were circular spots with a diameter on the retina of 430 μm.

TABLE 1. Calculation of xenon and tungsten light densities in equivalent photon densities at λ_{max} for red- and green-sensitive cones

For red-sensitive cones

	A Photon density transmitted by 644 nm filter (photons μm^{-2})		*B* Equivalent photon density of white light at 644 nm (photons μm^{-2})
Tungsten source (10 msec flash)	$1{\cdot}24 \times 10^5$	× 11·74 →	$1{\cdot}44 \times 10^6$
			× 190·5 ↓
Xenon flash	$2{\cdot}52 \times 10^7$		$2{\cdot}74 \times 10^8$

For green-sensitive cones

	A Photon density transmitted by 559 nm filter (photons μm^{-2})		*B* Equivalent photon density of white light at 559 nm (photons μm^{-2})
Tungsten source (10 msec flash)	$7{\cdot}12 \times 10^4$	× 11·35 →	$8{\cdot}08 \times 10^5$
			× 263 ↓
Xenon flash	$2{\cdot}11 \times 10^7$		$2{\cdot}12 \times 10^8$

The figures for the tungsten source in column *A* were derived from measurements with a radiometer and with a photodiode; those for the xenon source in column *A* with the photodiode only. The factors above the horizontal arrows (11·74 and 11·35) are from Baylor & Hodgkin (1973, Table 3); those to the right of the vertical arrows (190·5 and 263) were determined experimentally in the present study.

Calculation of equivalent light intensity for xenon flash

Since the xenon flashes were only just strong enough to give a near maximal E.R.P. it was not possible to use monochromatic light. The equivalent quantum density of the xenon flash for red- and green-sensitive cones was calculated from the data in Table 1. Column *A* shows that the 644 nm filter transmitted $1{\cdot}24 \times 10^5$ photons μm^{-2} in a 10 msec flash from the tungsten iodide lamp. From the measurements of Baylor & Hodgkin (1973) on red-sensitive cones using the same lamp and the same filter, a 10 msec flash of white light from the tungsten lamp is estimated as equivalent to $1{\cdot}24 \times 10^5 \times 11{\cdot}74$ photons μm^{-2} at 644 nm. To obtain a similar figure for the xenon flash the relative effectiveness of xenon flash and a 10 msec tungsten flash in generating the late receptor potential were determined in two experiments. This gave a factor of 190·5 and allowed the xenon flash to be expressed as equivalent for red-sensitive cones to $2{\cdot}74 \times 10^8$ photons μm^{-2} at 644 nm. The corresponding figure for green-sensitive cones was $2{\cdot}12 \times 10^8$ photons μm^{-2}.

Experimental procedure

As flashes which evoke a measurable E.R.P. bleached an appreciable amount of visual pigment, it was necessary to allow time for pigment regeneration between successive flashes. Preliminary tests showed that after a maximum xenon flash the E.R.P. recovered completely in 60–80 sec. Successive flashes were therefore delivered at intervals greater than 80 sec. In the bleaching and regeneration experiments considered on p. 749 a control flash was delivered after 120–180 sec of recovery in order to ensure that the E.R.P. had recovered completely; if the E.R.P. had regained its normal amplitude the next bleaching sequence was begun after a further interval of 120 sec.

RESULTS

Fig. 1 illustrates the general method used in studying the E.R.P. of turtle cones. Records *A* and *B* show the effect on a red-sensitive cone of a brief exponential flash, of time constant 1·5 msec, from a xenon tube. This flash delivered the equivalent of $2{\cdot}7 \times 10^8$ photons μm^{-2} at the optimum wave-length of 644 nm, which was about 200 times the equivalent quantity in a 10 msec flash from our standard tungsten-iodide source. The depolarizing phase R_1 of the E.R.P. can be clearly seen in the fast time base record *B*, but the hyperpolarizing component, R_2, merges into the late receptor potential (L.R.P.) from which it must be separated by some special procedure. The change in potential recorded when the microelectrode was outside the cone was always negligible compared to the change in internal potential during both L.R.P. and E.R.P. (records *E* and *F* in Fig. 1).

The method of separating E.R.P. from L.R.P., which is illustrated in records *C* and *D* of Fig. 1, was to superpose the xenon flash on a step of light from the tungsten lamp that was strong enough to saturate the late receptor potential. The E.R.P. can then be observed by itself on the flat plateau produced by the conditioning step. Record *D* was obtained in this way and may be compared with record *B* which was obtained with no

conditioning step and is clearly a mixture of E.R.P. and L.R.P., the latter beginning about 4 msec after the flash. In *B*, the circles which give the E.R.P. component were obtained from record *D*.

The method used to separate the responses to xenon flashes of increasing intensity into early and late receptor potentials is shown in Fig. 2. Column *A* are records produced by the xenon flash alone and column *B* by the

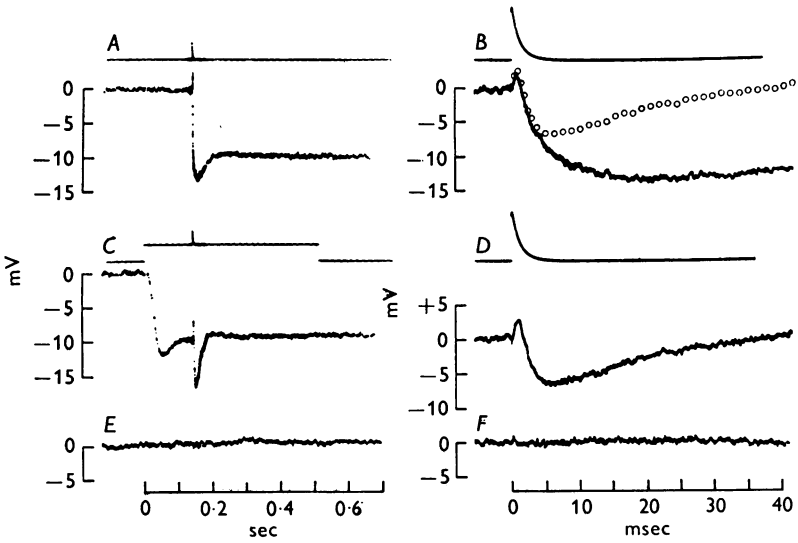

Fig. 1. Use of a conditioning step of light to separate E.R.P. from L.R.P., recorded from a red-sensitive cone with an internal electrode. *A*, effect of unattenuated xenon flash without conditioning light (slow time base). *B*, same but on faster time base. The response is a mixture of E.R.P. (shown by circles obtained from *D*) and L.R.P. *C*, effect of applying same xenon flash on conditioning step of light which saturates the L.R.P. mechanism. The response to the xenon flash in this record and in *D* is considered to be E.R.P. only. *D*, same as *C* but showing effect of xenon flash on faster time base-expansion of 0·13–0·18 sec of record *C*; the zero on the voltage scale corresponds to −10 mV in *C*. *E*, *F*, same stimulus as in *C* and *D* but recorded with electrode outside cone. The xenon flash was equivalent to $2{\cdot}74 \times 10^8$ photons μm^{-2} at 644 nm and the intensity of the conditioning step to about $1{\cdot}4 \times 10^6$ photons $\mu m^{-2}\,sec^{-1}$ at $\lambda = 644$ nm. After an unattenuated xenon flash the cone remained at the saturated potential for several seconds. Red-sensitive cone 20. Temperature 20·8° C.

same flash superposed on a conditioning light of an intensity sufficient to saturate, or nearly saturate, the late receptor potential, without causing appreciable bleaching ($< 0{\cdot}5\%$), Column *B* is considered to be E.R.P. alone and column *C*, which is the difference between *B* and *A*, should be the late receptor potential. In terms of present ideas, the records in *B* are produced by a mechanism which generates a small electric current for

each photoisomerization, whereas those in *C* result from the liberation of a blocking molecule which closes ionic channels and hyperpolarizes the cell to a saturating value. On this basis it is reasonable that the two components should add in an approximately linear manner. It is necessary to introduce the qualification 'approximately' because the method neglects the change in membrane resistance during the late receptor potential which should make the later stages of the E.R.P. larger when the xenon flash is

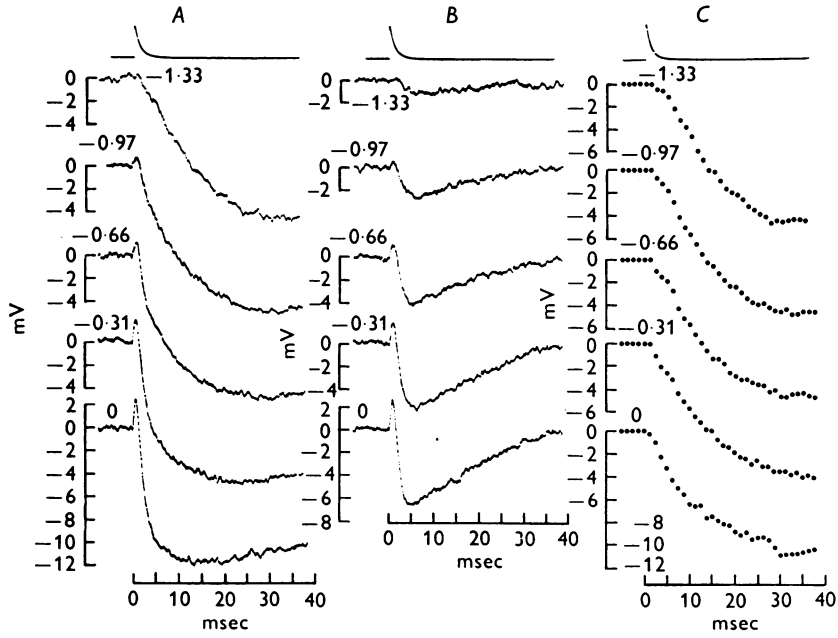

Fig. 2. Response of red-sensitive cone (Red 21) to xenon flashes of increasing intensity, in presence and absence of conditioning step. Column *A*, xenon flashes alone. Column *B*, xenon flashes superposed on conditioning step (about 0·1 sec after beginning); as in Fig. 1 *B* and *D*, only the E.R.P. part of the response is shown and the zero of the voltage scale has been shifted down about 10 mV. Column *C*, difference between responses in columns *A* and *B* which is taken as the L.R.P. generated by the xenon flash. Numbers give the attenuation of the xenon flash in $\log_{10}$ units; the unattenuated light was equivalent to $2{\cdot}74 \times 10^8$ photons μm^{-2} at 644 nm; the conditioning step was equivalent to about $1{\cdot}4 \times 10^6$ photons μm^{-2} sec^{-1}. Temperature 20·5° C.

superposed on a conditioning step. Another possible source of error is that the conditioning step may not have been strong enough to remove all the late receptor potential.

The form of the late receptor potential produced by strong flashes is in general agreement with the theory of Baylor, Hodgkin & Lamb (1974*b*). Thus Dr T. D. Lamb (personal communication) has shown that the theory

predicts a rise of the potential to half its maximal value in 10 msec after an exponential flash of time constant 1·5 msec which isomerizes 10^8 molecules; this is similar to the late receptor potentials calculated for the strongest flashes in Fig. 2.

General characteristics of the E.R.P. in turtle cones

A striking difference between early and late receptor potentials is that whereas the time course of the L.R.P. varied with the intensity of the flash the time course of the E.R.P. has essentially the same form for all

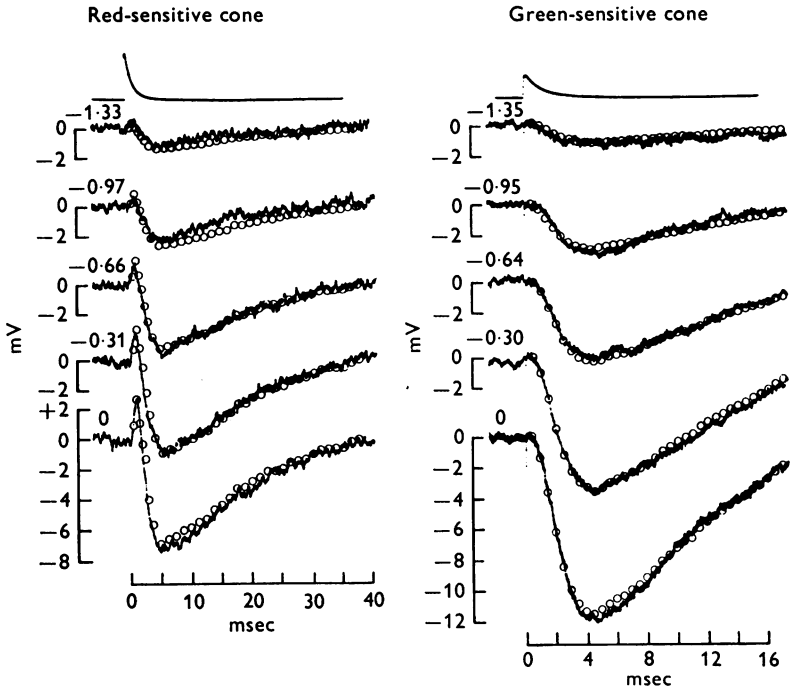

Fig. 3. Families of E.R.P. responses from a red- and a green-sensitive cone isolated from L.R.P. by the method shown in Fig. 1. The circles superimposed on the records were obtained by multiplying the mean response per photoisomerization by the number of photoisomerizations produced by a given flash. The fraction f isomerized by a flash of strength Q was calculated as $1-\exp(-Q/\bar{Q})$ with the $\bar{Q}$ values given in Table 3, i.e. $\bar{Q} = 6{\cdot}49 \times 10^7$ photons μm^{-2} for the red-sensitive cone (Red 20), and $\bar{Q} = 10^8$ for the green-sensitive cone (Green 2). The circles were then calculated as $f\Sigma v(t)/\Sigma f$. The unattenuated xenon flash was equivalent to $2{\cdot}74 \times 10^8$ photons μm^{-2} at 644 nm (red-sensitive cone) or $2{\cdot}12 \times 10^8$ photons μm^{-2} at 559 nm (green-sensitive cone). The conditioning light was equivalent to about $1{\cdot}4 \times 10^6$ photons μm^{-2} sec^{-1} at 644 nm for the red-sensitive cone or 8×10^5 photons μm^{-2} sec^{-1} at 559 nm for the green-sensitive cone. Temperature: Red 20, 20·8° C; Green 2, 21·5° C.

flashes, and within experimental error, curves for weak and strong flashes can be superposed by scaling. This is illustrated by Fig. 3 in which the circles are average values scaled in proportion to the calculated

TABLE 2. Characteristics of the E.R.P.

Cone	R_1 Peak (mV)	R_1 t_{peak} (msec)	R_2 Peak (mV)	R_2 t_{peak} (msec)	R_2 τ_{end}	L.R.P. max (mV)
Red-sensitive cones						
Red 1	—	—	11·3	6·0	15·3	19
2	2·3	1·25	8·7	9·0	17·0	18
3	1·35	1·25	6·0	6·25	8·0	8
4	2·5	1·0	7·6	6·75	14·0	20
5	—	—	4·0	5·0	13·8	17
6	—	—	6·6	5·75	13·0	16
7	—	—	6·4	10·0	17·8	16
8	1·8	1·25	7·8	9·3	27·0	21
9	1·3	1·0	8·3	7·75	13·5	14
10	2·7	1·3	7·0	7·0	14·0	16
11	2·5	1·5	7·2	10·0	21·0	18
12	1·5	1·2	5·5	7·0	18·3	9
13	1·0	1·25	6·0	6·0	15·5	8
14	0·8	1·2	7·6	7·0	19·0	8
15	2·1	1·2	10·0	7·5	16·0	18
16	2·7	1·25	6·5	7·0	17·8	19
17	2·1	1·25	7·6	6·5	17·8	14
18	2·4	1·0	7·6	7·0	14·0	19
19	2·3	0·7	6·2	6·5	13·3	14
20	3·4	0·8	8·0	6·5	13·5	20
21	2·4	0·5	6·7	5·0	15·3	20
Mean	2·04	1·09	7·38	7·05	15·9	
and S.E. of mean	±0·15	±0·06	±0·34	±0·3	±0·82	
Green-sensitive cones						
Green 1	< 0·2	—	10·6	5·5	—	7
2	0·4	—	13·5	4·0	7·0	7
3	< 0·2	—	11·8	5·0	15·3	5
4	< 0·2	—	13·0	4·5	17·5	7
5	< 0·2	—	13·8	3·1	8·3	4
6	0·2	—	11·9	6·0	12·5	13
7	< 0·2	—	13·8	3·8	8·8	16
Mean	—	—	12·6	4·56	12·8	—
and S.E. of mean	—	—	±0·47	±0·38	±1·9	—

R_1 is the positive (depolarizing) phase and R_2 the negative (hyperpolarizing) phase of the E.R.P. Peak values are those observed and not extrapolated maxima as in Table 3. t_{peak} gives time to peak and τ_{end} is the exponential time constant of the end of R_2. The maximum late receptor potential (L.R.P.) is included for comparison. Temperature 18–22° C.

number of photoisomerizations (see p. 746). The agreement with the experimental curves supports the idea that the amplitude of the E.R.P. is proportional to the number of photoisomerizations at all times. Further evidence is provided by the section on p. 751 which shows that the exponential constant $\bar{Q}$ of the strength amplitude relations is the same for different phases of the response.

As can be seen from Table 2 and Fig. 3, the E.R.P.s of green-sensitive cones differ from those in red-sensitive cones in the following respects. In green-sensitive cones R_1 is small or absent and R_2 reaches its peak amplitude earlier than in red-sensitive cones. It is possible that a delay in the input stage and micro-electrode of perhaps 500 μsec may have largely eliminated a very rapid R_1 component in green-sensitive cones. However, the difference between red- and green-sensitive cones is unlikely to be due to differences between electrodes because on another occasion records similar to those in Fig. 3 were obtained from adjacent red- and green-sensitive cones with the same electrode. The small size or absence of R_1 in green-sensitive cones is reminiscent of the finding of Murakami & Pak (1970) that in the rod-like receptors of *Gekko gekko* the internally recorded E.R.P. usually appears as a monophasic hyperpolarizing response with at most only a trace of an initial phase of depolarization.

An interesting difference between the mechanisms underlying E.R.P. and L.R.P. is that desensitization of the L.R.P. mechanism does not reduce the E.R.P. This was illustrated in Fig. 1 and provides the basis of the method used to separate the early and late components of the response. It was also found that some cones which gave small late receptor potentials (< 5 mV) still gave large E.R.P.s (10–15 mV). All this points to the existence of two current generating mechanisms with very different properties which contribute independently to the membrane potential.

Relation between strength of flash and amplitude of E.R.P.

Fig. 4 shows that the relation between E.R.P. amplitude (or potential at a fixed time) and the equivalent density of applied quanta is well fitted by the simple expression:

$$\frac{U}{U_{\max}} = 1 - e^{-Q/\bar{Q}}, \tag{1}$$

where $U_{\max}$ is the potential which the peak E.R.P. amplitude approaches asymptotically with strong flashes. Q is the strength of the light flash expressed as the equivalent quantum density in photons μm^{-2} at the optimum wave-length; $\bar{Q}$ is a constant with the same dimensions as Q. Values of $U_{\max}$ and $\bar{Q}$ in individual experiments were obtained by using the MLAB curve fitting programme (Knott & Reece, 1972) and are given in Table 3. In the theoretical section (pp. 751–757) it is shown that an

equation approximating to eqn. (1) is expected if the size of the E.R.P. is proportional to the number of photoisomerizations. For cones of low sensitivity the constant $\bar{Q}$ should be of the same order as $(\alpha\gamma)^{-1}$ where α is the molecular extinction coefficient and γ is the quantum efficiency of the isomerization. It is also shown that the quantity $\bar{N}$ obtained by multiplying $\bar{Q}$ by A_i^*, the effective collecting area of the cone, should be equal to the number of pigment molecules in the unbleached cone. If that is the case the peak amplitude E.R.P. from a single photoisomerization in an isolated cone should equal $U_{max}/\bar{N}$; from the mean values in Table 3 this

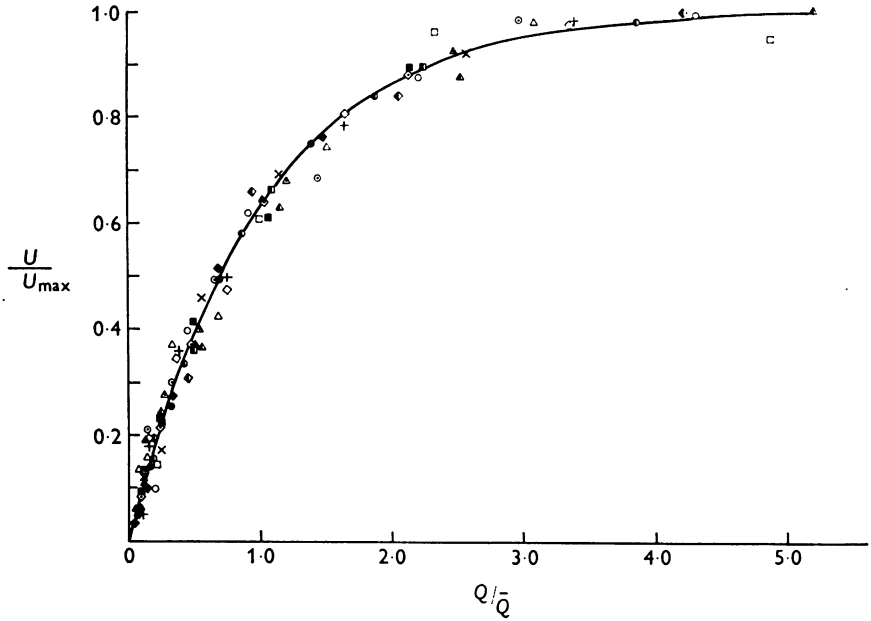

Fig. 4. Relation between E.R.P. amplitude, U (or voltage at 5 msec) and density of applied photons in thirteen red-sensitive cones (open and half-filled symbols and crosses) and four green-sensitive cones (filled symbols). Values of U_{max} and $\bar{Q}$ were obtained in individual experiments by a least-squares curve-fitting programme which fitted eqn. (1) to the points. The continuous curve is $1-e^{-Q/\bar{Q}}$.

quantity is found to be 7×10^{-11} V in red-sensitive cones. This may be compared with the value of $2{\cdot}5 \times 10^{-5}$ V estimated for the average peak of the late receptor potential after a single photoisomerization in an isolated cone.

Values of $\bar{N}$ were not calculated for green-sensitive cones as there is doubt about the right value to use for S_Φ, the voltage change per photoisomerization, which must be known before the effective collecting area of each cone can be calculated. If the figure of 21 μV per photoisomerization

obtained by Baylor & Fettiplace (1975), which rests on a single observation, is used, green-sensitive cones 6 and 8 give values of 184 and 290 × 10^6 molecules for $\bar{N}$. These values are 2–3 times the mean $\bar{N}$ in red-sensitive cones instead of about 60 % as expected from the histological measurements of Baylor & Fettiplace (1975).

The average value of $\bar{Q}$ obtained by measuring the amplitude of R_1, or

TABLE 3. Values of $\bar{Q}$ and $\bar{N}$ in cones

Cone	S_F (μV photon^{-1} μm^2)	A_i^* (μm^2)	L.R.P. max. (mV)	E.R.P. max. (mV)	$\bar{Q}$ (10^6 photons μm^{-2})	$\bar{N}$ (10^6 molecules)
Red-sensitive cones						
Red 1	29·9	1·2	19·1	11·5	66·6	79·6
2	52·6	2·1	18·2	8·9	89·6	188
3	4·9	0·2	8·2	7·8	81·1	16·2
4	57·4	2·3	20·3	6·3	61·4	141
5	29·9	1·2	17·3	4·4	51·8	62·1
6	19·7	0·8	15·5	6·7	92·9	73·1
7	15·5	0·6	16·5	6·9	112	69·1
10	9·6	0·38	15·5	6·9	103	39·0
16	62·0	2·48	18·5	6·6	81·1	201
17	68·2	2·73	14·0	7·8	71·2	194
18	117·7	4·71	20·1	7·3	52·9	249
19	16·6	0·66	19·8	6·9	125	82·1
20	17·8	0·71	12·6	7·1	64·9	46·2
21	10·1	0·4	13·6	6·3	71·2	28·8
Mean	36·6	1·45	16·4	7·2	80·3	104·9
Green-sensitive cones						
Green 1	—		7·5	14·1	153	
2	—		7·3	15·2	100	
6	18·4		12·5	18·5	210	
8	84·5		17·5	9·2	71·7	
Mean	—		11·2	14·3	133·7	

S_F is the flash sensitivity; A_i^* is the effective collecting area obtained by dividing the observed value of S_F by S_Φ which is taken as 25 μV photoisomerization^{-1} in red-sensitive cones, Baylor & Hodgkin (1973), Baylor & Fettiplace (1975). Column 4 gives the maximum value of the late receptor potential and column 5 is an estimate of the maximum early receptor potential obtained from the asymptote of an exponential curve drawn through the strength amplitude relation (see Fig. 4). $\bar{Q}$ is the exponential constant of the curve relating E.R.P. amplitude to photon density. $\bar{N}$ (which should equal the number of molecules in the cone) is $A_i^*\bar{Q}$ see text. Note that if γ the quantum efficiency is defined as the number of pigment molecules isomerized per photon absorbed (with dimensions molecule photon^{-1}) then A_i^* the effective collecting area has dimensions area molecule photon^{-1} and $\bar{N}$ has the dimensions of molecules, i.e. the number of pigment molecules per cone; for simplicity (but not consistency) A_i^* is given in its usual units of μm^2. Temperature 19–22° C.

the potential at a fixed time during R_2, was about the same as that obtained by measuring the peak amplitude of R_2. Thus in seven red-sensitive cones the mean $\bar{Q}$ for R_1 was $(9{\cdot}4 \pm 1{\cdot}2) \times 10^7$ photons μm^{-2} which is not significantly different from the mean $\bar{Q}$ of R_2 of $(8{\cdot}1 \pm 0{\cdot}9) \times 10^7$ for the same cones.

An alternative method of measuring $\bar{Q}$

The results in the previous section are consistent with the idea that the amplitude of the E.R.P. is proportional to the number of photoisomerizations. An alternative method of measuring $\bar{Q}$ is to apply the xenon flash

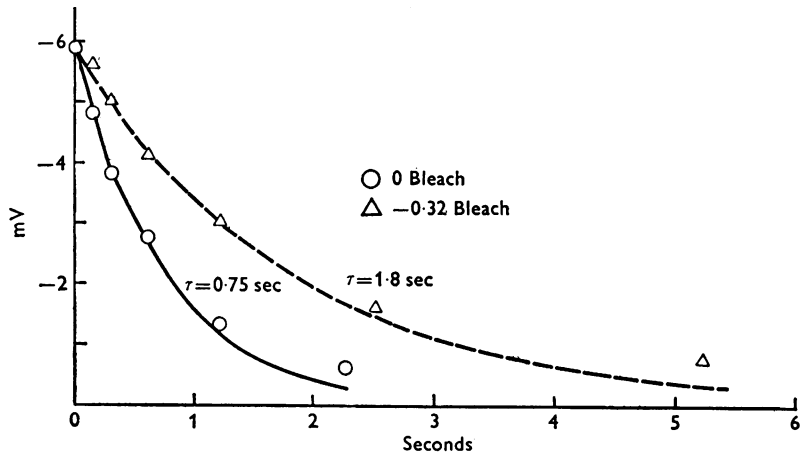

Fig. 5. Plot of E.R.P. amplitude (R_2) from the xenon flash as a function of time after the onset of a bleaching light (two separate bleaching intensities are shown) in a red-sensitive cone (Red 20). The unattenuated bleaching light was equivalent to $1{\cdot}44 \times 10^8$ photons μm^{-2} sec^{-1} at 644 nm and the xenon flash to $2{\cdot}74 \times 10^8$ photons μm^{-2} at 644 nm. The curves are exponential with a time constant given by the numbers near each curve. An interval of several minutes was left between measurements, each of which required the application of a xenon flash at time t after the onset of the bleach by the tungsten light. Temperature 20·8° C.

after the cone has been partly bleached by the tungsten light for 1–5 sec. This reduces the E.R.P. by lowering the number of unbleached pigment molecules which are available to contribute to it. When a series of such measurements were made with the xenon flash timed to occur after different durations of bleaching light, it was found that the E.R.P. declined exponentially with a time constant which decreased as the light intensity was increased. In such experiments an interval of several minutes must be left between successive measurements in order to allow the cone to recover from the combined effects of the conditioning light and test flash.

In Fig. 5 the amplitude of the E.R.P. was measured as a function of time during two sets of conditioning steps differing in intensity by a factor of 2·1. The curves for the fall of the E.R.P. are exponential in both cases and the exponential rate constants of the decline are proportional to the intensity of the light. This result is to be expected if the E.R.P. is proportional to unbleached pigment and the rate of photolysis of pigment is high compared to the rate of regeneration.

Table 4 shows that on the average there is fair agreement between the values of $\bar{Q}$ calculated from the strength amplitude relation (Fig. 4) and from experiments of the type illustrated in Fig. 5.

TABLE 4. Comparison of $\bar{Q}$ obtained by varying intensity of xenon flash (see Fig. 4) or by superposing xenon flash on bleaching light and determining the time constant τ_{bleach} with which the E.R.P. declined

Cone	Intensity of bleach (10^6 photons μm^{-2} sec^{-1})	τ_{bleach} (sec)	$\bar{Q}_b$ (10^6 photons μm^{-2})	$\bar{Q}$ (10^6 photons μm^{-2})
Red 18	144	0·4	57·6	52·9
19	144	1·2	172	125
20	144	0·75	108	64·9
	69·7	1·8	125	
Green 6	80·8	2·25	181	210
	20·1	8·0	161	
Red 10	—	—	127	103
Mean	—	—	129	112
and S.E.	—	—	±21	±28

$\bar{Q}$ was obtained from the relation between E.R.P. amplitude and the intensity of the xenon flash (Fig. 4) and $\bar{Q}_b$ from the time constant with which the E.R.P. declined when a steady light was applied. In the last experiment (Red 10) the duration of the bleaching light was fixed at 2·95 sec and its intensity was varied. In all cases $\bar{Q}_b$ represents the number of photons μm^{-2} (at the optimum wave-length) required to reduce the E.R.P. to $1/e$ of its maximum value. White light was used and intensities are given for the equivalent intensity of monochromatic light at the optimum wave-length. Temperature 20–21° C.

Recovery of the E.R.P. after different periods of bleaching

Fig. 6*A* shows the recovery of E.R.P. amplitude in a red-sensitive cone after a series of 10 sec bleaches which delivered the equivalent of $1{\cdot}44 \times 10^8$ photons μm^{-2} sec^{-1} at the optimum wave-length and should have bleached nearly all the pigment. The E.R.P. recovered in an approximately exponential way with a time constant of 23 sec. Similar results were obtained in two other experiments with 10 sec bleaches of the same intensity, the time constants being 19 and 24 sec. On the other hand in two experiments in which the cone was bleached for 80–90 sec, recovery was much slower, the time constant being about 100 sec. This behaviour which is illustrated

in Fig. 6*B* is reminiscent of that described by Baylor & Hodgkin (1974) in connexion with the recovery of L.R.P. sensitivity. A possible explanation is that the 20 sec recovery time constant depends on combination of cone opsin with stored 11-cis retinal whereas the 100 sec time constant

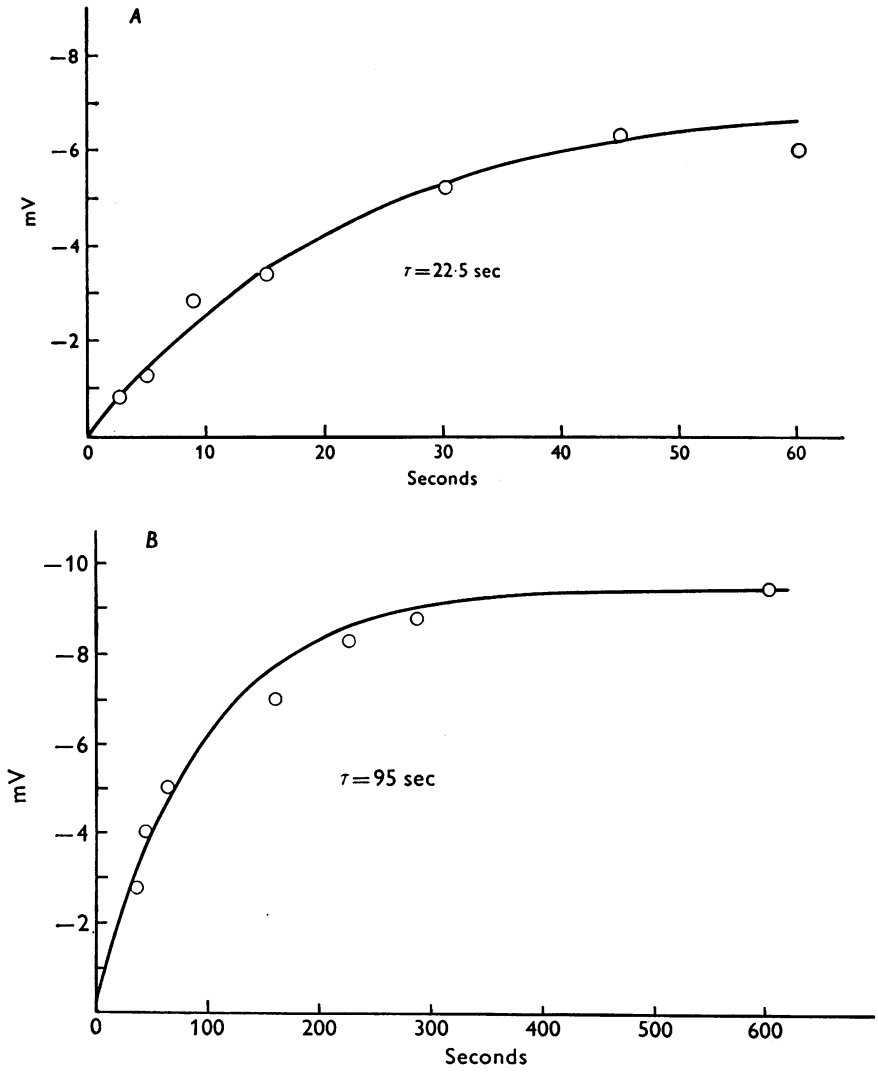

Fig. 6. Recovery of E.R.P. amplitude as a function of time after bleaches lasting 10 sec, *A*, or 80 sec, *B*. The continuous curves through the points are of the form $V = V_{max}(1-e^{-t/\tau})$. *A*, cone Red 1. *B*, cone Red 16. An interval of several minutes was left between measurements each of which required applications of the bleaching light and xenon flash. Temperature *A*, 20·0° C; *B*, 21·3° C.

depends mainly on replenishment of the 11-cis isomer. The same type of hypothesis has been proposed by Rushton & Henry (1968) and Goldstein & Price (1975) in order to account for a similar dependence of regeneration rate on bleaching time in human and frog cones. However, the time constants observed in our preliminary experiments were shorter than those usually obtained with cone pigment, e.g. 130–390 sec for frog cones at 20° C (Goldstein & Price, 1975).

Experiments with two flashes

Hagins (1955), Rushton (1964), Williams (1974) and others have shown that a brief very intense flash bleaches only about half the rhodopsin because second quantum hits convert metarhodopsin back into rhodopsin, or a mixture of rhodopsin and isorhodopsin. A second flash delivered a few msec after the first bleaches half the remaining pigment, and pulses longer than a few milliseconds give more complete bleaches. Whether the Hagins phenomenon is observed depends critically on flash duration and temperature and it is not clear if our exponential flashes of 1·5 msec time constant would or would not bleach most of the pigment when applied to cones at 20° C. Preliminary tests with two xenon flashes indicated that the strongest flashes probably did bleach most of the pigment (more than 80 %). This conclusion is supported by the approximate agreement between the values of $\bar{Q}$ obtained with xenon flashes and those found with longer pulses from the tungsten light (Table 4).

Since the xenon flashes contained substantial energy at short wave-lengths (Table 1) one might expect to see the very interesting phenomenon of a reversed E.R.P. when blue light falls on bleached rhodopsin (Cone, 1967). However, no such effect was observed when two unfiltered xenon flashes were applied to red-sensitive cones so there may be a difference between rods and cones or between A_1 and A_2 based pigment. Absorption of short wave-lengths in the oil droplet could contribute to this negative result but is unlikely to be of much importance unless the light was strictly parallel with the axis of the cone (see Baylor & Fettiplace, 1975).

ANALYSIS OF RESULTS

The relation between E.R.P. amplitude and intensity of flash

Theory

The results in Fig. 4 show that the relation between the quantity of light applied by the flash and the amplitude, U, of the early receptor potential is approximately described by the equation

$$\frac{U}{U_{\max}} = 1 - e^{-Q/\bar{Q}}, \tag{1}$$

where $U_{\max}$ is the maximum amplitude which is approached asymptotically with flashes of high intensity. Q is the quantity of light expressed as the equivalent density of photons of optimum wave-length in photons μm^{-2} (see Baylor, Hodgkin & Lamb, 1974*a*). $\bar{Q}$ is a constant with the same dimensions as Q.

This section is concerned with the basis of eqn. (1) and the physical

meaning of the constant $\bar{Q}$. The theoretical approach is similar to that first described by Dartnall, Goodeve & Lythgoe (1936). Initially, it will be assumed that the incident light is parallel to the axis of the cone and that the photoproducts have the same absorption as the unbleached pigment. Later (p. 755) a second model will be considered in which the photoproducts are assumed to be transparent. The real situation is intermediate between these two extremes, but as there is little difference between the predictions of the two models the precise assumption made about absorption by the photoproducts is evidently not a critical one. The main symbols used are:

i total light flux in cone in photons sec^{-1}

i_0 value of i at $x = 0$

x distance along outer segment with $x = 0$ at base and $x = l$ at tip of cone

t time from beginning of flash

A_x cross-sectional area of outer segment with A_0 at base and $\hat{A}$ mean cross-sectional area

A_c area over which light is captured by cone (i_0 = incident light intensity $\times A_c$)

A_i^* effective collecting area of cone as defined by Baylor & Hodgkin (1973) or Baylor & Fettiplace (1975); eqn. (21) gives the relation between A_i^* and A_c

c concentration of unbleached pigment at x and t

c_0 concentration at $t = 0$

$\hat{c}_\infty$ mean concentration of unbleached pigment at $t = \infty$, i.e. mean concentration at end of flash

q $\left(= \int_0^\infty i_0 \mathrm{d}t\right)$ quantity of light applied to base of cone in equivalent quanta at optimum λ

Q $\left(= \frac{q}{A_c}\right)$ equivalent photon density of light flash expressed in photons μm^{-2} (at λ_{max})

α molecular extinction coefficient (Napierian) of photopigment expressed in μm^2 per molecule

γ quantum efficiency (isomerizations per quantum absorbed)

l length of outer segment

L $= \alpha l c_0$

Photoreversal effects are ignored and it will be assumed that the amplitude U of the early receptor potential is proportional to the total photochemical change, that is to $c_0 - \hat{c}_\infty$. Hence

$$\frac{U}{U_{max}} = 1 - \frac{\hat{c}_\infty}{c_0} \tag{2}$$

so our object is to find $\hat{c}_\infty/c_0$ as a function of q. It is assumed that no light is lost from the outer segment, and that light is attenuated by absorption by pigment in both normal and bleached form. For the case where the photoproducts have the same absorbance as the unbleached pigment the basic equations are

$$-\frac{\partial i}{\partial x} = \alpha i c_0 \tag{3}$$

and

$$-\frac{\partial c}{\partial t} = \frac{\alpha\gamma i c}{A_x}. \tag{4}$$

These equations express the fact that in this model the absorption of light depends on the total amount of pigment, both bleached and unbleached, which is given by the constant c_0 whereas the rate of isomerization depends on the variable c which is the concentration of unbleached pigment. By integrating eqn. (3) we obtain

$$i = i_0 \exp(-\alpha c_0 x) \tag{5}$$

and on inserting (5) in (4) and integrating,

$$\frac{c_{x,\infty}}{c_0} = \exp\left\{-\frac{\alpha\gamma}{A_x}\left(\int_0^\infty i_0 \mathrm{d}t\right)[\exp(-\alpha c_0 x)]\right\}. \tag{6}$$

Replacing $\int_0^\infty i_0 \mathrm{d}t$ by q gives

$$\frac{c_{x,\infty}}{c_0} = \exp\left\{-\frac{\alpha\gamma q}{A_x}\exp(-\alpha c_0 x)\right\} \tag{7}$$

and the average concentration $\hat{c}_\infty$ after the flash is given by

$$\frac{\hat{c}_\infty}{c_0} = \frac{1}{\hat{A}l}\int_0^l A_x \exp\left\{-\frac{\alpha\gamma q}{A_x}\exp(-\alpha c_0 x)\right\}\mathrm{d}x. \tag{8}$$

Before evaluating the integral in eqn. (8) we must make some assumption about the amount of taper in the cone and will consider two simple cases which probably bracket the real situation. In the first case it is assumed that the outer segment tapers in such a way that all molecules have an equal chance of absorbing a quantum. This situation, which will be called critical taper, and which depends on complete internal reflexion causing a funnelling of light from the broad to the narrow end of the cone, requires that

$$A_x = A_0 \exp(-\alpha c_0 x). \tag{9}$$

From Liebman & Granda (1971) $(\alpha c_0)^{-1} \doteqdot 33\ \mu$m which implies that for critical taper the diameter of the cone decreases by 20% in 15 μm. On combining eqns. (8) and (9) and integrating, we obtain

$$\frac{\hat{c}_\infty}{c_0} = \exp\left(-\frac{\alpha\gamma q}{A_0}\right) \tag{10}$$

or
$$\frac{\hat{c}_\infty}{c_0} = \exp(-Q/\bar{Q}), \tag{11}$$

where
$$\bar{Q} = \frac{A_0}{A_c \alpha\gamma} \tag{12}$$

or
$$\bar{Q} = \frac{\hat{A}}{A_c \alpha\gamma}\left\{\frac{\alpha l c_0}{1-\exp(-\alpha l c_0)}\right\}. \tag{13}$$

A different simplifying assumption is to suppose that the outer segment is uniform in diameter, i.e.
$$A_x = A_0 = \hat{A}.$$
In this case integration of eqn. (8) and subsequent rearrangement in terms of Q, $\bar{Q}$ and L gives
$$\frac{\hat{c}_\infty}{c_0} = 1-\frac{Q}{\bar{Q}}+\frac{1}{2!}\left(\frac{Q}{\bar{Q}}\right)^2\frac{L(1-e^{-2L})}{2(1-e^{-L})^2}-\frac{1}{3!}\left(\frac{Q}{\bar{Q}}\right)^3\frac{L^2(1-e^{-3L})}{3(1-e^{-L})^3}, \tag{14}$$

TABLE 5. Theoretical relations between fraction of unbleached pigment at end of flash and quantity of light in flash calculated on three different assumptions

	$\hat{c}_\infty/c_0$		
$Q/\bar{Q}$	(1)	(2)	(3)
0	1·0000	1·0000	1·0000
0·1	0·9048	0·9049	0·9038
0·2	0·8187	0·8191	0·8151
0·5	0·6065	0·6081	0·5908
1·0	0·3679	0·3717	0·3345
1·5	0·2231	0·2283	0·1841
2·0	0·1353	0·1409	0·0996
2·5	0·0821	0·0874	0·0534
3·0	0·0498	0·0544	0·0285
3·5	0·0302	0·0341	0·0151
4·0	0·0183	0·0214	0·0080
5·0	0·0067	0·0085	0·0023

The table shows the mean concentration of unbleached pigment at the end of a flash as a function of the photon density of the flash; $\hat{c}_\infty$ is the average concentration of unbleached pigment at the end of the flash and c_0 is the initial concentration; $\hat{c}_\infty/c_0$ is the fraction bleached. Q is the photon density of the flash in photons μm^{-2} and $\bar{Q}$ is a constant given by eqn. (13). Column (1), an exponential, is calculated on the assumption that bleached pigment has the same absorbance as unbleached pigment and that the cone is critically tapered (eqn. (11)). Column (2) is calculated on the same basis as (1) but with a uniform cone (eqn. (14)). Column (3) is calculated for a uniform cone but with the bleached products transparent (eqn. (20)). Columns (2) and (3) were calculated with $L = 0{\cdot}5$ which corresponds to a cone length of 16·7 μm and a Napierian axial density (αc_0) of 0·03 μm^{-1} (see text).

where $Q = q/A_c$, $\bar{Q}$ is defined by eqn. (13) and $L = \alpha c_0 l$. (Note that eqns. (12) and (13) are equivalent only for the critically tapered cone and that for the other cases considered $\bar{Q}$ is defined by eqn. (13).) For $L < \frac{1}{2}$ eqn. (14)

is within 0·01 of eqn. (11) (see Table 5). If the cone outer segments are 15 μm long, $\alpha l c_0 = 0{\cdot}45$ and the term in brackets in eqn. (13) is 1·2. As a rough approximation we may therefore take $\bar{Q}$ as

$$\bar{Q} \doteqdot \frac{\hat{A}}{A_c \alpha\gamma}. \tag{15}$$

If the light is not axial and there is no funnelling of rays into the outer segment then $\hat{A} = A_c$ and

$$\bar{Q} \doteqdot \frac{1}{\alpha\gamma}. \tag{16}$$

Eqn. (16) should apply as an approximation provided the angle between the light and the axis of the cone is greater than about 10° (see Baylor & Fettiplace, 1975).

The third case considered was that in which the cone is uniform in its diameter and the photoproducts are transparent. The basic equations are the same as (3) and (4) except that the variable c replaces c_0 in eqn. (3). The solution of this pair of equations is

$$\frac{c}{c_0} = \tfrac{1}{2} + \tfrac{1}{2} \tanh\left(\frac{\alpha c_0 x - \ln B}{2}\right), \tag{17}$$

where

$$B = \exp\left(\alpha\gamma \int_0^t I_0 \, dt'\right) - 1$$

with $I_0 = i_0/\hat{A}$. For the case of steady light where I_0 is constant and $\alpha\gamma I_0 t \gg 1$, eqn. (17) reduces to

$$\frac{c}{c_0} = \tfrac{1}{2} + \tfrac{1}{2} \tanh\left(\frac{\alpha c_0 x - \alpha\gamma I_0 t}{2}\right). \tag{18}$$

Thus, in a very long tube, light clears a path for itself at a constant velocity given by $\gamma I_0/c_0$. However, this consideration is not relevant to the present problem for which we need the mean concentration $\hat{c}_\infty$ as a function of q, the total quantity of light in the flash. From eqn. (17), or more simply by integrating the modified eqn. (3) and eqn. (4) with respect to x we find

$$\frac{\hat{c}_\infty}{c_0} = \frac{1}{\alpha l c_0} \ln\left\{1 + (e^{\alpha l c_0} - 1)\, e^{-\alpha\gamma q/\hat{A}}\right\}. \tag{19}$$

On introducing $L = \alpha l c_0$, $Q = q/A_c$ and $\bar{Q}$ from eqn. (13) this becomes

$$\frac{\hat{c}_\infty}{c_0} = \frac{1}{L} \ln\left\{1 + (e^L - 1) \exp\left[-\frac{Q}{\bar{Q}}\left(\frac{L}{1 - e^{-L}}\right)\right]\right\}. \tag{20}$$

As can be seen from Table 5 both eqn. (20), column 3, and eqn. (14), column 2, approximate closely to exponentials, eqn. (11), column 1, if $\alpha l c_0 = \frac{1}{2}$. In all three cases the exponential constant $\bar{Q}$ is given by eqn. (13).

Even if $\alpha l c_0 \gg 1$ and the curves are not exponential eqn. (13) still applies to the experimental results if $\bar{Q}$ is measured as $\bar{Q} = U_{max}/(dU/dQ)_{Q \to 0}$.

The effective collecting area A_i^* (see Baylor & Fettiplace, 1975; Baylor & Hodgkin, 1973) is defined by the equation

$$A_i^* = A_c \gamma [1 - \exp(-\alpha l c_0)]. \tag{21}$$

From this relation and eqn. (13) for $\bar{Q}$ it follows that a quantity $\bar{N}$ calculated as

$$\bar{N} = \bar{Q} A_i^* = \hat{A} l c_0 \tag{22}$$

should be equal to the number of molecules in the cone. In Table 3, A_i^* in red-sensitive cones was estimated by the method described by Baylor & Hodgkin (1973) or Baylor & Fettiplace (1975) as

$$A_i^* = S_F/(25\,\mu\text{V photon}^{-1}), \tag{23}$$

where S_F is the observed flash sensitivity in μV photon^{-1} μm^2. The right hand column, in Table 3 which gives values of $\bar{N}$ calculated on this basis, is included as a test of consistency. It does not provide an independent measurement of the number of molecules in a cone since this information is implicit in the assumptions made by Baylor & Hodgkin (1973) or Baylor & Fettiplace (1975) about cone dimensions and the specific axial density of pigment. However, if the number of molecules in a given cone, or set of cones, were known, measurements of $\bar{Q}$ and the use of eqn. (22) might well be the best method of determining the effective collecting area A_i^* which at present has to be estimated by the rather uncertain methods described by Baylor & Hodgkin (1973) and Baylor & Fettiplace (1975).

Comparison with experiments

From the information provided about A_2-based pigments in Table 1 of Dartnall (1972) the quantum efficiency γ is taken as 0·64 and α as $1{\cdot}7 \times 10^{-8}$ μm^2 per molecule (a factor of 1·5 has been introduced in calculating α to allow for pigment orientation). From the finding of Liebman & Granda (1971) that the specific axial density of pigment in the outer segment of turtle cones is 0·013 μm^{-1}, which implies $\alpha c_0 = 0{\cdot}013 \times 2{\cdot}303 = 0{\cdot}03$ μm^{-1}, we take c_0 as $1{\cdot}8 \times 10^6$ molecules μm^{-3} or 3 mM. (This is higher than the value of 2 mM usually adopted but the value chosen is not critical for calculating $\bar{Q}$.) The length of the outer segment of red-sensitive cones is taken as 15 μm (Baylor & Fettiplace, 1975).

For cones of low sensitivity ($< 25\,\mu$V photon^{-1} μm^2) we assume no funnelling of light and take $A_c \doteqdot \hat{A}$ so that $\bar{Q} \doteqdot \frac{1}{\alpha\gamma} = 9{\cdot}2 \times 10^7$ molecule μm^{-2}, which is close to the average $\bar{Q}$ of $9{\cdot}3 \times 10^7$ molecule μm^{-2} in the seven red-sensitive cones with sensitivities below 25 μV photon^{-1} μm^2

(average value $13{\cdot}4\,\mu$V photon$^{-1}\mu$m^2). A different way of stating this result is that $\bar{Q}$ is approximately equal to the reciprocal of the photosensitivity ($\alpha\gamma$) of the pigment (see Dartnall, 1972). The calculated value of $\bar{N}$ of $5{\cdot}1\times10^7$ is also close to the expected number of molecules in the cone, about $7{\cdot}5\times10^7$ if $c_0 = 3$ mM, $\hat{A} = 3\,\mu$m^2 and $l = 15\,\mu$m. On the other hand for the red cones of higher sensitivity ($S_F > 25$, $\hat{S}_F = 60\,\mu$V photon$^{-1}\mu$m^2) where one might expect some funnelling of light into the cone the mean values $\bar{Q} = 6{\cdot}8\times10^7$ and $\bar{N} = 1{\cdot}6\times10^8$ are about twice the expected value. A possible explanation is that since the xenon flash contains more energy at short wave-lengths than the tungsten light used in estimating S_F, cells of high sensitivity give misleadingly high values of $\bar{Q}$ when the light is axial because the short wave-lengths are filtered off by the oil drop. Alternatively it may be supposed that in this series of experiments variations in $\hat{A}/A_c$ were not the main cause for the variability of S_F and that other factors such as cone size, pigment concentration and transduction efficiency were more important.

The time course of the E.R.P. recorded intracellularly

Theory

From the work of Cone & Pak (1971) and Rüppel & Hagins (1973) it will be assumed that an instantaneous flash of light, which isomerizes N_Φ molecules, generates a component $j_\Phi(t)$ of membrane current in the outer segment given by

$$j_\Phi(t) = -N_\Phi K\delta_0(t) + BN_\Phi K a_3 \mathrm{e}^{-a_3 t} \qquad (24)$$

when $\delta_0(t)$ is a unit impulse at $t = 0$, B, K and a_3 are constants and $j_\Phi(t)$ is an outward current assumed to be in parallel with the total capacity and resistance of the cone.

The first term on the right hand side, an inward current which depolarizes the cell and gives R_1, may be produced by the photochemical events leading to metarhodopsin I (or its equivalent in cones); the second term, an outward current which hyperpolarizes the cell during R_2 and declines with a time constant a_3^{-1} of about 1 msec, may correspond to the conversion of metarhodopsin I into metarhodopsin II.

From eqn. (24) it follows that the total electric charge q_{el} transferred outwards through the outer segment by a single photoisomerization is

$$q_{el} = -K + BK. \qquad (25)$$

Fig. 7 shows in more detail how an equation such as (24) might arise. At zero time a very short intense flash converts N_Φ molecules of unbleached pigment into M_I with negligible delay. M_I then decays exponentially with rate constant a_3 to give M_{II}.

It is assumed that P, M_1 and M_{II}, which are partially embedded in

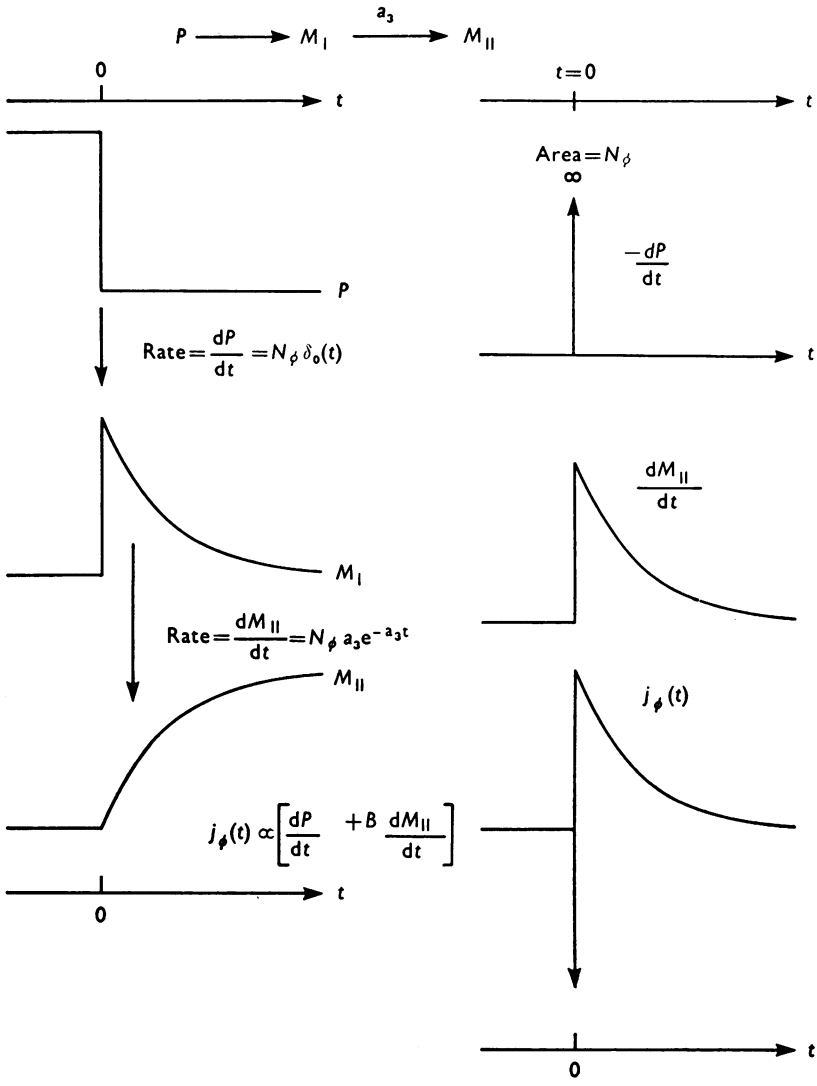

Fig. 7. Diagram illustrating possible origin of current $j_\Phi(t)$ which generates the E.R.P. P is unbleached cone pigment; M_I and M_{II} are the cone equivalents of metarhodopsin I and metarhodopsin II. The diagram illustrates the case where an instantaneous flash isomerizes N_Φ pigment molecules at $t = 0$. Outward current is shown upwards. Note that in the experiments the flash was not instantaneous but decayed exponentially with a time constant of 1·5 msec: allowance for this is made in deriving eqn. (3).

the membrane, have different electric dipoles or charge configurations and that transition from one form to the other involves current flow through the surface membrane of the outer segment. Hence

$$j_\Phi(t) = K\frac{dP}{dt} + BK\frac{dM_{II}}{dt}, \tag{26}$$

where K and BK are proportionality constants which depend on the difference in effective dipole movements between P and M_I in one case (K) and on the difference between M_{II} and M_I in the other (BK).

For a flash which isomerizes N_Φ molecules

$$\frac{dP}{dt} = -N_\Phi \delta_0(t) \tag{27}$$

and

$$\frac{dM_{II}}{dt} = N_\Phi a_3 e^{-a_3 t}. \tag{28}$$

Equation (24) is obtained when (27) and (28) are substituted in (26).

For simplicity it will be assumed that there are negligible potential differences between inner and outer segments (both inside and outside the cell) and that the cone can be represented by a parallel combination of a capacity c_m and resistance r_m. On this basis the differential equation relating the internal potential V to the component of current $j_\Phi(t)$ is

$$c_m\frac{dV}{dt} + \frac{V}{r_m} + j_\Phi(t) = 0. \tag{29}$$

This expresses the requirements of Kirchoff's Law that no net current enters or leaves the cell. Before solving it we must allow for the fact that the pulse of light was not a unit impulse $\delta_0(t)$ but an exponentially decaying pulse of rate constant a_1. Using the Laplace transform method and writing a_2 for $\frac{1}{r_m c_m}$ the equation for $V(s)$ becomes

$$V(s) = \frac{N_\Phi K}{c_m}\left(1 - \frac{a_3}{s+a_3}B\right)\left(\frac{a_1}{s+a_1}\right)\left(\frac{1}{s+a_2}\right) \tag{30}$$

of which the inverse transform is

$$\frac{V}{N_\Phi K/c_m} = \frac{a_1}{a_1-a_2}[e^{-a_2 t} - e^{-a_1 t}] + \frac{Ba_1a_3[(a_2-a_3)e^{-a_1 t} + (a_3-a_1)e^{-a_2 t} + (a_1-a_2)e^{-a_3 t}]}{(a_1-a_2)(a_2-a_3)(a_3-a_1)}. \tag{31}$$

Comparison with experiments

In fitting this expression to the experimental records, a_1^{-1} was taken at its measured value of 1·5 msec and the best value of the other four parameters B, a_2, a_3 and $N_\Phi K/c_m$ were found by a least squares fitting

programme, MLAB, at the National Institutes of Health (see Knott & Reece, 1972). Examples of the experimental points and fitted curves resulting from this procedure are shown in Fig. 8, and Table 6 gives the parameters obtained in the eight experiments analysed. No allowance was made for the finite rise time of the flash or for delay in the micro-electrode and input stage which, with full compensation possibly amounted to

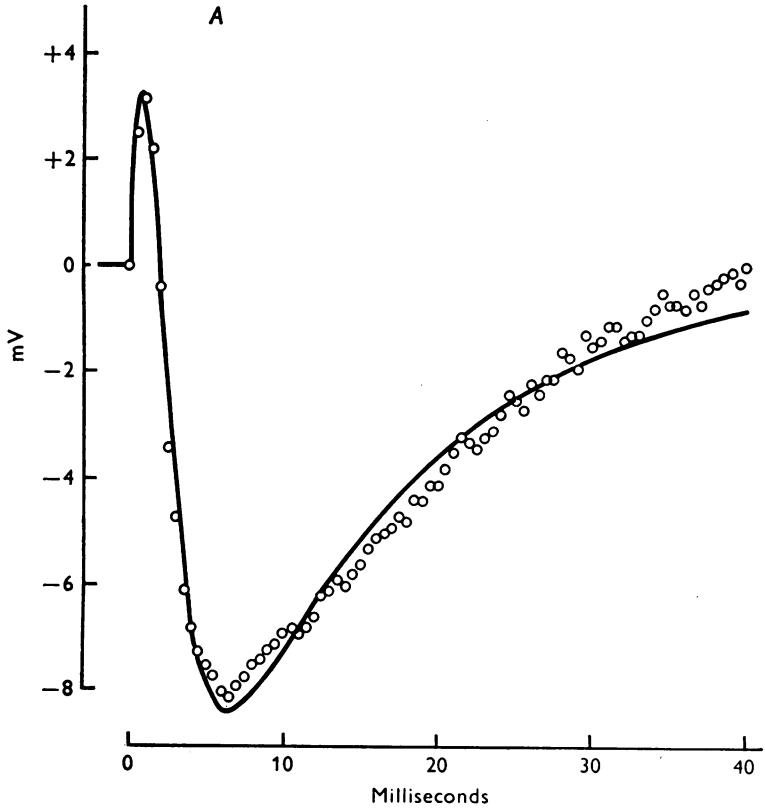

Fig. 8. Reconstruction of the E.R.P. in a red- (*A*) and green-sensitive cone (B). The continuous curves are the fits of eqn. (3) to the experimental values (open circles); see text. The xenon flash was equivalent to $2{\cdot}74 \times 10^8$ and $2{\cdot}12 \times 10^8$ photons μm^{-2} at λ_{max} for red- and green-sensitive cones respectively. Table 6 gives the values of constants used in plotting the theoretical curves. Cones: Red 20, and Green 6. Temperature: Red 20, 20·8° C; Green 6, 21·0° C.

0·5 msec, and must make the theory unreliable during the first half millisecond. The relatively small size and short duration of R_1 in green-sensitive cones is 'explained' by the relatively large values of the constant B calculated for these cells.

Both the goodness of fit (Fig. 8) and the values of the constants (Table 6) seem reasonable in view of the simplified nature of the theory and the number of constants to be determined. The cell time constant τ_2 of 10–15 msec derived from the analysis is larger than the values obtained by Baylor *et al.* (1974*a*) from passing rectangular pulses of current into red-sensitive cones. However, the likelihood that most of these cells were

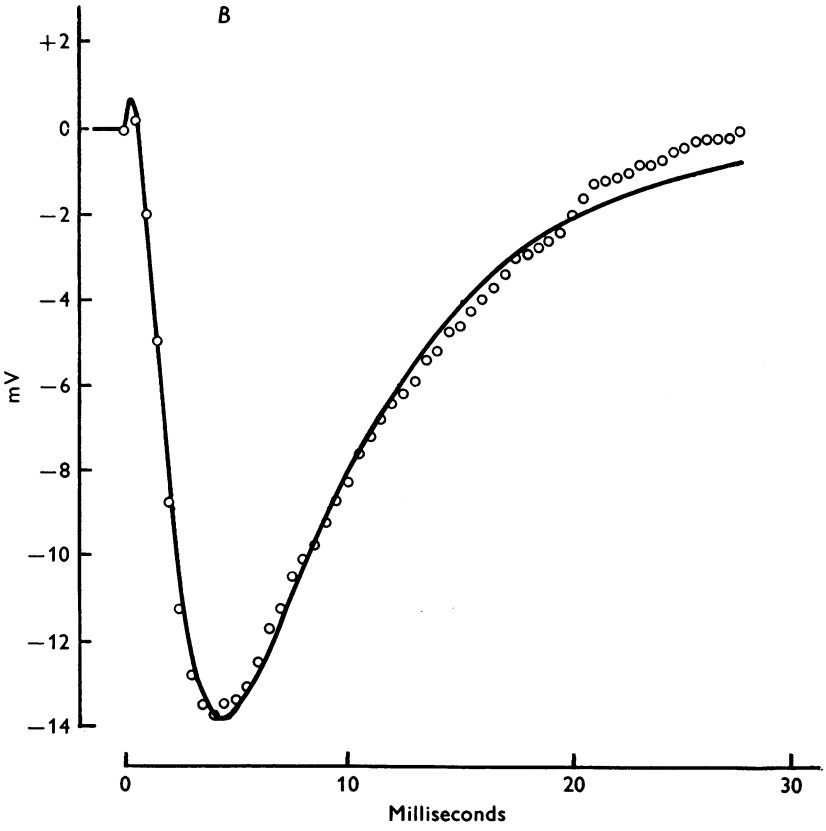

Fig. 8*B*. For legend see facing page.

electrically coupled makes it difficult to interpret these time constants, which varied between 5 and 8 msec in the light. A better comparison may be with the value of 8–10 msec obtained from the time constant with which the potential approached its maximum in strong light (Baylor *et al.* 1974*a*, pp. 709 and 712).

It is difficult to make any close comparison between the value of a_3 and that expected for the transition from metarhodopsin I to metarhodopsin II. There seems to be no direct information about the

rate of this reaction in cones and with rhodopsin the method of extracting the pigment has a substantial effect on the measured rate constant (Williams & Breil, 1968). It seems to us that the most relevant results are those of Baumann (1976) on isolated frog retinae who determined the time constant of the metarhodopsin I–II reaction as $0{\cdot}97 \pm 0{\cdot}16$ msec at 21° C; this should be compared with our values of about 1·2 msec at 20° C (range 0·5–2·0 msec). In this paper Baumann also estimated the time constant of decay of lumirhodopsin as 40–50 μsec which is sufficiently short to justify our assumption that an instantaneous light pulse would generate the cone equivalent of metarhodopsin I with negligible delay.

TABLE 6. Values of constants and derived quantities obtained by fitting eqn. (31) to observed E.R.P.s

1 Cone	2 $N_\Phi K/c_m$ (mV)	3 B	4 τ_2 $(= a_2^{-1})$ (msec)	5 τ_3 $(= a_3^{-1})$ (msec)	6 N_Φ (10^6 Φ)	7 K/c_m (pV/Φ)	8 BK/c_m (pV/Φ)
Red 1	12·61	2·198	15·17	1·44	77·4	163	358
Red 16	11·47	1·748	18·08	1·46	194·2	59	103
Red 19	10·59	1·813	13·45	1·35	72·9	145	263
Red 20	16·51	1·669	13·85	1·12	45·5	363	606
Red 21	20·97	1·420	14·42	0·53	28·2	744	1056
Mean	14·43	1·77	15·0	1·18	83·6	295	477
Green 2	11·49	2·848	5·81	0·73	44·0	261	741
Green 6	4·29	5·518	13·66	2·04	31·8	135	744
Green 7	9·26	3·330	7·29	0·82	—	—	—
Mean	8·35	3·90	8·92	1·20			

The four quantities derived by fitting eqn. (31) to the records are given in columns 2, 3, 4 and 5. $N_\Phi K/c_m$ is a scaling factor; B gives the ratio of the charge transfer during R_2 to that in R_1; τ_2 $(= a_2^{-1})$ is the cell time constant, $r_m c_m$; τ_3 $(= a_3^{-1})$ is the time constant of the reaction M_I to M_{II} in the model; a_1 was taken as its measured value of 0·667 msec^{-1}.

For red-sensitive cones N_Φ the number of photoisomerizations was calculated by the method described in the text from $Q = 2{\cdot}74 \times 10^8$ photons μm^{-2} and the values of $\bar{Q}$ and $\bar{N}$ in Table 3. For green-sensitive cones $\bar{N}$ was taken as 5×10^7 molecules per cone, i.e. about 60 % of the value for red-sensitive cones which is consistent with the histological observations of Baylor & Fettiplace (1975); Q was $2{\cdot}12 \times 10^8$ photons μm^{-2} and $\bar{Q}$ was taken from Table 2. Columns 7 and 8 give the scale factors per photoisomerization for R_1 and R_2 events in picovolts per photoisomerization. A record length of 40 msec was used in all experiments except Green 2 where it was 15 msec. Temperature 20–21·5° C.

The value of the scaling factor $N_\Phi K/c_m$ in this analysis is of interest since it enables a calculation to be made of the quantity of charge transferred by a single photoisomerization. Since the E.R.P. approached its

limiting value in an exponential manner it seems reasonable to take N_Φ, the number of photoisomerizations, produced by the flash as

$$N_\Phi = \bar{N}[1-\exp(-Q/\bar{Q})], \tag{32}$$

where $\bar{N}$ is the total number of pigment molecules which was estimated as $4{\cdot}62\times10^7$ in the cone of Fig. 8*A* (Red 20, Table 3), Q is the equivalent photon density of the flash at λ_{max} which was $2{\cdot}74\times10^8$ photons μm^{-2} and $\bar{Q}$ is the exponential constant which was found to be $6{\cdot}49\times10^7$ photons μm^{-2} (Table 3). From these figures and the result $N_\Phi K/c_m = 16{\cdot}51$ mV we obtain $K/c_m = 3{\cdot}63\times10^{-10}$ V per photoisomerization. The mean value of K/c_m in the five red-sensitive cones analysed was $2{\cdot}95\times10^{-10}$ V per photo-isomerization. Baylor *et al.* (1974*a*) consider that the capacity, c_m, of a single red-sensitive cone may be about 40 pF. Hence,

$$K = 1{\cdot}18\times10^{-20}\text{ C per photoisomerization}$$

$$\simeq 0{\cdot}07\text{ electronic charge per photoisomerization.}$$

This is the charge transfer during R_1; the transfer during R_2 is about 80% larger. Another way of describing these results is to say that the electrical events after a single isomerization correspond to the very rapid inward transfer of one positive electronic charge through about 7% of the membrane during R_1 and a subsequent, slower outward transfer of the same charge through 12% of the membrane during R_2. This estimate is of the same order as those made by Rüppel & Hagins (1973) and Cone (1969).

DISCUSSION

The observations in this paper reinforce the conclusions of previous authors that the early and late receptor potentials have completely different properties (Brown & Murakami, 1964; Murakami & Pak, 1970; Cone, 1969). As an example of this contrast the first point which might be mentioned is the difference between the sizes of the unitary events underlying the two kinds of response; in an isolated cone these are of the order of 10^{-10} V per photoisomerization for the E.R.P. and $2\text{–}5\times10^{-5}$ V per photoisomerization for the L.R.P. The implication is that the physical changes underlying the E.R.P. can be a relatively simple molecular rearrangement in the immediate vicinity of the photopigment molecule, whereas for the L.R.P. one needs a more elaborate mechanism in which one photoisomerization can affect the movement of some 10^5 ions through the surface membrane at an appreciable distance from the place where the photon is absorbed. For the L.R.P., but not for the E.R.P., it seems necessary to postulate an internal transmitter to explain how absorption

of a photon by any one of about 10^8 molecules can block roughly 1/1000 of the ionic channels in the outer segment.

As the number of photoisomerizations produced by the flash increases, the individual electrical events underlying the E.R.P. appear to add in a strictly linear way. The shape of the response does not alter with increasing flash intensity and the exponential approach to saturation is consistent with the amplitude of the E.R.P. being always proportional to the number of photoisomerizations. The half-saturation levels are completely different; the E.R.P. is half maximal at about 7×10^7 photoisomerizations per cone, when roughly half the molecules have been altered photochemically, whereas the L.R.P. is half saturated at about 1000 photoisomerizations per cone (assuming $S_\Phi = 25\,\mu V$ per photoisomerization and $V_{max} = 25$ mV). As is well known the L.R.P. changes in shape and size as the flash is increased, or if it is superimposed on a background, in a manner which suggests that the effect of photons interact in a complicated way unless the light intensity is very low. All this may be summarized by saying that the properties of the E.R.P. are consistent with a relatively simple physical system which has little functional significance and shows none of the complexities that appear when an important biological mechanism is subjected to a long period of natural selection. On the other hand the mechanism underlying the L.R.P. has several features, such as its high sensitivity and ability to operate over a wide range of light intensities, which show that it has been carefully adapted to form an efficient part of the transduction mechanism.

Although it is unlikely that the E.R.P. can have an important function under natural conditions, it may be wrong to conclude that it is without psychophysical effect in man. The E.R.P. recorded internally after a strong flash, which may be nearly 20 mV in amplitude and which lasts for 20–30 msec, is a conspicuous signal which may occur when the mechanism underlying the L.R.P. is saturated and incapable of an immediate response. We have observed that an E.R.P.-like signal can be recorded from horizontal cells and it would not be surprising to find conditions in which the E.R.P. might give rise to impulses in optic nerve fibres. However, it must be admitted that the main reason for investigating the E.R.P. rests not in its functional significance but on the information it may provide about the transduction mechanism. If the maximum amplitude of the E.R.P. is proportional to the number of unbleached molecules, as seems possible from our preliminary results, then investigation of the way in which the E.R.P. recovers after various types of bleach may provide useful information about light adaptation and the resynthesis of visual pigments under different conditions. Another possible application of E.R.P. measurement might be in determining the effective collecting

area of a cone, since on theoretical grounds this should be approximately equal to the number of molecules in the cone divided by the constant $\bar{Q}$ which can be determined from the strength-amplitude relation of the E.R.P. However, before placing weight on such methods we need to know more about photoreversal effects in cones and about the fraction of photopigment bleached by intense flashes of various durations.

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