*J. Physiol.* (1984), **346**, *pp.* 557–578
*With* 13 *text-figures*
*Printed in Great Britain*



# EFFECTS OF TEMPERATURE CHANGES ON TOAD ROD PHOTOCURRENTS

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(*Received 12 April 1983*)

SUMMARY

1. Rod current responses were measured over the range 5–30 °C.

2. Following a rapid decrease in temperature the amplitude of the dark current decreased without detectable delay (less than 3 s). Over a period of several minutes the amplitude of the dark current sometimes relaxed slightly towards its previous value. The rapid change cannot be accounted for simply by altered activity of the sodium pump and instead indicates that the conductance of the outer segment in darkness changes with temperature.

3. Over the range 10–30 °C the amplitude of the dark current increased approximately linearly with temperature, and the straight line of best fit extrapolated to zero current at about 5 °C. The few points available below 10 °C indicated that the relationship flattened out, but this could not be investigated properly.

4. The kinetics of responses to dim flashes accelerated with a $Q_{10}$ of about 2·2, and were well described by an Arrhenius plot with an activation energy of 13·8 kcal $mol^{-1}$ (HEPES Ringer solution).

5. The time course of recovery of dark current following a saturating flash showed a similar temperature dependence to that of the dim flash kinetics.

6. A simple explanation of the previous two findings is that the delays determining the time course of responses to both dim and bright flashes are largely determined by the fluidity of the disk membrane.

7. The sensitivity to dim flashes had a broad peak at about 22 °C, decreasing at both lower and higher temperatures.

8. The relative sensitivity to long wave-length light increased slightly with temperature. The sensitivity at 700 nm relative to that at 500 nm increased by 0·225 $\log_{10}$ units (1·68 times) upon a temperature increase from 11·5 to 29·3 °C (from approximately $-5{\cdot}0$ $\log_{10}$ units to approximately $-4{\cdot}8$ $\log_{10}$ units). This change appears to be approximately what would be expected theoretically.

## INTRODUCTION

It is well established that the responses of vertebrate photoreceptors are accelerated at higher temperatures (see, for example, Penn & Hagins, 1972; Baylor, Hodgkin & Lamb, 1974; Baylor, Matthews & Yau, 1980, 1983) and $Q_{10}$s ranging from 1·8–2·7 have been reported. Penn & Hagins (1972) showed that the magnitude of the rod

photocurrent is also quite strongly temperature dependent, and this has recently been investigated by Baylor *et al.* (1983).

The latter phenomenon might result from a direct effect of temperature on the dark conductance of the outer segment, or alternatively from an effect on the rate of operation of the sodium–potassium pump, or perhaps from both. In order to help distinguish between these possibilities a rapid temperature change system was developed which permitted temperature decrements of 10 °C within 30 s. These experiments show that the changes in dark current follow the applied temperature changes with negligible delay, and support the idea of a direct temperature dependence of the conductance. A preliminary report of these results has been published (Lamb, 1982*b*).

In addition the opportunity was taken to examine the effects of steady temperature on the kinetics, dark current and photocurrent. The results are in close agreement with those of Baylor *et al.* (1983) in relation to the kinetics, but significant differences were found in relation to dark current and sensitivity.

Temperature is expected to affect the relative sensitivity to very long wave-length light (where the photon energy $hc/\lambda$ is low) because the activation barrier to chromophore isomerization becomes smaller as $kT$ increases ($h$ = Planck's constant, $c$ = speed of light, $\lambda$ = wave-length, $k$ = Boltzmann's constant, $T$ = absolute temperature). Such an effect has been observed psychophysically in the cone system of man (de Vries, 1948; G. S. Brindley & P. R. Lewis, unpublished, cited in Lewis, 1955), behaviourally in frog (Denton & Pirenne, 1954) and electrophysiologically in *Limulus* lateral eye (Srebro, 1966) but the existence or magnitude of the phenomenon has apparently not previously been tested in recordings from vertebrate photoreceptors. I report here experiments which demonstrate the effect, and which show its magnitude to be roughly that predicted by the model of Stiles (1948).

## METHODS

The methods were similar to those of Baylor, Lamb & Yau (1979*a*, *b*) and Lamb, McNaughton & Yau (1981). The outer segment of a rod in the retina of *Bufo marinus* was drawn into a glass pipette so that its photocurrent could be measured.

Temperature control was provided by two mechanisms (Lamb, 1982*a*). A Peltier device attached to the microscope substage was used for controlling the steady temperature, and for slow temperature changes (less than 3 °C $min^{-1}$). Thermal contact between the substage and the glass chamber holding the retinal pieces was improved by using a thin coat of conducting grease (Heat-Sink Compound, R.S. Components). Rapid temperature decrements were accomplished by perfusing the chamber with Ringer solution cooled in an ice-water bath. The pumping rate was controllable from 0·5–5 ml $min^{-1}$ and permitted a maximum rate of temperature decrease of up to 50 °C $min^{-1}$. The calibrated thermistor used to monitor the temperature of the Ringer solution in the chamber was Teflon coated (YSI, 44108) in order to provide total electrical insulation from the Ringer solution. Cells were recorded only from pieces of retina which were close to the thermistor bead, within about 1–2 mm of the surface of the Teflon.

Experiments were performed using either bicarbonate-buffered Ringer solution (Cervetto, Pasino & Torre, 1977; Lamb *et al.* 1981) or HEPES-buffered Ringer solution (Baylor *et al.* 1979*a*). The effects of temperature on the recorded photocurrents were similar in the two solutions. The compositions in mM were:

| | NaCl | KCl | $CaCl_2$ | $MgCl_2$ | $NaHCO_3$ | HEPES | EDTA | Glucose |
|---|---|---|---|---|---|---|---|---|
| HEPES | 111 | 2·5 | 1·0 | 1·6 | — | 3 | 0·1 | 10 |
| Bicarbonate | 110 | 2·6 | 2·0 | 2·0 | 22 | — | 0·1 | 10 |

HEPES Ringer solution was bubbled with 100 % $O_2$ and had a pH at room temperature of 7·7–7·8; bicarbonate Ringer solution was bubbled with 5 % $CO_2$/95 % $O_2$ and had a pH at room temperature of 7·4.

The temperature dependence of the $pK_a$ of HEPES is given by Vega & Bates (1976) and Good, Winget, Winter, Connolly, Izawa & Singh (1966) as $\Delta pK_a/°C \simeq -0{\cdot}0125$. For a bicarbonate/$CO_2$ buffer the temperature dependence of pH depends on whether $CO_2$ is maintained at constant partial pressure or constant dissolved concentration. In these experiments the bicarbonate Ringer solution was saturated with 5 % $CO_2$ at room temperature. The solution was then warmed as it entered the test chamber, or cooled in a heat-exchanger just prior to this, so that the total $[CO_2]$ was fixed. Harned & Bonner (1945) give data for the ionization constant $K_a$ of $CO_2$/bicarbonate in 0·1 M-NaCl defined by

$$K_a = \frac{[H^+][HCO_3^-]}{[CO_2]},$$

where $[CO_2]$ is molarity rather than partial pressure. Expressed this way they obtained $pK_a = 6{\cdot}234$ (10 °C), 6·148 (20 °C), 6·089 (30 °C), indicating an approximate $\Delta pK_a/°C$ of −0·007. Hence, in rapidly dropping the temperature from 30 to 10 °C the pH would be expected to increase by about 0·25 with HEPES buffering, and by about 0·15 with bicarbonate buffering.

*Calibration of temperature*

It was found that the thermistor reading was slightly influenced by ambient temperature. As only about 1 cm of its length was immersed in the Ringer solution, and as it was Teflon insulated, conduction along the thin wire leads meant that the temperature of the bead was not exactly equal to that of the solution. Furthermore, during the fast perfusion used in the rapid cooling experiments temperature gradients would be expected to occur in the chamber. Because of these two factors an accurate calibration was needed of the temperature at the position of the recorded photoreceptors.

To accomplish this the electrical conductivity of the solution was monitored. The temperature dependence of the conductivity of dilute NaCl solution has been measured very accurately. Over the range 5–35 °C the conductivity is nearly linearly related to temperature, with a $Q_{10}$ (15–25 °C) of 1·250 (Benson & Gordon, 1945; Robinson & Stokes, 1959). The strength of this temperature effect is essentially independent of concentration (Hewitt, 1960).

A pipette with an orifice somewhat smaller than normal was used, so that a high proportion of the total electrode resistance would arise near the tip; the chamber and pipette were filled with 0·1 M-NaCl. Fig. 1 shows the conductance measured with such a pipette under conditions as close as possible to those in the rapid temperature change experiments of Fig. 3.

The heavy trace represents the temperature monitored by the thermistor, after correction as described below. The lighter noisy trace is the conductance measured with the pipette; the polarizing voltage of 0·8 mV was interrupted for 1 s at intervals of 12 s in order to check the zero level (not shown in this expanded Figure). The conductance and thermistor traces are in close agreement, except for an overshoot of approximately 1 °C during the cooling 'step'. The steep phases of the traces coincide partly because of two compensating delays; the insulated thermistor had a thermal time constant of about 3 s, but the pipette tip was 1–2 mm down-stream from the thermistor (as were the recorded cells) so the advancing front of cooled perfusate was delayed by a few seconds. With similar measurements directly under the thermistor the conductance change preceded the thermistor trace by about 3 s.

The ratio of the conductance measured at the times marked by the arrows was 1·29. After allowing for the resistance of the electrolyte path not exposed to the temperature change (measured as 0·25 MΩ), the ratio of conductances of the pipette tip at the two temperatures was 1·30. On the assumption that the thermistor monitored a constant fraction of the temperature displacement from ambient (21·0 °C in this case) the monitored temperatures of 25·0 and 14·8 °C were calculated to correspond to 25·8 and 13·6 °C respectively at the position of the pipette tip. This indicates that the thermistor registered only about 85 % of the displacement of the Ringer solution temperature from ambient. The temperature scale in Fig. 1 has been corrected according to these calculations.

The close agreement in form between the traces in Fig. 1 indicates that the thermistor readings provided an accurate monitor of the time course of the applied temperature changes. The corrected values are considered to have provided an accurate absolute measure of temperature at the location of the recorded cells; the corrected values have been used throughout this paper.

RESULTS

The general nature of the effect of temperature is shown by the families at 6·7 and 30·5 °C in Fig. 2. When plotted on the same horizontal and vertical scales the differences are dramatic. At 6·7 °C the saturating response amplitude was 7 pA and the time to peak of the dim flash response was greater than 2 s (bicarbonate Ringer solution). On raising the temperature to 30·5 °C the saturating amplitude

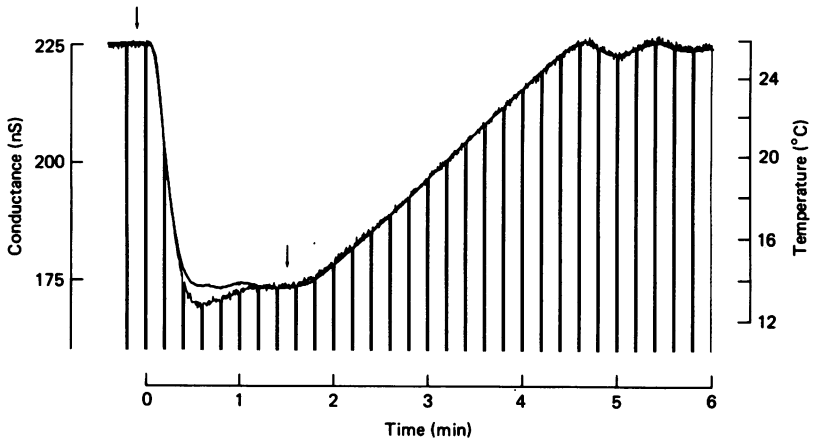


Fig. 1. Comparison of pipette conductance and thermistor reading during a rapid temperature decrement and subsequent re-warming. Heavy trace is temperature (ordinate at right) monitored by thermistor, corrected as described in the text. Lighter noisy trace is the conductance (ordinate at left) measured with a pipette under conditions as close as possible to those in the experiments of Fig. 3; band width d.c. to 25 Hz. The pipette had a small diameter lumen (*ca.* 3·5 $\mu$m) and was filled with 0·1 M-NaCl. The polarizing voltage of 0·8 mV was interrupted for 1 s at intervals of 12 s in order to check the zero level (off scale in this magnified plot). Ordinate is time after switching to cooled perfusate. After approximately 25 s the pump speed was reduced to provide a roughly constant temperature. After approximately 100 s the Peltier heating was turned back on and slow perfusion with solution at room temperature resumed.

increased more than fivefold to 39 pA while the linear response time to peak decreased by a factor of about four to around 0·5 s. The response *versus* intensity relations are plotted in Fig. 2*C*, and are discussed in detail later.

In the following sections an examination is made of the speed with which the dark current changes following a change in temperature, and of the dependence of dark current, response kinetics and sensitivity on temperature.

It was difficult to reduce the temperature much below 7 °C with the apparatus described, although 5 °C was eventually reached in a few experiments. In any case condensation on the chamber at lower temperatures would have interfered with the optical stimulus. Most of the results described were obtained in the range 8–30 °C.

### *Rapid temperature changes*

Fig. 3 illustrates the results of rapid temperature changes in four cells. In each part the upper trace with ordinate at the right is temperature, and the lower trace is

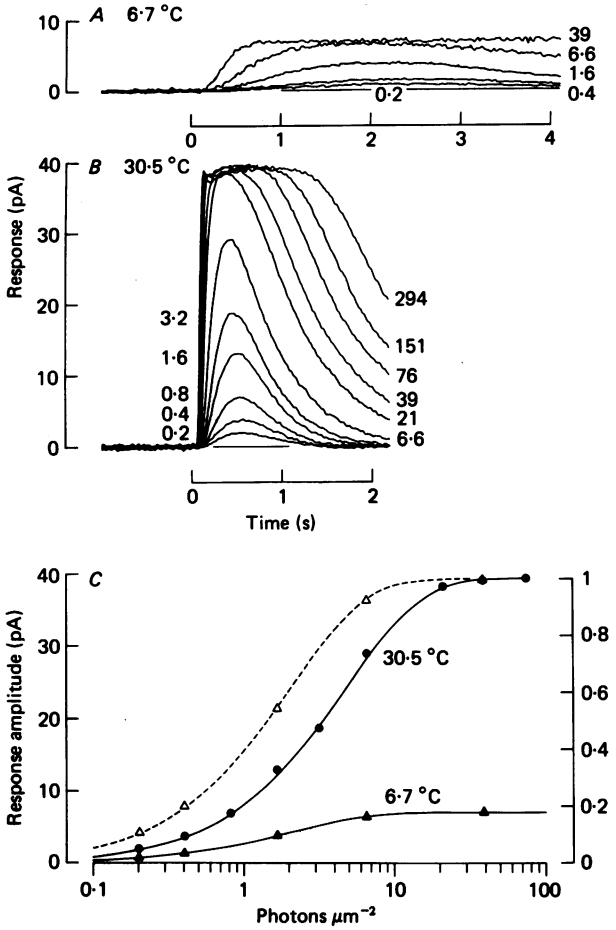


Fig. 2. Response families at two temperatures in one cell. *A*, 6·7 °C; *B*, 30·5 °C. Numbers alongside the traces give the intensities in photons $\mu m^{-2}$ of the flashes presented at time zero. For the lower intensities (up to 1·6 photons $\mu m^{-2}$) at least ten responses were averaged; for higher intensities at least two responses were averaged. *C*, response *versus* intensity relations. Filled symbols are response amplitudes in pA (ordinate at left). Open triangles (△) plot the normalized values at 6·7 °C on a common scale with the 30·5 °C points (ordinate at right). Continuous curve near the filled circles (●) drawn by eye; it is intermediate between a Michaelis curve and the form $1-e^{-x}$. Interrupted trace is the same curve shifted to the left 0·36 log units (2·3 times); it is redrawn as the lower continuous curve. Bicarbonate Ringer solution.

inward membrane current measured during presentation of saturation flashes at regular intervals (30 s in *A*, 60 s in *B–D*).

For ease of comparison with the temperature records the current traces have been inverted with respect to the normal convention, so that inward membrane current into the outer segment is plotted upwards. In each cell it is clear that the membrane current followed the applied temperature change nearly synchronously. It was not possible to resolve a delay between the sets of traces; it is expected that a difference

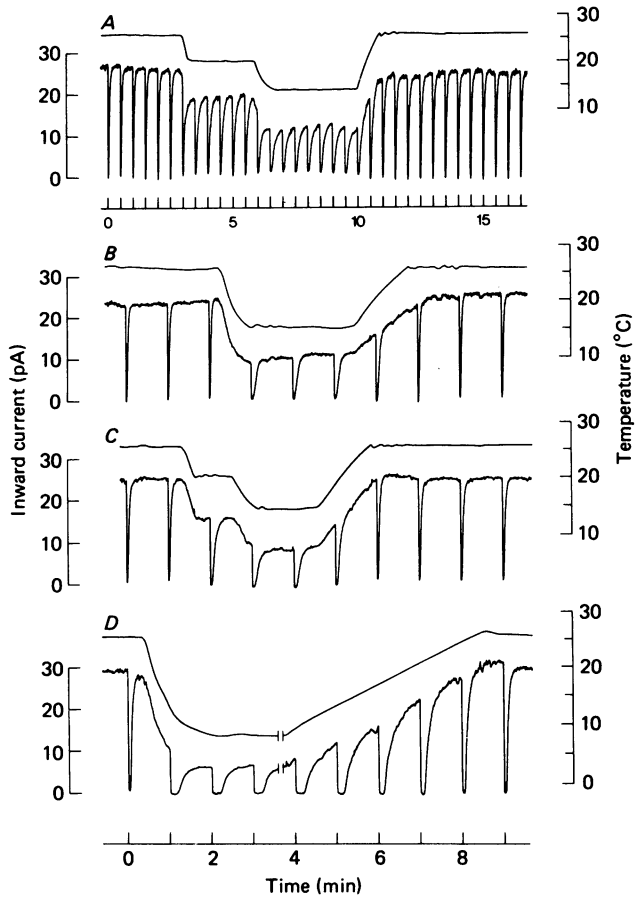


Fig. 3. Rapid temperature changes in four cells. In each case upper trace is temperature (ordinate at right) and lower trace is inward current (ordinate at left). Light monitor and time scale is shown below *A*, for which flashes were presented at 30 s intervals. For *B–D* flashes were at 60 s intervals, and are monitored by the bottom trace. Approximate steady temperatures were: *A*, 25·7, 19·8, 13·9, 25·7 °C; *B*, 25·8, 14·7, 25·7 °C; *C*, 25·6, 19·9, 13·8, 25·6 °C; *D*, 25·7, 7·7, 25·8 °C. *A* and *B* in bicarbonate Ringer solution, *C* and *D* in HEPES Ringer solution. Gap in *D* was 6 min. In *A–D* flashes delivered about 10, 43, 29 and 26 photons $\mu m^{-2}$ respectively, estimated to cause about 270, 1400, 680 and 880 isomerizations respectively.

of about 3 s would have been detectable. Similar experiments were performed on twenty-five cells, and in no case was a significant delay observed.

In each part of Fig. 3 (and most prominently in Fig. 3*B*) there is a suggestion that following the rapid temperature decrease the dark current slowly recovered slightly towards its previous level. Part of this effect may have resulted from the small overshoot in temperature shown in the calibration experiment of Fig. 1. In addition there may have been very slight movement of the cell within the pipette as a result of thermal volume changes, and it is also possible that the sealing resistance slowly changed perhaps because of cellular swelling. A slight sag was seen in almost all of

the cells recorded, and despite the uncertainties mentioned above it seems likely that at least part of this phenomenon represented a relaxation in dark current following a step change in temperature.

In Fig. 3 the zero level of current, at the peak of the responses to bright flashes of light, is nearly independent of temperature. In early experiments, however, this

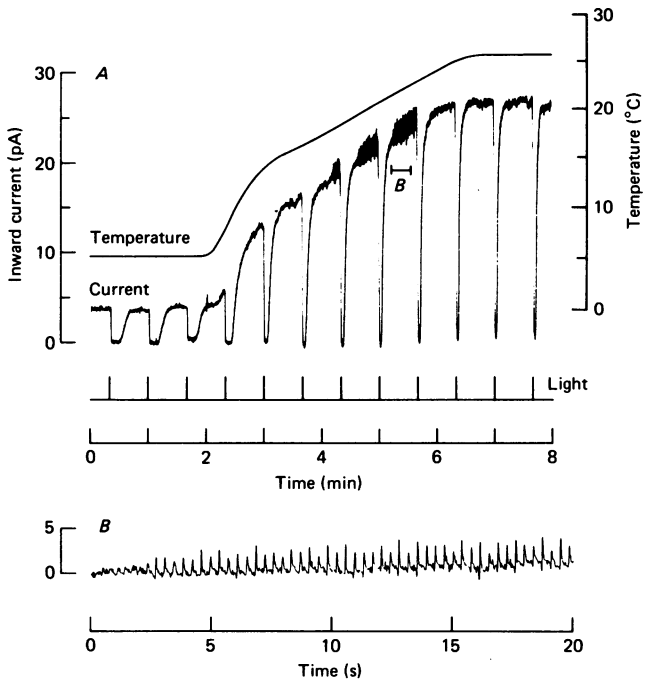


Fig. 4. Development of small amplitude oscillations during re-warming from 5 °C in bicarbonate Ringer solution. Ordinate at left is inward current; at right is temperature. Light suppressible oscillations appeared as the temperature reached about 18 °C and disappeared above 23 °C. The marked section in *A* is shown on an expanded time base in *B*. Flashes delivered 48 photons $\mu m^{-2}$ estimated to cause about 100 isomerizations per flash.

expected result did not occur, because of the strong temperature dependence of the Ringer solution's electrical conductivity. Prior to a temperature change experiment it was necessary to adjust the polarizing voltage (DC offset), so that the peaks of the responses corresponded to zero absolute current, in order to obtain flat base lines as in Fig. 3. This simply ensured that there was no steady polarizing voltage across the pipette which could cause a current artifact during the temperature-induced change in Ringer solution resistance.

*Oscillations*

An unusual feature was noticed during the re-warming of cells in bicarbonate Ringer solution which had been cooled to about 5 °C. As the temperature of the cell in Fig. 4 reached about 18 °C small amplitude oscillations appeared. These were temporarily suppressed by saturating flashes of light, and were not observed

beyond about 23 °C. On the expanded time base of Fig. 4*B*, the frequency of these events is seen to be about 2·6 Hz. Similar oscillations were prominent in three out of five cells in bicarbonate Ringer solution cooled below 8 °C, but they were not obvious in any other cases (e.g. not in Fig. 3*D* in HEPES Ringer solution). The origin of these oscillations is unknown, although similar but smaller events are occasionally noticed; see for example Baylor *et al.* (1979*b*) Fig. 2. It is possible that they arise in the inner segment.

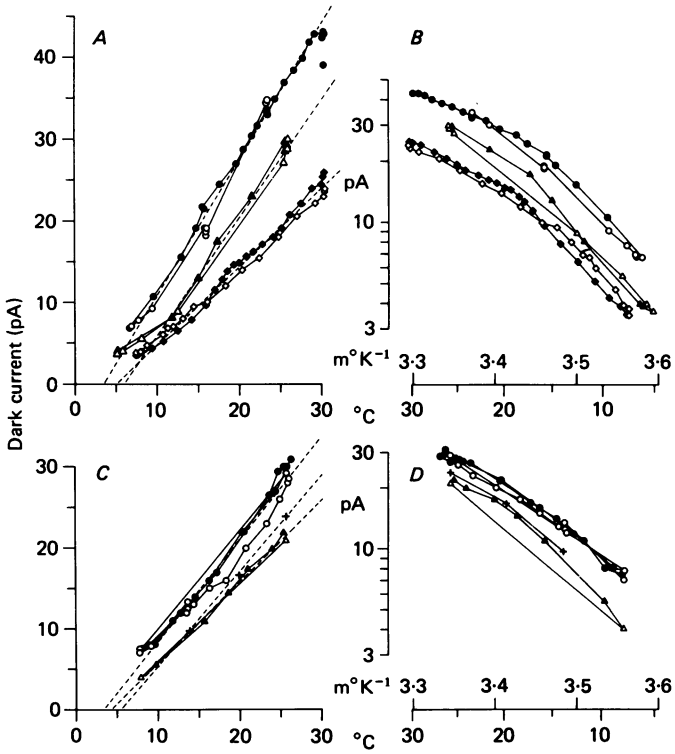


Fig. 5. Dark current as a function of temperature. Left, linear plots; right, Arrhenius plots. Saturating responses were measured at 60 s intervals, and the amplitudes are plotted against temperature; open symbols represent decreasing temperature, filled symbols increasing temperature. *A* and *B*, three cells in bicarbonate-buffered Ringer solution. *C* and *D*, eight cells in HEPES Ringer solution; circles are for cell of Fig. 3*D* followed over two cycles of change between 26 and 8 °C. Crosses (+) represent average values from six cells in HEPES Ringer solution stepped between just three temperatures (approx. 14, 20 and 26 °C).

### *Dark current*

The relationship between dark current and temperature is shown in Fig. 5 in both linear and Arrhenius coordinates for a number of cells in Ringer solution buffered with bicarbonate (above) and HEPES (below). The points were obtained from the amplitudes of the responses to bright flashes of light delivered at 60 s intervals during temperature changes, so the spacing between points gives a measure of the rate of temperature change. (The crosses (+) in *C* and *D* are an exception and represent

average values obtained from six cells which were stepped between just three temperatures.)

Over much of the range the experimental points are well-described by a linear relation between dark current and temperature. The interrupted straight lines in *A* and *C* have been fitted by eye to the data above 10 °C and provide a reasonable fit. These lines extrapolate to zero current at temperatures of between 4 and 6 °C in different cells, although at the lowest temperatures the experimental points tend to lie above these lines. These data are consistent with Fig. 2*A* of Baylor *et al.* (1983) which showed an approximately linear relationship extrapolating to zero at roughly 4–5 °C.

In the Arrhenius coordinates of Fig. 5*B* and *D* there is a marked curvature for most cells, with the slope at 10 °C being more than double that at 30 °C. Over a smaller temperature range (15–25 °C) Baylor *et al.* (1983) reported that a straight line provided a good fit in both coordinate systems, but the present results suggest that over a wider temperature range (5–30 °C) the behaviour is non-Arrhenius.

The cell with the greatest observed deviation from a linear current–temperature relation is shown by the circles in *C* and *D* (○, ●, HEPES). In the Arrhenius plot a straight line provides an approximate fit, although close examination shows the slope at 10 °C to be about 50 % greater than that at 25 °C.

A degree of hysteresis is apparent in the results plotted in Fig. 5. This is presumably related to the previously mentioned tendency for a slight relaxation to occur following the rapid phase of the response to a step change in temperature. Whether the hysteresis is a genuine phenomenon, perhaps indicating a slight influence of the rate of temperature change, or whether it is an artifact resulting from the measurement problems discussed on p. 562 is not clear.

### *Kinetics*

The effects of temperature on the kinetics of both dim and bright flash responses are shown for one cell in Fig. 6. In *A* averaged responses to dim flashes at 25·7, 19·8 and 13·9 °C are superimposed, while in *B* averaged responses to saturating flashes at the same temperatures are shown, plotted in both cases relative to the dark level. Clearly temperature has a profound effect on the response time course, with the time to peak of the linear responses decreasing by a factor of about 2·5 upon warming by 12 °C.

In Fig. 6*C* the linear responses from *A* have been scaled in both amplitude and time so that their peaks coincide. This leads to superposition of the entire wave forms, indicating that the response shape is unchanged at the three temperatures, and showing that all time constants involved in the linear responses change in a similar manner with temperature.

In Fig. 6*D* the bright flash responses from *B* have also been scaled in amplitude and time, with the constraint that the time scaling was the same as in *C*. With this procedure the recovery phases of the saturating responses at the three temperatures also superimpose, when shifted laterally by a small amount. This result indicates that the reactions governing recovery from bright flashes have the same temperature dependence as those involved in the responses to dim flashes. (The slight lateral shifts in Fig. 6*D* are consistent with the observations in the next section that the half-saturating intensity increases with temperature.)

*Dim flashes*

Collected results from sixteen cells are plotted in Arrhenius coordinates in Fig. 7 for the variation of $t_{peak}$, the time to peak of the dim flash response, as a function of temperature. For both HEPES- (*A*) and bicarbonate- (*B*) buffered Ringer solutions the results appear consistent with a linear dependence of ln $t_{peak}$ on reciprocal absolute temperature. The slope of the best fitting line in *A* gives an activation energy,

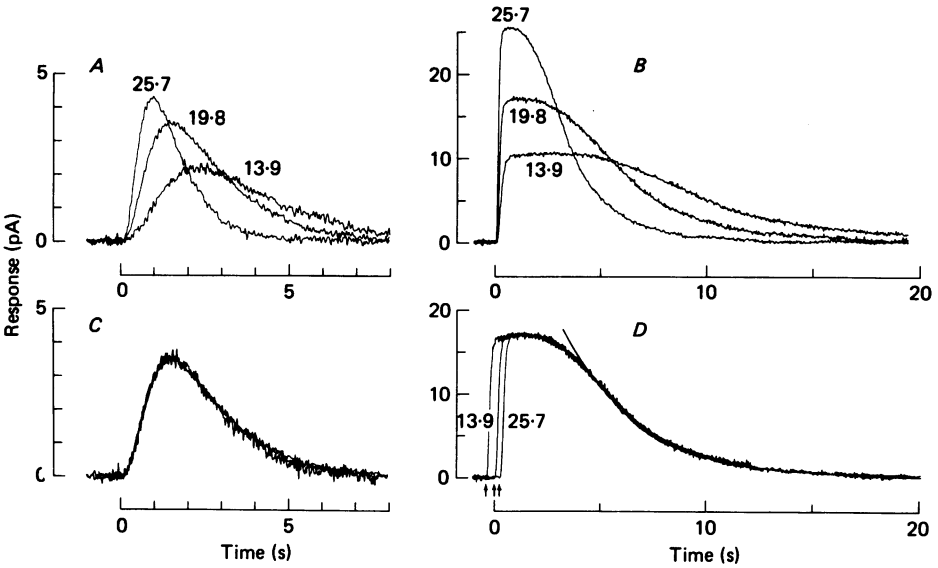


Fig. 6. Temperature dependence of dim and bright flash responses. *A* and *B*, raw responses to dim and bright flashes respectively, positioned to align at the dark resting level; flashes at time 0 delivered 0·47 (*A*) and 43 (*B*) photons $\mu m^{-2}$, estimated to isomerize 16 and 1500 rhodopsins. *C* and *D*, responses from *A* and *B* scaled in time and in amplitude; the responses at 19·8 °C are unaltered. Scaling factors were as follows:

| | Horizontal magnification (*C* and *D*) | Vertical magnification (*C*) | (*D*) |
|---|---|---|---|
| 13·9 °C | 0·65 | 1·62 | 1·62 |
| 19·8 °C | 1 | 1 | 1 |
| 25·7 °C | 1·67 | 0·82 | 0·675 |

Traces in *D* have been shifted horizontally as indicated by flash timing to align in the recovery phase. Smooth curve in *D* is an exponential with time constant 3·5 s. *A* and *C*, average of 10–21 responses; *B* and *D*, average of 4–10 responses. Bicarbonate Ringer solution. Same cell as Fig. 3*B*; symbol ○ in Fig. 7*B*.

$E_a$, of 13·8 kcal $mol^{-1}$ and a $Q_{10\,(15–25\,°C)}$ for $t_{peak}^{-1}$ of 2·2 for HEPES Ringer solution, and in *B* an $E_a$ of 11·7 kcal $mol^{-1}$ and a $Q_{10\,(15–25\,°C)}$ of 2·0 for bicarbonate Ringer solution. These results are broadly similar to those of previous workers. In the same preparation Baylor *et al.* (1983) obtained an $E_a$ of 16·8 kcal $mol^{-1}$ (HEPES Ringer solution), while in turtle cones Baylor *et al.* (1974) obtained 9·8 kcal $mol^{-1}$, and in rat rods Penn & Hagins (1972) obtained 15·8 kcal $mol^{-1}$.

*Time scaling.* For nine of the sixteen cells in Fig. 7 the responses to dim flashes at different temperatures had essentially the same shape simply scaled in time. However six out of seven cells taken below 10 °C showed significant discrepancies. Although the form of the scaled curves up until slightly after the peak was superimposable, the final recovery phase at the lowest temperatures was too fast. A possible

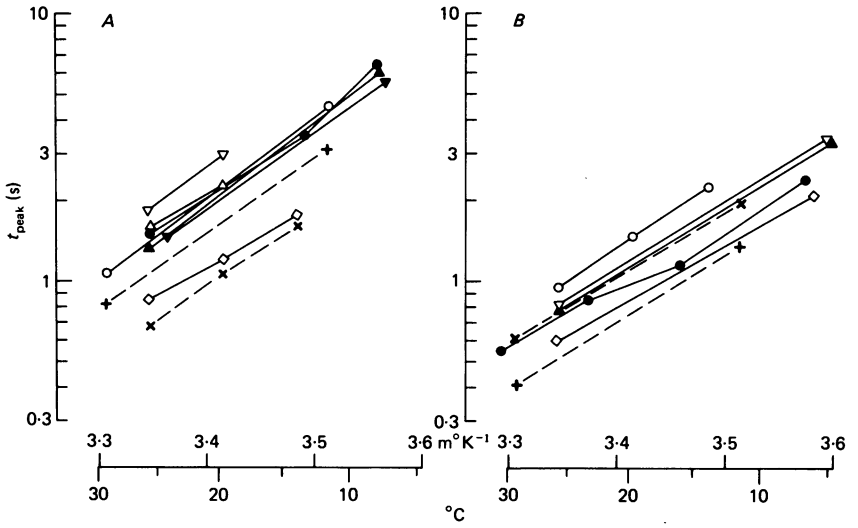


Fig. 7. Temperature dependence of $t_{peak}$ (time to peak of dim flash response) in Arrhenius coordinates. *A*, HEPES Ringer solution; *B*, bicarbonate Ringer solution. Crosses (+, ×) connected by interrupted lines in *A* and *B* are from four 'isolated' cells (outer segment with inner segment). Remaining symbols are from intact cells.

explanation might be that the flashes were too bright or too frequent, but on the other hand a similar phenomenon was also noted in the responses to bright flashes (next section). In one cell (+, HEPES) a poor fit was obtained upon scaling. An unchanged response shape was also found by Baylor *et al.* (1983).

*Bright flashes*

In Fig. 6*D* the common falling phase following bright flashes is closely described (after the first 20 % or so of recovery) by a single exponential with a time constant of 4·5 s at 19·8 °C. The same phenomenon is shown in another cell in Fig. 8 at three temperatures. Here the individual responses have been fitted with exponentials having time constants of 2·8 s (25·7 °C), 4·5 s (19·8 °C) and 6·5 s (13·9 °C). These time constants vary with temperature in much the same ratio as predicted by the data for the dim flash kinetics in Fig. 7. For all of the cells in Fig. 7 a single exponential provided a good fit to at least the final 50 % of recovery at all temperatures, and for thirteen of the sixteen cells it provided a good fit to all but the first 30 % of recovery.

The time constants are plotted in Arrhenius coordinates in Fig. 9*A* and *B*. At higher temperatures the slope is very similar to that seen in Fig. 7, but below about 15 °C there is a significant reduction in slope, indicating that at low temperature the time constant of recovery from bright flashes increased by less than would be expected for the activation energy describing the dim flash behaviour.

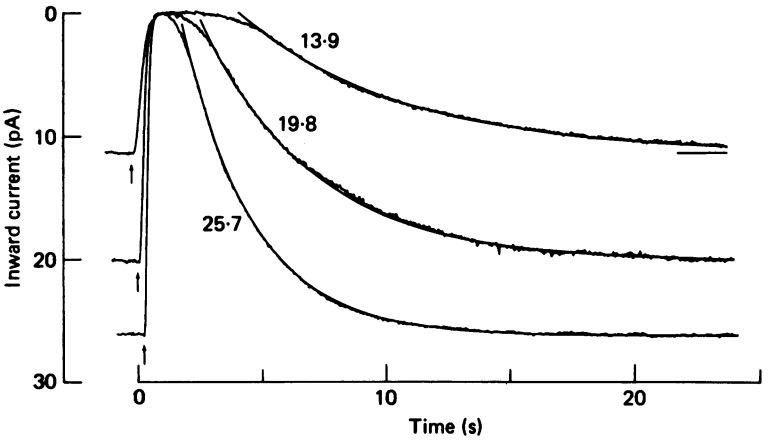


Fig. 8. Averaged bright flash responses from cell of Fig. 3*A*. Traces have been shifted to align initially at the saturating level; arrows show flash timing, 13·9 °C trace shifted 0·25 s to left, 25·7 °C trace shifted 0·2 s to right. Numbers of sweeps averaged: 13·9 °C, 12; 19·8 °C, 8; 25·7 °C, 24. Smooth curves are exponentials with time constants and final values (shown at right): 13·9 °C, 6·5 s, 11·5 pA; 19·8 °C, 4·5 s, 20 pA; 25·7 °C, 2·8 s, 26 pA. Flashes delivered 10 photons $\mu m^{-2}$ estimated to cause about 270 isomerizations.

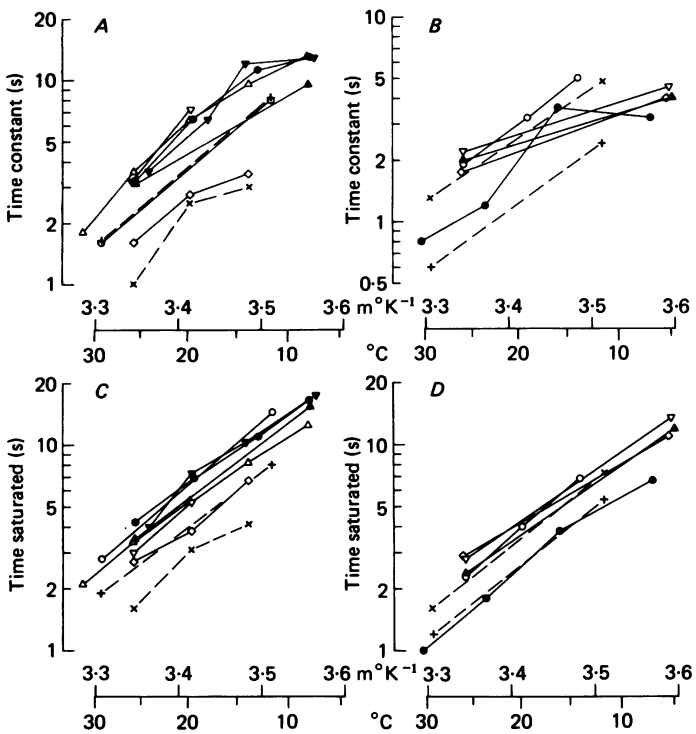


Fig. 9. Recovery following saturating flashes for the cells of Fig. 7. *A* and *B* plot time constant of recovery in Arrhenius coordinates for HEPES (*A*) and bicarbonate (*B*). *C* and *D* plot the time for which the response remains saturated, again in Arrhenius coordinates, for HEPES (*C*) and bicarbonate (*D*); the ordinate is measured as time from the flash until 20% of the original dark current was regained. Symbols represent same cells as in Figs. 7*A* and *B*.

Another parameter which describes the bright flash kinetics is the time for which the cell remained saturated after a flash. This was determined by measuring the time taken to recover to a level arbitrarily chosen at 20 % of the steady dark current. The temperature dependence of this parameter is plotted in Fig. 9*C* and *D* for the cells of Fig. 7. In both Ringer solutions the slope appears essentially independent of

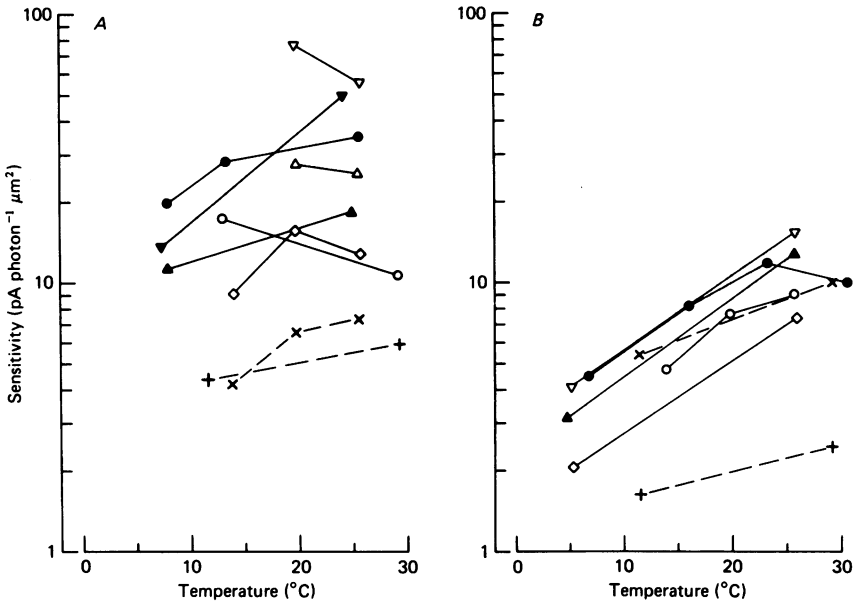


Fig. 10. Flash sensitivity as a function of temperature. *A*, HEPES Ringer solution; *B*, bicarbonate Ringer solution; symbols represent same cells as in Fig. 7 *A* and *B*. Responses were close to linear range, and have been corrected for Michaelis compression. 500 nm light plane polarized in the preferred orientation. Crosses (×, +) joined by interrupted lines in *A* and *B* are from four 'isolated' cells; other symbols are for intact cells.

temperature, and corresponds to an activation energy of 14·2 kcal mol$^{-1}$ (HEPES) and 13·2 kcal mol$^{-1}$ (bicarbonate), close to the values obtained for the dim flash kinetics.

In summary the experiments with saturating flashes of light indicate that the time for which the dark current remains suppressed has the same Arrhenius temperature dependence as the linear range flash kinetics. In addition the recovery is quite accurately described by a single exponential. At higher temperatures the time constant of this recovery has the same temperature dependence as the dim flash kinetics, but at low temperatures the time constant is shorter than expected.

### *Sensitivity*

Figs. 2 and 6*A* showed that in two cells an increase in temperature substantially increased the sensitivity to dim flashes. Collected results from sixteen cells (those of Fig. 7) are plotted in Fig. 10 and show a general trend towards higher sensitivity at higher temperatures. This result conflicts with the observation of Baylor *et al.* (1983) that in the same preparation warming usually reduced sensitivity with a $Q_{10}$ for the average effect of 1·6.

Inspection of Fig. 10 shows that in thirteen out of fourteen cells for which measurements were made below 20 °C the sensitivity at the lower temperatures was lower than that measured at or above 20 °C. This indicates a very reproducible tendency for sensitivity to increase with temperature, at least up to 20 °C. However of those cells recorded both near 20 °C and at a higher temperature, four out of five

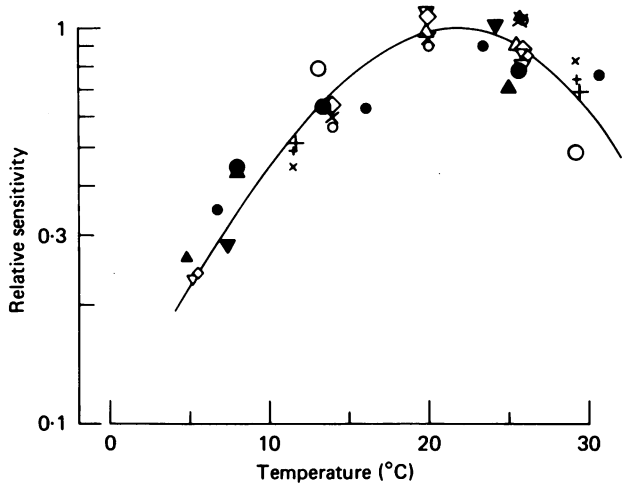


Fig. 11. Relative flash sensitivity as a function of temperature. See text for details. Points for different cells have been positioned vertically (logarithmic ordinate) to provide best fits to the curve. Symbols are as in Fig. 10, except that larger symbols are used for cells of Fig. 10*A* (HEPES), and smaller symbols for Fig. 10*B* (bicarbonate).

show a reduction in sensitivity at the higher temperature. Furthermore the negative effect of temperature on sensitivity reported by Baylor *et al.* (1983) appears to have been obtained only above 19 °C (see their Fig. 3). These results taken together strongly suggest that the flash sensitivity is maximal somewhere near 20 °C and declines on either side. Unfortunately though, neither this study nor that of Baylor *et al.* (1983) obtained controlled sensitivity measurements at many temperatures in an individual cell.

Based on the idea of a decline in sensitivity above and below roughly 20 °C an attempt was made to find a common curve which could account approximately for all the measurements. Fortunately most of the cells had been recorded at a temperature near 25 °C, and as an initial step the measurements for those cells were normalized to that value. A curve was fitted to the normalized data and then, by trial and error, the curve was adjusted to provide a reasonable fit to all of the data. Fig. 11 shows the curve obtained and the scaled data; the points for each cell have been shifted vertically (logarithmic ordinate) to provide individual fits. Although this may be a rather arbitrary procedure, the fit obtained is consistent with the notion that flash sensitivity has a broad maximum near 22 °C and declines at both higher and lower temperatures. It may be noted that the form of the curve above 19 °C describes reasonably accurately the data in Fig. 3 of Baylor *et al.* (1983).

*Response–intensity relation*

The increased sensitivity at higher temperatures can be seen in the response *versus* intensity relations plotted in Fig. 2*C*; the points at 20·5 °C (●) lie above those at 6·7 °C (▲) for all intensities. A continuous curve has been drawn to provide a fit to the data at 30·5 °C. (This curve, for the response *peaks*, is intermediate between a Michaelis compression and the expression $1-e^{-kI}$ found by Lamb *et al.* 1981, to fit rod responses at fixed times; see Baylor *et al.* 1974.) The same curve simply shifted laterally (interrupted curve) provides a good fit to the normalized data for 6·7 °C (△).

As found by Baylor *et al.* (1983) the required shift of the normalized curve is leftwards at lowered temperatures, indicating a smaller half-saturating intensity. For this temperature decrease from 30·5 to 6·7 °C the required shift was 0·36 log units, a factor of 2·3 times. Although complete intensity families were studied in only a few cells, the measurements of sensitivity and saturating response indicated a similar shift in all cells.

*Absolute sensitivities and time course*

It is apparent in Fig. 10 that substantial differences exist in absolute sensitivities between different cells. In this preparation Baylor *et al.* (1980) reported a powerful correlation between response kinetics and flash sensitivity ($S_F$), with $S_F \propto t_{peak}^{2.5}$, at fixed temperature. A broadly similar correlation may be seen by comparing Figs. 7 and 10; the more sensitive cells being generally slower.

Two other trends are apparent in these Figures. First, responses in bicarbonate-buffered Ringer solution tended to be faster and less sensitive than those in HEPES-buffered Ringer solution, as reported by Lamb *et al.* (1981). And secondly, responses from a few 'isolated' cells tended to be faster and less sensitive than those from cells still attached to the retina (in Figs. 7, 9 and 10, four isolated cells are indicated by crosses (+, ×) joined by interrupted lines).

For intact cells the mean values of $t_{peak}$ (time to peak) and $S_F$ (sensitivity) to dim flashes, interpolated to 20 °C, were:

HEPES Ringer solution, $t_{peak} = 2{\cdot}1$ s, $S_F = 38$ pA photon$^{-1}$ $\mu$m$^2$, ($n = 7$);

bicarbonate Ringer solution, $t_{peak} = 1{\cdot}2$ s, $S_F = 12$ pA photon$^{-1}$ $\mu$m$^2$, ($n = 5$).

The origin of the relatively wide variation in kinetics (or sensitivity) in a given Ringer solution at a fixed temperature is not known; it may partly be explained by the observation that as cells deteriorated with the passage of time, they usually gave slower responses.

*Comparison with effects of background light*

It is of interest to compare the effects of temperature and background light on the kinetics and sensitivity of the responses to dim flashes. As has been pointed out before (see Penn & Hagins, 1972; Baylor & Hodgkin, 1974; Baylor *et al.* 1974, 1980, 1983) the effects of temperature and background light are quite different, and Fig. 12 provides a useful illustration of the differences. In Fig. 12*A* responses are superimposed for flashes of fixed intensity at three temperatures, while in Fig. 12*B* responses in

another cell are superimposed for flashes of fixed intensity presented in darkness and on four background intensities. (Strictly, in Fig. 12*B*, responses have been scaled according to test flash intensity, as brighter flashes were employed on the backgrounds.)

Perhaps the most prominent difference between Fig. 12*A* and *B* concerns the rising phase of the response. With backgrounds of different intensity the responses begin

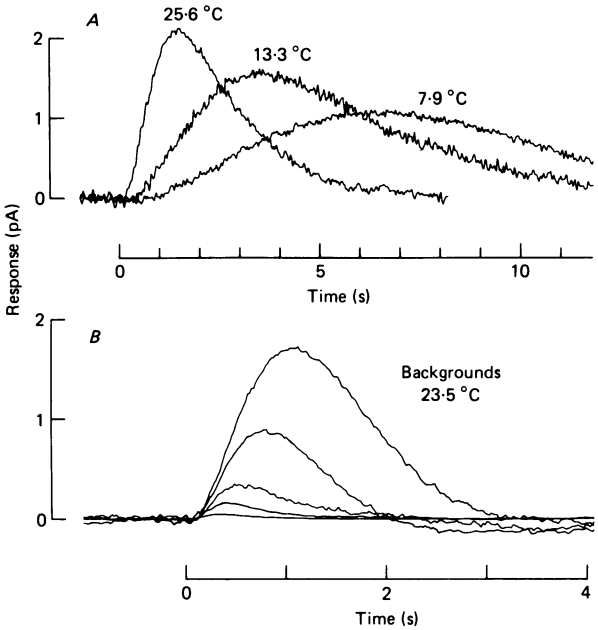


Fig. 12. Comparison of effects of temperature and background light on response to dim flashes. *A*, flashes delivered 0·07 photons $\mu m^{-2}$ estimated to cause mean of 2·2 isomerizations per flash. Average of 20–30 responses. HEPES Ringer solution, cell of Fig. 3*D*. Saturating response 28 pA (25·7 °C), 13 pA (13·3 °C), 7·5 pA (7·9 °C). *B*, backgrounds at 23·5 °C in another cell. Top trace is for flashes of 0·2 photons $\mu m^{-2}$ (estimated to cause seven isomerizations) presented in darkness. Remaining traces for backgrounds of 0·4, 1·7, 6·5 and 29 photons $\mu m^{-2} s^{-1}$. These traces were obtained with brighter flashes and have been scaled to the same test flash intensity as the top trace. Bicarbonate Ringer solution; saturating response 22 pA in darkness; backgrounds gave steady responses of 4, 7·4, 10·8 and 14·6 pA.

rising approximately on a common curve, as reported for turtle cones by Baylor & Hodgkin (1974). When temperature is varied, however, the rising phases differ drastically from each other.

When considered in terms of the circulating (dark) current, opposite effects of temperature and backgrounds are apparent. A reduction in circulating current brought about by decreased temperature is associated with slowed response kinetics, whereas a reduction in circulating current brought about by background light is associated with accelerated kinetics.

### *Long wave-length sensitivity*

An experiment to measure the effect of temperature on the relative sensitivity to long wave-lengths is illustrated in Fig. 13. In each pair of responses the lighter trace

represents the average response to 500 nm flashes of constant (arbitrary) intensity while the heavier trace is for 700 nm. At 29·3 °C (left) the intensity of the 700 nm flashes was adjusted to give approximately the same size response as for 500 nm, while at 11·4 °C (right) the 700 nm intensity was increased by nominally 0·3 log units. Calibrations showed that the actual density change at 700 nm was only 0·265 log units (1·84 times).

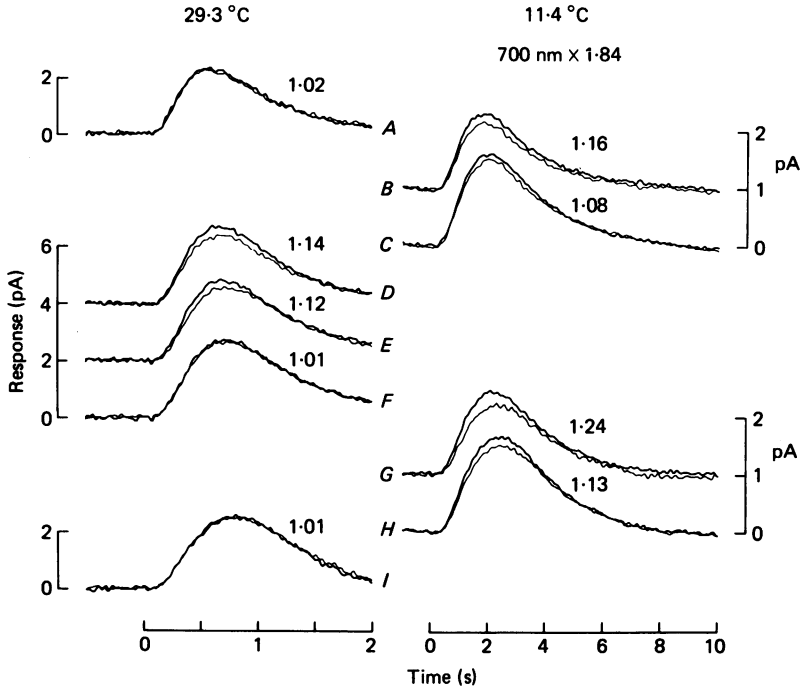


Fig. 13. Effect of temperature on sensitivity at 700 nm relative to 500 nm. In each pair the lighter trace was obtained with 500 nm light of fixed intensity 0·25 photons $\mu m^{-2}$ (estimated to cause approx. 7·5 isomerizations per flash). The heavier trace is for 700 nm light delivering: $1{\cdot}63 \times 10^4$ photons $\mu m^{-2}$ on left (29·3 °C), and $3{\cdot}0 \times 10^4$ photons $\mu m^{-2}$ on right (11·4 °C). (The change in filter density was nominally 0·3 log units, but was measured with a quantum photometer to be 1·84 times at 700 nm). Presented in sequence 20 flashes at 500 nm, 40 at 700 nm, 20 more at 500 nm. Responses obtained in the order *A–I*; this experiment took about 3 h. Numbers near responses are ratios of amplitude at 700 nm to amplitude at 500 nm after correction for Michaelis compression. Saturating response at 29·3 °C initially 25 pA and finally 19 pA; saturating response at 11·4 °C initially 7 pA and finally 6·5 pA. Bicarbonate Ringer solution; isolated cell; symbol (×) in Figs. 7*B*, 9*B* and 10*B*.

The experiment was performed in the sequence illustrated from top to bottom, and each pair of traces represents an interlaced set of measurements (20 flashes at 500 nm, 40 at 700 nm, 20 more at 500 nm; a total of 40 flashes at each wave-length). In each case the average response at 700 nm was at least as great as that at 500 nm, and the numbers against each pair of responses represent the ratio of amplitudes after correction for non-linearity; this correction had negligible effect.

For the five repetitions at 29·3 °C the mean amplitude ratio (700 nm:500 nm) for the selected intensities was 1·06, while for the four repetitions at 11·4 °C the ratio was

1·16 despite the fact that the red light was then 1·84 times more intense. Hence the increase in relative sensitivity ($S_F$ 700 nm/$S_F$ 500 nm) in going from 11·4 to 29·3 °C was $1{\cdot}84 \times 1{\cdot}06/1{\cdot}16 = 1{\cdot}69$, or about 0·23 log units.

This is quite a small change in relative sensitivity, and explains the need for presenting more than 700 flashes in obtaining Fig. 13. Slight inaccuracies in the measured sensitivities due to quantal fluctuations and other noise would easily have obscured the sensitivity change if only small numbers of flashes had been presented.

It might be thought that a larger effect could have been observed by using even longer wave-length light but there was insufficient energy in the stimulating beam for this to have been possible. At 700 nm the rod sensitivity is down by about 5 log units from its peak at 500 nm and it continues to fall steeply at longer wave-lengths. This restriction to wave-lengths shorter than about 700 nm also meant that accurate measurement of the *slope* of the log sensitivity curve at long wave-length was difficult, as quite closely spaced wave-lengths would have been required. The ratio of sensitivities would then have been even closer to unity and hence more prone to measurement error.

In two other cells for which comparable stability was obtained with a large number of flashes the change in relative sensitivity was 1·71 times (0·23 log units) and 1·64 times (0·21 log units) at almost exactly the same temperatures. The mean change in $S_F$ 700 nm:$S_F$ 500 nm for 29·3 °C compared with 11·5 °C was 1·68 times (0·225 log units).

It is believed that these measurements of *changes* in relative sensitivity are very accurate. However, the absolute magnitude of the sensitivity ratio $S_F$ 700 nm: $S_F$ 500 nm measured at either temperature is less reliable, and depends on the accuracy of absolute calibration of the light stimulus at the two wave-lenghts. The approximate values obtained for $S_F$ 700 nm:$S_F$ 500 nm were −4·8 log units at 29·3 °C and −5·0 log units at 11·5 °C. These values compare favourably with the value of approximately −4·8 log units obtained by Baylor *et al.* (1979*a*) at room temperature.

## DISCUSSION

### *Rapid temperature changes*

The absence of a detectable delay between a change in temperature and the resulting change in dark current seems to indicate that a temperature dependence of the dark conductance is responsible. The alternative, a change in driving force, can be ruled out because of the long time scale which would be involved. From the size of the dark current of about 30 pA at 20 °C, and the estimated outer segment cytoplasmic volume of about 900 $\mu m^3$, sodium ions would accumulate inside the cell at the rate of approximately 20 mM $min^{-1}$ in the absence of a sodium pump. Assuming the steady dark level of the intracellular sodium concentration ($[Na^+]_i$) with the pump operating to be in the region of 10 mM the turnover time for intracellular sodium would be 10 mM/20 mM $min^{-1}$ = 30 s; this is probably a lower limit. Hence during a rapid temperature decrement, when the sodium pumping rate might be expected to decrease, a constant dark current would increase $[Na^+]_i$ with a time constant of at least 30 s. Such a slow change could not be responsible for the nearly instantaneous effect of temperature on dark current, but might instead explain the slight and slow

relaxation which followed the initial rapid effects. It has been pointed out to me by Professor A. L. Hodgkin that if the dark conductance and pumping rates had exactly the same temperature dependence then a step change in temperature would result in a step change in dark current, without subsequent relaxation, and without alteration in $[Na^+]_i$.

It is not possible from these experiments to say whether the change in dark conductance is a *direct* effect of temperature on the light-sensitive 'channels', or whether it is mediated by some rapid intracellular change, for example by pH or pCa.

*Dark current*

The temperature dependence of the dark current seems to deviate significantly from simple Arrhenius behaviour over the range 5–30 °C, although the deviation is much less obvious over the range 15–25 °C. This deviation is not altogether surprising as there would seem to be no *a priori* reason for assuming the dark current to be controlled by a single process with constant activation energy. It seems likely that the dark conductance would be influenced by several temperature dependent factors (for example, open 'channel' permeability and number of open 'channels') in addition to any effects of driving force. Whether there is a special significance to the approximately linear relationship found between dark current and temperature is not known.

*Sensitivity*

The sensitivity to dim flashes declined at high temperature (in agreement with Baylor *et al.* 1983) and at low temperatures also. From the collected results it appears that sensitivity is maximal at around 22 °C. This may not be far from the normal body temperature of the animal, which inhabits tropical regions and is nocturnal; it is also approximately the temperature at which the animals were maintained (22–25 °C).

*Kinetics*

The temperature dependence of the dim flash response kinetics is very similar to that found by previous workers. All studies have found Arrhenius type behaviour and the activation energies were as follows: rat rods, 15·8 kcal $mol^{-1}$ (Penn & Hagins, 1972); turtle cones, 9·8 kcal $mol^{-1}$ (Baylor *et al.* 1974); toad rods, HEPES Ringer solution, 16·8 kcal $mol^{-1}$ (Baylor *et al.* 1983); toad rods, HEPES Ringer solution, 13·8 kcal $mol^{-1}$ (this study). As found by Baylor *et al.* (1983) the entire response wave form to dim flashes simply scaled in time (except perhaps at temperatures below 10 °C). In the 'chain of reactions' scheme of Baylor *et al.* (1974) this would indicate that all time constants determining the linear range response shape exhibited the same temperature dependence. On the single time constant model of Payne & Howard (1981) such scaling is implicit in the model. In the present study it was also found that the kinetics of the large signal responses varied in a similar manner with temperature as did the dim flash kinetics.

A simple explanation of these findings would be that the response kinetics (both small signal and large signal) are determined by diffusion of molecules within the disk and plasma membranes. On this idea a change in membrane fluidity would affect each

of the rate constants in a cascade of reactions in the same manner. In accord with this hypothesis, Cone (1972) and Liebman, Wiener & Drzymala (1982) have found that the disk membrane fluidity, measured from the rotational and lateral diffusion of visual pigment, has a $Q_{10}$ of about 2·5–3·0, quite similar to the $Q_{10}$ of the kinetics observed here. It should be possible to test this hypothesis by changing the fluidity of the lipid by other means; for example, by the incorporation of fatty acids (Orly & Schramm, 1975), alcohols (Chin & Goldstein, 1977) or small hydrocarbons.

It is possible to speculate on the nature of the four dominant reactions involved in the light response given the hypothesis that they are closely related to diffusional processes in the membrane. The following is an attempt to combine this hypothesis with both the cyclic nucleotide and calcium schemes of phototransduction, and stems from a suggestion by Dr E. N. Pugh, Jr. Three of the proposed reactions involve *lateral* diffusion in the disk membrane or at its surface, these are: lateral diffusion in the interactions (i) between isomerized rhodopsin and GTP-binding protein (GBP) (see Liebman & Pugh, 1981), (ii) between activated GBP and the phosphodiesterase, and (iii) between some kind of 'inactivator' protein and any one of the above species – for example, in phosphorylation of the activated rhodopsin. The fourth proposed reaction is the 'tumbling' diffusion of the $Na^+/Ca^{2+}$ exchanger within the membrane as $Ca^{2+}$ is extruded from the cytoplasm (see References in Kaupp & Schnetkamp, 1982). The time constants of each of these reactions should have approximately the same temperature dependence as the viscosity of the disk membrane, and hence each can be considered as a realistic candidate. A useful feature of this model is that two generation steps and two removal steps are accelerated as the temperature is raised. What is omitted from this scheme, however, is an explanation of the link between cyclic nucleotide hydrolysis and calcium release, but possible connexions are discussed in Miller (1981), George & Hagins (1983) and Liebman, Mueller & Pugh (1984).

The almost exactly exponential shape of the recovery following saturating flashes is not what would be expected on the simplest internal transmitter model (e.g. Baylor *et al.* 1974) where an approximately exponential decline of a chemical species is 'compressed' by the non-linearity of its binding to channels. On such a model the final tail of recovery is exponential, but a pronounced rounded shoulder dominates the first half of recovery. One modification which might explain the exponential decay would be very tight binding of internal transmitter substance to the channels, rather than the more commonly assumed weak binding. In this way (see Baylor *et al.* 1974) the relation between transmitter concentration and channel closure would be linear up to saturation.

*Long wave-length sensitivity*

Based on the measured decline of human spectral sensitivity in the far red, Stiles (1948) proposed a model in which the probability of absorption of a lower energy photon depended on the thermal energy of the absorbing rhodopsin molecule. This predicted a linear decline of log sensitivity with wave number in the far red, as observed experimentally, and also predicted that the limiting slope of the decline should vary inversely with absolute temperature.

In the present experiments reduction of the temperature from 29·3 °C (302·5 °K)

to 11·5 °C (284·7 °K) decreased the relative sensitivity $S_F$ 700 nm:$S_F$ 500 nm by 1·68 times (0·225 log units) from about −4·8 log units to about −5·0 log units. This respresents a fractional change of approximately 4·7 % in $\log_{10}$ sensitivity ratio upon a change in absolute temperature of 6·25 %. It must be stressed that this is a measure not of the change in slope of the log sensitivity curve at long wave-lengths, but simply of the change in attenuation from the peak sensitivity. Nevertheless the ratio 4·7 %/6·25 % = 0·75 is quite close to the factor of 0·79 obtained by Stiles (1948) as the ratio of measured to theoretically expected slopes in the long wave-length region for rods. This indicates that the present observations are broadly in line with theoretical expectation. It is conceivable that had it been possible to measure the change in long wave-length *slope* a factor of unity rather than 0·75 might have been found.

I wish to thank Professor A. L. Hodgkin and Drs D. A. Baylor, R. Fettiplace and E. N. Pugh for helpful comments on the manuscript. Supported by the Medical Research Council and by a Royal Society Locke Research Fellowship.

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