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MEMBRANE PROPERTIES OF A BARNACLE PHOTORECEPTOR EXAMINED BY THE VOLTAGE CLAMP TECHNIQUE

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SUMMARY

1. Electrical properties of the membrane of photoreceptor cells in the lateral ocelli of barnacles, *Balanus amphitrite* and *B. eburneus* were investigated by intracellular recording, polarization and voltage-clamp techniques.

2. The resting potential of a dark adapted cell was $36{\cdot}3 \pm 6{\cdot}6$ mV (S.D.) and depended mainly on the external K^+ concentration.

3. Current–voltage relations obtained from voltage–clamp experiments in the absence of light were non-linear and varied with time after the onset of a step change in membrane potential; the steady state was reached after about 0·5 sec.

4. Illumination resulted in a membrane potential change under current clamp and in a change of membrane current (light-initiated membrane current (L.I.C.): total membrane current with illumination minus current without illumination) under voltage–clamp conditions. Amplitudes and time course of L.I.C. depended on the light intensity as well as membrane potential.

5. The L.I.C.-voltage relation was non-linear and corresponded with a slope conductance increase with increasing positive membrane potential.

6. The reversal potential of L.I.C. was independent of the light intensity and the time after onset of illumination; the average value obtained in normal saline was $+26{\cdot}9 \pm 5{\cdot}0$ mV.

7. The membrane conductance estimated from instantaneous L.I.C.-

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voltage relations agreed with the chord conductance of the non-linear L.I.C.-voltage relation.

8. Decreasing external Na^+ concentration decreased the inward component of L.I.C. but not the outward component.

9. Decreasing external Ca^{2+} concentration increased the inward as well as the outward component of L.I.C.

10. The reversal potential shifted in the negative direction with decreasing external Na^+ concentration (the rate was 10–15 mV for a tenfold change in concentration) and the rate was augmented in the absence of Ca^{2+} but did not exceed 21 mV.

11. The change of reversal potential with changes of external Ca^{2+} concentration was negligible in normal Na^+ media but was significant in the absence of Na^+ (rate as high as 20 mV).

12. Alteration of the external K^+ or Cl^- concentrations did not affect the amplitude or reversal potential of L.I.C.

13. The results indicate that illumination increases the membrane permeability mainly to Na^+ ions and that the primary effect of Ca^{2+} ions is suppression of the permeability increase; Ca^{2+} permeability may increase slightly during illumination.

INTRODUCTION

Large photoreceptor cells in the lateral eye of the barnacle, originally described by Fales (1928), have stimulated interest in the membrane mechanisms that underlie the light-initiated, slow, depolarizing potential recorded from photoreceptors (Gwilliam, 1965; Brown, Meech, Sakata & Hagiwara, 1968; Brown, Meech, Koike & Hagiwara, 1969). Although regenerative activity does not usually occur in photoreceptors from this preparation, the characteristics of the slow potential change associated with illumination are similar to those of other Arthropods, being graded with light intensity and possessing a more complex wave form at high than at low light intensity (*Limulus* ommatidia: Hartline, Wagner & MacNichol, 1952; Kikuchi, Naito & Tanaka, 1962; crayfish ommatidia: Eguchi, 1965; *Limulus* ventral eye: Millecchia, Bradbury & Mauro, 1966; Smith, Stell & Brown, 1968; Smith, Stell, Brown, Freeman & Murray, 1968; insect ommatidia: Naka, 1961; Naka & Eguchi, 1962; Fuortes, 1963; Washizu, 1968; Fulpius & Baumann, 1969). Although little is known concerning the ionic events that are associated with the observed membrane potential change, there are two prevalent hypotheses of the membrane mechanism. From a linear analysis of experimental results obtained from the relatively small eccentric cells of the *Limulus* ommatidium, Fuortes (1959), and Rushton (1959) concluded that the potential changes arise due to a conductance change during illumination and membrane potential approaches a fixed value which

is independent of light intensity. However, from more recent work conducted on large cells in the *Limulus* ventral eye, it has been proposed that the membrane potential changes might be due to deactivation by light of a metabolic electrogenic sodium pump (Smith *et al.* 1968).

The present study of large photoreceptor cells in the barnacle ocellus was conducted to analyse the mechanism of potential change by the voltage clamp method and to describe the major ionic events that are associated with the receptor potential. The small size of most receptors has precluded analysis of membrane properties with the voltage clamp technique although this method has been used extensively to study membrane mechanisms of most other classes of excitable tissues. The relatively large size (about 80 μ diameter; Fahrenbach, 1965) of photoreceptor cells in the lateral ocellus of the barnacle has allowed us to overcome this fundamental problem. Preliminary reports of some experiments in this paper have been reported previously (Brown *et al.* 1968; Brown *et al.* 1969).

MATERIALS AND METHODS

Barnacles obtained from the Salton Sea in southern California (*Balanus amphitrite*) or from Woods Hole, Massachusetts (*B. eburneus*) were used in the present work with no evident differences.

Access to the ocellus was gained by first sawing through the exterior rostral and caudal plates of the barnacle with a small diamond abrasive wheel. The paired opercula, still attached at the apex, were severed by scissors yielding two half-shells each containing an ocellus with its attached ocellar nerve. The ocellar nerve was cut approximately 3 mm proximal to the ocellus and the ocellus was dissected free from the mantle connective tissue. The ocellus consists of a cup-shaped pigment epithelium, a layer of light-reflecting tapetal cells, and three photoreceptor cells (Fahrenbach, 1965). The photoreceptors were isolated by removing the epithelial and tapetal cells with fine forceps; the resilient strands of connective tissue remaining from the tapetal cells (Fales, 1928) were softened by incubation in a mixture of pronase and collagenase (30 mg/ml. each in barnacle saline) for 1 min. This procedure softened the connnective tissue sufficiently for relatively easy penetration of the micro-electrodes but had apparently no effect on the electrical properties of the cells (ascertained by recording electrical activity of cells not treated with enzyme and approached from the corneal side of the ocellus with micro-electrodes driven by a small electronic hammer). Thus prepared, the photoreceptors were held in place in a recording chamber (Fig. 1) and protruded through the opening of a 60 mesh platinum electron microscope grid held by means of a wire loop attached to a micromanipulator. This method provided free access of the cells to the continuously flowing saline (a and a′) yet held them securely despite the flow. The preparation was positioned, corneal side down, over a small (3 mm in diameter) fibre optics bundle (L) located immediately beneath a thin glass plate of the chamber on which the preparation rested. Light pulses were transmitted to the preparation via the fibre optics bundle from an electronically shuttered, 150 W quartz-iodine light source (maximum illuminance at the preparation approximately 10^5 lux); intensity was reduced as desired with neutral density filters. A photoreceptor was penetrated under visual control with two glass micro-electrodes filled with 3 M-KCl (resistance

measured in 3 M-KCl, about 3 MΩ). Membrane potential (E_m) was recorded on a cathode ray oscilloscope (CRO) as the potential difference between one intracellular electrode and a KCl-filled micro-electrode in the saline bath (left and middle electrodes in Fig. 1). The second internal electrode (right) was used to apply constant current pulses (current clamp, CC) or to control the membrane potential by the feed-back amplifier (A 1) between the potential and current passing electrodes (voltage clamp method, VC). A thin insulated phosphor-bronze sheet connected to ground (not

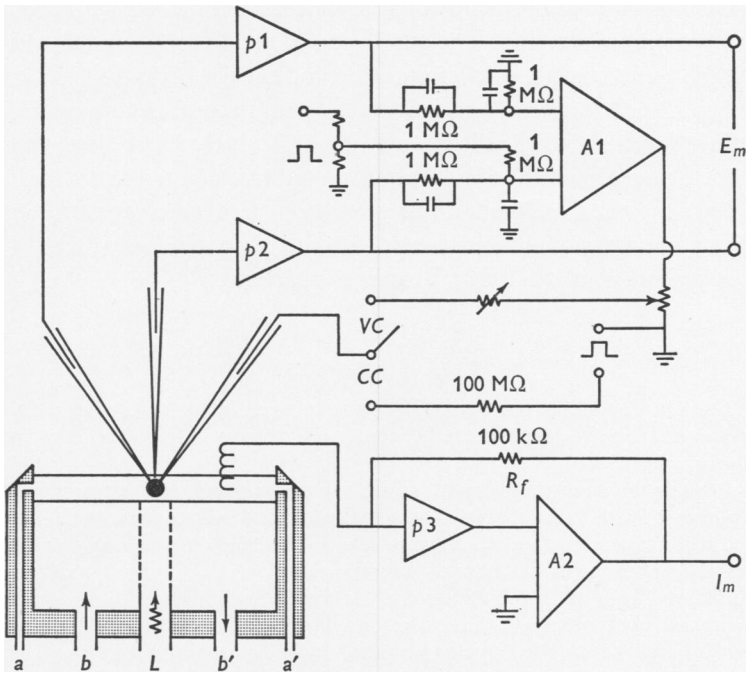

Fig. 1. Recording apparatus. Fibre optics bundle (L) for illumination of the cell (large filled circle). Inlets and outlets for continuous perfusion and water flow to cool the chamber are shown by a, a′ and b, b′ respectively. p 1, p 2 and p 3: unity gain preamplifiers. A 1: high gain DC amplifier to control membrane potential; A 2: operational amplifier used to measure membrane current (IR drop across R_f). VC–CC: switch to pass current from A 1 (voltage clamp) or from a constant current generator (current clamp).

illustrated) was introduced between the two internal electrodes to reduce capacitative coupling between them. The time required to clamp membrane potential to a final value with the system used was about 1·5–2·0 msec when a rectangular commanding voltage pulse was applied to the feed-back amplifier. The circuit for the voltage clamp was similar to that used by Hagiwara, Takahashi & Junge (1968). Membrane current (I_m) was displayed on the CRO as the voltage drop across the feed-back resistor (R_f) of the operational amplifier (A 2) used to hold the external saline at ground potential. The composition of saline used is shown in Table 1. All solutions were buffered to pH 7·7 with Tris-maleate and NaOH or Tris-OH and HCl. Experiments were carried out at room temperature (20–25° C).

TABLE 1. Composition of salines

	NaCl (mM)	KCl (mM)	$CaCl_2$ (mM)	$MgCl_2$ (mM)	Tris–OH (mM)	HCl (mM)	Sucrose (mM)	Na iso-thionate (mM)	Tris-maleate (mM)
Normal saline	462	8	20	12	—	—	—	—	10
Ca saline	462	8	32	—	—	—	—	—	10
Mg saline	462	8	—	32	—	—	—	—	10
Na-free Tris saline	—	8	20	12	540	387	—	—	10
Na-free sucrose saline	—	8	20	12	—	—	924	—	10
Low Cl saline	—	8	20	12	—	—	—	462	10
K saline	—	470	20	12	—	—	—	—	10
Na, Ca-free	—	8	—	32	540	387	—	— pH 7·7	10

RESULTS

Membrane properties examined by constant current polarization

The resting membrane potential of a receptor cell, dark adapted for a period of 5–10 min, was 30–40 mV, inside negative (average $36{\cdot}3 \pm 6{\cdot}6$ mV S.D. for thirty-five cells). The potential changes of the membrane associated with steps of illumination of different light intensities are shown in Fig. 2. Steps of light ranging from 65 lux (3 to 4 orders of magnitude greater than the intensity of the background light in the room) to 27,000 lux were applied at 10 sec intervals and the steady-state response was recorded after three or four flashes. The response to low light intensity (*e* in Fig. 2) was a depolarization with an approximately rectangular time course. At higher intensities (*c* and *d*) a transient peak phase emerged and the time to the peak occurred earlier at the higher light intensity. The membrane potential at the peak became more positive and exceeded the reference potential with increasing light intensity (*a* to *c*). Following the peak, the response decayed with time into a steady phase. At the higher intensities of illumination (*a* and *b*) a notch or trough was seen following the peak and the membrane potential at this notch became more negative the greater the light intensity.

Fig. 3 shows records obtained when the cell was illuminated during polarization of the membrane by constant current pulses applied through the second internal pipette. The current pulse produced a potential change of the cell membrane and its time course depended on the intensity and polarity of the current (total time course is not shown in Fig. 3). The additional potential change resulting from the light pulse was a large positive-going potential change when the membrane was hyperpolarized. The amplitude of the positive-going response became progressively less as the

membrane was depolarized and at a particular positive membrane potential almost no potential change was produced by light. At membrane potentials more positive than this particular value a negative-going potential change was obtained during illumination, i.e. there is a membrane potential at which the potential change to light reverses its sign.

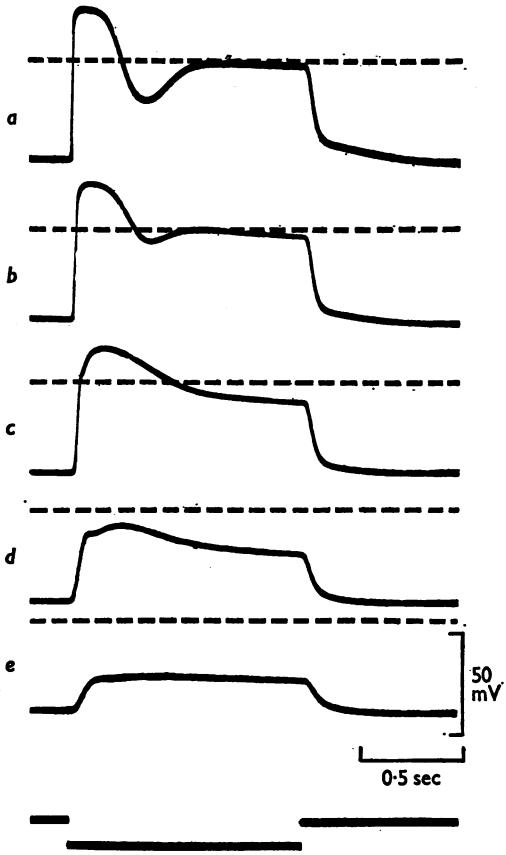

Fig. 2. Membrane potential changes associated with steps of illumination of different intensities: *a*, $2{\cdot}7 \times 10^4$ lux; *b*, $1{\cdot}9 \times 10^4$ lux; *c*, 5×10^3 lux; *d*, 1×10^3 lux; *e*, $6{\cdot}5 \times 10^1$ lux. The dashed horizontal lines represent the reference potential; negativity is downward from these lines. Duration of the light step is indicated by the downward deflexion of the trace at the bottom of the records.

The open symbols in Fig. 4*A* represent current–voltage relations of the same cell membrane during illumination, the circles and triangles representing the peak and steady phase of the light response respectively. Filled circles and triangles in Fig. 4*A* represent the relationship between membrane potential and membrane current in the absence of illumination

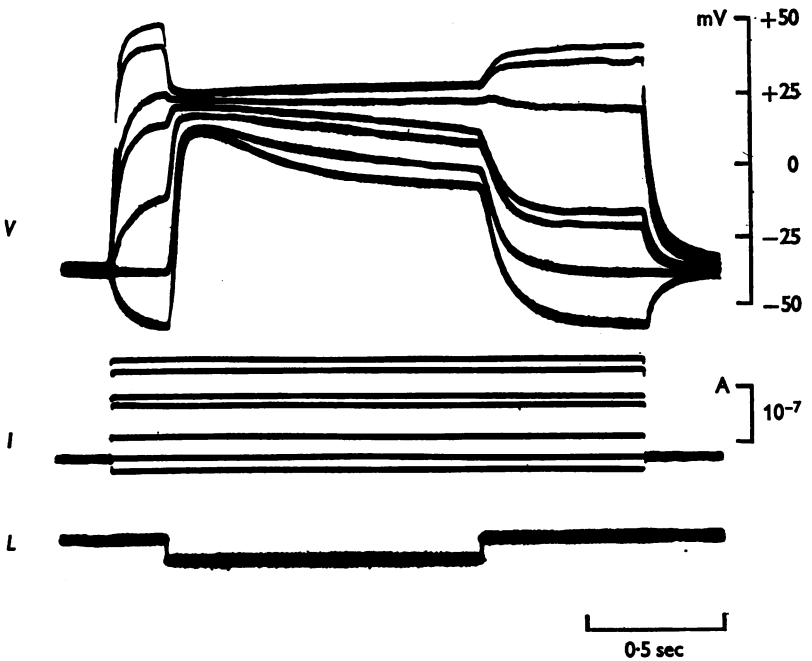

Fig. 3. Membrane potential changes (V) under current clamp conditions. Light pulses (L) were applied during application of current steps (I) of various strengths, outward current (electrode tip positive) is displayed upward.

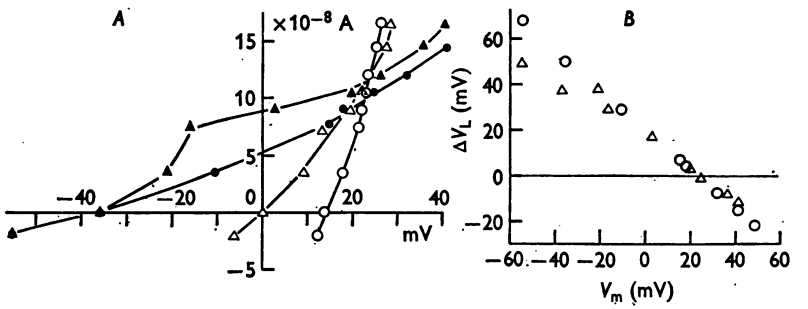

Fig. 4. Graphical representation of Fig. 3. *A*. Membrane potential (abscissa) as a function of applied current intensity (ordinate). Open circles and triangles represent the peak and steady phases of the light response 100 msec and 1 sec after initiation of the light pulse. Filled circles and triangles represent the relation without illumination corresponding to the peak and steady phase of response. *B*. Amplitude of the receptor potential produced by illumination (ΔV_L, ordinate) plotted against membrane potential in the absence of illumination (V_m, abscissa). Open circles: peak phase of the response to light; triangles: steady phase of the response.

obtained at times corresponding to the peak and steady phase of the light response. Although membrane potential was measured at times greater than the membrane time constant, the two relations are different and are non-linear in both cases. There was no evidence of regenerative activity such as spike potentials. The potential difference between these curves and the corresponding ones obtained during illumination represents the amplitude of the potential change (ΔV_L) produced by light at a given level of membrane current. When ΔV_L is plotted against the membrane potential (V_m) in the absence of illumination, instead of membrane current, the relation shown in Fig. 4*B* is obtained. The relations of both the peak (open circles) and steady phase (open triangles) are almost linear and cross the abscissa at about +22·5 mV. The linear property of this relation is similar to that found for membranes often referred to as 'electrically inexcitable membranes' such as the end-plate membrane (Fatt & Katz, 1951; Takeuchi & Takeuchi, 1959). Fig. 4*B* may imply that, and seems to be in agreement with the rather common notion that sensory membranes have linear electrical properties, i.e. behave in accordance with Ohms law. However, as described previously, the current–voltage relations of the membrane in the absence of illumination are not linear. Furthermore, as will be described later, the current–voltage relations of the light sensitive component are also non-linear. Therefore, the apparent linearity between ΔV_L and V_m is not due to the linear $I-V$ relations of the membrane but due to cancellation of non-linearities in the $I-V$ relations of the light sensitive and insensitive components of the receptor membrane.

Membrane properties examined with voltage clamp

Membrane properties in the absence of illumination. The experimental results obtained with constant current polarization revealed that there was time variation of membrane potential and that the $I-V$ relations with and without illumination were non-linear, indicating that time variation of membrane current would be expected under voltage–clamp conditions. Fig. 5*A* shows membrane currents of the receptor cell in the absence of illumination obtained at two different sweep speeds when the membrane potential was clamped from the resting potential (−34 mV) to four different potential levels. A voltage step more negative than the resting potential resulted in an inward capacitative current followed by a constant inward current (see traces for −60 mV and −56 mV). For voltage steps more positive than the resting potential an outward capacitative current was followed by an initially small outward current 2–3 msec after the onset of the voltage pulse; this was followed by an increased outward current, which decayed to a smaller steady value within about 100 msec. The larger transient component of outward current became more pro-

nounced and the peak was reached earlier as the membrane potential was clamped to more positive values (+5 mV, 7·5 msec; +33 mV, 4 msec). Fig. 5*B* shows current–voltage relations of the same cell membrane obtained at 5, 30, 100 msec and 1·8 sec after the onset of the voltage pulse. The four relations almost coincided for the range of membrane potentials more negative than the resting potential. The slope of the relation was, as a rule, greater for the late *I–V* relation (1·8 sec) than for the early one

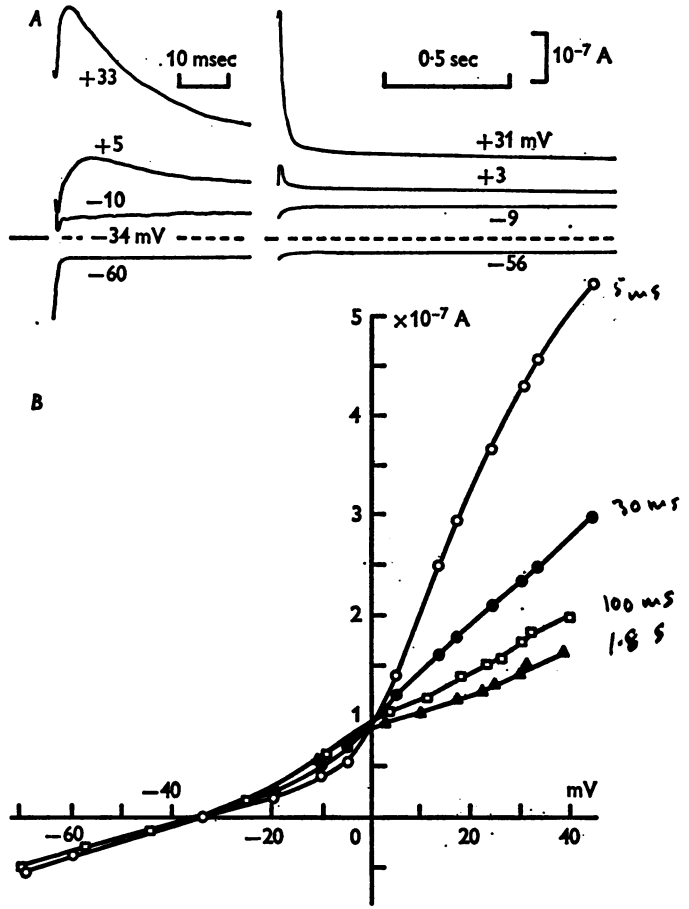

Fig. 5. Voltage clamp without illumination. *A*. Membrane current associated with step changes of membrane potential from −34 mV to the level indicated adjacent to each record. Left records: fast sweep velocity to show early changes of membrane current; right records: slow sweep to show membrane current during a protracted (1·8 sec) change of membrane potential. *B*. Relation of membrane current (ordinate) and membrane potential (abscissa) at several different times following step changes of membrane potential (○, 5 msec, ●, 30 msec, □, 100 msec, ▲, 1·8 sec.) Negative membrane potential and inward current are shown as negative.

(5 msec) between the resting potential and -10 mV. This tendency however, reversed for the range of membrane potentials above zero where the slope of the early relation exceeded that of the late by a factor of 6–7. The early current–voltage relation shows that slope increases with increasing positive membrane potential. This tendency is similar to the rectification of the K^+ component of membrane current in the squid giant axon membrane (Hodgkin & Huxley, 1952). The time dependent behaviour of the current–voltage relation shown in Fig. 5*B* indicates that this rectifying property becomes less pronounced with time after the onset of the voltage pulse. The late or the steady-state *I–V* relation was, as a rule, 'flattened', i.e. the slope of the relation tended to approach zero in the range of membrane potentials between 0 and $+20$ mV and again increased at more positive membrane potentials. Although this tendency is evident in the case shown in Fig. 5*B*, it was generally more pronounced (compare Fig. 5*B* with the late *I–V* relation obtained from the cell shown in Fig. 4*A*).

The effective membrane resistance, in the absence of illumination, measured from the slopes of current–voltage relations at membrane potentials more negative than the resting potential was $1{\cdot}65 \pm 0{\cdot}83 \times 10^6\,\Omega$ (S.D., $n = 21$). Considering the cell to be a sphere $100\,\mu$ in diameter, a figure of about $520\,\Omega\,cm^2$ is obtained for the 'apparent' specific membrane resistance. The time constant calculated from the time course of the membrane potential change produced by a small inward current step was 98 ± 43 msec (S.D., $n = 10$ cells). The average 'apparent' specific membrane capacity for the same cells was $180\,\mu F/cm^2$. The specific membrane resistance mentioned above is the same order of magnitude as that obtained in some other nerve cells (Hagiwara, 1960), although the estimate of membrane capacity tends to be higher. Membrane surface area may be underestimated by an indeterminate amount by assuming the cell to be a $100\,\mu$ sphere, because numerous microvilli with an unknown amount of surface area have been reported on the distal surface of the cell (Fahrenbach, 1965).

Membrane changes during illumination. Fig. 6 shows membrane currents associated with illumination during voltage clamp at various membrane potentials. The series of records in each of the three columns show experimental results from the same receptor cell at one of three different light intensities. The first row (*V*) shows potential changes during illumination obtained without voltage clamp and the remaining records show membrane current associated with a step voltage pulse from the resting membrane potential to the level indicated adjacent to the current traces; a light step was applied about 150 msec following the onset of the voltage pulse and was terminated about 300 msec preceding the end of the pulse. The light-initiated membrane current (total membrane current with illumination minus membrane current without illumination) was inward at the resting

potential (second row) and had a time course similar to that of the potential change obtained in the absence of voltage clamp (see the first row). The amplitude of the light-initiated inward current decreased as membrane potential was shifted to more positive values (reading downward in each column) and became zero at about +29 mV; above this membrane potential light-initiated membrane current changed sign from inward to outward.

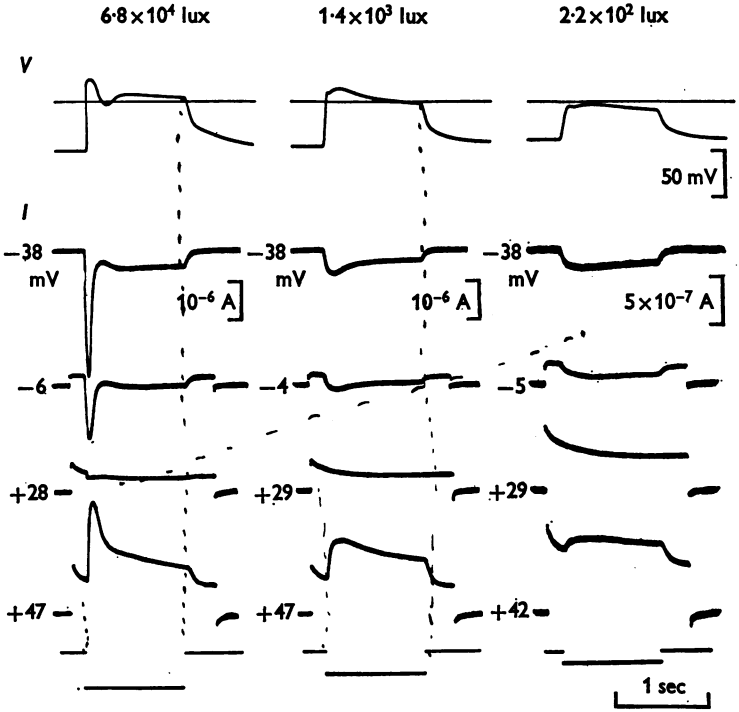

Fig. 6. Top row. Membrane potential changes (V) associated with three different intensities of illumination; light intensity is indicated above each record in lux. Zero membrane potential is indicated by the horizontal lines. I. Voltage clamp records of membrane current during step changes of membrane potential from −38 mV to the level indicated to the left of each record. Steps of illumination (bottom traces) were applied during the changes of membrane potential; inward current is displayed downward.

Current–voltage relations for the light-initiated current obtained at various times after the onset of a step of light are shown in Fig. 7*A*. The results show the following: (1) the current–voltage relation at a fixed time is always non-linear and (2) the slope conductance at a given membrane potential is time dependent, i.e. it increases from 50 to 100 msec and then decreases with time in the case of Fig. 7*A*; the particular time sequence is dependent upon light intensity. (3) All the relations intersect at approxi-

mately the same point on the potential axis. In other words, the membrane potential for zero net light-initiated current (reversal potential) for a given light intensity is constant and independent of the time after the onset of illumination.

A comparison of the *I–V* relations obtained from the same cell at a fixed time following the onset of illumination (50 msec) but at different light intensities is shown in Fig. 7*B*. Although the slope conductance at a given

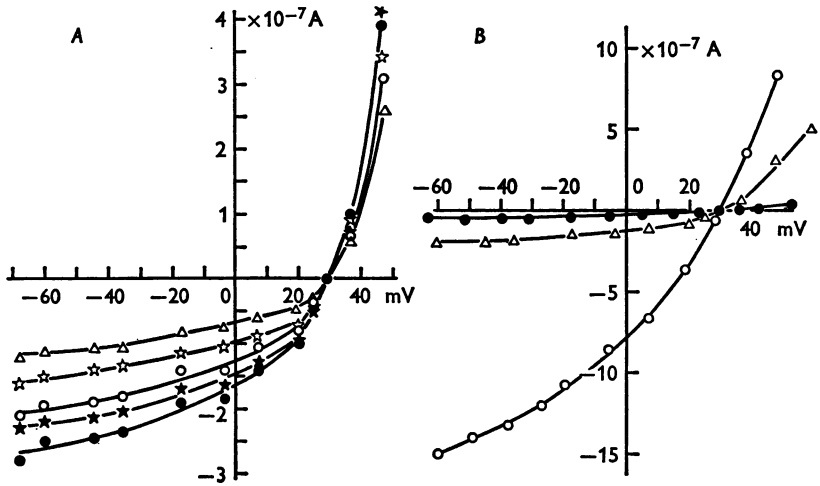

Fig. 7. *A*. The relationship between light-initiated membrane current and voltage-clamped membrane potential at different times following the onset of illumination (○, 50 msec, ●, 100 msec, ★, 200 msec, ☆, 400 msec, △, 1000 msec); light intensity, $1{\cdot}4 \times 10^3$ lux. *B*. Relationship between light-initiated membrane current and membrane potential, 50 msec after the onset of illumination, for three intensities of illumination (○, $6{\cdot}8 \times 10^4$ lux, △, $1{\cdot}4 \times 10^3$ lux, ●, $2{\cdot}2 \times 10^2$ lux).

membrane potential increased as light intensity was increased, the reversal potential remained constant and thus was independent of light intensity. From these results it is concluded that the membrane potential at which the light-initiated current reverses its sign is invariant with the light intensity as well as the time after the onset of illumination. The mean value of the reversal potential in normal barnacle saline was $+26{\cdot}9 \pm 5{\cdot}0$ mV (S.D., $n = 33$).

The foregoing results suggest that the membrane change during illumination is an increase of membrane conductance in series with a fixed membrane e.m.f. and, as a consequence of the conductance increase, membrane potential during illumination approaches a fixed value. Furthermore, the non-linear current–voltage relations indicate that the membrane conductance during illumination is membrane potential dependent.

In the experiments described above, the membrane potential had been altered before illumination and was maintained at a constant level throughout the period of illumination. In the following experiments attempts were made to obtain instantaneous current–voltage relations for the light-initiated current by altering the membrane potential during the period of illumination. The membrane potential was shifted from a constant level to various other levels at a given time after the onset

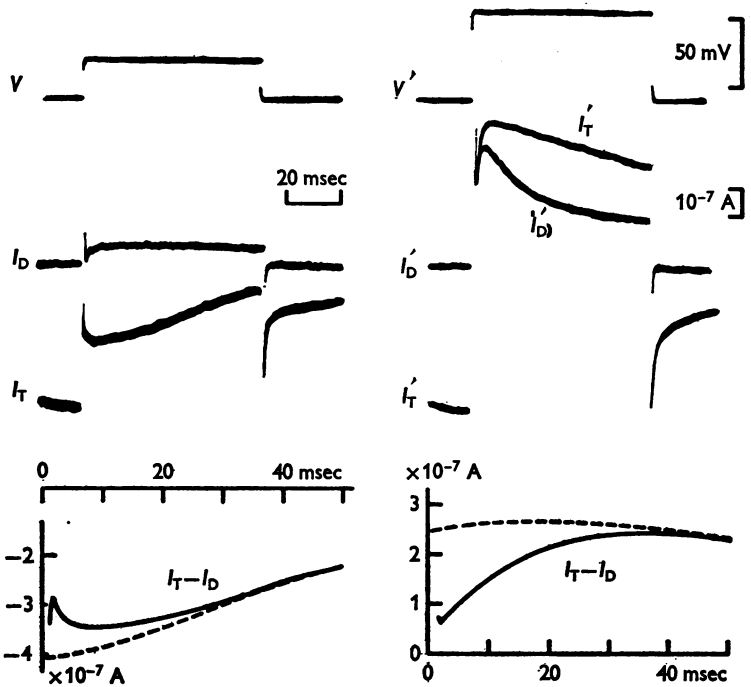

Fig. 8. The early time course of membrane current during step changes of membrane potential from the resting level to that shown in V and V'. I_D and I_D' show membrane current without illumination; I_T and I_T' show membrane current during illumination. Voltage steps were applied 60 msec after the onset of a light pulse. Continuous lines in the lower graphs show the early time course of light-induced current (I_T–I_D and I_T'–I_D'). The dashed curves represent the expected current if membrane potential was clamped to values shown in V and V' before and during the time of illumination.

of illumination. Membrane current due to illumination, immediately after the shift of membrane potential, was determined as a function of the membrane potential at the second level. The records in Fig. 8 show examples in which the first membrane potential level was the resting potential (−32 mV) and the voltage shift to two different levels was applied about 60 msec after the onset of illumination. In this experi-

ment the second step was maintained for the relatively brief period of 70 msec. The second potential levels, in V and V' were, -4 and $+31$ mV respectively. The light-initiated current following the shift of membrane potential to the second level was determined as before, i.e. by subtracting the membrane current without illumination (I_D or I_D') from total membrane current with illumination (I_T or I_T'). The light-initiated current thus determined from the two groups of records is shown by continuous lines in the graphs designated $I_T - I_D$ and $I_T' - I_D'$ in Fig. 8 below each set of

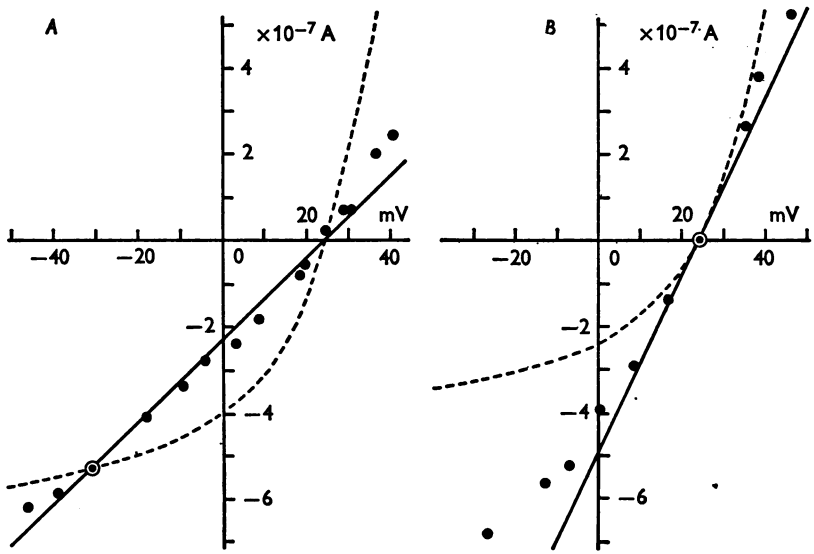

Fig. 9. Instantaneous $I-V$ relations during illumination. Current was measured 2·0 msec after membrane potential was shifted from the initial resting level (double circle in A), or from the reversal potential (double circle in B), to various new values of membrane potential (filled circles). Dashed curves in A and B represent $I-V$ relations obtained by the conventional voltage-clamp method (steady-state $I-V$ relations).

records. Since the membrane currents with and without illumination were associated with an identical potential change, the capacitative current should cancel out. Dashed lines in the bottom graphs in Fig. 8 show light-initiated membrane currents when the membrane potential was maintained at the level of the second step throughout the period of illumination (-4 mV and $+31$ mV). The continuous curves deviate significantly from the broken curves just after the shift of membrane potential to the second level and approach the level shown by the dashed curves in about 30–40 msec. Since the actual rise time of the membrane voltage step in these voltage clamp experiments was about 1·5 msec, the light-initiated membrane current, 2 msec after the onset of the second step, was measured and

plotted as a function of the second membrane potential as shown in Fig. 9 (filled circles and continuous line). The dashed curve in the same Figure shows the $I-V$ relation 60 msec after the onset of illumination obtained by the conventional voltage–clamp method in which the membrane potential was altered before the start of illumination and was maintained at a constant level throughout that period. The latter relation can, therefore, be called a 'steady-state $I-V$ relation' whereas the former can be referred to as an 'instantaneous $I-V$ relation'. The terms 'steady state' and 'instantaneous' as used here refer to time variation of membrane current with respect to the shift in membrane potential but not to illumination. The double circle in the instantaneous $I-V$ relation represents the current obtained when the membrane potential was kept at the first potential level (in Fig. 9*A* it was -32 mV) and, therefore, the instantaneous relation should coincide with the steady-state relation at this point. Fig. 9*A* shows that the instantaneous relation is almost linear and intersects the potential axis approximately at the reversal potential of the steady-state relation. Fig. 9*B* shows the results of an experiment performed in a similar manner except that the membrane potential was held at the reversal potential of the cell ($+24$ mV) and shifted from this potential, at which there was no light-initiated current, to various other levels. The instantaneous $I-V$ relation obtained from such a procedure is also linear and the slope is very close to that of the tangent of the steady-state relation at the reversal potential. If the $I-V$ relation was plotted at a time slightly greater than 2 msec after the onset of the second voltage step it deviated from the straight line in such a way that the relation tended to approach the steady-state relation. This tendency became more marked with increasing time after the onset of the second voltage step. In order to estimate the relaxation time of the membrane potential dependent conductance, the difference between the continuous and broken curves in each of the two graphs at the bottom of Fig. 8 was plotted on a logarithmic scale against the time after the onset of the second voltage step. The plots approximated a straight line and the time constant estimated from the slope ranged from 13 to 15 msec.

As shown in Fig. 9, membrane conductance during illumination depends on the membrane potential at which the membrane was clamped by the holding pulse and this conductance is in good agreement with the chord conductance obtained from the steady-state current–voltage relation. Thus from Fig. 7 it is evident that the chord conductance also depends upon light intensity and the time after the onset of illumination.

Chord conductance, calculated 50 msec after the onset of illumination as a function of membrane potential, is shown in Fig. 10 for three

different light intensities. The conductance increases as the membrane potential becomes more positive. Saturation of membrane conductance was not observed in the range of membrane potentials shown. At the resting potential in this cell (-38 mV) membrane conductance in the absence of illumination was 1×10^{-6} mho, whereas the light-initiated conductance for the three light intensities, 50 msec from the onset of the light pulse, was 1×10^{-6}, 2×10^{-6} and 20×10^{-6} mho respectively at the

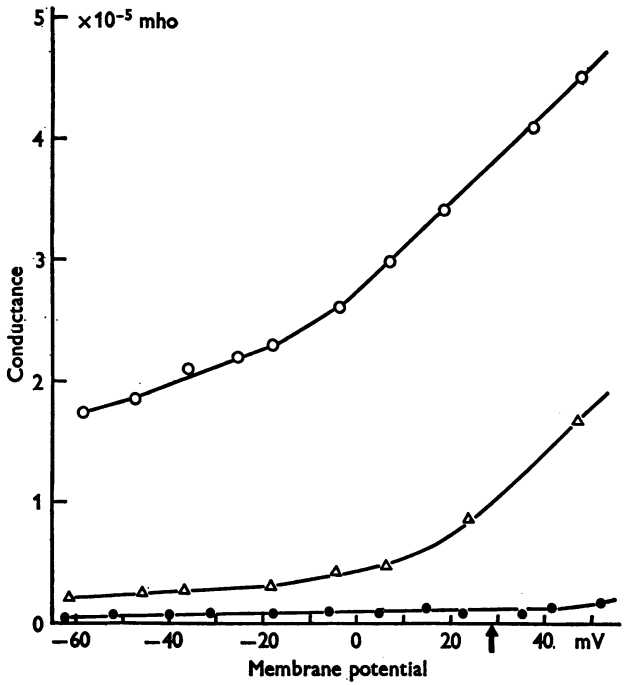

Fig. 10. Chord conductance [$g = I_L/(E_m - E_{rev})$] as a function of membrane potential, 50 msec after the onset of three intensities of illumination: open circles, $6{\cdot}8 \times 10^4$ lux; triangles, $1{\cdot}4 \times 10^3$ lux; filled circles $2{\cdot}2 \times 10^2$ lux. The arrow indicates the reversal potential.

same membrane potential. This indicates that the membrane conductance was increased by factors of 2, 3 and 21 for the three intensities of illumination.

Although the slope of the current–voltage relations at a given membrane potential varied with the light intensity as well as the time after the onset of illumination, the general shape of the relations under different conditions appeared similar. To examine the degree of similarity, I–V relations of the same cell at different light intensity and time after the onset of illumination were normalized by taking the slope at the reversal potential as unity. The results obtained showed that the agreement was usually not

satisfactory among such relations in that systematic deviations were obtained at early times during the response at all light intensities. In this paper, the details of this problem will not be discussed.

Effect of ion substitutions on the current–voltage relations

Sodium. The three records in Fig. 11 *A* designated *V* were obtained from the same receptor cell when the normal Na^+ concentration (462 mM) in the

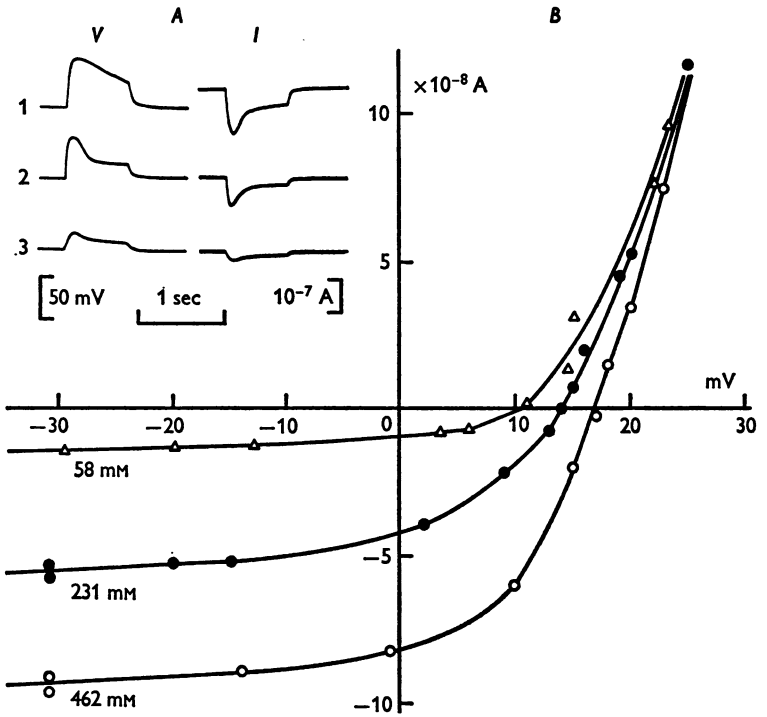

Fig. 11. *A.* Records of light-produced membrane potential changes (*V*) and membrane current (*I*) when the membrane was clamped at the resting potential in the presence of Na^+ concentrations of 462 mM (1), 231 mM (2), 58 mM (3). *B.* Relations of light-initiated membrane current, measured 100 msec after the onset of light, and voltage-clamped membrane potential at the Na^+ concentrations indicated adjacent to each curve.

external saline was reduced to 231 (2) and 58 mM (3) by replacing the appropriate amount of the normal barnacle saline with Tris-saline. Although no significant change was observed in the resting potential, the potential change produced by illumination of a given intensity was greatly reduced by reduction of the Na^+ concentration. In this experiment, the peak amplitude of the response became 82 % of that in normal saline at 231 mM and 37 % at 58 mM. The response was not abolished completely even when

the total external Na was replaced with Tris-Cl. As a rule, the peak and steady amplitude of the response in the Na-free saline was about 40 and 22 % of that found with the normal concentration of Na; however, in a few cases, the peak and steady phase were reduced to between 10 and 15 %, with complete recovery when returned to normal saline. The records designated *I* in the same Figure show membrane current obtained from the same cell at the same light intensity when the cell was voltage clamped near the resting potential level. The inward light-initiated current decreased as the concentration of external Na ions was decreased and this diminution was more marked than that found for the amplitude of membrane potential changes as Na^+ concentration was reduced. The changes described above always reached a steady state within 5 min after the application of the test solution and no progressive changes were observed within the following 30 min. The effects of reduced Na usually were reversible when normal barnacle saline was returned to the preparation.

Current–voltage relations obtained from another cell at three different Na^+ concentrations are shown in Fig. 11*B*. The light intensity was 10^4 lux and all the relations were taken 100 msec after the onset of illumination. These relations show the following. (1) The inward light-initiated current is greatly reduced by reduction of the external Na^+ concentration, and in contrast to this, (2) little change is found in the outward light-initiated current, and (3) the reversal potential for membrane current shifts in the negative direction as the external Na^+ concentration is decreased. In Fig. 13*A*, inward membrane current at -30 mV and outward membrane current at $+25$ mV were plotted against the external Na^+ concentration. Inward current (bottom graph) decreased linearly with decreasing external Na^+ concentration and, for this particular cell, the straight line extrapolated near the origin. In other words, inward current at a membrane potential sufficiently more negative than the reversal potential is proportional to the external Na^+ concentration. At the reversal potential currents carried by the inward movement of external ions and the outward movement of internal ions are equal in amplitude and opposite in sign. At a sufficiently negative membrane potential, the membrane current is mainly carried by inward movement of external cations and outward movement of internal anions; therefore, the above result suggests that the inward membrane current is carried almost exclusively by the inward movement of Na^+ ions. In some cases, however, there remained a significant amplitude of inward membrane current upon extrapolation of the curve to zero Na^+ concentration. The average amplitude of the residual inward current estimated for zero external Na^+ concentration in seven preparations was 16 ± 11 % (S.D.) of the current found in the normal Na^+ concentration. This corresponds to the fact that the response to light was

reduced significantly but not abolished even in the absence of external Na ions. In these experiments NaCl was replaced with Tris-Cl. Similar residual currents were also found when the NaCl was replaced by sucrose, urea and choline chloride.

In contrast to the behaviour of inward current as the external Na was changed, the outward current at +25 mV was almost independent of the Na^+ concentration. Since the reversal potential in normal saline was +17 mV in this particular cell, this membrane potential was only 8 mV more positive than the reversal potential. With the present voltage-clamp system it was not possible to obtain large outward membrane currents at membrane potentials substantially more positive than the reversal potential. Extrapolation of the current–voltage relations in Fig. 11*B* suggests that all three relations coincide at membrane potentials slightly more positive than it was possible to test.

The foregoing results suggest that the major change in the receptor membrane during illumination is a permeability increase to Na^+ ions. If the change is exclusively due to a permeability increase to Na^+ ions, the reversal potential of the membrane should behave like that of a Na electrode. The changes in the reversal potential from normal solution to various other external Na^+ concentrations are plotted in Fig. 14*A* (filled circles) as a function of the logarithm of the Na^+ concentration. These data were obtained from five cells. The slope corresponds to a 10–15 mV change in the reversal potential for a tenfold change of Na between 462 and 231 mM, and below these concentrations the slope tends to diminish. These values for the slope are much smaller than that expected from a Na electrode. The dependence of the reversal potential upon the Na^+ concentration was increased when the effect of reduced Na was examined in Ca-free media (20 mM-Ca in the normal saline replaced with Mg). Changes of the reversal potential obtained under this condition for two different cells are shown by open circles in Fig. 14*A*; changes in the reversal potential were determined with respect to the reversal potential in normal saline. The slope between 462 and 231 mM ranged between 16 and 21 mV for tenfold increase of Na^+ concentration. These values are still substantially smaller than the Nernst slope of 58 mV. The behaviour of the reversal potential of this membrane is quite different from that of the spike potential producing membrane of the squid axon in which the major inward current is also carried by Na^+ ions. Another difference between the light response of the barnacle photoreceptor cell and the spike potential of the squid was the effect of tetrodotoxin. Sodium permeability, which underlies production of a spike, is effectively suppressed by tetrodotoxin at a concentration as low as 5×10^{-8} g/ml. in the external solution (Narahashi, Deguchi & Urakawa & Ohkubo, 1960). By contrast the light response of the barnacle

photoreceptor was unaltered even when the concentration of tetrodotoxin in the external solution was raised to 10^{-4} g/ml.

Calcium. The records designated *V* in Fig. 12*A* show membrane potential changes to a light pulse when the photoreceptor was bathed in saline containing Ca concentrations of (1) 32, (2) 20, and (3) 2 mM. The con-

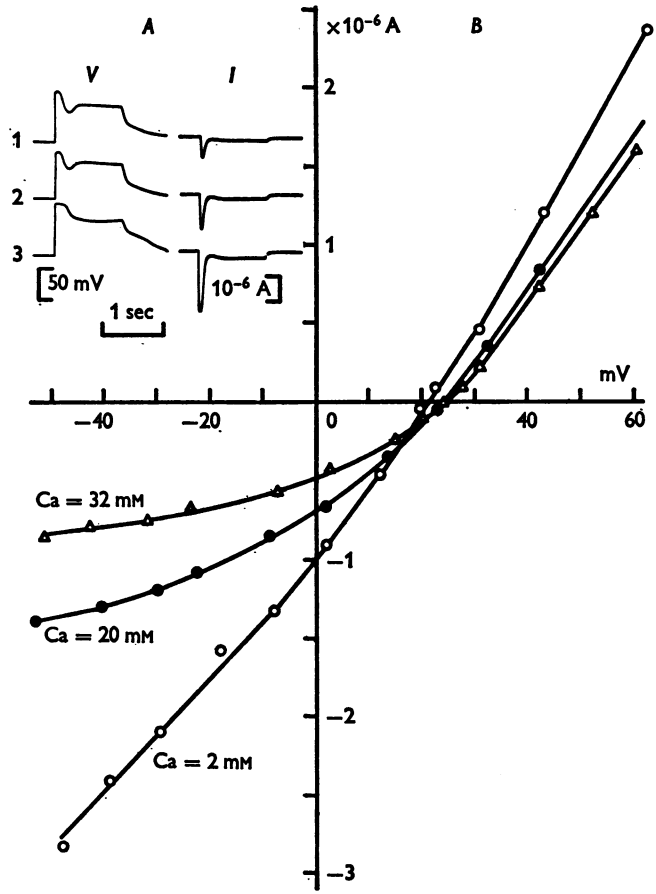

Fig. 12. *A.* Records of light-produced membrane potential changes (*V*) and membrane current (*I*) when the membrane was clamped at the resting potential in the presence of Ca^{2+} concentrations of 32 mM (1), 20 mM (2), and 2 mM (3). *B.* Relations of light-initiated membrane current, measured 5 msec after the onset of light, and voltage-clamped membrane potential at the Ca^{2+} concentrations indicated adjacent to each curve.

centration of Ca was altered by mixing Ca and Mg salines in appropriate amounts. Alteration of the external Ca^{2+} concentration in this range did not significantly change the resting membrane potential in the absence of illumination. The records indicated by *I* in the same Figure show membrane

currents obtained under voltage–clamp conditions near the resting membrane potential at the same three Ca^{2+} concentrations. The amplitude of the potential change was increased as the external Ca^{2+} concentration was decreased. It was most pronounced during the steady phase of the light response. In contrast to this, the membrane current at both the peak and the steady phase increased significantly.

Fig.12*B* shows current–voltage relations obtained from the same cell at three different Ca^{2+} concentrations. The light intensity was $6{\cdot}8 \times 10^4$ lux and all the relations were obtained from records 50 msec after the onset

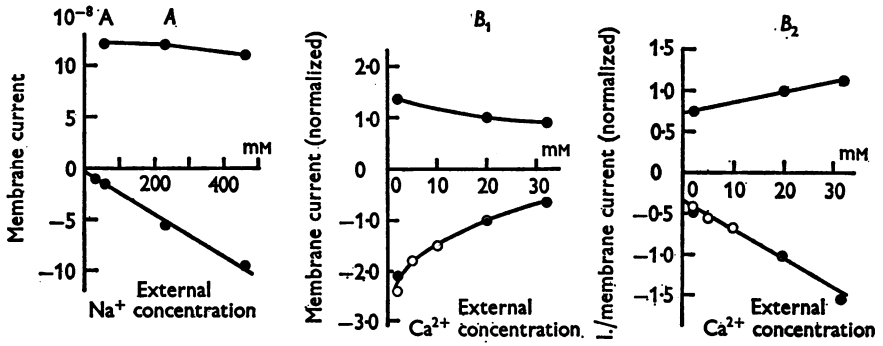

Fig. 13. *A*. Inward (negative) and outward (positive) membrane current measured at membrane potentials of -30 mV and $+25$ mV respectively as a function of external Na^+ concentration (from Fig. 11). B_1. Relative amounts of inward and outward membrane current at membrane potentials of -50 mV and $+55$ mV respectively as a function of Ca^{2+} concentration. Filled circles represent data from the I–V relation of Fig. 12; open circles are from another cell (normalized to membrane current in 20 mM Ca). B_2. Reciprocal of membrane current shown in B_1 as a function of Ca^{2+} concentration.

of illumination. These I–V relations show that (1) inward as well as outward current at a given membrane potential increases as Ca^{2+} concentration is decreased and (2) the reversal potential is altered very little by the change of Ca^{2+} concentration. The amplitude of inward and outward current at membrane potentials of -50 mV and $+55$ mV respectively were plotted against the external Ca^{2+} concentration in Fig. 13B_1 (filled circles). Inward current data from a second preparation (open circles) are also plotted in this Figure. Membrane currents were normalized by setting membrane current at 20 mM-Ca to unity. Since both inward and outward membrane current increased in low Ca^{2+} concentrations the primary action of external Ca seems to be suppression of membrane current. Fig. 13B_1 shows that the relationship between the membrane current and Ca^{2+} concentration is not linear but resembles a rectangular hyperbola. This suggests a possible mechanism of suppression. As shown previously, the major

inward current seems to be carried by Na^+ ions and from the linear relation between membrane current and Na^+ concentration it seems unlikely that Na^+ ions permeate a fixed charge membrane. Rather, the more likely mechanism of permeation is through neutral membrane sites or via neutral molecular carriers. If Ca^{2+} ions also bind competitively to the same carriers or sites with a much higher binding constant and if the binding follows the Langmuir isotherm the concentration, X_{Na}, of Na-carrier or Na-site complex should be given by:

$$\frac{\bar{X}}{X_{Na}} = \left(1 + \frac{k_{Na}}{[Na^+]_{out}}\right) + \frac{k_{Na}}{k_{Ca}[Na^+]_{out}} \cdot [Ca^{2+}]_{out}. \quad (1)$$

Here, $\bar{X}$ is the total concentration of carriers or sites; k_{Na} and k_{Ca} are the dissociation constants of Na^+ and Ca^{2+} to carriers or sites. The effect of Mg^{2+} is neglected. If membrane current is proportional to X_{Na}, the relation between membrane current and $[Ca^{2+}]_{out}$ should be a rectangular hyperbola since we are considering the case in which $[Ca^{2+}]_{out}$ is varied at constant $[Na^+]_{out}$. The same data shown in Fig. 13B_1 were replotted in Fig. 13B_2, the reciprocal of membrane current on the y-axis and the Ca^{2+} concentration on the x-axis. Each set of plots is a good approximation to a straight line.

In Fig. 14B the changes in the reversal potential from normal saline to various other Ca^{2+} concentrations are shown as a function of the logarithm of calcium concentration (filled circles); data is shown for two different cells. Although ΔE_{rev} is fairly independent of Ca^{2+} concentration, there seems to be a tendency of the reversal potential to change slightly in the negative direction as the external Ca^{2+} concentration was reduced. This tendency was greatly augmented when the Ca^{2+} concentration was altered in the absence of Na^+ ions (Tris saline) as illustrated by open circles in the same Figure. For three different cells, the slope obtained between 20 and 10 mM was 15, 17 and 20 mV for a tenfold increase in concentration. However, the slope had a tendency to become less as the Ca^{2+} concentration decreased. The 20 mV slope is not much smaller than that expected from a Ca electrode (29 mV).

Potassium and chloride. Changes in the external K^+ concentration altered the resting potential, i.e. the membrane potential in the absence of illumination. Fig. 15 shows the relation between the resting potential and the external K^+ concentration when an increasing amount of the NaCl in the normal saline was replaced with KCl. For the range of K^+ concentration between 50 and 250 mM the potential increased linearly with the logarithm of the K^+ concentration and the slope was about 45 mV for a tenfold increase in concentration. The membrane potential attained its final level within a few minutes after the test solution was applied. In a few cases

experiments were performed where the product between $[K^+]_{out}$ and $[Cl^-]_{out}$ was kept constant by substituting Cl^- with methane sulphonate and practically the same result was obtained. This evidence suggests that the Cl^- permeability of the resting receptor membrane is much smaller than the K^+ permeability.

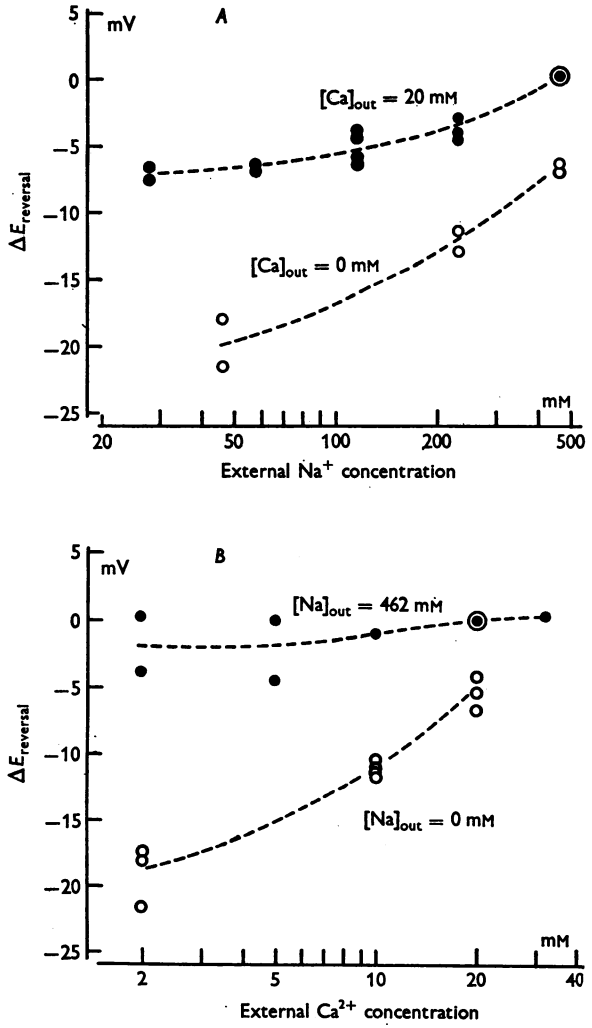

Fig. 14. *A*. Reversal potential changes ($\Delta E_{reversal}$) from the value obtained in normal saline (double circle) to various other concentrations of Na as a function of external Na^+ concentration. Filled circles: external Ca^{2+} concentration 20 mM; open circles: external Ca^{2+} concentration 0 mM. *B*. Same as *A* except Ca^{2+} concentration was changed. Filled circles: external Na^+ concentration 462 mM; open circles, 0 mM-Na. Dashed curves are drawn by eye.

Current–voltage relations for the light-initiated current were examined in the same cell before and after replacement of 462 mM-NaCl in the normal saline with Na isothionate. No detectable changes were found in the *I–V* curves. The effect of substitution of NaCl with KCl on the light-initiated current was also examined by using the voltage–clamp technique. The membrane potential was held at the resting level found in normal saline

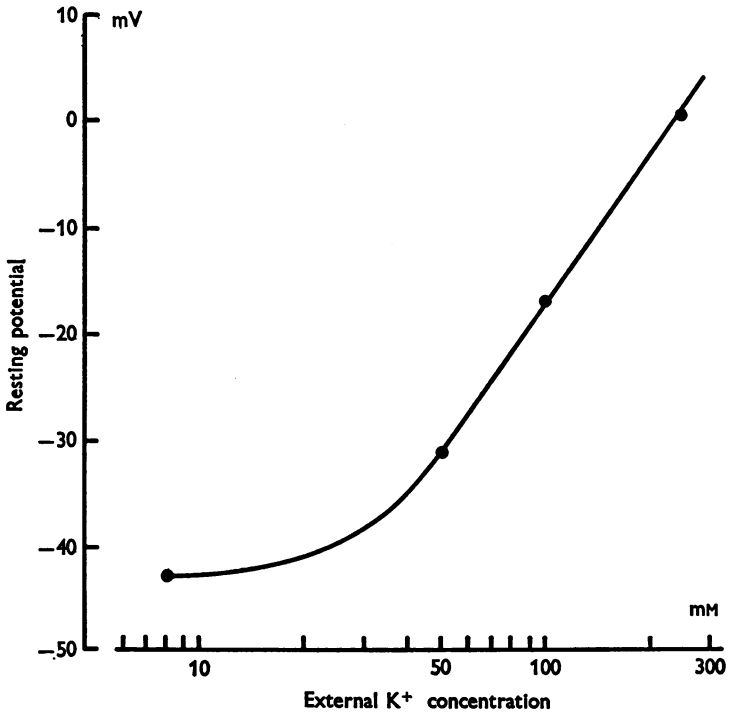

Fig. 15. Resting membrane potential as a function of the logarithm of external K^+ concentration; K^+ ions replaced with Na^+ ions.

throughout the experiment. The results showed that the effect of substituting NaCl with KCl (at least up to 250 mM) is practically the same as that obtained by substituting Tris-Cl or sucrose for NaCl. This suggests that no significant fraction of the light-initiated current is carried by the inward movement of external K^+ ions.

DISCUSSION

Recently the hypothesis has been proposed that the depolarizing receptor potential of photoreceptors in the *Limulus* ventral eye is produced by deactivation of an electrogenic Na pump (Smith *et al.* 1968). This hypothesis does not seem applicable to the barnacle photoreceptor membrane

for the following reasons. The instantaneous $I-V$ relationships (Fig. 9*A*, *B*) showed that light initiates a membrane change that has all the characteristics of a conductance change. If alteration of voltage dependent electrogenic pump current by light was the prevailing mechanism for the response in the barnacle photoreceptor, these results would not be expected. Rather, the instantaneous current–voltage relation should have had a zero slope, i.e. the light-initiated current should have been the same immediately following a voltage change if the voltage dependent electrogenic pump current had a sufficiently long relaxation time. However, if the relaxation time was brief, the relation should have followed the non-linear 'steady state $I-V$ relation'. Neither case was observed in the present experiments. The effects of ouabain on the receptor potential are also relevant to the present discussion. Ouabain at a concentration of 10^{-5} M abolished the afterhyperpolarization that has been observed to occur following cessation of illumination (Brown *et al.* 1968). Recent evidence indicates that this afterhyperpolarization is due to activation of an electrogenic Na pump (H. Koike, H. Mack Brown & S. Hagiwara, in preparation). However, the same concentration of ouabain has a negligible effect on the depolarizing receptor potential. Therefore, it can be concluded that operation of the 'pump' is not obligatory for the mechanism that generates the depolarizing receptor potential. Only after longer application or higher concentrations of ouabain can any effect on the depolarizing receptor potential be observed and this effect can be attributed in part to a reduced Na^+ concentration gradient.

A conductance increase during illumination was evident in barnacle photoreceptor cells from the results of the instantaneous voltage-clamp experiments. In photoreceptors of the *Limulus* ventral eye, there was no detectable conductance increase, or even a decrease, when conductance was measured during the steady phase of the depolarizing receptor potential by applying constant current pulses (Smith *et al.* 1968). It was difficult to detect a conductance increase in barnacle photoreceptors under the same experimental conditions. In this regard, it should be recalled from the present experiments that the relaxation time constant of the conductance change measured by the instantaneous voltage clamp is brief (15 msec) compared to the time constant of the resting membrane (100 msec). Therefore, membrane conductance measured by constant current methods is necessarily a measure of the slope conductance of the 'steady-state' $I-V$ relation. The slope conductance of the 'steady-state' $I-V$ relation for light-initiated current is significantly less than the 'instantaneous' conductance in the physiological range of membrane potentials (resting potential to about +10 mV, see Fig. 7), especially for the steady phase of the depolarizing receptor potential, whereas the slope of the $I-V$ relation

without illumination (Fig. 5) tends to be greater in the same membrane potential range. Therefore, 'total slope conductance' measured by applying constant current pulses is dominated by the slope conductance of the light insensitive component. However, in no case was a decrease in membrane conductance during illumination observed in the barnacle photoreceptor.

Photoreceptors in the *Limulus* ventral eye and the barnacle ocellus may operate on different principles. One exceptional experimental difference between them is their behaviour in Na-free media. In *Limulus*, the response to illumination diminishes or disappears upon removal of external Na^+ ions (replaced with Tris or Li) but this is always transient and the response reappears after 4–5 min (Millecchia *et al.* 1966; Smith *et al.* 1968). In the barnacle receptor, however, the response is not transiently abolished and there is no increase of the response with time in Na-free solution.

The experimental results show that inward current during illumination is linearly related to the concentration of Na^+ ions in the external medium and that the reversal potential for the light-initiated current decreases as external Na^+ concentration is decreased. These findings suggest that the conductance increase during illumination is due to a permeability increase to Na^+ ions. There is good general agreement from experiments on other receptors that Na is required for generation of receptor potentials (Diamond, Gray & Inman, 1958; Kikuchi *et al.* 1962; Edwards, Terzuolo & Washizu, 1963; Hamasaki, 1963; Ottoson, 1964; Calma, 1965; Obara & Grundfest, 1968). However, the experimental results in the present paper do not support the idea that the permeability increase is due exclusively to Na^+ ions. Although light-initiated inward current is approximately proportional to the Na^+ concentration, there is usually a small but significant residual current at zero Na^+ concentration in the external medium. Even though the dependence of the reversal potential upon the external Na^+ concentration was augmented from normal Ca to Ca-free media (from 10–15 to 16–21 mV for a tenfold change in concentration), this slope remains much smaller than that expected from the Nernst equation (58 mV). This indicates that during illumination the membrane becomes permeable not only to Na^+ ions but also to some other ion species. The Na^+ concentration was reduced by substituting NaCl with either Tris-Cl or sucrose and essentially the same changes in the reversal potential were obtained. This excludes a significant permeability to Tris ions, which is in contrast to the finding by Obara (1968) on the crayfish stretch receptor.

The primary effect of external Ca^{2+} ions appears to be suppression of membrane current. This agrees with the result obtained from other receptors (Fulpius & Baumann, 1969). Yet the fact that the dependence of the reversal potential on Ca^{2+} concentration in Na-free medium is as high as 20 mV for a tenfold increase in concentration may indicate that

the second permeable ion species in question is Ca. A small permeability increase to Ca^{2+} ions has been suggested previously for the active phase of the end-plate potential (Takeuchi, 1963) and a Ca^{2+} permeability increase has been proposed for the spike potential of the giant nerve cell in *Aplysia* by Junge (1967) and Geduldig & Junge (1968). The experiments of the latter workers showed that the dependence of the membrane potential at the peak of the spike upon Na^+ or Ca^{2+} concentration is substantially smaller than the Nernst slope if the concentration of one species is altered in the presence of the other. However, the slope approaches the Nernst slope if changes in either one of them is examined in the absence of the other. The results obtained from the barnacle photoreceptor agree with their results as far as the dependence upon Ca^{2+} ions is concerned; however, the dependence of the reversal potential on Na^+ ions is too low even when examined in Ca-free media. Since the slope tends to diminish as the external Na^+ concentration decreases, the above small dependence cannot be explained in terms of saturation of binding between Na^+ ions and carriers or sites of a finite concentration. A difficulty with assuming Ca^{2+} permeability is the following. The residual inward current found in Na-free media increases rather than decreases when the external Ca^{2+} concentration is reduced. This indicates that the inward current carried by Ca^{2+} ions is very small if it does exist. At the present stage we can safely conclude that the conductance increase during illumination is due mainly to a permeability increase to Na ions. However, further conclusions regarding the nature of the reversal potential changes in different concentrations of Na must await the completion of additional experiments.

A paper (Millechia & Mauro, 1969), 'The ventral photoreceptor cells of *Limulus*. III. A voltage–clamp study', has appeared while the present paper was in preparation dealing with a voltage–clamp study of the depolarizing receptor potential of photoreceptors in the *Limulus* ventral eye. The I–V relations obtained by their methods appear qualitatively similar to those of the barnacle photoreceptor.

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