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EFFECT OF SODIUM AND POTASSIUM IONS ON THE ELECTRICAL ACTIVITY OF SINGLE CELLS IN THE LATERAL EYE OF THE HORSESHOE CRAB

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It is generally accepted that sodium is responsible for the generation of the action potential in nerve and muscle fibres of many animals. However, other cations may be substituted for sodium under some experimental conditions (Lorente de Nó, 1949; Fatt & Katz, 1953; Lorente de Nó, Vidal & Larramendi, 1957; Fatt & Ginsborg, 1958; Koketsu, Cerf & Nishi, 1959). Moreover, it has been suggested that in certain insects a different mechanism of the generation of impulses may operate, since they contain only traces of sodium (Wigglesworth, 1947).

Recently Diamond, Gray & Inman (1958*b*) have shown that the amplitude and rate of rise of the receptor potential, recorded from the Pacinian corpuscles of the cat, were related in a graded manner to the concentration of sodium ions in the perfusing solutions. This evidence is consistent with the hypothesis that the receptor potential is due to a change in the permeability of the membrane to sodium ions or other ion species.

As has already been reported (Kikuchi & Tazawa, 1960), the retinal slow potential—termed by Hartline, Wagner & MacNichol (1952) the ommatidial action potential (OAP)—recorded from single ommatidia in the lateral eye of the horseshoe crab has many properties in common with the receptor potentials of Pacinian corpuscles in the cat (Gray & Sato, 1953) and of stretch receptor in the frog (Katz, 1950) and lobster and crayfish (Eyzaguirre & Kuffler, 1955).

Since the effect of sodium and potassium on the action potentials in nerve and muscle fibres has been studied by many authors, the aim of this investigation was to obtain clues to the ionic basis of the generation of the slow action potential and the spike potential arising within single ommatidia of the lateral eye of the horseshoe crab. In the present paper, however, attention was directed mainly to the slow action potential rather than the spike. A preliminary account of some of the experiments described here was given at a General Meeting of the Physiological Society of Japan (Kikuchi & Tanaka, 1956).

METHODS

Preparation. The horsehoe crabs (*Tachypleus tridentatus*) were supplied by the Kouchi Fishing Cooperative Association, Konoshima, Kasaoka. They were caught in the Inland Sea of Japan during May and September, sometimes in December, and kept in a large fish pond of sea water just beside their habitat. At intervals of 2 weeks several animals were sent to the laboratory and kept in a dark room in a small wooden pool of aerated artificial sea water, which was warmed at 12° C in the winter. It seemed to be important to maintain the temperature of the artificial sea water at between 10 and 20° C in order to keep the animals in good condition. Some of them were also kept in the Metropolitan Aquarium. The animals were usually used within 2 weeks after they were sent from their habitat.

The lateral eye of the horseshoe crab was used throughout these experiments. It is a compound eye, such as that of the *Limulus*, i.e. is composed of hundreds of ommatidia. Each ommatidium is made up of an eccentric cell, sometimes two, and retinular cells (cf. Figs. 6, 7 and 8 in Hartline *et al.* 1952; Fig. 1 in Tomita, Kikuchi & Tanaka, 1960). The structure of the ommatidium of *Tachypleus tridentatus* has not yet been so intensely studied as that of the *Limulus* (Miller, 1960). However, as far as has been studied, it seems that there is little difference in their basic structure.

The longitudinal surfaces of ommatidia were exposed by cutting the excised eye perpendicularly to the corneal surface with a sharp razor. The preparation was mounted with a lacquered metal eye-holder and immersed in a transparent Lucite bath which contained the saline for the horseshoe crab (Kikuchi & Tanaka, 1957). The ommatidia were viewed by reflected light. All experiments were carried out at room temperature. The temperature of the horseshoe-crab Ringer's solution in the bath was usually about 2° C lower than the reading of room temperature.

Solutions. The composition of the horseshoe-crab Ringer's solution, determined by analysis of the blood, was as follows (mM): NaCl 420, KCl 10, $CaCl_2$ 10, $MgCl_2$ 25. $NaHCO_3$ for adjustment of the pH of the solution was omitted in these experiments, since it was found that the removal of $NaHCO_3$ seemed to have little effect on the OAP under these experimental conditions. Sodium-free solutions were made by replacing sodium chloride with either choline chloride 500 mM ('choline-Ringer') or sucrose 840 mM ('sucrose-Ringer'), the other ions being maintained at their normal concentrations.

Replacement of the solutions. The removal of the external bathing solution was carried out by suction; the bath was then refilled with the new solution, except in the case of potassium-rich solution. By such a procedure about one-twentieth of the solution in the bath might remain and mix with the new solution. Therefore, in some experiments the replacement with the new solution was repeated.

For raising the external potassium concentration the following method was adopted, to avoid the possible movement of the micro-electrode by repetitive replacing of the solutions. A concentrated potassium solution which was made by dissolving solid potassium chloride in normal horseshoe-crab Ringer's solution was added quantitatively little by little to the external solutions in a known quantity and then the potassium concentration at each step of addition was calculated. The effect of the increase in the osmotic pressure of the solution was neglected.

Intracellular micro-electrodes filled with 3M-KCl were used. Their resistance ranged from 20 to 40 MΩ measured in 3M-KCl with alternating current (50 c/s) bridge. The values of micro-electrode resistance measured in normal Ringer's solution were about one and a half times that measured in 3M-KCl. The resistance of micro-electrodes by which good results were obtained usually was higher than 20 MΩ after the experiments.

The micro-electrode was set up on a micro-electrode manipulator designed by Tomita (cf. Fig. 17, Tomita & Torihama, 1956), which was mounted on the main manipulator made by attaching a small metal box for the input valve instead of the tube of a microscope. The

micro-electrode and the ommatidia were observed with a Greenough microscope (magnification $\times 72$). For the application of current across the membrane the micro-electrode for potential recording was used through a high resistance (Fig. 1).

Stimulation and recording system. The optical system used for the stimulation and recording system for resulting electrical responses was almost the same as that described by Tomita (1956). A magnetic shutter serving as a light valve in front of a slit was operated by a relay circuit, which was triggered by a saw-tooth wave of the sweep generator. The micro-electrode was connected to the cathode-follower input. The grid current of the input valve was less than 10^{-12} A. A conventional direct-coupled amplifier and cathode-ray oscilloscope were employed. As a calibrator, a potentiometer was connected between the bath for the preparation and earth.

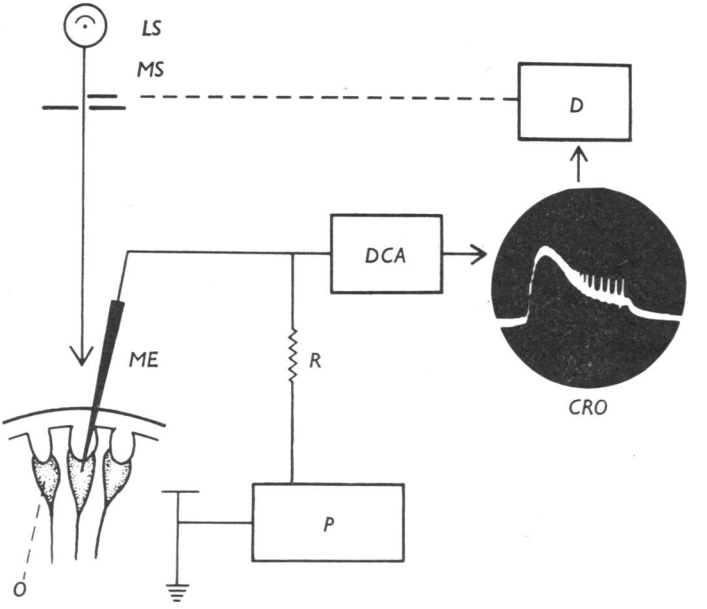

Fig. 1. Diagram of arrangement for illumination, application of polarizing currents and recording. *LS*, light source; *MS*, magnetic shutter; *D*, relay circuit; *ME*, micro-electrode; *R*, 200 MΩ resistance; DCA, DC amplifier; *P*, potentiometer; *O*, ommatidium; *CRO*, cathode-ray oscillograph. For details see text.

RESULTS

General description of OAP in normal medium

The penetration of the micro-electrode into a cell within a single ommatidium was indicated by the sudden appearance of the resting potential, ranging usually between 30 and 40 mV. The values of the resting potential were lower than those of other excitable tissues; this may be partly due to tip potential (Adrian, 1956) and to injury depolarization; since micro-electrodes of relatively high resistance are used without selection, the values of the resting potential were rather widely distributed, and further-

more spontaneous discharges were frequently observed following the penetration of the micro-electrodes.

The micro-electrode was not withdrawn during the course of an experiment on a unit. The resting potential was checked again in normal solution at the end of each experiment by withdrawing the micro-electrode from the ommatidium. The micro-electrode drift remained within several millivolts after about 2 hr. Therefore, the size of the resting potential during the course of an experiment cannot be presented with the same accuracy as the amplitude of the OAP.

By illumination of short duration the OAP appeared as a transient depolarization, with concomitant changes in the amplitude and frequency of the nerve impulses superimposed on the slow depolarization (Fig. 2*A*).

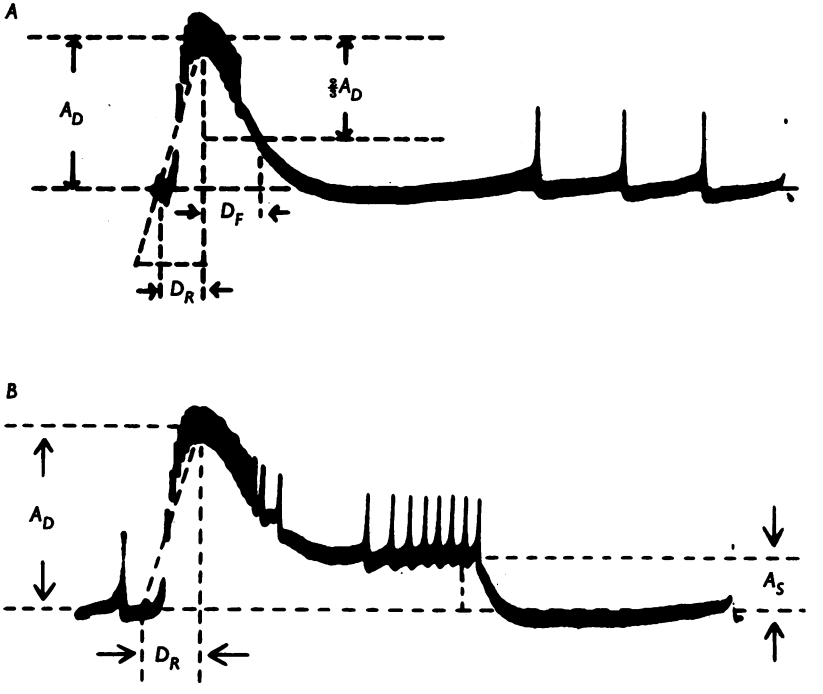

Fig. 2. Diagram illustrating the components of OAP. *A* and *B* were obtained from the same unit as that shown in Fig. 3. Upward and downward pips indicate 'on' and 'off' sign of illumination. In *A* a short flash (duration, 0·024 sec) was applied, and the downward 'off' sign appears before the rising portion of OAP. A_D, amplitude of 'dynamic phase', i.e. distance from base line to the peak of OAP; D_R, duration of rising portion of 'dynamic phase'; D_F, duration of falling portion during which 'dynamic phase' decreases to one third of the maximal amplitude of OAP; A_S, amplitude of 'static phase' measured at the downward 'off' pip. This is not always the same as that of the final depolarization by prolonged illumination, whose value is not influenced by the time in darkness before illumination.

As described before (Kikuchi & Tazawa, 1960), when the duration of illumination was longer than a certain period of time, the initial depolarization was followed by a lower and sustained depolarization (Fig. 2*B*). The form of the OAP was similar to that of stretch potentials recorded from the muscle spindle of the frog (Katz, 1950) and the stretch receptor of the lobster and crayfish (Eyzaguirre & Kuffler, 1955), though in the case of the muscle spindle of frogs the external recording was adopted. For convenience, the OAP was divided into three phases: an initial maximal depolarization or 'dynamic phase' (from the 'on' sign to the end of the falling portion of the initial maximal depolarization); a less but maintained depolarization or 'static phase'; a phase of repolarization or 'off-effect' (potential change following the 'off' sign). The effect of intensity, duration, interval and temperature on the OAP has been discussed elsewhere (Kikuchi & Tazawa, 1960; Kikuchi, Naito & Minagawa, 1961).

Some difficulties for the measurement of the latency, total duration and rate of rise and fall of the OAP were experienced for the following reasons. The OAP was usually complicated by propagated impulses and local responses or abortive impulses, and sometimes followed by a transient positive potential change or after-hyperpolarization as the stretch potential recorded from muscle spindle (Katz, 1950). The after-hyperpolarization often induced the production of local responses and spike potentials. Moreover, the rising portion of the OAP was rounded and not straight as that of the receptor potential of the Pacinian corpuscles (cf. Fig. 4 in Inman & Peruzzi, 1961).

The measurement of the falling portion of the 'dynamic phase' was more difficult, for its final part was followed by the 'static phase' when a prolonged illumination was applied. The separation of the 'dynamic phase' and 'static phase' was not always clear, especially in records obtained at low temperature or by illumination of low intensity. Hence the rate of fall was calculated only on responses obtained by short flashes of suitable intensity, and expedient measures were adopted, as seen in Fig. 2.

Procaine or other local anaesthetics were used in muscle spindle (Katz, 1950), stretch receptors of lobster and crayfish (Eyzaguirre & Kuffler, 1955) and Pacinian corpuscles (Gray & Sato, 1953) in order to abolish the all-or-nothing potential. In this experiment, however, they were not employed, since the OAP was often affected by anaesthetics more easily than the all-or-nothing potential, and there was no measure to determine the effect of anaesthetics on the OAP during the course of an experiment for more than 1 hr.

OAP in sodium-deficient media

In Fig. 3 a typical example of the experiments on the effect of sodium-deficient media upon the action potentials recorded inside from a single ommatidium is illustrated. Having taken a series of records in normal Ringer's solution after three different periods in darkness, which are shown in the top records in the figure, the external bathing solution was replaced with choline-Ringer. Responses to the stimulation of the same intensity, duration and interval were recorded successively at different times following the substitution of the solutions, and are shown on the second, third, fourth and fifth rows. In each column responses obtained after

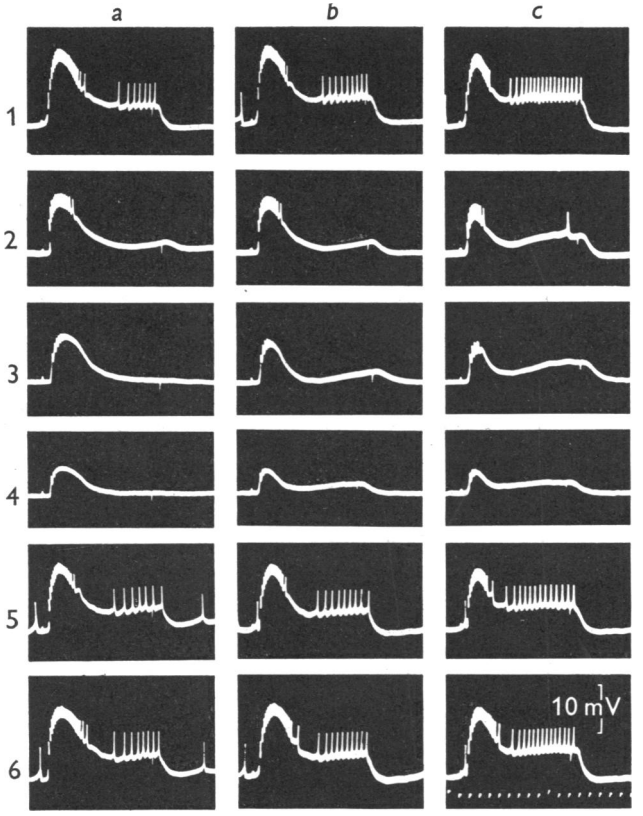

Fig. 3. Effect of sodium-deficient medium and of stimulus interval on OAP. Records in columns *a*, *b* and *c* are responses obtained after 60, 30 and 15 sec in darkness respectively. Records in row 1, in normal solution; responses in rows 2, 3 and 4, taken at 2, 24 and 46 min after replacing normal solution with a sodium-free solution; records in rows 5 and 6 were taken at 3 and 14 min after reapplication of normal solution. Upward and downward pips indicate 'on' and 'off' signs of illumination. Time, 0·1 + 1 sec. Temperature, 21° C.

three different periods in darkness are illustrated. The sodium lack produced a slow decrease in the amplitude of the OAP and in the frequency of the spike discharge initiated by the OAP and often by a slight depolarization due to the insertion of micro-electrode through the cell membrane. The spike potentials decrease in their height and finally residual or abortive impulses were found at the rising portion of the OAP as oscillations.

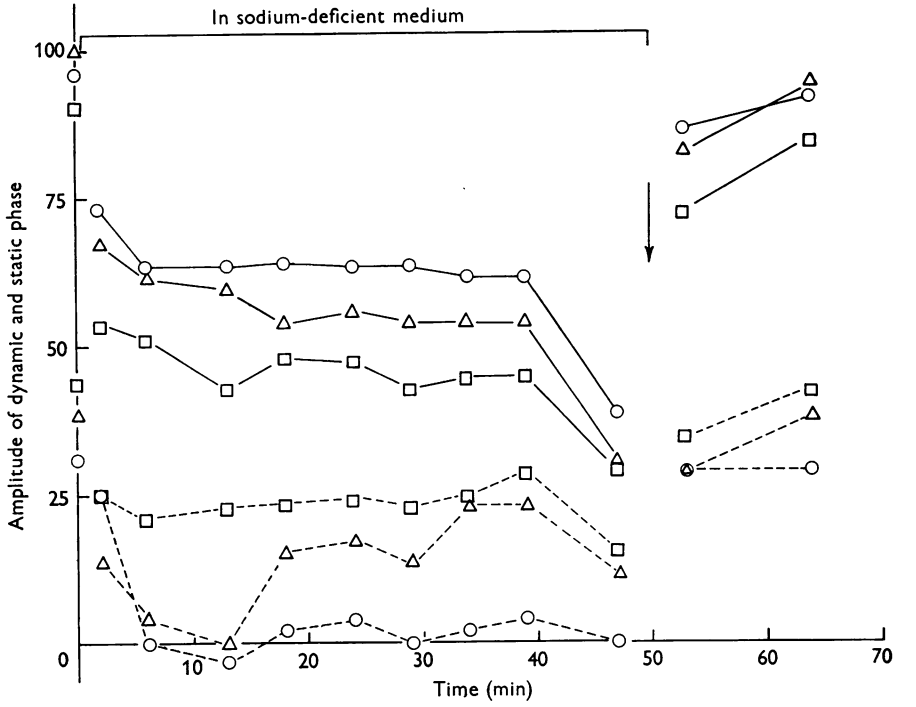

Fig. 4. Effect of sodium-deficient medium upon the amplitude of 'dynamic phase' and 'static phase' of OAP. This figure was drawn from data obtained in the experiment illustrated in Fig. 3. Solid line, amplitude of 'dynamic phase'; interrupted line, amplitude of 'static phase'. ○, △, □ show values of responses obtained after 60, 30 and 15 sec in darkness respectively. Arrow indicates the time when normal solution was reapplied. Ordinate; amplitude of 'dynamic' and 'static phase' expressed as a percentage of the maximal value of 'dynamic phase' in normal solution. Abscissa; time following replacement of normal solution with a sodium-free solution.

Changes in the amplitude of the OAP during the course of the experiment are plotted in Fig. 4. The amplitude of the 'dynamic phase' and 'static phase' were reduced by replacement of normal Ringer's solution with a sodium-free solution. These phases showed about 90–100% recovery following reapplication of normal solutions (records on the 5th and 6th rows).

Figure 5 was obtained from an experiment similar to that in Fig. 3. In this instance, however, short flashes were applied in order to obtain transient simple OAP which were convenient for the determination of the rate of fall. It will be seen in the figure that both the rate of rise and of fall of the OAP also declined following the change of the normal solution to a sodium-free solution, though the rate of rise was more affected than that of fall. Generally speaking the recovery was fairly good if the preparation

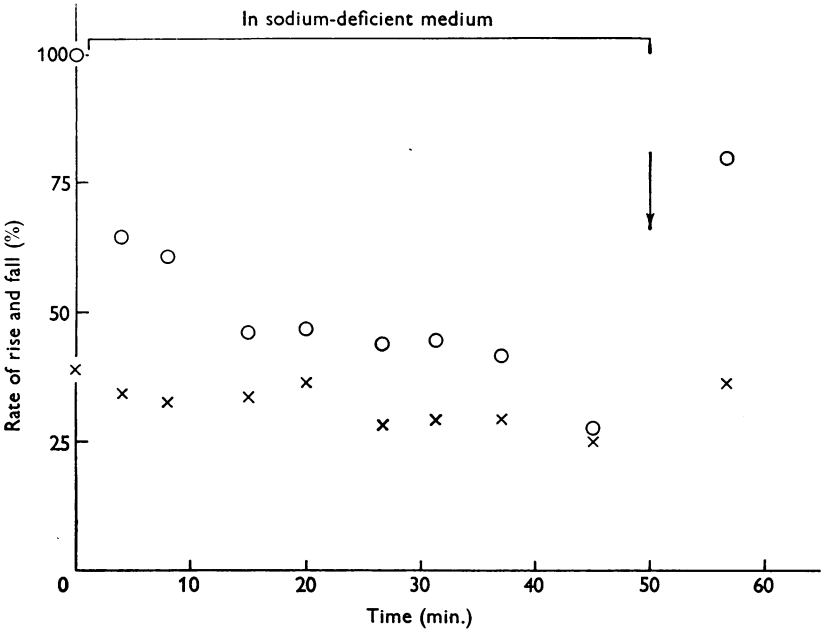

Fig. 5. Changes in rate of rise and fall of 'dynamic phase' after replacing normal solution with a sodium-free solution. Ordinate, rate of rise (○) and fall (×) expressed as percentage of the initial maximal value. Abscissa, time following the change of normal solution with a sodium-free solution. Arrow indicates the time of reapplication of normal solution. All values were obtained from the responses of the same unit as in Fig. 3, but flashes of 0·024 sec duration were applied after 30 sec in darkness.

was not soaked in the sodium-deficient medium for too long a period of time. Sodium-free solutions were not used for more than about 50 min. If a longer time was used, an irreversible effect on the cells was induced. Such a deleterious effect of the sodium-free solution, in which choline chloride was substituted for sodium chloride, is reported in Pacinian corpuscles (Diamond *et al.* 1958*b*). These authors ascribe the result to the pharmacological effect that choline might have on the receptors. However, we must at the same time recognize the fact that the metabolism of the

nervous tissue is much influenced in sodium- or potassium-free media (Takagaki & Tsukada, 1957).

The determination of the sodium concentration in the medium just outside the cell membrane could not be made. Therefore it is difficult to give a quantitative relationship between the OAP and the external sodium concentration. As stated above, up to 5% of the initial sodium may possibly remain in the bath after one replacement of the solution. Nevertheless, the rate of decrease in the amplitude of the OAP was smaller than expected. The lack of effect on the receptor potential of soaking Pacinian corpuscles in a sodium-free solution can be explained by the slow movement of sodium ions into Pacinian corpuscles (Gray & Sato, 1955; Diamond *et al.* 1958*b*). In the case of the lateral eye of the horseshoe crab the cells within the ommatidium are encapsulated; furthermore, the eccentric cell is surrounded by retinular cells. This peculiarity in the structure of the ommatidia seems to be related to the formation of the diffusion barrier for ions, as in the Pacinian corpuscles.

It should be mentioned that the time taken for the OAP to decline to about 50% in sodium-free solutions is always longer than that required for the OAP to recover to approximately the initial value. But this seems not to be a specific finding for the OAP, since a similar result was found in the receptor potential of the Pacinian corpuscles (cf. Text-fig. 4 in Diamond *et al.* 1958*b*).

Effect of time interval of stimuli. The amplitude and rate of rise of the 'dynamic phase' decreased as the time interval of illumination was shortened, unless the intensity of stimulus was too weak (Kikuchi & Tazawa, 1960). Similar effects were reported for the receptor potentials of Pacinian corpuscles (Diamond, Gray & Inman, 1958*a*; Loewenstein & Altamilano-Orrego, 1958). This 'depressive' effect of the time interval of the stimuli was also found in sodium-deficient media as in normal. Table 1 shows the rates of 'depression' of the 'dynamic phase' calculated from the data illustrated in Fig. 4. The 'depression' of the 'dynamic phase' increased in a low-sodium medium.

On the other hand, the final amplitude of the 'static phase' was not affected by the time in darkness before illumination, but by the intensity of illumination and temperature (unpublished observation) when a prolonged illumination was applied. However, when a stimulus of short duration was applied the time course and amplitude (measured at the 'off' sign) of the 'static phase' were influenced by the time in darkness before illumination. That is, the smaller the 'dynamic phase', the earlier the 'static phase' reached to the final depolarization. In this experiment, on account of experimental conditions, the duration of stimuli was too short to reach the final depolarization, but the amplitude of the 'static phase'

of the responses obtained after shorter time in darkness was larger than that after longer time in darkness. This relation was observed also in reduced responses recorded in sodium-deficient media (Figs. 3, 4).

TABLE 1. 'Depression' by illumination after shorter time in darkness in normal and low-sodium media. Magnitudes of the 'dynamic phase' obtained after 30 and 15 sec in darkness were expressed as percentage of those obtained after 60 sec in darkness at each recording. Recording 1 was taken in normal solution; recordings 2–10 taken successively in a low-sodium solution; recordings 11 and 12 obtained in normal solution again

Recording no.	After 60 sec in dark (%)	After 30 sec in dark (%)	After 15 sec in dark (%)
1	100	96·1	93·4
2	100	92·1	73·7
3	100	97·0	75·6
4	100	94·0	67·7
5	100	84·0	67·7
6	100	87·8	75·0
7	100	84·8	67·6
8	100	82·3	79·2
9	100	82·3	72·9
10	100	80·0	75·0
11	100	95·5	84·5
12	100	98·0	89·2

Application of direct current. The OAP was reversed in its polarity by strong depolarizing currents (Kikuchi & Tazawa, 1960) and the current necessary for the reversal of the 'static phase' was weaker than that for the 'dynamic phase'. This observation can be explained by supposing that the membrane potential exceeded the equilibrium potential of the OAP. In the experiment illustrated in Fig. 6 currents were applied through the micro-electrode 20 sec before the illumination and removed soon after the records were taken. Stimuli of a given intensity and duration were delivered at 60 sec intervals. No direct determination of the membrane potential under depolarizing currents could be made. The OAP was decreased and then reversed as the strength of the applied depolarizing current increased. The strength of the current at which the OAP was reversed in a normal sodium solution may be compared with that in sodium-deficient medium. As is illustrated in Figs. 6 and 7, the strength of current required to reverse the OAP was reduced in sodium-deficient medium and the current for the reversal of the 'static phase' was also weaker than that for the 'dynamic phase'. The OAP returned to about 80% of the initial value on reapplication of the normal solution. This decrease in the amplitude of the OAP found after reapplication of the normal solution seemed mainly to be caused by soaking the ommatidium in the sodium-deficient medium and not by the current application, since the OAP was not appreciably affected by depolarizing current in the control records, which were taken 60 sec after each record in Fig. 6 had been photographed.

As the effective resistance in the resting state, which was determined by applying a short anodal pulse, was not significantly changed in sodium-deficient medium, this result suggests that the membrane potential at which the OAP reverses depends upon the external sodium concentration. Thus, besides intensity, duration, time interval of the stimulus, membrane potential (Kikuchi & Tazawa, 1960) and temperature (Kikuchi *et al.* 1961),

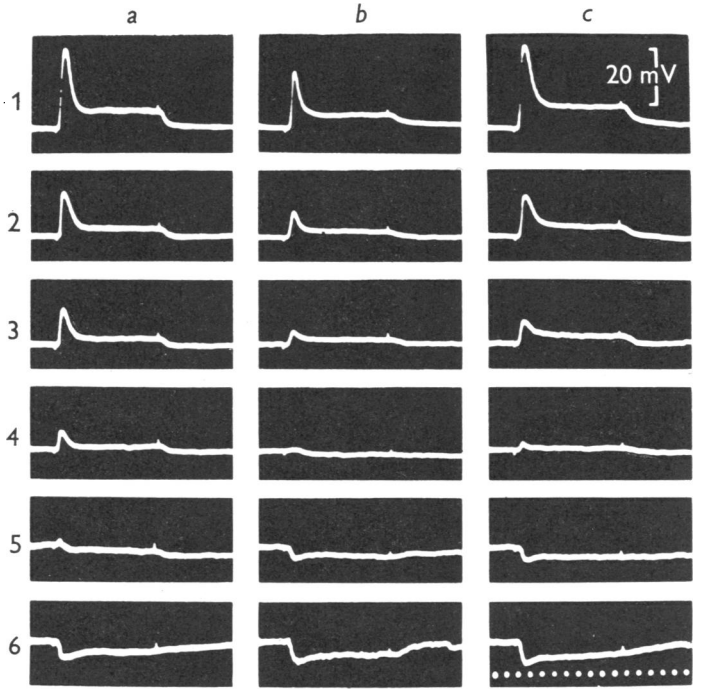

Fig. 6. Production of reversed OAP under depolarizing current in normal and sodium-deficient media. From top to the bottom: records in row 1, no current was applied; those in rows 2, 3, 4, 5 and 6 are responses obtained under depolarizing currents of 1, 2, 3, 4 and 5×10^{-9} A respectively. Records in column *a*, in normal solution; in column *b* were taken successively from 5 min after application of a sodium-free solution; in column *c*, were begun to be photographed at 8 min after reapplication of normal solution. All responses were obtained after 60 sec in darkness. Downward and upward pips show 'on' and 'off' signs of illumination. Time marker, 0·1 sec. Temperature, 23·5° C.

the sodium is an important factor for the production of the OAP. The action of sodium ions was not mimicked by lithium or tetraethylammonium ions (Kikuchi & Naito, 1958), but it probably was by barium ions, though barium ions induced a remarkable prolongation of the OAP at the same time (Kikuchi & Minagawa, 1961).

Sucrose-Ringer. When sucrose-Ringer was used instead of choline-Ringer, similar effects to those of choline-Ringer on both OAP and spike

potentials were obtained, i.e. a decrease in the amplitude and rate of rise of the OAP and abolition of spike potentials. Therefore, the effect of choline-Ringer on the OAP was not its specific one, but was due to the lack of sodium in the surrounding solution. However, in the case of

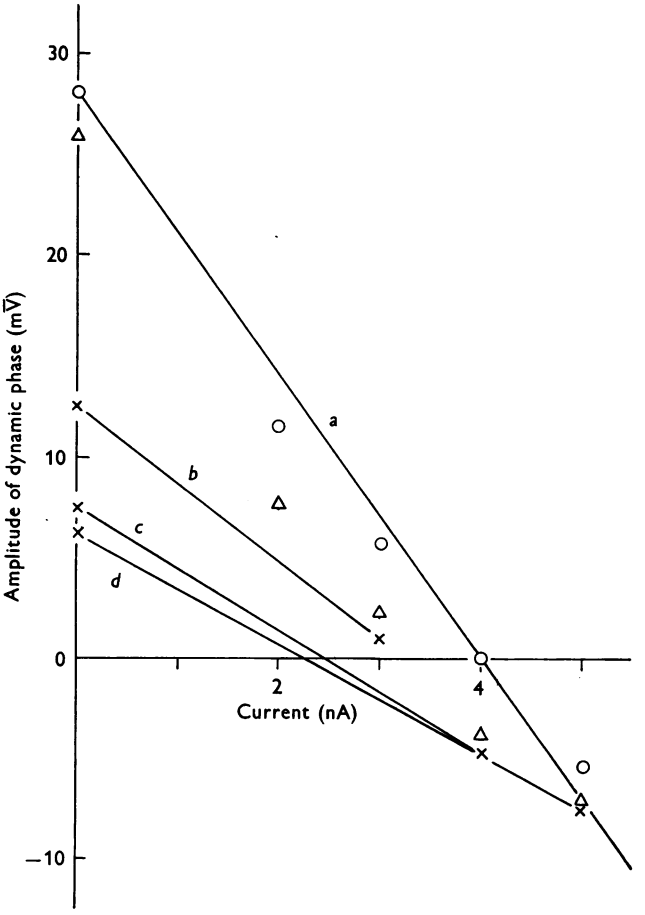

Fig. 7. Relation between maximal amplitude of 'dynamic phase' and applied depolarizing currents in normal and sodium-deficient media. Drawn from the data shown in Fig. 6. Line *a*, in normal solution (○); lines *b*, *c* and *d*, amplitude at 13, 15 and 17 min following treatment with a sodium-free solution with and without currents (×); △, amplitude in normal solution again.

sucrose-Ringer, a slight prolongation of the falling portions of the 'dynamic phase' and the 'off-effect', and moreover a less recovery, were usually found following reapplication of normal solution. This decrease seemed to be due to the diminution of the resting potential which was found at the end of the experiment on withdrawing the micro-electrode from the cell.

These side effects of sucrose-Ringer have not been reported in other excitable tissues and could not be easily explained. The lack of chloride ions may be partly responsible for these effects.

Spike potential in sodium-deficient media

As mentioned above, the first effect of sodium-deficient media on the spike potential was usually a decrease in the frequency of its discharge and then a decline in its amplitude. The effect of sodium deficiency did not always steadily progress. Often the reduced spontaneous spike disappeared abruptly. A few spikes of almost normal form could be evoked in some other experiments by illumination under hyperpolarization, even if the residual spike had totally disappeared. The spike was changed reversibly by replacing the external sodium-deficient solution with normal solution, as in the case of the OAP. Occasionally spikes of various sizes were found at the beginning stage in the recovery course of the spike potential following reapplication of normal solution.

One of us (Tanaka) has obtained graphically the first derivatives of the spike potentials recorded on faster time base at various steps of the experiment, and found that the maximal rate of rise of the spike potential declined progressively following the change from normal to sodium-free solution. As in the case of the OAP, no quantitative relationship between the concentration of external sodium ions and the amplitude and rate of rise of the spike potential could be obtained.

The effects of the surrounding sodium-deficient solution on the OAP and spike potential did not always run parallel. Sometimes the spike potential was affected earlier than the OAP, but in other cases they were little influenced for more than 50 min following the change of the solutions, even though the OAP had fallen considerably in amplitude. These facts seem to support the view presented by Tomita (1956, 1957) that the site of the OAP and spike potential are different. Such a possibility of the difference between the site of receptor potential and impulses is indicated in Pacinian corpuscles (Gray & Sato, 1953), stretch receptor in crayfish (Edwards & Ottson, 1958) and also in the observations on the muscle spindle of frog (Katz, 1950). It will be noticed that, in contrast to the action potentials from the single ommatidia, the impulses from the receptors mentioned above were more easily abolished than the receptor potentials by replacing normal solution with a sodium-free solution. The reason why the time of disappearance of the impulses in the eye of the horseshoe crab is sometimes long may be due to the structure of ommatidia. The distal part of the optic nerve of the horseshoe crab is encapsulated, together with the soma of the eccentric cell and retinular cells, and probably much less affected than the nerves from other receptors by the change of

the external solutions, when the spike potentials were recorded inside from a cell in the ommatidium.

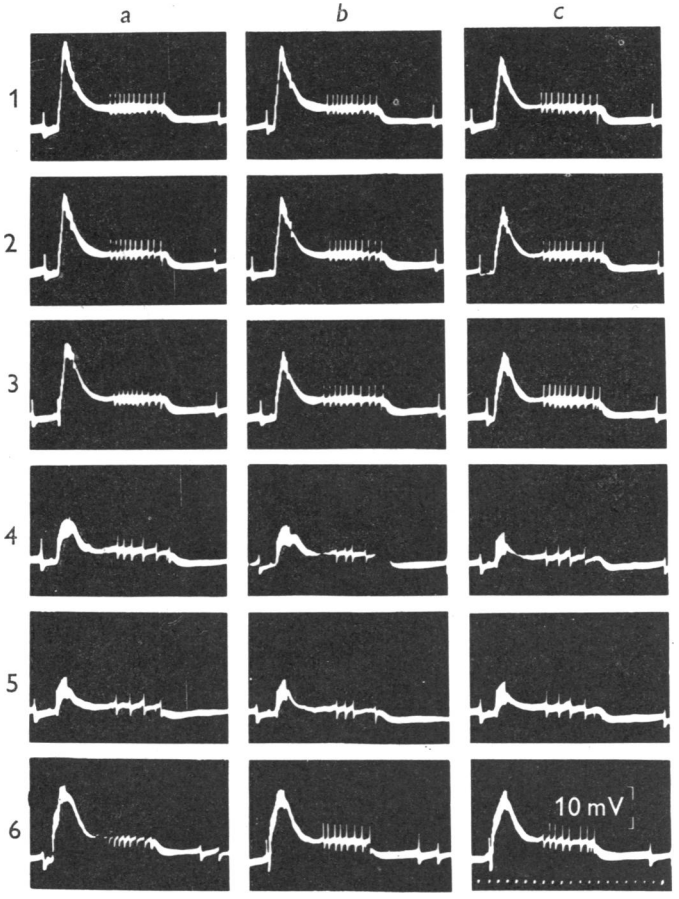

Fig. 8. Effect of potassium-rich solutions upon OAP recorded at different periods in darkness before illumination. Records in row 1, in normal solution; records in rows 2 and 3, taken in solutions of 18 and 32 mM-K respectively; in row 4 and 5 taken in the solution of 90 mM-K, but those in row 5 were obtained 5 min later than responses in row 4; in row 6, at 4 min after reapplication of normal solution. *a*, *b* and *c* mean the time in darkness before illumination, 60, 30 and 15 sec respectively. Pips indicate 'on' and 'off' signs of illumination. Time marker, 0·1 sec. Temperature, 21° C.

Effect of potassium-rich solution on OAP

The resting potential seemed to be only slightly affected by replacing the external normal solution with sodium-free solutions. On the other hand, the amplitude and spike potential declined in parallel with the decrease in the frequency of spike discharge as the external potassium concentration was raised. In Figs. 8 and 9 one of the results from such experiments is

illustrated. Records in row 5 were taken 5 min later than those in row 4 in a solution of the same potassium concentration. They were smaller than the OAP in row 4. This seems to indicate that it takes time to reach the new equilibrium following the increase in the external potassium concentration, as in the case of the change of normal solution with sodium-free solutions. The 'depressive' effect of the time interval of the stimulus was

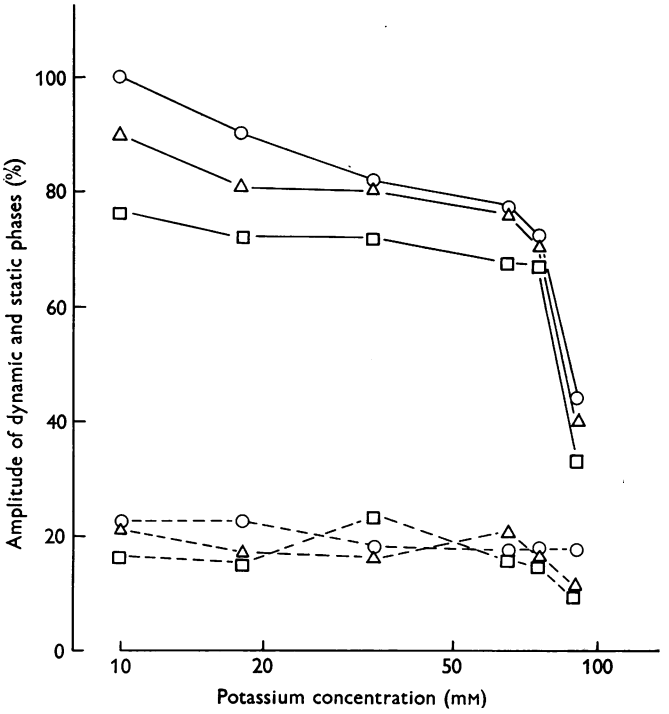

Fig. 9. Relation between potassium concentration and amplitude of 'dynamic' and 'static phase'. Each value was expressed as percentage of the maximum in normal solution, and was obtained from the same experiment as that in Fig. 7. ○, △, □ show responses obtained after 60, 30 and 15 sec in darkness. —— amplitude of 'dynamic phase'; – – – amplitude of 'static phase'.

decreased as the OAP declined. The 'static phase' was less affected by potassium-rich solution than the 'dynamic phase'. The resting potential was not precisely determined. However, a decrease in the resting potential was assured in a few preparations by withdrawing the micro-electrodes after the application of potassium-rich solutions. This decrease may be due to the depolarizing action of potassium and was similar to the effect of background illumination or depolarizing currents in this respect (Kikuchi & Tazawa, 1960).

As mentioned above, like the spike potential in the isolated giant axon of cephalopod, the OAP was followed by a positive phase or an after-hyperpolarization in units with relatively low resting potential or when the background illumination or depolarizing current was applied. It should be noticed that the after-hyperpolarization was influenced by potassium, as that in the spike potential recorded from ommatidia or the giant axon of the squid (Hodgkin & Katz, 1949), that is, it became smaller or disappeared under depolarization by potassium (Figs. 11*B* and 12).

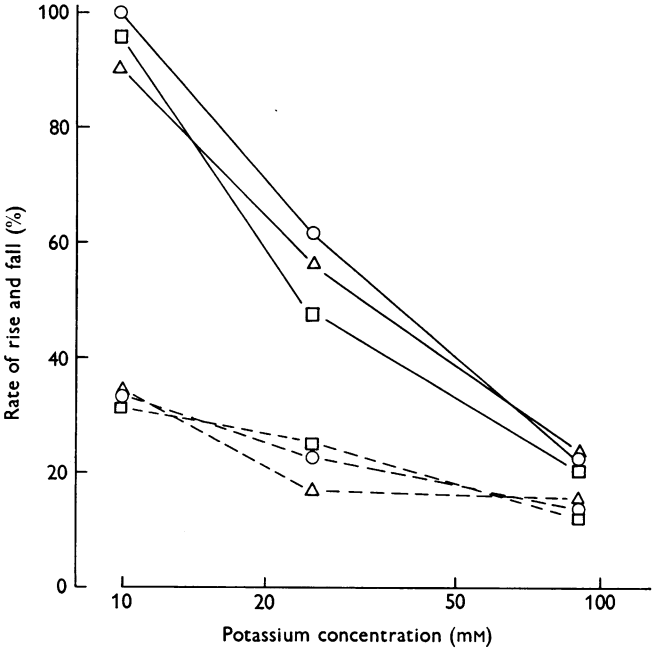

Fig. 10. Relation between potassium concentration and rate of rise and fall of OAP. ○, △ and □ show values obtained from responses after 60, 30 and 15 sec in darkness, respectively, expressed as percentage of the maximal value in normal solution. These values were obtained from the experiment on a unit to which flashes (duration, 0·024 sec) were applied. ——, rate of rise; – – –, rate of fall. Temperature, 21° C.

The rate of rise and fall of the OAP was also decreased in accordance with the change in its amplitude (Fig. 10). The rate of rise was more affected than the latter. In the preparation shown in Fig. 10, short flashes were applied, therefore the 'depression' by short stimulus intervals was not effective.

As seen in Figs. 10 and 11*B*, 60–80 % recovery could be obtained by reapplication of normal solution unless the concentration of potassium was too high. In a preparation 100 % recovery was obtained after the OAP had

declined to about 40% of the initial value, though the frequency and amplitude of the spike did not return to the value at the beginning. As might well be expected, owing to the possible diffusion barrier for ions around the cells in ommatidia, no clear relationship between the amplitude of the OAP and the potassium concentration was obtained.

Effect of hyperpolarizing current. The decreased OAP caused by application of potassium-rich solution was increased by hyperpolarizing currents, as was the spike potential. Fig. 11*A* illustrates such an effect of hyperpolarization. As seen in the records *c–h*, the OAP, decreased by treatment with potassium-rich solution, increased in amplitude as the strength of the applied hyperpolarizing current was increased. This evidence indicates that the polarizing current and potassium ions display similar effects on the action potentials under certain experimental conditions.

The 'depressive' effect of the time interval of the stimulus was also found in potassium-rich media and decreased as the amplitude of the OAP diminished on raising the potassium concentration.

Effect of potassium-rich solution on spike potential

The spike potential and its positive phase were diminished following the application of potassium-rich solutions, and then were abolished before the membrane was completely depolarized. The spike reappeared under hyperpolarizing current, though the after-hyperpolarization did not recover unless normal solution was reapplied (Fig. 11*B*). This relationship between potassium and polarizing current is similar to that on the OAP. As seen in Fig. 12, periodic grouped discharges occasionally appeared on hyperpolarizing the membrane in the preparation immersed in a solution of high potassium concentration. When the potassium concentration was further raised, hyperpolarization failed to restore the spike discharge. Spikes reappeared when the external solution was returned to normal and finally reached nearly the same height as in the controls. Such periodic discharges were occasionally observed in normal solution under strong depolarizing currents or following the administration of a small amount of strychnine (Kikuchi & Tazawa, unpublished observation) or by treatment of barium ions (Kikuchi & Minagawa, 1961). The mechanism of the generation of the grouped discharges may be not always similar in these cases. However, it seems likely that the grouped discharges in potassium-rich solution under hyperpolarizing currents are due to the increased after-depolarization of the spike potentials. Prolonged or trapezoid potentials reported in single myelinated nerve fibres (Mueller, 1958) have not been observed in the potassium-rich solutions, though they were recorded following application of tetraethylammonium ions (Kikuchi & Naito, 1958) or barium ions (Kikuchi & Minagawa, 1961).

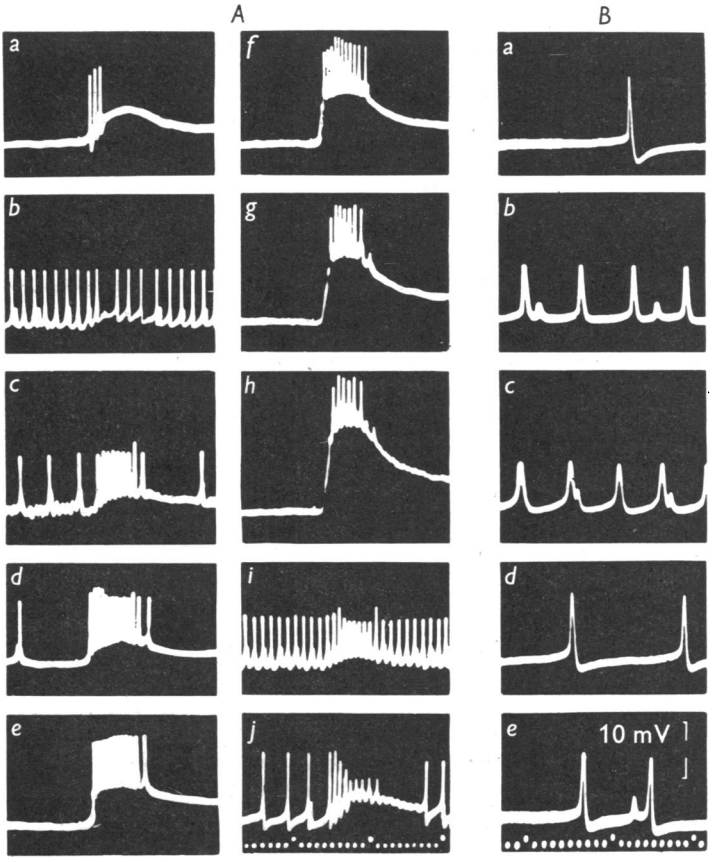

Fig. 11 *A*. Effect of hyperpolarizing currents on the OAP decreased by treatment with a potassium-rich solution. *a*, OAP in normal solution; *b*, in potassium-rich solution (317 mM); *c–h*, in a potassium-rich solution under hyperpolarizing currents of 2, 3, 4, 5, 6 and 7×10^{-9}A respectively; *i*, the last record in potassium-rich solution without polarizing current; *j*, recorded at 14 min after twice washing out with normal solution. All OAP were obtained after 60 sec in darkness. Only the first half of each is illustrated (retouched). Pips indicate the onset of illumination. Time marker, 0·1 sec. Temperature, 13·5° C.

B. Changes in the spike form in a potassium-rich solution. All records were taken during the experiment shown in *A*. *a*, spike evoked by illumination of very weak intensity in normal solution. *b* and *c*, spontaneous spike discharges recorded at 16 and 36 min following treatment with a potassium-rich solution—they correspond to records *b* and *i* in *A*, respectively. *d* and *e* taken at 3 and 13 min after twice washing with normal solution. Double discharges (Tomita *et al.* 1960) were recorded from this unit. Time marker, 0·02 sec.

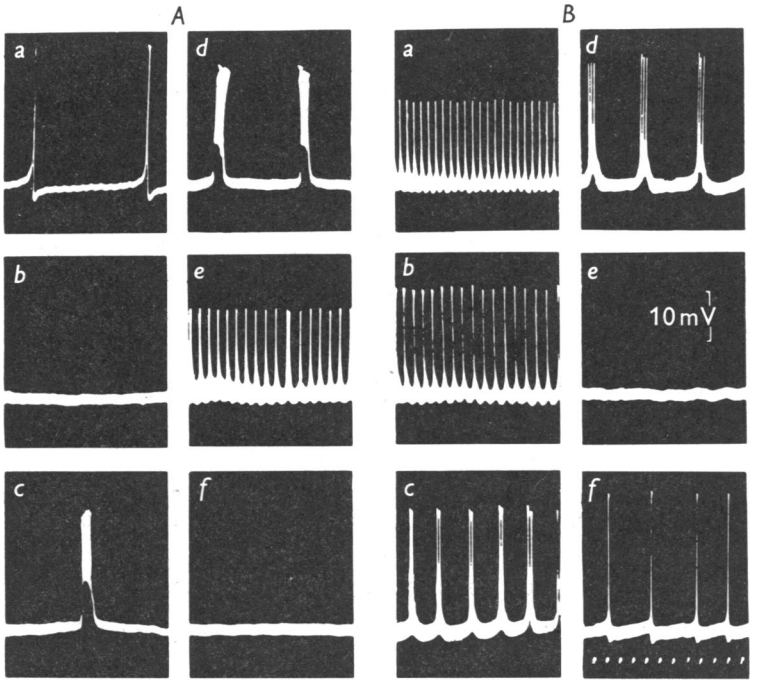

Fig. 12. Competitive effect of potassium-rich solution and hyperpolarizing current on spike potentials. *c–f* in *A*, *a–d* in *B* were recorded under hyperpolarizing currents of equal intensity (4×10^{-9} A). *A*: *a*, spike potentials in normal solution; *b*, disappearance of spontaneous discharge on raising the external potassium concentration to 90 mM; *c*, appearance of spontaneous grouped discharge on application of currents; *d* and *e*, increase in the frequency and decrease in the amplitude of spike potentials on further raising potassium concentration; *f*, spontaneous discharge was abolished again at potassium concentration of 150 mM. *B*: from *a* to *d* gradual recovery of spontaneous spike potentials following replacing potassium-rich solution with normal solution; spontaneous discharge disappeared on stopping hyperpolarizing current; *f*, reappearance of spike discharge in normal solution without application of current (at 25 min after washing with normal solution; retouched). Time marker, 0·1 sec. Temperature, 22° C. The upper parts of spikes in records *c* and *d* were faded out of the screen of cathode-ray tube.

DISCUSSION

The OAP is a graded potential change whose form depends upon the intensity, duration and interval of illumination, and generates the afferent impulses in the optic nerve and is not produced by the electrical stimulation. The site of the production of the OAP is still a matter of controversy (Tomita *et al.* 1960). Some of the properties of the cell membrane concerning the generation of the OAP have been shown to be similar to those of nerve and muscle fibres which initiate the all-or-nothing potential.

Tomita (1956) has obtained the following conclusions by the observation on the action potentials recorded intra- and extracellularly from single ommatidia, the effect of antidromic stimulation of the optic nerve and on changes in spike potentials superimposed on the OAP. The sites of generation of the OAP and spike potential are different; further, the resistance of the membrane through which the micro-electrode is penetrated decreases during illumination. Recently Fuortes (1959) demonstrated quantitatively the changes in membrane resistance during illumination, by two types of experiments. According to his results, the change in impedance was maximal at the initial maximal depolarization, gradually decreased during the steady depolarization, and the phase of repolarization, and returned to the resting value. And this impedance change should be ascribed to a change of membrane resistance. The decrease in membrane resistance during illumination was also shown by our own experiment in which rectangular anodal pulses were passed through the impaling electrode at various phases of the OAP. These results seem to support the permeability hypothesis, that the production of the OAP is due to the increase in the permeability of the membrane to one or more ion species, as suggested in the production of the receptor potential of the Pacinian corpuscles (Diamond *et al.* 1958*b*).

The amplitude and rate of rise of the 'dynamic phase' decreased gradually after replacing the external normal solution with sodium-free solutions, probably without significant change in the resting potential, although the illumination of fixed intensity and duration was applied. This fact indicates that sodium ions are related to the decrease in the membrane resistance during illumination. In other words, they may probably move down their electrochemical gradients, though other ions may be also responsible. This deduction becomes more comprehensible by rearranging the results of the effect of direct currents on the reversal of the OAP in normal and sodium-deficient media.

Figure 13 illustrates the relation between applied current and membrane-potential shift. It was drawn from the data in Fig. 6 and the control records to estimate the effect of sodium-deficiency on the membrane resistance of the cell in an ommatidium during illumination. It was assumed in this experiment that the membrane resistance, whose value was obtained from the potential drop across the membrane, did not change within the range of current applied. Thus the interrupted line, the relation between the applied current and membrane potential shift in darkness, was drawn. Then, starting from the interrupted line, the maximal amplitude of the OAP under depolarizing current of various intensities was plotted. The solid line joining the experimental points shows the membrane potential resulting from the combined effects of illumination and the extrinsic current.

Under the assumption mentioned above the slope of these lines shows the membrane resistance at the peak of the OAP. The two solid lines (*a* and *b*) show the membrane resistance, (*a*) in normal solution and (*b*) at 17 min after replacing normal solution with a sodium-free solution. As is seen in the figure, the gradient of the lines increases in a sodium-deficient medium, i.e. the change in the membrane resistance by illumination was diminished to a lesser extent by sodium lack, even though the same stimuli were applied.

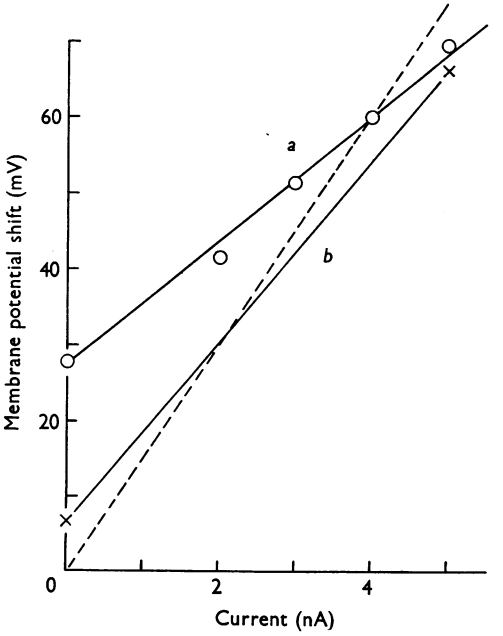

Fig. 13. Relation between change of membrane potential by illumination and depolarizing currents in normal and sodium-deficient media. Results of Fig. 6 were replotted, to take into consideration the membrane-potential shift caused by current in darkness. Interrupted line measures change of resting membrane potential caused by current. Solid lines *a* and *b* indicate the relation between amplitude of 'dynamic phase' (starting from the interrupted line) and current applied in normal and sodium-deficient media.

The solid line in a sodium-deficient medium (*b*) intersects the interrupted line at a lower level than that in normal solution (*a*). This indicates that the equilibrium potential of the OAP diminishes following the substitution of the solution, and depends upon the external sodium concentration.

The resting potential was estimated as about 40 mV at the beginning of the experiment shown in Fig. 13. Therefore, the equilibrium potential of the OAP in normal solution should be about 20 mV above the zero potential level. This deduction can be explained by assuming that sodium

permeability predominantly increases during the rising portion of the 'dynamic phase'. The estimated value is consistent with our observation that, as far as has been observed, the overshoot of the OAP never exceeded value.

In order to compare the effect of potassium ions on the membrane resistance at the peak of the 'dynamic phase' with that of sodium, a figure was drawn by reconstructing the experimental finding shown in Fig. 9*A* in a similar manner as in the case of the effect of sodium deficiency upon the membrane resistance in darkness and during illumination. This figure showed that the change in the external potassium concentration may not significantly affect the membrane resistance at the peak of the 'dynamic phase' of the OAP. In most excitable tissues the concentration of potassium inside the cell is higher than that outside, and the resting membrane potential is approximately determined by the ratio of the potassium concentration between inside and outside (Hodgkin, 1951). As mentioned above, the experiments on the effect of potassium-rich solutions upon the action potentials arising within single ommatidia and also that of hyperpolarizing currents upon them are related to the influences of background illumination and depolarizing currents upon them. These facts suggest that the effect of potassium-rich solution upon the action potentials may be mainly due to the decrease in the resting potential caused by the diminution of the potassium ratio between the inside and outside of the cell concerned within the ommatidium.

The falling phase of the receptor potential of the Pacinian corpuscles shows an exponential decay (Gray & Sato, 1953; Inman & Peruzzi, 1961) and no after-potential was recorded. On the other hand, the slow potential recorded from muscle spindle (Katz, 1950), or the OAP, was often followed by an after-hyperpolarization. According to our observations mentioned above this after-hyperpolarization was rather sensitive to the external potassium concentration, as was that of the action potentials of the squid giant axon. The falling portions of the 'dynamic phase' and the 'off-effect' have properties in common and are strictly not of passive nature. In addition, the phase of repolarization was sometimes modified by an after-depolarization when the OAP was recorded at high temperature and the resting potential was large (unpublished observation). These observations seem to suggest that their falling portions cannot be simply explained and the change in the potassium permeability may possibly be responsible for the formation of repolarization.

In connexion with these effects of sodium and potassium ions on the OAP it is natural to think that the change in the photochemical reaction, which probably intervenes between stimulus and electrical events, may possibly be affected by these ions. This problem, however, needs further investigation.

SUMMARY

1. The effect of sodium, potassium and polarizing currents on the action potentials arising within a single ommatidium of the horseshoe crab (*Tachypleus tridentatus*) have been studied by means of intracellular microelectrodes.

2. Amplitude and rate of rise of the retinal slow potential—ommatidial action potential (OAP)—was decreased in low-sodium solutions. And the effect was reversible, unless the preparation was soaked in low-sodium media for a long period of time.

3. The strength of depolarizing currents at which the initial maximal depolarization ('dynamic phase') of the OAP was reversed in its polarity was stronger than that of the subsequent lesser but sustained depolarization ('static phase'). Both of them decreased in sodium-deficient media.

4. The spike discharge decreased in its frequency, then in its amplitude, and finally disappeared, following application of a sodium-free solution. This effect did not always run parallel with the change of the OAP.

5. The 'depressive' effect of shortening the stimulus interval on the amplitude of the 'dynamic phase' of the OAP was also found in preparations immersed in sodium-deficient solutions.

6. The rate of fall of the 'dynamic phase' was affected to a much less extent than the rate of rise and the amplitude by reducing the external sodium concentration.

7. Amplitude and rate of rise and fall of the OAP were diminished as the external potassium concentration was raised. These changes were accompanied by a reduction of spike potentials and by an increase in their frequency. Initation of spike was inhibited by further increase in the potassium concentration. The decreased OAP and spike potential in potassium-rich media were restored by hyperpolarization, and a concomitant decrease in the frequency of the spike discharges was found.

8. The amplitude of the 'static phase' of the OAP was much less affected by external potassium concentration than that of the 'dynamic phase'.

9. These findings concerning the OAP and spike potential are discussed.

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