J. Physiol. (1952) 116, 473–496

THE COMPONENTS OF MEMBRANE CONDUCTANCE IN THE GIANT AXON OF *LOLIGO*

BY A. L. HODGKIN AND A. F. HUXLEY

From the Laboratory of the Marine Biological Association, Plymouth, and the Physiological Laboratory, University of Cambridge

(*Received* 24 *October* 1951)

The flow of current associated with depolarizations of the giant axon of *Loligo* has been described in two previous papers (Hodgkin, Huxley & Katz, 1952; Hodgkin & Huxley, 1952). These experiments were concerned with the effect of sudden displacements of the membrane potential from its resting level ($V=0$) to a new level ($V=V_1$). This paper describes the converse situation in which the membrane potential is suddenly restored from $V=V_1$ to $V=0$. It also deals with certain aspects of the more general case in which V is changed suddenly from V_1 to a new value V_2. The experiments may be conveniently divided into those in which the period of depolarization is brief compared to the time scale of the nerve and those in which it is relatively long. The first group is largely concerned with movements of sodium ions and the second with movements of potassium ions.

METHODS

The apparatus and method were similar to those described by Hodgkin *et al.* (1952). The only new technique employed was that on some occasions two pulses, beginning at the same moment but lasting for different times, were applied to the feed-back amplifier in order to give a wave form of the type shown in Fig. 6. The amplitude of the shorter pulse was proportional to $V_1 - V_2$, while the amplitude of the longer pulse was proportional to V_2. The resulting changes in membrane potential consisted of a step of amplitude V_1, during the period when the two pulses overlap, followed by a second step of amplitude V_2.

RESULTS

Experiments with relatively brief depolarizations

Discontinuities in the sodium current

The effect of restoring the membrane potential after a brief period of depolarization is illustrated by Fig. 1. Record *A* gives the current associated with a maintained depolarization of 41 mV. As in previous experiments, this consisted of a wave of inward current followed by a maintained phase of

outward current. Only the beginning of the second phase can be seen at the relatively high time base speed employed. At 0·85 msec. the ionic current reached a value of 1·4 mA./cm.^{2}. Record B shows the effect of cutting short the period of depolarization at this time. The sudden change in potential was associated with a rapid surge of capacity current which is barely visible on the time scale employed. This was followed by a 'tail' of ionic current which

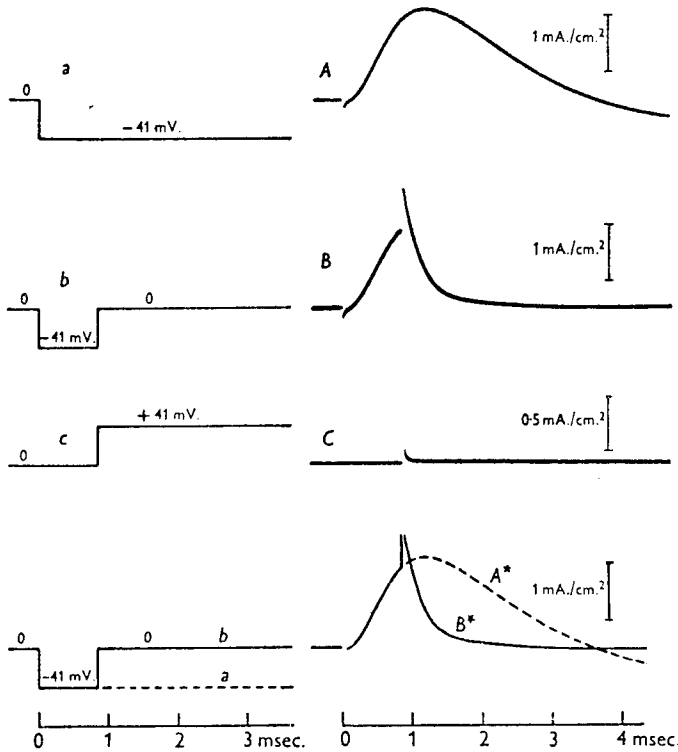

Fig. 1. Left-hand column: a, b, c, time course of potential difference between external and internal electrode. Right-hand column: A, B, C, records of membrane current associated with changes in membrane potential shown in left-hand column. (The amplification in C was 90% greater than that in A and B.) A^*, B^*, time course of ionic currents obtained by subtracting capacity current in C from A and B. Axon 25; temperature 5° C.; uncompensated feed-back. Inward current is shown upward in this and all other figures except Fig. 13.

started at about 2·2 mA./cm.^{2} and declined to zero with a time constant of 0·27 msec. The residual effects of the capacitative surge were small and could be eliminated by subtracting the record obtained with a corresponding anodal displacement (C). Curves corrected by this method are shown in A^* and B^*.

The first point which emerges from this experiment is that the total period of inward current is greatly reduced by cutting short the period of depolarization. This suggests that the process underlying the increase in sodium permeability is reversible, and that repolarization causes the sodium current to

fall more rapidly than it would with a maintained depolarization. Further experiments dealing with this phenomenon are described on p. 482. At present our principal concern is with the discontinuity in ionic current associated with a sudden change of membrane potential. Fig. 2*D* illustrates the discontinuity in a more striking manner. In this experiment the nerve was depolarized nearly to the sodium potential, so that the ionic current was relatively small during the pulse.

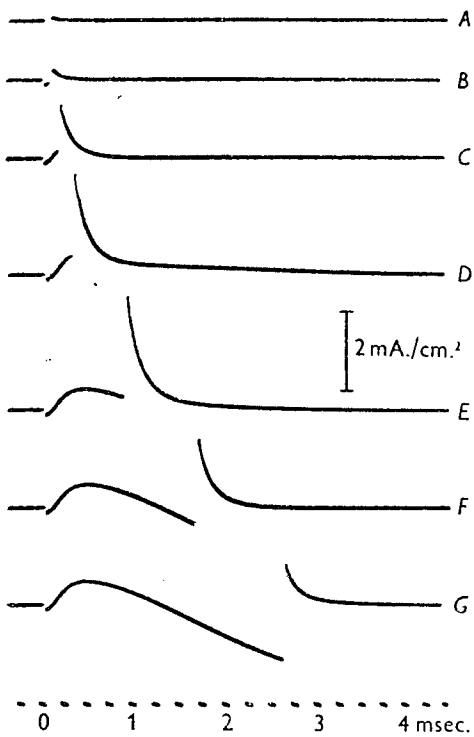

Fig. 2. Records of membrane current associated with depolarization of 97·5 mV. lasting, 0·05, 0·08, 0·19, 0·32, 0·91, 1·6 and 2·6 msec. The time and current calibration apply to all records. Axon 41; temperature 3·5° C.; compensated feed-back.

The other records in Fig. 2 illustrate the effect of altering the duration of the pulse. The surge of ionic current was small when the pulse was very short; it reached a maximum at a duration of 0·5 msec. and then declined with a time constant of about 1·4 msec. For durations less than 0·3 msec. the surge of ionic current was roughly proportional to the inward current at the end of the pulse. Since previous experiments suggest that this inward current is carried by sodium ions (Hodgkin & Huxley, 1952), it seems likely that the tail of inward current after the pulse also consists of sodium current. Fig. 3 illustrates an experiment to test this point. In *A*, the membrane was initially depolarized to the sodium potential. The ionic current was very small during the pulse but

the usual tail followed the restoration of the resting potential. The sequence of events was entirely different when choline was substituted for the sodium in the external fluid (Fig. 3*B*). In this case there was a phase of outward current during the pulse but no tail of ionic current when the membrane potential was restored. The absence of ionic current after the pulse is proved by the fact that the capacitative surges obtained with anodal and cathodal displacements were almost perfectly symmetrical (records *B* and *C*). These effects are explained quite simply by supposing that sodium permeability rises when the membrane is depolarized and falls exponentially after it has been repolarized.

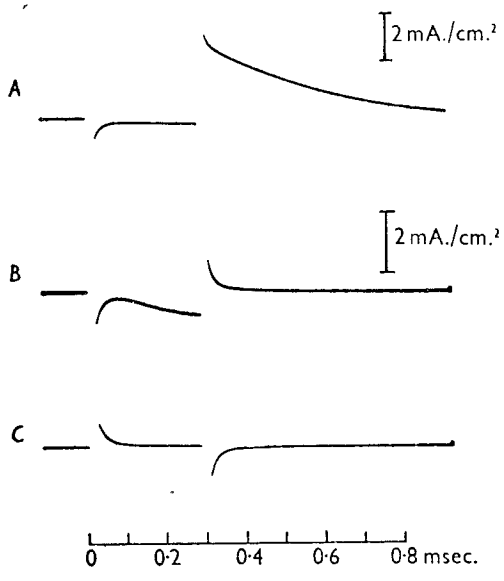

Fig. 3. *A*, membrane current associated with depolarization of 110 mV. lasting 0·28 msec.; nerve in sea water. *B*, same, but with nerve in choline sea water. *C*, membrane currents associated with an increase of 110 mV. in membrane potential; nerve in choline sea water. Axon 25; temperature 5° C.; uncompensated feed-back.

In record *A* the increase in permeability did not lead to any current during the pulse, since inward and outward movements of sodium are equal at the sodium potential. After the pulse the tendency of external sodium ions to enter the fibre is very much greater than that of internal sodium ions to leave. This means that there must be a large inward current after the pulse unless the sodium permeability reverts instantaneously to a low value. Record *B* is different because there were no external sodium ions to carry the current in an inward direction. The increase in sodium permeability therefore gave a substantial outward current during the period of depolarization but no inward current after the pulse. One might expect to see a 'tail' of outward current in *B* corresponding to the tail of inward current in *A*. However, the tendency of the internal sodium ions to leave the fibre against the resting

potential difference would be so small that the resulting outward current would be indistinguishable from the capacitative surge. According to the 'independence principle' (Hodgkin & Huxley, 1952, equation 12), the outward current in *B* should be only 1/97 of the inward current in *A*.

Continuity of sodium conductance

Discontinuities such as those in Figs. 1, 2 and 3*A* disappear if the results are expressed in terms of the sodium conductance (g_{Na}). This quantity was defined previously by the following equation:

$$g_{Na} = I_{Na}/(V - V_{Na}), \quad (1)$$

where V is the displacement of the membrane potential from its resting value and V_{Na} is the difference between the equilibrium potential for sodium ions and the resting potential (Hodgkin & Huxley, 1952).

The records in Fig. 4 allow g_{Na} to be estimated as a function of time. Curves α and *A* give the total ionic current for a nerve in sea water. Curve α was obtained with a maintained depolarization of 51 mV. and *A* with the same depolarization cut short at 1·1 msec. Curves β and *B* are a similar pair with the nerve in choline sea water. Curves γ and *C* give the sodium current obtained from the two previous curves by essentially the method used in the preceding paper (see Hodgkin & Huxley, 1952). In this experiment the depolarization was 51 mV. and the sodium potential was found to be −112 mV. To convert sodium current into sodium conductance the former must therefore be divided by 61 mV. during the depolarization or by 112 mV. after the pulse. Curves δ and *D* were obtained by this procedure and show that the conductance reverts to its resting level without any appreciable discontinuity at the end of the pulse. Fig. 5 illustrates the results of a similar analysis using the records shown in Fig. 2. In this experiment no tests were made in choline sea water, but the early part of the curve of sodium current was obtained by assuming that sodium current was zero initially and that the contribution of other ions remained at the level observed at the beginning of the pulse. Records made at the sodium potential (−117 mV.) indicated that the error introduced by this approximation should not exceed 5% for pulses shorter than 0·5 msec.

The instantaneous relation between ionic current and membrane potential

The results described in the preceding section suggest that the membrane obeys Ohm's law if the ionic current is measured immediately after a sudden change in membrane potential. In order to establish this point we carried out the more complicated experiment illustrated by Fig. 6. Two rectangular pulses were fed into the feed-back amplifier in order to produce a double step of membrane potential of the type shown inset in Fig. 6. The first step had a duration of 1·53 msec. and an amplitude of −29 mV. The second step was relatively long and its amplitude was varied between −60 mV. and +30 mV.

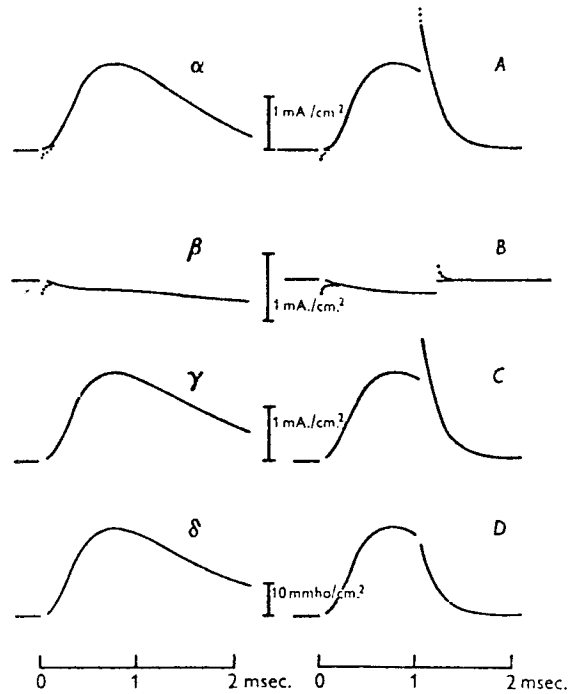

Fig. 4. α, ionic current in sea water associated with maintained depolarization of 51 mV. applied at $t=0$. (The dotted line shows the form of the original record before correcting for capacity current.) β, same in choline sea water. γ, sodium current estimated as $(\alpha-\beta)\times 0{\cdot}92$. δ, sodium conductance estimated as $\gamma/61$ mV. A, B, same as α and β respectively, but with depolarization lasting about 1·1 msec. C, sodium current estimated as $(A-B)\times 0{\cdot}92$ during pulse or $(A-B)\times 0{\cdot}99$ after pulse. D, sodium conductance estimated as $C/61$ mV. during pulse or $C/112$ mV. after pulse. The factors 0·92 and 0·99 allow for the outward sodium current in choline sea water and were obtained from the 'independence principle'. Axon 17; temperature 6° C.; V_{Na} in sea water $=-112$ mV.; uncompensated feed-back.

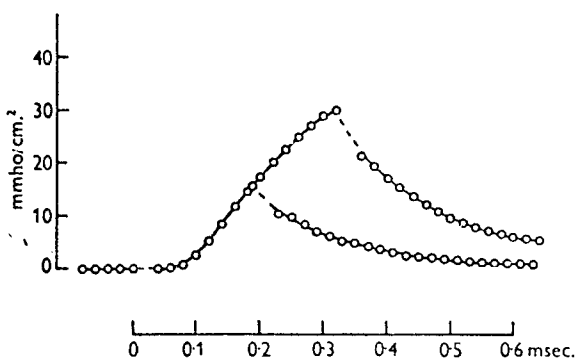

Fig. 5. Time course of sodium conductance estimated from records C and D (Fig. 2) by method described in text. At zero time the membrane potential was reduced by 97·5 mV. and was restored to its resting value at 0·19 msec. (lower curve) or 0·32 msec. (upper curve). The broken part of the curve has been interpolated in the region occupied by the capacitative surge. Axon 41; temperature 3·5° C.; compensated feed-back; $V_{Na}=-117$ mV.

The ordinate (I_2) is the ionic current at the beginning of the second step and the abscissa (V_2) is the potential during the second step. Measurement of I_2 depends on the extrapolation shown in Fig. 6*A*. This should introduce little error over most of the range but is uncertain for $V_2 > 0$, since the ionic current then declined so rapidly that it was initially obscured by capacity current. There was some variation in the magnitude of the current observed during the first pulse. This arose partly from progressive changes in the condition of the

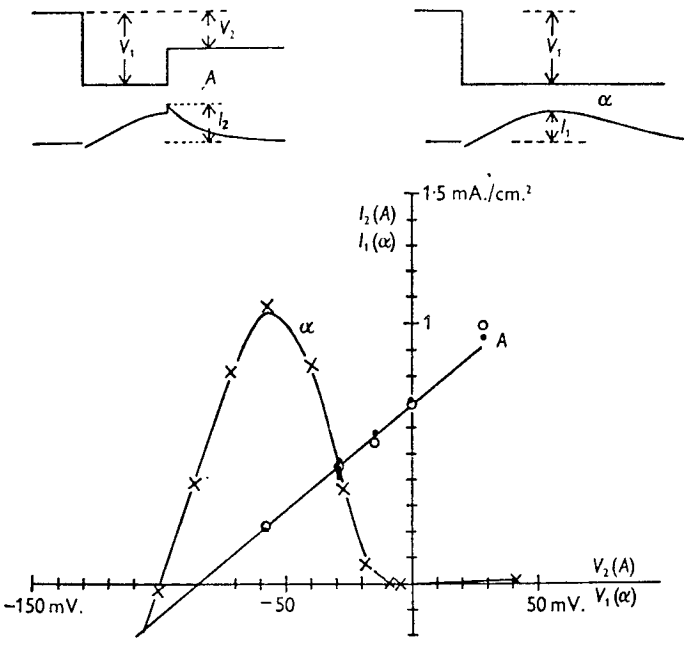

Fig. 6. Line *A*, instantaneous current-voltage relation. The first step had an amplitude of −29 mV. and a duration of 1·53 msec. The abscissa (V_2) gives the amplitude of the second step. The ordinate (I_2) is the ionic current at the beginning of the second step. The dots are observed currents. Hollow circles are these currents multiplied by factors which equalize the currents at the end of the first step. Inset *A*, method of measuring V_2 and I_2. Curve α and crosses, relation between maximum inward current (I_1) and membrane potential using single pulse of amplitude V_1. Inset α, method of measuring V_1 and I_1. Axon 31; temperature 4° C.; uncompensated feed-back.

nerve and partly from small changes in V_1 which cause large variations in current in the region of $V = -29$ mV. Both effects were allowed for by scaling all records so that the current had the same amplitude at the end of the first step. This procedure is justified by the fact that records made with $V_2 = 0$ show that the amplitude of the current immediately after the step was directly proportional to the current immediately before it.

The results are plotted in curve *A* and show that the relation between I_2 and V_2 is approximately linear. This is in striking contrast to the extremely non-

linear relation obtained when the current is measured at longer intervals. An example of the second type is provided by curve α which shows how the maximum inward current varied with membrane potential in the same axon. In this case only a single pulse of variable amplitude was employed and current was measured at times of 0·5–2·0 msec. Under these conditions the sodium conductance had time to reach the value appropriate to each depolarization and the current-voltage relation is therefore far from linear.

The line A and the curve α intersect at -29 mV. since the two methods of measurement are identical if $V_2 = V_1$. A second intersection occurs at -106 mV. which is close to the sodium potential in this fibre.

A similar pair of curves obtained with a larger initial depolarization is shown by A and α in Fig. 7. In this case the nerve was depolarized to the sodium potential so that one would expect the line A to be tangential to the curve α. This is approximately true, although any exact comparison is invalidated by the fact that the two curves could not be obtained at exactly the same time.

The instantaneous current-voltage relation in sodium-free solution

The measurements described in the preceding section indicate that the instantaneous behaviour of the membrane is linear when the nerve is in sea water. The conclusion cannot be expected to apply for all sodium concentrations. The method of defining a chord conductance breaks down altogether if there is no sodium in the external medium. In this case $V_{Na} = \infty$ and g_{Na} must be zero if the sodium current is to be finite. This condition could not be realized in practice but the theoretical possibility of its existence indicates that the concept of sodium conductance must be used with caution.

The lower part of Fig. 7 illustrates an attempt to determine the instantaneous current-voltage relation in a sodium-free solution. The upper curves (A and α) were measured in sea water and have already been described. The crosses in the lower part of the figure give the instantaneous currents in choline sea water, determined in the same way as the circles which give the corresponding relation in sea water. The effect of the change in resting potential has been allowed for by shifting the origin to the right by 4 mV. (see Hodgkin & Huxley, 1952). The series of records from which these measurements were made was started shortly after replacing normal sea water by choline sea water and was continued, in the order shown, with an interval of about 40 sec. between records. On analysis it was found that the earliest records (e.g. 1) showed a small inward current, whereas records taken later (e.g. 11 or 15) gave no such effect. It is evident that the series was started before all the sodium had diffused away from the nerve and that only the later records (e.g. 6–15) can be regarded as representative of a nerve in a sodium-free solution. Nevertheless, it is clear that the instantaneous current-voltage relation shows a marked curvature and is quite different from the linear relation in sea water.

The results are, in fact, reasonably close to those predicted by the 'independence principle'. This is illustrated by a comparison of the crosses in Fig. 7 with the theoretical curves *B* and *C* which were calculated from *A* on the assumption that the independence principle holds and that the sodium

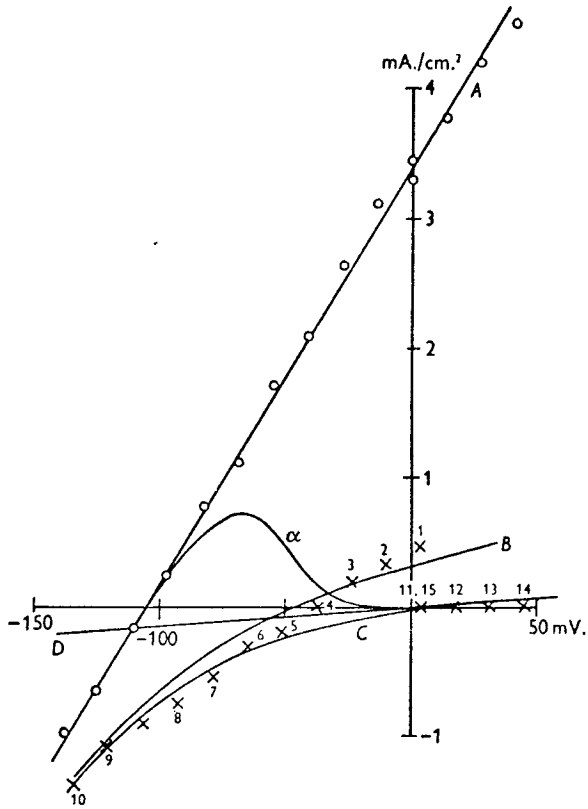

Fig. 7. Current-voltage relations in sea water and choline sea water. Ordinate: current density. Abscissa: displacement of membrane potential from resting potential in sea water. Line *A* and curve α were obtained in sea water in the same way as in Fig. 6, except that the current for α was not measured at the maximum but at a fixed time (0·28 msec.) after application of a single step. The initial depolarization for *A* was 110 mV. and the duration of the first step was 0·28 msec. The crosses give the instantaneous currents in choline sea water, determined in the same way as the circles in *A*. The numbers show the order in which the measurements were made. *B* and *C*, instantaneous current in 10% sodium sea water and in choline sea water respectively, derived from *A* by means of the 'independence principle' using the equations

$$\frac{(I_{Na})_B}{(I_{Na})_A} = \frac{0{\cdot}1 \exp{(V - V_{Na})/24} - 1}{\exp{(V - V_{Na})/24} - 1}$$

and

$$\frac{(I_{Na})_C}{(I_{Na})_A} = \frac{-1}{\exp{(V - V_{Na})/24} - 1},$$

V_{Na} = sodium potential in sea water = −110 mV. Sodium currents measured from the line *D* which passes through the origin and the point for the small current observed at the sodium potential in sea water. Axon 25; temperature 5° C.; uncompensated feed-back.

concentrations in the external solution were 10% (*B*) and 0% (*C*) of that in *A*. It will be seen that there is general agreement between calculated and observed results, although the change in external sodium and the possibility of progressive changes invalidates any exact comparison. In the preceding paper it was shown that the observed sodium currents in a choline solution were usually larger than those calculated from the independence principle. This deviation is not seen here, probably because the measurements in choline were made later than those in sea water and no attempt was made to correct for deterioration, which is likely to have reduced the currents by 30% between the sea water and choline runs.

The experiment described in the preceding paragraph indicates that the linear relation between current and voltage observed in sea water is not a general property of the membrane since it fails in sodium-free solutions. This does not greatly detract from the usefulness of the result, for the primary concern of this paper is to determine the laws governing ionic movements under conditions which allow a normal action potential to be propagated.

The reversible nature of the change in sodium conductance

The results described in the first part of this paper show that the sodium conductance reverts rapidly to a low value when the membrane potential is restored to its resting value. Figs. 2 and 5 suggest that this is true at all stages of the response and that the rate at which the conductance declines is roughly proportional to the value of the conductance. A rate constant (b_{Na}) can be defined by fitting a curve of the form $\exp(-b_{Na}t)$ to the experimental results. Values obtained by this method are given in Table 1.

In order to investigate the effect of repolarizing the membrane to different levels on the rate of decline of sodium conductance we carried out the experiment illustrated by Fig. 8. The curves in the left-hand column are tracings of the membrane current while those on the right give the sodium conductance, calculated on the assumption that the contribution of ions other than sodium is negligible (records made in a solution containing 10% of the normal sodium concentration show that the error introduced by this approximation should not exceed 5% of the maximum current). The initial depolarization was 29 mV. and the sodium conductance reached its maximum value in 1·53 msec. When the membrane potential was restored to its resting level the conductance fell towards zero with a rate constant of about 4·3 msec.$^{-1}$ (curve γ). If V_2 was made +28 mV. the rate constant increased to about 10 msec.$^{-1}$ and a further increase to 15 msec.$^{-1}$ occurred with $V_2 = +57$ mV. On the other hand, if V_2 was reduced to −14 mV. the conductance returned with a rate constant of only 1·6 msec.$^{-1}$. When $V_2 = -57$ mV. the conductance no longer fell but increased towards an 'equilibrium' value which was greater than that attained at −29 mV. (The curve cannot be followed beyond about 2 msec.

TABLE 1. Apparent values of rate constant determining decline of sodium conductance following repolarization to resting potential

Axon	Membrane potential during pulse (mV.)	Duration of pulse (msec.)	Temperature (° C.)	Average rate constant (msec.$^{-1}$)	Rate constant at 6° C. (msec.$^{-1}$)
15	−32	0·4–1·1	11	9·4	5·4
15	−91	0·1–0·5	11	9·0	5·2
17	−32	0·7–1·6	6	5·9	5·8
17	−51	0·2–1·0	6	6·7	6·6
24	−42	0·2	20	18·5	4·1
24	−84	0·1	20	17·2	3·9
25	−41	1·0	4	3·8	4·8
25	−110	0·3	4	3·3	4·0
31	−100	0·3	4	3·0	3·8
31	−29	1·5	4	4·2	5·3
32	−116	0·2	5	6·3*	6·9*
32	−67	0·7	5	6·3*	6·8*
41	−98	0·1–4	3	7·1*	9·6*
41	−117	0·1–3	3	7·7*	10·5*

Results marked with an asterisk were obtained with compensated feed-back. The last column is calculated on the assumption that the temperature coefficient (Q_{10}) of the rate constant is 3.

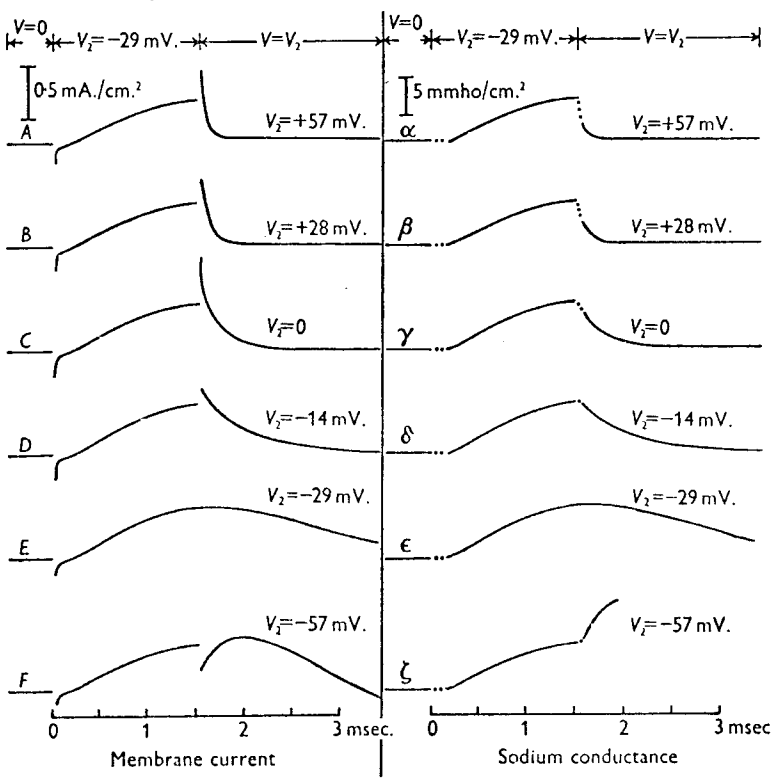

Fig. 8. *A–F*, time course of membrane current associated with change in membrane potential shown at top of figure. α–ζ; time course of sodium conductance obtained by dividing *A–F* by $V + 100$ mV. Axon 31; temperature 4° C.; uncompensated feed-back.

because the contribution of potassium ions soon becomes important at large depolarizations.) The whole family of curves suggests that the conductance reached at any depolarization depends on the balance of two processes occurring at rates which vary in opposite directions with membrane potential.

The observation that the rate constant increases with membrane potential does not depend on the details of the method used to estimate sodium conductance, for the tracings of current in the left-hand column of Fig. 8 show exactly the same phenomenon. Similar results were obtained in all the experiments of this type, and are plotted against V_2 in Fig. 9. It will be seen that there is good agreement between different experiments, and that a tenfold increase of rate constant occurs between $V_2 = -20$ and $+50$ mV.

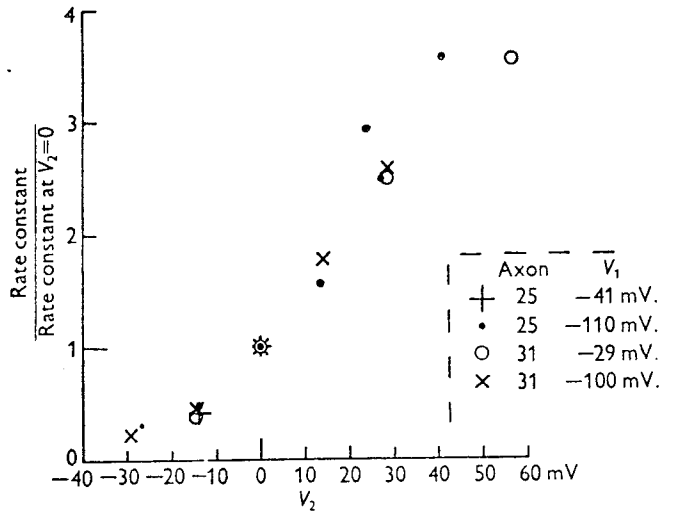

Fig. 9. Relation between rate constant determining decline of sodium conductance and potential to which membrane is repolarized. Abscissa: membrane potential during second step (V_2). Ordinate: relative value of rate constant.

Errors due to the series resistance

Most of the experiments in this paper were obtained with uncompensated feed-back and must therefore have been somewhat affected by the small resistance in series with the membrane (Hodgkin *et al.* 1952). The linearity of the relation between current and voltage illustrated by Figs. 6 and 7 can clearly stand, for the effect of a series resistance would simply be to change the slope of the straight line and not to introduce any curvature. From our estimates of the value of the series resistance it can be shown that the true slopes in these two figures should be 7 and 30% greater than those shown. A more serious error is introduced in the measurement of the rate constant. In Fig. 8 the total current at the beginning of the record was about 0·5 mA./cm.^{2}. This means that the true membrane potential was not zero but about -4 mV. At this potential the rate of return of membrane conductance would be slowed by about 8%. In some of the experiments used in compiling Table 1 this error may be as great as 50%. However, it should be small in axons 32 and 41 which were examined with compensated feed-back. We are also uncertain about the extent to which the rate of return of sodium conductance can be regarded as exponential. Axon 41, which was in excellent condition and was examined with compensated feed-back, showed clear depar-

tures from exponential behaviour in that the initial fall of conductance was too rapid (see Figs. 2 and 5). In all other experiments the curves of current against time were reasonably close to exponentials, but in many cases this may have been due to an error introduced by the series resistance.

Errors due to polarization effects

If the membrane was maintained in the depolarized condition for long periods of time the outward current declined as a result of a 'polarization effect' (Hodgkin *et al.* 1952). At the end of such a pulse a phase of inward current was observed and was found to be roughly proportional to the amount of 'polarization'. This was quite distinct from the inward current described in the preceding sections, since it only appeared with long pulses and was unaffected by removing external sodium. With the possible exception of Fig. 2*G*, the results described in the preceding sections are unlikely to have been affected by polarization since the duration of the pulse was always kept short.

The time course of the sodium conductance during a maintained depolarization

In a previous paper we showed that the time course of the sodium conductance could be obtained from records of membrane current in solutions of different sodium concentration (Hodgkin & Huxley, 1952). An alternative method of calculating these curves is illustrated by Fig. 10. The method

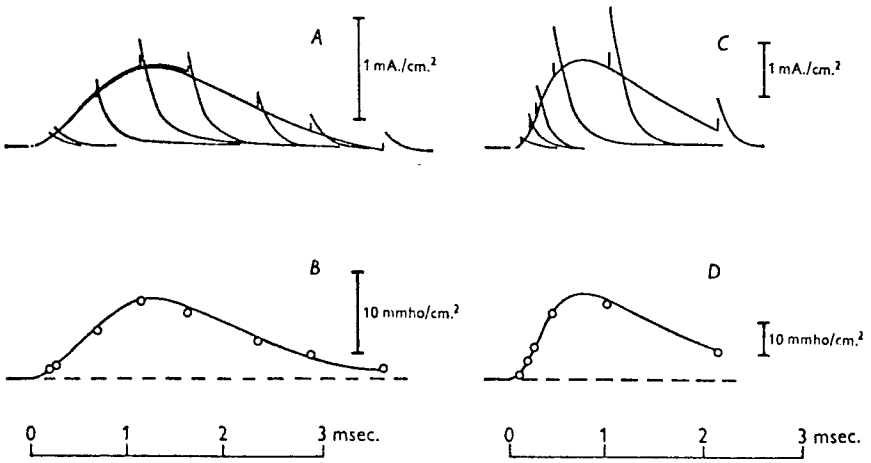

Fig. 10. *A*, time course of ionic currents associated with depolarizations of 32 mV. lasting from $t=0$ to times indicated by vertical strokes on tracings. Nerve in sea water. *B*, time course of sodium conductance. The circles were obtained by dividing the peak currents in *A* by 112 mV. and the continuous curve from the difference between the continuous curve in *A* and a similar curve in choline sea water (see text and legend to Fig. 4). *C*, same as *A* but employing depolarization of 51 mV. *D*, sodium conductance obtained from *C* by similar methods to those used for *B*. (The smooth curve is the same as that shown in Fig. 4.) Axon 17; temperature 6° C.; V_{Na} in sea water = − 112 mV.; uncompensated feed-back.

depends on the fact that the inward current immediately after a pulse is proportional to the sodium conductance at the end of the preceding depolarization. The variation of inward current with pulse duration is illustrated by the tracings in Fig. 10*A*. The time course of the sodium conductance can be measured by determining the maximum ionic current associated with repolar-

ization and dividing this quantity by the difference between the resting potential and the sodium potential (about −112 mV. in this experiment). A series of points obtained by this method is shown in Fig. 10*B*. These may be compared with the smooth curve, which represents sodium conductance obtained by the method described in the preceding paper (subtraction of ionic current in choline from that in sea water). Good agreement was obtained, and also when other depolarizations were employed, for example *C* and *D* at −51 mV. The only occasions on which the two methods did not agree were those in which the sodium conductance was measured at long times, with a large depolarization. In these experiments the subtraction method sometimes gave an apparent negative conductance which we regarded as an error due to slight differences between the potassium currents in the two records. This conclusion was confirmed by the fact that the alternative method never showed a 'negative conductance' but only a residual positive conductance.

Experiments with relatively long depolarizations

The instantaneous relation between potassium current and membrane potential

In a previous paper (Hodgkin & Huxley, 1952) we gave reasons for thinking that potassium ions were largely responsible for carrying the maintained outward current associated with prolonged depolarization of the membrane. In order to investigate the instantaneous relation between potassium current and membrane potential it is necessary to employ depolarizations lasting for much longer periods than those used to study sodium current. Polarization effects made such experiments difficult at 5° C., but errors from this cause could be greatly reduced by working at 20° C. In this case the polarization effect occurred at the same rate but the potassium conductance rose in about one-fifth of the time required at 5° C.

A typical experiment with a nerve in choline sea water is illustrated by Fig. 11. Its general object is to measure the ionic currents associated with repolarization of the membrane when the potassium conductance is much greater than the sodium conductance. The amplitude of the first step was −84 mV. and its duration 0·63 msec., which is equivalent to about 4 msec. at 5° C. Under these conditions 90–95% of the outward current should be potassium current and only 5–10% should be sodium current (see Fig. 10 for an indication of the rate of fall of sodium conductance from its initial maximum). After the pulse, sodium current should be negligible since the nerve was in choline sea water (see Fig. 3).

The simplest record in Fig. 11 is *E*, in which the membrane potential was restored to its resting value at the end of the first step. The sequence of events was as follows. At $t=0$ the membrane was depolarized by 84 mV. and was held at this level until $t=0{\cdot}63$ msec. The current was outward during the whole period and consisted of a hump of sodium current followed by a rise of

potassium current which reached 1·83 mA./cm.^{2} at $t=0·63$ msec. At this moment the membrane potential was restored to its resting value. The sudden increase in potential was associated with a brief capacity current in an inward direction. This was followed by an outward current which declined exponentially to zero. A record at higher amplification (*e*) shows this 'tail' of outward current more clearly. The dots give the ionic current extrapolated to

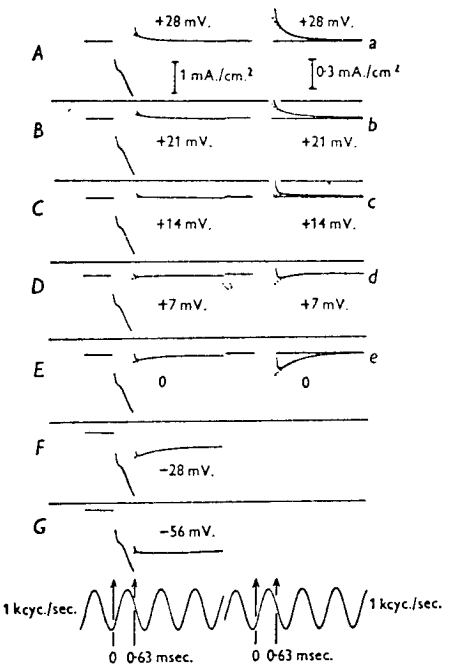

Fig. 11. Membrane currents associated with depolarization of 84 mV. followed by repolarization to value shown on each record. The duration of the first step was 0·63 msec. The second step lasted longer than these records. *A* to *G*, records at low amplification showing current during both steps. *a–e*, records at higher amplification showing only the current during the second step. The dots give the ionic current after correcting for capacity current. Axon 26 in choline sea water; temperature 20° C.; uncompensated feed-back.

$t=0·63$ msec. after correcting for the residual effect of the capacitative surge. The 'tail' of outward current can be explained by supposing that the equilibrium potential for potassium is about 12 mV. greater than the resting potential in choline sea water, and that the instantaneous value of the potassium conductance (g_K) is independent of V. At $t=0·63$ msec. the current is 1·83 mA./cm.^{2} when $V=-84$ mV., or 0·22 mA./cm.^{2} when $V=0$. Taking the potassium potential (V_K) as +12 mV. and neglecting the contribution of chloride and other ions it follows that the potassium conductance (g_K) was approximately the same in the two cases. Thus

$$g_K=I_K/(V-V_K), \tag{2}$$

so that

$$g_K = \frac{-1{\cdot}83 \text{ mA./cm.}^2}{-96 \text{ mV.}} = 19 \text{ m.mho/cm.}^2 \quad \text{when } V = -84 \text{ mV.,}$$

and

$$g_K = \frac{-0{\cdot}22 \text{ mA./cm.}^2}{-12 \text{ mV.}} = 18 \text{ m.mho/cm.}^2 \quad \text{when } V = 0.$$

As soon as the membrane potential is restored to its resting value the potassium conductance reverts exponentially to its resting level and therefore gives the tail of current seen in E and e. If this explanation is right, the exponential tail of current should disappear at $V = V_K$ and should be reversed in sign when $V > V_K$. Records a to d show that the current is inward for $V > 21$ mV. and is outward for $V < 7$ mV. There is practically no inward current at $V = 14$ mV. and 13 ± 1 mV. would seem to be a reasonable estimate of V_K.

This method of measuring the potassium potential depends on the assumption that potassium ions are the only charged particles responsible for the component of the current which varies with time after the end of the pulse. It is not affected by the fact that chloride and other ions may carry appreciable quantities of current, provided that the resistance to the motion of these ions is constant at any given value of membrane potential. The magnitude of the 'leak' due to chloride and other ions may be estimated from the current needed to maintain the membrane at the potassium potential. In the experiment illustrated by Fig. 11 this current was about 0·008 mA./cm.^{2} which is small compared with the maximum potassium current at $V = 0$ or $V = +28$ mV.

Fig. 12 was prepared from the records in Fig. 11 by essentially the same method as that used in studying the instantaneous relation between sodium current and membrane potential. Curve α gives the relation between current and voltage 0·63 msec. after the application of a single step of amplitude V_1. In curve A, V_1 was fixed at -84 mV. and the potential was changed suddenly to a new level V_2. The abscissa is V_2, while the ordinate is the ionic current immediately after the sudden change. The experimental points in A are seen to fall very close to a straight line which crosses curve α at the potassium potential ($+13$ mV.). In this experiment no measurements were made with $V_2 < V_1$ but records obtained with other fibres showed that the instantaneous current-voltage relation was linear for $V_2 < V_1$ as well as for $V_2 > V_1$.

In the experiment considered in the previous paragraphs the initial rise of potassium current was obscured by sodium current and the plateau was not reached because the pulse was kept short in order to reduce possible errors from the 'polarization effect'. A clearer picture of the sequence of events is provided by Fig. 13. In this experiment the amplitude of the pulse was -25 mV. and its duration nearly 5 msec. The polarization effect was not appreciable since the current density was relatively small. Sodium current was also small since the nerve was in choline sea water, and the depolarization

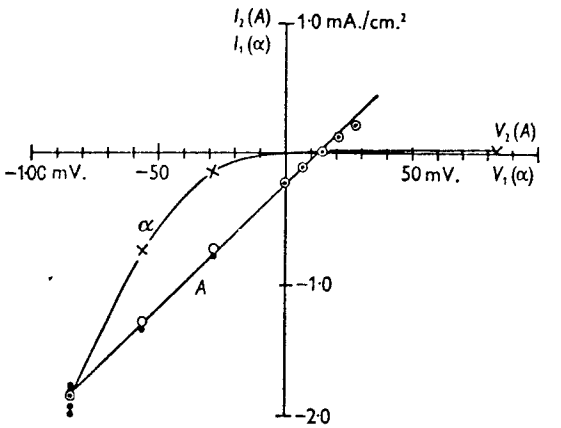

Fig. 12. Current-voltage relations during period of high potassium permeability. Line *A*, instantaneous current-voltage relation determined by changing membrane potential in two steps. The first step had a constant amplitude of −84 mV. and a constant duration of 0·63 msec. The abscissa (V_2) gives the amplitude of the second step in millivolts. The ordinate (I_2) is the ionic current density at the beginning of the second step. The dots are observed currents. Hollow circles are these currents multiplied by factors which equalize the currents at the end of the first step. Curve α and crosses, relation between current and membrane potential at 0·63 msec. after beginning of single step of amplitude V_1. Experimental details are as in Fig. 11.

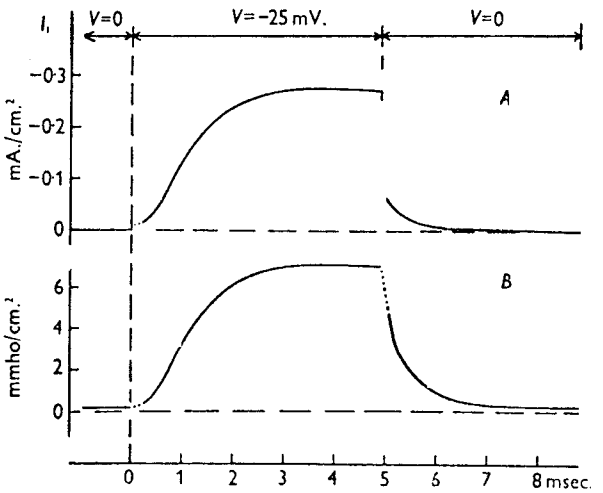

Fig. 13. *A*, ionic current associated with depolarization of 25 mV. lasting 4·9 msec. Axon 18 in choline sea water at a temperature of 21° C. The curve is a direct replot of the original current record except in the regions 0–0·3 msec. and 4·9–5·2 msec., where corrections for capacity current were made by the usual method. Outward current shown upward. *B*, potassium conductance estimated from *A* by the equation $g_K = I_K/(V - V_K)$, where V_K is 12 mV. and I_K is taken as the ionic current (I_i) minus a leakage current of 0·5 m.mho/cm.² × (V + 4 mV.).

was less than that at which a hump of outward sodium current first became appreciable. On the other hand, it was desirable to make a small correction for the leakage current due to ions other than sodium and potassium. The method of estimating this current at different voltages is indicated on p. 494. The experiment shows that whereas the potassium conductance rises with a marked delay it falls along an exponential type of curve which has no inflexion corresponding to that on the rising phase. This difference was present in all records except, possibly, those with very small depolarizations. It was also present in the curves calculated for the rise and fall of sodium conductance (e.g. Figs. 4, 5 and 8).

The rate constant determining the decline of potassium conductance

The experiments described in the preceding sections indicate that the potassium conductance returns to a low level when the membrane is repolarized to its resting value. The restoration of the condition of low conductance leads to a 'tail' of potassium current which can be fitted with reasonable accuracy by a curve of the form $\exp(-b_K t)$. Table 2 gives the values of the rate constant (b_K) determined by this method. It shows that b_K varies markedly with temperature and also to some extent with the amplitude of the step used to depolarize the axon. The second effect was particularly noticeable in axon 1 which was in poor condition and had a high potassium conductance in the resting state.

The effect of repolarizing the membrane to different levels is shown in Fig. 14. For $V_2 > -20$ mV. the rate constant increased with membrane potential but the relation is less steep than in the corresponding curve for sodium conductance (Fig. 9). Thus, changing V_2 from 0 to +40 mV. increases b_{Na} about 3·2-fold and b_K about 1·6-fold. Another important difference between the two processes is that b_{Na} is about 30 times greater than b_K at the resting potential.

The potassium potential

Table 3 summarizes a number of measurements of the potential at which potassium current reverses its direction. At 22° C. the apparent potassium potential is about 19 mV. higher than the resting potential if the axon is in sea water and about 13 mV. higher if it is in choline sea water. Corresponding figures at 6–11° C. are 13 mV. in sea water and 8 mV. in choline sea water. Since the resting potential is about 4 mV. higher in choline sea water (Hodgkin & Huxley, 1952) it seems likely that the absolute value of the potassium potential is unaffected by substituting choline for sodium ions. At 20° C. the absolute value of the potassium potential would be 80–85 mV. if the resting potential is taken as 60–65 mV. (Hodgkin & Huxley, 1952). This is nearly equal to the potential of 91 mV. estimated from chemical analyses

(Hodgkin, 1951). A similar conclusion applies to the results at 6–11° C. In squid fibres, cooling from 20 to 8° C. either has no effect or increases the resting potential by 1 or 2 mV. (Hodgkin & Katz, 1949). The observed

TABLE 2. Rate constant determining decline of potassium conductance following repolarization to resting potential

Axon	Depolarization (mV.)	Temperature (° C.)	Rate constant (msec.$^{-1}$)	Rate constant at 6° C. (msec.$^{-1}$)	Average rate constant at 6° C. (msec.$^{-1}$)
A 1	6	23	1·2	0·14	
1	13	23	1·3	0·15	
1	21	23	1·3	0·16	
15	13	11	0·36	0·19	
15	20	11	0·35	0·19	
17	10	6	0·20	0·20	
18	6	22	1·5	0·20	
18	13	22	1·6	0·22	0·17
20	21	6	0·17	0·17	
21	14	7	0·19	0·16	
38*	10	5	0·12	0·13	
39*	20	19	1·0	0·20	
39*	10	19	0·83	0·16	
39*	10	3	0·10	0·15	
41*	20	4	0·10	0·12	
41*	10	4	0·11	0·14	
B 1	36	23	1·5	0·18	
1	54	23	1·8	0·22	
18	50	22	2·0	0·27	
18	63	22	1·7	0·23	0·22
18	112	22	1·8	0·24	
27	28	21	1·4	0·22	
28	28	21	1·3	0·20	
C 15	13	11	0·49	0·26	
17	10	6	0·21	0·21	
18	6	22	1·6	0·21	0·23
18	13	22	1·7	0·23	
18	19	22	1·9	0·25	
D 18	25	22	2·0	0·27	
18	50	22	2·1	0·28	
18	63	22	2·1	0·28	
23	84	21	1·8	0·28	0·28
24	84	20	2·0	0·35	
26	84	20	1·8	0·30	
27	28	21	1·3	0·19	

Groups A and B in sea water; C and D in choline sea water. Groups A and C: depolarization less than 25 mV.; B and D: depolarization greater than 25 mV. An asterisk denotes the use of compensated feed-back. Rate constants at 6° C. are calculated for a Q_{10} of 3·5, which was found suitable for groups A and C.

potassium potential should therefore be taken as 75–80 mV. while the theoretical potassium potential would be reduced from 91 to 87 mV.

The effect of changing the external concentration of potassium on the potassium potential

Experimental determinations of V_K such as that illustrated by Fig. 11 were made in choline solutions containing different concentrations of potassium.

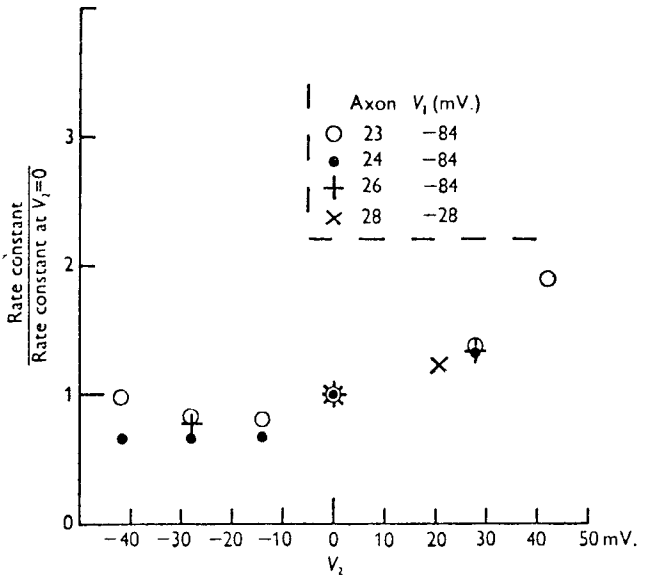

Fig. 14. Effect of membrane potential on the rate constant determining decline of potassium conductance. Abscissa (V_2): membrane potential during second step. Ordinate: relative value of rate constant.

TABLE 3. Apparent values of potassium potential

Axon	Medium	Temperature (°C.)	Potassium potential minus resting potential (mV.)	Average (mV.)
1	S	23	18	19
28	S	21	19	
15	S	11	14	11
20	S	6	10	
21	S	8	9	
18	C	22	12	13
23	C	21	14	
24	C	20	7	
26	C	20	13	
27	C	21	15	
28	C	21	16	
15	C	11	8	8
17	C	6	7	
20	0·7C:0·3S	6	8	
21	0·9C:0·1S	8	10	

S denotes sea water; C choline sea water. In axons 23–28 the potassium potential was found by the method illustrated by Fig. 12. In other cases it was taken as the potential at which the steady state current-voltage curve intersects the line joining the potassium currents before and after repolarization to the resting potential (e.g. line A and curve α intersect at 13 mV. in Fig. 12).

TABLE 4. Effect of potassium concentration on the apparent value of the potassium potential

Axon	Medium	Change in resting potential (ΔE_r) (mV.)	Change in applied potential at which I_K is zero (ΔV_K) (mV.)	Change in absolute membrane potential at which I_K is zero ($\Delta E_K = \Delta E_r + \Delta V_K$) (mV.)
27	A ($\frac{1}{2}$K)	+2	+6	+8
27	B (2K)	−3	−6	−9
28	B (2K)	−3	−7	−10
28	C (5K)	−13	−9	−22

Changes are given relative to the mean potentials observed in a 1K choline sea water before and after application of the test solution. The 1K choline sea water was identical with that described by Hodgkin & Huxley (1952) and contained choline at a concentration of 484 and potassium at 10 g.ions/kg. H_2O. The test solutions A, B, C and D were similar but contained potassium at concentrations of 5, 10, 20, 50 g.ions/kg. H_2O and correspondingly reduced concentrations of choline. Potentials are given as 'outside potential' minus 'inside potential'.

It was not possible to use a wide range of potassium concentrations, since squid axons tend to undergo irreversible changes if left in solutions containing high concentrations of potassium for any length of time. The results obtained with the two axons studied by this method are given in Table 4. They show that the potassium potential (E_K) is sensitive to the external concentration of potassium but that it changes by only about half the amount calculated for a concentration cell. Thus solutions *A*, *B*, *C* should give changes of +17, −17 and −41 mV. if E_K obeyed the ordinary equation for a concentration cell.

One possible explanation of this result is that potassium ions are not the only charged particles responsible for the delayed rise in conductance associated with depolarization (delayed rectification). Thus the discrepancy would be explained if choline or sodium, which are present in relatively high concentrations in the external solution, take part in the process with an affinity only 5% of that of potassium. This explanation might be consistent with the evidence which suggests that potassium ions are responsible for carrying most of the outward current through a depolarized membrane. For the concentration of potassium inside a fibre is about 10 times greater than that of sodium and the internal concentration of choline is almost certainly negligible. The participation of chloride ions in the process responsible for delayed rectification can probably be eliminated since one experiment showed that replacing all the choline chloride and two-thirds of the magnesium chloride in the choline sea water by dextrose gave an apparent *increase* of 3 mV. in the 'potassium potential'. The magnitude of the change was less certain in this experiment, since the solution employed gave a junction potential of 5–7 mV. which had to be allowed for in estimating the shift in resting potential. But it was clear that any change in E_K was small compared with the *reduction* of 45 mV. expected on the hypothesis that delayed rectification is entirely due to chloride ions.

Another way of accounting for the relatively small changes seen in Table 4 is to suppose that the potassium concentration in the immediate vicinity of the surface membrane is not the same as that in the external solution. Isolated cephalopod axons leak potassium ions at a fairly high rate and these must diffuse through layers of connective tissue and other structures between the excitable membrane and the external solution. This leakage is likely to increase in potassium-deficient solutions and to decrease in potassium-rich solutions. Hence the changes in effective potassium concentration might be less than those in Table 4. A related possibility is that the potassium concentration may be raised locally by the large outward currents used in these

experiments. This hypothesis is of interest since it might account for the slow polarization effect which is not otherwise explained except in terms of a complicated polarization process at the internal electrode. In a former paper (Hodgkin *et al.* 1952) we obtained evidence of an external layer with a resistance of about 3 Ω.cm.^{2}. The transient change in potassium concentration due to current cannot be calculated without knowing the thickness of this layer. The steady change due to leakage might be large enough to explain the deviations in Table 4, if the leakage of potassium had been several times greater than that found by Steinbach & Spiegelman (1943).

It may be asked why effects similar to those discussed in the preceding paragraph do not upset the relation between the external sodium concentration and V_{Na}. The answer, probably, is that similar effects are present but that they are small because the sodium concentration in sea water is 45 times greater than that of potassium. Changes in concentration due to current would also be smaller in the case of sodium because the sodium currents are of relatively short duration.

The contribution of ions other than sodium and potassium

The experimental results described in this series of papers point to the existence of special mechanisms which allow first sodium and then potassium to cross the membrane at a high rate when it is depolarized. In addition it is likely that charge can be carried through the membrane by other means. Steinbach's (1941) experiments suggest that chloride ions can cross the membrane and there is probably a small leakage of sodium, potassium and choline through cut branches

TABLE 5. Tentative values of leakage conductance and 'equilibrium' potential for leakage current. Five nerves in choline sea water at 6–22° C.

	Average	Range
Leakage conductance (g_l) (m.mho/cm.^{2})	0·26	0·13 to 0·50
Equilibrium potential for leakage current (V_l) (mV.)	−11	−4 to −22
Resting potassium conductance $(g_K)_r$ (m.mho/cm.^{2})	0·23	0·12 to 0·39
Equilibrium potential for potassium (V_K)	+10	+7 to +13

or through parts of the membrane other than those concerned with the selective system. All these minor currents may be thought of as contributing towards a leakage current (I_l) which has a conductance (g_l) and an apparent equilibrium potential (V_l) at which I_l is zero. In this leakage current we should probably also include ions transferred by metabolism against concentration gradients. So many processes may contribute towards a leakage current that measurement of its properties is unlikely to give useful information about the nature of the charged particles on which it depends. Nevertheless, a knowledge of the approximate magnitude of g_l and V_l is important since it is needed for any calculation of threshold or electrical stability. Various methods of measurement were tried but only the simplest will be considered since the orders of magnitude of g_l and V_l were unaffected by the precise method employed. In the experiment of Fig. 11 the steady current needed to maintain the membrane at the potassium potential (+13 mV.) was 8 μA./cm.^{2}. According to our definitions this inward current must have been almost entirely leakage current, for the nerve was in choline sea water and $I_K = 0$ when $V = V_K$. Hence

$$(13 \text{ mV.} - V_l)\, g_l = 8 \ \mu\text{A./cm.}^2.$$

In order to estimate g_l we make use of the fact that the inward current associated with $V = +84$ mV. was not appreciably affected by a fourfold change of potassium concentration (from 5 to 20 mM.). We therefore assume that the potassium conductance was reduced to a negligible value at this membrane potential and that the inward current of 24 μA./cm.^{2} was entirely leakage current. Hence

$$(84 \text{ mV.} - V_l)\, g_l = 24 \ \mu\text{A./cm.}^2.$$

From those two equations we find a value of −22 mV. for V_l and one of 0·23 m.mho/cm.^{2} for g_l. An estimate of the resting value of g_K may also be obtained by this method. At the resting potential in choline sea water

$$V_l g_l + V_K (g_K)_r = 0.$$

Hence

$$(g_K)_r = 0{\cdot}39 \text{ m.mho/cm.}^2.$$

Tentative values obtained by this type of method are given in Table 5.

DISCUSSION

At this stage all that will be attempted by way of a discussion is a brief comparison of the processes underlying the changes in sodium and potassium conductance. The main points of resemblance are: (1) both sodium and potassium conductances rise along an inflected curve when the membrane is depolarized and fall without any appreciable inflexion when the membrane is repolarized; (2) the rate of rise of conductance increases continuously as the membrane potential is reduced whereas the rate of fall associated with repolarization increases continuously as the membrane potential is raised; (3) the rates at which the conductances rise or fall have high temperature coefficients whereas the absolute values attained depend only slightly on temperature; (4) the instantaneous relation between sodium or potassium current and membrane potential normally consists of a straight line with zero current at the sodium or potassium potential.

The main differences are: (1) the rise and fall of sodium conductance occurs 10–30 times faster than the corresponding rates for potassium; (2) the variation of peak conductance with membrane potential is greater for sodium than for potassium; (3) if the axon is held in the depolarized condition the potassium conductance is maintained but the sodium conductance declines to a low level after reaching its peak.

SUMMARY

1. Repolarization of the giant axon of *Loligo* during the period of high sodium permeability is associated with a large inward current which declines rapidly along an approximately exponential curve.

2. The 'tail' of inward current disappears if sodium ions are removed from the external medium.

3. These results are explained quantitatively by supposing that the sodium conductance is a continuous function of time which rises when the membrane is depolarized and falls when it is repolarized.

4. For nerves in sea water the instantaneous relation between sodium current and membrane potential is a straight line passing through zero current about 110 mV. below the resting potential.

5. The rate at which sodium conductance is reduced when the fibre is repolarized increases markedly with membrane potential.

6. The time course of the sodium conductance during a voltage clamp can be calculated from the variation of the 'tail' of inward current with the duration of depolarization. The curves obtained by this method agree with those described in previous paper.

7. Repolarization of the membrane during the period of high potassium permeability is associated with a 'tail' of current which is outward at the

resting potential and inward above a critical potential about 10–20 mV. above the resting potential.

8. The instantaneous relation between potassium current and membrane potential is a straight line passing through zero at 10–20 mV. above the resting potential.

9. These results suggest that the potassium conductance is a continuous function of time which rises when the nerve is depolarized and falls when it is repolarized.

10. The rate at which the potassium conductance is reduced on repolarization increases with membrane potential.

11. The critical potential at which the 'potassium current' appears to reverse in sign varies with external potassium concentration but less steeply than the theoretical potential of a potassium electrode.

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