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THE EFFECT OF SODIUM IONS ON THE ELECTRICAL ACTIVITY OF THE GIANT AXON OF THE SQUID

BY A. L. HODGKIN AND B. KATZ

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

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Experiments with internal electrodes suggest that the nerve action potential is not a simple depolarisation of the kind postulated by Bernstein (1912) and Lillie (1923). In the giant axon of the squid, the resting membrane potential appears to be about -50 mV, whereas the action potential amplitude is of the order of 100 mV (Curtis & Cole, 1942; Hodgkin and Huxley, 1939, 1945). This result implies that the surface membrane undergoes a transient reversal in polarity during the passage of the nervous impulse. The magnitude of the reversal cannot be measured precisely, because of uncertainties concerning the liquid junction potential between the axoplasm and the internal recording electrode. But there is now little doubt that the membrane potential of certain types of nerve fibre does undergo an apparent reversal in polarity, which cannot be reconciled with the classical form of the membrane theory. Several attempts have been made to provide a theoretical basis for this result (Curtis & Cole, 1942; Hodgkin & Huxley, 1945; Hober, 1946; Grundfest, 1947), but the explanations so far advanced are speculative and suffer from the disadvantage that they are not easily subject to experimental test. A simpler type of hypothesis has recently been worked out, in collaboration with Mr Huxley, and forms the theoretical background of this paper. The hypothesis is based upon a comparison of the ionic composition of the axoplasm of a squid nerve with that of the seawater in which experimental preparations are normally immersed. The potassium concentration of fresh squid axoplasm appears to be some twenty to forty times greater than that in seawater, whereas the sodium and chloride ions may be present in concentrations which are less than one tenth of those in seawater (Steinbach, 1941; Steinbach & Spiegelman, 1943). The resting membrane potential is supposed to arise in a manner, which is essentially similar to that postulated in Bernstein's form of the membrane theory. The resting membrane is assumed to be permeable to potassium and possibly to chloride ions, but is only very sparingly permeable to sodium. There should, therefore, be a potential difference of the correct polarity (i.e. negative) and magnitude across the surface membrane of a resting nerve fibre.

According to the membrane theory excitation leads to a loss of the normal selectively permeable character of the membrane, with the result that the resting membrane potential depolarises towards zero during activity. This aspect of the theory is at variance with modern observations and must be rejected. However, a large reversal in polarity of membrane potential can be obtained if it is assumed that during an action potential the membrane does not lose its selective permeability, but reverses the resting conditions by becoming highly and specifically permeable to sodium. The resulting membrane potential at the peak of an action potential may approach +60 mV in a nerve with an internal sodium concentration equal to one-tenth of that outside according to the relationship $E_{Na} = 58 \log_{10}([Na]_o/[Na]_i)$. The essential point in the hypothesis is that the permeability to sodium must rise to a value, which is much higher than that to potassium and chloride. Unless this occurs the potential difference which should arise from the sodium concentration difference would be abolished by the contributions of potassium and chloride ions to the membrane potential. The hypothesis therefore presupposes the existence of a special mechanism, which allows sodium ions to traverse the membrane during an action potential at a much higher rate than either potassium or chloride ions.

A simple consequence of the hypothesis is that the amplitude of the action potential should be greatly influenced by the concentration of sodium in the external fluid. Thus the potential at the peak of an action potential should no longer be capable of exceeding 0 mV if the external sodium concentration were made equal to the internal concentration, since $E_{Na} = 0$ mV when $[Na]_o = [Na]_i$. On the other hand, an increase in the depolarisation of the membrane would occur if the external sodium concentration could be raised without damaging the axon by osmotic effects. Experiments of this kind are difficult to make when external electrodes are used for recording for the sodium content of the external medium cannot be varied without changing the electrical resistance of the extra-cellular fluid, and this would in itself cause a large alteration in the magnitude of the recorded action potential. We have therefore studied the influence of sodium concentration on the profile and amplitude of the action potential recorded with an internal electrode in the giant axon of the squid.

APPARATUS

The general plan of the equipment was essentially similar to that used by Hodgkin & Huxley (1945) and need not be described in detail. A diagram of the recording cell is shown (Fig 1) in order to facilitate description of the experimental procedure. The walls of the cell were made of glass or Perspex. The Perspex was at first coated with a thin film of paraffin wax, but no adverse effects were observed when this precaution

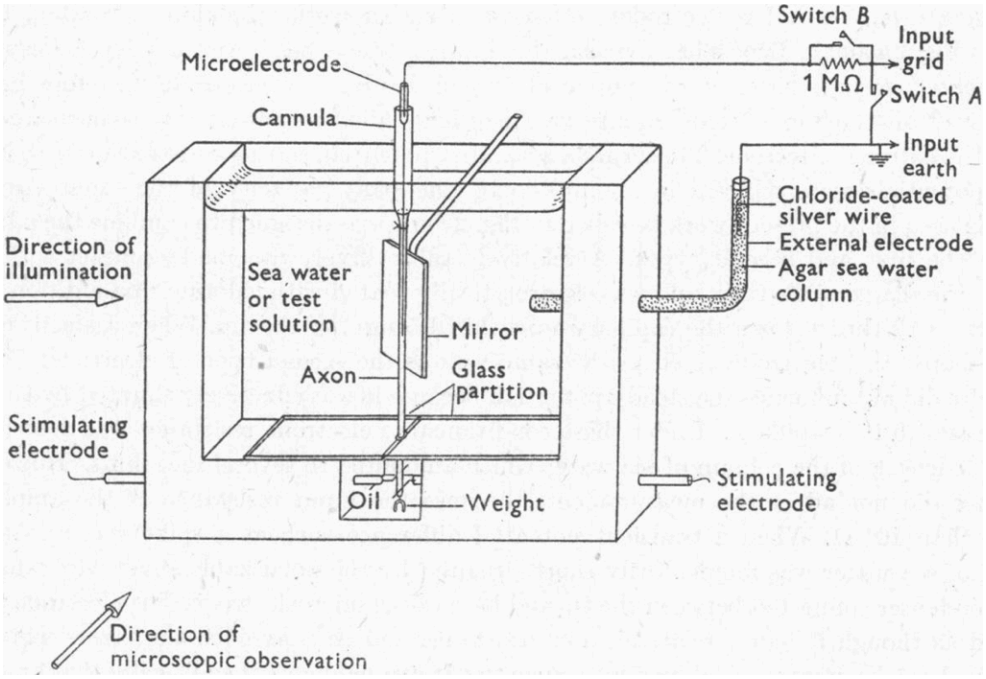

Fig 1. Simplified diagram of recording cell.

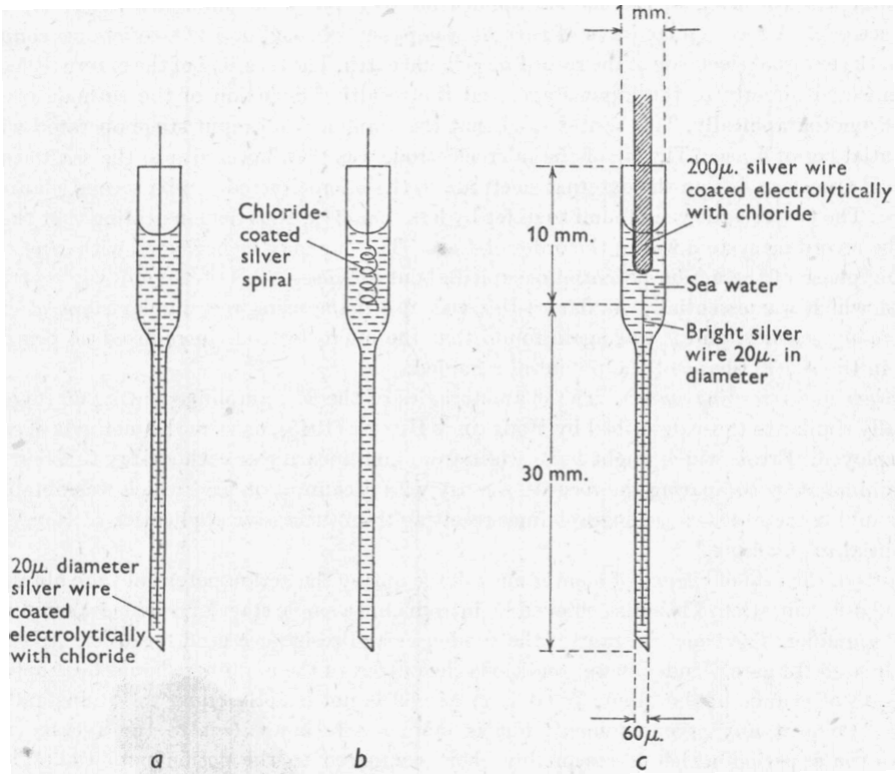

Fig 2. Diagrams of microelectrodes *a* and *b*, types formerly employed; *c*, type used in present work.

was omitted. The cell was illuminated from the side and this had the advantage that a double image of the axon could be obtained in the microscope by using a single mirror instead of the more complicated arrangement employed by Hodgkin & Huxley (1945). The mirror was removed from the cell as soon as the microelectrode had been inserted to the correct distance.

Microelectrode. The microelectrodes employed in earlier work (Hodgkin & Huxley, 1945) are shown in Fig 2*a* and *b*. Type *a* had a reasonably low resistance, but it was unsuitable for prolonged experiments because the electrode potential was unsteady. The electrode therefore had to be withdrawn from the fibre at frequent intervals in order to allow its potential to be measured against that of the external electrode. Type *b* gave a steadier potential, but its resistance was so high that action potentials were reduced in magnitude by the stray capacity of the input circuit. The electrode used in the present work is shown in Fig 2*c* and was designed to combine the advantages of both the first and second types. A relatively thick silver wire made contact with the seawater in the shank of the tube, and was electrolytically coated with chloride. In addition, a bright silver wire was thrust down the capillary to within 1-5 mm of the tip. When a steady potential was measured this electrode acted in the same way as the second type of electrode. The bright silver wire did not influence the steady potential, because it was effectively shunted by the column of seawater in the capillary. Under these conditions the electrode resistance was determined by the whole length of the column of seawater, which amounted to several megohms. However, this resistance did not affect the measurements, because the input resistance of the amplifier was greater than 10^9 Ω. When a transient potential difference such as a spike was measured, the column of seawater was momentarily short-circuited by the polarisable silver wire, which acted like a capacitor connected between the tip and base of the microelectrode. The electrode therefore behaved as though it had a relatively low resistance and thus avoided the errors, which would otherwise have been introduced by stray capacity. It can be shown theoretically that an electrode of this kind should not introduce any distortion of the action potential, provided that the polarisation capacity of the bright silver wire is large compared to the stray capacity of the input circuit. But these calculations need not be presented, since the performance of the electrode could be tested directly in a manner which will now be described.

The first test consisted in the sudden application of a potential difference to the tip of the microelectrode. A rectangular wave of current was passed through a 5 kΩ resistance connected between the external electrode of the recording cell and earth. The terminal of the external electrode was connected directly to the input stage, and the resulting deflection of the cathode ray tube recorded photographically. This test showed that the amplifier and input stage operated with an exponential lag of 4 μs. The tip of the microelectrode was then lowered into the seawater, the input lead transferred from the external electrode to the microelectrode and a second photograph obtained. The two records were found to differ by less than 5 %, thus demonstrating that the total lag of the recording system was of the order of 4 μs. This lag may be neglected with safety, since the rising phase of the action potential occupied about 200 μs.

A test, which was essentially similar to this, was also made using rectangular steps of current lasting many seconds, and it was again found that the microelectrode introduced no perceptible change in the size or shape of the potential recorded.

Amplifier and recording system. The characteristics of the d.c. amplifier and input stage were essentially similar to those described by Hodgkin & Huxley (1945), as were the methods of calibration employed. Errors which might have arisen from non-linearity or cathode-ray tube curvature were eliminated by comparing the records directly with a calibration grid which was obtained by photographing the series of oscillograph lines resulting from successive application of 10 mV steps of potential to the input.

Records of the rate of change of membrane voltage during the action potential were obtained by electrical differentiation. This was achieved by introducing a single stage of capacitative coupling into the d.c. amplifier. The time constant of the capacitor and resistance used for differentiating was approximately 13 ps. Under these conditions the output of the amplifier should be proportional to the rate of change of the input. What is measured is not exactly equal to the instantaneous rate of change at any given moment, but is more nearly equivalent to the average rate of change over a period which is reasonably short compared to the action potential. The rate circuit and amplifier were tested with rectangular inputs or sine waves of known frequency and amplitude. The rate amplifier was calibrated by comparing the absolute magnitude of the action potential in millivolts with the values obtained by graphical integration of the first phase of the rate record. The quotient of the two quantities gives the scale factor for the rate amplifier.

Rates were occasionally measured by graphical differentiation of the action potential. This procedure was laborious, and subject to considerable uncertainty unless the action potential was recorded on an expanded time-scale. The difference between electrical and graphical measurements usually amounted to about 10 %, and there may be an error of 10 % or even more in the absolute magnitude of the rates quoted in this paper. For the most part we shall be concerned not with absolute rates but with the relative magnitude of the rates in solutions of varying sodium concentration, and the error here is likely to be less than 5 %.

Artificial solutions. Test solutions were usually made by mixing isotonic solutions in different proportions. The values used in preparing isotonic solutions are given in Table 1 and were based on cryoscopic data in International Critical Tables. Concentrations were chosen to give a freezing point depression of 1.88°C which appears to be the correct value for seawater of salinity 3.45 % (Glazebrook, 1923). No cryoscopic data could be obtained for choline chloride and the figure of 0.6 M must be regarded as a guess.

Seawater was used as a normal medium, since no Ringer's solution applicable to the squid has yet been developed. An artificial seawater solution was also employed on certain occasions, and was made according to the formula in Table 1. No appreciable difference could be detected between the action of this solution and that of seawater.

Sodium-rich solutions were made by adding solid sodium chloride to seawater. Tests with indicators showed that all solutions employed had approximately the same pH as that of seawater.

Dextrose solutions were made up at frequent intervals and were stored in a refrigerator when not in use.

TABLE 1. Composition of artificial seawater (aSW)

	Stock (mM)	g/l	Parts per thousand	(mM)
NaCl	560	32.7	804	450
KCl	560	41.8	18	10
$CaCl_2$	380	42.1	28	10.6
$MgCl_2$	360	34.3	146	52.5
$NaHCO_3$	560	47.1	4.6	2.6
Choline chloride	600	83.6		
Dextrose	980	177		
Sucrose	930	319		

Liquid junction potentials in the external circuit. The action of a test solution was examined by sucking out the seawater from the recording cell and running in a new solution. The test solution did not alter the potential of the external electrode, since the silver chloride surface was separated from the recording cell by a long column of agar seawater. However, the test solution set up a small liquid junction potential at the edge of the agar seawater column and this had to be measured before the effect of a test solution on the resting potential could be evaluated.

Junction potentials were measured by dipping a silver chloride electrode (in some cases the microelectrode itself) into a beaker filled with seawater which was connected to the recording cell by means of a saturated KCl bridge. The system employed was, therefore,

Ag.AgCl	Seawater	Saturated KCl	Test solution in recording column	Agar seawater column in external electrode	AgCl.Ag

In measuring junction potentials an attempt was made to reproduce, as far as possible, the experimental conditions used in examining a living nerve. The data obtained are, therefore, not strictly comparable to those given by standard physicochemical methods, but should provide the right corrections for the present research. The saturated KCl bridge method is known to be unsatisfactory in certain respects, but it probably gives results of an accuracy sufficient for the present purpose.

TABLE 2. Liquid junction potentials measured by saturated KCl bridge method

Test solution	$E_{seawater} - E_{test\ solution}$ (mV)
1 part seawater: 1 part isotonic dextrose	-2.6
1 part seawater: 4 parts isotonic dextrose	-6.0
1000 c.c. seawater + 15 g NaCl	0.7
1 part seawater: 1 part 0.6 M choline chloride dextrose	0.7

Liquid junction potentials for solutions of intermediate strength (e.g. 0.7 seawater, 0.3 dextrose) were obtained by interpolation.

The results obtained are shown in Table 2, and give the corrections, which have to be subtracted from any apparent change in resting potential produced by the solution in question. No value is given for isotonic dextrose, since this solution gave an unsteady potential, which increased with time to a large value.

Experimental procedure. Giant axons, with a diameter of 500-700 μm, were obtained from the hindmost stellar nerve of *Loligo forbesi*. The methods of mounting the axon and of inserting the microelectrode require no description, since they were essentially similar to those employed by Hodgkin & Huxley (1945). Before introducing the microelectrode, a value was obtained for the small potential difference between the microelectrode and the external recording electrode. The potential difference was obtained by dipping the microelectrode into the seawater in the recording cell (which was normally connected to earth by the external electrode) and comparing the position of the base-line with the value obtained by 'earthing' the input lead. The potential difference between the two electrodes usually amounted to several millivolts and this value had to be added to the apparent resting potential. Errors which might have arisen from amplifier drift were avoided by repeated checks of the amplifier zero, but this procedure did not obviate errors caused by changes in the microelectrode potential. The microelectrode could not be withdrawn during the course of an experiment, and we therefore had to rely on the stability of its potential. In the most complete experiments the electrode did not drift by more than 4 mV in about 4 hr, but changes equivalent to 2 mV hr^{-1} were sometimes encountered. We attempted to allow for changes in microelectrode potential by a sliding correction, but measurements of the resting potential cannot be presented with the same confidence, as can those relating to the amplitude of the spike. The method of obtaining the amplifier zero requires comment since this was not such a simple operation as might at first be supposed. In the interests of stability it was desirable, first, that the input circuit should never be open-circuited; and secondly, that the nerve membrane should never be short-circuited. The following procedure was therefore adopted. First, a photographic record of the action potential and resting base line was obtained with switch *A* open and switch *B* closed (Fig 1). Switch *B* was opened then switch *A* closed and a second record obtained. This operation gave the amplifier zero but did not short-circuit the membrane, since this was protected first by the resistance of the microelectrode and secondly by the 1 MΩ resistance. The switching procedure was reversed when the amplifier zero had been obtained. In a few experiments, switch *B* was left open throughout. This increased the recording lag from 4 μs to 11 μs, but it did not cause any measurable change in the form of the action potential or its derivative.

The giant fibre was normally stimulated at 40 per min throughout the entire period of experimental test.

RESULTS

Electrical properties of axons immersed in seawater

The magnitudes of the action potential (AP), resting potential and after hyperpolarisation (AHP) were measured as a matter of routine at the beginning of each experiment, and are shown in Table 3. Approximate

values for the maximum rates of rise and fall of the spike are also included. A few axons gave action potentials less than 80 mV in amplitude, but were not used for quantitative measurements because they deteriorated rapidly. The values for action potential amplitude are in good agreement with those obtained by Hodgkin & Huxley (1945), but are considerably smaller than those reported by Curtis & Cole (1942). The average value for the resting potential (-48 mV) is slightly more

TABLE 3. Electrical properties of axons in seawater

	Temp (°C)	Resting potential (mV)	Action potential amplitude (mV)	Overshoot (mV)	AHP (mV)	Max rate of rise ($V\ sec^{-1}$)	Max rate of fall ($V\ sec^{-1}$)
	22	-46	85	39	14	-	-
	20	-52	93	39	11	-	-
	21	-52	86	34	10	490	290
	24	-51	83	32	13	580	380
	22	-50	86	36	15	650	400
	22	-49	93	44	15	770	460
	20	-40	87	47	15	560	330
	20	-51	98	47	15	630	390
	-	-48	87	39	15	520	340
	21	-46	89	43	16	600	360
	20	-53	99	46	14	1000*	530*
	20	-46	85	39	14	620	480
	19	-42	85	43	15	490	330
	20	-45	86	41	16	590	360
	21	-45	82	37	15	680*	350*
Av	21	-48	88	40	14	630	380

* Indicates that these values were obtained by graphical differentiation. The values for resting potential are those observed with a microelectrode containing seawater. No correction has been made for the junction potential between axoplasm and seawater.

depolarised than that given by Curtis & Cole (-51 mV), but a difference of this kind is to be expected since Curtis & Cole used KCl in the microelectrode, whereas we employed seawater. The average action potential was about 20 mV smaller in amplitude than that given by Curtis & Cole. But a more serious discrepancy arises from the fact that we have

never observed action potentials greater than 100 mV in amplitude at 18-23°C, whereas Curtis & Cole describe a spike as large as 168 mV in a fibre which gave a resting potential of -58 mV. The matter is not one that can be lightly dismissed, because the existence of a fibre capable of giving an overshoot of 110 mV, and therefore exceeding E_{Na}, has far-reaching implications. We are no longer inclined to think that our relatively small action potentials can be attributed to the poor condition of the experimental animals, since a number of the squids employed were extremely lively and in perfect condition. Nor does it seem likely that axons were damaged in the process of isolation, since microelectrodes were sometimes inserted into axons, which were still surrounded by a greater part of the nerve trunk and had been subjected to a minimum amount of dissection. Hodgkin & Huxley's (1945) experiments indicate that the process of inserting a microelectrode did not in itself reduce the action potential, so that the possibility of damage at this stage may also be reasonably dismissed. Curtis & Cole's experiments may have been made at a different temperature, but this does not account for the discrepancy, since the action potential amplitude increases by 5-10 % when the nerve is cooled from 20 to 0°C and decreases as the temperature is raised above 20°C (unpublished results). Apart from the possibility of instrumental error, the only explanation, which can be offered, is that there is a real difference between the properties of *L. peali* used at Woods Hole and *L. forbesi* used at Plymouth.

Sodium-free solutions

Many years ago Overton (1902) demonstrated that frog muscles became inexcitable when they were immersed in isotonic solutions containing less than 10 % of the normal sodium-chloride concentration. He also showed that chloride ions were not an essential constituent of Ringer's solution, since excitability was maintained in solutions of sodium nitrate, bromide, sulphate, phosphate, bicarbonate, benzoate, etc. On the other hand, lithium was found to be the only cation which provided a reasonably effective substitute for sodium. Overton was unable to repeat his experiment with a frog's sciatic nerve, which maintained its excitability for long periods of time in salt-free solutions. But it now seems likely that this result was due to retention of salt in the interstitial spaces of the nerve trunk. Thus Kato (1936) found that application of isotonic dextrose to single myelinated fibres of the frog caused a rapid but reversible loss of excitability, and a similar result was obtained by Erlanger & Blair (1938) on the sensory rootlets of the bullfrog. Kato's result has also been confirmed by Huxley & Stampfli (unpublished experiments), who applied both isotonic sucrose and isotonic dextrose to single myelinated fibres of the frog and found that

conduction is blocked reversibly within a few seconds when the saline content falls below about 11 mM. Katz (1947) has shown that isotonic sucrose mixtures abolish the action potential of *Carcinus* axons if the sodium chloride concentration is less than 10-15 % of that normally present in seawater. Further experiments on the effect of sodium-free solutions on *Carcinus* axons were made by one of us and will be summarized, because they provide a useful addition to the work with squid axons. In the first place, the action of isotonic dextrose on a single *Carcinus* axon is exceedingly rapid. The action potential is blocked in a few seconds and is restored in a similar space of time by restoration of saline. The speed at which these solutions act is not surprising, since solute molecules have to diffuse across a distance of only a few micra of loose connective tissue in order to reach the surface membrane of the axis cylinder. Further evidence can be obtained for the conclusion of Overton (1902) and Lorente de Nó (1944, 1947) that it is the sodium and not the chloride ion which is essential for propagation. Thus axons are blocked by a mixture of 50 % isotonic choline chloride and 50 % dextrose, although this solution contains three or four times as much chloride as that present in a solution containing the minimum amount of sodium in the form of sodium chloride. The blocking effect of the first solution is not due to any harmful property of choline, since propagation occurs satisfactorily through a mixture of 50 % choline chloride and 50 % seawater. Another point is that propagation is not affected by replacing

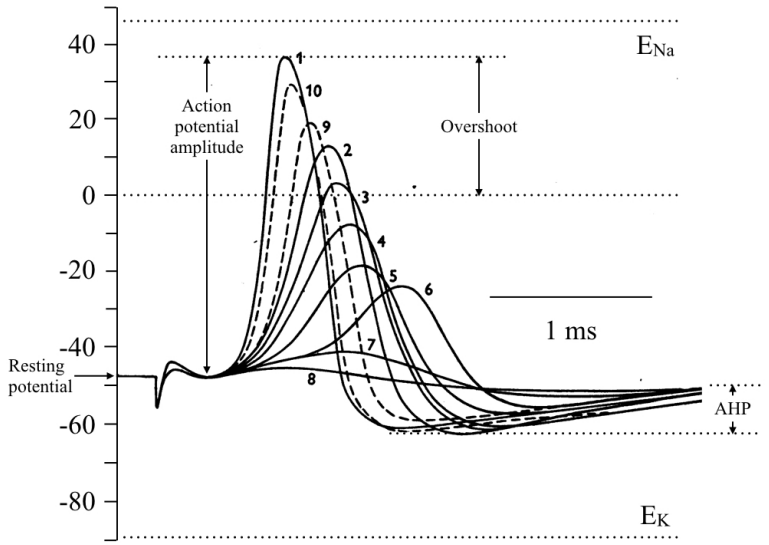

Fig 3. Action of isotonic dextrose. Record 1: action potential in seawater just before application of dextrose. 2-8: records taken at following times after arbitrary zero, defined as moment of application of dextrose: 2, 30 sec; 3, 46 sec; 4, 62 sec; 5, 86 sec; 6, 102 sec; 7, 107 sec; 8, 118 sec. Record 9 taken 30 sec after reapplication of seawater; 10, record at 90 and 500 sec after reapplication of seawater (only one curve is drawn since the responses at these times were almost identical). The action potential properties quantified in Table 3 are illustrated for Record 1.

the chloride in seawater with sulphate. All these experiments support the view that removal of sodium is the primary cause of block in salt-free solutions. A subsidiary factor may be the removal of calcium, since *Carcinus* axons do not survive for any length of time in a medium from which all traces of calcium have been removed.

The records in Fig 3 show what happens to the membrane action potential when Overton's experiment is repeated on the giant axon of the squid. Curve 1 shows the electrical response of an axon immersed in seawater. Isotonic dextrose was substituted for seawater as soon as this record had been obtained. The operation of changing solutions took 30-60 sec and the zero time to which subsequent records are referred was defined at a somewhat, arbitrary point during this process. Records 2-8 show how the action potential changed as the preparation came into diffusion equilibrium with the new medium. The action potential amplitude dropped progressively and eventually fell to a very small value (records 7 and 8). This residual deflection was almost certainly due to electrotonic spread from the part of the axon surrounded by oil, which was not affected by the test solution. Removal of salt had a very striking effect on the rate of rise of the action potential, which decreased to about one twelfth of its former amplitude after 107 sec. On the other hand, the rate of fall and the AHP changed much less rapidly. The resting potential appeared to hyperpolarise with time, but this effect may be attributed to the external liquid junction potential which could not be evaluated in this experiment; all records have therefore been traced from the same base-line. At 6 min after zero, seawater was restored, with the result that the action potential recovered rapidly to a value which was close to that observed initially. The effect of isotonic dextrose thus appears to be almost completely reversible.

The action potential was also found to be abolished reversibly by a mixture of 50 % isotonic dextrose and 50 % choline chloride. Only one satisfactory experiment was performed, but this gave a result, which was essentially similar to that in Fig 3. On the other hand, the action potential amplitude was maintained at a value of about 70 mV in a solution containing 50 % isotonic dextrose and 50 % seawater, or in one containing 50 % choline chloride and 50 % seawater. Axons from the squid therefore behave like those of *Carcinus*, in that a certain amount of external sodium is necessary for production of the action potential.

Fig 3 shows that the action of isotonic dextrose was considerably slower in the giant axon of the squid than it was in axons from *Carcinus*. The difference is not surprising since *Carcinus* axons are surrounded by only 3 μm of connective tissue, whereas the squid axons were rarely dissected cleanly and in this experiment the axon was left with a layer of

tissue about 110 μm in thickness. Such a thickness of external tissue is of the right order of magnitude to account for the delay in terms of diffusion. A detailed analysis of the process of equilibration has not been attempted, but a rough calculation suggests that the delay may reasonably be attributed to diffusion of sodium chloride from the adventitious tissue surrounding the axon into the large volume of isotonic dextrose in the recording cell. After the records in Fig 3 had been obtained, the axon was immersed in a solution containing 20 % seawater and 80 % isotonic dextrose. In this solution the action potential fell rapidly to a value corresponding to that in record 5, and underwent only a small reduction during the subsequent period of 14 min. It therefore appears that the action of salt-free solution was 80 % complete in about 90 sec. This figure can be used to calculate an apparent diffusion constant if it is assumed that the fluid outside the preparation was completely stirred and that the diffusion process operated in the same manner as for a single substance diffusing into a slab of tissue 10 μm in thickness. The value found for this experiment was about 0.1 cm^2 day^{-1} and values of this order of magnitude have been obtained in other cases. The diffusion constant for sodium chloride in water is 1.0 cm^2 day^{-1} and for dextrose 0.5 cm^2 day^{-1} (Landolt-Bernstein, 1931). The lag in the action of salt-free solutions can be explained if diffusion through the connective tissue and interstitial spaces in the remains of the nerve trunk are assumed to be about one seventh of those in water. This is a reasonable assumption, since Stella (1928) concluded that diffusion of phosphate through the extra-cellular part of the frog's sartorius muscle was very slow compared to that in free solution. Another factor which may have retarded diffusion in the later stages is that the external solution was not stirred mechanically after the initial process of applying the test solution had been completed.

The effect of solutions of reduced sodium content on the resting potential and action potential

The general action of solutions containing a low sodium concentration is illustrated by Fig 4. Record *a*1 shows the action potential of an axon immersed in seawater. The zero was determined by short-circuiting the amplifier input and subtracting the small difference of potential, which existed between the two recording electrodes. The zero therefore occurs at the potential, which would have been observed if the microelectrode had been withdrawn and placed in the seawater outside the axon. Record *a*2 shows the resting potential and action potential recorded after 16 min in a solution containing 33 % seawater and 67 % isotonic dextrose. The method of obtaining the resting potential was similar to that formerly employed, except that an additional correction for

the liquid junction potential has been introduced. The resting potential would have appeared to be 4.2 mV more hyperpolarised if no such allowance had been made. The zero on the record now corresponds to the potential, which would have been observed if the microelectrode had been withdrawn and connected to the solution in the recording cell by means of a saturated KCl bridge. Record *a*3 was obtained 14 min after

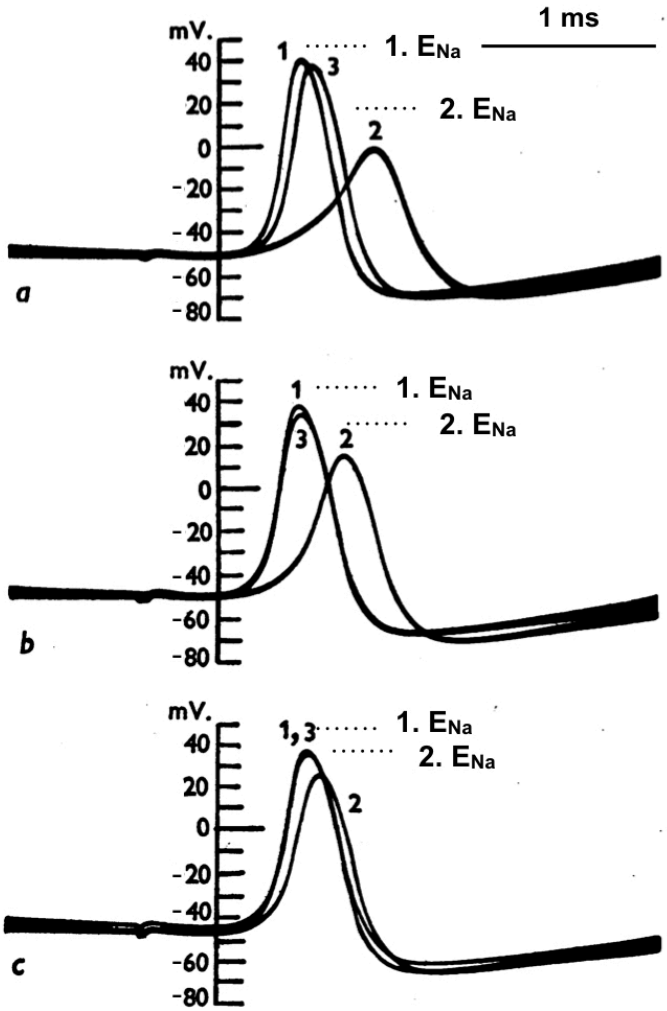

Fig 4. Action of sodium-deficient solutions on the resting and action potential. *a*l, response in seawater; *a*2, after 16 min in 33 % seawater, 67 % isotonic dextrose; *a*3, 13 min after reapplication of seawater. *b*1, response in seawater, *b*2, after 15 min in 50 % seawater, 50 % isotonic dextrose; *b*3, 6 min after reapplication of seawater. *c*1, response in seawater; *c*2, after 16 min in 71 % seawater, 29 % isotonic dextrose; *c*3, 7 min after reapplication of seawater. The scale gives the potential difference across the nerve membrane (inside - outside) with no allowance for the junction potential between the axoplasm and the seawater in the microelectrode. The calculated E_{Na} has been inserted, based on $[Na^+]_i$ of 72 mM and the appropriate $[Na^+]_o$.

replacing seawater in the recording cell. The action potential amplitude was 5 mV less than that at the beginning of the experiment, but the

difference was small compared to the decrease shown by *a*2. The action potential also arose with a greater delay, although the profile and rate of rise were close to those observed originally. An effect of this kind is inevitable because the test solution diffused from the upper part of the recording cell into the region of nerve surrounded by oil. The total conduction time was increased by this process, and the effect was only very slowly removed by application of seawater to the upper part of the recording cell. Records *b*l, *b*2, and *b*3 or *c*1, *c*2, and *c*3 were made in a comparable manner, except that the test solutions consisted of 50 or 71 % seawater. The effects produced by these solutions were smaller, but of the same general type as those illustrated by *a*l, *a*2 and *a*3.

This experiment illustrates a number of important points. In the first place it shows that dilution of seawater with isotonic dextrose caused a large and reversible decrease in the amplitude of the action potential. On the other hand the resting potential was altered to such a small extent that no difference can be seen in Fig 4. There was usually a small hyperpolarisation in resting potential, as may be seen from the figures in Table 4, but the change was always small compared to the change in action potential amplitude. The constancy of the resting potential means that removal of external sodium reduces the action potential by decreasing the overshoot. In fact, in 33 % seawater, the overshoot had disappeared and the membrane potential at the peak of

TABLE 4. Effects of replacing seawater with solutions containing different concentrations of sodium

$[Na^+]_o$ (mM)	$[Na^+]_{test}/[Na^+]_{sw}$	$\log_{10}([Na^+]_{test}/[Na^+]_{sw})$	Change in resting potential (mV)	Change in AP amplitude (mV)	Change in overshoot (mV)	Change in AHP (mV)	Max rate rise AP	Max rate fall AP	Norm AP
90	0.2	-0.699	-4	-57	-59	4	0.08*	0.25*	0.24
135	0.3	-0.523	-2	-42	-44	-1	0.22	0.51	0.54
225	0.5	-0.301	-2	-19	-21	-2	0.53	0.79	0.77
322	0.715	-0.146	0	-9	-9	-1	0.75	0.91	0.89
450	1	0.000	0	0	0	0	1	1	1.0
567	1.26	0.100	-1	4	3	-1	1.17	1.11	1.05
702	1.56	0.193	1	7	9	-1	1.3	1.16	1.09

All rates except those marked with an asterisk were obtained by electrical differentiation. Sodium-deficient solutions were made by diluting seawater with isotonic dextrose, sodium-rich solutions by adding solid sodium chloride to seawater. Changes were measured with reference to a normal value in seawater, which was obtained in each case from the mean of determinations made before and after application of a test solution. Last three columns are relative to normal e.g. 450 mM Na^+.

the action potential was then less than 0 mV. Another interesting point is that the rate of rise of the action potential was markedly affected by sodium-deficient solutions, whereas the rate of fall changed only in proportion to the amplitude. It can also be seen that the AHP was only slightly affected by removal of sodium.

The quantitative results obtained with sodium-deficient solutions are shown in Table 4. The principal difficulty in making these measurements was connected with the fact that diffusion times prevented the sodium-deficient solutions from acting instantaneously, and it was essential that quantitative measurements should not be made until equilibrium had been obtained. Photographic records were usually made at intervals of 2, 5, 10 and 15 min after application of the new solution.

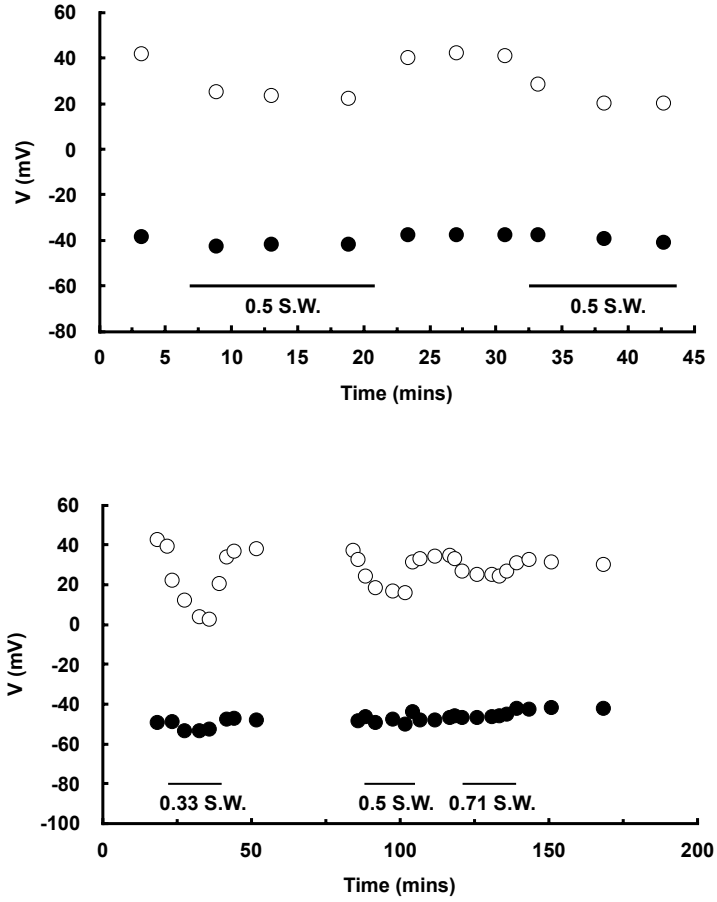

Fig 5. Time course of action of sodium-deficient solutions made by diluting seawater with isotonic dextrose. Resting potentials are apparent potentials corrected for liquid junction potentials in the external circuit, but not corrected for the junction potential between axoplasm and seawater at the tip of the microelectrode. Action potential (○) and resting potential (●) are illustrated.

This procedure gave satisfactory results when solutions containing more than 50 % seawater were employed. The results at 10 and 15 min rarely differed by more than 2 mV, and equilibrium was sometimes attained after 5 min. On the other hand, measurements in solutions containing less than 50 % sodium were unsatisfactory, because there was always a progressive decline in the action potential amplitude and depolarisation of the resting potential. This could not be attributed to diffusion in the space outside the axon, but may have been caused by a slow leakage of potassium chloride from the axon itself. The errors introduced by this progressive decline are not likely to be large, but it is certain that measurements of potentials in 30 and 20 % seawater cannot be regarded with the same confidence as can those in 50 and 70 % seawater.

The time course of the action of sodium-deficient solutions on both action potential amplitude and resting potential is shown by two experiments illustrated in Fig 5. The resting potential may be seen to undergo small and irregular variations and in general these were accentuated by the operation of changing the solution in the recording cell. Such variations are regarded as spurious, and an attempt has been made to minimize their effect by using average values and neglecting measurements made shortly after the solutions had been changed.

The effect of sodium-deficient solutions on action potential amplitude is illustrated by Fig 6, and the average effect on overshoot by Fig 7. The dotted line in these figures shows the relation described by the Nernst equation. In this case the potential difference across the active membrane should be given by

$$E = \frac{RT}{F} \ln \frac{[\mathrm{Na}]_o}{[\mathrm{Na}]_i} = 58 \text{ mV x } \log_{10} \frac{[\mathrm{Na}]_o}{[\mathrm{Na}]_i} \qquad (1)$$

where E_{Na} is the reversal potential for Na^+; R, T and F have their usual significance; $[Na^+]_i$ and $[Na^+]_o$ are sodium concentrations, or more strictly sodium activities, in the axoplasm and external solution. The change in peak action potential amplitude, which results from an alteration of external sodium should be given by equation 2, since it may be assumed that the internal concentration of sodium does not change, or changes only very slowly when the external sodium is altered.

$$\Delta E = E_{\text{test}} - E_{\text{sw}} = \frac{RT}{F} \ln \frac{[\mathrm{Na}^+]_{test}}{[\mathrm{Na}^+]_{sw}} \qquad (2)$$

Since the resting potential is only slightly altered by dilution of seawater, equation 2 should also apply to the change in action potential amplitude. The data in Table 4, Figs 6 and 7 show that equation 2 is obeyed reasonably by solutions containing 50 and 70 % of the normal sodium

concentration. The rough agreement must not be pressed, because the behaviour of the membrane during an action potential is likely to be much more complicated than a Nernstian relationship. Another reason

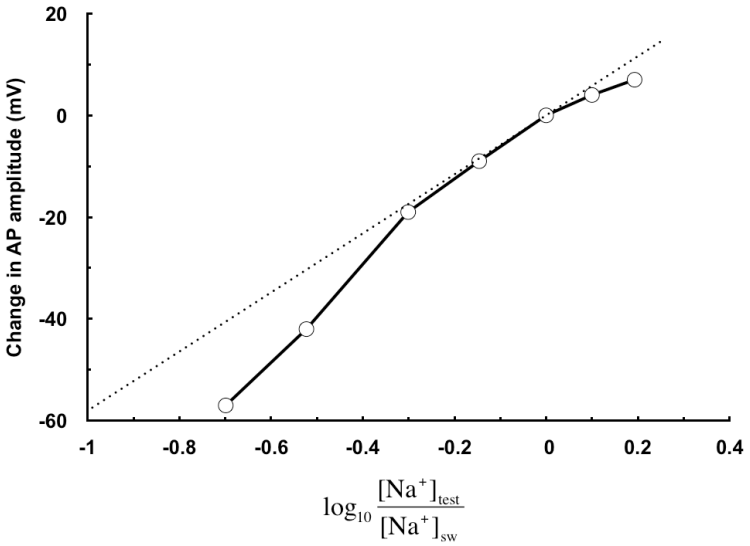

Fig 6. Change in amplitude of action potential (open circles) caused by alteration of external sodium concentration. The dotted line is drawn according to Equation 2 and plots the Nernstian change in action potential amplitude versus external sodium concentration (see Table 4 for data).

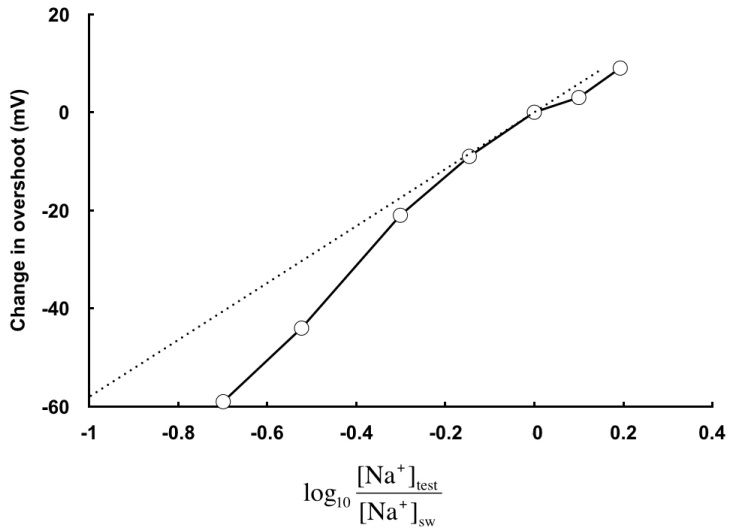

Fig 7. Average change in overshoot (open circles) caused by alteration of external sodium concentration. The dotted line is drawn according to equation 2 (see Table 4 for data).

for caution is that there is no certain information about the activity coefficient of the sodium ion in dextrose mixtures. Preliminary measurements indicate that an allowance for changes in activity coefficients would make the slope of the theoretical line about 10 % less

than that in Figs 6 and 7. But the corrections for activity coefficients were so uncertain that we have preferred to use concentrations in equation 2. The results in Figs 6 and 7 indicate that the depression of action potential amplitude was disproportionately large in solutions containing 20 and 33 % seawater. An effect of this kind can be explained if it is assumed that the permeability to sodium increases with the depolarisation of the membrane (* References). The action potentials in solutions of 20 or 33 % sodium were much smaller than normal, so that it is plausible to suppose that the mechanism responsible for transporting sodium might not be operating at full efficiency in these solutions.

Sodium-rich solutions

The experiments with sodium-deficient solutions were in good agreement with the simple hypothesis, which they were designed to test. But it might be argued that the results observed were due to the abnormal nature of the external media rather than to any specific effect of the sodium ion. The regular and reversible nature of the changes speaks against this view, but there is a more compelling reason for rejecting it. The concentration of sodium chloride was increased from 455 to 711 mM by dissolving 15 g of solid NaCl in 1 1itre of seawater. This solution was strongly hypertonic and damaged the axon by osmotic effects in 5-15 min. But before the osmotic effects became apparent the axon gave an increased action potential amplitude with characteristics, which were the converse of those in sodium-deficient solutions. The effect of sodium-rich solutions is best illustrated by the behaviour of an axon from which

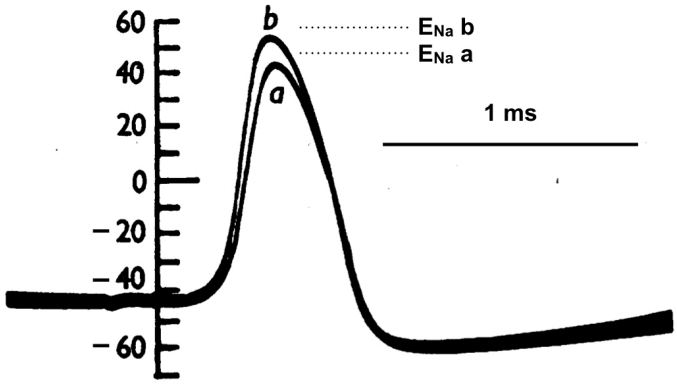

Fig 8. Action of sodium-rich solution on the resting and action potential. *a*, response in seawater; *b*, response 50 sec after application of seawater containing additional quantity of NaCl. (The sodium concentration of this solution was 1.56 times that of seawater.) The scale gives no allowance for the junction potential between the axoplasm and the seawater in the microelectrode. The calculated E_{Na} has been inserted, based on $[Na^+]_i$ of 72 mM and the appropriate $[Na^+]_o$.

almost all the external tissue had been removed by dissection. In this axon the thickness of the external tissue was about 25 μm so that diffusion times were relatively short. Fig 8a shows the action potential observed when this axon was immersed in seawater. A sodium-rich solution was applied 2 min after curve *a* had been obtained, and curve *b* recorded 50 sec later. The amplitude of the action potential, the overshoot and the rate of rise all show small but quite definite increases. Measurements indicate that the action potential increased in amplitude from 86 to 95 mV, while the overshoot changed from 42 to 53 mV. These values were maintained for 4 min and, on replacing seawater, returned to 84 and 41 mV. The changes are not large, but the increase in overshoot is close to that predicted by equation 2, as indicated by the dotted lines. The sodium concentration of this solution was 1.56 times that in seawater so that the theoretical change in overshoot is

$$\Delta E = E_{test} - E_{seawater} = 58 \log_{10} \frac{1.56}{1} = 11 \text{ mV}$$

A control with a solution containing 0.5 M dextrose dissolved in 1 1itre of seawater gave no immediate increase in spike height but only a very small and gradual decrease which must be regarded as an osmotic effect.

Other experiments with sodium-rich solutions gave results, which were essentially similar to those in Fig 8, although the changes observed were somewhat smaller, as may be seen in Table 4. It was also found that the period of increased action potential height was rarely maintained for more than a few minutes, and was followed by a period of progressive deterioration, which was only partially reversible. Control experiments with solutions containing extra dextrose showed the phase of progressive deterioration, but never gave the initial increase in action potential height, or rate of rise. There is therefore some reason for believing that the changes produced by excess of sodium would have been rather larger if the action of extra sodium could have been dissociated from the osmotic effect of the solutions.

The rate of rise of the action potential

The basic assumption in our hypothesis is that excitation causes the membrane to change from a condition in which the permeability to potassium is greater than the permeability to sodium, to a state in which the permeability to sodium exceeds that to potassium. The transition from the resting to the active state occurs as the resting nerve becomes depolarised by local circuits spreading from an adjacent region of active nerve. Because the inside of the axon contains a low concentration of sodium, external sodium ions should enter the axon at a relatively high rate when excitation occurs. In the absence of other processes, sodium

entry would continue until the inside of the axon became sufficiently positive to overcome the effect of the diffusion gradient i.e. where the theoretical E_{Na} is reached. The rate at which the membrane approaches its new equilibrium value should be determined by the rate at which the membrane capacity is discharged by entry of sodium. Our hypothesis therefore suggests that the rate of rise of the action potential should be determined by the rate of entry of sodium, and on a simple view it might be expected to be roughly proportional to the external concentration of sodium.

A quantitative basis for part of this argument can be provided in the following way. The membrane current during the action potential is proportional to the second derivative of potential with respect to time, and is therefore zero when the first derivative is at a maximum or a minimum. The current passing through the membrane consists of capacity current $(C\,\partial V/\partial t)$, which involves a change of ion density at its outer and inner surface, and an ionic current due to transport of ions across the membrane. These two components must be equal and opposite when the total membrane current is zero. The following relation therefore applies at the moments when the rate of change of membrane voltage is at a maximum or minimum

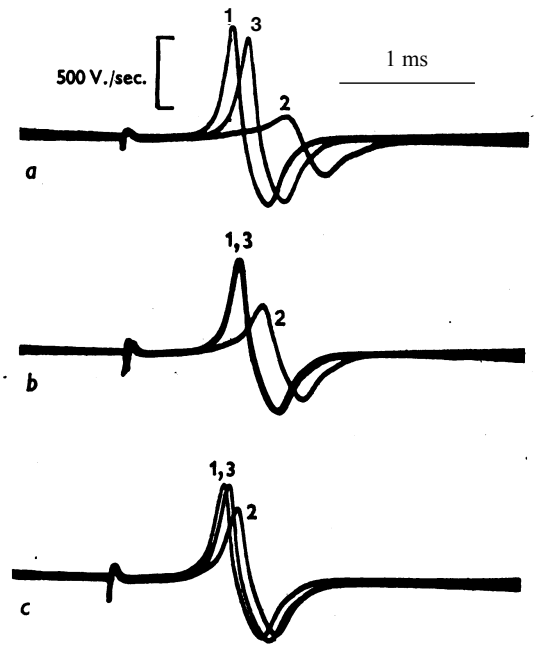

Fig 9. Action of sodium-deficient solutions on rate of change of membrane voltage. *a*2, *b*2, *c*2, response in 33, 50 and 71 % seawater, remainder in pure seawater. The times at which records were obtained are approximately the same as those in Fig 4. The calibration scale in *a* applies to all records.

$$-C\,\frac{\partial V}{\partial t} = I \qquad (3)$$

where I is the net inward current per cm^{-2} due to transfer of ions from outside to inside, C is the membrane capacity per cm^2, V is the potential difference across the nerve membrane.

The simple nature of equation (3) indicates that the most valuable type of rate measurement is a determination of the maximum rate. This can be obtained by graphical analysis of the action potential, but is best recorded directly by electrical differentiation. Typical records obtained by this procedure are given in Figs 9 and 10. These show two distinct phases and not three, as might have been expected from the diphasic character of the squid action potential. The reason for this is that the rate at which the AHP disappears is small compared to the rates at which the initial part of the action potential rises and falls. The peak of the rate record is proportional to the positive ionic current entering the axon and the trough to the positive ionic current leaving the axon. The absolute value of these currents can be estimated roughly, since the membrane capacity of the squid axon has been determined as 1.1 μF cm^{-2} (Cole & Curtis, 1938) or 1.8 μF cm^{-2} (Cole & Curtis, 1939), and may be taken as 1.5 μF cm^{-2}. The average values for the maximum rates of rise and fall of the spike were 630 and 380 V sec^{-1}, so that the ionic current entering the axon during the rising phase was of the order of 0.9 mA cm^{-2} (Q = 1.5e^{-6} F cm^{-2} x 630 V sec^{-1} = 0.9 mC cm^{-2} sec^{-1} = 0.9 mA cm^{-2}), whereas the ionic current leaving during the falling phase was of the order of 0.6

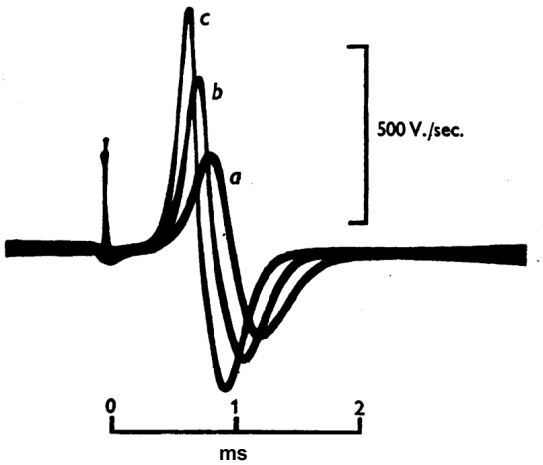

Fig 10. Rate of change of membrane voltage in solutions containing various concentrations of sodium: *a*, 12 min after application of 50 % seawater, 50 % isotonic dextrose; *b*, 16 min after application of seawater; *c*, 2 min after application of sodium-rich solution (the sodium concentration in this solution was 1.56 times that in seawater). The interval between record *a* and *b* was 18 min. and between *b* and *c* was 3 min.

mA cm^{-2} (Q = $1.5e^{-6}$ F cm^{-2} x 380 V cm^{-2} = 0.6 mC cm^{-2} sec^{-1} = 0.6 mA cm^{-2}). Corresponding figures in terms of the rate of entry or exit of a monovalent cation are 10^{-8} C (0.9 mC sec^{-1} cm^{-2}/ 96,500 C mol^{-1}) and 0.6 x 10^{-8} mol cm^{-2} sec^{-1} (0.6 mC sec^{-1} cm^{-2}/ 96,500 C mol^{-1}). Fig 9 shows how the first derivative of the action potential is affected by sodium-deficient solutions. These records were obtained from the same axon and under the same conditions as those in Fig 4; they show that the rate of rise of the action potential undergoes large and substantially reversible changes as a result of treatment with sodium-deficient solutions. Fig 10 shows the changes produced by successive application of solutions containing 50, 100 and 156 % of the normal sodium concentration. The action potential reached a constant value in the 50 and 100 % solutions so that the change in rate shown by record *c* was certainly a genuine increase and not merely a recovery from the previous immersion in the 50 % solution. Data from other axons are collected in Table 4 and plotted graphically in Fig 11. It will be seen that the rate of rise is proportional to sodium concentration over the range 50-100 % seawater, but that the rate falls off rapidly below 50 %. This effect is almost certainly related to the disproportionately large changes in action potential observed in solutions containing 20 and 33 % seawater.

The rates of rise showed substantial increases in solutions containing extra sodium, but the effects were no longer proportional to the sodium concentration. Thus the largest increase encountered in a solution containing 1.56 times the normal sodium was 1.39, and the average value was only 1.3. This result may be attributed to the deleterious action of the hypertonic solutions, but it is also possible that

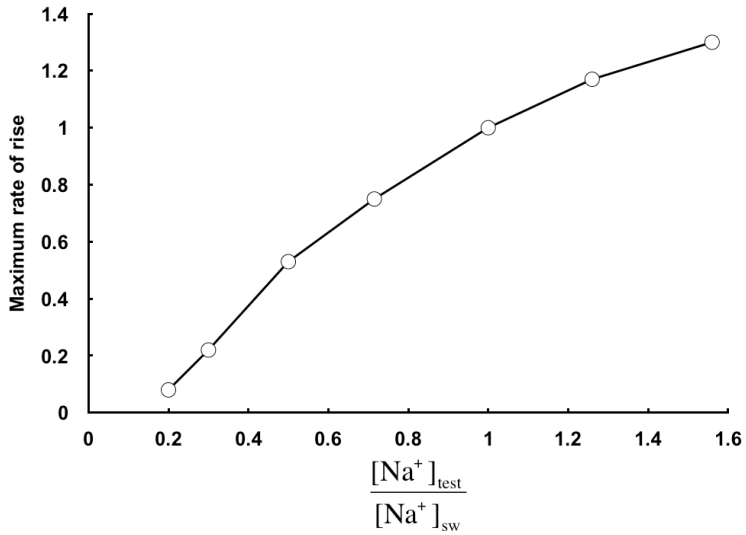

Fig 11. Ordinate: maximum rate of rise of spike in test solution/maximum rate of rise in seawater. Abscissa: sodium concentration of test solution/sodium concentration in seawater.

there may be a genuine lack of proportionality in solutions containing an excess of sodium. However, there is good evidence to show that simple proportionality does hold over a limited region and it is certain that the rate of rise is altered reversibly over a wide range by changes in the external sodium concentration.

The rate of fall of the action potential is also influenced by sodium, but to a lesser extent (Table 4). Thus the average change in rate of rise in a 50 % solution was 0.53, whereas the average change in rate of fall was 0.8. The rates of fall appear to change in proportion to the height of the action potential, as may be seen by comparing the average ratios in the last two columns of Table 4. This result suggests that changes in external sodium affect the rate of fall indirectly by altering the amplitude of the spike. A change in the rate of fall is a natural consequence of a change in spike height; for it is to be expected that the rate at which the potential reverts to its resting value should depend upon the extent to which activity has displaced the membrane from its resting level.

Changes in conduction velocity

The velocity of transmission should be reduced by sodium-deficient solutions, since these solutions lower the rate of rise of the action potential. This deduction could not be tested in the experiments with internal electrodes, since a large part of the conduction time arose in the lower part of the nerve, which was immersed in oil. A single experiment with external electrodes was made in order to find out if there was an appreciable change of velocity in a large volume of 50 % seawater. The axon was arranged in such a way that the conduction stretch between stimulating and recording electrodes could be dipped into seawater or into a test solution (cf. Hodgkin, 1939). With an arrangement of this kind the absolute changes in conduction time could be measured with considerable accuracy, but there was some uncertainty in determining the velocity because it was difficult to know which was the correct point on the action potential to choose for measurement. This source of error can be eliminated by the method used by Katz (1947), but shortage of time and material prevented us from making more than a single experiment of the simplest kind. The result left no doubt that there was a substantial decrease of conduction velocity in a solution containing 50 % seawater, and a tentative estimate is that the velocity in this solution was 0.7 of that in seawater. The change in velocity was evidently smaller than the average change in rate given in Table 4, but there is no reason to suppose that the velocity should change as the first power of the rate of rise, and in a simplified theoretical system it can be shown to vary with the square root of the rate of rise of the action potential. There is evidently a difference between this result and those recently reported by Katz (1947)

for *Carcinus* axons. Katz was primarily concerned with another aspect of the problem of conduction velocity, but two experiments are quoted which show a velocity decrease of only 5 % in a solution containing 50 % seawater and 50 % isotonic sucrose, and in other unpublished experiments of a similar kind the velocity change rarely exceeded 10 %. The data at present available are not sufficient to justify speculation into the nature of this apparent difference between crab and squid fibres. But the conduction velocity must depend upon processes occurring at threshold as well as upon the rate of rise or height of the action potential, and it is likely that dilution of seawater would give different overall effects in different types of axon. In this connection it should be remembered that dilution of seawater with sugar solutions alters the concentration of other ions besides that of sodium, and it is conceivable that the effect of sodium removal may sometimes be balanced by an increase in excitability resulting from the simultaneous reduction of calcium concentration. Apart from the numerical discrepancy, the results of Katz are in general agreement with those reported here. Thus the velocity of conduction in *Carcinus* axons was found to undergo a substantial decrease in solutions containing less than 30 % seawater and block occurred when the seawater content was less than 10 %.

Specificity of sodium action

The reduction in action potential height which results from mixing seawater with isotonic dextrose has been attributed to dilution of sodium, but it is conceivable that the observed effects might have been partly due to dilution of other ions such as Ca, K or Cl. This possibility was examined by comparing the effects of two solutions. The first solution was made by mixing artificial seawater with isotonic dextrose, while the second was made in such a way that all components except the sodium and chloride ions were maintained at about their normal level. The composition of the two solutions and the results obtained with them are given in Table 5. It will be seen that solution 2 gave a smaller action potential than solution 1, but that this drop was almost entirely due to a 5 mV depolarisation in resting potential which probably arose from the increased potassium concentration of the second solution. Since both solutions contained the same concentration of sodium, equation (2) predicts that the overshoot should remain constant and the figures in Table 5 show that this prediction is verified. The rate of rise in the second solution was 20 % less than that in the first, and this effect may be attributed either to the higher calcium and magnesium content of solution 2 or to the depolarised resting potential resulting from the increase in potassium concentration.

Table 5

Operation	Change in resting membrane potential (mV)	Change in action potential amplitude (mV)	Change in overshoot (mV)	Change in AHP (mV)	Max rate of rise relative to previous condition	Max rate of fall relative to previous condition	AP amplitude relative to previous condition
From aSW to soln 1	-4	-19	-23	-1	0.51	0.80	0.75
From soln 1 to soln 2	+5	-5	0	0	0.81	0.92	0.90
From aSW to soln 1	-3	-14	-17	-3	0.59	0.80	0.84
From soln 1 to soln 3	-1	2	1	0	1.17	1.09	1.03

The measurements in the first two rows were made on the same axon but were separated by a considerable time interval; measurements in the last two rows were obtained on the same axon at approximately the same time. All figures are average values determined in the usual way. The compositions of the solutions are given below:

		Concentration as a fraction of concentration in aSW					
Solution	Description	Na	K	Ca	Mg	Cl	HCO_3
1	aSW diluted 1:1 with isotonic dextrose	0.5	0.5	0.5	0.5	0.5	0.5
2	Solution 1 + K, Ca, Mg, HCO_3	0.5	1.0	1.0	1.0	0.5	1.0
3	aSW diluted 1:1 with 0,6 M choline chloride	0.5	0.5	0.5	0.5	1.0	0.5

The previous experiment indicates that the changes in action potential profile and amplitude were primarily due to alterations in the concentrations of either the chloride or the sodium ion. The effect of these two ions may be separated by diluting seawater with isotonic choline chloride instead of isotonic dextrose. The results obtained in a single experiment of this type are illustrated by the effects given in Table 5 for solutions 1 and 3. It will be seen that the general action of these solutions was similar and, in particular, that the overshoot differed by less than 1 mV, although the chloride concentrations of the two solutions were widely different. The only anomalous point is that the rate of rise was found to be appreciably greater in the solution containing choline chloride than in the one containing dextrose. Part of this difference may be attributed to a small change in resting potential, but it seems unreasonable to ascribe all the increase to this cause.

A single experiment with an artificial seawater solution containing lithium instead of sodium indicated that the action of these two ions was almost identical. This result is supported by unpublished experiments

with *Carcinus* axons, which show that propagation occurs satisfactorily for at least 1 hr in a solution containing lithium but no sodium. Gallego & Lorente de Nó (1947) report that myelinated nerve becomes depolarised and inexcitable after immersion in lithium solutions for several hours. We must therefore suppose either that the reactions of vertebrate nerve to lithium differ from those of invertebrate nerve, or that our experiments were not maintained for sufficient time to reveal the effects described by Gallego & Lorente de Nó.

Preliminary experiments with isotonic sucrose mixtures show that the action of this sugar was similar to that of dextrose.

No perceptible changes occurred when the oxygen tension of the seawater was increased fivefold.

Effect of varying potassium concentration

The action potential may be regarded as being made up of a component due to the resting potential, which is only slightly altered by dilution of seawater with isotonic dextrose, and an overshoot beyond 0 mV, which is directly influenced by the external sodium concentration. It is known that variations in the external potassium concentration alter the resting potential, and on a simple view it is to be expected that these variations would change the amplitude of the action potential but not the overshoot of the action potential. This hypothesis cannot be pressed, because increasing the potassium concentration causes nerve fibres to become inexcitable long before they are completely depolarised (Curtis & Cole, 1942). There is also the experimental difficulty that the changes in resting potential are small over the range in which excitability is retained. In practice, this meant that the values of resting potential and action potential amplitude had to be measured to a degree of precision which was near the experimental limit.

The effect of changing from a potassium-free solution to one containing the normal potassium content is shown in Fig 12. It will be seen that the action potential peak was slightly greater in the potassium-free solution than it was in seawater, and that this effect was largely due to a change in the resting potential. The numerical results obtained in this and other experiments are given in Table 6 and are more reliable than values obtained by comparison of single records of the type shown in Fig 12. They show that the action potential amplitude in a potassium-free solution was 4.5 mV greater than normal and that the resting potential was 3.1 mV more hyperpolarised. The action potential peak was therefore 1.4 mV larger in a potassium-free solution than in seawater. The sodium concentration of the first solution was 2.2 % greater than that of the second, so that 0.5 mV of the difference must be attributed to this cause

[58 x log(463/453)]. The remaining difference is not greater than the experimental error, but is probably real because the converse effect is seen with potassium-rich solutions. Thus, in a solution containing twice the usual amount of potassium, the depolarisation in resting potential

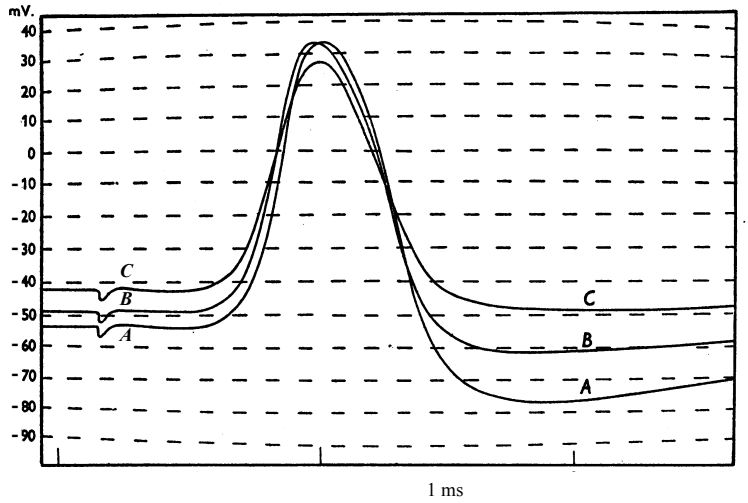

Fig 12. Effect of varying K concentration on action potential and resting potential. Curve *A*, K free artificial seawater (K = 0, Na = 463 mM.); *B*, artificial seawater (K = 10 mM, Na = 453 mM.); *C*, K rich artificial seawater (K = 20 mM, Na = 443 mM). The dotted lines were traced from a projection of the calibration grid and give the potential difference across the nerve membrane without correction for the junction potential between the axoplasm and the seawater in the microelectrode.

accounts for less than half the decrease in action potential amplitude, and further increases in potassium concentration cause the action potential to drop rapidly to zero, although there is still a substantial resting potential. These facts indicate that it is an over-simplification to suppose that the action potential peak always reaches the equilibrium potential for sodium. Instead, we must assume that the sodium permeability does not rise to a value high enough to swamp the contribution of potassium and chloride to the action potential. Under these circumstances anything which interferes with the sodium transport mechanism must result in a diminution of action potential amplitude. If this view is adopted the changes in Table 6 can be explained by supposing that the efficiency of the sodium transport mechanism depends upon the membrane potential of the resting nerve. Hyperpolarising the resting membrane potential may cause a slight increase in efficiency, whereas depolarising it leads to a rapidly augmenting drop in efficiency. A hypothesis of this kind is also needed to explain the fact that strong depolarising currents depress the peak of the action potential more than they decrease th

e resting potential of *Carcinus* axons. This phenomenon is conspicuous when a train of spikes is recorded from the stimulating electrode and is illustrated by Fig 1 of a recent paper (Hodgkin, 1948).

Small changes in potassium concentration have a marked effect on the AHP of the squid potential. In Fig 12 the AHP in K free solution amounted to 23 mV whereas in 2K it was only 7 mV. Table 6 shows that this action of potassium is consistent and repeatable. A theoretical explanation of the effect is given on p. 35.

TABLE 6.

Effect of solutions of varying potassium content

Characteristics in aSW [Na = 453, K = 10 mM (in mV)]

Resting Potential (mV)	Action Potential amplitude (mV)	Overshoot (mV)	AHP (mV)
-46	84	38	13

Change in potential on substituting test solution for aSW (in mV)

K-free [Na = 463 mM, K = 0]				1.5K [Na = 448 mM, K = 15 mM]				2K [Na = 443 mM, K = 20 mM]			
ΔRP	ΔAP	ΔOS	ΔAHP	ΔRP	ΔAP	ΔOS	ΔAHP	ΔRP	ΔAP	ΔOS	ΔAHP
-3.1	4.5	-1.4	10.2	2.3	-3.6	1.2	-3.7	3.7	-8.6	4.9	-5.9

DISCUSSION

The experiments described in this paper are clearly consistent with the view that during the action potential the membrane becomes selectively permeable to sodium, and thereby allows a reversal in membrane polarity during an action potential to be established. The evidence is indirect, and the sodium hypothesis cannot be pressed until more is known about the ionic exchanges associated with nervous transmission. But the hypothesis does provide a good working basis for future experiments, and it gives a satisfactory explanation of several observations, which cannot be reconciled with the classical membrane theory. On the other hand, the hypothesis encounters a number of difficulties of which only a few can be mentioned here. One of the most serious objections arises from the fact that Curtis & Cole (1942) describe an experiment in which the overshoot reached 110 mV during an action potential, whereas Steinbach & Spiegelman's (1943) figures indicate that the sodium concentration of fresh axoplasm is about one-tenth of that in seawater. The maximum overshoot allowed by a tenfold ratio is 58 mV and the ratio would have to be nearly 100 in order to produce an overshoot of 110 mV. The discrepancy is all the more serious because it is exceedingly unlikely that the membrane potential could reach the theoretical maximum for the E_{Na}. The difficulty does not arise in our experiments, since the overshoot has

always been well below the limit allowed by Steinbach & Spiegelman's figures. The only alternatives which remain if Curtis & Cole's figure of 110 mV is accepted are: first, that the sodium hypothesis is incorrect or incomplete; and secondly, that the sodium-ion activity in certain axons may be less than one-hundredth of that in the external fluid. Another possible criticism is that many agents affect the amplitude of the action potential without causing much change in the resting potential. Examples are afforded by cocaine or amyl alcohol, which block conduction at concentrations that cause a slight hyperpolarisation in resting potential (Bishop, 1932). Observations of this kind can be explained by assuming that the mechanism for transporting sodium is of a highly specialized nature, and is readily put out of action by agents, which have little effect on the resting potential. Another possibility is that certain substances may act on the secretory mechanism, which normally keeps the internal sodium at a low level.

For many years physiologists have known that the action potential of myelinated nerve is ultimately abolished by anoxia or by agents which interfere with oxidative processes (Gerard, 1932; Schmitt, 1930; Schmitt & Schmitt, 1931; Lorente de Nó, 1947). But agents of this type also depolarise the resting potential and, in such cases, the action potential of myelinated nerve can be restored by hyperpolarising the nerve (Lorente de Nó, 1947). There is therefore little reason to believe that the processes directly concerned with the generation of the action potential are of an oxidative nature. The converse view is expressed by Arvanitaki & Chalazonitis (1947) as a result of an interesting investigation into the effect of metabolic inhibitors on *Sepia* nerve. But the axons used in these experiments were surrounded by a relatively small amount of external fluid and stimulation frequencies of the order of 100 Hz were employed. Under these conditions secretory activity may be of great importance for the maintenance of ionic concentration differences, and hence for the maintenance of normal excitability. There is, in any case, no direct conflict between the views of Arvanitaki & Chalazonitis and our own, since it is conceivable that oxidative metabolism may be essential for the proper operation of the mechanism responsible for transport of sodium.

The last objection to be mentioned is of a different kind. It has been assumed that the resting membrane is permeable to potassium and to chloride, but impermeable or only sparingly permeable to sodium. This is a plausible assumption since sodium is a more heavily hydrated ion than potassium or chloride. On the other hand, it is much more difficult to accept the assumption that the membrane can become selectively permeable to sodium during an action potential. We therefore suggest that sodium does not cross the membrane in ionic form, but enters into

combination with a lipoid soluble carrier in the membrane, which is only free to move when the membrane is depolarised. Potassium ions cannot cross the membrane by this route, because their affinity for the carrier is assumed to be small. An assumption of this kind is speculative but not unreasonable, since there is already some indication that a specific, enzyme-like process is concerned with the transport of sodium through cell membranes (Davson & Reiner, 1942; Krogh, 1946; Ussing, 1947). In this connection it is interesting to read that the permeability of the erythrocyte of the cat to sodium may be five to ten times greater than the permeability to potassium (Davson & Reiner, 1942), and that sodium permeability is reduced to zero by concentrations of amyl alcohol which cause an increase in potassium permeability (Davson, 1940).

In formulating our hypothesis we have been careful to avoid making any quantitative assumptions about the relative permeabilities of the membrane to sodium and potassium. The resting membrane has been considered as more permeable to potassium than sodium, and this condition was regarded as reversed during activity. It is natural to inquire whether any limit can be set to the degree of selective permeability actually present in the resting and active membranes. Some light can be thrown on this problem if the observed potentials are compared with those predicted by a simple equation. In order to interpret the results in terms of selective permeability we need to know the potential difference, which would arise across a membrane separating different concentrations of potassium, chloride and sodium. Thermodynamic equations cannot be applied because the system is not in equilibrium, while the theories of Planck (1890*a*, *b*) and Henderson (1907, 1908) make assumptions which are almost certainly not valid for a thin membrane of high resistance. A simple equation has been derived by Goldman (1943). He assumes that the voltage gradient through the membrane may be regarded as constant and that ions move under the influence of diffusion and the electric field. Goldman also makes the tacit assumption that the concentrations of ions at the edges of the membrane are directly proportional to those in the aqueous solutions. In the Appendix we show that these assumptions give the following expression for the membrane potential:

$$E = \frac{RT}{F} \ln \left[\frac{P_{\mathrm{K}}[\mathrm{K}^+]_{\mathrm{o}} + P_{\mathrm{Na}}[\mathrm{Na}^+]_{\mathrm{o}} + P_{\mathrm{Cl}}[\mathrm{Cl}^-]_{\mathrm{i}}}{P_{\mathrm{K}}[\mathrm{K}^+]_{\mathrm{i}} + P_{\mathrm{Na}}[\mathrm{Na}^+]_{\mathrm{i}} + P_{\mathrm{Cl}}[\mathrm{Cl}^-]_{\mathrm{o}}} \right] \qquad (4)$$

where $[K^+]_i$, $[Na^+]_i$ and $[Cl^-]_i$ are activities inside the axon; $[K^+]_o$, $[Na^+]_o$ and $[Cl^-]_o$ are activities outside the axon; P_K, P_{Na} and P_{Cl} are permeability constants for the individual ions. The relative magnitudes of the permeability constants depend upon the relative mobilities and solubilities of the ions in the membrane. Thus

TABLE 7

State of nerve		Composition of test solution K	Na	Cl	Change in membrane potential on subtracting test solution for seawater or aSW Observed (mV)	Calculated (mV)	Permeability coefficients used in calculation P_K	P_{Na}	P_{Cl}
Resting	A	10	465	587	-3	-4.7	1	0.04	0.45
	B	15	450	587	2	2.2			
	C	20	445	587	4	4.1			
	D	7	324	384	0	0.1			
	E	5	227	270	-2	-0.6			
	F	3	152	180	-2	-1.6			
	G	2	91	108	-4	-2.1			
	H	10	573	658	-1	0.8			
	I	10	711	796	2	0.7			
AP peak	A	10	465	587	1	0.8	1	20	0.45
	B	15	450	587	-1	0.0			
	C	20	445	587	-5	-0.3			
	D	7	324	384	-9	-7.1			
	E	5	227	270	-21	-15.3			
	F	3	152	180	-44	-24.8			
	G	2	91	108	-59	-37.2			
	H	10	573	658	3	5.7			
	I	10	711	796	9	10.4			
Peak AHP	A	10	465	587	-13	-12.7	1.8	0	0.45
	B	15	450	587	6	4.6			
	C	20	445	587	10	8.4			
	D	7	324	384	-1	-0.4			
	E	5	227	270	-4	-1.1			
	F	3	152	180	-4	-2.3			
	G	2	91	108	0	-2.6			
	H	10	573	658	-1	-0.9			
	I	10	711	796	0	-2.5			
Membrane potential at rest in seawater					-48 + J	-59			
Membrane potential at AP peak					40 + J	38			
Membrane potential at max AHP					-62 + J	-74			
Action potential in seawater					88	97			
AHP in seawater					14	15			

Solutions A, B and C were tested against an artificial seawater solution containing 10 mM K, 455 mM Na, 587 mM Cl. Solutions D-I were tested against seawater containing approximately 10 mM K, 455 mM Na, 540 mM Cl. Calculated potentials were obtained from equation 4 using values of $[K^+]_i$ = 345 mM, $[Na^+]_i$ = 72 mM, $[Cl^-]_i$ = 61 mM. J is the liquid junction potential between the axoplasm and the seawater in the microelectrode.

$$P_K = \frac{RT}{Fa} u_K b_K; \quad P_{Na} = \frac{RT}{Fa} u_{Na} b_{Na}; \quad P_{Cl} = \frac{RT}{Fa} u_{Cl} b_{Cl},$$

where a is the thickness of the membrane; u_K, u_{Na} and u_{Cl}, are mobilities of the ions in the membrane; b_K, b_{Na} and b_{Cl} are the partition coefficients between the membrane and the aqueous solution. E is the potential difference across the membrane in the absence of any net ionic current.

There are many reasons for supposing that this equation is no more than a rough approximation, and it clearly cannot give exact results if ions enter into chemical combination with carrier molecules in the membrane or if appreciable quantities of current are transported by ions other than K, Na or Cl. On the other hand, the equation has two important advantages. In the first place it is extremely simple, and in the second it reduces to the thermodynamically correct forms when any one permeability constant is made large compared to the others.

In order to apply this equation we must first adopt values for the internal concentrations of K, Cl and Na, and for this purpose the data of Steinbach (1941) and Steinbach & Spiegelman (1943) will be employed. These writers give values for freshly isolated axons and for those treated with seawater for 2-4 hr. The physiological condition of the axons used in the present work is thought to be intermediate between these two conditions and we therefore propose that the following values should be used:

$[K^+]_i$ = 345 mM (mean of average values in table 4, in Steinbach & Spiegelman, 1943);

$[Na^+]_i$ = 72 mM (mean of average values in table 4, in Steinbach & Spiegelman, 1943);

$[Cl^-]_i$ = 61 mM (mean of tables 1 and 2 in Steinbach, 1941).

The experiments of Steinbach (1941) and Steinbach & Spiegelman (1943) suggest that the squid axon is permeable to chloride, sodium and potassium, but they give little information about the relative permeabilities to these three ions. It is extremely unlikely that the permeability ratios can be determined from electrical measurements with any degree of certainty, since the values adopted for the internal concentration are subject to considerable error, and equation 4 cannot be regarded as more than a rough approximation. Our object is to show that a large number of observations can be fitted into a coherent picture by the use of appropriate permeability ratios for resting, active and refractory nerve. The experimental data against which equation (4) must be tested are summarized in Table 7, which shows the average change in membrane potential produced by substituting a test solution for seawater

or artificial seawater. Solution A is potassium-free artificial seawater, solutions B and C are potassium-rich artificial seawater; D, E, F, G are seawater solutions diluted with isotonic dextrose while H and I were made by adding solid sodium chloride to seawater. It will be seen that there is reasonable agreement between all the results obtained with resting nerve and those predicted by the theory for P_K: P_{Na}: P_{Cl} =1: 0.04: 0.45. These coefficients were found by trial and error, and serious

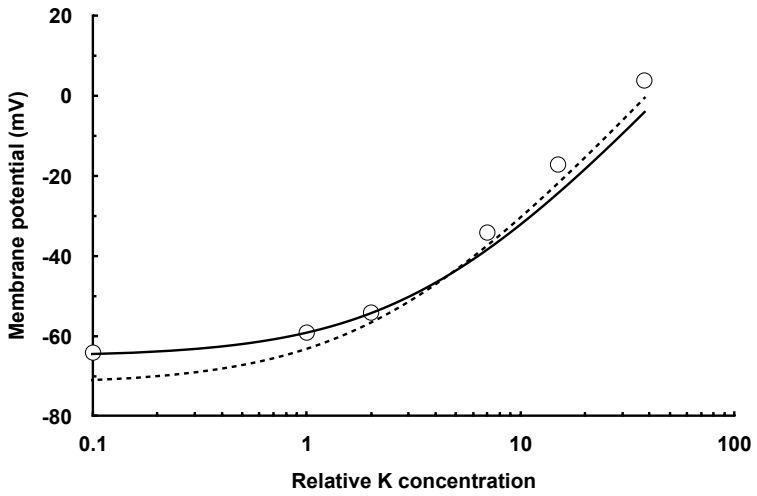

Fig 13. Data from Curtis & Cole (1942, fig 2). Ordinate: Membrane potential. Abscissa: potassium concentration of test solution in multiples of potassium concentration in standard solution (13 mM); logarithmic scale. Circles were obtained from Curtis & Cole's experimental points. Smooth curves were drawn according to equation 4 with P_K: P_{Na}: P_{Cl} = 1: 0.04: 0.45 (continuous line) and P_K: P_{Na}: P_{Cl} = 1: 0.025: 0.3 (dotted line). Internal concentrations assumed to remain constant; $[Cl^-]_i$ taken as 61 mM and $[Na^+]_o + [K^+]_o$ = 463 mM.

deviations occur if values differing by more than 50 % are employed. Thus, if the chloride permeability is made zero, a hyperpolarisation of 17 mV in the resting potential is predicted for a solution of 50 % seawater, while if it is made equal to the potassium permeability a depolarisation of 5 mV is predicted. The average change observed experimentally was 2 mV and this is the value predicted by the coefficients which have been adopted. The variations in resting potential were not large, because the external potassium concentration was kept well within the physiological range. However, the relation between potassium concentration and resting potential has been determined by Curtis & Cole (1942) and their data are supported by unpublished results obtained with Mr Huxley in 1939. Curtis & Cole's data are shown by the hollow circles in Fig 13, while they were plotted according to the theory for P_K: P_{Na}: P_{Cl} = 1: 0.04: 0.45 and 1: 0.025: 0.3, respectively. It will be seen that the first curve fits the data obtained in the physiological range, but that rather different

values are needed to cover the observations with high potassium concentration. However, the deviations are not large and are hardly surprising in view of the simplifications made in deriving equation 4. The absolute value of the resting potential predicted on the basis of P_K: P_{Na}: P_{Cl} = 1: 004: 0.45 is -59 mV, while the resting potential observed with a microelectrode containing seawater averaged -48 mV. The difference is most easily explained by supposing that there is a liquid junction potential of -11 mV between seawater and axoplasm. The magnitude of the liquid junction potential has not been measured experimentally and cannot be computed theoretically until more is known about the nature of the organic anions in the axoplasm. A tentative estimate can be obtained by making the assumption of Curtis & Cole (1942) that the anions are monovalent and have a mobility sufficient to give the axoplasm its measured value of 28 Ω cm. In this way Curtis & Cole obtained a value of -6 mV for the junction potential between isotonic KCl and axoplasm. A repetition of this calculation, using the figures for internal potassium, chloride and sodium adopted in this paper, gave a value of -14 mV for the junction potential between seawater and axoplasm.

The experiments described in this paper indicate that the action potential arises because the sodium permeability increases as the nerve membrane is depolarised. The absolute magnitude of the action potential can be calculated if values are assumed for the relative permeabilities of the active membrane to sodium, potassium and chloride ions. If the permeabilities are assumed to change from a resting condition in which P_K: P_{Na}: P_{Cl} = 1: 0.04: 0.45 to an active condition in which P_K: P_{Na}: P_{Cl} = 1: 20: 0.45 an action potential of 97 mV amplitude is predicted. This is 9 mV larger than the average value observed experimentally, but it must be remembered that equation 4 only applies if there is no external current through the membrane. The difference of 9 mV appears to be a safe allowance for the potential difference arising from current flow, since the membrane current density at the height of activity is about 0.2 mA cm^{-2} (Hodgkin & Huxley, 1945), and the active membrane resistance is about 25 Ω cm^2 (Cole & Curtis, 1939) (0.2 mA cm^{-2} x 25 Ω cm^2 = 5 mV). The new values of the permeability coefficients may be used to predict the changes in potential, which would arise from the applications of solutions A-I and these are compared with the average experimental results in Table 7. It will be seen that there is reasonable agreement over most of the range, but that deviations occur in the case of solution C (twice normal potassium), E (2/3 isotonic dextrose, 1/3 seawater) and F (4/5 isotonic dextrose, 1/5 seawater). The nature of these deviations has already been discussed and requires little further comment. In order to account for them we must assume that, for one reason or another, the

membrane during an action potential does not acquire its full sodium permeability. Thus a change of 58 mV would have been predicted for solution G if we had assumed that in this solution the permeability coefficients were 1: 2.5: 0.45 instead of 1: 20: 0.45 as in the normal active membrane. Although the sodium permeability has been assumed to be twenty times the potassium permeability, the peak action potential is still 8 mV more hyperpolarised than the theoretical maximum for E_{Na}. This indicates that equation 1 is useful only in so far as it gives an upper limit to the overshoot. On the other hand, equation 2 remains a reasonably good approximation since it predicts changes, which are within 10 % of the calculated values in Table 7.

The third block of figures in Table 7 give the changes in membrane potential recorded at the height of the AHP. In this condition the nerve is in a refractory state, so that there is no reason to assume that the permeability ratios are intermediate between those in the resting and active states. If the sodium permeability remained at its active level of 20, the nerve could show no recovery from the peak of the action potential. The sodium permeability must therefore be reduced by exhaustion or inactivation of the special mechanism which comes into play when the nerve is first depolarised. We now assume that the sodium permeability is reduced to zero and that it does not recover its normal value until the end of the relative refractory period. If this assumption is made, we find that the nerve would show an AHP of 10 mV. This is not far from that recorded experimentally, but there are still considerable deviations between theory and experiment which can be resolved by making P_K: P_{Na}: P_{Cl} = 1.8: 0: 0.45. These values have been adopted in Table 7 and give good agreement both with respect to the absolute magnitude of the AHP and to its variation in solutions of different potassium content. The agreement may be fortuitous and can hardly be used as evidence for a differential action on potassium and chloride permeability. On the other hand, the assumption that the sodium permeability is reduced to a subnormal value during the recovery process appears to be in keeping with the general nature of the refractory period, and provides a plausible explanation of the characteristic diphasic appearance of the squid action potential. The AHP is not seen in other single fibre preparations, but it must be remembered that the assumptions which have been made only lead to an AHP when there is an appreciable leakage of sodium in the resting condition i.e. where the resting membrane potential $\neq E_K$. A fibre with low sodium leakage and with potassium and chloride ions distributed according to a Donnan ratio would have a membrane potential close to the theoretical maximum for E_K predicted by the Nernst equation, which would be relatively insensitive to a decrease in the amount of

sodium leakage. In such fibres the action potential would return to the resting level without showing any appreciable AHP.

The preceding arguments suggest that an isolated squid axon is not in a steady state, but is gaining sodium and leaking potassium at a rate determined by the permeability of the membrane and the concentration differences across it. An exchange of this kind has been observed by Steinbach & Spiegelman (1943), and it is interesting to compare their result with that predicted by the constant field theory on which equation 4 is based. Steinbach & Spiegelman's figures show an average increase of 50 mM Na and an average decrease of 72 mM K during a period of 3 hr. These figures may be expressed in terms of the flow of ions through 1 cm^2 of membrane, since the average axon diameter cannot have been far from 500 μm. Adopting this value for the diameter we find that the entry of sodium through 1 cm^2 was 6 x 10^{-11} mol sec^{-1} while the exit of potassium was 8 x 10^{-11} mol sec^{-1}. In order to calculate a theoretical flow from the constant field theory we need to know the concentration differences across the membrane, the permeability ratios and the absolute value of the membrane conductance. The relation between these quantities is given by equation 7.0, 7.1 or 7.2 of the Appendix and numerical values can be obtained by adopting the concentrations and permeability coefficients used previously, with a value of 1000 Ω cm^2 for the membrane resistance (Cole & Hodgkin, 1939). The following theoretical rates are obtained: entry Na, 8.4 x 10^{-11} mol cm^{-2} sec^{-1}; exit K, 10.6 x10^{-11} mol cm^{-2} sec^{-1}; exit Cl, 2.2 x 10^{-11} mol cm^{-2} sec^{-1}. Steinbach & Spiegelman (1943) give no figures for the flow of chloride, but Steinbach (1941) states that the chloride concentration of squid axoplasm shows a rise from an initial value of 36 mM to one of about 75 mM at which level the concentration remains constant for long periods of time. If a value of 36 mM had been adopted for the chloride concentration a substantial entry of chloride would have been predicted, and this may explain the initial rise in chloride concentration observed by Steinbach. The difference between the theoretical rates for sodium and potassium and those observed by Steinbach & Spiegelman is not larger than would be expected from the nature of the calculations used in making the comparison. However, a difference of this kind is to be expected, since it is likely that entry of sodium would be partly compensated by the active extrusion process normally responsible for maintaining a low internal sodium concentration in the living animal.

The experiments described in this paper suggest that sodium ions enter the axon during the rising phase of the spike, and that the rate of rise is determined by the speed at which the charge on the membrane capacity is altered by entry of sodium. It is natural to inquire how large

the sodium permeability would have to be in order to give a rate of rise comparable to that observed experimentally. The problem may be formulated in a different way. The maximum rate of rise of the spike is of the order of 600 V sec^{-1} and, for a membrane capacity of 1.5 μF cm^{-2}, this corresponds to an ionic current density of 0.9 mA cm^{-2}. The maximum rate occurs approximately at zero membrane potential, and we may suppose that at this moment the permeability coefficients have already assumed their fully active values of P_K: P_{Na}: P_{Cl}, = 1: 20: 0.45. We are also given the fact that the resistance of the resting membrane is roughly 1000 Ω cm^2, and in this condition we assume as before that P_K: P_{Na}: P_{Cl} = 1: 0.04: 0.45. This information allows the total ionic current to be calculated by the methods described in the Appendix. We find

(1) an inward sodium current of 1.3 mA cm^{-2};

(2) an outward potassium current of 0.06 mA cm^{-2};

(3) an outward chloride current of 0.04 mA cm^{-2};

(4) a net inward current of 1.2 mA cm^{-2}.

The total inward current is of the same order as that obtained experimentally so that there is no difficulty in accounting for the rate of rise of the action potential in terms of our hypothesis.

The preceding calculation suggests that the inward sodium current greatly exceeds the outward potassium current during the rising phase of the action potential, and we should expect that this situation would be reversed during the falling phase of the spike. A minimum value for the quantity of sodium entering the axon can be obtained by assuming that the period of sodium entry does not overlap to any appreciable extent with the period of potassium exit. In this case the total quantity of sodium entering the axon would be given by the product of the membrane action potential and the membrane capacity divided by the Faraday constant. Thus 1.5 x 10^{-12} mol must be transferred through a membrane of capacity 1.5 μF in order to change its potential difference from -50 mV to 45 mV ((0.095 V x 1.5e^{-6} F cm^{-2})/96500 C mol^{-1}). More sodium would enter if there was a simultaneous exchange of potassium and sodium, but the quantity entering could not be less than 1.5 x 10^{-12} mol unless some other mechanism assists in the active process. A crucial test of the sodium hypothesis would therefore be to measure the quantity of sodium entering the axon in one impulse. This experiment has never been performed in a satisfactory way, although the work of Fenn & Cobb (1936), Tipton (1938) and v. Euler, v. Euler & Hevesy (1946) provides some indication of sodium entry during activity. The total charge moving out through the membrane during the falling phase must be approximately equal to the charge transferred during the rising phase. The outward charge would be

carried primarily by potassium ions if the permeability of the active membrane is greater to potassium than chloride. Under these conditions a minimum potassium leakage of 1.5 x 10^{-12} mol is to be expected. This is not far from the value obtained by Hodgkin & Huxley (1947), who gave an average value of 1.7 x 10^{-12} moles in *Carcinus* axons with an average membrane capacity of 1.3 μF cm^{-2}. The average action potential amplitude in *Carcinus* axons has been estimated at about 120 mV (Hodgkin, 1947) so that the theoretical minimum for the potassium leakage would be

$$\frac{120 \text{ mV} \times 1.3 \ \mu\text{F cm}^{-2}}{96{,}500 \text{ coulomb mol}^{-1}} = 1.6 \times 10^{-12} \text{ mol cm}^{-2}$$

The close agreement is unlikely to be more than a coincidence, but the similarity in order of magnitude may be significant, since Keynes (1948) has recently obtained comparable results by the use of radioactive tracers.

SUMMARY

The reversal of membrane potential during the action potential can be explained if it is assumed that the permeability conditions of the membrane during an action potential are the reverse of those in the resting state. The resting membrane is taken to be more permeable to potassium than sodium, and during the action potential the membrane becomes more permeable to sodium than to potassium. (It is suggested that the reversal of permeability is brought about by a large increase in sodium permeability and that the potassium permeability remains unaltered or undergoes a relatively small change.) A reversal in polarity of the membrane potential can arise in a system of this kind if the concentration of sodium in the external solution is greater than that in the axoplasm.

This hypothesis is supported by the following observations made with a microelectrode in squid giant axons:

1. The action potential is abolished by sodium-free solutions, but returns to its former value when seawater is replaced.

2. Dilution of seawater with isotonic dextrose produces a slight hyperpolarisation in resting potential, but a large and reversible decrease in the height of the action potential. The reversal in polarity of the membrane potential during an action potential depends upon the sodium concentration in the external fluid and is reduced to zero by solutions containing less than about 30 % of the normal sodium concentration.

3. The amplitude of the action potential is increased by a hypertonic solution containing additional sodium chloride, but is not

increased by addition of dextrose to seawater. The resting potential is unaffected or slightly depolarised by sodium-rich solutions.

4. The changes in peak action potential amplitude which result from increases or decreases of external sodium are of the same order of magnitude as those predicted by the Nernst equation.

5. The rate of rise of the action potential can be increased to 140 % of its normal value and reduced to 10 % by altering the concentration of sodium in the external solution. To a first approximation, the rate of rise is directly proportional to the external concentration of sodium.

6. The conduction velocity undergoes a substantial decrease in solutions of low-sodium content.

7. The changes produced by dilution of seawater with isotonic dextrose appear to be caused by reduction of the sodium concentration and not by alterations in the concentrations of other ions.

Removal of external potassium causes a small increase in action potential amplitude, which is almost entirely due to a hyperpolarisation in the resting potential, the overshoot of the action potential remaining substantially constant. Increasing the external potassium causes a depression of action potential amplitude and depolarisation of the resting potential, but the former is affected to a much greater extent than the latter. The AHP of the squid action potential is markedly increased by potassium-free solutions and decreased by potassium-rich solutions.

The effects of a large number of solutions on the membrane potential in the resting, active and refractory state are shown to be consistent with a quantitative formulation of the sodium hypothesis.

We wish to express our gratitude to the Rockefeller Foundation for Medical Research for financial aid; to the director and staff of the Laboratory of the Marine Biological Association, Plymouth, for their assistance at all stages of the experimental work; and to Mr A. F. Huxley for much helpful and stimulating discussion.

APPENDIX

This section contains a brief description of the way in which constant field equations may be derived and applied to practical problems. The treatment is essentially similar to that of Goldman (1943) but is summarized here for the convenience of the reader.

The basic assumptions are (1) that ions in the membrane move under the influence of diffusion and the electric field in a manner which is essentially similar to that in free solution; (2) that the electric field may

be regarded as constant throughout the membrane; (3) that the concentrations of ions at the edges of the membrane are directly proportional to those in the aqueous solutions bounding the membrane; and (4) that the membrane is homogeneous.

Assumption (1) leads to the following equations for the current carried by ions:

$$-I_K = RTu_K \frac{dC_K}{dx} + C_K u_K F \frac{d\psi}{dx} \tag{1.1}$$

$$-I_{Na} = RTu_{Na} \frac{dC_{Na}}{dx} + C_{Na} u_{Na} F \frac{d\psi}{dx} \tag{1.2}$$

$$-I_{Cl} = RTu_{Cl} \frac{dC_{Cl}}{dx} + C_{Cl} u_{Cl} F \frac{d\psi}{dx} \tag{1.2}$$

Here I_K, I_{Na} and I_{Cl} are the contributions of potassium, sodium and chloride to the total inward current density through the membrane. C_K, C_{Na} and C_{Cl} are the concentrations of ions in the membrane and u_K, u_{Na} and u_{Cl}, are their mobilities; x is the distance through the membrane from the outer boundary defined as $x = 0$. The inner boundary is defined as $x = a$. ψ is the potential at a point x; R, T and F have their usual significance. In the steady state I_K I_{Na} and I_{Cl} must be constant throughout the membrane; $d\psi/dx$ is also regarded as constant and equal to $-V/a$, where V is the potential of the outside solution minus that of the inside solution. Equations (1.1), (1.2) and (1.3) may therefore be integrated directly. Thus (1.1) gives

$$\left[\frac{aI_K e^{-VFx/RTa}}{VFu_K}\right]_0^a = \left[C_K e^{-VFx/RTa}\right]_0^a \tag{2.1}$$

Hence

$$I_K = \frac{u_K FV}{a} \frac{(C_K)_o - (C_K)_a e^{-VF/RT}}{1 - e^{-VF/RT}} \tag{2.2}$$

Now the concentration $(C_K)_o$ at the outer edge of the membrane is directly proportional to the concentration $(K)_o$ of potassium in the external fluid. Hence

$$(C_K)_o = \beta_K (K)_o \quad \text{and} \quad (C_K)_a = \beta_K (K)_i,$$

where β_K is the partition coefficient between the membrane and the aqueous solution; $(K)_i$ is the concentration in the axoplasm.

Equation (2.2) then becomes

$$I_K = P_K \frac{F^2 V}{RT} \frac{(K)_o - (K)_i e^{-VF/RT}}{1 - e^{-VF/RT}}, \tag{2.3}$$

where P_K is a permeability coefficient defined as $u_K \beta_K RT/aF$.

In a similar way we obtain

$$I_{Na} = P_{Na} \frac{F^2 V}{RT} \frac{(Na)_o - (Na)_i e^{-VF/RT}}{1 - e^{-VF/RT}}, \tag{2.4}$$

and

$$I_{Cl} = P_{Cl} \frac{F^2 V}{RT} \frac{(Cl)_i - (Cl)_o e^{-VF/RT}}{1 - e^{-VF/RT}}. \tag{2.5}$$

The total ionic current density through the membrane is therefore given by

$$I = \frac{F^2 V P_{\mathrm{K}}}{RT} \frac{w - y e^{-VF/RT}}{1 - e^{-VF/RT}}, \tag{3.0}$$

where

$$w = (\mathrm{K})_o + \frac{P_{\mathrm{Na}}}{P_{\mathrm{K}}}(\mathrm{Na})_o + \frac{P_{\mathrm{Cl}}}{P_{\mathrm{K}}}(\mathrm{Cl})_i,$$

$$y = (\mathrm{K})_i + \frac{P_{\mathrm{Na}}}{P_{\mathrm{K}}}(\mathrm{Na})_i + \frac{P_{\mathrm{Cl}}}{P_{\mathrm{K}}}(\mathrm{Cl})_o,$$

The potential difference across the membrane in the absence of ionic current will be designated E. $V = E$ when $I = 0$. Hence

$$E = \frac{RT}{F} \log_e \frac{y}{w}, \tag{4.0}$$

which is equivalent to equation (4) used in the text. The membrane conductance G is defined as $(dI/dV)_{I \to 0}$ and is given by

$$G = \frac{F^2 P_{\mathrm{K}}}{RT} \left\{ V \frac{d}{dV} \left[\frac{w - y e^{-VF/RT}}{1 - e^{-VF/RT}} \right] + \left[\frac{w - y e^{-VF/RT}}{1 - e^{-VF/RT}} \right] \right\} \tag{5.0}$$

The second term in this expression is zero when $I = 0$ and $V = E$. After differentiation V may be equated to E. Hence

$$G = \frac{F^3}{(RT)^2} E P_{\mathrm{K}} \left\{ \frac{wy}{y - w} \right\}. \tag{6.0}$$

This expression allows us to compute the numerical values of the permeability coefficient P_{K} provided that the ratios $P_{\mathrm{Na}}/P_{\mathrm{K}}$ and $P_{\mathrm{Cl}}/P_{\mathrm{K}}$ are known. For the case considered in the text P_{K} is found to be 1.8×10^{-6} cm sec^{-1}. The individual ionic currents may be determined by using this value in applying equations (2.3), (2.4), and (2.5) to any particular set of experimental conditions.

When $I = 0$ and $V = E$ a more convenient method is to use the following relations which may be obtained from (2.3), (2.4), (2.5), (4.0) and (6.0):

$$I_{\mathrm{K}} = \frac{RT}{F} G \left\{ \frac{(K)_o}{w} - \frac{(K)_i}{y} \right\}, \tag{7.0}$$

$$I_{\mathrm{Na}} = \frac{RT}{F} G \frac{P_{\mathrm{Na}}}{P_{\mathrm{K}}} \left\{ \frac{(\mathrm{Na})_o}{w} - \frac{(\mathrm{Na})_i}{y} \right\}, \tag{7.1}$$

$$I_{\mathrm{Cl}} = \frac{RT}{F} G \frac{P_{\mathrm{Cl}}}{P_{\mathrm{K}}} \left\{ \frac{(\mathrm{Cl})_i}{w} - \frac{(Cl)_o}{y} \right\}. \tag{7.2}$$

These equations were used in the calculation given on p. 71.

The constant field equations may be applied to the rising phase of the spike if it is assumed that the rate of change of potential is low enough to allow the ionic currents to attain their steady state value. At the moment when the rate of rise of the spike is at a maximum the total membrane current is zero, but there is a large ionic current which is equal

and opposite to the capacity current through the membrane dielectric. In this case we cannot use (7.0), (7.1) and (7.2), but must return to (2.3), (2.4) and (2.5). Since the maximum rate of rise occurs at approximately the time when $V = 0$ these equations may be simplified to

$$I_{\mathrm{K}} = P_{\mathrm{K}} F\left[(\mathrm{K})_o - (\mathrm{K})_i\right], \tag{8.0}$$

$$I_{\mathrm{Na}} = P_{\mathrm{Na}} F\left[(\mathrm{Na})_o - (\mathrm{Na})_i\right], \tag{8.1}$$

$$I_{\mathrm{Cl}} = P_{\mathrm{Cl}} F\left[(\mathrm{Cl})_i - (\mathrm{Cl})_o\right]. \tag{8.2}$$

In making the calculation on p. 37 we assumed that when $V = 0$, P_{K} and P_{Cl} had the same values as in the resting nerve, but that P_{Na} was $20P_{\mathrm{K}}$ instead of $0.04P_{\mathrm{K}}$.

REFERENCES

Arvanitaki, A. & Chalazonitis, N. (1947). *Arch. int. Physiol*. **54**, 406.
Bernstein, J. (1912). *Electrobiologie*. Braunschweig: Vieweg.
Bisjho, G. H. (1932). *J. cell. comp. Physiol*. **1**, 177.
Cole, K. S. & Curtis, H. J. (1938). *J. gen. Physiol*. **21**, 757.
Cole, K. S. & Curtis, H. J. (1939). *J. gen. Physiol*. **22**, 649.
Cole, K. S. & Hodgkin, A. L. (1939). *J. gen. Physiol.* **22**, 671.
Curtis, H. J. & Cole, K. S. (1942). *J. cell. comp. Physiol*. **19**, 135.
Davson, H. (1940). *J. cell. comp. Physiol*. **15**, 317.
Davson, H. & Reiner, M. (1942). *J. cell. comp. Physiol*. **20**, 325.
Erlanger, J. & Blair, E. A. (1938). *Amer. J. Physiol*. **124**, 341.
Fenn, W. O. & Cobb, D. M. (1936). *Amer. J. Physiol*. **115**, 345.
Gallego, A. & Lorente de Nó (1947). *J. cell. comp. Physiol*. **29**, 189.
Gerard, R. W. (1932). *Physiol. Rev*. **12**, 469.
Glazebrook, Sir R. (1923). *A Dictionary of Applied Physics*, vol. 3. London: Macmillan.
Goldman, D. E. (1943). *J. gen. Physiol*. **27**, 37.
Grundfest, H. (1947). *Ann. Rev. Physiol*. **9**, 477.
Henderson, P. (1907). *Z. phys. Chem*. **59**, 118.
Henderson, P. (1908). *Z. phys. Chem*. **63**, 325.
Hober, R. (1946). *Ann. N.Y. Acad. Sci*. **47**, 381.
Hodgkin, A. L. (1939). *J. Physiol*. **94**, 560.
Hodgkin, A. L. (1947). *J. Physiol*. **106**, 305.
Hodgkin, A. L. (1948). *J. Physiol*. **107**, 165.
Hodgkin, A. L. & Huxley, A. F. (1939). *Nature, Lond*., **144**, 710.
Hodgkin, A. L. & Huxley, A. F. (1945). *J. Physiol*. **104**, 176.
Hodgkin, A. L. & Huxley, A. F. (1947). *J. Physiol*. **106**, 341.
Kato, G. (1936). *Cold. Spr. Harb. Symp. quant. Biol*. **4**, 202.
Katz, B. (1947). *J. Physiol*. **106**, 411.
Keynes, R. D. (1948). *J. Physiol*. **107**, 35P.
Krogh, A. (1946). *Proc. Roy. Soc. B*, **133**, 123.
Landolt-Bernstein (1931). *Phyaicalisch-chemische Tabellen*, 5th ed. Erganzungsband IIa, 189. Berlin: Springer.
Lillie, R. S. (1923). *Protoplasmic Action and Nervous Action*. Chicago: University Press.

Lorente de Nó, R. (1944). *J. cell. comp. Physiol*. **24**, 85.
Lorente de Nó, R. (1947). *A Study of Nerve Physiology*, vols. 1 and 2 in *Studies from the Rockefeller Institute for Medical Research*, vols. **131** and **132**. New York.
Overton, E. (1902). *Pflug. Arch. ges. Physiol*. **92**, 346.
Planck, M. (1890 *a*). *Ann. Phys., Lpz*., **39**, 161.
Planck, M. (1890 *b*). *Ann. Phys., Lpz*., **40**, 561.
Schmitt, F. O. (1930). *Amer. J. Physiol*. **95**, 650.
Schmitt, F. O. & Schmitt, O. H. A. (1931). *Amer. J. Physiol*. **97**, 302.
Steinbach, H. B. (1941). *J. cell. comp. Physiol*. **17**, 57.
Steinbach, H. B. & Spiegelman, S. (1943). *J. cell. comp. Physiol*. **22**, 187.
Stella, G. (1928). *J. Physiol*. **66**, 19.
Tipton, S. R. (1938). *Amer. J. Physiol*. **124**, 322.
Ussing, H. H. (1947). *Nature, Lond*., **160**, 262.
v. Euler, H. V., v. Euler, U. S. & Hevesy, G. (1946). *Acta physiol. Scand*. **12**, 261.

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