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THE EFFECT OF INTERNAL AND EXTERNAL POTASSIUM CONCENTRATION ON THE MEMBRANE POTENTIAL OF FROG MUSCLE

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The ability of potassium to depolarize the membrane of excitable tissues is well known, and the fact that there is a linear relation between the resting potential and the logarithm of the external potassium concentration has been taken as evidence that this potential arises from the difference between the concentration of potassium inside and outside the cell (Hober, 1905; Bernstein, 1912; Hodgkin, 1951). In frog sartorius muscle this relation has been known for a long time, and recently internal microelectrodes (Graham & Gerard, 1946; Ling & Gerard, 1949) have been used to determine how well the experimental relation agrees with theoretical predictions (Ling & Gerard, 1950; Jenerick, 1953; Harris & Martins-Ferreira, 1955). The present paper describes experiments in which the membrane potential of frog sartorius muscle was measured in solutions of different potassium content, and in solutions which were designed to alter the inside concentration of potassium keeping the outside concentration constant. In order to be able to compare the membrane potentials in different solutions it is essential that the measurements are not altered by a junction potential at the tip of the electrode. Nastuk & Hodgkin (1950) filled microelectrodes with 3 M-KCl to reduce the electrical resistance, and they assumed provisionally that such electrodes measured the membrane potential without introducing any junction potential error. However, for some electrodes filled with 3 M-KCl, which on other grounds would appear perfectly suitable, this assumption may not be true (Nastuk, 1953; del Castillo & Katz, 1955). The first part of this paper describes experiments designed to explain this anomalous junction potential, and to find a way to select electrodes which give reliable results. In the second part the results of altering the ratio of potassium concentration across the muscle fibre membrane are given.

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METHODS

Materials

All the experiments were done on sartorii from male frogs. Small or medium-sized muscles (<100 mg wet weight) were used in order to keep the equilibration time for the extracellular space and the intracellular water within reasonable limits. Muscles were left attached to the pelvic bone, which was split when paired muscles were needed, and the cut ends of other muscles attached to the pelvis were carefully removed. The experiments were done between the months of February and December at room temperatures between 13 and 23° C. No seasonal variation was observed.

Apparatus

Microelectrodes were made by machine from Pyrex tubing of approximately 1 mm outside diameter. The electrode puller, built to Alexander & Nastuk's design (1953), produced electrodes with comparable characteristics at any given setting of the various controls. Electrodes were filled with 3 M-KCl by boiling for about 2 min at reduced pressure. The filling solution was carefully filtered before each batch of electrodes was filled. The resistance and tip junction potential (hereafter called tip potential) of each electrode were measured in Ringer's solution (see p. 634). Electrodes with resistances less than 5 MΩ and tip potentials more negative than −5 mV in Ringer's solution were rejected.

Electrical apparatus. The microelectrode was fixed by a short length of rubber tubing to a glass tube containing agar-Ringer in contact with a chlorided silver wire connected directly to the grid of the input cathode-follower. The glass tube was mounted in the end of the cathode screen of the cathode-follower probe, and the whole probe was attached to a Prior manipulator (Nastuk & Hodgkin, 1950; Keynes & Martins-Ferreira, 1953). A conventional differential d.c. amplifier and cathode-ray oscilloscope were used. When only resting potentials were measured the grid of one of the two cathode-followers was earthed. A low resistance calibrator, with a Weston cell as a reference potential, was connected between the Perspex muscle bath and earth by an agar-Ringer bridge and an Ag-AgCl wire. This calibrator produced potentials of up to 210 mV in steps of 1 and 10 mV, and was used to back off the deflexion of the oscilloscope trace which resulted from the penetration of a fibre. Membrane potentials were measured to the nearest mV, and unless otherwise stated eight penetrations were done on each muscle in each set of conditions. Measurement of the potentials by backing off made it possible to make a series of measurements in a very short time, and to use amplifications larger than could be used with photographic recording. Part of the output of the amplifier was taken to the grid of a thyratron oscillator driving a loudspeaker (Draper & Weidmann, 1951). The majority of impalements produced a very sharp change in the note from the loudspeaker, and those where the frequency change was not abrupt were rejected. The grid current of the input valve was 2×10^{-11} A, an amount too small to have produced measurable errors.

In order to overcome the difficulty of movement of the muscle when measuring the action potential, only a few fibres were stimulated at a time by a very small electrode. This electrode was made by sealing a 40 μ platinum wire into the end of a glass tube and grinding a small flat surface at the tip with the wire at its centre. By lowering this electrode, connected to the stimulator by an isolating transformer, into close contact with the surface of the muscle, the fibres which were stimulated could be observed. The stimulus was then short-circuited and the microelectrode was lowered into one of these fibres. On releasing the short-circuit key several action potentials could be obtained without the electrode pulling out of the fibre. Some decline in the resting potential was always observed, probably owing to leakage round the tip of the electrode. Only the first action potential was photographed for measurement. A stimulating electrode of this kind produces a large stimulus artifact. In order to reduce this, microelectrodes of similar resistance were attached to both cathode-follower probes, and the reference electrode was left just outside the muscle fibre close to the point of penetration of the internal electrode.

Solutions

Distilled water, A.R. electrolytes, and recrystallized sucrose were used throughout. The inorganic constituents were made up as molar, half-molar, or tenth-molar stock solutions, measured volumes of which were mixed together and made up to a final known volume. Sucrose was added either as a solid or as a molar solution. In the latter case the sucrose solution was made up freshly, and the final solutions which contained sucrose were stored in a refrigerator for not more than a week, after which they were discarded. All solutions which were used for treating muscles contained identical concentrations of $CaCl_2$ and phosphate buffer, which was a mixture of NaH_2PO_4 and Na_2HPO_4 calculated to give a pH of 7·2. The pH of solutions was checked with bromthymol-blue. Solutions of either KCl or NaCl alone were used in some of the experiments on the microelectrode tip potential. The composition of the normal Ringer's solution, which was made as simple as possible, is given in Table 1. To obtain higher concentrations of K^+ ion, KCl was substituted for NaCl unless otherwise stated.

TABLE 1. Composition of normal Ringer's solution; mM

KCl	NaCl	$CaCl_2$	Na_2HPO_4	NaH_2PO_4
2·5	115	1·8	2·15	0·85

Composition of sulphate solutions with 10, 50 and 190 m-equiv/l. of potassium; mM

K_2SO_4	Na_2SO_4	$CaCl_2$	Na_2HPO_4	NaH_2PO_4	Sucrose
5	55	1·8	2·15	0·85	72
25	35	1·8	2·15	0·85	72
95	—	1·8	2·15	0·85	—

Sulphate solutions were made from half-molar stock solutions of Na_2SO_4 and K_2SO_4 (with $CaCl_2$ and buffer). On the basis of lowering of freezing-point data 95 mM-K_2SO_4 is isotonic with normal Ringer's solution, and this was checked by weighing muscles in both solutions. Sulphate solutions with a potassium concentration of 100 m-equiv/l. or less contained 120 m-equiv/l. of sulphate, and the osmotic deficit was made up with 72 m-mole/l. of sucrose. Sulphate solutions with a potassium content of less than 10 m-equiv/l. were not used because muscles twitch spontaneously in these solutions. The full composition of sulphate solutions containing 10, 50 and 190 m-equiv/l. of potassium is given in Table 1. Muscles survive well in solutions containing sulphate (Katz, 1949). Even after prolonged treatment with isotonic K_2SO_4, the excitability of the outside fibres is restored within about 2 min of transferring the muscle to normal Ringer's solution. The resting potential reaches normal values within half an hour. This delay may be due to the diffusion of potassium from the extracellular space.

Estimation of intracellular potassium and sodium

Muscles were carefully cut from the pelvic bone and, after blotting with an ashless filter-paper (Whatman no. 542), were suspended from the arm of a 100 mg torsion balance by about 1 cm of fine cotton thread tied to the tendon. After weighing, each muscle was hung from a pin in the cork of a specimen tube and dried in an oven at 100° C. The dry muscle with its thread was weighed, and after cutting the muscle from its thread the dry and wet weights of the thread were found. The dry and wet weights of the muscle alone were expressed as a percentage, and from this, assuming the extracellular space was 12·5% of the wet weight (Desmedt, 1953), the fibre water was calculated. The dry muscles, in platinum crucibles, were put into a furnace overnight at 520–580° C. The ash was washed into Pyrex volumetric flasks, and the resulting solution was compared in an Evans Electroselenium flame photometer with a standard containing 100 μmole/l. KCl and 50 μmole/l. NaCl. Volumetric flasks of 50 or 100 ml. capacity were used to bring the final potassium concentration reasonably close to that of the standard. The concentration of potassium in the muscle (C_M) was expressed in m-mole/kg wet weight, and from it the intracellular

concentration ($C_{f.w.}$ in m-mole/kg fibre water) was found by means of the formula (Boyle, Conway, Kane & O'Reilly, 1941; Desmedt, 1953):

$$C_{f.w.} = \frac{C_M - 0{\cdot}125C_0}{1 - [(\text{Dry wt.}/\text{Wet wt.}) + 0{\cdot}125]},$$

where C_0 is the concentration of the particular ion in the external solution. The usual precautions against contamination of the samples, particularly by sodium from the glass of the flasks, were taken.

RESULTS

The influence of the tip potential on membrane potential measurements

Three observations on the behaviour of microelectrodes, made in the course of some preliminary experiments, showed that measurements with unselected electrodes could not be accepted uncritically. The first was that some electrodes with high resistance which penetrated the membrane of the muscle fibre without apparent damage gave very low resting potentials in normal Ringer's solution. In the most extreme case, the mean of nineteen impalements with an electrode of resistance 36 MΩ was $58{\cdot}7 \pm 0{\cdot}6$ mV (S.E. of mean). The second observation was that two electrodes might give very different mean values for the resting potential of the same muscle, and that this difference was statistically significant. The third observation was that breaking an electrode which gave low values for the resting potential, if it was still usable, immediately increased the measured resting potential to about 90 mV. In all these cases faults in the recording apparatus were carefully excluded. The possibility that some kind of junction potential could account for these observations was increased when it was shown that though the resting potential might be very low the total size of the action potential was not significantly altered. In fifteen impalements with four electrodes, five before breaking and ten after breaking, the mean resting potential before breaking was $64 \pm 2{\cdot}5$ mV, and after breaking $88 \pm 1{\cdot}8$ mV (t test, $P < 0{\cdot}001$). The corresponding values for the action potential were 119 ± 3 mV before breaking and 125 ± 2 mV after breaking ($P > 0{\cdot}1$).

In order to measure the tip potential of an electrode the Ag-AgCl wire connected to the grid of the input valve was surrounded by agar-Ringer (Fig. 1) as in the arrangement used by Nastuk & Hodgkin (1950). In preliminary experiments this wire made direct contact with the 3 M-KCl in the electrode, so that owing to the difference of concentration of chloride at the two Ag-AgCl wires, the grid of the input valve was always negative to earth. In the absence of an electrode, with the agar-Ringer in the cathode-follower probe in direct contact with the Ringer's solution in the bath, the potential of the grid did not differ from earth potential by more than 1 mV. This was checked regularly, and the agar-Ringer in the probe was replaced when necessary. The introduction of an electrode filled with 3 M-KCl should not alter the

potential of the grid as the junction potentials at the two new junctions should be equal. Frequently this is not the case and the grid may be as much as 60–70 mV negative to earth. Not only was the grid current too small (2×10^{-11} A) to account for this potential, it was in the wrong direction and would have made the grid positive to earth. If the tip of an electrode with a large tip potential is lowered on to the bottom of the bath so that it is broken (shown by a substantial fall in resistance) the potential is immediately abolished.

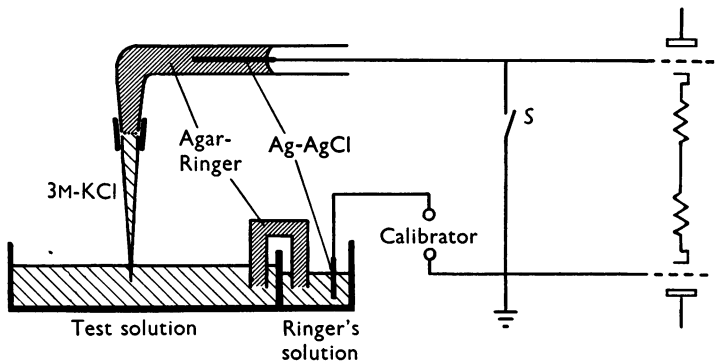

Fig. 1. Diagram of muscle bath and microelectrode mounting. To measure the tip potential the output of the calibrator was adjusted until closing switch *S* produced no deflexion of the oscilloscope trace.

Fourteen unselected electrodes, of known resistance (range 7–120 MΩ) and tip potential, were used to measure the resting potential of two muscles. In Fig. 2 the mean resting potential obtained with each electrode (± S.E. of mean) is plotted against its tip potential in Ringer's solution. On four occasions both the resistance and the tip potential rose suddenly to new stable values, and the mean resting potential before and after the change, attributed to blocking, are included. One electrode changed twice in this way. From the data of these two experiments correlation coefficients r were calculated for the three variables, tip potential (TP), microelectrode resistance (MeR), and resting potential (RP) treated as a positive quantity. The total and partial correlation coefficients are given in Table 2. Large variations of the resting potential measurement can be introduced by the microelectrode, and though this error seems to be directly related to the tip potential, electrodes of high resistance are more likely to give rise to this trouble than electrodes of low resistance.

The mechanism of the tip potential

The effect on the tip potential of solutions of different concentrations of KCl and NaCl outside the tip is shown in Fig. 3. The following system was used to measure the tip potential:

Ag-AgCl . agar-Ringer . test solution . microelectrode 3 M-KCl . agar-Ringer . Ag-AgCl.

The results were corrected for the measured junction potential at the junction of the agar-Ringer and test solution. The results in Fig. 3 are from a single electrode, and show that the inside of this microelectrode was always more negative when the outside ion was sodium rather than potassium. If the tip potentials in 125 mM-NaCl and KCl are assumed to represent roughly the tip potentials in Ringer's solution and intracellular fluid, then as the tip of the electrode passes from the outside to the inside of the cell, the measured resting potential will be the true resting potential minus the difference between the tip potentials in KCl and NaCl. To test this idea the tip potentials of a series of electrodes were measured in 100 mM-KCl and 100 mM-NaCl. In Fig. 4 the

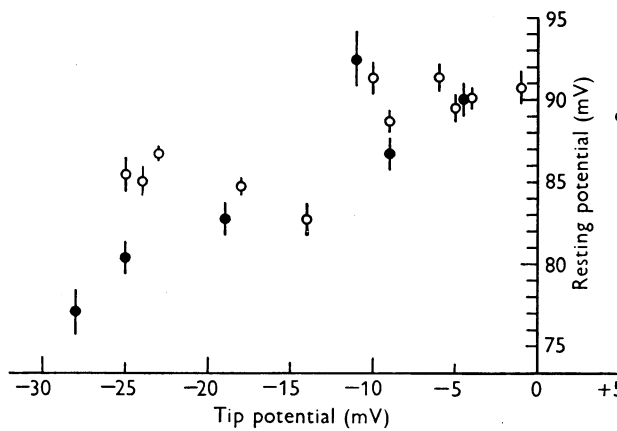

Fig. 2. The effect of the tip potential on the measured resting potential of two muscles in normal Ringer's solution. Abscissa: tip potential in mV. Ordinate: mean resting potential in mV ± the standard error of the mean.

TABLE 2. The correlation between resting potential, tip potential, and microelectrode resistance

$r_{TP, MeR}$	−0·766	Significant ($P<0·01$)
$r_{TP, RP}$	+0·857	Significant ($P<0·01$)
$r_{RP, MeR}$	−0·668	Significant ($P<0·01$)
Partial correlation coefficients		
$r_{RP, TP(MeR)}$	+0·702	Significant ($P<0·01$)
$r_{RP, MeR(TP)}$	−0·030	Not significant

difference ($TP_{KCl} - TP_{NaCl}$) is plotted against the tip potential in 100 mM-NaCl (closed circles). On the same graph the difference between the measured resting potential and an arbitrary value of 90 mV (90 – RP) is plotted against the tip potential in Ringer's solution (open circles; replotted from Fig. 2). While the agreement is not perfect, over a wide range of tip potentials both quantities are of the same order of magnitude.

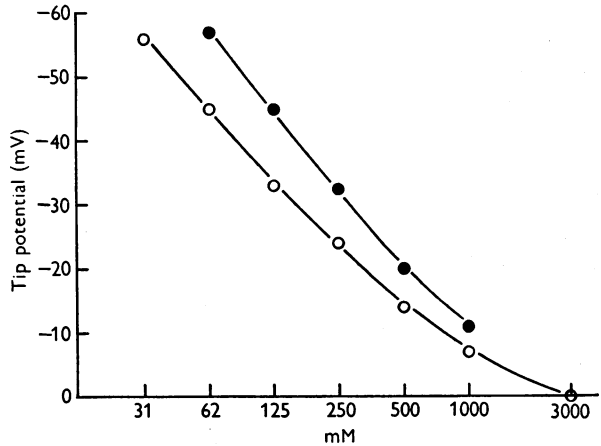

Fig. 3. The effect of solutions of NaCl and KCl on the tip potential of a single electrode. Abscissa: the concentration in mM (logarithmic scale) of NaCl (solid circles) and KCl (open circles). Ordinate: the potential of the inside of the electrode with respect to the surrounding solution in mV.

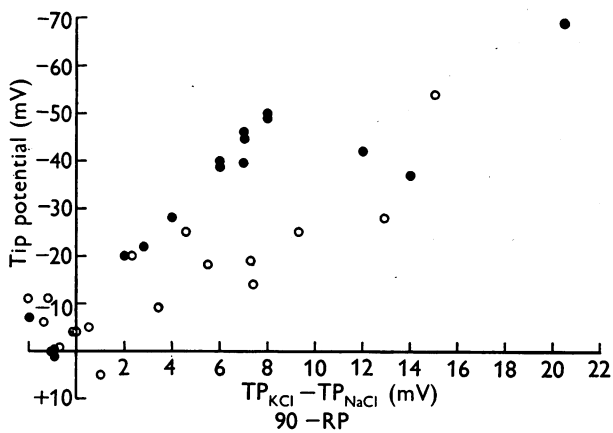

Fig. 4. The difference in tip potential in 100 mM-NaCl and KCl, $TP_{KCl} - TP_{NaCl}$, plotted against the tip potential in 100 mM-NaCl, and the deficit in resting potential, 90 – RP, plotted against the tip potential in Ringer's solution. Different electrodes were used for the two sets of measurements. Abscissa: $TP_{KCl} - TP_{NaCl}$: (solid circles) and 90 – RP (open circles) in mV. Ordinate: tip potential in mV.

If the Planck equation (Planck, 1890) is assumed to hold for the liquid junction at the tip of the electrode, then this equation can be used to calculate the expected size of the junction potential and to gain some idea of the possible causes of the tip potential. The Planck equation was chosen in preference to the Henderson equation as the latter applies only to junctions free from the effects of diffusion. Planck postulated a junction where the two solutions were separated by a layer of constant thickness in which diffusion of electrolyte from the two sides maintained a steady state. Using the mean activities of the solutions of KCl and NaCl and, as an approximation, the mobilities at infinite dilution, the Planck equation predicts that the potential of the inside of the microelectrode in either 100 mM-KCl or 100 mM-NaCl would not be more than +2 mV. If the mobility of the chloride ion in the tip of the electrode were reduced to zero but the mobilities K^+ and Na^+ were unchanged TP_{KCl} would be −78 mV and $TP_{KCl} - TP_{NaCl}$ would be +9·5 mV. In Fig. 3 the corresponding values are −37 mV and +11 mV. There appears to be a considerable discrepancy between the measured results and those predicted by assuming that only the mobility of the anion is affected. Apart from the uncertainty of the assumptions and the effect of diffusion from the tip of the electrode, a further possibility exists to explain this discrepancy. Michaelis (1926) and, more recently, Sollner, Dray, Grim & Neihof (1955) have produced collodion membranes which are not only impermeable to anions, but which also exaggerate the difference of mobility of cations. They suggest a porous structure for these membranes with a pore size which restricts the movement of the hydrated sodium ion more than the smaller hydrated potassium ion. The anion impermeability is supposed to be due to fixed negative charges in the walls of the pores. If a microelectrode were to become blocked with some substance, possibly a protein, which reproduced these conditions in the tip of the electrode, a system would be set up which would explain the large difference in tip potential in KCl and NaCl solutions and the relatively small absolute size of these potentials. If the mobilities of Cl^- and Na^+ were reduced to a third and a fifth respectively of their free solution values, approximate agreement would be obtained between the values for TP_{KCl} and TP_{NaCl} in 125 mM solutions predicted by the Planck equation and the values for the particular electrode of Fig. 3. That some form of blocking is essential for the production of a tip potential is suggested by the fact that the tip potential may suddenly change to a new value, an increased negativity being accompanied by an increase in resistance. Sudden changes in tip potential are particularly liable to occur when the electrode is being withdrawn from a muscle fibre. In this case the oscilloscope trace does not return to the base-line. Occasional very large resting potentials (110 mV in normal Ringer's solution) were seen, but they were never abruptly established and were rejected for this reason (del Castillo & Katz, 1955). It is possible that in these cases a tip potential is established on penetration and abolished on withdrawal.

In the experiments reported in the rest of this paper the uncertainty introduced by the tip potential has been minimized by using electrodes which had tip potentials between 0 and −5 mV. Each electrode was tested shortly after filling and only electrodes with tip potentials less negative than −5 mV and resistances more than 5 MΩ were retained for use. Only one out of about 250 electrodes had a positive tip potential (+5 mV, see Fig. 4). A proportion of the electrodes selected shortly after filling developed large tip potentials on storing, so before a series of impalements the tip potential was measured in the solution bathing the muscle. Once again electrodes with tip potentials more negative than −5 mV were rejected. (When the bathing solution contains sulphate as the anion there is a junction potential between the solution and the agar-Ringer bridge connecting the bath to the calibrator which must not be mistaken for a tip potential.) This selection procedure involved a large wastage of electrodes—just over half of all the electrodes with a resistance greater than 5 MΩ had to be rejected at the first testing—but the increased reliability of the results justified the time spent on it.

The resting potential in normal Ringer's solution

The resting potential of thirty-seven muscles from twenty-nine frogs was measured in normal Ringer's solution. These muscles had been soaked in normal Ringer's solution for between half and one hour from the completion of the dissection. Eight impalements were made on each muscle, and the mean resting potential and the standard error of the mean were calculated for each muscle to the nearest 0·1 mV. The average of the mean resting potentials was 92·2 mV (range 87·9 to 95·3 mV), and the average standard error of these means was ±0·5 mV (range ±0·3 to ±1·3 mV). An analysis of variance of the resting potentials of the eight pairs of muscles shows that while the differences between the pairs from different frogs are very highly significant ($P < 0{\cdot}001$), the differences between the means of muscles of a pair are not significant. The standard deviation of a single penetration on one muscle is ±1·9 mM, and the standard deviation of the difference between the means of muscles of a pair is ±0·7 mV. This estimate of the standard deviation of the difference between muscles of a pair is itself subject to error, but it cannot have been greater than ±1·7 mV, taking 5% confidence limits.

Resting potentials and action potentials were measured on six muscles. The results are given in Table 3. The internal potassium concentration of fourteen similarly treated muscles was estimated in order to find the value which corresponded with the normal Ringer's solution used in these experiments. The mean internal potassium concentration was 139 ± 2 m-mole/kg fibre water (S.E. of mean).

The resting potential and action potential found in these experiments do not differ greatly from the results of Nastuk & Hodgkin (1950), who used a

comparable Ringer's solution and found values of 88 ± 1 mV for the resting potential and 119 ± 1 mV for the action potential. Nastuk (1953), who mentions the existence of the junction potential at the tip of the electrode, and devised a method of filling which he says gives lower tip potentials than the boiling method, found a mean resting potential of $92{\cdot}8 \pm 0{\cdot}5$ mV and an action potential of $130 \pm 0{\cdot}5$ mV. Fatt & Katz (1951), measuring the resting potential at the end-plate in both curarized and non-curarized muscles in a Ringer's solution containing 2·0 mM-KCl and 113 mM-NaCl (with 3·6 mM-$CaCl_2$ and PO_4 buffer),

TABLE 3. Resting and action potentials in normal Ringer's solution for muscles from six frogs

Expt.	Resting potential (mV)	Action potential (mV)	No. of observations
55	$92{\cdot}2 \pm 0{\cdot}5$	$133{\cdot}1 \pm 0{\cdot}7$	8
57	$93{\cdot}1 \pm 1{\cdot}0$	$127{\cdot}1 \pm 1{\cdot}9$	9
58	$90{\cdot}0 \pm 0{\cdot}5$	$118{\cdot}9 \pm 1{\cdot}5$	9
	$90{\cdot}5 \pm 0{\cdot}5$	$126{\cdot}3 \pm 0{\cdot}7$	9
59	$93{\cdot}2 \pm 1{\cdot}3$	$119{\cdot}5 \pm 0{\cdot}5$	10
	$91{\cdot}7 \pm 0{\cdot}5$	$124{\cdot}9 \pm 0{\cdot}8$	9
Average	91·8	125·0	

$\pm$ the standard error of the mean.

found a mean value of 90 mV. Jenerick (1953) found a value of $82 \pm 0{\cdot}6$ mV for the mean resting potential of thirty fibres from seven muscles in a Ringer's solution containing 2·5 mM-KCl and 113 mM-NaCl. It seems possible that the results of many previous authors, except Nastuk (1953), may have been affected to a greater or less extent by an unrecognized tip potential effect.

Grundfest, Kao & Altamirano (1954), using a microelectrode technique, report that squid giant axons with resting potentials as low as 25 mV give propagated action potentials with overshoots of as much as 55 mV. This otherwise unexpected result would be explained if the zero potential had been shifted by the effect of the tip potential. Fig. 3 shows that in outside solutions of half molar strength $TP_{KCl} - TP_{NaCl}$ may be quite large. Moreover, TP_{NaCl} in Fig. 3 (-20 mV) is not the largest that has been observed in solutions of this concentration.

The effect of the external potassium concentration on the membrane potential

In order to determine the relation between the external potassium concentration, $[K]_0$, and the membrane potential, the normal Ringer's solution was modified by substituting equimolar quantities of KCl for NaCl. Boyle & Conway (1941) showed that though the fibre swells in solutions of this kind, the internal concentration of potassium, $[K]_i$, remains unchanged. Solutions containing more than 50 mM-KCl were not used because it was felt that the swelling produced when the sodium content was less than 70 mM would be likely to damage the fibre.

0·5–50 mM-*KCl.* The membrane potential in these solutions is plotted against log $[K]_0$ in Fig. 5. Each point is the average of the mean membrane potentials from several muscles. All muscles had been left for at least 10 min in each solution before measurement, and when the same muscle was used in different solutions, they were applied in order of increasing potassium content. For the external potassium concentrations less than 2·5 mM, muscles were washed for $\frac{3}{4}$–1 hr in K-free Ringer's solution. This treatment did not affect the resting potential measured subsequently in normal Ringer's solution, nor did it appear to reduce the internal potassium concentration by more than a few per cent. Of each of two pairs of muscles one was washed in K-free Ringer's solution for

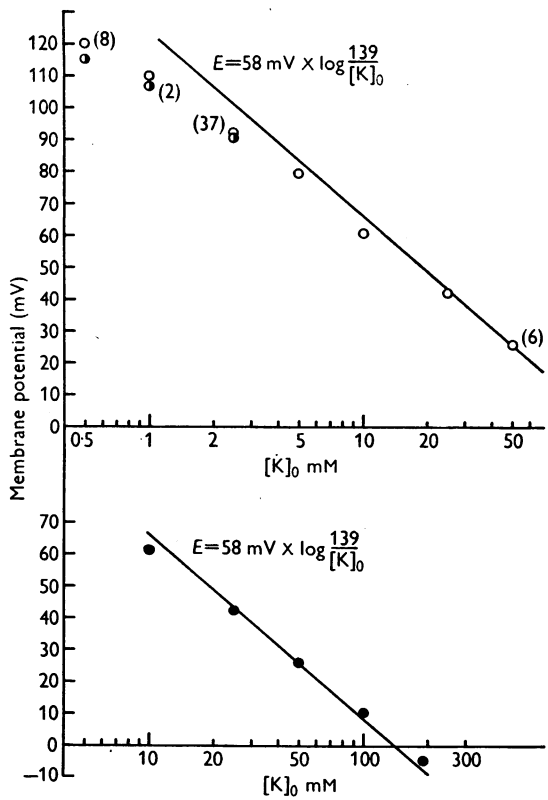

Fig. 5. The effect of external potassium concentration on the membrane potential of the sartorius muscle. Abscissa: potassium concentration in mM (logarithmic scale). Ordinate: membrane potential in mV. ○, membrane potential in chloride solutions; ●, membrane potential in sulphate solutions; ◑, membrane potential in chloride solutions with half the normal concentration of NaCl. Where the number of muscles is not indicated by a figure in brackets the point is the average value of four muscles. The lines are drawn according to the equation

$$E = \frac{RT}{F} \ln \frac{[K]_i}{[K]_o}.$$

1 hr, while the other was left in normal Ringer's solution. The washed muscles contained 145 and 143 m-mole/kg f.w. and the control muscles 148 and 145 m-mole/kg f.w. The line in Fig. 5 is drawn according to the equation

$$E = \frac{RT}{F} \ln \frac{139}{[K]_0}.$$

At concentrations of potassium below 25 mM the membrane potential is less than the equilibrium potential for potassium, though not by as much as has been previously reported (Ling & Gerard, 1949; Jenerick, 1953; Harris & Martins-Ferreira, 1955).

To confirm that the internal concentration is not changed by solutions in which potassium is substituted for sodium, two solutions were made up containing the same amount of potassium (50 m-equiv/l.) but different amounts of sodium (70 and 120 m-equiv/l.). These solutions correspond to the two ways of increasing the potassium content of Ringer's solution, by substitution of K^+ for Na^+, or by the addition of solid KCl to Ringer's solution. The weight change of a muscle was followed in each solution by suspending the muscle from the arm of a torsion balance and weighing without blotting at 5 min intervals. After the base line had been established—the muscles showed a slow loss of weight in normal Ringer's solution—the muscle was transferred to the new solution. In both solutions contracture occurred, followed by progressive relaxation, but in 50 mM-KCl and 70 mM-NaCl there was a large increase in weight, while in 50 mM-KCl and 120 mM-NaCl there was a small rapid loss of weight followed by a return to the base line. The membrane potential was followed in other muscles in the same solutions, and the internal potassium estimated. In the solution with 70 mM-NaCl the membrane potential was about 26 mV and $[K]_i$ was 144 m-mole/kg f.w. (normal 139 m-mole/kg f.w.) and in the solution with 120 mM-NaCl the membrane potential was 32–34 mV and $[K]_i$ 187 m-mole/kg f.w. The results are given in greater detail in Fig. 6. Assuming equal activity coefficients, the potentials predicted by the equation,

$$E = \frac{RT}{F} \ln \frac{[K]_i}{[K]_0}$$

are 26·6 and 33·2 mV.

5–95 mM-K_2SO_4. Solutions with sulphate as anion were used so that high external concentrations of potassium could be reached without allowing the fibre to swell or the internal potassium concentration to change. At external potassium concentrations of 10, 25 and 50 m-equiv/l. the membrane potential in the sulphate solution does not differ significantly from that in chloride solutions of the same potassium concentration (Fig. 5). At first sight it would appear that at concentrations of potassium greater than 10 m-equiv/l. chloride cannot contribute to the membrane potential, because its removal and replacement with impermeable sulphate does not alter the potential. However, it

must be remembered that the activity of K^+ in sulphate solutions is probably less than the activity of K^+ in chloride solutions. The fact that there is no difference between the membrane potential in Cl^- and SO_4^{2-} solutions may mean that the chloride contribution is equal and opposite to the effect of the smaller activity coefficient in the sulphate solutions (see Discussion).

The regression line of membrane potential on log $[K]_0$, calculated for the mean membrane potentials of the four muscles in sulphate solutions containing 25, 50 and 100 m-equiv/l. K^+, has a slope which corresponds to a $52{\cdot}3 \pm 0{\cdot}64$ mV (S.E.) change for a tenfold change in $[K]_0$ (the value of the membrane potential in 190 m-equiv/l. was excluded as the ionic strength of isotonic K_2SO_4 is

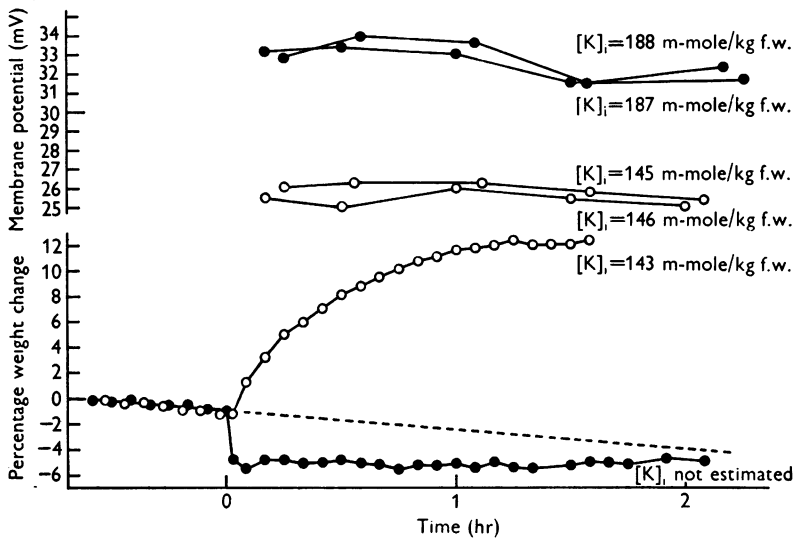

Fig. 6. The time-course of the weight change and the membrane potential of muscles in solutions containing 50 mM-KCl and 70 mM-NaCl (open circles), and 50 mM-KCl and 120 mM-NaCl (solid circles). Abscissa: time in hours. Ordinate: weight change as percentage of initial weight, and membrane potential in mV. The intracellular concentration of potassium at the end of the experiment is shown on the figure.

greater than the sulphate solutions of lower potassium content), and this value is significantly different from the theoretical value of 58 mV. It seemed possible that this difference might be explained if the muscles, which were exposed to the solutions in order of increasing potassium content, were able to expel Na^+ in exchange for K^+, and could maintain a lower internal sodium concentration when the external concentration of sodium was low. To test this point, the internal ionic concentrations of sixteen muscles were estimated, four from each of the sulphate solutions. The results are given in Table 4, together with the mean resting potentials in each solution (the measurements of the

membrane potential and internal concentration were made on different muscles). While the internal sodium concentration does appear to fall, there is no corresponding increase in the internal potassium concentration (mean of sixteen muscles 142 m-mole/kg f.w., cf. muscles from Ringer's solution 139 m-mole/kg f.w.: t test, $P > 0{\cdot}2$).

TABLE 4. The membrane potential and internal concentrations of muscles in sulphate solutions of high potassium concentration

$[K]_o$ (m-equiv/l.)	Membrane potential							
	Muscle 1		Muscle 2		Muscle 3		Muscle 4	
25	41·2 ±0·4		43·1 ±0·3		41·6 ±0·4		41·5 ±0·4	
50	26·1 ±0·2		26·6 ±0·3		26·4 ±0·3		25·4 ±0·4	
100	10·1 ±0·2		10·2 ±0·3		10·9 ±0·2		11·0 ±0·3	
190	4·3* ±0·3		4·4* ±0·2		4·5* ±0·2		4·0* ±0·2	
	Internal concentrations (m-mole/kg f.w.)							
	$[K]_o$	$[Na]_i$	$[K]_i$	$[Na]_i$	$[K]_i$	$[Na]_i$	$[K]_i$	$[Na]_i$
25	135	20	144	14	152	16	140	18
50	134	11	137	11	150	17	141	15
100	145	4	139	4	139	5	138	7
190	145	4	138	4	155	7	141	5

Brackets indicate paired muscles: ± standard error of the mean.
* Inside positive.

When the external potassium concentration exceeds the internal concentration, the membrane potential reverses sign, the inside of the fibre becoming positive to the outside. This has been shown to occur in squid giant axons (Curtis & Cole, 1942) and in *Sepia* axons (Hodgkin & Keynes, 1955*b*). Jenerick (1953), using frog sartorius, obtained a potential of 17 mV (inside negative) in 110 m-equiv/l. K^+, but he added solid KCl to his normal Ringer's solution so that $[K]_i$ would have been nearly twice the normal value. Harris & Martins-Ferreira (1955), using muscles of the South American frog *Leptodactylus ocellatus* which they state have an internal potassium concentration of 167–195 m-mole/kg f.w., found that the membrane potential in Ringer's solution plus 147 mM-KCl was 16 mV (inside negative) but in 147 mM-KCl alone was 8 mV (inside negative). It seems likely that this difference can be accounted for by the different internal potassium concentrations to be expected in the two cases.

The effect of the external concentration of sodium chloride on the membrane potential at low external potassium concentration

The deviation of the membrane potential from the theoretical potassium potential at low external potassium concentrations is likely to be due to the permeability of the membrane to ions other than potassium. The membrane potential was therefore measured in solutions in which half the NaCl had been

replaced by sucrose (109 mM-sucrose is osmotically equivalent to 60 mM-NaCl). This was done at three external potassium concentrations, 0·5, 1·0 and 2·5 mM-KCl, and in each case a fall in membrane potential was observed (Table 5, Fig. 5). Nastuk & Hodgkin (1950) obtained a similar effect substituting sucrose

TABLE 5. The effect of halving the external sodium chloride concentration (tonicity maintained with 109 mM/l. of sucrose) on the resting potential in low external potassium concentrations

Expt.	RP in 0·5 mM-KCl 120 mM-NaCl (mV)	$[K]_i$ (m-mole/kg f.w.)	$[Na]_i$ (m-mole/kg f.w.)	RP in 0·5 mM-KCl 60 mM-NaCl (mV)	$[K]_i$ (m-mole/kg f.w.)	$[Na]_i$ (m-mole/kg f.w.)
72	120·4 ± 0·5	135	31	—	—	—
	—	—	—	115·3 ± 0·5	138	23
	121·9 ± 0·6	138	25	—	—	—
	—	—	—	115·8 ± 0·5	141	17
73	120·5 ± 0·8	144	52	—	—	—
	—	—	—	116·4 ± 0·9	148	23
	119·5 ± 0·6	139	35	—	—	—
	—	—	—	115·5 ± 0·5	145	20
	1·0 mM-KCl 120 mM-NaCl			1·0 mM-KCl 60 mM-NaCl		
74	109·9 ± 0·5	137	32	—	—	—
	—	—	—	107·2 ± 0·5	147	27
	109·4 ± 0·7	139	34	—	—	—
	—	—	—	106·8 ± 0·8	143	32
	2·5 mM-KCl 120 mM-NaCl			2·5 mM-KCl 60 mM-NaCl		
75	93·2 ± 0·5	133	33	—	—	—
	—	—	—	91·6 ± 0·5	143	18
	94·0 ± 0·5	139	26	—	—	—
	—	—	—	93·1 ± 0·4	138	22
76	92·9 ± 0·5	144	19	—	—	—
	—	—	—	90·9 ± 0·8	140	15
	89·6 ± 0·5	149	16	—	—	—
	—	—	—	88·0 ± 0·7	149	18

Brackets indicate paired muscles: ± the standard error of the mean.

for NaCl, but in 2·5 mM-KCl substitution of choline chloride for NaCl produced a slight rise in potential. This would suggest that chloride contributes to the membrane potential at these low values of $[K]_0$. The effect of halving the ionic strength of the outside solution, unless it caused some change in the membrane itself, would be to lower the potential by rather less than 2 mV (see Discussion).

The effect of internal potassium concentration on the membrane potential

Variations of the internal potassium concentration were produced by treating the muscles in solutions of differing osmotic pressure but the same potassium concentration. The effect at high external potassium concentration was investigated in sulphate solutions containing 75 m-equiv/l. of K^+ and

45 m-equiv/l. of Na^+ to which 0, 50, 100 or 150 m-mole/l. of sucrose had been added. The addition of 72 m-mole/l. of sucrose would have made the solution isotonic with normal Ringer's solution. In the first experiment (Figs. 7, 8) four muscles were used, one at each sucrose concentration, and the membrane potential was measured at approximately 10 min intervals. For fibres on the outside of the muscle the half time of the potential change is about five minutes. Each muscle was left for 90 min in the test solution to allow the water movement in all the fibres to become complete, and the internal potassium concentration was then estimated in the usual way. In Fig. 8 the final value of the membrane potential of each muscle is plotted and through each point a line is drawn with the slope predicted by the Nernst equation (58 mV for a tenfold change in $[K]_0$). If the activity coefficients are equal, these lines should cut the abscissa when the internal and external concentrations of potassium are equal. The values for the internal potassium concentration obtained by analysis are marked by the arrows.

The validity of the figures for the internal concentration depends upon the following assumptions: (1) that no sucrose or sulphate enters the muscle fibres in the $1\frac{1}{2}$ hr soaking period (Johnson, 1955); (2) that the water movement is complete in this time; and (3) that the extracellular space remains a constant fraction of the wet weight. The following points suggest that these assumptions are justified. The membrane potential in the outside fibres, having reached a new value in about 20 min, remains constant over the $1\frac{1}{2}$ hr period. If a muscle is weighed in normal Ringer's solution to which 100 m-mole/l. of sucrose has been added, the weight loss, which is fully reversible, is complete within an hour and the weight does not increase after the new lower value has been reached. The third assumption is perhaps more uncertain, but when $[K]_0 =$ 75 m-equiv/l. quite large variations in the extracellular fraction will not greatly affect the calculated intracellular concentration. At low external potassium concentrations the uncertainty due to the extracellular space will be larger, but the changes in internal concentration caused by a given change in the osmotic pressure are in fair agreement whether the external concentration of potassium is 2·5 or 75 m-equiv/l.

The membrane potentials of twelve more muscles, three at each sucrose concentration, were measured after 30 min in the test solution, and the muscles were prepared for estimation after $1\frac{1}{2}$ hr. Four pairs of muscles were also used, but in this case the estimation was done on one of each pair which had been in normal Ringer's solution only, and the membrane potential was measured on the second of each pair in the sulphate solution containing 75 m-equiv/l. K^+ and 50 m-mole/l. of sucrose. This test solution is slightly hypotonic to normal Ringer's solution, and this is reflected in the fact that the mean internal concentration of the four muscles estimated directly from this solution ($130 \pm 2{\cdot}5$ m-mole/kg f.w.) is significantly lower than the internal

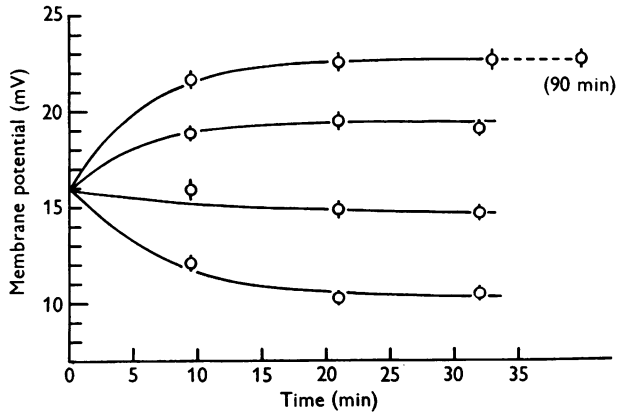

Fig. 7. The time-course of the potential change when muscles are transferred to sulphate solutions containing 0, 50, 100 and 150 mM-sucrose and 75 m-equiv/l. of potassium. Abscissa: time in minutes. Ordinate: membrane potential in mV. The origin of the curves, which were drawn by eye, is taken from Fig. 6 by interpolation.

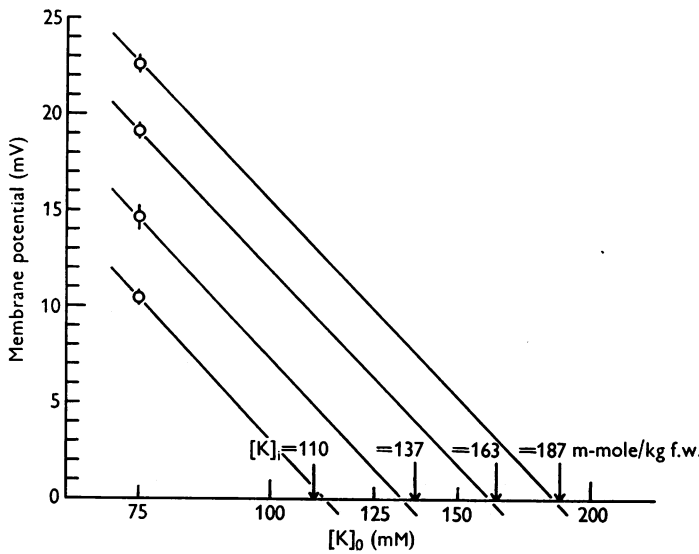

Fig. 8. The membrane potentials of four muscles of Expt. 51 in sulphate solutions containing 75 m-equiv/l. K and 0, 50, 100 and 150 mM-sucrose. Through each point the theoretical line is drawn to find the external concentration at which the membrane potential would be 0 mV. The arrows mark the internal concentrations of each muscle. Abscissa: potassium concentration mM. Ordinate: membrane potential in mV.

potassium concentration of muscles estimated from Ringer's solution (139 ± 2 m-mole/kg f.w., t test, $P = 0{\cdot}02$). The collected results are given in Table 6. In Fig. 9 the regression line of log $[K]_i$ on membrane potentials drawn for the data of Table 6, excluding the pairs of values obtained by estimating $[K]_i$ on paired muscles. This line has a slope which corresponds to a 50·2 mV change in membrane potential for a tenfold change in the internal concentration, and the internal concentration which gives a membrane potential of 0 mV in 75 m-equiv/l. K^+ is 64·8 m-mole/kg f.w. It is probable that both these facts can be accounted for by changes in the internal activity coefficient of potassium produced by changes of internal ionic strength.

In order to calculate the activity coefficient of K^+ in the external sulphate solution, which has an ionic strength (I) of 0·18, the Debye-Hückel equation (Glasstone, 1942)

$$\log f_K = \frac{-0{\cdot}509\sqrt{I}}{1+0{\cdot}329a\sqrt{I}}$$

was used. The value for a, the diameter of the hydrated potassium ion, was taken as 3 Å (Conway, 1952). On this basis the activity coefficient of the potassium ion is found to be 0·704. Using this value, one can calculate the internal activity coefficients which would correspond to plus and minus the standard error of the regression line in Fig. 9 by means of the equation

$$f_i = 0{\cdot}704\frac{[K]_0}{[K]_i}\exp(EF/RT).$$

The vertical lines in Fig. 10 join the two internal activity coefficients calculated in this way for each internal potassium concentration. The curve is the mean activity coefficient of solutions of KCl determined experimentally (from Conway, 1952) at the same potassium concentration. Since the total cation concentration inside is greater than that outside, it is impossible that all the internal anions are univalent, so that the ionic strength of the solution inside the fibre is always greater than the ionic strength of a solution of KCl of the same potassium concentration. The lower calculated activity coefficient for potassium at an internal concentration of 140 m-mole/kg f.w. corresponds to an internal ionic strength of 0·175. Boyle & Conway (1941) calculated an internal ionic strength of 0·16. The ionic strength of the experimental solutions containing chloride is 0·12 which gives an activity coefficient for potassium of 0·755 (Fig. 10). The two calculated activity coefficients for the potassium inside the fibre at normal concentration are 0·727 and 0·746, and these values give a ratio of internal to external activity coefficients of between 0·965 and 0·988. This is in satisfactory agreement with the usual assumption that the internal and external activity coefficients of potassium are equal when the muscle is in chloride solutions.

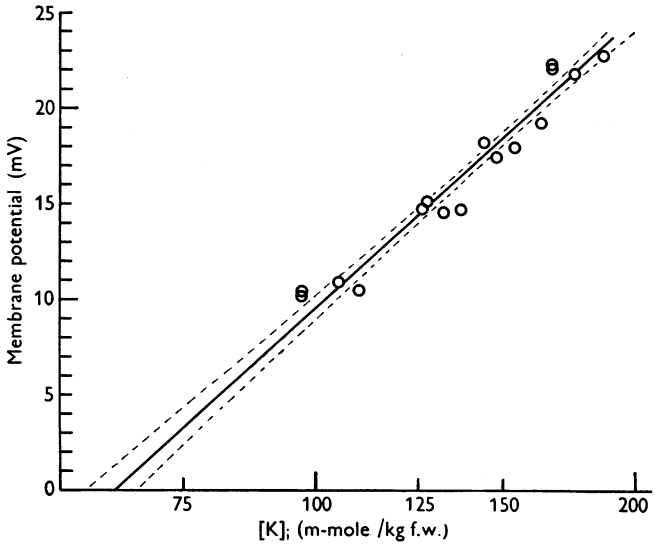

Fig. 9. The effect of the internal potassium concentration on the membrane potential in sulphate solutions containing 75 m-equiv/l. K^+ and 0, 50, 100 and 150 m-mole/l. sucrose. The data are from Table 6 excluding Expt. 49. The line is the regression line of log $[K]_i$ on membrane potential, and the dotted lines are ± one standard error. Abscissa: internal potassium concentration in m-mole/kg f.w. Ordinate: membrane potential in mV.

TABLE 6. The effect of external osmotic pressure on the internal potassium concentration and on the membrane potential in sulphate solutions containing 75 m-equiv/l. K

Expt.	Sucrose (mM)	Membrane potential (mV)	$[K]_i$ (m-mole/kg f.w.)	$[Na]_i$ (m-mole/kg f.w.)
51	0	10·4±0·2	110	6
61	0	10·2±0·3	97	9
	0	10·4±0·3	97	10
	0	10·9+0·3	105	11
49	50	16·2±0·3	133*	—
	50	15·6±0·3	142*	—
	50	15·1±0·2	139*	—
	50	15·5±0·3	126*	—
51	50	14·6±0·6	137	10
61	50	14·5±0·3	132	20
	50	14·6±0·5	126	20
	50	15·0±0·3	127	19
51	100	19·2±0·2	163	9
60	100	18·1±0·4	144	13
	100	17·3±0·3	148	13
	100	17·8±0·4	154	17
51	150	22·6±0·4	187	22
60	150	22·1±0·8	167	25
	150	22·0±0·8	167	27
	150	21·6±0·4	175	19

* $[K]_i$ estimated on paired muscle (see text): ± the standard error of the mean.

In a single preliminary experiment four pairs of muscles were soaked for 50 hr at 2–3° C in a solution containing 0·2 mM-KCl, 120 mM-NaCl with normal calcium and buffer concentrations. Desmedt (1953) showed that muscles under these conditions lose potassium and gain sodium. After the long soaking four of the muscles were prepared for estimation and the other four were transferred to sulphate solution containing 75 m-equiv/l. K^+, 45 m-equiv/l.

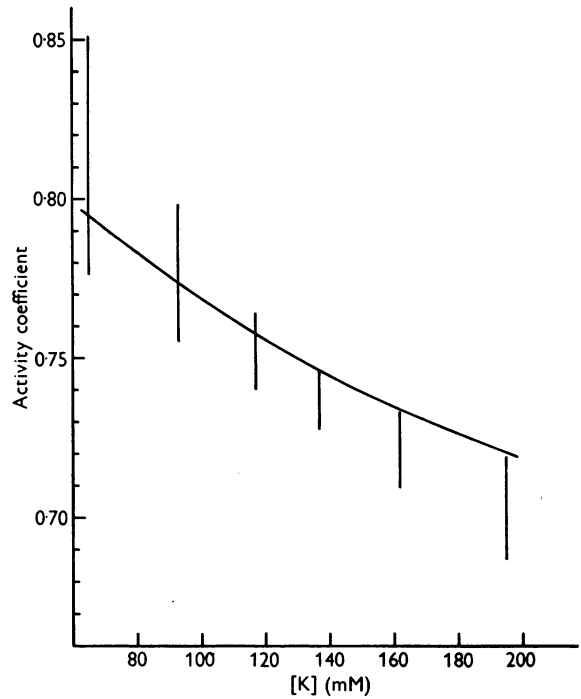

Fig. 10. The effect of concentration on the calculated internal activity coefficient of potassium. The vertical lines represent the range of internal potassium activity coefficient calculated by the method described in the text, and the continuous line is the mean activity coefficient for solutions of KCl. Abscissa: concentration in mM. Ordinate: activity coefficient.

Na^+, and 50 m-mole/l. of sucrose. Measurements of the membrane potential were begun within 5 min of the transfer to high potassium solution. The mean membrane potential of the muscle which was measured first was 25 mV, the inside of the fibre being positive to the outside. The scatter of the values for individual fibres was large, and a proportion of the fibres of each muscle were coagulated, so that the muscles could not be considered to be in very good condition, though all the muscles were excitable at the end of the soaking period. The muscles measured subsequently also showed reversed membrane potentials (inside positive), but after about 80 min all the muscles had membrane

potentials of about 10 mV, the inside of the fibre now being negative. The four muscles which were estimated at the end of the long soak in low potassium, had an average internal potassium concentration of 44 m-mole/kg f.w. and sodium concentration of 94 m-mole/kg f.w. This represents a much larger loss of potassium than Desmedt found, but this may possibly be due to the fact that in these experiments the muscles were soaked in large volumes of low potassium solutions and were exposed on both sides to the solution. These internal concentrations represent the average concentrations of all the fibres of the muscle, and it seems possible that the outside fibres may have had concentrations even lower. Uncertainties of this sort make quantitative interpretation of little value, but this experiment certainly indicates that when the internal concentration of potassium is made less than the outside concentration by replacing internal potassium by sodium the membrane potential reverses sign. The falling off of this reversed membrane potential and its replacement by a potential of the normal sign (negative inside) could be explained if potassium entered and sodium left the fibres under these conditions. The greater part of this movement could have been entirely passive, because when the soaked muscles were put into the high potassium–low sodium sulphate solution, the normal concentration gradients of both potassium and sodium were reversed.

At physiological external potassium concentration, 2·5 m-equiv/l., the effect of changing the internal potassium was investigated in the following manner. The resting potential of each of a pair of sartorii from the same frog was measured in normal Ringer's solution. The mean values from paired muscles did not differ by more than 1 mV. One of each pair was then prepared for estimation, and the other was transferred to normal Ringer's solution to which had been added 100 m-mole/l. of sucrose (Table 7). The resting potential was measured after half an hour, and the muscle prepared for estimation after $1\frac{1}{2}$ hr to allow for completion of water movement throughout the muscle. An increase of internal potassium concentration of $40{\cdot}5 \pm 1{\cdot}3$ m-mole/kg f.w. raises the resting potential by $4{\cdot}4 \pm 0{\cdot}3$ mV. This corresponds to a $39{\cdot}4 \pm 3{\cdot}4$ mV change for a tenfold change in $[K]_i$ (S.E. of means).

Table 8 gives the results of lowering the internal potassium concentration. In order to keep the external sodium concentration the same, the two comparison solutions contained, besides buffer and $CaCl_2$, in the one case 90 mM-NaCl and 60 mM-sucrose, and in the other 90 mM-NaCl. Both solutions contained 2·5 mM-KCl. The sucrose-containing solution was slightly hypertonic to normal Ringer's solution, as NaCl was replaced by sucrose on a stoichiometric basis, and this is reflected in the somewhat higher average value for $[K]_i$. The resting potential was not increased, but this is probably due to the fact that both sodium and chloride concentrations were lower than in normal Ringer's solution. The same experimental procedure was used as in the experiments of

Table 6. A decrease in the internal potassium concentration of $23{\cdot}2 \pm 3{\cdot}3$ m-mole/kg f.w. reduces the membrane potential by $2{\cdot}6 \pm 0{\cdot}34$ mV. This corresponds to a $35{\cdot}9 \pm 6{\cdot}9$ mV change for a tenfold change in $[K]_i$, (S.E. of means).

TABLE 7. The effect of raising the osmotic pressure on the internal potassium concentration and the resting potential in normal Ringer's solution (2·5 mM-KCl)

Expt.	RP in Ringer's solution (mV)	$[K]_i$ (m-mole/kg f.w.)	$[Na]_i$ (m-mole/kg f.w.)	RP in Ringer's solution +100 mM-sucrose (mV)	$[K]_i$ (m-mole/kg f.w.)	$[Na]_i$ (m-mole/kg f.w.)
52	92·1 ± 0·4	143	24	—	—	—
	91·3 ± 0·4	—	—	96·6 ± 0·5	183	35
	90·8 ± 0·3	137	22	—	—	—
	90·9 ± 0·4	—	—	95·8 ± 0·3	179	36
62	92·5 ± 0·5	131	24	—	—	—
	92·7 ± 0·6	—	—	96·3 ± 0·3	174	32
	93·1 ± 0·4	140	18	—	—	—
	92·5 ± 0·6	—	—	97·1 ± 0·3	177	32
	Av. 92·0	138	22	96·45	178	34

Brackets indicate paired muscles: ± the standard error of the mean.

TABLE 8. The effect of lowering the osmotic pressure on the internal potassium concentration and the membrane potential in solutions containing 90 m-mole/l. NaCl and 2·5 m-mole/l. KCl

Expt.	Membrane potential in 2·5 mM-KCl 90 mM-NaCl 60 mM-sucrose (mV)	$[K]_i$ (m-mole/kg f.w.)	$[Na]_i$ (m-mole/kg f.w.)	Membrane potential in 2·5 mM-KCl 90 mM-NaCl (mV)	$[K]_i$ (m-mole/kg. f.w.)	$[Na]_i$ (m-mole/kg. f.w.)
67	93·1 ± 0·5	149	19	—	—	—
	93·6 ± 0·8	—	—	89·8 ± 0·5	120	15
	90·4 ± 0·3	148	21	—	—	—
	90·5 ± 0·3	—	—	88·2 ± 0·5	120	12
69	90·2 ± 0·3	137	14	—	—	—
	89·3 ± 0·3	—	—	88·0 ± 0·5	116	5
	90·6 ± 0·3	142	18	—	—	—
	90·4 ± 0·4	—	—	87·8 ± 0·6	127	9
	Av. 91·0	144	18	88·45	121	10

Brackets indicate paired muscles: ± the standard error of the mean.

DISCUSSION

The main conclusion of this paper is that the membrane potential depends upon the internal and external concentrations of potassium, both at high external potassium concentration, and at the physiological concentration. Falk & Gerard (1954) found that the membrane potential of the sartorius muscle in Ringer's solution was not altered when 3 M solutions of KCl and NaCl were injected through a microelectrode. The discrepancy between their results and those of the present paper are unexplained, and more experiments are needed to resolve it.

At high external potassium concentrations the membrane of the sartorius muscle appears to obey the Nernst equation for a potassium electrode,

$$E = \frac{RT}{F} \ln \frac{f_i [K]_i}{f_0 [K]_0},$$

at least to a first approximation. This must mean that at high values of $[K]_0$, either the permeability of the membrane to potassium is higher than to other ions, or that any ion with a high permeability is distributed passively in accordance with the membrane potential within a very short time. There is unfortunately very little evidence to show which of these possibilities is the case. Levi & Ussing (1948) showed that radioactive chloride exchanges with inactive chloride with a half time of about 10 min. This exchange may have been considerably slowed by the rate of diffusion in the extracellular space (Harris & Burn, 1949; Keynes, 1954), so that the true half time may have been considerably shorter. Since the membrane potential measurements were made on the outside fibres after 10–15 min, it seems possible that the chloride redistribution might be almost complete in these fibres. Boyle & Conway (1941) showed that for solutions in which KCl replaces NaCl the volume changes of the whole muscle after 2 hr at room temperature (provided that the increase in volume was not greater than about 50%) agreed well with the volume changes predicted by their theory, an essential assumption of which was that

$$\frac{[K]_i}{[K]_0} = \frac{[Cl]_0}{[Cl]_i}.$$

When solid KCl was added to Ringer's solution, this condition was obeyed over a large range of KCl concentration (12–300 mM-KCl). The membrane potential in sulphate solutions does not differ from that in chloride solutions of the same potassium concentration, so that if the activity of the potassium ion in the two types of solution is taken into account, the contribution of chloride to the membrane potential cannot be greater than 2 mV

$$RT/F \ln (0{\cdot}755/0{\cdot}704).$$

Either the permeability of potassium is much greater than that of chloride, or the chloride ions redistribute themselves until the ratio of internal to external concentrations is very nearly equal to exp $(-EF/RT)$. There is no information about the intracellular concentration of chloride in solutions where the external anion is almost all sulphate.

The slope of the regression line of membrane potential on log $[K]_0$ in sulphate solutions corresponds to a 52·3 mV change for a tenfold change in $[K]_0$. A possible explanation for the difference between this value and the theoretical 58 mV is that the membrane permeability to sodium is about a thirtieth of the permeability to potassium (assuming that the chloride ion is distributed passively according to the potential). In the physiological range of external

potassium concentrations, making the same assumption about the distribution of chloride, the ratio of sodium to potassium permeability would have to be about 1:100. At first sight it seems odd that the selectivity of the membrane should fall with increasing depolarization, since the potassium conductance is thought to undergo a maintained increase and the sodium conductance a transient increase when the fibre is depolarized (Hodgkin & Huxley, 1952). However, if the inactivation of the increased sodium conductance were not complete a decreased selectivity might result.

There is little evidence to indicate how the permeabilities to potassium, sodium, or chloride vary with increasing external potassium concentration. At high external potassium concentrations the constant field equation for the membrane potential (Goldman, 1943; Hodgkin & Katz, 1949)

$$E = \frac{RT}{F} \ln \frac{P_K[K]_i + P_{Na}[Na]_i + P_{Cl}[Cl]_0}{P_K[K]_0 + P_{Na}[Na]_0 + P_{Cl}[Cl]_i}$$

is relatively insensitive to changes in P_{Na} so that it is difficult to decide from membrane potential measurements alone how P_{Na} or P_{Cl} vary in relation to P_K. Hodgkin & Katz (1949) found that a larger value of P_K was needed to fit the data of Curtis & Cole (1942) on the squid axon membrane potential in varying potassium concentrations, when the external potassium concentration was high. In *Sepia* axons, poisoned with DNP to make the fluxes of ions passive, Hodgkin & Keynes have shown that the potassium conductance, g_K, rises about 25-fold for a 20-fold increase in the external potassium concentration (calculated from Hodgkin & Keynes, 1955*b*, Table 8). The potassium conductance is defined as

$$g_K = I_K/(E - E_K),$$

where I_K is the membrane current transported by K^+ and is equal to F (influx − efflux), and E and E_K are the membrane potential and the potassium equilibrium potential respectively. On the assumptions of the constant field theory, g_K would be expected to increase with increasing $[K]_0$ even if P_K were constant, but it is difficult to say whether g_K increased by more than could be accounted for by this theory assuming a constant P_K. Harris & Burn (1949) showed that both the influx and efflux of potassium in the sartorius muscle increase when the external concentration of potassium is raised. However, since the influx at least included an active component and both fluxes are subject to a somewhat uncertain correction for diffusion, it is difficult to draw any conclusion about the rate of rise of g_K.

Jenerick (1953) concluded that the total membrane conductance, G_M, of the sartorius muscle did increase more rapidly than could be explained by the constant field equations, assuming a constant value for P_K, when $[K]_0$ was

raised. The constant field assumptions lead to the following expression for G_M (Hodgkin & Katz, 1949, equation 6·0):

$$G_M = \frac{F^3}{(RT)^2} EP \frac{wy}{y-w},$$

where

$$y = [K]_i + \frac{P_{Na}}{P_K}[Na]_i + \frac{P_{Cl}}{P_K}[Cl]_0,$$

$$w = [K]_0 + \frac{P_{Na}}{P_K}[Na]_0 + \frac{P_{Cl}}{P_K}[Cl]_i,$$

$$E = \frac{RT}{F} \ln \frac{y}{w}.$$

Jenerick used the above equation in the form

$$\exp\left(-\frac{EF}{RT}\right) - 1 = \frac{F^3}{(RT)^2} EP_K \frac{y}{G_M}.$$

G_M was measured by inserting two microelectrodes into the same fibre and finding the extrapolar spread of a square wave of current passed through one of them. P_{Na}/P_K and P_{Cl}/P_K were assumed to be constant, and they were found by fitting the constant field equation for the membrane potential to his data for E against log $[K]_0$. He then plotted exp $(-EF/RT) - 1$ against y/G_M and found a straight-line relation. From this he concluded that EP_K was constant and therefore that P_K must have increased as E fell. However, it appears that his values for the membrane potential may have been subject to junction potential errors (see p. 640), and he assumed a constant value of 125 mM/kg f.w. for $[K]_i$. But because he added solid KCl to his Ringer's solution, $[K]_i$ must have increased progressively, and this throws doubt not only on his values for P_{Na}/P_K and P_{Cl}/P_K, but also the value of y at the various external potassium concentrations. The latter must have increased more rapidly than he calculated, so that EP_K must have fallen as E decreased. The uncertainty of the membrane potential measurements and the degree of potassium accumulation does not allow any conclusion about the constancy of P_K to be drawn from Jenerick's data.

In the physiological range of external potassium concentration the membrane potential is below the potassium equilibrium potential. In normal Ringer's solution (2·5 mM-KCl) this difference is about 10 mV. If the fibre is in a steady state this must mean that either a substantial fraction of the intracellular potassium is bound, or that there is an active uptake of potassium by some metabolic process. There is evidence that part of the potassium influx is directly linked to the active sodium efflux both in *Sepia* (Hodgkin & Keynes, 1955*a*) and in frog muscle (Keynes, 1954). At low values of $[K]_0$ the variation of the membrane potential with log $[K]_0$ is less than the theoretical 58 mV for

a tenfold change of $[K]_0$. This is usually explained by the fact that the membrane is slightly permeable to sodium and chloride ions. The experiments in Table 4 suggest that chloride may be an important factor in the membrane potential at low external potassium concentrations, but it is impossible to fit the constant field equation to the data. Indeed it is impossible to fit this equation to the values of the membrane potential in the range of external potassium concentration 0·5–5 m-equiv/l. (Fig. 6). Taking the value for the membrane potential in 0·5 m-equiv/l. as 120 mV and in 2·5 m-equiv/l. as 92 mV, the values for the fraction

$$\frac{P_K[K]_i + P_{Na}[Na]_i + P_{Cl}[Cl]_0}{P_K[K]_0 + P_{Na}[Na]_0 + P_{Cl}[Cl]_i}$$

must be 117·5 and 38·6 respectively. Assuming the denominator remains constant and $[Cl]_i$ is constant, the value of $P_{Na}[Na]_0/P_K + P_{Cl}[Cl]_i/P_K^-$ would be 0·48. From this the denominator can be calculated and is found to be 115. But $[K]_i$ is 139 m-mole/kg f.w. so that the denominator can hardly be less than this figure. Nevertheless, even at low external potassium concentration, the membrane potential is not independent of the internal concentrations when these are changed by changing the osmotic pressure of the surrounding fluid. The inability of the constant field equation to describe the membrane potential adequately need not cause surprise or concern as it is based on a very simplified idea of the membrane. However, the discrepancy emphasizes the need to find out much more about the internal concentrations of potassium, sodium, chloride, and possibly hydrogen ion, and how they are maintained and controlled under various conditions.

SUMMARY

1. Conventional glass microelectrodes filled with 3 M-KCl are not free from junction potentials, and unless the electrodes are carefully selected, can introduce large errors into membrane potential measurements.

2. The observations on the tip junction potential are consistent with the view that this potential is produced by some substance blocking the tip of the electrode which reduces the mobility of the chloride ion and exaggerates the difference of mobility of the sodium and potassium ions.

3. The average resting potential of frog sartorius muscle in Ringer's solution containing 2·5 mM-KCl is 92·2 mV. Pairs of muscles from the same frog appear to have resting potentials more nearly equal than muscles from different frogs. The normal internal potassium concentration is 139 ± 2 mM/kg fibre water ($\pm$ S.E. of mean).

4. At external potassium concentrations greater than 25 m-equiv/l. the membrane potential depends primarily upon the ratio of the internal and external activities of potassium.

5. At physiological external concentrations of potassium the membrane potential is not independent of the internal concentration when this is altered by changing the external osmotic pressure.

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