

RUBIDIUM AND CESIUM FLUXES IN MUSCLE AS RELATED TO THE MEMBRANE POTENTIAL*

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ABSTRACT

The reduction of membrane potential in frog sartorius muscle produced by rubidium and cesium ions has been studied over a wide concentration range and compared with depolarization occasioned by potassium ions. The constant field theory of passive flux has been used to predict the potential changes observed. The potential data suggest certain permeability coefficient ratios and these are compared with ratios obtained from flux data using radioactive tracers. The agreement of the flux with the potential data is good if account is taken of the inhibition of potassium flux which occurs in the presence of rubidium and cesium ions. A high temperature dependence has been observed for cesium influx ($Q_{10} = 2.5$) which is correlated with the observation that cesium ions depolarize very little at low temperatures. The observations suggest that cesium ions behave more like sodium ions at low temperatures and more like potassium ions at room temperature with respect to their effect on the muscle cell resting potential. The constant field theory of passive ion flux appears to be in general agreement with the experimental results observed if account is taken of the dependence of permeability coefficients on the concentrations of ions used and of possible interactions between the permeabilities of ions.

INTRODUCTION

The effects of rubidium and cesium ions on excitable tissues resemble those of potassium ions, but a different order of effectiveness is observed for invertebrate and vertebrate material. The order for invertebrates seems to be $Rb > K > Cs$, that for vertebrates being $K > Rb > Cs$. Wilbrandt (1), for example, found rubidium ions more effective than potassium ions in depolarizing *Maia* nerve. Hodgkin (2) found these ions to increase the conductance of crayfish axons in the order given for invertebrates above. The respective relative concentrations for equal elevation of conductance were $Rb = 0.8$, $K = 1$, and $Cs = 2.2$. Guttman *et al.* (3) found rubidium to be more effective than potassium in inducing contraction upon rapid cooling in *Mytilus* muscle. As an example of the order observed in vertebrate material, Feng and Liu (4)

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found rubidium ions to be less effective than potassium ions in depolarizing frog nerve, a logarithmic relation being observed between depolarization and concentration. Sandow and Mandel (5) found the same to be true for frog muscle. The latter workers related their observations to mobilities within the membrane by using an estimate of the relative permeabilities of the ions from the results of Conway and Moore (6). A diffusion potential was assumed and fair agreement was demonstrated. The permeabilities observed in the work of Conway and Moore, however, were at different levels of depolarization and hence do not represent true mobilities.

The present investigation aims at a more complete study of the depolarization produced by rubidium and cesium ions in muscle fibers and an analysis in terms of ion fluxes, with due regard for the membrane potential, in order to obtain estimates of the mobility. To relate measured fluxes to ion mobilities requires a knowledge of the driving forces involved since ions are moving in a chemical as well as in an electrical gradient. The constant field approximation of Goldman (7) is used throughout this work. This approximate solution of the differential flux equation yields the following expression for the net flux of an ion along an electrochemical gradient:

$$\bar{m} = P \frac{EF}{RT} \frac{(C_o - e^{-EF/RT} C_i)}{1 - e^{-EF/RT}} \quad (1)$$

in which P , the permeability coefficient, is defined by the equation:

$$P = \frac{RT}{Fd} \beta U \quad (2)$$

The symbols R , T , and F have their usual meaning; d is the membrane thickness, U is the mobility inside the membrane, and β is a partition coefficient setting boundary conditions on the concentrations just inside the membrane on either side. The symbol E is the membrane potential, a positive value indicating negativity in the cell interior. The ion concentrations outside and inside the cell are given respectively by C_o and C_i . The following equation may be taken as the definition of β in this work.

$$C_m = \beta C_o \quad (3)$$

Equation (1) may be separated into two parts, an influx and an efflux such that $\bar{m} = m_i - m_o$ when

$$m_i = P \frac{F}{RT} \frac{EC_o}{(1 - e^{-EF/RT})} \quad (4)$$

and

$$m_o = P \frac{F}{RT} \frac{E(e^{-EF/RT})C_i}{(1 - e^{-EF/RT})} \quad (5)$$

For abbreviation, the terms involving E and its exponential in these equations will be designated f_i and f_o respectively so that $m_i = P(F/RT)f_iC_o$ and $m_o = P(F/RT)f_oC_i$. It is evident that P can be determined if the flux, potential, and concentration are known.

Methods

All potential measurements were made with 3 M KCl-filled microelectrodes, having low tip potentials and resistances between 5 and 10 Megohms, using a cathode fol-

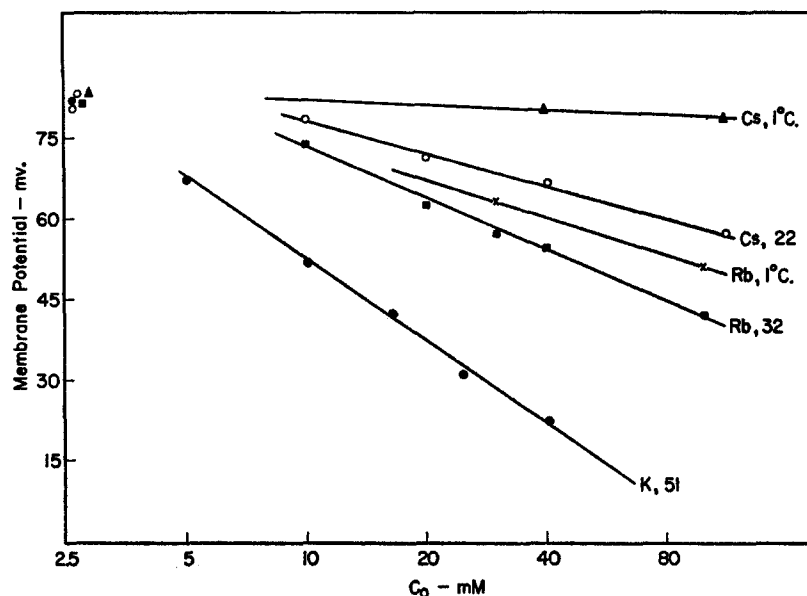


FIG. 1. The membrane potential measured at various external concentrations of potassium, rubidium, and cesium ions is plotted against the logarithm of the external concentration. The numbers appearing at the ends of the lines denote slopes in millivolts per tenfold change in concentration at 22°C. Also included are curves for rubidium and cesium determined at 1°C.

lower input and a differential d. c. amplifier of an oscilloscope. Cooling was effected by placing the muscles in a chamber surrounded by a water jacket through which a cold water-alcohol mixture circulated. Frog sartorii muscles were carefully dissected so as to leave one side as free as possible from connective tissue. The muscles were equilibrated for 15 minutes in the experimental solution, to allow for diffusion in the extracellular space prior to microelectrode penetration. Only potentials which developed instantaneously with puncture of the muscle cell were used in the data. It was found that about fifteen such penetrations could be obtained per muscle, each in a different surface fiber. In general four muscles from two animals were used at each concentration; this required the measurement of the potentials of from fifty to sixty cells. If agreement was satisfactory, no further measurement at a given con-

centration was made. In cases involving cooling, one of the pair of muscles was studied at room temperature. Two muscles from two animals were studied in the cold and compared with the others of the pairs at room temperature. Flux measurements were made by obtaining the uptake of radioactive ions *versus* time from solutions of known specific activity. The isotopes used were Rb^{86} , Cs^{134} , and K^{42} . Counting was performed with a scintillation well-type counter and a spectrometer.

Muscles were placed in the radioactive solution for 15 minutes and removed to obtain a zero time count. The extracellular space was found to be within 10 per cent of equilibration at the end of this period. Muscles were carefully blotted on clean filter paper and washed in a non-radioactive solution, having the same composition

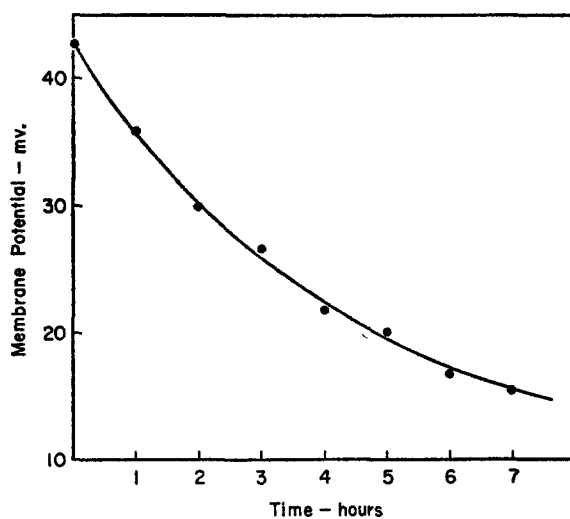


FIG. 2. The membrane potential measured in a solution containing rubidium ions at a concentration of 100 mM is plotted against the time in contact with the solution. Muscles were allowed to remain in contact with the solution for 15 minutes prior to measurement. Four microelectrode penetrations on two muscles were used to establish each point.

as the experimental solution, for 1 minute prior to counting. The tracer uptake was then followed by similarly washing and counting the same muscle every 30 minutes or every hour. Since the background count was of the order of 1 count per minute at the window width used, the muscles could be counted for as little as 1 minute to obtain each uptake point. Muscles used for the measurement of potential were never used in a flux experiment. Potential measurements could be made, however, on muscles on which a flux study had been completed. Temperatures were measured with a 200 K ohm thermistor making contact with the bathing solution and connected in a bridge circuit. A standard Ringer solution having the following composition was used: 111 mM NaCl, 2 mM KCl, 1.8 mM CaCl_2 , 2 mM NaHCO_3 , 0.1 mM NaH_2PO_4 , 10 mM glucose. Rubidium, cesium, and potassium ion concentration changes were made by substituting them for sodium ions.

In calculations requiring a knowledge of the intracellular potassium concentration, the figure used was 130 mM. The extracellular space was taken as 15 per cent of the muscle weight. The intrafibrillar water was taken as 70 per cent of the muscle weight corrected for extracellular space.

RESULTS

Potential Measurements.—The results of the study of the depolarization produced by potassium, rubidium, and cesium ions are summarized in Fig. 1. When the cells were appreciably depolarized by higher concentrations of rubidium and cesium, the measured potentials were found to be time-dependent, the cells becoming increasingly depolarized with time in contact with the depolarizing solutions. The time dependence with 100 mM rubidium is seen in Fig. 2. That this decline in potential is correlated with the entry of rubidium into the cells will be shown subsequently.

The influx of the foreign cation gives rise to a diffusion potential initially since the original condition of zero net current has been altered by inward current flow. The sign of the diffusion potential is such that the membrane becomes depolarized. Depolarization, however, brings the membrane potential further from the equilibrium potential for potassium ions and an increased efflux of K^+ ensues. The resulting outward current carried by K^+ restores the membrane to the initial condition of zero net current. The membrane potential may now be expected to remain stable until significant changes in intracellular ion concentrations occur. This potential may be predicted quantitatively by equating the influx of the foreign cation to the outflux of potassium ions. Since the cells are highly permeable to chloride ions, Cl^- may be expected to come rapidly to electrochemical equilibrium at any new potential.

From equations (4) and (5), setting $(m_i)_{Rb} = (m_o)_K$ the following relation for the potential is obtained:¹

$$e^{zF/RT} = P_K[K^+]_i/P_{Rb}[Rb^+]_o \quad (6)$$

¹ It has been demonstrated (Boyle and Conway (8)) that muscle cells, placed in solutions in which part of the sodium ions have been replaced by potassium ions, swell due to the entry of KCl. The KCl entry and swelling were always such that the Donnan ratios for potassium and chloride ions were obeyed. Adrian (9) points out that, since the Nernst equation is obeyed at high external potassium concentrations in sartorius muscle, the permeability of the membrane to potassium must either be much higher than to other ions, or any ion having a high permeability must become passively distributed in accordance with the membrane potential within a very short time. In the present experiments, in which the external sodium has been largely replaced in some instances with rubidium and cesium, the muscles were found to swell up to 20 per cent in 100 mM solutions of the foreign cations. This swelling must be due to the entry of RbCl or CsCl. Since Levi and Ussing (10) have demonstrated that radioactive chloride exchanges with inactive chloride with a half-time of about 10 minutes, it is possible that chloride ions have reached an

If E is written as a function of $[\text{Rb}^+]_o$, the concentration dependence of E can be seen more easily:

$$E = (RT/F) \ln [\text{K}^+]_i/[\text{Rb}^+]_o + (RT/F) \ln P_{\text{K}}/P_{\text{Rb}}$$

or at 20°C.,

$$E = 58 \log [\text{K}^+]_i/[\text{Rb}^+]_o + 58 \log P_{\text{K}}/P_{\text{Rb}} \quad (7)$$

This is seen to be the equation for the potassium electrode with $[\text{Rb}^+]_o$ replacing $[\text{K}^+]_o$ and with the additional term involving the permeability coefficients. E plotted *versus* $\log [\text{Rb}^+]_o$ should, theoretically, give a straight line with slope -58 mv. per tenfold concentration change. This line should be displaced by $58 \log P_{\text{K}}/P_{\text{Rb}}$ mv. above the line for the potassium electrode. It is seen that the slope of -58 mv. will not be demanded by equation (7) if the ratio $P_{\text{K}}/P_{\text{Rb}}$ is not constant over the concentration range employed. In particular, if the ratio $P_{\text{K}}/P_{\text{Rb}}$ becomes smaller at lower rubidium concentrations, the slope expected will be less than -58 mv. The same considerations apply to cesium.

The slopes shown in Fig. 1 suggest that the permeability ratios are, in fact, not constant over a wide concentration range. How this ratio might change with concentration will be considered in the section on fluxes. The potential obtained at a given concentration can be used to calculate a permeability ratio using equation (6). For 100 mM Rb and 113 mM Cs, the calculated ratios are: $P_{\text{K}}/P_{\text{Rb}}/P_{\text{Cs}} = 9/2.1/1$. The foregoing considerations may now be tested by measuring the fluxes of the ions concerned and calculating permeability ratios from flux data. These can then be compared with those calculated from potential data.

equilibrium distribution, at the potential set by the balance of Rb^+ or Cs^+ influx and K^+ efflux, by the time the potentials were measured. Prior to this time, the membrane potential would vary with the intracellular concentration of chloride ions and would be given by the equation:

$$E = \frac{RT}{F} \ln \frac{P_{\text{K}}[\text{K}^+]_i + P_{\text{Cl}}[\text{Cl}^-]_o}{P_{\text{Rb}}[\text{Rb}^+]_o + P_{\text{Cl}}[\text{Cl}^-]_i}$$

The contribution to the potential made by sodium ions is negligible since P_{Na} is relatively small and the external sodium is significantly replaced by the foreign cations. When chloride ions reach their equilibrium distribution, the terms involving P_{Cl} and Cl^- may be dropped and the above equation reduced to equation (6).

As time progresses and significant K^+ — Rb^+ exchange ensues, the potential will be given by:

$$E = \frac{RT}{F} \ln \frac{P_{\text{K}}[\text{K}^+]_i + P_{\text{Rb}}[\text{Rb}^+]_i}{P_{\text{Rb}}[\text{Rb}^+]_o}$$

The initial $[\text{Rb}^+]_i$ due to entry of RbCl is not large enough to introduce serious errors, however.

Flux Measurements.—The data for uptake of rubidium and cesium ions by the muscle cells are summarized in Figs. 3 and 4. To relate the measured uptake to permeability coefficients requires relating the measured uptake to the fluxes appearing in equations (4) and (5). Permeability coefficients can then be calculated.

The occurrence of muscle swelling in 100 mM solutions of RbCl and CsCl has been mentioned.¹ The maximum swelling observed in 100 mM RbCl was about 20 per cent for the uptake times used. In CsCl, the swelling was of the

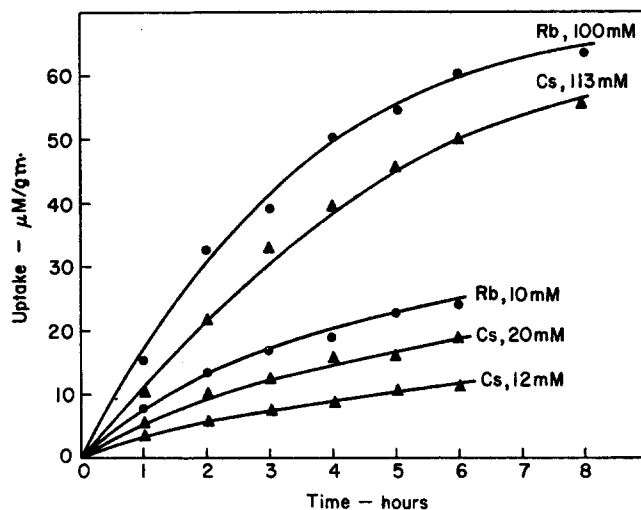


FIG. 3. The uptake of rubidium and cesium ions from radioactive solutions is plotted against the time in contact with the solution. The uptake is expressed as micromoles per hour per gram of muscle fiber (corrected for extracellular space). Each curve represents the averaged data from three experiments on three muscles. The external concentration used is shown on the curves.

order of one-half of that noted in RbCl over the same time interval. The bearing that the swelling might have on the results reported must be considered. If uptake is reported per final weight, the initial flux will be proportionately too low. If the initial weight is used, the final flux will be too high. Since one is really interested in estimating the flux across unit area of cells, it would appear better to use the correct muscle weight at each point of the uptake curve. The uptake curves in Figs. 3 and 4 were obtained in this way. The initial influx is of primary importance in the present treatment. Since swelling in 100 mM RbCl was only about 4 per cent during the 1st hour, the difference between expressing uptake per initial weight and per instantaneous weight is small and is within the experimental error of the flux determinations, during the 1st hour of uptake.

Another effect of swelling must be considered. Boyle and Conway (8) showed that in high KCl solutions, in which the normal tonicity was preserved by lowering the NaCl concentration proportionately, the swelling was accompanied by a net uptake of KCl. The swelling was always approximately in proportion to the additional KCl taken up so that the internal K^+ concentration remained essentially constant. It seems entirely likely that a net uptake of RbCl occurs in the present experiments in addition to the Rb^+ which exchanges for K^+ . The rubidium which enters the muscle as the ion pair $Rb^+ - Cl^-$ does not contribute to the initial depolarization but only to the later depolarization which is

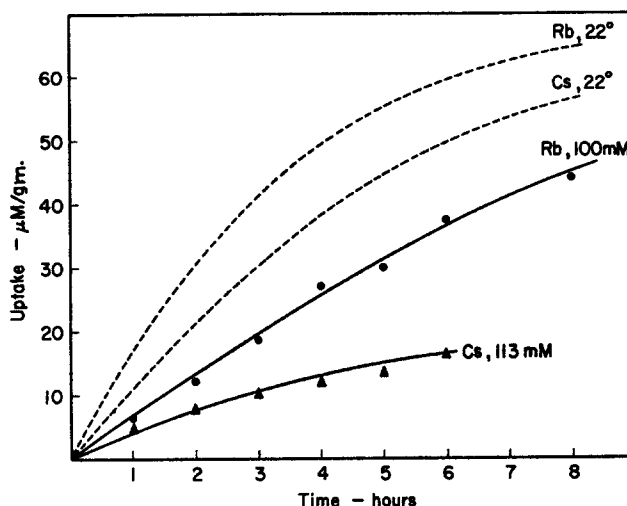


FIG. 4. The curves shown illustrate the effects of temperature on the uptake of rubidium and cesium ions by frog sartorius muscle. The upper two curves, shown as dotted lines, are curves obtained at 22°C. and are taken from Fig. 3. The lower curves were obtained at 1°C. using the same ion concentrations as were used to obtain the upper curves.

determined mainly by the extent to which the muscle membrane potential resembles a rubidium electrode. Hence, to be precise, the net uptake of RbCl which accompanies the swelling must be separated from the fraction exchanging for K^+ . The two fractions were estimated by means of an experiment in which a weight gain curve was obtained in 100 mM RbCl together with a simultaneous tracer Rb^+ uptake curve. At the termination of the experiment the muscles were analyzed for Rb^+ and for K^+ . It was then assumed that RbCl entry is similar to KCl entry and that the final $Rb^+ + K^+$ concentration is close to the initial K^+ concentration. This supposition was supported by the fact that final $[Rb^+] + [K^+]$ was found to agree with many normal values of the K^+ concentration of muscles. This estimate of the initial K^+ concentration was

used together with the weight gained during the 1st hour to calculate the amount of RbCl which entered the muscle in this period over and above the Rb^+ which exchanged for K^+ . The RbCl fraction determined in this way was found to represent about 20 per cent of the total Rb^+ entry which was obtained from the tracer uptake during the 1st hour. It thus seems likely that only 80 per cent of the rubidium flux during the 1st hour represents exchange with K ions. The effect of this on the permeability ratios deduced from potential measurements can be seen by setting $0.8 (m_i)_{Rb} = (m_o)_K$, and solving for P_K/P_{Rb} as in equation (6).

The net flux, \bar{m} , of ions entering the muscle cells is measured as the rate at which the cells become radioactive when placed in solutions of the penetrating ion of known specific activity. The amount of the ion taken into the cells after a time, t ; e.g., the uptake, is simply the time integral of the net flux over the interval. Quantitatively, $S = A \int_0^t \bar{m} dt$ in which S is the uptake in micromoles, A the total area of the muscle cells across which permeation occurs, and \bar{m} the net flux in micromoles/cm.² sec. The uptake expressed as a concentration inside the cells, C_i , is obtained by dividing the above integral by the intracellular water space available to the ions in question. For rubidium and cesium, this space is found to approximate that available to potassium ions to within experimental error. Also, Lubin and Schneider (11) have demonstrated complete exchangeability of intracellular K^+ for rubidium and cesium ions. To predict the uptake of an ion quantitatively requires first an expression for the net flux. Since the constant field approximation is used throughout this paper, the net flux is given by equation (1), which can now be rewritten as:

$$\bar{m} = (F/RT)PC_o f_i - (F/RT)PC_o f_o = (F/RT)PC_o f_i - (F/RT)P f_o (1/V_i)A \int_0^t \bar{m} dt,$$

in which V_i is the intracellular water space. Next we require the net flux as a function of time since the time integral of \bar{m} is sought. For the case in which E is constant throughout the uptake interval, $\bar{m}(t)$ can easily be obtained by differentiating the equation above with respect to time, which simply removes the integral sign from the last term: $d\bar{m}/dt = -(F/RT)P f_o A (1/V_i) \bar{m}$. This can be easily integrated to give $\bar{m}(t)$.

$$\bar{m}(t) = m_i \exp \left\{ - \frac{A}{V_i} \frac{F}{RT} P f_o t \right\} \quad (8)$$

in which m_i is the initial net flux, or, simply the influx given by

$$m_i = (F/RT)P f_i C_o.$$

Equation (8) shows that the measured tracer flux can be expected to decline exponentially from an initial value which is the true influx. The uptake, given

by $S = A \int \bar{m} dt$, is now obtained by inserting equation (8) into this integral. Integration yields the following equations for the uptake:

$$S = V_s C_o e^{EF/RT} (1 - e^{-kt}) \quad (9)$$

$$C_i = C_o e^{EF/RT} (1 - e^{-kt}) \quad (10)$$

in which

$$k = (A/V_s)(F/RT)f_o P.$$

It is seen that the equilibrium distribution is approached by the factor

$$(1 - e^{-kt}).$$

The time constant, $1/k$, can be found from the uptake curve and relative P values thus obtained.

The uptake of rubidium and cesium at concentrations which do not depolarize appreciably followed approximately the theoretical curve. It was found, however, that no great error was introduced by estimating the influx directly from the uptake over an interval small compared to $1/k$; *i.e.*, over an interval in which the outflux of the foreign cation can be neglected. Relative permeabilities can then be obtained directly from equation (4). This analysis must be regarded as approximate only as, actually, potential changes do occur when significant amounts of rubidium or cesium enter the cells. When concentrations of these ions that depolarize appreciably are employed, the analysis fails completely as the measured potential then becomes definitely time dependent. The above integration then becomes exceedingly difficult and, in general, computer methods must be used. One approximation method which can be applied is that of graphical integration. The membrane potential time dependence in depolarizing concentrations of rubidium is known from experiment. Fig. 2 may be used to obtain the constant field factors f_i and f_o at different times during the uptake, and the inside concentrations may be estimated from the tracer uptake curves. Since C_o is known, m_i and m_o may be calculated as functions of time from equations (4) and (5). The net flux, \bar{m} , may then be obtained by subtraction. The fluxes are known only relatively as P has not yet been estimated. One can roughly estimate m_i initially, however, and obtain an intercept on the flux axis. The \bar{m} curve can then be integrated graphically. The application of an appropriate volume constant should then yield the uptake curve C_i . By successive adjustment for best fit, the original estimate of the flux can then be made more accurate. This method can be easily applied when the time constant of the uptake is of the order of 2 or 3 hours. The method is illustrated in Table I and Fig. 5. The time integral of \bar{m} so obtained is seen to give a good fit to the experimental uptake curve. Also, it can be seen that the net flux over an early time interval approximates the influx. The slight correction required can be obtained from the graph. Relative permeabilities can be obtained by applying equation (4).

The relative P values for rubidium and cesium uptake at three external concentrations are shown in Table II, along with the respective influxes. The non-constancy of P over a wide range of concentrations is apparent from the data and is to be expected if the permeability barrier to ion penetration becomes saturated at higher concentrations. Table III shows potassium influxes at various outside concentrations and levels of depolarization along with calculated permeability coefficients. The permeability coefficients calculated from flux data for potassium, rubidium, and cesium ions at various external concentrations are summarized in Fig. 6. The decline in permeability with increasing concentration is apparent for rubidium and cesium. The permea-

TABLE I
Data for Graphical Integration of Rubidium Flux in 100 mM Solution

Time	E^*	f_i	f_o	C_i^\ddagger	C_o	m_i^\S	$m_o^ $
hrs.	mv.			mM	mM	$\mu\text{m/gm. hr.}$	
0	43	52.6	9.6	0	100	16.0	0
1	36	47.4	11.4	24	100	14.4	0.83
2	30	43.1	13.1	47	100	13.1	1.88
3	27	41.1	14.1	64	100	12.5	2.75
4	22	37.8	15.8	78	100	11.5	3.74
5	20	36.5	16.6	86	100	11.1	4.34
6	17	34.7	17.8	94	100	10.5	5.09
7	15	33.5	18.5	100	100	10.2	5.62
8	12	31.7	19.7	108	100	9.6	6.48

* The potential is obtained from the curve in Fig. 2.

‡ Calculated from experimentally determined uptake using intracellular water calculated as indicated in Methods section.

§ Calculated from equation (4).

|| Calculated from equation (5).

bility coefficient for potassium is seen to be relatively constant over a wide range of concentration. The permeability ratios suggested from the potential measurements can now be compared with the ratios obtained by flux measurements. The comparison for 100 mM Rb and 113 mM Cs shows that the agreement is very poor, (Table V), measured P_K being too high and P_{Rb} too low. Using Rb and Cs permeabilities estimated at lower concentrations is seen to give better agreement, though insufficient for accepting the theoretical basis for the depolarizations.

The question of the constancy of the potassium permeability in the presence of rubidium and cesium arose next. Is it permissible to assume that potassium permeability remains unaffected by the presence of rubidium and cesium ions or to assume that both ions equally affect the potassium permeability? To

estimate P_K in the presence of Rb and Cs required the use of efflux data for potassium.² The P_K deduced from measurements of potassium efflux are shown

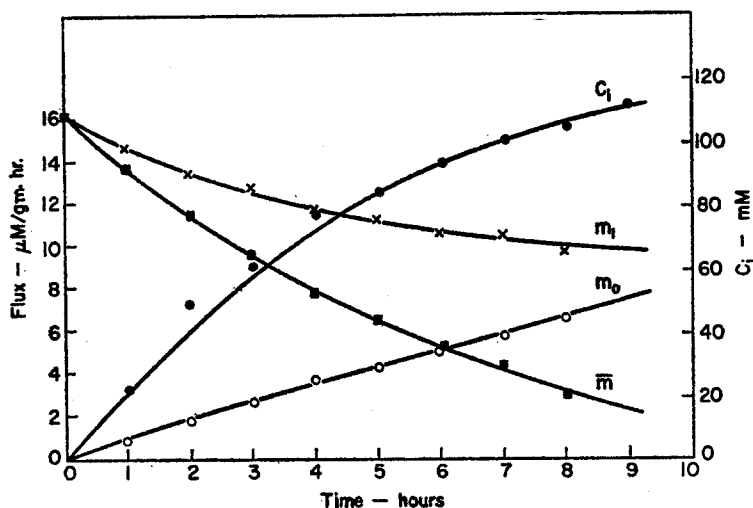


FIG. 5. These curves, together with Table I, illustrate the method used to obtain a graphical integration of the constant field flux equation (equation (1)) for the uptake of rubidium ions from a 100 mM solution. The calculated values of m_i and m_o tabulated in Table I are plotted against the time. The net flux, $m = m_i - m_o$. The rising smooth curve is the time integral of the net flux curve, obtained graphically, multiplied by the average weight in grams of the muscles used in the flux experiments (corrected for extracellular space) and divided by the average intrafibrillar water space in milliliters. This curve should represent the increase in internal concentration of rubidium ions, C_i , which takes place when muscles remain in the rubidium solution. The points represent the intracellular concentration of rubidium ions obtained from the experimental uptake curve. The theoretical smooth curve is seen to give a good fit to the experimentally determined points.

in Table IV. The permeability coefficients for potassium to be used in Rb and in Cs calculations are seen to be lower than the normal values and to be different for the two ions. Permeability ratios obtained in this way are seen from

² At concentrations of Rb^+ and Cs^+ that depolarize the membrane, there is a large, net efflux of potassium ions. It has been observed (Hodgkin and Keynes (13)) that potassium influx measurements made by the tracer technique are too low in such situations. In the present experiments, potassium permeabilities deduced from efflux measurements were always higher than those deduced from influx measurements when the membranes were significantly depolarized. Since potassium efflux is the relevant quantity in this work, and since the influx of an ion is difficult to estimate under conditions favoring a large net efflux of that ion, potassium permeability coefficients deduced from efflux measurements were used.

Table V to show good agreement with the ratios calculated from potential measurements.

TABLE II
Rubidium and Cesium Influxes vs. Concentration

	0.6 mM	2.5 mM	10 mM (Rb ⁺) 12 mM (Cs ⁺)	100 mM (Rb ⁺) 113 mM (Cs ⁺)
Rb ⁺ influx, $\mu\text{mole/gm. hr.}$	0.877	3.6	8.0	16.0
Relative P_{Rb}^*	167	166	101	30.4
Cs ⁺ influx, $\mu\text{mole/gm. hr.}$	0.229	—	4.3	10.9
Relative P_{Cs}	43.6	—	42.9	14.9

* All calculated permeability coefficients are given in comparable, relative units throughout this investigation. All contain the term $10 \times F/RT$ and, hence, must be multiplied by $0.1 \times RT/F$ if absolute units of cm./sec. are desired. The coefficient obtained must be corrected by factors which convert flux in micromoles/gm. hr. to flux expressed in moles/cm.² sec. and concentration in millimoles/liter to moles/cm.³ Since only ratios of permeability coefficients are considered in this work, relative P values are sufficient. As a calibration, the normal potassium permeability represented by a relative P_{K} of 320 corresponds to an absolute P_{K} of 42×10^{-8} cm./sec., using the factor 530 cm.³/gm. muscle fiber (Harris (12)).

TABLE III
Potassium Permeability vs. Outside Concentration

m_i	C_o	E	$f_i(E)$	$P \times 10F/RT$
$\mu\text{mole/gm. hr.}$	mM	mV.	constant field	
5.9*	2.0	91‡	93.5	316
7.3	2.5	90	92.6	316
11.3*	4.0	85	88.0	321
13.9	5.0	80	83.5	333
19.7*	8.0	70	74.7	329
22.0	10	60	66.1	333
30.0	15	50	58.0	345
39.0	20	45	54.1	360
64.1	40	32	44.5	360

* From data of Harris (12).

‡ All potentials used in this table were taken from Adrian (9) as the fluxes reported here were measured on English frogs.

The effects of temperature on the depolarization produced by rubidium and cesium ions (Fig. 1) suggest that the temperature coefficients for potassium, rubidium, and cesium permeabilities are different. Since rubidium and cesium ions depolarize less at low temperatures, equation (6) demands that P_{Rb} and P_{Cs} fall off more rapidly with decreasing temperature than does the potas-

sium permeability coefficient. This may be tested experimentally by determining the temperature coefficients of the rubidium and cesium permeabilities and comparing them with values available for the potassium permeability.

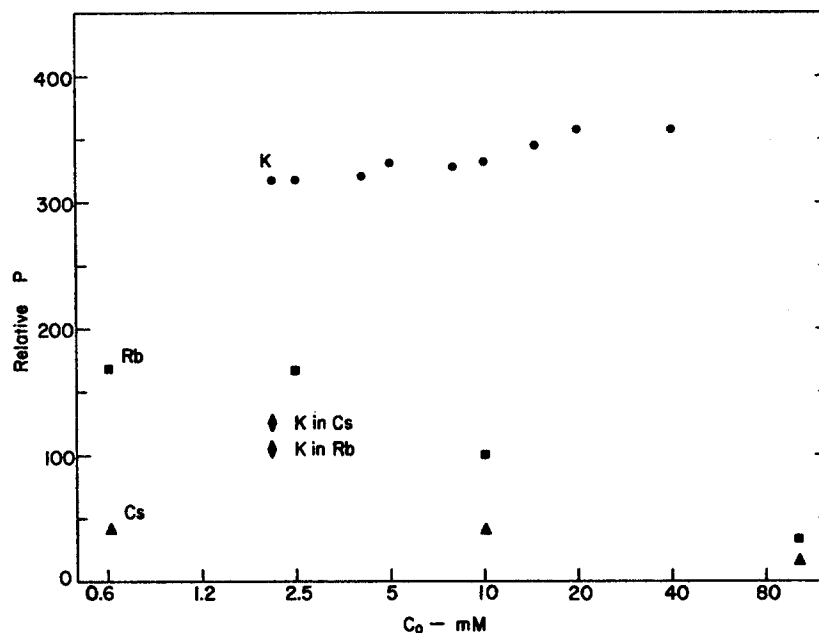


FIG. 6. The relative permeability coefficients calculated from flux data for potassium, rubidium, and cesium ions over a wide concentration range are shown plotted against the concentration of the ion in question. Also included are the permeability coefficients for potassium obtained in the presence of rubidium and cesium ions at concentrations of 100 mM and 113 mM, respectively.

TABLE IV
Inhibition of Potassium Efflux by Rubidium and Cesium

	2 mM K Ringer	2 mM K 100 mM Rb ⁺	2 mM K 113 mM Cs ⁺
K efflux, $\mu\text{mole/gm. hr.}$	13.4	12.7	10.2
Relative P_K	325	104	126

The fluxes of Rb and Cs observed at 1°C. (Fig. 4) may be used to calculate P_{Rb} and P_{Cs} at 1°C. The temperature coefficients calculated from flux data are found in Table VI where they are compared with those demanded by equation (6) for agreement with the potential data. Since the temperature dependence of the potassium flux was not measured in these experiments, a

Q_{10} of 1.7 is estimated for the potassium permeability from the data of Harris (14). This value is also used in the calculation of rubidium and cesium temperature coefficients from potential data employing equation (6). Again, agreement between potential data and flux data is seen to be adequate.

TABLE V
Summary of Permeability Ratios and Correlation with Potential Data

	P_K	P_{Rb}	P_{Cs}
Measured ratios in low concentration range using normal P_K	7.3	3.8	1
Potential measurements lead to ratios	8.7	2.1	1
Measured ratios in 100 mM concentration range using normal K permeability	22.2	2.0	1
Measured ratios in 100 mM concentration range using P_K determined in the presence of Rb and Cs	3.4	1	
Ratio calculated from potential data for rubidium with swelling taken into account by setting $0.8(m_i)_{Rb} = (m_o)_K$	8.5		1
	3.4	1	

TABLE VI
Temperature Coefficients of Permeabilities

Ion	Calculated Q_{10} from potential data	Measured Q_{10} from flux data
K	1.7*	1.7‡
Rb	2.0	1.9
Cs	2.6	2.5

* Assumed value from data of Harris.

‡ Inferred from data of Harris (14).

DISCUSSION

Passive flux theory seems to adequately describe the depolarizing action of rubidium and cesium in terms of their fluxes into the muscle cell. The points of special interest are the concentration and temperature dependence of the permeability coefficients and the inhibition of potassium flux in the presence of other cations. The permeability coefficient as defined in equation (2) involves the ionic mobility and the partition coefficient, β . The changes in permeability observed for a particular ion over a given concentration range can be attributed to changes in either the mobility or the partition coefficient. The mobility might be expected to be dependent upon the electric field and rather independent of concentration. The permeability changes observed for rubidium and cesium ions in the 1 to 10 mM concentration range occur in a

region where the potential is constant since very little depolarization takes place under these conditions. It seems more likely, therefore, that the permeability changes observed are a reflection of changes in β since it is known that partition coefficients are, in general, concentration-dependent. The partition coefficient may be expected to decrease as less space becomes available in the membrane for the ions in question.

Permeation of an ion into the cells seems likely to occur either by reaction with a "carrier" molecule, by moving through a specified number of sites with a certain potential energy barrier for each jump, or by moving through pores in single file. Whatever the permeation process, β would represent the number of carriers, sites, or pores available to the penetrating ions. An expression relating β to concentration may be derived from simple physical considerations. The pore model is used, but the formulation applies equally well to the other cases.

β_i = maximum limiting value of β at low concentrations

β = β as a function of concentration = $\beta(C_o)$

S = total number of pores available

Y = number of filled pores

$S - Y$ = number of unfilled pores

The first assumption is that β_i measures the total number of pores available.

$$\beta_i = kS$$

The number of filled pores will be proportional to the concentration of the ion in the membrane.

$$Y = k'C_m = k'\beta C_o$$

The second assumption is that $\beta(C_o)$ might be expected to be proportional to the number of pores yet available.

$$\beta = k(S - Y) = k\left(\frac{\beta_i}{k} - k'\beta C_o\right)$$

Solving for β :

$$\beta = \frac{\beta_i}{1 + kk'C_o} \quad (11)$$

This equation gives a good fit to the rubidium data in these experiments. At low concentrations β is seen to approach β_i .

The variation of β with temperature is more difficult to treat. On the "carrier" and site to site hypothesis, one must assume a very high activation energy for carrier reactions or diffusion jumps. It is possible that the very high Q_{10} seen for the cesium flux from 113 mM solutions might be due to its being measured in a region of low permeability, and that the Q_{10} at a lower concentration might be closer to that for potassium ions. Should this prove

to be the case, it would be difficult to understand why the activation energy should increase sharply as less carriers or sites become available, the activation energy being a property of individual molecules or permeation sites. Moreover, one must also assume a very different activation energy for potassium and cesium, an assumption which seems unlikely in view of the chemical similarity between these ions. The pore hypothesis offers the advantage of being able to account for very high temperature coefficients for ion fluxes in biological material. Relatively small changes in temperature may suffice to change a distribution of pore sizes considerably by way of thermal expansion effects. A relatively non-permeating ion such as cesium would have a low β , which would signify a small number of available pores. If these pores occurred near the tail of a pore size distribution, small temperature changes could have a large effect on the number of available pores. The temperature variation of β would then be stated as $\beta(T) = k(S(T) - Y)$.

The inhibition of potassium flux in the presence of other cations suggests competition for carriers, sites, or pores. Such competition has also been observed between potassium and rubidium in the red cell by Solomon (15). An ion will compete more with another for transit into cells the more access it has to the means of permeation used by the other ion. A more permeating ion may be expected to have more access to potassium permeation channels than a less permeating ion. This expectation is borne out in the present experiments, rubidium having a greater inhibitory effect on the potassium flux than cesium.

In terms of the formalism, it is possible to obtain an expression for the lowering of the potassium permeability in the presence of a competing cation. The pore model is again assumed, though the equations also apply to other permeation mechanisms. For K efflux one may write:

$$\beta_K = k_K(S_K - k'_K\beta_K[K^+]_i)$$

The rubidium ions may be assumed to have access to a certain fraction, a , of the potassium pores as well as to other pores not used by potassium. A fraction, b , of the pores occupied by rubidium ions will therefore be unavailable to potassium ions. The number of pores filled with Rb is:

$$Y_{Rb} = k'_{Rb}\beta_{Rb}[Rb^+]_o$$

The pores unavailable to K are given by (bY_{Rb}). The partition coefficient for K in the presence of Rb is then given by:

$$\beta_{K/Rb} = k_K(S_K - k'_K\beta_{K/Rb}[K^+]_i - bk'_{Rb}\beta_{Rb}[Rb^+]_o)$$

Solving for $\beta_{K/Rb}$ yields:

$$\beta_{K/Rb} = \frac{\beta_i}{1 + k_K k'_K [K^+]_i} - \frac{bk_K k'_{Rb} \beta_{Rb} [Rb^+]_o}{1 + k_K k'_K [K^+]_i}$$

The first term on the right is seen to be just β_K in the absence of any competition. Hence:

$$\beta_{K/Rb} = \beta_K - \frac{bk_K k'_{Rb} \beta_{Rb} [Rb^+]_o}{1 + k_K k'_K [K^+]_i} \quad (12)$$

β_K is seen to be decreased in the presence of Rb by a quantity involving β_{Rb} and $[Rb^+]_o$. The equation thus predicts an increasing inhibition of K flux for increasing external concentration and permeability of the competing cation. Equation (12) can be easily subjected to experimental test by studying the inhibition of potassium flux over a wide range of concentration of the competing cation.

Another observation which suggests penetration of ions through pores is the non-reversibility of the temperature effect. If depolarization is performed with cesium at room temperature and the muscle cells cooled when a stable potential is reached, very little increase in potential occurs. If carriers were involved, a rapid unloading would be predicted upon cooling, in accordance with the high Q_{10} and energy of activation, leading to less depolarization. The pore model would be consistent with the observation, however. Once ions have captured the available pores at room temperature, the presence of the ions will tend to stabilize the distribution of pores so that temperature changes are no longer as effective in altering the distribution. An observation of Sandow and Mandel (5) may be accounted for on a similar basis. They observed that after depolarization with rubidium ions and return to normal Ringer's solution as a wash, potassium ions were no longer as effective in depolarizing the cells, tending to act more like rubidium ions for some time. This would be expected if rubidium ions tended to be retained in pores leading to a persisting inhibition of potassium flux. It is difficult to plausibly account for such persistent effects with a carrier model.

Finally, some comment seems required on the apparent concentration dependence of β . The current trend in active transport studies seems to be the testing of flux ratios with the Ussing equation. When this equation is not obeyed, one concludes that an active transport process occurs. There is no difficulty with this approach when large deviations are met. It should be emphasized that a certain amount of deviation is to be expected if β is not the same on both sides of the cell membrane. If P is different on the two sides dividing equation (4) by equation (5) will not give the Ussing equation and a factor P_{in}/P_{out} will occur. Since a variation of β with concentration seems to occur in general, this consideration cannot be neglected. The potassium fluxes across the cell membrane may be expected to be affected by this mechanism, and in this case the potassium electrode equation will be modified by a permeability term and a concentration *vs.* potential slope of -58 mv. need not be expected. That many measurements of the membrane potential against

the logarithm of $[K^+]_o$ give slopes somewhat lower than -58 mv. suggests that such considerations may be relevant. A significant permeability to other ions not in an equilibrium distribution will, of course, also introduce departures from the slope predicted by the potassium electrode equation.

Membrane Conductance in Elevated K Solutions.—The finding of a relatively constant P_K over a wide range of outside K ion concentration seems somewhat surprising since the permeability to ions of excitable cells has been shown to be highly dependent on the membrane potential. Jenerick (16), for example, deduces from his conductance data in frog muscle that P_K is inversely related to the membrane potential. The equation for the conductance contribution of an ion in the steady state, $G_n = (F^2/RT)m_n$. (Hodgkin (17)), shows a linear dependence of the conductance on the flux in either direction. If one assumes that half the membrane conductance at the resting potential is due to K^+ , G_K can be estimated from the conductance data of Jenerick. If the G_K so estimated is plotted against the potassium concentration, the curve obtained should fit the flux curve if the system is in the steady state with respect to K^+ at each concentration. It can be shown that the conductances measured by Jenerick give a very good fit to the K^+ flux curve presented in this paper. It appears, therefore, that the data of Jenerick do not require the assumption of variations in P_K with membrane potential. Several difficulties arise, however, when correlations are attempted between conductance experiments and tracer experiments. Harris (18), for example, points out that the conductances predicted from flux measurements in muscle are too low by a factor of about 20. As a possible explanation, he points out that the measured fluxes may be too low because of long pore effects. Another explanation might be found in departures of the system from the steady state. A non-steady state conductance equation derived by Keynes (19) and one derived by the author³ can be used to estimate the conductance under such conditions. It is difficult to account for a factor of 20 in this way. In the author's equation, the term $(RT/F)\partial \ln P/\partial V$ is seen to enter the net current term. This could

³ If equation (1) is multiplied by F , to convert \bar{m} to current carried by univalent positive ions of a particular species, and differentiated with respect to E , an expression is obtained for the membrane conductance due to such ions. Use is made of equations (4) and (5), and their ratio, in the derivation. Also, P , the permeability coefficient, is not assumed to be constant in the differentiation and a term involving its derivative with respect to the potential results. If P is constant, the equation reduces to that obtained by Keynes (19) by a similar differentiation. For a positively charged, univalent ion, the conductance is given by:

$$G = \frac{F^2}{RT} \left\{ m_i + (m_i - m_o) \left[\frac{RT}{EF} - \frac{1}{(1 - e^{-EF/RT})} + \frac{RT}{F} \frac{\partial \ln P}{\partial E} \right] \right\}$$

serve to amplify any contribution to the conductance by non-steady state conditions. One might, therefore, postulate a transient value for $\partial \ln P / \partial V$ for even small voltage displacements. This would elevate the conductances calculated from flux measurements even though such measurements would not reveal any permeability potential dependence. The discrepancy in conductance-flux data might also be sought in more substantial contributions from other ions though this is difficult to visualize under resting conditions. It is, of course, possible that all the factors suggested contribute.

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APPENDIX

The terms *flux* and *permeability* are, strictly speaking, defined for single cells of known dimensions and surface to volume ratio. When ion uptake measurements are made on whole muscle, the fluxes determined obviously refer to a population of fiber sizes with varying surface to volume ratio. Any permeability parameter deduced from such data must represent an average over a population of fiber sizes. Creese, Neil, and Stephenson (20) present a distribution of surface to volume ratios for diaphragm muscle. Harris (12) found that no great error results when this distribution is approximated by assuming that all the fibers are of equal size and belong to the group in the distribution with size just greater than the most frequent value. If each single fiber shows exponential uptake kinetics, the entire distribution of fibers can then be expected to show very nearly the same kinetics. It can be shown that the same is true for the distribution of fiber sizes in frog sartorius muscle given by Carey and Conway (21). It would appear, therefore, that it is permissible to use whole sartorius muscle as an approximation to a single fiber without expecting gross anomalies in the observed kinetics. When only relative permeabilities are of importance, as in this investigation, such errors and anomalies as do result from this approximation may be expected to be the same for all the ions.

Using the factor 530 cm.³ per gm. fiber (Harris (12)), the relative permeabilities may be expressed in absolute units. A relative P_K of 320, the normal potassium permeability, represents an absolute P_K of 42×10^{-8} cm./sec. The corresponding K influx from a 2.5 mM K Ringer solution is 3.8 pmoles/cm.² sec. One might ask how these values would be expected to compare with those measured on single muscle fibers. Diffusion in the extracellular space would tend to make whole muscle fluxes lower than corresponding single cell fluxes (Harris and Burn (22); Keynes (23)). Keynes, for example, gives a value of 4.3 pmoles/cm.² sec. for the measured potassium influx in sartorius muscle (2.5 mM K) and states that the true flux may be as high as 12 pmoles/cm.² sec. The recent measurement of potassium fluxes in single frog muscle cells (Hodgkin and Horowicz (24)) provides a means for direct comparison. In fibers which varied between 90 μ and 137 μ in diameter, potassium influxes were measured (2.5 mM K) to give values varying between 3.1 and 8.1 pmoles/cm.² sec. The averaged value for five fibers was 5.4 pmoles/cm.² sec. It

is likely that the total flux deduced for the whole muscle using single cell data would be somewhat higher than this value as the average diameter of sartorius fibers (about 100 μ) lies at the lower end of the range of sizes used by Hodgkin and Horowicz. Using the distribution given by Carey and Conway (21), one can estimate that the whole muscle flux should be very close to 6 pmoles/cm.² sec. The diffusion effect factor is then seen to be around 1.5. Again it would appear that the frog sartorius muscle is a fairly good approximation to a single fiber.

In addition to errors which can arise from the above considerations, one must consider the finding that departures from the flux equations can result from the non-independence of influx and efflux as would be expected if penetration took place along long, single file pores (Hodgkin and Keynes (13)). This effect becomes amplified the more the flux ratio departs from unity and always acts to increase the flux ratio from that predicted by theory when there is a net gain of the ion in question and to decrease the flux ratio when there is a net loss. Since there is an increasing net gain of potassium ions in solutions in which more and more sodium is replaced with potassium, this effect may account for the slight but significant increase in measured P_K which results with increasing external K concentration (Fig. 6).

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