J. Physiol. (1961), **157**, *pp.* 289–305
With 5 text-figures
Printed in Great Britain

RUBIDIUM AND CAESIUM ENTRY, AND CATION INTERACTION IN FROG SKELETAL MUSCLE

By VALERIE BOLINGBROKE, E. J. HARRIS AND R. A. SJODIN*

From the Department of Biophysics, University College London

(*Received* 18 *January* 1961)

When a muscle is exposed to a solution containing Rb or Cs ions there is a gradual replacement of its K by the foreign ion (Lubin & Schneider, 1957; Sjodin, 1959). The aim of this paper is to present the kinetic results for the interchange of Rb or Cs and K, using a model proposed to explain the observed dependence of K-for-K exchange on external concentration (Harris & Sjodin, 1961). By 'interchange' is meant the equivalent-for-equivalent replacement of an ion by a chemically unlike ion; this process can be followed by analytical methods. By 'exchange' is meant the equivalent-for-equivalent replacement of an ion by a chemically identical ion; this can only be followed by the use of isotopically labelled material. Net movement of a cation can take place either as a consequence of interchange with a different cation, or along with a mobile anion. The model used allows uptakes from K-free solutions and from mixtures to be treated consistently. Values for the relative exchangeabilities of K with itself and with Rb and Cs are obtained.

METHODS

The uptakes of ^{42}K or ^{89}Rb or ordinary Rb were measured, using the sartorii of *Rana temporaria*. The Cs results are taken in part from Sjodin (1959), who used *Rana pipiens*. The tracer experiments were made by the technique described by Harris & Sjodin (1961); a series of readings of the radioactivity of the tissue was obtained after timed exposures to the radioactive solution. Before the measurements the muscles were blotted on filter paper moistened with an inactive solution chemically similar to the soak solution. At the end of each experiment the muscle was extracted overnight in dilute nitric acid and the extract used for analysis by flame photometry. In making most of the experiments depending on Rb or Cs analysis after timed exposure to a given solution, it was found convenient to apply a 1 min wash in Na methyl sulphate solution (0·11 M) at 0° C before extraction of the cations; this wash would remove much of the extracellular solution and so render less necessary a correction for the trapped Rb or Cs.

The analyses were made with a flame spectrophotometer using a prism monochromator, Beckman oxy-hydrogen burner and a photomultiplier tube with sensitivity extending beyond 8500 Å (E.M.I. type 9553B). It was confirmed that at the levels used the Rb–K

* Present address: Biophysics Department, University of Maryland, Baltimore, U.S.A.

interference was negligible. There was, however, some K in the Rb salts; this amounted to 1 part K to 50 Rb in the RbCl and 1 part K to 150 Rb in the Rb_2CO_3. The CsCl contained 1 part K to 250 Cs.

The experiments were started in solutions made up with mixtures of the alkali chlorides. These mixtures had [NaCl] = 80 − ½[RbCl]; [$NaHCO_3$] = 30 mM; [KCl] and [RbCl] as stated and [$CaCl_2$] = 2 mM. Up to Rb concentrations of 40 m-equiv/l. provided the K concentration did not exceed 5 m-equiv/l. there was little or no net gain of cation from the solutions; the sum (K + Rb) found after 4 hr immersion was between 75 and 97 m-equiv/kg, while the K content of fresh muscles was 75–85 m-equiv/kg. Hence in these cases it is justifiable to regard the Rb^+ (and labelled K^+) entry as occurring mainly in exchange for the muscle K^+. This was not so when 80 m-equiv Rb/l. was used, for then the (Rb + K) increased to about 130 m-equiv/kg tissue, presumably on account of entry of RbCl (and KCl when K was present). For this reason the later Rb experiments were all made in Cl-free mixtures of $RbHCO_3$ and K and Na methyl sulphates ($NaMeSO_4$). These had

$$[K + Rb + Na] = 115 \text{ m-equiv/l.}, \quad [HCO_3] = 20 \text{ m-equiv/l.}$$

or equal to [Rb] when [Rb] exceeded 20; [methyl sulphate] = 115 − [HCO_3] m-equiv/l.; [Ca acetate] = 2 mM. The use of high concentrations of $RbHCO_3$ equilibrated with 95% O_2 + 5% CO_2 led to high pH (8·3 when $RbHCO_3$ = 80 mM) but the K exchange results already described (Harris & Sjodin, 1961) show that in alternative solutions containing either high or normal [HCO_3] the K exchange follows the same course. This is the case despite the precipitation of calcium in the high bicarbonate solution. Confirmation that pH and Ca have little effect on the Rb entry was obtained by comparing the results obtained in the two kinds of solution, namely with and without Cl. The caesium salt solutions were made from mixtures of CsCl or $CsNO_3$ with Na salts and had [Cs + Na] = 120 m-equiv/l.

Some measurements of the space accessible to the dye naphthol green were made at the same time as certain analytical experiments by adding 0·5% of the dye to the solution. The muscle extracts were then made for 30 min in distilled water without added acid and the dye contents were read on a colorimeter. The extracts were then returned to the vessels containing the muscles and a drop of nitric acid added to assist cation release. The space measured in relatively fresh muscles (up to 30 min in isolation) was 0·11 ± 0·02 ml./g (S.D. for 11 results); after longer isolation values tended to be higher, for example, 0·14 ml./g after 2 hr.

RESULTS

Method of presentation

Penetration of the cations into the cell is assumed to occur through an outer compartment which has ion-exchange properties (Harris & Sjodin, 1961). In the absence of net movement accompanying chloride ions, the cations only move by exchange diffusion (Ussing, 1949) against ions already adsorbed. The equilibration of the compartment bounded by the ion exchange region then occurs as if from a source having a time-dependent concentration of the new ions deriving from the solution. Suppose a constant concentration $[Rb]_e$ is applied to the ion exchanger and no appreciable external concentration of the desorbed K ions is allowed to build up (by use of a large volume of solution) the equation governing the rate of exchange of adsorbed ions is

$$dRb_a/dt = k_{Rb}[Rb]_e(N\text{-}Rb_a). \qquad (1)$$

Here Rb_a denotes the activity of the adsorbed Rb per unit mass of tissue and N is the total activity of sites able to hold Rb ions, so (N-Rb_a) is the activity of the still unexchanged K ions in the adsorption region. k_{Rb} is the rate coefficient for Rb-K interchange.

The integral form of this equation for the condition that $Rb_a = 0$ at $t = 0$ is

$$Rb_a/N = \{1-\exp(-k_{Rb}[Rb]_e t)\} \quad (2)$$

in which N can be replaced by Rb_a ($t = \infty$) or by K_a ($t = 0$), provided that eventually all the adsorbed K is replaceable by Rb. This is an exponential process of build-up within the outer region with a rate constant equal to $k_{Rb}[Rb]_e$, that is, proportional to $[Rb]_e$. If, next, the outer region be regarded as the source from which diffusion into the cell interior occurs and provided the disturbance of the exponential process due to passage of ions from the cell interior is negligible, one can compare the course of the uptake into the cell interior with that of the appropriate diffusion process. When the rate constant (β) of equilibration of the source (the outer region) is so low that the quotient $a^2\beta/D < 1$, a being the radius of a cylindrical object or the half thickness of a sheet and D the internal diffusivity, then the shape of the object and the values of a and D become unimportant (Harris & Sjodin, 1961) and the fractional equilibration of the interior depends almost entirely on the value of βt. Since on our assumptions the rate constant β is identified with $k_{Rb}[Rb]_e$ we should find Rb uptakes from all K-free Rb solutions falling on a single curve when plotted against $[Rb]_e t$ or a function of this product. The omission of the factor k_{Rb} from the function used as abscissa corresponds to multiplication of the derivative of the uptake with respect to the abscissa by the appropriate function of k_{Rb}.

Rb uptakes in absence of external K

The amounts of Rb measured in the tracer experiments include the extracellular Rb, since no wash was given before taking the readings. Before plotting these quantities or those found by analysis of muscles which had not been washed a standard deduction of the Rb calculated to be held in an extracellular space of 0·1 ml./g was made. This correction was probably somewhat too small, particularly after long immersions. Analytical results obtained after a 1 min wash in a Rb-free solution were plotted directly. The abscissa against which the uptake was plotted was chosen to be $([Rb]_e t)^{\frac{1}{2}}$. Figure 1 shows that the results obtained over the range of concentrations 2·5–100 m-equiv Rb/l. fall along a single line; the part from 8 to 40 m-equiv Rb/kg tissue is nearly straight. The curve for Rb/K interchange is less steep than the corresponding one for K–K exchange, which is also indicated on the figure. The slope of the Rb-uptake

curve is 0·42 times that of the K-exchange curve. This corresponds to the coefficient k_{Rb} being $(0{\cdot}42)^2 = 0{\cdot}2$ times the coefficient k_K for K exchange.

The effect of temperature on Rb uptake was estimated by comparing the values of the product (concentration × time) required to give a particular

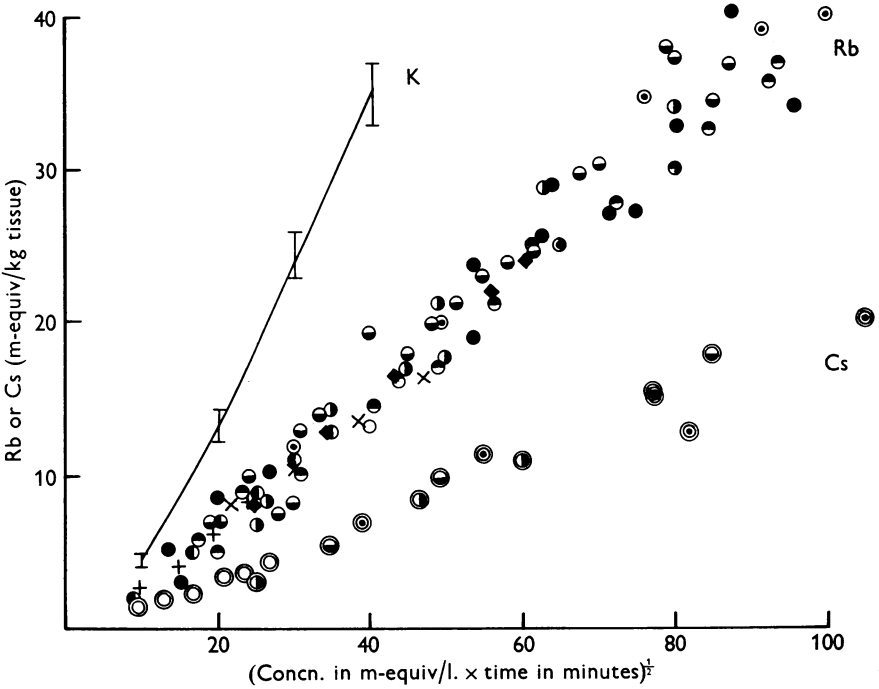

Fig. 1. (K) The line relating K exchanges at 20° C to $([K]_e \times \text{time in min})^{\frac{1}{2}}$ with an indication of the experimental scatter (from Harris & Sjodin, 1961, Fig. 3).

(Rb) The experimental observations described in this paper of the Rb uptakes from various Rb concentrations plotted against $([Rb]_e \times \text{time in min})^{\frac{1}{2}}$. Points for Cl-free mixtures using analytical method; ○ 5, ◑ 10, ◒ 20, ◓ 40, ● 80, ⊙ 100 m-equiv Rb/l. Points obtained in Cl mixtures using tracer method; ◐ 2·5, ◆ 20 m-equiv Rb/l. Points obtained in Cl mixtures using tracer method applied to muscles pre-loaded with ordinary Rb; + 5, × 20 m-equiv Rb/l. Values are corrected when necessary for the Rb in a space equal to 0·1 ml/g.

(Cs) Experimental observations of Cs uptakes from various Cs concentrations plotted against $([Cs]_e \times \text{time in min})^{\frac{1}{2}}$. Points for Cl mixtures using tracer method; ◎ 2·5, ◍ 5 m-equiv Cs/l. Points for Cl-free mixtures (using $CsNO_3$) obtained analytically; ◒ 20, ◎ 100 m-equiv Cs/l.

uptake at 0° C with the value interpolated from the 20° C curve. The ratio so found was 3·4:1 (from 9 readings at 0° C); this according to our model represents the ratio of the value of k_{Rb} at 20° C to that at 0° C. In the K exchange the ratio of the values of k_K at the two temperatures was 3·3:1 (Harris & Sjodin, 1961).

The question arose whether the Rb–Rb exchange might differ in rate from the Rb-K interchange: this was tested by preparing muscles by overnight immersion in a 100 mM-RbCl mixture before following the uptakes of labelled Rb. The muscles contained about 85 m-equiv. Rb/kg and 10 m-equiv K/kg. The results of these experiments fall on the same line (the points are distinguished in the figure) showing that the Rb^+–Rb^+ exchange occurs at practically the same rate as the Rb^+–K^+ interchange. In both Rb^+–K^+ and Rb^+–Rb^+ exchanges the postulated outer region becomes occupied by Rb^+ because in each case the solution contains only Rb ions.

The Rb uptake results did not show any regular difference between Cl–containing and Cl–free media. This suggests that there is little associated net movement of Rb^+ with Cl^- into the muscle even from raised RbCl concentrations, since this would have led to the observed uptakes for Cl mixtures exceeding the comparable ones from Cl–free media. The point was tested by comparing the analysis of a muscle soaked for 64 min in

100 mM-RbCl + 90 mM-NaCl + 20 mM-$NaHCO_3$

with that of the paired muscle soaked for the same time in a mixture of

100 mM-$RbHCO_3$ + 20 mM-$NaMeSO_4$.

The total [Rb + K] in the muscle from the Cl mixture was 112 m-equiv/kg, that from the Cl-free mixture was 104 m-equiv/kg, so here only an extra 8 m-equiv Rb/kg was gained. In the comparable case with 100 mM-KCl present net gains of 35–48 m-equiv K/kg tissue were found. That there is some entry of RbCl from mixtures containing high concentrations of the salt can also be shown by following the swelling which occurs when water accompanies the RbCl into the tissue from a mixture having Rb replacing equivalent Na. The time course of swelling of a muscle in 100 mM-RbCl + 20 mM-$NaHCO_3$ + 2 mM-$CaCl_2$ is shown in Fig. 2. A few points from the swelling curve found in a similar mixture but with 100 mM-KCl are indicated (from Harris & Sjodin, 1961, Fig. 5). The results confirm that the water movement and, by inference, the RbCl movement, is slower than when KCl is used. The slope of the line in KCl solution is to that in RbCl solution as 4·5 to 1; to reach the same degree of swelling the muscle in RbCl mixture has to be given $(4{\cdot}5)^2 = 20$ times as long as the one in the KCl mixture. The swelling in a caesium mixture was insignificant in 3 hr. This shows that there is a still greater disparity between the rates of net movement of the respective salts than exists between the rates of the cation exchanges.

Some points for Cs uptakes from solutions having different concentrations are plotted in Fig. 1. Like the Rb results these were obtained alternatively by tracer and analytical methods. The slope of the Cs–K curve is about 0·20 times that of the K exchange curve, which corresponds to k_{Cs} being $(0{\cdot}20)^2 = 0{\cdot}04$ times k_K.

Effect of net Rb gain on Rb uptake

The experiments made in RbCl solutions showed that net gain of the salt was slight. In order to obtain appreciable changes of total content of (K + Rb), use was made of muscles which had first been K-depleted by storage for 48 hr in a K-free solution at 4° C. This procedure follows that

used for obtaining a net K gain and has also been applied by Libin & Schneider (1957) to Rb and Cs uptakes. The point of the present experiments was to find out how the uptake of Rb occurring when the muscle Na content falls compares with the amount of Rb normally exchanging for K. The results obtained after various times of exposure to a mixture containing (in m-equiv/l.) [Rb] = 10, [Na] = 105, [methyl sulphate] = 95,

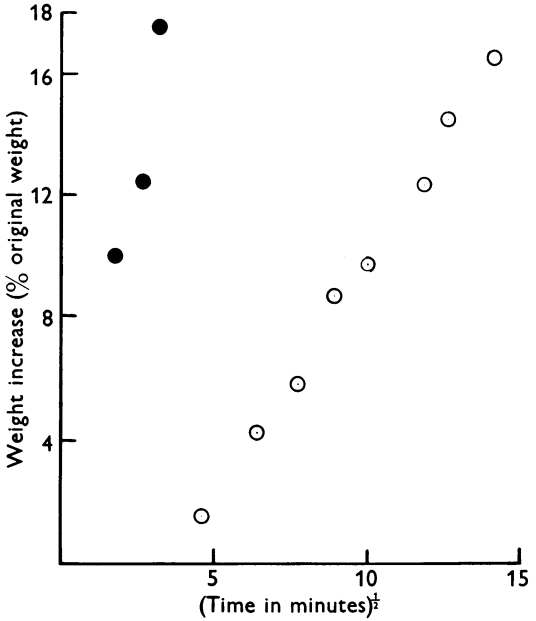

Fig. 2. ○, the swelling of a muscle at 20° C in a mixture having (mM): RbCl 100, $NaHCO_3$ 20, $CaCl_2$ 2. A few points (●) from the corresponding experiment in a KCl mixture are taken from Harris & Sjodin (1961, Fig. 5).

TABLE 1. The contents of Rb, K and Na found in muscles after soaking in a solution at 20° C with 10 m-equiv Rb/l. and 105 m-equiv Na/l., using K-depleted muscles and fresh muscles. A control K-depleted muscle had K = 38·3, Na = 70·4, sum = 108·7 m-equiv/kg. Mean of 5 fresh muscles K = 76·1, Na = 26·4, total 102·5 ± 5·2 (S.D.) m-equiv/kg

1	2	3	4	5	6	7	8	9	10
		Using K-depleted muscles				Using fresh muscles			
		Contents (m-equiv/kg) after 1 min wash in Rb-free soln							
						Means from 3–5 analyses ± S.D.			
Time (min)	$(10t)^{\frac{1}{2}}$	Rb	K	Na	Total	Rb	K	Na	Total
50	22·4	16·4	44·5	45·2	106·1	—	—	—	—
62	24·9	21·3	28·9	59·2	109·4	6·8 ± 0·7	66·5	32·2	105·5
121	34·8	33	36·8	37·7	107·5	10·3 ± 0·7	65·3	29·5	105·1
195	44·4	39·4	18·8	56·3	114·5	15·7 ± 2·3	58·0	27·9	101·6
244	49·5	34·9	42·0	40·8	117·7	18·0 ± 1·7	52·9	32·1	103·0
250	50	34·0	35·3	46·6	115·9	—	—	—	—
328	57·2	42·0	22·5	62·1	126·6	18·8 ± 1·7	55·5	35·0	109·3

$[HCO_3]$ = 20, and 2 mM-Ca acetate are given in Table 1 (col. 3); the Rb uptakes are much greater than usual (col. 7). This shows that there can be a relatively rapid entry of Rb linked with Na extrusion in addition to the Rb–K interchange.

Rb uptakes from mixtures of Rb and K

It is necessary to find out whether the tissue as a whole has any selectivity between K and Rb ions, because this determines the equilibrium levels to which the two ions tend when presented in a mixture. Selectivity within the outer region may be present but its analytical demonstration has not been possible. Lubin & Schneider (1957), using 5 m-equiv/l. of each of Rb and K, found that the Rb content of muscles exposed for 2 days at 25° C was slightly (8 m-equiv/kg cell water) in excess of the K content, while the disproportion in favour of Rb after 3 days was as much as 25 m-equiv/kg water. However, these authors further showed that when loss of K and Rb occurred from a muscle previously loaded with Rb it was the K which tended to escape faster. Since their muscles after 3 days loading contained considerably (35 m-equiv/kg cell water) more Na than at 2 days it is likely that some escape of K and Rb in exchange for Na had taken place. The faster movement of the K would then lead to an increase in the ratio Rb:K within the cell. It seems important to choose conditions, if this be possible, to avoid loss of K or Rb against Na in equilibration experiments. A further disturbing factor would be introduced if chloride were present, on account of the faster rate of KCl movement compared with that of RbCl (as illustrated in Fig. 2). We made our equilibration experiments in media having only non-penetrating anions (HCO_3 and methyl sulphate) present. In making the trials the muscles were frequently transferred to fresh portions of soaking solution to remove the K which had emerged from the cells and to discourage bacterial growth.

The cation contents of muscles which had been exposed for 16–48 hr to various mixtures of K, Rb and Na salts were determined after a preliminary 1 min wash in a cold methyl sulphate solution. The ratio [Rb]:[Rb + K] in the tissue was found to tend towards, but not to attain, the ratio pertaining in the solution (Table 2).

It is possible that the failure to reach equality of Rb:K ratios inside and outside the cell in the mixtures of Rb and K has its explanation in the same phenomenon as is encountered when attempting to exchange muscle K with labelled K. Long exposure of the isolated tissue leads to breakdown of the sodium exclusion. Once Na can enter and displace the internal K, the external ion, be it labelled K or Rb, meets additional competition for entry into the sites of the ion exchanger. Such interference has

been demonstrated using muscles which had been loaded with excess KCl (Harris & Sjodin, 1961, Fig. 9).

We shall proceed on the basis that there is no important difference between the final Rb–K distribution inside and outside the cell although the results in Table 2 only extend to about 85% equilibration. It does not follow that the activity coefficients of K and Rb are equal within the ion exchanger, because the effect of localized concentration differences would not be seen in the gross analysis. A model system in which activity

TABLE 2. The ratio [Rb]:[Rb+K] found for the cell cations after long exposures to media of given compositions at 20° C. The anions present were bicarbonate and methyl sulphate

	Solution composition (m-equiv/l.)			Time of exposure (hr)	Ratio: [Rb]:[Rb+K]		Total Rb+K (m-equiv/kg) In tissue
	Rb	K	Na		In soln.	In tissue	
1	10	10	100	21	0·50	0·39	75·2
2	20	20	80	21	0·50	0·41	87·7
3	20	20	80	27	0·50	0·44	87·0
4	40	40	40	21	0·50	0·43	100·1
5	49	53	23	22	0·48	0·41*	103*
6	50	50	20	18	0·50	0·43	92·3
7	60	55	1·5	21	0·52	0·44*	103·5*
Pre-treated	80	0	33	4	—	—	—
then							
8	49	53	23	17	0·50	0·44†	105†
9	42	11	70	19	0·79	0·66	82
10	42	11	70	26	0·79	0·67	82
11	42	11	70	48	0·79	0·75	100

* mean of 4; † mean of 3.

differences within the ion exchanger do lead to concentration ratios within the *exchanger* differing from those in the external media has been found (Sjodin, unpublished). However in the formulation of the exchange kinetics it is the activities of the ions in the exchanger which are important, they are respectively equal to the products of adsorbed concentration and local activity coefficient. Within the exchanger the respective activities must be proportional to the appropriate applied concentrations, since in solution the activity coefficients of K and Rb are similar; this must in turn lead eventually to the internal Rb:K ratio becoming equal to that outside when *intracellular* activity coefficients of the two ions are similar.

The rate of build up of the adsorbed Rb, in presence of K, can be written

$$\frac{d\mathrm{Rb}_a}{dt} = k_{\mathrm{Rb}}[\mathrm{Rb}]_e(N-\mathrm{Rb}_a) - k_{\mathrm{Rb}}[K]_e\mathrm{Rb}_a, \qquad (3)$$

where the quantities $\mathrm{K}_a = (N-\mathrm{Rb}_a)$ and Rb_a are the activities in the adsorption region. The second term on the right gives the rate of displace-

ment of absorbed Rb by external K. For constant $[Rb]_e$ and $[K]_e$ applied at $t = 0$ one has

$$\frac{Rb_a}{N} = \frac{[Rb]_e}{[Rb]_e + [K]_e}\{1 - \exp(-k_{Rb}([Rb]_e + [K]_e)\, t)\}. \quad (4)$$

Comparing this with the equations (Harris & Sjodin, 1961, eqn. (2); this paper eqn. (2)) for build-up of either labelled K or Rb in absence of the other ion, we see that the final concentration is reduced in the ratio $[Rb]_e : ([Rb]_e + [K]_e)$ and that the rate constant for the build-up is

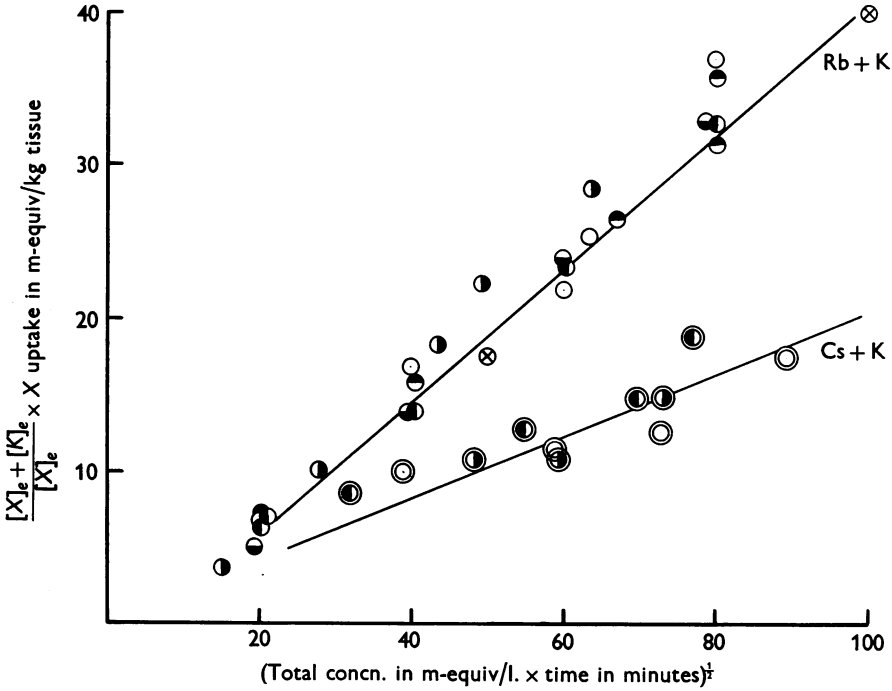

Fig. 3. Uptakes of Rb or Cs at 20° C from mixtures with K. Ordinates are content (m-equiv/kg) × $([X]_e + [K]_e)/[X]_e$ and abscissae are $\{([X]_e + [K]_e)\, t\}^{\frac{1}{2}}$, where X stands for Rb or Cs. Mixtures and methods used: (Rb) (tracer method), ⊗ 2 Rb + 100 K; ◑ 2·5 Rb + 5 K: (analytical method), ◒ 5 Rb + 10 K; ◐ 10 Rb + 10 K; ○ 20 Rb + 5 K; ◓ 40 Rb + 5 K; (Cs) (tracer method), ◎ 10 Cs + 10 K; ◉ 10 Cs + 20 K; (analytical method), ◐ 50 Cs + 50 K. The numbers indicate m-equiv/l.

k_{Rb} $([K]_e + [Rb]_e)$ instead of $k_K[K]_e$ or $k_{Rb}[Rb]_e$. At a given value of $([Rb]_e + [K]_e)\, t$, say V, the Rb content which is observed will then be only $[Rb]_e/([Rb]_e + [K]_e)$ of the content observed in absence of the external K at $[Rb]_e t = V$. To express the Rb uptakes from mixtures in a way consistent with the method used for the ions separately the uptakes are multiplied by $([Rb]_e + [K]_e)/[Rb]_e$ and the products plotted against $\{([K]_e + [Rb]_e)\, t\}^{\frac{1}{2}}$ in-

stead of $([K]_e t)^{\frac{1}{2}}$ or $([Rb]_e t)^{\frac{1}{2}}$ (Fig. 3). For a series of different mixtures of K and Rb this procedure leads to a curve which is the same within experimental variability as the curve obtained for Rb uptakes alone (Fig. 1). The slope of the linear part of the curve is a measure of the square root of the Rb exchange coefficient, $k_{Rb}^{\frac{1}{2}}$. The result can be used in reverse to calculate the Rb content of a muscle which has been exposed to a given K+Rb mixture for a given time.

Cs uptakes from mixtures of Cs and K

Measurements of the Cs content of muscles after various exposures to mixtures of Cs and K have been plotted in Fig. 3 in the same way as that described for the Rb uptakes from mixtures. The quantity

$$\{\text{Cs uptake} \times ([Cs]_e + [K]_e)/[Cs]_e\}$$

is related to $\{([Cs]_e + [K]_e)\, t\}^{\frac{1}{2}}$ in the same way as the Cs uptake from a K-free solution is related to $([Cs]_e \times t)^{\frac{1}{2}}$.

Rb and Cs uptakes from mixtures

When both Rb and Cs are presented to the tissue the two ions enter the outer region and mutual displacements of one by the other can occur. If, however, one considers the sum $(Rb_a + Cs_a)$, the mutual displacements do not matter and only the displacement of the original K is of importance. For the equilibration of the adsorbed ions one has

$$d(Rb_a + Cs_a)/dt = (k_{Rb}[Rb]_e + k_{Cs}[Cs]_e)\,(N - (Rb_a + Cs_a)) \qquad (5)$$

so that

$$\frac{Rb_a + Cs_a}{N} = \{1 - \exp(-(k_{Rb}[Rb]_e + k_{Cs}(Cs]_e)\, t)\}. \qquad (6)$$

Comparing this with equation (2) for build-up of adsorbed Rb one sees that the sum $(Rb_a + Cs_a)$ can be regarded as equivalent to Rb_a provided that the sum $([Rb]_e + (k_{Cs}/k_{Rb})\,[Cs]_e)$ is used instead of $[Rb]_e$. The rate of diffusion of the mixture into the cells can then be predicted from the Rb exchange curve.

In the test of this point shown in Table 3 analyses were made after various exposures to a mixture of 50 m-equiv/l. of both Rb and Cs at 20° C. The usual correction for extracellular ions was applied. Since the uptake curves of Fig. 1 lead to the ratio $k_{Cs}:k_{Rb} = 0{\cdot}04:0{\cdot}2 = 0{\cdot}2$ the mixture should give uptakes equal to those from 60 m-equiv Rb/l., and this is approximately the case. The analyses show that the tissue takes up about 1·5 times as much Rb as Cs. That the disproportion is not as great as that between the exchange coefficients demonstrates the consequence of interaction between the ions.

TABLE 3. Comparisons between observed sums of Rb + Cs uptakes from 50 m-equiv/l. each of Rb and Cs at given times and values predicted from the Rb curve (Fig. 1). 50 m-equiv Cs/l. is taken from the results of Fig. 1 as equivalent to 10 m-equiv Rb/l. The analyses are means of 3 results and have S.D. about ±2 u.

Time (min)	$(60t)^{\frac{1}{2}}$	Cations in tissue (m-equiv/kg) Rb	Cs	Sum	Value predicted from Rb exchange curve (m-equiv/kg)
10	24·5	5·3	2·5	7·8	8·3
25	38·7	8·2	5·7	13·9	14·8
60	60·0	13·7	10·2	23·9	24·0
100	77·4	18·1	9·8	27·9	31·6

Uptakes of labelled K in presence of Rb or Cs

At first sight it might seem that a simple solution would apply to the problem of labelled K uptakes from mixtures. However, this is not the case because there are three components in the system, namely ordinary K, labelled K and Rb (or Cs). The labelled K finds its way into a gradually diminishing reservoir of K and also encounters Rb (or Cs) ions in its passage.

Some K uptakes were measured by using muscles which had first been loaded overnight with Rb. Into these the K movement followed the Rb curve of Fig. 1, showing that the interchange coefficient k_{Rb} determines the rate whichever way round the experiment is performed. Using this one can approach the K uptake from K + Rb mixtures by writing the equation for equilibration of the outer region

$$\mathrm{d}K^*_a/\mathrm{d}t = k'[K]^*_e(N - K^*_a - Rb_a) - k_{\mathrm{Rb}}[Rb]_e K^*_a + k_{\mathrm{Rb}}[K]^*_e Rb_a. \qquad (7)$$

The second term on the right is the rate of loss by displacement of adsorbed K* by Rb and the last term is the rate of gain of labelled K by its displacement of adsorbed Rb. The equation cannot be integrated directly because it turns out that the K–K exchange coefficient in presence of Rb is a function of time, presumably because it depends on the Rb content of the outer region. Before proceeding evidence bearing on this will be given.

The course of uptake of labelled K from equal K concentrations with and without Rb present was compared without correction for extracellular ions. Figure 4, curves *A* and *B*, show that K uptake commences similarly from each solution. It is only after about 8 min when the tissue contains some 1·5 m-equiv K/kg that the curves diverge. At this time it can be calculated that the muscle in the Rb mixture contains just over 5 m-equiv Rb/kg. This quantity then gives an indication of how much tissue Rb is necessary for the K movement to be affected. The 1·5 m-equiv K/kg taken up exceeds by a factor of at least two the amount expected for extracellular material and the lag in Rb action cannot be attributed to its being delayed relative to the K in the extracellular space because in water both ions have

the same diffusivities. That the lag is really due to a requirement for a certain accumulation of Rb and is not a spurious effect stemming from extracellular K is further shown by the fact that exposure to Rb before the labelled K causes a perceptible reduction of even the earliest K uptake (Fig. 4, curve *C*).

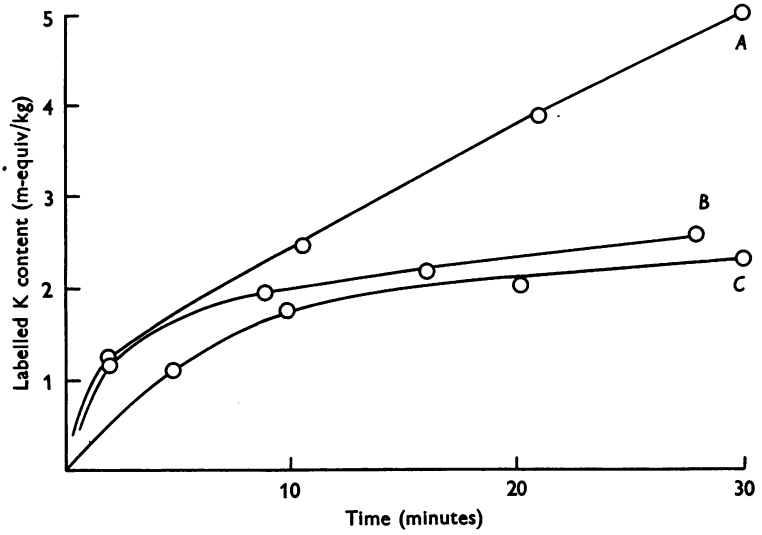

Fig. 4. The early parts of labelled K uptakes at 20° C from (*A*) 5 m-equiv K/l., (*B*) 5 m-equiv K/l. + 40 m-equiv Rb/l. applied at $t = 0$, and (*C*) from the same mixture as (*B*) but applied to a muscle pre-treated with 5 m-equiv K/l. + 40 m-equiv Rb/l. for 75 min before transfer to the labelled K mixture.

It was found that when experiments like that of Fig. 4*B*, in which the ratio of Rb:K applied was 4 or more, were treated in a way corresponding to that described for the Rb uptake from mixtures, by plotting the product of the labelled K content times $([K]_e + [Rb]_e)/[K]_e$ against $\{([K]_e + [Rb]_e)t\}^{\frac{1}{2}}$ the lines ran parallel to, but above, the standard Rb exchange line of Fig. 1. A rapid uptake of K at the beginning of the exposure was responsible for the upward displacement of the line, which took the longer to become parallel to the Rb curve the lower was the Rb concentration applied.

The kinetic results for labelled K uptakes were analysed and shown to fit the hypothesis that the exchange coefficient of K is ultimately reduced to equality to the K–Rb interchange coefficient k_{Rb} as the tissue Rb content increases. This was done in the following way. The actual uptake results for a number of mixtures was plotted (Fig. 5) and curves were computed by rough methods from the data for K and Rb movements already presented with an assumption about the relation between k' and the Rb

content of the tissue. If one integrates eqn. (7) over a short interval, so that k' can be taken as constant, the result is

$$\frac{K_a}{N} = \{1-\exp(-(k'[K]_e+k_{Rb}[Rb]_e)t)\} - \frac{[Rb]_e}{[Rb]_e+[K]_e}\{1-\exp(-k_{Rb}([K]_e+[Rb]_e)\,t)\}. \quad (8)$$

This is the difference between two exponential build-ups, so that the consequence of diffusion from this source can be obtained by taking the difference between the respective diffusions from (1st term) a source of K reaching a final concentration N with rate constant $(k'[K]_e+k_{Rb}[Rb]_e)$ and (2nd term) a source of K which reaches final concentration

$$N[Rb]_e/[Rb]_e+[K]_e$$

with rate constant $k_{Rb}([K]_e+[Rb]_e)$. Both quantities can be obtained from the K exchange curves, which are a particular form of transform of the function describing the source concentration. The quantities over the abscissae $\{(k'/k_K)\,[K]_e+(k_{Rb}/k_K)\,[Rb]_e)\,t\}^{\frac{1}{2}}$ correspond to the first term, while those over the abscissae $\{(k_{Rb}/k_K)\,([K]_e+[Rb]_e)\,t\}^{\frac{1}{2}}$ are reduced in the ratio $[Rb]_e/([Rb]_e+[K]_e)$ for the second term. It was arbitrarily chosen to make $k' = 0{\cdot}8\,k_K$ for such time as was necessary for the tissue to gain 6 m-equiv Rb/kg. This time was computed by the method used to plot Fig. 2 and values are listed in the legend of Fig. 5. In the interval between the tissue having 6 and 10 m-equiv Rb/kg the value of k' chosen was $0{\cdot}45\,k_K$, and finally for tissue Rb in excess of 10 m-equiv/kg k' was taken as equal to k_{Rb}.

The rough method of calculation shows that our results are consistent with the idea that the ease of K movement is progressively reduced to a final exchangeability equal to that of Rb as the tissue Rb content increases. If one had information about the quantity of Rb actually in the outer region, so that the law relating K exchange coefficient to the local Rb concentration was known, the problem could be applied to a computer.

Uptakes of K in presence of a given Cs concentration are similar to, or slightly higher than those found in presence of comparable concentrations of Rb. Despite its rate of entry being less than Rb, the Cs did not in the times used in our experiments exert a greater hindrance than Rb. This could be because its slower movement keeps the Cs concentration in the outer region at a level proportionately less than the Rb level. Two sets of the experimental points found in K+Cs mixtures have been shown on Fig. 5.

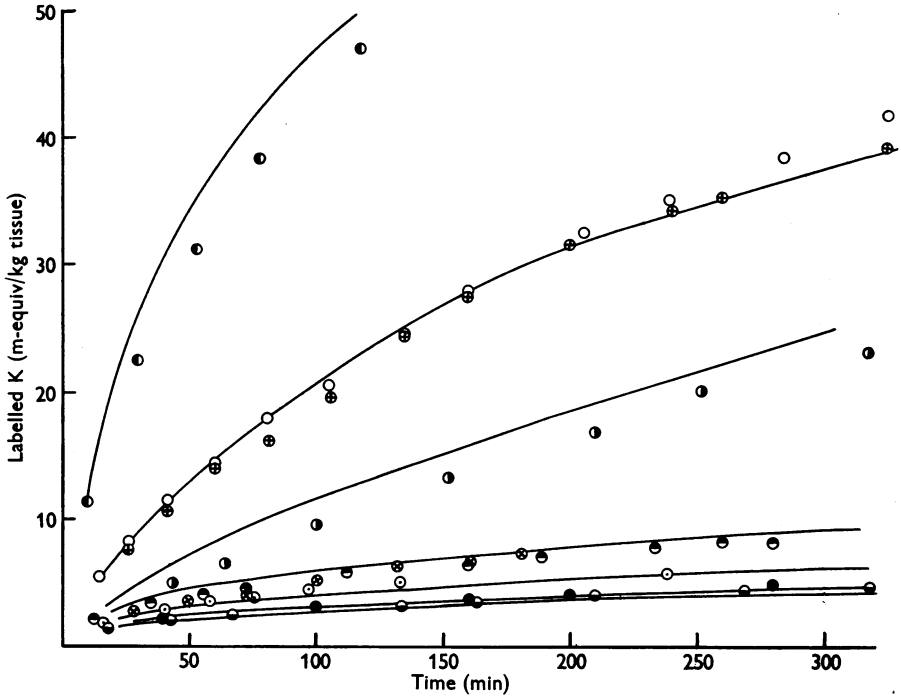

Fig. 5. Uptakes of labelled K at 20° C from mixtures with Rb or Cs corrected for the amount held in extracellular space equivalent to 0·1 ml./kg. The curves drawn have been computed according to the method described in the text which takes account of a reduction of the rate coefficient of K exchange as the tissue Rb content increases. The mean value of the coefficient, relative to that holding in Rb-free solution, was taken as 0·8 for Rb contents between 0 and 6 m-equiv/kg, 0·45 for Rb contents between 6 and 10 m-equiv/kg and 0·2 (the same as the Rb rate coefficient) for Rb contents exceeding 10 m-equiv/kg. The times at which the tissue Rb contents reached 6 and 10 m-equiv/kg were calculated by using in reverse the method applied for plotting Fig. 2.

Mixtures			Time (min) for Rb content to be:	
K	Rb (m-equiv/l.)	Symbol	6 m-equiv/kg	10 m-equiv/kg
46	21	◐	39	91
10	5	○	146	365
5	2·5	◑	291	730
5	20	◓	22	47
5	40	⊙	10	22
2·5	25	●	15	35
5	80	◒	5	11
	Cs			
10	5	⊕		
5	25	⊗		

No attempt to fit the results in the Cs mixtures was made, but it appears that a similar procedure would be applicable.

DISCUSSION

The nature of the results obtained has required their discussion in the body of this paper so here it is appropriate to refer to a few salient features. It can be said to be well known that K, Rb and Cs fall in a diminishing series of ease of penetration into muscle, and the figures derived from the relative exchange coefficients obtained by squaring the slopes of the linear parts of the curves in Fig. 1 are 1:0·2:0·04. It is suggestive that Rb and Cs not only compete with K but also reduce its exchangeability (as deduced earlier by Sjodin, 1959). Some such interaction, due in his view to a distortion of pore dimensions, has been proposed by Mullins (1956) to explain the action of ions which affect cell permeability. Our explanation of the effect rests rather on the greater adsorption of Rb and Cs on fixed sites along the channels through which the K must pass.

The model we have consistently used in presenting the K, Rb and Cs data requires that the cells 'outer region', which may be the membrane and reticular complex, should have a cation-exchange property holding not more than 10 m-equiv of K or K-like ion/kg tissue. Although various authors (e.g. Ling, 1960) have used competitive adsorption as the basis for interpretation of the interaction between cations entering muscle (not to mention the many papers concerned with other kinds of cell), our present approach extends this by attributing as much importance to the rate of equilibration of the ion-exchange region as to the ionic composition ultimately attained there.

In the results for exchange of muscle K, whether with labelled K (Harris & Sjodin, 1961) or with Rb, there has been no indication that the external Na concentration affects the process. On the other hand, the movement of the Na of high-Na muscles has been shown to be influenced by the external K concentration (Keynes, 1954; Edwards & Harris, 1957). That Na movement from high-Na muscle has an ion-exchange character may be inferred from the observation (Harris, 1950) that Na output to glucose solution is much slower than to an ion-containing medium. Keynes & Swan (1959), using media containing Li ions, have concluded that at least half the Na movement was an ion exchange. May we then regard the outer region proposed in our theory as accommodating both Na and K ions but in separate channels or on different sites? Since K movement is so insensitive to Na, it appears that the latter does not compete for the K sites, while the influence of K on Na movement, and the possibility of displacing muscle Na by K (Carey & Conway, 1954, Table 1) shows that K can occupy Na sites. It seems possible that the slowing of K movement by Rb ions may have the same mechanism as the acceleration of Na movement by K ions. In each case a group or line of ions may be involved and an essential inter-

change movement has a higher probability when a K ion is on a particular site than when either Rb or Na is present. That in some cells Na exchange may, in some instances, follow the same law as K and Rb exchanges follow in muscle, can be inferred from the curves for human erythrocytes given by Harris (1960, Figs. 5, 6). The slopes of the uptake curves plotted against $(\text{time})^{\frac{1}{2}}$ are roughly in proportion to the square root of [Na] applied.

The reduction that we find of the K exchangeability to the K–Rb value as the muscle Rb increases suggests that the adsorption sites in the outer region are present in sufficient depth to ensure that when 10 m-equiv Rb/kg has been reached there is at least one Rb ion in each pathway for K exchange.

It is notable that no conditions were found in the present K + Rb experiments in which the labelled K content would rise through a maximum and fall subsequently as Rb entered. From the beginning the uptake from a mixture is constrained to proceed towards its eventual equilibrium level with equal (or nearly equal) internal and external Rb:K ratios. This is itself evidence that the Rb:K ratio in the outer region is established early in the process and thereafter controls the ratio of Rb to K entering the tissue.

The impediment of K movement by Rb and Cs should have an effect on the electrical properties of the tissue. Since normally the Cl ion carries some $\frac{2}{3}$ of the current (Hodgkin & Horowicz, 1959; Hutter & Noble, 1960) any change of the cation component of conductance will best be seen in absence of Cl (or other penetrating anions). A qualitative experiment in which the internal–external resistance of few fibres from each of a pair of muscles in solution containing respectively 3 mM-K or Rb with Na methyl sulphate showed that the values in the Rb solution were 2–4 times those in the K solution. Experiments now in progress indicate that the Cl exchange process follows the same pattern as that we have described for the cations.

SUMMARY

1. The uptakes of Rb and Cs by frog sartorius muscles have been measured and the interaction between K, Rb and Cs has been studied.

2. The uptakes of Rb from various mixtures of Rb and Na salts and the uptakes of Cs from various mixtures of Cs and Na salts are made to fall along single curves for a given temperature by the expedient of plotting them against a function of (concentration × time of exposure).

3. By choosing the square root of (concentration × time) as abscissa the respective uptake curves for Rb and Cs can be compared with that previously obtained for K exchange.

4. The results are interpreted, using as model an interior compartment

fed from an outer region having ion-exchange properties. The relative exchangeabilities of K for K, Rb and Cs respectively are as 1:0·2:0·04.

5. Uptakes of Rb or Cs from mixtures with K or with each other are shown to be consistent with the predictions from the model. The results can be fitted to the curves obtained for the single ions.

6. Entry of Rb into previously K-depleted muscles so that Na is displaced by Rb is faster than the interchange of Rb with the muscle K.

7. Uptake of labelled K from mixtures with Rb or Cs follows a course consistent with the hypothesis that when Rb or Cs has entered the ion-exchange region the K exchange is slowed down. In the case of Rb+K mixtures the K exchange ultimately falls to the same rate as holds for Rb–K interchange, but Cs takes longer to exert its full inhibitory effect.

REFERENCES

CAREY, M. & CONWAY, E. J. (1954). Comparison of various media for immersing frog sartorii at room temperature and evidence for the regional distribution of fibre Na^+. *J. Physiol.* **125**, 232–250.

EDWARDS, C. & HARRIS, E. J. (1957). Factors influencing the Na movement in frog muscle with a discussion of the mechanism of Na movement. *J. Physiol.* **135**, 567–580.

HARRIS, E. J. (1950). Transfer of Na and K between muscle and the surrounding medium. 2. The Na flux. *Trans. Faraday Soc.* **46**, 872–882.

HARRIS, E. J. (1960). *Transport and Accumulation in Biological Systems*, 2nd ed. London: Butterworth.

HARRIS, E. J. & SJODIN, R. A. (1961). Kinetics of exchange and net movement of frog muscle K. *J. Physiol.* **155**, 221–245.

HODGKIN, A. L. & HOROWICZ, P. (1959). The influence of K and Cl ions on the membrane potential of single muscle fibres. *J. Physiol.* **148**, 127–160.

HUTTER, O. & NOBLE, D. (1960). The chloride conductance of frog skeletal muscle. *J. Physiol.* **151**, 89–102.

KEYNES, R. D. & SWAN, R. C. (1959). The effect of external Na concentration on the Na fluxes in frog skeletal muscle. *J. Physiol.* **147**, 591–625.

KEYNES, R. D. (1954). The ionic fluxes in frog muscle. *Proc. Roy. Soc.* B, **142**, 359–382.

LING, G. N. (1960). The interpretation of selective ionic permeability and cellular potentials in terms of the fixed charge induction hypothesis. *J. gen. Physiol.* **43**, Suppl. 149–174.

LUBIN, M. & SCHNEIDER, P. B. (1957). The exchange of potassium for caesium and rubidium in frog muscle. *J. Physiol.* **138**, 140–155.

MULLINS, L. J. (1956). The Structure of Nerve Cell Membranes, in *Molecular Structure and Functional Activity of Nerve Cells. Amer. Inst. Biol. Sc. Symp.* pp. 123–154.

SJODIN, R. A. (1959). Rubidium and caesium fluxes in muscle as related to the membrane potential. *J. gen. Physiol.* **42**, 983–1003.

USSING, H. H. (1949). Transport of ions across cellular membranes. *Physiol. Rev.* **29**, 127–155.