J. Physiol. (1973), **228**, *pp.* 1–11
With 3 text-figures
Printed in Great Britain

THE INFLUENCE OF EXTERNAL CAESIUM IONS ON POTASSIUM EFFLUX IN FROG SKELETAL MUSCLE

BY L. A. BEAUGÉ,* A. MEDICI AND R. A. SJODIN

From the Department of Biophysics, University of Maryland School of Medicine, Baltimore, Maryland 21201, U.S.A.

(*Received 27 March 1972*)

SUMMARY

1. At a concentration of 2·5 mM in the external solution, Cs ions reduced K efflux in muscles incubated in Na media. This effect was demonstrated in the presence or absence of 2·5 mM-K and in the presence or absence of 10^{-4} M ouabain.

2. In Ringer solution in which NaCl was replaced by an osmotic equivalent of $MgCl_2$, external Cs ions increased K efflux if the solution was K-free and decreased K efflux if the solution contained 2·5 mM-K.

3. External Cs ions reduced the inward rate of leakage of Na ions into muscle cells by about 25 % when the medium was K-free and contained 10^{-5} M ouabain.

4. The effects of 2·5 mM-K ions and 2·5 mM-Cs ions on the muscle fibre membrane potential were about the same. The influence of Cs ions on K efflux cannot be explained by changes in the resting membrane potential.

5. The results suggest that a large part of the K efflux from muscle cells is mediated by a K:K exchange mechanism that is inhibited by external Cs ions.

6. The results also suggest that a smaller part of the K efflux is due to K:Na exchange that is also inhibited by external Cs ions.

7. In the absence of either external K or Na ions for internal K to exchange with, Cs ions promote a small amount of K exchange, perhaps via both mechanisms.

INTRODUCTION

The presence of the foreign cations, Rb^+ and Cs^+, in the external medium has been shown to slow the movements of K ions across the muscle cell membrane and so reduce the rate of K exchange (Sjodin, 1959, 1961; Bolingbroke, Harris & Sjodin, 1961). These workers regarded the observed cation interactions as due to competition for fixed sites in the mem-

* Permanent address: Instituto de Investigación Médica, Mercedes y Martín Ferreyra, Casilla de Correo 389, Córdoba, Argentina.

brane at which K exchanges occur. Cs ions have been shown to exchange with muscle Na ions by an active process that is abolished by the drug strophanthidin (Beaugé & Sjodin, 1968). The rate of exchange of external Cs ions with muscle K was much lower than the rate of exchange with Na, indicating a very low permeability of the muscle cell membrane to Cs ions. The permeability of the muscle fibre membrane to Cs ions was reckoned to be nearly the same as the permeability to Na ions (Sjodin & Beaugé, 1967; Beaugé & Sjodin, 1968). In addition to these actions, Cs ions depolarize the muscle fibre membrane potential when applied externally (Sjodin, 1959). The rank order of cation permeability observed was $P_K > P_{Rb} > P_{Cs}$.

Cs ions, therefore, affect both passive and active processes in muscle cell membranes. The purpose of the present study was to further characterize the passive effect by determining the influence of externally applied Cs ions on K efflux and the resting membrane potential.

METHODS

All experiments were performed on carefully dissected sartorius muscles from the frog, *Rana pipiens*.

Solutions. The following solutions were used (mM): (*a*) K-free Na Ringer; NaCl 120; $CaCl_2$ 2; Tris-Cl, pH (20° C) 7·4, 1; (*b*) K-free Mg Ringer, which had the same composition as solution *a* except that all the Na had been replaced by an isosmotic equivalent of $MgCl_2$; (*c*) K, Cs or K+Cs Ringer formed by adding proper amounts of each cation to the K-free solutions to obtain the desired concentrations. All chemicals were reagent grade and deionized water was used in preparation of solutions. Strophanthidin and ouabain were obtained from Sigma Co., U.S.A., and stored in stock solutions at low temperature for no more than 1 week.

Potassium efflux. The conditions of loading were long enough to obtain at least 60–80 % isotopic equilibration. Fresh muscles were loaded 5 hr at room temperature in 5 mM-K Na Ringer. To obtain ^{42}K-loaded muscles with elevated Na contents, muscles were first loaded in the usual way and then were transferred to a 0·1 mM-K Na Ringer with the same specific activity and kept overnight at 4° C. The technique for efflux has been described in detail elsewhere (Sjodin & Henderson, 1964) and consisted in passing the muscles, tied to Pt frames, through a series of tubes with 5 ml. of the appropriate solutions. The rate constants for ^{42}K efflux were calculated from a semilogarithmic plot of the counts remaining in the muscle against time.

Cation content. The determination of Na and K contents of the muscles was performed using the method described by Sjodin & Beaugé (1973). The method involves extrapolation to zero of the semilogarithmic plots of Na and K loss as a function of time when muscles are in a K-free, Na-free, Mg Ringer with 10^{-4} M ouabain. This method gives the intracellular cation contents as distinct from the total cation contents. A specially designed flame photometer with Baird Atomic interference filters was used.

Membrane potentials. Resting membrane potentials were measured using glass micro-electrodes. Electrodes were filled with 3 M-KCl solution and had tip potentials below 5 mV. Values were read to the nearest millivolt with a Tektronix oscilloscope and a digital voltmeter, using a preamplifier having a grid current less than 10^{-12} A.

RESULTS

The effect of external Cs ions on K ion efflux

Addition of 2·5 mM-Cs^+ to the external medium reversibly reduced the efflux of K ions from muscles in Na-containing Ringer solution under all conditions employed. The results of typical experiments are shown in Figs. 1 and 2. The rate constants for loss of ^{42}K from muscles under different conditions are summarized in Table 1. Two facts deserve mention. Cs ions

TABLE 1. The influence of external Cs ions on K efflux in skeletal muscle incubated in Na media. Those values in the same row correspond to measurements made on the same muscle

	Rate constants for ^{42}K loss (hr^{-1})			
	K-free	2·5 mM-K	2·5 mM-Cs	2·5 mM-Cs + 2·5 mM-K
	0·0585	—	0·0431	0·0770
	0·0621	0·162	—	0·0815
	0·0668	0·145	0·0421	—
	0·0990	—	0·0770	—
	0·0517	—	—	—
	—	0·236	0·0954	—
	—	0·192	—	—
	0·0555	—	0·0489	0·0724
	0·0612	0·159	—	0·0789
	0·0678	0·161	0·0385	—
	0·0507	0·127	0·0440	—
	0·0770	—	0·0431	—
	0·0555	—	—	—
	—	0·129	0·0575	—
	0·0630	—	0·0363	0·0761
	0·0715	0·147	—	0·0825
	0·0583	0·154	0·0479	—
	0·0575	0·125	0·0415	—
	0·0488	—	0·0306	—
	0·0396	—	—	—
	0·0536	—	0·0354	0·0612
	0·0545	0·109	—	0·0583
	0·0583	0·142	0·0440	—
	0·0621	0·150	0·0593	—
	0·0668	—	0·0565	—
	—	0·140	0·0430	—
	—	0·119	—	—
	0·0470	0·105	0·0286	—
	0·0640	0·135	0·0360	—
	0·0660	—	0·0390	0·0580
	0·0530	—	0·0340	0·0490
	0·0520	0·096	—	0·0430
Mean	0·0601*	0·143**	0·0464*	0·0671**
S.E.	0·0026	0·0068	0·0033	0·0041

* $P < 0·002$, ** $P < 0·001$.

and K ions affect K efflux differently. When 2·5 mM-Cs^+ is substituted for 2·5 mM-K^+ in the medium, K efflux declines threefold, on the average. Also, when 2·5 mM-Cs^+ is added to the medium in the presence of 2·5 mM-K^+, K efflux declines to about the value observed in a K-free, Cs-free medium.

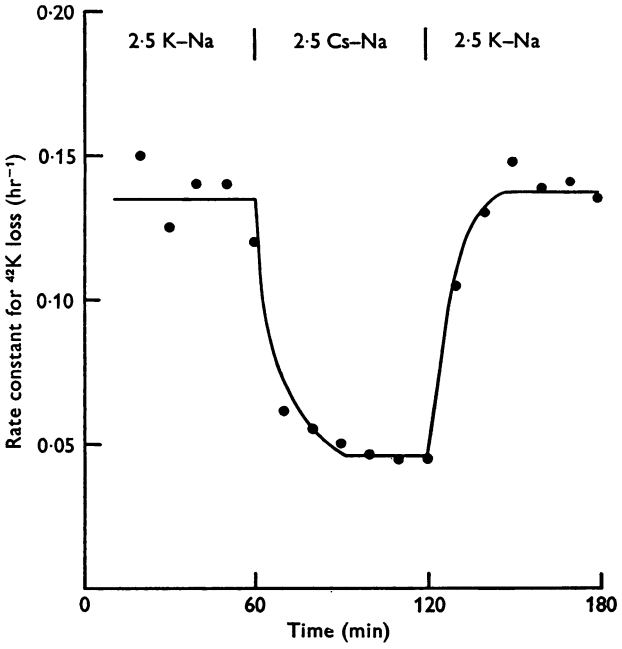

Fig. 1. The reversible effect on K efflux of substitution of 2·5 mM-Cs for 2·5 mM-K in Ringer solution.

The effect of external Cs ions on the resting membrane potential

As Cs^+ and K^+ had considerably different effects on K efflux, it was of interest to compare the effects of Cs^+ and K^+ on the resting membrane potential. The results of approximately 350 micro-electrode penetrations on several different muscles are shown in Table 2. Some measurements were also made in the presence of 10^{-5} M strophanthidin to remove any contributions to the membrane potential of an electrogenic Na pump. In marked contrast to the effects on potassium efflux, Cs^+ and K^+ had nearly the same effect on the resting potential. Using the potential in K-free Ringer solution as a reference, 2·5 mM-Cs^+ depolarized the membrane by 15·7 mV, on the average, compared with 18·5 mV of depolarization caused by 2·5 mM-K^+. The depolarizing action of 2·5 mM-Cs^+ on the muscle fibre membrane is not likely to be due to an inward Cs ionic current because the depolarization persists in the presence of 10^{-5} M strophanthidin, which

has been shown to reduce Cs^+ influx to very low values (Beaugé & Sjodin, 1968). The inward Cs current in the presence of strophanthidin is about one hundredth of the inward K current at the same external concentra-

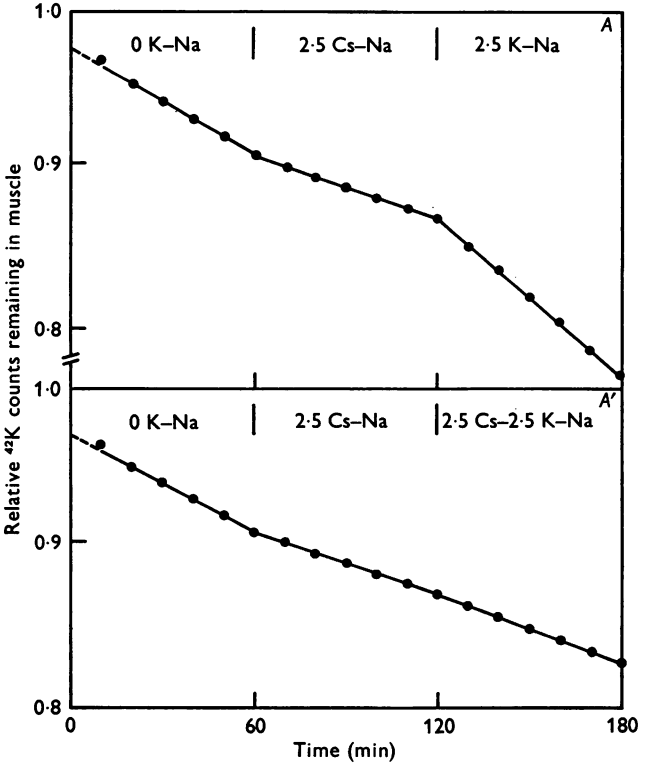

Fig. 2. Typical experiments showing the effect of 2·5 mM-Cs ions on the ^{42}K efflux in frog sartorius muscles incubated in Na Ringer solution. Muscles *A* and *A'* were dissected from the same frog.

TABLE 2. The effect of 2·5 mM-Cs on the muscle resting membrane potential compared with the effect of 2·5 mM-K. All measurements were made in Ringer solutions with the same normal Na ion concentration with $[K]_o$ or $[Cs]_o$ as indicated. *n* refers to the total number of micro-electrode penetrations made on several different muscles

	Membrane potentials (mV)				
	Control			10^{-5} M Strophanthidin	
	K-free	2·5 mM-K	2·5 mM-Cs	2·5 mM-K	2·5 mM-Cs
Mean	−107·3†	−88·8*	−91·6†*	−86·9**	−89·9**
S.E.	0·52	0·36	0·88	0·56	1·09
n	(110)	(104)	(95)	(22)	(24)

* $P < 0.005$; ** $P < 0.02$; † $P < 0.001$.

tion, yet the depolarizations produced in the two cases are comparable. In view of the fact that Cs^+ depresses K efflux, it seems that the depolarization induced by Cs^+ may result from a depressing action of caesium on the K ion permeability, P_K. Hodgkin & Horowicz (1959) found the resting potential of frog muscle fibres to be satisfactorily described by the following equation:

$$E = \frac{RT}{F} \ln \frac{[K]_o + \alpha[Na]_o}{[K]_i + \alpha[Na]_i}, \quad (1)$$

where R, T, and F have their usual meanings, square-bracketed quantities refer to ionic concentrations outside and inside the fibres and $\alpha = P_{Na}/P_K$, the ratio of the Na to the K ionic permeability. When $\alpha = 0{\cdot}012$, the resting potential calculated from eqn. (1) is -90 mV, which is close to the average measured value. It should be noticed that the chloride ion concentrations do not appear in eqn. (1) because it is assumed that chloride ions have reached an equilibrium distribution at the time potentials are recorded. When 2·5 mM external K^+ is replaced by 2·5 mM-Cs^+, eqn. (1) can be solved for α if the membrane potential is known. Using the measured resting potential in 2·5 mM-Cs Ringer, α_{Cs} is calculated to be 0·031. The small contribution due to the term $P_{Cs}[Cs]_o$ is negligible as P_{Cs} is approximately $10^{-2} P_K$. The conclusion is that the ratio P_{Na}/P_K has increased about threefold in the presence of 2·5 mM-Cs^+. The question arises as to how much of this change is due to a change in P_{Na} and how much to a change in P_K.

The influence of external Cs ions on the resting membrane permeability to Na ions

To answer this question, the effect of Cs on Na net influx was investigated. Na inward leakage rate was determined in a K-free medium containing 10^{-5} M strophanthidin to abolish the action of the Na pump. Net Na influx was measured by the method of Sjodin & Beaugé (1973). Experiments were performed on paired muscles from the same frog in the presence and absence of external Cs ions. Results are presented in Table 3. The presence of 10 mM external Cs^+ reduced net Na influx by 23 %, on the average. As 10 mM-Cs^+ in the medium causes a further depolarization of the membrane potential (Sjodin, 1959), some of the decreased influx could be due to this factor. Furthermore, when similar experiments were performed in 2·5 mM-Cs^+ solutions, the change in Na influx was considerably less and of dubious statistical significance for the same number of experiments. It is concluded that by far the greater part of the increase in α caused by 2·5 mM external Cs is due to a decrease in P_K. In fact, we conclude that P_K falls approximately threefold in the presence of external Cs ions, a conclusion also reached by Sjodin (1959).

TABLE 3. The effect of external Cs ions on Na net influx in muscles incubated in K-free, 120 mM-Na Ringer solution containing 10^{-4} M ouabain. Comparisons are made of final intracellular Na contents of paired muscles incubated for the same time interval when one muscle was in the absence of Cs and the paired muscle was in the presence of Cs. Net influxes were determined from a knowledge of final concentrations, the average initial Na concentration for over twelve muscles and the time interval

Expt. no.	Final $[Na]_i$ (μmole/g) 0 mM-Cs	Final $[Na]_i$ (μmole/g) 10 mM-Cs	$\Delta[Na]_i$ (μmole/g)	Δt (hr)	Na influx† (μmole/g.hr) 0 mM-Cs	Na influx† (μmole/g.hr) 10 mM-Cs
36*AA'*	23·68	20·66	−3·02	6	2·65	2·15
36*BB'*	22·67	21·90	0·77	6	2·48	2·35
36*CC'*	35·50	23·83	−1·67	6	2·95	2·67
37*AA'*	27·43	23·35	−4·08	5	3·93	3·11
37*BB'*	28·58	20·86	−7·72	5	4·16	2·61
37*CC'*	21·46	16·11	−5·35	5	2·73	1·66
Mean	24·89*	21·12*	−3·77	—	3·15**	2·43**
S.E.	1·14	1·13	—	—	0·29	0·20

* $P < 0·05$; ** $P < 0·05$.

† Taking $[Na]_i$ at zero time as the average internal Na in fresh muscles, which was found to be 7·79 μmole/g.

TABLE 4. The influence of Na ion concentrations and 10^{-4} M ouabain on the inhibitory action of Cs ions on K efflux. The term 'elevated' when referring to $[Na]_i$ means that the intracellular Na contents of muscles had been raised to over 3 times the normal value before the experiment. Muscles with Na contents in this range were previously shown to have uniform flux characteristics for both K and Na ions (Sjodin & Beaugé, 1968)

Conditions $[Na]_i$, $[Na]_o$	Conditions 10^{-4} M ouabain	Mean rate constants for ^{42}K loss (hr^{-1}) ± S.E. K-free	2·5 mM-K	2·5 mM-Cs	2·5 mM-K and 2·5 mM-Cs
Elevated, 120	−	0·0826 ± 0·0053	0·130 ± 0·008	0·0458 ± 0·0037	0·0720 ± 0·0014
Elevated, 120	+	0·0918 ± 0·0128	0·176 ± 0·012	0·0613 ± 0·0083	0·0968 ± 0·0027
Normal, 120	+	0·0636 ± 0·0013	0·170 ± 0·006	0·0448 ± 0·0056	0·121 ± 0·003
Normal, 0	−	0·0245 ± 0·0036	0·0887 ± 0·0093	0·0353 ± 0·0043	0·0530 ± 0·0110
Elevated, 0	−	0·0080 ± 0·0024	0·1035 ± 0·0153	0·0309 ± 0·0055	0·0845 ± 0·0255

The influence of Na ion concentrations and pumping rates on the inhibitory action of Cs on K efflux

As previously discussed, external Cs ions have at least two actions on muscle cell membranes, an effect on K movements and an activating effect on the Na pump. In an attempt to see if the two actions are in any way related or if one action can offset or modify the other, the influence of Cs ions on K efflux was studied under conditions in which the Na pumping rate would be expected to vary widely. Na pumping rates were increased

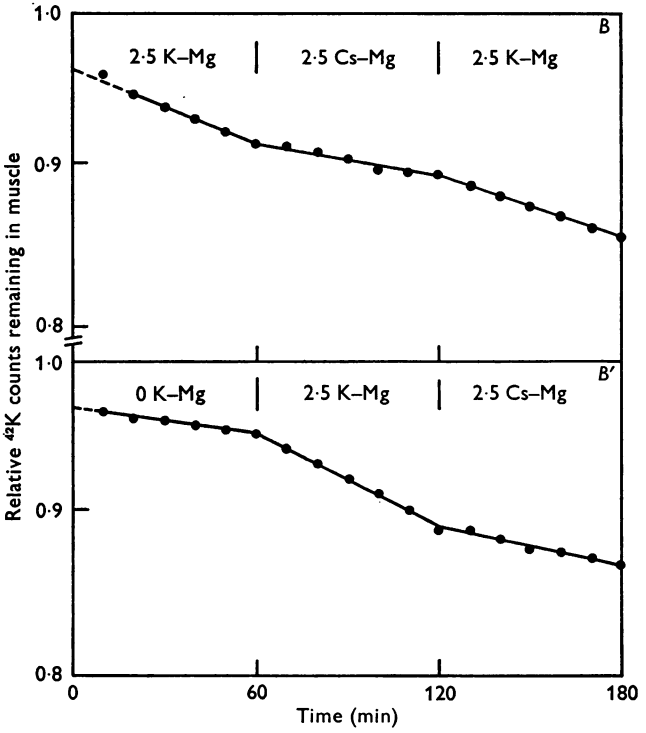

Fig. 3. Typical experiments showing the effect of 2·5 mM-Cs ions on the ^{42}K efflux in sartorius muscles incubated in Na-free, $MgCl_2$-substituted Ringer solutions. Muscles *B* and *B′* were dissected from the same frog.

by elevating the internal Na ion concentration as previously described (Sjodin & Beaugé, 1968; Sjodin, 1971). Na pumping rates were reduced by application externally of 10^{-4} M ouabain. Some experiments were also performed in Na-free $MgCl_2$ substituted solutions to determine the influence of the external Na ion concentration (Fig. 3). Results are summarized in Table 4. Under all conditions employed, external Cs ions reduced K efflux

significantly in the presence of 2·5 mM external K. In Na-free media, both the percentage and absolute reductions in K efflux occasioned by the presence of Cs ions were less. In addition, external Cs ions increased K efflux in Na-free media if K ions were also absent from the medium whereas, in Na Ringer solutions, Cs ions always reduced and never increased the K ion efflux. The effect of Cs ions on K efflux appears to be insensitive to the rate of Na ion pumping but dependent on the presence of external Na ions.

DISCUSSION

Replacement of 2·5 mM-K in Ringer solution with 2·5 mM-Cs reduced K ion efflux threefold and produced a small change in the resting membrane potential, a hyperpolarization of about 3 mV. Calculations of relative P_K from electrical potential measurements indicate that the external Cs concentration of 2·5 mM has also reduced P_K about threefold. Had K movement been under the control of the membrane potential alone during substitution of Cs, K efflux would be expected to decline only slightly for a 3 mV hyperpolarization as the K-dependent steady-state current–voltage relation in frog muscle is approximately linear (Adrian, Chandler & Hodgkin, 1970). This argument, however, is based on the principle of independence of the K ionic fluxes in the absence of Cs ions. There exists some experimental evidence in favour of this assumption (Sjodin, 1965).

As K fluxes are clearly not independent in the presence of Cs, one should examine the possibility that removal of external K ions *per se* has produced a change in K efflux that is separate from the effect of either Cs^+ or the membrane potential. In the absence of flux measurements during a voltage clamp, it is not possible to make a complete analysis. One can, however, examine the case in which K ions are removed from the medium in the absence of Cs ions. Under these conditions, K efflux declined by over twofold and the muscle fibre membranes became hyperpolarized by almost 20 mV. From these experiments alone, it is not possible to decide whether this change in K^+ efflux is due to the disappearance of a K:K exchange upon reducing $[K]_o$ to zero or to the membrane hyperpolarization or to some combination of both. When 2·5 mM-Cs^+ is added to K-free Ringer solution bathing muscles, the membrane potential is restored to within 3 mV of the value measured in 2·5 mM K Ringer solution. If K efflux were determined solely by the membrane potential, the rate constant for ^{42}K loss should have increased by approximately 2·5-fold. Actually, the rate constant was decreased 23 % by this experimental operation. Whatever the method applied to analyse the present data, it is evident that Cs ions have reduced P_K approximately threefold or produced an equivalent reduc-

tion in a K:K exchange mechanism. The fact that the P_K changes deduced from flux and membrane potential measurements agree rather well indicates that, should a K:K exchange mechanism be present in the muscle membrane, the permeability of the membrane to potassium ions is probably intimately related to the same mechanism.

In Na-free, $MgCl_2$-substituted Ringer solution, a further reduction in K efflux was observed. This reduction in efflux has been also reported by Sjodin & Beaugé (1973) and a similar reduction in Na efflux has been observed (Beaugé & Ortiz, 1970). This effect on K efflux indicates either a stimulating action of external Na ions or an inhibitory action of Mg ions or both. When Cs ions were added to K-free $MgCl_2$ Ringer solution, K efflux increased. The increase is most marked in muscles previously elevated in internal Na. This Cs^+-induced increase in K efflux is interesting because it was never observed in the presence of external Na ions.

A hypothesis that is consistent with all of these observations is that K efflux from muscle occurs by way of K:K and K:Na exchanges, possibly via the same membrane sites with differing affinities for K and Na. External Cs ions can be postulated to occupy these sites competitively but to exchange with muscle K at a rate lower than either the K:K or K:Na exchanges. Thus, in the presence of external K or Na, Cs ions would competitively inhibit the two kinds of exchange whereas in the absence of external K and Na, where neither type of exchange could occur, Cs ions would increase K efflux by inducing a K:Cs exchange. Though this interpretation of the results seems entirely reasonable, further experimentation would be required to establish firmly the postulated cationic exchanges.

This work was supported by U.S. Public Health Service Research Grant NS-07626 to R. A. Sjodin from the National Institute of Neurological Diseases and Stroke.

REFERENCES

ADRIAN, R. H., CHANDLER, W. K. & HODGKIN, A. L. (1970). Slow changes in potassium permeability in skeletal muscle. *J. Physiol.* **208**, 645–668.

BEAUGÉ, L. A. & ORTIZ, O. (1970). Lithium-stimulated sodium efflux in frog skeletal muscle. *Biochim. biophys. Acta* **219**, 479–483.

BEAUGÉ, L. A. & SJODIN, R. A. (1968). Transport of caesium in frog muscle. *J. Physiol.* **194**, 105–123.

BOLINGBROKE, V., HARRIS, E. J. & SJODIN, R. A. (1961). Rubidium and caesium entry and cation interaction in frog skeletal muscle. *J. Physiol.* **157**, 289–305.

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

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

SJODIN, R. A. (1961). Some cation interactions in muscle. *J. gen. Physiol.* **44**, 929–962.

SJODIN, R. A. (1965). The potassium flux ratio in skeletal muscle as a test for independent ion movement. *J. gen. Physiol.* **48**, 777–795.

SJODIN, R. A. (1971). The kinetics of sodium extrusion in striated muscle as functions of the external sodium and potassium ion concentrations. *J. gen. Physiol.* **57**, 164–187.

SJODIN, R. A. & BEAUGÉ, L. A. (1967). Strophanthidin-sensitive transport of cesium and sodium in muscle cells. *Science, N.Y.* **156**, 1248–1250.

SJODIN, R. A. & BEAUGÉ, L. A. (1968). Strophanthidin-sensitive components of potassium and sodium movements in skeletal muscle as influenced by the internal sodium concentration. *J. gen. Physiol.* **52**, 389–407.

SJODIN, R. A. & BEAUGÉ, L. A. (1973). An analysis of the leakages of sodium ions into and potassium ions out of striated muscle cells. *J. gen. Physiol.* (In the press.)

SJODIN, R. A. & HENDERSON, E. G. (1964). Tracer and non-tracer potassium fluxes in frog sartorius muscle and the kinetics of net potassium movement. *J. gen. Physiol.* **47**, 605–638.