The Dual Effect of Lithium Ions on Sodium Efflux in Skeletal Muscle

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ABSTRACT Sartorius muscle cells from the frog were stored in a K-free Ringer solution at 3°C until their average sodium contents rose to around 23 mM/kg fiber (about 40 mM/liter fiber water). Such muscles, when placed in Ringer's solution containing 60 mM LiCl and 50 mM NaCl at 20°C, extruded 9.8 mM/kg of sodium and gained an equivalent quantity of lithium in a 2 hr period. The presence of 10^{-5} M strophanthidin in the 60 mM LiCl/50 mM NaCl Ringer solution prevented the net extrusion of sodium from the muscles. Lithium ions were found to enter muscles with a lowered internal sodium concentration at a rate about half that for entry into sodium-enriched muscles. When sodium-enriched muscles labeled with radioactive sodium ions were transferred from Ringer's solution to a sodium-free lithium-substituted Ringer solution, an increase in the rate of tracer sodium output was observed. When the lithium-substituted Ringer solution contained 10^{-5} M strophanthidin, a large decrease in the rate of tracer sodium output was observed upon transferring labeled sodium-enriched muscles from Ringer's solution to the sodium-free medium. It is concluded that lithium ions have a direct stimulating action on the sodium pump in skeletal muscle cells and that a significantly large external sodium-dependent component of sodium efflux is present in muscles with an elevated sodium content. In the sodium-rich muscles, about 23% of the total sodium efflux was due to strophanthidin-insensitive Na-for-Na interchange, about 67% being due to strophanthidin-sensitive sodium pumping.

INTRODUCTION

Levi and Ussing (1948) and Ussing (1949) suggested that some sodium might cross the membrane of skeletal muscle cells by a sodium-for-sodium exchange process they termed "exchange diffusion." A feature of this process is that, as originally hypothesized, it does not involve the conversion of metabolic energy into secretory work. Some hint that such a process might be operative in

skeletal muscle cells was provided by the work of Keynes and Maisel (1954) which showed that the sodium efflux occurring in frog muscle cells is rather insensitive to the presence of various metabolic inhibitors. Keynes and Swan (1959 *a*) obtained more direct evidence that such a process is operative in muscle cells when they observed that the efflux of ^{24}Na from freshly dissected frog muscles is reversibly reduced to approximately one-half when the sodium in the external medium is replaced with lithium. These authors observed that the response of sodium efflux to a Na-free solution disappeared if muscles remained in normal Ringer's solution for more than 12 hr or in K-free Ringer's solution for about 1 or 2 hr. The disappearance of the Na-free effect was attributed to a rise in the internal sodium concentration. The previous paper, Sjodin and Beaugé (1968 *b*), showed that if freshly dissected muscles are allowed to lose sodium rather than gain sodium, the reduction in sodium efflux observed when Na in the external medium is replaced by Li becomes much greater than that observed when freshly dissected muscles are employed.

The main purpose of this investigation was to examine the question of why the reduction of sodium efflux in a solution with Na replaced by Li disappears as the internal sodium concentration rises. To demonstrate the existence of the exchange diffusion process previously proposed by Ussing (1949), a cation must be employed to replace sodium ions in the Ringer solution. The cation employed as a sodium substitute must meet two requirements; it must have a negligibly small affinity for the sites involved in the Na-for-Na exchange process and it must not have any significant action on the remaining outwardly directed sodium transport process or processes.

It is well-known that the presence of external potassium is a requisite for the operation of the mechanism responsible for the net outward extrusion of sodium from muscles against an electrochemical gradient (Steinbach, 1940; Conway and Hingerty, 1948; Desmedt, 1953). The potassium requirement of the sodium transport system is a general property found in a variety of cells as it has been observed in squid giant axons (Hodgkin and Keynes, 1955) in, erythrocytes (Glynn, 1956), in crab nerve (Baker and Connelly, 1966), and in giant muscle fibers from the barnacle (Beaugé and Sjodin, 1967). The ion selectivity of the sodium transport process normally requiring K ions is not absolute. Rubidium ions have a very significant K-like action on sodium efflux from muscle cells (Sjodin, 1961; Adrian and Slayman, 1966) and from squit giant axons (Sjodin and Beaugé, 1967 *a*, 1968 *a*). Cesium ions were found to be capable of promoting the extrusion of sodium from skeletal muscle cells (Sjodin and Beaugé, 1967 *b;* Beaugé and Sjodin, 1968) and from giant axons of the squid (Sjodin and Beaugé, 1967 *a*, 1968 *a*).

In view of the fact that two other group I alkali metal cations exert a significant K-like action on the sodium pump in muscle cells, it seemed wise to test

also the effect of lithium ions on sodium extrusion. If lithium ions exert a significant action on the sodium extrusion mechanism, it is clear that one of the requirements for use as a sodium replacement in demonstrating exchange diffusion is not met by lithium.

METHODS

The methods employed were very similar to those employed in the previous paper (Sjodin and Beaugé, 1968 *b*). To test the ability of lithium ions to promote the extrusion of sodium, pairs of sartorii were carefully dissected from specimens of *Rana pipiens* and were subsequently enriched with sodium by the technique reported in the previous paper. Again, 40 hr storage in K-free Ringer's solution at 3°C was employed. The average sodium content attained was 23.4 mM/kg. Making use of the average value of the extracellular space, 22%, and the average value of the fiber water content, 76%, the internal sodium concentration attained was, on the average, very close to 40 mM/liter fiber water. One of a pair of sodium-enriched sartorii was then prepared for analysis to obtain the initial sodium content. The other member of the pair was placed in Ringer's solution containing 60 mM LiCl and 50 mM NaCl and gently stirred at 20°C for a period of 2 hr, after which it was prepared for analysis. Before preparing for analysis, all muscles were rinsed for 10 min in a tris-substituted K-free and Na-free Ringer solution containing 2 mM $CaCl_2$ and Tris neutralized to a pH of 7.35. The Tris wash solution was osmotically matched to normal Ringer's solution. The 10 min rinse in the K- and Na-free medium removed the extracellular K and Na without significant alteration of the intracellular concentrations. Preparation for analysis was according to the method of Sjodin and Henderson (1964). Cation analyses were performed by flame photometry using optical filters with transmission at the following wavelengths: sodium (589 mμ), potassium (768 mμ), and lithium (671 mμ). The band widths were narrow enough to eliminate detectable interference. As neither the muscle wet weights, the total water contents, nor the extracellular spaces of members of a pair of muscles differed significantly, the amount of sodium extruded was obtained by subtraction of sodium contents.

In experiments using muscles with a lowered internal sodium concentration, the low sodium concentrations were obtained as before. After 40 hr storage in normal Ringer's solution containing 2.5 mM K at 3°C, muscles were placed in 5 mM K Ringer's solution at 20°C for 3 hr and subsequently used in a replacement type experiment.

In cases in which radioactive sodium efflux was determined, muscles were subjected to the previously described sodium enrichment procedure for 24 hr. At this time the sodium in the Na enrichment solution was labeled with ^{22}Na and the muscles kept in the labeled solution at 3°C for another 16 hr. After this period, sodium efflux was measured by the method previously described (Sjodin and Henderson, 1964). Rate constants were obtained from a semilogarithmic plot of the counts per minute remaining in the muscle vs. time. Replacement and isotope efflux experiments were performed at 20°C.

The following solutions were employed in the compositions indicated:

Solution	*Composition*
Normal Ringer's	NaCl, 105 mM; KCl, 2.5 mM; $CaCl_2$, 2 mM; tris (hydroxymethyl) aminomethane, 1 mM (Tris).
K-free Ringer's	NaCl, 105 mM; $CaCl_2$, 2 mM; Tris, 1 mM.
5 mM K Ringer's	NaCl, 105 mM; KCl, 5 mM; $CaCl_2$, 2 mM; Tris, 1 mM.
Li test solution	NaCl, 50 mM; LiCl, 60 mM; $CaCl_2$, 2 mM; Tris, 1 mM.
Low Na test solution	NaCl, 50 mM; $CaCl_2$, 2 mM; Tris, 1 mM; sucrose, 108 mM.
Li Ringer's	LiCl, 105 mM; KCl, 2.5 mM; $CaCl_2$, 2 mM; Tris, 1 mM.

When strophanthidin was employed, it was added to the solution from an alcoholic stock solution just before each use.

RESULTS

The Promotion of Sodium Extrusion against an Electrochemical Gradient by Lithium Ions To see whether, like rubidium and cesium, lithium can also substitute for potassium in promoting sodium extrusion, pairs of sartorii were enriched with sodium as indicated in Methods. The average intracellular sodium concentration attained was 23 mM/kg or around 40 mM/liter fiber water. As the affinity of the potassium sites for lithium is likely to be relatively low, a lithium ion concentration of 60 mM was selected for the recovery test solution. The sodium concentration in the recovery solution was accordingly lowered to 50 mM which is still higher than the sodium concentration within the cells. Thus, any net loss of sodium ions from the muscles to the lithium recovery solution must be against a concentration gradient and, of course, against a high electrochemical gradient as the cells maintain an internal electrical negativity of around −90 mv under these conditions. The sodium-rich muscles were equilibrated for 1 hr at 20°C in K-free Ringer's solution prior to the test. One of the pair was then analyzed to give the initial sodium content; the other muscle was placed in the lithium recovery solution for a period of 2 hr and then analyzed. The results of nine such experiments are presented in Table I. The table shows that net sodium extrusion has taken place in all nine cases. On the average, muscles in the lithium recovery solution have extruded 9.8 mM/kg of sodium and gained an equivalent amount of lithium. It is evident that the muscle cells have maintained a relatively constant potassium content during recovery. The magnitude ot the Na-Li interchange is statistically significant ($P < 0.001$). It is clear that lithium ions do promote the net extrusion of sodium ions in a manner similar to that of potassium ions. A quantitative comparison of the lithium and potassium effects will be deferred to the Discussion.

Before this conclusion could be made with absolute certainty, it was necessary to establish one more point. It has been suggested by Conway (1960), that sodium extrusion from muscle cells takes place according to whether or not the energy barrier for extrusion is less than or greater than a "critical energy barrier." In the experiments just reported, sodium extrusion occurred in a recovery solution with a lower than normal sodium concentration. It is possible that the extrusion took place merely because the energy barrier for extrusion was lowered. To answer the question, similar experiments were performed in which the recovery solution contained 50 mM NaCl, 2 mM $CaCl_2$,

TABLE I

THE PROMOTION OF NET SODIUM EXTRUSION BY LITHIUM IONS

	Before recovery			After recovery				Net changes (2 hr)	
	Na	K	Na+K	Na	K	Li	Na+K+Li	ΔNa	ΔK
					mM/kg muscle				
	17.8	58.8	76.6	11.3	57.6	6.1	75.0	−6.5	−1.2
	16.2	58.7	74.9	11.6	61.6	8.2	81.4	−4.6	+2.9
	20.2	56.8	77.0	10.5	58.0	7.9	76.4	−9.7	+1.2
	23.6	46.0	69.6	16.5	43.7	8.9	69.1	−7.1	−2.3
	30.3	44.3	74.6	17.3	49.8	10.5	77.6	−13.0	+5.5
	26.6	53.7	80.3	14.7	54.6	11.4	80.7	−11.9	+0.9
	34.2	40.5	74.7	16.4	43.6	15.5	75.5	−17.8	+3.1
	20.2	61.2	81.4	10.4	61.2	10.3	81.9	−9.8	0.0
	21.9	60.5	82.4	14.3	56.4	9.8	80.5	−7.6	−4.1
Mean	23.4*	53.4‡	76.8§	13.6*	54.1‡	9.8	77.6§	−9.8	+0.7
±SE	2.0	2.6	1.3	0.9	2.3	0.9	1.4	—	—

* $P < 0.001$.
‡ $P > 0.5$.
§ $P > 0.5$.

Tris buffer, and no potassium or lithium, with osmotic balance attained by the use of sucrose. The results are presented in Table II and show that no sodium extrusion occurs merely as a consequence of lowering the external sodium concentration.

The Prevention of Lithium-Induced Sodium Extrusion by 10^{-5} M *Strophanthidin*

It is known that the external application of various cardiac glycosides prevents the recovery of muscles from the sodium-rich state in the presence of cations which normally promote sodium extrusion. The external presence of 10^{-5} M strophanthidin, for example, reduces sodium efflux from Na-rich muscles stimulated by K or Cs ions essentially to values observed in K- and Cs-free solutions (Sjodin and Beaugé, 1967 *b*; Beaugé and Sjodin, 1968). Experiments

similar to those designed to demonstrate lithium-induced sodium extrusion were performed with 10^{-5} M strophanthidin added to the lithium recovery solution. The results appear in Table III and indicate that the lithium-stimulated sodium extrusion has now been abolished. The changes in sodium contents reported in the table are not statistically significant. The uptake rate of lithium ions has been lowered by strophanthidin but is still quite significant.

TABLE II

ABSENCE OF NET SODIUM EXTRUSION WHEN LITHIUM IS REPLACED BY SUCROSE

	Before recovery			After recovery			Net changes (2 hr)	
	Na	K	Na+K	Na	K	Na+K	ΔNa	ΔK
				mM/kg muscle				
	23.6	53.0	76.6	23.3	53.0	76.3	−0.3	0.0
	20.8	56.9	77.7	21.7	57.2	78.9	+0.9	+0.3
	16.5	58.2	74.7	17.1	60.2	77.3	+0.6	+1.8
	22.9	57.0	79.9	22.8	57.0	79.8	−0.1	0.0
	20.7	56.4	77.1	24.0	54.0	78.0	+3.3	−1.6
	18.4	53.1	71.5	18.2	56.8	75.0	−0.2	+3.7
Mean	20.5*	55.8‡	76.3§	21.2*	56.4‡	77.6§	+0.7	+0.7
±SE	1.1	0.9	1.2	1.2	1.0	0.7	—	—

* $P > 0.5$.
‡ $P > 0.5$.
§ $P > 0.2$.

TABLE III

INHIBITION BY 10^{-5} M STROPHANTHIDIN OF THE NET SODIUM EXTRUSION INDUCED BY LITHIUM IONS

	Before recovery			After recovery				Net changes (2 hr)	
	Na	K	Na+K	Na	K	Li	Na+K+Li	ΔNa	ΔK
				mM/kg muscle					
	15.2	62.9	78.1	14.9	61.8	4.4	81.1	−0.3	−1.1
	18.7	77.5	96.2	17.5	65.6	5.0	88.1	−1.2	−11.9
	26.4	39.2	65.6	25.3	36.3	10.5	72.1	−1.3	−2.9
	24.5	52.2	76.7	23.4	45.0	8.4	76.8	−1.1	−7.2
	25.8	54.6	80.4	25.3	48.5	6.4	77.6	−0.5	−6.1
	26.9	50.7	77.6	22.4	42.7	7.3	72.4	−4.5	−8.0
	29.7	47.5	77.2	31.1	39.0	6.1	76.2	+1.4	−8.5
Mean	23.9*	54.9‡	78.8§	22.8*	48.4‡	6.9	77.8§	−1.1	−6.5
±SE	1.9	4.6	3.4	2.0	4.2	0.8	2.1	—	—

* $P > 0.5$.
‡ $P > 0.2$.
§ $P > 0.8$.

It is of interest that, in the presence of strophanthidin, the gain of lithium is now compensated for by a loss of potassium rather than a loss of sodium.

Evidently the lithium-induced sodium extrusion presently observed is qualitatively similar to the sodium extrusion induced by potassium, rubidium, and cesium (Johnson, 1956; Edwards and Harris, 1957; Sjodin, 1961; Sjodin and Beaugé, 1967 *b*; Adrian and Slayman, 1966; Beaugé and Sjodin, 1968).

The Influence of the Intracellular Sodium Concentration on the Uptake of Lithium

A characteristic of the cations which have the capacity to promote net sodium extrusion from Na-rich muscles is that the rate at which they are taken up by muscle cells depends upon the sodium concentration inside the cells. Uptake

TABLE IV

LITHIUM UPTAKE IN LOW SODIUM MUSCLES

	Before recovery			After recovery				Net changes (2 hr)	
	Na	K	Na+K	Na	K	Li	Na+K+Li	ΔNa	ΔK
					mM/kg muscle				
	9.3	70.2	79.5	7.3	61.7	4.8	73.8	−2.0	−8.5
	10.2	76.3	86.5	8.4	69.8	2.9	81.1	−1.8	−6.5
	6.1	79.3	85.4	6.9	69.6	3.3	79.8	+0.8	−9.7
	6.9	76.2	83.1	5.6	65.3	3.9	74.8	−1.3	−10.9
	8.0	75.7	83.7	8.8	71.7	5.0	85.5	+0.8	−4.0
	7.0	74.6	82.7	6.6	63.2	4.1	73.9	−0.4	−11.4
	7.3	76.4	83.7	8.2	67.7	4.3	80.2	+0.9	−8.7
	7.4	71.2	78.8	6.2	62.8	4.3	73.3	−0.8	−8.4
Mean	7.8*	75.0‡	82.8§	7.3*	66.5‡	4.1	77.8§	−0.5	−8.5
±SE	0.5	1.0	0.9	0.4	1.3	0.3	1.6	—	—

* $P > 0.2$.
‡ $P < 0.001$.
§ $P < 0.02$.

becomes higher as the intracellular sodium concentration rises. This has been shown for potassium in the previous paper (Sjodin and Beaugé, 1968 *b*), for rubidium (Bolingbroke, Harris, and Sjodin, 1961; Adrian and Slayman, 1966) and for cesium (Beaugé and Sjodin, 1968). Experiments were performed to determine lithium uptake by muscles in which the intracellular sodium content had been lowered by the technique described in Methods. Changes in intracellular cation contents were determined as differences between the contents of members of a pair, one of which was analyzed initially while the other was analyzed after remaining for 2 hr in the 60 mM LiCl–50 mM NaCl Ringer solution described. The results are presented in Table IV and show that the rate at which lithium ions are taken up by the muscles with reduced sodium contents is only about 40% of the rate observed in muscles with an elevated sodium content. The sodium contents remain unchanged as would be expected if

lithium ions continue to stimulate sodium pumping to the rate at which it balances the inward leak of sodium. Potassium ions are lost from the cells, however, at a rate equal to about twice the rate of lithium gain. Potassium ions are lost from normal muscle cells when they are placed in a K-free Ringer solution. The rate of loss in such instances is between 4 and 5 mM/kg per hr (Sjodin and Henderson, 1964). This is about equal to the rate of loss presently observed. The passive permeability of the muscle membrane to lithium appears to be about equal to the permeability to sodium (Keynes and Swan, 1959 *b*). If lithium ions were present at the concentration of sodium ions in

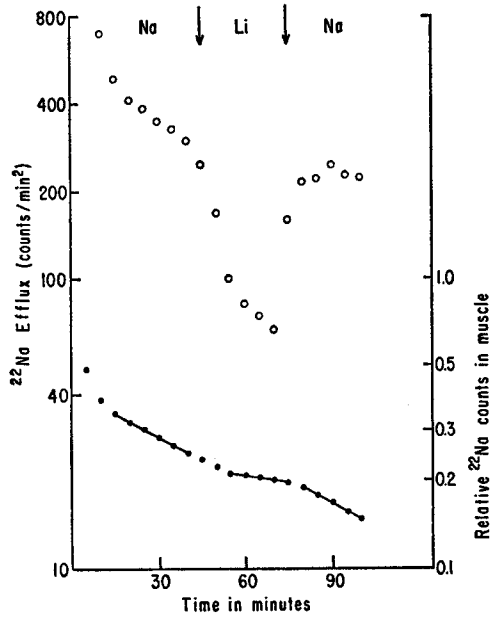

FIGURE 1. Sodium efflux from high sodium muscles into sodium-free lithium-substituted Ringer's solution containing 10^{-5} M strophanthidin is illustrated. The initial and final portions of efflux measurement took place in normal Ringer's solution which also contained 10^{-5} M strophanthidin. The left axis refers to the open circles. The right axis refers to the solid circles which represent the counts per minute remaining in the muscle plotted semilogarithmically against time.

Ringer's solution, the inward rate of lithium gain would be about equal to the rate of potassium loss.

The results presented in this section indicate that, as is the case for other cations which promote sodium extrusion, the lithium uptake rate is dependent on the intracellular sodium concentration.

The Demonstration of Na-for-Na Interchange in Muscles with Elevated Internal Sodium Concentrations It is evident that lithium is not an ideal choice as a sodium replacement when attempting to demonstrate the presence of Na-for-Na interchange in muscle cells. Two requirements that have to be met by the cation used to replace sodium in Ringer's solution were stated in the Introduction. Clearly, lithium fails to meet the second requirement as a considerable action of Li on strophanthidin-sensitive sodium transport has been demonstrated. The first requirement, a negligibly small affinity of Li for the Na-for-

Na membrane sites involved in the Na-for-Na interchange appears to be met rather well by lithium. Otherwise a very large reduction in sodium efflux would not take place when muscles with a low sodium content are transferred to lithium-substituted Ringer's solution. Fortunately, however, essentially all the action of lithium on sodium transport is abolished by application of strophanthidin. Clearly, if the reasoning is correct, it should be possible to use lithium to demonstrate a reduction in Na efflux due to Na-free conditions in muscles with elevated sodium contents if strophanthidin is present in the medium. Experiments were performed as follows. Muscles were enriched with sodium and labeled with ^{22}Na as indicated in Methods. Efflux of sodium was subsequently measured at 20°C in solutions all of which contained 10^{-5} M strophanthidin. Muscles were transferred from normal Ringer's solution con-

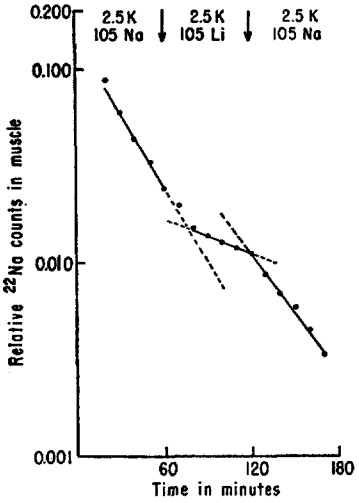

FIGURE 2. The effect of replacing sodium in Ringer's solution by lithium on sodium efflux from muscles with a reduced sodium content is illustrated. The reduction in sodium efflux which occurred in the lithium-substituted medium is over 4-fold.

taining strophanthidin to sodium-free lithium Ringer's solution containing strophanthidin and back to a solution with the initial composition. The results of a typical experiment are illustrated in Fig. 1 and indicate that a large drop in sodium efflux does indeed take place in the lithium-substituted Ringer's solution when 10^{-5} M strophanthidin is present. In the particular experiment illustrated the reduction in sodium efflux was 4-fold. A similar experiment performed on a muscle with a low sodium content in the absence of strophanthidin is illustrated in Fig. 2. It is evident that a muscle with an elevated sodium content behaves like a muscle with a low sodium content when transferred to lithium-substituted Ringer's solution if 10^{-5} M strophanthidin is present.

A final sodium efflux experiment was designed to further test the correctness of the reasoning. Pairs of sartorii were made sodium-rich and radioactively labeled as before. Sodium efflux was measured on one of the pair of muscles in normal Ringer's solution and then in lithium-substituted Ringer's solution.

Sodium efflux was measured on the other muscle of the pair in solutions of identical ionic composition but with 10^{-5} M strophanthidin present. For both muscles, the initial efflux points were obtained in a K-free medium to retain as many counts per minute as possible in the muscles during the period of rapid

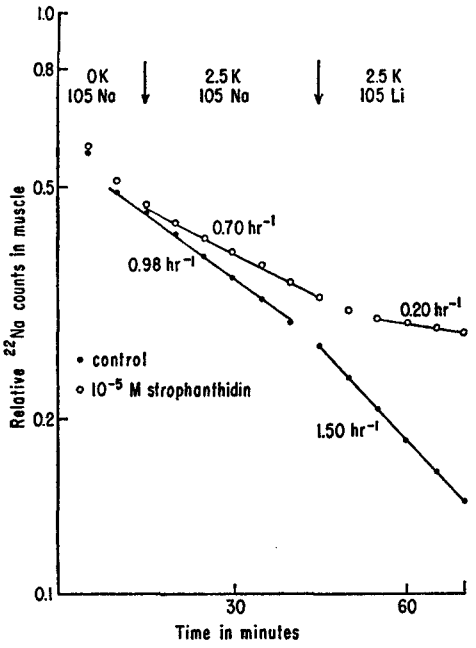

FIGURE 3. The effect of replacing sodium in Ringer's solution by lithium on sodium efflux from muscles with an elevated sodium content is illustrated. Open circles refer to efflux measured in the presence of 10^{-5} M strophanthidin, whereas filled circles refer to efflux measured in the absence of strophanthidin. The numbers above and below the curves refer to the rate constants determined from the straight lines drawn through the experimental points.

TABLE V

RATE CONSTANTS FOR ^{22}Na EFFLUX (HR^{-1})

	Solutions, *mM/liter*			
	Control		10^{-5} M strophanthidin	
Muscle	2.5 K–105 Na	2.5 K–105 Li	2.5 K–105 Na	2.5 K–105 Li
a	0.96	1.39	—	—
a′	—	—	0.72	0.18
b	0.92	1.45	—	—
b′	—	—	0.46	0.19
c	0.98	1.50	—	—
c′	—	—	0.70	0.20
Mean	0.95	1.45	0.63	0.19

^{22}Na washout. In view of the previous results, one would expect lithium ions to increase sodium efflux in the muscles not exposed to strophanthidin and to decrease sodium efflux in the muscles exposed to strophanthidin. The results of a typical experiment are illustrated in Fig. 3. The expectations were entirely borne out. This type of experiment shows, perhaps more clearly than any of

the others, the 2-fold action of lithium ions on sodium efflux, one action stimulating, the other inhibiting. The overall results reported in this section are summarized in Table V. In muscles with sodium contents above 20 mM/kg, it is evident that about 90% of sodium efflux can be accounted for as a combination of a strophanthidin-sensitive sodium pump and a process involving a sodium-for-sodium interchange.

DISCUSSION

A role of lithium ions has been demonstrated in which the extrusion of sodium from muscle cells is stimulated. This action of lithium does not appear to differ qualitatively from the similar action of K^+, Rb^+, and Cs^+ as it is abolished by application of strophanthidin and has the same sort of dependence on the intracellular sodium concentration. A potassium-like action of lithium in promoting the extrusion of sodium from red blood cells has been reported by Maizels (1968). Baker et al. (1967) observed that external lithium increased the sodium efflux from squid giant axons in the presence of external calcium. This stimulating action of lithium on sodium efflux differs from that observed in this investigation, however, in that the stimulation in squid giant axons is unaffected by glycosides (ouabain up to 10^{-3} M).

The possible role of external calcium ions in these experiments was not investigated. Cosmos and Harris (1961) have reported that the influx of calcium ions into skeletal muscle cells is increased when the external sodium concentration is reduced. As the replacement of internal sodium by external lithium during recovery was stoichiometric in the present experiments, and as the rate of calcium influx is rather low, calcium movement does not appear to play a large role.

Even though the sodium ions extruded against an electrochemical gradient were replaced with lithium ions, there is some question as to the mechanism for the stimulation of sodium extrusion. Baker and Connelly (1966) observed an inhibitory action of external sodium ions on the activation of the sodium pump in crab nerve by external potassium ions. A possible action of a low external sodium concentration on muscle cells is an enhancement of the pump stimulating action of the potassium ions leaking from the cells into a K-free medium. It is possible that some of the pump stimulation observed in the presence of external lithium ions was due to potassium ions via this effect. Lowering the external sodium concentration by replacement with an osmotic equivalent of sucrose did not lead to pump activation. In this case, however, there was only a negligible K leak from the cells. In the presence of 10^{-5} M strophanthidin, the K leak into 50 mM Na/60 mM Li Ringer's solution was quite significant (Table III) and was of about the same magnitude as the K leak into K-free Ringer's solution. There does not appear to be any certain way to assess the relative degrees of pump activation by K and by Li under the

conditions of our experiments. The fact that a considerable concentration of sodium was employed in the solutions in which recovery occurred tends to argue that a good deal of the pump activation is probably due to lithium. The sodium concentration was about halved in the present experiments. Under these conditions, Baker and Connelly (1966) observed a rather modest effect on pump activation by potassium. When the external sodium concentration was reduced 4-fold, on the other hand, the effect on pump activation by potassium was considerable.

It is of interest to compare the relative effectiveness of various cations in promoting the extrusion of sodium. This can be done on a very approximate basis by noting the amount of sodium extruded in a certain time with a given concentration of the stimulating cation present in the external medium. This is done below gathering data on sartorius muscle cells from various sources.

Stimulating cation	Concentration	Recovery time	ΔNa extruded	Reference
	mM	*hr*	*mM/kg*	
K	5	1.0	−12.0	Sjodin and Beaugé (unpublished data)
Rb	10	1.0	−14.0	Adrian and Slayman (1966)
Cs	25	1.5	−8.6	Beaugé and Sjodin (1968)
Li	60	2.0	−9.8	This investigation

Though the data do not permit the assignment of relative affinities of these cations for the transport system, the rank in order of increasing effectiveness at stimulating sodium extrusion appears to be $Li^+ < Cs^+ < Rb^+ < K^+$. The mechanism for the stimulation of sodium extrusion remains obscure. The Mg^{++}-activated ATPase extracted from membranes and studied by Skou (1960) might be involved as the in vitro activity of this enzyme is inhibitable by cardiac glycosides and is stimulated by monovalent cations according to the rank order noted above. Also, it can be noted that the depolarizing action of these cations on the muscle membrane potential is not likely to play a large role in bringing about extrusion. In the above table the recovery times and amounts of sodium extruded are rather comparable, yet the effects of the cations on the muscle membrane potential at the concentrations given vary widely (Keynes and Swan, 1959 *b*; Adrian and Slayman, 1966; Beaugé and Sjodin, 1968).

The results of this investigation are of more interest, however, than the fact that another cation has been added to the list of cations which exert a stimulating action on sodium extrusion in muscle cells. The reason for this is that the present results provide an explanation for the finding of Keynes and Swan (1959 *a*) that exchange diffusion more or less mysteriously disappears when the sodium concentration inside the fibers rises and reappears when the sodium

concentration is allowed to fall again. The basis for the explanation is the dual action of lithium on sodium efflux, one action decreasing Na efflux by providing a sodium-free external medium and one action increasing Na efflux. The action which increases sodium efflux is due to a stimulating action on the sodium pump and the magnitude of this effect will rise along with the magnitude of the strophanthidin-sensitive component of sodium efflux. The latter component rises as the internal sodium concentration rises (Sjodin and Beaugé 1968 *b*). A point will be reached where the internal sodium concentration is such that the Na efflux increasing action and decreasing action of lithium tend to balance one another. At this sodium concentration, external lithium ions will be without a net effect on total sodium efflux. In the absence of strophanthidin, one would conclude that the exchange diffusion component of sodium efflux has disappeared.

An explanation is also now available for why Keynes and Swan (1959 *a*) found that the effects on Na efflux of removing K and of removing Na from the external medium are not additive when lithium is used to replace sodium. The reason is obviously that, when Na is replaced by Li in a K-free medium, the Li will exert an action that is equivalent to reintroducing some K into the medium.

It is clear that one must exercise caution in giving the concept of exchange diffusion as visualized by Ussing (1949) an operational definition in terms of the change in sodium efflux observed when sodium in the medium is removed and replaced with another constituent. For example, on the basis of such a definition, the muscle not exposed to strophanthidin in the experiment illustrated by Fig. 3 is exhibiting negative exchange diffusion (Mullins and Frumento, 1963). It should be emphasized that the validity of the actual concept of exchange diffusion for muscle cells is not established by these experiments. All that we have established is that what has been observed in freshly dissected muscles and termed exchange diffusion (Keynes and Swan, 1959 *a*) can also be observed in muscles with an elevated internal sodium concentration under appropriate conditions. There is some question as to whether such sodium-free effects should be termed exchange diffusion for the reasons discussed in the preceding paper (Sjodin and Beaugé, 1968 *b*).

In view of the previous discussion, and bearing in mind the results of the preceding paper, it is probable that Keynes and Swan (1959 *a*) have somewhat underestimated the fraction of sodium efflux in freshly dissected muscles that is due to Na-for-Na interchange, though not seriously. In the preceding paper, a fractionation of the total efflux was given that holds rather well for muscles with less than half the normal sodium content; 78% could be attributed to Na-for-Na interchange, 10% to strophanthidin-sensitive sodium pumping, leaving 12% unaccounted for ("residual"). From Table V, a similar fractionation of sodium efflux can be made in the case of muscles with an elevated

sodium concentration. Using average values of the rate constants for ^{22}Na loss one obtains: 23% strophanthidin-insensitive Na-for-Na interchange, 67% strophanthidin-sensitive sodium pump, and 10% residual. (The rate constant in the 2.5 mM K–105 mM Li Ringer solution was taken as more or less maximally stimulated sodium transport. The efflux due to Na-for-Na interchange was added to this to obtain the total efflux on the assumption that the flux components are independent and additive (Keynes, 1966).

Looking at the present results and those of the previously reported investigation together, it is apparent that about 90% of the total sodium efflux can be accounted for by Na-for-Na interchange and strophanthidin-sensitive sodium pumping in both muscles with a low and with a high intracellular sodium concentration. When the internal sodium concentration is low (about one-half normal), sodium efflux is predominantly due to Na-for-Na interchange, a relatively minor component being due to pumping. When the internal sodium concentration becomes high (about twice normal), the situation reverses and Na-for-Na interchange, quantitatively speaking, assumes the minor role.

The type of Na-for-Na interchange observed in this investigation has been demonstrated only in the presence of a cardiac glycoside. This differs from the type of Na-for-Na exchange observed in red blood cells by Garrahan and Glynn (1967) which occurs in the absence but not in the presence of a cardiac glycoside. The experiments presently reported do not, however, prove the nonexistence in muscle cells of the red cell type of Na-for-Na interchange. This type of interchange would have been obscured, in the present experiments, by the presence of cardiac glycoside. In the absence of cardiac glycoside, however, this type of interchange would have been obscured by the positive action of lithium on the sodium pump that we have demonstrated. To establish the presence or absence of the red cell type of Na-for-Na interchange in muscle cells would require the use of a cation to replace external sodium that is inert with respect to the sodium pump. The Na-free effects observed in muscle cells differ from those observed in red cells in another respect. In red cells a glycoside-sensitive Na influx has been demonstrated and the Na-for-Na movement in both directions has been studied. In muscle cells one only knows the effects on sodium efflux of the removal of external sodium ions. More experimentation is required before the extent of the differences or similarities between sodium transport in muscle cells and in red cells will become apparent.

It was of interest to attempt the demonstration of the presently observed type of Na-for-Na interchange in "high sodium" muscles in the absence of a cardiac glycoside. One way that this might be accomplished would be to attempt the saturation of the Na pumping rate by elevating the external potassium concentration. In the presence of 5 mM K in the Ringer solution, lithium ions did not further increase the Na efflux. A reduction in sodium efflux was not observed in lithium Ringer's solution under these conditions, however,

indicating that pump stimulation may just compensate for the Na-free effect. The safest conclusion that can be drawn at present appears to be that, when the Na pumping rate is high, Na-for-Na exchange is absent or difficult to detect. When the Na pumping rate is very much reduced by either lowering the internal sodium concentration or applying strophanthidin externally, Na-for-Na exchange is readily demonstrable.

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