The Control of the Membrane

Potential of Muscle Fibers

by the Sodium Pump

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ABSTRACT   Frog sartorius muscles were made Na-rich by immersion in K-free sulfate Ringer's solution in the cold. The muscles were then loaded with Na^{24} and the extracellular space cleared of radioactivity. When such Na-rich muscles were transferred to lithium sulfate Ringer's solution at 20°C, Na efflux was observed to increase with time, to reach a maximum about 15 minutes after the transfer of the muscles to Li_2SO_4, and then to decline. The decline in efflux from these muscles was proportional to $([Na]_i)^3$ over a considerable range of $[Na]_i$. The membrane potential of Na-rich muscles was about -48 mv in K-free sulfate Ringer's at 4°C but changed to -76 mv in the same solution at 20°C and to -98 mv in Li_2SO_4 Ringer's at 20°C. By contrast, muscles with a normal $[Na]_i$ showed a fall in membrane potential when transferred from K-free sulfate Ringer's to Li_2SO_4 Ringer's solution. The general conclusions from this study are (a) that Na extrusion is capable of generating an electrical potential, and (b) that increases in $[Na]_i$ lead to reversible increases in P_{Na} of muscle fibers.

INTRODUCTION

Evidence of a variety of sorts (3, 4, 7) has been accumulating to suggest that the Na extrusion mechanism in muscle is not coupled 1:1 with K uptake and that therefore the Na pump is capable of generating an electrical potential in the course of its operation. Direct measurements of the membrane potentials of muscle fibers during Na extrusion (3) have been made upon muscles loaded with Na in Cl-containing Ringer's solutions but there remains some uncertainty about the role of Cl^- in affecting the membrane potential. Our measurements were, therefore, made in sulfate Ringer's solutions throughout. A disadvantage of sulfate Ringer's solutions is that muscle fibers do not tolerate prolonged contact with them nearly as well as they do with Cl Ringer's solutions; for this reason loading times must be shortened as much as possible and a quite large number of muscles must be discarded because of damage that shows up subsequent to loading.

Although previous measurements of Na efflux from Na-rich muscles in Cl

Ringer's solution have been made (6), it appeared essential to make these measurements in sulfate Ringer's solution in order to compare the behavior of Na efflux with the behavior of the membrane potential.

METHODS

Frog sartorius muscles were used exclusively in this study; the methods of dissecting, handling, and the automatic efflux apparatus are as described previously (6). The muscles used in this study were dissected in pairs; one muscle was placed in K-free sulfate Ringer's solution at 4°C for 12 hours while the other muscle was kept at 20°C in K-containing sulfate Ringer's solution for 12 hours. At this time, muscles were examined under the microscope for damaged fibers. The appearance of clots in 4 or more surface fibers led to the rejection of a muscle for further experimentation. As a further check on fiber damage, muscles showing a 5 per cent or greater change in water content were rejected. Both muscles were then loaded with Na^{24} for 3 hours (under K-free conditions at 4°C for the loaded muscle and in normal sulfate Ringer's at 20°C for the paired muscle). Next, both muscles were transferred to fresh solutions without Na^{24} for 1 hour and muscles were generally washed in 3 changes of inactive solution during this time in order to clear their extracellular spaces of radioactivity. Finally, both muscles were placed in Li_2SO_4 Ringer's solution with 2.5 mM K at 20°C (in some cases choline chloride or normal Ringer's was used) and the automatic efflux apparatus was started. The usual program for solution changes was ten 2 minute efflux measurements followed by ten of 5 minutes and ten of 10 minutes. At the end of the efflux run, muscles were removed from their mounting frames, weighed, dried, and reweighed and then analyzed for Na, K, and Na^{24}. The back addition of counts lost in the efflux samples as a function of time permitted the calculation of efflux in counts per minute squared as a function of Na concentration expressed as counts per minute, by methods previously described (6).

Muscles used for potential measurements were loaded with Na as described above but were not loaded with Na^{24}; the time taken for Na^{24} loading and for clearing the extracellular space of Na^{24} was added to the loading time of the muscles studied. Measurements of membrane potential were made as described by Mullins and Noda (7). About 20 measurements of potential on different fibers of a muscle were made while it was at 4°C in the loading solution, and a similar number of measurements were made on other fibers of the same muscle after its transfer to the efflux solution at 20°C. Such measurements could be made within 2 minutes after the cold solution bathing the muscle in a lucite chamber had been changed to fresh solution at 20°C. The composition of the Ringer's solutions used was as follows: sulfate Ringer's Na_2SO_4 55, K_2SO_4 1.25, $CaSO_4$ saturated, sucrose 65 mM. Tris (1 mM) adjusted with H_2SO_4 to pH 7.4 was sometimes added. Li_2SO_4 was substituted for Na_2SO_4 to make Na-free solutions.

RESULTS

The Effects of Na Efflux on Membrane Potential Normal muscles were equilibrated in sulfate Ringer's at 20°C for 12 hours and the fibers of these muscles were then impaled with microelectrodes in order to measure mem-

brane potentials under several experimental conditions. The measurements were made with the muscles at 4 °C in K-free sulfate Ringer's, when the muscles were warmed to 20 °C in the same solution, and when the muscles were placed in Li_2SO_4 Ringer's solution (2.5 mM K) at 20 °C. These experimental conditions were chosen to duplicate those encountered by muscles subjected to loading with Na, and such loaded muscles were compared with control muscles that had not been in K-free solutions in the cold. The results of po-

TABLE I

MEMBRANE POTENTIALS OF CONTROL AND Na-LOADED MUSCLES

| | K-free sulfate Ringer's | | Li_2SO_4 Ringer's, 20°C 2.5 mM [K] | |
	4°C	20°C	0.5–2.0 min.	90–130 min.
	mv	*mv*	*mv*	*mv*
Membrane potential for muscles in sulfate Ringer's 12 hrs. at 20 °C upon transfer to	−104±2 (6)*	−107±2 (6)	−88±1 (6)	−86±2 (6)
E_K (calculated)‡			−95	−89
Membrane potential for muscles in K-free sulfate Ringer's for 12 hrs. at 4 °C (Na-loaded) upon transfer to	−47±5 (10)	−76±4 (10)	−98±4 (10)	−83±3 (10)
E_K (calculated)			−82	−88

* Variability is given as ± sd. The number of muscles used is shown in parentheses.
‡ Data for this calculation were obtained from Table II; the calculation used the following relation $E_K = 58\{\log (\gamma_K)_o/(\gamma_K)_i\} + 58\{\log [K]_o/[K]_i\}$ where $(\gamma_K)_o = 0.7$ for sulfate Ringer's solution and $(\gamma_K)_i = 0.56$ (see reference 7).

tential measurements are shown in Table I. A point of some interest is that muscles with a normal $[Na]_i$ show only a slight increase in potential in going from 4 to 20°C (a change compatible with the temperature coefficient of a Donnan potential), while muscles loaded with Na show an increase in potential when the temperature is increased and the potential increases further when the solution bathing the muscle is changed to Li_2SO_4 Ringer's with 2.5 mM K. After 1 to 2 hours the membrane potential of loaded muscles declines to a lower value. An important point is that 0.5 to 2.0 minutes after the transfer of a loaded muscle to Li_2SO_4 Ringer's the membrane potential is 13 mv greater than the value of E_K deduced from analytical measurements, while after 1 to 2 hours in Li_2SO_4 Ringer's (when the Na efflux is very small) the membrane potential is now about 5 mv *less* than E_K. By contrast, non-loaded muscles have a value for membrane potential that is 3 mv less than E_K upon their transfer to Li_2SO_4 Ringer's, and this difference is maintained for 130 minutes. These findings confirm those of Kernan (3) and of Keynes and Rybova (4) although both these authors used elevated $[K]_o$ and extrusion of Na into Na

Ringer's so that the experimental conditions were not entirely comparable to those used in this investigation.

Analyses for electrolyte content were made on muscles that had undergone experimental treatments similar to those involved in membrane potential measurements and these results are shown in Table II. Sodium-loaded muscles that underwent efflux into Li_2SO_4 Ringer's had Na concentrations equal to muscles that had been in sulfate Ringer's but had not undergone Na-loading. In both cases, $[K]_i$ was somewhat low. Partly this is because of a significant Li^+ entry but it is also possible that the low $[Ca]_o$ that is unavoidable in sulfate Ringer's solution, leads to some electrolyte loss from the muscle fibers.

TABLE II

Na AND K CONTENT OF CONTROL AND Na-LOADED MUSCLES

	[K]		[Na]	
	μmole/gm	*mmole/ l.f.w.**	*μmole/gm*	*mmole/ l.f.w.*
Muscles loaded 12 hrs. in K-free sulfate Ringer's at 4°C followed by 130 min. in Li_2SO_4 Ringer's at 20°C	59±6 (43)	105	4±1 (43)	7
Muscles 12 hrs. in sulfate Ringer's (2.5 mM K) at 20°C followed by 130 min. in Li_2SO_4 Ringer's (2.5 mM K) at 20°C	61±5 (23)	109	4±1 (23)	7
Muscles loaded 12 hrs. in K-free sulfate Ringer's at 4°C	45±4 (6)	81	54±5 (6)	57
Muscles 12 hrs. in sulfate Ringer's at 20°C	75±3 (6)	134	31±2 (6)	17

* Concentration expressed as per liter fiber water. Extracellular space is 20 per cent (see reference 6) and water content is 70 per cent of fiber weight.

Reducing $[Na]_o$ (with an increase in sucrose for osmotic balance), however, gives poor Na-loading. A number of muscles that had been Na-loaded showed membrane potentials of about -90 mv in K-free sulfate Ringer's in the cold. Analysis for $[Na]_i$ in such muscles showed values around 30 mM; hence such measurements were discarded as not representative of fully Na-loaded muscles. Values for $[Na]_i$ and $[K]_i$ of muscles taken directly from sulfate Ringer's solution are shown in the last two lines of Table II.

Na Efflux into Sulfate Ringer's Solutions   Previous measurements of Na efflux from Na-loaded muscles have generally been into a Cl Ringer's solution with $choline^+$ or Li^+ replacing the Na^+ of Ringer's. Under these conditions, the muscles lose Na but it is not clear just what fraction of the Na extrusion is accompanied by Cl^- and what fraction is lost by exchange for extracellular cations. Because the movements of Cl^- in response to a potential generated by Na extrusion might be expected to act as a shunt on the system, it appeared necessary to make potential measurements in Na-loaded but Cl-free muscle fibers. This requirement necessitated Na flux measurements on such Cl-free

muscles in order to compare Na extrusion with that previously observed. Muscles were loaded for 12 hours in K-free Na_2SO_4 Ringer's at 4°C and then transferred to Li_2SO_4 Ringer's (2.5 mM [K]) at 20°C and Na efflux followed. The results obtained with 4 muscles are shown in Fig. 1. In each case the apparent efflux of Na^{24} rose from an initial value about half that ultimately reached and then declined in a regular way. While there are several possible explanations
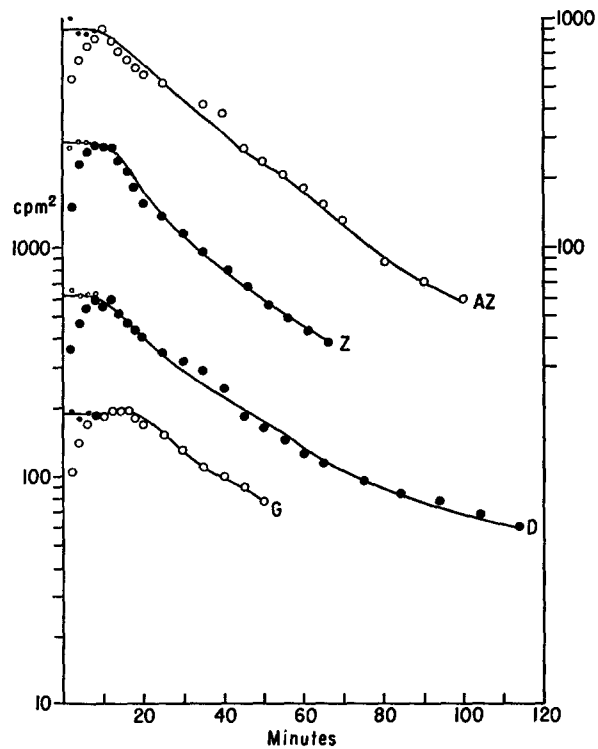
FIGURES 1.   The efflux of Na^{24} in cpm/min. is plotted against time for 4 muscles loaded in K-free sulfate Ringer's solution and transferred to Li_2SO_4 Ringer's at zero time. The large circles (open or filled) are the experimental points. The small circles (open or filled) are calculated from the equation in the text.

for this effect, a relatively simple assumption—that the absence of K^+ in the extracellular space depressed the output of Na^{24} from the fibers[1]—sufficed to make the initial efflux of Na^+ constant for the first 12 minutes of efflux (as has been observed in Cl Ringer's). The efflux of Na from the fibers was calculated on the assumption that when $[K]_o$ is 2.5 mM, efflux is at a maximum while when $[K]_o$ is zero, Na efflux is at a lower value (approximately half that when

[1] Some provisional data that we have suggest that Na efflux into Li_2SO_4 Ringer's is 1.2 times greater than it is into Na_2SO_4 Ringer's. Such observations would require the Na exchange diffusion to be negative in fully Na-loaded muscles.

$[K]_o$ is 2.5 mM). Since the extracellular space of the muscle washes out with a time constant of 2.5 minutes, the [K] at any time $[K]_o^t = [K]_o^\infty (1 - \exp - t/\tau)$. Knowing the zero time and maximum values of Na efflux, and assuming a linear relation between $[K]_o$ and increment of Na efflux, the efflux at any time is given by $m_{Na}^t = m_{Na}^\infty - (m_{Na}^\infty - m_{Na}^o) \exp - t/\tau$ where m_{Na}^t is the Na efflux at a given time, m_{Na}^o and m_{Na}^∞ are the zero time and peak values of Na efflux, while τ is the time constant for the extracellular space of the muscle.
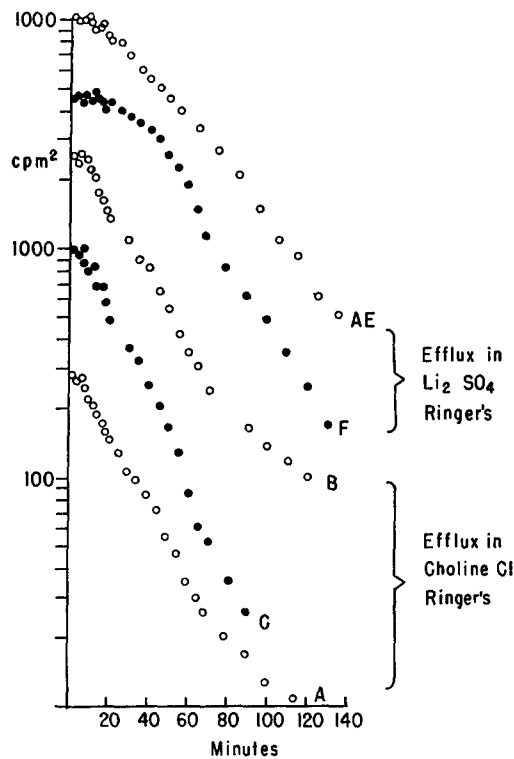

FIGURE 2. The efflux of Na^{24} in cpm/min. is plotted against time for muscles loaded in Cl Ringer's solution (K-free) and transferred at zero time to either Li$_2$SO$_4$ Ringer's (top two curves) or to choline Cl Ringer's solution.

The small circles on Fig. 1 are calculated values for m_{Na}^∞ using this relationship and the calculated Na efflux is constant with time.

When muscles have been loaded in K-free Cl Ringer's at 4°C and then changed to choline chloride Ringer's or to Li$_2$SO$_4$ Ringer's solutions at 20°C, the shape of the efflux vs. time curve is different. Some representative curves are shown in Fig. 2. Sodium efflux into Li$_2$SO$_4$ Ringer's solution may be expected to comprise a NaCl loss both because the solution is Cl-free and because the membrane potential of the fibers rises considerably. The efflux into a Cl-containing Ringer's fluid must also be expected to involve a NaCl loss because the change in membrane potential, incident to Na extrusion, will decrease $[Cl]_i$. If the explanation suggested for the apparent rise in Na^{24} efflux as shown in Fig. 1 is correct, it should also apply to the curves of Fig. 2. The fact

that efflux is initially constant suggests that a declining $[Cl]_o$ and a rising $[K]_o$ may interact in some way to produce a constant efflux. The lower three curves in Fig. 2 show Na efflux into choline Ringer's. These have quite short plateaus and no suggestion of a rise in Na^{24} efflux. The upper two curves in Fig. 2 are from muscles loaded in Cl Ringer's but unloaded in Li_2SO_4 Ringer's. They are not especially different from those where Cl^- was present throughout. In neither case is there the initial rise in efflux shown for muscles loaded and unloaded in SO_4 solutions.

A Comparison of the P_{Na} of Loaded and Control Muscles   The results of membrane potential measurements on Na-loaded muscles suggest that the P_{Na} of the muscle fibers has increased considerably. A very large passive leak of Na

TABLE III
Na CONTENT OF MUSCLES

	[Na]_i
	μmole/gm
Muscles loaded 12 hrs. in K-free sulfate Ringer's at 4°C	54±5 (6)
Muscles loaded 12 hrs. in K-free sulfate Ringer's at 4°C and transferred to 2.5 mm K sulfate Ringer's for:	
6 hrs. at 20°C	52±4 (8)
12 hrs. at 20°C	55±6 (8)

from the fibers could seriously confuse the efflux measurements that are designed to show how the Na pump responds to changes in $[Na]_i$. There are several ways by which the passive leak of Na can be examined. If loaded muscles are transferred to Na sulfate Ringer's (2.5 mm K^+) at 20°C they do not lose $[Na]_i$ as judged by analytical measurements (see Table III and reference 1); therefore the rate at which such muscles lose Na^{24} can be equated with the rate of operation of the Na pump at constant $[Na]_i$ plus contributions to Na^{24} loss given by Na diffusion from the fibers and a contribution from exchange diffusion. By comparing the rate constant for the loss of Na^{24} in Ringer's solution containing 2.5 mm K, from muscles with a normal $[Na]_i$ with that from muscles with an elevated $[Na]_i$, when in both cases $[Na]_i$ is constant with time, it is possible to calculate rates for Na loss. Since a time-invariant $[Na]_i$ implies that influx = efflux, a knowledge of rate constant for Na loss and $[Na]_i$ allows a direct estimate of influx for the muscles involved. While Na efflux involves contributions from Na pump, passive diffusion, and exchange diffusion, influx involves only passive diffusion and exchange diffusion. If, for the moment, exchange diffusion is ignored, then efflux for the two muscles is proportional to $[Na]_i k$, where k is the rate constant for Na loss. Measurements of the reciprocal rate constant (time constant τ) for Na loss for 8 muscles kept in sulfate Ringer's at 20°C for 12 hours prior to Na^{24} loading and efflux measurement are given in Table IV

together with values for Na-loaded muscles kept in K-free sulfate Ringer's for 12 hours followed by Na^{24} loading and at 20°C into normal sulfate Ringer's. The analytical values for $[Na]_i$ are from Table II, and the third column gives a number proportional to efflux (and to influx). Because influx has a component that is exchange diffusion for both muscles, it is necessary to correct for this. For normal muscles, exchange diffusion is in the range of 0.5 for fresh muscles with a low $[Na]_i$, to zero for muscles with an elevated $[Na]_i$ (see reference 5). The muscles used had an $[Na]_i$ of 17 mM by analysis (see Table II) and it is somewhat difficult to decide whether this is a low or high value because of the presence of appreciable amounts of non-exchangeable Na. Previous work (6) suggests that this non-exchangeable Na is ~ 5 mM so the analytical figure

TABLE IV

THE SODIUM PERMEABILITY OF NORMAL AND Na-LOADED
MUSCLES IN SULFATE RINGER'S SOLUTION

	τ	$[Na]_i$	Efflux $[Na]_i/\tau =$ influx	Influx corrected for exchange diffusion	Ratio $P_{Na}(\text{loaded})$ $P_{Na}(\text{normal})$
	min.	*m M*			
Na-loaded muscles	70±6	57	0.81	0.97	
					7
Normal muscles	62±2	17	0.27	0.135	

for $[Na]_i$ would be 12 mM or in the range where exchange diffusion is appreciable. We have therefore used 0.5 as a value with the reservation that it might be less than this. For Na-loaded muscles exchange diffusion is apparently negative (see footnote 1) and apparent influx must be multiplied by 1.2 to get true influx for Na-loaded muscles. These corrections are made in the fourth column of Table IV. The membrane potential of loaded or normal muscles in sulfate Ringer's solution is (within experimental variation) the same and very close to E_K so that no correction of influx is necessary. The last column of Table III gives the ratio of permeabilities for Na for loaded and normal muscles. The large value of the ratio is the basis for concluding that P_{Na} in loaded muscle has been increased.

A Comparison of the Concentration-Efflux Curves for Muscles in Chloride or Sulfate Ringer's As a further check on the validity of the assumption that muscles loaded in sulfate Ringer's solution and then transferred to Li_2SO_4 Ringer's for efflux behave as do muscles loaded in Cl-containing Ringer's solutions, our data on Na efflux in these various solutions was recalculated to yield cpm Na^{24} in muscle *vs.* $cpm^2 Na^{24}$ efflux. The results for 4 muscles are shown in Fig. 3. For unloaded muscles, a comparison of efflux *vs.* concentration reveals that both curves show a "cubic" relationship over an appreciable concentration

range (*i.e.* Na efflux is proportional to $([Na]_i)^3$) but that at low $[Na]_i$, muscles that have been equilibrated with sulfate for 12 hours prior to the measurements show a concentration-efflux relationship that is linear. This effect can also be demonstrated with unloaded muscles kept in Cl Ringer's solution but it occurs at much lower values of $[Na]_i$. A tentative conclusion is that for unloaded muscles, P_{Na} is somewhat greater if the muscle has been kept in sulfate Ringer's than if it has been kept in Cl Ringer's solution prior to efflux measurements. It is difficult to make this point a quantitative one because the variability among muscles is quite great. What can be said is that over a concentration
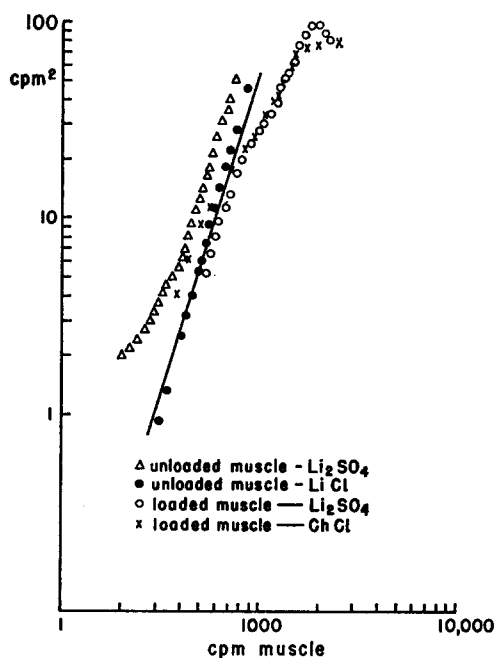

FIGURE 3. A log-log plot of Na^{24} efflux in cpm/min. against Na^{24} cpm in muscle. The line drawn has a slope of 3 and the points for loaded muscles have been displaced along the abscissa to facilitate their comparison with those for non-loaded muscles. All muscles are maintained either in Cl or SO_4 Ringer's throughout.

range of about 6 to 16 mM $[Na]_i$ the efflux of Na is cubic (or the contribution from leakage Na is quite small). Muscles loaded with Na in Cl and SO_4 Ringer's and unloaded in choline Cl Ringer's or Li_2SO_4 Ringer's were also compared. While the efflux clearly saturates at high $[Na]_i$, it also follows a cubic relationship between efflux and internal Na concentration over the lower part of the curve. Again there is no obvious difference between muscles loaded in Cl or sulfate Ringer's, a finding that suggests that efflux is largely that resulting from the operation of the Na pump.

The Effect of Temperature on Na Efflux A possible explanation for the slow rise of efflux shown in Fig. 1 is that the Na pump responds slowly to a change in temperature from 4–20°C. To look into this possibility, a number of muscles were loaded with Na in the usual way and then transferred to Li_2SO_4

Ringer's. Sodium efflux was followed in these muscles 10 minutes after their transfer to Li_2SO_4 Ringer's at 20°C (to avoid the complications of the efflux rise) and the muscles were then transferred to the same solution at 4°C at a time when Na efflux was judged to be just about maximal. A typical experiment is shown in Fig. 4 (left). The usual 2 minute efflux sampling periods were employed and Na efflux fell essentially to its final value in the first 2 minute period. It is clear, therefore, that the response of the Na efflux to temperature change is essentially instantaneous. The change in Na efflux in going from 20–4°C for 6 muscles measured averaged $1/2.2 \pm 0.3$ of the initial rate.
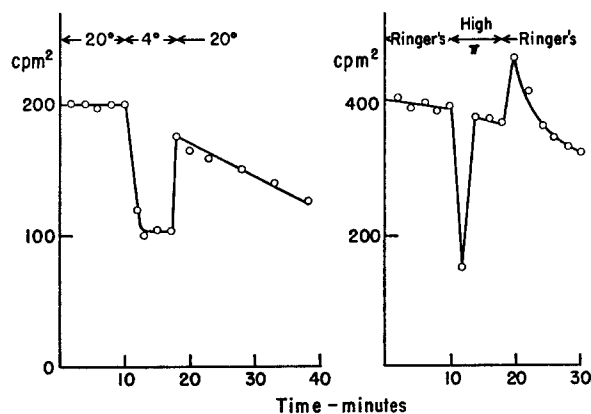

FIGURE 4. On the left is shown the response of the Na^{24} efflux (in cpm/min.) to a change in temperature (from 20–4°C) for a muscle loaded with Na in sulfate Ringer's. Zero time corresponds to the efflux 10 minutes after transfer to Li_2SO_4 Ringer's. On the right, Na^{24} efflux (in cpm/min.) from a Na-rich muscle in Li_2SO_4 Ringer's (zero time as above). After 10 minutes, Li_2SO_4 Ringer's containing 210 mM sucrose was added (high π) and efflux was followed for 8 minutes before Li_2SO_4 Ringer's was again applied.

The Effect of Osmotic Pressure Change on Na Efflux The efflux of Na from muscle when $[Na]_i$ is low has been shown to depend on the 3rd power of $[Na]_i$; thus small changes in $[Na]_i$ lead to large changes in efflux. If, however, $[Na]_i$ is high and the Na extrusion mechanism saturated, there should be no change in Na efflux when $[Na]_i$ is increased. A number of experiments were performed in which a Na-loaded muscle was changed from a Ringer's solution (2.5 mM K) to one containing 210 mM sucrose in addition to the usual salts. Such solutions had twice the normal osmotic pressure of Ringer's and both $[Na]_i$ and $[K]_i$ would be expected to increase. Fig. 4 (right) shows the result obtained for a muscle in choline chloride Ringer's solution. There was a momentary drop and then a return to a normal level of efflux, while a reverse rise of efflux took place when the muscle was returned to a fluid with a normal osmotic pressure. These transient flux changes can be explained by supposing that the sudden withdrawal of water from the fibers greatly dilutes and expands the extra-

cellular space and thus leads to a temporary diminution of Na^{24} efflux. Results with 8 muscles showed that (efflux in hypertonic Ringer's)/(efflux in normal Ringer's) = 1.0 ± 0.1. The point established by these measurements is that the efflux is not affected by a very considerable increase in $[Na]_i$, or that the pump is saturated. If an appreciable part of the Na efflux were by passive diffusion, efflux ought to increase upon the application of a hypertonic solution. The lack of an increase in efflux confirms previous measurements showing a negligible fraction of Na efflux to be passive. It might be argued that the concomitant increase in $[K]_i$ involved in this sort of measurement may change the membrane potential and thus affect the Na pump. In answer to this, it may be noted that the evidence from potential measurements is that the Na pump is in a large measure in control of the membrane potential (*e.g.* E_m is greater than E_K) and the few preliminary measurements we have made of membrane potentials under the experimental conditions described above showed that there was no change in membrane potential when hypertonic Ringer's solutions were applied.

The Effect of Increased $[K]_o$ *on Na Efflux*   It has been known since the original demonstration by Steinbach (9) that if muscles, loaded with Na by soaking in K-free Ringer's in the cold, are to regain K and lose Na it is necessary to raise the $[K]_o$ of the Ringer's into which muscles are placed for recovery. Our interest in the effect of increases in the $[K]_o$ of sulfate Ringer's solution on the Na efflux was to establish a value for the maximum rate of Na efflux. Muscles were loaded in the usual way in K-free SO_4 Ringer's at 4°C in pairs and were then transferred to Li_2SO_4 Ringer's at 20°C. After 10 minutes of efflux, one of the paired muscles was changed to Li_2SO_4 Ringer's with 25 mM [K] while the other muscle remained in Li_2SO_4 Ringer's. After 10 minutes of sample collection, the 25 mM [K] muscle was returned to Li_2SO_4 Ringer's for 6 minutes after which both muscles were analyzed for Na, K, and Na^{24}. From the specific activity of Na in muscle and an assumed value of 405 cm^2/gm it was possible to calculate the plateau efflux, and the efflux in high $[K]_o$. These values (for 8 muscles) were 21 ± 4 pmole/cm^2 sec. for Na efflux in 2.5 mM $[K]_o$ and 43 ± 9 pmole/cm^2 sec. for efflux in 25 mM $[K]_o$. The effect of high $[K]_o$ was thus to increase Na efflux by 2.1 times.

DISCUSSION

The finding that Cl-free muscle fibers with a high $[Na]_i$ have a membrane potential that during Na extrusion is substantially higher than E_K suggests that the extrusion mechanism itself is able to generate an EMF. Before making this conclusion a firm one, however, it is necessary to analyze the potential data of Table I in somewhat more detail. The membrane potential (−47 mv) for loaded fibers in K-free sulfate Ringer's at 4°C requires a ratio P_K/P_{Na} = 10

if the equation for membrane potential with net ion flow[2] is to be applied, while for unloaded fibers the membrane potential that is observed under similar circumstances (−104 mv) requires a permeability ratio of 80. Such a change in ratio may be compared with the data of Table III where it is suggested that P_{Na} increases sevenfold when a muscle is fully Na-loaded. If P_K were approximately constant in the two cases the P_{Na} values found are roughly in agreement with each other. On the other hand, no account is taken of the fact that there is appreciable Na extrusion at 4°C even under K-free conditions. We have no systematic measurements under these conditions but in Li_2SO_4 Ringer's (2.5 mM K) the change in Na efflux was 2.2-fold in going from 4–20°C, while from the measurements of Keynes and Swan (5), Na efflux was reduced to about 0.5 in loaded muscles transferred to K-free Ringer's. Combining these values suggests that Na efflux falls to 1/4.4 or 0.23 at 4°C under K-free conditions. As our value for Na efflux from Na-loaded muscles at 20°C and with 2.5 mM [K] is about 20 pmoles/cm² sec., there might be 4.6 pmoles of Na efflux under K-free conditions in the cold. This outward ion movement, if not coupled by pumping to an inward ion movement, would contribute to the membrane potential and the P_{Na} as calculated from the equation given in footnote 2 would be too low. When Na-loaded muscles are warmed to 20°C in K-free sulfate Ringer's, the muscles are clearly still gaining Na and losing K and the change from 4°C values may be taken to represent an increased Na efflux by pumping. Two pairs of muscles that we used for comparison purposes were transferred from K-free sulfate Ringer's at 20°C to normal (2.5 mM K) sulfate Ringer's at the same temperature and showed a mean membrane potential of −88 mv instead of the mean value of −98 mv shown in Table I for muscles transferred to Li_2SO_4 Ringer's. The former membrane potential is very close to E_K while values in Li are clearly higher than E_K and this finding suggests that P_{Na} is somewhat greater than P_{Li}. This conclusion, however, in no way makes it necessary to suppose that the membrane potential observed is the result of a slow passive inward movement of Li and a fast passive outward movement of Na. The high membrane potential would require an impossibly high ratio of P_{Na} to P_{Li} while the experimental data that are available suggest that P_{Na}/P_{Li} might lie in the region of 2 to 1. A relatively modest P_{Na}/P_{Li} ratio of 1.5 would suffice to explain the difference in potentials observed in Na and Li Ringer's. It is also clear, from Table II, that muscles soaked in Li Ringer's must gain appreciable quantities of Li (from the sums of Na + K for muscles in Li vs. Na Ringer's). It might be thought

[2] Membrane potential was calculated from the following:

$$E = 58\{\log (\gamma_K)_o/(\gamma_K)_i\} + 58 \{\log(P_K[K]_i + P_{Na}[Na]_i)/P_{Na}[Na]_o\}$$

with analytical data from the last two lines of Table II.

that the sum $[Na + K]_i$ is not a reliable index of Li penetration if intracellular cation is lost with accompanying anion. Such an effect, over a period of 12 hours in sulfate Ringer's amounts to about 5 per cent of cell water (7). Over the period of 130 minutes in Li Ringer's there is no detectable loss of intracellular anion and further, control muscles lose 14 μmole/gm K which suggests an equivalent entry of Li. This amounts to a value of 6.8 μmole/gm hour which is a value close to that for Na influx in muscle. Na-loaded muscles gain K and lose Na but the calculated Li gain is the same as that given above. This is not surprising if, over most of the 130 minute period, P_{Li} is at normal levels. It is important to note that the observed membrane potentials in Li_2SO_4 Ringer's cannot be explained on the basis of passive ion movement whatever the assumptions one might make about P_{Li}. The Li equilibrium potential upon transfer of muscles to Li Ringer's is positive and infinite, while the observed membrane potential is larger than E_K and negative. The only question is how large a shunt on the potential developed by Na pumping the inward movement of Li constitutes. The measurements suggest that it is of the same order of magnitude as but slightly less than that produced by Na^+.

When the Na extrusion process is largely over, the membrane potential of the fibers is -83 which agrees reasonably well with the potential calculated with $P_K/P_{Li} = 80$, or with the notion that the selectivity of the membrane for K^+ returns when $[Na]_i$ is low.

Muscles loaded with Na and then placed in sulfate Ringer's (2.5 mM K) neither lose nor gain Na in analytically detectable amounts (see Table III), and the membrane potential of their fibers is very close to E_K (see above) as it should be if Na fluxes are in balance and there is no coupling of Na efflux to K influx (see reference 7). Such muscles promptly lose Na if $[K]_o$ is increased tenfold. A partial explanation of this effect is that Na influx is diminished by the fall in membrane potential but by far the larger effect is the doubling of Na efflux brought about by the increased $[K]_o$. The mechanism for this action is far from apparent and notions regarding it might be grouped as follows: (a) Na-K coupling, (b) direct effect of membrane potential on Na pump, and (c) action of altered [K] on the pump mechanism. A very careful examination of K flux ratios in normal muscle (8) has shown that a 3:1 coupling ratio could have been detected so that 4:1 is the lowest ratio compatible with the data at hand. There is not, however, any comparable study on Na-loaded muscle and the most that can be said is that for such muscles coupling cannot be 1:1 because then the electrical effects observed in this study would not be apparent. A high coupling ratio is, unfortunately, a most difficult point to establish experimentally, because K fluxes are generally quite large and somewhat variable; and, in practice, large coupling ratios do not differ experimentally from no coupling at all. A variant of the notion of a high coupling ratio is that of having a coupling ratio dependent upon experimental conditions. At present so little

is known about the conditions that bring about coupling between Na and K fluxes in muscle that it is hardly profitable to discuss the point. Keynes and Rybova (4) point out in their note that K can be absorbed from Ringer's solutions when E_m is held down with 30 mM K even though E_m is now less than E_K. A direct effect of E_m on the Na pump would satisfactorily explain why pump efflux increases when $[K]_o$ is increased.[3] Indeed, a suggestion along these lines has been proposed by Conway (2) with the term "critical energy barrier." Briefly, the idea is that if the extrusion of Na requires more energy than about 2 kcal/mole, no extrusion will occur. The situation in muscle is not quite as simple as this because whether muscles in Na-Ringer's recovery solutions will lose Na depends to an important extent on P_{Na} which is high when $[Na]_i$ is high while the Na pump is saturated (e.g. its efflux is independent of concentration). Thus a loss of Na under these conditions depends entirely on having the pump efflux greater than Na influx and increased $[K]_o$ is able to do this. The experiments with Ringer's solutions with twice normal osmotic pressure, reported above, clearly show that Na efflux from loaded muscles is independent of $[Na]_i$ so that the effect of a high $[K]_o$ can be most directly considered as an effect of membrane potential on the pump mechanism (in this sense, Na-K coupling is only a special case of membrane potential–pump interaction). If there is a stoichiometry between energy substrate molecules and Na^+ pumped, then there must exist an electrochemical potential difference at which Na pumping is zero, together with a relationship between $(E_{Na} - E_m)$ and Na pump current for all values of $(E_{Na} - E_m)$ less than the zero current value. Under our experimental conditions, Na-loaded muscles have a E_{Na} of +17 mv and in Na_2SO_4 Ringer's are in a steady state with respect to Na at a membrane potential of −88 mv so that the electrochemical potential difference for Na is 105 mv while in control muscles in Na_2SO_4 Ringer's, E_{Na} is +47 mv and E_m is −88 or the electrochemical potential difference is 135 mv again for steady state Na. Both these values of electrochemical potential are much higher than the critical energy barrier of Conway (2 kcal $\sim$ 83 mv) but the muscles are not losing Na. The fluxes are in balance in both cases because in loaded muscles our measured value for initial flux is $\sim$ 20 pmole/cm² sec. and for influx to equal this value, P_{Na} must be 8 A/sec. while for unloaded muscles this value must be $\sim$ 1 A/sec. Finally, it may well be that the application of [K] to the outside of the membrane changes the [K] in the vicinity of the Na pump and that such changes in [K] affect the energy flow to the pump without involving any coupling of the K fluxes to those of Na.

[3] Note Added in Proof.   Consierable support for the notion that the membrane potential affects Na pumping is provided by two recent papers (Horowicz, P., and Gerber, C. J., J. Gen. Physiol., 1965, 48, 489 and 515).

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