The Kinetics of Sodium Extrusion in Striated Muscle As Functions of the External Sodium and Potassium Ion Concentrations

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ABSTRACT After a 20 min initial washout, the rate of loss of radioactively labeled sodium ions from sodium-enriched muscle cells is sensitive to the external sodium and potassium ion concentrations. In the absence of external potassium ions, the presence of external sodium ions increases the sodium efflux. In the presence of external potassium ions, the presence of external sodium ions decreases the sodium efflux. In the absence of external potassium ions about one-third of the Na^+ efflux that depends upon the external sodium ion concentration can be abolished by 10^{-5} M glycoside. The glycoside-insensitive but external sodium-dependent Na^+ efflux is uninfluenced by external potassium ions. In the absence of both external sodium and potassium ions the sodium efflux is relatively insensitive to the presence of 10^{-5} M glycoside. The maximal external sodium-dependent sodium efflux in the absence of external potassium ions is about 20% of the magnitude of the maximal potassium-dependent sodium efflux. The magnitude of the glycoside-sensitive sodium efflux in K-free Ringer solution is less than 10% of that observed when sodium efflux is maximally activated by potassium ions. The inhibition of the potassium-activated sodium efflux by external sodium ions is of the competitive type. Reducing the external sodium ion concentration displaces the plots of sodium extrusion rate vs. $[K]_o$ to the left and upwards.

INTRODUCTION

Sodium efflux from striated muscle cells is sensitive to the external potassium and sodium ion concentrations (Keynes, 1954; Keynes and Swan, 1959; Horowicz and Gerber, 1965). In general, it is possible in muscle cells to divide sodium efflux into potassium-requiring and sodium-requiring fractions (Keynes, 1966; Keynes and Steinhardt, 1968; Sjodin and Beaugé, 1968). The sodium-requiring fraction is subject to different interpretations. It is generally accepted, however, that the potassium-requiring fraction is a component of sodium efflux that also requires a metabolic energy supply and that

external potassium ions somehow activate a metabolically coupled mechanism to extrude sodium ions.

Some properties of the activation of sodium transport by external potassium ions deserve mention. Such activation is abolished by cardiac glycosides (Edwards and Harris, 1957; Horowicz and Gerber, 1965). In addition, the activation mechanism responds to cations other than potassium. The cations Rb^+, Cs^+, and Li^+ have been shown to exert a K-like action on sodium efflux in striated muscle cells (Adrian and Slayman, 1966; Beaugé and Sjodin, 1968 *a*,*b*).

Other properties of sodium transport activation by potassium ions have been studied in both muscle and nerve cells. The curve relating degree of activation of the sodium pump in muscle cells to the external potassium ion concentration has been observed to be sigmoidal in character in the presence of external sodium ions (Sjodin and Beaugé, 1968). Baker et al. (1969) have observed a similar relationship in squid giant axons. Squid giant axons, however, showed a nonsigmoidal activation by potassium ions in the absence of external sodium ions. In crab nerve, activation of sodium transport by potassium ions was consistent with a simple hyperbolic relationship (Baker and Connelly, 1966). Also external sodium ions had an inhibitory effect on activation by potassium ions in crab nerve. In mammalian nonmyelinated nerve, Rang and Ritchie (1968) have observed that activation of sodium pumping by external potassium ions can be fit by a rectangular hyperbolic relationship.

The purpose of the present work is to investigate the influence of sodium ions on the activation of sodium extrusion in muscle cells by potassium ions and to describe in detail the actions of external sodium ions and external potassium ions on sodium efflux separately and in combination.

METHODS

The details of the experimental methods employed are identical to those previously reported (Sjodin and Henderson, 1964; Sjodin and Beaugé, 1968). All experiments were performed on sartorius muscles from the frog, *Rana pipiens*. All muscles used for experimentation were first enriched with sodium by storage at 3°C in potassium-free Ringer solution for periods ranging between 24 and 48 hr. The time of storage depended on the time required to elevate the internal sodium concentration to a value of around 30 μmoles/g muscle. This time interval was subject to some seasonal variation but was rather uniform for muscles obtained from a given batch of animals. 12 hr before the time for experimentation, the potassium-free storage solution was labeled with ^{22}sodium ions. After labeling of the muscle sodium the efflux of radioactive sodium ions was measured as a function of time in solutions of specified composition. The radioactive sodium remaining in the muscles was plotted against time semilogarithmically. An initial rapid component of ^{22}Na washout that lasted about 20 min was always observed. After this period semilogarithmic plots became linear in a solu-

tion of specified composition. In experiments designed to measure the influence of the external potassium ion concentration or the influence of 10^{-5} M strophanthidin on sodium efflux, the uniform rate constant obtained in a K-free and glycoside-free medium was measured for a period of 40–60 min prior to addition of either potassium or strophanthidin to the medium. The potassium-stimulated sodium transport was then obtained as an increment in rate constant elicited by the presence of a given concentration of potassium ions. The glycoside-sensitive efflux was obtained as the decrement in rate constant observed in the presence of 10^{-5} M strophanthidin. In all measurements of the potassium-sensitive sodium efflux the same concentration of external sodium ions was employed throughout the entire experiment so that in no case was there a simultaneous change in both the external sodium and potassium ion concentrations. For example, the potassium-sensitive sodium efflux in Ringer solution with a sodium concentration of 30 mM/liter is obtained as the difference between the rate constant measured in 30 mM Na-Ringer solution with a given K^+ concentration and that measured in 30 mM Na-Ringer solution in the absence of external potassium ions.

The rate constant difference denoting potassium-sensitive sodium transport is designated m_{Na}. This quantity is plotted vs. ion concentration under Results. This quantity is plotted rather than sodium efflux because the magnitude of sodium efflux rapidly changes in most of the experiments reported. Muscles are not in a steady state because they are engaging in net sodium extrusion. The initial internal sodium ion concentration had an average value close to 30 mmoles/kg muscle (about 50 mM/liter of fiber water). The initial average K-sensitive sodium efflux, ϕ_{Na}, can be obtained from the relation $\phi_{Na} = m_{Na}\left(\frac{2}{r}\right)[Na]_i$ where r is the average fiber radius.

Solutions The purpose of the experiments requires the variation of the external sodium ion concentration from zero to 120 mM. The standard Ringer solution employed had the following composition (mM): NaCl, 120; $CaCl_2$, 2; Tris buffer, 1. The Tris-substituted Ringer solution employed was formulated as above with all the NaCl replaced with an osmotic equivalent of Tris neutralized with HCl to a pH of 7.35. Potassium ions were added to these solutions from an isotonic stock solution. The external sodium ion concentration was varied by varying the proportion of Tris Ringer solution to sodium Ringer solution. When it was desired to maintain the external sodium ion concentration at 120 mM, potassium was added by increasing the total osmotic pressure of the solution. The potassium-free control solutions, under these conditions, were adjusted to the same osmotic pressure by adding either Tris or sucrose. The osmotic pressure matches of various solutions were checked by the freezing point depression method.

All experiments were carried out in a water bath with the temperature regulated to 20°C ± 0.2°C.

RESULTS

The Influence of External Sodium Ions on Sodium Efflux in the Absence of External Potassium Ions

Before studying the influence of external sodium ions on activation of sodium pumping by potassium ions, the effects of sodium ions alone were studied.

The external sodium concentration was varied from zero to 120 mM. Experiments were performed in the absence and in the presence of 10^{-5} M strophanthidin. The results appear in Fig. 1 where the rate of sodium loss is plotted as the total measured rate in the absence of strophanthidin, the rate in the presence of strophanthidin, and the reduction in rate produced by strophanthidin for each muscle studied. At normal Ringer solution concentrations of

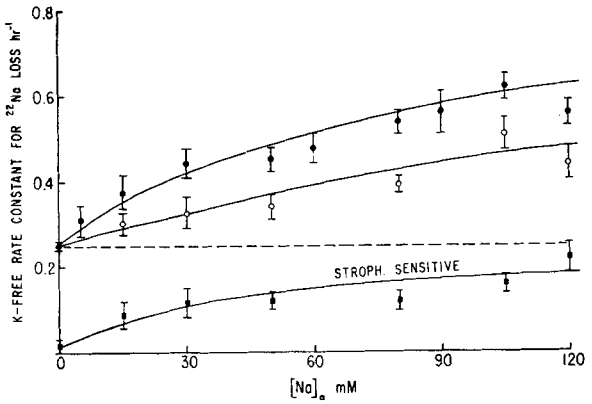

FIGURE 1. The rate constant for loss of radioactively labeled sodium ions from muscles in potassium-free media is plotted against the external sodium ion concentration. The top curve represents the total measured rate constant. The middle curve refers to measurements made in the presence of 10^{-5} M strophanthidin. The bottom curve refers to the amount of sodium efflux that is abolished by application of 10^{-5} M strophanthidin. Each point represents the average value ± 1 SE. Each point on the upper curve represents the average of 12–30 experiments; each point on the bottom two curves represents the average of between 6 and 10 experiments. The broken line refers to the average value of the rate constant measured in a K- and Na-free medium (44 experiments). The upper two curves were obtained on different muscles and the K-free and Na-free rate constants holding for the absence and presence of strophanthidin are not significantly different statistically. The bottom curve was constructed by using the conditions, absence and presence of strophanthidin, on the same muscles. In this case a small but statistically significant reduction in the K-free and Na-free rate constant is observed when 10^{-5} M strophanthidin is applied.

sodium ions, removal of sodium, and replacement with Tris brought about a reduction in sodium efflux to less than one-half of the value observed with sodium present (the broken line shows the average rate of loss observed in sodium- and potassium-free Tris Ringer solution for 44 muscles). At all external sodium ion concentrations above 15 mM, the addition of 10^{-5} M strophanthidin produced an approximately 25% reduction in sodium efflux. In Na- and K-free Tris Ringer solution, the addition of 10^{-5} M strophanthidin produced either no change in sodium efflux or a small reduction amounting to a few per cent. The effects of 10^{-5} M strophanthidin on sodium efflux from

individual muscles at different external sodium ion concentrations are summarized in Table I.

The results show that all components of efflux rise with increasing external sodium ion concentrations along uninflected curves that can be approxi-

TABLE I

THE INHIBITION OF SODIUM EFFLUX IN STRIATED MUSCLE CELLS BY STROPHANTHIDIN AT DIFFERENT EXTERNAL SODIUM ION CONCENTRATIONS IN THE ABSENCE OF EXTERNAL POTASSIUM IONS

	Rate constants		
$[Na]_o$	Control	10^{-5} M strophanthidin	Inhibition
mM	hr^{-1}	hr^{-1}	%
105	0.54	0.42	22
105	0.56	0.44	21
105	0.79	0.61	23
105	0.66	0.44	33
105	0.79	0.65	18
80	0.52	0.40	23
80	0.55	0.42	24
80	0.51	0.38	25
80	0.45	0.33	27
80	0.52	0.41	21
50	0.54	0.38	30
50	0.58	0.47	19
50	0.43	0.33	23
50	0.45	0.34	24
50	0.39	0.23	41
50	0.36	0.26	28
0	0.23	0.21	9
0	0.22	0.22	0
0	0.24	0.24	0
0	0.22	0.17	23
0	0.20	0.20	0
0	0.21	0.18	14
0	0.19	0.13	32
0	0.24	0.18	25
0	0.29	0.24	17
0	0.30	0.26	13

mated by a constant plus a saturating "Michaelis" term. In the case of the strophanthidin-sensitive component, the constant is nearly zero and the sodium ion concentration for half-maximal efflux is approximately 30 mM. The glycoside-insensitive component reaches a half-maximal value at an external sodium ion concentration of approximately 45 mM.

Activation of Sodium Extrusion by Potassium Ions in the Absence of External Sodium Ions

Removal of sodium ions from Ringer solution and replacement with Tris produced increases in the potassium-sensitive component of sodium efflux at all potassium ion concentrations studied. On a percentage of change basis, increases were greater the lower the external potassium ion concentration. The increased sensitivity of sodium efflux to potassium ions under sodium-free conditions is particularly well-revealed at $[K]_o$ = 0.25 mM. This potassium concentration produces no detectable activation of sodium extrusion in normal Ringer solution but a reproducible and easily detected activation in Tris Ringer solution. The activation produced by $[K]_o$ = 0.25 mM in Tris Ringer solution is equivalent to that produced by $[K]_o$ = 1.5 mM in normal Ringer solution.

In addition to increasing the sensitivity of sodium efflux to external potassium ions, sodium-free conditions convert the curve relating degree of pump activation produced by potassium to the external potassium ion concentration from a sigmoidal to a nonsigmoidal relation. In Ringer solution, the potassium-activated sodium extrusion rises with increasing potassium ion concentration along an S-shaped curve (Sjodin and Beaugé, 1968). The results obtained in the absence of external sodium can be fit very well by a rectangular hyperbolic relation of the form

$$m_{Na} = M_{Na}\left(\frac{[K]_o}{[K]_o + k_m}\right) \tag{1}$$

where m_{Na} is potassium-activated sodium efflux, M_{Na} is maximally activated efflux, $[K]_o$ is the external potassium ion concentration, and k_m is the Michaelis constant. The results obtained in Na-free Tris Ringer solution are plotted in Fig. 2 as the reciprocal of K-activated sodium efflux vs. the reciprocal of the potassium ion concentration. The line drawn through the points is a plot of the equation

$$\frac{1}{m_{Na}} = \frac{1}{M_{Na}} + \frac{k_m}{M_{Na}}\left(\frac{1}{[K]_o}\right) \tag{2}$$

with k_m = 3.3 mM and M_{Na} = 2.5 hr^{-1}.

The Influence of External Sodium Ions on Activation of the Sodium Pump by Potassium Ions

In the presence of external potassium ions, the action of external sodium ions on the rate of sodium extrusion was reversed from the types of action seen

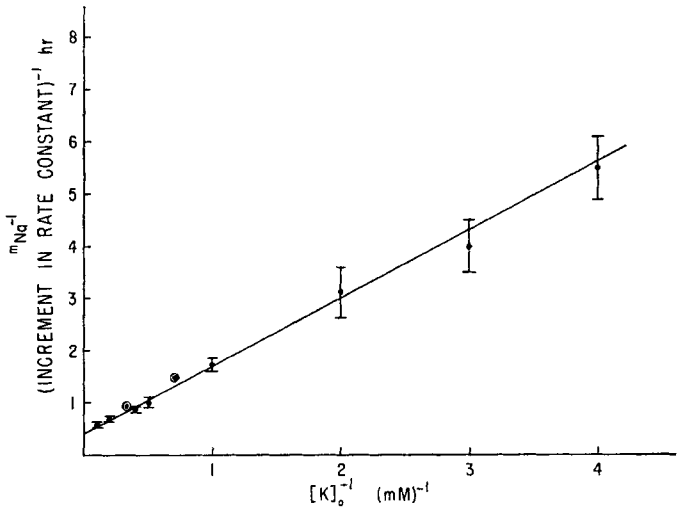

FIGURE 2. The reciprocal of the potassium-sensitive portion of the rate constant for labeled sodium loss in the absence of external sodium ions is plotted against the reciprocal of the external potassium ion concentration. Each point refers to the average of from six to nine experiments ±1 SE. The two points lacking standard errors are averages of two experiments. The straight line drawn is a plot of equation (2) with the constants given in the text.

in Fig. 1. Increasing external sodium ion concentrations produced diminutions in the rate of extrusion in the presence of external potassium ions. The activating effect of external potassium ions at different external sodium ion concentrations is shown in Fig. 3.[1] Increasing sodium ion concentrations displace the curves progressively to the right and downward. The effects of different external sodium and potassium ion concentrations on the total measured rate of labeled sodium loss from muscles are summarized in Table II.

Though the complete explanation of these effects is likely to be complex, the kinetics suggest that external sodium ions may act by competitively displacing potassium ions from sites at the outer membrane surface. If it is assumed that each activation site can bind three ions, the following reactions

[1] All the potassium-sensitive sodium efflux plotted is abolished by application of 10^{-5} M strophanthidin. The potassium-sensitive sodium efflux can be compared with the total glycoside-sensitive sodium efflux by referring to Fig. 1 where one can read the glycoside-sensitive efflux in the absence of external potassium ions at any external sodium ion concentration desired. Since the external sodium-dependent sodium efflux in the presence of 10^{-5} M glycoside is insensitive to external potassium, the total glycoside-sensitive efflux at any potassium ion concentration can be obtained by adding the potassium-sensitive efflux and the glycoside-sensitive efflux in the absence of external potassium at the appropriate external sodium ion concentration.

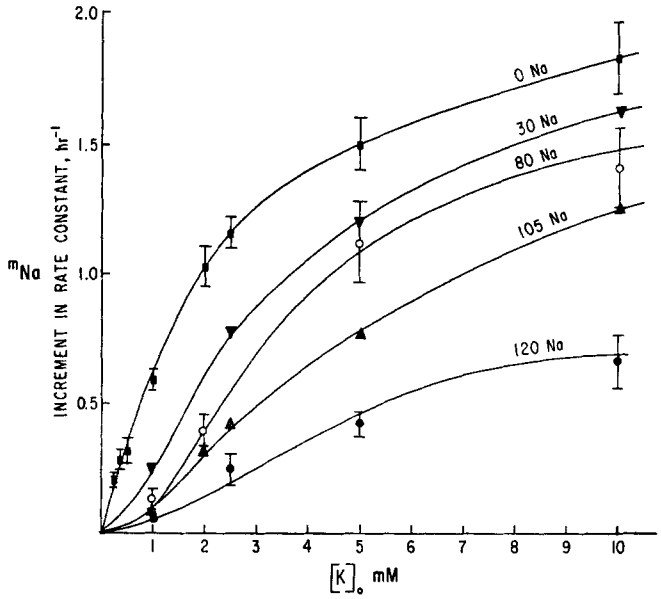

FIGURE 3. The potassium-sensitive portion of the rate constant for loss of labeled sodium ions from muscles is plotted against the external potassium ion concentration for different external sodium ion concentrations. From top to bottom the external sodium ion concentrations are 0, 30, 80, 105, and 120 mM. Each point represents the average of from six to nine experiments ±1 SE. For two of the curves, the standard errors have been omitted for clarity of the graphs. The standard errors omitted are of similar magnitude to those indicated. For all cases except the 80 mM sodium case, the curves were drawn to fit the data points. For the 80 mM sodium data, the curve drawn is a plot of equation (3) with the constants stated in the text.

TABLE II

THE TOTAL MEASURED RATE CONSTANT FOR LOSS OF RADIOACTIVELY LABELED SODIUM IONS FROM MUSCLES AT DIFFERENT EXTERNAL POTASSIUM AND SODIUM ION CONCENTRATIONS:

(Each entry is the average of at least six measurements.)

		$[Na]_o$, mM					
		0	30	60	80	105	120
		hr^{-1}					
	0	0.25	0.44	0.48	0.54	0.62	0.56
	0.5	0.63	—	—	—	—	—
	1.0	0.87	0.63	0.52	0.66	0.66	0.59
$[K]_o$,	2.0	1.32	—	—	0.96	0.84	—
mM	2.5	1.38	1.14	0.91	—	1.07	0.71
	5.0	1.60	1.46	1.07	1.66	1.25	0.92
	10.0	2.15	1.93	1.57	1.95	1.97	1.15
	20.0	2.40*	—	—	—	—	2.24

* A rate constant of 2.4 hr^{-1} represents an efflux of 37.5 pmoles/cm^2sec for a 75 μ diameter fiber with $[Na]_i$ = 30 mM/liter.

may occur at the outer membrane surface:

$$K_o + X\text{Na}_3 \underset{k_2}{\overset{k_1}{\rightleftharpoons}} X\text{Na}_2\text{K} + \text{Na}_o$$

$$K_o + X\text{Na}_2\text{K} \underset{k_4}{\overset{k_3}{\rightleftharpoons}} X\text{NaK}_2 + \text{Na}_o$$

$$K_o + X\text{NaK}_2 \underset{k_6}{\overset{k_5}{\rightleftharpoons}} X\text{K}_3 + \text{Na}_o .$$

If it is further assumed that $X\text{K}_3$ is the form that "activates" the transport mechanism to turn over one cycle, the potassium-activated portion of sodium efflux will be proportional to the fraction of sites in the $X\text{K}_3$ form. By solving for the steady-state concentration of $X\text{K}_3$ with the additional assumption that other reactions which might change $[X\text{K}_3]$ occur at rates much slower than the rates of the reactions given above, one obtains

$$m_{\text{Na}} = \frac{M_{\text{Na}}}{1 + a\frac{[\text{Na}]_o}{[\text{K}]_o} + b\left(\frac{[\text{Na}]_o}{[\text{K}]_o}\right)^2 + c\left(\frac{[\text{Na}]_o}{[\text{K}]_o}\right)^3} \tag{3}$$

where m_{Na} and M_{Na} have the same significance as before and where a, b, and c are given by the rate constant ratios as follows:

$$a = 3\frac{k_6}{k_5}$$

$$b = 3\frac{k_4 k_6}{k_3 k_5}$$

$$c = \frac{k_2 k_4 k_6}{k_1 k_3 k_5}.$$

The line drawn through the data points for activation in 80 mM sodium Ringer solution is a plot of equation (3) with $a = 3.75 \times 10^{-2}$, $b = 6.25 \times 10^{-4}$, $c = 2.34 \times 10^{-5}$, and $M_{\text{Na}} = 2.0$. The data are not fit as well if it is assumed that each carrier molecule must bind two potassium ions before activation occurs.

If the external sodium ion concentration is varied, equation (3) with the constants given above fits the data fairly well between sodium concentrations of 80 and 105 mM. At lower sodium ion concentrations, activation is less than predicted by equation (3) except at high potassium ion concentrations. For the case, $[\text{Na}]_o = 0$, equation (3) predicts maximal activation at all potassium ion concentrations. The data, however, show that the activation sites bind potassium ions according to equation (1) in the absence of external sodium ions. Equation (3) reduces to the correct form at $[\text{Na}]_o = 0$ if it is empirically

modified to the relation:

$$m_{Na} = \frac{M_{Na}}{1 + \frac{k_m + a[Na]_o}{[K]_o} + b\left(\frac{[Na]_o}{[K]_o}\right)^2 + c\left(\frac{[Na]_o}{[K]_o}\right)^3}. \quad (4)$$

Equation (4) gives a fairly satisfactory fit to the data obtained at external sodium ion concentrations of 105 mM and lower when k_m, a, b, and c have the values given previously and $M_{Na} = 2.5$.

External Sodium Ions As Competitive Inhibitors of Activation by Potassium Ions

For competitive action at a single activation site, the reciprocal of the rate is a linear function of the concentration of the competitively inhibiting species at constant concentration of the activating species. Reciprocal plots of pumping rate vs. $[Na]_o$ at three values of the external potassium ion concentration are shown in Fig. 4. The plots are approximately linear over restricted ranges

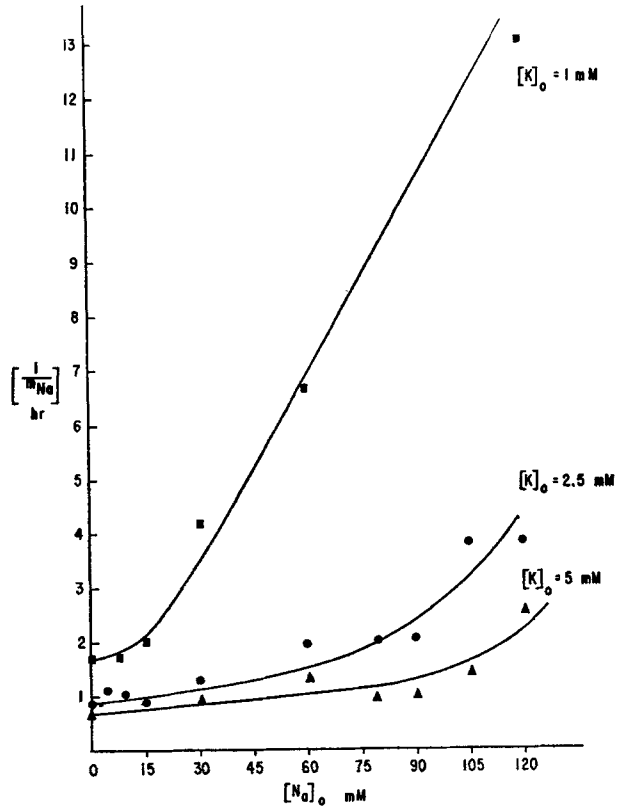

FIGURE 4. The reciprocal of the potassium-sensitive portion of the rate constant for loss of labeled sodium ions from muscles is plotted against the external sodium ion concentration at three external potassium ion concentrations: $[K]_o$ = 1, 2.5, and 5 mM (from top to bottom). Each point represents the average of from six to nine observations. Curves were drawn to fit the data points.

but deviate from linearity in general due to the fact that activation and competition occur according to a law containing the second and third powers of ionic concentrations.

Another mode of plotting that is useful in determining whether or not inhibition is of the competitive type is to plot the reciprocal of rate against the reciprocal of the activator concentration at different concentrations of the inhibitor. This type of plot is shown in Fig. 5 for four sodium ion con-

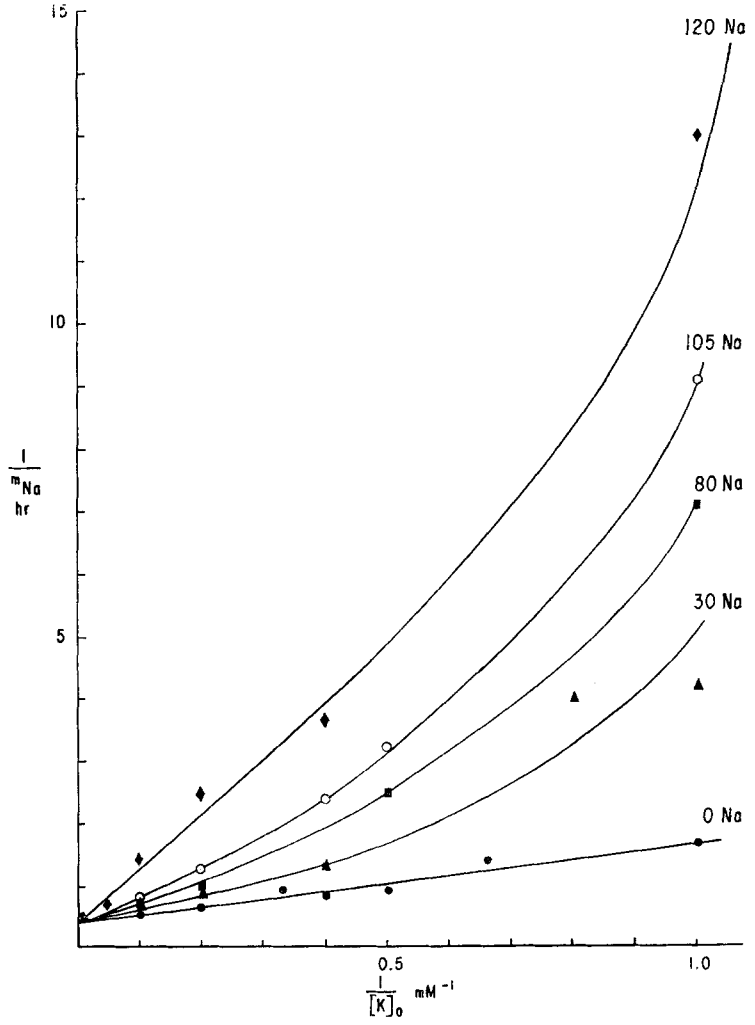

FIGURE 5. The reciprocal of the potassium-sensitive portion of the rate constant for loss of labeled sodium ions from muscles is plotted against the reciprocal of the external potassium ion concentration at different external sodium ion concentrations: 120, 105, 80, 30 MM, and 0 from top to bottom. Each point is the average of from six to nine observations. Curves were drawn to fit the data points.

centrations compared with the case in which the external sodium ion concentration has been reduced to zero. All curves are observed to extrapolate to the same point on the reciprocal rate axis. These results are consistent with the conclusion that the inhibition of rate due to sodium ions is of the competitive type.

That equation (4) is of the correct form to fit these data can be seen by rewriting the equation in reciprocal form to obtain:

$$\frac{1}{m_{Na}} = \frac{1}{M_{Na}} + \frac{1}{M_{Na}}\left(\frac{k_m}{[K]_o} + \frac{a}{[K]_o}[Na]_o + \frac{b}{[K]_o^2}[Na]_o^2 + \frac{c}{[K]_o^3}[Na]_o^3\right). \quad (5)$$

An obvious test is to see whether the curves in Fig. 4 meet the reciprocal rate axis at a slope given by $a/[K]_o$. Application of this test indicates that they do. Determined by this method, the constant a is evaluated to be 3.60×10^{-2} which compares with the value of 3.75×10^{-2} giving the best fit to activation at varying $[K]_o$ at a constant external sodium ion concentration of 80 mM. The apparent k_I (inhibitor constant) for sodium ions determined from Fig. 5 is 40 mM.

Activation of Sodium Extrusion at Constant $[Na]_o$:$[K]_o$ *Ratio*

Increasing external potassium ion concentrations produce increasing degrees of activation while increasing external sodium ion concentrations produce increasing amounts of inhibition. Increasing the concentrations of both ionic species in proportion should measure the extent to which these two processes stay in pace or fail to stay in pace with one another. Stated another way, the terms in equations (3) and (4) contain the ratio $[Na]_o$:$[K]_o$. Constancy of this term thus implies a constancy of the relative kinetic effect due to the presence of sodium ions.

Experiments were performed at constant $[Na]_o$:$[K]_o$ ratios equal to 6, 12, and 24. Results are plotted against $[Na]_o$ in Fig. 6 and against $[K]_o$ in Fig. 7. The broken lines in Fig. 6 are predicted values according to equation (4). The results show that the potassium-activated transport rate at constant $[Na]_o$:$[K]_o$ ratio passes through optima in nearly all cases studied. For all ratios studied the optima occur at an external sodium ion concentration between 90 and 100 mM. The optimal rate occurs at different external potassium ion concentrations for different values of the $[Na]_o$:$[K]_o$ ratio. When the ratio of $[Na]_o$ to $[K]_o$ is equal to 24, the optimal external potassium ion concentration is 3.75 mM.

The interpretation of these results in terms of the kinetics previously described is that, at low external sodium ion concentrations, the activating effect of $[K]_o$ predominates due to the large difference between k_m for potassium and k_I for sodium. When the external sodium ion concentration becomes

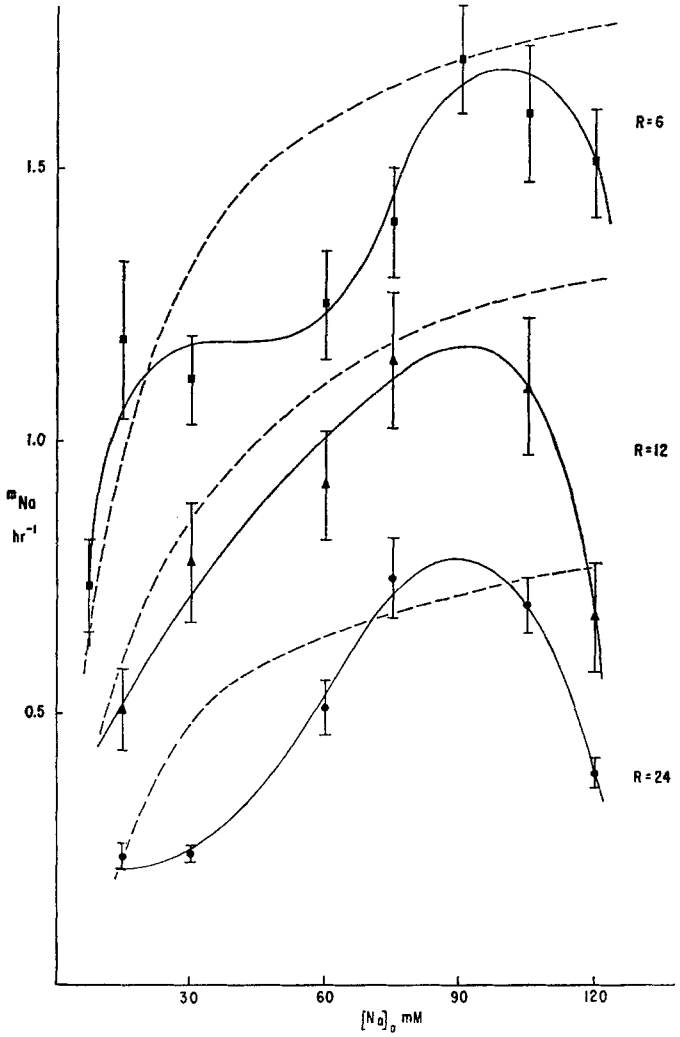

FIGURE 6. Rate of sodium extrusion is plotted against the external sodium ion concentration at constant ($[Na]_o$:$[K]_o$) ratio (R). From top to bottom: $R = 6$, $R = 12$, and $R = 24$. Solid curves were drawn to fit the data points. Broken curves were plotted from equation 4 with $M_{Na} = 2.5\ hr^{-1}$ and other constants as given in the text.

much larger than k_I, however, the competitive inhibition due to sodium ions becomes the strongest factor and the rate falls off with further increase in $[Na]_o$.

The predictions of equation (4) give a reasonable fit to the data in some ranges. The test of the equation is rather severe as $[K]_o$ was varied between 0.6 and 20 mM, $[Na]_o$ was varied between 15 and 120 mM, and the $[Na]_o$:$[K]_o$ ratio varies over a fourfold range. It would not be reasonable to expect a

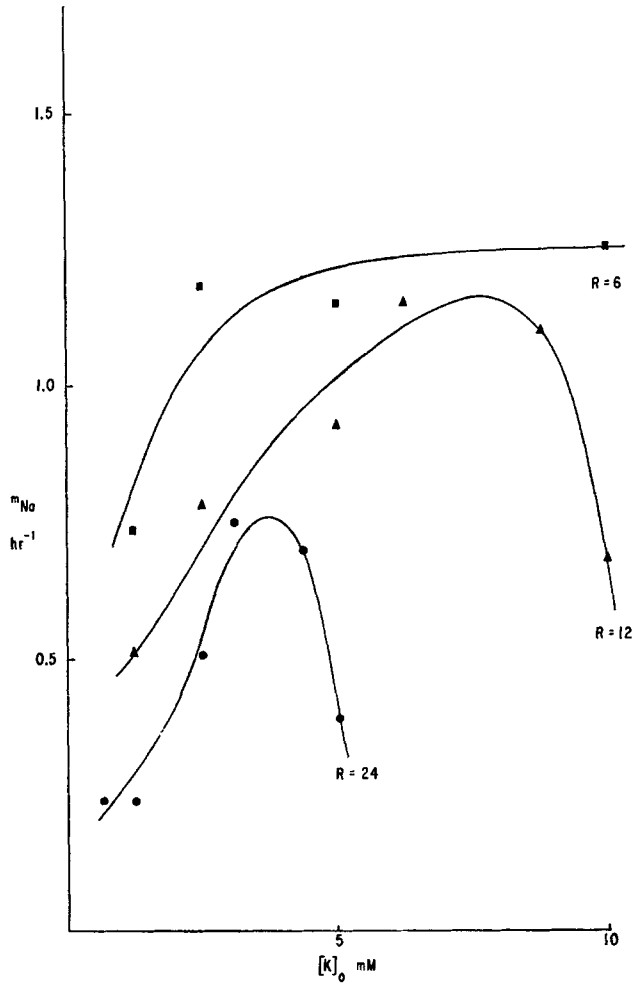

FIGURE 7. Rate of sodium extrusion is plotted against the external potassium ion concentration at constant ($[Na]_o$:$[K]_o$) ratio (R). From top to bottom: $R = 6$, $R = 12$, and $R = 24$. Curves were drawn to fit the data points.

model as simple as that upon which equation (4) is based to give a precise fit to the data over the entire range studied. It is interesting to note, however, that the most uniform weakness of the model is the failure to account for the large decline in rate between an external sodium ion concentration of 90 mM and one of 120 mM. The system behaves as though sodium ions become more effective competitors in this concentration range. A decline in the maximal transport rate observed at high $[K]_o$ apparently does not take place (Fig. 5) and hence cannot explain the decline in rate observed at high external sodium ion concentrations.

The Influence of the Internal Sodium Ion Concentration on the Rate of Sodium Extrusion in Tris-Substituted Ringer Solution

Keynes and Swan (1959) and Mullins and Frumento (1963) observed that the efflux of sodium ions from the muscle cells into lithium-substituted Ringer solution varied with the internal sodium ion concentration in a nonlinear manner as low intracellular sodium ion concentrations were approached. The results obtained by following sodium efflux in Tris Ringer solution to low intracellular sodium ion concentrations are shown in Fig. 8. In this particular case, the activation of sodium transport employed was that due to the presence of 10 mM external potassium. Similar results were obtained at other potassium ion concentrations. The behavior of Na efflux in Tris Ringer solution is similar to that observed by others using lithium Ringer solution. Sodium

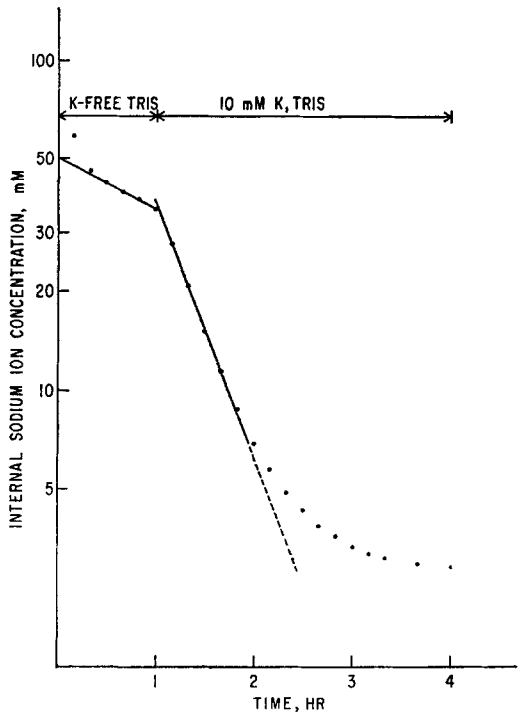

FIGURE 8. The internal sodium ion concentration is plotted semilogarithmically against time in contact with a sodium-free solution. The muscle was previously fully equilibrated with a Ringer solution containing labeled sodium ions at known specific activity. The internal sodium ion concentration was computed from a knowledge of the counts per minute of radioactivity remaining in the muscle, the specific activity of sodium ions, and the amount of intracellular water. For the first hour of efflux the bathing medium was K-freeTris-substituted Ringer solution. For the remaining 3 hr of efflux, the bathing medium contained potassium ions at a concentration of 10 mM.

efflux varies linearly with intracellular sodium concentration down to sodium concentrations equal to about 8 mM since rate constants obtained from semilogarithmic plots do not change in this range in media of constant composition. In all the experiments reported in this work, the cation-activated sodium efflux was always measured well within the linear region. The sodium efflux at any internal sodium ion concentration between 10 and 35 mM can be obtained from the present data by multiplying the internal sodium ion concentration by the rate constants reported and appropriate constant factors. The figure of 35 mM is cited because the cation-activated sodium efflux measurement generally began at that internal sodium ion concentration. Experiments were terminated well before an intracellular sodium ion concentration of 10 mM was reached, usually at a value between 15 and 20 mM.

The equation presented by Mullins and Frumento (1963) is of the correct form to fit the data in Fig. 8. The present theory can be extended to lower internal sodium ion concentrations by taking into account the functional relationship between the rate of sodium ion pumping and $[Na]_i$ observed by these authors.

The Potassium Concentration Threshold for Net Sodium Extrusion

In order to produce a net sodium extrusion from muscle cells, external potassium ions must activate the sodium pump to produce a pumped flux that is greater than the inward leak of sodium ions. The influence of external sodium ions on net sodium extrusion, therefore, is twofold. External sodium ions inhibit the pump at a given activating concentration of potassium ions and also produce an inward leakage that must be exceeded by the pump. Both factors contribute to an elevation of the minimum external potassium ion concentration required to produce net sodium extrusion at a given external sodium ion concentration.

It is clear that the activation of sodium pumping by external sodium ions in the absence of external potassium ions does not lead to a net extrusion of sodium ions. This type of sodium movement must be regarded as an exchange between internal and external sodium ions. The additional sodium pumping that occurs in the presence of external potassium ions can lead to a net extrusion of sodium if the potassium concentration and consequent activation are great enough. It is an experimental fact that an increment in the rate of sodium pumping brought about by external potassium ions does not lead to a net extrusion of sodium unless it exceeds a certain magnitude. This fact can be reconciled by assuming that the potassium-activated sodium pumping works against an inward leak of sodium ions that is in addition to the Na-for-Na interchange occurring in the absence of external potassium.

In the absence of direct measurements of the inward sodium leakage rate,

its magnitude is unknown and can only be estimated on the basis of additional assumptions. The assumptions applied are the simplest ones consistent with the data and are as follows: (*a*) The inward sodium ion leak is a linear function of the external sodium ion concentration. (*b*) The inward sodium ion leak is in addition to the sodium-for-sodium exchange. (*c*) The magnitude of the inward sodium leakage rate when $[Na]_o$ = 120 mM is assumed to be given by the potassium-activated sodium pump rate when $[K]_o$ = 5 mM. (*d*) Only K-activated sodium pumping results in net sodium extrusion in the absence of other K-like activators. The basis for the third assumption is the present experimental observation that Na-loaded muscles neither showed an appreciable net gain nor loss of sodium ions when equilibrated in 120 mM Na, 5 mM K Ringer solution.

By equating rate of pumping to the assumed magnitude of the inward leak, it is possible to compute the predicted potassium ion concentration threshold for producing net sodium ion extrusion as follows. Pumped flux = $m_{Na}[Na]_i\, r/2$ where $r/2$ is the fiber volume to surface ratio and where m_{Na} is given by equation (4). Inward leak = $k[Na]_o$. Equating the two and rearranging gives:

$$[Na]_o\left(1 + \frac{k_m}{[K]_o}\right) + a\frac{[Na]_o^2}{[K]_o} + b\frac{[Na]_o^3}{[K]_o^2} + c\frac{[Na]_o^4}{[K]_o^3} = Y \qquad (6)$$

where Y is constant for all internal sodium ion concentrations within the linear region of pumping rate vs. $[Na]_i$ and where other constants retain their previously given values. The constant Y is evaluated by knowing one point at which the pumped flux just balances the inward leak. The point known most accurately is that obtained in 120 mM Na, 5 mM K Ringer solution. The third assumption previously stated is used to evaluate the constant Y which is found to have the value Y = 750 mM.

These assumptions are tested by constructing an experimental threshold curve and making a comparison with the theoretical curve calculated from equation (6). The experimental curve is constructed by selecting an external sodium ion concentration and evaluating the leak rate from the assumption of linearity. This rate is then located on the pump rate axis on Fig. 3. Using the curve for the appropriate external sodium ion concentration, the potassium ion concentration that produces this rate of pumping is determined. External potassium ion concentration is plotted against external sodium ion concentration in this manner. The resulting curve (Fig. 9, open circles) represents the locus of $[K]_o$ values that produce pumping rates that just balance inward leak rates at different $[Na]_o$ values. Higher values of $[K]_o$ should lead to extrusion and lower $[K]_o$ values should lead to gain of intracellular sodium ions. The broken line in Fig. 9 is the relation predicted by equa-

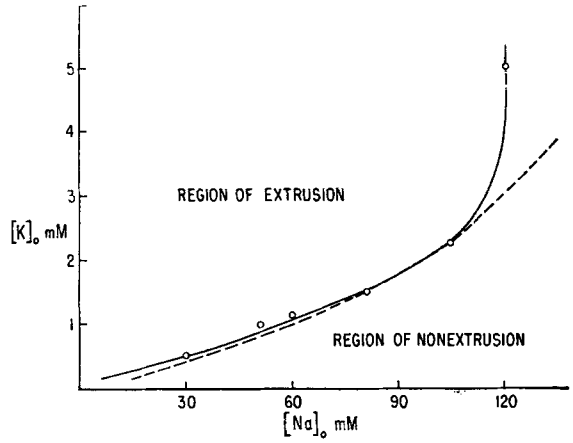

FIGURE 9. The threshold potassium ion concentration for producing a net extrusion of sodium ions from muscle cells is plotted against the external sodium ion concentration. The open circles refer to points obtained from experimental data applying the assumption that the inward leak of sodium ions occurs at a rate directly proportional to the external sodium ion concentration. The method for obtaining the points is discussed in the text. The points for $[Na]_o$ = 30, 80, 105, and 120 mM were obtained by use of the curves in Fig. 3. The point for $[Na]_o$ = 50 mM was obtained from Fig. 4, using the $[K]_o$ = 1 mM curve read at $[Na]_o$ = 50 mM. The point for $[Na]_o$ = 60 mM was obtained from separate experiments not reported in the other figures. The broken line represents the locus of solutions of equation (5) with the constants given in the text.

tion (5). Agreement is good up to an external sodium ion concentration of 100 mM. Above $[Na]_o$ = 105 mM, the experimental curve rises much more steeply than does the theoretical curve.

DISCUSSION

In the absence of external potassium ions, part of the external sodium-dependent efflux of sodium ions in muscle cells is abolished by application of glycosides. Behavior of this sort was first observed in partially poisoned squid giant axons by Caldwell et al. (1960) and in red blood cells by Garrahan and Glynn (1967) where, quantitatively, this type of exchange diffusion amounts to about one-half of the total glycoside-sensitive sodium efflux. The present results indicate that, in muscle cells, the maximal sodium efflux that is activated by external sodium ions and abolished by glycosides is about 10% of the magnitude of the maximally potassium-activated sodium efflux.

In red blood cells, the sodium-activated sodium efflux is accompanied by a sodium influx of equivalent magnitude that is abolished by application of glycosides. In muscle cells, Keynes and Steinhardt (1968) observed that both sodium influx and efflux were reduced about 20% by ouabain in the absence

of external potassium ions. It seems likely, therefore, that the sodium-activated sodium efflux presently observed is accompanied by a glycoside-sensitive sodium influx, though influx measurements were not made in the present study.

Horowicz and coworkers (1970) have identified a component of sodium efflux in muscle cells that has similar properties in that it shows activation by external sodium ions and is sensitive to the presence of strophanthidin. The authors identify this component of efflux as (SASS). It is possible that the (SASS) component of efflux is related to the presently observed sodium-activated and glycoside-sensitive sodium efflux. It is premature to state that the two components are related as the (SASS) component required prolonged exposure to sodium-free solutions for its appearance. Also, the present results have not yet been extended to lower internal sodium ion concentrations in the "nonlinear" region of sodium efflux.

Baker and coworkers (1969) have obtained evidence for a glycoside-sensitive Na:Na exchange in partially poisoned squid giant axons studied in K-free seawater. It is evident that, under certain conditions, red blood cells, striated muscle cells, and invertebrate giant axons all show this same type of Na:Na interchange. A possible interpretation is that the externally directed activation sites in the membrane have a finite affinity for sodium ions that becomes revealed in a K-free medium. Stated another way, in addition to Rb^+, Cs^+, and Li^+, external Na^+ also has a K-like activating effect on the sodium pump. In this regard, it is interesting that Keynes and Steinhardt (1968) find no glycoside-sensitive Na:Na interchange in striated muscle cells when the bathing medium contains 2.5 mM K. Whatever the mechanism, the effect is of limited physiological interest in muscle cells as it is only observed under nonphysiological conditions and occurs at a rate that is much lower than the potassium-activated sodium efflux. Also, it is clear that this mode of operation of the sodium pump cannot change the internal sodium ion concentration.

The main finding concerning the potassium-activated outward transport of sodium ions in muscle cells is that the presence of external sodium ions moves the activation vs. concentration curves to the right and downwards (Fig. 3). This effect has been observed in red blood cells by Post et al. (1960). More recently, a similar finding has been made by Baker et al. (1969) in squid giant axons. Using different techniques, Baker and Connelly (1966) observed that external sodium ions interfered with the activating effect of external potassium ions on the sodium pump in crab nerve membrane. All these effects could be satisfactorily interpreted on the basis that external sodium ions act as competitive inhibitors of the activation process. The present results indicate that the sodium transport mechanism in striated muscle cell membrane responds similarly to external sodium ions. Figs. 4

and 5 show that external sodium ions act on the rate of sodium pumping in the manner expected for a competitive inhibitor.

The action of external sodium ions on the activation of sodium transport in striated muscle cells shows at least three similarities to observations on squid giant axons (Baker et al., 1969):

1. The K activation curve is shifted to the left and upwards by reducing the external sodium ion concentration.
2. Reduction of the external sodium ion concentration produces K activation curves with a less sigmoidal character.
3. All curves tend to approach the same or a similar maximum rate.

A notable difference is that the sodium efflux into nominally K-free solutions is increased at low external sodium ion concentrations in squid giant axons whereas it is decreased under such conditions in striated muscle cells (Fig. 1). This difference, however, may only indicate that the actual potassium ion concentration at the activation sites in a nominally K-free medium is significant in the case of squid giant axons and negligible in the case of sodium-enriched muscle cells.

It would be possible to analyze the effects of external potassium and sodium ions on the rate of sodium transport in terms of the usual Michaelis-Menten kinetics for the case of the presence of a competitive inhibitor were it not for the pronounced sigmoidal nature of the activation curves in the presence of external sodium ions. There are several possible ways to interpret the sigmoidal curves. A cooperative type of binding of the activating cation, an ion-induced allosteric transformation, or a multivalent binding site will all offer possibilities for analysis. The fact that one model provides a fit to the data does not rule out the possibility that other models might provide a more accurate description. The alternative of a multivalent carrier site was selected because of its relative ease of manipulation compared with other alternatives. Baker and coworkers (1969) analyzed their results in terms of a carrier site with two identical ion-binding groups. With the present data, the influence of external sodium ions was somewhat stronger and three ion-binding groups gave a better fit than two ion-binding groups. Furthermore, different forms of the ion-binding groups were not assumed to have identical dissociation constants. The constants were selected to give the best fit to the curve for K activation in the presence of 80 mM external sodium. From the constants giving the best fit, however, two of the sites turn out to have comparable dissociation constants. One of the sites has a dissociation constant equal to about one-tenth of those for the other two groups. The affinity of the two comparable groups for potassium ions is about 70 times greater than their affinity for sodium ions.

In the absence of external sodium ions, the K activation curve for striated

muscle cells loses discernible sigmoidal character. It cannot be ruled out that a slight degree of sigmoidal character may remain that is undetectable by the methods employed. The data in the absence of external sodium ions can be adequately fit by a Michaelis equation with k_m = 3.3 mM. The equation is very similar to that describing K activation in mammalian nonmyelinated nerve fibers (Rang and Ritchie, 1968). In mammalian nonmyelinated fibers, however, the activation by K^+ was determined in a solution containing sodium ions.

Any model describing these effects should predict a loss of sigmoidal nature in the curves at very low external sodium ion concentrations. In the present interpretation, only one group need bind a potassium ion to achieve activation in the absence of external sodium ions.

One can visualize two forms of the carrier at the outer membrane surface corresponding to a "potassium conformation" and a "sodium conformation." The potassium conformation turns over at a rate corresponding to the maximal rate of activation by K^+ while the sodium conformation turns over at a rate corresponding to the maximal rate of activation by Na^+ in the absence of external potassium ions. The K form is evidently formed with ease in the absence of external sodium ions and the binding of a single potassium ion suffices to produce the K form. In the presence of external sodium ions, the K form is formed with difficulty as the binding of a single sodium ion suffices to prevent the occurrence of the K form. The sigmoidal activation curve in the presence of external sodium ions then represents the titration of these groups to produce XK_3 from XNa_2K and $XNaK_2$. These postulates can be summarized by considering those site forms which produce the potassium conformation and those which produce the sodium conformation. The case in which no subsite is occupied by a sodium or a potassium ion is denoted by ---. Permutations of these arrangements are not considered so that K-- equals - K- equals -- K. The following site forms correspond to the potassium conformation:

K--, KK-, KKK.

The following forms correspond to the sodium conformation:

Na--, NaNa-, NaNaNa,

NaKK, NaNaK.

The form represented by --- is postulated to be inert and to lead to a very low rate of turnover of the transport cycle. Experimentally, this case is approached by the sites in the presence of a K-free Tris medium. Glycoside-sensitive sodium efflux is zero or minimal in this case as observed experimentally. All these considerations, of course, apply only to the glycoside-sensitive portion of sodium efflux.

It has been assumed that both activation and inhibition by sodium ions occur at the same sites. This point has not been proven but the assumption is reasonable as the k_m for activation by sodium agrees fairly well with the k_I for inhibition by sodium. Both k_m(Na) and the apparent k_I(Na) are found to be between 30 and 40 mM.

As elevating the external sodium ion concentration lowers the rate of sodium extrusion while raising the rate of inward sodium ion leakage, it is clear that higher potassium ion concentrations are required to produce net sodium extrusion under these conditions. The threshold potassium concentration for net sodium extrusion at different external sodium ion concentrations has been determined in a separate series of net extrusion experiments. The results are in agreement with the curves in Fig. 9. For example, the following combinations give a detectable rate of net sodium extrusion: 25 mM Na, 0.5 mM K; 50 mM Na, 1 mM K; 105 mM Na, 2.5 mM K; 120 mM Na, 10 mM K. The steep rise in threshold potassium concentration above an external sodium ion concentration of 105 mM is unexplained. Conway et al. (1961) have attributed this behavior to a "critical energy barrier." Part of the steep rise region in the results presently observed can be attributed to the competitive inhibition of activation due to external sodium ions. The "three-site" model considered, however, will not account for all of the steepness of the curve above a sodium concentration of 105 mM without the introduction of additional assumptions. In formulating an expression for the potassium concentration threshold that produces a balance of the unidirectional sodium fluxes, it has been assumed that the permeability of the membrane to sodium ions does not depend on the sodium concentration. If the membrane becomes more permeable to sodium ions at elevated external sodium ion concentrations, a steeper rise in the threshold potassium concentration curve at high external sodium ion concentrations would be predicted.

In the constant $[Na]_o:[K]_o$ ratio experiments reported in Fig. 6, the present theory does not predict the decline in transport rate observed at very high external sodium ion concentrations unless the affinity of the sites for sodium ions increases in this region. There is no evidence that this is an impossibility and the somewhat poor fit in the very high $[Na]_o$ region does not invalidate the rather good fit obtained in other regions. There are also departures from the equations at very low potassium ion concentrations in the presence of external sodium ions. It should be emphasized that all ionic affinities employed in the equations have been assumed to remain constant over very wide concentration ranges. Cooperative effects at the sites may account for deviations.

The presently employed framework must be regarded as a useful approximation that is helpful in accounting for some of the behavior observed. Taking an over-all view, it is possible that the rate of sodium extrusion is controlled by three sites at the inner membrane surface sensitive to the inside sodium

ion concentration (Mullins and Frumento, 1963) and three sites at the outer membrane surface sensitive to both the external potassium and sodium ion concentrations.

The results obtained apply to muscles in which sodium transport has been maximally activated by the internal sodium ion concentration so that the rate constant for sodium loss is a function of the composition of the external medium only. It has been emphasized (Sjodin and Beaugé, 1968) that the sodium and potassium transport mechanisms in muscle cells are influenced considerably by the internal sodium ion concentration in some ranges of concentration. The study of activation is being extended to cases in which the rate constant for loss of labeled sodium ions is a function of $[Na]_i$ as well.

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