Effects of External Potassium and Strophanthidin on Sodium Fluxes in Frog Striated Muscle

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ABSTRACT Unidirectional Na fluxes in isolated fibers from the frog's semitendinosus muscle were measured in the presence of strophanthidin and increased external potassium ion concentrations. Strophanthidin at a concentration of 10^{-5} M inhibited about 80 per cent of the resting Na efflux without having any detectable effect on the resting Na influx. From this it is concluded that the major portion of the resting Na efflux is caused by active transport processes. External potassium concentrations from 2.5 to 7.5 mM had little effect on resting Na efflux. Above 7.5 mM and up to 15 mM external K, the Na efflux was markedly stimulated; with 15 mM K the Na influx was 250 to 300 per cent greater than normal. On the other hand, Na influx was unchanged with 15 mM K. The stimulated Na efflux with the higher concentrations was not appreciably reduced when choline or Li was substituted for external Na, but was completely inhibited by 10^{-5} M strophanthidin. From these findings it is concluded that the active transport of Na is stimulated by the higher concentrations of K. It is postulated that this effect on the Na "pump" is produced as a result of the depolarization of the muscle membranes and is related to the increased metabolism and heat production found under conditions of high external K.

INTRODUCTION

In the long run, excitability in muscle cells depends on, among other factors, the ability of the cell to extrude sodium ions which leak in slowly across the membrane during rest and more rapidly during activity (7, 8, 11, 12, 22, 25). The inward leakage of sodium is inevitable because sodium ions are permeant and are at a higher electrochemical potential outside the cell than they are inside. The outward extrusion of sodium is certain because (*a*) the cell maintains a nearly steady internal concentration of sodium at non-equilibrium levels for periods much longer than those required for the inward leakage to

bring sodium into equilibrium across the cell membrane and (*b*) the cell can produce large net sodium movements after it is loaded with sodium by soaking in a potassium-free medium (5, 6, 30–32).

Several different mechanisms have been proposed to account for the movement of sodium ions across the membranes of striated muscle. To begin with, sodium ions can move in either direction across the membrane by means of passive diffusion pathways. Thermal agitation in these pathways will give rise to the outward movement of Na ions despite the fact that the electrochemical gradient produces a *net* inward diffusion of Na. An estimate of the magnitude of the efflux of sodium through the leaks in the membrane can be obtained from a formula derived by Ussing and others (13, 33, 35). For independent diffusion of free ions, these derivations show that

$$\frac{\text{Efflux}}{\text{Influx}} = e^{(V_m - V_x)zF/RT} \qquad (1)$$

where V_m is the transmembrane potential with the external potential taken as the zero level, V_x is the equilibrium potential across the membrane for the ion in question, and z, F, R, and T have their usual significance. For Na ions in frog's striated muscle, $V_m = -92$ mv and $V_x = V_{Na} \backsimeq 58$ mv, which, when these values are substituted in the above equation, give a value of 0.0025 for the ratio. Since in normal physiological salt solutions the Na efflux and influx are nearly equal, it is clear that even if all the influx were ascribable to diffusion, only a negligible fraction of the efflux, at most, can be due to passive diffusion. If the ions move across the membrane by a single file mechanism, the ratio of the efflux to the influx is even smaller because the exponent on the right side of equation (1) is multiplied by a number n, the value of which is always greater than one; the exact value depends on the number of sites the ion has to pass in getting from one side of the membrane to the other (14). Consequently, an active transport system for sodium must be present.

There is, of course, no *a priori* reason why there should be only one passive mechanism and one active mechanism for the movement of Na across membranes. For example, Ussing has pointed out that an internal Na ion could move out passively without drawing on any free energy source by simply exchanging for a sodium ion in the external fluid (34, 36). He envisages that a one-for-one exchange of Na ions might occur by means of a carrier which becomes mobile when it binds sodium at either side of the membrane. Once it binds a Na ion on one side it can, as a result of thermal agitation, move to the other side and dissociate to give up the Na bound on the first side. The return to the first side can only occur if the carrier finds another Na ion on the second side. Some support for this "exchange diffusion" process, as it is called, has come from the recent experiments of Keynes and Swan (24) in

which they found in freshly dissected frog's sartorius that the Na efflux is reduced by about one-half when external Na is replaced by either lithium or choline ions. The presence of this component of Na efflux is variable since it slowly disappears with time after dissection. It can be made to reappear, however, after prolonged exposures to solutions free of Na ions. Although these observations are compatible with a passive exchange diffusion process, they are also compatible with an active transport mechanism for extrusion of Na which is dependent on the presence of external Na. Mullins (27) has recently proposed a model in which the exchange diffusion effect is a result of the Na concentration dependence and location in the membrane of the Na "pump." Whatever shall prove to be the correct interpretation for the findings of Keynes and Swan, it is nevertheless clear that there exists in frog's muscle a component of the Na efflux, variable in magnitude, which is dependent on external Na ions.

In the past, two different approaches have been used for studying the movement of sodium ions. In one approach the internal concentration of Na is determined as a function of time. This method provides a direct measure of the difference between the influx and efflux of Na across the membrane. In the other approach, the influx and efflux of Na across the membrane are measured separately by using radioactive isotopes of Na as markers for the individual fluxes. Since the latter approach provides a more direct and sensitive measure of the unidirectional fluxes, this experimental technique was chosen. In order to distinguish between the various mechanisms outlined, it was necessary to choose a muscle preparation in which both the Na efflux and the Na influx could be measured reliably under comparable conditions. For this purpose, small bundles of muscle fibers were isolated in an undamaged condition. With such preparations diffusion is sufficiently rapid that influx measurements of reasonably short duration could be made using solutions made from Na^{24} supplied at specific activities which are readily available.

The experiments reported in this paper deal with the effects of strophanthidin and increased external potassium concentrations (abbreviated $[K]_o$ in what follows) on the fluxes of sodium in frog's striated muscle. In the following paper, the effects of sodium azide on the fluxes of sodium will be described. It will become apparent that in our preparations the major fraction of the resting Na efflux must be ascribed to active transport and only a small fraction can be ascribed to exchange diffusion; that strophanthidin reversibly and specifically inhibits this active transport; that increased $[K]_o$ and azide produce a stimulation of the active transport of sodium; and that the stimulating effects of increased $[K]_o$ and azide are probably related to the increase in the internal potential (*i.e.* a depolarization) produced by these two agents. Preliminary accounts of this work have already appeared (15–17).

METHODS

Dissection and Mounting

Bundles, generally containing from 2 to 24 fibers, were isolated from the dorsal head of the semitendinosus muscle of the frog *Rana pipiens*. The dorsal head was divided into two nearly equal parts with fine scissors and the part with the nerve entry was removed. The bundle to be used was isolated from the remaining half of the muscle while immersed in Ringer's solution containing 10^{-5} gm tubocurarine/ml fluid. Most bundles had only two layers of fibers and in these all fibers were exposed on one side directly to Ringer's solution. Occasionally larger bundles had more than two layers of fibers.

After isolation, the bundle was left for about an hour in Ringer's solution so that any initially unobservable damage could become apparent. If, at this time, any fiber had signs of damage or if the bundle did not respond with a brisk twitch to electrical stimulation, the bundle was rejected. The transfer of an acceptable bundle from the dissecting dish to the isotope apparatus was made with a small glass spoon in order to avoid taking the fibers through an air-water interface. When the bundles were mounted in the isotope apparatus they were stretched to about 7/6 of their slack length. A final microscopic examination and excitability check were made at the end of each experiment. Only data obtained from bundles which were still excitable and had no damaged fibers at the end have been considered.

Solutions

The various solutions used had the compositions listed in Table I. Solutions with an increased potassium concentration were made by removing 1 mmole NaCl for each mmole KCl added. In this way, the tonicity and ionic strength of the solutions were kept constant.

The strophanthidin, for which a molecular weight of 404.5 was assumed, used in these experiments was obtained from the Sigma Chemical Company, St. Louis. Because strophanthidin is relatively insoluble in water, concentrated solutions of strophanthidin were first prepared in absolute ethanol. In the solutions finally applied to the fibers, the concentration of ethanol was kept constant at a value of 0.05 per cent (v/v). This concentration of ethanol had no measurable effect on the fluxes of Na.

The Na^{24} used in preparing radioactive solutions was obtained from two sources. In earlier experiments, which will be referred to with the letter A, the isotope was supplied as Na^{24} Cl in HCl solution from the Oak Ridge National Laboratory. In later experiments, which will be referred to with the letter B, the isotope was supplied as solid $Na_2^{24}CO_3$ from the Brookhaven National Laboratory. The solid $Na_2^{24}CO_3$ was converted to Na^{24} Cl by adding a slight excess of 0.5 N HCl. In both cases, the Na^{24} Cl in HCl solution was evaporated to dryness by first drying at 100°C and then heating at 200–300°C for 1 hour. After cooling, the Na^{24} Cl was weighed and made into radioactive solutions. These solutions were made by adding to the Na^{24} Cl an appropriate volume of a solution free of NaCl but containing the other constit-

TABLE I

COMPOSITION OF SOLUTIONS

Designation of solution in text	NaCl	Na*Cl‡	Choline Cl	LiCl	KCl	$CaCl_2$	Na_2HPO_4	K_2HPO_4	NaH_2PO_4	KH_2PO_4	Strophanthidin
					mmole/liter solution						
Ringer's solution	115	—	—	—	2.5	1.8	2.15	—	0.85	—	—
*x*mM K Ringer's solution	(117.5−*x*)	—	—	—	*x*	1.8	2.15	—	0.85	—	—
Choline solution	—	—	117.5	—	—	1.8	—	1.075	—	0.425	—
*x*mM K choline solution§	—	—	(120−*x*)	—	(*x*−2.5)	1.8	—	1.075	—	0.425	—
Lithium solution	—	—	—	117.5	—	1.8	—	1.075	—	0.425	—
*x*mM K lithium solution§	—	—	—	(120−*x*)	(*x*−2.5)	1.8	—	1.075	—	0.425	—
Na^{24} Ringer's solution	—	115	—	—	2.5	1.8	1.075	—	0.425	—	—
15 mM K Na^{24} solution	—	102.5	—	—	15	1.8	1.075	—	0.425	—	—
Na^{24} solution with strophanthidin	—	115	—	—	2.5	1.8	1.075	—	0.425	—	10^{-2}

‡ The Na^{24} used was not carrier-free.

§ In these solutions, the actual K concentration is 0.075 mM greater than that designated by *x*.

uents at the concentrations tabulated. The buffer had to be reduced to one-half its usual concentration in order to avoid precipitation of the calcium by the phosphate in the solutions free of NaCl.

Apparatus

The isotope apparatus used for the experiments reported in this paper was similar to that described by Hodgkin and Horowicz (11). Briefly, the apparatus consisted of two parts, an influx cell and an observation cell. The fibers, whose tendons were gripped in waxed forceps, were exposed to Na^{24} Ringer's solution in a V-shaped groove in the influx cell which was provided with platinum electrodes for stimulation. At the end of the exposure period, the Na^{24} Ringer's solution was removed by means of a syringe and both cells were filled to the top with inactive Ringer's solution. In order to minimize contamination of the observation cell with Na^{24}, the fluid flow was made rapid and was directed from the observation cell to the influx cell. The fibers were transferred from the influx cell to the observation cell under fluid through a special channel; during this transfer, the excess fluid was being removed by suction. When the bundle was in the observation cell, the influx cell was removed from the room so as to eliminate the large γ-ray background introduced by the decay of Na^{24} left adhering to the influx cell.

The observation cell was fastened to a brass plate beneath which was mounted a shielded Geiger counter, which will be referred to as the experimental detector. The brass plate was of sufficient thickness to absorb all the β-rays and was separated from the observation cell by a layer of mica 50 μ in thickness. A rectangular opening, 3 mm $\times$ 10 mm, was milled in this plate and served as a window for the β-rays of the Na^{24} decay to pass through. The bundles were mounted in a channel of the observation cell directly over this opening. Since the length of the fibers in any bundle was never less than 14 mm, only Na^{24} in the central 10 mm of the fibers was counted. In multiple determinations of influx on the same bundle, the fibers were located at precisely the same position over the mica window by providing rigid stops for the horizontal and vertical manipulators to which the bundle was attached. The adequacy of this method was tested by the positioning procedure employed in the work of Hodgkin and Horowicz (11). The background was measured with the fibers in position by means of a brass shutter, 3.2 mm thick, which closed the window above the experimental detector and absorbed the β-rays from the decay of Na^{24} in the fibers.

Flux Measurements

In most efflux experiments, the fibers first were exposed to Na^{24} Ringer's solution for about 4 minutes, during which time they were stimulated for 3 minutes at a frequency of 1 shock/second. Calculations based on data published by Hodgkin and Horowicz (11) indicate that such stimulation should have raised the internal Na concentration about 15 per cent. In a few experiments, the fibers were loaded with Na^{24} by placing them in the Na^{24} Ringer's solution for 6 minutes while at rest.

When fibers were located finally in the observation cell over the experimental detector, the efflux of isotope was determined from the decline of the counting rate

with time, after correction for decay and background, as inactive solutions flowed past the bundle. The solution flowed at rates close to 1 ml/minute through the channel which had a cross-sectional area of 9 mm^2. Generally, the fibers were counted for 3 minute intervals, with every third or fourth interval being a background count with the brass shutter closed. Towards the end of each experiment when the counting rates became low the time interval was increased to 5 or 10 minutes. With the shutter open, the total count accumulated in any interval was generally more than 1000 and always greater than 500. The ratio of fiber counts to background counts varied from about 10 at the start of an experiment to 4 at the end.

For Na influx, a single determination consisted of approximately a 6 minute soak in Na^{24} Ringer's solution; the total time of exposure was measured to the nearest second with a stop-watch. The count of Na^{24} in the bundle was started as soon as it was properly located over the experimental detector. The bundle was counted for a period of about 90 minutes and a single exponential function of time was fitted to the results. From this function the amount of Na^{24} in the bundle at the end of the exposure period was determined by extrapolation. This procedure was used not only for influx from Na^{24} Ringer's solution but also for influx from the Na^{24} solutions having high K concentrations. However, the values for the influx from high K solutions were increased by 9 per cent. The validity for using this procedure in the latter case rests on the finding that whenever a bundle was returned from a high K solution to normal Ringer's solution, the rate constant for loss of Na^{24} did not promptly return to a value characteristic for Na^{24} efflux in inactive Ringer's solution (see Figs. 2 to 4). Using efflux experiments similar to those illustrated in Figs. 2 to 4, it was found that the influx is underestimated, on the average, by 9 per cent when the exponential fitted to the later points in the efflux to inactive Ringer's solution was used for extrapolation. On the other hand, whenever the Na^{24} taken up by the bundle on exposure to a Na^{24} solution containing strophanthidin was to be determined an alternate procedure was used. In this situation, only the counting data of the first 15 or 20 minutes after the end of the exposure to Na^{24} solution were used for extrapolation. The observation that the recovery of the Na^{24} efflux in Ringer's solution was slow whenever the efflux had been reduced by strophanthidin provided the basis for this alternate procedure (see Figs. 1 and 5). An experiment illustrating the method used with strophanthidin is given in Fig. 1.

This experiment is also helpful in describing the method used for determining the uptake of isotope when Na^{24} was present from previous exposures. Since Na^{24} is lost exponentially, the equation governing the uptake of isotope is given by

$$\frac{dn^*}{dt} = M^*_{\text{in}} - kn^* \tag{2}$$

which, for M^*_{in} constant, on integration and solution gives

$$M^*_{\text{in}} = \left(\frac{kt}{1 - \exp(-kt)}\right) \times \left(\frac{n_t^* - n_o^* \exp(-kt)}{t}\right) \tag{3}$$

where

t is the total time of exposure to isotope in minutes,

k is the rate constant for loss of isotope in the exposure solution given in (minutes)$^{-1}$,

n_i^* is the total quantity of isotope in the fibers at the end of the exposure to isotope in counts/minute,

n_o^* is the total quantity of isotope present in the fibers at the start of the exposure to isotope in counts/minute, and

M_{in}^* is the influx of isotope in (counts/minute)/minute of exposure to isotope solution.

Equation (3) was used to calculate the influx of isotope. The experimental detector was calibrated through the use of an auxiliary Geiger counter in order to get a measure of the absolute flux. At the end of the experiment the fibers of the bundle were cut from the tendons and placed on a planchet. When they were dry they were counted by the auxiliary detector. The sensitivity of the auxiliary detector, S_a, was measured by appropriately diluting the Na^{24} solutions with H_2O and then drying a known aliquot of this diluted solution on a planchet. With precautions to ensure identical positioning of planchets with respect to the auxiliary detector, the known sample of Na^{24} was counted and the sensitivity, S_a, was calculated in units of mole Na per count/minute. The sensitivity of the experimental detector, S_e, was calculated from the formula,

$$S_e = r \cdot S_a \tag{4}$$

where r was the ratio of the bundle-counting rate with the auxiliary detector to the bundle-counting rate with the experimental detector at the time when the fibers were removed from the observation cell. The average influx of sodium per unit length of muscle fiber was calculated from the formula

$$M'_{in} = \frac{M^*_{in} \cdot S_e}{60\, N \cdot l} \tag{5}$$

where,

N is the number of fibers in the bundle,

l is the length of the bundle in centimeters,

M_{in}^* and S_e are given by equations (3) and (4) respectively, and

M'_{in} is the influx of sodium in (mole Na/cm)/second.

RESULTS

Comparison with Single Fiber Values

The experiments reported in this and the following paper involved the use of a small number of fibers isolated in bundles. In the course of these experiments a considerable amount of data was obtained on sodium movements using this type of preparation and the results obtained will first be compared with similar measurements of Hodgkin and Horowicz (11) on isolated single fibers.

The results for this comparison are collected in Table II. In the present series of experiments the entry of Na per unit length of fiber was measured. In order to express the results as Na entry per unit surface area of fiber, the diameters of the fibers were needed. However, in bundles having more than two or three fibers, it was not feasible to measure reliably all fiber diameters. Based on 12 randomly chosen fibers from this series of experiments, an average diameter of 68 ± 4 μ (mean ± SEM) was used for the comparison given in Table II. The conversion of the influx data was then made using the formula

$$M_{in} = M'_{in}/\pi d \tag{6}$$

TABLE II

COMPARISON OF SODIUM INFLUX DATA FROM BUNDLES WITH SIMILAR DATA FROM SINGLE FIBERS

	Bundle data‡		Single fiber data‡	
	Mean ± SEM	No. of bundles	Mean ± SEM	No. of fibers
Resting Na influx, (*pmole Na/cm*)/*sec.*	0.079±0.015	10	—	9
Resting Na influx§, (*pmole Na/cm²*)/*sec.*	3.7±0.7§		3.6±0.4	
Resting rate coefficient for loss of Na^{24}, *min.*$^{-1}$	0.0092±0.0011		0.0143±0.0016	
Average fiber diameter, *microns*	68±4	12	99±6	

‡ Bundle data from present experimental series. Single fiber data from Table 2 of Hodgkin and Horowicz (11).

§ This figure was calculated using equation (6) of the text, taking 68μ as the average diameter for the fibers.

where

M_{in} is the rate of Na entry per cm² surface area of fiber,

M'_{in} is the rate of Na entry per cm length of fiber, and

d is the average diameter of the fibers.

When the results for the resting sodium influx from the two sets of experiments are compared, there is good agreement between the findings.

In a single fiber the loss of Na^{24} was found to be given by a single exponential function of time in the experiments of Hodgkin and Horowicz (11). In the present series, however, it was unusual to find that the loss of Na^{24} to inactive Ringer's solution could be expressed by a single exponential function for the entire experimental period. Since each bundle had two or more fibers, each of which could be expected to have a slightly different rate constant for loss of Na^{24}, this finding was not surprising. Nevertheless, in any given bundle the loss of Na^{24} could be expressed by a single exponential for periods lasting one

or two time constants. Thus, in most experiments, a single exponential adequately described the loss of Na^{24} during the first 2 hours after the fibers were loaded with isotope.

The average rate constant, which is the reciprocal of the time constant, for the bundles on which the resting influx was measured, was 0.0092 min^{-1}. This, then, is to be compared with an average rate constant of 0.0143 found for single fibers (see Table I). The general order of magnitude is in reasonable agreement, although the difference is statistically significant. Pursuing this further, if the fibers are in a steady state, then the ratio of the internal concentration of Na is given by

$$\frac{[Na^b]_i}{[Na^s]_i} = \frac{M^b_{in} \cdot k^s \cdot r^s}{M^s_{in} \cdot k^b \cdot r^b} \qquad (7)$$

where

$[Na^b]_i$ and $[Na^s]_i$ are the internal sodium concentrations for the bundle and single fiber experiments respectively,

M^b_{in} and M^s_{in} are the sodium influxes for the bundle and single fiber experiments respectively,

k^b, k^s are the rate constants for loss of Na^{24} in the bundle and single fiber experiments respectively, and

r^b, r^s are the average fiber radii for the respective experiments.

When the values from Table II are substituted in the above formula, a ratio for $[Na^b]_i/[Na^s]_i$ of 1.9 is obtained. This gives a value for $[Na^b]_i$ = 17.5 mmole/liter if $[Na^s]_i$ = 9.2 mmole/liter, which is the value given by Hodgkin and Horowicz (11). Although the internal sodium concentration was not measured in the present series of experiments, the value of 17.5 mmole/liter agrees with recent measurements made on whole sartorius of *R. pipens* in this laboratory (18).

From these considerations, it appears that the results from bundles are in reasonable agreement with the results from single fiber experiments when due allowance is made for the differences in fiber geometry and species of frog.

Effects of Strophanthidin on Resting Sodium Fluxes

The experiments to be considered next were designed to measure the effects of strophanthidin on sodium movement in isolated fibers. In these experiments, the fibers were not in any way treated so as to increase their normal internal sodium concentration.

Fig. 1 illustrates the results from one such experiment. Initially, the four fibers isolated were exposed for about 6 minutes to Na^{24} Ringer's solution. The fibers were then transferred to the counting cell and were continuously washed by inactive Ringer's solution containing 10^{-5} M strophanthidin. Soon

after the transfer, the counting of Na^{24} in the central region of the fibers was started. It is seen that the Na^{24} which entered the fibers did not rapidly leave in the presence of 10^{-5} M strophanthidin. During the 90 minutes of washing, the loss of Na^{24} could be fitted by a single exponential having a time constant of 522 minutes. Extrapolating back to the end of the influx period it is found that the fibers took up 255 counts/minute of Na^{24}.

At the end of the first efflux period, the fibers were exposed for about 6

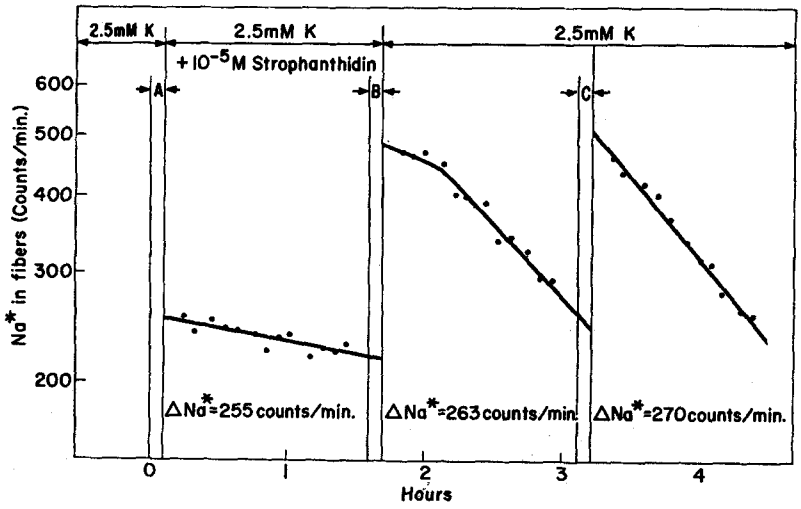

FIGURE 1. Effects of strophanthidin on sodium fluxes. The fibers, while kept at rest, were exposed to Na^{24} solutions during periods A (357 seconds), B (358 seconds), and C (354 seconds); at all other times, inactive Ringer's solution either with or without strophanthidin, as indicated, flowed past the fibers. Na^{24} Ringer's solution was used for periods A and C, while Na^{24} solution with strophanthidin was used for period B. Experiment B104; a bundle of four fibers. Temperature 20°C. 100 counts/minute in fibers are equivalent to 6.71 pmole of Na*/cm of fiber.

minutes to Na^{24} Ringer's solution containing 10^{-5} M strophanthidin; the fibers were then transferred to a flowing solution of inactive Ringer's solution without strophanthidin. The loss of Na^{24} into inactive Ringer's solution was slow at first and then progressively increased as the effects of the strophanthidin disappeared. Such a slow and progressive recovery of the efflux was seen in all experiments with strophanthidin (see Fig. 5). In the Na^{24} Ringer's solution with strophanthidin, the fibers picked up an additional 263 counts/minute of Na^{24}. A final control influx of Na^{24}, period C, after 90 minutes in inactive Ringer's solution gave an increment of 270 counts/minute. At the end of the experiment the Na^{24} was being lost with a time constant of 130 minutes, which was within the range of time constants for loss of Na^{24} found for untreated muscles. Thus, in this experiment the Na influx was insensitive to

strophanthidin while a large fraction of the Na efflux was strophanthidin-sensitive.

All the experiments in which the effect of 10^{-5} M strophanthidin on sodium influx was measured are collected in Table III. This concentration of strophanthidin was used because of its large effect on the sodium efflux. In the

TABLE III
EFFECT OF 10^{-5} MOLAR STROPHANTHIDIN ON SODIUM INFLUX

Experiment reference	No. of fibers	Temperature	Time from death of frog to start of first influx measurement	Influx before strophanthidin exposure‡	Influx in Ringer's +10^{-5} molar strophanthidin§	Influx after strophanthidin exposure‖	Na influx ratio = Influx in strophanthidin / Influx in Ringer's fluid¶
		°C	*min.*		(*pmole Na/cm*)/*sec.*		
B58	11	19	251	0.073 (69)	0.054 (9)	—	0.74
B62	24	20	224	—	‡‡ (9)	‡‡ (89)	1.35
B100	2	20	201	0.162	0.108 (89)	0.174 (88)	0.64
B104	4	20	152	0.048	0.049 (90)	0.051 (86)	0.99
B108	15	20	123	0.059	0.045 (91)	0.029 (84)	1.02
Mean ± SEM							0.95±0.12

‡ For experiment B58, the total time (in minutes) between the end of the first influx period and the beginning of the exposure to Ringer's fluid containing strophanthidin is given in parentheses; during this time the bundle was washed with inactive Ringer's fluid. For experiment B62, the first influx was from the Na^{24} solution with strophanthidin and was started after an initial 9 minutes in Ringer's fluid containing strophanthidin. In the last three experiments, after the first 6 minute influx period in Na^{24} Ringer's fluid the fibers were placed directly into Ringer's fluid containing strophanthidin (see Fig. 1).

§ The total time (in minutes) the fibers were exposed to Ringer's fluid containing strophanthidin before starting the influx measurement is given in parentheses. The influx period was nearly 6 minutes in duration.

‖ The total time (in minutes) between the end of the influx measurement with strophanthidin and the start of the influx measurement in Na^{24} Ringer's fluid is given in parentheses. During his interval fibers were kept in Ringer's fluid.

¶ When more than one measurement of influx was made, the average of the two determinations was used in calculating this ratio.

‡‡ In this experiment no calibrations were made. The ratio was calculated directly from the measured uptake of Na^{24} in units of counts per minute per second of influx.

first two experiments listed, the influx of Na^{24} was measured from the 9th to the 15th minute of the exposure to strophanthidin. This period was chosen because it was as early as one could be sure that the effect on the efflux was fully developed (see Fig. 5). In order to measure the effect of strophanthidin at a later time, the influx of Na^{24} was measured from the 90th to the 96th minute of the exposure to strophanthidin in the last three experiments listed. Since at neither time was there any measurable effect of strophanthidin on the sodium influx, all the data have been averaged.

The experiments in which the effect of strophanthidin on sodium efflux

was measured, are collected in Table IV. In these from 75 to 90 per cent of the sodium efflux was inhibited by strophanthidin. One experiment, B9, in which the bundle of fibers was kept overnight before beginning, showed an unusually large reduction in sodium efflux when strophanthidin was added. The mean, which does not include this experiment, will be used for com-

TABLE IV
EFFECT OF 10^{-5} MOLAR STROPHANTHIDIN ON SODIUM EFFLUX

Experiment reference	No. of fibers	Temperature	Time from death of frog to start of first influx measurement	Rate coefficient for Na^{24} loss $min.^{-1}$			Rate constant ratio = Rate constant in strophanthidin / Rate constant in Ringer's fluid ¶
				In Ringer's before strophanthidin‡	In Ringer's with strophanthidin§	In Ringer's after strophanthidin‖	
		°C	*min.*				
A135	6	21	349	0.0120 (84)	0.0024 (95)	0.0123 (50)	0.20
B9	30	18	1133	0.0230 (58)	0.0021 (80)	0.0208 (50)	0.10
B100	2	20	201	—	0.0027 (95)	0.0144 (60)	0.19
B104	4	20	152	—	0.0019 (96)	0.0077 (81)	0.25
B108	15	20	123	—	0.0025 (97)	0.0096 (87)	0.26
Mean ± SEM (all experiments)							0.20±0.03
Mean ± SEM (not including B9)							0.23±0.02

‡ For experiments A135 and B9, the total time (in minutes) that the fibers were in Ringer's fluid from the end of the influx period to the beginning of the exposure to the Ringer's fluid containing strophanthidin is given in parentheses. For further details concerning the Na^{24} loading for experiment A135 see Fig. 5. In the last three experiments, after the influx from Na^{24} Ringer's fluid the fibers were placed directly into Ringer's fluid containing strophanthidin.

§ The total time (in minutes) that the fibers were exposed to Ringer's fluid containing strophanthidin is given in parentheses. For the last three experiments, this figure includes the 6 minute influx period.

‖ The duration (in minutes) of the period for which the rate constant was determined is given in parentheses. The time intervening between exposure to strophanthidin and this final period varied from experiment to experiment as well as did the solutions to which the fibers were exposed. For experiment A135 see Fig. 5. In the last three experiments listed, the preparations were treated similarly. Experiment B104, which is illustrated in Fig. 1, typifies the procedures and durations used in these three experiments.

¶ When more than one measurement of the rate constant in Ringer's fluid was made, the average of the two determinations was used for calculating this ratio.

parison with other data determined on fresher fibers. From the data in these two tables, it is clear that 10^{-5} M strophanthidin did not significantly alter the Na influx while it did inhibit the Na efflux by 77 per cent.

Effects of Increased External Potassium on Sodium Efflux

An experiment, in which the effect on Na efflux produced by increasing $[K]_o$ was measured, is illustrated in Fig. 2. After the nine fibers were loaded with isotope, the initial loss of isotope to normal inactive Ringer's solution

was exponential, with a rate constant of 0.43×10^{-2} min^{-1}. The Na^{24} efflux increased when $[K]_o$ was increased to 10 mM, and after the first few minutes the rate constant for loss of Na^{24} for the last part of the 40 minute exposure period became 1.28×10^{-2} min^{-1}. When the fibers were returned to Ringer's solution, the rate constant gradually returned to a value ($k = 0.46 \times 10^{-2}$ min^{-1}) close to normal.

To test whether this stimulation was dependent on the external Na concen-

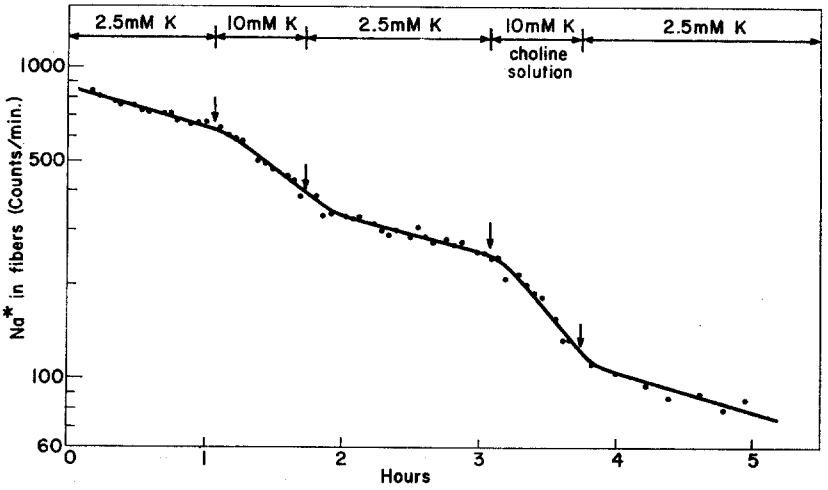

FIGURE 2. Effect of 10 mM external potassium on the efflux of sodium. The fibers were loaded with Na^{24} by exposing them to Na^{24} Ringer's solution for 5 minutes during which time they were stimulated 216 times at a frequency of about 1 shock/second. The fibers were removed from the active solution at the time which has been taken as zero in this figure. The inactive solution flowing past the fibers at any given moment is given by the legend above the data. Experiment A87; a bundle of nine fibers. Temperature 20°C. 100 counts/minute in fibers are equivalent to 3.94 pmole of Na*/cm of fiber.

tration, the fibers were then exposed to a Na-free solution made with choline in which $[K]_o$ was 10 mM. As before, the loss of Na^{24} was considerably increased. In this case, the rate constant became 1.96×10^{-2} min^{-1}. Again, when the fibers were returned to Ringer's solution the loss of Na^{24} returned to a value ($k = 0.41 \times 10^{-2}$ min^{-1}) close to normal. Another experiment, in which the external K was increased to 15 mM and the external Na was replaced by Li, is illustrated in Fig. 3. Results similar to those of the previous experiment were obtained.

These findings indicate that when $[K]_o$ was increased to 10 or 15 mM, the efflux of Na^{24} increased. In addition, the results obtained with high K media which were free of Na show that the increased Na^{24} efflux did not markedly depend on the external Na concentration, and was not related to an increase

in the internal Na concentration which might have resulted from the high K outside.

Effect of High External K Concentration on Na Influx

The effect of high external K on the Na efflux might be explained by an increased membrane permeability to Na ions; a possibility not eliminated by the results of the above experiments using high K media which were free of

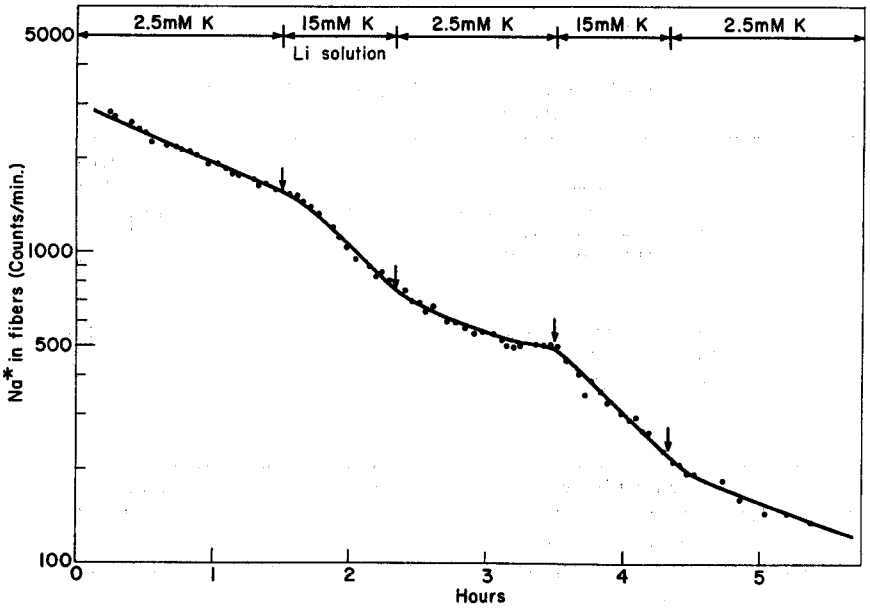

FIGURE 3. Effect of 15 mM external potassium on the efflux of sodium. Fibers stimulated 216 times at a frequency of about 1 shock/second while in Na^{24} Ringer's solution for 5 minutes. The exposure to the active solution was started 1 minute after the time taken as zero for this figure. Inactive solutions flowing past the fibers are given above the data. Experiment A125; a bundle of thirty two fibers. Temperature 21°C. 100 counts/minute in fibers are equivalent to 1.57 pmole of Na/cm of fiber.

Na. To test this hypothesis the Na^{24} influx from solutions containing high external K was measured. Since K stimulation of Na^{24} efflux takes time to develop, the delay being shorter at higher concentrations of external K (*vide infra*), a 9 minute soak in inactive test solution preceded the measurement of Na^{24} influx. Because consistently large effects on the Na efflux were obtained after 9 minutes of exposure to 15 mM K outside, this concentration of K was used for the experiments on influx.

A summary of the measurements on influx from three preparations is given in Table V. The average increment was small and was not statistically significant. From this finding, it can safely be concluded that the stimulated

Na efflux produced by high K outside was not caused by an increased membrane permeability to Na ions.

Effect of Strophanthidin on K-stimulated Na Efflux

Since the K-stimulated Na efflux was not accompanied by a significant increase in the Na influx, it seems probable that high external K stimulates active transport of Na. On the basis of this hypothesis, the effect of strophanthidin on the K-stimulated Na efflux was measured because this aglycone

TABLE V
EFFECT OF 15 mM EXTERNAL POTASSIUM ON SODIUM INFLUX

Experiment reference	No. of fibers	Temperature	Time from death of frog to start of first influx	Influx before high potassium exposure‡	Influx in solution with 15 mM K_o§	Influx after high potassium exposure‖	Na influx ratio = Influx in 15 mM K_o / Influx in Ringer's fluid
		°C	min.		(pmole Na/cm)/sec.		
B38	7	19	288	0.060 (83)	0.054	—	0.90
B49	11	19	320	0.056 (83)	0.064	—	1.14
B53	6	18	251	—	0.120	0.085 (105)	1.41
Mean ± SEM							1.15±0.15

‡ The total time (in minutes) between the end of the first influx period and start of exposure to 15 mM K Ringer's fluid is given in parentheses. For experiment B53, the first Na^{24} influx was measured from 15 mM K Ringer's fluid.

§ In all experiments, fibers were exposed to 15 mM K Ringer's fluid for 9 minutes prior to the Na^{24} influx measurement.

‖ For experiments B38 and B49 there was no final measurement of Na^{24} influx from Ringer's fluid. For experiment B53, the total time (in minutes) between the end of the influx measurement in 15 mM K Ringer's fluid and the start of the influx in Ringer's fluid is given in parentheses.

has been shown to inhibit the net movement of Na up an electrochemical gradient in frog muscle (20).

In the first part of the experiment illustrated by Fig. 4 there was a reversible threefold increase in the rate constant for loss of Na^{24} when $[K]_o$ was raised to 15 mM. After the Na^{24} efflux had returned to its initial value in normal Ringer's solution, the external K was increased to 15 mM in the presence of (10^{-6} molar) strophanthidin. In this situation, the increment in Na efflux was largely inhibited. The experiments given previously in Figs. 2 and 3 showed that, in the absence of strophanthidin, fibers did increase their Na efflux to a second application of high K outside after a comparable exposure to normal Ringer's solution.

In another type of experiment, illustrated by Fig. 5, after an initial period of Na^{24} efflux into inactive Ringer's solution, the fluid washing the fibers was

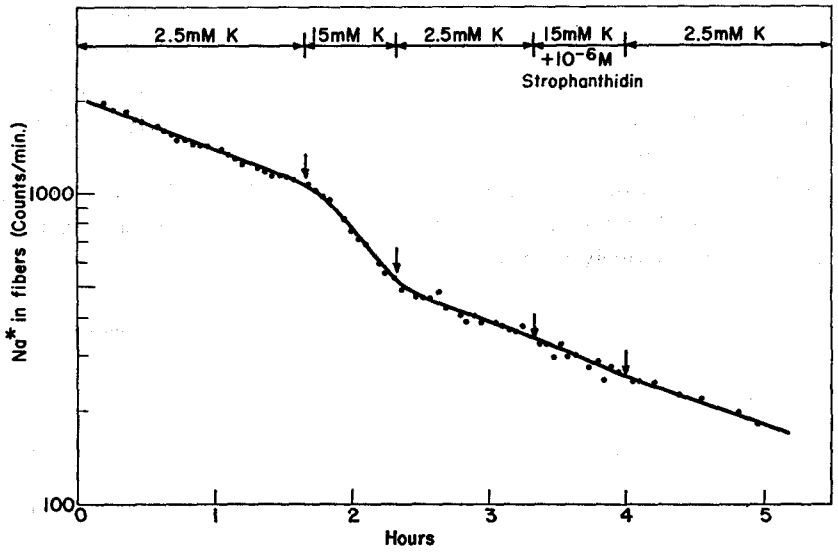

FIGURE 4. Effect of strophanthidin on potassium-stimulated sodium efflux. Fibers were stimulated 195 times at a frequency of about 1 shock/second while in Na^{24} Ringer's solution for 5 minutes. The exposure to the active solution started at the time taken as zero. Inactive solutions flowing past the fibers are given above the data. Experiment A130; a bundle of fourteen fibers. Temperature 20°C. 100 counts/minute in fibers are equivalent to 2.71 pmole of Na*/cm of fiber.

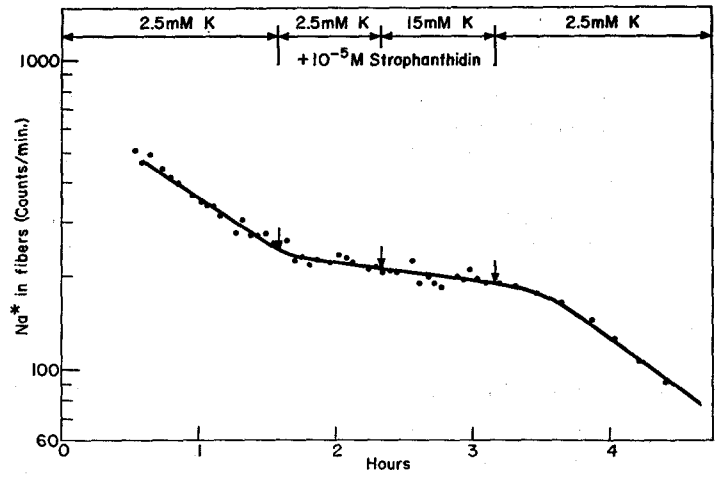

FIGURE 5. Effect of increasing external potassium on sodium efflux which was first inhibited by strophanthidin. Fibers were stimulated 210 times at a frequency of about 1 shock/second while in Na^{24} Ringer's solution for 4 minutes. The exposure to the active solution started 6 minutes after the time taken as zero. Inactive solutions flowing past the fibers at other times are given above the data. Experiment A135; a bundle of six fibers. Temperature 21°C. 100 counts/minute in fibers are equivalent to 7.48 pmole of Na*/cm of fiber.

changed to one containing 10^{-5} M strophanthidin. The rate of loss of Na^{24} was reduced by about a factor of 4 and could not be further stimulated by an increase of $[K]_o$ to 15 mM in the presence of 10^{-5} M strophanthidin. When Ringer's solution was readmitted, the efflux of Na^{24} gradually returned to a value close to normal.

From these experiments it is clear that the K-stimulated Na efflux was largely sensitive to the cardiac aglycone strophanthidin, whether it was applied prior to or simultaneously with the high K media.

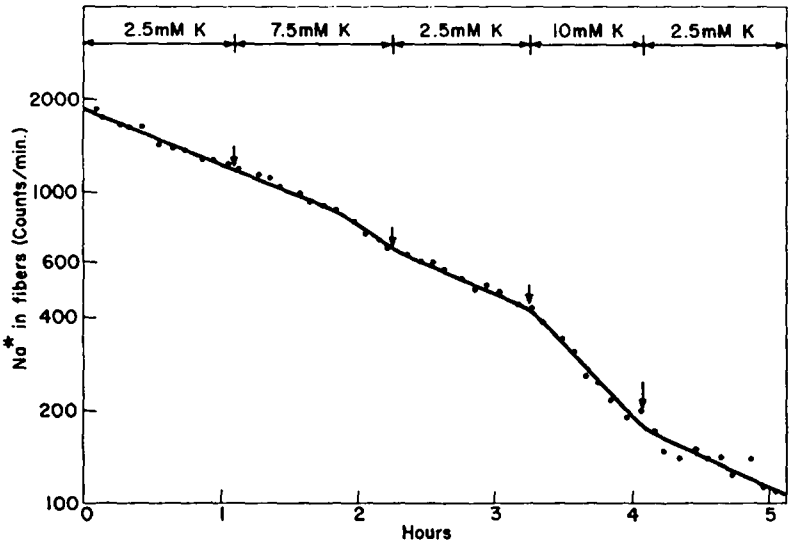

FIGURE 6. Effect of concentration on the onset of the potassium-stimulated sodium efflux. The fibers were stimulated 129 times at a frequency of about 1 shock/second while in Na^{24} Ringer's solution for 5.5 minutes. The end of the exposure to the active solution was at the time taken as zero for this figure. Inactive solutions flowing past the fibers at other times are given above the data. Experiment B130; a bundle of twenty-two fibers. Temperature 21°C. 100 counts/minute in fibers are equivalent to 1.50 pmole of Na*/cm of fiber.

Onset of K-Stimulated Na Efflux

It is rare for the Na efflux to respond promptly to an increase in $[K]_o$. When the external K was increased to 10 or 15 mM, the increase in the Na efflux was gradual and generally took from 3 to 10 minutes to develop fully (see Figs. 2 to 4). The transition period, although highly variable, was, on the average, shorter for the higher concentration of K.

The transition period of gradually increasing Na efflux could be quite long for concentrations of external K less than 10 mM. Fig. 6 shows an experiment in which, after an initial period of Na^{24} loss to Ringer's solution, $[K]_o$ was increased to 7.5 mM. After the change, it took approximately 45 minutes to

develop fully an increased Na efflux. Since the effect on Na efflux was much more promptly reversed when the fibers were returned to Ringer's solution, the long initial transition period cannot be ascribed purely to diffusion of K to the surface membranes in such a small bundle of fibers. After a recovery period of 1 hour in Ringer's solution, an increase in the external K to 10 mM produced a stimulation of Na efflux which developed much more rapidly than when the K was first increased to 7.5 mM. Again, the recovery of Na efflux was rapid when the fibers were returned to Ringer's solution.

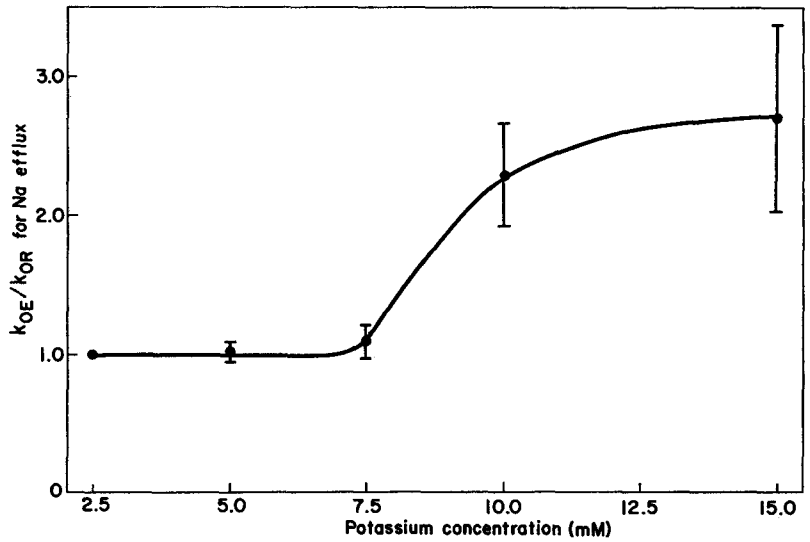

FIGURE 7. The relation between the external potassium concentration and the rate coefficient for loss of sodium. The value of the rate coefficient for sodium efflux at any given $[K]_o$ divided by the value of this coefficient found in normal Ringer's solution is plotted as the ordinate. The vertical bars at each point represent the mean ± *twice* the standard error of the mean. For details, see Table VI.

These findings suggest that the increase in Na efflux is not directly related to the increase in the K concentration at the external surface of the membrane; other factors apparently intervene between the increased K and the stimulated Na efflux.

Dependence of Na Efflux on External K Concentration

The experiments to be considered next deal with the dependence of the Na efflux on $[K]_o$. This dependence is of interest because it provides a clue to the mechanism involved in the stimulation of the Na efflux.

Fig. 7 shows how the rate constant for loss of Na^{24} varied with external K concentration for the range 2.5 to 15 mM. On the axis of ordinates is plotted the ratio of the rate constant in the high K solution to the rate constant in

TABLE VI

EFFECT OF VARIOUS EXTERNAL POTASSIUM CONCENTRATIONS ON SODIUM EFFLUX

Experiment reference	No. of fibers	Temp.	$(K)_o$	Rate coefficient for loss of Na^{24} min^{-1}. In Ringer's before high K (k_{OR})	In high K* (k_{OE})	In Ringer's after high K (k_{OR})	$\frac{k_{OE}}{k_{OR}}$‡
		°C	mM				
B122	18	21	5	0.0144	0.0135 (50)	0.0131	0.98
B142	20	21	5	0.0100	0.0088 (60)	0.0068	1.05
Mean ± SEM							1.02±0.03
B122	18	21	7.5	0.0113	0.0121 (69)	0.0115	1.06
B126	24	20	7.5	0.0090	0.0112 (60)	0.0097	1.20
B130	22	21	7.5	0.0067	0.0067 (40)	0.0068	1.00
Mean ± SEM							1.09±0.06
A87	9	20	10	0.0043	0.0128 (40)	0.0046	2.88
A105	24	19	10	0.0067	0.0133 (55)	—	1.99
A110	3	18	10	0.0149	0.0270 (40)	—	1.81
A120	10	21	10	0.0068	0.0121 (70)	0.0061	1.88
B4	1	19	10	0.0184	0.0483 (30)	0.0191	2.58
B130	22	21	10	0.0068	0.0184 (50)	0.0070	2.67
Mean ± SEM							2.30±0.19
A125	32	21	15	0.0067	0.0158 (50)	0.0068	2.34
A130	14	20	15	0.0071	0.0192 (40)	0.0056	3.02
Mean ± SEM							2.68±0.34

* The time interval (in minutes) for which the rate coefficient for Na^{24} loss was estimated is given in parentheses.

‡ When more than one determination of the efflux rate coefficient in normal Ringer's solution was made, the average was used for this calculation.

normal Ringer's solution containing 2.5 mM K. The average of the rate constants in Ringer's solution determined before and after exposure to the high K solution was used for this ratio. The experiments on which this figure is based are tabulated in Table VI.

It is evident that there was an abrupt change in the responsiveness of the Na efflux to external K at a concentration of 7.5 mM. Taking the resting Na efflux in Ringer's solution as 100 per cent, the average increase in Na efflux was 9 per cent on going from 2.5 mM K to 7.5 mM K Ringer's solution; while it was 121 per cent on going from 7.5 mM K to 10 mM K Ringer's solution. The average rate of increase was 2 ± 1 per cent/mM K (mean ± SEM) for the first interval and 48 ± 8 per cent/mM K (mean ± SEM) for the second

interval. As is easily seen in Fig. 7, for the range of K concentrations considered, the curve is clearly S-shaped.

DISCUSSION

Nature of Resting Na Fluxes

The first point to be discussed concerns the nature of the resting Na fluxes in fibers isolated from the frog semitendinosus muscle. The effects of strophanthidin on the unidirectional fluxes of Na provide the clearest information on this point. It was found that 77 per cent of the resting Na efflux was inhibited by a concentration (10^{-5} M) of strophanthidin which had no measurable effect on the Na influx. Since in resting single fibers isolated from the semitendinosus it was found that the influx is only about 20 per cent greater than the efflux (11), it can be assumed, as a first approximation, that the Na influx nearly equals the Na efflux in the small bundle preparations reported on here. On this basis it follows that 77 per cent of the resting Na efflux is not rigidly coupled to Na influx in these preparations, since the efflux can be disassociated readily from the influx by strophanthidin.

An exchange diffusion mechanism in its simplest form, for example, requires an obligatory inward movement of at least one Na for every Na coming out (34, 36). Any agent inhibiting exchange diffusion should inhibit both the influx and the efflux by the same amount. Thus, it is clear from the above experiments that at most 23 per cent of the unidirectional Na fluxes could possibly be ascribed to exchange diffusion.

As regards the active transport of Na, it has already been shown by Johnson (20) that strophanthidin at 10^{-6} M inhibits the net movement of Na out of the frog sartorius muscle under conditions where transport would otherwise have occurred against an electrochemical gradient. The findings reported in this paper are consonant with those of Johnson and in addition clearly indicate that strophanthidin at 10^{-5} M has its primary effect on Na efflux with no significant effect on the Na influx.

Since only a small fraction of the influx can possibly be ascribed to exchange diffusion in the experiments reported in this paper, it is also clear that at a concentration of 10^{-5} M, strophanthidin had no measurable effect on the passive leak of Na ions into muscle cells.

Nature of Na Efflux Stimulated by External K

Whenever the Na efflux is stimulated, a natural question which arises is whether the enhanced efflux is passive or whether it is active. As an initial hypothesis, it might be supposed that the increased Na efflux is the result of an increased rate of exchange diffusion caused by high external K concentrations. There are two observations, however, which make this hypothesis

untenable. First, there was no significant change in the K stimulation of the Na efflux when all the external Na was replaced by choline. Second, there was no significant change in the Na *influx* when $[K]_o$ was increased. The second observation provides the most compelling reason for rejecting the initial hypothesis. If the effect is caused by an increased rate of exchange diffusion, an increase of Na efflux by a factor of 2.7 (Table VI) on increasing the external K to 15 mM should be accompanied by an increase in the Na influx by a factor of 2.7. The data presented in Table V seem sufficient to rule out the existence of such an increment in the Na influx.

A second hypothesis might be that the increased Na efflux was the result of an increased membrane permeability to Na caused by increasing the external K. This hypothesis is also ruled out by the lack of any measurable effect on the Na influx by going to a solution containing *15* mM K on the outside.

It seems clear from this that there was induced a net movement of Na out of the fibers when the external K was increased to a concentration above 7.5 mM. Since this net movement occurs up an electrochemical gradient for Na ions, the processes stimulated must be active in nature. In agreement with this is the finding that strophanthidin inhibited the stimulated Na efflux. From the above discussion, it is also clear that this stimulated active Na efflux by high K was relatively specific and not complicated by any large change in the rates of Na movement due to either exchange diffusion or passive leaks.

Mechanism of the K-Stimulated Na Efflux

A couple of possibilities which could account for the stimulated Na efflux when the external K is increased can be readily eliminated. First, the stimulated Na efflux could not have been caused by an increased concentration of internal Na. Such an increase could not have occurred either for the case when Na was present outside, since the Na influx did not change, or for the case when external Na was replaced by choline; yet, in both situations the Na efflux was stimulated when the external K was higher than 7.5 mM. Second, the stimulated Na efflux could not have been caused by simple saturation of a carrier in a coupled pump in which a Na ion is extruded for a K ion. If the carrier mechanism was not saturated to begin with in normal Ringer's fluid, one would have expected that the stimulation of the Na efflux would be greater for an increase in the external K from 2.5 to 7.5 mM than for an increase of K from 7.5 to 10 mM; the data of Table VI and Fig. 7 do not conform to this expectation.

A simple explanation for the effects described in this paper is that the Na pump is controlled by the transmembrane potential and the depolarization produced by increasing the external potassium concentration is responsible for the stimulation of the Na pump. A more complete analysis of the present

data together with the data on the effects of NaN_3 from the point of view of this hypothesis is given in the following paper.

The effects of external K, for the range of concentrations considered in this paper, on oxygen consumption, heat production, glycolysis, and organophosphoryl levels in frog muscle have been extensively studied (4, 9, 10, 21, 23, 26, 28, 29). Hegnauer *et al.* (9), in an early and important paper, showed that, among other metabolic effects, the oxygen consumption was increased when the $[K]_o$ is increased. They found that between 0 and 6.5 mM external K, the oxygen consumption remained relatively constant while at 8.2 mM K the oxygen consumption was increased; the "threshold" for the effect was between these two values and is in reasonable agreement for the threshold K concentration of about 7.5 mM for stimulation of Na efflux reported here. There is a similar correspondence when the effect of K on heat production is considered. Solandt (29) found that between 0 and 6 mM K outside the heat production was constant, while at 8 mM the heat production was up over normal; again the threshold K concentration is in good agreement with that reported here. In more recent experiments of Hill and Howarth (10), they state that a potassium "concentration of 9 or 10 mM has little, if any, influence on the heat rate"; although in late autumn the threshold concentration "may be rather (perhaps 20%) less."

The actual increments in Na efflux and oxygen consumption produced by the high K are also comparable. Hegnauer *et al.* (9) found a three- to fivefold increase in O_2 consumption over the normal resting O_2 consumption with 15 mM K. This range of increment overlaps the increments found for Na efflux in the experiments reported in this paper. Keynes and Maisel (23) also found comparable increases in Na efflux and O_2 consumption when the external K was increased from 0 to 10 mM. The increments in heat production produced by high K, however, seem to be rather larger than the increments in the Na efflux. In 15 mM K heat production is up by a factor of 10 to 20 over its resting value (10, 29); this is well above the threefold increase in Na efflux. Since the heat measurements were made under conditions of drastically reduced extracellular fluid in which K, lactic acid, and other metabolites leaking from the cells could accumulate in appreciable quantities to affect the metabolism of the cells, the measurements of heat rate may not be strictly comparable to the determinations of O_2 consumption and Na efflux in which a more abundant extracellular fluid was present. What is significant for the discussion here is that heat production is increased sufficiently and at precisely the same K concentrations as give rise to the increased Na efflux. Many theories have been advanced to explain the increased metabolic rates produced by K ions (4, 10, 21, 23, 26, 28). The data presented in this and the following paper provide support for the idea originally advanced by Hill and Howarth (10) that the effects on the metabolism can be ascribed to the

depolarizations produced by the increased $[K]_o$. Whether the altered metabolism is due primarily to a stimulated rate of Na "pumping" leading to an increased energy demand or to some other process remains an unsettled problem.

Finally, the hypothesis that the transmembrane potential controls the rate of Na pumping offers a simple explanation not only for the dependence of Na efflux on $[K]_o$ but also for the delay and slow development of the stimulated Na efflux when $[K]_o$ is increased. In the presence of chloride ions, Hodgkin and Horowicz (12) showed that when $[K]_o$ is suddenly increased, the internal potential, at first, rapidly increases (*i.e.* the membrane is depolarized) and then drifts slowly to its final equilibrium value. Upon restoration of normal $[K]_o$, there is a small initial rapid repolarization followed again by a slow drift to its original value. With changes from 2.5 to 10 mM K and back again these slow drifts last from 30 to 50 minutes. The reason for the slow drifts in transmembrane potential associated with alterations in $[K]_o$ is that in striated muscles of the frog chloride is permeant and distributes itself, to a first approximation, passively (1–3, 12, 19). When $[K]_o$ is changed abruptly the transmembrane potential does not achieve its final steady state value until the Cl has had time to come to its new equilibrium distribution. For $[K]_o$ around 10 mM this can take from 30 to 50 minutes. If the transmembrane potential which is just threshold for stimulating the Na efflux is the steady state potential when $[K]_o$ equals 7.5 mM (*i.e.* $V_m \backsimeq -70$ mv), then it will take 30 to 50 minutes before the Na efflux increases appreciably, if it is to increase at all. Since for 7.5 mM K, one is close to the threshold potential in the steady state, reduction of $[K]_o$ to 2.5 mM will abruptly stop the stimulated Na efflux (see Fig. 6) because the early rapid repolarization will take it past the threshold potential back to "subthreshold" potentials. With changes to high $[K]_o$ the stimulated Na efflux should come on with a shorter delay and develop more rapidly because the transmembrane potential will pass the threshold value sooner.

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