

Fractionation of Sodium Efflux in Frog Sartorius Muscles by Strophanthidin and Removal of External Sodium

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ABSTRACT The influence of strophanthidin, ouabain, and the removal of external sodium on the sodium efflux from frog sartorius muscle was measured. In freshly dissected muscles strophanthidin and ouabain in maximally effective concentrations reduced the efflux of sodium by about 50%. Of the sodium efflux which is strophanthidin-insensitive about 75% is inhibited after complete replacement of external sodium by lithium. In the absence of strophanthidin replacement of external sodium by lithium, calcium, or magnesium produces an initial rise in the sodium efflux, followed by a fall in the efflux as the exposure of the muscles to sodium-free media is continued. When the muscles are exposed for prolonged periods in sodium-free media, the fraction of internal sodium lost per minute is higher when returned to normal Ringer fluid than it was initially. The activation of sodium efflux by external sodium after long periods in sodium-free solutions is partly strophanthidin-sensitive and partly strophanthidin-insensitive. The internal sodium concentration is an important factor in these effects. The effects of temperature on the sodium efflux were also measured. Above 7°C the Q_{10} of both the strophanthidin-sensitive and strophanthidin-insensitive sodium efflux is about 2.0. Below 7°C the strophanthidin-insensitive sodium efflux has a Q_{10} of about 7.4.

INTRODUCTION

Ever since the early demonstrations that muscles can extrude sodium ions against an electrochemical gradient (Conway and Hingerty, 1948; Desmedt, 1953; Steinbach, 1940, 1951, 1952) it has been accepted by most workers that the low sodium concentration in muscle cells is maintained by an active transport system. In most of these early experiments, and in many similar ones since then, muscles were aged in cold, potassium-deficient media for long periods in order to increase the internal sodium concentration. When such aged muscles are returned to warm, potassium-enriched media, the internal

sodium is rapidly extruded to levels near those found in freshly isolated muscles. Recently, Frumento (1965) has reported that such extrusion can occur in solutions containing the normal level of external potassium.

In order to study aspects of sodium ion transport that are not easily clarified by measurements on net movements another approach has been developed and widely used. In this approach unidirectional fluxes of sodium are measured by following the movements of the radioactive isotopes $^{22}\text{Na}^+$ and $^{24}\text{Na}^+$. Employing this method on frog muscle, Levi and Ussing (1948) obtained results which led them to suggest that a part of the measured sodium efflux might be the consequence of a process, called exchange diffusion, which, in theory, does not require free energy from metabolism. An important feature of this process is a one-for-one exchange of sodium ions between the two sides of the membrane (Ussing, 1949). Direct experimental evidence consistent with such a mechanism was obtained some years ago by Keynes and Swan (1959). When lithium replaced sodium in solutions bathing sartorius muscles only a few hours after the muscles were dissected from frogs, they found that the sodium efflux was reduced by about a half.

Since an exchange diffusion process does not contribute to net extrusion of sodium out of the cell, the relation between the active sodium transport mechanism and the exchange diffusion process has been the subject of investigation in several laboratories. The experimental approach has stemmed from the observation that strophanthidin and other cardiac glycosides can inhibit net extrusion of sodium from frog sartorius muscle (Johnson, 1956) as well as from other tissues. These agents have been used extensively to study the active transport mechanisms in various situations. As a result of such studies on frog striated muscle, some interesting problems have been revealed. One problem relates to the variable amount of sodium efflux that is inhibited by strophanthidin in different preparations. For example, using fibers isolated from semitendinosus muscle, Horowicz and Gerber (1965) reported that strophanthidin inhibited 77% of the sodium efflux. With freshly dissected sartorius muscles, on the other hand, only about 50% of the sodium efflux can be inhibited by strophanthidin or ouabain (Horowicz, 1965; Keynes, 1966; Keynes and Steinhardt, 1968). Recently, Sjodin and Beaugé (1968), using aged frog sartorius muscles, have investigated this matter further. In such preparations they find that the proportion of the strophanthidin-sensitive sodium efflux increases as the internal sodium concentration increases. This suggests that differences of internal sodium concentration in different preparations might be responsible for the differences in strophanthidin sensitivity. The situation in freshly isolated muscle, however, appears to be different in this respect from that found in aged muscles. In a freshly isolated sartorius, for example, the results given below show that the proportion of sodium efflux which is strophanthidin-sensitive increases as the internal sodium con-

centration decreases. This clearly differs from the results on aged muscles. Another unresolved problem of sodium transport in frog muscle relates to the variable effects produced on sodium efflux when external sodium is removed. In the experiments of Keynes and Swan (1959), sodium efflux fell promptly when external sodium was removed from freshly dissected muscles from *Rana temporaria*. After muscles were stored for some time, the initial effect of removing external sodium was an increase in sodium efflux (Keynes and Swan, 1959; Keynes and Steinhardt, 1968). Freshly isolated muscles from *Rana pipiens* also give an initial increase in sodium efflux when external sodium is removed. In this regard they behave like aged muscle from *R. temporaria*.

The results obtained on freshly dissected muscles from the American leopard frog are presented here so that the similarities with and differences from other preparations can be shown. The results with this preparation indicate that there is a significant portion of the sodium efflux which is not strophanthidin-sensitive and which is reduced by removal of external sodium ions. In addition, the results show that external sodium has both an inhibitory and an activating effect on the component of sodium efflux which is strophanthidin-sensitive. Consequently, the response of sodium efflux to removal of external sodium in an untreated muscle represents the result of at least three operationally distinct effects. It is possible, however, to find conditions in which one or the other of these effects predominates.

The experiments described herein were performed during the period from May, 1963 to August, 1965, and a brief report on the earlier findings has already been published (Horowicz, 1965).

METHODS

Whole sartorius muscles were dissected from frogs of the species *Rana pipiens*. The animals were visually examined for freedom from parasites and other signs of disease before they were used. Care was taken to damage as few fibers as possible during the dissection. If more than four fibers showed damage after a brief period in normal Ringer fluid the muscle was discarded. The pelvic end was cleared of all adhering bone and damaged fibers from other muscles. In most experiments both sartorius muscles were taken from the same frog so that comparisons could be made between different treatments.

The composition of the normal Ringer fluid was (mM): NaCl 115; KCl 2.5; CaCl₂ 1.8; NaHPO₄ 2.15; and NaH₂PO₄ 0.85. The Li⁺-Ringer solution had the composition: LiCl 117.5; CaCl₂ 1.8; K₂HPO₄ 1.075; and KH₂PO₄ 0.425. For the Ca⁺²- and Mg⁺²-Ringer solutions, osmotically equivalent amounts of CaCl₂ and MgCl₂ (81.5 mM), respectively, were substituted for the 115 mM NaCl, and Tris buffer was substituted for the phosphate buffer. The cardiac aglycone, strophanthidin, was added to all solutions in the form of a concentrated ethanol solution. In the solutions applied to the muscles, the ethanol concentration was kept at a constant value of 0.05% (v/v). This ethanol concentration had no detectable effect on the sodium efflux.

$^{22}\text{Na}^+$ was used in preparing radioactive solutions. It was added as carrier-free $^{22}\text{NaCl}$ to normal Ringer fluid; the final specific activity was 1–2 mCi per 10 ml of solution. In most cases, freshly dissected muscles were soaked in these solutions for a period of 2 hr.

After exposure to the radioactive solutions, the muscles were washed in inactive Ringer fluid and attached with short silk threads to glass frames that had not been exposed to the solution containing the isotope. The frame with muscle attached was gently agitated in a series of tubes containing 12 ml of solution of one type or another. The radioactivity coming from the muscle was collected for a period of 10 min and the muscle was then transferred to another tube. Whenever there was a change in the composition of solution, the first two collection tubes in the new solution were exposed for 5 min to the muscles and then the 10 min collecting period was reinstated.

The solutions were counted in a crystal well system sensitive to gamma radiations. At the end of the experiment the muscle was also counted. A correction factor allowing for differences in efficiencies in detecting the $^{22}\text{Na}^+$ was determined, and the counts lost by the muscle into the solutions were added to the counts, after correction, found in the muscle at the end. The results are presented in the form of the average fraction of $^{22}\text{Na}^+$ lost per min for each collection period.

Most experiments were performed at room temperatures which fell in the range from 19° to 23°C. For those experiments in which the effects of temperature were being studied specifically, the tubes containing the solutions and muscles were immersed in temperature-controlled baths whose temperatures were maintained to within 0.1°C for any value chosen.

RESULTS

Loss of $^{22}\text{Na}^+$ from Untreated Sartorius Muscle

Since most of the experiments which will be reported on here involve a succession of treatments extending over several hours, it is useful, for purposes of comparison, to have at the outset a typical example of the manner in which $^{22}\text{Na}^+$ leaves an untreated sartorius muscle over a long period. Fig. 1 illustrates

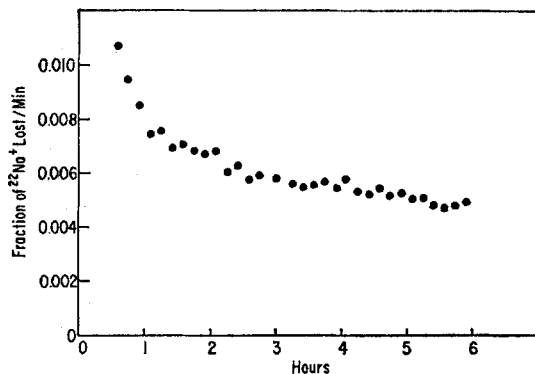


FIGURE 1. The behavior of sodium efflux from an isolated frog sartorius placed in inactive Ringer fluid for a period of 6 hr. The sodium efflux is expressed in terms of the average fraction of internal sodium lost per minute for each collection period of 10 min. Experiment of 14 July 1964.

the findings in one such experiment. After soaking for 2 hr in a Ringer fluid with $^{22}\text{Na}^+$, the muscle was transferred through a series of tubes containing inactive Ringer fluid. During the first hour of such an efflux experiment the fraction of $^{22}\text{Na}^+$ lost per minute declines very rapidly. After this initial stage is past, the fractional loss of $^{22}\text{Na}^+$ per unit time declines more slowly; the average rate of decline for this coefficient is about 10% per hr in this experiment. For 10 muscles used in control experiments of this type the slower decline of the rate coefficient ranged from 7 to 15% per hr, with the average being about 11% per hr.

Action of Glycosides and Aglycones on $^{22}\text{Na}^+$ Loss

When one employs an inhibitor, such as strophanthidin or ouabain, to fractionate the sodium efflux it is important to know how the sodium efflux depends on the concentration of inhibitor for a wide range of values in order to be certain that one is obtaining near maximal inhibition. In addition, the inhibitor has to be applied for a time sufficient to produce its maximal effect. Fig. 2 illustrates one of a series of experiments performed to study these points. In this experiment the sodium efflux was first inhibited by the aglycone at a concentration of 3×10^{-5} M and then the concentration was further increased to 3×10^{-4} M. When allowance is made for the slow drift in sodium efflux which normally occurs no further inhibition was detectable in this experiment when the concentration of the inhibitor was increased. 1 hr was more than sufficient to obtain a maximal effect at these concentrations.

A summary of the averages of several such experiments is given in Fig. 3 in the form of a concentration-response curve. The data can be adequately fitted by an equation of the form $i = \frac{I}{I + K_i}$ where i is the fraction of the

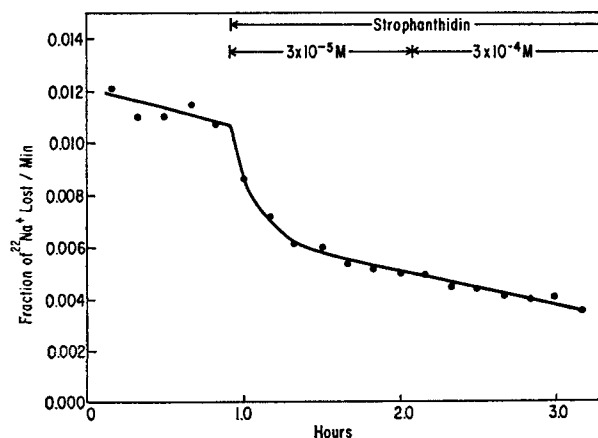


FIGURE 2. The effect of strophanthidin on sodium efflux from isolated sartorius muscles.

strophanthidin-sensitive sodium efflux, I is the concentration of strophanthidin in moles per liter, and K_i has a value of 1.8×10^{-6} M. If one takes this equation as being applicable, then at a concentration of 3×10^{-5} M one inhibits 94% of the total strophanthidin-sensitive sodium efflux. The scale on the right of Fig. 3 gives the actual measured fraction of the total Na^+ efflux which is inhibited by strophanthidin and it is important to note that 50% of the total Na^+ efflux in normal Ringer solution is sensitive to strophanthidin in these freshly isolated sartorius muscles.

A comparison was also made between the effect produced by strophanthidin

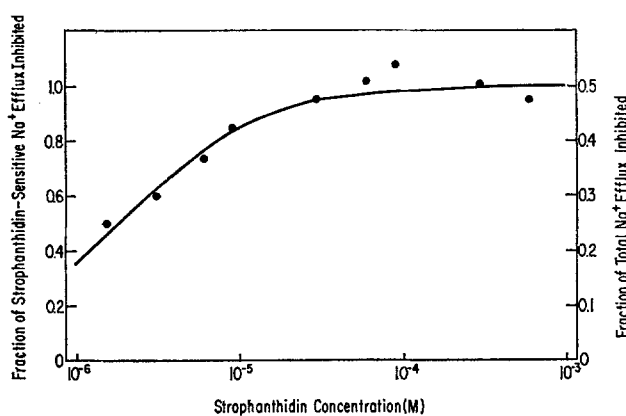


FIGURE 3. A summary plot of the inhibition produced by strophanthidin on the sodium efflux in frog muscle. The concentration axis has a logarithmic scale. Each point gives the average inhibition found at the given concentration in a number of experiments. The curve has been drawn on the basis of the equation $i = \frac{I}{I + K_i}$. See text for details.

and that produced by ouabain. We found that 10^{-4} M ouabain does not significantly inhibit more of the sodium efflux than does 3×10^{-5} M strophanthidin. An analysis for ouabain similar to that given above for strophanthidin yields a value for K_i of about 2×10^{-7} M.

Effect of Substituting Li^+ for Na^+ on Na^+ Efflux

When a maximal inhibition of sodium efflux is achieved by strophanthidin, such as depicted in Fig. 2, it is noteworthy that a large portion of the sodium efflux remains insensitive to the aglycone. The response of the strophanthidin-insensitive efflux to a substitution of lithium for sodium in the bathing medium is shown in the experiment of Fig. 4. In this experiment strophanthidin is first applied and then, after the rapid fall in Na^+ efflux associated with inhibition by the aglycone is over, lithium is substituted for sodium. This substitution always produces a prompt, large, and reversible reduction in the

strophanthidin-insensitive sodium efflux; only about 10% of the total sodium efflux remains after both the strophanthidin addition and the lithium substitution have been made. These results, reported by Horowicz (1965), have also been obtained by Keynes (1966) and Beaugé and Sjodin (1968). Since a similar effect is also obtained with the substitution of choline for sodium, it is clear that most of the strophanthidin-insensitive component is abolished on removal of external sodium. Therefore, this external sodium-activated, strophanthidin-insensitive (SASI) component of sodium efflux behaves, at least on prima facie evidence, as if it were a sodium-for-sodium exchange of the type postulated by Ussing (1949).

As was noted in the introduction, Keynes and Swan (1959), using *Rana*

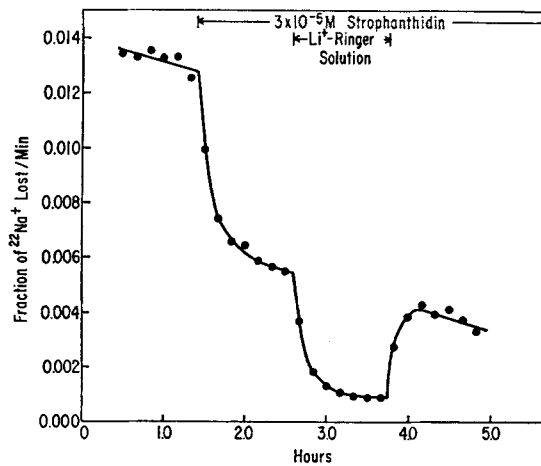


FIGURE 4. The effects of the substitution of lithium for sodium on the sodium efflux of a muscle treated first with 3×10^{-5} M strophanthidin. Experiment of 31 May 1963.

temporaria, reported that substitution of lithium for sodium in Ringer fluid produces a prompt reduction in sodium efflux in freshly dissected muscles. On occasion one obtains batches of *Rana pipiens* which give similar results. However, on most occasions we have found that a substitution of lithium for sodium in the external fluid produces at first a rise in sodium efflux. This rise is transient and is followed by a fall in the sodium efflux.

An experiment illustrating this response pattern is shown in Fig. 5. In this run two muscles from a single frog were used. When lithium was substituted for sodium in the fluid bathing muscle A, the rate coefficient for sodium efflux first increased and then declined. After 60 min muscle A was returned to sodium Ringer fluid and the rate coefficient for sodium efflux increased until strophanthidin was added. As usual, this produced a prompt reduction in sodium loss. With muscle B, strophanthidin was added to the bathing fluid at the time when lithium was substituted for sodium. This produced a prompt

and large reduction in the sodium efflux rate coefficient. Since lithium substitution produces no sign of a transient increase in sodium efflux when strophanthidin is present, both in this type of experiment and in the type depicted in Fig. 4, the inference is that this effect in muscle A is the response of a strophanthidin-sensitive mechanism.

There are several features in this experiment that require some additional comment. First, the final fractional rate of sodium loss in sodium solutions containing strophanthidin was essentially the same for both muscles. Second,

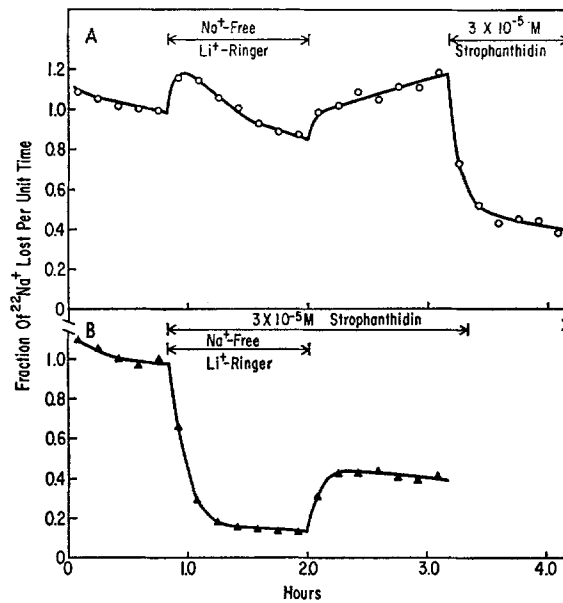


FIGURE 5. The effect of the substitution of lithium for sodium alone and with added strophanthidin on the sodium efflux from two sartorius muscles isolated from the same frog. In the experiment the fractional rate of sodium loss has been normalized for ease of comparison. For muscle A $\Gamma \equiv 0.0091 \text{ min}^{-1}$ and for muscle B $\Gamma \equiv 0.0094 \text{ min}^{-1}$. Experiment of 30 May 1963.

the increase in the rate coefficient for sodium efflux on returning to sodium Ringer fluid after exposure to lithium was about the same in the presence as in the absence of strophanthidin. As will be seen, this finding occurs only on occasion and represents an essentially adventitious result. Finally, one notes that in muscle A the rate coefficient for sodium efflux is greater in sodium Ringer fluid after the exposure to lithium containing Ringer than it was before. Although this "rebound with overshoot" occurs regularly after longer exposures to solutions without sodium, it is somewhat unusual to find such a pronounced effect after a relatively short exposure. The results obtained with long exposures to sodium-free solutions will be given below.

Action on Na⁺ Efflux of Partial Li⁺ Substitution for External Na⁺

The response of sodium efflux when external sodium is replaced by lithium in two steps rather than one is illustrated by the experiment shown in Fig. 6. With muscle A, which was not treated with strophanthidin, the first step was a 60% replacement of sodium by lithium. This step produced a transient increase in fractional sodium loss followed by a slow decrease. It is noteworthy that when the remaining 40% of external sodium was replaced by lithium in the second step there was a prompt decline in the rate coefficient for sodium

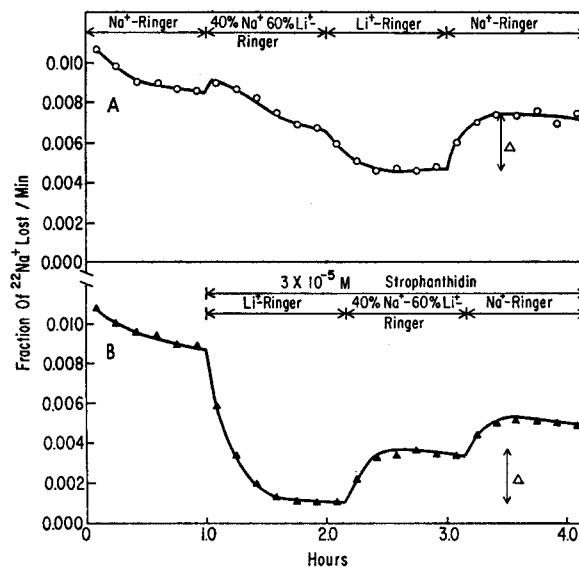


FIGURE 6. The effects of partial substitution of lithium for sodium on sodium efflux in the absence and presence of 3×10^{-5} M strophanthidin. Experiment of 27 June 1963.

efflux with no sign of a transient increase. When the muscle was finally returned to Na⁺-Ringer from Li⁺-Ringer the rate coefficient for sodium efflux recovered, with no sign of an initial decrease, to a level somewhat higher than that consistent with the kind of drift depicted in Fig. 1. One possible explanation for this response pattern will become apparent in the discussion section.

Muscle B from the other side was placed in Li⁺-Ringer with strophanthidin. This treatment reduced the rate coefficient for sodium loss by 88%. With strophanthidin kept constant for the rest of the experiment, increases in sodium efflux occurred whenever there was a step increase in external sodium.

For this experiment the increase in the sodium efflux rate coefficient on going from Li⁺-Ringer to Na⁺-Ringer is greater in the presence of strophanthidin than in its absence; without the aglycone the increase in efflux rate coefficient with total replacement is about equal to the increase in rate co-

efficient with 40% replacement with strophanthidin present (arrow labeled Δ in Fig. 6). This is what might be expected if external sodium activates a strophanthidin-insensitive component of sodium efflux and inhibits a component which is strophanthidin-sensitive. In the absence of aglycone, the increase in the strophanthidin-insensitive efflux on addition of external sodium is masked by inhibition produced by external sodium of the strophanthidin-sensitive efflux. This masking effect can be complete or partial depending upon how long the muscles have been in low-sodium solutions. This effect is commented on more fully in the discussion section.

A number of experiments of the type shown in Fig. 6 B, with different amounts of lithium substituted for sodium, were performed. The data from such experiments showing the dependence of the strophanthidin-insensitive efflux on external sodium are summarized in Fig. 7. For the range of sodium

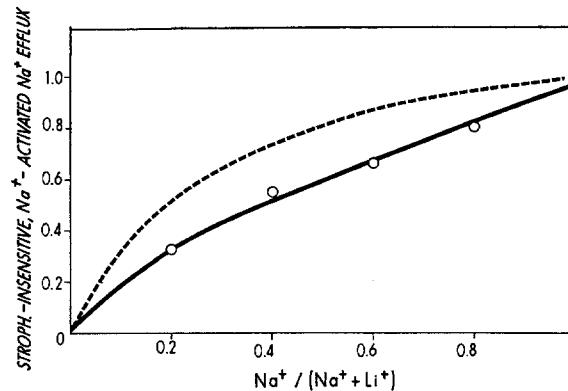


FIGURE 7. The dependence of the sodium-activated, strophanthidin-insensitive sodium efflux on the external sodium concentration. The sodium concentration is expressed as a fraction of the total sodium plus lithium concentration which was 120 mM in all solutions. For the significance of the broken curve see text.

and lithium concentrations studied, this strophanthidin-insensitive efflux, which is external sodium-activated, increases monotonically as the fraction of external sodium increases. The interrupted curve is taken from the data of Keynes and Swan (1959) on the effects of external sodium for unpoisoned muscles. This curve, which they use to fit their results, is of the Michaelis-Menten type with a half-saturation value of 38 mM for external sodium. The results of our experiments with strophanthidin-treated muscles fall consistently below this curve. It is also interesting to note that the relationship in Fig. 7 is quite different from that found for the ouabain-insensitive Na^+ efflux in squid axon (Baker, Blaustein, Hodgkin, and Steinhardt, 1969).

Action of Mg^{++} and Ca^{++} Substitution for External Na^+

The response of the sodium efflux when lithium is substituted for external sodium might be caused by either the addition of lithium or the removal of sodium or possibly, a combination of these two aspects of the substitution procedure. In order to resolve this problem, experiments were performed in which the NaCl in the Ringer fluid was replaced by osmotically equivalent amounts of $MgCl_2$ or $CaCl_2$ for one muscle of a pair and LiCl for the other.

One such experiment in which the effects produced by Li^+ -Ringer are compared to those produced by Mg^{++} -Ringer is illustrated in Fig. 8. In this

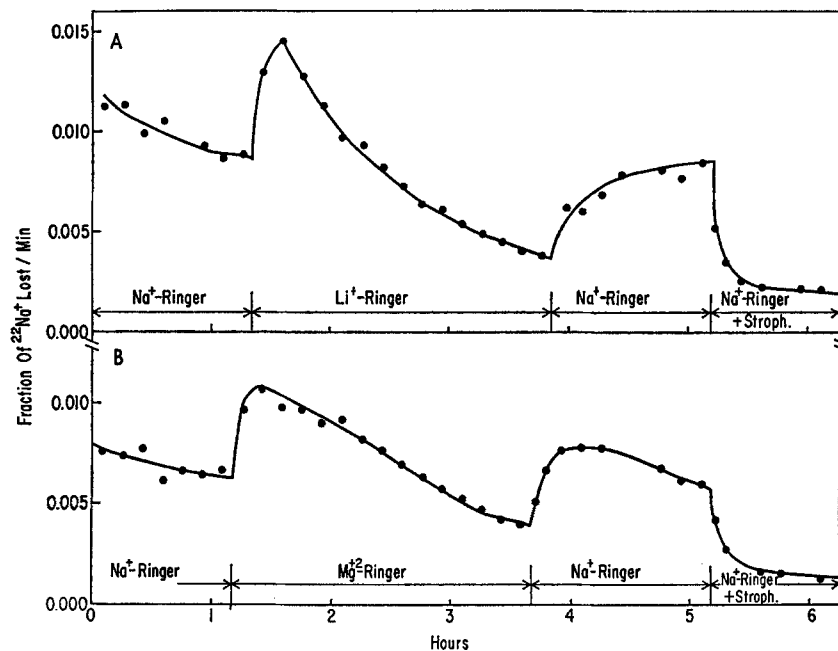


FIGURE 8. Comparison of the effects of the substitution of lithium for sodium on the sodium efflux with those of the substitution of magnesium for sodium. Muscle A and muscle B were dissected from the same frog. Experiment of 23 June 1965.

experiment, the exposure to the sodium-free solutions lasted for 2.5 hr before returning the muscles to normal Na^+ -Ringer fluid. The magnitude of the initial rise in the fractional sodium loss was about the same for the two solutions. In three such experiments, the ratio of the increment in Na^+ efflux produced by substitution of the divalent cation to the increment produced by Li^+ was 0.94, 0.93, and 0.99. The decline which followed the initial rise was, in this experiment, slower for the muscle in Mg^{++} -Ringer; this finding, however, is not invariable. On the average there was no significant difference in the rate of decline of

fractional sodium loss between muscles in Mg^{++} -Ringer and those in Li^+ -Ringer when one takes into account the variability in the response of sodium efflux in paired muscles to the same kind of Na^+ -free solution (see Fig. 9). When the muscles were returned to normal Ringer fluid the response of the sodium efflux was more rapid and pronounced for the muscle which was exposed to Mg^{++} -Ringer. For this muscle, the fractional rate of loss of sodium in normal Na^+ -Ringer fluid returned to values higher than those found prior to the exposure

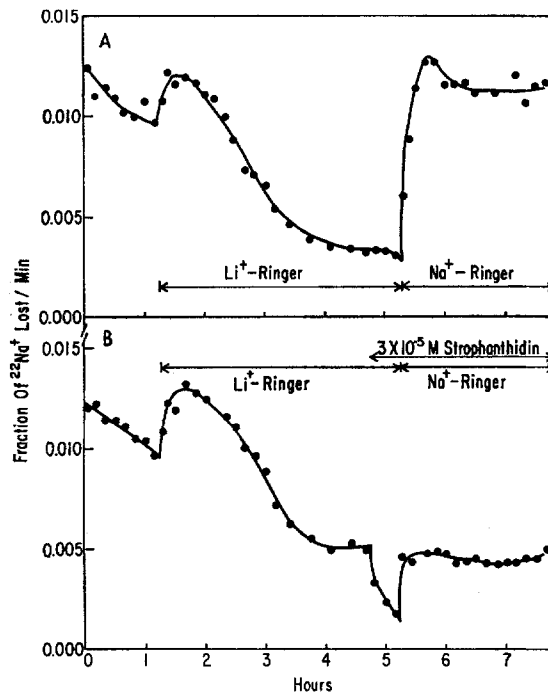


FIGURE 9. The effects of a long exposure to a sodium-free lithium-Ringer fluid on the sodium efflux from two sartorius muscles. The two muscles were dissected from the same frog. Experiment of 13 July 1965.

to Mg^{++} -Ringer. In the muscle exposed to Li^+ -Ringer the fractional rate of loss of sodium in normal Na^+ -Ringer fluid returned to about the same value it had before exposure to Li^+ -Ringer. In this experiment, at the end of the exposure to Na^+ -free solutions, 77% of the sodium efflux is strophanthidin-sensitive and about 50% of the sodium efflux is external sodium-dependent when assessed by the increase in sodium efflux on returning to Na^+ -Ringer fluid. Thus, the sum of the external sodium-dependent efflux and the strophanthidin-sensitive efflux is greater than the total sodium efflux from the muscle.

In other muscles which were exposed for 2 hr to Na^+ -free solutions, the

effects on Na^+ efflux produced by Ca^{++} -Ringer were not significantly different from those produced by Li^+ -Ringer. From these findings, it can be concluded that the effects produced by Li^+ -Ringer are largely ascribable to the removal of external sodium rather than to a stimulation specifically produced by lithium ions.

Effects on Na^+ Efflux of Long Exposure to Li^+ Solutions

In two of the experiments so far illustrated (Figs. 5 and 8) it was noted that the rate coefficient for Na^+ efflux in normal Na^+ -Ringer can be greater immediately after exposure to Na^+ -free solutions than it was prior to such an exposure. This effect can be obtained consistently after prolonged exposure to Li^+ -Ringer.

An experiment in which the response of the sodium efflux was measured in a pair of muscles exposed for 4 hr to Li^+ -Ringer is shown in Fig. 9. In both muscles, the sodium efflux responded in the usual manner when they were transferred to Li^+ -Ringer. After 4 hr in Li^+ -Ringer, muscle A was returned to normal Na^+ -Ringer fluid and the fractional rate of loss for sodium rebounded with an overshoot. On the other hand, after 3.5 hr in Li^+ -Ringer, muscle B was exposed to strophanthidin. After an additional 0.5 hr in Li^+ -Ringer plus strophanthidin, this muscle was returned to Na^+ -Ringer fluid containing strophanthidin. The Na^+ efflux increased in this muscle as well; however, the increment in sodium efflux upon adding external sodium was smaller in the presence of the aglycone than in its absence. In three such experiments, the increment in the fractional Na^+ loss on returning to Na^+ -Ringer in the absence of strophanthidin was, on the average, greater by a factor of 2.3 than the increment produced on returning to Na^+ -Ringer in the presence of strophanthidin. This finding can be contrasted with the result of the experiment depicted in Fig. 6. The natural conclusion to be drawn from the experiment shown in Fig. 9 is that after prolonged exposure to sodium-free solutions an external sodium-activated, strophanthidin-sensitive (SASS) component of efflux is revealed. Further comment will be deferred until the discussion section.

The Effect of Temperature on the Components of Na^+ Efflux

If the strophanthidin-insensitive sodium efflux is a process of the Na^+ -exchange-diffusion type which, in theory, need not draw on metabolic free energy sources, then it is of some interest to determine how this component depends on temperature. Fig. 10 illustrates how two muscles responded to cooling from 22.4° to 0.25°C. In this figure curve A shows how the total sodium efflux in an unpoisoned muscle responded to such cooling, while curve B shows how the sodium efflux in a muscle treated with strophanthidin responded. In both situations there is a marked reduction in the sodium efflux;

for the case illustrated, cooling to a temperature near 0°C, the proportionate reduction in sodium efflux is greater for the strophanthidin-treated muscle than it is for the untreated muscle.

A number of experiments of this type have been collected and presented together as an Arrhenius plot in Fig. 11. The curve drawn connects the data obtained with strophanthidin-treated muscles; thus, the curve shows how the strophanthidin-insensitive component of sodium efflux varies with tempera-

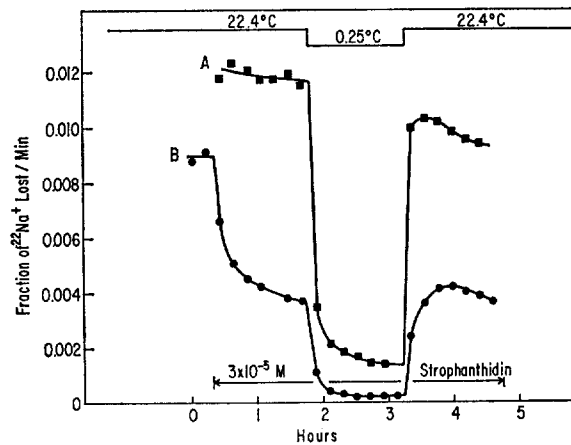


FIGURE 10. The effect of cooling on the total sodium efflux and the strophanthidin-insensitive sodium efflux from two sartorius muscles. Paired muscles from the same frog were used. Experiment of 21 September 1963.

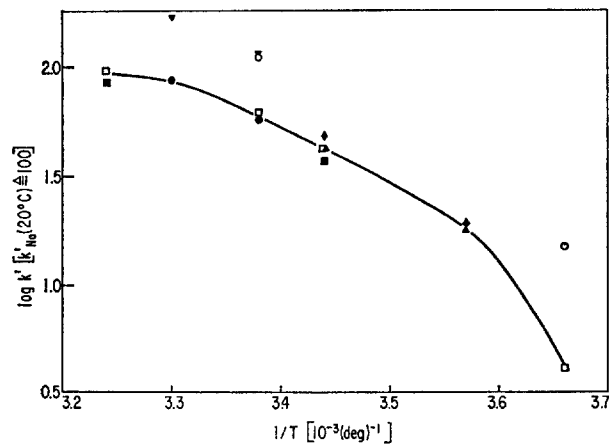


FIGURE 11. The dependence of the fractional rate of sodium loss (k') on the absolute temperature. The logarithm of the rate coefficient is plotted on the axis of ordinates; the reciprocal of the absolute temperature is plotted on the axis of abscissas. The data have been normalized with k' taken as 100 at 20°C. Each type of symbol represents the results from a single muscle.

ture. The temperatures investigated ranged from 0.25° to 36°C; over the entire range the relationship between the logarithm of the fractional rate coefficient for Na⁺ loss and the reciprocal of the absolute temperature is non-linear. However, for temperatures between 7° and 25°C, the relationship is approximately linear with an apparent "activation energy" of about 11,400 cal per mole or a Q_{10} of about 2.0; between 0° and 7°C the curve becomes considerably steeper with the apparent activation energy increasing to about 33,400 cal per mole or a Q_{10} of about 7.4.

The data at the higher temperatures for the total Na⁺ efflux in untreated muscles run parallel to the curve for the strophanthidin-insensitive components; near 0°C, however, the total Na⁺ efflux does not fall as sharply as do the strophanthidin-insensitive components. The temperature dependence of the strophanthidin-sensitive Na⁺ efflux can be reasonably approximated over the range from 0° to 30°C by a constant apparent activation energy of about 10,700 cal per mole or a Q_{10} of about 1.9.

DISCUSSION

Our purpose in these experiments was to investigate how the fractionation of sodium efflux by strophanthidin is related to the effects produced by the removal of external sodium in freshly isolated muscles. The fractionation produced by strophanthidin can be considered first. In these muscles about half of the sodium efflux can be inhibited by this aglycone and the inhibition appears to be of the simple noncompetitive type. At concentrations several times those producing half-maximal inhibition the effects are rapid; for such concentrations the times for inhibition are of the order required for diffusion to take place through the whole muscle. Ouabain produces the same amount of inhibition as does strophanthidin and can be presumed to affect the same mechanisms as does strophanthidin.

The strophanthidin-insensitive fraction of the sodium efflux can be largely eliminated by substituting lithium or choline for sodium in the bathing medium both in fresh and cold-stored muscles (see Fig. 4 this paper; Horowicz, 1965; Keynes and Steinhardt, 1968, and Beaugé and Sjodin, 1968). If we judge by the data giving the dependence of this sodium-activated, strophanthidin-insensitive (SASI) component on the external sodium (see Fig. 7), the strophanthidin-insensitive fraction of the sodium efflux is not fully activated at the sodium levels present in Ringer solution. From the experiments of LeBlanc and Erlj (1969), using ethacrynic acid, it appears that this SASI component represents a sodium-for-sodium exchange process. After complete replacement of external sodium by either lithium or choline, about 25% of the strophanthidin-insensitive sodium efflux (or 13% of the total) sodium efflux remains. This residual strophanthidin-insensitive (RESI) component of the sodium efflux is not affected by removal of external calcium (Hays and Horowicz,

unpublished observations) and probably is not related to the ouabain-insensitive, sodium-for-calcium exchange found in squid giant axons exposed to lithium seawater (Baker et al., 1969).

From the experiments presented in this paper it is clear that external sodium has two effects on the strophanthidin-sensitive fraction of the sodium efflux. First, one can consider what happens to sodium efflux during the first 15–20 min of an exposure to a sodium-free solution. The experimental findings are that in some preparations the sodium efflux decreases and in others it increases. The factors which determine this difference in the response of sodium efflux between preparations will be considered below. For the moment we shall focus attention on the second type of response pattern since this is the one we have found to occur most commonly in muscles freshly isolated from *R. pipiens*. The increase in sodium efflux found in fresh muscles after immersion in sodium-free solutions is prevented by strophanthidin. The natural conclusion is that in these muscles external sodium produces a significant inhibition of the strophanthidin-sensitive sodium efflux; removal of external sodium releases this inhibited efflux. The fact that the magnitude of the response is the same whether lithium, calcium, or magnesium replaces sodium implies that the response is referable to the removal of sodium rather than to specific stimulatory effects produced by any of these substitutes. In some respects the inhibition of the strophanthidin-sensitive efflux by external sodium in frog muscles resembles the reduction by external sodium of a ouabain-sensitive sodium efflux in squid axons placed in O-K⁺ dextrose seawater (Baker, Blaustein, Keynes, Manil, Shaw, and Steinhardt, 1969). There is a difference in that for the squid axon lithium is as effective as sodium in reducing this efflux of sodium. This is not the case in frog muscles because the substitution of lithium for sodium does result in an increase of the strophanthidin-sensitive sodium efflux.

The increase in sodium efflux on removal of external sodium also occurs in aged muscles with high [Na⁺]_i (Keynes and Steinhardt, 1968; Beaugé and Sjodin, 1968). Beaugé and Sjodin (1968) considered that this effect resulted from a specific stimulation by Li⁺ since partial replacement of NaCl by sucrose does not produce the net extrusion found in cold-stored muscles when LiCl is the replacement. The lack of extrusion in the presence of sucrose could be due to an inhibition produced by either the sucrose or the lowered ionic strength. It would be interesting to know whether sodium extrusion occurs in aged muscles with high [Na⁺]_i when either choline, calcium, or magnesium replaces sodium.

When one considers the response of the sodium efflux after prolonged exposure to sodium-free solutions a quite different effect of external sodium becomes apparent. After such exposures the increment in sodium efflux upon adding external sodium is considerably greater in the absence of strophanthidin.

thidin than in its presence (see Fig. 9). If external sodium had no effect on the strophanthidin-sensitive sodium efflux then the increments should be the same with or without strophanthidin. On the other hand, if external sodium inhibits the strophanthidin-sensitive sodium efflux, then the increment in sodium efflux on adding external sodium should be less in the absence of the aglycone than in its presence, as is found in the experiments after relatively short periods in sodium-free solutions (see Fig. 6). The only conclusion that can be drawn from experiments like those depicted in Fig. 9, is that an external sodium-activated, strophanthidin-sensitive (SASS) component of sodium efflux becomes prominent in frog muscle after long periods in sodium-free solutions. The same conclusion has to be drawn from experiments of shorter exposure to sodium-free solutions in which the sum of the external sodium-activated sodium efflux and the strophanthidin-sensitive efflux is greater than the total Na^+ efflux from the muscle (see Fig. 8).

It is possible that the SASS component of sodium efflux might be a strophanthidin-sensitive sodium-for-sodium exchange mechanism similar to the ouabain-sensitive, sodium-for-sodium exchange which has been found for special conditions in red cells (Garrahan and Glynn, 1967 *a*; Garrahan and Glynn, 1967 *b*) and squid axons (Baker et al., 1969). However, our experiments provide no direct evidence to support this notion. Nevertheless, it is noteworthy that the SASS component of sodium efflux in frog muscle and the ouabain-sensitive, sodium-for-sodium exchange in red cells both become prominent when $[\text{Na}^+]_i$ is low.

Since the substitution of lithium, calcium, and magnesium for external sodium gives not only the same initial effect but also, within experimental variation, the same long-term effects, it seems probable that the long-term effects of sodium-free solutions are the result of a reduction in internal sodium concentration. This suggests that a useful way of summarizing the results is to plot the fractional rate coefficient for sodium loss as a function of the internal sodium concentration. For this purpose advantage can be taken of the fact that for muscles bathed in sodium-free solutions the loss of total $^{22}\text{Na}^+$ is a direct measure of the loss of the internal sodium ions (Keynes and Swan, 1959).

Experimental findings similar to those shown in Figs. 8 and 9 have been analyzed in terms of the efflux rate coefficient and internal sodium concentration at the start of the period in sodium-free solutions. The result of such an analysis is shown in Fig. 12. In each experiment, the internal sodium concentration and efflux rate coefficient at the beginning of the exposure to sodium-free solution were given the value of 1.0. In all cases allowance was made for the slow decline in the efflux rate coefficient which occurs over long periods of time (see Fig. 1).

When external sodium is first removed the sodium efflux increases. As can

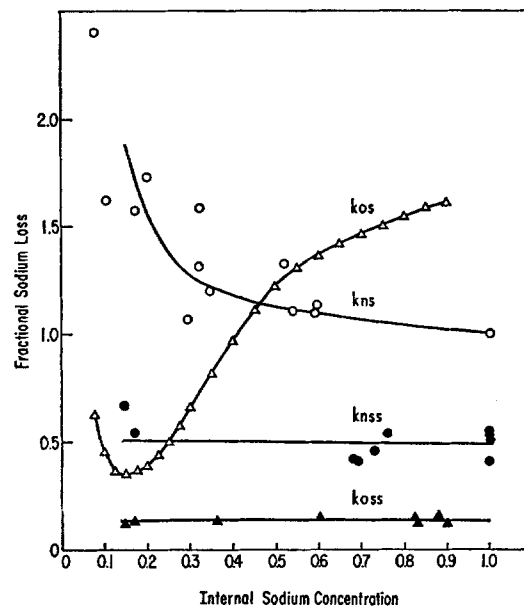


FIGURE 12. The relations between the fractional sodium loss and the internal sodium concentration for various experimental conditions. Results are from muscles exposed to sodium-free solutions. In each experiment the fractional sodium loss and internal sodium content were assigned a value of 1.0 at the start of the period in solutions with zero external sodium. Open triangles and the curve through them, labeled k_{0s} , give the average values of the parameters plotted taken from six experiments when the muscles were in sodium-free solutions. In only two experiments was the internal sodium run to values below 0.15 and the rise in fractional sodium loss given for these low values is from these two experiments. The filled triangles and the curve through them, labeled k_{0ss} , were taken from experiments in which the external solution was sodium-free and contained 3×10^{-5} M strophanthidin. The results were obtained from experiments in which muscles were exposed to strophanthidin for 1 hr or less (see Figs. 6 and 9). The filled circles and the curve through them, labeled k_{Nss} , were taken from experiments such as those shown in Figs. 5 and 9 in which external sodium was replaced after a period in sodium-free solutions; in all cases muscles were exposed to 3×10^{-5} M strophanthidin for 1.5 hr or less. The points for an internal sodium of 1.0 were from experiments in which strophanthidin was added to normal Ringer fluid. The open circles are the values of fractional sodium loss 15 min after reimmersion of sodium-depleted muscles in normal Ringer fluid. The curve, labeled k_{Ns} , is drawn according to equation (5) of the text. The value of 1.0 for internal sodium is equivalent to 26 mM, the value of 1.0 for the fractional sodium loss is equivalent to 0.0084 min^{-1} .

be seen in Fig. 12 (curve k_{0s}), the efflux rate coefficient subsequently falls as the internal sodium concentration declines. For values of internal sodium concentration between 0.15 and 1.0 the behavior of the efflux rate coefficient is qualitatively consistent with the findings of Mullins and Frumento (1963) and Keynes (1965). For values of internal sodium concentration less than

0.15 the efflux rate coefficient increases as the internal sodium continues to fall. When strophanthidin is added to sodium-free solutions the efflux rate coefficient is reduced to a value of about 0.13; this appears to be constant for levels of internal sodium above 0.15 (curve k_{OSS}). The implication of this finding is that the residual strophanthidin-insensitive (RESI) component has an efflux which increases linearly with increasing $[\text{Na}^+]_i$ over the range studied. The rate coefficient under these circumstances will be denoted as k_{OSS} or k_{RESI} . When sodium is added in the presence of strophanthidin, the efflux rate coefficient increases to a value of about 0.50 and appears to remain constant for values of internal sodium above 0.15 (curve k_{NSS}). The difference between the efflux rate coefficient in solutions containing normal sodium with strophanthidin, denoted by k_{NSS} , and k_{RESI} is a measure of the sodium-activated, strophanthidin-insensitive (SASI) component. Thus, the efflux rate coefficient for this component is given by the relation

$$k_{\text{SASI}} = k_{\text{NSS}} - k_{\text{RESI}} = k_{\text{NSS}} - k_{\text{OSS}} \quad (1)$$

Since this efflux rate coefficient also remains constant over the range of internal sodium concentrations considered, the sodium efflux through the sodium-activated, strophanthidin-insensitive (SASI) component is a linear function of $[\text{Na}^+]_i$.

The dependence of the efflux rate coefficient on the internal sodium concentration when the external solution is the normal Ringer solution can be considered next. The efflux rate coefficient for this solution will be denoted by k_{NS} . An examination of the data points (open circles) in Fig. 12 indicates that this rate coefficient gradually increases as the internal sodium concentration decreases. When one examines the strophanthidin sensitivity of this efflux rate coefficient (i.e., the difference $k_{\text{NS}} - k_{\text{NSS}}$) one notes that the increase in the rate coefficient as $[\text{Na}^+]_i$ decreases is completely strophanthidin-sensitive.

A comparison of the strophanthidin sensitivity of the efflux rate coefficient in the absence and presence of external sodium reveals some interesting features of the systems responsible for sodium efflux. When $[\text{Na}^+]_i$ is near 1.0 the efflux is more strophanthidin-sensitive in the absence of external sodium than in its presence (compare $[k_{\text{OS}} - k_{\text{OSS}}]$ with $[k_{\text{NS}} - k_{\text{NSS}}]$). It is clear that for high $[\text{Na}^+]_i$ the net effect of external sodium is to inhibit the strophanthidin-sensitive efflux and for low $[\text{Na}^+]_i$ the net effect of external sodium is to activate the strophanthidin-sensitive efflux (compare $[k_{\text{OS}} - k_{\text{OSS}}]$ with $[k_{\text{NS}} - k_{\text{NSS}}]$ when $[\text{Na}^+]_i \approx 0.15$). These effects are undoubtedly related in that both are strophanthidin-sensitive; however, the mechanisms involved are not clear at present.

With aged muscles Sjodin and Beaugé (1968) report that strophanthidin sensitivity of sodium efflux decreases as $[\text{Na}^+]_i$ decreases. With freshly isolated

muscles, on the other hand, we find that the percentage of efflux which is strophanthidin-sensitive consistently increases from its initial value of about 50% whenever the muscles are treated with Na^+ solutions. This is seen clearly in Figs. 5, 8, 9, and 12. The explanation for this difference is unclear. One possibility is that the SASS component of the Na^+ efflux perishes during aging. Another possibility is that reducing $[\text{Na}^+]_i$ by pretreatment with elevated $[\text{K}^+]_o$ eliminates the SASS component.

When we return to a consideration of the strophanthidin-sensitive components in fresh muscle, it is reasonable to suppose that both the external sodium-activated and the external sodium-inhibited strophanthidin-sensitive components are present for all values of $[\text{Na}^+]_i$. If there were no external sodium-activated, strophanthidin-sensitive (SASS) component for high $[\text{Na}^+]_i$, then the amount of inhibition produced by external sodium in Ringer fluid on the strophanthidin-sensitive (SISS) component could be estimated from the ratio of the strophanthidin-sensitive effluxes in the presence and absence of external sodium. For example, in Fig. 12 one finds that at an $[\text{Na}^+]_i$ of 0.9 the strophanthidin-sensitive efflux in the presence of external sodium is only 30% of the strophanthidin-sensitive efflux in its absence; thus, the inhibition by external sodium in Ringer fluid seems to be about 70%. From one point of view this represents the actual inhibition. From another point of view this represents a minimum since the actual amount of inhibition is masked in part by the component which is activated by external sodium and is also strophanthidin-sensitive.

Some estimate has to be made of the amount of the sodium-activated, strophanthidin-sensitive (SASS) component present at high internal sodium concentrations. One explanation for the observation that the efflux rate coefficient associated with the SASS component increases as the $[\text{Na}^+]_i$ decreases is that the efflux through this component saturates at very low $[\text{Na}^+]_i$ and remains constant for values of $[\text{Na}^+]_i$ above 0.15. For such a component the efflux rate coefficient varies inversely as the internal sodium concentration; that is,

$$k_{\text{SASS}} = \frac{\alpha}{[\text{Na}^+]_i} \quad (2)$$

where α is a constant.

When we turn to the external sodium-inhibited, strophanthidin-sensitive (SISS) component of efflux, it seems reasonable to assume that external sodium inhibits the strophanthidin-sensitive efflux by the same fraction regardless of the value of $[\text{Na}^+]_i$; that is,

$$k_{\text{SISS}} = \beta(k_{\text{OS}} - k_{\text{RESI}}) = \beta(k_{\text{OS}} - k_{\text{OSS}}) \quad (3)$$

for all values of $[\text{Na}^+]_i$, where β is a constant. On the further assumption that

the residual strophanthidin-insensitive (RESI), the external sodium-activated strophanthidin-insensitive (SASI), the external sodium-inhibited strophanthidin-sensitive (SISS), and the external sodium-activated strophanthidin-sensitive (SASS) components are additive, one can write

$$k_{NS} = k_{RESI} + k_{SASI} + k_{SISS} + k_{SASS} \quad (4)$$

which on substituting equations 1-3 becomes

$$k_{NS} = k_{NSS} + \frac{\alpha}{[Na^+]_i} + \beta(k_{OS} - k_{OSS}) \quad (5)$$

It is important to note that there are only two arbitrary constants in equation (5); α and β . The rate coefficients, k_{NSS} , k_{OS} , and k_{OSS} , are obtained from the data in Fig. 12. The curve drawn through the data points in Fig. 12 for normal Ringer fluid (curve k_{ns}) was obtained by using equation (5) with $\alpha = 0.20$ and $\beta = 0.21$. Since the curve fits the points reasonably well, the assumptions outlined in deriving equation (5) are at least consistent with the data at hand. The percentage inhibition of the SISS component is given by $(1 - \beta)100$, which, with $\beta = 0.21$, amounts to 79%. Thus, depending upon the assumptions made, the external sodium in normal Ringer fluid inhibits about 70-79% of the strophanthidin-sensitive component of sodium efflux.

A consequence of the above analysis is that when the internal sodium concentration has a value of 1—that is, when the muscle is at its initial steady-state value in normal Ringer fluid—about 20% of the sodium efflux goes through the sodium-activated, strophanthidin-sensitive (SASS) component. If this component represents a sodium-for-sodium exchange and the muscle is essentially in a steady state then strophanthidin should reduce the sodium influx by about 20%. In earlier experiments on fibers isolated from the semitendinosus muscles of *R. pipiens*, Horowicz and Gerber (1965) found no significant reduction of the sodium influx on applying 10^{-5} M strophanthidin. However, the scatter in the results was such that a 20% reduction in the influx would not have been detected. Keynes and Steinhardt (1968), using sartorius muscles from *R. temporaria*, were also unable to detect any reduction in sodium influx when 10^{-4} M ouabain was added to muscles immersed in Ringer fluid containing 2.5 mM K^+ , although in K^+ -free solutions the influx was reduced by about 20% when ouabain was applied. However, when placed in sodium-free solutions a freshly isolated sartorius muscle from *R. pipiens* responds in a different manner than does a similar preparation isolated from *R. temporaria*. Hence, for this situation, a direct comparison between the two species may not be valid.

The initial $[Na^+]_i$ is probably the most important factor accounting for the difference in the response patterns mentioned above. The $[Na^+]_i$, measured on

sartorius muscles, isolated in our laboratory from other animals in no obvious way different from the ones used for the experiments reported on in this paper had an average value of 26 mM (Varga and Horowicz, unpublished results). On the other hand, for isolated sartorius muscles of *R. temporaria*, Keynes and Swann (1959) report an internal sodium concentration of 8.3 mmole/liter myoplasm. Hodgkin and Horowicz (1959) report a value of 9.2 mmole/kg fiber water obtained with single fibers isolated from the semitendinosus muscle of *R. temporaria*. It seems necessary, therefore, in making comparisons between the results reported on *R. pipiens* and those on *R. temporaria* to allow for a 2.5- to 3-fold difference in the initial $[Na^+]_i$.

Fig. 13 summarizes in schematic form for a few experimental procedures

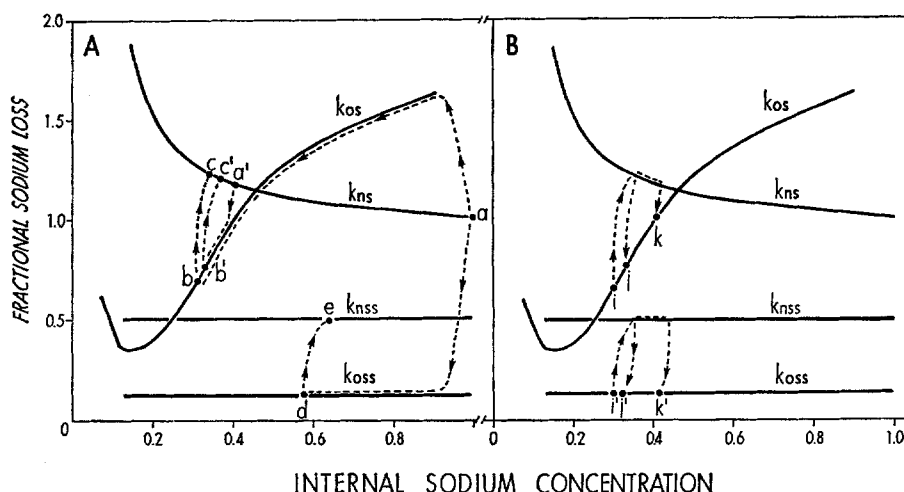


FIGURE 13. The response paths of fractional sodium loss for different experimental sequences. For the significance of the coordinates see legend to Fig. 12. See text for a description of the paths depicted.

the paths taken on plots of the internal sodium vs. fractional sodium loss. Since a value of 1.0 for the internal sodium concentration in the figure is equivalent to 26 mM, a freshly isolated sartorius muscle from *R. temporaria* would be operating at some point like a' in Fig. 13 A. During a brief exposure to a sodium-free solution the fractional sodium loss would fall along the broken curve to a point such as b' on the curve relating the rate coefficient to internal sodium with no external sodium present (see Harris, 1965). When the muscle is exposed to normal Ringer fluid the rate coefficient increases to a point like c' on the curve relating the rate coefficient to the internal sodium with normal external sodium. On the other hand, with a sartorius dissected from *R. pipiens*, exposure to a sodium-free solution for an extended period produces the response path for fractional sodium loss depicted by the broken curve ab . Upon reimmersion in normal Ringer fluid the fractional sodium loss increases

from *b* to *c*. In addition, one can consider the response path of a muscle immersed in a sodium-free solution with added strophanthidin, such as *ad* in Fig. 13 A. When sodium is reintroduced in the presence of strophanthidin the fractional sodium loss increases as the muscle passes to the point *e*. From such conditions it is clear that only a chance occurrence will make the change in rate coefficient on going from *b* to *c* or from *b'* to *c'* equal to the change on going from *d* to *e*.

The results presented above also provide an alternative explanation to experimental findings which Keynes and Steinhardt (1968) regard as support for a multicompartment system for internal sodium. After reducing the internal sodium, they exposed muscles to Na⁺-Ringer for different periods in the presence and absence of ouabain. Upon returning to Li⁺-Ringer, the decay, in unpoisoned muscles, of the rate coefficient for Na⁺ efflux to its initial value in Li⁺-Ringer was appreciably delayed in the muscles exposed for longer periods to Na⁺-Ringer; in poisoned muscles the decay was rapid and independent of the period of time in Na⁺-Ringer (see Fig. 8 of Keynes and Steinhardt, 1968). Fig. 13 B illustrates the response paths for these experiments and shows why this behavior occurs. With a low initial [Na⁺]_i in the absence of ouabain, the Na⁺ efflux from the muscle can be assumed to be operating at a point such as *i* on the *k*_{OS} curve. On exposure to Na⁺-Ringer the operating point switches to the *k*_{NS} curve at a rate determined by diffusion in the extracellular space. During this period there is a slight increase in [Na⁺]_i. The longer the muscle stays in Na⁺-Ringer the higher the [Na⁺]_i becomes. After a brief period in Na⁺-Ringer, return to Li⁺-Ringer brings the operating point to *j*; after a longer period in Na⁺-Ringer, the operating point drops to *k* in Li⁺-Ringer. The final return from *j* to *i* or from *k* to *i* is determined by the time taken to reduce [Na⁺]_i; this is considerably longer than the diffusion times for clearing out the extracellular space. On the other hand, with a low initial [Na⁺]_i in the presence of ouabain, the operating point at the start is *i'* on the *k*_{OSS} curve. On exposure to Na⁺-Ringer with added ouabain the operating point switches to the *k*_{NSS} curve, the switching time again being determined by diffusion in the extracellular space. After a short exposure to Na⁺-Ringer the operating point returns to *j'*; after a longer exposure it returns to *k'*. The return from *j'* to *i'* or from *k'* to *i'* is not seen as a variation of the rate coefficient in time because in the presence of ouabain or strophanthidin the rate coefficient is independent of [Na⁺]_i and is a true rate constant; that is, the Na⁺ efflux in the presence of ouabain is a linear function of [Na⁺]_i. In the absence of ouabain the rate coefficient varies because the Na⁺ efflux is a nonlinear function of [Na⁺]_i.

In the experiments on the effects of temperature the strophanthidin-insensitive fraction of the Na⁺ efflux had a higher *Q*₁₀ than did the strophanthidin-sensitive components below 7°C. This observation provides an explanation

for the finding of Keynes and Steinhardt (1968) that at 1.5°C removal of external Na^+ caused an increase in Na^+ efflux rather than the usual decrease that occurs in freshly isolated muscles of *R. temporaria*. The proportionately greater reduction in the external sodium-activated, strophanthidin-insensitive (SASI) component at low temperatures unmasks the presence of the external sodium-inhibited, strophanthidin-sensitive (SISS) component of efflux. In addition, any slight increase in $[\text{Na}^+]$; that occurs as the result of the low temperature tends to reduce the fractional contribution of the external sodium-activated, strophanthidin-sensitive (SASS) component. This also will unmask the SISS component.

In summary, it can be said that external sodium affects the sodium efflux in at least three operationally distinct ways: (a) it inhibits a strophanthidin-sensitive (SISS) component of efflux; (b) it activates a strophanthidin-sensitive (SASS) component of efflux; and (c) it activates a strophanthidin-insensitive (SASI) component of efflux. The SISS component is prominent in freshly dissected sartorius muscles of *R. pipiens* and in muscles from *R. temporaria* that have been loaded with sodium. The SASS component is prominent in muscles that have been exposed for a few hours to sodium-free solutions. The SASI component seems to be present in significant amounts under most conditions tested to date.

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REFERENCES

- BAKER, P. F., M. P. BLAUSTEIN, A. L. HODGKIN, and R. A. STEINHARDT. 1969. The influence of calcium on sodium efflux in squid axons. *J. Physiol. (London)*. **200**:431.
- BAKER, P. F., M. P. BLAUSTEIN, R. D. KEYNES, J. MANIL, T. I. SHAW, and R. A. STEINHARDT. 1969. The ouabain-sensitive fluxes of sodium and potassium in squid giant axons. *J. Physiol. (London)*. **200**:459.
- BEAUGÉ, L. A., and R. A. SJODIN. 1968. The dual effect of lithium ions on sodium efflux in skeletal muscle. *J. Gen. Physiol.* **52**:408.
- CONWAY, E. J., and D. HINGERTY. 1948. Relations between potassium and sodium levels in mammalian muscle and blood plasma. *Biochem. J.* **42**:372.
- DESMEDT, J. E. 1953. Electrical activity and intracellular sodium concentration in frog muscle. *J. Physiol. (London)*. **121**:191.
- FRUMENTO, A. S. 1965. Sodium pump: Its electrical effects in skeletal muscle. *Science (Washington)*. **147**:1442.
- GARRAHAN, P. J., and I. M. GLYNN. 1967 *a*. The behaviour of the sodium pump in red cells in the absence of external potassium. *J. Physiol. (London)*. **192**:159.
- GARRAHAN, P. J., and I. M. GLYNN. 1967 *b*. Factors affecting the relative magnitudes of the sodium:potassium and sodium:sodium exchanges catalyzed by the sodium pump. *J. Physiol. (London)*. **192**:189.
- HARRIS, E. J. 1965. The dependence of efflux of sodium from frog muscle on internal sodium and external potassium. *J. Physiol. (London)*. **177**:355.
- HODGKIN, A. L., and HOROWICZ, P. 1959. Movements of Na and K in single muscle fibres. *J. Physiol. (London)*. **145**:405.

- HOROWICZ, P. 1965. Sodium movements in frog's sartorius muscle. *Acta Physiol. Acad. Sci. Hung. (Suppl.)* **26**:14.
- HOROWICZ, P., and C. J. GERBER. 1965. Effects of external potassium and strophanthidin on sodium fluxes in frog striated muscle. *J. Gen. Physiol.* **48**:489.
- JOHNSON, J. A. 1956. Influence of ouabain, strophanthidin and dihydrostrophanthidin on sodium and potassium transport in frog sartorii. *Amer. J. Physiol.* **187**:328.
- KEYNES, R. D. 1965. Some further observations on the sodium efflux in frog muscle. *J. Physiol. (London)*. **178**:308.
- KEYNES, R. D. 1966. Exchange diffusion of sodium in frog muscle. *J. Physiol. (London)*. **184**:31.
- KEYNES, R. D., and R. A. STEINHARDT. 1968. The components of the sodium efflux in frog muscle. *J. Physiol. (London)*. **198**:581.
- KEYNES, R. D., and R. C. SWAN. 1959. The effect of external sodium concentration on the sodium fluxes in frog skeletal muscle. *J. Physiol. (London)*. **147**:591.
- LEBLANC, G., and D. ERLIJ. 1969. Effects of ethacrynic acid on sodium fluxes in frog sartorius muscle. *Biochim. Biophys. Acta.* **173**:149.
- LEVI, H., and H. H. USSING. 1948. The exchange of sodium and chloride ions across the fibre membrane of the isolated frog sartorius. *Acta Physiol. Scand.* **16**:232.
- MULLINS, L. J., and A. S. FRUMENTO. 1963. The concentration dependence of sodium efflux from muscle. *J. Gen. Physiol.* **46**:629.
- SJODIN, R. A., and L. A. BEAUGÉ. 1968. Strophanthidin-sensitive components of potassium and sodium movements in skeletal muscle as influenced by the internal sodium concentration. *J. Gen. Physiol.* **52**:389.
- STEINBACH, H. B. 1940. Sodium and potassium in frog muscle. *J. Biol. Chem.* **133**:695.
- STEINBACH, H. B. 1951. Sodium extrusion from isolated frog muscle. *Amer. J. Physiol.* **167**:284.
- STEINBACH, H. B. 1952. On the sodium and potassium balance of isolated frog muscles. *Proc. Nat. Acad. Sci. U. S. A.* **38**:451.
- USSING, H. H. 1949. Transport of ions across cellular membranes. *Physiol. Rev.* **29**:127.