Sodium Efflux in Myxicola Giant Axons

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ABSTRACT Several properties of the Na pump in giant axons from the marine annelid Myxicola infundibulum have been determined in an attempt to characterize this preparation for membrane transport studies. Both Na\textsubscript{0} and K\textsubscript{0} activated the Na pump of normal microinjected Myxicola axons. In this preparation, the K\textsubscript{0} activation was less and the Na\textsubscript{0} activation much greater than that found in the squid giant axon. However, when the intracellular ATP:ADP ratio of the Myxicola axon was elevated by injection of an extraneous phosphagen system, the K sensitivity of Na efflux increased to the magnitude characteristic of squid axons and the activating effect of Na\textsubscript{0} disappeared. Several axons were injected with Na\textsubscript{2}SO\textsubscript{4} in order to determine the effect of elevated Na\textsubscript{0} on the Na efflux. Increasing Na\textsubscript{0} enhanced a component of Na efflux which was insensitive to ouabain and dependent on [Ca] in Na-free (Li) seawater. After subtracting the Ca\textsubscript{0}-dependent fraction, Na efflux was related linearly to [Na\textsubscript{0}] in all solutions except in K-free (Li) seawater, where it appeared to reach saturation at high [Na\textsubscript{0}].

INTRODUCTION

Myxicola giant axons show mechanisms for membrane excitation that are similar to those observed in giant axons from the squid (Binstock and Goldman, 1969, 1971; Goldman and Binstock, 1969; Goldman and Schauf, 1972). As in the squid, the membranes of Myxicola have resting potentials which indicate a high selectivity for K relative to Na (Goldman, 1968). The ionic composition of axoplasm from fresh Myxicola giant axons is also similar to that of squid but [Na\textsubscript{0}] is lower in Myxicola, ~15 mM compared to ~50 mM in squid (Gilbert, 1975).

Studies of ion transport across the membranes of Myxicola giant axons have not been as extensive as electrical studies. Sodium efflux in dialyzed Myxicola axons has been observed to be reduced by application of strophanthidin, by removal of external K, and by replacement of external Na with Li or choline (Forbush, 1974). The transport of Na across the squid giant axon membrane has been investigated in considerable detail (Hodgkin and Keynes, 1955; Caldwell et al., 1960a, b; Mullins and Brinley, 1967; Brinley and Mullins, 1968; Baker et al., 1969a, b). The present work is an attempt to extend the knowledge of the Na pump in Myxicola giant axons to a level more comparable with what is known about Na transport in squid. The purpose is twofold: (a) to provide information on Na transport in a preparation gaining wider usage; and (b) to see if any characteristic differences in behavior are noted between the Na pump in Myxicola and that in squid giant axons. The method chosen was that of intracellular microinjection of \textsuperscript{22}Na. A preliminary report of this investigation has been made (Abercrombie and Sjodin, 1976).
MATERIALS AND METHODS

Experimental Materials

Myxicola were obtained from Marine Research Associates, New Brunswick, Canada. Animals were shipped to the laboratory by air freight and were maintained in a seawater aquarium at 9°C. The method used for dissection has been described (Binstock and Goldman, 1969). Axons were carefully cleaned of surrounding tissue after dissection and were mounted, after cannulation, in a microinjection chamber. The method and apparatus for microinjection of isotopes has been previously described (Hodgkin and Keynes, 1956; Brinley and Mullins, 1965; Sjodin, 1966). The injection medium was 0.5 M K₂SO₄ and 0.005 M EDTA to which ³²Na was added. Where appropriate, the injection medium was modified as stated in the text. At the termination of an experiment, the injected region was cut from the axon and subsequently weighed, ashed, and dissolved in nitric acid. A sample was taken for determination of radioactivity. Efflux samples and axoplasm were counted in a Beckman low level beta counter (Beckman Instruments, Inc., Fullerton, Calif.) after samples had been dried in planchets. Self-absorption corrections were applied to obtain the data from which rate constants were determined. In some cases, axoplasmic Na and K contents were determined by flame photometry. The axoplasm samples were taken from axons washed for 15 min in Mg-mannitol solution. A longitudinal slit was made on the axon, which enabled a sample of axoplasm to be pulled away with forceps. Preparation for analysis and flame photometry were performed as previously described (Sjodin and Henderson, 1964).

Solutions

The artificial seawater solutions used are listed in Table I. The numbers in the shorthand notation for the solutions refer to millimolar concentrations. Solutions with different [K] were prepared by mixing 0 K and 50 mM K seawater in varying proportions. In cases where [K] was elevated to 100 mM, the additional [K] replaced an osmotic equivalent amount of Na or Mg-mannitol in the seawater. When ouabain was added to solutions, it was present at a concentration of 10⁻⁴ M. Efflux experiments were performed at 10°-12°C. The pH of each solution was adjusted to 7.5 ± 0.05 at 10°C. The temperature coefficient of HEPES buffer is ~0.014 pH units/°C. Osmolarity of the solutions was checked on a freezing point osmometer and was found to be within 3% of 950 mosM.

ATP and ADP Analysis

ATP was analyzed according to the method of Bucher (1947) using the coupled enzymatic reactions:

\[
3\text{-phosphoglycerate} + \text{ATP} \rightarrow 1,3\text{-diphosphoglycerate} + \text{ADP}
\]

\[
1,3\text{-diphosphoglycerate} + \text{NADH} \rightarrow \text{glyceraldehyde-3-P} + \text{NAD} + \text{P}.
\]

Formation of NAD was monitored spectrophotometrically at 3,400 Å.

ADP was analyzed by using the coupled reaction sequence:

\[
\text{ADP} + \text{phospho(enol)pyruvate} \rightarrow \text{ATP} + \text{pyruvate}
\]

\[
\text{pyruvate} + \text{NADH} \rightarrow \text{lactate} + \text{NAD}.
\]

The second reaction was followed spectrophotometrically at 3,400 Å. A detailed description of the method is given in Adam (1965). The chemical reagents were obtained from Sigma Chemical Co., St. Louis, Mo.

Injected Chemicals

The Mg salt of ATP, the Tris salt of creatine phosphate (CP), and creatine phosphokinase (CPK) were obtained from Sigma Chemical Co. and were stored frozen before use.
**RESULTS**

**The K₀ and Ouabain Sensitivity of Na Efflux**

Removal of external K or application of ouabain (or strophanthidin) reduces Na efflux in squid giant axons (Mullins and Brinley, 1967; Brinley and Mullins, 1968; Sjodin and Beaugé, 1969; Baker et al., 1969b). An experiment to test for the presence of similar effects in *Myxicola* giant axons is illustrated in Fig. 1. Removal of K₀ decreased Na efflux by 50% while 10⁻⁴ M ouabain brought about a greater reduction, to 29% of the control Na efflux. On the average, removal of K₀ from a normal 10 mM [K]₀ medium reduced Na efflux to 64% of the normal value while application of 10⁻⁴ M ouabain reduced Na efflux to 35% of the value.

### Table I

**Artificial Seawater Solutions**

<table>
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<tr>
<th></th>
<th>CaCl₂</th>
<th>MgSO₄</th>
<th>NaCl</th>
<th>KCl</th>
<th>MgCl</th>
<th>Mannitol</th>
<th>LiCl</th>
<th>HEPES</th>
<th>EDTA</th>
</tr>
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<tbody>
<tr>
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<td>10</td>
<td>25</td>
<td>400</td>
<td>30.6</td>
<td>50</td>
<td>5</td>
<td>0.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>50 K (Na)</td>
<td>10</td>
<td>25</td>
<td>400</td>
<td>50</td>
<td>14</td>
<td>5</td>
<td>0.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 K (Mg + mannitol)</td>
<td>10</td>
<td>25</td>
<td>400</td>
<td>164</td>
<td>450</td>
<td>5</td>
<td>0.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>50 K (Mg + mannitol)</td>
<td>10</td>
<td>25</td>
<td>400</td>
<td>147.4</td>
<td>400</td>
<td>5</td>
<td>0.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 K (Li)</td>
<td>10</td>
<td>25</td>
<td>400</td>
<td>50</td>
<td>50</td>
<td>400</td>
<td>5</td>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td>50 K (Li)</td>
<td>10</td>
<td>25</td>
<td>400</td>
<td>50</td>
<td>50</td>
<td>14</td>
<td>0</td>
<td>5</td>
<td>0.5</td>
</tr>
<tr>
<td>0 K (Li) 0 Ca++</td>
<td>10</td>
<td>25</td>
<td>400</td>
<td>40.6</td>
<td>400</td>
<td>5</td>
<td>0.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>50 K (Li) 0 Ca</td>
<td>10</td>
<td>25</td>
<td>400</td>
<td>50</td>
<td>24</td>
<td>0</td>
<td>0</td>
<td>5</td>
<td>0.5</td>
</tr>
</tbody>
</table>

*Figure 1.* Effect of K-free seawater and ouabain-containing seawater on the Na efflux from a single nerve fiber of *Myxicola*. The vertical axis represents the rate constant for Na efflux; the horizontal axis is the time of duration of the experiment. Open circles: K-free seawater. Filled circles: 10 K seawater. Temperature: 11°C. Axon 110874.
in 10 mM [K]₀ seawater. When [K]₀ was varied in Na seawater in both the absence and the presence of 10⁻⁴ M ouabain, the results shown in Fig. 2 were obtained. Increasing [K]₀ brought about an activation of the Na pump which increased to saturation at about [K]₀ = 50 mM. In the presence of ouabain, increasing [K]₀ caused a much smaller increase in Na efflux to a value also showing saturation at about [K]₀ = 50 mM. The maximal value of Na efflux at high [K]₀ in ouabain was 30% of the maximal value in the absence of ouabain.

![Graph showing normalized rate constant for Na efflux measured in Na seawater plotted against [K]₀.](image)

**Figure 2.** The normalized rate constant for Na efflux measured in Na seawater is plotted against [K]₀. The upper curve (filled circles) was obtained in the absence and the lower curve (filled squares) in the presence of 10⁻⁴ M ouabain.¹

**The Influence of Na₀ on Activation of the Na Pump**

A mixture of Mg and mannitol (Mg-mannitol seawater) was used as a substitute for Na to reduce [Na]₀ to zero and to vary [Na]₀. When all Na₀ was replaced with Mg-mannitol, three classes of Na-free effect were observed, depending upon the value of [K]₀. When the outside solution was nominally K-free, reducing [Na]₀ to zero increased Na efflux about 25%. When [K]₀ was in the range of 20–30 mM, in most of the following graphs, the results are given in terms of a normalized Na efflux rate constant; i.e., relative to the rate constant in 10 K, 400 Na seawater. The actual Na efflux in 10 K, 400 Na seawater can be calculated from the rate constant, the [Na]₀, and the fiber diameter. An average of 15.8 ± 3.2 pmol/cm² s was obtained from data acquired in this work. The efflux in other seawater solutions can therefore easily be obtained as a fractional amount of 16 pmol/cm² s.

¹ In most of the following graphs, the results are given in terms of a normalized Na efflux rate constant; i.e., relative to the rate constant in 10 K, 400 Na seawater. The actual Na efflux in 10 K, 400 Na seawater can be calculated from the rate constant, the [Na]₀, and the fiber diameter. An average of 15.8 ± 3.2 pmol/cm² s was obtained from data acquired in this work. The efflux in other seawater solutions can therefore easily be obtained as a fractional amount of 16 pmol/cm² s.
removing Na\textsubscript{e} reduced Na efflux by about 20%. At [K]\textsubscript{0} values of 50 mM and above, there was no change in Na efflux upon removal of Na\textsubscript{e}. Results in Na-free media are shown in Fig. 3 together with the data from Fig. 2. Also included are data obtained in the presence of 10^{-4} M ouabain. In ouabain, the Na efflux was lower in Mg-mannitol seawater than in Na seawater; however, the data in Fig. 3 show that the Na efflux reductions caused by a change to Na-free solutions are largely in the ouabain-sensitive fraction. A possible interpretation of the results is that, in normal intact *Myxicola* giant axons, a significant amount of activation of the Na pump is by external Na.

The results of several experiments in which the axon was immersed in mixtures of Na- and Mg-mannitol seawater are shown in Fig. 4. In the presence of 20 mM K\textsubscript{0} (Fig. 4 A), increasing [Na] activates additional Na efflux. The activation is approximately hyperbolic and half-maximal when Na\textsubscript{e} \approx 75 mM. By comparison, increasing Na\textsubscript{e} inhibits Na efflux in the absence of K\textsubscript{0} (Fig. 4 B). These experiments extend the previous results and show that Na\textsubscript{e} inhibits at low concentrations of K\textsubscript{0} and activates at intermediate concentrations of K\textsubscript{0}. 

**Figure 3.** Figure represents a collection of all results showing the activation by K\textsuperscript{+} of the Na efflux in two types of ASW solutions with and without ouabain: Na and Mg + mannitol. Abscissa: [K]\textsubscript{0}; ordinate: \textsuperscript{22}Na efflux relative to normal 10 K/400 Na ASW. Interpretation of these results is given in the discussion. Filled symbols: Na ASW; open symbols: Mg + mannitol ASW; circles: without ouabain; squares: with ouabain.
The Dependence of Na Sensitivity of the Na Pump on the Intracellular ATP and ADP Levels

In contrast to normal squid axons, where only K₀ activates the Na pump, both K₀ and Na₀ apparently activate the Na pump of normal microinjected *Myxicola* axons. However, the activation by Na₀ (and reduced activation by K₀) seen in *Myxicola* bears a close resemblance to results obtained with squid axons which have an artificially reduced ratio of ATP:ADP (Caldwell et al., 1960b; Baker et al., 1969b; De Weer, 1970). Therefore, it seemed plausible that the difference in behavior between the Na pump in *Myxicola* and that in squid giant axons might be due to a lower ATP:ADP ratio in the *Myxicola* cells.

Measured intracellular concentrations of ATP and ADP (see Materials and Methods) are shown in Table II. An ATP:ADP ratio of approximately 5, as suggested by this data, is somewhat lower than the value of 11 obtained in squid axoplasm (Mullins and Brinley, 1967). Should the lower ATP:ADP ratio be responsible for the Na₀-dependent Na efflux in normal *Myxicola* giant axons, elevating this ratio should decrease Na₀ sensitivity and increase sensitivity to K₀.

![Graphs showing the relative rate constant for Na efflux](image-url)
MgATP was injected into some axons to elevate [ATP]i, in an attempt to establish a causal relationship between the ATP:ADP ratio on one hand, and the reduced sensitivity to $K_0$ and increased $Na_0$ sensitivity on the other. Data shown in Table III indicate that MgATP injection slightly increased the $K_0$ sensitivity. Comparing the values in the $\Delta[ATP]$ column with the [ATP]i and [ADP]i measurements of Table II indicates that the ATP:ADP ratio must have been elevated several-fold in these axons.

In other experiments, creatine phosphate (CP) was injected into axons which had been preinjected with phosphocreatine kinase (CPK). The ADP level in such axons was reduced and maintained by the reaction $CP + ADP \rightarrow C + ATP$. This technique has been shown to be effective in controlling the ATP:ADP ratio in squid giant axons (De Weer, 1970). Results of a typical experiment are illustrated in Fig. 5. Injection of CP caused: (a) a fall in Na efflux in K-free, Na seawater (which is consistent with a decreased $Na_0$ activation); and (b) an apparent increase in the activation by $K_0$. Results on three axons studied in this way (Table III)
IV) indicate that the K-free effects observed in Myxicola giant axons injected with CPK and CP are comparable to those in squid.

In additional experiments, sensitivity to Na was determined in CPK- and CP-injected axons at constant K = 10 mM. The results given in Table V demonstrate that activation by Na was abolished in the treated axons. This effect has to be regarded as a composite one in which the activation and inhibitory effects of Na now balance one another.

![Graph](https://example.com/figure5.png)

**Figure 5.** Effect of increasing the intracellular ATP:ADP ratio by injection of an extraneous phosphagen system. The axon was preloaded with CPK and ^22Na. Closed circles: efflux in 10 K artificial seawater. Open circles: efflux in K-free artificial seawater. At the time designated by the arrow, CP was injected into the fiber. Final concentration of CP was 20 mM; final activity of CPK was 200 U/ml. Temperature: 11°C. Axon: 111275.

**Measurements in Li-Substituted Solutions and Summary of Ouabain-Sensitive Na Efflux**

When Li was substituted for Na, results differed considerably from those in which Mg-mannitol was the Na substitute. With K at either 0 or 10 mM, no significant change in Na efflux occurred when replacing Na with Li; whereas, at 50 mM K, changing from Na to Li seawater reduced Na efflux.

We also determined the activation of Na efflux by K in the presence of 10^-4 M ouabain in Na, Li, and Mg-mannitol seawater. In Fig. 6, the ouabain-sensitive fraction of Na efflux is plotted against K for the different solutions employed.
Ca-Activated Na Efflux in Myxicola Giant Axons

Squid giant axon membranes show a Ca₀-dependent Na efflux under conditions favoring a counter exchange of Ca₀ for Naᵢ, namely when [Naᵢ] is elevated and [Na₀]₀ is low or zero (Baker et al., 1969a). This fraction of Na efflux is insensitive to ouabain and in squid giant axons is apparent in Li seawater. To see if a similar component of Na efflux exists in Myxicola giant axons, the effect of Ca₀ removal in Li seawater was determined. Measurements were made in the presence of ouabain to obtain better resolution as the base line is lower and ouabain does not affect this component of efflux. Several experiments performed on normal injected axons ([Naᵢ] = 25 mM/kg) revealed no change in Na efflux upon removal of external Ca. As the effect is favored by high [Naᵢ] in squid giant axons, unlabeled Na was added to the ²²Na in the injection fluid such that the [Naᵢ] after injection was elevated to about 75 mM/kg. In the experimentally produced high [Naᵢ] axons, removal of Ca₀ reversibly reduced Na efflux by about one-third. A typical experiment is illustrated in Fig. 7. It is evident, when [Naᵢ] is elevated, that Myxicola giant axons show a Ca₀-dependent component of Na efflux that is similar to that observed in squid giant axons. Results on several axons are tabulated in Table VI.

<table>
<thead>
<tr>
<th>Table IV</th>
<th>EFFECT OF INJECTIONS OF CREATINE PHOSPHATE (CP) ON AXONS PRELOADED WITH PHOSPHOCREATINE KINASE (CPK)</th>
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<tr>
<td>Rate constant relative to normal 10 K, 400 Na ASW</td>
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<tr>
<td>Axon reference</td>
<td>0 K, 400 Na</td>
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<tr>
<td>111175</td>
<td>0.46</td>
</tr>
<tr>
<td>111275</td>
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<tr>
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<td>Mean</td>
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<table>
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<th>Table V</th>
<th>Na-FREE EFFECT IN 10 K ASW AFTER INJECTION OF PHOSPHOCREATINE KINASE AND PHOSPHOCREATINE</th>
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<tr>
<td>Rate constant x 10⁻⁶</td>
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<td>± SEM</td>
<td>0.08</td>
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Figure 6. The ouabain-sensitive fraction of the rate constant for $^{22}$Na efflux is plotted against $[K]_0$ for various external media. The curves are labeled with the solutions used.

Figure 7. Ca$^{2+}$-dependent Na efflux in an axon with elevated Na$_i$ in Li artificial seawater. Increase in Na$_i$: 60 mM. Temperature: 10°C. Axon: 021176.
The Dependence of Na Efflux on $[\text{Na}]_i$

In the course of the experimentation reported in the previous section, data were accumulated on values of Na efflux at various levels of $[\text{Na}]_i$ in different solutions. The rate constants for Na efflux were observed to be constant, and hence independent of $[\text{Na}]_i$ over a wide range (25–115 mM/kg). The magnitude of Na efflux (rate constant $\times [\text{Na}]_i$) is plotted against $[\text{Na}]_i$ in Fig. 8. When a correction was applied for the ouabain-insensitive, Ca-activated Na efflux, linear relationships were obtained for all cases except in K-free Li seawater. In the excepted case, Na efflux appears to reach saturation at high $[\text{Na}]_i$.

### Table VI

<table>
<thead>
<tr>
<th>Axon reference</th>
<th>Increase in $[\text{Na}]_i$ (mM)</th>
<th>10 mM Ca</th>
<th>Ca-free</th>
<th>Change</th>
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<tbody>
<tr>
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<td>10</td>
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<td>0.03</td>
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</table>

**Figure 8.** The "corrected" Na efflux represents the total Na efflux minus the Ca$^{++}$-dependent fraction, which is insensitive to ouabain. The curve representing Na efflux vs. $[\text{Na}]_i$ in 0 K, 400 Li artificial seawater is not linear. Open squares: 10 K, Li; filled squares: 10 K, Na; open circles: 0 K, Li; closed circles: 0 K, Na.
DISCUSSION

Three phenomena seem to be special properties of normal microinjected Myxicola giant axons: (a) Na$_0$-activated Na efflux; (b) a naturally low ATP:ADP ratio; and (c) an additional activation by K$_0$ of Na efflux which occurs in Mg-mannitol seawater when K$_0$ is raised above 20 mM.

Properties (a) and (b) appear to be related. Injection of CP + CPK demonstrated that when the ATP:ADP ratio of Myxicola axoplasm is elevated, the Na$_0$-activated Na efflux is eliminated. This confirms, in a second nerve preparation, the observation made with squid that Na$_0$-stimulated Na efflux is promoted by a lower ATP:ADP ratio.

The methods used for elevating the ATP:ADP ratio in this investigation offer an interesting comparison. Of the two, CP + CPK injection had a larger effect than simple injection of ATP (see Tables IV and V). This may mean that reducing ADP is more effective than elevating ATP in reducing the Na$_0$-dependent Na efflux in Myxicola.

Property (c), the S-shaped curve relating Na efflux to K$_0$ in Mg-mannitol seawater, is also of interest. This characteristic shape was present when the ouabain-sensitive fraction as well as the total Na efflux was plotted as a function of K$_0$. This result contrasts sharply with published data for the squid giant axon. Whereas the Na efflux from squid axons bathed in Na-free (choline, dextrose, or Mg-mannitol) seawater becomes saturated by very small amounts of K$_0$, the Na efflux from Myxicola does not. As [K]$_0$ is elevated, Na efflux from Myxicola axons bathed in Mg-mannitol solutions has an initial plateau and then increases to a maximum when [K]$_0$ reaches 50 mM. A reasonable possibility is that the secondary activation by K$_0$ in Mg-mannitol seawater is due to activation of a second class of sites with a lower affinity for K. Since the sensitivity to Na$_0$ disappears in the range of high [K]$_0$, the postulated low-affinity sites may be normally activated by Na$_0$. In this view, the Na efflux in 0 K Mg-mannitol seawater would be due to residual K in the diffusion-limited extracellular spaces of Myxicola. (For an equivalent interpretation in squid, see Baker et al., 1969b.)

Another interesting interpretation of these results was suggested to us by Professor L. J. Mullins. According to this scheme, only a single external site is required; however, the site engages in three different modes of Na transport with different maximum rates of Na efflux, or $V_{\text{max}}$, for each of the three modes. In seawater containing no K and the usual amount of Na, Na efflux would be due to an exchange of internal for external Na through the pump. This would account for the large ouabain-sensitive Na efflux in 0 K, 400 Na seawater. As K is increased up to 50 mM, the pump mode changes to the more usual Na-K exchange and the level of Na efflux increases accordingly. In Na-free, K-free (Mg-mannitol) seawater, the Na efflux is ascribed to an additional mode of the Na pump. This is an efflux of Na uncoupled to either Na or K outside the cell. An uncoupled Na efflux has been described in red blood cells by Glynn and Karlish (1976) and may account for the ouabain-sensitive Na efflux in 0 K, 0 Na (Mg-mannitol) seawater in this preparation. As K$_0$ is increased in these Na-free solutions, two things may happen: (a) the amount of uncoupled efflux may be attenuated; and (b) the pump may change to the Na-K mode of transport with a $V_{\text{max}}$ equal to the $V_{\text{max}}$ in high K, 400 Na seawater. The initial plateau
phase in Mg-mannitol seawater and the subsequent S-shaped activation by K may therefore be due to the transition between two modes of Na transport: an uncoupled Na efflux, which is dominant at low K, and an Na-K exchange with a higher \( V_{\text{max}} \), which prevails as K is increased to 50 mM.

While it may not be possible to decide between the alternatives at the present time, several possible explanations can be ruled out. The biphasic effect of K on the Na efflux in Mg-mannitol seawater may suggest a possible effect of membrane potential (Horowicz and Gerber, 1965). However, from the work of Goldman (1968), one would expect the membrane potential at a given \([K]\) to be approximately the same in Na, Li, or Mg-mannitol solutions. The absence of a biphasic activation by \( K_0 \) in either Li or Na seawater is, therefore, a drawback of this explanation. Another possibility is that in the Mg-mannitol solutions, Mg ions inhibit the activation site in a complicated way so that the appearance of the secondary activation represents an antagonism by K of this inhibitory effect of Mg ions. Were this the case, removal of Mg should release the inhibition and allow the Na efflux to reach its maximum value. In some experiments, mannitol alone was used to replace Na in the absence of Mg. The Na efflux under these conditions was far below the maximum uninhibited value one would expect according to this hypothesis. The ouabain-insensitive, \( Ca^2+ \)-dependent, Na efflux mechanism cannot be responsible for the biphasic effect described above because: (a) the \( Ca^2+ \)-dependent component was not observed in axons with normal \( Na^+ \); and (b) the biphasic characteristic is also present when the ouabain-sensitive flux is plotted (Fig. 6).

Sensitivity of Na efflux to external Ca was found in ouabain-poisoned axons when \([Na]_o \) was artificially high and the fiber was bathed in Li seawater. This finding is similar to observations on squid giant axons (Baker et al., 1969b). Myxicola axons thus have a \( Ca^2+:Na^+ \) exchange system with characteristics similar to that in squid. Clearly, the Myxicola giant axon is quite suitable for ion transport studies and several components of Na efflux are present which can be studied in detail that goes beyond the scope of this introductory investigation.

This work was supported by grants from the National Institute of Neurological and Communicative Disorders and Stroke, United States Public Health Service (NS-07626) and the National Science Foundation (BMS 74-12243).

Received for publication 1 November 1976.

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