Sodium Flux in the Smooth Muscle of Frog Stomach

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ABSTRACT Sodium efflux from rings of frog stomach muscle was measured at 5° and 15°C in three different steady states. After incubation in normal, K-free, or ouabain (10^{-4} M) solutions, intracellular cations stabilized at markedly differing levels. At 5°C, inhibition of Na extrusion was shown in the rate coefficients for ^22Na efflux, which were slightly smaller in K-free than in normal solutions, and much smaller in ouabain. Due to the intracellular Na concentration differences, total Na efflux was similar in K-free and ouabain solutions, and only 1/3 as large in normal solution. At 15°C, normal total Na flux was only 1/10 of that in inhibitors, and may be underestimated. The total flux differences may involve dependence of the Na pump and Na permeation on internal Na concentration. The Q_{10} of the steady-state fluxes was 3.7 in ouabain, 2.8 in K-free solution, and 1.9 in normal solution. The high temperature dependence of influx as well as efflux suggests transport mechanisms other than simple diffusion. Sodium turnover in the cell water was 46-66 nM/hr in inhibitors at 15°C, and a high rate of Na extrusion in normal muscle is suggested. However, cell volume:surface ratio is only 1.6 μ and all estimates of Na flux were under 3 pmoles/cm² per sec, indicating low Na permeability.

INTRODUCTION

The osmotic survival and normal function of vertebrate smooth muscle, as in other excitable tissues, must depend on membrane ion transport. The large surface:volume ratio of these small fibers implies that for a given sodium permeability and electrochemical gradient, the minimum work required per kilogram cell water for active sodium extrusion would be much greater than in larger cells, such as skeletal muscle fibers. However, the ion transport mechanisms have not been well-characterized, and the ionic basis of the electrical properties of this spontaneously active tissue has not been clearly delineated (7, 33). Sodium flux has been difficult to evaluate, due to the rapidity of tracer sodium exchange at ordinary temperatures and uncertainties about the free sodium concentration in the cell water. The low membrane potential of mammalian intestinal muscle has been attributed to high...
sodium permeability, and early estimates of sodium flux were extraordinarily high (e.g. reference 14). However, reassessment of the distribution of tissue sodium has given estimates similar to those in mammalian skeletal muscle (4), and recently extremely low values of the sodium flux component of interest have been reported in mammalian arterial muscle (26). These studies were complicated by the presence of "bound" sodium components with similar exchange rates at normal temperatures, and an unsteady state during temperature reduction.

Frog stomach muscle maintains low intracellular levels of exchanging sodium over a wide temperature range, and the bound sodium exchanges with tracer at an extremely slow rate (35). Consequently, in this preparation the exchange of free cell sodium can be studied under steady-state conditions at reduced temperatures. In the present experiments, sodium efflux was estimated at two temperatures in three different steady states which followed (a) normal incubation, (b) potassium removal, and (c) treatment with the cardiac glycoside ouabain. The improved resolution of extracellular and cellular exchange obtained at the lower temperatures permitted characterization of some features of transmembrane exchange in this tissue.

A preliminary report of portions of this work has been presented (36).

METHODS

Rings of circular muscle from the stomach of Rana pipiens were studied (mucosa removed). Storage of the frogs and preparation of the tissue have been described previously (35). Each ring was suspended in a holder between two glass hooks, one fixed and one in series with a small spring, for maintenance near normal fiber length and ease of handling without trauma. Muscle rings were incubated for washout experiments and correlated uptake studies in two ways: (a) Individual baths were continuously gased, providing stirring, oxygenation, and buffering, either through the fritted glass filter of small Buchner funnels or through thin polyethylene tubing in small glass vials. (b) Small vials each containing a mounted muscle ring in well-gassed solution were refrigerated (5-6°C) in a gas-filled precooled desiccator. Most of the efflux experiments reported employed the second loading procedure. In all efflux measurements, each Na-loaded muscle on its holder was blotted lightly and transferred through a series of vials containing 1.5 ml inactive solution (~50 volumes) gased (and stirred) through polyethylene tubing. Tracer loss was followed for 2-4 hr. At the end of all experiments, the rings were removed from their holders onto moistened filter paper, lightly blotted, and weighed in foil to 0.1 mg.

The standard Ringer solution contained (mM/liter) NaCl 92.5, NaHCO3 18.5, KCl 2.0, CaCl2 1.0, MgCl2 1.0, and was equilibrated with humidified 95 % O2-5 % CO2. In K-free solution, KCl was omitted, and in ouabain-Ringer solution ouabain was added to 10^-4 M. For dissection solution, NaCl replaced NaHCO3. Solutions were labeled with sucrose-14C UL (Nuclear-Chicago Corp., Des Plaines, Ill.) by addition of concentrated stock solution, stored frozen, to give approximately 2.4 mM/liter sucrose, 35 μC/mM. Solutions were labeled with NaCl (Abbott Laboratories,
North Chicago, Ill.) by the addition of high specific activity stock solution to give about 0.8 \( \mu \text{c/mm} \) for uptake experiments and 7.5 \( \mu \text{c/mm} \) for washout experiments.

The determinations of Na and K contents (by flame photometry) and sucrose-\(^{14}\)C and \(^{22}\)Na contents (by liquid scintillation counting) of nondried tissue have been described previously (35). Sucrose-\(^{14}\)C content was determined simultaneously with \(^{22}\)Na uptake in the same muscles from double-labeled solutions. Dry weight was determined in separate experiments. The sucrose-\(^{14}\)C space or relative activity (R. A.) of \(^{22}\)Na is given by: net disintegrations per minute per gram muscle/net disintegrations per minute per milliliter loading solution \( \times 100 \) for \%. Efficiency variations between muscle and appropriate dilutions of its individual loading solution were always monitored, and were so small that counts per minute rather than disintegrations per minute ratios could be utilized. Exchanging Na (in mm/kg wet weight) is given by fractional R. A. \(^{22}\)Na times mm Na/liter loading solution (N\(_{\text{a}}\)). Exchanging cell Na in mm/kg cell water (N\(_{\text{a}}\)) is calculated as

\[
\text{Exchanging cell Na} = \frac{(\text{R. A.} \times \text{ECS}) \times \text{N}_{\text{a}}}{\text{Wet wt} \times (1 - \text{dry wt} - \text{ECS})}
\]

where the extracellular space (ECS) was given by the fractional sucrose-\(^{14}\)C space or by the difference between total exchanging Na and the cellular Na component determined from the intercepts of washout plots (see Results).

Activity remaining in the muscle during washout was back-added from the final muscle counts and the activity of the wash solutions, and was expressed as R. A. \(^{22}\)Na. First-order rate coefficients \( (k_o) \) were calculated for each washout interval from dpm lost per minute/mean dpm remaining, and averaged over the linear portion of semilog plots of activity remaining. Sodium flux was calculated as \( k_o \times \text{Na}_{\text{a}} \), in mm/kg cell water/hr. These values were converted to pmol/cm\(^2\) per sec with an approximate value for volume:surface ratio obtained by measuring fiber diameter and applying the formula for \( V/S \) of a cylinder. Diameters were measured adjacent to the nucleus on longitudinal sections of tissue fixed in Dalton’s osmium tetroxide fixative at pH 7.4 for 1 hr at room temperature, washed for 30 min in several changes of 10% ethanol, and dehydrated rapidly in graded ethanols and propylene oxide. The specimens were embedded in Epon 812, and 1 \( \mu \) sections were examined by phase contrast microscopy at a magnification of 1200, with suitable calibration. The author is indebted to Dr. M. M. Cassidy for these preparations. The width of 50 fibers was determined in normal and in ouabain-incubated tissue; these did not differ significantly.

Results are expressed as the mean \( \pm \) standard deviation, followed by the number of muscles or muscle pairs. The significance of differences was evaluated with Student’s \( t \) test and expressed as a probability; \( P < 0.05 \) was considered significant.

**RESULTS**

\(^{22}\)Na Exchange in Normal Ringer Solution Preincubated frog stomach muscle has been shown to equilibrate with \(^{22}\)Na-Ringer solution rapidly; \(^{22}\)Na, Na, and K content change very little after about 30 min at room temperature,
although there is an extremely slowly exchanging Na component (35). In this series of experiments, muscles were loaded directly in cold $^{22}$Na-Ringer solution, unstirred but saturated with gas mixture, for 20 hr at 5–6°C. Tracer loss into normal solution, stirred by gassing, was measured at 5°C and 15°C. The time course of tracer loss is shown in Fig. 1; log relative activity (R. A.) remaining is plotted against time for one representative muscle out of four in
each experiment (from the same frog batch). The later loss of tracer at 5°C regularly approximated a single exponential function of time, although the calculated rate coefficients decreased slightly; the extrapolated intercept of this line on the ordinate was small. Tracer loss at 15°C was more rapid and also more complex; later loss could be divided into a faster and a slower phase. The faster phase often had a high intercept (see below), and the lower mean rate coefficient, obtained by following the slower phase back as far as the points fell near a single line, was taken as a more conservative estimate.

**Table I**

### CATION CONTENTS UNDER WASHOUT CONDITIONS IN NORMAL RINGER SOLUTION

<table>
<thead>
<tr>
<th>Incubation conditions</th>
<th>3 hr* (24-27°C)</th>
<th>18-20 hr (5-6°C)</th>
<th>20 + 2 hr (5-6°C + 5°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Content</strong> (mM/kg wet wt)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Na</td>
<td>57.9±5.3 (12)</td>
<td>51.3±6.0 (6)</td>
<td>—</td>
</tr>
<tr>
<td>Change in Na</td>
<td>—5.0±4.6 (6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>K</td>
<td>64.6±5.8 (12)</td>
<td>69.8±5.4 (6)</td>
<td>—</td>
</tr>
<tr>
<td>Change in K</td>
<td>+2.3±10.0 (6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Exchanging Na§</td>
<td>49.0±5.0 (26)</td>
<td>47.9±6.2 (46)</td>
<td>45.9±5.7 (6)</td>
</tr>
<tr>
<td>Change in Na§</td>
<td>+2.2±5.0 (6)</td>
<td></td>
<td>—3.1±9.3 (6)</td>
</tr>
</tbody>
</table>

Results of experiments with three different frog batches were pooled. Variation is expressed as the standard deviation followed by the number of determinations in parentheses. Change in content is the difference between paired muscles, in the same batch.

* I hr preincubation plus 2 hr in labeled or unlabeled solution, all gassed.
† P < 0.05 for pair difference.
§ Determined from 22Na uptake and the Na concentration of the bath.

In this pair of experiments, the mean rate coefficient at 15°C, 0.91 ± 0.08 (4) hr⁻¹, was 2.0 times the mean rate coefficient at 5°C, 0.45 ± 0.04 (4) hr⁻¹. The mean rate coefficients of these and additional experiments are collected in Table VI.

Analysis and interpretation of the tracer loss depend on the net ionic state of the tissue during the efflux measurements. Muscle rings paired to efflux muscles (from the same stomach) were assayed for 22Na and sucrose-14C uptake after the same loading procedure, and in separate experiments, 22Na or Na and K content were measured under conditions pertinent to efflux measurements. Table I shows that muscles incubated in normal solution in the cold for 20 hr had essentially the same Na, K, and exchanging Na content as control muscles incubated in gassed normal solution at room temperature for 3 hr; total Na may have been slightly lower. Subsequent incubation in
stirred solution, corresponding to washout conditions, caused no change. Therefore, during washout the tissue was in a steady state which was similar to that at room temperature in stirred (gassed) solution.

\[ ^{22}\text{Na Exchange in K-Free Ringer Solution} \]

Stomach muscle rings gain Na and lose K in K-free solution, following preincubation in normal solution, al-

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

**Figure 2.** Typical loss of \(^{22}\text{Na}\) from muscle rings loaded in K-free solution at 5–6°C for 20 hr, and washed out in K-free solution at 5°C (○) or 15°C (X). R. A. \(^{22}\text{Na}\) remanining is plotted as a function of time. (Muscles were from the same frog batch.)

though the changes observed under these conditions are small (35). Armstrong (1) measured much higher Na and lower K in sheets or strips of frog stomach muscle placed directly in K-free solution and incubated overnight in the cold. Mounted muscle rings were loaded in this way in the experiments described below, and were washed out at 5°C and 15°C, into K-free solution.

Fig. 2 illustrates the time course of \(^{22}\text{Na}\) loss from muscles loaded in K-free solution in the cold without preincubation and washed out in K-free solution. Each curve represents one muscle of four in each experiment, all from the
same frog batch. As in normal solution, at 5°C, tracer loss after about 30 min approximated a single exponential; this loss presumably represents the cellular Na component, and the extrapolated relative activity in the tissue at zero washout time was considerably larger than in normal solution. The rapid tracer loss at 15°C usually had two slower components, as in normal tissue, and the rate coefficient was estimated in the same way. In this pair of experiments the mean rate coefficient at 15°C, $1.40 \pm 0.18 \ (4) \ hr^{-1}$, was 2.6 times the mean rate coefficient at 5°C, $0.54 \pm 0.02 \ (4) \ hr^{-1}$. These values and

<table>
<thead>
<tr>
<th>TABLE II</th>
</tr>
</thead>
<tbody>
<tr>
<td>CATION CHANGES IN K-FREE SOLUTION</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Incubation conditions</th>
<th>Total Na</th>
<th>Change in Na</th>
<th>Total K</th>
<th>Change in K</th>
<th>Exchanging Na</th>
</tr>
</thead>
<tbody>
<tr>
<td>NR, 20 hr 5-6°C</td>
<td>59.5±4.2 (6)</td>
<td>—</td>
<td>61.7±4.4 (6)</td>
<td>—</td>
<td>49.3±5.2 (18)</td>
</tr>
<tr>
<td>K-free, 19-20 hr 5-6°C</td>
<td>66.2±4.9 (12)</td>
<td>50.8±6.7 (12)</td>
<td>63.8±9.3 (60)</td>
<td>+11.2±4.7 (6)</td>
<td>46.9±6.8 (26)</td>
</tr>
<tr>
<td>Postincubation NR*</td>
<td>55.0±4.1 (6)</td>
<td>—10.8±3.0 (6)</td>
<td>60.1±5.9 (6)</td>
<td>+9.2±7.1 (6)</td>
<td>+6.8±7.2 (6)</td>
</tr>
</tbody>
</table>

Incubation conditions are described in the text. Results from five different batches of frogs are pooled for compact presentation; variation, expressed as the standard deviation, includes variation between batches. However, the differences shown are between paired muscles from a single batch; these all are significant ($P < 0.05$) except for the value marked*.

* Following incubation as in the preceding column.

† Change from condition in the preceding column.

‡ Determined from the relative activity of $^{24}$Na and the Na concentration of the medium.

§ Change from column 1; this pair difference is not significant, but the difference calculated as exchanging cell Na (mM/kg cell water) is significant.

those from similar experiments under these conditions are summarized in Table VI.

The rate coefficients observed in K-free solution at 5°C did not differ markedly from those in normal solution. However, when two experiments performed with the same frog batch were compared, the mean rate coefficient in K-free solution, $0.40 \pm 0.03 \ (4) \ hr^{-1}$, was significantly lower than that in normal solution, $0.61 \pm 0.03 \ (4) \ hr^{-1} \ (P < 0.01)$.

Muscles incubated in K-free solution as described above contained more Na, less K, and more exchanging Na than tissue in normal solution. Columns 1-3 of Table II show these values compared in the same frog batches. These changes were practically complete by the time tracer efflux measure-
ments were made: Table III shows a comparison between muscles incubated for 20 hr as described above, and paired muscles transferred to gassed vials for an additional 2 or 4 hr in K-free solution at 5° or 15°C (simulating washout conditions). The pair difference for an additional 4 hr at 15°C is not statistically significant, but this difference calculated for exchanging cell Na (using the simultaneously measured sucrose-¹⁴C space) was significant \((P < 0.05)\); the mean change, however, was less than 1 mM/kg wet weight per hr. Therefore the tissue was close to a new steady state, with altered internal Na and K levels, during tracer efflux measurements in K-free solution.

### Table III

<table>
<thead>
<tr>
<th>Incubation time</th>
<th>Temperature</th>
<th>Exchanging Na*</th>
<th>Change in Na*; ‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>hr</td>
<td>°C</td>
<td>mM/kg wet wt</td>
<td>mM/kg wet wt</td>
</tr>
<tr>
<td>20</td>
<td>5–6</td>
<td>63.8±9.3 (60)</td>
<td></td>
</tr>
<tr>
<td>20+2</td>
<td>+5</td>
<td>67.4±4.8 (6)</td>
<td>+0.7±3.1 (6)</td>
</tr>
<tr>
<td>20+4</td>
<td>+5</td>
<td>58.9±4.1 (4)</td>
<td>+0.3±4.6 (6)</td>
</tr>
<tr>
<td>20+2</td>
<td>+15</td>
<td>63.4±6.3 (6)</td>
<td>+0.4±3.8 (6)</td>
</tr>
<tr>
<td>20+4</td>
<td>+15</td>
<td>65.0±10.2 (6)</td>
<td>+0.6±6.8 (6) §</td>
</tr>
</tbody>
</table>

Incubation conditions are described in the text. Results from three different batches are pooled for compact presentation; the standard deviation of large \(n\) includes batch variation. Only differences between paired muscles in a single batch are shown.

* Determined from the relative activity of Na and the Na concentration of the medium.
‡ Change from condition in first row.
§ This pair difference is not significant, but the difference calculated as exchanging cell Na (mM/kg cell water) is significant.

The moderate cation shift in K-free solution under these incubation conditions was reversed by subsequent incubation, still in the cold, in normal Ringer solution (2 mM K); comparison of paired muscle rings, shown in the last column of Table II, indicated significant Na extrusion and K uptake returning these contents to normal levels. The time course of tracer loss also was compared under these conditions: muscle rings were loaded in K-free ²²Na solution and washed out into either normal or K-free solution, at 5°C. In normal solution, the tissue was in an unsteady state. Curves obtained from one representative muscle of four in each experiment, all from the same frog batch, are shown in Fig. 3; the mean rate coefficient in normal solution, \(0.65 ± 0.07 (4) \) hr⁻¹, significantly exceeded that for loss into K-free solution, \(0.45 ± 0.06 (4) \) hr⁻¹. In an additional experiment, 25 min of washout into normal solution was interposed during washout into K-free solution, but the erratic changes in rate were not significant.
**Na Exchange in Ouabain-Ringer Solution**  
Preincubated stomach muscle rings treated with ouabain ($10^{-4} M$) gain large amounts of Na and lose equivalent amounts of K. These net fluxes have a positive temperature dependence between $5^\circ$ and $30^\circ$C, but the $Q_{10}$ of the component unidirectional fluxes could not be determined satisfactorily at $20^\circ$ and $30^\circ$C (35). In the present experiments, muscles were tracer-loaded in $^{23}$Na-ouabain solution for 20 hr in the cold, without preincubation, and washed out at $5^\circ$ or $15^\circ$C.

The time course of $^{23}$Na loss in ouabain-treated tissue is illustrated in Fig. 4. Again, each curve represents one of four in each experiment, all from the same frog batch. The rate coefficients were much smaller than in K-free solution, and the later tracer loss approximated a single exponential function at $15^\circ$ as well as $5^\circ$C. As in the other solutions, rate tended to decrease slightly
with time, even at 5°C. Mean rate coefficients for this pair of experiments were 0.65 ± 0.11 (4) hr⁻¹ at 15°C and 0.20 ± 0.03 (4) hr⁻¹ at 5°C, with a Q₁₀ of 3.2. Thus even under conditions of appreciable inhibition of active Na transport and high internal Na levels, tracer loss showed a high degree of temperature dependence. Some efflux experiments at 5°C were continued for 3 or 4 hr; these results resembled the curves shown, except that the tendency for rate to decrease slightly with time could be seen more easily. In two experiments, muscles loaded in the same way were washed out into unbuffered

Li-ouabain Ringer solution (LiCl substituted for NaCl and NaHCO₃, gassed with air). At 15°C, tracer loss was very similar to the average results in Na; no Na experiments were performed in that batch. At 5°C, the curves of activity remaining during a 4-hr washout were superposable on those from washout in Na in the same batch. The rate coefficients in this batch were smaller than average. Under these conditions in Li (5°C), total Na fell from 94.0 ± 7.0 (6) to 36.6 ± 4.8 (6) mM/kg, and total K fell from 22.4 ± 8.0 (6) to 15.5 ± 4.7 (6) mM/kg. The decrease in K between paired muscles, 6.8 ± 4.2 (6) mM/kg, was significant, an effect not found in Na-ouabain solution under these conditions (see below). The cellular Na + K loss was about 41 mM/kg cell water, when extracellular Na loss and cell water were estimated from the sucrose-¹⁴C space under comparable conditions.

The rate coefficients from these and additional experiments are collected in Table VI. Averaged over all experiments, the rate coefficients in ouabain were almost half those in K-free solution at 15°C, and less than half at 5°C. When
comparisons were made within the same frog batch, the ratio was 0.6 at both 5°C (batch 11/11) and 15°C (batch 12/28).

As with other loading conditions, the net ionic changes in the tissue during loading and washout in 10⁻⁴ M ouabain solution were assessed. Table IV shows that a large cation shift occurred which was essentially complete when tracer efflux was measured. Paired muscles were incubated for 20 hr in the cold, and then one of each pair was incubated for an additional period in

| Table IV |
| CATION CONTENTS IN OUABAIN RINGER SOLUTION UNDER WASHOUT CONDITIONS |

<table>
<thead>
<tr>
<th>Content</th>
<th>NR, 3 hr</th>
<th>OR, 20 hr 5-6°C</th>
<th>OR, 20 + 2 hr</th>
<th>OR, 20 + 4 hr</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mM/kg wet wt.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Na</td>
<td>53.7±4.2 (6)</td>
<td>93.2±7.1 (12)</td>
<td>95.0±11.5 (6)</td>
<td>95.0±11.5 (6)</td>
</tr>
<tr>
<td>Change in Na</td>
<td>39.5±4.2 (6)</td>
<td>+2.6±7.2 (6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>K</td>
<td>64.6±4.1 (6)</td>
<td>23.2±8.0 (12)</td>
<td>22.8±8.0 (6)</td>
<td></td>
</tr>
<tr>
<td>Change in K</td>
<td>41.4±4.1 (6)</td>
<td></td>
<td></td>
<td>+1.2±4.3 (6)</td>
</tr>
<tr>
<td>Exchanging Na</td>
<td>49.3±4.9 (12)</td>
<td>91.3±7.3 (49)</td>
<td>84.6±8.4 (4)</td>
<td>87.0±5.2 (6)</td>
</tr>
<tr>
<td>Change in Na</td>
<td>4.0±4.9 (12)</td>
<td>98.3±8.1 (5)§</td>
<td>96.1±4.4 (6)§</td>
<td>+3.9±6.3 (5)§</td>
</tr>
</tbody>
</table>

Results of experiments with four different frog batches were pooled. Variation is expressed as the SD. In four additional batches, mean exchanging Na ranged from 45.3 to 56.1 mM/kg wet wt after 3 hr in normal Ringer (NR) at room temperature and from 86.5 to 96.5 mM/kg wet wt after 20 hr in ouabain-Ringer (OR) at 5-6°C. Ouabain concentration was 10⁻⁴ M. Change in content is the change from column 2, and indicates the difference between paired muscles from the same frog batch.

* 1-hr preincubation plus 2 hr in unlabeled or labeled NR, all gassed.
‡ Incubated as in column 2, plus additional time in gassed solution at 5°C, unless otherwise noted.
§ Additional incubation time in gassed solution at 15°C.

gassed ouabain solution, simulating washout conditions. None of the pair differences was statistically significant, and the tissue was in a steady state.

**Tracer Efflux after Loading with Preincubation and Stirring** When muscle rings were preincubated in gassed normal solution and then gassed during tracer loading, Na efflux at 5°C usually showed a pronounced alternation of fast and slow rates. That is, semilog or linear plots of tracer remaining against time showed several inflections. Initial measurements in normal solution were made in this way, and the effect was tentatively related to rapidity of exchange of the very small cellular component. However, later experiments with these loading conditions showed a similar effect with the larger slower cellular component.
components in K-free or ouabain solutions, using frog batches in which smooth curves resulted with the usual loading procedure. The rate coefficients of the slow phases were usually similar to those of the smooth curves from tissue which had not been preincubated or gassed during loading. The loading solutions and the washout procedures used were the same in all cases; no methodological difference during the efflux period itself appeared to account for the complex time course of tracer loss. Separate experiments indicated that in each solution the tissue was in a steady state, over-all, though possibly slightly different than in the previously described experiments. In the steady state, tracer in each compartment is a monotone decreasing function (19), and no combination of compartment sizes and exchange rates can cause such efflux variations. Thus changes in rate coefficients during the washout period are indicated, perhaps due to transient variations in efflux, influx (backflux), or extracellular diffusion.

Estimation of Total Na Efflux. The rate coefficients of $^{22}$Na loss from nonpreincubated muscle rings loaded without gassing were used to estimate transmembrane efflux. The values of intracellular Na concentrations required for flux estimates were derived from the size of the slow $^{22}$Na efflux component when feasible, and from the simultaneously determined $^{22}$Na and sucrose-$^{14}$C contents of tissue from the same batch loaded under the same conditions. Total water content was determined periodically.

Sucrose-$^{14}$C space was employed as an approximation of extracellular Na and extracellular water on the basis of two types of evidence. Most important was the reasonably good agreement in ouabain and K-free solutions between the exchanging cellular Na component estimated from the sucrose-$^{14}$C space with the size of the slow component of $^{22}$Na efflux at 5 °C. In Table V the intercepts of the slow component of relative activity $^{22}$Na remaining, extrapolated to zero washout time, are compared with the differences between relative activity $^{22}$Na and sucrose-$^{14}$C space ("cellular $^{22}$Na space") in tissue from the same batch and from paired muscles. The two methods of estimation usually gave similar cell Na values, over a wide range of Na contents, when the later loss of tracer was approximately exponential and sufficiently slower than the earlier, presumably extracellular, loss. In normal Ringer solution the slow component intercept at 5°C was very small, and in these batches the difference between relative activity $^{22}$Na and sucrose-$^{14}$C space was not significant under these loading conditions. In an earlier uptake experiment a mean difference of 6.8 ± 1.4 (6) % R. A. was observed. Within the accuracy of estimation by the double-label uptake method, the cellular exchanging Na in these batches could be nonzero but small. If sucrose-$^{14}$C space in these measurements represents an overestimate of extracellular Na, the amount of overestimation must be very small by the criterion of the size of the washout slow component (see Discussion).
Under most experimental conditions, no significant changes in sucrose-14C space were found, although mean values were somewhat larger than in previous studies (2, 35). Sucrose-14C uptake measurements were collected from all experiments connected with this series; preincubation per se and the type of (isotonic) incubation solution caused no significant differences, and the data were pooled on the basis of incubation time and whether continuous stirring was maintained or not. In the few frog batches in which the difference between 22Na and sucrose-14C content was not significant in muscles loaded as

**Table V**

<table>
<thead>
<tr>
<th>Incubation solution</th>
<th>Intercept of slow component</th>
<th>(22Na-sucrose-14C) space Paired muscles</th>
<th>(22Na-sucrose-14C) space Batch mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ouabain-R</td>
<td>45.5±3.2 (4)</td>
<td>47.9±6.6 (4)</td>
<td>45.7±6.7 (21)</td>
</tr>
<tr>
<td></td>
<td>34.4±2.9 (4)</td>
<td>41.2±4.3 (4)</td>
<td>38.3±4.4 (8)</td>
</tr>
<tr>
<td></td>
<td>34.6±3.2 (4)</td>
<td>37.0±6.1 (4)</td>
<td>37.5±5.7 (10)</td>
</tr>
<tr>
<td></td>
<td>36.1±11.2 (4)</td>
<td>27.8±5.7 (4)</td>
<td>31.2±5.7 (10)</td>
</tr>
<tr>
<td>K-free R</td>
<td>19.6±2.2 (4)</td>
<td>—</td>
<td>10.4±1.9 (12)</td>
</tr>
<tr>
<td></td>
<td>10.6±1.7 (4)</td>
<td>—</td>
<td>11.3±4.5 (12)</td>
</tr>
<tr>
<td></td>
<td>17.4±2.4 (4)</td>
<td>16.6±4.4 (4)</td>
<td>13.4±4.9 (16)</td>
</tr>
<tr>
<td></td>
<td>12.3±1.7 (2)</td>
<td>6.2±2.2 (4)</td>
<td>10.4±3.6 (16)</td>
</tr>
<tr>
<td></td>
<td>17.8±2.3 (4)</td>
<td>11.8±3.9 (4)</td>
<td>13.1±3.2 (10)</td>
</tr>
<tr>
<td>Normal R</td>
<td>2.8±0.4 (4)</td>
<td>0.0±1.6 (4)</td>
<td>0.0±1.1 (14)</td>
</tr>
<tr>
<td></td>
<td>2.8±0.8 (4)§</td>
<td>-0.4±1.8 (4)§</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>2.8±0.6 (4)§</td>
<td>0.00 (4)</td>
<td>0.0±1.7 (26)</td>
</tr>
</tbody>
</table>

Tracer-loading conditions are described in the text. Variation is expressed as the SD.
* Sucrose-14C uptake was measured simultaneously with 22Na uptake.
† Paired muscles designate rings from the same stomach as rings used for efflux measurements, and loaded with tracer at the same time.
§ These muscles were preincubated before loading with tracer.

for normal washout (described above), sucrose-14C space was significantly larger in nonpreincubated tissue loaded in the cold in a desiccator (as for washout) than in paired muscles which were preincubated and gassed continuously. These results were unusual and not borne out in the collected data. Mean values of sucrose-14C space after 2–26 hr in stirred or unstirred solutions ranged only from 40.2 to 43.8 % space (total n = 465). In normal solution gassed at room temperature, the mean space after 2 hr was 40.2 % (n = 118); gassed at 5°C, the mean space was 42.0 % (24) after 2 hr. After 20 hr of incubation in the cold, the space found with continuously stirred solution was 42.2 % (n = 40), and the space with desiccator incubation was 42.7 % (191).
After 24 hr of incubation, essentially identical results were obtained. Additional means at 4, 6, 16, 22, and 26 hr in the cold showed no differences. It is clear that on the whole, sucrose-$^{14}$C space did not vary significantly with loading conditions, and sucrose uptake did not increase significantly with prolonged incubation.

Estimates of total Na flux at 5°C and 15°C are given in Table VI together with the estimated ionic intracellular Na concentrations and rate coefficients for $^{22}$Na loss from which they were made. Intracellular Na concentrations were based on an average of the size of the slow component of $^{22}$Na loss and the difference between $^{22}$Na and sucrose-$^{14}$C uptake, for washout experiments at 5°C in K-free and ouabain solution and at 15°C in ouabain solution. The tracer uptake difference alone was used for experiments at 15°C in K-free solution, since the washout kinetics did not give an unequivocal value for the size of the cellular component. For the washout experiments in normal Ringer solution, the best estimate seemed to be the size of the slow component of tracer loss at 5°C; i.e., the intercept of the later exponential, applied to experiments at both temperatures (see Discussion). (The extracellular water in this case was calculated from the difference between total relative activity of $^{22}$Na and the cellular component.) Since it has been shown that tracer efflux was measured under approximately steady-state conditions in all three solutions, the apparent Na efflux in mm/kg cell water per hr is the simple product of Na and $k_\alpha$. It may be noted here also that this steady-state efflux must equal the influx.

Table VI shows that sodium efflux at 5°C was the same in ouabain and in K-free solution; the rate coefficients differed more than twofold between these conditions, but the intracellular Na varied reciprocally. At 15°C, these fluxes were fairly similar; if the intermediate phase of the more complex tracer loss in K-free solution at 15°C were used to estimate rate, instead of the slowest phase, these fluxes would be very similar. In normal solution, on the other hand, the total Na flux appeared to be markedly lower than in the inhibitors: at 5°C, normal efflux was only 1/5 of the other estimates, and at 15°C only 1/10 of the efflux in ouabain. From the steady-state conditions, it follows that influx also was apparently smaller in normal solution than in the inhibitor solutions (see Discussion).

Comparison of the upper and lower parts of Table VI shows that Na efflux in each solution had a large temperature coefficient, as must follow from the $Q_{10}$ of the rate coefficients and the steady-state conditions. The average ratio of efflux at 15°C to that at 5°C was 3.7 in ouabain, 2.8 in K-free solution, and 1.9 in normal Ringer solution. The steady-state conditions again indicate that the large temperature dependence must have been a feature of the influx of Na as well.
In Table VI the steady-state flux is estimated both as mM per kg cell water per hr and as pmoles per cm² per sec. Surface flux was calculated using a volume-to-surface ratio of 1.6 μ; this was obtained from the mean fiber diameter, measured at 5.7 μ and corrected for shrinkage during fixation to 6.3 μ.

**Table VI**

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Solution</th>
<th>Exchanging Na⁺</th>
<th>Na efflux</th>
<th>Na efflux</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>mM/kg c.w.</td>
<td>hr⁻¹</td>
<td>mM/kg c.w. per hr</td>
</tr>
<tr>
<td>5°C</td>
<td>Normal</td>
<td>6.8⁺</td>
<td>0.44±0.04 (8)</td>
<td>3.0 (8)</td>
</tr>
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<td></td>
<td>7.1⁺</td>
<td>0.61±0.03 (4)</td>
<td>4.3 (4)</td>
<td>0.19 (4)</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>6.9</td>
<td>0.50 (12)</td>
<td>3.4 (12)</td>
</tr>
<tr>
<td></td>
<td>K-free</td>
<td>31.6§</td>
<td>0.51±0.06 (11)</td>
<td>16.3 (11)</td>
</tr>
<tr>
<td></td>
<td>47.1</td>
<td>0.32±0.02 (4)</td>
<td>15.1 (4)</td>
<td>0.66 (4)</td>
</tr>
<tr>
<td></td>
<td>45.2§</td>
<td>0.40±0.03 (4)</td>
<td>18.0 (4)</td>
<td>0.79 (4)</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>39.4</td>
<td>0.45 (19)</td>
<td>16.7 (19)</td>
</tr>
<tr>
<td></td>
<td>Ouabain</td>
<td>96.9§</td>
<td>0.20±0.03 (12)</td>
<td>19.4 (12)</td>
</tr>
<tr>
<td></td>
<td>96.3</td>
<td>0.12±0.03 (3)</td>
<td>11.6 (3)</td>
<td>0.51 (3)</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>96.8</td>
<td>0.18 (15)</td>
<td>17.8 (15)</td>
</tr>
<tr>
<td>15°C</td>
<td>Normal</td>
<td>6.8⁺</td>
<td>0.95±0.08 (8)</td>
<td>6.5 (8)</td>
</tr>
<tr>
<td></td>
<td>K-free</td>
<td>33.3</td>
<td>1.40±0.18 (4)</td>
<td>46.6 (4)</td>
</tr>
<tr>
<td></td>
<td>44.6</td>
<td>1.04±0.07 (4)</td>
<td>46.4 (4)</td>
<td>2.04 (4)</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>39.0</td>
<td>1.22 (8)</td>
<td>46.5 (8)</td>
</tr>
<tr>
<td></td>
<td>Ouabain</td>
<td>100.1</td>
<td>0.64±0.09 (6)</td>
<td>66.3 (6)</td>
</tr>
</tbody>
</table>

The conditions of the experiments and calculation of the estimates are described in the text. Results of experiments with 10 different batches of frogs were pooled when differences did not appear significant. Variation is expressed as the sn.

* Estimates were averaged from Na and sucrose-[¹⁴C] space and from the intercepts of the slow component at 5°C (and 15°C in ouabain), unless otherwise indicated.
+ Estimated from the slow component intercepts at 5°C.
§ Uptake experiments gave erratic values in some batches; these values were not used to calculate Na⁺.

(see Methods). A ratio of 1.5 μ has been estimated for the guinea pig taenia coli (14). At 15°C, the fluxes in ouabain and K-free solution expressed as turnover in the cell water were large, 46–66 mM/hr, while the apparent flux in normal solution was only 6–7 mM/hr. However, the estimated fluxes in terms of exchange per unit surface area were small in all cases, ranging from about 3 pmoles/cm² per sec in ouabain down to only 0.3 pmole in normal solution at 15°C. At 5°C, surface flux was well below 1 pmole in all cases.
DISCUSSION

The present studies define several characteristics of Na transport in this smooth muscle which resemble those in skeletal muscle, and others which do not. The sodium efflux mechanism has a large temperature dependence, and is inhibited by removal of external K and markedly by the cardiac glycoside ouabain. Sodium influx must also have a large temperature dependence, and thus the mechanism of passive movement is not ordinary simple diffusion. Sodium flux per unit surface area, reflecting "permeability," is small, at least as low as in resting frog skeletal muscle, and probably lower. However, due to the fiber surface:volume ratios, the rate of turnover in the cell water is at least as large, and probably larger than in skeletal muscle. Before analyzing these results and the transport mechanisms which may underlie them, the flux estimation per se will be discussed.

Evaluation of Na Efflux

Under suitable loading conditions, the later loss of $^{23}$Na in the three different steady states approximated a simple exponential time course at 5°C, and in ouabain-treated tissue at 15°C. In normal or K-free solutions at 15°C, a faster middle phase occurred, which could reflect additional components. In the steady state, extrusion probably was not actually decreasing as a function of time. A dispersion of fiber sizes or permeabilities seems unlikely because this would cause similar effects at 5°C and in ouabain. One possibility is that the fibers are not homogeneous with respect to their Na pump capacity; at the higher rates of pump activity in K-free and normal solutions at 15°C, such dispersion would be more apparent. This factor is especially plausible in K-free solution, since extracellular K is unlikely to fall to zero in the more central regions of the tissue (17, 21); we have recently found that the net cation shifts occur largely in more peripheral fibers (unpublished data). The slowest component at 15°C tended to be more linear and larger, and was used to give a minimum estimate of transmembrane exchange rate. If the faster efflux component in normal or K-free solution represents a group of more active fibers, the average rate must be somewhat higher at 15°C.

A further point to be considered is the effect of extracellular diffusion delays. For frog skeletal muscle, Harris and Burn (17) and Keynes (27) made approximate corrections for diffusional and backflux effects for $^{4}$K exchange, but they took Na rate constants directly, in some cases empirically utilizing an early faster phase of tracer loss. In the present experiments, at 5°C, the "exponential" portion of extracellular loss had a rate coefficient 50-60 times the cellular rate coefficient in ouabain solution, and 20-25 times $k_{o}$ $^{23}$Na in normal and K-free solutions. At 15°C, however, the ratio was 10 or less in K-free and normal solutions with the slowest phase used for reference. More-
over, the early loss of tracer may not completely describe the decline of extracellular specific activity, since sucrose washout is heterogeneous (3). At 15°C, especially in normal solution, some recirculation of tracer within the tissue compartments seems likely; this would mean that the true rate coefficient is higher than the observed value. In ouabain at 15°C, and in all cases at 5°C, extracellular loss was sufficiently faster than cellular loss to minimize back-flux effects; with the possible exception of normal solution, the observed rate coefficients should be good approximations of the transmembrane exchange.

Intracellular Na was estimated from the size of the slow component of tracer loss and from the difference between 2Na and sucrose-14C uptake under equivalent loading conditions. The use of sucrose to measure extracellular space has been questioned (2), but considerable evidence suggests that smaller molecules reflect more accurately the extracellular water available to ions than do larger probe molecules (15). This conclusion is supported by the present results. Sucrose-14C equilibrated fairly rapidly with a limited fraction of the tissue water, and the cellular 2Na estimated using this fraction was close to that estimated from the slow component of 2Na efflux at 5°C (Table V). According to Huxley's analysis (24), the intercept of the slow component of remaining activity tends to overestimate intracellular tracer at the start of washout, by a fraction which depends on the relative rates of fast and slow exponentials. Thus the intercept represents an upper limit of exchanging cell Na. In ouabain and K-free solutions at 5°C, Na, estimated with the sucrose space corresponded with this upper limit, and estimates using the appreciably smaller extracellular space of a large probe molecule would exceed it. At 5°C, Huxley's treatment indicates that the true value should be about 96% of the intercept in ouabain, and about 90% of the intercept in K-free and normal solutions. The similarity sometimes seen between 2Na and sucrose-14C uptake in normal solution under washout conditions (overnight loading) may be due in part to a slight real reduction in Na (Table I); in any case, the 2Na washout intercepts were so small (2-3% R.A.) that narrow limits are set on both Na, and the possible error in 2Na-sucrose-14C uptake in a few batches. Therefore, under washout conditions which give an independent estimate of Na, the sucrose-14C space approximates the extracellular space for Na in this tissue.

Temperature Dependence of Na Flux The large temperature dependence of Na efflux observed between 5 and 15°C is consistent with the effects of metabolic inhibitors on this tissue (1) and the metabolic dependence of active Na transport in other smooth muscle (7, 10, 33). This is not evidence by itself, of course, for active transport. The largest Q10, 3.7, was seen in ouabain-treated tissue, in which active transport is partially inhibited (13), and the
smaller $Q_{10}$, 1.9, was seen in normal tissue, in which efflux should be predominantly active. A genuine increase in $Q_{10}$ with inhibition would not be anticipated. However, efflux at 15°C (and therefore $Q_{10}$) should be estimated most accurately in ouabain-treated tissue, and in fact the temperature dependence observed in the three solutions varied as the ratios of extracellular to cellular rates of tracer loss at 15°C. For this reason, the smaller apparent $Q_{10}$ in normal solution suggests again that the true $k_e$ at 15°C may be larger than the observed value. In toad stomach muscle sheets, $^{22}$Na loss in normal solution is much slower, with a half-time of 1.3 hr at 15°C, and the temperature dependence ($-2$ to $15°C$) is almost as high as that seen here in ouabain (6). The large $Q_{10}$ probably extends well beyond 15°C; previous minimum estimates of Na flux in ouabain at 30°C (35) were more than three times the present values at 15°C.

The ionic stability of this tissue in the cold implies a priori a similar $Q_{10}$ for influx and efflux; this was indicated also by the positive $Q_{10}$ of the net flux in ouabain shown previously (35). In the present experiments, since efflux was measured under steady-state conditions, the high temperature dependence applies also to influx. The large $Q_{10}$ of influx, corresponding to an activation energy of 20.8 kcal/mol in ouabain-treated tissue, must reflect mechanistic differences between passive membrane permeation and diffusion in bulk solution. Three types of process might underlie this temperature dependence. (a) Diffusion through narrow membrane channels filled with highly structured water could vary with the anomalous viscosity of the “ice-like” water. In toad bladder, for example, both $T_2$O diffusion and bulk water flow have a high activation energy in the absence of vasopressin (18). (b) Diffusion through narrow channels of critical geometry could vary with the “melting” of quasi-crystalline regions in the membrane matrix. This effect of thermal motion characterizes physicochemical changes in gels (20) and phospholipid films (9), but must be more complex in heterogeneous natural membranes. (c) Transport by way of a binding reaction, possibly to a carrier molecule, could vary with the activation energy of the reaction or the carrier movement. Both facilitated diffusion of sugar (34) and passive Na movement (12) in red blood cells have a high $Q_{10}$. The data do not exclude any of these alternatives, but neither the first nor the second alone seems likely to account for the observed features of Na influx. A determinant role for the viscosity of membrane water is not consistent with the apparent increase in influx with Na$_i$ (see below). An increase in the ratio Na$_i$/K$_i$ might change the ionic composition of aqueous membrane channels correspondingly, but this would tend to increase water structure and decrease $D_{Na}$ (16). In the second case, the required thermal change in channel geometry seems quantitatively improbable. Since the driving force in ouabain was the same, the $Q_{10}$ for influx would be proportional to the ratio of diffusion coefficients and thus to the
ratio of channel cross-sectional areas; i.e., $D_{r_2}/D_{r_1} = r_2^2/r_1^2$. Correction of
the measured $Q_{10}$ of 3.7 for free diffusion leaves a ratio of 3 to be accounted
for by change of radius; if the $Q_{10}$ is constant over the range 5 to 30°C, the
ratio of $r^2$ required would be $\sim 15.5$. With a minimum radius at 5°C of
1.78 Å (the hydrated radius of Na), this gives a minimum radius at 30°C
of 6.9 Å. Channels of this size seem unlikely because sucrose (equivalent diffusion
radius 4.4 Å) does not permeate at 30°C (35). An implicit assumption
in this treatment, however, is that temperature does not affect a spectrum of
channels asymmetrically. Several types of carrier mechanism have been
described, and a little indirect evidence bears on these. The large $Q_{10}$ in
ouabain makes Na-Na exchange through the pump, i.e. the "ouabain-
sensitive Na-Na exchange" seen in red cells (11) unlikely, but further infor-
mation is needed to exclude a pump process in this tissue. The results to date
on $^{22}Na$ efflux into Li- and Na-ouabain solutions in the present and previous
work (35) suggest that ouabain-insensitive Na-Na exchange is not important
here. In the taenia coli also, tracer Na loss is hardly altered in Li (4). Another
possible carrier is a mechanism associated with electrical activity; in squid
axons, the conductances involved in the action potential have a large $Q_{10}$
(22). Sodium has been related to slow wave activity in other visceral smooth
muscle; while recent observations suggest that calcium flux may be important
in spike generation (5, 31, 32), Na movement is not excluded. In arterial
muscle, Na flux dominates spike generation (25, 26). Associated flux and
electrical measurements would clarify the role of this mechanism in stomach
muscle. In theory at least the contributions of these possible influx mecha-
nisms might be assessed with a series of nonelectrolyte probe molecules.
Influx would have a high $Q_{10}$ in the first two cases, but if the state of mem-
brane water were the primary determinant, the limiting molecular size for
permeation would be independent of temperature. A low $Q_{10}$ for nonelec-
trolyte entry would suggest a dominant role for a binding reaction specific
for cations if not for Na.

Alterations in Na Flux At 5°C, the reductions in rate coefficient for
tracer loss in K-free and ouabain solutions are consistent with the specific
effects of K and cardiac glycosides on active Na transport observed in other
tissues (13). These reductions directly relate to efflux the net changes in
Na and K contents which have been observed in this tissue (1, 35) and in the
taenia coli (8). The effect of K removal on $k_{e^{Na}}$ was much smaller than that
of ouabain; this is in accord with the finding that in K-free solution the
steady state was reached at cell Na and K levels which were much closer
to normal. The actual extracellular K concentration within the tissue block
probably does not fall to zero, as mentioned above. In the taenia coli, only a
small effect of K on tracer Na efflux could be detected with temperature
manipulation (4). In the present steady-state data, the extent of inhibition
cannot be specified until the functional relation between Na efflux and Na, has been described quantitatively, without pump inhibition as a variable. The steady-state tracer rate coefficient is necessarily first-order, while efflux dependence on concentration is probably more complex. In frog skeletal muscle, efflux varies with (Na0) at low Na0, linearly at moderate Na0, and not at all at high Na0, when pump efflux becomes constant (28, 30). In stomach muscle, if efflux varied with (Na0) up to 10–12 mM and then linearly, the total efflux and ko Na (= M0/Na0) in normal solution would be considerably larger than the values observed in K-free solution. Then the inhibition of Na extrusion in K-free solution would be greater than indicated by the differences in ko Na at 5°C. If pump efflux became constant at high Na0, as in frog skeletal muscle, ko Na would be lower, but not as low as the value observed in ouabain. At 15°C, ko Na in normal solution actually appeared to be smaller than in K-free solution, again suggesting that backflux effects are appreciable in normal solution.

Total Na flux was similar in K-free and ouabain solutions, especially at 5°C, while flux in normal solution was only one-fifth of these at 5°C and one-seventh–one-tenth at 15°C. Factors which might contribute to the differences between normal and inhibited fluxes are (a) dependence of active Na extrusion on Na0, (b) variation of influx with Na0, and (c) underestimation of normal flux, particularly at 15°C. The net operation of active transport at 5°C is displayed by the reversal of K-free effects in normal solution, and by the loss of additional K from ouabain-treated muscle in Li solution. The amounts of steady-state active transport cannot be quantitated from the present data, but should equal the difference between the passive and total efflux. The passive unidirectional flux ratios may be roughly predicted from Ussing's equation (37), using literature values for E and the present estimates of Na0. The principal assumption made in derivation and application of this equation is that the passive flux asymmetry is due entirely to asymmetry of the electrochemical driving forces, while ion mobility is the same in both directions; the molecular details of passive movement are not specified. Normal E in frog stomach muscle is 50–60 mV (29), similar to that in the taenia coli (7). After ouabain treatment or temperature reduction (with large cation shifts), E in the taenia coli drops to 20–30 mV (4, 8). The passive flux ratios calculated with these values for E suggest that essentially all the efflux in normal solution, and more than half the efflux in ouabain, may be active. Most of the efflux in K-free solution, even assuming depolarization, may be active. Mullins and Frumento (30) showed that the pump efflux in the frog sartorius (20°C) increases eight times from 8 to 30 mM Na. An analogous concentration dependence of pump activity in stomach muscle could explain the higher total Na efflux in partially inhibited tissue at 5°C.
At 15°C, this explanation seems inadequate, and a higher true value of normal flux again seems indicated.

From the steady-state relation, normal influx at 15°C is an equally small fraction of that in inhibitors, although the electrical driving force must have been larger in normal than in inhibited tissue. Mullins and Frumento (30) suggested that \( P_{Na} \) increases 2.6 times in skeletal muscle with high Na\(_i\). A 7- to 10-fold increase in permeability in stomach muscle seems improbably large; a simple increase in pore radius, specifically, is unlikely, since the required radius would exceed that of sucrose, as shown previously in relation to temperature dependence. Consideration of influx thus also suggests some underestimation of normal flux at 15°C. In skeletal muscle loaded with tracer in K-free solution, Na-Na exchange might contribute to the increased exchange rate; in stomach muscle, however, unless the influx in K-free and in ouabain solutions at 5°C is identical fortuitously, Na-Na exchange seems unlikely to play a major role. These fluxes in inhibitors at 5°C therefore imply that \( P_{Na} \) may increase markedly from 7 to 40 mM Na\(_i\), and then remain nearly constant. Normal flux at 5°C is likely to be estimated fairly closely, and part of the effect of Na\(_i\) on influx appears to be real.

The Size of the Transmembrane Na Flux. The observed fluxes serve as limiting values for the normal flux in stomach muscle. The estimates in normal solution form a lower limit, since if tracer backflux is appreciable, particularly at 15°C, the rate of transmembrane efflux may in fact be higher than observed. A major contribution of Na-Na exchange to this efflux seems unlikely, as discussed above. The estimates in inhibitors represent an upper limit, due to the dependence of efflux (and influx) on Na\(_i\). Within these limits, the turnover of Na in the cell water can be very large. The experimental estimate in normal solution at 15°C, 6.5 mM/kg cell water per hr, falls within the range of 2 to 10 mM observed in frog skeletal muscle; however, tracer backflux is most likely to affect this estimate. If the normal flux at 5°C is extrapolated to 15°C using the \( Q_{10} \) observed in ouabain (and in normal toad stomach muscle), the normal turnover in the cell water at 15–20°C would be 13–23 mM/hr. The turnover in inhibited tissue at 15°C was 5–10 times that in normal skeletal muscle, and in K-free solution, at least, a large fraction of the efflux must be active extrusion. From these considerations, it seems likely that the active Na extrusion involved in normal Na turnover at room temperature is at least twice as large as that in frog skeletal muscle, and possibly larger.

Due to the large surface:volume ratio of these cells, however, all estimates of flux per unit surface area were strikingly small. The highest value, 2.9 pmoles/cm\(^2\) per sec in ouabain at 15°C, was at the bottom of the range of literature values for frog skeletal muscle at 15–20°C, about 3–10 pmoles/cm\(^2\)
per sec (21, 23, 27), while the estimate in normal solution was an order of magnitude smaller. Comparably low Li permeability is suggested by the rate of net Na + K loss into Li-ouabain solution at 5°C; if equivalent Li entry is assumed, it is then somewhat smaller than Na flux in that batch under these conditions. In frog skeletal muscle, permeability to Na and to Li is similar (28). In the taenia coli, the recent Na flux estimate of 30 pmol/cm² per sec at 35°C (4) resembles the flux seen here in ouabain, with a Q10 of about 3 applied. Resting Na flux in sheep arteries recently has been estimated at only 0.18 pmol/cm² per sec at 35–39°C (26). These estimates, which involved analysis and subtraction of extraneous flux components, are in qualitative accord with the present findings. The surface permeability of vertebrate smooth muscle to Na appears to be as low or lower than in skeletal muscle, and the low membrane potential in smooth muscle may be related to unusually low K permeability.

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BIBLIOGRAPHY