The Effect of Calcium on Sodium Transport by Frog Skin

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ABSTRACT Calcium added to the solution bathing the outside of isolated frog skin caused a reversible decrease in net sodium transport across the skin. At constant sodium concentration, the inhibition of transport increased with increasing calcium concentration, but approached a limiting value. This maximum degree of inhibition was found to depend on sodium concentration; sodium transport could be inhibited by 60 per cent at 96 mM sodium, but by only 18 per cent at 19 mM sodium. The relative effectiveness of a given calcium concentration was also greater the higher the sodium concentration. The unidirectional flux of chloride across the short-circuited skin was decreased by calcium to approximately the same degree as active sodium transport. The results have been interpreted in terms of a relatively non-specific decrease in permeability of the outward facing membrane of the transporting cells. The resulting decrease in sodium permeability apparently causes a decrease in active sodium transport by reducing the availability of sodium to the trans- porting system.

Calcium has a marked influence on the permeability of excitable membranes and appears to be associated in some way with the permeability changes occurring during activity (1, 2). It also influences properties of non-excitable membranes whose primary function is net transport of ions, as illustrated by the recent observation that elevated calcium concentration depresses net active sodium transport by rat small intestine (3). However, only limited information is available for such epithelial membranes, and it is not clear whether calcium affects active transport directly or indirectly through changes in membrane permeability. In excitable membranes, the major effect seems to be on permeability properties rather than on active transport. Several authors (1, 2, 4) have recently discussed the probable importance, in these effects, of calcium binding either at the surface or within the membrane. The
The present experiments represent an attempt to obtain further information for non-excitable membranes by studying the influence of calcium on active sodium transport across the isolated frog skin.

**METHODS**

The abdominal skin of *Rana temporaria* was dissected off and mounted as a flat sheet between lucite chambers. The area of skin exposed was 7.1 cm². The chambers and auxiliary equipment used, as well as the method of short-circuiting the skin, have been described in detail by Ussing and Zerahn (5). Net sodium transport across the skin, as measured by the short-circuit current (5), was determined as a function of the calcium concentration of the bathing solutions. Most experiments involved changes in calcium content of the solution bathing the outside surface of the skin while the inside solution had “zero” calcium concentration (no CaCl₂ added to the solution). The sodium and chloride concentrations in the solution bathing the inside of the skin were always the same as those in the outside solution.

The solutions bathing the skin were modifications of a frog-Ringer’s solution in which different fractions of the sodium chloride were replaced by choline chloride.

### Table 1a

<table>
<thead>
<tr>
<th>Solution</th>
<th>Sodium (mM)</th>
<th>Choline (mM)</th>
<th>Chloride (mM)</th>
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<tbody>
<tr>
<td>A</td>
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<td>17.4</td>
<td>113.0</td>
</tr>
<tr>
<td>B</td>
<td>85.0</td>
<td>28.4</td>
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</tr>
<tr>
<td>C</td>
<td>62.0</td>
<td>51.4</td>
<td>113.0</td>
</tr>
<tr>
<td>D</td>
<td>31.0</td>
<td>82.4</td>
<td>113.0</td>
</tr>
<tr>
<td>E</td>
<td>19.0</td>
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<td>113.0</td>
</tr>
<tr>
<td>F</td>
<td>6.0</td>
<td>107.4</td>
<td>113.0</td>
</tr>
</tbody>
</table>

Other ions present: K 2.0 mM, HCO₃ 2.4 mM

### Table 1b

<table>
<thead>
<tr>
<th>Solution</th>
<th>Sodium (mM)</th>
<th>Choline (mM)</th>
<th>Calcium (mM)</th>
<th>Chloride (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C (control)</td>
<td>62.0</td>
<td>51.4</td>
<td>0</td>
<td>113.0</td>
</tr>
<tr>
<td>C-1</td>
<td>62.0</td>
<td>47.8</td>
<td>2.8</td>
<td>113.0</td>
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<tr>
<td>C-2</td>
<td>62.0</td>
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<td>113.0</td>
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<tr>
<td>C-4</td>
<td>62.0</td>
<td>25.0</td>
<td>13.7</td>
<td>113.0</td>
</tr>
</tbody>
</table>

Other ions present: K 2.0 mM, HCO₃ 2.4 mM
The calcium content of these solutions was then altered by replacing choline chloride with calcium chloride so that changes in calcium concentration were made at constant sodium concentration. Five different sodium concentrations were studied. The composition of all control solutions (containing no calcium) is given in Table Ia, and the composition of the test solutions used at one sodium concentration is given in Table Ib. The skin was mounted in the control solution having the sodium concentration to be studied and was allowed to equilibrate for at least 1 hour before beginning the experiment. In some experiments, barium and magnesium were used instead of calcium. The effect of magnesium was also tested using a Ringer's solution in which all chloride was replaced by sulfate. The sodium and calcium concentrations of the solutions were checked periodically with a Zeiss flame photometer. The trisodium salt of ethylenediaminetetraacetic acid (EDTA) was added to the control solutions which bathed the outside of the skin to give a concentration of 0.5 mM. The presence of EDTA gave more consistent results and better recovery after treatment with calcium but appeared to have very little additional effect at this concentration. Solutions were changed rapidly by washing approximately 50 ml of new solution through the chamber. Control experiments indicated that more than 99 per cent of the old solution was replaced by this procedure.

Unidirectional sodium and chloride fluxes were occasionally measured in short-circuited skins using $^{22}$Na and $^{36}$Cl. Radioactive tracer was added to one side of the skin and samples were removed every 30 minutes from the solution on the opposite side for counting. The samples were dried on aluminum planchets and counted to at least 3000 counts with a Tracerlab thin window gas flow counter equipped with an automatic sample changer. Flux in the absence of calcium was first determined for two 30 minute periods; calcium was then added and flux determined for two more periods.

In one series of experiments, the effect of calcium on electrical potential difference and on total skin conductance was measured. A small direct current (usually 20 μamp) was passed through the skin, first in one direction and then in the other, and the accompanying changes in potential difference were measured. Conductance was calculated from the current and the change in potential difference, and conductance was taken as the mean of results obtained with current flow in the two directions. In each experiment, the effect of 8.3 mM calcium was tested at several sodium concentrations. Each test period was bracketed by control periods, during which the outside solution contained no calcium.

**RESULTS**

**The Effect of Calcium on Short-Circuit Current**

In early experiments, calcium was added to solutions bathing both sides of the skin. Short-circuit current usually decreased, but large transient effects were observed. Consequently, four experiments were carried out to examine separately the effects of 16 mM calcium on each side of the same skin. Sodium concentration was 80 mM in these experiments. The addition of calcium to the
outside solution caused an average decrease in current of 51 ± 5 per cent with no appreciable transient effect. Addition of calcium to the inside solution caused a large transient increase in current. After about 1 hour, the current had again fallen to a steady level averaging 107 ± 9 per cent of the control level. In all subsequent experiments, the effect of calcium added to the outside solution was studied keeping calcium concentration of the inside solution "zero."

As shown by the results presented in Fig. 1, bathing the skin for relatively long periods in calcium-free solutions had no adverse effects. In the experiment illustrated here, the skin from one frog was divided into two parts. One piece was mounted in Ringer's solution containing 1.0 mM calcium, and the other was mounted in the same solution but with no calcium. The results show that there was no marked difference between the two skins over a 5 hour period. The short-circuit current, open circuit potential difference, and conductance were all somewhat lower for the skin in the calcium-containing

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1 Errors are given as the standard deviation.
solution, but this is consistent with the effect of calcium on these properties as described below.

In a typical experiment, the effect on current of four different calcium concentrations was measured at constant sodium concentration. Since the effect was completely reversible, each test period could be bracketed by control periods in which the bathing solution contained zero calcium. The results of one experiment at 62 mM sodium are illustrated in Fig. 2. Addition of calcium resulted in a decrease in current which was greater the higher the calcium concentration. Similar results were obtained in experiments in which the order of testing different calcium concentrations was varied. The upward

![Figure 2](image-url)

**Figure 2.** Effect of varying calcium concentration on short-circuit current at constant sodium concentration (62 mM) in a single skin. The calcium concentration is shown at the top; calcium concentration of control solutions was nominally zero.

drift of control current with time shown in Fig. 2 was not consistently observed. A decrease in current with time also occurred frequently. As shown in Fig. 2, the decrease in current caused by calcium took place quickly and was usually 80 to 90 per cent complete in 10 minutes. The first reading of current was usually made 2 to 3 minutes after beginning to change solutions, but in some experiments, measurements were made as rapidly as possible. In 30 seconds, the current had dropped appreciably, indicating that there is relatively little delay in onset of the effect.

Experiments similar to the one illustrated in Fig. 2 were carried out at five different sodium concentrations using solutions A through E as given in Table I a. The results of this series of experiments testing the effect of calcium at constant sodium concentration may be compared in terms of the fraction of current inhibited. Fractional inhibition will be defined as $1 - I/I_0$, in which $I$ is current in the presence of calcium and $I_0$ is control current. Fractional inhibition of current will hereafter be denoted by the symbol $\alpha$. The use of this parameter eliminated differences due to variation in the level of control cur-
rent in different skins. In Fig. 3, $\alpha$ is plotted against calcium concentration for five different sodium concentrations. The curves represent the averaged results of four to six experiments at each sodium concentration. The fractional inhibition approaches a limit as calcium concentration is increased indicating that sodium transport cannot be completely inhibited by calcium. This limit is greater the higher the sodium concentration.

In order to evaluate these results, it is more convenient to consider only that portion of the current which can be affected by calcium. This requires evaluation of the limiting value of $\alpha$ at high calcium concentration. The curves of Fig. 3 suggest that $\alpha$ is a hyperbolic function of calcium concentration and, therefore, a plot of $1/\alpha$ against $1/[\text{Ca}]_0$ should give a straight line with an intercept equal to the reciprocal of the maximum value of $\alpha$. Fig. 4 shows the data from Fig. 3 plotted in such a manner; straight lines are obtained having slopes and intercepts which depend on sodium concentration. The values of maximum inhibition thus obtained in this series of experiments range from 18 per cent at 19 mM sodium to 60 per cent at 96 mM sodium.

Similar straight lines were obtained for individual experiments so that maximum inhibition could be determined for each experiment. The total current could then be divided into two fractions, one sensitive to and one insensitive to the addition of calcium. The fractional inhibition of the calcium-sensitive current ($\alpha'$) could then be calculated. $\alpha'$ may be defined by Equation 1.

$$\alpha' = 1 - \frac{I - I_{\alpha}}{I_0 - I_{\alpha}} = \frac{I_0 - I}{I_0 - I_{\alpha}}$$  (1)
in which $I_0$ is control current, $I$ is current at any calcium concentration, and $I_\infty$ is current which is insensitive to calcium. Equation 1 may be rearranged to yield

$$\alpha' = \frac{\alpha}{1 - I_\infty/I_0} = \frac{\alpha}{\alpha_m}$$

in which $\alpha_m$ is the maximum value of $\alpha$ obtained from intercepts of lines such as those shown in Fig. 4. Fig. 5 shows the average values of $\alpha'$ as functions of calcium concentration. The effect of calcium added to the outside solution is relatively greater the higher the sodium concentration of the solution. The data in Fig. 5 show that approximately five times as much calcium is needed to give the same inhibition of calcium-sensitive current at 19 mM sodium as at 85 mM sodium. There are, thus, two differences, probably interrelated, in the effect of calcium at different sodium concentrations. First, the higher the sodium concentration, the larger is the fraction of the total sodium transport which is sensitive to calcium. Second, when only that portion of sodium transport which is sensitive to calcium is considered, a given calcium concentration causes a larger inhibition the higher the sodium concentration.

The Effect of Calcium on Unidirectional Ion Fluxes

Ussing and Zerahn (5) have shown that the short-circuit current of the frog skin is equal to the net sodium transport under normal conditions. Since it is possible that calcium disturbs this equality between current and sodium transport, some experiments were carried out to measure changes in unidirectional
sodium fluxes. The effect of 8.3 mM calcium was tested at two sodium concentrations (96 and 19 mM). The results are summarized in Table II. The addition of calcium to the outside solution resulted in a marked decrease in sodium influx. The fractional inhibition of flux was approximately the same as that of the current at both sodium concentrations. In three experiments at the higher sodium concentration, calcium appeared to have little effect on sodium outflux. The low values of sodium outflux and possible inaccuracies in measuring outflux when the outside bathing solution must be changed made an exact determination difficult. However, the data in Table II do suggest that the effect of calcium on sodium outflux must be small relative to the effect on influx. The decrease in short-circuit current can, therefore, be attributed almost entirely to a decrease in sodium influx, and the contribution to short-circuit current by net movement of other ions must be relatively small in the presence of calcium. The results of similar experiments testing the effect of calcium on chloride influx are also given in Table II. Although chloride is not actively transported, chloride influx across the short-circuited skin was inhibited to approximately the same degree as sodium influx under similar conditions.

The results at 19 mM sodium given in Table II show a fractional inhibition of sodium transport which is appreciably greater than that shown for a different series of experiments in Fig. 3. The experiments at 19 mM sodium in Table II were carried out on freshly caught spring frogs while those shown in Fig. 3 were carried out on winter frogs which had been stored for some time. There appeared to be a decreased sensitivity to calcium in the winter frogs. Indication of this decreased sensitivity has also been observed by one of the authors (P. F. C., unpublished data) in experiments on *Rana pipiens.*
The Effect of Calcium on Skin Conductance and Electrical Potential Difference

The results of seven experiments testing the effect of calcium on skin conductance and electrical potential difference are summarized in Table III. In each experiment, the effect of 8.3 mM calcium was tested at five different sodium concentrations. Addition of calcium decreased both potential difference and conductance with the greatest absolute changes occurring at the highest sodium concentration. The fractional decrease in potential difference depends on sodium concentration, but the fractional decrease in conductance does not vary appreciably with changes in sodium except at the two lowest concentrations. This observation appears to be at variance with the conclusion that the

<table>
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<tr>
<th>Flux measured</th>
<th>Sodium concentration</th>
<th>Flux*</th>
<th>Short-circuit current†</th>
<th>Fractional inhibition</th>
<th>Current</th>
</tr>
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<td>mm</td>
<td>µg/hr.</td>
<td>µg/hr.</td>
<td></td>
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<td>Na influx</td>
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<td>12.3</td>
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<td>11.7  4.1</td>
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<td></td>
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<td></td>
<td></td>
<td>9.8</td>
<td>5.3</td>
<td>9.3  4.8</td>
<td>0.46  0.48</td>
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<td></td>
<td></td>
<td>11.8</td>
<td>5.7</td>
<td>9.7  5.1</td>
<td>0.52  0.48</td>
</tr>
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<td>Na influx</td>
<td>19</td>
<td>4.2</td>
<td>2.7</td>
<td>4.0  2.7</td>
<td>0.36  0.33</td>
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<td></td>
<td></td>
<td>5.6</td>
<td>3.8</td>
<td>5.1  3.6</td>
<td>0.34  0.30</td>
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<td></td>
<td></td>
<td>6.4</td>
<td>5.4</td>
<td>6.0  5.2</td>
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<td></td>
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<td>5.1</td>
<td>5.3  4.4</td>
<td>0.21  0.17</td>
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<td>Na outflux</td>
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<td>0.29</td>
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<td>0.09  0.83</td>
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<td></td>
<td>0.35</td>
<td>0.34</td>
<td>11.2  8.5</td>
<td>0.03  0.24</td>
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<td></td>
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<td>0.26</td>
<td>0.30</td>
<td>5.6  3.4</td>
<td>-0.15 0.40</td>
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<tr>
<td>Cl influx</td>
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<td>1.2</td>
<td>0.8</td>
<td>3.8  2.1</td>
<td>0.33  0.46</td>
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<tr>
<td></td>
<td></td>
<td>1.4</td>
<td>0.9</td>
<td>3.5  2.1</td>
<td>0.35  0.40</td>
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<td></td>
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<td>1.4</td>
<td>11.6  6.8</td>
<td>0.46  0.40</td>
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<td></td>
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<td>1.7</td>
<td>4.3  2.7</td>
<td>0.41  0.37</td>
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<td></td>
<td></td>
<td>2.2</td>
<td>0.9</td>
<td>5.2  2.2</td>
<td>0.59  0.57</td>
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<td>Cl influx</td>
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<td>1.1</td>
<td>0.9</td>
<td>2.4  2.0</td>
<td>0.18  0.17</td>
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<td></td>
<td></td>
<td>1.7</td>
<td>1.1</td>
<td>2.1  1.6</td>
<td>0.35  0.24</td>
</tr>
</tbody>
</table>

* Skin area, 7.1 cm².
† Current is given in chemically equivalent values. The measured current represents the movement of positive charge from outside toward inside of the skin.
‡ Fractional inhibition of flux was calculated as 1 - Φ/Φ₀, in which Φ is flux in the presence of calcium and Φ₀ is control flux.
effect of calcium is dependent on sodium concentration. Possible explanations of this difference in effects on conductance and on sodium transport will be considered in the discussion.

Changes in chloride partial conductance of the skin caused by calcium can be calculated from chloride fluxes measured in other experiments (Table II) using the equation given by Hodgkin (Equation 19, reference 7). At a sodium concentration of 96 mM, chloride conductance is reduced from 0.30 to 0.16 mmho/cm² by 8.3 mM calcium, and at 19 mM sodium, chloride conductance is reduced from 0.20 to 0.14 mmho/cm². Thus, at the two sodium concentrations for which data are available, the change in chloride conductance caused by calcium averages approximately 25 per cent of the total change in conductance.

In the present series of experiments, the short-circuit current or net sodium transport can be estimated from the product of the measured values of conductance and potential difference, assuming that the potential difference is a linear function of current. (Control experiments have indicated that such an estimate might be in error by 10 per cent.) Fig. 6 shows the values of short-circuit current calculated from potential differences and conductances measured in one of these experiments. The relationship between short-circuit current and sodium concentration under control conditions is similar to that observed by direct measurement (8, 9). The fractional inhibition of sodium transport by calcium (α) estimated in this manner agrees fairly well with the values which might be expected from the data presented in Fig. 3. Thus, these somewhat indirect results indicate that the dependence of α on sodium concentration can also be observed in experiments in which sodium concentra-
Effect of Calcium on Sodium Transport by Frog Skin

Figure 6. Effect of 8.3 mM calcium on short-circuit current at varying sodium concentrations. Control current was measured with calcium concentration nominally zero. Current has been calculated from total skin conductance and potential difference as explained in the text. Lines have been drawn by eye.

Other Divalent Ions

In four experiments, the effects on current of calcium, barium, and magnesium were compared by testing all three ions on the same skin under similar conditions. The results are summarized in Table IV. Calcium and barium appear to be nearly equally effective in inhibiting sodium transport while magnesium is only 65 per cent as effective as calcium. In five experiments, the effect of magnesium was tested in sulfate-Ringer's solution using 8.3 mM magnesium and 96 mM sodium. Calcium could not be used because of the low solubility of CaSO₄, but MgSO₄ is sufficiently soluble. In these experiments, the average

<table>
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<tr>
<th>Experiment</th>
<th>Fractional inhibition of current</th>
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<tr>
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<td>Calcium</td>
</tr>
<tr>
<td>1</td>
<td>0.66</td>
</tr>
<tr>
<td>2</td>
<td>0.60</td>
</tr>
<tr>
<td>3</td>
<td>0.70</td>
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<tr>
<td>4</td>
<td>0.55</td>
</tr>
<tr>
<td>Average</td>
<td>0.63 ± 0.07</td>
</tr>
</tbody>
</table>

* In all experiments, sodium concentration was 96 mM and divalent ion concentration was 8.3 mM.
fractional inhibition of current was 0.37 ± 0.07 which compares well with the value of 0.42 ± 0.03 observed for the effect of magnesium at the same concentration in chloride-Ringer's solution. Thus, the inhibition of sodium transport does not depend on the presence of chloride ion in the bathing solution.

DISCUSSION

The effect of calcium on sodium transport by rat intestine (3, 10) seems to be at least qualitatively similar to the effect on frog skin. In both tissues, net sodium transport decreases with increasing calcium concentration in the solution bathing the "outside" surface (lumen of the intestine), but transport cannot be completely inhibited by calcium. The two sets of results cannot be compared quantitatively since the experiments on intestine were not carried out at constant sodium concentration. Bentley (11) has studied the effect of calcium on sodium transport across the isolated toad bladder. Increasing the calcium concentration of the solution bathing the serosal side of the bladder caused an increase in short-circuit current. A similar increase has been observed in the present experiments, but the effect was transient. An increase in calcium concentration at the mucosal surface of the bladder from 2.7 to 8.7 mM did not alter short-circuit current suggesting that sodium transport is not affected by calcium in the mucosal solution. However, the data given in Fig. 3 indicate that such a change in calcium concentration would cause a change in current of less than 10 per cent in frog skin. Thus, the use of different experimental methods makes comparison of results on toad bladder and frog skin difficult at present.

Net sodium transport by intestine is inhibited at very low calcium concentration both in vivo (3) and in vitro (10), and Dumont, Curran, and Solomon (3) have suggested that calcium may be essential for active sodium transport. When the toad bladder is bathed in calcium-free solutions, short-circuit current is decreased (11). Results with nerve and muscle also suggest that some calcium is necessary for normal function (12–14). In contrast, the frog skin is able to transport sodium normally for long periods when bathed in calcium-free Ringer's solution. However, Curran, Zadunaisky, and Gill (15) have found that the addition of small amounts of EDTA to the solution bathing the inside of the skin results in a large and rather rapid drop in potential difference, and a smaller decrease in short-circuit current. The effect seems to be due to removal of calcium since it can be completely reversed by subsequent addition of CaCl₂. Thus, although addition of calcium to the outside of the skin decreases sodium transport, some calcium is necessary at the inside if the skin is to retain its normal properties. Apparently, the skin loses calcium very slowly when bathed in calcium-free solution while other tissues lose essential calcium more rapidly under these conditions.
Increase of calcium concentration in the solution bathing the outside of the skin causes a decrease in diffusion of the passively transported chloride ion as well as a decrease in the rate of active sodium transport. These effects of calcium will be discussed, as far as possible, in terms of the model of the frog skin proposed by Koefoed-Johnsen and Ussing (16) since it appears to offer a reasonable explanation of the sodium transport system. They have suggested that the outward facing membrane of the transporting cells of the skin is permeable to sodium and chloride but not to potassium, while the inward facing membrane is permeable to potassium and chloride but not to sodium. Active transport of sodium is assumed to take place at the inner membrane, sodium in the cell being exchanged for potassium in the inside bathing solution, while sodium enters the cell from outside by diffusion through the sodium-permeable membrane. The result is a net transport of sodium from the outside solution into the inside solution.

On the basis of this model, calcium in the outside solution appears to act on the outer membrane rather than directly on the system responsible for active sodium transport. The effect on current is relatively rapid, beginning almost as soon as calcium is added and reaching 80 to 90 per cent completion in approximately 10 minutes. Since calcium usually penetrates cells quite slowly (17, 18), it seems unlikely that calcium could enter the transporting cells rapidly enough to account for the observed effects by inhibition of the active transport mechanism if it is located at the inner membrane. Further, the results indicate that both sodium and chloride influx are reduced to approximately the same degree. Both sodium and chloride are assumed to diffuse passively across the outer membrane, and a relatively non-specific "tightening" of this membrane would be expected to alter both fluxes in a similar manner. A direct effect on active sodium transport need not necessarily influence chloride flux at all. Gill and Nedergaard (19) have found that calcium added to the outside solution decreased net flow of water across the skin under influence of an osmotic pressure gradient. Since the primary barrier to water movement is located at the outside of the skin (20) this observation offers further evidence that calcium acts on the outer membrane.

This conclusion suggests that calcium probably affects net sodium transport across the skin by reducing sodium permeability of the outward facing membrane rather than by a direct effect on the active transport system itself. Such a direct effect on active transport cannot be entirely ruled out at present, but on the basis of the working model of the skin under consideration, it seems relatively unlikely. However, the action of calcium need not be directly on the outward facing membrane of the transporting cells. The layer of the skin between the epithelial cells and the outside solution may play a role in con-
trolling sodium entry into the cells and calcium could act by altering the 
properties of this layer. Thus, until further information becomes available, 
the term “outer membrane” may be taken to include all that part of the skin 
between the outer surface and the cytoplasm of the transporting cells.

On the basis of these considerations, the following hypothesis can be pro-
posed to explain the effect of calcium on sodium transport. The addition of 
calcium to the solution bathing the outside of the skin causes a decrease in 
sodium permeability of the outer membrane. This permeability change leads 
to a decrease in the rate of sodium diffusion into the epithelial cells and thus 
reduces the supply of sodium to the active transport system which is located 
at the inner border of the cell. The over-all effect of this change should be a 
reduction in the sodium concentration within the cell with a resulting de-
crease in the rate of active sodium transport to a new steady level. A similar 
mechanism has been suggested by Skou and Zerahn (21) to account for the 
increase in sodium transport observed in the presence of procaine and other 
local anesthetics. They have proposed that these agents increase sodium per-
meability of the outer membrane. This would lead to an increase in cell so-
dium concentration and hence to an increase in active sodium transport. This 
hypothesis suggests that the sodium permeability of the outer membrane plays 
an important part in controlling the rate of net sodium transport across the 
skin. It further suggests that the active transport system itself is not saturated 
at the sodium concentration normally found in the cells since the hypothesis 
requires that the rate of transport must vary with changes in the sodium pool 
in the cells. This hypothesis seems to offer a reasonable explanation of the 
present results and at least some features of it would seem to be open to further 
experimental test.

The observation, in three experiments, that sodium outflux appears to be 
little affected by calcium does not seem to be consistent with this hypothesis. 
The considerations of Schoffeniels (22) regarding tracer fluxes in such a system 
indicate that the fractional decrease in sodium outflux should be approxi-
mately the same as the fractional decrease in net sodium transport. This ap-
parent discrepancy might be ascribed to difficulties in measuring outflux 
accurately under present conditions when the outside solution must be 
changed and to the very limited number of observations. However, failure to 
observe such an effect does not necessarily invalidate the present hypothesis of 
calcium action. If sodium outflux followed a different path through the skin 
than that affected by calcium, outflux should be independent of calcium. For 
example, outward movement of sodium could take place between the trans-
porting cells rather than through them. This seems quite possible since the 
observations of Koeffoed-Johnsen and Ussing (16) indicate that the inner 
membrane of the transporting cells must have an extremely low sodium per-
This hypothesis of calcium action suggests that both the ion permeability of the outer membrane and ion concentration in the transporting cells will be altered by addition of calcium. Since the potential difference across the skin depends on these properties (16), a change might be expected. However, quantitative prediction of this effect cannot be made without specific information concerning the alteration of those parameters which determine the potential difference. Further, addition of calcium to the outside solution should result in a decrease in conductance of the outer membrane due to the reduction in sodium and chloride permeability of this membrane. This change in the outer membrane cannot be measured directly, but should be reflected by a decrease in total skin conductance which has been observed.

The observed effect of calcium on total conductance does not, however, appear to be consistent with the effect on sodium transport. The data presented in Fig. 3 indicate that, under the present conditions, the effect of calcium on sodium transport is relatively greater the higher the sodium concentration of the bathing solutions. However, the fractional decrease in skin conductance caused by calcium does not show any appreciable dependence on sodium at the higher sodium concentrations. On the basis of the present hypothesis of calcium action at the outer membrane, the change in conductance of this membrane should follow a pattern relatively similar to that observed for sodium transport. This discrepancy could be explained if the fractional decrease in total skin conductance does not accurately reflect conductance changes at the outer membrane alone. In the simplest possible case, the total skin conductance must be made up of at least two conductances in series, one representing the outer membrane and one the inner membrane. It will, therefore, be a relatively complex function of ionic mobilities in both membranes and ion concentrations in the transporting cells and the bathing solutions. The conductance of both membranes could change upon addition of calcium even if the direct effect is on the outer membrane. In this case, the relative change in total conductance would be given by the following expression:

\[
\text{Fractional decrease in conductance} = 1 - \frac{G_o' G_i' \left[ G_o + G_i \right]}{G_o G_i' \left[ G_o' + G_i' \right]}
\]

in which \(G_o\) and \(G_i\) are conductances of the outer and inner membranes, respectively, under control conditions and \(G_o'\) and \(G_i'\) are conductances in the presence of calcium. Since values of individual conductances in this expression are not known, the relationship between the fractional change in total conductance and the change at the outer membrane \((1 - G_o'/G_o)\) cannot be pre-
dicted. In particular, for some values of conductances, the change in the above expression could be much smaller than a change in $G_\alpha/G_\infty$. Thus, without additional information, the data in Table III cannot be taken as indicating that the change in conductance of the outer membrane itself is independent of sodium concentration.

There is at present, no completely adequate explanation of the observation that the effect of calcium on sodium transport is dependent on sodium concentration. On the basis of the hypothesis outlined above, it seems reasonable to assume that calcium acts by combining with certain sites on or in the outer membrane, thereby changing permeability properties. The data presented in Fig. 3 would then suggest that fewer sites of calcium action are available at lowered sodium concentrations. This cannot be explained by a direct competition between sodium and calcium since, in such a case, more sites should be available to calcium at low sodium concentrations. An effect similar to that observed by Bianchi and Shanes (23) in muscle could explain the results shown in Fig. 3. They found that some of the calcium bound to muscle fibers could be released by increasing the sodium concentration of the bathing solution. If the loss of calcium from the outer membrane of the skin under our control conditions (no calcium in the bathing solution) were greater at high sodium concentration, more sites would then be available during subsequent treatment with calcium and a larger effect would be expected. However, the data presented in Fig. 5 suggest that at low sodium concentration those sites which are available combine less readily with calcium than at high sodium concentration. This observation cannot be explained on such a simple basis, and a complete description of the relationship between sodium and calcium must await further, more detailed information.

The mechanism by which calcium might influence ion permeability is also unclear, but a relatively non-specific tightening of the outer membrane seems to take place. If the membrane of the transporting cells is a porous structure, it is possible that calcium bound to negatively charged sites at the pore wall partially blocks the pores, thereby reducing diffusion through them. If so, calcium does not appear to block the pores entirely, since it cannot completely inhibit sodium transport. Further information concerning possible sites and mechanisms of calcium action might be obtained by comparison of its effects with those of other agents which seem to affect permeability of the outer membrane of the skin, such as antidiuretic hormone (20) and procaine (21).

In conclusion, the present results indicate that the action of calcium on the outside of the frog skin is due to a relatively non-specific tightening in some region, leading to decreased permeability to both sodium and chloride. This reduction in sodium permeability leads to a decrease in active transport of sodium, but calcium does not seem to affect the active transport mechanism directly. The effect seems to depend on a decrease in the rate of sodium entry...
into the epithelial cells as a result of the lowered permeability of the outer membrane. The results, therefore, suggest that the rate of sodium transport may be controlled, at least in part, by the permeability of the outer membrane, rather than by the active transport system itself.

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