Calcium and Strontium in Rat Small Intestine
Their Fluxes and Their Effect on Na Flux

P. A. DUMONT, PETER F. CURRAN, and A. K. SOLOMON
From the Biophysical Laboratory of the Harvard Medical School, Boston, Massachusetts
Dr. Dumont's present address is Department of Zoophysiology, Institute of Zoology, University of Louvain, Belgium

ABSTRACT Studies have been carried out on movements of Ca and Sr ions in rat small intestine, using the in vivo preparation developed by Curran and Solomon (5). In the concentration range of 0 to 25 mM, Sr flux appears to be passive, though restricted. Ca transport may not, however, be ascribed to passive independent movement of these ions since at higher concentrations (12.5 and 25 mM) Ca return from blood to intestinal lumen increases more than expected. An apparent diffusion coefficient of Ca and Sr ions in the membrane has been calculated and the influence of negative charges within the membrane on cation diffusion has been examined in a semiquantitative manner.

Both Ca and Sr ions exercise a drastic effect on active Na absorption from intestine and on concomitant passive water movement. From 0 to 1 mM, Ca and Sr ions cause a sharp increase in Na and water efflux from the lumen. This rising phase is interpreted in terms of combination of the divalent cation with the Na carrier system following Michaelis-Menten kinetics. At concentrations higher than 1 mM, the effect of Ca and Sr ions is reversed and Na and water absorption decreases slowly as Ca or Sr concentration is increased. This falling phase is ascribed to a non-specific Ca effect which produces a general “stiffening” of the membrane.

The present study is concerned with cation transport across the small intestinal mucosa in the rat—specifically with Ca and Sr fluxes and the effect exercised by these ions on Na flux. Though the intestinal absorption of Ca has long been studied (1-4), no specific mechanisms of absorption have yet been demonstrated. The experiments comprise an in vivo study of the absorp-

This work has been supported in part by the Atomic Energy Commission and in part by a research grant A-1824 (CI) from the National Institute of Arthritis and Metabolic Diseases, Public Health Service.

Dr. Dumont is a Research Fellow of the Institut Interuniversitaire des Sciences Nucleaires, Belgium.
Dr. Curran is a Public Health Service Research Fellow of the National Heart Institute.
Received for publication, November 12, 1959.
tion of Ca, Sr, Na, and water from segments of intestine perfused with NaCl solutions to which graded amounts of Ca and Sr have been added. The use of radioactive isotopes coupled with chemical analysis permits measurement of the flux of ions into and out of the intestine following the method previously described by Curran and Solomon (5). The net flux of water across the intestine has also been measured, as has the effect of Ca and Sr on the potential difference between lumen and plasma.

EXPERIMENTAL METHODS

Male Wistar rats, weighing from 300 to 400 gm. were fed with standard pellets which contain 1 gm. Ca and 20 I. U. of vitamin D per 100 gm. of feed. All animals were kept on this diet for a period of at least 30 days before being fasted overnight prior to use.

Minor modifications have been introduced into the procedure used by Curran and Solomon (5) in order to adapt it to the study of Ca and Sr permeability. In the previous experiments, segments of ileum about 10 cm. in length were cannulated at both ends with glass tubes, in vivo. Since the absorption of Ca proceeds at a much slower rate than that of Na, it was necessary to extend the length of the perfused segment to 45 to 55 cm. The distal cannula was inserted within 5 to 10 cm. of the ileocecal valve so that the preparation included all of the ileum and part of the jejunum. During the perfusion, the intestine remained outside of the body and was kept moist with saline-soaked paper tissues. The whole animal (anesthetized with nembutal, 7 mg./100 gm.) was kept in a 37°C. constant temperature box. The entering perfusion fluid was brought to 37°C. and pumped through the intestine with a peristaltic action pump (American Instrument Co.) which was necessary to overcome the impedance of the long length of intestine. A vertical glass tube, a few centimeters high, was connected to the perfusion circuit to detect any pressure rise consequent upon any interference with normal fluid flow. The normal perfusion pressure measured at the entrance to the intestine was about 5 cm. of water, which produced a flow rate of about 0.5 ml./min.

The perfusion solutions contained CaCl$_2$ or SrCl$_2$ at concentrations$^1$ varying from 0 to 25 mM. The solutions were slightly buffered with NaHCO$_3$ at a concentration of 10 mM and made up with NaCl to a total osmotic concentration equal to NaCl at 150 mM. Human hemoglobin at a concentration of about 3 X 10$^{-5}$ M served as an indicator of water absorption. Trace amounts of Ca$^{45}$Cl$_2$ and Sr$^{85}$Cl$_2$ were added as required. In a typical experiment 4 to 6 solutions containing increasing concentrations of CaCl$_2$ or SrCl$_2$ were passed through the intestine in succession. For each, the effluent collected during the first 15 minutes was discarded. This period of time appeared to be sufficient to displace the previous perfusion fluid completely and to allow the establishment of a new steady state. In the succeeding 15 minutes, the flow rate was measured and the total sample collected for analysis. A complete experiment thus lasted 2 to 3 hours.

The absorption of water was measured by colorimetric analysis of hemoglobin at

$^1$ A few preliminary experiments in which the Ca concentration was set at 50 and 75 mM/liter produced evident signs of damage to the intestinal mucosa.
411 m\(\mu\) as previously described. Control experiments indicated that the amount of hemoglobin that entered the intestine was completely recovered. \(CaCl_2\), \(SrCl_2\), and \(NaHCO_3\) did not affect the optical density. Na and Sr were measured on the flame photometer of Solomon and Caton (6) at wave lengths of 589 and 460.7 m\(\mu\) respectively. The standard deviation in sets of 10 replicate measurements was 1.8 per cent for Na and 1.0 per cent for Sr. At the Sr wave length, the interference of Ca and Na with the Sr signal was negligible (the standard solutions contained the same concentrations of the interfering cations).

Calcium was determined by the titrimetric method of Wallach et al. (7) to an accuracy of 0.2 per cent. This method uses versene as a titrating agent and 3,6 dihydroxy-2,4-bis \(\left[N,N'\right]-\text{dicarboxymethyl-aminomethyl}\) fluoran as a fluorescent indicator (kindly supplied by Dr. D. F. H. Wallach). Since this method does not discriminate between Sr and Ca, these two cations could be determined simultaneously using the titrimetric method to determine their sum, coupled with a flame photometric determination of Sr.

**Table I**

<table>
<thead>
<tr>
<th>Ca Concentration</th>
<th>pH Before perfusion</th>
<th>Experiment 9</th>
<th>Experiment 10</th>
</tr>
</thead>
<tbody>
<tr>
<td>mM</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>8.1</td>
<td>8.1</td>
<td>8.6</td>
</tr>
<tr>
<td>0.53</td>
<td>8.1</td>
<td>8.0</td>
<td>8.4</td>
</tr>
<tr>
<td>2</td>
<td>8.1</td>
<td>8.7</td>
<td>8.2</td>
</tr>
<tr>
<td>4.94</td>
<td>7.9</td>
<td>8.3</td>
<td>8.4</td>
</tr>
<tr>
<td>12.3</td>
<td>7.7</td>
<td>8.2</td>
<td>8.3</td>
</tr>
<tr>
<td>25</td>
<td>7.5</td>
<td>8.6</td>
<td>8.2</td>
</tr>
</tbody>
</table>

Radioactivity was measured in a Robinson flow proportional counter (8), on samples prepared by the method of Hunter and Commerford (9). No self-absorption correction was found necessary since the sample weight was constant and small compared to the weight of lens paper and sucrose in the planchet. The accuracy of the radioactive determinations was 1.5 per cent for \(Ca^{45}\) and 1.1 per cent for \(Sr^{89}\).

The potential difference between lumen and peritoneal cavity was measured with 1 m KCl agar bridges, calomel electrodes, and a Keithley model 200 B electrometer, as previously described. In two experiments, pH was measured as a function of the Ca concentration in the perfusing solution. The results are given in Table I. It can be seen that the pH of the collected perfusion solution shows no relationship to the Ca concentration of the solution that enters the intestine. Thus the effects ascribed to changes in Ca concentration are not caused by pH changes.

Ca and Sr fluxes were calculated according to the equations previously given (5) for the study of Na and Cl fluxes, which rest on several assumptions. The most important is the assumption of a two compartment system, in which the rate of absorption of water is linear with time, and in which the back flow of \(Ca^{45}\) (or \(Sr^{89}\)) from
the body into the intestine is considered to be negligible. Curran and Solomon tested these assumptions by carrying out experiments in which the same fluid was continuously circulated through the intestine for a 2 hour period. Such experiments tested the validity of the following equations (their Equations 5 and 10) for \( v_p \), the volume of the lumen compartment, and for \( k_{\text{eff}} \), the rate constant for Na efflux from the lumen of the intestine to plasma.

\[
v_p = v_{p0}(1 - \lambda t) \tag{1}
\]

\[
k_{\text{eff}} = v_{p0} \lambda \left[ \frac{\ln (p/p_0)}{\ln (1 - \lambda t)} + 1 \right] \tag{2}
\]

**Figure 1.** Logarithm of \( p/p_0 \) as a function of the logarithm of \((1 - \lambda t)\). The x's are values obtained in our experiments with no second addition of Ca\(^{45}\) to the fluid circulating in the lumen. The circles are values obtained when Ca\(^{45}\) has been added at the time indicated by the arrow (50 minutes after the beginning of the experiment) to the solution circulating in the lumen. It will be seen that the second addition of Ca\(^{45}\) leads to a linear relationship as required by Equation 2. The curves have been drawn by eye.

in which \( p \) is the Na\(^{24}\) concentration in the lumen, and \( \lambda \), a constant, is the fraction of water absorbed from the lumen per unit time. The subscript \( \sigma \) refers to initial conditions. In the study reported here recirculation experiments were carried out at an initial Ca concentration of 5 mm. The requirement of Equation 1 that water absorption be linear with time was satisfied. Equation 2 requires the \( \ln (p/p_0) \) to be linear with the \( \ln (1 - \lambda t) \); Fig. 1 shows that this relationship was satisfied only for the initial 50 minutes of the experiment. It appeared possible that the discrepancy could be ascribed to some reentrance of Ca\(^{45}\) into the lumen as the experiment continued, since Ca\(^{45}\) disappearance from the lumen is very much slower than that of Na\(^{24}\). Consequently two recirculation experiments were carried out in which addi-
tional increments of Ca$^{45}$ tracer were added to the solution 60 minutes after the experiment began in order to raise the lumen Ca$^{45}$ concentration fivefold. Under these conditions the linear relationship between ln ($p/p_0$) and ln ($1 - \lambda t$) was found to obtain for the full 2 hour period, as shown in Fig. 1. As a result the standard experiments were designed so that the total perfusion time for a single solution never exceeded 40 minutes. Furthermore, the radioactivity in each succeeding perfusion solution was increased over that of the preceding solution according to the ratio $1:2:3:...$. In spite of these additional precautions, it seems clear that the assumptions of a two compartment system and zero tracer backflow are not entirely valid for Ca. The results of the recirculation experiments do not provide information as to which assumption is not satisfied. In consequence the effect of a possible third compartment must be considered in the interpretation of the experimental results. The fact that Equation 2 is satisfied over the time period used in these experiments, supports the validity of calculating fluxes in these periods according to the equations of Curran and Solomon.

The equations also assume explicitly that the influx to the lumen is independent of concentration in the lumen, and implicitly that the potential difference does not change significantly during any perfusion period. In the previous experiments (5) the influx into the lumen was found to change by no more than 3 per cent in a perfusion period, an effect which was considered negligible; in the present series the effect is less than 5 per cent and is also considered negligible. The change in potential difference in any perfusion period is less than 3 per cent—also small enough to be neglected.

RESULTS AND DISCUSSION

Effect of Ca and Sr on the Potential Difference

At each level of Ca concentration in the range of 0 to 25 mM/liter the potential difference between the lumen and the plasma reached a steady and definite value within a few minutes. Table II shows that the potential difference, measured in 66 experimental periods, is determined primarily by the concentration of NaCl within the lumen. There is clearly no difference between the data obtained in the present studies with CaCl$_2$ in the lumen and the previous ones in which the solution in the lumen was made isosmotic by the addition of mannitol. The differences observed when SrCl$_2$ is placed in the intestine are of small magnitude. Since the potential difference never exceeded 2.5 mV, a range in which experimental uncertainty is very great, observations over a larger range of Na concentrations would be required to determine whether any real effect may be ascribed to the presence of Sr.

Calcium and Strontium Fluxes

Tables III and IV present the results of measurements of Ca and Sr fluxes in the concentration range of 0 to 25 mM/liter. It is instructive to compare the
TABLE II
LUMEN TO PLASMA POTENTIAL DIFFERENCE*

<table>
<thead>
<tr>
<th>Potential difference (lumen − plasma)</th>
<th>Previously observed (5) when solution made isosmotic with mannitol</th>
<th>Observed when solution made isosmotic with CaCl₂</th>
<th>Observed when solution made isosmotic with SrCl₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>[Na] mm</td>
<td>no.</td>
<td>mm</td>
<td>no.</td>
</tr>
<tr>
<td>151</td>
<td>−0.4</td>
<td>−0.5 ± 0.2 (5)</td>
<td>−1.6 ± 0.5 (9)</td>
</tr>
<tr>
<td>148</td>
<td>−0.4</td>
<td>−1.6 ± 0.4 (9)</td>
<td>+0.1 ± 0.3 (9)</td>
</tr>
<tr>
<td>146</td>
<td>−0.3</td>
<td>−0.1 ± 0.2 (5)</td>
<td>−0.7 ± 0.6 (9)</td>
</tr>
<tr>
<td>141</td>
<td>−0.2</td>
<td>+0.2 ± 0.4 (4)</td>
<td>+1.2 ± 0.4 (8)</td>
</tr>
<tr>
<td>130</td>
<td>+0.2</td>
<td>+2.1 ± 0.3 (9)</td>
<td>+2.4 ± 0.5 (8)</td>
</tr>
<tr>
<td>111</td>
<td>+2.4</td>
<td>+2.4 ± 0.3 (9)</td>
<td>+2.4 ± 0.5 (8)</td>
</tr>
</tbody>
</table>

* Errors are standard errors of the mean. The number of experimental periods is given in parentheses. The potential difference was measured between lumen and peritoneal cavity and is assumed to be equivalent to that between lumen and plasma.

TABLE III
CALCIUM FLUXES ACROSS THE INTESTINAL MUCOSA OF THE RAT

<table>
<thead>
<tr>
<th>[Ca] in lumen</th>
<th>Influx</th>
<th>Efflux</th>
<th>Net efflux</th>
<th>No. of experiments</th>
</tr>
</thead>
<tbody>
<tr>
<td>mm</td>
<td>μM/hr. cm.</td>
<td>μM/hr. cm.</td>
<td>μM/hr. cm.</td>
<td></td>
</tr>
<tr>
<td>0.06 ± 0.01</td>
<td>0.022 ± 0.006</td>
<td>0.004 ± 0.006</td>
<td>−0.050 ± 0.005</td>
<td>11</td>
</tr>
<tr>
<td>0.53 ± 0.01</td>
<td>0.02 ± 0.01</td>
<td>0.05 ± 0.01</td>
<td>+0.03 ± 0.01</td>
<td>15</td>
</tr>
<tr>
<td>1.05 ± 0.02</td>
<td>0.03</td>
<td>0.06 ± 0.03</td>
<td>+0.01 ± 0.01</td>
<td>13</td>
</tr>
<tr>
<td>2.00 ± 0.03</td>
<td>0.02 ± 0.01</td>
<td>0.05 ± 0.01</td>
<td>+0.03 ± 0.01</td>
<td>13</td>
</tr>
<tr>
<td>4.94 ± 0.06</td>
<td>0.03 ± 0.03</td>
<td>0.06 ± 0.03</td>
<td>+0.01 ± 0.01</td>
<td>13</td>
</tr>
<tr>
<td>12.3 ± 0.2</td>
<td>0.16 ± 0.08</td>
<td>0.35 ± 0.06</td>
<td>+0.19 ± 0.05</td>
<td>10</td>
</tr>
<tr>
<td>25.0 ± 0.2</td>
<td>0.32 ± 0.10</td>
<td>0.9 ± 0.2</td>
<td>+0.6 ± 0.1</td>
<td>13</td>
</tr>
</tbody>
</table>

* 13 net efflux experiments.

TABLE IV
STRONTIUM FLUXES ACROSS THE INTESTINAL MUCOSA OF THE RAT

<table>
<thead>
<tr>
<th>[Sr] in lumen</th>
<th>Influx</th>
<th>Efflux</th>
<th>Net efflux</th>
<th>Net flux</th>
</tr>
</thead>
<tbody>
<tr>
<td>mm</td>
<td>μM/hr. cm.</td>
<td>μM/hr. cm.</td>
<td>μM/hr. cm.</td>
<td></td>
</tr>
<tr>
<td>0.49 ± 0.01</td>
<td>0 ± 0.01</td>
<td>0.03 ± 0.02</td>
<td>0.03 ± 0.01</td>
<td>4</td>
</tr>
<tr>
<td>1.96 ± 0.04</td>
<td>−0.02 ± 0.01</td>
<td>0.14 ± 0.05</td>
<td>0.11 ± 0.02</td>
<td>9</td>
</tr>
<tr>
<td>4.97 ± 0.01</td>
<td>−0.05 ± 0.03</td>
<td>0.21 ± 0.05</td>
<td>0.27 ± 0.05</td>
<td>9</td>
</tr>
<tr>
<td>12.5 ± 0.1</td>
<td>0 ± 0.05</td>
<td>0.5 ± 0.2</td>
<td>0.5 ± 0.2</td>
<td>9</td>
</tr>
<tr>
<td>25.1 ± 1.5</td>
<td>0.11 ± 0.15</td>
<td>1.2 ± 0.1</td>
<td>1.0 ± 0.1</td>
<td>7</td>
</tr>
</tbody>
</table>

* 13 net flux experiments.
Ca flux as a function of intestinal Ca concentration with the Sr flux as a function of Sr concentration, as has been done in Fig. 2. It will be seen that some similarities exist in the behavior of the two elements.

The efflux of Sr from the intestine increases linearly with the concentration in the lumen which is compatible with a passive diffusion process. Since Sr is not a normal constituent of the plasma, it is not surprising that the influx is nowhere statistically different from zero (the slope of the least squares line through the influx points also does not differ significantly from zero). The efflux of Ca is comparable to that of Sr. The net efflux curve cuts the zero flux axis at a Ca concentration of 1.7 ± 0.3 mM/liter, a value in agreement with the figure of 1.6 ± 0.1 mM/liter given by Benjamin and Hess (10) for the concentration of diffusible Ca in rat plasma. Almost all of the diffusible Ca may be considered to exist in an ionized form (11).
There is no evidence of net transport up an electrochemical potential gradient at any luminal concentration that we have studied, so that the transport of Ca would be considered passive according to Rosenberg's criterion (12). However, a somewhat different conclusion is reached when Ussing's criterion (13) is applied, as shown by the data presented in Table V. At concentrations lower than 5 mM Ca, transport appears to be passive since the difference between the calculated and observed flux ratios (Table V, columns 4 and 5) is not significant. At the higher concentrations, however,

### Table V

<table>
<thead>
<tr>
<th>[Ca] in lumen (mM)</th>
<th>[Ca] (lumen)</th>
<th>[Ca] (plasma)</th>
<th>ΔΨ</th>
<th>Calculated flux ratio</th>
<th>Observed flux ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>[Ca]</td>
<td>Lumen - plasma</td>
<td>φ_φ^Ca_φ^Ca</td>
<td>φ_φ^Ca_φ^Ca</td>
<td>φ_φ^Ca_φ^Ca</td>
<td>φ_φ^Ca_φ^Ca</td>
</tr>
<tr>
<td>0.53</td>
<td>0.33</td>
<td>-0.5</td>
<td>0.3</td>
<td>0.2 ± 0.3</td>
<td></td>
</tr>
<tr>
<td>1.05</td>
<td>0.66</td>
<td>-0.3</td>
<td>0.6</td>
<td>3 ± 14</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>1.25</td>
<td>-0.1</td>
<td>1.2</td>
<td>2.5 ± 1.8</td>
<td></td>
</tr>
<tr>
<td>4.94</td>
<td>3.09</td>
<td>0</td>
<td>3.1</td>
<td>2.7 ± 3.2</td>
<td></td>
</tr>
<tr>
<td>12.3</td>
<td>7.7</td>
<td>+0.2</td>
<td>7.8</td>
<td>2.2 ± 1.3</td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>15.6</td>
<td>+2.4</td>
<td>18.7</td>
<td>2.8 ± 1.5</td>
<td></td>
</tr>
</tbody>
</table>

*If we assume that the activity of the water in the lumen equals that in the plasma, the passive flux ratio will be given by the following equation (13):

\[
\frac{\Phi_φ^Ca}{\Phi_φ^Ca} = \frac{[Ca]_φ}{[Ca]_p} e^{F(\Psi_l - \Psi_p)/RT}
\]

in which z is the ionic charge, F the Faraday, R the gas constant, and T the absolute temperature. The subscript, φ, refers to concentration in the plasma, and the subscript, p, refers to concentration in the intestinal lumen. In deriving Equation 3, it has been assumed that the activity coefficient of the ionized Ca in the lumen is the same as in the plasma.

the observed flux ratios differ significantly from those predicted. Thus, our data indicate that the flux ratios at Ca concentrations of 12.3 and 25 mM may not be explained on the basis of simple independent diffusion of Ca ions.

The possibility exists that, at the higher concentrations of Ca, absorption of Ca from the intestine is sufficient to raise the Ca concentration in the blood flowing past the perfused intestinal segment. The data in Table V permit us to calculate that the diffusible Ca in the blood stream would have to rise to 9.4 mM for the influx to be passive according to the criterion of Ussing. It can be shown that this value is unreasonable on the basis of the measured fluxes, if the intestinal blood flow data of Atkinson, Parsons, and Smyth (14)
in dog may be taken as applicable to the rat. On this basis, we might expect a diffusible Ca concentration in the blood stream of only 2.1 mM, a rise of only 0.5 mM above the normal diffusible Ca concentration. Thus, it seems unlikely that the extra influx of Ca into the intestine can be ascribed to a local increase of Ca in the plasma. This conclusion is strengthened by the difference between the behavior of Ca and Sr in this respect, as illustrated in Fig. 2. We may conclude that an extra transport process is involved in the flux of Ca across the mucosa at the higher levels of intestinal Ca concentration.

As discussed by Durbin, Curran, and Solomon (17), the intestinal wall is a complex structure which includes several cellular layers. The specific cells responsible for ion transport have not been identified. In the discussion that follows we shall assume that the active transport processes are confined to a single cell face and shall use "membrane" to refer to the structure that separates the cell contents from the cellular environment on that face alone. In the frog skin, Koefoed-Johnsen and Ussing (18) have put forth evidence which leads to the conclusion that the ion transport system is located in the membrane at the inner face of the epithelial cells.

From Fig. 2, it appears that the intestinal mucosa responds to increased Ca levels in the intestine by returning more and more of the absorbed Ca into the intestine. The mechanism by which the process is regulated is not clear. Possibly the cells in the mucosa respond to increased intracellular Ca levels by activating a return process, or possibly a third compartment becomes important at these higher Ca concentrations. Alternatively, we may be observing a transconcentration effect as described for the gastric mucosa by Heinz and Durbin (19). This effect depends upon the presence of a carrier complex confined to the membrane phase. The concentration of the carrier complex, and hence its efficiency in transport, depends upon the dissociation of the complex into carrier and ion at both faces of the membrane. Hence, the ionic concentration at the face towards which the transport is directed can affect the flux coming towards it. Furthermore, the departure of the observed flux ratio from the predicted one shown in Table V is in a direction compatible with such an exchange diffusion process. Indeed, the data available from the present experiments are quantitatively compatible with an exchange diffusion process which begins to play a significant role when the luminal Ca concentration exceeds 5 mM.

Schachter and Rosen (20) have concluded that Ca is transported actively from the intestinal lumen to the serosal surface in preparations of everted intestine from the pyloric end of the intestine in rabbit, rat, and guinea pig.

---

In the rat 1 cm. of small intestine weighs 30 mg, according to Curran and Schwartz (15). The diffusible Ca concentration in the blood has been taken as 0.6 of the total Ca as determined for man (16).
In the rabbit the first 15 cm., proximal to the pylorus, are the most effective; in the rat, the length of the effective segment is not specified. These results are not comparable to the present ones, since different segments of the intestine were probably involved. Furthermore, Schachter and Rosen made no measurements of inorganic Ca in the solutions on either side of the mucosa, nor of potential difference. Thus their observation of an asymmetric distribution of Ca\(^{45}\) may not be taken as proof that an active process is indeed operative.

**TABLE VI**

DIFFUSION COEFFICIENTS OF Ca AND Sr

<table>
<thead>
<tr>
<th>Concentration in lumen (mM)</th>
<th>(D_{37}^\text{calcuated}) (\times 10^6) cm(^2)/sec</th>
<th>(D_{37}^\text{in free solution}) (\times 10^6) cm(^2)/sec</th>
<th>(\frac{\text{D}<em>{37}^\text{calcuated}}{\text{D}</em>{37}^\text{free solution}}) per cent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ca</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.05</td>
<td>0.42</td>
<td>1.500</td>
<td>28</td>
</tr>
<tr>
<td>4.94</td>
<td>0.19</td>
<td>1.439</td>
<td>13</td>
</tr>
<tr>
<td>12.3</td>
<td>0.25</td>
<td>1.413</td>
<td>18</td>
</tr>
<tr>
<td>25</td>
<td>0.25</td>
<td>1.383</td>
<td>18</td>
</tr>
<tr>
<td>Sr</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.49</td>
<td>0.54</td>
<td>1.496</td>
<td>36</td>
</tr>
<tr>
<td>1.98</td>
<td>0.59</td>
<td>1.480</td>
<td>40</td>
</tr>
<tr>
<td>4.97</td>
<td>0.33</td>
<td>1.447</td>
<td>23</td>
</tr>
<tr>
<td>12.5</td>
<td>0.30</td>
<td>1.410</td>
<td>21</td>
</tr>
<tr>
<td>25.1</td>
<td>0.32</td>
<td>1.390</td>
<td>23</td>
</tr>
</tbody>
</table>

* Ca data obtained from Lyons and Riley (22) and Harned and Parker (23). Sr data obtained from Harned and Polestra (24). A temperature coefficient of 0.01913/degree has been obtained from measurements of the temperature coefficient of Ca diffusion in 0.5 per cent agar (25) and used to calculate the 37\(^\circ\) figures from the initial observations which were made at 25\(^\circ\)C. in both cases.

**Effect of Charges in the Membrane Pore**

It is possible to calculate the apparent diffusion coefficient of Ca and Sr for the efflux process using the method described by Curran and Solomon (5). They have estimated the apparent membrane diffusion coefficient of Na to be 84 per cent of its free diffusion coefficient, and that of Cl to be 25 per cent of its free diffusion coefficient. This difference has been interpreted as a reflection of a negative charge barrier within the pores of the membrane, and Curran (21, 5) has estimated the charge density within the pores to lie between 0.1 and 0.6 m.eq./ml. In the course of their calculations, Curran and Solomon have given a value of 35.2 cm. per 10 cm. of intestine for \(A_p/\Delta x\), the apparent pore area divided by the path length through the membrane. If this figure may be considered representative for the length of intestine used in the pres-
ent experiments, it is possible to calculate the apparent membrane diffusion coefficient for Ca and Sr as has been done in Table VI. These figures represent maximum values, since they perforce include any contribution that may arise from exchange diffusion. Two conclusions may be drawn from the table: first, the apparent membrane diffusion coefficients are much lower than that for Na, being in the range of 18 to 40 per cent of the free diffusion co-

**Figure 3.** Donnan ratio within the pores of the small intestine as a function of the fixed membrane charge. The inset shows the Donnan ratio as a function of the Ca concentration inside the intestine for a fixed charge density of 400 m.eq./liter. In making the calculations it has been assumed that no additional water can enter the pores as a result of the osmotic pressure incident to the Donnan effect, and that the pores are accessible to the solution in the lumen.

efficient, and second, that Sr appears to be able to move more freely than does Ca. The difference between the apparent membrane diffusion coefficients is a reflection of the difference in the efflux of Ca and Sr, as shown in Fig. 2.

The ionic radii as calculated from the diffusion coefficient in free solution are 2.18 Å for Ca (22, 23) and 2.24 Å for Sr (24) which are not much larger than the radius for Na of 1.77 Å. If the apparent pore radius of 36 Å given by Curran and Solomon is representative of all the pores, steric hindrance would hardly play a part in restricting the diffusion of the alkaline earths
relative to Na. However, it is not unlikely that many of the pores are smaller than the mean since the measurements did not permit any conclusion concerning the homogeneity of the apparent pore radius. A complex structure such as the intestinal mucosa could hardly be expected to contain only a single set of homogeneous pores; rather it might be characterized by sets of pores of several diameters, some in parallel and some in series. Thus, for example, the gastric mucosa has been shown to be heteroporous by Durbin, Frank, and Solomon (26), who concluded that 7 per cent of the pores had an equivalent pore radius of 60 Å while 93 per cent had an equivalent pore radius of 2.5 Å.

A consideration of the charge distribution within the pores of the membrane gives further information on the mechanism of Ca and Sr transport. When the solution in the lumen contains Na alone, the negative charges on the pores are balanced by Na counter-ions, if the pores are accessible to the solution in the lumen. However, when the perfusing solution contains Ca or Sr, these ions will displace some Na from the charged sites within the pores. The Na displacement may be calculated on the basis of the Gibbs-Donnan equation, as shown in Fig. 3. At a charge density of 400 m.eq./liter and a Ca concentration of 10 mM/liter in the lumen, the Ca counter-ion concentration is 65 mM/liter. Thus, the concentration of divalent counter-ions at the pore wall far outweighs the concentration of these ions in the free solution flowing through the pores. As a consequence, the distribution of counter-ions could play a determining role in the passage of divalent ions across the intestinal mucosa. Passage across the lumen would presumably involve the repeated transfer of an ion from site to site as it progressed along the pore.

**Relationship between Na and Water Flux**

Net Na and water fluxes were measured, as routine, in all the experiments in which Ca flux was measured. The relationship between net fluxes of Na and Ca and net water flux has been fitted by the method of least squares, leading to the following equation:

$$\Phi_w^* = (5.9 \pm 0.6)(\Phi_N^Na + 1.5\Phi_Ca^Na) + (0.01 \pm 0.01)\text{ [ml./hr.cm.]}$$

Equation 3 may be compared with the following equation given previously by Curran and Solomon (5):

$$\Phi_w^* = (6.2 \pm 0.7)(\Phi_N^Na + 0.5\Phi_Ca^Na) + (0.001 \pm 0.008)\text{ [ml./hr.cm.]}$$

in which the superscript $m$ refers to mannitol. In both equations the errors given are standard deviations, and it can be seen that the two equations do not differ from one another statistically.
Both Ca and Sr appear to have a considerable effect on net water and Na fluxes as shown in Fig. 4. Small concentrations of either ion cause a sharp increase in the net flux of water across the intestine, Sr appearing to be more effective than Ca in this respect. The shape of the curve is most unusual since it rises rapidly to a peak as the divalent ion concentration in the lumen is increased to 1 mM and then falls off much more slowly as the divalent ion concentration is increased until it reaches 25 mM. The effect of changes in NaCl concentration in the lumen on net Na flux may be calculated from Figs. 6 and 7 of reference 9. These calculations indicate that there is no significant change in net Na flux between concentrations of 100 and 150 mM Na in the lumen. Consequently, it is not necessary to correct the observations in Fig. 6 for changes in luminal Na.

The net water flux observed in the present series of experiments is 0.026 ml./(hr. cm. length) when the intestine is perfused with isotonic NaCl solution, in the absence of added Ca. This figure is considerably smaller than the value of 0.12 ml./(hr. cm. length) observed by Curran and Solomon (5)
on a shorter length of ileum. The reason for this difference is not known. It may arise from the additional trauma involved in perfusing a much longer segment of intestine, or from other differences in the detailed experimental technique. Our observations of an increased water uptake in the presence of Ca are in accord with previous observations of Rabinovitch (27) in dog intestine. He found that the addition of 2.2 mM CaCl₂ to an 0.9 per cent NaCl solution in the intestine caused an increased water absorption of the same order of magnitude as we have observed.

A working hypothesis may be advanced to account for the rising and falling phases of the curve in Fig. 4. According to this hypothesis, the rising phase of the curve is caused by an association between Ca (or Sr) and a Na carrier mechanism. The result of this association is an increased net flux of Na and water out of the intestine. The details of the association are unknown; the Ca may be attached to the carrier complex directly and make it more mobile, or may interact with the mechanism in some other fashion. Whatever the effect, it is apparent that the Ca is not transferred all the way across the mucosa in one to one apposition to the Na, since such an increase would not escape detection in Fig. 2. Consequently this limited Na-Ca process is presumably separate from the transport mechanism for Ca itself. The kinetics of the process may be treated in terms of association between the alkaline earth and an unspecified site. Such a treatment is equivalent to the usual Michaelis-Menten enzyme substrate kinetics, but is preferred in the present instance because it makes less specific implications about the nature of the site. The forward reaction, the rate of condensation of Ca on the site, is given by

\[ v_c = k_1[Ca](1 - \Theta) \]  

where \( \Theta \) is the fraction of the forward reaction sites occupied by Ca, and \( 1 - \Theta \), is the fraction unoccupied. The rate of evaporation is given by

\[ v_e = k_{-1}\Theta \]  

At equilibrium, the rates are equal, and

\[ \Theta/(1 - \Theta) = (k_1/k_{-1})[Ca] = K[Ca] \]  

It will be seen that \( K \) is the association constant for binding by the forward reaction sites. On the basis of the hypothesis, the rate of active transport of Na is proportional to the number of occupied sites, so

\[ \Phi_{Na}^a = k_2K[Ca]/(1 + K[Ca]) \]  

in which \( k_2 \) is the proportionality constant. Equation 8 predicts that the function \([Ca]/\Phi_{Na}^a\) should depend linearly on \([Ca]\). Fig. 5 shows that this
requirement is satisfied in the concentration range of 0 to 1 mM Ca. Since water flux is passive, driven by net Na flux, a similar relationship would be expected for \([Ca]/\Phi_{\text{Na}}^{0}\) as also shown in Fig. 5. Equation 8 predicts that the net efflux of Na would fall to zero in the absence of Ca in the lumen, and that Ca is therefore necessary for active Na transport. This cannot be tested under the present conditions, since the leakage of Ca from plasma into the lumen is sufficient to raise the concentration to 0.06 mM Ca, the value observed when no Ca is added to the perfusing solution. The method of least squares applied to the data for net Na flux as presented in Fig. 5 yields values of 9.2 liters/mM for \(K\) and 7.9 \(\mu\)M/(hr. cm.) for \(k_5\). In Fig. 4 these values have been used to obtain the curve plotted in the concentration range between 0 and 1 mM Ca. An analogous procedure has been used to draw the curve for net water flux between the same concentrations. A figure of 9.2 liters/mM for \(K\) corresponds to a high degree of association between Ca and site so that at a Ca concentration of 1 mM 90 per cent of the sites are filled.

When the sites for the Na forward reaction process are filled, additional Ca in the membrane is free to settle on other sites. This additional Ca in the membrane could cause a general "stiffening" of the membrane producing
an increased resistance to carrier complex movement. We may define this impedance to movement, $I$, as follows, over the range of 1 mM Ca/liter to 25 mM Ca/liter.

$$I = \frac{\Phi_n^{Na}(2\text{mM Ca})}{\Phi_n^{Na}}$$

A concentration of 2 mM has been chosen for normalization since data are available at this concentration for both Ca and Sr. Since the impedance is presumed to be dependent upon the non-specific absorption of Ca in the membrane, $I$ should be proportional to the number of sites covered by Ca, and given by:

$$I = k'_i K'[Ca]/(1 + K'[Ca]) \quad \text{1 mM < [Ca] < 25 mM (9)}$$

analogous to Equation 8. If this equation is satisfied, $([Ca] \cdot \Phi_n^{Na})$ which is proportional to $([Ca]/I)$, should bear a linear relationship to $[Ca]$, which is shown to be the case in Fig. 6. A similar relationship holds for $\Phi_n^{H_2O}$ over the concentration range 1 to 25 mM for both Sr and Ca. The values for the inhibition of Na flux have been obtained by least squares: 0.23 liters/mm for $K'$, and 3.5 for $k'_i$. Since the value of $K'$ is so much lower for the non-specific absorption process than for the specific one, it appears satisfactory to segregate the processes, and to fit the first part of the curve with one equation, and the latter part of the curve with another. The completed curve, in which the region below 1 mM Ca is fitted with Equation 8, and the region above...

**Figure 6.** Fit of Equation 9 to the experimental data.
1 mM Ca is fitted with Equation 9, is drawn in Fig. 4. It will be seen that the fit to the experimental data is quite satisfactory. Since the working hypothesis makes quite specific predictions about Na flux in the region of low Ca concentration, it should be possible to test it by further experiments. In particular, in vitro experiments should make it possible to see whether the Na flux does indeed go to zero in the absence of Ca.

Though the mechanisms of the two processes probably differ, the relationship presented between Ca concentration and Na flux bears a resemblance to the observations that have been made in nerve fibers both myelinated and unmyelinated (28). Frankenhaeuser (29) has shown that the myelinated frog nerve fiber becomes inexcitable when a Ca-free medium is applied. The effect is reversible and small traces of Ca (<0.01 mM) are sufficient to restore excitability. This observation is analogous to the prediction that Na transport in the intestine should cease in the absence of Ca. In squid axons, Frankenhaeuser and Hodgkin (30) also find that treatment with Ca-free solutions causes inactivation of the Na-carrying system. In this case, the effect is not so immediate and higher Ca concentrations, in the range of 2 to 4 mM, are required to restore conduction. In both fibers, inactivation has also been observed at high external Ca concentrations, similar to the findings in the intestine. The inactivation of the Na-carrying system in nerve is greater in low Ca than in high Ca. This observation is also analogous to the present findings: zero Ca should theoretically reduce the Na flux to zero, whereas, as shown in Fig. 4, Ca concentrations as high as 25 mM only reduce the Na flux by 74 per cent. These interesting similarities raise the question of whether any common sites exist in these otherwise dissimilar processes.

BIBLIOGRAPHY