Amino Acid and Sugar Transport in Rabbit Ileum

STANLEY G. SCHULTZ, ROBERT E. FUISZ, and PETER F. CURRAN

From the Biophysical Laboratory, Harvard Medical School, Boston

ABSTRACT L-Alanine and 3-O-methyl-D-glucose accumulation by mucosal strips from rabbit ileum has been investigated with particular emphasis on the interaction between Na and these transport processes. L-Alanine is rapidly accumulated by mucosal tissue and intracellular concentrations of approximately 50 mM are reached within 30 min when extracellular L-alanine concentration is 5 mM. Evidence is presented that intracellular alanine exists in an unbound, osmotically active form and that accumulation is an active transport process. In the absence of extracellular Na, the final ratio of intracellular to extracellular L-alanine does not differ significantly from unity and the rate of net uptake is markedly inhibited. Amino acid accumulation is also inhibited by 5 X 10^{-5} M ouabain. 3-O-methyl-D-glucose accumulation by this preparation is similarly affected by ouabain and by incubation in a Na-free medium. The effects of amino acid accumulation, of ouabain, and of incubation in a Na-free medium on cell water content and intracellular Na and K concentrations have also been investigated. These results are discussed with reference to the two hypotheses which have been suggested to explain the interaction between Na and intestinal nonelectrolyte transport.

In vitro preparations of mammalian small intestine are capable of transporting sugars and amino acids from low concentrations in the mucosal solution to higher concentrations in the solution bathing the serosal side. Further, sugars and amino acids are accumulated by intestinal tissue and the concentrations of these solutes in tissue water may reach more than ten times those in the surrounding media. These observations have led to the widely held view that intestinal sugar and amino acid absorption is the result of carrier-mediated processes which bring about uphill transport from the mucosal solution into the cell followed by downhill exit from the cell across the serosal tissues.

In recent years, interactions between Na and the transport of sugars and amino acids have been demonstrated. Thus, intestinal transport (transmural)
(4, 6, 7) and accumulation (4, 8) of these solutes require the presence of Na in the mucosal bathing solution. Similar observations have been reported for sugar (9) and amino acid (10–12) accumulation by a variety of tissues other than the intestine. In addition, active transport of sugars and/or amino acids is associated with an increase in the rate of Na transport from mucosa to serosa in rabbit (13, 14), rat (15), and human (16) small intestines. An increase in the transmural potential difference and/or short-circuit current in the presence of actively transported sugars and amino acids has been reported for several other species (17–20), but the relation between these bioelectric phenomena and Na transport has not been clearly established.

Although several hypotheses have been suggested to explain the role of Na in nonelectrolyte transport, present evidence is as yet inconclusive. This study is concerned with amino acid and sugar accumulation by mucosal tissue from rabbit ileum and was undertaken for the purpose of further clarifying the interaction between Na and these processes.

**METHODS**

Male and female white rabbits (2.5 to 4 kg) which had been maintained on normal food intake were sacrificed by intravenous administration of Nembutal. A segment of terminal ileum was rapidly resected, opened along the mesenteric border, and washed free of intestinal contents with normal buffer. The intestinal segment was then separated into a "mucosal strip" which consisted of the epithelial layer and underlying connective tissue (Fig. 1a), and a "serosal strip" which contained the muscularis mucosa, submucosa, muscular layers, and serosa (Fig. 1b). The stripping procedure was carried out with glass microscope slides and was essentially that described by Dickens and Weil-Malherbe (21). The mucosal tissue was separated into small sheets weighing 30 to 40 mg which were incubated at 37°C in appropriate buffer solution containing tritiated inulin (inulin-methoxy-H<sub>a</sub>, New England Nuclear Corp.). The tissue to medium ratio did not exceed 100 to 150 mg wet weight of tissue per 5 ml buffer. The medium was bubbled with a humidified mixture of 95% O<sub>2</sub>-5% CO<sub>2</sub> throughout the incubation period.

Carbon-14-labeled L-alanine or C<sup>14</sup> 3-O-methyl-D-glucose was added to the incubation medium after 15 to 30 min. The initial incubation period permitted stabilization of intracellular cation concentrations as well as equilibration of inulin in the extracellular space. At varying times after the addition of amino acid or sugar, tissue was removed, gently blotted on Whatman No. 1 filter paper, and cut into two portions of approximately equal size each of which was immediately weighed on a quartz-helix microbalance (Misco). This entire procedure was usually completed within 1 min and water loss due to evaporation during this period was shown to be negligible. One portion of tissue was used for determination of the dry-to-wet weight ratio after drying at 105°C for 48 hr. The second portion was extracted for 48 hr in 2.0 ml 8 mM Li<sub>2</sub>SO<sub>4</sub> at 4°C. Examination of the residue indicated that less than 5% of the total C<sup>14</sup> remained in the tissue. This fraction could not be eluted by longer periods of extraction. Aliquots of extract and suitably diluted aliquots of incubation medium
Figure 1. (a) Section of mucosal strip obtained by the method of Dickens and Weil-Malherbe (21). (b) Section of "unstripped" rabbit ileum. Tissue was fixed in Bouin's solution and stained with hematoxylin and cosin. \( \times 120 \). Comparison of Figs. 1a and 1b reveals that the mucosal strip consists of the epithelial lining and the underlying lamina propria. The stripping procedure has removed the muscularis mucosa, submucosa, muscularis externa, and serosa. The authors are indebted to Dr. S. Ito, Department of Anatomy, Harvard Medical School, for assistance in obtaining these photomicrographs.
were assayed simultaneously for C¹⁴ and H² content using a liquid scintillation spectrometer (Nuclear Chicago Corp., model 6801) as well as for Na and K content using an internal standard flame photometer (Instrumentation Laboratory, Inc., model 143). The combined error in the determination of cell solute content arising from both incomplete isotope separation and differential quenching of extract and medium did not exceed 4%. Cell concentrations of amino acid or sugar, Na, and K were calculated after correction for the contents of the inulin space, assuming that the concentrations in the inulin space are equal to those in the incubation medium. Four experiments were carried out in each of which 5 pieces of tissue were treated in identical manner. The average standard errors of the intracellular concentrations of alanine, Na, and K were ± 4.0%, ± 8.5%, and ± 3.6% respectively.

The composition of the normal buffer was NaCl 140 mM; KHCO₃ 10 mM; K₂HPO₄ 1.2 mM; KH₂PO₄ 0.2 mM; CaCl₂ 1.2 mM; and MgCl₂ 1.2 mM. Several investigators have shown that this relatively high K concentration (13 mM) is optimal for both uptake and transport of sugars and amino acids (11, 22) as well as for respiratory activity by isolated rat intestinal cells (23). The final pH was 7.2 after equilibration with 95% O₂-5% CO₂. Na-free media were prepared by replacing NaCl with either choline chloride or KCl or with its osmotic equivalent of mannitol. None of the media employed in this study contained glucose; the only nonelectrolytes present at any time were either L-alanine (Nutritional Biochemical Co.) or 3-O-methyl-D-glucose (Calbiochem).

Results and Discussion

Extracellular Space

The calculation of intracellular solute concentrations, as described above, rests heavily on the assumption that the inulin space is a reliable measure of the tissue extracellular space. Ideally, any molecule used for the determination of the extracellular space of a complex tissue should be completely excluded from the cells and yet should equilibrate with the entire extracellular volume. In the present study, inulin spaces determined on more than 250 mucosal strips incubated for 15 to 85 min ranged in value between 0.18 and 0.40 ml/g wet weight; the average value of 161 determinations was 0.253 ± 0.006 (±σ) ml/g wet weight and the space was independent of the duration of incubation. The range of values encountered may be attributed largely to the variability of the blotting procedure. Spaces determined using C¹⁴-inulin and H²-inulin simultaneously in two experiments agreed to within 4%. The observation that the inulin space is both time-independent and considerably less than the total tissue water is strong evidence that inulin is excluded from the cells. Other investigators have arrived at similar conclusions using intestinal tissue from rabbits and other species (4, 24).

The extent to which inulin permeates the entire extracellular volume is much more difficult to establish. Page (25), in a detailed study of the extracellular space of papillary muscle, has demonstrated that both inulin and
mannitol are excluded from the cells, but that the mannitol space is considerably greater than the inulin space. Table I shows a comparison of the inulin and mannitol spaces of 4 mucosal preparations determined using C-14-inulin and H-2-mannitol simultaneously. Cell water contents and intracellular Na and K concentrations calculated using these different spaces are also shown. The mannitol space is significantly greater than the inulin space. The possibility that mannitol is not completely excluded by the cell and that its rate of entrance could be influenced by our experimental procedures made it unsuitable for use in the present study. Thus in the present experiments the "true" extracellular space may be somewhat larger than the inulin space employed in our calculations and is probably between 0.25 and 0.35 ml/g wet weight. As indicated by the data in Table I, the effect of under-

**TABLE I**

**COMPARISON OF INULIN AND MANNITOL SPACES**

<table>
<thead>
<tr>
<th>Space</th>
<th>Cell water</th>
<th>[Na]</th>
<th>[K]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ml/g wet weight</td>
<td>ml/g dry weight</td>
<td>mmol/liter cell H2O</td>
</tr>
<tr>
<td>C-14-inulin</td>
<td>0.22±0.01</td>
<td>3.91±0.13</td>
<td>66±5</td>
</tr>
<tr>
<td>H-2-mannitol</td>
<td>0.35±0.01</td>
<td>2.89±0.04</td>
<td>48±4</td>
</tr>
</tbody>
</table>

* Cell water, Na, and K corrected for size and content of the inulin or mannitol space assuming the space to be identical in composition with the incubation medium. The average dry-to-wet weight ratio was 0.16±0.01. All errors are standard errors of the mean.

estimating the true extracellular space is to lower the intracellular concentration of all substances concentrated by the cell and to raise the intracellular concentration of all substances whose concentration in the cell is lower than that in the medium.

**Recovery of C-14-Alanine**

Calculation of cell concentrations also involves the assumption that tissue C-14 content is a valid measure of amino acid or sugar content. There is abundant evidence that 3-O-methyl-D-glucose is not metabolized by mammalian tissue and that it may be recovered from intestinal tissue in unaltered form (6). There are, however, conflicting reports in the literature regarding the metabolism of l-alanine by intestinal tissue. Finch and Hird (3), Matthews and Laster (26), and Lin et al. (27) have reported that more than 90% of the initial l-alanine could be recovered following 30 to 60 min incubations with intestinal preparations from the rat and hamster. Larsen, Ross, and Tapley (28) have reported, however, that uniformly labeled C-14-l-alanine was metabolically altered during transport across everted sacs of rat small intestine; the fraction of the initial alanine which was metabolized is not indicated. Because of this uncertainty, recovery of C-14-l-alanine was checked
by ascending paper chromatography using a n-butanol–acetic acid–water solvent system (29). Tissue was incubated with C\textsuperscript{14}-L-alanine and extracted as described above. Samples of extract and incubation medium were chromatographed together with L-alanine as control. An end window paper chromatograph scanner was used to locate the C\textsuperscript{14} label and the alanine was identified by the use of the ninhydrin color reaction. In both the extract and incubation media, at least 95\% of the C\textsuperscript{14} label migrated with the amino acid.

**Intracellular Na and K in the Absence of Amino Acid or Sugar**

The first row in Table II shows the mean cell contents of water, Na, and K as well as intracellular Na and K concentrations determined on mucosal strips incubated for 60 min in normal buffer but in the absence of either amino acid or sugar. Fewer determinations on samples incubated for 30 and 45 min gave similar values. However, tissue removed prior to 20 min after the onset of incubation had somewhat higher intracellular Na concentrations and correspondingly lower intracellular K concentrations, suggesting that the cells gain Na and lose K during manipulation at room temperature but that these movements are reversed following restoration of more nearly physiological conditions. The ability of the tissue to maintain an intracellular K concentration which is much greater than that in the external medium and a relatively low cell Na concentration in the absence of exogenous substrate is probably attributable to the metabolism of endogenous nutrient which has been described by Newey et al. (30) for rat small intestine. These observations are consistent with the finding that isolated rabbit ileum will actively transport Na from mucosa to serosa in the absence of added substrate for periods exceeding 2 hr (31).

Gilles-Baillien and Schoffeniels (32) have recently demonstrated that in

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**Table II**

<table>
<thead>
<tr>
<th>Cell contents</th>
<th>Intracellular concentrations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>Na</td>
</tr>
<tr>
<td>(\mu l/mg) dry weight</td>
<td>(\mu mol/mg) dry weight</td>
</tr>
<tr>
<td>Control</td>
<td>3.38±0.09</td>
</tr>
<tr>
<td>L-alanine(5 mM)</td>
<td>3.78±0.06</td>
</tr>
<tr>
<td>(P)</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

* Data based on 19 paired samples from 5 animals. Control and L-alanine tissues were incubated simultaneously in normal buffer containing 140 mM Na and 13 mM K. L-Alanine was added after an initial incubation period of 30 min duration and the tissue was removed after an additional 30-min period had elapsed.
the small intestine from the Greek tortoise the cell interior is electrically negative with respect to both the serosal and mucosal solutions. Preliminary studies carried out in this laboratory suggest a similar electrical profile for in vitro rabbit ileum (33). Together with the observation that the intracellular Na concentration is lower than that in the surrounding medium, these findings lead to the conclusion that Na movement across the mucosal membrane into the cell takes place in the direction of its electrochemical potential gradient, and that the process responsible for active Na transport across the tissue is located on or near the serosal membrane. This location is also sufficient to account for the observation that ouabain rapidly abolishes active transmural Na transport only when added to the serosal solution (31).

Uptake of L-Alanine

A typical time course of L-alanine uptake by mucosal strips incubated in normal buffer containing 5 mM L-alanine is shown in Fig. 2. The initial rate of uptake is very rapid and the intracellular concentration approaches that of the external medium within 2 min. The steady-state intracellular L-alanine
concentration is attained between 15 and 30 min and, in 39 observations, averaged $47 \pm 3$ mM (or approximately 9 times that in the incubation medium). The steady-state level is relatively time-independent since intracellular concentrations determined between 30 and 60 min of incubation did not differ significantly from each other.

The conclusion that L-alanine accumulation by intestinal cells is an active process requires the demonstration that the amino acid exists in free solution in the cell water and that it is not bound or adsorbed within the cell. The second row in Table II shows the intracellular water, Na, and K contents as well as Na, K, and L-alanine concentrations of mucosal tissue incubated for 30 min in normal buffer containing 5 mM L-alanine. The data given in the first row of this table, which were previously discussed, were obtained simultaneously on tissue from the same animals. After 30 min the intracellular L-alanine concentration achieved a level of 51 mM. Concomitantly there is a significant increase in the cell water content per milligram dry weight ($P < 0.01$) and decreases in both the intracellular Na and K concentrations. The intracellular Na and K contents of tissue which had accumulated L-alanine do not differ significantly from those in the tissue which was incubated under identical conditions but in the absence of amino acid. If all L-alanine entering the cell compartment is assumed to be osmotically active, the predicted final cell water content is $3.90 \pm 0.10 \mu l/mg$ dry weight which does not differ significantly from the observed value of $3.78 \pm 0.06 \mu l/mg$ dry weight. Thus, L-alanine exists within the cell in an unbound, osmotically active form and its accumulation is accompanied by an increase in cell water content of sufficient magnitude to maintain isosmolarity. Together with our chromatographic evidence that L-alanine is not metabolically altered within the cell, these data permit the unequivocal conclusion that L-alanine accumulation by intestinal cells is an active transport process. Christensen and Riggs (34) have similarly demonstrated that glycine accumulation by Ehrlich ascites tumor cells is accompanied by an increase in cell water content, but a clear demonstration of this phenomenon for the intestine has not been reported previously.

### Table III

<table>
<thead>
<tr>
<th>Alanine concentration</th>
<th>mmol/liter total tissue water</th>
<th>mmol/liter nonulin space water</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mucosal strip</td>
<td>40±2</td>
<td>60±5</td>
</tr>
<tr>
<td>Serosal strip</td>
<td>8±1</td>
<td>11±2</td>
</tr>
<tr>
<td>Whole intestine</td>
<td>38±3</td>
<td>45±4</td>
</tr>
</tbody>
</table>

* Tissue obtained from the same animal incubated for 30 to 40 min in normal buffer containing 5 mM L-alanine.
Finally, it is of interest to examine the steady-state intracellular L-alanine concentrations of the mucosal strip, the serosal strip, and an adjacent portion of "total intestine" obtained from the same animal and incubated simultaneously in the presence of 5 mM L-alanine. Such a comparison is useful for the interpretation of previously reported data on amino acid uptake of whole intestine (3, 4) as well as for the evaluation of possible errors in the present data arising from nonepithelial tissues in the mucosal strip which exclude inulin and thus contribute to the "extracellular space." The data presented in Table III indicate that L-alanine concentration in "noninulin space" of the total tissue is approximately 75% of that in the mucosal strip, and that the serosal strip, which consists primarily of the smooth muscle layers, achieves an intracellular concentration twice that of the incubation medium. These results clearly demonstrate that contamination of our preparation with non-epithelial elements leads, at worst, to an underestimate of the intracellular L-alanine concentration in the transporting cells.

**Effects of Na and Ouabain on L-Alanine Uptake**

Results of experiments in which tissue was incubated for 30 min in Na-free buffer solutions and in normal buffer containing 5 × 10⁻⁶ M ouabain, are given in Fig. 3 and Table IV. As shown in Fig. 3, incubation in the normal buffer resulted in an average intracellular L-alanine concentration which was 8.5 times that in the medium while the ratio of intracellular to extracellular L-alanine concentrations following incubation in the Na-free, choline medium or in the presence of ouabain did not differ significantly from unity. Incubation in the KCl medium resulted in ratios significantly less than 1.0. Table IV gives the cell water content and intracellular Na and K concentrations for the experiments shown in Fig. 3. Incubation in Na-free media results in a rapid depletion of intracellular Na. The results given are those obtained after 45 to 60 min of incubation in these media; similar intracellular Na concentrations are observed after 20 min. When Na is replaced with choline, intracellular K concentration does not differ significantly from that observed in the presence of normal buffer, but cell water content is significantly reduced. These results suggest that choline does not readily enter the cells and that the decrease in cell water content is the result of a net loss of Na and some K accompanied by anions, presumably Cl. When Na is replaced by K there is a marked increase in intracellular K concentration and cell water content. Cell swelling in the presence of high K media has been observed with many tissues including intestine (35) and kidney slices (11) and is attributed to a net gain of KCl by the cells. The average net uptake of L-alanine during a 30 min incubation in high K medium expressed relative to tissue dry weight (14.9 µmol/g dry weight) does not differ significantly from uptake in the choline medium (14.6 µmol/g dry weight). Thus, the lower intracellular L-alanine concentrations observed in the presence of K are entirely the result
Figure 3. L-Alanine concentration ratios under different conditions. All tissues were preincubated for 20 min under the conditions indicated and then for 30 min in the presence of 5 mM L-alanine. Bars indicate ±1 se of the mean. Number of experiments are shown in parentheses.

of differences in cell water content, and cannot be ascribed to a specific inhibitory effect of K on alanine movement.

Ouabain brings about a marked and rapid increase in cell Na so that after 30 min the ratio of intracellular to extracellular Na concentrations does not

<table>
<thead>
<tr>
<th>TABLE IV</th>
<th>CELL WATER CONTENT AND CATION CONCENTRATIONS</th>
<th>DURING L-ALANINE UPTAKE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cell water</td>
<td>[Na]</td>
</tr>
<tr>
<td></td>
<td>ml/g dry weight</td>
<td>mol/liter cell water</td>
</tr>
<tr>
<td>Normal buffer (20)</td>
<td>4.02±0.17</td>
<td>51±5</td>
</tr>
<tr>
<td>KCl medium (4)</td>
<td>8.32±0.46</td>
<td>11±3</td>
</tr>
<tr>
<td>Choline medium (5)</td>
<td>2.93±0.24</td>
<td>11±3</td>
</tr>
<tr>
<td>Ouabain (5×10⁻⁶ M) (9)</td>
<td>4.06±0.23</td>
<td>152±7</td>
</tr>
</tbody>
</table>
differ significantly from unity. Concomitantly there is an increase in cell water content compared to control tissue incubated without L-alanine (Table II, column 1). There is also a decrease in cell K concentration, but at a time when the Na concentration difference has been abolished, the cell K concentration is still significantly greater than that in the incubation medium (13 mM). The effect of ouabain on cell cation concentrations is entirely consistent with observations on a large variety of tissues (36) and with the finding that active transport of Na across isolated segments of rabbit ileum is completely inhibited in the presence of this glycoside (31).

![Graph showing time course of L-alanine uptake](image)

**Figure 4.** Time course of L-alanine uptake from Na-free choline medium and from normal buffer containing $5 \times 10^{-4}$ M ouabain. Medium L-alanine concentration was 5 mM.

The effect on L-alanine uptake of incubation in a Na-free choline medium cannot be attributed to irreversible tissue damage. In 2 experiments, tissue was incubated for 20 min in choline medium and was then placed in normal buffer containing 142 mM Na and 5 mM L-alanine. After 15 min the intracellular L-alanine concentration averaged 40 mM and did not differ significantly from that of tissue from the same animal which was preincubated in normal buffer and not exposed to a Na-free medium.

The time course of L-alanine uptake in Na-free choline medium is shown in Fig. 4. Uptake of L-alanine from a Na-free medium and from normal buffer differs in two respects. First, in the absence of Na the ratio of intracellular to extracellular L-alanine concentrations approaches a limiting value of 1.0 in contrast to a value of 8 to 9 for the control. Second, the rate of net uptake from Na-free medium is considerably slower than the rate of uptake from the normal buffer. The effect of ouabain on the time course of L-alanine uptake
from normal buffer is also shown in Fig. 4. These results clearly indicate that Na replacement and ouabain not only prevent accumulation of high concentrations of amino acid within the tissue but also markedly reduce the rate of net uptake of L-alanine. The data shown in Figs. 2 and 4 cannot be used to estimate meaningful unidirectional fluxes of L-alanine for two reasons. First, the amino acid is moving across both the mucosal and serosal membranes of the cells and, with the methods used, there is no way of evaluating the contributions of these separate processes. Second, kinetic analysis of these nonsteady state experiments requires some assumption with respect to the relation between unidirectional fluxes and intracellular alanine concentration; there is, at present, no information on which to base such an assumption.

**Uptake of 3-O-Methyl-d-Glucose**

Fig. 5 and Table V show the results of incubating mucosal strips for 30 min in normal buffer containing 2 mM 3-O-methyl-d-glucose (3-MG) after an initial preincubation of 20 min duration. The effects of Na replacement with choline, K, or mannitol, and the effects of ouabain and phlorizin are also shown. The intracellular to extracellular 3-MG concentration ratio of 2.76 ± 0.43 is in good agreement with that reported by Crane and Mandelstam (2).
for 3-MG uptake by intact strips of hamster small intestine. The effects of Na replacement and ouabain on 3-MG uptake are similar to those observed with L-alanine except that, after 30 min of incubation, intracellular 3-MG concentrations were still significantly less than that in the medium. Since, in this preparation, net accumulation is the result of movements across both the serosal and mucosal aspects of the tissue, these quantitative differences between L-alanine and 3-MG uptake cannot be further clarified in the absence of knowledge of the bidirectional fluxes of these species across the different surfaces of the cell.

The inhibitory effect of $10^{-4}$ M phlorizin on 3-MG uptake by rabbit mucosal strips (Fig. 5) is in complete agreement with the results reported by Crane and Mandelstam (2) for the effect of this agent on sugar uptake by hamster intestine. Newey et al. (30) have demonstrated that, at low concentrations, phlorizin inhibits glucose entry into the intestinal cell across the mucosal membrane without affecting tissue metabolism; at higher concentrations, however, phlorizin inhibits endogenous tissue metabolism. As is seen in Table V, the presence of phlorizin resulted in a significant increase in cell water content and a decrease in the intracellular K concentration. However, since this concentration of phlorizin did not inhibit L-alanine uptake by the mucosal strip, the effect on 3-MG uptake may be attributed to specific interference with sugar transport rather than inhibition of metabolic processes.

**CONCLUSIONS**

Studies of transport across rabbit ileum *in vitro* have demonstrated that: (a) sugar and amino acid transport takes place from the mucosal to the serosal solution in the absence of, or against, chemical potential differences; and (b) net transport of sugar and amino acid requires the presence of Na in the mucosal solution, and is abolished by low concentrations of ouabain added to the serosal solution (4, 37). The present results indicate that the effects of

**TABLE V**

**CELL WATER AND CATION CONCENTRATIONS DURING 3-O-METHYL-D-GLUCOSE UPTAKE**

<table>
<thead>
<tr>
<th></th>
<th>Cell water</th>
<th>[Na]</th>
<th>[K]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ml/g dry weight</td>
<td>mmol/liter cell water</td>
<td></td>
</tr>
<tr>
<td>Normal buffer (20)</td>
<td>3.81±0.22</td>
<td>54±5</td>
<td>147±9</td>
</tr>
<tr>
<td>KCl medium (12)</td>
<td>8.38±0.33</td>
<td>7±1</td>
<td>171±11</td>
</tr>
<tr>
<td>Choline medium (3)</td>
<td>3.07±0.29</td>
<td>8±3</td>
<td>130±18</td>
</tr>
<tr>
<td>Mannitol medium (3)</td>
<td>2.51±0.44</td>
<td>14±4</td>
<td>155±15</td>
</tr>
<tr>
<td>Phlorizin (10⁻⁴M) (6)</td>
<td>4.76±0.17</td>
<td>56±5</td>
<td>116±4</td>
</tr>
<tr>
<td>Ouabain (5 X 10⁻⁵M) (6)</td>
<td>4.41±0.28</td>
<td>148±4</td>
<td>48±11</td>
</tr>
</tbody>
</table>
Na and ouabain on sugar and amino acid accumulation by the mucosal cells are analogous to their effects on the transmural transport of these non-electrolytes. They lend further support to the current hypothesis that absorption is the result of sequential processes consisting of (a) carrier-mediated processes transporting amino acids or sugars across the brush-border from a low concentration in the mucosal solution to a higher concentration in the cell water, (b) accumulation of the solute within the cell in an unbound osmotically active form, and (c) downhill movement of solute from the cell, across the serosal membrane into the subepithelial tissues. There is at present little information bearing on the nature of the exit process and studies of the unidirectional fluxes of these nonelectrolytes across the serosal membrane are required to determine whether these movements can be attributed to simple diffusion or whether carrier-mediated processes must be invoked.

The recently published autoradiographic study of Kinter and Wilson (38) suggests the presence of carrier-mediated processes at the serosal border which, under the conditions of their study, are capable of uphill transport of sugars and amino acids across the serosal membrane into the cell. The significance of this finding with respect to the absorptive process is unclear. Since most cell membranes are only slightly permeable to nonelectrolytes having five or more carbon atoms (39), some carrier process is probably involved in the movement of large, and in some cases charged, amino acids across the serosal membrane.

Although previous investigators have demonstrated amino acid accumulation by intestinal tissue (3, 4), their use of preparations containing the muscle and serosal layers has raised some question as to the location of the amino acid within the tissue. Thus it would be possible, as suggested, in part, by Newey and Smyth (40), that the concentration of amino acid within the transporting cell is quite low; that the site for uphill transport is on or near the serosal membrane; and that the observed accumulation is due to retention of amino acid within the subepithelial spaces by diffusion barriers. The absence of formed subepithelial elements in the present studies (Fig. 1 a) makes this interpretation improbable. Furthermore, the autoradiographic study of Kinter and Wilson (38), and Huang’s (41) recent demonstration of amino acid accumulation by isolated intestinal cells leave little doubt that the location of the major amino acid pool is within the epithelial cell.

The removal of Na from the incubation medium has two effects on sugar and amino acid uptake by the mucosal preparation. First, uphill transport is abolished and the final intracellular concentration achieved does not significantly exceed that in the incubation medium. Second, the rate at which a concentration ratio of unity is achieved is markedly reduced so that the rate of net uptake is slowed even when this movement is in a downhill direction. These findings are qualitatively similar to those which have been reported.
for sugar (8, 42) and amino acid (4) uptake by hamster and rabbit intestine and are sufficient to account for the inability of the tissue to accomplish active transmural transport of these solutes in the absence of Na, providing the only processes capable of uphill transport are those located at the mucosal border of the cell.2

Two hypotheses have been suggested to explain the Na requirement for active sugar and amino acid transport by intestine. Crane (43) has proposed that sugars enter the intestinal cell by means of a ternary sugar-Na-carrier complex; that accumulation is dependent, in part, upon the difference between intracellular and extracellular Na concentrations; and that the driving force for sugar accumulation is derived from the Na concentration gradient which is maintained by a ouabain-sensitive extrusion mechanism. According to this hypothesis, the major effect of Na is to increase the affinity of the carrier for sugar. Thus, in a Na-free medium, carrier transport of sugar is drastically reduced in the presence of relatively low sugar concentrations. Csáky, on the other hand, has argued that nonelectrolyte active transport processes are the results of interactions between two systems: (a) carrier sites with which substrates combine and which are responsible for the translocation of hydrophilic solutes across a lipid barrier; and (b) a system which couples energy to the carrier process and thereby enables it to accomplish osmotic work (44). Csáky suggests that it is the energy-yielding system which requires intracellular Na for its activation and which is inhibited by the cardiac glycosides. According to this hypothesis, removal of Na from the mucosal solution inhibits nonelectrolyte active transport because it results in a depletion of the intracellular Na pool. The translocation mechanism, according to Csáky, does not itself require Na, but in the absence of Na is incapable of uphill transport and behaves as a “facilitated diffusion” system (44).

Since the removal of Na from the incubation medium leads to a depletion of intracellular Na (and thus disappearance of the Na concentration difference), both models predict a steady-state intracellular solute concentration which, under these conditions, does not differ from that in the surrounding medium. Thus, this observation does not distinguish between them. A decreased rate of net solute uptake in the absence of Na is also predicted by both models. Assuming, for the moment, that net movements across the serosal and lateral aspects of the cell are unimportant, the rate of net uptake is the difference between unidirectional influx and efflux across the mucosal membrane. According to Crane’s model, unidirectional influx across the mucosal membrane would be inhibited in the absence of Na. Csáky’s hypothesis is con-

3 In this discussion we do not explicitly raise the possibility that more than one transport mechanism for L-alanine or for 3-O-methyl-β-glucose is present at the mucosal border of the cell. While several transport mechanisms having different Na dependencies for each of these substrates are conceivable, this possibility does not influence the interpretation of the present results.
sistent with a model which could easily predict that the inhibition of the energy-yielding reaction, which provides the asymmetry for the carrier process, leads to either an increased carrier-mediated efflux or a decreased influx across the mucosal membrane.

This latter point may be illustrated by considering an expression for carrier flux derived by Wilbrandt and Rosenberg (45). Their expression (equation 20) for a carrier system linked to metabolism takes the form

\[ V = V_m \left[ \frac{S_o}{S_o + K_m(1 + K_o)} - \frac{S_i}{S_i + K_m(1 + K_i)} \right] \]

in which \( V \) is the rate of net transport, \( V_m \) is its maximum value, \( S_o \) and \( S_i \) are substrate concentrations outside and inside the cell respectively, and \( K_m \) is the so called Michaelis constant of the carrier system. \( K_i \) and \( K_o \) are equilibrium constants for metabolic reactions leading to conversion of carrier to a nonactive form and the reverse. A number of assumptions are involved in the derivation of this expression, but they are not important for the present case. The major points of interest are as follows. If the effect of metabolism is at the inner side of the membrane only, \( K_o \) = 0, \( K_i \) > 0; inhibition of the metabolic step by Na removal would decrease \( K_i \) and unidirectional efflux would increase. If the metabolic event is at the outside of the membrane, \( K_o \) > 0, \( K_i \) = 0; inhibition would increase \( K_o \) and influx would decrease. It is of interest to note that in this latter case the effect of Na removal would be to markedly increase the "effective \( K_m \)" for influx of sugar or amino acid. Thus Crane's model predicts a decreased influx, Csáky's model is consistent with an increased efflux or a decreased influx, and both models are consistent with the experimental finding of a decreased rate of net uptake. Further, under certain rather restricted conditions, both models could predict a marked influence of Na on the "\( K_m \)" for sugar or amino acid influx. Thus it appears that kinetic studies and observations such as those reported here are not ideally suited for distinguishing between these two hypotheses. The observation that ouabain abolishes the difference between intracellular and extracellular Na concentrations and inhibits accumulation of both sugars and amino acids is consistent with the hypothesis that uphill transport of sugars and amino acids is coupled to the presence of a Na activity difference across the mucosal membrane. One cannot, however, rule out Csáky's suggestion that ouabain directly affects the transport mechanism for sugars and amino acids in addition to its effect on the Na extrusion mechanism.

Crane's demonstration of uphill sugar transport out of mucosal cells when intracellular Na concentration is higher than that in the surrounding medium (46) as well as the observation that active sugar and amino acid transport is accompanied by an increased rate of active Na transport (13, 14) is strong evidence in favor of his hypothesis. However, on the basis of the above con-
siderations, we come to the conclusion that studies of the unidirectional fluxes of both sugars and amino acids across the mucosal border of the cell during the process of net uptake, together with simultaneous studies of unidirectional influx of Na, are necessary before the two models can be clearly distinguished. Studies of the exchange of labeled sugars (42) or amino acids (4) with tissues that have been preloaded with the nonelectrolyte in both the presence and absence of external Na have shown decreased rates of influx in the absence of Na. The interpretation of these findings is complicated by the possibility of "transconcentration effects" (45). Thus the unidirectional influx in the absence of Na may be low because the cells have a low steady-state level of the transported solute as opposed to a high level in the presence of Na. These studies are also complicated by the fact that in the preparations used there is no way of distinguishing between influx across the mucosal and serosal faces of the cells. Until information on the properties of these separate membranes is available, studies of total fluxes between the cell and its environment must be interpreted with caution.

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