Relations among Transepithelial Sodium Transport, Potassium Exchange, and Cell Volume in Rabbit Ileum

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ABSTRACT The relation between active transepithelial Na transport across rabbit ileum and 42K exchange from the serosal solution across the basolateral membranes has been explored. Although 42K influx across the basolateral membranes is inhibited by ouabain and by complete depletion of cell Na, it is not affected when transepithelial Na transport is abolished (i.e. in the presence of an Na-free mucosal solution) or stimulated (i.e. when glucose or alanine is added to the mucosal solution). We are unable to detect any relation between the ouabain-sensitive Na-K exchange mechanism responsible for the maintenance of intracellular Na and K concentrations and active transcellular Na transport. In addition, the maintenance of cell volume (water content) does not appear to be dependent upon transepithelial Na transport or the ouabain-sensitive Na-K exchange pump. Although the results of these studies cannot be considered conclusive, they raise serious questions regarding the role of the Na-K exchange pump, located at the basolateral membranes, in active transepithelial Na transport and the maintenance of cell volume.

In 1958, Koefoed-Johnsen and Ussing proposed a model for Na transport by isolated frog skin that has provided a major conceptual framework for the interpretation of studies on a variety of epithelia (1, 2). A central feature of this model is the notion that the same mechanism is responsible for homocellular and transcellular Na transport. That is, an active Na-K exchange mechanism located at the inner membrane was presumed to be responsible for maintaining the low intracellular Na concentration and the high intracellular K concentration characteristic of most animal cells, as well as active transcellular Na transport.

Some findings on in vitro rabbit ileum are consistent with this notion. Thus, ouabain inhibits active Na absorption only when it is present in the serosal solution; the presence of this glycoside in the mucosal solution alone is ineffective (3). And, concomitant with the inhibition of transepithelial Na transport there is a loss of cell K and a gain of cell Na (4). Further, autoradiographic studies (5) and enzymic analyses of membrane fragments isolated from intestinal cells (6, 7) have localized ouabain binding sites and the ouabain-sensitive, Na-K stimulated ATPase to the basolateral membranes; little or no ouabain binding or Na-K-ATPase activity is found in the brush border.
Rose and Schultz (8) and Frizzell and Schultz (9) have presented evidence that the active Na extrusion mechanism at the basolateral membranes of rabbit ileum is rheogenic (or electrogenic) and thus cannot involve a one-for-one exchange of cell Na for K in the serosal solution. However, this conclusion per se does not exclude the possibility that Na extrusion from the cell is mediated by an Na-K exchange mechanism since it is well established for erythrocytes (10) and nerve and muscle (11) that, in general, the stoichiometry of the Na-K exchange pump is not one-for-one but that the Na:K ratio often exceeds unity.

Finally, Schultz and Zalusky (3) reported that active Na transport by rabbit ileum is not affected when the tissue is bathed for more than 1 h by "nominally" K-free media (<0.3 mM). The interpretation of this finding was complicated by the possibility that leakage of K from the cells resulted in a local K concentration within the villus core that was greater than that in the serosal solution. These authors concluded that, barring this reservation, their results suggested that active Na transport by rabbit ileum may not be necessarily coupled to K transport and may be electrogenic. The present study was undertaken in order to examine directly the relation between transepithelial Na transport and K uptake across the basolateral membranes of rabbit ileum.

MATERIALS AND METHODS

Male white rabbits (2–4 kg) were sacrificed with intravenous pentobarbital and a section of terminal ileum was excised, opened along the mesenteric border, and rinsed free of intestinal contents with a Ringer solution that contained (mM): NaCl, 119; NaHCO3, 21; K2HPO4, 2.4; KH2PO4, 0.6; CaCl2, 1.2; MgCl2, 1.2. The pH of this solution, when gassed with a mixture of 95% O2-5% CO2 at 37°C, is 7.4. The serosal musculature and connective tissues were carefully removed with a pair of fine forceps as described previously (12); the remaining mucosal strip consists of only the epithelial cell layer the underlying lamina propria and scattered fragments of muscularis mucosa (see Fig. 1 in reference 12). The mucosal strips were then mounted serosal surface up on a supporting layer of nylon mesh between the two half-chambers shown in Fig. 1. This apparatus permits exposure of the mucosal and serosal surfaces of the tissue to readily accessible solutions of desired composition. The serosal solution (upper chamber) was vigorously bubbled with 95% O2-5% CO2 and the mucosal solution (lower chamber) was gently bubbled with the same gas mixture and rapidly stirred with a magnetic stirrer. All experiments were carried out at 37°C in a warm room and the apparatus permitted simultaneous studies of the effects of different experimental conditions on eight segments of tissue (each 1.13 cm², serosal area) from the same animal; the compiled results of such studies are referred to as "paired determinations."

The general approach used for the determination of 42K uptake from the serosal solution across the basolateral membranes is as follows (detailed descriptions will be presented in Results): (a) after mounting, the tissues were bathed by mucosal and serosal solutions of desired composition for varying periods; (b) 14C injulin was added to the serosal solution and 3H injulin was added to the mucosal solution. In every instance the tissue was exposed to these extracellular markers for at least 10 min — this is sufficient to achieve more than 80% equilibration with the total inulin space (see below); (c) 42K was then added to the serosal solution; (d) the tissue was exposed to all three radioactive isotopes for varying periods of time, and at the end of the exposure period samples of both bathing media were withdrawn and the two chambers were emptied. The exposed area of tissue was punched out with a steel punch, gently blotted, weighed in tared
containers to the nearest 0.1 mg, and then extracted in 0.1 N HNO₃ for 20 h. Preliminary studies in which the extracted tissues were homogenized and re-extracted indicated that more than 98% of chemical and radioactive K and Na as well as labeled inulin is extracted within 20 h. These findings are in agreement with those reported previously (4).

Aliquots of the extract and bathing media were assayed simultaneously for ³H, ¹⁴C, and ⁴²K by use of a triple-channel liquid scintillation counter. The Na and K contents of the tissue extract were determined with an internal Li-standard flame photometer. The volumes of the extracellular spaces (ECS) accessible from the mucosal and from the serosal solutions were calculated from the ³H and ¹⁴C contents of the bathing media and extract; the total extracellular space is simply the sum of these two spaces.

The volume of intracellular water was calculated from the wet weight and the total ECS by using a dry weight:wet weight ratio of 0.180 ± 0.002 (SEM) derived from an analysis of 102 mucosal strips. Previous studies (4, 13) and observations in the present study indicate that this value is essentially invariant even under conditions that lead to changes in intracellular volume. Cell swelling or shrinking appear to influence the size of the ECS but do not significantly affect the dry weight:wet weight ratio.

Intracellular Na and K concentrations ([Na]ᵢ and [K]ᵢ) were calculated from the Na and K contents of the extract after correction for the Na and K contents of the mucosal and serosal inulin spaces on the assumption that the concentrations of these ions in each ECS are equal to those in the adjacent bathing media. The values are expressed in mM on the assumption that 1 kg cell water is equivalent to 1 liter cell water and that all cell water is accessible to these ions.

Intracellular ⁴²K content was calculated from the isotope content of the extract after correction for the isotope content of the appropriate ECS. The specific activity of intracellular ⁴²K, (K*ᵢ), is the ratio of cell ⁴²K (Kᵢ), to cell K content (Kᵢ), and is independent of cell volume.

Na-free solutions were prepared by replacing NaCl and NaHCO₃ with the choline salts. K-free solutions were prepared by replacing K₂HPO₄ and KH₂PO₄ with the Na salts.
$^{22}$Na, $^{42}$K, labeled inulin (mol wt 5,000-5,500), mannitol, and polyethylene glycol (mol wt 4,000) were obtained from New England Nuclear (Boston, Mass.); all other reagents were of the highest grade available commercially. The two-tailed Student t-test was employed to determine statistical significance of differences and all errors are expressed as the standard error of the mean (SEM).

Finally, efforts aimed at defining unilateral uptake by using the present approach may be complicated by "recycling" of isotope; i.e. isotope added to the serosal solution may cross the tissue and enter (or re-enter) the epithelial cells across the mucosal membranes. In order to minimize this complication, experiments were rejected if: (a) the $[^{14}$C]inulin activity of the mucosal solution and/or the $[^{3}$H]inulin activity of the serosal solution at the conclusion of the experiment significantly exceeded background (these isotopes were originally added to the opposite solutions so that any statistically significant cross-contamination, particularly after brief exposures, suggests a perforation in the tissue); and/or (b) the specific activity of $^{42}$K in the mucosal solution at the end of the experiment exceeded 2% of that in the originally labeled solution.

RESULTS AND DISCUSSION

The Extracellular Spaces

The time courses of equilibration of labeled inulin with tissue spaces accessible from the mucosal and serosal solutions, and the total inulin space, are illustrated in Fig. 2. The time courses over the initial 40 min clearly display at least two phases. There is a rapid uptake during the first 10 min which results in 75–80% equilibration of the mucosal, serosal, and total inulin spaces. Thereafter, there are slow increases, particularly in the serosal and total inulin spaces, which appear to reach maximal levels at 40 min. The apparent decline in the serosal and the total inulin spaces between 40 and 60 min may be the result of cell swelling but additional studies are required at time periods between 40 and 60 min to verify this point. The maximum value for the total inulin space achieved at 40 min corresponds to approximately 25% of the wet weight and is in excellent agreement with the value of 26% reported previously for mucosal strips of rabbit ileum prepared by scraping off the epithelium with a glass microscope slide (4).

The adequacy of inulin as a measure of the extracellular space in a tissue characterized by a complex geometry is an unsettled point. There is good reason to believe that if inulin enters the intestinal cells it does so very slowly (4, 14, 15). However, there is a reasonable likelihood that there are small spaces which, though extracellular, are inaccessible to this polymer. Recent autoradiographic studies by Stirling (14) clearly indicate that after 30 min of exposure of mucosal strips of rabbit ileum to $[^{3}$H]inulin, exposed grains are found in the intercellular spaces but perhaps not at the same density found in the villus core. It is, of course, possible that these grains are not due to $[^{3}$H]inulin but represent the presence of smaller tritiated breakdown products of the parent compound; if so, these products appear to be restricted to the extracellular spaces so that calculation of the volume of the space on the assumption that all radioactivity is due to inulin is valid.

Studies carried out with $[^{14}$C]- and $[^{3}$H]polyethylene glycol (mol wt = 4,000) yielded results identical to those obtained with inulin. On the other hand, studies carried out with $[^{14}$C]- and $[^{3}$H]mannitol (Fig. 3) disclosed a rapid uptake by the
tissue during the first 10 min that paralleled the rapid phase of inulin uptake. However, thereafter, mannitol uptake continued to increase slowly with no indication of saturation. After 60 min the mannitol spaces accounted for more than 30% of the tissue wet weight and almost 50% of the tissue water in

![Figure 2](image2.png)

**Figure 2.** The extracellular spaces (ECS) accessible to inulin from the mucosal solution (■), the serosal solution (▲), and the total ECS (●) as a function of time. The ordinate on the left gives the ECS as the percent of total tissue water and the ordinate on the right gives the ECS in microliters per square centimeter (serosal area) tissue. The numbers in parentheses are the number of determinations.

![Figure 3](image3.png)

**Figure 3.** The extracellular spaces (ECS) accessible to mannitol. See legend to Fig. 2 for further details. All data are the results of 10 determinations on paired tissues from the same animals.

agreement with previous observations (4). Thus, it seems that mannitol slowly enters the intracellular space and is unsuitable for use as an extracellular marker during long incubation periods. Similar findings have been reported by Armstrong et al. (16) for bullfrog small intestine.
In the light of these considerations, the distribution of inulin was chosen as a measure of the ECS. Although it is likely that this resulted in an underestimate of the true ECS, as will be pointed out below, this error cannot significantly affect our main conclusions.

**Effect of Extracellular Na and K**

The effects of unilateral and bilateral exposure of the tissue to Na-free choline-Ringer are given in Table I. In these experiments, the Na-free media were exchanged every 10 min to ensure that the solutions remained nominally Na free; the Na concentration in the Na-free solutions determined at the conclusion of the experiments was always less than 0.6 mM. In the presence of the normal Ringer solution, the intracellular concentrations of Na and K ([Na]$_i$ and [K]$_i$) are in excellent agreement with the values reported previously for mucosal strips of rabbit ileum after incubation of the tissue for 45–60 min in a similar Ringer solution with $^3$Hinulin as the extracellular space marker (4, 13). Exposure of

| Incubation conditions | Intracellular composition | [Na]$_i$ (mM) | [Na]$_e$ (mM) | H$_2$O (μl/cm$^2$) | [Na]$_i$ (nM) | [K]$_i$ (nM) |
|-----------------------|---------------------------|---------------|---------------|------------------|---------------|-------------|
| Na$_m$ 140 Na$_s$ 140 | 25±1 61±1                 | 190±5         |
| Na$_m$ 0 Na$_s$ 140  | 24±2 24±2                 | 139±7         |
| Na$_m$ 140 Na$_s$ 0  | 24±1 30±2                 | 117±5         |
| Na$_m$ 0 Na$_s$ 0   | 17±1* 7±0*                | 135±5         |

*Tissues were incubated for 40 min under the above conditions and the extracellular markers ([$^{14}$C]- and [3H]inulin) were added to the bathing media 10 min before the removal of the tissue. Data from six experiments on paired tissues.*

*Significantly different from control at $P < 0.01$.

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mM), $[\text{Na}]_e$ and $[\text{K}]_e$ are in excellent agreement with the values reported in Table I; these values are not affected by removal of K from the mucosal solution alone. However, replacement of K in the serosal solution alone results in a significant decline in $[\text{K}]_e$, and a further decline is observed when both media are K free. These data suggest that the mucosal membrane is impermeable to K and that maintenance of cell K is dependent upon K in the serosal solution alone; the fact that complete removal of K from the bathing media has a greater effect on $[\text{K}]_e$ than removal from the serosal solution alone may be attributable to movement of K from the mucosal solution into the villus core (primarily via an extracellular route [17, 18]) and recycling across the basolateral membranes. Finally, associated with the decline in $[\text{K}]_e$ resulting from removal of K from both solutions, there is a significant increase in $[\text{Na}]_e$. In no instance was cell H2O content significantly affected.

**Table II**

| Incubation conditions | Intracellular composition |
|-----------------------|---------------------------|
| $[\text{K}]_m$ | $[\text{K}]_s$ | $[\text{Na}]_e$ | $[\text{K}]_e$ |
| mM | mM | μL/cm² | mM | mM |
| 5 | 5 | 23±4 | 60±3 | 136±8 |
| 0 | 5 | 21±3 | 56±1 | 141±8 |
| 5 | 0 | 21±3 | 62±5 | 119±4* |
| 0 | 0 | 19±3 | 83±2† | 107±5‡ |

Tissues were incubated for 60 min under the above conditions and the extracellular markers ([14C]- and [3H]inulin) were added to the bathing media 10 min before the removal of the tissue. Data from six experiments on paired tissues.

* Significantly different from control at $P < 0.05$.
† Significantly different from control at $P < 0.01$.

**Exchange of Cell K**

The intracellular K concentrations determined by using either inulin or mannitol as the extracellular space markers are given as functions of time of incubation in Fig. 4. $[\text{K}]_e$ calculated with the inulin ECS remained relatively constant for 60 min and ranged between 120 and 135 mM. In contrast, when mannitol was used to measure the ECS, $[\text{K}]_e$ was significantly greater than the values observed with inulin and increased considerably over 1 h; after 90 min (not shown) $[\text{K}]_e$ averaged 215 ± 15 mM ($n = 8$). It seems likely that the steady increase in $[\text{K}]_e$ calculated with mannitol as the ECS marker is due to slow entry of mannitol into the cells which results in a spuriously low calculated intracellular water volume.

The time course of exchange of cell K with $^{42}\text{K}$ in the serosal solution alone is shown in Fig. 5 (solid circles) where $[\text{K}^*]_e/[\text{K}^*]_s$ is the ratio of the specific activity of intracellular K to that in the serosal solution. Also shown is the time course of K exchange when both bathing media contained $^{42}\text{K}$ at the same specific activity (open circles). It is clear from the latter experiments that after 1 h, less than 40% of cell K has exchanged and that the process appears to be plateauing, suggesting that only a fraction total cell K is readily exchangeable with $^{42}\text{K}$ in both
media. As shown in the appendix, if the total cell K is designated as K_c and the fraction that is exchangeable from both solutions is αK_c, then when both solutions contain 42K a plot of ln [1 - ([K^*]_c/[K^*]_m)/R_s] vs. time (t) should yield a straight line with a slope equal to -(f_{em} + f_{es})/αK_c; where R_s is the steady-state

![Figure 4](image_url)

**Figure 4.** The intracellular K concentration as a function of time calculated by using the total inulin ECS (●) and the total mannitol ECS (○).

![Figure 5](image_url)

**Figure 5.** The ratio of the cell 42K specific activity to that in the bathing medium as a function of time. The closed circles (●) are the results when 42K was present only in the serosal solution (each point is the mean ± SEM of 10 experiments). The open circles (○) are the results when 42K was present in both bathing media at equal specific activity (each point is the mean ± SEM of 16 experiments). The curves are the theoretical curves derived from the semilogarithmic plots shown in Fig. 6.
specific activity ratio; \( m, c, \) and \( s \) designate the mucosal, intracellular, and serosal compartments, respectively, and \( J_{ik} \) designates the unidirectional flux from compartment \( i \) to compartment \( k \). In addition, when \( ^{42}\text{K} \) is present only in the serosal solution a plot of \( \ln \left(1 - \frac{(K^*_c)/(K^*_s)}{R_a}\right) \) vs. \( t \) should yield a straight line whose slope is also equal to \(-\left(J_{cm} + J_{cs}\right)/\alpha K_c\). Further, when \( ^{42}\text{K} \) is present in both solutions \( R_a = \alpha \) and when \( ^{42}\text{K} \) is present only in the serosal solution \( R_a = \alpha J_{sc}/(J_{cm} + J_{cs}) \) (19).

Semilogarithmic plots of the data given in Fig. 5 are given in Fig. 6. In both instances \( R_a \) was initially estimated by eye (from Fig. 5) and then repeatedly adjusted to obtain the best least-squares lines; the lines shown in Fig. 6 correspond to \( R_a = 0.4 \) (open circles) and \( R_a = 0.25 \) (solid circles). Both lines have correlation coefficients greater than 0.95 and the slopes do not differ significantly; the half-time for exchange with \( ^{42}\text{K} \) in both media is 21 min and the half-time for exchange with \( ^{42}\text{K} \) in the serosal solution alone is 22.5 min. Thus the data are consistent with the notion that 40% of the cell \( K(\alpha) \) is contained within a single compartment that is accessible to \( ^{42}\text{K} \) in both solutions. From the value of \( R_a \) when \( ^{42}\text{K} \) is present only in the serosal solution (0.25) and the average slope of the two lines (−0.3/min) values of \( J_{mc} = J_{cm} = 0.9 \mu \text{Eq/cm}^2 \text{h} \) and \( J_{sc} = J_{cs} = 1.4 \mu \text{Eq/cm}^2 \text{h} \) can be calculated by employing the relations given above and in the appendix.

It should be stressed that although the data given in Figs. 5 and 6 are consistent with the notion of a single intracellular \( K \) compartment that is readily
(within 1 h) exchangeable with $^{42}\text{K}$ in both solutions and that this is the simplest interpretation, there are other alternatives. For example, 25% of cell K may be accessible only from the serosal solution and 15% only from the mucosal solution; such a two-compartment system could not be distinguished from the one-compartment system if the rates of exchange across the mucosal and serosal membranes were similar. The important point, for the present purpose, is that at least 80% of the cell K accessible from the serosal solution alone or from both solutions is exchanged with $^{42}\text{K}$ in the serosal solution within 60 min. If one assumes that the remaining cell K is exchangeable, the rate of exchange is so slow that it cannot be discerned kinetically over a 60-min period of study.

Finally, these studies obviously cannot disclose whether the rapidly exchangeable cell K is contained in one or more of the cell types that comprise this heterogeneous epithelium and the slowly exchangeable K in other cell types, or whether K within single cells is compartmentalized. Lee and Armstrong (20) have reported that the thermodynamic activity coefficient of K in bullfrog small intestinal cells is close to unity; that is, K activity did not differ markedly from $[\text{K}]_c$ determined on mucosal strips with inulin as the ECS marker. If the same is true for rabbit ileal cells, intracellular compartmentalization of K would seem unlikely (but not impossible) and compartmentalization within different cell types would seem to be the most reasonable explanation for our findings.

**Relation between Transepithelial Na Transport and Serosal K Uptake**

The effect of $10^{-3}$ M ouabain, added only to the serosal solution, on $[\text{Na}]_c$, $[\text{K}]_c$, and $^{42}\text{K}$ exchange across the basolateral membranes is given in Table III. As reported previously (4), exposure of the tissue to this glycoside for 30 min results in a marked increase in cell Na, an equivalent decrease in cell K, and no significant change in cell water content. As shown in the last two columns of Table III, after 10 min the relative specific activity of cell K in the presence of ouabain did not differ from that observed under control conditions in paired tissues from the same animals in spite of the fact that cell K declined by more than 50%. Thus, after 10 min the amount of cell K that equilibrated with $^{42}\text{K}$ in the serosal solution under control conditions is more than twice the amount which equilibrated in the presence of ouabain. The final column gives the approximate K influx across the basolateral membranes, $J_{\text{K, net}}$, calculated from the $^{42}\text{K}$ content of the intracellular space, and $[\text{K}]_e$ on the assumption that uptake is essentially linear for the first 10 min. Although this assumption is not strictly correct, the data in Fig. 5 indicate that it is a valid first approximation. We see that under control conditions $J_{\text{K, net}}$ is approximately 1.7 $\mu\text{Eq/cm}^2\text{h}$, whereas in the presence of ouabain it is significantly reduced to a value of only 0.6 $\mu\text{Eq/cm}^2\text{h}$.

The effects of unilateral and bilateral Na replacement with choline on $^{42}\text{K}$ exchange across the basolateral membranes are given in Table IV. As reported in Table I, removal of Na from either the mucosal or the serosal solution results in a reduction in $[\text{Na}]_c$, but neither $[\text{K}]_e/[\text{K}]_c$ nor $J_{\text{K, net}}$ differs significantly from the control values. However, when both solutions are rendered Na free, leading to a near-complete depletion of cell Na (Tables I and IV), $[\text{K}]_e/[\text{K}]_c$ and $J_{\text{K, net}}$ are significantly reduced.
The findings reported in Tables I-IV are entirely consistent with the notion that a significant fraction of K influx across the basolateral membranes is mediated by a ouabain-sensitive Na-K exchange. Thus, the presence of ouabain in the serosal solution or virtually complete depletion of cell Na reduces \( J_{\text{K}} \) from control levels to essentially the same value. These findings further suggest that when only one bathing solution is Na-free the "residual" cell Na is sufficient to sustain the Na-K exchange at a level that does not differ significantly from that observed under control conditions. It may have been argued that the inhibition of \( J_{\text{K}} \) observed in the presence of ouabain was secondary to a decline in \( [K]_c \) and a reduction in a K:K exchange process. However, the same inhibited level of \( J_{\text{K}} \) is observed when intracellular Na is depleted but \( [K]_c \) is maintained at control levels. Finally, these data indicate that when the Na-K exchange mechanism is inhibited by exposure to ouabain or K-free bathing media there is a one-for-one loss of cell K and gain in cell Na with no change in cell H\(_2\)O. However, when the Na-K exchange is inhibited by depletion of cell Na, \( [K]_c \) is maintained at control levels. Further, a series of studies comparing \( [K]_c \) and \( J_{\text{K}} \) under control conditions, and in the presence of (a) Na-free bathing media, (b) a ouabain-contain-

**Table III**

**EFFECT OF OUABAIN ON INTRACELLULAR COMPOSITION AND SEROSAL UPTAKE OF POTASSIUM**

| H\(_2\)O | [Na\(_c\)] | [K\(_c\)] | \( [K^+]_c/[K^+]* \) | \( J_{\text{K}} \) |
|---------|-----------|-----------|-----------------|-------------|
| control | 30±1 | 61±0  | 116±4 | 0.11±0.01 | 1.7±0.1 |
| + 10\(^{-3}\) M ouabain | 27±1 | 122±3* | 48±3* | 0.11±0.02 | 0.6±0.0* |

Tissues were incubated for 30 min under the above conditions and the extracellular markers ([\(^{14}\text{C}\)]-
and [\(^{3}\text{H}\)]inulin) were added to the bathing media 10 min before the addition of \(^{42}\text{K}\) to the serosal solution. The tissues were removed after 10 min of exposure to \(^{42}\text{K}\). Data from 16 experiments on paired tissues.
* Significantly different from control at \( P < 0.01 \).

**Table IV**

**EFFECT OF EXTRACELLULAR SODIUM ON POTASSIUM UPTAKE ACROSS THE BASOLATERAL MEMBRANES**

| Incubation conditions | [Na\(_c\)] | [Na\(_c\)] | H\(_2\)O | [Na\(_c\)] | [K\(_c\)] | \( [K^+]_c/[K^+]* \) | \( J_{\text{K}} \) |
|-----------------------|-----------|-----------|---------|-----------|-----------|-----------------|-------------|
| mM | mM | \( \mu\text{m}^3 \) | mM | mM | \( \mu\text{m}^3 \) | mM | \( \mu\text{m}^3 \) |
| 140 | 140 | 24±2 | 57±3 | 118±2 | 0.09±0.01 | 1.3±0.1 |
| 0 | 140 | 25±2 | 24±2* | 122±2 | 0.07±0.01 | 1.1±0.1 |
| 140 | 0 | 25±2 | 31±3* | 119±2 | 0.06±0.01 | 1.3±0.1 |
| 0 | 0 | 20±1 | 4±0* | 120±3 | 0.05±0.01 | 0.6±0.1* |

Tissues were incubated for 40 min under the above conditions; the extracellular markers were added 10 min before the addition of \(^{42}\text{K}\) to the serosal solution and the tissue was exposed to \(^{42}\text{K}\) for 10 min before removal. Data from eight experiments on paired tissues.
* Significantly different from control at \( P < 0.02 \).
ing Na-Ringer, and (c) Na-free media containing ouabain, indicated that the effects of Na-free media and ouabain on $J_{\mathrm{e}}^{\mathit{Na}}$ are nonadditive and that $[\mathit{K}]_{\mathit{e}}$ is maintained at control levels in the absence of Na even when ouabain is present in the serosal solution. Hence, the loss of cell K when the Na-K exchange is inhibited appears to require the presence of an exchangeable cation in one or both bathing media and choline apparently does not fulfill this requirement.¹

Finally, it is of interest that in this and previous (4) studies exposure of the tissue to ouabain for 40 min resulted in a loss of more than 50% of cell K. Yet as shown in Fig. 5, less than 40% of cell K exchanges with ⁴²K in both media within 1 h. Thus it seems that slowly exchangeable K can be rapidly lost from the cell in the presence of ouabain and Na. We have no ready explanation for this unexpected finding.²

The finding that part of K influx from the serosal solution across the basolateral membrane is mediated by a ouabain-sensitive mechanism that requires the presence of intracellular Na and serosal K is not unexpected; the results discussed thus far serve largely to establish the validity of the technique employed to evaluate K influx. However, one noteworthy observation in Table IV is that cell H₂O, $[\mathit{K}]_{\mathit{e}}$, and $J_{\mathit{e}}^{\mathit{Na}}$ are not affected when the mucosal solution alone is rendered Na free in spite of the fact that under these conditions the intracellular “Na transport pool”³ must be depleted and transepithelial active Na transport is abolished. To explore this apparent dissociation between K influx and transepithelial Na transport further, the effects of actively transported sugars and amino acids in the mucosal solution on serosal K exchange were examined. This approach has the advantage that the rate of active transepithelial Na transport can be markedly stimulated without changing the ionic composition of the bathing media and the effect of sugars and amino acids on the electrical potential profile across this tissue is known (8, 21).

The effect of 20 mM L-alanine or 10 mM D-glucose in the mucosal solution on $[\mathit{Na}]_{\mathit{e}}$, $[\mathit{K}]_{\mathit{e}}$, the specific activity ratio, and $J_{\mathit{e}}^{\mathit{Na}}$ during a 3-min exposure are given in Table V; clearly, none of these parameters is affected significantly. The effect of glucose on K exchange over a longer exposure period and in the presence or absence of ouabain is given in Table VI. Clearly, glucose has no effect on the specific activity ratio or the approximate K influx, and ouabain reduced both of these parameters to the same level in the presence and absence of glucose. Thus,

¹ As discussed above, when the Na-K exchange is inhibited by replacement of Na in both bathing media there is a loss of cell K, presumably accompanied by anions (e.g., Cl), and the loss of an isotonic equivalent of cell H₂O. The loss of K may be limited by the availability of diffusible intracellular anions, and further K loss appears to depend upon the presence of an exchangeable extracellular cation.

² A possibly related finding has recently been reported by Lee and Armstrong (20). These investigators found that when frog skeletal muscle is incubated for 48 h in a K-free medium at 5°C, there is a loss of cell K and an increase in cell Na. However, the loss of cell K is significantly greater than the decrease in intracellular K activity determined with ion-sensitive microelectrodes and the gain in cell Na is significantly greater than the increase in intracellular Na activity. Thus, some of the K that is lost appears to originate from a “compartment” that is not “sensed” by the cation-selective microelectrode, and some of the Na gained by the cell enters a “compartment” that is “inaccessible” to these electrodes.

³ The “Na transport pool” is defined as Na derived from the mucosal solution, that is “in contact” with the mechanism responsible for transepithelial transport and is “awaiting” transport.
the ouabain-sensitive portion of K influx is not affected by glucose in spite of the fact that this agent markedly increases the rate of active transepithelial Na transport.

Finally, in order to exclude the possibility that the negative effects of alanine and glucose reported in Table V and VI were due to the fact that in these experiments the serosal surface of the tissue was exposed to $^{42}$K for relatively brief periods (see below), experiments were carried out in which $^{42}$K exchange was evaluated over a 45-min interval. In the absence of sugar or amino acid $[K]_c$

| TABLE V | EFFECT OF GLUCOSE AND ALANINE ON INTRACELLULAR CONCENTRATIONS AND SEROSAL UPTAKE OF SODIUM AND POTASSIUM* |
|---------|------------------------------------------------------------------------------------------------------------|
| $[Na]_i$ | $[K]_o$ | $[K^+][K^+]_i$ | $\mu E_{[K]}$/h |
| Control (8) | 55±3 | 113±4 | 0.03±0.00 | 1.9±0.2 |
| + 20 mM alanine | 60±2 | 114±1 | 0.03±0.00 | 1.9±0.1 |
| Control (10) | 59±4 | 124±7 | 0.03±0.00 | 1.7±0.1 |
| + 10 mM glucose | 61±4 | 124±6 | 0.03±0.00 | 1.8±0.1 |

* Tissues were preincubated for 15 min in the presence of extracellular space markers with or without alanine or glucose. The values for relative specific activities and serosal influxes of Na and K are based on a 3-min exposure of the serosal surface to $^{42}$K. The number of paired experiments is given in parentheses.

| TABLE VI | POTASSIUM UPTAKE IN THE PRESENCE AND ABSENCE OF GLUCOSE AND OUABAIN |
|----------|---------------------------------------------------------------------|
| Condition | $[K]_c$ | $[K^+][K^+]_i$ | $\mu E_{[K]}$/h |
| Control | 158±6 | 0.112±0.011 | 1.3±0.1 |
| + 10 mM glucose | 140±5 | 0.111±0.004 | 1.4±0.1 |
| + $10^{-3}$M ouabain | 68±3 | 0.073±0.006 | 0.4±0.0 |
| 10 mM glucose and $10^{-3}$M ouabain | 68±3 | 0.074±0.007 | 0.4±0.0 |

Tissues were incubated for 45 min under the above conditions; the extracellular space markers were added to both bathing media and $^{42}$K was added to the serosal solution 15 min before removal of the tissue. Data derived from four paired experiments.

averaged $130 \pm 7$ mM and $[K^+][K^+]_i$ averaged $0.19 \pm 0.03$; in the presence of 10 mM d-glucose these values were $122 \pm 7$ mM and $0.21 \pm 0.02$, respectively; in the presence of 20 mM L-alanine they were $120 \pm 6$ mM and $0.21 \pm 0.03$, respectively. The small decline in $[K]_i$ after the prolonged exposure to sugars or amino acids has been noted previously (4, 16) and is attributable to cell swelling secondary to the accumulation of these osmotically active solutes (4, 16, 22).

The compiled data on $^{42}$K exchange from the serosal solution in the presence and absence of d-glucose or L-alanine are illustrated in Fig. 7. The curve represents the exponential function calculated from the data given in Figs. 5 and 6 and it clearly provides an adequate description of data obtained on three different sets of paired tissues. Thus we conclude that neither glucose nor
alanine has any discernible effect on the rate of $^{42}K$ exchange with the intracellular K compartment that is rapidly accessible from the serosal solution.

**CONCLUSIONS**

The purpose of this investigation was to examine the notion that transepithelial Na transport by rabbit ileum is mediated by an Na-K exchange pump at the basolateral membranes and is, therefore, coupled to K influx across that membrane. The results are consistent with other evidence for the presence of an ouabain-sensitive Na-K exchange process at the serosal surface of the epithelium which is responsible, at least in part, for the maintenance of intracellular Na and K composition (3-7). However, we are unable to detect any relation between active transepithelial Na transport and either K influx across the basolateral membranes or the K content of the epithelial cells. Thus, neither abolition of active Na transport by rendering the mucosal solution Na-free nor stimulation of Na transport by the addition of glucose or alanine to the mucosal solution significantly affects $[K]_c$ or the influx of $^{42}K$ across the basolateral membranes. Before these findings are discussed further, three possible sources of error should be considered.

(a) **MEASUREMENT OF THE ECS** As discussed above, it is quite likely that the use of inulin as an extracellular marker results in an underestimate of the "true" ECS; on the other hand, the use of mannitol would almost certainly have led to an overestimate of this space particularly after prolonged incubations. However, it can be readily shown that although uncertainties with respect to the size of the ECS significantly influence determinations of intracellular Na con-

![Graph](https://via.placeholder.com/150)

**Figure 7.** The ratio of the specific activity of cell $^{42}K$ to that of the serosal solution as a function of time under control conditions (●) in the presence of 10 mM d-glucose (○) and in the presence of 20 mM L-alanine (□). The curve is the theoretical curve derived from the semilogarithmic plot shown in Fig. 6 for $^{42}K$ exchange from the serosal solution alone.
tent, $[\text{Na}]_c$, and $[\text{K}]_c$ (Fig. 4), they do not significantly affect determinations of cell K content or the specific activity ratio. For example, using inulin as the ECS marker we find that for a mucosal strip ($1.13 \text{ cm}^2$) with a wet weight of approximately 50 mg, the ECS is approximately 12.5 $\mu\text{l}$, the cell K content is 3.71 $\mu\text{eq}$, $[\text{K}]_c = 130 \text{ mM}$, and $[\text{K}^*]_c/[\text{K}^*]_n = 0.10$ after 10-min exposure of the serosal surface to $^{42}\text{K}$. If the "true" ECS were twice the inulin space (i.e. 50% of the wet weight), the cell K content would be 3.66 $\mu\text{eq}$, $[\text{K}]_c$ would be 229 mM, and $[\text{K}^*]_c$ would be 0.09 (rather than 0.10). Thus, because the cell K concentration is so much greater than that in the ECS, differences in the size of ECS almost exclusively affect the estimate of cell water content and, thus, $[\text{K}]_c$, but only minimally affect cell K content or the specific activity ratio. The above example represents extreme limits (i.e. the mannitol space after 1 h is less than 50% of the wet weight) and serves to demonstrate that our major findings are not likely to be seriously affected by uncertainties with respect to the true ECS.

It follows from these considerations that most if not all of the calculated $^{42}\text{K}$ uptake must have crossed cell membranes and that part of this influx process must be mediated by a ouabain-sensitive Na-K exchange mechanism; the effects of Na depletion and ouabain cannot be attributed to changes in the ECS.

(b) MULTIPLE CELL TYPES The epithelial cell layer of rabbit ileum contains five distinct cell types (23). The villus cells are the most numerous and certainly account for a major fraction of the intracellular water. Photomicrographs of rabbit ileum (see Fig. 1 of reference 12) reveal that the height of the villus cells is 20-25 $\mu\text{m}$ and that the villi result in an eightfold increase in the surface area. Hence the volume of the villus cells is approximately 18 $\mu\text{l}/\text{cm}^2$ (serosal area). If we assume that water content is approximately 75% of total volume, it follows that the villus cells contain 14 $\mu\text{l}$ H$_2\text{O}/\text{cm}^2$, or more than 50% of the total cell water (based on the inulin space) in 1 cm$^2$ tissue. Barring major differences in ionic composition among different cell types it is likely that the villus cells also contain 50-60% of the total cell K and Na of the epithelium. These cells appear to be responsible for active sugar and amino acid transport (14, 15) so that it seems certain that they are also responsible for the enhanced active Na transport that accompanies the absorption of these nonelectrolytes. Further, autoradiographic studies have clearly demonstrated that virtually all of the cells on the villus participate in active sugar and amino acid transport (14, 15).

The crypt cells are the next most numerous population. their role in active transepithelial Na transport is entirely unclear but it is of interest that they appear to contain a significantly lower specific activity of Na-K ATPase than the villus cells (24).

The results of the present study are unavoidably complicated by the fact that the crypt cells are situated closer to the serosal solution than are the villus cells and thus are the first major group of cells to be exposed to serosal $^{42}\text{K}$. However, the results of the kinetic studies illustrated in Figs. 5 and 6 seem to diminish the seriousness of this complication. These data are consistent with the notion that serosal $^{42}\text{K}$ equilibrates with more than 80% of the cell K that is accessible to either the serosal alone, or both bathing media, within 45-60 min. If $J_{\text{K}}^S$
predominantly reflects exchange with crypt cells, then the rate of exchange with the villus cells must be so slow as to preclude significant coupling to transepithelial Na transport. On the other hand, if \( J_{\text{K}} \) reflects exchange with villus cells alone, or a combination of villus and nonvillus cells, the findings that it is not affected by Na-free mucosal media or by the addition of glucose or alanine to the mucosal solution would also seem to preclude significant coupling to transepithelial Na transport.

(c) "Masking" of Effects If \( J_{\text{K}} \) were large compared to the rate of Na transport, the effect of changes in Na transport on \( J_{\text{K}} \) could be obscured by experimental error. However, under control conditions the rate of active Na transport across this preparation is approximately 2–3 \( \mu \)eq/cm²h (18); glucose or alanine in the mucosal solution increases this rate to 4–5 \( \mu \)eq/cm²h (18, 21). \( J_{\text{K}} \) is approximately 1–2 \( \mu \)eq/cm²h so that one would expect to see highly significant changes in \( J_{\text{K}} \) when Na transport is abolished or doubled if these two processes are tightly coupled. Since the error in determining \( J_{\text{K}} \) is approximately 0.1–0.2 \( \mu \)eq/cm²h, an Na:K coupling ratio greater than 8:1 would not be detectable; however the Na:K ratio of approximately 3:2 that has been reported for some systems (10, 11) should be readily detectable.

An additional finding that is difficult to reconcile with the notion of coupling between transepithelial Na transport and carrier-mediated K influx across the basolateral membranes is that neither cell K nor \([K]_{c}\) is affected when the tissue is exposed to an Na-free mucosal solution which abolishes Na absorption and must deplete the "Na transport pool." For example, assuming a Na:K exchange ratio of 3:2, active Na extrusion from the transport pool at a rate of 3 \( \mu \)eq/cm²h should be associated with coupled K influx at a rate of 2 \( \mu \)eq/cm²h. The total cell K is approximately 2.5–3 \( \mu \)eq/cm². Thus, if 50% of the total K is located in cells responsible for active Na absorption (e.g., the villus cells), this entire pool would be expected to turn over in 1 h due to coupling to the Na extrusion mechanism at the basolateral membranes, and one would predict that the cessation of transepithelial Na transport would lead to a rapid and readily detectable decline in cell K content. However, cell K content did not differ significantly from control after a 40-min exposure of the tissue to an Na-free mucosal solution. The errors involved in the determination of total cell K content are such that a 50% decline in a pool that comprises only 20% of the total cell K would have been readily detected.

At face value, these findings are difficult to reconcile with any model featuring an obligatory, tight coupling between transepithelial Na transport and active K accumulation by the epithelial cells unless (a) the Na:K exchange ratio is considerably greater than that reported for any other cell studied to date, and/or (b) K influx coupled to Na extrusion exchanges with a cell K pool that is considerably smaller than the cell K content of the villus cells. For example, we might not be able to detect coupling if only a small fraction of the villus cells are responsible for Na absorption, but the autoradiographs of Kinter and Wilson (14) and Stirling and Kinter (15) would seem to preclude this possibility at least for the case of sugar- and amino acid-stimulated Na absorption. Alternatively, it is quite possible that the Na transport pool in the villus cells comprises only a small fraction of the total Na content of these cells. If so, extrusion of Na from this
pool could be coupled to K influx across the basolateral membranes into a very small K pool. Equilibration of serosal $^{42}$K with this pool could be extremely rapid and undetectable with the present techniques. Changes in cell K content in response to changes in transepithelial Na transport could also be masked. Thus we cannot exclude the possibility that Na extrusion from the cell is coupled to K influx that enters and rapidly exchanges with a K pool that comprises only a small percent of the total cell K. However, if so, the mechanism(s) responsible for homocellular Na and K transport and that (those) responsible for transcellular Na transport are clearly dissociated. In any event we are unaware of any approach that would resolve the uncertainties that preclude a more positive interpretation of our negative findings. Experiments on isolated villus cells may prove useful but the isolation techniques developed to date yield rather leaky preparations and, of course, unilateral exposure to desired media and agents is not possible.

The strongest evidence favoring direct coupling between Na transport and K influx in rabbit ileum is the asymmetric localization of the ouabain-sensitive Na-K ATPase (5-7) and the fact that serosal ouabain completely inhibits active Na absorption (3). It is of interest in this respect that Na-K ATPase activity of the avian salt gland is also located at the basolateral membranes (25, 26) in spite of the fact that this gland secretes Na across the apical membrane; thus, it is difficult to draw definitive conclusions which relate the localization of this enzyme activity to the direction of transepithelial Na transport.

Possible alternative explanations for the fact that Na absorption is completely inhibited by serosal ouabain are:

(a) the effect of ouabain could be the result of inhibition of the Na-K exchange mechanism responsible for the maintenance of normal cell Na and K contents. The resulting increase in cell Na and decrease in cell K could indirectly affect the active Na transport mechanism perhaps by impairing metabolic process (see Addendum);

(b) In recent years it has become clear that the Na-K exchange mechanism in erythrocytes is highly versatile and can, under some conditions, mediate an Na-Na exchange, a K-K exchange, and an Na efflux that is apparently not coupled to the influx of cations (27, 28). Although this uncoupled Na efflux from red cell ghosts is manifest only under "unphysiologic" conditions (e.g., an Na- and K-free incubation medium), it appears to be a feasible mode of operation of the ouabain-sensitive Na-K pump. Goldin and Tong (29) have recently reported that lipid vesicles "doped" with Na-K ATPase purified from canine renal medulla appear to be capable of actively transporting Na in the presence of ATP. This "apparent active transport" of Na was blocked by ouabain but was not associated with an exchange of Na for K; instead, it appears that electroneutrality was preserved by cotransport of Cl. Thus, there are findings consistent with the possibility that a ouabain-sensitive, Na-dependent ATPase may mediate uncoupled (rheogenic) Na efflux from the enterocyte across the basolateral membranes.4

4 Proverbio et al. (54) have recently reported the finding of an Na-stimulated ATPase activity in an aged microsomal preparation from guinea pig kidney cortex that (a) is not dependent upon the presence of K; (b) is insensitive to ouabain; and (c) is inhibited by ethacrylic acid.
Further speculation on this point is unwarranted. However, it should be clear that the fact that ouabain completely inhibits active Na absorption need not necessarily directly implicate an Na-K exchange.

The relation between active transcellular Na transport and K influx across the inner or basolateral membranes of other epithelia is as yet unsettled. Evidence has been presented suggesting that transepithelial Na transport by toad urinary bladder (30, 31), isolated frog skin (32, 33), and Necturus kidney (34) is not tightly coupled to K uptake across the serosal or inner membranes. On the other hand, some of the results reported by Biber et al. (35) appear to be consistent with a coupling between transepithelial Na transport and K influx across the inner membranes of isolated frog skin, whereas other results reported by these authors are difficult to reconcile with this notion (e.g., the finding that K influx was not affected when transepithelial Na transport was abolished by amiloride). Further, Finn and Nellans (36) have concluded that transepithelial Na transport across toad urinary bladder is coupled to K influx across the basolateral membranes; however, the findings of Macknight et al. (37) seriously question the validity of the kinetic analysis employed by Finn et al. (36, 38). In all instances the interpretations of the results are complicated (as in the present study) by the heterogeneity of the tissues (39) and, in some instances, by the indirect methods employed to evaluate K exchange. The question, at present, must be considered unresolved.

Finally, Macknight et al. (37, 40) reported that the K and H2O contents of isolated epithelial cells from toad urinary bladder are not affected when active transepithelial Na transport is abolished either by rendering the mucosal solution Na free or by treatment with amiloride. These results are in complete agreement with our findings (Tables I and IV) and, as pointed out by these authors, "this complete dissociation of cellular K content from transepithelial transport seems inconsistent with any hypothesis that there is necessarily specific tight coupling between cellular potassium accumulation and transepithelial sodium transport" (see Addendum).

The Maintenance of Cell Volume

It has been widely accepted for many years that volume regulation by cells that do not possess rigid walls requires active pump mechanisms capable of extruding solutes so as to overcome the osmotic forces due to intracellular macromolecules (41, 42). In 1960, Tosteson and Hoffman (43) proposed a model in which regulation of cell volume by erythrocytes could be accomplished by a neutral Na-K exchange pump. It could be readily shown that impairment of this pump would result in a one-for-one exchange of cell K for extracellular Na, down their respective electrochemical potential gradients, and an additional net entry of Na, Cl, and H2O which results in cell swelling. However, more recently, a number of investigators have presented evidence that cell volume regulation is the result of a mechanism that brings about the extrusion of Na and Cl from the cell but which is distinct from the Na-K exchange pump inasmuch as it is not inhibited by ouabain or by K-free bathing media (cf. references 34, 40, and 44). The "dual pump hypothesis" suggested by Whittembury and his collaborators (cf. reference 44) dissociates volume regulation from homocellular Na and K
regulation but both processes are linked, by this model, to transepithelial Na transport.

The present results suggest that cell water content in rabbit ileum is not dependent upon active transepithelial Na transport or the operation of the ouabain-sensitive Na-K exchange pump. Thus, exposure of the tissue to an Na-free mucosal solution for more than 40 min has no effect on cell water content (Tables I and IV) in spite of the fact that active transepithelial Na transport is obviously precluded. Further, inhibition of the Na-K exchange by ouabain (Table III) or by removal of K from the serosal solution (Table II) did not significantly affect cell H$_2$O content. In both instances the gain in cell Na was equal to the loss of cell K and, if anything, there was a small but statistically insignificant loss of cell H$_2$O. When the Na-K exchange was inhibited by depletion of cell Na, there was a significant loss of cell H$_2$O due to an isotonic loss of K and some anion, probably Cl.

As discussed above, if only a small fraction of the epithelial cells are responsible for active transepithelial Na transport, a change in cell volume after exposure of the mucosal surface to an Na-free solution could be obscured. However, it seems reasonably to infer that all of the cells in the mucosal strip regulate cell Na and K content by means of an Na-K exchange pump. Thus the finding that ouabain did not affect total cell H$_2$O content dissociates the operation of this pump from cell volume regulation by cells that are involved in transepithelial Na transport as well as those that are not.

Identical conclusions have been reported by Macknight et al. (37, 40) for toad urinary bladder epithelial cells. In their studies, inhibition of transepithelial Na transport by exposure of the mucosal surface to an Na-free solution or amiloride had no effect on cell H$_2$O content, nor did treatment of the tissue with ouabain which blocks both transcellular Na transport and the Na-K exchange mechanism.

ADDENDUM

Since the completion of this manuscript, Robinson and Macknight (45-47) have reported that only approximately 30% of the K content of epithelial cells isolated from toad urinary bladder is readily exchangeable with $^{42}$K in the serosal solution; the remainder exchanges with a rate so slow that it could not possibly be coupled to transepithelial Na transport. The readily exchangeable K compartment is reduced by ouabain but does not appear to be related to transepithelial Na transport inasmuch as: (a) although abolishing transepithelial Na transport with amiloride slightly reduced the rate of exchange of K in this compartment with $^{42}$K in the serosal solution, vasopressin at a concentration sufficient to double the rate of transepithelial Na transport had no effect on the rate of exchange; and (b) the rate of transepithelial Na transport is 3-40 times (average = 10) the rate of K influx into the readily exchangeable intracellular compartment. These findings together with the earlier findings (37, 40) that abolishing transepithelial Na transport by removal of Na from the mucosal solution or treatment with amiloride has no effect on total epithelial cell K content closely parallel our findings on rabbit ileum.

Finally, Robinson and Macknight (45, 46) also presented evidence suggesting
that the inhibition of transepithelial Na transport observed when the serosal solution is rendered K free is due to a decrease in cell K, as suggested by Essig and Leaf (30, 31), and not to the absence of serosal K per se needed for an Na-K exchange. This observation raises the intriguing possibility that the effect of ouabain on transepithelial Na transport need not be direct but could be the indirect result of a decline in cell K.

APPENDIX

Assume a three-compartment system where m, c, and s designate the mucosal, intracellular, and serosal compartments, respectively. Let \( J_{ik} \) represent the unidirectional (tracer) flux from compartment i to compartment k. \( K_i \) and \( K'_i \) designate the chemical K and \(^{42}\text{K} \) contents of the ith compartment and \( [K^*]_i \) is the specific activity of \(^{42}\text{K} \) defined as \( K^*/K_i \). Then, when \( [K^*]_m = [K^*]_s \) and both bathing solutions are essentially infinite reservoirs,

\[
\frac{dK^*_c}{dt} = (J_{mc} + J_{sc})[K^*]_m - (J_{cm} + J_{cs})[K^*]_c. \tag{1 A}
\]

Under short-circuit conditions, there is no net transport of K across rabbit ileum (M. Field and R. Schooley, personal communication; and unpublished observations from this laboratory) so that \( J_{mc} = J_{cm} \) and \( J_{sc} = J_{es} \). Thus,

\[
K_c \left( \frac{d[K^*]_c}{dt} \right) = (J_{mc} + J_{sc})([K^*]_s - [K^*]_c). \tag{2 A}
\]

Since \( [K^*]_s \) is constant during the course of the experiment, Eq. 2 A can be readily integrated from \( t = 0 \) to \( t = t \) to yield

\[
\frac{[K^*]_c}{[K^*]_s} = 1 - \exp \left( \frac{-(J_{mc} + J_{sc})t}{K_c} \right). \tag{3 A}
\]

Now, if only a fraction of the total cell K, \( \alpha K_c \), is exchangeable, then at \( t = \infty \), \( [K^*]_c/[K^*]_s = \alpha \), and Eq. 3 A can be written

\[
\frac{[K^*]_c}{[K^*]_s} = 1 - \exp \left[ -\left( \frac{J_{mc} + J_{sc}}{\alpha K_c} \right)t \right].
\]

where \( R_w \), the specific activity ratio when \( t = \infty \), is simply equal to \( \alpha \).

Thus, if \( \ln \left[ 1 - \frac{[K^*]_c/[K^*]_s}{R_w} \right] \) is plotted vs. \( t \), the slope should be \( -(J_{mc} + J_{sc})/K_c \).

When \(^{42}\text{K} \) is present only in the serosal solution, \( [K^*]_m = 0 \) and Eq. 1 A reduces to

\[
\frac{d[K^*]_c}{dt} = J_{sc}[K^*]_s - (J_{cm} + J_{cs})[K^*]_c, \tag{4 A}
\]

which upon integration yields

\[
1 - \frac{[J_{cm} + J_{cs}]t}{[K^*]_s} = \exp \left[ -\left( \frac{J_{cm} + J_{cs}}{K_c} \right)t \right].
\]

Thus \( R_w = J_{sc}/(J_{cm} + J_{cs}) \) and a plot of \( \ln \left[ 1 - \frac{[K^*]_c/[K^*]_s}{R_w} \right] \) vs. \( t \) should also have a slope given by \( -(J_{cm} + J_{cs})/K_c \).

If only \( \alpha K_c \) is exchangeable, \( R_w = \alpha J_{sc}/(J_{cm} + J_{cs}) \) and the slope of the semilogarithmic plots is \( -(J_{cm} + J_{cs})/\alpha K_c \).

Thus if a single intracellular compartment is accessible to \(^{42}\text{K} \) in both bathing media, an analysis of the kinetics of exchange yields \( \alpha, (J_{cm} + J_{cs}) \) and \( J_{sc}/(J_{cm} + J_{cs}) \). Recalling that \( J_{mc} = J_{cm} \) and \( J_{ac} = J_{cs} \), it is obvious that this information is sufficient to calculate all four fluxes.
The authors gratefully acknowledge the technical assistance of Miss Barbara Jennings.
This investigation was supported by research grants from the United States Public Health Service, National Institutes of Health, National Institute of Arthritis, Metabolism and Digestive Diseases (AM-16275), and the American Heart Association (70-633). During part of this investigation Hugh N. Nellans was a Public Health Service Postdoctoral Research Fellow (AM-52248).

Received for publication 7 November 1975.

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