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# Sodium-Coupled Amino Acid and Sugar Transport by *Necturus* Small Intestine

## An Equivalent Electrical Circuit Analysis of a Rheogenic Co-Transport System

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**Summary.** *Necturus* small intestine actively absorbs sugars and amino acids by Na-coupled mechanisms that result in increases in the transepithelial electrical potential difference ($\psi^{ms}$) and the short-circuit current ($I_{sc}$) which can be attributed entirely to an increase in the rate of active Na absorption. Studies employing conventional microelectrodes indicate that the addition of alanine or galactose to the mucosal solution is followed by a biphasic response. Initially, there is a rapid depolarization of the electrical potential difference across the apical membrane ($\psi^{mc}$) which reverses polarity (i.e., cell interior becomes positive with respect to the mucosal solution) and a marked decrease in the ratio of the effective resistance of the mucosal membrane to that of the serosal membrane ($R^m/R^s$); these events do not appear to be dependent on the availability of metabolic energy. These initial, rapid events are followed by a slow increase in ($R^m/R^s$) toward control values which is paralleled by a repolarization of $\psi^{mc}$ and increases in $\psi^{ms}$ and $I_{sc}$; this slow series of events is dependent upon the availability of metabolic energy.

The results of these studies indicate that: (i) the Na-coupled mechanisms that mediate the entry of sugars and amino acids across the apical membrane are "rheogenic" (conductive) and result in a decrease in $R^m$ and a depolarization of $\psi^{mc}$; and (ii) the subsequent increase in ($R^m/R^s$) and repolarization of $\psi^{mc}$ are the results of a decrease in $R^s$ which is associated with an increase in the activity of the Na pump at the basolateral membrane.

The physiologic implications of these findings are discussed and an equivalent electrical circuit model for "rheogenic" Na-coupled solute transport processes is analyzed.

**Key words** *Necturus* small intestine · Na-coupled transport · alanine · galactose · electrophysiology

## Introduction

The electrophysiological responses associated with Na-coupled sugar and amino acid transport have been described previously for the small intestine (Gilles-Ballien & Schoffeniels, 1965; Wright, 1966; Lyon & Sheerin, 1971; Rose & Schultz, 1971; White & Armstrong, 1971; Barry & Eggenton, 1972; Okada, Tsuchiya, Irimajiri & Inouye, 1977*b*) and renal proximal tubule (Maruyama & Hoshi, 1972; Hoshi, Sudo & Suzuki, 1976; Frömter, 1977; 1979) of a number of species. With only a few exceptions (Gilles-Ballien & Schoffeniels, 1965; Wright, 1966; Lyon & Sheerin, 1971), these responses have been characterized by an abrupt and sustained depolarization of the electrical potential difference across the apical membrane ($\psi^{mc}$) and a concomitant increase in the transepithelial electrical potential difference (serosa positive). Several lines of evidence from a number of laboratories (Rose & Schultz, 1971; White & Armstrong, 1971; Hoshi et al., 1976; Okada et al., 1977*b*; Frömter, 1979) support the notion that the depolarization of $\psi^{mc}$ results from rheogenic Na-coupled solute influx across the apical membrane. The relation between the changes in the transepithelial and transapical electrical potential differences, however, is less clear. The increase in the transepithelial potential difference has been attributed to changes in the electrical properties of the apical membrane alone (White & Armstrong, 1971; Maruyama & Hoshi, 1972; Frömter, 1979) or to changes in the properties of both the apical and the basolateral membranes (Rose & Schultz, 1971; Schultz, Frizzell & Nellans, 1974; Okada, Irimajiri & Inouye, 1977*a*).

In the course of a study of the electrophysiology of *Necturus* small intestine, we found, quite unexpectedly, that the addition of sugars or amino acids to the mucosal bathing solution elicits a protracted transient response. In view of the earlier observations cited above, we have investigated this response in an attempt to gain further insight into the mechanisms operating at the two limiting membranes that are ultimately responsible for the overall response of the tissue. The results have been presented previously in abstract form (Gunter-Smith, Grasset & Schultz, 1980).

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## Materials and Methods

### *Animals and Solutions*

Mudpuppies, *Necturus maculosa* (Mogul-Ed, Oshkosh, Wisc., and Connecticut Valley Biological Supply Co., Southampton, Me.) were stored in tapwater at 4 °C. The animals were either stunned by a blow to the head or anesthetized by immersion in tapwater containing tricaine methylsulfonate (Sigma), 660 mg/liter, until reflexes were suppressed. The entire length of the small intestine distal to the attachment of the pancreas was excised and the proximal one-third was used for experiments. The intestinal segment was stripped of its underlying musculature by blunt dissection (White, 1977)[1], opened along its mesenteric border, and mounted in the appropriate chambers described below.

Standard Ringer's solution contained (mM): 100, Na; 104.8, Cl; 2.5, K; 1.2, Ca; 1.2, Mg; 1.2, $HPO_4$; 0.3, $H_2PO_4$; and 10, mannitol. For solutions containing alanine or galactose, 10 mM L-alanine (Sigma) or 10 mM D-galactose (Sigma) replaced mannitol. The pH of this solution when gassed with air is 7.4.

All experiments were carried out at room temperature (23 °C).

### *Flux Measurements*

The transepithelial unidirectional fluxes of Na and alanine from mucosa-to-serosa ($J^{ms}_{Na}, J^{ms}_{ala}$) and serosa-to-mucosa ($J^{sm}_{Na}, J^{sm}_{ala}$) were determined using paired intestinal segments isolated from the same animal; the net flux is given by $J = J^{ms} - J^{sm}$. The tissues were mounted in Ussing chambers exposing 0.64 $cm^2$ of surface area and both surfaces were bathed by the standard Ringer's solution without mannitol. After the transepithelial potential difference, $\psi^{ms}$, stabilized (generally within 30 min), the tissue was short circuited as described previously (Schultz & Zalusky, 1964*a*), correcting for the fluid resistance between the voltage-sensing electrodes. The tissue was continuously short circuited except for brief periods to record the open-circuit transepithelial electrical potential difference and resistance. $^{22}Na$ was added to the appropriate reservoir and, after a 40-min equilibration period, $J^{ms}_{Na}$ and $J^{sm}_{Na}$ were determined during four 20-min periods as described previously (Schultz & Zalusky, 1964*a*). After the final "control" measurements, alanine was added to all reservoirs to a final concentration of 10 mM. $^{14}C$-alanine was then added to the same reservoir to which $^{22}Na$ had been previously added. After a 20-min equilibration period, $J^{ms}_{Na}$, $J^{sm}_{Na}$, $J^{ms}_{ala}$, and $J^{sm}_{ala}$ were determined during four additional 20-min flux periods.

The samples were analyzed for $^{22}Na$ and $^{14}C$-alanine simultaneously using a three-channel liquid scintillation counter (Tracor Analytic).

$^{22}Na$ and $^{14}C$-alanine were obtained from New England Nuclear, Boston, Mass.

### *Intracellular Electrical Potential Measurements*

Stripped intestinal segments were clamped between two halves of a Lucite chamber exposing 0.13 $cm^2$ of tissue. The mucosal and serosal surfaces were continuously superfused with Ringer's solution by gravity-feed from reservoirs. The flow was adjusted so that the volumes of the mucosal and serosal solutions were replaced every sec.

The electrical measuring system and the preparation of conventional microelectrodes were identical to those described in detail previously by Gunter-Smith and Schultz (1982). Briefly, the transepithelial electrical potential difference with respect to the mucosal solution, $\psi^{ms}$, or the transepithelial current, $I^{ms}$, was monitored using an automatic voltage-clamp in contact with the solutions via calomel cells and Ringer-agar bridges. In some experiments, the tissues were open-circuited ($I^{ms}=0$) and bipolar constant-current pulses of $\pm 150\,\mu A/cm^2$ were passed across the tissue at 10-sec intervals; the duration of each pulse was 2 sec. In other experiments, the tissues were maintained under short-circuit conditions ($\psi^{ms}=0$; $I^{ms}=I_{sc}$) and bipolar current pulses were passed across the tissue at 10-sec intervals sufficient to voltage-clamp the preparation at $\pm 10$ mV for 2 sec.

The electrical potential difference across the apical membrane with respect to the mucosal solution, $\psi^{mc}$, was measured using conventional microelectrodes filled with 0.5 M KCl having a resistance of $\approx 100\,M\Omega$ with the tip immersed in 0.5 M KCl; the rationale for the use of such electrodes has been discussed by Fromm and Schultz (1981). The output of the microelectrode with respect to ground was monitored by a high impedance electrometer (WP Instruments, F-23B) whose output with respect to a calomel cell in contact with the mucosal solution was monitored by another electrometer (WP Instruments, 750). $\psi^{mc}$ and either $\psi^{ms}$ or $I_{sc}$ were recorded on a Gould chart recorder (Model 2400).

The ratio of the effective resistances of the mucosal and serosal membranes, $(R^m/R^s)$, was calculated from the plateau values of the deflections of $\psi^{mc}$ and $\psi^{ms}$ (between the "on-off" spikes) produced by the bipolar transepithelial current pulses.[2]

The results are expressed as the mean ± the standard error of the mean (SEM). The significance of the difference between two means was determined using the paired or unpaired Student *t*-test with a value of $P<0.05$ accepted as the level of significance.

## Results

### *Transepithelial Na and Alanine Fluxes*

The unidirectional transepithelial fluxes of Na and alanine across isolated segments of *Necturus* small intestine are given in Table 1. $J_{Na}$ in the absence of alanine (control) averaged $1.3 \pm 0.3\,\mu eq/cm^2 hr$. $J_{Na}$ is greater than the $I_{sc}$, suggesting the net transport of some other ion under these conditions.[3] In the presence of 10 mM alanine in both bathing solutions, $J_{Na}$ increased to $3.7 \pm 0.6\,\mu eq/cm^2 hr$. This increase could be attributed entirely to an increase in $J^{ms}$; there was no significant change in $J^{sm}$. The increase in $J_{Na}$ closely corresponds to the net transepithelial alanine flux, $J_{ala}$ (Fig. 1*A*). Furthermore, the increase in short-circuit current, $\Delta I_{sc}$, elicited by alanine can

[1] The stripping procedure removes all but a thin layer of muscle immediately below the lamina propria.

[2] After each successful cell impalement, the microelectrode was advanced through the cell and $(R^m/R^s)$ was calculated from the deflections in $\psi^{ms}$ recorded with the tip of the electrode immediately below the cell. This procedure eliminates possible contributions to $R^s$ from the series resistance offered by the connective tissue in the villous core.

[3] The finding that $J_{Na}$ is greater than $I_{sc}$ is consistent with the presence of a neutral NaCl absorptive mechanism as has been reported for small intestine from a wide variety of species (Frizzell, Field & Schultz, 1979).

**Table 1.** Sodium and alanine fluxes under short-circuit conditions[a]

| | $J^{ms}_{Na}$ | $J^{sm}_{Na}$ | $J_{Na}$ | $J^{ms}_{ala}$ | $J^{sm}_{ala}$ | $J_{ala}$ | $I_{sc}$ | $G_t$ |
|---|---|---|---|---|---|---|---|---|
| Control | 5.4 | 4.1 | 1.3 | – | – | – | 0.4 | 9.3 |
| | ± | ± | ± | – | – | – | ± | ± |
| | 0.3 | 0.4 | 0.3 | – | – | – | 0.1 | 0.1 |
| Alanine | 7.4 | 3.7 | 3.7 | 2.9 | 0.3 | 2.6 | 1.9 | 12.0 |
| | ± | ± | ± | ± | ± | ± | ± | ± |
| | 0.6 | 0.4 | 0.6 | 0.6 | 0.1 | 0.6 | 0.2 | 1.4 |
| $\Delta$ | 1.9[b] | 0.4 | 2.4[b] | 2.9 | 0.3 | 2.6 | 1.4[b] | 2.7[b] |

[a] $J_i$ is the net flux of species $i$ given by $J_i = J_i^{ms} - J_i^{sm}$. The $J$'s and $I_{sc}$ are in μeq/cm² hr; $G_t$ is in mS/cm²; $n = 7$.
[b] Significant difference with $P < 0.5$.

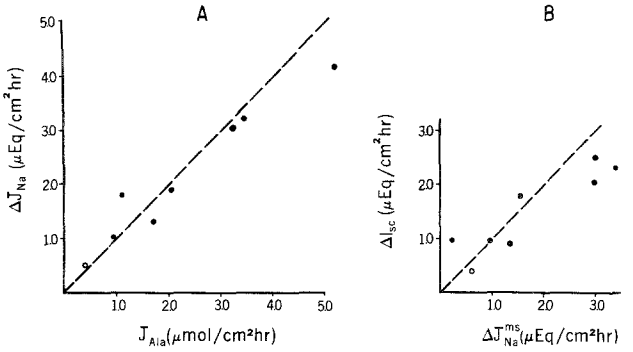


**Fig. 1.** (*A*): Relation between $\Delta J_{Na}$ (μeq/cm²hr) and $J_{ala}$ (μmol/cm²hr) determined for 8 tissues. 1 mM (○) or 10 mM (●) alanine was added to the mucosal solution. The dashed line indicates a 1:1 relation. A least-squares regression analysis of the data yields the relation

$$\Delta J_{Na} = 0.8\, J_{Ala} + 0.2 \; (r = 0.976,\ P < 0.01);$$

this does not differ significantly from the line of identity. (*B*): Relation between $\Delta J^{ms}_{Na}$ and $\Delta I_{sc}$ in μeq/cm²hr elicited by 1 mM (○) or 10 mM (●) alanine. The dashed line indicates a 1:1 relation

largely be accounted for by the increase in $J^{ms}_{Na}$ (Fig. 1*B*). These results are similar to those reported for small intestine and renal proximal tubule from a wide variety of animal species throughout the phylogenetic scale (Schultz, 1977). They are consistent with the notions that the increase in active Na absorption is the result of the coupled entry of Na and alanine across the apical membrane and that the coupling-coefficient of this co-transport process, under these conditions, *may* be unity.

The transepithelial conductance, $G_t$, under control conditions averaged 9.3 ± 1.0 mS/cm². This value is in agreement with that reported for *Amphiuma* small intestine (White, 1977). Alanine elicited a small but significant increase in $G_t$ to an average value of 12.0 ± 1.4 mS/cm².

### *Effect of Alanine on Membrane Potentials of Villus Cells*[4]

Successful punctures with conventional microelectrodes fulfilled the criteria previously adopted by this laboratory (Rose & Schultz, 1971; Duffey, Turnheim, Frizzell & Schultz, 1978); namely, (i) an abrupt negative deflection upon advancing into the cell; (ii) stability of the potential for at least 30 sec; and, (iii) a return of the potential to baseline when the electrode is retracted from the cell. Such impalements usually could be held for 20 min, permitting the assessment of effects of solution changes on $\psi^{mc}$ on a given cell. The results of a typical impalement of a villus cell and the response to the addition of 10 mM alanine to the mucosal solution are illustrated in Fig. 2. As shown, the addition of 10 mM alanine to the mucosal solution elicited a rapid depolarization of $\psi^{mc}$ and a reduction of $(R^m/R^s)$. The magnitude of the depolarization exceeded that of the initial $\psi^{mc}$, so that the cell interior became electrically positive with respect to the bathing solutions. In spite of the large magnitude of the change in $\psi^{mc}$, changes in $I_{sc}$ and $\psi^{ms}$ (not shown in Fig. 2) were minimal. After the abrupt depolarization of $\psi^{mc}$ there ensued a much slower repolarization associated with an increase in $(R^m/R^s)$ and parallel increases in $\psi^{ms}$ and $I_{sc}$. The peak changes in $I_{sc}$ and $\psi^{ms}$ were not reached until $\psi^{mc}$ and $(R^m/R^s)$ returned toward their initial values. Thus, the increases in the transepithelial electrical parameters paralleled the slow (about 10 min) repolarization of $\psi^{mc}$ rather than the initial abrupt depolarization. The steady-state values of $\psi^{mc}$, $(R^m/R^s)$, $\psi^{ms}$ and $I_{sc}$ were maintained as long as the Ringer's solution containing alanine superfused the mucosa.

The results of similar studies on 11 tissues are summarized in Table 2. In the absence of alanine, $\psi^{mc}$ averaged −39 ± 2 mV in agreement with values obtained for the small intestine of other species (Rose & Schultz, 1971; White & Armstrong, 1971; Okada et al., 1977*a*) and $(R^m/R^s)$ averaged 1.67 ± 0.35. Alanine initially depolarized $\psi^{mc}$ by +50 ± 3 mV to an average value of 11 ± 3 mV; the steady-state value of $\psi^{mc}$ in the presence of alanine was −27 ± 3 mV, significantly lower than the initial $\psi^{mc}$.

Returning the mucosal superfusate to the standard (alanine-free) solution ("washout") after the

[4] In this study we have confined our impalements to the cells on the upper portion of the villus. Gunter-Smith and White (1979) found that the increases in $\psi^{ms}$ and $I_{sc}$ elicited by actively transported sugars and amino acids arise primarily from the villous cells of *Amphiuma* small intestine and not from the intervillus cells.

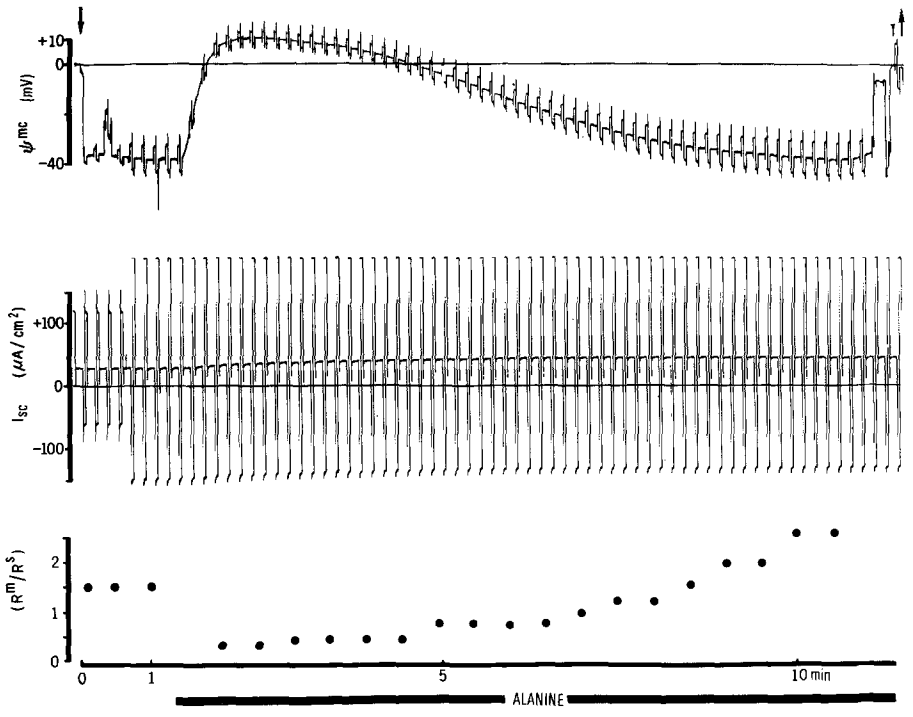


**Fig. 2.** Changes in $\psi^{mc}$ (upper tracing), $I_{sc}$ (middle tracing) and $(R^m/R^s)$ (lower tracing) elicited by the addition of 10 mM alanine to the solution bathing the mucosal surface. (↓) indicates impalement of a villus cell, (*T*) indicates advancement of the microelectrode through the epithelium; and (↑) retraction of the microelectrode

**Table 2.** Effects of alanine[a]

| Control | | | | "Peak"[b] | | "Steady-state"[c] | | | | Washout | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| $\psi^{ms}$ | $I_{sc}$ | $\psi^{mc}$ | $R^m/R^s$ | $\psi^{mc}$ | $R^m/R^s$ | $\psi^{ms}$ | $I_{sc}$ | $\psi^{mc}$ | $R^m/R^s$ | $\psi^{mc}$ | $R^m/R^s$ |
| 1.8 | 0.94 | −39 | 1.67 | +11 | 0.37 | 5.6 | 2.7 | −27 | 1.51 | −49 | 2.04 |
| ± | ± | ± | ± | ± | ± | ± | ± | ± | ± | ± | ± |
| 0.3 | 0.15 | 2 | 0.35 | 3 | 0.03 | 1.0 | 0.3 | 2.5 | 0.17 | 3.3 | 0.19 |
| (9) | (8) | (16) | (16) | (16) | (16) | (9) | (8) | (14) | (14) | (6) | (6) |
| Metabolic inhibition[d] | | | | | | | | | | | |
| 1.0 | 0.64 | −45 | 1.06 | −6 | 0.36 | 2.5 | 0.71 | −14 | 0.42 | −42 | 0.95 |
| ± | ± | ± | ± | ± | ± | ± | ± | ± | ± | ± | ± |
| 1.0 | – | 1 | 0.50 | 6 | 0.07 | 1.5 | – | 3 | 0.13 | 2 | 0.53 |
| (3) | (1) | (4) | (4) | (4) | (4) | (3) | (1) | (4) | (4) | (4) | (4) |

[a] $\psi^{ms}$ and $\psi^{mc}$ are in mV; $I_{sc}$ is given in μeq/cm$^2$ hr; the number of tissues studied is given in parentheses.
[b] Peak refers to values observed at the maximal depolarization.
[c] Steady-state values in presence of alanine.
[d] Metabolic inhibitors cyanide ($10^{-3}$ M) and iodoacetamide ($10^{-3}$ M) were added 1 hr prior to the measurements.

steady-state had been achieved resulted in a hyperpolarization of $\psi^{mc}$ and an increase in $(R^m/R^s)$ to values *above* those observed initially as illustrated in Fig. 3 and summarized in Table 2. These effects were transient and within 10–15 min $\psi^{mc}$, $(R^m/R^s)$, $\psi^{ms}$ and $I_{sc}$ slowly returned to pre-alanine ("control") values.

These observations suggested that at least two events with different time courses are associated with the increments in $\psi^{ms}$ and $I_{sc}$ produced by alanine, each associated with a change in the resistance of one or both limiting membranes. The following experiments were performed to gain further insight into these events.

### *Response to Alanine in the Presence of Metabolic Inhibitors*

The addition of metabolic inhibitors, cyanide ($10^{-3}$ M) and iodoacetamide ($10^{-3}$ M), to the serosal solution slowly reduced $\psi^{ms}$ and $I_{sc}$; after 30–60 min,

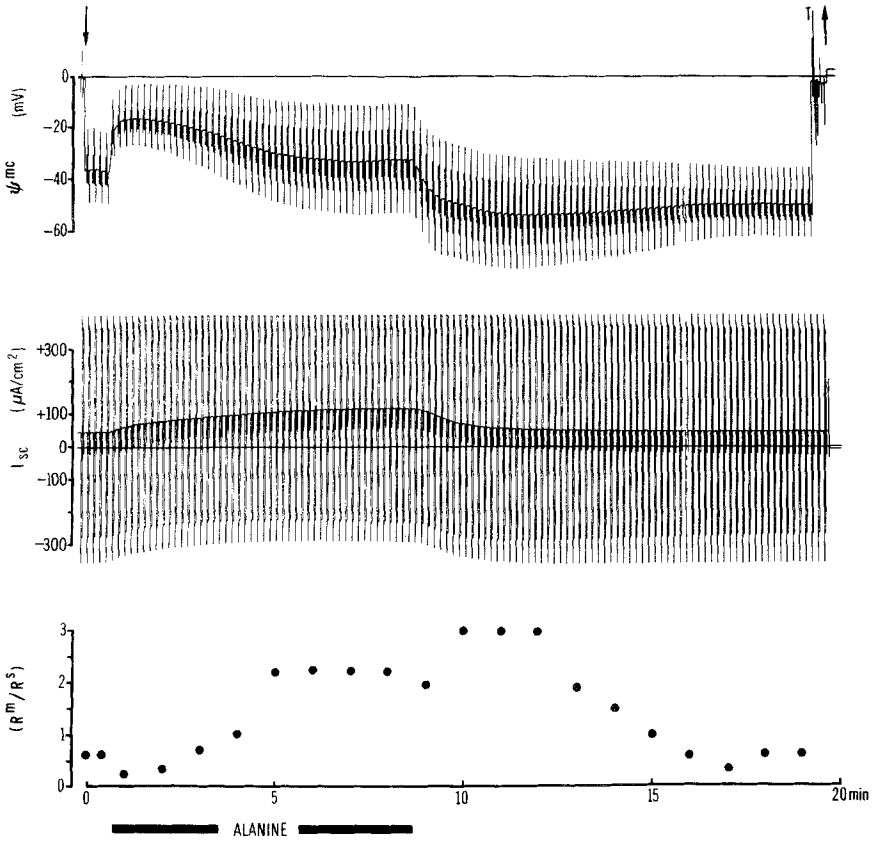


**Fig. 3.** Effects of 10 mM alanine addition to and washout from the mucosal solution on $\psi^{mc}$, $I_{sc}$, and $(R^m/R^s)$

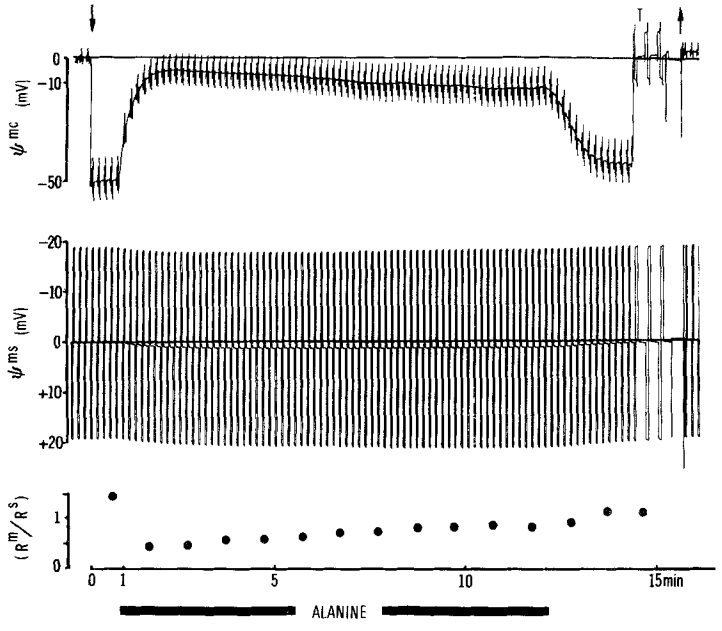


**Fig. 4.** Effect of alanine on $\psi^{mc}$, $\psi^{ms}$, and $(R^m/R^s)$ of a tissue bathed in Ringer's containing $10^{-3}$ M cyanide and $10^{-3}$ M iodoacetamide in the serosal solution for 1 hr prior to the impalement

$\psi^{ms}$ was reduced by at least 50%. A typical response to the addition of 10 mM alanine to the mucosal solution 1 hr after adding the metabolic inhibitors is illustrated in Fig. 4, and the results of such studies on four tissues are summarized in Table 2. Alanine elicited a rapid depolarization of $\psi^{mc}$ and a reduction in $(R^m/R^s)$ as observed in the absence of the metabolic inhibitors. However, there was no change in the polarity of $\psi^{mc}$. Furthermore, $\psi^{mc}$ did not repolarize (as in nonpoisoned tissues), and the re-

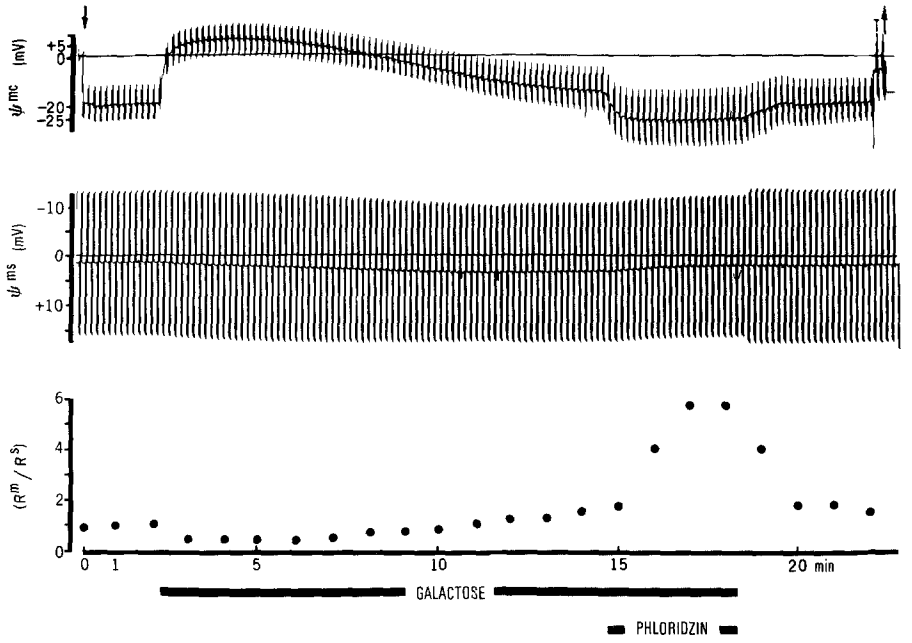


**Fig. 5.** Effect of 10 mM galactose on $\psi^{mc}$, $\psi^{ms}$, and $(R^m/R^s)$. Phloridzin ($3 \times 10^{-6}$ M) was added after a steady-state had been reached.

ductions of $\psi^{mc}$ and $(R^m/R^s)$ were essentially *sustained* until alanine was removed from the mucosal superfusate. The alanine-induced increases in $\psi^{ms}$ and $I_{sc}$ were less than 50% of those observed in nonpoisoned tissues. Continued incubation of tissues in the presence of the metabolic inhibitors gradually reduced the initial $\psi^{mc}$ and $(R^m/R^s)$. After 2 hr, $\psi^{mc}$ was $\simeq -15$ mV. Even under these conditions, alanine induced a small ($\simeq 3$ mV) sustained depolarization of $\psi^{mc}$.

These results suggest that the repolarization of $\psi^{mc}$ and the accompanying increase in $(R^m/R^s)$ are associated with some event(s) requiring metabolic energy. The depolarization $\psi^{mc}$ and the reduction of $(R^m/R^s)$ elicited by alanine do not appear to have such a requirement. A similar conclusion was reached for the case of rabbit ileum (Rose & Schultz, 1971) where metabolic inhibitors did not prevent the alanine-induced depolarization of $\psi^{mc}$ but markedly reduced the increment in $\psi^{ms}$.

*Response to Galactose and Phloridzin*

Results similar to those described above for alanine were observed when 10 mM galactose was added to the mucosal solution (Fig. 5). The addition of $3 \times 10^{-6}$ M phloridzin to the mucosal solution after a steady state had been reached in the presence of galactose ($\psi^{mc}$ had repolarized) resulted in a hyperpolarization of $\psi^{mc}$ and an increase in $(R^m/R^s)$ *above* pregalactose values. At this concentration the effect of phloridzin was reversible and $\psi^{mc}$ and $\psi^{ms}$ returned to control values after replacing the galactose-phloridzin Ringer's with the standard Ringer's solution.

**Table 3.** Effects of galactose on $\psi^{mc}$ and $(R^m/R^s)$[a]

| Control | | Peak | | Steady-state | | Phloridzin[b] | |
|---|---|---|---|---|---|---|---|
| $\psi^{mc}$ | $R^m/R^s$ | $\psi^{mc}$ | $R^m/R^s$ | $\psi^{mc}$ | $R^m/R^s$ | $\psi^{mc}$ | $R^m/R^s$ |
| −37 | 1.69 | +11 | 0.39 | −17 | 1.39 | – | – |
| ± | ± | ± | ± | ± | ± | – | – |
| 3 | 0.49 | 6 | 0.03 | 4 | 0.25 | | |
| (8) | (8) | (8) | (8) | (8) | (8) | | |
| −31 | 1.39 | −11 | 0.38 | −27 | 0.96 | −36 | 3.06 |
| ± | ± | ± | ± | ± | ± | ± | ± |
| 3 | 0.49 | 2 | 0.08 | 3 | 0.19 | 3 | 0.72 |
| (7) | (7) | (7) | (7) | (7) | (7) | (7) | (7) |
| Effects of galactose after metabolic inhibition | | | | | | | |
| −30 | 0.73 | −5 | 0.37 | −8 | 0.42 | −29 | 0.77 |
| ± | ± | ± | ± | ± | ± | ± | ± |
| 10 | 0.31 | 6 | 0.06 | 4 | 0.11 | 8 | 0.35 |
| (2) | (2) | (2) | (2) | (2) | (2) | (2) | (2) |

[a] $\psi^{mc}$ in mV. The number of tissues studied is given in parentheses.

[b] The concentration of phloridzin used in this study was $3 \times 10^{-6}$ M.

The results of such studies on 15 tissues are similar to those observed following addition of alanine to the mucosal superfusate and are summarized in Table 3. Of particular importance is the finding that the addition of phloridzin in the presence of galactose after the steady state was achieved resulted in an increase in $(R^m/R^s)$ to a value significantly

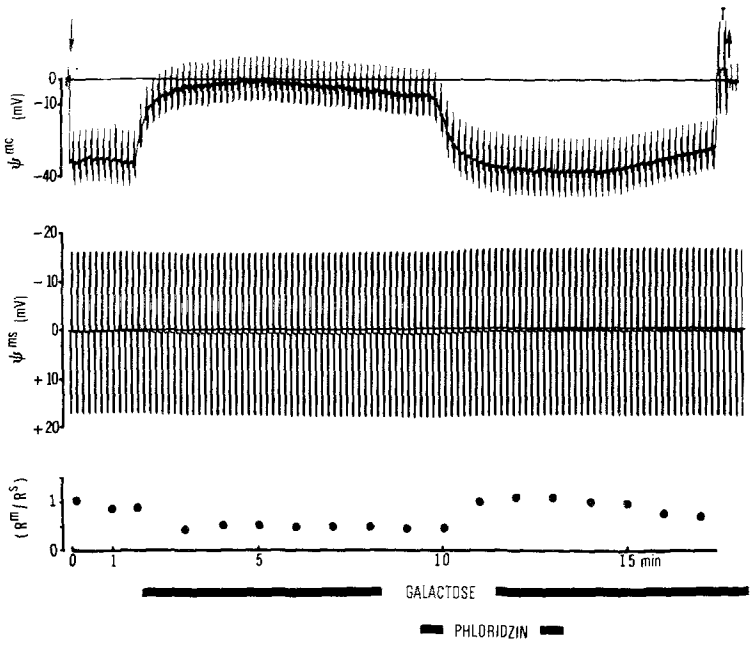


**Fig. 6.** Effect of galactose and phloridzin on $\psi^{mc}$, $\psi^{ms}$, and $R^m/R^s$ of tissue bathed in Ringer's containing cyanide and iodoacetamide in the serosal solution for one hour before the impalement

greater than that observed prior to the addition of galactose to the mucosal solution.

The effect of galactose and phloridzin on tissues exposed to metabolic inhibitors is shown in Fig. 6. Galactose was added to the mucosal solution one hour after the introduction of $10^{-3}$ M cyanide and $10^{-3}$ M iodoacetamide to the serosal solution. As observed with alanine, galactose elicited a *sustained* depolarization of $\psi^{mc}$ and decrease in $(R^m/R^s)$; $\psi^{ms}$ and $I_{sc}$ increased only slightly. The addition of phloridzin to the mucosal solution in the presence of galactose had effects identical to those seen when galactose was removed from the solution, i.e., $\psi^{mc}$ and $(R^m/R^s)$ returned to control values.

The results of these experiments are summarized in Table 3. It should be noted that in poisoned tissues the addition of phloridzin restored $\psi^{mc}$ and $(R^m/R^s)$ to control values, *not* to values significantly greater than control as observed in nonpoisoned tissues.

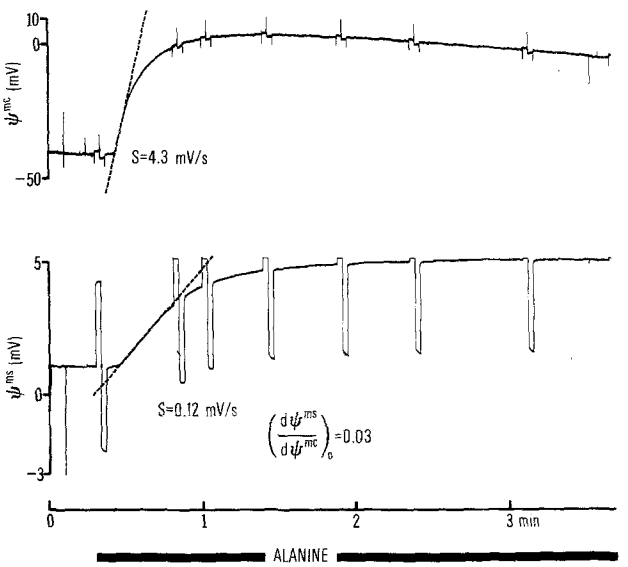


**Fig. 7.** Comparison of the initial rates of change of $\psi^{ms}$ and $\psi^{mc}$ elicited by alanine in a nonpoisoned tissue. *S* is the slope of the dashed line

### *Relation Between Initial Changes in Transapical and Transepithelial Electrical Potential Differences*

As noted above, the marked depolarization of $\psi^{mc}$ in response to the addition of galactose or alanine to the mucosal solution was accompanied by a minimal increase in $\psi^{ms}$. For reasons that will be discussed below, it was important to determine, as accurately as possible, the relation between $\Delta\psi^{ms}$ and $\Delta\psi^{mc}$ as soon as possible after the addition of galactose or alanine to the mucosal solution. To this end, a series of experiments was carried out in which alanine was rapidly introduced into the mucosal solution bathing normal or metabolically inhibited tissues and $\Delta\psi^{mc}$ and $\Delta\psi^{ms}$ were recorded simultaneously using a chart-speed of 1 mm $sec^{-1}$. A typical recording is shown in Fig. 7 and the results of this series of experiments are tabulated in Table 4 where $(d\psi^{ms}/d\psi^{mc})_o$ is the ratio of the initial rates of change of $\psi^{ms}$ and $\psi^{mc}$ and $(\Delta\psi^{ms}/\Delta\psi^{mc})_p$ is the ratio of the *increments* of these values at the "peak" of the depolarization. Clearly, the values of $(d\psi^{ms}/d\psi^{mc})_o$ do

**Table 4.** Relation between $(d\psi^{ms}/d\psi^{mc})_0$ and $(\Delta\psi^{ms}/\Delta\psi^{mc})_p$ under control and metabolically inhibited conditions[a]

| | $(d\psi^{ms}/d\psi^{mc})_0$ | $(\Delta\psi^{ms}/\Delta\psi^{mc})_p$ |
|---|---|---|
| Control | $0.03 \pm 0.01$ (7) | $0.06 \pm 0.02$ (7) |
| Metabolic inhibition[b] | $0.03 \pm 0.01$ (4) | $0.03 \pm 0.01$ (4) |

[a] $(d\psi^{ms}/d\psi^{mc})_0$ and $(\Delta\psi^{ms}/\Delta\psi^{mc})_p$ are defined in the text. The number of tissues studied is given in parentheses.
[b] Tissues were exposed to cyanide ($10^{-3}$ M) and iodoacetamide ($10^{-3}$ M).

not differ significantly in the presence or absence of metabolic inhibitors. However, in the absence of metabolic inhibitors, $(\Delta\psi^{ms}/\Delta\psi^{mc})_p$ is twice $(d\psi^{ms}/d\psi^{mc})_o$ whereas in the presence of metabolic inhibitors $(\Delta\psi^{ms}/\Delta\psi^{mc})_p$ is virtually identical to $(d\psi^{ms}/d\psi^{mc})_o$.

The implications of these findings will be discussed below.

## Discussion

In 1971, Rose and Schultz (1971) and White and Armstrong (1971) independently reported that the addition of sugars and/or amino acids to the solution bathing the mucosal surface of rabbit ileum and bullfrog small intestine, respectively, results in a prompt depolarization of the electrical potential difference across the apical membrane ($\psi^{mc}$) and simultaneous increases in $\psi^{ms}$ and $I_{sc}$. These observations have subsequently been confirmed for small intestine (Okada et al., 1977*b*) and renal proximal tubule (Maruyama & Hoshi, 1972; Hoshi et al., 1976; Frömter, 1977; 1979) from several species. Rose and Schultz (1971) also argued that because of the low resistance shunt pathway in these "leaky" epithelia the increase in the transepithelial electrical potential difference ($\psi^{ms}$) could not be attributed to events at the apical membrane alone but must be largely the result of an increase in pump activity at the basolateral membrane. Okada et al. (1977*a*) have arrived at a similar conclusion for the case of rat small intestine.

The problem, however, in pursuing this issue further stemmed from the fact that, in all of the previous studies, the electrophysiological responses (i.e., the depolarization of $\psi^{mc}$ and hyperpolarization of $\psi^{ms}$) were rapid and sustained so that events taking place at the apical and basolateral membranes could not be separated temporally and conclusions regarding the individual contribution of these barriers to the overall response were based on indirect inferences.

We have no explanation for the protracted transient responses following the addition of alanine or galactose to the solution bathing the mucosal surface of *Necturus* small intestine except to suggest that it may be related to the large size of these cells. Nevertheless, this transient permits a temporal separation of events at the apical and basolateral membranes and an approach toward a quantitative assessment of these events.

The time-course of the electrophysiological responses to the addition of alanine or galactose to the mucosal solution may be summarized as follows:

(*a*) The "immediate" response to the addition of the amino acid or sugar, limited only by the "dead space" of the perfusion system and the efficacy of stirring, is a marked depolarization of $\psi^{mc}$, which most often actually reversed its polarity, and a concomitant fourfold decline in the value of $(R^m/R^s)$. At the same time, the increase in $\psi^{ms}$ was minimal and the ratio $(\Delta\psi^{ms}/\Delta\psi^{mc})$ at the "peak" was only 0.06.

(*b*) The "peak" response is followed by a gradual repolarization of $\psi^{mc}$ that is paralleled by an increase in the value of $(R^m/R^s)$ and gradual increases in the values of $I_{sc}$ and $\psi^{ms}$.

(*c*) When the steady-state is achieved, the value of $\psi^{mc}$ is less negative than its value prior to the addition of sugar or acid amino to the mucosal solution; the value of $(R^m/R^s)$ does not differ significantly from that under control conditions; and, the increments in $I_{sc}$ and $\psi^{ms}$ are maximal.

It should be emphasized that in the previously reported studies, phases *a* and *b* could not be temporally resolved and the system appeared to enter phase *c* immediately.[5]

In order to assess the role of the Na-pump mechanism at the basolateral membrane in this transient response we initially attempted to inhibit the pump with ouabain. Unfortunately, this preparation, like some other amphibian epithelia (e.g., Degnan & Zadunaisky, 1976; DeLong & Civan, 1978), proved to be rather resistant to this glycoside and, after one hour with 1 mM ouabain in the serosal bathing solution, the $I_{sc}$ and the $\psi^{ms}$ were only slightly affected. Consequently, we were forced to employ metabolic inhibitors to accomplish this end. The results indicate that after exposure of the tissue to $10^{-3}$ M cyanide and $10^{-3}$ M iodoacetamide for one hour the subsequent addition of alanine or galactose to the mucosal solution brings about a depolarization of $\psi^{mc}$ and a decrease in the value of $(R^m/R^s)$ similar to those observed in the absence of metabolic inhibitors; but the repolarization of $\psi^{mc}$, the increase in the value of $(R^m/R^s)$, and the increases in the values of $I_{sc}$ and $\psi^{ms}$ were markedly inhibited.

[5] The depolarization of $\psi^{mc}$ in response to sugars or amino acids is followed by a small repolarization in *Amphiuma* small intestine (J.F. White, *personal communication*).

Finally, in order to determine whether the increase in the value of $(R^m/R^s)$ after achieving its minimum at the "peak" is due to an increase in $R^m$, a decrease in $R^s$, or both we employed phloridzin, a nontransported, competitive inhibitor of the Na-coupled sugar entry mechanism (Stirling, 1967), to block the apical entry step. If the only action of phloridzin is to block the Na-coupled entry step, this agent should increase $R^m$ to its original value and, if $R^s$ had not been affected, restore $(R^m/R^s)$ to its pregalactose value. As shown in Table 3, inhibition of the Na-coupled galactose entry pathway after a steady state was achieved resulted in an increase in the value of $(R^m/R^s)$ to a value *twice* that observed in the absence of galactose. However, as also shown in Table 3, in the presence of metabolic inhibitors, the addition of phloridzin simply restored the value of $(R^m/R^s)$ to that observed prior to the addition of galactose.

These findings indicate, conclusively, that the increase in $(R^m/R^s)$ after the peak of the transient is not due to an increase in $R^m$ but to a decrease in $R^s$ that is dependent upon the availability of metabolic energy.

### *An Equivalent Electrical Circuit Analysis*

In an initial attempt to analyze these data quantitatively, we employ the equivalent electrical circuit model of a villus cell illustrated in Fig. 8 where $R_i^m$ is the lumped (Thévenin) resistance of the apical membrane to the movements of all ions, $i$, and $E_i$ is the lumped (Thévenin) electromotive force acting on all $i$ across that barrier (for a discussion of these Thévenin equivalents *see* Schultz, 1980); $R^s$ and $E^s$ are the Thévenin resistance and electromotive force, respectively, across the basolateral (serosal) membrane; $R_{SNa}^m$ is the resistance offered by the mucosal membrane to the rheogenic transport of an unchanged solute ($S$) coupled to the transport of Na across that barrier and $E_{SNa}^m$ is the electromotive force of that transport process; and, $R^p$ is the resistance of the paracellular pathway. A similar model has been proposed by Frömter (1979) for the Na-coupled transport of sugars and amino acids across the luminal membrane of renal proximal tubule.

However, there is no *a priori* justification for the use of an electromotive force in series with a resistor to describe the relation between current flow resulting from a rheogenic, co-transport process and the "thermodynamic" driving force of that process. As discussed previously (Finkelstein & Mauro, 1963; Schultz, 1979, 1980; Sten-Kundsen, 1978), electrodiffusional processes that can be kinetically described by an integrated form of the Nernst-Planck equation

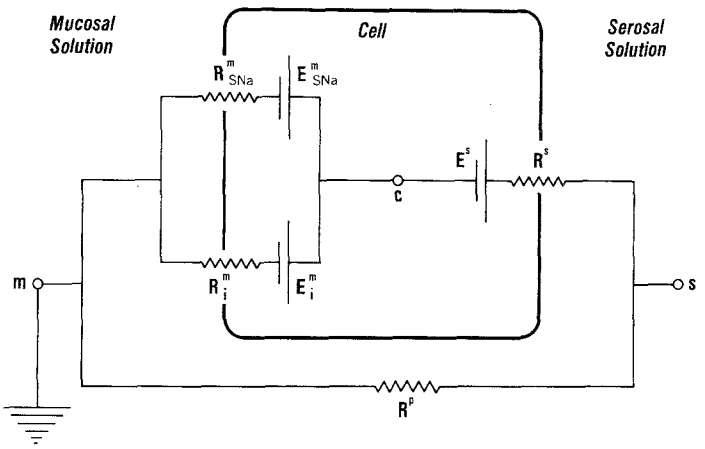


**Fig. 8.** An equivalent electrical circuit model of an intestinal villus cell, $m$, $c$, and $s$ designate the mucosal solution, intracellular compartment and serosal solution, respectively. Resistances and electromotive forces are described in the text

can be represented in equivalent electrical circuit models using resistors and batteries (electromotive forces) that have explicit physical meanings (*see* Appendix). The question is: can a rheogenic co-transport process that is presumably "carrier-mediated" be represented by an analogous linear relation[6] between flow and driving force given by

$$I_{SNa}^m = (E_{SNa}^m - \psi^{mc})/R_{SNa}^m \tag{1}$$

as inferred by the model illustrated in Fig. 8? And, if so, what are the physical meanings of $R_{SNa}^m$ and $E_{SNa}^m$?

Let us start by considering the meaning of $E_{SNa}^m$ which, by definition, is the equilibrium potential ("reversal potential") of the co-transport process; *or*, stated in other words, that value of $\psi^{mc}$ at which $I_{SNa}^m = 0$ (Schultz, 1979, 1980). From thermodynamic considerations, it follows that the equilibrium condition for a Na-coupled co-transport process across the apical membrane is given by (Schultz, 1977)

$$\nu\{[RT/\mathscr{F}] \ln[(\mathrm{Na})_m/(\mathrm{Na})_c] - \psi^{mc}\} = [RT/\mathscr{F}] \ln[(S)_c/(S)_m] \tag{2}$$

where the terms in parentheses are thermodynamic activities; $S$ stands for an uncharged solute; the subscripts $m$ and $c$ designate the mucosal solution and the intracellular compartment, respectively; $R$, $T$,

[6] Although relations between flows and driving forces of the type given in Eq. (1) are often referred to as "linear" or "Ohmic" it should be emphasized that the generalized resistance relating the flow and the driving force (in this case $R_{SNa}^m$) is, in general, a function of the properties (state) of the system (i.e., $E_{SNa}^m$, $\psi^{mc}$). Thus, the relation may be better characterized by the term "first order."

and $\mathscr{F}$ have their usual meanings; and $v$ is the coupling or stoichiometric coefficient of the co-transport process (i.e., the number of Na ions coupled to the transport of 1 molecule of $S$). In the following discussion and the Appendix we will assume, for the sake of simplicity, that $v=1$. It follows that:

$$E^m_{SNa}=[RT/\mathscr{F}]\ln[(Na)_m/(Na)_c] - [(RT/\mathscr{F}]\ln[(S)_c/(S)_m] \quad (3)$$

or

$$E^m_{SNa}=E^m_{Na}-E^m_S=[RT/\mathscr{F}]\ln[(Na)_m(S)_m/(Na)_c(S)_c] \quad (4)$$

where

$$E^m_{Na}=[RT/\mathscr{F}]\ln[(Na)_m/(Na)_c]$$

and

$$E^m_S=[RT/\mathscr{F}]\ln[(S)_c/(S)_m]. \quad (5)$$

[It should be noted that, in general, $E^m_{SNa}=E^m_{Na}-(E^m_s/v)$]. In addition, it can be shown that the electrophysiologic expression for $I^m_{SNa}$ given by Eq. (1) which is based on *thermodynamic* considerations and assumes that there is a linear (Ohmic) relation[6] between the flow of Na coupled to $S$ across the apical membrane and the conjugate driving force $(E^m_{SNa}-\psi^{mc})$ is, under some conditions, isomorphic with *kinetic* models of Na coupled co-transport processes and that $R^m_{SNa}$ can be expressed in physically meaningful terms (Appendix).

Solving this equivalent electrical circuit for $\psi^{ms}$ and $\psi^{mc}$ we obtain

$$\psi^{ms}=R^p(E^m+E^s)/(R^p+R^m+R^s) \quad (6)$$

and

$$\psi^{mc}=[(R^s+R^p)E^m-R^mE^s]/(R^p+R^m+R^s) \quad (7)$$

where $E^m$ and $R^m$ are the lumped electromotive force and resistance of the apical membrane respectively given by

$$E^m=(R^m/R^m_i)E^m_i+(R^m/R^m_{SNa})E^m_{SNa} \quad (8)$$

and

$$R^m=R^m_iR^m_{SNa}/(R^m_i+R^m_{SNa}). \quad (9)$$

We assume that prior to the addition of $S$ to the mucosal solution, $R^m_{SNa}=\infty$ so that $(R^m)_0=R^m_i$.[7] Now, the moment $S$ is added to the mucosal solution two events take place which lead to the rapid depolarization of $\psi^{mc}$: First, a *new* conductive pathway, $R^m_{SNa}$, is opened across the apical membrane; and, second, a *new* electromotive force is established across that barrier given by $E^m_{SNa}$. It can be readily shown that if the change in $\psi^{mc}$ is due to a change in $R^m$ and/or $E^m$ *alone* (before there is any change in $R^s$, $E^s$, etc.) then

$$(d\psi^{ms}/d\psi^{mc})_0=1/[1+(R^s/R^p)]. \quad (10)$$

This relation can be readily derived from Eqs. (6) and (7) by partial differentiation of $\psi^{ms}$ and $\psi^{mc}$ with respect to $R^m$ and $E^m$; then, $(\partial^2\psi^{ms}/\partial R^m\,\partial E^m)/(\partial^2\psi^{mc}/\partial R^m\,\partial E^m)$ yields Eq. (10).

As discussed above, the most rapid measurements of $(d\psi^{ms}/d\psi^{mc})_0$ possible using our recording technique yield values of 0.03 in the absence and presence of metabolic inhibitors. (It is evident from the data given in Table 4 that a significant decrease in $R^s$ had already taken place in nonpoisoned tissues by the time the "peak" response is reached.) Thus, from Eq. (10) we conclude that $R^s=32.33\,R^p$. Since in this leaky epithelium $R^p\simeq R_t=100\,\Omega\text{cm}^2$, it follows that $(R^s)_0\simeq 3233\,\Omega\text{cm}^2$. Further, since $(R^m/R^s)_0=1.7$ (Tables 2 and 3), it follows that $(R^m)_0=(R_i)=5496\,\Omega\text{cm}^2$. Inserting these values together with those from Table 2 into Eqs. (6) and (7), we can solve for $(E^m)_0$ and $(E^s)_0$ which are 59 and 100 mV, respectively. In this respect it is of interest that Garcia-Diaz, O'Doherty and Armstrong (1978) have reported that the intracellular Na and K activities in *Necturus* small intestine are 6 and 108 mM, respectively. Thus, $E_{Na}=[RT/\mathscr{F}]\ln[(Na)_m/(Na)_c]=65$ mV and, similarly, $E_K=81$ mV. These values, particularly the former, are in reasonable agreement with our estimates for $E^m$ and $E^s$ and are consistent with the "classical" notion that the apical membrane is predominantly Na-selective and that the basolateral membrane is predominantly K-selective.[8]

Finally, our data permit an independent test of the validity of this analysis. Thus, under control conditions,

$$\psi^{mc}=E^m-I^mR^m_i$$

where $I^m$ is the current across the apical membrane. From Tables 1 and 2 we see that the $I_{sc}$ averages

[7] The subscript 0 indicates values prior to the addition of sugar or amino acid (control) and the subscript $\infty$ denotes steady-state values.

[8] In the experiments reported by Garcia-Diaz et al. (1978) the K concentration in the bathing solutions was 5.4 mM. Preliminary measurements of intracellular K activity of *Necturus* small intestine bathed by solutions containing 1.9 mM K (activity) indicate that $(K)_c=68$ mM so that $E_K=91$ mV; this value is in good agreement with the estimate for $E^s$ of 100 mV (E. Grasset, P.J. Gunter-Smith and S.G. Schultz, *unpublished observations*).

approximately $0.75\,\mu eq/cm^2\,hr$ or approximately $19\,\mu A/cm^2$. Thus, $I^m R_i^m = (5.5 \times 10^3\,\Omega cm^2) \times (19\,\mu A/cm^2) = 105\,mV$. Hence, the predicted value of $\psi^{mc}$ is $(59-105) = -46\,mV$ which is in reasonable agreement with the observed value of $-39\,mV$.

We can also estimate the steady-state value of $(R^s)_\infty$, assuming that phloridzin abruptly closes the Na-coupled galactose entry (conductive) pathway and restores $(R^m)_\infty$ to $(R_i)_0$ from the observation that shortly after the addition of phloridzin to the mucosal solution $(R^m/R^s)_\infty = 3.1$. If this assumption is correct, the steady-state value of $(R^s)_\infty$ is $1773\,\Omega cm^2$ or almost half its original value. Further, since the steady-state value of $(R^m/R^s)_\infty$ in the presence of galactose is 0.96 (Table 3) we can conclude that $(R^m)_\infty$ is approximately $1702\,\Omega cm^2$ (approximately one-third its original value) and that $(R^m_{SNa})_\infty$ is approximately $2466\,\Omega cm^2$.

It should be emphasized that these steady-state estimates are based on the assumption that $R_i$ is essentially constant over the range $-40 < \psi^{mc} < -25\,mV$ (i.e., the range of initial and steady-state values). It is not possible to extend this analysis to the values observed at the peak of the response because it may not be reasonable to assume that $R_i$ and $R^s$ are voltage-independent over the range of $-40$ to $+10\,mV$.

## Conclusions

### *An Electrochemical Feedback Model for Na-Coupled Solute Transport*

In 1977, Schultz (1977) suggested that the role of the active Na extrusion mechanism ("pump") at the basolateral membrane of small intestine with respect to rheogenic Na-coupled solute transport is twofold. First, as postulated earlier (Schultz & Zalusky, 1964*b*), it serves to maintain a low intracellular Na activity and thus the *chemical* driving force for Na across the apical membrane, $E^m_{Na}$. Second, if the pump is rheogenic as suggested by the data of Rose and Schultz (1971), an increase in pump activity would serve to hyperpolarize $\psi^{mc}$ and thus maintain the *electrical* driving force for the Na-coupled entry process. The present findings provide strong support for the notion that an increase in pump activity results in a hyperpolarization of $\psi^{mc}$ and provides new insight into the underlying mechanism of this response.

As discussed above, the addition of sugars or amino acids to the mucosal solution results in the "insertion" of a new conductive pathway across the apical membrane, $R^m_{SNa}$, and a new electromotive force, $E^m_{SNa}$, oriented so that the cell interior is electrically positive.[9] From Eq. (7) it should be clear that an increase in $E^m$ and a decrease in $R^m$ both serve to depolarize $\psi^{mc}$ which, in these studies, actually reversed polarity. However, this is, so-to-speak, "self-defeating" inasmuch as the electrical contribution to the driving force for the rheogenic Na-coupled entry process as well as for any other conductive Na entry process uncoupled to nonelectrolytes is reduced by approximately 50 mV.

The repolarization of $\psi^{mc}$ is the result of at least two processes. The first is related to the accumulation of $S$ within the cell which decreases the value of $E^m_{SNa}$ and, ultimately, when $(S)_c > (S)_m$ reverses the orientation of $E^m_S$. This is, in part, the reason for the hyperpolarization of $\psi^{mc}$ (Fig. 2 and Table 2) observed when the mucosal solution is rendered alanine-free after a steady-state intracellular accumulation is achieved.[10]

The second, and apparently the most important, reason for the repolarization is an energy-dependent decrease in $R^s$ to a value estimated to be approximately one-half of its original value. It is clear from Eq. (7) that a decrease in $R^s$ alone will lead to a hyperpolarization of $\psi^{mc}$ and that this effect will be greater if it is also accompanied by a decrease in $E^m$ (*see* above). It is not clear at this time whether one must also invoke an energy-dependent increase in $E^s$ to quantitatively satisfy our data.

Finally, as illustrated in Figs. 2, 3 and 5, the *physiologically* important event, namely the rate of active Na absorption as reflected by the $I_{sc}$, increases in parallel with the repolarization of $\psi^{mc}$ and the re-establishment of a favorable *electrical* driving force for Na entry into the cell via conductive pathways whether coupled to nonelectrolytes or not.[11] Clearly, immediately after adding alanine or galactose to the mucosal solution, the chemical gradients

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[9] Inasmuch as the concentration of $S$ in the cell is likely to be negligible under control conditions, immediately after addition of $S$ to the mucosal solution, $E^m_{SNa} \to \infty$.

[10] By analogy with the argument in footnote 9, when the mucosal solution is rendered $S$-free, $E^m_{SNa} \to -\infty$ and $I^m_{SNa}$ is directed from the cell to the mucosal solution.

[11] Although the increment in $I_{sc}$ once the steady-state has been achieved can be attributed to an increase in the rate of active Na absorption as a result of the coupled entry step ($I^m_{SNa}$) (Fig. 1), the ionic basis of the $I_{sc}$ during the transient is unclear. Clearly, when $\psi^{mc}$ reverses polarity there is a considerable driving force for the diffusional flow of K out of the cell (presumably across the basolateral membrane) and the flow of Cl into the cell (if conductive pathways are present for this ion at either membrane). At the same time one can state with reasonable confidence that conductive movements of Na into the cell, whether coupled to the nonelectrolyte or not, must be impeded and must increase as cell negativity is restored.

of Na and $S$ favoring the coupled entry process are maximal and decline as $(S)_c$ and, perhaps, $(Na)_c$ increase toward their final steady-state values.[12] Nonethe-less, at this time the increment in $I_{sc}$ is minimal. It is not until $\psi^{mc}$ has repolarized that $\Delta I_{sc}$ or $I^m_{SNa}$ reaches its maximum in spite of the fact that at this time the chemical driving force for this flow is reduced. Thus, it appears that it is the *electrical feedback* between the basolateral membrane and the apical membrane, which results in an increase in the negativity of the cell interior, that plays a dominant role in the overall co-transport process (*see* Appendix).

### *The Relation between the "Pump" and "Leak" Properties of the Basolateral Membrane*

The most direct interpretation of the parallelism between the increase in $I_{sc}$ and the decrease in $R^s$ is that an increase in Na pump activity at the basolateral membrane is associated with a near parallel decrease in the electrical resistance of that barrier. Higgins, Gebler and Frömter (1977) arrived at the same conclusion for the case of *Necturus* urinary bladder and suggested that this could be explained "... if the passive K-leak permeability were an inherent property of the Na−K pump unit or were regulated by an intracellular feedback mechanism." Clearly, if the coupling ratio of the Na−K exchange pump is invariant, such a mechanism would serve to prevent large changes in cell K activity in the face of major *physiological* variations in the rate of transcellular Na transport (or pump activity). Indeed, Lee and Armstrong (1972) reported that the intracellular K activity, $(K)_c$, in bullfrog small intestine determined using ion-selective microelectrodes decreased following addition of 3-O-methylglucose to the mucosal solution, and, using similar methods, White (1976) has reported that $(K)_c$ in *Amphiuma* small intestine is the same in the presence and absence of 10 mM glucose; although the rate of Na absorption was not measured in these studies, it is fair to assume that it was increased in the presence of these sugars (Schultz, 1977). Recently, Kristensen (1980) has reported that rheogenic Na-alanine co-transport by isolated rat hepatocytes is associated with a five-fold increase in the permeability of the hepatocyte membrane to K and no significant change in cell K content.

The notion of a parallel relation between the activity of the Na−K pump and the K-leak is also supported by studies in which pump activity is inhibited. Thus, 20 years ago, MacRobbie and Ussing (1961) reported that exposure of the inner membrane of frog skin to ouabain decreased the permeability of that barrier to KCl and concluded: "It may be that any interference with the pump mechanism is accompanied by a decrease in the passive permeabilities to ions and that the active ion transport and the passive fluxes are not entirely independent." More recently, Helman, Nagel and Fisher (1979) demonstrated that addition of ouabain to the solution bathing the inner surface of frog skin is followed by a marked increase in the resistance of the inner membrane; this finding is consistent with the notion that inhibition of pump activity results in a decrease in the permeability of that barrier to K.

The mechanism responsible for the parallel increase in the permeability of the basolateral membrane with increasing Na pump activity is unclear, but the two possibilities raised by Higgins et al. (1977) warrant brief consideration.

The notion that "leaks" are an inherent property of the "pump" and that an increase in Na transport involves the recruitment of more pump-leak units, while highly speculative, derives some support from the arguments marshalled by Lew and Beauge (1979) to the effect that "passive" (dissipative) cation fluxes across the erythrocyte membrane are mediated by the pump mechanism (the "all pump" model).

The notion that the parallelism between pump activity and K permeability may be mediated by an intracellular "feedback" messenger also has some experimental precedent. It is well established that intracellular Ca in the physiological range markedly influences the K permeability of erythrocytes and several other cell types (the "Gardos effect") (Lew & Beauge, 1979). Further, there is evidence that the sensitivity of the K channel to Ca is modulated by intracellular levels of ATP or some other metabolite (Lew & Beauge, 1979); a decrease in ATP appears to increase the sensitivity of the K channels to Ca. It is intriguing to speculate that an increase in cell Ca and/or a decrease in ATP associated with increased Na transport mediates the parallel increase in K permeability of the basolateral membrane. Clearly, this admittedly speculative notion is more amenable to experimental test than the former.

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[12] At present it is unclear whether $(Na)_c$ changes significantly in the presence of sugars or amino acids in spite of the fact that $J_{Na}$ is markedly increased. Lee and Armstrong (1972) did not observe an increase in $(Na)_c$ in bullfrog small intestine cells following stimulation of Na absorption with galactose.

## Appendix

It can be readily shown that electrodiffusional processes that can be described by an integrated form of the Nernst-Planck equation can be represented in equivalent electrical terms by the equation:

$$I_i R_i = E_i - \Delta\psi \tag{A1}$$

where $I_i$ is the current due to the flow of $i$; $E_i$ is the electromotive force given by the Nernst equation, i.e., $E_i = (RT/\mathscr{F})\,\Delta \ln(i)$; $\Delta\psi$ is the observed electrical potential difference across the barrier; and $R_i$ is the "integral resistance" of the barrier to $i$ given by

$$R_i = \int_{x=0}^{x=x} (dx/z^2\mathscr{F}^2 u_i c_i) \tag{A2}$$

where $u_i$ is the mobility of $i$; $c_i$ is the activity of $i$ at some point within the barrier; and, $x$ is the thickness of the barrier (Finkelstein & Mauro, 1963; Sten-Knudsen, 1978; Schultz, 1980).

We now enquire whether the kinetic equations that describe rheogenic Na-coupled co-transport are also isomorphic with Eq. (A1)?

To do so, let us consider the kinetic model illustrated in Fig. 9 where $X$ is a neutral carrier molecule confined to the membrane. We assume that Na can bind to $X$ at the interfaces to form a cationic binary complex, Na$X$, and that then the sugar or amino acid ($S$) can bind to form the ternary complex $S$Na$X$. We further assume that only the free carrier, $X$, and the ternary complex, $S$Na$X$, can translocate across the membrane; this is the simplest such model and ensures a coupling-coefficient, $\nu$, of unity. $P$ is the first-order rate constant for the translocation of the neutral carrier and is independent of $\psi^{mc}$. The translocation rate constant for the movements of $[S\mathrm{Na}X]_m$ from $m$ to $c$ and $[S\mathrm{Na}X]_c$ from $c$ to $m$ are given by $P\xi$ and $P\xi^{-1}$, respectively, where $\xi = \exp(-\mathscr{F}\psi^{mc}/2RT)$. This, in essence, assumes that the electrical potential profile is antisymmetric about the midpoint of the membrane (for further discussion of this point, *see* Schultz, 1980). Finally we assume that translocation is rate limiting so that the association and dissociation reactions at the interfaces can be described by the equilibrium (dissociation) constants $K_1$ and $K_2$ as shown in Fig. 9.

Solving the equations that describe this system (Schultz, 1980), we obtain

$$J^m_{S\mathrm{Na}} = P[X]_t\{\xi K_1 K_2 (\mathrm{Na})_m (S)_m - \xi^{-1} K_1 K_2 (\mathrm{Na})_c (S)_c\}/D \tag{A3}$$

where $J^m_{S\mathrm{Na}}$ is the net flow of $S$ and Na and is positive when directed from $m$ to $c$; $[X]_t$ is the total concentration of $X$ in all forms per cm$^2$ membrane; and

$$\begin{aligned} D = {} & \{[\xi + \xi^{-1}]\,(\mathrm{Na})_m (\mathrm{Na})_c (S)_m (S)_c + [1 + \xi^{-1}]\,K_1 K_2 (\mathrm{Na})_c (S)_c] \\ & + [1 + \xi]\,K_1 K_2 (\mathrm{Na})_m (S)_m + K_2 (\mathrm{Na})_m (\mathrm{Na})_c [\xi (S)_m + \xi^{-1} (S)_c] \\ & + K_1 K_2^2 [(\mathrm{Na})_m + (\mathrm{Na})_c] + 2K_1^2 K_2^2\}. \end{aligned} \tag{A4}$$

Clearly, $J^m_{S\mathrm{Na}} = 0$ when

$$[(\mathrm{Na})_m (S)_m / (\mathrm{Na})_c (S)_c] = \xi^{-2} = \exp(\mathscr{F}\psi^{mc}/RT)$$

as demanded by the thermodynamic considerations described by Eqs. (1), (2) and (3) in the text.

Now, using the definition of $E^m_{S\mathrm{Na}}$ given by Eqs. (3) and (4) and simple algebraic manipulation, it can be shown that Eq. (A3) can be reduced to

$$I^m_{S\mathrm{Na}} = \frac{\mathscr{F}\{\exp(\mathscr{F}E^m_{S\mathrm{Na}}/RT) - \exp(\mathscr{F}\psi^{mc}/RT)\}}{D/[\xi K_1 K_2 P[X]_t (\mathrm{Na})_c (S)_c]}. \tag{A5}$$

Clearly, $I^m_{S\mathrm{Na}} = 0$ when $E^m_{S\mathrm{Na}} = \psi^{mc}$ [Eq. (1)]. Further using the Taylor series expansion for $e^x$, Eq. (A5) reduces to

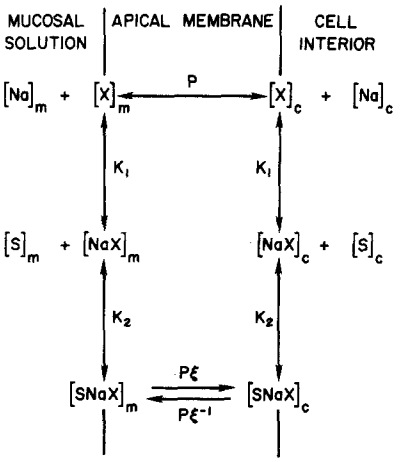


**Fig. 9.** Kinetic model for Na-coupled solute transport

$$I^m_{S\mathrm{Na}} = \frac{(\mathscr{F}^2/RT)\,(E^m_{S\mathrm{Na}} - \psi^{mc}) + \mathscr{R}}{D/[\xi K_1 K_2 P[X]_t (\mathrm{Na})_c (S)_c]} \tag{A6}$$

where $\mathscr{R}$, the remainder, is the summation of all of the terms of second order and higher and is given by (at $T = 22\,^\circ$C)

$$\mathscr{R} = \mathscr{F} \sum_{n=1}^{n=\infty} \{(E^m_{S\mathrm{Na}}/25.2)^{n+1} - (\psi^{mc}/25.2)^{n+1})\}\,! \tag{A7}$$

Clearly when $\mathscr{R} \ll \{(E^m_{S\mathrm{Na}} - \psi^{mc})/25.2\}$, Eq. (A6) reduces to

$$I^m_{S\mathrm{Na}} = \frac{E^m_{S\mathrm{Na}} - \psi^{mc}}{RTD/[\mathscr{F}^2 \xi K_1 K_2 P[X]_t (\mathrm{Na})_c (S)_c]}. \tag{A8}$$

Equation (A8) is clearly isomorphic with Eqs. (1) and (A1) where $R^m_{S\mathrm{Na}}$ is given by the denominator. It is a simple matter to show that: (i) the denominator has units of $\Omega \cdot \mathrm{cm}^2$; and (ii) $R^m_{S\mathrm{Na}}$ is inversely related to the total concentration of carrier in charged form (i.e., $[S\mathrm{Na}X]_m + [S\mathrm{Na}X]_c$) and $P$ as expected on intuitive grounds and inferred by Eq. (A2).

We now address two important questions:

(1) Is the isomorphism between the kinetic expression for $J^m_{S\mathrm{Na}}$ and $I^m_{S\mathrm{Na}}$ given by Eqs. (A3) and (A8) dependent upon the particular model we chose to examine (Fig. 9)? The answer to this question is: *No*. It can readily be shown that reversing the order of association (i.e., $S$ first then Na) would only affect the value of $D$ without affecting the numerator of Eq. (A3). The same is true if we assume that the free carrier, $X$, is anionic and the ternary complex, $S$Na$X$, is neutral (Schultz, 1980). Finally it should be intuitively obvious that regardless of the complexity of the *kinetic* model, the numerator must satisfy the *thermodynamic* condition that $J_{S\mathrm{Na}} = 0$ when $E^m_{S\mathrm{Na}} = \psi^{mc}$ and thus *must* be of the general form $\{f(E^m_{S\mathrm{Na}}) - f(\psi^{mc})\}$.

(2) Can one generally assume that $\mathscr{R}$ in Eq. (A6) can be ignored so that this equation *always* reduces to the form of Eqs. (A8) or (A1)? The answer is: *No*. That is, in general, the flow ($J^m_{S\mathrm{Na}}$ or $I^m_{S\mathrm{Na}}$) is not a first-order function of the driving force ($E^m_{S\mathrm{Na}} - \psi^{mc}$) [*see* Eqs. (A6) and (A7)]. Consequently, the rheogenic Na-coupled entry process cannot, in general, be represented in an equivalent electrical circuit model as a resistor ($R^m_{S\mathrm{Na}}$) in series with an electromotive force ($E^m_{S\mathrm{Na}}$) which obeys Eqs. (1) or (A1). However, there are conditions where the contribution of $\mathscr{R}$ to the numerator of Eq. (A6) is minimal (i.e., less than 10%) and under those, empirically determined, conditions, Eq. (A6) is isomorphic with Eqs. (1) or (A1).

The relations between $J^m_{S\mathrm{Na}}$ (or $I^m_{S\mathrm{Na}}$) and $\psi^{mc}$ given by Eqs. (A3) and (A4) are illustrated in Fig. 10 for the conditions

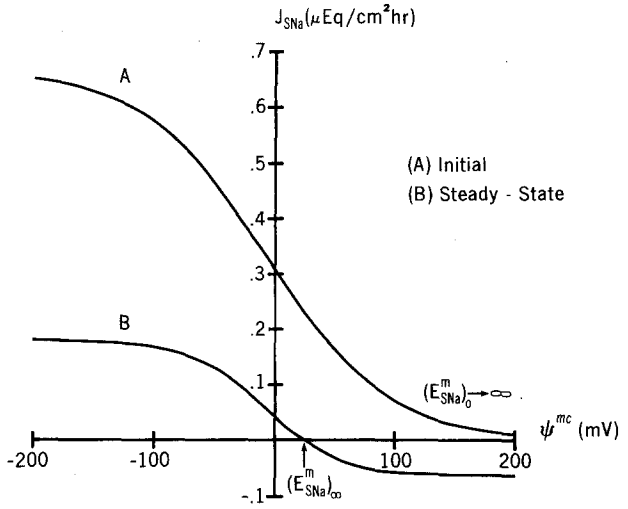


**Fig. 10.** Current-voltage relation predicted by the kinetic model for Na-coupled solute transport shown in Fig. 9. *A* represents the "initial" relation when $(S)_c = 0$ and *B* represents the "steady-state" relation assuming $(S)_c = 40$ mM

where $(Na)_m = 100$ mM; $(Na)_c = 10$ mM; $(S)_m = 10$ mM; $K_1 = 20$ mM; $K_2 = 5$ mM; $P[X]_t = 1$; and $(S)_c \cong 0$ (initial) or $(S)_c = 40$ mM (steady state). The values for $(Na)_c$, $K_1, K_2$ and the steady-state value of $(S)_c$ are reasonable estimates drawn from the literature (Schultz & Curran, 1970).

The important points are: (i) in the presence of extreme hyperpolarized or depolarized values of $\psi^{mc}$, $J^m_{SNa}$ is independent of $\psi^{mc}$ and the co-transport system behaves like a "constant-current" generator; however, (ii) over the range $-50\,\text{mV} < \psi^{mc} < 50\,\text{mV}$, $J^m_{SNa}$ is, within the limits of experimental error, a linear function of $\psi^{mc}$ and thus behaves like a constant source of electromotive force (zero internal resistance) in series with a finite resistance. Stark (1973) has derived similar current-voltage relations for "carrier-mediated" ion transport using a somewhat different, more general, approach. Clearly, in the "steady-state", $I^m_{SNa}$ is a linear function of $\psi^{mc}$ between $\psi^{mc} = 24\,\text{mV} \cong E^m_{SNa}$ and $\psi^{mc} = -40$ mV and thus can be described, over this range, by Eq. (1) or (A1) with constant $R^m_{SNa}$.

Finally, inspection of Fig. 10 reveals that the co-transport process is very sensitive to $\psi^{mc}$ over the range observed in these studies, regardless of the chemical driving force. Thus, when $(S)_c \cong 0$ (initial), $E^m_{SNa}$ given by Eq. (3) is very large (approaches infinity). Nonetheless, there is a marked decrease in $J^m_{SNa}$ when $\psi^{mc}$ goes from $-50$ to $+50$ mV. Stated in other words: The *kinetic* relation given by Eq. (A3) predicts a marked effect of a *finite* change in $\psi^{mc}$ on $J^m_{SNa}$ even when the *thermodynamic* chemical driving force $E^m_{SNa} \to \infty$. This is, of course, consistent with our finding that $\Delta I_{sc}$ is minimal at the "peak" and increases as $\psi^{mc}$ repolarizes.

## References


Barry, R.J.L., Eggenton, J. 1972. Membrane potentials of epithelial cells in rat small intestine *J. Physiol. (London)* **277**:201–216

Degnan, K.J., Zadunaisky, J.A. 1976. The electrical properties and active ion transport across the urinary bladder of the urodele, *Amphiuma* means. *J. Physiol. (London)* **265**:207–230

DeLong, J., Civan, M.M. 1978. Dissociation of cellular $K^+$ accumulation from net $Na^+$ transport by toad urinary bladder. *J. Membrane Biol.* **42**:19–43

Duffey, M.E., Turnheim, K., Frizzell, R.A., Schultz, S.G. 1978. Intracellular chloride activities in rabbit gallbladder: Direct evidence for the role of the sodium gradient in energizing "uphill" chloride transport. *J. Membrane Biol.* **42**:229–245

Finkelstein, A., Mauro, A. 1963. Equivalent circuits as related to ionic systems. *Biophys. J.* **3**:215–233

Frizzell, R.A., Field, M., Schultz, S.G. 1979. Sodium-coupled chloride transport by epithelia tissues. *Am. J. Physiol.* **236**:F1–F8

Fromm, M., Schultz, S.G. 1981. Some properties of KCl-filled microelectrodes: Correlation of potassium "leakage" with tip resistance. *J. Membrane Biol.* **62**:239–244

Frömter, E. 1977. Magnitude and significance of the paracellular shunt path in rat kidney proximal tubule. *In:* Intestinal Permeation. H. Kramer and E. Lauterbach, editors. pp. 166–178. Excerpta Medica, Amsterdam

Frömter, E. 1979. Solute transport across epithelia: What can we learn from micropuncture studies on kidney tubules? *J. Physiol. (London)* **288**:1–31

Garcia-Diaz, J.F., O'Doherty, J., Armstrong, W.McD. 1978. Potential profile, K and Na activities in *Necturus* small intestine. *Physiologist* **21**:41

Gilles-Ballien, M., Schoffeniels, E. 1965. Site of action of L-alanine and D-glucose on the potential differences across the intestine. *Arch. Int. Physiol. Biochim.* **73**:355–357

Gunter-Smith, P.J., Grasset, E., Schultz, S.G. 1980. The electrophysiology of Na-coupled alanine transport in *Necturus* small intestine. *J. Gen. Physiol.* **74**:11*a*

Gunter-Smith, P.J., Schultz, S.G. 1982. Potassium transport and intracellular potassium activities in rabbit gallbladder. *J. Membrane Biol. (in press)*

Gunter-Smith, P.J., White, J.F. 1979. Contributions of villus and intervillus epithelium to intestinal transmural p.d. and response to theophylline and sugar. *Biochim. Biophys. Acta* **557**:425–435

Helman, SI.., Nagel, W., Fisher, R.S. 1979. Ouabain on active transepithelial sodium transport in frog skin. Studies with microelectrodes. *J. Gen. Physiol.* **74**:105–127

Higgins, T.J., Gebler, B., Frömter, E. 1977. Electrical properties of amphibian urinary bladder epithelia. I. The cell potential profile of *Necturus maculosus*. *Pfluegers Arch.* **371**:87–97

Hoshi, T., Sudo, K., Suzuki, Y. 1976. Characteristics of changes in the intracellular potential associated with transport of neutral, dibasic and acidic amino acids in *Triturus* proximal tubule. *Biochim. Biophys. Acta* **448**:492–504

Kristensen, L.O. 1980. Energization of alanine transport in isolated rat hepatocytes. *J. Biol. Chem.* **255**:5236–5243

Lee, C.O., Armstrong, W.McD. 1972. Activities of sodium and potassium ions in epithelial cells of small intestine. *Science* **175**:1261–1264

Lew, V.L., Beauge, L. 1979. Passive cation fluxes in red cell membranes. *In:* Membrane Transport in Biology. G. Giebisch, D.C. Tosteson, and H.H. Ussing, editors. Vol. 2, pp. 81–115. Springer-Verlag, Berlin

Lyon, I., Sheerin, H.E. 1971. Studies on transmural potentials *in vitro* in relation to intestinal absorption. VI. The effect of sugars on electrical potential profiles in jejunum and ileum. *Biochim. Biophys. Acta* **249**:1–4

MacRobbie, E.A.C., Ussing, H.H. 1961. Osmotic behaviour of the epithelial cells of frog skin. *Acta Physiol. Scand.* **53**:348–365

Maruyama, T., Hoshi, T. 1972. The effect of D-glucose on the electrical potential profile across the proximal tubule of newt kidney. *Biochim. Biophys. Acta* **282**:214–225

Okada, Y., Irimajiri, A., Inouye, A. 1977*a*. Electrical properties and active solute transport in rat small intestine: II. Conductive properties of transepithelial routes. *J. Membrane Biol.* **31**:221–232

Okada, Y., Tsuchiya, W., Irimajiri, A., Inouye, A. 1977*b*. Electri-

cal properties and active solute transport in rat small intestine: I. Potential profile changes associated with sugar and amino acid transport. *J. Membrane Biol.* **31**:205–219

Rose, R.C., Schultz, S.G. 1971. Studies on the electrical potential profile across rabbit ileum. Effects of sugars and amino acids on transmural and transmucosal electrical potential differences. *J. Gen. Physiol.* **57**:639–663

Schultz, S.G. 1977. Sodium-coupled solute transport by small intestine: A status report. *Am. J. Physiol.* **233**:*E*249–*E*254

Schultz, S.G. 1979. Application of equivalent electrical circuit models to study of sodium transport across epithelial tissues. *Fed. Proc.* **38**:2024–2029

Schultz, S.G. 1980. Basic Principles of Membrane Transport. Cambridge University Press, New York

Schultz, S.G., Curran, P.F. 1970. Coupled transport of sodium and organic solute. *Physiol. Rev.* **50**:637–718

Schultz, S.G., Frizzell, R.A., Nellans, HN.. 1974. Ion transport by mammalian small intestine. *Annu. Rev. Physiol.* **36**:51–91

Schultz, S.G., Zalusky, R. 1964*a*. Ion transport in isolated rabbit ileum. I. Short-circuit current and Na fluxes. *J. Gen. Physiol.* **47**:567–584

Schultz, S.G., Zalusky, R. 1964*b*. Ion transport in isolated rabbit ileum. II. The interaction between active sodium transport and active sugar transport. *J. Gen. Physiol.* **47**:1043–1059

Stark, G. 1973. Rectification phenomena in carrier-mediated ion transport. *Biochim. Biophys. Acta* **289**:323–332

Sten-Knudsen, O. 1978. Passive Transport Processes. *In*: Membrane Transport in Biology. G. Giebisch, D.C. Tosteson, and H.H. Ussing, editors. Vol. 1, pp. 5–113. Springer-Verlag, Berlin

Stirling, C.E. 1967. High-resolution radioautography of phloridzin $-H^3$ in rings of hamster intestine. *J. Cell Biol.* **35**:605–618

White, J.F. 1976. Intracellular potassium activities in *Amphiuma* small intestine. *Am. J. Physiol.* **231**:1214–1219

White, J.F. 1977. Alterations in electrophysiology of isolated amphibian small intestine produced by removing the muscle layers. *Biochim. Biophys. Acta* **467**:91–102

White, J.F., Armstrong, W.McD. 1971. Effect of transported solutes on membrane potentials in bullfrog small intestine. *Am. J. Physiol.* **221**:194–201

Wright, E.M. 1966. The origin of the glucose dependent increase in the potential difference across the tortoise small intestine. *J. Physiol.* (*London*) **185**:486–500

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