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Current-Voltage Relations of Sodium-Coupled Sugar Transport Across the Apical Membrane of *Necturus* Small Intestine

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Summary. The current-voltage (*I-V*) relations of the rheogenic Na-sugar cotransport mechanism at the apical membrane of *Necturus* small intestine were determined from the relations between the electrical potential difference across the apical membrane, ψ^{mc}, and that across the entire epithelium, ψ^{ms}, when the latter was varied over the range ±200 mV, (i) under steady conditions in the presence of galactose and (ii) after the current across the apical membrane carried by the cotransporter, I^m_{SNa}, is blocked by the addition of phloridzin to the mucosal solution. I^m_{SNa} was found to be strongly dependent upon ψ^{mc} over the range -50 mV $< \psi^{mc} < E^m_{SNa}$ where E^m_{SNa} is the "zero current" or "reversal" potential. Over the range of values of ψ^{mc} encountered under physiological conditions the cotransporter may be modeled as a conductance in series with an electromotive force so that $I^m_{SNa} = g^m_{SNa}(E^m_{SNa} - \psi^{mc})$ where g^m_{SNa} is the contribution of this mechanism to the conductance of the apical membrane and is "near constant." In several instances I^m_{SNa} "saturated" at large hyperpolarizing or depolarizing values of ψ^{mc}.

The values of E^m_{SNa} determined in the presence of 1, 5, and 15 mM galactose strongly suggest that if the Na-galactose cotransporters are kinetically homogeneous, the stoichiometry of this coupled process is unity.

Finally, the shapes of the observed *I-V* relations are consistent with the predictions of a simple kinetic model which conforms with current notions regarding the mechanico-kinetic properties of this cotransport process.

Key Words small intestine · Na-sugar cotransport · current-voltage relations · stoichiometry · kinetic model

Introduction

It is well established that Na-coupled sugar cotransport across the apical membranes of small intestine and renal proximal tubule from a variety of species is *both* rheogenic *and* conductive. Thus, the addition of sugar to the solution bathing the apical surface depolarizes the electrical potential difference across the apical membrane, ψ^{mc}, and decreases the ratio of the slope resistance of the apical membrane with respect to that of the basolateral membrane, (r^m/r^s), in *Necturus* small intestine (Gunter-Smith, Grasset & Schultz, 1982) as well as rat (Frömter, 1982), frog (Lang et al., 1984) and rabbit (Lapointe, Laprade & Cardinal, 1984) proximal tubule. Further, the rates of Na-coupled sugar uptake by isolated chicken enterocytes (*cf.* Kimmich, Randles, Restrepo & Montrose, 1985; Restrepo & Kimmich, 1985) and brush border membrane vesicles isolated from rabbit small intestine (Kessler & Semenza, 1983) appear to be voltage dependent. Finally, several groups employing potential-sensitive fluorescent probes have demonstrated that Na-coupled sugar uptake by brush border membrane vesicles is associated with a depolarization of the intravesicular space (Burckhardt & Murer, 1980; Wright, 1984).

Indirect estimates of the effect of varying membrane potentials on the rates of Na-coupled sugar uptake by isolated enterocytes (Kimmich et al., 1985; Restrepo & Kimmich, 1985) and Na-coupled amino acid uptake by pancreatic acinar cells (Iwatsuki & Peterson, 1980) have been reported. However, the current-voltage (*I-V*) relations of these Na-coupled cotransport processes have not been *directly* established for any system. Such *I-V* relations can provide three important items of information. First, they provide insight into how the rate of the cotransport process is affected by changes in ψ^{mc} over the physiological range. Second, the "reversal" or "zero current" potential is a measure of the electromotive force of the cotransport process so that knowing the values of the intracellular and extracellular Na and sugar concentrations one can derive the stoichiometry of the coupled process. And, finally, the shape of the *I-V* relation is a feature that kinetic models of this cotransport process must conform to and thus may provide grounds for

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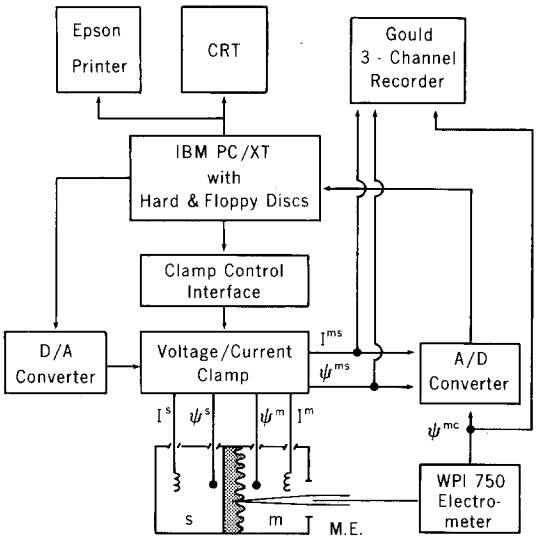

Fig. 1. Schematic of the apparatus employed in these studies: *s* and *m* designate the serosal and mucosal solutions, respectively; I^s and I^m represent the electrodes employed to pass the transepithelial current, I^{ms}; and, ψ^s and ψ^m are the electrodes employed to record the transepithelial electrical potential difference, ψ^{ms}. *M.E.* designates the microelectrode

distinguishing among different kinetic models of these processes.

In the present study we describe a general method for the determination of the *I-V* relations across the membranes of "leaky epithelia" and apply this method to the Na-galactose cotransport process at the apical membrane of *Necturus* small intestine. The results strongly suggest a Na : galactose coupling stoichiometry of unity and the shapes of the *I-V* relations are *consistent* with the predictions of a simple kinetic model of a negatively charged carrier (or gated pore) in which the translocation steps are rate limiting and the rate of translocation of the "free carrier" is significantly greater than that of the "ternary complex."

Glossary of Symbols

ψ^{ms}	The transepithelial electrical potential difference with reference to the mucosal solution (mV).
ψ^{mc}	The electrical potential difference across the apical membrane with reference to the mucosal solution (mV).
${}_0\psi^{mc}$	The electrical potential difference across the apical membrane when $\psi^{ms} = 0$ (i.e., under short-circuit conditions) (mV).
$\Delta\psi^{mc}$	The difference between ψ^{mc} in the absence and presence of phloridzin at any given value of ψ^{ms} (mV).
I_{sc}	The short-circuit current in $\mu A/cm^2$ defined as positive for the movement of a cation from the mucosal to the serosal solution.
r^m, r^s	The slope resistances of the apical and basolateral membranes, respectively, per cm^2 nominal serosal area (Ω cm^2).
g^m, g^s	The slope conductances of the apical and basolateral membranes, respectively, per cm^2 nominal serosal area (mS/cm^2).
g_i^m	The slope conductance of the apical membrane in the presence of phloridzin (mS/cm^2).
C^m, C^s	The capacitances of the apical and basolateral membranes, respectively, per cm^2 nominal serosal area ($\mu F/cm^2$).
f_r	$\equiv (\delta\psi^{mc}/\delta\psi^{ms}) \equiv [r_m/(r^m + r^s)]$; i.e., the "voltage divider ratio" which is assumed to be equal to the fractional resistance of the apical membrane. $\delta\psi^{mc}$ and $\delta\psi^{ms}$ are the displacements in ψ^{mc} and ψ^{ms} resulting from the intermittent current pulses.
f_c	$\equiv [C^s/C^m + C^s)]$; i.e., the fractional capacitance of the basolateral membrane.
I^m_{SNa}	The Na current across the apical membrane coupled to the entry of sugar ($\mu A/cm^2$).
E^m_{SNa}	The electromotive force of the Na-coupled sugar entry step (mV).
ν	The number of Na ions coupled to the entry of one sugar molecule across the apical membrane.
$(Na)_m$, $(Na)_c$	The Na activities in the mucosal solution and the intracellular compartment, respectively (mM).
$(S)_m$, $(S)_c$	The sugar activites in the mucosal solution and intracellular compartment, respectively (mM); $(S)_m$ is assumed to be equal to its concentration.

The subscript 0 designates values observed when $\psi^{ms} = 0$; i.e., when the tissue is short circuited.

Materials and Methods

Determination of *I-V* Relations

Segments of small intestine from *Necturus maculosa,* stripped of the underlying musculature and connective tissues were obtained and mounted, mucosal surface up, in a perfusion chamber having an exposed serosal surface area of 0.08 cm^2 as described previously (Gunter-Smith et al., 1982). The composition of the control solution perfusing both surfaces of the tissue was (mM): Na, 110; K, 2.5; Ca, 1.0; Mg, 1.0; Cl, 117; HEPES, 2.5; and mannitol, 15. The mucosal perfusates containing galactose had the same electrolyte composition as the control solution with 1, 5, or 15 mM galactose replacing the same amounts of mannitol. The pH of all solutions, gassed with air, was adjusted to 7.4 by the addition of Tris base. All experiments were carried out at room temperature (23°C).

After mounting in the chamber, the tissues were short circuited using an automatic voltage clamp that corrected for fluid resistance. At 20-sec intervals, bipolar current pulses of 1 sec duration were passed across the tissue sufficient to clamp the transepithelial electrical potential difference, ψ^{ms}, to ± 10 mV. The voltage clamp was interfaced with a microcomputer (IBM, PC-XT) so that the tissues could be open circuited, short circuited or subjected to a transepithelial current pulse train by commands from the keyboard. The system employed closely resembles that described by Thompson, Suzuki and Schultz (1982) and is illustrated schematically in Fig. 1. The transepithelial electrical potential difference with respect to the mucosal solution, ψ^{ms}, was measured using 0.5 M KCl-agar bridges and

calomel electrodes; the current-passing electrodes were Ag-AgCl pellets embedded in the chambers. The electrical potential difference across the apical membrane was measured employing glass microelectrodes filled with 0.5 M KCl and connected to a high-impedance electrometer (WPI, Model 750); these electrodes were fabricated using a horizontal puller (Brown & Flaming, Model P-77) and had resistances between 50–100 MΩ when dipped into a 0.5-M KCl solution.

The experimental procedure was as follows: After mounting the tissue in the chamber, both surfaces were perfused with the control, galactose-free, solution until the short-circuit current (I_{sc}) stabilized (approximately 10 min). Then the solution perfusing the mucosal surface of the tissue was switched to one containing either 1, 5, or 15 mM galactose and the stimulation of the I_{sc} was recorded (*see* Fig. 2). When I_{sc} reached a new steady state, a cell on a plicae[1] was impaled through the apical membrane with a microelectrode and ψ^{mc} and the deflections in ψ^{mc} in response to the periodic current pulses, $\delta\psi^{mc}$, were recorded; the fractional resistance of the apical membrane, $f_r = [r^m/(r^m + r^s)]$, was calculated from $(\delta\psi^{mc}/\delta\psi^{ms})$. As discussed by Boulpaep and Sackin (1980), because of possible distributed resistive effects in the lateral intercellular spaces, the voltage divider ratio, $(\delta\psi^{mc}/\delta\psi^{ms})$, in general, will not be equal to the fractional resistance of the apical membrane, $[r^m/(r^m + r^s)]$, particularly in "leaky" epithelia. However, there is no practical way around this potential problem and, as will be discssed below, the assumption that $[r^m/(r^m + r^s)] = (\delta\psi^{mc}/\delta\psi^{ms})$ does not appear to distort our results.

When ψ^{mc} and f_r reached stable values (2–10 min) a train of bipolar current pulses was passed across the tissue sufficient to clamp ψ^{ms} over the range from 0 (the short-circuited state) to ±200 mV in 10-mV increments; i.e., ψ^{ms} = 0, +10, 0, −10, 0, +20, 0, −20, . . . 0, +200, 0, −200 mV (*see* Fig. 3). Each pulse had a duration of 200 msec and the interval between two pulses was 300 msec. During each pulse, ψ^{mc}, ψ^{ms} and the transepithelial current, I^{ms}, were sampled ten times at equal intervals from 20 to 200 msec by the A-D converter and stored for later processing.

Then, 10^5 M phloridzin was added to the mucosal solution and when ψ^{mc} stabilized at a new, hyperpolarized, value a second current pulse train was passed across the tissue (*see* Fig. 3). In some instances several such pulse trains were passed across the tissue during the ensuing minutes in order to detect possible secondary changes in ψ^{mc} and f_r.

In a separate series of 12 experiments, the relations between ψ^{mc} and ψ^{ms} were first determined before and then again 1–2 min after the addition of 15 mM galactose to the mucosal perfusate.

Estimation of Intracellular Galactose Concentrations

Intracellular galactose concentrations were estimated by isotopic methods employing ^{14}C-galactose and ^{3}H-inulin; the latter served as a marker of the extracellular fluid volume. The methods employed are similar to those described by Schultz, Fuisz and Curran (1966).

Briefly, plicae were carefully dissected off the intestinal strip and incubated in solutions containing 5 or 15 mM galactose plus ^{14}C-galactose (20 and 6.7 μCi/mmol, respectively) and ^{3}H-inulin (0.5 μCi/ml) at 23°C for 2 hr. The tissues were then removed, blotted, weighed and disrupted by suspension in 1 ml distilled water for 1 hr. The tissue extracts were assayed in a liquid scintillation spectrometer for ^{14}C and ^{3}H simultaneously using a standard dual-window procedure. The extracellular fluid volume was calculated from the ^{3}H-inulin content of the extract and was used to calculate the extracellular galactose content of the extract. The intracellular galactose content can then be derived in units of μmol per gram of tissue wet wt.

In a separate series of experiments the cell water content was determined as a function of tissue wet wt from measurements of tissue wet wt, dry wt and extracellular fluid content. The cell water content averaged 85 ± 1% (n = 6) of the tissue wet wt.

The intracellular galactose concentration can thus be estimated in units of mM.

Results are expressed as the means ± SEM. Statistical analyses were performed employing either the Student t test or an analysis of variance with $P < 0.05$ chosen as the level of significance.

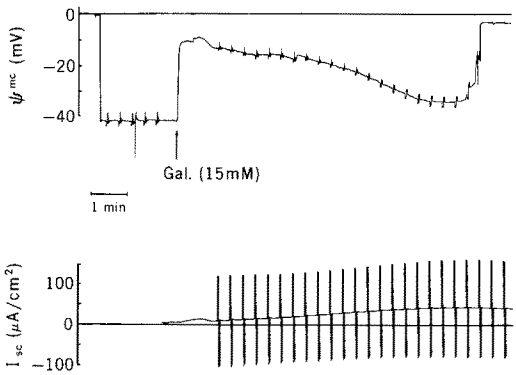

Fig. 2. The upper panel illustrates a typical response of ψ^{mc} to the addition of 15 mM galactose to the mucosal solution; the increase in the I_{sc} is illustrated in the lower panel. The intermittent bipolar displacements in the I_{sc} tracing represent the current pulses necessary to displace ψ^{ms} by ±10 mV. Two points should be noted: First, the increase in I_{sc} parallels the repolarization of ψ^{mc}. Second, following the addition of galactose to the mucosal solution there is a transient decrease in $(\delta\psi^{mc}/\delta\psi^{ms})$ followed by a gradual increase in that value

Results

Electrophysiological Responses to the Addition of Galactose and Phloridzin to the Mucosal Solution

The effects of the addition of galactose to the mucosal solution on ψ^{mc}, (r^m/r^s) and I_{sc} are illustrated in Fig. 2. Immediately after the introduction of the

[1] The *Necturus* small intestine is characterized by plical "outfoldings" and interplical "valleys." Gunter-Smith and White (1979) have demonstrated that cells on the upper portion of the plicae of *Amphiuma* small intestine are responsible for Na-coupled sugar and amino acid absorption as is the case for the villi of mammalian small intestine (Kinter & Wilson, 1965). Thus, the present studies were limited to cells in the uppermost regions of the plicae.

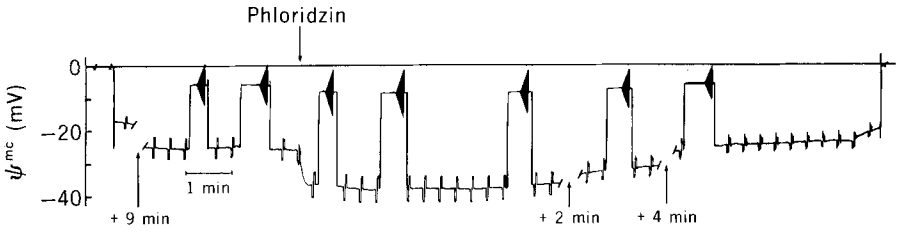

Fig. 3. Example of the effect of phloridzin on ψ^{mc} and f_r when this agent is added to the mucosal solution bathing tissues that were exposed to 15 mM galactose in the mucosal solution for at least 30 min

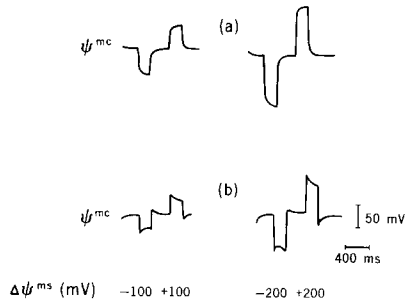

Fig. 4. Examples of "undershoots" and "overshoots" in ψ^{mc} in response to rapidly (less than 2 msec) clamping ψ^{ms} to ±100 mV and ±200 mV

sugar to the mucosal solution there is an abrupt depolarization of ψ^{mc} and a decrease in (r^m/r^s) (top panel). This initial response is followed by a gradual, partial repolarization of ψ^{mc} and an increase in (r^m/r^s) to a value greater than that observed under control conditions. These effects closely resemble those reported previously (Gunter-Smith et al., 1982; Grasset, Gunter-Smith & Schultz, 1983; Hudson & Schultz, 1984) and similar biphasic responses in ψ^{mc} following the addition of sugars or amino acids to the luminal solution have been observed in goldfish intestine (Albus, Bakker & van Heukelom, 1983), and rabbit (Lapointe et al., 1984) and frog (Messner, Oberleithner & Lang, 1985) renal proximal tubule.

As discussed previously (Gunter-Smith et al., 1982), the initial response can be attributed to the activation of rheogenic and conductive transport processes that mediate the coupled entry of Na and galactose into the cell across the apical membrane; the later response is due, at least in part, to an increase in conductance of the basolateral membrane to K (Grasset et al., 1983; Lau, Hudson & Schultz, 1984; Brown & Sepulveda, 1985).

As shown in the lower panel, galactose elicits an increase in the I_{sc}, but it is gradual and tends to parallel the repolarization of ψ^{mc}; as discussed previously (Gunter-Smith et al., 1982), this parallelism is to be expected if the Na-coupled entry process is affected by ψ^{mc}.

The effects of the addition of phloridzin to the mucosal solution after a steady state is achieved in the presence of galactose are illustrated in Fig. 3. This agent brings about a prompt hyperpolarization of ψ^{mc} and a significant increase in (r^m/r^s). These new values of ψ^{mc} and (r^m/r^s) are sustained for several min; but, as shown, approximately 5 min after introducing phloridzin there is a gradual depolarization of ψ^{mc} and a decrease in (r^m/r^s). These findings are consistent with the notion that the slow increase in the K conductance of the basolateral membrane, g^s_K, which follows the addition of galactose to the mucosal solution is slowly reversed when Na-coupled galactose entry across the apical membrane is blocked with phloridzin.

Finally, as demonstrated previously (Gunter-Smith et al., 1982), phloridzin completely abolished the increase in I_{sc} resulting from the addition of galactose to the mucosal perfusate (*data not shown*).

Capacitative Transients: Determination of the Fractional Capacitance

Examples of the time course of ψ^{mc}, $\psi^{mc}(t)$, following a rapid displacement of ψ^{ms} from the "holding" value, $\psi^{ms} = 0$, to a new value are illustrated in Fig. 4. In 63 experiments, prior to the addition of phloridzin to the mucosal solution, 43% were characterized by an "undershoot" (Fig. 4*a*), 43% by an "overshoot" (Fig. 4*b*) and 14% were considered "square." Thus, it was necessary to take steps designed to determine the optimal time to sample ψ^{mc} for the purposes of this study; i.e., that time after capacitative transients are essentially disspated but before secondary effects (e.g. polarization) take place. This was accomplished as follows: Assuming that, to a first approximation, the transcellular pathway can be represented by two linear resistance-capacitance (*RC*) circuits arranged in series, then when a current pulse is passed across the tissue sufficient to clamp ψ^{ms} from zero to a new value $(\delta\psi^{ms})$ (within 2 msec) ψ^{mc} will change with a time course given by (Schultz et al., 1984; DeLong & Civan, 1984; Garcia-Diaz & Essig, 1985)

$$f(t) = [\delta\psi^{mc}(t)/\delta\psi^{ms}] = f_r[1 - \exp(-t/\tau)] + f_c \exp(-t/\tau) \quad (1)$$

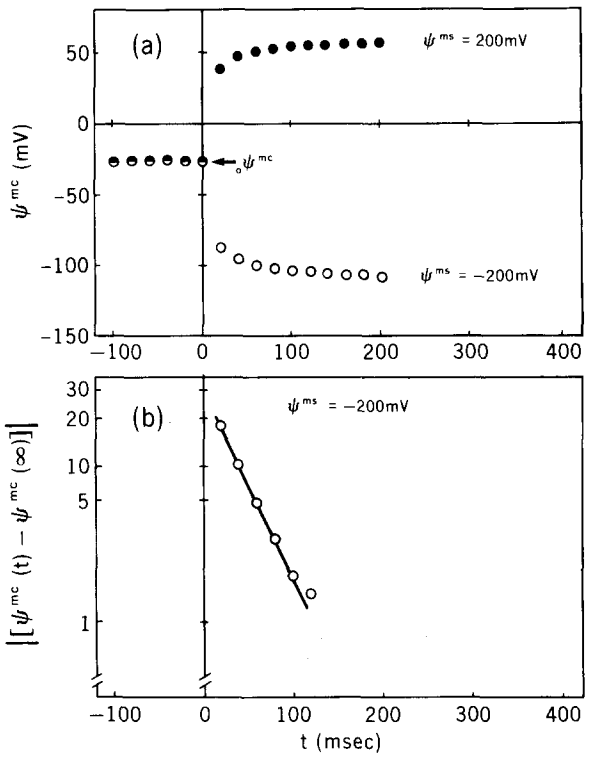

Fig. 5. (*a*) The time course of ψ^{mc} in response to rapidly clamping ψ^{ms} to +200 mV (●) and −200 (○) mV for the case of an initial "undershoot." (*b*) A plot of the time course of ψ^{mc} when ψ^{ms} is clamped at −200 mV employing Eq. (2)

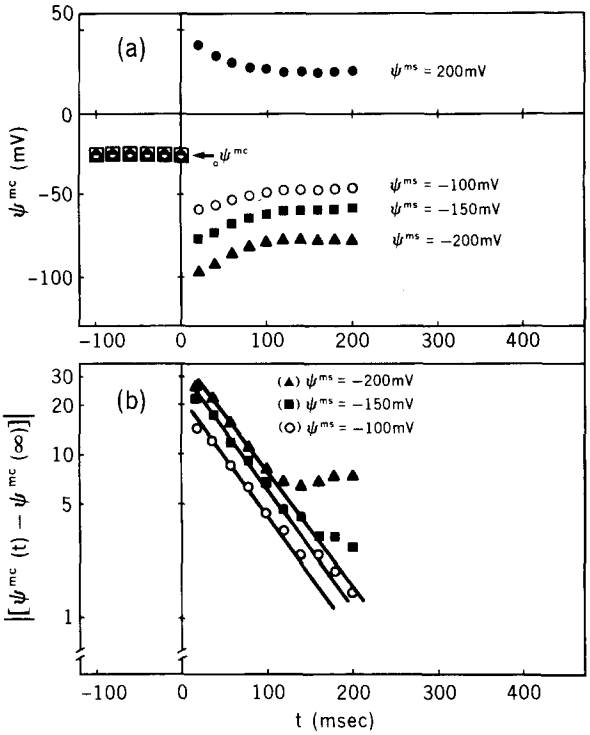

Fig. 6. (*a*) The time course of ψ^{mc} in response to clamping ψ^{ms} to the values shown for the case of an initial "overshoot." (*b*) Plots of $\psi^{mc}(t)$ when ψ^{ms} is clamped to −100, −150 and −200 mV employing Eq. (2); note that the lines are parallel and thus yield the same value of τ

where $f_r = [r^m/(r^m + r^s)]$, $f_c = [C^s/(C^s + C^m)]$ and $\tau = [(C^s + C^m)/(g^s + g^m)] = f_r r^s\ (C^s + C^m)$; τ is clearly the time required for dissipation of approximately 63% of the capacitative transient. (*See* Glossary for definition of symbols.)

Thus, at $t = 0$, $f(t)$ is equal to f_c (i.e., the fractional capacitance of the basolateral membrane) and when $t = \infty$, $f(t) = f_r$ (i.e., the fractional resistance of the apical membrane).

It follows from Eq. (1) that $\psi^{mc}(0)$ may either overshoot or undershoot the final steady-state value, $\psi^{mc}(\infty)$, depending on the relation between f_c and f_r. When $f_r > f_c$, $\delta\psi^{mc}$ will be characterized by an initial "undershoot," whereas an "overshoot" will be observed when $f_r < f_c$. As illustrated in Fig. 4 both types of time courses of $\psi^{mc}(t)$ were encountered in these studies, suggesting that *on the average* $f_r \approx f_c$.

Rearranging Eq. (1) it can be shown that

$$\ln |[\psi^{mc}(t) - \psi^{mc}(\infty)]| = -(t/\tau) + \ln |[\psi^{mc}(0) - \psi^{mc}(\infty)]|. \quad (2)$$

Thus, a plot of $\ln |[\psi^{mc}(t) - \psi^{mc}(\infty)]|$ *vs.* t should be linear with a slope of $(-1/\tau)$ and an intercept given by $\ln |[\psi^{mc}(0) - \psi^{mc}(\infty)]|$.

The digitized values of $\psi^{mc}(t)$ in response to clamping ψ^{ms} to ±200 mV for 200 msec are plotted as functions of time for the case of an initial "undershoot" of $\psi^{mc}(0)$ in Fig. 5*a*. A plot of $\ln |[\psi^{mc}(t) - \psi^{mc}(\infty)]|$ *vs.* t for these data, choosing the value of ψ^{mc} achieved after 120 msec (i.e., −106 mV) for $\psi^{mc}(\infty)$ is shown in Fig. 5*b*. Clearly, the points between 20 and 100 msec can be described by a straight line. The value of τ derived from the slope of this line is 33 msec. Further, from the intercept on the ordinate (−30 mV) we can calculate that $\psi^{mc}(0) = -76$ mV. Since $_0\psi^{mc}$ was −28 mV, $f(0) = f_c = (\delta\psi^{mc}(0)/\delta\psi^{ms}) = [(76 - 28)/200] = 0.24$. Further, $f(\infty) = f_r = [(106 - 28)/200] = 0.39$.

The digitized values of $\psi^{mc}(t)$ for the case of an initial "overshoot" are plotted in Fig. 6*a*. For all positive values of ψ^{ms} and negative values of ψ^{ms} up to −100 mV, excellent exponentials could be obtained using a value of $\psi^{mc}(\infty)$ corresponding to the values of ψ^{mc} observed between 100–160 msec. However, for values of ψ^{ms} between −100 and −200 mV a secondary hyperpolarization was frequently observed (*see* Fig. 4*b*). This is apparent in Fig. 6*b* where there is a considerable difference between the values of $\psi^{mc}(\infty)$ needed to obtain an good expo-

nential fit for the points between 20 and 100 msec and the values of $\psi^{mc}(t)$ observed between 100–200 msec.

The results of this analysis for those paired experiments in which the capacitative relaxation of $\psi^{mc}(t)$ was sufficiently large to permit reasonably accurate estimates of τ and f_c may be summarized as follows: The addition of 15 mM galactose to a previously galactose-free mucosal perfusate (n = 9)) resulted in a decrease in τ from 59 ± 7 msec to 46 ± 5 msec and an increase in f_c from 0.37 ± 0.02 to 0.44 ± 0.02 (both changes are significant at $P < 0.05$, employing the paired t-test). A decrease in τ is to be expected as a result of a decrease in f_r [*see* Eq. (1)]; the increase in f_c (corresponding to an increase in (C^s/C^m) from 0.6 to 0.8) is a finding that awaits further study.[2] The values of τ and f_c when a steady state had been achieved in the presence of 15 (n = 4), 5 (n = 7) and 1 (n = 7) mM galactose and shortly following the addition of phloridzin to the mucosal perfusates did not differ significantly and averaged 33 ± 3 msec and 0.29 ± 0.02, respectively. The fact that we were unable to detect a significant decrease in τ before and after blocking the conductive Na-coupled galactose entry step, particularly in the presence of 15 mM galactose, is an unexpected finding that also awaits further study. Finally, as illustrated in Fig. 6*b*, τ was independent of the clamping potential ($\delta\psi^{ms}$). This observation supports the assumption that, to a first approximation, the system can be treated as two linear *RC* circuits arranged in series.[3]

Because of the presence of secondary transient phenomena in some of the experiments, care was exercised to use only those experiments in which $\psi^{mc}(\infty)$ could be accurately evaluated for the determinations of the relations between ψ^{mc} and ψ^{ms} reported below. The time required for $\psi^{mc}(t)$ to achieve a steady state was evaluated in each experiment and most often corresponded to the value of $\psi^{mc}(t)$ observed between 100 and 160 msec.

The Relations Between ψ^{mc} and ψ^{ms} in the Presence of Galactose Before and After the Addition of Phloridzin to the Mucosal Solution

Typical examples of the relations between ψ^{mc} and ψ^{ms} when the tissues had reached a steady state in the presence of 15, 5, or 1 mM galactose in the mucosal solution and, then, within the first min after the addition of phloridzin to the mucosal solution are illustrated in Figs. 7(*a–c*).

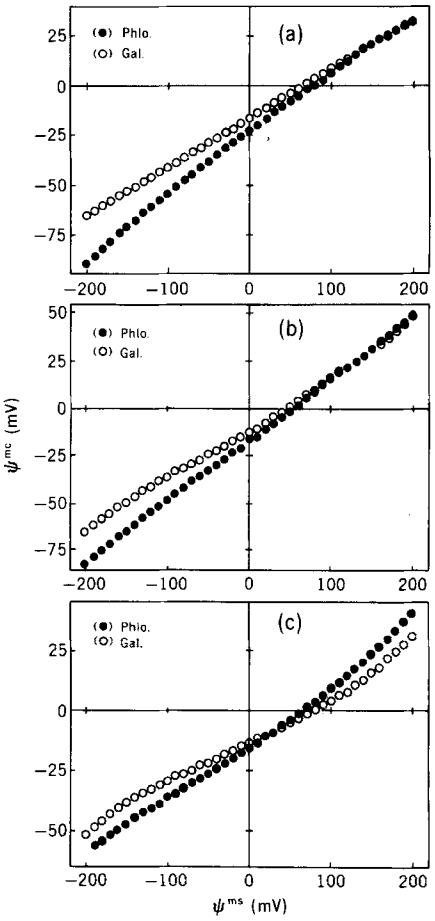

Fig. 7. The relations between ψ^{mc} and ψ^{ms} before and after the addition of phloridzin to the mucosal solution in the presence of (*a*) 15 mM, (*b*) 5 mM and (*c*) 1 mM galactose

Under short-circuit conditions in the presence of 15 mM galactose, ${}_0\psi^{mc}$ averaged −22 ± 1 mV and f_r averaged 0.37 ± 0.03 (n = 13). Following the addition of 10^5 M phloridzin to the mucosal solution, ${}_0\psi^{mc}$ hyperpolarized by 10 ± 2 mV and f_r increased to 0.46 ± 0.04. These values are comparable to those reported previously by Gunter-Smith et al. (1982).

In general, the *shapes* of the relations between ψ^{mc} and ψ^{ms} in the presence of 15, 5, and 1 mM galactose solution did not differ markedly. The principal differences were in the values of ψ^{mc} at which the relations between ψ^{mc} and ψ^{ms} in the absence and presence of phloridzin intersected; these values were 28 ± 4 mV (n = 13), 11 ± 5 mV (n = 11) and 6 ± 4 mV (n = 9) in the presence of 15, 5, and 1 mM galactose, respectively. An analysis of variance indicated that the average values observed in the presence of 5 and 1 mM galctose differ significantly

[2] In this respect it is of interest that Warnke and Lindemann (1980) have reported that stimulation of transcellular Na absorption across toad urinary bladder by ADH is accompanied by decreases in the resistances of *both* the apical and the basolateral membranes as well as increases in the capacitances of *both* of these barriers.

[3] Note that r^m must be essentially independent of ψ^{mc} only over the range of the capacitative transient.

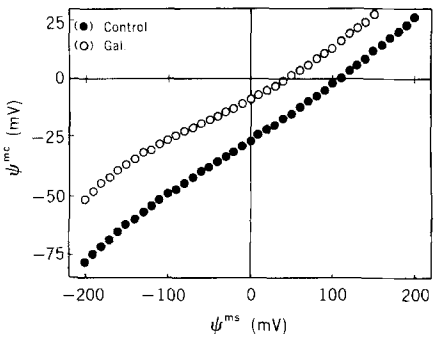

Fig. 8. The relation between ψ^{mc} and ψ^{ms} before and 30 sec after the addition of 15 mM galactose to the mucosal bathing solution

($P < 0.05$) from that observed in the presence of 15 mM galactose but do not differ significantly from each other.

The significance of this value will be discussed below.

Finally, a series of studies was carried out in which the relations between ψ^{mc} and ψ^{ms} were determined in the absence of galactose and then within 1 min after the addition of this sugar to the mucosal solution. A typical result is illustrated in Fig. 8. It should be noted that 15 mM galactose brought about a significant depolarization of ${}_0\psi^{mc}$ from −26 to −10 mV and a decrease in ${}_0f_r$. However, with increasing positive values of ψ^{mc} and ψ^{ms} the two curves tend to parallel each other and do not intersect over the range of values studied.

Derivations of the *I-V* Relations of the Na-Galactose Transporter: Theory and Results

Theory

The approach employed in previous studies reported by this laboratory dealing with the *I-V* relations of the amiloride-inhibitable Na entry step across the apical membranes of rabbit descending colon (Thompson et al., 1982) and *Necturus* urinary bladder (Thomas, Suzuki, Thompson & Schultz, 1983) was based on determining the changes in *transcellular current* (I^c) resulting from the addition of amiloride to the mucosal solution at different, clamped values of the transepithelial electrical potential difference, ψ^{ms}. This approach is applicable to "tight" or "moderately tight" epithelia where a significant fraction of the transepithelial current, when $\psi^{ms} \neq 0$, is transcellular. This approach, however, cannot be applied to a "leaky" epithelium, such as *Necturus* small intestine, where the transepithelial conductance is dominated by those of the paracellular pathways (Gunter-Smith et al., 1982) so that when ψ^{ms} is displayed from 0 changes in I^c are negligible compared to changes in paracellular current. Thus, the determination of the *I-V* relations of conductive pathways across the apical or basolateral membranes of "leaky epithelia" must employ approaches that circumvent direct measurements of I^c.

The rationale for the approaches employed in these studies is as follows: As shown in Fig. 3, the addition of phloridzin to a mucosal solution containing a Na-cotransported sugar results in a hyperpolarization of ψ^{mc} because the inward Na-current coupled to the carrier-mediated entry of the sugar, I^m_{SNa}, is suddenly blocked (Gunter-Smith et al., 1982). Clearly, the magnitude of this hyperpolarization will be a function of I^m_{SNa} and the membrane conductances. Further, Hopfer, Nelson, Perrotto & Isselbacher (1973) have shown that phloridzin blocks Na-coupled sugar efflux from preloaded brush border membrane vesicles isolated from rat small intestine. Thus, this agent blocks I^m_{SNa} in both directions across the apical membrane. Employing the equivalent electrical circuit model for this epithelium proposed by Gunter-Smith et al. (1982) and illustrated in Fig. 9, it can be shown that if phloridzin specifically blocks I^m_{SNa} and has no effect on g^m_i or g^s then, at any given value of ψ^{ms},

$$I^m_{SNa} = (g^m_i + g^s)(\psi^{mc} - \psi^{mc'}) = (g^m_i + g^s)\,\Delta\psi^{mc} \qquad (3)$$

where: ψ^{mc} is the electrical potential difference across the apical membrane in the absence of phloridzin; $\Delta\psi^{mc'}$ is the electrical potential difference across the apical membrane in the presence of phloridzin; $\psi^{mc} = (\psi^{mc} - \psi^{mc'})$; g^m_i is the slope conductance of the apical membrane to all current flow in the presence of phloridzin; and, g^s is the slope conductance of the basolateral membrane. Thus, if g^m_i and g^s remain constant shortly after the addition of phloridzin to the mucosal solution, and are voltage independent, I^m_{SNa} will be directly proportional to $\Delta\psi^{mc}$ so that a plot of $\Delta\psi^{mc}$ *vs.* ψ^{mc} provides the *shape* of the *I-V* relation of the cotransport mechanism.

It also follows from Eq. (3) that when $\Delta\psi^{mc} = 0$, $I^m_{SNa} = 0$ regardless of the behavior of g^m_i and g^s. Thus, the value of ψ^{mc} at which $\Delta\psi^{mc} = 0$ (i.e., that value of ψ^{mc} at which the ψ^{mc} *vs.* ψ^{ms} relations illustrated in Figs. 7 intersect) is the "reversal" or "zero-current" potential; or, alternatively, the "electromotive force" of the coupled transport process across the apical membrane, E^m_{SNa}.

Another approach toward obtaining the *I-V* relation of the cotransporter is to compare the *slopes* of the relations between ψ^{mc} and ψ^{ms} before and after the addition of phloridzin to the mucosal solution. These slopes, f_r and f'_r, are measures of the fractional resistances of the apical membrane [i.e., $r^m/(r^m + r^s)$] at a given value of ψ^{mc} before and after

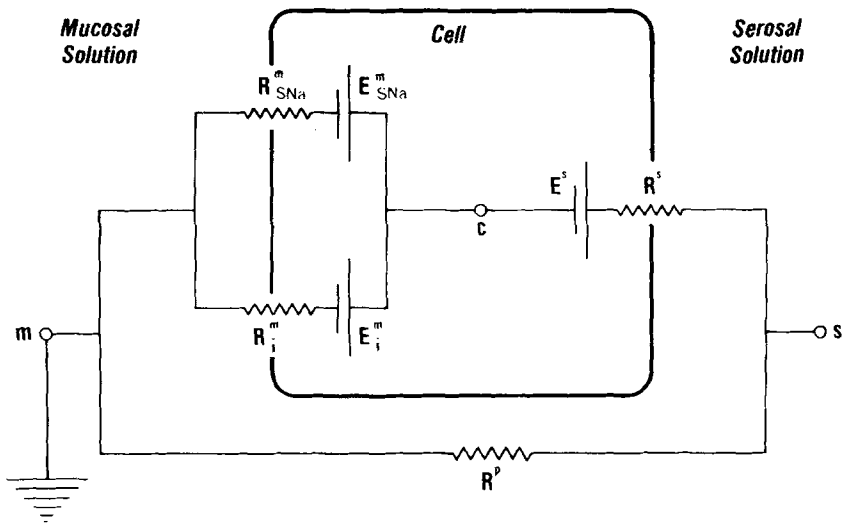

Fig. 9. Equivalent electrical circuit model proposed by Gunter-Smith et al. (1982) for the coupled entry of Na and a neutral solute across the apical membrane. Note that in this figure we refer to the chord resistances R^m_{SNa}, R^m_i and R^s, whereas in the present study we deal with the slope resistances or conductances; Thompson (1986) has recently considered the relations between chord and slope conductances and their equivalent electromotive forces

blocking the contribution of the cotransporter to the conductance of the apical membrane, g^m_{SNa}. It can be readily shown that if g^s is not affected by phloridzin within the time frame of these studies

$$g^m_{SNa} = (f_r' - f_r)g^s/(f_r' f_r). \quad (4)$$

Knowing f_r and f_r' at any value of ψ^{mc} and assuming that g^s is voltage independent we can relate g^m_{SNa}, the slope of the relation between I^m_{SNa} and ψ^{mc}, to g^s. Then, employing an arbitrary value for g^s, we can construct the relation between I^m_{SNa} and ψ^{mc} starting at the "reversal potential" (i.e., that value of ψ^{mc} at which $\Delta\psi^{mc}$ and, thus, $I^m_{SNa} = 0$). Then, the current (I^m_{SNa}) axis is scaled so that I^m_{SNa} at that value of ${}_0\psi^{mc}$ (when $\psi^{ms} = 0$) is equal to the steady-state increase in the I_{sc} following the addition of the sugar to the mucosal solution.

Finally, rearranging Eq. (3) we obtain

$$g^s = I^m_{SNa} f_r'/\Delta\psi^{mc} \quad (5)$$

and, substituting this expression into Eq. (4) we obtain

$$g^m_{SNa} = (dI^m_{SNa}/d\psi^{mc}) = I^m_{SNa}(f_r' - f_r)/(\Delta\psi^{mc} \cdot f_r). \quad (6)$$

This relation is independent of any voltage dependence of g^m_i and g^s and only assumes that these conductances are not affected by phloridzin shortly after it is added to the mucosal solution; this assumption is supported by the data illustrated in Fig. 3 where the relation between ψ^{mc} and ψ^{ms} is shown to be constant for 1–3 min after the addition of phloridzin.

Employing Eq. (6), the relation between I^m_{SNa} and ψ^{mc} can be constructed from the values of $\Delta\psi^{mc}$, f_r and f_r' at each value of ψ^{mc}. This equation, in general, has two unknowns, i.e., g^m_{SNa} and I^m_{SNa}. But, it is uniquely defined at that value of ψ^{mc} corresponding to ${}_0\psi^{mc}$ (i.e., ψ^{mc} when $\psi^{ms} = 0$) where the value of I^m_{SNa} is given by the increase in I_{sc} resulting from the addition of galactose to the mucosal solution. Thus, g^m_{SNa} can be calculated at that point and an initial segment of the *I-V* relation can be drawn between ${}_0\psi^{mc}$ and $({}_0\psi^{mc} \pm \Delta/2)$ where Δ is the difference between ${}_0\psi^{mc}$ and the consecutive value of ψ^{mc}. The value of I^m_{SNa} corresponding to this consecutive value of ψ^{mc} is extrapolated and reintroduced into Eq. (6), together with the values of f_r, f_r' and $\Delta\psi^{mc}$ at that value of ψ^{mc}. Using this newly estimated value of g^m_{SNa}, I^m_{SNa} is reevaluated and reintroduced into Eq. (6). After a few such iterations, the value of I^m_{SNa} needed to satisfy Eq. (6) is precisely found and the same procedure is applied to the next (consecutive) value of ψ^{mc}.

It should be noted that Eq. (6) has a singularity when $\Delta\psi^{mc} = 0$ which corresponds to that value of ψ^{mc} where $I^m_{SNa} = 0$ (i.e., the reversal potential). However, in this range the calculated value of I^m_{SNa} is very sensitive to small changes in $\Delta\psi^{mc}$ and inasmuch as this measurement is subject to error (±0.5 mV) large variations in the slope may be obtained. To prevent the repercussion of such erroneous estimates of g^m_{SNa} on all subsequent calculations of I^m_{SNa}, I^m_{SNa} was set to zero when the absolute value of $\Delta\psi^{mc}$ was less than ±0.5 mV. Beyond this point, when $\Delta\psi^{mc}$ becomes more negative than −0.5 mV, a new value of I^m_{SNa} is calculated using the proportion between I^m_{SNa} and $\Delta\psi^{mc}$ observed at ${}_0\psi^{mc}$ and the iterative procedure is continued as described above.

Results

Analyses of the relations between ψ^{mc} and ψ^{ms} in the presence of 15 mM galactose before and after the addition of phloridzin to the mucosal solution (Fig.

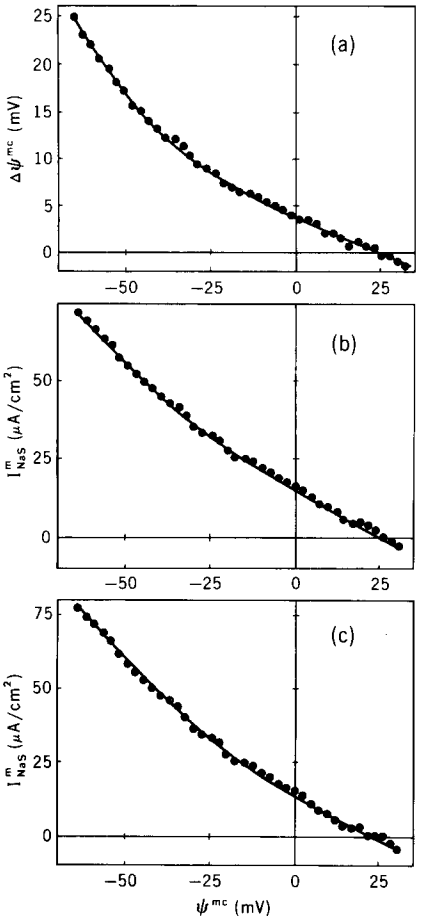

Fig. 10. Relations between (*a*) $\Delta\psi^{mc}$ and ψ^{mc} [*see* Eq. (3)] and [(*b*) and (*c*)] between I^m_{SNa} and ψ^{mc} determined employing Eqs. (4) and (6)

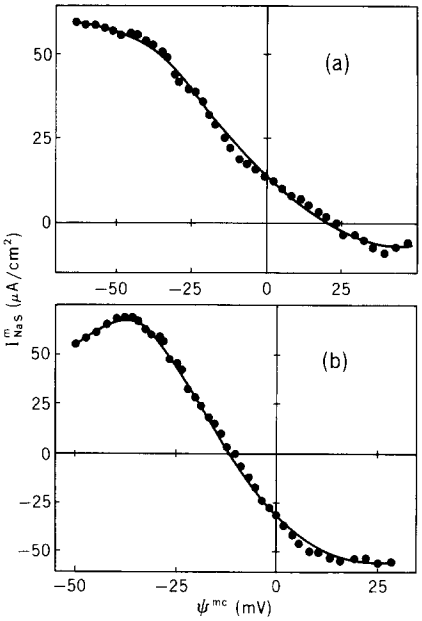

Fig. 11. Examples of "saturation" of the relations between I^m_{SNa} and ψ^{mc}

7*a*) employing Eqs. (3), (4) and (6) are illustrated in Fig. 10(*a–c*). In this experiment, ${}_0\psi^{mc}$ was −17 mV; the stimulation of the I_{sc} by galactose was 25 μA/cm^2; and, E^m_{SNa} was 24 mV. It should be noted that the curves have similar shapes supporting the assumptions employed in the first two approaches that g^m_i and g^s are not markedly voltage dependent. It should be further noted that the relation illustrated in Fig. 10*a* [derived employing Eq. (3)] does not rely upon the assumption that $f_r = (\delta\psi^{mc}/\delta\psi^{ms}) = [r^m/(r^m + r^s)]$, whereas the relations illustrated in Fig. 10*b* and *c* do rely upon this assumption. The fact that these relations have virtually superimposable shapes suggests that a "distributed resistance" in the lateral intercellular spaces (Boulpaep & Sackin, 1980) does not markedly distort the relation between $(\delta\psi^{mc}/\delta\psi^{ms})$ and $[r^m/(r^m + r^s)]$.

Under short-circuit conditions in the presence of 15 mM galactose, g^m_{SNa}, calculated from Eq. (6), averaged 0.9 ± 0.1 mS/cm^2 (n = 13). In the same experiments, the average value of ${}_0(r^m/r^s)$ increased from 0.66 ± 0.09 to 1.00 ± 0.18 following the addition of phloridzin to the mucosal solution. If this increase is attributable entirely to the blocking of the conductance of the cotransporter, ${}_0(g^m_{SNa})$, and if the value of ${}_0(r^m/r^s)$ is not underestimated due to a distributed resistance in the paracellular pathway (Boulpaep & Sackin, 1980), then the average values of ${}_0g^m_i$ and ${}_0g^s$ can be calculated to be 2.2 mS/cm^2 and 1.9 mS/cm^2, respectively.

The majority of the *I-V* relations obtained in the presence of 15, 5, and 1 mM galactose had shapes that resemble those illustrated in Fig. 10(*a–c*). In five experiments, however, the relation between I^m_{SNa} and ψ^{mc} either saturated (i.e., I^m_{SNa} was independent of ψ^{mc}) or exhibited a "negative conductance" (i.e., I^m_{SNa} decreased with increasing hyperpolarization or depolarization of ψ^{mc}). Examples of these phenomena are illustrated in Fig. 11(*a,b*).

Measurements of Intracellular Galactose Concentrations

The average values of the intracellular contents of galactose in plicae cells in the presence of 15 and 5 mM galactose in the suspension medium were 41 ± 4 (n = 6) and 33 ± 3 (n = 6) mmol/g tissue wet wt, respectively. Expressing these value in terms of estimated cell water content, they are 48 ± 5 mM and 41 ± 4 mM, respectively.

Discussion

To date, there have been few reportes of directly determined *I-V* relations of ion-coupled nonelectrolyte transport mechanisms across biological mem-

branes and these have been limited to the results of studies of proton-coupled processes in plant cells, algae and bacteria (*cf.* Hansen, Gradmann, Sanders & Slayman, 1981). Heretofore there have been no reported direct studies of the *I-V* relations of Na-coupled nonelectrolyte transport mechanisms possessed by many animal cell membranes. For the case of Na-coupled sugar transport the principle obstacle confronting the determination of these *I-V* relations stems from the fact that these processes appear to be present *only* in "leaky" epithelia such as small intestine and renal proximal tubule. Thus, as discussed above, when ψ^{ms} is displaced from 0, more than 90% of the change in the transepithelial current bypasses the transcellular route so that any attempt to detect a change in the transcellular current in response to the activation or inhibition of Na-coupled sugar or amino acid entry processes is doomed by the highly unfavorable signal-to-noise ratio.

The fact that measurements of the transepithelial currents necessary to voltage clamp a "leaky" epithelium over the range $\psi^{ms} = 0$ to $\psi^{ms} = \pm 200$ mV provide almost no "cellular information" compelled us to devise a method for deriving the *I-V* relations of the process responsible for the rheogenic and conductive coupled transport of Na and galactose across the apical membrane of *Necturus* small intestine that circumvents the need to measure I^c directly. This approach has the advantage that all of the information needed to construct the *shape* of the *I-V* relations can be derived from the relations between ψ^{mc} and ψ^{ms} *obtained from the impalement of a single cell* before and after the addition of phloridzin to the mucosal solution. No assumptions need be made regarding the properties of the paracellular pathways or electrical coupling between the impaled cell and adjacent cells. It should be noted that this approach is applicable to any conductive pathway across the apical or basolateral membranes of "tight" as well as "leaky" epithelia providing one can rapidly activate or block this pathway and the assumptions discussed above are valid.

Capactitative Transients and "Effective Membrane Areas"[4]

As indicated above, the values of ψ^{mc} that must be employed in these relations are those values observed after capacitative transients are dissipated but before possible secondary effects take place. By averaged 33 msec so that by 100 msec ($3 \times \tau$) capacitative transients are essentially (95%) dissipated. Employing a similar approach, Garcia-Diaz and Essig (1985) have found that τ is approximately 50 msec for *Necturus* gallbladder cells.

Inasmuch as, in the presence of phloridzin, $\tau = (C^m + C^s)/(g_i^m + g^s)$, using the values of ${}_0g_i^m$ (2.2 mS/cm^2) and ${}_0g^s$ (1.9 mS/cm^2), calculated above, and assuming the nominal value of 1 μF/cm^2 for membrane capacitance, we calculate that the "effective" membrane area per cm^2 of nominal serosal surface area (i.e., $C^m + C^s$) is 135 cm^2; this is consistent with a considerable amplification of the apical membrane area due to microvilli and of the basolateral membrane due to infoldings and projections. Further, the average fractional capacitance of the basolateral membrane, $f_c = [C^s/(C^m + C^s)]$, was 0.29. Thus, if the two membranes have the same specific capacitance, the effective area of the apical membrane is 2–3 times that of the basolateral membrane and corresponds to approximately 95 cm^2 per cm^2 of serosal surface area. Electron micrographs of *Necturus* small intestine (kindly provided by Dr. Karl Karnaky) indicate that the plical cells have a width of approximately 10 μm and a height of approximately 75 μm. The apical membrane possesses a uniform population of well-developed microvilli that have a diameter of approximately 0.1 μm, a height of approximately 4 μm, and are separated by a distance of approximately 0.1 μm; one can readily calculate that this array results in approximately a 30-fold amplification of the apical membrane surface. The additional threefold amplification suggested by our data could be the result of the plical folds, which further increase the total apical membrane surface per cm^2 of serosal surface area.

The Electromotive Force and Stoichiometry of the Na-Galactose Cotransporter

The *stoichiometry* or *coupling coefficient* (ν) of the Na-sugar cotransporters in the brush border membranes of small intestine and renal proximal tubule is a kinetically and energetically important parameter but is, as yet, an unsettled matter (Schultz, 1986). Goldner, Schultz and Curran (1969) reported a stoichiometry of 1 : 1 for Na-coupled sugar influx across the apical membrane of intact rabbit ileum. Hopfer and Groseclose (1980) reported that $\nu = 1$ for Na-glucose cotransport by brush border membrane vesicles isolated from rabbit small intestine (and kidney), whereas the results of Kaunitz, Gunther and Wright (1982), employing brush border

[4] The term "effective membrane area" denotes the total membrane area that serves as a pathway for transcellular current flow and thus contributes to the total capacitance, $(C^m + C^s)$. In view of the complex geometries of these cells (e.g. closely packed apical membrane microvilli and the complex projections and infoldings of the basolateral membranes), the "effective" area need not correspond with the anatomical area.

sampling ψ^{mc} at 20-msec intervals during the 200-msec pulse and analyzing these data employing Eqs. (1) and (2), we determined that the value of τ membrane vesicles isolated from rabbit small intestine, apparently using the same methods employed by Hopfer and Groseclose (1980), appear to be consistent with a coupling coefficient equal to or greater than 2. Kimmich and Randles (1980) have reported a value of $\nu = 2$ for Na-coupled sugar uptake by isolated chicken enterocytes (*see also* Kimmich et al., 1985). Turner and Moran (1982*a*,*b*) have reported the presence of two Na-sugar cotransport mechanisms in rabbit nephron; brush border vesicles isolated from the outer cortex are characterized by a $\nu = 1$, whereas vesicles isolated from the outer medulla appear to display a $\nu = 2$ (*see also* Turner, 1985). Kaunitz and Wright (1984) found that brush border vesicles from bovine small intestine possess a major, low affinity, high capacity Na-sugar cotransport mechanism with a $\nu = 1$ and a minor, high affinity, low capacity system with a $\nu = 3$. Finally, the electrophysiological data reported by Frömter (1982) are consistent with a coupling coefficient of unity for Na-sugar influx across the apical membrane of rat proximal tubule.

As discussed above, one value that can be obtained *directly* from the relations between ψ^{mc} and ψ^{ms} before and after the addition of phloridzin to the mucosal solution is the steady-state value of E^m_{SNa} in the presence of a given concentration of galactose in the mucosal solution. This value is equal to the value of ψ^{mc} at which the two relations intersect so that $\Delta\psi^{mc}$ and $I^m_{SNa} = 0$. The only underlying assumption is that phloridzin, at a concentration of 10^{-5} M, does not affect any conductive pathways other than the Na-coupled sugar entry step within 1 min after addition to the mucosal solution; this assumption is strongly supported by the fact that phloridzin has no effect on any of the electrophysiological properties of small intestine in the absence of sugars that interact with the Na-coupled entry mechanism.

Assuming that the Na-sugar cotransport process is driven entirely by the electrochemical potential differences of the cotransported solutes across the apical membrane, it follows that (Schultz, 1986)

$$(\psi^{mc})_{I^m_{SNa}=0} = E^m_{SNa} = (RT/\nu F)\ \ln\ [(Na)^\nu_m(S)_m]/[(Na)^\nu_c(S)_c] \qquad (7)$$

where (Na) and (S) designate the activities of Na and the cotransported solute, respectively; the subscripts m and c designate the mucosal solution and the intracellular compartment, respectively; and, ν is the coupling, or stoichiometric, coefficient given by the number of Na ions coupled to the entry of one cotransported solute molecule. It should be emphasized that Eq. (7) is based on classical thermodynamics and thus is independent of kinetic considerations or underlying mechanisms (pathways).

Recent results from this laboratory indicate that the steady-state value of $(Na)_c$ in *Necturus* small intestine in the presence of 15 mM galactose, determined employing Na-selective microelectrodes, averaged 14 mM and did not differ significantly from the value of $(Na)_c$ in the absence of sugar (Hudson & Schultz, 1984). Using the value $(Na)_m = 80$ mM and assuming that $(Na)_c = 14$ mM and is independent of $(S)_m$, as suggested by the findings of Hudson and Schultz (1984), the steady-state values of $(S)_c$ predicted by Eq. (7) for different values of ν can be calculated from the values of E^m_{SNa} determined when $(S)_m = 15$, 5 and 1 mM.

The values predicted by Eq. (7) for $(S)_c$ for the *individual experiments* assuming $\nu = 1$ average 32 ± 5 mM ($n = 13$), 22 ± 4 mM ($n = 11$) and 5 ± 1 mM ($n = 9$) when $(S)_m = 15$, 5 and 1 mM, respectively. The corresponding values predicted by Eq. (7) assuming $\nu = 2$ average 182 ± 29 mM, 123 ± 22 mM and 29 ± 5 mM, respectively.

The values of $(S)_c$ estimated employing isotopic techniques in the presence of 15 and 5 mM galactose were 48 ± 5 mM and 41 ± 4 mM, respectively. Further, inasmuch as these values were obtained under conditions when both surfaces of the tissue were exposed to galactose they must be greater than the steady-state values of $(S)_c$ attained when galactose is absent from the serosal solution. Indeed, if the rate of galactose exit across the basolateral membrane is a linear function of $[(S)_c - (S)_s]$ where $(S)_s$ is the concentration of S in the serosal solution (i.e., the exit mechanism is not saturated at these concentrations), then the values of $(S)_c$ estimated when *both* surfaces of the cells were exposed to 15 or 5 mM galactose (i.e., 48 and 41 mM, respectively) would be 15 and 5 mM greater than the values of $(S)_c$ when $(S)_s = 0$. These "corrected" values are 35 and 36 mM, respectively.

Clearly, the average values of $(S)_c$ predicted by Eq. (7) assuming that $\nu = 1$ are in reasonable agreement with those estimated employing isotopic techniques whereas those predicted assuming that $\nu = 2$ are unreasonably high.

The relations between the average predicted values of $(S)_c$ derived from Eq. (7) assuming that $\nu = 1$ and the average of the increments in the I_{sc}, (ΔI_{sc}), in the presence of 15, 5 and 1 mM galactose are illustrated in Fig. 12. This close parallelism is to be expected if our estimates of E^m_{SNa} are accurate and if the rate of exit of galactose from the cell across the basolateral membrane is a linear function of $(S)_c$.

Thus, if the cotransporters that mediate the coupled influx of Na and sugar across the apical

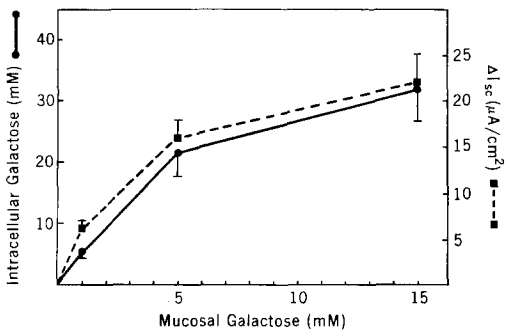

Fig. 12. The relation between the average values of $(S)_c$ derived from Eq. (7) employing the experimentally observed values of E^m_{SNa} (assuming that $\nu = 1$) and the observed steady-state increase in I_{sc} (ΔI_{sc})

membrane of *Necturus* small intestine are kinetically homogeneous, the present data strongly support the conclusion that the coupling coefficient is unity; a value of $\nu = 2$ is highly unlikely.

Finally, as illustrated in Fig. 8, the relations between ψ^{mc} and ψ^{ms} before and shortly (within 1 min) after the addition of galactose to the mucosal solution do not intersect within the range of values studied and indeed closely parallel each other when $\psi^{mc} > 0$; this is precisely what is to be expected inasmuch as when $(S)_c \to 0$, $E^m_{SNa} \to \infty$.

The Relations Between I^m_{SNa} and ψ^{mc}

Three approaches were employed to analyze the relations between ψ^{mc} and ψ^{ms} in the presence of galactose before and after the addition of phloridzin to the mucosal perfusate. The first, employing Eq. (3) assumes that g^m_i and g^s are not affected directly or indirectly by phloridzin within 1 min and that both are voltage independent over the range of values of ψ^{mc} (and ψ^{cs}) encountered in these studies. The second, employing Eq. (4), eliminates the requirement for the voltage independence of g^m_i. And, the third, employing Eq. (6), eliminates the need to make any assumption regarding the effect of voltage on g^m_i and g^s. It is well established that phloridzin, a highly specific inhibitor of Na-coupled sugar transport across the apical membrane of small intestine and renal proximal tubule, does not *directly* affect any other transcellular or paracellular conductive pathways. The findings that (i) the relations between ψ^{mc} and ψ^{ms} after the addition of phloridzin to the mucosal solution remained constant for several min (Fig. 3) and (ii) the *I-V* relations illustrated in Fig. 10 have similar shapes further support the assumptions underlying the derivation of these relations.

Clearly, the rate of Na-coupled galactose entry across the apical membrane is strongly dependent upon ψ^{mc} over a wide range of values including the physiologically important range between ${}_0\psi^{mc}$ and E^m_{SNa}. Indeed, over the range ${}_0\psi^{mc} < \psi^{mc} < E^m_{SNa}$ the relation between I^m_{SNa} and ψ^{mc} is close to linear and can thus be described by the relation $I^m_{SNa} = g^m_{SNa}(E^m_{SNa} - \psi^{mc})$ with a "near constant" g^m_{SNa}. In other words, over the "physiological range" this rheogenic cotransport mechanism can be modelled, using equivalent electrical circuit analogues, as an electromotive force in series with a near constant conductance. This conclusion is consistent with that arrived at by Gunter-Smith et al. (1982) (*see* Fig. 9) on the basis of theoretical considerations. Any model that portrays this cotransporter as a constant-current source is clearly inconsistent with these experimental findings.

Comparison of Observed *I-V* Relations with the Predictions of a Simple Kinetic Model

The results of recent studies on the properties of the Na-sugar cotransporter in brush border membrane vesicles isolated from rabbit small intestine provide a reasonable foundation for initial attempts at constructing kinetic models of this process. This matter has been the subject of several recent reviews (Kessler & Semenza, 1983; Semenza et al., 1985; Wright & Peerce, 1985; Schultz, 1986).

Briefly, it appears that the cotransporter is an integral membrane protein with a molecular weight of approximately 75 kD that spans the apical membrane and is structurally asymmetric with respect to the plane of that membrane; that portion of the "free carrier" or "gated channel" (Läuger, 1980; Kessler & Semenza, 1983) involved in the translocation step appears to bear a net negative charge(s). Na binds to the carrier first and brings about a conformational change that permits the subsequent binding of the sugar. The Na-carrier complex is "immobile"; that is, it does not mediate the entry of Na *alone* into the cell. The binding of sugar to the Na-carrier complex "opens the gate" and permits translocation of both ligands across the membrane. The order of "debinding" at the cytoplasmic side of the apical membrane is not well established, but the findings of Hopfer and Groseclose (1980) are consistent with the notion of "glide symmetry"; i.e., first-on, first-off.

As discussed previously (Schultz, 1986), the simplest kinetic model consistent with these notions is illustrated in Fig. 13. For the reasons discussed previously (Schultz, 1986) we assume that the first-order rate constants for translocation (P_1 and P_2)

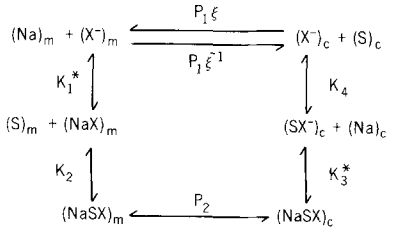

Fig. 13. Kinetic model of the Na-galactose cotransport process described by Schultz (1986)

are slow compared to the rates of the association-dissociation reactions so that the latter may be represented by the ("pseudoequilibrium") dissociation constants K_1, K_2, K_3 and K_4.

Further, while many previous attempts at modeling rheogenic carrier-mediated cotransport processes have restricted the voltage dependency of these processes to the translocation (or reorientation) step of the charged form of the "carrier," (*cf.* Jacquez, 1972; Geck & Heinz, 1976; Heinz & Geck, 1978; Hansen et al., 1981; Sanders, Hansen, Gradmann & Slayman, 1984), it is clear that if the "gated channel" notion is correct there are, in general, at least two additional steps that could be voltage dependent; namely, the rates of association and dissociation of Na with and from its binding site *within* the channel (Schultz, 1986). Assuming that the electrical field across the channel is constant, it follows that $\xi = \exp(-\alpha F\psi^{mc}/2RT)$; $K_1^* = K_1 \exp(\beta F\psi^{mc}/RT)$; and $K_3^* = K_3 \exp(-\gamma F\psi^{mc}/RT)$. Where K_1 is the dissociation constant of the Na-carrier complex, $(NaX)_m$, at the surface of the membrane facing the mucosal solution; K_3 is the dissociation constant of the ternary complex, $(NaSX)_c$, at the cytoplasmic surface of the membrane; and, $\alpha + \beta + \gamma = 1$. Thus, α, β and γ essentially distribute the overall effect of ψ^{mc} among the three possible voltage-dependent steps in this rheogenic process.[5]

The solution of this kinetic model is (Schultz, 1986)

$$J_{SNa} = P_1P_2(X)_t[\xi K_3^*K_4(Na)_m(S)_m - \xi^{-1}K_1^*K_2(Na)_c(S)_c]/D \tag{8}$$

where $(X)_t$ is the total concentration of carrier in all forms per unit membrane area and

[5] Restrepo and Kimmich (1985) have considered kinetic models in which translocation is the voltage-dependent step as well as a "sodium-well model" in which the Na binding constants are voltage dependent. They did not consider a "hybrid" model in which binding as well as translocation may be voltage dependent.

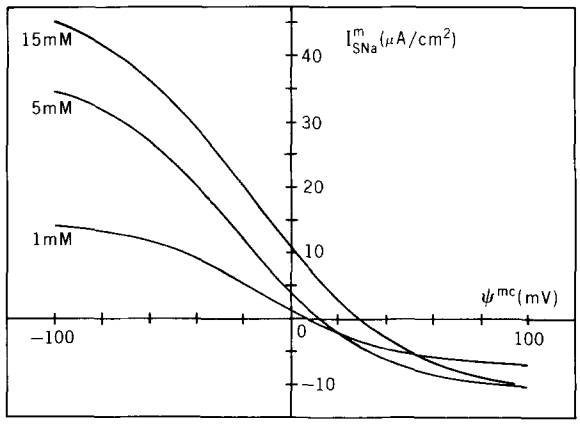

Fig. 14. Predicted relations between I^m_{SNa} and ψ^{mc} when $(S)_m = 1$, 5 and 15 mM for the conditions described in the text

$$D = (P_2 + P_1\xi)K_3^*K_4(Na)_m(S)_m + (P_2 + P_1\xi^{-1})K_1^*K_2(Na)_c(S)_c + 2P_2(Na)_m(Na)_c(S)_m(S)_c + P_1K_1^*K_2K_3^*K_4(\xi + \xi^{-1}) + P_2K_3^*(Na)_m(S)_m(S)_c + P_1\xi^{-1}K_1^*K_2K_3^*(S)_c. \tag{9}$$

Equation (8) contains seven independent variables. Thus, in an attempt to compare the predictions of that equation with our experimental results we first chose a "reasonable" initial set of conditions and values for (P_1/P_2), $K_1 = K_3$, $K_2 = K_4$, α and β. The "fixed values" were $(Na)_m$ = 80 mM, $(Na)_c$ = 14 mM (Hudson & Schultz, 1984) and the values for $(S)_c$ when $(S)_m$ = 1, 5 or 15 mM were those predicted by the observed values of E^m_{SNa} (*see above*). The aims of this exercise were: (i) to reproduce the *shapes* of the observed *I-V* relations; (ii) to determine which of the independent variables have little or no effects on these *shapes*; and (iii) to narrow-down on those parameters that best conformed to the observed values of the steady-state ΔI_{sc} in the presence of 1, 5 and 15 mM galactose in the mucosal solution.

Figure 14 illustrates the predictions of Eq. (8) for the conditions $\alpha = 1$; $K_1 = K_3 = 50$ mM; $K_2 = K_4 = 5$ mM and $(P_1/P_2) = 10$. The shapes of the curves conform with the general features of the experimentally determined *I-V* relations in that: (i) the conductance of the cotransporter increases as ψ^{mc} decreases (becomes less positive) in the regions around E^m_{SNa} and (ii) I^m_{SNa} can saturate at very negative values of ψ^{mc}. The agreement between the predicted values of ΔI_{sc} (or ${}_0I^m_{SNa}$) and the observed values is shown in Fig. 15. It is of some interest that if the interaction of Na with its binding site at both sides of the membrane is affected by 25% of the electrical field (i.e., $\beta = \gamma = 0.25$), the predicted *I-V* relations (when $K_1 = K_3 = 50$ mM) are drastically linearized between E^m_{SNa} and $\psi^{mc} = -100$ mV. And, if $\beta = \gamma = 0.5$, so that the entire effect of the electrical

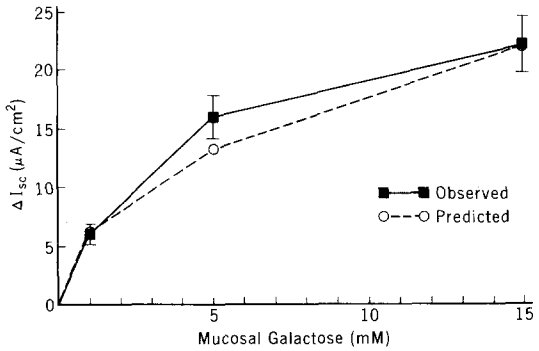

Fig. 15. Relation between the observed values of ΔI_{sc} (i.e., ${}_0I^m_{SNa}$) and those predicted by the *I-V* relations illustrated in Fig. 14

field is on the association-dissociation reaction of Na with its binding site (i.e., no effect of the field on translocation), the curvature of the predicted *I-V* relations is inverted compared to those observed so that the conductance of the cotransporter *decreases* with increasingly negative values of ψ^{mc} in the region surrounding E^m_{SNa}. Even by varying the parameters (P_1/P_2) and $K_1 = K_3$ we were unable to satisfy the experimentally observed shapes of the *I-V* relation *and, at the same time*, obtain good agreement between the observed and predicted values of ΔI_{sc} when $\beta = \gamma = 0.5$.

Thus, our experimental observations on intact small intestine are compatible with the simplest mechanico-kinetic model for Na-coupled sugar cotransport across the apical membrane of small intestine emerging from studies on isolated brush border membrane vesicles. It must be emphasized that this "compatibility" does not exclude other possible models of this cotransport process. The present data are equally likely to be compatible with a model in which the "free carrier" is neutral; but, this notion would be inconsistent with a considerable body of evidence derived from studies on brush border membrane vesicles (*cf.* Schultz, 1986). Further, while the values assigned above for the independent variables seem reasonable and provide a good fit to the experimental data, they are most unlikely to be unique; given the fact that there are at least seven independent variables in this *most simplified* model, it seems certain that there are other "combinations" that would be equally consistent with our data. Thus, the results of this exericse should not be viewed as a "final statement" but rather as an "initial effort" that must be subjected to further experimental tests. The most obvious next step is to determine whether the parameters that describe the *I-V* relations when $(S)_m$ is varied in the presence of constant $(Na)_m$ (present study) also describe the *I-V* relations in the presence of a fixed $(S)_m$ when $(Na)_m$ is varied; while the methods for such studies are "on hand" this is not a trivial undertaking in terms of time and effort.

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Note Added in Proof

After the submission of the revised form of this manuscript, a paper by Läuger and Jauch was published in this journal (*J. Membrane Biol.* **91:**275–284, 1986) in which the authors carefully analyze the effects of voltage on ion-driven cotransport processes and examine the effects of voltage on binding of the charged species within an "ion well" as well as translocation of the charged complex employing transition-rate theory. As they point out, our finding that the entire effect of ψ^{mc} can be attributed to the translocation step does not imply that the Na binding site actually moves across the entire thickness of the lipid bilayer. Instead, if our analysis is correct, it is more likely that the movements of Na to and from its binding site take place through wide "access channels" that have low electrical resistances so that only a very small fraction of the total transmembrane electrical field is dissipated along these routes. The translocation of the charged complex from one "access channel" to the other probably involves a short distance through a high resistance "gate."