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Electrophysiology of *Necturus* Urinary Bladder: I. "Instantaneous" Current-Voltage Relations in the Presence of Varying Mucosal Sodium Concentrations

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Summary. The "instantaneous" transepithelial current-voltage (*I-V*) relations of *Necturus* urinary bladder were determined under short-circuit conditions during impalement of a cell with a microelectrode as described previously (Thompson et al., *J. Membrane Biol.* **66**:41–54, 1982). These studies were performed in the presence of 5, 15 and 45 mM Na in the mucosal solution, $[Na]_m$, and in the absence and presence of a maximally effective dose of amiloride.

The *I-V* relations of the amiloride-sensitive Na-entry step at the apical membrane conformed closely to that predicted by the Goldman-Hodgkin-Katz (GHK) "constant field" flux equation over a wide range under all conditions. From these *I-V* relations we calculated: (a) the permeability of the apical membrane to Na, P^m_{Na}; (b) the *chord* conductance of the apical membrane to Na under short-circuit conditions, ${}_0G^m_{Na}$; and, (c) the intracellular Na *activity*, $(Na)_c$. In addition, from the *I-V* relations in the absence and presence of amiloride and the voltage-divider ratio, we determined the *slope* conductances of the transcellular pathway (g^c) and the apical (g^m) and basolateral (g^s) membranes at each different steady state. Our findings indicate that:

(a) While the rate of active Na transport (I_{sc}) increases hyperbolically with increasing $[Na]_m$, $(Na)_c$ is maintained constant at approximately 6 mM and is independent of both $[Na]_m$ and the I_{sc}.

(b) The increase in I_{sc} with increasing $[Na]_m$ is entirely attributable to an increase in ${}_0G^m_{Na}$; the thermodynamic driving force for Na-entry across the apical membrane is maintained constant under these conditions.

(c) P^m_{Na} decreases with increasing $[Na]_m$.

(d) g^c, g^m and g^s increase linearly with increasing I_{sc}.

A possible mechanism that could account for *both* the increase in basolateral pump activity in the face of a constant $(Na)_c$ *and* the increase in g^s that parallels the increase in pump activity is the "recruitment" of additional "pump-leak units" to the basolateral membrane with increasing $[Na]_m$. Other possibilities are discussed.

Key Words sodium transport · electrophysiology · urinary bladder · current-voltage relations · epithelial transport

Introduction

In previous publications from this laboratory, a method was described for the determination of the "instantaneous" transepithelial current-voltage (*I-V*) relations of rabbit colonic epithelium during impalement of an absorptive cell with a microelectrode.[1] We demonstrated that the *I-V* relations determined before and after the addition of a maximally effective concentration of amiloride to the mucosal solution provide sufficient information to define the *I-V* relation of the amiloride-inhibitable Na-entry step across the apical membrane as well as the *I-V* relations of the basolateral membrane and the pathways that parallel the amiloride-inhibitable pathway (Thompson, Suzuki & Schultz, 1982*a*, *b*). The results indicate that the *I-V* relation of the amiloride-inhibitable Na-entry step across the apical membrane conforms to the Goldman-Hodgkin-Katz (Goldman, 1943; Hodgkin & Katz, 1949) (GHK) "constant-field" flux equation for a single ion over a 150-mV range. From the fit of the experimental data to the GHK equation one can derive the intracellular Na activity, $(Na)_c$; the permeability of the apical membrane to Na, P^m_{Na}; and the *chord* conductance of the apical membrane to Na under short-circuit conditions, ${}_0G^m_{Na}$.[2]

[1] As discussed previously (Schultz et al. 1981) the term "instantaneous" infers that *I-V* measurements were made before cell composition can change significantly, in spite of the fact that ionic profiles within the apical and basolateral membranes will have had sufficient time to achieve new steady states.

[2] For an excellent discussion of the distinction between *chord* (G) and *slope* (g) conductances, the reader is referred to Finkelstein and Mauro (1963, 1977). Briefly, the *slope* conductance at a point on the *I-V* relation of a barrier is the value of $(\Delta I/\Delta V)$ determined after the ionic profile within the membrane has had sufficient time to achieve a new steady state in response to the change in V (Schultz, 1981); this is what is *actually* measured when brief current pulses are passed across the barrier. The *chord* conductance at a point on the *I-V* relation of a barrier is the value of $(\delta I/\delta V)$ at "zero time" before there is any change in intramembrane ionic profile in response to the change in V; this *cannot* be measured but can only be derived from the *I-V* relation determined from the point of interest to the "reversal potential." In both instances it is assumed that measurements are made before there is any significant change in the ionic composition of the bathing solutions. It should be stressed that it is the *chord* conductance that directly relates the diffusional flow of an ion to it's thermodynamic driving force (Hodgkin & Huxley, 1952, p. 641).

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In this paper, we describe the results of a series of experiments in which we employed this technique to examine the electrophysiology of *Necturus* urinary bladder under steady-state short-circuit conditions when the Na concentration in the mucosal solution was varied over the range 5–45 mM.

Glossary of Symbols and Nomenclature

ψ^{ms}	Transepithelial electrical potential difference; serosal solution with respect to the mucosal solution, $\psi^s - \psi^m$ (mV).
ψ^{mc}	Electrical potential difference across the apical membrane; cellular compartment with respect to the mucosal solution, $\psi^c - \psi^m$ (mV).
ψ^{cs}	Electrical potential difference across the basolateral membrane, serosal solution with respect to the cellular compartment, $\psi^s - \psi^c$ (mV).
I^{ms}	Total transepithelial current defined as positive for cation movement from the mucosal to the serosal bath ($\mu A/cm^2$).
E	Equivalent electromotive force or zero current potential (mV).
I	Current ($\mu A/cm^2$).
R, r	Effective *chord and slope* resistances, respectively, uncorrected for *actual* membrane area (Ωcm^2).
G, g	*Chord and slope* conductances, respectively, uncorrected for actual membrane area (mS/cm^2).
f	$\equiv r^m/(r^m + r^s)$
$\Delta\mu, \Delta\tilde{\mu}$	Chemical and electrochemical potential differences, respectively (Joules/equiv).

Superscripts

m	Mucosal or apical membrane.
s	Serosal or basolateral membrane.
c	Cellular pathway, refers only to the amiloride-sensitive cells.
p	Parallel pathway, includes all pathways, cellular or paracellular, which are electrically isolated from and in parallel with the amiloride-sensitive absorptive cells.
$'$	Primes denote data obtained in the presence of amiloride

Subscripts

Na, K, i	Sodium, potassium, or unidentified ionic species, respectively (e.g., I^m_{Na} is the Na current across the apical membrane).
0	Preceeding a term indicates the value of that term when $\psi^{ms} = 0$ (e.g., ${}_0I^m_{Na}$).
ψ^{ms}	Values obtained at a given value of ψ^{ms}.

Materials and Methods

All experiments were performed using male *Necturus maculosa* obtained from Mogul-Ed, Oshkosh, Wisc., or Connecticut Valley Biological Supply Co., Southampton, Me., and stored in tap water containing methylene blue at 4 °C. The animals were anesthetized by immersion in tap water containing 660 mg/liter tricaine methylsulfonate (Sigma), and the urinary bladder was removed, opened, and mounted as a stretched, flat sheet on a Lucite ring as described previously (Thompson et al., 1982*a*).[3] These "tissue cartridges" were mounted in a chamber with an exposed tissue area of 0.07 cm^2, the fluid depth above and below the tissue was 3 mm. In a small series of studies (4 tissues) the cells were impaled across the apical or mucosal membrane. In a larger series of studies (8 tissues), the cells were impaled from the serosal (basolateral) surface after a small patch of underlying submucosal connective tissue and muscle was removed using a fine forceps and iris scissors. Finally, the results of a series of studies on a group of animals, with very low spontaneous rates of Na absorption are summarized in the Appendix.

The two surfaces of the tissue were superfused from reservoirs at room temperature using a gravity-feed system. The rate of perfusion was sufficient to replace the volumes of the mucosal and serosal chambers every second. The serosal bathing solution always consisted of (mM): 110, Na; 115, Cl; 2.5, K; 1.2, Ca; 1.2, Mg; 1.2, HPO_4; 0.3, H_2PO_4; and, 5 glucose. The mucosal solution contained either 5, 15, or 45 mM Na with the remainder, up to the total of 110 mM, made up by tetraethylammonium ion; the justification and rationale for this choice of replacement ion will be given below. All solutions had a pH of 7.4 when gassed with 100% O_2 at room temperature (23 °C).

The experimental procedure and apparatus employed have been described in detail (Thompson et al., 1982*a*). Briefly, after mounting, the tissue was automatically short-circuited using an automatic voltage-clamp apparatus. When the short-circuit current (I_{sc}) stabilized, a cell was impaled with a microelectrode filled with 0.5 M KCl and having a tip resistance of 100–150 MΩ (when immersed in 0.5 M KCl); the rationale for the use of these electrodes was discussed by Fromm and Schultz (1981). The criteria for a successful impalement have been given previously (Thompson et al., 1982*a*). During this time, the transepithelial electrical potential difference, ψ^{ms}, was intermittently pulsed to 20 mV by the voltage clamp. The transepithelial slope resistance, r_t, was calculated from the necessary clamping current, and the fractional resistance of the apical membrane, f, was calculated from the ratio of the deflection in the electrical potential difference across the apical membrane, $\Delta\psi^{mc}$, to that across the entire tissue, $\Delta\psi^{ms}$ ("voltage-divider ratio"). Thus,

$$f = \Delta\psi^{mc}/\Delta\psi^{ms} = r^m/(r^m + r^s) \quad (1)$$

where r^m and r^s are the *slope* resistances of the apical and basolateral membranes, respectively.

The experimental protocol is illustrated in Fig. 1.

In general, the tissue was initially perfused with a mucosal solution containing 5 mM Na. When I_{sc}, ${}_0\psi^{mc}$ and f achieved stable values, a computer-generated pulse train was relayed via a digital-to-analog converter to the voltage-clamp device which, in turn, passed current pulses across the tissue sufficient to clamp ψ^{ms} over the range of 0 to ±200 mV in 20-mV increments (e.g., 0, +20, 0, −20, 0, +40, 0, −40, 0, ... +200, 0, −200 mV), each pulse had a duration of 100 msec, and the interval between pulses was 500 msec. During the pulse train, the transepithelial current (I^{ms}), the electrical potential difference across the apical membrane (ψ^{mc}) and the electrical potential difference across the tissue (ψ^{ms}) were recorded on a Gould (Model 2400) 3-channel chart recorder, sampled by an analog-to-digital converter, and relayed to the computer for storage and processing. Five samples of each signal were taken 16 msec after the upstroke of each pulse and again at the end of the

[3] In urine collected from seven bladders, the average osmolarity was 27 ± 4 mOsm (range 11–51 mOsm); the average Na concentration was 1.1 ± 0.7 mM (range 0.1–5.6 mM); and the average K concentration was 1.1 ± 0.5 mM (range 0.1–3.9 mM).

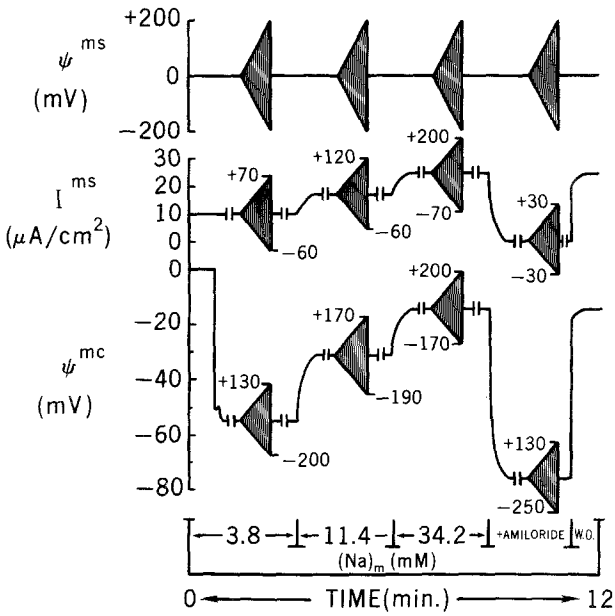

Fig. 1. Outline of the experimental protocol described in the text. $(Na)_m$ is the Na activity in the mucosal solution. The values of ψ^{ms}, I^{ms} and ψ^{mc} are representative. At the "breaks" the gain of the recorder was reduced tenfold and the shaded triangles in I^{ms} represent the sequence of current pulses needed to clamp ψ^{ms} over the range 0 to ±200 mV. In several experiments amiloride was washed out (*W.O.*) of the mucosal solution, and the values of I^{ms} and ψ^{mc} returned to the pre-amiloride values

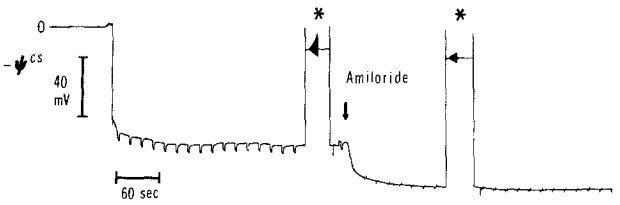

Fig. 2. Typical impalement from the serosal surface when the mucosal solution contained 5 mM Na. During the periods marked by asterisks the gain of the recorder was reduced and the dark triangles represent the response of ψ^{cs} to the sequence of current pulses. The small periodic displacements in ψ^{cs} are the responses to current pulses required to displace ψ^{ms} by 20 mV. Note that following the addition of amiloride to the mucosal perfusate there was an abrupt hyperpolarization and the periodic displacements in ψ^{cs} essentially vanished, indicating that the fractional resistance of the apical membrane was close to unity

pulse (100 msec). Each set of five samples was averaged to give a single value at 16 msec and another at 100 msec. For the case of the transepithelial and transapical *I-V* relations no substantive differences between these two sets of data were found and only the data obtained at 16 msec will be presented.

When the "instantaneous" *I-V* relations were completed in the presence of a mucosal solution containing 5 mM Na, the mucosal solution was switched to one containing 15 mM Na, and, after a new steady state was achieved, the voltage-clamping procedure described above was repeated. Then, the mucosal perfusate was switched to one containing 45 mM Na, and the *I-V* relations were obtained once again. Finally, the mucosal perfusate was switched to one containing 10^{-4} M amiloride, and the *I-V* relations were determined in the presence of this agent.

Several points should be added. (a) These experiments generally lasted 6–12 min, and in every instance the microelectrode was maintained within the impaled cell throughout that duration. (b) In three experiments the order of mucosal perfusion was 5 mM Na, 15 mM Na, 45 mM Na and then 5 mM Na (again) prior to the addition of amiloride to the perfusate; the data obtained in the presence of 5 mM Na at the beginning and end of the study did not differ significantly. (c) In two experiments the order of perfusion was reversed, i.e., 45 mM Na, 15 mM Na, 5 mM Na; the results do not differ from those obtained using the opposite order. In short, there is no reason to suspect that the duration of the experiment or the order of changing the mucosal perfusates significantly influenced the results.

For the purposes of these experiments it was essential that the ion employed to replace Na: (a) does not permeate the apical membrane; (b) does not affect the resistance of the paracellular shunt pathway or any other pathway in parallel with the amiloride-sensitive Na entry step; and (c) does not affect the rate of active Na transport. Three ions, namely, choline, tetraethylammonium, and tetramethylammonium satisfied these three criteria inasmuch as: (i) when the mucosal surface of the tissue was perfused with solutions in which all of the Na was replaced with any of these ions, r_t, f and ψ^{mc} were not affected by amiloride and did not differ from those values observed when the tissue was perfused with the normal Ringer solution containing 10^{-4} M amiloride, (ii) when Na in the mucosal solution alone was partially replaced with any of these ions, the I_{sc} was entirely inhibited by amiloride, and (iii) there was no significant difference in the I_{sc} in the presence of 45 mM Na when the remainder (65 mM) was made up with any of these ions. Replacement of Na with K fulfilled criteria (a) and (b) but partial replacement of Na with K consistently resulted in a lower I_{sc} than when any of the organic ions was employed. Thus it appears that high mucosal K inhibits Na transport by this preparation as appears to be the case in toad urinary bladder (Frazier, 1964) and frog skin (Lindemann, 1970; Rotunno, Villalonga, Fernandez & Cereijido, 1970; Mandel & Curran, 1973) in spite of the fact that it does not appear to enter the cell via the amiloride-sensitive Na entry step.

Results are expressed as the mean ± the standard error of the mean (SEM) and statistical analyses were performed using the Student t test with a value of $P<0.05$ established as the level of significance. Graphics analysis of the data was carried out using a graphics terminal (Hewlett-Packard 2647A).

Results and Discussion

A typical impalement from the serosal surface of the tissue is shown in Fig. 2. Clearly, following the addition of amiloride to the mucosal solution, there was a marked hyperpolarization of ψ^{cs} and an increase in f. The results of all impalements from the serosal surface of the tissue are given in Table 1 where: $(Na)_m$ is the Na *activity in the mucosal solution*; r_t is the tissue resistance in Ωcm^2 determined from the current needed to displace ψ^{ms} briefly by 20 mV; r^p is the tissue resistance (Ωcm^2) in the presence of amiloride and is assumed to reflect, predominantly, the resistance of the parallel pathways (*see* below); f and f' are the frac-

Table 1. Effects of mucosal Na on electrophysiologic properties of *Necturus* urinary bladder[a]

$(Na)_m$ (mM)	r_t (Ωcm^2)	r^p	f	${}_0g^m$ (mS/cm^2)	${}_0g^s$	${}_0\psi^{mc}$ (mV)	I_{sc} ($\mu A/cm^2$)	$(Na)_c$ (mM)	${}_0G^m_{Na}$ (mS/cm^2)	P^m_{Na} (cm/hr $\times 10^2$)	${}_0\psi^{mc\prime}$ (mV)	f'
3.8 (11)	3,947	14,341	0.91	0.20	2.1	−55	10.3	5.1	0.22	5.1	−70	0.96
SEM	411	2,126	0.01	0.03	0.3	4	1.1	0.6	0.03	0.8		0.01
11.4 (8)	2,441	14,326	0.87	0.41	2.9	−31	17.2	5.8	0.39	4.1	−75	0.96
SEM	356	3,171	0.02	0.06	0.5	7	2.0	0.9	0.05	0.6	2	0.01
34.2 (8)	1,818	13,974	0.84	0.68	3.8	−14	25.0	8.6	0.53	2.7	−74	0.96
SEM	293	3,032	0.02	0.05	0.8	6	2.5	1.4	0.08	0.5	2	0.01

[a] Symbols and units are defined in the Glossary. Number of data points at each $(Na)_m$ are given in parentheses.

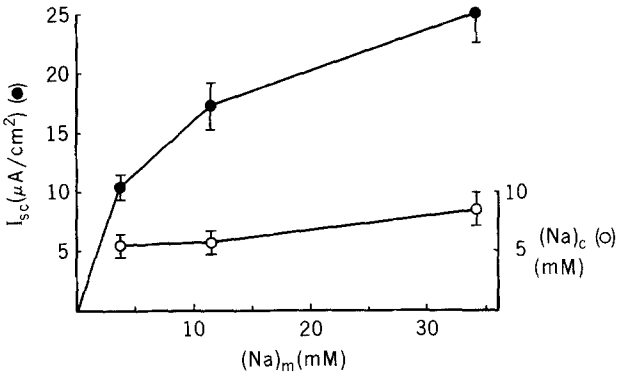

Fig. 3. Relations among $(Na)_m$, I_{sc} (●) and $(Na)_c$ (○)

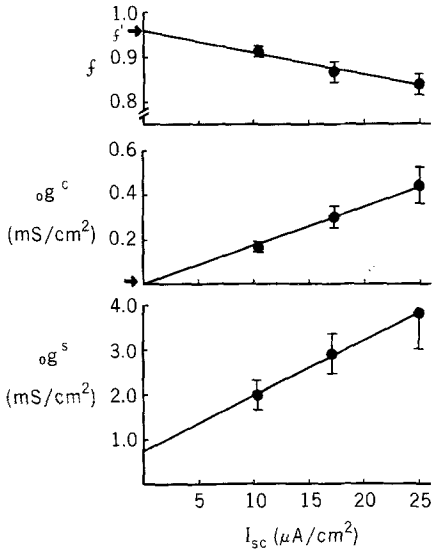

Fig. 4. Relations among I_{sc} and f, ${}_0g^c$ and ${}_0g^s$

tional resistances of the apical membrane in the absence and presence of amiloride; I_{sc} is the short-circuit current in $\mu A/cm^2$; and ${}_0\psi^{mc}$ and ${}_0\psi^{mc\prime}$ are the electrical potential differences across the apical membrane under short-circuit conditions in the absence and presence of amiloride (mV). The definitions and methods of calculation of the other values will be discussed below.

Relation between $(Na)_m$ and I_{sc}

The relation between $(Na)_m$ and I_{sc} is shown in Fig. 3. Clearly, I_{sc} saturates with increasing $(Na)_m$ and the data can be described reasonably well by Michaelis-Menten kinetics with a maximal I_{sc} of 24 $\mu A/cm^2$ and a half-maximal I_{sc} observed when $(Na)_m = 5$ mM. Similar findings have been reported by others for frog skin, toad urinary bladder and several other Na-transporting epithelia (*cf.* Macknight, DiBona & Leaf, 1980).

Relations among I_{sc}, f and the Slope Conductances of the Transcellular Pathway

As illustrated in Fig. 4, f decreases linearly with increasing I_{sc}. The line extrapolates to a value of 0.96 when $(Na)_m$ and, thus, I_{sc} are zero. This value is identical to that observed in the presence of amiloride.

As shown in Table 1, the total tissue resistance, r_t, decreased with increasing $(Na)_m$ but the value of the transepithelial resistance in the presence of amiloride (r^p) was not affected by changes in $(Na)_m$ (and thus by the different concentrations of TEA employed to replace Na) and was approximately 14 kΩ cm^2. Inasmuch as $f = 0.96$, it is reasonable to assume, as is generally done, that r^p is a good estimate of the resistance of the pathways that parallel the Na-transporting cells. Assuming that r^p is not affected by amiloride, the slope resistance of the transcellular pathway can be calculated from the relation

$$r^c = r_t\, r^p/(r^p - r_t)$$

and, because $r^c = r^m + r^s$, it follows that $r^m = fr^c$ and $r^s = (1-f)r^c$.

The values of ${}_0g^m$ (i.e., $1/{}_0r^m$) and ${}_0g^s$ (i.e., $1/{}_0r^s$) in the presence of the three different mucosal Na concentrations are given in Table 1 and the relations among ${}_0g^c$ (i.e., $1/{}_0r^c$), ${}_0g^s$ and I_{sc} are illustrated in Fig. 4. Clearly, ${}_0g^s$ is 6–10 times greater than ${}_0g^m$ and the ratio $({}_0g^s/{}_0g^m)$ decreases with increasing $(Na)_m$.

As shown in Fig. 4, both ${}_0g^c$ and ${}_0g^s$ increase linearly with increasing I_{sc}. Using the values of ${}_0g^s = 0.75$ mS/cm^2 and $f = 0.96$ when I_{sc} *or* $(Na)_m = 0$, we can calculate that in the absence of mucosal Na, ${}_0r^c = 33$ kΩ·cm^2 so that ${}_0g^c = 0.03$ mS/cm^2; the latter does not differ significantly from the intercept of the plot shown in Fig. 4b.

Similiar relations were observed when the tissue was impaled from the apical surface; however, f and f' were significantly lower than when the cells were impaled from the serosal surface, f averaged 0.82 in the absence of amiloride and only 0.91 in its presence.

These results confirm and extend those reported by Higgins et al. (Higgins, Cesaro, Gebler- & Frömter, 1975; Higgins, Gebler & Frömter, 1977) and Frömter and Gebler (1977) for open-circuited *Necturus* urinary bladders with spontaneously varying I_{sc} (calculated from (ψ^{ms}/r_t)) in the presence of 109 mM Na; namely, direct relations among I_{sc} and g^c, g^m and g^s. In their studies f was approximately 0.93 and f' averaged 0.985 (Frömter, Higgins & Gebler, 1981).

Relations between I_{sc} and ${}_0\psi^{mc}$

The relations between the values of I_{sc} and ${}_0\psi^{mc}$ under steady-state conditions in the presence of 5, 15 and 45 mM Na are illustrated in Fig. 5. Clearly, the relations obtained from both apical and serosal impalements are essentially linear and the intercepts on the abscissa do not differ significantly from the values of ${}_0\psi^{mc}$ observed in the presence of amiloride (arrows).

Transepithelial Current-Voltage (I-V) Relations and the I-V Relations of the Sodium Entry Step Across the Apical Membrane

Typical transepithelial I^{ms} *vs.* ψ^{ms} relations in the presence of 5, 15, and 45 mM Na in the mucosal solution before (*C*) and after (*A*) amiloride are shown in Fig. 6; the data shown were obtained 16 msec after the onset of the current pulse. In the absence of amiloride, the relations between I^{ms} and ψ^{ms} are decidedly nonlinear with the departure from linearity becoming more marked as $(Na)_m$ decreases. On the other hand, in the presence of

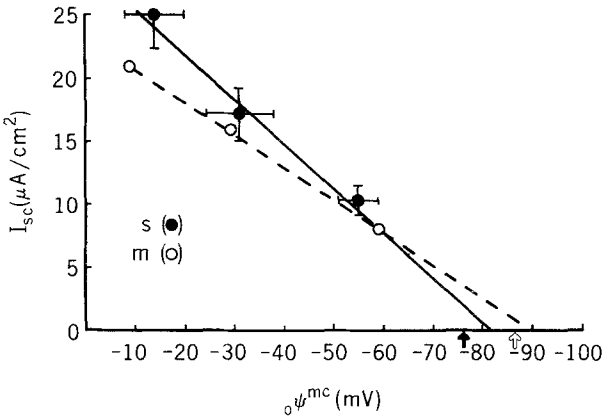

Fig. 5. Relation between I_{sc} and ${}_0\psi^{mc}$ (or $-{}_0\psi^{cs}$) for impalements from the serosal surface (●) and mucosal surface (○). The arrows indicate the observed values of $\psi^{mc'}$ in the presence of amiloride in these two series of studies

amiloride, the relations between $I^{ms'}$ and $\psi^{ms'}$ are strictly linear over the range ±200 mV. Clearly, in the presence of amiloride the I_{sc} is abolished and r_t is markedly increased. The point at which the curves in the absence (*C*) and presence (*A*) of amiloride intersect is that value of ψ^{ms} at which transapical and hence transcellular Na flow is abolished; this value of ψ^{ms} may be defined as the "instantaneous E_{Na}" of the tissue (Schultz, Thompson & Suzuki, 1981) and its significance will be discussed below.

The relations between ψ^{mc} and ψ^{ms} for the experiments shown in Fig. 6, are given in Fig. 7. In one of the experiments shown, the microelectrode was dislodged by the switching to a mucosal solution containing amiloride so that only the pre-amiloride data are available. Clearly, these relations are very close to linear over a wide range. The slope of the line in the absence of amiloride corresponds to f; in the presence of amiloride, the slope (f') increases.

As discussed previously (Thompson et al., 1982*a*), if the apical membrane is exclusively permeable to Na and if 10^{-4} M amiloride completely blocks the Na entry step across that barrier, then the Na current across the apical membrane at any value of ψ^{ms} is given by

$$(I^m_{Na})_{\psi^{ms}} = (I^{ms} - I^{ms'})_{\psi^{ms}} = (\Delta I^{ms})_{\psi^{ms}}. \quad (2)$$

On the other hand, if there is another conductive pathway across the apical membrane and/or the action of amiloride is incomplete, then, providing certain conditions are met (Thompson et al., 1982*a*, *b*)

$$(I^m_{Na})_{\psi^{ms}} = (\Delta I^{ms}/f')_{\psi^{ms}}. \quad (3)$$

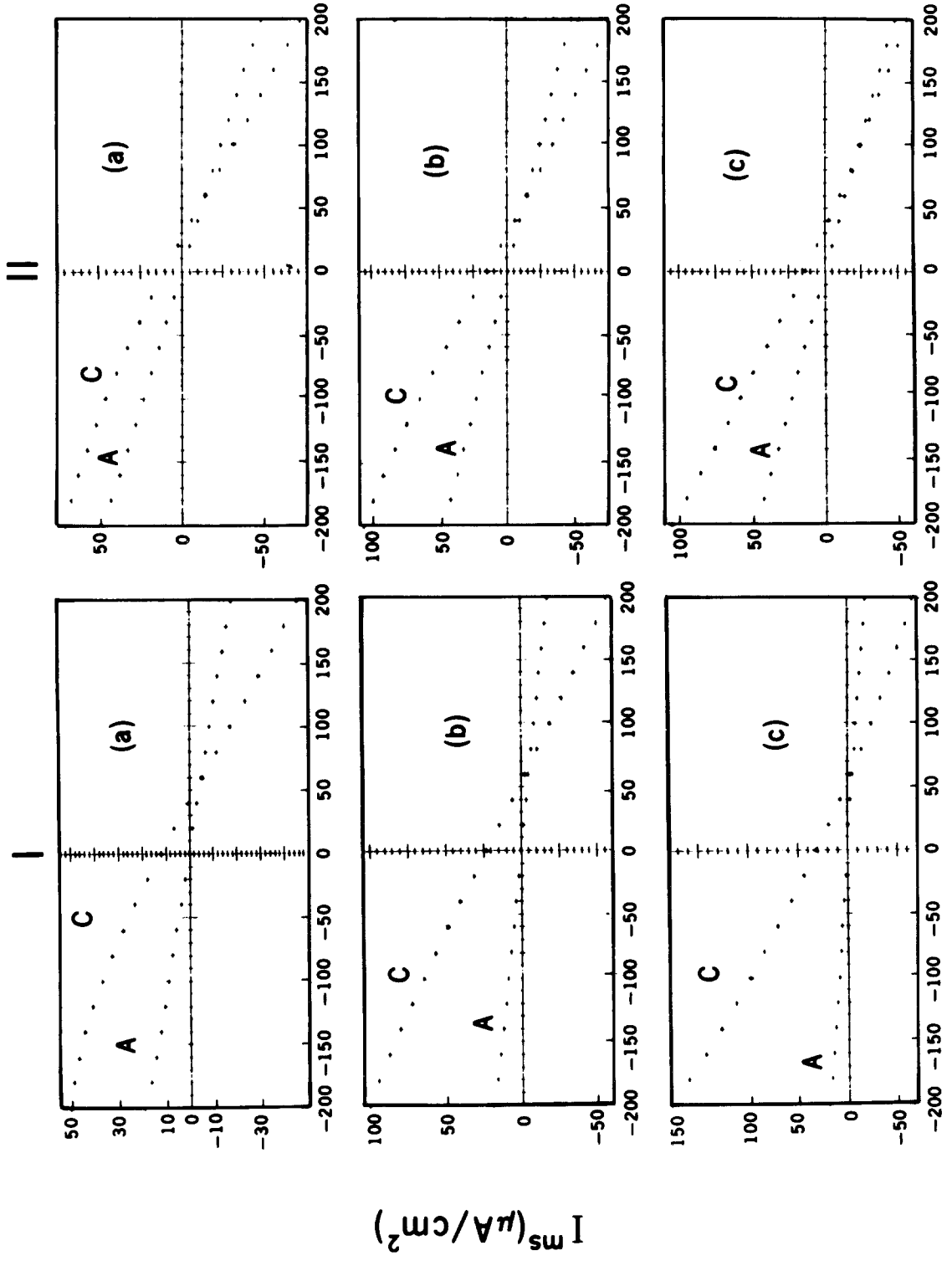

Fig. 6. Two (best and worst cases) examples of the relations between I^{ms} and ψ^{ms} in the presence of (*a*) 5 mM, (*b*) 15 mM and (*c*) 45 mM Na in the mucosal solution in the absence (*C*) and presence (*A*) of amiloride. Note that under control conditions these *I-V* relations are nonlinear whereas they are strictly linear in the presence of amiloride. The vertical displacement between I^{ms} and $I^{ms\prime}$ at any value of ψ^{ms} is defined as I^{m}_{Na} at that value of ψ^{ms} (Eq. (2))

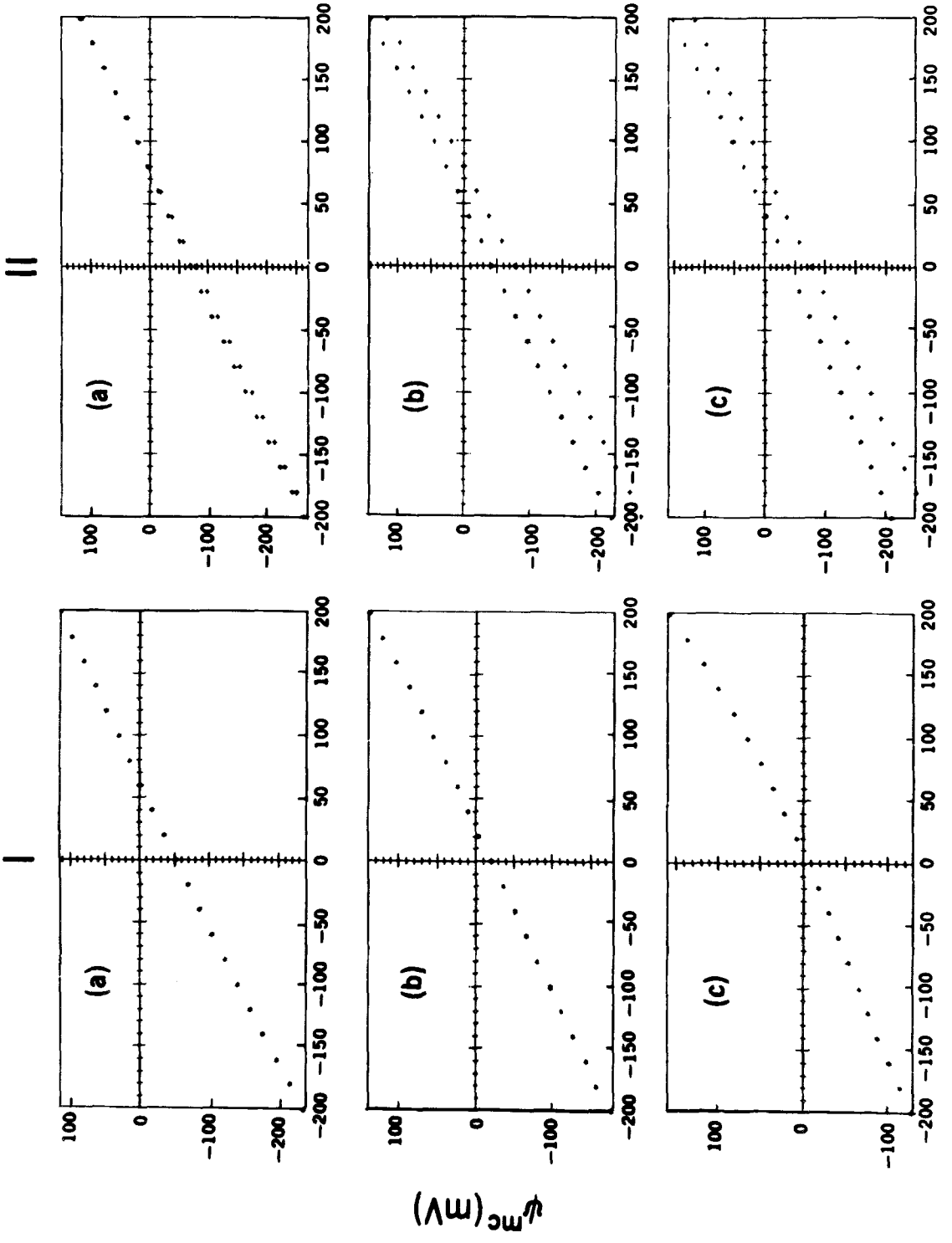

Fig. 7. Relations between ψ^{mc} and ψ^{ms} for the experiments illustrated in Fig. 6. In experiment *I*, the microelectrode was dislodged when the mucosal solution was switched to one containing amiloride; in experiment *II*, the values obtained in the presence of amiloride are below those obtained in the absence of this agent. Note that the values of f given by the slopes of the relations (i.e., $d\psi^{mc}/d\psi^{ms}$) are nearly constant and increase in the presence of amiloride

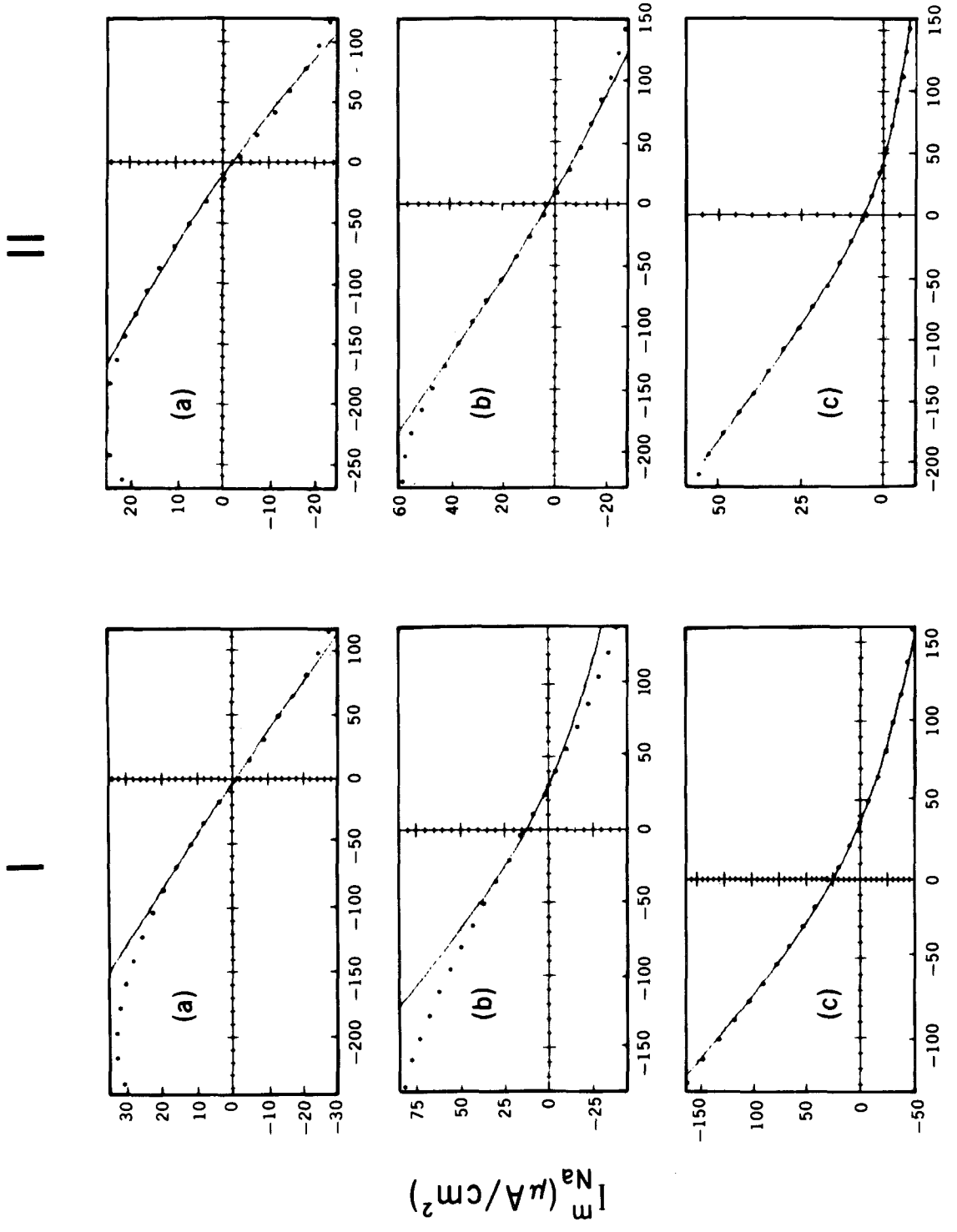

Fig. 8. The relations between I^m_{Na} and ψ^{mc} for the experiments illustrated in Figs. 6 and 7. The curves are those predicted by the GHK "constant-field" flux equation for a single cation fit to the experimental data over the range ± 50 mV by a least-squares nonlinear algorithm

Since f' averaged 0.96 the error involved in using Eq. (2) rather than Eq. (3) to calculate $(I^m_{Na})_{\psi^{ms}}$ is small. Thus, Eq. (2) was employed to calculate $(I^m_{Na})_{\psi^{ms}}$ in this study. This simplification will be further justfied below.

Inasmuch as ψ^{mc} is also known at any value of ψ^{ms} (Fig. 7), we can readily determine the relation between $(I^m_{Na})_{\psi^{ms}}$ and $(\psi^{mc})_{\psi^{ms}}$;, or, the *I-V* relation of the amiloride-inhibitable Na entry step across the apical membrane.

The relations between I^m_{Na} and ψ^{mc} when the Na concentration in the mucosal solution was 5, 15 or 45 mM for the experiments illustrated in Figs. 6 and 7 are given in Fig. 8. The curves shown are the best fits of the GHK "constant field" flux equation for a monovalent cation (Goldman, 1943; Hodgkin & Katz, 1949) to the data determined using a nonlinear least-squares curve-fitting algorithm constrained to the range $\psi^{mc} = \pm 50$ mV; in essence, the program solves for the values of $(Na)_c$ and P^m_{Na} that minimize the sum of the squared deviations of the experimental data from the theoretical curve over that range. Clearly, the experimental data conform closely to the GHK equation (most often well beyond the range chosen for the curve-fitting algorithm) and the conformity of the fit increases with increasing $(Na)_m$; thus, when the concentration of Na in the mucosal solution was 45 mM, the relation between I^m_{Na} and ψ^{mc} conformed closely to the GHK equation over a 250–300 mV range.

Frömter et al. (1981) have reported that the *I-V* relation of the Na entry step across the apical membrane of open-circuited *Necturus* urinary bladder in the presence of 109 mM Na conforms to the GHK equation, but a good fit was observed only over the relatively small range between $\psi^{mc} = 0$ and the "reversal potential" (i.e., $I^m_{Na} = 0$). These data were derived from 200-msec current pulses and this may be the source of the difference between our results and theirs.

Fuchs, Larsen and Lindemann (1977) have also found that the amiloride inhibitable Na current across the apical membrane of frog skin exposed to a high-K serosal solution conforms to the GHK equation over a limited range with particularly significant departures observed beyond the "reversal potential." Similar findings have been reported for toad urinary bladder also exposed to a high-K serosal solution (Li, Palmer, Edelman & Lindemann, 1982; Palmer, Edelman & Lindemann, 1980; Palmer, Li, Lindemann & Edelman, 1982).

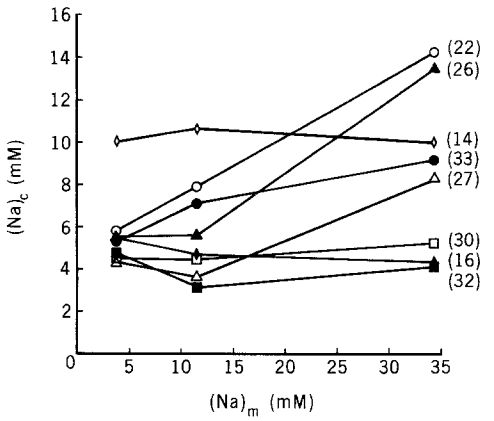

Fig. 9. Relations between $(Na)_m$ and $(Na)_c$ for eight studies involving impalements from the serosal surface of the tissue. The individual values of I_{sc} for each tissue when $(Na)_m = 34.2$ mM are given in parenthesis adjacent to the value of $(Na)_c$.

Relation Among Mucosal Sodium Activity and Cell Sodium Activity

Inasmuch as Na entry into the cell across the apical membrane appears to be driven solely by its conjugate thermodynamic driving force (i.e., the electrochemical potential difference for Na across that barrier), the "reversal potential" of the relation between I^m_{Na} and ψ^{mc} (i.e., that value of ψ^{mc} at which $I^m_{Na} = 0$) is given by $E^m_{Na} = (RT/\mathcal{F}) \ln [(Na)_m/(Na)_c]$, where R, T and $\mathcal{F}$ have their usual meanings.

The values of $(Na)_c$ determined from the "reversal potentials" of the GHK fits to the experimental data in the presence of 5, 15 or 45 mM Na in the mucosal solution are given in Table 1 and illustrated for the individual experiments in Fig. 9. Clearly, $(Na)_c$ does not increase significantly when $(Na)_m$ is increased from 3.8 to 11.4 mM. In three experiments there was an increase in $(Na)_c$ when $(Na)_m$ was increased from 11.4 to 34.2 mM, but in five experiments no significant increase was observed over this range. The average values of $(Na)_c$ when $(Na)_m$ is varied over the range 11.4 to 34.2 mM do not differ significantly at the $P < 0.05$ level. Further, as is also shown in Fig. 9, there is no relation between $(Na)_c$ and I_{sc} when $(Na)_m = 34.2$ mM.

The results obtained from the apical impalements were $(Na)_c = 5.2 \pm 0.7$ ($n = 4$), 5.4 ± 0.6 ($n = 7$), and 4.3 ± 0.5 ($n = 4$) when $(Na)_m$ was 3.8, 11.4 and 34.2 mM, respectively; none of these values differ significantly.

Thus, there is little or no change in $(Na)_c$ when $(Na)_m$ is varied over a ninefold range; and, as

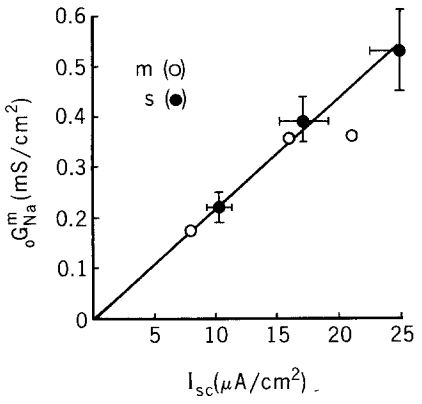

Fig. 10. The relation between I_{sc} and $_0G^m_{Na}$ for impalements from the serosal (●) and apical (o) surfaces

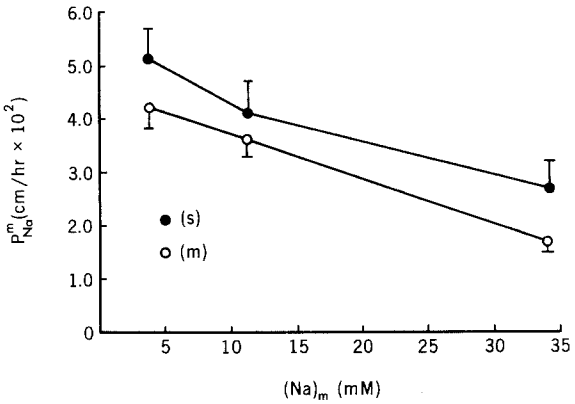

Fig. 11. Relations between $(Na)_m$ and P^m_{Na} for impalements from the serosal (●) and apical (o) surfaces

illustrated in Figs. 3 and 9, there is no apparent relation between $(Na)_c$ and I_{sc}.

In the study reported by Frömter et al. (1981) the intracellular Na concentration in the presence of 109 mM Na was 5–6 mM. This value is in excellent agreement with those found in the present study and suggests that $(Na)_c$ is maintained relatively constant when the mucosal Na concentration is varied over the range from 5–109 mM.

Relation Between I_{sc} and the Chord Conductance of the Apical Membrane to Sodium

When the relation between I^m_{Na} and ψ^{mc} is determined, the *chord,* or "instantaneous" conductance[2] of the apical membrane to Na under short-circuit conditions can be *derived* from the expression

$$_0G^m_{Na} = [(_0E^m_{Na} - {}_0\psi^{mc})/I_{sc}]^{-1} = [(\Delta\, {}_0\tilde{\mu}^m_{Na}/\mathcal{F})/I_{sc}]^{-1} \quad (4)$$

where $(\Delta\tilde{\mu}^m_{Na}/\mathcal{F})$ is the electrochemical potential difference for Na across the apical membrane in mV, and the subscript 0 indicates that the values pertain to the short-circuit condition (i.e., $\psi^{ms} = 0$).

The values of $_0G^m_{Na}$ at the three Na concentrations studied are given in Table 1 and the relation between $_0G^m_{Na}$ and I_{sc} is illustrated in Fig. 10. Clearly, the data are well described by a straight line indicating that variations in I_{sc}, resulting from variations in $(Na)_m$, are due *solely* to changes in the Na conductance of the apical membrane; the thermodynamic driving force across the apical membrane $(\Delta\, {}_0\tilde{\mu}^m_{Na}/\mathcal{F})$ is constant and, in these experiments, averaged 48 mV.

Relation Between $(Na)_m$ and the Permeability of the Apical Membrane to Sodium

The average values of the permeability of the apical membrane to Na, P^m_{Na}, at the three different mucosal Na concentrations, derived from the non-linear least-squares fit of the GHK equation to the experimental data, are given in Table 1 and plotted as a function of $(Na)_m$ in Fig. 11. Clearly, in spite of the relatively large variance, there is a significant decrease in P^m_{Na} with increasing $(Na)_m$. A similar relation was observed in the studies involving apical impalements where the values of P^m_{Na} (in 10^{-2} cm/hr) averaged: 4.2 ± 0.4 $(n=4)$, 3.6 ± 0.3 $(n=7)$ and 1.7 ± 0.2 $(n=4)$ when $(Na)_m$ was 3.8, 11.4 and 34.2 mM, respectively. This relation is also illustrated in Fig. 11.

Lindemann and his collaborators have provided convincing evidence for an inhibitory effect of $(Na)_m$ on P^m_{Na} in frog skin (Lindemann & Voute, 1976; Fuchs et al., 1977) and toad urinary bladder (Palmer et al., 1980). The present data are consistent with this notion inasmuch as the decrease in P^m_{Na} with increasing $(Na)_m$ cannot be attributed to an increase in $(Na)_c$ which, in fact, remained essentially unchanged; this matter will be discussed further below.

Finally, it is of interest to note that the values of P^m_{Na} determined in these studies are in good agreement with those reported by Fuchs et al. (1977) for frog skin and Palmer et al. (1980) for toad urinary bladder in the presence of low mucosal Na concentrations. Frömter et al. (1981) reported a much lower value of $P^m_{Na} = 0.2 \times 10^{-2}$ cm/hr for *Necturus* urinary bladder in the presence of 109 mM Na; this value is consistent with the inverse relation between $(Na)_m$ and P^m_{Na}.

Relation between Apical Membrane Potential and $(Na)_m$

The relation between the electrical potential difference across the apical membrane under short-circuit conditions, $_0\psi^{mc}$, and $(Na)_m$ is shown in Fig. 12. Clearly there is a linear relation between $_0\psi^{mc}$ and log $(Na)_m$. For the impalements from

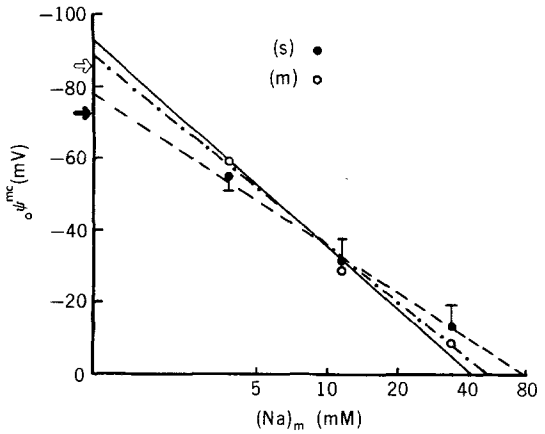

Fig. 12. Relations between $_0\psi^{mc}$ and log $(Na)_m$ for impalements from the serosal (•) and apical (o) surfaces of the tissue. The predicted (solid) line when $(Na)_c = 6$ mM and $(I_{sc}/{}_0G^m_{Na}) = 48$ mV does not differ significantly from those observed. The arrows indicate the observed values of $_0\psi^{mc'}$ in the presence of amiloride

the serosal surface (•) the line has a slope of 43 mV for a 10-fold change in $(Na)_m$, and for the apical impalements (o) the slope is 53 mV. The intercepts of these lines with the ordinate (i.e., $(Na)_m = 0$) are -79 ± 5 mV and -88 ± 7 mV, respectively; these values are in excellent agreement with the values of $_0\psi^{mc'}$ observed in the presence of amiloride which averaged -73 and -83 mV, respectively (arrows).

According to Eq. (4)

$$_0I^m_{Na} = {}_0G^m_{Na}\ (_0E^m_{Na} - {}_0\psi^{mc}). \quad (5)$$

However, as was illustrated in Fig. 10, $(_0E^m_{Na} - {}_0\psi^{mc})$ is independent $_0I^m_{Na}$ and averages 48 mV. Thus,

$$_0\psi^{mc} = 58.4 \log (Na)_m - [58.4 \log (Na)_c + 48]. \quad (6)$$

Since $(Na)_c$ is essentially independent of $(Na)_m$, the relation between $_0\psi^{mc}$ and log $(Na)_m$ should be linear with a slope of 58 mV, and, using the average value of 6 mM for $(Na)_c$, we obtain the predicted line shown in Fig. 12; this line does not differ significantly from those observed.

Nagel (1977) found a linear relation between ψ^{mc} and log $(Na)_m$ in frog skin, under open-circuit conditions, with a slope of 33 mV for a 10-fold change in $(Na)_m$, and Narvarte and Finn (1980*a*) reported changes in ψ^{mc} of only 11–23 mV for a 10-fold change in $(Na)_m$ in toad urinary bladder also studied under open-circuit conditions. These investigators expressed some concern over the difference between the observed slopes and the *expected* 58-mV slope. However, it is clear from Eq. (6) that:

(a) A linear relation with a 58-mV change in ψ^{mc} in response to a 10-fold change in $(Na)_m$ is to be *expected only* when (i) $(Na)_c$ is constant or independent of $(Na)_m$ *and* (ii) $I^m_{Na}\ R^m_{Na}$ (or I^m_{Na}/G^m_{Na}) is constant or zero. If these conditions are not met, the relation between ψ^{mc} and log $(Na)_m$ *may* be nonlinear and the slope(s) *will* be less than 58 mV.

(b) The finding of a 58-mV change in ψ^{mc} (or $_0\psi^{mc}$) in response to a 10-fold change in $(Na)_m$ does *not* imply that the barrier is permselective for Na to the exclusion of other ions. Such a relation would be obtained even if the apical membrane is significantly permeable to other ions, providing that these ions are passively distributed across that barrier and the conditions in (a) are met.

Conclusions

In the present study we have determined the "instantaneous" current-voltage relations of the transcellular and paracellular pathways and the apical membrane of *Necturus* urinary bladder when the steady-state rate of active Na transport, I_{sc}, was varied by varying the Na activity in the mucosal solution, $(Na)_m$.

The analysis of these data depends on three assumptions.

The first is that amiloride does not affect the resistance of the parallel (shunt) pathway, r^p, within 1–2 min following the addition of this agent to the mucosal perfusate. This assumption is supported by the findings that amiloride does not affect the serosa-to-mucosa fluxes of small ions (e.g., Na, K and Cl) that are believed to take place through pathways that parallel the amiloride-sensitive absorptive cells of other epithelia (Saito, Lief & Essig, 1974; Frizzell, Koch & Schultz, 1976; Finn & Bright, 1978). In addition, we have demonstrated in the present studies that amiloride has no effect on r_t when $(Na)_m = 0$.

The second assumption is that replacement of Na in the mucosal solution alone with TEA (or, in some instances, choline) does not affect r^p; evidence supporting this assumption was given in the *Methods* section. In addition, the findings that amiloride completely abolished the ψ^{ms} and I_{sc} in the presence of large transepithelial concentration gradients of Na and TEA and that r_t was not affected by replacing Na in the mucosal solution with TEA strongly suggests that the parallel pathways are either equally conductive to these ions, or more likely, that they are only sparingly permeable to them.

The third assumption is that there are no conductive pathways for ions other than Na across the apical membrane. This is not strictly true inasmuch as f' was significantly less than unity even when the cells were impaled from the serosal sur-

face. Thus as pointed out by Frömter and Gebler (1977) there must be a "natural" conductive pathway across the apical membrane for ion(s), *i*, other than Na; a similar conclusion was arrived at by Narvarte and Finn (1980b) for toad urinary bladder. Therefore, as discussed earlier by Thompson et al. (1982*a*), strictly speaking, $I^m_{Na} \neq I^{ms} - I^{ms\prime}$. However, inasmuch as f averaged 0.96 ± 0.01 the error introduced by employing Eq. (2) to calculate I^m_{Na} is small. Further, it can be readily shown that while any error introduced by assuming that $I^m_{Na} = \Delta I^{ms}$ may affect the *shape* of the *I-V* relation, particularly at large hyperpolarizing or depolarizing values of ψ^{mc}, it does not affect the value of the reversal potential, E^m_{Na}. That is, the value of ψ^{ms} at which $\psi^{mc} = E^m_{Na}$ (so that $I^m_{Na} = 0$) is *also* that value of ψ^{ms} at which amiloride has no effect on I^{ms} (Fig. 6); it follows that at that value of ψ^{ms}, any other transapical current (I^m_i) must be the *same* in the presence and absence of amiloride.

Thus, in the analysis of the present data we assumed that $I^m_{Na} = \Delta I^{ms}$ at any value of ψ^{ms}.

Properties of the Na Entry Step Across the Apical Membrane

As shown in Fig. 8, the relations between I^m_{Na} and ψ^{mc} conform closely to the GHK constant-field flux equation for a single ion over a wide range so that Na entry across the apical membrane can be attributed to simple electrodiffusion through homogeneous pores. This conformity was particularly striking in the presence of 45 mM Na where an excellent fit was observed over a range that exceeded 300 mV. The range of the fits were consistently less wide in the presence of 15 mM Na and still narrower in the presence of 5 mM Na. Two possible explanations may be offered for these departures. First, as discussed above, since $f \neq 1.0$, $I^m_{Na} \neq \Delta I^{ms}$. The error introduced by assuming that $I^m_{Na} = \Delta I^{ms}$ will be magnified in the presence of low mucosal Na activities since, at any value of ψ^{mc}, I^m_{Na} will be smaller so that I^m_i will account for a larger fraction of the transapical current. This will be particularly true at large hyperpolarizing or depolarizing values of ψ^{mc} that may be far removed from E^m_i.[4]

Another possibility is that Na movement across the apical membrane does not strictly conform to the "independence principle" (Hodgkin & Huxley, 1952) which underlies the derivation of the GHK equation; indeed, as discussed by Hille (1975) strict "independence" is unlikely in any *real* biological channel. Departures from independent flows will be manifested by saturating currents at large hyperpolarizing or depolarizing potentials but reasonable conformity with the GHK equation may be observed at smaller potentials (Schultz, 1980). If this is the explanation, however, it is not immediately apparent why such excellent conformity was observed in the presence of 45 mM Na over a very wide range.

Thompson et al. (1982*a*) demonstrated that the relation between I^m_{Na} and ψ^{mc} in rabbit colon also conforms to the GHK equation over a wide range. Frömter et al. (1981) reported similar findings for *Necturus* urinary bladder in the presence of 109 mM Na, although these authors pointed out that the fit "... was less good particularly in the range beyond the reversal potential." Finally, Lindemann and co-workers have reported that Na entry across the apical membrane of frog skin (Fuchs et al., 1977) and toad urinary bladder (Palmer et al., 1980; Li et al., 1982) conforms to the GHK equation using preparations in which the electrical contribution of the inner or basolateral membranes was minimized or abolished by exposing these surfaces to a high-K bathing solution.

Thus, it seems safe to conclude that this is a general property of amiloride-sensitive apical Na channels.

Thompson et al. (1982*a*) have also shown that in rabbit colon there is a linear relation between the *chord* conductance of the apical membrane to Na when the tissue is short-circuited ($_0G^m_{Na}$) and spontaneous variations in the short-circuit current in the presence of 140 mM Na. As pointed out by these investigators, this means that the electrochemical potential difference for Na across the apical membrane (i.e., the thermodynamic driving force for Na entry) is constant and that the spontaneous variations in I_{sc} are attributable entirely to changes in the conductance of the entry mechanism. As pointed out by Schultz (1981) linear relations between the *slope* conductance of the apical membrane and I_{sc} can be derived from published data dealing with frog skin and *Necturus* urinary bladder; and Clausen, Lewis and Diamond (1979) derived a linear relation between the *slope* conductance of the apical membrane and I_{sc} in rabbit urinary bladder from AC impedance analysis of the tissue. It should be emphasized, however, that while g^m should, in general, parallel G^m, it is the

[4] The ionic pathway(s) responsible for the small "residual" apical conductance in the presence of amiloride is (are) unknown. One possibility is that the action of amiloride is incomplete so that a small $_0g^m_{Na}$ persists. The other major possibilities are conductive pathways for K and/or Cl. The former seems unlikely on the basis of the present studies and the results recently reported by Palmer (1982). Narvarte and Finn (1980*b*) have presented evidence for a Cl conductance in the apical membrane of toad urinary bladder.

latter that *directly* relates the flow of an ion to its thermodynamic driving force.[2]

An unexpected finding that emerged from these studies is the linear relation between I_{sc} and ${}_0G^m_{Na}$ (Fig. 10) when I_{sc} is varied by varying $(Na)_m$. Under these conditions *both* E^m_{Na} *and* ${}_0\psi^{mc}$ are functions of $(Na)_m$ (Table 1) *but* $(\Delta\, {}_0\tilde{\mu}^m_{Na}/\mathcal{F}) = ({}_0E^m_{Na} - {}_0\psi^{mc})$ *remains constant* at an average value of 48 mV. Thus, even under these conditions, the thermodynamic driving force for Na entry is, somehow, maintained constant and the rate of entry is determined *solely* by the conductance of the entry step.

We now ask: "What is the mechanism responsible for the increase in ${}_0G^m_{Na}$ with increasing $(Na)_m$?"

As shown in Fig. 11, P^m_{Na} decreases with increasing $(Na)_m$. Further, inasmuch as $(Na)_c$ remains essentially constant under these conditions, the decrease in P^m_{Na} (recall that it is not *directly* dependent on ψ^{mc}) must be related in some way to the increase in $(Na)_m$. This "self-inhibition" effect of $(Na)_m$ on P^m_{Na} has been reported by Lindemann and coworkers for frog skin (Fuchs et al., 1977; Lindemann & Voute, 1976) and toad urinary bladder (Li et al., 1982). Further, fluctuation analysis of the Na-entry step across the apical membrane of frog skin when the outer bathing solution contained 6–60 mM Na indicated that increasing $(Na)_m$ *decreases the number of open or active channels* in a hyperbolic fashion but, at the same time, the single-channel current and conductance increased linearly with increasing $(Na)_m$ (van Driessche & Lindemann, 1979). It should be noted, again, that these studies were carried out on a preparation in which the electrical contributions of the basolateral (inner) membranes were presumably minimized or eliminated by exposing those barriers to a high-K solution.

The present results are consistent with the notion that the decrease in P^m_{Na} with increasing $(Na)_m$ is the result of a decrease in the number of open or active, amiloride-inhibitable Na channels which, in turn, is responsible for the saturation of I_{sc} and ${}_0G^m_{Na}$ with increasing $(Na)_m$ (Fig. 13). At the same time, the preparation employed in our studies is considerably more complicated than those studied by Lindemann et al. inasmuch as the apical membrane is *not* voltage-clamped (presumably at zero) but, instead, ${}_0\psi^{mc}$ is a strong function of $(Na)_m$ (Fig. 12). This point is important in view of the recent evidence suggesting that cell Ca is, somehow, directly responsible for the "negative feedback" of $(Na)_c$ on P^m_{Na} (Chase & Al-Awqati, 1981; Taylor, 1981). According to this notion, cytoplasmic free Ca, $(Ca)_c$, is regulated, at least in part, by a Na-Ca countertransport mechanism located at the basolateral membranes whereby the downhill movement of Na into the cell energizes Ca extrusion from the cell; direct evidence for such a mechanism has been provided by Hildmann et al. (1982) for small intestine and Chase and Al-Awqati (1981) for toad urinary bladder. Consequently, an increase in $(Na)_c$ would decrease the electrochemical driving force for the downhill movement of Na and result in an increase in the steady-state level of $(Ca)_c$. In the present studies, while $(Na)_c$ did not vary significantly with changes in $(Na)_m$, ${}_0\psi^{mc}$ or $-{}_0\psi^{cs}$ did; the cell interior became progressively less negative with increasing $(Na)_m$ (Fig. 12). Inasmuch as the Na-Ca countertransport mechanism is rheogenic, presumably involving an exchange of 3 Na for each Ca (Blaustein, 1974; Mullins, 1981), this change in ${}_0\psi^{mc}$, alone, would lead to a decrease in the electrical driving force for the Na-Ca exchange mechanism and, possibly, an increase in $(Ca)_c$. Thus, in spite of the compelling evidence for "self-inhibition" of $(Na)_m$ on P^m_{Na} derived from studies on what is, presumably, electrophysiologically a "single membrane system," the issue, as yet, cannot be resolved with certainty for intact cell systems.

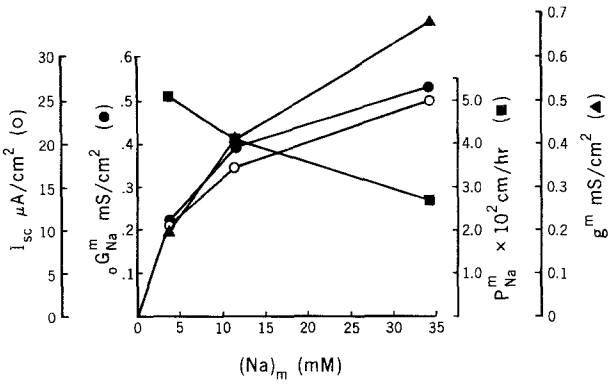

Fig. 13. Relations among $(Na)_m$ and I_{sc} (○), ${}_0G^m_{Na}$ (●), P^m_{Na} (■) and ${}_0g^m$ (▲). Note that I_{sc} and ${}_0G^m_{Na}$ increase hyperbolically with increasing $(Na)_m$ and approach a maximum while P^m_{Na} decreases. As discussed in the test, these findings are consistent with the notion that the single-channel conductance of open channels increases with increasing $(Na)_m$ while the number of open channels, as reflected by the P^m_{Na}, decreases

In short, we cannot conclude from the results of the present studies that the decrease in P^m_{Na} with increasing $(Na)_m$ is due to a direct effect of the Na in the mucosal solution on the entry mechanism.

The "Sodium Transport Pool"

One of the unstated, but strongly suggested, implications of the Koefoed-Johnsen-Ussing double

membrane model of Na-transporting epithelia is that $(Na)_c$ or the "intracellular Na transport pool" is the "line of communication" between the apical and basolateral membranes; that is, an increased rate of Na entry across the apical membrane leads to an increase in $(Na)_c$ which, in turn, leads to an increase in "pump rate" at the basolateral membrane (and *vice versa*). Indeed this notion, which at first glance is intuitively attractive, became "the conventional wisdom," and many efforts have been made to identify this "pool" and relate its size to the rate of transcellular Na transport (*cf.* Macknight & Leaf, 1978; Macknight et al., 1980).

One of the somewhat surprising results of the present study is the finding that there is *no* significant relation between $(Na)_c$ and either $(Na)_m$ or, more important, I_{sc} when the former varies over a ninefold range and the latter varies over a two- to threefold range. Frömter, Higgins and Gebler (1981) reported a value of 5–6 mM for $(Na)_c$ in open-circuited *Necturus* urinary bladder in the presence of 109 mM Na; combining their results with ours suggests that $(Na)_c$ is invariant over the range of mucosal Na concentrations from 5–109 mM. Further, as discussed in the Appendix, where we summarize the data obtained on "low Na-transporting bladders," the value of $(Na)_c$ appears to be independent of I_{sc} over a tenfold range.

There are relatively few studies dealing with the relation between $(Na)_m$ and $(Na)_c$ in epithelia where Na-entry is mediated by an amiloride-sensitive channel. $(Na)_c$ has been found to increase hyperbolically with increasing $(Na)_m$ in toad urinary bladder (Palmer et al., 1982) and rabbit descending colon (K. Turnheim, S.M. Thompson and S.G. Schultz, *unpublished*); but, in these studies, $(Na)_c$ was calculated from the reversal potentials determined from *I-V* relations of tissues exposed to a high-K serosal solution.[5]

There are also relatively few studies dealing with the relation between $(Na)_c$ and the rate of active Na transport by epithelia. Eaton (1981) has reported an increase in $(Na)_c$ in open-circuited rabbit urinary bladder following treatment with aldosterone, and there was a sigmoidal relation between $(Na)_c$ and I_{sc} in these tissues as well as among tissues with spontaneously varying rates of Na absorption. On the other hand, Wills and Lewis (1980) found that $(Na)_c$ in rabbit urinary bladders of control animals and animals maintained on a low-Na diet did not differ significantly and averaged 6–7 mM in spite of the fact that the I_{sc} of animals maintained on the low-Na diet was twice that of control animals. Further, Palmer et al. (1982) and Li et al. (1982) found small effects of aldosterone and antidiuretic hormone on $(Na)_c$ of toad urinary bladder in spite of the fact that these agents brought about marked increases in P_{Na}^m and I_{sc}. Finally, Lee and Armstrong (1972) found that the addition of 3-O-methylglucose to the solution bathing the mucosal surface of frog small intestine results, if anything, in a small *decrease* in $(Na)_c$ in spite of the fact that there is a significant increase in the rate of active Na absorption.

Clearly, additional studies are needed to clarify this important issue and resolve apparent conflicts. Although there is no doubt that an increase in $(Na)_c$ will result in an increase in pump activity (Graf & Giebisch, 1979; Lewis & Wills, 1981; Turnheim, Thompson & Schultz, *unpublished*), it seems equally clear that pump activity can increase markedly in the *absence* of a significant increase in $(Na)_c$.

Given the present results, which were obtained during a *single* impalement of a cell subjected to *three* different steady-state conditions, the obvious question that arises is: What *signals* the basolateral pump to increase its activity (i.e., the pump rate as given by the I_{sc}) with increasing $(Na)_m$?

Three possibilities should be considered.

First, it is possible that the relation between $(Na)_c$ and I_{sc} is so steep that a small increase in $(Na)_c$, below the level that can be established with statistical confidence, can result in a many-fold increase in I_{sc}. Lewis and Wills (1981) and Eaton (1981) have reported sigmoidal relations between $(Na)_c$ and I_{sc} in rabbit urinary bladder and a similar relation has been observed in rabbit colon (Turnheim, Thompson & Schultz, *unpublished*); but, these data cannot be readily reconciled quantitatively with our findings.

Second, in recent years a reasonably compelling body of evidence has accrued suggesting that the Na-K exchange pumps at the basolateral membranes of epithelial cells are non-neutral or rheogenic (*cf.*, Schultz, 1981) as appears to be the case for a number of nonepithelial cells (Thomas, 1972). If so, the basolateral pump rate *must* be influenced by the electrical potential difference across that barrier over some, as yet undefined, range (*cf.*

[5] Preliminary results obtained by R. Thomas, S. Thompson and S.G. Schultz in this laboratory suggest that $(Na)_c$ increases with increasing $(Na)_m$ in short-circuited *Necturus* urinary bladder *when the basolateral membrane is exposed to a high-K serosal solution*. Thus, it is possible that exposure of the tissue to a high-K serosal solution impairs the mechanism(s) responsible for maintaining $(Na)_c$ constant in the "electrically intact" cell under short-circuit conditions.

Hansen, Gradmann, Sanders & Slayman, 1981). The data given in Table 1 and illustrated in Fig. 5 indicate that there is an inverse relation between ${}_0\psi^{cs}$ (or $-{}_0\psi^{mc}$) and pump activity (or I_{sc}); these observations raise the possibility that the increase in pump rate with increasing $(Na)_m$ is due to the concomitant decrease in the electrical potential energy barrier that the rheogenic pump must overcome. However, in order to test this possibility the *I-V* relation of the basolateral Na-K pump must be determined; this has not been accomplished for any epithelium.

Finally, one cannot exclude the possibility that the increase in I_{sc} in response to an increase in $(Na)_m$ is the result of the *recruitment* of additional pumps in the basolateral membrane so that total pump activity can increase in the presence of the same $(Na)_c$. This notion will be discussed further below.

Properties of the Basolateral Membrane

One of the important findings of this study is that the conductance of the basolateral membrane under short-circuit conditions (${}_0g^s$) is a linear function of I_{sc} and increases, almost in parallel, with the conductance of the apical membrane (Fig. 4). Higgins et al. (1977) arrived at the same conclusion from studies on open-circuited *Necturus* urinary bladders whose rates of active Na transport in the presence of 109 mM Na varied spontaneously. In this respect, the present results are somewhat more compelling inasmuch as the relation between ${}_0g^s$ and I_{sc} was observed in the *same tissue during a single impalement*.[6] Narvarte and Finn (1980*a*) have reported a direct relation between g^s and $(Na)_m$ in toad urinary bladder (I_{sc} was not determined in those studies) and Davis and Finn (1982) have observed a decrease in g^s in toad and frog urinary bladder in the presence of amiloride that can be attributed to a decrease in the K permeability of that barrier. Further, a direct relation between ${}_0g^s$ and I_{sc} can be derived from the data reported by Helman and Fisher (1977) on isolated frog skin (Schultz, 1981). Finally, Gunter-Smith, Grasset and Schultz (1982) have demonstrated an increase in ${}_0g^s$ of *Necturus* small intestine that parallels the increase in the rate of Na absorption elicited by the addition of actively transported sugars or amino acids to the mucosal solution and, more recently, Grasset, Gunter-Smith and Schultz (1983) provided evidence that this is due, primarily if not entirely, to an increase in the conductance of the basolateral membrane to K, G^s_K.

Thus, it seems quite clear that, at least in some tissues,[7] an increase in basolateral pump activity is associated directly or indirectly with an increase in G^s_K and *vice versa*. As discussed previously (Schultz, 1981; Grasset et al., 1983; Gunter-Smith et al., 1982) this homocellular regulatory mechanism serves to prevent an inordinate increase in cell K with increasing activity of the basolateral Na-K exchange pump. In addition, in "leaky epithelia" (where $r^p \ll r^c$) or in "tight epithelia" under short-circuit conditions (which is equivalent to setting $r^p = 0$) an increase in G^s_K would tend to hyperpolarize the cell interior (more negative) and thus preserve the electrical driving force for any conductive Na entry process (Schultz, 1981).

The mechanism responsible for the increase in G^s_K with increasing I_{sc} is unknown. However, there are two *general* possibilities that should be considered.

The first is that the conductance of a *fixed number of pre-existing* basolateral K channels is increased. Two possible mechanisms that could bring about such an increase are:

(a) If the relation between the diffusional current of K across the basolateral membrane, I^s_K, and the electrical potential difference across that barrier, ψ^{cs}, conforms to the GHK flux equation, then G^s_K will be dependent upon ψ^{cs} and will increase as ψ^{cs} decreases. As shown in Figs. 5 and 12, ψ^{cs} does, in fact, decrease with increasing $(Na)_m$ and, thus, I_{sc}. However, in the studies reported by Higgins et al. (1977), which were carried out under open-circuit conditions, an increase in g^s was observed when ψ^{cs} remained essentially constant. Further, in the studies by Gunter-Smith et al. (1982) and Grasset et al. (1983) the increase in G^s_K associated with an increase in I_{sc} cannot be attributed simply to the direct result of changes in ψ^{cs}. Thus, while the direction of the change in ψ^{cs} with increasing $(Na)_m$ and I_{sc} favors an increase in G^s_K, this may be a contributory but, at the same time, is an insufficient explanation. Clearly, further studies are needed to resolve this issue definitively, including studies of the *I-V* relation of the basolateral K leak pathway.

[6] A possible explanation for the findings of Higgins et al. (1977) is that spontaneous variations in I_{sc} are due to parallel variations in the *number* of Na-absorbing cells per unit area of bladder; this would result in a constant f while g^c and I_{sc} vary. This explanation could not reasonably apply to the present observations.

[7] There does not appear to be a direct relation between I_{sc} and ${}_0g^s$ in rabbit colon (Thompson et al., 1982b) and rabbit urinary bladder (Lewis, Eaton & Diamond, 1976). The explanation for this apparent difference between these two "mammalian epithelia" and "amphibian epithelia" is obscure.

b) In recent years it has become clear that cell Ca is an important physiological mediator (or activator) of membrane K conductance in a number of cell systems (the so-called "Gardos effect") (Lew & Ferreira, 1979). If *Necturus* urinary bladder possesses a rheogenic Na-Ca countertransport mechanism at the basolateral membrane, then, as discussed above, an increase in $(Na)_c$ and/or a decrease in ${}_0\psi^{cs}$ could result in an increase in $(Ca)_c$. This, in turn, may be responsible for an increase in G_K^s in response to increasing $(Na)_m$ and I_{sc}.

The second *general* possibility is that the increase in G_K^s with increasing I_{sc} is the result of the recruitment or accretion of *additional* ("new") active pump-leak pathways into the basolateral membrane. This possibility, which was raised by Higgins et al. (1977), is particularly attractive in the light of the findings that (a) there is an increase in I_{sc} with increasing $(Na)_m$ in spite of the fact that $(Na)_c$ does not increase significantly; and (b) the increase in ${}_0g^s$ (presumably G_K^s) is proportional to the increase in I_{sc}. This, *the single explanation* that could most easily account for these two findings is that the increases in I_{sc} and ${}_0g^s$ are the results of an increase in the number of functional "pump-leak units" in the basolateral membrane. This could occur in two ways:

(a) An increase in active Na transport may be associated with cell-swelling, resulting in an expansion of the basolateral membrane and the exposure of previously unexposed ("infolded") portions of this barrier to the serosal solution. This explanation is not very attractive inasmuch as it would require a near twofold expansion of the "effective" basolateral membrane area; but, it cannot be excluded.

b) An increase in active Na transport resulting from an increase in the rate of Na entry across the apical membrane may be associated with the insertion of new (active) "Na-K pump and K-leak units" into the basolateral membrane. There is evidence that the insertion of intracellular membranous elements into the apical membrane is involved in the hydro-osmotic response of toad urinary bladder to ADH (Muller et al., 1980; Wade et al., 1981); the stimulation of proton secretion by gastric parietal cells (Forte et al., 1977) and turtle urinary bladder (Gluck et al., 1982); and, Na absorption by rabbit urinary bladder (Lewis & deMoura, 1982; Lewis, 1982). The possibility that similar events are responsible for an increase in pump-leak activity at the basolateral membrane in response to an increase in the Na entry cannot be dismissed at this time and is certainly worthy of further investigation.

Finally, the notion that there may be a direct relation between Na entry across the apical membrane and Na pump activity at the basolateral membrane derives some support from the recent findings of Petty, Kokko and Marver (1981). These investigators found that the Na-K ATPase activity of isolated rabbit renal cortical collecting tubules is markedly decreased following adrenalectomy and can be restored to normal, within 3 hr, by injection of physiological doses of aldosterone. However, pretreatment of the adrenalectomized animals with amiloride blocked the increase in Na-K ATPase activity following the injection of aldosterone. Thus, the increase in Na-K ATPase activity appears to be a secondary response to an aldosterone-induced increase in the rate of Na entry into the cell through newly recruited channels (Palmer et al., 1982). These intriguing findings suggest a direct relation between Na entry across the apical membrane and the *number* of Na-K pumps at the basolateral membrane which could account for the increases in overall pump *activity* in the absence of increases in $(Na)_c$ observed in the studies of Wills and Lewis (1980). It should be emphasized, however, that in the studies of Petty et al. (1981) an increase in Na-K ATPase activity was observed 2–3 hr after the administration of aldosterone; it is not clear whether this is peculiar to mineralocorticoid-stimulated Na absorption and, if not, how quickly it can occur in response to an increase in the rate of Na entry from other causes.

In short, there may be a "cross-talk" between the rate of Na entry across the apical membrane and the activities of the Na-K pump and K-leak at the basolateral membrane such that wide variations in the rate of active, transcellular Na transport need not be accompanied by major changes in cell Na and K composition. If so, the signal(s) that link Na entry rate to basolateral pump and leak activities remain to be defined.

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Appendix

During the course of these studies we encountered a group of animals that displayed spontaneously low rates of active Na transport but otherwise did not differ in appearance or activity from the *Necturi* used in the studies reported above. In each instance the relations between I_{Na}^m and ψ^{mc} conformed to the GHK equation over at least a 100-mV range.

Table A1. Effects of mucosal Na on electrophysiologic properties of *Necturus* urinary bladder ("low transporters")

$(Na)_m$	r_t	r^p	f	${}_0g^m$	${}_0g^s$	${}_0\psi^{mc}$	I_{sc}	$(Na)_c$	${}_0G^m_{Na}$	P^m_{Na}	${}_0\psi^{mc}$	f'
7.6 (4)	7,405	10,073	0.93	0.04	0.51	−44	1.9	6.1	0.03	0.35	−55	0.96
SEM	926	1,381	0.02	0.00	0.08	4	0.6	0.7	0.01	0.07	6	0.01
15.2 (7)	6,149	8,989	0.91	0.05	0.60	−36	2.6	5.9	0.04	0.42	−51	0.96
SEM	940	1,450	0.02	0.01	0.07	2	0.4	0.8	0.01	0.04	1	0.01
41.8 (5)	5,998	9,619	0.92	0.07	0.77	−20	3.4	6.5	0.05	0.22	−51	0.96
SEM	1,030	2,031	0.01	0.01	0.07	6	0.5	2.1	0.01	0.03	3	0.01

See Table 1 for definition of symbols and units.

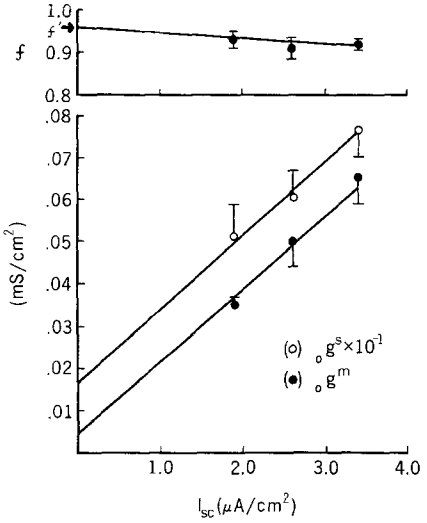

Fig. A1. Relations among I_{sc} and f, ${}_0g^s$ (o) and ${}_0g^m$ (●)

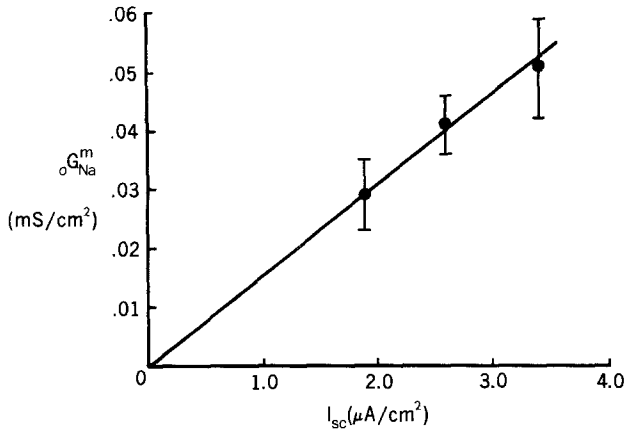

Fig. A2. Relation between I_{sc} and ${}_0G^m_{Na}$

It is of interest to compare, briefly, the data obtained on these "low Na-transporters" with those presented above.

Table A1 summarizes these findings in the presence of 10, 20 and 50 mM Na in the mucosal solution. Clearly the values of I_{sc} of the these "low Na-transporters" are approximately an order of magnitude less than those reported in Table 1, and, at the same time ${}_0g^m$, ${}_0G^m_{Na}$ and P^m_{Na} are also approximately an order of magnitude lower than those values given in Table 1.

The relations among f, ${}_0g^m$ and ${}_0g^s$ and the I_{sc} are plotted in Fig. A1 these relations resemble those illustrated in Fig. 4. The relation between ${}_0G^m_{Na}$ and I_{sc} is plotted in Fig. A2; clearly there is a linear relation indicating that the changes in I_{sc} with increasing $(Na)_m$ are attributable entirely to changes in ${}_0G^m_{Na}$; the electrochemical potential difference across the apical membrane is maintained constant at a value of approximately 65 mV.

Finally, the values of $(Na)_c$ determined from the "reversal potentials" of the relations between P^m_{Na} and ψ^{mc}, given in Table A1, are independent of $(Na)_m$ and I_{sc} and averaged approximately 6 mM, a value in excellent agreement with that found in "spontaneously high-transporters."

Thus, the primary difference between the spontaneously "low" and "high" Na-transporters appears to reside in the conductance of the apical membrane to Na. The lower values of ${}_0g^s$ observed in "low" transporters are entirely consistent with the relation between ${}_0g^s$ and I_{sc} discussed in the main body of this paper. Finally, the finding that $(Na)_c$ is the same in "low" and "high" transporters supports the notion that pump activity is not necessarily dependent upon parallel changes in $(Na)_c$; indeed, combining these studies indicates that pump activity can increase 10-fold in the presence of a constant $(Na)_c$.

As discussed in Footnote 6, one possible explanation for these findings is that the spontaneously "low transporters" simply have fewer active, Na absorbing cells per unit tissue area with each cell identical to those found in the "high transporters." Assuming that the "low" and "high" transporters have the same number of functioning absorptive cells per unit area, these results suggest that the limiting factor in transcellular Na transport is the ease with which Na can enter the cell across the apical membrane driven by a constant electrochemical potential difference and that the activity of the Na-K pump and the K-leak at the basolateral membrane are "geared" to this entry rate.

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