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Electrophysiology of *Necturus* Urinary Bladder: II. Time-Dependent Current-Voltage Relations of the Basolateral Membranes

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Summary. As reported previously (S.R. Thomas et al., *J. Membrane Biol.* **73**:157–175, 1983) the current-voltage (*I-V*) relations of the Na-entry step across the apical membrane of short-circuited *Necturus* urinary bladder in the presence of varying mucosal Na concentrations are (i) time-independent between 20–90 msec and (ii) conform to the Goldman-Hodgkin-Katz constant field flux equation for a single cation over a wide range of voltages.

In contrast, the *I-V* relations of the basolateral membrane under these conditions are (i) essentially linear between the steady-state, short-circuited condition and the reversal potential (E^s); and (ii) are decidedly time-dependent with E^s increasing and the slope conductance, g^s, decreasing between 20 and 90 msec after displacing the transepithelial electrical potential difference. Evidence is presented that this time-dependence cannot be attributed entirely to the electrical capacitance of the tissue.

The values of g^s determined at 20 msec are linear functions of the short-circuit current, I_{sc}, confirming the relations reported previously, which were obtained using a more indirect approach.

The values of E^s determined at 20 msec are significantly lower than any reasonable estimate of the electromotive force for K across the basolateral membrane, indicating that this barrier possesses a significant conductance to other ions which may exceed that to K. In addition, these values increase linearly with decreasing I_{sc} and approach the value of the electrical potential difference across the basolateral membrane observed when Na entry across the apical membrane is blocked with amiloride or when Na is removed from the mucosal solution.

A possible explanation for the time-dependence of E^s and g^s is offered and the implications of these findings regarding the interpretation of previous microelectrophysiologic studies of epithelia are discussed.

Key Words current-voltage relations · basolateral membranes · *Necturus* urinary bladder · membrane capacitance · time-dependent *I-V* relations

Introduction

In recent years, electrophysiological studies have provided considerable insight into the properties of the Na-entry step across the apical membranes of several Na-absorbing epithelia; e.g. frog skin (Fuchs, Larson & Lindemann, 1977; Lindemann & van Driessche, 1977; Van Driessche & Lindemann, 1979), toad urinary bladder (Palmer, Edelman & Lindemann, 1980, 1982; Li, Palmer, Edelman & Lindemann, 1982; Garty, Edelman & Lindemann, 1983), rabbit descending colon (Thompson, Suzuki & Schultz, 1982; Turnheim, Thompson & Schults, 1983), and *Necturus* urinary bladder (Frömter, Higgins & Gebler, 1981; Thomas, Suzuki, Thompson & Shultz, 1983). The results of these studies are consistent with the notion that Na entry into the absorbing cells conforms to the Goldman-Hodgkin-Katz (GHK) (Goldman, 1943; Hodgkin & Katz, 1949) "constant-field flux equation" over a reasonable range of electrical potential differences and can be attributed to simple electrodiffusion through pores or channels.[1] This conclusion is supported by the findings that the bidirectional fluxes of Na across the apical membranes of frog skin (Benos, Hyde & Latorre, 1983) and toad urinary bladder (Palmer, 1982) conform reasonably well with the Ussing flux-ratio equation (Ussing, 1949) for simple electrodiffusion uncomplicated by single-filing, exchange-diffusion, etc. These bidirectional Na fluxes thus conform to the "independence principle" which underlies the GHK flux equation (*cf.* Schultz, 1980).

In contrast, the properties of the basolateral membranes of these epithelia have not been exam-

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[1] The microscopic structure of pores consistent with the GHK flux equation has recently been considered by Lindemann (1982).

ined extensively and are, as yet, poorly understood. Wills, Eaton, Lewis and Ifshin (1979) determined the current-voltage (*I-V*) relations of the basolateral membranes of rabbit colon but only after disrupting the apical membranes with nystatin, so that it is not clear whether their results apply only to the intact *Na-absorbing* cells of this multicellular epithelium. Lewis, Wills and Eaton (1978) have estimated the permselective properties of the basolateral membrane of rabbit urinary bladder, but these estimates are based on the assumptions that this barrier is permeable only to Na, K and Cl and that the diffusional movements of these ions across that barrier conform to the GHK equation; there is no direct evidence supporting those assumptions. In short, we are unaware of any direct studies of the *I-V* relations of the basolateral membranes of intact Na-absorbing cells that might permit an evaluation of the transport properties of those barriers.

Between April 1981 and January 1982, a series of studies on the *I-V* relations of *Necturus* urinary bladder employing intracellular microelectrodes was carried out in this laboratory. These studies enabled us to obtain the *I-V* relations of both the apical and basolateral membranes of the Na-absorbing cells as well as that of the pathways that parallel those cells. The results dealing with the electrophysiologic properties of the apical membrane have been published (Thomas et al., 1983). This paper will deal primarily with the *I-V* relations of the basolateral membrane; but, for reasons that will become obvious, data dealing with the time-dependence of the apical and the paracellular (parallel) *I-V* relations will also be presented and discussed.

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 (μA/cm^2).
I_{sc}	Short-circuit current or ${}_0I^{ms}$ (μA/cm^2).
E	Equivalent electromotive force or zero-current potential (mV).
I	Current (μA/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	$\equiv r^m/(r^m + r^s)$.
C	Effective membrane capacitance uncorrected for actual membrane area (μF/cm^2).

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	Preceding a term indicates the value of that term when $\psi^{ms} = 0$ (e.g., ${}_0I^m_{Na}$).
ψ	Values obtained at a given value of ψ^{ms}.

Materials and Methods

The methods employed were described in detail by Thomas et al. (1983). Briefly, after impaling a *Necturus* urinary bladder cell from either the apical (m) or the serosal (s) surface under short-circuit conditions, sufficient current was passed across the tissue by means of a computer-driven voltage clamp to clamp the transepithelial electrical potential difference (ψ^{ms}) over the range 0 to ±200 mV in 20-mV increments (i.e., 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. The intracellular electrical potential with respect to the mucosal solution (ψ^{mc}), ψ^{ms} and the clamping-current, I^{ms}, were monitored at 20 msec and again at 90 msec and relayed to the computer via an *A-D* converter for storage and processing. These *I-V* relations were determined during a single impalement when the mucosal bathing solution contained 5, 15 or 45 mM Na and finally in the presence of a maximally effective concentration of amiloride (10^{-4} M). Subtracting the transepithelial clamping current in the presence of amiloride at any value of ψ^{ms}, $(I^{ms'})_{\psi^{ms}}$, from the current in the absence of amiloride at *that* value of ψ^{ms}, $(I^{ms})_{\psi^{ms}}$, yields the transcellular current at *that* value of ψ^{ms}, $(I^c)_{\psi^{ms}}$. The latter is assumed to be equivalent to the Na current across the apical membrane $(I^m_{Na})_{\psi^{ms}}$ as well as the *total* current across the basolateral membrane $(I^s)_{\psi^{ms}}$. Since ψ^{mc} and the electrical potential difference across the basolateral membrane, ψ^{cs}, are also known at any value of ψ^{ms}, it is a simple matter to derive the relations between I^m_{Na} and ψ^{mc} as well as those between I^s and ψ^{cs} under the different conditions studied.

The reader is referred to our earlier paper for experimental details and a discussion and justification of the underlying assumptions (Thomas et al., 1983).

Results and Discussion

Previously (Thomas et al., 1983) we presented the *I-V* relations of the apical membrane and parallel pathway(s) determined 16–20 msec after the onset

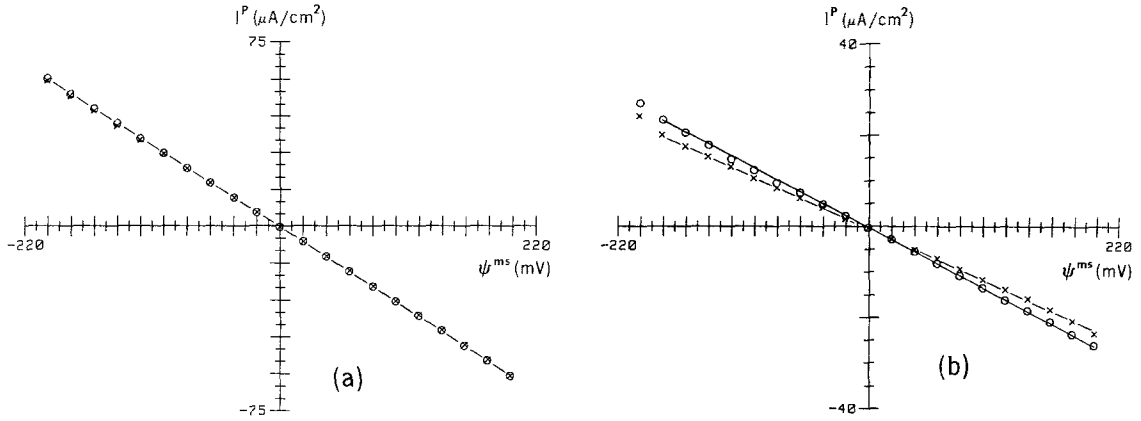

Fig. 1. Examples of the relation between I^p and ψ^{ms} at 20 (○) and 90 (×) msec. (*a*): r^p = 3.3 kΩ cm²; (*b*): r^p increased from 7.7 to 8.6 kΩ between 20–90 msec

of the clamping-current pulse and noted that these relations did not differ significantly from those observed 90 msec after the onset of the pulse (top, p. 159). For reasons that will shortly become evident, we will briefly document and elaborate upon these points.

I-V Relations of the Parallel Pathway(s)

Two examples of the relation between $I^{ms'}$ or (I^p) and ψ^{ms} are illustrated in Fig. 1. In all instances these relations were linear over the range ± 200 mV. Further, in most cases the relations observed at 20 and 90 msec were superimposable (Fig. 1*a*); in some instances there was a small *increase* in the resistance of the parallel pathway(s), r^p, between 20 and 90 msec (Fig. 1*b*); in no instance was there a decrease in r^p with time.

In the experiments carried out in the spring of 1981, r^p averaged 5–6 kΩ cm², whereas in the experiments carried out in the fall–winter of 1981 r^p averaged 14 kΩ cm². The most probable explanation for this difference is seasonal variation, which has been frequently observed in this species. A less likely, but possible, explanation is that the difference is due to the fact that different investigators performed these experiments employing slightly different chambers and mounting techniques.[2]

The results of studies on frog skin (Mandel & Curran, 1972) and toad urinary bladder (Bindslev, Tormey, Pietras & Wright, 1974; Bobrycki, Mills, Macknight & DiBona, 1981) indicate that voltage-clamping can result in an increase in the permeability of paracellular pathways and marked alterations in the morphology ("blistering") of the "tight" junctions. The results of the present studies indicate that voltage-clamping *Necturus* urinary bladder over the range ± 200 mV for 90 msec does not significantly affect the conductance of these pathways and whenever small effects were noted they were in the direction of an increase in r^p.

The I-V Relations of the Apical Membrane

The relations between I^m_{Na} and ψ^{mc} at 20 and 90 msec after the onset of the clamping currents were most often superimposable; two examples are illustrated in Fig. 2. Small differences were sometimes noted when $\psi^{mc} < -75$ mV, but in every instance: (i) the relation conformed closely to the GHK flux equation over, at least, a 100-mV range; and, (ii) the permeability of the apical membrane to Na, P^m_{Na}, and the intracellular Na activity, $(Na)_c$, determined from a least-squares, nonlinear regression analysis of the *I-V* relations at 20 and 90 msec after the onset of the clamping-current, did not differ significantly.[3]

[2] It should be noted that in a small series of preliminary studies r^p was not affected by replacing Na in the bathing media with K, choline, tetramethylammonium, or tetraethylammonium. On the other hand, replacing Cl in the bathing solutions with gluconate (two experiments) resulted in a significant increase in r^p. These findings suggest that: (i) either the parallel pathway(s) are equally permeable to a variety of cations with considerably different hydrated radii or, more likely, that they are only sparingly permeable to these cations; and (ii) these pathways are significantly permeable to Cl.

[3] By the use of the word "superimposable" we do not wish to infer that the values of ψ^{mc} at a given ψ^{ms} were the *same* at 20 and 90 msec. As discussed in the Appendix, since ψ^{ms} is constant over this time period but ψ^{cs} changes, ψ^{mc} must also change equally but in an opposite direction. The important point is that the *relation* between I^m_{Na} and ψ^{mc} determined at 20 and 90 msec did not differ so that the *properties* of the apical membrane that determined that relation appear to be time-independent over that period.

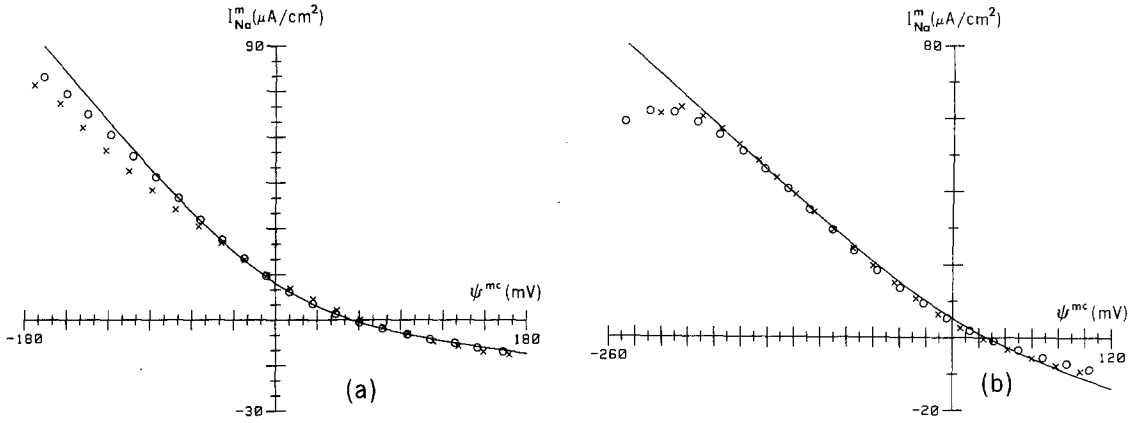

Fig. 2. Examples of the relations between I^m_{Na} and ψ^{mc} at 20 (○) and 90 (×) msec. The solid curves correspond to the GHK equation

Thus, the *properties* of the system that determine the *I-V* relations of the Na entry step across the apical membrane and the conductive pathways that parallel that barrier appear to be essentially time-independent between 20–90 msec.

The I-V Relations of the Basolateral Membrane

Because the resistance of the apical membrane accounts for 80–90% of the total transcellular resistance, the relations between I^s and ψ^{cs} could only be obtained over a relatively narrow range of voltages, particularly in the presence of 5 mM Na when f was generally greater than 0.85. Nonetheless, we could always obtain the relation between I^s and ψ^{cs} over the range ($\psi^{cs} = {}_0\psi^{cs}$, $I^s = I_{sc}$) to ($\psi^{cs} = E^s$, $I^s = 0$) (*see* Fig. 7*c*). Typical examples of these *I-V* relations when the Na *activities* in the mucosal solution, $(Na)_m$, were 3.8, 11.4 or 34.2 mM are illustrated in Fig. 3.

Clearly, the *I-V* relations of the basolateral membrane differ from those of the Na entry step across the apical membrane in two respects.

First, they are essentially linear over the range $\psi^{cs} = {}_0\psi^{cs}$ to $\psi^{cs} = E^s$; thus the conductance of the basolateral membrane determined 20 msec after displacing ψ^{cs} is voltage-independent over that range so that the slope (g^s) and chord (G^s) conductances are identical.

Second, the relations between I^s and ψ^{cs} are clearly time-dependent; thus, when ψ^{ms} is clamped at any value other than the *steady-state* value of zero, E^s increases and g^s decreases with time between the initial readings at 20 msec and the final readings at 90 msec.

For reasons that will be discussed and justified below we will consider the values of g^s and E^s determined at 20 msec to *most closely* represent the steady-state properties of the system under short-circuit conditions; these values will be designated ${}_0g^s$ and ${}_0E^s$.

The values of ${}_0g^s$ and I_{sc} when $(Na)_m$ = 3.8, 11.4 or 34.2 mM are given in the Table and illustrated in Fig. 4 for the studies involving impalements from the apical (mucosal) and basolateral (serosal) surfaces of the tissue. Clearly there is a close linear relation between I_{sc} and ${}_0g^s$ in both sets of studies. It should be noted that in the studies involving impalements from the serosal surface of the tissue ${}_0g^s$ was almost twice the values observed when the cells were impaled from the mucosal surface. The origin of this difference is not apparent. One possible explanation is, of course, "impalement damage"; another is "seasonal variation." In either case, these differences do not materially affect the interpretation of these findings.

A positive relation between I_{sc} and ${}_0g^s$ was reported previously (Thomas et al., 1983), but in that paper ${}_0g^s$ was calculated from the "voltage-divider ratio" determined 20 msec after the onset of a 20-mV transepithelial pulse employing the following relations:

$$ {}_0f = (\Delta\psi^{mc}/\Delta\psi^{ms}) = {}_0r^m/({}_0r^m + {}_0r^s) \quad (1) $$

$$ {}_0r^c = r_t r^p/(r^p - r_t) \quad (2) $$

where $\Delta\psi^{mc}$ is the change in ψ^{mc} in response to a 20-mV increase in ψ^{ms} (i.e., $\Delta\psi^{ms}$); r_t is the transepithelial resistance determined from the value of I^{ms} required to displace ψ^{ms} from 0 to +20 mV; and, r^p is the transepithelial resistance in the presence of 10^{-4} M amiloride. Since $r^c = r^m + r^s$ it follows that

$$ {}_0r^m = {}_0f\,{}_0r^c \quad \text{and} \quad {}_0r^s = (1 - {}_0f)\,{}_0r^c. \quad (3) $$

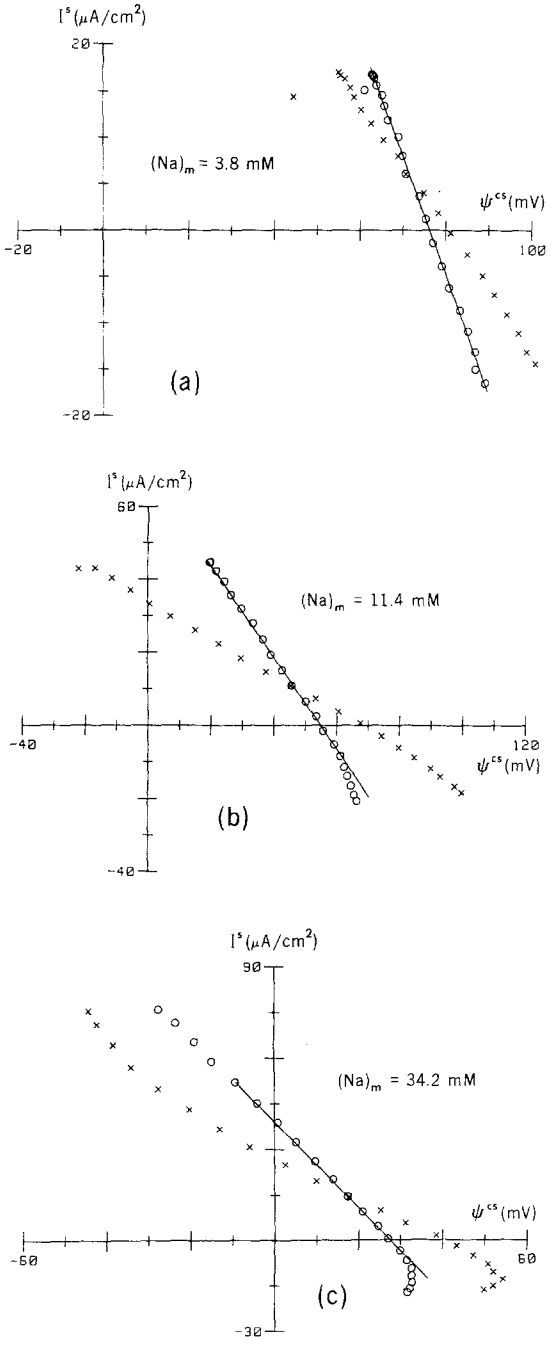

Fig. 3. Examples of the relations between I^s and ψ^{cs} at 20 (○) and 90 (×) msec when $(Na)_m$ = 3.8 (*a*), 11.4 (*b*) or 34.2 (*c*) mM

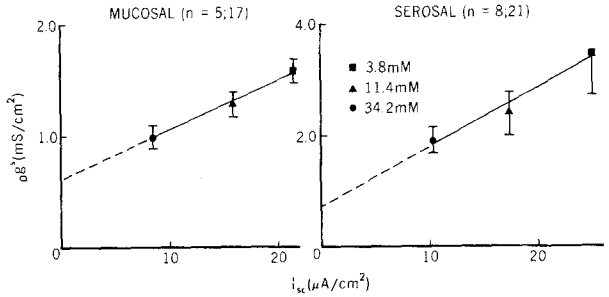

Fig. 4. Relations between $_0g^s$ and I_{sc} for mucosal and serosal impalements

This approach is based upon the assumption that $(\Delta\psi^{mc}/\Delta\psi^{ms})$ is, in fact, a good approximation of the fractional resistance $(_0r^m/_0r^c)$. This assumption has been questioned (Boulpaep & Sackin, 1980; Nagel, Garcia-Diaz & Essig, 1983) at least for "leaky" epithelia where the resistance of the intercellular spaces may contribute significantly to the total paracellular resistance; but, it would seem to be reasonable for an epithelium characterized by wide intercellular spaces (*unpublished observations*) where the total paracellular resistance ranges between 5–14 kΩ cm^2. This approach is also subject to large statistical uncertainties inasmuch as when f approaches unity the value of $(1 - f)$ is subject to considerable error. The excellent agreement between the values of $_0g^s$ determined *directly* from the relation between I^s and ψ^{cs} and those *calculated indirectly* from the values of f, r_t and r^p indicates that these two approaches are internally consistent.

Further, inasmuch as the relations between I^m_{Na} and ψ^{mc} closely conform to the GHK flux equation, we can calculate $_0g^m$ from the relation $_0g^m_{Na} = (\partial I^m_{Na}/\partial\psi^{mc})$ so that

$$_0g^m_{Na} = -\left[\frac{P^m_{Na}\mathcal{F}^2}{RT}\right]\left[\frac{(Na)_m - (Na)_c\xi(1 + ln\xi)(1 - \xi) + \xi\, ln\xi\, (Na)_m - (Na)_c\xi}{(1 - \xi)^2}\right] \quad (4)$$

where $\xi = \exp(\mathcal{F}\psi^{mc}/RT)$.

The values of $_0g^m_{Na}$ when $(Na)_m$ = 3.8, 11.4 and 34.2 mM calculated using Eq. (4) employing the values for P^m_{Na}, $_0\psi^{mc}$ and $(Na)_c$ reported by Thomas et al. (1983; Table 1 and text) are also given in the Table and are in excellent agreement with those calculated using Eqs. (1)–(3). It follows that the values of $_0f$ determined *directly* from the *I-V* relations of the apical and basolateral membranes are in excellent agreement with those calculated using the "voltage-divider ratio" $(\Delta\psi^{mc}/\Delta\psi^{ms})$ (Table); these findings further support the internal consistency of the data and analyses.

A typical example of the relations between ψ^{mc} and ψ^{ms} determined at 20 and 90 msec is illustrated in Fig. 5. The slopes of the lines are $f = (\Delta\psi^{mc}/\Delta\psi^{ms})$; clearly the decrease in f between 20–90 msec is consistent with the observation that r^m (at any value of ψ^{ms}) during this period is constant (Fig. 2) whereas r^s increases.

The relations between the values of $_0E^s$ determined at 20 msec and the values of I_{sc} when $(Na)_m$

Table. Electrical properties of the basolateral membranes

$(Na)_m$		I_{sc}	${}_0\psi^{cs}$	${}_0E^s$	${}_0g^{s*}$	${}_0G^{s**}$	${}_0g^{s\dagger}$	${}_0g^{m\dagger}$	${}_0g^{m\dagger\dagger}$	$({}_0f\dagger/{}_0f\dagger\dagger)$
3.8	*m*	8.5	59	71	0.99	0.71	1.00	0.20	0.20	1.00
		1.0	4	8	0.11		0.10	0.02		
	s	10.3	55	62	1.92	1.75	2.10	0.20	0.22	1.01
		1.1	4	5	0.26		0.30	0.03		
11.4	*m*	16.0	29	45	1.28	1.00	1.33	0.35	0.36	1.00
		1.4	3	7	0.11		0.10	0.03		
	s	17.2	31	41	2.39	2.16	2.90	0.41	0.42	1.02
		2.0	7	8	0.39		0.50	0.06		
34.2	*m*	21.5	9	23	1.58	1.53	1.89	0.40	0.39	1.02
		2.8	2	3	0.10		0.24	0.03		
	s	25.0	14	22	3.49	3.50	3.80	0.68	0.68	1.00
		2.5	6	7	0.76		0.80	0.05		

Units are given in the Glossary. *m* and *s* designate impalements from the mucosal or serosal surfaces, respectively. Values are means ± SEM (below). * Determined from the slopes of the relations between I^s and ψ^{cs}; ** calculated from the relation ${}_{0SIA}I^s = {}_0G^s({}_0E^s - {}_0\psi^{cs})$; † Calculated from Eqs. (1)–(3); †† calculated using Eq. (4). $({}_0f\dagger/{}_0f\dagger\dagger)$ is the ratio of the fractional resistances determined from the "voltage-divider ratio" and those calculated from the *I-V* relations of the apical and basolateral membranes as described in the text.

= 3.8, 11.4 and 34.2 mM are given in the Table and illustrated in Fig. 6; also shown in Fig. 6 are the values of E^s determined at 90 msec. The solid lines were determined from least-squares regression analyses. For the studies involving impalements from the mucosal surface the line intersects the ordinate at $({}_0E^s)_{I_{sc}=0}$ = 103 mV; the average value of ψ^{cs} in the presence of amiloride ($\psi^{cs'}$) in these studies was 86 mV (arrow). For the studies involving impalements from the serosal surface of the tissue, $({}_0E^s)_{I_{sc}=0}$ = 90 mV and the average value of $\psi^{cs'}$ was 76 mV. The dashed lines through the values of E^s determined at 90 msec were drawn by eye and constrained to pass through the same intercepts on the ordinates; the rationale for this choice will be discussed below.

Conclusions

The Origin(s) of the Time-Dependent Behavior of the Basolateral Membrane

There are, in general, three sources of time-dependent responses to a displacement of the electrical potential difference across biological membranes: (i) "electrical capacitance" arising from the dielectric properties of the membrane (~1 μF/cm^2); (ii) "chemical relaxation" resulting from the redistribution of ions *across* the membrane in response to a change in the electrical potential difference; and, (iii) time-dependent changes in ionic permeabilities (or pump activity) that are *directly* or *indirectly* the result of changes in the electrical potential difference.[4] The latter phenomena have long been recognized in excitable membranes and have recently been described in several epithelia (Larsen & Kristensen, 1978; Zeiske & Van Driessche, 1981; Maruyama, Gallacher & Peterson, 1983).

There are several compelling reasons to believe that the time-dependent behavior of the *I-V* relations of the basolateral membranes observed in these studies cannot be entirely attributed to *electrical capacitance*.

First, as demonstrated in the Appendix, inasmuch as the cells are electrically clamped to a constant ψ^{ms}, well within 2 msec of the onset of the command pulse, the capacitative responses of both the apical and basolateral membranes must be characterized by a *single* time-constant (τ). Thus, the finding that the relation between I^m_{Na} and ψ^{mc} is essentially time-independent rules out any significant distortion of this relation by capacitative effects and suggests that the same should be true for the basolateral membranes. In other words, although the *values* of I^m_{Na} and ψ^{mc} change between 20 and 90 msec, they fall on the same GHK curve so that the *properties* of the apical membrane that determine the relation between I^m_{Na} and ψ^{mc} are not distorted by capacitative transients. In contrast, while the values of I^s (= I^m_{Na}) and ψ^{cs} (= $\psi^{ms} - \psi^{mc}$) also change between 20 and 90 msec, with the same time constant as that of the apical membrane, they do

[4] A change in voltage may *directly* affect "voltage-gated channels" in a time-dependent manner. Alternatively, a change in voltage may result in a time-dependent change in the intracellular (or extracellular) activity of an ion that affects the conductance of "chemically-gated channels."

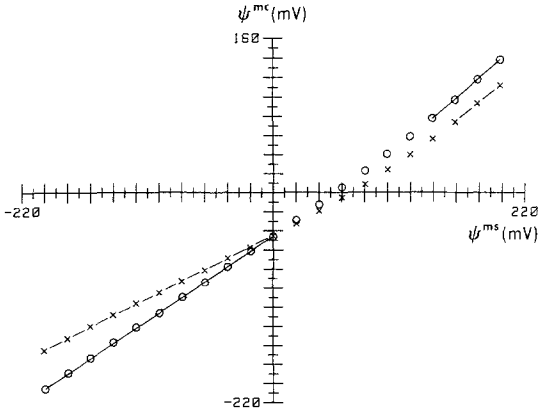

Fig. 5. Relations between ψ^{mc} and ψ^{ms} at 20 (○) and 90 (×) msec. Note that over the range -200 mV $< \psi^{ms} < 80$ mV, f decreased from a value of 0.80 to 0.59 between 20 and 90 msec. When $\psi^{ms} >$ 100 mV both slopes approach unity because when ψ^{mc} exceeds E^m_{Na}, r^m approaches ∞ (Fig. 2)

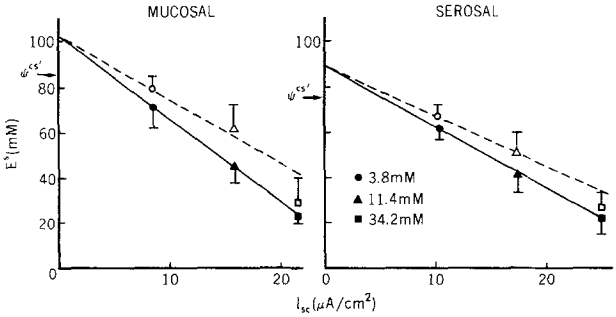

Fig. 6. Relations between E^s and I_{sc} for mucosal and serosal impalements. Solid symbols connected by the solid lines are values at 20 msec; open symbols connected by dashed lines are values of E^s at 90 msec

not fall on the same line but instead, *their relation changes.*

Second, as shown in the Appendix, the time-dependence of the fractional resistance, $f(t)$, is given by

$$f(t) = f[1 - \exp(-t/\tau)] \quad (5)$$

where the time constant, τ is given by

$$\tau = r^m r^s(C^m + C^s)/(r^m + r^s) = fr^s(C^m + C^s). \quad (6)$$

Thus, the effects of electrical capacitance should lead to an *increase* in $f(t)$ with time; instead, as discussed above, the *observed* values of f decrease with time between 20–90 msec.

Third, according to Eqs. (5) and (6), τ, and thus the influence of electrical capacitance on the time-dependence of the *I-V* relations of *both* membranes, should increase when f increases. However, the *I-V* relations of the apical membrane were essentially superimposable in the region around $\psi^{mc} = E^m_{Na}$ (Fig. 2) where r^m and f are maximal.

Fourth, using Eq. (5) we can make a "worst-case estimate" of the maximum value of τ by assuming that the "true steady-state value of f" *in all instances is unity so that*

$$f(t) = 1 - \exp(-t/\tau) \quad (7)$$

where $\tau = r^s(C^m + C^s)$. Using the values of f determined experimentally when $t = 20$ msec (Table), the values of τ calculated from Eq. (7) range between 8–13 msec. Thus, when $t = 20$ msec, $f(t)$ is *at least* 80–92% of the steady-state value of f.

Finally, recent electron-microscopic studies of *Necturus* urinary bladder indicate that the cells have a width of approximately 10 μm and a depth (length) of approximately 75 μm (*unpublished observations*). Assuming that these cells can be approximated by rectanguloids, the total membrane area associated with 1 cm^2 of mucosal surface is approximately 32 cm^2. Assuming further that the electrical capacitance of these membranes is 1 μF/cm^2 and using the values for r^m and r^s measured directly or indirectly (Table), Eq. (6) predicts a time constant of approximately 15 msec.[5]

In short, each of these arguments strongly suggests that the effects of electrical capacitance *alone* cannot account for either the *direction* or the *magnitude* of the observed changes in the *I-V* relations of the basolateral membranes between 20 and 90 msec.

Qualitative Assessment of E^s

Since, by definition, E^s is that value of ψ^{cs} at which $I^s = 0$ we may write the general expression:

$$I^s = G^s(E^s - \psi^{cs}) = I^s_K + I^s_i + I_p$$
$$= G^s_K(E^s_K - \psi^{cs}) + \Sigma[G^s_i(E^s_i - \psi^{cs})] + I_p \quad (8)$$

where I^s_K and I^s_i are the currents of K and all other

[5] The findings of Clausen and Wills (1981) on rabbit urinary bladder and of Schifferdecker and Frömter (1978) on *Necturus* gallbladder indicate excellent agreement between membrane capacitances determined from AC impedance studies and membrane areas estimated from morphometric measurements, assuming relatively simple geometries of the cells. Further, it should be noted that while the value of 1 μF/cm^2 has been generally accepted as a "universal constant" for biological membranes, actual values range between 0.5 μF/cm^2 and 1.5 μF/cm^2 (Schanne & P.-Ceretti, 1978).

ionic species, i, across the basolateral membrane; G^s_K and G^s_i are the conductances of that barrier to those ions; E^s_K and E^s_i are the electromotive forces for these ions across the basolateral membrane given by the Nernst equation; and, I_p is the current attributable to the Na-K exchange pump (Schultz, 1980). Solving for E^s we obtain

$$E^s = (G^s_K/G^s)E^s_K + \Sigma(G^s_i/G^s)E_i{}^s + (I_p/G^s). \quad (9)$$

Most measurements of intracellular K activities made in a number of epithelia using K-selective microelectrodes fall in the range between 60–90 mM (*cf.* De Long & Civan, 1983; Lewis et al., 1978; Kubota, Biagi & Giebisch, 1983; Grasset, Gunter-Smith & Schultz, 1983; Garcia-Diaz, O'Doherty & Armstrong, 1978; Reuss & Weinman, 1979). Assuming that $(K)_c$ in *Necturus* urinary bladder falls within this range, the corresponding value of E^s_K would be between 87–97 mV, or an average value of approximately 92 mV. This value is significantly greater than the observed values of ${}_0E^s$, particularly when $(Na)_m$ = 11.4 or 34.2 mM, indicating that the basolateral membrane must possess a very significant conductance to at least one other major ion. The most prominent candidate, of course, is Cl. In this respect, Lewis et al. (1978) have reported that Cl is passively distributed across the basolateral membrane of rabbit urinary bladder and that the permeability of this barrier to Cl *exceeds* that to K (P^s_{Cl}/P^s_K = 1.17). Macknight (1977) has reported that ^{36}Cl exchanges rapidly across the basolateral membranes of toad urinary bladder and a significant Cl permeability of the inner or basolateral membrane of frog skin (MacRobbie & Ussing, 1961; Ussing, Biber & Bricker, 1965) and frog urinary bladder (Davis & Finn, 1982) is *suggested* by the results of studies dealing with the volume responses of these epithelia to changes in the composition of the serosal bathing solution.

If, as in the case of rabbit urinary bladder, the basolateral membrane of *Necturus* urinary bladder has a relatively high conductance to Cl and this anion is passively distributed across that barrier, then under short-circuit steady-state conditions, since $E^s_{Cl} = {}_0\psi^{cs}$, Cl will not contribute to ${}_0I^s$ or ${}_0\psi^{cs}$ (Eq. (8)). But, when an attempt is made to measure r^c or r^s by perturbing ψ^{ms} (and therefore ψ^{cs}), I^s_{Cl} will contribute to I^s and the total conductance of the basolateral membrane will include the contribution from g^s_{Cl}. In short, any measurement of r^c will be influenced by the conductance of the basolateral membrane to an ion that does not contribute to the steady-state current across that barrier under short-circuit conditions.

Clearly, further speculation on this point is unwarranted in the absence of direct measurements of $(Cl)_c$ in the presence of varying $(Na)_m$. Suffice it to say, at this time, that our directly determined values of ${}_0E^s$ are not consistent with the notion that the basolateral membrane of this epithelium is predominantly permeable to K.[6,7]

The Time Dependence of E^s and g^s

As argued above, it seems very unlikely that the time-dependent increases in E^s and decreases in g^s can be attributed to the effects of electrical capacitance alone. The two other possibilities are chemical relaxation (also referred to as "diffusion polarization" (Läuger & Neumcke, 1973; Attwell, 1979)) and/or the *inactivation* of ionic conductances due directly or indirectly to changes in ψ^{cs}. The finding that the basolateral membrane of this epithelium possesses a major conductance to ion(s) other than K strongly suggests that a time-dependent redistribution of these ions will occur and could contribute to the time-dependent changes in E^s and g^s. However, it is not clear whether such a redistribution could significantly affect E^s in the brief period between 20–90 msec.[8]

Nevertheless, a compelling argument can be made with the aid of Figs. 6 and 7 that these changes in E^s and g^s are entirely predictable regardless of underlying mechanism.

This argument is based on the assumption that

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[6] If we assume, as did Lewis et al. (1978), that the basolateral membrane is permeable to only Na, K and Cl, that the diffusional movements of these ions conform to the GHK flux equation, and that Cl is passively distributed across that barrier then it is a simple matter to show that the observed values of ${}_0E^s$ are consistent with the following set of data: $(Na)_c$ = 5 mM; $(K)_c$ = 60 mM; $(Cl)_c = (Cl)_s \exp(-\mathcal{F}_0\psi^{cs}/RT)$; $P^s_{Na} = 5 \times 10^{-8}$ cm/sec; $P^s_K = 5 \times 10^{-6}$ cm/sec; and, $P^s_{Cl} = 4 \times 10^{-6}$ cm/sec (so that $P^s_{Na}/P^s_K/P^s_{Cl}$ = 0.01 : 1.0 : 0.8). Under these conditions the *I-V* relations of the basolateral membrane are linear and the values of g^s are in reasonable agreements with those observed (Table).

[7] It should be noted that the original notion that the basolateral membrane is permselective to K stemmed from studies in which the inner surface of frog skin was bathed with Cl-free, SO_4 medium (Koefoed-Johnsen & Ussing, 1958; also *see*, Lindley & Hoshiko, 1964).

[8] The problem arises from the fact that the inner and outer solutions bathing the basolateral membrane must be considered essentially unstirred. Thus, although the total amounts of ions that redistribute across that barrier between 20–90 msec in response to a displacement in ψ^{cs} may be very small, changes in the activities of these ions in the unstirred layers adjacent to this barrier may be quite large and difficult to assess.

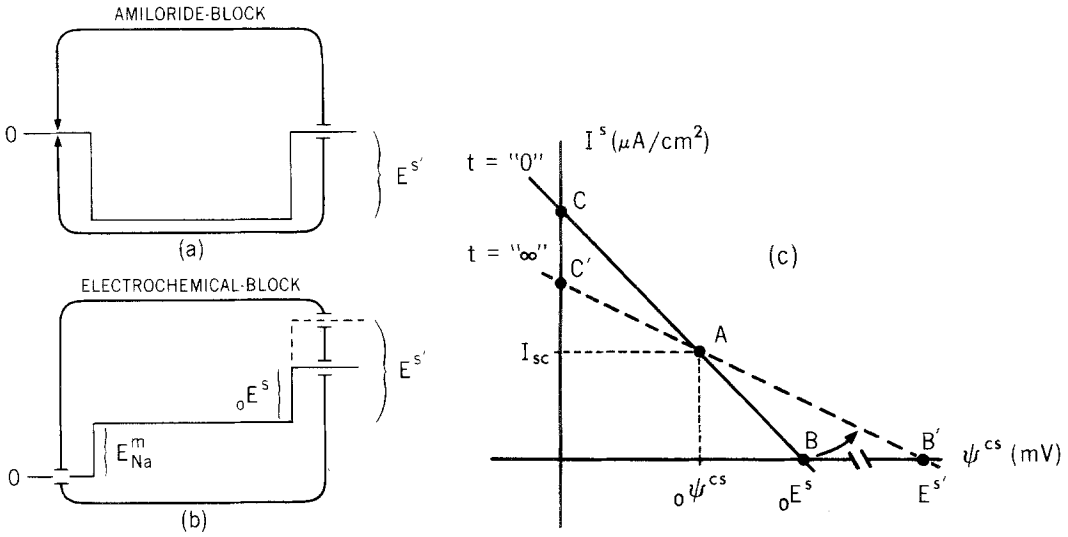

Fig. 7. (*a*): Electrical potential profile across cell when Na entry is blocked with amiloride or when $(Na)_m = 0$. (*b*): Electrical potential profile across cell when $\psi^{mc} = E^m_{Na}$ so that I^m_{Na} (and I^s) = 0. The dashed line indicates the predicted final state of the system. (*c*): Simplified representations of the relations between I^s and ψ^{cs}. Point *A* is the steady-state, short-circuit condition corresponding to the coordinates ($\psi^{cs} = {}_0\psi^{cs}$, $I^s = I_{sc}$). Point *B* corresponds to the condition ($\psi^{cs} = {}_0E^s$, $I^s = 0$); the slope of $|AB| = -{}_0g^s = -{}_0G^s$. In the region $|AB|$ passively distributed anions will leave the cell whereas passively distributed cations will enter; the opposite holds for the region $|CA|$; throughout the range $|CB|$ the passive flow of K will be directed out of the cell

$I^m = I^m_{Na} = I^s$; this assumption appears to be entirely reasonable in the light of the arguments presented above and the data reportd by Frömter et al. (1981) and Thomas et al. (1983), which indicate that in the presence of amiloride, f' averages between 0.97 and 0.99. It follows from this assumption that $\psi^{cs} = E^s$ at that value of ψ^{ms} at which $\psi^{mc} = E^m_{Na}$ so that $I^s = 0$ when $I^m_{Na} = 0$.

There are three ways by which I^m_{Na} and, thus, I^s can be abolished; namely, (i) the addition of a maximally effective concentration of amiloride to the mucosal bathing solution; (ii) removal of Na from the mucosal bathing solution; or (iii) clamping the electrical potential difference across the apical membrane (ψ^{mc}) to the value of E^m_{Na} so that the driving force for the diffusional entry of Na is abolished.

Now:

a) As reported previously (Thomas et al., 1983), in the presence of a maximally effective concentration of amiloride, $\psi^{cs'}$ ($=E^{s'}$) averages between 76–86 mV and is independent of $(Na)_m$ or $_0\psi^{mc}$ ($={}_0\psi^{cs}$); the latter ranged between −9 to −58 mV. Thus, the "quasi-steady-state" achieved in the presence of amiloride, which persists for at least 10 min (Fig. 2 in Thomas et al., 1983), is independent of the initial steady state.

b) In addition, there is a linear relation between I_{sc} and $_0\psi^{cs}$ which approaches $\psi^{cs'}$ as I_{sc} (or $(Na)_m$) approach zero (Figs. 5 and 12 of Thomas et al., 1983).

Therefore, when I^m_{Na} and, thus, I^s are abolished either by inhibiting Na entry with amiloride or by reducing $(Na)_m$ to zero, the steady-state values of $\psi^{cs'}$ ($=E^{s'}$) are the same and range between 76–86 mV and the electrical potential profile across the cell is that illustrated in Fig. 7*a*.

As illustrated in Fig. 6, when I^m_{Na} and, thus, I^s are abolished by clamping ψ^{mc} to E^m_{Na}, E^s determined at 20 msec is a linear function of I_{sc} which approaches $\psi^{cs'}$ or ($E^{s'}$) as I_{sc} (or $(Na)_m$) approach zero. Subsequently, E^s increases with time and, if our reasoning is correct, must approach the same value of $\psi^{cs'}$ observed when Na entry is blocked with amiloride or when $(Na)_m = 0$ (Fig. 7*b*). Thus, referring to Fig. 7*c*, since the short-circuit condition is our steady-state operating point (point *A*), the near-linear relation between I^s and ψ^{cs} must rotate counter-clockwise around point *A* between the value $\psi^{cs} = {}_0E^s$ and the value $\psi^{cs} = \psi^{cs'} = E^{s'}$ with an attendant decrease in G^s (or g^s). And, according to this reasoning, the solid lines illustrated in Fig. 6 should rotate counter-clockwise around the intercept on the ordinate and, in time, become horizontal. In other words, if our reasoning is correct, the same final value of E^s (i.e., $E^{s'} = \psi^{cs'}$) should be attained regardless of the initial values of $(Na)_m$, I_{sc} or $_0\psi^{cs}$; the final quasi-steady-state of the system

when Na entry across the mucosal membrane is abolished by clamping $\psi^{mc} = E^m_{Na}$ should be the same as when Na entry is abolished with amiloride or by removing Na from the mucosal solution.[9]

Implications of the Present Findings

The determination of *I-V* relations across membranes is confronted by two limiting problems in the time domain. Truly *"instantaneous"* measurements of a voltage change in response to a current pulse are complicated by the effects of the electrical capacitance of the membrane so that the truly conductive properties of the barrier cannot be readily extracted from the total admittance. At the same time, noninstantaneous measurements will, in general, be complicated by shifts in the ionic activities of the solutions bathing the two interfaces of the membrane and/or time-dependent changes in ionic permeabilities directly or indirectly due to the voltage change.[4] These problems are particularly acute in the case of epithelia where (i) the electrical capacitance of the tissue per unit nominal membrane area may be multiplied several-fold by apical projections, basolateral infoldings, etc.; and, (ii) the solution bathing the inner surface of the apical membrane and *both* solutions surrounding the basolateral membranes must be considered essentially unstirred.

It follows that DC *I-V* measurements should be made as soon as possible *after* most if not all of the capacitative transients have decayed so that the *I-V* relation reflects the original, unperturbed state of the system as closely as possible.

Unfortunately, direct measurements of the capacitances of the apical and basolateral membranes which could provide some guidance in this matter are relatively sparse. Clausen and Wills (1981) have estimated, using AC analysis, that for rabbit colon $C^m = 17\ \mu F/cm^2$ and $C^s = 9.4\ \mu F/cm^2$; using their values for r^m (510 Ω cm^2) and r^s (97 Ω cm^2), Eq. (6) predicts a value of $\tau = 2$ msec.[10] AC impedance studies on *Necturus* gallbladder by Schifferdecker and Frömter (1978) yielded the values: $C^m = 8\ \mu F/cm^2$, $C^s = 26\ \mu F/cm^2$, $r^m = 1220\ \Omega\ cm^2$ and $r^s = 210\ \Omega\ cm^2$ so that $\tau = 6.1$ msec; the values of the apical and basolateral membrane capacitances reported in that study were in good agreement with morphometric studies of the epithelium. For frog skin, several groups have reported a *total tissue* capacitance (C_t) of approximately 2–3 $\mu F/cm^2$ (Finkelstein, 1964; Brown & Kastella, 1965; Cuthbert & Painter, 1969; Fishman & Macey, 1969; Smith, 1971; Smith, 1975). Direct measurements of C^m and C^s are not available for frog skin but, Cuthbert and Painter (1969) estimated that $C^m = 2\ \mu F/cm^2$ and $C^s = 16\ \mu F/cm^2$ while Smith (1971) estimated that $C^m = 1.6\ \mu F/cm^2$ and $C^s = 78\ \mu F/cm^2$. Using these values and the directly measured values for r^m (~3000 Ω cm^2) and r^s (~1000 Ω cm^2) provided by Helman and Fisher (1977), τ is between 15–60 msec. Finally, Warncke and Lindemann (1981) have reported preliminary data dealing with the capacitances of toad urinary bladder determined from AC analysis; the values extracted from Fig. 3 of their paper are: $C^m \cong 2\ \mu F/cm^2$; $C^s \cong 27\ \mu F/cm^2$; $r^m \cong 4 \times 10^3\ \Omega\ cm^2$ and $r^s \cong 3 \times 10^3\ \Omega\ cm^2$ so that $\tau \cong 50$ msec. The value of $C_t = 0.5\ \mu F/cm^2$ determined by Bindslev et al. (1974) from the transepithelial voltage response of toad urinary bladder to a constant current pulse seems unrealistically low.

Thus, some values of τ for voltage-clamp studies are below 15 msec so that transients arising from electrical capacitance should be at least 90% complete by 35 msec. Assuming the larger values of τ for frog skin and toad urinary bladder, these transients would be 90% complete by approximately 120 msec. Yet, in many voltage-clamp studies employing microelectrodes, *I-V* relations were determined from measurements made long after these capacitative transients should have essentially vanished. For examples, in the studies by Helman and his collaborators (*cf.* Helman & Fisher, 1977; Helman, Nagel & Fisher, 1979; Els & Helman, 1981) and Nagel and his collaborators (*cf.* Nagel, 1978; Nagel & Crabbé, 1980; Nagel & Essig, 1982; Goudeau et al., 1982) on frog skin, most measurements were made between 580–600 msec, and in the study by Nagel and Essig (1982) the *I-V* relations were determined between 100–250 msec after the onset of the current-pulse. The rationale provided by these in-

[9] The only difference between these conditions, as illustrated in Fig. 7*a* and *b*, is the value of ψ^{mc}. But, inasmuch as the conductance of the apical membrane to ions other then Na is very small and is negligible in comparison with that of the basolateral membrane, this difference in ψ^{mc} should not affect our conclusion. Further, if we assume that G^s_{Na} is very small (so that $I_p \approx 0$ when $I^m_{Na} = 0$) and that all major ions other than Na and K are passively distributed across the basolateral membrane (so that $E^s_l = \psi^{cs}$), the quasi-steady state that will be achieved when $I^s = 0$ will be $E^{s'} \iota \psi^{cs'} = E^{s'}_K$, regardless of the means employed to bring about this zero-current condition (Eq. (8)). The values of $\psi^{cs'}$ ($=E^{s'}$) given in Fig. 6 are in good agreement with the predicted value of E^s_K (p. 264).

[10] It should be recalled that this value of τ applies only to voltage-clamp studies. *I-V* relations determined by employing constant current pulses ("current-clamping") will be characterized by at least two time constants (Suzuki, Kottra, Kampmann & Frömter, 1982).

vestigators for the "timing" of these measurements is that it corresponds with the achievement of ". . . the steady-state values of voltage and current . . ." (*cf.* Helman & Fisher, 1977; Nagel, 1978).

However, from the above considerations it seems very likely that this new ". . . steady-state . . ." may not simply be the result of the relaxation of capacitative transients but, instead, may be the result of the redistribution of ions across the limiting membranes and/or other time- and voltage-dependent events. If so, the values of f, r^s and perhaps r^m determined in those studies cannot be related to the values of I_{sc} and ψ^{mc} determined *before* the system was perturbed. It follows that conclusions drawn with respect to the relation, or lack thereof, between I_{sc} and r^s (e.g., Nagel, 1978; Els & Helman, 1981) are open to some reservations until this issue is resolved.

In short, at present microelectrophysiologic studies of epithelial cells are faced with a difficult and perplexing conundrum. Because of the large capacitance per unit nominal membrane area, the time constants for voltage-clamp studies due, inescapably, to membrane capacitance are large. At the same time, the cells are characterized by the presence of unavoidable unstirred layers adjacent to the limiting membranes whose ionic composition will be affected, with time, by changes in the electrical potential differences across those barriers. This is particularly serious for the basolateral membranes because the conductance of this barrier is much greater than that of the apical membrane and both interfacial solutions must be considered essentially unstirred.[11] It follows that a judicious compromise must be made in designing the "timing" of *I-V* determinations such that (i) electrical capacitance transients are "almost over"; and (ii) "chemical relaxation" and other voltage-dependent changes in membrane properties are still minimal. This will introduce some unavoidable errors in the estimates of f, r^s and perhaps r^m. But, certainly, steady-state *I-V* relations determined long after capacitance transients have relaxed may reflect the properties of a "new state" that may differ quite markedly from the original steady state so that attempts to relate the findings of the *I-V* analysis to the original steady state may be invalid. Indeed it is quite possible that in epithelia with long capacitative transients and relatively high basolateral membrane conductances it may not be possible to determine the *I-V* relations of that barrier from simple DC studies and that more complex studies of AC impedance will be required for this purpose.

[11] Laüger and Neumke (1973) and Attwell (1979) have considered this problem quantitatively.

Finally, although this discussion has focused on microelectrophysiologic studies, it should be appreciated that the problems raised also affect the results and interpretations of "black box" transepithelial electrophysiological studies. Clearly inasmuch as E^s and g^s are time-dependent, the transcellular resistance ($r^m + r^s$) (often referred to as R_{Na}) and the transcellular, effective, electromotive force ($E^m_{Na} + E^s$) (sometimes referred to as E_{Na}) must also be time-dependent. Consequently, previously published values of R_{Na} and E_{Na} must be viewed with some skepticism (*cf.* Schultz, Thompson & Suzuki, 1981).

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References

Attwell, D. 1979. Problems in the interpretation of membrane current-voltage relations. *In:* Membrane Transport Processes. Vol. 3, pp. 29–41. C.F. Stevens and R.W. Tsien, editors. Raven, New York

Benos, D.J., Hyde, B.A., Latorre, R. 1983. Sodium flux ratio through the amiloride-sensitive entry pathway in frog skin. *J. Gen. Physiol.* **81**:667–685

Bindslev, N., Tormey, J. McD., Pietras, R.J., Wright, E.M. 1974. Electrically and osmotically induced changes in permeability and structure of toad urinary bladder. *Biochim. Biophys. Acta* **332**:286–297

Bobrycki, V.A., Mills, J.W., Macknight, A.D.C., DiBona, D.R. 1981. Structural responses to voltage-clamping in the toad urinary bladder. I. The principle role of granular cells in the active transport of sodium. *J. Membrane Biol.* **60**:21–33

Boulpaep, E.L., Sackin, H. 1980. Electrical analysis of intraepithelial barriers. *In:* Current Topics in Membranes and Transport. Vol. 13, pp. 169–197. E.L. Boulpaep, editor. Academic, New York

Brown, A.C., Kastella, K.G. 1965. The AC impedance of frog skin and its relation to active transport. *Biophys. J.* **5**:591–606

Clausen, C., Wills, N.K. 1981. Impedance analysis in epithelia. *In:* Ion Transport by Epithelia. S.G. Schultz, editor. pp. 79–92. Raven, New York

Cuthbert, A.W., Painter, E. 1969. Capacitance changes in frog skin caused by theophylline and antidiuretic hormone. *Br. J. Pharmacol. Chemother.* **37**:314–324

Davis, C.W., Finn, A.L. 1982. Sodium transport inhibition reduces basolateral membrane conductance in tight epithelia. *Science* **216**:525–527

DeLong, J., Civian, M.M. 1983. Microelectrode study of K^+ accumulation by tight epithelia: I. Baseline values of split

frog skin and toad urinary bladder. *J. Membrane Biol.* **72**:183–193

Els, W.J., Helman, S.I. 1981. Vasopressin, theophylline, PGE_2 and indomethacin on active Na transport in frog skin: Studies with microelectrodes. *Am. J. Physiol.* **241**:*F*279–*F*288

Finkelstein, A. 1964. Electrical excitability of isolated frog skin and toad urinary bladder, *J. Gen. Physiol.* **47**:545–565

Fishman, H.M., Macey, R.I. 1969. The *N*-shaped current-potential characteristic in frog skin: I. Time development during step voltage clamp. *Biophys. J.* **9**:127–139

Frömter, E., Higgins, J.T., Gebler, B. 1981. Electrical properties of amphibian urinary bladder epithelia: IV. The current-voltage relationship of the sodium channels in the apical cell membrane. *In:* Ion Transport by Epithelia. S.G. Schultz, editor. pp. 31–45. Raven, New York

Fuchs, W., Larsen, E.H., Lindemann, B. 1977. Current-voltage curve of sodium channels and concentration dependence of sodium permeability in frog skin. *J. Physiol. (London)* **267**:137–166

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

Garty, H., Edelman, I.S., Lindemann, B. 1983. Metabolic regulation of apical sodium permeability in toad urinary bladder in the presence and absence of aldosterone. *J. Membrane Biol.* **74**:15–24

Goldman, D.E. 1943. Potential, impedance and rectification in membranes. *J. Gen. Physiol.* **27**:37–60

Goudeau, H., Weitzerbin, J., Mintz, E., Gingold, M.P., Nagel, W. 1982. Microelectrode studies of the effect of lanthanum on the electrical potential and resistance of outer and inner membranes of isolated frog skin. *J. Membrane Biol.* **66**:123–132

Grasset, E., Gunter-Smith, P., Schultz, S.G. 1983. Effects of Na-coupled alanine transport on intracellular K activities and the K conductance of the basolateral membranes of *Necturus* small intestine. *J. Membrane Biol.* **71**:89–94

Helman, S.I., Fisher, R.S. 1977. Microelectrode studies of the active Na transport pathway of frog skin. *J. Gen. Physiol.* **69**:571–604

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

Hodgkin, A.L., Katz, B. 1949. The effect of sodium ions on the electrical activity of the giant axon of the squid. *J. Physiol. (London)* **108**:37–77

Koefoed-Johnsen, V., Ussing, H.H. 1958. The nature of the frog skin potential. *Acta Physiol. Scand.* **42**:298–308

Kubota, T., Biagi, B.A., Giebisch, G. 1983. Intracellular potassium activity measurements in single proximal tubules of *Necturus* kidney. *J. Membrane Biol.* **73**:51–60

Larsen, E-H., Kristensen, P. 1978. Properties of a conductive cellular chloride pathway in the skin of the toad (*Bufo bufo*). *Acta Physiol. Scand.* **102**:1–21

Läuger, P., Neumcke, B. 1973. Ion conductances in lipid bilayers. *In:* Membranes: A Series of Advances. Volume 2, pp. 1–59. G. Eisenman, editor. Marcell Dekker, New York

Lewis, S.A., Wills, N.K., Eaton, D.C. 1978. Basolateral membrane potential of a tight epithelium: Ionic diffusion and electrogenic pumps. *J. Membrane Biol.* **41**:117–148

Li, J.H.-Y., Palmer, L.G., Edelman, I.S., Lindemann, B. 1982. The role of sodium-channel density in the natriferic response of the toad urinary bladder to an antidiuretic hormone. *J. Membrane Biol.* **64**:77–89

Lindemann, B. 1982. Dependence of ion flow through channels on the density of fixed charges at the channel opening. *Biophys. J.* **39**:15–22

Lindemann, B., Driesche, W. van. 1977. Sodium-specific membrane channels of frog skin are pores: Current fluctuations reveal high turnover. *Science* **195**:292–294

Lindley, B.D., Hoshiko, T. 1964. The effects of alkali metal cations and common anions on the frog skin potential. *J. Gen. Physiol.* **47**:749–771

Macknight, A.D.C. 1977. Contribution of mucosal chloride to chloride in toad bladder epithelial cells. *J. Membrane Biol.* **36**:55–63

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

Mandel, L.J., Curran, P.F. 1972. Response of the frog skin to steady-state voltage clamping. I. The shunt pathway. *J. Gen. Physiol.* **59**:503–518

Maruyama, Y., Gallacher, D.V., Peterson, O.H. 1983. Voltage and Ca-activated K-channel in baso-lateral acinar cell membranes of mammalian salivary glands. *Nature (London)* **302**:827–829

Nagel, W. 1978. Effects of antidiuretic hormone upon electrical potential and resistance of apical and basolateral membranes of frog skin. *J. Membrane Biol.* **42**:99–122

Nagel, W., Crabbé, J. 1980. Mechanism of action of aldosterone on active sodium transport across toad skin. *Pflueger's Arch.* **385**:181–187

Nagel, W., Essig, A. 1982. Relationship of transepithelial electrical potential to membrane potentials and conductance ratios in frog skin. *J. Membrane Biol.* **69**:125–136

Nagel, W., Garcia-Diaz, J.F., Essig, A. 1983. Contribution of junctional conductance to the cellular voltage-divider ratio in frog skin. *Pflueger's Arch.* **399**:336–341

Palmer, L.G. 1982. Na transport and flux ratio through apical channels in toad urinary bladder. *Nature (London)* **297**:688–690

Palmer, L.G., Edelman, I.S., Lindemann, B. 1980. Current-voltage analysis of apical sodium transport in toad urinary bladder: Effects of inhibitors of transport and metabolism. *J. Membrane Biol.* **57**:59–71

Palmer, L.G., Li, J.H.-Y., Lindemann, B., Edelman, I.S. 1982. Aldosterone control of the density of sodium channels in the toad urinary bladder. *J. Membrane Biol.* **64**:91–102

Reuss, L., Weinman, S.A. 1979. Intracellular ionic activities and transmembrane electrochemical potential differences in gallbladder epithelium. *J. Membrane Biol.* **49**:345–362

Schanne, O.F., P.-Ceretti, E.R. 1978. Impedance Measurements in Biological Cells. John Wiley & Sons, New York

Schifferdecker, E., Frömter, E. 1978. The AC impedance of *Necturus* gallbladder epithelium. *Pfluegers Arch.* **377**:125–133

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

Schultz, S.G., Thompson, S.M., Suzuki, Y. 1981. Equivalent electrical circuit models and the study of Na transport across epithelia: Nonsteady-state current-voltage relations. *Fed. Proc.* **40**:2443–2449

Smith, P.G. 1971. The low frequency electrical impedance of the isolated frog skin. *Acta Physiol. Scand.* **81**:355–366

Smith, P.G. 1975. Aldosterone-induced moulting in amphibian skin and its effect on electrical capacitance. *J. Membrane Biol.* **22**:165–181

Suzuki, Y., Kottra, G., Kampmann, L., Frömter, E. 1982. Square wave pulse analysis of cellular and paracellular con-

ductance pathways in *Necturus* gallbladder epithelium *Pfluegers Arch.* **394**:302–312

Thompson, S.M., Suzuki, Y., Schultz, S.G. 1982. The electrophysiology of rabbit descending colon. I. Instantaneous transepithelial current-voltage relations and the current-voltage relation of the Na-entry mechanism. *J. Membrane Biol.* **66**:41–54

Thomas, S.R., Suzuki, Y., Thompson, S.M., Schultz, S.G. 1983. Electrophysiology of *Necturus* urinary bladder: I. "Instantaneous" current-voltage relations in the presence of varying mucosal sodium concentrations. *J. Membrane Biol.* **73**:157–175

Turnheim, K., Thompson, S.M., Schultz, S.G. 1983. Relation between intracellular sodium and active sodium transport in rabbit colon: Current-voltage relations of the apical sodium entry mechanism in the presence of varying luminal sodium concentrations. *J. Membrane Biol.* **76**:299–309

Ussing, H.H. 1949. The distinction by means of tracers between active transport and diffusion. *Acta Physiol. Scand.* **19**:43–56

Ussing, H.H., Biber, T.U.L., Bricker, N.S. 1965. Exposure of the isolated frog skin to high potassium concentrations at the internal surface: II. Changes in epithelial cell volume, resistance and response to antidiuretic hormone. *J. Gen. Physiol.* **48**:425–433

Van Driessche, W., Lindemann, B. 1979. Concentration dependence of currents through single sodium-selective pores in frog skin. *Nature (London)* **282**:519–520

Warncke, J., Lindemann, B. 1981. Effect of ADH on the capacitance of apical epithelial membranes. *In:* Advances in Physiological Sciences. Vol. 3: Physiology of Non-Excitable Cells. J. Salanki, editor. pp. 129–133. Pergamon, New York

Wills, N.K., Eaton, D.C., Lewis, S.A., Ifshin, M.S. 1979. Current-voltage relationship of the basolateral membrane of a tight epithelium. *Biochim. Biophys. Acta* **555**:519–523

Zeiske, W., Driessche, W. van. 1981. Apical K-channels in frog skin (*Rana temporaria*): Cation adsorption and voltage influence gating kinetics. *Pfluegers Arch.* **390**:22–29

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Appendix

In this section, we derive an expression for the time-dependence of the fractional resistance, f, for the circuit illustrated in Fig. A1 under conditions where ψ^{ms} is "instantaneously" clamped from zero to a new "holding voltage" assuming that r^m, C^m, r^s and C^s are constant for small changes in ψ^{ms}. For the sake of simplicity we have omitted the electromotive forces from this circuit so that when $\psi^{ms} = 0$, I^c and $\psi^{mc} = 0$.

Since the transcellular current is assumed to be conserved,

$$I^c(t) = g^m\psi^{mc}(t) + C^m(d\psi^{mc}/dt) = g^s\psi^{cs}(t) + C^s(d\psi^{cs}/dt). \tag{A1}$$

Since ψ^{ms} achieves a constant value within 2 msec, thereafter

$$(d\psi^{ms}/dt) = (d\psi^{mc}/dt) + (d\psi^{cs}/dt) = 0 \tag{A2}$$

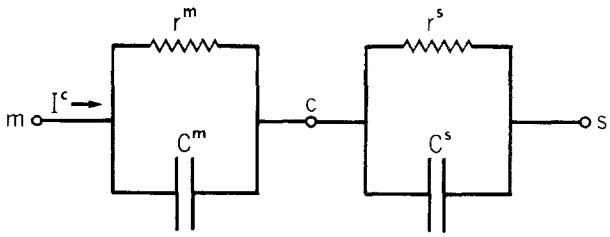

Fig. A1. Equivalent electrical circuit model of the Na-absorbing cell consisting of two parallel *RC* circuits linked in series; the electromotive forces across the two membranes have been omitted for simplicity. The resistance of the fluid in series with the apical and basolateral membranes are compensated for by the voltage clamp and thus can be ignored. Inasmuch as *both* the transcellular and parallel pathways are clamped to a constant ψ^{ms} within 2 msec and the latter does not appear to possess a significant capacitance, the inclusion of a series resistance in this circuit diagram is unnecessary

so that $(d\psi^{mc}/dt) = -(d\psi^{cs}/dt)$. Substituting this relation into Eq. (A1) and rearranging yields

$$(C^m + C^s)(d\psi^{mc}/dt) + (g^m + g^s)\psi^{mc} = g^s\psi^{ms}. \tag{A3}$$

Equation (A3) is a simple, first order, linear differential equation of the form $(dy/dx) + P(x)y = Q(x)$ whose solution from $t = 0$ to $t = \infty$ is

$$\psi^{mc}(t) = \left[\frac{g^s\psi^{ms}}{g^m + g^s}\right][1 - \exp(-t/\tau)] \tag{A4}$$

where $\tau = (C^m + C^s)/(g^m + g^s)$.

Converting from g's to r's we obtain the more familiar relations

$$(\psi^{mc}(t)/\psi^{ms}) = f(t) = f[1 - \exp(-t/\tau)] \tag{A5}$$

where $f = r^m/(r^m + r^s)$ and $\tau = f\,r^s(C^m + C^s)$.

The application of this equation is based on two assumptions. First, that there are no additional sources of capacitance arising from the experimental set-up that are not dissipated well within the time-domain of interest in these studies. The finding that there is no significant time-dependence of the *I-V* relations in the presence of amiloride (Fig. 1) strongly supports this contention.

The second assumption is that r^m and r^s are essentially voltage-independent for small pertubations of ψ^{ms}. This is not strictly true inasmuch as while r^s *is* voltage-independent r^m *is not* (Fig. 2). Nonetheless, the change in r^m over the range $\Delta\psi^{ms} = \pm 20$ mV is small and since $r^m \gg r^s$, the ratio $r^m/(r^m + r^s)$ scarcely changes over this range of $\Delta\psi^{ms}$. In other words, when $r^m \gg r^s$, τ is influenced predominantly by r^s (Eq. A5).

Strictly speaking, Eq. (A3) should be solved after substituting for g^m the expression for the voltage-dependence of this parameter given by Eq. (4); but simple inspection suggests that the resulting expression would not be readily, if at all, integrable and, even if so, the exercise would not yield significantly different results.