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The Electrophysiology of Rabbit Descending Colon

I. Instantaneous Transepithelial Current-Voltage Relations and the Current-Voltage Relations of the Na-Entry Mechanism

Stephen M. Thompson, Yuichi Suzuki*, and Stanley G. Schultz

Department of Physiology and Cell Biology, University of Texas Medical School, Houston, Texas 77025

Summary. A method is described for determining the "instantaneous" transepithelial current-voltage (I-V) relations across rabbit descending colon and deriving the I-V relations of the amiloride-sensitive Na-entry step across the apical membrane. The latter conforms closely to the predictions of the Goldman-Hodgkin-Katz "constant-field" flux equation over a wide range of values of the transapical electrical potential difference (−120 to +50 mV), suggesting that Na entry is the result of simple electrodiffusion through homogeneous pores or channels. The permeability of the apical membrane to Na averaged 0.012 cm/hr, and the intracellular Na activity averaged 10 mM. In these studies, the rate of Na entry across the apical membrane varied, spontaneously, over a fourfold range; this variation is entirely attributable to parallel variations in the partial conductance of the apical membrane to Na with no change in the driving force for this movement.

Bathing the serosal surface of the tissue with a high-K solution abolishes the electrical potential difference across the baso-lateral membrane and markedly reduces the resistance of that barrier. Under these conditions, the I-V relations of the amiloride-sensitive Na-entry step across the apical membrane also conform closely to the predictions of the "constant-field" flux equation.

Finally, the significance of the point at which the transepithelial I-V relations in the absence and presence of amiloride intersect ("E_{Na}") and the origin of the "bends" in these I-V relations at or around this point are discussed. We demonstrate that the point of intersection is simply that value of the transepithelial electrical potential difference at which Na entry is abolished and has no direct bearing on the energetics of the baso-lateral pump. The "bend" in the I-V relations appears to be due to an increase in the conductance of a pathway in the apical membrane that parallels the Na-entry pathway as well as an increase in the conductance of the paracellular pathway; thus, this "bend" does not appear to be directly related to changes in the "active Na transport pathway".

Key words colon · Na entry · electrophysiology · current-voltage relations · apical membrane · amiloride

Introduction

Rabbit descending colon resembles several other Na-transporting epithelia inasmuch as: (i) the transepithelial electrical potential difference and short-circuit current are, normally, entirely attributable to active Na transport from the mucosal to the serosal solution (Frizzell, Koch & Schultz, 1976); (ii) active Na absorption is stimulated by aldosterone, *in vitro* (Frizzell & Schultz, 1978); and (iii) Na absorption is inhibited by the pyrazine diuretic amiloride (Frizzell et al., 1976) which, as in other epithelia, acts by blocking Na entry across the apical membrane (Frizzell & Turnheim, 1978).

The electrophysiology of the active Na transport pathway across rabbit descending colon has been examined previously under "open-circuit" conditions (Schultz, Frizzell & Nellans, 1977). The purpose of the present study is to extend these observations by determining the "instantaneous" current-voltage relations of the barriers for ion movements across this epithelium under short-circuit conditions. This paper (I) will describe the methods employed and the I-V relations of the entire tissue and of the amiloride-sensitive Na-entry step across the apical membrane. The accompanying paper (II, Thompson, Suzuki & Schultz, 1982) will deal with the I-V relations of the baso-lateral membrane, an amiloride-insensitive leak pathway across the apical membrane, and pathways in parallel with the amiloride-sensitive absorptive cells.

Materials and Methods

Adult New Zealand white rabbits (2–4 kg) were sacrifieced by intravenous injection of pentobarbital, and a segment of the descending colon was removed, opened along the mesenteric border to form a flat sheet, and rinsed free of intestinal contents with a physiologic saline solution.

A segment of tissue was stripped of its underlying musculature and connective tissue using a glass microscope slide (Frizzell et al., 1976) and was stretched over a Lucite ring and held in place by means of an "O" ring. The tissue "cartridge" was then mounted horizontally between two halves of a Lucite chamber

* *Present address:* Department of Physiology, Yamagata University, School of Medicine, Yamagata, Japan 990-23.

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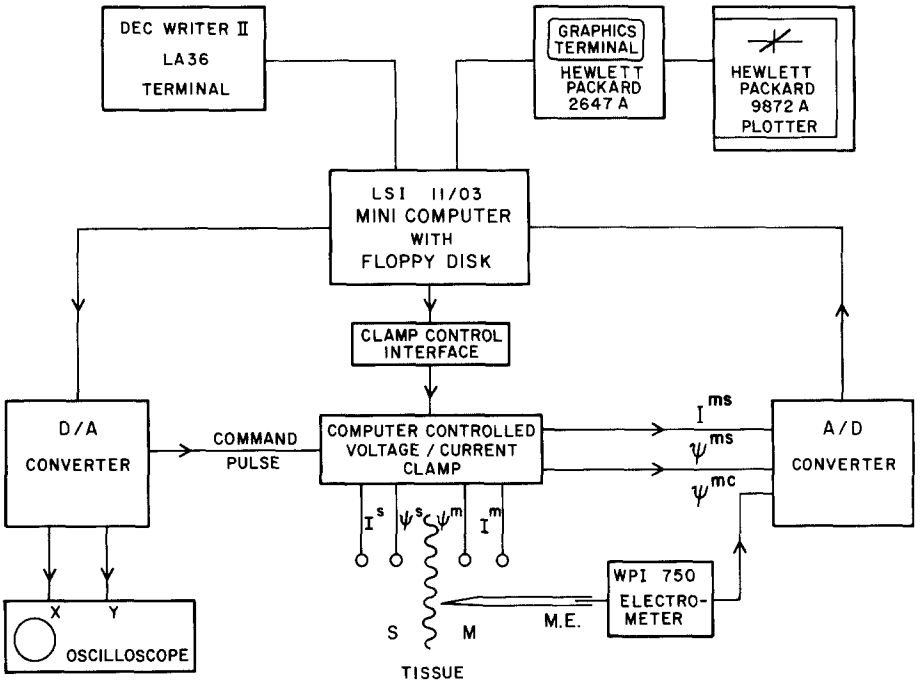

Fig. 1. Schematic of the experimental set-up. *M.E.* is the microelectrode and *s* and *m* designate the serosal and mucosal solutions, respectively

with either the mucosal or surosal side facing upwards. A fine nylon mesh was used to support the tissue on its lower side and a silicone grease mixture was used to provide a seal against the upper half chamber. The grease mixture contained a 1:1 mixture of Dow-Corning stopcock grease and high vacuum grease. A small amount of carbon decolorizing alkaline (Norit-A) was added to enable the formation of the seal with the chamber to be visualized. The resistance of those tissues mounted with the mucosal side facing down against the nylon mesh was generally lower than the resistance of tissues mounted mucosal side up against the silicone grease seal, indicating the presence of some edge damage in the former (*see* Results). The chamber had an exposed tissue area of 0.07 cm^2, and the fluid depth above and below the tissue was approximately 3 mm. The tissue was continuously superfused on both sides by an electrolyte solution flowing at ~1.3 ml/min or approximately one "chamber-volume" per second. In eight experiments both sides of the tissue were perfused with the "normal" electrolyte solution which contained (mM): Na, 140; K, 5.4; Cl, 124; HCO_3, 21; HPO_4, 2.4; H_2PO_4, 0.6; Mg, 1.2; Ca, 1.2; and glucose, 10. In five additional experiments, the serosal side only was perfused with a high-K solution containing (mM): K, 143; SO_4, 62; HCO_3, 21; HPO_4, 2.4; H_2PO_4, 0.6; Ca, 1.2; Mg, 1.2; glucose, 10; and mannitol, 95. All serosal perfusates contained verapamil (2×10^{-5} M) in order to inhibit movement due to contraction of any remaining musculature. Preliminary studies indicated that verapamil has no effect on either the short-circuit current or the tissue conductance. The mucosal perfusate could be switched to one containing 10^{-4} M amiloride by means of a rotary valve located near the inlet to the chamber. Each solution had a pH of 7.4 at 37 °C when gassed with a mixture of 95 % O_2 and 5 % CO_2. The temperature of the reservoirs was set at 40 °C so that the fluid entering the chamber by gravity feed was maintained at 36–37 °C.

A schematic diagram of the experimental setup is shown in Fig. 1. The transepithelial electrical potential difference, ψ^{ms}, and the transepithelial current, I^{ms}, were determined using a voltage/current clamp designed and constructed in these laboratories which provided automatic correction for the fluid (series) resistance between the voltage-sensing electrodes and the tissue; this series resistance was less than 2 % of the transepithelial resistance. The potential-sensing electrodes were calomel cells (Coleman B-710) connected to the chamber via 3 M KCl-agar filled bridges which were positioned near the center of the chamber close to the surfaces of the tissue. The current-passing electrodes were sintered Ag–AgCl pellets (In Vivo Metrics) which were mounted in the outflow lines of the chamber. This electrode configuration provided a reasonably uniform electrical field across the tissue inasmuch as ψ^{ms} varied by less than 3 mV over the surface of the tissue when I^{ms} was as high as 600 μA/cm^2 and ψ^{ms} was ~200 mV. The intracellular electrical potential difference across the apical membrane with reference to the mucosal solution, ψ^{mc}, was determined using glass microelectrodes connected to a high input impedance electrometer (WPI-750). The voltage clamp was under control of a mini-computer (LSI 11/03, Digital Equipment Corp.) so that the tissue could be open circuited, voltage clamped, current clamped or subjected to a voltage pulse train by commands from the console terminal. The measured values of I^{ms}, ψ^{ms} and ψ^{mc} were recorded using a three-channel chart recorder (Gould Inc., Model 2400) and a storage oscilloscope (Tektronic, Model 5114).

The glass microelectrodes were pulled from 1.2 mm (OD) filament lined capillary tubing (WP Instruments Inc.) using a horizontal puller (Brown and Flaming, Sutter Instruments). Unless otherwise stated, electrodes were backfilled using a 0.5 M KCl solution and had a resistance of 100–190 MΩ when dipped in the normal electrolyte solution (corresponding to 20–40 MΩ when filled with 3 M KCl and dipped in 3 M KCl). The rationale for the use of these electrodes has been discussed (Fromm & Schultz, 1981).

The criteria for a successful impalement were (*see* Results): (a) an abrupt deflection reaching a plateau value; (b) maintenance of the plateau value with no more than a 10 % variation for at least 30 sec; (c) a tip potential change of less than 5 mV after withdrawal of the electrode from the cell; and (d) no change in electrode tip-resistance after withdrawal from the cell.

The experimental protocol was as follows: After mounting, the tissues were voltage clamped to the short-circuit condition ($\psi^{ms}=0$) and monitored until the short-circuit current (I_{sc}) stabil-

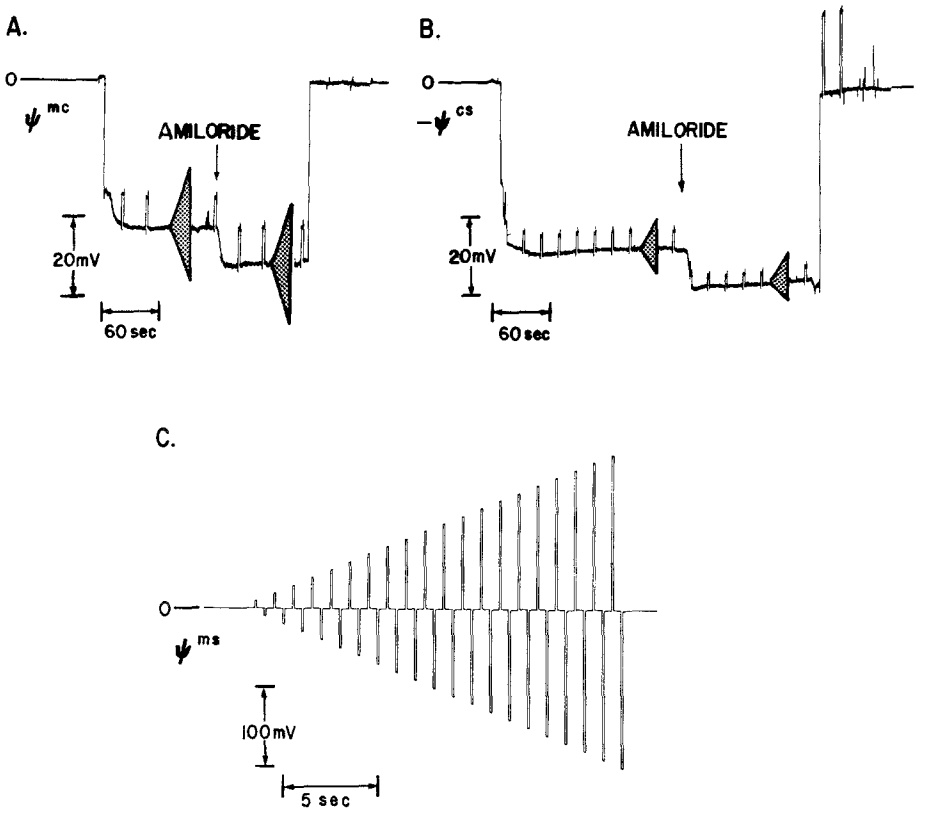

Fig. 2. Typical recordings of intracellular potential in which the impalement was made through either (*A*) the apical membrane or (*B*) the basolateral membrane. The dot-filled triangles represent the changes in ψ^{mc} or ψ^{cs} (drawn to 1/10 scale) associated with voltage-clamping ψ^{ms} according to the pulse train shown in (*C*)

ized (~20 min). A microelectrode was then advanced into the cell using a hydraulic microdrive (Narashige, MO-8) while ψ^{ms} was intermittently pulsed to 10 mV. When the microelectrode tip penetrated the cell membrane there was an abrupt negative deflection in the recorded value of ψ^{mc} and a large increase in the magnitude of the deflection when ψ^{ms} was pulsed to 10 mV, indicating that the microelectrode tip had passed a resistive barrier (Fig. 2*A*, *B*). The ratio of the deflection in the transapical electrical potential difference ($\Delta\psi^{mc}$) to the deflection in the transepithelial electrical potential ($\Delta\psi^{ms}$) is the fractional resistance defined as $f = r^m/(r^m + r^s)$ where r^m and r^s are the slope resistances of the apical and basolateral membranes, respectively.[1] When ψ^{mc} and f reached stable values (*see* below) a computer-generated pulse train was relayed via the digital-to-analog converter to the voltage-clamp device which passed alternating polarity current pulses across the tissue sufficient to clamp ψ^{ms} over the range 0 to ±200 mV in 10-mV increments (e.g., $\psi^{ms} = 0, +10, 0, -10, 0, +20, 0, -20, 0 \ldots 0, +200, 0, -200$ mV); each pulse had a duration of 100 msec, and the interval between pulses was 500 msec (Fig. 2*C*). During the pulse train the transepithelial current (I^{ms}), the transapical electrical potential difference (ψ^{mc}) and the clamped transepithelial electrical potential difference (ψ^{ms}) were recorded on the chart recorder and were sampled by an analog-to-digital converter and relayed to the computer for processing and storage. Five samples of each signal were taken 16 msec after the upstroke of each pulse and again at the end of the pulse (100 msec). Each set of five values were averaged to give a single value at 16 msec and another at 100 msec. No substantive differences between these two sets of data were found and only the data at 16 msec will be presented. Immediately following the "control" pulse train the mucosal perfusate was switched to one containing 10^{-4} M amiloride. When the blocking effect of amiloride on the short-circuit current was complete a second pulse train was generated (Fig. 2) and each parameter was recorded as before. Thus, the transepithelial current-voltage (*I-V*) relations and corresponding intracellular potentials were recorded both before and after inhibition of the amiloride-sensitive Na transport pathway.

Graphics analysis of the data was carried out using a graphics terminal (Hewlett-Packard) and hard copy was obtained using a four-pen *X-Y* plotter (Hewlett-Packard 9872 A).

[1] For an excellent discussion of the distinction between *chord* (*G*) and *slope* (*g*) conductances the reader is referred to Finkelstein and Mauro (1963, 1977).

Glossary of Symbols and Nomenclature

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

Superscripts

m	Mucosal or apical membrane
s	Serosal or baso-lateral 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}

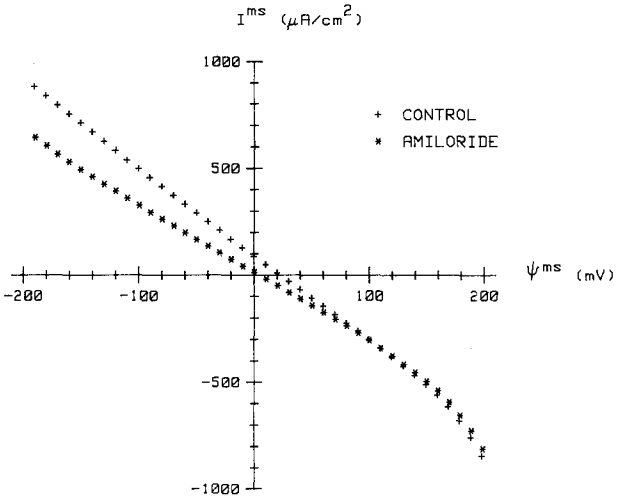

Fig. 3. Transepithelial *I-V* relation measured in the absence (+) and presence of amiloride (*)

Results and Interpretation

The Transepithelial I-V Relations

Typical transepithelial *I-V* relations in which the values of I^{ms} were determined 16 msec after a step change in ψ^{ms} are illustrated in Fig. 3. The *I-V* relations appear to be nearly linear over a wide range of ψ^{ms} under control conditions and in the presence of 10^{-4} M amiloride, and both curves make a decided downward bend at or near the value of ψ^{ms} at which the two curves intersect ($\psi^{ms} \simeq 100$ mV). Similar results have been reported for frog skin (Helman & Fisher, 1977; Helman, O'Neil & Fisher, 1975); toad urinary bladder (Civan, 1970; Macchia & Helman, 1979), and toad colon (Macchia & Helman, 1979). In the present studies the "bend" in the *I-V* curve when $\psi^{ms} > 100$ mV was a consistent finding and always occurred in the "downward" direction (i.e., direction of increasing conductance); this is unlike the findings in the frog skin where the *I-V* curve appears to "break" in either direction (Helman & Fisher, 1977). The value of ψ^{ms} where the two *I-V* relations intersected averaged 115 ± 5 mV.

Clearly, both the short-circuit current (I_{sc} or the value of I^{ms} when $\psi^{ms}=0$) and the slope conductance of the tissue were reduced by amiloride. The value of I_{sc} in eight such experiments averaged $61 \pm 13\,\mu A/cm^2$ under control conditions and $0 \pm 7\,\mu A/cm^2$ in the presence of amiloride. The conductance of tissues mounted mucosal side up averaged 4.7 ± 0.3 mS/cm^2 under control conditions and declined to 3.9 ± 0.2 mS/cm^2 in the presence of amiloride.

Intracellular Potentials and the Fractional Resistance

As discussed recently by several investigators (Lindemann, 1975; Nelson, Ehrenfeld & Lindemann, 1978; Suzuki & Frömter, 1977; Lewis & Graf, 1979), intracellular potentials determined using microelectrodes may be influenced by three sources of artifacts; namely (i) "impalement damage" of the penetrated membrane, (ii) "pre-tip potentials" resulting from the effect of cytoplasmic fixed-negative charges on the mobilities of K and Cl (Nelson et al., 1978), and (iii) KCl diffusion out of the tip which could lead to cell swelling and time-dependent changes in ψ^{mc} (Nelson et al., 1978).

The time course of two typical impalements from the mucosal surface of the epithelium are illustrated in Fig. 4. We see that the initial abrupt negative deflection is followed by a small, rapid depolarization and then by a slower hyperpolarization which ultimately (30–90 sec) reaches a stable value equal to or exceeding the amplitude of the initial deflection. When the fractional resistance ($f = \Delta\psi^{mc}/\Delta\psi^{ms}$) determined at different times during the slow hyperpolarization phase is plotted versus ${}_0\psi^{mc}$ measured at those times, a linear relation is obtained which has an intercept near the origin (Fig. 5). Linear regression analysis of seven such impalements yielded an average intercept of 0.3 ± 0.8 mV and an average correlation coefficient of 0.98 ± 0.07.

As discussed by others (Suzuki & Frömter, 1977; Lewis & Graf, 1979), this finding suggests that the slow hyperpolarization of ψ^{mc} is due to "resealing" of the apical membrane around the microelectrode tip. The finding that the line extrapolates to the origin indicates that there is no significant "pre-tip" potential. Evidence suggesting the absence of significant "impalement damage" of the apical membrane will be presented below.

The relationships between ψ^{mc} and ψ^{ms} obtained before and after amiloride for the experiment illus-

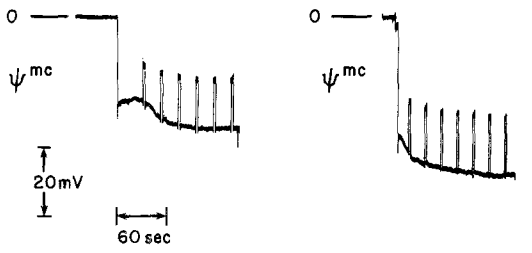

Fig. 4. Time-course of two typical determinations of ψ^{mc}. The square pulses show the change in ψ^{mc} resulting from pulsing ψ^{ms} from 0 to 20 mV

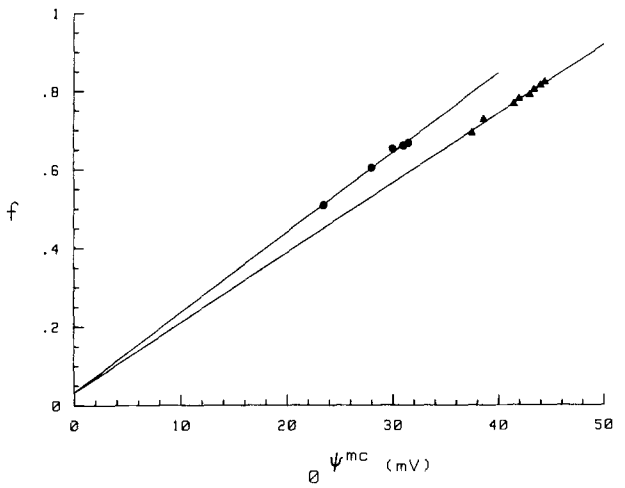

Fig. 5. Plots of the fractional resistance, $f=\Delta\psi^{mc}/\Delta\psi^{ms}$, against ψ^{mc} for the two impalements shown in Fig. 4. The solid lines were obtained by least-squares linear regression analysis

trated in Fig. 3 are given in Fig. 6. The slope of the curves at any value of ψ^{ms} or ψ^{mc} is a measure of the fractional resistance, f or f', at those values. In eight experiments, the value of f when $\psi^{ms}=0$ averaged 0.76 ± 0.02; this value is in reasonable agreement with that of 0.83 reported previously for this tissue under open-circuit conditions (Schultz et al., 1977; Wills, Lewis & Eaton, 1979*b*). In the presence of amiloride, f' when $\psi^{ms}=0$ averaged 0.88 ± 0.01; this value is also in reasonable agreement with the value of 0.91 reported previously (Schultz et al., 1977; Wills et al., 1979*b*). Similarly, the mean values of ${}_0\psi^{mc}$ (-39 ± 2 mV) and ${}_0\psi^{mc'}$ (-49 ± 1 mV) in the eight experiments for which *I-V* relations were obtained are in good agreement with those reported previously (Schultz et al., 1977).

Also shown in Fig. 6 are the corresponding values of the electrical potential difference across the basolateral membranes before, ψ^{cs}, and after, $\psi^{cs'}$, amiloride. Clearly, as ψ^{ms} is pulsed over the range 0 to ±200 mV, ψ^{cs} traverses the much smaller range $-45\,\text{mV}\leqq\psi^{cs}\leqq85\,\text{mV}$; and, in the presence of amiloride, the range of $\psi^{cs'}$ is reduced to $0\,\text{mV}\leqq\psi^{cs'}\leqq85\,\text{mV}$.

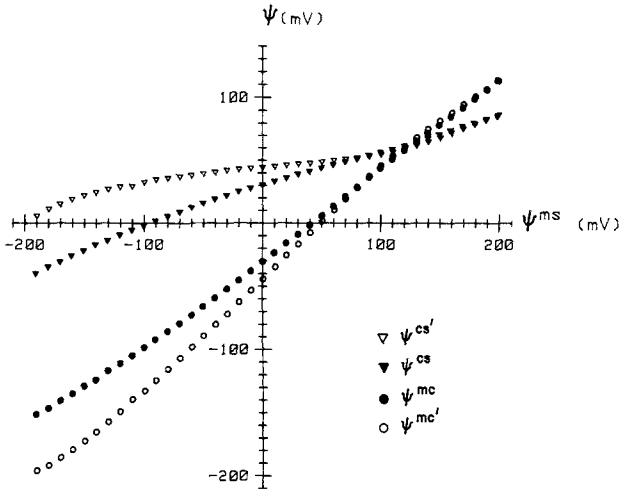

Fig. 6. Relations between ψ^{ms} and ψ^{mc} (●), $\psi^{mc'}$ (○), ψ^{cs} (▼), and $\psi^{cs'}$ (▽), where primes designate values obtained in the presence of amiloride

Table 1. Comparison of impalements from the mucosal or serosal surfaces

		${}_0\psi^{mc}$	${}_0\psi^{mc'}$	f	f'	(r^m/r^s)	$(r^m/r^s)'$
Mucosal	(10)	−42	−53	0.81	0.89	4.3	9.1
Serosal	(8)	−43	−51	0.82	0.88	4.6	7.3

Comparison of Impalements of the Apical and Basolateral Membranes

The finding that f' is significantly less than unity suggested that *either* the apical membrane retains a significant "normal" ionic conductance in the presence of 10^{-4} M amiloride (due to leak pathways for ions other than Na or an incomplete effect of amiloride) *or* that impalement of the cell damaged the apical membrane. Higgins, Gebler and Frömter (1977) found that the fractional resistance of the apical membrane of *Necturus* urinary bladder was significantly lower when the microelectrode was advanced into the cell from the mucosal surface than when the microelectrode was advanced into the cell from the serosal surface even though stable intracellular potentials were recorded in both instances. These investigators concluded that penetration of the apical membrane resulted in "impalement artifacts."

In order to assess this possibility for this tissue, a series of experiments was performed in which cells were impaled either from the mucosal or the serosal surface of the epithelium. The results are given in Table 1. Clearly, the values of ${}_0\psi^{mc}$ and of f before and after amiloride determined from impalements through either surface are in excellent agreement. Inasmuch as the resistance of the apical membrane

is approximately four times greater than that of the basolateral membrane in the absence of amiloride and increases to a value approximately eight times greater than that of the basolateral membrane in the presence of amiloride, it follows that impalement damage of the apical membrane should have a much greater effect on f and f' than *equivalent* impalement damage to the basolateral membrane and, in the presence of significant impalement damage one would expect both of these values to be greater when the tissue is impaled from the serosal surface. Consequently, the data reported in Table 1 strongly suggest that our measurements of ψ^{mc} and f are not significantly affected by impalement artifacts and that the finding that $f'<1$ implies a residual "natural" conductance of the apical membrane in the presence of 10^{-4} M amiloride. The same conclusion was reached previously by Wills et al. (1979*b*) using a very different experimental approach.

Data Reduction and Interpretation

If the only conductive pathway across the apical membrane of rabbit colon is the amiloride-inhibitable Na entry process, and if 10^{-4} M amiloride completely blocks this process without affecting any conductive pathways in parallel with the amiloride-sensitive cells (hereafter referred to as "parallel pathways"), the difference between the transepithelial currents before and after amiloride ($\Delta I^{ms}=I^{ms}-I^{ms'}$) at any value of ψ^{ms} would represent the Na current across the apical membrane, I^m_{Na}, at that value of ψ^{ms}. Since the values of ψ^{mc} are also known at any value of ψ^{ms}, a plot of I^m_{Na} *vs.* ψ^{mc} – or the *I-V* relation of the Na entry step – could be obtained *directly* from the data illustrated in Figs. 3 and 6.

However, the finding that f' differs significantly from unity precludes this simple approach. This finding indicates a significant "natural" leak conductance across the apical membrane, and under these conditions $(I^m_{Na})_{\psi^{ms}}$ is not, in general, equal to $(I^{ms}-I^{ms'})_{\psi^{ms}}$. The reason for this is straightforward. When I^m_{Na} is inhibited by amiloride, ψ^{mc} hyperpolarizes significantly (Schultz et al., 1977 and present data). Consequently, the driving force for ion movements across the amiloride-insensitive leak pathway and, in turn, the current across that pathway in the presence of amiloride will differ from that in the absence of amiloride. Thus, it follows that, in general, $\Delta I^{ms}\neq I^m_{Na}$.

An equivalent electrical circuit model of the rabbit colonic epithelial cell that is consistent with our observations is illustrated in Fig. 7; Wills et al. (1979*b*) have proposed a very similar model based on results obtained using a very different approach.

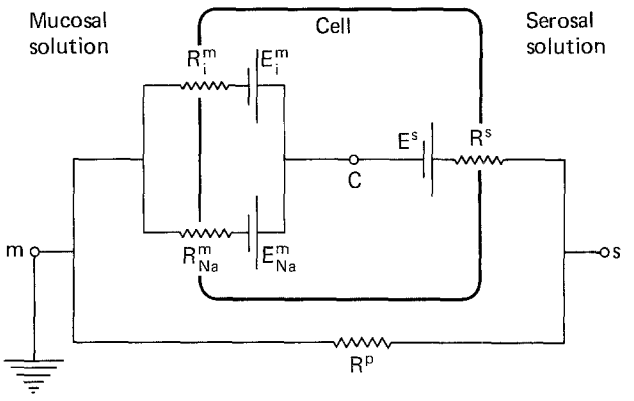

Fig. 7. Equivalent electrical circuit model of rabbit descending colon

In this model, R^m_{Na} is the *chord* resistance of the apical membrane to Na and E^m_{Na} is the electromotive force for Na across that barrier; R^m_i and E^m_i are the lumped chord resistance and electromotive force of the leak pathway across the apical membrane; R^s and E^s are the analogous circuit parameters of the basolateral membrane; and, R^p is the lumped chord resistance of the parallel pathways.[2]

It can be readily shown (Appendix) that, according to this very general model,

$$(I^m_{Na})_{\psi^{ms}}=[(I^{ms}-I^{ms'})/f']_{\psi^{ms}} \quad (1)$$

(where the subscript, ψ^{ms}, indicates that the values pertain to a given ψ^{ms}), providing that: (i) in the presence of amiloride R^m_{Na} is infinite; (ii) amiloride does not significantly affect R^m_i, E^m_i, R^s, E^s or R^p within the time-frame of these studies; and, (iii) R^m_i and R^s are voltage-independent over the range of interest (so that the slope and chord resistances are equal). Support for these assumptions will be provided in the Discussion and in the following manuscript (II – Thompson et al., 1982). Clearly, according to Eq. (1), I^m_{Na} approaches ΔI^{ms} as f' approaches unity.

Applying Eq. (1) to the data given in Figs. 3 and 6, we obtain the relation between I^m_{Na} and ψ^{mc} illustrated in Fig. 8. The *I-V* relation of the amiloride-blockable pathway across the apical cell membrane of rabbit descending colon is clearly nonlinear. The solid curve represents the Goldman-Hodgkin-Katz (GHK) constant field flux equation for a *single* permeant ion, i.e.,

$$I^m_{Na}=-\left[\frac{P^m_{Na}\mathscr{F}^2\psi^{mc}}{RT}\right]\left[\frac{(Na)_m-(Na)_c\exp(\mathscr{F}\psi^{mc}/RT)}{1-\exp(\mathscr{F}\psi^{mc}/RT)}\right] \quad (2)$$

[2] We have chosen to treat the paracellular pathway and the amiloride-insensitive cellular pathway (Wills et al., 1979*b*) as a single resistive pathway. The consequences of this are discussed in the following manuscript (II – Thompson et al., 1982).

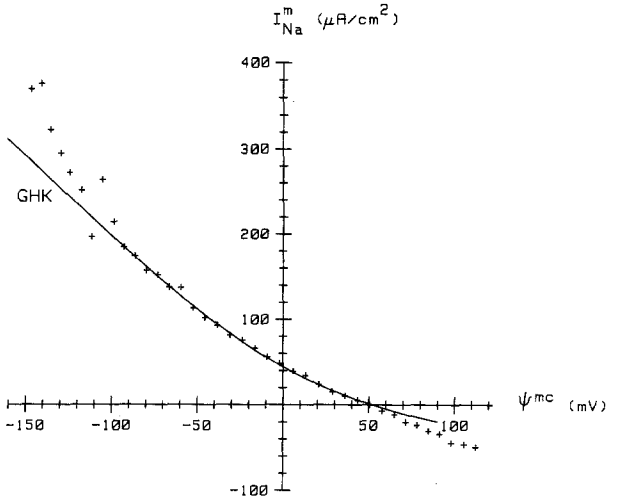

Fig. 8. *I-V* relation of the amiloride-blockable pathway in the apical membrane. I^m_{Na} is calculated using Eq. (1) and is plotted versus ψ^{mc}

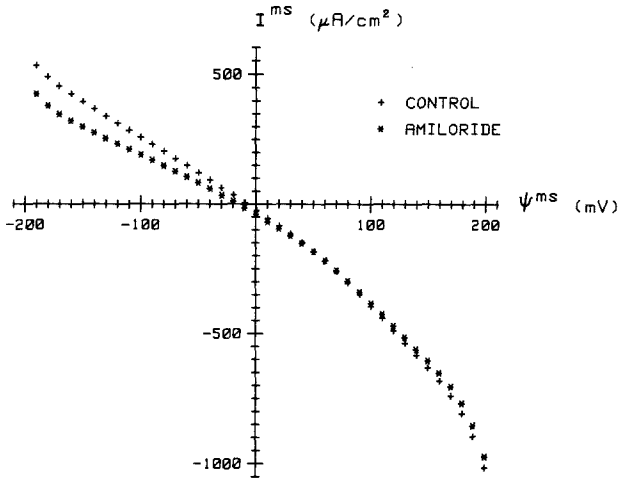

Fig. 9. Transepithelial *I-V* relations obtained when the basolateral membrane was exposed to a solution containing 86 mM K in the absence (+) and presence (*) of amiloride (10^{-4} M) in the mucosal perfusate

where: (Na) is the Na activity and the subscripts m and c designate the mucosal solution and intracellular compartment, respectively; P^m_{Na} is the permeability of the amiloride-inhibitable Na entry step across the apical membrane; and R, T and $\mathcal{F}$ have their usual meanings. This curve was obtained by constraining Eq. (2) to fit two conditions; namely, the value of I^m_{Na} when $\psi^{mc}=0$ and the value of I^m_{Na} when $\psi^{mc}={}_0\psi^{mc}$ (i.e., when $\psi^{ms}=0$). The resulting two simultaneous equations were solved by the computer for P^m_{Na} and $(Na)_c$, which, in turn, were substituted into Eq. (2) to generate the curve shown. Clearly the data conform closely to the GHK relation over the range $-120\,\text{mV} \leqq \psi^{mc} \leqq 65\,\text{mV}$.

In the experiment illustrated in Fig. 8, the values $P^m_{Na}=0.018$ cm/hr and $(Na)_c=14.6$ mM provided the best fit to the experimental data. In every experiment, the data analyzed as described above, conformed to the GHK flux equation over a wide range (generally $-120\text{mV} \leqq \psi^{mc} \leqq 50\text{mV}$). The data departed from this relation only in the extreme ranges where the assumptions underlying Eq. (1) are no longer valid. The average value of P^m_{Na} was 0.012 ± 0.002 cm/hr; the average value of $(Na)_c$ was 10 ± 2 mM; and, the corresponding average value of E^m_{Na} calculated using the Nernst equation was 66 ± 5 mV.

I-V Relations Determined in the Presence of High Serosal Potassium

An alternative approach which has been used to determine the *I-V* relation of the amiloride-blockable Na-entry pathway in two other Na transporting epithelia (Fuchs, Hviid Larsen & Lindemann, 1977; Palmer, Edelman & Lindemann, 1980) involves only the measurement of transepithelial currents and voltages. According to this technique, when the K-selective basolateral membrane is depolarized by elevating the K concentration in the serosal bathing medium, the resistance of the basolateral membrane is effectively eliminated so that the electrical properties of the cell are largely determined by the apical membrane. Under these conditions, the amiloride-blockable Na current across the apical membrane may be computed directly from the difference in transepithelial currents at each clamping potential; i.e.,

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

The results of an experiment in which we have directly compared this "extracellular" technique with the "intracellular" technique described above are illustrated in Figs. 9 and 10. In this experiment the tissue was bathed on the serosal side with an isotonic solution in which the K concentration was increased to 143 mM and all Cl was replaced by SO_4 (*see* Methods). An epithelial cell was impaled from the mucosal side and the pulse train sequences were executed as before. Since under these conditions ψ^{mc} is nearly zero (*see* below), a successful impalement was identified by a sudden increase in f to a stable value, indicating that the tip had penetrated a resistive barrier. Further advancement of the microelectrode resulted in a subsequent, sudden increase in f to a value of unity, indicating that the tip had passed through the basolateral membrane.

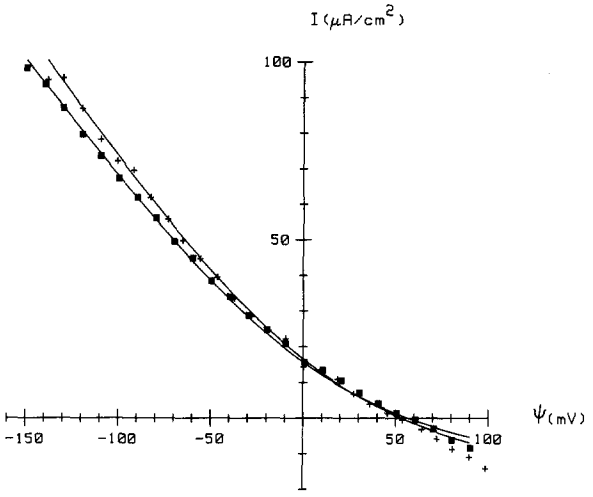

Fig. 10. Comparison of the *I-V* relations of the amiloride-sensitive pathway plotted either as ΔI^{ms} *vs.* ψ^{ms} (■) or I^{m}_{Na} *vs.* ψ^{mc} (+)

A typical transepithelial *I-V* relation obtained in the presence of high serosal K is illustrated in Fig. 9. Under these conditions there appear to be two regions of inflection in the range $\psi^{ms}>0$; one where the two curves first intersect ($\psi^{ms}\cong 50$ mV) and another when $\psi^{ms}\cong 150$–160 mV. The significance of these observations will be discused below.

As can be seen in Fig. 10, nearly identical results are obtained either when (i) I^{m}_{Na} is calculated using Eq. (1) and is plotted *vs.* the corresponding ψ^{mc}; or (ii) when I^{m}_{Na} is calculated as $(\Delta I^{ms})_{\psi^{ms}}$ and is plotted *vs.* the corresponding transepithelial electrical potential difference, ψ^{ms}. Clearly, if both the electromotive force (E^s) and resistance (R^s) across the basolateral membrane are reduced to zero by high K, the two sets of data should coincide. The small disparity between these two sets of data indicates that either E^s and/or R^s are nonzero; our estimates indicate that R^s is reduced by $\sim 50\,\%$ to $100\,\Omega\,cm^2$. The solid curves are drawn according to the GHK equation (2) and provide excellent fits to the data over nearly a 200-mV range.

The results of five such experiments in the presence of high serosal K are summarized and compared with values obtained under control conditions in Table 2; in each experiment, the relation between I^{m}_{Na} and either ψ^{mc} or ψ^{ms} closely conformed to the constant-field flux equation (2). Clearly in the presence of the high-K serosal solution, ${}_0\psi^{cs}$ is close to zero consistent with the notion that the basolateral membrane is predominantly permeable to K (Wills et al., 1979*a*, *b*) and that the electrical potential difference across that barrier is largely a K-diffusion potential. Other noteworthy points are that (i) $(Na)_c$ does not differ significantly from that observed when both sides of the tissue are perfused with the control saline solution and (ii) P^{m}_{Na} is significantly lower in the presence of the high-K serosal solution than under control conditions (0.007 and 0.012 cm/hr, respectively). Possible explanations for the latter finding will be presented below.

Discussion

A method has been described for determining the current-voltage relations of the barriers to ion movements across rabbit descending colon. Because brief, bipolar pulses were employed, these *I-V* relations may be considered "instantaneous" with respect to the epithelium inasmuch as intracellular ionic composition is not likely to be affected. At the same time, the pulses are of sufficient duration so that the ionic profiles *within* the limiting membranes, and hence the conductance of these barriers, will have achieved new steady-states at each different value of the transmembrane electrical potential difference. Thus, the method permits us to examine the electrical properties of the barriers without altering the steady-state composition of the cell. The ramifications of this point with respect to the interpretation of the electrophysiologic data have been discussed elsewhere (Schultz, Thompson & Suzuki, 1981*a*).

Transepithelial I-V Relations

The transepithelial *I-V* relation (Fig. 3) under control conditions is approximately linear over a range from -200 to $+100$ mV consistent with observations in other Na-transporting epithelia such as toad colon (Macchia & Helman, 1979), toad urinary bladder (Civan, 1970; Saito, Lief & Essig, 1974; Spooner & Edelman, 1975; Macchia & Helman, 1979; Palmer et al., 1980), frog skin (Helman & Fisher, 1977; this laboratory, *unpublished*), and *Nec-*

Table 2. Effect of high serosal K^+

	(*n*)	I_{sc}	g_t	${}_0\psi^{cs}$ (mV)	P^{m}_{Na} (cm/hr)	E^{m}_{Na} (mV)	*f*	r^m/r^s
Control	(8)	61 ± 13[a]	6.1 ± 0.7[a]	39 ± 2	0.012 ± 0.002	66 ± 5	0.75 ± 0.02	3
High-K^+	(5)	-49 ± 22	5.0 ± 1.5	1 ± 1	0.007 ± 0.001	70 ± 13	0.87 ± 0.02	6.7

[a] For only those tissues mounted mucosal side up the average values were $I_{sc}=79\pm13\,\mu A/cm^2$ and $g_t=4.7\pm0.3$ mS/cm^2.

turus urinary bladder (this laboratory, *unpublished*). In the presence of amiloride the transepithelial *I-V* relation is also nearly linear over the same range of ψ^{ms} but the slope is decreased due to inhibition of the Na conductance across the apical membrane.

The intersection of the transepithelial *I-V* relations obtained in the absence and presence of amiloride ($\psi^{ms} \cong 120$ mV) occurs at or near the value of ψ^{ms} where a decided curvilinearity in the direction of the increasing tissue conductance (g_t) begins; thereafter, the two *I-V* relations almost overlap. Similar bends or "breaks" in the transepithelial *I-V* relations have been reported for frog skin (Helman & Fisher, 1977), toad colon (Macchia & Helman, 1979), and toad urinary bladder (Civan, 1970; Macchia & Helman, 1979; Palmer et al., 1980). For the case of frog skin (Helman & Fisher, 1977, this laboratory, *unpublished observations*) breaks sometimes occur in the direction of decreased g_t; in the case of rabbit colon, the bends were always in the direction of increasing g_t.[3]

We will consider the significance of the point of intersection and the mechanism of the inflection separately for reasons that will become clear.

The value of ψ^{ms} at which the transepithelial *I-V* relations in the absence and presence of amiloride intersect is clearly that value of ψ^{ms} at which I^{ms} is not affected by inhibition of Na entry into the cell across the apical membrane. In other words, the intersection occurs at that value of ψ^{ms} at which $I^{m}_{Na} = 0$ and $\psi^{mc} = E^{m}_{Na}$ (*see* Fig. 8). Thus the point of intersection is a function of f (which influences ψ^{mc} at any value of ψ^{ms}) and $(Na)_c$ (which determines E^{m}_{Na}). Although $(Na)_c$ is certainly determined, in part, by the activity of the pump mechanism at the basolateral membrane, it is clear that the value of ψ^{ms} where the instantaneous transepithelial *I-V* relations in the absence and presence of amiloride intersect, the "instantaneous" E_{Na}, is not directly related to the energetics of the pump. Clearly, transcellular Na transport must cease when Na entry ceases regardless of the properties of the pump.

The mechanism responsible for the "inflection" in the *I-V* relation is not entirely clear, but inspection of Figs. 3 and 6 indicate that it occurs at a point on the *I-V* relations where several events take place, namely: (i) $g_t = g_t'$ and both begin to increase (Fig. 3); (ii) $f = f'$ and both begin to decrease (Fig. 6); (iii) $\psi^{mc} = \psi^{mc'} = \psi^{cs} = \psi^{cs'} \cong \psi^{ms}/2$; (iv) $\psi^{mc} = \psi^{mc'} = E^{m}_{Na}$; and (v) thereafter, ($\psi^{mc} \cong \psi^{mc'}$) and is greater than ($\psi^{cs} \cong \psi^{cs'}$). Clearly part of the reason for the increase in g_t is a decrease in r^m as indicated by the decrease in f. Inasmuch as f' decreases to the same extent, the decrease in r^m must involve a pathway other than the amiloride-sensitive Na pathway. However, it can be readily shown that the decrease in f, and hence r^m, is insufficient to account for the entire increase in g_t when $\psi^{ms} > 160$ mV. The only other alternative is a decrease in r^p at large hyperpolarizing values of ψ^{ms} as has been reported by Bindslev, Tormey, Pietras and Wright (1974) and Finn and Rogenes (1980) for toad urinary bladder.

These interpretations are strongly supported by the *I-V* relations determined when the serosal surface of the tissue was bathed with a high-K solution. As illustrated in Fig. 9, under these conditions there are two inflections in the direction of increasing g_t in the range $\psi^{ms} > 0$. The first is small, but distinct, and occurs when $\psi^{ms} \cong 55$ mV where the two curves intersect. Since under these conditions r^s and ψ^{cs} are very small, this point corresponds to the potential where $\psi^{ms} \cong \psi^{mc} \cong E^{m}_{Na}$ (*see* Fig. 10). The fact that this inflection point is displaced to the left by approximately 55 mV (*compare* Fig. 3) when the basolateral membrane is depolarized indicates that this rectification is the result of a decrease in the resistance of a barrier *in series* with the basolateral membrane; namely, a decrease in r^m. Further, the finding that, as under "control" conditions, the inflections in the absence and presence of amiloride are identical (i.e., the two curves overlap) means that the decrease in r^m is due to a decrease in the resistance of a pathway in parallel with the amiloride-sensitive pathway. Palmer et al. (1980) arrived at a similar conclusion concerning the rectifying element in the apical membrane from their studies of the transepithelial *I-V* relation of toad urinary bladder under control conditions and in the presence of a high (depolarizing) serosal K concentration. The second, much sharper inflection begins when $\psi^{ms} \geqq 150$ mV, as was found in the "control" tissues (Fig. 3), suggesting that the barrier responsible for this increase in g_t must be *in parallel* with the basolateral membrane of the amiloride-sensitive cells; in short, this must be due to a decrease in r^p.

Civan (1970) and, more recently, Helman and his collaborators (Helman, O'Neil & Fisher, 1975; Helman & Fisher, 1977; Macchia & Helman, 1979) have identified the point of inflection of the transepithelial *I-V* relations with the E_{Na} of the tissue (Ussing & Zerahn, 1951) or the value at which ψ^{ms} must be clamped to abolish transcellular active Na transport. Indeed, many direct estimates of this "static head ψ^{ms}" fall in the range 100–150 mV where the bend begins (Ussing & Zerahn, 1951; Ussing, 1960;

[3] Helman and Fisher (1977) report sharp "breaks" in the transepithelial *I-V* relations across isolated frog skin. We and others have observed gradual bends with an indistinct inflection point. In this discussion we will employ "bends" and "breaks" synonymously.

Saito et al., 1974; Chen & Walser, 1975; Helman et al., 1975; Wolff & Essig, 1977). For rabbit colon, Schultz et al. (1977) estimated a value of $E_{Na} = 100$ mV from studies of steady-state transepithelial fluxes of Na when the tissue was clamped at -50, 0, and $+50$ mV. This estimate is in reasonable agreement with the "instantaneous E_{Na}" of 115 ± 5 mV found in the present studies. However, as discussed above and elsewhere (Schultz et al., 1981*a*), the most that can be confidently concluded from these observations is that the values of E_{Na} determined from instantaneous *I-V* relations as well as those determined under steady-state conditions are simply the values of ψ^{ms} at which $\psi^{mc} = E^m_{Na}$; there need not be any direct relation between these values and the potential energy of the pump. Further, although it seems that the bend in the transepithelial *I-V* relations begins at or near this value of ψ^{ms} ("E_{Na}"), the increase in slope conductance does not appear to be due to changes in the resistance of the basolateral membrane or the amiloride-sensitive Na entry step, and thus does not appear to reflect changes in the properties of the active Na transport pathway. Nevertheless, it would be premature to conclude that the fact that the bend begins at or near the value of ψ^{ms} when $\psi^{mc} = E^m_{Na}$ is simply a fortuitous coincidence. Clearly addtional studies are needed to elucidate the ionic pathway responsible for this rectification and its functional significance.

In summary: The departure from linearity ("break") in the transepithelial *I-V* relations occurs at a point where there is an interesting confluence of electrophysiological events and cannot be fully explained at this time. Suffice it to say that at or near this point $\psi^{mc} = E^m_{Na}$ so that Na entry is abolished; this can account for the inhibition of transcellular Na transport without having to invoke any effect of ψ^{ms} (or ψ^{cs}) on the pump mechanism. The increase in transepithelial slope conductance observed at this point does not appear to involve steps generally considered to be part of the active transcellular Na transport pathway.

The I-V Relation of the Sodium Entry Step

As illustrated in Fig. 8, the relation between I^m_{Na}, determined using Eq. (1), and ψ^{mc} conforms closely to the Goldman-Hodgkin-Katz (Goldman, 1943; Hodgkin & Katz, 1949) constant-field flux equation over a wide range (-120 mV $< \psi^{mc} < +50$ mV). The permeability of the apical membrane to Na (P^m_{Na}) in these experiments averaged 0.012 cm/hr in excellent agreement with that calculated previously (0.011 cm/hr) by Schultz (1979) based on data obtained using another approach (Schultz et al., 1977). The value of E^m_{Na}, the reversal potential, averaged 66 mV; this value is also in excellent agreement with the values of 64 and 60 mV reported by Schultz et al. (1977) and Wills et al. (1979*b*) for this epithelium and the value of ~ 60 mV reported by Frömter and Gebler (1977) for *Necturus* urinary bladder. The corresponding value of the intracellular Na activity, $(Na)_c$, in rabbit colon, calculated using the Nernst equation, which assumes that Na entry is driven entirely by its electrochemical potential difference and is uninfluenced by nonconjugate driving forces, is ~ 10 mM. This value is in excellent agreement with the values of $(Na)_c$ determined for bullfrog (Lee & Armstrong, 1972) and *Necturus* (O'Doherty, Garcia-Diaz & Armstrong, 1979) small intestine, rabbit urinary bladder (Lewis & Wills, 1980; Wills & Lewis, 1980), and *Necturus* gallbladder (Graf & Giebish, 1979) using ion-selective microelectrodes. It is also in reasonable agreement with the values of the intracellular Na *concentration* determined for frog skin (Rick, Dorge, Von Arnim & Thurau, 1978) and toad urinary bladder (Rick & Thurau, 1978; Civan, Hall & Gupta, 1980) using electron microprobe analysis.

As noted above, the relation between I^m_{Na} and ψ^{mc} illustrated in Fig. 8 employs values of I^m_{Na} derived using Eq. (1). The derivation of this equation from the equivalent electrical circuit model illustrated in Fig. 7 is based on a number of assumptions (Appendix). One is that the parallel "shunt" resistance is not affected by amiloride over the timeframe of these studies; this assumption is supported by the finding that the unidirectional flux of Na from the serosal to the mucosal solution and the bidirectional tracer fluxes of K are not affected by this agent (Frizzell et al., 1976). Similar conclusions have been drawn for frog skin (O'Neil & Helman, 1976). The other assumptions (p. 46) will be justified in the subsequent paper (II – Thompson et al., 1982).

Additional support for our approach is derived from the results obtained when the tissue was bathed with a serosal solution containing 143 mM K. The latter approach was introduced by Lindemann and his collaborators and is based on the rationale that if the basolateral membrane is predominantly K-selective, then bathing that surface with a solution containing a high K concentration should depolarize that barrier and markedly reduce its resistance. Under these circumstances, the electrical properties of the epithelium will be dominated by the apical membrane and, in essence, the series "double-membrane" array is reduced to a single resistive barrier. Experimental support for this approach had been provided earlier by Rawlins, Mateu, Fragachan and

Whittembury (1970), and this approach was employed successfully in the studies of Morel and LeBlanc (1975) dealing with the properties of the apical membrane of frog skin. Using this technique, Fuchs et al. (1977) demonstrated that the *I–V* relation of the amiloride-sensitive entry step across the outer-facing membrane of frog skin conforms to the constant-field flux equation over the range $0 < \psi^{mc} \leqq 60$ mV; the value of P_{Na}^m reported in these studies was approximately 0.03 cm/hr when the Na concentration in the outer bathing solution was 27 mM.[4] More recently Palmer et al. (1980) applied this technique to toad urinary bladder and also found that the *I-V* relation of the Na-entry step conformed to the constant-field flux equation over the range $-20\,\text{mV} < \psi^{mc} < +60\,\text{mV}$. These investigators further demonstrated that when the serosal bathing solution consisted of a normal Ringer's, the addition of nystatin to that solution markedly reduced ψ^{ms} and r_t. In contrast, when the serosal surface of the tissue was bathed with a high-K solution, the addition of nystatin had no significant effect on these parameters. These findings lend support to the notion that in the presence of high-K serosal solution the baso-lateral membrane resistance was very low and that this barrier was essentially depolarized.

The data reported in Table 2 indicate that when the serosal surface of rabbit colon is bathed with a high-K solution, r^s is reduced significantly and ${}_0\psi^{cs}$ is essentially zero so that $\psi^{mc} \cong \psi^{ms}$ at all values of I^{ms}. These findings are in accord with, and support, the conclusion of Wills et al. (1979*a*, *b*) that the baso-lateral membrane of this epithelium is predominantly K-selective. Clearly, under these conditions the amiloride-insensitive apical leak pathway (R_i^m) can be lumped together with the parallel pathways and I_{Na}^m can be determined directly from ΔI^{ms}, providing that r^p is not affected by amiloride. The close conformity between I_{Na}^m and ψ^{mc} (or ψ^{ms}) and the GHK equation in the presence of high serosal K (Fig. 10) re-enforces the validity of employing the equivalent electrical circuit model (Fig. 7) and Eq. (1) to derive the values I_{Na}^m under control conditions (Fig. 8). Our findings also provide the first *direct* support for the approach employed by Lindemann and his collaborators.

As shown in Table 2, the value of E_{Na}^m and hence $(Na)_c$ determined in the presence of high serosal K are in remarkably good agreement with those observed under control conditions. However, in the presence of high serosal K, P_{Na}^m appears to be nearly 40% smaller (0.007 cm/hr) than under control conditions (0.012 cm/hr). One plausible explanation is that the reduced permeability is secondary to some alteration of the cytoplasmic ionic composition resulting from the sustained depolarization of the apical and basolateral membranes and/or changing the chemical gradients across the basolateral membrane. In this regard, it is a longstanding observation that removal of Na from the serosal bathing solution reduces Na transport by some epithelia (Bently, 1960; Navarte & Finn, 1980; Mandel & Curran, 1973). This has been attributed to a decrease in apical Na permeability due to an increase in cytosolic Ca resulting from the inhibition of a Na/Ca exchange mechanism located in the basolateral membrane (Grinstein & Erlij, 1978; Taylor & Windhager, 1979; Taylor, 1981). If an exchange mechanism is operative in the basolateral membrane of rabbit colon whereby Ca extrusion is driven (at least in part) by the electrochemical potential difference for Na, then removal of serosal Na and depolarization by K will certainly reduce, if not reverse, the driving force for Ca extrusion. Thus intracellular Ca may increase in the high K experiments and effect, in some manner, a decrease in P_{Na}^m.

Mechanism of Sodium Entry and the Regulation of Transcellular Na Transport

It is reasonably well established for several "tight" or "moderately tight" Na-transporting epithelia that, under physiologic conditions, the rate of Na entry across the apical membrane is the rate-limiting step in overall transcellular Na transport. That is, under most physiological conditions, the pump rate is not maximal or rate-limiting so that the fine regulation of transcellular Na transport, in the final analysis, is determined by the rate of Na entry across the apical membrane (Turnheim, Frizzell & Schultz, 1978; MacKnight, DiBona & Leaf, 1980).

The finding that the relation between I_{Na}^m and ψ^{mc} conforms to the GHK equation over a wide range suggests that the mechanism of entry is electrodiffusion via homogeneous, amiloride-sensitive pores or channels. As pointed out elsewhere (Schultz et al., 1981*b*), this finding does not preclude the possibility that entry is mediated by a "carrier mechanism." However, the findings that the single-site conductance of the amiloride-sensitive Na-entry mechanism at the apical membranes of frog skin (Lindemann & Van Driessche, 1977) and toad urinary bladder (Li, Palmer, Edelman & Lindemann,

[4] Considering the fact that the experiments on frog skin were performed in the presence of low Na concentrations as well as possible differences in effective surface area, the values for P_{Na}^m for frog skin and rabbit descending colon are in remarkably good agreement.

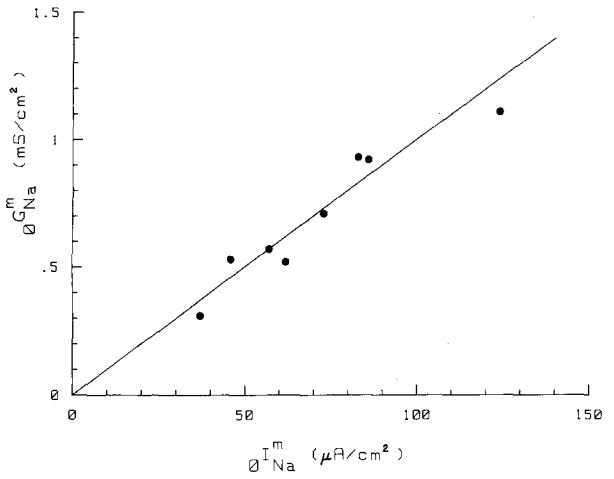

Fig. 11. $_0G^m_{Na}$ *vs.* $_0I^m_{Na}$ under steady-state conditions. The solid line is drawn from the origin through the mean values of $_0G^m_{Na}$ and $_0I^m_{Na}$ and has a reciprocal slope: $(E^m_{Na}-\psi^{mc})=100$ mV

1979) is in excess of 10^6 Na/sec favors the electrodiffusion model.

These findings also indicate that the rate of Na entry and hence the rate of transcellular Na transport under steady-state short-circuit conditions are given by

$$_0I^m_{Na}={}_0G^m_{Na}(E^m_{Na}-{}_0\psi^{mc})={}_0G^m_{Na}(\Delta\tilde{\mu}^m_{Na}/\mathcal{F}) \quad (3)$$

where $\Delta\tilde{\mu}^m_{Na}$ is the electrochemical potential difference for Na across the apical membrane in Joules/mole and $(\Delta\tilde{\mu}^m_{Na}/\mathcal{F})$ is that electrochemical potential difference expressed in mV. Clearly, then, $_0I^m_{Na}$ or I_{sc} may be influenced by changes in the Na conductance of the apical membrane *or* the driving force for Na entry *or* some combination of both.

In these studies, the values of $_0I^m_{Na}$ varied spontaneously over the range 37 to 124 μA/cm². Figure 11 gives the relation between $_0I^m_{Na}$ and $_0G^m_{Na}$ derived from the plots I^m_{Na} *vs.* ψ^{mc} for these eight tissues. Clearly, there is a close linear relation between these two variables ($r=0.95$; $p<0.001$).[5] This finding indicates that, under short-circuit conditions, spontaneous variations in the rate of active Na transport by this tissue, over a fourfold range, are *entirely* attributable to parallel variations in the conductance of the apical membrane to Na and that the "driving force" for the entry process (100 mV) remains essentially constant. As discussed elsewhere (Schultz, 1981), a similar conclusion can be drawn from published data dealing with rabbit urinary bladder (Lewis & Diamond, 1976; Lewis et al., 1976; Clausen, Lewis & Diamond, 1979), *Necturus* urinary bladder (Frömter & Gebler, 1977), and frog skin (Helman & Fisher, 1977).

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Appendix

Derivation of I^m_{Na}

We derive here an expression for I^m_{Na} which corrects for the change in current through the apical leak pathway when amiloride is applied.

According to the equivalent circuit model (Fig. 7) we may write the transepithelial current in the absence and presence (′) of amiloride as

$$I^{ms}=I^c+I^p=I^m_{Na}+I^m_i+I^p \quad (A1)$$

and

$$I^{ms'}=I^{c'}+I^p=I^{m'}_i+I^p \quad (A2)$$

so that

$$\Delta I^{ms}=\Delta I^c=I^m_{Na}+\Delta I^m_i \quad (A3)$$

where $\Delta I^m_i=I^m_i-I^{m'}_i$ (*see* Glossary for definition of terms).

Equation (A3) is based on the assumptions that during the time course of these studies amiloride increased R^m_{Na} to infinity but had no effect on R^p so that $I^p=I^{p'}$ at each ΔI^{ms}. Solving Eq. (A3) for I^m_{Na}, it is clear that I^m_{Na} differs from ΔI^{ms} by an amount equal to the change in current through the apical leak pathway, i.e.,

$$I^m_{Na}=\Delta I^{ms}-\Delta I^m_i \quad (A4)$$

where each term is evaluated at the same ψ^{ms}. The term ΔI^m_i may be expressed as

$$\Delta I^m_i=G^m_i(E^m_i-\psi^{mc})-G^{m'}_i(E^{m'}_i-\psi^{mc'}) \quad (A5)$$

which, assuming that $G^{m'}_i=G^m_i$, reduces to

$$\Delta I^m_i=G^m_i(\Delta E^m_i-\Delta\psi^{mc}). \quad (A6)$$

It should be noted that since amiloride causes a hyperpolarization of ψ^{mc} over a wide range of ψ^{ms} this assumption constrains our final expression for I^m_{Na} to the range of ψ^{ms} for which the *I-V* relation of the apical membrane in the presence of amiloride ($I^{c'}$ *vs.* ψ^{mc}) is linear; this relation is discussed in detail in the following paper (II).

Equation (A6) may be further simplified as follows:

$$\psi^{mc}=\psi^{ms}-\psi^{cs}=\psi^{ms}-E^s+I^cR^s \quad (A7)$$

and

$$\psi^{mc'}=\psi^{ms}-\psi^{cs'}=\psi^{ms}-E^{s'}+I^{c'}R^{s'} \quad (A8)$$

so that at constant ψ^{ms} and assuming $R^{s'}=R^s$

$$\Delta\psi^{mc}=-\Delta E^s+\Delta I^cR^s. \quad (A9)$$

The assumption that $R^{s'}=R^s$ further restricts the use of our final expression for I^m_{Na} to the range of ψ^{ms} for which the *I-V* relation of the basolateral membrane is linear; however, as will be demonstrated in (II) the range of ψ^{ms} over which this assumption is reasonable is quite broad. Thus from Eqs. (A3), (A4), (A6) and (A9) we may write:

$$I^m_{Na}=\Delta I^{ms}(1+R^s/R^m_i)-(\Delta E^m_i+\Delta E^s)/R^m_i. \quad (A10)$$

[5] Plots of $_0I^m_{Na}$ *vs.* $_0g^m_{Na}$ or P^m_{Na} (not shown) are also linear with correlation coefficients of 0.95 and 0.95, respectively.

Further, as will be shown in paper II, over the range of ψ^{ms} for which the above assumptions are valid, R^s and R_t^m are equal to their respective slope resistances so that we may write

$$1+R^s/R_t^m=1+r^s/r_t^m=1/f'. \tag{A11}$$

Finally, assuming that amiloride does not substantially affect E_t^m or E^s it follows that

$$I_{Na}^m \cong \Delta I^{ms}/f' \tag{A12}$$

where each parameter is measured at the same ψ^{ms}. Clearly, in the absence of a leak pathway in the apical membrane (i.e., as $R_t^m \to \infty$) both Eqs. (A10) and (A12) reduce to $I_{Na}^m = \Delta I^{ms}$ as required.

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