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Ion Transport by Rabbit Colon: II. Unidirectional Sodium Influx and the Effects of Amphotericin B and Amiloride

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Summary. The unidirectional influx of Na from the mucosal solution into the epithelium of *in vitro* descending rabbit colon (J^{Na}_{me}) determined under short-circuit conditions, is comprised of two components: one represents entry of Na into transporting epithelial cells and is abolished by amiloride which also abolishes Na absorption (J^{Na}_{net}). The other represents diffusional Na entry into paracellular pathways traversing the epithelium. In all instances, exposure of the mucosal surface to amphotericin B increased tissue conductance and J^{Na}_{me} and elicited K secretion. Tissues showing a spontaneous I_{sc} of approximately 4 μeq/cm²hr did not respond to amphotericin B with increased I_{sc} and J^{Na}_{net}. However, in tissues characterized by a lower I_{sc} under control conditions, amphotericin B increased I_{sc} and J^{Na}_{net} to approximately 4 μeq/cm²hr. These findings suggest that amphotericin increases J^{Na}_{net} and elicits K secretion by disrupting the normal permselectivity of the mucosal membrane. Under these conditions the extrusion of Na from cell-to-serosal solution becomes the rate limiting step in transepithelial Na transport. Finally, a close correlation between J^{Na}_{me} and J^{Na}_{net} was observed when the rate of Na absorption varied either spontaneously or experimentally with amiloride, suggesting that the backflux of Na from cell-to-mucosal solution is undetectably small.

The results of previous studies from this laboratory [13, 25] indicate that the electrical potential difference (ψ_{ms}) and short-circuit current (I_{sc}) across descending rabbit colon *in vitro* can be attributed entirely to the rate of active Na absorption (J^{Na}_{net}) from mucosa to serosa. Although Cl is actively absorbed, this appears to be the result of an electrically neutral anion-exchange process which may involve simultaneous HCO_3 secretion. Transepithelial movements of K appear to be restricted to diffusion through paracellular pathways, a finding which was attributed to a low mucosal membrane K permeability.

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The present studies were designed to examine further the properties of Na and K transport by this tissue. The unidirectional influx of Na from the mucosal solution into the epithelium was determined, and the effects of amiloride and amphotericin B on Na entry were evaluated. Under conditions where the rate of active Na absorption varied either spontaneously or consequent to treatment with amiloride a one-for-one correlation between unidirectional Na influx and net transcellular Na transport was observed. The effects of amphotericin on transepithelial Na and K transport indicate that this agent elicits active K secretion and induces a maximal rate of Na absorption which is consistent with saturation[1] of the active Na transport mechanism at the basolateral membranes. These effects appear to be explained solely by a disruption of the normal permselective properties of the mucosal membrane as a consequence of interaction with the antibiotic; there is no indication that this agent directly or indirectly affects the maximum activity of the basolateral Na pump.

Materials and Methods

Segments of descending colon were obtained from white rabbits (2–3 kg) of either sex which were sacrificed with pentobarbital. The tissue was opened along its mesenteric border and rinsed free of intestinal contents with a standard electrolyte solution containing (mM): Na, 140; Cl, 124; HCO_3, 21; K, 5.4; HPO_4, 2.4; H_2PO_4, 0.6; Mg, 1.2; Ca, 1.2; glucose, 10. This solution was employed for all studies except those (Figs. 3, 4 and 5) in which the Na concentration of the bathing media was reduced by isosmotic replacement of NaCl with choline Cl. The pH of these solutions was 7.4 at 37 °C when gassed with 95 % O_2 – 5 % CO_2.

Transepithelial Fluxes

Four segments of a "partial mucosal strip" preparation were mounted in the apparatus described by Schultz and Zalusky [26] for determination of simultaneous, bidirectional fluxes of ^{22}Na and ^{42}K under short-circuit conditions, as previously described [13]. Fluxes were evaluated during an initial 40-min period in the standard electrolyte solution (control), following which amphotericin (15 μg/ml) was added to the mucosal solution alone. After a 20-min equilibration period, which assured the achievement of a steady state *(see below)*, fluxes were determined for an additional 40-min period in the presence of amphotericin. This approach is justified by previous findings [13] that steady-state values of I_{sc}, tissue conductance (G_t) and the bidirectional fluxes of Na and K across this tissue under control conditions are maintained for at least 3 hr.

1 The term *saturation* does not necessarily refer to the maximal kinetic velocity of the basolateral Na pump. In the present context, saturation implies that Na extrusion from cell-to-serosal solution is the rate-limiting process in transepithelial Na transport, i.e., that this mechanism is operating at its maximal rate in the presence of the standard Ringer's solution.

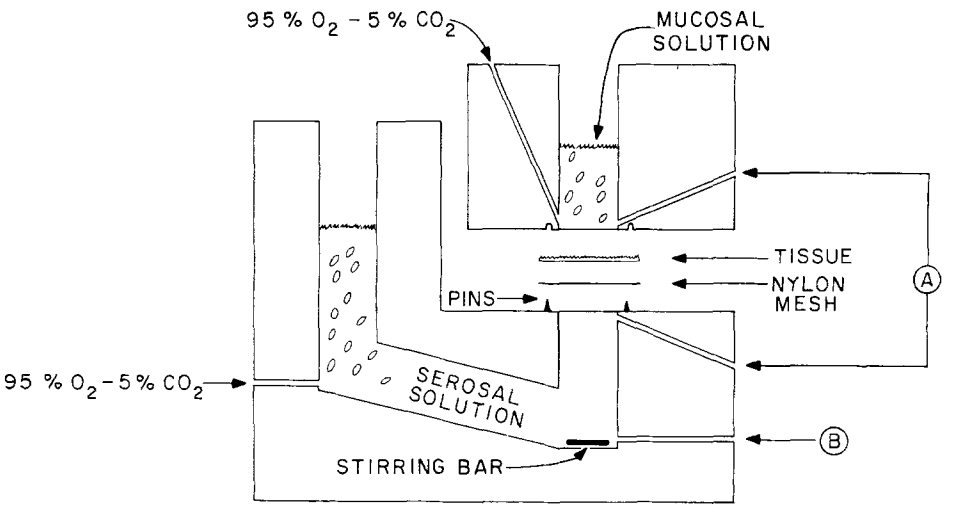

Fig. 1. Schematic of a portion of the chamber employed for determination of unidirectional Na influx under short-circuit conditions. Side view of a single port is shown; the entire chamber consisted of four ports in a row. Using two chambers, eight influx determinations were routinely performed on tissue from a single animal

Unidirectional Na Influxes

Segments of the "partial mucosal strip" preparation were mounted mucosal surface up in the chamber shown schematically in Fig. 1. This apparatus permitted exposure of defined areas (1.13 cm^2) of the mucosal surface to solutions of desired composition; the serosal surface rested on nylon mesh in contact with a serosal solution whose composition was identical to that bathing the mucosal surface. Both media were vigorously gassed with 95 % O_2 – 5 % CO_2, and in some experiments the serosal compartment was stirred with an externally driven magnetic stirring bar; paired studies using tissue from the same animal indicated that tissue viability was maintained for at least 1 hr with gassing alone. All experiments were performed at 37 °C.

The spontaneous transepithelial potential difference (ψ_{ms}) was monitored using Ringer's-agar bridges adjacent to each surface of the tissue (inlets *A*) and matched calomel electrodes leading to a Kiethley electrometer, model 600B. External current, sufficient to bring ψ_{ms} to zero, was applied via Ringer's-agar bridges inserted into inlet *B* and directly ino the mucosal solution.

The techniques employed to measure the unidirectional influx of Na from the mucosal solution into the epithelium (J_{me}^{Na}) have been previously described [14, 24]. Tissues were preincubated for approximately 30 min with mucosal *and* serosal solutions of composition identical to that used for the subsequent influx determination. The following procedure was then applied, sequentially, to each tissue: The spontaneous ψ_{ms} was recorded and the tissue was short-circuited. The mucosal preincubation solution was withdrawn and replaced by a test solution containing ^{3}H-PEG (1000 mol wt) and ^{22}Na via a fluid inlet to the mucosal compartment adjacent to inlet *A*, Fig. 1. Following a predetermined exposure time, the test solution was withdrawn and the mucosal compartment was flushed with ice-cold, isotonic mannitol solution (1–2 sec). The exposed tissue was cut out with a steel punch, washed briefly (1-2 sec) in ice-cold mannitol solution and

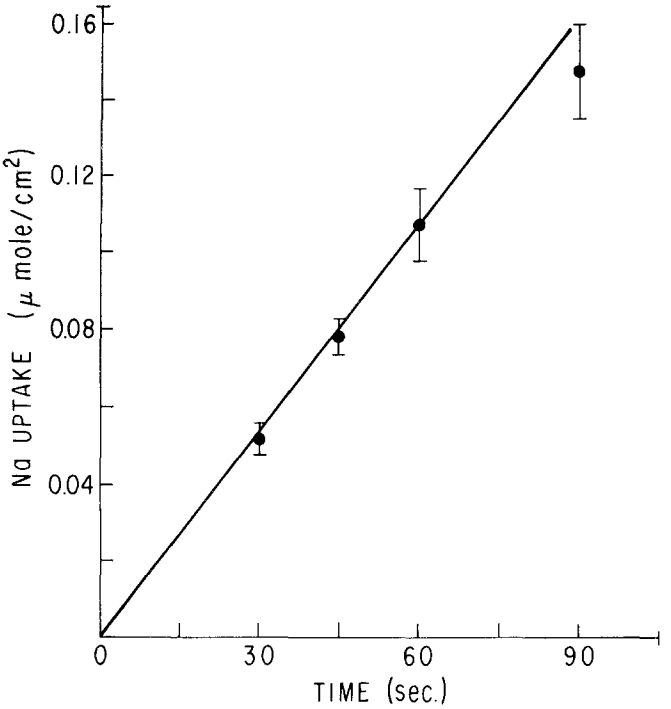

Fig. 2. Time course of Na uptake from the mucosal solution into the epithelium. Each point represents the mean of eight determinations

placed in 2 ml of 0.1 N HNO_3 for extraction with shaking (at least 2 hr). Na uptake was calculated from the ^{22}Na content of the tissue extract after correction for the volume of adherent test solution not removed by the mannitol washes, given by the ^{3}H-PEG "space."[2]

The uptake of Na from the mucosal test solution as a function of exposure time is illustrated in Fig. 2. As previously discussed [24], the zero intercept and linear time-course indicate that PEG is a reliable marker of the adherent mucosal volume and that the unidirectional influx of Na may be estimated from the rate of ^{22}Na uptake during exposure times up to 1 min. Thus, in subsequent experiments the duration of exposure to the mucosal test media was routinely 45 sec. Analysis of samples taken from the serosal solution immediately prior to terminating the influx measurement indicated that tracer had not crossed the tissue during this brief test period.

In some experiments, amphotericin (15 μg/ml) was added to alternate mucosal solutions for the entire 30-min preincubation period. In others, amiloride (10^{-4} M) was

2 If a significant amount of ^{22}Na left the tissue during the mannitol wash, influx would be underestimated. However, the duration of exposure to the labeled test media (45 sec) was considerably longer than the subsequent wash in ice-cold mannitol (3–4 sec). Therefore, an underestimation of less than 10% would be expected even *if* ^{22}Na were lost from the tissue at a rate equal to the rate of uptake. Since the Q_{10} of this process is probably large [24], it is likely that the mannitol wash does not complicate these measurements and that J_{me}^{Na} is reliably estimated with this technique.

added to alternate tissues at the end of the preincubation period, and sufficient time (1–2 min) was allowed for I_{sc} to achieve a new, stable value prior to the influx determination. In both instances, amphotericin or amiloride were also included in the test solutions to which those tissues were subsequently exposed.

Amphotericin was obtained as Fungizone® from Squibb. This preparation contains Na deoxycholate for solubilization of the antibiotic in aqueous media; in the amphotericin-containing solutions employed for most of the present studies, the final concentration of deoxycholate was 29 μM. Incubation of tissues with this concentration of deoxycholate alone had no effect on the short-circuit current or tissue conductance in several preliminary studies. Amiloride was a generous gift of Merck, Sharp and Dohme, West Point, Pa; ^{22}Na and ^{42}K were obtained from ICN Pharmaceuticals, and ^{3}H-PEG from New England Nuclear; other chemicals were of reagent grade.

Results are expressed as the mean ± SEM. The statistical significance of differences between mean values was analyzed using the Student t-test; a value of $p<0.05$ was considered significant.

Results

Unidirectional Na Influx and the Effect of Amiloride

The relation between the unidirectional influx of Na from the mucosal solution into the epithelium (J_{me}^{Na}) under short-circuit conditions and the Na concentration of the mucosal solution is illustrated in Fig. 3. J_{me}^{Na} is a curvilinear function of mucosal Na concentration, in agreement with the results obtained on other Na-transporting epithelia using similar techniques [3, 4, 7, 9, 20]. The results of prior studies [13] indicated that the entire serosa-to-mucosa flux of Na across rabbit colon conformed to the characteristics of a diffusional process, traversing a paracellular pathway. Thus, the measured J_{me}^{Na} should be comprised of this diffusional, paracellular component ($_dJ_{me}^{Na}$), as well as a component which represents entry of Na across the mucosal membranes of the epithelial cells (J_{mc}^{Na}). The lower dashed line shown in Fig. 3 represents the *expected* relation between $_dJ_{me}^{Na}$ and the Na concentration of the mucosal solution under short-circuit conditions. The equation describing this relation is:

$$_{0d}J_{me}^{Na} = 0.008\,[Na]_m$$

where $_{0d}J_{me}^{Na}$ represents Na influx into the paracellular pathways when $\psi_{ms}=0$; $[Na]_m$ is the Na concentration of the mucosal solution; and 0.008 is the Na permeability of the passive conductance pathways across rabbit colon expressed in cm/hr, obtained from prior studies [13]. The remainder of J_{me}^{Na} (following subtraction of the linear component ($_{0d}J_{me}^{Na}$) obeys Michaelis-Menten kinetics and represents entry of Na into the

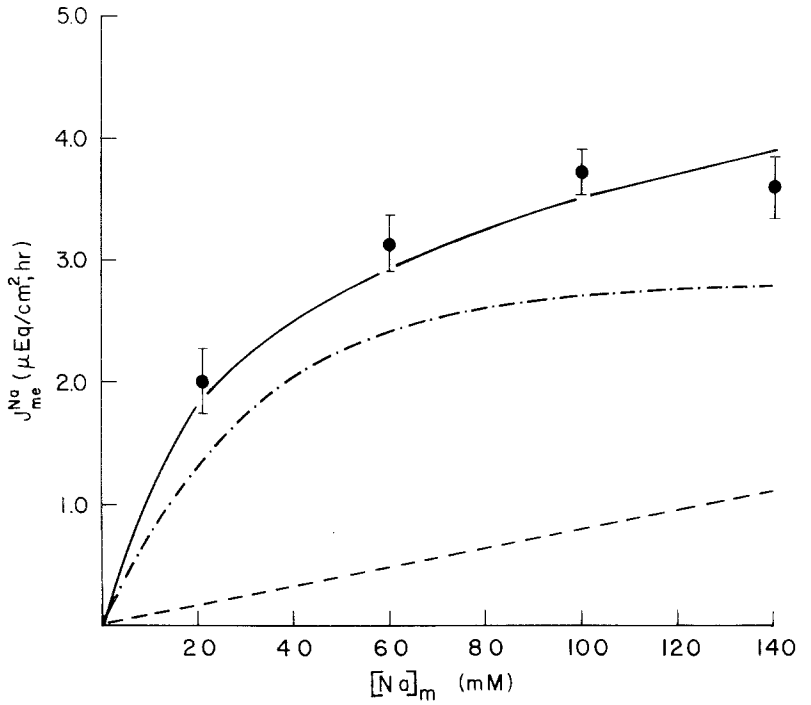

Fig. 3. Unidirectional Na influx as a function of the Na concentration of the mucosal solution. The dashed line (---) represents influx into paracellular pathways, ${}_{0d}J^{Na}_{me}$. The remainder of Na influx (-·-) following subtraction of the paracellular component, represents entry into Na-transporting epithelial cells, J^{Na}_{mc} (*see text* for further explanation). Each point is the mean of nine determinations

epithelial cells (J^{Na}_{mc}) *(see below)*; it is described by the relation

$$J^{Na}_{mc} = 3.2\,[Na]_m/(20 + [Na]_m),$$

and is also illustrated in Fig.3.

The inhibitory effect of amiloride on Na influx under short-circuit conditions is illustrated in Fig. 4, where the decrease in J^{Na}_{me} and I_{sc} elicited by amiloride (ΔJ^{Na}_{me} and ΔI_{sc}) are plotted as functions of the mucosal Na concentration. The amiloride-sensitive Na influx and I_{sc} are saturable functions of the mucosal Na concentration and the kinetic constants employed for plotting the curve shown in Fig. 4 and those describing the saturable component of total Na influx (Fig. 3) are virtually identical. The maximal amiloride-sensitive Na influx (Fig. 4) is 2.9 μeq/cm^2 hr and the maximal influx associated with the saturable component of J^{Na}_{me} (Fig. 3) is 3.2 μeq/cm^2 hr. In both instances, the Na concentration at which half-maximal influx is observed is 20 mM. These

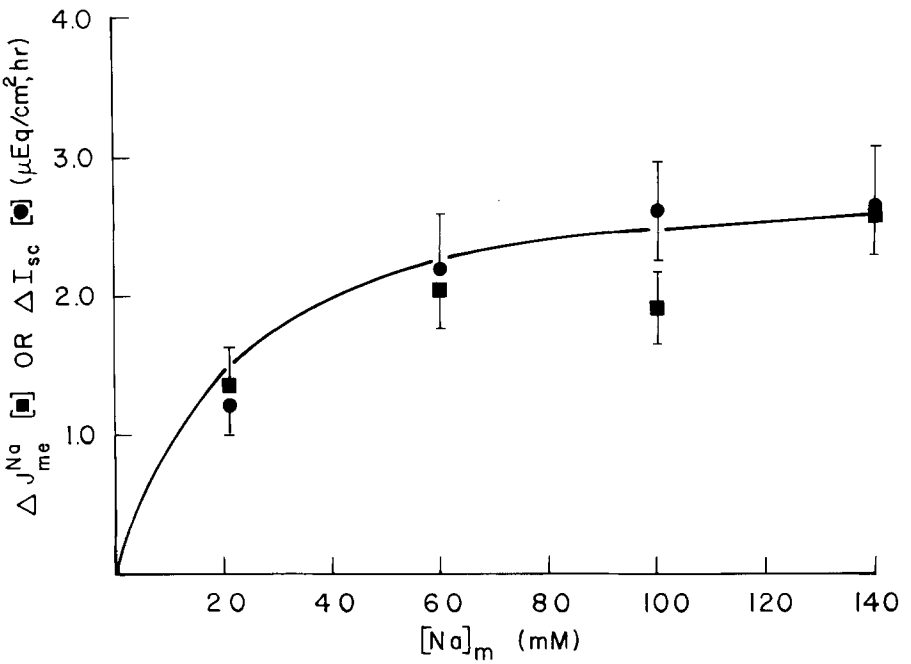

Fig. 4. Difference in unidirectional Na influx or short-circuit current in the presence and absence of amiloride (10^{-4} M) as a function of mucosal solution Na concentration. Each point represents the mean of differences obtained from nine tissue pairs, one of which was exposed to amiloride

findings strongly suggest that amiloride completely inhibits the saturable component of Na influx and that this component exclusively represents entry into Na-transporting epithelial cells, i.e., $\Delta J^{Na}_{me} = J^{Na}_{mc}$.

In addition, since both the I_{sc} and net Na transport across this tissue are abolished by amiloride [13, 25], the values of ΔI_{sc} plotted on the ordinate of Fig. 4 represent the rate of net Na absorption as a function of mucosal Na concentration. Under steady-state conditions, net Na movement across the mucosal membrane must equal the rate of net transepithelial Na transport. The close agreement between ΔI_{sc} and the amiloride-sensitive Na influx (ΔJ^{Na}_{me}) strongly suggests that, within experimental error, the efflux of Na from cell-to-mucosal solution, J^{Na}_{cm}, is undetectably small.

This close correlation between unidirectional Na influx and net Na transport (I_{sc}) is apparent when data at individual Na concentrations are evaluated. The spontaneous variations in I_{sc} from animal to animal observed in this and prior studies [13] affords an appraisal of the relation between J^{Na}_{me} and I_{sc}. Figure 5 illustrates the data obtained using

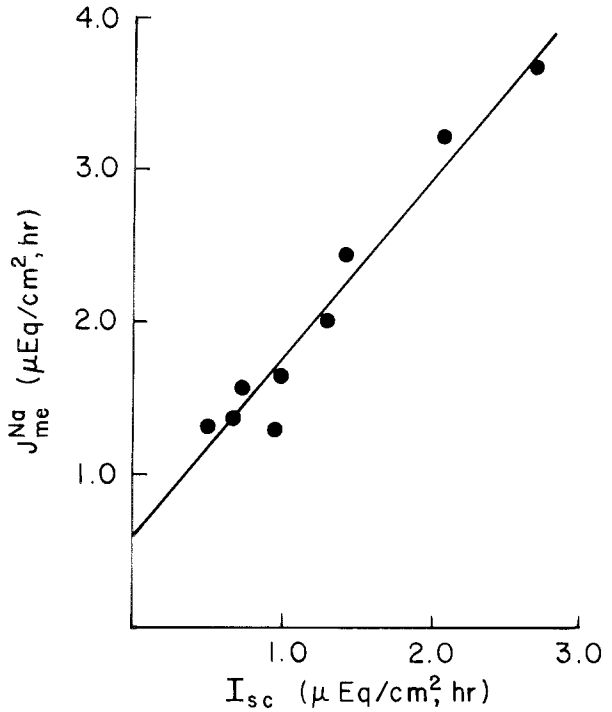

Fig. 5. Unidirectional Na influx as a function of the simultaneously-determined short-circuit current. Individual determinations from nine animals are shown. Mucosal solution Na concentration was 21 mM; the mean of the values of J^{Na}_{me} is that shown in Fig. 3 at this $[Na]_m$

tissues from nine animals in which the short-circuit current spontaneously varied between 0.5 and 2.7 μeq/cm^2 hr. Clearly, a close correlation between Na influx and I_{sc} is observed; linear regression analysis indicates that the data are described by the equation,

$$J^{Na}_{me} = (1.2 \pm 0.2)\, I_{sc} + (0.6 \pm 0.3),$$

whose slope does not differ significantly from unity. The small, but significant, intercept on the ordinate represents that fraction of Na influx which is not related to net Na transport and probably reflects Na entry into paracellular pathways, ${}_{0d}J^{Na}_{me}$, in agreement with the analysis presented above.

Effects of Amphotericin B

The effects of amphotericin on I_{sc} and G_t are illustrated as a function of the amphotericin concentration of the mucosal solution in Fig. 6. With

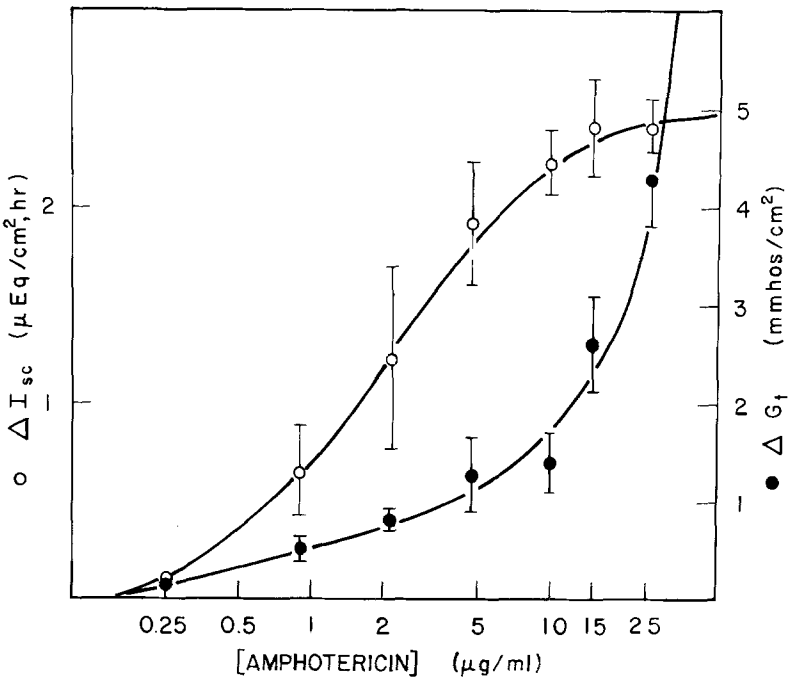

Fig. 6. Steady-state changes in short-circuit current (o) and tissue conductance (●) as a function of the amphotericin concentration of the mucosal solution. Both ΔI_{sc} and ΔG_t were determined using ten tissues at each amphotericin concentration; $[Na]_{m,s}=140$ mM

increasing amphotericin concentration, tissue conductance rose steadily. In contrast, the short-circuit current increased to a plateau level and further elevation of the amphotericin concentration produced no additional increase in I_{sc}. A maximal increment in the I_{sc} could be achieved in the presence of 15 μg/ml of the antibiotic, and this was the concentration chosen for all subsequent studies. The significance of the limiting value of I_{sc} achieved at this amphotericin concentration will be discussed further below. Addition of the antibiotic to the serosal solution alone had no effect on the I_{sc} or on G_t.

The time course of the increase in short-circuit current elicited by addition of 15 μg/ml amphotericin to the mucosal solution alone is illustrated in Fig. 7. Increases in tissue conductance followed a similar time course. Although a transient stimulation of the I_{sc} has been reported for a variety of other tissues [5, 12, 23], in rabbit colon amphotericin elicited a twofold increase in the short-circuit current which was maintained for a period of time sufficient to determine steady-state bidirectional ionic fluxes across the epithelium under short-circuit conditions. Transepithelial fluxes of Na and K were simultaneously de-

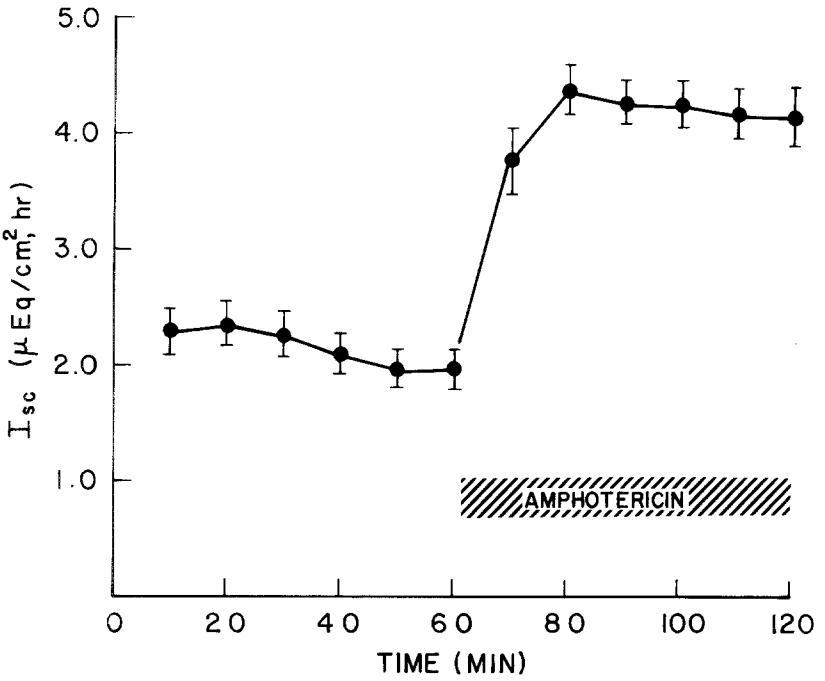

Fig. 7. Short-circuit current as a function of time for 28 tissues before and after addition of 15 μg/ml amphotericin to the mucosal solution; $[Na]_{m,s} = 140$ mM

Table 1. Effect of amphotericin on Na and K fluxes across rabbit colon[a]

	J^{Na}_{ms}	J^{Na}_{sm}	J^{Na}_{net}	J^{K}_{ms}	J^{K}_{sm}	J^{K}_{net}	I_{sc}	G_t
Control [14]	3.8 ± 0.3	1.5 ± 0.1	2.3 ± 0.3	0.39 ± 0.06	0.45 ± 0.07	−0.06 ± 0.12	2.1 ± 0.2	4.7 ± 0.
Ampho-tericin 15 μg/ml	6.5 ± 0.4	2.0 ± 0.1	4.5 ± 0.3	0.32 ± 0.04	1.10 ± 0.07	−0.78 ± 0.10	4.3 ± 0.2	6.9 ± 0.

[a] Na and K fluxes were determined simultaneously during an initial control period and following addition of amphotericin to the mucosal solution alone. All values are expressed in μeq/cm^2 hr, except G_t which is in mmhos/cm^2. The number of experiments is given in parenthesis.

termined before (20 to 60-min period) and after (80 to 120-min period) addition of amphotericin to the mucosal solution alone; the results of these studies are presented in Table 1.

As shown in Table 1, under control conditions there was good agreement between the rate of net Na absorption and the I_{sc}; net K transport was not observed. In the presence of amphotericin, both I_{sc} and J^{Na}_{net} increased twofold. The increase in active Na absorption could be attributed almost entirely to an increase J^{Na}_{ms}. Addition of amphotericin to

Table 2. Effect of amphotericin on Na influx[a]

	I_{sc}	J_{me}^{Na}	G_t
Tissues not responding			
Control [13]	3.3 ± 0.2	3.9 ± 0.3	3.5 ± 0.2
Amphotericin	3.5 ± 0.2	5.1 ± 0.4	4.1 ± 0.3
Tissues responding			
Control [14]	1.5 ± 0.3	3.2 ± 0.3	3.8 ± 0.1
Amphotericin	3.5 ± 0.2	6.0 ± 0.5	4.4 ± 0.3

[a] Amphotericin (15 μg/ml) was added to the mucosal solution alone of alternate, paired tissues 20 min prior to the influx determination. The criteria employed for designating tissues as "responding" or "not responding" are given in the text. I_{sc} and J_{me}^{Na} are expressed in μeq/cm² hr; G_t in mmhos/cm². The number of tissues studied is given in parentheses.

the mucosal bathing solution also elicited net K secretion under short-circuit conditions which was due entirely to an increase in J_{sm}^{K}. Comparing mean values, there was still close agreement between J_{net}^{Na} and I_{sc}. However, K secretion may contribute directly to the short-circuit current in the presence of amphotericin. Given the errors involved in these determinations and the fact that J_{net}^{K} is relatively small compared to J_{net}^{Na}, the relation $I_{sc} = J_{net}^{Na} + J_{net}^{K}$ cannot be excluded. Alternatively, amphotericin may result in other ion movements (e.g., Cl loss from the tissue) which tend to obscure the current associated with K secretion. Amphotericin has been reported to increase the Cl permeability of the mucosal or outer membranes of toad bladder [19] and frog skin [21].

The effects of amphotericin on G_t and J_{net}^{K} (Table 1) were observed in *all* tissues employed in this study; however, four tissues did not respond with increases in I_{sc} and J_{net}^{Na}. The short-circuit current of these four tissues under control conditions averaged 4.1 μeq/cm² hr, a value in close agreement with that observed for all tissues in the presence of amphotericin. This relation between the control I_{sc} and its response to amphotericin was more apparent in another series of experiments, designed to evaluate the effects of amphotericin on J_{me}^{Na}. Tissues characterized by a low spontaneous I_{sc} responded to amphotericin with an increase in I_{sc}; however, the antibiotic had no significant effect on I_{sc} when it was spontaneously high. These data are summarized in Table 2, where the following criteria were employed to divide the tissues into two populations: If, using paired tissues from the same animal, the I_{sc} in the

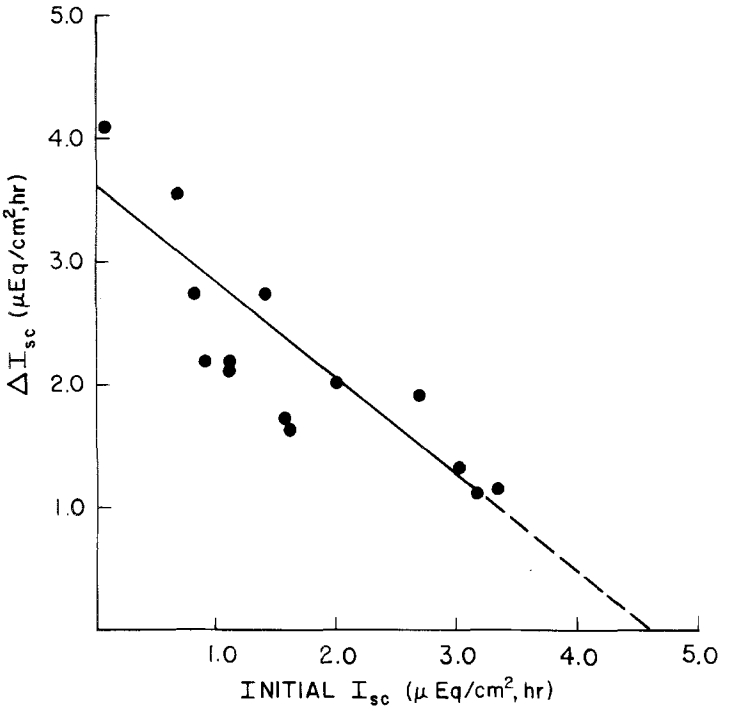

Fig. 8. Relation between the difference in $I_{sc}(\Delta I_{sc})$ of paired control and amphotericin-treated tissues and the I_{sc} of the controls (initial I_{sc}). Data derived from the 14 tissue pairs which responded to amphotericin (Table 2)

presence of amphotericin did not differ by at least 25 % from that of its corresponding control, the tissues were characterized as "not responding". Those tissues showing a greater than 25 % increase in I_{sc} were regarded as "responding" to amphotericin. In this series of experiments, roughly 50 % of the tissues fell into each group. The results indicate that the final I_{sc} of those tissues which responded to amphotericin was in good agreement with that observed in nonresponding tissues prior to addition of the antibiotic. However, regardless of its influence on the I_{sc}, amphotericin increased J_{me}^{Na} and G_t of both groups. Thus, the absence of an effect of amphotericin on the I_{sc} of tissues that displayed high control I_{sc}'s cannot be attributed to an inability of amphotericin to enhance Na entry into those tissues, rather, some other step in the overall transport process must be rate-limiting under these conditions.

The increase in I_{sc} observed in those tissues which responded to amphotericin was compared with the spontaneous or "initial" I_{sc} of their paired controls; the resulting relation is illustrated in Fig. 8. There is an

inverse relation between the extent to which amphotericin increased the $I_{sc}(\Delta I_{sc})$ and the I_{sc} observed in the absence of the antibiotic, which indicates that when the spontaneous I_{sc} is 4–5 μeq/cm² hr, amphotericin cannot increase it further. Similarly, when the I_{sc} is spontaneously close to zero, the increase elicited by amphotericin is roughly 4 μeq/cm² hr. The slope of the regression line which describes the data is −0.80 and does not differ significantly from unity. Thus, over a considerable range of values, the extent to which amphotericin can increase the I_{sc} is determined, in an inverse manner, by the spontaneous rate of Na transport under control conditions.

Discussion

Unidirectional Sodium Influx (J_{me}^{Na})

The purpose of the present study was to examine the first step in the process of Na absorption by descending rabbit colon: the unidirectional influx of Na from the mucosal solution into the epithelium. Two parallel components of J_{me}^{Na} can be identified (Figs. 3 and 5): (i) a diffusional component, ${}_{0d}J_{me}^{Na}$, which is not related to J_{net}^{Na} and whose magnitide is consistent with previous estimates of Na movements via transepithelial, paracellular pathways under short-circuit conditions [13], and (ii) a component, J_{mc}^{Na}, which represents Na uptake across the mucosal membranes of the Na-transporting epithelial cells and which saturates with increasing $[Na]_m$. Thus, in analogy with the formulation employed by Biber and Curran [3] for isolated frog skin, total Na influx under short-circuit conditions, J_{me}^{Na}, can be described by the relations:

$$J_{me}^{Na} = J_{mc}^{Na} + {}_{0d}J_{me}^{Na};$$

$$J_{me}^{Na} = \frac{(J_{mc}^{Na})_{max} \cdot [Na]_m}{K_t + [Na]_m} + P_{Na}[Na]_m;$$

where $[Na]_m$ is the Na concentration of the mucosal solution; $(J_{mc}^{Na})_{max}$ represents the maximal influx across the mucosal membranes of the epithelial cells; K_t is the $[Na]_m$ at which this rate is half-maximal and P_{Na} is the permeability of the paracellular pathways to Na. In these studies, $(J_{mc}^{Na})_{max}$ averaged 3 μeq/cm² hr and K_t averaged 20 mM; the latter is in good agreement with data obtained on other Na-transporting epithelia [3, 9, 18, 20].

The present findings disclose a strict relationship between the rate of Na absorption and J_{mc}^{Na}. Both spontaneous variations in I_{sc} (Fig. 5) and amiloride-induced reductions in I_{sc} (Fig. 4) are paralleled by changes in J_{mc}^{Na} on a one-for-one fashion. This equivalent relation between unidirectional Na influx and the simultaneously determined I_{sc} has been observed in frog skin by Biber [2], Rick *et al.* [22] and Leblanc and Morel [17] and in toad colon by Dawson and Curran [7]. In frog skin, Cruz and Biber [6] found a similar relation between transepithelial Na transport and unidirectional Na influx when these movements were stimulated by novobiocin. In addition, we have recently shown that aldosterone elicits a marked increase in Na absorption (and I_{sc}) across rabbit colon which is accompanied by an equivalent increase in J_{mc}^{Na} [15]. The agreement between J_{mc}^{Na} and J_{net}^{Na} under steady-state conditions suggests that unidirectional Na efflux from cell to mucosal solution, J_{cm}^{Na}, is undetectably small. If the movement of Na across the mucosal membrane is diffusional, the relative magnitude of J_{cm}^{Na} can be predicted from the Ussing flux-ratio equation, given our previous estimates of the electrical potential difference across the mucosal membrane under short-circuit conditions (ca. 45 mV, cell interior negative) and of cell Na activity (one-tenth that of the mucosal solution) obtained from electrophysiologic studies [25]. Using these values, the predicted ratio of efflux to influx (J_{cm}^{Na}/J_{mc}^{Na}) is 0.02, so that a difference between J_{mc}^{Na} and J_{net}^{Na} could not be detected experimentally. This conclusion is consistent with the kinetics of Na washout from the Na transport pool of frog skin determined by Dorge and Nagel [8], who estimated that the flux ratio across the outer barrier (analogous to J_{mc}^{Na}/J_{cm}^{Na}) is in the range of 20–40. Indeed, there is only one instance in which changes in J_{me}^{Na} and I_{sc} are not correlated: in tissues characterized by a high spontaneous I_{sc}, amphotericin increases J_{mc}^{Na} without significantly affecting the simultaneously-determined I_{sc}. A possible explanation for this dissociation between unidirectional Na influx and net Na transport will be discussed below.

Effect of Amiloride on J_{mc}^{Na}

Previous studies have demonstrated that addition of amiloride to the mucosal solution abolishes J_{net}^{Na} and I_{sc} across rabbit colon [13]. As shown in Fig. 4, amiloride abolishes Na absorption by inhibiting Na influx from the mucosal solution into the epithelial cells as observed for other Na-transporting epithelia [2, 8, 9, 20]. In addition, the agreement

between amiloride-sensitive J_{me}^{Na} and I_{sc} illustrated in Fig. 4 further supports the conclusion that there is a strict one-for-one relation between the unidirectional influx of Na across the mucosal membranes (J_{mc}^{Na}) and the rate of transepithelial Na transport so that all of the Na that enters the cells across the apical membrane is destined for active transcellular transport. In other words, none of the Na that crosses the mucosal membrane enters cells or compartments that are not involved in active transcellular Na transport.

Effects of Amphotericin B

Results derived from studies of lipid bilayers and biological membranes doped with amphotericin indicate that this substance forms relatively nonselective pores of 3–4 Å radius which permit passage of ions and small nonelectrolytes across these barriers [10]; only sterol-containing membranes are affected in this manner. Several investigators have demonstrated that addition of amphotericin to the solution bathing the mucosal (outer) surface of Na-transporting epithelia results in increased tissue conductance and enhanced Na absorption [1, 19]. These findings were interpreted as indicating that amphotericin increased the ease with which mucosal solution Na gained access to the Na "pump" at the basolateral (inner) membrane. Thus, according to the classical, series-membrane model of epithelial cells [16], an amphotericin-induced increase in Na absorption would indicate that the entry of Na across the mucosal membrane is normally the rate-limiting step in transepithelial Na transport. Conversely, if the Na pump at the basolateral membrane were already saturated (rate-limiting), a further increase in Na entry would not be expected to affect J_{net}^{Na}. The results of the present study are consistent with the interpretation that the rate-limiting step in Na absorption by rabbit colon may reside at either the mucosal or basolateral membrane, determined by variations in mucosal membrane Na permeability. These observations are as follows: (i) as the concentration of amphotericin bathing the mucosal surface is increased, I_{sc} reaches a maximal, plateau value but tissue conductance continues to rise (Fig. 6); (ii) amphotericin has no effect on the I_{sc} of tissues absorbing Na at a rate of approximately 4 μeq/cm^2 hr under control conditions, but increases the I_{sc} of tissues spontaneously transporting Na at "submaximal" rates to this value (Table 2); (iii) amphotericin increases J_{me}^{Na} and G_t and elicits K secretion regardless of whether it affects the I_{sc} (Table 2 and [15]); (iv)

an inverse relationship exists between the extent to which amphotericin increases I_{sc} and the basal rate of Na absorption by paired tissues not exposed to the antibiotic (Fig. 8); (v) exposure of the mucosal surface to certain sulfonated anions (e.g., isethionate) increases amiloride-sensitive J_{mc}^{Na} and may elicit a maximal rate of Na absorption which does not differ from that observed in the presence of amphotericin [28]. If I_{sc} is elevated to the maximal value with isethionate, subsequent addition of amphotericin has no additional effect on the I_{sc} [28]. Thus, amphotericin increases the conductance of the mucosal membrane and saturates the activity of the transport mechanism responsible for Na extrusion, from the cell to the serosal solution, across the basolateral membrane and, in this tissue, is a useful probe with which to evaluate the maximum activity of the Na pump.[3] Since this maximal rate of transport can be observed spontaneously, entry of Na across the mucosal membrane appears to be the process which, physiologically, governs the rate of Na absorption by this tissue.[4] It is of interest in this respect that Singer *et al.* [27] also noted an inverse relation between the response to amphotericin and the initial ψ_{ms} in toad urinary bladder, but because of the highly variable behavior of this tissue (e.g., seasonal), no firm interpretation of this finding was offered. The response of rabbit colon to amphotericin is quite predictable and the interpretation of our results are uncomplicated.

These conclusions are entirely consistent with those of Lichtenstein and Leaf [19] who observed that addition of amphotericin to the mucosal solution increased the rate of Na absorption by toad urinary bladder. However, in a later study, Finn [11] was unable to reproduce this finding. A possible explanation for these divergent results is as follows: The control rate of Na absorption determined by Lichtenstein and Leaf was 0.8 μeq/cm² hr and increased to 1.5 μeq/cm² hr in the

3 In the presence of amphotericin, steady-state values of I_{sc} and J_{net}^{Na} are maintained for at least 1 hr (Fig. 7 and Table 1). In addition, the observations that amphotericin has no effect on Na absorption by tissues spontaneously transporting at maximal rates or experimentally brought to this level by treatment with sulfonated anions [28] implies that amphotericin has no direct effect on the activity of the Na pump at the basolateral membranes and does not impair the delivery of metabolic energy to this active transport process.

4 The response to amphotericin has proved to be useful in evaluating the mechanism by which aldosterone increases Na absorption by rabbit colon [15]. Addition of amphotericin to aldosterone-treated tissues did not result in a further elevation of I_{sc} or J_{net}^{Na}, whereas the antibiotic increased Na absorption by control tissues to the level observed in the aldosterone-treated preparations. These findings were interpreted as indicating that aldosterone increases the permeability of the mucosal membranes to Na without markedly affecting the maximal capacity of the Na pump at the basolateral membranes.

presence of amphotericin, whereas the control rate of absorption in the studies of Finn was 1.6 μeq/cm² hr and was not affected by the antibiotic. Thus, the tissues employed by Finn were spontaneously absorbing Na at a rate equal to that observed by Lichtenstein and Leaf in the presence of amphotericin, and failure to observe an increase in J_{net}^{Na} may be due to the fact that the pump mechanism was operating at its maximal rate under control conditions.

Potassium Transport and the Effect of Amphotericin B

Whereas short-circuited rabbit colon shows no net K transport under normal conditions, exposure of the mucosal surface of this tissue to amphotericin results in active K secretion. The conclusion that the net appearance of ^{42}K in the mucosal solution in the presence of amphotericin represents steady-state transcellular secretion is supported by the following line of reasoning: The measured net K flux under these conditions averaged 0.8 μeq/cm² hr, whereas the exchangeable cell K content is 0.9 μeq/cm² (Frizzell, *manuscript in preparation*). Therefore, if unreplenished K leakage from the cells accounted for the observed secretory flux, exchangeable K levels would be depleted by 90 % during the first hour following exposure to the antibiotic. Two observations argue against this possibility. (i) Steady-state bidirectional K fluxes are observed during the period 20–60 min following amphotericin addition. It is extremely unlikely that a 90 % depletion of cell K during this period would be consistent with the maintenance of steady-state fluxes. (ii) Treatment of tissues with amphotericin results in only a 10 % decline in total cell K content, which could, at most, represent an 18 % decline in exchangeable K (Frizzell, *manuscript in preparation*). These conclusions are entirely consistent with those of Nielsen [21] who found that exposure of the outer surface of frog skin to amphotericin elicited active K secretion (from inner to outer solution) under short-circuit conditions. Lichtenstein and Leaf [19] also reported a marked stimulation of serosa-to-mucosa K flux across toad urinary bladder in response to this agent which they ascribed to an increase in K permeability.

The results of recent studies (Frizzell, *manuscript in preparation*) suggest that high cellular K concentrations in rabbit colon (120–140 mM) are maintained by a ouabain-sensitive K uptake mechanism at the basolateral membranes. The absence of net K secretion by control tissues under short-circuit conditions appears to be due to low mucosal mem-

brane permeability for K, as suggested previously [13]. This notion is supported by the results obtained with amphotericin, and also by recent observations of the rate of cell K exchange with ^{42}K in the mucosal and/or serosal solutions (Frizzell, *manuscript in preparation*). Less than 10 % of cell K is exchangeable with ^{42}K in the mucosal solution. Indeed, much of the K which gains access to the cell from the mucosal solution may do so by entering the paracellular pathways, which are relatively permeable to K [13], and then crossing the basolateral membranes. Thus, all of these findings taken together strongly suggest that under normal conditions the transepithelial movements of K across rabbit colon are restricted to paracellular pathways. Under open-circuit conditions, K would be secreted by diffusion, in accordance with the transepithelial electrical potential difference (lumen negative) established by the active absorption of Na.

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