J. Membrane Biol. 39, 1–26 (1978)

Effect of Aldosterone on Ion Transport by Rabbit Colon *In Vitro*

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Received 15 March 1977; revised 8 June 1977

Summary. Segments of descending colon obtained from rabbits, that had been maintained on drinking water containing 25 mM NaCl and an artificial diet which contains 1% Na and is nominally K-free, respond to aldosterone in vitro (after a 30 to 60-min lag period) with a marked increase in the short-circuit current (I_{sc}), an equivalent increase in the rate of active Na absorption (J_{net}^{Na}) and a decline in tissue resistance (R_t). Aldosterone also brings about a marked increase in the unidirectional influx of Na into the cells across the mucosal membrane ("zero-time" rate of uptake) which does not differ significantly from the increase in I_{sc}. Treatment of control tissues with amphotericin B brings about sustained increases in I_{sc} and J_{net}^{Na} *to levels observed in aldosterone-treated tissues.* However, addition of amphotericin B to the mucosal solution of aldosterone-treated tissues does not result in a sustained increase in I_{sc} or J_{net}^{Na} and these values do not differ markedly from those observed in control tissues treated with amphotericin B. These findings, together with other evidence that Na entry in the presence of amphotericin B is sufficiently rapid to saturate the active Na extrusion mechanism at the baso-lateral membrane, are consistent with the notion that the aldosterone-induced protein increases the permeability of the mucosal membrane to Na but does not increase the "saturation level" of the active Na "pump" within the time-frame of these studies (3 hr).

Finally, aldosterone has no effect on the bidirectional or net transepithelial movements of K under short-circuit conditions, suggesting that the enhanced secretion of K observed in vivo is the result of increased diffusion of K from plasma to lumen via paracellular pathways in response to an increased transepithelial electrical potential difference (lumen negative).

Aldosterone stimulates Na absorption by mammalian distal nephron, colon, sweat glands, and salivary glands; this action is associated with an increase in the rates of K and/or H secretion into the luminal or tubular fluid (Sharp & Leaf, 1973).

However, the mechanism of action of this potent mineralocorticoid has been most extensively studied using in vitro toad urinary bladder, a tissue in which active Na absorption is not coupled to or associated with K and/or H secretion (at least under the conditions of those studies, *vide*

0022-2631/78/0039-0001 $5.20

infra). The results of those studies appear to have established the initial phases of the mechanism of aldosterone action. Thus, after entry into the toad bladder epithelial cells, aldosterone is bound within the nuclei where it promotes DNA-dependent synthesis of messenger RNA, which, in turn, results in the synthesis of protein(s) (AIP) (Feldman, Funder & Edelman, 1972; Sharp & Leaf, 1973). The results of recent studies (Sapirstein & Scott, 1975; Scott & Sapirstein, 1975) indicate that this process takes place in the mitochondria-rich cells, which only comprise approximately 10–20% of the total epithelial cell population of toad urinary bladder (Choi, 1963). Evidence for a transcriptional effect of aldosterone has been accrued for a number of tissues that are "target organs" for this agent; on the other hand, specific binding of aldosterone has not been demonstrated in tissues that are nonresponsive to mineralocorticoids (Feldman *et al.*, 1972).

Assuming that the transcriptional role of aldosterone has been established, there are two major questions that remain to be resolved. The first is concerned with the role of the newly synthesized protein(s) (AIP) in the enhancement of active Na absorption. The second is concerned with the relation between the stimulation of Na absorption and the concomitant increase in K and/or H secretion by mammalian epithelia in vivo.

The results reported in this paper describe the effects of aldosterone on Na *and* K transport by a mammalian epithelium (i.e., rabbit descending colon) in vitro. Our data indicate that (i) under short-circuit conditions, aldosterone stimulates active Na absorption but has no effect on transepithelial K movements; and, (ii) aldosterone enhances the unidirectional influx of Na into the epithelial cell. Our data also suggest that aldosterone does not increase the "saturation level" of the mechanism responsible for the active extrusion of Na from the cells across the baso-lateral membranes within the time-frame of these studies.

Materials and Methods

All experiments were performed using "partial mucosal strips"[1] of rabbit descending colon, obtained as described previously (Frizzell, Koch & Schultz, 1976). The solutions bathing the mucosal and serosal surfaces of the tissue contained (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; and glucose, 10; this solution had a pH of 7.4 at 37 °C when gassed with a mixture of 95% O_2 and 5% CO_2. Aldosterone was introduced into the serosal solution by adding 10 μl of a solution of D-

1 The "partial mucosal strip" consists of the epithelial cell layer and the underlying *muscularis mucosa;* the thick outer muscle layers are teased-away with fine optical forceps.

aldosterone in ethanol so that the final concentration was 10^{-6} M; at the same time 10 µl of ethanol was added to the serosal solution of paired, control tissues.

Initial studies indicated that the responsiveness of tissues, mounted in the short-circuit apparatus described by Schultz and Zalusky (1964), to aldosterone was sporadic; approximately 10%–20% of the tissues responded with an increase in the open-circuit transepithelial electrical potential difference (ψ_{ms}) and the short-circuit current (I_{sc}). "Sodium-loading" of the rabbits by addition of NaCl (25 mM) to their drinking water in an effort to decrease endogenous aldosterone levels did not influence the responsiveness of the descending colon to aldosterone in vitro. However, feeding the rabbits with a completely artificial diet (prepared by Zeigler Bros., Inc., Gardner, Pa.) which contains 1% Na and is nominally K-free *plus* the addition of NaCl (25 mM) to their drinking water for at least 5 days resulted in a marked increase in the average urinary (Na/K) ratio ($\cong 8$) as compared to controls which were not fed the artificial diet ($Na/K \cong 0.4$)[2]; tissues obtained from these animals almost always responded to aldosterone, in vitro, after a lag period of approximately 1 hr.

Bidirectional transepithelial fluxes of Na and K were determined simultaneously under short-circuit conditions (interrupted at 10-min intervals for measurement of ψ_{ms}) using the apparatus and technique described by Schultz and Zalusky (1964). Four chambers were employed in all experiments; two were used to determine the bidirectional fluxes of Na^{22} and K^{42} under control conditions and the other two were used to determine these fluxes in the presence of aldosterone. Advantage was taken of the characteristic delay before the onset of action of aldosterone to internally control these studies. That is, aldosterone (10^{-6} M) was added to the serosal bathing solution of two tissues 25 min after mounting; at the same time 10 µl of ethanol were added to the serosal solutions of the other two tissues. Four samples of the initially unlabelled bathing solutions were withdrawn at 10-min intervals between 40 and 70 min (after a steady state is achieved but before the onset of a significant aldosterone effect) and 5 samples were withdrawn at 20-min intervals between 90 and 170 min (after the onset of action) (*see* Fig. 1). The control tissues that were not exposed to aldosterone simply served to rule out any possible effects due to the duration of the experiments (or ethanol). Samples were assayed for Na^{22} *plus* K^{42} using a liquid scintillation spectrometer on the day of the experiment and were reassayed for Na^{22} two weeks later after essentially all of the K^{42} had decayed.

The unidirectional influx (or "zero-time" rate of uptake) of Na^{22} from the mucosal solution into the epithelium across the mucosal membrane was determined under short-circuit conditions using a modification of the influx apparatus described by Frizzell and Schultz (1972) and the technique described by Schultz *et al.* (1967). Briefly, 8 partial-mucosal strips of descending colon from the same animal were mounted in the chambers; four served as controls and four were exposed to aldosterone (10^{-6} M) in the serosal solution. The transepithelial electrical potential difference (ψ_{ms}) was monitored. When the ψ_{ms} of aldosterone-treated tissues reached their peak values, one of the tissues was short-circuited. Then, the mucosal solution bathing this tissue was removed and replaced with an identical solution containing Na^{22} and H^3-polyethylene glycol (PEG) mol wt 1,000). The mucosal surface of the tissue (1.13 cm^2) was exposed to this radioactive solution for

2 Rabbits maintained on normal chow had average urinary Na and K concentrations of 125 mM and 350 mM, respectively; rabbits on the special regimen had average urinary Na and K concentrations of 115 mM and 15 mM, respectively. Thus, it seems as if in vitro responsiveness to aldosterone was elicited by K-depletion rather than by Na-loading. This is an interesting finding in view of the fact that, in nature, these herbivorous animals generally have a surfeit of K and are relatively Na-deprived. It appears that endogenous aldosterone secretion may be more "keyed" to excreting excess K than preserving Na.

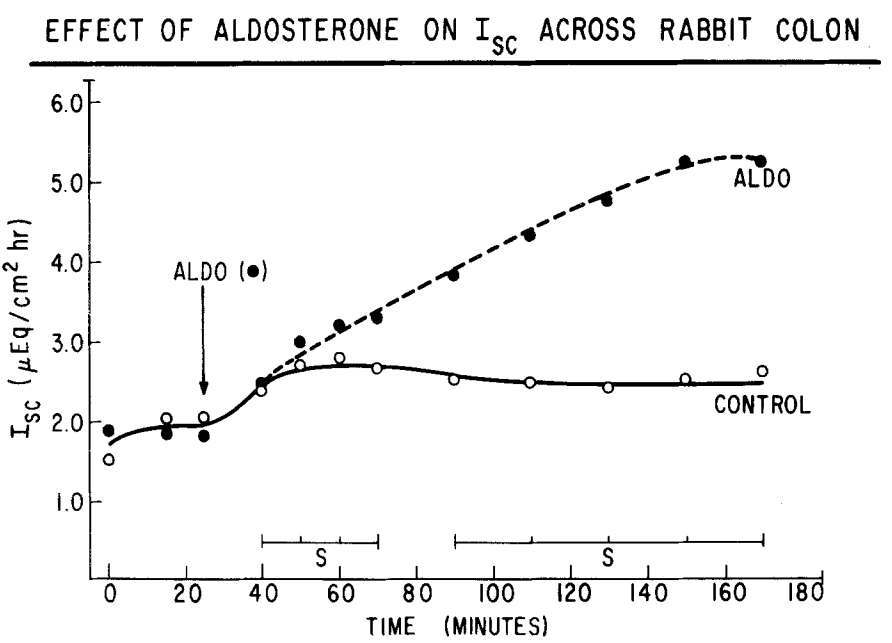

Fig. 1. Time-course of the effect of aldosterone on the short-circuit current (I_{sc}) across in vitro rabbit descending colon. Aldosterone (10^{-6} M) was added to the serosal solutions 25 min after the tissues were mounted. Data obtained on aldosterone-treated tissues are designated by the symbol ●; data obtained on paired, control tissues are designated by the symbol ○. The horizontal bars labeled *S* indicate the sampling periods

40 sec. The mucosal solution was then withdrawn, the chamber was flushed with ice-cold isotonic mannitol solution, and then the exposed tissue was punched-out, washed briefly (1–2 sec) in ice-cold isotonic mannitol solution and extracted for at least 2 hr in 0.1 N HNO_3. This procedure was repeated on all eight tissues in alternating sequence between control and aldosterone-treated so as to eliminate the factor of time. The extracts were assayed for Na^{22} and H^3-PEG using a triple channel liquid scintillation spectrometer. The uptake of Na^{22} by the tissues across the mucosal membranes was calculated after correction for the volume of adherent radioactive solution (not removed by the mannitol washes) given by the PEG space. Previous studies have shown that Na^{22} uptake by the tissue is a linear function of time for at least 60 sec so that the uptake after 40 sec is a valid approximation of the unidirectional influx across the mucosal membrane (Frizzell & Turnheim, *manuscript submitted*).

D-aldosterone was obtained from Sigma Chemical Co., St. Louis; amphotericin B was obtained from Squibb (as Fungizone®); Na^{22} from Amersham/Searle; K^{42} from ICN Pharmaceuticals and H^3-PEG from New England Nuclear.

In each experiment, the average tissue resistance was calculated from the ψ_{ms} determined at 10- or 20-min intervals and the I_{sc} determined immediately before and after the tissue was open-circuited. This procedure is justified by our previous demonstration that the tissue behaves as an ohmic resistor over the range ± 50 mV (Schultz, Frizzell & Nellans, 1977).

All errors are expressed as the SEM calculated using the number of animals studied. A

value of $p < 0.05$ calculated using the two-tail t test is considered a statistically significant difference.

Results

Effect of Aldosterone on Transepithelial Fluxes of Na and K

The time-course of the effect of aldosterone on the I_{sc} across rabbit colon is illustrated in Fig. 1; the points represent the average values from 5 experiments involving 10 control and 10 aldosterone-treated tissues. A small but statistically insignificant effect on I_{sc} is already evident 25 min after the addition of aldosterone to the serosal solution. However, a statistically significant enhancement of I_{sc} is not observed until approximately 1 hr has elapsed. The horizontal lines, labelled *S*, indicate the sampling periods.

The effects of aldosterone on Na and K fluxes across short-circuited rabbit colon are given in Table 1 where R_t is the tissue resistance in ohm · cm^2, J^i_{ms} is the unidirectional flux of i from the mucosal solution to the serosal solution, J^i_{sm} is the unidirectional flux in the opposite direction and $J^i_{\text{net}} = J^i_{ms} - J^i_{sm}$. Between 40–70 min there is excellent agreement between control tissues and aldosterone-treated tissues with respect to ψ_{ms}, I_{sc}, R_t and the bidirectional and net fluxes of Na and K. As reported previously (Frizzell *et al.*, 1976), Na is actively absorbed, whereas there is no significant net transepithelial transport of K under short-circuit conditions. Although there is fair agreement between the I_{sc} and $J^{\text{Na}}_{\text{net}}$, much closer agreement was reported in our previous studies (Frizzell *et al.*, 1976). A possible explanation for this finding is that some of the tissues from rabbits whose endogenous aldosterone secretion was, presumably, markedly suppressed displayed extremely low electrical potential differences (in some instances 0.5–2 mV); under these conditions small asymmetries in the calomel electrodes and/or junction potentials arising from the agar bridges, which are ordinarily negligible, can introduce an error in the determination of I_{sc}.

After the onset of aldosterone action (fluxes determined between 90–170 min), the average values obtained for the control tissues are in excellent agreement with those observed earlier (40–70 min), confirming our previous observation that steady-state fluxes across this in vitro preparation are sustained for at least 3–4 hr. In contrast, the aldosterone-treated tissues exhibited highly significant increases in ψ_{ms}, I_{sc}, J^{Na}_{ms} and $J^{\text{Na}}_{\text{net}}$ and a decrease in R_t. J^{Na}_{sm} is not affected nor are the bidirectional or net fluxes of K. Further, after the onset of aldosterone action, there is

Table 1. Effect of aldosterone on sodium and potassium fluxes across short circuited rabbit colon[a]

	ψ_{ms}	I_{sc}	R_t	J^{Na}_{ms}	J^{Na}_{sm}	J^{Na}_{net}	J^{K}_{ms}	J^{K}_{sm}	J^{K}_{net}
40–70 minutes									
Control	13±2	2.2±0.3	203±6	4.5±0.4	1.5±0.1	3.0±0.4	0.4±0.1	0.5±0.1	−0.1±0.1
Aldosterone	13±2	2.4±0.4	200±10	4.1±0.5	1.4±0.1	2.7±0.5	0.4±0.1	0.5±0.1	−0.1±0.1
90–170 minutes									
Control	14±1	2.3±0.2	208±5	4.4±0.4	1.5±0.1	2.9±0.3	0.4±0.1	0.6±0.1	−0.2±0.1
Aldosterone	22±1[b]	4.1±0.3[b]	184±7[b]	5.5±0.5[b]	1.5±0.1	4.0±0.1[b]	0.5±0.1	0.6±0.1	−0.1±0.1

Effect of Aldosterone:[c] $\Delta I_{sc} = 1.6 \pm 0.4$; $\Delta J^{Na}_{ms} = 1.5 \pm 0.6$; $\Delta J^{Na}_{net} = 1.4 \pm 0.3$

[a] I_{sc} and J's are in μeq/cm² hr; ψ_{ms} is in mV; and, R_t is in ohm · cm².

[b] Significantly different from control at $p < 0.01$. Data derived from 18 paired studies on tissues from 9 rabbits.

[c] These values represent the change in the aldosterone-treated tissues *minus* the change in control tissues to correct for changes that might be time-dependent.

excellent agreement between J_{net}^{Na} and I_{sc}. Finally as shown in Table 1, the increments in I_{sc}, J_{ms}^{Na} and J_{net}^{Na} induced by aldosterone do not differ significantly.

Effect of Aldosterone on the Unidirectional Influx of Na into the Epithelium

The effect of aldosterone on the unidirectional influx of Na across the mucosal membrane of short-circuited rabbit colon (J_{mc}^{Na}) is summarized in Table 2. The aldosterone-treated tissues display highly significant increases in ψ_{ms}, I_{sc} and J_{mc}^{Na} compared to control tissues. Further, the increase in J_{mc}^{Na} is in excellent agreement with the increase in I_{sc}. Although, the average R_t in the presence of aldosterone is less than that observed in control tissues, these values do not differ significantly because of relatively large scatter.

The relation between J_{mc}^{Na} and I_{sc} for control and aldosterone-treated tissues is shown in Fig. 2. Least-squares, linear regression analysis of these data indicate that: (i) for the 28 control tissues $J_{mc}^{Na}=(1.1\pm0.2)I_{sc}+0.48$; (ii) for the 28 aldosterone-treated tissues, $J_{mc}^{Na}=(1.1\pm0.5)I_{sc}+0.53$; and, (iii) for all 56 tissues, $J_{mc}^{Na}=(1.1\pm0.2)I_{sc}+0.58$. In all instances, $r>0.9$. This close agreement between J_{mc}^{Na} and I_{sc} or J_{net}^{Na} has been observed under a variety of conditions (Frizzell & Turnheim, *manuscript submitted*) and suggests that the backflux of Na from the cell across the mucosal membrane (J_{cm}^{Na}) is undetectably small under control conditions and in the presence of aldosterone. The nonzero intercept on the ordinate probably reflects Na influx into paracellular pathways.

Table 2. Effect of aldosterone on unidirectional influx of sodium (J_{mc}^{Na}) across the mucosal membrane of short circuited rabbit colon[a]

	ψ_{ms}	I_{sc}	R_t	J_{mc}^{Na}
Control	17 ± 2	2.2 ± 0.2	275 ± 17	2.8 ± 0.2
Aldosterone	29 ± 2[b]	3.9 ± 0.2[b]	250 ± 17	4.6 ± 0.3[b]

Effect of aldosterone: $\Delta I_{sc}=1.7\pm0.3$; $\Delta J_{mc}^{Na}=1.8\pm0.4$

Data derived from 28 influx determinations on paired tissues from 7 rabbits.

[a] ψ_{ms} is in mV; R_t is in ohm · cm^2; and, I_{sc} and J_{mc}^{Na} are in μeq/cm^2 hr.

[b] Significantly different from control at $p<0.01$.

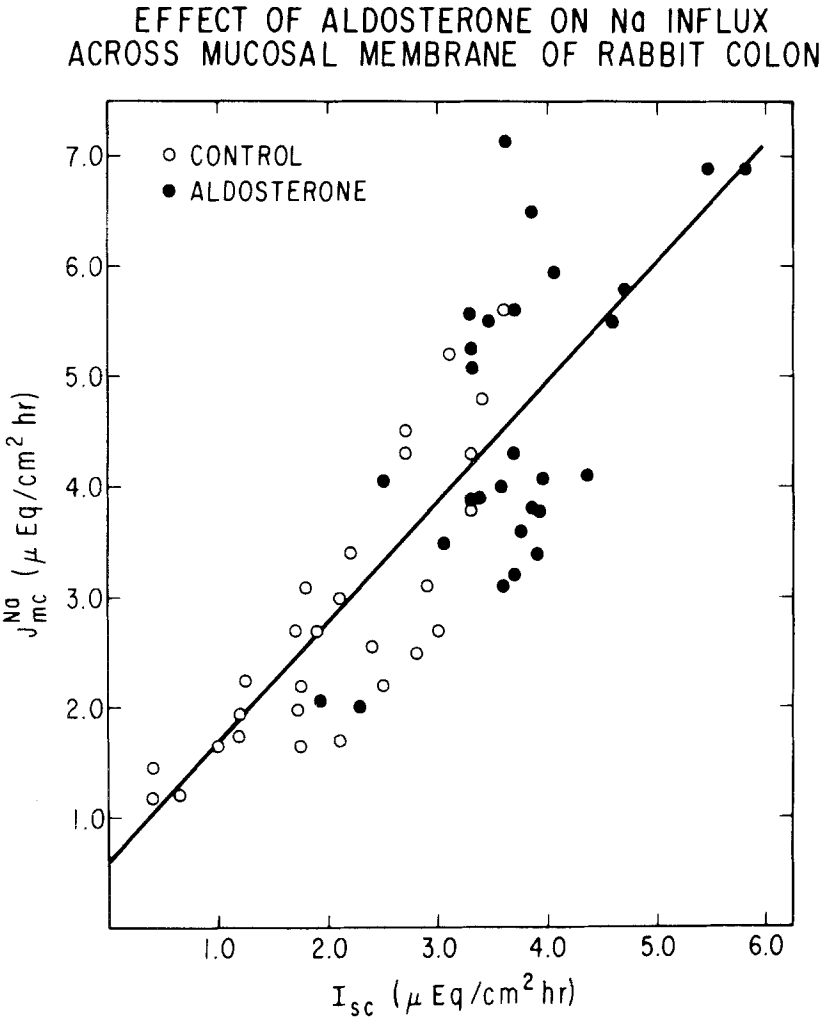

Fig. 2. Relation between I_{sc} and J^{Na}_{mc} in control tissues (○) and aldosterone-treated tissues (●)

Effect of Amphotericin B on Control and Aldosterone-Treated Tissues

In 6 of the 9 experiments reported in Table 1, amphotericin B was added to the mucosal solution (final concentration, 15 μg/ml) immediately after the 170-min sample was withdrawn and the bidirectional fluxes of Na and K were determined at 5 to 10-min intervals for an additional 15–30 min. This concentration of amphotericin B is sufficient to elicit a sustained maximal response of I_{sc}, J^{Na}_{net} and J^{Na}_{mc} under control conditions (Frizzell & Schultz, 1976; Frizzell & Turnheim, *manuscript sub-*

Table 3. Effect of amphotericin B on Na and K fluxes across short circuited rabbit colon in the presence and absence of aldosterone[a]

	ψ_{ms}	I_{sc}	G_t	J^{Na}_{ms}	J^{Na}_{sm}	J^{Na}_{net}	J^{K}_{ms}	J^{K}_{sm}	J^{K}_{net}
Control	12±1	2.1±0.2	4.8±0.1	3.9±0.3	1.5±0.2	2.4±0.3	0.4±0.0	0.6±0.1	−0.2±0.1
+Ampho.	17±1	3.6±0.3	6.1±0.2	6.1±0.5	2.7±0.4	3.4±0.3	0.4±0.0	1.0±0.2	−0.6±0.2
Δ	5±2[b]	1.5±0.4[b]	1.3±0.2[b]	2.2±0.6[b]	1.2±0.4[b]	1.0±0.4[b]	0	0.4±0.2[b]	−0.4±0.2[b]
Aldosterone	20±1	3.7±0.2	5.4±0.2	5.3±0.7	1.5±0.2	3.8±0.6	0.5±0.1	0.6±0.1	−0.1±0.1
+Ampho.	18±1	4.1±0.2	6.7±0.4	6.5±0.8	2.5±0.2	4.0±0.7	0.4±0.1	1.0±0.2	−0.6±0.2
Δ	−2±1	0.4±0.3	1.3±0.4[b]	1.2±1.0	1.0±0.3[b]	0.2±0.9	−0.1±0.1	0.4±0.2[b]	−0.5±0.2[b]

[a] I_{sc} and J's in μeq/cm² hr; ψ_{ms} in mV; and G_t in mmhos/cm². Ampho = Amphotericin B (15 μg/ml).
[b] Significantly different from the corresponding "pre-amphotericin" value at $p<0.05$.

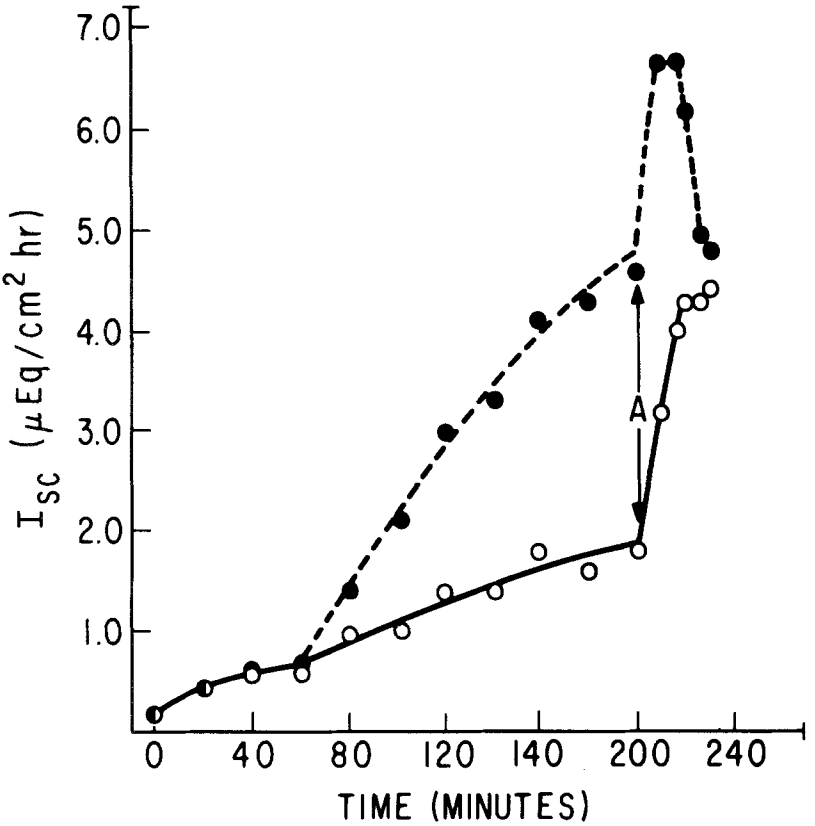

Fig. 3. An example of the transient effect of amphotericin B on the I_{sc} across an aldosterone-treated tissue. Aldosterone (10^{-6} M) was added to the serosal bathing solution of one tissue (x) at $t=0$. Between 60–80 min thereafter, the I_{sc} of the aldosterone-treated tissue began to increase. At 200 min, amphotericin B (15 μg/ml) was added to the mucosal solution of the aldosterone-treated (●) and control (○) tissues

mitted). The results of these studies are summarized in Table 3. In the control tissues, amphotericin B brought about prompt increases in ψ_{ms}, I_{sc}, J^{Na}_{ms}, J^{Na}_{sm}, and J^{Na}_{net} and a significant increase in the tissue conductance, G_t. In the aldosterone-treated tissue, amphotericin B brought about a significant increase in G_t and *equivalent* increases in J^{Na}_{ms} and J^{Na}_{sm}. It is important to note that: (i) in all instances, there is excellent agreement between J^{Na}_{net} and I_{sc}; (ii) in contrast with the control tissues, amphotericin B did not bring about significant increases in ψ_{ms}, I_{sc} or J^{Na}_{net} in the aldosterone-treated tissues; and (iii) the I_{sc} and J^{Na}_{net} in control tissues treated with amphotericin do not differ significantly from those values observed in aldosterone-treated tissues *either before or after* the addition of amphotericin B to the mucosal solution.

In the course of these experiments we noted that the addition of amphotericin B to the mucosal solution of aldosterone-treated tissues either had no significant effect on the I_{sc} (4 instances) or brought about a transient increase in I_{sc} which then declined to the pre-amphotericin value (2 instances). In contrast, in control tissues, amphotericin B always elicited a sustained increase in I_{sc}. To explore this point further, a series of experiments was carried out in which we simply monitored the time-course of the I_{sc} response of control and aldosterone-treated tissues to amphotericin B. A striking example of the transient response is illustrated in Fig. 3. We see a highly significant effect of aldosterone commencing between 60–80 min. The addition of amphotericin B to the mucosal solution (at 200 min) resulted in a prompt increase in the I_{sc} of the control tissue which was sustained for 40 min. In contrast, in the aldosterone-treated tissue, amphotericin B brought about a prompt increase in the I_{sc} which, after reaching its peak value, declined to the pre-amphotericin level. Further, it is important to note that the I_{sc} elicited by amphotericin B in control tissues does not differ significantly from the I_{sc} of the aldosterone-treated tissue before the addition of amphotericin or after the transient decayed.

Finally, as shown in Table 3, exposure of both control and aldosterone-treated tissues to amphotericin B resulted in a significant rate of K secretion. This phenomenon has been observed previously in rabbit colon (Frizzell & Schultz, 1976; Frizzell & Turnheim, *manuscript submitted*) and frog skin (Nielsen, 1971)[3].

Discussion

Ion Transport by Mammalian Colon

It is generally accepted that mammalian colon in vivo absorbs Na and Cl and secretes K and HCO_3 (Phillips, 1969; Turnberg, 1970; Powell, 1977). Further, in vivo studies on dog (Cooperstein & Brockman, 1959; Phillips & Code, 1966), rat (Curran & Schwartz, 1960; Edmonds, 1967*a–c*) and human (Edmonds & Godfrey, 1970) colon have disclosed significant transepithelial electrical potential differences; although the

3 In view of the fact that amphotericin B elicits K secretion, the close agreement between I_{sc} and J_{net}^{Na} requires some comment. First, it should be noted that J_{net}^{K} is small compared with J_{net}^{Na}. Second, amphotericin B apparently increases the permeability of the mucosal or outer membranes of toad bladder and frog skin to Cl (Lichtenstein & Leaf, 1965; Nielsen, 1971). Thus a leak of Cl from the cell into the mucosal solution might off-set the K current.

values recorded in vivo often display extreme scatter, the plasma is invariably electrically positive with respect to the luminal fluid. Thus, it seems clear that Na is actively absorbed by these tissues. However, because the net movements of Cl and K are in the direction of a favorable electrical potential difference it is difficult to draw firm conclusions regarding the mechanisms responsible for these movements from in vivo studies.

For the case of K, it has been argued that the high K concentration of human feces, determined by in vivo dialysis (Wrong *et al.*, 1965) or by the method described by Edmonds (*see* Edmonds & Godfrey, 1970), of approximately 100 mM cannot be attributed entirely to a passive distribution but that active secretion must be involved. However, the possibility that these high K values are influenced by the presence of bacteria, exfoliated epithelial cells and/or undigested vegetable matter cannot be excluded. The same criticism applies to the value of 60 mM for canine feces reported by Berger, Kanzaki and Steele (1959). Phillips and Code (1966) reported that the "equilibrium concentration" of K in the luminal fluid perfusing canine large intestine is approximately 17 mM and that this cannot be accounted for by the PD which had a mean value of 9.1 mV (range 0.1–15.4 mV); these authors concluded that active K secretion "may occur in the colon". On the other hand, Cooperstein and Brockman (1959) suggested that K secretion by canine colon was attributable to the transepithelial PD. Edmonds (1967*c*) found that the "equilibrium concentration" of K in the fluid perfusing in vivo rat colon (20–30 mM) could not be accounted for by the PD (approximately 10 mV) and that the bidirectional fluxes of K did not conform to the Ussing flux-ratio equation; he concluded that rat colonic mucosa actively secretes K into the lumen.

There have been relatively few studies of ion transport by mammalian large intestine in vitro using the short-circuit technique. Frizzell *et al.* (1976) reported that Na and Cl are actively absorbed by short-circuited, partial-mucosal strips of rabbit colon but that the I_{sc} and ψ_{ms} are entirely attributable to the rate of active Na transport; active Cl absorption is electrically silent and is, in all likelihood, coupled to HCO_3 secretion (*c.f.* Phillips, 1969; Turnberg, 1970; Hubel, 1968; Phillips & Schmalz, 1970). Bidirectional fluxes of K did not differ significantly, so that $J^K_{net} \cong 0$, and the relation between J^K_{sm} and ψ_{ms} conformed precisely to that predicted for strict ionic diffusion through a paracellular pathway. These results differ from those reported by Yorio and Bentley (1977) using short-circuited *unstripped* rabbit colon. These investigators found

that (i) Na was actively absorbed and was equivalent to 109% of the I_{sc}; (ii) there was no net transport of Cl under short-circuit conditions; and (iii) there was a small but statistically significant net secretion of K under short-circuit conditions. It is possible that some of the differences between these results and those reported by Frizzell *et al.* (1976) stem from the fact that Yorio and Bentley employed full-thickness (unstripped) colon. The outer muscle layers of rabbit colon comprise approximately 50% of the thickness of the unstripped preparation (*see* Fig. 1, Frizzell et al., 1976) and contribute significantly to the electrical resistance of the tissue (in a small series of studies we found that the resistance of unstripped rabbit colon was approximately 25% greater than that of the partial mucosal strip; thus when these tissues are short-circuited a significant PD may still be present across the epithelial cell layer particularly when the open-circuit PD is large). Frizzell *et al.* (1974) demonstrated that the outer musculature of rabbit ileum is a significant barrier to oxygen diffusion from the serosal solution to the epithelial cell layer; the rate of oxygen consumption of the stripped epithelial cell layer *alone* significantly exceeded the rate of oxygen consumption of the epithelial cell layer *plus* the underlying musculature. Since the thickness of the outer musculature in rabbit ileum is considerably less than that of rabbit colon, it is possible that the unstripped preparation of rabbit colon is also oxygen-limited. If active Cl absorption is coupled to the secretion of HCO_3 which is produced within the epithelial cells, oxygen-limitation could severely impair this exchange process.[4]

The finding by Yorio and Bentley (1977) that *unstripped* rabbit colon actively secretes K under short-circuit conditions ($J^K_{ms}=0.05$, $J^K_{sm}=0.39$, $J^K_{net}=-0.34$) is in accord with the findings of Bentley and Smith (1975) on short-circuited, unstripped preparations of the helicoidal colon of the new-born pig ($J^K_{ms}=0.12$, $J^K_{sm}=0.17$, $J^K_{net}=-0.05$) and with the findings of Archampong, Harris and Clark (1972) on short-circuited, stripped preparations of normal human colon ($J^K_{ms}=0.118$, $J^K_{sm}=0.137$, $J^K_{net}=-0.019$). Thus, the majority of published studies on K transport across in vitro, short-circuited mammalian colon have reported very low, but statistically significant, rates of active K secretion as suggested by the in vivo studies cited above. It is difficult to assess the physiological significance of these small rates of active K secretion since "apparent" K secretion

4 It is of interest in this respect that Schultz, Zalusky and Gass (1964) were unable to detect active Cl absorption by *unstripped* preparations of rabbit ileum, whereas numerous investigators have subsequently shown that Cl is actively absorbed by preparations of rabbit ileum stripped of the underlying musculature (Schultz, Frizzell & Nellans, 1974).

would be expected if (i) the epithelial cell layer of unstripped preparations were not fully short-circuited because of the series resistance offered by the muscle layers; and/or (ii) damage to some or all of the cells (e.g., anoxia) rendered the mucosal membrane leaky to K. In the present studies, J^{K}_{net} did not differ significantly from zero; however, in all instances the mean values were negative. Thus we cannot *exclude* the possibility that there is a very low rate of active K secretion by rabbit colon that is statistically masked by much larger paracellular diffusional movements of K. If so, the rate of active K secretion could not exceed approximately 0.1 μeq/cm^2 hr; this value is approximately 3–5% of the rate of active Na absorption and would not significantly affect the I_{sc}.

Effect of Aldosterone on Sodium and Potassium Transport

It is well established that mineralocorticoids increase the rates of Na absorption and K secretion by human (Levitan & Ingelfinger, 1965; Edmonds & Godfrey, 1970), canine (Berger, Kanzaki & Steele, 1960), and rat (Edmonds, 1967*c*; Edmonds & Marriott, 1967; Lange *et al.*, 1974) colon. However, although it is indisputably clear that aldosterone enhances the rate of active transcellular Na transport, the mechanism(s) and forces responsible for the enhanced K secretion are unresolved. There are three possible explanations for the enhanced rate of K secretion observed in vivo: (i) stimulation of active K secretion; (ii) an increase in the rate of diffusion of K from plasma (serosal solution) to lumen (mucosal solution) secondary to the increase in ψ_{ms} which, in turn, is a consequence of the increased rate of active Na absorption; and (iii) some combination of possibilities (i) and (ii).

Clearly, in principle, this issue could be resolved by studies on short-circuited in vitro preparations where the influence of ψ_{ms} on the movements of K are eliminated. Edmonds and Marriot (1968) found that stripped in vitro preparations of descending colon from Na-depleted rats had significantly higher ψ_{ms} and I_{sc} than did tissue from normal or adrenalectomized rats, presumably due to endogeneous aldosterone secretion; however, their preparation deteriorated rapidly (as judged by a steady decline in ψ_{ms} with time) and they were unable to detect any effect of aldosterone in vitro. Although Cofre and Crabbe (1967) clearly demonstrated a stimulation of active Na absorption by in vitro toad colon and close agreement between J^{Na}_{net} and I_{sc}, the effect of aldosterone on K transport was not investigated (*also see* Dawson & Curran, 1976). Lewis and Diamond (1976) have recently reported that aldosterone markedly increases the I_{sc} and the transepithelial conductance across

rabbit urinary bladder in vitro; however, the effect of this agent on transepithelial K movements was not explored.

Thus, to the best of our knowledge, the present studies are the first in which the effect of aldosterone on K transport by a mammalian epithelium has been examined in vitro under short-circuit conditions. The results reported in Table 1 indicate that during the period (90–170 min) in which aldosterone markedly increased the rate of active Na absorption there was no significant effect on the simultaneously determined bidirectional or net fluxes of K under short-circuit conditions. Even if there is a small (and statistically undiscernible) rate of active K secretion under control conditions we are unable to detect any effect of aldosterone on this movement. Thus, our findings strongly suggest that the increase in K secretion by mammalian colon elicited by aldosterone in vivo is entirely attributable to an increase in paracellular diffusional movements of K driven by the increased ψ_{ms} which, in turn, is a consequence of an increased rate of active Na absorption.[5]

Finally, Frizzell *et al.* (1976) found that the permeability of the passive conductance pathways across rabbit descending colon (which are, in all likelihood, paracellular) to K is at least ten times greater than the permeabilities of these pathways to Na or Cl; thus, these "shunt" pathways are highly permselective for K. This is an ideal property for a tissue in which the rate of Na absorption and, in turn, the transepithelial *PD* are regulated by changes in the properties of the active, transcellular Na transport pathway (*vide infra*) and the rate of K secretion is concomitantly regulated by the *PD* acting across a K-selective, paracellular, passive conductance pathway.

The Mechanism of Action of Aldosterone on Sodium Transport by Rabbit Colon

According to the classical, double-membrane model of epithelial cells (*c.f.* Koefoed-Johnsen & Ussing, 1958; Leaf, 1965) transepithelial Na transport can be stimulated by at least two "primary" actions; *viz:*

1) A *primary* increase in the rate of Na entry into the transporting

5 When the tissue is bathed by identical solutions

$$J^{K}_{net} = (P_K \mathscr{F} [K] \psi_{ms})/RT.$$

Since $P_K = 0.12$ cm/hr (Frizzell *et al.*, 1976) the rate of K secretion by open-circuited control tissues would be 0.3 μeq/cm² hr and this would increase to 0.5 μeq/cm² hr following treatment with aldosterone (Table 1). Further, when the concentration of K in the serosal solution (plasma) is 5.4 mM, the "equilibrium concentration" in the mucosal (luminal) fluid will be 9 mM for control tissues and 12 mM for aldosterone-treated tissues.

cells (e.g., an increase in the Na permeability or a decrease in the resistance to Na movement across the mucosal membrane). This would bring about an increase in the Na activity of the intracellular "transport pool" which, in turn, would result in an increase in the rate of transepithelial Na transport *if* the carrier-mechanism responsible for Na extrusion across the baso-lateral membranes is not saturated. The findings that amphotericin B added to the outer or mucosal bathing solutions stimulates active transepithelial Na transport across toad urinary bladder (Lichtenstein & Leaf, 1965), frog skin (Nielsen, 1971), and rabbit colon (Frizzell & Schultz, 1976; Frizzell & Turnheim, *manuscript submitted*) appear to be pure examples of this mechanism of action. This polyene antibiotic appears to form relatively nondiscriminating pores across sterol-containing biological and artificial membranes, thereby enhancing the permeabilities of these barriers to ions, water, and small nonelectrolytes (Kinsky, Luse & Van Deenen, 1966; Lichtenstein & Leaf, 1965; Holz & Finkelstein, 1970; Andreoli & Monahan, 1968; Nielsen, 1971). Evidence obtained for rabbit colon indicates quite conclusively that amphotericin B does not *directly* affect the Na "pump" mechanism at the baso-lateral membranes (Frizzell & Turnheim, *manuscript submitted*; Turnheim et al., 1977).

2) A *primary* increase in the activity of the mechanism responsible for the active extrusion of Na from the cell across the baso-lateral membrane due to either an increase in the number or activity of pump sites *or* an increased availability of energy supplies to energy-limited pumps. This would bring about a decrease in the Na activity of the transport pool and secondarily enhance Na entry across the mucosal membrane.

3) An enhancement of both Na entry and Na extrusion resulting from *direct* actions at both the mucosal and the baso-lateral membranes.

The results of the studies reported in Table 2 indicate the treatment of rabbit colon with aldosterone brings about a significant increase in the *unidirectional* influx of Na from the mucosal solution into the transporting cells. Further, as illustrated in Fig. 2, this increase in influx is closely paralleled by the increase in the I_{sc} or J_{net}^{Na}. These results, taken at face value, suggest that the effect of aldosterone can be attributed entirely to an enhancement of the rate of Na entry into the transporting epithelial cells across the mucosal membranes and that the decrease in R_t (Table 1) is the result of a decrease in the resistance of the mucosal membrane to Na entry.[6] The notion that aldosterone or AIP increases the ease with

6 Previous studies have shown that the electrophysiologic properties of rabbit colon are consistent with a relatively simple equivalent electrical circuit model in which R_t

which Na can enter the epithelial cell across the mucosal membrane is consistent with a considerable body of evidence summarized by Sharp and Leaf (1966, 1973) and with the recent finding of Cuthbert and Shum (1975) that treatment of toad urinary bladder with aldosterone, in vitro, doubles the amiloride-binding capacity of the tissue which is presumably a measure of the number of Na-entry sites at the mucosal membrane (Cuthbert, 1973).

However, a definitive interpretation of these observations is complicated by evidence which suggests that there is a negative feedback between the size of the intracellular Na transport pool and the Na-conductance or permeability of the mucosal membrane. For examples, the results of the studies by Essig and Leaf (1963) on toad urinary bladder, Biber (1971), Moreno *et al.* (1973), Rick, Dörge and Nagel (1975), and Larsen (1973) on isolated frog skin, and Lewis, Eaton and Diamond (1976) on in vitro rabbit urinary bladders are consistent with the notion that an increase in the intracellular Na transport pool resulting from maneuvers that inhibit pump activity brings about a decrease in the permeability (increase in the resistance) of the outer or mucosal membrane to Na. Evidence recently accrued in our laboratory indicates that procedures that increase cell Na (e.g., treatment with ouabain or amphotericin B) lead to a decrease in the amiloride-sensitive J_{mc}^{Na} whereas procedures that decrease cell Na result in a marked increase in the amiloride-sensitive J_{mc}^{Na}. Thus, a decrease in the Na transport pool due to a *primary* stimulation of the baso-lateral pump in aldosterone-

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$=R_{Na}R_L/(R_{Na}+R_L)$ where R_{Na} is the total resistance of the transcellular active Na transport pathway and is equal to $(R_{Na}^m+R_{Na}^s)$ where the superscripts m and s designate the mucosal and serosal membranes (Schultz *et al.*, 1977, Fig. 8). R_L is the resistance of the "passive conductance pathways" across the tissue which appear to be paracellular and can be viewed as a "shunt" which parallels the active Na transport pathway. We have also demonstrated that $(1/R_L)=G_L\cong 0.85\,G_t$; that is, the shunt conductance is approximately 85% of the total tissue conductance (Frizzell et al., 1976). In addition, the results of our previous studies are consistent with the notion that $R_{Na}=1700$–2200 ohm $\cdot$ cm^2 and that $R_{Na}^s<0.1\,R_{Na}^m$ (Schultz *et al.*, 1977).

The data given in Table 1 indicate that in the presence of aldosterone R_t decreased from an average value of 208 ohm cm^2 to an average value of 184 ohm cm^2; i.e., G_t increased from 4.8 mmhos/cm^2 to 5.4 mmhos/cm^2. In view of the fact that neither J_{sm}^{Na} nor J_{sm}^{K} are affected by aldosterone it is reasonable to conclude that the resistance of the passive conductance or shunt pathway(s) is not affected by this hormone and that the decrease in R_t is entirely attributable to a decrease in R_{Na}; Saito and Essig (1973) arrived at the same conclusion in the case of toad urinary bladder.

If this line of reasoning is correct it can be readily shown that in the present studies under control conditions $R_{Na}=2440$ ohm cm^2 and in the presence of aldosterone $R_{Na}=2180$ ohm cm^2. Since, under control conditions $R_{Na}^s<0.1\,R_{Na}^m$, the decrease in R_t or R_{Na} must involve a decrease in R_{Na}^m and cannot to be due to a decrease in R_{Na}^s *alone.*

treated tissues could result in a *secondary* increase in the permeability of the mucosal membrane to Na and the observed increase in J_{mc}^{Na}.[7]

However, the effect of aldosterone on the size of the Na transport pool is not completely resolved so that the possibility of a negative feedback cannot be evaluated. Aldosterone does not affect the cell Na concentration in rat (Edmonds, 1972; Dolman, Edmonds & Salas-Coll, 1976) or rabbit colon *(unpublished observations)*; however, it is difficult to place much faith in these *overall* concentrations in a complex tissue comprised of several cell types. For the case of toad urinary bladder this question is also somewhat unsettled (*c.f.* Handler, Preston & Orloff, 1972; Lipton & Edelman, 1971; Leaf & Macknight, 1972).

The interpretation of the finding that J_{mc}^{Na} is increased by aldosterone is also complicated by the possibility that it is the result of a change in the electrical potential difference across the mucosal membrane (ψ_{mc}). Thus, as discussed previously (Schultz *et al.*, 1977), stimulation of pump activity could lead to a hyperpolarization of ψ_{mc}, which in turn would increase rheogenic Na entry (J_{mc}^{Na}).[8]

Thus, in principle, the finding that J_{mc}^{Na} is increased by aldosterone *could* be entirely attributable to an increase in pump activity which results in (i) a decrease in pool size with a feedback effect on the resistance of the entry step and/or (ii) a hyperpolarization of ψ_{mc}. There are no compelling data at this time which definitively exclude either of these possibilities.

Effect of Amphotericin B on Aldosterone-Treated Tissues

Perhaps the most *suggestive* evidence that aldosterone does not affect the pump mechanism in rabbit colon but that its action can be attributed solely to an increase in the ease of Na entry into the cell, derives from the

7 Recent results reported by Robinson and Macknight (1976) suggest that the inhibition of active Na transport by toad urinary bladder resulting from exposure to a K-free serosal solution may be due to a decrease in cell K content with no change in the size of the intracellular Na compartment accessible from the mucosal solution. These results suggest that the increase in the resistance of the mucosal membrane to Na entry observed when active Na transport is inhibited by ouabain, metabolic inhibitors or K-free serosal solutions may be due to a decline in intracellular K content and need not involve a "negative feedback" between an enlarged Na transport pool and the Na permeability of the mucosal membrane.

8 Clearly inhibition of the baso-lateral pump could lead to a depolarization of ψ_{mc} and an inhibition of Na entry. Thus the "feedback" effects on Na entry following inhibition of the pump mechanism cited above need not be (entirely) attributable to an increase in the resistance of the entry step secondary to an increase in $[Na]_c$. Clearly, in the presence of a finite shunt pathway there may be electrical coupling between the two limiting membranes such that a change in pump rate will tend to exert a "concerted" effect on the rate of Na entry.

results of our studies on the effect of amphotericin B (Table 3, Fig. 3). Previous studies have shown that the addition of this agent to the mucosal solution brings about steady-state increases in ψ_{ms}, I_{sc}, G_t, J^{Na}_{net} and J^{Na}_{mc}, and elicits K secretion. Further, the I_{sc} in the presence of amphotericin B (over the control value) is not amiloridesensitive (Frizzell & Turnheim, *manuscript submitted*). These results are similar to those reported by Nielsen (1971) for isolated frog skin and can be attributed to the formation of relatively nonspecific permeation pathways across the mucosal membrane and a breakdown of the normal permselective properties of this barrier. The effect of this antibiotic on K transport is consistent with the notion that, normally, the mucosal membrane is essentially impermeable to K and that under control conditions transepithelial K movements are restricted to the paracellular pathway(s) (Frizzell *et al.*, 1976; Frizzell & Schultz, 1976; Frizzell & Turnheim, *in preparation*).

However, in the course of these earlier and more recent (Turnheim *et al.*, 1977) studies we observed that the effect of amphotericin B on ψ_{ms}, I_{sc} and J^{Na}_{net} was *inversely related* to the values observed prior to the addition of this agent to the mucosal solution. That is, when the control rates of I_{sc} and J^{Na}_{net} were low (1.5–2.0 $\mu eq/cm^2$ hr), the addition of amphotericin increased these rates to approximately 4–5 $\mu eq/cm^2$ hr. However, as the control rates approached 4–5 $\mu eq/cm^2$ hr, the ability of amphotericin B to increase I_{sc} and J^{Na}_{net} diminished; and, when the rate of active Na absorption by the control tissue was approximately 4–5 $\mu eq/cm^2$ hr, the subsequent addition of amphotericin B had no effect on I_{sc} or J^{Na}_{net}. However, under all circumstances amphotericin B increased J^{Na}_{mc}, G_t and elicited K secretion. These findings strongly suggest that under *all* conditions amphotericin B brings about an increase in the Na permeability of the mucosal membrane through a strictly physico-chemical interaction, but that (for what ever reason) the baso-lateral Na pump mechanism saturates at a mean value of approximately 5 $\mu eq/cm^2$ hr. Thus, as the I_{sc} and J^{Na}_{net} of control tissue approach this "maximal" rate, the effect of amphotericin B diminishes in spite of the fact that the permeability of the mucosal membrane to Na is significantly increased (Frizzell & Turnheim, *manuscript submitted*). Lichtenstein and Leaf (1965) demonstrated that, whereas amphotericin B elicited an increase in the I_{sc} across toad urinary bladder *after* treatment with vasopressin, vasopressin had no effect on the I_{sc} *after* maximal stimulation by amphotericin B (12 μg/ml). These investigators concluded that "the limit to the increase of short-circuit current after amphotericin B and vasopressin may be dictated by the capacity of the pump and, therefore, not indicate the true

extent to which these agents have affected the mucosal permeability". Thus, the findings of these investigators suggest that the increase in Na entry across the mucosal membrane elicited by amphotericin B is sufficient to saturate the baso-lateral pump mechanism; our findings are entirely consistent with this conclusion. Finally, our findings that (i) amphotericin B does not affect the "maximal" rate of J_{net}^{Na} when added to tissues that are already transporting Na at the maximal rate; and (ii) in the presence of amphotericin B, maximal rates of J_{net}^{Na} are sustained for more than 1 hr, strongly suggest that this agent does not *directly* (e.g., by interaction with the baso-lateral pump) or *indirectly* (e.g., by leading to alterations in cell ion contents or loss of essential metabolic intermediates) affect the Na pump at the baso-lateral membranes. In short, all of our findings to date strongly support the notion that the J_{net}^{Na} in the presence of amphotericin B is a valid measure of the maximal rate or "saturation level" of the Na pump.

The rationale for the studies reported in Table 3 was based on our earlier, exasperating, attempts to determine why the response of rabbit colon, even in Na-loaded animals, was sporadic. During the course of these studies we found that tissues that did not respond to aldosterone after 100–140 min, in general, did not respond to the subsequent addition of amphotericin B. These preliminary results suggested that in these tissues, the pump mechanism might be rate-limiting. It follows that, if aldosterone stimulates the pump either directly or by increasing the availability of high-energy intermediates, the rate of active Na absorption observed in the presence of aldosterone *plus* amphotericin B should exceed the rate of active Na absorption observed in the presence of amphotericin B alone. However, as shown in Table 3 (i) the values of I_{sc} and J_{net}^{Na} observed in control tissues in the presence of amphotericin B do not differ significantly from those values observed in aldosterone-treated tissues *prior* to the addition of amphotericin B; (ii) the addition of amphotericin B to aldosterone-treated tissues did not elicit significant steady state increases in I_{sc} or J_{net}^{Na} and these values are in excellent agreement with previous estimates of the "normal saturation level" of the pump; and (iii) the J_{ms}^{Na}, I_{sc} and J_{net}^{Na} of control tissues in the presence of amphotericin B do not differ markedly from those values observed in aldosterone-treated tissues in the presence of amphotericin B.[9] Thus, if

9 As shown in Table 3, amphotericin B elicited equivalent increases in J_{ms}^{Na} and J_{sm}^{Na} in aldosterone-treated tissues. These increases do not differ significantly from the increase in J_{sm}^{Na} elicited by amphotericin B in control tissues and in all likelihood are attributable to an increase in shunt conductance.

our reasoning is correct, it does not appear that aldosterone increases the "saturation level" of the pump mechanism, but that its action can be explained entirely by a primary effect on the Na permeability of the mucosal membrane leading to an increase in J_{mc}^{Na} and that this increase in entry rate is sufficient to saturate the pump. However, it should be stressed that these conclusions are only *suggestive*, inasmuch as the direct and indirect effects of amphotericin B may be complex and are not completely understood. Suffice it to say that, if aldosterone markedly stimulated the pump activity of this preparation, one might have expected that the J_{ms}^{Na} and J_{net}^{Na} of aldosterone-treated tissues after exposure to amphotericin would have significantly exceeded those values in amphotericin-treated control tissues.

The occasional transient response of aldosterone-treated rabbit colon to amphotericin B resembles the findings of Finn (1970) on isolated toad urinary bladder. This investigator demonstrated that, when active transepithelial Na transport is abolished by rendering the serosal solution K-free, the addition of amphotericin B to the mucosal solution elicited a marked increase in J_{sm}^{Na}, a prompt but *transient* increase in I_{sc}, but little or no increase in J_{ms}^{Na}; at the same time there was an increase in intracellular Na content and a decrease in cell K content. When K was subsequently added to the serosal solution there was an increase in J_{ms}^{Na} and cell K content and a decrease in cell Na content. Finn concluded that, when the active Na transport mechanism at the serosal membrane is inhibited, the transient increase in I_{sc} in response to amphotericin B is, at least in part, the result of diffusion potentials across the "damaged" mucosal membrane. The increase in J_{ms}^{Na} following the addition of K to the serosal solution was attributed to a restoration of pump activity. An analogous interpretation may apply to the transient responses observed occasionally in the present studies. Thus, if the aldosterone-induced increase in Na entry is sufficient to saturate the baso-lateral pump a further increase in the Na permeability of the mucosal membrane could result in a transient Na diffusion potential and, therefore, in an increase in the measured I_{sc} with no concomitant increase in J_{net}^{Na}; when the transient is completed and a steady state is restored, the I_{sc} might once again closely reflect the "saturation level" of the active Na pump mechanism. When the pump mechanism is not saturated, increased Na entry into the Na transport pool induced by amphotericin B could rapidly result in a new steady state without a significant transient, as observed in the present (control tissues) and previous (Frizzell & Turnheim, *manuscript submitted*) studies. Although it is possible to offer

speculative explanations for the fact that a transient response was only occasionally observed in aldosterone-treated tissues, this does not seem warranted.

Other Studies: The Possibility of a Time-Dependent Dual-Effect of Aldosterone

Although the results of the present studies are consistent with the notion that the primary effect of aldosterone is to increase the ease of entry of Na into the cell across the mucosal membrane, there is evidence from other studies suggesting that aldosterone may also enhance the activity of the baso-lateral pump mechanism, but only after a delay of several hours.

In 1961, Crabbe reported that the increment in I_{sc} resulting from the addition of vasopressin to aldosterone-treated toad urinary bladder did not differ from that observed in control tissues; these results suggested that the effects of vasopressin and aldosterone are strictly additive and that the baso-lateral pump is not affected by the mineralocorticoid. However, Sharp and Leaf (1966) reported that vasopressin had a greater stimulatory effect on the I_{sc} of bladders that had been exposed to aldosterone for several hours compared to that observed in control tissues or during the initial response to aldosterone; these investigators attributed this effect to a glucocorticoid action of aldosterone. Fanestil, Porter and Edelman (1967) and Handler, Preston and Orloff (1969) confirmed the enhanced response of the I_{sc} to vasopressin in bladders that were exposed to aldosterone for several hours, and the latter group presented evidence that this delayed effect is due to the mineralocorticoid action of aldosterone. The notion that the initial response to aldosterone is the result of a direct effect at the apical membrane which enhances Na entry but that there is a later direct or indirect effect which enhances the activity of the baso-lateral pump mechanism is consistent with the observations of Lipton and Edelman (1971), Crabbe (1972, 1974), Saito, Essig and Caplan (1973), Lang, Caplan and Essig (1975), and Spooner and Edelman (1975) on amphibian epithelia.

Thus, whereas the results of the present studies suggest an early effect of aldosterone on the Na entry process alone, the possibility of a later direct or indirect effect which enhances the pump mechanism at the baso-lateral membranes of rabbit colon cannot be excluded and awaits further study.

This research was supported by grants from the U.S. Public Health Service, National Institutes of Health, NIAMDD (AM16275 and AM18199), the Western Pennsylvania

Heart Association, and the Wechsler Research Foundation. The expert technical assistance of Barbara Jennings and Dennis Clayton is gratefully acknowledged.

Dr. Frizzell is the recipient of an NIH Research Career Award (AM-00173).

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