

# Basolateral Potassium Channel in Turtle Colon

## *Evidence for Single-File Ion Flow*

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**ABSTRACT** Treatment of the apical surface of the isolated, ouabain-inhibited turtle colon with the polyene antibiotic amphotericin B permitted the properties of a barium-sensitive potassium conductance in the basolateral membrane to be discerned from the measurements of transepithelial fluxes and electrical currents. Simultaneous measurements of potassium currents and  $^{42}\text{K}$  fluxes showed that the movement of potassium was not in accord with simple diffusion. Two other cations, thallium and rubidium, were also permeable and, in addition, exhibited strong interactions with the potassium tracer fluxes. The results indicate that permeant cations exhibit positive coupling, which is consistent with a single-file mechanism of ion translocation through a membrane channel.

### INTRODUCTION

Recently (21), we showed that the basolateral membrane of the turtle colon contains a potassium conductance that is blocked by barium. The properties of this conductance can be studied by measuring ionic flows across portions of ouabain-treated colon in which the apical membrane has been rendered highly permeable to potassium by treatment with the polyene antibiotic amphotericin B. This preparation offers the unique opportunity to measure simultaneously both current flow and tracer fluxes through a biological cation conductance. We present here the results of studies which show that the barium-sensitive basolateral conductance is permeable to at least three cations: potassium, thallium, and rubidium. Furthermore, the results indicate that permeant cations exhibit a positive coupling that is consistent with single-file ion movement through a membrane channel.

### METHODS

For transmural measurements, colons were removed from turtles (*Pseudemys scripta*; Lemberger Co., Germantown, WI), stripped of musculature, and mounted in Ussing chambers ( $A = 5.2 \text{ cm}^2$ ) as previously described (10, 21). The provisions for measurement of transepithelial potential and voltage clamping have been described in

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detail (10). In these studies, both sides of the isolated tissue were bathed initially by solutions that contained (mM); 102 Na, 100 benzenesulfonate, 1 Ca, 2 Cl, 2.5 HCO<sub>3</sub>, 2.5 K, 5 D-mannitol, and 5 D-glucose. We used benzenesulfonate to replace chloride as the major anion for these studies because tissues treated with amphotericin B in the presence of chloride-rich solutions developed inordinately high conductances. This presumably reflects a significant chloride permeability of the polyene channel (6, 25, 26), which allows net salt and water entry into the cells sufficient to produce cell swelling and lysis. Benzenesulfonate was chosen as the substitute anion because tissues continue to actively absorb Na<sup>+</sup> in the presence of benzenesulfonate solutions and because benzenesulfonate has a binding affinity for calcium similar to that of chloride (7). All tissues were initially bathed on both sides by identical solutions and active sodium absorption was abolished by ouabain (0.1 mM, serosal). Transmural cation gradients were produced by adding small volumes of concentrated salt solution to the mucosal or serosal baths either before or after the addition of amphotericin B to the mucosal bath. Although this procedure resulted in a small (~60 mosmol) osmotic gradient across the tissue, it had the advantage of resulting in only one cation gradient (isosmotic ion substitution results in two), and allowed us to establish ion gradients conveniently without otherwise disturbing the bathing solutions. Experiments with potassium gradients showed no difference in currents produced by the chloride, methylsulfate, or nitrate salt of this ion, i.e., small amounts of chloride (~30 mM) did not result in cell damage. Furthermore, identical currents were obtained if potassium gradients were established by isosmotic substitution for sodium. The potassium content of all solutions was verified by flame photometry. In experiments in which the currents due to thallium or rubidium were measured, the tissues were bathed in potassium-free Ringers. All solutions were stirred and aerated by a stream of air bubbles so that the pH at room temperature was ~8.3.

Transmural fluxes of <sup>42</sup>K were measured simultaneously with those of [<sup>14</sup>C]-mannitol using a sample-and-replace paradigm (10). At least 1 h was allowed to achieve steady state tracer flow, and the flux period immediately following any experimental maneuver was always omitted from data analysis to avoid problems of non-steady tracer flow. Samples were counted in a liquid scintillation spectrometer and the net counts due to <sup>14</sup>C and <sup>42</sup>K were obtained. In most instances the data are presented as the cellular component of the transmural potassium flux that was obtained by correcting the total transmural potassium flux using the mannitol flux as a measure of the permeability of the paracellular shunt path (10). Other experiments (16) showed that the movements of potassium and mannitol in the paracellular path are in accord with simple diffusion. The magnitude of this correction never exceeded 30% of the total transmural flow.

In one series of experiments, the uptake of potassium or rubidium across the apical membrane was measured using the technique of Thompson and Dawson, which has been described in detail (35). Preliminary experiments indicated that in the presence or the absence of amphotericin B, the uptake of the <sup>42</sup>K or <sup>86</sup>Rb was a linear function of time for at least the first 60 s of exposure to the isotope. Thus, the uptake of tracer at 30–40 s was used to calculate the unidirectional influx across the apical membrane.

## RESULTS

### *Potassium Fluxes*

Table I summarizes the results of experiments in which transmural fluxes of <sup>42</sup>K and [<sup>14</sup>C]mannitol were measured in the presence of a mucosal-to-serosal

(M-to-S) potassium gradient ( $\sim 11:1$ ). The control values characterize the isolated colon after the basolateral Na/K pump has been blocked by ouabain and the apical sodium channel blocked with amiloride. The table shows that, as previously reported, mucosal amphotericin B induced a substantial  $I_{sc}$ , which was abolished by addition of serosal barium (5 mM). The M-to-S potassium flux behaved in an identical manner and a comparison of either the barium-induced inhibition or the amphotericin-induced increases in these quantities leads to the conclusion that the currents were entirely attributable to net potassium flow down an electrochemical potential gradient.

The values in Table I also permit a calculation of the ratio of the barium-sensitive transmembrane fluxes. For simple diffusion, Ussing's flux ratio (36) predicts that under short-circuit conditions ( $V_{ms} = 0$ ):

$$\Delta J_{ms}^K / \Delta J_{sm}^K = [K]_m / [K]_s, \quad (1)$$

where  $\Delta J_{ms}^K$  and  $\Delta J_{sm}^K$  are the barium-sensitive potassium fluxes.

TABLE I  
*Transcellular Potassium Fluxes in the Presence of Amphotericin B and Ouabain*

	$I_{sc}$ ( $n = 8$ )	$(J_{ms}^K)_{cell}$ ( $n = 4$ )	$(J_{sm}^K)_{cell}$ ( $n = 4$ )
(1) Pre-amphotericin	$0.92 \pm 0.08$	$0.38 \pm 0.05$	$0.03 \pm 0.01$
(2) Amphotericin	$6.44 \pm 0.52$	$5.95 \pm 0.78$	$0.17 \pm 0.03$
(3) Amphotericin + $Ba^{++}$	$1.48 \pm 0.28$	$0.72 \pm 0.06$	$0.04 \pm 0.01$
$\Delta(2 - 3)$	$4.95 \pm 0.67$	$5.24 \pm 0.77$	$0.13 \pm 0.04$

Units =  $\mu\text{Eq}/5.2 \text{ cm}^2$ .  $\bar{X} \pm \text{SEM}$ .

The cellular portion of the transmembrane potassium flow was obtained by using the simultaneously measured transmembrane mannitol flow to estimate the permeability of paracellular paths. Tissues were bathed on both sides by Na-benzenesulfonate Ringers and treated with serosal ouabain (0.1 mM) and mucosal amiloride (0.1 mM). The mucosal solution contained, in addition, 33 mM KCl and the serosal solution 3 mM KCl. Because the values of  $I_{sc}$  did not vary greatly between pairs the values have been averaged.

The expectation for simple diffusion is, therefore, a flux ratio of  $\sim 11$ , whereas the actual flux ratio was  $\sim 40$ , nearly fourfold in excess of the diffusional value. Additional insight into this result can be obtained by examining the behavior of the potassium rate coefficients. Since each of the fluxes in Eq. 1 can be written as the product of a rate coefficient and a bath concentration, we obtain by substitution:

$$\lambda_{ms}^K [K]_m / \lambda_{sm}^K [K]_s = [K]_m / [K]_s, \quad (2)$$

where  $\lambda_{ms}^K$  and  $\lambda_{sm}^K$  are the barium-sensitive, transmembrane rate coefficients for potassium flow from M to S and S to M, respectively.

Eq. 2 serves to emphasize the important point that for simple diffusional flow the rate coefficients should be identical (since  $V_{ms}$  is zero) and independent of the magnitude or direction of the transmembrane potassium gradient (9,

11). The results indicate that, contrary to the expectation for simple diffusion, there was a marked asymmetry in the rate coefficients such that

$$\lambda_{ms}^K > \lambda_{sm}^K,$$

which suggests that some nonconjugate force was coupled to tracer flow, which either facilitated tracer flow in the M-to-S direction or retarded tracer flow in the S-to-M direction, or both. In the absence of metabolic coupling and other significant ion gradients, these results suggest positive coupling between the flows of labeled and unlabeled potassium (9, 11).

A graphic demonstration of this positive coupling is provided by Fig. 1, which shows the behavior of the potassium tracer rate coefficients in the

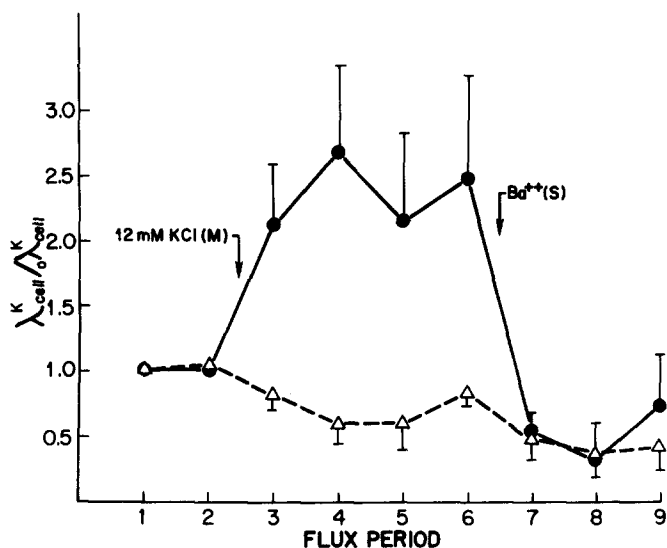


FIGURE 1. Rate coefficients for transmural potassium flows across portions of turtle colon treated with serosal ouabain (0.1 mM) and mucosal amphotericin B (15  $\mu$ M) in the presence and absence of a transmural potassium gradient. Initially, both solutions contained 2.5 mM K. As indicated by the arrows, mucosal KCl (12 mM) was added to create a transmural potassium gradient. ●,  $(\lambda_{cell}^K)_{ms}$ ; Δ,  $(\lambda_{cell}^K)_{sm}$ . Cellular rate coefficients were obtained by correcting according to the transmural mannitol flow and are presented as normalized values relative to the initial flux period. Flux periods were 20 min in duration and each value represents a mean from four tissues. Error bars indicate SEM.

presence and in the absence of a transmural potassium gradient. The addition of 12 mM KCl to M produced a dramatic increase in the M-to-S rate coefficient and a smaller decrease in the S-to-M rate coefficient. Note that the increase in the M-to-S rate coefficient was abolished by serosal barium. These results clearly suggest a strong interaction between tracer and abundant potassium.

*Single-File Transport*

There are a variety of kinetic schemes that could be invoked to account for the observation of positive coupling. One of the simplest is the model suggested by Hodgkin and Keynes (20) to explain anomalous potassium flux ratios in the squid giant axon: single-file diffusion. This model proposes that cations traversing a channel interact because the average channel contains more than one ion and these ions cannot pass each other. This model has recently been applied to studies of potassium channels in nerve membrane (3, 4, 19) and to ion and water flow in gramicidin channels (1, 14, 24, 30), and its attractive simplicity has led us to use it to analyze ion flow through the basolateral potassium conductance.

The expected flux ratio for a single-file channel under short-circuit conditions is given by:

$$J_{12}^K/J_{21}^K = ([K]_1/[K]_2)^n, \quad (3)$$

where  $J_{12}^K$  and  $J_{21}^K$  are the unidirectional fluxes through the channel. In simple single-file models in which ions move by a knock-on mechanism, the value of  $n$  is related to the number of ions in the pore (11, 19, 20). The behavior of the basolateral potassium conductance was compared with the predictions of this equation by measuring unidirectional potassium fluxes in the presence of different gradients of abundant potassium.

Fig. 2 shows the ratio of the barium-sensitive transmembrane potassium fluxes plotted vs. the transmembrane potassium concentration ratio on a log-log plot. The flux ratio was calculated by measuring in each tissue the barium-sensitive

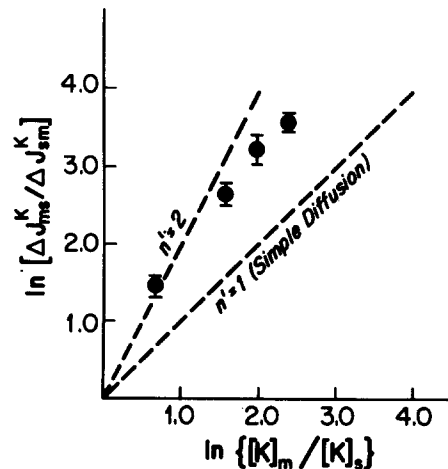


FIGURE 2. The logarithm of the ratio of the transcellular potassium fluxes plotted vs. the logarithm of the transmembrane potassium concentration ratio. Since the barium-sensitive  $I_{sc}$  is equal to the net potassium flux, potassium flux ratios were calculated for each tissue from the simultaneously measured barium-sensitive current and S-to-M potassium flux. Each point represents the mean and SEM for measurements of four to eight tissues.

current,  $\Delta I_{sc}$ , and the barium-sensitive S-to-M flux so that the flux ratio is given by:

$$\Delta J_{ms}^K / \Delta J_{sm}^K = (\Delta I_{sc} + \Delta J_{sm}^K) / \Delta J_{sm}^K. \quad (4)$$

On a log-log plot the expectation for simple diffusion is a straight line with a unity slope indicated by  $n' = 1$ . In each case, however, the measured flux ratio is well above the unity line. The points are in fact more consistent with an  $n'$  of 2. Thus, the behavior of the potassium flux ratios is qualitatively in accord with a single-file model.

#### Cation Selectivity

Since our ability to study ionic interactions in the potassium conductance was a function of the number of permeant ions, we investigated its ion selectivity.

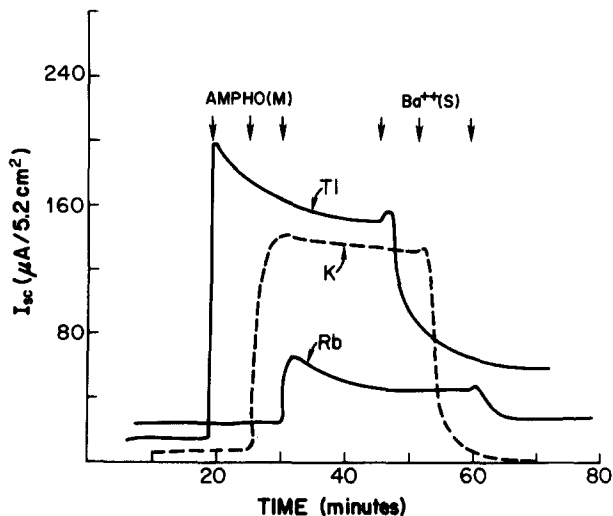


FIGURE 3. Representative polyene-induced currents in the presence of 10:1, M-to-S, transmural gradients of potassium, thallium, and rubidium. All tissues were initially bathed by potassium-free, sodium-benzenesulfonate Ringers with serosal ouabain (0.1 mM) and mucosal amiloride (0.1 mM). Cation gradients were established by adding appropriate amounts of the nitrate salts of potassium or thallium or the chloride salt of rubidium to the mucosal and serosal baths.

Fig. 3 shows the results of a representative experiment in which tissues were exposed to a 10:1 gradient of potassium, thallium, or rubidium. In each case, mucosal addition of amphotericin B induced a current that was partially (Tl) or totally (K, Rb) abolished by barium. In the case of thallium, additional barium (up to 10 mM) did not produce further inhibition of  $I_{sc}$ , which suggests the possibility of a barium-insensitive path for thallium movement across the basolateral membrane. Table II shows average values for a series of such experiments. If the barium-sensitive values are used for comparison, the apparent thallium permeability is approximately equal to that of potassium, whereas that of rubidium is  $\sim 1/10$  that of potassium. Note that in

TABLE II  
Cation Currents in the Presence of Amphotericin B and Ouabain

	Potassium (n = 23)	Thallium (n = 6)	Rubidium (n = 4)
(1) Pre-amphotericin	13.6±1.7	16.5±2.6	17.0±3.7
(2) Amphotericin	121.5±9.0	127.5±9.8	33.8±7.3
(3) Amphotericin + Ba <sup>++</sup>	16.7±3.6	36.8±12.2	21.8±8.0
$\Delta(2 - 3)$	104.3±9.0	90.7±11.3	12.0±3.3

Units =  $\mu\text{A}/5.2 \text{ cm}^2$ .  $[I]_m/[I]_s = 10$ .

Tissues were bathed on both sides by Na-benzenesulfonate Ringers, which for the measurement of thallium and rubidium currents was potassium free. 10-fold cation gradients were applied by adding ~30 mM of the appropriate salt to the mucosal bath and 3 mM to the serosal bath as a small volume of concentrated solutions.

each case the direction of the polyene-induced current was determined by the orientation of the imposed ionic emf; i.e., if the cation gradient was reversed, so was the direction of the current (Fig. 5). In similar experiments using cesium, ammonium, or lithium, there was no detectable barium-sensitive current.

#### Cation Interaction and a "Knock-On" Model

The simplest test for interaction between permeant cations is to examine the effect of substitute cations on the ionic current caused by potassium. Fig. 4 shows an example of one such experiment in which the effect of mucosal or serosal rubidium on an M-to-S potassium current was examined. At the arrows, 5 mM rubidium was added to the mucosal or the serosal bath. Serosal (*trans*) addition produced a marked inhibition of  $I_{sc}$ , whereas mucosal (*cis*) addition was virtually without effect. In view of the relatively low permeability of rubidium (Table II), this result cannot be attributed to rubidium current.

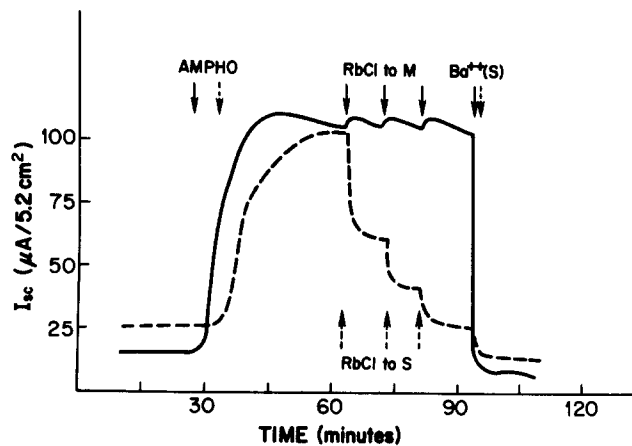


FIGURE 4. Effect of *trans* or *cis* addition of RbCl on amphotericin-induced transmural potassium current. Each arrow indicates the addition of 5 mM RbCl<sub>2</sub>.  $[K]_m$ , 17.5 mM;  $[K]_s$ , 2.5 mM.

In six flux measurements in the presence of an M-to-S potassium gradient, the reduction in  $I_{sc}$  caused by *trans* rubidium (15 mM) was  $2.6 \pm 0.5 \mu\text{Eq}/5.2 \text{ cm}^2 \cdot \text{h}$  and the reduction in net potassium flux was  $2.4 \pm 0.4 \mu\text{Eq}/5.2 \text{ cm}^2 \cdot \text{h}$ .

Fig. 5 shows that the *trans* inhibition by rubidium was independent of the direction of the potassium current. A potassium current was induced from S to M (by an S-to-M K gradient) and this current was virtually unaffected by serosal rubidium but was blocked by mucosal (cellular) rubidium. Regardless of the direction of net potassium flow, the predominant effect was *trans* inhibition.

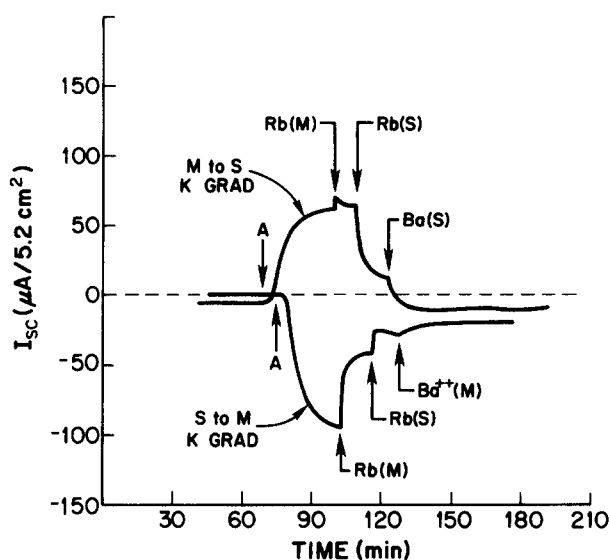


FIGURE 5. Effect of *trans* or *cis* addition of RbCl on M-to-S and S-to-M potassium currents induced by M-to-S or S-to-M gradients of KCl (30:3).

To interpret qualitatively the asymmetric effect of rubidium, we have adopted the simplest possible variant of single-file schemes: the "knock-on" model introduced by Hodgkin and Keynes (20). We chose the knock-on model, not because it offers profound physical insights, but because it has great heuristic value in relation to a qualitative assessment of modes of ionic interaction. The knock-on model places single filing in a mechanical context in which ion translocation through the channel consists of two steps: first the ion must enter the channel, which is a random event. Once inside the channel, translocation and exit from the channel are governed not only by thermal energy but also by the number of "hits" the ion receives from each side. The number of hits on either side is directly proportional to the concentration of permeant ions on either side. This billiard-ball type of interaction creates positive coupling between the flows of permeant ions.

This simple mechanical scheme suggests two possible types of interaction between permeant ions, "block" and "knock." An ion can "block" by entering the channel and "sticking" there so that the translocation of a second ion in



either direction is impeded. In addition, an entering ion, regardless of its intrinsic mobility in the channel, could exert a "knock" effect by accelerating the exit of ions residing in the channel toward the other side. The utility of this simplistic dichotomy lies in the fact that these two effects can be distinguished experimentally. The "knock" effect will lead to positive coupling between permeant species such that it should be possible to drive the net flux of one ion with the gradient of a second ion. In contrast, a simple block effect on tracer flow should be revealed by symmetric addition of the substitute ion so that no source of free energy exists.

Fig. 6 shows the results of a direct test for a knock-on interaction between permeant cations. Transmural potassium fluxes were measured in the absence

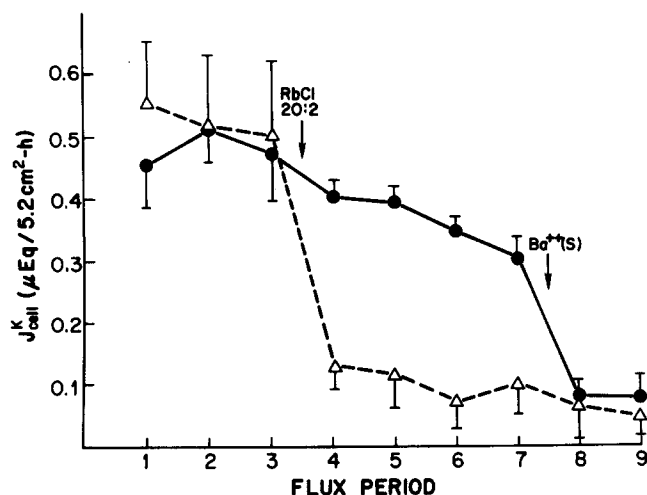


FIGURE 6. Net potassium flow induced by a rubidium gradient in the absence of a potassium gradient. Opposing  $^{42}\text{K}$  fluxes were measured on paired tissues in the absence and in the presence of a 20–2 mM M-to-S transmural rubidium gradient. ●,  $J_{ms}^K$ ; Δ,  $J_{sm}^K$ .  $[\text{K}]_m = [\text{K}]_s = 5$  mM. Cellular fluxes were obtained by correcting according to the simultaneously measured transmural mannitol flux. Values for M-to-S and S-to-M fluxes represent means from determinations on seven and five tissues, respectively.

of a potassium gradient, with and without a rubidium gradient. Before the addition of rubidium, the transmural K fluxes were virtually identical. The application of an M-to-S rubidium gradient induced a net potassium flux from M to S, largely by virtue of a marked inhibition of the S-to-M potassium flux. Fig. 7 shows the results of a similar experiment in which the imposition of a transmural thallium gradient produced net potassium flow by virtue of a substantial increase in the M-to-S flux and a substantial decrease in the S-to-M flux. Both results provide compelling evidence for positive coupling between the flows of permeant cations.

If equal concentrations of a second permeant ion are added to both sides of a single-file channel there should be no net "knock" effect since there is

no source of free energy to drive net ionic flow. Any effect of this addition on tracer flow should be attributable to blockade of the channel, i.e., entry of the second ion reducing the mobility of the first or making channels unavailable for the first ion. Fig. 8 shows the effect of symmetrical rubidium addition on potassium fluxes measured in the absence of a transmural potassium gradient. Rubidium, which has only 1/10 the intrinsic permeability of potassium, is an effective blocker of potassium fluxes. Fig. 9 shows a representative experiment with symmetrical thallium addition. Thallium, which is at least as permeable as potassium and which exerts an appreciable knock effect (Fig. 6), also blocks tracer potassium flow (28).

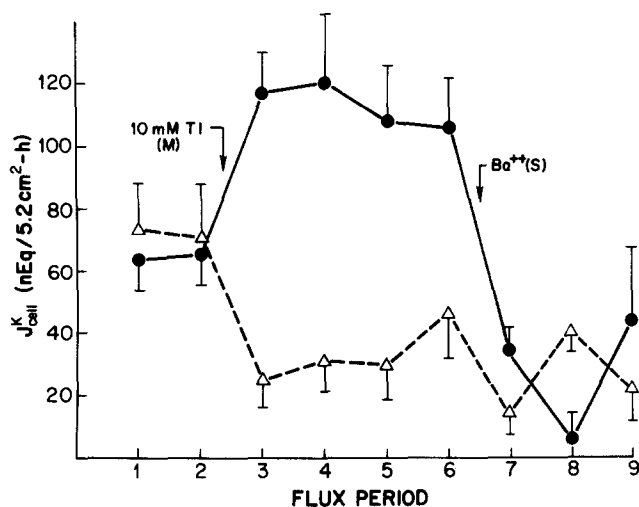


FIGURE 7. Net potassium flow induced by a thallium gradient in the absence of a potassium gradient. Cellular components of the transmural potassium fluxes were determined before and after the addition of 10 mM  $TlNO_3$  to the mucosal bath. ●,  $J_{ms}^K$ ; △,  $J_{sm}^K$ . Mucosal and serosal bathing solutions were sodium-benzenesulfonate Ringers containing 2.5 mM potassium. Each point represents the mean  $\pm$  SEM from five tissues.

#### *Cation-Cation Interactions Do Not Occur in the Polyene Channel*

All of the results presented were obtained from measurements on a series membrane system. Hence, it is necessary to consider the possibility that all or part of the observed cation-cation interactions might reflect properties of the amphotericin B channels in the apical membranes of the epithelial cells. We addressed this issue by measuring the unidirectional influx of  $^{42}K$  from the mucosal bathing solution into the cells of the colon in the presence of amphotericin B and a transmural potassium gradient. Table III shows the results. As expected, addition of the polyene increased  $I_{sc}$  and potassium influx. Serosal addition of barium or rubidium markedly reduced  $I_{sc}$  but potassium influx was virtually unaffected, as expected if barium and rubidium

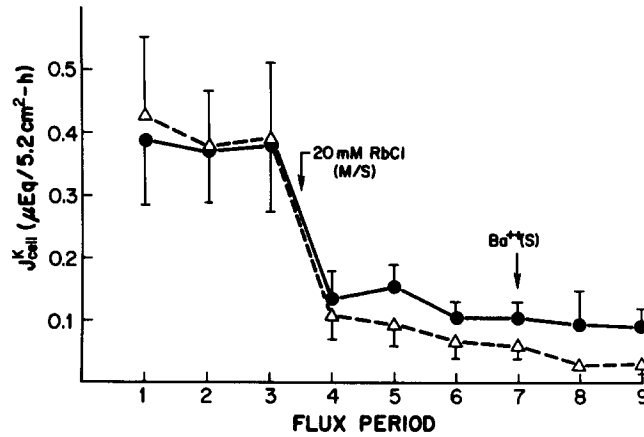


FIGURE 8. Rubidium blockade of cellular potassium fluxes. Mucosal and serosal potassium concentrations were 5 mM. ●,  $(J_{cell}^K)_{ms}$ ; △,  $(J_{cell}^K)_{sm}$ . Cellular fluxes were measured before and after the addition of 20 mM RbCl to both bathing solutions ( $n = 3$ ,  $x \pm \text{SEM}$ ).

block potassium movement at the basolateral membrane of the epithelial cells.

Using the amphotericin-induced increases in  $I_{sc}$  and  $J_i^K$  it is possible to calculate the flux ratio across the apical membrane, i.e.,

$$J_{mc}^K/J_{cm}^K = J_{mc}^K/(J_{mc}^K - I_{sc}),$$

where  $J_{mc}^K$  and  $J_{cm}^K$  are the unidirectional fluxes across the apical membrane. The values from Table III yield a flux ratio of 1.6 for the apical

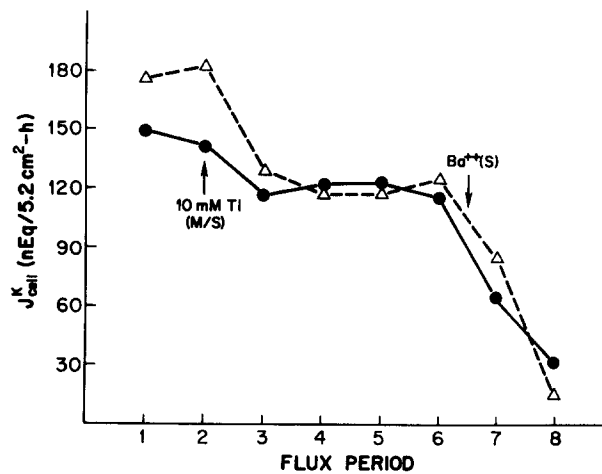


FIGURE 9. Representative experiment showing the effect of symmetric thallium addition on cellular potassium fluxes. Symbols are as in Fig. 8.

TABLE III  
Potassium Influx: Effects of  $Ba^{++}$  and  $Rb^+$

	Control (n = 16)	Amphotericin (n = 24)	Amphotericin + $Ba^{++}$ (n = 7)	Amphotericin + $Rb^+$ (n = 6)
$J_i^K$	0.31±0.08	3.57±0.29	3.21±1.43	2.99±1.26
$I_{sc}$	0.19±0.02	1.42±0.10	0.19±0.09	0.35±0.15

Units =  $\mu\text{Eq}/\text{cm}^2 \cdot \text{h}$ .  $\bar{X} \pm \text{SEM}$ .

Potassium influx was determined in tissues exposed to Na-benzenesulfonate Ringers containing ouabain (serosal, 0.1 mM) and amiloride (mucosal, 0.1 mM) in the presence of a 30–3 mM, M-to-S potassium gradient. Barium chloride (5 mM) and rubidium chloride (15 mM) were added to the serosal bath.

membrane. The transmural flux ratio can be expressed in terms of the flux ratios at the apical and basolateral membranes by assuming a three-compartment model (see Appendix), which yields:

$$J_{ms}^K/J_{sm}^K = (J_{mc}^K/J_{cm}^K)(J_{cs}^K/J_{sc}^K),$$

where  $J_{cs}^K$  and  $J_{sc}^K$  are the fluxes from cell to serosal bath and serosal bath to cell, respectively. The basolateral flux ratio,  $J_{cs}^K/J_{sc}^K$ , can thus be calculated to be 25, a value that is more than twice as large as the maximum expected for simple diffusion even if the total transmural driving force appears across the basolateral membrane. The observed nondiffusional flux ratio is, therefore, almost totally attributable to the basolateral membrane. The radius of the polyene channel ( $\sim 4 \text{ \AA}$ ) and weak ion selectivity also argue against the polyene pore as a site of cation coupling. Net water flows induced by the small osmotic gradient, if present, would produce effects opposite in direction to those observed.

The relatively low flux ratio for potassium at the apical membrane is in accord with the notion that the major barrier to transmural potassium flow resides in the basolateral membrane. A similar result emerges from a consideration of the rubidium influx shown in Table IV. The amphotericin-induced increase in  $J_i^{Rb}$  is similar to that seen for potassium even though the transmural current is substantially less. Taken together, these observations show that the ion selectivity, the flux ratio, and blocking effects observed in transmural flow measurements are properties of the basolateral membrane.

TABLE IV  
Rubidium Influx Across the Apical Membrane in the Presence of Amphotericin B

	Control (n = 11)	Amphotericin (n = 14)
$J_i^{Rb}$	0.30±0.07	3.79±0.46
$I_{sc}$	0.08±0.02	0.37±0.05

Units:  $\mu\text{Eq}/\text{cm}^2 \cdot \text{h}$ .  $[Rb]_m = 30 \text{ mM}$ ;  $[Rb]_s = 3 \text{ mM}$ . Amphotericin, 15  $\mu\text{M}$ .  $\bar{X} \pm \text{SEM}$ .

## DISCUSSION

The results of this study indicate that the basolateral membrane of the turtle colon contains a potassium conductance with properties that are similar to those of potassium channels in nerve and muscle. These channels are blocked by barium and exhibit a selectivity sequence for the monovalent cations that is similar to that measured in the turtle colon (2, 5, 8, 13, 15, 17, 18, 29, 32). In addition, channels in nerve and muscle are characterized by strong interactions between permeant cations which are reflected in anomalous tracer flux ratios and blocking effects (3–5, 19, 20, 31, 34). These similarities have led us tentatively to attribute the potassium conductance of the basolateral membrane to a channel or pore through which potassium ions move in response to the transmembrane electrochemical potential gradient. The present results are not sufficient to establish the pore-like nature of the basolateral potassium conductance, but the concept offers an economical working hypothesis that provides a useful framework for interpreting the data. With this reservation, for the remainder of the discussion we will use the term "channel" to refer to the site of the basolateral conductance.

*Barium Blockade*

There is now a considerable body of evidence indicating that barium ion is a potent blocker of potassium channels in muscle (32, 33), nerve (2, 13), and epithelial cells (12, 21, 27, 37, 38). This effect has been demonstrated for epithelial potassium channels in apical (12) as well as basolateral cell membranes (21, 27). The simplest hypothesis for barium blockade recognizes the fact that the crystal radius for barium ion is quite close to that of potassium (27). Barium ions might thus enter the channel but would not traverse the pore because of the electrostatic energy barrier created by its two charges. Two studies of barium blockade of the delayed rectifier potassium conductance in squid giant axon are consistent with this simple hypothesis (2, 13). Recently, Van Driessche and Zeiske (37) analyzed fluctuations in apical potassium conductance in frog skin that were thought to be induced by reversible barium blockade of a sort expected if barium moved in and out of the channel.

*Potassium Flux Ratios*

Potassium flux ratios have been determined for the giant axon of the squid in the presence of different electrochemical gradients (3, 4, 20). In all cases, the flux ratio has been nondiffusional, or in the framework of Eq. 3, a value of  $n'$  greater than unity was required to describe the results. Hodgkin and Keynes (20) found an  $n'$  of 2.5, while in a more recent study, Begenisich and De Weer (3, 4) found  $n'$  to vary, generally between 2 and 3. Spalding et al. (31) found a potassium flux ratio exponent of 2 in depolarized frog skeletal muscle when the external potassium concentration exceeded  $\sim 70$  mM. In the present study, the value of  $n'$  varied from  $\sim 1.5$  to 2, depending on the value of the potassium concentration ratio. The issue of principal importance is that in the turtle colon, as in the squid axon, the flux ratios are markedly

nondiffusional. Even at the lowest apparent value for  $n'$  (1.5), the flux ratio is  $\sim 4.5$  times that predicted for simple diffusion. This qualitative conclusion, since it is based on the analysis of flux ratios, is independent of problems caused by unstirred layers or series membranes (9, 11). On the other hand, the actual value of the flux ratio obtained in the present experiments must be interpreted with caution because all measurements pertain to a series membrane system. Although it was demonstrated that the polyene-treated apical membrane is not the site of cation-cation interactions, the value of the flux ratio will be affected by the fractional conductance of the basolateral membrane. It is readily demonstrated (see Appendix) that the value of  $n'$  obtained from transcellular flux measurements is a lower limit for the actual flux ratio at the basolateral membrane.

#### *Cation-Cation Interactions in Potassium Channels*

The simplest expression of cation-cation interaction is the blockade of the channel to one ion by virtue of the presence of a second permeant ion. The present results show that rubidium, which is  $\sim 1/10$  as permeant as potassium in the basolateral channel, is a potent blocker of potassium movement. Similarly, rubidium was found not only to be less permeable than potassium in squid axon (5) and frog skeletal muscle (34), but also to block potassium flow.

The ability to measure simultaneously electrical current flow and tracer fluxes across the polyene-treated colon enabled us to perform the most direct test for cation-cation interaction in the basolateral channel, the coupling of the flows of permeant ions. The fact that, in the absence of a transmural potassium gradient, net flow of potassium can be induced by gradients of either rubidium or thallium represents compelling evidence for positive coupling between the flows of these ions such as would be expected if both ions can occupy a single channel at the same time.

At least two physical mechanisms have been suggested that can, in principle, account for this effect. The repulsive Coulombic force exerted by the second ion could hasten the exit of the tracer from the opposite end of the channel (19, 22–24). A second possibility is strict single filing of ions and water in which the movements of two occupying ions are coupled via the intervening water molecules (1, 14, 24). The latter mechanism is conceptually similar to a simple knock-on scheme.

#### *Biological Significance of the Basolateral Potassium Channel*

The results of the present study describe the properties of a potassium channel in the basolateral membrane of the turtle colon. The relation between the properties of this channel and the conductive properties of the basolateral membrane of normally transporting tissue, however, has yet to be resolved. The treatments employed to functionally isolate the basolateral membrane (amphotericin, ouabain) are likely to cause alterations in cell composition and cell volume that could result in quantitative or even qualitative changes in the properties of the basolateral membrane, i.e., the potassium conductance detected in this altered state may have been induced by

the very maneuvers used to make the measurements. Further studies are required to determine whether this potassium conductance plays a role in normal epithelial cell function or in the response of epithelial cells to perturbations in cellular composition.

#### APPENDIX

##### *Interpretation of Flux-Ratio Exponent ( $n'$ ) in the Presence of Series Membranes*

Consider a three-compartment model where compartments 1, 2, and 3 represent the mucosal bathing solution, the cell interior, and the serosal bathing solution, respectively. Let the flux ratio at the apical membrane be in accord with simple diffusion and that at the basolateral membrane be single-file transport. The two flux ratios are given by (9, 11, 35):

$$\begin{aligned} J_{12}/J_{21} &= \exp(\Delta\tilde{\mu}_{12}/RT); \\ J_{23}/J_{32} &= \exp(N'\Delta\tilde{\mu}_{23}/RT), \end{aligned} \quad (\text{A1})$$

where  $\Delta\tilde{\mu}_{12}$  and  $\Delta\tilde{\mu}_{23}$  are the electrochemical potential difference across the apical and basolateral membranes, respectively, and  $N'$  is the flux-ratio exponent for the single-file process.

To obtain the measured flux ratio we note that

$$J_{13}/J_{31} = (J_{12}/J_{21})(J_{23}/J_{32}). \quad (\text{A2})$$

In addition, since the electrochemical potential differences at the two membranes must add to give the transmural electrochemical potential difference, we have:

$$\Delta\tilde{\mu}_{13} = \Delta\tilde{\mu}_{12} + \Delta\tilde{\mu}_{23}; \quad (\text{A3})$$

$$1 = \Delta\tilde{\mu}_{12}/\Delta\tilde{\mu}_{13} + \Delta\tilde{\mu}_{23}/\Delta\tilde{\mu}_{13}. \quad (\text{A4})$$

Thus, we write:

$$\Delta\tilde{\mu}_{23} = \beta\Delta\tilde{\mu}_{13}; \quad (\text{A5})$$

$$\Delta\tilde{\mu}_{12} = (1 - \beta)\Delta\tilde{\mu}_{13}, \quad (\text{A6})$$

where  $\beta = \Delta\tilde{\mu}_{23}/\Delta\tilde{\mu}_{13}$ .

Combining Eqs. A1, A2, A5, and A6, we obtain for the transmural flux ratio

$$J_{13}/J_{31} = \exp\{[1 + \beta(N' - 1)]\Delta\tilde{\mu}_{13}/RT\}. \quad (\text{A7})$$

By inspection of Eqs. A1 and A7 we see that the measured flux-ratio exponent for a two-barrier system,  $n'$ , is related to the actual exponent at the basolateral membrane by:

$$n' = 1 + \beta(N' - 1), \quad (\text{A8})$$

where  $n' \leq N'$ . Thus,  $n'$  is a minimum estimate of  $N'$ . The relation between the two values is determined by  $\beta$ , which is the fractional conductance of the basolateral membrane. If  $\beta = 1$ , the values of  $n'$  and  $N'$  are identical because the contribution of the apical membrane is negligible.

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