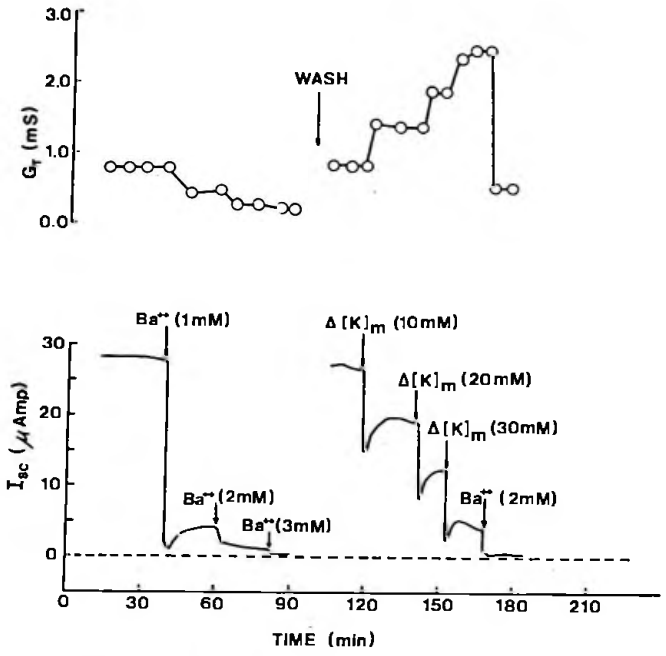

Fig. 3.--Reversible inhibition of I_{sc} by mucosal barium and effect on I_{sc} and G_T of increasing mucosal potassium concentration.

periments indicate that R_{cell} is linearly related to the reciprocal of $[K]_m$ whereas E_T decreases linearly with the log of $[K]_m$. The electrical behavior of the flounder bladder is thus at least qualitatively consistent with the presence in the apical membrane of a cation channel which is highly selective for potassium and is blocked by barium. Furthermore, this behavior is consistent with the notion that the sole driving force for potassium exit from the cell is the electrochemical potential gradient of this ion across the apical membrane. This research was supported by a grant from the NIH-NIAMDD (AM18776) and D.C.D. was the recipient of a Research Career Development Award from NIH-NIAMDD (AM00702).

POTASSIUM TRANSPORT BY THE INTESTINE OF THE WINTER FLOUNDER, SPEUDOPLEURONECTES AMERICANUS: EVIDENCE FOR KCl CO-TRANSPORT

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Prior studies of electrolyte transport across flounder intestine under short-circuit conditions revealed active transport of Na and Cl from mucosa to serosa. The short-circuit current could be attributed to Na and Cl absorption, according to the relation, $I_{sc} = J^{Na}_{net} - J^{Cl}_{net}$. However, the high Na permeability of this tissue produces uncertainty with regard to the absolute magnitude of the net Na flux and evaluation of the relation between I_{sc} and net ion movements. In the present study, the possibility that flounder intestine carries out net K transport was examined. The finding of net K secretion led to an examination of the permeability properties of flounder intestinal cells to K and Cl.

Methods

Transepithelial ion fluxes were determined under short-circuit conditions as previously described (Field, et al., J. Memb. Biol. 41: 265, 1978). Care was taken to assure that the unidirectional fluxes of K and Rb were measured

under steady-state conditions: a one-hour equilibration period following isotope addition was required to obtain a time-independent flux from serosa to mucosa; this protocol was adopted for subsequent studies. Conventional microelectrodes were employed to determine the electrical potential profile of the epithelial cells as described by Duffey, et al., (J. Memb. Biol. 50: 331, 1979).

Results and Discussion

Unidirectional fluxes of K and Rb across flounder intestine maintained under short-circuit conditions are given in Table 1. Potassium fluxes were measured using the standard Ringers solution (Field et al., J. Memb. Biol. 41:265, 1978)

TABLE 1.--UNIDIRECTIONAL K OR Rb FLUXES ACROSS FLOUNDER INTESTINE

J^{K}_{ms}	J^{K}_{sm}	J^{K}_{net}	I_{sc}	G_t
0.40 ± 0.05	1.1 ± 0.10	-0.73 ± 0.12	-3.7 ± 0.2	25 ± 3
J^{Rb}_{ms}	J^{Rb}_{sm}	J^{Rb}_{net}	I_{sc}	G_t
0.40 ± 0.05	1.3 ± 0.05	- .90 ± 0.10	- .35 ± 0.4	30 ± 2

All values are in μEq/cm^{2}hr except G_t in mS/cm^2, means + SEM for 6 K and 4 Rb flux experiments. The standard electrolyte solution (Field, et al., J. Memb. Biol. 41: 265, 1978) was employed for the K flux studies, and for the Rb flux studies Rb replaced K on an equimolar basis.

and Rb fluxes were determined using a Ringers solution in which K was replaced by Rb on an equimolar basis. Tissues maintained normal values of I_{sc} and tissue conductance (G_t) when incubated in Rb-Ringers for a 3-4 hr period. The bidirectional fluxes of K and Rb were indistinguishable and both ions were secreted at rates that did not differ significantly. Therefore, in subsequent flux studies Rb was employed as a substitute for K to avoid technical problems associated with the use of the short-lived ^{42}K. The results given in Table 1 justify this approach.

The finding that flounder intestine actively secretes K (and Rb) indicates that the I_{sc} across this tissue is not solely determined by the difference in the absorptive fluxes of Na and Cl and that the secretory flux of K must also enter into the balance equation. However, both J^{Na}_{net} and J^{K}_{net} are small relative to J^{Cl}_{net}, and the magnitude of K secretion is less than the standard error encountered in the determination of J^{Na}_{net}. Thus the I_{sc} across this tissue is largely determined by the rate of active Cl absorption.

The capacity for active K secretion raised the possibility that the apical membrane of flounder intestinal cells is permeable to K. This was examined using ion-replacement studies. The effect of altering the K and Cl concentrations of the mucosal and serosal solutions on the electrical potential differences across the apical (ψ_a) and basolateral (ψ_b) membranes were determined and the results of these studies are presented in Table 2. Elevation of mucosal solution K concentration, $[K]_m$, from 5 to 75 mM rapidly depolarized ψ_a by 48 mV. A more complete relation between ψ_a and $[K]_m$ is provided in the companion paper by Smith et al. (Bull. MDIBL. 20: 1980). Elevation of $[K]_m$ resulted in a comparatively small change in transepithelial electrical potential difference, ψ_t (c.a. 5 mV) indicating substandial shunting of the electromotive force generated across the apical membrane to the basolateral membrane, as would be expected for this low resistance epithelium. These findings are in agreement with those of Helman and Beyenbach (Bull. MDIBL. 18: 51, 1978), who also demonstrated little if any effect of varying mucosal solution Na and Cl concentrations on ψ_a. Thus, the permselective properties of the apical

TABLE 2.--EFFECTS OF K, Cl AND Ba ON APICAL (ψ_a) AND BASOLATERAL (ψ_b) MEMBRANE POTENTIALS

Mucosal Solution	ψ_a	Serosal Solution	ψ_b
5mM K	-59 ± 2 (26)	5mM K	55 ± 3 (11)
75mM K	-10 ± 1 (9)	50mM K	54 ± 3 (12)
5mM K + 5mM Ba	-37 ± 2 (27)	5mM K	47 ± 1 (6)
75mM K + 5mM Ba	-24 ± 1 (11)	5mM K + 5mM Ba	44 ± 1 (6)
		150mM Cl	63 ± 1 (6)
		0mM Cl	61 ± 1 (4)

Values of ψ_a and ψ_b given in mV, mean + SEM. The standard electrolyte solution (5mM K, 150mM Cl) was used as control; K replaced Na in high K media; Cl replaced by SO_4 and mannitol in Cl-free media. Similar results were obtained with gluconate replacing Cl. Number of observations given in parentheses.

membrane are dominated by its K conductance. Addition of Ba to the mucosal solution also depolarized ψ_a. Barium has been shown to reduce the K permeability of the basolateral membranes of Na-transporting epithelia (Nagel, Biochim. Biophys. Acta, 552: 356 1979; Kirk et al., Nature, 287: 237, 1980). The present findings demonstrate that Ba is also capable of blocking K channels traversing the apical membrane since the depolarization of ψ_a elicited by elevated $[K]_m$ was markedly reduced in the presence of Ba (Table 2).

Elevation of serosal solution K concentration from 5 to 50 mM or omission of Cl from the serosal bathing solution had no significant effect on ψ_b. In these studies, sufficient time was allowed for the changes in ψ_t induced by differences in the K and Cl concentrations of the mucosal and serosal solutions to come to steady values. This assured that a change in the ionic composition of the solutions bathing the basolateral membranes of the epithelial cells had been produced. Finally, addition of Ba to the serosal bathing solution alone had no effect on ψ_b or ψ_t. These results strongly suggest that the conductance of the basolateral membrane to K and Cl is very low.

The effects of Ba on unidirectional Rb fluxes under short-circuit conditions are given in Table 3. Since Ba was added to the mucosal or serosal solution following a control flux period, Rb fluxes were also determined during sequential control flux periods to assure that time-dependent changes in Rb fluxes do not complicate interpretation of the effects of Ba. Addition of Ba to the mucosal solution alone decreased I_{sc}, abolished net Rb secretion and unmasked a modest rate of Rb absorption. The reduction in I_{sc} elicited by mucosal Ba could not be entirely attributed to the change in J^{Rb}_{net}. Results of preliminary studies indicate that a reduction in Cl absorption may be responsible for the remainder of the decrease in I_{sc}; however, further studies are necessary to verify this. In addition, the possibility that the Rb absorption revealed by mucosal Ba might be sensitive to addition of ouabain to the mucosal solution should be tested.

A revised model for electrolyte transport across flounder intestine is illustrated in Figure 1. Chloride entry into the cell from the mucosal solution is mediated by NaCl co-transport; Na that enters with Cl is subsequently excluded across the basolateral membrane by the Na-K-pump. The predominant cation selectivity of the paracellular pathway permits a fraction of the transported sodium to recycle to the mucosal solution under

TABLE 3.--EFFECT OF Ba ON Rb FLUXES ACROSS FLOUNDER INTESTINE

	J^{Rb}_{ms}	J^{Rb}_{sm}	J^{Rb}_{net}	I_{sc}	G_t
Control	0.40 ± 0.05	1.3 ± 0.05	-0.90 ± 0.10	-3.5 ± 0.4	30 ± 2
Control	0.43 ± 0.05	1.2 ± 0.07	-0.77 ± 0.09	-2.8 ± 0.5	31 ± 1
Control	0.41 ± 0.05	1.2 ± 0.22	-0.81 ± 0.26	-3.2 ± 0.3	28 ± 3
+ 5mM Ba (m)	1.1 ± 0.24*	0.57 ± 0.09*	0.54 ± 0.29*	-0.66 ± 0.11*	22 ± 3
Control	0.41 ± 0.06	1.3 ± 0.19	-0.86 ± 0.22	-3.2 ± 0.3	30 ± 0
+ 5mM Ba (s)	0.35 ± 0.04	1.2 ± 0.26	-0.88 ± 0.29	-3.1 ± 0.1	28 ± 2

All values in $\mu Eq/cm^2 hr$ except G_t in mS/cm^2, mean ± SEM of 6 experiments. $BaCl_2$, 5mM, added to the mucosal (m) or serosal (s) solution alone following the control flux period. See Table 1 for solution composition.

*$p < 0.05$.

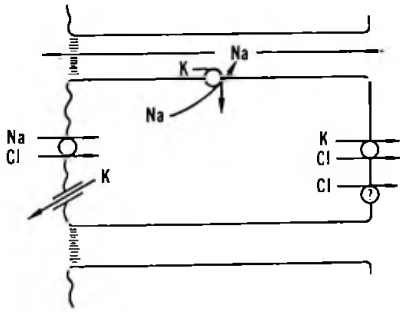

Figure 1.--Revised model for ion transport by flounder intestine. See text for details.

short-circuit conditions. These features of the model have been previously discussed (Field et al., J. Memb. Biol. 41: 265, 1978; Frizzel et al., J. Memb. Biol. 46: 27, 1979).

The results of the present study suggest that the apical membrane is characterized by a Ba-sensitive K conductance which is the major determinant of ψ_a and permits net K secretion across the apical membrane down its electrochemical potential difference. Intracellular K activity averages approximately 85 mM (Smith et al., Bull. MDIBL. 20: 1980), so that the net driving force favoring K secretion from cell to mucosal solution is approximately 20 mV. Addition of Ba to the mucosal solution blocks the K conductance of the apical membrane, depolarizes ψ_a and abolishes K secretion. In addition, the K and Cl conductances of the basolateral membrane appear to be negligible, so that diffusional movements of K and Cl cannot contribute to net transport of these ions across this barrier. Thus, a substantial portion of net Cl movement from cell to serosal solution may result from Kcl co-transport. This conclusion is in agreement with findings obtained from both rabbit (Duffey et al. J. Memb. Biol. 42: 229, 1978) and Necturus (Reuss et al., J. Memb. Biol. 47: 239, 1979) gallbladders, suggesting that the Cl conductance of the basolateral membrane is insufficient to account for the observed rate of net Cl transport across this barrier.

Since Cl entry and exist across the limiting membranes of the epithelial cell are non-conductive, the critical driving forces determining net Cl movement are the chemical potential differences for Cl across the apical and basolateral memberanes; neutral NaCl or KCl co-transport processes would not be influenced by trans-membrane electrical potential differences. At the apical membrane, both Na and Cl enter the cell down their concentration differences; however, at the basolateral membrane Cl exists from the cell against its concentration difference. The energy for Cl exit could be derived from the concentration difference for K via Kcl co-transport and would represent the "uphill" step in Cl absorption. Determination of cell K and Cl activities (Smith et al. Bull. MDIBL 20: 1980) indicates that the energy inherent in the chemical potential difference of K across the basolateral membrane is approximately 75 mV, which would be sufficient to drive Cl out of the cell against its chemical potential difference of approximaterly 40 mV.

Finally, comparison of the rates of net Na, Cl and K transport across the limiting membranes of flounder intestinal cells indicates that KCl co-transport may not be the sole determinant of Cl exit from the cell. The rate of NaCl co-transport across the apical membrane averages approximately 5 μEq/cm^{2}hr, so that sodium must be extruded from the cell at an equal rate. If the Na-K pump stoichiometry is 3:2 then K enters the cell at a rate of approximately 3.5 μEq/cm^{2}hr. Of this, approximately 0.5 μEq/cm^{2}hr is secreted leaving 3.0 μEq/cm^{2}hr to "recycle" across the basolateral membrane via KCl co-transport. Therefore, 3/5 or 60% of Cl absorption could be derived from KCl co-transport if these assumptions are correct. Other co-transport or counter-transport processes, also electrically neutral, presumably account for the remainder of net Cl movement across the basolateral membrane. Supported by grants from the NIH (AM 27524 and AM 21345) and Merck and Co. PLS was supported by a National Research Service Award (AM 05973), MJW by a National Pulmonary Faculty Training Award (HL 07159) and RAF by a Research Career Development Award (AM 00173); from the NIH.

CHLORIDE ABSORPTION BY THE INTESTINE OF THE WINTER FLOUNDER PSEUDOPLEURONECTES AMERICANUS: MECHANISM OF INHIBITION BY REDUCED pH

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A revised model for NaCl absorption by flounder intestine is discussed in the companion paper by Stewart et al (Bull. MDIBL., 20: 1980). This model includes features previously discussed in detail (Field et al., J. Memb. Biol.,