When g_K^a is low as a result of spontaneous variation or experimentally-induced reduction of g_K^a, inhibition of Cl absorption either by exposure to Cl-free mucosal solutions or by addition of 10^{-5}M bumetanide to the mucosal solution increased g_K^a and hyperpolarized ψa. This effect was more pronounced in tissues showing spontaneously high Cl absorption rates. These findings suggest that the K conductance of the apical membrane varies inversely with the rate of Cl absorption, but the mechanistic details of this relationship remain to be defined.

The hyperpolarization of ψa induced by Cl replacement or bumetanide is given as a function of the initial ψa in Figure 4. The effect of Cl-free media or bumetanide is small when ψa approaches -70 to -80 mV since g_K^a would be near its maximal value. The E_K across the apical membrane of flounder intestinal cells, calculated from cellular and mucosal solution K activities using the Nernst equation, is -77 $\pm$ 3 mV (Smith et al., Bull. MDIBL 20:96, 1980) suggesting that ψa approaches E_K as g_K^a increases. Thus, inhibition of Cl absorption by exposure to Cl-free media or bumetanide increases g_K^a so that ψa approaches E_K. Supported by grants from the NIH (AM 27524) and Merck and Co.

POTASSIUM DEPENDENCE OF CHLORIDE TRANSPORT IN THE INTESTINE OF THE FLOUNDER, PSEUDOPLEURONECTES AMERICANUS

44 Michael Field, Leigh S. Kimberg, Stephanie A. Orellana and Raymond A. Frizzell, Departments of Medicine and Pharmacological and Physiological Sciences, University of Chicago, Chicago, Il., and Department of Physiology, University of Alabama, Birmingham, Al.

Barium-stimulated net Rb absorption[x] and unidirectional Rb influx across the brush border (initial rate of Rb intake from lumen-to-epithelium) are inhibited by furosemide and by replacement of medium Cl with gluconate, suggesting possible co-transport of K and Cl across the brush border (Musch et al, MDIBL, 1981). To further test this possibility we have determined the effects of removing K from the mucosal bathing medium on transepithelial Cl fluxes and on Cl influx.

In addition to possible coupling at the brush border, K and Cl fluxes may also be coupled at the basolateral membrane. Electrophysiological data suggest that the conductance of the basolateral membrane is insufficient to account for a large passive Cl flux, suggesting that Cl movement across that membrane is electroneutral. The most obvious possibility would be KCl cotransport, which would link the large difference in K concentration to Cl efflux across the serosal border. To examine this possibility, we determined the effect of increasing medium K concentration on transepithelial Cl fluxes. [x] ^{86}Rb and ^{42}K fluxes have been shown to be identical in flounder intestine (Stewart et al., Bull. MDIBL 20:92, 1980).

METHODS

Cl fluxes across and into flounder intestine stripped of muscle were determined as previously described (Figle et al., J. Membr. Biol. 41:265, 1978; Frizzell et al., J. Membr. Biol. 46:27, 1979). Bathing solutions used were the same as those described elsewhere in this bulletin (see Musch et al) except that 5 mM KCl replaced 5 mM $RbNO_3$. When K concentration was increased to 50 mM, Na concentration was decreased by 45 mM.

RESULTS

When K was removed from the mucosal medium, net Cl absorption was reduced by 60% (Table 1). This reduction of J_{net}^{Cl} was due entirely to a decrease in J_{ms}^{Cl}. To examine this phenomenon further, we determined the dependence of Cl influx on mucosal K (Table 2). The furosemideinhibitable portion of Cl influx was reduced by 5.4 $\mu Eq \cdot h^{-1} \cdot cm^{-2}$ or 70% when K was removed from the mucosal medium. Total Cl influx was also reduced. The relative magnitudes of the K-dependent Cl influx and the Cl-dependent K influx (musch et al., Bull. MDIBL 1981) suggest 2:1 stoichiometry.

Table 1.--Dependence of Transepithelial Cl Fluxes on Mucosal [K]

$[K]_m$	$[K]_s$	J_{ms}	J_{sm}	J_{net}	I_{sc}
5	5	6.17 ± .48	2.63 ± .33	3.54 ± .32	1.78 ± .16
ϕ	5	3.93 ± .62*	2.49 ± .44	1.44 ± .56*	0.93 ± .10*

Mean $\mu Eq \cdot h^{-1} \cdot cm^{-2}$ ± 1 SE for 9 paired experiments.
*Less than control, $p < .05$.

Table 2.--Dependence of Cl Influx on Mucosal [K]

$[K]_m$	$[K]_s$	control	+ furosemide	Δ
5	5	15.83 ± 2.09	8.20 ± 1.75	7.63 ± 0.80
ϕ	5	12.24 ± 1.29*	9.97 ± 1.42	2.27 ± 0.56*

Means ± 1 SE for 9 paired experiments.
*Less than control, $p < .01$.

In order to examine the influence of serosal [K] on Cl efflux across the serosal border, we measured unidirectional and net Cl fluxes at 5 and 50 mM serosal K (mucosal [K] kept at 5 mM). The results shown in Table 3A

Table 3.--Effects of High Medium [K] on Transepithelial Cl Fluxes

	$[K]_m$	$[K]_s$	J_{ms}	J_{sm}	J_{net}	I_{sc}	G
A. 50 mM serosal K^+ (n = 12)							
	5	5	8.04 ± .91	2.54 ± .37	5.49 ± .74	-2.44 ± .280	19.8 ± .9
	5	50	9.03 ± .46	4.65 ± .40*	4.39 ± .59	-5.22 ± .391*	25.1 ± 1.3*
B. 50 mM mucosal and serosal K^+ (n = 5)							
	5	5	9.05 ± 1.1	3.21 ± .66	5.85 ± .52	-2.91 ± . 27	18.3 ± 1.3
	50	50	19.82 ± 2.9*	10.71 ± .94*	9.11 ± 2.8	-2.49 ± . 29	33.4 ± 5.2*

Means ± 1 SE for (n) paired experiments. Fluxes are in $\mu Eq \cdot h^{-1} \cdot cm^{-2}$ and G is in $mS \cdot cm^{-2}$. In all instances, K was substituted for Na. *Different from control, $p < .05$.

indicate no change in net Cl flux but significant increases in J_{sm}^{Cl} and G. When [K] was increased on both sides of the epithelium, J_{net}^{Cl} was again not significantly altered but J_{sm}^{Cl} and G were markedly increased. We were therefore unable to verify the presence of a KCl cotransporter in the basolateral membrane.

DISCUSSION

The present study together with that of Musch et al., in this bulletin (1981) and of Frizzell et al., (J. Membr. Biol. 46:27, 1979) describe a K, Na, Cl cotransport system with roughly 1:2:2 stoichiometry. The influxes of all 3 ions are inhibitable by furosemide. Since the system appears to carry a positive charge, the mem-

brane hyperpolarization caused by furosemide is accounted for. These results differ from those obtained with Ehrlich cells in that the latter cotransport exhibits K:Na:2Cl stoichiometry and appears to be electrically neutral (Pietrzyk et al., Biochim. Biophys. Acta 600:432, 1980).

The failure of high serosal K to reduce net Cl absorption argues against KCl cotransport as a means for absorbing Cl across the serosal border. At 50 mM serosal K, the outwardly directed K concentration gradient is probably less that the inwardly directed gradient of Cl concentration. This is only probable because the effects of high serosal K on cell K and Cl activities have not yet been determined. It should be noted, however, that both J_{sm}^{Cl} and G increased, which would be consistent with the development of Cl conductance in the basolateral membrane. Thus KCl cotransport may operate under baseline conditions, but, when the K gradient is appreciably diminished, conductive Cl movement may replace cotransport. The increase in J_{sm}^{Cl} is even more striking when both the mucosal and serosal K concentrations are elevated. Since elevating mucosal K depolarizes the cell, it may be that a voltage-sensitive Cl channel is present in the basolateral membrane. Although serosal K does not affect cell potential when the former is elevated for only 5010 min (Stewart et al., Bull. MDIBL 20:92, 1980), it is possible - although unproven - that longer exposure to high serosal K does depolarize. We had previously observed that ouabain, which causes a marked cell depolarization (Field et al., J. Membr. Biol. 55:157, 1980), also greatly increases unidirectional Cl fluxes. This work was supported by NIH research grants AM21345 (to Michael Field) and AM27524 (to Raymond A. Frizzell).

ACTIVE K^+ TRANSPORT BY THE INTESTINE OF THE FLOUNDER, PSEUDOPLEURONECTES AMERICANUS: EVIDENCE FOR COTRANSPORT WITH Na AND Cl

Mark W. Musch, Michael Field and Raymond A. Frizzell, Departments of Pharmacological and Physiological Sciences and of Medicine, University of Chicago, Chicago, Il., and Department of Physiology, University of Alabama, Birmingham, Al.

Flounder intestinal epithelium, in addition to actively absorbing Na and Cl (Field et al., J. Membr. Biol. 41:265, 1978), actively secretes K (Stewart et al., Bull. MDIBL 20:92, 1980). This was established by measuring transepithelial unidirectional fluxes of ^{42}K (in 5 mM K-Ringer) and ^{86}Rb (in 5 mM Rb-Ringer) across the short-circuited mucosa. The fluxes of the two cations were in fact identical and the transport properties of the tissues were well maintained in both Ringer solutions, justifying our subsequent use of Rb-Ringer and ^{86}Rb, which has a much longer half-life than does ^{42}K, to study the K transport mechanism.

Electrophysiologic measurements (Stewart et al., Bull. MDIBL 20:92, 1980; Smith et al., Bull. MIDBL 20:96, 1980) indicated that (1) the conductance of the flounder intestinal brush border is almost exclusively a K conductance and (2) intracellular K activity is 20-30 mV above electrochemical equilibrium. Thus net K secretion appeared to result from active uptake coupled to active Na extrusion at the serosal border (Na, K-activated ATPase) and passive efflux through a K conductance channel in the brush border. Adding Ba (to 5 mM) to the mucosal bathing solution markedly diminished apical K conductance and abolished net K secretion, providing thereby further evidence to support the above hypothesis for K secretion.

Not only did Ba abolish K secretion, however, but it also produced net K absorption (under short-circuit conditions), indicating that K transport by flounder intestine is more complex than we had initially thought. The present study was undertaken to further explore the mechanisms involved. Specifically, we wished to determine (1) the dependence of K secretion of Na influx across the brush border; (2) the K concentration dependence of K secretion; (3) the sensitivity (if any) of K absorption of mucosal ouabain; (4) the Na dependence of K absorption; and (5) the K permeability properties of the brush border determined through measurements of ^{86}Rb influx.