Table 3 shows the time course of ouabain binding to homogenates of basal and stimulated rectal glands. Ouabain

Table 3

Ouabain binding by whole homogenates of basal and stimulated rectal glands.

	10^{-8}[^{3}H]-ouabain	
	Basal	Stimulated
0	0.06±0.02(4)	0.04±0.02(5)
15	0.88±0.13(4)	0.63±0.32(5)
30	1.82±0.15(5)	1.15±0.30(5)*
60	3.36±0.26(5)	2.04±0.25(5)*
120	5.72±0.56(5)	3.87±0.32(5)*
180	7.74±1.04(5)	5.23±0.54(5)*
240	9.33±1.11(5)	6.50±0.76(5)*
300	10.41±1.30(5)	7.23±0.86(5)*

Units are picomoles of ouabain bound per milligram of protein. Values are Mean ± SE(n)
* $p < 0.05$

binding was not increased in stimulated glands but rather decreased, indicating that intact cells are necessary for the cyclic AMP induced increase of ouabain binding to become manifest. Cell homogenization may expose all enzymatic sites within the cell to binding by ouabain, whereas only those on the surface of the cell are available in intact cells.

From these experiments we conclude that the stimulation of ouabain binding induced by theophylline and cyclic AMP is independent of the entry of sodium into the cell. The process does not appear to involve the formation of microtubules inasmuch as it cannot be blocked by colchicine. The stimulation of ouabain binding is apparent in intact cells but cannot be demonstrated in whole homogenates of rectal gland in which the cellular architecture has been destroyed.

INHIBITION OF K-INDEPENDENT Na/Cl UPTAKE INCREASES APICAL MEMBRANE K CONDUCTANCE IN FLOUNDER INTESTINE.

Dan R. Halm, Edward J. Krasny, Jr., and Raymond A. Frizzell, Department of Physiology and Biophysics, University of Alabama Medical Center, Birmingham, Al.

Studies of electrolyte transport across isolated flounder intestine have provided evidence for a coupled Na/K/Cl uptake process and for a barium-sensitive K conductance at the apical membrane (Musch et al., Bull MDIBL 21:95, 1981; Field et al., Bull. MDIBL 21:93, 1981; Stewart et al., Bull. MDIBL 20:92, 1980). Inhibition of Cl absorption was shown to hyperpolarize the apical membrane electrical PD (ψ_a) suggesting that the apical membrane K conductance increases, even in the presence of barium (Halm et al., Bull. MDIBL, 21:88, 1982). The results presented here further extend the description of membrane conductive properties and their relationship with salt absorption.

METHODS--Conventional microelectrodes were employed to measure ψ_a. The criteria for successful impalement have been described by Duffey et al (J. Memb. Biol. 50:331, 1979); 3 to 6 values of ψ_a were obtained under each experimental condition.

RESULTS--Figure 1a shows the dependence of ψ_a on mucosal solution K concentration, which is consistent with a high apical membrane K conductance. Addition of barium (2 mM) to the mucosal solution depolarized ψ_a and reduced the dependence of ψ_a on mucosal solution [K], consistent with barium blockade of apical K conductance. As reported previously (Halm et al., Bull MDIBL 21:88, 1981), addition of bumetanide (0.1 mM) to the mucosal solution hyperpolarized ψ_a to near the control value, even though barium was present. Return of the steep dependence of ψ_a on mucosal solution [K] indicates that apical membrane K conductance increased relative to basolateral membrane conductance. Figure 1b shows that the basolateral membrane is conductive to Cl, since

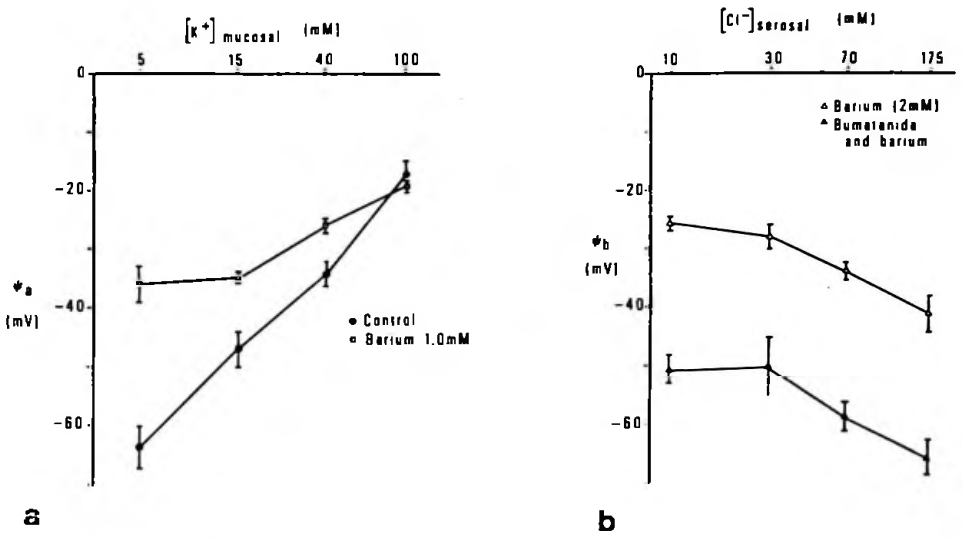

Figure 1.--Apical (ψ_a) and basolateral (ψ_b) membrane potentials as functions of external K and Cl, respectively. Points are mean ± SD of 3-6 impalements during each change in bathing solution ion concentration from a single experiment. Control recordings were obtained between each experimental perturbation; barium (2 mM(and bumetanide (0.1 mM) were added to the mucosal solution. a) ψ_a vs. mucosal solution [K]; K was replaced with Na. b) ψ_b vs. serosal solution [Cl]; Cl replaced with gluconate.

decreasing serosal solution [Cl] depolarized the basolateral membrane electrical PD (ψ_b). The inhibition of Cl absorption with mucosal solution bumetanide did not alter the dependence of ψ_b on serosal solution [Cl], suggesting that the Cl conductance of the basolateral membrane does not change with inhibition of transport. This result also suggests that the relative increase in apical membrane K conductance was not due to a decrease in basolateral membrane Cl conductance, but rather to an absolute increase in K conductance of the apical membrane.

Table 1 shows that ψ_a hyperpolarized when NaCl absorption was inhibited by Na-free or Cl-free media or

Table 1.--Hyperpolarization of ψ_a ($\Delta\psi_a$) in the presence of agents or conditions that inhibit Na/Cl absorption

	control $\Delta\psi_a$ (mV)	n	mucosal barium $\Delta\psi_a$ (mV)	n
bumetanide	-12±2*	8	-19±2*	13
Cl replacement	-14±4*	4	-24±3*	8
Na replacement	-15±4*	2	-14±5*	4
K replacement	-20±3*	3	- 3±2	3
cAMP	---		0±2	4
cGMP	-12±4*	2	-21±1*	1

*Means ± SEM for (n) experiments. Asterisks indicate significant differences from zero. Bumetanide (0.1 mM) was added to the mucosal solution. Gluconate replaced Cl in the mucosal solution. N-methyl-D-glucamine replaced Na in the mucosal and serosal solutions. Na replaced K in the mucosal solution. The 8-bromo forms of the cyclic nucleotides were added to the serosal solution, 0.5 mM cAMP and 0.1 mM cGMP.

by bumetanide. Hyperpolarization ($\Delta\psi_a$) after K replacement might be explained simply by the apical membrane K conductance. In the presence of mucosal solution barium K replacement evoked no hyperpolarization, consistent with barium blockade of apical membrane K channels. This suggests that the hyperpolarization ($\Delta\psi_a$) results from

inhibition of a K-independent Na/Cl uptake process in the apical membrane. The relation between $\Delta\psi_a$ and mucosal [Na] or [Cl] provide apparent dissociation constants of Na (45 mM) and Cl (75 mM) for the K-independent uptake process. These K_m values are much higher than those of the K-dependent uptake process (Musch et al., Bull MDIBL. this issue). In addition, the kinetics of hyperpolarization as a funct ion of mucosal [Cl] are simple hyperbolic, consistent with the binding of a single Cl ion for uptake; this contrasts with the sigmoidal kinetics of the K-dependent uptake process which implies interaction with two Cl ions. Cyclic AMP has been shown to inhibit NaCl absorption (Field et al., J. Memb. Biol. 55:157, 1980) and the influxes of Cl (Frizzell et al., J. Memb. Biol. 46:27, 1979) and Rb (Musch et al., Bull MDIBL, this issue) across the apical membrane. Cyclic GMP has also been shown to inhibit Cl and Rb uptake (Rao et al., Bull MDIBL, this issue). These findings together with the significant hyperpolarization of ψ_a induced by cGMP (Table 1), suggests that both K-dependent and K-independent Na/Cl uptake mechanisms are sensitive to cGMP. The lack of a cAMP-induced $\Delta\psi_a$ suggests that the K-independent Na/Cl uptake mechanism may be insensitive to cAMP.

In summary, the conductive properties of the flounder intestine consist of an apical membrane K conductance and a basolateral membrane Cl conductance. Apical K conductance increases when a K-independent Na/Cl uptake process is inhibited by removal of mucosal solution Na or Cl or by addition of bumetanide. The extent of K-independent Na/Cl uptake and its contribution to Na/Cl absorption has not been defined with certainty.

REGULATION OF PARACELLULAR PERMSELECTIVITY IN FLOUNDER INTESTINE

Edward J. Krasny, Jr., Amy I. DiBona and Raymond A. Frizzell, Department of Physiology and Biophysics, University of Alabama in Birmingham, Birmingham, Al.

The paracellular pathway that traverses the intestinal mucosa of the winter flounder, Pseudopleuronectes americanus, is responsible for its low transepithelial resistance and is more permeable to cations than anions. The serosa-to-mucosa flux of Na is 5 times that of Cl and the high tissue conductance is largely dependent on the presence of Na in the bathing media. These observations led Field et al., J. Memb. Biol. 55:157, 1978) to propose that the serosa-negative transepithelial potential difference (ψ_t) originates, at least in part, from a diffusion potential across cation-selective tight junctions. The purpose of our study was to evaluate the permselective properties of the paracellular pathway under conditions that affect the rate of NaCl absorption (e.g., inhibitors of salt transport) or physically alter the dimensions of the lateral intercellular space (e.g., osmotic water flow, voltage-clamping).

METHODS--The procedures for dissection and mounting of the tissue, measurement of electrical parameters, and determination of isotopic fluxes were described previously (Field et al., 1978; Frizzell et al., J. Memb. Biol. 46:27, 1979). Dilution (diffusion) potentials are expressed as the change in ψ_t resulting from a 10% reduction in mucosal solution [NaCl] (isosmotic replacement with mannitol); values were corrected for liquid junction potentials. Bathing solutions were made hyperosmotic by addition of 330 mM mannitol to the normal electrolyte solution (doubling its osmolarity).

RESULTS AND DISCUSSION--The resistance of the paracellular shunt pathway is the sum of the tight junction resistance (R_{TJ}) and the series resistance of the lateral intercellular space (R_{LIS}). Whereas R_{TJ} is dominated by the cation-selective properties of the tight junction, R_{LIS} should be determined by free-solution ionic mobilities to the extent that this is a watery pathway for salt diffusion. Thus, one predicts that increases in R_{LIS} induced by dimensional restrictions would reduce the cation selectivity of the paracellular pathway as free-solution mobilities ($\lambda_{Cl} > \lambda_{Na}$) are approached. Conditions and agents known to affect the geometry of the lateral intercellular space were tested and dilution potentials were monitored as indicative of paracellular selectivity. Results are presented in Table 1.

Dilution potentials measured in control tissues range from 1.9 - 2.3 mV (serosa negative) indicating that the paracellular pathway is cation selective. When ion transport is inhibited by barium, bumetanide, ouabain, or cyclic GMP (see Rao et al., this volume, and below), there are no significant effects on either transepithelial resistance