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., 41: 265, 1978; Frizzel et al., J. Memb. Biol., 46: 17, 1979): (1) NaCl cotransport at the apical membrane, (2) active Na extrusion across the basolateral membrane via the Na-K-pump, and (3) predominant cation selectivity of the paracellular (junctional) pathway. Its novel features are (1) KCl co-transport across the basolateral membrane contributing to non-conductive Cl exit from the cell, and (2) conductive K exit across the apical membrane leading to net K secretion.

Previous studies have shown that reduced bathing solution pH decreases transepithelial electrical potential difference (ψ_t), short-circuit current (I_{sc}) and net Cl absorption (Field et al., J. Memb. Biol., 41: 265, 1978; Field et al., Bull. MDIBL., 18: 44, 1978; Smith et al., Bull. MDIBL., 19: 24, 1979). The effect of reduced pH can be demonstrated at either constant HCO_3 concentration or constant P_{CO_2}. According to the model summarized above, the decrease in Cl absorption with reduced pH could result from: (1) a reduction in NaCl co-transport across the apical membrane, (2) increased Cl permeability of the apical membrane which would compromise net Cl entry since the electrochemical potential difference for Cl across this barrier is oriented from cell to mucosal solution, (3) decreased Cl exit across the basolateral membrane by a direct effect on KCl co-transport or on other mechanism(s) responsible for Cl exit, or (4) inhibition of the Na-K-pump, at the basolateral membrane which would indirectly affect both NaCl and KCl co transport processes. The maintenance of a low intracellular Na activity and a high intracellular K activity would be required to optimize net Cl transport across the apical and basolateral membranes, respectively.

The present study was designed to evaluate the site(s) and mechanism(s) responsible for the decrease in Cl absorption with reduced pH using both flux studies and electrophysiologic techniques.

Methods

Transepithelial Rb fluxes were determined as described in the companion paper Stewart, et al., Bull. MDIBL., 20: 1980, and Cl influxes as described by Frizzell et al., J. Memb. Biol., 46: 27, 1979. Measurement of the electrical potential profile and intracellular Cl activities was similar to that described by Duffey et al., J. Memb. Biol., 50: 331 1979. The techniques for constructing and calibrating the K-selective microelectrodes were identical to those used for the Cl selective microelectrodes except that Corning exchanger 477317, was employed as the ion-exchange resin. Intracellular K activity (a_c^K) was calculated from the equation:

$$\Delta E_t = \psi_a + S \ln \frac{a_c^K}{a_m^K + K_{K,Na}\, a_m^{Na}}$$

where ΔE_t is the electrical potential difference between the cell interior (c) and the mucosal solution (m) sensed by the K-selective microelectrode, ψ_a is the electrical potential difference between the cell interior and the mucosal solution recorded with a conventional microelectrode, S is the slope of the relation between the electrical output of the K-selective microelectrode and ambient K activity (obtained from calibration) and $K_{K,Na}$ is the selectivity coefficient of the electrode for potassium vs. sodium. Calibration of the electrodes used in this study yielded values of S averaging 26.0 mV per decade change in K activity, in good agreement with the predicted value of 25.4 mV. The selectivity coefficient for these electrodes was approximately 50, which is in agreement with values obtained by other investigators (Armstrong et al., Fed. Proc., 39: 2851, 1980). No correction was made for interference by intracellular Na in the calculation of a_c^K; this should add only a small error since a_c^K is probably ten times a_c^{Na}.

Results and Discussion

Chloride influx from the mucosal solution into the epithelium (J_{me}^{Cl}) was determined in the presence and absence of Na at both normal and reduced pH. The results of these studies are given in Table 1. Reduced pH had no effect on J_{me}^{Cl} in the presence or absence of sodium. The results of prior studies (Smith et al., Bull. MDIBL 19: 24, 1979), indicated that the furosemide-sensitive fraction of J_{me}^{Cl} is also not affected by reduced pH. Since furosemide is a selective inhibitor of NaCl co-transport by flounder intestine, these findings indicate that the reduction in Cl absorption elicited by a reduced pH cannot be attributed to a decrease in NaCl co-transport across the apical membrane. Inasmuch as the sodium-independent fraction of Cl entry was not affected by acidification of the bathing media (Table 1), it also does not appear that an increase in apical membrane Cl permeability can account for the reduction in Cl absorption.

Table 1.--Effect of pH on Cl influx in the presence or absence of Na

	pH 8.0		pH 7.2	
	J_{me}^{Cl}	I_{sc}	J_{me}^{Cl}	I_{sc}
CONTROL	2.3 ± 0.51	-0.70 ± 0.26	2.3 ± 0.10	-0.50 ± 0.09
Na-FREE	1.1 ± 0.17*	-0.04 ± 0.13*	1.3 ± 0.15*	-0.02 ± 0.03*

All values are in μEq/cm^{2}hr, mean + S.E.M. for 5 experiments in which all four conditions were tested using tissues from the same animal. Control solutions contained 20 mM NaCl; Na-free solutions contained 20 mM choline Cl. Both solutions contained (mM): K_2SO_4, 2.5; $CaSO_4$, 1.25; $MgSO_4$, 1.1; mannitol, 300; and Tris-HEPES, 6; ratio adjusted to yield desired pH.

*$p < 0.05$.

In an attempt to further localize the site of inhibition of Cl absorption, cell Cl activity was determined at both normal and reduced pH. The results are provided in Table 2, together with values of I_{sc}, ψ_a and a_c^K. Reduced pH

Table 2.--Effects of pH on intracellular Cl and K activities and electrical properties of flounder intestine

	ψ_t (mV)	ψ_a (mV)	I_{sc} (μAmps/cm^2)	a_c^{Cl} (mM)	a_c^K (mM)
1% CO_2, pH 8.0	-4.5 ± 0.2	-65 ± 3	-118 ± 7	22 ± 4	82 ± 8
5% CO_2, pH 7.2	-2.8 ± 0.4*	-44 ± 4*	- 68 ± 11*	35 ± 4*	56 ± 5*

Mean + S.E.M. for 7 experiments. In each experiment, 4-6 measurements were obtained with Cl- and K-selective microelectrodes at each pH. These were preceded and followed by 4-6 measurements of ψ_a using conventional microelectrodes. The standard electrolyte solution was employed (Field et al., J. Membrane Biol. 41: 265, 1978).

*$p < 0.05$.

elicited: (1) a decrease in I_{sc} indicating that Cl absorption was inhibited, (2) depolarization of the apical membrane potential, ψ_a, from 65 to 44 mV, cell interior negative, (3) an increase in a_c^{Cl} from 22 to 35 mM, (4) a decrease in a_c^K from 82 to 52 mM. The increase in a_c^{Cl} with reduced pH suggests that the reduction in Cl absorption results from an inhibition of net Cl transport across the basolateral membrane. The effects of reduced pH on ψ_a and a_c^K will be discussed below.

In the companion paper (Stewart et al., Bull. MDIBL 20: 1980), we show that ψ_a is largely determined by the high K permeability of the apical membrane. Thus the decrease in ψ_a which results from reduced bathing solution pH may result from a reduction in apical membrane K permeability or an increase in permeability to some other ion. This was evaluated by determining the effect of elevated mucosal solution K concentration on ψ_a at both normal and reduced pH. The results of these studies are shown in Figure 1. The depolarization of ψ_a elicited by a ten-fold increase in $[K]_m$ was 29 mV at pH 8.0 and 9 mV at pH 7.2. Stewart et al., Bull. MDIBL 20: 1980, also show that bidirectional Rb fluxes across flounder intestine are indistinguishable from those of K. The effects of reduced pH on

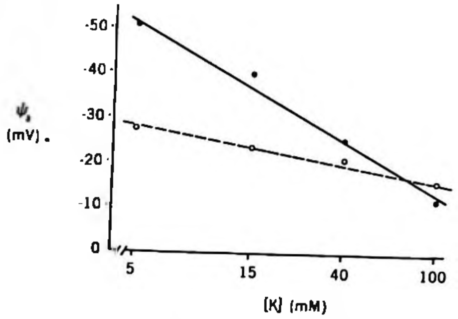

Fig. 1.--Effect of bathing solution pH on the relation between mucosal solution. K concentration and the electrical potential difference across the apical membrane, ψ_a. Filled circles, pH 8.0, 1% CO_2; open circles pH 7.8, 5% CO_2. Each point represents the mean of 4-8 observations.

Table 3.--Effects of pH on Rb fluxes across flounder intestine

	J_{ms}^{Rb}	J_{sm}^{Rb}	J_{net}^{Rb}	I_{sc}	G_t
1% CO_2, pH 8.0	0.40 ± 0.05	1.3 ± 0.05	-0.90 ± 0.05	-3.5 ± 0.4	30 ± 1.9
1% CO_2, pH 8.0	0.43 ± 0.05	1.2 ± 0.07	-0.77 ± 0.09	-2.8 ± 0.6	31 ± 1.4
1% CO_2, pH 8.0	0.29 ± 0.02	1.2 ± 0.05	-0.95 ± 0.06	-3.0 ± 0.2	24 ± 1.6
5% CO_2, pH 7.2	0.41 ± 0.05	0.66 ± 0.06*	-0.26 ± 0.05*	-0.3 ± 0.1*	18 ± 1.1*

All values are in $\mu Eq/cm^2 hr$ except G_t in mS/cm^2, mean ± S.E.M. for 4 animals in which all conditions were examined. Solutions of standard composition, except that K was replaced by Rb (see Stewart et al., Bull. MDIBL 20: 1980).

$*p < 0.05$.

unidirectional Rb fluxes are given in Table 3. Acidification of the bathing media inhibited Rb secretion by approximately 2/3, due entirely to a decrease in the unidirectional Rb flux from serosa to mucosa. The inhibition of Rb secretion, together with the results of the studies illustrated in Figure 1, indicate that reduced pH decreases the K permeability of the apical membrane of flounder intestinal cells. This reduction in K permeability is probably responsible for the depolarization of ψ_a observed at pH 7.2 (Table 2).

In the experiments described above, the pH of both bathing media was reduced by elevating their P_{CO_2} from 1 to 5%. However, the decrease in Cl absorption which results from reduced pH is due solely to a decrease in the pH of the serosal solution alone (Field, et al., Bull. MDIBL. 18: 44, 1978). To determine whether the reduction in apical membrane K permeability demonstrated here results from acidification of the mucosal solution, the pH of the mucosal solution alone was reduced from 8.0 to 7.2 by decreasing its HCO_3 concentration while the pH of the serosal solution remained at 8.0. The effect of elevating $[K]_m$ on ψ_a was used as a measure of apical membrane K permeability. Increasing $[K]_m$ from 5 to 75 mM depolarized ψ_a by 43 mV at pH 8.0 and by 39 mV when the pH of the mucosal solution alone was reduced to 7.2. These results indicate that both inhibition of Cl absorption and reduced apical membrane K permeability are due to acidification of the serosal solution.

The reduction in apical membrane K permeability elicited by acidification of the bathing media would be expected to increase cell K activity. However, the concurrent decrease in a_c^K (Table 2) suggests that Na-K-pump activity is inhibited by reduced pH. Support for this argument is provided by the changes in electrical potential profile and intracellular K and Cl activities induced by addition of ouabain to the serosal bathing solution alone. Ouabain, a specific inhibitor of the Na-K-pump, inhibits Cl absorption by flounder intestine (Field et al., J. Memb. Biol. 41: 265, 1978), as does reduced pH. The results of these studies are given in Table 4. In brief, ouabain elicited changes in the electrical potential profile and in cell K and Cl activities that were qualitatively similar to those observed with acidification of the bathing solutions (compare Tables 2 and 4). As with reduced pH, the depolarization of ψ_a elicited by increased $[K]_m$ (5 to 75 mV) was decreased from 45 to 6 mV during the inhibition by ouabain. Thus, ouabain also reduced the permeability of the apical membrane to K and decreases intracellular K activity.

Table 4.--Effects of ouabain on intracellular Cl and K activities and electrical properties of flounder intestine

	ψ_t (mV)	ψ_a (mV)	I_{sc} (μAmps/cm^2)	a_c^{Cl} (mM)	a_c^K (mM)
CONTROL	-3.2 ± 0.4*	-64 ± 2	-78 ± 15	19 ± 2	72 ± 2
OUABAIN, 10^{-4} M	-0.2 ± 0.4*	-13 ± 1*	- 7 ± 8*	64 ± 3*	18 ± 2*

Mean ± S.E.M. for 4 experiments. See Table 2 for details. $^*p < 0.05$.

The results of this study are interpreted within the framework of the model presented by Stewart et al., Bull. MDIBL. 20: 1980. According to this model, transcellular Cl transport is an electrically neutral process at both the apical and basolateral membrane. NaCl co-transport accounts for Cl entry across the apical cell membrane and is not influenced by reduced pH. Cell chloride activity increases with acidification of the bathing media, suggesting that net Cl exit across the basolateral membrane is impaired. The reduction in cell K activity, in spite of a decrease in apical membrane K permeability, suggests inhibition of Na-K-pump activity at the basolateral membrane by reduced pH. This decrease in cell K activity would diminish the rate of KCl co-transport across the basolateral membranes causing cell Cl activity to rise. Finally, we estimate that KCl co-transport across the basolateral membrane could account for as much as 60% of net Cl absorption (Stewart et al., Bull. MDIBL. 20: 1980). It is of interest that this is the approximate degree of inhibition produced by acidification of the bathing media. Supported by grants from NIH (AM 27524 and AM 21345) and from Merck and Co. PLS was supported by a National Research Service Award (AM 05973), MJW by a National Pulmonary Faculty Training Award (HL07159), and RAF by a Research Career Development Award (AM 00713); from the NIH.

INTRACELLULAR CHLORIDE ACTIVITIES IN THE ISOLATED PERFUSED SHARK RECTAL GLAND

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The rectal gland of the spiny dogfish secretes a fluid rich in NaCl in the interest of maintaining salt homeostasis (Burger and Hess, Science, 131:670, 1960). Based on studies of Cl secretion by the isolated perfused rectal gland, Silva et al., Am. J. Physiol., 233:F298, 1977, proposed a model that appears to apply to a wide variety of secretory epithelia (Frizzell et al., Am. J. Physiol., 236:F1, 1979); this model is illustrated in Fig. 1. Two critical features of this model are an intracellular Cl activity greater than the value expected for electrochemical equilibrium and a Na-coupled entry of Cl into the cell across the basolateral membrane. To test these components of the model for the rectal gland, we used ion-selective microelectrodes to measure intracellular Cl activity under baseline conditions, when secretion was stimulated and when the gland was perfused with Na-free media.

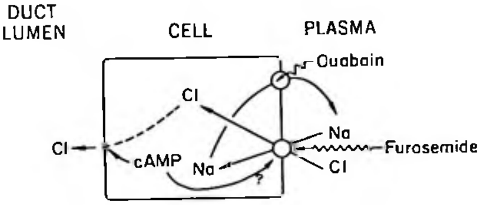

Fig. 1.--Model for chloride secretion. See text for details.

METHODS

Rectal glands were obtained and perfused as previously described (Silva et al., Am. J. Physiol., 233:F298-F306, 1977). The perfusion medium contained (mM): Na, 288; K, 5; Mg, 2.5: Ca, 2.5; Cl, 293; HCO_3, 8; SO_4, 0.5; H_2PO_4, 1; and urea, 350. The solution was bubbled with 99% O_2 - 1% CO_2 and maintained at 15°C. Sodium-free solutions were prepared by substituting choline for Na. Secretion was stimulated by addition of 1 mM theophylline plus 0.05 mM 8-Bromo-cAMP to the perfusate.

A small area of capsule (10-30 mm^2) was removed to permit impalement of the epithelial cells with microelectrodes. The electrical potential difference between periglandular fluid and duct fluid (ψ_t) and the rate of secretion (J_v) were measured as previously described (Silva et al., Am. J. Physiol., 233:F298, 1977). Microelectrodes were advanced manually to the gland surface with a micromanipulator and cellular impalements were made by rapidly advancing the microelectrode with a piezo-electric driver (Physik Instruments, Berlin). The electrical potential