Despite the apparent decrease in transcellular NaCl transport elicited by this agent, TAP produced a significant increase in the rate of active Na absorption and reduced the ratio of net Cl to Na transport to a value not significantly different from unity. Thus, by decreasing junctional Na permeability, TAP tends to equalize the absorptive fluxes of Na and Cl across flounder intestine. These findings lend additional support to the notion that the Na-permselective tight junctions constitute the pathway through which transported Na recycles to the mucosal solution (Figure 1), thus, leading to the predominance of Cl over Na absorption that is normally observed under short-circuit conditions. Supported by research grants from the NIH-NIAMDD (AM-18199 and AM-21345).

ROLE OF HCO_3 IN THE REGULATION OF Cl TRANSPORT BY FLOUNDER INTESTINE

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Prior *in vitro* studies show that active salt absorption by the intestine of flounder [Field et al., J. Memb. Biol. 41:265, 1978] and other teleosts [Oide, Comp. Biochem. Physiol. 46A:639, 1973] can be enhanced by alkalinizing the bathing medium. The mechanism for this effect has not been established. In the present study we have altered HCO_3 concentration at constant pCO_2 to determine (a) whether mucosal or serosal HCO_3 (or both) is (are) critical and (b) whether the associated change in transepithelial Cl flux can be attributed to a change in the Cl permeability of the luminal or contraluminal border of the epithelium.

Flounder weighing between 250 and 600 gm were netted near Mt. Desert Island and maintained (without feeding) for at least two days in running seawater at 15-17°. The intestine was stripped of muscle and mounted in chambers for measuring either transepithelial fluxes (standard Ussing chamber) or influxes across the luminal border (influx chamber). The techniques employed were those previously described [Field et al., J. Memb. Biol. 41:265, 1978 and Frizzell et al., J. Memb. Biol., in press]. The standard Ringer solution contained 20 mM HCO_3 and the following other ions in mmoles/l: Na=168, Cl=150, K=5, HPO_4/H_2PO_4=2, Ca=1, and Mg=1. HCO_3 concentration was reduced to either 2 or 0.4 mM by replacement with equimolar amounts of SO_4 and mannitol (9 or 9.8 mmol/l). All solutions were bubbled with 1% CO_2 in O_2 and maintained at 15°. Glucose (10 µmol/ml) was added to the serosal side and an equimolar amount of mannitol to the mucosal side. In all experiments, tissues were initially mounted in 20 mM HCO_3-Ringer and, after the PD had stabilized (10-20 min), solutions were changed as needed.

In the standard Ussing chamber, reduction of serosal HCO_3 from 20 to 2 mM produced a gradual decrease in PD and Isc, a new plateau being reached in 20-30 min. When serosal HCO_3 was increased again to 20 mM, the Isc also increased. In contrast to these results, PD and Isc were not affected when mucosal HCO_3 was reduced. As shown in Table 1, net Cl absorption was inhibited by reducing serosal HCO_3 but not by reducing mucosal HCO_3. This inhibition was due wholly to a reduction in the m-to-s unidirectional flux. The reduction in J^{Cl}_{net} was roughly proportional to the reduction Isc, suggesting no significant change in the relative amounts of Na and Cl transported under short-circuit condition.

The observed inhibition of net Cl absorption could have resulted from inhibition of either Cl transfer across the basolateral cell border or Cl influx across the luminal border. Enhancement of Cl efflux across the luminal border is unlikely since J^{Cl}_{sm} did not increase. Table 2 shows the effects of serosal acidification on Cl influx across the brush border (J^{Cl}_{me}) in the presence and absence of 1 mM furosemide, which was employed as a measure of the Na-coupled portion of Cl influx (see Frizzell et al., Bull. MDIBL, 1977 and J. Memb. Biol., in press). It proved far more difficult to reduce Isc by

Table 1. Effects of [HCO_3] on Transepithelial Cl Fluxes

mM HCO_3 M	S	J^{Cl}_{ms}	J^{Cl}_{sm}	J^{Cl}_{net}	Isc	G
A. Mucosal HCO_3 = 20mM (N = 4)						
20	20	8.6 ± .89	3.4 ± .69	5.2 ± .70	2.9 ± .31	30 ± 1.7
20	2	4.4 ± .10	2.4 ± .66	2.0 ± .58	1.4 ± .21	24 ± 1.7
	P<	.001	ns	.001	.001	.001
B. Mucosal HCO_3 = 2mM (N = 5)						
2	20	8.9 ± 1.0	3.4 ± 1.1	5.6 ± .72	2.5 ± .35	27 ± 1.7
2	2	3.5 ± .19	1.8 ± .33	1.6 ± .45	1.0 ± .16	20 ± 1.1
	P<	.001	.02	.001	.001	.001

Means ± 1 SEM for N experiments. Probabilities are for paired differences. Both sides bubbled with 1% CO_2 in O_2. Fluxes and Isc are in μEq/h·cm^2 and conductances (G) are in mmhos/cm^2.

Table 2. Effect of [HCO_3] on Cl Influx (J^{Cl}_{me})

	$[HCO_3]_s$ = 20mM			$[HCO_3]_s$ = 0.4mM		
	J^{Cl}_{me}	Isc	G	J^{Cl}_{me}	Isc	G
control	8.4 ± .95	5.0 ± .68	33 ± 3.3	8.4 ± .74	2.8 ± .39	30 ± 3.4
+furosemide	4.9 ± .67	0.2 ± .13	30 ± 2.9	4.1 ± .34	0.6 ± .30	25 ± 1.2

Means ± 1 SEM for 5 experiments in which all 4 conditions were tested. Furosemide (1 μmol/ml) added to mucosal medium 5 min before flux measurement. Both sides bubbled with 1% CO_2 in O_2.

serosal acidification in the influx chamber than in the standard Ussing chamber. This was probably the case because of poorer mixing at the serosal surface. In order to reliably reduce Isc in the influx chamber, it was necessary to decrease serosal HCO_3 to 0.4 mM and to wait 45 min with solution changes every 10 min. Even then the reduction in Isc (about 45%) was not as great as that observed at a higher HCO_3 concentration (2 mM) in the standard Ussing chamber (see Table 1). Nonetheless, it is apparent from Table 2 that serosal acidification had no effect on Na-coupled (i.e., furosemide-inhibited) Cl influx. We therefore conclude that reducing serosal HCO_3 concentration inhibits net Cl absorption through an inhibitory effect on the Cl transfer process at the serosal border. The mechanism for this inhibition remains to be elucidated. This work was supported by NIH grants AM-21345 and AM-18199.