Skate (30 g), dogfish (100 g) or flounder liver (12 g) was perfused with ice-cold 0.25 M sucrose-Tris HCl buffer (0.005M, pH 8), minced and homogenized in the same buffer (3 ml/g liver). The homogenate was centrifuged at 4°C at 900 x g for 10 min. The pellet was washed once and resuspended in the homogenization buffer ("Nuclear fraction"). The wash was added to the supernatant ("Postnuclear fraction).

UDPglucuronate glucuronyl transferase was assayed as described before (Bilirubin Glucuronidation in Fish. I. this Bulletin). Bilirubin glucuronoside glucuronosyl transferase activity was assayed as described by Jansen et al (J. Biol. Chem. 1977. 252:2710-2716). Protein was measured according to Lowry et al. Distribution of UDPglucuronate glucuronyl transferase and bilirubin glucuronoside blucuronosyl transferase was different in subcellular fractions (Fig. 1).

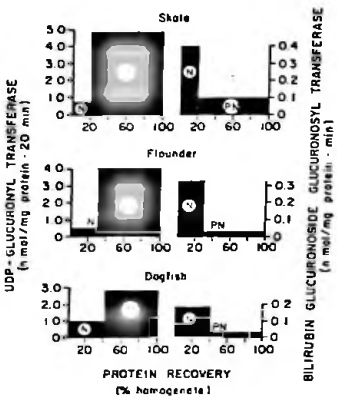

Figure 1. Protein recovery and enzyme specific activities in nuclear (N) and post-nuclear (PN) fractions of skate, flounder and dogfish liver. Protein recovery as percentage of total homogenate protein is shown in the abscissa. UDPglucuronate glucuronyl transferase activity and bilirubin glucuronoside glucuronosyl transferase activity were assayed as described in "Material and methods" and are expressed as nmol/mg protein. 20 min and nmol/mg protein.min, respectively (ordinate).

This suggests that, as in rats (Jansen et al. 1977), the two enzyme activities are concentrated in different organelles of the liver cells.

ROLE OF MEDIUM pH IN THE REGULATION OF CHLORIDE TRANSPORT IN THE INTESTINE OF THE WINTER FLOUNDER, PSEUDOPLEURONECTES AMERICANUS

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Salt absorption in flounder intestine increases as medium pH increases (Field et al, J. Memb. Biol. 41:265, 1978; Field et al, Bull. MDIBL 18:44, 1978). Whether this effect is due to HCO_3 per se or to pH has not been determined. Furthermore, the step or steps in transepithelial Cl transport that are pH (or HCO_3)-dependent have not been elucidated, although preliminary experiments suggest that NaCl co-transport across the brush border is not affected (Field et al, Bull. MDIBL 18:44, 1978). The present study was undertaken to further explore the regulation of intestinal salt absorption by medium pH and [HCO_3].

Methods

Methods for maintaining fish and for in vitro determinations of electrical properties and both transepithelial and trans-luminal border (J_{me}) ion fluxes are those previously described (Field et al, J. Memb. Biol. 41:265, 1978; Frizzell et al, J. Memb. Biol. 46:27, 1979). Ringer solutions employed were the standard 20mM HCO_3-Ringer (Field et al, J. Memb. Biol. 41:265, 1978); 4mM HCO_3-Ringer, which was the same as the standard Ringer except that 8mM each of sulfate and mannitol were substituted for 16mM HCO_3; and HEPES-Ringer (pH 8.2), which was also the same as the standard Ringer except that 20mM N-2-Hydroxyethylpiperazine-N'-2-ethanesulfonic acid titrated with NaOH was substituted for $NaHCO_3$. Solutions were gassed with either 1% or 5% CO_2 in O_2 or, in the case of HEPES-Ringer, with 100% O_2. D-Glucose and D-Mannitol, 10μmol/ml, were always added to serosal and mucosal sides, respectively.

Intracellular [Cl] was determined with ^{36}Cl in the "influx" chambers so that electrical properties could be simultaneously monitored. Extra-cellular space was estimated with ^{3}H-polyethylene glycol (2-10mCi/g, mw 900, NEN, Boston). Equal concentrations of radioisotopes were added to both mucosal and serosal media and allowed to equilibrate with the tissue for 55-65 min. Tissues were then quickly removed, rinsed in cold 300 mM mannitol, blotted and weighed. After 2-6 h at 95°C, tissues were reweighed to obtain the dry weights. Radioactivity was extracted from the dried tissues with 0.1N HNO_3 for 12-16 h and measured by liquid scintillation spectrometry.

Results and Discussion

At constant HCO_3 (20mM), an increase in pCO_2 from 7.6 to 38mm Hg (the measured change in pH was 8.0 to 7.2) caused a decrease in transepithelial electrical potential difference (PD) and short-circuit current (I_{sc}), a new steady state having been reached in about 30 min; when pCO_2 was restored to 7.6mm Hg, PD and I_{sc} again increased (data not shown). The increase in pCO_2 also decreased net Cl absorption (J^{Cl}_{net}) from 5.9 to 1.5μEq/h · cm^2, due entirely to a decrease in mucosa (m)-to-serosa (s) Cl flux (Table 1). Similar results were previously obtained when serosal HCO_3 concentration was

TABLE 1

Effects of pH on Transepithelial Cl Fluxes

pH_o	J^{Cl}_{ms}	J^{Cl}_{sm}	J^{Cl}_{net}	I_{sc}	G_t
8.0	8.5 ± 1.04	2.7 ± 0.72	5.9 ± 0.56	-3.3 ± 0.27	21.4 ± 1.8
7.2	4.6 ± 0.49	3.1 ± 0.70	1.5 ± 0.44	-1.5 ± 0.15	17.8 ± 1.3
p <	0.01	ns	0.001	0.001	0.05

Means ± 1 SEM for 6 paired experiments. Rissues were bathed in 20mM HCO_3-Ringer and gassed with oxygen containing either 1% CO_2 (pH 8.0) or 5% CO_2 (pH 7.2.). Fluxes and I_{sc} are in μEq/h·cm^2 and conductances (G_t) are in mmhos/cm^2.

reduced from 20 to 2mM at constant (1%) CO_2 (Field et al, Bull. MDIBL 18:44, 1978). [In general, Cl transport can more readily be inhibited by increasing medium pCO_2 than by decreasing medium HCO_3. Some groups of fish are more susceptible to the latter alteration than others (compare, for example, Table 1 in Bull. MDIBL 18:45, 1978 to Table 4 in this article.] These results suggest, therefore, that pH and not HCO_3 *per se* regulates Cl transport. This conclusion is reinforced by the data in Table 2 which reveals a high rate of Cl absorption in HCO_3-free HEPES buffer.

TABLE 2

Transepithelial Cl Fluxes in HCO_3-free HEPES Ringer (pH 8.2)

J^{Cl}_{ms}	J^{Cl}_{sm}	J^{Cl}_{net}	I_{sc}	G_t
10.0 ± 1.6	3.8 ± 0.7	6.2 ± 1.3	-3.5 ± 0.50	24.3 ± 0.6

Means ± 1 SEM for 5 experiments. Fluxes and I_{sc} are in μEq/h·cm^2 and G_t is in mmhos/cm^2.

A priori, the effect of medium pH on J^{Cl}_{net} could have been due to (1) a decrease in Na-coupled Cl influx across the brush border; (2) an increase in Na-independent Cl permeability of the brush border; (3) a decrease in Cl permeability of the serosal border; or (4) direct inhibition of the Na pump. In order to examine the first two possibilities, we determined Cl influx across the brush border (J^{Cl}_{me}) at both pH's and in the presence and absence of furosemide. Furosemide was previously shown to inhibit Na-coupled Cl influx (Frizzell et al, J. Memb. Biol. 46:27, 1979) and, accordingly, was used as a measure of this process. Furosemide-inhibitable Cl influx was found to be the same at both pH's, indicating that NaCl co-transport across the brush border is not altered over this pH range (Table 3).

TABLE 3

Effects of pH on Cl Influx

	1% CO_2, pH = 8.0		5% CO_2, pH = 7.2	
	J^{Cl}_{me}	I_{sc}	J^{Cl}_{me}	I_{sc}
control	6.4 ± 0.92	3.7 ± 0.52	9.2 ± 1.09*	-1.8 ± 0.30‡
+ furosemide	3.6 ± 0.30	0.3 ± 0.21	6.0 ± 1.06	-0.7 ± 0.17
p <	0.05	0.01	0.01	0.02

Means ± 1 SEM for 7 experiments in which all four conditions were tested. Furosemide (1μmol/ml) was added to the mucosal medium 5 min prior to the flux measurement. Fluxes and I_{sc} are in $\mu Eq/h \cdot cm^2$ and G_t is in $mmhos/cm^2$.

*$p < 0.05$ 5% CO_2 vs 1% CO_2 ‡$p < 0.01$ 5% CO_2 vs 1% CO_2

This result agrees with our prior observations at fixed pCO_2 and altered HCO_3 concentration (Field et al, Bull. MDIBL 18:44, 1978). It is of interest, however, that the Na-independent portion of Cl influx increased with the decrease in pH, suggesting an increase in luminal Cl permeability as a possible explanation for the change in J^{Cl}_{net}. The implications of this change with respect to Cl efflux from cell-to-lumen and intracellular [Cl] remain to be explored.

The third possible explanation for the decrease in J^{Cl}_{net} is a decrease in serosal Cl permeability. This change, if it were the predominant one, should result in an increase in cellular Cl concentration (assuming no major change in intracellular electric potential). In order to examine this possibility, therefore, we estimated intracellular Cl concentration bu determining the equilibrium concentration of ^{36}Cl in cell water (see Methods). As shown in Table 4, when medium

TABLE 4

Effect of Extracellular pH and [HCO_3] on Intracellular [Cl]

Medium composition HCO_3 (mM)	$\%CO_2$	pH_o	$[Cl]_i$	I_{sc}
20	1%	8.00	40.1 ± 3.9	-3.8 ± 0.89
20	5%	7.22	28.5 ± 2.5	-1.5 ± 0.24
		p <	0.05	0.05
20	1%	8.00	36.2 ± 2.8	-2.7 ± 0.51
4	1%	7.38	40.6 ± 4.8	-2.9 ± 0.43
		p <	ns	ns

Means ± 1SEM for 3 (upper half) or 4 (lower half) paired experiments. I_{sc} is in $\mu Eq/h \cdot cm^2$.

pH was decreased from 8.0 to 7.2 by increasing pCO_2 at fixed $[HCO_3]$, intracellular [Cl] decreased from 40 to 28mM. There is close agreement between these determinations of intracellular [Cl] and those made with Cl-selective microelectrodes (see Duffey et al, Bull. MDIBL 18:70, 1978).] In contrast, when medium pH was decreased from 8.0 to 7.4 by decreasing medium $[HCO_3]$ at fixed pCO_2, intracellular [Cl] did not change. The former maneuver also resulted in a decrease in I_{sc} (and therefore J^{Cl}_{net}) whereas the latter maneuver did not. Thus cell [Cl] is directly rather than inversely proportional to J^{Cl}_{net}, excluding a decrease in serosal Cl permeability. The remaining explanation for the decrease in J^{Cl}_{net} is a pH-dependent limitation of the Na pump.

In summary, salt absorption in flounder intestine is regulated in part by medium pH and this effect is due partly or wholly to a pH-dependent increase in luminal Cl permeability. An effect of pH on the coupling of energy metabolism to active Na transport is another possibility that remains to be explored. This work was supported by NIH grant AM-21345 and NIH post-doctoral fellowship AM-05973 to P. L. Smith.

INTRACELLULAR pH AND CL TRANSPORT IN THE INTESTINE OF THE WINTER FLOUNDER, PSEUDOPLEURONECTES AMERICANUS: STUDIES WITH DIMETHYLOXAZOLIDINE-2, 4-DIONE (DMO)

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Active Cl absorption by flounder intestine is inversely proportional to the pH of the bathing medium (Field et al, Jl. Memb. Biol. 41:265, 1978; Field et al, Bull. MDIBL 18:44, 1978 and Smith et al, present volume). This inhibition is most likely due to a pH-dependent increase in luminal membrane permeability to Cl (Smith et al, present volume). In order to evaluate the role of intracellular pH and of the medium-to-cell pH gradient in the regulation of Cl permeability and transport, we estimated intracellular pH with ^{14}C-DMO. We have also measured transepithelial DMO fluxes in order to test the validity of using DMO to measure intracellular pH.

Methods for maintaining fish and for in vitro determinations of electrical properties and solute fluxes are those described in the companion paper (Smith et al, present volume). Intracellular pH was determined in the "influx" chambers to permit simultaneous monitoring of short-circuit current (I_{sc}). ^{14}C-DMO (47 mCi/mmol, NEN, Boston), yielding a medium concentration of 7×10^{-6}M, was added to both sides of the epithelium and, after 60 min equilibration, tissue DMO concentration was measured as described for ^{36}Cl in our companion paper, ^{3}H-polyethylene glycol again being added to measure extracellular space. Intracellular pH was estimated by the following equation, in which it is assumed that the concentrations of undissociated DMO in medium and in intracellular water are equal:

$$pH_i = pK + \log \left| \frac{\frac{^{14}C\ (TW)}{^{14}C\ (0)} - ecs}{(TW - ecs)} \cdot \frac{1 + 10^{pH_o - pK}}{} - 1 \right|$$

where i, 0 and TW refer to cell water, medium, and tissue water, respectively; ecs is the extracellular space and pK is 6.15 (the pK of DMO).

The effects of medium pH on pH_i, and ΔpH ($pH_o - pH_i$), as measured with DMO, are shown in Table 1; included also are values for I_{sc}. The data indicate that (1) pH_i is less than pH_o; (2) ΔpH decreases when pH_o is decreased; and (3) the decrease in ΔpH is the same whether pH_o is decreased by increasing pCO_2 or by decreasing $[HCO_3]_o$. Since only the former maneuver caused a decrease in I_{sc} and, therefore, in J^{Cl}_{net}, a change in ΔpH does not appear to be responsible for the change in transepithelial Cl transport. Thus, the effect of pH_o on Cl transport cannot be readily explained by an effect on either pH_i or ΔpH.