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.

TABLE 1

Effects of Extracellular pH and [HCO_3] on Intracellular pH

Medium Composition HCO_3 (mM)	%CO_2	pH	pH_i	ΔpH	I_{sc}
20	1%	8.00	7.64 ± 0.03	-0.37 ± 0.03	-3.8 ± 0.89
20	5%	7.22	7.02 ± 0.03	-0.20 ± 0.03	-1.5 ± 0.24
		p <	0.01	0.05	0.05
20	1%	8.00	7.46 ± 0.07	-0.54 ± 0.07	2.7 ± 0.51
4	1%	7.38	7.03 ± 0.05	-0.35 ± 0.05	2.9 ± 0.43
		p <	0.01	0.05	ns

Means ± 1 SEM for 8 paired experiments. I_{sc} is in $\mu Eq/h \cdot cm^2$.

The validity of using DMO to measure intracellular pH is contingent on the correctness of several assumptions including (1) the cell membrane is far more permeable to undissociated than to dissociated DMO and (2) the equilibrium concentration of undissociated DMO in the cells is the same as in the medium, i.e. no active transport of this species occurs. In order to test these assumptions we determined unidirectional transepithelial fluxes of ^{14}C-DMO across the short-circuited mucosa at pH 8.0 (pCO_2 = 7.6 mmHg) and at pH 7.2 (pCO_2 = 48 mmHg) (Table 2).

TABLE 2

Effects of Extracellular pH on DMO Fluxes

pH_o	n	J^{DMO}_{ms}	J^{DMO}_{sm}	J^{DMO}_{net}	I_{sc}
8.0	5	46.2 ± 1.1	67.1 ± 4.8	-20.9 ± 5.6	-3.5 ± 0.27
7.2	4	76.8 ± 2.8	83.8 ± 2.7	- 7.0 ± 5.0	-1.0 ± 0.12
p <		0.001	0.05	ns	0.01

Means ± 1 SEM for n experiments. Probabilities are for unpaired differences. Tissues were bathed in 20mM HCO_3-Ringer and bubbled with oxygen containing either 1% CO_2 (pH 8.0) or 5% CO_2 (pH 7.2). DMO fluxes are in $\mu l/h \cdot cm^2$ (cpm transferred divided by cpm/μl on the initially labelled side). I_{sc} is in $\mu Eq/h \cdot cm^2$.

The results are not reassuring about either assumption. If the only appreciable transepithelial transport of DMO were by simple diffusion of the undissociated species, then unidirectional fluxes should have changed about 6-fold in accordance with the 6-fold change in undissociated DMO concentration. Furthermore, at pH 8.0, a significant net secretion of DMO was observed. This secretion suggests the presence of transmural HCO_3 (or OH) secretion which would tend to change the pH of the unstirred layers, decreasing pH in the serosal layer and increasing pH in the mucosal layer, with a resulting serosa (s)-to-mucosa (m) concentration difference for undissociated DMO. The use of DMO to measure intracellular pH in intestinal mucosa is problematic, therefore, and the DMO determinations need to be compared with

measurements made under the same circumstances by other means, such as pH-sensitive microelectrodes or the equilibrium distribution of a weak base, such as nicotine. In addition, the possibility that flounder intestine secretes HCO_3 when pH_o is 8.0 needs to be evaluated. In prior studies, the absence of a residual ion flux ($I_{sc} - J^{Na}_{net} + J^{Cl}_{net}$) suggested an absence of HCO_3 secretion, but residual flux measurements are by their nature extremely inaccurate. This work was supported by NIH grant AM-21345 and NIH postdoctoral fellowship AM-05973 to P. L. Smith.

PRELIMINARY OBSERVATIONS ON MORPHOLOGY AND ELECTRICAL PROPERTIES OF GALLBLADDER EPITHELIUM FROM THE WINTER FLOUNDER, PSEUDOPLEURONECTUS AMERICANUS

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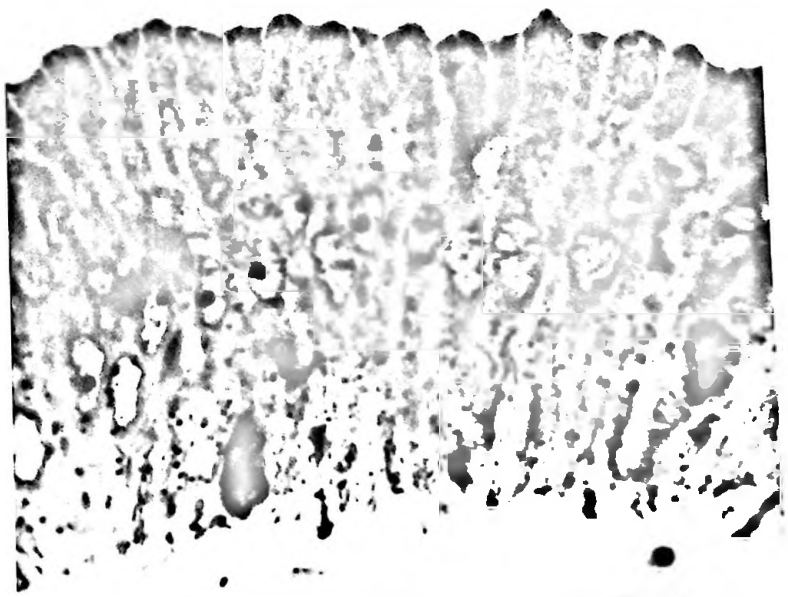
We report here our initial experiments with isolated gallbladder epithelium from the flounder. Methods and Ringer solution (20mM HCO_3-Ringer) were the same as those we have previously employed for flounder intestine (Field et al, J. Membrane Biol. 41:265, 1978). The Ringer solution was gassed with either 1% CO_2 in O_2 (pH 8.0) or 5% CO_2 in O_2 (pH 7.22). The gallbladder was stripped of muscle with fine, curved forceps. For morphology, gallbladder mucosa was fixed in gluteraldehyde immediately after removal from the Ussing chamber; it was post-fixed in osmium tetroxide and embedded in epoxy resin. Sections were cut 1μm thick and stained with toluidine blue.

After mounting, the PD across the gallbladder increased (in absolute value) steadily for 20-40 min, stabilizing at -6 to -9mV, mucosal reference. The resistance was 50 to 70 $-\Omega \cdot cm^2$. Serosal addition of theophylline (5mM) alone or together with 8-Br-cAMP (0.2mM) decreased the PD down to -1 to -2mV gradually over 20-30 min. Reduction of medium pH from 8.0 to 7.2 caused a similar decline in PD. Dilution potentials were the same under control conditions, after theophylline and 8-Br-cAMP, and after acidification: 10% dilution of salts in the mucosal medium with isotonic mannitol produced an abrupt -2.0mV change in PD; serosal dilution gradually reversed the prior PD change (15-20 min). Selectivity ratio (R) of monovalent cations to monovalent anions was calculated from the constant-field equation as follows:

$$R = (e^{(F/RT)\,\Delta PD}[-]m - [-]s)/([+]m - e^{F/RT)\,\Delta PD}[+]s)$$

where [-] and [+] are the concentrations of monovalent anions and cations respectively and the other symbols have their usual meanings. Cation selectivity of the mucosa was about 8:1.

As shown in Figure 1, the cells are long (~55 μm) and narrow (~4μm) with centrolly to basally located nuclei and infrequent goblet cells.



igure 1. Light micrograph of flounder allbladder magnified 1300X.