RELATION BETWEEN NA AND CL TRANSPORT PROCESSES IN TELEOST INTESTINE: POSSIBLE MODIFICATIONS OF TRANSCELLULAR ION FLUXES IN THE PARACELLULAR SHUNT PATHWAY.

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The intestines of certain marine teleosts such as the eel and flounder have been found to sustain in vitro a serosa-negative electric potential difference (PD) and, under short circuit condition, to transport appreciably more Cl than Na (Huang and Chen, Am. J. Physiol., 220:1734, 1971; Ando et al., Comp. Biochem. Physiol., 51A:27, 1975; Smith et al., Pflügers Arch., 357:303, 1975), suggesting an electrogenic Cl pump. Our own examination of ion transport across the isolated intestinal mucosa of the winter flounder, *Pseudopleuronectes americanus* (Field and Smith, MDIBL Bull., Vol. 15) indicate, however, that Cl absorption is tightly coupled to the transport of Na: Although, in NaCl Ringer under short-circuit condition, the net Cl flux ($_oJ^{Cl}_{net}$) was almost three times $_oJ^{Na}_{net}$, replacement of all Na with choline abolished J^{Cl}_{net} and replacement of all Cl with SO_4 abolished J^{Na}_{net}. Furthermore, ouabain (0.5 mM) abolished all NaCl transport within 30 min. These experiments suggest that the Cl absorptive mechanism in flounder intestine is fundamentally similar to that already demonstrated for mammalian (Nellans et al., Am. J. Physiol., 225:467, 1973) and amphibian (Quay and Armstrong, Am. J. Physiol., 217:694, 1969) small intestine and mammalian (Frizzel et al., J. Gen. Physiol., 65:769, 1975) and teleost (Diamond, J. Physiol. [London], 161:474, 1962) gallbladder, namely, facilitated diffusion of NaCl across the luminal border with net absorption of Cl resulting from the Na gradient (i.e., efflux of NaCl from cell to lumen restricted by low intracellular Na activity).

How can the evidence for coupled NaCl transport in flounder intestine be reconciled with the serosa-negative PD and the preponderance of $_oJ^{Cl}_{net}$ over $_oJ^{Na}_{net}$? This paradox becomes explicable once we consider that measured transepithelial fluxes may differ from transcellular fluxes if the latter are modified by the perm-selective and resistive properties of the paracellular shunt pathway.

It is clear from the relative magnitudes of $_oJ^{Na}_{sm}$ and $_oJ^{Cl}_{sm}$ that we previously reported ($_oJ^{Na}_{sm} = 3.9\,_oJ^{Cl}_{sm}$) that the paracellular pathway in flounder intestine is highly cation selective. The site of cation selectivity is undoubtedly the tight junction, across which a Na diffusion potential should develop since active transport is thought to elevate the salt concentration in the lateral space above that in the bathing media. A smaller Cl diffusion potential should also develop across the lateral space since the mobility of Cl in free solution is 50% greater than that of Na. Both diffusion potentials would contribute to the serosa-negativity of the transmural PD, their magnitudes being determined by the degree of cation selectivity in the tight junction and by the size of the salt concentration gradient in the lateral space. The latter is determined in part by the resistance to ionic diffusion along the lateral intercellular space, which can become significant relative to junctional resistance when the space width falls below 0.1 μm (see Frömter, J. Membrane Biol., 8:259, 1972). Microscopic examination of flounder mucosa reveals that lateral space dilitation due to active fluid absorption seldom extends into the apical 12 μm of the space, a region where many mitochondria are clustered (see Karnaky et al., elsewhere in this bulletin). Thus the PD across flounder intestine could arise as a diffusion potential in the paracellular pathway and it is therefore not necessary to postulate an electrogenic Cl pump.

These considerations about the origin of the transmural PD also have consequences for the relative rates of Na and Cl absorption across the short-circuited epithelium. If the resistance of the lateral space to Na movement is higher than that of the "tight" junction then most of the Na pumped

into the apical portion of the space from the cells will diffuse back into the luminal bathing medium. In contrast, since the Cl permeability of the tight junction is low, most of the Cl pumped into the space will diffuse into the serosal medium. To illustrate this I have analyzed the fluxes previously determined (Field and Smith, MDIBL Bull., Vol. 15) in terms of a simple model which assumes the lateral space to be a tissue compartment into which the cell transports equal amounts of Na and Cl and out of which these ions can diffuse into both mucosal and serosal bathing solutions (see Figure 1). I have also assumed (a) that Na and Cl are the only ions transported and are both present in the

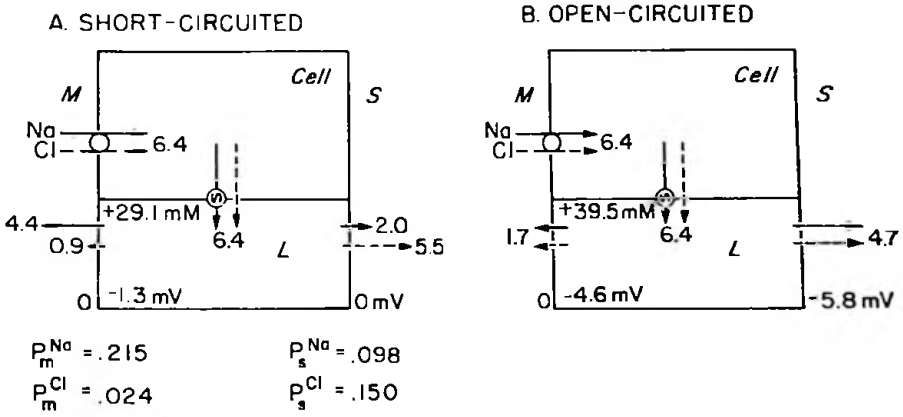

Figure 1. Compartmental analysis of Na and Cl fluxes through the lateral space (L). See text for details.

bathing medium at 150 mM concentration; (b) that the Na and Cl permeabilities of the contraluminal boundary of the lateral space relate to each other as do their free solution mobilities, i.e., $p_s^{Cl} = 1.52P_s^{Na}$; and (c) that the permeabilities of luminal and basolateral faces of the epithelial cells are sufficiently low that transcellular ion diffusion is negligible and therefore that J_{sm}^{Na} and J_{sm}^{Cl} are strictly extracellular. Applying the Goldman constant-field equation to ion movements across the postulated mucosal and serosal boundaries of the lateral space yields, for net fluxes,

$$ {}_oJ_j^i = p_j^i \cdot Z \cdot U \cdot (C_o + \frac{\Delta C}{1 - e^{-Z \cdot U}} \qquad (1) $$

where i refers to Na or Cl, j to m or s, o to the short-circuited state, P is a permeability constant, Z the valence of Na or Cl, $U = (F/RT) \times \Delta\Psi_L$, $\Delta\Psi_L$ being equal to $\Psi_L - \Psi_o$ (L referring to the lateral space), Co = 150 mM and $\Delta C = C_L - C_o$. For unidirectional s-to-m fluxes,

$$ {}_oJ_{sm}^i = \frac{-C_o \cdot Z \cdot U}{1 - e^{Z \cdot U}} \times \frac{p_m^i \cdot p_s^i}{p_m^i + p_s^i} \; . \qquad (2) $$

Finally, if the cell transports equal amounts of Na and Cl into the space, then, in the steady-state, Na and Cl effluxes out of the space must also be equal, i.e.,

$$ {}_oJ_m^{Na} + {}_oJ_s^{Na} = {}_oJ_s^{Cl} + {}_oJ_s^{Cl} \quad . \tag{3} $$

For $J_{net}^{Na} = 2.0$, $J_{net}^{Cl} = 5.5$, $J_{sm}^{Na} = 10.4$ and $J_{sm}^{Cl} = 3.0$, the values shown in Figure 1A for ΔC, $\Delta\Psi$, p_j^i and J_j^i can be obtained. If short-circuiting doesn't change the cellular output of NaCl, then relevant values for the open-circuited state can also be predicted (see Figure 1B).

Although more complex and realistic analyses could probably be made, the present one serves to illustrate the potential influence of the paracellular pathway on transepithelial ion transport. In general, if lateral space resistances to ion movements are significant relative to junctional resistances, it becomes hazardous to extrapolate from transmural PD and flux measurements to transport events at the cellular level. A serosa-negative PD has also been noted in the thick ascending limb of Henle's loop in rat kidney (Burg and Green, Am. J. Physiol., 224:659, 1973). It may be worthwhile to consider the interpretation offered here as an alternative to an electrogenic Cl pump at that site. The role of lateral space diffusion potentials in modifying transepithelial ion transport has previously been pointed out by Machen and Diamond (J. Membrane Biol., 1:194, 1969) and by Armstrong (in Intestinal Ion Transport, MTP press, 1976).

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CHARACTERIZATION OF CYCLIC AMP-MEDIATED CHLORIDE PERMEABILITY CHANGE IN THE INTESTINAL EPITHELIUM OF THE FLOUNDER, *Pseudopleuronectes americanus*

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We previously reported (Field and Smith, MDIBL Bull., Vol. 15) that cyclic 3',5'-AMP (cAMP) increases the unidirectional serosa (s)-to-mucosa (m) Cl flux across short-circuited flounder intestinal mucosa ($_oJ_{sm}^{Cl}$) without producing appreciable changes in net Cl flux ($_oJ_{net}^{Cl}$) or in unidirectional or net Na fluxes, suggesting a specific effect on passive Cl permeability. In the present study we have further characterized this effect. Experiments were designed to answer the following three questions: (1) In the absence of an increase in intracellular cAMP concentration, does $_oJ_{sm}^{Cl}$ result exclusively from diffusion over the paracellular shunt pathway or does it contain a transcellular component? (2) Does the increase in $_oJ_{sm}^{Cl}$ caused by cyclic AMP result from an alteration of Cl permeability in the paracellular pathway or in a transcellular pathway? (3) Finally, since stimulation by cAMP of active Cl secretion in mammalian intestine appears to require both exogenous Na and a functioning Na pump, does active Na transport also play an essential role in the cyclic AMP-induced Cl permeability change in flounder intestine?

In order to evaluate the transcellular and paracellular components of $_oJ_{sm}^{Cl}$ that are normally present, we measured this flux at different extracellular Cl concentrations and at different applied transmural PDs. Figure 1 shows the effect of [Cl] on $_oJ_{sm}^{Cl}$ and on short-circuit current (Isc). Over the range 27 to 120 mM, $_oJ_{sm}^{Cl}$ varies linearly with [Cl], although Isc, which should be proportional to J_{net}^{Cl}, approaches saturation. Thus, in the absence of cAMP, $_oJ_{sm}^{Cl}$ does not appear to traverse the saturable, presumably transcellular limb of the pathway for $_oJ_{net}^{Cl}$.