rotated 90° for ease of freezing. The bladders, still tied to the rings, were frozen in liquid propane; freeze-dry plastic section autoradiography was then carried out.

Figures 1-3 show the bladder epithelial cells (largely granular cells) after 15 days of emulsion development. ^{14}C sucrose is almost completely excluded from the epithelial cells. ^{14}C urea, on the other hand, labels the cells in a relatively uniform manner both in the presence and absence of vasopressin. An unexpected finding is the failure to find more numerous grains in the presence of vasopressin.

The findings suggest that there are no specialized epithelial cells involved in urea transport, and that urea, like water, traverses the granular cell. The "independent pathways," then, would be separate sites in the granular cell membrane.

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EFFECTS OF TRIAMINOPYRIMIDINE (TAP) ON Na AND Cl TRANSPORT BY *Pseudopleuronectes americanus* INTESTINE

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Field et al. (J. Memb. Biol. 41:265, 1978) proposed a model for Na and Cl transport by flounder intestine whose essential features are depicted in Figure 1. According to this model, neutral (one-

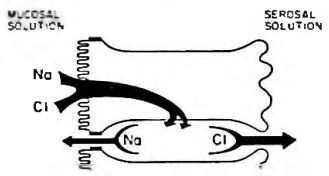

Figure 1. Model for Na and Cl transport by flounder intestine under short-circuit conditions.

for-one) transcellular NaCl transport is obscured by the permselective characteristics of the paracellular pathway, such that much of the Na transported into the lateral intercellular space recycles to the mucosal solution. Under open-circuit conditions, a diffusion potential develops across the tight junction, accounting for the spontaneous, serosa-negative electrical potential difference that is observed across this tissue, and under short-circuit conditions, a preponderance of Cl over Na absorption is observed (Field et al., J. Memb. Biol. 41:265, 1978).

Support for the notion of neutral transcellular NaCl transport was derived from studies by Frizzell et al. (J. Memb. Biol., in press) who identified an obligatory NaCl co-transport process at the brush border membranes of the absorptive cells. To address the question of whether the tight junctions represent the site of Na recycling, the effects of triaminopyrimidine (TAP) on NaCl transport by flounder intestine were evaluated. Moreno (J. Gen. Physiol. 66:97, 1975) demonstrated that addition of TAP to the solution bathing the mucosal surface of several epithelia elicited a reversible increase in tissue resistance that was due to a decrease in Na permeability of the paracellular pathway; the permselective properties of this pathway are dominated by those of the tight junction (Moreno and Diamond, In: Membranes--A Series of Advances (G. Eisenman, ed.), Dekker: New York, 1976). TAP is most active in this regard when present as the monovalent cation, which predominates at pHs below its pK_1 (6.7). However, flounder intestine requires the presence of HCO_3 in the bathing media for optimal rates of Na and Cl absorption. The results of studies reported elsewhere in this bulletin

(Field, Smith, Clayton and Frizzell) indicate that it is the HCO_3 concentration of the serosal solution that determines the rate of Cl absorption. Reducing the HCO_3 concentration (and pH) of the mucosal solution alone has no effect, so that the mucosal surface could be exposed to acidified, TAP-containing solutions without compromising electrolyte transport (see below).

Preliminary studies were performed to evaluate the effect of TAP, added to the mucosal solution, on the electrical resistance of the tissue. Mucosal solution pH was varied by adjusting the HCO_3 concentration at fixed pCO_2. The results of these studies confirmed the findings of Moreno: At a mucosal solution pH of 8.0 (20 mM HCO_3) TAP produced a 25% increase in tissue resistance (R_t). At pH 7.0 (2 mM HCO_3), TAP increased R_t by 40%, and at pH 6.3 (0.4 mM HCO_3), R_t was increased nearly twofold.

The last of these conditions was chosen for evaluating the effects of TAP on transepithelial Na and Cl fluxes. The results of these studies are given in Table 1; the legend to this table provides

TABLE I

EFFECT OF TAP ON SODIUM AND CHLORIDE FLUXES

ACROSS FLOUNDER INTESTINE

	J^{Na}_{ms}	J^{Na}_{sm}	J^{Na}_{net}	J^{Cl}_{ms}	J^{Cl}_{sm}	J^{Cl}_{net}	I_{sc}	G_t	$J^{Cl}_{net}/J^{Na}_{net}$
Control	13.6±0.7	12.5±0.5	1.1±0.8	6.5±0.3	2.7±0.4	3.8±0.4	-3.0±0.2	25±1	3.5±0.3
+TAP	9.8±0.6*	7.6±0.4*	2.2±0.6*	4.2±0.2*	2.2±0.4	2.0±0.3*	-2.0±0.2*	13±1*	0.9±0.5*

All values in $\mu Eq/cm^2 hr$ except G_t in $mmhos/cm^2$. J_{ms} represents the unidirectional flux from mucosa-to-serosa; J_{sm} that from serosa-to-mucosa; $J_{net}=J_{ms}-J_{sm}$. Fluxes determined during 30-min control period, TAP (20 mM) then added to mucosal solution; mannitol (20 mM) to serosal solution; a 20-min equilibration period was followed by a 30-min experimental flux period in the presence of TAP. All serosal media consisted of (mM): Na, 165; Cl, 150; HCO_3, 20; K, 5; HPO_4-H_2PO_4, 2; Ca, 1; Mg, 1; pH, 8.0; gassed with 99% O_2 - 5% CO_2. The pH of the mucosal solutions (both control and experimental) was lowered to 6.3 by reducing HCO_3 concentration to 0.4 mM ($NaHCO_3$ replaced by Na_2SO_4 and mannitol). * designates a significant difference from control value, $p < 0.05$ by paired t analysis. Results obtained from experiments performed using paired tissues from 7 animals.

details of the solution composition. Despite acidification of the mucosal solution, the rates of Na and Cl absorption observed under "control" conditions (in the absence of TAP) are in excellent agreement with those reported previously. The ratio of net Na to Cl absorption averaged 3.5. As previously discussed (Field et al., J. Memb. Biol. 41:265, 1978), the Na permselectivity of this tissue is reflected by the relative magnitudes of the serosa-to-mucosa Na and Cl fluxes ($J^{Na}_{sm}/J^{Cl}_{sm} = 4.6$).

Addition of TAP to the mucosal bathing solution elicited a 50% reduction in tissue conductance (R_t doubled), which was paralleled by decreases in the bidirectional fluxes of Na across the epithelium. A reduction in the diffusional transepithelial fluxes of Na, which in leaky epithelia are largely, if not entirely, restricted to the paracellular pathway (Frizzell and Schultz, J. Gen. Physiol. 59:318, 1972) is the most reasonable explanation for these findings. The lack of any effect of TAP on J^{Cl}_{sm} is consistent with the findings of Moreno, suggesting that this agent does not influence diffusional anion movements. However, TAP reduced the rate of net Cl absorption by nearly 50% due to a decrease in the unidirectional Cl flux from mucosa-to-serosa. Therefore, in addition to its effects on paracellular ion movement, TAP appears to inhibit active, transcellular NaCl transport. The results of other studies also suggest that TAP may interfere with cellular transport mechanisms. In frog skin (Zieske, Pflugers Arch. 359:R127, 1975) and rat kidney (Bowman et al., J. Pharm. Exp. Ther. 206:207, 1978) TAP was found to have amiloride-like effects on Na transport. In guinea pig gallbladder, Savorymuttu and Wood (J. Physiol. 266:68P, 1976) observed a 50% reduction in fluid absorption with TAP. The decrease in J^{Cl}_{net} & J^{Cl}_{ms} across flounder intestine suggests that TAP, added to the mucosal solution, may interfere with NaCl co-transport across the mucosal membrane, but additional studies are necessary to evaluate this possibility.

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