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# Ion Transport Across the Isolated Intestinal Mucosa of the Winter Flounder, *Pseudopleuronectes americanus:* II. Effects of Cyclic AMP

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**Summary.** Addition of cyclic AMP and theophylline to the intestinal mucosa of the winter flounder, *Pseudopleuronectes americanus* decreased short-circuit current and net Na and Cl absorption and increased total conductance and the serosa-to-mucosa unidirectional Cl flux ($J_{sm}^{Cl}$). The last two changes were independent of the original rate of NaCl absorption and persisted even when net absorption of Na and Cl had been abolished by ouabain. Voltage-clamp experiments revealed that the increment in $J_{sm}^{Cl}$ produced by cyclic AMP is PD-insensitive and therefore not due to an increase in the Cl conductance of the paracellular shunt. Cyclic AMP appears, therefore, both to inhibit net NaCl absorption and to increase the Cl permeability and total conductance of the intestinal epithelial cells; its failure to stimulate secretion (in contrast to its action on mammalian intestine) may be related to the absence of crypts in flounder intestinal epithelium.

We recently described structural and ion transport properties of the intestine of a marine teleost, the winter flounder [7]. This preparation differs from mammalian small intestine in at least two significant ways: it contains no crypts or other multicellular gland-like structures; and, when bathed on both sides with the same $HCO_3$-buffered Ringer solution, it generates a serosa-negative electric potential difference (PD) and, under short-circuit condition, absorbs almost 3 times as much Cl as Na. Despite this disparity, Cl transport is dependent upon Na in the bathing medium and is inhibited by ouabain, suggesting that the Cl absorptive process in flounder intestine is the same at the cellular level as that in rabbit gallbladder [8], i.e., that its driving force is the Na gradient and that direct coupling between Na and Cl fluxes occurs in the brush border membrane. Both the transepithelial PD and the predominance under short-circuit condition of the net Cl flux over the net Na flux can be explained by permselective and resistive properties of the paracellular shunt, i.e., a junctional complex which is highly cation-selective and which offers less resistance to Na diffusion than does the lateral intercellular channel. Thus, under short-circuit condition, most of the Na pumped from cell to lateral space may diffuse across the junctional complex back to the luminal solution. Two essential features of this model have recently been verified: coupling between Na and Cl influxes across the brush border has been demonstrated by measuring initial rates of uptake [10] and a Na-dependent intracellular accumulation of Cl above electrochemical equilibrium has been demonstrated with Cl-sensitive microelectrodes [5].

In mammalian small intestine, net absorption of water and electrolytes disappears and net secretion develops upon addition of cyclic 3′,5′-AMP (cAMP) or an agent which increases its cellular concentration [6]. Studies with rabbit ileum suggest two separable effects of the nucleotide: (i) inhibition of coupled NaCl uptake across the brush border membrane [15] and (ii) stimulation of active anion secretion in the crypts of Lieberkuhn [6]. Since flounder intestine is devoid of crypts and since it possesses a coupled NaCl uptake process, we felt it would be of interest to examine the effects of cAMP on ion transport in this epithelium.

## Materials and Methods

Methods for obtaining fish, stripping and mounting of intestine, and for measurements of PD, short-circuit current ($I_{sc}$), conduc-

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tance ($G_t$), unidirectional mucosa-to-serosa ($J_{ms}$) and serosa-to-mucosa ($J_{sm}$) and net fluxes ($J_{net}$) were previously described [7]. The Ringer solutions employed were those previously described [7]. All solutions were bubbled with 1 % $CO_2$ in $O_2$ and maintained at 15°. Adaptation of fish to 15 % seawater was carried out by progressively diluting seawater at 48–72 hr intervals, the sequence being 100, 50, 25, and 15 %. Fish were maintained at 15° during adaptation and were not used before at least 48 hr in 15 % seawater.

Results are expressed as means ± SEM. Statistical comparisons are by Student's $t$ test for paired or unpaired variates.

## Results

### *Effects of cAMP on Na and Cl Fluxes, $I_{sc}$ and $G_t$*

In order to insure a high intracellular concentration of cAMP, theophylline (5 μmol/ml) and cAMP (2 μmol/ml) were added together to the serosal bathing medium. Results for both normal and 15 % seawater-adapted fish and for both high (20 mM) and low (5 mM) $HCO_3$-Ringer solutions are shown in Table 1. Consistent with prior observations [1, 18], adaptation to 15 % seawater resulted in a reduction of $I_{sc}$ and ${}_oJ^{Cl}_{net}$ [1]. A decrease in the mean value for ${}_oJ^{Na}_{net}$ also occurred but was not statistically significant.

In high $HCO_3$-Ringer, theophylline and cAMP produced a gradual (15–20 min) decrease in the absolute value of the $I_{sc}$. Subsequent Na and Cl flux measurements revealed decreases in the means for both ${}_oJ^{Cl}_{net}$ and ${}_oJ^{Na}_{net}$, the former change being larger than the latter and the ratio of the two remaining unchanged. These changes were observed with tissues from both normal and 15 % seawater-adapted fish. Based on an analysis of pooled data for both groups of fish, the changes in $I_{sc}$ and ${}_oJ^{Cl}_{net}$ are highly significant ($P<0.001$ for both) but the change in ${}_oJ^{Na}_{net}$ is not ($0.05<P<0.1$). Small changes in ${}_oJ^{Na}_{net}$ are difficult to ascertain from measurements of large unidirectional Na fluxes. If, however, ${}_oJ^{Na}_{net}$ is assumed equal to the sum of the $I_{sc}$ and ${}_oJ^{Cl}_{net}$ [7], then a significant diminution in ${}_oJ^{Na}_{net}$ becomes apparent (i.e., cAMP and theophylline produced a greater change in ${}_oJ^{Cl}_{net}$ than in $I_{sc}$, $P<0.02$).

Cyclic AMP and theophylline also decreased the mean values for $I_{sc}$, ${}_oJ^{Cl}_{net}$ and ${}_oJ^{Na}_{net}$ in low $HCO_3$-Ringer, but, since the control fluxes were low, none of these changes were statistically significant.

Under all conditions, cAMP and theophylline significantly increased both ${}_oJ^{Cl}_{sm}$ and total conductance. The decreases in ${}_oJ^{Cl}_{net}$ were not in fact associated with decreases in ${}_oJ^{Cl}_{ms}$ (which actually increased slightly) but with increases in ${}_oJ^{Cl}_{sm}$. The increase in ${}_oJ^{Cl}_{sm}$ was approximately the same in magnitude under all conditions shown in Table 1 and therefore was not a function of the pre-existing net Cl flux. The increase in ${}_oJ^{Cl}_{sm}$ was also not associated with any change in ${}_oJ^{Na}_{sm}$, indicating that cAMP does not produce a general increase in the ionic conductances of the paracellular pathway.

In order to exclude the possibility that the above changes were due to an action of theophylline other

[1] The subscripted prefix "$o$" refers to the short-circuited state.

**Table 1.** Effects of cAMP on Na and Cl fluxes, $I_{sc}$ and conductance ($G_t$)

| | ${}_oJ^{Na}_{ms}$ | ${}_oJ^{Na}_{sm}$ | ${}_oJ^{Na}_{net}$ | ${}_oJ^{Cl}_{ms}$ | ${}_oJ^{Cl}_{sm}$ | ${}_oJ^{Cl}_{net}$ | $I_{sc}$ | $G_t$ |
|---|---|---|---|---|---|---|---|---|
| A) High $HCO_3$-Ringer (20 mM $HCO_3$, 1 % $CO_2$, pH 8.2) | | | | | | | | |
| 1) Normal fish (5) | | | | | | | | |
| Control | 15.9±0.87 | 13.8±1.13 | 2.17±0.46 | 9.58±0.76 | 3.06±0.68 | 6.52±0.32 | −3.57±0.41 | 25.5±2.58 |
| +cAMP | 14.4±0.88 | 13.2±1.02 | 1.28±0.78 | 9.74±0.61 | 6.43[a]±0.63 | 3.32[a]±0.77 | −1.16[a]±0.54 | 28.4±2.02 |
| 2) 15% seawater-adapted fish (4) | | | | | | | | |
| Control | 13.5±1.19 | 12.5±0.92 | 1.04±0.90 | 6.96±0.69 | 3.30±0.77 | 3.66±0.89 | −1.51±0.15 | 21.5±0.21 |
| +cAMP | 10.3±1.93 | 9.53±1.77 | 0.82±0.85 | 8.19±1.58 | 8.08[a]±1.62 | 0.11[a]±0.85 | −0.16[a]±0.42 | 24.4[a]±0.35 |
| 3) Pooled results (9) | | | | | | | | |
| Control | 14.9±0.79 | 13.2±0.74 | 1.67±0.48 | 8.15±0.70 | 3.17±0.48 | 5.25±0.64 | −2.61±0.44 | 23.7±1.54 |
| +cAMP | 12.6[a]±1.16 | 11.5±1.10 | 1.08±0.54 | 9.05±0.77 | 7.16[b]±0.79 | 1.89[b]±0.78 | −0.71[b]±0.34 | 26.6[a]±1.28 |
| B) Low $HCO_3$ Ringer (5 mM $HCO_3$, 1 % $CO_2$, pH 7.6); normal fish (7) | | | | | | | | |
| Control | 12.1±0.79 | 10.7±0.71 | 1.35±0.59 | 4.99±0.63 | 2.97±0.32 | 2.02±0.65 | −1.43±0.16 | 18.4±1.01 |
| +cAMP | 11.9±0.72 | 11.8±1.25 | 0.06±0.85 | 9.15[b]±0.87 | 7.72[b]±0.44 | 1.44±0.70 | −0.99±0.07 | 22.1[a]±0.78 |

All experiments were conducted at 15 °C. Values are means ± 1.SE for ($n$) experiments. Fluxes and $I_{sc}$ are in μeq/hr cm² and $G_t$ is in mmhos/cm². Cyclic AMP (2 μmol/ml) was added to the serosal medium together with theophylline (5 μmol/ml).
Statistical differences from control values: [a] $P<0.02$; [b] $P<0.001$.

**Table 2.** Na and Cl fluxes in the presence of ouabain and cAMP

| | ${}_oJ_{ms}$ | ${}_oJ_{sm}$ | ${}_oJ_{net}$ |
|---|---|---|---|
| Na | 9.62±0.40 | 9.11±0.46 | 0.52±0.54 |
| Cl | 10.50±0.63 | 10.30±0.54 | 0.22±0.57 |

Fluxes are in μeq/hr cm². Values shown are for 18 experiments, 8 with tissues bathed in low $HCO_3$-Ringer and 10 in high $HCO_3$-Ringer. Results for the two groups did not differ significantly and therefore were pooled (${}_oJ^{Na}_{net}$ and ${}_oJ^{Cl}_{net}$ for high $HCO_3$-Ringer alone were −0.04±0.90 and −0.29±0.98, respectively). $G_t$ for all tissues was 21.2 ± 0.74 mmhos/cm². Ouabain (0.5 μmol/ml) was added to the serosal medium 30–40 min after tissues were mounted *in vitro*. Thirty min later, cAMP (2.5 μmol/ml) and theophylline (5 μmol/ml) were added to the serosal reservoir. Flux measurements were begun 1 hr thereafter.

than inhibition of cAMP phosphodiesterase, we determined ${}_oJ^{Cl}_{sm}$ and $I_{sc}$ in the presence of a higher concentration of cAMP alone (7.5 mM; 2 experiments) and also in the presence of dibutyryl cAMP (0.25 mM; 3 experiments). The changes observed (data not shown) were similar to those described in Table 1. In the remainder of this paper, therefore, we attribute to cAMP alone effects produced by adding both cAMP and theophylline.

### *Effect of cAMP on Cl Fluxes in the Presence of Ouabain*

The increase in ${}_oJ^{Cl}_{sm}$ produced by cAMP could represent an increase in a passive flux or stimulation of active secretion. Although cAMP did not produce net Cl secretion, it could nonetheless have stimulated an active secretion which was more than offset by a normally present active absorption. In order to examine this possibility, we determined the effect of cAMP on Na and Cl fluxes in tissues treated with ouabain (0.5 mM), which inhibits active Cl absorption in flounder intestine [7]. In ouabain-treated tissues, cAMP did not alter PD (which remained at zero) nor stimulate a net flux of Cl (Table 2). Cyclic AMP did, however, increase ${}_oJ^{Cl}_{sm}$ in ouabain-treated as well as in control tissues (Table 3). Ouabain itself caused a marked increase in ${}_oJ^{Cl}_{sm}$, the reason for which is uncertain[2], but the combination of ouabain and cAMP produced a significantly greater increase in ${}_oJ^{Cl}_{sm}$ than did either agent alone. Thus cAMP can increase ${}_oJ^{Cl}_{sm}$ even when all active Na and Cl transport has been abolished, implying an effect of the nucleotide on passive Cl permeability. Cyclic AMP also increased $G_t$ in ouabain-treated tissues (Table 3), as it does in normal tissues (Table 1).

### *Variation of $J^{Cl}_{sm}$ with PD in cAMP-Treated and Control Tissues*

In principle the cAMP-induced increase in ${}_oJ^{Cl}_{sm}$ could have resulted from an increase in the Cl permeability of an epithelial cell membrane (brush border or basolateral) or from an increase in the Cl conductance of the paracellular pathway. In order to distinguish between these two possibilities, we examined $J^{Cl}_{sm}$ as a function of imposed transmural voltage. A PD-insensitive component of $J^{Cl}_{sm}$ would almost certainly be transcellular in origin.

Each tissue was clamped at 0 mV (short-circuited) and also at either +10 or −15 mV (mucosal reference), the clamping order being randomized. To re-

[2] A large, nonspecific increase in passive permeability is unlikely since unidirectional Na fluxes were not increased. Elimination of intracellular electronegativity would increase transcellular diffusion of $^{36}Cl$ (*see* Eq. (8) in our preceding paper [7]), but this alone is unlikely to account for the full increase in ${}_oJ^{Cl}_{sm}$ since, under normal conditions, transcellular Cl diffusion appears to be small [7].

**Table 3.** Effect of cAMP on ${}_oJ^{Cl}_{sm}$ and $G_t$ in the presence and absence of ouabain

| | −Ouabain | | | +Ouabain | | |
|---|---|---|---|---|---|---|
| | *n* | ${}_oJ^{Cl}_{sm}$ | $G_t$ | *n* | ${}_oJ^{Cl}_{sm}$ | $G_t$ |
| −cAMP | 8 | 4.7±0.55 | 27.4±1.66 | 9 | 11.6±0.68 | 23.1±1.46 |
| +cAMP | 6 | 10.3±0.62 | 32.4±1.92 | 9 | 14.6±1.06 | 27.3±1.88 |
| *P* | 5 | <0.005 | NS | 8 | <0.02 | <0.05 |

Values are means ±SEM for *n* experiments in high $HCO_3$-Ringer. Units for ${}_oJ^{Cl}_{sm}$ and $G_t$ are μEq/hr cm² and mmhos/cm², respectively. Probabilities (*P*) are for paired differences in *n* experiments, unpaired experiments having been excluded. Ouabain (0.5 μmol/ml) and/or cAMP (2 μmol/ml), together with theophylline (5 μmol/ml), were added to the serosal reservoir shortly after mounting tissues. Radioisotope ($^{36}Cl$) was added about 30 min later and flux measurements were begun 30 min after that. Although cAMP appeared to increase ${}_oJ^{Cl}_{sm}$ more in the absence than in the presence of ouabain, this difference was not statistically significant.

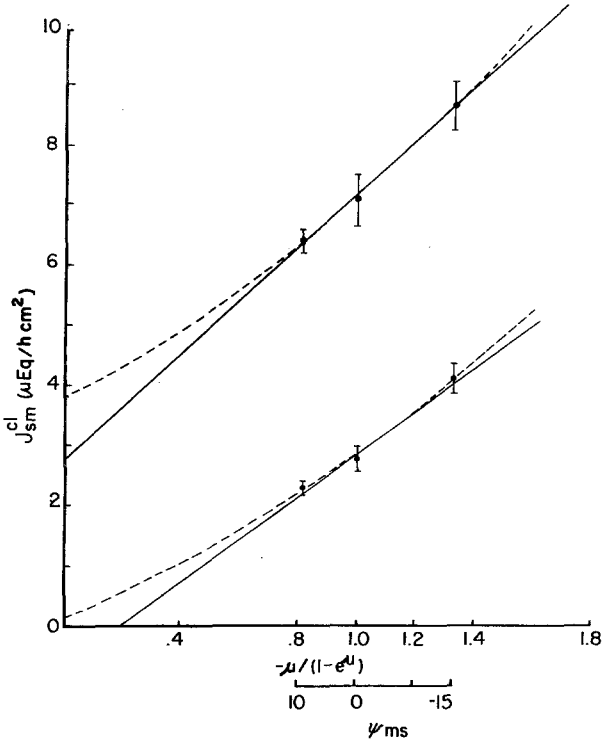


**Fig. 1.** Variation of $J^{Cl}_{sm}$ with PD in cAMP-treated (upper line and curve) and control intestine. $\mu=(F/RT)\times\Psi_{ms}$. Results are means $\pm 1$ SE. Number of experiments at each PD for cAMP-treated (listed first) and control tissues (listed second) were as follows : PD $=\emptyset$ (29, 27); $+10$ mV (14, 13); and $-15$ mV (15, 14). Each solid, least squares regression line predicts the relation between $X$ and $Y$ axes for diffusion across a single membrane. The dashed lines are those predicted for diffusion across two membranes in series. *See* text for further details

duce the effect of tissue variability, fluxes measured at $+10$ and $-15$ mV were normalized against fluxes measured in the same tissues at 0 mV, as previously described [7]. In Fig. 1, $J^{Cl}_{sm}$ is plotted against $-u/(1-e^{u})$, where $u=(F/RT)\times\Psi ms$. Data are shown for both cAMP-treated and control tissues.[3] Simple ionic diffusion across a single homogenous membrane would vary linearly with the above function [17] and a positive $y$-intercept would suggest a PD-insensitive component of the total flux. The least squares regression line for these points provides a close fit of the data and intercepts the $y$ axis at 2.74 μeq/hr cm², which is significantly greater than zero ($P<01$).[4] The calculated diffusional flux at 0 mV is 4.42. In contrast, the $y$-intercept and slope of the regression line for control tissues are $-0.70$ and 3.54, respectively. Comparison suggests that cAMP induced a PD-insensitive Cl flux without significantly altering the preexisting diffusional flux.

For reasons previously presented [7], diffusion across the paracellular pathway is better described by an equation for diffusion across two membranes in series than by one for diffusion across a single membrane. Equation (8) from our prior paper [7] is the basis for the curvilinear relation in Fig. 1, which was determined as described in the appendix to that paper. When calculated in this way, a $y$-intercept of 3.76 and a diffusional flux at 0 mV of 3.38 are obtained for cAMP-treated tissues. The corresponding values for control tissues are 0.14 and 2.73.

Large clamping currents ($<320$ μA/cm²) have been shown to alter tissue conductance in gallbladder due to electro-osmotically-induced structural changes in the paracellular pathway [3]. Since such alterations, if they also developed in flounder intestine, would influence the voltage-flux relationship, we compared the conductances of the cAMP-treated tissues measured at each clamping voltage. The currents passed at $+10$, $\emptyset$, and $-15$ mV were $252\pm11$, $25\pm3$, and $-324\pm10$ μA/cm², respectively; conductances at these three PDs were $23.2\pm0.82$, $23.0\pm0.57$, and $23.9\pm0.75$ mmhos/cm², respectively. Although the latter two conductances differ significantly ($P<0.005$), a 4% change is too small to detectably alter $J^{Cl}_{sm}$. We previously reported similar results for the control tissues [7].

## Discussion

### *Effects of cAMP on Net Na and Cl Fluxes*

Determination of initial rates of Na and Cl uptake across the brush border of flounder intestine indicate the presence there of an obligatory cotransport of NaCl with approximately 1:1 stoichiometry [10]. Similar observations have been made with rabbit ileum [15] and gallbladder [8]. Nonetheless, flounder intestine exhibits a serosa-negative PD and, under short-circuit condition, transports almost three times as much Cl as Na. To explain this paradox we proposed that an electrically-neutral transcellular NaCl transport process is transformed into an electrogenic transepithelial process by the permselective and resistive properties of the paracellular pathway, i.e., the open-circuit PD can be attributed largely to salt diffusion across a cation-selective "tight" junction and, under short-circuit condition, most of the Na transported into the lateral space from the cells recycles back to the mucosal solution.

[3] The voltage-flux data for controls are those presented in our preceding paper [7].

[4] The significance of the Y-intercept was determined by testing the null hypothesis that the regression line passes through $Y=0$ [19].

Addition of cAMP reduced the transepithelial PD, $I_{sc}$, ${}_oJ^{Cl}_{net}$ and, to a lesser extent, ${}_oJ^{Na}_{net}$ (although the last change was not in itself statistically significant, its validity is supported by the related observation that the change of ${}_oJ^{Cl}_{net}$ significantly exceeded the change in $I_{sc}$). These changes are consistent with an inhibitory effect of cAMP on NaCl cotransport across the brush border, an effect of cAMP which has already been demonstrated to occur in rabbit ileum [15] and gallbladder [8]. In flounder intestine, direct measurements of Na and Cl influxes have demonstrated inhibition of coupled influx by furosemide, which also inhibits $I_{sc}$ and ${}_oJ^{Cl}_{net}$ [10]. Cyclic AMP did not significantly reduce total Cl influx, but it reduced by two-thirds the furosemide-inhibitable portion, which is synonymous with the Na-coupled portion. Thus, in flounder intestine, as in rabbit ileum and gallbladder, cAMP appears to inhibit NaCl cotransport across the brush border. Cyclic AMP may also increase the Na-independent Cl permeability of the brush border membrane, although more influx measurements are needed to be confident of this. This particular permeability change would also be consistent with the effect of cAMP on ${}_oJ^{Cl}_{sm}$.

It is of interest that cAMP diminished NaCl absorption in flounder intestine but did not stimulate its secretion. The same result has been obtained with rabbit gallbladder [8], and the responses of both these tissues to cAMP contrast to those of rabbit ileum and colon, in both of which net secretion develops [6, 9]. Flounder intestine [7] and rabbit gallbladder [11] are devoid of crypts, whereas, in mammalian small intestine and colon, crypts are prominent and they contain cells which differ morphologically from the remaining epithelial cells [21]. These correlations suggest that active secretion in mammalian intestine originates in crypts. This conclusion is supported by the observation that selective destruction of villus cells by osmotic shock does not appreciably diminish cholera toxin-induced secretion [16] and also by the observation that cholera toxin-induced net secretion does not develop unless the toxin has been left in the lumen long enough to increase adenylate cyclase activity in crypt cells [4]. Briefer exposure to toxin, although it increases villus cell cyclase and abolishes net absorption, does not produce net secretion. It will be interesting to relate the presence or absence of active secretion with morphological features in intestinal preparations from other animal species.

### *Effect of cAMP on Passive Cl Permeability*

Two features of the present data, namely the cAMP-induced increases in ${}_oJ^{Cl}_{sm}$ and $G_t$, require explanation. Since these effects were observed even when ${}_oJ^{Cl}_{net}$ was reduced to zero by ouabain, they cannot have been due to stimulation of active Cl secretion. Furthermore, since ${}_oJ^{Na}_{sm}$ was not affected and since the increase in $J^{Cl}_{sm}$ was PD-insensitive (*see* Fig. 1), these effects of cAMP cannot be attributed to a change in either the overall conductance or the permselectivity of the paracellular pathway. Cyclic AMP appears therefore to increase the Cl permeability of the transcellular pathway. The extent to which changes in luminal and contraluminal membrane permeabilities contribute to the overall transcellular permeability change remains to be evaluated. Although it may seem paradoxical that an increase in cellular permeability to Cl can give rise to both a PD-insensitive unidirectional Cl flux and an increase in $G_t$, these observations are not inconsistent. Consider, for example, the following related possibilities: (i) cAMP increases the Cl-conductance of the mucosal (brush border) membrane; (ii) as a result, the electrical resistance of the mucosal membrane becomes much less than that of the serosal (basolateral) membrane so that imposed changes in transepithelial voltage do not significantly alter the PD across the mucosal membrane; and (iii) serosal Cl permeability is largely nonconductive, i.e., due to anion exchange or obligatory coupling to a cation flux. In these circumstances, the cAMP-induced increase in unidirectional transcellular Cl flux would be PD-insensitive and yet associated with an increase in transcellular conductance. Whether or not or to what extent these possibilities are realistic is at present unknown, but *a priori* they constitute one possible resolution of the above paradox.

### *Estimation of the cAMP-Induced Transcellular Component of $J^{Cl}_{sm}$*

If cellular and paracellular routes of transepithelial Cl permeation were to constitute two parallel and independent pathways, then the cAMP-induced transcellular portion of $J^{Cl}_{sm}$ would be approximated by the $y$-intercept of the curve shown in Fig. 1, i.e., 3.8 μeq/hr cm$^2$. To explain our prior observations on ion transport across flounder intestine, we modeled this epithelium as shown in Fig. 2. We treated the lateral space ($L$) as a compartment with a long cell border and with luminal and serosal limiting membranes (the latter membrane could more realistically be viewed as an integrated resistance over the lateral space). We also assumed that the cell communicates with the serosal bathing medium exclusively by way of the lateral space, i.e., across the serosal border of $L$. Since more than 98 % of the flounder intestinal

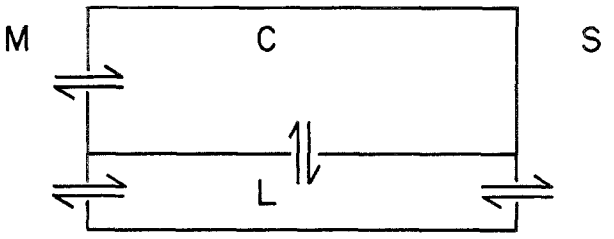


**Fig. 2.** Pathways for transepithelial ion transport in flounder intestine. $M$ and $S$ = mucosal and serosal bathing solutions, $C$ = cell and $L$ = lateral intercellular space. The lateral space is treated as a tissue compartment

epithelial cell's contraluminal surface borders on $L$ [7], this assumption seems reasonable. Use of this model enabled us to reconcile NaCl cotransport by the cells with the predominance of ${}_oJ^{Cl}_{net}$ over ${}_oJ^{Na}_{net}$ for the epithelium as whole, i.e., under short-circuit condition most of the Na pumped from the cell ($c$) to $L$ recycles to the luminal bathing medium across the "tight" junction. This can only happen, of course, if the Na permeability of the mucosal border of $L$ exceeds that of the serosal border of $L$. The curves shown in Fig. 1 for both cAMP and control conditions are those obtained with this compartmental analysis, the assumption being made that the Cl permeability of the lateral cell border is negligible relative to that of the paracellular pathway itself (*see* reference [7] for details).

In light of the voltage-insensitive nature of the cAMP-induced increment in $J^{Cl}_{sm}$, however, the assumption that the cell is Cl impermeable and therefore that cellular and paracellular pathways for Cl diffusion are independent is no longer tenable. We therefore need a more general equation for $J^{Cl}_{sm}$ which takes into account a transcellular Cl flux from $L$ to $m$. It can readily be shown that, in the steady state,

$$J_{sm} = \frac{J_{sL}(J_{Lcm} + J_{Lm})}{(J_{Lcm} + J_{Lm} + J_{Ls})} \tag{1}$$

where $J_{Lcm}$ is the transcellular $L-m$ flux and is equal to $J_{Lc}J_{cm}/(J_{cm}+J_{cL})$. We assume that $J_{Lcm}$ is not altered by voltage clamping and that cAMP does not alter the Cl permeabilities of the $m$ and $s$ borders of $L$. By applying the constant-field equation to unidirectional fluxes (setting trans-concentrations to zero)[5] and using the steady-state condition that the sums of fluxes into and out of $L$ must be equal, it can be shown that

$$J^{Cl}_{sm} = \frac{W^{Cl}_s J^{Cl}_{Lcm} + J^{Cl}_{spm}(J^{Cl}_c + W^{Cl}_m + W^{Cl}_s)}{J^{Cl}_{Lcm} + J^{Cl}_c + W^{Cl}_m + W^{Cl}_s} \tag{2}$$

[5] The relevant equations are given in reference 7 and are equivalent to those derived in a different way in reference 17.

where

$$W^{Cl}_j = C_o P^{Cl}_j \mu_j/(1-e^{-\mu_j}),$$

$$\mu = (F/RT)(\Psi_L - \Psi_j) \quad \text{with } \Psi_m = \emptyset,$$

$p^{Cl}_j$ = Cl permeability of $m$ or $s$ boundries of $L$, $C_0 = 150$ mM, $J^{Cl}_c$ is the net Cl flux from $C$ to $L$, and $J^{Cl}_{spm}$ is the paracellular component of $J^{Cl}_{sm}$, i.e., $J^{Cl}_{sm}$ for the condition $J^{Cl}_{Lcm} = \emptyset$.

Since, under short-circuit condition, $\mu_j/(1-e^{-\mu_j}) \cong 1$ (at least for cAMP-treated tissues), Eq. (2) can be rearranged and simplified:

$$J^{Cl}_{Lcm} \cong \frac{({}_oJ^{Cl}_{sm} - {}_oJ^{Cl}_{spm})(J_c + C_0[p^{Cl}_m + p^{Cl}_s])}{(C_0 P^{Cl}_s - {}_oJ^{Cl}_{sm})}. \tag{3}$$

Since $({}_oJ^{Cl}_{sm} - {}_oJ^{Cl}_{spm})$ is the $Y$-intercept of the upper curve shown in Fig. 1, it is obvious that $J^{Cl}_{Lcm}$ exceeds the $Y$-intercept since $(J_c + CoP^{Cl}_s)/(CoP^{Cl}_s - {}_oJ^{Cl}_{sm})$ must be greater than 1.0. Reasonable estimates for $J_c$, $P^{Cl}_m$ and $P^{Cl}_s$ yield a ratio greater than 2.0, suggesting that $J^{Cl}_{Lcm}$ is more than twice the $Y$-intercept[6]. Thus, following addition of cAMP, transcellular Cl permeability is considerably higher than paracellular Cl permeability. Some of this transcellular flux could represent exchange diffusion.

Equation (2) can also be used to generate a curve relating $J^{Cl}_{sm}$ to $-\mu(1-e^{\mu})$. This curve closely approximates the measured fluxes at $+10$ and $-15$ mV; its slope would be increased by a voltage-dependent $J^{Cl}_{Lcm}$, providing a less satisfactory fit of the data.

### *Relation to Effects of cAMP on Other Epithelia*

Inhibition by cAMP of NaCl cotransport across the luminal border of gallbladder [10] and intestine [15] has been cited above. Cyclic AMP has also been shown to enhance cell membrane permeabilities to water and small aqueous solutes in toad bladder, frog skin, and renal collecting tubule [20]. Of particular relevance to the present study, Mandel [14] has shown that ADH and theophylline increase transcellular Cl permeability in frog skin and that this effect is independent of active Na transport; i.e., it persists in the presence of ouabain. Whether this effect of cAMP is exerted on the outer, inner, or both border(s) of the frog skin epithelial cell is not known.

Electrophysiologic studies of rabbit corneal epi-

[6] Assume $J^{Cl}_c = 1.9$, which is 6.4, the value calculated in our prior study [7], multiplied by the ratio of $I_{sc}$ for cAMP-treated tissues in the present study and $I_{sc}$ for control tissues in the prior study; assume also that $P^{Cl}_m = 0.0216$ and $P^{Cl}_s = 0.135$, the permeabilities previously obtained for control tissues [7] reduced by 10% since ${}_oJ^{Cl}_{sm}$ for controls in Fig. 1 is 10% lower than the previously reported value. The calculated value for $J^{Cl}_{Lcm}$ is then 8.6 $\mu$eq/hr cm$^2$.

thelium, which actively secretes Cl when stimulated by epinephrine, indicate that addition of epinephrine decreases the resistance of the outer (tear side) cell membrane and that this resistance change does not develop when Cl is replaced with $SO_4$ [12]. Presumably this effect of epinephrine is mediated by cAMP. Thus, in this and probably other secretory epithelia, cAMP appears to increase apical membrane Cl conductance. Measurements of cell Cl and cell membrane potentials indicate that Cl efflux across the outer corneal membrane is downhill [12]. By increasing the Cl conductance of this membrane, therefore, cAMP increases Cl secretion. The relationship on the molecular level between these two effects of cAMP, i.e., inhibition of NaCl cotransport across the apical membrane of certain absorptive cells and enhancement of apical Cl conductance of certain secretory and possibly also absorptive cells, is as yet poorly understood. Cyclic AMP is not the only intracellular mediator for such effects since Ca appears to decrease apical membrane resistance and stimulate secretion in insect salivary gland [2] and to decrease NaCl co-transport across the brush border of rabbit ileum (P.L. Smith and M. Field, *unpublished*).

## Addendum

Recently MacKay and Lahlou [13] reported effects of theophylline on ion transport across the intestine of the European flounder, *Platichthys Flesus*. Their observations were similar to our own: $J_{net}^{Cl}$ and $I_{sc}$ decreased and $J_{sm}^{Cl}$ increased.

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