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# BICARBONATE TRANSPORT SYSTEMS IN THE INTESTINE OF THE SEAWATER EEL

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## Summary

Utilizing a pH-stat method, the rates of mucosal and serosal alkalinization were measured separately in the seawater eel intestine. These two rates were dependent on contralateral $HCO_3^-$ concentration and were inhibited by contralateral application of DIDS, an inhibitor of $HCO_3^-$ transport, indicating that the mucosal and serosal alkalinization are due to $HCO_3^-$ secretion and absorption, respectively. The mucosal alkalinization was enhanced after inhibiting $Na^+/K^+/Cl^-$ cotransport by treatment with bumetanide, furosemide or $Ba^{2+}$, with a latent period of more than 10 min, suggesting that $HCO_3^-$ absorption from mucosa to serosa depends on $Na^+/K^+/Cl^-$ cotransport. The serosal alkalinization caused by $HCO_3^-$ absorption was completely abolished after mucosal application of bumetanide. After pretreatment with bumetanide, mucosal omission of $Cl^-$ halved the enhanced rate of mucosal alkalinization, and $Na^+$ omission had no effect on it; this indicates that the exit of $HCO_3^-$ into the lumen depends on luminal $Cl^-$, i.e. on the existence of the usual $Cl^-/HCO_3^-$ exchange on the brush-border membrane. When serosal $Na^+$ was removed under the same conditions, mucosal alkalinization was reduced, indicating that $HCO_3^-$ entry from the serosal fluid depends on $Na^+$. Serosal omission of $Cl^-$ did not reduce mucosal alkalinization. In addition, serosal alkalinization was enhanced by serosal removal of $Na^+$ but not of $Cl^-$. These results suggest that there is a $Na^+/HCO_3^-$ cotransport on the basolateral membrane. A possible model for $HCO_3^-$ transport systems in the seawater eel intestine is proposed, and a possible role for these transport systems is discussed in relation to $Na^+$, $Cl^-$ and water transport.

## Introduction

In the preceding paper (Ando, 1990), it was proposed that $HCO_3^-$ transport systems may contribute to a homeostasis in the intracellular $H^+$ concentration (pHi), which will control $Na^+/K^+/Cl^-$ cotransport *via* pHi-sensitive $K^+$ channels on the brush-border membrane of the epithelium in the intestine of the seawater eel. The present study aimed to elucidate how $HCO_3^-$ is transported across

Key words: $HCO_3^-$, $Cl^-/HCO_3^-$ exchange, $Na^+/HCO_3^-$ cotransport, $Na^+/K^+/Cl^-$ cotransport, pH-stat, eel intestine.

the intestinal epithelium. However, the $HCO_3^-$ flux cannot be detected directly by using radioisotopes, because labels on $HCO_3^-$ are promptly dispersed into $CO_2$ and $H_2O$. Therefore, in the present study, the $HCO_3^-$ transport rate was estimated from the rate of alkalinization of the bathing fluid.

$HCO_3^-$ transport in the fish intestine has been little studied. So far as we know, the only study is that of Dixon and Loretz (1986), who observed $HCO_3^-$ secretion in the goby intestine using a pH-stat method. However, they clamped the pH manually, and therefore they were not able to analyse precisely the time course of $HCO_3^-$ secretion. Using an automatic pH-stat, we analysed more precisely the time course of $HCO_3^-$ secretion as well as $HCO_3^-$ absorption, and examined the effects of $Na^+$, $Cl^-$, 4,4′-diisothiocyanostilbene-2,2′-disulphonic acid (DIDS) and bumetanide on $HCO_3^-$ transport. The results obtained indicate that some $HCO_3^-$ absorption is linked with the $Na^+/K^+/Cl^-$ cotransport system, and that at least two kinds of $HCO_3^-$ transport system exist in the seawater eel intestine.

## Materials and methods

Japanese cultured eels *Anguilla japonica*, weighing 200–240 g, were kept in seawater aquaria (20 °C) for more than 1 week before use. After decapitation, the intestine was removed and stripped of its serosal muscle layers. The stripped intestine was opened by cutting longitudinally and mounted as a flat sheet in an Ussing–Rehm chamber with an exposed area of 0.785 $cm^2$. One side of the intestine was bathed with normal $HCO_3^-$ Ringer's solution (6.5 ml), and the other side was bathed with an unbuffered Ringer's solution (5.0 ml). Both solutions were kept at 20 °C and circulated continuously; they were gassed with a 95 % $O_2$/5 % $CO_2$ gas mixture or 100 % $O_2$.

Table 1 shows the composition of the Ringer's solutions used in this experiment. Solution A is the normal $HCO_3^-$ Ringer's solution. In $Na^+$-free Ringer's solution (solution B), all $Na^+$ was replaced with choline$^+$. $Cl^-$-free Ringer's solution (solution C) was made by replacing NaCl, KCl and $CaCl_2$ with sodium gluconate, $KNO_3$ and $Ca(NO_3)_2$, respectively. These $HCO_3^-$-buffered Ringer's solutions were bubbled with a 95 % $O_2$/5 % $CO_2$ gas mixture (pH 7.4). Solution D is phosphate-buffered Ringer's solution, gassed with 100 % $O_2$ (pH 7.4). Solution E is the standard unbuffered Ringer's solution, in which $HCO_3^-$ is replaced with gluconate and acetate, and $MgCl_2$ is substituted for $MgSO_4$. In low-$Na^+$ unbuffered Ringer's solution (solution F), $Na^+$ was replaced with choline$^+$, and this solution was used within 1 week. $Cl^-$-free unbuffered Ringer's solution (solution G) was made by replacing NaCl, KCl, $CaCl_2$ and $MgCl_2$ with sodium gluconate, $KNO_3$, $Ca(NO_3)_2$ and magnesium acetate, respectively. These unbuffered solutions were gassed with 100 % $O_2$ and the pH was clamped at 7.4 using a pH-stat (TOA, HSM-10A).

The rate of alkalinization ($J^{OH}$) was calculated from the amount of 20 mmol $l^{-1}$ HCl titrated automatically to clamp the unbuffered fluid pH at 7.4 using the pH-stat. The amount of HCl titrated was recorded automatically (TOA, EPR-

Table 1. *Composition of experimental solutions (mmol $l^{-1}$)*

| | A $HCO_3^-$ | B $Na^+$-free | C $Cl^-$-free | D Phosphate | E Unbuffered | F Low-$Na^+$ | G $Cl^-$-free |
|---|---|---|---|---|---|---|---|
| NaCl | 118.5 | | | 137.4 | 118.5 | | |
| Choline chloride | | 118.5 | | | | 118.5 | |
| Sodium gluconate | | | 118.5 | | 24.3 | 24.3 | 142.8 |
| KCl | 4.7 | 4.7 | | 4.7 | 2.3 | 2.3 | |
| $KNO_3$ | | | 4.7 | | | | 4.7 |
| Potassium acetate | | | | | 3.6 | 3.6 | 3.6 |
| $CaCl_2$ | 3.0 | 3.0 | | 3.0 | 3.0 | 3.6 | |
| $Ca(NO_3)_2$ | | | 3.0 | | | | 3.0 |
| $KH_2PO_4$ | 1.2 | 1.2 | 1.2 | 0.6 | | | |
| $MgSO_4$ | 1.2 | 1.2 | 1.2 | 1.2 | | | |
| $MgCl_2$ | | | | | 1.2 | 1.2 | |
| Magnesium acetate | | | | | | | 1.2 |
| $NaHCO_3$ | 24.9 | | 24.9 | | | | |
| Choline bicarbonate | | 24.9 | | | | | |
| $Na_2HPO_4$ | | | | 2.5 | | | |
| D-Glucose | 5.0 | 5.0 | 5.0 | 5.0 | 5.0 | 5.0 | 5.0 |
| L-Alanine | 5.0 | 5.0 | 5.0 | 5.0 | 5.0 | 5.0 | 5.0 |

121A) and the pH in the unbuffered medium was monitored throughout the experiment with a polyrecorder (TOA, EPR-10A). A similar technique has been used for measuring $H^+$ secretion rate in the eel stomach (Ando *et al.* 1986). The transepithelial potential difference (PD) was recorded with the polyrecorder (TOA, EPR-121A) as the serosal potential with respect to the mucosa through a pair of calomel electrodes (A. H. Thomas Co.). The PD was short-circuited every 10 min for less than 10 s and the tissue resistance ($R_t$) was calculated from the ratio of the PD to the short-circuit current ($I_{sc}$). Under short-circuit conditions, current flow from mucosa to serosa is reported as a positive $I_{sc}$. The fluid resistance was 18.8 $\Omega cm^2$ and this factor was also used to correct each $I_{sc}$ and $R_t$ value as usual.

After these four variables had reached steady levels under the standard condition, 4-4′-diisothiocyanostilbene-2-2′-disulphonic acid (DIDS, Sigma), acetazolamide (Sigma) or bumetanide (a gift from Sankyo Co., Tokyo) was added to either the serosal or the mucosal fluid.

## Results

### *Mucosal and serosal alkalinization are due to $HCO_3^-$ transport*

When the mucosa was bathed with standard unbuffered Ringer's solution (solution E), while the serosa was bathed with normal $HCO_3^-$ Ringer's solution (solution A), the mucosal fluid was alkalinized at a constant rate (Fig. 1). The serosa-negative PD and $I_{sc}$ were maintained under these conditions. After replacement of the $HCO_3^-$-buffered solution with phosphate-buffered solution,

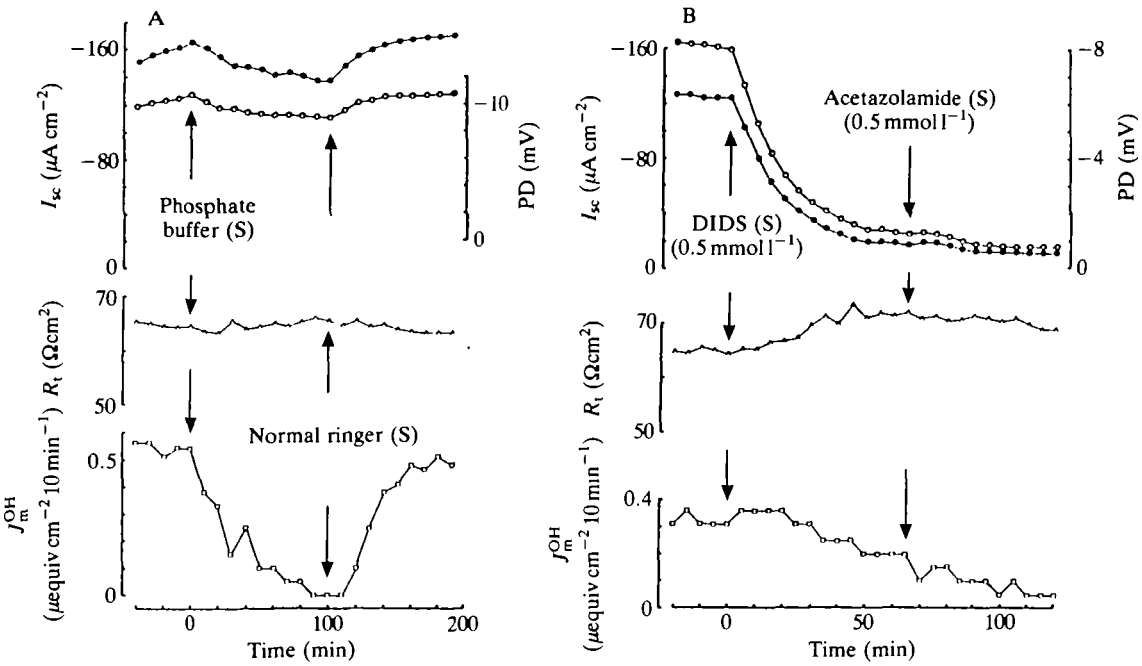


Fig. 1. Effects of serosal $HCO_3^-$ and DIDS on the rate of mucosal alkalinization ($J_m^{OH}$, □), the transepithelial potential (PD, ○), the short-circuit current ($I_{sc}$, ●) and the tissue resistance ($R_t$, △). (A) After bathing the mucosa and the serosa of the intestine with the standard unbuffered solution (solution E) and normal $HCO_3^-$ Ringer's solution (solution A), respectively, the serosal fluid was replaced with phosphate-buffered Ringer's solution (solution D) at time zero. At the second arrows, $HCO_3^-$ was reintroduced to the serosal fluid. S in parentheses denotes that the serosal fluid is replaced. (B) After a steady state had been reached, 0.5 mmol $l^{-1}$ DIDS was added to the serosal fluid (first arrows). At the second arrows, 0.5 mmol $l^{-1}$ acetazolamide was further added to the serosal medium. S in parentheses denotes that each drug is applied to the serosal side of the intestine.

the rate of mucosal alkalinization ($J_m^{OH}$) was reduced to zero, accompanied by a decrease in PD and $I_{sc}$. The tissue resistance ($R_t$) tended to increase.

When DIDS, an inhibitor of $HCO_3^-$ transport, was added to the serosal fluid under the same conditions, $J_m^{OH}$ decreased gradually, accompanied by a decrease in PD and $I_{sc}$ and by an increase in $R_t$ (Fig. 1B). Addition of acetazolamide, an inhibitor of carbonic anhydrase, enhanced the inhibitory effects of DIDS. When DIDS was applied to the mucosal fluid under the same conditions, $J_m^{OH}$ decreased slightly, accompanied by a slight decrease in PD and $I_{sc}$, whereas $R_t$ did not change significantly (data not shown).

Similar experiments were performed after bathing the mucosa and the serosa with normal $HCO_3^-$ Ringer and standard unbuffered Ringer, respectively (Fig. 2). After removal of $HCO_3^-$ from the mucosal fluid, the rate of serosal alkalinization ($J_s^{OH}$) was reduced to zero, accompanied by a decrease in PD and $I_{sc}$

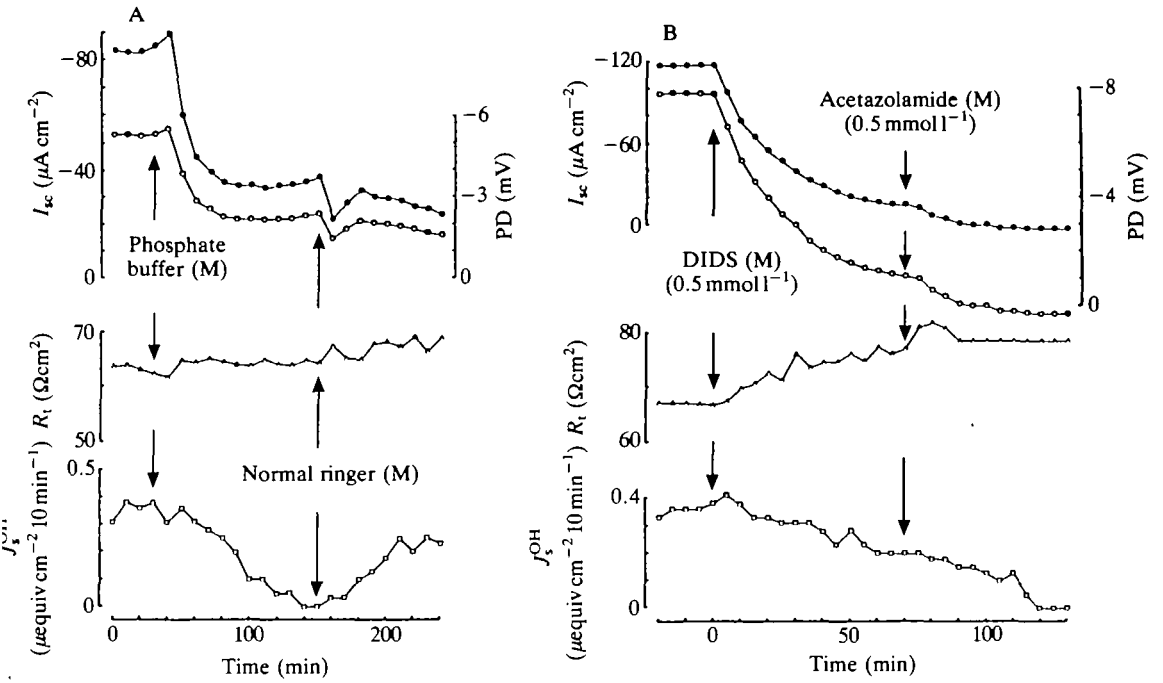


Fig. 2. Effects of mucosal $HCO_3^-$ and DIDS on the rate of serosal alkalinization ($J_s^{OH}$, □), PD (○), $I_{sc}$ (●) and $R_t$ (△). (A) After bathing the mucosa and the serosa with solution A and solution E, respectively, the mucosal fluid was replaced with phosphate-buffered Ringer's solution (solution D) at the first arrows. After 150 min (second arrows), $HCO_3^-$ was reintroduced to the mucosal fluid. M in parentheses denotes that the mucosal fluid is replaced. (B) At time zero, 0.5 mmol $l^{-1}$ DIDS was added to the mucosal fluid (first arrows). At the second arrows, 0.5 mmol $l^{-1}$ acetazolamide was added to the mucosal fluid.

and by an increase in $R_t$ (Fig. 2A). When DIDS was added to the mucosal fluid, $J_s^{OH}$ decreased gradually (Fig. 2B). PD and $I_{sc}$ also decreased after treatment with DIDS, accompanied by an increase in $R_t$. Acetazolamide also enhanced the inhibitory effects of mucosal DIDS on these four parameters. Serosal addition of DIDS inhibited $J_s^{OH}$ slightly, accompanied by a slight decrease in PD and $I_{sc}$ (data not shown).

### *Effects of inhibition of $Na^+/K^+/Cl^-$ cotransport*

To clarify the relationship between $HCO_3^-$ transport and $Na^+/K^+/Cl^-$ cotransport, the following experiments were performed. Whilst bathing the mucosa and the serosa with standard unbuffered Ringer's solution and normal $HCO_3^-$ Ringer's solution, respectively, 1 μmol $l^{-1}$ bumetanide, an inhibitor of $Na^+/K^+/Cl^-$ cotransport, was added to the mucosal fluid (Fig. 3A). After addition of bumetanide, PD and $I_{sc}$ decreased immediately and $R_t$ increased more slowly, indicating that $Na^+/K^+/Cl^-$ cotransport is blocked by this drug and that the luminal $K^+$ channels are blocked secondarily. The mucosal alkalinization

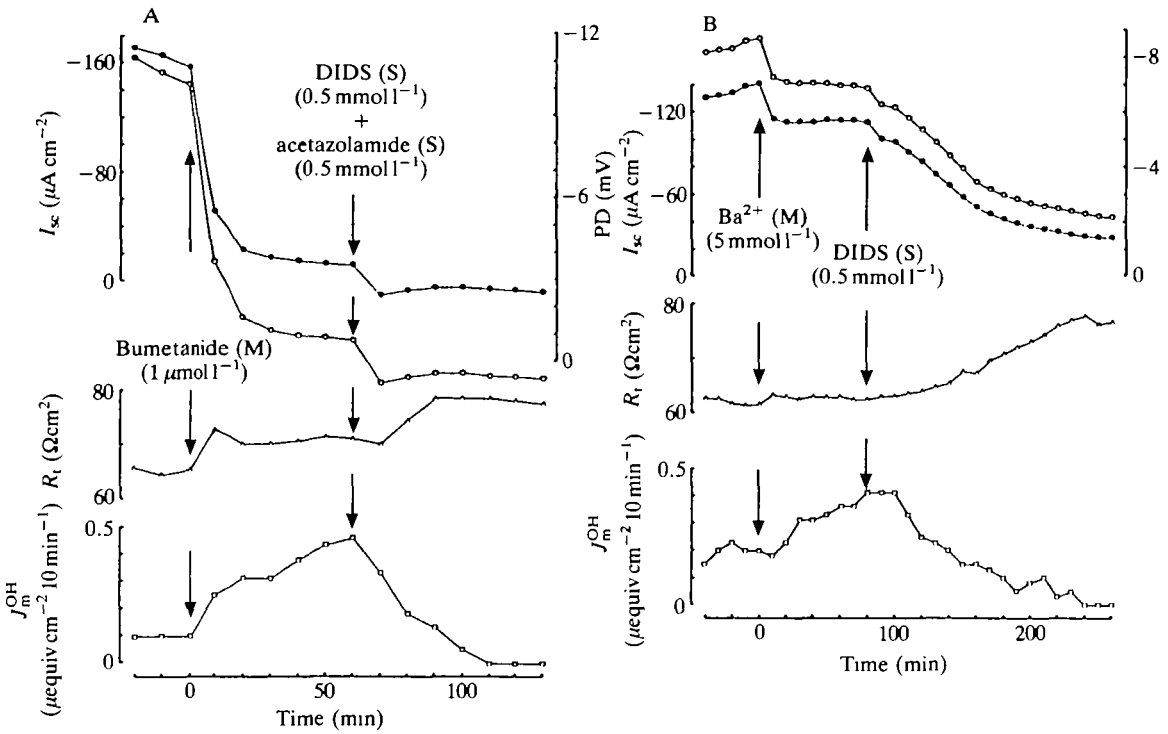


Fig. 3. Effects of bumetanide and $Ba^{2+}$ on mucosal alkalinization ($J_m^{OH}$, □), PD (○), $I_{sc}$ (●) and $R_t$ (△). (A) After bathing the mucosa and the serosa with solution E and solution A, respectively, 1 μmol $l^{-1}$ bumetanide was added to the mucosal fluid (first arrows). At the second arrows, 0.5 mmol $l^{-1}$ DIDS and 0.5 mmol $l^{-1}$ acetazolamide were added to the serosal fluid. (B) At time zero, 5 mmol $l^{-1}$ $BaCl_2$ was added to the mucosal fluid (first arrows). At the second arrows, 0.5 mmol $l^{-1}$ DIDS was added to the serosal fluid.

($J_m^{OH}$) increased gradually after a latent period of 10.0±1.0 min ($N$=14). This enhancement in $J_m^{OH}$ was completely blocked by DIDS and acetazolamide added to the serosal fluid. A similar increase in DIDS-sensitive $J_m^{OH}$ was also observed after application of furosemide (10 μmol $l^{-1}$) to the mucosal fluid. When $Ba^{2+}$, a well-known blocker of $K^+$ channels, was added to the mucosal fluid, the DIDS-sensitive $J_m^{OH}$ was also enhanced with a latent period of 18.8±1.9 min ($N$=5). However, PD and $I_{sc}$ decreased immediately, accompanied by an immediate increase in $R_t$ (Fig. 3B). Since bumetanide, furosemide and $Ba^{2+}$ are known to inhibit $Na^+/K^+/Cl^-$ cotransport, these results suggest that inhibition of the cotransport either stimulates $HCO_3^-$ secretion or inhibits $HCO_3^-$ absorption. The following result supports the latter explanation.

Fig. 4 shows the 'sidedness' of the effects of bumetanide. In this experiment, the serosal $HCO_3^-$ was omitted and bumetanide was added either to the serosal side or to the mucosal side. Although serosal addition of bumetanide had no effects on any of the four parameters (PD, $I_{sc}$, $R_t$ and $J_s^{OH}$), mucosal application abolished

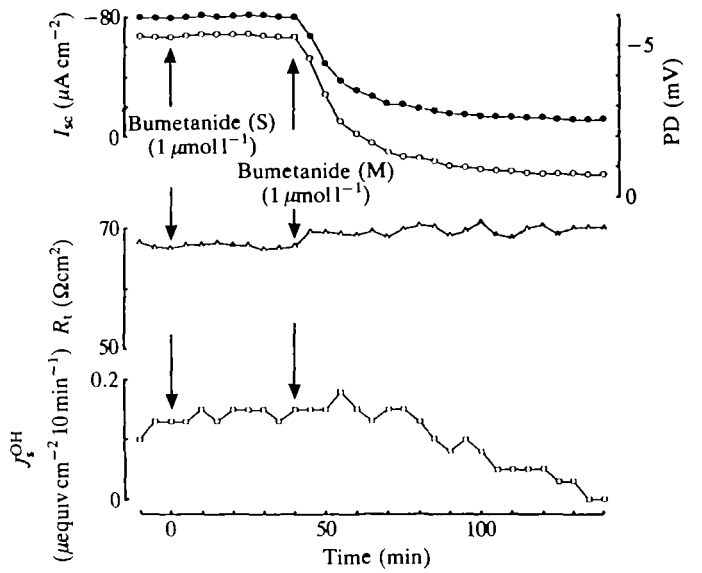


Fig. 4. The 'sidedness' of the effect of bumetanide on serosal alkalinization ($J_s^{OH}$, □), PD (○), $I_{sc}$ (●) and $R_t$ (△). After bathing the mucosa and the serosa with solution A and solution E, respectively, 1 $\mu$mol l$^{-1}$ bumetanide was added to the serosal fluid (first arrows). At the second arrows, 1 $\mu$mol l$^{-1}$ bumetanide was added to the mucosal fluid.

$J_s^{OH}$, reduced PD and $I_{sc}$, and caused an increase in $R_t$. These changes in the electrical parameters were similar to those shown in Fig. 3A.

### *Effects of $Na^+$ and $Cl^-$ on $HCO_3^-$ transport systems*

Since $HCO_3^-$ reabsorption was blocked by mucosal bumetanide, as shown in Figs 3 and 4, the following experiments were designed to clarify the mechanisms of $HCO_3^-$ secretion in the presence of bumetanide. Fig. 5A shows the effects of removal of mucosal $Cl^-$ on mucosal alkalinization ($J_m^{OH}$) after pretreatment with bumetanide. When $Cl^-$ was omitted from the mucosal solution, $J_m^{OH}$ was reduced by 50 %; it recovered after the reintroduction of $Cl^-$ into the mucosal fluid. In the absence of $Cl^-$ in the mucosal fluid, PD and $I_{sc}$ shifted their polarity to become serosa-positive and $R_t$ increased significantly. These three electrical parameters recovered to their original levels after reintroduction of $Cl^-$ into the mucosal fluid.

The effects of mucosal $Na^+$ on mucosal alkalinization were also examined (Fig. 5B). $J_m^{OH}$ was not affected by lowering the mucosal $Na^+$ concentration. When the mucosal $Na^+$ concentration was lowered, the serosa-negative PD and $I_{sc}$ increased dramatically and $R_t$ also increased significantly. These three electrical parameters returned to their original levels after reintroduction of the standard solution into the mucosal fluid.

Under the same conditions, when serosal $Na^+$ was removed, however, $J_m^{OH}$ was gradually reduced by 40 % (Fig. 6A). PD and $I_{sc}$ became more serosa-positive and

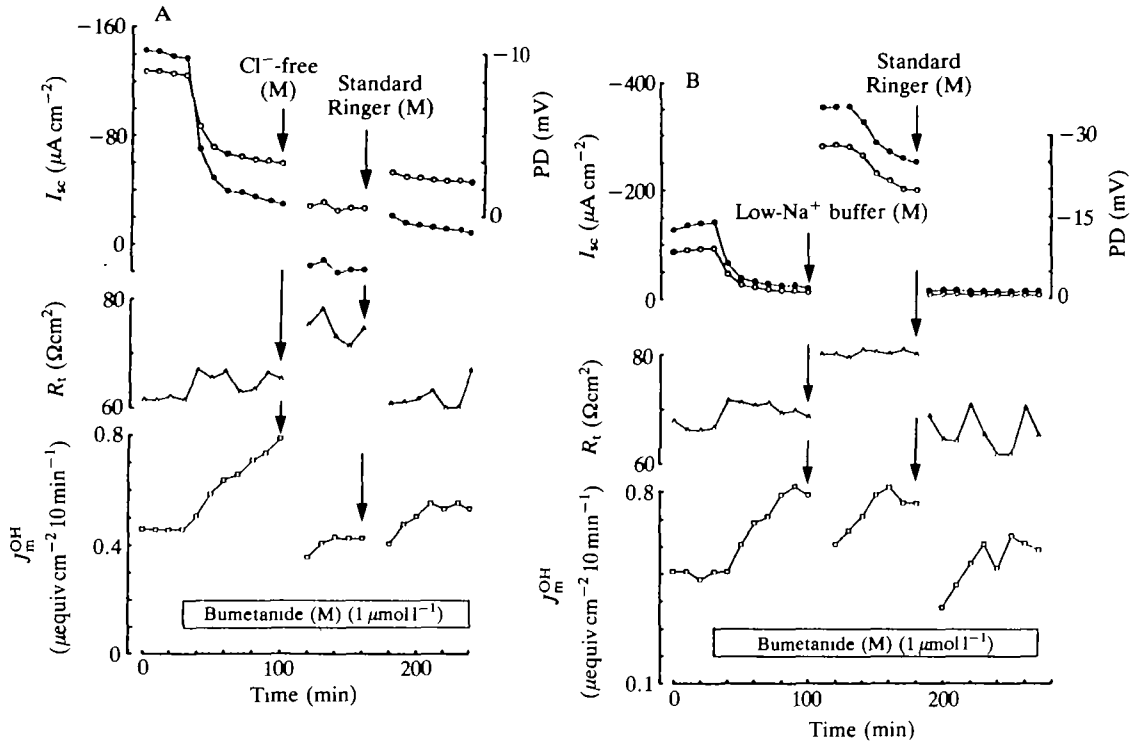


Fig. 5. Effects of mucosal $Cl^-$ and $Na^+$ on the mucosal alkalinization ($J_m^{OH}$, □), PD (○), $I_{sc}$ (●) and $R_t$ (△). (A) After bathing the mucosa and the serosa with solution E and solution A, respectively, 1 μmol $l^{-1}$ bumetanide was applied to the mucosal fluid at 30 min. In the presence of bumetanide, mucosal $Cl^-$ was removed by replacement with solution G (first arrows). At the second arrows, the standard unbuffered Ringer's solution (solution E) was reintroduced to the mucosal side. Discontinuous lines denote that measurements were interrupted for more than 20 min, which is the time required until titration starts, since the pH in the unbuffered fluid is lower than 7.0. (B) After pretreatment with bumetanide (1 μmol $l^{-1}$), the mucosal fluid (solution E) was replaced with low-$Na^+$ Ringer's solution (solution F) at 100 min. At the second arrows, the standard unbuffered Ringer's solution (solution E) was reintroduced to the mucosal side.

$R_t$ increased significantly. After reintroduction of $Na^+$ into the serosal fluid, all these four parameters returned to their original levels.

In contrast, serosal omission of $Cl^-$ did not affect mucosal alkalinization (Fig. 6B). PD and $I_{sc}$ increased gradually and $R_t$ increased dramatically after removal of $Cl^-$ from the serosal fluid. When normal Ringer's solution was reintroduced, these electrical parameters recovered to their original levels.

After bathing the mucosa and the serosa with normal $HCO_3^-$ Ringer's solution and with standard unbuffered Ringer's solution, respectively, the effects of serosal $Na^+$ or $Cl^-$ on serosal alkalinization ($J_m^{OH}$) were examined (Fig. 7). When the serosal $Na^+$ concentration was lowered from 142.8 to 24.3 mmol $l^{-1}$, $J_s^{OH}$

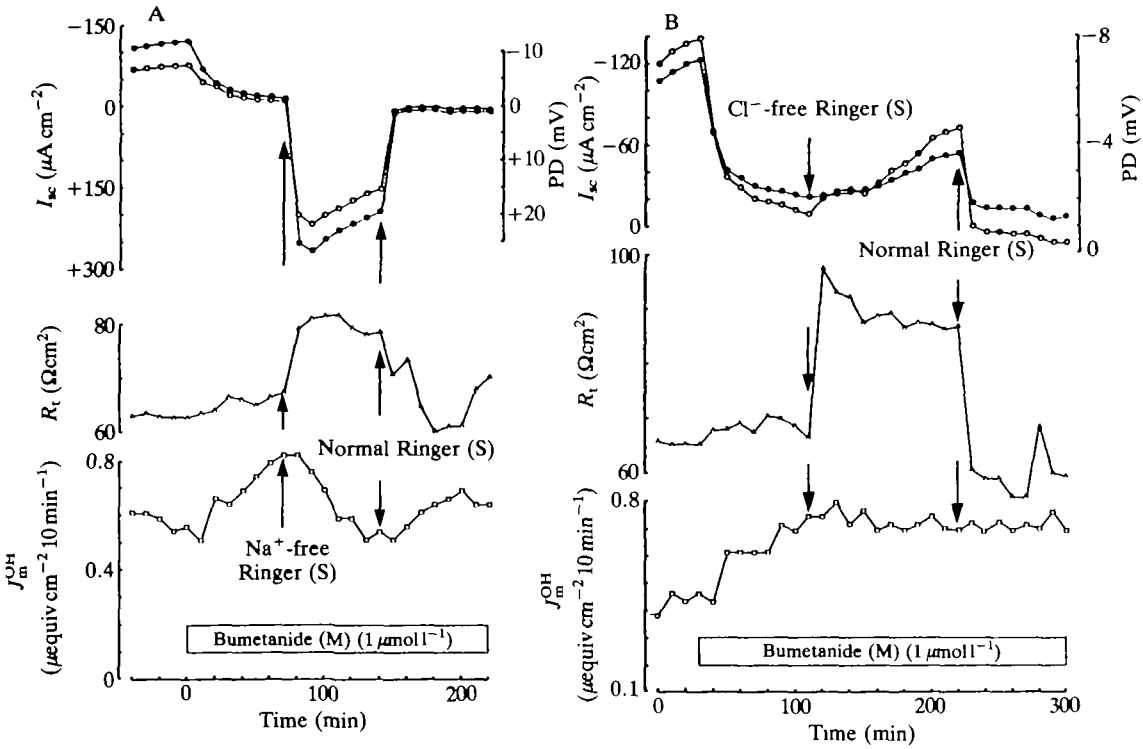


Fig. 6. Effects of serosal $Na^+$ and $Cl^-$ on mucosal alkalinization ($J_m^{OH}$, □), PD (○), $I_{sc}$ (●) and $R_t$ (△). (A) After pretreatment with bumetanide (1 μmol $l^{-1}$), the serosal fluid (solution A) was replaced with $Na^+$-free Ringer's solution (solution B) at the first arrows. After 70 min normal $HCO_3^-$ Ringer's solution (solution A) was reintroduced to the serosal side. (B) After pretreatment with bumetanide (1 μmol $l^{-1}$), the serosal fluid (solution A) was replaced with $Cl^-$-free Ringer's solution (solution C) at the first arrows. After 110 min, solution A was reintroduced to the serosal side.

increased significantly (Fig. 7A). PD and $I_{sc}$ become more serosa-positive and $R_t$ also increased significantly. When the standard solution was reintroduced into the serosal fluid, all these four parameters returned to their initial levels.

In contrast, when serosal $Cl^-$ was omitted, $J_s^{OH}$ was not affected (Fig. 7B). PD and $R_t$ increased significantly but $I_{sc}$ increased only slightly. After reintroduction of the standard solution into the serosal fluid, $R_t$ returned to its original level, but PD and $I_{sc}$ were slightly lower than their original values.

## Discussion

The present study demonstrates that mucosal and serosal alkalinization in the seawater eel intestine are due to $HCO_3^-$ secretion and absorption, respectively, since these two rates of alkalinization depend on contralateral $HCO_3^-$ concentration and are inhibited by contralateral DIDS, an inhibitor of $HCO_3^-$ transport

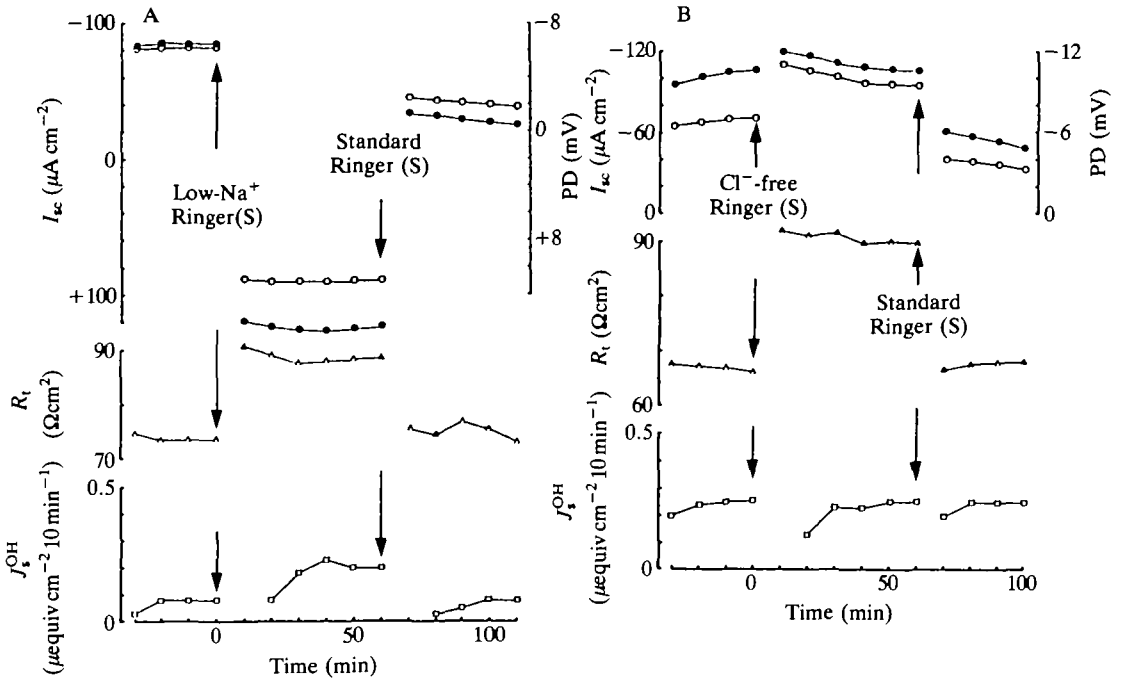


Fig. 7. Effects of serosal $Na^+$ and $Cl^-$ on serosal alkalinization ($J_s^{OH}$, □), PD (○), $I_{sc}$ (●) and $R_t$ (△). (A) After bathing the mucosa and the serosa with solution A and solution E, respectively, the serosal fluid was replaced with low-$Na^+$ Ringer's solution (solution F) at time zero. At the second arrows, standard unbuffered solution (solution E) was reintroduced to the serosal side. (B) After bathing the mucosa and the serosa with solution A and solution E, respectively, the serosal fluid was replaced with $Cl^-$-free Ringer's solution (solution G) at time zero. At the second arrows, solution E was reintroduced to the serosal side.

(Cabantchik and Rothstein, 1972; Marsh and Spring, 1985; Jentsch *et al.* 1988). Acetazolamide, an inhibitor of carbonic anhydrase, enhanced these inhibitory effects of DIDS. When $HCO_3^-$ transport was inhibited in both directions, the serosa-negative PD and $I_{sc}$ decreased and $R_t$ increased simultaneously. These phenomena may be explained by an inhibition of luminal $K^+$ channels, since the serosa-negative PD is mostly due to $K^+$ leakage from the cell to the lumen in the seawater eel intestine (Ando and Utida, 1986).

Mucosal alkalinization was enhanced by the addition of bumetanide to the mucosal fluid. Since mucosal bumetanide blocks $HCO_3^-$ absorption from mucosa to serosa (Fig. 4), this enhanced $J_m^{OH}$ seems to be due to the inhibition of $HCO_3^-$ reuptake from the luminal fluid. Similar enhancement in $J_m^{OH}$ was also observed after the addition of furosemide or $Ba^{2+}$ to the mucosal fluid. Since these three drugs are known inhibitors of the $Na^+/K^+/Cl^-$ cotransport system, these results suggest that the $HCO_3^-$ reuptake processes are closely linked with $Na^+/K^+/Cl^-$ cotransport. However, it is unlikely that the cotransport itself carries $HCO_3^-$,

because the inhibition of $HCO_3^-$ reuptake (enhancement of $J_m^{OH}$) is delayed by more than 10 min after the initiation of changes in PD, $I_{sc}$ and $R_t$.

After blocking the $HCO_3^-$ reuptake processes with bumetanide, omission of $Cl^-$ from the mucosal side halved the enhanced $J_m^{OH}$ but $Na^+$ omission had no effect on it, indicating that the movement of $HCO_3^-$ into the lumen depends on luminal $Cl^-$. In other words, this suggests that there is $Cl^-/HCO_3^-$ exchange on the brush-border membrane: this idea is also supported by the inhibitory effect of mucosal DIDS on $J_m^{OH}$, since DIDS is known to inhibit $Cl^-/HCO_3^-$ exchange.

Mucosal alkalinization was reduced by removing $Na^+$ from the serosal fluid but not by removing $Cl^-$ (Fig. 6), and blocked by serosal DIDS (Fig. 1). In addition, serosal alkalinization ($J_s^{OH}$) was enhanced by lowering serosal $Na^+$ concentration, but not by removing serosal $Cl^-$ (Fig. 7). These results indicate that $HCO_3^-$ entry from the serosal fluid depends on $Na^+$ but not on $Cl^-$, and suggest that there is a DIDS-sensitive $Na^+/HCO_3^-$ cotransporter which may be driven by the $Na^+$ gradient across the basolateral membrane. Similar DIDS-sensitive $Na^+/(HCO_3^-)_n$ cotransport has been reported in the renal tubules of amphibians (Boron and Boulpaep, 1983; Guggino *et al.* 1983; Wang *et al.* 1987) and mammals (Good *et al.* 1984; Alpern, 1985; Good, 1985; Yoshitomi *et al.* 1985; Akiba *et al.* 1986; Biagi and Sohtell, 1986; Grassl and Aronson, 1986; Jentsch *et al.* 1986*a*,*b*; Grassl *et al.* 1987; Kondo and Frömter, 1987; Sasaki *et al.* 1987; Ullrich and Papavassiliou, 1987), in the frog gastric fundus (Curci *et al.* 1987) and in bovine corneal endothelial cells (Jentsch *et al.* 1984, 1985; Wiederholt *et al.* 1985).

Although the relationship between $Na^+/K^+/Cl^-$ cotransport and $HCO_3^-$ reuptake across the brush-border membrane is not clear yet, a plausible explanation is a coupling between $HCO_3^-$ reuptake and $Cl^-$ movement out of the cell, such as $Cl^-/HCO_3^-$ exchange, since $HCO_3^-$ absorption ($J_s^{OH}$) is blocked by mucosal DIDS (Fig. 2B). Considering driving forces for such $Cl^-/HCO_3^-$ exchange, however, the exchanger must be driven by other force(s), such as the $Na^+$ gradient. Such a DIDS-sensitive $Na^+/(HCO_3^-)_n/Cl^-$ transport has been reported in *Necturus* proximal tubule (Guggino *et al.* 1983; Matsumura *et al.* 1984) and in invertebrate cells (Thomas, 1977; Boron *et al.* 1981). We have no direct information about how $HCO_3^-$ moves from the cell into the serosal fluid, except that this process is independent of serosal $Cl^-$ and inhibited by serosal DIDS.

All the responses of the electrical parameters (PD, $I_{sc}$ and $R_t$) observed after replacement of $Na^+$ or $Cl^-$ indicate that this tissue is substantially permeable not only to $Na^+$ but also to $Cl^-$, although the permeation pathways are not clear from this study.

Fig. 8 shows a possible model for $HCO_3^-$ transport systems in the seawater eel intestine: the $HCO_3^-$ absorption process ($Na^+/HCO_3^-/Cl^-$ exchange and $HCO_3^-$ conductance) is based on speculation from circumstantial evidence. Since NaCl and water absorption depend on $HCO_3^-$ transport (Ando, 1990) and $HCO_3^-$ transport also depends on $Na^+/K^+/Cl^-$ cotransport (present study), all these transport systems appear to be mutually interrelated. The $HCO_3^-$ transport systems discussed in this paper will control the pHi homeostasis in the intestinal

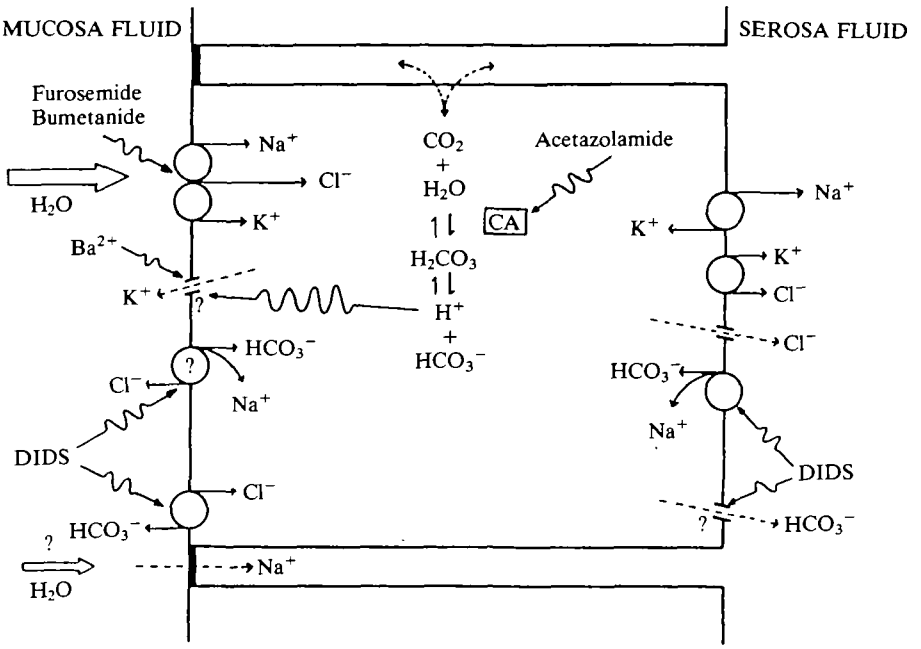


Fig. 8. A possible model for $HCO_3^-$ transport systems in the seawater eel intestine in relation to $Na^+$, $Cl^-$ and water transport. The direction of each ion flux is indicated by solid arrows and the actions of inhibitors are shown as wavy lines. Dotted arrows indicate diffusional ion fluxes. Water flux is represented by open arrows. Question marks mean that these processes were not directly demonstrated, but are based on speculation from circumstantial evidence. $Na^+$, $K^+$, $Cl^-$ and water fluxes are all taken from Ando and Utida (1986). CA, carbonic anhydrase.

epithelium. Although other intracellular organic osmolytes may also control the pHi homeostasis, their contribution may be smaller than that of the $HCO_3^-/CO_2$ buffer system, since amino acid metabolism is very active in this tissue (Ando, 1988). The amino acid metabolism may continuously acidify the cytoplasm. Intracellular pH may control $K^+$ channels on the brush-border membrane, and secondarily regulate $Na^+/K^+/Cl^-$ cotransport, as discussed in the preceding paper (Ando, 1990). Among these $HCO_3^-$ transport systems, the $Na^+/HCO_3^-$ cotransport system on the basolateral membrane might be the most important in controlling pHi, since serosal deficiency of $HCO_3^-$ and serosal addition of DIDS effectively inhibit the serosa-negative PD and water absorption (Ando, 1990).

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