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Potassium-Dependent Chloride and Water Transport across the Seawater Eel Intestine

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Summary. Simultaneous measurements of net ion and water fluxes and transepithelial potential difference (PD) were made in the stripped intestine of the seawater eel, and it was examined whether Cl^- was driven following electrochemical gradient for Na^+ across the brush border membrane of the epithelium or not. When mucosal Na^+ was completely replaced with K^+, while the serosa was being bathed with normal Ringer's solution, net Cl^- and water fluxes were maintained as high as those in normal Ringer's solution. After serosal Na^+ was completely replaced with choline$^+$ while the mucosa was being bathed with Na^+-free KCl Ringer's solution, 80% of the original Cl^- and water fluxes still persisted, indicating significant Na^+-independent Cl^- and water transport. These results are against a hypothesis that Cl^- is driven by electrochemical gradient of Na^+ across the brush border membrane. The Na^+-independent Cl^- and water fluxes were a saturable function of mucosal K^+ concentration, suggesting K^+-dependent Cl^- and water transport. A possible mechanism of Cl^- transport is discussed in relation to K^+ transport. On the other hand, a good correlation was observed between the net Cl^- and water fluxes. This suggests that water transport depends on Cl^- transport system; NaCl and/or KCl cotransport.

Key Words Cl^- transport · NaCl transport · KCl transport · water transport · eel · intestine

Introduction

Seawater eel intestine shows serosa-negative transepithelial potential difference (PD) in normal Ringer's solution (Ando, 1975; 1980; Ando, Utida & Nagahama, 1975; Ando & Kobayashi, 1978) and greater net Cl^- flux than net Na^+ flux under short-circuited condition (Ando et al., 1975), which are very similar to observations in flounder intestine (Huang & Chen, 1971; Field et al., 1978).

Recently, in the seawater eel intestine, it has also been demonstrated that Na^+ and Cl^- transport depend on each other and water transport is linked to the coupled NaCl transport (Ando & Kobayashi, 1978; Ando, 1980; 1981). Such interdependence of Na^+ and Cl^- transport has also been demonstrated in the flounder intestine, in which NaCl cotransport system across the brush border membrane of the epithelium has been proposed by Field et al. (1978). The model is the same as for the NaCl cotransport system proposed in rabbit ileum and gallbladder (Schultz, Frizzell & Nellans, 1974; Frizzell, 1976) or other tissues (*see* Frizzell, Field & Schultz, 1979). Their model emphasizes that Cl^- is driven following the electrochemical gradient of Na^+ across the brush border membrane of the epithelium, and the serosa-negative PD is due to diffusion potential of Na^+ through the tight junction. However, evidence for the NaCl cotransport is not direct yet.

In order to clarify whether Cl^- is driven following the sodium gradient or not, Cl^- flux was measured under reduced Na^+ gradient by replacing mucosal Na^+ with various cations. If Cl^- is driven following the Na^+ gradient, all of these treatments will reduce the Cl^- transport.

The results indicate that Cl^- flux depends not only on Na^+ flux but also on K^+ flux across the brush border membrane of the epithelium in the seawater eel intestine.

Materials and Methods

Japanese cultured eels, *Anguilla japonica,* weighing about 150 g were obtained from a commercial fish pond and kept in seawater aquaria at 20 °C for more than one week before use. They were sacrificed by decapitation. After opening the abdomen, the intestine was excised and the outer muscle layers were stripped off. The intestinal sac was filled with Ringer's solution (0.5 to 1 ml) and incubated in 30 ml Ringer's solution at 20 °C. The net water flux was measured gravimetrically by weighing the sac every 5 min for 30 or 50 min, and described as positive in case of absorption from mucosa to serosa. The PD was recorded as serosal potential with respect to the mucosal side. Details of the simultaneous measurement of the net water flux and the PD are described previously (Ando & Kobayashi, 1978).

In the present study, net Na^+, K^+ and Cl^- fluxes were also measured simultaneously. The net ion flux (J_{net}^{ion}) was calculated as following:

$$J_{net}^{ion} = C_i V_i - C_f V_f$$

where C is the mucosal ionic concentration, V is the mucosal volume, and subcripts i and f mean initial and final, respectively. Incubation time was 50 min, since both the PD and the net water flux were constant for 50 min in normal Ringer's solution (Ando, 1980). Sodium and K^+ concentration was measured with flame photometry (Hiranuma, FPF-2A) and Cl^- concentration was determined with a chloride counter (Hiranuma, CL-5M). Initial and final volume were obtained gravimetrically by subtracting the tare which was weighed after emptying the sac.

Normal Ringer's solution contained, in mM: 118.5 NaCl, 4.7 KCl, 3.0 $CaCl_2$, 1.2 $MgSO_4$, 1.2 KH_2PO_4, 24.9 $NaHCO_3$ and 5 glucose. In other test solutions, Na^+ was replaced with choline$^+$, Tris$^+$, Li^+, Rb^+ or K^+. Sodium bicarbonate was replaced with choline HCO_3 or $KHCO_3$ in case of Na^+-free Ringer's soltuion. All these solutions were kept at the same osmolality (290 mOsm/kg), and at the same pH (7.3) by bubbling with 95% O_2–5% CO_2 gas mixture. When the bathing solution was replaced with a test solution, the intestinal sac was rinsed five times with the new solution. Junction potential, arising between a Ringer's-agar bridge and the mucosal or serosal Ringer's solution, was measured separately by bathing a calomel electrode (A.H. Thomas Co.), which made contact directly with the test solution through 3 M KCl solution, into the mucosal or serosal test solution. The difference between these mucosal and serosal junction potentials was subtracted from the observed transepithelial potential, and the corrected value was used as the PD.

Results

Effects of Replacement of Na^+ with Various Cations

Figure 1 illustrates typical effects of Na^+ on the PD and the net water flow. When mucosal Na^+ was replaced with choline$^+$, the serosa-negative PD increased immediately to −40 mV, and the net water flux represented as the slope of the water flow decreased to zero. After returning to normal Ringer's solution, the initial PD and the net water flux were restored. On the other hand, when serosal Na^+ was replaced with choline$^+$, the serosa-negative PD reversed to serosa-positive without significant change in the net water flux. Thus, the presence of Na^+ in the mucosal side seems to be essential for water absorption from mucosa to serosa.

Relationship between the mucosal Na^+ concentration and the net water flux is shown in Fig. 2. In this experiment, mucosal Na^+ concentration was decreased progressively by mixing normal Ringer's solution with each test solution, while the serosa was being bathed with normal Ringer's solution. When choline$^+$ or Tris$^+$ was substituted for the mucosal Na^+, the net water flux decreased progressively to nearly zero with the diminution of Na^+ concentration. On the other hand, after complete substitution of Li^+ for the mucosal Na^+ the net water flux was diminished to about 30% of the initial water flux. Similar results were obtained by replacing the mucosal Na^+ with Rb^+. In contrast, when the mucosal Na^+ was replaced with K^+, the net water flux was maintained as

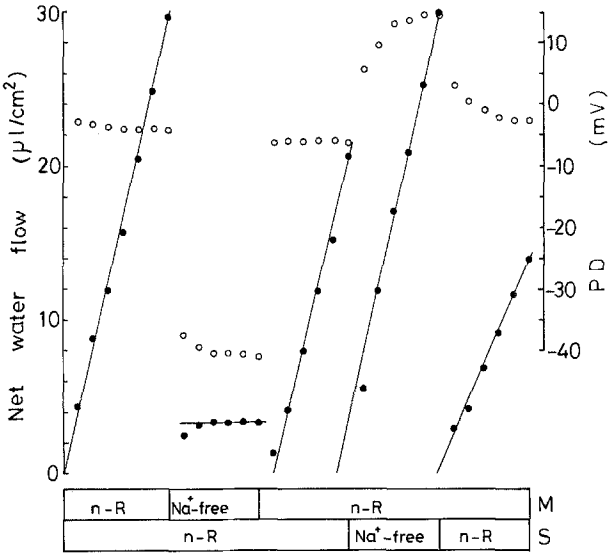

Fig. 1. Effects of replacement of Na^+ with choline$^+$ in mucosal (*M*) or serosal (*S*) fluid on PD (o) and net water flow (•) across the middle part of the seawater eel intestine. The slope represents net water flux. n-R, normal Ringer's solution; Na^+-free, choline$^+$ Ringer's solution

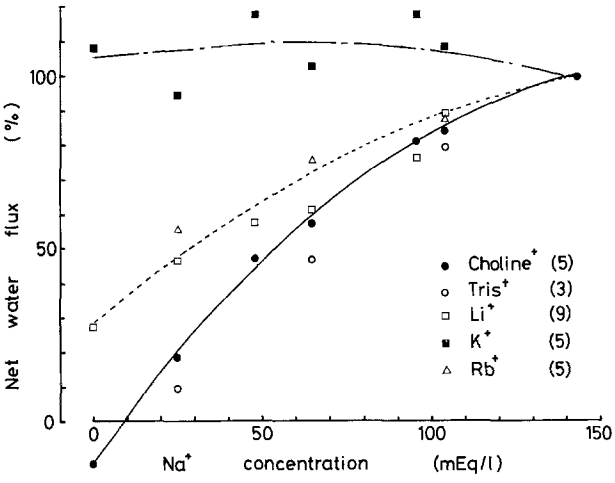

Fig. 2. Effects of mucosal Na^+ concentration on net water flux across the middle part of the seawater eel intestine. The net water flux is expressed as percentage of the initial value in normal Ringer's solution. Mucosal Na^+ was replaced with choline$^+$ or Tris$^+$, solid line; with Li^+ or Rb^+, dotted line; with K^+, dashed line. Each point represents the mean value. The number of preparations is indicated in the parentheses

high as that in normal Ringer's solution. The original values were mostly restored after reintroducing normal Ringer's solution at the end of these experiments except for the case of Li^+ substitution.

Figure 3 shows, the relationship between mucosal Na^+ concentration and PD. The PD was estimated after 30 min, when a steady level had been attained (*cf.* Fig. 1). When mucosal Na^+ was replaced with choline$^+$ or Tris$^+$, the serosa-negative PD increased progressively with the diminution of mucosal Na^+ concentration, mainly due to diffusion of Na^+ from serosa to mucosa as explained previously (Ando & Kobayashi, 1978). On the other hand, the PD did not change when Li^+ was substituted for mucosal Na^+. When the mucosal Na^+ was replaced with K^+ or Rb^+, in contrast, the serosa-negative PD decreased progressively to around zero, or reversed to serosa-positive in case of Rb^+ substitution. Although the PD could be complicated by two components, active and passive diffusion potentials, the change in PD might reflect mainly a biionic potential, thus permeability sequence across the eel intestine could be estimated as $Rb^+ \geqq K^+ > Na^+ \geqq Li^+ \gg$ choline$^+$ = Tris$^+$, which is very similar to that in the "tight junction" of the rabbit gallbladder (Moreno & Diamond, 1974). After reintroducing normal Ringer's solution at the end of the experiments, the PD was restored to the original value in cases of choline$^+$ and Tris$^+$ substitution, but was less than half of the control level in cases of Li^+, K^+ and Rb^+ substitution.

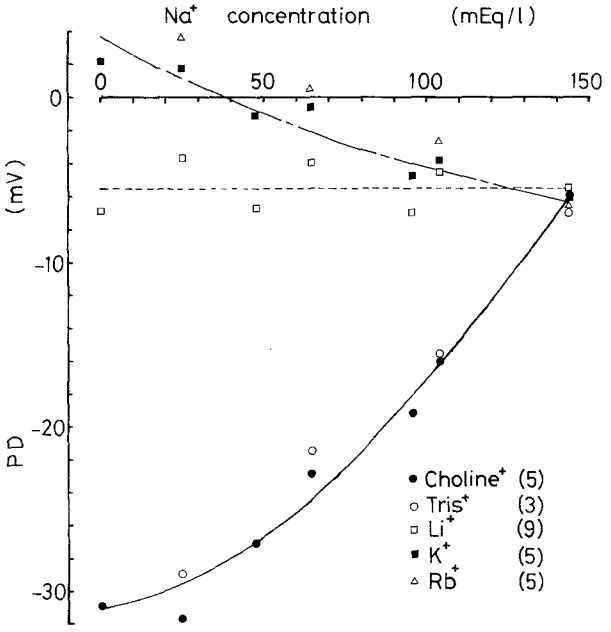

Fig. 3. Effects of mucosal Na^+ concentration on PD in the middle part of the seawater eel intestine. Each point represents the mean value. All symbols are the same as in Fig. 2

Ion and Water Fluxes in KCl-Ringer's solution

Since among cations substituted for mucosal Na^+ only K^+ retained the water transport as high as Na^+ did, net ion fluxes were measured in KCl Ringer's solution in the following experiments. As shown in Table 1, the net Na^+ and Cl^- fluxes were almost identical and the net K^+ flux was negligible in normal Ringer's solution, which confirms previous results (Oide & Utida, 1967; Utida et al., 1972). When the mucosal side of the intestine was bathed with KCl Ringer's solution, while the serosa was being bathed with normal Ringer's solution, net Na^+ secretion from serosa to mucosa and high net K^+ absorption were observed. However, net Cl^- and water fluxes were similar to those in normal Ringer's solution. On the other hand, when the serosal Na^+ was replaced with K^+, while the mucosa was being bathed with normal Ringer's solution, net Na^+ absorption increased and significant K^+ secretion was observed. At the same time, the net Cl^- and water fluxes decreased to almost half of the control level in normal Ringer's solution. When both the mucosa and serosa were bathed with KCl Ringer's solution, the Na^+, K^+, Cl^- and water fluxes were all diminished to appro-

Table 1. Effects of Na^+ replacement with K^+ on the transepithelial potential difference (PD), net ion fluxes (J^{Na}_{net}, J^{K}_{net} and J^{Cl}_{net}) and net water flux ($J^{H_2O}_{net}$) in the seawater eel intestine. All Ringer's solutions were buffered with $NaHCO_3$.

Mucosal solution	Serosal solution	No. of eels	PD (mV)	J^{Na}_{net}	J^{K}_{net} (μeq/cm^2·h)	J^{Cl}_{net}	$J^{H_2O}_{net}$ (μl/cm^2·h)
NaCl	NaCl	16	−6.2±0.4[a]	7.1±0.4	0.1±0.0	7.1±0.5	42.9±3.1
KCl	NaCl	9	−0.8±0.6	−4.6±0.5	12.3±0.5	8.5±0.4	46.3±3.2
NaCl	KCl	4	−3.5±0.3	9.8±0.5	−6.7±1.1	3.7±0.4	27.3±3.2
KCl	KCl	3	−0.8±0.0	−0.9±0.2	1.4±0.7	0.3±0.3	2.1±3.1
NaCl	NaCl[b]	3	−1.5±0.3	7.1±0.3	−0.1±0.0	7.5±0.6	44.3±6.7

[a] Mean ± SEM.
[b] These values were obtained after pretreatment of both sides with KCl Ringer's solution.

Table 2. Effects of Na^+-free Ringer's solution on the transepithelial potential difference (PD), net ion fluxes (J^{Na}_{net}, J^{K}_{net} and J^{Cl}_{net}) and net water flux ($J^{H_2O}_{net}$) in the seawater eel intestine. The mucosa and serosa were bathed with KCl Ringer's solution buffered with $KHCO_3$ and choline Cl Ringer's solution buffered with choline HCO_3, respectively.

Mucosal solution	Serosal solution	PD (mV)	J^{Na}_{net}	J^{K}_{net} ($\mu eq/cm^2 \cdot h$)	J^{Cl}_{net}	$H^{H_2O}_{net}$ ($\mu l/cm^2 \cdot h$)
NaCl	NaCl	-9.8 ± 1.1[a]	8.7 ± 0.6	0.3 ± 0.0	8.8 ± 0.6	59.0 ± 4.7
KCl	NaCl	-3.1 ± 2.0	-5.8 ± 1.0	15.4 ± 0.5	9.4 ± 0.6	60.5 ± 6.0
KCl	Choline Cl	19.5 ± 2.6	-0.3 ± 0.1	11.0 ± 1.1	6.7 ± 0.4	41.1 ± 2.6
KCl	NaCl	-1.2 ± 0.8	-9.7 ± 1.2	18.5 ± 0.6	9.4 ± 1.2	55.5 ± 7.4

[a] Mean $\pm$ SEM ($n=6$).

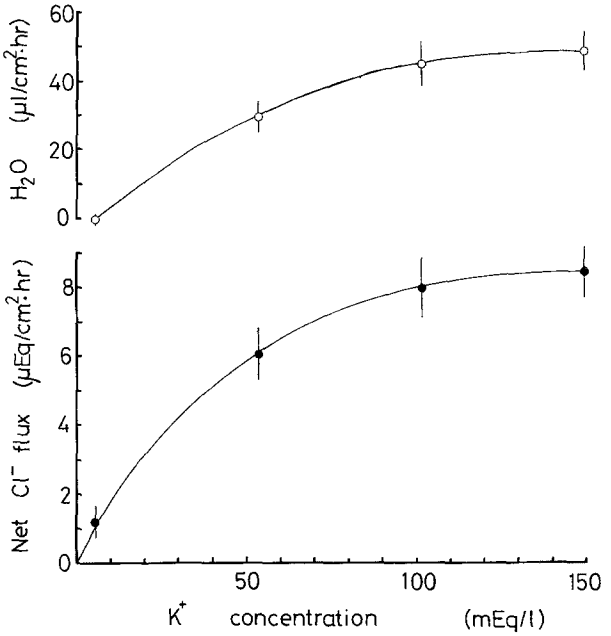

Fig. 4. Relationship between mucosal K^+ concentration and net Cl^- (•) and water (o) fluxes. Mucosal K^+ concentration was diminished progressively by mixing Na^+-free KCl Ringer's solution with choline Cl Ringer's solution (Na^+-free), while the serosa was being bathed with normal Ringer's solution. Each point represents the mean value, and vertical bars indicate standard error of the mean ($n=7$)

ximately zero. After reintroducing normal Ringer's solution at the end of each treatment with KCl Ringer's solution, the ion and water fluxes were all restored to the control level, although recovery of the PD was incomplete.

Since it might be possible that Na^+ residue was enough to maintain the Cl^- and water fluxes, in the next experiments, Na^+-free KCl Ringer's solution was introduced into mucosa (Table 2). Changes in the PD, the net ion and water fluxes were all similar to those in Table 1. When the serosal Na^+ was replaced with choline$^+$ (Na^+-free) while the mucosa was being bathed with Na^+-free KCl Ringer's solution, the net K^+, Cl^- and water fluxes decreased barely, by about 20% of the control level. In spite of the absence of Na^+ absorption under this condition, 80% of the Cl^- and water fluxes were still maintained, indicating significant sodium-independent Cl^- and water transport.

Figure 4 shows the relationship between mucosal K^+ concentration and net Cl^- and water fluxes. Initially, the mucosa was bathed with Na^+-free KCl Ringer's solution, and then the mucosal K^+ concentration was diminished progressively by mixing the KCl Ringer's solution with choline Cl (Na^+-free) Ringer's solution. During the experiments, the serosa was being bathed with normal Ringer's solution. With the diminution of mucosal K^+ concentration, the net Cl^- and water fluxes decreased progressively to zero; they were a saturable function of mucosal K^+ concentration, suggesting potassium-dependent Cl^- and water transport.

Relationship between Cl^- and Water Fluxes

As already shown in Tables 1 and 2 and Fig. 4, change in Cl^- transport was parallel to change in water transport. Figure 5, in addition, shows a good correlation between individual net Cl^- and water fluxes. The net Cl^- and water fluxes were obtained simultaneously from one preparation bathed either in normal or in Na^+-free (mucosa and serosa were bathed with K^+ and choline$^+$, respectively, as described in Table 2) Ringer's solution. Under both conditions, an identical regression line was obtained ($J^{H_2O}_{net} = 6.6\ J^{Cl}_{net} - 3.5$; $r = 0.94$, $p < 0.001$). In normal Ringer's solution, the net Na^+ and Cl^- fluxes were almost identical with no K^+ absorption (Tables 1 and 2), suggesting that water transport depends on NaCl transport. On the other hand, in Na^+-free Ringer's solution the net K^+ and Cl^- fluxes were observed with no Na^+ absorption (Table 2), suggesting that water transport depends on KCl transport. These results may imply that water transport depends on Cl^- transport system (NaCl and/or KCl cotransport) in the seawater eel intestine.

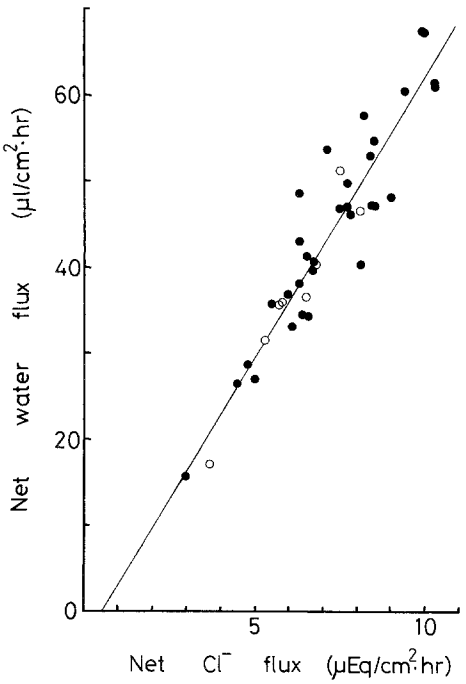

Fig. 5. Correlation between net Cl^- and water fluxes. The net Cl^- and water fluxes were measured simultaneously from one preparation bathed either in normal Ringer's solution (●) or in Na^+-free Ringer's solution (○), in which the mucosa and serosa were bathed with KCl and choline Cl Ringer's solution, respectively, as described in Table 2

Discussion

A sodium chloride cotransport system has been proposed by many workers (Field et al., 1978; Duffey, Thompson, Frizzell & Schultz, 1979; Frizzell, Smith, Vosburg & Field, 1979; Frizzell, Field & Schultz, 1979; Eveloff et al., 1980) in the flounder intestine. In their model, Cl^- transport is secondary active and is driven by electrochemical potential difference for Na^+ across the brush border membrane of the epithelium. The present results do not exclude the existence of such a NaCl cotransport system across the brush border membrane of the epithelium in the seawater eel intestine bathed in normal Ringer's solution. However, it is clearly shown in the present study that the electrochemical gradient of Na^+ is not the only driving force for Cl^- transport; substitution of K^+ for mucosal Na^+ also maintained Cl^- transport at a high level. Furthermore, when both sides of the intestine were bathed with Na^+-free Ringer's solution, significant Cl^- absorption still remained with no Na^+ absorption (Table 2). The sodium-independent Cl^- transport was a saturable function of mucosal K^+ concentration (Fig. 4). Therefore, it is most likely that Cl^- transport in the seawater eel intestine depends on Na^+ and/or K^+ transport.

The dependence of Cl^- flux on K^+ flux might be explained by the other way. Because K^+ diffuses faster than Na^+ and thus induces serosa-positive PD, Cl^- can follow the K^+ flux through the PD. If it were true, mucosal substitution of Rb^+ should maintain Cl^- transport, and thus water transport, as high as those in mucosal K^+ substitution, since Rb^+ could diffuse as fast as K^+ (Fig. 3). However, mucosal Rb^+ substitution diminished the water flux to 30% of the control level (Fig. 2), suggesting that K^+ flux drags 3 times more Cl^- than Rb^+ does. Moreover, it is also against this explanation that the Cl^- transport under K^+ concentration gradient persists in the absence of serosa-positive PD (Tables 1 and 2). In addition, it has been recently observed that mucosal furosemide inhibits both K^+ and Cl^- fluxes under K^+ concentration gradient (M. Ando, *in preparation*). If furosemide inhibits specifically Cl^- transport, this phenomenon will mean that a part of the K^+ flux also depends on Cl^- transport, suggesting KCl cotransport across the brush border membrane of the epithelium.

Although the reason for serosal K^+ to inhibit Cl^- transport is unclear in this study, this phenomenon may suggest another KCl cotransport driven through K^+ concentration gradient across the basolateral membrane, as suggested in rabbit (Duffey et al., 1978) and *Necturus* (Reuss, 1979) gallbladder and flounder intestine (Stewart et al., 1981).

The water transport was always in parallel with the net Cl^- flux, and regression line between these two parameters was $J_{net}^{H_2O} = 6.6\, J_{net}^{Cl} - 3.5$. The value of 3.5 μl/cm²·h is too low to detect by the present method, and may be considered as approximately zero, since the net water flux is nearly zero in Cl^--free Ringer's solution (Ando, 1980). From the regression line, the absorbed Cl^- concentration can be calculated as $1000/6.6 = 151.5$ meq/liter, which is only slightly higher than that in the experimental solutions (129.2 meq/liter). The nearly unity among the absorbed and bathing Cl^- concentration suggests that water is transported almost isosmotically by a Cl^- transport system (NaCl and/or KCl cotransport) in the seawater eel intestine.

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