Ion transport across intestinal mucosa of winter flounder, *Pseudopleuronectes americanus*

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**METHODS**

Winter flounder (*Pseudopleuronectes americanus*) were caught in the Gulf of Maine during the summer period of July and August. They were kept alive in a cart submerged in the ocean water for more than a week before used in experiments at which time the intestinal contents consisted mainly of fluid. A piece of small intestine, located about 2 cm distal to the pylorus, was removed, washed with Ringer solution and mounted in the apertures of a Lucite chamber with an area of 2.3 x 0.55 cm or 1.3 cm². The design of the chamber was similar to that described by Ussing et al. (23) and by Gonzalez et al. (10), except smaller in size. Seven milliliters of bathing fluid were placed in each side of the chamber, a quantity sufficient to cover the mounted intestinal membrane. Two calomel electrodes were attached to a pair of glass adapters, the tip of which was filled with NaCl Ringer agar and was placed close to the intestinal membrane to measure the PD. Current-sending electrodes consisted of metallic lead plus agar layers of lead acetate, saturated KCl solution, and NaCl Ringer, where the latter was the layer in contact with the bathing solution and was changed to either Na₂SO₄ Ringer or choline Ringer agar to correspond with the Ringer used as bathing solution. The PD and Iₑ were recorded on an Esterline-Augus recorder. The input impedance of the recorder for PD measurements was 100 kilohms. A modified Forster (9) teleost Ringer was used as the bathing solution. Unless otherwise specified, this solution will be referred to as normal NaCl ringer and con-
tained: NaCl 115 mM, K acetate 10 mM, CaCl₂ 2.5 mM, MgSO₄ 1.1 mM, and NaHCO₃ 25 mM. In Na₂SO₄, LiCl, choline chloride, and choline methylsulfate Ringer solutions, a 115 mM corresponding salt was used to replace the 115 mM NaCl in the normal NaCl Ringer without change of other constituents. Glucose was added, if necessary, to keep the osmolarity of all solutions at 300 mosm/liter. The solutions were freshly prepared and aerated with 95% O₂-5% CO₂ gas for at least 30 min before each experiment. During the experiment, the bathing fluid was bubbled continuously with the same gas which was saturated with water vapor. All experiments were carried out with the chamber in a constant temperature incubator at 26-27°C.

The transport of water and salt by the flounder intestine was determined by a technique similar to that described by Diamond (6) using gall bladder. A section of the intestine 2 cm distal to the pylorus and 5 cm proximal to the anus was removed and the lumen was washed at least 3 times with normal NaCl Ringer. Then the section of the intestine was tied at one end, filled with 5-6 ml of normal 115 mM NaCl Ringer or 90 mM NaCl Ringer with glucose added to maintain the same osmolarity, and the other end closed around a small glass tube filled with agar. This agar bridge could be connected to a calomel electrode for the purpose of measuring the electrical potential during the experiment. The intestine was suspended in a beaker containing 1 liter 115 mM NaCl Ringer. The solution was stirred throughout the experiment by a stream of vapor-saturated O₂-CO₂ bubbles. The intestine was weighed before incubation and at half-hour periods, and it was found that the weight decreased continuously over a 4-hour period. The pH, Na⁺, K⁺, and Cl⁻ and HCO₃⁻ of the pre- and postincubation solution were analyzed by the use of flame photometry, Buchler-Cotlove chloridometry (4), and the Van Slyke method (15) (for total CO₂).

Ion flux measurements were done with either ⁵²Na, ³⁵Cl, or ⁶⁸SO₄ by placing the isotope in the bathing solution on either side of the chamber after PD and Iₑ reached steady-state levels. Two neighboring sections of small intestine from one fish were mounted in identical chambers, one for the study of mucosal-to-serosal flux, and the other for serosal-to-mucosal flux of the same isotope. In each determination 50-µl samples were collected at 30-min or hourly intervals, dissolved in Bray's scintillation fluid (1) and counted in an automatic liquid scintillation counter. The procedure and calculations were identical to those described by Gonzalez et al. (10).

**RESULTS**

PD and Iₑ. When the mucosa and serosa of flounder intestine were bathing in identical normal NaCl Ringer solutions, a potential ranging from 1 to 5 mV was observed and oriented so that the serosal side was electronegative relative to the mucosal side. This potential drifted very little over a period of 1 to 2 hr. In 10 individual fish intestines, the PD was −2.9 ± 0.42 mV (mean ± se) and the Iₑ was −24.3 ± 2.5 µamps. The addition of glucose to the bathing solution in a final concentration of 5.5 mM increased the negativity of the PD and Iₑ to −3.35 ± 1.46 mV and −26.5 ± 13.3 µamps, respectively (n = 18). At a final glucose concentration of 22.5 mM the PD was −3.41 ± 0.83 mV and Iₑ was −29.0 ± 4.86 µamps (n = 7). After replacement of CO₂-O₂ with N₂ as the bubbling gas, both PD and Iₑ dropped to zero within 10-15 min, then recovered on restoration of CO₂-O₂ aeration.

Figure 1 presents results of two separate experiments. In the fish intestine bathing with normal NaCl Ringer, the serosa was electronegative to the mucosa and the PD and Iₑ were constant for at least 20 min. Then the mucosal and serosal bathing solutions were replaced with Na₂SO₄ Ringer and a gradual reversal of PD and Iₑ from negative to constant positive values was observed. This positive PD and Iₑ could be reversed by replacing the Na₂SO₄ bathing solution with NaCl Ringer. In seven individual experiments employing Na₂SO₄ Ringer, the average PD was +1.14 ± 0.14 mV and the average Iₑ was +7.3 ± 0.99 µamps. When the flounder intestine was bathed in either choline chloride Ringer or LiCl Ringer, a very high negative PD of −5 to −15 mV and Iₑ of −40 to −60 µamps were usually observed, which drifted gradually to smaller but constant negative values. In seven individual experiments wherein choline chloride Ringer was the bathing fluid for a 1 hr period the average PD was −8.3 ± 2.2 mV, and the average Iₑ was −51.5 ± 14.0 µamps. However, the negative PD and Iₑ were reduced greatly when the bathing fluids on both sides of the intestinal membrane were replaced by either choline methylsulfate Ringer with the same bicarbonate concentration or choline chloride-phosphate buffer Ringer without bicarbonate. This effect was reversed when the bathing fluids were changed back to choline chloride-bicarbonate buffer Ringer. These results suggest that chloride is actively transported from mucosa to serosa and that the presence of bicarbonate ion is required for the transport process.

Isotopic measurement of ion fluxes. Table 1 shows the average mucosal-to-serosal (Jₘₛ) and serosal-to-mucosal (Jₘₛ) fluxes of sodium, sulfate, and chloride ion determined using radioactive isotopes in sodium sulfate, choline chloride, and NaCl Ringer solutions. In 27 sets of determinations on nine different fish intestines bathed in Na₂SO₄ Ringer, a net mucosal-to-serosal sodium flux was clearly demonstrated; this net flux was greater than the ion flux calculated from the short-circuit current, Iₑ. In 48 sets of determinations on 12 fish
ion fluxes across flounder intestine

<table>
<thead>
<tr>
<th>Bathing Solution</th>
<th>Isotope</th>
<th>( J_{\text{Na}} )</th>
<th>( J_{\text{Cl}} )</th>
<th>( J_{\text{net}} )</th>
<th>( I_{\text{se}} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaCl Ringer</td>
<td>(^{23}\text{Na})</td>
<td>5.25 ± 0.60 (9)</td>
<td>4.23 ± 0.63 (9)</td>
<td>1.00 ± 0.35 (9)</td>
<td>0.196 ± 0.023 (9)</td>
</tr>
<tr>
<td></td>
<td>(^{35}\text{Cl})</td>
<td>0.230 ± 0.13 (6)</td>
<td>0.224 ± 0.13 (6)</td>
<td>0.005 ± 0.020 (6)</td>
<td>0.185 ± 0.016 (6)</td>
</tr>
<tr>
<td>Choline chloride Ringer</td>
<td>(^{23}\text{Na})</td>
<td>4.30 ± 0.33 (12)</td>
<td>2.95 ± 0.29 (12)</td>
<td>1.35 ± 0.41 (12)</td>
<td>1.05 ± 0.13 (12)</td>
</tr>
<tr>
<td>NaCl Ringer</td>
<td>(^{35}\text{Cl})</td>
<td>5.16 ± 0.80 (7)</td>
<td>2.98 ± 0.70 (7)</td>
<td>2.88 ± 0.77 (7)</td>
<td>0.88 ± 0.15 (7)</td>
</tr>
</tbody>
</table>

Numbers in parentheses are the numbers of fish. *Mean ± SE.

Fluid and electrolyte transport. Control studies for experiments in which the rates of water absorption and electrolyte transport were measured are illustrated in Fig. 2. It can be seen here that the weight steadily decreased in the first 2 hr and then leveled off gradually in the last 2 hr. Table 2 summarizes the results obtained from experiments in which the weight loss and change of ions were determined at pre- and postincubation periods only. In these experiments, the PD across the intestinal sac was measured at half-hour periods and found to be negative. In the first set of experiments both mucosal and serosal solutions were 115 mM normal NaCl Ringer solution. Assuming the weight loss is due to fluid loss, an average loss of fluid at the end of 4-hr periods was 21.5% of the preincubation value. There was a significant decrease in chloride and sodium concentration in the final mucosal fluid accompanied by an increase in HCO\(_3\) and K ions. Acetazolamide added to the bathing solution at a final concentration of 2 mM produced an average volume loss equal to 10.76% of the preincubation value. The HCO\(_3\) concentration in the final mucosal fluid was practically unchanged while the chloride concentration was decreased, but to a much lesser degree than in the control.

In five fish experiments in which the mucosal bathing fluid was low NaCl (90 mM) Ringer (osmolarity maintained at 300 mOsm by addition of glucose) and the serosal fluid was normal NaCl Ringer (115 mM), a mucosal volume loss averaging 9.5% of the preincubation volume was still observed at the end of 4 hr. Although the chloride and sodium concentrations in the final mucosal fluid were changed in a much lesser degree, the calculated total loss of chloride and sodium ions was substantial, suggesting the existence of a process of transport against a concentration gradient.

Kinetic studies of Cl\(^-\) transport. In six individual experiments the intestines were originally bathed with \(^{23}\text{NaSO}_4\) Ringer solution so that in the sodium concentration of the bathing fluid should be relatively constant so that the change of \( I_{\text{se}} \), \( \Delta I_{\text{se}} \), (the observed \( I_{\text{se}} \) minus the control \( I_{\text{se}} \)) would be directly due to the increase of chloride ions in the bathing fluid because the constant transport rate of Na\(^+\) would be cancelled out. The results are presented in Fig. 3A, which shows that as the Cl concentration increased in the bathing fluid, so did the magnitude of \( \Delta I_{\text{se}} \); saturation was reached at a Cl concentration of 110 mM. When the data are plotted in the manner suggested by Dowd and Riggs (8), i.e., S/\( \Delta I_{\text{se}} \) vs. S, a linear relationship is observed as seen in Fig. 3B where the line was calculated by the least-squares technique. The calculated \( K_m \) was 59 mM.

**Discussion**

Data presented here demonstrate that a potential exists across the small intestine of winter flounder, a marine teleost, orientated such that the serosa was electronegative to the mucosa in the presence of normal teleost fish Ringer solution; the PD changed to positive when Na sulfate Ringer solution was used as bathing fluid. These results establish three important points concerning electrolyte transport across the flounder intestine: first, there must be a net transmural movement of anion(s) and cation(s) across the flounder fish intestine with the net transport rate of anion(s) being greater than that of cation(s). The ion flux measurements with radioisotopes provide evidence in support of this conclusion. As seen in Table 1, under short-circuit current conditions there was a net mucosal-to-serosal flux of sodium and chloride ions across the flounder intestine. The average net Na flux was 1.00 \( \mu\text{Eq cm}^{-2}\text{hr}^{-1} \) in sodium sulfate Ringer.
ION TRANSPORT ACROSS FLOUNDER INTESTINE

TABLE 2. Fluid and ions transport across noneverted sacs of flounder intestine and effect of acetazolamide

<table>
<thead>
<tr>
<th>Preincubated Bathing Solution</th>
<th>Postincubated Mucosal Solution</th>
<th>PD, mV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mucosal</td>
<td>Serosal</td>
<td>No. of Fish</td>
</tr>
<tr>
<td>NaCl Ringer</td>
<td>NaCl Ringer</td>
<td></td>
</tr>
<tr>
<td>115 mM</td>
<td>115 mM</td>
<td>12</td>
</tr>
<tr>
<td>Control</td>
<td>NaCl Ringer</td>
<td></td>
</tr>
<tr>
<td>2 mM Acetazolamide</td>
<td>110 mM</td>
<td>9</td>
</tr>
<tr>
<td>NaCl Ringer†</td>
<td>NaCl Ringer</td>
<td></td>
</tr>
</tbody>
</table>

(+) Indicates the net gain, (−) the net loss. *Mean± SE. †Glucose was added to maintain the solution isosmolar (300 mOsm).

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medium which exceeds the value calculated from the short-circuit current. However, when the intestine was bathed in choline chloride Ringer medium, a net transmural chloride flux averaging 1.35 μEq cm<sup>−2</sup>hr<sup>−1</sup> was observed; this value accounts for most of the short-circuit current. This suggests that sodium transport is independent of Cl<sup>−</sup> transport and that Cl<sup>−</sup> transport is also independent of Na<sup>+</sup> transport.

Second, our findings from the electrical measurements across flounder fish intestine differ not only from the results found in freshwater goldfish intestine (20, 21), but also from those obtained from the intestine of Cottus scürpus, another marine teleost (11). In their report, the authors recorded no PD across the Cottus intestinal membrane, despite the demonstration of an active transport of sodium ion from mucosal to serosal surface. Since neither anion flux measurements or different bathing fluids were included in the experiments on Cottus intestine, the results reported cannot rule out the possibility of a simultaneous transmural transport of Na and Cl ion in this species. Our recent work on freshwater catfish intestine (2) showed that D-glucose and L-tyrosine were transported across the intestinal mucosa against a concentration gradient and that there was a simultaneous net transmural transport of Na ion and Cl ion. Thus, species variation appears to play a role in determining whether there is cation transport only as in goldfish intestine or a simultaneous cation and anion transport as in catfish. This may also apply to flounder fish intestine and Cottus intestine.

Third, the results of our study on fluid and ion movement with the noneverted intestinal sac technique have demonstrated a transmural chloride transport against electrochemical potential gradients accompanied by a transmural sodium and water transport. The net transmucosal chloride transport was statistically significant in the presence of either normal NaCl Ringer or low NaCl Ringer as mucosal medium. The chloride ion lost from the mucosal fluid during the 4-hr incubation was at least partially replaced by bicarbonate ion. As seen in Table 2, the bicarbonate concentration of the final mucosal fluid was increased significantly.

The addition of acetazolamide at a concentration of 2 mM decreased the mucosal fluid loss from 21.5 to 10.7% of the initial volume during the 4-hr incubation period. Chloride loss and bicarbonate gain by the mucosal fluid were also reduced. The effect of acetazolamide, a potent carbonic anhydrase inhibitor, on electrolyte transport in the mammalian intestine has been reported by Parsons (14) and Kinney and Code (13). Preliminary studies in this labora-
tory have shown the intestinal mucosa of flounder fish to contain 35–50 units of carbonic anhydrase per gram wet weight. Therefore, it is reasonable to assume that carbonic anhydrase may play a role in chloride transport across the fish intestine, probably in a Cl–HCO₃ exchange process.

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