Bicarbonate secretion plays a role in chloride and water absorption of the European flounder intestine


You might find this additional information useful...

This article cites 26 articles, 11 of which you can access free at:
http://ajpregu.physiology.org/cgi/content/full/288/4/R936#BIBL

Updated information and services including high-resolution figures, can be found at:
http://ajpregu.physiology.org/cgi/content/full/288/4/R936

Additional material and information about *American Journal of Physiology - Regulatory, Integrative and Comparative Physiology* can be found at:
http://www.the-aps.org/publications/ajpregu

This information is current as of May 19, 2005.
Bicarbonate secretion plays a role in chloride and water absorption of the European flounder intestine

M. Grosell,1 C. M. Wood,1,2 R. W. Wilson,3 N. R. Bury,4 C. Hogstrand,1,4 C. Rankin,5 and F. B. Jensen5

1RSMAS, University of Miami, Miami, Florida; 2McMaster University, Hamilton, Ontario, Canada; 3Exeter University, Exeter; 4Kings College, London, United Kingdom; and 5University of Southern Denmark, Odense, Denmark

Submitted 1 December 2003; accepted in final form 1 December 2004

Grosell, M., C. M. Wood, R. W. Wilson, N. R. Bury, C. Hogstrand, C. Rankin, and F. B. Jensen. Bicarbonate secretion plays a role in chloride and water absorption of the European flounder intestine. Am J Physiol Regul Integr Comp Physiol 288: R936–R946, 2005. First published December 2, 2004; doi:10.1152/ajpregu.00684.2003.—Experiments performed on isolated intestinal segments from the marine teleost fish, the European flounder (Platichthys flesus), revealed that the intestinal epithelium is capable of secondary active HCO3− secretion in the order of 0.2–0.3 μmol·cm−2·h−1 against an apparent electrochemical gradient. The HCO3− secretion occurs via anion exchange, is dependent on mucosal Cl−, results in very high mucosal HCO3− concentrations, and contributes significantly to Cl− and fluid absorption. This present study was conducted under vivo-like conditions, with mucosal saline resembling intestinal fluids in vivo. These conditions result in a transmucosal potential of ∼−16.2 mV (serosal side negative), which is very different from the −2.2 mV observed under symmetrical conditions. Under these conditions, we found a significant part of the HCO3− secretion is fueled by endogenous epithelial CO2 hydration mediated by carbonic anhydrase because acetazolamide (10−4 M) was found to inhibit HCO3− secretion and removal of serosal CO2 was found not to influence HCO3− secretion. Reversal of the epithelial electrochemical gradient for Cl− (removal of serosal Cl−) and elevation of serosal HCO3− resulted in enhanced HCO3− secretion and enhanced Cl− and fluid absorption. Cl− secretion via an anion exchange system appears to partly drive fluid absorption across the intestine in the absence of net Na+ absorption.

HCO3− secretion; chloride absorption; carbonic anhydrase; osmoregulation; marine teleost

It is well established that marine teleosts drink seawater and that the intestine has a vital role in osmoregulation by performing water absorption (18, 24, 25) necessary to compensate for the continuous osmotic water loss to the marine environment. This water absorption is generally accepted to be coupled to Na+ and Cl− absorption driven by Na+/Cl− and Na+/K+−2Cl− cotransporters (15, 18), but an additional, perhaps major contribution to Cl− uptake and thereby fluid absorption by anion exchange has so far largely been ignored.

High HCO3− concentrations in the intestinal fluids of marine teleost fish were first suggested by Shehadeh and Gordon (24) more than three decades ago and subsequently documented for the first time by Walsh et al. (26) more than 10 yr ago. Only recently, however, has this unique phenomenon been investigated in greater detail (7, 8, 9, 10, 28–31). Cl−/HCO3− exchange across the apical membrane of the marine teleost intestine is responsible for the extremely high HCO3− concentrations found in the intestinal lumen (8, 10, 28, 30, 31). It can result in HCO3− concentrations in excess of 100 mM, which is much higher than the 5–10 mM found in the extracellular fluids of these water-breathing vertebrates. This substantial chemical gradient and the low blood side negative transepithelial potential (TEP) of marine teleost intestinal epithelia (18) strongly suggest active bicarbonate secretion, but this remains to be documented.

Active HCO3− secretion is perhaps best known to occur from the exocrine pancreatic ducts, which can secrete fluid containing HCO3− concentrations up to 140 mM (22), i.e., concentrations very similar to those found in the marine teleost intestine. Pancreatic exocrine HCO3− secretion serves digestion; however, this is clearly not the case for the marine teleost intestine (28, 30, 31). Rather, in these marine animals, HCO3− secretion is perhaps involved in osmoregulation by reducing the osmolarity of the intestinal fluids by cation-carbonate precipitation, thereby aiding fluid absorption indirectly and by contributing to Cl− and perhaps fluid absorption directly through the anion exchange process (31). Marine fish live in a hypercalcemic environment, so the precipitation (and subsequent rectal excretion) of imbibed calcium as insoluble carbonates also minimizes the intestinal absorption of excess calcium, indicating a further role for HCO3− secretion in calcium homeostasis (31).

Fluid absorption linked to NaCl absorption via Na+/Cl− and Na+/K+−2Cl− cotransporters relies on the electrochemical gradient for Na+, which in turn is generated by the basolateral electrogenic Na+/K+−ATPase. Although this mechanism accounts for part of the intestinal Cl− absorption, simultaneous measurements of net Na+ and Cl− absorption on several species of marine teleost fish have revealed that Cl− absorption rates in all cases greatly exceed the corresponding rates for Na+ (5, 7, 8, 10, 19, 20). The stoichiometry of Na+/K+−2Cl− cotransporters can contribute to Cl− absorption exceeding Na+ absorption, with K+ absorption making up for any Na+/Cl− gap. However, it is likely that Cl−/HCO3− exchange also contributes to the higher Cl− absorption rates: net HCO3− secretion rates, when measured (7, 8, 10), were similar in magnitude to the gap between net Na+ and net Cl− absorption rates (5, 7, 8, 10, 19, 20).

In the present study performed on the European flounder, Platichthys flesus, one objective was to determine whether the intestinal epithelium is capable of performing truly active HCO3− transport. This objective was pursued by measuring both HCO3− transport rates and TEP across isolated intestinal segments at a range of transepithelial HCO3− gradients. The Cl− dependence of intestinal HCO3− secretion was tested by experiments with Cl−-free solutions on the luminal and the mucosal sides.
serosal side of the epithelium. An additional objective was to determine whether HCO₃⁻ secretion via anion exchange contributes significantly to Cl⁻ and thus water absorption. Experiments employing acetazolamide, a carbonic anhydrase inhibitor, were designed to test the hypothesis of involvement of carbonic anhydrase in HCO₃⁻ secretion and to potentially inhibit HCO₃⁻ secretion. Attempts to inhibit and stimulate intestinal HCO₃⁻ secretion to test the potential contribution to Cl⁻ absorption also included experiments with reduced and elevated serosal CO₂ levels. These experiments also served to address the question regarding the source for intestinal HCO₃⁻ secretion. Our hypothesis was that reduced serosal CO₂ might result in reduced HCO₃⁻ secretion if the normal origin was serosal rather than endogenous, due to substrate depletion, and that this in turn might reduce Cl⁻ and fluid absorption. Conversely, elevated serosal CO₂ was hypothesized to stimulate intestinal HCO₃⁻ secretion and thereby Cl⁻ and fluid absorption.

MATERIALS AND METHODS

Experimental animals. European flounder, Platichthys flesus, were obtained by local fisherman from inshore areas of variable salinity and acclimated to laboratory holding conditions at a salinity of 20‰ for a minimum of 5 days at the marine station in Kerteminde, Fyn, Denmark. Fish were then transferred to 33‰ salinity water (full-strength seawater) and were allowed to acclimate for an additional 3 days before experimentation. Fish were not fed during holding and acclimation, and experimental temperature was kept constant at 12°C via climate-controlled laboratory facilities.

General experimental approach. Fish were killed by an overdose of tricaine methanesulfonate (MS-222; 0.25 g/l), and the entire intestine was obtained by dissection. The intestine was separated from the stomach just posterior to the pyloric sphincter. Care was taken not to include the distal part of the gastrointestinal tract (the rectum), which is separated from the posterior intestine by a distinct sphincter. A short length of heat-flared polyethylene (PE) tubing was tied in the anterior end of the intestine, and the entire intestine was flushed with 20 ml of the appropriate mucosal saline (see Table 1). Subsequently, sacs were prepared from the anterior, the mid-, and the posterior segments of the intestine, each ~ 30 mm in length. Each segment was fitted with a PE-tubing catheter (PE-60) tied in the proximal end with double silk thread to fill and drain the sac preparation. The PE catheter was thereafter sealed, and the sac preparation was blotted dry with paper towels. The mass of the preparation (consisting of intestinal tissue and mucosal saline) was determined to the nearest 100 mg.

Table 1. General saline composition

<table>
<thead>
<tr>
<th></th>
<th>Mucosal Saline</th>
<th>Serosal Saline</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Cl⁻ free</td>
</tr>
<tr>
<td>CaCl₂(2H₂O)</td>
<td>5.0</td>
<td>5.0</td>
</tr>
<tr>
<td>Calcium gluconate</td>
<td>5.0</td>
<td>5.0</td>
</tr>
<tr>
<td>Glucose</td>
<td>11.0</td>
<td>11.0</td>
</tr>
<tr>
<td>NaCl</td>
<td>100.0</td>
<td>100.0</td>
</tr>
<tr>
<td>MgCl₂(7H₂O)</td>
<td>22.5</td>
<td>22.5</td>
</tr>
<tr>
<td>MgSO₄</td>
<td>100.0</td>
<td>100.0</td>
</tr>
<tr>
<td>NaCl</td>
<td>49.0</td>
<td>49.0</td>
</tr>
<tr>
<td>Sodium gluconate</td>
<td>11.0</td>
<td>11.0</td>
</tr>
<tr>
<td>NaHCO₃</td>
<td>10.0</td>
<td>10.0</td>
</tr>
<tr>
<td>NaH₂PO₄(H₂O₂)</td>
<td>10.0</td>
<td>10.0</td>
</tr>
<tr>
<td>Choline-HCO₃</td>
<td>8.0</td>
<td>8.0</td>
</tr>
<tr>
<td>pH*</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Osmolality†</td>
<td>308</td>
<td>308</td>
</tr>
</tbody>
</table>

All values are in mM, except osmolality (mosmol/kg H₂O) and pH. *When gassed with 0.5% CO₂ in O₂; †adjusted with mannitol.
vation by performing three subsequent measurements on each sac preparation, randomizing the order of mucosal and serosal HCO₃⁻ concentrations (3 intestinal segments from each of 4 fish). In all cases, salines were osmotically compensated with mannitol to ensure the same osmolality of mucosal and serosal salines regardless of HCO₃⁻ concentrations.

TEP measurements were performed under the above conditions on sac preparations of all three segments from a total of three individual fish. The TEP was measured under traditional symmetrical conditions with serosal saline (which resembled the blood plasma composition; see Table 1) on both surfaces and also with mucosal saline (which approached the in vivo intestinal fluid composition; see Table 1) on the mucosal surface and serosal saline on the serosal surface. Under the latter condition, HCO₃⁻ concentrations were manipulated. As above, the order of experimental conditions was randomized throughout. We performed TEP measurements using agar/salt bridges (3 M KCl in 4% agar) connected through Ag/AgCl electrodes (World Precision Instruments) to a Radiometer (Copenhagen, Denmark) PHM 84 high-impedance electrometer. We expressed all TEP values using a high-impedance electrometer (BMS3 system), with the signal displayed on a PHM 73 blood-gas monitor. We measured total CO₂ in all salines using a 7-bulb blood gas analyzer (Mettler Toledo 965). The concentration of HCO₃⁻ equivalents was calculated from these measurements according to the Henderson-Hasselbalch equation. Initially, HCO₃⁻ concentrations were determined from measurements of total CO₂ and pH using the following rearrangement of the Henderson-Hasselbalch equation:

\[
[HCO_3^-] = [total \ CO_2]/(1 + 10^{pK_a-pH})
\]

where brackets indicate concentration and where \( pK_a \) is the appropriate ionic strength (1/3 seawater), obtained from Ref. 27. Subsequently, the contribution of molecular CO₂ was determined as

\[
[\text{Molecular } \ CO_2] = [HCO_3^-]/(1 + 10^{pK_a-pH})
\]

using the apparent \( pK_a \) for ionic strength equivalent of teleos plasma at 12°C for the measured pH from Boutillier et al. (3).

Thus \( [total \ CO_2] = [\text{molecular } \ CO_2] + [HCO_3^-] + [CO_2^-] \). In practice the molecular CO₂ is generally very low (<10⁻⁶ M) at the pH values used in the present experiments; thus

\[
[CO_2^-] = [total \ CO_2] - [HCO_3^-]
\]

From the estimated concentrations of HCO₃⁻ and CO₂⁻, we determined the total HCO₃⁻ equivalents present in a saline sample as [HCO₃⁻] + 2[CO₂⁻].

The validity of this approach has been established previously (7), which showed a correlation coefficient \( r^2 \) of 0.999 between HCO₃⁻ equivalents determined as described above from measurements of pH and total CO₂ and HCO₃⁻ determined by double-endpoint titration (titratable alkalinity). For simplicity, hereafter in the text “concentration of HCO₃⁻ equivalents” is referred to as “HCO₃⁻ concentrations.”

The internal mucosal saline volumes of each individual sac preparation at the beginning and end of the experiments were determined by subtracting the mass of the empty sac preparation from the corresponding initial or final total mass of the filled preparation. Net fluid transport was determined from the difference in total mass over the experimental period, taking into account the gross surface area of exposed epithelium and time elapsed. Net fluxes of Na⁺, Cl⁻, and HCO₃⁻ were calculated from the differences in mucosal saline volume and ion concentration at the beginning and end of each flux period, again taking into account the gross surface area and time elapsed.

Unidirectional mucosal-to-serosal Cl⁻ transport was calculated from the appearance of the \( ^{36}\text{Cl} \) isotope in the serosal saline, and unidirectional Na⁺ transport was calculated from the disappearance of
Na from the mucosal saline. For both ions, the mean of the corresponding specific activities in the mucosal saline at the start and the end of the flux period allowed for calculation of absolute ion flux rates. Unidirectional serosal-to-mucosal Na⁺ and Cl⁻ transports were determined from the individual differences between the corresponding net flux and unidirectional mucosal-to-serosal flux rate.

**RESULTS**

**Preparation viability and regional differences.** Based on constant net flux of HCO₃⁻, Na⁺, and Cl⁻ as well as unidirectional Na⁺ and Cl⁻ flux rates over three subsequent 2-h flux periods, the intestinal preparations appeared viable and stable at least 6 h after initial preparation (Table 2). In agreement with previous reports (7, 8, 10), there were no significant differences in net ion transport, unidirectional Na⁺ and Cl⁻ flux rates, or fluid absorption rates among the three intestinal segments used in the present study (Table 3). Combining measurements from control preparations in all experiments further supported this observation (data not shown), with overall mean values being $-0.28 \pm 0.02 \mu\text{mol} \cdot \text{cm}^{-2} \cdot \text{h}^{-1}$ for net HCO₃⁻ secretion, $2.12 \pm 0.30 \mu\text{mol} \cdot \text{cm}^{-2} \cdot \text{h}^{-1}$ for fluid absorption, and $0.49 \pm 0.04 \mu\text{mol} \cdot \text{cm}^{-2} \cdot \text{h}^{-1}$ for net Cl⁻ absorption ($n = 99$). Unexpectedly, net Na⁺ transport was characterized by secretion in the order of $-0.37 \pm 0.04 \mu\text{mol} \cdot \text{cm}^{-2} \cdot \text{h}^{-1}$. Because of the homogeneity among intestinal segments, data obtained from the anterior, mid-, and posterior segments were combined and presented as one in Figs. 1–7.

**Effects of HCO₃⁻ gradients.** Intestinal HCO₃⁻ secretion was stimulated by elevated serosal HCO₃⁻ concentrations (Fig. 1). Net Cl⁻ absorption and fluid movement tended to be higher with higher serosal HCO₃⁻ and thus HCO₃⁻ secretion rates. Interestingly, HCO₃⁻ secretion was present even with a serosal HCO₃⁻ concentration as low as 1 mM and with a mucosal HCO₃⁻ concentration of 11 mM at the beginning of the flux period (Fig. 1A). Conversely, elevated mucosal HCO₃⁻ concentrations reduced intestinal net HCO₃⁻ secretion in the presence of 11 mM serosal HCO₃⁻, whereas low mucosal HCO₃⁻ concentrations resulted in highly elevated HCO₃⁻ secretion (Fig. 2).

When the preparations were exposed to serosal saline (which duplicated blood plasma composition) on both sides (symmetrical conditions), TEP was only $-2.2 \pm 0.6 \text{ mV}$. However, when the preparations were exposed to in vivo-like conditions (mucosal saline in the lumen, Table 1), the TEP decreased markedly to $-16.2 \pm 2.3 \text{ mV}$. Varying the transepithelial HCO₃⁻ gradient under in vivo-like conditions did not significantly influence TEP (Fig. 3).

**Influence of extracellular CO₂.** Changing the gassing of serosal saline from 0.5% CO₂-99.5% O₂ to 0% CO₂-100% O₂ did not influence HCO₃⁻ secretion, unidirectional and net fluxes of Na⁺ and Cl⁻, or fluid absorption (Fig. 4). Elevating CO₂ to 2%, however, approximately doubled HCO₃⁻ secretion rates and elevated net Cl⁻ and fluid absorption. In addition, the net Na⁺ loss seen under control conditions was reduced (Fig. 4).

**Effects of acetazolamide.** The carbonic anhydrase inhibitor acetazolamide when applied at $10^{-4} \text{ M}$ reduced intestinal HCO₃⁻ secretion by 30–40% but did not influence any of the other measured intestinal transport parameters (Fig. 5).

**DISCUSSION**

**Active HCO₃⁻ secretion?** In isolated flounder intestinal segments, HCO₃⁻ secretion occurs against a chemical gradient, with a TEP of $-16.2 \text{ mV}$ serosal side negative. The recorded TEP under in vivo-like conditions was much higher than the corresponding value obtained under symmetrical conditions (serosal saline on both sides of the epithelium). This is consistent with previous reports for intestinal preparations of the European flounder (30) and the winter flounder, *Pseudopleuronectes americanus* (12), and the gall bladder epithelium of...
Table 3. Ion and water flux rates in three intestinal segments

<table>
<thead>
<tr>
<th>Segment</th>
<th>HCO₃⁻ net flux</th>
<th>Cl⁻ net flux</th>
<th>Cl⁻ influx</th>
<th>Cl⁻ efflux</th>
<th>Na⁺ net flux</th>
<th>Na⁺ influx</th>
<th>Na⁺ efflux</th>
<th>Water Transport Rate (H₂O net flux), μl·cm⁻²·h⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ant</td>
<td>-0.27±0.05</td>
<td>-0.30±0.32</td>
<td>3.28±0.51</td>
<td>-3.58±0.55</td>
<td>-0.68±0.16</td>
<td>1.34±0.22</td>
<td>-2.02±0.24</td>
<td>1.69±0.93</td>
</tr>
<tr>
<td>Mid</td>
<td>-0.33±0.04</td>
<td>0.44±0.40</td>
<td>2.40±0.40</td>
<td>-1.96±0.55</td>
<td>-0.53±0.23</td>
<td>2.01±0.35</td>
<td>-2.54±0.34</td>
<td>1.80±1.49</td>
</tr>
<tr>
<td>Post</td>
<td>-0.44±0.06</td>
<td>0.67±0.24</td>
<td>2.27±0.33</td>
<td>-1.60±0.32</td>
<td>-0.22±0.18</td>
<td>2.17±0.44</td>
<td>-2.40±0.34</td>
<td>1.65±1.74</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 15 measurements in all cases. Net flux of HCO₃⁻ (negative to indicate secretion), net flux and influx rates, and efflux rates of Cl⁻ and Na⁺ as well as net fluid movement in isolated intestinal segments of the anterior (Ant), mid-, and posterior (Post) regions of the intestine from the European flounder are shown. There were no significant differences among regions (P < 0.05).

Fig. 1. Net flux of HCO₃⁻ (A), unidirectional efflux and influx rates (open bars) and net flux rates (hatched bars) of Cl⁻ (B) and Na⁺ (C), and net water movement (D) in isolated intestinal segments from the European flounder at different serosal HCO₃⁻ concentrations ([HCO₃⁻]). Mucosal [HCO₃⁻] was 11 mM. Results are means ± SE; n = 12 measurements in all cases. Means labeled with different letters are significantly different (P < 0.05).

Fig. 2. Net flux of HCO₃⁻ (A), unidirectional efflux and influx rates (open bars) and net flux rates (hatched bars) of Cl⁻ (B) and Na⁺ (C), and net water movement (D) in isolated intestinal segments from the European flounder at different mucosal [HCO₃⁻]. Serosal [HCO₃⁻] was 11 mM. Results are means ± SE; n = 12 measurements in all cases. Means labeled with different letters are significantly different (P < 0.05).
the HCO₃⁻ concentration (up to 100 mM) is consistent with previous reports of limitation compared with in vivo gradients (luminal concentration). This becomes clear when considering individual HCO₃⁻ secretion rates, as hypothesized. Because HCO₃⁻ secretion occurs via apical anion exchange (see below), which is not in itself ATP consuming, this secretion must be of secondary active nature. Varying the mucosal HCO₃⁻ concentration, but even the combined effect of altering pH and HCO₃⁻ concentration did not significantly alter TEP. The Nernst equation provides the equilibrium potential (E) required to sustain a given chemical gradient across an epithelium: 

\[ E = \frac{(RT)}{2.303F} \log \left( \frac{\text{serosal} [X]}{\text{mucosal} [X]} \right) \]

where X represents the ion in question on the outer (o) and inner (i) side of the epithelium, z is the valence of this ion, and R, T, and F have their usual meanings. When isolated segments were bathed in 1 mM HCO₃⁻ in the serosal saline and in 11 mM HCO₃⁻ in the mucosal saline (Fig. 1), the epithelial potential required, according to the Nernst equation, for continuous secretion of HCO₃⁻ would have to be lower than −58.9 mV serosal side negative for nonactive transport to occur. This predicted equilibrium potential is much more negative than the recorded TEP under those conditions (Fig. 3), demonstrating that the intestinal epithelium is capable of performing (secondary) active HCO₃⁻ secretion as hypothesized. Because HCO₃⁻ secretion occurs via apical anion exchange (see below), which is not in itself ATP consuming, this secretion must be of secondary active nature. Varying the mucosal HCO₃⁻ concentration revealed that, although HCO₃⁻ secretion can occur against an electrochemical gradient, it is almost completely abolished when mucosal HCO₃⁻ concentrations are elevated to 25 mM (Fig. 2).

Considering individual HCO₃⁻ secretion rates, measured in the HCO₃⁻ gradient experiments, as a function of the corresponding mean HCO₃⁻ gradient across the epithelium, it appears that transepithelial HCO₃⁻ secretion can occur against an absolute gradient of up to 17 mM (Fig. 8A). This apparent limitation compared with in vivo gradients (luminal concentrations up to 100 mM) is consistent with previous reports of lower HCO₃⁻ secretion rates in isolated epithelia than those seen in intact animals (31). Because the isolated intestinal preparation appears stable and healthy for the duration of these experiments, this may suggest that the HCO₃⁻ secretory process is not fully stimulated or activated in these preparations. Nevertheless, the isolated intestinal segments are clearly capable of active HCO₃⁻ secretion even in this nonstimulated state. This becomes clear when considering individual HCO₃⁻ secretion rates now as a function of transepithelial HCO₃⁻ concentration ratio, which is thermodynamically relevant (see the Nernst equation), rather than absolute concentration differences. HCO₃⁻ secretion in the intestinal sac preparations continued until log (serosal [HCO₃⁻]/mucosal [HCO₃⁻]) became as low as −1.7 (Fig. 8B), which corresponds to an equilibrium potential of −96 mV, clearly much more negative than the TEP measured under the same conditions (Fig. 3).

Source of HCO₃⁻. The experiments with elevated and reduced serosal HCO₃⁻ concentrations (Fig. 1) suggest that serosal HCO₃⁻ may provide substrate for intestinal HCO₃⁻ secretion. HCO₃⁻ could enter the intestinal epithelial cells across the basolateral Cl⁻/HCO₃⁻ exchanger (AE) as first proposed by the rainbow trout, Oncorhynchus mykiss (11). Although the type of mucosal saline influences TEP, varying the HCO₃⁻ gradient did not appear to have an effect (Fig. 5). The pH varied with HCO₃⁻ concentration, but even the combined effect of altering pH and HCO₃⁻ concentration did not significantly alter HCO₃⁻ secretion rates, measured in the HCO₃⁻ gradient experiments, as a function of the corresponding mean HCO₃⁻ gradient across the epithelium, it appears that transepithelial HCO₃⁻ secretion can occur against an absolute gradient of up to 17 mM (Fig. 8A). This apparent limitation compared with in vivo gradients (luminal concentrations up to 100 mM) is consistent with previous reports of lower HCO₃⁻ secretion rates in isolated epithelia than those seen in intact animals (31). Because the isolated intestinal preparation appears stable and healthy for the duration of these experiments, this may suggest that the HCO₃⁻ secretory process is not fully stimulated or activated in these preparations. Nevertheless, the isolated intestinal segments are clearly capable of active HCO₃⁻ secretion even in this nonstimulated state. This becomes clear when considering individual HCO₃⁻ secretion rates now as a function of transepithelial HCO₃⁻ concentration ratio, which is thermodynamically relevant (see the Nernst equation), rather than absolute concentration differences. HCO₃⁻ secretion in the intestinal sac preparations continued until log (serosal [HCO₃⁻]/mucosal [HCO₃⁻]) became as low as −1.7 (Fig. 8B), which corresponds to an equilibrium potential of −96 mV, clearly much more negative than the TEP measured under the same conditions (Fig. 3).

Source of HCO₃⁻. The experiments with elevated and reduced serosal HCO₃⁻ concentrations (Fig. 1) suggest that serosal HCO₃⁻ may provide substrate for intestinal HCO₃⁻ secretion. HCO₃⁻ could enter the intestinal epithelial cells across the basolateral Cl⁻/HCO₃⁻ exchanger (AE) as first proposed by
Dixon and Loretz (4). Additional potential basolateral HCO₃⁻ carriers include the Na⁺-HCO₃⁻ cotransporter (NBC), as seen in guinea pig and human pancreatic ducts (1, 13), and the electroneutral Na⁺-driven Cl⁻/HCO₃⁻ exchanger (NDCBE) identified from human brain (6). However, the previously demonstrated lack of sensitivity of intestinal HCO₃⁻ secretion to serosal DIDS (10⁻³ M) in the European flounder (8) does not support the involvement of basolateral AE, NBC, or NDCBE, as these transporters are sensitive to stilbenes (6, 23). Nevertheless, a negative result with DIDS does not conclusively exclude the possible involvement of AE, NBC, and NDCBE, as species-specific differences in DIDS sensitivity do occur (14). The involvement of NDCBE offers an appealing hypothesis because this transporter effectively exchanges NaHCO₃ for HCl (6). Such an exchange would aid not only HCO₃⁻...
import but also proton extrusion across the basolateral membrane. The latter would prevent reversal of the carbonic anhydrase-mediated CO₂ hydration (10), which seems to be important for HCO₃⁻ secretion (Figs. 4 and 5, see also Fig. 9). In support of the involvement of NDCBE are previous observations of intestinal HCO₃⁻ secretion in marine teleosts being dependent on serosal Na⁺ (2, 10).

Although the observations of increased HCO₃⁻ secretion with increased serosal HCO₃⁻ concentrations strongly suggest transepithelial HCO₃⁻ transport, it cannot be ruled out that the pH in the serosal fluids, which depends on the HCO₃⁻ concentration, might have influenced HCO₃⁻ secretion. The pH in the serosal salines containing 1, 11, and 25 mM HCO₃⁻ was 6.545 ± 0.021, 7.810 ± 0.028, and 8.166 ± 0.071, respectively. Because the HCO₃⁻ secretion likely depends on proton extrusion across the basolateral membrane (Fig. 9) and because this extrusion would have occurred against a lower serosal proton concentration in experiments with high serosal HCO₃⁻ concentrations, elevated basolateral proton extrusion rather than, or in combination with, increased basolateral HCO₃⁻ import could be responsible for the observed stimulation of HCO₃⁻ secretion.

A recent report on HCO₃⁻ secretion in the European flounder did not find evidence for transepithelial HCO₃⁻ transport (30), which is in contrast to the present study. The discrepancy between these two studies, performed on the same species and using very similar salines but different techniques for measuring HCO₃⁻ secretion, could perhaps be explained by the different temperatures at which these studies were conducted. The present study was conducted at a slightly lower temperature (12°C compared with 14°C), and it seems likely that the higher temperature in the previous study (30) would increase epithelial metabolic rate and thereby CO₂ production, reducing the
need for basolateral HCO$_3^\text{-}$ import to sustain luminal secretion. An additional possible explanation for the discrepancy is that, whereas the serosal pH varied with the serosal HCO$_3^\text{-}$ concentration in the present study, the previous study was conducted in the presence and absence of serosal HCO$_3^\text{-}$ but at constant pH (30). The reduced pH in serosal salines containing 1 mM HCO$_3^\text{-}$ in the present study may have impaired proton secretion and thus HCO$_3^\text{-}$ secretion, whereas this would not have been the case in the previous study, which used HEPES buffering in the HCO$_3^\text{-}$-free serosal saline.

It seems that elevated serosal CO$_2$ (2%) may fuel CO$_2$ hydration, which provides HCO$_3^\text{-}$ for luminal secretion (Fig. 4). Elevating serosal CO$_2$ from 0.5 to 2.0% resulted in a slightly increased serosal HCO$_3^\text{-}$ concentration (measured values at start of flux period of 9.62 ± 0.10 and 10.27 ± 0.05, respectively) and a change in pH from 7.77 to 7.22 at the beginning of the flux period. Reduced serosal pH cannot explain the increased HCO$_3^\text{-}$ secretion seen in these experiments, as elevated serosal proton concentrations would lead to reduced basolateral proton extrusion and thus reduced apical HCO$_3^\text{-}$ secretion (Fig. 9). Furthermore, the slight increase in serosal HCO$_3^\text{-}$ concentration is not sufficient to explain the twofold elevation in HCO$_3^\text{-}$ secretion rate, which must therefore be the result of elevated CO$_2$. However, it is also clear that endogenous CO$_2$ production is sufficient to provide most of the CO$_2$ for hydration under physiological conditions (0.5% CO$_2$) because removal of this serosal CO$_2$ does not lead to reduced HCO$_3^\text{-}$ secretion. These latter findings are in agreement with recent studies on the European flounder, which concluded that endogenous CO$_2$ was fueling the HCO$_3^\text{-}$ secretion (30). Previous estimates of the metabolic rate of the intestinal tissue of a marine teleost in the context of HCO$_3^\text{-}$ secretion suggested that endogenous CO$_2$ production rates may be insufficient in sustaining the observed HCO$_3^\text{-}$ secretion metabolic rate (10). However, it should be noted that these estimates were not based on actual measurements of intestinal epithelium metabolic rate. Because the metabolic rate of the intestinal epithelium can be expected to be high, compared with whole animal metabolic rate, which formed the basis for the above estimates, these estimates might have underestimated the potential contribution of endogenous metabolic CO$_2$.

Carbonic anhydrase appears to facilitate CO$_2$ hydration, which fuels HCO$_3^\text{-}$ secretion in that application of the carbonic anhydrase inhibitor acetazolamide led to a reduction in HCO$_3^\text{-}$ secretion rate (Fig. 7). This observation is in agreement with a previous report of reduced intestinal HCO$_3^\text{-}$ secretion rate caused by acetazolamide treatment in seawater-acclimated rainbow trout (29). Neither in the present study nor in the study performed on rainbow trout did inhibition of carbonic anhydrase completely abolish HCO$_3^\text{-}$ secretion, suggesting that noncatalyzed CO$_2$ hydration is sufficient to support part of the intestinal HCO$_3^\text{-}$ secretion.

**Interactions between HCO$_3^\text{-}$ secretion and Cl$^\text{-}$.** Cl$^\text{-}$-free mucosal saline greatly reduced but did not completely abolish HCO$_3^\text{-}$ secretion (Fig. 6), which is in agreement with previous reports from several marine teleost fish (2, 10, 29). Measurements of Cl$^\text{-}$ in the mucosal “Cl$^\text{-}$-free” saline revealed an average of 3.5 mM Cl$^\text{-}$ and 20.7 mM Cl$^\text{-}$ in samples obtained at the beginning and at the end of the flux periods, respectively. Low levels of Cl$^\text{-}$ in the Cl$^\text{-}$-free saline, presumably coming from incomplete rinsing of the intestinal lumen at the beginning and from serosal-to-mucosal Cl$^\text{-}$ diffusion during the experiments, is perhaps the reason for the remaining HCO$_3^\text{-}$ secretion, mediated by apical anion exchange, observed under these conditions. A slight net Na$^\text{+}$ loss seen in control preparations was greatly increased under Cl$^\text{-}$-free mucosal conditions, clearly showing the involvement of Na$^\text{+}$-Cl$^\text{-}$ and/or Na$^\text{+}$-K$^\text{+}$-2Cl$^\text{-}$ cotransporters in intestinal NaCl absorption.

Removal of serosal Cl$^\text{-}$ enhanced HCO$_3^\text{-}$ secretion (Fig. 6), which is in contrast to previous studies performed on the Pacific sandbunch, where removal of serosal Cl$^\text{-}$ had no effect on HCO$_3^\text{-}$ secretion (10). The reason for this discrepancy is unknown, but the difference likely reflects that different transport mechanisms are involved in HCO$_3^\text{-}$ secretion in these two species. Removal of serosal Cl$^\text{-}$ effectively reverses the electrochemical gradient for Cl$^\text{-}$ across the intestinal epithelium and resulted in a more negative TEP (Fig. 7), which may have contributed to the enhanced HCO$_3^\text{-}$ secretion. In the European flounder, serosal Cl$^\text{-}$ removal not only increased the net absorption of Cl$^\text{-}$, and thereby the HCO$_3^\text{-}$ secretion, driven by apical anion exchange but also reversed the net Na$^\text{+}$ loss seen in control preparations to a slight net Na$^\text{+}$ uptake (Fig. 6), again showing the involvement of NaCl cotransporters.

**Contribution of Cl$^\text{-}$/HCO$_3^\text{-}$ exchange to Cl$^\text{-}$ and water transport.** All control preparations with the exception of those displayed in Fig. 2 exhibited significant water absorption (overall mean of 2.12 ± 0.30 µl·cm$^{-2}$·h$^{-1}$, n = 99). The reason for the lack of water absorption in the control preparations of Fig. 2 is unknown. The present data suggest that anion exchange may contribute significantly to Cl$^\text{-}$ and water absorption across the intestinal epithelium. Elevated HCO$_3^\text{-}$ secretion caused by increased serosal HCO$_3^\text{-}$ concentration, Cl$^\text{-}$-free serosal salines, and increased CO$_2$ results in increased net Cl$^\text{-}$ and fluid absorption (Figs. 1, 4, and 6), although this was not statistically significant in the case of fluid absorption during exposure to elevated serosal HCO$_3^\text{-}$. Reduction of HCO$_3^\text{-}$ secretion by increased mucosal-serosal HCO$_3^\text{-}$ gradients (Fig. 2) or exposure to the carbonic anhydrase inhibitor acetazolamide, however, did not result in significant reduction of net Cl$^\text{-}$ and fluid absorption. Removal of mucosal Cl$^\text{-}$, depleting both the anion exchange process and the Na$^\text{+}$-Cl$^\text{-}$ and/or Na$^\text{+}$-K$^\text{+}$-2Cl$^\text{-}$ cotransport systems, however, resulted in reduced HCO$_3^\text{-}$ secretion and fluid absorption as well as increased net Na$^\text{+}$ loss. Conversely, removal of serosal Cl$^\text{-}$ stimulated HCO$_3^\text{-}$ secretion as well as net Na$^\text{+}$ and fluid absorption. It thus appears that two parallel systems exist for Cl$^\text{-}$ and fluid absorption across the marine teleost intestine, one being the traditionally accepted Na$^\text{+}$-Cl$^\text{-}$ and/or Na$^\text{+}$-K$^\text{+}$-2Cl$^\text{-}$ cotransport pathway and the other being an anion-exchange-mediated system. From the present study, it appears that the relative importance of these two systems may depend on luminal chemistry and perhaps other parameters. In the case of reduced HCO$_3^\text{-}$ secretion, Cl$^\text{-}$ and fluid absorption still persist, presumably mediated by the Na$^\text{+}$-Cl$^\text{-}$ and/or Na$^\text{+}$-K$^\text{+}$-2Cl$^\text{-}$ system. However, in the case of stimulated HCO$_3^\text{-}$ secretion, it appears that both Cl$^\text{-}$ and fluid absorption are also stimulated, suggesting an important role of this system in marine teleost osmoregulation.

It is not possible from the present data to accurately separate the relative contributions of these two systems. It should be noted, however, that the HCO$_3^\text{-}$ secretion rates observed in the isolated intestinal segments are lower than the corresponding...
rates observed in vivo and that stimulated HCO$_3^-$ secretion rates in the present study (approaching those seen in vivo) stimulate Cl$^-$ and fluid absorption rates. This may suggest that the anion exchange is relatively more important under in vivo conditions. Indeed, an in situ intestinal perfusion study performed on the lemon sole, Parophrys vetulus, revealed Cl$^-$ absorption rates twice as high as the corresponding rates for Na$^+$, with the difference accounted for by HCO$_3^-$ secretion rates. This was interpreted to mean that the Cl$^-$/HCO$_3^-$ exchange system accounted for ~50% of the total Cl$^-$ absorption (7). Furthermore, when Na$^+$ uptake was completely inhibited by treatment with silver, substantial Cl$^-$ absorption persisted and matched perfectly with HCO$_3^-$ secretion, and no reduction in fluid absorption was observed (7). It thus appears that intestinal anion exchange may play a quantitatively important role in Cl$^-$ and fluid absorption across marine teleost intestinal epithelia.

The mechanism(s) of water transport continues to be a controversial topic, but there seems to be general consensus that fluid movement is driven by NaCl transport and that the absorbate is isotonic (16, 17). In the present study, in almost all cases, significant water absorption was observed in the absence of net Na$^+$ absorption, which contrasts with this general view. Water absorption occurred despite no transepithelial difference in osmotic pressure. It is clear, however, that fluid absorption across the European flounder intestine is linked to active net Cl$^-$ uptake (Figs. 1, 4, and 6). Cl$^-$ absorption must be associated with accompanying cation absorption or anion secretion to maintain charge balance in the absorbed fluid. Considering all control measurements in the present study, mean net Cl$^-$ absorption was 0.58 ± 0.04 μmol·cm$^{-2}$·h$^{-1}$ and net HCO$_3^-$ secretion was 0.28 ± 0.02 μmol·cm$^{-2}$·h$^{-1}$ (n = 99). This suggests that 0.3 μeq of cation cm$^{-2}$·h$^{-1}$ (different from Na$^+$) must have been absorbed in conjunction with the absorbed Cl$^-$, K$^+$, Ca$^{2+}$, and Mg$^{2+}$ are possible candidates, but the net transport rates of these ions were not measured in the present study. The observed active HCO$_3^-$ secretion must be associated with proton extrusion to prevent reversal of the (carbonic anhydrase-mediated) CO$_2$ hydration reaction. It has been argued that this proton extrusion must occur predominantly across the basolateral membrane because the intestinal epithelium exhibits net base secretion (10) (Fig. 9). Basolateral proton extrusion may account for part of the “missing cation” required for charge balance, which would result in an acidic absorbate. The mechanism of basolateral H$^+$ extrusion is presently unknown and offers an exciting area for future studies.

**Perspectives**

In conclusion, the intestinal epithelium of the European flounder is capable of secondary active HCO$_3^-$ secretion, and this may be a general phenomenon of marine teleost fish. This secretion occurs via anion exchange, contributes significantly to Cl$^-$ absorption, and provides an explanation for fluid absorption in the absence of net Na$^+$ absorption. The marine teleost intestine offers an interesting comparison to other fluid-absorbing epithelia, including the mammalian duodenum and the vertebrate gall bladder and renal tubules, and is an easily accessible organ of practical size for experimentation. Furthermore, the ability of the intestinal epithelium to perform secondary active HCO$_3^-$ secretion, yielding luminal concentrations in excess of 100 mM, greatly resembles that of the ducts of the exocrine pancreas, an organ which is far from fully understood (22). The marine teleost intestine may thus be an interesting model for studies of active epithelial HCO$_3^-$ secretion and may offer insight into the mechanisms of this transport system not only in fish intestines but also in other organ systems of higher vertebrates.

**GRANTS**

This work was supported by a National Science Foundation Grant (0416440) to M. Grosell and a National Sciences and Engineering Research Council discovery grant to C. M. Wood. C. M. Wood is supported by the Canadian Research Chair Program.

**REFERENCES**


