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# Intestinal $HCO_3^-$ secretion in marine teleost fish: evidence for an apical rather than a basolateral $Cl^-/HCO_3^-$ exchanger

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**Abstract**

Intestinal fluid was collected from 11 marine teleost fish from the Baltic sea and the Pacific ocean. The anterior, mid and posterior segments of the intestine contained 33–110 mM of $HCO_3^-$ equivalents (with exception of the Atlantic cod which contained only 5–15 mM). Considering literature values of transepithelial potentials and concentration gradients, these high levels of $HCO_3^-$ equivalents are probably the result of active $HCO_3^-$ transport. Possible $HCO_3^-$ transport mechanisms were studied in the Pacific sanddab (*Citharichthys sordidus*) *in vitro*. Measurements of net secretion of $HCO_3^-$ equivalents across the intestinal epithelium revealed mucosal DIDS sensitivity ($10^{-4}$ M) and $Cl^-$-dependence of the $HCO_3^-$ equivalent net flux, but no serosal DIDS ($10^{-4}$ M) sensitivity. Net $Na^+$ uptake was abolished in the absence of $Cl^-$, but some $Cl^-$ uptake persisted in the absence of $Na^+$, at a rate similar to that of net $HCO_3^-$ secretion. Anterior, mid and posterior segments of the intestine performed similarly. These observations support the presence of an apical rather than a basolateral $Cl^-/HCO_3^-$ exchanger and thus contrast the currently accepted model for intestinal $HCO_3^-$ secretion. This apical $Cl^-/HCO_3^-$ exchanger alone, however, is not sufficient for maintaining the observed $HCO_3^-$ equivalents gradient *in vivo*. We suggest a coupling of cytosolic carbonic anhydrase, a basolateral proton pump and the apical $Cl^-/HCO_3^-$ exchanger to explain the intestinal $HCO_3^-$ transport.

## Introduction

The intestine of marine teleost fish plays an important role in osmoregulation (Smith, 1930). Due to the surrounding hyper-osmotic environment, marine teleosts are constantly losing fluid and thus face dehydration. To compensate for this fluid loss, marine teleosts drink seawater. The seawater is diluted in the anterior regions by NaCl absorption of the gastrointestinal tract (GIT) so as to reach a tonicity equivalent to approximately 1/3 seawater, iso-osmotic to the extracellular fluid, at the point of the anterior intestine. Water uptake from the intestine follows the active trans-epithelial transport of $Na^+$ and $Cl^-$. The consequent salt gain is subsequently eliminated across the gills.

More recently, an additional role of the intestine in acid-base regulation has been investigated. Observations of high $HCO_3^-$ concentrations in the intestinal fluids of a marine teleost, the gulf toadfish (*Opsanus beta*) (Walsh et al. 1991) led to the hypothesis that the intestine may contribute to acid-base regulation through secretion of considerable amounts of $HCO_3^-$ across the intestinal epithelium followed by rectal excretion. This hypothesis was first tested by Wilson and co-workers who found that rectal base excretion contributed significantly to whole body acid-base status in seawater acclimated rainbow trout (*Oncorhynchus*

*mykiss*) (Wilson et al. 1996; see Wilson 1999 for review).

In the present study, we document high levels of $HCO_3^-$ in intestinal fluids from 10 out of 11 investigated species. Intestinal $HCO_3^-$ secretion thus appears to be a generally occurring phenomenon in marine teleosts. The intestinal epithelium is characterized as a 'leaky epithelium' and thus exhibits low (3–8 mV, blood side negative) trans-epithelial potentails (TEP) (Kirschner 1991; Ando 1990). Typical levels of $HCO_3^-$ in intestinal fluids range from 30 to more than 100 mM, whereas the blood plasma $HCO_3^-$ levels are typically 4–10 mM (Wilson 1999; Grosell et al. 1999) and the blood to lumen $HCO_3^-$ gradient cannot be sustained by the low blood side negative TEP, indicating $HCO_3^-$ secretion against an electrochemical gradient by the intestinal epithelium.

In a recent review of ion and water transport processes in marine teleost intestinal epithelium, Loretz (1995) proposed a $Cl^-/HCO_3^-$ exchanger located at the basolateral membrane. Typical criteria for the presence of a $Cl^-/HCO_3^-$ exchanger are $Cl^-$-dependence of $HCO_3^-$ transport and DIDS (4,4′diisothiocyanostilbene-2,2′-disulphonic acid) sensitivity. DIDS is a well-known inhibitor of $Cl^-/HCO_3^-$ exchange in erythrocytes and other cells (Nikinmaa 1990). The proposed localization of the $Cl^-/HCO_3^-$ exchanger was based on the original observations of $Cl^-$-dependence and serosal DIDS sensitivity of luminal alkalinization in Ussing-chamber experiments with anterior intestinal segments from *Gillichthys mirabilis* (Dixon and Loretz 1986). Later, however, Ando and Subramanyam (1990) reported $Cl^-$-dependence and both serosal and mucosal DIDS ($5 \times 10^{-4}$ M) sensitivity of luminal alkalinization in Ussing-chamber experiments with intestinal epithelium of the Japanese eel (*Anguilla japonica*). The latter study thus provides evidence for an apical $Cl^-/HCO_3^-$ exchanger. The study by Wilson et al. (1996) reported mucosal $Cl^-$ dependence of $HCO_3^-$ secretion *in vivo* in seawater-acclimated rainbow trout but no sensitivity to mucosal DIDS at $2 \times 10^{-5}$ M. Recently, Grosell and Jensen (1999) found that intestinal $HCO_3^-$ secretion *in vitro* in the European flounder (*Plathichthys flesus*) was sensitive to DIDS applied to the mucosal solution ($10^{-3}$ M) but not to the serosal solution. This indicates the presence of an apical $Cl^-/HCO_3^-$ exchanger.

Based on this somewhat conflicting available information, it appears that there may be a $Cl^-/HCO_3^-$ exchanger present in the apical membrane of at least some species of marine teleost fish and that not all species have a $Cl^-/HCO_3^-$ exchanger in the basolateral membrane of the intestinal epithelium. Consequently, we set out to investigate the presence of $Cl^-/HCO_3^-$ exchangers in both the apical and basolateral membrane of the Pacific sanddab (*Citharichthys sordidus*) intestine using freshly isolated segments of the anterior, mid and posterior part. We here report $Cl^-$-dependence and DIDS sensitivity, indicating the presence of a $Cl^-/HCO_3^-$ exchanger in the apical but not the basolateral membrane.

## Materials and methods

Fish for the survey of intestinal fluid composition were collected either as by-catch by commercial shrimp fishing boats in the vicinity of Bamfield, British Columbia, Canada during the summer of 1997 and 1999 or from a commercial fishing boat in the vicinity of Kerteminde, Funen, Denmark during the summer of 1998. Fish collected around Bamfield were held in 400-l fiberglass tanks with a flow-through of aerated Bamfield Marine Station seawater (salinity 30 ppt, temperature 14 °C) for at least 5 days prior to sampling. Fish collected around Kerteminde was held in 400 l-PVC tanks with a flow-through of aerated Odense University Aquatic Research Center seawater (salinity 11–24 ppt, temperature 6–10 °C) for 6–8 days prior to sampling.

Pacific Sanddab (*Citharichthys sordidus*) for studies of localization of intestinal $Cl^-/HCO_3^-$ exchangers were obtained by hook and line angling just off Brady's Beach, Bamfield, British Columbia, Canada during the summer of 1999 and were held at Bamfield Marine Station as described above for at least 3 days prior to experimentation.

### *Spot sampling of rectal and intestinal fluids*

Whether at Bamfield Marine Station or Odense University Aquatic Research Center, fish were anaesthetized with 0.1 g $l^{-1}$ tricainemethanesulphonate (MS-222) and subsequently killed by spinalectomy. Rectal fluid was collected with a syringe fitted with a short length of polyethylene tubing (PE90). Subsequently, the gastro-intestinal tract (GIT) was exposed by dissecting away the musculature on one side of the body. The intestine was then ligated in four places - at the pyloric sphincter, one third and two thirds towards

the anus, and immediately anterior to the anus. The intestine was removed from the body cavity and fluids were obtained from the three areas isolated by the ligatures. Samples were immediately analyzed for pH and total $CO_2$ as described below and stored at −20 °C for later ion analysis.

*Localization of $HCO_3^-$ carriers in the intestine of the Pacific sanddab*

Pacific sanddab were anaesthetized and killed as above, and the GIT was obtained by dissection and placed on ice. 'Gut bags' was made according to Grosell et al. (1999). In brief, an inflow catheter (6 cm length of PE60 flared at the tip) was inserted at the anterior end of the intestine and tied in place using two silk ligatures. The intestine was flushed gently with approximately 20 ml of the appropriate mucosal saline (see below for composition) to displace any intestinal fluid and solids. The anterior, mid and posterior segments of the intestine were then separated. The mid and posterior segments were fitted with catheters as above. All gut segments were tied of by two silk ligatures. The resulting gut bags were filled with the appropriate mucosal saline and placed in glass vials containing 15 ml of the appropriate serosal solution, according to the experimental protocol outlined below.

At the beginning of a flux period, the gut bags were carefully blotted dry, weighed and again placed in the vials. During the flux period, the serosal saline was aerated with a 0.3% $CO_2$ in $O_2$ mix ($P_{CO2} = 2.3$ torr). To maintain a constant temperature of 14 ± 1 °C, the vials were partly submerged in a water bath supplied with a flow-through of Bamfield Marine Station seawater. Samples of mucosal and serosal solution were obtained at the start of the flux period. The flux period was in all cases 4 h, after which, the weight of each individual gut bag was determined as above. Flux periods of 3–4 h have been applied previously in identical preparations from different teleost fish species (Grosell et al. 1999; Grosell and Jensen 1999). These preparations maintained fairly constant flux rates up to over four hours of $Na^+$ and $Cl^-$ that were affected only by the changes in the gradients of these ions as a result of the active transport. In a recent study, transport rates of water, $HCO_3^-$, $Na^+$ and $Cl^-$ remained constant for up to 6 h when salines were changed every 2 h (Grosell et al., unpublished). Subsequently, samples of both the mucosal and serosal salines were obtained. Total $CO_2$ and pH in the serosal and mucosal saline were determined immediately and sub-samples of saline were stored at −20 °C for later analysis of $Na^+$ and $Cl^-$ concentrations as described below. The gut bags were then opened by a longitudinal incision and blotted dry on both surfaces. The weight of the empty gut bag was determined gravimetrically. The tissue was stored overnight at 4 °C to allow muscle relaxation and then the exposed gross surface area of each gut bag was measured using graph paper.

The composition of the basic mucosal saline (given in Table 1) was identical to the mucosal saline used in a previous study of ion transport in the Lemon sole (*Parophrys vetulus*) intestine (Grosell et al. 1999).

The initial volume of mucosal saline was determined by subtracting the weight of the empty gut bag from the initial weight of the gut bag + mucosal saline. The net water transport was evaluated based on the difference in weight of the gut bag including mucosal saline between the start and the end of the flux period. In both cases, 1 g was assumed equal to 1 ml of mucosal fluid.

Net transport of $HCO_3^-$ equivalents (see below), $Na^+$, and $Cl^-$ was calculated by dividing the difference in content (i.e., volume × concentration) of the mucosal saline between beginning and end of the flux period by the surface area and time elapsed.

*DIDS sensitivity experiments*

For the DIDS sensitivity experiments, L-15 (Leibovitz's L-15 medium, containing L-glutamine; Gibco) was used as serosal saline. The L-15 medium was supplemented with 5 mM $NaHCO_3$, resulting in approximately 5 mM total $CO_2$ and a pH of approximately 7.80 when aerated with the above mentioned $CO_2/O_2$ mix. The L-15 medium was used because it was found to improve the viability of a similar preparation from European flounder (Grosell and Jensen 1999).

For all experiments, DIDS was dissolved in dimethylsulphoxide (DMSO) and applied to a final concentration of $10^{-4}$ M and 0.1% DMSO. All control measurements were performed in the presence of 0.1% DMSO (vehicle control). All DIDS-DMSO solutions were made up fresh daily. In the experiments with serosal DIDS, an *in vivo* pre-incubation procedure was employed to ensure the desired local concentration of the drug in this preparation, where the serosal muscle layers remained intact. Fish were injected into the caudal artery (1 ml $kg^{-1}$) with a saline (Cortland saline, gassed as above, adjusted to 159 mM $Na^+$ with NaCl for sea water teleosts, Wolf, 1963 - see Table 1 for

*Table 1.* pH and ionic composition of mucosal and serosal salines. Concentrations given in mM.

| | Muscosal | | | Serosal | | |
|---|---|---|---|---|---|---|
| | control | $Cl^-$ free | $Na^+$ free | control | $Cl^-$ free | $Na^+$ free |
| $CaCl_2(2H_2O)$ | 2.5 | – | 2.5 | 1.6 | – | 1.6 |
| Ca-gluconate | – | 2.5 | – | – | 1.6 | – |
| Choline-$HCO_3$ | – | – | – | – | – | 11.9 |
| Glucose | – | – | – | 5.6 | 5.6 | 5.6 |
| KCl | 5.0 | – | 4.0 | 5.0 | – | 2.0 |
| K-gluconate | – | 5.0 | – | – | 5.0 | – |
| $KHCO_3$ | – | – | 1.0 | – | – | – |
| $KH_2PO_4$ | – | – | – | – | – | 3.0 |
| $MgCl_2$ | 17.5 | – | 17.5 | – | – | – |
| Mg-gluconate | – | 17.5 | – | – | – | – |
| $MgSO_4$ $(7H_2O)$ | 62.5 | 62.5 | 62.5 | 0.9 | 0.9 | 0.9 |
| NaCl | 99.0 | – | – | 144.1 | – | – |
| Na-gluconate | – | 99.0 | – | – | 144.0 | – |
| $NaHCO_3$ | 1.0 | 1.0 | – | 11.9 | 11.9 | – |
| $NaH_2PO_4(H_2O)$ | – | – | – | 3.0 | 3.0 | – |
| N-methyl-D-glucamine | – | – | 100 | – | – | 147.1 |
| pH* | 7.6-7–9 | 7.6–7.9 | 7.6–8.0 | 7.8–7.9 | 7.8–7.9 | 7.8–7.9 |

* pH when gassed with 0.3% $CO_2$ in $O_2$.

composition) containing DIDS dissolved in DMSO (15 mg $ml^{-1}$) to achieve a final DIDS concentration in the extracellular compartment (ECFV) of $10^{-4}$ M, 30 minutes prior to dissection. The ECFV was assumed to represent 30% of the body weight. Control fish were sham-injected with saline and DMSO as above but with no DIDS.

*Ion-replacement experiments*

Having established mucosal DIDS sensitivity of $HCO_3^-$ secretion, we set out to establish whether this $HCO_3^-$ transport was $Cl^-$-dependent. Pilot experiments with $Cl^-$-free mucosal saline revealed a large back flux of $Cl^-$ from the serosal saline (L-15 medium) resulting in substantial $Cl^-$ concentrations in the intended $Cl^-$-free mucosal saline. Consequently, in order to perform experiments under $Cl^-$-free conditions we employed $Cl^-$-free salines on both the mucosal and the serosal sides (see Table 1 for composition). In order to have $Cl^-$-free serosal conditions, we used as a basis a modified Cortland saline (Wolf 1963) rather than the L-15 medium as the serosal saline (see Table 1 for composition). All corresponding controls were performed with the modified Cortland saline as the serosal medium. To establish whether the $Cl^-$-free serosal medium alone influenced the $HCO_3^-$ secretion, positive control measurements of $HCO_3^-$ flux in the presence of $Cl^-$ in the mucosal saline and the absence of $Cl^-$ in the serosal saline were performed.

Since Ando and Subramanyam (1990) reported $Na^+$-dependent $HCO_3^-$ flux in the intestine of seawater acclimated Japanese eel, we also investigated whether $HCO_3^-$ secretion in the Pacific sanddab required the presence of $Na^+$. The experimental approach was identical to the one applied for the $Cl^-$-free experiments above.

*Analytical techniques*

Intestinal fluids collected at Bamfield Marine Station and saline samples from the gut bag experiments were analyzed for total $CO_2$ using a total $CO_2$ analyzer (CMT965, Corning Medical and Scientific). The pH was measured using a micro-capillary pH electrode (Radiometer G279/G2) coupled with a PHM71 meter. The $Cl^-$ concentrations were analyzed using either colorimetric assay (Zall et al. 1956) or coulometric titration (Radiometer CMT-10). Cations were analyzed by standard atomic absorption spectrophotometry (Varian 1275) using standard operating conditions.

Intestinal fluids collected at Odense University Aquatic Research Center were analyzed for total $CO_2$

by the method of Cameron (1971) and pH was measured with the capillary pH electrode of a Radiometer (Copenhagen, Denmark) BMS3 system with the signal displayed on a PHM 73 monitor and REC 80 recorder. The $Cl^-$ concentrations were measured by coulometric titration (Radiometer CMT-10). The $Na^+$ concentration was measured by flame photometry (Instrumentation Laboratory 243) and other cations were analyzed by atomic absorption spectrophotometry (Perkin-Elmer 2380) using standard operating conditions.

The concentration of $HCO_3^-$ equivalents was calculated according to the Henderson-Hasselbach equation as $[HCO_3^-] + 2[CO_3^{2-}]$ using values for $CO_2$ solubility and $pk^I$ and $pk^{II}$ in one-third seawater at the appropriate temperature from Walton Smith (1974). This approach have previously been shown to correlate very well with values obtained by titration (Grosell et al. 1999).

*Statistical evaluation and data presentation*

All data are expressed as absolute values, means ± SEM (N). Significant differences between values obtained from preparations with DIDS and their corresponding vehicle controls were evaluated using unpaired Student's *t*-test (two-tailed). For the ion-replacement studies, the data obtained from $Cl^-$-free and $Na^+$-free preparations were compared to the control values obtained in the presence of control mucosal and serosal saline. These data were also compared to the positive control values obtained in the absence of the relevant ion ($Cl^-$ or $Na^+$) in the serosal saline but with control mucosal saline, using unpaired Student's *t*-test (two-tailed). Furthermore, the values obtained from the control ($Na^+$ or $Cl^-$ present in both the mucosal and serosal saline) and the positive control ($Na^+$ and $Cl^-$ present in the mucosal saline but absent in the serosal saline) were compared. In all cases, groups were considered significantly different at $p < 0.05$.

## Results

*Composition of intestinal and rectal fluids*

Intestinal and rectal fluids from most of the species investigated were characterized by high $HCO_3^-$ equivalent and pH levels. Only the Atlantic cod (*Gadus morhua*) exhibited slightly acidic to neutral pH in the intestinal fluids, and $HCO_3^-$ equivalent levels comparable to plasma levels. Overall, the [$HCO_3^-$ equivalents] were typically 30–40 mM in fluids obtained from the anterior segment of the intestine. Values obtained from the more distal segments and the rectum were generally slightly higher (Table 2). These values were therefore 5–10× higher than typical blood plasma [$HCO_3^-$]. In parallel, pH was highest in samples obtained from the more distal segments of the intestine and the rectum, in most cases exceeding 8.2 (Table 2).

$Cl^-$ concentrations in fluids obtained from the anterior segment of the intestine were typically 120–130 mM, but the Atlantic cod and the European flounder (*Platichthys flesus*) exhibited even lower $Cl^-$ concentrations - 90 and 65 mM, respectively. Generally, there was a tendency to slightly decreased $Cl^-$ concentration in fluids obtained from more distal segments of the GIT (Table 2). In parallel, $Na^+$ concentrations, though more variable, tended to decrease from between 40 and 110 mM in the anterior intestine to values between 30 and 100 mM in the most distal segments of the GIT. With a few exceptions, $K^+$ concentrations were lower than 15 mM in the intestinal fluids and showed no trend to decrease or increase in the more distal segments of the intestine (Table 2). $Ca^{2+}$ concentrations was generally lower than 10 mM and as for $K^+$ there was no tendency for the $Ca^{2+}$ concentration to decrease or increase in the more distal segments (Table 2). In contrast to $Cl^-$ , $Na^+$, $K^+$, and $Ca^{2+}$ ion concentrations, which were all considerably lower than corresponding seawater levels, $Mg^{2+}$ concentrations in the intestinal fluids were equal to or greater than ambient levels (Table 2 and Grosell et al. 1999). Furthermore, $Mg^{2+}$ was concentrated from 20–100 mM in the anterior segment to as high as 140 mM in the more distal segments of the GIT.

*Localization of $HCO_3^-$ carriers in the intestine of the Pacific sanddab*

Mean control $HCO_3^-$ equivalent flux rates (from serosa to mucosa) ranged from 0.40 to 0.65 $\mu$mol $cm^{-2}$ $h^{-1}$ with L-15 as serosal saline and 0.70–0.85 $\mu$mol $cm^{-2}$ $h^{-1}$ when the modified Cortland saline was used as serosal saline. The net flux of $HCO_3^-$ equivalents resulted in a parallel increase in pH in the mucosal saline (from 7.8 up to 8.3, data not shown).

DIDS ($10^{-4}$ M) applied to the mucosal saline and $Cl^-$-free mucosal saline both significantly reduced $HCO_3^-$ equivalent secretion (Figures 1A and 2A). In contrast, DIDS applied to the serosal saline (following

*Table 2.* pH and ionic composition (in mM) of intestinal and rectal fluids

| | | pH | $HCO_3^-$ | $Cl^-$ | $Na^+$ | $K^+$ | $Ca^{2+}$ | $Mg^{2+}$ |
|---|---|---|---|---|---|---|---|---|
| *Pleuronectes platessa* | Ant | 8.122±0.059(10) | 31.3±2.5(11) | 132.9±6.4(10) | 60.5±7.7(8) | 10.4±3.8(8) | 4.7 ±0.9(8) | 33.8±6.1(8) |
| | Mid | 8.135±0.107(9) | 34.9±2.2(9) | 128.6±12.9(5) | 77.2±2.9(2) | 13.5±9.1(2) | 5.4 ±2.3(2) | 64.2±12.5(2) |
| | Post | 8.299±0.064(8) | 49.3±4.0(9) | 129.1±15.8(8) | 64.2±31.1(6) | 13.7±3.8(6) | 3.02±1.0(6) | 78.3±13.5(6) |
| | Rect | 8.351±0.041(7) | 42.2±2.2(6) | 141.4±21.6(8) | 62.1±17.9(6) | 11.4±3.6(6) | 2.6 ±0.7(6) | 91.2±28.5(6) |
| *Scophthalmus maximus* | Ant | 7.408±0.324(2) | 20.2±4.1(4) | 134.4±10.0(3) | 103.2±5.5(3) | 13.6±3.3(3) | 4.9 ±1.1(3) | 24.7±6.4(3) |
| | Mid | 8.206±0.173(4) | 27.5±7.5(5) | 132.6±14.3(4) | 96.6±8.4(2) | 6.4±1.2(2) | 3.8 ±1.7(2) | 34.0±7.1(2) |
| | Post | 8.064±0.120(4) | 30.4±7.1(5) | 125.0±9.5(4) | 73.6±13.2(4) | 4.8±1.0(4) | 4.1 ±0.8(4) | 46.2±17.8(4) |
| | Rect | 8.318±0.119(8) | 52.2±7.1(6) | 115.0±14.2(7) | 47.3±8.0(5) | 9.2±6.1(5) | 5.8 ±1.3(5) | 49.1±7.2(5) |
| *Scophthalmus rhombus* | Ant | 7.970±0.219(4) | 34.7±6.3(5) | 120.2±14.5(5) | 62.7±6.7(4) | 8.3±0.7(4) | 2.5 ±0.3(4) | 58.3±8.2(4) |
| | Mid | 8.341±0.067(3) | 49.1±9.8(5) | 112.8±8.4(4) | 73.3±11.3(3) | 13.2±5.3(3) | 4.3 ±0.5(3) | 116.6±27.3(3) |
| | Post | 8.234(1) | 65.8±17.3(2) | 73.4±8.2(2) | | | | |
| | Rect | 8.505±0.082(4) | 43.4±8.4(4) | 128.4±9.0(4) | 28.0±13.2(2) | 3.7±1.8(2) | 3.6 ±2.1(2) | 101.3±11.1(2) |
| *Limanda limanda* | Ant | 7.993±0.320(4) | 40.3±7.6(5) | 198.7±7.3(5) | 44.0±7.3(3) | 10.9±4.3(3) | 4.8 ±1.0(3) | 78.4±58.3(3) |
| | Mid | 8.194±0.092(4) | 35.8±6.1(4) | 150.4±33.3(3) | 38.6±11.5(2) | 22.1±16.5(2) | 5.5 ±2.2(2) | 112.3±34.9(2) |
| | Post | 8.511±0.127(2) | 33.2±11.0(4) | 149.0±16.3(4) | | | | |
| | Rect | 8.269±0.099(3) | 20.2±3.3(4) | 117.2±12.2(3) | 58.8±4.7(3) | 54.8±19.9(3) | 5.1 ±1.9(3) | 35.6±14.2(3) |
| *Cyclopterus lumpus* | Ant | 7.779(1) | 35.2(1) | 135.5(1) | 114.8(1) | 14.3(1) | 2.6(1) | 20.9(1) |
| | Mid | 7.921(1) | 34.6(1) | 127.3(1) | 138.9(1) | 13.1(1) | 2.6(1) | 21.7(1) |
| | Post | 8.010(1) | 24.1(1) | 146.3(1) | 118.6(1) | 13.1(1) | 2.0(1) | 21.7(1) |
| | Rect | 7.580(1) | 29.9(1) | 146.9(1) | 97.1(1) | 11.7(1) | 5.2(1) | 45.0(1) |

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| | | pH | $HCO_3^-$ | $Cl^-$ | $Na^+$ | $K^+$ | $Ca^{2+}$ | $Mg^{2+}$ |
|---|---|---|---|---|---|---|---|---|
| *Myoxocephalus scorpius* | Ant | 7.718±0.087(2) | 36.3±3.7(3) | 96.0±5.9(3) | 93.3±1.8(2) | 24.3±13.1(2) | 6.4±3.0(2) | 22.4±17.7(2) |
| | Mid | 8.062(1) | 42.4±5.1(3) | 95.9±13.4(4) | 121.6(1) | 12.8(1) | 2.8(1) | 22.3(1) |
| | Post | 7.900±0.200(4) | 34.0±5.6(5) | 116.4±10.8(5) | 113.3±19.6(5) | 10.6±4.2(5) | 10.1±5.8(5) | 57.4±12.5(5) |
| | Rect | 7.947±0.312(4) | 31.7±4.3(5) | 108.3±6.4(2) | 104.9±0.6(2) | 4.5±0.4(2) | 3.3±0.8(2) | 39.3±10.3(2) |
| *Zoarces viviparus* | Ant | 7.938±0.654(2) | 15.4±3.4(7) | 145.5±16.4(8) | 111.2±8.9(2) | 4.6±2.3(2) | 7.2±1.5(2) | 34.6±3.4(2) |
| | Mid | 7.911(1) | 24.9±5.9(5) | 180.9±28.7(3) | 84.0±20.1(2) | 8.8±1.3(2) | 8.2±0.2(2) | 39.0±2.8(2) |
| | Post | 8.461±0.214(2) | 28.3±6.8(2) | 173.8±23.1(2) | 57.5(1) | 5.8(1) | 6.0(1) | 28.1(1) |
| | Rect | 8.508±0.004(3) | 26.2±8.4(3) | 142.2±27.0(4) | 33.8(1) | 6.7(1) | 21.4(1) | 139.9(1) |
| *Gadus morhua* | Ant | 6.733(1) | 7.5(1) | 89.3(1) | | | | |
| | Mid | 6.659±0.072(3) | 5.1±2.0(3) | 111.2±9.3(2) | 91.1±16.1(2) | 17.9±3.3(2) | 16.5±9.4(2) | 26.3±8.8(2) |
| | Post | 6.735±0.290(2) | 10.2±4.3(2) | 116.5(1) | 112.3(1) | 23(1) | 16.5(1) | 24.7(1) |
| | Rect | 7.228±0.154(3) | 15.8±2.8(6) | 117.9±8.8(4) | | | | |
| *Platichthys flesus*[(a)] | Ant | 8.499±0.055(6) | 68.8±6.4(6) | 64.4±5.5(6) | 37.3±5.3(6) | 1.1±0.2(6) | 4.2±1.2(6) | 26.3±5.6(6) |
| | Mid | 8.628±0.014(6) | 87.7±5.7(6) | 57.2±11.3(6) | 31.0±4.8(6) | 1.0±0.31(6) | 3.4±0.4(6) | 27.5±3.6(6) |
| | Post | 8.671±0.012(6) | 97.7±3.3(6) | 38.2±6.9(6) | 25.8±4.0(6) | 2.3±1.7(6) | 2.1±4.0(6) | 26.0±2.1(6) |
| *Parophrys vetulus*[(b)] | Ant | 7.906±0.149(8) | 22.7±5.3(8) | 135.8±6.9(5) | 68.6±12.4(5) | 7.6±1.5(3) | 4.0±0.4(4) | 97.0±17.0(4) |
| | Mid | 8.264±0.047(8) | 30.1±4.6(8) | 128.9±8.1(7) | 66.5±10.9(6) | 10.5±3.9(4) | 3.9±0.5(5) | 123.0±8.0(5) |
| | Post | 8.383±0.042(8) | 36.0±5.3(8) | 124.0±11.5(5) | 56.6±17.1(5) | 17.4±6.2(4) | 2.5±0.2(4) | 165.0±5.0(4) |
| | Rect | 8.437±0.122(8) | 41.9±7.9(8) | 103.0±8.7(6) | 41.8±19.3(6) | 7.1±2.8(5) | 1.8±0.3(5) | 117.0±26(5) |
| *Citharichthys sordidus* | Ant | 7.873±0.177(6) | 41.3±4.1(7) | 128.6±11.3(8) | 42.1±4.7(8) | 36.0±4.5(8) | 6.7±0.7(8) | 78.0±5.8(8) |
| | Mid | 8.152±0.060(5) | 43.4±5.9(6) | 137.5±19.2(8) | 33.5±4.5(8) | 19.6±5.7(8) | 7.1±1.3(8) | 103.6±13.6(8) |
| | Post | 8.213±0.075(7) | 44.9±4.9(7) | 126.9±11.2(8) | 30.0±3.9(8) | 15.8±4.1(8) | 6.5±0.4(8) | 112.5±15.6(8) |

(a): Grosell and Jensen (1999).
(b): Grosell et al. (1999).

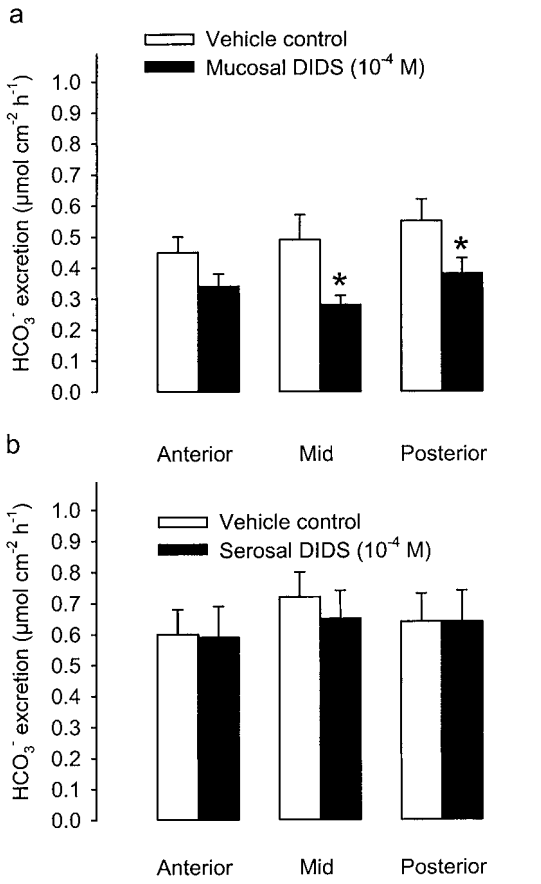


*Figure 1.* DIDS-sensitivity of net flux rates of $HCO_3^-$ ($\mu$mol $cm^{-2}$ $h^{-1}$) in the anterior, mid and posterior segments of freshly isolated Pacific sanddab intestine during a four hour flux period. White bars are vehicle control (dimethylsulphoxide) values, black bars are values obtained in the presence of $10^{-4}$ M DIDS in the mucosal solution (a) and in the presence of $10^{-4}$ M DIDS in the serosal solution (b). In the latter, fish were injected *in vivo* with DIDS (resulting in $10^{-4}$ M DIDS in the extracellular fluid) 30 minutes prior to dissection and control fish were sham-injected with dimethylsulphoxide medium (see text for further details). Values are mean±SEM, n = 6-7. * indicates significant difference from the corresponding control value (unpaired *t*-test, $p < 0.05$).

*in vivo* pre-treatment) and $Cl^-$ free serosal saline did not significantly reduce $HCO_3^-$ equivalent flux rates (Figures 1B and 2A).

Removal of $Na^+$ from both the mucosal and the serosal saline significantly reduced $HCO_3^-$ equivalent flux rates (Figure 2B). The flux rates when $Na^+$ was absent on both the mucosal and serosal sides were however, not significantly lower than values obtained when $Na^+$ was absent on the serosal side but present on the mucosal side (Figure 2B).

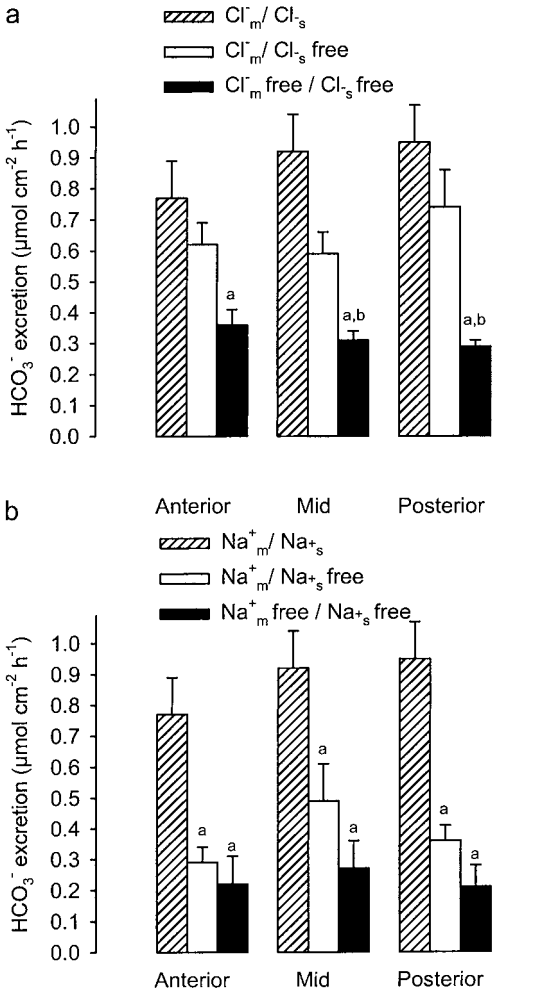


*Figure 2.* $Na^+$ and $Cl^-$-dependence of net flux rates of $HCO_3^-$ ($\mu$mol $cm^{-2}$ $h^{-1}$) in the anterior, mid and posterior segments of freshly isolated Pacific sanddab intestine during a four hour flux period. Top panel (a): Hatched bars are control values obtained in the presence of control mucosal saline and control serosal saline (n = 8 for the anterior segment and 9 for the mid and posterior segments). White bars are values obtained in the presence of $Cl^-$ in the mucosal saline and absence of $Cl^-$ in the serosal saline (n = 6). Black bars are values obtained in the absence of $Cl^-$ in both the mucosal and serosal saline (n = 7). Bottom panel (b): Hatched bars are control values obtained in the presence of control mucosal saline and control serosal saline (n = 8 for the anterior segment and 9 for the mid and posterior segments). White bars are values obtained in the presence of $Na^+$ in the mucosal saline and absence of $Na^+$ in the serosal saline (n = 5). Black bars are values obtained in the absence of $Na^+$ in both the mucosal and serosal saline (n = 5 for the anterior and posterior segments and n = 4 for the mid segment). Values are mean±SEM. An 'a' denotes statistically significant difference from the control values obtained in the presence of both $Cl^-$ and $Na^+$ in the mucosal and serosal solution. A 'b' denotes statistically significant difference from the positive control values obtained in the absence of $Cl^-$ (top panel) or absence of $Na^+$ (bottom panel) from the serosal solution but presence of both $Cl^-$ and $Na^+$ in the mucosal solution (unpaired *t*-test, $p < 0.05$).

*Table 3.* Net fluxes $Na^+$, $Cl^-$ and water in control and DIDS treated gut segments

| Net flux | Vehicle control I (0.1% DMSO in mucosal solution | Mucosal DIDS ($10^{-4}$ M) | Vehicle control II (0.1% DMSO in serosal solution) | Serosal DIDS ($10^{-4}$ M) |
|---|---|---|---|---|
| $Na^+$ ($\mu$mol cm$^{-2}$ h$^{-1}$) | | | | |
| Anterior | 0.18±0.33(6) | −0.60±0.15(6) | 0.29±0.46(6) | 0.23±0.48(7) |
| Mid | 0.48±0.53(6) | −0.60±0.13(6) | 0.59±0.25(6) | 0.64±0.46(7) |
| Posterior | 0.68±0.28(6) | −0.74±0.31(6)* | 0.86±0.29(6) | 0.42±0.63(7) |
| $Cl^-$ ($\mu$mol cm$^{-2}$ h$^{-1}$) | | | | |
| Anterior | 0.28±0.09(6) | 0.28±0.37(6) | 0.77±0.33(6) | 0.83±0.33(7) |
| Mid | 0.18±0.17(6) | −0.14±0.29(6) | 0.94±0.34(6) | 0.91±0.48(7) |
| Posterior | 1.11±0.43(6) | −0.18±0.16(6)* | 0.88±0.35(6) | 0.89±0.30(7) |
| Water ($\mu$l cm$^{-2}$ h$^{-1}$) | | | | |
| Anterior | 0.24±1.08(6) | 0.13±1.75(6) | 2.28±2.15(6) | 3.45±1.66(7) |
| Mid | −0.49±0.79(6) | −1.80±1.22(6) | 1.52±0.60(6) | 3.66±2.65(7) |
| Posterior | 3.31±3.25(6) | −3.63±0.56(6) | 1.95±1.67(6) | 2.36±1.86(7) |

*Significant difference from corresponding control ($t$-test, $p < 0.05$)

Net uptake flux of $Na^+$, $Cl^-$ and water tended to be lower in the presence of mucosal DIDS compared to the corresponding vehicle control. This was statistically significant only for the net flux of $Na^+$ and $Cl^-$ in the posterior segment of the intestine (Table 3). DIDS applied to the serosal saline did not affect net flux of $Na^+$, $Cl^-$ or water (Table 3).

Removal of either $Na^+$ or $Cl^-$ from both the mucosal and serosal saline significantly reduced net water transport across the intestinal epithelium (Figure 3C and 4C). When $Na^+$ and $Cl^-$ were present in the mucosal saline but absent from the serosal saline, net water fluxes tended to be lower, but were not significantly different from the corresponding control values.

$Na^+$ net flux was completely abolished in the absence of $Cl^-$ in the mucosal and serosal saline, but was not significantly different from control conditions when $Cl^-$ was present in the mucosal saline but absent from the serosal saline (Figure 3A).

$Cl^-$ net flux was significantly reduced in the absence of $Na^+$ in both the mucosal and serosal saline. In contrast to $Na^+$ net flux, which was completely abolished in absence of $Cl^-$, some $Cl^-$ net flux still persisted in the absence of $Na^+$, at a rate comparable to $HCO_3^-$ secretion rate. When $Na^+$ was absent from the serosal saline but present in the mucosal saline, net $Cl^-$ flux remained not significantly different from the corresponding control values (Figure 4B).

Net flux of both $Na^+$ and $Cl^-$ under conditions where both ions were present in the mucosal saline but where $Na^+$ and $Cl^-$, respectively, were absent from the serosal saline remained similar to control values where both ions were present in both the mucosal and serosal saline (Figure 3B and 4A).

## Discussion

### *Ionic composition of intestinal fluids*

High levels of $HCO_3^-$ equivalents accompanied by high pH were found in the intestinal fluids of all but one (Atlantic cod, *Gadus morhua*) of the investigated species (Table 3). Considering the low serosal side negative TEP −3–8 mV; (cf. Introduction), the serosal to mucosal $HCO_3^-$ equivalent gradient (typically 5–10 fold higher [$HCO_3^-$] in serosal fluids than in blood plasma) indicates that $HCO_3^-$ is not distributed passively across the intestinal epithelium. $Na^+$ and $Cl^-$ concentrations in the intestinal fluids were generally much lower than in the surrounding seawater. This reduction is the product of desalinization of the imbibed seawater in the anterior segments of the gastro-intestinal tract, primarily the esophagus (Loretz 1995). The transport of isotonic fluids across the intestinal epithelium does not appear to cause dramatic reductions in the concentration of $Na^+$ and $Cl^-$ along the intestine but leaves, especially $Mg^{2+}$, more concentrated in fluids obtained from the distal parts of the intestine.

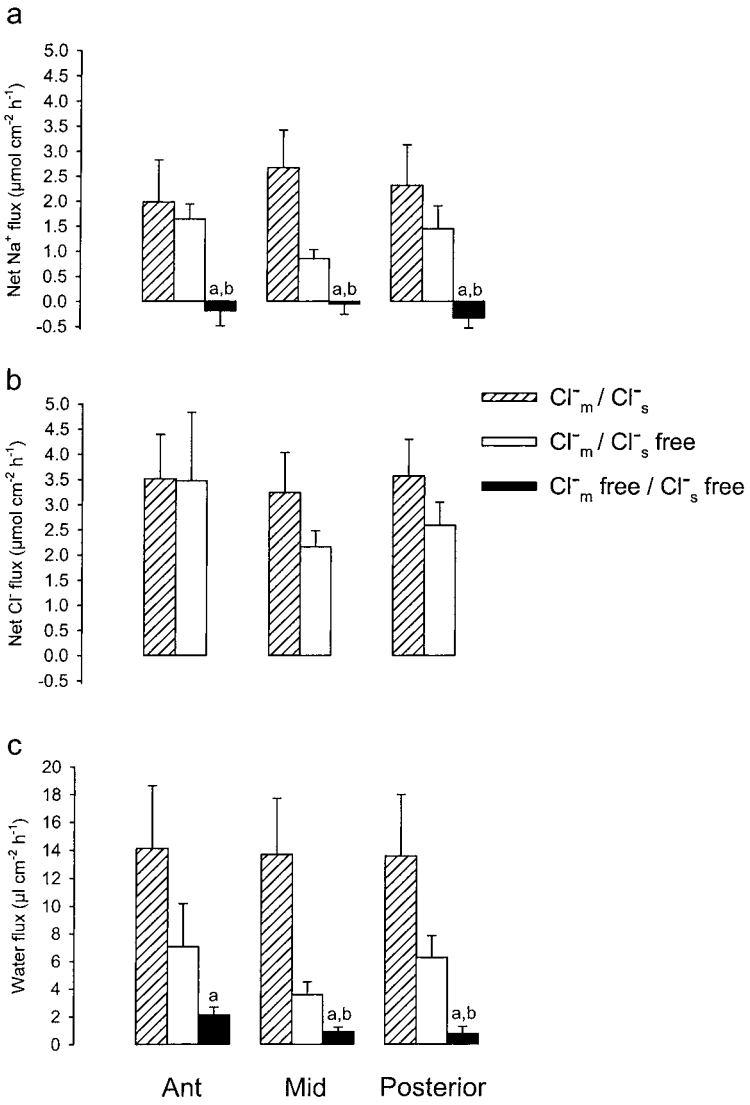


*Figure 3.* $Cl^-$-dependence of net flux rates of $Na^+$ (a) ($\mu$mol $cm^{-2}$ $h^{-1}$), $Cl^-$ (b) ($\mu$mol $cm^{-2}$ $h^{-1}$) and water (c) ($\mu$l $cm^{-2}$ $h^{-1}$) in the anterior, mid and posterior segments of freshly isolated Pacific sanddab intestine during a four hour flux period. Hatched bars are control values obtained in the presence of control mucosal saline and control serosal saline (n = 8 for the anterior segment and 9 for the mid and posterior segments). White bars are values obtained in the presence of $Cl^-$ (control saline) in the mucosal saline and absence of $Cl^-$ from the serosal saline (n = 6). Black bars are values obtained in the absence of $Cl^-$ from both the mucosal and serosal salines (n = 7). Values are mean±SEM. An 'a' denotes statistically significant difference from the control values obtained in the presence of $Cl^-$ in the mucosal and serosal solution. A 'b' denotes statistically significant difference from the positive control values obtained from the absence of $Cl^-$ in the serosal solution but presence of $Cl^-$ in the mucosal solution (unpaired *t*-test, $p < 0.05$).

*Localization of the $Cl^-/HCO_3^-$ exchanger*

Based on mucosal DIDS sensitivity and mucosal $Cl^-$-dependence of $HCO_3^-$ equivalent secretion by the intestinal tissue, the present results on the Pacific sanddab support the idea that a $Cl^-/HCO_3^-$ exchanger is present in the apical membrane of the intestinal epithelium. Furthermore, in contrast to the model proposed by Loretz (1995), it appears that there is no $Cl^-/HCO_3^-$ exchanger in the basolateral membrane. We can, however, not conclusively exclude the presence of a $Cl^-/HCO_3^-$ exchanger isoform with a low DIDS sensitivity in the basolateral membrane. The presence of an anion exchange mechanism in the apical membrane of the intestinal epithelium agrees with data on the European flounder (Grosell et al. 1999) and

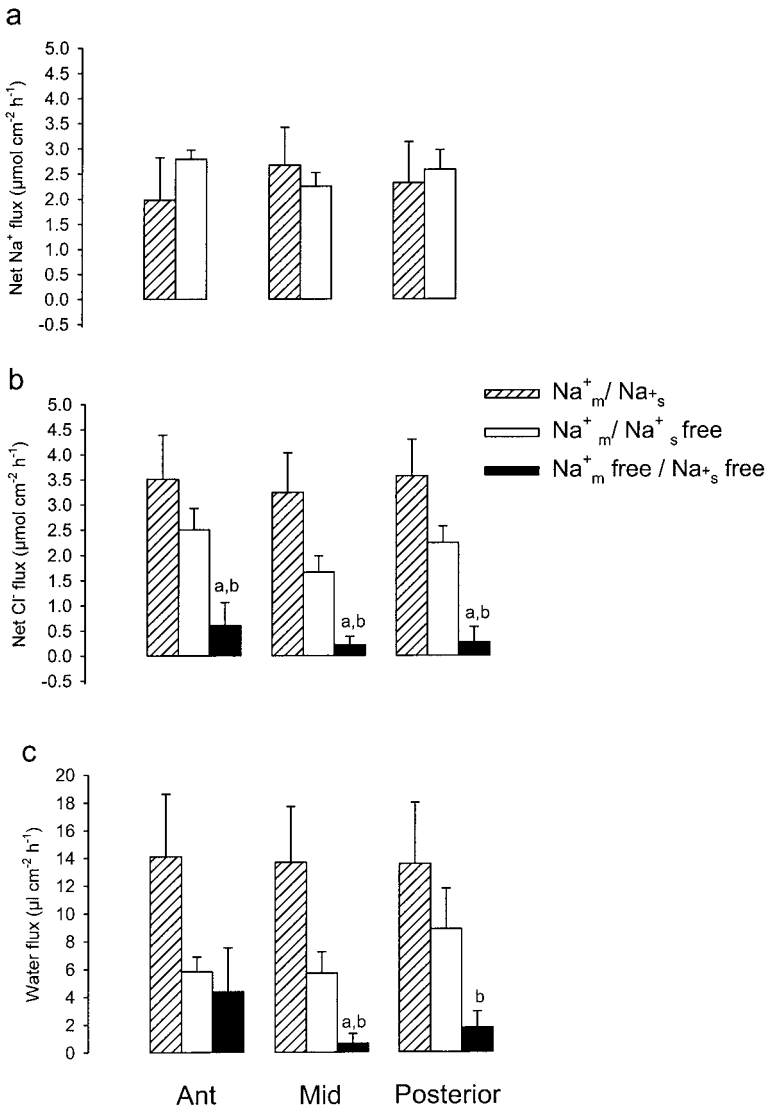


*Figure 4.* $Na^+$-dependence of net flux rates of $Na^+$ (a) ($\mu$mol $cm^{-2}$ $h^{-1}$), $Cl^-$ (b) ($\mu$mol $cm^{-2}$ $h^{-1}$) and water (c) ($\mu$l $cm^{-2}$ $h^{-1}$) in the anterior, mid and posterior segments of freshly isolated Pacific sanddab intestine during a four hour flux period. Hatched bars are control values obtained in the presence of control mucosal saline and control serosal saline (n = 8 for the anterior segment and 9 for the mid and posterior segments). White bars are values obtained in the presence of $Na^+$ (control saline) in the mucosal saline and absence of $Na^+$ from the serosal saline (n = 6). Black bars are values obtained in the absence of $Na^+$ from both the mucosal and serosal saline (n = 7). Values are mean±SEM. An 'a' denotes statistically significant difference from the control values obtained in the presence of $Na^+$ in the mucosal and serosal solution. A 'b' denotes statistically significant difference from the positive control values obtained in the absence of $Na^+$ from the serosal solution but presence of $Cl^-$ in the mucosal solution (unpaired *t*-test, $p < 0.05$).

the Japanese eel (Ando and Subramanyam 1990). This is also supported by the work of Wilson et al. (1996) on seawater-acclimated rainbow trout, where $HCO_3^-$ secretion was greatly reduced in the absence of $Cl^-$ in the mucosal medium, and where $Cl^-$ uptake and $HCO_3^-$ secretion were directly correlated. The latter study reported no mucosal DIDS sensitivity but employed lower DIDS concentration than all of the above mentioned studies ($2 \times 10^{-5}$ M compared to values ranging from $10^{-3}$ to $10^{-4}$ M) and a shorter exposure period than either the present study or the study on European flounder ($\leq$80 min compared to $\geq$4 h).

Our findings of no $Cl^-/HCO_3^-$ exchanger in the basolateral membrane of the intestinal epithelium are in contrast to the findings from the Japanese eel (Ando and Subramanyam 1990) of evidence for a $Cl^-/HCO_3^-$

exchanger in the basolateral as well as the apical membrane. The present findings also differ from the report of a $Cl^-/HCO_3^-$ exchanger only in the basolateral and not in the apical membrane of the goby, *Gillichtys mirabilis* (Dixon and Loretz 1986).

The apparent $Na^+$-dependence of $Cl^-/HCO_3^-$ exchange in the present study is in agreement with the findings from the Japanese eel (Ando and Subramanyam 1990). However, since this apparent $Na^+$-dependence of the apical $Cl^-/HCO_3^-$ exchanger was also evident in situations with $Na^+$ in the mucosal saline and absence of $Na^+$ in the serosal saline, it is possible that this is a non-specific effect of $Na^+$ removal on the intestinal epithelium. Net $Na^+$ transport was reduced in presence of mucosal DIDS where $HCO_3^-$ secretion was also reduced. This could indicate a coupling of $Na^+$ uptake and $HCO_3^-$ secretion, but unidirectional ion flux measurements are needed to verify this possible coupling.

Based on the above, it appears that the presence of a $Cl^-/HCO_3^-$ exchanger in the apical membrane of the intestinal epithelium may be a general feature (with one exception, *Gillichthys mirabilis*, Dixon and Loretz 1986) of marine teleost fish. Whether this $Cl^-/HCO_3^-$ exchanger is similar to the red blood cell $Cl^-/HCO_3^-$ exchanger or whether it is $Na^+$ dependent remains to be investigated. However, substantial cross reactivity between the apical membrane of the anterior intestine in seawater adapted Coho salmon and the trout AE1 antibody (Band 3-like protein) has been shown (personal communication with Jonathan Wilson).

### *Can the observed $HCO_3^-$ equivalent gradient be sustained by the apical $Cl^-/HCO_3^-$ exchanger?*

The large $HCO_3^-$ equivalent gradient across the intestinal epithelium implies active transport of $HCO_3^-$. Traditionally, the $Cl^-/HCO_3^-$ exchanger which is electrogenically silent and ATP-independent, is not associated with active transport. However, active $Cl^-$ transport by an apical $Cl^-/HCO_3^-$ exchanger has been documented extensively in turtle urinary bladder ionocytes (see Hviid Larsen 1991 for a review). In the following we attempt to analyze whether the observed $HCO_3^-$ gradient can be explained simply by the presence of an apical $Cl^-/HCO_3^-$ exchanger.

The equilibrium potential for any given ion can be described by the Nernst equation:

$$E = \big((RT)/(zF)\big)\big(\ln([X_o]/[X_i])\big)$$

where $X$ represent the concentrations (or activities) of the ion on the outer ($_o$) or the inner ($_i$) side of the membrane, $z$ is the valence of the ion in question and $R$, $T$ and $F$ have their usual meanings. For the $Cl^-/HCO_3^-$ exchanger, the equilibrium potential for the exchange process can be calculated as the sum of the equilibrium potential of $Cl^-$ and $HCO_3^-$ as follows:

$$E_{\text{exchange}} = \Big(\big((RT)/(zF)\big)\big(\ln([Cl_o^-]/[Cl_i^-])\big)\Big) + \Big(-\big((RT)/(zF)\big)\big(\ln([HCO_{3o}^-]/[HCO_{3i}^-])\big)\Big).$$

Note that the $HCO_3^-$ contribution is negative since the transport is in the opposite direction to that of $Cl^-$. The exchange process will occur until '$E_{\text{exchange}}$' becomes zero (0). Solving the equation for '$E_{\text{exchange}}$' = 0 gives:

$$\Big(\big((RT)/(zF)\big)\big(\ln([Cl_o^-]/[Cl_i^-])\big)\Big) = \Big(\big((RT)/(zF)\big)\big(\ln([HCO_{3o}^-]/[HCO_{3i}^-])\big)\Big)$$

and since the valence is the same for the two ions in question, '$\big((RT)/(zF)\big)$' is an identical constant on both sides of the equation. The formulation can then conveniently be reduced to:

$$\big([Cl_o^-]/[Cl_i^-]\big) = \big([HCO_{3o}^-]/[HCO_{3i}^-]\big).$$

$[Cl_o^-]$ and $[HCO_{3o}^-]$ were measured in the present study, and cytoplasmic $Cl^-$ ($[Cl_i^-]$) in intestinal epithelial cells has been estimated to be approximately 30 mM (Duffey 1979; Smith et al. 1980). Assuming $[HCO_{3o}^-]$ = 41 mM and $[Cl_o^-]$ = 128 mM (Table 2) as in the present study, the above equation predicts a cytosolic $HCO_3^-$ concentration ($[HCO_{3i}^-]$) in the Pacific sanddab of 9.1 mM at '$E_{\text{exchange}}$' = 0. The above calculation are based on $[Cl_i^-]$ from a different species obtained under different conditions. However, it follows from the equation, that if the $Cl^-$ gradient was higher than assumed, it would be able to sustain a higher $HCO_3^-$ gradient via the $Cl^-/HCO_3^-$ exchanger and *vice versa*.

The basolateral membrane potential has been estimated to be in the range of 50–80 mV, cytosol negative (Loretz 1995) and sustaining a cytosolic $[HCO_3^-]$ of 9.1 mM simply by diffusion of $HCO_3^-$ from the plasma across the basolateral membrane is thus not possible. Furthermore, the cytosolic $[HCO_3^-]$ can be estimated to be 1.5 mM $HCO_3^-$ from the Henderson–Hasselbalch equation using $pk^I$ and $\alpha CO_2$ values from (Boutilier et al. 1984) and assuming intracellular pH = 7.4 and intracellular $PCO_2$ = 2.3 torr. Based on these assumptions, this estimated cytosolic $[HCO_3^-]$ is not sufficient

to drive the $Cl^-/HCO_3^-$ exchange. Recently, however, Wang and co-workers (1998) showed 4–5 fold higher white muscle intracellular resting $P_{CO2}$ in rainbow trout perfused tail trunks compared to the perfusate saline. This indicates that even in their resting perfused preparation there appeared to be a diffusion limitation causing higher intracellular $P_{CO2}$ than would be expected from the $P_{CO2}$ in the perfusate saline. Similarly, in the present study, the intracellular $P_{CO2}$ in the metabolically active intestinal epithelial may well have been substantially higher than in the serosal saline. In fact, the lack of perfusion in the present study could have resulted in an even bigger difference between intracellular $P_{CO2}$ and $P_{CO2}$ in the serosal saline in the present study than that seen in the study of Wang and co-workers (1998).

Following this argument, the main source of $HCO_3^-$ for intestinal secretion would be intestinal endogenous metabolic $CO_2$ production. Using whole animal oxygen consumption rates from the Starry flounder of 0.458 ml $kg^{-1}$ $min^{-1}$ (Wood et al. 1979) and assuming a $O_2$ to $CO_2$ conversion coefficient of 1, this hypothesis can be tested by calculating the expected $CO_2$ production of the intestinal tissue. The preparations from the present study weighed 0.089 g $cm^{-2}$ (data not shown) which translates to a $CO_2/HCO_3^-$ production rate of 0.1 $\mu$mol $cm^{-2}$ $h^{-1}$. This is lower than the average $HCO_3^-$ secretion rates of 0.5–0.8 $\mu$mol $cm^{-2}$ $h^{-1}$ but is based on the assumption that the metabolic rate of the intestinal epithelial cells is similar to the metabolic rate of the whole animal. The metabolic rate of cells in a transport epithelium is likely higher than the metabolic rate of the whole animal and the above assumption thus underestimates the $CO_2/HCO_3^-$ production rate. In addition, the above Starry flounder metabolic rate was measured at 7.5–10.5 °C (Wood et al. 1979) which is considerably lower than the 14 °C in the present study. This temperature difference also underestimates the intestinal epithelial cell metabolic rate and thus $CO_2/HCO_3^-$ production rate.

Take together, the above considerations support that a significant part of intestinal $HCO_3^-$ secretion could be a supported by endogenous metabolic $CO_2$ production. Metabolic $CO_2$ production combined with a $CO_2$ diffusion limitation could well result in a high intracellular $P_{CO2}$. A high intracellular $P_{CO2}$ could, via the combined activity of a proton pump (to export $H^+$ to the serosal side) and cytosolic carbonic anhydrase (to sustain the supply of $HCO_3^-$ and $H^+$), increase the cytosolic $[HCO_3^-]$ and thus facilitate $Cl^-/HCO_3^-$ exchange. Linkage between a proton pump and $Cl^-/HCO_3^-$ exchange by carbonic anhydrase has been reviewed in detail by Hviid Larsen (1991) and Hviid Larsen et al. (1996). In the teleost intestine where a considerable net base flux into the mucosal compartment prevails, the proton pump must be located at the basolateral membrane (opposite the $Cl^-/HCO_3^-$ exchanger) (Figure 5). According to this scenario, secondary active $HCO_3^-$ secretion via $Cl^-/HCO_3^-$ exchange in the teleost intestine is possible through the combined action of cytosolic carbonic anhydrase and $H^+$ extrusion via a basolateral ATP-dependant proton pump, increasing the cytosolic $[HCO_3^-]$ (Figure 5). Such a transport model has been documented for $\beta$-type ion transporting cells of high resistance epithelia from higher vertebrates (reviewed by Hviid Larsen 1991). These cells have a capacity for base secretion (Lombard et al. 1983; McKinney and Burg 1977, 1978) via active, $Cl^-$-dependant $HCO_3^-$ secretion (Star et al. 1985).

The proposed model is supported by findings presented by Wilson and co-workers (1996) of reduced net base secretion in the intestine of seawater rainbow trout in the presence of the carbonic anhydrase inhibitor acetazolamide ($10^{-4}$ M). That study reports a minor (20%) inhibition of base flux, but the applied mucosal saline contained only 1 mM $HCO_3^-$ and a significant contribution of diffusion, from the blood to the intestinal lumen, to the overall net $HCO_3^-$ flux is likely under the conditions of their experiment where mucosal $HCO_3^-$ was kept low. This diffusive component could have partly clouded the effect of acetazolamide on the secondary active component of the net base flux. Furthermore, the proposed model could be supported by recent findings of $H^+$ pump expression (Northern blot with mRNA) in the intestine of several teleost fish including the European flounder although basolateral localization remains to be documented (Seidelin, Madsen and Jensen, unpublished).

Assuming constant cytosolic $[Cl_i^-]$ and $[HCO_{3i}^-]$, a consequence of the above equation would be that with lower lumenal $[Cl^-]$ and higher lumenal $[HCO_3^-]$ as observed in more distal segment of the intestine there will be less favorable conditions for $Cl^-/HCO_3^-$ exchange in posterior compared to anterior segments of the intestine. This is in agreement with the observed $[HCO_3^-]$ in intestinal fluids in most of the investigated species. The $[HCO_3^-]$ in fluids from the anterior segment of the intestine is already high, whereas

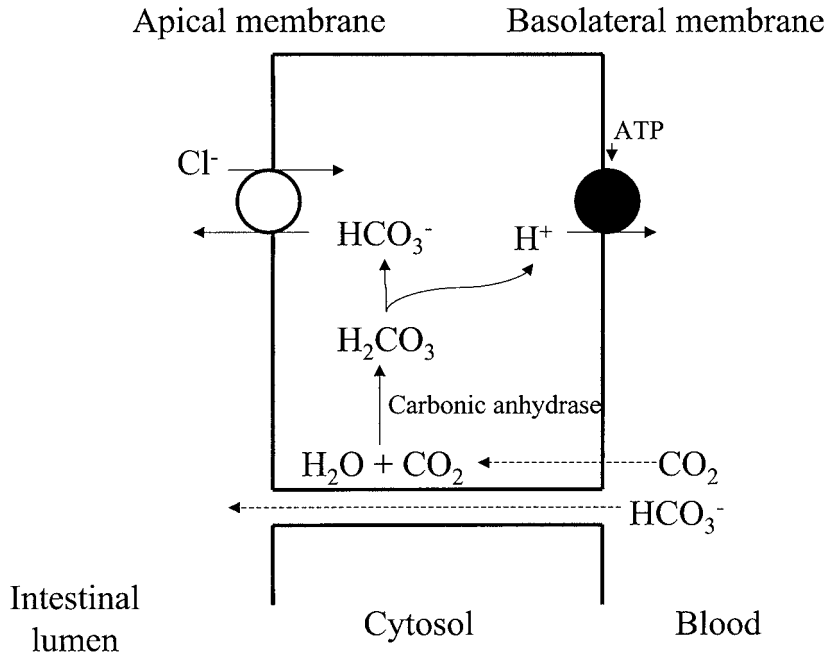


*Figure 5.* Proposed conceptual model for transepithelial $HCO_3^-$ transport by the teleost intestine. Directions of fluxes are depicted. Solid lines indicate enzymatic processes or $Cl^-/HCO_3^-$ exchange and the dotted line indicates simple diffusion occurring under conditions with low luminal $HCO_3^-$ concentration. The combined activity of cytosolic carbonic anhydrase and a basolateral ATP-dependent proton pump increase the cytosolic $HCO_3^-$ concentration to favor apical $Cl^-/HCO_3^-$ exchange and thereby $HCO_3^-$ secretion. See text for further details.

the $[HCO_3^-]$ in mid and posterior segments are only slightly higher, indicating that most of the $HCO_3^-$ secretion occurs in the anterior segment of the intestine. *In vitro*, however, anterior, mid and posterior segments, exhibit similar $HCO_3^-$ flux rates when isolated and exposed to the same lumenal saline (present study Figures 1–4; Grosell et al. 1999; Grosell and Jensen 1999). It thus appears that the $Cl^-/HCO_3^-$ exchanger is present in all segments of the intestine of at least the Pacific sanddab, European flounder, and lemon sole, but that the conditions *in vivo* favor $Cl^-/HCO_3^-$ exchange less in the mid and posterior segment than in the anterior segment due to lower lumenal $[Cl^-]$ and higher lumenal $[HCO_3^-]$. Under our experimental conditions, considerable $HCO_3^-$ equivalent secretion prevails even in the presence of DIDS or the absence of $Cl^-$ (Figures 1 and 2). This however, is not surprising considering the low $HCO_3^-$ in the mucosal saline and an estimated TEP of −3–8 mV, blood side negative (see Introduction), resulting in an electrochemical gradient favoring simple $HCO_3^-$ ion diffusion from serosa to mucosa.

*Ion and water transport by the intestinal epithelium*

$Na^+$ transport was completely abolished in the absence of $Cl^-$. This is not surprising because $Na^+$ entry across the apical membrane via the Na/Cl and Na/K/2Cl co-transporters will depend directly on the availability of $Cl^-$ (reviewed by Loretz 1995). Even though $Cl^-$ transport was greatly reduced in the absence of $Na^+$, some $Cl^-$ transport still remained under $Na^+$-free conditions. The magnitude of this $Cl^-$ flux was comparable to the $HCO_3^-$ flux in the very same preparations (Figures 2 and 4) and thus could be explained by transport of $Cl^-$ by the $Cl^-/HCO_3^-$ exchanger under $Na^+$-free conditions. This net $Cl^-$ flux must be active since it occurs against an electrochemical gradient (blood side negative TEP). Active $Cl^-$ transport by a $Cl^-/HCO_3^-$ exchanger in higher vertebrates has been documented extensively (reviewed by Hviid Larsen 1991; Hviid Larsen et al. 1996). As expected, water transport was strongly dependant on presence of both $Na^+$ and $Cl^-$ in the mucosal fluids.

## Conclusions

An apical $Cl^-/HCO_3^-$ exchanger seems to be present in most of the marine teleost species investigated so far. The transport of $HCO_3^-$ needed to establish the high $HCO_3^-$ concentrations in the intestinal lumen of most marine teleosts could be explained by endogenous metabolic $CO_2$ production combined with a low $CO_2$ permeability resulting in high intracellular $P_{CO2}$, and the combined action of cytosolic carbonic anhydrase, a basolateral proton pump and an apical $Cl^-/HCO_3^-$ exchanger.

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