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# Intestinal $HCO_3^-$ Secretion in *Amphiuma*: Stimulation by Mucosal $Cl^-$ and Serosal $Na^+$

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**Summary.** The requirement for $Na^+$ and $Cl^-$ in the bathing media to obtain a maximal $HCO_3^-$ secretory flux ($J^{HCO_3^-}$) across isolated short-circuited *Amphiuma* duodenum was investigated using titration techniques and ion substitution. Upon substitution of media $Na^+$ with choline, $HCO_3^-$ secretion was markedly reduced. Replacement of media $Cl^-$ produced a smaller reduction of $J^{HCO_3^-}$. The presence of $Cl^-$ enhanced $HCO_3^-$ secretion only if $Na^+$ was also in the media. Elevation of media $Na^+$ or $Cl^-$ in the presence of the other ion produced a saturable increase of $J^{HCO_3^-}$. In the presence of $Na^+$, $Cl^-$ stimulated $J^{HCO_3^-}$ when added to the mucosal but not the serosal medium. In the presence of $Cl^-$, $Na^+$ elevated $J^{HCO_3^-}$ when added to the serosal but not the mucosal medium. The ability of mucosal $Cl^-$ to stimulate $J^{HCO_3^-}$ was not apparently dependent on mucosal $Na^+$. Simultaneous addition of 10 mM $Cl^-$ to the $Na^+$-free mucosal medium and 10 mM $Na^+$ to the $Cl^-$-free serosal medium stimulated $J^{HCO_3^-}$ above levels produced by serosal $Na^+$ alone. In conclusion, intestinal $HCO_3^-$ secretion required mucosal $Cl^-$ and serosal $Na^+$ and did not involve mucosal NaCl cotransport. The results are consistent with a mucosal $Cl^-$ absorptive mechanism in series with parallel basolateral $Na^+$-$H^+$ and $Cl^-$-$HCO_3^-$ exchange mechanisms.

**Key Words** chloride · sodium · bicarbonate · intestine · *amphiuma* · cotransport

## Introduction

The secretion of $HCO_3^-$ linked to the absorption of $Cl^-$ has been observed in a wide variety of epithelia including frog skin [3], turtle urinary bladder [1, 13, 20], stomach [4], gallbladder [9], and the large [5] and small intestine [10, 17, 19]. While the term $Cl^-$-$HCO_3^-$ exchange is often attached to this process, the implication of linked, passive transport is often contrary to the observations. When examined under rigorous *in vitro* conditions the movements of both ions are against their electrochemical gradients [1, 3, 5, 17]. Moreover, although the exchange is independent of the presence of $Na^+$ in the medium bathing turtle urinary bladder [1, 11] and rabbit colon [5], $Cl^-$-$HCO_3^-$ exchange in guinea pig gallbladder [9] is reported to be $Na^+$-dependent.

The small intestine of the urodele *Amphiuma* secretes $HCO_3^-$ actively *in vitro* at a rate which is optimal only when $Na^+$ and $Cl^-$ are present in the bathing medium [12]. Replacement of $Na^+$ with choline or $Cl^-$ with $SO_4^{2-}$ reduced the rate of $HCO_3^-$ secretion by short-circuited segments of isolated duodenum. In this same preparation we have also reported that active electrogenic $Cl^-$ absorption occurs if $Na^+$ and $HCO_3^-$ are present in the medium [23]. Complete replacement of either ion eliminated $Cl^-$ absorption. One possible interpretation of these observations is that $Cl^-$ absorption is linked to $Na^+$ uptake via Na-Cl cotransport at the mucosal membrane, as reported in other intestinal preparations [7, 15], and that $Cl^-$ accumulated within the cytoplasm exchanges with $HCO_3^-$ at the serosal membrane. Indeed, serosal application of the stilbene SITS, the inhibitor of anion exchange in erythrocytes [2], eliminated $Cl^-$ absorption [23] and $HCO_3^-$ secretion [12] in *Amphiuma* intestine. This finding supports the second step in the proposed scheme, i.e., exchange of $Cl^-$ and $HCO_3^-$ at the serosal membrane. However, mucosal Na-Cl cotransport was not favored in an earlier report [23] because $Cl^-$ transport is electrogenic and because there was no relationship between unidirectional or net fluxes of isotopically labeled $Na^+$ and $Cl^-$.

The present study utilized ion substitution procedures to characterize the roles of $Na^+$ and $Cl^-$ in $HCO_3^-$ secretion and the possible role of Na-Cl cotransport in this process.

$HCO_3^-$ secretion was observed to be divisible into three components: a $Na^+$ and $Cl^-$-independent component, a $Na^+$-dependent component, and a component that required the presence of both $Na^+$ and $Cl^-$. $Na^+$ was required in the serosal medium, while $Cl^-$ was required in the mucosal fluid. The results do not support Na-Cl cotransport in the process of $HCO_3^-$ secretion. More likely, $Cl^-$-$HCO_3^-$ and $Na^+$-$H^+$ exchange are operating.

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**Table 1.** Bathing media

| | Normal media | | $Na^+$-free media | | $Cl^-$-free media | | $Na^+$, Cl-free media | |
|---|---|---|---|---|---|---|---|---|
| | *B* | *U* | *B* | *U* | *B* | *U* | *B* | *U* |
| $Na^+$ | 95 | 95 | 0 | 0 | 95 | 95 | 0 | 0 |
| Choline$^+$ | 0 | 0 | 95 | 70 | 0 | 0 | 95 | 95 |
| $Cl^-$ | 76.3 | 76.3 | 70 | 70 | 0 | 0 | 0 | 0 |
| $SO_4^{-2}$ | 0 | 12.5 | 2.25 | 2.25 | 37.25 | 49.75 | 37.25 | 49.75 |
| $HCO_3^-$ | 25 | 0 | 25 | 0 | 25 | 0 | 25 | 0 |
| $K^+$ | 2.5 | 2.5 | 2.5 | 2.5 | 2.5 | 2.5 | 2.5 | 2.5 |
| $Ca^{+2}$ | 0.9 | 0.9 | 0.9 | 0.9 | 0.9 | 0.9 | 0.9 | 0.9 |
| $Mg^{+2}$ | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 |
| Gluconate | 0 | 0 | 1.8 | 1.8 | 1.8 | 1.8 | 1.8 | 1.8 |
| Mannitol | 20 | 32.5 | 20 | 70 | 55 | 67.5 | 56.55 | 62.1 |

*B* – buffered serosal medium; *U* – unbuffered mucosal medium. Ion concentrations are in meq/liter. Media $Na^+$ concentration was varied from 1 to 25.5 mM by substitution with choline. Media $Cl^-$ concentration was varied from 1 to 25.5 mM by substitution with $SO_4^{2-}$ with adjustment of the mannitol concentration to maintain the osmolarity.

## Materials and Methods

### *Animals and Tissue Preparations*

Adult *Amphiuma* were maintained and anesthetized as described previously [23]. Proximal segments of small intestine, which will be referred to as duodenum, were excised and stripped of their outer muscle layers while bathed in oxygenated media. Two adjacent duodenal segments were each opened and mounted as a sheet in separate plexiglass chambers.

### *Bathing Media*

The composition of the bathing media used in this study is seen in Table 1. The $HCO_3^-$-buffered medium used in the serosal compartment was gassed vigorously with humidified 95% $O_2$/5% $CO_2$. The companion unbuffered medium employed in the mucosal compartment was gassed with $O_2$ passed through a solution of NaOH to remove $CO_2$. The choline sulfate employed in the $Na^+$ and $Cl^-$-free medium (Table 1) was prepared by titration of choline $HCO_3^-$ to pH 7.4 with $H_2SO_4$.

### *Experimental Procedure*

Before sacrificing the animal two identical tissue chambers were assembled and pre-equilibrated with the gas mixture. The chambers, as previously described [23], accommodated 1.77 $cm^2$ of mucosa and allowed the continual measurement of the rate of $HCO_3^-$ secretion, while the tissue was voltage clamped to eliminate the transepithelial potential. When the sodium concentration of the medium was varied, the potential-sensing and current-passing agar bridges were formed with saturated KCl and unbuffered Na-free (choline) media, respectively; when the chloride concentration was varied, the bridges were made with 1 M Na formate and unbuffered $Cl^-$-free ($SO_4^{2-}$) media, respectively. When both $Na^+$ and $Cl^-$ were replaced, all agar bridges were formed with unbuffered choline $SO_4^{2-}$ media. After the tissue was in place both compartments of the tissue chamber were rinsed frequently with fresh medium for 1–2 hr before titration commenced. The paired duodenal segments were randomized between two automatic titrating units.

### *Measurement of $HCO_3^-$ secretion*

Intestinal segments bathed on their serosal surface with buffered medium (10 ml) alkalinized the unbuffered mucosal medium (5 ml). The $HCO_3^-$ secretory flux was measured by titrating the mucosal medium with 0.05 N $H_2SO_4$ by means of a Radiometer titration system (The London Co., Cleveland, Ohio). The slope of the titration curve was calculated automatically at 20-min intervals using a Hewlett-Packard Digitizer in line with a Calculator Plotter. The mucosal pH was held constant at the pH of the serosal medium (7.38–7.42). The rate of titration, referred to as the $HCO_3^-$ secretory flux $J^{HCO_3^-}$, was expressed in μeq/hr $cm^2$. The titrant was standardized with potassium hydrogen phthalate (National Bureau of Standards, Washington, D.C.). The total volume of titrant added was maximally about 0.20 ml.

### *Electrical Measurements*

The transmural potential difference and the current required to reduce the potential to zero, the short-circuit current ($I_{sc}$), were measured with an automatic voltage-clamp device which automatically compensated for the solution resistance [23]. While all of the measurements were performed with the transepithelial potential clamped at 0 mV, the $I_{sc}$ is only reported in an initial study because the interpretation of subsequent measurements were complicated by the presence of diffusion potentials.

### *Sodium Flux Measurements*

The serosa-to-mucosa flux of $Na^+$ was determined using $^{22}Na$. The isotope was added to the serosal fluid (1 μCi/ml) immediately following addition of cold $Na^+$ to elevate the serosal [$Na^+$] to 10 meq/liter. After 1 hr 100 μl samples were withdrawn from the mucosal fluid every 20 min, the volume of the mucosal compartment being maintained by subsequent addition of unbuffered medium. $^{22}Na$ was counted with a Packard liquid scintillation spectrometer and the $Na^+$ flux calculated by standard methods employed previously [23].

### *Statistical Tests*

Statistical significance was examined using Student's *t*-test for paired or unpaired comparisons. All errors are expressed as the standard error of the mean.

## Results

### *Substitution of $Na^+$ and $Cl^-$*

The time course of the $HCO_3^-$ secretory flux $J^{HCO_3^-}$ across short-circuited duodenal segments is seen in Fig. 1. $J^{HCO_3^-}$ was greatest when $Na^+$ and $Cl^-$ were present in the mucosal and serosal media (Table 1, normal media) and was reduced when either $Na^+$ or $Cl^-$ was substituted. When $Cl^-$ was replaced with $SO_4^{2-}$, $J^{HCO_3^-}$ was lower than in paired segments bathed in the normal media. The value of $J^{HCO_3^-}$ in $Cl^-$-free media approached the value in the normal media after 4 hr. $J^{HCO_3^-}$ was lowest when tissues were bathed in media in which both $Na^+$ and $Cl^-$ were replaced. Tissues bathed in $Na^+$-free media were also

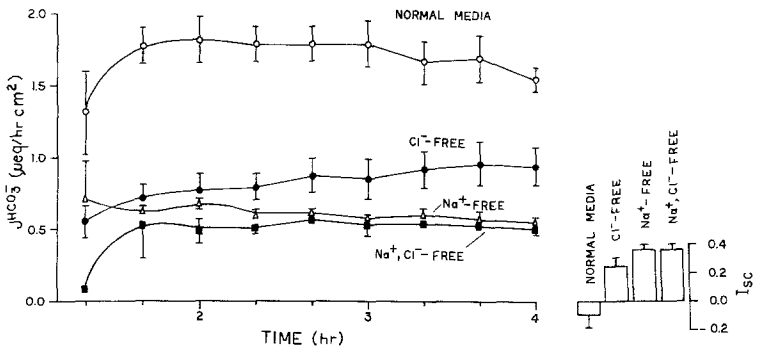


**Fig. 1.** Average time course of $HCO_3^-$ secretory flux ($J^{HCO_3^-}$) in media of different composition. The short-circuit ($I_{sc}$) at 4 h is provided for comparison. Paired tissues from the same animal ($n=4$) were used to compare normal media with $Cl^-$-free media. Additional paired segments ($n=3$) were used to compare $Na^+$-free with $Na^+$, $Cl^-$-free media

secreting $HCO_3^-$ at a relatively slow rate after 4 hr. Thus $HCO_3^-$ secretion was maximal in the presence of both $Na^+$ and $Cl^-$; media $Na^+$ supported secretion in the presence or absence of $Cl^-$, while the effects of media $Cl^-$ was marked only in the presence of $Na^+$. Comparison of the time course of $HCO_3^-$ secretion in normal media with that in $Cl^-$-free media suggested that the stimulating effect of $Cl^-$ was greatest early and declined steadily with time. Additional support for this view is given below. Also seen in Fig. 1 the short-circuit current ($I_{sc}$) at 4 hr was slightly negative in normal media and differed significantly from $J^{HCO_3^-}$ ($P<0.01$) in agreement with earlier observations [23]. $J^{HCO_3^-}$ was somewhat greater than $I_{sc}$ in $Cl^-$-free media as well ($P<0.05$).

### *Relationship between Media $Na^+$ and $J^{HCO_3^-}$*

The rate of $HCO_3^-$ secretion was measured in $Cl^-$-containing media as the [$Na^+$] of the media was varied from 0 to 95 meq/liter by substitution with choline. This was done in either of two ways. In the first series adjacent segments of tissue from the same animal were exposed to media containing $Na^+$ at a single concentration and $J^{HCO_3^-}$ was measured at 4 hr. In the second series segments were rinsed in $Na^+$-free media then exposed to a series of media of increasing [$Na^+$] for 60–80 min intervals. This required simultaneous replacement of the unbuffered mucosal and buffered serosal medium. A new stable rate of $HCO_3^-$ secretion usually developed within 40 min. Since the results of both protocols were practically identical, they were pooled and are illustrated in Fig. 2. $J^{HCO_3^-}$ was not influenced by elevation of $Na^+$ to 1 meq/liter, but a detectable increase was observed at 5 meq/liter and saturation was evident at 25 meq/liter $Na^+$.

### *Media $Cl^-$ vs. $J^{HCO_3^-}$*

The influence of the $Cl^-$ concentration of the media on $J^{HCO_3^-}$ is also illustrated in Fig. 2. In this series segments initially bathed in $Cl^-$-free media (Table 1) were exposed on both surfaces to a series of solutions

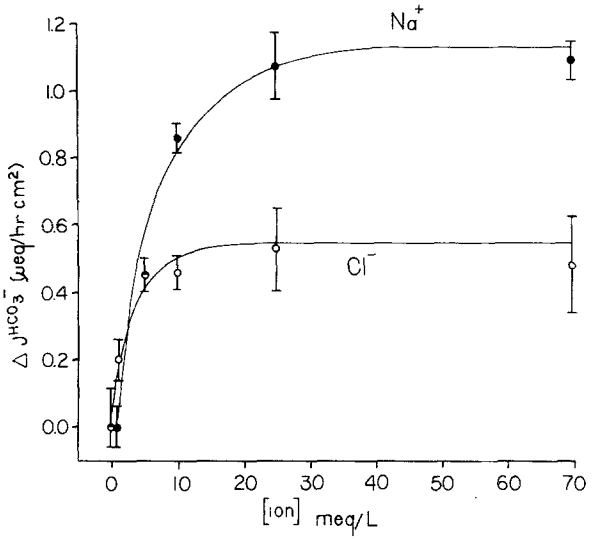


**Fig. 2.** The $HCO_3^-$ secretory flux $J^{HCO_3^-}$ as $Na^+$ or $Cl^-$ was increased simultaneously in both mucosal and serosal bathing fluid by substitution of media choline or $SO_4^{2-}$, respectively. Each point is the average of six ($Na^+$) or eight ($Cl^-$) measurements. The curves were drawn by eye

of increasing [$Cl^-$]. In contrast with the results observed on addition of $Na^+$, $J^{HCO_3^-}$ was enhanced measureably when only 1 meq/liter of $Cl^-$ was present in the bathing media and was elevated further as $Cl^-$ was raised to 25.5 mM. Further increases in medium $Cl^-$ did not increase $J^{HCO_3^-}$. The [$Cl^-$] of the final solution (70 mM) was equivalent to normal media. The smaller stimulation of $J^{HCO_3^-}$ following $Cl^-$ addition as compared with $Na^+$ addition seen in Fig. 2 was consistent with the results in Fig. 1, since at 4 hr the difference in $J^{HCO_3^-}$ between normal media and $Na^+$-free media exceeded that between normal *vs.* $Cl^-$-free media.

### *Sidedness of $Na^+$ Stimulation*

In order to determine whether $Na^+$ was more effective at one surface of the epithelium, the mucosa was exposed on the luminal face to a medium of normal [$Na^+$] while the serosal medium was $Na^+$-free. For the companion tissue only the mucosal medium was

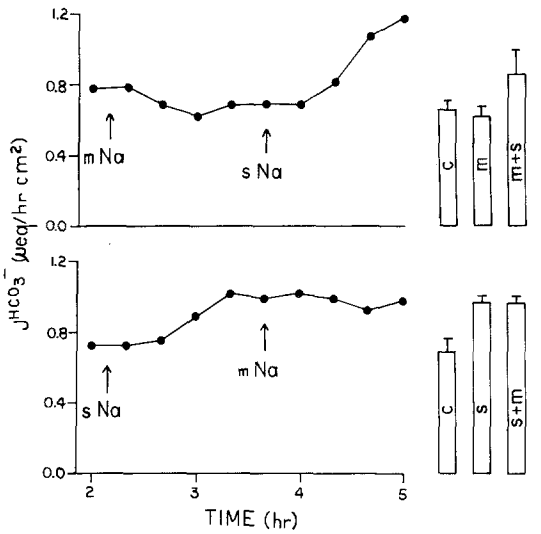


**Fig. 3.** Typical response in paired segments to addition at the arrow of sodium (as NaCl) to the mucosal fluid (*m Na*) or serosal fluid (*s Na*). The bar diagram to the right illustrates the average control $J^{HCO_3^-}$ (*c*) and the value after unilateral then bilateral addition of $Na^+$ in paired adjacent duodenal segments from four animals. $Cl^-$ was present at 76.4 mM before the addition

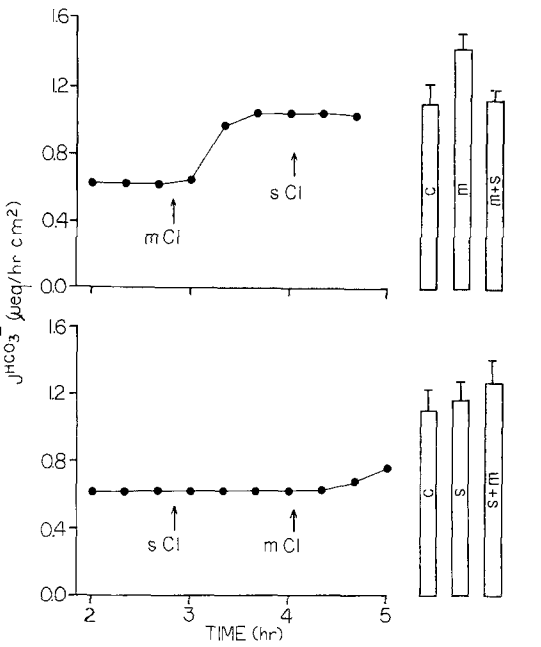


**Fig. 4.** Typical response in paired segments to addition of $Cl^-$ (as NaCl). The bar diagram to the right illustrates the control $J^{HCO_3^-}$ (*c*) and the response to $Cl^-$ addition in paired tissues from six animals at 4 hr. Na was present initially at 95 mM

$Na^+$-free. No detectable difference in $J^{HCO_3^-}$ was observed in preliminary observations, possibly because of the high tissue conductance to $Na^+$. To circumvent this potential difficulty, intestinal segments were bathed in $Na^+$-free media and after a constant rate of $HCO_3^-$ secretion had developed a small volume of NaCl was added to raise the $[Na^+]$ to 10 mM. As seen in Fig. 3, within 80 min after serosal $Na^+$ addition $J^{HCO_3^-}$ had risen to a new stable value. In contrast, when NaCl was added to the mucosal side to the same final concentration $HCO_3^-$ secretion was not enhanced. $J^{HCO_3^-}$ was measureably stimulated (Fig. 3) only after subsequent addition of NaCl to the serosal medium. In four animals addition of serosal $Na^+$ increased $J^{HCO_3^-}$ by $0.27 \pm 0.04$ μeq/hr $cm^2$ compared to a very slight reduction in $J^{HCO_3^-}$ ($-0.04 \pm 0.02$ μeq/hr $cm^2$) following mucosal $Na^+$ addition in paired adjacent segments. The effect of serosal $Na^+$ was highly significant ($P < 0.01$).

### *Sidedness of $Cl^-$* Stimulation

A similar protocol was used to investigate the sidedness of $Cl^-$ stimulation. Paired segments initially bathed in $Cl^-$-free media (Table 1) were exposed to $Cl^-$ on either the mucosal or serosal surface. As seen in Fig. 4 for a typical tissue, mucosal addition of $Cl^-$ (as NaCl) to a final concentration of 10 mM elevated $J^{HCO_3^-}$ while serosal addition of $Cl^-$ was completely ineffective. In six tissues averaged in Fig. 4, $J^{HCO_3^-}$ was significantly stimulated ($P < 0.01$) by addition of $Cl^-$ on the mucosal side. It is also evident in the figure and adjacent bar graph that early addition of $Cl^-$ to the mucosal medium was more stimulatory than when added later after serosal addition of $Cl^-$. This was probably due to a temporal decline in sensitivity to $Cl^-$ as discussed in regard to Fig. 1.

### *Role of $Na^+$ in $Cl^-$ Stimulation*

Since $Cl^-$ was able to stimulate $HCO_3^-$ secretion only in the presence of $Na^+$, the possibility that this stimulatory effect of mucosal $Cl^-$ depended on Na-Cl cotransport was examined in two series. In the first, paired segments were rinsed in $Na^+$-free media ($Cl^- = 70$ mM) for 60 min. The $[Na^+]$ of the medium bathing each surface of one segment was then raised to 10 mM by addition of $Na_2SO_4$, while for the adjacent segment only the $[Na^+]$ of the serosal medium was elevated. As seen in Table 2, although $HCO_3^-$ secretion was enhanced in both segments, the addition of $Na^+$ to mucosal and serosal media did not stimulate $HCO_3^-$ secretion over that produced by elevation of serosal $Na^+$ alone. The response in paired segments was not statistically different ($P > 0.05$).

In the second series paired segments were rinsed for 60 min in $Na^+$ and $Cl^-$-free media. At 1 hr the $[Na^+]$ of the serosal medium bathing both segments was elevated to 10 mM by addition of $Na_2SO_4$. At 140 min NaCl was added to the mucosal medium

**Table 2.** Stimulation of $HCO_3^-$ secretion by mucosal+serosal $Na^+$ *vs.* serosal $Na^+$

| | Control | Addition | $\Delta J^{HCO_3^-}$ |
|---|---|---|---|
| $J^{HCO_3^-}$ | Choline $Cl^-$ | Mucosal+serosal $Na^+$ | |
| | $0.64 \pm 0.04$ | $0.93 \pm 0.04$ | $0.29 \pm 0.04$ |
| $J^{HCO_3^-}$ | Choline $Cl^-$ | Serosal $Na^+$ | |
| | $0.58 \pm 0.04$ | $0.96 \pm 0.12$ | $0.38 \pm 0.10$ |

$HCO_3^-$ fluxes are in μeq/hr $cm^2$. Control rates were obtained in tissues incubated in Na-free (choline) media with 70 mM $Cl^-$. $Na^+$ was added as $Na_2SO_4$. The response of paired tissues from four animals was averaged. Additions were made at approximately 140 min. The final $[Na^+] = 10$ mM.

**Table 3.** Stimulation of $HCO_3^-$ secretion by mucosal $Cl^-$ with or without $Na^+$

| | Control | Mucosal addition | $\Delta J^{HCO_3^-}$ |
|---|---|---|---|
| $J^{HCO_3^-}$ | 10 mM serosal $Na^+$ | 10 mM NaCl | |
| | $0.62 \pm 0.04$ | $1.16 \pm 0.04$ | $0.54 \pm 0.06$ |
| $J^{HCO_3^-}$ | 10 mM serosal $Na^+$ | 10 mM choline Cl | |
| | $0.56 \pm 0.04$ | $0.99 \pm 0.05$ | $0.43 \pm 0.06$ |

$HCO_3^-$ fluxes are in μeq/hr $cm^2$. Segments were incubated in Na, Cl-free media for 1 hr. Control rates were obtained after addition of $Na^+$ (as $Na_2SO_4$) to the serosal medium to a final concentration of 10 mM. The response of paired tissues from four animals was averaged. Additions were made at approximately 140 min.

bathing one segment to raise the NaCl concentration to 10 mM. In the adjacent segment choline $Cl^-$ was added to the same final concentration. The results in Table 3 illustrate that $HCO_3^-$ secretion was stimulated in both segments about equally. Comparison of the relative degree of stimulation in the paired segments indicate that the presence of $Na^+$ along with $Cl^-$ on the mucosal side did not significantly accelerate $HCO_3^-$ secretion ($P > 0.05$) over segments exposed to choline along with $Cl^-$. Subsequent addition of NaCl on the mucosal side of the tissue previously exposed to choline $Cl^-$ failed to further stimulate secretion.

### *Mucosal $Cl^-$ Stimulation of $J^{HCO_3^-}$ in Absence of Mucosal Na*

Stimulation of $HCO_3^-$ secretion by mucosal $Cl^-$ in the absence of mucosal $Na^+$ was demonstrated in the next series. Paired segments were rinsed in $Na^+$ and $Cl^-$-free media for 1 hr then serosal $Na^+$ was elevated to 10 mM by addition of $Na_2SO_4$. At the same time choline $Cl^-$ was added to the mucosal medium of one segment to raise the $[Cl^-]$ to 10 mM. As seen in Fig. 5, $J^{HCO_3^-}$ was markedly greater in the segment exposed to mucosal $Cl^-$. The difference was significant ($P > 0.05$) as seen in the averaged results of five tissues. Thus while the ability of mucosal $Cl^-$

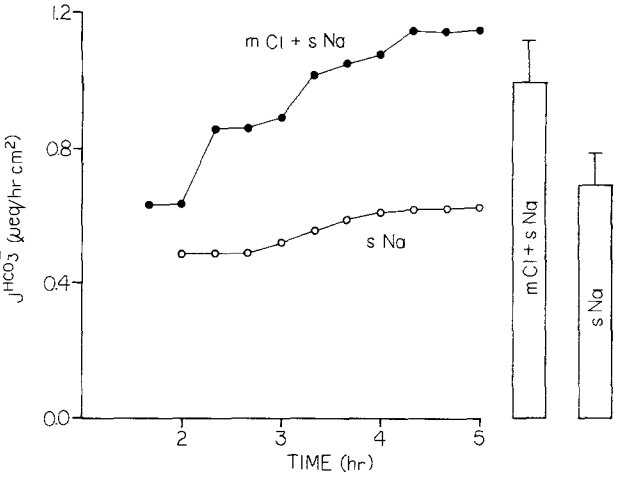


**Fig. 5.** Typical response in paired segments to addition of 10 mM $Na^+$ serosally (*sNa*) compared with simultaneous addition of 10 mM $Cl^-$ mucosally and 10 mM $Na^+$ serosally (*mCl+sNa*). The bar diagram illustrates the average $J^{HCO_3^-}$ of paired tissues from five animals at 4 hr

to enhance $HCO_3^-$ secretion was dependent on the presence of $Na^+$, these results indicate that it was serosal $Na^+$ which was necessary and sufficient – there was no obvious requirement for $Na^+$ in the mucosal medium.

### *Estimate of $Na^+$ Leak to Mucosal Medium*

To examine whether appreciable amounts of $Na^+$ could diffuse into the mucosal medium to support cotransport with $Cl^-$, $^{22}Na$ was added to the serosal medium simultaneous with elevation of the $[Na^+]$ to 10 meq/liter. In four tissues the addition of $Na^+$ elevated $J^{HCO_3^-}$ significantly by $0.23 \pm 0.04$ μeq/hr $cm^2$. The calculated $[Na^+]$ of the mucosal medium rose slowly to 0.16 meq/liter after 160 min. Since, as seen in Fig. 2, $HCO_3^-$ secretion was not stimulated after raising mucosal and serosal $Na^+$ to 1 meq/liter, this low concentration of $Na^+$ in the mucosal fluid could not account for the increase in $J^{HCO_3^-}$. Therefore, serosal $Na^+$ alone was sufficient to stimulate $HCO_3^-$ secretion.

## Discussion

Duodenal $HCO_3^-$ secretion in *Amphiuma* is an active transport process that is dependent upon metabolism and is linked to the transport of $Na^+$ and $Cl^-$ [12, 23]. By using the titration technique, the dependence of $HCO_3^-$ secretion on the presence of $Na^+$ and $Cl^-$ in the media, as reported previously [12], was confirmed and examined further in this study. Specifically, the possibility was examined that the dependence on both $Na^+$ and $Cl^-$ reflected the operation of a mucosal Na-Cl cotransport system, e.g., one pro-

viding intracellular $Cl^-$ for exchange with serosal $HCO_3^-$. This hypothesis was in keeping with luminal Na-Cl cotransport reported for rabbit ileum and flounder small intestine [7, 15]. Substitution of $Na^+$ and $Cl^-$ produced results, however, that could not be explained so simply. Although $HCO_3^-$ secretion was lower in the absence of $Na^+$ as seen in Fig. 1, a sizeable secretion occurred in $Cl^-$-free media. Clearly $HCO_3^-$ secretion was not primarily $Cl^-$ dependent. Nevertheless, $Cl^-$ was able to stimulate secretion but only in the presence of $Na^+$ (Fig. 1) which left open the possibility of a role discussed further below, for Na-Cl cotransport in $HCO_3^-$ secretion. Recently $Cl^-$-dependent $HCO_3^-$ secretion by guinea pig gallbladder was reported by Heintze et al. to be $Na^+$-dependent [9]. These findings in *Amphiuma* and those reported for guinea pig gallbladder contrast with results in bullfrog duodenum [18] and rabbit cortical collecting tubules [14] in which $HCO_3^-$ secretion is not $Cl^-$-dependent.

Duodenal $HCO_3^-$ secretion is a saturable function of media $Na^+$ and $Cl^-$ in *Amphiuma*, suggesting the involvement of carrier-mediated mechanisms in the interactions. Therefore, although $HCO_3^-$ secretion occurs in the direction of the chemical gradient, it cannot be due solely to passive leakage through a paracellular pathway but instead must involve a transcellular route. A transcellular route is also evident from previous observations [12] that $HCO_3^-$ secretion is saturable and is reduced by acetazolamide, ouabain, and SITS, the inhibitor of erythrocyte $Cl^-/HCO_3^-$ exchange [2]. Moreover, $HCO_3^-$ secretion is eliminated in the presence of 100% $N_2$ despite the presence of a $HCO_3^-$ gradient [12] indicating that passive leakage of $HCO_3^-$ is virtually negligible. The nature of the $Na^+$ and $Cl^-$ independent secretion, amounting to about 0.5 μeq/hr $cm^2$ (Fig. 1), remains to be determined.

While the normal plasma level of $HCO_3^-$ is about 14 mM in *Amphiuma* [8], a medium bicarbonate concentration of 25 mM was employed in the present study since at this concentration the rate of $HCO_3^-$ secretion was maximal in a previous study [12].

Determining whether $Na^+$ and $Cl^-$ stimulated $HCO_3^-$ secretion more effectively when added to one or the other side of the mucosa was not possible at the normal media concentrations of these ions, possibly because the passive permeability of the intestinal mucosa for the ions was high. This problem was circumvented by using lower concentrations of $Na^+$ and $Cl^-$. By this approach submaximal but significant stimulation of $HCO_3^-$ secretion was produced by $Cl^-$ added to the mucosal medium, as observed in gastric mucosa [4], or $Na^+$ added to the serosal medium, as reported in bullfrog duodenum [18]. That $HCO_3^-$ secretion was stimulated by serosal rather than mucosal $Na^+$ argues against mucosal Na-Cl cotransport. However, $Cl^-$-stimulated $HCO_3^-$ secretion, which requires mucosal $Cl^-$ (Fig. 4), could involve Na-Cl cotransport since $Na^+$ was also present in the mucosal medium. This was tested in two series. In the first, it was observed in $Cl^-$-containing media that $HCO_3^-$ secretion following addition of 10 mM $Na^+$ to the serosal fluid was not greater if the same concentration of $Na^+$ was added simultaneously to the mucosal fluid (Table 2). In the second series, when NaCl was added to a final concentration of 10 mM in the mucosal medium $HCO_3^-$ secretion was not enhanced over controls exposed similarly to 10 mM choline $Cl^-$ (Table 3). Since $Cl^-$ stimulation of $HCO_3^-$ secretion was marked at 10 mM $Cl^-$ (Fig. 2), the involvement of Na-Cl cotransport would have been evident in both series unless the affinity for $Na^+$ binding to a Na-Cl carrier was very much smaller than that for $Cl^-$.

Direct evidence that stimulation of $J^{HCO_3^-}$ by mucosal $Cl^-$ is independent of mucosal $Na^+$ was obtained when $HCO_3^-$ secretion was observed to be markedly greater in tissues exposed simultaneously to mucosal 10 mM $Cl^-$ (as choline $Cl^-$) and serosal 10 mM $Na^+$ (as $Na_2SO_4$) over tissues exposed to serosal $Na^+$ alone (Fig. 5). Assuming that $Na^+$ did not enter the mucosal fluid from the serosal fluid in sufficient amounts to support cotransport, $HCO_3^-$ secretion in the absence of added mucosal $Na^+$ argues very strongly against the involvement of mucosal Na-Cl cotransport in Na+Cl co-dependent $HCO_3^-$ secretion. Direct measurement of the leak of $Na^+$ from serosa to mucosa indicated that the $[Na^+]$ of the mucosal medium did not exceed 0.1 meq/liter after 1 hr. Since $J^{HCO_3^-}$ in those tissues exposed to mucosal $Cl^-$ was significantly above that of control tissues even before 1 hr, as suggested in Fig. 5, and elevation of media $Na^+$ to 1 mM does not stimulate $HCO_3^-$ secretion (Fig. 2), mucosal $Na^+$ could not be supporting $Cl^-$-dependent $HCO_3^-$ secretion unless the $[Na^+]$ in the unstirred layers at the vicinity of the brush border exceeds 1 mM. Although ion gradients have been reported between the bulk solution and the unstirred layers adjacent to the brush border when perfusion of the tissue chamber was slow [21, 23], since the mucosal medium was vigorously mixed in this study, sodium of serosal orgin was unlikely to support Na-Cl cotransport.

The role of serosal $Na^+$ and mucosal $Cl^-$ to enhance $HCO_3^-$ secretion could not be a result of their movement as counter ions to the secreted $HCO_3^-$ since these measurements were conducted under short-circuited conditions and thus in the absence of an electrical gradient. Furthermore, the results of Fig. 2 were

obtained on tissue after symmetrical additions of $Na^+$ or $Cl^-$, ruling out the role of transepithelial diffusional fluxes in these observations.

The transport of $Na^+$ or $Cl^-$ may elevate metabolism and stimulate $HCO_3^-$ secretion indirectly rather than through a direct transport link. This is not likely for $Cl^-$ since a direct link between $Cl^-$ transport and $HCO_3^-$ secretion is supported from the dependency of $Cl^-$ absorption on media $HCO_3^-$ and the sensitivity of $Cl^-$ absorption and $HCO_3^-$ secretion to acetazolamide and serosally applied SITS [12, 23]. However, the active accumulation of $Cl^-$ across the mucosal membrane may involve a direct utilization of energy which enhances metabolism and $HCO_3^-$ secretion at least to some extent.

The likelihood that $Na^+$ stimulates $HCO_3^-$ secretion by enhancing metabolism is more remote. While the brush border membrane is believed to possess a finite passive permeability to $Na^+$ [16], the basolateral membrane of the intestinal absorptive cell is poorly permeable to $Na^+$ [6]. The observed stimulation of $HCO_3^-$ secretion with serosal rather than mucosal $Na^+$ is not in keeping with enhanced $Na^+$ transport and suggests that $Na^+$-stimulated $HCO_3^-$ secretion is not related directly to the metabolic requirement for $Na^+$ transport.

The link between $HCO_3^-$ secretion and serosal $Na^+$ is consistent with $Na^+$-$H^+$ exchange across the serosal membrane. In an earlier study net $Na^+$ transport in *Amphiuma* small intestine did not correlate with net $Cl^-$ transport but correlated inversely with the residual flux, that portion of the short-circuit current not attributable to net $Na^+$ and $Cl^-$ fluxes [23]. It was concluded that $Na^+$-$H^+$ rather than Na-Cl cotransport was operating, although the earlier conjecture that this process was localized in the mucosal membrane is not consistent with the present results. If a $Na^+$-$H^+$ exchange process exists it appears to reside in the basolateral membrane. Sherrin and Field localized $Na^+$-$H^+$ exchange to this membrane in rabbit ileum [17]. Similarly, serosal but not mucosal $Na^+$ stimulated duodenal $HCO_3^-$ secretion in bullfrog using the same technique employed in this study [18]. In contrast $Cl^-$-$HCO_3^-$ exchange in turtle urinary bladder [11] and rabbit colon [5] are not $Na^+$-dependent.

Net $Cl^-$ absorption requires the presence of $HCO_3^-$ in the medium bathing *Amphiuma* small intestine [23]. This observation taken with the complete inhibition of $Cl^-$ absorption [23] and the partial inhibition of $HCO_3^-$ secretion [12] by serosal but not mucosal application of the stilbene SITS led to the conclusion that $Cl^-$-$HCO_3^-$ exchange occurs at the basolateral membrane of the intestinal villus cells. The present results demonstrate that $Na^+$ is also required for this exchange process. It was previously demonstrated that in the absence of $Na^+$ net $Cl^-$ absorption failed to occur and intracellular $Cl^-$ activity measured with $Cl^-$-sensitive microelectrodes was not accumulated above electrochemical equilibrium by the absorptive cell [22, 23]. It was proposed that media $Na^+$ was essential for $Cl^-$ absorption by maintaining intracellular pH via $Na^+$-$H^+$ exchange. This exchange would serve to influence cytoplasmic pH and the level of intracellular $HCO_3^-$. Possibly the outward diffusion of $HCO_3^-$ across the mucosal membrane energize $Cl^-$ accumulation by a brush border anion exchange process. However, SITS added to the mucosal fluid does not inhibit $Cl^-$ absorption or $HCO_3^-$ secretion [12, 23]. Furthermore, $Cl^-$ absorption is not electrically neutral. Instead, it is associated with a serosa negative electrical potential [23]. A more likely model is depicted in Fig. 6. $Cl^-$ is accumulated into the cell electrogenically across the mucosal membrane. Intracellular $HCO_3^-$ generated from serosal ion exchange processes ($Na^+$-$H^+$, $Cl^-$-$HCO_3^-$), diffuses down a favorable electrochemical gradient into the luminal media. If intracellular pH (or $HCO_3^-$) regulates the rate of $Cl^-$ uptake or influences the diffusional permeability of $Cl^-$ at the mucosal membrane, as suggested previously [23], then acidification of the cytoplasm following inhibition of $Na^+$-$H^+$ exchange (by $Na^+$ replacement) would limit the availability of cytoplasmic $Cl^-$ for serosal exchange. In this way the dependence on $Na^+$ for active $Cl^-$ accumulation and absorption [23] as well as $Cl^-$-dependent $HCO_3^-$ secretion, observed in this study, would be satisfied.

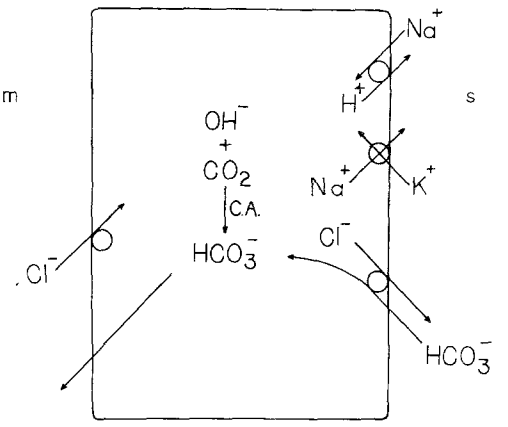


**Fig. 6.** Working model which suggests a role for $Na^+$ and $Cl^-$ in $HCO_3^-$ secretion from mucosal (*m*) to serosal (*s*) medium in *Amphiuma* duodenum. Parallel serosal ion exchange processes control intracellular pH and maintain a gradient for $HCO_3^-$ across the mucosal membrane

In summary, the $Na^+$ dependence of $Cl^-$-enhanced $HCO_3^-$ secretion was related to the serosal effects of $Na^+$ rather than mucosal NaCl cotransport.

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## References

1. Brodsky, W.A., Durham, J., Ehrenspeck, G. 1979. The effects of a disulfonic stilbene on chloride and bicarbonate transport in the turtle bladder. *J. Physiol. (London)* **287**:559–573
2. Cabantchik, Z.I., Rothstein, A. 1972. The nature of the membrane sites controlling anion permeability of human red blood cells as determined by studies with disulfonic stilbene derivatives. *J. Membrane Biol.* **10**:311–330
3. Ehrenfeld, J., Garcia-Romeu, F. 1978. Coupling between chloride absorption and base excretion in isolated skin of *Rana esculenta*. *Am. J. Physiol.* **235**:*F*33–*F*39
4. Flemstrom, G. 1980. $Cl^-$ dependence of $HCO_3^-$ transport in frog gastric mucosa. *Upsala J. Med. Sci.* **85**:303–309
5. Frizzell, R.A., Koch, M.J., Schultz, S.G. 1976. Ion transport by rabbit colon: I. Active and passive components. *J. Membrane Biol.* **27**:297–316
6. Frizzell, R.A., Schultz, S.G. 1972. Ionic conductances of extracellular shunt pathway in rabbit ileum: Influence of shunt on transmural sodium transport and electrical potential difference. *J. Gen. Physiol.* **59**:318–346
7. Frizzell, R.A., Smith, P.L., Vosburgh, E., Field, M. 1979. Coupled sodium-chloride influx across brush border of flounder intestine. *J. Membrane Biol.* **46**:27–39
8. Gunter-Smith, P.J., White, J.F. 1979. Response of *Amphiuma* small intestine to theophylline: Effect on bicarbonate transport. *Am J. Physiol.* **236**:*E*775–*E*783
9. Heintze, K., Petersen, K.U., Olles, P., Saverymuttu, S.H., Wood, J.R. 1979. Effects of bicarbonate on fluid and electrolyte transport by the guinea pig gallbladder: A bicarbonate-chloride exchange. *J. Membrane Biol.* **45**:43–59
10. Hubel, K.A. 1967. Bicarbonate secretion in rat ileum and its dependence on intraluminal chloride. *Am J. Physiol.* **213**:1409–1413
11. Husted, R.F., Eyman, E. 1980. Chloride-bicarbonate exchange in the urinary bladder of the turtle. *Biochim. Biophys. Acta* **595**:305–308
12. Imon, M.A., White, J.F. 1981. Intestinal $HCO_3^-$ secretion in *Amphiuma* measured by pH stat *in vitro*: Relationship with metabolism and transport of sodium and chloride ions. *J. Physiol. (London)* **314**:429–443
13. Leslie, B.R., Schwartz, J.H., Steinmetz, P.R. 1973. Coupling between $Cl^-$ absorption and $HCO_3^-$ secretion in turtle urinary bladder. *Am. J. Physiol.* **225**:610–617
14. McKinney, T.D., Burg, M.B. 1978. Bicarbonate secretion by rabbit cortical collecting tubules *in vitro*. *J. Clin. Invest.* **61**:1421–1427
15. Nellans, H.N., Frizzell, R.A., Schultz, S.G. 1973. Coupled sodium-chloride influx across the brush border of rabbit ileum. *Am. J. Physiol.* **225**:467–475
16. Schultz, S.G., Frizzell, R.A., Nellans, H.N. 1974. Ion transport by mammalian small intestine. *Annu. Rev. Physiol.* **36**:51–91
17. Sheerin, H.E., Field, M. 1975. Ileal $HCO_3^-$ secretion: Relationship to Na and Cl transport and effect of theophylline. *Am. J. Physiol.* **228**:1065–1074
18. Simson, J.N.L., Merhav, A., Silen, W. 1981. Alkaline secretion by amphibian duodenum. I. General characteristics. *Am. J. Physiol.* **240**:*G*401–*G*408
19. Turnberg, L.A., Bieberdorf, F.A., Morawski, S.G., Fordtran, J.S. 1970. Interrelationship of chloride, bicarbonate, sodium and hydrogen transport in the human ileum. *J. Clin. Invest.* **49**:557–567
20. Weiner, M.W. 1980. The effects of bicarbonate and hydroxyl ions on chloride transport by toad bladders. *Biochim. Biophys. Acta* **596**:292–314
21. White, J.F. 1976. Intracellular potassium activities in *Amphiuma* small intestine. *Am. J. Physiol.* **231**:1214–1219
22. White, J.F. 1977. Activity of chloride in absorptive cells of *Amphiuma* small intestine. *Am. J. Physiol.* **232**:*E*553–*E*559
23. White, J.F. 1980. Bicarbonate-dependent chloride absorption in small intestine: Ion fluxes and intracellular chloride activities. *J. Membrane Biol.* **53**:95–107

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