Intracellular pH Regulation in the Renal Proximal Tubule of the Salamander

*Basolateral $\text{HCO}_3^-$ Transport*

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**ABSTRACT** We have used pH-, Na-, and Cl-sensitive microelectrodes to study basolateral $\text{HCO}_3^-$ transport in isolated, perfused proximal tubules of the tiger salamander *Ambystoma tigrinum*. In one series of experiments, we lowered basolateral pH ($pH_b$) from 7.5 to 6.8 by reducing $[\text{HCO}_3^-]_b$ from 10 to 2 mM at a constant $p\text{CO}_2$. This reduction of $pH_b$ and $[\text{HCO}_3^-]_b$ causes a large (~0.35), rapid fall in $pH_i$ as well as a transient depolarization of the basolateral membrane. Returning $pH_b$ and $[\text{HCO}_3^-]_b$ to normal has the opposite effects. Similar reductions of luminal pH ($pH_l$) and $[\text{HCO}_3^-]_l$ have only minor effects. The reduction of $[\text{HCO}_3^-]_b$ and $pH_b$ also produces a reversible fall in $a_{\text{Na}}^-$. In a second series of experiments, we reduced $[\text{Na}^+]_b$ at constant $[\text{HCO}_3^-]_b$ and $pH_b$, and also observed a rapid fall in $pH_i$ and a transient basolateral depolarization. These changes are reversed by returning $[\text{Na}^+]_b$ to normal. The effects of altering $[\text{Na}^+]_b$ in the presence of $\text{HCO}_3^-$, or of altering $[\text{Na}^+]_b$ in the nominal absence of $\text{HCO}_3^-$, are substantially less. Although the effects on $pH_i$ and basolateral membrane potential of altering either $[\text{HCO}_3^-]_b$ or $[\text{Na}^+]_b$ are largely blocked by 4-acetamido-4-isothiocyanostilbene-2,2'-disulphonate (SITS), they are not affected by removal of $\text{Cl}^-$, nor are there accompanying changes in $a_{\text{Na}}^-$. The aforementioned changes are apparently mediated by a single transport system, not involving $\text{Cl}^-$. We conclude that $\text{HCO}_3^-$ transport is restricted to the basolateral membrane, and that $\text{HCO}_3^-$ fluxes are linked to those of $\text{Na}^+$. The data are compatible with an electrogenic $\text{Na}/\text{HCO}_3$ transporter that carries $\text{Na}^+$, $\text{HCO}_3^-$, and net negative charge in the same direction.

**INTRODUCTION**

There is considerable evidence from experiments on intact renal tubules supporting a linkage between acid secretion and $\text{Na}^+$ reabsorption in the proximal tubule (see Warnock and Rector, 1979, for a review). Studies on brush-border (i.e., luminal) membrane vesicles prepared from renal tubules...
have identified a Na-H exchanger (Murer et al., 1976), sensitive to amiloride (Kinsella and Aronson, 1980), with attributes consistent with the long-hypothesized luminal Na-H exchanger. In the first paper of this series (Boron and Boulpaep, 1983), we used ion-sensitive microelectrodes and isolated, perfused proximal tubules of the salamander to study, for the first time, Na-H exchange in intact epithelial cells. We found that the salamander proximal-tubule cells indeed possess a luminal Na-H exchanger. Quite unexpectedly, we found that these cells possess a Na-H exchanger at the basolateral membrane as well. Both luminal and basolateral Na-H exchangers serve to regulate intracellular pH (pHᵢ), much as do comparable transport mechanisms in nerve and muscle cells. The properties of the luminal Na-H exchanger would also enable it to participate in acid secretion. The basolateral Na-H exchanger, however, is physiologically oriented in the wrong direction to effect the basolateral uptake of acid necessary for transcellular acid secretion.

In view of the HCO₃⁻ dependence of proximal-tubule acid secretion (see Warnock and Rector, 1979), it has long been supposed that the basolateral step in acid secretion is actually brought about by the exit of HCO₃⁻. Until now, the most direct evidence for basolateral HCO₃⁻ transport came from the studies of Frömter and his colleagues (Frömter, 1975; Burckhardt and Frömter, 1980), who inferred a HCO₃⁻ or OH⁻ conductance from transient changes in basolateral membrane potential, and those of Ullrich and his colleagues (Radtke et al., 1972; Ullrich et al., 1971, 1975, 1977), who studied the reabsorption of non-HCO₃⁻ buffers. The aforementioned studies, however, have not provided unambiguous evidence for basolateral HCO₃⁻ transport. We now report a direct study of basolateral HCO₃⁻ transport in which we used isolated, perfused proximal tubules of the tiger salamander Ambystoma tigrinum together with microelectrodes for measuring cell membrane potential and intracellular activities of H⁺, Na⁺, or Cl⁻. The results indicate that there is a pathway for HCO₃⁻ transport that is confined to the basolateral membrane. Furthermore, this basolateral HCO₃⁻ transport appears to be linked to Na⁺. This linkage can be accounted for by an electrogenic Na/HCO₃⁻ transporter which carries Na⁺, HCO₃⁻, and net negative charge in the same direction.

We propose that proximal-tubule acid secretion is a byproduct of intracellular pH (pHᵢ) regulation by the proximal tubule cells: basolateral HCO₃⁻ efflux lowers pHᵢ and thereby stimulates both luminal and basolateral Na-H exchange. Net transcellular acid secretion proceeds to the extent that H⁺ extrusion occurs across the luminal rather than the basolateral membrane.

Portions of this work have been reported in preliminary form (Boron and Boulpaep, 1981a, b, 1982).

METHODS

General

We used isolated, perfused proximal tubules of the tiger salamander Ambystoma tigrinum. The details are presented in the preceding paper (Boron and Boulpaep, 1983).
Solutions

The compositions of the Ringer’s solutions are given in Table I. Note that the numbering of solutions 1–8 is consistent with the numbering pattern used in the previous paper (Boron and Boulpaep, 1983). Solution 8 was used only for the dissection of tubules. For solution 11, [Ca] was increased threefold to compensate for Ca, which may have been chelated by the substituting anions. Solution 14 (low-substrate/HCO₃⁻), in which lactate and amino acids were deleted, was used for basolateral solutions in experiments in which SITS was used. Additional solutions,

TABLE I

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*Composition given in millimolar unless otherwise noted. M⁺ is a monovalent cation: either H₂(2-hydroxyethyl) dimethylammonium (BDA⁺), tetramethylammonium (TMA⁺), or N-methyl-D-glucammonium (NMDG⁺). For solutions 10 and 13, that portion of M⁺ accompanying HCO₃⁻ was always 10 mM NMDG⁺. X⁻ is a monovalent anion, either cyclamate or glucuronate. PVP is polyvinyl pyrrolidone (average molecular weight, 40,000).
All solutions were delivered to pipettes or chamber by gravity through CO₂-impermeable Saran tubing (Clarkson Equipment and Controls, Detroit, MI).

**Electrodes and Electronics**

The Na-sensitive and pH-sensitive microelectrodes were of the recessed-tip design of Thomas (1970, 1974). The Cl-sensitive microelectrodes were of the liquid-ion exchanger type and used the Corning resin (477315; Dow Corning Corp., Midland, MI). The details concerning the construction and use of these electrodes are given in the previous paper (Boron and Boulpaep, 1983).

**Curve-fitting Procedure**

Rate constants of exponential pHᵢ recoveries were obtained by using an iterative, least-squares curve-fitting procedure to fit the data to an equation of the form pHᵢ = A - B exp(-kt), where k is the rate constant, and t is the time. Details are given in the previous paper (Boron and Boulpaep, 1983).

All mean values are given ± standard error.

**RESULTS**

In the preceding paper (Boron and Boulpaep, 1983), we examined Na-H exchange in renal proximal tubule cells. To avoid the contribution that the flux of HCO₃⁻ (or an equivalent species) might have made to the pHᵢ transients, we purposely performed the preceding experiments in nominally HCO₃⁻-free solutions. The present study is devoted to an examination of basolateral HC₀₃⁻ transport. Thus, the reference solution for most of these experiments was standard HC₀₃⁻ Ringer (solution 7). As noted in Table II of the preceding paper, when HEPES Ringer (solution 1) is replaced by standard HC₀₃⁻ Ringer, the steady state pHᵢ falls, and the steady state basolateral membrane potential (Vᵢ) becomes more positive. This transition is illustrated in Fig. 1A. The application of HC₀₃⁻ Ringer causes an abrupt decrease of pHᵢ, because of the influx of CO₂, its hydration to H₂CO₃, and the subsequent dissociation to H⁺ plus HC₀₃⁻. This represents an acute intracellular acid load. In the previous paper (Boron and Boulpaep, 1983), however, we showed that a similar degree of intracellular acid loading in pH 7.5 HEPES Ringer would accelerate Na-H exchange and thereby restore pHᵢ to its initial level. Here, instead, with the tubule bathed in pH 7.5 HC₀₃⁻ Ringer, the fall in pHᵢ is sustained. This failure of pHᵢ to recover indicates that some HC₀₃⁻- or CO₂-dependent process continually loads the cell with acid as rapidly as the aforementioned Na-H exchangers can extrude the acid. Our evidence (discussed below) indicates that this process is the basolateral efflux of HC₀₃⁻ and/or a related species.

The application of HC₀₃⁻ Ringer also produces changes in Vᵢ, two patterns of which were observed. In most cases there was a monotonic and sustained basolateral depolarization (Fig. 1A); in others, this depolarization was preceded by a small, rapid hyperpolarization (see Fig. 1A, inset). The hyperpolarization is probably due to the instantaneous establishment of a highly negative diffusion potential for HC₀₃⁻ or an equivalent species. As CO₂ enters the cell and generates intracellular HC₀₃⁻, this diffusion potential relaxes to a value more positive than Vᵢ. Inasmuch as CO₂ diffusion is rapid as compared
with solution mixing in the chamber and the response time of our recording equipment, it is not surprising that the initial hyperpolarizing transient was often missed. The net, steady state basolateral depolarization is thus the combined result of the introduction of an \( \text{HCO}_3^- \) diffusion potential together with possible changes in other \( p\text{H}_i \)-sensitive ionic conductances.

**Figure 1.** Effect of \( \text{CO}_2 \)-containing Ringer and of low extracellular \( p\text{H} \). A. Transition from \( \text{HCO}_3^- \)-free to \( \text{HCO}_3^- \)-containing Ringer. \( V_l \) refers to basolateral membrane potential, and \( V_3 \) to transepithelial potential difference, both referenced to the bath. In the first portion of the experiment, the tubule was exposed (lumen and bath) to a nominally \( \text{HCO}_3^- \)-free Ringer buffered with HEPES to \( p\text{H} 7.5 \) (solution 1). At the indicated time, the luminal and basolateral solutions were replaced with Ringer of the same \( p\text{H} \), but buffered with 10 mM \( \text{HCO}_3^-/1.5\% \text{CO}_2 \) in \( \text{O}_2 \) (solution 7). This is one of 10 similar experiments, each on a separate tubule. B. Basolateral or luminal acidification in the absence of \( \text{HCO}_3^- \). During the indicated intervals, the \( p\text{H} \) of either the basolateral or luminal solutions was reduced from 7.5 to 6.8 (solutions 1 to 3). A total of four such experiments were performed on two tubules. C. Basolateral or luminal acidification in the presence of \( \text{HCO}_3^- \). During the indicated intervals, the \( p\text{H} \) of either the basolateral or luminal solution was reduced from 7.6 to 6.8 by reducing [\( \text{HCO}_3^- \)] from 10 to 2 mM at a constant \( \text{CO}_2 \) of 1.5% (solutions 7 to 9). All experiments, except for the one in the inset, were performed on the same tubule. A total of 41 such experiments was performed on 15 different tubules.

At least three phenomena contribute to the \( p\text{H}_i \) changes that accompany the replacement of HEPES buffer with a \( \text{HCO}_3^- \) buffer: (a) the influx of \( \text{CO}_2 \), (b) first the influx and then the efflux of \( \text{HCO}_3^- \), and (c) the regulatory
response of the Na-H exchangers. Because of the complexity of these events, simultaneously changing pCO₂ and [HCO₃⁻] is not a useful tool for studying basolateral HCO₃⁻ transport. Therefore, in our first series of experiments (see Basolateral HCO₃⁻ Effect below), we opted for a protocol in which we replaced one variable (i.e., pCO₂) with another (i.e., pH): we altered extracellular pH (pH₀) and [HCO₃⁻] at constant pCO₂. As will be seen, this approach greatly simplifies the interpretation of pHᵢ transients.

Hypothesis

In the first series of experiments, we simultaneously reduced basolateral pH (pH₀) and basolateral [HCO₃⁻] ([HCO₃⁻]₀) at constant pCO₂ while monitoring concomitant changes in pHᵢ, intracellular Cl⁻ activity (aᵢCl⁻), intracellular Na⁺ activity (aᵢNa⁺), Vᵢ, and transepithelial voltage (Vₑ). The following hypothesis emerged from these experiments. We propose that a SITS-sensitive carrier in the basolateral membrane transports HCO₃⁻ (or an equivalent species), Na⁺, and negative charge out of the cell when [HCO₃⁻]₀ is reduced. This electrogenic Na/HCO₃ transporter would mediate the opposite movements when [HCO₃⁻]₀ is returned to its initial value. For achieving such a net movement of negative charge, the ratio of HCO₃ to Na⁺ fluxes would have to exceed 1; the simplest stoichiometry is two HCO₃ moving together with one Na⁺. This model suggested a second series of experiments in which the effects of altering [Na⁺]₀ were examined. The predictions of the hypothesis for both series of experiments are given in Table II. Clearly, these predictions are only qualitative. Other transport systems could modify the magnitude or direction of the effects.

The remainder of the Results is divided into two parts. The first part tests predictions a–d, as well as the sensitivity to SITS and dependence on Cl⁻. The second part tests predictions e, g, and h, as well as the SITS sensitivity and Cl⁻ dependence. Prediction f has been verified in another study (Sackin et al., 1981). Moreover, a portion of effect f is inhibited by SITS (Sackin, Boron, and Boulpaep, unpublished data). A prediction not included in Table II concerns the effect of altering Vᵢ on pHᵢ, aᵢNa⁺, and aᵢCl⁻. However, in a leaky epithelium it is impossible to clamp the basolateral membrane independently of the luminal membrane, thus making it difficult to interpret the results. Hence this prediction has not been examined in this paper.

Basolateral HCO₃⁻ Effect

General Description  Figs. 1B and C compare the effects of acidifying the luminal or basolateral solutions in HCO₃⁻-free Ringer with those of similar acidifications in HCO₃⁻-containing Ringer. With the tubule bathed in nominally HCO₃⁻-free Ringer (solution 1), lowering either pH₀ or pHᵢ to 6.8 (solution 3) has only a modest effect on pHᵢ, producing decreases of ~0.15 and ~0.10, respectively (Fig. 1B). When either pH₀ or pHᵢ is restored to 7.5, pHᵢ recovers (Fig. 1B). With the tubule bathed in HCO₃⁻-containing Ringer (Fig. 1C), lowering pH₀ to 6.8 (i.e., lowering [HCO₃⁻]₀ to 2 mM; solution 9) has a much larger effect than in HCO₃⁻-free Ringer, reducing pHᵢ by ~0.40.
When $[\text{HCO}_3^-]_b$ and $\text{pH}_b$ are returned to their initial values, $\text{pH}_i$ recovers along an exponential time course (Fig. 1C). In 25 experiments on 11 tubules, the mean rate constant was $1.73 \pm 0.09$ min$^{-1}$. Reducing luminal $\text{pH}$ and $[\text{HCO}_3^-]$ produces only a small, slow acidification. Four aspects of the experiment of Figs. 1B and C are of particular interest.

(a) Presence of basolateral $\text{HCO}_3^-$ transport. Basolateral acidification leads to a larger fall of $\text{pH}_i$ in a $\text{HCO}_3^-$-containing than in a $\text{HCO}_3^-$-free medium (Figs. 1B and C). In both cases, the fall of $\text{pH}_i$ is probably the result of one or more of the following four events: (i) $\text{H}^+$ permeability, which probably makes a rather small contribution because of the low concentration of $\text{H}^+$; (ii) $\text{HCO}_3^-$ permeability, which in theory could produce a fall in $\text{pH}_i$; (iii) $\text{Na}/\text{HCO}_3^-$ transport, which would also produce a decrease in $\text{pH}_i$; (iv) inhibition of $\text{Na}$-$\text{H}$ exchange by the reduction of $\text{pH}_b$; however, the eventual decline of $\text{pH}_i$ would secondarily stimulate luminal $\text{Na}$-$\text{H}$ exchange; (v) HEPES permeability, particularly that of the neutral weak acid, whose concentration rises at low $\text{pH}$. The balance among the aforementioned five events will determine the new steady state $\text{pH}_i$. The much larger fall of $\text{pH}_i$ in pH 6.8 $\text{HCO}_3^-$ Ringer indicates that either basolateral permeability to $\text{HCO}_3^-$ or the $\text{Na}/\text{HCO}_3^-$ transport rate must be high.

The recovery of $\text{pH}_i$, when $[\text{HCO}_3^-]_b$ and $\text{pH}_b$ are returned to their initial values, is the result of the interaction of the first four of the aforementioned mechanisms. (i) The passive flux of $\text{H}^+$ cannot contribute to this rise of $\text{pH}_i$, since the electrochemical gradients still favor $\text{H}^+$ influx across both luminal and basolateral membranes. (ii) The rise in $\text{pH}_i$ cannot be accounted for by a passive influx of $\text{HCO}_3^-$ per se, since the basolateral electrochemical gradient favors $\text{HCO}_3^-$ efflux. (iii) However, $\text{HCO}_3^-$ (or a related species) may be carried into the cell by the hypothesized $\text{Na}/\text{HCO}_3^-$ transporter. (iv) The $\text{pH}_i$ recovery could in part be the result of luminal and basolateral $\text{Na}$-$\text{H}$ exchange. The contribution of $\text{Na}$-$\text{H}$ exchange will be examined in subsection d below.

(b) Absence of luminal $\text{HCO}_3^-$ transport. Luminal acidification has very little effect on $\text{pH}_i$ when the cells are bathed in $\text{HCO}_3^-$ Ringer, even though a similar maneuver in $\text{HCO}_3^-$-free (HEPES) Ringer produces a small but

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**TABLE II**

**PREDICTIONS OF HYPOTHESIS**

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<th>Basolateral $\text{HCO}_3^-$ effect</th>
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</tbody>
</table>

All the above changes are (i) blocked by SITS and (j) independent of $\text{Cl}^-$.

* $\uparrow$ = increase, $\downarrow$ = decrease, 0 = no change, + = depolarization, - = hyperpolarization.
significant decline in pH_i (Figs. 1B and C). The much smaller and slower fall of pH_i that accompanies the reduction of luminal pH, as opposed to basolateral pH, indicates that the net flux of H^+ and/or HCO_3^- across the luminal membrane is much smaller than across the basolateral membrane. The small, slow fall of pH_i that occurs with luminal acidification probably reflects an inhibition of luminal Na-H exchange in the face of continued basolateral HCO_3^- efflux. The much larger fall of pH_i in 6.8 HEPES Ringer is thus probably due to the permeation by one of the members of the HEPES conjugate pair. We conclude that there is HCO_3^- transport in these cells and that it is limited to the basolateral membrane.

(c) Electrogenic nature of basolateral HCO_3^- transport. As shown in Figs. 1B and C, extracellular acidifications produce characteristic changes in V_i, which are larger for basolateral than for luminal acidifications. Generally, these V_i changes have a triphasic time course. In nominally HCO_3^- free experiments, reducing pH_b produces (i) an abrupt depolarization of ~10 mV, often followed by (ii) a partial recovery, and finally followed by (iii) an additional slow, sustained depolarization of ~2 mV and, exceptionally (as in Fig. 1B), up to 10 mV. Phase i could be caused by changes in pH_b-sensitive conductances such as K^+ (Steels and Boulpaep, 1976), and changes in the diffusion potentials of charged buffer species, such as the HEPES anion or residual HCO_3^- . Phase ii may be due to a secondary fall in the intracellular concentrations of a buffer anion. Phase iii could be due to any determinant of V_i (e.g., an ion activity, an ion conductance, or an electrogenic transport system), which slowly responds to changes in pH_i.

For experiments in HCO_3^- Ringer, basolateral acidification (i.e., reduced [HCO_3^-]_b) generally produces a similar triphasic time course of V_i, though the initial depolarization (phase i) is generally much larger, 25–30 mV. The partial recovery of V_i (phase ii) was present in about half the cases (see Figs. 2 and 5) and amounted to 2–4 mV. The slow, sustained depolarization (phase iii), also observed in about half the cases, amounts to only 2–5 mV. These three phases of V_i in the HCO_3^- Ringer would, at least in part, have the same origin as the changes in the HCO_3^- -free experiments, except for lack of HEPES contribution, a greater contribution by HCO_3^-, and a greater contribution from pH_i-sensitive determinants of V_i. These explanations, however, cannot account quantitatively for the observed initial depolarizations (phase i) of 30 mV on the basis of either alterations in single-ion conductances or single-ion diffusion potentials. The H^+ conductance is unlikely to contribute significantly to V_i. Since no chemical potential changes occur for Na^+, K^+, or Cl^-, these ions could only contribute to the depolarization if their permeabilities were very pH_i sensitive. Although such permeability changes could theoretically account for the initial depolarization in HCO_3^- -free Ringer, they cannot explain the threefold-larger depolarization in HCO_3^- -containing Ringer. This difference is due to the presence of HCO_3^-, although not to the diffusion potential of the bicarbonate ion. 1 Although other ions (e.g., phosphate,

1 Reducing pH_b from 7.5 to 6.8 in HCO_3^- Ringer (Fig. 1C) instantaneously shifts the HCO_3^- equilibrium potential (E_{HCO_3^-}) from ~12 to +12 mV, the proper direction for explaining a basolateral depolarization. To determine whether the magnitude of the E_{HCO_3^-} shift is sufficient
NaCO₃, or organic weak acids and bases) undergo large fractional changes in concentration during alterations in pHb, their concentrations are too low to influence V₁ in the absence of extraordinarily high permeabilities. We conclude that a large portion of the initial depolarization is caused by the electrogenic Na/HCO₃ transporter, which must carry net positive current into the cell when [HCO₃]ᵢ is lowered.

When pHb is returned to 7.5 in HCO₃ Ringer (Fig. 1C) there is a triphasic shift in V₁ that is a mirror image of the initial one: (i) an abrupt hyperpolarization, followed by (ii) a slight recovery of 2–4 mV, followed by (iii) a further hyperpolarization of ~5 mV. The same sort of analysis applied above to the initial depolarization can now be applied to the initial hyperpolarization. We conclude that a large portion of the initial hyperpolarization is caused by the Na/HCO₃ transporter, which must carry net positive current out of the cell when [HCO₃]ᵢ is raised.

Although basolateral depolarizations also occur during luminal acidification, in HCO₃-free or HCO₃-containing Ringer, these are rather small (e.g., ~5 mV). They may be the result of changes in external ion composition and/or pH₁-sensitive conductances at either the luminal membrane or shunt, or could be due to changes at the basolateral membrane, secondary to changes of intracellular composition. As to the changes in the transepithelial potential difference (V₃), these are of the same sign as those in V₁, though much smaller, and may reflect changes at the basolateral membrane, as expected for a leaky epithelium.

(d) Contribution of Na-H exchange to pHᵢ changes. Although the above pHᵢ and V₁ changes, taken together, can only be accounted for by the hypothesized Na/HCO₃ transporter, the pHᵢ changes should also be influenced by luminal and basolateral Na-H exchange. The experiment of Fig. 2 was performed to examine the contribution of Na-H exchange to the pHᵢ recovery that follows restoration of [HCO₃]ᵢ and pHb to normal. The tubule is exposed to HCO₃ Ringer throughout. During six separate intervals, pHb and [HCO₃]ᵢ are simultaneously lowered (solution 9) and then restored. During the first two restorations of normal pHb and [HCO₃]ᵢ, the recoveries of pHᵢ are quite rapid (rate constants, 1.94 and 2.70 min⁻¹). During the third interval of basolateral acidification, after pHᵢ had fallen to a new steady level, 2 mM amiloride is applied to the bath and lumen. This level of amiloride blocks ~75% of Na-H exchange in these cells (Boron and Boulpaep, 1983). The application of amiloride causes a slight rise in pHᵢ, which may be due to amiloride's acting as a weak base. When pHb is subsequently returned to 7.5, pHᵢ recovers at a lower rate (1.30 min⁻¹) than in the two previous controls and in the succeeding
one. During the fifth basolateral acidification, the amiloride test is repeated, and the pHr recovery is once again slower than in the bracketing controls (0.94 min\(^{-1}\) vs. 2.00 and 1.77 min\(^{-1}\)). We conclude that about half the pHr recovery rate triggered by returning pHb to 7.5 is due to an amiloride-sensitive Na-H exchange. The balance presumably represents the basolateral uptake of HCO\(_3\) (or an equivalent species) mediated by the hypothetical Na/HCO\(_3\) transporter.

**INHIBITION BY SITS** The results of the previous section suggest that the movement of HCO\(_3\) (or of a related species) may occur via a Na/HCO\(_3\) transporter. Inasmuch as SITS is known to block a variety of anion transporters, we tested the effect of adding SITS (0.5 mM) to the basolateral solution. Fig. 3 illustrates an experiment in which [HCO\(_3\)] and pHb were lowered three times. In the first case (i.e., the control condition) pHb basolateral membrane potential, and the transepithelial potential difference change in the usual way. The subsequent application of SITS, though not shown in the figure, slightly increases pHr and hyperpolarizes the basolateral membrane. After a 10-min pretreatment with SITS, the pHr, V1, and V3 changes elicited by reduction of [HCO\(_3\)] and pHb are greatly attenuated. In the presence of SITS the changes in pHr and the initial changes in V1 closely resemble those described above in the absence of HCO\(_3\) (see Fig. 1B). These results are
consistent with inhibition by SITS of the hypothesized Na/HCO₃ transporter. The level of sustained basolateral depolarization (phase iii) is also reduced by SITS. This may have two explanations. In the first place, the plateau value of \( V_I \) may be pHᵢ dependent and thus mirror the plateau value of pHᵢ. Second, the magnitude of the sustained depolarization may normally be determined by the continued transport of charge by the Na/HCO₃ transporter, and thus may be reduced when the transporter is inhibited by SITS.

**Figure 3.** Effect of SITS on basolateral-acidification-induced changes. \( V_I \) and \( V_3 \) represent basolateral membrane potential and transepithelial potential, respectively. During the indicated three intervals, pHᵢ was lowered from 7.5 to 6.8 by reducing [HCO₃]ᵢ from 10 to 2 mM at constant pCO₂ (solutions 7 to 9). The rate constants for the pHᵢ recovery from the basolateral acidifications (units: min⁻¹) are given in parentheses. Beginning 5 min before, and continuing throughout the final two pH 6.8 pulses, the tubule was exposed to 0.5 mM SITS in the basolateral solution. This is one of six such experiments on three separate tubules.

**Lack of Cl⁻ Involvement** Although the hypothesized, electrogenic Na/HCO₃ cotransporter seems necessary to account for the data of the previous two sections, we have not yet ruled out that a portion of the HCO₃⁻ movement is mediated by an electroneutral Cl⁻-HCO₃ exchange or by a Na/HCO₃-Cl⁻/H exchange. The possibility of a linkage of Cl⁻ to HCO₃⁻ is raised by the observation that the steady state \( a_{Cl}^i \) is higher in the nominal absence than in the presence of HCO₃⁻ (Boron and Boulpaep, 1983; Guggino et al., 1982).
(a) pH\textsubscript{i} changes. In the experiment of Fig. 4, the effect of basolateral acidification is tested in the absence of Cl\textsuperscript{−} (HCO\textsubscript{3}{−} present throughout). The tubule is first subjected to a basolateral acidification in the presence of Cl\textsuperscript{−}, which produces the usual changes in pH\textsubscript{i} and V\textsubscript{3}. Removal of Cl\textsuperscript{−} from the bath and lumen (solution 11; Cl\textsuperscript{−} replaced by cyclamate) causes pH\textsubscript{i} to slowly decrease by ~0.2. If HCO\textsubscript{3}{−}-Cl or Na/HCO\textsubscript{3}{−}-Cl/H exchange were a major

![Figure 4](image_url)

**Figure 4.** Effect of Cl\textsuperscript{−} removal on basolateral-acidification-induced changes. V\textsubscript{1} and V\textsubscript{3} represent basolateral membrane potential and transepithelial potential, respectively. During the indicated intervals, pH\textsubscript{b} was reduced from 7.5 to 6.8 by lowering [HCO\textsubscript{3}{−}]\textsubscript{b} from 10 to 2 mM at constant CO\textsubscript{2} tension. Before and during the second and third acidifications, Cl\textsuperscript{−} was replaced, bath and lumen, with cyclamate (solution 11). The rate constants for the pH\textsubscript{i} recoveries (units: min\textsuperscript{-1}) are given in parentheses. Although the phase ii hyperpolarization is not evident in the absence of Cl\textsuperscript{−} in this experiment, it has been observed in other experiments (not shown). A total of eight similar experiments was performed on four separate tubules.

pathway for basolateral HCO\textsubscript{3}{−} flux, Cl\textsuperscript{−} removal should instead have raised pH\textsubscript{i}. After 10 min in Cl-free Ringer (a period sufficient to remove most intracellular Cl\textsuperscript{−}; see Fig. 5), reducing pH\textsubscript{b} and [HCO\textsubscript{3}{−}]\textsubscript{b} has about the same effect on pH\textsubscript{i} as under control conditions. Returning pH\textsubscript{b} and [HCO\textsubscript{3}{−}]\textsubscript{b} to normal causes a pH\textsubscript{i} recovery which is about as rapid (k = 2.59 and 2.23 min\textsuperscript{-1}) as in the presence of Cl\textsuperscript{−} (k = 2.55 min\textsuperscript{-1}). In six paired experiments on three tubules, the mean rate constant in Cl-containing solutions was 2.07 ± 0.26 min\textsuperscript{-1}, not significantly different from 1.91 ± 0.28 min\textsuperscript{-1}, the value in
Cl⁻-free solutions (paired t test, \(P = 0.33\)). The experiment of Fig. 4 has been repeated while replacing Cl⁻ with either glucuronate (solution 11) or SO₄²⁻ (solution 12), with similar results. These data indicate that most of the movement of HCO₃⁻ (or an equivalent species) and the movement of charge across the basolateral membrane are not dependent on Cl⁻. We cannot, however, rule out a small component of HCO₃⁻-Cl⁻ exchange.

(b) Voltage changes. The removal of Cl⁻ (replaced with cyclamate) causes a basolateral hyperpolarization, as observed by others (Anagnostopoulos and Planelles, 1979; Guggino et al., 1982) when Cl⁻ is removed in solutions of near-constant Ca²⁺ activity. When Cl⁻ is replaced with glucuronate, as in the experiment of Fig. 5, the hyperpolarization is only transient. The magnitude of the subsequent basolateral-acidification-induced depolarization (phase i) is unaffected by Cl⁻ removal. These two results are consistent with a low basolateral Cl⁻ conductance. In contrast, a high paracellular Cl⁻ conductance

The sign of the \(V_l\) change upon Cl⁻ removal is opposite to that expected of a membrane with a high Cl⁻ conductance. In addition, the magnitudes of the basolateral acidification-induced depolarizations are the same with and without Cl⁻. Because the decreases in \(pH_b\) and therefore the amount of current carried by the hypothesized Na/HCO₃ transporter, are the same in the two cases, the basolateral-membrane resistance must also have been the same with and without Cl⁻. Hence, Cl⁻ conductance is low.
is indicated by the transepithelial hyperpolarization caused by removal of Cl⁻, as previously noted (Sackin and Boulpaep, 1981b). The larger basolateral-acidification-induced V₃ changes seen in Cl-free as opposed to Cl-containing solutions are also consistent with a larger IR drop due to an increased paracellular resistance.

(c) aₐCl changes. As a final test for the involvement of Cl⁻, we monitored changes in aₐCl during four periods in which pH₈ and [HCO₃]₈ were lowered in HCO₃ Ringer (Fig. 5). Basolateral acidification produces an abrupt 1–2 mM increase in aₐCl, followed a slower increase of another ~2 mM over 5 min. When pH₈ and [HCO₃]₈ are returned to normal, aₐCl falls over the course of several minutes. To verify that the Cl⁻ microelectrode was functioning properly, we exposed the tubule, from both the bath and lumen, to a Cl-free solution (solution 11). This causes the apparent aₐCl to fall to ~5 mM. In seven such experiments, the minimal apparent aₐCl in Cl-free Ringer was 5.5 ± 0.7 mM; the residual Cl⁻ signal may represent cross-sensitivity of the Cl⁻ electrode to other anions. When comparing the aₐCl changes of Fig. 5 with the pH₈ changes of Figs. 1–4, it is important to note that when [HCO₃]₈ and pH₈ are reduced, the slow phase of the aₐCl increase has a much longer time course than the fall in pH₈. Thus, this slow phase of the aₐCl rise is probably not caused by the same mechanism responsible for the rapid fall of pH₈. It may be due to another HCO₃-linked transporter or to a change in pH₈-sensitive Cl⁻ transport. As for the rapid phase of the aₐCl increase, its magnitude is far too small to account for the observed fall in pH₈ on the basis of either a one-for-one Cl⁻/HCO₃ exchange or a Na/HCO₃-Cl/H exchange (which is equivalent to one Cl⁻ for two HCO₃).

Involvement of Na⁺. In the experiment of Fig. 6, we monitored aₐNa while simultaneously reducing pH₈ from 7.5 to 6.8 and [HCO₃]₈ from 10 to 2 mM. During both basolateral acidifications of Fig. 6A, aₐNa declines with about the same course as the fall in pH₈ that occurs under these conditions (see Figs. 1–4). As the basolateral acidification is maintained, aₐNa gradually recovers. The initial fall of aₐNa could result from either decreased Na⁺ entry or increased Na⁺ exit. Decreased, presumably luminal, entry of Na⁺ could be caused by the depolarization of the luminal membrane, which can be inferred from the changes in V₁ and V₃. However, we would expect this to lead to a monotonic fall of aₐNa as the declining Na⁺ pump rate once again comes into balance with the reduced Na⁺ influx. The biphasic nature of the fall in aₐNa is better explained by a sudden, transient exit of Na⁺, which is somehow linked to the reduction of either [HCO₃]₈, or pH₈. It is unlikely that a pH₈-sensitive

₃ Taking the intrinsic intracellular buffering power (βₙ) as 36 mM (Boron and Boulpaep, 1983) and the CO₂ buffering power (βCO₂) as 2.3 [HCO₃]₈ (see Roos and Boron, 1981) or 9 mM, the total intracellular buffering power (βT = βₙ + βCO₂) comes to 45 mM. Thus, a pH decrease of 0.4 corresponds to the net exit of 45 x 0.4 = 18 mmol HCO₃ (or an equivalent species) per liter intracellular fluid, substantially greater than the observed increment in aₐCl. Since this comparison neglects the possible contribution of other Cl⁻ pathways for regulating aₐCl, the slow rise in aₐCl of Fig. 5 may underestimate the Cl⁻ influx mediated by Cl-HCO₃ exchange or Na/HCO₃-Cl/H exchange.
Na-K pump would exhibit a biphasic response in view of the monotonic fall in pHi. We suspect that the sudden exit of Na⁺ is caused by the hypothesized Na/HCO₃ transporter. A portion of the recovery of aNa may be due to stimulation of luminal Na-H exchange, secondary to the fall in pHi. Restoring pHb to 7.5 produces an overshoot of aNa followed by a gradual return of aNa to its initial value. The overshoot of aNa is probably caused by a rapid and transient entry of Na⁺, associated with the simultaneous, basolateral entry of HCO₃⁻. Subsequently, aNa returns to its initial value as the activity of the Na-K pump overtakes the now-declining Na⁺ entry. Fig. 6B illustrates the effect of SITS on these aNa transients. During the first period of basolateral acidification, in the absence of SITS, the usual changes in aNa, V₁, and V₃ are observed. With the addition of SITS to the basolateral solution, however, basolateral acidification produces no change in aNa. We do not show here an experiment in which we found that removal of Cl⁻ has no effect on these changes in aNa. The fall of aNa associated with basolateral acidification, and the blockade of this fall by SITS, suggests but does not prove that basolateral Na⁺ and HCO₃⁻ transport are coupled.

**Basolateral Na⁺ Effect**

**General Description** To determine whether the hypothesized electrogenic HCO₃⁻ transporter is, indeed, linked to Na⁺, we performed experiments in which we removed Na⁺ from the bath or the lumen, while continuously exposing the tubule to pH 7.5 HCO₃⁻ Ringer. In the experiment of Fig. 7A, Na⁺ was absent (replaced with TMA⁺) from the lumen throughout (solution 10). Subsequent removal of basolateral Na⁺ causes pHi to fall by >0.3 and produces a large, transient depolarization of the basolateral membrane. Returning Na⁺ to the bath causes a rapid recovery of pHi and a large, transient basolateral hyperpolarization, which in some cases reached ~140 mV. The experiment of Fig. 7B, on a second tubule, compares the effects of removing luminal and basolateral Na⁺. Removal of luminal Na⁺ causes a small, slow fall in pHi, followed by a recovery. In addition, there is a basolateral hyperpolarization (amounting to ~20 mV in this case), followed by a partial recovery, as well as a gradual transepithelial depolarization.

(a) Presence of basolateral Na/HCO₃ transport. One would expect that removing basolateral Na⁺ should lower pHi in virtue of blocking basolateral Na-H exchange. Similarly, returning basolateral Na⁺ should restore basolateral Na-H exchange and thereby return pHi to normal. However, changes in the basolateral Na-H exchange rate are unlikely to explain fully the pHi changes elicited by altering [Na⁺]b. First, the fall in pHi caused by removing basolateral Na⁺ is much more striking than that produced by removing luminal Na⁺. The evidence for Na-H exchange in HCO₃⁻-free Ringer (Boron and Boulpaep, 1983) indicates that Na⁺ removal should be only slightly more effective from the basolateral than from the luminal side. Second, the pHi recoveries of Figs. 7A and B have rate constants of ~2.0 and ~2.6 min⁻¹, respectively, substantially higher than expected for Na-H exchange alone, ~1.0 min⁻¹ (Boron and Boulpaep, 1983). Therefore, these pHi changes must be mediated in part by
another mechanism, presumably the electrogenic Na/HCO$_3$ transporter, in accord with prediction $e$ of Table II.

(b) Absence of luminal Na/HCO$_3$ transport. The difference between the pH$_i$ changes produced by basolateral vs. luminal Na$^+$ removal indicate a lack of a substantial Na/HCO$_3$ flux across the luminal membrane. In Fig. 7B, the modest fall of pH$_i$ caused by luminal Na$^+$ removal is probably caused by blockage of luminal Na$_-$H exchange. In addition, the accompanying basolateral hyperpolarization, which reflects luminal Na$^+$ conductance in this leaky epithelium (Sackin and Boulpaep, 1981$b$), may enhance basolateral Na/HCO$_3$ efflux. The subsequent pH$_i$ recovery (i.e., preceding basolateral Na$^+$ removal) may be due to increased basolateral Na-H exchange secondary to the fall of $a_Na^+$, as well as to a slowing or reversal of basolateral Na/HCO$_3$ transport secondary to decreases of pH$_i$ and a$Na^+$.

(c) Electrogenic nature of Na/HCO$_3$ transporter. The results of Figs. 7A and B confirm prediction $g$ of Table II, that removal of basolateral Na$^+$ should produce an abrupt basolateral depolarization. Furthermore, as the exit of HCO$_3^-$, Na$^+$, and net negative charge gradually slows, the depolarization should decay. The opposite fluxes and charge movement should occur when basolateral Na$^+$ is restored, thereby producing the opposite changes in V$_l$.

An alternative explanation for the changes in V$_l$ produced by these alterations of [Na$^+$]$_b$ is that the V$_l$ changes reflect alterations of the electrogenic Na-K pump rate. To test this hypothesis, we performed the experiment of Fig. 8, in which Na$^+$ was absent from the lumen throughout. In the first two sequences, basolateral Na$^+$ is removed as usual, and the standard changes in V$_l$ are observed. During the third removal of Na$^+$, ouabain (10$^{-4}$ M) is added to the basolateral solution. When Na$^+$ is finally added back to the basolateral solution, after a 20-min pretreatment with ouabain, the spiking basolateral hyperpolarization still occurs, even though the Na-K pump is presumably blocked. It is interesting to note that with the Na-K pump blocked, and therefore presumably with a higher-than-normal $a_Na^+$, the subsequent basolateral removal of Na$^+$ causes a larger initial basolateral depolarization (Fig. 8, the fourth and fifth Na$^+$ removals). This is expected if the Na/HCO$_3$ efflux is sensitive to the Na$^+$ electrochemical gradient.

Inhibition by SITS  Fig. 9 illustrates the effect of SITS on the pH$_i$ and V$_l$ changes induced by the basolateral removal and reapplication of Na$^+$. Note that Na$^+$ is present in the lumen throughout. Under control conditions, changes in basolateral Na$^+$ have the same general effects as pointed out for

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**Figure 6.** (opposite) A. Intracellular Na$^+$ activity during basolateral acidification. V$_l$ and V$_e$ represent basolateral membrane potential and transepithelial potential, respectively. Twice pH$_b$ was lowered from 7.5 to 6.8 by lowering [HCO$_3^-$] from 10 to 2 mM at constant CO$_2$ tension (solutions 7 to 9). A total of 18 similar experiments was performed on six separate tubules. B. Effect of SITS on Na$^+$ activity changes induced by basolateral acidification. This second tubule was treated with 0.5 mM basolateral SITS for ~5 min before the second and third pH 6.8 pulses. The gap represents a period of 13 min. A total of three experiments was performed on two separate tubules.
Fig. 7, where Na\(^+\) was absent from the lumen. With SITS present in the bath, however, removal of basolateral Na\(^+\) does not elicit the rapid fall in pH\(_i\) normally observed or the usual changes in \(V_1\) and \(V_3\). This suggests that SITS blocks the electrogenic Na/HCO\(_3\) transporter, in agreement with prediction i of Table II.

**Figure 7.** A. Effect of basolateral Na\(^+\) removal. \(V_1\) and \(V_3\) represent basolateral membrane potential and transepithelial potential, respectively. Luminal Na\(^+\) was replaced with TMA\(^+\) (solution 10) 15 min before Na\(^+\) was removed from the bath. Although not pronounced in this example, in many experiments the removal and readdition of basolateral Na\(^+\) produced triphasic shifts in \(V_1\), much as did basolateral reduction and restoration of [HCO\(_3\)]\(_\text{b}\). B. Comparison of luminal and basolateral Na\(^+\) removal (second tubule). pH was 7.5 throughout.

A total of 63 similar experiments was performed on 36 separate tubules.

In the absence of SITS (Fig. 9, first pulse), Na\(^+\) removal causes a transient depolarization followed by a slower phase of depolarization (phase iii), in contrast to Fig. 7. The slower depolarization in Fig. 9 is due to the presence of Na\(^+\) in the lumen and, therefore, to a higher \(a_i\) Na\(^+\), which sustains a higher rate of electrogenic Na/HCO\(_3\) exit throughout the period of basolateral Na\(^+\)
removal. This is manifested by a greater phase iii basolateral depolarization as well as by a transepithelial depolarization. In the presence of SITS, Na⁺ removal causes a transient basolateral hyperpolarization, rather than the usual depolarization. Also, the transient transepithelial depolarization is replaced by a sustained hyperpolarization. These changes may be due to Na⁺ diffusion potentials across both the shunt and the basolateral membrane; these effects are of opposite sign to those induced by the electrogenic Na/HCO₃ transport.

**Figure 8.** Effect of ouabain on basolateral membrane potential changes induced by changes in basolateral [Na⁺]. V₁ and V₃ represent membrane potential and transepithelial potential, respectively. Luminal Na⁺ was absent throughout (replaced with NMDG⁺). The first two intervals of basolateral Na⁺ removal (solutions 7 to 10) were performed in the usual way. After the third basolateral Na⁺ removal, however, 10⁻⁴ M ouabain was added to the basolateral solution. Inasmuch as aNa was already probably very low before the application of ouabain, the ouabain probably did not lead to any build-up of intracellular Na⁺ before the third reintroduction of Na⁺. Na⁺ was removed and readmitted twice more in the continued presence of ouabain. This was the only such experiment performed.

Whereas the experiment of Fig. 9 demonstrates inhibition by SITS of Na/HCO₃ exit, that of Fig. 10 is designed to determine whether SITS also inhibits the Na/HCO₃ entry. Removing Na⁺ first from the lumen and then from the bath causes the usual changes in pHᵢ (see Fig. 7), followed by a rapid pHᵢ recovery (rate constant, 2.13 min⁻¹) upon restoration of basolateral Na⁺. After a second removal of basolateral Na⁺, SITS is added to the bath. Reapplication of Na⁺ now elicits a pHᵢ recovery that is slower than in the absence of SITS.
(rate constant, 1.08 min⁻¹). Table III summarizes a total of seven such experiments, and shows that the mean rate constant for pHᵢ recovery in the absence of SITS ($k_{\text{HCO}_3}$) is about twice as great as in the presence of SITS ($k_{\text{SITs}}$). This difference is statistically significant. The value of $k_{\text{SITs}}$ is close to 0.96 ± 0.02, the rate constant for basolateral Na-H exchange, which is SITS insensitive (Boron and Boulpaep, 1983). These results suggest that the pHᵢ recovery in the presence of SITS is mediated by basolateral Na-H exchange alone, whereas the pHᵢ recovery in the absence of SITS is mediated by two parallel basolateral events, Na-H exchange and the electrogenic Na/HCO₃ influx.

When the basolateral Na⁺ is finally removed, as shown in Fig. 10, in the presence of SITS, pHᵢ slowly falls. This is in contrast to the stability of pHᵢ in the experiment of Fig. 9. The discrepancy may be due to the presence of

![Figure 9](image-url)
luminal Na\(^+\), and thus of luminal Na-H exchange, in Fig. 9. The presence or absence of Na\(^+\) in the lumen may also account for the discrepancies in voltage changes between Figs. 9 and 10.

![Diagram](https://example.com/diagram.png)

**Figure 10.** Effect of SITS on pHi recovery accompanying restoration of 100 mM basolateral Na\(^+\). \(V_1\) and \(V_3\) represent basolateral membrane potential and transepithelial potential, respectively. Na\(^+\) was first removed (solutions 7 to 10) from the luminal (replaced by TMA\(^+\)) and then the basolateral solution. Restoring \([\text{Na}^+]_b\) to 100 mM caused a rapid pHi recovery (rate constant, 2.13 min\(^{-1}\)). After the second removal of basolateral Na\(^+\), SITS was applied. The pHi recovery upon restoring \([\text{Na}^+]_b\) to 100 mM this time was much slower (rate constant, 1.08 min\(^{-1}\)). pHi and pHl were 7.5 throughout. A total of 15 experiments similar to the ones in this figure and Fig. 9 was performed on 14 separate tubules.

4 The reapplication of basolateral Na\(^+\) in the presence of SITS causes a basolateral hyperpolarization in Fig. 10, as opposed to a smaller depolarization in Fig. 9. There are two major differences between the experiments of Fig. 9 and 10 with regard to the readmittance of basolateral Na\(^+\). (i) In Fig. 9 there is 100 mM Na\(^+\) present in the lumen, whereas Na\(^+\) is absent in Fig. 10. (ii) In Fig. 10 there is a pHi recovery, whereas in Fig. 9 there is none. The hyperpolarization of Fig. 10 is probably related to the simultaneous recovery of pHi, and was also observed in the terminal portion of Fig. 5 in the preceding paper (Boron and Boulpaep,
INVolvEMENT OF HCO₃⁻  The electrogenic Na/HCO₃ hypothesis predicts that the basolateral [Na⁺] effect should depend on HCO₃⁻. In the first half of Fig. 11, simultaneous removal of luminal and basolateral Na⁺ in HCO₃⁻-free Ringer causes pHᵢ to fall slowly by nearly 0.40 over a 10-min period. This decrease is probably due to abolition of Na-H exchange and/or reversal of the Na-H exchangers. Reintroduction of basolateral Na⁺ causes pHᵢ to recover with a rate constant of 0.37 min⁻¹, probably because of basolateral Na-H exchange alone. The failure of pHᵢ to return to its initial value probably reflects a rather high rate of metabolic acid production in this tubule. The basolateral addition of Na⁺ in HCO₃⁻-free Ringer also causes a slow, sustained hyperpolarization, in contrast to the hyperpolarizing V₁ spike normally seen in HCO₃⁻ Ringer (Fig. 7), but similar to the sustained hyperpolarization seen with SITS in HCO₃⁻ Ringer (Fig. 10). The subsequent readdition of luminal Na⁺ causes a further recovery of pHᵢ. The transient basolateral depolarization produced by reapplication of Na⁺ to the lumen probably reflects a luminal Na⁺ conductance.

In the second half of the experiment of Fig. 11, the HCO₃⁻-free Ringer is replaced with standard HCO₃⁻ Ringer, leading to the usual sustained fall in pHᵢ and the depolarization of V₁. Simultaneous removal of luminal and basolateral Na⁺ now leads to a fall in pHᵢ, which, when compared with the sequence in HCO₃⁻-free Ringer, is both more rapid (initial rate, 0.166 vs. 0.072 pH units/min) and of greater magnitude (0.50 vs. 0.40). In addition, there is a transient basolateral depolarization rather than a hyperpolarization. These transepithelial voltage changes associated with alterations of [Na⁺]b in Fig. 10 are probably due to changes in the Na⁺ diffusion potential at the shunt.

<table>
<thead>
<tr>
<th>Table III</th>
<th>EFFECT OF SITS ON pHᵢ RECOVERY AFTER RETURNING BASOLATERAL Na⁺*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tubule</td>
<td>k_HCO₃⁻ min⁻¹</td>
</tr>
<tr>
<td>-----------</td>
<td>---------------</td>
</tr>
<tr>
<td>080180A</td>
<td>1.28</td>
</tr>
<tr>
<td>080180B</td>
<td>2.39</td>
</tr>
<tr>
<td>080180C</td>
<td>2.13</td>
</tr>
<tr>
<td>080580A</td>
<td>1.33</td>
</tr>
<tr>
<td>080580B</td>
<td>1.40</td>
</tr>
<tr>
<td>080680C</td>
<td>1.34</td>
</tr>
<tr>
<td>080780A</td>
<td>1.46</td>
</tr>
<tr>
<td>1.62±0.17</td>
<td>0.82±0.10</td>
</tr>
</tbody>
</table>

* k_HCO₃⁻ is the control rate constant in HCO₃⁻ Ringer; k_SITS is the rate constant in HCO₃⁻ Ringer containing 0.5 mM SITS in the bath. One rate constant for each was measured per tubule. The mean k_HCO₃⁻ is significantly different from the mean k_SITS (paired t test; P < 0.0004). The mean k_HCO₃⁻/k_SITS was obtained assuming a log-normal distribution. Luminal solutions were all Na-free so that the pHᵢ recovery rates were minimally contaminated by luminal events.
differences are probably due to basolateral Na/HCO₃ efflux. When Na⁺ is now readmitted to the basolateral solution, pHi recovers more rapidly than it had in the absence of HCO₃⁻ (rate constant, 1.05 vs. 0.37 min⁻¹). There is also a transient basolateral hyperpolarization instead of a depolarization.

**Figure 11.** Dependence on HCO₃⁻ of pHᵢ recovery accompanying restoration of [Na⁺]ₐ to 100 mM. V₁ and V₃ represent basolateral membrane potential and transepithelial potential, respectively. In the first part of the experiment, the Ringer was nominally HCO₃⁻-free HEPES (solution 1). Na⁺ removal (solution 4) from both bath and lumen (replaced with TMA⁺) produces a slow fall in pHi and a transient basolateral hyperpolarization. The latter is probably due to a luminal Na⁺ conductance. A hyperpolarization was also observed when luminal Na⁺ was removed in the first part of Fig. 10. Returning Na⁺ to the bath caused pHi to recover slowly (rate constant, 0.37 min⁻¹). Na⁺ was later returned to the lumen. The HEPES Ringer was then replaced with 1.5% CO₂/10 mM HCO₃⁻ Ringer at the same pH (7.50) in both the bath and lumen (solution 7), causing a fall in pHi. After simultaneous removal of luminal and basolateral Na⁺ (solution 10) and subsequent restoration of [Na⁺]ₐ to 100 mM, pHi recovered rapidly (rate constant, 1.05 min⁻¹). A total of 17 similar experiments was performed on 10 separate tubules.

Table IV summarizes the mean rate constants for pHᵢ recovery in HCO₃⁻-free (k_HEPES) vs. HCO₃⁻-containing (k_HCO₃⁻) Ringer. The paired difference between k_HEPES and k_HCO₃⁻ is statistically significant. When adjusted for the
difference in intracellular buffering power, the mean \( k_{\text{HCO}_3} \) is about twice as large as the mean \( k_{\text{HEPES}} \). This is close to the \( k_{\text{HCO}_3}/k_{\text{HEPES}} \) ratio of Table III, 2.01, and provides further support for the hypothesis that the \( p\text{H}_i \) recovery produced by the reintroduction of basolateral \( \text{Na}^+ \) in \( \text{HCO}_3^- \) Ringer is mediated by two parallel processes, basolateral \( \text{Na}-\text{H} \) exchange and basolateral \( \text{Na}/\text{HCO}_3 \) uptake.

**Lack of Cl\(^-\) Involvement**  
The hypothesis of the electronegenic \( \text{Na}/\text{HCO}_3 \) transporter predicts that the changes of \( p\text{H}_i \) induced by alterations of \( [\text{Na}^+]_b \) should not be affected by removal of \( \text{Cl}^- \) (Table II, prediction j), and should not be accompanied by changes of \( q_{\text{Cl}} \) (prediction h). These predictions were tested in two series of experiments. In the first (Fig. 12), we tested whether \( \text{Cl}^- \) is necessary for the \( p\text{H}_i \) changes that normally occur as \( [\text{Na}^+]_b \) is altered. The first and third basolateral removals of \( \text{Na}^+ \) produce the same effects as pointed out for Fig. 7A. Readdition of basolateral \( \text{Na}^+ \) causes \( p\text{H}_i \) to recover with rate constants of 0.70 and 0.47 min\(^{-1}\), respectively. When \( \text{Cl}^- \) was removed from both the bath and lumen, the rate constant of the \( p\text{H}_i \) recovery upon readdition of \( \text{Na}^+ \) was 0.51 min\(^{-1}\), only slightly less than the preceding control, and actually somewhat greater than the succeeding control. Table V summarizes the results of similar experiments on a total of five tubules. The mean rate constants for \( p\text{H}_i \) recovery in the presence (\( k_{\text{HCO}_3} \)) and in the absence

\[ ^* \text{rate constant of the } p\text{H}_i \text{ recovery is inversely proportional to the total intracellular buffering power } (\beta_T). \text{ In } \text{HCO}_3^-\text{free (HEPES) Ringer, } \beta_T \text{ is simply the buffering power of intrinsic intracellular buffers, } \sim 36 \text{ mM (Boron and Boulpaep, 1983). However, when the cells are bathed in } 1.5\% \text{ CO}_2 \text{ Ringer, the presence of } \sim 4 \text{ mM } \text{HCO}_3^- \text{ in the intracellular fluid raises } \beta_T \text{ to } \sim 45 \text{ mM. Therefore, the ratio of } k_{\text{HEPES}} \text{ to } k_{\text{HCO}_3} \text{ must be corrected by the factor } 45/36 = 1.25. The mean of the ratio } k_{\text{HCO}_3}/k_{\text{HEPES}} \text{ (corrected for the } \beta_T \text{ discrepancy) was 2.20, assuming a log-normal distribution.}\]
(k0,Cl) of Cl− were not significantly different, which suggests that Cl− does not participate in the operation of the electrogenic Na/HCO3 transporter.

In a second series of experiments we monitored aCl while altering [Na+]b. In the experiment of Fig. 13, Na+, initially present in the lumen and bath, is removed from the basolateral solution. This causes aCl to rise rather rapidly over the first ~40 s, and then more slowly over the duration of the basolateral

![Diagram](image)

**Figure 12.** Effect of Cl− removal on pHi and voltage changes induced by alterations of [Na+]b. V1 and V3 represent basolateral membrane potential and transepithelial potential, respectively. Basolateral Na+ is removed (replaced by NMDG+) during three different intervals. 12 min before the second 0-Na interval, Cl− was removed (replaced by glucuronate) from both the bath and lumen. The first gap in the record represents 8.3 min, the second represents 25 min. Throughout the experiment, pH was 7.5 and solutions were buffered with 10 mM HCO3/1.5% CO2. A total of 13 similar experiments was performed on 5 separate tubules.

Na+ removal. The initial rate of aCl rise (i.e., over the first 40 s) is 2.1 mM/min. This contrasts with an initial equivalent HCO3 flux of 12.6 mM/min calculated from Fig. 9 under identical conditions. During the first 140 s of basolateral Na+ removal, a period sufficient for the accompanying pHi fall to be complete (Fig. 9), aCl rises by a total of 3.9 mM. In this same time interval in Fig. 9, the equivalent loss of HCO3− was 10.9 mM. This latter figure is a
minimal estimate, since the pH decline is blunted by luminal Na-H exchange. After the first 140 s, however, \( a_{\text{Cl}}^{\text{in}} \) continues to rise, achieving a total increase of 8.2 mM after ~7 min. Thus, because of the large discrepancy between the initial Cl\(^-\) flux and the initial equivalent HCO\(_3^-\) flux, as well as the substantial

### Table V

Cl\(^-\) Dependence of pH\(_i\) Recovery After Returning Basolateral Na\(^+\)

<table>
<thead>
<tr>
<th>Tubule</th>
<th>( k_{\text{HCO}_3} )</th>
<th>( k_{\text{Cl}} )</th>
<th>( k_{\text{Cl}}/k_{\text{HCO}_3} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>071481</td>
<td>0.51±0.07 (2)</td>
<td>0.46±0.08 (4)</td>
<td>0.89</td>
</tr>
<tr>
<td>071581</td>
<td>0.53±0.09 (3)</td>
<td>0.48±0.03 (2)</td>
<td>0.90</td>
</tr>
<tr>
<td>071681</td>
<td>0.74±0.02 (2)</td>
<td>0.74±0.05 (3)</td>
<td>1.00</td>
</tr>
<tr>
<td>071781A</td>
<td>1.56±0.14 (2)</td>
<td>1.54±0.04 (2)</td>
<td>0.98</td>
</tr>
<tr>
<td>071781B</td>
<td>1.13±0.17 (1)</td>
<td>0.75±0.05 (2)</td>
<td>0.67</td>
</tr>
<tr>
<td></td>
<td>0.91±0.17</td>
<td>0.85±0.17</td>
<td>0.91</td>
</tr>
</tbody>
</table>

* \( k_{\text{HCO}_3} \) is the rate constant in Cl-containing HCO\(_3^-\) Ringer; \( k_{\text{Cl}} \) is the rate constant in Cl-free HCO\(_3^-\) Ringer. The mean \( k_{\text{HCO}_3} \) is not significantly different from the mean \( k_{\text{Cl}} \) (paired t test; \( P = 0.36 \)). The mean \( k_{\text{Cl}}/k_{\text{HCO}_3} \) was obtained assuming a log-normal distribution. The number of experiments per tubule given in parentheses. Luminal solutions were all Na-free so that the pH\(_i\) recoveries were minimally contaminated by luminal events.

**Figure 13.** Intracellular Cl\(^-\) activity during changes in [Na\(^+\)]\(_b\). \( V_1 \) and \( V_3 \) represent basolateral membrane potential and transepithelial potential, respectively. During the first interval of basolateral Na\(^+\) removal, both Na\(^+\) and Cl\(^-\) were present in the lumen. During the second, Cl\(^-\) was absent from the lumen only. During the third 0-[Na\(^+\)]\(_b\) interval, Na\(^+\) was absent from the lumen. Finally, during the fourth interval, both Na\(^+\) and Cl\(^-\) were present in the lumen. Throughout the experiment, pH was 7.5 and solutions were buffered with 10 mM HCO\(_3^-\)/1.5% CO\(_2\). A total of six similar experiments, incorporating one or more elements of Fig. 13, was performed, each on a separate tubule.

difference in the overall time course of \( a_{\text{Cl}}^{\text{in}} \) and pH\(_i\), the basolateral equivalent HCO\(_3^-\) flux cannot all be linked to Cl\(^-\). The subsequent return of Na\(^+\) to the basolateral solution causes \( a_{\text{Cl}}^{\text{in}} \) to return to about its initial value over a period
BORON AND BOULPAEP Intracellular pH Regulation in the Renal Proximal Tubule II.

of ~20 min, which indicates once again a discrepancy between the time courses of Cl⁻ and equivalent HCO₃⁻ fluxes, because the pHᵢ recovery is essentially complete in 2–3 min.

Several mechanisms might be invoked to explain the observed rise in \( a_{\text{Cl}} \) in the experiment of Fig 13. (i) Basolateral or luminal Cl⁻-HCO₃⁻ (or Cl-OH) exchange cannot account for the rise of \( a_{\text{Cl}} \), since the observed fall of pHᵢ could only serve to reduce Cl⁻ influx. (ii) A basolateral, electroneutral Na⁺/HCO₃⁻-Cl⁻/H exchange, such as identified in invertebrate nerve and muscle, could account for the rise of \( a_{\text{Cl}} \). Indeed, such a transporter has been postulated to account for Cl⁻ transport across the basolateral membrane of the Necturus proximal tubule (Guggino et al., 1982). And finally, (iii) the increase of \( a_{\text{Cl}} \) could result in part from an entry of Cl⁻ across the luminal membrane. For example, if lowering [Na⁺]₀ reduces \( a_{\text{Na}} \), as is known to be the case (Sackin et al., 1981), then Cl⁻ would be expected to enter across the luminal membrane via an electroneutral NaCl cotransporter.

To test this last possibility, we moved from the lumen either Cl⁻ or Na⁺ and repeated the basolateral Na⁺ removal experiment (Fig. 13). The luminal removal of Cl⁻ produces a slow fall of \( a_{\text{Cl}} \). During this period of declining \( a_{\text{Cl}} \), we removed basolateral Na⁺ and found that \( a_{\text{Cl}} \) increased by only ~0.6 mM after 5 min. Correcting for the declining \( a_{\text{Cl}} \) baseline, the true increase in \( a_{\text{Cl}} \) is probably closer to 1.0 mM, substantially less than the rise of \( a_{\text{Cl}} \) that occurred in a comparable time under control conditions, 6.3 mM. Some of this depression of the \( a_{\text{Cl}} \) increase is probably due to the absence of unidirectional Cl⁻ influx via a luminal NaCl cotransporter. To further test this possibility, we returned Cl⁻ to the lumen (note the rise in \( a_{\text{Cl}} \)) and subsequently removed Na⁺ from the lumen (note the fall in \( a_{\text{Cl}} \)). When Na⁺ is now removed from the bath, in the absence of luminal Na⁺, \( a_{\text{Cl}} \) increases by 3.9 mM after ~7 min (correcting for the declining \( a_{\text{Cl}} \) baseline), which is substantially less than after a comparable time under control conditions, 8.2 mM. The inability of luminal Na⁺ removal to completely inhibit the rise of \( a_{\text{Cl}} \) suggests that there is another mode of Cl⁻ entry besides luminal NaCl cotransport. Moreover, the leveling off of this rise in \( a_{\text{Cl}} \), as opposed to the continuing rise under control conditions, suggests a mechanism of Cl⁻ entry that is limited by the depletion of intracellular Na⁺. This could well be basolateral Na/HCO₃⁻-Cl/H exchange.

Finally, Na⁺ is returned to the lumen, causing a slow rise of \( a_{\text{Cl}} \), which may reflect the activity of a luminal NaCl cotransporter. When Na⁺ is subsequently removed from the basolateral solution, as in the initial control experiments, \( a_{\text{Cl}} \) again increases. The rise amounts to 9.6 mM over ~7 min, comparable to the previous value, 8.2 mM. Similarly, the return of Na⁺ to the bath leads to a slow recovery of \( a_{\text{Cl}} \).

**DISCUSSION**

**Basolateral HCO₃⁻ Transport**

In the preceding paper (Boron and Boulpaep, 1983), we showed that the salamander proximal tubule cell possesses Na-H exchangers on both the luminal and basolateral membranes. The cell must, of course, have some
asymmetry of its H+ and/or HCO₃⁻ transporters if it is to engage in net, transcellular acid secretion. The results of the present study show that the requisite asymmetry of the tubule cell, with respect to acid transport, is conferred by a pathway for HCO₃⁻ (or an equivalent species), which is confined to the basolateral membrane. Evidence for a basolateral pathway for HCO₃⁻ movement comes from three observations in the experiment of Fig. 1. (i) Application of CO₂/HCO₃⁻ Ringer causes a fall in pHᵢ from which the cell fails to recover. Although the CO₂-induced fall in pHᵢ undoubtedly stimulates Na-H exchange, this increased rate of acid extrusion is balanced by the newly established efflux of HCO₃⁻ and/or a related species. (ii) Reducing pHᵢ causes a much larger fall in pHᵢ when the basolateral solution contains HCO₃⁻ (at constant pCO₂) than when it is nominally HCO₃⁻ free. (iii) In HCO₃⁻ Ringer, reducing pHᵢ at constant pCO₂ has a substantially larger effect on pHᵢ than reducing pHᵢ, which indicates that the pathway for HCO₃⁻ must be limited to the basolateral membrane.

Although the changes in pHᵢ induced by changes in [HCO₃⁻]ᵢ are indicative of a basolateral pathway for HCO₃⁻ (or an equivalent species), this pathway is not the sole contributor to the pHᵢ changes in the aforementioned experiments. In fact, the decline in pHᵢ produced by lowering [HCO₃⁻]ᵢ and pHᵢ (at constant pCO₂) probably has two major causes: (i) a reduction in the basolateral Na-H exchange rate in the face of continued intracellular acid loading, and (ii) an increased net efflux of HCO₃⁻ and/or a related species. Similarly, returning [HCO₃⁻]ᵢ and pHᵢ to normal produces a very rapid recovery of pHᵢ, which apparently has two major components: (i) an increased rate of basolateral Na-H exchange, and (ii) an influx of HCO₃⁻ and/or a related species. The contribution of Na-H exchange is clearly demonstrated by the inhibitory effect of amiloride on the pHᵢ recovery (see Fig. 2). The component of the pHᵢ recovery remaining after application of amiloride, which largely blocks Na-H exchange, is presumably due to basolateral HCO₃⁻ transport.

The transport of HCO₃⁻ (or of an equivalent species) across the basolateral membrane could theoretically be effected by any of the five transport mechanisms listed in Fig. 14. The following observations must be accounted for: (i) pHᵢ falls when [HCO₃⁻]ᵢ is lowered from 10 to 2 mM (i.e., pHᵢ is lowered from 7.5 to 6.8), and rises when the reverse solution change is made. (ii) aᵣNa falls when [HCO₃⁻]ᵢ is lowered, and rises when the reverse solution change is made. (iii) The basolateral membrane depolarizes, usually transiently, upon reducing [HCO₃⁻]ᵢ, and hyperpolarizes, always transiently, upon restoring [HCO₃⁻]ᵢ to a normal level. (iv) There are only small changes in aᵣCl when [HCO₃⁻]ᵢ is altered. (v) pHᵢ falls when [Na⁺]ᵢ is reduced to zero and rises when [Na⁺]ᵢ is raised to normal. (vi) aᵣNa correspondingly falls and rises when [Na⁺]ᵢ is altered. (vii) The basolateral membrane transiently depolarizes upon reduction of [Na⁺]ᵢ and transiently hyperpolarizes upon restoration of [Na⁺]ᵢ. (viii) Changes in aᵣCl induced by alterations in [Na⁺]ᵢ appear not to be directly related to the aforementioned changes in pHᵢ, aᵣNa, or Vᵢ. (ix) The aforementioned changes in pHᵢ, aᵣNa, and Vᵢ are blocked by SITS, but are not inhibited by removal of Cl⁻.
Each of the five models is examined in detail for the six most important experimental conditions of the Results. The first three conditions pertain to the experiment in which $[\text{HCO}_3^-]_b$ is reduced (as in Fig. 1C), and the final three pertain to the experiment in which $[\text{Na}^+]_b$ is reduced (as in Fig. 7A): (i) the standard control condition, (ii) at the instant $[\text{HCO}_3^-]_b$ is reduced from 10 to 2 mM, (iii) at the instant $[\text{HCO}_3^-]_b$ is restored to 10 mM, (iv) the new control condition in which $[\text{Na}^+]_i$ is 0 mM, (v) at the instant $[\text{Na}^+]_b$ is reduced from 100 to 0 mM, and (vi) at the instant $[\text{Na}^+]_b$ is restored to 100 mM.

These conditions, and the ion activities and voltages pertaining to them, are summarized in Table VI for each of the five models of Fig. 14. The Appendix contains the thermodynamic calculations necessary for determining whether each of the models can account for the observed changes of intracellular ion activities and voltages for each of the six aforementioned conditions. (a) Conductive path for $\text{HCO}_3^-$. As shown in the Appendix, a simple conductive path for $\text{HCO}_3^-$ can account for the presumed efflux of $\text{HCO}_3^-$ (or an equivalent species) in the control condition $i$ and is consistent with the observations for condition $iv$, but cannot account by itself for the pH changes in conditions $ii$, $iii$, $v$, or $vi$. A small basolateral conductance for $\text{HCO}_3^-$ cannot
be ruled out. However, as indicated in the Results, the observed resting $V_1$ as well as the $V_1$ changes induced by alterations of $[\text{HCO}_3^-]_b$ are not consistent with a high partial conductance for $\text{HCO}_3^-$.

(b) Cl-HCO$_3$ exchange. As shown in the Appendix, an electroneutral Cl-HCO$_3$ exchanger could account for the observations in conditions i, ii, and iv, but cannot explain the activity changes occurring during conditions iii, v, and vi. Furthermore, since it predicts electroneutrality, it also cannot explain the observed changes in $V_1$ induced by altering either $[\text{HCO}_3^-]_b$ or $[\text{Na}^+]_b$. Although we cannot rule out a small component of Cl-HCO$_3$ exchange, we conclude that this process cannot by itself account for our data.

(c) Na-H exchange in parallel with Cl-HCO$_3$ exchange. As detailed in the Appendix, the parallel exchanger model makes an indeterminate prediction concerning conditions i and iv, and would satisfy condition v, but cannot account for the activity changes of conditions ii, iii, and vi. Furthermore, parallel exchangers cannot account for the observed voltage changes. We conclude that this hypothesis cannot by itself account for the data. Note, however, that basolateral Na-H has been identified in this preparation (Boron and Boulpaep, 1983).

Inasmuch as several investigators have suggested that electroneutral NaCl entry at the luminal membrane is mediated by parallel Na-H and Cl-HCO$_3$ exchangers, it is instructive to analyze the thermodynamic predictions of such a hypothesis. The free-energy changes for luminal Na-H and Cl-HCO$_3$ exchangers are the same as those noted above for basolateral exchangers in the control condition i: the $\Delta G_{\text{net}}$ for both exchangers is such that net $\text{Na}^+$ and Cl$^-$ entry would occur at the luminal membrane. Thus, the parallel exchange model can at least qualitatively account for luminal NaCl uptake. Only in the fortuitous case in which overall transport is isohydric, however, would $\text{Na}^+$ and Cl$^-$ move in equimolar amounts. The results of two additional experiments, performed by others on the Necturus proximal tubule (Spring and Kimura, 1978; Kimura and Spring, 1979) and by us in the present study, provide the basis for a more detailed examination of the parallel exchanger model. In one experiment $[\text{Cl}^-]_i$ only was lowered, and in the other, $[\text{Na}^+]_i$ only. In both cases, however, $a_{\text{Cl}}^i$ and $a_{\text{Na}}^i$ both fall. This linkage between Cl$^-$ and Na$^+$ could be achieved in any of four ways: (i) a direct linkage at the luminal membrane (e.g., NaCl cotransport), (ii) an indirect linkage at the luminal membrane (e.g., parallel Na-H and Cl-HCO$_3$ exchangers linked by changes in pH$_i$), (iii) a direct linkage at the basolateral membrane, and (iv) an indirect linkage at the basolateral membrane. The $a_{\text{Cl}}^i$ and $a_{\text{Na}}^i$ decreases in the aforementioned two experiments are certainly consistent with mechanisms i and iv. Possibility ii cannot account for the data because the requisite pH$_i$ changes do not occur. For example, lowering $[\text{Cl}^-]_i$ could reverse a Cl-HCO$_3$ exchanger (Cl$^-$ out, Na$^+$ in), but could not reverse the Na-H exchanger (Na$^+$ out, H$^+$ in) unless pH$_i$ would rise above 7.99. Although we have yet to lower $[\text{Cl}^-]$ in the lumen only, simultaneously removing Cl$^-$ from lumen and bath caused pH$_i$ to fall or remain unchanged. Conversely, lowering $[\text{Na}^+]_i$ could reverse a Na-H exchanger, but could not reverse a Cl-HCO$_3$ exchanger unless pH$_i$ would fall below 6.85. Fig. 7B shows that luminal Na$^+$ removal causes
only a transient fall of pH_i by 0.1 to a value of ~7.2; the steady state pH_i is unchanged. Possibility iii also cannot explain the data. It predicts that lowering [Cl^-]_i should reduce a^{Cl}_i but raise a^{Na}_i, and that lowering [Na^+]_i should lower a^{Na}_i but raise a^{Cl}_i. Such increases in a^{Na}_i and a^{Cl}_i have not been observed. These data do not rule out parallel Na-H and Cl-HCO_3 exchangers at the luminal membrane. However, they indicate that the simultaneous reversal of such exchangers cannot account for the decreases of a^{Cl}_i and a^{Na}_i observed when either [Cl^-]_i or [Na^+]_i is reduced.

(d) Na/HCO_3-Cl/H exchange. In squid axons (Russell and Boron, 1982), snail neurons (Thomas, 1977), and barnacle muscle (Boron et al., 1979, 1981), pH_i is regulated by a system that exchanges external HCO_3^- and Na^+ for internal Cl^- (and possibly H^+). The stoichiometry is one Na^+ entering for each Cl^- leaving, and each pair of protons neutralized intracellularly (Russell and Boron, 1982). Thomas (1977) has suggested model d1 of Fig. 14. This is not distinguishable thermodynamically from models d2-d4, which are also presented.

As detailed in the Appendix, this model fails to account for our observations for all experimental conditions other than iv. Note, however, that the discrepancies for conditions ii, iii, v, and vi all bear on the involvement of Cl^-_. We recognize that the transporter could have such a high affinity for Cl^- that it could be difficult to demonstrate a Cl^- dependence, and that a^{Cl}_i could be so well regulated by other transport systems that large changes of a^{Cl}_i would not occur. However, even if the interpretation of the Cl^- data were in error, this electroneutral model would still fail to explain the voltage changes. We conclude that an Na/HCO_3-Cl/H exchanger cannot by itself account for our data, though we cannot rule out the participation of such an exchanger in the a^- shifts.

(e) Electrogenic Na/HCO_3 transport. Fig. 14 lists four thermodynamically indistinguishable variants that would result in the equivalent of electrogenic Na/HCO_3 transport. Although the stoichiometry predicted by these models is equivalent to two HCO_3^- for each Na^+, we emphasize that our data only require that the ratio of net fluxes of HCO_3^- to Na^+ be greater than unity. Models e1-e4 are the same as d1-d4, except for the lack of Cl^- involvement. As described in the Appendix, models e1-e4 account for all the a^{Na}_i and pH_i data for the six experimental conditions. The models also account for all V_i transients that follow changes in [HCO_3^-]_b and [Na^+]_b, as well as the depolarization accompanying application of CO_2/HCO_3^- Ringer.

The reversal potential for the electrogenic Na/HCO_3 transport system is the equilibrium potential for the anion pair in model e4, E_{NaCO_3}:

\[ E_{NaCO_3} = \frac{RT}{F} \ln \frac{[Na^+]_i [HCO_3^-]^2}{[Na^+]_o [HCO_3^-]_o^2} \]

The values of ΔG described in the Appendix are related to E_{NaCO_3} by the equation

\[ ΔG = F(V_i - E_{NaCO_3}) \]
It is instructive to examine the relationship between $E_{\text{NaCO}_3}$ and $V_1$ for each of the six experimental conditions described above. (i) In the normal control condition, $E_{\text{NaCO}_3}$ is $-52.4$ mV, compared with a $V_1$ in HCO$_3$ Ringer of about $-60$ mV. Thus, the transport system would carry negative current outward and thereby tend to depolarize the basolateral membrane. This explains why the basolateral membrane depolarizes when the tubule is transferred from HEPES to HCO$_3$ Ringer (i.e., from solution 9 to 1). (ii) When $[\text{HCO}_3]$$_b$ is reduced to 2 mM, $E_{\text{NaCO}_3}$ instantly becomes $+29.3$ mV, explaining the observed, basolateral depolarization. (iii) When $[\text{HCO}_3]$$_b$ is subsequently restored to 10 mM, $E_{\text{NaCO}_3}$ instantly becomes $-98.2$ mV, which accounts for the observed basolateral hyperpolarization. (iv) In the new control condition (with a $[\text{Na}^+]_b$ of 0 mM), $E_{\text{NaCO}_3}$ is $-72.2$ mV close to the prevailing value of $V_1$ (approximately $-70$ mV). (v) When $[\text{Na}^+]_b$ is reduced to 0 mM, $E_{\text{NaCO}_3}$ instantly approaches $+\infty$, accounting for the abrupt basolateral depolarization. (vi) When $[\text{Na}^+]_b$ is subsequently returned to 100 mM, $E_{\text{NaCO}_3}$ instantly becomes $-151.1$ mV, which again explains hyperpolarizations of the basolateral membrane which, in some cases, reached $-140$ mV.

If the electrogenic Na/HCO$_3$ transporter is indeed responsible for a portion of the pH$_i$ and $V_1$ changes associated with the alteration of either $[\text{HCO}_3]$$_b$ or $[\text{Na}^+]_b$, then the magnitude of a $V_1$ change should be related in a predictable way to the rate of pH$_i$ change. Consider as an example the experiment of Fig. 7A, in which Na$^+$ is removed from and then returned to the bath. The rate of pH$_i$ recovery upon restoring Na$^+$ to the bath is $\sim 0.014$ pH units·s$^{-1}$. If the pH$_i$ recovery is due entirely to the entry of HCO$_3^-$ and Na$^+$ in a 2:1 ratio, then it can be shown$^6$ that the expected initial basolateral hyperpolarization is 34 mV. This value should be interpreted with caution since, on the one hand, the rather slowly responding pH microelectrode probably underestimates the very rapid, initial pH$_i$ recovery rate, while on the other, a portion of the pH$_i$ recovery is probably due to electroneutral Na-H exchange. Assuming that these opposing influences approximately cancel each other, then the calculated hyperpolarization is in reasonable agreement with that actually observed,$^7$ 38 mV. Thus, it would seem that a single Na/HCO$_3$ transporter could account for both the $V_1$ and pH$_i$ changes.

With the present data, we cannot distinguish among models $e1$--$e4$. The last

$^6$ The product of this pH$_i$ recovery rate (i.e., 0.014 pH·s$^{-1}$) and the total intracellular buffering power at pH$_i$ = 7.0 (i.e., 43 mM/pH; see Boron and Boulpaep, 1983) is the equivalent net influx of HCO$_3^-$ across the basolateral membrane, 0.602 mmol·s$^{-1}$·(liter cell volume)$^{-1}$. If the stoichiometry is two HCO$_3^-$ for each Na$^+$, this corresponds to a flux of 0.301 meq·s$^{-1}$·(liter cell volume)$^{-1}$, or to 29.0 amp·(liter cell volume)$^{-1}$. The ratio of cell volume to luminal surface area for the Ambystoma proximal tubule is $2.54 \times 10^{-3}$ cm, based on a morphometric analysis (Maunsbach and Boulpaep, unpublished). Thus, the current flow through the Na/HCO$_3$ transporter would be 73.7 $\mu$A·cm$^{-2}$. Given a transepithelial resistance for the Ambystoma of 52.1 $\Omega$·cm$^2$ (Sackin and Boulpaep, 1981a), a basolateral membrane resistance of 591 $\Omega$·cm$^2$ (Sackin et al., 1982), and a luminal membrane resistance of 2,305 $\Omega$·cm$^2$ (Sackin and Boulpaep, unpublished), then the calculated basolateral hyperpolarization (taking current loops into account), comes to 34 mV.

$^7$ The magnitude of the hyperpolarization is taken as the degree to which $V_1$ transiently undershoots the final steady state $V_1$. This is the portion which is blocked by SITS.
one, a channel or a carrier for the ion pair, is perhaps the simplest inasmuch as only a single species need cross the membrane. \( \text{NaCO}_3 \) is known to exist, though its expected concentration is very low. The data of Garrels et al. (1961), which were obtained for seawater and therefore can provide only a rough estimate, predict a \( [\text{NaCO}_3]_b \) of only \( \sim 25 \mu \text{M} \). This would require a carrier of high affinity or a channel of extraordinary permeability.

**Unifying Model for Basolateral Na\(^+\), HCO\(_3\)\(^-\), and Cl\(^-\) Transport**

The simultaneous, transcellular reabsorption of HCO\(_3\) and Cl\(^-\) requires the basolateral efflux of both HCO\(_3\) and Cl\(^-\). The present study documents an electrogenic Na/HCO\(_3\) transporter capable of high transport rates, and which carries most of the HCO\(_3\) and a fraction of the Na\(^+\) out across the basolateral membrane. The mechanism of Cl\(^-\) efflux at the basolateral membrane is unsettled. Basolateral Cl\(^-\) conductance in the *Necturus* proximal tubule is probably too low (Guggino et al., 1982) to mediate the efflux, and an electroneutral Cl/HCO\(_3\) exchanger would normally be poised in the direction of basolateral Cl\(^-\) entry. However, Guggino et al. (1980) have identified a basolateral Cl\(^-\) transporter with properties expected of model d of Fig. 14. As is evident from the analyses in the Appendix, independent Cl\(^-\) and HCO\(_3\) systems are a necessity for the simultaneous basolateral efflux of Cl\(^-\) and HCO\(_3\). However, the electrogenic Na/HCO\(_3\) transporter shares several characteristics with the postulated Cl\(^-\) system: (i) involvement of Na\(^+\), (ii) involvement of HCO\(_3\), and (iii) sensitivity to SITS. We suggest a scheme (Fig. 15) in which a single carrier could accomplish the two apparently disparate tasks. The upper loop in Fig. 15, described by the directions \( k_1 \) and \( k_{-2} \), represents the hypothesized electrogenic Na/HCO\(_3\) transporter operating in the direction of HCO\(_3\) reabsorption. The large loop, in the direction described by \( k_{-1} \) and \( k_3 \), represents the Na/HCO\(_3\)-Cl/H exchanger operating in the direction of a classic pH\(_i\) regulator (i.e., Cl\(^-\) reabsorption). The lower loop, in the direction described by \( k_5 \) and \( k_{-2} \), is equivalent to an electrogenic Cl\(^-\) carrier operating in the direction of Cl\(^-\) reabsorption. All three loops have been described in the directions appropriate for exergonic reactions under normal conditions. Depending upon the values chosen for the various rate constants, this system could mediate pure Na/HCO\(_3\) reabsorption, pure Cl\(^-\) reabsorption, any combination of the two, or even Cl\(^-\) reabsorption with the opposite movement of Na/HCO\(_3\). When concentrations or voltages are altered, the net directions of one or more loops could be reversed and thereby produce a wide variety of apparent interdependencies among the fluxes of the ions and net negative charge.

**Model of Renal Acid Secretion**

Nerve and muscle cells actively regulate their pH\(_i\) by extruding acid from the cell (Roos and Boron, 1981). The rate of acid extrusion in barnacle muscle approaches zero at pH\(_i\) values at or above a certain threshold, and gradually increases as pH\(_i\) falls below this threshold. The Na-H exchanger of the proximal tubule cells apparently exhibits a similar sensitivity to intracellular
acid loading, regulating pH\textsubscript{i} much as it would in a nerve or muscle cell (Boron and Boulpaep, 1983). In the absence of a basolateral pathway for HCO\textsubscript{3}\textsuperscript{-} (as can be achieved by nominal removal of HCO\textsubscript{3}\textsuperscript{-}, or by addition of SITS), the tubule cells function much the same as a nerve or muscle cell with respect to pH\textsubscript{i} regulation. It is basolateral HCO\textsubscript{3}\textsuperscript{-} transport that endows these cells with the potential for transcellular acid secretion. It should be noted that acid secretion has yet to be demonstrated in this tubule segment. Nevertheless, the identification of HCO\textsubscript{3}\textsuperscript{-} and H\textsuperscript{+} transport systems in this and the companion study (Boron and Boulpaep, 1983), makes it likely that such acid secretion can indeed occur. In the control state, there is a net efflux of Na/HCO\textsubscript{3} across the basolateral membrane. This efflux lowers pH\textsubscript{i} and a\textsubscript{Na} and therefore stimulates the pH\textsubscript{i}-regulating mechanism: luminal and basolateral Na-H exchange. The extrusion of H\textsuperscript{+} across the basolateral membrane represents a substantial inefficiency with respect to the presumed acid secretory activity of the tubule, since this extrusion short-circuits a portion of the basolateral HCO\textsubscript{3}\textsuperscript{-} efflux. H\textsuperscript{+} extrusion across the luminal membrane is identical to the presumed unidirectional, transcellular acid secretion.

Our model of proximal-tubule acid secretion represents a unifying theory.
of intracellular pH regulation in epithelial and nonepithelial cells. However, it differs somewhat from the prevailing viewpoint, which has luminal acid extrusion (and therefore a rise in pHₜ) as the primary event in acid secretion, and the basolateral HCO₃⁻ efflux merely following to keep pHₜ from rising too high. Cited as evidence for this latter view is the observation that pHₜ in proximal tubule cells is rather alkaline, ~7.4. The crucial parameter, however, is not the absolute value of the steady state pHₜ, but rather the degree to which pHₜ is below the threshold for activating the pHₜ-regulating mechanism. We estimate that the pHₜ threshold of the proximal tubule cells' Na-H exchanger is at or somewhat above 7.43, the mean pHₜ in HEPES Ringer (Boron and Boulpaep, 1983). In the absence of basolateral HCO₃⁻ efflux, the rate of intracellular acid loading is probably very low in these amphibian cells, and the unopposed pHₜ-regulating system (i.e., the Na-H exchangers) drives pHₜ upward to ~7.43, at which point the Na-H exchangers are, or are nearly, inactive. The normal pHₜ of these cells at a physiologic [HCO₃⁻]ₜ and pCO₂, however, is ~0.17 lower than 7.43. This represents a substantial intracellular acid load, which must therefore greatly stimulate Na-H exchange. Thus, we feel that the primary event in acid secretion is the basolateral efflux of HCO₃⁻, which decreases pHₜ below the threshold for activating the pHₜ-regulating mechanism. For the salamander, in which basolateral HCO₃⁻ transport is mediated by the Na/HCO₃⁻ transR, or transporter (see Fig. 16), basolateral HCO₃⁻ efflux is also accompanied by a fall in aᵢNa, which may also enhance Na-H exchange. The rate of transcellular acid secretion (i.e., luminal Na-H exchange) is therefore directly regulated by pHₜ and, to a certain extent, aᵢNa. We predict that the proximal tubule's rate of transcellular acid secretion should be increased by any treatment which lowers pHₜ: raising pCO₂ (at constant external pH), lowering [HCO₃⁻]ₜ (at constant pHₜ), and selectively inhibiting basolateral Na-H exchange.

**APPENDIX**

*Thermodynamic Analysis of Basolateral HCO₃⁻ Transport*

The hypothetical movements of the HCO₃⁻ (or an equivalent species) can be analyzed for six relevant experimental conditions. The first three pertain to the experiment in which [HCO₃⁻]ₜ is reduced (see Fig. 1C), and the final three pertain to the experiment in which [Na⁺]ₜ is reduced (see Fig. 7A): (i) the control condition, (ii) at the instant [HCO₃⁻]ₜ is reduced from 10 to 2 mM, (iii) at the instant [HCO₃⁻]ₜ is restored to 10 mM, (iv) the new control condition in which [Na⁺]ₜ is 0 mM, (v) at the instant [Na⁺]ₜ is reduced from 100 to 0 mM, and (vi) at the instant [Na⁺]ₜ is restored to 100 mM. These conditions are summarized in Table VI.

**Conductive Path for HCO₃⁻** The net free energy change for HCO₃⁻ as it exits across the basolateral membrane is:

$$\Delta G_{\text{net}} = RT \ln \frac{[\text{HCO}_3^-]_t}{[\text{HCO}_3^-]_i} + FV_i,$$

where $\Delta G_{\text{net}} < 0$ indicates a net HCO₃⁻ efflux. In addition, the HCO₃⁻ flux through an idealized channel can be calculated from the constant field equation (Goldman, 1943;
Hodgkin and Katz, 1949):

\[ J_{\text{net}}^{\text{HCO}_3} = P \cdot \frac{V_i F}{RT} \cdot \frac{[\text{HCO}_3^-]_b - [\text{HCO}_3^-]}{1 - \epsilon}, \]

where \( J_{\text{net}}^{\text{HCO}_3} \) is the net \( \text{HCO}_3^- \) influx; \( P \) is the permeability to \( \text{HCO}_3^- \); \( F, R, \) and \( T \) have their usual meanings; and \( \epsilon = \exp(-V_i F/RT) \).

(i) Under standard control conditions (see Table VI), \( \Delta G_{\text{net}} \) is \(-1.9RT\) and \( J_{\text{net}}^{\text{HCO}_3} \) comes to \(-14.0 \, P \) (units: \( 10^{-6} \text{ mol cm}^{-2} \text{ s}^{-1} \)); the negative signs signify a net efflux. This is in agreement with the sustained fall in \( pHi \), presumably because of the net efflux of \( \text{HCO}_3^- \) (or an equivalent species), actually observed when \( \text{HCO}_3^- \)-free is replaced with \( \text{HCO}_3^- \)-containing Ringer (see Fig. 1A). (ii) When \([\text{HCO}_3^-]_b\) is reduced from 10 to 2 mM, there is an immediate basolateral depolarization of \(-20 \text{ mV} \). The instantaneous \( \Delta G_{\text{net}} \) is therefore \(-2.7RT\), which predicts an increased gradient for \( \text{HCO}_3^- \) efflux. The calculated, instantaneous \( J_{\text{net}}^{\text{HCO}_3} \), however, is actually reduced somewhat to \(-11.7P \). This occurs because \( V_i \) appears as an exponential term in the expression for \( J_{\text{net}}^{\text{HCO}_3} \), causing the effect of the depolarization to outweigh that of lowering \( [\text{HCO}_3^-]_b \). Therefore, unless \( P \) increases substantially as \( pHi \) is reduced from 7.5 to 6.8, the observed fall in \( pHi \) cannot be explained by an increased passive \( \text{HCO}_3^- \) efflux. (iii) Just before \([\text{HCO}_3^-]_b\) is returned to 10 mM, \( \text{pH}_i \) is 2.1 mM (assuming that \( \text{pH}_i \) had previously fallen by 0.35). When \([\text{HCO}_3^-]_b\) is raised, \( V_i \) instantly hyperpolarizes to about \(-60 \text{ mV} \). The initial \( \Delta G_{\text{net}} \) is \(-1.1RT\), which predicts a continued net \( \text{HCO}_3^- \) efflux. The initial

![Figure 16. Model of acid secretion in the salamander proximal tubule.](image-url)
$J_{\text{HCO}_3^-}$ comes to $-4.8P$, a net efflux which would be smaller than that prevailing during the reduction of $[\text{HCO}_3^-]_b$ (condition ii), but not the net influx required to explain the data. (iv) In the new control condition in which $[\text{Na}^+]_i$ is $0 \text{ mM}$, the basolateral membrane is hyperpolarized compared with condition i, and $V_t$ will be assumed to be $-70 \text{ mV}$. $\Delta G_{\text{net}}$ comes to $-2.3RT$ and predicts a net efflux. $J_{\text{HCO}_3^-}$ is $-16.7P$, describing the magnitude of this efflux. The data of Fig. 7B are consistent with a diminished net $\text{HCO}_3^-$ efflux or even an influx. (v) When $[\text{Na}^+]_b$ is reduced to $0 \text{ mM}$, the basolateral membrane instantly hyperpolarizes by $-40 \text{ mV}$; $V_t$ is assumed to be $-30 \text{ mV}$. The initial $\Delta G_{\text{net}}$ is $-0.7RT$, which predicts a continued $\text{HCO}_3^-$ efflux. However, $J_{\text{HCO}_3^-}$ is only $-5.5P$, which predicts that this efflux should be reduced by two-thirds compared with the control. Thus, this model cannot account for the observed fall of $pH_i$ (i.e., efflux of $\text{HCO}_3^-$ or an equivalent species) unless there is a concomitant change in $P$. (vi) When $[\text{Na}^+]_b$ is restored to $100 \text{ mM}$, the basolateral membrane initially hyperpolarizes, usually to beyond $-100 \text{ mV}$. Assuming $V_t$ is $-100 \text{ mV}$ and $[\text{HCO}_3^-]_b$ is $2.8 \text{ mM}$, the initial $\Delta G_{\text{net}}$ is $-2.7RT$, which predicts a net $\text{HCO}_3^-$ efflux, not the influx required to explain the observed rise of $pH_i$. $J_{\text{HCO}_3^-}$ comes to $-10.4P$, which predicts that the $\text{HCO}_3^-$ efflux should be sizeable.

**Table VI**

Predicted free energy changes for five models of basolateral $\text{HCO}_3^-$ transport* and parameter values assumed in calculations‡

<table>
<thead>
<tr>
<th>Model</th>
<th>$[\text{Na}^+]_b = 100 \text{ mM}$</th>
<th>$[\text{Na}^+]_b = 0 \text{ mM}$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>$[\text{HCO}_3^-]_b$</td>
</tr>
<tr>
<td>(a) $\text{HCO}_3^-$</td>
<td>$-1.9RT$</td>
<td>$-2.7RT$</td>
</tr>
<tr>
<td>(b) $\text{Cl-HCO}_3^-$</td>
<td>$-1.0$</td>
<td>$-2.6$</td>
</tr>
<tr>
<td>(c) $\text{Na-H}$</td>
<td>$-1.6$</td>
<td>$0.0$</td>
</tr>
<tr>
<td>(d) $\text{Na/NaCl}$</td>
<td>$-0.5$</td>
<td>$+2.6$</td>
</tr>
<tr>
<td>(e) $\text{Na/HCO}_3$</td>
<td>$-0.3$</td>
<td>$-2.7$</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Parameter values</th>
</tr>
</thead>
<tbody>
<tr>
<td>$pH_i$</td>
<td>$7.30$</td>
</tr>
<tr>
<td>$pH_b$</td>
<td>$7.50$</td>
</tr>
<tr>
<td>$[\text{HCO}_3^-]$</td>
<td>$4.7$</td>
</tr>
<tr>
<td>$[\text{H}_2\text{CO}_3]$</td>
<td>$7.5$</td>
</tr>
<tr>
<td>$[\text{Na}^+]$</td>
<td>$24$</td>
</tr>
<tr>
<td>$[\text{Cl}^-]$</td>
<td>$75$</td>
</tr>
<tr>
<td>$[\text{HCO}_3^-]$</td>
<td>$16$</td>
</tr>
<tr>
<td>$[\text{H}_2\text{CO}_3]$</td>
<td>$71$</td>
</tr>
<tr>
<td>$V_t$</td>
<td>$-0.060V$</td>
</tr>
</tbody>
</table>

* The reactions are written so that they proceed spontaneously (i.e., $\Delta G_{\text{net}} < 0$) under control condition i. The calculations of $\Delta G_{\text{net}}$ for conditions ii, iii, iv, and vi are made at the instant the external solution change is made.

‡ Conditions ii, iii, iv, and vi are given for the instant at which the external solution change is made, assuming that $V_t$ has had time to shift, but that all other intracellular parameters are unaltered from the previous steady state.
CL-HCO₃ EXCHANGE

For an electroneutral Cl-HCO₃ exchanger, the net change in free energy is:

$$\Delta G_{\text{net}} = RT \ln \frac{[\text{HCO}_3^-]_b [\text{Cl}^-]_i}{[\text{HCO}_3^-]_i [\text{Cl}^-]_b},$$

where a negative value for $\Delta G_{\text{net}}$ indicates net Cl⁻ influx and HCO₃ efflux. Activities are understood in place of concentrations. It is possible to calculate $\Delta G_{\text{net}}$ for the same six conditions as above, during changes in either $[\text{HCO}_3^-]_b$ or $[\text{Na}^+]_b$. (i) Under standard control conditions (see Table VI), the values for $d_1$ and $d_2$ are ~71 and 16 mM, respectively. Thus, $\Delta G_{\text{net}}$ is -1.0RT, and HCO₃ would leave the cell in exchange for Cl⁻. This model would therefore account for the sustained fall in pHı observed after applying CO₂/HCO₃ Ringer (Fig. 1A). (ii) When $[\text{HCO}_3^-]_b$ is reduced from 10 to 2 mM, $\Delta G_{\text{net}}$ rises to -2.6RT. Thus, if anything, HCO₃ efflux would increase, consistent with the observed pHı decrease. However, $d_1^2$ should rise in proportion to the net intracellular alkali loss, which was not observed, as noted in the analysis of Fig. 5. (iii) When $[\text{HCO}_3^-]_b$ is returned to 10 mM, $\Delta G_{\text{net}}$ starts off at ~19 mM. The initial $\Delta G_{\text{net}}$ is 0.0RT, which predicts no net HCO₃ flux. Therefore, the Cl-HCO₃ exchange model cannot account for the rise of pHı, presumably caused by the net influx of HCO₃ or an equivalent species, which occurs upon raising $[\text{HCO}_3^-]_b$. (iv) In the new control condition in which $[\text{Na}^+]_i$ is 0 mM, $d_1^3$ is assumed to be 16 mM. $\Delta G_{\text{net}}$ comes to -1.0RT, which predicts a net efflux of HCO₃ (or an equivalent species). As noted in the Results, the data of Fig. 7B are consistent with a slowing or a reversal of the net HCO₃ efflux. (v) At the instant $[\text{Na}^+]_b$ is reduced to 0 mM, there are no changes in any of the relevant activities, and $\Delta G_{\text{net}}$ remains at -1.0RT, predicting a net HCO₃ efflux unchanged from the control state. Thus, this model cannot account for the observed rapid rise in pHı. (vi) Just before $[\text{Na}^+]_b$ is restored to 100 mM, $\Delta G_{\text{net}}$ is ~20 mM. The $\Delta G_{\text{net}}$, at the instant $[\text{Na}^+]_b$ is raised, is no different from that value prevailing immediately before the $[\text{Na}^+]_b$ increase, 0.0RT. Thus, this model predicts no net HCO₃ flux, and therefore cannot account for the observed, rapid rise of pHı.

NA-H EXCHANGE IN PARALLEL WITH CL-HCO₃ EXCHANGE

For an electroneutral, basolateral Na-H exchanger, the net change in free energy is:

$$\Delta G_{\text{net}} = RT \ln \frac{[\text{Na}^+]_i [\text{H}^+]_b}{[\text{Na}^+]_b [\text{H}^+]_i},$$

where a negative value for $\Delta G_{\text{net}}$ indicates net Na⁺ influx and H⁺ efflux. The free energy change for a Cl-HCO₃ exchanger is given above. (i) In the standard control condition with 100 mM Na⁺ in the lumen (see Table VI), $\Delta G_{\text{net}}$ (Na-H) is -1.6RT, whereas, as pointed out above, $\Delta G_{\text{net}}$ (Cl-HCO₃) is -1.0RT. The net effect of the parallel operation of the two exchangers is net Na⁺ and Cl⁻ influx, and net H⁺ and HCO₃ efflux. If the transport rates of the two exchangers were fortuitously identical, the net result would be isohydric NaCl influx; such a mechanism has previously been postulated for the luminal membrane (Liedtke and Hopfer, 1977). If, on the other hand, the two rates were not identical, the net effect of the two transport processes would be either an increase or a decrease in pHı. (ii) When $[\text{HCO}_3^-]_b$ is reduced from 10 to 2 mM, the instantaneous $\Delta G_{\text{net}}$ (Na-H) becomes zero, whereas $\Delta G_{\text{net}}$ (Cl-HCO₃) increases to -2.6RT. Thus, for this experimental maneuver, the parallel exchangers would behave as would a single Cl-HCO₃ exchanger (see CL-HCO₃ EXCHANGE above). (iii) At the instant $[\text{HCO}_3^-]_b$ is restored to 10 mM, $\Delta G_{\text{net}}$ (Na-H) becomes -2.6RT, whereas $\Delta G_{\text{net}}$ (Cl-HCO₃) becomes zero. Thus, for condition iii, the parallel exchangers would behave as a single Na-H exchanger. However, our pHı recovery data indicate
a rate constant approximately twice as great as that expected for pure Na-H exchange. Furthermore, the pH recovery was only inhibited about half by 2 mM amiloride, a treatment which should have eliminated 80% of Na-H exchange. (iv) In the new control condition with $[Na^+]_i = 0$ mM, $d_{Na}$ is $\sim 11$ mM (Sackin et al., 1981). Thus, $\Delta G_{net}$ (Na-H) across the basolateral membrane is $-2.4RT$, and $\Delta G_{net}$ (Cl-HCO$_3$) is $-1.0RT$. This condition is similar to i above with respect to net Na$^+$ and Cl$^-$ gain and the effect on pH$_i$. (v) When $[Na^+]_b$ is also reduced to 0 mM, the instantaneous $\Delta G_{net}$ (Na-H) becomes $+\infty$, whereas $\Delta G_{net}$ (Cl-HCO$_3$) is unchanged at $-1.0RT$. Thus, there would be a Cl$^-$ gain and a Na$^+$ loss combined with a fall in pH$_i$, secondary to both H$^+$ gain and HCO$_3^-$ loss. These predictions qualitatively agree with our data. (vi) Just before $[Na^+]_b$ is restored to 100 mM, $d_{Na}$ is $\sim 2.5$ mM (Sackin et al., 1981). At the instant $[Na^+]_b$ is raised, $\Delta G_{net}$ (Na-H) is $-4.7RT$ and $\Delta G_{net}$ (Cl-HCO$_3$) is zero. Thus, the parallel exchanger hypothesis predicts that pH$_i$ should recover because of Na-H exchange alone. Our data, however, contradict this prediction on three counts: first, the rate constant of the pH$_i$ recovery exceeds that for pure Na-H exchange; second, the recovery is sensitive to SITS; and third, the pH$_i$ recovery is HCO$_3^-$ dependent.

**NA/HCO$_3^$/CL/H EXCHANGE**

The predicted free energy change is:

$$\Delta G_{net} = RT \ln \frac{[Na^+]_i [HCO_3^-]_i [Cl^-]_b [H^+]_b}{[Na^+]_b [HCO_3^-]_b [Cl^-]_i [H^+]_i},$$

where a negative value for $\Delta G_{net}$ indicates a net influx of Na$^+$ and HCO$_3^-$, and a net efflux of Cl$^-$ and H$^+$. (i) Under standard control conditions (see Table VI), $\Delta G_{net}$ is $-0.6RT$, which predicts a net uptake of Na$^+$ and HCO$_3^-$, and a net loss of Cl$^-$ and H$^+$. This is opposite to the expected net HCO$_3^-$ efflux, based on the sustained fall in pH$_i$ which accompanies application of CO$_2$/HCO$_3^-$ Ringer (Fig. 1A). (ii) When $[HCO_3^-]_b$ is reduced to 2 mM, the initial $\Delta G_{net}$ is $+2.6RT$. This correctly predicts a net Na$^+$ and HCO$_3^-$ influx. It also implies an absolute dependence on Cl$^-$, as well as a net Cl$^-$ influx which should amount to half of the total alkali efflux plus acid influx. Our data, however, do not support these last two predictions. In the first place, an absolute dependence on Cl$^-$ was not observed when Cl$^-$ was replaced by cyclamate, glucuronate, or sulfate in the [HCO$_3^-$]$_b$ reduction experiments. Second, concomitant change in $d_{Cl}$ did not have a time course and magnitude consistent with the changes in pH$_i$ and $d_{Na}$. (iii) At the instant $[HCO_3^-]_b$ is restored to 10 mM, $\Delta G_{net}$ becomes $-2.5RT$, which correctly predicts a net uptake of Na$^+$ and HCO$_3^-$. It also predicts a dependence on Cl$^-$ as well as a Cl$^-$ efflux, which are not supported by our data (see ii above). (iv) In the new control condition in which $[Na^+]_i$ is zero, $\Delta G_{net}$ becomes $-1.4RT$, which predicts a net uptake of Na$^+$ and HCO$_3^-$, and a net loss of Cl$^-$ and H$^+$. The predicted pH$_i$ change is thus not inconsistent with the data of Fig. 7B, which indicates that the net HCO$_3^-$ efflux should be slowed or even reversed. (v) When $[Na^+]_b$ is reduced to zero, $\Delta G_{net}$ becomes $+\infty$, correctly predicting a net Na$^+$ and HCO$_3^-$ influx. This model also predicts an absolute dependence on Cl$^-$ as well as a simultaneous uptake of Cl$^-$, which should amount to half the total alkali leaving plus acid entering the cell. The Cl$^-$ dependence was not observed, and, as pointed out in Results, both the time course and the magnitude of the $d_{Cl}$ increase were inappropriate. (vi) At the instant $[Na^+]_b$ is restored to 100 mM, $\Delta G_{net}$ becomes $-4.7RT$, which correctly predicts a net influx of Na$^+$ and HCO$_3^-$. However, this model also predicts an absolute requirement for Cl$^-$ as well as a simultaneous efflux of Cl$^-$, which should amount to half the total alkali gained plus acid lost from the cell. The Cl$^-$ dependence was not observed, and, as pointed out in Results, both the time course and magnitude of the $d_{Cl}$ decrease were inappropriate.
ELECTROGENIC Na/HCO₃ TRANSPORT  The models e₁–e₄ are thermodynamically equivalent. Their common predicted free energy change, when expressed in the form for model e₁, is:

\[ \Delta G_{\text{net}} = RT \ln \frac{[\text{Na}^+]_b [\text{HCO}_3^-]_b}{[\text{Na}^+]_i [\text{HCO}_3^-]_i} + FV_1, \]

where a negative value for \( \Delta G_{\text{net}} \) signifies net Na⁺ and equivalent HCO₃⁻ efflux. (i) Under standard control conditions (Table VI), this model predicts \( \Delta G_{\text{net}} = -0.3RT \). The efflux of HCO₃⁻ thus predicted is consistent with the sustained fall in pHi observed after application of CO₂/HCO₃⁻ Ringer. (ii) Reducing [HCO₃⁻]₀ from 10 to 2 mM would bring \( \Delta G_{\text{net}} \) to \(-2.7RT \), which predicts an increase in both Na⁺ and HCO₃⁻ efflux, consistent with the \( d_{\text{Na}} \) and pHi data. (iii) When [HCO₃⁻]₀ is returned to 10 mM, \( \Delta G_{\text{net}} \) becomes \(+1.45RT \). This would probably produce an influx of both Na⁺ and HCO₃⁻, once again consistent with the data. (iv) In the new control condition when [Na⁺]₀ is 0 mM, \( \Delta G_{\text{net}} \) is very slightly positive, \(+0.1RT \). This is consistent with the earlier explanation for the pHi changes of Fig. 7B, in which we pointed out that removing luminal Na⁺ would slow or even reverse the electrogenic Na/HCO₃ system. (v) Reducing [Na⁺]₀ to zero in the absence of luminal Na⁺ causes a substantial basolateral depolarization and causes \( \Delta G_{\text{net}} \) to approach \(-\infty \), favoring net Na⁺ and HCO₃⁻ efflux. This is consistent with the observed fall of pHi. (vi) When [Na⁺]₀ is returned to 100 mM, \( V_1 \) reaches \(-100 \) mV, causing \( \Delta G_{\text{net}} \) to rise to \(+2.0RT \), favoring net Na⁺ and HCO₃⁻ uptake. These predictions are also consistent with the \( d_{\text{Na}} \) and pHi data.

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