Intracellular pH Regulation in the Renal Proximal Tubule of the Salamander

**Basolateral HCO₃⁻ Transport**

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**ABSTRACT** We have used pH-, Na-, and Cl-sensitive microelectrodes to study basolateral HCO₃⁻ transport in isolated, perfused proximal tubules of the tiger salamander *Ambystoma tigrinum*. In one series of experiments, we lowered basolateral pH (pH₄) from 7.5 to 6.8 by reducing [HCO₃⁻]₄ from 10 to 2 mM at a constant pCO₂. This reduction of pH₄ and [HCO₃⁻]₄ causes a large (~0.35), rapid fall in pHi as well as a transient depolarization of the basolateral membrane. Returning pH₄ and [HCO₃⁻]₄ to normal has the opposite effects. Similar reductions of luminal pH (pH₁) and [HCO₃⁻]₁ have only minor effects. The reduction of [HCO₃⁻]₄ and pH₄ also produces a reversible fall in aNa. In a second series of experiments, we reduced [Na⁺]₄ at constant [HCO₃⁻]₄ and pH₄, and also observed a rapid fall in pHi and a transient basolateral depolarization. These changes are reversed by returning [Na⁺]₄ to normal. The effects of altering [Na⁺]₄ in the presence of HCO₃⁻, or of altering [Na⁺]₄ in the nominal absence of HCO₃⁻, are substantially less. Although the effects on pHi and basolateral membrane potential of altering either [HCO₃⁻]₄ or [Na⁺]₄ are largely blocked by 4-acetamido-4-isothiocyanostilbene-2,2'-disulphonate (SITS), they are not affected by removal of Cl⁻, nor are there accompanying changes in aNa⁺ consistent with a tight linkage between Cl⁻ fluxes and those of Na⁺ and HCO₃⁻. The aforementioned changes are apparently mediated by a single transport system, not involving Cl⁻. We conclude that HCO₃⁻ transport is restricted to the basolateral membrane, and that HCO₃⁻ fluxes are linked to those of Na⁺. The data are compatible with an electrogenic Na/HCO₃ transporter that carries Na⁺, HCO₃⁻, 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 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

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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 HC03 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 HC03. Until now, the most direct evidence for basolateral HC03 transport came from the studies of Frömter and his colleagues (Frömter, 1975; Burckhardt and Frömter, 1980), who inferred a HC03 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-HC03 buffers.

The aforementioned studies, however, have not provided unambiguous evidence for basolateral HC03 transport. We now report a direct study of basolateral HC03 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 HC03 transport that is confined to the basolateral membrane. Furthermore, this basolateral HC03 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).
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,
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 HCO₃⁻ transport. Thus, the reference solution for most of these experiments was standard HCO₃⁻ Ringer (solution 7). As noted in Table II of the preceding paper, when HEPES Ringer (solution 1) is replaced by standard HCO₃⁻ Ringer, the steady state pHᵢ falls, and the steady state basolateral membrane potential (Vₑ) becomes more positive. This transition is illustrated in Fig. 1A. The application of HCO₃⁻ Ringer causes an abrupt decrease of pHᵢ, because of the influx of CO₂, its hydration to H₂CO₃, and the subsequent dissociation to H⁺ plus HCO₃⁻. 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 HCO₃⁻ Ringer, the fall in pHᵢ is sustained. This failure of pHᵢ to recover indicates that some HCO₃⁻- 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 HCO₃⁻ and/or a related species.

The application of HCO₃⁻ Ringer also produces changes in Vₑ, 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 HCO₃⁻ or an equivalent species. As CO₂ enters the cell and generates intracellular HCO₃⁻, this diffusion potential relaxes to a value more positive than Vₑ. 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 HCO₃⁻ diffusion potential together with possible changes in other pH₁-sensitive ionic conductances.

**Figure 1.** Effect of CO₂-containing Ringer and of low extracellular pH. A. Transition from HCO₃⁻-free to HCO₃⁻-containing Ringer. V₁ refers to basolateral membrane potential, and V₅ 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 HCO₃⁻-free Ringer buffered with HEPES to pH 7.5 (solution 1). At the indicated time, the luminal and basolateral solutions were replaced with Ringer of the same pH, but buffered with 10 mM HCO₃⁻/1.5% CO₂ in O₂ (solution 7). This is one of 10 similar experiments, each on a separate tubule. B. Basolateral or luminal acidification in the absence of HCO₃⁻. During the indicated intervals, the pH 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 HCO₃⁻. During the indicated intervals, the pH of either the basolateral or luminal solution was reduced from 7.6 to 6.8 by reducing [HCO₃⁻] from 10 to 2 mM at a constant CO₂ 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 pH₁ changes that accompany the replacement of HEPES buffer with a HCO₃⁻ buffer: (a) the influx of CO₂, (b) first the influx and then the efflux of HCO₃⁻, 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 (pHb) and basolateral [HCO3] ([HCO3]b) at constant pCO2 while monitoring concomitant changes in pHi, intracellular Cl- activity (aCl), intracellular Na+ activity (aNa), V1, and transepithelial voltage (V3). 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]b is reduced. This electrogenic Na/HCO3 transporter would mediate the opposite movements when [HCO3]b 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+]b 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 V1 on pHb, aNa, and aCl. 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 pHb 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 pHb or pHi is restored to 7.5, pHi recovers (Fig. 1B). With the tubule bathed in HCO3-containing Ringer (Fig. 1C), lowering pHb to 6.8 (i.e., lowering [HCO3]b 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 $1.73 \pm 0.09 \text{ min}^{-1}$. Reducing luminal 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 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 Na-H exchange. The contribution of Na-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* |
|----------------------------|
| $\downarrow [\text{HCO}_3^-]_b$ | $\uparrow [\text{HCO}_3^-]_b$ | $\downarrow [\text{Na}^+]_b$ | $\uparrow [\text{Na}^+]_b$ |
| (a) $\text{pH}_i$ | $\downarrow$ | $\uparrow$ | $\downarrow$ | $\uparrow$ |
| (b) $\Delta \text{V}_i$ | $\downarrow$ | $\uparrow$ | $\downarrow$ | $\uparrow$ |
| (c) $\Delta \text{V}_i$ | $+$ | $-$ | $+$ | $-$ |
| (d) $\Delta \text{V}_i$ | $0$ | $0$ | $0$ | $0$ |

* $\uparrow = \text{increase}, \downarrow = \text{decrease}, 0 = \text{no change}, + = \text{depolarization}, - = \text{hyperpolarization}.$
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_{b} 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.\footnote{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 to explain the observed basolateral depolarization, a more complete model of the basolateral membrane is needed.} Although other ions (e.g., phosphate,
NaCO₃, or organic weak acids and bases) undergo large fractional changes in concentration during alterations in pHb, their concentrations are too low to influence Vl 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]b is lowered.

When pHb is returned to 7.5 in HCO₃ Ringer (Fig. 1C) there is a triphasic shift in Vl 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]b 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 pH-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 Vl, though much smaller, and may reflect changes at the basolateral membrane, as expected for a leaky epithelium.

(d) Contribution of Na-H exchange to pH changes. Although the above pHb and Vl changes, taken together, can only be accounted for by the hypothesized Na/HCO₃ transporter, the pHb 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 pHb recovery that follows restoration of [HCO₃]b and pHb to normal. The tubule is exposed to HCO₃ Ringer throughout. During six separate intervals, pHb and [HCO₃]b are simultaneously lowered (solution 9) and then restored. During the first two restorations of normal pHb and [HCO₃]b, the recoveries of pHb are quite rapid (rate constants, 1.94 and 2.70 min⁻¹). During the third interval of basolateral acidification, after pHb 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 pHb, which may be due to amiloride's acting as a weak base. When pHb is subsequently returned to 7.5, pHb recovers at a lower rate (1.30 min⁻¹) than in the two previous controls and in the succeeding to account for the observed depolarization, we calculated a Vl from the equation of Goldman (1943) and Hodgkin and Katz (1949). In pH 7.5 Ringer, [K⁺]b = 2.5, [K⁺]l = 100, [HCO₃]b = 10, and [HCO₃]l = 6.3 mM (calculated on the basis of pHb = 7.3). Assuming that K⁺ and HCO₃ are the sole permeant ions and, moreover, that PHCO₃ is even as large as PK, the Goldman equation predicts a Vl of ~64 mV, not far from the mean control value of ~56 mV (Boron and Boulpaep, 1983). When [HCO₃]b is now suddenly reduced to 2 mM, the predicted Vl changes by only 2 mV to ~62 mV. This is a small fraction of the observed, initial depolarization. Thus, unless the reduction of pH simultaneously produces an extraordinary increase in PHCO₃, the shift of EHCO₃ could not account for the large shift of Vl.
one. During the fifth basolateral acidification, the amiloride test is repeated, and the pHi 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 pHi 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 HCO₃⁻ (or an equivalent species) mediated by the hypothetical Na/HCO₃ transporter.

**INHIBITION BY SITS** The results of the previous section suggest that the movement of HCO₃⁻ (or of a related species) may occur via a Na/HCO₃ 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 [HCO₃]b 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 pHb and hyperpolarizes the basolateral membrane. After a 10-min pretreatment with SITS, the pHb, V₁, and V₃ changes elicited by reduction of [HCO₃]b and pHb are greatly attenuated. In the presence of SITS the changes in pHb and the initial changes in V₁ closely resemble those described above in the absence of HCO₃⁻ (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 \( V_1 \) may be pH₃ 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. \( V_1 \) and \( V_3 \) represent basolateral membrane potential and transepithelial potential, respectively. During the indicated three intervals, pH₃ was lowered from 7.5 to 6.8 by reducing \([\text{HCO}_3^-]\)₃ 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 exchanger or by a Na/HCO₃-Cl/H exchanger. The possibility of a linkage of Cl⁻ to HCO₃⁻ is raised by the observation that the steady state \( a_{\text{Cl}} \) is higher in the nominal absence than in the presence of HCO₃⁻ (Boron and Boulpaep, 1983; Guggino et al., 1982).
(a) pH$_i$ changes. In the experiment of Fig. 4, the effect of basolateral acidification is tested in the absence of Cl$^-$ (HCO$_3^-$ present throughout). The tubule is first subjected to a basolateral acidification in the presence of Cl$^-$, which produces the usual changes in pH$_i$ and $V_1$. Removal of Cl$^-$ from the bath and lumen (solution 11; Cl$^-$ replaced by cyclamate) causes pH$_i$ to slowly decrease by $\sim$0.2. If HCO$_3^-$-Cl or Na/HCO$_3^-$-Cl/H exchange were a major pathway for basolateral HCO$_3^-$ flux, Cl$^-$ removal should instead have raised pH$_i$. After 10 min in Cl-free Ringer (a period sufficient to remove most intracellular Cl$^-$; see Fig. 5), reducing pH$_b$ and [HCO$_3^-$]$_b$ has about the same effect on pH$_i$ as under control conditions. Returning pH$_b$ and [HCO$_3^-$]$_b$ to normal causes a pH$_i$ recovery which is about as rapid ($k = 2.59$ and $2.23 \text{ min}^{-1}$) as in the presence of Cl$^-$ ($k = 2.55 \text{ min}^{-1}$). In six paired experiments on three tubules, the mean rate constant in Cl-containing solutions was $2.07 \pm 0.26 \text{ min}^{-1}$, not significantly different from $1.91 \pm 0.28 \text{ min}^{-1}$, the value in
Cl\textsuperscript{-}-free solutions (paired t test, \(P = 0.33\)). The experiment of Fig. 4 has been repeated while replacing Cl\textsuperscript{-} with either glucuronate (solution 11) or SO\textsubscript{4}\textsuperscript{2-} (solution 12), with similar results. These data indicate that most of the movement of HCO\textsubscript{3}\textsuperscript{-} (or an equivalent species) and the movement of charge across the basolateral membrane are not dependent on Cl\textsuperscript{-}. We cannot, however, rule out a small component of HCO\textsubscript{3}\textsuperscript{-}-Cl\textsuperscript{-} exchange.

(b) \textit{Voltage changes.} The removal of Cl\textsuperscript{-} (replaced with cyclamate) causes a basolateral hyperpolarization, as observed by others (Anagnostopoulos and Planelles, 1979; Guggino et al., 1982) when Cl\textsuperscript{-} is removed in solutions of near-constant Ca\textsuperscript{2+} activity. When Cl\textsuperscript{-} 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\textsuperscript{-} removal. These two results are consistent with a low basolateral Cl\textsuperscript{-} conductance.\textsuperscript{2} In contrast, a high paracellular Cl\textsuperscript{-} conductance

\footnote{The sign of the \(V_1\) change upon Cl\textsuperscript{-} removal is opposite to that expected of a membrane with a high Cl\textsuperscript{-} conductance. In addition, the magnitudes of the basolateral acidification-induced depolarizations are the same with and without Cl\textsuperscript{-}. Because the decreases in pH\textsubscript{b} and therefore the amount of current carried by the hypothesized Na/HCO\textsubscript{3} transporter, are the same in the two cases, the basolateral-membrane resistance must also have been the same with and without Cl\textsuperscript{-}. Hence, Cl\textsuperscript{-} conductance is low.}
is indicated by the transepithelial hyperpolarization caused by removal of Cl\(^{-}\), as previously noted (Sackin and Boulpaep, 1981a). The larger basolateral-acidification-induced \(V_3\) 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.

\((c)\) \(a_{\text{Cl}}^i\) changes. As a final test for the involvement of Cl\(^{-}\), we monitored changes in \(a_{\text{Cl}}^i\) during four periods in which pH\(_b\) and [HCO\(_3\)]\(_b\) were lowered in HCO\(_3\) Ringer (Fig. 5). Basolateral acidification produces an abrupt 1–2 mM increase in \(a_{\text{Cl}}^i\), followed a slower increase of another ~2 mM over 5 min. When pH\(_b\) and [HCO\(_3\)]\(_b\) are returned to normal, \(a_{\text{Cl}}^i\) 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 \(a_{\text{Cl}}^i\) to fall to ~5 mM. In seven such experiments, the minimal apparent \(a_{\text{Cl}}^i\) 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 \(a_{\text{Cl}}^i\) changes of Fig. 5 with the pH\(_i\) changes of Figs. 1–4, it is important to note that when [HCO\(_3\)]\(_b\) and pH\(_b\) are reduced, the slow phase of the \(a_{\text{Cl}}^i\) increase has a much longer time course than the fall in pH\(_i\). Thus, this slow phase of the \(a_{\text{Cl}}^i\) rise is probably not caused by the same mechanism responsible for the rapid fall of pH\(_i\). It may be due to another HCO\(_3\)-linked transporter or to a change in pH\(_i\)-sensitive Cl\(^{-}\) transport. As for the rapid phase of the \(a_{\text{Cl}}^i\) increase, its magnitude is far too small to account for the observed fall in pH\(_i\) on the basis of either a one-for-one Cl-HCO\(_3\) exchange or a Na/HCO\(_3\)-Cl/H exchange (which is equivalent to one Cl\(^{-}\) for two HCO\(_3\)).

**Involvement of Na\(^{+}\)** In the experiment of Fig. 6, we monitored \(a_{\text{Na}}^i\) while simultaneously reducing pH\(_b\) from 7.5 to 6.8 and [HCO\(_3\)]\(_b\) from 10 to 2 mM. During both basolateral acidifications of Fig. 6A, \(a_{\text{Na}}^i\) declines with about the same course as the fall in pH\(_i\) that occurs under these conditions (see Figs. 1–4). As the basolateral acidification is maintained, \(a_{\text{Na}}^i\) gradually recovers. The initial fall of \(a_{\text{Na}}^i\) 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 \(V_1\) and \(V_3\). However, we would expect this to lead to a monotonic fall of \(a_{\text{Na}}^i\) as the declining Na\(^{+}\) pump rate once again comes into balance with the reduced Na\(^{+}\) influx. The biphasic nature of the fall in \(a_{\text{Na}}^i\) is better explained by a sudden, transient exit of Na\(^{+}\), which is somehow linked to the reduction of either [HCO\(_3\)]\(_b\), or pH\(_b\). It is unlikely that a pH\(_i\)-sensitive

\(^3\) Taking the intrinsic intracellular buffering power (\(\beta_i\)) as 36 mM (Boron and Boulpaep, 1983) and the CO\(_2\) buffering power (\(\beta_{\text{CO}_2}\)) as 2.3 ([HCO\(_3\)]\(_i\)) (see Roos and Boron, 1981) or 9 mM, the total intracellular buffering power (\(\beta_T = \beta_i + \beta_{\text{CO}_2}\)) comes to 45 mM. Thus, a pH\(_i\) decrease of 0.4 corresponds to the net exit of \(45 \times 0.4 = 18\) mmol HCO\(_3\) (or an equivalent species) per liter intracellular fluid, substantially greater than the observed increment in \(a_{\text{Cl}}^i\). Since this comparison neglects the possible contribution of other Cl\(^{-}\) pathways for regulating \(a_{\text{Cl}}^i\), the slow rise in \(a_{\text{Cl}}^i\) of Fig. 5 may underestimate the Cl\(^{-}\) influx mediated by Cl-HCO\(_3\) exchange or Na/HCO\(_3\)-Cl/H exchange.
Na-K pump would exhibit a biphasic response in view of the monotonic fall in pH. We suspect that the sudden exit of Na⁺ is caused by the hypothesized Na/HCO₃ transporter. A portion of the recovery of aN⁺ may be due to stimulation of luminal Na-H exchange, secondary to the fall in pH. Restoring pHb to 7.5 produces an overshoot of aN⁺, followed by a gradual return of aN⁺ to its initial value. The overshoot of aN⁺ is probably caused by a rapid and transient entry of Na⁺, associated with the simultaneous, basolateral entry of HCO₃⁻. Subsequently, aN⁺ 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 aN⁺ transients. During the first period of basolateral acidification, in the absence of SITS, the usual changes in aN⁺ are observed. With the addition of SITS to the basolateral solution, however, basolateral acidification produces no change in aN⁺. We do not show here an experiment in which we found that removal of Cl⁻ has no effect on these changes in aN⁺. The fall of aN⁺ 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 pH 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 pH 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 pH, 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 pH in virtue of blocking basolateral Na-H exchange. Similarly, returning basolateral Na⁺ should restore basolateral Na-H exchange and thereby return pH to normal. However, changes in the basolateral Na-H exchange rate are unlikely to explain fully the pH changes elicited by altering [Na⁺]b. First, the fall in pH 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 pH 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 pH changes must be mediated in part by
another mechanism, presumably the electrogenic Na/HCO₃ transporter, in accord with prediction e of Table II.

(b) Absence of luminal Na/HCO₃ transport. The difference between the pHᵢ changes produced by basolateral vs. luminal Na⁺ removal indicate a lack of a substantial Na/HCO₃ flux across the luminal membrane. In Fig. 7B, the modest fall of pHᵢ 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, 1981b), may enhance basolateral Na/HCO₃ efflux. The subsequent pHᵢ recovery (i.e., preceding basolateral Na⁺ removal) may be due to increased basolateral Na-H exchange secondary to the fall of aᵣNa, as well as to a slowing or reversal of basolateral Na/HCO₃ transport secondary to decreases of pHᵢ and aᵣNa.

(c) Electrogenic nature of Na/HCO₃ 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 HCO₃⁻, 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 V₁.

An alternative explanation for the changes in V₁ produced by these alterations of [Na⁺]ᵣ is that the V₁ 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 V₁ 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 aᵣNa, 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/HCO₃ efflux is sensitive to the Na⁺ electrochemical gradient.

INHIBITION BY SITS Fig. 9 illustrates the effect of SITS on the pHᵢ and V₁ 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

**Figure 6.** (opposite) A. Intracellular Na⁺ activity during basolateral acidification. V₁ and V₃ represent basolateral membrane potential and transepithelial potential, respectively. Twice pHᵢ was lowered from 7.5 to 6.8 by lowering [HCO₃⁻] from 10 to 2 mM at constant CO₂ 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.

![Fig. 7](image)

**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\(_3\)]. 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 \(d_i\)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/HCO$_3$ transporter.

Figure 8. Effect of ouabain on basolateral membrane potential changes induced by changes in basolateral [Na$^+$]. $V_1$ and $V_3$ 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 $a_{Na}^+$ 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/HCO$_3$ exit, that of Fig. 10 is designed to determine whether SITS also inhibits the Na/HCO$_3$ entry. Removing Na$^+$ first from the lumen and then from the bath causes the usual changes in pH$_i$ (see Fig. 7), followed by a rapid pH$_i$ 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 pH$_i$ 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 pH\(_i\) recovery in the absence of SITS (\(k_{HCO_3}\)) is about twice as great as in the presence of SITS (\(k_{SR_3}\)). This difference is statistically significant. The value of \(k_{SR_3}\) 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 pH\(_i\) recovery in the presence of SITS is mediated by basolateral Na-H exchange alone, whereas the pH\(_i\) recovery in the absence of SITS is mediated by two parallel basolateral events, Na-H exchange and the electrogenic Na/HCO\(_3\) influx.

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

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**Figure 9.** Effect of SITS on changes induced by changes in basolateral [Na\(^+\)]. \(V_1\) and \(V_3\) represent basolateral membrane potential and transepithelial potential, respectively. In the first portion of the experiment, basolateral Na\(^+\) was removed in the usual way (replaced by BDA\(^+\)). The gap represents an interval of 38 min. Basolateral SITS (0.5 mM) was applied 33 min before the second period of Na\(^+\) removal. pH\(_b\) and pH\(_i\) were 7.5 throughout. Note that 100 mM Na\(^+\) was present in the lumen. A total of 15 experiments similar to the ones in this figure and Fig. 10 was performed on 14 separate tubules.
luminal Na\(^+\), and thus of luminal Na-H exchange, in Fig. 9. The presence or absence of Na\(^+\) in the lumen may also account\(^4\) for the discrepancies in voltage changes between Figs. 9 and 10.

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 pH\(_i\) recovery, whereas in Fig. 9 there is none. The hyperpolarization of Fig. 10 is probably related to the simultaneous recovery of pH\(_i\), and was also observed in the terminal portion of Fig. 5 in the preceding paper (Boron and Boulpaep,
Involvement of \( \text{HCO}_3^- \) The electrogenic Na/\( \text{HCO}_3^- \) hypothesis predicts that the basolateral [\( \text{Na}^+ \)] effect should depend on \( \text{HCO}_3^- \). In the first half of Fig. 11, simultaneous removal of luminal and basolateral \( \text{Na}^+ \) in \( \text{HCO}_3^- \)-free Ringer causes \( \text{pH}_i \) to fall slowly by nearly 0.40 over a 10-min period. This decrease is probably due to abolition of \( \text{Na}-\text{H} \) exchange and/or reversal of the \( \text{Na}-\text{H} \) exchangers. Reintroduction of basolateral \( \text{Na}^+ \) causes \( \text{pH}_i \) to recover with a rate constant of 0.37 min\(^{-1}\), probably because of basolateral \( \text{Na}-\text{H} \) exchange alone. The failure of \( \text{pH}_i \) to return to its initial value probably reflects a rather high rate of metabolic acid production in this tubule. The basolateral addition of \( \text{Na}^+ \) in \( \text{HCO}_3^- \)-free Ringer also causes a slow, sustained hyperpolarization, in contrast to the hyperpolarizing \( V_1 \) spike normally seen in \( \text{HCO}_3^- \) Ringer (Fig. 7), but similar to the sustained hyperpolarization seen with SITS in \( \text{HCO}_3^- \) Ringer (Fig. 10). The subsequent readdition of luminal \( \text{Na}^+ \) causes a further recovery of \( \text{pH}_i \). The transient basolateral depolarization produced by reapplication of \( \text{Na}^+ \) to the lumen probably reflects a luminal \( \text{Na}^+ \) conductance.

In the second half of the experiment of Fig. 11, the \( \text{HCO}_3^- \)-free Ringer is replaced with standard \( \text{HCO}_3^- \) Ringer, leading to the usual sustained fall in \( \text{pH}_i \) and the depolarization of \( V_1 \). Simultaneous removal of luminal and basolateral \( \text{Na}^+ \) now leads to a fall in \( \text{pH}_i \), which, when compared with the sequence in \( \text{HCO}_3^- \)-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

### Table III

| Tubule      | \( k_{\text{HCO}_3^-} \) | \( k_{\text{SITS}} \) | \( k_{\text{HCO}_3^-}/k_{\text{SITS}} \) |
|-------------|----------------|----------------|----------------|
| 080180A     | 1.28           | 1.00           | 1.28           |
| 080180B     | 2.39           | 1.10           | 2.17           |
| 080180C     | 2.13           | 1.08           | 1.97           |
| 080580A     | 1.33           | 0.41           | 3.24           |
| 080580B     | 1.40           | 0.84           | 1.67           |
| 080680C     | 1.34           | 0.75           | 1.79           |
| 080780A     | 1.46           | 0.59           | 2.46           |
|             | 1.62±0.17      | 0.82±0.10      | 2.01           |

*\( k_{\text{HCO}_3^-} \) is the control rate constant in \( \text{HCO}_3^- \) Ringer; \( k_{\text{SITS}} \) is the rate constant in \( \text{HCO}_3^- \) Ringer containing 0.5 mM SITS in the bath. One rate constant for each was measured per tubule. The mean \( k_{\text{HCO}_3^-} \) is significantly different from the mean \( k_{\text{SITS}} \) (paired t test; \( P < 0.0004 \)). The mean \( k_{\text{HCO}_3^-}/k_{\text{SITS}} \) was obtained assuming a log-normal distribution. Luminal solutions were all \( \text{Na}^- \) free so that the \( \text{pH}_i \) 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](image-url)

**Figure 11.** Dependence on HCO₃⁻ of pHᵢ recovery accompanying restoration of [Na⁺]₀ 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⁺]₀ 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 (kₜHEPES) vs. HCO₃⁻-containing (kₜHCO₃) Ringer. The paired difference between kₜHEPES and kₜHCO₃ 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 $k_{\text{HCO}_3}/k_{\text{HCO}_3}$ ratio of Table III, 2.01, and provides further support for the hypothesis that the pH$_i$ recovery produced by the reintroduction of basolateral Na$^+$ in HCO$_3^-$ Ringer is mediated by two parallel processes, basolateral Na-H exchange and basolateral Na/HCO$_3$ uptake.

**Lack of Cl$^-$ Involvement** The hypothesis of the electronegic Na/HCO$_3$ transporter predicts that the changes of pH$_i$ induced by alterations of [Na$^+$]$_b$ should not be affected by removal of Cl$^-$ (Table II, prediction j), and should not be accompanied by changes of act (prediction h). These predictions were tested in two series of experiments. In the first (Fig. 12), we tested whether Cl$^-$ is necessary for the pH$_i$ changes that normally occur as [Na$^+$]$_b$ is altered. The first and third basolateral removals of Na$^+$ produce the same effects as pointed out for Fig. 7A. Readdition of basolateral Na$^+$ causes pH$_i$ to recover with rate constants of 0.70 and 0.47 min$^{-1}$, respectively. When Cl$^-$ was removed from both the bath and lumen, the rate constant of the pH$_i$ recovery upon readdition of 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 pH$_i$ recovery in the presence ($k_{\text{HCO}_3}$) and in the absence

| Tubule   | $k_{\text{HCO}_3}$ | $k_{\text{HCO}_3}$ | Corrected $k_{\text{HCO}_3}/k_{\text{HEPES}}$ |
|----------|---------------------|---------------------|-----------------------------------------------|
|          |                     |                     |                                               |
| 081380A  | 2.49±0.25 (4)       | 0.94±0.46 (2)       | 3.31                                          |
| 081480A  | 3.08                | 2.06                | 1.87                                          |
| 081480B  | 1.23                | 1.30                | 1.18                                          |
| 082080A  | 1.16±0.18 (3)       | 0.54                | 2.69                                          |
| 082180A  | 0.94±0.05 (4)       | 0.44±0.03 (4)       | 2.67                                          |
| 082180B  | 1.33±0.03 (2)       | 0.77±0.24 (2)       | 2.16                                          |
(k_0, Cl) of Cl^- were not significantly different, which suggests that Cl^- does not participate in the operation of the electronegic Na/HCO_3 transporter.

In a second series of experiments we monitored a_i^{Cl} 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 a_i^{Cl} to rise rather rapidly over the first ~40 s, and then more slowly over the duration of the basolateral

Na^+ removal. The initial rate of a_i^{Cl} rise (i.e., over the first 40 s) is 2.1 mM/min. This contrasts with an initial equivalent HCO_3^- 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 pH_i fall to be complete (Fig. 9), a_i^{Cl} rises by a total of 3.9 mM. In this same time interval in Fig. 9, the equivalent loss of HCO_3^- 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, $a_{Cl}^i$ 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 difference in the overall time course of $a_{Cl}^i$ and pH$_b$, the basolateral equivalent HCO$_3^-$ flux cannot all be linked to Cl$^-$. The subsequent return of Na$^+$ to the basolateral solution causes $a_{Cl}^i$ to return to about its initial value over a period.

### Table V

| Tubule   | $k_{HCO_3}$ | $k_{HCO_3}$ | $k_{Cl}/k_{HCO_3}$ |
|----------|-------------|-------------|---------------------|
| 071481   | 0.51±0.07 (2) | 0.46±0.08 (4) | 0.89                |
| 071581   | 0.53±0.09 (3) | 0.48±0.03 (2) | 0.90                |
| 071681   | 0.74±0.02 (2) | 0.74±0.05 (3) | 1.00                |
| 071781A  | 1.56±0.14 (2) | 1.54±0.04 (2) | 0.98                |
| 071781B  | 1.13±0.17 (1) | 0.75±0.05 (2) | 0.67                |
| 0.91±0.17 | 0.85±0.17 | 0.91            |

* $k_{HCO_3}$ is the rate constant in Cl-containing HCO$_3^-$ Ringer; $k_{Cl}$ is the rate constant in Cl-free HCO$_3^-$ Ringer. The mean $k_{HCO_3}$ is not significantly different from the mean $k_{Cl}$ (paired t test; $P = 0.36$). The mean $k_{Cl}/k_{HCO_3}$ 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 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.
of ~20 min, which indicates once again a discrepancy between the time courses of Cl\(^{-}\) and equivalent HCO\(_3\)\(^{-}\) fluxes, because the pH\(_i\) 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-HCO\(_3\) (or Cl-OH) exchange cannot account for the rise of \(a_{\text{Cl}}\), since the observed fall of pH\(_i\) could only serve to reduce Cl\(^{-}\) influx. (ii) A basolateral, electroneutral Na/HCO\(_3\)-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\(^{+}\)]\(_b\) 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/HCO\(_3\)-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 HCO\(_3\)\(^{-}\) 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 HCO\(_3^-\) 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 HCO\(_3^-\) (or an equivalent species), which is confined to the basolateral membrane. Evidence for a basolateral pathway for HCO\(_3^-\) movement comes from three observations in the experiment of Fig. 1. (i) Application of CO\(_2\)/HCO\(_3^-\) Ringer causes a fall in pH\(_i\) from which the cell fails to recover. Although the CO\(_2\)-induced fall in pH\(_i\) undoubtedly stimulates Na-H exchange, this increased rate of acid extrusion is balanced by the newly established efflux of HCO\(_3^-\) and/or a related species. (ii) Reducing pH\(_b\) causes a much larger fall in pH\(_i\) when the basolateral solution contains HCO\(_3^-\) (at constant pCO\(_2\)) than when it is nominally HCO\(_3^-\) free. (iii) In HCO\(_3^-\) Ringer, reducing pH\(_b\) at constant pCO\(_2\) has a substantially larger effect on pH\(_i\) than reducing pH\(_i\), which indicates that the pathway for HCO\(_3^-\) must be limited to the basolateral membrane.

Although the changes in pH\(_i\) induced by changes in [HCO\(_3^-\)]\(_b\) are indicative of a basolateral pathway for HCO\(_3^-\) (or an equivalent species), this pathway is not the sole contributor to the pH\(_i\) changes in the aforementioned experiments. In fact, the decline in pH\(_i\) produced by lowering [HCO\(_3^-\)]\(_b\) and pH\(_b\) (at constant pCO\(_2\)) 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 HCO\(_3^-\) and/or a related species. Similarly, returning [HCO\(_3^-\)]\(_b\) and pH\(_b\) to normal produces a very rapid recovery of pH\(_i\), which apparently has two major components: (i) an increased rate of basolateral Na-H exchange, and (ii) an influx of HCO\(_3^-\) and/or a related species. The contribution of Na-H exchange is clearly demonstrated by the inhibitory effect of amiloride on the pH\(_i\) recovery (see Fig. 2). The component of the pH\(_i\) recovery remaining after application of amiloride, which largely blocks Na-H exchange, is presumably due to basolateral HCO\(_3^-\) transport.

The transport of HCO\(_3^-\) (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) pH\(_i\) falls when [HCO\(_3^-\)]\(_b\) is lowered from 10 to 2 mM (i.e., pH\(_b\) is lowered from 7.5 to 6.8), and rises when the reverse solution change is made. (ii) \(a_{\text{Na}}\) falls when [HCO\(_3^-\)]\(_b\) is lowered, and rises when the reverse solution change is made. (iii) The basolateral membrane depolarizes, usually transiently, upon reducing [HCO\(_3^-\)]\(_b\), and hyperpolarizes, always transiently, upon restoring [HCO\(_3^-\)]\(_b\) to a normal level. (iv) There are only small changes in \(a_{\text{Cl}}\) when [HCO\(_3^-\)]\(_b\) is altered. (v) pH\(_i\) falls when [Na\(^+\)]\(_b\) is reduced to zero and rises when [Na\(^+\)]\(_b\) is raised to normal. (vi) \(a_{\text{Na}}\) correspondingly falls and rises when [Na\(^+\)]\(_b\) is altered. (vii) The basolateral membrane transiently depolarizes upon reduction of [Na\(^+\)]\(_b\) and transiently hyperpolarizes upon restoration of [Na\(^+\)]\(_b\). (viii) Changes in \(a_{\text{Cl}}\) induced by alterations in [Na\(^+\)]\(_b\) appear not to be directly related to the aforementioned changes in pH\(_i\), \(a_{\text{Na}}\), or \(V_i\). (ix) The aforementioned changes in pH\(_i\), \(a_{\text{Na}}\), and \(V_i\) 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 $[\text{HCO}_3^-]$ is reduced (as in Fig. 1C), and the final three pertain to the experiment in which $[\text{Na}^+]$ is reduced (as in Fig. 7A): (i) the standard control condition, (ii) at the instant $[\text{HCO}_3^-]$ is reduced from 10 to 2 mM, (iii) at the instant $[\text{HCO}_3^-]$ is restored to 10 mM, (iv) the new control condition in which $[\text{Na}^+]$ is 0 mM, (v) at the instant $[\text{Na}^+]$ is reduced from 100 to 0 mM, and (vi) at the instant $[\text{Na}^+]$ 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$_i$ 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 \( V_i \) as well as the \( V_i \) changes induced by alterations of \([\text{HCO}_3^-]_b\) are not consistent with a high partial conductance for \( \text{HCO}_3^- \).

(b) \( \text{Cl}-\text{HCO}_3^- \) exchange. As shown in the Appendix, an electroneutral \( \text{Cl}-\text{HCO}_3^- \) 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 \( V_i \) induced by altering either \([\text{HCO}_3^-]_b\) or \([\text{Na}^+]_b\). Although we cannot rule out a small component of \( \text{Cl}-\text{HCO}_3^- \) exchange, we conclude that this process cannot by itself account for our data.

(c) \( \text{Na}-\text{H} \) exchange in parallel with \( \text{Cl}-\text{HCO}_3^- \) 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 \( \text{Na}-\text{H} \) has been identified in this preparation (Boron and Boulpaep, 1983).

Inasmuch as several investigators have suggested that electroneutral \( \text{NaCl} \) entry at the luminal membrane is mediated by parallel \( \text{Na}-\text{H} \) and \( \text{Cl}-\text{HCO}_3^- \) exchangers, it is instructive to analyze the thermodynamic predictions of such a hypothesis. The free-energy changes for luminal \( \text{Na}-\text{H} \) and \( \text{Cl}-\text{HCO}_3^- \) 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 \( \text{Cl}^- \) entry would occur at the luminal membrane. Thus, the parallel exchange model can at least qualitatively account for luminal \( \text{NaCl} \) uptake. Only in the fortuitous case in which overall transport is isohydric, however, would \( \text{Na}^+ \) and \( \text{Cl}^- \) move in equimolar amounts. The results of two additional experiments, performed by others on the \textit{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, \( a^{\text{Cl}} \) and \( a^{\text{Na}} \) both fall. This linkage between \( \text{Cl}^- \) and \( \text{Na}^+ \) could be achieved in any of four ways: \( i \) a direct linkage at the luminal membrane (e.g., \( \text{NaCl} \) cotransport), \( ii \) an indirect linkage at the luminal membrane (e.g., parallel \( \text{Na}-\text{H} \) and \( \text{Cl}-\text{HCO}_3^- \) 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 \( a^{\text{Cl}} \) and \( a^{\text{Na}} \) 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 \( \text{Cl}-\text{HCO}_3^- \) exchanger (\( \text{Cl}^- \) out, \( \text{Na}^+ \) in), but could not reverse the \( \text{Na}-\text{H} \) exchanger (\( \text{Na}^+ \) out, \( H^+ \) in) unless \( pH_i \) would rise above 7.99. Although we have yet to lower \([\text{Cl}^-]_i \) in the lumen only, simultaneously removing \( \text{Cl}^- \) from lumen and bath caused \( pH_i \) to fall or remain unchanged. Conversely, lowering \([\text{Na}^+]_i \) would reverse a \( \text{Na}-\text{H} \) exchanger, but could not reverse a \( \text{Cl}-\text{HCO}_3^- \) exchanger unless \( pH_i \) would fall below 6.85. Fig. 7B shows that luminal \( \text{Na}^+ \) removal causes
only a transient fall of pH by 0.1 to a value of ~7.2; the steady state pH is unchanged. Possibility iii also cannot explain the data. It predicts that lowering [Cl\textsuperscript{−}]\textsubscript{i} should reduce a\textsuperscript{Cl}\textsubscript{i} but raise a\textsuperscript{Na}\textsubscript{i}, and that lowering [Na\textsuperscript{+}]\textsubscript{i} should lower a\textsuperscript{Na}\textsubscript{i} but raise a\textsuperscript{Cl}\textsubscript{i}. Such increases in a\textsuperscript{Na}\textsubscript{i} and a\textsuperscript{Cl}\textsubscript{i} have not been observed. These data do not rule out parallel Na-H and Cl-HCO\textsubscript{3}\textsuperscript{−} exchangers at the luminal membrane. However, they indicate that the simultaneous reversal of such exchangers cannot account for the decreases of a\textsuperscript{Cl}\textsubscript{i} and a\textsuperscript{Na}\textsubscript{i} observed when either [Cl\textsuperscript{−}]\textsubscript{i} or [Na\textsuperscript{+}]\textsubscript{i} is reduced.

(d) Na/\text{HCO}_{3}\textsuperscript{−}-Cl/H exchange. In squid axons (Russell and Boron, 1982), snail neurons (Thomas, 1977), and barnacle muscle (Boronet al., 1979, 1981), pH\textsubscript{i} is regulated by a system that exchanges external HCO\textsubscript{3}\textsuperscript{−} and Na\textsuperscript{+} for internal Cl\textsuperscript{−} (and possibly H\textsuperscript{+}). The stoichiometry is one Na\textsuperscript{+} entering for each Cl\textsuperscript{−} 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\textsuperscript{−}. We recognize that the transporter could have such a high affinity for Cl\textsuperscript{−} that it could be difficult to demonstrate a Cl\textsuperscript{−} dependence, and that a\textsuperscript{Cl}\textsubscript{i} could be so well regulated by other transport systems that large changes of a\textsuperscript{Cl}\textsubscript{i} would not occur. However, even if the interpretation of the Cl\textsuperscript{−} data were in error, this electroneutral model would still fail to explain the voltage changes. We conclude that an Na/\text{HCO}_{3}\textsuperscript{−}-Cl/H exchanger cannot by itself account for our data, though we cannot rule out the participation of such an exchanger in the a\textsuperscript{Cl}\textsubscript{i} shifts.

(e) Electrogenic Na/\text{HCO}_{3}\textsuperscript{−} transport. Fig. 14 lists four thermodynamically indistinguishable variants that would result in the equivalent of electrogenic Na/\text{HCO}_{3}\textsuperscript{−} transport. Although the stoichiometry predicted by these models is equivalent to two HCO\textsubscript{3}\textsuperscript{−} for each Na\textsuperscript{+}, we emphasize that our data only require that the ratio of net fluxes of HCO\textsubscript{3}\textsuperscript{−} to Na\textsuperscript{+} be greater than unity. Models e1–e4 are the same as d1–d4, except for the lack of Cl\textsuperscript{−} involvement. As described in the Appendix, models e1–e4 account for all the a\textsuperscript{Na}\textsubscript{i} and pH\textsubscript{i} data for the six experimental conditions. The models also account for all \textit{V}_\textit{i} transients that follow changes in [HCO\textsubscript{3}\textsuperscript{−}]\textsubscript{b} and [Na\textsuperscript{+}]\textsubscript{b}, as well as the depolarization accompanying application of CO\textsubscript{2}/HCO\textsubscript{3}\textsuperscript{−} Ringer.

The reversal potential for the electrogenic Na/\text{HCO}_{3}\textsuperscript{−} transport system is the equilibrium potential for the anion pair in model e4, \(E_{NaCO_3}\):

\[
E_{NaCO_3} = \frac{RT}{F} \ln \left( \frac{[Na^+]_i \cdot [HCO_3^-]^2}{[Na^+]_0 \cdot [HCO_3^-]^2} \right)
\]

The values of \(\Delta G\) described in the Appendix are related to \(E_{NaCO_3}\) by the equation

\[
\Delta G = F(V_1 - E_{NaCO_3})
\]
It is instructive to examine the relationship between $E_{\text{NaHCO}_3}$ and $V_1$ for each of the six experimental conditions described above. (i) In the normal control condition, $E_{\text{NaHCO}_3}$ 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 [HCO$_3^-$]$_b$ is reduced to 2 mM, $E_{\text{NaHCO}_3}$ instantly becomes +29.3 mV, explaining the observed, basolateral depolarization. (iii) When [HCO$_3^-$]$_b$ is subsequently restored to 10 mM, $E_{\text{NaHCO}_3}$ instantly becomes -98.2 mV, which accounts for the observed basolateral hyperpolarization. (iv) In the new control condition (with a [Na$^+$]$_b$ of 0 mM), $E_{\text{NaHCO}_3}$ is -72.2 mV close to the prevailing value of $V_1$ (approximately -70 mV). (v) When [Na$^+$]$_b$ is reduced to 0 mM, $E_{\text{NaHCO}_3}$ instantly approaches $+\infty$, accounting for the abrupt basolateral depolarization. (vi) When [Na$^+$]$_b$ is subsequently returned to 100 mM, $E_{\text{NaHCO}_3}$ 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 [HCO$_3^-$]$_b$ or [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 ~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 $\ell_1$-$\ell_4$. The last

$^6$ The product of this pH$_i$ recovery rate (i.e., 0.014 pH units 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 s$^{-1}$ (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$^3$, based on a morphometric analysis (Maunsbach and Boulpaep, unpublished). Thus, the current flow through the Na/HCO$_3^-$ transporter would be 73.7 pA 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. NaCO₃ 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 [NaCO₃]₀ of only ~25 μM. This would require a carrier of high affinity or a channel of extraordinary permeability.

**Unifying Model for Basolateral Na⁺, HCO₃⁻, and Cl⁻ Transport**

The simultaneous, transcellular reabsorption of HCO₃⁻ and Cl⁻ requires the basolateral efflux of both HCO₃⁻ and Cl⁻. The present study documents an electrogenic Na/HCO₃ transporter capable of high transport rates, and which carries most of the HCO₃⁻ 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⁻-HCO₃ 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 HCO₃ systems are a necessity for the simultaneous basolateral efflux of Cl⁻ and HCO₃⁻. However, the electrogenic Na/HCO₃ transporter shares several characteristics with the postulated Cl⁻ system: (i) involvement of Na⁺, (ii) involvement of HCO₃⁻, 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 k₁ and k₂, represents the hypothesized electrogenic Na/HCO₃ transporter operating in the direction of HCO₃⁻ reabsorption. The large loop, in the direction described by k₁ and k₃, represents the Na/HCO₃-Cl/H exchanger operating in the direction of a classic pHi regulator (i.e., Cl⁻ reabsorption). The lower loop, in the direction described by k₃ and k₂, 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/HCO₃ reabsorption, pure Cl⁻ reabsorption, any combination of the two, or even Cl⁻ reabsorption with the opposite movement of Na/HCO₃. 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 pHi by extruding acid from the cell (Roos and Boron, 1981). The rate of acid extrusion in barnacle muscle approaches zero at pHi values at or above a certain threshold, and gradually increases as pHi 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 $a_{i}^{Na}$ 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 pH$_i$) as the primary event in acid secretion, and the basolateral HCO$_3^-$ efflux merely following to keep pH$_i$ from rising too high. Cited as evidence for this latter view is the observation that pH$_i$ in proximal tubule cells is rather alkaline, ~7.4. The crucial parameter, however, is not the absolute value of the steady state pH$_i$, but rather the degree to which pH$_i$ is below the threshold for activating the pH$_i$-regulating mechanism. We estimate that the pH$_i$ threshold of the proximal tubule cells' Na-H exchanger is at or somewhat above 7.43, the mean pH$_i$ in HEPES Ringer (Boron and Boulpaep, 1983). In the absence of basolateral HCO$_3^-$ efflux, the rate of intracellular acid loading is probably very low in these amphibian cells, and the unopposed pH$_i$-regulating system (i.e., the Na-H exchangers) drives pH$_i$ upward to ~7.43, at which point the Na-H exchangers are, or are nearly, inactive. The normal pH$_i$ of these cells at a physiologic [HCO$_3^-$]$_b$ and pCO$_2$, 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 HCO$_3^-$, which decreases pH$_i$ below the threshold for activating the pH$_i$-regulating mechanism. For the salamander, in which basolateral HCO$_3^-$ transport is mediated by the Na/HCO$_3^-$ transporter (see Fig. 16), basolateral HCO$_3^-$ efflux is also accompanied by a fall in $a^Na$, which may also enhance Na-H exchange. The rate of transcellular acid secretion (i.e., luminal Na-H exchange) is therefore directly regulated by pH$_i$ and, to a certain extent, $a^Na$. We predict that the proximal tubule's rate of transcellular acid secretion should be increased by any treatment which lowers pH$_i$: raising pCO$_2$ (at constant external pH), lowering [HCO$_3^-$]$_b$ (at constant pH$_i$), and selectively inhibiting basolateral Na-H exchange.

**APPENDIX**

*Thermodynamic Analysis of Basolateral HCO$_3^-$ Transport*

The hypothetical movements of the HCO$_3^-$ (or an equivalent species) can be analyzed for six relevant experimental conditions. The first three pertain to the experiment in which [HCO$_3^-$]$_b$ is reduced (see Fig. 1C), and the final three pertain to the experiment in which [Na$^+$]$_b$ is reduced (see Fig. 7A): (i) the control condition, (ii) at the instant [HCO$_3^-$]$_b$ is reduced from 10 to 2 mM, (iii) at the instant [HCO$_3^-$]$_b$ is restored to 10 mM, (iv) the new control condition in which [Na$^+$]$_b$ is 0 mM, (v) at the instant [Na$^+$]$_b$ is reduced from 100 to 0 mM, and (vi) at the instant [Na$^+$]$_b$ is restored to 100 mM. These conditions are summarized in Table VI.

**Conductive Path for HCO$_3^-$** The net free energy change for HCO$_3^-$ as it exits across the basolateral membrane is:

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

where $\Delta G_{\text{net}} < 0$ indicates a net HCO$_3^-$ efflux. In addition, the HCO$_3^-$ flux through an idealized channel can be calculated from the constant field equation (Goldman, 1943;
Hodgkin and Katz, 1949):

$$J_{\text{net}}^{\text{HCtO}_3} = P \frac{V_F}{RT} \frac{[\text{HCtO}_3]_b - [\text{HCtO}_3]}{1 - e},$$

where $J_{\text{net}}^{\text{HCtO}_3}$ is the net $\text{HCtO}_3$ influx; $P$ is the permeability to $\text{HCtO}_3$; $F$, $R$, and $T$ have their usual meanings; and $e = \exp(-V/F/RT)$. (i) Under standard control conditions (see Table VI), $\Delta G_{\text{net}}$ is $-1.9RT$ and $J_{\text{net}}^{\text{HCtO}_3}$ comes to $-14.0$ P (units: $10^{-6}$ mol cm$^{-2}$ s$^{-1}$); the negative signs signify a net efflux. This is in agreement with the sustained fall in pH$_i$, presumably because of the net efflux of $\text{HCtO}_3$ (or an equivalent species), actually observed when $\text{HCtO}_3$-free is replaced with $\text{HCtO}_3$-containing Ringer (see Fig. 1A). (ii) When $[\text{HCtO}_3]_b$ is reduced from 10 to 2 mM, there is an immediate basolateral depolarization of ~20 mV. The instantaneous $\Delta G_{\text{net}}$ is therefore $-2.7RT$, which predicts an increased gradient for $\text{HCtO}_3$ efflux. The calculated, instantaneous $J_{\text{net}}^{\text{HCtO}_3}$, however, is actually reduced somewhat to $-11.7P$. This occurs because $V_1$ appears as an exponential term in the expression for $J_{\text{net}}^{\text{HCtO}_3}$, causing the effect of the depolarization to outweigh that of lowering $a[\text{HCtO}_3]$. Therefore, unless $P$ increases substantially as pH$_b$ is reduced from 7.5 to 6.8, the observed fall in pH$_i$ cannot be explained by an increased passive $\text{HCtO}_3$ efflux. (iii) Just before $[\text{HCtO}_3]_b$ is returned to 10 mM, $a[\text{HCtO}_3]$ is 2.1 mM (assuming that pH$_i$ had previously fallen by 0.35). When $[\text{HCtO}_3]_b$ is raised, $V_1$ instantly hyperpolarizes to about $-60$ mV. The initial $\Delta G_{\text{net}}$ is $-1.1RT$, which predicts a continued net $\text{HCtO}_3$ efflux. The initial

![Diagram 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 mM, the basolateral membrane is hyperpolarized compared with condition i, and $V_t$ will be assumed to be $-70$ 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 $[\text{Na}^+]_o$ is reduced to 0 mM, the basolateral membrane instantly hyperpolarizes by $-40$ mV; $V_t$ is assumed to be $-30$ 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 $p\text{H}_i$ (i.e., efflux of $\text{HCO}_3^-$ or an equivalent species) unless there is a concomitant change in $P$. (vi) When $[\text{Na}^+]_o$ is restored to 100 mM, the basolateral membrane initially hyperpolarizes, usually to beyond $-100$ mV. Assuming $V_t$ is $-100$ mV and $[\text{HCO}_3^-]_b$ is 2.8 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 $p\text{H}_i$. $J_{\text{HCO}_3}$ 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‡

| Model          | (i)  | (ii) | (iii) | (iv)  | (v)  |
|----------------|------|------|-------|-------|------|
|                |     |      |       |       |      |
| (a) $\text{HCO}_3^-$ | $-1.9RT$ | $-2.7RT$ | $-1.1RT$ | $-2.3RT$ | $-0.7RT$ | $-2.7RT$ |
| (b) Cl-$\text{HCO}_3$ | $-1.0$ | $-2.6$ | $0.0$ | $-2.4$ | $+\infty$ | $-4.7$ |
| (c) Na-H       | $-1.6$ | $0.0$ | $-2.6$ | $-1.0$ | $-1.0$ | $0.0$ |
| (d) Na/$\text{HCO}_3$-Cl/H | $-0.6$ | $+2.6$ | $-2.5$ | $-1.4$ | $+\infty$ | $-4.7$ |
| (e) Na/$\text{HCO}_3$-H/H | $-0.3$ | $-2.7$ | $+1.5$ | $-0.1$ | $-\infty$ | $+2.0$ |

*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, v, and vi are made at the instant the external solution change is made.

‡Conditions ii, iii, v, 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.

0 mM, the basolateral membrane instantly hyperpolarizes by $\sim40$ mV; $V_t$ is assumed to be $-30$ 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 $p\text{H}_i$ (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 mM, the basolateral membrane initially hyperpolarizes, usually to beyond $-100$ mV. Assuming $V_t$ is $-100$ mV and $[\text{HCO}_3^-]_b$ is 2.8 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 $p\text{H}_i$. $J_{\text{HCO}_3}$ comes to $-10.4P$, which predicts that the $\text{HCO}_3^-$ efflux should be sizeable.

| Parameters | Parameter values |
|------------|------------------|
| pH_i       | 7.30             |
| pH_b       | 7.50             |
| $a_{\text{HCO}_3}$ | 4.7  |
| $a_{\text{HCO}_3}$ | 4.7  |
| $a_{\text{HCO}_3}$ | 2.1  |
| $a_{\text{HCO}_3}$ | 7.5  |
| $a_{\text{HCO}_3}$ | 7.5  |
| $a_{\text{HCO}_3}$ | 7.5  |
| $a_{\text{HCO}_3}$ | 11  |
| $a_{\text{HCO}_3}$ | 11  |
| $a_{\text{HCO}_3}$ | 20  |
| $a_{\text{HCO}_3}$ | 20  |
| $a_{\text{HCO}_3}$ | 75  |
| $a_{\text{HCO}_3}$ | 75  |
| $a_{\text{HCO}_3}$ | 75  |
| $a_{\text{HCO}_3}$ | 16  |
| $a_{\text{HCO}_3}$ | 16  |
| $a_{\text{HCO}_3}$ | 16  |
| $a_{\text{HCO}_3}$ | 20  |
| $a_{\text{HCO}_3}$ | 71  |
| $a_{\text{HCO}_3}$ | 71  |
| $a_{\text{HCO}_3}$ | 71  |
| $a_{\text{HCO}_3}$ | 71  |
| $a_{\text{HCO}_3}$ | 71  |
| $V_t$       | $-0.060V$         |
| $V_t$       | $-0.040V$         |
| $V_t$       | $-0.060V$         |
| $V_t$       | $-0.070V$         |
| $V_t$       | $-0.030V$         |
| $V_t$       | $-0.100V$         |

* 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, v, and vi are made at the instant the external solution change is made.

‡ Conditions ii, iii, v, 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:

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

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 $d_i^\text{Cl}$ and $d_b^\text{HCO}_3$ 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 pH 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 pH decrease. However, $d_i^\text{HCO}_3$ 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, $d_i^\text{Cl}$ starts off at ~19 mM. The initial $\Delta G_{\text{net}}$ is $0.0RT$, which predicts no net HCO₃⁻ flux. Therefore, the Cl-HCO₃ exchanger model cannot account for the rise of pH, 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, $d_i^\text{Na}$ 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 fall in pH. (vi) Just before $[\text{Na}^+]_b$ is restored to 100 mM, $d_i^\text{Na}$ 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 pH.

NA-H EXCHANGE IN PARALLEL WITH CL-HCO₃ EXCHANGE

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

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

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 pH. (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 pH recovery data indicate
a rate constant approximately twice as great as that expected for pure Na-H exchange. Furthermore, the pH$_i$ 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, $d_{Na}^{Na}$ 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, $d_{Na}^{Na}$ 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:

$$\Delta G_{net} = RT \times \ln \left( \frac{[Na^+]_i[Na^+]_b[Cl^-]_b[H^+]_b}{[Na^+]_b[HCO_3^+]_b[Cl^-]_i[H^+]_i} \right)$$

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$_3^-$ efflux. 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 $d_{Na}^{Cl}$ did not have a time course and magnitude consistent with the changes in pH$_i$ and $d_{Na}^{Na}$. (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$_3^-$, and net loss of Cl$^-$ and H$^+$. This prediction is not inconsistent with the data of Fig. 7B, which indicates that the net HCO$_3^-$ efflux should be slowed or even reversed. (iv) When [Na$^+$]$_b$ is reduced to zero, $\Delta G_{net}$ becomes $+\infty$, correctly predicting a net Na$^+$ and HCO$_3^-$ efflux. This model also predicts an absolute dependence on Cl$^-$ as well as a simultaneous uptake of Cl$^-$, which should amount to half of 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 magnitude of the $d_{Cl}^{Cl}$ increase were inappropriate. (v) At the instant [Na$^+$]$_b$ is restored to 100 mM, $\Delta G_{net}$ becomes $-4.7RT$, which correctly predicts a net efflux of Na$^+$ and HCO$_3^-$, and net gain of Cl$^-$ and HCO$_3^-$, which should amount to half of 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 $d_{Cl}^{Cl}$ decrease were inappropriate.
ELECTROGENIC Na/HCO₃ TRANSPORT The models el-e4 are thermodynamically equivalent. Their common predicted free energy change, when expressed in the form for model el, is:

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

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 \( \Delta G_{\text{net}} \) 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 \(-∞\), favoring net Na⁺ and HCO₃⁻ efflux. This is consistent with the observed fall of pHᵢ. (vi) When [Na⁺]₀ is returned to 100 mM, \( V_i \) reaches \(-100 \text{ mV} \), causing \( \Delta G_{\text{net}} \) to rise to \(+2.0RT\), favoring net Na⁺ and HCO₃⁻ uptake. These predictions are also consistent with the \( \Delta G_{\text{net}} \) and pHᵢ data.

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