Control of Active Proton Transport in Turtle Urinary Bladder by Cell pH

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ABSTRACT
The rate of active H⁺ secretion (J_H) across the luminal cell membrane of the turtle bladder decreases linearly with the chemical (∆pH) or electrical potential gradient (∆Ψ) against which secretion occurs. To examine the control of J_H from the cell side of the pump, acid-base changes were imposed on the cellular compartment by increasing serosal [HCO₃⁻] at constant PCO₂ or by varying PCO₂ at constant [HCO₃⁻]. When serosal [HCO₃⁻] was increased from 0 to 60 mM, cell [H⁺] decreased, as estimated by the 5,5-dimethyloxazolidine-2,4-dione method. J_H was a saturable function of cell [H⁺], with an apparent Kₘ of 25 nM. When PCO₂ was varied between 1 and 20% at various serosal [HCO₃⁻], the PCO₂ required to reach a maximal J_H increased with [HCO₃⁻] so that J_H was a function of cell [H⁺] rather than of cell [HCO₃⁻] or CO₂. The proton pump was controlled asymmetrically with respect to the pH component of the electrochemical potential for protons, μ_H. On the cell side of the pump, a ∆pH of <1 U was required to vary J_H between maximal and zero values, whereas on the luminal side a ∆pH of 3 U was required. Cell [H⁺] regulates J_H by determining the availability of H⁺ to the pump in a relationship resembling Michaelis-Menten kinetics. Increasing luminal [H⁺] generates an energy barrier at a luminal pH near 4.4 that equals the free energy (per H⁺ translocated) of the metabolic driving reaction.

INTRODUCTION
The isolated turtle bladder acidifies its urinary compartment by active proton transport across the luminal cell membrane (Steinmetz, 1967 and 1974; Schwartz et al., 1974; Al-Awqati, 1978). Because the luminal cell membrane and the tight junctions between cells have a very low passive permeability to protons (Steinmetz, 1969; Steinmetz and Lawson, 1971; Beauwens and Al-Awqati, 1976), the maximal pH gradient that can be generated by the proton pump is almost entirely determined by the characteristics of the active transport system. Al-Awqati et al. (1977) defined the transport system further by showing that active proton secretion (J_H) is inhibited to about the same extent by an opposing pH gradient (∆pH) as by an equivalent electrical potential gradient (∆Ψ). This is consistent with other evidence that the H⁺ pump is electrogeneric (Steinmetz et al., 1967). Thus, J_H is reduced to zero by either a ∆pH of 3 U or a ∆Ψ of ~180 mV. A similar equivalency of ∆pH and
∆Ψ has been observed in energy transductions in bacterial membranes (Kaback, 1976) and in mitochondria and chloroplasts (Racker, 1976). In contrast to these organelles, however, the polar epithelial cells of the turtle bladder operate the transport processes of acidification and alkalinization across two cell membranes in series so that three compartments must be considered to define the control of the proton pump by the electrochemical potential for H+ (μH). The luminal compartment is normally subject to large changes in μH in vivo. Similarly, the electrochemical potential gradients that have been applied in vitro were imposed primarily by changing μH in the luminal compartment. Thus, in the studies by Steinmetz and Lawson (1971) and Al-Awqati et al. (1977) the serosal compartment was kept at pH 7.4 and the ΔpH was established by lowering luminal pH. Much less is known about the effect of acid-base changes within the cellular compartment. Because the basolateral cell membrane is highly permeable to HCO3− and CO2 (Steinmetz, 1969 and 1974) acid-base changes can be imposed in the cellular compartment by altering the serosal medium. In the present study this experimental approach was taken to determine how active proton secretion is controlled from the cell side of the pump.

### TABLE I

**COMPOSITION OF SEROSAL AND LUMINAL BATHING MEDIA**

| Serosal | Luminal |
|---------|---------|
| HCO3−   | SO4−    | Cl−   | Sucrose |
| mM      | mM      | mM    | mL     |
| 10      | 0       | 109   | 0       | 0       | 5     | 109   | 5     |
| 20      | 0       | 99    | 0       | 0       | 10    | 99    | 10    |
| 30      | 0       | 89    | 0       | 0       | 15    | 89    | 15    |
| 40      | 0       | 79    | 0       | 0       | 20    | 79    | 20    |
| 60      | 0       | 59    | 0       | 0       | 30    | 59    | 30    |

Bladders were initially bathed on both sides with the luminal solutions indicated. After the addition of HCO3−-containing solution to the serosal compartment, both solutions remained symmetrical with respect to osmolality and the composition of electrolytes, other than HCO3− and SO4−.

### METHODS

Urinary bladders from the freshwater turtle *Pseudemys scripta* were mounted in Lucite chambers and maintained in the short-circuited state by voltage clamping. The composition of the bathing solutions with respect to SO4−, [HCO3−], sucrose, and Cl− is shown in Table I. All solutions contained in mmol/liter: Na+, 114.4; Mg2+, 0.5; HPO4−, 2.0; Ca2+, 1.8. Bladders were initially bathed on both sides with a symmetrical HCO3−-free Ringer's solution of various SO4− and Cl− concentrations. The serosal (S) gas phase was 5% CO2 in air and the luminal (L) gas phase was CO2-free air. The S pH was initially held at 5.6 to keep HCO3− concentration low. The L pH was initially held at 7.40. The pH of both solutions was maintained by manual titration with either 0.1 N HCl or 0.1 N NaOH.

The rate of H+ secretion (JH+) was measured as the short-circuit current in bladders.
in which net sodium transport was abolished by serosal addition of 0.5 mM ouabain. 

\( J_H \) was recorded first at "level secretion" in the absence of exogenous \([\text{HCO}_3^-] \) and, then, under asymmetric conditions with \([\text{HCO}_3^-] \) present in the serosal (S) solution. Fig. 1 shows a representative experiment in which the inhibition of \( J_H \) by an acute increase in the S \text{HCO}_3^- concentration is shown and in which the small asymmetry current resulting from \text{HCO}_3^- diffusion is indicated separately on the right. The control current 2 h after ouabain was 79 \( \mu \)A. Replacement of the S solution with Ringer’s solution containing 40 mM \text{HCO}_3^- decreased the current to 44 \( \mu \)A. The decrease was reversible when the \text{HCO}_3^- was removed. The asymmetry current was measured by reintroducing 40 mM \text{HCO}_3^- into S after \( J_H \) was brought to zero. As shown, the current due to the diffusion of \([\text{HCO}_3^-] \) was small, 8 \( \mu \)A, so that the net inhibition of \( J_H \) by \text{HCO}_3^- was 27 \( \mu \)A or 34%.

In this experiment, total tissue resistance (4,078 ohm \( \times \) cm\(^2\)) was little affected by the addition of \text{HCO}_3^- (4,324 ohm \( \times \) cm\(^2\)). In the entire group of 31 bladders

**Figure 1.** Inhibition of the H\(^+\) current after serosal addition of \text{HCO}_3^- to a final concentration of 40 mM. On the right, the \text{HCO}_3^- diffusion current is assessed after the H\(^+\) current was brought to zero (\( J_H = 0 \)) by lowering M pH. Resistance measurements were also similar before and after \text{HCO}_3^- addition (3,705 ± 235 vs. 3,285 ± 197 ohm \( \times \) cm\(^2\)).

To ensure that the diffusion current was the same at (L pH)\(_{20} \) and L pH 7.40, \( J_H \) was nearly abolished in a separate group of bladders by S addition of 0.5 mM SITS (4-acetamido-4'-isothiocyanato-2,2'-disulfonic stilbene). In about one-third of bladders treated in this way, \( J_H \) could be reduced to 2 ± 1 \( \mu \)A. In these bladders it was, therefore, possible to determine whether the diffusion current changed by changing L pH since \( J_H \) was only a small fraction of the diffusion current.

In another series of experiments we determined whether the inhibition of \( J_H \) produced by S addition of \text{HCO}_3^- could be influenced by a change in the percent \text{CO}_2 of the S gas phase. Hemibladders were bathed with Ringer’s solution containing 0, 20, 40, or 60 mM \text{HCO}_3^- and the percent \text{CO}_2 in the gas phase was either increased from 1 to 20 or decreased from 20 to 1. \( J_H \) was measured at each \text{CO}_2 tension after ~30 min when the rate had become constant.

To assess whether a change in the percent \text{CO}_2 of the gas phase altered the (L
pH)jH=0, bladders were initially bubbled with 1 or 10% CO2. The (L pH)jH=0 was determined, after which the S solution was replaced with one containing 40 mM HCO3-. When the diffusion current was stable, the S gas phase was changed to 5 and 10% or to 5 and 1% CO2 depending on the initial conditions, and the current was continuously monitored.

In the final series of experiments, cell pH was estimated from the distribution of the weak acid 5,5-dimethylloxazoladine-2,4-dione ([14C]DMO). [14C]DMO was added to both the S and L solutions of bladders bathed on the S side with 10, 20, 40, and 60 mM HCO3- and bubbled with 5% CO2. The L pH was adjusted with 0.1 N HCl or 0.1 N NaOH to equal the S pH. After 2 h of exposure to [14C]DMO, the bladders were removed from the chambers, blotted dry on filter paper, and weighed and dried overnight at 100°C. After dry weight was measured, the isotope was extracted for 24 h in 0.1 N HNO3 and counted in a liquid scintillation counter. The counts were corrected for the [14C]inulin space determined in a separate series of bladders. Isotopes were obtained from New England Nuclear, Boston, Mass. The validity of this technique for the estimation of overall cell pH in turtle bladder has been discussed previously (Steinmetz, 1969). Cell pH was calculated according to Waddell and Butler (1959).

RESULTS

Inhibition of H+ Current (JH) with Increasing Serosal [HCO3-]

The H+ current was inhibited by increasing S [HCO3-] according to the protocol shown in Fig. 1. In Fig. 2, the results of five groups of experiments are summarized. The solid line indicates the decrements in current after serosal addition of 10 mM (n = 6), 20 mM (n = 7), 30 mM (n = 7), 40 mM (n = 7), and 60 mM (n = 5) HCO3-. The dashed line represents the asymmetry
current measured in the same bladders after $J_H$ was stopped by lowering the luminal pH. This asymmetry current was a small component of the measured decrements and, interestingly, remained relatively constant as S [HCO$_3^-$] was increased from 20 to 60 mM.

Because the asymmetry current was measured at low luminal pH (range for $J_H = 0$ was 4.3–5.0) rather than at pH 7.4, similar measurements were made after $J_H$ was abolished by S addition of the disulfonic stilbene SITS (0.5 mM) at luminal pH 7.4. In five bladders in which the H$^+$ current was virtually abolished by SITS, the asymmetry current induced by S addition of 20 mM HCO$_3^-$ was not different at luminal pH 7.4 and 5.0.

In Fig. 3, the percent inhibition of the corrected values of $J_H$ is shown. The inhibition increased in linear fashion from 7% at 10 mM HCO$_3^-$ to 55% at 60 mM. The intercept of the regression line with the abscissa was at 110 mM HCO$_3^-$.

**Estimate of Cellular Alkalinity with Increasing Serosal [HCO$_3^-$]**

To gain further information on the intracellular acid-base status under these conditions, we measured the apparent cell pH by the DMO method. Table II shows that the DMO pH increased linearly with serosal pH, with a slope of 0.76.

In Fig. 4, the relationship between $J_H$ and cell [H$^+$] is shown on the basis of the DMO data. Cell [H$^+$] was altered by changing S [HCO$_3^-$] at a constant PCO$_2$ of 5%. Because at low S [HCO$_3^-$] $J_H$ is maximal at this CO$_2$ tension (Schwartz, 1976), $J_H$ was normalized to this value. The solid circles represent the cellular H$^+$ concentrations obtained at 10, 20, 40 and 60 mM S
[HCO$_3^-$]. $J_H$ was markedly reduced as cell [H$^+$] was reduced by increasing S [HCO$_3^-$]. The open circles are obtained from the data of Fig. 3 at 0 and 30 mM S HCO$_3^-$ and the relationship between cell pH and S pH as given in Table II. Although these results do not yield absolute [H$^+$] values for the population of H$^+$ secreting cells, they do suggest that $J_H$ is a hyperbolic function of cell [H$^+$]. As estimated from the DMO data, $J_H$ was half maximal at a cell [H$^+$] of ~25 nM. The observed maximal rates occurred at a cell [H$^+$] near 190 nM.

**Effect of Acute Changes in PCO$_2$: Alternative Method of Altering Cell [H$^+$]**

An alternative way of changing cell [H$^+$] is to change the CO$_2$ tension at constant S [HCO$_3^-$]. As shown in Fig. 5, the serosal CO$_2$ tension was varied from 1 to 20% CO$_2$ in air at various serosal HCO$_3^-$ concentrations. $J_H$ was normalized to the value at 5% CO$_2$ in the absence of exogenous HCO$_3^-$, which represented a maximal rate as shown by the open circles, a result in accord with the study of Schwartz (1976). At 20 mM HCO$_3^-$ (solid squares), a maximal $J_H$ was reached at CO$_2$ tensions between 10 and 15% CO$_2$. At 40 and 60 mM HCO$_3^-$, $J_H$ was still increasing at 20% CO$_2$. These results indicate that as the serosal HCO$_3^-$ concentrations increase, the CO$_2$ tensions required to stimulate $J_H$ maximally also increase. The maximal rate of transport is not determined by the HCO$_3^-$ or the CO$_2$ concentration per se, but rather by

**Table II**

| Serosal HCO$_3^-$ (mM) | Serosal pH | Cell pH |
|------------------------|------------|---------|
| 10 (n=6)               | 6.96 ± 0.02| 7.04 ± 0.12|
| 20 (n=7)               | 7.26 ± 0.02| 7.28 ± 0.03|
| 40 (n=5)               | 7.56 ± 0.01| 7.50 ± 0.03|
| 60 (n=5)               | 7.74 ± 0.01| 7.63 ± 0.08|

Cell pH ($y$) was a linear function of serosal pH ($x$): $y = 0.76x + 1.79; r = 0.99$. The serosal gas phase was 5% CO$_2$ in air.

**FIGURE 4.** Effect on $J_H$ of changing cell [H$^+$] by varying serosal [HCO$_3^-$]. See text.
combinations of the two in such a way that the H⁺ concentration appears to be the principal determinant of J_H.

Effect of Changes in Cell pH on the Luminal pH at which J_H = 0.

To determine whether inhibition of J_H by cellular alkalinity affected the minimal pH that could be generated by the proton pump, we determined this pH level in eight bladders with and without 40 mM S HCO₃⁻ at CO₂ tensions ranging from 1 to 10% CO₂. As shown in Table III, in this group of bladders, there was no significant HCO₃⁻ diffusion current. At the minimal luminal pH of 4.61 ± 0.12 obtained without HCO₃⁻ the current induced by HCO₃⁻ addition was not different from zero, and no significant change occurred when cell pH was changed by varying the percent CO₂ between 1 and 10. These results suggest that the apparent protonmotive force was not reduced by cell alkalinity. It might have increased slightly, as judged from the ΔpH between lumen and cytoplasm.

DISCUSSION

Transcellular Pathway for H⁺ Transport and OH⁻ Disposal

The acidification pump at the luminal cell membrane of the turtle bladder appears to be a proton-translocating ATPase (Dixon and Al-Awqati, 1979)
with characteristics somewhat different from those of mitochondrial ATPase (Steinmetz et al., 1980). Because the detailed mechanisms of H⁺ secretion remain unknown, we describe the process operationally as one in which H⁺ is translocated from the cellular to the luminal compartment. If H₂O is the immediate source of H⁺, the result of H⁺ extrusion is the formation of OH⁻ within the cell. This OH⁻ is thought to react with CO₂ and to form HCO₃⁻, which moves passively down an electrochemical potential gradient across the basolateral cell membrane into the serosal compartment.¹

These previous studies suggest that the availability of CO₂ and the disposition of OH⁻ and HCO₃⁻ are factors in controlling the rate of H⁺ transport (Schwartz and Steinmetz, 1971; Schwartz et al., 1972; Cohen et al., 1978). The present study provides a more systematic examination of how the pump rate for protons is controlled from the cell side of the pump.

**Control of H⁺ Transport by Cellular Acid-Base Conditions**

Our studies demonstrate that J_H is reduced progressively when cellular alkalinity is increased. The most extensive data were obtained by increasing serosal [HCO₃⁻] from 0 to 60 mM. J_H was an inverse linear function of serosal [HCO₃⁻] as well as of mean cellular [OH⁻] measured by the DMO method. When J_H was plotted against cell [H⁺], a hyperbolic function was obtained (Fig. 4). J_H was half maximal at 25 nM and maximal at a cell [H⁺] of 186 nM. A similar relationship was obtained when the percent CO₂ of the serosal gas phase was varied in the presence of constant serosal [HCO₃⁻] between 1 and 20% (Fig. 5).

To compare the effects on J_H of the two acid-base maneuvers, changing serosal [HCO₃⁻] and changing PCO₂, we replotted the data of Fig. 5 in such a way that each CO₂ tension is represented by a bar giving a potential range of cell [H⁺]. In Fig. 6 the zero (open) end of the bar indicates the cell [H⁺] that would have resulted if the PCO₂ change caused a [H⁺] change without any compensatory regulation. In most tissues the CO₂-induced pH changes are reduced by altered proton extrusion and by other regulatory mechanisms (Boron et al., 1979; Thomas, 1976). Although the extent of compensation varies, depending on the tissues and the experimental conditions, the reported values usually range between 30 and 50% (Waddell and Bates, 1969). Struyvenberg et al. (1968) observed 42% compensation in response to changes in PCO₂ in canine renal tubules. The bars of Fig. 6 represent a range of cellular H⁺ concentrations from 0 to 50% compensation. The solid parts of the bars cover the likely range of compensation from 30 to 50%. In all three groups of experiments, the relationship between J_H and the H⁺ concentrations represented by the bars appears to be hyperbolic. For comparison, the curve of Fig. 4, in which J_H was varied by changing S [HCO₃⁻], is again indicated. A

¹ There is as yet no agreement on the ion that is actively translocated across the luminal membrane. Schilb and Brodsky (1972) and Schilb (1978) have suggested that HCO₃⁻ is the transported ion. In our view the bulk of the evidence (Schwartz et al., 1974) supports the H⁺ pump model. There is agreement that HCO₃⁻ is the ion transported across the basolateral cell membrane and that the overall transport process is associated with the transfer of charge.
reasonable agreement between results obtained by changing $[\text{HCO}_3^-]$ and by changing $\text{PCO}_2$ is obtained, especially if the degree of compensation is assumed to be near 50%. Although in this analysis the actual $H^+$ concentrations in the $H^+$ secreting cells remain only estimates, the observed behavior of $J_H$ in response to experimental changes in cell $[H^+]$ can be evaluated without making major assumptions. These results suggest that $J_H$ is determined by the cellular concentration of $H^+$ rather than by that of $\text{HCO}_3^-$ or free $\text{CO}_2$.

A dependence of $H^+$ secretion on cell $[H^+]$ rather than $\text{PCO}_2$ per se is consistent with studies on the rate of transport in the kidney during hypercapnia. The stimulatory effect of $\text{CO}_2$ is best demonstrated in the presence of a high ambient $\text{HCO}_3^-$ when cell $[H^+]$ is well below the level required for a

![Figure 6. Effect on $J_H$ of changing cell $[H^+]$ by varying $\text{PCO}_2$. $\text{PCO}_2$ was varied between 1 and 20% $\text{CO}_2$ in the serosal gas phase, with serosal $[\text{HCO}_3^-]$ of 60 mM (top panel), 40 mM (middle panel), and 20 mM (bottom panel). The bars encompass cell $[H^+]$ values for a range of from 0 to 50% compensation to the changes in $\text{CO}_2$ tension. The solid part of the bar represents the likely range of compensation (see text). The curve of Fig. 4 obtained at 5% $\text{CO}_2$ is given for comparison.](image)

maximal rate of transport (Malnic et al., 1972; Mello-Aires and Malnic, 1975; Rector, 1973).

Several mechanisms could play a role in the stimulation of $J_H$. First, the transport enzyme could have a $pH$ optimum at a cellular $[H^+]$ near 200 nM. It is of interest that the $pH$ optimum of the $H^+$-ATPase of plasma membranes of *Neurospora* and certain yeasts is near 6.0, whereas the mitochondrial ATPase has a higher optimum, between 8.0 and 9.0 (Bowman and Slayman, 1977; Willsky, 1979). Because ATP hydrolysis by a cell membrane ATPase causes the net translocation of protons, this possibility is difficult to distinguish from the second mechanism, in which protons serve as substrate for a proton-translocating enzyme. According to this mechanism, $J_H$ is related to cell $[H^+]$
in a Michaelis-Menten manner. This interpretation is attractive because of its simplicity and is consistent with the results obtained by equivalent circuit analysis, which indicate that the experimental maneuvers (changing serosal CO$_2$ or HCO$_3^-$) alter the conductance in the active pathway and not the force of the pump (Al-Awqati et al., 1977). Other factors to be considered are pH effects on enzymes that play a role in the supply of metabolic energy to the pump. Until the coupling between transport and metabolism is better defined, it will be difficult to determine to what extent the supply of metabolic energy is affected by cell pH. In our view it is more likely that the pump rate for protons is a function of the availability of protons and that the metabolic rate is paced by the transport rate. Al-Awqati (1978) has provided evidence that the stimulation of $J_H$ by aldosterone results from a primary stimulation of transport followed by adaptive changes in metabolism. Such a sequence of events in the stimulation of $H^+$ secretion would be comparable to that described by Whittam (1961), who showed how the sodium transport rate serves as a pacemaker for the rate of respiration. The simplest explanation of our data, therefore, remains that the $H^+$ concentration on the cell side of the pump is the primary determinant of the transport rate. The kinetics of $H^+$ secretion observed when cell [H$^+$] is altered by different maneuvers suggest that the transport system is saturable. At a certain cellular acidity all pump sites are in operation at a maximal turnover rate. Such saturation of the $H^+$ transport system resembles the saturation observed for sodium transport when more sodium is made available to the sodium pump. The kinetic effects of altering cell pH should be distinguished from any effects on the force of the pump. It is of interest that the apparent protonmotive force was barely altered by changes in cell pH.

**Sidedness of the Pump and Apparent Protonmotive Force**

These studies indicate that the proton pump is far more sensitive to pH changes on the internal surface than to changes on the external surface of the luminal cell membrane. At a reference cell pH of 7.28, corresponding to our experiments carried out at 5% CO$_2$ and 20 mM HCO$_3^-$ in the serosal compartment, it takes an opposing $\Delta p$H on the luminal side of the pump of $\sim$3 U to stop $H^+$ transport but only 0.6 U to stop transport from the cytoplasmic side. Fig. 7 shows the asymmetry in the pH control of $J_H$ under these conditions. What is the significance of the difference in the pH control on the two sides of the proton pump? As pointed out above, the simplest explanation is that kinetic or supply factors determine the transport rate from the cell side. Because of the striking linear dependence of $J_H$ on $\Delta p$H or $\Delta \Psi$ on the luminal side of the pump (Steinmetz and Lawson, 1971; Al-Awqati et al., 1977), it is necessary to at least consider the possibility that control might in fact remain symmetrical if both the electrical and the chemical components of the potential gradient, $\Delta \Psi_H$, were considered. In our example of Fig. 7, serosal addition of HCO$_3^-$ would stop transport by the time cell pH increased by 0.6 U. To account for this inhibition by a $\Delta \Psi_H$ effect per se, the cell would have to be hyperpolarized by $\sim$140 mV as a result of the HCO$_3^-$ addition. It is most unlikely that an increase in the serosal HCO$_3^-$ concentration would
cause a $\Delta \Psi$ of that magnitude. The serosal cell membrane has a high \( \text{HCO}_3^- \) permeability and the electrical resistance of this membrane is low compared with that of the luminal membrane (Hirshhorn and Frazier, 1971). Fröchter et al. (1976) similarly observed that the basolateral cell membrane of acid-secreting epithelial cells of the proximal tubule is highly permeable to \( \text{HCO}_3^- \). Increases in peritubular \( \text{HCO}_3^- \) concentration caused only very transient hyperpolarization of the cell interior.

More compelling than these considerations is the fact that after inhibition of \( J_H \) by cellular alkalinity (Table III) the luminal pH at which \( J_H \) was brought to zero was unaltered. The achievable $\Delta p$H across the pump pathway, therefore, was not reduced by cellular alkalinity. If any change occurred, the apparent force of the pump, as judged from $\Delta p$H, increased somewhat with increased cell alkalinity.\(^2\)

For the purposes of our analysis we may conclude that among the acid-base factors controlling \( J_H \) from the cell side of the pump the cell $[H^+]$ is the major determinant. Although it is possible that CO2 is required at some minimal level, the effects of CO2 and \( \text{HCO}_3^- \) on the \( H^+ \) pump are mediated through the \( H^+ \) activity. The asymmetry of pH control of the pump rate may be described in mechanistic terms. On the cell side the metabolic energy is most likely derived from ATP hydrolysis (Dixon and Al-Awqati, 1979) and protons serve as the substrate to a transport system that is saturable. On the outside of the luminal membrane the transport rate is less sensitive to pH. It takes a change in $\mu_H$ of $\approx 180$ mV or 3 pH units to stop the translocation of protons.

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\(^2\) One explanation for this small increase may be that the free energy of ATP hydrolysis increases with cell pH (Alberty, 1968).
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