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# Electrogenic Proton Secretion in the Hindgut of the Desert Locust, *Schistocerca gregaria*

Robert B. Thomson and John E. Phillips
Department of Zoology, University of British Columbia, Vancouver, British Columbia, Canada V6T 2A9

**Summary.** The cellular mechanisms responsible for rectal acidification in the desert locust, *Schistocerca gregaria*, were investigated in isolated recta mounted as flat sheets in modified Ussing chambers. Previous studies conducted in the nominal absence of exogenous $CO_2$ and $HCO_3^-$ suggested that the acidification was due to a proton-secretory rather than bicarbonate-reabsorptive mechanism (Thomson, R.B., Speight, J.D., Phillips, J.E. 1988. *J. Insect Physiol.* **34**:829–837). This conclusion was confirmed in the present study by demonstrating that metabolic $CO_2$ could not contribute sufficient $HCO_3^-$ to the lumen to account for the rates of rectal acidification observed under the nominally $CO_2$/$HCO_3^-$-free conditions used in these investigations.

Rates of luminal acidification ($J_{H^+}$) were completely unaffected by changes in contraluminal pH, but could be progressively reduced (and eventually abolished) by imposition of either transepithelial pH gradients (lumen acid) or transepithelial electrical gradients (lumen positive). Under short-circuit current conditions, the bulk of $J_{H^+}$ was not dependent on $Na^+$, $K^+$, $Cl^-$, $Mg^{2+}$, or $Ca^{2+}$ and was due to a primary electrogenic proton translocating mechanism located on the apical membrane. A small component (10–16%) of $J_{H^+}$ measured under these conditions could be attributed to an apical amiloride-inhibitable $Na^+/H^+$ exchange mechanism.

**Key Words** pH · carbon dioxide · bicarbonate · proton secretion · excretion · locust

## Introduction

Although pH regulation in terrestrial insects is poorly understood, preliminary observations suggest that long-term pH homeostasis is maintained by controlled excretion or reabsorption of nonvolatile acids and bases in the Malpighian tubule-hindgut complex (Phillips et al., 1986; Stagg, Harrison & Phillips, 1991). A primary isosmotic urine containing most blood solutes and reflecting the pH status of the hemolymph is formed in the Malpighian tubules, and selective addition or removal of solutes and water occurs in both the anterior (ileum) and posterior (rectum) segments of the hindgut (for detailed reviews of the functional morphology of the locust excretory system, *see* Phillips, 1981; Phillips et al., 1986). Acidification of luminal contents, ammonia secretion, and bicarbonate reabsorption have all been reported to occur to varying extents in both the ileum (Lechleitner, 1988; Thomson, Audsley & Phillips, 1991) and rectum (Thomson & Phillips, 1985; Thomson, Speight & Phillips, 1988*a*; Thomson, Thomson & Phillips, 1988*b*). To date, however, the only acid/base transport mechanism in the locust excretory system to be characterized at the cellular level is an apical, amiloride-sensitive $Na^+/NH_4^+$ antiporter located in the rectum (Thomson, Thomson & Phillips, 1988*b*).

In the present study we have focused our attention on the cellular mechanisms responsible for mediating the luminal pH changes observed in the rectal segment of the hindgut. The rectum is a major site of solute and water recovery in the locust excretory system. $Na^+$, $K^+$, $Cl^-$, $H_2O$, and metabolites are all reabsorbed from a KCl-rich fluid which enters the lumen from the anterior hindgut. The rectum consists of six radially arranged longitudinal pads covered with a selectively permeable cuticular intima (*see* Phillips et al., 1986 for review). The pads are composed almost entirely of large columnar "principal" cells (17 × 100 $\mu$m) and occasional small secondary "B" cells. Beneath the rectal pads is a distinct subepithelial space and a smaller secondary cell layer. The size of the principal cells, degree of membrane differentiation, number of mitochondria, extent of tracheal development, and measurements of intracellular parameters all indicate that the principal cells of the primary cell layer are predominantly responsible for the transepithelial transport of solutes and water observed in the rectum (Phillips et al., 1986). Cable analysis of the epithelium also indicates that

the rectum is a moderately tight to tight epithelium where 60–96% of the transepithelial conductance is transcellular, depending on experimental conditions (Hanrahan & Phillips, 1984*b*).

Using isolated recta mounted as flat sheets in modified Ussing chambers we previously demonstrated that the rectal epithelium was capable of generating and maintaining transepithelial pH gradients similar to those observed in the intact hindgut *in situ* (≈1.8 pH units; Thomson et al., 1988*a*) and that those gradients could be formed in the nominal absence of exogenous phosphate or $CO_2$/$HCO_3^-$. Measurements of intracellular pH and apical and basolateral membrane potentials indicate that luminal acidification is mediated by an active transport process and that acid/base transfer typically occurs against electrochemical gradients of at least 79 mV at the apical membrane. The results of that initial study suggested that most, if not all, of the observed luminal acidification was due to active proton secretion rather than selective movements of phosphate, bicarbonate or some unidentified buffer component.

The continuous production of metabolic $CO_2$ made it very difficult for us to completely rule out the possibility that at least some component of the observed acidification might have been due to $HCO_3^-$ reabsorption from the lumen. Even though the solutions were initially $CO_2$/$HCO_3^-$-free and precautions were taken to prevent $CO_2$/$HCO_3^-$ accumulation in the bathing solutions over the course of an experiment, the possibility existed that sufficient $HCO_3^-$ to account for a significant fraction of the rectal acidification might have been formed in the unstirred layers adjacent to the epithelium without necessarily being detected in the bulk solution. A similar argument for $HCO_3^-$-reabsorptive mediated luminal acidification in the absence of exogenous $CO_2$/$HCO_3^-$ has been repeatedly suggested for the turtle bladder (Brodsky & Schilb, 1974; Schilb, 1978; Schilb, Durham & Brodsky, 1988).

In the present study we re-examine this issue in much more detail and conclude that our experimental protocol is more than adequate for maintaining a nominally $CO_2$/$HCO_3^-$-free state in the luminal bath and that the observed rates of luminal acidification could not be mediated by the reabsorption of the trace levels of $HCO_3^-$ which might be present in the unstirred layers under the bilaterally $CO_2$/$HCO_3^-$-free conditions used in these experiments. Ion substitutions, inhibitor studies, and manipulation of transepithelial electrochemical proton gradients all suggest that the bulk of rectal acidification is due to a primary electrogenic proton pump located on the apical membrane.

## Materials and Methods

### Animals

Adult female desert locusts (*Schistocerca gregaria* Forskal) 14–22 days beyond their final moult were used for all experiments. They were maintained at 28°C and 60% r.h. on a 12 : 12 hr light : dark cycle and fed daily on fresh lettuce and a dried mixture of bran, alfalfa, and powdered milk.

### In Vitro Rectal Preparation

Isolated recta were mounted as flat sheets in miniaturized Ussing chambers as described previously (Thomson et al., 1988*a*) with 2.0 ml of solution per chamber. Saline was constantly circulated and oxygenated in each chamber by means of a gas lift pump which maintained constant gas tension and circulation regardless of perfusion flow rates. Provision was made for gravity-fed perfusion (4–5 ml/min) of each chamber as protocol dictated. Typically, recta were brought to steady-state conditions (as defined by stable short-circuit current or transepithelial potential; approximately 2 hr) under bilateral perfusion, and then perfusion was stopped unilaterally for the experimental period.

Transepithelial potential ($V_t$) and short-circuit current ($I_{sc}$) were determined as described by Hanrahan et al., (1984). Briefly, $V_t$ was measured as the potential difference between luminal and contraluminal 3 M KCl agar bridges. Short-circuit current was applied with a dual-channel automatic voltage clamp which allowed for compensation of saline resistance and variable voltage-clamp settings (*see* Hanrahan et al., 1984 for complete circuit description). $V_t$ and $I_{sc}$ were monitored and recorded on dual-channel strip-chart recorders (1242; Soltec, Sun Valley, CA).

### Measurement of Rectal Acidification

Recta were mounted and perfused as above. Rates of luminal acidification ($J_{H^+}$) were determined with a pH-stat technique (PHM 84 research pH meter, TTT 80 titrator, ABU 80 autoburette; Radiometer, Copenhagen, Denmark). $J_{H^+}$ was calculated as the rate of titrant addition (0.01 N NaOH) required to maintain the initial pH.

### Total $CO_2$ Measurement

Saline samples (100 μl) were acidified in gas-tight Hamilton syringes containing 2 ml of 0.1 N HCl and 2 ml of nitrogen gas (sample size and relative quantities of HCl and $N_2$ were varied to give optimal detection at extremely low $CO_2$ concentrations). After 5 min the gas phase was assayed for $CO_2$ content by standard gas chromatography using a Carle 1111 gas chromatograph (Hach, Loveland, CO). $CO_2$ standards were prepared immediately before use with $NaHCO_3$ and $N_2$ saturated distilled water. With very careful sample handling and $CO_2$ standard preparation, $CO_2$ concentrations could be determined repeatedly within 1–2 μM at low $CO_2$ concentrations. The practical detection limit for this method was approximately 10 μM, below which repeatability and accuracy deteriorated rapidly. This detection limit appeared to be more a function of standard preparation and sample handling than

a performance limitation imposed by the gas chromatograph *per se*.

## Intracellular Voltage Measurement

Recta were mounted as flat sheets in specially designed microelectrode chambers (*see* Hanrahan et al., 1984) requiring continuous bilateral perfusion (8–10 ml/min; gravity fed) to maintain required gas tensions. To facilitate apical impalement, the cuticular intima was removed from the rectal pad with fine forceps and irridectomy scissors. This treatment does not affect epithelial transport as judged by $I_{sc}$, $V_t$, and transepithelial resistance (*see* Hanrahan et al., 1984). Microelectrodes with tip diameters $\leq 1$ $\mu$m were fabricated with a Brown and Flaming horizontal electrode puller (model P-77; Sutter Instruments, San Diego, CA). Microelectrodes were advanced manually via hydraulic microdrive (MO-8; Narashige, Tokyo, Japan) at an angle of 30–40° to the plane of the epithelium.

Apical and basolateral membrane potentials ($V_a$ and $V_b$) were measured as the potential difference between the reference barrel and a luminal or contraluminal 3 M KCl agar bridge, respectively, with a high-input impedence differential electrometer ($10^{15}$ Ω; FD 223; WPI, New Haven, CT). Signals were monitored on a storage oscilloscope (D15 Tektronics, Beaverton, OR) and recorded on a three-channel strip-chart recorder (1243; Soltec, Sun Valley, CA).

Transepithelial resistance ($R_t$) and voltage-divider ratios ($\Delta R_a/\Delta R_b$) were calculated from deflections in $V_t$, $V_a$, and $V_b$ produced by transepithelial current pulses. Voltage deflections were corrected for saline resistance by subtracting background deflection values from experimental values. Background values were determined in chambers with saline only (i.e., no tissue) by positioning the microelectrode tip in the same plane of focus as when the tissue was present and passing a train of current pulses. Constant current pulses (13 $\mu$A, 1.5-sec duration, 0.2 Hz) were generated by two operational amplifers configured as a voltage-controlled constant current source (LF 356, National Semiconductor, Santa Clara, CA). The pulses were triggered with an LM 555 timer (National Semiconductor). Current pulses were measured as the voltage drop across a 1 KΩ resistor in series.

Acceptable impalements were characterized by (i) abrupt monotonic deflections in membrane potentials, (ii) stable membrane potentials (±1 mV) for at least 60 sec, (iii) constant voltage divider ratios, (iv) constant $R_t$, and (v) immediate return to baseline potential after electrode withdrawal (±2 mV).

## Solutions

The composition of experimental salines was based on the content of locust hemolymph and Malpighian tubule fluid (Chamberlin & Phillips, 1982; Hanrahan, 1982) and, unless otherwise stated, contained (in mM); 100 NaCl, 5 $K_2SO_4$, 10 $MgSO_4$, 10 $Na^+$-isethionate, 10 glucose, 100 sucrose (to adjust osmolarity), 5 $CaCl_2$, 2 MOPS (3-[N-morpholino]-propanesulphonic acid; $pK_a$ = 7.20 at 20°C), 1.0 arginine, 1.5 serine, 13.1 proline, 1.3 asparagine, 11.4 glycine, 2.9 alanine, 1.8 valine, 5.0 glutamine, 1.0 tyrosine, 1.4 lysine, and 1.4 histidine. Salines titrated to pH < 6.50 also contained 2 mM 2(*N*-morpholino)ethanesulfonic acid (MES; $pK_a$ 6.1 at 25°C) to ensure adequate buffering capacity at all saline pH values used throughout the study. Solutions were vigorously aerated with 100% $O_2$ for at least 2 hr prior to use, and likewise, perfusion reservoirs were continuously aerated throughout the entire experimental period. pH electrodes were calibrated with Radiometer Precision Buffer Solutions (pH ± 0.005), and experimental salines were manually titrated to the desired pH using concentrated nitric acid or sodium hydroxide crystals. Experiments were performed at 23 ± 1°C. All tissues were brought to a steady state in the standard pH 7.00, $CO_2/HCO_3^-$-free saline, regardless of ensuing experimental protocols.

$Na^+$- and $Cl^-$-free salines were prepared by substitution with choline or gluconate salts, respectively. $K^+$-free salines were prepared by replacement with isosmotic concentrations of sucrose. Background levels of $Na^+$ and $K^+$ in the respective ion-substituted salines were measured by atomic absorption spectrophotometry (Techtron AA 120; Varian, Melbourne, Australia). Background $Cl^-$ levels were measured by the titrimetric method described by Ramsay, Brown and Croghan (1955).

In all ion-substitution experiments, the composition of agar bridges reflected the salt composition of the respective bathing salines to maintain the nominally $Na^+$-, $K^+$-, or $Cl^-$-free state. During $Na^+$-substitution experiments, the pH-stat titrant was changed from 0.01 N NaOH to 0.01 N KOH. Luminal $K^+$ levels rose by less than 0.04 mM during the 15-min pH-stat measurement period and appeared to have no significant effect on rectal transport processes as judged by stable $V_t$ and $I_{sc}$ values.

All inhibitors were made up immediately before use and added to the preparation at least 30 min prior to the experimental period to allow complete exposure of the membrane to the inhibitor. Omeprazole, 4-acetamido-4′-isothiocyanatostilbene-2,2′-disulfonic acid (SITS), and 4,4′-diisothiocyanatostilbene-2,2′-disulfonic acid (DIDS) were presolubilized in dimethyl sulfoxide (DMSO) immediately before use. The volume of DMSO (+ inhibitor) added to experimental salines was always <1% of the final saline volume. Similar volumes of DMSO were added to control salines to ensure that DMSO alone was not affecting $J_{H^+}$, $V_t$, or $I_{sc}$. Salines containing SITS and DIDS were amino acid free (replaced with sucrose) as suggested by Strange and Phillips (1984). Acetazolamide and vanadate were dissolved directly in the standard $CO_2/HCO_3^-$-free saline used throughout the study. Amiloride was made up in sulfate-free salines. Omeprazole was a generous gift from Hässle, Mölndal, Sweden. SITS, DIDS, vanadate and acetazolamide were obtained from Sigma Chemical; St. Louis, MO. Amiloride was a generous gift from Merk Frosst Canada, Pointe-Claire, Quebec.

## Calculations and Statistics

Acid ($J_{H^+}$) secretion rates were calculated as apparent flux rates per $cm^2$ of tissue per hour.

Bicarbonate concentrations were calculated from total $CO_2$ measurements using the Henderson-Hasselbach equation (pK = 6.1695). Saline pK values were calculated from Siggaard-Andersen (1976) for a salt solution at 22°C with an ionic strength of 0.18 mol · $kg^{-1}$.

Apparent proton motive force (PMF) values were calculated as the transepithelial electrochemical potential for $H^+$ ($\Delta\bar{\mu}_H/F$) at which $J_{H^+} = 0$. Electrochemical potentials were calculated as:

$$\Delta\bar{\mu}_H/F = (RT\ln(a_o/a_i))/F + V_t$$

where $a_o$ is the proton activity in the outside compartment, $a_i$ is the proton activity in the inside compartment, $V_t$ is the transepi-

thelial membrane potential, and $F$, $R$, and $T$ have their usual meanings.

All values are reported as means ± SE. Statistical significance was determined using paired or nonpaired $t$ tests. Differences were considered statistically significant if $P < 0.05$.

## Results

### Acid Secretion *Versus* Bicarbonate Reabsorption

To determine whether the previously reported rectal acidification observed under nominally $CO_2$/$HCO_3^-$-free conditions (Thomson et al., 1988*a*) could have been sustained by reabsorption of metabolically derived $HCO_3^-$, we first reassessed our ability to maintain a truly $CO_2$/$HCO_3^-$-free state in the solutions bathing the epithelium (in the absence of exogenously added $CO_2$/$HCO_3^-$) and then attempted to stimulate $J_{H^+}$ by indirectly adding trace levels of $CO_2$ and $HCO_3^-$ to the rectal lumen. Recta were mounted as indicated above and bathed bilaterally in the standard $CO_2$/$HCO_3^-$-free saline (pH 7.00). Contraluminal perfusion was maintained throughout the entire experiment, and luminal perfusion was discontinued once a steady state (as defined by a stable $I_{sc}$ and $V_t$) had been reached. As a first approximation of the efficacy of our attempts to blow off metabolic $CO_2$, we measured the total $CO_2$ content of the initial saline (before exposure to the tissue), the contraluminal perfusate, and the luminal saline after 4-hr exposure to the tissue. All samples contained <10 μM total $CO_2$ (this represented the practical lower limit of the $CO_2$ assay used in this study; *see* Materials and Methods; $n = 6$ preparations). Although this demonstrated that the experimental protocol was effective for maintaining a $CO_2$-free state in the bulk solution, it could be argued that a significant $CO_2$/$HCO_3^-$ concentration might exist in the unstirred layers adjacent to the apical membrane without necessarily being detected in the bulk solution. If this was true and rectal acidification occurred by $HCO_3^-$ reabsorption rather than proton secretion, one would predict that increasing the $P_{CO_2}$ (and hence the $HCO_3^-$ concentration) of the unstirred layers should result in an increase in net rectal acidification.[1]

This hypothesis was tested by adding 1.5 mM $HCO_3^-$ to the contraluminal perfusate and aerating the contraluminal chamber with 1% $CO_2$/99% $O_2$.[2] The luminal bath was continuously aerated with 100% $O_2$, and the luminal pH was maintained at 7.00 by pH stat. Based on the typical $CO_2$ permeabilities reported in the literature (*see* Schwartz et al. (1981) for example), this maneuver would be expected to increase both the intracellular $P_{CO_2}$ and the $P_{CO_2}$ (and concomitant $HCO_3^-$ concentrations) of the unstirred layers next to the apical membrane. Measurements of the total $CO_2$ content of the initial luminal saline (before exposure to the tissue) and the luminal saline after 4-hr exposure to the tissue (in the presence of contraluminal 1.5 mM $HCO_3^-$/1% $CO_2$) were again always < 10 μM. This chamber design clearly permitted vigorous enough stirring (with 100% $O_2$ in the absence of luminal perfusion) to maintain a nominal $CO_2$-free state in the bulk solution in the presence of a significant transepithelial $P_{CO_2}$ gradient. Consistent with the previous observations, the addition or removal of contraluminal 1% $CO_2$/1.5 mM $HCO_3^-$ had no effect on $J_{H^+}$ or $I_{sc}$ (Table 1).

The possibility remained, however remote, that $CO_2$-induced changes in $J_{H^+}$ were mitigated by diffusion of contraluminal $HCO_3^-$ into the lumen (*see* Schwartz & Steinmetz, 1971). This hypothesis was tested by repeating the above experiment in the absence of exogenously added $HCO_3^-$ at a contraluminal pH of 5.50 (luminal saline was $CO_2$/$HCO_3^-$-free; pH 7.00). At this pH, equilibrium levels of $HCO_3^-$ should be several orders of magnitude less than in the previous experiment and corresponding rates of $HCO_3^-$ diffusion into the lumen should be substantially lower. Under similar control conditions (i.e., bilateral $CO_2$/$HCO_3^-$-free; lumen pH 7.00) a decrease in contraluminal pH from 7.50 to 5.00 had no effect on $J_{H^+}$, $V_t$ or $I_{sc}$ (*see* Fig. 1). The addition of contraluminal 1% $CO_2$ at pH 5.50 (lumen pH maintained at 7.00) also had no discernible effects on $J_{H^+}$ or $I_{sc}$ (Table 1). It would appear, therefore, that $HCO_3^-$ diffusion was not a factor in the previous experiment and that the addition of contraluminal $CO_2$/$HCO_3^-$ at these low levels had no effect on rectal acidification. In view of these results it is unlikely that the trace

[1] This assumes that if $J_{H^+}$ was mediated by $HCO_3^-$ reabsorption that it would not be maximally stimulated by the submicromolar quantities of $HCO_3^-$ present in the luminal bath when the tissue was bathed bilaterally with nominally $CO_2$/$HCO_3^-$-free salines (*see* Schwartz & Steinmetz, 1971).

[2] $CO_2$ and $HCO_3^-$ were not added directly to the luminal bath due to limitations of the pH-stat measurement technique described above. The volatile nature of the $CO_2$/$HCO_3^-$ buffer pair and the much increased saline buffering capacity associated with $CO_2$/$HCO_3^-$ addition (remember that the amount of tissue present in the Ussing chamber is very small; 0.196 $cm^2$) made accurate detection of rectal acidification very difficult. $CO_2$ and $HCO_3^-$ were added to the contraluminal bath instead in an effort to increase the $HCO_3^-$ concentration of the unstirred layers adjacent to the apical membrane without significantly affecting the buffering capacity of the luminal bath.

**Table 1.** Changes in rates of rectal acidification ($\Delta J_H$·) induced by contraluminal addition of $CO_2/HCO_3$

| Contraluminal bath | | | Luminal bath | $\Delta J_H$· ($\mu$equiv·cm$^{-2}$·hr$^{-1}$) | $J_{HCO_3}$ ($\mu$equiv·cm$^{-2}$·hr$^{-1}$) |
|---|---|---|---|---|---|
| %$CO_2$ | $HCO_3$ (mM) | pH | Total $CO_2$ ($\mu$M) | | |
| 1 | 1.5 | 7.15 | <10 | 0.003 ± 0.011 | — |
| 1 | 0.0 | 5.50 | <10 | −0.011 ± 0.014 | — |
| 2 | 5.0 | 7.09 | 19.5 ± 1.6[a,k] | 0.160 ± 0.009[e,g,k] | 0.173 ± 0.014[e,g,k] |
| 2 | 0.0 | 5.50 | 20.2 ± 0.9[a,k] | 0.171 ± 0.012[e,h,k] | 0.180 ± 0.008[e,h,k] |
| 5 | 10.0 | 7.03 | 26.8 ± 1.7[b,k] | 0.228 ± 0.011[d,i,k] | 0.239 ± 0.015[f,i,k] |
| 5 | 0.0 | 5.50 | 24.9 ± 1.4[b,k] | 0.219 ± 0.014[d,i,k] | 0.221 ± 0.012[f,i,k] |

$I_{sc}$ was completely unaffected by contraluminal $CO_2/HCO_3$ changes in every preparation tested.

$\Delta J_H$· denotes the change in $J_H$· observed after the contraluminal bath was switched from the control $CO_2/HCO_3$-free saline (pH 7.00) to the various contraluminal treatments listed in the table and was calculated as: final $J_H$· measured in the presence of contraluminal $CO_2/HCO_3^-$ minus the initial $J_H$· measured under $CO_2/HCO_3$-free conditions. $J_{HCO_3}$ denotes the apparent rate of bicarbonate formation in the lumen and was calculated as final $HCO_3^-$ concentration minus initial $HCO_3^-$ concentration and expressed per $cm^2$ of tissue per hour. Luminal total $CO_2$ and $\Delta J_H$· measurements were made 1 hr after contraluminal $CO_2$ or $CO_2/HCO_3$ additions. The detection limit for total $CO_2$ measurement with the assay used in this study was 10 $\mu$M (*see* Materials and Methods); $J_{HCO_3}$ was not calculated when total $CO_2$ concentrations were below this threshold. Values are means ± SE; $n = 6$ for each treatment. The mean rate of rectal acidification ($J_H$·) measured under $CO_2/HCO_3$-free conditions (for all preparations included in this table) was 1.61 ± 0.09 $\mu$equiv·$cm^{-2}$·$hr^{-1}$.

[a,b,c,d,e,f] Values with common symbols not significantly different by Student's *t* test ($P > 0.05$).

[g,h,i,j] Values with common symbols not significantly different by paired *t* test ($P > 0.05$).

[k] Significantly different from control values (determined under bilateral $CO_2/HCO_3$-free conditions; bilateral pH 7.00) by paired *t* test ($P < 0.05$).

levels of metabolic $CO_2$ produced by the rectum under $CO_2/HCO_3^-$-free conditions could generate sufficient $HCO_3^-$ to account for the observed rates of rectal acidification.

It was possible to induce significant increases in $J_H$· and luminal total $CO_2$ concentration by increasing contraluminal $CO_2$ tensions to 2 or 5% (Table 1). Based on the increased total $CO_2$ content of the lumen 1 hr after contraluminal $CO_2/HCO_3$ addition, it is clear that $CO_2$ was entering the luminal bath faster than it could be removed (i.e., "blown off") by vigorous aeration with 100% $O_2$. As observed in the previous experiment with 1% $CO_2$ and 1.5 mM $HCO_3^-$, removal of contraluminal $HCO_3$ (while maintaining a constant $CO_2$ tension) had no effect on $J_H$·, $I_{sc}$ or luminal total $CO_2$ content (Table 1). This suggested that there was not a significant diffusional pathway for $HCO_3^-$ in the contraluminal to luminal direction under these experimental conditions and that the increase in luminal total $CO_2$ content was due solely to $CO_2$ diffusion across the epithelium.

Without concomitant measurements of pH*i* it is difficult to determine whether the apparent increases in $J_H$· ($\Delta J_H$·) were due to increased rates of $CO_2$ hydration in the lumen alone or in conjunction with increased rates of luminal proton secretion or $HCO_3^-$ reabsorption. If $\Delta J_H$· was augmented by elevated levels of proton secretion, the apparent rate of bicarbonate formation in the lumen ($J_{HCO_3}$) should have been consistently less than $\Delta J_H$·. Likewise if $\Delta J_H$· was due to bicarbonate reabsorption from the lumen, rates of apparent $HCO_3$ formation should again have been less than $\Delta J_H$·. Since $J_{HCO_3}$ is calculated as a change in luminal $HCO_3$ concentration, $J_{HCO_3}$ will only reflect true rates of $HCO_3$ formation if $HCO_3$ is not removed from the system. If $HCO_3$ is removed from the system (e.g., by epithelial transport), $J_{HCO_3}$ will underestimate actual rates of $HCO_3$ formation (by the rate at which $HCO_3$ is removed from the system) and will be less than the rate of proton addition to the system by $CO_2$ hydration (in this scenario estimated as $\Delta J_H$·). The results clearly do not support either of these hypotheses (Table 1). In all cases, apparent rates of bicarbonate formation ($J_{HCO_3}$) were not significantly different from the apparent increases in luminal acidification rates ($\Delta J_H$·). The simplest explanation for this observation is that $\Delta J_H$· was merely due to $CO_2$ hydration in the lumen and was not the result of an epithelial acid/base transport mechanism being stimulated by contraluminal (or luminal) $CO_2$ or the presence of luminal $HCO_3$. Since even measurable quantities of luminal $HCO_3$ did not appear to directly affect the apical acid/base transporter, it is difficult to believe that luminal acidification under bilateral $CO_2/HCO_3$-free conditions could be due to a bicarbonate-reabsorptive mechanism.

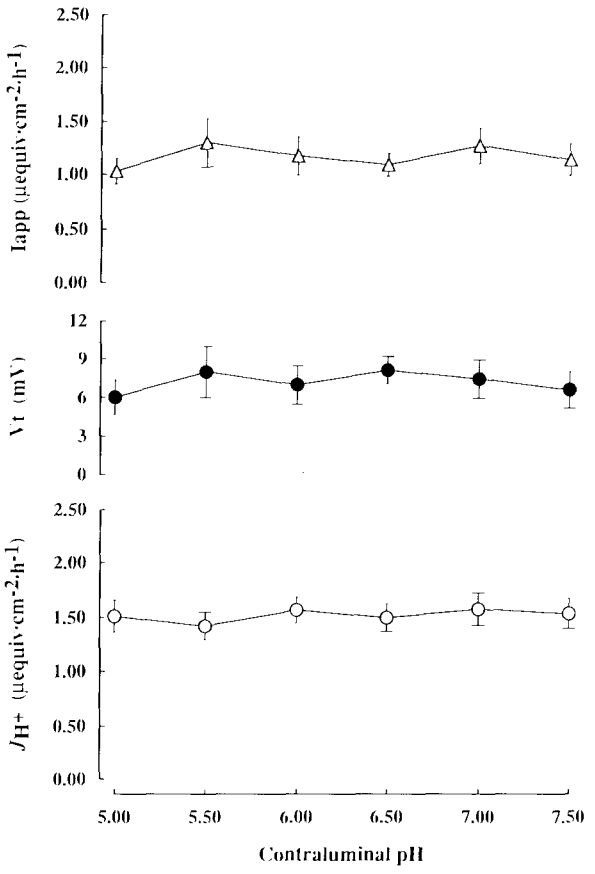



**Fig. 1.** Effect of contraluminal pH on $J_{H^+}$, $V_t$, and $I_{app}$. $J_{H^+}$, rate of rectal acidification. $V_t$, transepithelial potential (lumen relative to hemocoel). $I_{app}$, current required to clamp $V_t = 0$ mV (a positive value indicates net cation movement into or anion movement out of the lumen). All experiments performed with $V_t$ clamped at 0 mV; open-circuit values of $V_t$ determined at end of each experimental period. Values are means ± SE; $n = 6$ recta.

## Ionic Requirements of Rectal Acid Secretion

### *Sodium*

Although $Na^+$ is known to be actively reabsorbed from the rectal lumen at rates of 1.5 to 2.5 $\mu$equiv · cm $^{-2}$ · h $^{-1}$ (Black et al., 1987), the actual mechanisms by which $Na^+$ crosses the apical membrane are still largely unknown. Hanrahan and Phillips (1984*b*) have shown that under similar experimental conditions to those used in the present study (i.e., $I_{sc}$; bilateral 110 mM $Na^+$) there is an electrochemical potential of 123 mV favoring passive $Na^+$ uptake at the apical membrane. The magnitude of this electrochemical gradient conceivably represents a very significant energy source for driving active movements of other ions and solutes across the apical membrane (via $Na^+$ coupling). $Na^+/NH_4^+$ exchange (Thomson et al., 1988*b*) and $Na^+$/glycine cotransport (Balshin & Phillips, 1971) are the only $Na^+$-coupled mechanisms identified to date, and together account for less than 40% of net $Na^+$ uptake. Electrophysiological measurements made in the absence of luminal organic substrates suggest that close to 50% of the $Na^+$ crossing the apical membrane does so by an organic-independent electrogenic pathway (i.e., not amino acid or glucose coupled; Black et al., 1987). Although an apical $Na^+$ channel has been postulated, cotransport or exchange with other ions (apart from $Cl^-$; Black et al., 1987) has by no means been ruled out.

To test the possibility that luminal proton secretion might somehow be coupled to transepithelial $Na^+$ flux, recta were mounted in Ussing chambers and subjected to long-term bilateral $Na^+$ removal. All experiments were performed under short-circuit current conditions with $CO_2/HCO_3^-$-free salines (bilateral pH = 7.00). Long-term (4-hr) bilateral $Na^+$ replacement under these conditions caused a significant decrease (40%) in net acid secretion (Table 2). The fact that such a large component of $J_{H^+}$ remained after bilateral $Na^+$ removal suggests several possibilities: (i) the apical proton pump has a very high affinity for $Na^+$ and luminal $Na^+$ was not reduced to levels low enough to completely abolish $J_{H^+}$, (ii) the apical proton pump does not require luminal $Na^+$, but long-term bilateral $Na^+$ removal is affecting other epithelial parameters which subsequently indirectly affect $J_{H^+}$, or (iii) rectal acid secretion has several components, only one of which is $Na^+$ dependent.

Background $Na^+$ levels were continuously monitored in all $Na^+$-free salines used in this portion of the study. Perfusate samples ranged from 12 to 56 $\mu$M $Na^+$. To insure that bath $Na^+$ levels did not rise significantly, tissues were continuously perfused (bilaterally) at 10–14 ml/min, except for two brief periods when luminal flow was stopped for 15 min to allow a pH-stat measurement (*see* Table 2). Luminal saline samples taken immediately after each 15-min pH-stat sampling period contained from 13 to 69 $\mu$M $Na^+$ ($n = 6$ preparations). Hanrahan and Phillips (1984*b*) have reported that passive $Na^+$ entry across the apical membrane cannot continue once luminal $Na^+$ levels drop below 1 mM because the electrochemical gradient for $Na^+$ at the apical membrane decreases to the point where it can no longer drive $Na^+$ uptake. At the micromolar levels of luminal $Na^+$ maintained in the present study, $Na^+$ coupling could not energize active proton secretion into the lumen. If rectal acid secretion was entirely dependent on the energy potential supplied by the apical $Na^+$ electrochemical gradient, $J_{H^+}$ should have been

**Table 2.** Effect of bilateral and luminal $Na^+$ removal on rectal acid secretion ($J_{H^+}$) under short-circuit current conditions

| | $J_{H^+}$ ($\mu$equiv · cm$^{-2}$ · hr$^{-1}$) | $V_t$ (mV) | $I_{sc}$ ($\mu$equiv · cm$^{-2}$ · hr$^{-1}$) | $R_t$ ($\Omega$ cm$^2$) |
|---|---|---|---|---|
| Control[a] | 1.62 ± 0.11 | 6.9 ± 1.4 | 1.22 ± 0.24 | 211.1 ± 10.0 |
| 30-min Bilateral $Na^+$ replacement[a] | 1.17 ± 0.08[c] | 14.3 ± 1.8[c] | 2.02 ± 0.27[c] | 264.2 ± 10.7[c] |
| 4-hr Bilateral $Na^+$ replacement[a] | 0.98 ± 0.09[c] | 5.9 ± 0.7 | 1.26 ± 0.20 | 175.9 ± 8.5[c] |
| Control[b] | 1.54 ± 0.14 | 8.7 ± 1.3 | 1.35 ± 0.19 | 239.4 ± 9.5 |
| 30-min Luminal $Na^+$ replacement[b] | 1.34 ± 0.12[c] | 19.0 ± 1.1[c] | 2.30 ± 0.25[c,d] | 308.0 ± 11.4[c] |
| 4-hr Luminal $Na^+$ replacement[b] | 1.30 ± 0.11[c] | 17.8 ± 0.8[c] | 2.27 ± 0.24[c,d] | 292.5 ± 10.9[c] |

$J_{H^+}$, rate of rectal acidification. $V_t$, transepithelial potential (lumen relative to hemocoel; measured at end of each experimental period). $I_{sc}$, short-circuit current (a positive value indicates cation movement into or anion movement out of the lumen). $R_t$, transepithelial resistance (calculated from $V_t$ and $I_{sc}$ by Ohm's Law). Contraluminal pH was maintained at 7.00 by continuous perfusion; luminal pH was maintained at 7.00 by continuous perfusion except at 30 min and 4 hr after $Na^+$ removal when it was maintained at 7.00 by pH stat for 15-min sampling periods. Control values of $J_{H^+}$, $V_t$, $I_{sc}$, and $R_t$ were determined on each tissue immediately before bilateral or luminal $Na^+$ replacement. Values are mean ± SE; $n = 6$ for each treatment.

[a,b] Control and experimental values for 30-min and 4-hr time periods collected from same preparations.

[b] Contraluminal bath contained 110 mM $Na^+$ throughout the entire experimental period.

[c] Significantly different from control values by paired $t$ test ($P < 0.05$).

[d] Current required to clamp $V_t = 0$ in the presence of the transepithelial $Na^+$ gradient (i.e., not true $I_{sc}$).

abolished by luminal $Na^+$ removal. That this did not happen, indicates that the bulk of active luminal proton secretion is not coupled to apical $Na^+$ flux.

Hanrahan and Phillips (1984*a*) reported that long-term bilateral $Na^+$ removal reduced cAMP-stimulated $Cl^-$ flux in the locust rectum by 42%. They could find no relationship between $Cl^-$ movements and trace levels of $Na^+$ in the luminal bath or electrochemical gradients for $Na^+$ across the apical membrane. They concluded that the inhibitory effect of long-term $Na^+$ removal was probably due to a generalized nonspecific effect (e.g., reduced metabolite uptake, volume regulatory defect, or $pH_i$ perturbation) rather than a direct requirement of the Cl transport mechanism for $Na^+$.

This possibility was addressed in the present study by comparing transepithelial electrical parameters and rates of proton secretion measured at 30 min after bilateral $Na^+$ removal with the same parameters measured 4 hr after $Na^+$ removal (Table 2). Although $J_{H^+}$ did not change dramatically over the 3.5-hr period, $V_t$, $I_{sc}$, and $R_t$ were all much reduced after prolonged exposure to $Na^+$-free salines. These observations are consistent with those reported by Hanrahan and Phillips (1984*a*) and support their conclusion that long-term $Na^+$ removal has a generalized adverse effect on the epithelium as a whole.

To circumvent this problem, the above experiments (i.e., luminal $Na^+$ removal; $V_t$ clamped at 0 mV) were repeated with 110 mM $Na^+$ present in the contraluminal bath (Table 2). Background levels of $Na^+$ were monitored in the freshly prepared salines before each experiment and in the luminal bath immediately after each 15-min pH-stat sampling period (*see above*). Salines contained from 7 to 48 $\mu$M $Na^+$ before exposure to the tissues ($n = 6$ preparations) and from 32 to 73 $\mu$M $Na^+$ after the pH-stat sampling period. Although the trace levels of $Na^+$ increased significantly during the 15-min period when luminal perfusion was stopped, luminal $Na^+$ concentrations never rose to the point where the electrochemical gradient for $Na^+$ at the apical membrane favored passive $Na^+$ uptake (*see above*). $J_{H^+}$, $V_t$, and $R_t$ were all virtually unchanged over the 3.5-hr period over which measurements were taken, indicating that this protocol was effective in preventing the decrease in viability observed with long-term bilateral $Na^+$ removal. Under these conditions, net proton secretion was only reduced by 13–16% as compared to the 40% reduction in $J_{H^+}$ observed with bilateral $Na^+$ removal. The bulk of net luminal proton secretion (>80%) is clearly not coupled to transepithelial $Na^+$ fluxes and is not energized by the large electrochemical gradient for $Na^+$ at the apical membrane.

The results from the previous set of experiments raise the possibility that a significant fraction of rectal acid secretion (≈20%) might require luminal $Na^+$. The presence of a $Na^+/NH_4^+$ exchange mechanism on the apical membrane (Thomson et al., 1988*b*) and the occurrence of $Na^+/H^+$ exchangers in many of the acid/base transporting epithelia studied to date (reviewed by Seifter & Aronson, 1986), suggests that the ubiquitous $Na^+/H^+$ exchanger might also be present in the locust rectum. To explore this possibility further, recta were exposed to 1 mM amiloride under short-circuit current condi-

**Table 3.** Effect of amiloride on rectal acid secretion ($J_{H^+}$) under short-circuit current conditions

| | $J_{H^+}$ ($\mu$equiv · cm$^{-2}$ · hr$^{-1}$) | $V_t$ (mV) | $I_{sc}$ ($\mu$equiv · cm$^{-2}$ · hr$^{-1}$) |
|---|---|---|---|
| 110 mM $Na^+$ saline[a] | 1.49 ± 0.11[c,e] | 6.5 ± 0.8 | 0.98 ± 0.16 |
| + 1 mM Luminal amiloride | 1.21 ± 0.07[c] | 7.2 ± 1.2 | 1.01 ± 0.12 |
| 20 mM $Na^+$ saline[b] | 1.41 ± 0.09[d,e] | 4.7 ± 1.0 | 1.05 ± 0.11 |
| + 1 mM Luminal amiloride | 1.25 ± 0.10[d] | 5.1 ± 1.6 | 1.11 ± 0.09 |

[a] Tissues were brought to steady state in sulphate- and $CO_2/HCO_3^-$-free saline with an otherwise full ion complement (including 110 mM $Na^+$; *see* Materials and Methods).
[b] Tissues were brought to steady state in the same saline used in the previous trial except that bilateral $Na^+$ concentrations were 20 rather than 110 mM (the $Cl^-$ deficit was made up with choline chloride). Bilateral pH was maintained at 7.00 by contraluminal perfusion and luminal pH stat. $V_t$ was measured at the end of each experimental period. Control values of $J_{H^+}$, $V_t$, and $I_{sc}$ were determined on each tissue immediately before luminal addition of 1 mM amiloride. Values are mean ± SE; $n = 6$ for each treatment.
[c,d] Values marked with these common symbols were significantly different by paired $t$ test ($P < 0.05$).
[e] Values marked with this symbol were not significantly different by Student's $t$ test ($P > 0.50$).

tions in the presence of either 110 or 20 mM bilateral $Na^+$ (Table 3).[3] Consistent with the results of the previous experiment, amiloride inhibited $J_{H^+}$ by 11–19% (20 and 110 mM $Na^+$ salines, respectively; both inhibitions significant by paired $t$ test; $P < 0.05$). $V_t$ and $I_{sc}$ were completely unaffected by the addition of amiloride in either of the $Na^+$ salines tested. Taken together, the results from the amiloride and $Na^+$ substitution studies support a model in which 15–20% of net active proton secretion occurs by electroneutral $Na^+/H^+$ exchange on the apical membrane and the remainder occurs by an as yet unidentified proton secretory mechanism.

### *Potassium*

The bulk of net $K^+$ uptake in the rectum of the desert locust is passive and electrically coupled to active, electrogenic $Cl^-$ reabsorption (*see* Phillips et al., 1986 for review). Under open-circuit conditions, net $K^+$ flux is equivalent to net $Cl^-$ flux both before and during cAMP exposure (0.9 and 4.5 $\mu$equiv · cm$^{-2}$ · hr$^{-1}$, respectively; Hanrahan, 1982). Under short-circuit current conditions, active $K^+$ reabsorption in unstimulated recta has been estimated at 0 to 0.2 $\mu$equiv · cm$^{-2}$ · hr$^{-1}$ (Hanrahan & Phillips, 1983; Williams et al., 1977, respectively). The similarity of $J_{H^+}$ under both open- and short-circuit current conditions (Thomson et al., 1991), despite the changes in net $K^+$ flux under these same conditions, implies that active proton secretion is not directly coupled to net $K^+$ flux at the apical membrane.

Under both open- and short-circuit current conditions, the electrochemical gradient for $K^+$ across the apical membrane is near zero (Hanrahan & Phillips, 1984*b*), indicating that $K^+$-coupled influx could not provide the driving force for active proton secretion across the apical membrane (the electrochemical gradient opposing active proton secretion at the apical membrane is approximately 79 mV; Thomson et al., 1988*a*). However, the possibility remains that luminal potassium might be required for net acid secretion in a manner similar to that proposed for active $Cl^-$ reabsorption in the locust rectum (e.g., as an enzyme activator; reviewed by Phillips et al., 1986) or HCl secretion in the vertebrate gastric muscosa (reviewed by Forte & Machen, 1987).

To test the possibility that proton secretion might be dependent on the presence of luminal or contraluminal $K^+$, recta were mounted in Ussing chambers and subjected to long-term (4-hr) bilateral $K^+$ removal under short-circuit current conditions. Hanrahan and Phillips (1984*b*) have shown that under these conditions, $V_a$ and $V_b$ depolarize by 25–30 mV and intracellular $K^+$ activities fall from 61 to <5 mM. In the present study, this treatment reduced net acid secretion by approximately 65% (Table 4). Consistent with observations reported by Hanrahan and Phillips (1984*a*), $V_t$ and $I_{sc}$ were similar to control values after the 4-hr $K^+$-free period; $R_t$, on the other hand, was significantly increased (19% in this study as compared to 25% reported by Hanrahan & Phillips, 1984*a*). On the basis of these results and the findings of Hanrahan and Phillips (1984*b*), it does not appear that long-term $K^+$ removal reduces

[3] Due to the competitive nature of amiloride inhibition of $Na^+/H^+$ exchange, it is sometimes possible to improve the efficacy of inhibition by decreasing the concentration of the competing ion (in this case $Na^+$). It is important not to decrease the $Na^+$ concentration to a level where $Na^+/H^+$ exchange is noticeably affected before amiloride addition.

**Table 4.** Effect of bilateral and luminal $K^+$ removal on rectal acid secretion ($J_{H^+}$) under short-circuit current conditions

| | $J_{H^+}$ ($\mu$equiv · cm$^{-2}$ · hr$^{-1}$) | $V_t$ (mV) | $I_{sc}$ ($\mu$equiv · cm$^{-2}$ · hr$^{-1}$) | $R_t$ ($\Omega$ · cm$^2$) |
|---|---|---|---|---|
| Control[a] | 1.52 ± 0.17 | 5.7 ± 1.2 | 0.94 ± 0.20 | 228.3 ± 9.6 |
| Bilateral $K^+$ replacement[a] | 0.53 ± 0.03[c] | 4.1 ± 0.9 | 0.57 ± 0.22 | 271.0 ± 11.2 |
| Control[b] | 1.59 ± 0.15 | 6.6 ± 1.2 | 1.33 ± 0.17 | 186.2 ± 8.4 |
| Luminal $K^+$ replacement[b] | 1.12 ± 0.14[c] | 27.0 ± 2.1 | 4.06 ± 0.34[d] | 248.3 ± 12.9 |

Contraluminal pH was maintained at 7.00 by continuous perfusion; luminal pH was maintained at 7.00 by continuous perfusion until 4 hr after $K^+$ removal, after which time it was maintained at 7.00 by pH stat. Control values of $J_{H^+}$, $V_t$, $I_{sc}$, and $R_t$ were determined on each tissue immediately before bilateral or luminal $K^+$ replacement; all other measurements were made 4 hr after commencement of the experimental treatment. $R_t$ was calculated from Ohm's Law using $V_t$ and $I_{sc}$. Values are mean ± SE; $n = 6$ for each treatment.

[a,b] Control and experimental values from sample preparations.

[b] Contraluminal bath contained 10 mM $K^+$ throughout the entire experimental period.

[c] Significantly different from control values by paired $t$ test ($P < 0.01$).

[d] Current required to clamp $V_t = 0$ in the presence of the transepithelial $K^+$ gradient (i.e., not true $I_{sc}$).

$J_{H^+}$ simply by altering the electrochemical gradient opposing net acid secretion at the apical membrane. If this was the case, one would expect the depolarization of the apical membrane associated with bilateral $K^+$ removal to increase rather than decrease $J_{H^+}$ (assuming $pH_i$ was not indirectly affected by $K^+$ removal).

The possibility existed that the decrease in $J_{H^+}$ was the result of nonspecific effects associated with long-term $K^+$ removal rather than a direct effect on the $H^+$-translocating mechanism *per se* (similar to that postulated for long-term $Na^+$ removal). To test this hypothesis, $J_{H^+}$ was measured in recta where $K^+$ was removed from the luminal bath and contraluminal $K^+$ concentrations were maintained at 10 mM. Hanrahan and Phillips (1984*b*) reported that intracellular levels of $K^+$ did not decrease significantly under these conditions. In the present study, $J_{H^+}$ decreased by only 30% when luminal $K^+$ was removed and contraluminal $K^+$ was left at 10 mM (significant by paired $t$ test; $P < 0.01$; Table 4). $V_t$ and $R_t$ were significantly greater than corresponding control values. The substantial increase in the current required to clamp $V_t = 0$ was due to the $K^+$ diffusion potential generated by the enlarged concentration gradient for $K^+$ across the apical membrane when luminal $K^+$ was removed (*see* Hanrahan, 1982). Again, there does not appear to be any relationship between the electrochemical driving force for $K^+$ across the apical membrane and net proton movements.

Background levels of $K^+$ were monitored carefully to ensure that $K^+$ contamination of nominally $K^+$-free salines did not occur. Luminal and contraluminal bath samples taken immediately before the pH-stat measurement period (*see* previous section) ranged from 15 to 45 $\mu$M $K^+$ ($n = 6$). Saline samples taken from the luminal bath immediately after the 15-min pH-stat measurement period ranged from 51 to 87 $\mu$M $K^+$ ($n = 6$). This increase was significant by paired $t$ test ($P < 0.01$), but the electrochemical gradient for $K^+$ at the apical membrane still did not favor $K^+$ uptake and could not drive active proton secretion into the lumen. Although the bulk of active proton secretion is clearly not dependent on luminal $K^+$, a significant fraction of $J_{H^+}$ (30%) was affected when luminal $K^+$ was removed. At this point, it is not apparent whether the $K^+$-dependent fraction can be attributed to generalized nonspecific effects of luminal $K^+$ removal, a stimulatory role for luminal $K^+$ similar to that observed for $Cl^-$ uptake, or simply a small $K^+$-coupled component of net acid secretion.

Apical, electroneutral $H^+/K^+$ exchange has been described in a number of vertebrate gastric mucosae (*see* Forte & Machen, 1987 for review). In many of these preparations, the substituted benzimidazole, omeprazole, has been shown to significantly inhibit both rates of acid secretion and net $K^+$ reabsorption. In an effort to identify the nature of the remaining $K^+$-dependent proton flux, 1 mM omeprazole was added bilaterally to recta mounted in Ussing chambers and bathed bilaterally in the standard $CO_2/HCO_3^-$-free saline used throughout this study. As evident from Table 5, this treatment had absolutely no effect on $V_t$, $I_{sc}$, or $J_{H^+}$. Although the lack of an effect does not necessarily rule out the existence of such a mechanism, taken with the incomplete inhibition of $J_{H^+}$ observed with bilateral $K^+$ removal, it is at least consistent with the conclusion that the bulk of net acid secretion in the locust rectum occurs by a $K^+$-independent mechanism.

**Table 5.** Effect of omeprazole on rectal acid secretion ($J_{H^+}$) under short-circuit current conditions

| | $J_{H^+}$ ($\mu$equiv · cm$^{-2}$ · hr$^{-1}$) | $V_t$ (mV) | $I_{sc}$ ($\mu$equiv · cm$^{-2}$ · hr$^{-1}$) |
|---|---|---|---|
| Control | 1.62 ± 0.12 | 8.4 ± 1.1 | 1.44 ± 0.17 |
| + 1 mM Bilateral omeprazole[a] | 1.66 ± 0.12[b] | 7.8 ± 1.0[b] | 1.35 ± 0.17[b] |

Control values of $J_{H^+}$, $V_t$, and $I_{sc}$ were determined on each tissue immediately before bilateral omeprazole addition.

[a] Measurements made 1 hr after bilateral omeprazole addition. Values are mean ± SE; $n = 6$ for each treatment.

[b] Values not significantly different from control by paired $t$ test ($P > 0.2$).

*Chloride*

$Cl^-$ is actively reabsorbed from the lumen of the locust rectum by a cAMP-stimulated electrogenic transport mechanism located on the apical membrane (reviewed by Phillips et al., 1986). Under unstimulated short-circuit current conditions, net $Cl^-$ uptake has been estimated at 0.85 $\mu$equiv · cm$^{-2}$ · hr$^{-1}$; contraluminal addition of 1 mM cAMP causes net $Cl^-$ flux to increase to 9.46 $\mu$equiv · cm$^{-2}$ · hr$^{-1}$ (Hanrahan & Phillips, 1984*a*). Although Hanrahan (1982) has shown that the cAMP-stimulated component of transepithelial net $Cl^-$ flux is not linked to transepithelial proton gradients, he has not ruled out the possibility that acid/base transport in the rectum might somehow be dependent on local $Cl^-$ movements across either the apical or basolateral membrane. The widespread involvement of $Cl^-$ in acid/base transport in a number of different cell types (e.g., mosquito salt gland: Strange & Phillips, 1985; turtle urinary bladder: Fischer, Husted & Steinmetz, 1983; erythrocytes: Knauf, 1987) suggests that this possibility be carefully considered in the locust rectum.

The first step in assessing the role of chloride in active proton secretion was the bilateral removal of $Cl^-$ under both open- and short-circuit current conditions (salines were $CO_2/HCO_3^-$-free; bilateral pH = 7.00). In light of the extremely high $Cl^-$ affinity observed for the $Cl^-/HCO_3^-$ exchanger found on the basolateral membrane of the turtle bladder ($K_m$ = 200 $\mu$M; Fischer et al., 1983), it was imperative that background $Cl^-$ levels in the nominally "$Cl^-$-free" salines used in the present study remain low throughout the entire experimental period. Luminal and contraluminal bath samples taken immediately after measurements of $J_{H^+}$ ranged from 24 to 61 $\mu$M $Cl^-$ ($n$ = 6 preparations). Four-hr bilateral removal of chloride had no significant effect on net proton secretion in unstimulated recta under both open- and short-circuit current conditions (Table 6). Although $V_t$ and $I_{sc}$ were very similar to control values for both treatments, $R_t$ increased significantly ($P < 0.01$; paired $t$ test) after bilateral $Cl^-$ removal under both open- and short-circuit current conditions. Unless $Cl^-$ affinities are submicromolar, it would appear that active proton secretion (under $CO_2/HCO_3^-$-free conditions) has no requirement whatsoever for extracellular $Cl^-$. Hanrahan and Phillips (1983) have reported that intracellular $Cl^-$ activities in the locust rectum fell to < 5 mM after exposure to $Cl^-$-free salines for 3 hr. Considering that recta in the present study were exposed to $Cl^-$-free salines for 4 hr before measurement of $J_{H^+}$, intracellular $Cl^-$ activities would be expected to fall to at least the same levels, if not lower. Based on this assumption and the lack of effect of $Cl^-$ removal on $J_{H^+}$, it would appear that active proton secretion also has no requirement for intracellular chloride.

*Divalent Cations*

Mechanisms of $Ca^{2+}$ and $Mg^{2+}$ uptake in the rectum of the desert locust are completely unknown. Rates of net absorption of both $Ca^{2+}$ and $Mg^{2+}$ were too low to be measured reliably in everted rectal sacs by atomic absorption (reviewed by Phillips et al., 1986). Nevertheless, considering the widespread involvement of calcium in cellular control processes and magnesium in enzyme function and protein synthesis, one would expect that closely regulated membrane transport mechanisms for these ions exist in most cell types. If the large fraction of active proton secretion in the locust rectum unaccounted for by $Na^+$, $K^+$, or $Cl^-$ dependence occurs by an electroneutral mechanism, proton movements across the apical membrane must be linked to movements of either $Ca^{2+}$ or $Mg^{2+}$. To test this possibility, recta were mounted in Ussing chambers and exposed to $Ca^{2+}$- or $Mg^{2+}$-free salines for 1 hr under short-circuit current conditions (Table 7). Bilateral removal of $Ca^{2+}$ (with or without 5 mM EGTA) had no significant effects on rectal acid secretion. However,

**Table 6.** Effect of bilateral $Cl^-$ removal on rectal acid secretion ($J_{H^+}$) under open- and short-circuit current conditions

| | $J_{H^+}$ ($\mu$equiv · cm$^{-2}$ · hr$^{-1}$) | $V_t$ (mV) | $I_{sc}$ ($\mu$equiv · cm$^{-2}$ · hr$^{-1}$) | $R_t$ ($\Omega \cdot cm^2$) |
|---|---|---|---|---|
| Control[a] | | | | |
| $I_{sc}$ conditions | 1.69 ± 0.11 | 8.0 ± 1.3[f] | 1.19 ± 0.17 | 251.4 ± 8.8[d] |
| Bilateral $Cl^-$ replacement[a] | | | | |
| $I_{sc}$ conditions | 1.76 ± 0.11[c] | 9.2 ± 1.1[f] | 1.02 ± 0.18 | 337.2 ± 9.5[d] |
| Control[b] | | | | |
| Open-circuit conditions | 1.58 ± 0.13 | 5.6 ± 1.5 | 0.96 ± 0.21[g] | 218.2 ± 9.7[e] |
| Bilateral $Cl^-$ replacement[b] | | | | |
| Open-circuit conditions | 1.53 ± 0.09[c] | 6.6 ± 1.7 | 0.83 ± 0.20[g] | 297.6 ± 10.1[e] |

Contraluminal pH was maintained at 7.00 by continuous perfusion; luminal pH was maintained at 7.00 by continuous perfusion until 4 hr after $Cl^-$ removal, after which time it was maintained at 7.00 by pH stat. Control values of $J_{H^+}$, $V_t$, $I_{sc}$, and $R_t$ were determined on each tissue immediately before bilateral $Cl^-$ replacement; all other measurements were made 4 hr after commencement of the experimental treatment. Values are mean ± SE; $n = 6$ for each treatment.

[a,b] Control and experimental values from same tissues.

[c] Not significantly different from control values by paired $t$ test ($P > 0.3$).

[d] Calculated from $V_t$ and $I_{sc}$ using Ohm's Law.

[e] Determined by passing transepithelial constant current pulsed (13.6 $\mu$A).

[f] Measured at end of each treatment period.

[g] Calculated from $V_t$ and $R_t$ using Ohm's Law.

**Table 7.** Effect of bilateral removal of $Ca^{2+}$ or $Mg^{2+}$ on rectal acid secretion ($J_{H^+}$) under short-circuit current conditions

| | $J_{H^+}$ ($\mu$equiv · cm$^{-2}$ · hr$^{-1}$) | $V_t$ (mV) | $I_{sc}$ ($\mu$equiv · cm$^{-2}$ · hr$^{-1}$) | $R_t$ ($\Omega \cdot cm^2$) |
|---|---|---|---|---|
| Control[a] | 1.38 ± 0.16 | 8.1 ± 0.9 | 1.05 ± 0.19 | 289.1 ± 6.6 |
| Bilateral $Ca^{2+}$ replacement[a] | 1.25 ± 0.14[e] | 10.9 ± 1.4[d] | 1.43 ± 0.21[d] | 284.6 ± 6.1 |
| Control[b] | 1.24 ± 0.11 | 6.6 ± 1.1 | 0.82 ± 0.13 | 301.8 ± 9.7 |
| Bilateral $Ca^{2+}$ replacement[b] + 5 mM EGTA | 1.20 ± 0.11[e] | 7.8 ± 1.2[d] | 0.95 ± 0.15[d] | 307.9 ± 9.4 |
| Control[c] | 1.48 ± 0.20 | 11.1 ± 2.2 | 1.29 ± 0.18 | 319.2 ± 8.8 |
| Bilateral $Mg^{2+}$ replacement[c] | 1.52 ± 0.18[e] | 10.2 ± 1.7 | 1.18 ± 0.16 | 315.8 ± 8.5 |

Contraluminal pH was maintained at 7.00 by continuous perfusion; luminal pH was maintained at 7.00 by continuous perfusion until 1 hr after either $Ca^{2+}$ or $Mg^{2+}$ removal, after which time it was maintained at 7.00 by pH stat. Control values of $J_{H^+}$, $V_t$, $I_{sc}$, and $R_t$ were determined on each tissue immediately before bilateral ion replacement; all other measurements were made 1 hr after commencement of the experimental treatment.

[a,b,c] Control and experimental values from same tissues. Values are mean ± SE; $n = 6$ recta for each treatment.

[d] Significantly different from control values by paired $t$ test ($P < 0.01$).

[e] Not significantly different from control values by paired $t$ test ($P > 0.1$).

a small, but consistently significant increase in $V_t$ and $I_{sc}$ was observed immediately after $Ca^{2+}$ removal; control values of $V_t$ and $I_{sc}$ were likewise restored immediately after return to control salines with the full ion complement. Although the increases in $V_t$ and $I_{sc}$ are clearly not related to net acid secretion, they are consistent with an increase in net cation movement into the lumen (loss of intracellular $Ca^{2+}$?) or an increase in net anion uptake from the lumen (increase of $Cl^-$ reabsorption under unstimulated conditions?). Bilateral removal of magnesium had no significant effects on $J_{H^+}$, $V_t$, $I_{sc}$, or $R_t$ under these experimental conditions.

Unless a very subtle form of local ion recycling is involved (with extremely low ion affinities), it would appear that the bulk of net active proton secretion in the locust rectum is not directly dependent on the presence of $Na^+$, $K^+$, $Cl^-$, $Ca^{2+}$, or $Mg^{2+}$ under short-circuit current conditions. The results from the ion-substitution studies as a whole consistently support the conclusion that the bulk of active proton secretion across the apical membrane

(apart from the small component of $Na^+/H^+$ exchange) occurs by an electrogenic transport mechanism.

## Effect of Transepithelial Electrochemical Gradients on Rectal Acid Secretion

Measurements of $J_{H^+}$ have thus far been largely confined to conditions where both luminal and contraluminal pH have been set to 7.00. Although this arrangement is essential for obtaining accurate measurements of rectal acid secretion under true short-circuit current conditions (i.e., no transepithelial electrochemical gradients), it does not necessarily reflect the conditions found in the intact animal. Phillips (1961) and Speight (1967) consistently measured significant transepithelial pH gradients in vivo with rectal pH values often less than 5.0. To study the effect of similar pH gradients on rectal acid secretion in vitro (under $CO_2/HCO_3^-$-free conditions), isolated recta mounted in Ussing chambers were exposed to a series of luminal and contraluminal pH changes. To obviate the effects of associated transepithelial electrical gradients, experiments were conducted with $V_t$ clamped at 0 mV, except for brief intervals to allow measurement of open-circuit transepithelial membrane potentials.

In the first series of experiments, luminal pH was maintained at 7.00 by pH stat and contraluminal pH was varied from 5.00 to 7.50 by rapid perfusion (15 ml/min). This manipulation had no effect on $J_{H^+}$, $V_t$, or $I_{app}$ (the current required to clamp $V_t$ = 0 mV) over the entire range of contraluminal pH values tested (Fig. 1). This observation suggests that under these conditions, there is not a significant paracellular leak pathway for protons (or hydroxyl ions) in this epithelium. Moreover, it implies that either the basolateral border is extremely impermeable to $H^-/OH^-$ or that $pH_i$ is regulated very closely by some unidentified mechanism located at the basolateral membrane.

In the second set of experiments, contraluminal pH was maintained at 7.00 by continuous perfusion and luminal pH was varied by a combination of rapid perfusion (15 ml/min) and pH stat. $J_{H^+}$ decreased linearly to zero as luminal pH was lowered from 7.00 to 5.25 (Fig. 2; the actual point at which $J_{H^+}$ equaled zero is estimated at 5.27 by regression analysis; $r^2 = 0.9904$). Further luminal pH reductions beyond 5.25 forced net acid movements in the opposite direction (i.e., net acid absorption). Based on the observations in the previous set of experiments, it appears that acid influx must have been occurring across the apical membrane rather than through an unspecified paracellular route. It is not clear at this

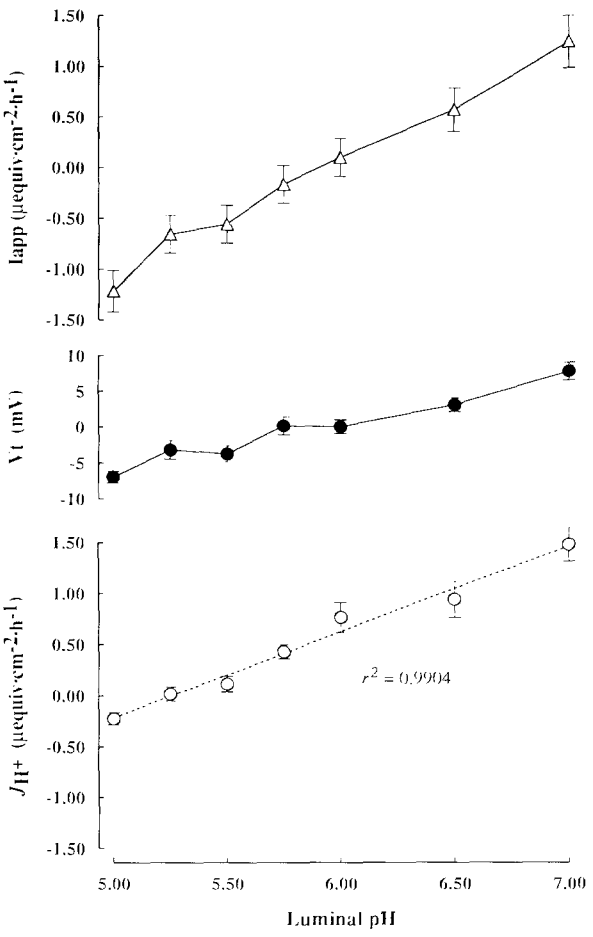



**Fig. 2.** Effect of luminal pH on $J_{H^+}$, $V_t$, and $I_{app}$. All experiments performed with $V_t$ clamped at 0 mV; open-circuit values of $V_t$ determined at end of each experimental period. Values are means ± SE; $n$ = 6 recta. Dashed line fit by regression analysis to facilitate determination of apparent PMF (least-squares method; $r^2$, coefficient of determination).

point, however, whether the acid influx was occurring through the apical proton transport mechanism *per se* (i.e., operating in reverse) or an unidentified parallel pathway across the apical membrane.

Under certain conditions it is possible to estimate the true force of an active ion pump from the transmembrane electrochemical gradient at which net flux equals zero. An accurate estimate requires a low parallel conductance for the ion in question and a reliable measurement of the transmembrane electrochemical gradient. Since there are no measurements of $pH_i$, $V_a$, or parallel proton conductance for the conditions used in this portion of the study, it is difficult to assess how accurately an apparent proton motive force (PMF) calculated from transepithelial rather than transmembrane parameters reflects the true PMF of the proton pump on the apical

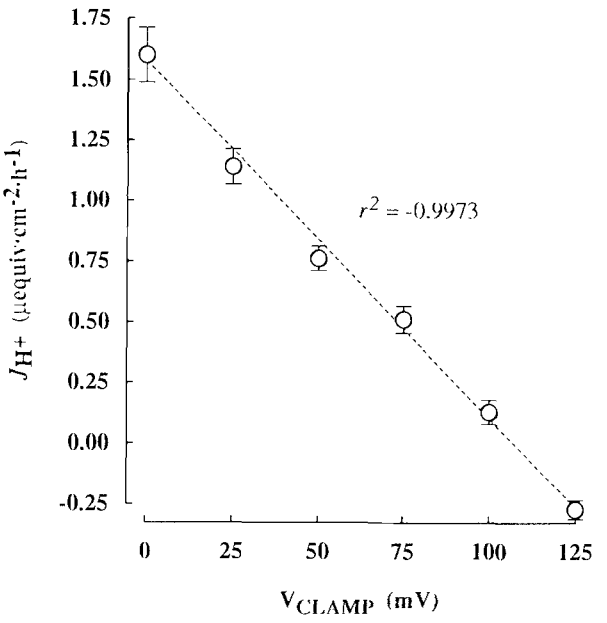



**Fig. 3.** Effect of transepithelial membrane potential on net acid secretion. $V_{CLAMP}$, transepithelial potential set by voltage clamp (lumen relative to hemocoel). Contraluminal pH maintained at 7.00 by continuous perfusion; luminal pH maintained at 7.00 by pH stat. Values are means ± SE; $n$ = 6 recta. Dashed line fit by regression analysis (least-squares method; $r^2$, coefficient of determination).

membrane. Nevertheless, one could argue that a PMF based on net transport rates rather than actual pump rates *per se* is a better indicator of the proton transport capabilities of the epithelium as a whole. An 'apparent' transepithelial PMF of 101 mV was calculated from a regression analysis of the data in Fig. 2.

When luminal pH was reduced from 7.00 to 5.00 in the previous set of experiments, $V_t$ and $I_{app}$ decreased concomitantly with $J_{H^-}$ (Fig. 2), indicating that this maneuver was affecting net charge movement across the apical membrane. If the changes in $V_t$ and $I_{app}$ were at least partially due to proton-conductive movements (in either direction), it should be possible to abolish net proton flux by increasing the opposing electrical gradient across the epithelium. This hypothesis was tested by voltage clamping recta at progressively greater transepithelial potentials (lumen positive) in the absence of a transepithelial pH gradient and measuring resultant rates of luminal acid secretion. Under these conditions, $J_{H^-}$ decreased linearly to zero as the luminal bath was made progressively more positive (Fig. 3). A regression analysis of the data estimates the transepithelial potential at which $J_{H^-} = 0$ (the apparent transepithelial PMF) to be 107 mV ($r^2 = -0.9973$). As observed with the pH gradient study above, when the electrical gradient was increased beyond the apparent PMF, net acid movement was forced to proceed in the opposite direction (i.e., absorption). The modulating effect of applied electrical or pH gradients, the very good agreement between the apparent PMF values measured under the two different conditions (i.e., electrical or chemical transepithelial gradients), and the ionic independence of $J_{H^+}$ consistently support a model in which the bulk of net acid secretion occurs via an apical electrogenic proton-translocating mechanism.

## Electrogenicity of Rectal Acid Secretion

The decrease in net acid secretion induced by application of a transepithelial pH gradient is paralleled by changes in transepithelial membrane potential and the current required to clamp $V_t$ at 0 mV (*see* Fig. 2). This and the ionic independence of $J_{H^-}$ under short-circuit current conditions intimates that the changes in $V_t$ and $I_{app}$ induced by transepithelial pH gradients are largely due to changes in net proton-conductive movements across the apical membrane. This proposal was investigated by monitoring apical and basolateral membrane potentials and transepithelial resistance (with intracellular microelectrodes) both before and after application of transepithelial pH gradients. All experiments were performed under open-circuit conditions with $CO_2$/$HCO_3$ -free salines. Although contraluminal pH was maintained at 7.00 at all times, luminal pH was rapidly changed from 7.00 to the pH value required to reduce net proton secretion to zero (i.e., the apparent PMF). Luminal bath changes were complete in less than 5 sec (as judged by dye removal; perfusion flow rate 55–60 ml/min; chamber volume 1.5 ml), and luminal pH was maintained at the value required to abolish net acid secretion for less than 20 sec at a time to guard against large shifts in $pH_i$. Under these conditions, reductions in luminal pH induced significant depolarizations of $V_t$, $V_a$, and $V_b$ which could be completely reversed by restoring luminal pH to 7.00 (*see* Fig. 4 for typical trace).

To determine if a component of the $V_t$ and $I_{app}$ changes could be attributed to pH effects on other ion conductances, these experiments were also performed in the absence of luminal $Na^+$, $K^+$, or $Cl^-$ (full ion complement maintained in the contraluminal bath at all times). Since luminal ion substitutions under open-circuit conditions substantially altered transepithelial membrane potentials, it was necessary to do a preliminary series of $J_{H^-}$ measurements under varying transepithelial pH gradients with each ion substitution to determine the respective luminal pH value at which net acid flux equaled zero (*see*

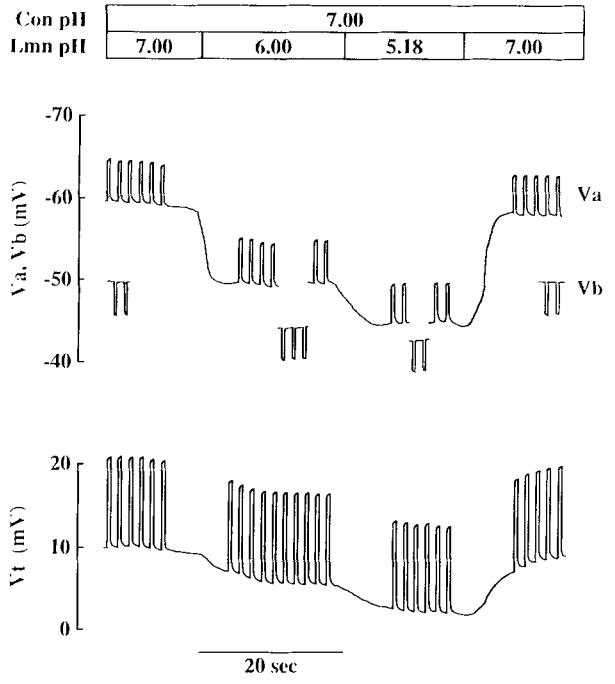



**Fig. 4.** Typical recording of $V_a$, $V_b$, and $V_t$ under open-circuit conditions when luminal pH was decreased stepwise from 7.00 to the value required to abolish net acid secretion. *Con*, contraluminal. *Lmn*, luminal. $V_a$ and $V_b$, apical and basolateral membrane potentials (cytoplasm relative to luminal or contraluminal bath, respectively). Voltage deflections are the result of transepithelial constant current pulses used to calculate voltage divider ratios and transepithelial resistance (13.2 $\mu$A; pulse frequency: 0.7 Hz; pulse duration: 0.5 sec).

Table 8). Calculated apparent PMF values under these conditions (i.e., transepithelial pH gradient; open circuit) agree well with those observed in earlier experiments when $V_t$ was clamped at 0 mV (*see* Table 8 and Fig. 2 for comparison).

$V_t$, $V_a$, $V_b$, $R_t$, and $I_{app}$ were all significantly reduced when luminal pH was decreased from 7.00 to the value required to abolish net acid secretion (with every treatment tested; Table 9). The similarity of the reductions in each of these parameters between the control and ion-substituted salines suggests that the changes are largely due to $H^+/OH^-$-conductive movements as predicted, rather than altered apical-conductive movements of $Na^+$, $K^+$, or $Cl^-$. Moreover, as predicted for a model in which the bulk of net acid secretion occurs by a primary electrogenic proton-translocating mechanism, the electrically determined values of $J_{H^+}$ (in this case estimated by $\Delta I_{app}$) agree remarkably well with $J_{H^+}$ values determined by the pH-stat method (*see* Tables 2, 4, and 6 for comparison).

The application of a transepithelial pH gradient could abolish net electrogenic proton secretion by

**Table 8.** Transepithelial pH gradients required to abolish net acid secretion after luminal ion substitution under open-circuit conditions

| Luminal bath | | $J_{H^+}$ ($\mu$equiv · cm$^{-2}$ · hr$^{-1}$) | $V_t$ (mV) | PMF (mV) |
|---|---|---|---|---|
| Saline | pH | | | |
| Control | 5.18 | −0.02 ± 0.01 | 0.4 ± 0.3 | 107 |
| $Na^+$-free | 5.40 | −0.01 ± 0.01 | 8.3 ± 1.2 | 102 |
| $K^+$-free | 5.44 | 0.01 ± 0.01 | 19.7 ± 1.6 | 111 |
| $Cl^-$-free | 5.37 | 0.03 ± 0.02 | −0.1 ± 0.3 | 95 |

Negative values of $J_{H^+}$ indicate net proton movements in the opposite direction (i.e., absorption). PMF, transepithelial proton motive force (calculated from mean $V_t$ values and transepithelial pH gradients). All experiments were performed under open-circuit conditions. The contraluminal surface of each epithelium was continuously perfused with the standard $CO_2/HCO_3^-$-free saline (full ion complement; pH 7.00) in all treatments. Luminal bath changes made by rapid perfusion (*see* text for details). Recta were exposed to each transepithelial pH gradient for 10–15 min to allow accurate measurements of $J_{H^+}$. Values are mean ± SE; $n = 6$ for each treatment.

directly inhibiting the proton pump *per se* or by increasing the conductive movement of protons in the opposite direction (i.e., increased backflux). If the abolition of $J_{H^+}$ in the locust rectum was due solely to a direct inhibition of the electrogenic proton pump, one would expect to see a decrease in $V_t$ and an increase in $R_t$. Under the present experimental conditions, $R_t$ did not increase concomitantly with the decrease in $V_t$ as predicted. Rather, $R_t$ decreased by 5–7 $\Omega \cdot cm^2$ in every preparation examined (significant by paired $t$ test; $P < 0.001$; *see* Table 9). The similarity of the change in $R_t$, regardless of luminal ion substitutions, suggests that the decrease in $J_{H^+}$ was at least partially due to an increased backflux of protons from the lumen. If this is true, $J_{H^+}$ will be an underestimate of the actual pump secretion rates.

### Effect of Putative Acid/Base Transport Inhibitors on Luminal Acid Secretion

Acetazolamide (ACTZ) has been shown to significantly inhibit net acid/base transport in a wide variety of epithelial cell types (e.g., mosquito salt gland, Strange & Phillips, 1984; turtle bladder, Steinmetz, 1969; mammalian gastric mucosa, reviewed by Forte & Machen, 1987). ACTZ is a potent inhibitor of carbonic anhydrase activity and has in the past been proposed to inhibit net acid secretion by forcing the accumulation of hydroxyl equivalents behind the ACTZ-blocked enzyme (the resulting increase in $pH_i$ was proposed to be the factor responsible for inhibi-

**Table 9.** Effect of transepithelial pH gradients and luminal ion substitutions on membrane potentials, $R_t$, and $I_{app}$

| Luminal bath | | $V_t$ (mV) | $V_a$ (mV) | $V_b$ (mV) | $R_t$ ($\Omega \cdot cm^2$) | $I_{app}$ ($\mu equiv \cdot cm^{-2} \cdot hr^{-1}$) |
|---|---|---|---|---|---|---|
| Saline | pH | | | | | |
| Control | 7.00 | 8.5 ± 0.5 | 59.7 ± 1.1 | 51.2 ± 1.2 | 188.0 ± 4.9 | 1.72 ± 0.08 |
| Control | 5.18 | 0.7 ± 0.6 | 44.6 ± 0.8 | 44.0 ± 1.2 | 183.0 ± 6.7 | 0.17 ± 0.11 |
| **Δ Control**[a] | | **−7.8 ± 0.4** | **−15.1 ± 0.8** | **−7.2 ± 0.6** | **−5.0 ± 0.5** | **−1.55 ± 0.07** |
| $Na^+$-free | 7.00 | 19.3 ± 0.8 | 62.6 ± 1.1 | 43.2 ± 0.6 | 312.6 ± 5.7 | 2.33 ± 0.11 |
| $Na^+$-free | 5.40 | 6.7 ± 0.7 | 43.6 ± 1.2 | 37.2 ± 0.7 | 306.9 ± 5.6 | 0.81 ± 0.09 |
| **Δ $Na^+$-free**[a] | | **−12.6 ± 0.7** | **−19.0 ± 0.9** | **−6.0 ± 0.5** | **−5.7 ± 0.6** | **−1.52 ± 0.08**[b] |
| $K^+$-free | 7.00 | 30.1 ± 0.9 | 89.6 ± 1.0 | 59.5 ± 0.9 | 249.5 ± 7.6 | 4.49 ± 0.13 |
| $K^+$-free | 5.44 | 22.5 ± 0.7 | 75.0 ± 2.1 | 52.7 ± 1.9 | 242.6 ± 8.4 | 3.46 ± 0.15 |
| **Δ $K^+$-free**[a] | | **−7.6 ± 0.6** | **−14.6 ± 0.9** | **−6.8 ± 0.7** | **−6.9 ± 0.8** | **−1.03 ± 0.10**[c] |
| $Cl^-$-free | 7.00 | 10.2 ± 0.4 | 62.2 ± 1.9 | 51.9 ± 1.2 | 252.0 ± 4.3 | 1.51 ± 0.05 |
| $Cl^-$-free | 5.37 | 0.5 ± 0.3 | 43.8 ± 1.0 | 43.2 ± 0.9 | 244.4 ± 3.8 | 0.08 ± 0.04 |
| **Δ $Cl^-$-free**[a] | | **−9.7 ± 0.3** | **−18.4 ± 0.8** | **−8.7 ± 0.6** | **−7.6 ± 0.8** | **−1.43 ± 0.03**[d] |

$V_a$ and $V_b$, apical and basolateral membrane potentials (cytoplasm relative to luminal or contraluminal bath, respectively). $R_t$ calculated from deflections in $V_t$ produced by transepithelial constant current pulses. $I_{app}$, applied current which would be required to clamp $V_t$ = 0 mV (calculated from $V_t$ and $R_t$). All experiments were performed under open-circuit conditions. The contraluminal surface of each epithelium was continuously bathed with the standard $CO_2/HCO_3^-$-free saline (full ion complement; pH 7.00) in all treatments.

[a] Values represent the mean difference ± SE of the respective parameters at pH 7.00 and pH 5.20. All other values are means ± SE; $n$ = 40–85 cells per treatment (six recta per treatment). All parameters measured at the luminal pH values required to abolish $J_{H^+}$ were significantly different than their respective values measured at pH 7.00 in every treatment tested (paired $t$ test; $P < 0.001$).

[b,d] Not significantly different from control by Student's $t$ test ($P > 0.10$).

[c] Significantly different from control by Student's $t$ test ($P < 0.001$).

**Table 10.** Effect of acetazolamide on rectal acid secretion ($J_{H^+}$) under short-circuit current conditions

| | $J_{H^+}$ ($\mu equiv \cdot cm^{-2} \cdot hr^{-1}$) | $V_t$ (mV) | $I_{sc}$ ($\mu equiv \cdot cm^{-2} \cdot hr^{-1}$) |
|---|---|---|---|
| Control | 2.08 ± 0.18 | 5.8 ± 1.1 | 0.99 ± 0.13 |
| + 1 mM Bilateral acetazolamide[a] | 1.28 ± 0.11[b] | 7.5 ± 1.4[b] | 1.21 ± 0.15[b] |

$V_t$ was measured at the end of each experimental period. Control values of $J_{H^+}$, $V_t$, and $I_{sc}$ were determined on each tissue immediately before bilateral acetazolamide addition.

[a] Measurements made 1 hr after bilateral acetazolamide addition. Values are mean ± SE; $n$ = 6 for each treatment.

[b] Values significantly different from controls by paired $t$ test ($P < 0.05$).

tion of net acid secretion). The effect of this inhibitor on net proton secretion in the locust rectum was investigated by exposing isolated recta (mounted in Ussing chambers; $CO_2/HCO_3^-$-free saline; bilateral pH 7.00) to 1 mM bilateral ACTZ. Although this treatment reduced $J_{H^+}$ by approximately 39%, both $V_t$ and $I_{sc}$ increased slightly (all changes significant by paired $t$ test; $P < 0.05$; Table 10). If the ACTZ effect was restricted to inhibition of electrogenic proton secretion, one would expect to see a decrease in $V_t$ and $I_{sc}$ concomitant with the decline in the activity of the electrogenic proton pump (unless the inhibitory effect was restricted to the $Na^+/H^+$ exchanger). That both $V_t$ and $I_{sc}$ increased with application of this compound suggests that conductive pathways for other ions are also affected.

The mechanisms of contraluminal alkalinization (Thomson et al., 1991) in the locust rectum are as yet unknown. Measurements of electrochemical gradients for protons across the basolateral membrane indicate that an active step at that membrane is not required to drive the observed rates of contraluminal alkalinization (Thomson et al., 1988*a*). Preliminary studies with rectal sacs (incubated in $CO_2/HCO_3^-$-containing salines) have shown that contraluminal application of 1 mM DIDS completely inhibits $CO_2/HCO_3^-$ uptake from the rectal lumen (Thomson & Phillips, 1985). DIDS is known to inhibit $Cl^-/HCO_3^-$ exchange and $Na^+/HCO_3^-$ cotransport in a wide range of epithelial cell types (e.g., $Cl^-/HCO_3^-$ exchange: turtle bladder, Steinmetz, 1974; rabbit medullary collecting ducts, Zeidel, Silva &

**Table 11.** Effect of inhibitors on rectal acid secretion ($J_{H^+}$) under short-circuit current conditions

| | $J_{H^+}$ ($\mu$equiv · cm$^{-2}$ · hr$^{-1}$) | $V_t$ (mV) | $I_{sc}$ ($\mu$equiv · cm$^{-2}$ · hr$^{-1}$) |
|---|---|---|---|
| Control | 1.43 ± 0.13 | 4.6 ± 1.3 | 0.82 ± 0.23 |
| + 1 mM Contraluminal SITS | 1.39 ± 0.13 | 4.2 ± 1.3 | 0.76 ± 0.22 |
| Control | 1.56 ± 0.10 | 8.3 ± 1.7 | 1.29 ± 0.18 |
| + 1 mM Contraluminal DIDS | 1.58 ± 0.09 | 8.6 ± 1.6 | 1.31 ± 0.18 |
| Control | 1.73 ± 0.13 | 9.7 ± 1.5 | 1.95 ± 0.26 |
| + 1 mM Bilateral vanadate | 1.77 ± 0.12 | 9.1 ± 1.5 | 1.83 ± 0.25 |

SITS, 4-acetamido-4′-isothiocyanatostilbene-2,2′-disulfonic acid. DIDS, 4,4′-diisothiocyanatostilbene-2,2′-disulfonic acid. Bilateral pH was maintained at 7.00 by contraluminal perfusion and luminal pH stat. Contraluminal amino acids were replaced with osmotically equivalent amounts of sucrose for both the control and experimental periods in the experiments with SITS and DIDS. Control values of $J_{H^+}$, $V_t$, and $I_{sc}$ were determined on each tissue immediately before inhibitor addition. All other measurements were made 1 hr after inhibitor addition. Values are mean ± SE; $n = 6$ for each treatment. Neither SITS, DIDS nor vanadate had a significant effect on $J_{H^+}$, $V_t$, or $I_{sc}$ by paired $t$ test ($P > 0.50$).

Seifter, 1986*b*; *Necturus* gall bladder, Reuss & Constantin, 1984; mosquito salt gland, Strange & Phillips, 1984; $Na^+/HCO_3^-$ cotransport: rabbit proximal tubules, Grassl & Aronson, 1986; bovine cornea, Jentsch et al., 1984; frog gastric parietal cells, Curci, Debellis & Fromter, 1987). To test whether base efflux across the basolateral membrane occurs by the same mechanism under both conditions (i.e., with or without exogenous $CO_2/HCO_3^-$), recta were mounted in Ussing chambers and exposed to 1 mM contraluminal SITS or DIDS (under $CO_2/HCO_3^-$-free conditions). Neither SITS nor DIDS had a significant effect on $J_{H^+}$, $V_t$, or $I_{sc}$ (Table 11), suggesting that either the mechanisms for electrogenic proton secretion and $CO_2/HCO_3^-$ reabsorption are completely unrelated, or that under $CO_2/HCO_3^-$-free conditions, base equivalents cross the basolateral membrane in a different manner than when $CO_2$ is present (e.g., $OH^-$ efflux rather than $Cl^-/HCO_3^-$ exchange).

Active electrogenic proton transport in the locust rectum occurs against electrochemical gradients of up to 107 mV at the apical membrane (Thomson et al., 1988*a*). Ion-substitution studies (*see above*) have clearly shown that the bulk of this transport is not linked to passive fluxes of other ions across the apical membrane (i.e., active electrogenic $H^+$ transport is not energized by the electrochemical potentials of other ions); therefore, one must postulate a direct metabolically dependent mechanism to account for this transfer (e.g., proton ATPase or redox pump). Plasma membrane proton ATPases have been proposed in a number of acid-secreting epithelia (e.g., toad bladder, Beuwens, Crabbe & Rentmeesters, 1981; turtle bladder, Gluck, Cannon & Al-Awqati, 1982; rat proximal tubule, Kline-Saffran & Kinne, 1986). Vanadate, a putative inhibitor of the so-called "P" class of ATPase (those ATPases which form a covalent phosphorylated intermediate as part of their reaction cycle; formerly referred to as "E1–E2" type ATPases; *see* Pedersen & Carafoli, 1987), has been shown to inhibit active proton secretion (presumably by inhibiting an apical proton ATPase) in both the toad bladder (Beauwens et al., 1981) and the turtle bladder (Arruda, Sabatini & Westenfelder, 1981; Steinmetz et al., 1981). The possibility that active proton secretion in the locust rectum might be mediated by a similar vanadate-sensitive mechanism was tested by exposing recta mounted in Ussing chambers ($CO_2/HCO_3^-$-free; bilateral pH 7.00; short-circuit current conditions) to 1 mM bilateral vanadate. Under these conditions, vanadate had no significant effects on $J_{H^+}$, $V_t$, or $I_{sc}$ (Table 11), suggesting that active proton secretion in the locust rectum was not mediated by the typical "P" class plasma membrane proton ATPase.

## Discussion

### Proton Secretion *Versus* Bicarbonate Reabsorption

The identification of the exact mechanism responsible for mediating pH changes in acid/base transporting epithelia (i.e., acid secretion or base reabsorption) has historically been one of the most difficult (and often controversial) issues to resolve in acid/base physiology (*see*, for examples Rector, Carter & Seldin 1965; Brodsky & Schilb, 1974; Steinmetz, 1974; DuBose, 1983). The volatile nature

of the $CO_2/HCO_3^-$ buffer system, the interactions of free protons and hydroxyl ions with the primary solvent, and the continued production of metabolic $CO_2$ all make it particularly awkward to apply standard ion-transport techniques to the solution of this problem.

In the present study, we distinguished between acid secretion and $HCO_3^-$ reabsorption by demonstrating that significant rates of luminal acidification persisted despite removal of exogenous $CO_2$ and $HCO_3^-$. To circumvent the problem of metabolic $CO_2$ addition, we vigorously aerated the luminal chamber with 100% $O_2$ and monitored luminal total $CO_2$ concentrations both before and after each experimental period. This protocol effectively maintained the initial $CO_2/HCO_3^-$-free state even in the presence of significant transepithelial $P_{CO_2}$ gradients (*see* Table 1).

Although we could clearly maintain a $CO_2$-free state in the bulk solution bathing the epithelium, we could not directly rule out the possibility that $HCO_3^-$ might be formed in the unstirred layers without necessarily being detected in the bulk solution and that this low level of $HCO_3^-$ might be sufficient to bring about the observed rates of rectal acidification. This implied a transport mechanism with an extremely high affinity for $HCO_3^-$, but it was not inconceivable considering the affinities which had to be postulated for a proton-translocating mechanism given the very much lower concentrations of protons present under typical physiological conditions.

This possibility was tested by indirectly increasing the $P_{CO_2}$ (and hence the $HCO_3^-$ concentration) of the unstirred layers until trace levels of $CO_2$ and $HCO_3^-$ could actually be detected in the luminal bath. Under those conditions, increases in rates of rectal acidification were eventually observed, but this appeared to be due to increased rates of $CO_2$ hydration in the lumen (with its attendant formation of $H^+$) rather than direct stimulation of an apical acid/base transport mechanism. In support of this interpretation was the observation that there were no changes in $I_{sc}$ with the contraluminal additions of either $CO_2$ or $HCO_3^-$. Subsequent ion-substitution studies clearly showed that rectal acidification was due to an apical electrogenic-transport mechanism; therefore, if the increased levels of luminal $HCO_3^-$ had directly stimulated the apical transporter, one would expect to see an increase in $I_{sc}$. That this did not happen is further evidence that rectal acidification under $CO_2/HCO_3^-$-free conditions is mediated by proton secretion rather than bicarbonate reabsorption.

For $HCO_3^-$ reabsorption to be the source of $J_{H^+}$ under exogenous $CO_2/HCO_3^-$-free conditions, metabolic $CO_2$ would have to be produced by the epithelium at rates at least commensurate with the observed rates of rectal acidification. Under similar experimental conditions, Chamberlin (1981) has shown that isolated recta consume oxygen at approximately 16.07 $nmol \cdot min^{-1} \cdot mg^{-1}$ dry weight. An average dry rectal weight of 1.5 mg and a mean surface area of 0.64 $cm^2 \cdot rectum^{-1}$ allows a conversion of Chamberlin's oxygen consumption value to 2.3 $\mu mol \cdot cm^{-2} \cdot hr^{-1}$. If one assumes an RQ ratio of 1 (for maximum conversion to $CO_2$), an equivalent rate of $CO_2$ diffusion across the apical and basolateral membranes (*see* Schwartz et al., 1974), a luminal pH of 7, an instantaneous hydration of $CO_2$ to $HCO_3^-$, and no significant loss of $CO_2$ from the system (i.e., no stirring with 100% $O_2$), there would only be enough $HCO_3^-$ formed in the lumen to account for approximately 75% of $J_{H^+}$ (i.e., 1.15 $\mu mol$ $CO_2 \cdot cm^{-2} \cdot hr^{-1}$ as compared to $J_{H^+}$ values of 1.54 $\mu equiv \cdot cm^{-2} \cdot hr^{-1}$). Obviously these are optimistic assumptions. Taking into account a more realistic RQ value (0.8–0.9), a much lower pH in the unstirred layer, and a loss of at least 30% of the $CO_2$ from the system (Schwartz et al., 1974, were reporting $CO_2$ losses of at least 50% with a similar flat-sheet preparation), rates of luminal $HCO_3^-$ formation would be substantially lower. Under these conditions, the likelihood that sufficient $HCO_3^-$ could be generated in the unstirred layers to account for the observed rates of rectal acidification is extremely remote.

## Characterization of Active Proton Secretion

Net acid secretion in the isolated locust rectum is extremely sensitive to both electrical and chemical driving forces for protons across the apical membrane. $J_{H^+}$ could be abolished by reducing luminal pH to 5.27 ($V_t$ clamped at 0 mV; Fig. 2) or clamping $V_t$ at 107 mV lumen positive (bilateral pH = 7.00; Fig. 3). The striking similarity between the rates of inhibition of $J_{H^+}$ and apparent transepithelial PMF values obtained by applying either an electrical or chemical transepithelial gradient (Fig. 5) implies that both treatments are modulating the same transport process. The ability to eliminate net acid secretion by application of electrical or chemical transepithelial gradients has also been observed in the turtle bladder (Al-Awqati, Mueller & Steinmetz, 1977) and is considered indicative of a proton-conductive pathway in the epithelium. The fact that contraluminal pH did not affect $J_{H^+}$, $V_t$, or $I_{app}$ (Fig. 1) suggests that this pathway does not have a paracellular component (as would be expected for a tight epithelium).

Inhibition of net acid secretion by application of either chemical or electrical transepithelial gradients

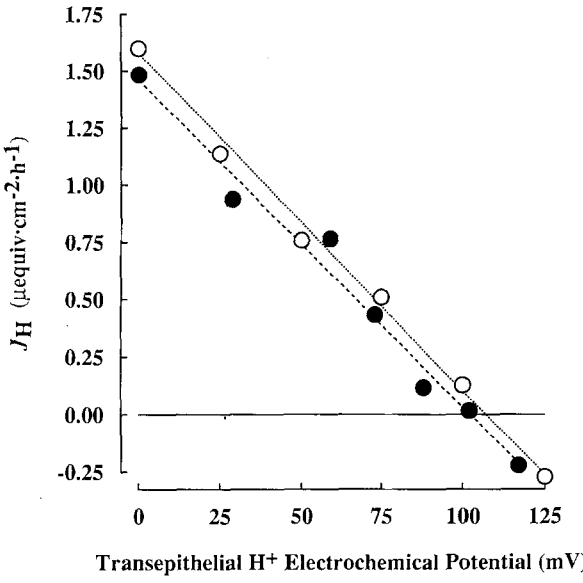



**Fig. 5.** Effect of transepithelial electrochemical proton gradients on rates of net acid secretion. Negative values of $J_{H^+}$ indicate acid uptake rather than aicd secretion. Data points obtained by converting abscissa values from Fig. 2 ($J_{H^+}$ *vs.* ΔpH) and Fig. 3 ($J_{H^+}$ *vs.* $\Delta V_t$) to transepithelial $H^+$ electrochemical gradients ($\Delta\bar{\mu}_H/F$). Open and filled circles represent values measured when the transepithelial $\Delta\bar{\mu}_H/F$ was created by imposing transepithelial voltage or pH gradients, respectively. Dashed and dotted lines fit by regression analysis (least-squares method) and correspond to filled and open circles, respectively.

(i.e., ΔpH or $\Delta V_t$) suggests that net acid secretion might occur by an electrogenic-transport mechanism on the apical membrane. From this observation alone, however, it is difficult to distinguish between the effects on the active proton-secretory mechanism and passive proton movements in the opposite direction. The observed changes in $J_{H^+}$, $V_t$, and $I_{app}$ could be easily explained by an increase in passive proton-conductive movements from the lumen to the cell without having to propose an electrogenic proton-secretory mechanism on the apical membrane.

The electrogenic nature of active proton secretion was confirmed by demonstrating that $J_{H^+}$ was largely independent of $Na^+$, $K^+$, $Cl^-$, $Ca^{2+}$, or $Mg^{2+}$. The only ions which had any effect at all on rates of net acid secretion when they were removed from the bathing salines were $Na^+$ and $K^+$. The 10–20% inhibition of $J_{H^+}$ observed after luminal $Na^+$ removal could be completely accounted for by an electroneutral, amiloride-sensitive $Na^+/H^+$ exchange mechanism on the apical membrane (*see* Tables 2 and 3). This conclusion is entirely consistent with the apical $Na^+/NH_4^+$ exchange mechanism reported by Thomson et al. (1988*b*). Kinsella and Aronson (1980) have shown that this class of transporter is fully able to exchange either protons or ammonium ions for external sodium in isolated renal microvillus membranes. It remains to be seen whether there are distinct populations of $Na^+/H^+$ and $Na^+/NH_4^+$ exchangers in the locust rectum or whether the same exchangers pump either $Na^+$ or $H^+$ depending on which ion interacts with the appropriate binding site.

Hanrahan and Phillips (1984*b*) reported that $K^+$ removal reduced active, electrogenic $Cl^-$ flux by up to 70% in the locust rectum under short-circuit current conditions. On the basis of changes in electrochemical gradients for $Cl^-$ and $K^+$ across the apical membrane, they concluded that the $K^+$ effect was due to a direct interaction of $K^+$ with the apical $Cl^-$ pump rather than an indirect effect of $K^+$ on intracellular membrane potentials or a strict requirement for a $K^+/Cl^-$-cotransport mechanism. The reduction in $J_{H^+}$ observed after long-term bilateral $K^+$ removal (65% reduction; $I_{sc}$ conditions; Table 4) could not be directly attributed to a $K^+$-induced modulation of apical membrane potentials. Under these conditions, intracellular $K^+$ activities have been shown to decrease to < 5 mM and $V_a$ and $V_b$ depolarize by 25–30 mV (Hanrahan & Phillips, 1984*b*). Since $J_{H^+}$ did not increase as would be predicted by the decrease in $V_a$, long-term $K^+$ removal must have either affected the $H^+$-transport mechanism directly in a manner similar to that proposed for the electrogenic $Cl^-$ pump or indirectly by unspecified effects similar to those proposed for long-term $Na^+$ removal (*see above* and Hanrahan & Phillips, 1984*b*). When the above experiments were repeated with $K^+$ present in the contraluminal bath (luminal bath $K^+$ free; $V_t$ clamped at 0 mV), $J_{H^+}$ was reduced by only 30%. Since intracellular $K^+$ activities were maintained at control levels by maintaining 10 mM $K^+$ in the contraluminal bath (60–65 mM; Hanrahan & Phillips, 1984*b*), it is likely that most cell functions were not adversely affected by this treatment. When luminal $K^+$ was removed under open-circuit conditions, $V_a$ and $V_b$ hyperpolarized to 90 and 60 mV, respectively (*see* Table 9) and the voltage divider ratio rose to 2.38 ± 0.05 ($n$ = 38 cells in six recta). On the basis of this voltage divider ratio, when $V_t$ was clamped at 0 mV (Table 4), $V_a$ and $V_b$ should have equaled ≈ 69 mV and the electrochemical gradient opposing active proton secretion at the apical membrane should have increased by ≈ 15% (assuming that $pH_i$ did not change significantly). Under these conditions ($K^+$ removed from the luminal bath only), it is reasonable to propose that the inhibition of $J_{H^+}$ could be attributed almost entirely to $K^+$ modulation of the apical membrane potential, and hence, the apical $\Delta\bar{\mu}_H/F$ rather than a

**Table 12.** The source of short-circuit current in unstimulated locust recta

| Ion | Net flux ($\mu$equiv · cm$^{-2}$ · hr$^{-1}$) | Source |
|---|---|---|
| $Cl^-$ | +0.85 | Hanrahan and Phillips (1984*a*) |
| $Na^+$ | −2.0 | Black et al. (1987) |
| $K^+$ | 0 | Hanrahan and Phillips (1983) |
| $NH_4^+$ | +0.6 | Thomson et al. (1988*b*) |
| $H^+$ | +1.5 | Present study |
| Expected $I_{sc}$ | +0.95 | Calculated from net flux values |
| Observed $I_{sc}$ | +0.82 to +1.95 | Range observed by present authors and Thomson (1990) and Thomson et al. (1988*a*,*b*) |

All flux values determined under short-circuit current conditions. Positive flux or $I_{sc}$ value indicates movement of positive charges into or negative charges out of the lumen.

direct requirement of the proton pump for $K^+$ (as proposed for the $Cl^-$ pump) or nonspecific cellular effects as proposed above for long-term bilateral $K^+$ removal.

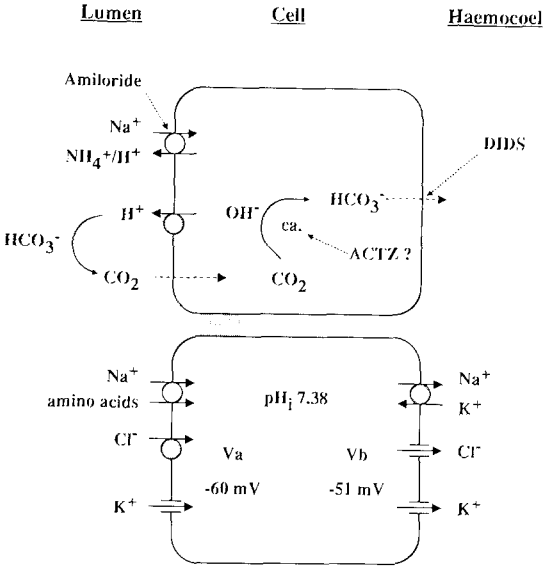
Having ruled out other ion gradients as the energy source for active proton secretion, one is forced to consider the possibility of either a redox pump or a proton ATPase. Redox pumps similar to those described in bacteria, chloroplasts, and mitochondria (*see* for respective reviews Harold & Altendorf, 1974; Dilley & Giaquinta, 1975; Wikstrom, 1982) have in the past been proposed for renal brush-border membranes (Gimenez-Gallego et al., 1980) and gastric mucosa (Rehm, 1972). Although the proton pump in the gastric mucosa has since been shown to be an ATPase (reviewed by Forte & Machen, 1987), it has been difficult to completely rule out the existence of the redox pump in the renal brush border (*see* Aronson, 1983). Nevertheless, the current view is that primary active transport of protons in eukaryote plasma membranes is facilitated by proton ATPases rather than redox pumps. Plasma membrane proton ATPases have been proposed for a variety of acid/base transporting epithelia including gastric mucosa (reviewed by Forte & Machen, 1987), turtle bladder (reviewed by Steinmetz & Andersen, 1982), rabbit renal medullary collecting ducts (Zeidel, Silva & Seifter, 1986*a*) and rabbit proximal tubules (Kuwahara et al., 1989). The fact that vanadate had no effect on net acid secretion in the locust rectum by no means rules out the possibility that a proton ATPase is present on the apical membrane. Vanadate-insensitive plasma membrane proton ATPases have previously been reported in other tissues (e.g., tobacco hornworm midgut, Schweikl et al., 1989; rat renal brush border, Turrini et al., 1989), and the possibility exists that vanadate might not even have reached the target site or that it was somehow being inactivated in the epithelium (*see* Thomson, 1990).

Acetazolamide (ACTZ) and DIDS have been reported to inhibit $HCO_3^-$ uptake in everted rectal sacs incubated in exogenous $CO_2$ and $HCO_3^-$ by 37 and 98%, respectively (Thomson & Phillips, 1985). The similar degree of inhibition of net acid secretion in the flat-sheet preparation under $CO_2/HCO_3^-$-free conditions with ACTZ (39%; Table 10) implies that both transport processes share a common pathway or that $HCO_3^-$ reabsorption may depend on active proton secretion as proposed for the vertebrate kidney (*see* Giebisch & Aronson, 1987). The lack of effect of contraluminal DIDS on net acid secretion under $CO_2/HCO_3^-$-free conditions, on the other hand, suggests fundamental differences depending on whether $CO_2$ and $HCO_3^-$ are present or not. DIDS has been shown to reduce net $HCO_3^-$ uptake in vertebrate proximal tubule cells by inhibiting the basolateral exit step, whereas ACTZ is thought to act by modulating the intracellular concentrations of $HCO_3^-$ and hydroxyl ions (reviewed by Preisig & Alpern, 1989). The disparate responses to contraluminal DIDS in the locus rectum suggest a difference in the basolateral exit step depending on the availability of intracellular $HCO_3^-$. In the vertebrate proximal tubule, the principal means of base efflux across the basolateral membrane when exogenous $CO_2$ and $HCO_3^-$ are present is a SITS- and DIDS-inhibitable $Na^+/HCO_3^-$-cotransport mechanism (reviewed by Preisig & Alpern, 1989). Under $CO_2/HCO_3^-$-free conditions, base efflux is maintained, but sensitivity to disulfonic stilbenes is lost. Burckhardt and Fromter (1987) have proposed a parallel stilbene-insensitive $H^+/OH^-$-conductive pathway on the basolateral membrane (which is normally silent when exogenous $CO_2/HCO_3^-$ is present) to account for this observation. It is possible that a similar phenomenon is also occurring in the locust rectum. Specific experiments aimed directly at this question must be performed before any conclusions can be drawn.

## Source of Short-Circuit Current in Unstimulated Locust Recta

Williams et al. (1977) reported that net fluxes of $Na^+$, $K^+$, and $Cl^-$ could not account for the short-circuit current values observed in unstimulated locust recta. On the basis of the magnitude of the unidentified component of the short-circuit current ($\approx 3.1$ $\mu$equiv $\cdot$ cm$^{-2}$ $\cdot$ hr$^{-1}$), the direction of the current (indicative of net cation movement into or net anion movement out of the lumen), and the rates of luminal acidification reported by Speight (1967; 2.15 $\mu$equiv $\cdot$ cm$^{-2}$ $\cdot$ hr$^{-1}$), they proposed that the unidentified component of $I_{sc}$ might be due to electrogenic movements of protons or hydroxyl ions across the rectal epithelium.

In the present study we have shown that the bulk of net acid secretion is due to an active, electrogenic proton-secretory mechanism located at the apical membrane. Under short-circuit current conditions, rates of net acid secretion were $1.53 \pm 0.03$ $\mu$equiv $\cdot$ cm$^{-2}$ $\cdot$ hr$^{-1}$ ($n = 90$ recta). Although this value is approximately half of that required to account for the unidentified component of $I_{sc}$ observed by Williams et al. (1977) the $I_{sc}$ and net flux values for $Na^+$, $K^+$, and $Cl^-$ reported by them are substantially larger than corresponding values reported by other researchers (e.g., Hanrahan & Phillips, 1984*a*; Black et al., 1987). Different experimental salines, measurement of short-circuit current without compensation for saline resistance, and data collection prior to steady-state conditions have all been suggested as possible reasons for this discrepancy (Hanrahan, 1982). To date, no one person has measured $Na^+$, $K^+$, $Cl^-$, and $H^+$ fluxes under exactly the same conditions with the same population of experimental animals. This and the significant variability associated with measurement of very small ion fluxes makes it very difficult to collectively reconcile reported flux values with observed short-circuit currents. Nevertheless, if one considers only those flux values obtained from more or less similar preparations, it would appear that active movements of $Na^+$, $Cl^-$, $H^+$, and $NH_4^+$ can account for most (if not all) of the observed short-circuit current in unstimulated locust recta (Table 12).




| | Net Electrochemical Potentials (mV) (Apical) | (Basolateral) |
|---|---|---|
| $H^+$ | -82 | 73 |
| $Na^+$ | -128 | 119 |
| $Cl^-$ | 32 | -23.3 |
| $K^+$ | -3.3 | -3.9 |

**Fig. 6.** Proposed model of acid/base transport in the principal cells of the locust rectum. DIDS, 4,4'-diisothiocyanatostilbene-2,2'-disulfonic acid; ACTZ, acetazolamide; *ca.*, carbonic anhydrase. Primary ion pumps indicated by shaded circles; secondarily active transport systems (co- or countertransport) indicated by open circles; passive conductive pathways (e.g., channels) indicated by arrows through gaps in the plasma membrane; postulated diffusive pathways indicated by dashed arrows. *Upper cell*: postulated mechanism of luminal acidification in the rectum (*see* text for details). *Lower cell*: transport pathways for $Na^+$, $K^+$, and $Cl^-$ identified in the principal cells thus far. $V_a$ and $V_b$ are referenced to the lumen and hemocoel, respectively. The values of $V_a$, $V_b$, and $pH_i$ reflect the experimental conditions used in the present study (i.e., $CO_2/HCO_3^-$-free; bilateral pH = 7.00; Thomson et al., 1988*a*). Net electrochemical potentials for $H^+$ (Thomson et al., 1988*a*), $K^+$, $Na^+$, and $Cl^-$ (Hanrahan & Phillips, 1984*b*) were determined under open-circuit conditions with a preparation similar to that used above. Negative values of electrochemical potentials indicate favorable gradients for net ion movement in the lumen to hemocoel direction, whereas positive values indicate opposing gradients.

## Summary

Isolated locust recta are able to maintain net acid secretion against transepithelial $H^+$ electrochemical gradients of up to 105 mV under $CO_2/HCO_3^-$-free conditions. The possibility of a proton-diffusional pathway on the apical membrane suggests that apparent PMF values calculated from net acid secretion rates might significantly underestimate the true PMF which can be generated by the active proton-secretory mechanism. As proposed for vertebrate proximal tubule cells (Kuwahara et al., 1989), net acid secretion in the locust rectum appears to be a composite of electroneutral $Na^+/H^+$ exchange and electrogenic proton secretion (ATPase?); the major difference between the two systems being that

$Na^+/H^+$ exchange accounts for the bulk of net acid secretion in the vertebrate kidney, whereas electrogenic proton secretion accounts for the major fraction of acid secretion in the locust rectum. Concomitant reports of $HCO_3$ reabsorption and active proton secretion in the locust rectum (Thomson & Phillips, 1985) suggest, that like most vertebrate urinary epithelia, locust recta reabsorb $HCO_3$ by titration of luminal $HCO_3$ to $CO_2$, subsequent $CO_2$ diffusion into the cell, carbonic anhydrase catalyzed hydration of intracellular $CO_2$ to $HCO_3$, and $HCO_3$ exit from the cell across the basolateral membrane through a stilbene-sensitive pathway (Fig. 6).

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