

# Na<sup>+</sup>-H<sup>+</sup> Exchange at the Apical Membrane of *Necturus* Gallbladder

## *Extracellular and Intracellular pH Studies*

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**ABSTRACT** The mechanism of luminal solution acidification was studied in *Necturus* gallbladder by measurement of mucosal solution and intracellular pH with glass electrodes. When the gallbladder was bathed by Na-Ringer's solution it acidified the luminal side by a Na<sup>+</sup>-dependent, amiloride-inhibitable process. In the presence of ouabain, acidification was reduced but could be stimulated to a rate greater than that under control conditions by the imposition of an inwardly directed Na<sup>+</sup> gradient. These results suggest that luminal acidification results from Na<sup>+</sup>-H<sup>+</sup> exchange at the apical membrane and not by diffusion of metabolic CO<sub>2</sub>. Li<sup>+</sup> can substitute for Na<sup>+</sup> but K<sup>+</sup>, Rb<sup>+</sup>, Cs<sup>+</sup>, and tetramethylammonium (TMA<sup>+</sup>) cannot. The maximal rate of exchange was about five times greater for Na<sup>+</sup> than for Li<sup>+</sup>. Intracellular pH (pH<sub>i</sub>) was measured with recessed-tip glass microelectrodes; with the tissue bathed in Na-Ringer's solution (pH 7.75), pH<sub>i</sub> was 7.51 ± 0.04. After inhibition of Na<sup>+</sup>-H<sup>+</sup> exchange by mucosal perfusion with amiloride (1 mM) or by complete Na<sup>+</sup> replacement with TMA<sup>+</sup>, pH<sub>i</sub> fell reversibly by 0.15 and 0.22 pH units, respectively. These results support the conclusion that Na<sup>+</sup>-H<sup>+</sup> exchange at the apical membrane is the mechanism of luminal acidification and is involved in the maintenance of steady state pH<sub>i</sub>.

### INTRODUCTION

The function of gallbladder epithelium is to concentrate the bile by isotonic fluid transport from the lumen to the serosal side (Diamond, 1968). Several studies in rabbit (Whitlock and Wheeler, 1969; Sullivan and Berndt, 1973a, b; Cremaschi et al., 1979) and guinea pig gallbladders (Heintze et al., 1981) have demonstrated acidification of the luminal fluid in vitro. In rabbit gallbladder, acidification was inhibited by Na<sup>+</sup> removal or by ouabain. Although the mechanism of acidification could be not determined, it was felt that a Na<sup>+</sup>-H<sup>+</sup> exchange process was possible, but non-ionic diffusion of endogenously produced CO<sub>2</sub> could not be ruled out.

Na<sup>+</sup>-H<sup>+</sup> exchange has been identified as a mechanism of acid extrusion in a number of systems including fertilized sea urchin eggs (Johnson et al., 1976),

mouse soleus muscle fibers (Aickin and Thomas, 1977), *Amphiuma* red blood cells (Cala, 1980), renal brush border vesicles from rat (Murer et al., 1976) and rabbit (Kinsella and Aronson, 1980), rat intestinal brush border vesicles (Murer et al., 1976), and *Ambystoma* proximal tubule (Boron and Boulpaep, 1982).

The existence of  $\text{Na}^+\text{-H}^+$  exchange at the luminal membrane might help to explain the two phenomena of (a) coupled NaCl entry as observed in gallbladder (Frizzell et al., 1975; Duffey et al., 1978; Reuss and Grady, 1979; Garcia-Diaz and Armstrong, 1980) and small intestine (Nellans et al., 1973), and (b) acidification of the luminal solution. Both phenomena could be linked if coupled NaCl entry were the result of simultaneously operating  $\text{Na}^+\text{-H}^+$  and  $\text{Cl}^- \text{-HCO}_3^-$  exchanges (Petersen et al., 1981; Liedtke and Hopfer, 1977, 1982a, b).

In the present investigation, the mechanism of luminal solution acidification was studied in *Necturus* gallbladder. Measurements of intracellular and mucosal fluid pH under various conditions have demonstrated that this acidification is caused by an amiloride-sensitive  $\text{Na}^+\text{-H}^+$  exchange and not by passive diffusion of  $\text{CO}_2$ .  $\text{Na}^+\text{-H}^+$  exchange operates continuously when the tissue is bathed on both sides with Na-Ringer's solution. Preliminary reports of these studies have appeared (Weinman et al., 1981; Weinman and Reuss, 1982).

#### MATERIALS AND METHODS

Mudpuppies (*Necturus maculosus*) were purchased from Riverside Biologicals (Somerset, WI) or Carolina Biologicals (Burlington, NC), kept in aquaria at 5–10°C, and fed live fish. Gallbladders were removed and mounted horizontally, mucosal side up, in a modified Ussing chamber at room temperature (~23°C) as previously described (Reuss and Finn, 1975a, 1977). The mucosal solution had a volume of 100–300  $\mu\text{l}$  and was exposed to the air. Na-Ringer's solution had the following composition: 109.2 mM NaCl; 2.5 mM KCl; 1.0 mM  $\text{CaCl}_2$ ; 1.0 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES). KOH was added to a final pH of 7.7–8.0 after equilibration with room air. For  $\text{Na}^+$  replacement studies, NaCl was replaced by isomolar substitution with tetramethylammonium ( $\text{TMA}^+$ ) chloride, KCl, LiCl, RbCl, or CsCl. Amiloride (a generous gift of Merck, Sharpe and Dohme, West Point, PA) or ouabain (0.1 mM, Sigma Chemical, St. Louis, MO) was added to Na-Ringer's solution as indicated.

#### *Electrical Potential Measurements*

Transepithelial ( $V_{ms}$ ), apical membrane ( $V_{mc}$ ), and basolateral membrane ( $V_{cs}$ ), potentials were measured as described previously (Reuss and Finn, 1975a, 1977). The serosal reference electrode was an Ag-AgCl pellet connected to the serosal solution by a Na-Ringer's-agar bridge and the mucosal reference electrode as a calomel electrode connected to the mucosal solution by either a 3-M KCl or a Ringer-agar bridge. Transepithelial current pulses of 40–100  $\mu\text{A}\cdot\text{cm}^{-2}$  were applied, and intracellular microelectrodes, filled with 3 M KCl and having resistances of 10–50  $\text{M}\Omega$ , were used as previously described (Reuss and Finn, 1975a, 1977).

*Mucosal pH Measurements*

Mucosal pH was measured with glass pH electrodes. These were constructed from lead glass pipettes to which a bulb of H<sup>+</sup>-selective glass (Clarke Electromedical Instruments, Reading, England) was fused. The bulb diameter varied from 0.5 to 1.0 mm. The electrodes were filled with Na-phosphate buffer (5 mM) plus NaCl (100 mM), pH 7.0, and were connected to the probe of a high-input impedance electrometer (model FD223; WP Instruments, New Haven, CT) by an Ag-AgCl wire and had time constants of ~1.0 s.  $V_{ms}$  and the potential difference between the pH electrode and the serosal reference ( $V^*$ ) were recorded as described previously (Reuss and Weinman, 1979). The pH of the Ringer's solution was measured with a pH meter (Radiometer, Copenhagen). Mucosal solution pH changes were determined from the changes of  $V_{ms}$  and  $V^*$  ( $\Delta V_{ms}$  and  $\Delta V^*$ ), according to the equation  $\Delta \text{pH} = \Delta V_{H^+} / S$ , where  $\Delta V_{H^+} = (\Delta V^*) - (\Delta V_{ms})$  and  $S =$  electrode slope (mV/pH unit).

Large transients in  $V^*$  were observed when Rb<sup>+</sup> or Cs<sup>+</sup> were rapidly substituted for either Na<sup>+</sup> or TMA<sup>+</sup>. This effect was seen even in the absence of a tissue and therefore, in experiments in which Rb<sup>+</sup> and Cs<sup>+</sup> were substituted for Na<sup>+</sup>, a liquid ion-exchanger H<sup>+</sup>-selective microelectrode (Ammann et al., 1981) was substituted for the glass electrode. These microelectrodes had tip sizes of ~1.0–2.0  $\mu\text{m}$  and slopes of ~60 mV/pH unit.

Two different protocols were used to measure the effect of mucosal cation substitutions on acid secretion. In protocol 1, steady state acidification, the pH electrode was observed under the microscope and positioned within 100  $\mu\text{m}$  of the mucosal surface. After at least 5 min of continuous mucosal perfusion with the test solution, the mucosal perfusion was abruptly stopped for 5–15 min and then restarted. The position of the electrode was kept constant during the experiment and solution pH was recorded continuously. In protocol 2, transient acidification, the pH electrode was positioned identically. The mucosal side was perfused with Na<sup>+</sup>-free TMA-Ringer's for at least 10 min and then the mucosal perfusion was abruptly stopped. After 2 min, the composition of the mucosal solution was changed either by rapid addition of a known volume of another solution or by perfusion with replacement solution for 3 s. After an additional 2–4 min without perfusion, mucosal perfusion with TMA-Ringer's was resumed. The initial rate of acidification was determined by the rate of change of  $V_{H^+}$  during the interval from 0.1 to 0.3 min after the mucosal solution change. Since the instantaneous potential changes were not used in the measurement of the slope, rapidly changing junction potentials did not contribute to the calculated rate of pH change.

Solution buffering power ( $\beta$ ) was calculated according to the equation of Koppel and Spiro (see Bates, 1973; Roos and Boron, 1980):

$$\beta = \frac{2.303 K H C}{(K + H)^2}$$

where  $K$  is the dissociation constant of the buffer,  $C$  is the total concentration of buffer (moles/liter),  $H$  is the hydrogen ion concentration (moles/liter), and  $\beta = \frac{db}{d\text{pH}}$  where

$db$  is an increment of strong base (moles/liter) and  $d\text{pH}$  is the resulting increment of pH. The calculated values of  $\beta$  agreed well with those determined experimentally by titration. Acidification rate ( $\Delta H$ ) of the solution surrounding the pH electrodes (moles H<sup>+</sup> · liter<sup>-1</sup> · min<sup>-1</sup>) was calculated according to  $\Delta H = \beta \cdot \Delta \text{pH} / t$ , where  $t =$  time in min.

For measurement of net  $H^+$  flux into the mucosal solution, the pH electrode was placed 1–2 mm from the tissue, acidification was allowed to proceed according to protocol 1, and the solution was mixed by repeated withdrawal into and expulsion from a hand-held pipette. Solution pH was measured immediately after mixing and the  $H^+$  flux was determined according to

$$J_H = \beta \cdot \Delta pH \cdot V / (A \cdot t)$$

where  $J_H$  is the net  $H^+$  flux ( $\text{mol} \cdot \text{min}^{-1} \text{cm}^{-2}$ ),  $\beta$  is the buffering power ( $\text{mol} \cdot \text{liter}^{-1} [\text{pH unit}]^{-1}$ ),  $\Delta \text{pH}$  is the pH change,  $V$  is the mucosal volume (liters), and  $A$  is the exposed tissue area ( $0.5 \text{ cm}^2$ ). This method provides a rough estimate of the net  $H^+$  flux and is likely to be an underestimate for the reasons described in the Discussion.

#### *Intracellular pH Measurements*

Intracellular pH was measured using recessed-tip glass pH microelectrodes of tip size 0.5–1.0  $\mu\text{m}$ , slope 55–59 mV/pH unit, and response time  $\sim 30$  s (Thomas, 1978). Simultaneous impalements with KCl-filled microelectrodes and pH microelectrodes were performed as was described for  $\text{Na}^+$ ,  $\text{K}^+$ , and  $\text{Cl}^-$  electrodes (Reuss and Weinman, 1979; Reuss et al., 1980). Criteria used to validate the impalements were those described earlier (Reuss and Weinman, 1979): (a) potentials recorded by the pH electrode were the same before and after impalement; (b) upon impalement the change in recorded potential was initially abrupt and monotonic; (c) the intracellular record was stable for at least 1 min after reaching a steady state value; (d) trans-epithelial current pulses produced identical steady state voltage deflections in both conventional and pH electrodes; (e) changes in ionic composition of the mucosal solution resulted in rapid, identical changes in the potentials measured by both electrodes.

#### *Data Analysis*

Dependence of acidification on  $\text{Na}^+$  and  $\text{Li}^+$  concentration was determined by a nonlinear least-squares fit of the initial rates of acidification, determined by protocol 2, to the Michaelis-Menten equation. Results are reported as mean  $\pm$  SE. Comparisons were made by conventional paired-data analysis.

### RESULTS

#### *Acidification of the Mucosal Solution*

Tissues were perfused on both sides with Na-Ringer's, and after the mucosal solution pH was found to be constant for several minutes, the superfusion was abruptly stopped for 5–6 min and then restarted. In each of 36 experiments performed according to this protocol (protocol 1), mucosal acidification was observed. The change of  $V_{H^+}$  ranged from +1 to +15 mV, corresponding to a pH change ( $\Delta \text{pH}$ ) ranging from  $-0.02$  to  $-0.27$  pH units. The magnitude of  $\Delta \text{pH}$  was dependent on the position of the pH electrode. After cessation of superfusion, pH gradients developed in the mucosal solution with greatest acidification observed when the electrode was closest to the tissue. Upon restarting the mucosal superfusion, the pH returned to the control value. Typical records of the observed changes in mucosal pH and  $V_{ms}$  during such an experiment are shown in Fig. 1.

To determine the net  $H^+$  flux in these experiments, in which pH gradients

existed in the luminal solution, mucosal perfusion was stopped, the pH of the solution was measured immediately after mixing, and  $J_H$  was calculated. In 44 measurements made in 6 tissues bathed by control Na-Ringer's (pH 7.7–7.9),  $J_H$  was  $1.45 \pm 0.27 \text{ nmol} \cdot \text{min}^{-1} \cdot \text{cm}^{-2}$ , or  $87.0 \pm 16.2 \text{ nmol} \cdot \text{h}^{-1} \cdot \text{cm}^{-2}$ .

#### *Sodium Requirement for Steady State Acidification*

The cation requirement of the mucosal acidification was determined by complete mucosal Na<sup>+</sup> substitution with TMA<sup>+</sup>, K<sup>+</sup>, or Li<sup>+</sup>. The mucosal side of the tissue was perfused with the test solution for 15 min and then the rate of acidification was measured according to protocol 1. During the entire

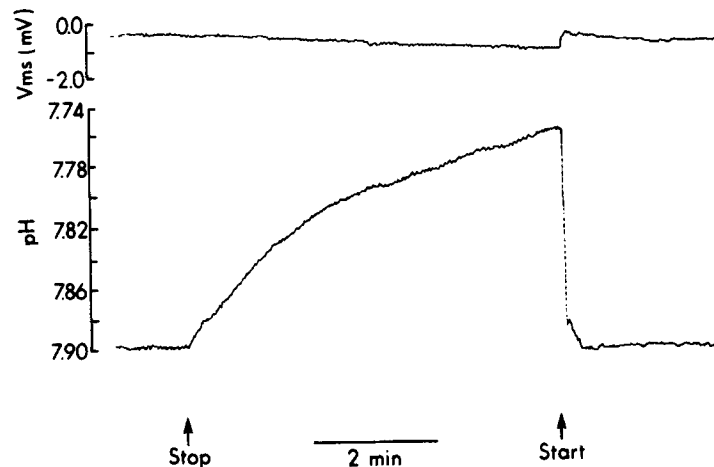


FIGURE 1. Mucosal solution pH (lower trace) and transepithelial potential difference (upper trace) during cessation of mucosal superfusion. The gallbladder was perfused on both sides with Na-Ringer's solution and acidification was measured according to protocol 1. Mucosal superfusion was stopped at the first arrow and resumed at the second arrow.

period of acidification, the serosal side was perfused with control Na-Ringer's. The effect of Na<sup>+</sup> substitution on steady state mucosal acidification is shown in Fig. 2. Na<sup>+</sup> replacement with each of the three ions inhibited acidification significantly and reversibly.

#### *Effect of Amiloride on Acidification*

Amiloride, at low concentrations ( $\sim 10^{-5} \text{ M}$ ), is known to inhibit apical membrane Na<sup>+</sup> entry in tight epithelia. At higher concentrations ( $\sim 10^{-3} \text{ M}$ ), it inhibits Na<sup>+</sup>-H<sup>+</sup> exchange in several systems, such as sea urchin eggs (Johnson et al., 1976), mouse soleus muscle (Aickin and Thomas, 1977), *Amphiuma* red blood cells (Cala, 1980), renal brush border vesicles (Kinsella and Aronson, 1981a), and *Ambystoma* proximal tubule (Boron and Boulpaep, 1982).

When acidification of the mucosal solution was measured according to

protocol 1, the addition of either 1.0 or 0.1 mM amiloride to the mucosal superfusion significantly reduced acidification to  $10 \pm 7\%$  and  $66 \pm 10\%$  of the control value, respectively (Fig. 3).

*Effects of Amiloride on Epithelial Electrical Parameters*

When amiloride (1.0 mM) was added to the mucosal solution, two effects on the electrical properties of the tissue were observed, as shown in Fig. 4. There was an immediate increase in transepithelial resistance ( $R_t$ ) and a slower depolarization of both cell membranes. Upon withdrawal of amiloride,  $R_t$  returned to its control value faster than did  $V_{mc}$  or  $V_{cs}$ . The effects of amiloride

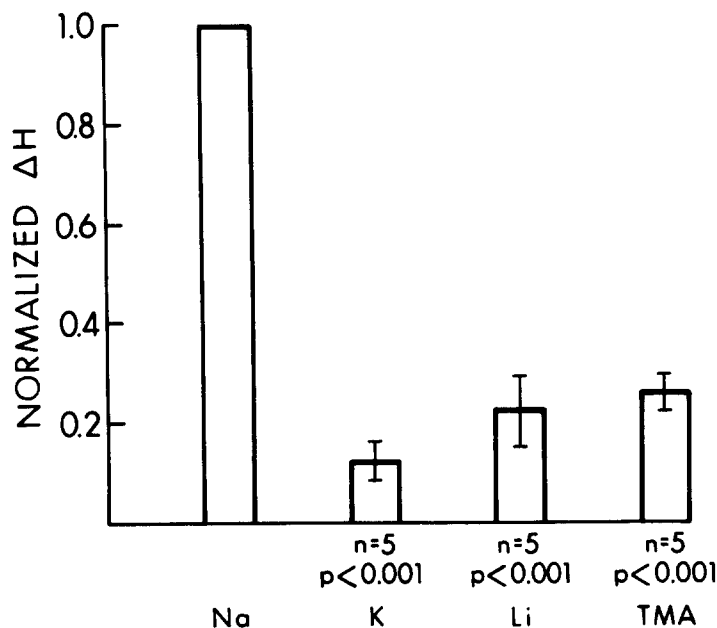


FIGURE 2. Effects of prolonged mucosal solution  $\text{Na}^+$  substitutions on luminal solution acidification. Mucosal solution acidification was measured according to protocol 1. Tissues were perfused with Na-Ringer's on the serosal side and either Na-Ringer's, K-Ringer's, Li-Ringer's, or TMA-Ringer's on the mucosal side. Rates of acidification ( $\Delta H$ ), for  $\text{K}^+$ ,  $\text{Li}^+$ , and  $\text{TMA}^+$  solutions were calculated as described in Methods and normalized to the respective rates in Na-Ringer's determined either immediately before or immediately after ionic substitution. *P* values compare the rate for each substitution to that in Na-Ringer's solution.

on cell membrane potentials and resistances are presented in Table I. Because the apical membrane is predominantly  $\text{K}^+$  permeable, a likely explanation for the cell membrane depolarization produced by amiloride is a decrease of  $\text{K}^+$  permeability. This was investigated by measuring the effect of  $\text{K}^+$  for  $\text{Na}^+$  substitutions on membrane potentials under control conditions and in the presence of amiloride. As shown in Table II, the changes in  $V_{mc}$  and  $V_{cs}$  produced by mucosal  $\text{K}^+$  for  $\text{Na}^+$  substitution were less during the presence

of amiloride than in its absence. This demonstrates that the relative K<sup>+</sup> permeability of the apical membrane decreased in the presence of the drug. The observation that  $R_a/R_b$  decreases during exposure to amiloride (Table I) suggests that the basolateral membrane conductance decreases as well.

#### *Effect of Ouabain on Acidification*

The inhibition of acidification by Na<sup>+</sup> replacement or amiloride is consistent with Na<sup>+</sup>-H<sup>+</sup> exchange, but it might also be explained by an effect of Na<sup>+</sup>

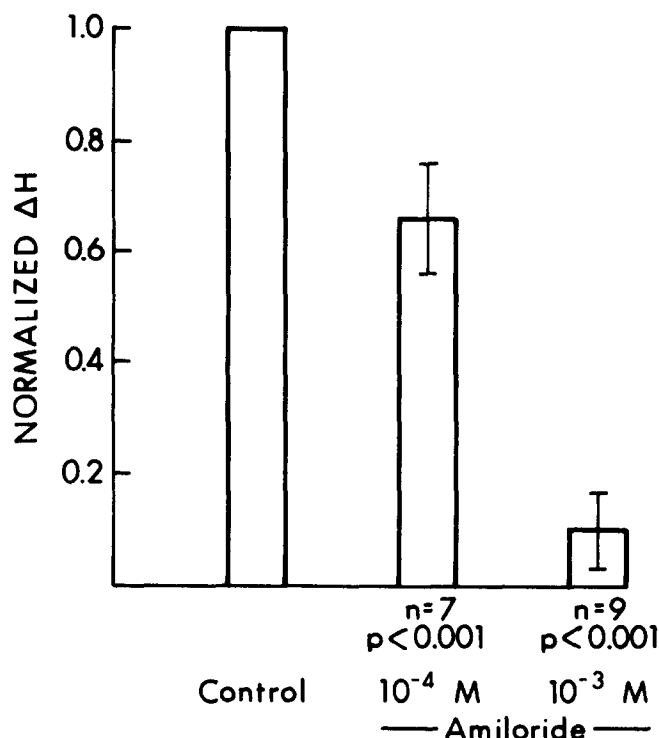


FIGURE 3. Effect of amiloride addition to the mucosal solution on luminal acidification. Rates of acidification with Na-Ringer's solution or Na-Ringer's plus amiloride (1.0 or 0.1 mM) bathing the mucosal side were determined according to protocol 1. Rates were normalized and compared as in Fig. 2.

entry on the rate of metabolic CO<sub>2</sub> production. Na<sup>+</sup> removal or exposure to amiloride might decrease the rate of Na<sup>+</sup> entry into the cells, which might reduce sodium pump rate and subsequently decrease ATP consumption and metabolic CO<sub>2</sub> production. To distinguish between these two possibilities, mucosal acidification was studied under conditions in which the activity of the sodium pump was inhibited by the serosal application of 10<sup>-4</sup> M ouabain.

After 60 min of serosal exposure to ouabain, mucosal acidification was reduced ~60% of the control rate (Fig. 5). The remaining acidification was still Na<sup>+</sup> dependent. Complete Na<sup>+</sup> replacement with TMA<sup>+</sup> after 1 h of exposure to ouabain changed the acidification rate from  $+6.27 \pm 0.99$  to

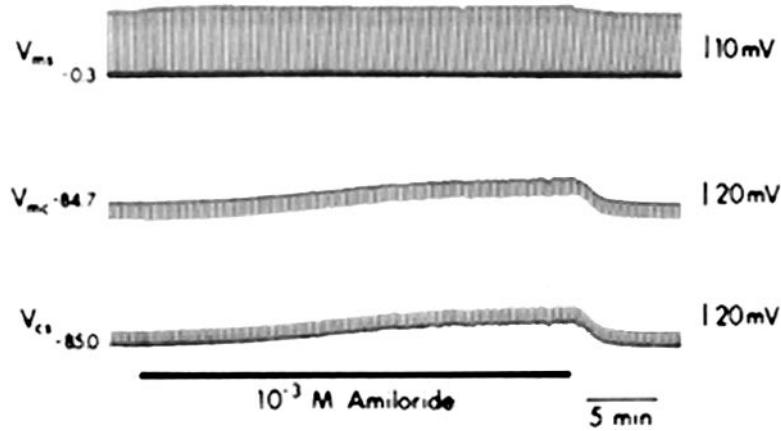


FIGURE 4. Effect of amiloride on membrane potentials. The records begin with a KCl-filled microelectrode in a cell. The numbers to the left of each record represent the initial values of the potentials. The vertical deflections were produced by transepithelial current pulses ( $100 \mu\text{A}/\text{cm}^2$ , 1 s duration). During the period indicated by the bar,  $1.0 \text{ mM}$  amiloride was added to the mucosal perfusion solution. The upward drifts seen in the records of  $V_{mc}$  and  $V_{cs}$  during amiloride exposure represent changes of voltage to more positive values. Note the changes in the heights of the deflections produced in  $V_{ms}$  by transepithelial current pulses. These reflect changes in transepithelial resistance.

TABLE I  
EFFECTS OF AMILORIDE ON POTENTIALS AND RESISTANCES

	Transepithelial potential ( $V_{ms}$ )	Apical membrane potential ( $V_{mc}$ )	Basolateral membrane potential ( $V_{cs}$ )	Ratio of membrane resistances ( $R_a/R_b$ )	Transepithelial resistance
	$mV$	$mV$	$mV$		$\Omega \cdot \text{cm}^2$
Na-Ringer's	$-0.4 \pm 0.2$	$-69.7 \pm 3.9$	$-70.1 \pm 3.9$	$2.88 \pm 0.28$	$158 \pm 20$
Na-Ringer's + amiloride ( $1.0 \text{ mM}$ )	$-0.3 \pm 0.2$	$-60.7 \pm 3.9$	$-61.0 \pm 3.9$	$2.03 \pm 0.40$	$210 \pm 26$
<i>P</i>	NS	$<0.001$	$<0.001$	$<0.001$	$<0.001$

Values in Na-Ringer's containing amiloride were recorded after 10 min of exposure to the drug.  $n =$  eight tissues. NS, not significant.

$-1.91 \pm 0.92 \mu\text{mol H}^+ \cdot \text{liter}^{-1} \cdot \text{min}^{-1}$  ( $n = 9$ ,  $P < 0.001$ ). In other words, during prolonged exposure to ouabain,  $\text{Na}^+$  replacement with  $\text{TMA}^+$  caused net alkalinization of the mucosal solution.

*Effects of Rapid Luminal Ionic Replacement: Cationic Selectivity and Concentration Dependence of Acidification*

The first protocol for measuring mucosal acidification has the disadvantage of requiring that the tissue be perfused for some time with the test solution before the perfusion is stopped so the acidification can be measured. During this preincubation period, intracellular ionic composition may change so that acidification or lack thereof may be a result of changes in intracellular as well



as mucosal solution parameters. To circumvent this problem, a second protocol was used to measure mucosal acidification. The mucosal side of the tissue was superfused with TMA-Ringer's for 10 min, after which the mucosal superfusion was stopped. 2 min later, the test cation was added either by pipetting

TABLE II  
EFFECT OF AMILORIDE ON APICAL MEMBRANE K<sup>+</sup>  
SELECTIVITY

	$\Delta V_{ms}$	$\Delta V_{mc}$	$\Delta V_{cs}$
	<i>mV</i>	<i>mV</i>	<i>mV</i>
Na-Ringer's	$-6.8 \pm 1.1$	$70.7 \pm 3.8$	$63.9 \pm 3.0$
Na-Ringer's + amiloride (1.0 mM)	$-5.7 \pm 0.9$	$57.9 \pm 2.6$	$52.1 \pm 2.0$
<i>P</i>	NS	<0.005	<0.025

Values shown are the changes in transepithelial potential ( $\Delta V_{ms}$ ), apical membrane potential ( $\Delta V_{mc}$ ), and basolateral membrane potential ( $\Delta V_{cs}$ ) produced by complete luminal replacement of Na<sup>+</sup> with K<sup>+</sup>. *n* = six tissues.

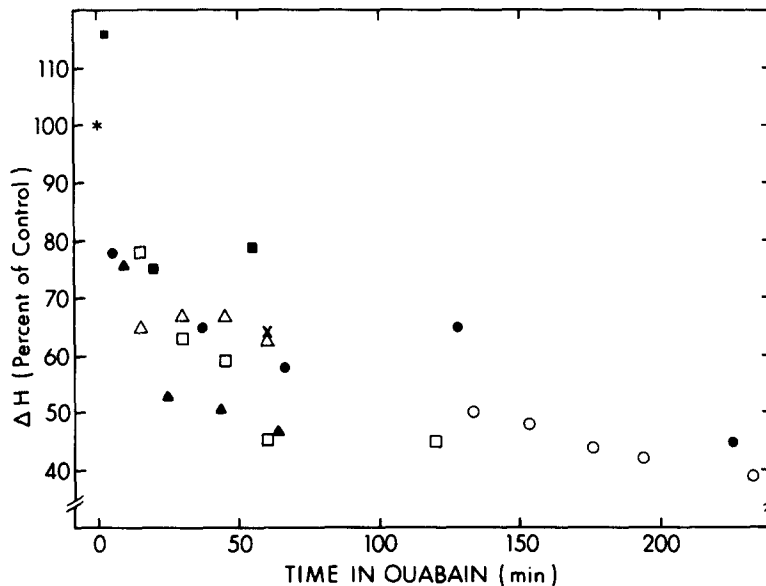


FIGURE 5. Effect of ouabain on mucosal solution acidification. At  $t = 0$ , the serosal perfusion was replaced by Na-Ringer's containing 0.1 mM ouabain and the mucosal acidification rate ( $\Delta H$ ) was measured according to protocol 1, with Na-Ringer's on the mucosal side, at the indicated times. Acidification rate is presented as percent of the control value before ouabain addition. The asterisk at 100% represents the control value for all experiments. Each of the other symbols represents a different tissue ( $n = 7$ ).

directly onto the nonperfused mucosal solution or by rapidly (<3 s) replacing the TMA-Ringer's on the mucosal side with a solution containing various concentrations of the cation to be tested. In this way, different mucosal ionic substitutions could be performed with the same initial intracellular composi-

tion. The effect of a change in the mucosal composition alone could therefore be studied.

Fig. 6 is an example of experiments in which tissues were first exposed to ouabain ( $10^{-4}$  M) on the serosal side for 1 h, after which the effects of  $\text{Na}^+$ ,  $\text{Li}^+$ , or  $\text{K}^+$  on mucosal pH were studied according to protocol 2. The sudden addition of  $\text{Na}^+$  or  $\text{Li}^+$  but not  $\text{K}^+$  caused a rapid acidification of the mucosal solution. In seven experiments performed in the presence of serosal ouabain

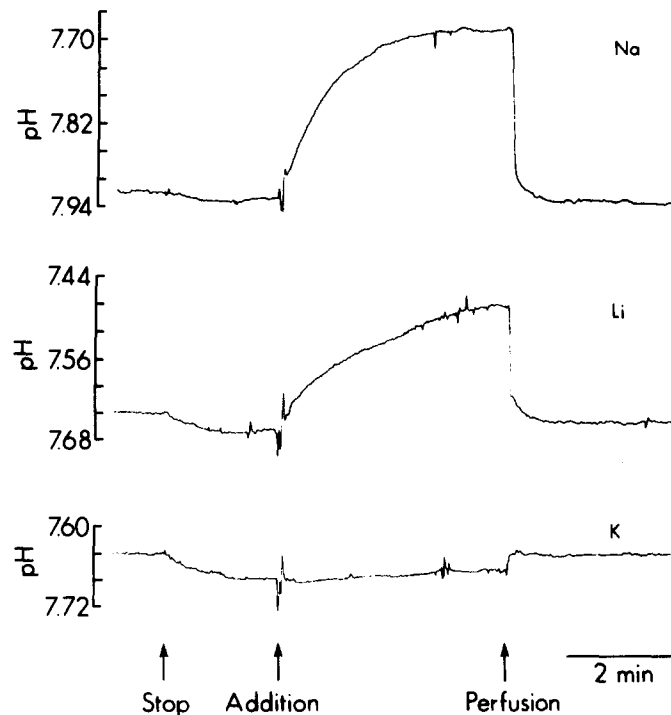


FIGURE 6. Mucosal solution acidification resulting from rapid cation addition to ouabain-treated tissues. Gallbladders were perfused on the serosal side for at least 60 min with Na-Ringer's + 0.1 mM ouabain and on the mucosal side for at least 10 min with TMA-Ringer's solution. The records represent mucosal solution pH as a function of time. At the first arrow, mucosal superfusion was stopped. At the second arrow, an aliquot of either Na-Ringer's, Li-Ringer's, or K-Ringer's was added to the mucosal solution to give final concentrations of 18.2, 9.9, and 9.9 mM, respectively. At the third arrow, mucosal superfusion with TMA-Ringer's was resumed. For each of the three experiments represented, the pH of the TMA-Ringer's and the added solution were identical.

( $10^{-4}$  M), the rapid exposure to mucosal  $\text{Na}^+$  (7.8–12.1 mM) after 10–15 min of  $\text{Na}^+$ -free mucosal perfusion (protocol 2) resulted in an acidification rate of  $64.4 \pm 24.9 \mu\text{mol H}^+ \cdot \text{liter}^{-1} \cdot \text{min}^{-1}$ . In the same tissues, the rate of acidification in Na-Ringer's solution (protocol 1) before ouabain was  $24.0 \pm 4.4 \mu\text{mol H}^+ \cdot \text{liter}^{-1} \cdot \text{min}^{-1}$ . This result demonstrates that in ouabain-treated tissues, acidification is stimulated by the presence of an inwardly directed  $\text{Na}^+$  gradient.

The effect on mucosal solution pH of sudden replacement of TMA<sup>+</sup> by each of the five alkali metal cations is presented in Table III. In this series, the tissues were not exposed to ouabain. Both Na<sup>+</sup> and Li<sup>+</sup> caused mucosal acidification, but K<sup>+</sup>, Rb<sup>+</sup>, and Cs<sup>+</sup> did not. In the same five tissues, the rate of acidification during continuous exposure to Na-Ringer's (protocol 1) was  $69.8 \pm 19.8 \mu\text{mol H}^+ \cdot \text{liter}^{-1} \cdot \text{min}^{-1}$ . This rate was only 11% of the one obtained when Na<sup>+</sup> was added after luminal superfusion with TMA-Ringer's ( $609.8 \pm 60.8$ ;  $P < 0.001$ ).

The dependence of the initial rate of acidification on Na<sup>+</sup> and Li<sup>+</sup> concentration was studied using protocol 2. Although the actual kinetics of this process are not known, an approximate cation affinity of the net acidification process was obtained by fitting normalized pooled data to the Michaelis-Menten equation as described in the legend to Fig. 7. The results presented in Fig. 7 show that the apparent affinities of the acidification process were 11.3 mM for Na<sup>+</sup> and 2.3 mM for Li<sup>+</sup>. The maximal rate of acidification, however, was about five times greater for Na<sup>+</sup> than for Li<sup>+</sup> (Table III).

TABLE III  
EFFECTS OF RAPID ION REPLACEMENT ON INITIAL RATE OF MUCOSAL ACIDIFICATION

	Na <sup>+</sup>	Li <sup>+</sup>	K <sup>+</sup>	Rb <sup>+</sup>	Cs <sup>+</sup>	TMA <sup>+</sup>
Mean	609.8	112.5	5.1	6.8	-14.9	-2.3
SE	±60.8	±12.5	±4.9	±4.6	±17.3	±5.0
n	5	5	4	5	5	5

Values are given in  $\mu\text{mol H}^+ \cdot \text{liter}^{-1} \cdot \text{min}^{-1}$ . Tissues were first exposed to mucosal TMA-Ringer's and then to Ringer's solution containing the indicated cation (109.2 mM, protocol 2). Rate of acidification in Na<sup>+</sup> is significantly greater than that in Li<sup>+</sup> ( $P < 0.001$ ). Rates of acidification in K<sup>+</sup>, Rb<sup>+</sup>, Cs<sup>+</sup>, and TMA<sup>+</sup> are not significantly different from zero but are significantly different from those in both Na<sup>+</sup> and Li<sup>+</sup> ( $P < 0.01$ ). In these five tissues, the rate of acidification during continuous mucosal exposure to Na-Ringer's (protocol 1) was  $69.8 \pm 19.8$ .

#### *Mucosal Acidification at Different Apical Membrane Potentials*

The influence of apical membrane potential on acidification rate was tested under conditions in which mucosal Na<sup>+</sup> concentration was kept constant while  $V_{mc}$  was changed. Gallbladders were superfused on the mucosal side with TMA-Ringer's for 10 min and then superfusion was stopped and the mucosal solution was replaced with a solution containing 10.9 mM Na<sup>+</sup> and either 98.3 mM TMA<sup>+</sup> or 98.3 mM K<sup>+</sup>. The results, presented in Table IV, demonstrate that although the mean  $V_{mc}$  values differ by ~77 mV in these two conditions, the rates of acidification were not significantly different.

#### *Intracellular pH: Effects of Amiloride and Luminal Na<sup>+</sup> Substitution*

Intracellular pH (pH<sub>i</sub>) was measured with recessed-tip glass pH microelectrodes. Due to the relatively large tip size of these electrodes and their slow response time, it was necessary to establish objective criteria to validate the impalements. The validation criteria used were those reported previously

(Reuss and Weinman, 1979). The potential change upon impalement was initially abrupt and was followed by a slower hyperpolarization until a stable value was reached; the changes in potential were monotonic. In general, two cells in a given tissue were impaled and recorded from simultaneously, one with a pH microelectrode and the other with a KCl-filled conventional

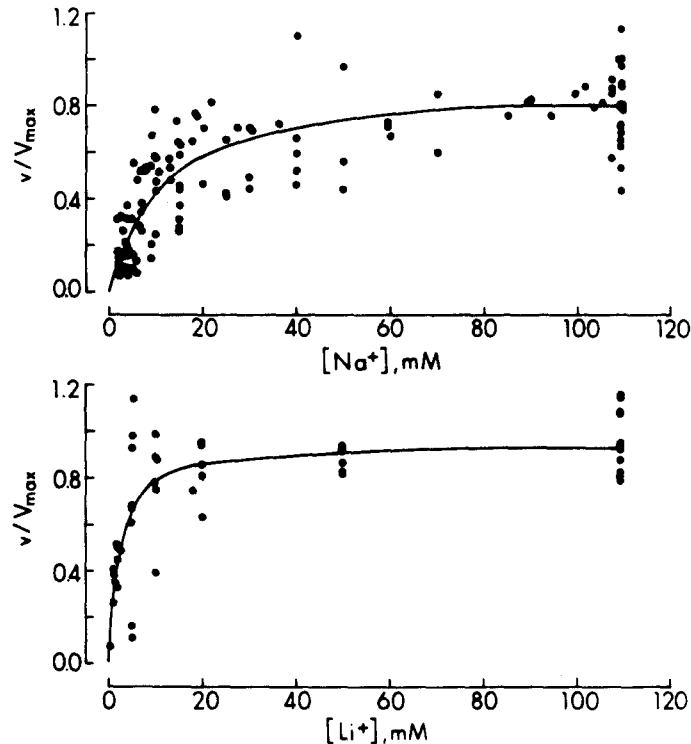


FIGURE 7. Dependence of transient acidification on  $Na^+$  and  $Li^+$  concentration. Gallbladders were perfused with Na-Ringer's on the serosal side and TMA-Ringer's on the mucosal side. Initial acidification rates after  $Na^+$  or  $Li^+$  addition were measured according to protocol 2. Since  $V_{max}$  varied from tissue to tissue, results for each tissue were analyzed by a nonlinear least-squares fit to the Michaelis-Menten equation; for each tissue, the rates of acidification were expressed as  $v/V_{max}$ . Relative rates from several tissues were then pooled and plotted vs. final  $Na^+$  or  $Li^+$  concentration. The points represent the normalized data and the curves represent the nonlinear least-squares fit of the pooled data to the Michaelis-Menten equation. The best fit values for  $K_m$  and normalized  $V_{max}$  for  $Na^+$  (upper panel,  $n = 11$ ) are  $11.3 \pm 0.2$  mM and  $0.91 \pm 0.43$ , respectively; those for  $Li^+$  (lower panel,  $n = 6$ ) are  $2.3 \pm 0.6$  mM and  $0.95 \pm 0.4$ , respectively.

microelectrode. Aside from allowing continuous measurement of intracellular pH, simultaneous impalements allowed us to compare the membrane properties of the two cells. For a pH impalement to be accepted, the same steady state voltage deflections had to be produced across the basolateral membranes

of both cells when transepithelial current pulses were passed. This criterion depends upon the two impaled cells having the same apparent ratio of cell membrane resistances and was applied in all cases. In some cases, the additional requirement that both cells depolarize by the same amount when exposed briefly to high K<sup>+</sup> on the mucosal side was used. Examples of these two validation criteria are shown in Fig. 8. Under control conditions, i.e., in tissues perfused continuously on both sides with Na-Ringer's (pH 7.75 ± 0.01), pH<sub>i</sub> was 7.51 ± 0.04 (*n* = 15 preparations).

Intracellular pH measurements before, during, and after changes in mucosal solution composition provided additional information about the mechanism of mucosal acidification. If extracellular acidification results from metabolic CO<sub>2</sub> production and CO<sub>2</sub> diffusion into the luminal solution, then the inhibitory effects of amiloride and of Na<sup>+</sup> removal should result either in

TABLE IV  
RELATIONSHIP BETWEEN ACIDIFICATION RATE AND APICAL  
MEMBRANE POTENTIAL

Test solution	Apical membrane potential ( <i>V</i> <sub>mc</sub> )	Acidification rate
	<i>mV</i>	$\mu\text{mol H}^+ \cdot \text{liter}^{-1} \cdot \text{min}^{-1}$
10.9 mM Na <sup>+</sup> 98.3 mM TMA <sup>+</sup>	-92.6 ± 4.8	259 ± 52
10.9 mM Na <sup>+</sup> 98.3 mM K <sup>+</sup>	-16.0 ± 2.1	267 ± 71
<i>P</i>	<0.001	NS

Acidification rates were measured according to protocol 2 either before or after potentials were measured with KCl-filled microelectrodes. The test solutions were Ringer's solutions in which 98.3 mM NaCl was replaced by isomolar substitution with TMACl or KCl. When Na-Ringer's (109.2 mM Na<sup>+</sup>) was used as the test solution, *V*<sub>mc</sub> and acidification rate were -79.0 ± 4.5 mV and 611 ± 107 μmol H<sup>+</sup> · liter<sup>-1</sup> · min<sup>-1</sup>, respectively. *n* = five preparations for all values reported.

intracellular alkalization or in no change in pH<sub>i</sub>. However, if acidification were a result of Na<sup>+</sup>-H<sup>+</sup> exchange, then amiloride and Na<sup>+</sup> replacement should cause intracellular acidification.

To test these possibilities, pH<sub>i</sub> was continuously measured for at least 10 min, during which time the mucosal perfusion was changed from Na-Ringer's to an experimental solution and back to Na-Ringer's. Fig. 9 shows examples of experiments in which we studied the effects on pH<sub>i</sub> of amiloride (1.0 mM) and complete mucosal Na<sup>+</sup> replacement with TMA<sup>+</sup> and Li<sup>+</sup>. A summary of the effects of these substitutions on pH<sub>i</sub> is presented in Fig. 10. Amiloride (1.0 mM) or Na<sup>+</sup> replacement with either TMA<sup>+</sup> or Li<sup>+</sup> caused reversible intracellular acidification. These results are consistent with the steady state operation of a Na<sup>+</sup>-H<sup>+</sup> exchange process at the apical membrane of the epithelial cells.

## DISCUSSION

The results presented in this paper demonstrate the existence of a  $\text{Na}^+$ -dependent, amiloride-sensitive luminal acidification process in *Necturus* gallbladder. Acidification occurs continuously when the tissue is bathed on both sides by Na-Ringer's.

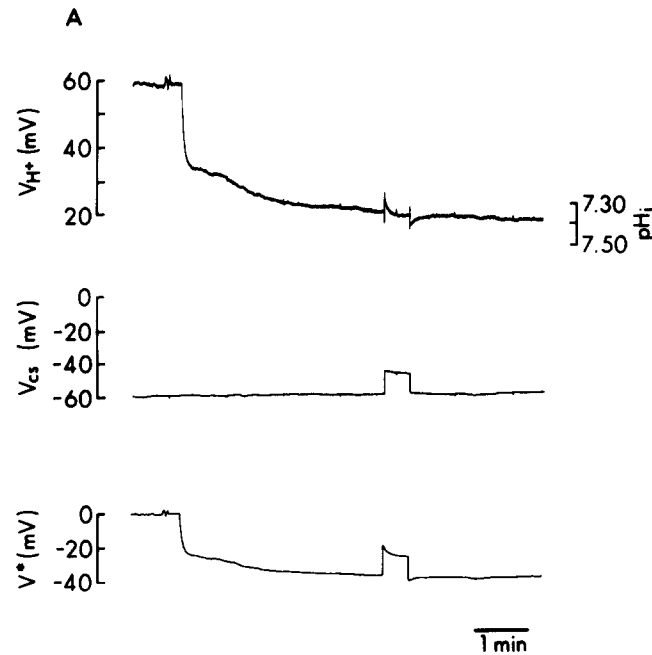
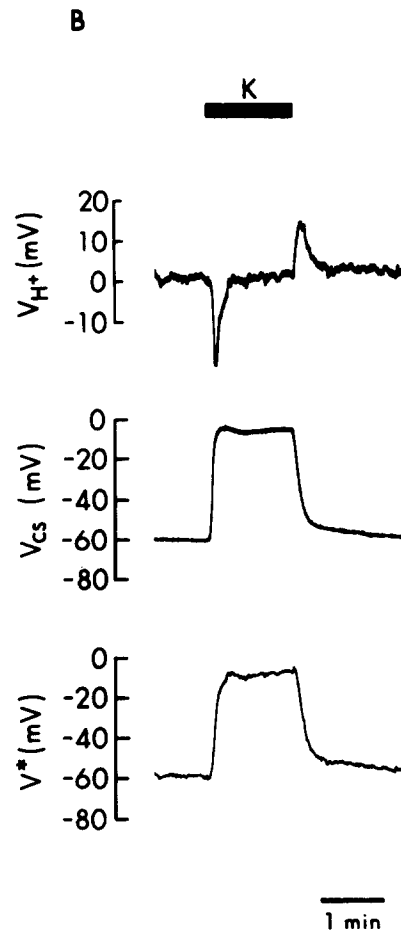


FIGURE 8. Validation criteria for impalements with recessed-tip pH microelectrodes. In each panel, the lower trace is the potential recorded by the pH microelectrode ( $V^*$ ). The middle trace is the basolateral membrane potential ( $V_{cs}$ ) and the upper trace is the difference ( $V_{H^+}$ ) = ( $V^*$ ) - ( $V_{cs}$ ). A. The record begins with the KCl-filled microelectrode in a cell. After  $\sim 1$  min another cell was impaled with the pH microelectrode. After an additional 3.5 min a square transepithelial current pulse (30 s duration) was passed. The figure demonstrates that the steady state voltage deflections produced across the basolateral membranes of the two impaled cells were identical. B. The record begins with both electrodes in cells. During the period indicated by the bar the mucosal superfusion was changed from Na-Ringer's to K-Ringer's. The figure demonstrates that the steady state depolarization produced by brief mucosal  $\text{K}^+$  for  $\text{Na}^+$  substitution was identical in both cells. The intracellular pH was 7.67.

#### Mechanism of Luminal Acidification

Na-dependent acidification in gallbladder has been observed previously (Sullivan and Berndt, 1973*a, b*; Whitlock and Wheeler, 1969; Cremaschi et al., 1979), but could have been explained either by  $\text{Na}^+$ - $\text{H}^+$  exchange or by a nonspecific acid transport process such as  $\text{CO}_2$  diffusion, in which the rate of acid production by the epithelial cells is  $\text{Na}^+$  dependent.

The results of this study demonstrate that the mechanism of acid secretion in *Necturus* gallbladder is Na<sup>+</sup>-H<sup>+</sup> exchange, or an equivalent process such as Na<sup>+</sup>-OH<sup>-</sup> cotransport, and not metabolic CO<sub>2</sub> diffusion, for the following reasons. (a) Mucosal solution acidification depends on luminal Na<sup>+</sup> concentration even when the tissue is poisoned by ouabain. Under these conditions fluid transport ceases (Reuss et al., 1979; van Os and Slegers, 1975), and it is



therefore unlikely that, in the presence of ouabain, Na<sup>+</sup> stimulates metabolic CO<sub>2</sub> production. (b) Intracellular pH falls when acidification is inhibited by Na<sup>+</sup> replacement or amiloride addition. This suggests that inhibition of acidification is caused by a reduction of H<sup>+</sup> transport across the apical membrane and not by a reduction of the rate of H<sup>+</sup> production. (c) Even in the presence of ouabain, the rate of mucosal acidification depends on the Na<sup>+</sup> gradient across the apical membrane and not just the presence of Na<sup>+</sup> in the

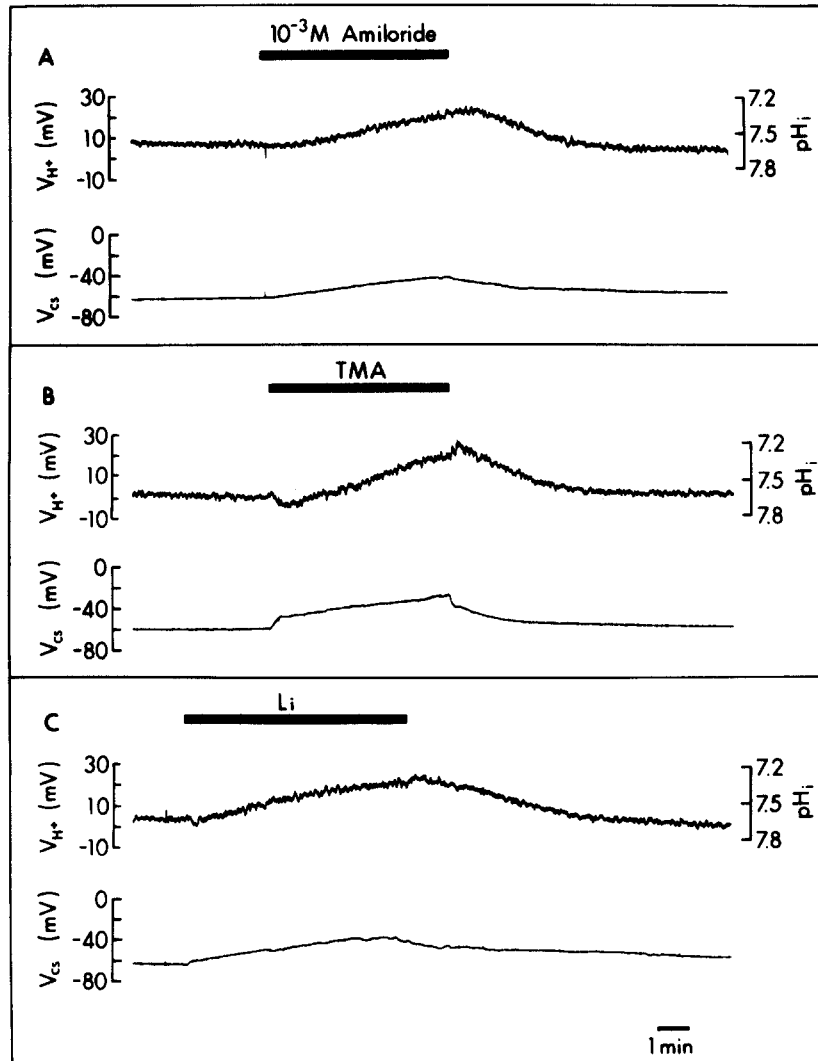


FIGURE 9. Effects of amiloride and  $\text{Na}^+$  replacement on  $\text{pH}_i$ . The lower record of each pair represents the basolateral membrane potential ( $V_{cs}$ ). The upper record of each pair ( $V_{H^+}$ ) represents the difference between the potential measured by the pH microelectrode and  $V_{cs}$ . All records begin with both microelectrodes in cells. The serosal side was perfused with Na-Ringer's. During the periods indicated by the bars the mucosal perfusion solution was changed from Na-Ringer's to one of the following test solutions: Na-Ringer's containing 1.0 mM amiloride (A), TMA-Ringer's (B), and Li-Ringer's (C).

mucosal solution. When ouabain-treated tissues were perfused with TMA-Ringer's for 10 min and then suddenly exposed to  $\sim 10$  mM  $\text{Na}^+$  on the mucosal side, they acidified the mucosal solution about two to three times as rapidly as they did when they were exposed to Na-Ringer's continuously. This



result is a direct prediction of the Na<sup>+</sup>-H<sup>+</sup> exchange model because tissues exposed to low-Na<sup>+</sup> solutions on the mucosal side have low intracellular Na<sup>+</sup> (Garcia-Diaz and Armstrong, 1980; Weinman and Reuss, 1982) and elevated intracellular H<sup>+</sup> activity (Fig. 10). Sudden re-exposure to Na<sup>+</sup>-containing media results in a large inward Na<sup>+</sup> gradient and an increased outward H<sup>+</sup> gradient. One would therefore expect a greater rate of H<sup>+</sup> efflux than under control conditions.

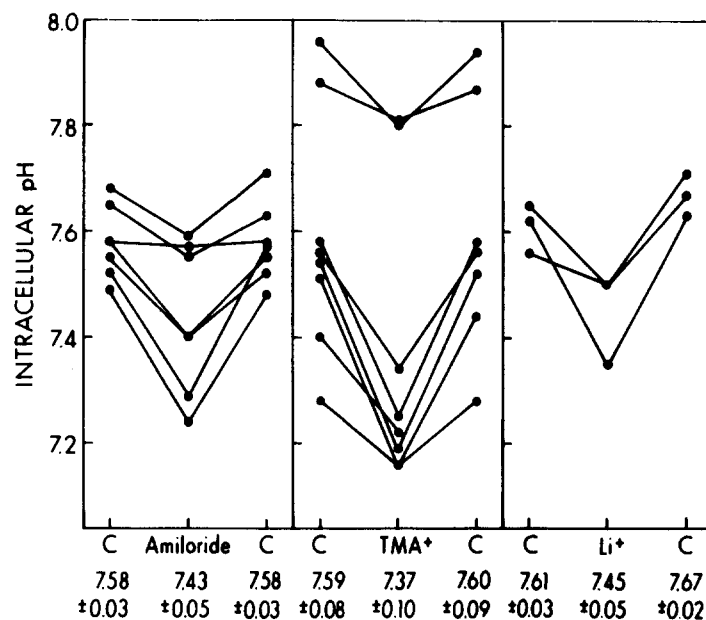


FIGURE 10. Effects of amiloride and luminal Na<sup>+</sup> removal on pHi. Intracellular pH was measured in gallbladders that were perfused on both sides with control solution (Na-Ringer's). After a stable value was obtained the mucosal solution was substituted with either Na-Ringer's + amiloride (1.0 mM), TMA-Ringer's, or Li-Ringer's, as shown in Fig. 9; after 5-10 min the mucosal side was perfused with Na-Ringer's again. Points represent values of pHi determined before, during, and after the mucosal solution changes. Lines connect values in individual tissues. Mean pHi ± SE for the different conditions are given at the bottom of the figure. pHi values in the presence of amiloride and TMA<sup>+</sup> were significantly different from control,  $P < 0.005$  and  $P < 0.001$ , respectively.

#### *Characteristics of Na<sup>+</sup>-H<sup>+</sup> Exchange*

The Na<sup>+</sup>-H<sup>+</sup> exchange at the luminal membrane of *Necturus* gallbladder appears to share many properties with Na<sup>+</sup>-H<sup>+</sup> exchange in other tissues. It is 90% inhibited by amiloride (1.0 mM). This inhibition cannot be explained by electrogenic H<sup>+</sup> transport altered by the effect of amiloride on cell membrane potentials because the amiloride-induced cell depolarization would be expected to increase the rate of a putative electrogenic H<sup>+</sup> secretion. Amiloride,

instead, is likely to act as a competitive inhibitor of  $\text{Na}^+\text{-H}^+$  exchange (Kinsella and Aronson, 1981a). Inhibition of gallbladder  $\text{Na}^+\text{-H}^+$  exchange by amiloride is similar in concentration dependence and reversibility to that reported for mouse soleus muscle fibers (Aickin and Thomas, 1977), sea urchin eggs (Johnson et al., 1976), *Amphiuma* red blood cells (Cala, 1980), rabbit renal cortical microvillus membrane vesicles (Kinsella and Aronson, 1981a), and salamander proximal tubule (Boron and Boulpaep, 1982).

It is interesting to note that  $\text{Na}^+$  substitution with  $\text{Li}^+$  inhibited acidification under steady state conditions (Fig. 2), whereas rapid  $\text{Li}^+$  addition to  $\text{Na}^+$ -depleted tissues induced acidification (Fig. 6 and Table III). These results imply that  $\text{Li}^+$  can substitute for  $\text{Na}^+$  in the exchange process but cannot replace  $\text{Na}^+$  in the maintenance of steady state acidification. This phenomenon would be explained if gallbladder transport of  $\text{Li}^+$  were similar to that in frog skin, where  $\text{Li}^+$  enters the cells but is not transported by the  $\text{Na}^+\text{-K}^+$  ATPase (Zerahn, 1955; Morel and Leblanc, 1975).  $\text{Li}^+$  would therefore be accumulated inside the cell under steady state conditions and the absence of a  $\text{Li}^+$  gradient across the apical membrane would prevent steady state mucosal acidification.

$\text{Na}^+\text{-H}^+$  exchange in *Necturus* gallbladder also has alkali metal ion requirements similar to those seen in other systems (Kinsella and Aronson, 1981b; Ives et al., 1982). Both  $\text{Na}^+$  and  $\text{Li}^+$  exchange for  $\text{H}^+$ , but  $\text{K}^+$ ,  $\text{Rb}^+$ , and  $\text{Cs}^+$  do not (Table III). Although the kinetics of the process are unknown, half-maximal acidification in *Necturus* gallbladder is obtained at  $\text{Na}^+$  and  $\text{Li}^+$  concentrations of 11.3 and 2.3 mM, respectively. These values must be considered approximations, however, because the technique of measurement of extracellular acidification has inherent errors which include small changes in electrode position and possible alterations of intracellular ionic composition over the course of the several hours that are required to examine a concentration range from 0 to 100 mM. Although our data fully support the conclusion that  $\text{Na}^+$  and  $\text{Li}^+$ , but not the other cations tested, can exchange for  $\text{H}^+$ , the quantitative estimate of the affinities of the exchanges for  $\text{Na}^+$  and  $\text{Li}^+$  is less certain. In experiments on  $\text{Na}^+\text{-H}^+$  exchange in renal brush border vesicles, Kinsella and Aronson (1981b) determined the affinities for  $\text{Na}^+$  and  $\text{Li}^+$  to be  $6.1 \pm 0.7$  and  $1.9 \pm 0.2$  mM, respectively, and Ives et al. (1982) obtained values of  $13.9 \pm 2.6$  and  $1.8 \pm 0.4$ . The results of the present study are in reasonable agreement with those values.

The  $\text{Na}^+\text{-H}^+$  exchange in *Necturus* gallbladder appears to be a neutral process like that reported in other systems (Kinsella and Aronson, 1980; Murer et al., 1976; Burnham et al., 1981). This conclusion is supported by the observation that at a given mucosal solution  $\text{Na}^+$  concentration, the rate of acidification remains the same when the apical membrane potential is changed by almost 80 mV (Table IV). It is therefore unlikely that  $\text{H}^+$  movement across the apical membrane is dependent on  $V_{mc}$ .

Prolonged exposure to ouabain reduces but does not abolish mucosal acidification when measured according to protocol 1 (Fig. 5). The reduction can be explained, at least in part, by the elevated intracellular  $\text{Na}^+$  activity

that results from the inhibition of the Na<sup>+</sup>-K<sup>+</sup> pump. The reduced Na<sup>+</sup> gradient would result in a lower rate of Na<sup>+</sup>-H<sup>+</sup> exchange. The existence of a residual rate of acidification even after prolonged exposure to ouabain may also be explained by the Na<sup>+</sup> and H<sup>+</sup> gradients. Because the cells are more acid than the mucosal solution, even if ouabain were to abolish the Na<sup>+</sup> gradient completely, acidification might still be driven by the apical membrane H<sup>+</sup> gradient. It is interesting to note that complete mucosal Na<sup>+</sup> replacement with TMA<sup>+</sup>, after prolonged exposure to ouabain, caused net mucosal alkalinization. This might be explained by the combination of sudden removal of external Na<sup>+</sup> and elevated intracellular Na<sup>+</sup> activity; the resulting reversed, outwardly directed Na<sup>+</sup> gradient may be large enough to drive H<sup>+</sup> into the cell.

#### *Intracellular pH in Necturus Gallbladder*

When *Necturus* gallbladder is bathed in Na-Ringer's, pH 7.75, pH<sub>i</sub> is 7.51. The cells are more acidic than the bathing medium, but H<sup>+</sup> activity is less than that predicted from equilibrium distribution (pH ~6.6). This result is in agreement with other microelectrode measurements of pH<sub>i</sub> in epithelia. Fujimoto et al. (1980) determined pH<sub>i</sub> to be 7.49 in bullfrog proximal tubules bathed in pH 7.66 solution, and Boron and Boulpaep (1982) measured a pH<sub>i</sub> of 7.43 in salamander proximal tubules bathed in pH 7.50 solution. As is the case for nonepithelial cells (see Roos and Boron, 1981, for a review), pH<sub>i</sub> determined by the above investigators is less than that of the extracellular medium, but H<sup>+</sup> is below electrochemical equilibrium.

The present investigation shows that apical membrane Na<sup>+</sup>-H<sup>+</sup> exchange is involved in maintaining low intracellular H<sup>+</sup> activity; when this process is inhibited the cells acidify (Figs. 9 and 10). Boron and Boulpaep (1982) have also demonstrated a direct role of Na<sup>+</sup>-H<sup>+</sup> exchange in epithelial pH<sub>i</sub> regulation similar to that reported here. In the salamander proximal tubule, pH<sub>i</sub> recovery from an acid load is Na<sup>+</sup> dependent and amiloride inhibitable and appears to result, at least in part, from Na<sup>+</sup>-H<sup>+</sup> exchange at the luminal membrane.

Mucosal addition of amiloride (1.0 mM) causes cell depolarization with a time course similar to that of intracellular acidification (see Fig. 9A). Cell depolarization may be causally related to intracellular acidification if the reduction of pH<sub>i</sub> decreases apical membrane K<sup>+</sup> permeability, just as apical membrane K<sup>+</sup> permeability decreases when the pH of the mucosal bathing solution is lowered (Reuss et al., 1981). Such a reduction in relative K<sup>+</sup> permeability was observed in these studies (Table II), and should have a substantial effect on membrane potentials, especially since the Na<sup>+</sup> conductance of the apical membrane is small (Reuss and Finn, 1975b).

#### *Physiological Significance of Na<sup>+</sup>-H<sup>+</sup> Exchange*

It is clear from the data presented in Fig. 10 that the maintenance of intracellular pH requires a functioning Na<sup>+</sup>-H<sup>+</sup> exchange. It is not clear, however, whether Na<sup>+</sup>-H<sup>+</sup> exchange serves other functions as well.

NaCl absorption by the gallbladder is known to depend on neutral NaCl uptake at the apical membrane (Frizzell et al., 1975; Duffey et al., 1978; Reuss and Grady, 1979; Garcia-Diaz and Armstrong, 1980; Rose and Nahrwold, 1980). Two possible explanations for coupled transport are the operation of a single NaCl carrier (Frizzell et al., 1975; Frizzell et al., 1979; Cremaschi et al., 1979) or the tandem operation of  $\text{Na}^+\text{-H}^+$  and  $\text{Cl}^-\text{-HCO}_3^-$  exchanges (Liedtke and Hopfer, 1982*a, b*; Heintze et al., 1981; Petersen et al., 1981). Spring and colleagues (Ericson and Spring, 1982*a, b*) have suggested, from measurement of changes in cell volume, that both a NaCl carrier and a double exchanger ( $\text{Na}^+\text{-H}^+$  and  $\text{Cl}^-\text{-HCO}_3^-$  exchangers) exist in the apical membrane of *Necturus* gallbladder. They claim that apical  $\text{Na}^+$  entry for steady state NaCl transport results solely from coupled NaCl cotransport and that  $\text{Na}^+\text{-H}^+$  and  $\text{Cl}^-\text{-HCO}_3^-$  exchangers operate when cell volume is perturbed.

The present investigation has focused on  $\text{Na}^+\text{-H}^+$  exchange and does not address itself directly to the growing controversy about the mechanism of apical membrane NaCl transport. It does, however, relate to the possibility that  $\text{Na}^+\text{-H}^+$  exchange could be responsible for a fraction of steady state apical membrane  $\text{Na}^+$  entry. The net rate of  $\text{H}^+$  secretion measured as extracellular acidification is  $1.45 \pm 0.27 \text{ nmol} \cdot \text{min}^{-1} \cdot \text{cm}^{-2}$ . If this  $\text{H}^+$  were to exchange 1:1 with  $\text{Na}^+$ , it would account for only ~7% of the net rate of  $\text{Na}^+$  absorption estimated from the fluid transport rate measured previously in this laboratory (Reuss et al., 1979). The net rate of  $\text{H}^+$  secretion, however, is likely to be an underestimate of the cell-to-lumen  $\text{H}^+$  flux, for the following reasons. First,  $\text{H}^+$  equivalents are lost into the air as  $\text{CO}_2$  in the open perfusion system used in this study. Second,  $\text{H}^+$  may be recycled across the apical membrane or the paracellular pathway either by electrodiffusion or by shifting the  $\text{HCO}_3^- \rightleftharpoons \text{H}_2\text{CO}_3$  equilibrium and allowing  $\text{H}_2\text{CO}_3$  or  $\text{CO}_2$  diffusion. Although no  $\text{HCO}_3^-$  was actually added to the solutions, at pH 7.75 the air-equilibrated solutions contained ~0.2 mM  $\text{HCO}_3^-$ . Third, if  $\text{Cl}^-\text{-HCO}_3^-$  exchange occurred along with  $\text{Na}^+\text{-H}^+$  exchange, the  $\text{H}^+$  would be partially neutralized by the  $\text{HCO}_3^-$  secreted into the lumen. In this case, the calculated  $\text{H}^+$  flux would also be an underestimate because the buffering power of the mucosal unstirred layer would be greater than that of the bulk solution.

In addition, the fluid transport rate measured previously (Reuss et al., 1979) may overestimate the rate expected in the present experiments since  $\text{HCO}_3^-$  is known to stimulate fluid transport, at least in mammalian gallbladder (Cremaschi et al., 1979; Heintze et al., 1981). The present studies were performed with 0.2 mM  $\text{HCO}_3^-$ , whereas the previous measurements (Reuss et al., 1979) were conducted at 2.4 mM  $\text{HCO}_3^-$ . Because of these uncertainties, 7% may be a minimal estimate of the contribution of  $\text{Na}^+\text{-H}^+$  exchange to overall apical membrane  $\text{Na}^+$  entry.

The results reported here do not seem to agree with the conclusion of Ericson and Spring (1982*a, b*) that  $\text{Na}^+\text{-H}^+$  exchange does not provide a route of  $\text{Na}^+$  entry for transepithelial transport. In the present study  $\text{Na}^+\text{-H}^+$  exchange has been found to operate in the absence of cell volume perturbation and in a nominally  $\text{HCO}_3^-$ -free medium.  $\text{Na}^+\text{-H}^+$  exchange must therefore be

responsible for at least a fraction of steady state apical Na<sup>+</sup> entry. This conclusion is supported by a preliminary report that mucosal application of amiloride to guinea pig gallbladder reduces the transepithelial fluid transport rate (Petersen, et al., 1978). The existence of Na<sup>+</sup>-H<sup>+</sup> exchange, however, in no way precludes the existence of other apical membrane Na<sup>+</sup> transport pathways.

In summary, the results of this study demonstrate that mucosal solution acidification by the *Necturus* gallbladder is the result of neutral Na<sup>+</sup>-H<sup>+</sup> exchange and not metabolic CO<sub>2</sub> production. Na<sup>+</sup>-H<sup>+</sup> exchange is necessary for the maintenance of steady state intracellular pH and could account for at least 7% of the Na<sup>+</sup> entry required for transepithelial fluid transport. Measurements of intracellular sodium and its dependence on the Na<sup>+</sup>-H<sup>+</sup> exchange process are in progress. These will provide further information on the contribution of Na<sup>+</sup>-H<sup>+</sup> exchange to apical membrane Na<sup>+</sup> entry.

We wish to thank R. Abercrombie and E. Bello-Reuss for help in the construction of electrodes, and A. Roos and K.-U. Petersen for constructive comments on a preliminary version of this paper. Amiloride was a generous gift from Dr. C. A. Stone (Merck, Sharpe and Dohme), and liquid H<sup>+</sup> exchanger was a generous gift from Dr. D. Ammann. We also appreciate the technical assistance of J. Costantin and the secretarial help of S. Eads.

This work was supported by grant AM-19580 from the National Institute of Arthritis, Metabolism and Digestive Diseases and National Research Service Award GM 07200 from the National Institute of General Medical Sciences.

*Received for publication 16 February 1982 and in revised form 17 May 1982.*

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