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Membrane Potentials, Resistances, and Conductances of Toad Bladder during $Na^+ - H^+$ Transport and H^+ Transport (41404)

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Abstract. This study was undertaken to define the electrical characteristics imparted to toad bladder by H^+ secretion in absence of Na^+ transport, and to determine Na^+ and H^+ conductances of the apical bladder cell membrane. Cellular membrane potentials, conductances and resistances, and paracellular resistance were studied with microelectrode methods during $Na^+ - H^+$ transport and with H^+ secretion after Na^+ transport was stopped by withdrawal of mucosal Na^+ and addition of mucosal amiloride. Epithelial potential reversed from 26 mV mucosa negative to 14 mV mucosa positive. Apical P.D. reversed from 13.7 mV cell positive to 16 mV cell negative to mucosa. Basal-lateral P.D. decreased to 3.8 mV, but cell remained negative to serosa. With Na^+ and H^+ transport and residual cationic and anionic conductances (probably K^+ and Cl^-), voltage divider ratio was 1.90, apical resistance was 2181 ohm·cm^2, shunt resistance was 12,387, and basal-lateral resistance was 1162. After stopping Na^+ transport, $\Delta\Psi a/\Delta\Psi b$ increased to 3.54, R_a to 3397, and R_s to 23,963 ohm·cm^2. R_b was unchanged. A mucosa–serosa H^+ gradient of 1000/1 raised R_a to 4629 ohm·cm^2 which suggested rectification of H^+ current. With 100 m*M* mucosal KCl, $\Delta\Psi a/\Delta\Psi b$ was 5.80 and R_a was 6714 ohm·cm^2. From these values, apical membrane conductances were calculated. Apical G_{Na+} of 168 μmhos·cm^2 was only slightly greater than the apical G_{H^+} of 151. Results are consistent with an electrogenic apical H^+ pump and electrogenic basal-lateral Na^+ pump.

The urinary bladder of the toad *Bufo marinus* actively transports sodium from mucosal to serosal surfaces and can maintain large transepithelial Na^+ concentration gradients (1). Sodium transport gives rise to a transepithelial electrical potential which is the sum of the potentials of apical and basal-lateral cell membranes. The epithelium has a high resistance. That of the cell is from 4000 to 5000 ohm·cm^2 and the paracellular or shunt resistance is 10,000 to 12,000 ohm·cm^2 (2–5). The high shunt resistance facilitates mucosal to serosal Na^+ transport by minimizing serosal to mucosal Na^+ flux. In addition to Na^+ transport, toad bladder also secretes H^+ to acidify the mucosal fluid (6–9). Proton secretion is facilitated by the presence of mucosal bicarbonate buffer (9, 10). The source of H^+ is hydration of CO_2, which is supplied either by gassing of ambient solutions or from endogenous production associated with Na^+ transport (11). The electrical characteristics of H^+ secretion can be delineated after sodium transport is stopped by amiloride and removal of mucosal sodium (9, 12). Both the polarity of the epithelial potential and the direction of the short circuit current are reversed by this maneuver. The purposes of this study were twofold: first, to delineate the membrane potentials and resistances and the paracellular shunt resistance during conditions of H^+ secretion in the absence of Na^+ transport; second, by utilization of this phenomenon, to delineate Na^+ and H^+ conductances (G) of apical membrane. The results showed that H^+ secretion in the absence of Na^+ transport reversed the polarity of the apical membrane potential, but the polarity of the basal-lateral potential remained unchanged. This was associated with a twofold increase in paracellular resistance and a 50% increase in the resistance of the apical membrane. The results indicated that the apical membrane was a high-resistance structure, with G for Na^+ and H^+ about equal.

Materials and Methods. Experiments were performed on the urinary bladder of

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Bufo marinus of Mexican origin. The horizontal mounting and support of hemibladders in a modified Ussing chamber, solution perfusion of serosal and mucosal hemichambers, fabrication of microelectrodes, bladder cell impalement under stereomicroscopic observation, circuitry of the microelectrode–macroelectrodes, and recording of potentials have been previously described (4, 5). Three potentials were obtained: Ψ_t (epithelial), Ψ_a (apical—microelectrode to mucosal macroelectrode), and Ψ_b (basal-lateral—microelectrode to serosal macroelectrode). Amphibian Ringer solution was gassed with 5% CO_2–95% O_2 and contained 100 m*M* Na^+, 4 m*M* K^+, 1.7 m*M* Ca^{2+}, 0.8 m*M* Mg^{2+}, 89 m*M* Cl^-, 18 m*M* HCO_3^-, 1 m*M* HPO_4^{2-}, and 10 m*M* glucose. Solutions were made free of sodium by isosmolal choline substitution. Solutions were made bicarbonate free with Cl^- substitution. Sodium transport was stopped by choline substitution for mucosal fluid Na^+, and addition of $1.25 \cdot 10^{-4}$ *M* amiloride to the mucosal solution. The electrical effects of proton secretion by toad bladder, namely reversed epithelial potential and reversed short circuit current are effectively abolished by 10^{-3} *M* serosal acetazolamide (9, 13). This technique together with 100% O_2 gassing of solutions and substitution of the solution buffers HCO_3^- and HPO_4^{2-} with Cl^- were utilized to abolish the electrical effects of H^+ secretion. In all experiments, the epithelial potential became zero. Despite these maneuvers, some residual CO_2 of tissue origin may have been available for proton production (11). If such was the case, quantitative proton secretion must have been extremely small in the absence of any reversed epithelial potential which could be measured in millivolts.

When Ψ_t became stable, the microelectrode was lowered into the mucosal solution and the potential microelectrode to serosal solution was compared to Ψ_t measured between the calomel electrodes in the serosal and mucosal solutions. These values had to be identical in order to accept the microelectrode, KCl–agar bridge, and amplifier circuitry. After cell impalement, adequacy of the circuitry of the experimental equipment was demonstrated by $\Psi_a + \Psi_b = \Psi_t$. Through silver–silver chloride electrodes in mucosal and serosal hemichambers, 1-sec pulses of 11.3 $\mu A \cdot cm^{-2}$ were delivered to the bladder from an external current source. One criterion for satisfactory impalement was the demonstration of voltage deflections of Ψ_a and $\Psi_b <$ voltage deflection of Ψ_t with a current pulse. Other criteria were an abrupt increase in microelectrode-mucosal potential upon impalement with the cell positive with mucosal Na^+, accompanied by an abrupt decrease in the microelectrode-serosal potential with the cell negative. Upon withdrawing the microelectrode, the potential microelectrode-mucosa became zero and the microelectrode-serosa potential became equal to Ψ_t measured by the calomel electrodes. The siphon effect of serosal solution flow and the supporting tantalum mesh enabled recording of stable and acceptable potentials for up to 90 min. Similar temporal stability is demonstrable in frog skin (14).

Resistances. Transepithelial resistance (R_t), apical resistance (R_a), and basal-lateral resistance (R_b) were measured by methods described previously (2, 3). Shunt resistance was calculated from

$$R_s = \frac{R_t \cdot R'_t \cdot (a' - a)}{R_t \cdot (a' + 1) - R'_t \cdot (a + 1)}.$$

R_t was calculated from the voltage deflection ($\Delta\Psi_t$) and current density (11.3 $\mu A \cdot cm^{-2}$). $a = \Delta\Psi_a/\Delta\Psi_b = R_a/R_b$. R'_t and a' are experimental values determined with choline chloride–amiloride mucosal Ringer solution and sodium chloride–acetazolamide serosal Ringer solution, both gassed with O_2. Under these conditions, the electrical potential and the short circuit current which result from Na^+ and H^+ transport are eliminated. After experimental determination of R_t and R_a/R_b and calculation of R_s, apical and basal-lateral resistances were calculated from the equivalent circuitry of toad bladder.

$$R_t = \frac{(R_a + R_b) R_s}{R_a + R_b + R_s}.$$

Mean R'_t was 5239 ohm·cm² ± SE 234 and a' was 5.32 ± 0.50. In a previous study (4)

in which R'_t and a' were determined in the absence of acetazolamide but in the presence of serosal HCO_3^-, respective values were 5115 ± 311 ohm·cm² and 5.40 ± 0.69. In another report (5), R'_t was 5301 ± 308 ohm·cm² and a' was 5.43 ± 0.33 in the absence of both serosal HCO_3^- and acetazolamide. As there are no statistical differences between these values in the three experimental conditions, the presence or absence of serosal HCO_3^- and acetazolamide does not affect R'_t or a'. Without a change in the voltage divider ratio, it is impossible for either a change in serosal fluid composition or acetazolamide to affect only basal-lateral resistance. A proportionally similar change in R_a would be required. Thus the entire cellular resistance ($R_a + R_b$) would either increase or decrease, and in order that R'_t remain unchanged, a reciprocal change would be required in R_s. These conditions would seem to be extremely unlikely, if not impossible. For these reasons, it was considered acceptable to utilize R'_t and a' determined in the presence of serosal acetazolamide and absence of HCO_3^- for the calculations of R_s, R_a, and R_b. Further, there was no effect on R_b alone, and hence it is acceptable to utilize the mean value so obtained to calculate R_a and R_s from experimentally determined R_t and voltage divider ratios in other experimental conditions.

Potentials and resistances with H^+ and Na^+ transport. Microelectrode impalement was performed with $NaHCO_3$ Ringer solution gassed with 5% CO_2 in both serosal and mucosal hemichambers. Potentials and resistances obtained under these conditions reflect mucosal to serosal Na^+ transport, cell to mucosa H^+ secretion, and residual cationic and anionic (probably K^+ and Cl^-) conductances. Subsequently, further potentials and resistances were obtained after Na^+ transport was stopped by changing the mucosal solution to CO_2 gassed choline HCO_3^- Ringer solution with amiloride. The latter has been shown to stop Na^+ penetration of the apical membrane and hence Na^+ transport (15, 16). Ten bladders were studied. The data reflected potentials caused by H^+ secretion, and the resistances were reflections of H^+ and residual cationic and anionic conductances. At least three cellular impalements were made under each condition, and the three or more potentials and voltage divider ratios were averaged.

Resistances with H^+ gradient. In another series of nine bladders, an H^+ gradient of 1000/1 was created by titrimetric acidification of amiloride–choline-Cl mucosal solution with 1 *N* HCl. The serosal fluid was NaCl Ringer solution with acetazolamide, and both solutions were gassed with O_2. pH was determined with an Orion digital pH meter. The serosal solution pH was 7.6 and the mucosal solution was 4.6. Periodic determinations during the experiments demonstrated no changes in pH of both solutions over 60 min. Voltage divider ratios and resistances were obtained under these conditions. The absence of CO_2, mucosal buffer, and Na^+ transport enabled delineation of cellular and epithelial resistances in the presence of a large H^+ concentration gradient and in the absence of the electrical potential sequelae of Na^+ and H^+ transport.

Resistances with mucosal KCl. A further nine bladders were studied to investigate the residual conductance after elimination of the electrical potential manifestations of both Na^+ and H^+ transport. Resistances and voltage divider ratios were obtained with 100 m*M* KCl mucosal Ringer solution and 100 m*M* NaCl acetazolamide serosal solution. Both solutions were gassed with O_2. Values obtained were considered due to K^+ and Cl^- conductances.

Results. During conditions of Na^+ and H^+ transport with 18 m*M* HCO_3^- −100 m*M* Na^+ mucosal fluid and CO_2 gassing, the mean Ψ_t was 26.3 mV ± SE 6.1. The mucosa was negative to serosa. When Na^+ transport was stopped by mucosal amiloride and choline substitution for mucosal Na^+, the epithelial potential reversed polarity and mucosa became 14.3 mV ± 3.6 positive to serosa. Similar data have been reported by Ludens and Fanestil (9), and are representative of the reversal of epithelial potential by H^+ secretion in the absence of Na^+ transport. The membrane potentials are shown in Fig. 1. With Na^+ and H^+ transport, mean Ψ_a was 13.7 ± 4.9

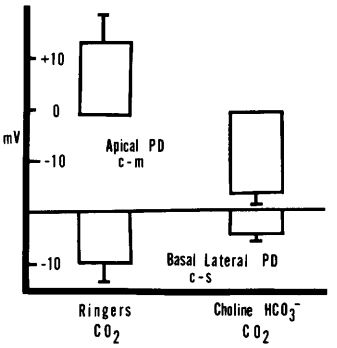

FIG. 1. Potentials of apical and basal-lateral membranes during $Na^+ - H^+$ transport with $NaHCO_3$ Ringers and with H^+ transport after mucosal choline substitution for Na^+ and amiloride addition had blocked Na^+ transport. Reference was mucosal solution for apical P.D. and serosal solution for basal-lateral P.D. H^+ transport reversed polarity of apical membrane, but not of basal-lateral membrane. Values are means ± SE.

mV, cell positive to mucosa. Ψ_b was 10.0 ± 4.1 mV with cell electronegative to serosa. With continuing H^+ transport but cessation of Na^+ transport by mucosal addition of amiloride and choline substitution for Na^+, there was a reversal of polarity of Ψ_a and cell became 16.0 ± 2.7 mV negative to mucosa. However, there was no change in the electrical polarity of the basal-lateral membrane and the cell remained negative to serosal solution. Ψ_b was 3.8 ± 0.5 mV. Thus H^+ secretion in the absence of Na^+ transport creates separation of charge and electronegativity of the cell to mucosal solution.

Resistance studies are shown in Figs. 2 and 3. With mucosal Na^+, K^+, Cl^-, HCO_3^-, and CO_2 gassing, resistances should reflect Na^+, H^+, and residual cationic and anionic (K^+ and Cl^-) conductances. The mean voltage divider ratio was 1.90 ± .14. Mean epithelial resistance was 2610 ± 117 ohm·cm², apical resistance was 2181 ± 108, and basal-lateral resistance was 1162 ± 142. Shunt resistance shown in Fig. 3 was 12,387 ± 882 ohm·cm². When H^+ secretion continued in the absence of Na^+ transport (which had been stopped with mucosal amiloride and choline substitution for Na^+), R_t increased to 3637 ± 156 (Fig. 2). The voltage divider ratio increased to 3.54 ± 0.09. This was accompanied by a significant increase in R_a to 3397 ± 255 ohm·cm². There was no significant change in R_b which was 960 ± 44. Figure 3 shows a striking increase in R_s to 23,963 ± 1417 ohm·cm².

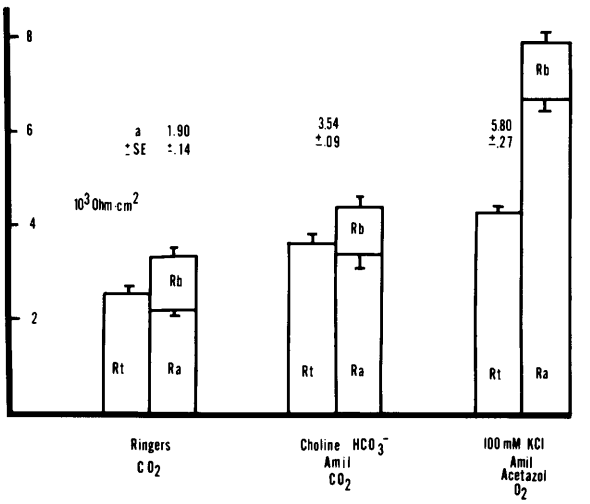

FIG. 2. Mean values ± SE for epithelial (R_t), apical (R_a), and basal-lateral (R_b) resistances during $Na^+ - H^+$ transport with $NaHCO_3$ Ringers, and H^+ transport after mucosal amiloride addition and choline substitution for mucosal Na^+. There was a significant increase in R_a from 2181 ± 108 to 3397 ± 255 ohm·cm². KCl resistance is shown in the right-hand columns where K^+ substitution for mucosal Na^+ and Cl^- substitution for mucosal HCO_3^- were made together with mucosal amiloride and serosal acetazolamide. R_a increased further to 6714 ± 304 ohm·cm².

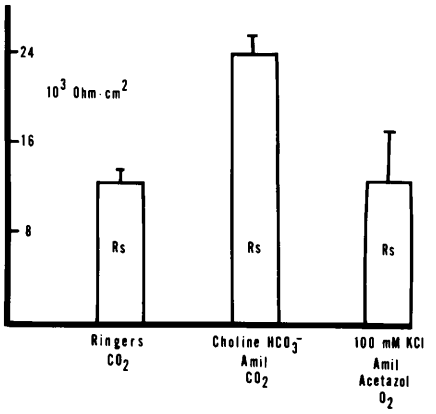

FIG. 3. Shunt resistances (R_s) during $Na^+ - H^+$ transport with mucosal $NaHCO_3$, with H^+ transport after mucosal amiloride and choline substitution for Na^+, and with mucosal K^+ substitution for Na^+ and Cl^- substitution for HCO_3^-, and amiloride. H^+ transport was stopped with serosal acetazolamide, and R_s reflects $G_{K^+-Cl^-}$. With H^+ transport, R_s increased from 12,387 to 23,963 ohm·cm^2. Values are means ± SE.

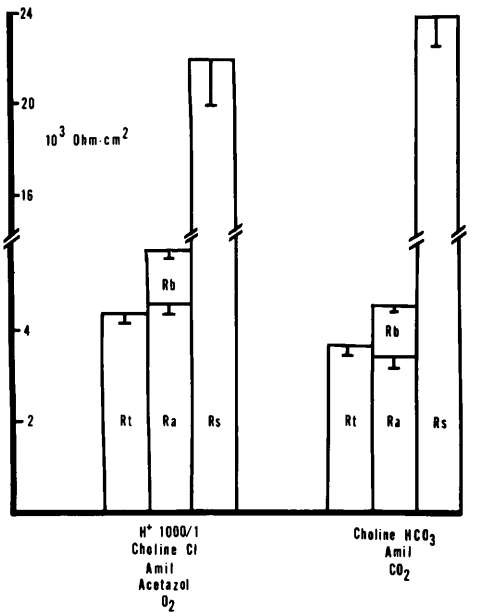

FIG. 4. Comparison of mean ± SE values of epithelial (R_t), apical (R_a), basal-lateral (R_b), and paracellular (R_s) resistances with 1000/1 mucosal/serosal H^+ gradient to similar values during H^+ transport. R_a increased from 3397 ± 255 to 4629 ± 218 ohm·cm^2, but R_s was comparable.

The epithelial resistance and voltage divider ratio were obtained under conditions of 1000/1 H^+ mucosal to serosal gradient. Mucosal acidification was accomplished by addition of HCl to 100 m*M* choline Cl–amiloride mucosal solution. The serosal fluid was NaCl Ringer solution with acetazolamide, and both solutions were gassed with O_2. The acetazolamide and the absence of CO_2 and mucosal buffer were designed to stop all but residual H^+ secretion. Rectification of H^+ conductance in the apical membrane with the H^+ gradient could then be tested. The mean R_b of 1162 ohm·cm^2 derived from the $Na^+ - H^+$ studies was utilized together with the experimentally determined R_t and $\Delta\Psi_a/\Delta\Psi b$ to calculate R_a and R_s. As seen in Figure 4, the mean R_t was 4415 ± 107 ohm·cm^2. The mean voltage divider ratio was 3.98 ± 0.16. Both of these values were significantly increased compared to R_t of 3637 ± 156 ohm·cm^2 and 3.54 ± 0.09 obtained during active H^+ secretion. There was also a significant increase in R_a from 3397 ± 255 to 4629 ± 218 ohm·cm^2 with the H^+ gradient. The paracellular resistance of 21,877 ± 4260 with the gradient was not significantly different from R_s of 23,963 ± 1417 during active H^+ secretion.

Voltage divider ratios and R_t were obtained in nine bladders with 100 m*M* mucosal KCl and amiloride. Serosal fluid was 100 m*M* NaCl Ringer solution with acetazolamide, and both solutions were gassed with O_2. There is evidence that high mucosal K^+ concentration has no effect on basal-lateral conductance (2). Therefore, the mean R_b of 1162 ohm·cm^2 obtained with Na^+ and H^+ transport was utilized together with the experimentally determined R_t and $\Delta\Psi a/\Delta\Psi b$ to calculate R_a and R_s. These resistances reflect residual conductance without influence from Na^+ and H^+ transport. The major contributors to residual conductance are probably K^+ and Cl^-. An apical membrane Cl^- conductance has been demonstrated (17). Mean voltage divider ratio was 5.80 ± 0.27. As shown in Fig. 2, mean R_t was 4297 ± 71 ohm·cm^2. There was a significant increase in R_a when compared to conditions of $Na^+ - H^+$ transport and H^+ transport. Mean value was 6714 ± 304. Whereas there had been about a twofold increase in R_s when Na^+ transport was

stopped, under conditions of $K^+ - Cl^+$ conductance, the shunt resistance returned to 12,408 ± 4640 ohm·cm², a value comparable to that seen with Na^+ and H^+ transport (Fig. 3).

Apical conductances were calculated as $G = 1/R$ and are shown in Fig. 5. With conditions of $Na^+ - H^+$ transport, apical conductance was a function of both of these ions plus residual $K^+ - Cl^-$ conductance. The mean value was 470 ± 25 μmhos·cm². After eliminating Na^+ transport, apical G was a function of H^+ and $K^+ - Cl^-$. The calculated mean value was 302 ± 53 μmhos·cm². Residual apical $K^+ - Cl^-$ conductance was 151 ± 7 μmhos·cm². Thus G_{H^+} is 151 and G_{Na^+} is 168. With 1000/1 mucosa–serosa H^+ gradient, apical G was 217 ± 8 μmhos·cm². The gradient reduced apical G_{H^+} from 151 to 66 μmhos·cm², which suggests rectification of apical G_{H^+}.

Discussion. It has been postulated that an apical membrane high-conductance paramicroelectrode shunt could produce low Ψ_a and R_a (18, 19), and that this could be avoided by cell impalement through the basal-lateral membrane (15, 20). However, comparison of potentials and resistances obtained by basal-lateral impalement with those obtained by apical impalement showed no difference (21). If apical impalement produced a high-conductance shunt, sudden removal of mucosal Na^+ and addition of amiloride should produce an instantaneous change of Ψ_a, similar to the response to a current pulse. The response, however, is delayed for 60 to 180 sec. Thus the postulation of a high-conductance paramicroelectrode shunt is not valid.

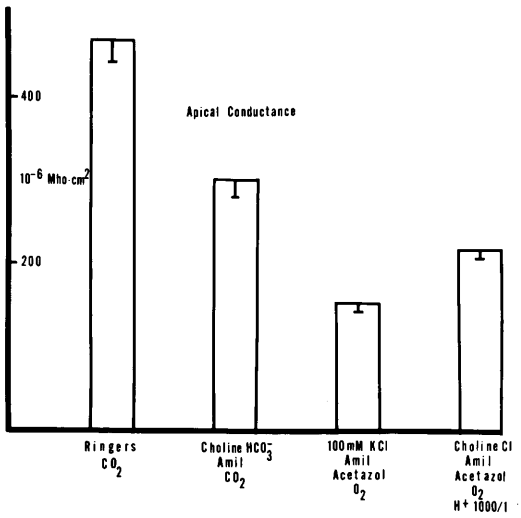

FIG. 5. Mean apical conductances ± SE derived from resistance data. With $Na^+ - H^+$ transport, apical G was 470 ± 25 μmhos·cm². With H^+ transport, apical G was 302 ± 53 μmhos·cm². Conductance was 151 ± 7 μmhos·cm² with mucosal KCl and amiloride, and serosal acetazolamide. An H^+ gradient in the absence of $Na^+ - H^+$ transport produced apical conductance of 217 ± 8 μmhos·cm².

Removal of mucosal sodium or addition of amiloride will stop Na^+ transport in Colombian toad bladder (15, 16), and reverse the polarities of epithelial potential and short circuit current. The electrical phenomena are the sequelae of acidification of the mucosal fluid (6, 7, 9–11, 13). There is a close quantitative correlation between reversed short-circuit current and the rate of urinary acidification (9, 13). Proton secretion is dependent on exogenous or endogenous CO_2, is independent of Na^+ transport (11), is stimulated by aldosterone and mucosal buffer HCO_3^- (13, 22), and is inhibited by carbonic anhydrase inhibitors (23).

The reversal of polarity of epithelial potential and short-circuit current together with the dependence of the magnitude of the reversals on the availability of CO_2 suggests a model of proton secretion for this epithelium in which a rheogenic pump is located near the apical membrane. The proton substrate for the pump is derived from the carbonic anhydrase-catalyzed hydration of CO_2. It might be predicted that proton secretion from cell to mucosa would be accompanied by HCO_3^- passage from cell to serosa. Proton secretion would create an apical potential with cell polarity negative to mucosa, a reversal of polarity observed during active Na^+ transport. Bicarbonate leaving the cell for the serosal medium could lead to separation of charge, and the polarity of the basal-lateral membrane, which is usually cell negative to serosa during Na^+ transport, would become cell positive. The reversal of apical polarity

was clearly demonstrated. However, there was no change in polarity of Ψ_b. The small residual Ψ_b of 3 mV was probably due to K^+ and Cl^- diffusion potentials. The reversal of epithelial polarity is entirely due to reversal of polarity of Ψ_a.

Toad bladder is composed of four types of epithelial cells: mitochondria-rich, granular, globular, and basal (24–27). The basal cells are adjacent to the basement membrane and do not reach the mucosal surface. The primary functions of granular cells are Na^+ transport and the osmotic flow of water (26–28). The mitochondria-rich cells are primarily concerned with proton secretion (29). The Dominican toad bladder does not secrete H^+ and has a relative paucity (13%) of mitochondria-rich cells (26). Colombian toad bladder does secrete H^+. Mexican toad bladders also secrete H^+ and are probably closely related to Colombian toad bladders. The percentage of mitochondria-rich cells in Colombian toad bladder is unsettled. In some studies the proportion is as high as 25–30% (26, 30), but in others the proportion is 12–16%, even when the animal is made acidotic (31, 32). These lower percentages together with the observation that the Colombian bladder has six times the number of cells with carbonic anhydrase activity than the Dominican bladder raises the possibility of proton secretion by granular cells. This has not been experimentally excluded. Nevertheless, it might seem prerequisite to impale the mitochondria-rich cells in order to delineate the active and passive electrical characteristics of toad bladder during H^+ secretion. Although the microscopic magnification and resolution were sufficient to delineate cell outlines, identification of cell type could not be made. High-conductance gap junctions exist between cells that are capable of transmitting current throughout an epithelial sheet of cells, and thereby impart a uniform membrane potential in each cell (33–38). Freeze fracture studies have demonstrated gap junctions between granular cells and adjacent basal cells (27), but not between adjacent granular cells and between adjacent granular and mitochondria-rich cells. The lack of morphological evidence of intercellular junctions between granular and mitochondria-rich cells does not necessarily exclude functional coupling. The definitive experiment to exclude functional coupling would be the exclusion of fluorescein from mitochondria-rich cells after microinjection into granular cells. Although the origin and function of basal cells are poorly understood, they may be precursors of both granular and mitochondria-rich cells, which would require intercellular communication. Toad bladder was among the first tissues in which intercellular electrical coupling was demonstrated (33), and it is likely that all cells are electrically coupled. Thus the voltage created by Na^+ transport in granular cells could be transmitted to mitochondria-rich cells, and the voltage from proton secretion in the absence of Na^+ transport could be transmitted from the mitochondria-rich cells to granular cells.

During conditions of Na^+ and H^+ transport, epithelial resistance was 2610 ohms·cm^2, apical resistance was 2181, basal-lateral was 1162, and shunt was 12,387. These values are comparable to previous data (2–5). With H^+ secretion alone, there was a significant increase in R_a to 3397 ohms·cm^2 and R_s to 23,963. The increase in R_s was similar to that found with low mucosal Na^+ concentrations (3, 5). Thus it would seem that the increase in shunt resistance was a mechanism to minimize J_{H^+} m → s, and maintain a large transepithelial H^+ concentration gradient. This conclusion was confirmed with the finding of a similar paracellular resistance of 21,877 ohms·cm^2 with 1000/1 H^+ transepithelial gradient. The increase in R_a reflects the loss of Na^+ conductance. Thus the difference between the two apical conductances (470–302 μmhos·cm^2) of 168 μmhos·cm^2 represents sodium conductance of apical membrane. With the H^+ gradient, R_a increased from 3397 during H^+ secretion to 4629 ohms·cm^2, and apical G_{H+} decreased from 151 to 66 μmhos·cm^2. This suggests rectification of H^+ current and conductance in the apical membrane. The increase in paracellular resistance and H^+ rectification in the apical membrane would

enable the epithelium to maintain the large concentration gradient. An earlier paper showed that mucosal acidification to pH 4.5 increased the short-circuit current (39). As proton secretion should reduce the magnitude of SCC during Na^+ transport, it is not surprising that a large H^+ gradient would stop H^+ secretion and augment SCC.

Mucosal K^+ has been shown to have little or no effect on R_b (2). Studies with potassium-sensitive liquid ion exchanger microelectrodes demonstrated intracellular K^+ activity of 41–54 m*M* (40). Some studies have suggested that K^+ traverses the mucosal membrane (41, 42). A twofold concentration gradient of K^+ across the apical membrane should be adequate for determination of apical K^+ conductance, together with apical anionic conductance. The major contributor to anion conductance in the apical membrane should be Cl^- (17). With an applied potential of 50 mV serosa positive, the m → s Cl^- flux is about 85% of K^+ s → m flux (43). With mucosal KCl, R_a was 6714 ohms·cm^2. In the absence of Na^+ and H^+ transport, the calculated residual apical K^+ – Cl^- conductance was 151 μmhos·cm^2. Thus the difference between this conductance and that calculated during H^+ transport (302 – 151 = 151 μmhos·cm^2) would represent apical H^+ conductance.

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