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Research Article

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The rate of secretion of hydrogen ion was not affected by replacement of the sodium in the bathing media by cesium, or by inhibition of sodium transport by dinitrophenol. Acidification continued when chloride in the solutions was replaced by sulfate, or when potassium or calcium was removed from the solution bathing the mucosal surface.

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Independence of Hydrogen Ion Secretion and Transport of Other Electrolytes in Turtle Bladder *

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Abstract. The relationship between hydrogen ion secretion and the transport of other electrloytes was examined in the isolated urinary bladder of the water turtle. Symmetrical solutions which were free from exogenous carbon dioxide and bicarbonate bathed the two surfaces of the preparation, and the spontaneous electrical potential of the bladder was nullified by a voltage clamp. Active transport of sodium from mucosal to serosal medium was confirmed by simultaneous bidirectional flux measurements and found to be slightly, but not significantly, greater than the short-circuit current. In the absence of sodium in the bathing solutions, the normal potential difference across the bladder reversed and the current required to nullify this reversed potential difference had the same magnitude as the simultaneously measured rate of hydrogen ion secretion. The results indicate that, under these experimental conditions, the bladder transports sodium and hydrogen ion actively, but that chloride movement does not contribute to the short-circuit current.

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Secretion of hydrogen ion by the turtle bladder is not dependent on the simultaneous transport of other electrolytes across the bladder.

Introduction

In the preceding article, evidence was presented indicating that H^+ transport in the urinary bladder of the fresh water turtle cannot be accounted for by passive driving forces (2). Acidification of the mucosal medium, therefore, must either be linked directly to metabolic energy production or be coupled to the active transport of another ion. Mechanisms for the active transport of Na^+ and, under some experimental conditions, for Cl^- have been demonstrataed in the bladder in vitro (3–5). The purpose of the present study is to investigate the possibility of coupling between the transport of H^+ ion and that of Na^+ and Cl^-.

The results indicate that the transport of H^+ in the turtle bladder is not directly coupled to the

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transport of Na^+. The rate of H^+ secretion was little affected by the removal of Na^+ from the bathing solution or by the reduction of Na^+ transport which followed treatment with dinitrophenol. Removal of Cl^- from the bathing solutions was associated with the continuation of both H^+ and Na^+ transport, although the rates were somewhat reduced. In the absence of Na^+ the mucosal side of the bladder became positive with respect to the serosal side and the current required to nullify this potential difference (PD) had the same magnitude as the rate of H^+ secretion. This result suggests that Cl^- is not actively transported across the bladder under these experimental conditions.

Methods

Experimental design. Although these studies were concerned primarily with an examination of coupling between H^+ secretion and transport of Na^+ or Cl^-, it proved useful to identify the ionic flows that contributed to the short-circuit current (SCC). The ions to be considered are the following: (*a*) Na^+, which is transported actively from the mucosal (M) to serosal (S) medium (3, 4), (*b*) Cl^-, which is transported actively from M to S under some experimental conditions (5), and (*c*) H^+, which is transported actively into M (2).

For the purposes of this study, active transport is defined as net movement of an ion across the bladder in the absence of gradients of concentration or electrical potential. All experiments were performed with the bladder in the short-circuited state and, unless specifically noted, with identical solutions bathing both surfaces of the bladder. Active transport of Na^+ was determined as the difference between the simultaneously measured fluxes of ^{24}Na and ^{22}Na. Since two different radioactive isotopes of Cl^- were not available, measurement of active Cl^- transport by the definitive double-label experiment was not feasible. An indirect method was used instead. Control of electrochemical gradients for H^+ was obtained by the use of a pH stat for the solution bathing one surface of the bladder, with frequent changes in the bathing solution of the other. When isotopic flux measurements were made, the mucosal solution was maintained at constant pH by the pH stat. The pH of the serosal solution was monitored with an additional glass electrode and small volumes of 0.01 M HCl were added as required to maintain constant pH. In the experiments in which sulfate Ringer's solution was used, the pH of the serosal solution did not deviate by more than 0.2 pH units from the original value, so no acid was added.

In our hands the variation in transport behavior in two portions of the same turtle bladder was comparable to that shown by two bladders from different animals. For this reason, we performed two general types of experiments: one in which a preparation served as its own control, and one which involved a comparison of two sets of preparations from different turtles.

Two additional features of our experiments require comment. The measurement of Na^+ fluxes by the isotopic method assumes constant specific activity of the labeled Na^+ in the transport pool of the epithelial cells. Paine and Foulkes (6) have reported that mixing of Na^+ in the turtle bladder preparation is slow. At a Na^+ concentration similar to the one employed in our experiments they observed that after 30 min 71% of cellular Na^+ had equilibrated with isotopic Na^+. This fraction was only slightly increased to 76% after 120 min. Under our experimental conditions, 30–40 min of exposure to ^{24}Na were required before the specific activity of the transported Na^+ reached a constant value. Therefore, the experimental design included a period of 50 min between the addition of isotopic Na^+ and the beginning of flux measurements. In all experiments, the bladder was washed three times in the incubation medium over a period of 1 hr before the addition of the isotope, or the measurement of SCC or H^+ secretion.

Since the turtle bladder has a relatively low DC resistance, appreciable current were required from the automatic voltage clamp in order to nullify the potential between the sensing salt bridges on the two sides of the bladder. Although special efforts were made to position the tips of the sensing bridges close to the surfaces of the bladder, the resistance of the fluid layer in a series of determinations was occasionally as large as 30 ohms. As a result, there was a residual potential difference of the usual orientation across the bladder even in the short-circuited state, the size of which varied with the SCC and the exact position of the sensing bridges, but which usually was considerably below the maximum value of 10 mv. The available information does not permit derivation of a rigorous expression which can be used to correct the two passive and one active flows of Na^+ for this deviation from the condition of zero electrical driving force. An approximate correction was calculated by making use of Ussing's flux ratio equation (7) and two assumptions: that the maximal gradient against which the Na^+ pump can operate is 170 mv (8); and that for small electrical gradients, the change in active transport is linearly related to the change in voltage across the bladder. The calculated correction yielded a small increase in net Na^+ flux and SCC. Since the difference between the two quantities was the figure of interest, and since the corrections did not change this difference significantly, the interpretation of our results was unaffected. As demonstrated in our previous paper, the rate of H^+ secretion was so little affected by the presence or absence of the spontaneous transbladder potential that no correction of this flux was required in the evaluation of the SCC. In the experiments with CsCl Ringer's solution, the short-circuiting error was avoided by application, to the input of the voltage clamp, of an offset potential which was approximately equal to the potential drop in the fluid layer between the sensing bridges.

Experimental procedure. The experimental methods have been described in the previous article (2). The same standard NaCl Ringer's solution was employed. In addition to the standard Ringer's solution, Na-free

TABLE I

*Comparison of simultaneous Na^+ fluxes, H^+ secretion, and short-circuit current (SCC) in turtle bladder**

Solution	^{24}Na M → S	^{22}Na S → M	Na net M → S	H^+ → M	SCC
			μa		
NaCl Ringer's, 30 periods, 9 turtles	349 ± 26	53 ± 11	296 ± 25	26 ± 2	280 ± 24
Na_2SO_4 Ringer's, 27 periods, 11 turtles	253 ± 20	78 ± 13	175 ± 20	13 ± 1	168 ± 20

M, mucosa; S, serosa.
* All experimental periods were 30 min.
‡ Statistical variation is expressed as ±1 SEM.

and Cl-free solutions of the following composition were prepared: CsCl Ringer's (mmoles/liter): Cs^+, 114.4; K^+, 4.1; Ca^{++}, 0.9; Cl^-, 119.8; $HPO_4^=$, 0.3; and glucose 2.0; osmolality, 227 mOsm/kg H_2O. Na_2SO_4 Ringer's (mmoles/liter): Na^+, 116.6; K^+, 3.6; $SO_4^=$, 59.8; $HPO_4^=$, 0.3; $CaSO_4$, saturated solution; glucose, 2.0; sucrose to bring osmolality to 227 mOsm/kg H_2O. To change the bathing solutions, we drained both half chambers and filled them with fresh Ringer's solution four times over a 50 min period to remove either Na^+ or Cl^- from the bathing media and to reduce the tissue concentration of these ions. In the pH stat experiments the Na^+ concentration in the bathing solution was determined by flame photometry and the Cl^- concentration by electrometric titration. At the beginning of the pH stat period in the Na-free and Cl-free Ringer's solution the concentrations in the bathing media of these excluded ions were below detection limits of 0.1 mmole/liter; at the end of the pH stat period the concentrations were less than 1 mmole/liter. In the experiments in which the effect of inhibition of Na^+ transport on H^+ secretion was examined, both bathing solutions were exchanged for new Ringer's solution containing 1×10^{-5} M dinitrophenol. All solutions were gassed with air which had passed through an alkali trap and were brought to a final pH of 7.40 ± 0.10. As in the preceding paper, identical solutions and gas phases were employed on both sides of the bladder, all flux periods were of 30 min duration unless otherwise noted, and fluxes were calculated as μmoles/hour or amperes. As titrant for the pH stat assembly either 0.01 N NaOH or 0.01 N KOH was employed. The volume of titrant added in a given flux period was less than 1.4% of the volume of incubation medium. ^{24}Na and ^{22}Na were obtained as the Cl salt from Cambridge Nuclear Corporation (Cambridge, Mass.). Both isotopes were counted in a Nuclear-Chicago model 4218 well counter, ^{24}Na at settings which excluded ^{22}Na activity, and ^{22}Na after a 2 wk delay to permit loss by decay of ^{24}Na activity.

Results

Short-circuit current in the turtle bladder. The simultaneously determined values of bidirectional Na^+ fluxes, rate of H^+ secretion, and SCC, in the presence and absence of Cl^-, are shown in Table I. The mean values for net Na^+ flux from M to S were close to the SCC, the former being slightly greater in both series of experiments. If H^+ secretion into the mucosal fluid involved the transfer of a positive charge, then net Na^+ transport minus H^+ transport should account for the SCC in the absence of ambient Cl^-. Subtraction of the rate of H^+ transport from net Na^+ transport resulted in a value slightly less than the SCC, the SCC value being straddled by the values for net Na^+ flux alone and net Na^+ flux minus H^+ secretion. The algebraic sum of the flux of measured ions differs from the simultaneously measured SCC, but the discrepancies are within the error of the measurement of the net Na^+ flux. For this reason, the results of these experiments performed in the presence and absence of Cl^- do not permit any conclusion regarding the existence of an active transport mechanism for Cl^-. In conformity with the findings of others, however, these results indicate that in the absence of passive driving forces net Na^+ transport is the major constituent of the SCC (3, 9). This fact supports the interpretation that large reductions in the SCC after

TABLE II

Effect of H^+ secretion of substituting Cs^+ for Na^+ in Ringer's solutions bathing turtle bladder

Turtle	H^+ secretion* Initial control	Cesium	Final control
		μmoles/hr	
1	2.66	2.16	2.42
2	1.00	0.71	0.40
3	1.30	0.69	0.46
4	1.67	1.30	1.80
5	0.69	0.77	0.71
6	1.20	1.10	1.20
7	0.84	0.80	0.65
Mean	1.33	1.08	1.09
±SEM	±0.25	±0.20	±0.29

* The experimental periods were of 30–40 min duration.

TABLE III

The effects of dinitrophenol (DNP) on acid secretion and short-circuit current (SCC) in the turtle bladder

Turtle	H^+ secretion		SCC	
	Control	DNP*	Control	DNP
	μmoles/hr		*μa*	
1	1.75	1.70	235	107
2	0.76	0.92	173	81
3	0.83	0.89	153	100
4	0.51	0.76	74	37
5	0.79	0.82	131	95
6	1.49	1.40	210	130
Mean	1.02	1.08	163	92
±SEM	+0.20	±0.15	±23	±13

* DNP was added to both bathing solutions in a concentration of 1×10^{-5} mole/liter. The H^+ secretion rate represents the mean of a 30 min period, which began 15 min after addition of DNP. The SCC values were obtained 45 min after the addition of DNP.

experimental manipulation are the result of similar changes in net Na^+ flux. It is only in this restricted and qualitative sense that the measurements of SCC in the presence of Na^+ are to be used in this study.

Effects on H^+ secretion of altering the rate of Na^+ transport. The results in Table II show the effects on H^+ secretion of substituting Cs^+ for Na^+ in the Ringer's solutions of both sides of the epithelium. The concentration of Na^+ in the medium was 115 mEq/liter during the control period and less than 1 mEq/liter at the end of the period with CsCl Ringer's solution. H^+ secretion continued after removal of Na^+ in all experiments with only slight reductions in the rate of secretion in some. Replacement of Na^+ failed to cause a consistent increase in secretion rate despite steep increases in Na^+ transport as judged from the SCC.

If acidification occurs by a process of exchange for Na^+, these experimental results require that the mechanism be saturated with respect to Na^+ when the concentration of the latter is in the micromolar range. This requirement and the experimental results make the existence of such coupling unlikely.

Since dinitrophenol has been shown to cause marked inhibition of Na^+ transport in the turtle bladder (10), the independence of H^+ secretion and Na^+ transport was further tested by exposing the bladder to standard NaCl Ringer's solutions containing 1×10^{-5} M dinitrophenol. In Table III, H^+ secretion is shown to be unaffected despite marked decreases in the SCC, and hence the active transport of Na^+. The decrease in SCC was that observed at 45 min; at 60 min the decrease was 65%. The inhibitory effect of dinitrophenol on the SCC was reversed when the bladder was exposed to fresh standard Ringer's solution. The rate of H^+ secretion remained relatively constant throughout.

Relationship between H^+ secretion and electrical activity of turtle bladder in Na-free Ringer's solution. Removal of Na^+ by repeated washes in CsCl Ringer's solution caused the orientation of the PD across the bladder to reverse, the serosal solution becoming negative with respect to the mucosal solution. The PD in the 2nd and 3rd hr of exposure to CsCl Ringer's solution ranged from − 1 to − 10 mv, the mean PD being − 4 mv. In most experiments the normal electrical orientation could be restored promptly by replacement of CsCl with NaCl Ringer's solution.

In Table IV six experiments are presented in which simultaneous measurements were made of the rate of H^+ secretion and of the reversed SCC. The rate of H^+ secretion expressed in μamperes closely approximated the reversed SCC. Deoxygenation of the bathing media caused a reduction

TABLE IV

Simultaneous measurement of H^+ secretion and short-circuit current (SCC) in turtle bladder bathed in sodium-free Ringer's solution

Turtle	H^+ secretion	Reversed SCC	Difference, H^+ secretion − SCC
		μa	
1 air	20	25	−5
N_2	18	18	0
2 air	14	10	4
N_2	7	6	1
3 air	13	11	2
N_2	0	7	−7
air	20	14	6
4 air	27	23	4
N_2	19	17	2
air	39	32	7
5 air	15	20	−5
N_2	0	6	−6
air	19	14	5
6 air	12	13	−1
N_2	0	5	−5
air	20	11	9
Mean			−0.7
±SEM			±1.2

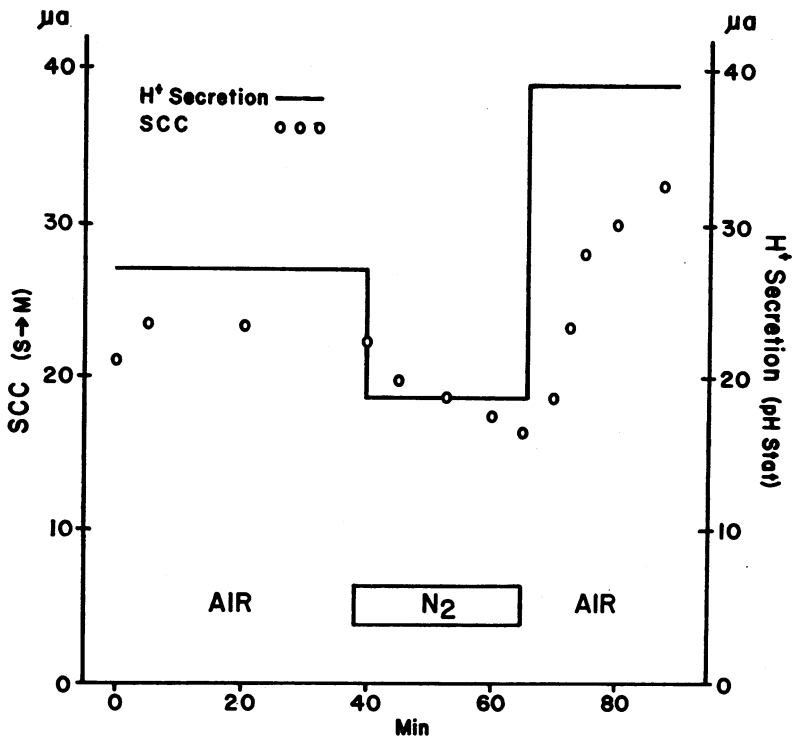

FIG. 1. SIMULTANEOUS MEASUREMENT OF THE RATE OF H^+ SECRETION BY THE pH STAT METHOD AND THE SHORT-CIRCUIT CURRENT (*SCC*). CsCl Ringer's solution was present on both surfaces of the bladder. The bathing solutions were deoxygenated with nitrogen during the interval indicated. The short-circuit current has the polarity of positive charge transfer across the bladder from serosa (*S*) to mucosa (*M*).

of both the rate of H^+ secretion and the SCC. In four experiments the bathing media were again equilibrated with air; both the SCC and the rate of H^+ secretion increased significantly. The course of one such experiment is demonstrated in Fig. 1. In all six experiments restoration of Na^+ concentration to 115 mmoles/liter at the end of the period in Na-free Ringer's solution resulted in a prompt return of the positive PD across the bladder.

Effect of removal of Cl^-, K^+, or Ca^{++} from the bathing solutions. The data in Table I, which gives an account of the SCC in NaCl and Na_2SO_4 Ringer's solutions indicate a significant difference between the rate of H^+ secretion in the two series of bladders. Although H^+ secretion was not abolished in any of the experiments in Cl-free Ringer's solution, the mean rate was about half to two-thirds the rate in standard NaCl Ringer's solution. The results of alternative experiments, in which each individual preparation was exposed sequentially to both Cl^- and $SO_4^=$, are summarized in Table V. The initial rate of H^+ secretion after a wash period of about 30 min in Na_2SO_4 Ringer's solution was close to the control rate. During the 2nd hr, however, the rate tended to decrease and become constant at a lower level.

TABLE V

Effect of H^+ secretion of substitution of $SO_4^=$ for Cl^- in solutions bathing turtle bladder

Turtle	H^+ secretion*			
	Initial control	$SO_4^=$ 1st hr	$SO_4^=$ 2nd hr	Final control
	μmoles/hr			
1	1.40	1.51	0.74	1.70
2	0.70	0.57	0.27	0.65
3	1.00	0.85	0.40	1.30
4	0.65	0.65	0.40	0.55
5	1.52	1.20	1.20	1.55
6	0.68	1.00	0.55	0.75

* The experimental periods were of 30–40 min duration. The $SO_4^=$ periods were at the end of the 1st and 2nd hr of exposure to Na_2SO_4 Ringer's solution.

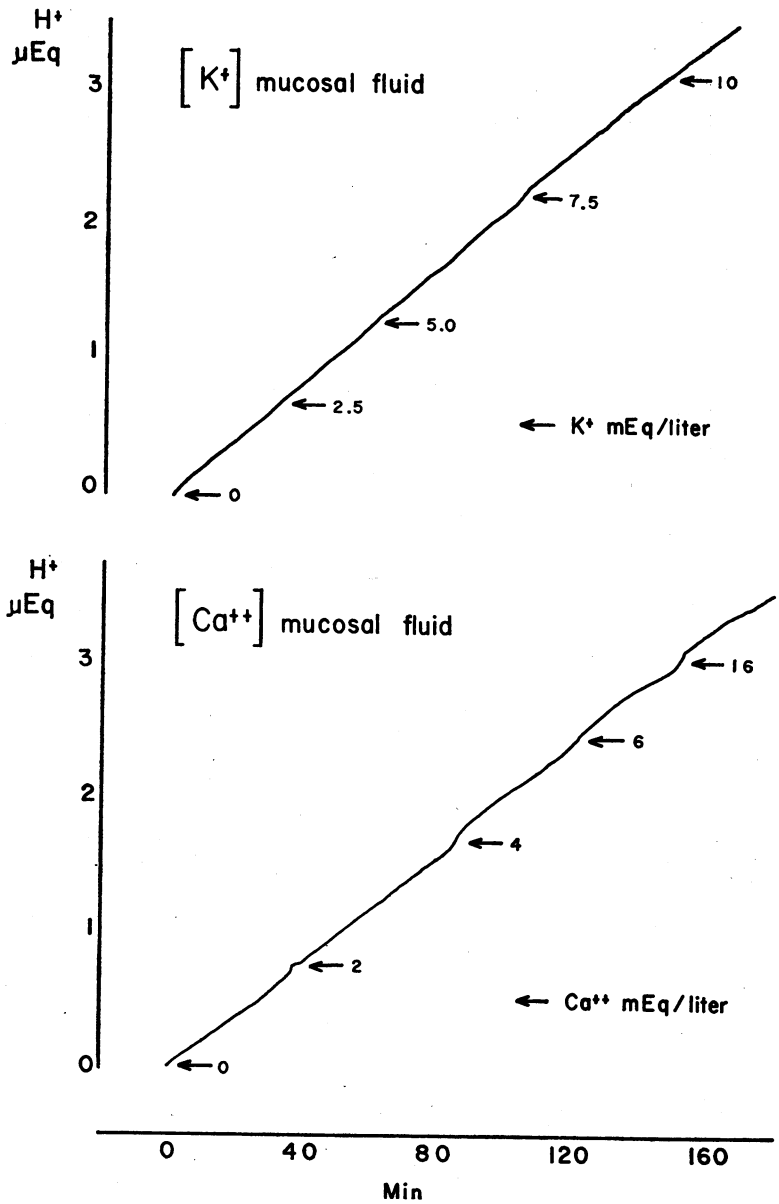

FIG. 2. EFFECT ON CUMULATIVE H^+ SECRETION OF VARIATION OF K^+ OR Ca^{++} CONCENTRATION IN THE MUCOSAL FLUID. The mucosal surface of the bladder was washed three times with a Ringer's solution free from the test cation. After measurement of the control rate of H^+ secretion, small volumes of a solution of the test cation were added to the mucosal medium at intervals to yield the final concentrations shown in the figure.

Replacement of Cl^- in the media was followed by a return of the rate of H^+ secretion to initial levels.

The influence of Ca^{++} and K^+ in the mucosal medium on the rate of H^+ secretion was examined in a series of five experiments. The mucosal surface was washed three times with a solution which was free from Ca^{++} or K^+, but which was otherwise identical with the normal Ringer's solution which bathed the serosal surface. The bladder was than short-circuited and a control rate of H^+ secretion was determined with either K^+ or Ca^{++} absent from the mucosal medium. At intervals of

20–40 min small amounts of KCl- or $CaCl_2$-containing solution were added to the mucosal medium to bring about a stepwise increase in the concentration of one of these ions.

Fig. 2 illustrates the results of two such experiments. Alteration of K^+ concentration in the range of 0–10 mEq/liter failed to change the rate of H^+ secretion significantly. Although these experimental results cannot eliminate the possibility of tightly coupled Ca^{++} or K^+ exchange as the basis of H^+ secretion, they make such a mechanism extremely unlikely.

Discussion

Passive driving forces fail to account for H^+ transport in the isolated urinary bladder of the turtle (2). The alternative explanations for the process invoke coupling either to one of the energy-yielding reactions of intermediary metabolism or to the flow of another actively transported ion. The present study was designed to test the latter possibility.

The active transport of Na^+ from mucosal to serosal medium was eliminated by removal of Na^+ from the ambient solution or inhibited by exposure of the bladder to dinitrophenol. Since neither maneuver altered the rate of H^+ secretion significantly, we have concluded that the energy for H^+ transport does not derive from the countertransport of Na^+ across the bladder.

The absence of coupling between Na^+ reabsorption and H^+ secretion in the isolated urinary bladder of the turtle is of interest because of the many physiologic and clinical observations in the intact kidney which indicate that part of Na^+ reabsorption in the renal tubule is linked to H^+ secretion (11–15). Although the cellular mechanisms of urinary acidification may well be different in the turtle bladder and the mammalian renal tubule, there are important similarities in the orientation of H^+ and Na^+ transport and in the maximal transcellular gradient of pH that can be reached in the two epithelia.

The absence of direct coupling between the transport of Na^+ and H^+ does not preclude an indirect relation between flows of the two ions. Thus, it should be emphasized that our comparisons of the rate of H^+ secretion in the presence and absence of Na^+ were made in the short-circuited state. In our previous paper we showed that the spontaneous PD generated by the active transport of Na^+ is a driving force which makes a small but definite contribution to the rate of H^+ secretion. In studies of the intact kidney such an effect might be considerably magnified, since only a small fraction of total H^+ secretion appears in the final urine and an even smaller fraction of the filtered Na^+ escapes reabsorption along the tubule.

The original reason for comparing net Na^+ flux and SCC was to determine whether the latter could be used as a convenient and instantaneous measure of the former. In 57 periods in which the bladder was short-circuited and bathed in identical solutions, the difference between the simultaneously determined, unidirectional Na^+ fluxes exceeded the SCC by less than 6%, both in the presence and absence of ambient Cl^-. This result is the justification for our use of the SCC as an indicator of changes in Na^+ transport.

Klahr and Bricker, who employed a Ringer's solution of similar composition, found that the net flux of Na^+ was 11% less than the SCC (3).

On the other hand, Gonzalez and his associates reported in an abstract that the net flux of Na^+ was 1.5 times the SCC (9). The basis for these discrepancies is not apparent at this time. There is agreement, however, that Na^+ is actively transported by the bladder in vitro and that net transport of Na^+ makes up the bulk of the SCC.

Gonzalez and associates (5) have demonstrated active transport of Cl^- from M to S in the isolated turtle bladder. In principle, this process might be coupled in some way to that of H^+ transport and provide the driving force for the latter. Our demonstration that H^+ ion secretion continued despite virtual absence of Cl^- from the medium is strong evidence against a coupling mechanism that depends on simultaneous and equivalent transport of Cl^- from M to S and H^+ ion from S to M.

As a by-product of the investigation of the relation between Na^+ transport and H^+ ion secretion, acid secretion was compared with the SCC in the presence of Cl^- and the absence of Na^+. The agreement between H^+ secretion and SCC in Table IV indicates that acidification accounts for all of the asymmetrical charge transport across the bladder epithelium, and hence, constitutes evidence against the active transport of Cl^- under these experimental conditions. The basis for the apparent discrepancy between this result and the

findings of Gonzalez et al. (5) has not been investigated, but there are several differences in the design of the two sets of experiments.

The coupling reaction responsible for H^+ secretion might involve the active transport of a mobile ion which is cycled across only one face of the cell and is not itself transported across the epithelium. Potassium, for example, has the role of mobile ion in the original model of active Na^+ transport in frog skin (16). For the reasons outlined in our previous study, the mechanism of H^+ secretion is probably located at the mucosal surface of the cell. Besides Na^+, the only extracellular cations available for exchange at this site are K^+ and Ca^{++}. Wide variation in the concentration of either of these ions in the mucosal medium was without significant effect on the rate of H^+ secretion.

A variant of this model is the ion-pair pump, which in this instance would bring about movement of H^+ across the mucosal boundary of the epithelial cell as an undissociated acid. The failure to demonstrate an increase in the buffer capacity of the mucosal medium after prolonged periods of acid secretion constitutes good evidence against such a mechanism (2).

At the present time, H^+ secretion is most satisfactorily described as a process which is located at the mucosal boundary of the epithelial cell, is directly coupled to metabolic energy production, and which in the short-circuited preparation transports the proton across the mucosal membrane without ion-pair formation or cationic exchange.

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