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Research Article

Aldosterone stimulates not only Na⁺ absorption but also urinary acidification. In this investigation the effects of aldosterone on H⁺ transport are examined in vitro in turtle bladder, a urinary membrane in which several of the factors controlling H⁺ transport have been defined. H⁺ transport was increased in bladder halves exposed to aldosterone compared to control halves. Stimulation of H⁺ secretion was observed as early as 1 h after addition of aldosterone and occurred before that of Na⁺ transport. In bladders depleted of endogenous substrate addition of glucose increased H⁺ transport more in aldosterone-treated halves (10.0±1.3 nmol/min) than in control halves (6.8±2.3). Addition of pyruvate failed to increase H⁺ transport (-0.3±0.7) in control halves but caused significant increments (2.4±0.5) in aldosterone-treated halves. In aldosterone-treated bladders glucose caused larger increments (16.5±2.7) in H⁺ transport than pyruvate (9.3±2.0) when halves of the same bladders were compared. Na⁺ transport, however, was equally increased by the two substrates. Despite the differences in time course and substrate requirements between the stimulation of H⁺ and Na⁺ transport, both increases were abolished by actinomycin-D. To examine the effect of aldosterone on the force of the H⁺ pump, protonmotive force, the pH gradient that would nullify the transport rate was determined with and without aldosterone. Aldosterone did not alter protonmotive force but significantly increased the slope of the H⁺ [...]

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Characteristics of Stimulation of H⁺ Transport by Aldosterone in Turtle Urinary Bladder

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ABSTRACT Aldosterone stimulates not only Na⁺ absorption but also urinary acidification. In this investigation the effects of aldosterone on H⁺ transport are examined in vitro in turtle bladder, a urinary membrane in which several of the factors controlling H⁺ transport have been defined. H⁺ transport was increased in bladder halves exposed to aldosterone compared to control halves. Stimulation of H⁺ secretion was observed as early as 1 h after addition of aldosterone and occurred before that of Na⁺ transport. In bladders depleted of endogenous substrate addition of glucose increased H⁺ transport more in aldosterone-treated halves (10.0±1.3 nmol/min) than in control halves (6.8±2.3). Addition of pyruvate failed to increase H⁺ transport (-0.3±0.7) in control halves but caused significant increments (2.4±0.5) in aldosterone-treated halves. In aldosterone-treated bladders glucose caused larger increments (16.5±2.7) in H⁺ transport than pyruvate (9.3±2.0) when halves of the same bladders were compared. Na⁺ transport, however, was equally increased by the two substrates. Despite the differences in time course and substrate requirements between the stimulation of H⁺ and Na⁺ transport, both increases were abolished by actinomycin-D.

To examine the effect of aldosterone on the force of the H⁺ pump, protonmotive force, the pH gradient that would nullify the transport rate was determined with and without aldosterone. Aldosterone did not alter protonmotive force but significantly increased the slope of the H⁺ transport rate on the applied pH gradient. It

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is concluded that aldosterone stimulates H⁺ transport independently of Na⁺ transport. It increases the responsiveness of the transport rate to glucose and to a lesser extent pyruvate, an effect probably secondary to the increased transport rate. Equivalent circuit analysis indicates that aldosterone facilitates the flow of protons through the active transport pathway but does not increase the force of the pump.

INTRODUCTION

Several lines of evidence indicate that aldosterone not only stimulates the renal absorption of sodium but also the process of urinary acidification. To which extent the increased acidification is a function of accelerated sodium absorption remains to be determined. At least some studies suggest that acidification may be stimulated independently of sodium transport. Lifschitz et al. (1) provided some evidence in the dog that renal H⁺ secretion is stimulated by a pathway that does not involve DNA-dependent synthesis of RNA. Ludens and Fanestil (2) reported that aldosterone increased the rate of acidification in the urinary bladder of the Colombian toad, as judged from the reversed short-circuit current after inhibition of sodium transport; they did not attempt, however, to explore the transport step in acidification that was stimulated by the hormone.

Since turtle urinary bladder responds to aldosterone (3) and is capable of urinary acidification under conditions that permit close examination of the transport components (4), this preparation was selected for a study of the mode of action of aldosterone on the process of acidification.

The studies indicate that aldosterone stimulates H⁺ secretion directly and that this stimulation precedes that of Na⁺ transport. The stimulatory effect is markedly enhanced by the presence of substrates. Analysis of the stimulation of H⁺ transport by means of an equivalent

TABLE I
Stimulation of J_H by Aldosterone

	J_H , nmol/min		
	0	2 h	$\Delta \pm SE$
Fresh			
Aldosterone	3.8	6.8	3.0 ± 0.5
Control	4.2	5.2	1.0 ± 0.5
Depleted			
Aldosterone	4.9	5.8	$+0.9 \pm 0.3$
Control	4.9	4.3	-0.6 ± 0.2

Paired hemibladders were exposed to $0.5 \mu\text{M}$ aldosterone or diluent (control), either after mounting (fresh; $n = 7$) or after 18 h of incubation in the absence of exogenous substrate (depleted; $n = 17$). The gassing mixture was CO_2 -free air.

circuit model indicates that aldosterone increases the conductance in the active transport pathway and not the force of the pump. The increased conductance in this analysis would be consistent with the formation of new pump sites, an increase in the accessibility of existing pump sites or with other factors facilitating the flow of protons at a site close to the pump.

METHODS

Hemibladders of fresh water turtles, *Pseudemys scripta*, obtained from Mogul Ed, Oshkosh, Wis., were mounted in pairs of Lucite chambers so that one half served as control for the other which was exposed to aldosterone. The turtles were soaked for 24–48 h in 0.6% NaCl to reduce endogenous mineralo-corticoid production. The experiments were carried out in the short-circuited state, and the rate of H^+ secretion was measured by the pH stat method as described previously (5). The rate of Na^+ transport was estimated as the sum of the short-circuit current and the rate of H^+ transport. In one series of experiments (Table

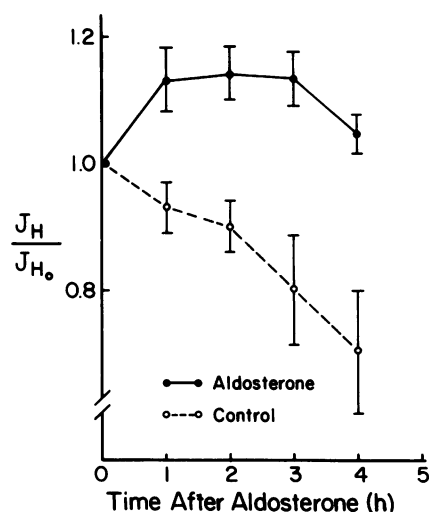


FIGURE 1 Time course of stimulation of H^+ secretion by aldosterone in seven substrate-depleted bladders.

VIII) carried out in bladders exposed to 0.5 mM ouabain, the rate of H^+ transport was considered to be equal to the short-circuit current. The evidence for this equality has recently been reviewed (4). For the purposes of this study a series of eight experiments was carried out in the presence of 1% CO_2 and 0.5 mM ouabain in the serosal solution; a plot of the pH stat rate against the simultaneously determined short-circuit current yielded a value of 1.01 ± 0.01 for the least-squares line.

The Ringer's solution used in these studies contained in mmol per liter: Na, 115; K, 3.5; Ca, 1.8; Cl, 119.7; HPO_4 , 0.3; the pH was 7.4, and the gas phase was CO_2 -free air unless specified otherwise. D-aldosterone (Sigma Chemical Co., St. Louis, Mo.) was added in a final concentration of $0.5 \mu\text{M}$ to the serosal side only. Similarly, the substrates, glucose and pyruvate were added to the serosal side in a final concentration of 5 mM in the substrate experiments. In all experiments two hemibladders from the same turtle were used in identical chambers with an exposed membrane area of 8 cm^2 . Aldosterone was added in $50 \mu\text{l}$ of methanol to the experimental halves, and $50 \mu\text{l}$ of methanol was added to the control hemibladders. Statistical analysis was carried out by the paired *t*-test, and differences were considered significant at $P < 0.05$. Results are expressed as means \pm SEM for an area of 8 cm^2 .

RESULTS

(a) *Rate limiting factors for H^+ secretion (J_H)*. Before examining the effects of aldosterone on acidification the rate limiting factors for J_H must be considered. They are the electrochemical gradient for H^+ across the epithelium (6), the presence of CO_2 (7), and the availability of metabolic fuels.

Metabolic fuel becomes limiting only in substrate-depleted bladders. In such bladders CO_2 is no longer rate limiting in the absence of exogenous substrate as is shown in the following experiments. In five bladders that had been depleted of substrate for at least 18 h J_H was 3.57 nmol/min when the gas phase was CO_2 -free air. After changing the gas phase to 1% CO_2 in air, J_H was 3.53 nmol/min ; the difference of $0.04 \pm 0.94 \text{ nmol/min}$ was not significant. Subsequent addition of 5 mM glucose to the serosal solution increased J_H by $4.00 \pm 1.48 \text{ nmol/min}$ ($P < 0.05$).

If, on the other hand, glucose is added before CO_2 is made available, J_H is increased, and an additional increment results when CO_2 is added (see control experiments of Tables II and VII).

In substrate-depleted bladders, therefore, the secretion rate of H^+ is limited by the availability of metabolic fuel rather than CO_2 . An opposing electrochemical gradient, however, will reduce J_H under all circumstances.

(b) *Stimulation of H^+ secretion by aldosterone*. The effects of aldosterone on J_H were examined in urinary bladders of water turtles that had been kept in dilute saline to suppress the activity of endogenous mineralo-corticoid hormone. In Table I, J_H is compared in hemibladders exposed to $0.5 \mu\text{M}$ aldosterone and control halves of the same bladders in two series of experi-

ments: in fresh bladders of turtles that had been quickly pithed to minimize the secretion of endogenous hormone and in bladders that had been depleted of substrate as well as endogenous hormone by overnight incubation in Ussing chambers. In both groups of experiments, J_H was increased 2 h after exposure to aldosterone, and the observed differences between the aldosterone-treated bladders and the control halves were significant. In the substrate-depleted bladders, however, the control rates of H^+ secretion and the magnitude of stimulation by aldosterone were variable. As will be shown later (Table II), the stimulation of J_H by aldosterone is increased by addition of substrate, especially glucose.

The time course of the effect of aldosterone in substrate-depleted bladders is given in Fig. 1. Initial J_H was 6.1 ± 1.0 nmol/min in seven test bladders and 5.5 ± 1.0 in the control halves. The ratios of J_H at the 1st, 2nd, 3rd, and 4th h over the initial rate, J_{H_0} , are presented. J_H in the control halves (dashed line) declined slowly with time. In contrast, J_H increased in the aldosterone-treated hemibladders. The ratio of J_H over J_{H_0} was significantly different from that in the controls as early as 1 h after aldosterone addition.

(c) *The effect on J_H of substrates and stimulation by aldosterone.* In bladders depleted of endogenous substrate J_H is increased by the addition of glucose. Table II shows that the addition of 5 mM glucose caused a marked increase in J_H from 4.3 to 11.2 nmol/min. It is of interest (lower half of Table II) that pyruvate failed to stimulate J_H in the absence of aldosterone. All measurements of J_H were made in the absence of exogenous CO_2 by the pH stat method.

In aldosterone-treated bladders a synergistic effect is demonstrated when glucose is added 4 h after the addition of aldosterone. Aldosterone plus glucose caused a large increment in J_H that was significantly greater than that caused by glucose alone. Pyruvate, which failed to stimulate J_H in the control bladders, also caused a significant increment in the aldosterone-treated bladders.

To obtain a better comparison of the effects of glucose and pyruvate, paired hemibladders were incubated overnight in media containing 5×10^{-7} M aldosterone and no substrate. Since changes in sodium transport may affect J_H via changes in the availability of CO_2 , the serosal gas phase contained 1% CO_2 . The stimulation of J_H by glucose and pyruvate in halves of the same bladders is shown in Table III. Glucose caused an increment of 16.5 ± 2.7 nmol/min, whereas pyruvate caused a significantly smaller increment of 9.3 ± 2.0 nmol/min.

The results of Tables II and III indicate that aldosterone increases the responsiveness of the transport system for H^+ to substrate and that under the experimental conditions used glucose stimulates J_H more than equimolar quantities of pyruvate.

TABLE II
Effect of Aldosterone and Exogenous Substrates on J_H

	J_H , nmol/min		
	No substrate	Glucose	Δ Substrate
Aldosterone	5.8	15.8	10.0 ± 1.3
Control	4.3	11.2	6.8 ± 2.3
Δ (A-C)			3.2 ± 0.8
	No substrate	Pyruvate	Substrate
Aldosterone	3.3	5.7	2.4 ± 0.5
Control	3.4	3.1	-0.3 ± 0.7
Δ (A-C)			2.7 ± 0.8

Paired hemibladders depleted of endogenous substrate were exposed to $0.5 \mu M$ aldosterone or diluent (control) for 4 h. At this time J_H was measured (no substrate). Glucose ($n = 7$) or pyruvate ($n = 5$) was then added to the serosal side, and the maximal values for J_H over the next 2 h were recorded. The gassing mixture was CO_2 -free air.

The effect of aldosterone on J_H was also examined 2–3 h after addition of either glucose or pyruvate to substrate-depleted bladders. Aldosterone caused a sustained increase in H^+ secretion in the presence of glucose ($n = 6$), but failed to bring about a consistent increase in the presence of pyruvate ($n = 6$). In both sets of experiments J_{Na} was markedly increased in the aldosterone-treated bladder halves compared to the control halves.

These results suggest that the stimulation of H^+ secretion is best supported by glucose as the substrate. Although pyruvate consistently enhanced the stimulation of J_{Na} , its effects on the stimulation of J_H were variable.

(d) *Comparison of stimulation of J_H and J_{Na} .* Fig. 2 shows the effect of aldosterone on J_{Na} , estimated as the sum of the short-circuit current and the current equivalent of J_H , in the seven substrate-depleted bladders of Fig. 1. In the hemibladders exposed to aldosterone, J_{Na} was increased compared to the control halves. In contrast to the increase in J_H (Fig. 1), which was signifi-

TABLE III
Comparison of the Effects of Glucose and Pyruvate on J_H in the Presence of Aldosterone in Paired Hemibladders

	J_H , nmol/min		
	0	+	$\Delta \pm SE$
Substrate	0	+	$\Delta \pm SE$
Glucose	6.0	22.5	16.5 ± 2.7
Pyruvate	5.5	14.8	9.3 ± 2.0

Six paired hemibladders were incubated for 18–20 h in media containing $0.5 \mu M$ aldosterone but no exogenous substrate. J_H was then measured, and one substrate was added to each hemibladder. The maximal values over the next 2 h were recorded. The serosal gas phase was 1% CO_2 in air.

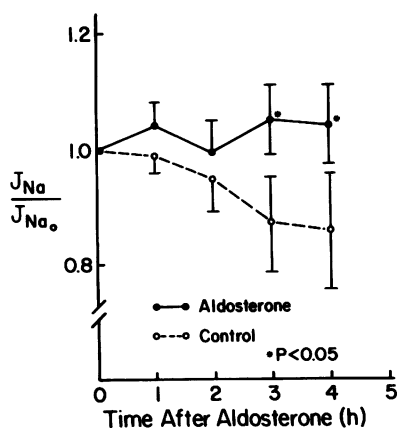


FIGURE 2 Time course of stimulation of J_{Na} in the seven bladders of Fig. 1.

cant after 1 h of aldosterone, the increase in J_{Na} became significant only after 3 h.¹ This result is comparable to observations by Lefèvre (3), who reported that the short-circuit current in turtle bladder was increased by aldosterone after an interval of 3 h. She found that the maximal stimulation occurred at about 7 h in substrate-depleted bladders.

The stimulation of J_{Na} by aldosterone was enhanced in depleted bladders by the addition of substrate. In the experiments of Table II both glucose and pyruvate increased the difference in J_{Na} between aldosterone-treated and control halves. In both, J_{Na} was approximately doubled in the presence of aldosterone. Table IV corresponds to Table III and compares the increments in J_{Na} caused by equimolar amounts of glucose and pyruvate. The increments in J_{Na} were comparable with the two substrates whereas the increments in J_H (Table III) had been significantly larger with glucose.

As indicated above, addition of aldosterone 2–3 h after substrate addition to depleted bladders caused marked stimulation of J_{Na} in the presence of either glucose or pyruvate. J_{Na} was still increasing during the 6th-h of observation. In contrast, J_H was stimulated only in the presence of glucose and reached an apparent steady level between 4 and 6 h.

These results suggest that the stimulation of J_H precedes that of J_{Na} : first, in substrate-depleted bladders stimulation of J_H began before that of J_{Na} ; and second, in the presence of glucose J_H reached a steady level be-

¹To examine the independence of the stimulation of J_H from that of J_{Na} with respect to time the data of Fig. 1 and 2 were subjected to multivariate analysis of variance. According to this analysis J_H is stimulated significantly ($P < 0.05$) while J_{Na} was not ($P > 0.05$) over the 4-h time period. More importantly, there was no significant interaction between J_H and J_{Na} . We are indebted to Dr. J. Kondo, Biostatistics Section, Department of Preventive Medicine, University of Iowa, for performing this analysis.

TABLE IV

Comparison of the Effects of Glucose and Pyruvate on J_{Na} in the Presence of Aldosterone in Paired Hemibladders

Substrate	J_{Na} , μA		
	0	+	$\Delta \pm SE$
Glucose	87	145	58 ± 9
Pyruvate	64	125	61 ± 18

Six paired hemibladders were incubated for 18–20 h in media containing $0.5 \mu M$ aldosterone but no exogenous substrate. J_{Na} was then measured, and one substrate was added to each hemibladder. The serosal gas phase was 1% CO_2 in air.

tween 4 and 6 h at a time that J_{Na} was still increasing. Under comparable conditions Lefèvre (3) observed the maximal increase in short-circuit current at 13 h. Aside from the difference in time course between the stimulation of the two transport systems, there is a difference in the substrate requirements, J_H being best supported by glucose and J_{Na} by either glucose or pyruvate.

To examine whether the stimulation of the two transport systems might have different mechanisms and whether these might be dissociated by an inhibitor of DNA-dependent RNA synthesis, the effects of aldosterone were studied in bladders pretreated with actinomycin-D. Table V shows that substrate-depleted bladders exposed to actinomycin-D in a final concentration of $2 \mu g/ml$ in the presence of 5 mM glucose in the media and 1% CO_2 in the gas phase fail to respond to aldosterone. Stimulation of neither J_H nor J_{Na} could be demonstrated. J_H and J_{Na} declined with time in both control and aldosterone-treated hemibladders. There was no significant difference between control and aldosterone bladders. Since the stimulation of J_{Na} and J_H by aldosterone was demonstrated in a consistent manner in the presence of glucose, these results are consistent with the

TABLE V

Effect of Aldosterone on J_H and J_{Na} in Paired Hemibladders Pretreated with Actinomycin-D

Time	0	1	2	3	4
J_H , nmol/min					
Aldosterone	14.8 ± 3.2	12.8 ± 2.2	10.0 ± 1.4	7.9 ± 1.9	7.1 ± 2.0
Control	14.2 ± 4.3	13.5 ± 4.1	11.0 ± 4.1	10.1 ± 4.5	10.2 ± 4.4
J_{Na} , μA					
Aldosterone	196 ± 70	164 ± 150	123 ± 36	99 ± 24	85 ± 17
Control	96 ± 38	91 ± 40	81 ± 39	78 ± 42	78 ± 44

2 h before the addition of aldosterone or diluent the bladders ($n = 5$) were exposed to actinomycin-D ($2 \mu g/ml$) and 5 mM glucose. The serosal gas phase was 1% CO_2 in air.

interpretation that both processes of stimulation depend on DNA-dependent RNA synthesis.

(e) *Effect of substrate at two different levels of J_H .* The enhanced responsiveness of the transport rate to metabolic substrates in the presence of aldosterone could be a reflection of the increased transport rate per se rather than of an effect of aldosterone on metabolism. To examine this point the effects of glucose on J_H were studied at two secretion rates in depleted bladders in the absence of aldosterone. The rate of transport was varied by lowering luminal pH from 7.0 to 6.0. It had been previously shown (6) that luminal acidification decreases the rate of active H^+ transport. Table VI shows that serosal addition of glucose caused an increment in J_H that was almost three times as great at the higher rate of transport (luminal pH = 7) as at the low rate of transport (luminal pH = 6). The transport rate per se, therefore, appears to be a determinant of the extent to which substrates will increase J_H .

(f) *Stimulation of J_H by aldosterone is not mediated by CO_2 .* Aldosterone could stimulate J_H either directly or by increasing the production of metabolic CO_2 through a mechanism unrelated to transport. The latter explanation is unlikely since the experiments of section (a) have shown that exogenous CO_2 fails to increase J_H in substrate-depleted bladders. Hence, even if aldosterone increased the CO_2 production by the tissue, the H^+ transport system would not have responded. To demonstrate further that the increase in J_H caused by aldosterone was not mediated through increased CO_2 production, the effects of addition of exogenous CO_2 were explored in aldosterone-treated and control hemibladders. Table VII shows that H^+ secretion was greater in the aldosterone-treated hemibladders before addition of CO_2 . Addition of CO_2 caused a larger increment in the aldosterone-treated hemibladders than in the control halves. Both rates were approximately doubled. These results indicate that aldosterone increases J_H by some mechanism other than an increase in P_{CO_2} .

TABLE VI
Effect of Glucose at Two Levels of J_H

	J_H , nmol/min		
	No substrate	Glucose	Δ
Luminal pH = 6.0	12.8	17.2	4.4 ± 1.6
Luminal pH = 7.0	18.5	30.9	12.4 ± 2.7
Δ			8.0 ± 2.2

J_H was measured in six substrate-depleted bladders in the absence of aldosterone at luminal pH = 6 and at pH = 7; the measurements were repeated after addition of glucose. Serosal pH was kept constant at 7.4. The gassing mixture was 1% CO_2 .

TABLE VII
Effect of Exogenous CO_2 on J_H

	J_H , nmol/min		
	CO_2 -free	1% CO_2	Δ
Aldosterone	6.5	12.5	6.0 ± 1.9
Control	3.9	8.0	4.1 ± 1.0
Δ (A-C)			1.9 ± 0.8

J_H was measured in four substrate-depleted bladders 4 h after addition of pyruvate and 0.5 μM aldosterone or diluent (control). The serosal gas phase was then changed from CO_2 -free air to 1% CO_2 in air.

(g) *Effect of aldosterone on the force of the H^+ pump.* To evaluate the site of action of aldosterone on H^+ transport, we will consider a general model of an energy converter in which metabolic energy is linked to a transport step. In such a model aldosterone could either facilitate the flow of protons through the transport system or alter the driving metabolic reaction in such a way that the transfer of chemical energy is promoted. It has been shown previously (6) that application of an unfavorable pH gradient across the bladder results in a decrease in J_H .² Following Ussing and Zerahn (8), it can be argued that the magnitude of the gradient sufficient to nullify the transport rate is equivalent to the force of the H^+ pump (the protonmotive force, [PMF]).³ Using a model of H^+ transport (see Discussion for details), it can be shown that the slope of J_H on the applied pH gradient gives the parameter that describes the ease with which protons flow through the system.⁴ The value of the PMF is a composite function which is determined, in part, by the free energy of the driving metabolic reaction. To determine these two parameters experiments were performed in paired hemibladders using the short-circuit current as the measure of H^+ transport. The luminal pH was lowered in steps in paired hemibladders in the presence of 1% CO_2 and 5 mM glucose in the media. The serosal pH was kept at 7.4.

² Steinmetz and Lawson (6) showed that the passive transepithelial H^+ permeability is negligible over the range of spontaneous acidification. The decrease in J_H with opposing H^+ gradient, therefore, reflects a decrease in the forward flux of H^+ .

³ *Abbreviation used in this paper:* PMF, protonmotive force.

⁴ For this analysis it is assumed that the conductance of the other ions is not affected by lowering of the luminal pH. Steinmetz and Lawson (6) observed that in ouabain-treated bladders (as were used for the experiments of Table VIII) the Na^+ conductance is not affected by luminal acidification over the range used. Likewise, luminal acidification had no effect on the permeabilities of Cl^- , Cs^+ , and K^+ in short-circuited control bladders (6).

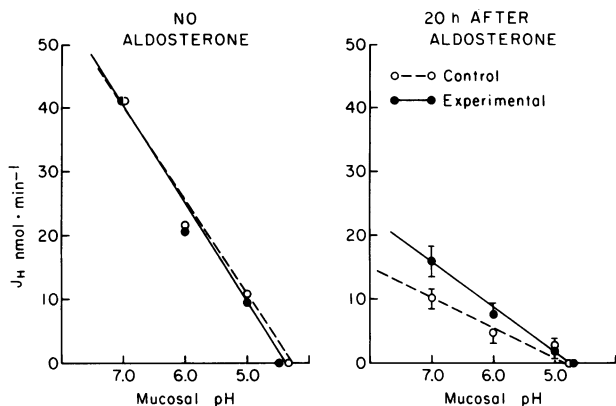


FIGURE 3 Failure of aldosterone to alter the force of the H^+ pump. In the left panel J_H was reduced to zero by lowering of mucosal pH before addition of aldosterone. The panel on the right shows the results of the same maneuver 20 h after addition of aldosterone or diluent (based on results of Table VIII).

Fig. 3 shows how J_H decreased with decreasing luminal pH. The left panel demonstrates that J_H decreased in similar manner in both hemibladders before addition of aldosterone. The panel on the right gives the results 20 h after addition of aldosterone or diluent to the hemibladders. J_H was increased in the tissues exposed to aldosterone, but the luminal pH at which J_H became zero (PMF) was the same for both groups of hemibladders. The slope of J_H on the applied chemical gradient, therefore, was increased by aldosterone. The values for J_H , PMF, and the slope $\partial J_H / \partial \Delta pH$ are given in Table VIII for the 1st day on the left and after aldosterone on the right. Aldosterone caused no significant change in PMF while J_H and the slope were clearly increased.

The results suggest that aldosterone does not increase the force of the pump directly but increases J_H by increasing the conductance in series with the active trans-

port pathway or by a thermodynamically equivalent mechanism.

DISCUSSION

The present study indicates that aldosterone stimulates H^+ secretion as well as Na^+ transport by the isolated turtle bladder. It confirms the observation by LeFèvre (3) that the response of sodium transport to aldosterone is slow in turtle bladder, and it supports the results obtained by Ludens and Fanestil (2) on urinary acidification by the Colombian toad bladder. Several characteristics of the stimulation of H^+ secretion in turtle bladder are defined. It is shown that H^+ secretion is stimulated independently of the stimulation of Na^+ transport and that stimulation is demonstrable in the presence as well as in the absence of the two known rate-limiting substrates for H^+ secretion: exogenous CO_2 and metabolic substrates. Finally, the mode of action of aldosterone is analysed in an equivalent circuit model of H^+ transport.

Several lines of evidence indicate that the stimulation of H^+ secretion is independent of that of Na^+ transport. First of all, the stimulation of H^+ secretion precedes that of Na^+ transport by about 2 h. Secondly, in aldosterone-treated bladders the stimulation of H^+ secretion has different substrate requirements than the stimulation of Na^+ transport. H^+ secretion was increased twice as much by addition of glucose as by addition of pyruvate, whereas Na^+ transport was increased to the same extent by these two substrates. Thirdly, H^+ secretion was stimulated by aldosterone in bladders in which Na^+ transport was abolished by ouabain (Table VIII). Since aldosterone is thought to exert its effect on sodium transport through a process involving DNA-dependent RNA synthesis (9), it would be conceivable that the early stimulation of H^+ secretion would be mediated by some other mechanism. This possibility, in fact, was suggested by Lifschitz et al. (1) who observed that actinomycin-D inhibits the aldosterone-induced increase in

TABLE VIII
The Force of the H^+ Pump before and after Addition of Aldosterone

	Before addition			20 h after addition		
	J_H	PMF	$\partial J_H / \partial \Delta pH$	J_H	PMF	$\partial J_H / \partial \Delta pH$
Aldosterone	41.2	3.00	15.9	16.0	2.75	6.9
Control	41.0	3.11	15.1	10.1	2.87	4.1
Δ (A-C)				5.9	-0.12	2.8
				± 2.2	± 0.18	± 0.6

J_H is expressed as nmol/min and PMF as pH units. The results of the left panel were obtained when the bladder halves were fresh; the results of the right panel were obtained either after 20 h of exposure to aldosterone or after depletion of endogenous hormone (control). All experiments were carried out in the presence of 0.5 mM ouabain, 5 mM glucose, and 1% CO_2 in the gas phase.

sodium absorption but not in H^+ secretion in the dog. In the isolated turtle bladder both the early stimulation of H^+ secretion and the late stimulation of Na^+ transport were abolished by actinomycin-D. The separate stimulation of H^+ secretion and Na^+ absorption and their specific substrate dependence could reflect the synthesis of two different proteins or merely inherent differences in the characteristics of the two transport systems.

The results of Tables II and III clearly indicate that the presence of aldosterone increases the responsiveness of substrate-depleted bladders to the addition of glucose or pyruvate. Both H^+ secretion and sodium transport showed this increased responsiveness, but for H^+ secretion glucose was preferred over pyruvate as a substrate. This synergism between aldosterone and substrate is well known for the sodium transport system in the toad bladder (9, 10). Its mechanism, however, remains only partially understood. Sharp and Leaf (10) have provided evidence that the stimulation of metabolism is secondary to stimulation of Na^+ transport, whereas Fanestil et al. (9) have suggested that aldosterone has a primary effect on the metabolic reactions coupled to sodium transport.

If the stimulation of H^+ transport by metabolic substrates is secondary to a primary acceleration of the transport step by aldosterone, then one should be able to demonstrate such a substrate effect following other maneuvers that primarily accelerate transport. Changes in the pH gradient against which H^+ secretion occurs alter the transport rate instantaneously (6). It was shown in Table VI that in depleted bladders not exposed to aldosterone the response of J_H to glucose addition was a function of the basal transport rate. The response was considerably greater at the higher basal transport rate observed at a mucosal pH of 7 than at the reduced rate at pH 6. This apparent dependence of the substrate effect on the basal transport rate is consistent with the interpretation that the stimulation of metabolism by aldosterone is probably secondary to its effect on the transport system.

The secretion of H^+ by the turtle bladder may be outlined by the following working description (4): protons, perhaps originating from splitting of water molecules, are translocated across the luminal border of the epithelium in a process that requires the input of metabolic energy. The hydroxyl ions remaining are disposed of with the aid of cytoplasmic carbonic anhydrase by combining with CO_2 ; the HCO_3^- so formed then diffuses down an electrochemical gradient into the serosal side. By analogy with the sodium transport system (8, 11) this process can be represented by an equivalent circuit made up of a battery representing the proton pump, a resistance in series with the pump and a shunt resistance in parallel with the pump. The force of the pump, the

PMF, can be evaluated following Ussing and Zerahn (8), as the transepithelial gradient necessary to bring net transport to zero. It follows then that for a constant flow of current through the parallel limb, the series conductance is approximated by the slope of the H^+ transport rate on the applied gradient.⁴ In evaluating these two parameters of the equivalent circuit, Table VIII shows that aldosterone does not change the PMF but significantly increases the conductance in the active pathway.

In this representation, what do the elements of the circuit represent? The PMF is considered to be a function of the chemical reaction that leads to charge separation. It includes a term that is equivalent to the free energy (ΔG) of the reaction that is tightly linked to transport. In many transport systems it is believed that ATP hydrolysis is an important "energy donor"; hence, the free energy available from ATP hydrolysis would be a determining factor in the value of the PMF. The PMF, however, depends not only on the free energy of the chemical reaction but also on its relation to the flow and translocation of protons through the system (12).

The conductance in the active transport pathway reflects the facility with which either protons or OH^- ions flow from the site of dissociation. This extensive function also includes, in descriptive terms, the number of pump sites available for the dissociation reaction. The parallel conductance for H^+ in turtle bladder is low as judged from the studies of Steinmetz and Lawson (6) and from recent evidence (13).

Essig and Caplan (12) have given a formal description of this kind of analysis in terms of linear irreversible thermodynamics. The formalism requires that the flow of the transported ion species (J_H) and the flow of metabolism (J_r) are coupled to the conjugate forces of the ion (X_H), and of metabolism (A , the affinity of the driving chemical reaction i.e. the ΔG noted above).

$$J_H = L_H X_H + L_{Hr} A$$

$$J_r = L_{rH} X_H + L_r A$$

where L_H and L_r are "straight coefficients" relating the flow of one species to its driving force, and L_{Hr} and L_{rH} are cross coefficients relating the flow of one species to the driving force of the other species. Over the pH range examined both J_H and J_r ⁵ have been shown to be a linear function of the transepithelial pH gradient (6, 13, 14). The two cross coefficients, following Onsager (15), are considered to be equal. The PMF in this

⁵ J_r was measured continuously as the rate of $^{14}CO_2$ production from $[U-^{14}C]$ glucose in ouabain-treated bladders (13, 14). Because of the linearity of J_r and X_H , the affinity may be considered constant during the period of experimental observation.

analysis is $(X_H)_{J_{H=0}}$ and is equal to

$$(X_H)_{J_{H=0}} = [L_{rH}/L_H]A$$

and

$$L_H = [\partial J_H / \partial X_H]_A$$

Since the affinity is probably constant during the period of the experiment, L_H is equivalent to the slope shown in Fig. 3. The constancy of the PMF could reflect a constant affinity or, alternatively, an increased affinity balanced by a decrease in the fraction L_{rH}/L_H . Recently, Saito et al. (16) have shown in the frog skin that the affinity may indeed increase after prolonged incubation with aldosterone.

It follows from this analysis that aldosterone increases the conductance of protons through the active pathway and not the PMF in the system. As discussed, the increased active conductance could represent a number of changes in the overall transport operation of the system. Among the more attractive possibilities are the formation of new pump sites or a structural change in the luminal membrane that would provide access to an increased number of existing pump sites. These changes would be mediated one way or another by the synthesis of new proteins (17). Alternatively, the increased conductance could be a manifestation of a change in the physical state of the membrane that would increase the mobility of protons through the active pathway. Recently, Goodman et al. (18) have provided evidence that aldosterone changes the lipid composition of the membrane in a way that would increase its "fluidity."

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