Effect of Aldosterone on the Coupling between H^+ Transport and Glucose Oxidation

QAIS AL-AWQATI, *Department of Internal Medicine, University of Iowa, Iowa City, Iowa 52242*

ABSTRACT The mode of action of aldosterone on the energetics of H^+ transport in the turtle bladder was examined with the rate of glucose oxidation as an index of the metabolic activity of the epithelium (we show that H^+ transport is not coupled to fatty acid oxidation).

Within 6 h of addition of aldosterone H^+, transport increased; so did glucose oxidation. The amount of H^+ transport per mole of $^{14}CO_2$ produced from glucose oxidation was 15.6 $eq \cdot mol^{-1}$ in the control hemibladder, while in the aldosterone-treated bladder it was 13.6, $\Delta = 2.0 \pm 4.0$ ($n = 6$). However, in bladders exposed to aldosterone for 20 h, the relation of transport to glucose oxidation was significantly altered: control 10.8, aldosterone 16.4, $\Delta = 4.5 \pm 2.5$, $P < 0.02$, $n = 7$. The slope of H^+ transport on the applied electrochemical gradient was steeper during both short- and long-term incubations. However, the maximum gradient necessary to nullify the net rate of secretion was unaltered in both experiments. Evidence is presented that aldosterone does not alter the passive backflux into the cell. In five additional experiments where aldosterone produced no significant stimulation of H^+ transport, no change was noted in any of the metabolic or transport characteristics measured, suggesting that the alterations discussed above are dependent on the stimulation of H^+ transport by the hormone.

These results, along with some thermodynamic considerations, suggest that the effect of aldosterone is primarily exerted on the transport process rather than on metabolism. Further, it appears that prolonged stimulation of transport work leads to secondary alterations in the metabolic pathways reminiscent of the changes that occur in skeletal muscles of athletes undergoing physical conditioning.

Dr. Al-Awqati's present address is Department of Medicine, Columbia University College of Physicians & Surgeons, New York 10032.

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INTRODUCTION

Two hypotheses are usually proposed to explain the mode of action of aldosterone on sodium transport in epithelia: (*a*) aldosterone increases the passive entry of Na^+ into the cell, thus leading to increased availability of the ion to the pump (1); and (*b*) the hormone stimulates directly the energetics of active extrusion either by supplying more high-energy intermediates or by some other process (2). Most investigators agree that an increase in Na^+ entry is essential to the effect. Intracellular Na^+ is increased (3–5 and our unpublished observations). The electrical resistance of the toad bladder and frog skin is decreased (6–8), and more specifically, the conductance in the active Na^+ transport pathway was found to be increased (9, 10). The force of the Na^+ pump (E_{Na})[1] remains unaltered by aldosterone (9, 10). However, the driving force of metabolic reactions coupled to transport (the thermodynamic affinity) increased on prolonged incubation with the hormone (11). Hence, it was suggested that aldosterone might affect both the induction of a "permease" as well as having an "energetic" effect (11, 12).

With these considerations in mind, we attempted previously to investigate the mechanism of action of aldosterone on H^+ transport in the turtle bladder (13). We found that aldosterone stimulated H^+ transport but had no effect on the maximum chemical gradient necessary to nullify net transport. Thus, aldosterone did not appear to affect the force of the H^+ pump, but rather increased the conductance for protons in the active transport pathway (13). Equivalent circuit analysis would lead one to believe that the conductance of the active pathway is a passive circuit element, probably representing the ease with which protons flowed through the active pathway (or OH^- were disposed of behind the pump). It was thus quite surprising when we recently found that a number of maneuvers that

[1] *Abbreviations used in this paper*: E_{Na}, force of the Na^+ pump; $J^*_{CO_2}$, rate of $^{14}CO_2$ production; J_H, H^+ transport rate.

aimed to change the metabolic activity of the tissue had large effects on the conductance (14). A variety of metabolic inhibitors decreased the conductance in the active pathway, and feeding glucose to substrate-depleted tissues increased it. Hence, aldosterone might conceivably have a metabolic (i.e., energetic) effect that could be reflected in a change in the conductance. Clearly, the role of energetic factors had to be directly examined to arrive at a more definitive result.

I examined the effect of aldosterone on the coupling between H^+ transport and glucose oxidation and found that initially the hormone had no effect on the relation of transport to substrate utilization despite a substantial increase in the rate of H^+ transport. However, prolonged incubation with the hormone led to an increase in the number of H^+ transported per mole of glucose oxidized. These results suggest that the effect of aldosterone is primarily exerted on the transport apparatus, but that prolonged stimulation leads to secondary changes in metabolism that may be adaptive in nature.

METHODS

The methods used have been published in detail previously (13, 15). Paired hemibladders from fresh-water turtles were mounted in Ussing chambers and short-circuited. The turtles in some cases were soaked for 1–3 days in 0.6% NaCl, and others were injected with aminoglutethimide, 100 mg i.m. twice per day for 2–3 days. The bladders were incubated for 15–18 h in media containing 0.5 mM ouabain but no metabolic substrate. After that period, 10 mM glucose was added to the serosal side, and the gassing mixture was changed from air to 1% CO_2 in air. The experiments in Table I, however, were performed without the overnight incubation.

The bladders were then washed for 30 min with Ringer's solution containing penicillin, gentamicin, colistin, and 10 mM glucose. After the final wash, a solution containing 10 mM glucose, antibiotics, and uniformly labeled [^{14}C]glucose was added to the serosal side, and the $^{14}CO_2$ production was continuously measured by an ionization chamber technique as described previously (15). In one set of experiments, the same process was used except that 4 mM butyrate and [1-^{14}C]butyrate were substituted for glucose. After the $^{14}CO_2$ production reached a steady state (2 h), the short-circuit current (J_H), the $^{14}CO_2$ production ($J^*_{CO_2}$), and the pH of the luminal medium were noted. The pH of the serosal bath was between 6.5 and 7.0. The luminal pH was changed by addition of HCl or NaOH, and the system was allowed to reach a new steady state. In each bladder, 2–10 such periods were performed. At all times during the experiment, the bladder was short-circuited. Aldosterone (final concentration 0.5 μM) was added to the serosal side in 15 μl of methanol at different times. The control hemibladder received 15 μl of methanol. After each experiment, the exposed area of the bladder was cut and dried to a constant weight in a hot-air oven. The average dry weight was 20.7±10.7 mg (SD). The $^{14}CO_2$ production was always corrected for the glucose oxidation produced by microorganisms in the medium at the end of the experiment, as discussed previously (15). In all experiments [^{14}C]glucose was added after the overnight incubation to keep the bacterial growth to its minimum. In some experiments, H^+ transport or $^{14}CO_2$ production after reaching an apparent steady state started to decline progressively. These experiments were not used in the calculation of the final results.

Statistical analysis was performed on the data using the paired t test (16). All results are normalized to the dry weight and are given as means±1 SE.

RESULTS

Coupling of H^+ transport to oxidation of respiratory fuels

We examined the relation of H^+ transport to the oxidation of two important respiratory fuels, glucose and butyrate. We had previously demonstrated in a number of experiments under a variety of circumstances that H^+ transport is tightly coupled to oxidation of ^{14}C-labeled glucose. In an additional set of eight experiments, we found that when H^+ transport was decreased by applying an adverse pH gradient, $^{14}CO_2$ production from glucose oxidation decreased by 0.163 ±0.054 nmol·min^{-1}·mg dw^{-1} (Table I). In another set of seven experiments, oxidation of sodium [^{14}C]-butyrate was followed. Decreasing the rate of transport by an amount equivalent to that seen in the glucose experiments resulted in no change in $^{14}CO_2$ production from butyrate oxidation (Table I). Note that the amount of $^{14}CO_2$ produced from butyrate is 3 times that produced from glucose. These experiments, performed without endogenous substrate depletion, had only one exogenous substrate present in any one bladder. The data indicate that butyrate is avidly utilized in pathways unrelated to H^+ transport, while glucose is the preferred substrate for the transport-linked pathway.

TABLE I

Coupling of H^+ Transport to Oxidation of Respiratory Fuels

	J_H	$J^*_{CO_2}$
	nmol·min^{-1}·mg dw^{-1}	nmol·min^{-1}·mg dw^{-1}
A. [^{14}C]Glucose, n = 8	0.690	0.386
	0.076	0.223
Δ±SE	0.614±0.106*	0.163±0.054*
B. [^{14}C]Butyrate, n = 7	0.576	0.691
	0.052	0.690
Δ±SE	0.524±0.07*	0.001±0.016

Coupling of H^+ transport to oxidation of respiratory fuels. 10 mM [^{14}C]glucose or 4 mM [^{14}C]butyrate were present on the serosal side in the two sets of experiments. After a steady rate of H^+ transport and $^{14}CO_2$ production was obtained, the luminal pH was decreased by 1.2 pH units and a new steady state was allowed to develop. The data presented are the steady-state values of these two periods.

* $P < 0.01$.

The effect of aldosterone on H^+ transport and glucose oxidation

The effect of aldosterone on H^+ transport, like that on Na^+ transport in toad bladders, is sometimes difficult to demonstrate. In part, this is due to the difficulty of adrenalectomy in these animals—the presence of endogenous hormone leading to no further effects on in vitro addition. Apocryphal anecdotes point to the importance of doing these experiments in the correct season (frequently defined as that season when aldosterone exerts its effect).

In a previous study (13), we showed that aldosterone stimulates H^+ transport in a series of unselected bladders. In the present study, 18 paired experiments were completed. In five of them, the rate of H^+ transport (in the absence of electrochemical gradients) was the same or lower in the aldosterone-treated hemibladder. The results of these experiments will be presented as a separate group. The justification for this segregation is that the purpose of the present study is to investigate the metabolic changes, if any, that occur with the stimulation of H^+ transport. The results of the experiments which showed no stimulation form a convenient control group.

In all experiments, paired hemibladders were incubated for 20 h in the absence of exogenous substrate. In one set of experiments, 0.5 μM aldosterone was present in the experimental hemibladder throughout the 20 h of incubation, while in the other it was added 1 h before the measurements began. In all experiments, uniformly labeled [^{14}C]glucose was added to the serosal medium with a final concentration of 10 mM. When the rate of $^{14}CO_2$ production reached a steady state, the H^+ transport rate (J_H), the rate of $^{14}CO_2$ production (J^*CO_2), and the luminal pH were measured and recorded. The luminal pH was changed and the system allowed to reach a new steady state. This was repeated two to seven times in each bladder.

Effect of aldosterone on H^+ transport in the absence of electrochemical gradients. In the set of bladders exposed to aldosterone for 1 h, the rate of H^+ transport before addition of the hormone was 1.21 nmol·min^{-1}·mg dw^{-1} in the control hemibladder, a value similar to 1.19 nmol·min^{-1}·mg dw^{-1} in the bladder to receive aldosterone ($\Delta = 0.02 \pm 0.22$). The average J_H in the first 6 h after addition was 1.33 nmol·min^{-1}·mg dw^{-1} in the control bladder, while in the aldosterone-treated bladder it was 2.49 nmol·min^{-1}·mg dw^{-1} ($\Delta = 1.15 \pm 0.50$, $P < 0.05$) (Table II A, $n = 6$).

In the experiments where the bladders were exposed to aldosterone for 20 h, J_H before the addition of the hormone was 1.01 nmol·min^{-1}·mg dw^{-1} in the control side and 1.04 nmol·min^{-1}·mg dw^{-1} in the side to receive aldosterone, $\Delta = 0.03 \pm 0.05$. On the second day the average J_H in the absence of electrochemical gradients was 0.89 nmol·min^{-1}·mg dw^{-1}, while in the aldosterone-treated bladder it was 1.33 nmol·min^{-1}·mg dw^{-1}, $\Delta = 0.44 \pm 0.10$, $P < 0.01$, $n = 7$.

Effect of aldosterone on glucose oxidation and its relation to H^+ transport. Aldosterone increased the transport-related $^{14}CO_2$ production in the first 6 h of its effect. Measurements were obtained in the period extending from 1 h after addition of the hormone to 5 h later. By contrast, exposure to the hormone for 20 h caused no significant change in this moiety of glucose

TABLE II
Effect of Aldosterone on H^+ Transport

	1–6 h $n = 6$	20 h $n = 7$	No response $n = 5$
A. $(J_H)_{\Delta\mu H=0}$, *nmol·min^{-1}·mg dw^{-1}*			
Control	1.334	0.891	1.827
Aldosterone	2.486	1.326	1.528
Δ±SE	1.151±0.5*	0.435±0.1*	−0.289±0.161
B. $\partial J_H/\partial \Delta pH$, *nmol·min^{-1}·mg dw^{-1}*			
Control	0.508	0.355	0.699
Aldosterone	0.935	0.535	0.547
Δ±SE	0.427±0.174*	0.18±0.05*	−0.153±0.07*
C. $(\Delta pH)_{J_H=0}$, *pH units*			
Control	2.735	2.355	2.930
Aldosterone	2.546	2.352	2.859
Δ±SE	−0.17±0.13	0.002±0.29	−0.071±0.53

Effect of aldosterone on the rate of H^+ transport in the absence of electrochemical gradients $(J_H)_{\Delta\mu H=0}$, the slope of J_H on the pH difference across the membrane ($\partial J_H/\partial \Delta pH$), and on the driving force of the H^+ pump evaluated as the maximum gradient necessary to nullify H^+ transport $(\Delta pH)_{J_H=0}$.
* $P < 0.05$.

TABLE III
Effect of Aldosterone on Glucose Oxidation

	1–6 h $n = 6$	20 h $n = 7$	No response $n = 5$
A. Suprabasal $(J^*_{CO_2})_{\Delta pH=0}$, $nmol \cdot min^{-1} \cdot mg\ du^{-1}$			
Control	0.107	0.108	0.134
Aldosterone	0.191	0.133	0.304
Δ±SE	0.084±0.03*	0.025±0.018	0.171±0.11
B. Basal, $(J^*_{CO_2})_{J_H=0}$			
Control	0.178	0.212	0.345
Aldosterone	0.193	0.222	0.321
Δ±SE	0.015±0.01	0.01±0.05	0.024±0.05
C. $\partial J_H/\partial J^*_{CO_2}$, by the ΔpH method, $eq \cdot mol^{-1}$			
Control	15.6	10.8	12.2
Aldosterone	13.6	16.4	8.0
Δ±SE	−2.0±4.0	5.6±2.5*	4.2±3.9
D. Affinity, $kcal \cdot mol^{-1}$ *ATP hydrolyzed*			
Control	16.55	6.80	8.73
Aldosterone	10.34	9.7	4.69
Δ±SE	−6.21±2.14	+2.9±1.0*	4.03±3.78

Effect of aldosterone on glucose oxidation in the active pathway (suprabasal $J^*_{CO_2}$); on glucose oxidation unrelated to H^+ transport (basal CO_2 production); on the slope of H^+ transport on glucose oxidation ($\partial J_H/\partial J_{CO_2}$); and on the driving force of the metabolic reaction tightly coupled to transport (the affinity).
* $P < 0.05$.

oxidation, despite a 50% increase in H^+ transport (Table III A). Hence, either the intrinsic "stoichiometry" of the pump has changed so that more H^+ could be transported per mole of glucose oxidized, or alternatively, another substrate previously not used in this pathway is now supporting transport.

Both J_H and J^*CO_2 decreased in the presence of adverse pH gradients. Their relation to the applied gradient and to each other was linear. Fig. 1 shows an experiment that illustrates these relations. The lines were drawn by linear regression analysis. The slope of J_H on J^*CO_2 obtained by this method was found to be unchanged in the bladders exposed to aldosterone for 6 h; control 15.6 $eq \cdot mol^{-1}$, aldosterone 13.6, $\Delta = -2.0 \pm 4.0$ (Table III C). By contrast, bladders exposed for 20 h to the hormone had significantly higher values; control 10.8 $eq \cdot mol^{-1}$, aldosterone 16.4, $\Delta = 5.6 \pm 2.5$, $P < 0.01$ (Table III C and Fig. 1).

Another method of evaluating the "glucose cost" of H^+ transport is to compare the increment in H^+ transport induced by aldosterone to that in $^{14}CO_2$ production in the absence of electrochemical gradients. This $\Delta J_H/\Delta J^*CO_2$ agreed well with the conclusions of the slope method (16.6±7.3 $eq \cdot mol^{-1}$). Although this may appear redundant, I wish to point out that these results are not the same. This follows from the fact that the measured J_H is a net rate, i.e., the sum of two opposing unidirectional fluxes. In the presence of opposing concentration gradients, it will be expected that the "passive" flux from lumen to serosa will become larger if the permeability to protons is significant. Hence, the relation of J_H to J^*CO_2 in the presence of gradients will not be that characteristic of the H^+ pump unless the passive permeability of the membrane is negligible. Obviously, in the absence of gradients, J_H represents the active pathway only; hence, $\Delta J_H/\Delta J^*CO_2$ is the intrinsic ratio of the pump. The equivalence of these two ratios can be used as an argument that the passive permeability to protons is negligible (15). Further, we can state that aldosterone does not increase the passive membrane permeability to protons.

Effect of aldosterone on the "basal metabolism." The basal metabolism is that amount of glucose oxidized in non-transport-related pathways and is evaluated from the intercept of the regression plot as J^*CO_2 when $J_H = 0$. Table III shows that in both sets of experiments there was no change in this moiety of metabolism. This suggests that the changes observed are limited to the metabolic pathways linked to H^+ transport and that aldosterone does not have a generalized effect on glucose oxidation.

Effect of aldosterone on the relation of J_H to the

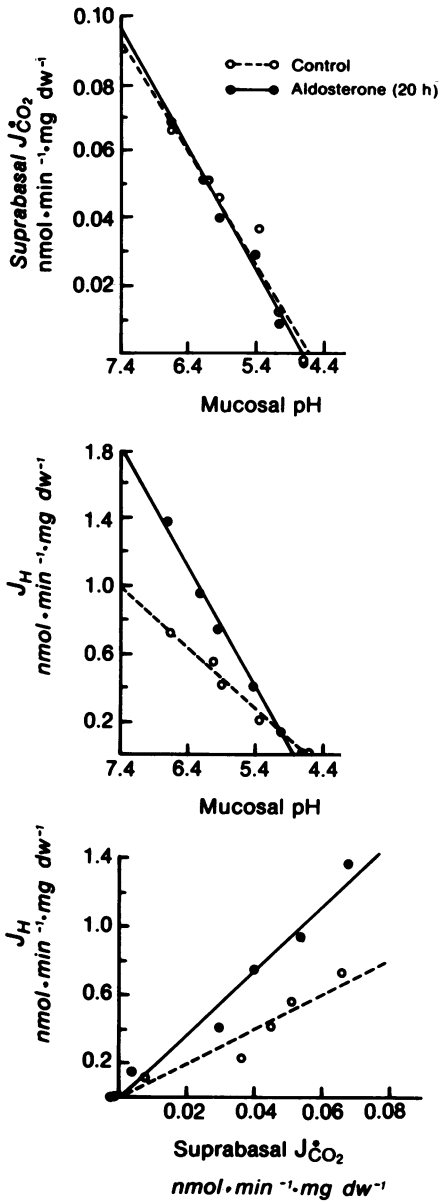

FIGURE 1 The relation of H^+ transport (J_H) and glucose oxidation (J^*CO_2) to the luminal pH (at constant serosal pH) and to each other in paired hemibladders one of which was exposed to aldosterone for 20 h.

applied gradient. The slope of J_H on ΔpH was increased by aldosterone in both sets of experiments (Table II and Fig. 1). However, the maximum gradient necessary to nullify net transport was unchanged (Table II and Fig. 1). This confirms our previous observations (13) and extends them to include the short-term effects of aldosterone.

Relation of J_H and J^*CO_2 in the experiments where aldosterone did not result in an increase in the rate of transport. In the five experiments where no significant stimulation of H^+ transport occurred, no change in the various parameters studied occurred (Tables II and III). (There was a significant decrease in $\partial J_H/\partial \Delta pH$.)

DISCUSSION

The results of Tables II and III show that when aldosterone stimulates H^+ transport, the oxidation of one respiratory fuel (viz., glucose) increases in proportion to the magnitude of stimulation. But after prolonged stimulation the transport system utilizes less glucose per equivalent of H^+ transported. These changes appear to be limited to the transport-related metabolic pathways, since the basal $^{14}CO_2$ production does not change nor is there any metabolic alteration in the absence of stimulation of H^+ transport. Hence, it appears that prolonged stimulation of transport leads to a change in the "gearing" ratio between transport and glucose oxidation.

The advantage of using glucose oxidation instead of oxygen consumption is that, in the former, one has knowledge of events in one subset of the respiratory pathways. The disadvantage is that it is not known a priori whether this pathway is a major route through which energy is channeled to the transport apparatus. A further problem is that when a change in the coupling between transport and glucose oxidation is shown (as in the long-term incubation studies), it is not clear whether that reflects a change in the "gearing" ratio of the pump or the induction of alternate pathways.

It is important to recall that all of the studies reported here were performed in the steady state. This implies that the label is present not only in glucose metabolites but also in the products that participate with glucose or its metabolites in isotope exchange. These latter will include glycogen, amino acids, and fats. In fact, when Weidemann and Krebs (17) fed uniformly labeled glucose to kidney cortex slices, they found that as much label was present in these metabolites as in CO_2. Thus the observation that $^{14}CO_2$ from glucose is coupled to H^+ transport while that from butyrate is not suggests that in the turtle bladder there is metabolic compartmentalization. Although the contribution of amino acids to the fuel of respiration in the transport-linked pathways has not been examined, it is unlikely that it will be significant, since there were no amino acids in the medium and it is doubtful that there are large endogenous pools of amino acids that can be easily recruited for use in the pathways coupled to transport. In the compartment feeding the H^+ pump, glucose therefore appears to be the major preferred substrate.

To what extent does aldosterone alter the metabolic pathways such that glucose is no longer the major metabolite? Although this cannot be ascertained by the present studies, it is critically dependent on the presence

of endogenous stores of other metabolites. Most of the experiments were performed in bladders that have been incubated overnight in the absence of exogenous substrates. Thus, it would be expected that their glycogen and fat contents will be lower than in the "fresh" state. The depletion of these endogenous stores is, however, not expected to be complete, since the bladders were incubated in the presence of ouabain, an agent that abolishes the utilization of that fraction of endogenous substrate coupled to sodium transport. Nevertheless, in this partially depleted state, exogenous substrate will be oxidized to a greater extent than that in the "fresh" state. Thus the rate of oxidation of exogenous glucose in these partially depleted bladders will approach that of the total oxidative activity of the tissue. Since fatty acid oxidation even in the fresh state is not coupled to H^+ transport and the contribution of amino acids is bound to be small, glucose oxidation is probably a reasonable index of the total metabolic activity of the tissue in the pathways coupled to transport. However, some doubt regarding this point must remain until more definitive experiments can be performed.

It is emphasized that the effect of aldosterone on the relation between H^+ transport and glucose oxidation can be interpreted without much ambiguity. It is only in the generalization of these effects to other metabolic pathways that caution should be exercised.

In discussing the mechanism of action of aldosterone on transport, it is customary to think of the hormone as having either a "permeability" effect (1, 3–6) or an "energetic" effect (2) or both (11, 12). For H^+ transport, using the familiar equivalent circuit, we found previously that the conductance in the active pathway is increased (13). In equivalent circuits, this conductance is assumed to represent a passive element. However, we have recently demonstrated that this parameter is highly dependent upon metabolism. In substrate-depleted bladders, addition of glucose stimulates H^+ transport and increases the conductance in the active pathway. Further, a variety of metabolic inhibitors decrease it to the same extent that they decrease H^+ transport (14). Hence, the increase in conductance induced by aldosterone may not represent only an increase in number of pumping sites or increases in permeability of protons by either a change in the pump or its lipid environment. It could easily represent some change in metabolism.

Aldosterone could affect metabolism by changing the coupling of transport to utilization of some high-energy intermediate such that, for a given amount of metabolite utilized, more H^+ can be transported. This does not appear to be the case for H^+ transport, since during stimulation of transport no such change occurred, at least in glucose utilization. Alternatively, the fundamental driving force of active transport, i.e., the $-\Delta G$ of the driving reaction, could be increased. This quantity is a function of the logarithm of the ratio of activities of the reactants to the products of the reaction. Presumably, the reaction as in other transport-linked reactions is the hydrolysis of ATP. Hence, $-\Delta G$ is proportional to the logarithm of the ratio of $ATP:ADP \cdot P_i$ (inorganic phosphate). Handler et al. (3) measured the ratio of ATP:ADP and creatine phosphate:creatine and found the latter to decrease, suggesting that the stimulation of transport is primary, leading to utilization of high-energy intermediates and a decrease in the ratio. For a complete measurement of the driving force, the ratio of all the reactants and products should be performed. Recently, Ross et al. (18) have found that in the isolated perfused kidney there was a parallel change between the $-\Delta G$ and sodium reabsorption. A further issue in these measurements is that it is the $-\Delta G$ in the vicinity of the pump that is the quantity of interest. Available techniques can only measure average tissue concentrations; these values may have little bearing on the activities adjacent to the pump, especially in a complex tissue such as an epithelium where cellular heterogeneity is a particularly vexing problem. This is highlighted by the observed metabolic compartmentalization in this epithelium. Despite these difficulties, it would be important to measure the levels of the high-energy intermediates. One can tentatively conclude that, initially at least, no change in glucose oxidative pathways coupled to H^+ transport seems to occur. After prolonged stimulation the transport system utilizes less glucose, and to the extent that this reflects less total metabolite oxidation, it has become more "efficient."

The cause of this secondary change in glucose utilization is not evident. Perhaps it is related to an adaptation of the tissue to a steady increase in the work load. This kind of adaptation to increased work occurs in skeletal muscle. ATP contents of muscle obtained from conditioned athletes are higher (19). Of interest is the observation that the ATP and creatine phosphate depletion for a given amount of work appears to be less for conditioned muscle than for muscle from sedentary individuals (19). This latter observation may bear some resemblance to the finding that after prolonged incubation with aldosterone, the "glucose cost" of H^+ transport was decreased. This may then be a general phenomenon whereby cells adapt to increased work load by decreasing the demand for high-energy intermediates through a change in the "gearing ratio" that couples the work performed to the amount of substrate utilized. Moreover, the metabolic pathways may be altered in such a way that the supply of energy-rich intermediates is increased.

Another approach to the measurement of the metabolic driving force is the thermodynamic analysis of Essig and Caplan (20) based on the work of Kedem (21). I realize that this approach is phenomenological.

Despite that, I think it is a useful initial approach. Since active transport is an example of energy conversion, an energetic (i.e., thermodynamic) approach is not inappropriate. For definitive descriptions of active transport, the direct and the thermodynamic approaches will have to be related to each other.

In this analysis, the rate of active H^+ transport (J_H) can be driven by its own gradient ($\Delta\tilde{\mu}_H$) or by the driving force of metabolism, the affinity A. Similarly, the rate of metabolism J_r (in this case the rate of glucose oxidation) is driven primarily by the affinity, but also by $\Delta\tilde{\mu}_H$ (see Fig. 1). The following equations can be written:

$$\begin{aligned} J_H &= L_H\,\Delta\tilde{\mu}_H + L_{Hr}\,A \\ Jr &= L_{rH}\,\Delta\tilde{\mu}_H + L_r\,A \end{aligned} \qquad (1)$$

where L_H and L_r are "straight" coefficients relating the flow of transport or metabolism to their respective conjugate driving forces, and L_{Hr} and L_{rH} are cross-coefficients relating the flow of one species to the force of the other. Following Onsager, they are considered to be equal.

To evaluate the affinity:

$$A = [(J_H)_{\Delta\tilde{\mu}_H=0}][\partial J_r/\partial\Delta\tilde{\mu}_H]_A^{-1} \qquad (2)$$

To calculate the affinity by this method, two assumptions are usually made; the cross-coefficients are assumed to be equal, and the affinity is supposed to be independent of the electrochemical potential difference during the relatively short period of experimental perturbation. Recently, Rottenberg has shown that the two cross-coefficients are equal in mitochondrial oxidative phosphorylation (22). The independence of the affinity from the transepithelial electrochemical potential is suggested by the linearity of J^*CO_2 and J_H in ΔpH (Fig. 1; [15]). Furthermore, since the affinity is some function of the concentration of high-energy intermediates, it seems reasonable to consider it to be independent of the pH difference across the membrane, especially since these metabolites are highly regulated functions. Using Eq. 2, the affinity will have units of kilocalories per mole of CO_2 produced from glucose oxidation. If we assume a P:O ratio of 3, we can convert this value to kilocalories per mole of ATP hydrolyzed. (The reason for choosing this conversion is to allow speculation on the quantitative aspects of this function.) The values of the affinity were quite variable. Ideally, one should have measured it in each bladder before adding aldosterone, then again after addition of the hormone or diluent. However, the constraints placed on the duration of experiments by bacterial growth in the chamber prevented the performance of this protocol. Thus these changes should be interpreted with caution. The results, expressed per mole of ATP, are presented in Table III D. It is seen that during the first 6 h of exposure to aldosterone, the affinity declined significantly, while 20 h later it increased. The five pairs of bladders showing no response in H^+ transport showed no significant change. The long-term incubation results are similar to those of Saito et al. (11) who showed that for sodium transport in frog skin, overnight incubation with aldosterone results in an increase in the affinity.

It is important to note that if aldosterone stimulated transport by increasing the driving force of metabolism, we would have observed an increase in the affinity at the time that the H^+ transport activity was increased. The results show that the affinity was decreased initially even though the rate of transport was significantly increased. The data can be interpreted to mean that the hormone stimulates H^+ transport by stimulating the transport apparatus itself by such processes as increasing the number of sites or facilitating the flow of protons through the active transport pathway. The increase in transport leads to increased utilization of high-energy intermediates and consequent decline in the affinity. On prolonged stimulation the affinity increases probably as a result of adaptation to increased work loads (though it may merely reflect the utilization of new substrates[2]). However, because of the imperfect protocol as well as the possible contribution of other unmeasured fuels to the support of H^+ transport, these conclusions must be considered only tentative at this time. Further, they have to be corroborated by more direct methods of measurement.

The value of the affinity given here can be interpreted quantitatively provided the reader is willing to relax his or her customary skepticism toward wanton speculation. Assume that the epithelium is an "equivalent" cell with only glucose being utilized in pathways that are coupled to ATP hydrolysis or synthesis. The affinity, or $-\Delta G$ of the whole system, will approximate the free energy of ATP hydrolysis, since it is likely that that is the major determinant of the overall ΔG in the transport-linked reactions. Recent elegant studies by Wilson et al. have shown that $-\Delta G$ for ATP in liver cells to be about 11 kcal/mol (23). Ross et al. (18) found a similar value in the isolated kidney perfused with glucose-containing media. Most of the values in Table III are in this range. This concordance is encouraging (unless it turns out to be coincidental).

In conclusion, aldosterone stimulates H^+ transport by a mechanism that acts primarily on the transport apparatus itself as opposed to stimulating the energy supply to the pump. Further, prolonged stimulation results in adaptive changes in the mechanism of cou-

[2] If a new metabolite was being utilized, $\partial J^*CO_2/\partial\Delta$pH will be an underestimate of the slope of total CO_2 production on ΔpH. Since this slope is in the denominator of Eq. 2, the affinity will be an overestimate of the total affinity.

pling of transport to glucose utilization. This latter is similar to the adaptive change that occurs in skeletal muscle undergoing prolonged exercise and, hence, may represent a general response of biological systems to prolonged continuous work.

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