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Metabolic Pathways Coupled to H^+ Transport in Turtle Urinary Bladder

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Summary. Active H^+ transport in the turtle urinary bladder is mediated by an ATPase. Although the source of ATP is usually mitochondrial oxidative phosphorylation, it is possible because of intracellular compartmentalization or cellular heterogeneity that one metabolic pathway exclusively provides ATP to the pump. To examine this we performed several types of experiments. In one, the coupling between the rate of transport and the rate of oxidation of ^{14}C-labeled substrates was studied. We found that there was coupling between H^+ transport and glucose, butyrate, oleate, and β-OH-butyrate oxidation. In another set of experiments we depleted turtle bladders of their endogenous substrates and tested the effect of a number of substrates on the rate of transport. We found that glucose, pyruvate, lactate, actetate, butyrate and β-OH butyrate all stimulated H^+ transport. In a third set of experiments we found no coupling between H^+ transport and lactate production. Finally, we found that reduction of H^+ transport by mucosal acidification resulted in an increase in epithelial cell ATP concentrations and a decrease in ADP levels.

These results suggest that the H^+ pump receives its ATP from carbohydrate and fatty acid oxidation. The changes in ATP and ADP levels provide an initial explanation for the coupling of H^+ transport to the rate of cellular oxidative metabolism.

Since active transport is capable of establishing electrochemical gradients across a membrane, it follows that an input of energy must fuel it. The source of this energy is cellular metabolism. The relation between ion transport and the rate of that metabolic reaction thus assumes a central importance in understanding the details of active transport. In epithelia, this is traditionally studied by measuring the rate of oxygen consumption or CO_2 production and ion transport, preferably in the same tissue under a variety of interesting physiological settings. For the case of active H^+ transport in the turtle urinary bladder, previous studies have shown that a change in H^+ transport was accompanied by a simultaneous change in CO_2 production in the same direction [4]. Recent studies by Dixon and Al-Awqati have shown that the H^+ pump in the turtle urinary bladder is a reversible ATPase [7]. While mitochondrial oxidative phosphorylation is the usual source of ATP, it is possible that other reactions because of the location of their enzymes in sites adjacent to the H^+ pump might contribute more ATP than would be anticipated from looking at a metabolic map of cellular ATP-producing reactions. Further, in the turtle bladder (as in some other urinary epithelia such as collecting tubules) a number of cell types are present [15]. It has been assumed that different transport processes might occur in different cell types. Each of these cell types might have different metabolic "machinery". In this paper we attempt an initital search for preferred metabolic pathways. Although a number of methods are available to investigate this problem, no one method is without its inherent disadvantages. Metabolic inhibitors, especially those purported to be specific, can be very useful in identifying a class of pathways that fuels the pump. One disadvantage is that inhibitors may not be as specific as claimed and that secondary effects due to reduction of cellular energy stores might dominate the picture. Using this method, Steinmetz et al. [16, 18] have shown that H^+ transport is dependent on oxidative metabolism but that under scrupulous anaerobic conditions there is still a substantial rate of transport. Simultaneous measurement of the rate of transport and the rate of one metabolic reaction offers the chance to identify specific reactions that energize the pump. In this paper, we present results on the coupling between H^+ transport and the oxidation of a number of important

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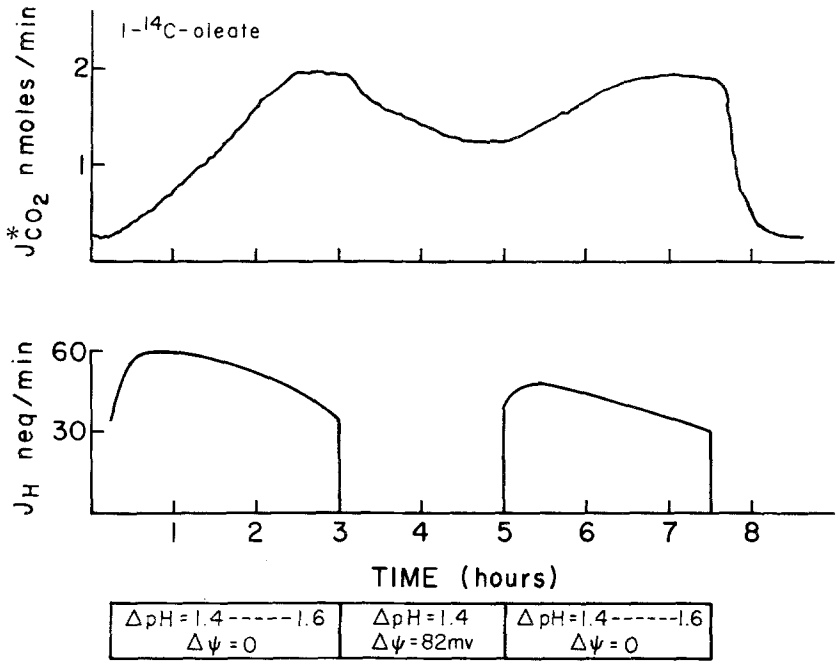

Fig. 1. The relation between H^+ transport (J_H) and $^{14}CO_2$ production from oleate in turtle bladder. The serosal pH was kept constant at 7.1. Oleate was added to the luminal medium

respiratory fuels and come to the qualitative conclusion that no single metabolic reaction is excluded from participation in the support of H^+ transport. Further, we present results of another approach where the effect on the rate of H^+ transport of addition of a number of exogenous fuels is tested. It is seen that a number of carbohydrates and fats can stimulate H^+ transport in starved bladders. These results demonstrate that the H^+ pump has catholic tastes when it comes to the source of ATP.

Materials and Methods

All experiments were performed on urinary bladders isolated from the fresh water turtle *Pseydemys scripta* elegans (Mogul Ed. Corp., Oshkosh, Wisc.). Bladders were mounted in Ussing chambers with the exposed areas of the membrane measuring 8–9 cm^2. After mounting, ouabain (1 mM) was added to the serosal side and amiloride (0.1 mM) was added to the mucosal side. The short-circuit current rapidly reversed in direction to reach a steady level in ~1 hr. In this state the short-circuit current has previously been found to be identical to the net rate of H^+ addition to the luminal medium as measured by the pH stat method [2, 4]. In all of the experiments reported here we used the short-circuit current as an index of the rate of H^+ transport.

a) Measurement of the Coupling between H^+ Transport and Metabolite Oxidation

This method has been described in detail previously [4]. In brief, bladders were mounted in Ussing chambers that had large areas of 9.2 cm^2 and small volumes, ~3–5 ml on each side. The membrane was bathed on both surfaces by a modified Ringer's solution containing in mmoles/liter: NaCl, 110; KCl, 3.5; $CaCl_2$, 1.0; $MgCl_2$, 0.5; Na_2HPO_4, 2.0; and titrated to pH 7.4 using HCl. All media were first filtered through milipore filters and contained 40 μg/ml of gentamicin, 0.1 mg/ml of penicillin and 0.2–0.5 mg/ml carbonic anhydrase. The medium was gassed with 1% CO_2 in air. The gas effluent from the chamber was passed through 2 drying chambers and then into an ionization chamber across whose poles 90 V were placed. The ionization chamber was connected to a Cary-Varian Vibrating Reed electrometer (Varian Instruments, Cupertino, Calif.) and a recorder. The ionization chamber was calibrated using Ba $^{14}CO_3$. The gas flow through the apparatus was measured using a flowmeter. After obtaining a background reading, l-^{14}C-butyrate or l-^{14}C-oleate was placed in the mucosal chamber while β-OH-^{14}C-butyrate or uniformly labeled ^{14}C-glucose was placed in the serosal chambers. For the oleate experiments, bovine serum albumin was initially defatted using the method of Chen [5] and mixed with ^{14}C-oleate and "cold" oleate in a molar ratio of albumin to fatty acid of 0.25. The albumin-oleate mixture was allowed to equilibrate for at least 24 hr. To prevent foaming in the albumin experiments, the air flow to the mucosal chamber was reduced. The final substrate concentrations were 2 mM for butyrate, 2 mM for β-OH-butyrate, 10 mM for glucose, and 0.24 mM for oleate.

After the addition of labeled substrate to the mucosal or serosal medium, a $^{14}CO_2$ signal appeared within a few minutes and reached a steady-state rate of production within 3–4 hr (Fig. 1). On reaching this steady rate the mucosal and serosal pH's were measured and the rate of H^+ and air flow was noted. The bladder was then voltage-clamped with a potential, mucosal side positive, that was calculated to bring the net transepithelial electrochemical gradient, i.e., $\Delta pH + \Delta\psi$, to 180 mV. This $\Delta\tilde{\mu}_H$ has been previously found to reduce the rate of transport to zero. On application of this adverse gradient, the rate of $^{14}CO_2$ production declined to reach a new steady state. In this period the $\Delta\mu_H$ once set did not change. Another 20–30 min of measurement was allowed, then the mucosal pH was measured and the bladder was returned to the short circuited state for another period of measurement. In the glucose and β-OH-butyrate experiments the rate of transport was altered by changing the luminal pH [4]. At the conclusion of this

last period, the exposed area of the bladder was cut and dried in a hot air oven. The mucosal medium, containing the isotope, was replaced in the chamber and $^{14}CO_2$ production from the medium was measured for an additional hour. Unlike our previous result [4], we did not find any bacterial growth. We ascribe this to the close attention paid to sterilizing the chambers and washing the bladder thrice with filtered antibiotic-containing media. The chambers were stored in 10% glutaraldehyde. The $^{14}CO_2$ flux was calculated as before [4], assuming that the complete oxidation of 1 mole of butyrate or β-OH-butyrate liberated 4 moles of CO_2 while that of oleate liberated 18 moles of CO_2 and glucose 6 moles of CO_2.

b) Effect of Various Substrates on the Rate of H^+ Transport in Depleted Bladders

The urinary bladders were mounted in Ussing chambers and bathed by a Ringer's solution that contained (in mmol/liter): NaCl, 85; KCl, 3.5; $CaCl_2$, 1.0; $MgCl_2$, 0.5; $NaHCO_3$, 25; Na_2HPO_4, 1.65; NaH_2PO_4, 0.3; and gassed with 5% CO_2 in air, final pH 7.4. After addition of 0.5 mM ouabain and 0.1 mM amiloride the short-circuit current was measured in the steady state. The bladder was then allowed to remain in the chamber for 12–16 hr in media that were HCO_3-free and gassed with air. After this period of depletion the media were replaced by the HCO_3-CO_2 containing solution and the short-circuit current was allowed to reach a new steady state. Substrates were then added to the serosal or mucosal media and the short-circuit current was recorded for an hour. At the end of the experiment 5 mM glucose was added to the serosal medium and the peak short-circuit current was recorded.

c) Coupling of H^+ Transport to Lactate Production

Turtle bladders were mounted in Ussing chambers and bathed in a Ringer's solution containing (in mmol/liter): NaCl, 110; KCl, 3.5; $CaCl_2$, 1.0; $MgCl_2$, 0.5; Na_2HPO_4, 2.0; glucose, 5.0; penicillin, 100 mg and gentamicin 40 mg. The pH was ~6.0 when the media were gassed with 5% CO_2 in air. On addition of ouabain (0.5 mM) and amiloride (0.1 mM) and attainment of the steady state, the solutions were changed three times. In 3 of the 7 experiments the luminal pH was titrated to a level sufficiently low to abolish the H^+ current. Thirty minutes later the solutions were changed twice and a 1-hr collection period was started at the end of which the media were removed and frozen. The bladder was washed three times again, and the lumenal pH in 4 of the 7 experiments was titrated to levels that would abolish the H^+ current. In the 3 experiments where the lumenal pH was low it was titrated back up to 7.4. Thirty minutes later the media were changed twice and another 1-hr long collection period was started. The media were collected and frozen at the end of that period.

The frozen media were lyophilized and redissolved in 1 ml of distilled water and lactate was determined enzymatically [9].

d) Measurement of Adenine Nucleotides

Paired hemibladders were glued onto Teflon rings with Eastman 910 glue and mounted in Ussing chambers. The bathing solutions contained (in mmol/liter): NaCl, 105; KCl, 3.5; $CaCl_2$, 1.0; $MgCl_2$, 0.5; $NaHCO_3$, 5; and were gassed with 1% CO_2 in air. After addition of ouabain (0.5 mM) and amiloride (0.1 mM) and attainment of the steady state, the mucosal pH of one member of each pair was acidified to pH 4.6, a maneuver that reduces the rate of H^+ transport. One hour later the bladders were removed and immediately dipped in liquid nitrogen. The mucosal surface was scraped using a precooled razor and the scrapings were placed over 3 ml of frozen 2% perchloric acid. This was slowly thawed with frequent mixing in a vortex. The sample was centrifuged at 15,000 rpm for 10 min in a refrigerated centrifuge. The pellet was used for protein measurement by a modified Lowry method [10]. The supernatant was alkalinized with a solution containing 7.5 N KOH and 100 mM Na_2HPO_4 and then placed in an ice bath for 5 min. This was then centrifuged at 400 rpm for 5 min. The supernatant was then used for adenine nucleotide measurements using the firefly luciferin-luciferase assay after the method of Kimmich [11]. In separate experiments bladders were incubated in media which contained ^{3}H-inulin. One hour later the bladders were vigorously blotted and the mucosal cells were scraped with a glass slide. The scrapings were weighed before and after drying in a hot air oven overnight at 90 °C. The dry scrapings were extracted in distilled water overnight. The intracellular volume of these cells was 2.84 ± 0.2 µl/mg dry wt ($n=8$). The protein content of dried epithelial cells was 0.67 mg/mg dry wt.

Table 1. Relation between H^+ transport (J_H) and $^{14}CO_2$ production ($J^x_{CO_2}$) from labeled substrates in turtle urinary bladder[a]

^{14}C-substrate	n	J_H (nmol/min)	$J^x_{CO_2}$	$\partial J_H / \partial J^x_{CO_2}$ (eq/mole)
1-^{14}C-butyrate	5	33.91	19.03	
		0	16.86	
		29.02	19.01	28.54 ± 6.86
1-^{14}C-oleate	6	35.92	4.22	
		0	2.37	
		30.27	3.49	29.47 ± 6.1
1-^{14}C-β-OH-butyrate	8	60.77	17.67	
		6.47	14.02	
		83.9	17.46	18.54 ± 3.6
U-^{14}C-glucose	6	68.6	9.15	
		0	4.73	
		69.0	9.20	15.42 ± 2.8

[a] For each substrate three periods were obtained, the middle one being at zero net transport rates.

Results

1. Coupling of H^+ Transport to Oxidation of Respiratory Fuels

Four substrates were used, uniformly labeled ^{14}C-glucose, 1-^{14}C-β-OH-butyrate, 1-^{14}C-butyrate and 1-^{14}C-oleate. The results of these experiments are given in Table 1 and Fig. 1. The effect of the adverse gradient on ^{14}C-glucose oxidation confirmed our previous findings. Using 1-^{14}C-β-OH-butyrate we find also that there is coupling between oxidation of this ketone body and H^+ transport. Both of these substrates were present in the serosal medium. We had previously reported that when ^{14}C-butyrate was present in the serosal medium a large amount of $^{14}CO_2$ was produced which was not affected by changes in the rate of H^+ transport. We dismissed the possibility that ^{14}C-butyrate was not gaining access to the epithe-

lial cell on the grounds that there was $^{14}CO_2$ produced from this substrate which was larger in amount than the $^{14}CO_2$ produced from glucose oxidation. We concluded that fatty acid oxidation was not coupled to H^+ transport [1]. We were wrong. In Table 1 we show that addition of ^{14}C-butyrate to the *mucosal* medium results in a large $^{14}CO_2$ production. When the membrane is voltage- or pH-clamped the rate of transport declines and so does the rate of butyrate-oxidation. Additional experiments, not shown here, confirmed our earlier finding that serosal addition of butyrate does not result in $^{14}CO_2$ production that is coupled to H^+ transport. Similar studies were performed with l-^{14}C-oleate placed this time in the mucosal medium (we learned our lesson). Care was taken to equilibrate the ^{14}C-oleate with defatted bovine serum albumin. Again we show that there is coupling between oleate oxidation and H^+ transport. For both oleate and butyrate the achievement of the steady state took longer than usual. Whereas with glucose it was usually a matter of ~2 hr, it was ~3–4 hr with oleate and butyrate. As always, after the end of the experiment the tissue was cut out and the isotope-containing fluid was returned to the chamber to measure its rate of $^{14}CO_2$ production. In no case was there any significant $^{14}CO_2$ production. This was due to the new procedure of sterilizing the chambers which we have recently implemented (*see* Methods).

To reduce the rate of H^+ transport we usually acidified the luminal medium and measured the effect on $^{14}CO_2$ production. When fatty acids are present in the luminal medium acidification will increase the dissociation of the acid from albumin with consequent increase in the aqueous concentration. Further, acidification will increase the fraction present as the protonated form which is more diffusible. Both of these changes will tend to increase entry of the acid into the cell and might affect $^{14}CO_2$ production. To guard against this the rate of H^+ transport in the oleate-^{14}C experiment was decreased by voltage clamping. In the butyrate-^{14}C experiments both voltage and pH clamping was performed with no discernible difference between the two types.

The quantitative relationship between ΔJ_H and ΔJ_{CO_2} is presented in Table 1 for all the substrates. These represent the average of the ratios obtained in individual bladders and as expected from the small number of experiments it often differed from the ratio of the averages.

2. *Effect of Addition of Substrates on the Rate of H^+ Transport*

Addition of exogenous substrates to freshly excised turtle bladders has no effect on the rate of H^+ transport. This is probably due to their high glycogen content [12]. However, incubation for ~15 hr results in a decline in the rate of transport which appears to be due, in part at least, to depletion of endogenous substrate. Addition of glucose or pyruvate to the serosal medium results in an increase in the rate of transport [3]. We extended these preliminary observations to a number of other substrates. Because of the likelihood that a lack of response might mean only a dying preparation rather than independence of the rate of transport from this substrate, we always added 5 mM glucose at the end of the experiments. Only bladders that responded to glucose by an increase in the rate of transport were included in this analysis. The rate of transport after depletion was quite variable and the increase induced by the substrates was also variable in magnitude. This well-known but ill-understood variability prevents us from drawing anything but qualitative conclusions.

Table 2. Effect of substrates on H^+ transport (J_H) in depleted turtle bladders[a]

Substrate (mM)	n	J_H (nmoles/min)		
		After depletion	Change induced by substrate	Change induced by glucose
Glucose, 5	7	5.2	+12.8±6.2	
Pyruvate, 5	6	17.4	+15.0±3.1	0 ± 1.2
Lactate, 5	4	13.9	+10.4±3.7	1 ± 1.5
Acetate, 5	5	13.2	+ 2.9±0.7	+10.6± 4.4
Butyrate, 2	6	11.4	+ 2.8±1.3	+28 ± 8.7
Oleate, 0.36	5	20.9	− 3.4±2.2	+25 ±12
β-OH butyrate, 5	6	12.8	+ 8.4±3.1	− 0.5± 0.5
Glutamate, 5	4	20.3	− 2.6±1.3	+13.8± 2.8
Alanine, 5	4	21.0	− 5.5±1.6	+12.8± 5.6

[a] With the exception of butyrate and oleate all the other substrates were added to the serosal medium.

Table 2 shows that glucose, pyruvate, lactate, acetate, butyrate and β-OH-butyrate increased H^+ transport while alanine, glutamate and oleate did not. It is interesting to note that mucosal addition of butyrate increased H^+ transport while serosal addition did not. The effect of oleate was highly variable; in about 40% of the bladders there was a definite and large stimulation. The rest showed either no stimulation or a decline even though addition of 5 mM glucose to the serosal medium resulted in an increase in the rate of transport.

3. *The Relation between Lactate Production and H^+ Transport*

We measured the total lactate production before and after acidifying the lumenal medium to pH 4.5. Two

problems were encountered, one was due to the effect of the thick serosal unstirred layer and the other was due to the effect of the presence of bicarbonate on lactate production. We noted that when the rate of transport was changed there was a large difference in the lactate production one hour after the change as compared to two hours. Hence after the bladders were equilibrated and a steady rate of H^+ transport has been achieved, the media were changed and lactate was measured in the subsequent hour. When these media were substituted by fresh media, an hour later, the media were changed again and lactate measured in the subsequent hour. In 4 of the experiments reported the luminal pH was 4.5 in the first period and 7.4 in the second while in the other 3 experiments the order was reversed. There was no difference in the results of the two groups; hence they were pooled. In all experiments ouabain was present in concentration of 0.5 mM, a dose sufficient to nullify sodium transport. We found, in initial experiments, that the presence of serosal HCO_3 affected the rate of lactate production.

The average rate of H^+ transport was 2.06 ± 0.79 μeq/hr. Lactate production was higher from the serosal side than from the mucosal side. Total lactate production in the presence of H^+ transport was 0.214 ± 0.08 μmol/hr while at zero H^+ transport it was 0.355 ± 0.16; $\Delta = 0.141 \pm 0.153$, $n = 7$. While there does not appear to be significant coupling between the rate of transport and lactate production, there was a possible negative relation between the change in H^+ transport and the change in lactate production. When the rate of transport was low, lactate production appeared to increase on reduction of the rate of transport. When transport was high there was hardly a change and in one instance a reduction in the rate of glycolysis on decreasing H^+ transport.

4. Relation of Intracellular Adenine Nucleotides to the Rate of Transport

Measurement of steady-state concentrations of ATP, ADP, and AMP showed that the concentration of these nucleotides is reasonably similar to other epithelia. However, ATP and ATP/ADP ratios are higher than kidney and liver. Table 3 shows that inhibition of H^+ transport by mucosal acidification results in an increase in ATP concentrations and a reduction in ADP levels. These results demonstrate coupling between H^+ transport and ATP hydrolysis.

Table 3. Effect of luminal acidification on H^+ transport and adenine nucleotide phosphates concentration in turtle urinary bladder[a]

	J_H (neq/min cm^2)	ATP (mM)	ADP (mM)	ATP/ADP
Luminal pH 7.4	5.8	3.74	1.46	4.13
Luminal pH 5.0	0.47	5.26	0.61	11.26
Δ	5.33	1.52	0.85	7.13
±SE	±1.22	±0.41	±0.24	±1.93

[a] All the nucleotides were measured as nmol/mg protein. The average cell volume of turtle bladder epithelial cells measured by equilibration with inulin was 4.5 μl/mg protein.

Discussion

The present results show that no single metabolic pathway supplies the ATP required for the function of the H^+ pump. The major ATP-generating reactions, glucose oxidation and fatty acid oxidation were shown to be coupled to H^+ transport by the two methods presented in this paper. These findings should not, however, be interpreted as showing that no pathway is preferentially used; they only show that no pathway is exclusively coupled. In order to examine this question further, quantitative analysis will have to be performed on the fraction of oxidative metabolism that is affected by changes in transport. This problem is very difficult to resolve in the turtle bladder for several reasons. The presence of very large endogenous substrate stores that are not depleted by prolonged incubation [12] makes the dependence of cellular metabolism on exogenous substrates of much less importance than in the kidney. To arrive at any quantitative estimate of the contribution of each pathway, it is necessary to measure the specific activity of a crucial metabolite in the epithelial cell. Since the amount of cells in each bladder is quite small, detection by existent methods in cells harvested from a single bladder has proved unsurmountable, at least to us. Further, even when obtained, the presence of cellular heterogeneity will always leave some ambiguity [15]. Finally to evaluate the fractional contribution of the pathway to be studied, simultaneous measurement of total CO_2 and $^{14}CO_2$ production is needed as well as adequate knowledge of the respiratory quotient. While the $^{14}CO_2$ method described here and elsewhere is reproducible and relatively simple the total CO_2 method of Maffly et al. [6] which we used previously is at best a capricious method and we have never had consistent performance from this method. More difficult is the fact that in order to measure the total CO_2 it is necessary to use CO_2-free air. This will drastically reduce the rate of H^+ transport, making the changes induced in the metabolic rate by changes in transport, already a small fraction under the best circumstances, quite low. Furthermore, in the presence of CO_2-free air the cell pH increases measurably [17], rendering any conclusion about preferred pathways suspect.

The lack of coupling between lactate production and H^+ transport is probably due to the fact that what was measured is total tissue lactate production rather than epithelial cell lactate production. It is not sufficiently realized that the epithelial cells of turtle and toad bladders form only a small fraction of the cellular elements of these epithelia. For instance, in the toad bladder MacKnight et al. [13] has estimated that the epithelial cells accounted for only 17% of the intracellular space. The situation in the turtle bladder, whose adventitial coat is much thicker, is likely to be worse. Further, under aerobic conditions lactate will be metabolized to CO_2, which will further reduce the amount of lactate secreted into the medium. Recent studies by Steinmetz have demonstrated that H^+ transport can continue, albeit at a low rate, in the complete absence of oxygen, suggesting that, at least under this condition, glycolysis also contributes to the support of the H^+ pump [18].

Previous studies on the relation of H^+ transport to metabolism have shown that deoxygenation results in a large decline in the rate of transport [16]. More recently Norby and Schwartz have suggested that the pentose pathway is the major donor of energy to the H^+ pump [14]. These latter results were obtained by comparing the $^{14}CO_2$ produced from 1-^{14}C-glucose to that produced from 6-^{14}C-glucose. The $^{14}CO_2$ was allowed to reach a steady state and the rate of transport was changed by a variety of maneuvers. The results of these experiments are not readily interpretable since the $^{14}CO_2$ was measured not as initital rates, but in the steady state. Indeed, it is not clear at the moment that one can measure the contribution of the pentose pathway by even initial rates of $^{14}CO_2$ production from glucose labeled in different carbons since the rate and extent of "scrambling" is unknown, i.e., because of isotope exchange what was initially 1-^{14}C-glucose may rapidly become 2-^{14}C-glucose or 6-^{14}C-glucose. Recent studies using ^{13}C-NMR have unequivocally shown that this "scrambling" indeed does occur and that its rate is quite fast [20]. This problem affects the interpretation of isotopic studies of oxidation of fatty acid and amino acids as well as glucose. In the steady state, $^{14}CO_2$ will be evolved from the dominant pathway that supports transport regardless of the species that carried the isotope initially.

A reduction in the rate of H^+ transport by luminal acidification resulted in large changes in the steady-state concentrations of ATP and ADP. Recently, we have shown that a number of maneuvers that change H^+ transport change not only ATP and ADP but also intracellular inorganic phosphate concentrations [8]. These results suggest a mechanism that will explain the changes in $^{14}CO_2$ production seen in Fig. 1. Recently Thayer et al. showed that increasing the external ΔG_{ATP} reduces the oxygen consumption of isolated mitochondria [19]. If the mitochondria of turtle bladder behave similarly, then decreases in ion transport will cause an increase in the ΔG_{ATP} [8] which in turn will reduce oxidative metabolism. In this way ion transport could act as the pacemaker of cellular metabolism [21].

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