Relationship between the Rate of H^+ Transport and Pathways of Glucose Metabolism by Turtle Urinary Bladder

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ABSTRACT The urinary bladder of the fresh-water turtle acidifies its contents by actively transporting H^+ ions across the luminal membrane. It is known that the H^+ transport system is dependent upon oxidative metabolism and the substrate glucose; however, the specific biochemical events resulting in H^+ translocation have not been identified.

This study examines the relationship between active H^+ transport and a specific oxidative pathway of glucose metabolism, the pentose phosphate shunt. To investigate this relationship the metabolic and transport rates were simultaneously measured under several well-defined conditions. When H^+ transport was inhibited by either the application of an opposing pH gradient or by acetazolamide, glucose metabolism by the pentose phosphate shunt declined. Conversely, stimulation of H^+ transport by either imposing a more favorable pH gradient or by CO_2 addition resulted in an increase in pentose phosphate shunt metabolism. Glycolytic activity, in contrast, was invariant with the maneuvers which altered the rate of H^+ transport. Additional experiments localized pentose phosphate shunt enzyme activity to the mucosal fraction of the bladder which is the cell layer responsible for acid secretion. The finding that the rate of glucose metabolism by the pentose phosphate shunt is related to the rate of H^+ transport suggests but does not prove that the pentose phosphate shunt may be an important metabolic pathway for H^+ transport by the turtle urinary bladder.

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INTRODUCTION

Several investigators have shown an association between ion transport and metabolic activity in urinary epithelia (1–8). These studies have been primarily concerned with the ratio of ion transported to total O_2 consumed or CO_2 produced. There have been few studies in urinary epithelia that simultaneously measure and compare the activity of a specific metabolic pathway and the rate of ion transport (9). In the present study we examined the relationship between glucose metabolism by the pentose phosphate shunt and H^+ secretion in the isolated turtle urinary bladder. In this epithelium, both the transport and metabolic rates can be carefully measured under well-defined conditions (10). Previous work has shown that (*a*) H^+ transport by turtle bladder is primarily dependent upon oxidative metabolism (11), (*b*) glucose is preferred over pyruvate as a metabolic substrate (11, 12), and (*c*) the rate of H^+ transport is linearly related to the rate of glucose metabolism (8). In addition, preliminary studies from our laboratory demonstrated that $[^{14}C]CO_2$ production from [1-^{14}C]glucose is greater than from [6-^{14}C]glucose by spontaneously acidifying turtle bladders (13). These observations suggested the possibility of coupling between pentose phosphate shunt metabolism and H^+ transport in isolated turtle urinary bladder.

To investigate this possibility we have measured the rate of glucose metabolism by the pentose phosphate shunt during manipulations in the rate of H^+ transport. When H^+ transport was stimulated, glucose metabolism by the pentose phosphate shunt increased. After inhibition of acidification, pentose phosphate shunt metabolism declined. Glycolytic activity, in contrast, was unaffected by changes in the rate of H^+ transport. From these data it was concluded that the rate of glucose metabolism by the pentose phosphate shunt was related to the rate of H^+ transport. Additional studies localized pentose phosphate shunt enzyme activity to

only the mucosal fraction of the bladder which is the cell layer responsible for acidification.

METHODS

Paired hemibladders were carefully removed from the turtle and mounted in identical Lucite (DuPont de Nemours & Co., Inc., Wilmington, Del.) chambers that provided a surface area of 8 cm^2. The tissues were initially bathed in a substrate-free Ringer's solution containing in millimoles per liter: Na, 115;K, 3.5;Ca, 1.0;Mg, 1.0;Cl, 119.5; and HPO_4, 1.5. The osmolality was 230 mosmol per kg H_2O. The pH was adjusted to 5.9 with 0.1 N HCl to minimize trapping of $[^{14}C]CO_2$ as $[^{14}C]HCO_3^-$. All bathing media contained 0.1 mg/ml of penicillin and streptomycin. The solutions were stirred and oxygenated with air that had been passed through 3 M KOH traps to remove all detectable CO_2. In one series of experiments the serosal solution was bubbled with 2.5% CO_2 in air (Matheson Gas Products, East Rutherford, N. J.).

Bladders that failed to maintain a potential difference greater than 20 mV during the 1st h were discarded. Thereafter the bladders were continuously short-circuited. H^+ transport was measured as the short-circuit current after sodium transport was inhibited with 0.1 mM ouabain. The technique has previously been validated as an accurate measure of H^+ transport rate in turtle bladder (8, 14, 15). After the acidification rate had stabilized for 2 h, the bathing media were changed to Ringer's solution containing 5 mM glucose. One hemibladder from each pair was exposed to 10 μCi of [1-^{14}C]glucose and the other to 10 μCi of [6-^{14}C]glucose (New England Nuclear, Boston, Mass.). $[^{14}C]CO_2$ production was measured by passing the gas effluent from the chambers through 5 ml Hyamine Hydroxide (New England Nuclear) that was later quantitatively transferred for liquid scintillation counting. A preliminary control experiment with a synthetic membrane demonstrated 96% recovery of $[^{14}C]CO_2$ from HCl titration of $[^{14}C]HCO_3^-$. Initial experiments demonstrated that $[^{14}C]CO_2$ production by bladders was linear over the time interval of 30 min to 3 h after exposure to [^{14}C]glucose. The protocol for subsequent studies consisted of a 30-min control period during which the rate of $[^{14}C]CO_2$ production and H^+ transport were continuously and simultaneously measured. After control observations the rate of H^+ transport was altered and the same measurements were obtained during a 30-min experimental period. At the end of each experiment the exposed tissues were removed from the chambers and dry weights were obtained. H^+ transport is expressed as micromoles per gram dry weight per hour. The metabolic rates are expressed as micromoles of glucose utilized per gram dry weight per hour. The bladder weights in these experiments averaged 10±1 mg.

To estimate the relative pathways of glucose metabolism we assumed that $[^{14}C]CO_2$ evolution from [6-^{14}C]glucose originates from the glycolytic pathway and that $[^{14}C]CO_2$ evolution from [1-^{14}C]glucose results from both glycolysis and pentose phosphate shunt metabolism. An estimate of $[^{14}C]CO_2$ produced by the latter pathway is obtained by subtracting $[^{14}C]CO_2$ of [6-^{14}C]glucose from that of [1-^{14}C]glucose. This simple and convenient approach has previously been used to make qualitative estimates of changes in glucose metabolism in toad bladder (9, 16–18). To validate the quantitative aspect of this simpler method we also reestimated the pathways of glucose metabolism according to a method of Katz and Wood (19). This technique requires independent measurements of $[^{14}C]CO_2$ production and glucose utilization. The utilization measurements were obtained by incubating an additional large segment from each hemibladder under similar conditions and determining the rate at which [^{14}C]glucose disappeared from the medium as indicated by the loss of radioactivity. Over the time course of these experiments, 10–15% of the added substrate was utilized. The assumptions underlying the method of Katz and Wood have been described in detail elsewhere (19).

Because the turtle bladder consists of a mesothelium, smooth muscle, connective tissue, and mucosal epithelial cells, it was considered important to demonstrate pentose phosphate shunt enzyme activity in the mucosal cell fraction of the tissue which is the fraction responsible for acidification. In these experiments, bladders were removed from the animal, washed in cold Ringer's solution, and placed in a large petri dish. The mucosal surface was lightly scraped with a glass microslide. Microscopic examination of Papanicolaou-stained scrapings showed predominantly epithelial cells. After three washes in cold Ringer's solution containing 10 μm $NADP^+$, the bladder scrapings were ultrasonicated and freeze thawed. The mixture was then centrifuged at 4°C and 7,000 rpm for 45 min. The supernate fraction was removed and assayed for glucose 6-phosphate dehydrogenase activity by a spectrophotometric method (20). The reaction measured the rate of $NADP^+$ reduction at pH 7.6 with 1 mM glucose 6-phosphate as substrate. In the absence of supernate or substrate there was no change in absorbance. The reaction velocity was a linear function of the amount of supernate fraction added. The extinction coefficient of NADP was assumed to be 6.22 A/μmol NADPH per ml. The protein concentration of supernate was measured according to Lowry et al. (21). Enzyme activity is expressed as IU per gram protein where 1 IU is equivalent to 1 μmol of $NADP^+$ reduced per min.

All results are given as the means±SEM. Statistical analysis was by paired t test and differences were considered significant at $P < 0.05$.

RESULTS

Time course of [^{14}C]glucose decarboxylation during spontaneous acidification. Before examining the relationship between H^+ transport (J^H)[1] and pathways of glucose metabolism it was considered important to demonstrate that $[^{14}C]CO_2$ production was constant in the absence of any alteration in J^H. This insures that differences in $[^{14}C]CO_2$ production during control and experimental periods reflect the influence of the experimental maneuver and are not simply the result of spontaneous changes in isotope equilibrium or metabolism with time. In these studies 12 bladders were treated with ouabain and incubated in substrate-free Ringer's solution. After the acidification rate was stable for 2 h, the bathing medium was changed to one containing 5 mM glucose and 10 μCi of labeled substrate. $[^{14}C]CO_2$ was collected in sequential 15-min intervals for 3 h after isotope addition. The results are presented in Fig. 1. Total counts of $[^{14}C]CO_2$ increased in a linear fashion with time. The equation for the regression line was $y = 172x - 1439$ ($r = 0.99$). In subsequent studies, control and experimental measurements were obtained during the time interval when $[^{14}C]CO_2$ production was

[1] *Abbreviations used in this paper*: G6P, glucose-6-phosphate; G6PD, glucose-6-phosphate dehydrogenase; J^H, H^+ transport.

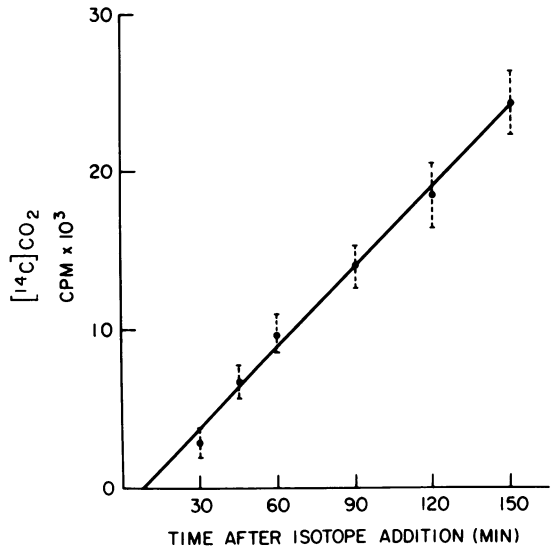

FIGURE 1 Time course of [^{14}C]glucose decarboxylation during spontaneous acidification. Results are means±SEM.

shown to be constant in these time-control experiments. These results are in close agreement with those previously published by Beauwens and Al-Awqati who also demonstrated constancy of CO_2 production by turtle bladder when the rate of spontaneous acidification was stable (8).

Changes in glucose metabolism during inhibition of J^H by acetazolamide. Table I shows the effects of 0.1 mM acetazolamide on J^H and glucose metabolism. H^+ secretion averaged 65 μmol/g dry wt per h during the control period and declined to a value not different from zero after acetazolamide. Complete inhibition of J^H by 0.1 mM acetazolamide is in agreement with previous reports (10, 22). [1-^{14}C]Glucose utilization during the control period was 2.72 μmol/g dry wt per h and decreased significantly after acetazolamide to 1.76 μmol/g dry wt per h. In contrast, [^{14}C]CO_2 production from [6-^{14}C]glucose did not significantly change after inhibition of J^H by acetazolamide. Subtracting [^{14}C]CO_2 production of [6-^{14}C]glucose from that of [1-^{14}C]glucose gives an estimate of glucose metabolism by nonglycolytic pathways, the principle one of which is the pentose phosphate shunt (16, 17). After acetazolamide this value declined from 1.96 to 1.04 μmol/g dry wt per h. These results show that inhibition of J^H by acetazolamide is associated with a decline in glucose metabolism by the pentose phosphate shunt.

Effects of varying mucosal pH on J^H and glucose metabolism. The rate of net acidification by isolated turtle bladder is a linear function of the mucosal solution to cell pH gradient (10). The back leak of H^+ is negligible over the mucosal pH range of 4.5–7.0, hence the net rate of acidification approximates the rate of active J^H (8, 10). In the next series of experiments we determined the effect of inhibition of J^H by mucosal acidification on pentose phosphate shunt metabolism. The results are presented in Table II. In these eight experiments, mucosal pH was lowered from 5.9 in the control period to 4.8 during experimental period. In response to the reduction in mucosal pH, J^H decreased from 70 to 7 μmol/g dry wt per h. [1-^{14}C]Glucose utilization declined from 3.11 to 1.92 μmol/g dry wt per h whereas [6-^{14}C]glucose metabolism was not significantly changed. Pentose phosphate shunt metabolism, estimated as the difference of [^{14}C]CO_2 from [1-^{14}C] minus [6-^{14}C]glucose, decreased significantly during inhibition of J^H. These results are similar to those obtained with acetazolamide and provide additional support for a relationship between pentose phosphate shunt metabolism and urinary acidification.

In a separate group of bladders, mucosal pH was increased from 5.9 in the control period to 7.5 except for a brief interval at the end of the experimental period when it was lowered to 4.9 to remove any [^{14}C]CO_2 trapped as [^{14}C]HCO_3^-. These results are shown in Table III. In response to the increase in mucosal pH, J^H increased from 63 to 105 μmol/g dry wt per h. [1-^{14}C]Glucose utilization increased from 2.91 to 4.24 μmol/g dry

TABLE I
Changes in Glucose Metabolism during Inhibition of J^H by Acetazolamide

	[1-^{14}C]Glucose	[6-^{14}C]Glucose	C-1 – C-6	J^H
	μmol/g dry wt/h			
Control	2.72	0.76	1.96	65
Acetazolamide	1.76	0.72	1.04	4
Δ±SE	0.96±0.20*	0.04±0.03	0.92±0.21*	61±12

Acetazolamide, 0.1 mM, was added to the serosal solution after the control period. (n = 6)
* $P < 0.05$.

TABLE II
Changes in Glucose Metabolism during Inhibition of J^H by Applied pH Gradient

	[1-^{14}C]Glucose	[6-^{14}C]Glucose	C-1 – C-6	J^H
	μmol/g dry wt/h			
Control	3.11	0.82	2.30	70
pH Gradient	1.92	0.69	1.22	7
Δ±SE	1.19±0.37*	0.13±0.12	1.08±0.23*	63±6*

Mucosal pH was lowered from 5.9 to 4.8 after the control period. (n = 8)
* $P < 0.05$.

TABLE III
Changes in Glucose Metabolism during Stimulation of J^H by Mucosal Alkalinization

	[1-^{14}C]Glucose	[6-^{14}C]Glucose	C-1 – C-6	J^H
		μmol/g dry wt/h		
Control	2.91	0.64	2.27	63
Alkalinization	4.24	0.72	3.52	105
Δ±SE	1.22±0.51*	0.08±0.07	1.25±0.42*	42±6*

Mucosal pH was increased from 5.9 to 7.5 after the control period. (n = 8)
* $P < 0.05$.

wt per h. [6-^{14}C]Glucose utilization was not statistically affected by the increase in mucosal pH. Glucose metabolism by the pentose phosphate shunt increased significantly during stimulation of J^H.

Effect of CO_2 enhancement of J^H on glucose metabolism. The relationship between J^H and glucose metabolism by the pentose phosphate shunt was also examined in a group of bladders before and after exposure to exogenous CO_2. Previous work has shown that the availability of CO_2 is rate limiting for J^H in substrate-replete bladders with no transepithelial pH gradient (10). In these seven experiments the serosal gas phase was changed from CO_2-free air to 2.5% CO_2 in air during the experimental period. The results are shown in Table IV. J^H increased from 0.77 to 1.23 μmol/g dry wt per h after addition of exogenous CO_2. The increase in transport rate was associated with a marked increment in [1-^{14}C]glucose utilization. The rate of [6-^{14}C]glucose utilization did not change significantly after addition of exogenous CO_2. The fraction of glucose metabolized by the pentose phosphate shunt increased significantly after enhancement of J^H by CO_2 addition.

Calculation of pentose phosphate shunt metabolism by a method of Katz and Wood (19). Use of absolute yields of [^{14}C]CO_2 to evaluate the pathway of glucose metabolism fails to consider recycling of hexose phosphates, metabolism of glucose by nontriose phosphate pathways, and incomplete oxidation of triose phosphates to CO_2. Katz and Wood have provided a method which considers these possibilities in estimating the relative pathways of glucose metabolism from [^{14}C]CO_2 evolution studies (19). Table V shows the results of the present studies analyzed according to this technique. The specific yield refers to that fraction of utilized glucose which appears as [^{14}C]CO_2. In hemibladders exposed to [1-^{14}C]glucose, the specific yield of [^{14}C]CO_2 increased during stimulation of J^H and decreased when acidification was inhibited. In contrast, the specific yield of [^{14}C]CO_2 from bladders exposed to [6-^{14}C]glucose was invariant with maneuvers which significantly altered J^H. The changes in the contribution of the pentose phosphate shunt to glucose metabolism were similar to the changes in the specific yield of [^{14}C]CO_2 from [1-^{14}C]glucose. During stimulation of J^H by 2.5% CO_2, pentose phosphate shunt metabolism increased from 29 to 59%. A similar response was seen after J^H was increased by raising mucosal pH. When J^H was inhibited by acetazolamide, calculated pentose phosphate shunt metabolism declined from 35 to 10%. The response of the pentose phosphate shunt metabolism to inhibition of J^H by an imposed pH gradient was virtually identical to that of acetazolamide.

TABLE IV
Changes in Glucose Metabolism during Stimulation of J^H by CO_2

	[1-^{14}C]Glucose	[6-^{14}C]Glucose	C-1 – C-6	J^H
		μmol/g dry wt/h		
Control	3.84	0.86	2.97	77
2.5% CO_2	6.71	1.55	5.16	123
Δ±SE	2.87±0.50*	0.69±0.37	2.17±0.66*	46±7*

The serosal gas phase was changed to 2.5% CO_2 after the control period. (n = 6)
* $P < 0.05$.

TABLE V
Changes in Glucose Metabolism by Pentose Phosphate Shunt (PS) during Alterations in J^H

	Specific yield [1-^{14}C]glucose	Specific yield [6-^{14}C]glucose	PS	J^H
			%	*μmol/g dry wt/h*
Control	0.66±0.05	0.24±0.03	29±6	77±10
Addition CO_2	0.86±0.03*	0.26±0.03	57±7*	123±13
Control	0.65±0.10	0.27±0.03	28±11	63±10
Mucosal pH 7.5	0.77±0.09*	0.28±0.02	42±14*	105±16
Control	0.17±0.09	0.24±0.04	35±11	65±4
Acetazolamide	0.43±0.05*	0.25±0.03	10±2*	4±2
Control	0.76±0.08	0.24±0.04	42±10	70±4
Mucosal pH 4.9	0.43±0.05*	0.25±0.04	10±2*	7±3*

Paired hemibladders were exposed to either [1-^{14}C]- or [6-^{14}C]glucose and [^{14}C]CO_2 production was determined before and after alterations in J^H. The percent contribution of PS to glucose metabolism was calculated according to a method of Katz and Wood where specific yield refers to that fraction of utilized glucose which appears as [^{14}C]CO_2 (19). (n = 8)
* $P < 0.05$.

Demonstration of pentose phosphate shunt dehydrogenase activity in mucosal homogenates. To exclude the possibility that changes in $[^{14}C]CO_2$ production resulted from changes in pentose phosphate shunt metabolism in nonacid secreting portions of the bladder, we measured the activity and distribution of glucose 6-phosphate dehydrogenase (G6PD) in mucosal and nonmucosal fractions of the bladder. G6PD activity of 16 single-bladder homogenates averaged 346±44 IU per g protein. Turtle erythrocyte G6PD activity was 1.0±0.2 IU per g Hb. Less than 2% of G6PD activity in mucosal homogenates could be accounted for by erythrocyte contamination. G6PD activity was not found in nonmucosal bladder fractions.

In a second group of eight bladders the reaction velocity was analyzed as a function of substrate (glucose-6-phosphate, G6P) concentration. A double reciprocal plot of the results is presented in Fig. 2. The calculated Michaelis constant (K_m G6P) was 0.0001 and maximal enzyme activity was 248 IU per g protein. This K_m is less than physiologic concentrations of the substrate G6P. It is also lower than the K_m G6P of 1 mM previously reported for rat gastric mucosa (23). In this gastric acid secreting tissue the pentose shunt metabolism accounts for a large fraction of O_2 consumption (23). In additional studies the range of pH optimum for turtle bladder G6PD was found to be 7.1–8.0.

DISCUSSION

Compared to our understanding of the energy requirements for sodium transport, the energetics of J^H in urinary epithelia have not been as thoroughly defined. In a recent study Beauwens and Al-Awqati were able to demonstrate tight coupling between J^H and glucose oxidation in isolated turtle bladder (8). Other investigations have shown that urinary acidification by turtle bladder is primarily dependent upon aerobic metabolism (11) and that glucose is the preferred metabolic substrate for support of H^+ secretion (11, 12). The substrate specificity for glucose and dependence upon aerobic metabolism suggest that glucose supports J^H by turtle bladder through some mechanism other than glycolytic metabolism or the Krebs cycle.

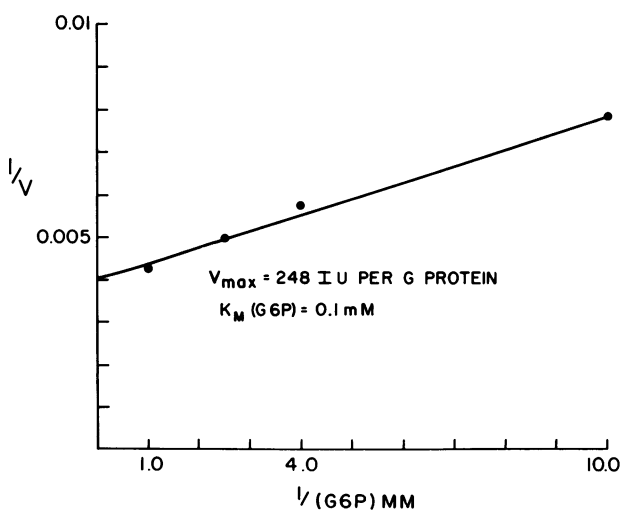

FIGURE 2 Double reciprocal plot of reaction velocity as a function of G6P concentration. The reaction velocity measures the rate of $NADP^+$ reduction. The equation for the regression line is $y = 0.000394\ (\pm 0.00011)\ x + 0.004\ (\pm 0.0003)$ ($r = 0.99$, $n = 8$). The K_m, 0.1±0.02 mM, was estimated from the slope and intercept of the regression line with the ordinate.

The major alternative pathway for glucose oxidation is the pentose phosphate shunt. Therefore, we sought to determine if this pathway was important in urinary acidification by isolated turtle bladder. In the present study it is demonstrated that the rate of pentose phosphate shunt metabolism by isolated turtle bladder is related to the rate of J^H. Inhibition of urinary acidification by two different maneuvers resulted in identical decreases in glucose metabolism by the pentose phosphate shunt. Conversely, when H^+ secretion was stimulated either by adding exogenous CO_2 or by imposing a more favorable pH gradient pentose phosphate shunt metabolism increased. Glycolytic activity, in contrast, was not statistically changed by the maneuvers which altered the rate of acidification and pentose phosphate shunt metabolism. Because of the magnitude of change in $[^{14}C]CO_2$ production from [1-^{14}C]glucose as opposed to that from [6-^{14}C]glucose with changes in the acidification rate, we considered it unlikely that these results represented factors other than changes in pentose phosphate shunt metabolism. To confirm this impression, we also used the more quantitative method of Katz and Wood to estimate the pathways of glucose metabolism (19). These results (Table V) support the conclusion that the rate of pentose phosphate shunt metabolism varies with the rate of J^H. Furthermore, the possibility that changes in $[^{14}C]CO_2$ production resulted from changes in pentose phosphate shunt metabolism in nonacid secretory portions of the bladder is excluded by the localization of pentose phosphate shunt enzyme activity to mucosal epithelial cells.

Thus it would appear that the previously described linear coupling between glucose metabolism and J^H (8) probably represents a coupling between J^H and glucose oxidation by the pentose phosphate shunt. Although it is attractive to conclude that J^H is dependent upon pentose shunt metabolism, it is also possible that these two apparently coupled activities of the bladder epithelial cells are not functionally dependent upon one another, but only linked by a common variable altered by the maneuvers used to change H^+ transport. The most likely factor common to both functions that could indirectly link these rates would be intracellular pH. For example, the alteration in H^+ transport may

change intracellular pH which then changes the rate of pentose shunt metabolism. This particular possibility, however, is improbable. Intracellular pH does not appreciably change after lowering mucosal pH or after addition of CO_2 or acetazolamide (24). Also, the range of pH optimum for G6PD is well within the range of change in intracellular pH of turtle bladder after these maneuvers.

A dependence of J^H upon pentose phosphate shunt metabolism may not be unique to the turtle bladder. Sernka and Harris have presented evidence suggesting that acid secretion by rat gastric mucosa is dependent upon glucose metabolism by this pathway (23). Dies and Lotspeich have made a similar proposal for acidification by rat kidney (25). The present study, however, represents the first report of simultaneous measurements of the rate of metabolism by this specific pathway and the rate of J^H. In each group of experiments the rate of pentose phosphate shunt metabolism varied with the rate of J^H. This finding provides additional support for the idea that pentose phosphate shunt metabolism is in some way coupled to the cellular process of acid secretion.

The nature of the coupling between the pentose shunt pathway for glucose metabolism and active J^H is not readily apparent. The oxidative and decarboxylation reactions catalyzed by G6PD are probably not directly related to the transport step because these enzymes are localized in the cytosol of this tissue[2] and in other tissues (26). Perhaps the pentose shunt produces a specific substrate that is required for J^H. The major end product of this pathway is NADPH. The dependence of J^H upon NADPH could be indirect, that is, NADPH is utilized for the synthesis of some other class of substrate not present in the in vitro system. One possibility is that fatty acids are important metabolic fuels for J^H by the turtle bladder. The function of the pentose shunt would be to produce reducing equivalents, NADPH, which are required for the biosynthesis of fatty acids. Although this possibility was not directly examined in the present study, we view it as unlikely because exogenous fatty acids fail to stimulate J^H in substrate-depleted bladders and the metabolism of fatty acids is not coupled to the rate of J^H (27). Another explanation is that the end product of the pentose phosphate shunt, NADPH, provides the source of protons for translocation across the luminal membrane. This proposal is similar to the one offered by Dies and Lotspeich (25) and represents a modification of the Conway redox pump theory (28) or the Mitchell chemiosmotic pump theory (29). According to this schema NADPH is oxidized by a luminal bound cytochrome or its equivalent resulting in H^+ secretion into the lumen and OH^- generation in the cell.

This latter proposal requires that 1 mol of NADPH be consumed for each mole of H^+ transported. The complete oxidation of each mole of glucose by the pentose shunt results in the formation of 12 mol of NADPH. Thus, the rate of J^H should be no more than 12 times the rate of glucose metabolized by the pentose shunt. In this study the ratio of change in the rate of J^H to change in pentose shunt metabolism of glucose varied between 14.7 and 66.5, with a mean value of 44.2 ± 10.1. It has been previously reported that metabolism of exogenous glucose accounts for one third or less of total CO_2 produced by the isolated turtle bladder (8). If the bulk of endogenous substrate utilized is glucose derived from tissue glycogen and this glucose is metabolized proportionately at the same rate by the pentose shunt as exogenous glucose, this ratio may be as low as 14. This estimated value approximates the 12:1 ratio required by our last proposal. Therefore, the rate of NADPH production and utilization as a source of protons for transport could account for the bulk of the acidification rate.

[2] Unpublished observations.

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