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Research Article

Addition of HCO3- to the serosal side (S) of the isolated turtle bladder results in a HCO3- flow from S to the mucosal side (M) which markedly reduces the net rate of acid secretion. To characterize the driving forces for this downhill HCO3- flow, the effects of metabolic inhibitors and substrates were examined. In short-circuited bladders with the M pH lowered to the point of zero net H+ secretion, the rate of HCO3- entry into M in response to a 20-mM HCO3- gradient was measured by pH stat titration. Deoxygenation reduced the HCO3- flux from 1.24 plus or minus 0.1 mum/h/8 cm2 (SEM) to 0.50 plus or minus 0.1 muM/h with glucose (2 times 10-3 M) AND FROM 1.32 PLUS OR MINUS TO 0.47 PLUS OR MINUS 0.1 MUM/h without glucose. A similar reduction (61 per cent) was observed in the presence of 1 per cent C92. Dinitrophenol (10-4 M), cyanide (10-3 M), and deoxyglucose (10-2 M) inhibited the HCO3- flux by 39 per cent, 37 per cent, and 38 per cent, respectively. The combination of any of these inhibitors with N2 caused the same inhibition as N2 alone. In […]

Energy Dependence of Urinary Bicarbonate Secretion in Turtle Bladder

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ABSTRACT Addition of HCO_3^- to the serosal side (S) of the isolated turtle bladder results in a HCO_3^- flow from S to the mucosal side (M) which markedly reduces the net rate of acid secretion. To characterize the driving forces for this downhill HCO_3^- flow, the effects of metabolic inhibitors and substrates were examined. In short-circuited bladders with the M pH lowered to the point of zero net H^+ secretion, the rate of HCO_3^- entry into M in response to a 20-mM HCO_3^- gradient was measured by pH stat titration. Deoxygenation reduced the HCO_3^- flux from 1.24±0.1 μM/h/8 cm^2 (SEM) to 0.50±0.1 μM/h with glucose (2×10^{-3} M) and from 1.32±0.1 to 0.47±0.1 μM/h without glucose. A similar reduction (61%) was observed in the presence of 1% CO_2. Dinitrophenol (10^{-4} M), cyanide (10^{-3} M), and deoxyglucose (10^{-2} M) inhibited the HCO_3^- flux by 39%, 37%, and 38%, respectively. The combination of any of these inhibitors with N_2 caused the same inhibition as N_2 alone. In bladders depleted of substrate, pyruvate (5×10^{-3} M) increased the HCO_3^- flux from 0.36 ±0.05 to 0.58±0.01 μM/h ($P < 0.005$); the increment was abolished by deoxygenation.

The results indicate that the bulk of the downhill HCO_3^- flow in this system is dependent on metabolic energy derived primarily from oxidative sources, and that this energy-dependent flow approximates the electroneutral component of HCO_3^- secretion that is coupled to Cl^- absorption.

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INTRODUCTION

Previous studies (1, 2) have shown that in the absence of exogenous CO_2 and HCO_3^- the isolated bladder of the water turtle acidifies the solution, bathing its urinary surface by the secretion of hydrogen ion. The addition of HCO_3^- to the (S)[1] side of the bladder reduces the net rate of acid secretion by a mechanism of HCO_3^- transport from the S to the urinary compartment (3). This downhill transport of HCO_3^- has been characterized by Leslie, Schwartz, and Steinmetz (4) under conditions of zero net H^+ secretion. In these studies, net H^+ secretion was reduced to zero by lowering the luminal pH. Bicarbonate entry into the urinary compartment was then measured by pH stat titration after addition of HCO_3^- to the S solution. It was shown that the bulk of the HCO_3^- flow into the urinary compartment occurred by an electroneutral mechanism of exchange for absorbed Cl^-, and that only a small fraction, less than 20%, diffused across the bladder in the ionic form. Since the experimental design depended on pH stat titration of HCO_3^-, transport under conditions of zero net H^+ secretion and, therefore, necessarily included a concentration gradient for HCO_3^-, it was not clear whether this concentration driving force was responsible for both components of the HCO_3^- flow or whether the electroneutral flow was driven by an additional force derived from a metabolic reaction.

The present study explores the possibility that the exchange flows of HCO_3^- and Cl^- are coupled to metabolic energy. The results indicate that inhibition of oxidative metabolism causes a marked reduction in HCO_3^- secretion[2] and Cl^- absorption. HCO_3^- secretion was inhibited by dinitrophenol, cyanide, and deoxy-D-

[1] *Abbreviations used in this paper:* DNP, 2,4-dinitrophenol; M, mucosal; S, serosal; PD, spontaneous electrical potential difference.

[2] The term HCO_3^- secretion is used interchangeably with HCO_3^- flux from serosa to mucosa. Since the diffusional component is small, HCO_3^- secretion refers mainly to the electroneutral flow which is coupled to Cl^- absorption (4).

TABLE I

Effect of Deoxygenation on S to M HCO_3^- Flux

Substrate	HCO_3^- flux 1 Control	HCO_3^- flux 2 N_2	HCO_3^- flux 3 Recontrol	Δ±SE (2-1)	% inhibition by N_2
	μmol/h			*μmol/h*	
Glucose	1.24	0.50	1.24	−0.74±0.10*	59±4
None	1.32	0.47	1.13	−0.85±0.15*	62±6

Mean values for eight experiments in each group. Glucose was present (2×10^{-3} M) in the M and S solutions. In four bladders exposed to 1% CO_2, deoxygenation at constant P_{CO_2} caused a similar reduction of HCO_3^- flux, 61±5% (see text).

* $P < 0.001$.

glucose, and was increased by pyruvate in bladders that were depleted of substrates.

METHODS

Urinary bladders of adult fresh-water turtles (*Pseudemys scripta*) were removed with minimal handling, washed with Ringer's solution, and mounted in Lucite chambers as previously described (1). All experiments were performed with the bladders in the short-circuited state by means of a voltage clamp except for brief intervals during which the spontaneous electrical potential difference (PD) was measured (1). At the outset the two sides of the bladder were bathed with HCO_3^--free Ringer's solution and changed twice at 30-min intervals. Bladders were considered viable if the PD remained greater than 10 mV during this wash period.

Two Ringer's solutions with identical chloride concentrations were used. The first, free of HCO_3^-, contained in millimoles per liter; NaCl, 94.4; Na_2SO_4, 13.3; KCl, 3.5; $CaCl_2$, 1.8; Na_2HPO_4, 0.3. The second contained: NaCl, 94.4; $NaHCO_3$, 20.0; KCl, 3.5; $CaCl_2$, 1.8; Na_2HPO_4, 0.3. Their osmolalities ranged from 215 to 230 mosmol per Kg H_2O. The solutions in the mucosal (M) and S compartments were stirred and oxygenated by bubbling with air from which CO_2 had been removed by passage through three 3 M KOH traps (4). Unidirectional $^{36}Cl^-$ fluxes were performed as previously described (5).

To measure the rate of alkali entrance into M after the addition of HCO_3^- to S, the pH stat method was employed (1). Since measurement of the S to M HCO_3^- flux requires the absence of net H^+ secretion, the M solution for each bladder was acidified to the pH at which net acid secretion was zero (pH range 4.5–5.2), while the pH of S was adjusted with NaOH to 8.5 in the absence of exogenous CO_2 and HCO_3 (4). The S solution was then exchanged for 20 mM HCO_3^- Ringer's solutions, and the HCO_3^- entry into M was determined by pH stat titration (4). A transfer rate of HCO_3^- from S to M greater than 0.5 μmol/h was required for bladders to be included in the studies. About 10% of the bladders mounted were discarded for failure to meet this criterion.

The effect of deoxygenation on the S to M HCO_3^- flux was examined by replacement of CO_2-free air by 100% N_2 for most experiments; for four experiments, air with 1% CO_2 was replaced by 99% N_2 with 1% CO_2 (Medical Technical Gases Inc., Medford, Mass.). Deoxygenation was limited to 30 min because longer periods often cause an irreversible decrease in the electrical resistance indicative of tissue leakiness. This leakiness was evidenced by large increments in the S to M HCO_3^- flow. Such a sequence of events was accepted as evidence of tissue damage and a basis for discarding the experiment. Since this response was occasionally observed after only 30 min of deoxygenation in the absence of glucose, the bathing media contained 2-mM glucose in all experiments except when otherwise indicated.

TABLE II

Effect of Deoxygenation on M to S $^{36}Cl^-$ Flux and S to M HCO_3^- Flux in the Same Bladders

Turtle	HCO_3^- flux Control	HCO_3^- flux N_2	HCO_3^- flux Δ	$^{36}Cl^-$ flux Control	$^{36}Cl^-$ flux N_2	$^{36}Cl^-$ flux Δ
	μmol/h		*μmol/h*	*μmol/h*		*μmol/h*
1	1.24	0.38	−0.86	2.46	1.95	−0.51
2	0.78	0.38	−0.40	2.76	1.91	−0.85
3	0.88	0.44	−0.44	1.95	1.71	−0.24
4	1.16	0.37	−0.79	1.33	0.90	−0.43
Mean			−0.62			−0.50
±SE			0.11			0.12

Δ, difference between control and N_2 periods.

The production of lactate by the bladders was not measured in the present study in which the deoxygenation periods were short. To provide an estimate of changes in lactate production in our system, we include data on the rate of lactate production before and after deoxygenation in a series of different experiments (Schwartz and Steinmetz, unpublished observations) in which the gas phase was changed in identical manner and lactate accumulation was measured in the bathing solutions over 1-h periods. The experimental conditions were the same except for the absence of the imposed HCO_3^- gradient. Lactate production in nine bladders (dry weight of 13.9±0.6 mg) was 0.09±0.01 μmol/h in air and 0.17±0.04 μmol/h in N_2. In nine other bladders (dry weight of 14.2±1.2 mg) lactate production was 0.16±0.04 μmol/h in air with 1% CO_2 and 0.26±0.07 μmol/h in N_2 with 1% CO_2.

For the experiments in which bladders were depleted of substrate, hemibladder sacs were tied (M surface inward) to a piece of plastic tubing. The bladder was filled with Ringer's solution and suspended in a beaker of aerated Ringer's solution free of substrate and containing 5 mg/100 ml of streptomycin to depress bacterial contamination. After a period of depletion of 20–24 h the bladders were mounted in chambers as indicated above. The sac preparation was elected for the long depletion experiments to minimize edge damage.

All experiments were carried out at room temperature and as in previous studies (4, 5), all rates are expressed as micromoles per hour per 8 cm^2 of membrane area ± the SE of the mean. This membrane area represented a mean dry weight of 14.7±0.6 mg per 8 cm^2 in a previous study carried out with the same technique (4).

RESULTS

Effect of deoxygenation on S to M HCO_3^- flux. Table I shows the effect of deoxygenation on the rate of HCO_3^- entry into M in the presence of 20-mM HCO_3^- Ringer's solution in S in short-circuited bladders. In eight experiments carried out with 2 mM glucose in the bathing media, the initial rate averaged 1.24 μmol/h. Removal of O_2 from the system by replacement of the air with 100% N_2 for 25–30 min resulted in an inhibition of the rates in all bladders, ranging from 45 to 80% and averaging 59%. This inhibition was rapidly reversible if air was reinstituted. Deoxygenation had a similar effect in the absence of glucose, the extent of inhibition being the same.

Since our system was exposed to a gas phase free of exogenous CO_2, deoxygenation caused inhibition not only of the energy-supplying reactions of aerobic metabolism, but also of metabolic CO_2 production. To avoid changes in the CO_2 tension, the effects of deoxygenation were also examined in four bladders exposed to 1% CO_2 in the S solution. In these experiments deoxygenation at a constant CO_2 tension of 1% reduced the HCO_3^- flux from 0.93±0.09 μmol/h to 0.36±0.08 μmol/h. After reoxygenation with 1% CO_2 in air the rate returned to 0.82±0.03 μmol/h. A final rate of HCO_3^- flux with CO_2-free air as the gas phase was 0.74±0.06 μmol/h. The inhibition of HCO_3^- transport at constant CO_2 tension was 61±5%, comparable to that observed in the absence of exogenous CO_2. Although the CO_2 tension of the epithelial cells may influence the transepithelial transport of HCO_3^- under certain conditions, these results indicate that the effect of deoxygenation is not mediated by a reduction in the CO_2 tension.[3]

TABLE III

Effect of Pyruvate on S to M HCO_3^- Flux in Depleted Bladders

	HCO_3^- flux			
	Depleted	Pyruvate		
Turtle	Air	Air	N_2	Air
	μmol/h		μmol/h	
1	0.32	0.57	0.26	0.57
2	0.25	0.55	0.27	0.52
3	0.18	0.57	0.47	0.65
4	0.53	0.63	0.49	0.75
5	0.47	0.63	0.47	0.67
6	0.45	0.54	0.47	0.62
Mean	0.36	0.58*	0.40	0.63
±SE	0.05	0.01	0.04	0.03

Pyruvate (5×10^{-3} M) was added to the S and M solutions.
* $P < 0.005$, difference from the depletion measurements.

Effect of deoxygenation on S to M HCO_3^- and M to S Cl^- fluxes in the same bladders. To determine whether deoxygenation reduces the rate of Cl^- absorption along with the rate of HCO_3^- secretion, a number of simultaneous measurements were made of the unidirectional Cl^- flux before and after deoxygenation. As shown in Table II, both fluxes were reduced by about equal decrements. To allow time to obtain steady-state Cl^- fluxes after deoxygenation, the flux measurements of Table II were taken during the 2nd half hour of deoxygenation in bladders in which the pH stat rate of HCO_3^- secretion remained constant during this period. Since net Cl^- fluxes could not be measured in these individual bladders, only the unidirectional Cl^- fluxes are given. The observed inhibition of the M to S Cl^- flux by deoxygenation is consistent with the O_2 dependence of Cl^- absorption in bladder sacs reported by Brodsky and Schilb (7). These results suggest that both HCO_3^- secretion and Cl^- absorption depend on aerobic energy.

[3] In the absence of exogenous CO_2, the cellular CO_2 tension maintained by metabolic CO_2 production has been estimated as less than 2-mm Hg (2, 6), a value well below that provided by exposure to 1% exogenous CO_2. It would be expected that the cellular [HCO_3^-] would be affected by the cellular CO_2 tension as well as by the S [HCO_3^-]. Since changes in the CO_2 tension from 0 to 1% had little effect on the HCO_3^- flux, it is likely that the S [HCO_3^-] of 20 mM was the principal determinant of the cellular [HCO_3^-] under our experimental conditions.

TABLE IV

Effect of DNP on S to M HCO_3^- Flux

HCO_3^- flux						
Control	N_2	Recontrol	DNP	DNP + N_2	Δ±SE	*P*
		μmol/h			*μmol/h*	
1.33	0.49				−0.84±0.18	<0.01
		1.35	0.87		−0.47±0.08	<0.01
			0.87	0.55	−0.32±0.11	<0.05

Mean values for six experiments. DNP (1×10^{-4} M) was added to S.

Dependence of HCO_3^- secretion on metabolic substrate. To explore further the dependence of HCO_3^- secretion on aerobic energy bladders were depleted of endogenous substrate for a period of 20 to 24 h. As shown in Table III, depleted bladders had low rates of HCO_3^- secretion. Addition of pyruvate increased the rate from an average of 0.36 μmol/h—0.58 μmol/h after 2 h. Subsequent deoxygenation abolished this increase. The increase was restored by reoxygenation. Addition of glucose only inconsistently increased HCO_3^- secretion.

Effects of metabolic inhibitors on HCO_3^- secretion. To define further the participation of different oxidative pathways in the support of HCO_3^- secretion, the inhibitory effects of 2,4-dinitrophenol (DNP) and cyanide were examined in separate groups of experiments. In each bladder, the effect of deoxygenation was tested before the addition of the inhibitor, so as to permit comparisons of the extent of inhibition.

Table IV shows the average inhibition observed after S addition of 10^{-4} M DNP. The rate of HCO_3^- secretion was reduced from 1.35 μmol/h to 0.87 μmol/h when the DNP effect had become maximal and steady, after 45 min of exposure. In these experiments, the inhibition by DNP was 39% compared to 62% by deoxygenation. The effect of DNP plus deoxygenation was the same as the effect of deoxygenation alone.

Inhibition of secretion by 10^{-3} M NaCN in the S solution was 37% in a group of eight bladders; the extent of inhibition was the same as with DNP.

Although deoxygenation occasionally decreased HCO_3^- secretion by as much as 80%, the average inhibition was about 60% in several groups of experiments, and the possibility is raised that part of HCO_3^- secretion may be supported by energy from anaerobic glycolysis. To examine this possibility the effects of iodoacetic acid and 2-deoxy-D-glucose were explored. Iodoacetic acid caused a marked decrease in the electrical resistance and an increase rather than a decrease in the S to M HCO_3^- flux. This large HCO_3^- flow was no longer inhibitable by deoxygenation and was considered to represent simple diffusion across a leaky membrane as a result of the concentration gradient. Data obtained with 2-deoxy-D-glucose are presented in Table V. The bladders were bathed in glucose-free Ringer's solution before and during addition of deoxyglucose. The initial inhibition of HCO_3^- secretion by deoxygenation, 59%, was similar to that in other groups of experiments, and reversed by reinstitution of air. About 1 h after the addition of deoxyglucose, the secretion rate reached a new steady level of maximal inhibition. The average inhibition was 38%, and the inhibition by N_2 plus deoxyglucose was the same as the inhibition by N_2 alone.

To determine whether HCO_3^- secretion was constant during the time of these experimental maneuvers, the rate of HCO_3^- secretion was followed in two groups of five bladders, one with and one without glucose in the media, for a period of 3–4 h after the standard period of deoxygenation. No significant differences were ob-

TABLE V

Effect of 2-Deoxy-D-Glucose on S to M HCO_3^- Flux

HCO_3^- flux						
Control	N_2	Recontrol	DG	DG + N_2	Δ±SE	*P*
		μmol/h			*μmol/h*	
1.15	0.56				−0.59±0.07	<0.001
		1.18	0.75		−0.42±0.05	<0.001
			0.75	0.56	−0.19±0.04	<0.01

Mean values for six experiments. 2-Deoxy-D-glucose (1×10^{-2} M) was added to the S solution; in four of the experiments it was added also to the M solution.

served between the initial and final rates of HCO_3^- secretion in either of these groups. In two experiments in which prolonged observations were made in glucose-free media, the secretion rates began declining spontaneously after the 5th h.

Electrical activity during inhibition of metabolism. Since the bulk of the HCO_3^- flux is electroneutral (4), and since all flux measurements were made in the short-circuited state, the electrical behavior of the bladders is described only briefly to provide an estimate of changes in the overall electrical resistance. In the eight experiments of Table I that were carried out in the presence of glucose, deoxygenation reduced the PD from 42±5 mV to 24±5 mV. The electrical resistance was 218 Ω per 8 cm^2 during the control period and 223 Ω after deoxygenation; the increment of 5±19 Ω was not significant. In the eight experiments (Table I) carried out in the absence of glucose, deoxygenation reduced the PD from 46±7 to 21±4 mV; the control resistance was 289 Ω per 8 cm^2 and 278 Ω during deoxygenation, the decrement of 11±32 Ω not being significant. Addition of dinitrophenol, cyanide, or deoxyglucose likewise caused no significant change in the electrical resistance.

DISCUSSION

Among the driving forces that could be responsible for the transport of HCO_3^- from the S to the urinary side of the bladder, three must be considered. The first is the electrochemical potential difference for HCO_3^- across the epithelium. Although the electrical driving force was eliminated in our experiments, the design of the studies included 20-mM concentration gradient for HCO_3^- which was required for the pH stat measurements of HCO_3^- transport under conditions of zero net H^+ secretion. The second driving force would be a metabolic reaction that is coupled directly to the transport system for HCO_3^-. The third is coupling to the flow of another ion that is actively transported. Since Leslie, Schwartz, and Steinmetz (4) have shown that HCO_3^- secretion and Cl^- absorption are closely coupled, the second and third possibility are difficult to distinguish and are considered together for the purposes of our analysis.

The inquiry, therefore, may be simplified: Is the concentration driving force for HCO_3^- responsible for HCO_3^- secretion as well as the coupled Cl^- absorption? Or, is the anion exchange dependent on an energy-supplying metabolic reaction? In examining the latter possibility no attempt will be made to determine whether there is metabolic coupling to Cl^- transport, to HCO_3^- transport, or to a common component of the exchange transport system.

The present study strongly suggests that the concentration force alone does not account for the electroneutral HCO_3^- flow. At constant concentration gradients for HCO_3^-, the HCO_3^- flow was markedly reduced by deoxygenation, cyanide, dinitrophenol, and deoxy-D-glucose; and the flow was increased by pyruvate in substrate-depleted bladders. These results suggest that the transport system is dependent on metabolic energy, but two alternative possibilities must be considered. First of all, the experimental maneuvers that inhibit HCO_3^- secretion could reduce the passive HCO_3^- permeability and, thereby, the flow of HCO_3^- for a given concentration gradient. This possibility is rather unlikely since the overall electrical resistance did not change during the inhibition experiments. Furthermore, increased HCO_3^- secretion after pyruvate addition in depleted bladders would be difficult to attribute to a simple permeability change. A second possibility would be that the metabolic inhibitors caused reductions in the availability of CO_2 which might critically interfere with HCO_3^- transport in our system that was gassed without exogenous CO_2. Because the Cl-HCO_3 exchange is inhibited by acetazolamide (4), it would have been conceivable that the observed inhibition of the transport system was CO_2 mediated. The observation, however, that HCO_3^- secretion was inhibited to the same extent in the presence of 1% exogenous CO_2 makes this explanation insufficient.

The downhill flow of HCO_3^- in our system, therefore, is coupled in one way or another to an energy-supplying metabolic reaction, and the bulk of this flow occurs by a mechanism of active transport by the Kedem criteria (8).

In several bladders, deoxygenation inhibited as much as 80% of the total HCO_3^- flow, a fraction approaching the electroneutral component which was 82% in the study by Leslie et al. (4). Although the average inhibition by deoxygenation was somewhat less, about 60% in our study, it should be stated that deoxygenation was not necessarily complete. Inhibitors of respiration or glycolysis, however, had no effect additional to that of deoxygenation alone. The results suggest that in all likelihood the entire electroneutral component of HCO_3^- secretion is dependent on metabolic energy and that much of this energy is derived from aerobic processes.

These inhibitor studies have dealt primarily with the HCO_3^- transport component of the exchanger. It must be assumed that the Cl^- component was similarly affected. Deoxygenation caused comparable decrements in HCO_3^- secretion and the unidirectional Cl^- flux in the absorptive direction. Studies by Brodsky and Schilb (8) also provide evidence for the dependence of Cl^- absorption on metabolic energy. These investigators reported that in the bladder sac preparation[4] Cl^- absorption was reduced in a number of experiments carried

[4] In this study (7) the S HCO_3^- concentration was 17.7 mM, comparable to the concentration employed in our study. Measurements of Cl^- absorption in the absence of O_2 and glucose were obtained under conditions that differed from ours, i.e., during incubation periods from 5 to 11 h.

out in the absence of O_2 and glucose. These lines of evidence with respect to both Cl^- and HCO_3^- transport indicate that one of these flows or a common component of the transport system must be coupled to an energy-supplying reaction.

It is of interest that the turtle bladder has in addition to this anion exchange pump two other energy-dependent transport systems, one for Na^+ and one for H^+. The three pump systems are not coupled directly to each other. Ouabain, which inhibits Na^+ transport, has no direct effect on either H^+ secretion or $Cl\text{-}HCO_3$ exchange transport (4). The present study suggests that the anion exchange pump depends largely on aerobic energy. H^+ secretion and Na^+ absorption, on the other hand, can be supported to an appreciable extent by anaerobic energy. Thus, Steinmetz, Omachi, and Frazier (9) observed that dinitrophenol failed to inhibit H^+ secretion in their system in which exogenous CO_2 was absent. Similarly H^+ secretion was reduced only moderately[5] by deoxygenation carried out at a constant CO_2 tension of 1% (10). Klahr and Bricker (11) and Nakagawa, Klahr, and Bricker (12) showed that Na^+ transport is not abolished during deoxygenation and exposure to KCN.

Although $Cl\text{-}HCO_3$ exchange has been described in a wide variety of tissues (for a review see reference 4), there is little information available on its occurrence in urinary epithelia other than turtle bladder. Studies by Coulson and Hernandez (13) suggest that HCO_3^- secretion may occur in the renal tubule of the alligator, another reptile. It is of interest that in the alligator acetazolamide causes an inhibition of HCO_3^- excretion that is associated with increased Cl^- excretion (13). The bladder of an amphibian, the Colombian toad, which has a capacity for urinary acidification somewhat lower than turtle bladder (14), has recently been reported (15) to secrete HCO_3^- under certain experimental conditions by an electroneutral mechanism.

In the distal nephron of the mammalian kidney the possibility of HCO_3^- secretion has not been explored. At the CO_2 tension prevailing in vivo such a process might be masked by a high rate of H^+ secretion. Several recent studies, however, indicate that Cl^- is actively reabsorbed in the distal tubule (16, 17) and collecting duct (18). Whether this process of Cl^- absorption is coupled to HCO_3^- secretion or an independent transport process remains to be determined.

[5] When H^+ secretion is stimulated by higher CO_2 tensions, its dependence on aerobic energy is increased (Schwartz, J. H. and P. R. Steinmetz, unpublished observations).

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