Regulation of Glutamine Metabolism In Vitro by Bicarbonate Ion and pH

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ABSTRACT The effect of variations of medium pH and bicarbonate concentration on glutamine oxidation was studied in slices and mitochondria from dog renal cortex. Decreasing pH and bicarbonate concentration increased the rate of oxidation of glutamine-U-^{14}C to $^{14}CO_2$ in both slices and mitochondria, an effect comparable to the acute stimulation of glutamine utilization produced by metabolic acidosis. Decreases in the concentration of glutamate and α-ketoglutarate, which accompany metabolic acidosis in the intact animal, also occurred in tissue slices when pH and [HCO_3^-] were lowered; decrease in α-ketoglutarate but not in glutamate content occurred in mitochondria under these conditions. Study of independent variations of medium pH and [HCO_3^-] showed that simultaneous changes in both pH and [HCO_3^-] produced a greater effect on glutamine metabolism than did change in either of these parameters alone.

The rate of glutamine oxidation was also compared in tissue preparations from pairs of litter-mate dogs with chronic metabolic acidosis and alkalosis. No significant difference in the rate of glutamine oxidation was present in mitochondria from the two sets of animals. Slices from animals with chronic metabolic acidosis consistently oxidized glutamine at a more rapid rate than slices from alkalotic dogs both at high and at low concentrations of bicarbonate in the medium. We believe this difference is a result of the same mechanism which leads to the delayed increase in ammonium excretion during induction of metabolic acidosis.

The close parallel between the effects demonstrated here and the changes in ammonium production and glutamine utilization in the intact animal with metabolic acidosis suggest that the observed in vitro changes accurately represent the operation of the physiologic mechanism by which acid-base changes regulate ammonium excretion. The similarity between the changes in glutamine oxidation observed in this study and those described previously for citrate suggests that one control mechanism affects the metabolism of both citrate and glutamine. Thus, we believe that the increase in citrate clearance in metabolic alkalosis and the increase in glutamine utilization and ammonium production in metabolic acidosis reflect the operation of the same underlying biochemical mechanism. This mechanism permits changes in pH and [HCO_3^-] in the cellular environment to regulate the rate of mitochondrial uptake and oxidation of several physiologically important substrates.

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INTRODUCTION

In chronic metabolic acidosis the rate of ammonium excretion is several times normal (1, 2). The major renal compensatory mechanism responsible for increased ammonium excretion in this circumstance is increased formation of ammonia from glutamine in cells of the renal cortex (3–5). The ammonia thus produced rapidly diffuses throughout the kidney. In the distal tubule and collecting duct where tubular fluid pH is markedly diminished, it reacts with secreted hydrogen ions to form large quantities of ammonium (6, 7).

The mechanism by which changes in systemic acid-base balance control glutamine metabolism and thus renal ammonium production has been the subject of considerable speculation, but so far there is no clear understanding of this homeostatic mechanism. In this article we describe in vitro studies related to the regulation of glutamine oxidation in the kidney by changes in acid-base balance. The effects of changes in pH and bicarbonate concentration of the medium on glutamine oxidation by slices and mitochondria of renal cortex are shown to resemble closely those previously described

in a similar study of citrate metabolism (8). In that study the physiologic increase in citrate clearance which occurs in metabolic alkalosis was found to be related to control of citrate oxidation in kidney mitochondria by bicarbonate and pH. The present experiments relate the increased glutamine utilization of metabolic acidosis to a similar regulation. Thus, we suggest that the increased glutamine utilization in metabolic acidosis and the increased citrate clearance in metabolic alkalosis reflect the operation of the same fundamental biochemical control. By regulating renal ammonium production in response to changes in acid-base balance, this mechanism plays a basic role in acid-base homeostasis.

METHODS

Tissue preparation and incubation. Dogs were killed by injecting a bolus of isotonic potassium chloride intravenously. The kidneys were rapidly removed, cut in half, and placed in ice cold 0.14 M NaCl–0.01 M KCl solution. The methods for preparing tissue slices and mitochondria and the general incubation technique have been described previously (8). 100 mg of slices was used in each flask in the slice experiments. In experiments with isolated mitochondria quantities of mitochondrial suspension containing 2–3 mg of protein were added to each flask. Incubation media contained 120 mM NaCl plus $NaHCO_3$, 10 mM KCl, 2.5 mM $CaCl_2$, 1.0 mM $MgSO_4$, 1.0 mM KH_2PO_4, 2 mM Na L-malate, 2 mM Na pyruvate, 0.5 mM Na citrate, and 0.5 mM glutamine. Mitochondrial media contained 115 mM KCl plus $KHCO_3$, 3 mM $MgSO_4$, 4 mM adenosine triphosphate (ATP), 10 mM P_i buffer, 2 mM K L-malate, 2 mM K pyruvate, 0.5 mM K citrate and 0.5 mM glutamine. The total volume of tissue preparation plus medium was 2.0 ml in each flask. In experiments in which pCO_2 was held constant 4% CO_2–96% O_2 saturated with water vapor was used as the gas phase. When pH and $[HCO_3^-]$ were varied independently, appropriate concentrations of bicarbonate were calculated from the Henderson-Hasselbalch equation using a pK of 6.10, an α-value of 0.030, and a pCO_2 of 728 × (per cent CO_2 of gas phase). Proper pH of the P_i buffer was also calculated in this manner. Bath temperature was 37°C in all experiments. After equilibrating the flasks with a gas mixture, the tissue preparation was introduced and gassing continued for 2.5 min, at which point the flasks were closed and 25 μl of glutamine-U-^{14}C [1] (or citrate-1,5-^{14}C [2] in a few experiments) were added to each medium. Incubation was stopped 10 or 12 min after introducing the isotope when tissue slices were used and 6 min afterwards when mitochondria were used. $^{14}CO_2$ was recovered from the flasks as previously described (8). Mitochondrial protein was determined by the biuret technique (9).

In experiments in which glutamate and α-ketoglutarate were measured, the incubation was terminated by opening the flask and adding 0.6 ml of 1.2 N perchloric acid. Slices were homogenized in a Potter-Elvehjem homogenizer. The homogenate or mitochondrial suspension was centrifuged and the supernatant used for the assays. Glutamate and α-ketoglutarate were measured fluorometrically using glutamate dehydrogenase as described, respectively, by Graham and Aprison (10) and by Goldberg, Passonneau, and Lowry (11).

We assume that the rate of glutamine oxidation is related to the rate of $^{14}CO_2$ production in these experiments by a constant, but undetermined, factor. The experimental conditions were chosen so that no more than 20%, and in most cases less than 10%, of the counts added as glutamine-^{14}C were converted to $^{14}CO_2$. Because all of the carbon atoms of glutamine were labeled, considerable isotope was present in products other than CO_2 at the end of the experiment. Therefore, it is not possible to calculate the absolute rates of glutamine utilization from these data in which only recovery of $^{14}CO_2$ was determined, nor can the relative contributions of the different labeled carbon atoms to the $^{14}CO_2$ recovered be specified. Results of the experiments in which glutamine-U-^{14}C was used are expressed as total counts per minute recovered as $^{14}CO_2$. Unless noted otherwise, each experimental point reported represents the mean of two flasks containing identical media.

Chronic metabolic acidosis and alkalosis experiments. Glutamine metabolism was compared in tissues from chronically acidotic and alkalotic animals. For these experiments pairs of litter-mate dogs were prepared for 7–9 days before sacrifice by feeding 8 mEq/kg of ammonium chloride or 18 mEq/kg of sodium bicarbonate daily. The salt was mixed with their regular food and the pans checked carefully to ensure complete consumption. (In experiment 1 of this series the acidotic dog was fed 18 mEq/kg of ammonium chloride for 7 days, but food consumption was incomplete). Pairs of animals were sacrificed at the same time and slices and mitochondria prepared for incubation simultaneously. Blood for determination of plasma bicarbonate concentration was obtained at the time of sacrifice.

RESULTS

Effect of variation in pH and $[HCO_3^-]$ on glutamine oxidation. Fig. 1 shows the results of four experiments in which tissue slices were used and the rate of glutamine oxidation determined in media of varying pH and $[HCO_3^-]$. As the pH and $[HCO_3^-]$ increased, $^{14}CO_2$ production from glutamine-U-^{14}C declined progressively. At 10 mM HCO_3^- the rate of $^{14}CO_2$ production was about two-thirds greater than at 30 mM HCO_3^-.

Similar experiments in which mitochondria were used are shown in Fig. 2. In mitochondria we found considerable variation between animals in the rate of glutamine oxidation and in the slope and shape of the curve of $^{14}CO_2$ production vs. $[HCO_3^-]$, as illustrated by the four experiments in this figure. However, in each instance glutamine oxidation was highest at the lowest bicarbonate concentration and declined progressively as pH and $[HCO_3^-]$ increased.

Fig. 3 illustrates an experiment in which we compared the effect of varying pH and $[HCO_3^-]$ on the metabolism of glutamine-U-^{14}C and citrate-1,5-^{14}C by renal mitochondria. With each substrate, increasing pH and $[HCO_3^-]$ had an inhibitory effect on the rate of $^{14}CO_2$ formation. The general similarity of the two responses is apparent.

Effect of independent variations of pH and $[HCO_3^-]$

[1] Calbiochem, Los Angeles, Calif.

[2] New England Nuclear Corp., Boston, Mass.

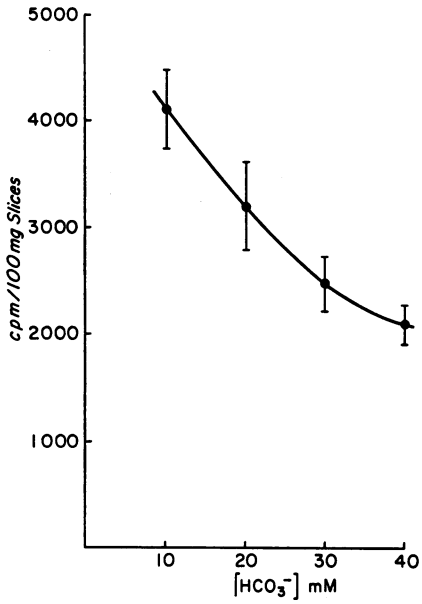

FIGURE 1 Effect of variation of $[HCO_3^-]$ and pH on glutamine-U-^{14}C oxidation by slices of dog renal cortex. In each flask 100-mg slices were incubated for 10 min in media containing varying concentrations of HCO_3^- and approximately 30,000 cpm glutamine-U-^{14}C. Gas phase was 4% CO_2, 96% O_2. The data represent cpm (mean ±SD) recovered as $^{14}CO_2$ in four experiments. In each experiment two flasks containing each $[HCO_3^-]$ were used.

on glutamine-U-^{14}C oxidation. We performed 22 experiments, under a variety of conditions, in order to compare the effect of change in $[HCO_3^-]$ without change in pH and the effect of change in pH without change

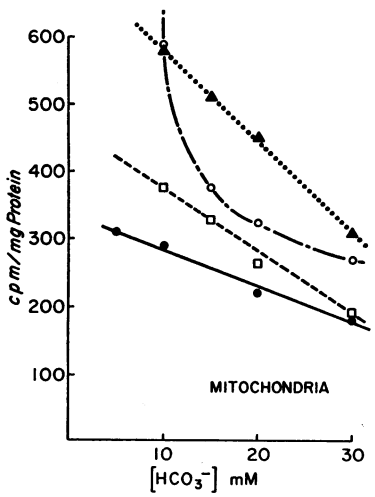

FIGURE 2 Effect of variation of $[HCO_3^-]$ and pH on glutamine-U-^{14}C oxidation to $^{14}CO_2$ by mitochondria. The results of four experiments are shown. Each point represents the mean cpm recovered as $^{14}CO_2$ from two flasks containing identical media.

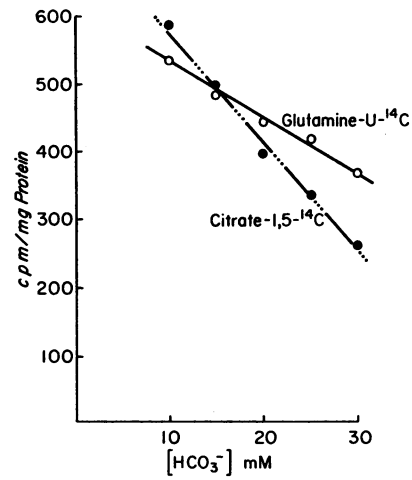

FIGURE 3 Comparison of the influence of $[HCO_3^-]$ and pH on oxidation of glutamine and citrate. Each point represents the $^{14}CO_2$ produced from ^{14}C-labeled glutamine or citrate by mitochondria incubated with varying concentrations of HCO_3^- in the medium.

in $[HCO_3^-]$ on glutamine oxidation by mitochondria. Examples which illustrate the pattern of the results of these experiments are shown in Fig. 4. In the experiment shown in Fig. 4 *a* the effect of a simultaneous change in both pH and $[HCO_3^-]$ was measured by determining the rate of glutamine oxidation at 10 and 30 mM HCO_3^- (pH 7.17 and 7.64, respectively) using 4% CO_2 in the gas phase; the results of these measurements are

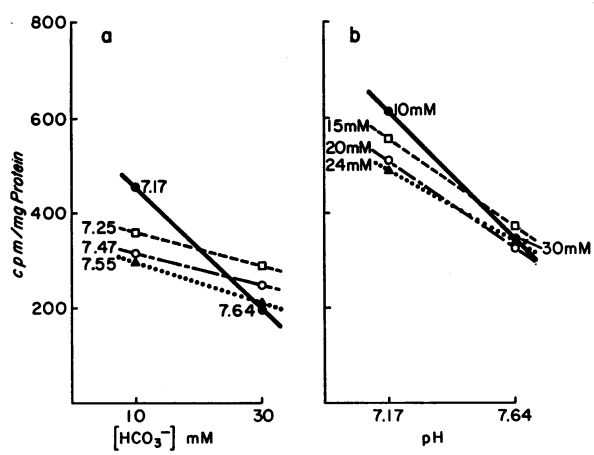

FIGURE 4 Effect of independent variations of pH and $[HCO_3^-]$ on glutamine oxidation by mitochondria. Each point represents the data from a pair of flasks. (*a*) $[HCO_3^-]$ was varied between 10 and 30 mmoles/liter at three different constant pH levels. For comparison the heavy line connects a pair of points in which both pH and $[HCO_3^-]$ changed (4% CO_2). (*b*) Medium pH varied between 7.17 and 7.64 while $[HCO_3^-]$ was held constant. The effect of simultaneous variation in both pH and $[HCO_3^-]$ is shown by the points connected by the heavy line.

connected by the heavy line in the figure. In other flasks glutamine oxidation was measured in media in which the pH was constant at 7.25 but the HCO_3^- concentration changed from 9 to 30 mmoles/liter. In other media constant pH values of 7.47 and 7.55 were maintained and the $[HCO_3^-]$ varied. Thus, the effect of independent variation of $[HCO_3^-]$ on glutamine oxidation was determined at three different constant pH levels for comparison with the effect of variation of both pH and $[HCO_3^-]$ over the same range of bicarbonate concentration. In six experiments of this type, when both pH and $[HCO_3^-]$ varied, glutamine oxidation was 2.18 ±0.61 times greater at 10 than at 30 mM HCO_3^-. This difference between low and high $[HCO_3^-]$ was only 66 ±19% as great when pH was constant and $[HCO_3^-]$ varied over the same range. In Fig. 4 *b* results of a similar experiment are shown in which pH was varied between 7.17 and 7.64 and glutamine oxidation measured. When $[HCO_3^-]$ also varied (heavy line) the increase in the rate of glutamine oxidation between high and low pH was greater than that found when $[HCO_3^-]$ was held constant at concentrations of 15, 20, or 24 mmoles/liter. In six such experiments glutamine oxidation was 2.43 ±0.70 times greater at low than at high pH when both pH and $[HCO_3^-]$ varied together and change in pH alone produced 81 ±16% as much effect as did simultaneous change in both variables.

In both types of experiments illustrated in Fig. 4, decrease of either pH or $[HCO_3^-]$ alone caused an increase in the rate of glutamine oxidation, but the effect was less than that obtained when both pH and $[HCO_3^-]$ were altered together. Thus, both $[HCO_3^-]$ and $[H^+]$ exert independent effects on glutamine metabolism in this system. These results are in contrast to those obtained previously in rabbit kidney when similar experiments were performed using citrate-1,5-^{14}C (8). In rabbit mitochondria the entire effect of pH and HCO_3^- on citrate metabolism was reproduced by variation in $[HCO_3^-]$ while pH was held constant; change in pH alone had no effect on the rate of citrate oxidation. In dog kidney mitochondria, as shown in Fig. 4, both moieties of the carbonic acid system affected glutamine oxidation. When similar experiments were repeated on dog mitochondria using citrate-1,5-^{14}C instead of glutamine-U-^{14}C, results entirely similar to those described here for glutamine were obtained.[8] Thus, there is a species difference between dog and rabbit kidney related to the independent effects of HCO_3^- on mitochondrial substrate metabolism.

Measurement of glutamate and α-ketoglutarate content at different levels of pH and HCO_3^-. Table I shows the results of two experiments in which the content of glutamate and of α-ketoglutarate was measured in slices and mitochondria incubated in media containing 10 or 30 mM HCO_3^-. In slices glutamate content was about 30% greater at 30 than at 10 mM HCO_3^-. No such difference was apparent in mitochondria incubated under these conditions. α-ketoglutarate content, on the other hand, was considerably greater at the higher bicarbonate concentration in both slices and mitochondria. The relative amounts of glutamate and α-ketoglutarate were also quite different in the two types of tissue preparation. In slices of renal cortex several times as much glutamate was present as α-ketoglutarate, whereas in mitochondria the content of α-ketoglutarate exceeded that of glutamate. This suggests that the high glutamate content present in renal cortex (5, 12), as well as in slices, is due to cytoplasmic rather than mitochondrial accumulation of this amino acid.

TABLE I

Glutamate and α-Ketoglutarate Content of Slices and Mitochondria Incubated with High or Low Concentrations of Bicarbonate

Tissue	$[HCO_3^-]$	Flask No.	Total glutamate		Total α-ketoglutarate	
			Experiment 1	Experiment 2	Experiment 1	Experiment 2
	mmoles/liter		*μmoles*		*μmoles*	
Slices	10	1	0.28	0.31	0.049	0.035
		2	0.26	0.31	0.053	0.035
		3	0.29	0.30	0.061	0.037
		Mean	0.28	0.31	0.054	0.036
	30	4	0.37	0.35	0.13	0.067
		5	0.40	0.39	0.14	0.061
		6	0.37	0.41	0.13	0.064
		Mean	0.38	0.38	0.13	0.064
Mitochondria	10	7	0.16	0.21	0.25	0.33
		8	0.23	0.21	0.25	0.32
		9	0.23	0.23	0.26	0.32
		Mean	0.20	0.22	0.25	0.32
	30	10	0.22	0.22	0.39	0.49
		11	0.19	0.22	0.44	0.50
		12	0.19	0.21	0.46	0.48
		Mean	0.20	0.22	0.43	0.49

Studies in tissue from dogs with chronic metabolic acidosis and alkalosis. Glutamine oxidation was compared in slices and mitochondria prepared from renal cortex of six pairs of litter-mate dogs which were made alkalotic or acidotic by feeding sodium bicarbonate or ammonium chloride for 7–9 days (Table II). The

[8] Simpson, D. P., and D. J. Sherrard. Unpublished observation.

TABLE II

Glutamine Oxidation by Slices and Mitochondria from Renal Cortex of Pairs of Dogs with Chronic Metabolic Acidosis or Alkalosis

Tissue	Experiment No.	$^{14}CO_2$ Recovered					
		$[HCO_3^-]$ = 10 mmoles/liter			$[HCO_3^-]$ = 30 mmoles/liter		
		Acidotic	Alkalotic	Ratio*	Acidotic	Alkalotic	Ratio*
		cpm × 10^{-3}/*100 mg slices*			*cpm* × 10^{-3}/*100 mg slices*		
Slices	1	3.69	2.52	1.46	3.29	1.90	1.72
	2	3.61	2.95	1.22	3.02	1.99	1.52
	3	3.10	2.39	1.28	2.48	1.47	1.68
	4	3.02	2.27	1.37	2.62	1.28	2.04
	5	2.89	2.17	1.33	2.32	1.58	1.47
	6	2.92	2.39	1.22	2.41	1.57	1.54
	Mean	3.20	2.45	1.31	2.69	1.63	1.66
	SD±	0.33	0.25	0.13	0.35	0.25	0.21
	P		< 0.001			< 0.002	
		cpm/mg protein			*cpm/mg protein*		
Mitochondria	1	436	420	1.04	316	267	1.18
	2	366	362	1.01	281	262	1.07
	3	230	288	0.81	96	183	0.52
	4	375	407	0.92	281	253	1.11
	5	262	383	0.68	155	218	0.71
	6	400	451	0.89	201	262	0.77
	Mean	344	385	0.89	222	241	0.89
	SD±	78	53	0.123	75	29	0.25
	P		> 0.10			> 0.05	

* Ratio = (cpm produced by tissue from acidotic dog)/(cpm produced by tissue from alkalotic dog).

mean plasma bicarbonate concentration of the acidotic animals at the time of sacrifice was 14.5 ±2.1 mmoles/liter; that of the alkalotic dogs was 27.4 ±3.1 mmoles/liter. The latter is not entirely representative of the alkalotic state of these animals because the blood samples were obtained about 24 hr after the last feeding of sodium bicarbonate. A few bicarbonate measurements made during the day before sacrifice showed bicarbonates in the range of 30–40 mmoles/liter; by the following morning excretion of much of the alkali load had lowered the plasma bicarbonate to almost normal levels.

In slices incubated at high (30 mmoles/liter) and at low (10 mmoles/liter) concentrations of bicarbonate the rate of oxidation of glutamine was higher in the dogs with chronic metabolic acidosis in all experiments. At 10 mM HCO_3^- glutamine oxidation was about one-third greater in the acidotic than in the alkalotic animals and was about two-thirds greater at 30 mM HCO_3^-. In the mitochondrial preparations no such difference was discernible between acidotic and alkalotic preparations. Considerable variation in the results between experiments was present but no consistent difference between the two groups of animals was evident at either bicarbonate concentration. Thus, there is an adaptive effect of chronic metabolic acidosis which enhances the rate of glutamine oxidation at a fixed pH and bicarbonate concentration. This effect does not appear to reside in the mitochondria but must be related to changes in the cell membrane or cytoplasm.

These results are in contrast to those reported by Rector and Orloff in similar experiments (13). These investigators found no difference in the ability of slices from chronically acidotic or alkalotic rats to utilize glutamine. However, in this latter study glutamine was present in an amount (49 mmoles/liter) approximately 100 times the physiologic concentration in body fluids. In other experiments we have shown that the difference between the rate of glutamine oxidation at 10 and that at 30 mM HCO_3^- diminishes as the concentration of glutamine in the medium increases.[4] Thus, the very large substrate concentration used by Rector and Orloff would mask the effect of pH and bicarbonate on glutamine utilization. In the experiments reported here we chose an amount of glutamine, 0.5 mmole/liter, in the

[4] Simpson, D. P., and D. J. Sherrard. Unpublished observation.

medium which approximates the normal plasma concentration.

DISCUSSION

When the daily nonvolatile hydrogen ion load is increased severalfold by feeding ammonium chloride, the kidneys respond by increasing hydrogen ion excretion in order to achieve a new steady state of acid-base balance. Ammonia provides the majority of the buffer for elimination of this excess hydrogen ion and after several days of acidosis a constant high rate of ammonium excretion is achieved (1, 2). A small part of this greatly increased ammonium excretion is the result of increased nonionic diffusion of ammonia into urine with a pH somewhat lower than that which preceded the acidosis (2). Most of the increase, however, is due to increased ammonia formation in the kidney from the amide, and to a lesser extent, the amine groups of glutamine as demonstrated by the studies of Pitts and associates (5, 6, 14). Thus, metabolic acidosis in some manner stimulates the deamidation and deamination of glutamine.

Several important characteristics of this response should be noted. When metabolic acidosis is acutely induced in dogs, an immediate increase in glutamine utilization occurs (15). In addition after the first few days of acidosis a fairly constant degree of extracellular acidosis develops but ammonium excretion continues to increase for several more days. In man, for example, a steady state of ammonium excretion is not reached until 5 or 6 days after starting to take ammonium chloride, although the maximum fall in plasma bicarbonate and pH occurs in about 3 days (1, 2). Thus, in addition to an acute effect of metabolic acidosis, there is a more gradual adaptive effect which increases glutamine utilization over a period of several days despite a stable acidosis.

In contrast to the very striking effect of chronic metabolic acidosis on ammonium excretion and glutamine utilization, chronic respiratory acidosis causes only a modest increase in renal ammonium production. Polak, Haynie, Hays, and Schwartz, in a study of chronic respiratory acidosis in dogs, found an increase of about 50% in ammonium excretion (16). Carter, Seldin, and Teng found a small increase in NH_4^+ excretion during the first few days of respiratory acidosis in rats followed by a decline to almost normal levels during chronic acidosis (17). In metabolic acidosis, on the other hand, increases of ammonium excretion of 500–600% may occur. Thus, metabolic acidosis provides a far greater stimulus to ammonium excretion than a comparable degree of respiratory acidosis.

In the present study we have attempted to demonstrate in an in vitro system metabolic effects on glutamine oxidation which are comparable to the physiologic effects of metabolic acidosis in the intact animal. The results described indicate that changes in pH and $[HCO_3^-]$ within the physiologic range exert a regulatory effect on glutamine oxidation both in intact cells and in isolated mitochondria. Under the same conditions of low pH and $[HCO_3^-]$ in which glutamine oxidation was increased the content of glutamate and of α-ketoglutarate in the slices was reduced. These changes parallel the reduction in concentrations of these substances in renal cortex which occurs in metabolic acidosis in the intact animal (5, 12, 18). Therefore we believe this in vitro effect corresponds to the acute increase in renal glutamine utilization which is induced by metabolic acidosis in vivo, and which results in enhanced ammonium production and excretion.

The experiments with tissue from dogs with chronic acidosis and alkalosis show that metabolic acidosis induces a change in the ability of cells of renal cortex to oxidize glutamine. Thus at the same $[HCO_3^-]$ and pH in the medium, slices from acidotic dogs produced one-third to two-thirds more CO_2 from glutamine than those from their alkalotic litter mates (Table II). Since mitochondria from the same kidneys did not exhibit this effect, it is apparently related to a change in the cell membrane or cytoplasm. A possible explanation for this observation is that chronic acidosis increased the rate of active transport of glutamine into the cell. Because concentrations of glutamine in renal cortex considerably exceed those in plasma (5), active accumulation of glutamine in the cell presumably occurs. If metabolic acidosis stimulated this process more glutamine would be available inside the cell for utilization. Another explanation for the increase in glutamine oxidation by slices from acidotic dogs would be that a change in the cytoplasm occurs which increases glutamine oxidation in the mitochondria. For example, if intracellular pH and $[HCO_3^-]$ were lower in the slices from the acidotic dogs, glutamine oxidation would be enhanced. Whatever the cause, this in vitro effect appears to reflect the adaptive mechanism which causes the delayed increase in ammonium production from glutamine after the first few days of metabolic acidosis.

Two routes exist in renal cortex by which ammonia may be produced from glutamine by deamidation and deamination. First, in mitochondria glutamine is deamidated by glutaminase, forming glutamate which can then be converted to α-ketoglutarate either by deamination by glutamic dehydrogenase or by transamination with pyruvate or oxalacetate. By a second route glutamine may be transaminated by glutamine–amino acid transferase forming α-ketoglutaramate, an unstable compound which is deamidated by ω-amidase to yield α-ketoglutarate. The enzymes involved in this latter pathway are probably cytoplasmic in location (19). The relative

contributions of these routes to ammonia production from glutamine are unknown but the concentration of glutaminase in renal cortex is about 25 times greater than that of glutamine-ketoacid transferase (20). By either route glutamine is metabolized to α-ketoglutarate which undergoes oxidative decarboxylation in the citric acid cycle. These biochemical considerations provide the basis for the use of glutamine-^{14}C oxidation as an index of glutamine utilization.

Until glutamine utilization and ammonia production are measured directly some uncertainty must remain in relating $^{14}CO_2$ production to glutamine utilization. Two other possibilities must be considered which could affect the rate of CO_2 formation from labeled glutamine. First, a change in the rate of de novo glutamine or glutamate synthesis could theoretically alter $^{14}CO_2$ production from glutamine-^{14}C without a change in the over-all rate of glutamine utilization. However, little glutamine is synthesized by dog kidney (21) and the concentration of glutamine synthetase is low in dog renal cortex (20). In addition amination of ketoglutarate to form glutamate does not take place to an appreciable extent in dog kidney (22). While some glutamate is undoubtedly synthesized by transamination with endogenous alanine or asparagine, such processes must provide little net glutamate in these experiments where only organic acid substrates and glutamine were added to the media. Second, an increase in $^{14}CO_2$ production from glutamine might be the result of a shift of glutamine or glutamate away from some nonoxidative pathway and into the route of decarboxylation. Although glutamine and glutamate participate in many metabolic reactions it is doubtful that their utilization by other routes, such as purine or protein synthesis, would occur at the high rates necessary to account for the observed changes in glutamine oxidation, particularly in mitochondrial preparations. In addition in a previous study (8) we showed that both citrate and α-ketoglutarate utilization, as well as citrate-^{14}C oxidation, were affected by changes in pH and HCO_3^- in the same way as glutamine oxidation was altered in the present experiments. Thus pH and [HCO_3^-] regulate both the utilization and oxidation of some mitochondrial substrates. For these reasons we believe that changes in $^{14}CO_2$ production reflected changes in glutamine utilization in the present experiments.

A number of hypotheses have been suggested in the past to explain the regulation of ammonium excretion by acid-base changes. The observation by Davies and Yudkin (23) and by others (24, 25) that glutaminase activity increased in acidotic rats suggested that induction of formation of this enzyme might be the controlling factor. However, Rector and Orloff (13) failed to observe such a change in glutaminase content in acidotic dog kidney. In addition, Goldstein was able to inhibit glutaminase induction with actinomycin D without preventing the increase in ammonium excretion in acidosis (26). These observations make glutaminase induction an unsatisfactory explanation for this phenomenon.

Another hypothesis, first used to explain the effect of metabolic alkalosis on citrate clearance (27) and subsequently extended to the effect of metabolic acidosis on glutamine utilization (28), is based on the observation that the activity of citrate synthase increases with increasing pH (29). According to this concept, decreased citrate synthase activity in acidosis would lead to the accumulation of oxalacetate and pyruvate in the cell and these in turn, by transaminase reactions, would increase the utilization of glutamine and glutamate. This theory, however, does not account for the marked difference between the effects of metabolic and of respiratory acidosis on renal ammonium excretion, a difference which we believe is related to a fundamental regulatory role of bicarbonate ion in this process.

Goodman, Fuisz, and Cahill have found that metabolic acidosis stimulates glucose formation from glutamine or citric acid cycle intermediates in slices of renal cortex (30). This observation has been confirmed by others (31) and, recently, in vivo evidence for increased gluconeogenesis during metabolic acidosis has been obtained (32). Because of these findings a regulatory role has been postulated for gluconeogenesis in controlling glutamine utilization in response to acid-base changes. The site of this effect is stated to be between oxalacetate and glucose (30, 31, 18). Our evidence, however, indicates that the effect of pH and [HCO_3^-] on glutamine utilization takes place in isolated mitochondria, eliminating cytoplasmically located enzymes, such as those concerned with gluconeogenesis, from consideration. It is possible that enhanced gluconeogenesis might account for the adaptive effect of chronic metabolic acidosis on glutamine oxidation which we found in studies on litter-mate dogs (Table II). This effect, unlike the basic effect of pH and HCO_3^- on glutamine oxidation, is present in slices but not in mitochondria and hence could be related to cytoplasmic enzymes. However, the studies of the effect of acidosis on gluconeogenesis have not yet shown that this phenomenon has a primary regulatory effect on glutamine utilization. Thus, the increased gluconeogenesis produced by acidosis could be secondary to stimulation of glutamine and citric acid cycle oxidation in mitochondria; or acidosis could have separate effects on gluconeogenesis and on glutamine metabolism.

In a previous study of citrate metabolism (8) we obtained results which correspond precisely with those noted here for glutamine. Increases in pH and [HCO_3^-] in the medium inhibited the oxidation and utilization of citrate by slices and mitochondria and increased the citrate content of these preparations. We interpreted the physiologic increases in citrate clearance and renal citrate concentration which occur in metabolic alkalo-

sis (27, 33–35). Thus under normal conditions of acid-base balance most filtered citrate is reabsorbed and oxidized by the cells of the tubules. In metabolic alkalosis, citrate oxidation in renal cortex is inhibited by the increase in pH and [HCO_3^-], resulting in decreased citrate reabsorption and increased citrate excretion. In the present investigation we have related similar in vitro observations to the increase in glutamine utilization produced by metabolic acidosis. The fall in systemic pH and [HCO_3^-] in the latter acid-base disturbance results in increased glutamine oxidation by mitochondria, producing more ammonia for buffering hydrogen ions in the tubular fluid.

Because of the similarities of the results of the two investigations we suggest, that the same underlying biochemical mechanism accounts for the effect of changes in pH and [HCO_3^-] on the mitochondrial oxidation of citrate, glutamine, and probably other substrates. Furthermore we postulate that this mechanism is responsible in the intact animal for two distinct physiologic phenomena observed at opposite extremes of acid-base balance, namely the increased glutamine utilization and ammonium excretion in metabolic acidosis and the increased citrate clearance in metabolic alkalosis.

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