The Effects of Acidosis and Alkalosis on the Metabolism of Glutamine and Glutamate in Renal Cortex Slices

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ABSTRACT Studies of the metabolism of glutamine and glutamate by renal cortex slices from acidotic, alkalotic, and control rats were performed. 88–95% of the glutamine and 104–115% of the glutamate taken up from the medium could be accounted for by the products found. Acidosis increased glutamine uptake and conversion to ammonia, CO_2, glucose, lactate, pyruvate, lipid, and protein. The increase in glutamine conversion to ammonia after acidosis could be completely accounted for by the associated increase in its conversion to glucose, glutamate, lactate, and pyruvate. When glutamate metabolism was examined, acidosis did not affect substrate uptake but did increase its conversion to ammonia, glucose, lactate, CO_2, and lipid. The increase in $^{14}CO_2$ from U-^{14}C-glutamine and U-^{14}C-glutamate found with cortex slices from acidotic animals could be explained by the CO_2 production calculated to be associated with the enhanced conversion of these substrates to other products during acidosis. $^{14}CO_2$ production from 1,2-^{14}C-acetate was found to be significantly increased in alkalosis rather than acidosis. These studies suggest that in the rat, the rate at which glutamine is completely oxidized in the Krebs cycle is not a factor regulating renal ammonia production. A comparison of the effects of acid-base status on glutamine and glutamate metabolism suggests that either glutamine transport or glutamine transaminase activity are significantly increased by acidosis.

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INTRODUCTION

The importance of glutamine as the major precursor of renal ammonia production, first described by Van Slyke, Phillips, Hamilton, Archibald, Futcher, and Hiller in 1943 (1), has been well decomuented (2–4). Despite numerous investigations over the past quarter century, the mechanism regulating the increase in ammonia production from glutamine during acidosis remains the subject of continued research. Many control mechanisms have been suggested including: glutamine transport (5), the balance between glutaminase I and glutamine synthetase (6), glutamine transaminase (7, 8), mitochondrial oxidation (9, 10), PEP-carboxykinase (11–13), and the "redox state" (14, 15).

To obtain further information on the mechanism through which acidosis increases renal ammonia production, studies of glutamine uptake and conversion to ammonia, CO_2, glucose, and other products by renal cortex slices from acidotic, normal, and alkalotic animals have been made. Because of the importance of glutamate as an intermediate of glutamine metabolism, similar studies have also been performed using this amino acid as substrate. These experiments demonstrate marked effects of acid-base status on glutamine, and to a lesser extent, glutamate metabolism. A comparison of the effects of acidosis on glutamate and glutamine metabolism suggests that either glutamine transport or glutamine transaminase activity are significantly increased during acidosis. Studies of $^{14}CO_2$ production from U-^{14}C-glutamine, U-^{14}C-glutamate, and 1,2-^{14}C-acetate suggest that in the rat, the increase in renal ammonia production found during acidosis is not secondary to an increase in the rate at which glutamine is completely oxidized in the Krebs cycle.

METHODS

Feeding protocol. Sprague-Dawley male rats (Holtzman) weighing 250–350 g were used in all experiments.

During the 48 hr before an experiment, all animals were deprived of solid food and tube-fed twice a day 15 ml of a 15% glucose solution containing either 200 mM NH_4Cl (acidotic), 200 mM NaCl (control), or 200 mM $NaHCO_3$ (alkalotic). Animals were allowed to drink 75 mM NH_4Cl (acidotic), 75 mM NaCl (control), or 75 mM $NaHCO_3$ (alkalotic) ad lib.

In vitro studies of renal cortical metabolism. On completion of the various tube-feeding regimens, rats were decapitated, blood was collected for the determination of plasma CO_2, and the kidneys were removed. Approximately 150 mg of renal cortical slices, prepared with a Stadie-Riggs microtome, were incubated for 90 min in Krebs-Ringer bicarbonate medium at pH 7.4 by a method previously described in detail (16). Cortex slices from each animal were incubated in separate flasks containing either no added substrate or 10 mM glutamine and U-^{14}C-glutamine (61,300–135,000 dpm/μmole) or 10 mM glutamate and U-^{14}C-glutamate (46,800–117,000 dpm/μmole). Glutamine and glutamate were added to the medium from solutions in which the pH had been adjusted to 7.4 with 0.01 N NaOH. The amount of NaCl added to the medium was adjusted so that the sodium concentrations in all media were equivalent. U-^{14}C-glutamic acid (New England Nuclear Corp., Boston, Mass.) was found to be 97% pure when chromatographed on Eastman Cellulose Chromagrams (Eastman Kodak Co., Rochester, N. Y.) using a solvent system containing isopropyl alcohol, formic acid, and water (75/12.5/12.5, v/v) (17).

When U-^{14}C-glutamine (New England Nuclear Corp.) was examined in the same chromatographic system as U-^{14}C-glutamate (17) it was found to be 90–93% pure. The main contaminant of U-^{14}C-glutamine was pyrollidone carboxylate, which contained 4–7% of the radioactivity. Glutamate contained 0.3–1.0%, and other amino acids, a total of 1–2% of the radioactivity. When U-^{14}C-glutamine was examined in the thin-layer system just described with the modification that solvent 1 was used in both directions, it was found that 4–5% of the radioactivity present in the glutamine spot after migration in the first direction appeared in the pyrollidone carboxylate spot when migrated in the second direction. Thus, the purity of U-^{14}C-glutamine present in the incubation flask before exposure to the chromatographic solvents is probably about 95%. To determine the impurities present as organic acids, U-^{14}C-glutamine was also examined in the thin-layer system described by Whereat, Snydman, and Barness (18). Using this method, pyrollidone carboxylate contained 3.5–4.0%, lactate + pyruvate 0.1–0.2% and all other organic acids 0.6–0.8% of the radioactivity. The radioactivity isolated in pyrollidone carboxylate was similar before and after incubation with slices. This finding is consistent with the observations of Weil-Malherbe and Krebs (19) in which pyrollidone carboxylate was not found to be metabolized by renal cortex slices. In the present studies, therefore, the radioactivity found in pyrollidone carboxylate at the end of an experiment was not considered a product of glutamine metabolism by cortex slices.

At the end of the 90 min incubation period, separate portions of the medium were deproteinized with 5% zinc sulfate heptahydrate and 0.3 N barium hydroxide, 6% perchloric acid, or 5% trichloroacetic acid (TCA) for later analyses. Determinations for ammonia and $^{14}CO_2$, and extraction of lipids were started immediately from untreated medium. Glucose was determined from the zinc sulfate-barium hydroxide filtrates using a glucose oxidase technique (20). Lactate and pyruvate were determined enzymatically in neutralized perchlorate filtrates (21, 22). Medium ammonia concentration was determined in duplicate by the Conway microdiffusion method (23). The ammonia found in glutamine medium incubated without slices was subtracted in the calculation of ammonia production.

Glutamate concentration was determined in the TCA filtrates using the fluorometric procedure described by Graham, Werman, and Aprison (24). Glutamine was determined in TCA or perchlorate filtrates, or in media diluted with 0.2 M acetate buffer at pH 4.8 and kept frozen overnight, by a variation of the glutaminase method described by Addae and Lotspeich (25). In this analysis, 1 ml of diluted medium or medium filtrate was added to 1 ml of 0.2 M acetate buffer containing 1 U of glutaminase (Sigma Chemical Co., St. Louis, Mo.). A similar portion was added to 1 ml of 0.2 M acetate buffer without glutaminase. The ammonia found in this sample was subtracted in the calculation of glutamine concentration. After incubation for 1 hr at 37°C, 1 ml of the phenol reagent (20 g phenol plus 0.1 g nitroferricyanide/liter) was added, followed by 1 ml of the alkaline hypochlorite solution (10 g NaOH plus 0.84 g sodium hypochlorite dissolved in 1 liter of pH 12 0.2 M sodium phosphate buffer). After incubation at room temperature for 30 min, the samples were read on a Gilford spectrophotometer (Gilford Instrument Laboratories, Inc., Oberlin, Ohio) at 625 mμ. Recovery studies demonstrated complete recovery of glutamine added to media, or to TCA and perchlorate filtrates of media, obtained after incubation with slices from acidotic, control, or alkalotic animals.

Since the glutaminase reaction is inhibited by glutamate, medium from the glutamate studies was passed through a column containing an anion exchanger (AG1-X8, Bio-Rad Laboratories, Richmond, Calif.) to separate glutamate from glutamine. Because of the low glutamine and high NH_4 + in the eluate, glutamine could not be accurately determined with the glutaminase/indolphenol method described above. Instead, glutamine was first converted to glutamate by acid hydrolysis (26) and glutamate determined with the fluorometric assay already described. Glutamine standards were also assayed as glutamate after similar treatment. Glutamine and glutamate utilization by cortex slices was determined by comparing substrate concentration present at the end of incubation with that found in medium incubated at 37°C without slices.

To determine the conversion of ^{14}C-substrates to CO_2, 1 ml of untreated medium was placed in a 25 ml Erlenmeyer flask and the medium in the stoppered flask was acidified with 0.2 ml of 10 N H_2SO_4. The evolved CO_2 was collected, during 3 hr of gentle shaking, in a polypropylene center well (Kontes Glass Co., Vineland, N. J.) containing 0.2 ml of phenethylamine. The center well was then dropped into a scintillation vial and counted in a Nucear-Chicago Mark I liquid scintillation counter (Nuclear-Chicago, Des Plaines, Ill.) after the addition of 15 ml of the liquid scintillation counting fluid described by Gupta (27). The radioactivity found in a blank $^{14}CO_2$ determination made with medium incubated without slices was subtracted in the calculation of $^{14}CO_2$ production. A correction was made for the CO_2 calculated to be present in the gas phase of the incubation flask. This correction was based on the measured flask volume, an assumed molar gas volume at 37°C of 25.4 μl/μmole and studies of medium P_{CO_2} and total CO_2 made anaerobically at the end of incubation (see Appendix). Using the calculated gas phase CO_2 in the determination of total flask CO_2, the recovery in CO_2 of $NaH^{14}CO_3$ added to eight flasks containing Krebs-bicarbonate buffer was 105.4% (range 100–109%).

The conversion of ^{14}C-substrates to glucose was determined from the specific activity of glucose isolated as the phenylosazone derivative and combusted using a modification of the in-vial combustion method described by Gupta (27). The glucosazone crystals were dissolved in about 2.5 ml of near boiling ethanol. 2 ml of this solution were pipetted into a tared tube, and 0.1 ml into a platinum-irridium wire stand and cup. This stand had previously been placed in a scintillation vial and contained a cotton pellet and siliconized lens paper blackened with India ink. After the vial had been dried for at least 4 hr in a vented oven at 60°C, phenethylamine, 0.1 ml, was added to a glass fiber disc (Reeve Angel 934 AH; H. Reeve Angel & Co., Clifton, N. J.) previously placed in the vial. The vial was then flushed with 100% O_2 and capped tightly with a foil-lined cap. The lens paper was then ignited by focusing a strong beam of light from a projector lamp on the blackened spot. 3 hr later, the vial was opened, 15 ml of counting solution was introduced; and the vial was tightly recapped. After allowing at least 3 hr for the carbamate formed between the CO_2 and phenethylamine to dissolve, the samples were counted in a liquid scintillation counter. The amount of material combusted was calculated from the osazone found in the dried tared tube. When the osazone was prepared with cold glucose and U-^{14}C-glucose, recovery of the isotope in the osazone was 96–104%. No radioactivity was recovered in the osazone when the derivative was prepared with cold glucose in the presence of 1,2-^{14}C-pyruvate, U-^{14}C-glutamine medium incubated without slices, or U-^{14}C-glutamate medium incubated without slices.

In the ^{14}C-substrate studies summarized in Table I all of the data, except those for glucose production, were calculated according to equation 1, and are presented as μmoles of substrate in product.

$$\mu\text{moles of substrate in product} = \frac{\text{dpm in product per gram dry weight}}{\text{dpm per } \mu\text{mole of substrate}} \quad (1)$$

The μmoles of glucose formed from substrate were calculated by equation 2. This calculation allows glucose production from glutamine and glutamate, determined with ^{14}C, to be directly compared with glucose production determined with glucose oxidase. Equation 2 is based on the assumption that only three of the five carbons of uniformly labeled glutamine or glutamate are converted to phosphoenolpyruvate (Fig. 1), and that the effective specific activity of the uniformly labeled glutamine and glutamate converted to glucose is therefore only $\frac{3}{5}$ the specific activity of the five carbon compound. Thus, the μmoles of phosphoenolpyruvate, and therefore of glutamine or glutamate, from which the glucose was derived is equal to the μmoles of substrate found in glucose according to equation 1 multiplied by 5/3. 2 μmoles of phosphoenolpyruvate are required for the synthesis of 1 μmole of glucose. Thus:

$$\mu\text{moles of glucose formed from substrate} = \mu\text{moles of substrate in glucose} \times \frac{5}{3} \times \frac{1}{2} \quad (2)$$

For extraction of lipids, slices were placed in a 2:1 chloroform-methanol mixture. The first crude extract was decanted and fresh chloroform-methanol added. The two crude extracts were combined and treated as described by Folch, Lees, and Sloane-Stanley (28). A portion of the final solution was placed in a liquid scintillation vial and counted after evaporation of the solvent. To determine the amount of ^{14}C-substrate converted to protein, the tissue remaining after the lipid extraction was homogenized and purified as described by Bignall, Elebute, and Lotspeich (29) with the exception that the final solubilization of the protein was in 1 N NaOH instead of hyamine. Protein was determined by the method of Lowry, Rosebrough, Farr, and Randall (30). $\frac{1}{2}$ ml of the protein solution was pipetted into a scintillation vial, neutralized with HCl, and counted in Bray's solution containing 5% Cab-O-Sil (Cabot Corp., Boston, Mass.). In our earlier experiments the radioactivity present in medium protein and lipid was determined and found not to be

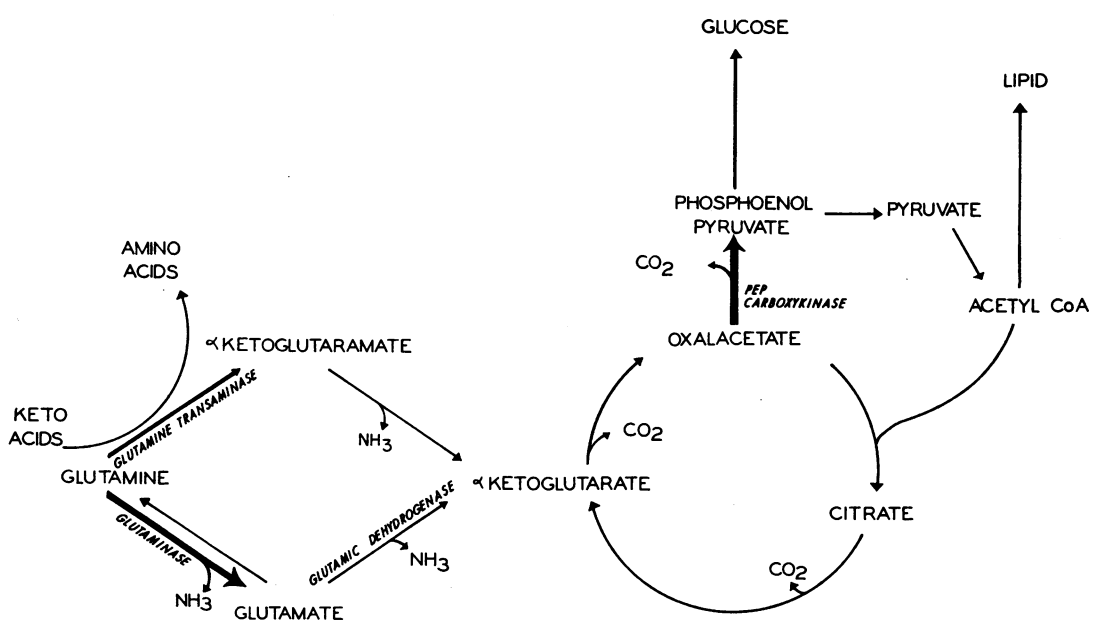

FIGURE 1 Pathways of glutamine and glutamate metabolism in renal cortex.

TABLE I

Effect of Acidosis and Alkalosis on Glutamate and Glutamine Metabolism in Renal Cortical Slices

	Units	Alkalotic		Control		Acidotic	
Glutamate Studies							
Substrate uptake	μmoles per g dry wt	718 ±38	(33)	728 ±43	(32)	797 ±49	(26)
Substrate conversion to:							
Ammonia	μmoles of product‡ per g dry wt	183 ±7.3**	(33)	319 ±8.1	(33)	436 ±9.6**	(27)
Glucose		108 ±4.1**	(33)	154 ±4.5	(33)	204 ±7.1**	(27)
Glutamine		181 ±23**	(13)	53 ±6.8	(13)	22.9 ±4.0**	(13)
Lactate		15.7 ±1.2	(29)	18.9 ±1.6	(24)	28.2 ±3.8*	(24)
Pyruvate		2.62±0.33	(10)	2.71±0.42	(10)	2.45±0.49	(8)
Glucose	μmoles of glucose from glutamate§ per g dry wt	87.6 ±3.4**	(33)	131 ±4.8	(33)	192 ±6.1**	(27)
Carbon dioxide	μmoles of glutamate§ in product per g dry wt	349 ±10.2	(33)	367 ±11.1	(33)	401 ±10.3*	(27)
Alanine		16.0 ±1.5	(12)	16.8 ±0.7	(13)	14.9 ±1.4	(11)
Glycine		<10	(12)	<10	(13)	<10	(11)
Serine		22 ±3.8	(12)	22.6 ±2.6	(13)	18.5 ±2.4	(11)
Slice							
Protein		16.2 ±0.66*	(12)	14.5 ±0.39	(12)	15.6 ±0.53	(10)
Nonprotein		106 ±4.85	(5)	91.4 ±3.12	(5)	115 ±3.51	(5)
Lipid		2.8 ±0.10	(12)	3.2 ±0.11	(12)	5.0 ±0.16**	(10)
Glutamine Studies							
Substrate uptake	μmoles per g dry wt	856 ±44*	(31)	1031 ±58	(33)	1559 ±73**	(31)
Substrate conversion to:							
Ammonia	μmoles of product‡ per g dry wt	819 ±29**	(25)	1186 ±31.1	(26)	1779 ±52.5**	(25)
Glucose		70 ±5.1**	(23)	136 ±6.3	(26)	241 ±9.6**	(25)
Glutamate		264 ±18.2	(19)	291 ±18.2	(19)	402 ±14.2**	(19)
Lactate		19.0 ±1.4*	(32)	24.2 ±1.7	(29)	53.2 ±4.9**	(24)
Pyruvate		3.64±0.46	(13)	4.22±0.66	(13)	7.07±1.3*	(11)
Glucose	μmoles of glucose from glutamine§ per g dry wt	49.6 ±1.9**	(25)	98.6 ±3.1	(26)	197 ±9.2**	(25)
Carbon dioxide	μmoles of glutamine§ in product per g dry wt	268 ±7.7**	(25)	336 ±9.9	(26)	429 ±16.2**	(25)
Alanine		16.3 ±2.5	(7)	13.2 ±2.4	(7)	19.9 ±2.0	(6)
Aspartate		20.0 ±3.3	(7)	18.1 ±2.4	(6)	16.0 ±2.4	(6)
Glycine		25.0 ±1.7	(7)	24.4 ±4.5	(7)	26.4 ±5.6	(6)
Serine		15.3 ±1.5	(7)	11.9 ±1.4	(7)	13.7 ±3.1	(6)
Slice							
Protein		7.38±0.31	(13)	7.8 ±2.53	(13)	12.8 ±0.35**	(12)
Nonprotein		111 ±6.0	(5)	96.8 ±3.84	(5)	113 ±5.36	(5)
Lipid		1.25±0.05**	(14)	1.6 ±0.05	(14)	3.22±0.15**	(12)

* Significantly different from control $*P < 0.05$, $**P < 0.01$.

‡ Determined by measurement of products.

§ Determined with ^{14}C.

significantly different from background. In this report, therefore, only the incorporation of ^{14}C-substrates into tissue protein and lipid has been considered.

The incorporation of ^{14}C-substrates into medium amino acids was determined by thin-layer chromatography using the bidirectional solvent system described by White (17) on Eastman Cellulose Chromagrams. In preliminary studies, the only amino acids produced from ^{14}C-glutamine and -glutamate in renal cortex slices in significant amounts were alanine, aspartate acid, glutamate, glutamine, glycine, and serine. The sensitivity of this method is not sufficient to rule out conversion of glutamate and glutamine to other amino acids in amounts less than 10 μmoles of substrate/g dry weight. When glutamate was substrate, its incorporation into aspartate could not be determined because of overlap of the aspartate spot with the large glutamate spot. After location of the amino acids by cochromatography, the appropriate spots were cut out and counted in liquid scintillation fluid containing 10.5 g PPO, 0.45 g POPOP, and 15 g naphthalene made up to 1500 ml with dioxane, to which water was added to give a total volume of 1800 ml. The loss of counts secondary to the ninhydrin used for

identification by cochromatography varied from 4 to 16% and was predictable for a given amino acid. All samples were counted long enough to give 5% accuracy. In several experiments, the incorporation of ^{14}C-substrates into the nonprotein components of the slice was also determined. For this determination, after incubation, slices were blotted briefly on damp filter paper and homogenized in 0.5 ml of 6% perchloric acid. A portion of the neutralized perchlorate filtrate was counted in the liquid scintillation fluid described above for use in the analysis of ^{14}C in amino acids. With the exception of glucose production, the results of the ^{14}C-substrate studies are expressed as μmoles of substrate converted to products per gram dry weight. In those studies in which the incorporation of ^{14}C-substrate into slices was determined, the dry weight were estimated from accurately determined wet weights and the wet weight: dry weight ratio of cortex slices from the same animal incubated without added substrate.

RESULTS

The observations on glutamate and glutamine metabolism summarized in Table I were derived from 24 experiments. Each experiment included acidotic, control, and alkalotic animals, with 3–7 animals per group. All of the products listed in the table were not determined in each experiment. The production of glucose and ammonia by slices incubated without added substrate has been subtracted in the calculation of net glucose and ammonia production from glutamine and glutamate. The production of glutamaine, glutamate, lactate, and pyruvate by slices incubated without added substrate was insignificant. The observations on the incorporation of ^{14}C-substrates into products are based only on the analysis of flasks containing 10 mM glutamine and U-^{14}C-glutamine or 10 mM glutamate and U-^{14}C-glutamate.

Glutamate studies. The effects of in vivo acid-base status on the metabolism of glutamate in renal cortical slices are summarized in the upper part of Table I. The mean ±SE is given and the number of observations is shown in parentheses. Glutamate uptake was 728±43 μmoles/g dry weight with slices from control animals and was not significantly affected by acid-base status. It can be seen that most of the glutamate taken up was converted to CO_2, glucose, and glutamine with smaller amounts appearing in lactate, pyruvate, alanine, serine, lipid, and protein. Acidosis increased the conversion of glutamate to ammonia, glucose, and lipid ($P < 0.01$), probably increased conversion to CO_2 and lactate ($P < 0.05$), and decreased the production of glutamine ($P < 0.01$). Alkalosis significantly decreased ammonia and glucose production ($P < 0.01$), and increased the conversion of glutamate to glutamine ($P < 0.01$) and protein ($P < 0.05$). Glucose production determined from the incorporation of U-^{14}C-glutamate into glucose correlated in a linear manner with glucose production determined with glucose oxidase. (Glucose production [^{14}C] = 0.84 glucose production [glucose oxidase] + 4.0, r = 0.84.])

Glutamine studies. The effects of in vivo acid-base status on the metabolism of glutamine in renal cortical slices are shown in the lower part of Table I. Glutamine uptake was significantly greater than glutamate uptake with slices from control animals ($P < 0.01$) and was, in addition, markedly influenced by acid-base status. With slices from acidotic animals glutamine uptake increased to 1559 μmoles/g dry weight, a value almost double the glutamate uptake of 797 μmoles. Conversely, with slices from alkalotic animals, glutamine uptake dropped to 819 μmoles, a value approaching the glutamate uptake of 719 μmoles. Most of the glutamine taken up from the medium was converted to CO_2, glucose, glutamate, and lactate with smaller amounts appearing in pyruvate, alanine, aspartate, glycine, serine, lipid, and protein. Acidosis significantly enhanced the conversion of glutamine to ammonia, glucose, lactate, CO_2, lipid, and protein ($P < 0.01$) and probably increased pyruvate production ($P < 0.05$). Overall, the effects of alkalosis on glutamine metabolism were opposite those of acidosis.

Glucose production determined from the incorporation of U-^{14}C-glutamine in glucose correlated in a linear manner with glucose production determined with glucose oxidase (glucose production [^{14}C] = 0.81 glucose production [glucose oxidase] − 4.0, r = 0.94). With either method, the effects of acid-base status on gluconeogenesis were found to be more marked in the glutamine than in the glutamate studies.

Substrate recovery. In Table II the data, which had been expressed as μmoles of product, or μmoles of substrate in product in Table I, have all been expressed in a common unit, μmoles of substrate carbon or nitrogen, so that they may be summed and the recovery of substrate carbon or nitrogen in products calculated. The total recovery of glutamate carbon and nitrogen observed was 104–115% and 79–98%, respectively, of the glutamate taken up from the media. The recovery of glutamine carbon and nitrogen in products was 88–95% and 82–87%, respectively.

$^{14}CO_2$ production from U-^{14}C-glutamine, U-^{14}C-glutamate, and 1,2-^{14}C-acetate. The conversion of glutamine and glutamate to products along the pathways shown in Fig. 1 is associated with predictable quantities of CO_2 production. Fig. 2 is an analysis of the contribution of the calculated product associated $^{14}CO_2$ production to total $^{14}CO_2$ production, using data derived from the values given in Table I. The total height of the bars represents total $^{14}CO_2$ production. The upper part of each bar represents the $^{14}CO_2$ calculated to be produced in association with the conversion of glutamate or glutamine to glucose, pyruvate, lipid, protein, and amino acids. When the $^{14}CO_2$ associated with the formation of these products is sub-

TABLE II

*Recovery of Glutamate and Glutamine Carbon and Nitrogen in Products**

	Glutamate studies						Glutamine studies					
	Alkalosis		Control		Acidosis		Alkalosis		Control		Acidosis	
	Carbon	Nitrogen	Carbon	Nitrogen	Carbon	Nitrogen	Carbon	Nitrogen	Carbon	Nitrogen	Carbon	Nitrogen
NH_3		183		319		436		928		1268		1860
CO_2	1745		1835		2005		1340		1680		2145	
Glucose	650		920		1225		420		815		1445	
Lactate	50		55		85		55		70		160	
Pyruvate	10		10		5		10		10		20	
Glutamine	905	362	265	106	115	46						
Glutamate							1320	264	1455	292	2010	402
Alanine	70	23	75	25	70	23	80	28	65	22	90	30
Aspartate							130	34	90	24	100	26
Glycine							125	64	130	66	145	74
Serine	80	27	85	28	75	25	65	22	60	20	75	26
Slice												
Protein	80	20	75	19	80	20	35	10	40	10	65	16
Nonprotein	530	90	455	77	575	97	490	102	485	101	565	118
Total recovery	4120	705	3775	574	4235	647	4070	1452	4900	1803	6820	2552
Observed uptake	3590	718	3640	728	3985	797	4280	1716	5155	2062	7795	3118
% Recovery	115	98	104	79	106	81	95	85	95	87	88	82

* In the calculation of the values used in this table, the products of glutamine metabolism shown in Table I were assumed to contain equivalents of substrate carbon as follows: glutamate, 5 μmoles; lactate and pyruvate, 3 μmoles; and glucose, 6 μmoles of glutamine carbon/μmoles of product found. The data for CO_2, alanine, aspartate, glycine, serine and slice contents, which were expressed as μmoles of substrate in each product in Table I, have been multiplied by 5 to convert them to μmoles of substrate carbon. In calculating the recovery of glutamine nitrogen, 1 mole of glutamine is considered to have 2 moles of glutamine nitrogen. Thus, each μmole of glutamate or ammonia found was considered the recovery of 1 μmole of glutamine nitrogen. Substrate nitrogen recovered in protein was calculated from the carbon recovery, assuming a ratio for carbon: nitrogen of 3:1 (31). The calculations for the glutamate studies were similar except that 1 mole of glutamate was considered to have 1 mole of glutamate nitrogen. Thus, one μmole of ammonia would indicate the recovery of one μmole of glutamate nitrogen and one μmole of glutamine would indicate the recovery of 2 μmoles of glutamate nitrogen. Nitrogen recovery in the nonprotein components of the slice was calculated from the carbon recovery, assuming a ratio for carbon:nitrogen of 4.8:1 in the glutamine studies and 5.9:1 in the glutamate studies. These ratios were based on studies of the incorporation of ^{14}C-glutamine and ^{14}C-glutamate into the amino acid, organic acid, lipid, and glucose + glycogen content of the slice. Incorporation of radioactivity into organic acids was determined using thin-layer chromatography (36). The percentage distribution of radioactivity in the glutamate studies (n = 6) were as follows: alanine + serine, 1.3; aspartate, 4.7; glutamate, 57; glutamine, 1.5; glycine, 0.7; organic acids, 9; lipid, 5.6; glucose + glycogen, 3.9; and undetermined 16.3%. Similar results were obtained in the glutamine studies except that glutamate contained 44, glutamine 19, and the undetermined fraction 10.3% of the radioactivity.

tracted from the total $^{14}CO_2$, the remainder, represented by the lower hatched portion of the bar is a measure of the extent to which the substrate is completely oxidized to CO_2 and presumably reflects substrate oxidation in the Krebs cycle. This figure demonstrates graphically the small changes in U-^{14}C-glutamate conversion to $^{14}CO_2$ and large changes in U-^{14}C-glutamine conversion to $^{14}CO_2$ already described in Table I, which occur following acidosis and alkalosis. When the product associated $^{14}CO_2$ is subtracted from total $^{14}CO_2$ production, the remaining $^{14}CO_2$ production is unaffected by acid-base status. These observations suggest that the complete oxidation of glutamine and glutamate to CO_2 in the Krebs cycle is not increased by acidosis.

Since acetate is oxidized in the Krebs cycle but would not give rise to CO_2 when converted to other products, studies of the effects of acid-base status on $^{14}CO_2$ production from 1,2-^{14}C-acetate were also performed (Fig. 3). Each point in Fig. 3 represents three to six observations on $^{14}CO_2$ production by cortex slices from different animals incubated in Krebs-Ringer bicarbonate medium containing 25 mM and 1,2-^{14}C-acetate (12,200–110,000 dpm/μmole). Also shown is the mean ±SE for all observations with alkalotic (n = 26), control (n = 27), and acidotic (n = 24) animals. With slices from alkalotic animals, the conversion of ^{14}C-acetate to $^{14}CO_2$ was 774±40 μmoles of CO_2/g dry weight, a value significantly higher ($P < 0.05$) than the values of 660±28 and 642±44 found with slices from control and acidotic animals, respectively. With the exception of one experiment, CO_2 production was higher after alkalosis, and lower after acidosis, when compared with the data from control animals. To determine if the results shown in Fig. 3 were related to the method employed, in which flasks were opened at the end of incubation, two similar experiments, not shown in Fig. 3, were done in which the conversion of 1,2-^{14}C-acetate to $^{14}CO_2$ (24,900–32,800 dpm/μmole) was determined without opening the flasks. At the end of incubation, 0.2 ml of phenethylamine was injected through the rubber stopper into a center well and 1 ml of 10 N H_2SO_4 was injected into the medium. $^{14}CO_2$ was then determined as described under Methods. Recovery of $NaH^{14}CO_3$ added to control flasks was 98–103%. CO_2 production from acetate with cortex slices

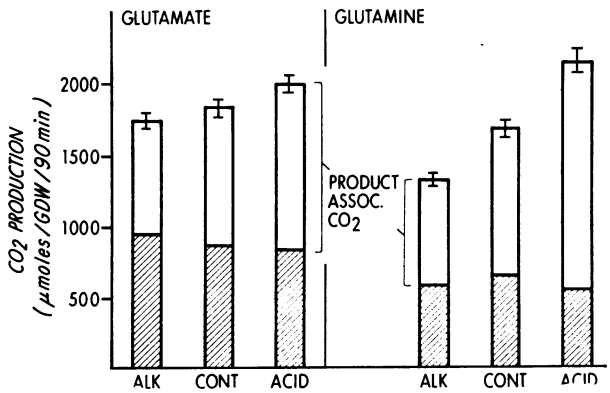

FIGURE 2 The effect of in vivo acid-base status on the conversion of U-^{14}C-glutamate and U-^{14}C-glutamine to $^{14}CO_2$. The data are expressed as μmoles of substrate carbon in CO_2 per gram dry weight (GDW) per 90 min. The total height of the columns represents total conversion of U-^{14}C-glutamate or U-^{14}C-glutamine to $^{14}CO_2$. The upper segment of each bar represents the $^{14}CO_2$ calculated to be produced as a result of the conversion of substrate to other products.

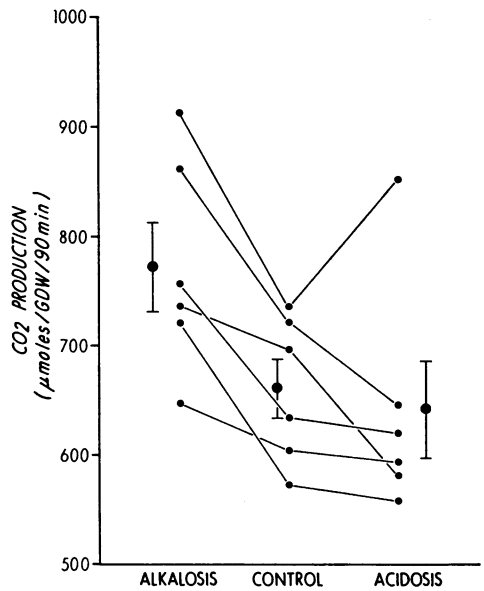

FIGURE 3 The effect of in vivo acid-base status on the conversion of 1,2-^{14}C-acetate to $^{14}CO_2$. Each point represents 3–6 observations on $^{14}CO_2$ production by cortex slices from different animals. Also, shown is the mean ± SE for all observations with alkalotic (n = 26), control (n = 27), and acidotic (n = 24) animals.

from normal animals was 542±19 μmoles/g dry weight per 90 min (n = 9). Acidosis reduced CO_2 production from acetate to 462±25 (n = 9, $P < 0.05$) and alkalosis increased it to 607±7.6 (n = 11, $P < 0.02$). With either method, it appears that acetate conversion to CO_2, unlike that of glutamine, is stimulated by alkalosis rather than acidosis.

Relation between glutamine conversion to ammonia and its conversion to other products. In Fig. 4 we have attempted to determine the extent to which the products of glutamine metabolism associated with ammonia production have been measured. The values shown have been derived from the data presented in Table I. In this analysis it has been assumed that all of the products have been derived from glutamine. Conversion of 1 mole of glutamine to a product not containing nitrogen is assumed to give rise to 2 moles of ammonia; conversion to 1 mole of the amino acids measured in this study is assumed to result in the production of 1 mole of ammonia. The predicted NH_3, shown on the ordinate, and graphed for the studies with slices from alkalotic, control, and acidotic animals, is plotted against the observed NH_3 production on the abscissa. The distribution of products associated with NH_3 production is shown on the right. The conversion of glutamine to glucose, lactate, pyruvate, and amino acids is associated with the production of both ammonia and CO_2. In Fig. 4 the ammonia produced in association with this CO_2 is already accounted for by the conversion to these products. Accordingly, the ammonia predicted to be produced in association with the conversion of glutamine to $^{14}CO_2$ is calculated from the total $^{14}CO_2$ production minus the $^{14}CO_2$ calculated to be produced in association with the conversion of glutamine to other products. If predicted and observed NH_3 production were the same, the top of each column would fall on the line of identity.

As shown in the Fig. 4, predicted ammonia production is about 200–250 μmoles greater than the observed ammonia production. Predicted ammonia may exceed observed ammonia for several reasons. Endogenous substrates containing no nitrogen may be converted to glucose, lactate, etc., without resulting in ammonia production. Conversely, glutamine converted to the products used to predict ammonia production may have donated its nitrogen to products not determined in these studies. The slope for the regression of predicted on observed ammonia production is close to one. This indicates that we are probably measuring all of the major products of glutamine metabolism in renal cortex slices associated with the change in ammonia production that occurs as a result of acidosis.

The data shown in Fig. 4 and Table I indicate that the products of glutamine metabolism, significantly affected by prior acid-base status, which could be associated with increased NH_3 production are glucose, lactate, pyruvate, lipid, protein, and glutamate. In Table III we have examined the extent to which the increased conversion of glutamine to these products can account for the enhanced CO_2 and ammonia production found during acidosis. The upper part of Table III shows the effect of acidosis on the conversion of glutamine to

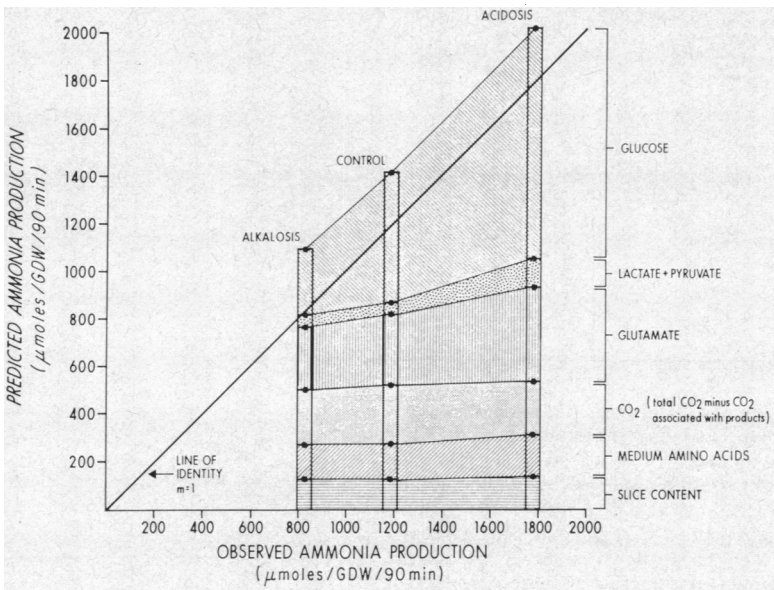

FIGURE 4 Relation between predicted and observed ammonia production. The predicted ammonia production, based on the conversion of glutamine to other products, is shown on the vertical axis and graphed for the studies with slices from alkalotic, control and acidotic animals. The distribution of products associated with ammonia production is shown on the right. The observed ammonia production during these studies is plotted on the horizontal axis (see text for details).

TABLE III

A Comparison of the Observed ΔCO_2 and ΔNH_3 following Acidosis and the Predicted ΔCO_2 and ΔNH_3, Based on the Changes in Glutamine and Glutamate Conversion to Other Products

	Acidosis minus control			Acidosis minus alkalosis		
	Observed	Predicted		Observed	Predicted	
	Δ Products	ΔCO_2	ΔNH_3	Δ Products	ΔCO_2	ΔNH_3
	μmoles of prod/ GDW	*μmoles of subst/ GDW*	*μmoles/GDW*	*μmoles of prod/ GDW*	*μmoles of subst/ GDW*	*μmoles/GDW*
Glutamine studies						
Glucose	105	84	420	171	137	684
Lactate + pyruvate	32	13	64	37	15	74
Lipid	1*	2	3	2*	3	10
Protein	5*	1	6	6*	2	8
Glutamate	111	0	111	138	0	138
Predicted total Δ		100	604		157	914
Observed total Δ		93	593		161	962
Observed Δ/predicted Δ		0.93	0.98		1.02	1.05
Glutamate studies						
Glucose	50	40	100	96	76	192
Lactate + pyruvate	9	4	9	13	5	13
Lipid	2*	3	5	2*	3	5
Protein	1*	0	0	0	0	0
Glutamine	−30	0	30	−158	0	158
Predicted total Δ		47	144		84	368
Observed total Δ		34	117		52	253
Observed Δ/predicted Δ		0.72	0.81		0.62	0.69

* Expressed as μmoles of substrate in product.

these products when compared with their production by slices from control (Δ acid — cont) and alkalotic animals (Δ acid — alk). The Δ NH_3 and Δ CO_2 predicted from the change in conversion to these products is also given. It can be seen that the ratio of observed: predicted $^{14}CO_2$ and ammonia production is close to one when $^{14}CO_2$ and ammonia production from glutamine in slices from acidotic animals is compared with that found in slices from either control (Δ acid — cont) or alkalotic animals (Δ acid — alk).

Relation between ammonia and CO_2 production from glutamate and its conversion to other products. As shown in Table I, the products of glutamate metabolism, other than ammonia and $^{14}CO_2$, which changed significantly as a result of acidosis were glucose, lactate, lipid, protein, and glutamine. In the lower part of Table III, the change in glutamate conversion to these products, and to ammonia and $^{14}CO_2$ after acidosis, has been analyzed in the same manner as that described in the previous section for the glutamine studies. In this analysis a decrease in glutamine production from glutamate is assumed to predict an equimolar increase in NH_3 production. The data indicate that the observed change in the conversion of glutamate to the products shown after acidosis, more than accounted for the observed increases in NH_3 and $^{14}CO_2$ production. As was suggested for the glutamine studies shown in Fig. 4, this finding may be due to conversion of endogenous substrates, which do not give rise to ammonia, to the products shown in Table III, or to the conversion of glutamate to these products and nitrogen containing products other than ammonia, which were not determined in these studies. Another possibility, suggested by the data shown in Fig. 3, is that following acidosis a decrease in the complete oxidation of glutamate to CO_2 and ammonia occurs.

Relation between the conversion of glutamate to glutamine and its conversion to glucose, lactate, and ammonia. In the glutamate studies, glucose, lactate, $^{14}CO_2$, and NH_3 production increased significantly after acidosis. Since acidosis did not enhance glutamate uptake, the increase in glucose, lactate, $^{14}CO_2$, and ammonia production can only be accounted for by a decrease in the conversion of glutamate to other products. It is apparent from Tables I and III that glutamine is the major product of glutamate metabolism which decreases after acidosis. As shown in the lower part of Table III (Δ acid — alk) the increase in glucose and lactate production after acidosis was 96 and 13 μmoles, respectively. Since 2 μmoles of glutamate are required to produce 1 μmole of glucose, this indicates that an additional 205 μmoles of glutamate were converted to these products as a result of acidosis. This diversion of glutamate to glucose and lactate during acidosis may, by making less glutamate available, explain the quantitatively similar decrease in conversion of glutamate to glutamine of 158 μmoles (Table III, Δ acid — alk). An alternate explanation, not ruled out by these studies, is that the primary event in acidosis is a net decrease in glutamine production from glutamate which secondarily leads to increased conversion of glutamate to glucose, lactate, CO_2, and ammonia.

DISCUSSION

These studies demonstrate that in renal cortex slices there is a marked dependence of glutamine metabolism, and to a lesser extent glutamate metabolism, on prior acid-base status. When compared with slices from alkalotic animals, slices from acidotic animals demonstrated a twofold increase in glutamine uptake. Glutamate uptake, on the other hand, was not significantly affected by prior acidosis or alkalosis. Although acid-base status did not affect glutamate uptake, it markedly influenced its metabolic fate. In the absence of changes in glutamate uptake, it must be concluded that acid-base status alters the intracellular metabolism of glutamate. Although an effect of acid-base status on glutamine transport into cells is not ruled out by these studies, the effects of acid-base status on glutamate metabolism suggests that the increase in NH_3 production from glutamine during acidosis is at least in part related to changes in its intracellular metabolism.

The data summarized in Fig. 4 indicate that all of the major products of glutamine metabolism associated with NH_3 production in rat renal cortex slices were determined in the present studies. They confirm our previous observation (32) that about three-quarters of the increment in NH_3 production from glutamine in rat renal cortex after acidosis can be accounted for by increased glucose production, and demonstrate that the remaining one-fourth can be explained by enhanced conversion of glutamine to lactate, pyruvate, lipid, protein, and glutamate. These experiments also indicate that most of the increase in glucose production as a result of acidosis was derived from glutamine, since the rise in glucose production determined from the incorporation of U-^{14}C-glutamine into glucose can account for 85% of the increase in glucose production determined with glucose oxidase.

With slices from control animals, glutamine uptake exceeded that of glutamate by 303 μmoles/g dry weight per 90 min, an amount approximately equal to, and presumably explained by the conversion of glutamine to glutamate (Table I). The fourfold greater ammonia production from glutamine when compared to glutamate (control studies, Table I) can also be explained in terms of the products summarized in Table I. The metabolism of glutamine to nonnitrogen containing products would result in twice as much ammonia as would the metabolism of a similar amount of glutamate. In addi-

tion, ammonia production is increased to the extent that glutamine is converted to glutamate. Conversely, with glutamate as substrate, conversion to glutamine reduces ammonia production.

Glucose and lactate production from glutamine was similar to that from glutamate when the metabolism of slices from control animals was examined. After acidosis however the increase in glucose and lactate production from glutamine was 171 and 37 μmoles/g dry weight per 90 min, respectively (Table III, Δ acid − alk). These values are significantly greater ($P < 0.01$) than the increase in glucose and lactate production of 96 and 13 μmoles which occurred from glutamate after acidosis (Table III, Δ acid − alk). The mechanism of the greater effect of acidosis on glutamine metabolism is not apparent from these studies. Possible explanations are: (*a*) stimulation by acidosis of glutamine transport but not of glutamate transport into kidney cells, and (*b*) increased glutamine metabolism along pathways, such as the glutamine transaminase-Ω-amidase pathway, in which glutamate is not an intermediate.

The conversion of glutamate to glutamine was increased by alkalosis and decreased by acidosis. These observations are consistent with the studies of Damian and Pitts (6) in which alkalosis increased glutamine synthetase activity and decreased glutaminase I activity in vivo in the rat kidney. They concluded that glutaminase I and glutamine synthetase are influenced by acid-base status in opposite ways and form an operationally reversible system which acts to control renal ammonia production. Increased net glutamine synthesis during alkalosis could, by decreasing glutamate concentration, make it less available for conversion to glucose and lactate. It is also possible that acidosis primarily stimulates glutamate conversion to glucose and lactate and that this subsequently leads to decreased glutamate availability for glutamine synthesis. By either mechanism, ammonia production would be decreased during alkalosis, both by reduced conversion of glutamate to glucose, lactate, CO_2, and ammonia, and because of increased conversion of free ammonia and glutamate to glutamine.

Simpson and Sherrard (9) have demonstrated that the conversion of U-^{14}C-glutamine to $^{14}CO_2$ is increased in renal cortical slices from acidotic dogs and in cortical slices from normal dogs incubated at a reduced medium pH. They suggest that during acidosis, increased glutamine utilization, presumably in mitochondria, leads to enhanced conversion of glutamine to ammonia and CO_2. Our experiments suggest that in renal cortex from acidotic rats, increased $^{14}CO_2$ production from U-^{14}C-glutamine is not secondary to stimulation of the complete oxidation of glutamine. Rather, it appears to be related to an acidosis-stimulated conversion of glutamine to glucose, lactate, pyruvate and lipid, products associated with obligatory CO_2 production when they are derived from glutamine. The conversion of 1,2-^{14}C-acetate to $^{14}CO_2$ was increased by alkalosis, and appeared to be decreased by acidosis. Nagata and Rasmussen (10) have found $^{14}CO_2$ production from both 1-^{14}C-pyruvate and 2-^{14}C-pyruvate to be decreased when isolated renal cortical tubules from normal rats were incubated at a reduced medium pH. These studies of acetate and pyruvate metabolism suggest, but do not prove, that in rat renal cortex, acetyl CoA entry into the Krebs cycle is decreased by acidosis. The increase in phosphoenolpyruvate carboxykinase activity known to be present in renal cortex from acidotic rats is a possible explanation for these observations on $^{14}CO_2$ production. Stimulation of oxalacetate conversion to phosphoenolpyruvate during acidosis would limit the complete oxidation of glutamine and glutamate in the Krebs cycle and make less oxalacetate available for a combination with acetyl CoA. In a previous study Goorno, Rector, and Seldin (33) found ^{14}C-acetate conversion to $^{14}CO_2$ by rat renal cortex to be unaffected by acid-base status. The data presented by Goorno et al. are based on studies with renal cortical slices from three acidotic and three alkalotic animals. The difference in our results may therefore be related to the limited number of observations employed by these investigators. As shown in Fig. 3, the effect of acid-base status on 1,2-^{14}C-acetate conversion to $^{14}CO_2$ was small in several experiments. A significant increase after alkalosis was only apparent when experiments were combined.

The effects of acid-base status on renal glutamine metabolism have also been examined by Pilkington and O'Donovon (34). These investigators found the conversion of glutamine to CO_2 to be increased to a greater extent than could be accounted for by the changes in glucose production when dog renal cortical slices were incubated at a reduced pH. Since our experiments were performed with rat renal cortex, and evaluated the effects of acidosis or alkalosis produced in vivo over a 48 hr period, a direct comparison with their studies is not possible. Renal cortical metabolism in the dog is different in several respects from that found in the rat. In the rat kidney glutamine synthetase activity in vivo (6), and glutaminase I activity in vivo (6) and in vitro (35), vary significantly during changes in acid-base status. In the dog kidney however adaptation of glutaminase I does not occur during acidosis (36) and glutamine synthetase is not detectable either in vivo (37) or in vitro (38). In addition, glucose production from 10 mM glutamine in rat renal cortex is about fivefold that found in dog renal cortex (32, 34).

Since O_2 utilization and total CO_2 production were not determined in the present experiments, these studies do not critically evaluate the effects of acid-base status on

total Krebs cycle activity. Previous studies of O_2 utilization and CO_2 production in the literature present conflicting results. Okabe and Kodama (39) determined the O_2 utilization and CO_2 production of rabbit renal cortex slices incubated in Ringers-bicarbonate medium. They found both parameters to increase when medium pH was increased from 7.4 to 7.8, and to decrease when medium pH was reduced to 7.0. Cohen (40) has examined gas metabolism across the dog kidney in vivo. In his studies acidosis significantly decreased CO_2 production, but did not effect O_2 utilization. In other studies, Preuss (41) has found increased O_2 utilization when isolated dog renal tubules were incubated at a reduced medium pH. Because of species differences and variation in the experimental technique none of these studies are directly comparable with our experiments.

Phosphoenolpyruvate is a precursor of glucose, pyruvate, lactate, and lipid synthesis. Increased PEP-carboxykinase activity therefore is a possible explanation for the increased conversion of glutamine to these products which was found in our studies with slices from acidotic animals. The only major product of glutamine metabolism stimulated by acidosis which cannot be explained in terms of PEP-carboxykinase activity is glutamate. Increased conversion of glutamine to glutamate during acidosis may be related to the changes in glutaminase I and glutamine synthetase activity described above. It is also possible that the production of glutamate in large amounts is an artifact of our experimental protocol in which kidney slice metabolism is examined in the presence of high concentrations of glutamine. There is no evidence that acidosis increases the conversion of glutamine to glutamic acid in the kidney in vivo (2, 3).

The present experiments demonstrate a quantitative relation between ammonia production from glutamine and the conversion of glutamine to products associated with PEP-carboxykinase activity. They do not however, establish that a cause and effect relation between PEP-carboxykinase activity and ammonia production is present. Recent studies by Pagliara and Goodman (42) and Preuss (14) have demonstrated a dissociation between glucose and ammonia production by renal cortex slices when incubations are carried out at various pHs. In addition, Simpson and Sherrard (9) have found pH effects on glutamine metabolism in mitochondria despite the fact that PEP-carboxykinase is located primarily in the cytoplasm (13). Although the present experiments do not provide evidence that PEP-carboxykinase is a primary regulator of renal ammonia production, they suggest that changes in its activity may be an important part of the response of renal cortex to acidosis.

The effect of medium pH on hepatic glutamine and glutamate metabolism has recently been examined by Lueck and Miller in the isolated perfused rat liver (43). Livers perfused at pH 7.15 had decreased glutamine utilization and conversion to CO_2 when compared with livers perfused at pH 7.45. Although these results are opposite those found with renal cortex slices in the present experiments, they are not completely unexpected since it has previously been demonstrated that liver and kidney metabolism respond differently to changes in acid-base status (44). The effects of acid-base status on glutamate metabolism however, appear to be similar in liver and kidney. In both our studies, and in those of Lueck and Miller, glutamate uptake and conversion to CO_2 were relatively unaffected by pH. In addition, in both tissues glutamate conversion to glutamine appears to be enhanced by alkalosis.

APPENDIX

Studies performed to determine the correction factor for gas phase CO_2

Slices from four acidotic, four control; and four alkalotic rats were incubated at 37°C for 90 min in 10 ml of Krebs-Ringer bicarbonate buffer containing 10 mM glutamine (12 flasks) and 10 mM glutamate (12 flasks). At the end of incubation, determinations for medium P_{CO_2} and total CO_2 were made on samples obtained anaerobically. Total CO_2 was determined with a Natelson microgasometer and P_{CO_2} using a Radiometer P_{CO_2} electrode and pH Meter 27. Flask volume was determined by weighing the stoppered flasks before and after the addition of distilled water. Separate factors were calculated to determine gas phase CO_2 for studies with slices from acidotic, control, and alkalotic rats using the following equations:

$$\text{Medium } H_2CO_3\ (\mu\text{moles/ml}) = 0.03\ P_{CO_2} \quad \text{(A1)}$$

$$\text{Gas phase } CO_2\ (\mu\text{moles/flask}) = \frac{\text{total flask volume (ml)} - 10\text{ ml} \times P_{CO_2}/760}{0.0254\text{ ml}/\mu\text{mole}} \quad \text{(A2)}$$

$$\text{Correction factor} = \frac{\text{medium total } CO_2\text{/flask} + \text{gas phase } CO_2\text{/flask}}{\text{medium total } CO_2\text{/flask} - \text{medium } H_2CO_3\text{/flask}} \quad \text{(A3)}$$

Sample calculation:

Medium total CO_2 = 25.5 μmoles/ml
Medium P_{CO_2} = 38 mm Hg
Total flask volume = 55.5 ml

$$\text{Gas phase } CO_2 = (55.5\text{ ml} - 10\text{ ml}) \times \frac{38}{760} \Big/ 0.0254\text{ ml}/\mu\text{mole} = 89.6\ \mu\text{moles/flask}$$

Correction factor

$$= \frac{255\ \mu\text{moles}/10\ \text{ml} + 89.6\ \mu\text{moles}}{255\ \mu\text{moles} - 10(0.03 \times 38)}$$
$$= 1.42$$

In an experimental sample in which the dry weight of tissue in the flask was 25.3 mg and the conversion of glutamine to CO_2 determined with U-^{14}C-glutamine was 0.61 μmoles/ml of medium:

CO_2 production from glutamine

$$= 1.42 \frac{0.61\ \mu\text{moles/ml} \times 10}{0.0253\ \text{g}}$$
$$= 342\ \mu\text{moles/g dry weight}$$

Equation A3 assumes that when experimental samples are analyzed, all of the medium H_2CO_3 (free CO_2) is lost when the flask is opened and the sample pipetted. In practice only part of the medium H_2CO_3 is lost. If none of the H_2CO_3 were lost the calculated flask CO_2 would be 5% higher than the true flask CO_2. This probably accounts in part for the greater than 100% recovery observed in our control studies.

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REFERENCES

1. Van Slyke, D. D., R. A. Phillips, P. B. Hamilton, R. M. Archibald, P. H. Futcher, and A. Hiller. 1943. Glutamine as source material of urinary ammonia. *J. Biol. Chem.* **150**: 481.
2. Owen, E. E., and R. R. Robinson. 1963. Amino acid extraction and ammonia metabolism by the human kidney during the prolonged administration of ammonium chloride. *J. Clin. Invest.* **42**: 263.
3. Shalhoub, R., W. Webber, S. Glabman, M. Canessa-Fischer, J. Klein, J. de Haas, and R. F. Pitts. 1963. Extraction of amino acids from and their addition to renal blood plasma. *Amer. J. Physiol.* **204**: 181.
4. Stone, W. J., S. Balagura, and R. F. Pitts. 1967. Diffusion equilibrium for ammonia in the kidney of the acidotic dog. *J. Clin. Invest.* **46**: 1603.
5. Churchill, P. C., and R. L. Malvin. 1970. Relation of renal gluconeogenesis to ammonia production in the dog. *Amer. J. Physiol.* **218**: 241.
6. Damian, A. C., and R. F. Pitts. 1970. Rates of glutaminase I and glutamine synthetase reactions in rat kidney in vivo. *Amer. J. Physiol.* **218**: 1249.
7. Stone, W. J., and R. F. Pitts. 1967. Pathways of ammonia metabolism in the intact functioning kidney of the dog. *J. Clin. Invest.* **46**: 1141.
8. Alleyne, G. A. O. 1970. Renal metabolic response to acid-base changes. II. The early effects of metabolic acidosis on renal metabolism in the rat. *J. Clin. Invest.* **49**: 943.
9. Simpson, D. P., and D. J. Sherrard. 1969. Regulation of glutamine metabolism in vitro by bicarbonate ion and pH. *J. Clin. Invest.* **48**: 1088.
10. Nagata, N., and H. Rasmussen. 1970. Renal gluconeogenesis: effects of Ca^{2+} and H^+. *Biochim. Biophys. Acta.* **215**: 1.
11. Goodman, A. D., R. E. Fuisz, and G. F. Cahill, Jr. 1966. Renal gluconeogenesis in acidosis, alkalosis, and potassium deficiency: its possible role in regulation of renal ammonia production. *J. Clin. Invest.* **45**: 612.
12. Goldstein, L. 1966. Relation of glutamate to ammonia production in rat kidney. *Amer. J. Physiol.* **210**: 661.
13. Alleyne, G. A. O., and G. H. Scullard. 1969. Renal metabolic response to acid base changes. I. Enzymatic control of ammoniagenesis in the rat. *J. Clin. Invest.* **48**: 364.
14. Preuss, H. G. 1969. Renal glutamate metabolism in acute metabolic acidosis. *Nephron.* **6**: 235.
15. Preuss, H. G. 1968. Pyridine nucleotides in renal ammonia metabolism. *J. Lab. Clin. Med.* **72**: 370.
16. Kamm, D. E., R. E. Fuisz, A. D. Goodman, and G. F. Cahill, Jr. 1967. Acid-base alterations and renal gluconeogenesis: effect of pH, bicarbonate concentration and P_{CO_2}. *J. Clin. Invest.* **46**: 1172.
17. White, H. H. 1968. Separation of amino acids in physiologic fluids by two-dimentional thin-layer chromatography. *Clin. Chim. Acta.* **21**: 297.
18. Whereat, A. F., D. R. Snydman, and L. A. Barness. 1968. Thin-layer chromatography of citric acid cycle intermediates, pyruvate and lactate. *J. Chromatog.* **36**: 390.
19. Weil-Malherbe, H., and H. A. Krebs. 1935. CCXLVI. Metabolism of aminoacids. V. The conversion of proline into glutamic acid in kidney. *Biochem. J.* **29**: 2077.
20. Hugget, A. St. G., and D. A. Nixon. 1957. Use of glucose oxidase, peroxidase and 0-dianisidine in determination of blood and urinary glucose. *Lancet.* **2**: 368.
21. Bucher, T., R. Czok, W. Lamprecht, and E. Latzko. 1965. Pyruvate. *In* Methods of Enzymatic Analysis. H. U. Bergmeyer, editor. Academic Press, Inc., New York. 2nd printing, revised. 253.
22. Hohorst, H. J. 1965. Lactate. *In* Methods of Enzymatic Analysis. H. U. Bergmeyer, editor. Academic Press, Inc., New York. 2nd printing, revised. 266.
23. Conway, E. J. 1947. Microdiffusion Analysis and Volumetric Error. Crosby Lockwood, London. 2nd edition.
24. Graham, L. T., R. Werman, and M. H. Aprison. 1965. Microdetermination of glutamate in single cat spinal roots. *Life Sci.* **4**: 1085.
25. Addae, S. K., and W. D. Lotspeich. 1968. Relation betwen glutamine utilization and production in metabolic acidosis. *Amer. J. Physiol.* **215**: 269.
26. Pfleiderer, G. 1965. Glutamate. *In* Methods of Enzymatic Analysis. H. U. Bergmeyer, editor. Academic Press, Inc. New York. 2nd printing, revised. 396.
27. Gupta, G. H. 1966. A simple in-vial combustion method for assay of hydrogen-3, carbon-14, and sulfur-35 in biological, biochemical, and organic material. *Anal. Chem.* **38**: 1356.
28. Folch, J., M. Lees, and C. H. Sloane-Stanley. 1957. A simple method for the isolation and purification of total lipids from animal tissues. *J. Biol. Chem.* **226**: 497.
29. Bignall, M. C., O. Elebute, and W. D. Lotspeich. 1968. Renal protein and ammonia biochemistry in NH_4Cl acidosis and after nephrectomy. *Amer. J. Physiol.* **215**: 289.

30. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193**: 265.
31. West, E. S., W. R. Todd, H. S. Mason, and J. T. Van Bruggen. 1966. Textbook of Biochemistry. The Macmillan Company, New York. 4th edition. 262.
32. Kamm, D. E., and R. R. Asher. 1970. Relation between glucose and ammonia production in renal cortical slices. *Amer. J. Physiol.* **218**: 1161.
33. Goorno, W. E., F. C. Rector, Jr., and D. W. Seldin. 1967. Relation of renal gluconeogenesis to ammonia production in the dog and rat. *Amer. J. Physiol.* **213**: 969.
34. Pilkington, L. A., and D. J. O'Donovan. 1971. Metabolism of glutamine in cortex slices from dog kidney during acid-base alterations. *Amer. J. Physiol.* **220**: 1634.
35. Rector, F. C., Jr., D. W. Seldin, and J. H. Copenhaver. 1954. The mechanism of ammonia excretion during ammonium chloride acidosis. *J. Clin. Invest.* **34**: 20.
36. Rector, F. C., Jr., and J. Orloff. 1959. The effect of the administration of sodium bicarbonate and ammonium chloride on the excretion and production of ammonia. The absence of alterations in the activity of renal ammonia-producing enzymes in the dog. *J. Clin. Invest.* **38**: 366.
37. Lyon, M. L., and R. F. Pitts. 1969. Species differences in renal glutamine synthesis in vivo. *Amer. J. Physiol.* **216**: 117.
38. Krebs, H. A. 1935. CCXXX. Metabolism of amino-acids. IV. The synthesis of glutamine from glutamic acid and ammonia, and the enzymic hydrolysis of glutamine in animal tissues. *Biochem. J.* **29**: 1951.
39. Okabe, T., and S. Kodama. 1934. Studies on gas metabolism of tissue in vitro. VII. Tohoku. *J. Exp. Med.* **23**: 273.
40. Cohen, J. J. 1960. High respiratory quotient of dog kidney in vivo. *Amer. J. Physiol.* **199**: 560.
41. Preuss, H. G. 1971. Ammonia production from glutamine and glutamate in isolated dog renal tubules. *Amer. J. Physiol.* **220**: 54.
42. Pagliara, A. S., and A. D. Goodman. 1970. Relation of renal cortical gluconeogenesis, glutamate content, and production of ammonia. *J. Clin. Invest.* **49**: 1967.
43. Lueck, J. D., and L. L. Miller. 1970. The effect of perfusate pH on glutamine metabolism in the isolated perfused rat liver. *J. Biol. Chem.* **245**: 5491
44. Kamm, D. E., and G. F. Cahill, Jr. 1969. Effect of acid-base status on renal and hepatic gluconeogensis in diabetes and fasting. *Amer. J. Physiol.* **216**: 1207.