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### Research Article

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In the present study, administration of ammonium chloride to rats in an amount inadequate to decrease cortical glutamate increased the capacity of cortex to produce ammonia from glutamine in vitro and increased cortical PDG. Similarly, cortex from potassium-depleted rats had an increased capacity to produce ammonia and an increase in PDG, but glutamate content was normal. The glutamate content of cortical slices incubated at pH 7.1 was decreased, and that at 7.7 was increased, compared to slices incubated at 7.4, yet ammonia production was the same at all three pH levels. These observations suggest that cortical glutamate concentration is not the major determinant of ammonia production.

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# Relation of Renal Cortical Gluconeogenesis, Glutamate Content, and Production of Ammonia

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**ABSTRACT** Glutamate is an inhibitor of phosphate dependent glutaminase (PDG), and renal cortical glutamate is decreased in metabolic acidosis. It has been postulated previously that the rise in renal production of ammonia from glutamine in metabolic acidosis is due primarily to activation of cortical PDG as a consequence of the fall in glutamate. The decrease in cortical glutamate has been attributed to the increase in the capacity of cortex to convert glutamate to glucose in acidosis.

In the present study, administration of ammonium chloride to rats in an amount inadequate to decrease cortical glutamate increased the capacity of cortex to produce ammonia from glutamine *in vitro* and increased cortical PDG. Similarly, cortex from potassium-depleted rats had an increased capacity to produce ammonia and an increase in PDG, but glutamate content was normal. The glutamate content of cortical slices incubated at pH 7.1 was decreased, and that at 7.7 was increased, compared to slices incubated at 7.4, yet ammonia production was the same at all three pH levels. These observations suggest that cortical glutamate concentration is not the major determinant of ammonia production.

In potassium-depleted rats there was a 90% increase in the capacity of cortex to convert glutamate to glucose, yet cortical glutamate was not decreased. *In vitro*, calcium more than doubled conversion of glutamate to glucose by cortical slices without affecting the glutamate content of the slices, and theophylline suppressed conversion of glutamate to glucose yet decreased glutamate content. These observations indicate that the rate of cortical gluconeogenesis is not the sole determinant of cortical glutamate concentration.

The increase in cortical gluconeogenesis in acidosis and potassium depletion probably is not the primary

cause of the increase in ammonia production in these states, but the rise in gluconeogenesis may contribute importantly to the maintenance of increased ammoniagenesis by accelerating removal of the products of glutamine degradation.

## INTRODUCTION

In metabolic acidosis there is a rise in renal ammonia production (1, 2), an increase in the capacity of renal cortex to produce glucose from glutamate and other substrates (3), and a decrease in cortical glutamate concentration (4, 5). We and others have suggested that the changes in ammonia production, gluconeogenesis, and glutamate content are causally related (3, 4, 6). According to this hypothesis, in acidosis the increased conversion of glutamate to glucose is the cause of the fall in cortical glutamate, and since glutamate is an inhibitor of phosphate-dependent glutaminase (4), the fall in glutamate causes activation of this enzyme, thereby increasing ammoniagenesis from glutamine. Consistent with this hypothesis, in metabolic alkalosis both cortical gluconeogenic capacity and ammonia production are decreased and renal glutamate content is increased (3, 4, 7). Further, although the effect of potassium deficiency on cortical glutamate has not been studied previously, it has been noted that in potassium-depleted rats there is an increase in cortical gluconeogenesis as well as in ammonia production (3, 8). Additional support for the hypothesis has been derived from studies of the effects of 3',5'-adenosine monophosphate (cyclic AMP), 3',5'-inosine monophosphate (cyclic IMP), and 5'-inosine monophosphate (5'-IMP) on the metabolism of cortical slices; these nucleotides, which enhance cortical glucose production, also lower glutamate content and increase production of ammonia from glutamine (9, 10).

In the present study we have investigated the constancy of the relationship between renal cortical gluconeogenesis, ammonia production, and glutamate con-

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tent, by studying these variables in a number of experimental situations in the rat. It was observed that under appropriate conditions the cortical capacity for production of ammonia and glucose can be increased in acidosis and decreased in alkalosis, in the absence of any change in cortical glutamate content. Further, in potassium-deficient rats there was a marked increase in the capacity of the cortex to produce ammonia and glucose, without alteration in glutamate content. In *in vitro* studies rat cortical slices were exposed to factors previously observed to affect gluconeogenesis, including alteration of pH of the incubation medium, alteration in the calcium content of the medium, and theophylline. It was observed that under certain conditions significant change could be effected in cortical gluconeogenesis without an associated change in glutamate content, and that in other experiments substantial changes in gluconeogenesis and glutamate content could be produced without an associated change in ammonia production. These findings have led us to reconsider our initial hypothesis concerning the relationship of cortical gluconeogenesis, ammonia production, and glutamate content.

## METHODS

*Effects of metabolic acidosis and alkalosis on cortical glutamate concentration and on the capacity of cortex to produce glucose and ammonia.* Male Sprague-Dawley rats, 220–260 g, obtained from Holtzman Company, Madison, Wis., were used in all experiments.

A group of rats was deprived of solid food. Half of the animals were tube fed three times daily for 3 days 10 ml of a 10% dextrose solution containing 230 mmoles/liter NaCl and 230 mmoles/liter  $\text{NH}_4\text{Cl}$  (27 mmol/kg per day); the other half was given in the same manner a 10% dextrose solution containing 230 mmoles/liter NaCl but devoid of  $\text{NH}_4\text{Cl}$ . Both groups were allowed isotonic saline *ad lib*. On the morning of the 4th day, 12 hr after the last tube feeding, some of the rats from both groups were killed by a blow on the head, a piece of renal cortex was excised within 10 sec, and the tissue was frozen immediately in liquid nitrogen for subsequent determination of glutamate content. Blood was collected from the remainder of the animals by decapitation for determination of plasma  $\text{CO}_2$  by an automated technique (11).

In a second experiment, lasting only 2 days, rats were deprived of solid food and divided into three subgroups. One subgroup was tube fed 20% dextrose in water, 8 ml twice daily for 2 days; the second group was fed in the same manner a 20% dextrose solution containing 300 mmoles/liter  $\text{NH}_4\text{Cl}$  (22 mmol/kg per day); and the third was given in the same manner a 20% dextrose solution containing 450 mmoles/liter  $\text{NaHCO}_3$  (33 mmol/kg per day). The first two groups were allowed isotonic saline *ad lib*, and the third was allowed a solution containing 75 mmoles/liter NaCl and 75 mmoles/liter  $\text{NaHCO}_3$ . On the morning of the 3rd day, 15 hr after the last tube feeding, some of the rats from each group were decapitated and blood was collected for determination of plasma  $\text{CO}_2$ . The remainder of the animals from each group were sacrificed by a blow

on the head and cortical tissue was obtained and frozen immediately for subsequent determination of glutamate and of phosphate-dependent glutaminase.

In addition, some of the remaining cortex was sliced and the slices incubated in glucose-free modified Krebs-Ringer bicarbonate buffer (pH 7.4) containing 10 mM glutamine, as described elsewhere (3). After 90 min the slices were frozen promptly in liquid nitrogen and the glutamate content determined; in some flasks the glutamate content of the slices was measured after only 10 min of incubation. After 90 min of incubation the glucose and ammonia in the media were determined by previously described methods (3, 9). Krebs-Ringer buffer containing 10 mM glutamine was also incubated for 90 min in the absence of tissue, and the ammonia content determined. In this and the succeeding experiments, in the calculation of the production of ammonia by cortical slices the amount of ammonia produced in the flasks devoid of tissue was subtracted from the ammonia produced in the flasks containing cortex; the production of ammonia in the flasks without tissue was only 5% of that observed in the flasks containing slices from the control animals. In calculating glucose and ammonia production in this and the succeeding experiments, we did not subtract production by slices incubated in medium free of glutamine from production by slices incubated in the presence of substrate, in contrast with the procedure in an earlier publication (3).

Cortical glutamate was determined by a previously described enzymatic technique (5). The glutamate content of cortical slices was expressed in terms of the wet weight of the slices prior to incubation. Phosphate-dependent glutaminase activity of renal cortex was calculated as the difference between glutaminase activity of cortical homogenate incubated in medium containing 50 mM phosphate ("total" glutaminase), and that of homogenate incubated in phosphate-free medium (phosphate-independent glutaminase). In the incubations in the phosphate medium, 0.3 ml of aqueous 2% cortical homogenate was added to a vial to which had been added 0.6 ml of 0.05 M Tris-HCl buffer, (pH 7.4), 0.3 ml of 0.1 M glutamine, 0.3 ml  $\text{H}_2\text{O}$ , and 1.5 ml of 0.1 M sodium phosphate buffer (pH 7.4), and the vials were shaken in a water bath at 37°C for 30 min. The incubations in phosphate-free medium were carried out in identical fashion except that 1.5 ml of water was substituted for the phosphate buffer. At the end of the incubations the ammonia content of the incubation media was determined by the micro-diffusion method of Seligson and Hiralhara (12). Generation of ammonia due to spontaneous breakdown of glutamine during the incubation and diffusion was determined by measuring ammonia production in the absence of homogenate (glutamine blank), and generation of ammonia from the tissue *per se* was determined by incubating homogenate in the absence of glutamine (homogenate blank). In the calculation of total glutaminase and of phosphate-independent glutaminase, the glutamine blank and homogenate blank were subtracted.

*Effects of potassium deficiency on cortical glutamate concentration and on the capacity of cortex to produce glucose and ammonia.* In studying the effect of potassium deficiency, a protocol was employed which ensured an identical nutritional intake for both potassium-depleted and control animals. A group of rats was placed on a synthetic diet low in potassium<sup>1</sup> for 21 days, and was allowed isotonic saline

<sup>1</sup>"Low potassium and sodium diet" of Nutritional Biochemical Corp., Cleveland, Ohio, to which NaCl, 170 mmol/kg of diet, was added.

ad lib. They were then deprived of the food and saline, and half were tube fed twice daily 10 ml of 20% dextrose for 3 days, while the other half were tube fed in the same manner 20% dextrose solution containing 200 mM KCl. On the 4th day some of the rats in both groups were tube fed 10 ml of water and placed in metabolic cages. Urine was collected from each of the rats for 4 hr in tubes containing toluene, for determination of urine pH using a Radiometer pH electrode and for determination of ammonia content by the method of Seligson and Hiralhara (12). The remainder of the rats in both groups were sacrificed by a blow on the head, and renal cortex was obtained and frozen immediately in liquid nitrogen for determination of glutamate. Some of the remaining renal cortex was sliced and incubated in glucose-free modified Krebs-Ringer bicarbonate solution (pH 7.4), containing glutamine (10 mmoles/liter). At the end of the 90 min incubation period the glucose and ammonia content of the medium was determined, and the slices were frozen immediately for determination of glutamate. Also, cortical slices were incubated in medium containing monosodium glutamate (10 mmoles/liter), and after 90 min the glucose content of the medium was determined.

To assess the effect of the potassium depletion protocol on the plasma potassium, in a separate study a group of rats was subjected to the same protocol and at the end of the tube feeding period the rats were anesthetized with sodium amobarbital, 75 mg/kg intraperitoneally, and blood was collected from the aorta in heparinized syringes for determination of plasma potassium. In the rats tube fed dextrose alone ("potassium-depleted group") the plasma potassium was  $2.6 \pm 0.1$  (SE) mEq/liter, and in the rats given dextrose plus KCl ("control group") it was  $3.4 \pm 0.1$  ( $P < 0.01$ ). Mean plasma potassium after an overnight fast in rats on a standard chow diet in our laboratory is  $3.8 \pm 0.1$ .

*Effect of alteration of pH of the incubation medium on glutamate content and glucose and ammonia production of cortical slices.* Three forms of modified Krebs-Ringer buffer were prepared in which the bicarbonate concentration was 12, 24, and 48 mmoles/liter. All three were gassed with 95% O<sub>2</sub>-5% CO<sub>2</sub>, and the pH of these media, as determined with a Radiometer pH meter, was approximately 7.1, 7.4, and 7.7 respectively. The sodium concentration of the three media was adjusted to 144 mEq/liter by altering the amount of NaCl added to the media. Glutamine was added in a concentration of 10 mmoles/liter.

Rats fasted for 18 hr were killed by decapitation and cortical slices from each rat were incubated at pH 7.1, 7.4, and 7.7. After a 90 min incubation, the slices were frozen immediately for determination of glutamate content and the media were analyzed for glucose and ammonia. In addition, each of the three media were incubated without cortical slices, and in calculating ammonia production by cortex, we subtracted the amount of ammonia produced in the absence of tissue at each pH level from the ammonia produced by the cortical slices at that pH; ammonia production in the flasks devoid of tissue was only 4% of that produced in the flasks containing cortex at pH 7.1, 5% at pH 7.4, and 6% at pH 7.7.

*Effect of calcium content of the incubation medium on glutamate content and glucose and ammonia production of cortical slices.* It has been found previously that omission of calcium from the incubation medium decreases the capacity of cortical slices to produce glucose from fumarate and lactate (13). In the present investigation a study has been made of the effect of omission of calcium on aspects of cortical metabolism of glutamine and glutamate. Two

forms of modified Krebs-Ringer bicarbonate were prepared, in which the calcium content was either 0 or 2.5 mEq/liter. Glutamine was added to both of the media in a concentration of 10 mmoles/liter. Rats fasted for 18 hr were sacrificed by decapitation, and cortical slices from each of the rats were incubated in each of the media. After a 90 min incubation period the slices were frozen immediately for determination of glutamate, and the glucose and ammonia content of the media was measured. In some experiments 10 mM glutamate was added to the media instead of glutamine, and the glucose content of the media was determined after a 90 min incubation.

*Effect of theophylline in the incubation medium on the glutamate content, and glucose and ammonia production of cortical slices.* We have observed previously in unpublished studies that theophylline, 0.5 mmoles/liter, decreases the capacity of rat cortical slices to produce glucose from  $\alpha$ -ketoglutarate and oxalacetate, but not from glycerol or fructose.<sup>2</sup> In the present study rats that had been fasted for 18 hr were killed by decapitation, and cortical slices were incubated in modified Krebs-Ringer bicarbonate solution (pH 7.4) containing 10 mM glutamine, with or without theophylline (0.5 mmole/liter). After 90 min incubation the slices were frozen immediately for determination of glutamate, and the glucose and ammonia content of the media was measured. In some experiments 10 mM glutamate was added to the media instead of glutamine, and the glucose content of the media was determined after a 90 min incubation.

## RESULTS

*Effects of metabolic acidosis and alkalosis on cortical glutamate content and on the capacity of cortex to produce glucose and ammonia.* In seven rats fed NH<sub>4</sub>Cl, 27 mmoles/kg per day for 3 days, cortical glutamate was  $3.5 \pm 0.2$  (SE)  $\mu$ moles/wet weight, whereas in seven control rats it was  $5.0 \pm 0.1$  ( $P < 0.001$ ), consistent with previous observations that metabolic acidosis can reduce renal glutamate content (4). Plasma CO<sub>2</sub> in NH<sub>4</sub>Cl-fed rats was  $14.3 \pm 0.3$  (SE) mmoles/liter, and in the control rats it was  $26.2 \pm 0.9$  mmoles/liter.

In rats fed NH<sub>4</sub>Cl, 22 mmoles/kg for only 2 days, plasma CO<sub>2</sub> was  $19.6 \pm 1.0$  (SE) mmoles/liter at the time of sacrifice, as compared to  $24.5 \pm 1.0$  in the control group and  $32.4 \pm 1.0$  in the group fed NaHCO<sub>3</sub>. Mean cortical glutamate was not affected by the feeding of NH<sub>4</sub>Cl or NaHCO<sub>3</sub> in this study (Table I), but nevertheless the cortical capacity for production of ammonia and glucose from glutamine, and the cortical level of phosphate-dependent glutaminase were significantly increased in the NH<sub>4</sub>Cl-fed animals and decreased in the alkali-fed group. These observations indicate that the changes in ammonia production caused by acid-base changes are independent, at least in part, of alterations

<sup>2</sup> The observation that theophylline suppresses cortical gluconeogenesis is surprising, since cyclic AMP stimulates cortical gluconeogenesis (9) and theophylline inhibits the degradation of cyclic AMP. The mechanism by which theophylline decreases cortical gluconeogenesis has not yet been elucidated.

TABLE I  
Effect of Administration of  $\text{NH}_4\text{Cl}$  and  $\text{NaHCO}_3$  for 2 days, on Cortical Production of Ammonia and Glucose In Vitro, Phosphate-Dependent Glutaminase Activity, and Glutamate\*

Experimental group	Production of ammonia from glutamine‡§	Production of glucose from glutamine‡	Phosphate-dependent glutaminase activity	Cortical glutamate concentration		
				At time of sacrifice	After 10 min incubation‡	After 90 min incubation‡
	$\mu\text{moles/g dry wt per 90 min}$	$\mu\text{moles/g dry wt per 90 min}$	$\text{mmoles NH}_3 \text{ produced/g dry wt per 30 min}$	$\mu\text{moles/g wet wt}$	$\mu\text{moles/g wet wt}$	$\mu\text{moles/g wet wt}$
Acidosis ( $\text{NH}_4\text{Cl}$ , 300 mmoles per liter in 20% dextrose, 8 ml twice daily) (n = 6)¶	1535 ± 36 (P < 0.01)¶	264 ± 18 (P < 0.01)	2.5 ± 0.2 (P < 0.001)	5.4 ± 0.4 (NS)	6.8 ± 0.2 (P < 0.01)	8.5 ± 0.1 (P < 0.001)
Control (20% dextrose, 8 ml twice daily) (n = 6)¶	1220 ± 41	153 ± 9	1.3 ± 0.1	5.6 ± 0.3	6.0 ± 0.1	7.0 ± 0.2
Alkalosis ( $\text{NaHCO}_3$ , 450 mmoles per liter in 20% dextrose, 8 ml twice daily) (n = 6)¶	1080 ± 32 (P < 0.02)	115 ± 9 (P < 0.02)	0.79 ± 0.09 (P < 0.01)	6.4 ± 0.2 (NS)	5.2 ± 0.2 (P < 0.01)	6.3 ± 0.2 (P < 0.02)

NS = not significant.

\* All values ± SE.

‡ Incubation in medium containing glutamine, 10 mmoles/liter.

§ Values represent the difference between ammonia production in flasks with cortical slices and ammonia production in flasks without cortical slices.

¶ n indicates the number of rats in each group.

¶ P for difference from control group.

in cortical glutamate content. Interestingly, both 10 min and 90 min after initiation of incubation, the glutamate content of the slices from the  $\text{NH}_4\text{Cl}$ -fed rats was found to be greater than that in the control slices, and glutamate in the slices from the alkali-fed group was sig-

nificantly less than in the control slices; we suspect that the relative rise in glutamate in the slices of the  $\text{NH}_4\text{Cl}$ -fed animals during incubation may have been due to increased production of glutamate from glutamine, secondary to increased glutaminase activity in acidosis, and

TABLE II  
Effect of Potassium Depletion on Urinary Ammonium Excretion, and on Cortical Production of Ammonia and Glucose In Vitro, Phosphate-Dependent Glutaminase Activity, and Glutamate\*

Experimental group	Urinary ammonium excretion	Urine pH	Production of ammonia from glutamine‡§	Production of glucose from glutamine‡	Production of glucose from glutamate	Phosphate-dependent glutaminase activity	Cortical glutamate concentration	
							At time of sacrifice	After 90 min incubation‡
	$\mu\text{moles/4 hr}$		$\mu\text{moles/g dry wt per 90 min}$	$\mu\text{moles/g dry wt per 90 min}$	$\mu\text{moles/g dry wt per 90 min}$	$\text{mmoles NH}_3 \text{ produced/g dry wt per 30 min}$	$\mu\text{moles/g wet wt}$	$\mu\text{moles/g wet wt}$
Control (n = 10)¶	65 ± 9	6.05–6.70	714 ± 21	81 ± 5	84 ± 6	2.1 ± 0.3	4.8 ± 0.2	7.1 ± 0.3
Potassium depleted (n = 10)¶	147 ± 12 (P < 0.001)	6.80–7.30	1100 ± 46 (P < 0.001)	200 ± 12 (P < 0.001)	157 ± 10 (P < 0.001)	3.4 ± 0.3 (P < 0.01)	4.3 ± 0.2 (NS)	9.6 ± 0.2 (P < 0.001)

\* All values ± SE.

‡ Incubation in medium containing glutamine, 10 mmoles/liter.

§ Values represent the difference between ammonia production in flasks with cortical slices and ammonia production in flasks without cortical slices.

|| Incubation in medium containing glutamate, 10 mmoles/liter.

¶ n indicates the number of rats in each group.

TABLE III  
*Effect of pH of the Incubation Medium on Production of Glucose and Ammonia,  
and on Glutamate Content of Rat Cortical Slices\**

pH of incubation medium	Production of ammonia from glutamine‡§	Production of glucose from glutamine‡	Cortical glutamate concentration after 90 min incubation‡
	$\mu\text{moles/g dry wt per 90 min}$	$\mu\text{moles/g dry wt per 90 min}$	$\mu\text{moles/g wet wt}$
7.1 (n = 12)	1138 $\pm$ 26 (NS)¶	152 $\pm$ 5 (P < 0.01)	4.7 $\pm$ 0.1 (P < 0.01)
7.4 (n = 12)	1185 $\pm$ 41	126 $\pm$ 6	5.9 $\pm$ 0.2
7.7 (n = 12)	1161 $\pm$ 25 (NS)	86 $\pm$ 6 (P < 0.001)	7.0 $\pm$ 0.2 (P < 0.01)

\* All values  $\pm$  SE.

‡ Incubation in medium containing glutamine, 10 mmoles/liter.

§ Values represent the difference between ammonia production in flasks with cortical slices and ammonia production in flasks without cortical slices.

|| n indicates number of flasks in each group.

¶ P for difference from flasks incubated at pH 7.4.

that the converse occurred in the slices from the alkalotic animals.

*Effects of potassium deficiency in vivo on cortical glutamate concentration and on the capacity of cortex to produce glucose and ammonia in vitro.* Cortical glutamate in rats depleted of potassium was not significantly different from that of the control group (Table II). However, urinary ammonium excretion in the potassium-depleted group was greatly increased, there was a marked elevation in the cortical capacity to produce ammonia and glucose from glutamine in vitro, and there was an increase in cortical phosphate-dependent glutaminase.<sup>8</sup> These observations indicate that the increase in production of ammonia in potassium depletion is not mediated by a change in cortical glutamate. The glutamate content of the slices at the end of the incubation in glutamine was considerably higher in the potassium-depleted group as compared to the control group, possibly reflecting an increase in the rate of conversion of glutamine to glutamate secondary to increased glutaminase activity in potassium depletion. In the experiments in which the slices were incubated in medium containing glutamate, the conversion of glutamate to glucose was increased in the potassium-deficient group.

\* The cortical slices of the control group (i.e. potassium-repleted group) in this experiment produced considerably less glucose and ammonia from glutamine than did the control group slices in the experiment described in Table I. This may have been due to the marked difference in the diets of the rats in the two studies. In the potassium deficiency experiment the control group was fed a synthetic diet for 21 days before being placed on the tube feeding regimen, whereas the control rats described in Table I were given a standard laboratory chow (Wayne Lab-Blox, Allied Mills, Chicago, Ill.) until initiation of the tube feeding.

*Effect of alteration of pH of the incubation medium on the glutamate content and glucose and ammonia production of rat cortical slices.* Cortical slices incubated in the presence of 10 mM glutamine, produced more glucose when incubated at pH 7.1 and less at pH 7.7 than at pH 7.4 (Table III), as observed previously (14). The glutamate content of the slices at pH 7.1 was significantly decreased, and at pH 7.7 it was significantly increased, yet ammonia production was not affected by alteration of medium pH. These findings suggest that glutamate content is not an important determinant of ammonia production in vitro.

*Effect of calcium content of the incubation medium on glutamate content and glucose and ammonia production of rat cortical slices.* Omission of calcium from the incubation medium sharply decreased cortical glucose production from glutamine and glutamate, but had no effect on the glutamate content of the slices, or on production of ammonia from glutamine (Table IV). This observation indicates that alteration in the rate of cortical gluconeogenesis does not invariably affect glutamate content and production of ammonia.

*Effect of theophylline in the incubation medium on the glutamate content and glucose and ammonia production of cortical slices.* The addition of 0.5 mM theophylline to the incubation medium decreased production of glucose from glutamine and glutamate, yet caused a small but significant decrease in the glutamate content of the cortical slices, and had no effect on production of ammonia (Table V). This experiment illustrates again that alteration in the rate of cortical glucose production does not invariably cause an inverse change in cortical glutamate and affect the rate of ammonia production.

TABLE IV  
*Effect of the Calcium Content of the Incubation Medium on Production of Glucose and Ammonia, and on Glutamate Content of Rat Renal Cortical Slices\**

Calcium concentration of incubation medium	Production of glucose from glutamine‡	Production of glucose from glutamate§	Cortical glutamate content after 90 min incubation‡	Production of ammonia from glutamine‡
	$\mu\text{moles/g dry wt per 90 min}$	$\mu\text{moles/g dry wt per 90 min}$	$\mu\text{moles/g wet wt}$	$\mu\text{moles/g dry wt per 90 min}$
0 (n = 8)¶	52 ± 4	50 ± 9	6.9 ± 0.2	990 ± 26
2.5 mEq/liter (n = 8)	132 ± 9 (P < 0.001)**	110 ± 5 (P < 0.001)	7.5 ± 0.3 (NS)	1070 ± 23 (NS)

\* All values ± SE.

‡ Incubation in medium containing glutamine, 10 mmoles/liter.

§ Incubation in medium containing glutamate, 10 mmoles/liter.

|| Values represent the difference between ammonia production in flasks with cortical slices and ammonia production in flasks without cortical slices.

¶ n indicates number of flasks in each group.

\*\* P for difference from flasks devoid of calcium.

## DISCUSSION

Glutamate is an inhibitor of phosphate-dependent glutaminase (4). It has been suggested previously that the effects of acid-base changes and potassium depletion on ammonia production may be due to alteration in glutaminase activity secondary to changes in renal glutamate content (3, 4, 6). In the present study we have confirmed previous observations that cortical glutamate falls in rats fed substantial doses of acid (4). However, it was observed that doses of acid that were insufficient to decrease cortical glutamate, caused a significant increase in cortical ammonia production, and that feed-

ing of alkali can decrease ammonia production in the absence of a change in glutamate (Table I). Further, potassium depletion caused a substantial increase in urinary ammonia excretion and cortical production of ammonia, in the absence of a change in cortical glutamate (Table II). These observations suggest that the effects of acid-base changes and potassium depletion on renal ammonia production are not dependent upon activation of phosphate-dependent glutaminase secondary to a decrease in cortical glutamate content. However, one cannot exclude the possibility that in mild metabolic acidosis and in potassium depletion there may occur a

TABLE V  
*Effect of Theophylline on Glucose and Ammonia Production and on Glutamate Content of Rat Renal Cortical Slices\**

Theophylline content of incubation medium	Production of glucose from glutamine‡	Production of glucose from glutamate§	Cortical glutamate content after 90 min incubation‡	Production of ammonia from glutamine‡
	$\mu\text{moles/g dry wt per 90 min}$	$\mu\text{moles/g dry wt per 90 min}$	$\mu\text{moles/g wet wt}^\ddagger$	$\mu\text{moles/g dry wt per 90 min}$
0 (n = 12)¶	116 ± 4	140 ± 5	7.3 ± 0.1	1053 ± 15
0.5 mmole/liter (n = 12)	90 ± 4 (P < 0.01)**	114 ± 6 (P < 0.01)	6.5 ± 0.1 (P < 0.001)	1055 ± 28 (NS)

\* All values ± SE.

‡ Incubation in medium containing glutamine, 10 mmoles/liter.

§ Incubation in medium containing glutamate, 10 mmoles/liter.

|| Values represent the difference between ammonia production in flasks with cortical slices and ammonia production in flasks without cortical slices.

¶ n indicates number of rats in each group.

\*\* P for difference from flasks devoid of theophylline.

significant decrease in the concentration of glutamate within the cortical mitochondrial compartment in which phosphate-dependent glutaminase is located (15), that is not reflected in total cortical glutamate concentration.

The concept that cortical glutamate concentration is an important direct determinant of ammonia production from glutamine, is weakened further by our observations on the effects of medium pH and of theophylline on cortical glutamate content and ammoniogenesis (Table III). When cortical slices were incubated at pH 7.1, tissue glutamate content was 40% lower than that in slices incubated at pH 7.7, yet ammonia production at both pH levels was essentially the same (Table III). Similarly, when slices were incubated in the presence of theophylline, cortical glutamate content fell but ammonia production remained unchanged (Table V). Of course, these in vitro studies do not exclude the possibility that cortical glutamate concentration directly affects production of ammonia in vivo. Further, since these studies were of short duration they do not exclude the possibility, suggested by Goldstein and Copenhaver (16), that a prolonged fall in cortical glutamate content, such as occurs in severe metabolic acidosis, could increase ammonia production by accelerating the synthesis of phosphate-dependent glutaminase.

There are at least two possible explanations of the effects of acidosis and potassium deficiency on renal production of ammonia from glutamine. First, a fall in intracellular pH or potassium concentration, by stimulating a key gluconeogenic enzyme such as phosphoenolpyruvate carboxykinase (6, 17), may alter the concentration of a cellular metabolite other than glutamate, which affects the synthesis or specific activity of one of the glutaminase isozymes or glutamine transaminase. Second, a fall in intracellular pH or potassium concentration may directly increase the synthesis or specific activity of these enzymes. Even if the latter hypothesis is correct, in potassium deficiency and acidosis the enhanced conversion to glucose of the immediate or distant products of glutamine degradation may contribute importantly to maintaining increased ammonia production, by preventing the rise in the cellular concentration of these products which might occur otherwise; for accumulation of the products of the glutaminase and glutamine transaminase reactions might limit net ammonia production either by "product repression" of the synthesis of these enzymes (18), by inhibition of phosphate-dependent glutaminase secondary to a marked rise in the cellular concentration of glutamate or one of its derivatives (4), or by accelerated uptake of ammonia through the glutamine synthetase reaction secondary to increased availability of glutamate (19).

It has been suggested previously that the fall in cortical glutamate provoked by severe metabolic acidosis

(4, 5), and by exposure of cortical slices to cyclic AMP, cyclic IMP, or 5'-IMP (9, 10), is due to the fact that these factors enhance conversion of glutamate to glucose. However, in the present study it was observed that in some experimental situations cortical gluconeogenesis can be altered substantially without an associated inverse change in glutamate. For example, cortex from potassium-deficient rats was found to have a 90% increase in the capacity to convert glutamate to glucose, yet cortical glutamate in these rats was not significantly different from that in the control group (Table II). Further, in in vitro experiments the inclusion of calcium in the incubation medium caused a substantial increase in cortical conversion of glutamate to glucose without decreasing glutamate content (Table IV); and in the experiment depicted in Table V, conversion of glutamate to glucose was higher in the medium devoid of theophylline, yet cortical glutamate was increased rather than decreased. These observations do not exclude the possibility that the decrease in cortical glutamate effected by severe metabolic acidosis and certain nucleotides is due to the increase in gluconeogenesis provoked by these factors, but the present studies indicate that in some situations processes other than gluconeogenesis play a major role in determining cortical glutamate content.

It may be pertinent that in kidney cortex both the Krebs cycle and the hexose monophosphate shunt appear to be operative (20, 21), and therefore it is likely that glutamate can be removed by oxidation to  $\text{CO}_2$  through these pathways as well as by conversion to glucose. Presumably the intracellular concentration of glutamate is determined in part by the rate of removal of glutamate, and in some situations the over-all removal rate could remain unchanged or even decrease despite increased conversion of glutamate to glucose, if there were a concomitant fall in the rate of oxidation of glutamate to  $\text{CO}_2$ . A second possible explanation for the lack of a constant inverse relationship between cortical gluconeogenesis and glutamate content is that in some situations there may occur a change in the cortical NAD/NADH<sub>2</sub> ratio, which could affect glutamate concentration independent of changes in rate of gluconeogenesis (22). A third factor which may affect cortical glutamate concentration is the rate of generation of glutamate from glutamine. For example, in potassium deficiency the failure of cortical glutamate to fall, in the face of a marked increase in the capacity of the cortex to convert glutamate to glucose (Table II), may be due to increased generation of glutamate from glutamine in this state, secondary to increased glutaminase activity.

In summary, the findings in the present study suggest that cortical glutamate concentration is not a major determinant of the rate of production of ammonia from glutamine. Further, it appears that factors other than



gluconeogenesis may play a significant role in the regulation of cortical glutamate content. Nevertheless, the increase in cortical gluconeogenesis effected by metabolic acidosis, potassium deficiency, and certain adenosine and inosine nucleotides, may contribute importantly to the increase in ammonia production induced by these factors, by enhancing removal of the products of glutamine degradation.

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