The Effect of Glutamine Administration on Urinary Ammonium Excretion in Normal Subjects and Patients with Renal Disease

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ABSTRACT The effect of acute changes in the delivery rate of glutamine to the kidney on urinary ammonium excretion was studied in man. Healthy subjects and patients with intrinsic renal disease were studied under three different acid-base conditions: unaltered acid-base balance; NH_4Cl-induced acidosis; and $NaHCO_3$-induced alkalosis. Anhydrous L-glutamine was administered orally in a single dose of 260 mmoles during each of these three acid-base states. We found that endogenous venous plasma glutamine concentration fell during acidosis and rose during alkalosis in both healthy subjects and patients with renal disease. In healthy subjects, orally administered glutamine raised plasma glutamine concentration markedly over a 2–3 hr period. This was accompanied by an increase in urinary ammonium excretion and a rise in urine pH under normal acid-base conditions and during metabolic acidosis. No increase in ammonium excretion occurred when glutamine was administered during metabolic alkalosis in spite of an equivalent rise in plasma glutamine concentration. In patients with renal disease, endogenous venous plasma glutamine concentration was lower than in healthy subjects, perhaps as a result of mild metabolic acidosis. Acute oral glutamine loading failed to increase urinary ammonium excretion significantly during either unaltered acid-base conditions or after NH_4Cl-induced acidosis, even though plasma glutamine rose as high as in healthy subjects. We conclude from these observations that glutamine delivery to the kidney is a rate-limiting factor for ammonium excretion in healthy subjects, both before and after cellular enzyme adaptation induced by metabolic acidosis. In contrast, in patients with renal disease, glutamine delivery is not rate-limiting for ammonium excretion. Presumably other factors, such as surviving renal mass and the activity of intracellular enzymes necessary for ammonia synthesis limit ammonium excretion in these patients.

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INTRODUCTION

Glutamine is the major substrate for ammonia produced by the kidney of rat (1), dog (2), and man (3). Under normal acid-base conditions, it is consistently extracted by the kidney, and during metabolic acidosis induced by ammonium chloride, renal extraction of glutamine rises so that its two nitrogens can account for all or almost all of the total ammonia produced by the kidney of man and dog (3–6). I.v. infusion of glutamine in dogs results in a rise in renal ammonium excretion under normal acid-base conditions and metabolic acidosis (6–9). Since virtually all of the glutamine is reabsorbed by the dog kidney under these loading conditions (9), the increased availability of glutamine to the tubular cells is presumably responsible for the rise in ammonia production.

Less is known about the effect of variations in the amount of glutamine delivered to the kidney on ammonium excretion in man. Madison and Seldin (10) administered a number of different amino acids orally, including glutamine, to normal male subjects maintained on chronic ammonium chloride loads of various magnitudes. They found that glycine, D-alanine, L-alanine, L-leucine, glutamine, and asparagine all significantly increased urinary ammonium excretion under these experimental conditions. Because plasma levels of the amino acids were not measured, however, it is not possible to evaluate the effect of liver metabolism on the ingested amino acids and thus the change in delivery rate of the specific amino acid to the kidney. Precursor administration studies have not been carried out in patients

TABLE I

Clinical Features of the Patients with Renal Disease

Patient	Age	Duration of disease	BP	Proteinuria	BUN	Endogenous creatinine clearance	Plasma CO_2	Diagnosis
	yr	*yr*	*mm Hg*	*g/24 hr*	*mg/100 ml*	*ml/min*	*mmole/liter*	
R. G.	58	>9	150/100	3.5	78	12	13.9	Chronic glomerulonephritis*
E. R.	35	>6	145/95	4.8	81	21	23.5	Chronic glomerulonephritis*
N. S.	31	3½	120/90	1.2	76	11	21.7	Chronic glomerulonephritis‡
E. L.	40	7	140/95	3.9	32	68	24.7	Irregular glomerular sclerosis*
L. M.	32	>1	160/110	4.8	79	8	21.1	Chronic glomerulonephritis‡

* Clinical diagnosis.

‡ Biopsy diagnosis.

with intrinsic renal disease. Such patients often have marked impairment in their ability to excrete ammonium (11–13), in spite of the presence of a chronic metabolic acidosis and a low urine pH, both of which should enhance ammonium excretion (14, 15). The low rate of ammonium excretion has been attributed to a reduced number of intact functioning nephrons (16, 17). Since renal ammonia production in the dog varies directly with changes in glomerular filtration rate (GFR)[1] and renal blood flow (RBF) (9), it seems possible that a contributing factor in these patients is a reduced rate of delivery of glutamine to the kidney, due to the altered renal hemodynamics.

The purpose of the present investigation was to study the effect of acute oral administration of glutamine on blood levels of glutamine in normal subjects and patients with advanced renal disease, and to determine the effects of this load on ammonium excretion under various acid-base states. We found that orally administered glutamine raises venous plasma glutamine concentration equally in healthy subjects and patients with renal disease. In the healthy subjects, ammonium excretion increased during normal acid-base balance and metabolic acidosis. In contrast, the patients with intrinsic renal disease failed to respond to the glutamine, even after ammonium chloride ingestion for 4 days. These observations suggest that glutamine delivery to the kidney is a rate-limiting factor for ammonia production in normal subjects, but not in patients with advanced intrinsic renal disease.

METHODS

Studies were carried out in six normal male volunteers (ages 25–35) and in five patients with a history and laboratory evidence of advanced intrinsic renal disease. The cardinal clinical features of the five patients with renal disease are presented in Table I. These patients demonstrated no evidence of congestive heart failure, pulmonary disease, or diabetes mellitus. Routine liver function tests were normal. None of the patients had undergone peritoneal dialysis or hemodialysis before the study. They took no medications during the time of this study.

All studies were carried out in the Clinical Research Center of Bellevue Hospital. The subjects were allowed regular diets except during the 12 hr period preceding glutamine administration, when food but not fluids was withheld. An initial period of 3 or 4 days after admission to the Research Center was used to obtain baseline laboratory values. During this period, 24-hr urine samples were collected under oil with thymol preservative for determination of creatinine, pH, and ammonium. Venous blood was drawn daily from an antecubital vein after release of the tourniquet for measurements of total CO_2, pH, creatinine, and glutamine concentration. Since red blood cell glutamine concentrations in man are quite close to plasma concentrations (18), rapid separation of the red cells was not critical. Nevertheless, blood samples were centrifuged within 5 min after collection.

Stage I: unaltered acid-base balance. After the baseline period of 3–4 days, food was withheld overnight and on the following morning the subject was given 1 liter of water to drink over a 20 min period. Timed urine collections and blood samples were then obtained at regular intervals over the next 3 hr. The subject was then given 260 mmole of anhydrous L-glutamine dissolved in 1 liter of water to drink over a 15–20 min period. Additional timed urine collections and blood samples were then collected over the following 3 hr.

Stage II: metabolic acidosis. On the day after completion of stage I, each healthy subject ingested 12 g of ammonium chloride daily in divided doses for 4 days. Of the patients with renal disease E. R. and E. L. received the same amount of ammonium chloride whereas L. M. and N. S. developed a moderate degree of acidosis (total CO_2 = 15 mmole/liter) on smaller amounts. The dose in these latter two patients was therefore varied from day to day in an attempt to maintain plasma CO_2 at a level of approximately 15 mmole/liter. R. G. had been taking small amounts of $NaHCO_3$ before admission to the study. When this was stopped he gradually reached a steady level of acidosis with a plasma CO_2 of 14 mmole/liter, so that ammonium chloride was not given to this patient.

24-hr urine collections were made during the 4 days of NH_4Cl ingestion, and daily blood samples were obtained. On the morning of the 5th day after ammonium chloride ingestion had been started, the subjects were hydrated orally

[1] *Abbreviations used in this paper:* C_{H_2O}, free water clearance; C_{osm}, osmolar clearance; GFR, glomerular filtration rate; RBF, renal blood flow.

with 1 liter of water and control urine collections and blood specimen were obtained over a 3 hr period, as described for stage I. L-glutamine, 260 mmole, was then given by mouth, and additional urine and blood samples were collected for the next 3 hr period. After this day of acute studies with glutamine loading, NH_4Cl administration was stopped and 24-hr urine collections and blood samples were obtained for an additional 5 days of recovery.

Stage III: metabolic alkalosis. In the third stage of the study, the healthy subjects and one of the patients with renal disease (R. G.) were given 8 g of $NaHCO_3$ by mouth daily in divided doses for 4 days. During this period, 24-hr urine and venous blood specimens were collected, as in stages I and II. On the morning of the 5th day, control urine and blood samples were obtained as described above, after which 260 mmole L-glutamine was given by mouth. Additional urine and blood samples were collected for the next 3 hr.

All urine samples obtained on the days of glutamine administration and all plasma samples were analyzed immediately. 24-hr urine specimens were frozen until analyzed. Urinary ammonium was determined in triplicate by a modification of the Berthellot reaction (19). Urine and blood pH was measured with a Beckman pH meter (Beckman Instruments, Inc., Fullerton, Calif.). Plasma total CO_2 was measured manometrically with a Natelson gas analyzer (Scientific Industries, Inc., Springfield, Mass.) (20) and urinary sodium and potassium were measured by flame photometry. In two of the healthy subjects, urine and plasma osmolality was measured by freezing point depression before and after glutamine loading. Osmolar clearance was calculated as $C_{osm} = U/P_{osm} \times V$ and free water clearance was calculated as $C_{H_2O} = V - C_{osm}$. Plasma glutamine concentration was measured as follows: 1 ml of plasma was incubated at 37°C in 0.5 ml acetate buffer (pH 4.9) containing sufficient glutaminase enzyme (glutaminase obtained from Worthington Biochemical Corp., Freehold, N. J.); another 1 ml of plasma, without enzyme, served as a blank. After 30 min of incubation, proteins were precipitated using tungstic acid (21). Duplicate 1 ml portions of the two plasma preparations were pipetted into 1 ml phenol, followed by 1 ml of NaOH reagent (22). The mixture was incubated at 37°C and read at 630 mμ. Standards were composed of 1 μmole/ml NH_4^+ and 1 μmole/ml glutamine. Glutamine was calculated by subtracting the plasma blank and enzyme blank from glutamine NH_3 optical density. The difference in optical density after subtracting the blanks represents the contribution of amide nitrogen of glutamine (23). Creatinine in plasma and urine was measured by the method of Bonsnes and Taussky (24) and endogenous creatinine clearance (U/P creatinine × urine flow rate) was taken as an approximation of GFR.

In the statistical analyses which appear in the text, paired *t* test was employed for observations in individual subjects, as each subject served as his own control for the acid-base alterations and glutamine loading. Where comparisons are made between the two groups of subjects, unpaired *t* test analysis was employed. Data are expressed as mean ±SEM.

RESULTS

In Fig. 1 are plotted the data on plasma CO_2, endogenous plasma glutamine concentration, and 24 hr urinary ammonium excretion in the healthy subjects and patients with renal disease before and during the 4 days of NH_4Cl ingestion, and 5 days of recovery. The first points on the figure represent the means of control data obtained before NH_4Cl ingestion. As can be seen, NH_4Cl ingestion by the healthy subjects caused a moderate fall in plasma CO_2 from a mean control value of 26.8 mmoles/liter to a low of 22.3 mmoles/liter on the 4th day. Urinary ammonium excretion rose significantly from a control value of 39±7 mmoles/day to 107±6 mmoles/day on the 2nd day and 156±8 mmoles/day on the 4th day of ingestion. After NH_4Cl administration was stopped, plasma CO_2 and urinary ammonium excretion returned gradually to their control levels over the next 3–5 days. Endogenous venous plasma glutamine concentration averaged 0.544±0.056 μmole/ml during the control days. Ammonium chloride loading caused a significant ($P < 0.01$) fall in plasma glutamine to a low of 0.305 μmole/ml on the 4th day. The level returned gradually to the control value 4 days after NH_4Cl ingestion had been stopped. It should be noted that the lowest plasma glutamine level corresponded in time with the highest rate of urinary ammonium excretion.

In the patients with renal disease, the control plasma CO_2 level averaged 21.4±2.2 mmoles/liter and fell to a low of 13.7±0.4 mmoles/liter on the day after NH_4Cl loading had been completed. In contrast to the healthy subjects, the patients with renal disease excreted only 13±3 mmoles ammonium per day and were unable to increase 24 hr urinary ammonium excretion significantly during NH_4Cl loading. Endogenous plasma glutamine concentration was significantly ($P < 0.02$) lower in the patients with renal disease than in the healthy subjects during the control and recovery periods (0.422±0.056 vs. 0.544±0.056 μmole/ml), and fell further during NH_4Cl loading. As can be seen, the fall in plasma glutamine was not associated with any detectable change in urinary ammonium excretion.

In Fig. 2 are plotted the relationship between urinary ammonium excretion and pH in the healthy subjects and the patients with renal disease. The urine samples were timed collections obtained over the 3 hr period just before oral glutamine loading, during the three different stages of the study. It can be seen that the patients with renal disease excreted considerably less ammonium than the healthy subjects over the entire range of urinary pH. The healthy subjects increased their ammonium excretion markedly after 4 days of NH_4Cl ingestion. In contrast, the patients with renal disease demonstrated only a small but statistically insignificant ($P > 0.1$) rise in ammonium excretion after 4 days of NH_4Cl, even though they were able to acidify their urine to a pH as low as 5.0.

The relationship between urinary ammonium excretion, urine pH, and renal mass, as approximated by endogenous creatinine clearance, is shown in Fig. 3. These data were obtained from the same urine collections as shown

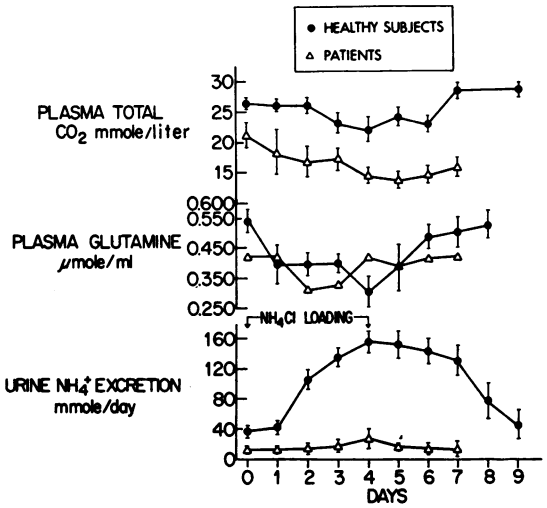

FIGURE 1 The effect of chronic NH_4Cl ingestion on endogenous plasma glutamine and 24 hr urinary ammonium excretion in healthy subjects and patients with renal disease. The vertical bars represent ±1 SEM.

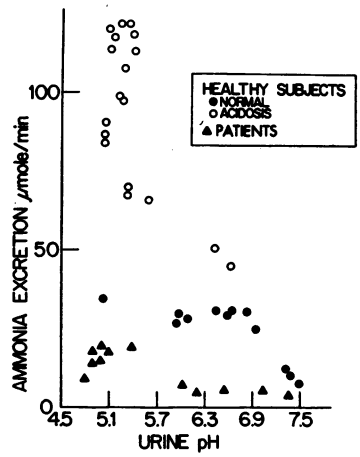

FIGURE 2 The relationship between urinary pH and ammonium excretion in healthy subjects and patients with renal disease. The data were obtained during spontaneous acid-base balance, during NH_4Cl ingestion and $NaHCO_3$ ingestion in both groups of subjects.

in Fig. 2. Under normal acid-base conditions, healthy subjects excreted a mean of 0.21±0.02 μmole of ammonium per ml of creatinine clearance, and had a mean urine pH of 5.87±0.24. Chronic oral NH_4Cl loading resulted in an almost four-fold rise in ammonium excretion to 0.79±0.12 μmole/ml creatinine clearance and a fall in mean urine pH to 5.05±0.10. Chronic $NaHCO_3$ administration resulted in a fall in ammonium excretion to 0.097 μmole/ml creatinine clearance and a rise in urine pH to 7.43. In the patients with renal disease, ammonium excretion averaged 0.67±0.15 μmole/ml creatinine clearance and urine pH was 6.01±0.30 under their ordinary conditions of acid-base balance. The difference in urine pH between the healthy subjects and patients with renal disease (5.87 vs. 6.01) was not statistically significant whereas the difference in ammonium excretion per milliliter creatinine clearance was ($P < 0.001$). The higher ammonium excretion per unit of creatinine clearance in the patients with renal disease may have been due in part to a moderate degree of metabolic acidosis (plasma CO_2 23.1 mmoles/liter vs. 26.8 mmoles/liter). After 4 days of NH_4Cl ingestion, plasma CO_2 was 15.8±1.3 mmoles/liter in the patients with renal disease on the day of the acute studies, and urine pH averaged 5.46±0.31. Urinary ammonium excretion increased slightly from 0.67±0.15 to 1.00±0.15 μmole/ml creatinine clearance, but the change was not statistically significant ($P > 0.05$). Sodium bicarbonate loading in one patient led to a rise in urine pH and a fall in urinary ammonium excretion to the same levels as seen in the healthy subjects. The observations shown in this figure indicate that under unaltered acid-base conditions (control), patients with renal disease excrete much more ammonium per unit of functioning renal mass than healthy subjects. After adaptation to acidosis, or alkalosis, however, the values for the two groups of subjects become more nearly comparable if not identical.

The effect of acute oral administration of glutamine on venous plasma glutamine levels in healthy subjects during the three different acid-base conditions is shown in Fig. 4. Initially, before ingestion of glutamine, plasma glutamine concentration was significantly elevated during metabolic alkalosis (0.680±0.044 vs. 0.544±0.056 μmole/ml) and was significantly reduced during metabolic acidosis (0.367±0.066 μmole/ml) ($P < 0.01$). After glutamine ingestion, plasma glutamine levels rose

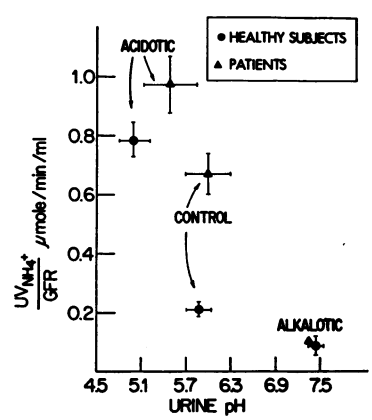

FIGURE 3 Urinary ammonium excretion divided by GFR (endogenous creatinine clearance). The vertical and horizontal bars represent ±1 SEM. The data were obtained during spontaneous acid-base balance (control), and during NH_4Cl acidosis and $NaHCO_3$ alkalosis.

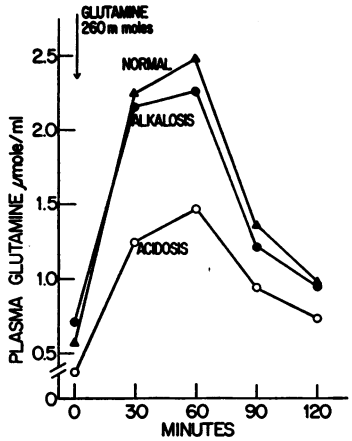

FIGURE 4 The effect of ingestion of L-glutamine on plasma glutamine concentration in healthy subjects during three different acid-base states.

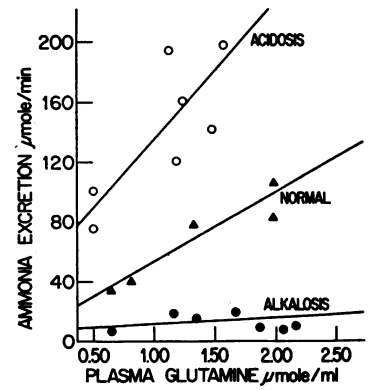

FIGURE 5 The effect of acute increases in plasma glutamine concentration on urinary ammonium excretion in healthy subjects during three different acid-base states.

rapidly in all instances, reaching peak levels between 30 and 60 min after ingestion had been completed. The levels then declined to near control values over a 2 hr period. During the first 60 min, plasma glutamine was significantly higher under conditions of normal acid-base balance or alkalosis than during metabolic acidosis. These observations demonstrate that large amounts of anhydrous L-glutamine can be absorbed from the intestinal tract and acutely raise the peripheral venous plasma glutamine level. Presumably, liver metabolism did not prevent the absorbed glutamine from reaching the peripheral circulation.

The effect of the acute glutamine load on plasma CO_2, urine pH, and ammonium excretion in the healthy subjects is summarized in Fig. 5 and Tables II and III. As can be seen in Table II, plasma CO_2 showed a slight tendency to rise after glutamine administration in stages I and II, but the changes were not statistically significant. Urine pH also rose slightly after glutamine administration. This was associated with an increase in urine flow rate, probably the consequence of the ingestion of 1 liter of fluid with the glutamine. In spite of the rise in urine pH, urinary ammonium excretion increased markedly under both normal acid-base conditions and during chronic metabolic acidosis. In sharp contrast, urinary ammonium excretion was unaltered by glutamine ingestion in the presence of $NaHCO_3$-induced metabolic

TABLE II

Effect of Oral Glutamine Administration on Urine NH_4^+ Excretion, Plasma Glutamine and Plasma CO_2 in Healthy Subjects

		Plasma		Urine		
		CO_2	Glutamine	Flow	pH	$U_{NH_4}V$
		mmole/liter	*μmole/ml*	*ml/min*		*μmole/min*
Stage I (Normal)	Preload*	26.8±1.1	0.544±0.056	2.4±0.5	5.87±0.24	27.2±3.3
	Postload‡	27.3±1.8	1.360±0.286‡	4.2±0.6	6.22±0.22	49.9±7.8‡
Stage II (Acidosis)	Preload	20.9±2.7	0.367±0.066	1.7±0.6	5.05±0.10	104.8±14
	Postload	22.1±4.0	1.022±0.059‡	3.0±1.1	5.31±0.10‡	152.3±23‡
Stage III (Alkalosis)	Preload	28.4±0.4	0.680±0.044	2.2±0.4	7.41±0.10	7.85±2.43
	Postload	28.2±0.9	1.350±0.295‡	4.5±0.2	7.56±0.03	7.92±1.67

* Preload values represent mean±SEM of measurements immediately before oral glutamine.

‡ Postload values represent mean±SEM of measurements during 180 min after ingestion of 260 mmole glutamine.

§ Difference between pre- and postload periods significant $P < 0.05$.

TABLE III

Typical Response of a Healthy Subject to Oral Glutamine Loading

Time	Plasma		Urine					
	CO_2	Glutamine	Flow	pH	$U_{NH_4}V$	Creatinine clearance	C_{osm}	C_{H_2O}
min	*mmole/liter*	*μmole/ml*	*ml/min*		*μmole/min*	*ml/min*	*ml/min*	*ml/min*
0–180	25.7	0.535	2.3	6.00	22.3	106	2.4	−0.1
185–205: 260 mmole L-glutamine in 1 liter water ingested								
205–265			4.1	6.21	49.2	138	2.8	1.3
265–325	29.5	1.320	10.0	6.37	79.0	106	2.9	7.1
325–385			6.8	6.48	91.5	115	2.7	4.1

alkalosis, in spite of a comparable rise in plasma glutamine concentration. A more detailed analysis of a typical response to glutamine administration in a healthy subject under normal acid-base conditions is shown in Table III. As can be seen, plasma CO_2 and urine pH rose after glutamine administration, whereas creatinine clearance and osmolar clearance (C_{osm}) remained relatively constant. Free water clearance (C_{H_2O}) increased sharply. Ammonium excretion increased from 22.3 to 91.5 μmoles/min during the 3rd hr after glutamine ingestion, in spite of the rise in plasma CO_2 and urine pH.

The relationship between acute changes in plasma glutamine concentration and urinary ammonium excretion in the healthy subjects is shown in more detail in Fig. 5. The lines are the calculated linear regression lines. The slope of the regression line was 0.48 under normal acid-base conditions and was 1.00 during metabolic acidosis. In contrast, no significant relationship between urinary ammonium excretion and plasma glutamine was apparent during $NaHCO_3$-induced alkalosis. These observations suggest that cellular adaptations to acid-base changes modify the renal response to glutamine in healthy subjects.

The results of the acute glutamine loading experiments in the patients with renal disease are shown in Table IV. In contrast to the healthy subjects, no change in plasma CO_2 was obsreved after glutamine ingestion in any of the three stages of acid-base balance studied. A second difference between these patients and the healthy subjects is that urine pH showed no tendency to rise after glutamine administration. Finally, urinary ammonium excretion, which was much less in absolute amount than in the healthy subjects, showed only a slight tendency to rise after glutamine ingestion but the change was not statistically significant during either unaltered acid-base conditions or after NH_4Cl loading for 4 days. Ammonium chloride administration per se resulted in some increase in ammonium excretion, from 16.7 to 31.4 μmoles/min, but because of the relatively large variability, the difference was not statistically significant.

TABLE IV

Effect of Oral Glutamine Administration on Urinary NH_4^+ Excretion, Plasma Glutamine, and Plasma CO_2 in Five Subjects with Advanced Renal Disease

		Plasma		Urine		
		CO_2	Glutamine	Flow	pH	$U_{NH_4}V$
		mmole/liter	*μmole/ml*	*ml/min*		*μmole/min*
Stage I	Preload	22.8±1.0	0.439±0.031	1.1±0.1	6.22±0.22	16.7±8.9
	Postload	22.7±1.2	1.612±0.293*	2.4±0.9	6.10±0.15	24.3±14.0
Stage II (Acidosis)	Preload	15.8±1.3	0.349±0.031	1.3±0.1	5.61±0.35	31.4±15.0
	Postload	15.9±1.6	1.610±0.288*	2.0±0.6	5.31±0.29	39.5±22.8
Stage III (Alkalosis)	Preload	29.1	0.312	1.7	7.27	1.38
	Postload	27.8	2.140	1.7	7.36	4.70

* Difference between pre- and postload values significant $P < 0.05$.

DISCUSSION

It is well established that patients with advance intrinsic renal disease often have an impairment in their ability to excrete ammonium in the urine, in spite of a normal ability to acidify the urine (11–13, 25). All the patients in the present study demonstrated this defect (Fig. 2), although only one of them (R. G.) had a clinically significant degree of metabolic acidosis. Since the degree of renal insufficiency was no worse in this patient than in two others (N. S. and L. M.) who were able to maintain a higher plasma [HCO_3], it is possible that R. G. had some form of "proximal" renal tubular acidosis (26). Detailed studies were not made, however, to establish this diagnosis, and in the other patients there was no clinical evidence to suggest overt renal tubular acidosis. Thus, with the possible exception of R. G., the metabolic defect in ammonium excretion observed in our patients seemed to be similar to that which has been described in other patients with advanced intrinsic renal disease.

The cause of the impairment in ammonium excretion in patients with renal disease is unknown, but several possibilities can be considered. First, it is conceivable that ammonia production by the diseased kidney is not reduced, but that for some reason relatively more of the NH_3 produced in the tubular cells diffuses into the peritubular capillaries and venous blood and less into the tubular urine. To our knowledge, total ammonia production rates have not been measured in patients with renal disease. However, this possibility seems somewhat remote for several reasons. The distribution of NH_3 between the renal blood and urine is thought to occur by a passive diffusion process dependent largely upon pH gradients (5, 15). Since NH_3 is highly diffusible, and since blood and urine pH were approximately the same in the patients as in the healthy subjects, there is no reason to suspect that there would be relatively greater diffusion into the vascular compartment than into the urine. In fact, the opposite might be the case. It has been found in normal subjects that urea or mannitol diuresis causes an acute rise in urinary ammonium excretion (27). The mechanism probably involves enhanced trapping of ammonium in the tubular lumen due to the osmotic effects of the two solutes (27). If the surviving nephrons of patients with renal disease are undergoing an endogenous osmotic diuresis, this might also favor diffusion of NH_3 into the tubular urine at the expense of diffusion into the blood. In addition, Owen and Robinson (3) found that chronic metabolic acidosis in normal man causes an increase in renal NH_3 production, but that the increase is manifested by a rise in ammonium excretion without any increase in renal vein ammonium. Since patients with renal disease are often mildly acidotic, a similar distribution of ammonia favoring urinary excretion might occur. Finally, as shown in Fig. 3, ammonium excretion per unit of functioning renal mass was considerably higher in the patients with renal disease than in the control subjects. If there were increased shunting of ammonia into the renal venous blood, ammonia production per unit of functioning mass would have to be even higher than is suggested by the data shown in this figure.

A second possible cause of decreased ammonium excretion in patients with renal disease is that limitations in urine flow rate might limit ammonium excretion. From the observations shown in Table IV, it is clear that the patients' urine flow rates did not rise as much after the glutamine and water load as did the healthy subjects. However, changes in water excretion per se do not have any marked effect on ammonium excretion in normal man (27) or dog (15), at least in the acid range of urine pH. Thus, it seems unlikely that differences in urine flow rate between the healthy subjects and the patients can account for the differences in ammonium excretion.

A third possible cause for diminished ammonium excretion is the reduced RBF and GFR that occurs in patients with renal disease. Pilkington, Young, and Pitts (9) found that ammonia production by the dog kidney varies directly with changes in RBF and GFR induced acutely by a balloon in the abdominal aorta. Thus, the rate of delivery to the kidney of substrates necessary for ammonia production, principally glutamine, appears to limit ammonia production in the dog kidney (6, 9) and might play an important role in the impairment of ammonium excretion in patients with renal disease. It was to test this possibility that large glutamine loads were administered to the patients in this study and to a group of healthy control subjects.

As shown in Fig. 4 and Table IV, orally administered glutamine effectively raised venous plasma glutamine concentration approximately four-fold in both the healthy subjects and the patients. In the healthy subjects, metabolic acidosis prevented the blood level from reaching as high a concentration as in normal acid-base balance or metabolic alkalosis (Fig. 4). The reason for this is uncertain, but might relate to the demonstrated increase in renal extraction and utilization of glutamine during metabolic acidosis in healthy man (3). The peak blood glutamine levels in the patients with renal disease were as high or even higher than in the normal subjects (Table IV vs. Table II) and in contrast to the healthy subjects did not seem to be affected by the presence of metabolic acidosis. The response to the glutamine load, however, was quite different in the two groups of subjects.

In the healthy subjects, urinary ammonium excretion increased promptly, and the increase was correlated with the venous plasma level of glutamine. Pitts and

Pilkington (6) found a similar correlation between arterial blood glutamine concentrations and ammonium excretion in dogs given acute loads of glutamine i.v. As can be seen in Fig. 5, a greater rise in urinary ammonium occurred for any given blood level of glutamine in the acidotic subjects than in the same subjects when they were studied under normal acid-base conditions. Kidney tissue glutamine level reflects plasma levels after glutamine loading in the rat (28) and presumably a rise in renal tissue glutamine also occurred in the healthy subjects in the present study. The greater rise in ammonium excretion at each level of plasma glutamine in the acidotic subjects is in accord with the observation that glutamine extraction by the kidney is increased in acidotic man (3) and dog (9). In the face of a four- to five-fold increase in substrate availability, ammonia production would most likely be limited by cellular enzyme activity. In support of this interpretation, glutaminase I activity in the rat kidney is greatly reduced in metabolic alkalosis (29), and in our studies ammonium excretion did not increase in response to the glutamine load in the presence of alkalosis. Madison and Seldin (10) also found that precursor administration failed to increase ammonium excretion in alkalotic human subjects. The observations shown in Fig. 5 are thus consistent with the view that in normal man, the level of enzyme activity plays a major role in setting the level of ammonium excretion. During normal acid-base conditions and metabolic acidosis, the amount of glutamine available to the kidney can alter the rate of ammonium excretion within the limits set by cellular enzyme activity.

In contrast to the healthy subjects, the patients with renal disease responded only minimally to the orally administered glutamine. This was true under both spontaneous acid-base conditions and after 4 days of ammonium chloride-induced acidosis. These observations suggest that factors other than the availability of substrate limit ammonia production in patients with renal disease. The possibility must be considered, however, that the diseased kidney is unable to extract glutamine, and that this accounts for impaired ammonium excretion. Although this possibility cannot be excluded without direct measurements of glutamine extraction, two observations argue against such an interpretation. First, endogenous glutamine levels in the peripheral venous blood were lower rather than higher in the patients with renal disease. Impaired glutamine extraction might be expected to lead to higher venous blood levels. Second, from the data shown in Fig. 3, ammonium excretion per unit of functioning renal mass was much higher in the patients than in the control subjects. If glutamine is the main precursor of ammonia production in the presence of renal disease, these observations suggest that endogenous glutamine uptake by the surviving nephrons is not impaired and may actually be enhanced. An acute increase in the delivery rate of glutamine to the kidney under these conditions might be expected to elevate urinary ammonium excretion provided that the glutamine metabolizing system is not already saturated by endogenous glutamine. The fact that this did not occur leads us to conclude that a reduced supply of glutamine was not the rate limiting factor for ammonium excretion in these patients. The data shown in Fig. 3 seem most consistent with the view that ammonia production is enhanced to near maximal levels in the surviving nephrons of the diseased kidney. That this enhancement might be due to cellular enzyme adaptation is suggested by the fact that after enzyme adaptation in the normal subjects (NH_4Cl loading), ammonium excretion per unit of functioning renal mass increased to values almost identical to those in the patients. Similarly, after $NaHCO_3$-induced alkalosis, ammonium excretion per unit of functioning mass became closely comparable in the two groups (Fig. 3). Van Slyke and co-workers (2) found that renal extraction of glutamine is essentially zero in the presence of alkalosis, and this might relate to the marked reduction in renal glutaminase I activity induced by alkalosis (29). Thus, the diseased kidney behaves as though enzyme activity in the surviving nephrons is capable of adapting to acid-base changes. The degree of cellular enzyme activity seems to be the rate-limiting factor in determining ammonium excretion rather than the supply of glutamine.

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