Renal Ammoniagenesis in an Early Stage of Metabolic Acidosis in Man

ALBERTO TIZIANELLO, GIACOMO DEFERRARI, GIACOMO GARIBOTTO, CRISTINA ROBAUDO, NICOLA ACQUARONE, and GIAN MARCO GHIGGERI, *Istituto Scientifico di Medicina Interna, Servizio di Nefrologia, University of Genoa, 16132, Genoa, Italy*

ABSTRACT Total renal ammonia production and ammonia precursor utilization were evaluated in patients under normal acid-base balance and in patients with 24-h NH_4Cl acidosis by measuring (*a*) ammonia excreted with urine and that added to renal venous blood, and (*b*) amino acid exchange across the kidney. In 24-h acidosis not only urinary ammonia excretion is increased, but also total ammonia production is augmented ($P < 0.005$) in comparison with controls. By evaluating the individual role of acid-base parameters, urine pH and urine flow in influencing renal ammonia production, it was shown that the degree of acidosis and urine flow are likely major factors stimulating ammoniagenesis. Both urine pH and urine flow are determinant in the preferential shift of ammonia into urine. In 1-d acidosis, renal extraction of glutamine was not increased and the total ammonia produced/glutamine N extracted ratio was higher than in controls ($P < 0.005$) and was inversely correlated with the log of arterial bicarbonate concentration ($P < 0.001$). In the same condition, renal glycine and ornithine uptake took place; the more severe the acidosis, the greater was the renal extraction of these amino acids ($P < 0.001$). These data indicate that at the early stages of metabolic acidosis, in spite of a brisk increase in ammonia production, the mechanisms responsible for the increased glutamine use, which are operative in chronic acidosis, are not activated and other ammonia precursors, besides glutamine, are probably used for ammonia production.

INTRODUCTION

It is well known that in man and in other mammals chronic metabolic acidosis induces an increase in total renal ammonia production (1–4) and that this increase is associated with an augmented extraction of glutamine by the kidney (1–6). However, mechanisms controlling renal ammoniagenesis in metabolic acidosis and the development of the renal adaptive response to acidosis are still controversial (7–9).

In man, urinary ammonia excretion is already significantly increased 2 h after the administration of an acid load (10–12), but it attains the highest values only within 5–6 d after the onset of acidosis (10, 13). Because renal production of ammonia, namely that excreted plus that added to renal venous blood, was never measured in man at the initial stages of metabolic acidosis, it is still uncertain whether the increase in urinary ammonia excretion is merely the consequence of an enhanced trapping of ammonia into urine, or if a rise in renal ammonia production takes place as well. Moreover, renal utilization of ammonia precursors, mainly glutamine, in these circumstances is unexplored so far. A number of investigations carried out in animals with acute metabolic acidosis provided conflicting results that also depended on species differences (14–21).

Some factors, such as urine flow, urine pH, and arterial acid-base parameters, which may influence ammonia excretion in acute metabolic acidosis have been investigated in the past. An inverse correlation between urinary ammonia excretion and urine pH was detected (11, 22, 23); furthermore, an increase in urine flow per se caused an increased ammonia excretion (12, 24). It is poorly understood if, and to what extent, these factors are able to stimulate renal ammonia production. Acidemia seems to be the major factor increasing ammonia production in the intact rat (17), whereas urine pH has a critical role in increasing ammoniagenesis in isolated perfused kidney preparations (25). In vitro studies, on the other hand, demonstrate that ammonia production from glutamine by both rat renal tissue slices and isolated mitochondria

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is unmodified or reduced (26–32) when an acidic incubation medium (pH 7.0) is used.

Studies reported here were carried out in order to investigate total renal ammonia production and ammonia precursor use in man with normal renal function in an early stage of metabolic acidosis. The study was performed by measuring renal ammonia production and amino acid (AA)[1] exchange across the kidney. The individual role of some factors that may affect ammonia production, such as arterial acid-base parameters, urine pH, and urine flow, has also been evaluated.

METHODS

Patients. 19 hypertensive patients, aged 21–60 yr, were studied. Their mean blood pressure ranged from 140 to 160 mm Hg. They had no history or evidence of congestive heart failure, hepatic or pulmonary diseases, or diabetes mellitus. Routine laboratory tests, serum and urine electrolyte concentration, acid-base measurements, urinalysis, and renal function tests were normal. The rapid sequence intravenous pyelogram showed a unilateral delayed appearance time and a persistent hyperconcentration of the contrast material. However, no significant difference in renal size was present. All patients were on a standard diet that provided 30–35 kcal and 0.70–0.85 g of protein/kg body weight per d. In all patients, a renal vein catheterization was considered necessary for diagnostic purposes to determine plasma renin activity. Medications were discontinued at least 15 d before renal vein catheterization. The final diagnosis was benign essential hypertension. 10 of 19 patients received 159±12 (111–223) mmol/m^2 body surface of NH_4Cl orally during the 24 h before the study. The acid load was given in three fractional doses, the last of which was administered 6 h before the study. The control and acidotic groups were evenly matched for age, sex, body surface, and blood pressure. Some data obtained in 6 of 9 patients presented here as controls were also reported elsewhere (33).

All patients were informed of the nature, purpose, procedure, and possible risks before obtaining their voluntary consent.

Procedure. Patients were studied in the recumbent position in the postabsorptive state. A Teflon catheter was inserted percutaneously into a peripheral artery to measure acid-base status and arterial ammonia and AA levels. A Cobra no. 6 or 7 S catheter was then guided under fluoroscopic control through a femoral vein to a renal vein. The catheter was kept patent by intermittent saline flushes. Catheter position was ascertained visually with image intensification before each blood withdrawal.

An intravenous infusion of Na thiosulphate and Na para-aminohippurate (PAH) was started after the administration of a priming dose of PAH (2.5–3 mmol). The infusion was kept at a constant flow rate (Na thiosulphate 0.8 mmol/min and PAH 0.09 mmol/min) and two or three sequential clearance periods of 20 min each were obtained. The correct position of the catheter in the renal veins was verified by calculating the renal extraction of PAH and oxygen. Oxygen extraction, calculated as $(\text{oxygen}_a - \text{oxygen}_v)/\text{oxygen}_a \times 100$, was used during catheterization as a quick test for checking, with a good approximation, the correct position of the catheter. During each clearance period, one set of blood samples was obtained simultaneously from a peripheral artery and a renal vein for the measurement of arterial-renal venous (A-V) differences of ammonia and AA. Blood samples were taken from renal veins of both kidneys. When samples were undoubtedly obtained from renal veins, PAH extraction and ammonia and AA A-V differences were not different between the two kidneys.

Blood was withdrawn by heparinized syringes kept in ice. During the study, urine was collected via a cannula under mineral oil and then stored at −25°C in bottles that contained thymol.

Analytical methods. For ammonia determination on whole blood, samples were deproteinized at +4°C with 0.3 mol/liter Na tungstate and 0.5 mol/liter sulphuric acid immediately after the withdrawal. The protein-free supernate was stored at −25°C and ammonia measured according to Chaney and Marbach (34) within 12 h. An eightfold concentration of phenol and hypochlorite reagents was used. The same method was followed for the measurement of ammonia in urine.

AA were determined on whole blood. Proteins were precipitated with cold 0.75 mol/liter perchloric acid within 60 min from the blood withdrawal. An aliquot of supernate was neutralized with a buffered solution, stored at −25°C, and used for enzymatic assay of glutamine, glutamate, and aspartate within 30 h. Another aliquot was stored at −25°C and used within 3 mo for the determination of 19 additional AA. Specimen storage at −25°C for such a period of time does not affect AA levels (33, 35). AA analyses were performed at least in triplicate by automated ion-exchange chromatography (Multichrom B AA analyzer, Beckman Instruments, Inc., Fullerton, Calif.). Sodium buffers were used; the supernate was neutralized and treated with Na sulphite immediately before assay to remove glutathione. This procedure causes a loss of methionine and cysteine. Glutamine was determined enzymatically according to the method reported by Lund (36), modified for fluorometer measurements (Farrand A 4, Farrand Optical Co., Inc., Valhalla, N. Y.). Recovery of glutamine, added to whole blood, was measured for each assay; the recovery ranged from 96.5 to 102%. Glutamate and aspartate were determined according to Graham and Aprison (37). Aspartate was measured enzymatically only in seven controls and in five patients with NH_4Cl-induced acidosis. Because values obtained enzymatically were not different from those provided by ion-exchange chromatography, only the latter are reported in the result section. For enzymatic assays of glutamine, glutamate and aspartate, samples, blanks, and standards were done in triplicate. AA in urine were determined by ion-exchange chromatography on a Multichrom B analyzer after deproteination.

Glomerular filtration rate (GFR) was measured with the Na tiosulphate method (38). Renal plasma flow was determined with PAH according to Smith et al. (39).

Blood and urine pH and $PaCO_2$ were estimated at 37°C with PHM 72/BMS 3 apparatus (Radiometer Co., Copenhagen, Denmark). Blood HCO_3^- was calculated using the Henderson–Hasselbalch equation. Urine titratable acidity was measured by titration with 0.1 mol/liter NaOH up to arterial pH. SaO_2 was measured with an Hellige oximeter (American Optical Corp., Scientific Instrument Div., Buffalo, N. Y.). Hematocrit was determined by a microcapillary procedure.

Calculations. Renal arterial plasma flow was calculated

[1] *Abbreviations used in this paper*: AA, amino acid; A-V, arterial-renal venous; GFR, glomerular filtration rate; PAH, Na para-aminohippurate, RBF, true renal blood flow.

from clearance and extraction of PAH using the Wolf equation (40). True renal arterial blood flow (RBF) was calculated from renal plasma flow and arterial hematocrit.

Net extraction (+) or net release (−) of individual AA and ammonia by the kidney were calculated by the following formula: $M = (F_a \times S_a) - S_v (F_a - F_u) - (F_u \times S_u)$, where: M = net uptake or release (μmol/min · 1.73 m^2), F_a = RBF (ml/min · 1.73 m^2), F_u = urine flow (ml/min · 1.73 m^2), S_a = arterial level of metabolite (μmol/ml), S_v = venous level of metabolite (μmol/ml), and S_u = urinary level of metabolite (μmol/ml).

The total ammonia production was obtained by summing the values for renal venous ammonia release and urinary ammonia excretion.

The N balance across the kidney reported here was calculated by subtracting N contributed by individual AA significantly released plus N contributed by total ammonia produced, from N contributed by individual AA significantly extracted.

Patients and controls with NH_4Cl acidosis were compared by analysis of variance by using a completely randomized design. A randomized block design was applied to the analysis of variance for the paired data (41). Analysis of simple regression and correlation (41) was used to evaluate the dependence of ammonia production and the ammonia-excreted/ammonia-produced ratio on NH_4Cl load, arterial blood acid-base parameters, PNH_3 in renal venous blood and urine, urine flow, and log of urine H^+. The same procedure was followed to evaluate the relationships between the following variables: renal ammonia production and extraction of glutamine and other AA; ammonia production/glutamine N extraction ratio and arterial HCO_3^-; exchange of N contributed by glycine plus ornithine and arterial HCO_3^-; and citrulline extraction and arginine output. Analysis of multiple regression (41) was used in order to estimate the dependence of ammonia production (*a*) and the ammonia-excreted/ammonia-produced ratio (*b*) on the independent variables that showed a significant simple correlation with (a) and (b). Values are given as mean±1 SEM.

RESULTS

Table I reports GFR, RBF, blood and urine acid-base parameters, urine flow, and ammonia excreted and added to renal venous blood in patients under normal acid-base balance and in patients with NH_4Cl-induced acidosis. The acid load caused a significant fall in arterial HCO_3^-, which was inversely correlated with the amount of acid administered per square meter of body surface ($r = -0.924$, $P < 0.001$). In acidotic patients urine pH was lower in comparison with controls, while titratable acidity and urine flow was higher. The amount of ammonia excreted was increased (+129%), whereas that added to renal venous blood was not different from controls. It follows that total ammonia production was augmented in the acidotic group (+63%), and the fraction excreted of total ammonia produced was increased. Ammonia production was directly correlated with the dose of acid load ($r = 0.817$, $P < 0.001$). Arterial ammonia levels were not different in the two groups of patients (44±6.9 μmol/liter in controls and 48±4.5 in acidotic patients).

There was a direct correlation between renal ammonia production and arterial H^+ ($r = 0.807$, $P < 0.001$), urine H^+ ($r = 0.607$, $P < 0.01$), and urine flow ($r = 0.788$, $P < 0.001$) (Fig. 1). In addition, ammonia production was inversely correlated with arterial HCO_3^- ($r = -0.829$, $P < 0.001$) (Fig. 2) and $PaCO_2$ ($r = -0.776$, $P < 0.001$). However, when multiple regression analysis was carried out with arterial HCO_3^-, urine H^+, and urine flow, as independent variables, ammonia production correlated significantly only with arterial HCO_3^- ($P < 0.01$) and urine flow ($P < 0.001$). The same results were obtained when arterial H^+ or $PaCO_2$, instead of arterial HCO_3^-, were considered. No correlation was detected between ammonia production and PNH_3 of renal venous blood and urine.

A correlation between the ammonia excretion/ammonia production ratio and urine flow ($r = 0.673$, $P < 0.01$) (Fig. 3), the log of urine H^+ ($r = 0.709$, $P < 0.001$) (Fig. 4), and arterial HCO_3^- ($r = -0.779$; $P < 0.001$) was found. Analysis of multiple regression confirmed the dependence of the ammonia excretion/ammonia production ratio upon both urine flow ($P < 0.001$) and the log of urine H^+ ($P < 0.001$), but not upon arterial HCO_3^-.

In Table II, arterial blood levels, rates of renal uptake and release, and urinary excretion of 19 free AA in controls and in patients with metabolic acidosis are reported. Arterial AA levels and urinary excretion were not different in the two groups, with the exception of the arterial histidine level which increased in acidosis ($P < 0.05$). Under normal acid-base balance, the kidney extracted glutamine, proline, citrulline and phenylalanine, and released serine, arginine, taurine, ornithine, tyrosine, threonine, lysine, histidine, and perhaps alanine and glutamate. After 24 h of acidosis, the renal exchange of most AA, including glutamine, was not different from controls. The only exceptions were glycine and ornithine, which were significantly extracted by the kidney only in the acidotic group; in addition, the release of histidine disappeared. Both in controls and in patients with acidosis, citrulline extracted was directly correlated with arginine released by the kidney ($r = 0.577$, $P < 0.02$). No correlation was detected between ammonia production and glutamine extraction in either group.

Nitrogen contributed by AA extracted by the kidney (110±15.2 μmol/min · 1.73 m^2 in controls and 111±5.7 in acidosis) was well balanced with N contributed by AA and ammonia released by the kidney (138±19.2 μmol/min · 1.73 m^2 in controls and 135±11.6 in acidosis).

Table III summarizes the data on renal production of ammonia and on the use of ammonia precursors in the two groups of patients. In acidotic patients, the

TABLE I

*Effects of 24-h Metabolic Acidosis on GFR, RBF, Blood and Urine Acid-base Parameters, Urine Flow, Total Ammonia Production, and Bidirectional Ammonia Release by the Kidney**

	GFR	RBF	pH_a	HCO_{3a}^-	Urine pH	Titratable acidity	Urine flow	Urinary NH_4^+	NH_4^+ added to the renal, veins	Total NH_4^+ production	Urinary NH_4^+ / Total NH_4^+ production
	ml/min·1.73 m²	*ml/min·1.73 m²*		*mmol/liter*		*μeq/min·1.73 m²*	*ml/min·1.73 m²*	*μmol/min·1.73 m²*	*μmol/min·1.73 m²*	*μmol/min·1.73 m²*	
Controls (9)	145±6.1	1098±79.0	7.41±0.010	22.6±0.69	5.58±0.134	15.6±2.75	1.83±0.227	22.5±1.99	18.2±1.45	40.6±2.34	0.55±0.030
24-h NH_4Cl acidosis (10)	139±7.3	956±69.8	7.33±0.010‖	15.1±0.98‖	4.74±0.058‖	37.8±1.50‖	3.09±0.492‡	51.5±6.05‖	14.6±1.33	66.1±5.84§	0.76±0.027‖

Abbreviations used in this table: GFR, glomerular filtration rate; RBF, true renal blood flow.

* Values are given as mean±SEM.

Significance of difference from the corresponding value in controls:

‡ $P < 0.01$.

§ $P < 0.005$.

‖ $P < 0.001$.

amount of N supplied by glutamine extraction was not changed despite the increase in total ammonia production; the ammonia production/glutamine N extraction ratio doubled and was inversely correlated with the log of arterial HCO_3^- ($r = -0.750$, $P < 0.001$) (Fig. 5). Further, in acidosis, a significant uptake of N contributed by glycine and ornithine took place. Finally, the exchange of N contributed by glycine and ornithine was inversely correlated with arterial HCO_3^- ($r = -0.816$, $P < 0.001$) (Fig. 6).

DISCUSSION

Data presented here demonstrate that in man in an early stage of metabolic acidosis, not only urinary ammonia excretion increases, as already known (10–12),

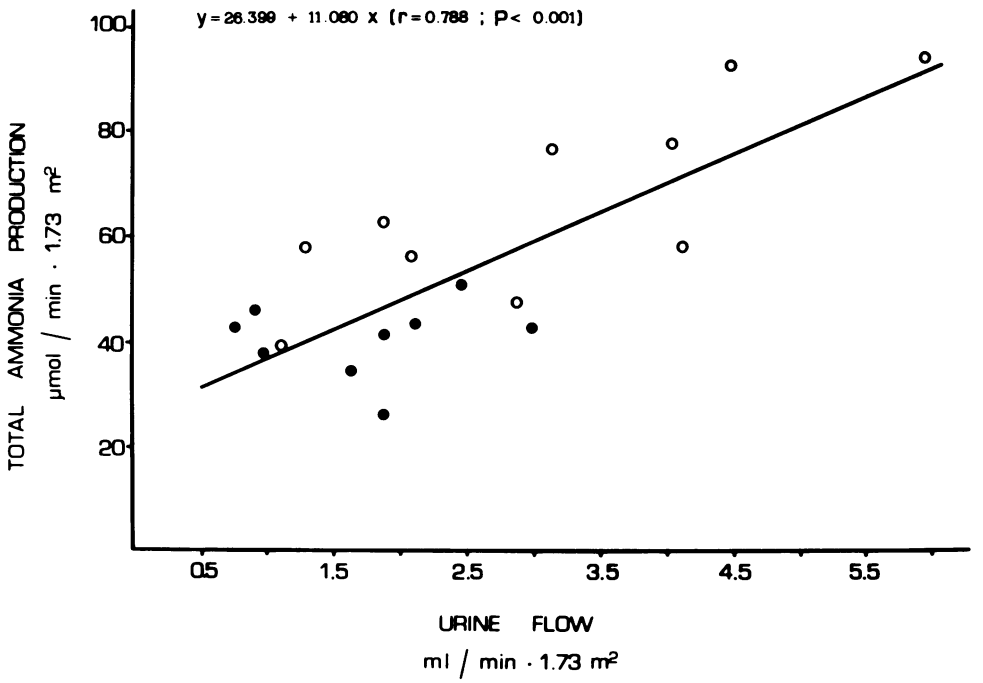

FIGURE 1 Relationships between total renal ammonia production and urine flow in 9 patients with normal acid-base balance (solid circles) and in 10 patients with 24-h NH_4Cl acidosis (open circles).

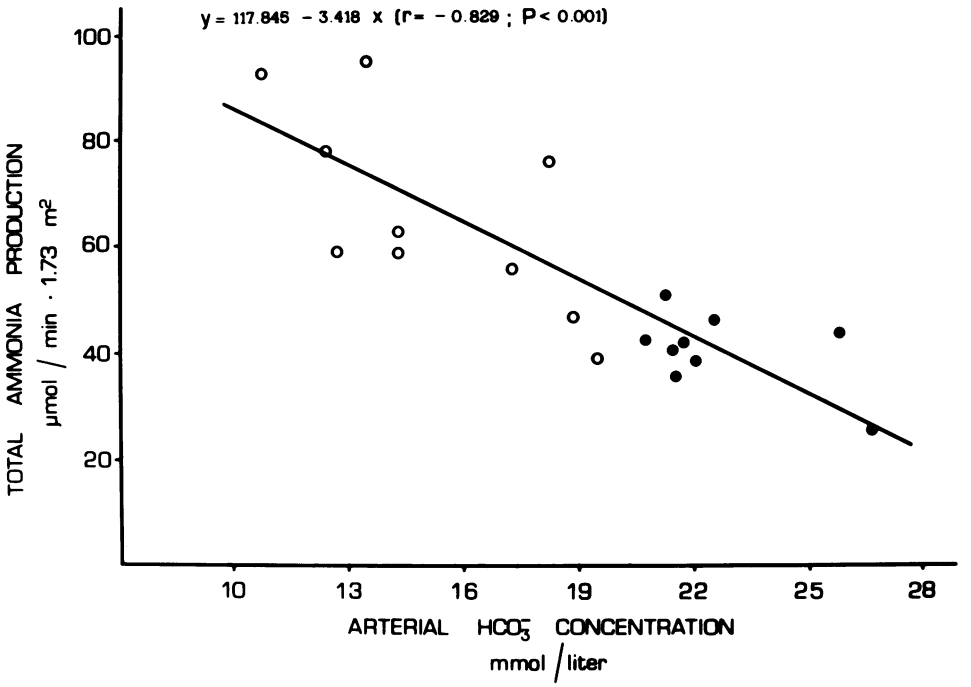

FIGURE 2 Relationships between total renal ammonia production and arterial HCO_3^- concentration in 9 patients with normal acid-base balance (solid circles) and in 10 patients with 24-h NH_4Cl acidosis (open circles).

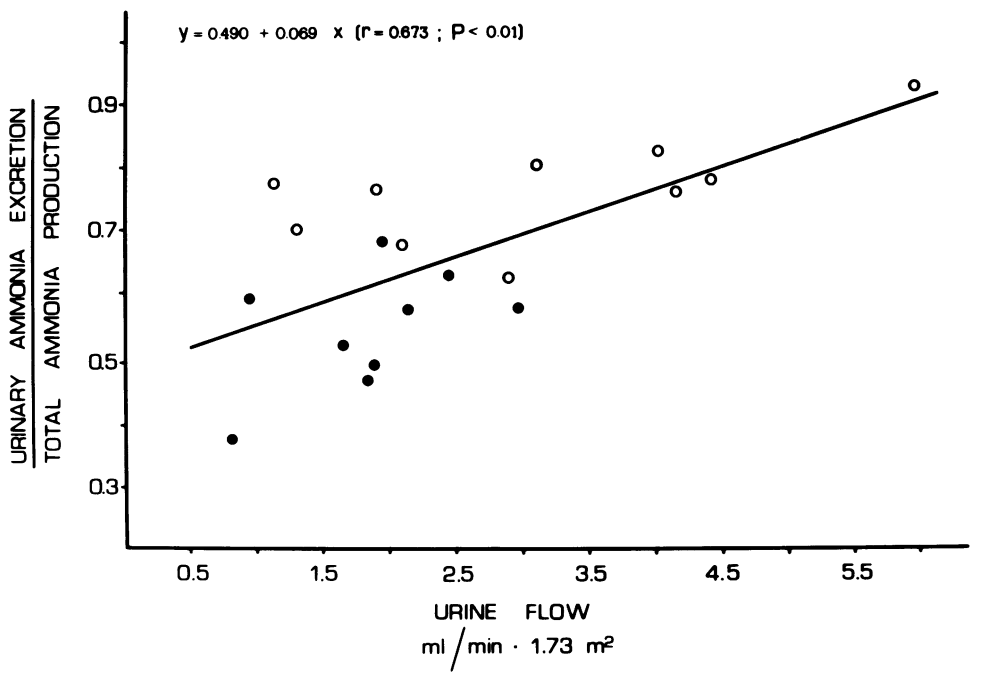

FIGURE 3 Relationships between the urinary ammonia excretion/total ammonia production ratio and urine flow in 9 patients with normal acid-base balance (solid circles) and in 10 patients with 24-h NH_4Cl acidosis (open circles).

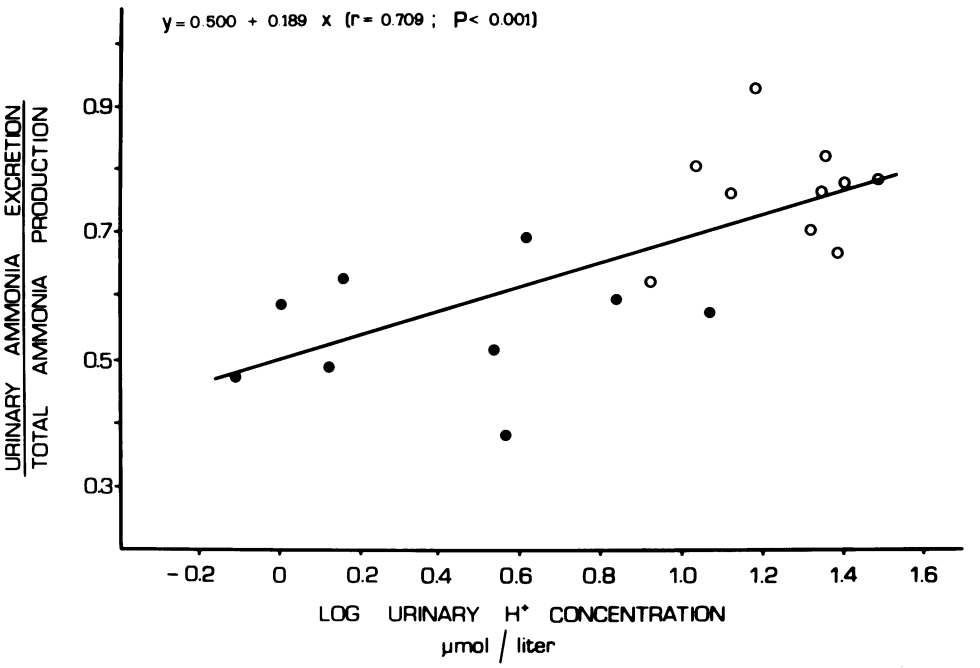

FIGURE 4 Relationships between the urinary ammonia excretion/total ammonia production ratio and the log of urinary H^+ concentration in 9 patients with normal acid-base balance (solid circles) and in 10 patients with 24-h NH_4Cl acidosis (open circles).

but also total renal ammonia production is augmented. Thus, metabolic acidosis induces a precocious adaptation of renal ammoniagenesis and, consequently, larger amounts of buffer become available to meet the greater need to excrete fixed acids.

Recent studies in the isolated perfused rat kidney have indicated that the fall of urine pH, rather than the decrease of HCO_3^- in the perfusion fluid, is the critical stimulus for increased ammonia production (25). Conversely, results reported here suggest that, in man, at the onset of metabolic acidosis, the degree of acidosis and the changes in urine flow, rather than the fall of urine pH, are the major factors stimulating the increase in ammonia production. The importance of acidemia has also been outlined in the intact rat with acute metabolic acidosis, whereas urine pH seemed to be meaningless (17). Moreover, no correlation was detected between ammonia production and urine pH both in dog and in man under normal acid-base balance and in chronic NH_4Cl-induced acidosis (1, 42, 43). It has been shown that intracellular pH of rat tubular cells is linearly correlated with extracellular pH (44). Thus, acidemia may induce a fall of pH within tubular cells and consequently, promote an increased ammoniagenesis.

A quite novel finding is the apparent role of urine flow as a stimulus for ammonia production in acute acidosis. One can hypothesize that in this condition the increased urine flow and the consequent enhanced washout of urinary ammonia are more effective than the fall in urine pH in facilitating diffusion of ammonia from cells into tubular lumen, hence, in lowering ammonia concentration in tubular cells, and eventually, in influencing renal ammonia formation. Ammonia production, on the other hand, seems to be independent of PNH_3 in renal venous blood and urine in the experimental conditions reported here.

The simultaneous measurement of ammonia excreted with urine and that added to renal venous blood carried out in this research allows one to estimate the actual role of urine pH and urine flow in the partition of ammonia produced between urine and blood. Findings obtained provide evidence that in an early stage of metabolic acidosis, the increase in both urine H^+ and urine flow is responsible for the preferential shifting of ammonia into urine. In fact, the fraction excreted of total ammonia produced increases proportionally to urine flow and to the log of urine H^+.

An important finding in the present study is that in man, at the onset of metabolic acidosis, renal glutamine extraction is not different from that measured in normal conditions in spite of a 63% increase in ammonia production. In addition the ammonia production/glutamine N extraction ratio rises proportionally to the fall of arterial HCO_3^-; these data indicate that the discrepancy between ammonia production and

TABLE II

*Arterial Levels, A-V Differences, Net Renal Uptake (+) or Release (−), and Urinary Excretion of Free Amino Acids in Nine Controls and in Seven Patients with 24-h NH_4Cl Acidosis**

	Arterial level		A-V difference		Rate		Urinary excretion	
	Controls	Acidosis	Controls	Acidosis	Controls	Acidosis	Controls	Acidosis
	μmol/liter		*μmol/liter*		*μmol/min · 1.73 m²*		*μmol/min · 1.73 m²*	
Taurine	221.1±18.28	216.5±18.45	−4.0±1.80••	−9.1±3.14••	−5.2±1.74	−8.2±3.11	0.31±0.069	0.60±0.188
Aspartate	134.5±15.78	138.5±21.38	−0.6±2.15	−0.1±1.27	−0.7±2.60	−0.3±1.42	not determined	
Threonine	104.8±7.94	112.7±7.15	−3.7±1.36••	−2.0±1.00¶	−4.0±1.56	−2.1±1.00	0.14±0.031	0.13±0.020
Serine	129.4±8.41	114.9±9.56	−20.8±2.65≠	−23.0±3.00≠	−22.8±3.77	−21.8±2.58	0.34±0.078	0.24±0.026
Glutamate‡	168.6±16.52	130.2±1.77	−3.8±1.80¶	−6.0±1.71‡‡	−3.3±1.63	−4.9±1.45	0.02±0.006	0.03±0.003
Glutamine‡	519.2±25.85	472.0±27.13	+31.8±4.52≠	+29.5±3.32≠	+33.5±4.58	+27.3±2.68	0.39±0.062	0.38±0.046
Proline	163.9±15.35	205.2±43.64	+11.9±4.39‡‡	+16.8±5.00‡‡	+14.1±5.78	+17.1±4.38	0.02±0.005	0.02±0.007
Glycine	272.6±20.53	261.1±11.32	+4.0±3.74	+12.0±2.12§§	+2.1±3.40	+11.4±1.68§	1.07±0.241	0.69±0.150
Alanine	237.1±23.07	275.3±29.42	−8.3±4.48¶	−7.5±2.79••	−8.8±5.40	−6.5±2.33	0.13±0.021	0.15±0.036
Citrulline	29.4±1.64	31.9±1.79	+6.9±1.09≠	+6.4±0.91≠	+7.7±1.39	+6.1±0.71	0.01±0.002	0.02±0.005
Valine	177.2±15.39	177.9±13.50	−1.1±2.35	−2.1±3.95	−0.9±2.81	−2.9±3.83	0.04±0.005	0.04±0.004
Isoleucine	53.3±4.37	51.1±4.75	+1.1±0.98	−0.6±1.18	+1.3±1.23	−0.4±1.12	0.03±0.013	0.02±0.006
Leucine	92.8±8.03	95.0±5.70	−1.6±1.61	−1.3±1.41	−1.3±1.96	−1.1±1.21	0.03±0.009	0.06±0.004
Tyrosine	42.9±3.26	43.5±3.87	−3.7±0.88§§	−5.1±1.20§§	−4.2±1.21	−4.7±0.90	0.09±0.031	0.07±0.012
Phenylalanine	35.5±2.50	37.0±3.55	+1.2±0.44••	+1.4±0.70¶	+1.3±0.52	+1.3±0.68	0.04±0.005	0.06±0.013
Ornithine	86.7±6.30	86.2±8.67	−4.3±0.89••	+2.9±0.91••	−4.6±1.16	+3.3±1.05‖	0.04±0.012	0.03±0.004
Lysine	157.1±7.26	137.6±6.04	−3.4±0.95‡‡	−3.3±1.70¶	−3.4±1.08	−3.4±1.90	0.18±0.058	0.12±0.013
Histidine	61.0±3.14	70.9±3.36§	−2.6±1.06••	−0.1±1.34	−3.3±1.38	−0.6±1.41	0.45±0.062	0.65±0.090
Arginine	68.7±2.77	68.0±5.50	−5.8±0.80≠	−5.1±1.58§§	−6.4±1.20	−4.4±1.25	0.02±0.003	0.02±0.005

• Values are given as mean±SEM.

‡ Determined enzymatically. Three additional patients with 24-h metabolic acidosis were used for glutamine and glutamate exchange across the kidney.

Probability that patients with metabolic acidosis do not differ from controls:

§ $P < 0.05$.

‖ $P < 0.01$.

Probability that A-V difference does not differ from zero:

¶ $P < 0.1$.

•• $P < 0.05$.

‡‡ $P < 0.025$.

§§ $P < 0.005$.

≠ $P < 0.001$.

TABLE III

*Renal Production of Ammonia and Utilization of Glutamine, Glycine, and Ornithine in Controls and in Patients with 24-h Metabolic Acidosis**

	Total NH_4^+ production	Gln N extraction	NH_4^+ production / Gln N extraction	Gln N reabsorbed	Gly N + Orn N exchange
	μmol/min·1.73 m²	*μmol/min·1.73 m²*		*μmol/min·1.73 m²*	*μmol/min·1.73 m²*
Controls (9)	40.6±2.34	67.0±9.16	0.68±0.083	150.2±10.73	−6.6±4.03
24-h NH_4Cl acidosis (10)	66.1±5.84§	54.5±5.37	1.29±0.150§	129.8±9.81	+17.7±3.32‡§

Abbreviations used in this table: Gln, glutamine; Gly, glycine; Orn, ornithine.

* Values are given as mean±SEM.

‡ Determined only in seven subjects.

§ Significantly different from the corresponding value in controls, $P < 0.005$.

glutamine N extraction increases along with the degree of acidosis. Conversely, in subjects with NH_4Cl-induced chronic acidosis, the ammonia production/glutamine N extraction ratio is not different from that determined in subjects under normal acid-base balance, as one can calculate from data reported elsewhere (1, 2, 33). Furthermore, assuming that glutamine extracted by the kidney is the major source of alanine and glutamate in renal tissue (45), a portion of N contributed by renal glutamine extraction is probably not used for ammonia formation. Thus in acute metabolic acidosis, in contrast to normal acid-base conditions and to chronic acidosis, the entire amount of ammonia produced by the kidney can hardly be accounted for by the glutamine extracted alone, even assuming a more complete utilization of N groups of glutamine for ammoniagenesis. All these data suggest that in man, in an early stage of metabolic acidosis, glutamine fails to maintain its role as the nearly exclusive ammonia precursor.

The reason why renal glutamine extraction does not increase at the onset of metabolic acidosis is so far

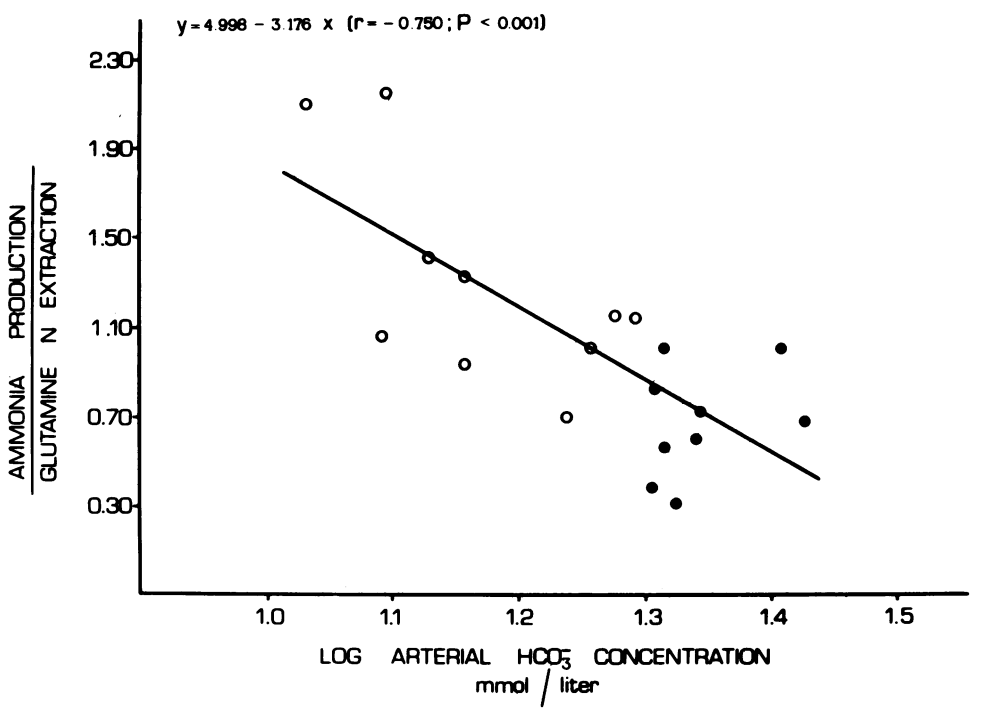

FIGURE 5 Relationships between the renal ammonia production/glutamine N extraction ratio and the log of arterial HCO_3^- concentration in 9 patients with normal acid-base balance (solid circles) and in 10 patients with 24-h NH_4Cl acidosis (open circles).

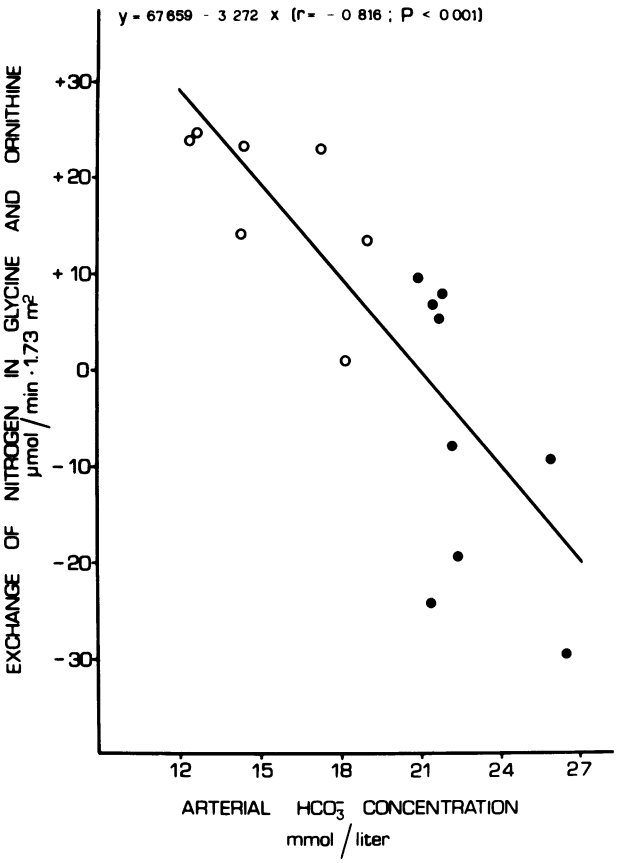

FIGURE 6 Relationships between the renal exchange of N contributed by glycine and ornithine and arterial HCO_3^- concentration in nine patients with normal acid-base balance (solid circles) and in seven patients with 24-h NH_4Cl acidosis (open circles).

obscure. Glutamine supply to tubular cells is not changed, because the amount of glutamine filtered and reabsorbed is by far in excess of glutamine extracted (Table III). Most studies in which the effect of pH on ammoniagenesis was evaluated in vitro agree that both ammonia production from glutamine and glutamine use by rat renal cortical slices or mitochondria are unchanged or depressed when incubation is carried out at low pH (26–32). Moreover, acute acidification does not stimulate rat renal phosphate-dependent glutaminase (E.C. 3.5.1.2.) (46–49) and intramitochondrial glutamate dehydrogenase (E.C. 1.4.1.2.) (50) activities. All the data obtained in vitro may be consistent with the failure of renal glutamine extraction to increase in man in 1-d metabolic acidosis. Because in this condition, despite the increased ammonia production, glutamine extraction is unchanged, additional ammonia precursors may come into play. Among them glutamate probably cannot be taken into account because the release of this AA is unchanged in acidotic patients. Conversely, acute acidification modifies the exchange of some AA across the kidney, that is, a significant glycine and ornithine extraction takes place. Particularly noteworthy is the effect of acidosis in influencing renal glycine and ornithine metabolism. The more severe the acidosis, the greater is the extraction of N contributed by glycine and ornithine. It is interesting to note that N contributed by glycine and ornithine extracted may cover the amount of ammonia not accounted for by the glutamine extracted. The data presented here cannot provide direct evidence that glycine and ornithine extracted are used for ammonia formation in acute acidosis. However, glycine can be used for ammoniagenesis in the intact dog (51, 52), and ornithine can produce ammonia by rat renal cortical slices (53) through conversion to glutamate. Two other AA, namely proline and citrulline, are extracted by the kidney in acute acidosis at the same rates as in controls. However, citrulline cannot be considered an ammonia precursor inasmuch as this AA is utilized by the kidney for arginine production in normal acid-base balance (33, 54) and probably in patients with 1-d metabolic acidosis studied here; in addition, citrulline does not produce ammonia in vitro (unpublished observations). Proline may be an ammonia precursor (52) through conversion to glutamate; however, because proline extraction does not increase in acute acidosis, its contribution to augmented ammoniagenesis in this condition is probably minor.

In conclusion, studies reported here demonstrate that in man the adaptation of renal ammoniagenesis to metabolic acidosis is a precocious event. At an early stage of acidosis, acidemia and increased urine flow seem to play a major role in stimulating ammonia production. In this stage, the adaptive mechanisms responsible for the increased renal glutamine use that are operative in the chronic condition are not yet activated; other substrates, besides glutamine extracted from the arterial blood, are probably utilized for ammonia production.

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