Amino Acid Handling in Uremic Rats: Citrulline, a Reliable Marker of Renal Insufficiency and Proximal Tubular Dysfunction

Olivier Levillain, Philippe Parvy, and Christine Hassler

The kidney is involved in amino acid reabsorption and metabolism; consequently, in renal insufficiency, these important functions are disturbed, as has been reported in animals and patients. In a first experimental series, rats were subjected to degrees of nephrectomy (NX) varying between 10% and 90%. Three weeks later, amino acid levels were measured in plasma to correlate the levels with the degree of NX. The results indicate that in the range of 33% to 74% NX, the plasma concentration of only three to four amino acids was modified, whereas in rats with 84% NX, the concentration of 11 amino acids was disturbed, compared with sham-operated rats. Citrullinemia was enhanced in uremic rats and correlated with the degree of NX. More interestingly, citrullinemia was increased in the range of 10% to 33% NX without any changes in uremia and creatininemia, two well-known markers of uremic states. A second experimental series was designed to study the time course of changes in aminoacidemia to find a marker for the onset of renal failure. Rats were subjected to 36% NX for a period of 1 to 21 days. Uremia and creatininemia peaked 24 to 48 hours after NX, and creatinine clearance (Cl_{creat}) concomitantly diminished. Unfortunately, these three markers of uremic states returned to control values during the next few days before increasing during the last 2 weeks. In contrast, citrullinemia increased twofold 48 hours after NX and plateaued over the next 20 days. We conclude that in rats, citrullinemia could be used (1) to detect acute and chronic renal failure, (2) as a specific marker of normal function of the proximal tubule, and (3) to estimate the degree of renal damage. From this study, renal insufficiency might be easily detected by measuring citrullinemia.

AMINO ACIDS are important nitrogenous compounds because they are involved in protein synthesis, nitrogen balance, energy production, and several metabolic pathways, including the synthesis of purines, pyrimidines, and other amino acids. Essential amino acids must be consumed in the food, whereas nonessential amino acids are synthesized in the body. Several organs are involved in the production and/or utilization of amino acids; among them, the most important, which possess a great number of enzymatic pathways of amino acid metabolism, are the liver, intestine, kidneys, brain, and skeletal muscle.

It is well known that almost all amino acids can be metabolized by the kidney, which contains numerous pathways of amino acid metabolism, and that filtered amino acids are virtually completely reabsorbed in the proximal convoluted tubule (PCT) in normal physiological states.[1-3] It is therefore not surprising that in chronic renal failure, a reduction of the functional renal tissue modifies normal renal function such as water, urea, and salt excretion, hormone synthesis, and both renal uptake and release of amino acids. This might result in alterations of blood amino acid concentrations, which probably also reflect disturbances of muscle, kidney, and liver amino acid metabolism and dietary changes.[4] An elevation in plasma citrulline and a decrease in plasma serine concentration seem to be constant findings in chronic renal failure, whereas changes in plasma concentrations of other amino acids appear to vary from one study to another.[2,5-9] But to our knowledge, despite the well-known increase in citrullinemia in uremic animals or patients,[5-7,10-15] it has never been reported whether citrullinemia could be correlated with the degree of renal insufficiency. No attempt has been made to establish correlations between changes in plasma amino acids and the degree of nephrectomy (NX) and to determine whether amino acids could be used as markers to detect the onset of renal failure either in human or in animal models of renal failure.

This is why we were interested to determine whether such correlations exist in rats with various degrees of NX obtained using an adaptation of the model of five-sixths NX,[16] and to know what percentage of renal failure induces a disturbance in the renal conversion of citrulline to arginine (Arg), which takes place mainly in the PCT.[17,18] For this, concentrations of amino acids and some biochemical parameters were measured in the plasma of groups of rats in which the degree of initial renal insufficiency varied from 10% to 90%. The other important goal of this study was to find a plasma amino acid that could be used as a marker to detect the onset of renal failure. For this, the time course of changes in aminoacidemia was evaluated in rats subjected to a low degree of renal insufficiency of about 36% for a period of 1 to 21 days.

The obvious conclusion of this study is that citrulline is the only amino acid that could be used as a marker of renal insufficiency for the following reasons: (1) citrullinemia was significantly enhanced 48 hours after NX and remained about twofold higher than the control level for 20 days; (2) citrullinemia was correlated with the degree of renal insufficiency in the range of 10% to 90%; and (3) a low degree of 10% to 33% NX induced an increase in citrullinemia without any changes in uremia and creatininemia. These experiments demonstrate that citrullinemia was a more reliable marker than uremia, creatinine clearance (Cl_{creat}), and creatininemia despite its nonsignificant increase 24 hours after NX. Renal insufficiency might be easily detected by measuring citrullinemia.

From the Laboratoire de Physiopathologie Métabolique et Rénale, Faculté de Médecine Lyon R.T.H. Laennec, INSERM CRI 950201, Lyon; Laboratoire de Biochimie B, Hôpital Necker, Paris; and Unité 169 INSERM, Villejuif, France.

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Address reprint requests to Olivier Levillain, PhD, Laboratoire de Physiopathologie Métabolique et Rénale, Faculté de Médecine Lyon R.T.H. Laennec, INSERM CRI 950201, 12 rue G. Paradin, 69372 Lyon Cedex 08, France; email levillai@cimac_res.univ_lyon1.fr.

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MATERIALS AND METHODS

Male Sprague-Dawley rats (Iffa Credo, L'Arbresle sur Orge, France) initially weighing 190 to 220 g were anesthetized intraperitoneally with 0.1 mL Nembutal (6%; Clin Midy, Paris, France) per 100 g body weight and subjected to renal insufficiency.

Depending on the theoretical degree of renal damage induced by ligation of branches of the renal artery belonging to either the right or the left kidney and/or removal of the right kidney, the rats were submitted to one of four surgical protocols: group I, control rats were sham-operated; group II, by ligating the branches of the left renal artery of these rats, the functional renal mass was reduced by 20% to 40%; group III, in rats with 50% NX, either the right kidney was removed or branches of the right and left renal arteries were ligated; and groups IV and V, the right kidney was removed and branches of the left renal artery were ligated to reduce the renal mass by 70% to 90%.

Experimental Determination of the Degree of Renal Insufficiency

In a preliminary study, two different methods were compared to determine the degree of renal insufficiency in the same rat: (1) weighing the healthy and necrotic portions of the kidney; and (2) visually estimating the degree of necrosis. (The ischemic part appears darker than the nonischemic part of the kidney.) We estimated the volume of the necrotic zone of the kidney compared with the total volume of the same kidney. Rats were anesthetized (0.1 mg Nembutal 6%/100 g body weight) and submitted to NX as described earlier, and the kidneys were removed 5 to 10 minutes later. We have defined that one kidney corresponds to 50% NX. This is justified, since in our control rats, the mass of the right kidney did not differ significantly from that of the left kidney, an observation also reported by Kaufman et al.[16]

Influence of the Degree of Renal Insufficiency on the Plasma Concentration of Amino Acids

In the first study, 45 rats were submitted to NX and divided into five groups (I to V) of nine animals. Rats were housed individually for 3 weeks and had free access to tap water and standard rat chow (M 25 Extralabo; Pietrement, Provins, France). The degree of necrosis was estimated by the visual method as described earlier.

Three weeks after surgery, the animals were weighed and anesthetized. Samples of arterial blood were taken from the abdominal aorta and transferred into lithium heparinized vacutainer tubes and maintained at 4°C. The remaining kidney(s) was/were removed, decapsulated, and weighed. Several rats from each group (I to V) were individually housed in metabolic cages for a period of 24 hours to collect the urine.

The blood samples were centrifuged at 4,000 × *g* for 20 minutes at 4°C, and a fraction of the plasma was stored frozen at −50°C until analyses were performed, whereas the remaining fraction was deproteinized with 40% sulfosalicylic acid (SSA) at a proportion of 100 μL SSA/mL plasma. After 20 minutes centrifugation at 4,000 × *g* at 4°C, the supernatant was sampled and frozen at −50°C until amino acid analysis was performed.

Urine volume was measured, and samples of urine were frozen at −50°C until determination of amino acid concentrations. Urinary concentration of creatinine was measured to calculate Cl_{creat} as an index of the glomerular filtration rate (GFR) despite the tubular secretion of creatinine, which is more marked in uremic animals and patients than in healthy controls.[19,20] However, in our experiments, inulin infusion would have modified plasma concentrations of amino acids and the other parameters.

Time Course of Changes in Creatininemia, Uremia, Creatinine Clearance, and Aminoacidemia in Response to 36% Renal Insufficiency

In a second study, 35 rats were subjected to approximately 36% NX and five control rats were sham-operated. Before sacrifice, each rat was individually housed in a metabolic cage for an adaptative period of 3 days. Rats had free access to tap water and standard rat chow in the form of powder (Souffirat, Genthon, France). Urine samples were collected during the last 24-hour period preceding sacrifice. Uremic rats were killed 1, 2, 4, 7, 11, 16, or 21 days after NX, and control rats 24 hours after sham-operation surgery. Rats were anesthetized and weighed. Samples of arterial blood were taken from the abdominal aorta to measure the plasma concentration of amino acids, urea, and creatinine. Samples were treated as described earlier. The volume of urine and the concentration of creatinine were measured to calculate Cl_{creat}. The right kidney and the nonischemic part of the left kidney were weighed.

Determination of Biochemical Parameters

Urea and creatinine concentrations were determined using an Astra 8 Beckman analyzer (Beckman Instruments, Irvine, CA).

Plasma amino acids were determined in SSA-deproteinized samples by ion-exchange chromatography using a Beckman 6300 amino acid analyzer and the manufacturer's methodology. Before analysis, samples were half-diluted with buffers (Beckman) containing two amino acid standards: D-glucosaminic acid and 2-amino-ethyl cysteine (Sigma Chemical, St Louis, MO). Plasma from the National Quality Control for amino acid analysis was regularly used to check our method.

Determination of urinary amino acids required a dilution of the samples that depended on the level of urinary creatinine. Thereafter, the samples were treated as described for the plasma samples.

Calculations and Statistics

Results are presented as the mean ± SE. Statistically significant differences were calculated by ANOVA (one factor, Statview II SE; MID, Paris, France) and Dunnett's *t* test. Results in Table 3 were analyzed by ANOVA (one factor, Statview II SE) and the Fisher paired least-significant difference test (PLSD). For correlation analyses, the correlation coefficient *r* was calculated and *P* was determined from the tables at the 95% and 99% level of significance.

RESULTS

Experimental Determination of the Degree of Renal Insufficiency

There was no statistically significant difference between the visual estimation of the proportion of necrosis and the weight determination of healthy and necrotic parts of kidney tissue (Fig 1). Therefore, the degree of renal injury was exclusively determined by the visual method.

Influence of the Degree of Renal Insufficiency on the Plasma Concentration of Amino Acids

Body and kidney weights. The mean body weight of control and uremic rats was not statistically different (Table 1).

In group II, the weight of the remnant kidneys did not statistically differ from the weight of the kidneys of control rats (group I), indicating that renal hypertrophy completely compensated for the initial destruction of 33% of the left kidney (Table

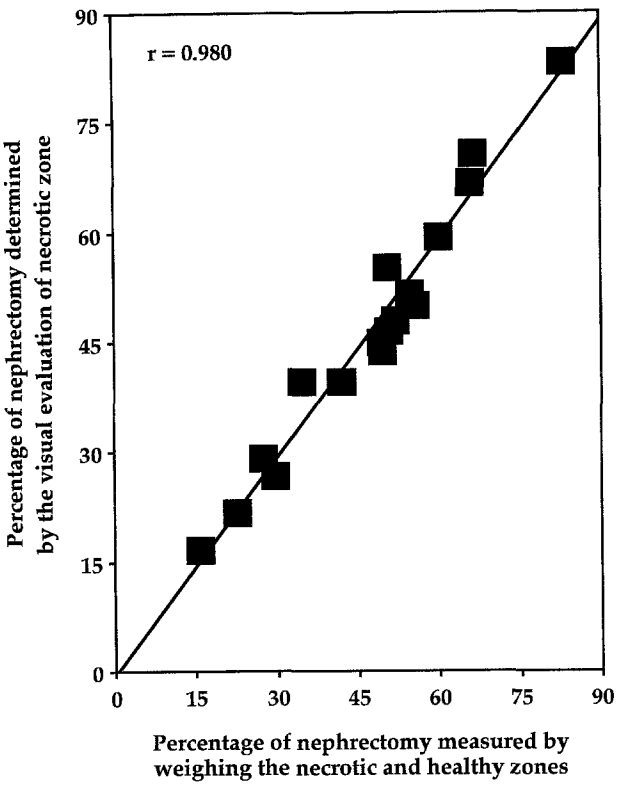

Fig 1. Evaluation of the degree of NX. Branches of the renal arteries from 18 kidneys were ligated, and the degree of NX was evaluated either by visual evaluation or by weighing the necrotic zone.

1). Renal hypertrophy was induced as soon as a small fraction of the kidney was destroyed. In the other groups of uremic rats (III, IV, and V), renal mass was lower than in the controls (by 20%, 39%, and 40%, respectively) despite significant hypertrophy of the remaining renal tissue (157%, 229%, and 376%, respectively). In these last three groups, renal hypertrophy did not completely compensate for lost tissue. The final kidney weight was linearly correlated with the severity of NX ($r = .852$, $P < .01$, data not shown).

Uremia and creatininemia. In rats with 33% NX, uremia remained unchanged as compared with that in the controls. Uremia was enhanced by 32% ($P < .05$) in rats with 49% NX. A twofold and threefold increase in uremia was observed in rats with 74% and 84% NX, respectively. In NX rats, uremia and creatininemia were exponentially increased in parallel (Table 1: $r = .857$ and $r = .865$, respectively, for the five groups of rats (I to V), $P < .01$; also see Fig 2), leading to a positive linear relationship ($r = .929$, $P < .01$, data not shown).

Plasma concentration of amino acids. Table 2 shows changes in individual amino acids in each group of uremic rats. The concentration of most amino acids was unaffected by the degree of NX. In rats with 33%, 49%, or 74% NX, specifically, plasma levels of taurine, aspartate, threonine, citrulline, and lysine were enhanced. In contrast, the plasma concentration of 11 amino acids was significantly increased or decreased in 84% NX rats compared with the control group.

Among these 24 amino acids, the plasma level of citrulline was markedly enhanced in each of the five groups of uremic rats. Citrullinemia progressively increased with the degree of renal insufficiency, leading to a good correlation (Table 2: $r = .860$ for the five groups of rats (I to V), $P < .01$; also see

Table 1. Biological Parameters in Control and Uremic Rats

Parameter	Experimental Group				
	I	II	III	IV	V
Degree of nephrectomy (%)	0	33 ± 2	49 ± 0	74 ± 1	84 ± 1
BW (g)	377 ± 28	360 ± 18	358 ± 13	357 ± 9	342 ± 12
Right kidney weight					
Absolute (mg)	1,265 ± 41	1,552 ± 50†	1,198 ± 51§	—	—
Relative (mg/100 g BW)	343 ± 16	433 ± 18†	376 ± 19§	—	—
Left kidney weight					
Absolute (mg)	1,232 ± 42	828 ± 55†	923 ± 87§ 1,899 ± 76‖	1,489 ± 76*	1,501 ± 92*
Relative (mg/100 g BW)	335 ± 17	230 ± 16†	294 ± 42§ 489 ± 18‖	418 ± 20*	437 ± 18†
Kidney(s) weight					
Absolute (mg)	2,497 ± 80	2,379 ± 88	2,121 ± 85§ 1,899 ± 76‖	1,489 ± 76‡	1,501 ± 92‡
Relative (mg/100 g BW)	678 ± 33	663 ± 30	670 ± 57§ 489 ± 18‖	418 ± 20‡	437 ± 18‡
Urea (mmol/L)	6.3 ± 0.2	7.7 ± 0.5	9.2 ± 0.4*	13.1 ± 1.3‡	18.3 ± 1.2‡
Creatinine (μmol/L)	44 ± 2	50 ± 1	63 ± 3†	77 ± 4‡	111 ± 9‡

NOTE. Results are the mean ± SE; n = 9 rats per group. Results were statistically analyzed by 1-factor ANOVA and Dunnett's *t* test, except for group III, where it was not applicable.

Abbreviation: BW, body weight.

*$P < .05$, †$P < .01$, ‡$P < .001$: *v* control group.

§Four rats.

‖Five rats.

Table 2. Amino Acid Concentrations in Plasma of Control and Uremic Rats

	Experimental Group				
	I	II	III	IV	V
Degree of NX (%)	0	33 ± 2	49 ± 0	74 ± 1	84 ± 1
Taurine (μmol/L)	99 ± 9	149 ± 18*	149 ± 14*	163 ± 20*	142 ± 20
Aspartate (μmol/L)	11 ± 1	13 ± 1	15 ± 2*	13 ± 1	15 ± 2*
Hydroxyproline (μmol/L)	75 ± 3	98 ± 11	102 ± 10	121 ± 13†	124 ± 16†
Threonine (μmol/L)	227 ± 6	291 ± 11*	248 ± 16	261 ± 15	255 ± 25
Serine (μmol/L)	238 ± 9	246 ± 7	212 ± 8	212 ± 11	205 ± 15*
Asparagine (μmol/L)	88 ± 6	81 ± 5	78 ± 4	81 ± 10	72 ± 9
Glutamate (μmol/L)	71 ± 9	87 ± 9	81 ± 5	71 ± 7	72 ± 7
Glutamine (μmol/L)	560 ± 17	592 ± 31	557 ± 21	542 ± 28	485 ± 29*
Proline (μmol/L)	234 ± 11	282 ± 15	273 ± 18	239 ± 17	291 ± 24*
Glycine (μmol/L)	351 ± 20	374 ± 21	383 ± 19	430 ± 29	465 ± 43†
Alanine (μmol/L)	420 ± 18	482 ± 15	502 ± 19	507 ± 42	519 ± 57
Citrulline (μmol/L)	65 ± 2	96 ± 5†	102 ± 4†	135 ± 12‡	158 ± 12‡
Valine (μmol/L)	164 ± 10	185 ± 7	178 ± 10	159 ± 6	159 ± 21
Cystine (μmol/L)	88 ± 2	95 ± 3	86 ± 3	76 ± 4	74 ± 6*
Methionine (μmol/L)	59 ± 3	66 ± 2	67 ± 4	66 ± 2	76 ± 7*
Isoleucine (μmol/L)	83 ± 6	86 ± 3	89 ± 6	75 ± 3	78 ± 10
Leucine (μmol/L)	117 ± 7	127 ± 5	126 ± 8	110 ± 4	111 ± 14
Tyrosine (μmol/L)	59 ± 5	67 ± 3	63 ± 4	61 ± 3	54 ± 6
Phenylalanine (μmol/L)	57 ± 3	59 ± 3	61 ± 3	63 ± 5	46 ± 2*
Ornithine (μmol/L)	53 ± 3	65 ± 7	55 ± 2	49 ± 3	51 ± 5
Lysine (μmol/L)	354 ± 24	447 ± 18*	413 ± 27	474 ± 28†	377 ± 26
Histidine (μmol/L)	54 ± 4	57 ± 3	63 ± 3	61 ± 3	49 ± 6
3-Methylhistidine (μmol/L)	3 ± 1	5 ± 1	4 ± 1	7 ± 2	9 ± 2†
Arginine (μmol/L)	179 ± 9	187 ± 10	183 ± 6	172 ± 8	159 ± 14

NOTE. Results are the mean ± SE; n = 9 rats per group. (For cystine, n = 7, 6, 6, 6, and 9 rats in groups I, II, III, IV, and V, respectively.) Results were statistically analyzed by 1-factor ANOVA and Dunnett's *t* test.

*$P < .05$, †$P < .01$, ‡$P < .001$: *v* the control group.

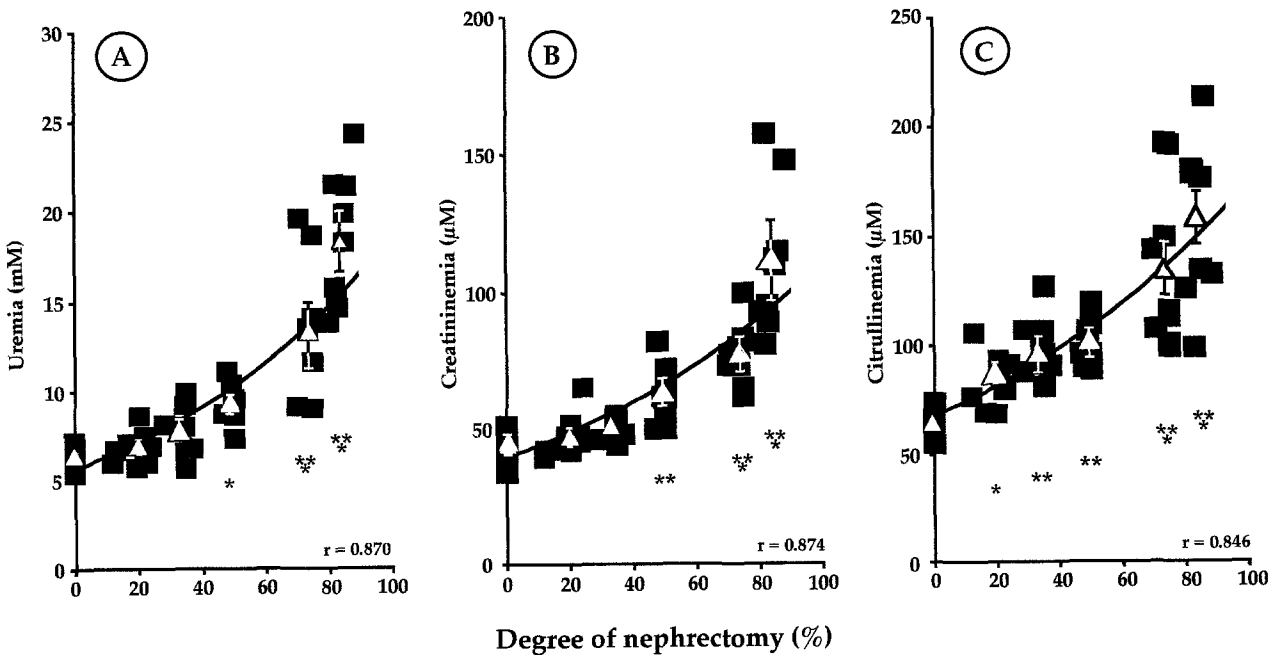

Fig 2. Influence of the degree of NX on uremia, creatininemia, and citrullinemia. Uremia (A), creatininemia (B), and citrullinemia (C) are correlated with the degree of NX. (■) individual value (1 rat); (△) mean ± SE for each group of 9 rats. Results obtained in the 5 groups of rats (I to V) and the additional experiment (rats with 20% NX) are included. *r* was calculated from the 6 groups of NX rats. The mean value of each uremic group of rats was compared with that of the control and analyzed by ANOVA 1-factor and Dunnett's *t* test: *$P < .05$, **$P < .01$, ***$P < .001$; n = 54 rats.

Fig 2). For the other amino acids, no correlation between the plasma concentration and the degree of NX was found.

Cl_{creat} and urinary parameters. Urine volume tended to increase with the degree of NX (Table 3). Cl_{creat} diminished with the degree of NX, indicating a significant decrease in GFR in each group of uremic rats (Table 3). These results indicate that 3 weeks after NX, group II had a weak renal insufficiency, since its Cl_{creat} was reduced by 18% compared with that in the control group ($P < .05$).

Generally, urinary excretion of most of the amino acids remained unchanged 3 weeks after NX. In rats with greater than 70% NX, the daily excretion of several amino acids such as hydroxyproline and methylhistidine was increased, whereas that of threonine and serine was diminished (data not shown). In control rats, low amounts of citrulline were excreted compared with most of the other amino acids (Table 3); however, citrullinuria was doubled in rats with 33% NX ($P < .05$). In the other groups of uremic rats, citrullinuria remained unchanged despite a sharp increase in citrullinemia (Tables 2 and 3). Most of the filtered citrulline in groups I, III, IV, and V was reabsorbed along the nephron (97% to 98%), whereas in group II, citrulline reabsorption was weakly diminished (92%, $P < .05$).

Additional study with 20% induced NX. In view of these results, we verified whether a degree of nephrectomy less than 33% would induce an increase in citrullinemia without any modifications of plasma urea and creatininemia. A new group of nine rats was subjected to degree of NX varying between 10% and 25%. The experimental protocol was similar to that already described.

The results showed no modifications in uremia (7.1 ± 0.3 mmol/L) and creatininemia (47 ± 3 μmol/L), but a significant increase in citrullinemia (88 ± 5 μmol/L, $P < .05$). For these three parameters, each individual value and the mean ± SE for this group of rats were included in Fig 2. Compared with the control levels, Cl_{creat} was reduced by 15.3% (2.06 ± 0.14 mL/min, $P < .052$), suggesting renal insufficiency. Citrullinuria did not differ from the control values, and 95% of the filtered citrulline was reabsorbed.

Time Course of Changes in Creatininemia, Uremia, Cl_{creat}, and Aminoacidemia in Response to 36% Renal Insufficiency

Biological parameters. The degree of NX (36.1% ± 0.5%) was not statistically different in the seven groups of uremic rats. The right kidney grew linearly throughout the experimental period of 3 weeks ($r = .951$, $P < .01$, data not shown), whereas a small increase in the left kidney weight was observed between days 16 and 21 (data not shown). This demonstrates that the renal hypertrophy was a linear but not step-by-step process.

Figure 3 indicates that uremia and Cl_{creat} showed important fluctuations, whereas creatininemia seemed more stable with time.

Twenty-four hours after NX, a sharp increase in uremia (+74%, $P < .005$) and creatininemia (+29%, $P < .005$) was observed (Fig 3A and B). A concomitant decrease in Cl_{creat} reflected a reduction of GFR (Fig 3C). The destruction of 24,000 glomeruli (one kidney contains ≈33,000 nephrons) decreased the GFR and was probably responsible for the plasma peaks of urea and creatinine.

During the next 3 days (day 1 to day 4), as a consequence of a reduction in the number of glomeruli, a hyperfiltration probably resulting from modifications of hemodynamic determinants[21] led to an enhancement of Cl_{creat} and, in parallel, to a better elimination of urea and creatinine from the plasma (Fig 3A, B, and C). It is of interest that uremia, creatininemia, and Cl_{creat} returned to control values on the fourth day.

During the next 17 days of the experiment, the time course of each of the three usual markers showed its own evolution. Cl_{creat} widely varied over this period. A stabilizing period of 3 days (day 4 to day 7) was followed by a slow but significant increase in Cl_{creat} for 11 days, and thereafter it remained stable during the last 5 days at a level 65% higher than the initial value ($P < .001$). This suggests that GFR has been partially restored due to renal growth and hypertrophy. The trend of uremia was to increase slowly until the eleventh day; thereafter, uremia seemed more stable and plateaued at a level 40% higher than the control value. Except on the sixteenth day, uremia was not different from the control value.

The second increase in creatininemia observed on the seventh

Table 3. Biological Parameters in Control and Uremic Rats

	Experimental Group				
	I	II	III	IV	V
Degree of NX (%)	0	33 ± 2	49 ± 0	74 ± 1	84 ± 1
Cl_{creat} (mL/min)	2.43 ± 0.14*	2.00 ± 0.19†	1.59 ± 0.07‡	1.47 ± 0.10§	0.81 ± 0.12*
Urine volume (mL)	14.9 ± 1.4‖	19.3 ± 1.2‖	19.9 ± 1.6‖	37.3 ± 4.8¶	33.9 ± 4.3¶
Citrullinuria (μmol/24 h)	0.36 ± 0.12	0.75 ± 0.17*	0.17 ± 0.09	0.15 ± 0.09	0.08 ± 0.04
Citrulline clearance (μL/min)	69.9 ± 20.0	157.0 ± 48.6*	34.1 ± 18.7	42.2 ± 25.2	16.7 ± 8.8
Fraction reabsorbed (%)	97.3 ± 0.7	92.7 ± 1.6*	97.7 ± 1.3	97.1 ± 1.0	97.3 ± 1.6

NOTE. Results are the mean ± SE for 7, 5, 5, 6, and 6 rats in groups I, II, III, IV, and V, respectively. Results were statistically analyzed by ANOVA with Fisher's PLSD at $P < .05$.

*Different from the other groups.

†Different from I, IV, and V.

‡Different from I and V.

§Different from I, II, and V.

‖Different from IV and V.

¶Different from I, II, and III.

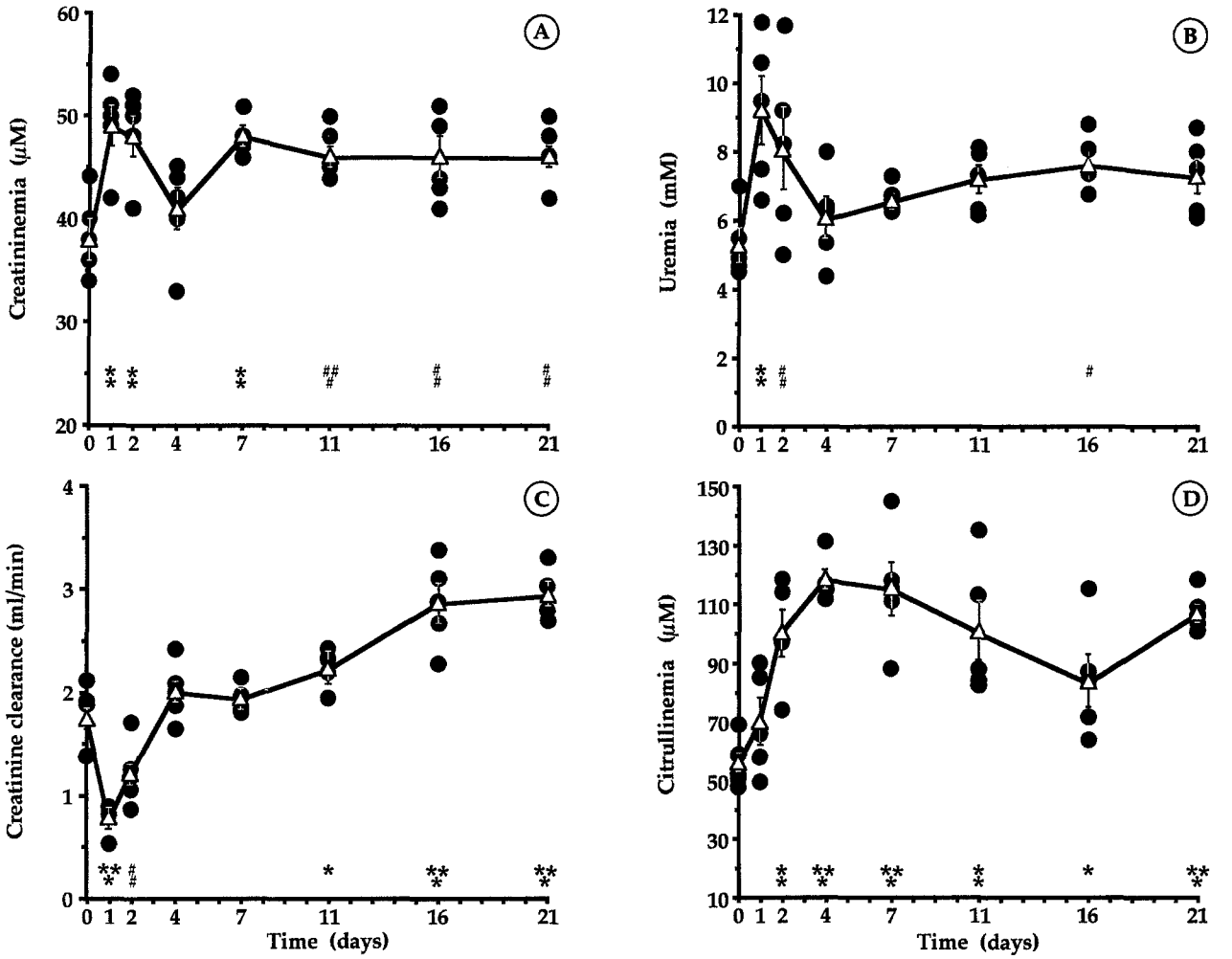

Fig 3. Time course of changes in creatininemia (A), uremia (B), Cl_{creat} (C), and citrullinemia (D) in response to 36% renal insufficiency. (●) individual value (1 rat); (△) mean ± SE for each group of 5 rats. The mean value of each uremic group of rats was compared with that of the control and analyzed by ANOVA 1-factor and Dunnett's *t* test: #$P < .05$, ##$P < .02$, ###$P < .01$, *$P < .025$, **$P < .005$, and ***$P < .001$; n = 45 rats.

day was followed by a plateau over the next 2 weeks. Creatininemia was about 20% higher than the control value ($P < .02$).

Plasma concentration of amino acids. The plasma concentration of each of 24 amino acids measured was significantly affected by NX. Most of the changes (enhancement or reduction) occurred within the first 96 hours and generally paralleled variations in the GFR (data not shown).

Citrullinemia tended to increase 24 hours after NX and seemed independent of variations in GFR (Fig 3). Thereafter, citrullinemia rapidly increased and plateaued to values twofold higher than the control level ($P < .005$ or greater) except on the day 16 (1.5-fold, $P < .025$). It is noteworthy that citrullinemia never returned to control values as observed for several other amino acids, and the three usual markers of uremic states. This suggests that there was no or only a partial recovery of citrulline conversion to arginine in the proximal tubule, despite renal growth and hypertrophy. It is possible that the intestine, liver, and other organs might also modify both Arg and citrulline metabolism in uremic rats.

Argininemia was reduced by 49% ($P < .001$) 24 hours after NX and returned to control levels during the following 24 hours. Throughout the next 20 days, argininemia did not differ from the control values.

DISCUSSION

At the present time, diagnosis of renal dysfunction in patients is based on the measurement of uremia, creatininemia, and GFR. Since the kidney, mainly the PCT, is involved in amino acid reabsorption and metabolism, we hypothesized that amino acids might be also good indicators of renal dysfunction. Consequently, the present study was designed to ascertain whether amino acids could be used as better markers than the classic markers of uremia to detect the onset of renal insufficiency and, if possible, also to reflect the extent of renal damage.

Among the natural amino acids, serine and arginine, which are synthesized in the proximal tubule,[17,18,22,23] and their respective metabolic precursor glycine, hydroxyproline, and citrulline might be good potential candidates. It is noteworthy

that in uremic patients and animals a constant increase in citrullinemia[5-7,10-15] and a decrease in plasma serine concentration[2] have been reported. However, it remains unknown whether the plasma concentration of citrulline or of another amino acid could be correlated with the degree of renal failure and used as a marker to detect renal dysfunction.

The present study clearly demonstrates that, at least in the rat, none of the four amino acids, serine, hydroxyproline, glycine, and arginine, could be used as markers of uremic states, since their plasma level did not vary in proportion to the degree of renal insufficiency. In contrast, citrulline seemed to be a more reliable and sensitive marker of uremia than the usual classic markers (ie, urea, creatinine, and Cl_{creat}).

In the first part of this study, we confirmed the enhancement of citrullinemia in uremic rats, but a more interesting result was the demonstration of a correlation between the degree of renal insufficiency and citrullinemia. Moreover, for the first time, we observed that in the range of 10% to 33% NX, citrullinemia was significantly increased without any changes in uremia and creatininemia (Fig 2). Thus, it seems that destruction of a small portion of the kidney induces an accumulation of citrulline in the plasma. Based on our observations, an important conclusion is that 3 weeks after NX—a situation simulating chronic renal failure—citrullinemia was a more sensitive marker of renal damage than plasma urea and creatinine concentration. Since citrulline is metabolized into arginine mainly in the PCT and to a lesser extent in the straight proximal tubule,[17] we conclude that citrulline could be used as a specific marker of normal function of the proximal tubule and, at the same time, to quantify the extent of renal damage by using a standard abacus.

That citrullinemia was highly enhanced in uremic rats suggests there was no increase in the ability of the remaining proximal tubules to synthesize arginine. Indeed, it is surprising that in rats with 33% NX citrullinemia increased despite a complete compensatory hypertrophy of the remaining kidney (Table 2); the kidney weight was similar to that of the control rats. Moreover, whatever the degree of renal damage, the low citrullinuria in control and uremic rats suggests that most of the filtered citrulline was reabsorbed along the PCT (93% to 98%; Table 3).[24] Thus, the excess of plasma citrulline might originate from a reduction of the arginine synthase complex activity in the kidney of uremic rats.[13] At short-term, this possible defect of enzymatic regulation associated with no modification in citrullinemia might be responsible for the sharp decrease in argininemia 24 hours after NX. Moreover, the unsignificant change in citrullinemia might be explained by the parallel enhancement of citrulline concentration in the liver and muscles, as recently reported by Swendseid et al[11] and Chan et al.[13] In uremia, the concentration of citrulline seems to be increased in the different tissular compartments. In contrast, at longer-term evaluation, since renal arginine synthesis depends on the availability of the substrate citrulline,[18,25] a possible lack of enzyme regulation might be compensated for by the enhancement in citrullinemia.[15] Indeed, we noted that over the next 20 days of the experiment, argininemia remained in the range of the control level. We do not exclude that other factors such as hepatic arginase activity[13] and citrulline synthesis in the small intestine[26,27] might also regulate citrullinemia. Further studies are needed to understand these findings.

The second part of the study was designed to know whether citrulline was also a marker of the onset of renal failure. The time course of changes in uremia, creatininemia, aminoacidemia, and Cl_{creat} was evaluated over 21 days, a situation simulating both acute and chronic renal insufficiency. In this experiment, we attempted to induce 30% to 33% NX, as in group II, to enhance only citrullinemia. Unfortunately, the mean degree of NX was 36.1% ± 0.5%. This percentage did not statistically differ from that of group II (32.9 ± 0.6% NX, experiment I). However, 3 weeks after NX, creatininemia was 20% higher than the control value of the second experimental series ($P < .02$). It is therefore likely that the range of 33% to 36% NX might be the upper limit where citrullinemia increases without any variations of creatininemia. This second part of our study shows that neither uremia nor Cl_{creat} could be used as markers of renal insufficiency, since too many variations occurred during the first 3 weeks following NX and since the levels often did not differ from control values. The use of creatininemia to detect renal failure requires caution for the following reasons: (1) creatininemia remained unchanged in the range of 10% to 33% NX, (2) creatininemia increased slightly (≈20%) in chronically uremic rats with 36% NX, and (3) during the first week (mainly on day 4; Fig 3), creatininemia was unchanged despite the presence of renal damage. In contrast, we demonstrate that among 24 natural amino acids, only citrulline could be used as a marker of both acute and chronic renal insufficiency. Safety in the use of citrulline to detect the onset of renal insufficiency is guaranteed by the reliability of the high-performance liquid chromatography method used to measure citrulline levels and the large increase in its plasma concentration (between 50% and 96%).

These results require caution before being extrapolated to humans, because amino acid handling in human and rat kidney could differ. However, it should be noted that an increase in citrullinemia has always been reported in uremic patients.[5-7,10,12,28] Some clinical data suggest that in patients, citrullinemia is also correlated with the degree of renal damage calculated on the basis of GFR.[12,28,29] In these patients, such an increase in citrulline plasma concentration can be distinguished from the rare hereditary disorder of the urea cycle caused by a deficient activity of argininosuccinate synthetase ([ASS] EC 6.3.4.5). In the latter metabolic disorder, a hypercitrullinemia is associated with a hyperammonemia in patients with quantitatively abnormal ASS in the liver but active ASS in the kidney.[30,31]

In conclusion, we have established, at least in the rat, that citrulline could be used as a good plasma marker of glomerular and tubular dysfunction and thus allows estimation of the amount of renal tissue that has been destroyed. These findings indicate that citrullinemia might be a better marker than the conventional markers used, ie, uremia, Cl_{creat}, and creatininemia.

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