Plasma and Urinary Amino Acids in Primary Gout, with Special Reference to Glutamine

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Abstract Measurement of the plasma free amino acids by column chromatography (AutoAnalyzer) in 32 patients with primary gout showed statistically significant increases or decreases in several components when compared with the spectrum in 18 control subjects, but the absolute amounts involved were small and the mean total plasma amino acid concentrations in both groups were the same. In the urine all major amino acid components, notably glutamine, serine, threonine, and leucine, were lower in our gouty than in our nongouty subjects, as were also the corresponding renal clearance ratios. These deficits could be reproduced by restricting dietary protein, so appear to be due largely to the somewhat reduced mean dietary protein intake of our gouty subjects. However, the low renal clearance of glutamine, the most striking and consistent of the deficits in urinary amino acids noted, could not be accounted for by dietary or other relevant factors, and is interpreted as indicating increased tubular reabsorption of glutamine in primary gout. This interpretation was supported by the results of glutamine loading. The possible compensatory relationship of the abnormality in renal handling of glutamine to the deficiency in renal production of ammonia previously reported is discussed.

INTRODUCTION

This study was undertaken because of the predominant role of certain amino acids in *de novo* purine biosynthesis and consequently in overproduction of uric acid, an important factor in the pathogenesis of gout. The relevant purine precursors are glutamine, which donates its amide nitrogen to form N-9 and N-3 of the purine ring; glycine which is incorporated to give C-4, C-5, and N-7; aspartic acid, which contributes N-1; and serine, which transfers its beta carbon by way of tetrahydrofolate derivatives to yield C-2 and C-8. These amino acids are utilized in increased amounts for formation of uric acid in gout, at least in gouty overproducers of uric acid, and one of the objectives of this inquiry was to determine what, if any, impact this diversion into *de novo* purine biosynthesis in primary gout might have on the plasma and urinary amino acid concentrations.

Special attention was paid to glutamine because abnormalities in the metabolic fate of this amino acid have been noted in primary gout (1, 2), and it has been suggested that these irregularities may initiate overproduction of uric acid since glutamine is the sole amino acid substrate of the committed, presumably rate-regulating reaction of *de novo* purine biosynthesis. In normal man, glutamine is present in plasma in concentrations greater than that of any other amino acid, and it is also one of the major amino acid components of the urine. In gouty subjects, conflicting data have been recorded for glutamine levels in plasma (3, 4) and none could be found in the literature for glutamine in urine: prior reports on the spectrum of plasma and urinary amino acids in gout (5–7) give no specific figures for glutamine.

METHODS

18 nongouty men, aged 20–64 (mean, 35 yr), served as controls for the studies on 32 men with primary gout, aged 17–73 (mean, 41 yr). The mean plasma urate in the nongouty was 5.7 mg%, that of the gouty was 10.3 mg%. 23 of the gouty subjects were overexcretors of uric acid (urinary uric acid excretion > 800 mg/24 hr). The mean creatinine clearance was 115 ml/min in the gouty, 124 ml/min in the nongouty; only subjects free of overt renal disease were selected for study. Drug treatment of the gouty subjects was discontinued, except for colchicine prophylaxis, well in advance of the study. Dietary protein intake was not strictly controlled. The mean urinary total nitrogen excretion in

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the nongouty subjects was 11.0 mg/min, that of the gouty was 9.9 mg/min. The impact of the differences in diet on the spectrum of plasma and urinary amino acids will be considered under Results.

The subjects omitted breakfast on the morning blood and urine samples were procured. Blood drawn from the antecubital vein was promptly centrifuged at 5°C, the plasma proteins precipitated with sulfosalicylic acid, and supernatant equivalent to 1.0 ml plasma was applied to the column of an amino acid AutoAnalyzer. For the studies on urine a freshly passed 2-hr morning sample was collected, the subject having taken enough water to ensure urine flow of 0.5–1.5 ml/min. The protein-free urine was adjusted to pH 2 and an aliquot equivalent to 0.3–0.5 min urine flow was applied to the column. Usually the plasma and urine chromatograms were initiated on the same day that the samples were procured; when this was not possible the protein-free plasma supernatant or the acidified urine was stored at −20°C.

For glutamine loading tests, three nongouty and eight of the gouty subjects were given 195 mg glutamine/kg body weight as a single oral dose in the morning before breakfast. Blood and urine samples were obtained before and at hourly intervals thereafter.

For determination of free amino acids in plasma and urine a Technicon model NC-1 standard 21-hr amino acid AutoAnalyzer was employed, equipped with 130 × 0.63 cm columns which were charged with Chromo-beads resin type B (nominal 8% cross-linked, spherical particles 17 ±1 μ in diameter). Plasma and urine samples were run simultaneously in two parallel analytical systems using two coils in the same heating bath. Norleucine, 0.25 μmole/0.1 ml in 1% HCl, was added to both columns as internal standard. The columns were operated at 30°C for the 1st 4 hr to minimize loss of glutamine (8), then at 60°C for 17 hr. The flow rate was set at 0.5 ml/min to ensure delivery of the citrate buffers of pH 2.875, 3.8, and 5.0 through the nine-chamber Autograd in a smooth pH gradient, and also a continuous Na gradient between 0.2 and 0.8 M. The effluents were reacted with ninhydrin-hydrindantin reagent and the optical densities recorded at 570 and 440 mμ. The respective integration constants were computed and the results expressed in terms of norleucine color equivalents. Recoveries and norleucine color equivalents of individual amino acids were checked with standard 0.25 μmole amino acid mixtures when the columns were regenerated and at other appropriate intervals. Difficulties in quantification due to incomplete separation of certain peaks in the chromatograms will be commented on under Results.

As a further check on glutamine levels in plasma, this amino acid was also determined enzymatically in 16 patients with primary gout and six control subjects. The Archibald method as modified by Segal and Wyngaarden (4) was employed, using purified glutaminase I (Worthington Biochemical Corp.). A few such checks on glutamine and asparagine levels in urine were also made, by chromatographic analysis before and after incubation with glutaminase-I or asparaginase.

To estimate total bound amino acids in urine, 1.0 ml of urine was hydrolyzed with 1 ml concentrated HCl in a sealed vial for 18 hr at 100°C. After evaporating the hydrolysate to dryness the residue was taken up in water and aliquots chromatographed. For measurement of bound glutamine the difference between the glutamic acid in the hydrolysate and the free glutamine plus glutamic acid in the unhydrolyzed urine was taken to represent glutamine in conjugated or otherwise bound form. Bound glycine and aspartate were similarly estimated.

To measure glutamine bound to phenylacetic acid in urine, the urine was first acidified to pH 2 with HCl and an aliquot (25–30 ml) subjected to continuous extraction with 100 ml of a 1:1 mixture of ethyl acetate and ethyl ether, using fresh solvent for each of three 72-hr extraction periods. (Extraction in this way of known quantities of phenylacetylglutamine added to urine was found to be virtually complete, with minimal contamination with free glutamine, glutamic acid, or other free amino acids.) The combined extracts were evaporated to dryness at 40°C, then taken up in 5 ml water. A 1.0 ml aliquot was then subjected to acid hydrolysis in a sealed vial and further treated as described for unextracted urine. Bound glutamine not conjugated with phenylacetic acid was estimated by direct measurement in the aqueous phase of the extracted urine.

RESULTS

Free amino acids in plasma. Table I records the mean levels of 20 amino acids as found in the venous plasma of 18 nongouty control subjects and 32 patients with primary gout. Proline is omitted because quantification was unsatisfactory due to frequently incomplete separation from the glutamic acid peak and the low color equivalent of proline; in a few favorable chromatograms proline was estimated to be present in concentrations of the order of 0.2–0.25 μmole/ml. Aspartic acid does not separate cleanly from serine when chromatographed at 30°C. In several runs initiated at 60°C the plasma concentration of free aspartic acid approximated 0.01 μmole/ml.

Despite operation of the columns at 30°C for the initial 4 hr to minimize losses of glutamine by conversion to pyrrolidonecarboxylic acid and glutamic acid, and average recoveries of 88% of known amounts of glutamine, the mean values of plasma glutamine shown in Table I are somewhat lower than those found by others. Using amino acid AutoAnalyzers Siegel, Roach, and Pomeroy (9) and Swendseid et al. (10) reported means of 0.577 and 0.601 μmole glutamine/ml plasma in normal men. The results with purified preparations of glutaminase-1 and of acid hydrolysis also are somewhat higher. Archibald (11), applying enzymatic analysis, recorded a range of 6–10 mg% glutamine (0.411–0.685 μmole/ml) in normal human plasma, with which subsequent investigators (3, 4, 12–16) essentially agree. In gouty subjects, Örström and Örström (3), using acid hydrolysis, reported a mean of only 1.40 mg% glutamine in whole blood as opposed to their normal mean of 9.95 mg%, whereas Segal and Wyngaarden (4) found normal plasma glutamine levels in patients with gout: means of 8.4 and 8.6 mg% by enzymatic analysis and acid hydrolysis, respectively, as compared to normal means of 8.9 and 9.5 mg%. Our own results with a purified preparation of glutaminase 1 likewise gave mean values of 0.534 μmole/ml (7.8 mg%, range 6.4–9.7 mg%) in 16

TABLE I

*Mean P_{aa} in Gouty and Nongouty Control Subjects**

	Controls (N = 18)	Gouty (N = 32)	P‡
	μmoles/ml ±SD	*μmoles/ml* ±SD	
Glutamine	0.496 ±0.080	0.495 ±0.075	
Alanine	0.379 ±0.057	0.422 ±0.076	$0.05 > P > 0.02$
Glycine	0.257 ±0.051	0.217 ±0.038	$0.01 > P > 0.001$
Valine	0.253 ±0.042	0.265 ±0.039	
Lysine	0.234 ±0.033	0.250 ±0.041	
Leucine	0.148 ±0.026	0.162 ±0.021	$0.1 > P > 0.05$
Threonine	0.145 ±0.027	0.129 ±0.020	0.05
Serine	0.119 ±0.019	0.105 ±0.021	$0.05 > P > 0.02$
Histidine	0.118 ±0.022	0.108 ±0.020	
Arginine	0.095 ±0.017	0.093 ±0.017	
Ornithine	0.095 ±0.035	0.088 ±0.015	
Isoleucine	0.071 ±0.014	0.081 ±0.012	$0.02 > P > 0.01$
Tyrosine	0.065 ±0.013	0.068 ±0.013	
Phenylalanine	0.056 ±0.008	0.057 ±0.007	
Cystine, half	0.055 ±0.012	0.063 ±0.012	
Glutamic acid	0.051 ±0.015	0.072 ±0.012	<0.001
Taurine	0.040 ±0.009	0.041 ±0.015	
Citrulline	0.035 ±0.007	0.037±0.009	
Methionine	0.027 ±0.005	0.025 ±0.005	
α NH_2-N-butyric acid	0.023 ±0.007	0.021 ±0.007	
Total	2.758 ±0.285	2.797 ±0.274	

N = Number of subjects.

* Proline and aspartic acid not included for reasons stated in the text.

‡ Only significant *P* values indicated.

patients with primary gout and 0.562 μmole/ml (8.2 mg%, range 6.3–10.4 mg%) in six nongouty control subjects. When plasma glutamine was determined both enzymatically and by AutoAnalyzer in replicates, the results with the latter procedure were consistently 10–15% lower in both the gouty and the nongouty.

The arrangement of amino acids in Table I (and subsequent tables) is in descending order in the nongouty control subjects but the sequence is much the same in the gouty subjects. In both, glutamine heads the list, at the same mean plasma concentrations in the nongouty and the gouty. The only highly significant statistical differences observed in the gouty were in the mean levels of plasma glycine (decreased) and glutamic acid (increased). Of lesser or borderline statistical significance were the somewhat higher mean figures of alanine and isoleucine, and the somewhat lower mean figures for serine and threonine. The differences in all other mean amino acid concentrations in the gouty were statistically insignificant. The mean totals for free amino acids in the plasma were 2.758 ±0.285 μmoles/ml in the control subjects and 2.797 ±0.274 μmoles/ml in the gouty, not a significant difference. If our tentative figures for proline and aspartic acid are included in the totals they would approximate 3.0 μmoles/ml in both groups.

Free amino acids in urine. Quantification of many amino acids is less reliable in the urine than in the plasma because of the multiplicity of ninhydrin-reactive components and incomplete resolution of some peaks (17–19). In our hands this applied particularly to the basic amino acids: separation of lysine from 1-methylhistidine and of histidine from 3-methylhistidine was frequently unsatisfactory, except in a few runs in which the pH 5.0 buffer was replaced with pH 4.75 buffer, as suggested by Efron (20). We give estimates for lysine (although probably too high) and for histidine but not for the methylhistidines. There were occasional difficulties in localization of leucine and isoleucine peaks because of adjacent minor peaks, some unidentified. Aspartic acid is included in the serine peak when chromatographed at 30°C but in such small quantities in fresh, unhydrolyzed urine samples that we consider the estimates for serine to be acceptable. No estimates for asparagine are given because of often incomplete separation from glutamine, even at 30°C, and the relatively low color equivalent of asparagine at 570 mμ. After incubating the urine with glutaminase 1, asparagine appeared to be present in amounts comprising 10–20% of the glutamine. The error in glutamine estimates incurred by asparagine interference consequently is small at 570 mμ, and we

believe that the figures for glutamine cited in Table II are reliable within the limits previously indicated.

Table II records the mean urinary excretion of 17 free amino acids in 13 nongouty control subjects and 29 patients with primary gout. All the major amino acid components of the urine were found to be excreted at less than normal rates, with deficits of 0.10 μmole/min or more in glycine, histidine, taurine, lysine, glutamine, and serine. However, the differences were statistically significant only for glutamine and serine ($P < 0.001$), due to the very large individual variations and overlap in excretion of the other amino acids, as noted in normal persons by previous investigators [reviewed by Soupart (21) and Scriver (22)]. The decreases in threonine and leucine were small but highly significant statistically ($P < 0.001$). The most striking of these various amino acid deficits in the urine was in glutamine, not only in absolute terms but also in consistency; the standard deviations for glutamine were relatively small and there was virtually no overlap between the gouty and the nongouty.

Renal clearance ratios of free amino acids. As would follow from their essentially normal plasma levels but more or less reduced rates of urinary excretion, the renal clearance ratios for amino acids in the gouty tended to be lower than in the control subjects (Table III). The decreases in C_{aa}/glomerular filtration rate (GFR) were neither as pronounced nor as consistent, however, as in $U_{aa}V$. The only deficits of statistical significance were in glutamine ($P < 0.001$), leucine ($P = 0.001$), serine, and threonine ($0.01 > P > 0.001$ for both).

Effect of dietary protein restriction. Some of the patients with primary gout included in the group under study were taking a low protein diet, thus affording opportunity to analyze the effect of protein restriction on the spectrum of plasma and urinary amino acids. Table IV summarizes the results in seven such patients whose mean urinary total nitrogen excretion was only 7.1 mg/min (range, 6.6–8.0 mg/min). When $U_{aa}V$ in these subjects is compared with the means shown in Table II for the composite group of 29 gouty subjects whose average urinary total nitrogen excretion was 9.9 mg/min, a decline in all the major amino acid components of the urine is apparent. The decreases exceeded 0.15 μmole/min for glycine, histidine, and lysine, and approximated 0.06 μmole/min for taurine and glutamine; the remaining urinary amino acids showed lesser declines. In contrast, as previously reported by Swendseid et al. (10), plasma amino acid concentrations were but little affected by restrictions in dietary protein intake of this degree (cf. Table I). The mean total of plasma amino acids, omitting proline and aspartic acid, was only slightly decreased: 2.725 ±0.225 μmoles/ml as compared to 2.797 ±0.274 μmoles/ml in the group of 29 gouty subjects as a whole. The renal clearance ratios for amino acids in the seven gouty subjects consequently tended to be lower than in the composite group of 29 patients with primary gout (cf. Tables III and IV) but the differ-

TABLE II

*Mean $U_{aa}V$ in Gouty and Nongouty Control Subjects**

	Controls (N = 13)	Gouty (N = 29)	P‡
	μmoles/min ±SD	*μmoles/min* ±SD	
Glycine	0.785 ±0.355	0.572 ±0.318	
Histidine	0.717 ±0.162	0.598 ±0.253	
Taurine	0.710 ±0.336	0.556 ±0.358	
Lysine	0.591 ±0.237	0.448 ±0.276	
Glutamine	0.440 ±0.078	0.239 ±0.058	<0.001
Serine	0.260 ±0.035	0.158 ±0.032	<0.001
Alanine	0.198 ±0.028	0.180 ±0.055	
Threonine	0.134 ±0.045	0.076 ±0.020	<0.001
Tyrosine	0.098 ±0.025	0.086 ±0.029	
Phenylalanine	0.058 ±0.014	0.047 ±0.019	
Leucine	0.045 ±0.009	0.031 ±0.008	<0.001
Cystine, half	0.044 ±0.018	0.048 ±0.016	
Valine	0.033 ±0.012	0.029 ±0.008	
Isoleucine	0.032 ±0.017	0.031 ±0.014	
Glutamic acid	0.027 ±0.006	0.033 ±0.018	
Methionine	0.025 ±0.007	0.023 ±0.006	
Arginine	0.021 ±0.005	0.026 ±0.015	

N = Number of subjects.

* Methylhistidines, ornithine, asparagine, and aspartic acid not included for reasons stated in the text.

‡ Only significant P values indicated.

TABLE III

*Mean C_{aa}/GFR in Gouty and Nongouty Control Subjects**

	Controls (N = 13)	Gouty (N = 29)	P‡
	% ±SD	% ±SD	
Taurine	14.21 ±7.41	12.98 ±8.66	
Histidine	5.77 ±1.72	4.44 ±2.24	
Glycine	2.48 ±0.99	2.28 ±1.06	
Lysine	2.17 ±0.94	1.73 ±1.25	
Serine	1.85 ±0.39	1.46 ±0.42	$0.01 > P > 0.001$
Tyrosine	1.32 ±0.26	1.15 ±0.48	
Phenylalanine	0.84 ±0.18	0.73 ±0.35	
Methionine	0.82 ±0.25	0.85 ±0.30	
Threonine	0.77 ±0.26	0.54 ±0.21	$0.01 > P > 0.001$
Glutamine	0.74 ±0.14	0.42 ±0.12	<0.001
Cystine, half	0.67 ±0.26	0.68 ±0.28	
Alanine	0.45 ±0.12	0.39 ±0.14	
Glutamic acid	0.43 ±0.20	0.37 ±0.17	
Isoleucine	0.38 ±0.16	0.38 ±0.19	
Leucine	0.28 ±0.05	0.18 ±0.06	0.001
Arginine	0.18 ±0.04	0.20 ±0.07	
Valine	0.11 ±0.05	0.10 ±0.03	

N = Number of subjects.

* Omissions as noted in footnotes to Tables II and III.

‡ Only significant *P* values indicated.

ences, with few exceptions, were not of statistical significance. Thus the generally lower $U_{aa}V$ and C_{aa}/GFR found in our patients with primary gout (Tables II and III), whose mean urinary total nitrogen excretion was somewhat less than that of our nongouty control subjects (9.9 vs. 11.0 mg/min), could be reproduced by restrictions in dietary protein intake.

However, these dietary factors did not altogether

TABLE IV

Mean $U_{aa}V$, P_{aa}, and C_{aa}/GFR of Seven Patients with Primary Gout Taking a Diet Restricted in Proteins

	$U_{aa}V$	P_{aa}	C_{aa}/GFR
	μmoles/min ±SD	*μmoles/ml* ±SD	% ±SD
Taurine	0.495 ±0.299	0.036 ±0.007	12.57 ±7.03
Histidine	0.417 ±0.117	0.106 ±0.016	3.52 ±0.92
Glycine	0.369 ±0.108	0.200 ±0.024	1.65 ±0.49
Lysine	0.296 ±0.155	0.232 ±0.034	1.18 ±0.70
Glutamine	0.181 ±0.034	0.473 ±0.082	0.36 ±0.12
Alanine	0.172 ±0.052	0.446 ±0.065	0.35 ±0.14
Serine	0.131 ±0.022	0.093 ±0.022	1.06 ±0.41
Tyrosine	0.070 ±0.013	0.066 ±0.015	1.03 ±0.39
Threonine	0.063 ±0.022	0.117 ±0.019	0.50 ±0.21
Cystine, half	0.043 ±0.015	0.064 ±0.011	0.60 ±0.18
Phenylalanine	0.038 ±0.018	0.057 ±0.009	0.60 ±0.31
Glutamic acid	0.037 ±0.001	0.069 ±0.010	0.50 ±0.09
Arginine	0.031 ±0.011	0.087 ±0.010	0.23 ±0.07
Valine	0.029 ±0.004	0.263 ±0.031	0.10 ±0.02
Methionine	0.028 ±0.009	0.023 ±0.009	1.09 ±0.44
Leucine	0.026 ±0.006	0.160 ±0.021	0.15 ±0.05
Isoleucine	0.023 ±0.005	0.080 ±0.014	0.26 ±0.08
Ornithine		0.088 ±0.023	
Citrulline		0.039 ±0.008	
α NH_2-N-butyric acid		0.020 ±0.005	

account for the differences noted. Deficits in UV and C/GFR in some degree persisted in glutamine, serine, and threonine when 19 gouty subjects with a mean urinary total nitrogen excretion of 11.0 mg/min were separated out from the rest and paired with the nongouty control subjects. By far the most conspicuous deficit found in the gouty in this comparison was in urinary glutamine excretion (mean deficit, 0.20 μmole/min).

Effect of urine pH. The urine in many patients with primary gout is unduly acid and less subject than normal to postcibal "alkaline tides" (23, 24). To determine what effect urine pH might have, the amino acid chromatograms of 25 gouty and 6 nongouty subjects with early morning urine pH of 4.8–5.6 were compared with those of four gouty and seven nongouty subjects with urine pH 5.7–6.2. Differences in urine pH over this range were found not to affect $U_{aa}V$ significantly in either the gouty or the nongouty.

Seven patients with primary gout were given sodium bicarbonate to increase their urine pH from 4.9–5.5 to 5.9–7.4. Alkalinization of the urine caused small but not statistically significant increases in the urinary output of several amino acids. It is concluded that the more acid urine pH of our patients with primary gout played little or no part in their generally somewhat lower $U_{aa}V$.

Effect of glutamine loading. Because of the strikingly decreased renal excretion of glutamine found in our patients with primary gout the response to glutamine, given in a single oral dose of 195 mg/kg body weight, was compared in eight gouty and three nongouty control subjects (Fig. 1). It is well established that glutamine is absorbed intact from the gastrointestinal tract (25) and that in normal man plasma glutamine levels rise promptly after oral administration (26). In both our gouty and nongouty subjects the plasma glutamine increased from initial mean levels of approximately 0.45 μmole/ml to equal mean levels in excess of 0.70 μmole/min at the end of ½ hr; at 1½ hr the values were declining, and at 2½ hr had almost returned to the initial levels. In contrast, the findings in the urine differed in the gouty and the nongouty. In the gouty, $U_{glutamine}V$ rose from a low mean of 0.207 ±0.033 μmole/min in the control period to a peak mean of 0.406 ±0.078 μmole/min (an increase of 0.20 μmole/min), whereas in the nongouty controls $U_{glutamine}V$ rose from 0.366 ±0.048 to a peak of 0.670 ±0.129 μmole/min, an increase of 0.30 μmole/min. $C_{glutamine}$/GFR in the nongouty increased from 0.67% initially to 0.93% whereas in the gouty the increase was from 0.39% initially to only 0.54%.

In contrast to the muted effect of glutamine loading on urinary excretion of glutamine in these gouty subjects, their elimination of uric acid was unduly augmented, from a mean of 0.68 mg/min initially to 1.02 mg/min, as compared to a rise from 0.50 to 0.65 mg/min in the nongouty. Mean urinary ammonia excretion increased equally in both groups (approximately 10 μmoles/min) but remained lower in the gouty subjects. The initial figures were 30.7 and 20.7 μmoles/min in the nongouty and gouty respectively, with mean peak increases to 40.7 and 31.4 μmoles/min respectively.

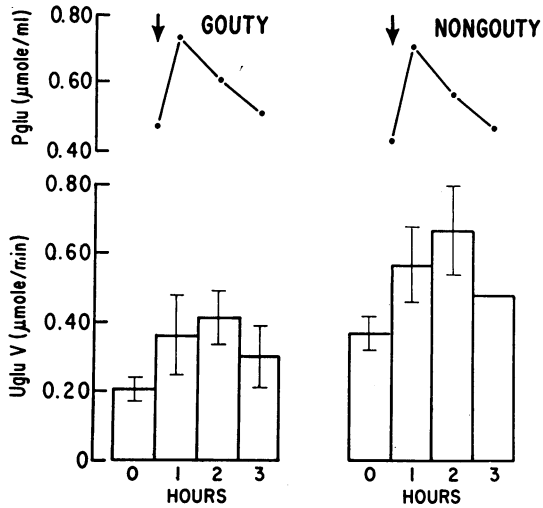

FIGURE 1 Response to oral glutamine load (arrow). Top, prompt rise in plasma glutamine to the same levels in the gouty and nongouty, with return toward initial concentrations in the third hour. Bottom, significantly less renal excretion of glutamine in the gouty than in the nongouty (SD indicated). The renal clearance of glutamine, accordingly, was significantly lower in the gouty (see text).

Bound amino acids in urine. A number of amino acids, notably glycine, glutamic acid (glutamine), and aspartic acid, are excreted in the urine not only as free amino acids but also, and in larger amounts, in conjugated or otherwise bound form (27–29). To determine whether the decreased renal excretion of free glutamine noted in our patients with primary gout was accompanied by a commensurate increase in bound glutamine, measurements were made before and after hydrolysis. Acid hydrolysis of ethyl acetate–ethyl ether extracts of urine, containing the phenylacetylglutamine present, yielded means of 0.869 ±0.086 μmole glutamic acid/min in five gouty subjects and 1.378 ±0.213 μmole glutamic acid/min in four nongouty control subjects ($0.01 > P > 0.001$). Thus excretion of glutamine in the phenylacetylglutamine fraction, as well as the free amino acid, appears to be significantly decreased in primary gout. The mean figures for hydrolyzed bound glutamine in the aqueous residue were 0.441 ±0.039 μmole/min in the gouty and 0.491 ±0.079 μmole/min in the nongouty. After correcting for the free glutamine and glutamic

acid initially present, the respective values were 0.184 ± 0.108 μmole/min and 0.081 ±0.057 μmole/min; this difference is not statistically significant.

When the glycine conjugates in the urine of these subjects were similarly estimated, acid hydrolysis of the organic solvent phase yielded means of 2.69 ±1.50 μmole glycine/min in the gouty and 2.36 ±0.60 μmole glycine/min in the nongouty, not a significant difference. Nor did the two groups differ with respect to the bound glycine in the aqueous phase: 1.18 ±0.31 μmole/min and 1.33 ±0.247 μmole/min respectively. After subtracting the free glycine initially present the respective figures were 0.812 ±0.134 and 0.405 ±0.042 μmole glycine/min ($P = 0.02$).

Similarly, no significant difference in respect to bound aspartic acid was found in the gouty and the nongouty: in the aqueous residue, which contained virtually all the bound aspartic acid, 0.622 ±0.192 μmole/min in the gouty and 0.580 ±0.041 μmole/min in the nongouty.

DISCUSSION

Comparison of the free amino acid concentrations in the peripheral venous plasma of gouty and nongouty subjects revealed no consistent trend of deviation in the gouty. The figures for plasma glutamine, the amino acid present in highest concentration, 6–10 mg%, were all within the normal range, as previously noted by Segal and Wyngaarden (4). In the gouty there was a statistically highly significant decrease in the mean plasma glycine level and a small but highly significant increase in the mean glutamic acid. Other increases or decreases were for the most part minor and appeared to be chiefly within the range of individual variations and experimental error. The mean total plasma amino acid concentrations in both the gouty and nongouty groups approximated 3.0 μmoles/ml—we did not find the hyperaminoacidemia reported in gout by others (6).

In the urine, all the major amino acid components were found to be excreted at a lower rate in our patients with primary gout than in the nongouty control subjects. The deficits were highly significant statistically ($P < 0.001$) for glutamine, serine, threonine, and leucine, not significant for the remaining amino acids. The renal clearance ratios also tended to be lower in the gouty subjects, although less consistently, but with highly significant differences for glutamine, leucine, serine, and threonine. Both in terms of absolute quantities and consistency the deficits in UV and C/GFR were more striking for glutamine than for any other amino acid.

These deficits in renal excretion of amino acids in our patients with primary gout are ascribed largely to their more restricted mean consumption of dietary proteins and their somewhat lower mean GFR. It was shown that even moderate restrictions in protein intake, such as are likely to be encountered in gouty populations under treatment, tend to decrease the renal excretion of many amino acids, and also their renal clearance since the plasma amino acid concentrations are affected little if at all. This response presumably reflects the normal regulation of reabsorption and elimination of amino acids by the kidneys to maintain appropriate plasma amino acid levels, by the operation of several distinct, genetically controlled reabsorptive systems (30). Such an interpretation seems to us more likely than the assumption of an abnormality in renal tubular membrane permeability (7) to explain the hyperaminoacidemia reported to be present in gout (6). As already mentioned, our data do not reveal any general hyperaminoacidemia.

In considering the significance of the most striking abnormalities found in the gouty subjects, namely the uniformly decreased urinary excretion of glutamine and the generally decreased concentration of plasma glycine, one possibility is that they reflect increased utilization of these amino acids in *de novo* purine biosynthesis. This is not certain, however, since the additional consumption of these amino acids in overproduction of uric acid in primary gout is small in relation to their utilization in other pathways.

Glutamine apparently represents a special case in the renal handling of amino acids in primary gout. In the gouty, despite normal glutamine filtered loads there was a decrease of 0.22 μmole/min (41%) in the mean $U_{glutamine}V$ (0.239 ±0.058 μmole/min vs. 0.458 ±0.061 μmole/min in the nongouty, $P < 0.001$), with a mean $C_{glutamine}$/GFR of 0.42 ±0.12% vs. 0.74 ±0.14% in the nongouty, $P < 0.001$. Subject to the limitations in method noted, the determinations appear to be reliable, the standard deviations are relatively small, and virtually without overlap between the gouty and the nongouty. The deficit persisted despite absolute changes in glutamine excretion as the protein intake was increased or decreased, so could not be ascribed to variations in the diet. After oral loading with glutamine the plasma glutamine promptly increased to the same degree in the gouty and the nongouty subjects but the mean peak urinary excretion of glutamine was only 0.406 ±0.078 μmole/min in the gouty as compared to 0.670 ±0.129 μmole/min in the nongouty, and $C_{glutamine}$/GFR increased only to 0.54% as compared to 0.93% in the nongouty. The reduced renal elimination of free glutamine in the gouty was found not to be compensated for by any increase in glutamine conjugates in the urine (in fact, excretion of glutamine in the phenylacetylglutamine fraction was decreased), nor could it be related to differences in urine pH over the range encountered.

It seems reasonable to surmise that the discriminating

tubular reabsorption of glutamine in increased amounts in primary gout is related in some way, perhaps compensatorily, to the deficiency in urinary ammonium excretion (in acid urine) previously reported in this disorder (23, 24). The first indication of the defect in renal production of ammonia was obtained when, after giving glycine-^{15}N, only about 1% of the total ^{15}N dose appeared as ammonium-^{15}N in the initial 24 hr urine in the gouty as compared to a mean of 2.7% in normal man (1, 2). This deficit was found to be due to less ammonium eliminated in the urine, not to less than normal ^{15}N enrichment of the urinary ammonium. Subsequent studies (23, 24) on a large gouty population, screened to remove those with reduced GFR or otherwise detectable renal disease, disclosed a mean deficit of 8 μmoles/min in urinary ammonium excretion, in relation to (acid) urine pH, in the fasting state, a deficiency of some 25–30%. It would appear that this deficit in formation of ammonia buffer by the kidneys is largely responsible for the undue and persistent acidity of the urine characteristic of many patients with primary gout (24), and this in turn is believed to play a major role in the prevalence of uric acid nephrolithiasis in gouty subjects, of the order of 1000 times greater than that in the population at large.[1]

All the gouty subjects of the present study had reduced urinary excretion of glutamine (overexcretors and normoexcretors of uric acid alike) and most of them also had reduced urinary ammonium excretion in relation to urine pH. Beyond this general correspondence no simple correlation could be made out between the degree of reduction in urinary excretion of glutamine and the degree of reduction in urinary excretion of ammonium. After glutamine loading the increase in urinary excretion of glutamine in the gouty was significantly less than in the nongouty, as already mentioned, whereas the peak increment in urinary ammonium excretion was about the same in both groups (mean, approximately 10 μmoles/min), although the total ammonium excretion remained lower in the gouty. The increase in urinary excretion of uric acid after glutamine loading was uniformly greater in the gouty subjects.

Glutamine being the predominant source of the ammonia formed by the kidneys (32), the imputed defect in renal production of ammonia in primary gout might be due to impaired extraction of glutamine by the kidneys. The net uptake of glutamine by the kidneys was found by Owen and Robinson (16) to average 47.4 μmoles/min in six normal human subjects under ordinary conditions of ammoniogenesis. No comparable figures are available for primary gout but the results of the present study do permit an estimate of the quantity of glutamine made available to the kidneys by way of tubular reabsorption of the filtered glutamine. In normal man, if the mean free glutamine of the arterial plasma as determined by AutoAnalyzers is approximated in round numbers as 0.5 μmole/ml and the GFR is taken to be 120 ml/min, the free glutamine filtered at the glomerulus would be 60 μmoles/min of which, according to our figures, a mean of 99.2% (59.5 μmole/min) would be reabsorbed (cf. 15, 33). Thus, although reabsorption of the filtered glutamine presumably represents only a minor fraction of the glutamine delivered to the tubules, more than enough is reabsorbed to account for all the ammonium ordinarily excreted in the urine if both the amide and amino nitrogen of all the glutamine were fully utilized for the purpose; indeed almost enough to supply all the glutamine required for total renal production of ammonia. In primary gout the filtered glutamine loads are the same but, according to our data, 99.6% is reabsorbed. Extraction of glutamine from the luminal surface of the tubular cells is therefore not only not reduced in primary gout but in fact somewhat in excess of the normal. To be sure, the extra glutamine thus made available to the kidneys is small, some 0.20 μmole/min; in fact the extra glutamine that could be made available by 100% tubular reabsorption of the filtered glutamine would be only of the order of 0.5 μmole/min. Moreover, there is no assurance that the extra glutamine reabsorbed enters a common glutamine pool and is utilized by the kidneys for ammonia production.

There remains the possibility that impaired production of ammonia by the kidneys in patients with primary gout and intact renal mass may be the result of deficient extraction of glutamine at the pericapillary border of the tubular cells, or of a defect in renal enzymatic liberation of ammonia from glutamine. Deficient extraction at the pericapillary border could not be ascribed to less than normal arterial plasma glutamine concentrations, which are within the normal range to judge by peripheral venous plasma concentrations and a few direct determinations in arterial blood we have made. Renal plasma flow is somewhat reduced in patients with primary gout, for reasons unexplained, but not ordinarily to a degree sufficient to account for the deficit in ammonia production, nor would the resulting deficits be confined to ammonia production. The possibility of a defect in transfer of glutamine across the pericapillary border of the tubular cells has not yet been subjected to experimental examination.

The only data on renal glutaminase-1 available at present are the microassays of Pollak and Mattenheimer (34) in four cases of gout subjected to kidney biopsy. These

[1] It should be pointed out that there is no general agreement as to whether urinary ammonium excretion in gout is or is not deficient. Most relevant studies concern patients with uric acid stone, with or without accompanying hyperuricemia, some reporting urinary ammonium excretion low in relation to the degree of acidity of the urine, others not. A detailed discussion of these discrepant findings is given elsewhere (24, 31).

investigators demonstrated the presence of the enzyme but their normal control values were so variable, because of the technical difficulties involved, that the question of a deficiency of the order in question in gout remains in abeyance. An indirect approach to the problem might be made by determination of glutamine concentrations in renal venous plasma (our figures refer to antecubital venous plasma). If renal extraction of glutamine is unimpaired in gouty subjects, but less glutamine is utilized for ammonia formation because of an enzyme deficiency, renal tissue concentrations of glutamine should be augmented and the glutamine returned to the renal vein should be increased. The anticipated increase would be small, however. In normal man Owen and Robinson (16) found a mean arterial-renal venous difference in glutamine concentration of only 0.0615 μmole/ml, sufficient to effect the large renal extraction of glutamine by virtue of the high rate of renal blood flow. On the basis of our data in primary gout the increase in renal venous glutamine expected would be but a minor fraction of the normal arterial-renal venous difference in glutamine concentration.

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