

Effect of Glycine Loading on Plasma and Urinary Uric Acid and Amino Acids in Normal and Gouty Subjects

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A single oral dose of glycine, 100 mg/kg body weight, a "physiologic" load, was given to ten normal men and seventeen patients with primary gout. The ensuing uricosuria, chiefly due to increased renal clearance of uric acid, was relatively greater in the gouty subjects. That the uricosuria was not due to competition between glycine and uric acid for a common reabsorptive mechanism was shown by giving probenecid, which increased renal excretion of uric acid but not of glycine or other amino acids. Pyrazinamide abolished the glycine-induced uricosuria, which is therefore assumed to be due to enhanced tubular secretion of uric acid. Glycine loading increased urinary excretion of ammonium to about the same degree in gouty and nongouty subjects; there does not seem to be any defect in utilization of glycine for renal formation of ammonia in gout. In the six normal and seven gouty subjects so examined, glycine loading evoked a similar marked increase in plasma glycine and serine, and a significant increase in urinary excretion not only of glycine and serine but also of a variety of other monoamino-monocarboxylic acids, consistent with a common tubular reabsorptive mechanism for these amino acids. The gouty subjects showed no statistically significant aberration in the response of plasma and urinary free amino acids to glycine loading.

A previous comparison of free amino acid concentrations in the plasma and urine of normal and gouty subjects [1] revealed a statistically significant mean decrease in plasma glycine in gouty subjects. Since glycine is a uric acid precursor, contributing C-4, C-5 and N-7 in de novo purine biosynthesis, this finding would appear to be meaningful and deserving of further study. We now record the effects of oral glycine loading.

It has long been known that glycine loading increases urinary excretion of uric acid in man [2-4]. At first this uricosuria was believed to reflect augmented metabolic formation of uric acid [2], but subsequent evidence [4,5] implicates a chiefly renal effect. The results of our present study in both normal and gouty man are in agreement with this latter view but suggest that the uricosuria is not due to inhibition of tubular reabsorption of filtered urate, such as is characteristic of uricosuric drugs generally [6], but predominantly to enhancement of tubular secretion of urate.

It has also been known for some time that rapid intravenous infusion of glycine in the dog causes an increase in urinary excretion not only of glycine but also of many other amino acids [7]. Documentation of this effect in man, particularly gouty man, is sparse and conflicting [5,8-10]. The data here presented indicate that when normal or gouty man is given glycine orally, even in the moderate quantities present in ordinary meals that include gelatine-rich foods [11], a statistically significant increase in the renal clearance of a number of monoamino-monocarboxylic acids ensues.

METHODS

Glycine loading tests were carried out in ten normal men, aged twenty-five to sixty-five years (mean, forty-five years), and seventeen male patients with primary gout, aged twenty-eight to fifty-eight years (mean, forty-three years). All were free of detectable renal disease. The mean endogenous creatinine clearance in the nongouty subjects was 118 ± 22 ml/minute and 114 ± 10 ml in the gouty subjects. The mean urinary total nitrogen excretion in the gouty subjects, many of whom were on a restricted diet,

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TABLE I Effect of Glycine Load on Plasma and Urinary Uric Acid and Urinary Excretion of Ammonium, Titratable Acidity, Urea and Total Nitrogen (means \pm S.D.)

Time (hr)	C_{cr} (ml/min)	P_{ur} (mg %)	$U_{ur}V$ (mg/min)	pH	NH_4^+ (μ M/min)	T.A. (μ M/min)	Urea N (mg/min)	Total N (mg/min)
Normal Subjects (N = 10)								
0	118 \pm 22	5.7 \pm 0.6	0.49 \pm 0.09	5.5	35.0 \pm 8.1	24.0 \pm 6.9	9.6 \pm 2.3	11.3 \pm 2.8
1	122 \pm 15	5.7 \pm 0.6	0.63 \pm 0.14	6.0	35.7 \pm 17.2	19.2 \pm 11.0	9.8 \pm 2.8	11.2 \pm 2.9
2	122 \pm 18	5.6 \pm 0.6	0.73 \pm 0.12	6.2	39.5 \pm 13.8	14.9 \pm 6.8	11.3 \pm 2.8	13.1 \pm 2.8
3	116 \pm 15	5.6 \pm 0.7	0.61 \pm 0.16	5.9	41.1 \pm 9.7	19.7 \pm 4.8	10.6 \pm 2.2	12.1 \pm 2.3
4	116 \pm 18	5.4 \pm 0.4	0.52 \pm 0.09	5.5	38.2 \pm 8.8	25.9 \pm 4.5	10.1 \pm 1.7	11.5 \pm 1.7
Gouty Subjects (N = 17)								
0	114 \pm 10	10.5 \pm 1.3	0.58 \pm 0.15	5.2	28.6 \pm 7.1	27.4 \pm 10.6	8.3 \pm 1.2	9.7 \pm 1.5
1	113 \pm 16	10.4 \pm 1.2	0.81 \pm 0.21	5.4	32.6 \pm 11.9	24.4 \pm 9.4	8.9 \pm 1.1	10.2 \pm 1.5
2	117 \pm 12	10.3 \pm 1.2	1.06 \pm 0.23	5.7	37.8 \pm 15.9	22.8 \pm 11.6	10.5 \pm 1.6	12.2 \pm 1.7
3	107 \pm 12	10.3 \pm 1.2	0.77 \pm 0.14	5.6	29.3 \pm 10.8	22.1 \pm 11.9	9.3 \pm 1.6	10.7 \pm 1.6
4	113 \pm 8	10.7 \pm 0.9	0.66 \pm 0.16	5.2	27.9 \pm 5.6	27.6 \pm 8.3	8.9 \pm 1.6	10.2 \pm 1.8

NOTE: C_{cr} = creatinine clearance; P_{ur} = plasma uric acid; $U_{ur}V$ = urinary uric acid excretion; T.A. = titratable acidity.

was less than in the normal controls, 9.4 versus 12.2 gm/day. On this dietary intake ten of the patients with gout excreted less than 800 mg uric acid/twenty-four hours and were classified as gouty normoexcretors of uric acid, the remaining seven excreted more and were classified as overexcretors. Except for colchicine prophylaxis, drug treatment of the patients with gout was discontinued well in advance of the study.

After an overnight fast, a preloading two hour urine sample was collected, and blood was drawn from an antecubital vein. Each subject was then given a single oral dose of 100 mg glycine/kg body weight in 250 ml of milk; the average total glycine dose was 7.9 and 8.9 gm for the normal and gouty subjects, respectively. Hourly blood and spontaneously voided urine collections were then made for two to four hours.

Plasma and urinary uric acid, and urinary excretion of ammonium, titratable acid, urea and total nitrogen were measured in all ten normal and seventeen gouty subjects before and after glycine loading. In six of the normal and seven of the gouty subjects plasma and urinary free amino acids were also measured. In further observations on one normal and two gouty subjects the same protocol was used, except that pyrazinamide, 3 gm, was given orally first and then a glycine load two hours later. Two additional normal and four gouty subjects received only probenecid, 2 gm orally, to observe the effects of this uricosuric drug on the amino acids in the plasma and urine.

A Technicon Model NC-1 standard twenty-one hour amino acid autoanalyzer was used to estimate the free amino acids in plasma and urine, usually on the day of procurement. Plasma and urine samples were prepared and applied as previously described [1]. To minimize degradation of glutamine the columns were operated at 30°C for the first four or five hours, then at the conventional 60°C. Quantification of proline, aspartic acid, asparagine and methylhistidines was unsatisfactory for reasons stated elsewhere [1], and no estimates for these components are given in the tables. The figures for histidine in the urine are lower in this study than in those previously cited [1] because, by more frequent use of Efron's buffer system, overlap with 3-methylhistidine could be avoided.

The methods for determining uric acid, creatinine, ammonia, titratable acid, urea and total nitrogen were the same as previously indicated [12].

RESULTS

Impact of Glycine Load on Urinary Uric Acid Excretion. The glycine loads administered elicited no significant change in plasma urate concentrations in either the normal or gouty subjects tested, but in both groups there was a uniform increase in urinary excretion of uric acid, hence in renal clearance of uric acid (Table I). In ten **normal** subjects, the initial mean urinary uric acid excretion ($U_{ur}V$) of 0.49 \pm 0.09 mg/minute rose after one hour to 0.63 \pm 0.14 mg/minute, reached a mean peak of 0.73 \pm 0.12 mg/minute in two hours ($p < 0.001$) and then declined. Concomitantly, the ratio of uric acid clearance to creatinine clearance ($C_{ur} : C_{cr}$) increased from an initial mean of 7.6 \pm 1.6 per cent to a mean second hour peak of 11.0 \pm 2.8 per cent ($0.01 > p > 0.001$), then declined. In ten **gouty normoexcretors** of uric acid the initial mean $U_{ur}V$ of 0.47 \pm 0.09 mg/minute rose to a mean second hour peak of 0.94 \pm 0.20 mg/minute ($p < 0.001$), a peak rate somewhat higher than in the normal subjects, but the difference is not statistically significant. $C_{ur} : C_{cr}$ increased from an initial mean of 4.3 \pm 0.9 per cent to a mean peak of 8.2 \pm 1.4 per cent ($p < 0.001$). Both the initial and peak clearance rates were significantly lower than normal ($p < 0.001$ and $0.02 > p > 0.01$, respectively). In seven **gouty overexcretors** of uric acid the initial mean $U_{ur}V$ of 0.74 \pm 0.08 mg/minute rose to a mean second hour peak of 1.22 \pm 0.15 mg/minute ($p < 0.001$), both figures significantly higher than normal ($p < 0.001$ in each instance). $C_{ur} : C_{cr}$ increased from 5.6 \pm 0.9 to 9.6 \pm 1.7 per cent ($p < 0.001$); these clearance ratios are both somewhat lower than in the normal subjects, but the differences are statistically significant only for the initial clearance ratio ($p = 0.01$).

Probenecid was administered to four gouty subjects to ascertain whether this uricosuric drug would also augment the urinary excretion of glycine and other amino acids. As

TABLE II Comparison of Effect of Probenecid on Renal Clearances of Urate and Glycine in Four Patients with Primary Gout

P_{ur} (mg %)	$C_{ur}:C_{cr}$ (%)	$P_{glycine}$ (μ M/ml)	$C_{glycine}:C_{cr}$ (%)
Control (means)			
10.4	5.4	0.221	2.21
Two Hours After Probenecid, 2 gm Oral Dose (means)			
8.9	34.4	0.200	2.60

shown in Table II, probenecid in dosage sufficient to increase $C_{ur}:C_{cr}$ from 5.4 to 34.4 per cent had no appreciable effect on the renal clearance of glycine; an insignificant response was noted also in the renal clearance of alanine, glutamine, serine, threonine and other amino acids. Similar results were obtained in two normal subjects.

Table III summarizes an experiment in which a 3 gm oral dose of pyrazinamide was given to a gouty subject, resulting in a characteristic sharp decline in renal elimination of uric acid, $C_{ur}:C_{cr}$ falling from 6.8 to 0.9 per cent in the second hour. There was no concomitant effect on the renal clearance of glycine or other amino acids measured simultaneously. A glycine load was then given, with a prompt rise in plasma and urinary levels of glycine; however, the increase in renal clearance of uric acid which would ordinarily occur after glycine loading was not in evidence, being nullified by the prior administration of pyrazinamide. Similar results were obtained in a normal subject and another patient with primary gout.

Impact of Glycine Load on Urine pH and Urinary Excretion of Ammonium, Titratable Acid, Urea and Total Nitrogen. Glycine serves as a minor source of the ammonia formed by the kidneys. In the acidotic dog, 3 to 4 per cent of the urinary ammonium was found to derive from glycine [13], a proportion that increased roughly as a linear function of plasma glycine when glycine was infused [13]. As shown in Table I, administration of glycine to man likewise is followed by a slight increase in urinary excretion of ammonium [14], with an accompanying rise in urine pH and decline in titratable acidity. In the normal subjects, the initial mean urinary

TABLE III Renal Clearances of Urate and Glycine After Administration of Pyrazinamide, Followed by Glycine Load, in a Gouty Subject

Time (hr)	P_{ur} (mg %)	$C_{ur}:C_{cr}$ (%)	$P_{glycine}$ (μ M/ml)	$C_{glycine}:C_{cr}$ (%)
-0.5	8.5	6.8	0.195	1.60
Pyrazinamide (3 gm orally)				
0 to 1	8.1	4.9	0.180	1.26
1 to 2	8.5	0.9	0.197	1.57
Glycine (100 mg/kg orally)				
2 to 3	8.1	0.9	1.00	7.73
3 to 4	8.3	0.8	0.62	4.78

ammonium excretion of $35.0 \pm 8.1 \mu$ M/minute reached a peak of $41.1 \pm 9.7 \mu$ M/minute in the third hour, and the urine pH rose from a mean of 5.5 to a mean peak of 6.2. In the gouty subjects the initial pH tended to be more acid (mean 5.2), but the urinary excretion of ammonium was nevertheless somewhat less than normal (mean, $28.6 \pm 7.1 \mu$ M/minute) ($p = 0.05$), as previously noted [12,15]. In response to glycine loading the urinary excretion of ammonium increased substantially to a mean peak of $37.8 \pm 15.9 \mu$ M/minute, not significantly different from that in the normal subjects. The urine pH also rose, but only to 5.7, lower than the normal peak. The peak increase in urinary total nitrogen in both groups occurred in the second hour after glycine loading and was predominantly due to the increased elimination of glycine nitrogen as urea.

Impact of Glycine Load on Free Amino Acids in the Plasma (Table IV). In the normal subjects the mean plasma glycine level, initially $0.270 \pm 0.056 \mu$ M/ml, rose to $0.827 \pm 0.129 \mu$ M/ml in the first hour after a single oral dose of 100 mg glycine/kg body weight, reached a mean fourfold peak of $1.031 \pm 0.331 \mu$ M/ml in the second hour, then declined. Also, the initial mean plasma serine concentration of $0.121 \pm 0.023 \mu$ M/ml rose to $0.147 \pm 0.032 \mu$ M/ml in the first hour and to a mean peak of $0.220 \pm 0.036 \mu$ M/ml in the second hour ($p < 0.001$). There was, in addition, some increase in the mean levels of plasma alanine, threonine, valine, glutamic acid and several other amino acids, but none of these were of statistical significance.

In the gouty subjects the initial mean plasma glycine of $0.214 \pm 0.027 \mu$ M/ml increased to a mean fivefold peak of $1.091 \pm 0.162 \mu$ M in the first hour and remained at $1.034 \pm 0.182 \mu$ M/ml in the second hour, then declined. The initial mean plasma serine level of $0.109 \pm 0.019 \mu$ M/ml rose to $0.158 \pm 0.023 \mu$ M/ml in the first hour and to a mean peak of $0.204 \pm 0.026 \mu$ M/ml in the second hour ($p < 0.001$). There were also smaller increases, of less statistical significance, in several other amino acids: in plasma alanine ($0.02 > p > 0.01$), threonine ($0.05 > p > 0.01$), glutamic acid ($0.05 > p > 0.02$), taurine ($p = 0.02$), methionine ($0.02 > p > 0.01$), isoleucine ($0.02 > p > 0.01$) and leucine ($0.05 > p > 0.02$). Increases in plasma glutamine and valine were not statistically significant.

When the peak increments in plasma free amino acids after glycine loading are compared in the normal and gouty subjects, few differences of statistical significance are noted. Higher in the gouty subjects were the mean peak plasma isoleucine ($p = 0.001$), glutamic acid ($p = 0.01$), tyrosine ($0.05 > p > 0.02$) and alanine ($p = 0.05$). Differences in mean peak increments in plasma glycine, serine or any other amino acid except those mentioned, were not of statistical significance.

Impact of Glycine Load on Free Amino Acids in the Urine (Tables V and VI). With rising filtered glycine loads both tubular reabsorption and urinary excretion of the filtered glycine increased, the latter disproportionately [cf. 8]. In our normal subjects, the filtered glycine load initially averaged 34.34μ M/minute of which a mean of 33.5μ M/minute was

TABLE IV Mean P_{aa} (expressed in $\mu\text{M}/\text{ML}$) in Normal and Gouty Subjects Before and at Hourly Intervals After Glycine Load

Amino Acids	Normal Subjects (N = 6)			Gouty Subjects (N = 7)				
	0 hour	1 hour	2 hours	3 hours	0 hour	1 hour	2 hours	3 hours
Taurine	0.040 ± 0.010	0.059 ± 0.028	0.046 ± 0.004	0.037 ± 0.002	0.044 ± 0.010	0.064 ± 0.014	0.057 ± 0.007	0.044 ± 0.002
Threonine	0.149 ± 0.027	0.174 ± 0.034	0.176 ± 0.028	0.102 ± 0.024	0.132 ± 0.020	0.181 ± 0.052	0.147 ± 0.018	0.162 ± 0.010
Serine	0.121 ± 0.023	0.147 ± 0.032	0.220 ± 0.036	0.145 ± 0.010	0.109 ± 0.019	0.158 ± 0.023	0.204 ± 0.026	0.190 ± 0.023
Glutamine	0.493 ± 0.063	0.466 ± 0.038	0.477 ± 0.044	0.432 ± 0.015	0.430 ± 0.041	0.482 ± 0.099	0.426 ± 0.045	0.472 ± 0.088
Glutamic acid	0.053 ± 0.014	0.070 ± 0.025	0.073 ± 0.025	0.039 ± 0.005	0.082 ± 0.030	0.129 ± 0.036	0.106 ± 0.035	0.099 ± 0.044
Citrulline	0.033 ± 0.003	0.035 ± 0.001	0.030 ± 0.005	0.035 ± 0.001	0.040 ± 0.008	0.046 ± 0.009	0.042 ± 0.007	0.039 ± 0.004
Glycine	0.270 ± 0.056	0.827 ± 0.129	1.031 ± 0.331	0.472 ± 0.034	0.214 ± 0.027	1.091 ± 0.162	1.034 ± 0.182	0.630 ± 0.113
Alanine	0.337 ± 0.077	0.374 ± 0.070	0.402 ± 0.078	0.337 ± 0.004	0.409 ± 0.056	0.497 ± 0.074	0.497 ± 0.049	0.444 ± 0.027
αNH_2 butyric acid	0.024 ± 0.006	0.024 ± 0.006	0.025 ± 0.007	0.017 ± 0.001	0.024 ± 0.005	0.033 ± 0.010	0.029 ± 0.008	0.035 ± 0.001
Valine	0.246 ± 0.028	0.274 ± 0.035	0.266 ± 0.026	0.211 ± 0.005	0.271 ± 0.025	0.318 ± 0.048	0.280 ± 0.032	0.326 ± 0.007
Cystine, half	0.057 ± 0.017	0.057 ± 0.009	0.063 ± 0.013	0.062 ± 0.002	0.054 ± 0.015	0.057 ± 0.020	0.057 ± 0.014	0.058 ± 0.010
Methionine	0.026 ± 0.005	0.027 ± 0.007	0.029 ± 0.005	0.016 ± 0.003	0.026 ± 0.003	0.032 ± 0.004	0.028 ± 0.002	0.033 ± 0.001
Isoleucine	0.063 ± 0.008	0.076 ± 0.016	0.068 ± 0.013	0.053 ± 0.013	0.085 ± 0.016	0.105 ± 0.006	0.088 ± 0.012	0.089 ± 0.006
Leucine	0.145 ± 0.013	0.156 ± 0.032	0.150 ± 0.022	0.112 ± 0.003	0.164 ± 0.015	0.197 ± 0.029	0.168 ± 0.020	0.184 ± 0.013
Tyrosine	0.068 ± 0.012	0.065 ± 0.007	0.062 ± 0.012	0.049 ± 0.005	0.072 ± 0.014	0.078 ± 0.008	0.072 ± 0.013	0.070 ± 0.006
Phenylalanine	0.061 ± 0.004	0.064 ± 0.006	0.060 ± 0.007	0.052 ± 0.001	0.060 ± 0.006	0.067 ± 0.009	0.060 ± 0.008	0.057 ± 0.012
Ornithine	0.081 ± 0.027	0.097 ± 0.017	0.079 ± 0.012	0.073 ± 0.003	0.094 ± 0.013	0.098 ± 0.008	0.082 ± 0.017	0.088 ± 0.011
Lysine	0.241 ± 0.039	0.239 ± 0.038	0.264 ± 0.048	0.195 ± 0.013	0.251 ± 0.037	0.293 ± 0.047	0.261 ± 0.033	0.310 ± 0.008
Histidine	0.108 ± 0.018	0.113 ± 0.025	0.111 ± 0.018	0.082 ± 0.001	0.111 ± 0.022	0.114 ± 0.019	0.103 ± 0.015	0.098 ± 0.006
Arginine	0.109 ± 0.008	0.104 ± 0.010	0.106 ± 0.011	0.095 ± 0.014	0.086 ± 0.018	0.098 ± 0.028	0.093 ± 0.011	0.143 ± 0.001

TABLE V Mean U_{aa}V (expressed in $\mu\text{M}/\text{minute} \pm \text{S.D.}$) in Normal and Gouty Subjects Before and at Hourly Intervals After Glycine Load

Amino Acids	Normal Subjects (N = 6)			Gouty Subjects (N = 7)				
	0 hour	1 hour	2 hours	3 hours	0 hour	1 hour	2 hours	3 hours
Taurine	0.776 ± 0.175	1.201 ± 0.318	0.908 ± 0.284	0.870 ± 0.316	0.906 ± 0.276	1.125 ± 0.387	0.934 ± 0.335	0.983 ± 0.149
Threonine	0.119 ± 0.022	0.225 ± 0.084	0.362 ± 0.120	0.193 ± 0.032	0.071 ± 0.024	0.145 ± 0.064	0.213 ± 0.076	0.189 ± 0.045
Serine	0.228 ± 0.066	0.605 ± 0.178	1.766 ± 0.906	0.766 ± 0.062	0.153 ± 0.032	0.456 ± 0.179	1.152 ± 0.446	0.684 ± 0.254
Glutamine	0.391 ± 0.082	0.612 ± 0.204	1.001 ± 0.271	0.628 ± 0.124	0.191 ± 0.054	0.332 ± 0.101	0.585 ± 0.188	0.440 ± 0.084
Glycine	0.835 ± 0.238	8.519 ± 5.204	15.542 ± 8.766	5.273 ± 1.360	0.493 ± 0.185	7.618 ± 2.414	10.772 ± 3.872	4.544 ± 2.046
Alanine	0.214 ± 0.069	0.365 ± 0.076	0.543 ± 0.198	0.455 ± 0.015	0.185 ± 0.084	0.310 ± 0.129	0.499 ± 0.141	0.271 ± 0.026
Valine	0.037 ± 0.014	0.043 ± 0.012	0.040 ± 0.003	0.045 ± 0.005	0.034 ± 0.012	0.042 ± 0.020	0.045 ± 0.019	0.056 ± 0.015
Cystine, half	0.044 ± 0.025	0.079 ± 0.046	0.069 ± 0.042	0.088 ± 0.012	0.036 ± 0.003	0.053 ± 0.025	0.048 ± 0.019	0.090 ± 0.003
Methionine	0.028 ± 0.006	0.032 ± 0.013	0.028 ± 0.013	0.019 ± 0.010	0.022 ± 0.004	0.029 ± 0.015	0.038 ± 0.013	0.021 ± 0.009
Isoleucine	0.029 ± 0.012	0.042 ± 0.012	0.046 ± 0.028	0.042 ± 0.012	0.027 ± 0.009	0.026 ± 0.009	0.029 ± 0.010	0.036 ± 0.009
Leucine	0.042 ± 0.008	0.054 ± 0.013	0.050 ± 0.007	0.034 ± 0.008	0.028 ± 0.012	0.038 ± 0.016	0.049 ± 0.012	0.023 ± 0.005
Tyrosine	0.100 ± 0.026	0.133 ± 0.048	0.128 ± 0.029	0.090 ± 0.009	0.072 ± 0.022	0.108 ± 0.052	0.118 ± 0.035	0.093 ± 0.021
Phenylalanine	0.050 ± 0.008	0.074 ± 0.024	0.080 ± 0.020	0.078 ± 0.020	0.037 ± 0.011	0.048 ± 0.019	0.044 ± 0.017	0.044 ± 0.012
Lysine	0.682 ± 0.287	0.982 ± 0.312	0.880 ± 0.323	0.753 ± 0.350	0.495 ± 0.209	0.606 ± 0.111	0.645 ± 0.149	0.464 ± 0.148
Histidine	0.689 ± 0.159	1.155 ± 0.323	1.280 ± 0.292	0.708 ± 0.101	0.442 ± 0.121	0.649 ± 0.191	0.881 ± 0.281	0.577 ± 0.279
Arginine	0.024 ± 0.018	0.040 ± 0.014	0.056 ± 0.008	0.032 ± 0.013	0.032 ± 0.013	0.038 ± 0.009	0.038 ± 0.009	...

TABLE VI Mean $C_{\text{max}}:C_{\text{cr}}$ (expressed in per cent \pm S.D.) in Normal and Gouty Subjects Before and at Hourly Intervals After Glycine Load

Amino Acids	Normal Subjects (N = 6)						Gouty Subjects (N = 7)					
	0 hour	1 hour	2 hours	3 hours	0 hour	1 hour	2 hours	3 hours	0 hour	1 hour	2 hours	3 hours
Taurine	14.70 \pm 3.74	19.62 \pm 9.34	16.30 \pm 4.97	21.07 \pm 7.54	18.00 \pm 7.15	14.03 \pm 5.15	13.18 \pm 4.66	22.34 \pm 3.66	0.48 \pm 0.19	0.77 \pm 0.43	1.25 \pm 0.55	1.25 \pm 0.30
Threonine	0.66 \pm 0.15	1.02 \pm 0.28	1.65 \pm 0.43	1.70 \pm 0.06	1.30 \pm 0.46	2.63 \pm 1.18	4.97 \pm 2.14	3.72 \pm 0.74	0.41 \pm 0.15	0.69 \pm 0.34	1.18 \pm 0.38	0.97 \pm 0.28
Serine	1.63 \pm 0.70	3.45 \pm 1.11	6.59 \pm 2.97	4.69 \pm 0.54	1.72 \pm 0.44	1.70 \pm 0.66	1.70 \pm 0.66	3.72 \pm 0.74	0.41 \pm 0.15	0.69 \pm 0.34	1.18 \pm 0.38	0.97 \pm 0.28
Glutamine	0.65 \pm 0.10	1.01 \pm 0.23	1.72 \pm 0.44	1.70 \pm 0.66	1.72 \pm 0.44	1.70 \pm 0.66	1.70 \pm 0.66	0.97 \pm 0.28	0.41 \pm 0.15	0.69 \pm 0.34	1.18 \pm 0.38	0.97 \pm 0.28
Glycine	2.51 \pm 0.67	8.79 \pm 4.79	10.48 \pm 5.21	9.90 \pm 2.55	2.08 \pm 0.76	7.13 \pm 2.83	8.85 \pm 4.51	7.39 \pm 2.10	2.08 \pm 0.76	7.13 \pm 2.83	8.85 \pm 4.51	7.39 \pm 2.10
Alanine	0.55 \pm 0.19	0.81 \pm 0.26	1.09 \pm 0.27	1.20 \pm 0.02	0.40 \pm 0.17	0.55 \pm 0.30	0.86 \pm 0.27	0.62 \pm 0.12	0.40 \pm 0.17	0.55 \pm 0.30	0.86 \pm 0.27	0.62 \pm 0.12
Valine	0.12 \pm 0.05	0.14 \pm 0.06	0.14 \pm 0.03	0.19 \pm 0.02	0.10 \pm 0.04	0.12 \pm 0.07	0.14 \pm 0.07	0.12 \pm 0.05	0.10 \pm 0.04	0.12 \pm 0.07	0.14 \pm 0.07	0.12 \pm 0.05
Cystine, half	0.63 \pm 0.34	1.02 \pm 0.51	1.06 \pm 0.39	1.42 \pm 0.25	0.63 \pm 0.20	0.93 \pm 0.56	0.77 \pm 0.22	1.65 \pm 0.11	0.63 \pm 0.20	0.93 \pm 0.56	0.77 \pm 0.22	1.65 \pm 0.11
Methionine	0.88 \pm 0.25	0.96 \pm 0.47	0.75 \pm 0.19	0.97 \pm 0.44	0.98 \pm 0.56	0.90 \pm 0.42	1.13 \pm 0.37	1.03 \pm 0.61	0.98 \pm 0.56	0.90 \pm 0.42	1.13 \pm 0.37	1.03 \pm 0.61
Isoleucine	0.37 \pm 0.19	0.51 \pm 0.21	0.62 \pm 0.41	0.74 \pm 0.10	0.27 \pm 0.13	0.23 \pm 0.09	0.30 \pm 0.14	0.42 \pm 0.10	0.27 \pm 0.13	0.23 \pm 0.09	0.30 \pm 0.14	0.42 \pm 0.10
Leucine	0.24 \pm 0.06	0.29 \pm 0.10	0.27 \pm 0.05	0.27 \pm 0.06	0.15 \pm 0.07	0.19 \pm 0.12	0.24 \pm 0.07	0.13 \pm 0.01	0.15 \pm 0.07	0.19 \pm 0.12	0.24 \pm 0.07	0.13 \pm 0.01
Tyrosine	1.21 \pm 0.24	1.48 \pm 0.39	1.69 \pm 0.41	1.64 \pm 0.06	0.89 \pm 0.31	1.31 \pm 0.64	1.24 \pm 0.37	1.43 \pm 0.09	0.89 \pm 0.31	1.31 \pm 0.64	1.24 \pm 0.37	1.43 \pm 0.09
Phenylalanine	0.66 \pm 0.06	0.89 \pm 0.43	1.08 \pm 0.26	1.34 \pm 0.36	0.55 \pm 0.21	0.70 \pm 0.35	0.63 \pm 0.27	0.77 \pm 0.23	0.55 \pm 0.21	0.70 \pm 0.35	0.63 \pm 0.27	0.77 \pm 0.23
Lysine	2.12 \pm 0.78	3.19 \pm 0.41	2.75 \pm 0.83	3.36 \pm 1.47	1.69 \pm 0.60	1.82 \pm 0.69	1.76 \pm 0.33	1.22 \pm 0.12	1.69 \pm 0.60	1.82 \pm 0.69	1.76 \pm 0.33	1.22 \pm 0.12
Histidine	5.13 \pm 0.72	7.68 \pm 2.09	9.32 \pm 1.59	7.70 \pm 1.45	4.27 \pm 2.78	5.52 \pm 3.42	7.48 \pm 2.49	5.96 \pm 2.08	4.27 \pm 2.78	5.52 \pm 3.42	7.48 \pm 2.49	5.96 \pm 2.08
Arginine	0.18 \pm 0.12	0.33 \pm 0.17	0.41 \pm 0.03	0.41 \pm 0.03	0.36 \pm 0.15	0.38 \pm 0.05	0.38 \pm 0.05	0.38 \pm 0.05	0.36 \pm 0.15	0.38 \pm 0.05	0.38 \pm 0.05	0.38 \pm 0.05

reabsorbed (97.5 per cent) and $0.835 \pm 0.238 \mu\text{M}/\text{minute}$ was excreted; in the first hour $93.3 \mu\text{M}/\text{minute}$ were reabsorbed (91.2 per cent) and $8.52 \pm 5.20 \mu\text{M}/\text{minute}$ were excreted; in the second hour, when the filtered glycine load reached a peak of $126.6 \mu\text{M}/\text{minute}$, a mean of $111.1 \mu\text{M}/\text{minute}$ was reabsorbed (89.5 per cent) and $15.54 \pm 8.76 \mu\text{M}/\text{minute}$ was excreted. The clearance ratio of glycine to creatinine ($C_{\text{glycine}} : C_{\text{cr}}$) progressively increased from a mean of 2.51 ± 0.67 per cent initially to 8.79 ± 4.79 per cent in the first hour and 10.48 ± 5.21 per cent in the second hour. Similarly in our **gouty** subjects, the filtered glycine load averaged $25.07 \mu\text{M}/\text{minute}$ initially, of which a mean of $24.58 \mu\text{M}/\text{minute}$ was reabsorbed (97.9 per cent) and $0.493 \pm 0.185 \mu\text{M}/\text{minute}$ was excreted. At the peak filtered glycine load of $136.5 \mu\text{M}/\text{minute}$, a mean of $125.75 \mu\text{M}/\text{minute}$ was reabsorbed (91.1 per cent) and $10.77 \pm 3.87 \mu\text{M}/\text{minute}$ was excreted. $C_{\text{glycine}} : C_{\text{cr}}$ rose from an initial mean of 2.08 ± 0.76 per cent to a mean peak of 8.85 ± 4.51 per cent. The differences in the mean increments in urinary glycine excretion ($U_{\text{glycine}}V$) and $C_{\text{glycine}} : C_{\text{cr}}$ in the gouty and nongouty subjects are not of statistical significance. On the average, approximately 1.5 per cent of the total glycine dose administered was recovered as free glycine in the urine over the first three hour period in the normal subjects, 1.1 per cent in the patients with gout.

With the doubling of plasma serine after glycine loading in both the gouty and the nongouty subjects, without appreciable change in glomerular filtration rate, the filtered load of serine correspondingly doubled and, as in the case of glycine, both tubular reabsorption and rejection of serine increased, the latter disproportionately. In the **normal** subjects the filtered serine level rose from a mean of $15.30 \mu\text{M}/\text{minute}$ initially to a mean peak of $27.71 \mu\text{M}/\text{minute}$ in the second hour, the reabsorbed serine increased from an initial mean of $15.07 \mu\text{M}/\text{minute}$ (98.4 per cent) to a mean peak of $25.94 \mu\text{M}/\text{minute}$ (93.4 per cent), the urinary serine excretion increased from an initial mean of $0.228 \pm 0.066 \mu\text{M}/\text{minute}$ to a mean eightfold peak of $1.766 \pm 0.906 \mu\text{M}/\text{minute}$. The clearance ratio of serine to creatinine ($C_{\text{serine}} : C_{\text{cr}}$) rose from an initial mean of 1.63 ± 0.70 per cent to a mean peak of 6.59 ± 2.97 per cent ($0.01 > p > 0.001$). Similarly in the **gouty** subjects the filtered serine level rose from a mean of $12.69 \mu\text{M}/\text{minute}$ initially to a mean peak of $24.38 \mu\text{M}/\text{minute}$ in the second hour, the reabsorbed serine increased from an initial mean of $12.54 \mu\text{M}/\text{minute}$ (98.7 per cent) to a mean peak of $23.23 \mu\text{M}/\text{minute}$ (95.0 per cent), the urinary serine excretion increased from an initial mean of $0.153 \pm 0.032 \mu\text{M}/\text{minute}$ to a mean eightfold peak of $1.152 \pm 0.446 \mu\text{M}/\text{minute}$. $C_{\text{serine}} : C_{\text{cr}}$ correspondingly rose from an initial mean of 1.30 ± 0.46 per cent to a mean peak of 4.97 ± 2.14 per cent ($p = 0.001$). The differences in mean peak urinary serine excretion ($U_{\text{serine}}V$) and $C_{\text{serine}} : C_{\text{cr}}$ in the gouty and nongouty subjects are not statistically significant.

In addition to the increase in renal excretion of glycine (mean, some twentyfold) and serine (mean, about eightfold) in both the normal and gouty subjects after glycine loading,

there was in both groups a mean peak twofold or more increase in the renal excretion of threonine ($p < 0.001$), glutamine ($p < 0.001$), alanine ($p = 0.001$) and histidine ($p = 0.01$). There were also lesser increases in urinary excretion, of borderline or no statistical significance, in several other monoamino-monocarboxylic acids.

The urinary excretion of many amino acids was less before glycine loading in the gouty subjects than in the normal subjects, and the mean peak renal excretion after glycine loading also tended to be somewhat lower in the gouty subjects. These differences at mean peak levels were not statistically significant, however, except for glutamine and threonine. The mean initial glutamine excretion in the gouty subjects was $0.191 \pm 0.054 \mu\text{M}/\text{minute}$ as compared to $0.391 \pm 0.082 \mu\text{M}/\text{minute}$ in the nongouty subjects ($p < 0.001$), and the mean $U_{\text{glutamine}}V$ peak in the gouty subjects after glycine loading was only $0.585 \pm 0.188 \mu\text{M}/\text{minute}$ as compared to $1.001 \pm 0.271 \mu\text{M}/\text{minute}$ in the nongouty subjects ($p = 0.01$). The mean peak $U_{\text{threonine}}V$ was also significantly less in the gouty subjects ($0.05 > p > 0.02$).

The twofold or more increases in urinary excretion of threonine, glutamine, alanine and histidine were associated with twofold or more increases in renal clearance of these amino acids in both the normal and gouty subjects. Thus the ratio of threonine clearance to creatinine clearance ($C_{\text{threonine}} : C_{\text{cr}}$) in the nongouty subjects increased from an initial mean of 0.66 ± 0.15 per cent to a mean peak of 1.65 ± 0.43 per cent ($p < 0.001$), in the gouty subjects from 0.48 ± 0.19 per cent to a mean peak of 1.25 ± 0.55 per cent ($0.01 > p > 0.001$). The clearance ratio of glutamine to creatinine ($C_{\text{glutamine}} : C_{\text{cr}}$) in the nongouty subjects increased from an initial mean of 0.65 ± 0.10 per cent to a mean peak of 1.72 ± 0.44 per cent ($p < 0.001$), in the gouty subjects from 0.41 ± 0.15 per cent to 1.18 ± 0.38 per cent ($p < 0.001$). The clearance ratio of alanine to creatinine ($C_{\text{alanine}} : C_{\text{cr}}$) in the nongouty subjects increased from an initial mean of 0.55 ± 0.19 per cent to a mean peak of 1.20 ± 0.02 per cent ($0.01 > p > 0.001$), in the gouty subjects from 0.40 ± 0.17 per cent to 0.86 ± 0.27 per cent ($0.01 > p > 0.001$). The clearance ratio of histidine to creatinine ($C_{\text{histidine}} : C_{\text{cr}}$) in the nongouty increased from a mean of 5.13 ± 0.72 per cent to a mean peak of 9.32 ± 1.59 per cent ($p < 0.001$), in the gouty subjects from 4.27 ± 2.78 per cent to 7.48 ± 2.49 per cent ($p = 0.05$). There were modest increases in the renal clearance of several other monoamino-monocarboxylic acids, but these were of borderline or no statistical significance.

When the mean peak increments in renal clearance of amino acids after glycine loading are compared in the normal and gouty subjects, the only difference of any statistical significance is the lower renal clearance ratio of glutamine in the gouty subjects ($p = 0.05$).

COMMENTS

The results of our study support the view of Friedman [4] and Kaplan et al. [5] that the uricosuria elicited by glycine

loading is a chiefly renal effect. In both normal and gouty subjects the renal clearance of uric acid increased significantly ($p < 0.001$) to peak levels in two hours, reflecting a significant increase in urinary excretion of uric acid ($p < 0.001$ in both groups) without any measurable rise in plasma urate levels. It is doubtful that augmented production of uric acid contributed appreciably, even in gouty overproducers of uric acid; although there is some labeling of the urinary uric acid within four hours after giving glycine- ^{15}N orally in the same dosage as here employed, peak labeling does not occur until many hours later [16].

It has been inferred [4,5] that the uricosuria following glycine loading is due to successful competition of the excess glycine with filtered urate for tubular reabsorption. This implies a common tubular reabsorptive mechanism for glycine and uric acid. To test this possibility, in our study probenecid, which markedly inhibits tubular reabsorption of the filtered urate [6], was administered in 2 gm dosage. The urinary uric acid excretion increased an average of about sixfold, but there was little or no effect on the renal elimination of glycine or other free amino acids, indicating that uric acid is reabsorbed by a distinct tubular transfer mechanism.

Moreover, evidence was adduced that the increased renal clearance of uric acid after glycine loading is more likely due to increased tubular secretion of uric acid, not to decreased reabsorption. Administration of pyrazinamide, which for reasons given elsewhere [17] is believed to suppress tubular secretion of uric acid in man, caused a sharp reduction in urinary excretion of uric acid, without any change in the renal clearance of glycine or other amino acids. While the pyrazinamide effect continued, a glycine load was administered. Plasma and urinary levels of glycine rose rapidly, but the customary accompanying increase in renal clearance of uric acid was abolished so long as tubular secretion of uric acid was suppressed by pyrazinamide. The mechanism by which glycine loading increases tubular secretion of uric acid is not clear, but it is known that regulation of the rate of secretory transport of uric acid is susceptible to a variety of influences [17-19]. Nor is it apparent why in the gouty subjects after glycine loading the mean increment in $U_{\text{ur}}V$ was twice as large as in the normal subjects (0.48 versus 0.24 mg/minute), a statistically significant difference.

In respect to augmented renal formation of ammonia after glycine loading, this response appeared to be quite normal in our gouty subjects and does not suggest any lack of enzymes for conversion of glycine amino nitrogen to ammonia in the kidneys. This is in contrast to the defect in renal utilization of glutamine for the purpose, which we have postulated to account for the generally lower mean urinary ammonium excretion and urine pH in patients with primary gout [12]. However the data are inadequate for any firm conclusion since ammonia concentrations in the renal venous return were not measured. The varying rise in urine pH in the normal and gouty subjects, and hence in the hydrogen ion concentration gradient, would variably favor augmented diffusion of ammonia into the renal vein. It is

known that blood ammonia may rise to high levels when glycine is infused very rapidly [8,20].

Our prior report on thirty-two patients with primary gout and eighteen normal men [1], who received no glycine loads, pointed to significant differences in both plasma and urinary free amino acids. In the **plasma** of the gouty subjects there were small but statistically significant **decreases** in the mean concentrations of glycine ($0.01 > p > 0.001$), serine ($0.05 > p > 0.02$) and threonine ($p = 0.05$), and small but statistically significant **increases** in glutamic acid ($p < 0.001$), alanine ($0.05 > p > 0.02$) and isoleucine ($0.02 > p > 0.01$). Similarly in the present study of seven patients with primary gout and six normal men the mean control plasma concentrations of glycine, threonine and serine were somewhat lower in the gouty subjects (differences of statistical significance in this smaller series only for glycine, $p = 0.05$), and somewhat higher for glutamic acid ($p = 0.05$), isoleucine ($p = 0.01$) and leucine ($p = 0.05$). The mean plasma total free amino acid concentrations, including estimates for proline and aspartic acid [1], consistently approximated $3.0 \mu\text{M}/\text{ml}$ in both the gouty and nongouty subjects. These results are in general accord with the reports of Derrick and Hanley [9] and Pagliara and Goodman [21] who noted normal levels of plasma α -amino nitrogen in their patients with gout. With respect to aberrations in individual plasma amino acids, Derrick and Hanley [9], who used paper chromatography for semi-quantitative estimations, found "generally similar" amino acid spectrums in their normal and gouty subjects. Pagliara and Goodman [21] employed enzymatic methods to measure only plasma glutamate, which they also noted to be significantly increased, and plasma glutamine, which they also found to be present in normal concentrations. In contrast, Kaplan et al. [22] recorded statistically significant elevations in their gouty subjects not only of serum alanine, isoleucine and leucine, like ourselves, but also of serum glycine, aspartic acid, valine, tyrosine, phenylalanine and lysine, with over-all hyperaminoacidemia.

Our prior report [1] further indicated that **urinary excretion** of virtually all the major free amino acids was less in our gouty than in our normal subjects, with statistically highly significant decreases in urinary excretion and clearance ratios for glutamine, leucine, serine and threonine. In the present series also there were statistically significant decreases in the control figures of the gouty subjects (Table V) for renal excretion of glutamine ($p < 0.001$), threonine ($0.01 > p > 0.001$), leucine ($0.05 > p > 0.01$), serine ($0.05 > p > 0.02$), glycine ($0.05 > p > 0.01$) and histidine ($p = 0.02$). Renal clearance ratios again tended to be lower in the gouty subjects (Table VI) but the only differences of statistical significance were in glutamine ($0.01 > p > 0.001$) and leucine ($p = 0.05$). For reasons given elsewhere [1], these discrepancies are ascribed to the generally lower dietary protein intake of our gouty subjects (mean urinary total nitrogen excretion/twenty-four hours, 9.4 versus 12.2 gm). The lower urinary excretion and renal clearance of amino acids probably reflect the operation of normal homeostatic mechanisms to main-

tain plasma amino acid concentrations within normal limits by regulation of the rate of renal excretion. In the extreme case of glutamine, however, this explanation did not seem to suffice and it was concluded that there is persistently increased tubular reabsorption of this amino acid in primary gout. Kaplan et al. [22] noted diminished renal excretion of threonine, serine, glutamic acid, glycine and alanine in their gouty subjects (glutamine was not measured), but the differences were not statistically significant. In a subsequent publication [5] Kaplan et al. reported statistically significant reductions in the renal clearance ratios of glycine, valine, histidine, glutamic acid, tyrosine and phenylalanine in patients with gout. These results, taken in conjunction with their finding of hyperaminoacidemia in their gouty subjects, were interpreted as signifying an intrinsic defect in tubular transport of amino acids in this disorder.

In our present study of the effects of glycine loading on the plasma and urinary free amino acids, the responses of the gouty subjects did not seem to differ appreciably from the normal. The plasma glycine concentration in both groups rapidly rose to about the same peak level, slightly in excess of $1.0 \mu\text{M}/\text{ml}$. In the urine, Kaplan et al. [5,23] found much smaller increments of glycine in their gouty subjects as compared to their normouricemic subjects ($p < 0.01$), a finding considered to support their view that patients with primary gout have a "defect in transport mechanisms leading to a reduction in secretion of both uric acid and certain amino acids." We also noted a lower mean peak urinary elimination of glycine in our gouty subjects, $10.77 \pm 3.87 \mu\text{M}/\text{minute}$ as compared to $15.54 \pm 8.76 \mu\text{M}/\text{minute}$ in the nongouty subjects, but this difference is not statistically significant. We are not aware of any evidence for secretion of amino acids by the renal tubules in man, nor does the present study offer any.

Concomitant with the mean fourfold or fivefold peak in plasma glycine there was a mean twofold peak increase in plasma serine ($p < 0.001$) in both the gouty and the nongouty subjects. The increase in plasma serine reflects a quantitatively major biosynthetic pathway of glycine metabolism after glycine loading (addition of a hydroxymethyl group by transfer from $\text{N}^5, \text{N}^{10}$ -methylenetetrahydrofolate through the action of L-serine hydroxymethyltransferase), and glycine loading has therefore been employed to test the operation of this enzyme system [24,25]; it is evidently intact in primary gout. There was also some increase in other plasma amino acid concentrations, but these were not statistically significant in the normal subjects and of lesser statistical significance in the gouty subjects, many of whom, as already mentioned, were following a diet restricted in protein intake.

Even in the "physiologic" dosage of glycine administered in this study the maximum tubular capacity for reabsorption of filtered glycine and serine seemed to be approached, as indicated by declines in the ratios reabsorbed : filtered; in the normal subjects from an initial mean of 97.5 to 89.5 per cent for glycine and from 98.4 to 93.4 per cent for serine. The renal clearance ratios accordingly rose, from an initial mean of 2.51 ± 0.67 to a mean peak of 10.48 ± 5.21 per

cent for glycine and from 1.63 ± 0.70 to 6.59 ± 2.97 per cent for serine, with an ensuing mean approximately twentyfold increase in urinary elimination of glycine and a mean approximately eightfold increase in urinary elimination of serine. (Similar responses were obtained in the gouty subjects; the differences were not of statistical significance.*) Near saturation of the tubular reabsorptive capacity for glycine and serine was accompanied in both gouty and

*Derrick and Hanley [9] earlier described the sharp increase in plasma serine in both gouty and normal subjects, with lesser increments in plasma glutamic acid, alanine and glutamine, and the increased urinary elimination not only of serine but also of alanine, glutamine and a variety of other monoamino-monocarboxylic acids. So far as can be ascertained from their semiquantitative tabulations, there were no striking differences in the responses of the gouty and the nongouty subjects. Doolan et al. [8], in normal men, found increased renal clearance of glycine, serine and threonine, but not of other amino acids, after glycine loading.

nongouty subjects by statistically significant increases in the renal clearance of a number of monoamino-monocarboxylic acids, notably glutamine, threonine, histidine and alanine, despite only minor increases in their plasma concentrations and filtered loads. The results imply competition for a common tubular reabsorptive mechanism and are in accord with the view that glycine is reabsorbed, at least in part, by the tubular system for active transport of monoamino-monocarboxylic acids [26,27]. As shown by Scriver et al. [10] glycine is also reabsorbed by the imino acid tubular transport system, which explains the absence of glycinuria in the monoamino-monocarboxylic aciduria characteristic of Hartnup disease [28], and otherwise so closely reproduced by glycine loading in normal and gouty man. We were unable to detect any change in urinary excretion of imino acids after glycine loading, nor did Scriver [29], who however found, conversely, that loading with proline or hydroxyproline increased the renal clearance of glycine.

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